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DOCTORAL THESIS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

STUDY OF THE MECHANISMS OF DRUG PASSAGE THROUGH BIOLOGICAL BARRIERS AIMED TO OPTIMIZE BIOAVAILABILITY AND/OR BLOOD-BRAIN BARRIER PERMEATION

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It has been said that every scientist
sees the truth through his experiments.
I see the truth through the eyes
of Simona. My love for her is in every single page
of this manuscript
as it is
in everything I do.

TABLE OF CONTENTS

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1.0 INTRODUCTION

In order for a drug to be effective, it must cross one or more biological membranes. Over one fourth of the new drug candidate pharmaceutical development failures occur due to unsatisfactorily pharmacokinetic properties [Van de Waterbeemd and Testa, 1987]. Therefore, much effort is put into the optimization of the pharmacokinetic properties of new chemical entities (NCEs), as well as into the achievement of desirable pharmacodynamics (PD) features. Furthermore, some barriers have peculiar features that enable them to selectively regulate the uptake of nutrients and other substances. The most selective and extensively studied biological barrier is the Blood-Brain barrier (BBB), that protects the integrity of the Central Nervous System (CNS); the BBB is probably the most common target for the medicinal chemists who wish to design new CNS active drug/prodrug. Beside, the intestinal epithelium is a single-cell layer that constitutes the largest and most important barrier against the external environment. It acts as a selectively permeable barrier, permitting the absorption of nutrients, electrolytes, and water while maintaining an effective defense against intraluminal toxins, antigens, and enteric flora.

Membrane barrier passage of drugs can occur either by paracellular (through the gaps that separate adjacent cells) or by transcellular pathway [Avdeef et al., 2012]. While a contribution of first pathway is likely for small hydrophilic molecules only, the latter is common for the majority of substances and can realize by active (ATP-dependent) or passive mechanisms. Although several compounds are known to cross biological membranes by active transport mechanisms, most of the drugs are absorbed mainly by passive diffusion. The latter is described by the Fick's first law (equation (1)):

$$\frac{dQ}{dt} = \frac{D \times A \times K}{h} \times (C_{out} - C_{in}) \qquad (1)$$

In the equation above reported (*dQ/dt*) is the diffusion speed through the barrier; *D* is the diffusion coefficient; *A* is the extension of the membrane; *K* is the partition coefficient; *h* is the thickness of the membrane and $(C_{out} - C_{in})$ is the difference of the solute concentration between the inner and the outer side of the membrane. It should be noted that in this equation the terms *A* and *h* are mainly dependent on the properties of the barrier considered, whereas *D* and *K* vary according to the chemical nature of the solutes taken into account. The value of the diffusion coefficient *D* is almost constant for small molecules but it drops dramatically for bulky compounds having molecular weights higher than 450

Da, consequently the parameter that affects predominantly the diffusion speed through the membrane is the partition coefficient *K*. Although many attempts of measuring *in vivo* this value were performed, its direct determination has always been challenging due to the poor reproducibility of the measures.

1.1 In vitro determination of partition coefficient

Historically, many techniques were performed to measure *in vitro* indexes as surrogates of membrane barrier partition coefficients. In particular, the three most regarded systems are:

- Aqueous-organic phase partition;
- Liposome-water partition;
- Chromatographic partition systems based on octadecylsilyl silica (ODS) gel.

1.1.1 Aqueous-organic phase partition studies

Aqueous-organic phase partition studies had been performed since Overton and Mayer, at the beginning of the twentieth century, demonstrated a strong relationship between the potency of general anesthetics and their olive oil-water partition coefficients [Meyer, 1899; Overton, 1901].

Among the different solvent investigated in these studies, *n*-octanol has always been regarded as the one mimicking more closely the interactions actually occurring between drugs and biological membranes; *n*-octanol/water partitioning direct measures are generally performed by *shake-flask* method [Hansch and Clayton, 1973; Takács-Novák and Avdeef, 1996] and the logarithms of the ratio of the concentration that the analyte realizes in *n*-octanol and that it does in water yield log P values. However, these values describe closely the phenomena involved in biological membrane passive diffusion of drugs only if the electrostatic interactions occurring between the analytes and the barrier play a negligible role. The *shake-flask* method has various drawbacks, being a tedious and time-consuming technique as well as requiring the analytes to be of high purity and UV visible. Furthermore, the measures may have high uncertainty when the analytes of interest have extremely low or extremely high lipophilicity values.

1.1.2 Liposome-water partition system

Liposomes have clearly the advantage of resembling much more closely membrane bilayers. They model both the polar and the apolar interactions occurring between the

solute and the biological membranes. In fact, they are vesicles made of various phospholipids with a small amount (in general no more than 8-10%) of cholesterol added in order to improve the stability of the system. However, working with liposomes requires a more considerable care, compared to *n*-octanol/water partitioning experiments. Handling of liposomes should be ideally done under an inert atmosphere at reduced temperatures and prepared suspensions ought to be stored frozen when not used. Air oxidation of *cis*-double bonds is facile as well as hydrolysis of esters to form free fatty acid [Vogel, 2006]. Furthermore, the comparisons among liposome-water partition coefficients determined in different laboratories could be misleading due to high inter-laboratory variability and hard standardization of the liposome preparation techniques.

1.1.3 Chromatographic partition systems based on octadecylsilyl silica gel (ODS)

Although HPLC is generally performed for separation and quantitation purposes, the measure of the affinity that analytes have for ODS stationary phases can be a good approximation of the lipophilic-hydrophobic component involved in the total interaction drug/biomembranes. Its superior speed, accuracy and reproducibility, compared to biochemical and pharmacological methods, make the HPLC technique suitable for rapid screenings of large libraries of compounds. The retention factor *k* is generally calculated according to the following equation (equation (2)):

$$k = \frac{t_r - t_0}{t_0} \qquad (2)$$

in the expression above reported, t_0 is the retention time of an unretained compound and t_r is the retention time of the analyte taken into account. Apart from their higher reproducibility, the HPLC methods have many advantages. In fact, the presence of low levels of impurities and the poor solubility of the compounds do not have a relevant impact on the determination of the retention factors. In addition, this technique is valuable and convenient because it allows to perform the determinations even having very low amount of substance and to measure the *k* values even for extremely lipophilic or extremely polar compounds by setting up specific experimental conditions makes. It should be also underlined that the selectivity of the method allows the analyst to determine various compounds at the same time. The retention factor k is a direct measure of the retention coefficient between the mobile phase and the stationary phase, as described by equation (3):

$$k = \frac{V_s}{V_m} \times K \quad (3)$$

In the equation above reported, V_m is total volume of the solvent flushing through the chromatographic column and V_s is the volume of interphase of the binded stationary phase. Since the ratio V_s/V_m is constant for each column, there's no need to measure V_s. Therefore, *k* can be assumed as a direct measure of K.

However, the partition phases based on ODS or n-octanol cannot accurately reproduce the chemical composition of the biological membranes and therefore do not mirror the interactions actually occurring between the analyte and the phospholipidic bilayer. In fact, the serious lack of predictivity of the lipophilic-hydrophobic component in modeling the drug *in vivo* behavior is evident when ionizable or structurally unrelated compounds are taken into account. For these reasons, there is a growing interest toward stationary phases consisting of Immobilized Artificial Membranes (IAM).

1.2 The IAM-HPLC technique

The use of IAM stationary phases combines the advantages of the rapidity, efficiency and reproducibility of the HPLC methods to the increased similarity (and therefore predictivity) of the membrane phospholipids. They have indeed the potential to model the interactions taking place in water-liposomes partition systems but the reproducibility and flexibility of a high performance liquid chromatography technique [Barbato et al., 1996] (Figure 1). IAM stationary phases consist of phospholipids, covalently binded to a propylamino silica core (Figure 2). They allow to study the polar-electrostatic interactions occurring between the solute and the biological membranes and consequently to gain original information differing from that expressed by *n*-octanol water partition coefficients. Albeit similar for their chemical composition, IAM stationary phases differ markedly from liposome vesicles because of the different surface density. In fact, for each head group the surface area is about 62 Å in liposomes, but 85 Å and 105 Å in single-chain and double-chain IAM stationary phases, respectively. In spite of this difference, several authors pointed out that the polar moieties of these stationary phases mimic rather closely the physico-chemical properties of the fluid membranes [Sheng et al., 1995]. These authors highlighted also how the hydrocarbon moieties have physico-chemical properties similar to fluid membranes. As stated by Pidgeon and others [Pidgeon et al., 1991], the inability of such phases to emulate faithfully the fluid membrane dynamics, is compensated by the increased stability arising

from a solid surface that binds covalently phospholipids. Therefore, IAM stationary phases can mimic efficiently the partition phenomena actually occurring in liposome phospholipid bilayers. Various partition studies were performed by employing IAM-HPLC technique. These studies underlined how the derived interaction scales are original and different from the "classical" *n*-octanol/water lipophilicity scale (log P).

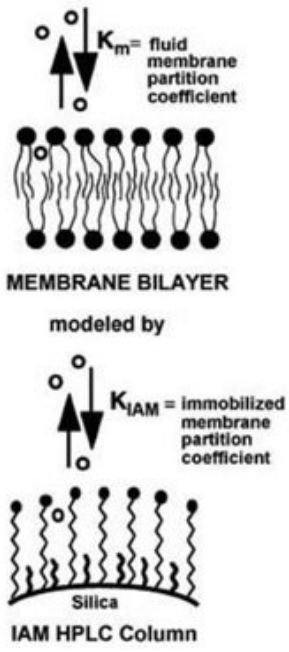

Figure 1. Schematic representation of a fluid membrane bilayer in comparison with an IAM stationary phases.

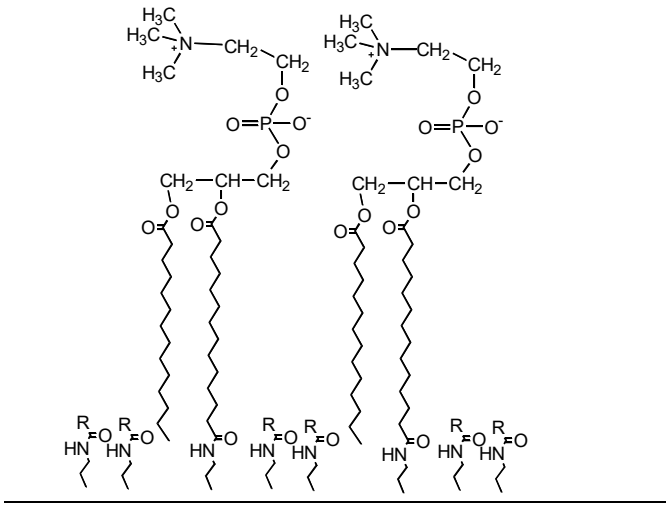

Figure 2. General structure of an IAM stationary phase.

From one hand, strong relationships between IAM partition coefficients and *n*-octanol/water partition coefficients were found for neutral compounds, on the other hand, ionizable compounds showed distinctive partition behavior on IAM stationary phases, particularly for basic compounds. Numerous studies aimed at elucidating the peculiar partitioning behavior of the IAM-HPLC technique were performed in the Department of Pharmacy, University of Naples Federico II. It should be noted that while the first experiments were performed on IAM.PC.MG stationary phases, the first stationary phases to be available on the market, more recent studies regarded also the performance of IAM.PC.DD and IAM.PC.DD2. Indeed, three different kinds of IAM stationary phases have been marketed. As shown in Figure 3, in the IAM.PC.MG stationary phase, the end-capping is performed by methylglicolate giving an outer layer rich in free hydroxyl groups, whereas in the IAM.PC.DD and IAM.PC.DD2 the endcapping of free propylamino cores is performed with C3 and C10 carboxylic acid anhydrides. The IAM.PC.DD does not present any glycerol moiety and therefore is indicated as “single chain” compared to the two others that are instead regarded as “double chain”; it is no longer available on the market due to increased instability and poor reproducibility of the results [Taillardat-Bertschinger et al., 2003].

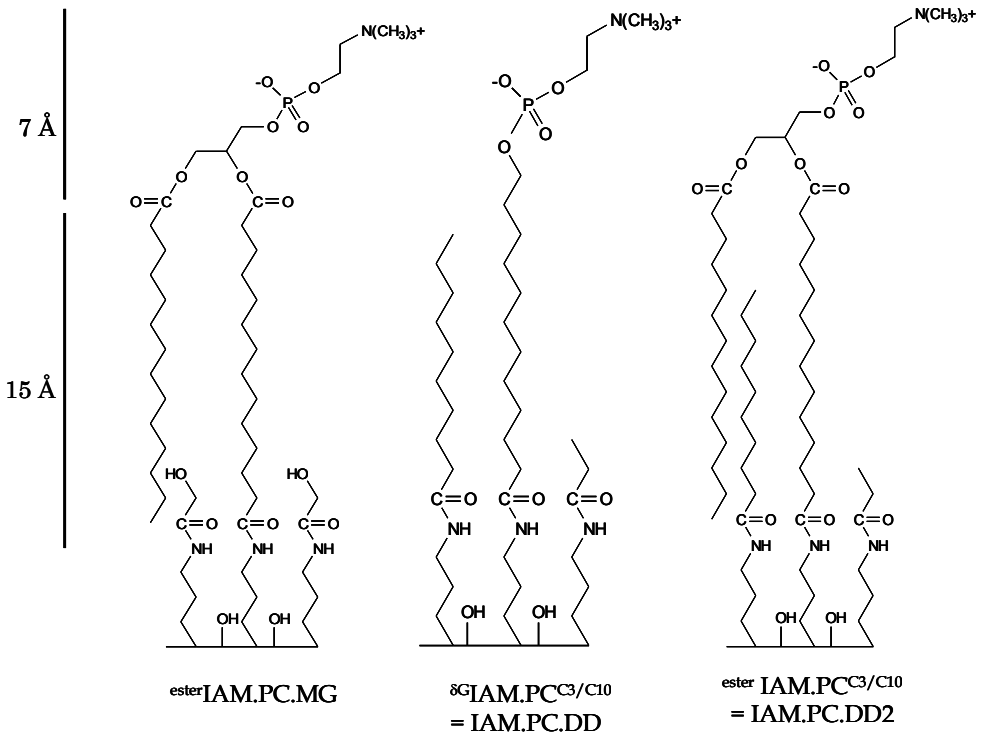

Figure 3. Immobilized Artificial Membrane stationary phases

Most of the results obtained in the present studies are based on the knowledge gained in the past; therefore, it is worth summarizing the most relevant findings. First, in order to obtain a consistent scale of drug/phospholipid affinity it was necessary to obtain retention coefficients employing a fully aqueous medium; in fact, it was noted that employing various percentages of organic modifiers (acetonitrile or methanol) produced rather different scales of interactions. Therefore, the determinations were performed either employing a 0.10 M phosphate buffered solution at pH 7.0 or employing the same buffered solution added to various percentages of organic modifier (acetonitrile) so as to derive the drug phospholipid affinity indexes in fully aqueous medium by an extrapolation method [Braumann et al., 1983]. The choice of employing a pH 7.0 buffered solution arises from the experiences of possible premature aging of the stationary phases caused by employing pH 7.4 buffers giving increased financial expenses for replacing them and poor reproducibility of results.

The first results were those obtained by a study concerning nine calcium-channel blocker dihydropyridines (DHP) [Barbato et al., 1996] performed at the Department of Pharmacy, Federico II University of Naples (formely known as Pharmaceutical Chemistry and Toxicology Department). Among these compounds, seven were neutral compounds and two (amlodipine and nicardipine) bases, because of the presence of a primary and tertiary

amino group moiety in the side chains, respectively. Log k_w^{IAM} values (i.e. logarithms of chromatographic retention coefficients measured or extrapolated to 100% aqueous phase on IAM stationary phases) of the seven neutral dihydropyridines related significantly with *n*-octanol/water lipophilicity values (log P). Surprisingly, log k_w^{IAM} of nicardipine fitted quite well this relationship, although it is ionized for 24% at the experimental pH (pH = 7.0) therefore it would have been more likely that it related better with log $D^{7.4}$, i.e. the lipophilicity of the mixture of neutral and ionized forms at the experimental pH, determined by the same authors at a pH value close to the one employed for the HPLC experiments. It should be pointed out that log D takes into account the ionization degree of the analytes and regards the partitioning of the mixture ionized/neutral form at a given pH. Since nicardipine log k_w^{IAM} related with its log P value better than with its log D value, it was clear that drug phospholipid interaction could be affected in any extent by the protonation of the amino function, probably because of the shielding effect of the polar heads of phosphatidylcholine, strongly binded to the ionized moieties of the molecule. The results of amlodipine were even more astonishing: it had a much higher phospholipid affinity compared to that of an isolipophilic molecule. This led to hypothesize an extra polar interaction between amlodipine and the electrically charged heads of phospholipids, cooperatively acting with the lipophilic-hydrophobic interaction. The hypothesis was consisted with the observation of Austin and coll., who measured a liposome vesicle partition coefficient of amlodipine much higher than that expected on the basis of its log P value [Austin et al., 1995]. It is noteworthy to underline that in the study above mentioned, it was demonstrated that ion-pair mechanisms were not involved in such peculiar interactions in any extent. Interaction mechanisms involved in lipophilicity as measured on different systems are listed in Figure 4.

<table>
<tr><th colspan="2">INTERACTION MECHANISMS</th><th colspan="2">LIPOPHILICITY</th></tr>
<tr><td colspan="2"></td><td>In liposomes, IAM e micelles</td><td>In n-ottanol/ water and RP-HPLC</td></tr>
<tr><td colspan="2">Electrostatic interactions
Charge transfer and aryl/aryl interactions</td><td></td><td></td></tr>
<tr><td colspan="2">Ionic bonds
Ion-dipole forces (permanent, induced)
Charge reinforced H-bonding
Normal H-bonding</td><td rowspan="2">Polarity</td><td rowspan="2">Polarity</td></tr>
<tr><td>Van der Waals Forces</td><td>Orientation forces (permanent dipole – permanent dipole)
Induction forces (permanent dipole – induced dipole)</td></tr>
<tr><td></td><td>Dispersion forces (instantaneous dipole – inducted dipole)
Hydrophobic Interactions</td><td>Hydrophobicity</td><td>Hydrophobicity</td></tr>
</table>

Figure 4. Interaction mechanisms involved in lipophilicity as measured on different systems.

New interesting insight were offered by a study carried out later on that involved 17 structurally unrelated non steroidal anti-inflammatory drugs [Barbato et al., 1997]. The dataset consisted of 16 compounds supporting a carboxyl moiety and of piroxicam, an amphoteric compound. Their phospholipid affinity was measured on IAM stationary phases and it related much better with their log P value rather than with their log $D^{7.0}$ values. This hypothesis was confirmed by the observation that only one equation was able to describe the IAM retention mechanism for structurally unrelated analytes; such equation was

derived employing the log k_w^{IAM} of the seven neutral dihydropyridines and three aromatic hydrocarbons (benzene, toluene and naphthalene) (equation (4)):

$$\log k_w^{IAM} = 0.816\ (\pm 0.035)\ \log P - 1.055\ (\pm 0.140) \qquad (4)$$

$n = 10 \qquad r = 0.993 \qquad s = 0.111$

In this, as well as in the following equations, *n* is the number of observations, *r* is the correlation coefficient and *s* is the standard deviation of the estimates. In parentheses, 95% confidence level are reported. Such relationship was demonstrated also plotting the other 10 acidic compounds whose carboxy groups where not direcly binded to the aromatic ring. This IAM interaction scale was original if compared to the chromatographic retention coefficient measured on ODS stationary phases, for which linear relationships between log k_w^{IAM} and log P were observed for structurally related analytes only.

For the compounds having the carboxy group directly binded to the aromatic ring and for ibuprofen, the regression line had the same slope as the equation 4, but different intercept, being shifted downwards compared to it. This behavior suggests that, for these molecules (identified as "outliers"), the interaction is prevalently driven by repulsive mechanisms. The latter was attributed to the peculiar structural features of these analytes supporting a carboxy group directly binded to the aromatic ring, except for ibuprofen. To rationalize this behavior a hypothesis was casted according to which this particular structure feature interfered negatively in the lipophilic-hydrophobic interaction between the aromatic moieties of the analytes and the phospholipids immobilized on the silica core of the IAM stationary phase. The repulsion given by a negative electrical charge in the lipophilic-hydrophobic interaction was balanced by the polar moieties of the IAM stationary phase; in fact if, instead of the log P values in equation 4, log $D^{7.0}$ values are considered, the log k_w^{IAM} calculated are much lower than the experimental ones. Regarding ibuprofen, no any chemical feature was identified to support its distinctive chromatographic retention pattern.

Equation (4) was also able to predict almost all the chromatographic retention coefficients of a following work that took into account 13 local anesthetics [Barbato et al., 1997]. Among these compounds, only two of them (i.e. tocainide and W36017), showed retention behavior not accurately predicted by equation (4). Indeed, for tocainide extra interactions with the phospholipid moieties were observed: it is worth to note that tocainide together with amlodipine were the only primary amines taken into account. For W36017 a rather weak extra interaction with phospholipids was observed: its inclusion in the equation that relates phospholipid affinity data with *n*-octanol lipophilicity values lowered the statistical

regression coefficients only a little. Subsequently, a study carried out taking into account 23 amines (primary, secondary and tertiary amines) highlighted how the retention on IAM.PC.MG related with *n*-octanol log P scale much better than with log $D^{7.0}$ scale, even if most of the amines were ionized at the experimental pH (7.0). The IAM retention coefficients of only 13 out of 23 amines fitted quite well the regression line generated by equation (4), whereas for the remaining 10 amines much higher retention coefficients were observed. Therefore, the whole dataset was split into two different subgroups: "outliers" that incorporates all the analytes whose IAM retention coefficients were higher than those expected by taking into account equation (4), and the remaining amines whose phospholipid affinity indexes were accurately predicted by the same equation. The former subgroup consisted of endocyclic nitrogen supporting amines and primary amines not completely ionized at the experimental pH (7.0) [Amato et al., 2000]. The latter included secondary and tertiary amines, regardless their ionization degree at pH 7.0, and primary amines completely ionized at the experimental pH. The fact that the relationship between log k_w^{IAM} and log P was better than that with log $D^{7.0}$ supports the evidence according to which phospholipids are able to shield the electric charge on the amino moiety and to interact equally or even more strongly than neutral compounds having lipophilicity values equal to that of the neutral form of each amine.

Such peculiar behavior was explained by the "pH piston hypothesis" [Avdeef et al., 1998] formulated by Avdeef and coll, in an attempt to rationalize the different partition of neutral and ionized compounds into liposomes. According to this hypothesis, the interaction of the analytes with phospholipids occurs in two steps: during the first step, the positive electric charge of the analyte interact with phosphate residues of polar phospholipid heads that are negatively charged so as to relocate, in a second step, into an optimal pathway to diffuse through the hydrophobic tails of phospholipids involving mainly lipophilic-hydrophobic interactions. Such mechanism would occur differently for acidic compounds since the anions interact with positive electrical charge of the choline residue that is located on the outer surface of phospholipid network allowing a relocation that hinder the diffusion through the apolar moieties of phospholipids (Figure 5).

The PhD research project I dealt with is part of a wider research field that has been carrying out at the Department of Pharmacy and involves the design and development of new *in vitro* tools aimed at evaluating the most important pharmacokinetic properties. In a first step, the meaning of this original biochromatographic scale was looked into and carefully investigated, especially in terms of its industrial applicability, because of the emerging need of nover high-throughput methods aimed at evaluating the pharmacokinetic properties of

new chemical entities (NCE) in an accurate way as well as in short time. In a second step, we investigated about the opportunity of combining the phospholipid affinity indexes with other physico-chemical parameters such as *n*-octanol water lipophilicity (log P) or apparent lipophilicity (log D^{pH}) in an attempt to offer new interesting insights into passive diffusion of solutes through biological membranes and predict/surrogate absorption data measured *in vivo*. Indeed, this approach could also lead to identify the structural features required for optimizing the bioavailability and/or the Blood-Brain barrier penetration of new drug candidates.

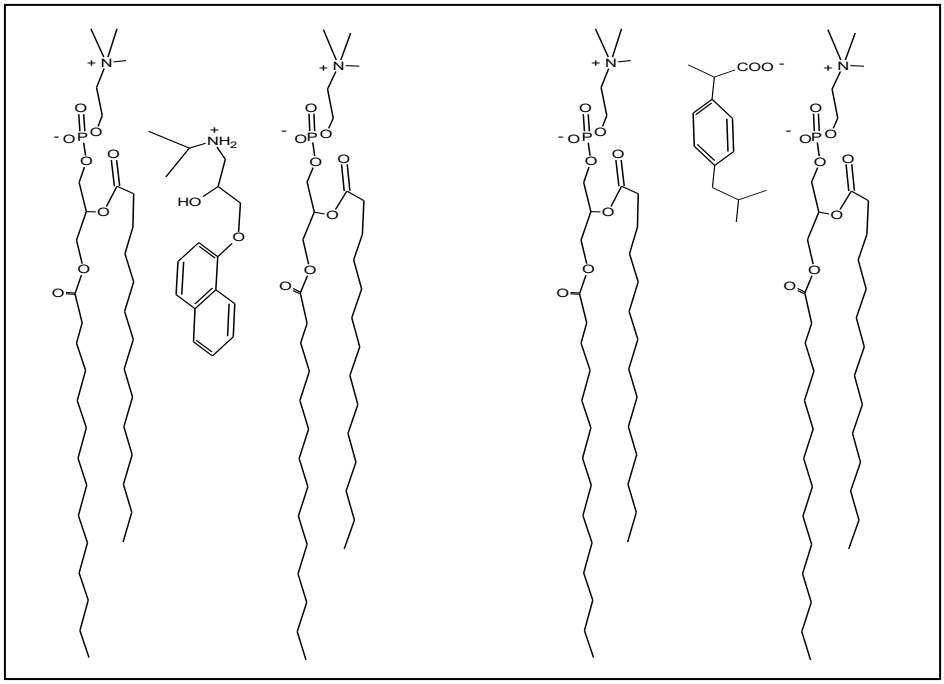

Figure 5. Schematic representation of the interactions between a basic molecule (on the left) and of an acidic molecule (on the right) and phospholipids according to the "pH piston hypothesis".

1.3 Computational Chemistry aided research

Subsequently a computational chemistry aided research leading at evaluating the most important physico-chemical descriptors involved in the phospholipophilicity was performed during a joint project in collaboration with the Drug Design Laboratory of the University "Statale" in Milan, under the supervision of Prof. Dr. Alessandro Pedretti and Prof. Dr. Giulio Vistoli. The development of partial-least-squares (PLS) based statistic models aimed at predicting drug phospholipophilicity offered indeed an interesting opportunity to

predict/surrogate also pharmacokinetic data and see how far these estimates were from actual values to eventually validate the proposed method.

1.4 Micellar liquid chromatography and IAM-HPLC/MS

Furthermore, near the end of my PhD studies, I had the opportunity to spend a seven-month period at the Separation Science Group, University of Ghent, working at the analytical laboratories of Prof. Dr. Frederic Lynen. My activity was focused for the first time on analytical determinations performed by Micellar liquid chromatography (MLC). In this reversed-phase chromatography, a surfactant is added to the aqueous mobile phase at concentrations higher than its critical micelle concentration (CMC), i.e. the concentration at which the surfactant monomers start forming micelles. Therefore, the interactions that the analytes undergo in this kind of chromatography are at least three:

- Partitioning of the analyte into the micellar dispersion;
- Retention of the analyte on the stationary phase modified by the free surfactant monomers;
- Ion-pair interactions, depending on the ionic strength of the mobile phase and on the possible presence of an organic modifier.

Such interactions are described in Figure 6. I had also the opportunity of consolidating my interested in IAM Liquid Chromatography (LC). Indeed, the chromatographic conditions of these experiments were carefully studied and optimized such as to achieve the retention coefficients of the analytes in a relatively short time and meet the demands of pharmaceutical companies in look for high-throughput screening methods. In a second step, the throughput of the technique was further enhanced by the employment of MS detection. The higher selectivity, given by m/z, allowed to analyse simultaneously the analytes of interest, up to 10 or even more in a mixture, thus shortening considerably the working times. The compounds selected for building up the dataset were the ones whose penetration of the Blood-Brain Barrier (BBB) was known from the literature in terms of log BB values. Log BB is the logarithm of the ratio of the concentration that the analyte of interest realizes in brain tissues and that it does in the blood ($\log BB = \frac{C_{Brain}}{C_{Blood}}$). Such measures are universally regarded as the ones more closely resembling what actually happens *in vivo* but are in general affected by poor reproducibility and high uncertainty that make the log BB values measured in different laboratories hardly comparable.

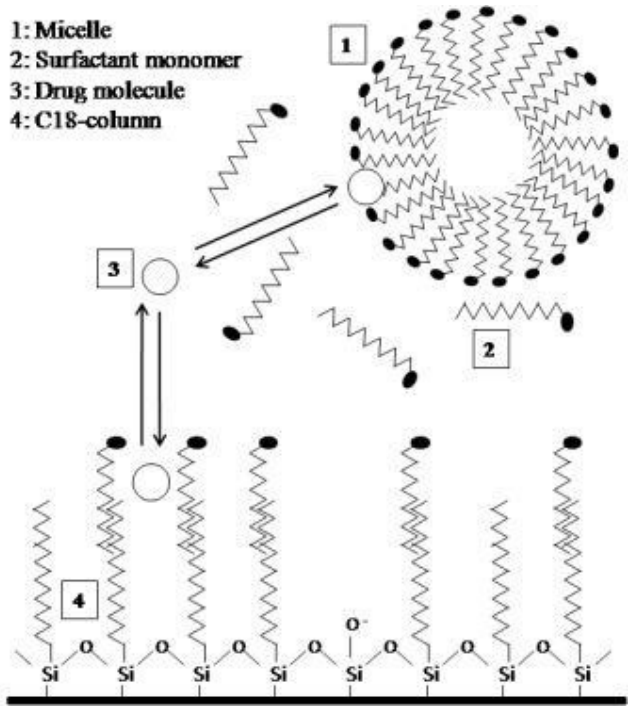

Figure 6. Molecular mechanisms involved in MLC.

1.5 Physico-chemical indexes in BBB partitioning of drugs

The choice of elucidating the mechanisms involved in BBB barrier permeation was due to the fact that the BBB is by far the most selective and extensively studied biological barriers of the human body because it acts hindering the passage of possible injurious substances such as toxins, but providing the uptake of nutrients and physiologically relevant solutes. The way such barrier acts to identify the solutes and which structural requisites are requested for an optimal BBB passage is still a matter of debate. However, it is interesting to note that most of the CNS active drugs support one or more basic moieties and phospholipid affinity studies had far demonstrated an enhanced partitioning of bases compared to neutral isolipophilic compounds giving a distinctive scale in comparison with log P scale obtained by the "*shake-flask*" method. This barrier results from the selectivity of the tight junctions between endothelial cells in CNS vessels that restricts the passage of solutes. At the interface between blood and the brain, endothelial cells are stitched together by these tight junctions, which are composed of smaller subunits, frequently biochemical dimers, that are transmembrane proteins such as occludins, claudins, junctional adhesion molecule, for example. The BBB (Figure 7) is composed of high-density cells restricting passage of substances from the bloodstream much more than the

endothelial cells in capillaries do elsewhere in the body. Astrocyte cell projections called astrocytic feet (also known as "*glia limitans*") surround the endothelial cells of the BBB, providing biochemical support to those cells. It should be also underlined that a solute undergoing passive diffusion can be metabolized by the enzymes possible present in the cells or pumped out the cells by the efflux mechanisms operated, for instance, by the p-glycoprotein. Furthermore, the BBB is not a homogeneous system, because it has portions included in a highly anisotropic phospholipid bilayer and the phospholipid chain mobility is relatively low in the aqueous portion (the blood) and remarkably higher in the hydrophobic core of the phospholipid bilayer (the brain). For a long time, hydrophilic solutes, especially ionized ones, were supposed not able to cross biological membranes; however, this statement strongly contrasts with the experimental evidence that recently demonstrated the passive diffusion also of charged species [Aasmundstad et al., 1995; Krämer et al., 1998]. Plenty of studies were performed in an attempt to elucidate BBB permeation. One of the first QSPR (Quantitative structure-property relationship) performed indicated that the lipophilicity was the key feature to assess in the prediction of drug passage across biological barriers. Hansch and coworkers [Hansch and Clayton, 1973] reported that the ideal log P value for biological membrane permeation was about 2 (log P scale). As a consequence, a minimal lipophilicity principle was formulated according to which the actives designed for a peripheral action have to be as hydrophilic as possible to avoid possible central untoward effects [Hansch et al., 1987]. Another similar study [Dishino et al., 1983] pointed out how for optimal BBB passage it would be necessary that the active has a log P value in the range 0.9 – 2.5; in particular, it was observed a parabolic relationship between log BB and the log P values for the dataset taken into account. A further study, performed on cultured cerebrovascular endothelial cells, demonstrated a parabolic trend between log BB and log D values rather than between log BB and log P values [van Bree et al., 1988] for a set of β-blockers and anti inflammatory drugs. Such relationship was confirmed also by a study on chinolons, amphiphilic molecules, whose permeability was poor for analytes having log D < 0, but increased noticeably for more lipophilic molecules ($0 \geq \log D \geq 2$) [Jaehde et al., 1993].

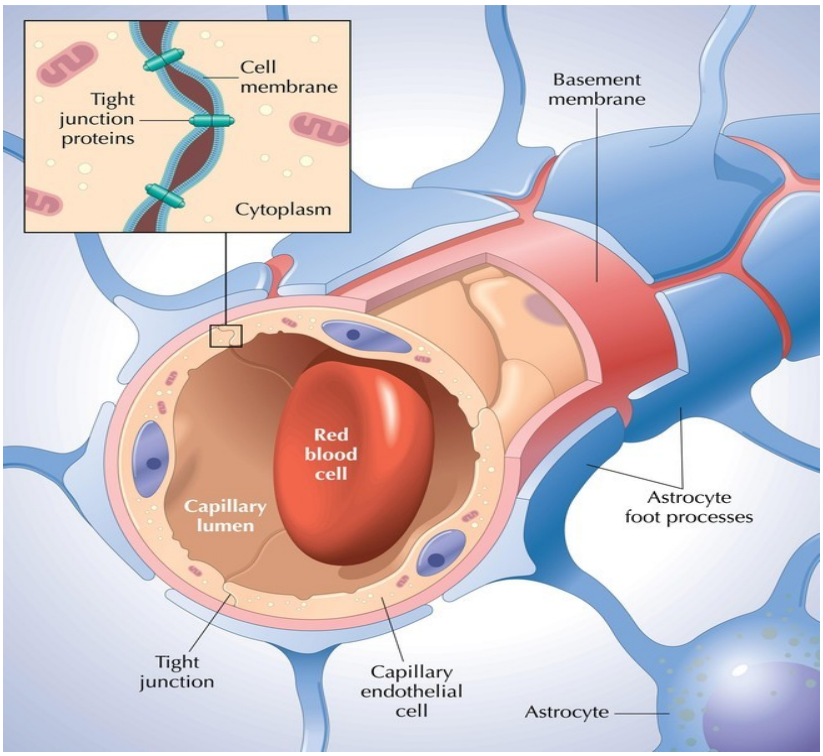

Figure 7. The Blood-Brain Barrier

Another molecular property that affects BBB permeation is for sure the mass weight of the solute; in fact, bulky molecule could be severely hindered in their membrane passage and indeed, in scientific literature, it was reported that CNS active drugs have lower m.w. compared to other drugs. As a consequence, it was hypothesized a mass weight cutoff of 450 Da, according to which molecules heavier than 450 Da are impaired in their membrane crossing. Another molecular properties affecting barrier passage of drugs is for sure, their capability of H-bonding, that is in general estimated by Δlog P, essentially a measure of the H-donor compound capability. Δlog P is the difference between the partitioning in *n*-octanol/water system and that in hydrocarbon/water system, since it is impossible to determine the partitioning between *n*-octanol and hydrocarbon being the two phases miscible.

In a study involving twenty H2 antagonists, $\Delta \log P_{oct-cyc}$ (cyc = cycloesane) was proved as inversely related to the activity toward CNS [Caron et al., 1999]. H-donor capability was also related to the CNS entering potential of seven phenylalanine oligomers esterificated with carboxylic moieties [Pliška et al., 1996]. The biological activity indexes, either measured *in vitro* or *in vivo*, well related to two lipophilicity parameters considered: log P determined in an eptane/ethylene glycol system and $\Delta \log P$. One possible explanation for such behavior is that phospholipids have two H-bonding acceptor moieties that could slower the diffusion

of H-bonding donor moiety supporting analytes. Indeed, the lipophilicity can also be seen as the balance of two different molecular interactions, as reported in equation (5):

- Molecular volume proportional interactions (Hydrophobicity)
- Functional group associated interactions (Polarity)

$$log\ P = a * V - \Lambda \quad (5)$$

The hydrophobicity of a molecule, V, is basically function of its molecular volume and therefore of its mass weight, since these properties are roughly proportional. Polarity, instead, depends on these parameters:

π*, a measure of polarity/polarizability of the molecule

α, a measure of H-bonding donor capability of the molecule (H-bond donor acidity)

β, a measure of the H-bonding acceptor capability of the molecule (H-bond acceptor basicity)

The terms **π***, **α** and **β**, defined as "solvatochromic parameters", are reported in the equation (6):

$$\log P = aV + b\pi * + c\beta + d\alpha + e \quad (6)$$

a, b, c, d and e represent numerical constants that indicate in what extent log P is dependent on the above mentioned parameters. It is possible to determine log P value of an analyte employing a solvent different from *n*-octanol: if the experiments are performed in eptane, a partition index (log P_{ept}) different from the log P gained in the *n*-octanol/water system will be achieved. The different physico-chemical meaning lies in the fact that, differently from *n*-octanol, hydrocarbons are not capable of H-bonding and as a consequence a molecule having considerable polarity will partition in hydrocarbon less than in *n*-octanol. The relationship of either log P or log P_{ept} and the solvatochromic parameters is expressed by the equations (7) and (8):

log P = 5.83 (± 0.53) V/100 – 0.74 (± 0.31) π* - 0.15 (± 0.23) α – 3.51 (± 0.38) β – 0.02 (± 0.34)

(7)

n = 78 r^2 = 0.922 *s* = 0.293

$\log P_{ept}$ = 6.78 (± 0.69) V/100 – 1.02 (± 0.39) π* - 3.54 (± 0.30) α – 5.35 (± 0.50) β – 0.06 (± 0.43) (8)

n=75 r^2 =0.955 *s*=0.360

As it is evident from the above reported equations, *n*-octanol partitioning (log P) is more dependent on V/100 and β, whereas log P_{ept} is dependent on π* and, in much larger extent, on α [Chikhale et al., 1994].

Therefore H-bonding capability is the most distinctive factor between the scales and can be used to gain a further key parameter, just computing the difference between two log Ps, called $\Delta \log P_{oct-cyc}$.

$$\Delta \log P = \log P_{oct} - \log P_{HC} = log \frac{[drug]_{oct}}{[drug]_{aq}} - log \frac{[drug]_{HC}}{[drug]_{aq}} = log \frac{\frac{[drug]_{oct}}{[drug]_{aq}}}{\frac{[drug]_{HC}}{[drug]_{aq}}} = \log \frac{[drug]_{oct}}{[drug]_{HC}} \quad (9)$$

In the above reported equations, the term log P_{HC} refers to the log P values determined in a hydrocarbon (HC)/water partition system. Therefore, Δlog P is the partition coefficient *n*-octanol/hydrocarbon, that is not experimentally achievable being the two phases miscible and indicates drug's H-bonding capability, especially its H-bond donor acidity (α).

It has been widely demonstrated [Abraham et al., 1994] that the BBB crossing potential of a drug is inversely proportional to its H bonding capability, and therefore to its Δlog P. In fact, such parameter could be of use in BBB penetration potential oriented screenings of new drug candidates. This evidence is supported by the "Lipinsky rule of five", according to which for optimal BBB penetration a drug must not support more than five H donor groups and more than ten H acceptor groups, must not be heavier than 500 Da and not have log P higher than five. When two of these criteria are not fulfilled, a poor permeation of this biological barrier is reasonably expected. However, it is known that log P values was not efficient in predicting the partitioning of charged species, for which a prevalent role is played by electrostatic interaction forces. Such forces cannot be neglected because according to a recent estimates reported on the World Drug Index in 2001 about 62.9 % of the drugs on the market are ionizable and of this percentage 14.5% are acids, 67.6% are bases and 17.9 % are ampholytes.

Consequently, the prediction of whichever pharmacokinetic (PK) or pharmacodynamic (PD) property has to take into account also the ionization degree of the compounds of interest; so IAM biochromatography represent an excellent approach for observing the electrostatic forces as well as the lipophilic-hydrophobic ones involved in the whole drug/phospholipids interaction. A research project carried out by the same research group I worked with was aimed at elucidating the mechanisms behind transdermal passage of solutes, a complex phenomenon involving drug-phospholipid interactions, using phospholipid affinity indexes [Barbato et al., 1998]. The dataset consisted of twelve structurally unrelated molecules (acidic, basic and neutral compounds) and no any significant relationship with transdermal absorption capability was observed by taking into account either log P or log k_w^{IAM} values and any improvement was not observed even correcting the log k_w^{IAM} with the mass weights of the analytes. Since other authors found out that existed a significant relationship between the permeability coefficient K_p and $\Delta \log P_{oct-hep}$, a novel physico-chemical parameter, $\Delta\log k_w^{IAM}$, was calculated, encoding mainly (but not exclusively) for the electrostatic interaction forces between drugs and phospholipids. Graphically, this parameter corresponds to the difference from the observed retention behavior and that expected by taking into account the equation (4).

As can be seen in the graph reported in Figure 8, the transdermal permeability decreased at increasing $\Delta\log k_w^{IAM}$ values for all the analytes except for Hydrocortisone and Griseofulvin. Since the latter are the only non-ionizable analytes in the dataset, the extra-interaction observed was probably due to H-bonding.

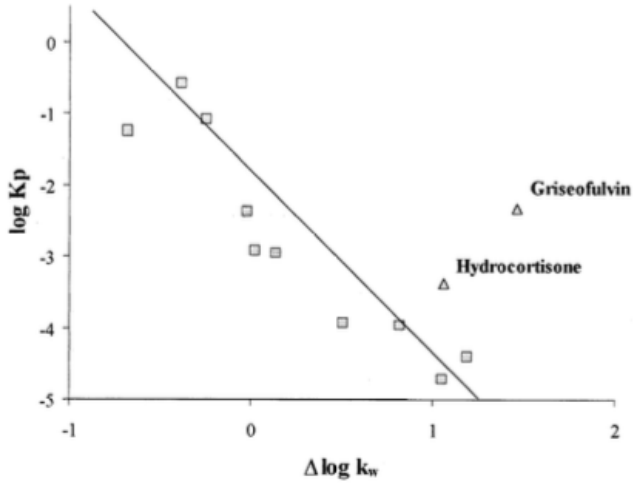

Figure 8. Plot of log K_p (permeability coefficient, in cm/h) vs $\Delta\log k_w^{IAM}$ for a set of 12 compounds.

The results suggest that the molecules having negative or very low values of Δlog k_w^{IAM} can cross the *stratum corneum* more efficiently compared to analytes having high Δlog k_w^{IAM} values. A possible explanation could be that the attractive electrostatic interactions could cause a decreased permeability, while the repulsive ones would provide a permeability enhancing effect.

The soundness of Δlog k_w^{IAM} at describing the mechanisms involved in transdermal passage of drugs was subsequently verified also in terms of BBB penetration potential, since albeit physiologically diverse, membrane passage has been suggested as being a universal process regardless the different composition or function of the membranes considered [Lennernäs et al., 1997].

On IAM.PC.MG and IAM.PC.DD2, log k_w^{IAM} for neutral structurally unrelated compounds relate unambiguously with log P values. Such relationships are even more significant considering analytes having polar surface area (PSA) equal to zero (see chapter 4). However, such evidence led to formulate the equations (10) and (11) generated by the analysis of 36 not ionizable analytes.

$$\log k_w^{IAM.MG} = 0.792\ (\pm 0.038)\ \log P - 0.732\ (\pm 0.105) \qquad (10)$$

$n = 36 \qquad r^2 = 0.926 \qquad s = 0.247$

$$\log k_w^{IAM.DD2} = 0.934\ (\pm 0.038)\ \log P - 0.883\ (\pm 0.104) \qquad (11)$$

$n = 36 \qquad r^2 = 0.946 \qquad s = 0.246$

For each analyte, it is possible to estimate the chromatographic coefficient expected for a neutral molecule having the same log P by the above reported equation. For compounds having positive Δlog k_w^{IAM}, therefore retained in the experimental conditions longer than expected, an extra-interaction, reasonably based on electrostatic interaction forces, can be hypothesized. Such behavior was observed for bases extensively ionized at the experimental pH, for which a stabilization operated by the phosphate moiety, negatively charged, of the phospholipid heads, could be supposed. On the contrary, negative values of Δlog k_w^{IAM}, observed for instance for some acidic anti-inflammatory drugs, indicate that these compounds are retained lower than expected and such evidence can be attributed to a repulsive electrostatic interaction.

Highly significant inverse linear relationships between Δlogk_w^{IAM} and log BB values were observed in a recent work [Grumetto et al., 2012] taking into account 14 structurally unrelated basic drugs, underlining how the excess of the polar and/or electrostatic

component involved in drug/phospholipids acted as a trapping force in drug permeation. In a more recent work, such relationships were also observed for eight acidic compounds [Grumetto et al., 2013], but the excess of the polar and/or electrostatic component was calculated by taking into account log $D^{7.4}$ values, rather than log P, yielding Δ'log k_w^{IAM}. The relationships between log BB values and Δ/Δ'log k_w^{IAM} are shown in Figure 9.

Such results are consistent with the *flip-flop* model of membrane barrier passage according to which, both neutral and ionized forms, in dynamic equilibrium, are involved in the passage of membrane phospholipid bilayer [Gurtovenko et al., 2007; Krämer et al., 2009].

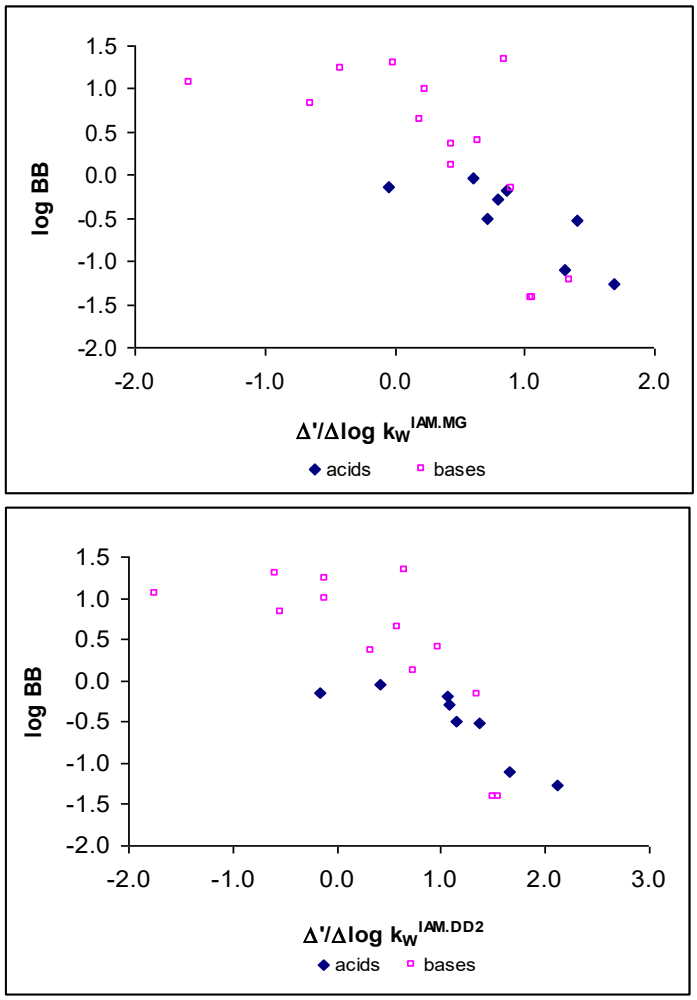

Figure 9. Relationships between log BB values and Δ/Δ'log k_w^{IAM} for a set of 22 structurally unrelated basic and acidic compounds.

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2.0 RELATIONSHIPS BETWEEN IAM DERIVED PARAMETERS AND *IN VIVO* BBB PERMEATION DATA

2.1 Introduction

Mainly based on the observation that the large majority of marketed drugs are ionizable [Comer and Tam, 2001], the recent works of the research team I worked with had focused the attention on the effects of an electric charge supported by the analyte on membrane interactions [Grumetto et al., 2012, 2013].

They proposed a fast and simple method to unravel the total interaction forces between drugs and membranes in a lipophilic/hydrophobic component and a polar/electrostatic one. Quantitation of such forces was achieved by combining phospholipophilicity and *n*-octanol lipophilicity data. Phospholipophilicity was assumed as a measure of the total interaction drug/membrane; it was measured by IAM-HPLC and was expressed as $\log k_w^{IAM}$ (logarithm of chromatographic retention factor measured or extrapolated to 100% aqueous eluent). *n*-octanol lipophilicity data were assumed as a measure of lipophilic/hydrophobic forces and were expressed as either $\log P^N$ or $\log D^{pH}$ values, i.e. the logarithm of partition coefficient of either the neutral form or the mixture of neutral and ionized forms at a given pH, respectively [Leo et al., 1971]. The differences between $\log k_w^{IAM}$ and $\log P^N$ ($\log D^{7.4}$ for acids, due to the different level of membrane interaction) were assumed as indexes of the polar/electrostatic forces occurring in membrane interaction and were expressed as $\Delta\log k_w^{IAM}$ and $\Delta'\log k_w^{IAM}$, for bases and acids, respectively [Grumetto et al., 2012, 2013], as already mentioned in Introduction.

According to the "pH-piston Hypothesis" [Avdeef et al., 1998], electrostatic forces can positively contribute to drug/membrane interactions, as demonstrated in previous works of the same research group [Barbato et al., 1996, 1997a, 1997b, 1998, 2004, 2005, 2007; Amato et al., 2000; Barbato 2006]. However, in the recent studies on neutral, basic, and acidic drugs above mentioned [Grumetto et al., 2012, 2013], these forces were found inversely related to BBB permeation.

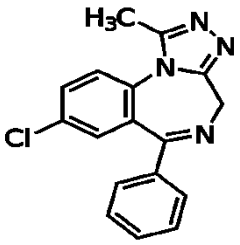
These results are consistent with the "flip-flop" model of membrane passage [Gurtovenko and Vattulainen, 2007; Krämer et al., 2009] where the first step of permeation, i.e. partition in the hydrophilic moieties of phospholipids at membrane surface, is promoted (in some cases unaffected) by ionization, whereas the second step, i.e. the passage through the

lipophilic inner moieties of phospholipid bilayer, is performed by the neutral forms in dynamic equilibrium. Actually, when the equilibrium ionized/neutral form cannot occur in the microenvironment of membrane bilayer, as for quaternary ammonium salts, no permeation can occur despite high partition.

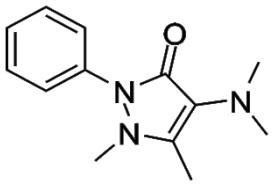
According to this model, BBB permeation was found inversely related to both $\Delta\log k_w^{IAM}$ and $\Delta'\log k_w^{IAM}$ values and, for bases only, directly related to log P^N, i.e. the lipophilicity of the neutral form, but not to log $D^{7.4}$, the lipophilicity of the mixture ionized/neutral forms at the physiological pH. These results suggest that the correction of lipophilicity on pH, at least as expressed by log D parameter, is not able to account for the interactions actually occurring with biological membranes.

In the present study we took into account twenty-one drugs whose BBB permeation capability is reported in the literature as log BB values, i.e. the ratios between brain and blood concentrations [Platts et al., 2001] (Scheme 1). The compounds are believed to cross BBB by passive mechanism. To confirm the model proposed in the previous studies of our research group [Grumetto et al., 2012, 2013] we measured the affinity values for phospholipids by IAM-HPLC technique on two different phospholipid stationary phases, i.e. IAM.PC.MG and IAM.PC.DD2. On the basis of lipophilicity values in *n*-octanol, expressed as either log P^N or log $D^{7.4}$, we calculated $\Delta\log k_w^{IAM}$ and $\Delta'\log k_w^{IAM}$ values, for bases and acids, respectively. Possible relationships between the various physico-chemical parameters, as well as between physico-chemical parameters and BBB permeation data, were investigated.

Finally, we assembled in a single set the data achieved in the present work (21 compounds) and the analogous data reported in previous works [Grumetto et al., 2012, 2013]. This set of 42 compounds was used to support statistically the model proposed.



Alprazolam



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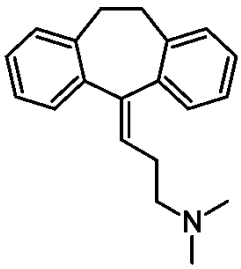

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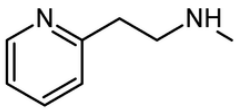
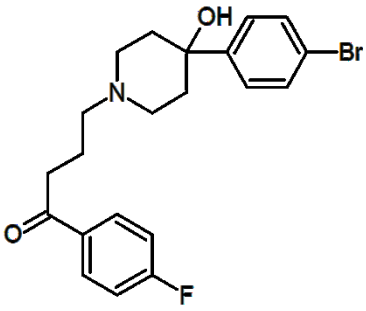
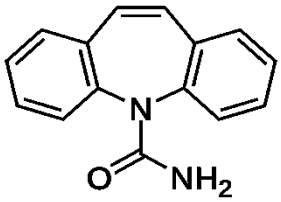


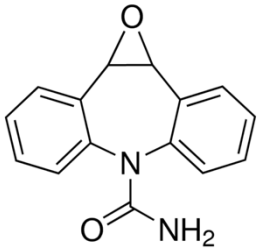
Betahistine



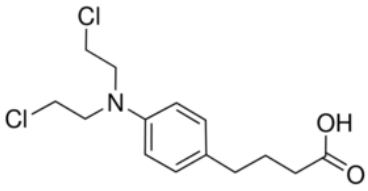
Bromperidol



Carbamazepine



Carbamazepine 10,11-epoxide



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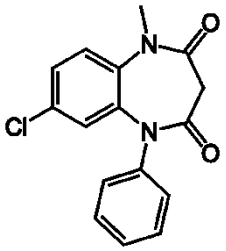
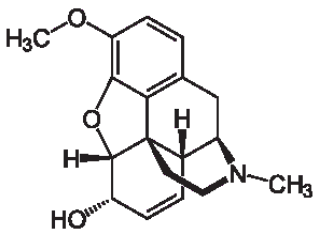
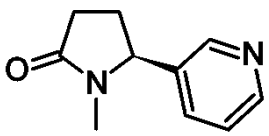


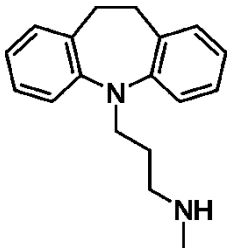
Clobazam



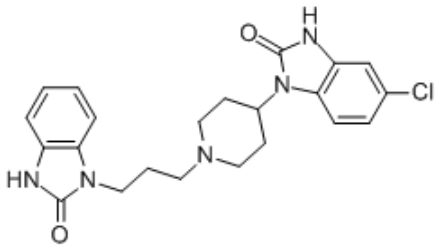
Codeine



Cotinine



Desipramine



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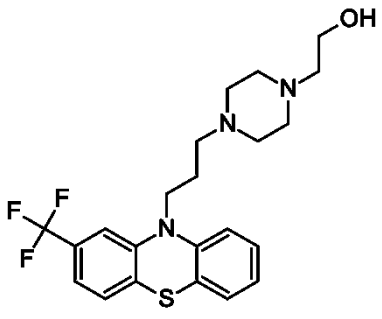
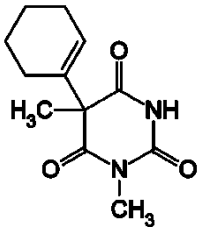


Fluphenazine



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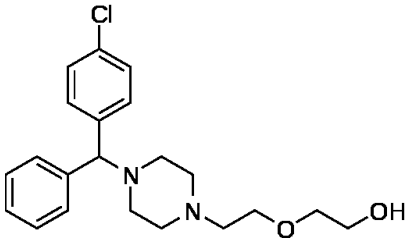
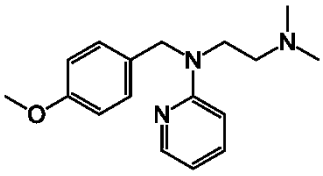
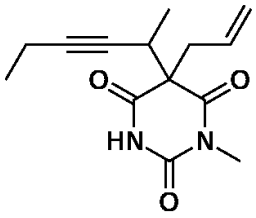


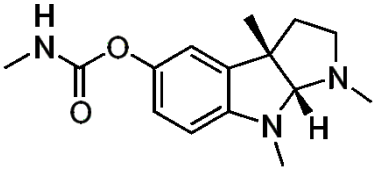
Hydroxyzine



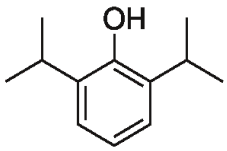
Mepyramine



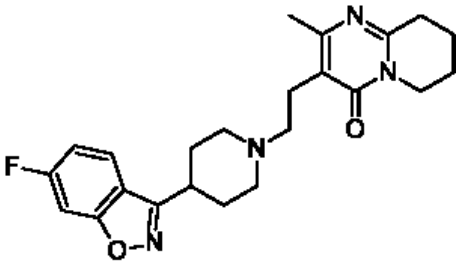
Methohexital



Physostigmine



Propofol



Risperidone

Scheme 1. Chemical structures of the compounds considered.

2.2 Materials and methods

All samples were obtained from commercial source. All chemicals were of HPLC grade and used without further purification.

Chromatographic system:

LC-10AD liquid chromatographic apparatus (Shimadzu Corporation, Kyoto, Japan); SPD-10AV UV detector (Shimadzu), set at λ of maximum absorbance for each compound; 7725 Rheodyne injection valve (fitted with a 20 µl loop).

Data processing: Cromatoplus software for personal computer, version 2009 (Shimadzu).

Analytical HPLC columns:

- IAM.PC.MG (4.6 mm x 150 mm; 12 µm, 300Å; Regis Chemical Company, Morton Grove, IL);
- IAM.PC.DD2 (4.6 mm x 100 mm, 10 µm, 300Å; Regis Chemical Company, Morton Grove, IL).

Chromatographic conditions:

The analyses were performed at room temperature with 0.1 M phosphate buffer at pH 7.0 in mixture with acetonitrile at various percentages. The flow rate was selected according to retention time of each analyte (1.0, 2.0, and 3.0 mL/min).

Sample preparation: each analyte was dissolved in the mobile phase or in methanol to *ca*. 10^{-4} M concentration. Chromatographic retention data are reported as log k (the logarithm of the retention factor), calculated by the expression: $\log k = \log [(t_r - t_0)/t_0]$ where t_r and t_0 are the retention times of the drug and a non-retained compound (acetone), respectively. Direct measurements of log k values in fully aqueous mobile phases ($\log k_w^{IAM}$) were only possible for the compounds eluting within 20 min, whereas for the solutes requiring the addition of acetonitrile in the eluent, the $\log k_w^{IAM}$ values were calculated by an extrapolation method [Braumann et al., 1983]: logk values were determined at four different mobile phases varying in acetonitrile percentages (φ) (from 10 to 30% v/v) and the intercept values of the linear relationships between log k and φ values, found for all compounds in the range of eluent composition examined ($r^2 \geq 0.99$), were assumed as $\log k_w^{IAM}$ values.

All reported log k values are the average of at least three measurements; for each log k value the 95% confidence interval associated with each value never exceeded 0.04. To avoid that the experimental measurements were affected by retention changes due to column ageing, the retention times of five test compounds (amlodipine, p-nitroaniline, toluene, isradipine, and ketoprofen) were weekly checked. No correction was done to the

experimental retention values since no retention value of test compounds changed more than 4% during the study

Lipophilic parameters:

log P^N values, i.e. partition coefficients *n*-octanol/aqueous phase of the neutral form of analytes, were either from the literature [Aravagiri et al., 1998; Avdeef 2012; Drug Bank, 2014; Gambaro et al., 2014; Ganellin and Triggle, 1996; Lombardo et al., 2000; Thatipamula et al., 2011; Hazardous Substances Data Bank, 2014] or calculated (clog P) by the program ClogP for Windows version 2.0 (Biobyte Corp., Claremont, CA). The *n*-octanol/aqueous buffer at pH 7.4 distribution coefficients (log $D^{7.4}$) were taken from the literature [Avdeef 2012; Hou et al., 2007] for alprazolam, amitriptyline, betahistine, chlorambucil, codeine, desipramine, and hydroxyzine. They express the partition of the mixture of neutral and ionized forms existing at this pH of the aqueous phase. For the other analytes the contribution of the ionized forms to their partition in *n*-octanol is negligible, being the pKa values < 9 for bases and > 8 for acids. Therefore, the log $D^{7.4}$ values were calculated according to the following equations:

$$\log D^{7.4} = \log P - \log (1 + 10^{7.4 - pKa}) \quad \text{(for acids)}$$

$$\log D^{7.4} = \log P - \log (1 + 10^{pKa - 7.4}) \quad \text{(for bases)}$$

Log BB values were from the literature [Platts et al., 2001].

Statistical analysis:

Linear regression analysis was performed by a commercially available statistical package for personal computer observing the requirements of significant regression analysis.

2.3 Results and discussions

The set of twenty-one molecules considered included eleven bases partially ionized at the experimental pH 7.0 (amitriptyline, betahistine, bromperidol, codeine, desipramine, domperidone, fluphenazine, hydroxyzine, mepyramine, physostigmine, risperidone), two bases negligibly ionized at pH 7.0 (alprazolam and aminophenazone), four neutral compounds (carbamazepine, carbamazepine 10, 11-epoxide, clobazam, and propofol), two acids (hexobarbital and methohexital), and the ordinary ampholyte chlorambucil. Table 1 summarizes the values of log P^N, pK_a, log $D^{7.4}$, and membrane phospholipid affinity on IAM.PC.MG and IAM.PC.DD2 (indicated as $\log k_w^{IAM.MG}$ and $\log k_w^{IAM.DD2}$, respectively). As can be seen, the compounds considered span a very large range of log P^N values (0.07 – 5.90).

Compound	log P^{N}	pK_a	log $D^{7.4}$	log $k_w^{IAM.MG}$	log $k_w^{IAM.DD2}$	Reference
Cotinine	0.07	4.79	0.07	0.450	0.167	Drug Bank, 2014
Betahistine	0.68	10.10	-2.90[b]	0.244	0.279	Drug Bank, 2014; Hou et al., 2007
Aminophenazone	1.00	4.50	0.99	0.980	0.771	Drug Bank, 2014
Codeine	1.39	8.21	0.22	0.855	1.290	Avdeef 2012;, Gambaro et al., 2014
Hexobarbital	1.49	8.20	1.43	0.366	0,959	Ganellin and Triggle, 1996
Carbamazepine 10,11-epoxide	1.58	-	1.58	1.118	1.213	Drug Bank, 2014
Physostigmine	1.58	8.32	0.61	0.902	1.151	Drug Bank, 2014
Methohexital	1.80	8.73	1.75	1.039	1.569	Drug Bank, 2014
Alprazolam	2.09	2.37	2.08	1.330	1.935	Avdeef 2012
Carbamazepine	2.19	-	2.19	1.039	1.717	Lombardo et al., 2000
Clobazam	2.62[a]	-	2.62	1.296	1.946	-
Risperidone	3.04	8.76	1.66	2.189	2.028	Aravagiri et al., 1998
Domperidone	3.10	7.90	2.48	2.790	3.213	Thatipamula et al., 2011
Mepyramine	3.27	8.85	1.80	2.109	1.893	Drug Bank, 2014
Chlorambucil	3.41	4.82/4.62	0.61	1.288	1.897	Avdeef 2012
Propofol	3.79	-	3.79	2.073	2.991	Drug Bank, 2014
Hydroxyzine	4.16	7.82	3.15	2.908	2.965	Ganellin and Triggle, 1996, Avdeef 2012
Bromperidol	4.45	8.04	3.72	2.893	3.053	Hazardous Substances Data Bank, 2014
Desipramine	4.90	10.40	1.38	2.826	2.741	Drug Bank, 2014, Avdeef 2012
Amitriptyline	4.92	9.18	2.80	2.881	3.122	Drug Bank, 2014, Avdeef 2012

Fluphenazine	5.90[a]	7.90	5.28	3.588	3.957	-

Table 1. Logarithms of lipophilicity values in *n*-octanol, of acidity constants, and of chromatographic retention factors on IAM phases for the compounds considered.

[a]log P values calculated; [b]log $D^{6.5}$;

The use of two different stationary phases was aimed at verifying that IAM data actually mirrored phospholipid interactions and were not affected in an appreciable extent by secondary mechanisms. Indeed, as already underlined, IAM.PC.MG and IAM.PC.DD2 differ from each other in the end-capping of residual amino groups of the silica-propylamine core; IAM.PC.MG supports hydroxy groups (being end-capped by methyl glycolate) whereas IAM.PC.DD2 supports C_{10} and C_3 alkyl chains (being end-capped by both decanoic and propionic anhydrides). The values of $\log k_w^{IAM.MG}$ and $\log k_w^{IAM.DD2}$ of the compounds considered strongly interrelate by an equation with slope near to unit (Equation (1)) suggesting that retention on both IAM phases is only negligibly affected by secondary mechanisms.

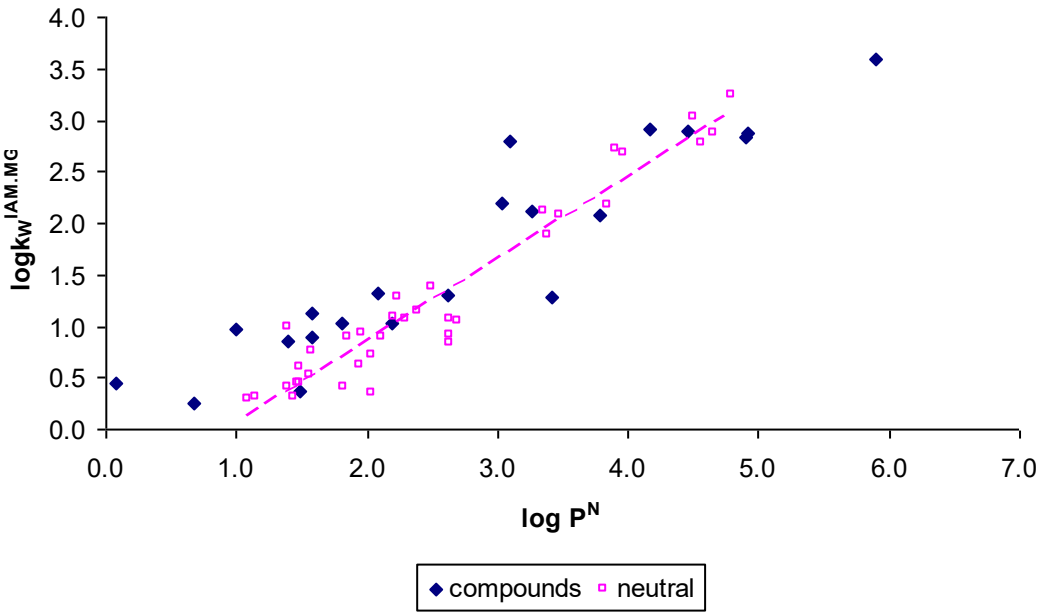
$$\log k_w^{IAM.DD2} = 0.971\ (\pm 0.080)\ \log k_w^{IAM.MG} + 0.319\ (\pm 0.154) \qquad (1)$$

$n = 21$ $\quad r^2 = 0.886$ $\quad s = 0.356$

As reported in previous studies from our laboratory [Grumetto et al., 2012, 2013], IAM retention data on both IAM phases relate unambiguously with log P values of neutral compounds, even structurally non-related, in the log P range 1.0 – 4.8. The relationships are expressed by the equations (10) and (11) reported in paragraph 1.5.

The plots $\log k_w^{IAM}$ *vs* log P^N of the compounds considered, superimposed to the plots of the neutral compounds, are reported in Fig 1.

A



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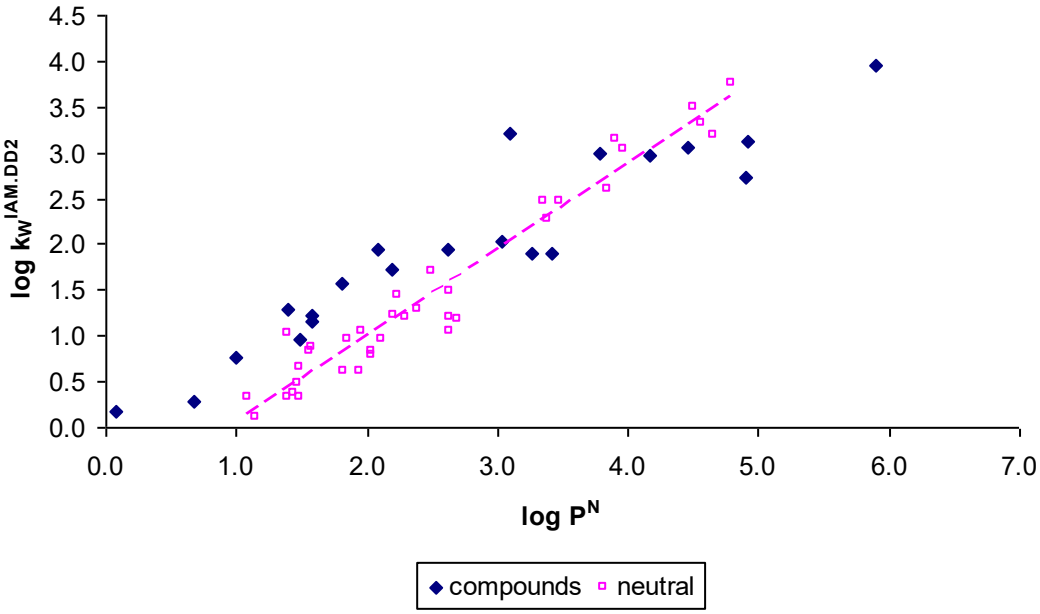


Figure 1. Relationships between either log $k_w^{IAM.MG}$ (A) or log $k_w^{IAM.DD2}$ (B) and log P^N values for the 21 compounds considered in comparison to the plots of 36 neutral compounds.

In the plot reporting $\log k_w^{IAM.MG}$ *vs* log P^N (Fig 1A), all points are close to the regression line or shifted upward, but the point relative to chlorambucil. In the plot reporting $\log k_w^{IAM.DD2}$ *vs* log P^N (Fig 1B), most points are shifted upward with respect to the regression line of neutral compounds; however, not only chlorambucil but also amitriptyline, fluphenazine, and desipramine lie below the regression line. Chlorambucil is an ordinary ampholyte, since the carboxy function has pKa = 4.82 and the amino function has pKa = 4.32 (pKa^{acidic} > pKa^{basic}). Therefore, at the experimental pH 7.0, it behaves as an acid, existing predominantly as an anion, while the cation percentage is negligible (< 1%). As to amitriptyline, fluphenazine, and desipramine, their behaviour confirms what already observed on IAM.PC.DD2 phase for strongly lipophilic bases [Grumetto et al., 2012]. The behaviour of the other bases is consistent with that already observed on phospholipid stationary phases [Amato et al., 2000; Barbato et al., 1996, 1997b, 2005; Grumetto et al., 2012].

The distance of the points from the regression line is expressed as $\Delta\log k_w^{IAM.MG}$ and $\Delta\log k_w^{IAM.DD2}$, for IAM.PC.MG and IAM.PC.DD2 phases, respectively. Therefore, $\Delta\log k_w^{IAM.MG}$ were the differences between the experimentally measured $\log k_w^{IAM.MG}$ values and the values calculated from log P^N by equation (10 of paragraph 1.5); $\Delta\log k_w^{IAM.DD2}$ were the differences between the experimentally measured $\log k_w^{IAM.DD2}$ values and the values calculated from log P^N by equation (11 of paragraph 1.5). Chlorambucil was an exception, being the only analyte interacting with phospholipids mainly as an anion. The distance from the regression line in Figure 1 was calculated taking into account its log $D^{7.4}$ value generating $\Delta'\log k_w^{IAM.MG}$ and $\Delta'\log k_w^{IAM.DD2}$ values [Grumetto et al., 2013]. Analogously, for the other two acid compounds, methohexital and hexobarbital, $\Delta'\log k_w^{IAM}$ values were also considered; however, these values were only negligibly different from $\Delta\log k_w^{IAM}$, due to their very low degree of ionization. When the combination of $\Delta\log k_w^{IAM}$ and $\Delta'\log k_w^{IAM}$ values is considered, it will be indicated as $\Delta/\Delta'\log k_w^{IAM}$.

Table 2 summarizes $\Delta/\Delta'\log k_w^{IAM.MG}$, $\Delta/\Delta'\log k_w^{IAM.DD2}$, and log BB values for the compounds considered.

Compound	$\Delta/\Delta' \log k_w^{IAM.MG}$	$\Delta/\Delta' \log k_w^{IAM.DD2}$	log BB
Cotinine	1.127	0.985	-0.320
Betahistine	0.438	0.527	-0.300
Aminophenazone	0.930	0.720	0.000
Codeine	0.486	0.875	0.550
Hexobarbital	-0.035	0.417	0.100
Carbamazepine 10,11- epoxide	0.599	0.620	-0.337
Physostigmine	0.382	0.558	0.079
Methohexital	0.385	0.819	-0.060
Alprazolam	0.407	0.865	0.044
Carbamazepine	0.036	0.554	0.000
Clobazam	-0.047	0.382	0.350
Risperidone	0.513	0.071	0.490
Domperidone	1.067	1.201	-0.780
Mepyramine	0.251	-0.278	0.490
Chlorambucil	1.537	2.211	-1.700
Propofol	-0.197	0.334	0.480
Hydroxyzine	0.345	-0.038	0.390
Bromperidol	0.101	-0.220	1.380
Desipramine	-0.323	-0.952	1.200
Amitriptyline	-0.284	-0.591	0.980
Fluphenazine	-0.353	-0.671	1.510

Table 2. Values of the differences between observed and expected logarithms of retention factors on IAM.PC.MG and IAM.PC.DD2 stationary phases ($\Delta/\Delta' \log k_w^{IAM.MG}$ and $\Delta/\Delta' \log k_w^{IAM.DD2}$, respectively) and logarithms of the ratio brain concentration/blood concentration for the compounds considered.

Δ/Δ' log k_w^{IAM} values on both IAM phases did not relate with log P^N values (r^2 = 0.406 and 0.656 for IAM.PC.MG and IAM.PC.DD2, respectively).

log BB values did not relate with lipophilicity values, either log P^N or log $D^{7.4}$. The combination of log P^N values, for bases, and log $D^{7.4}$ values, for acids (i.e. chlorambucil, hexobarbital, and methohexital), only negligibly improved the relationship (Figure 2). However, while the plot log BB *vs* log $D^{7.4}$ appear markedly scattered, a closer look to the plot log BB *vs* log P^N/log $D^{7.4}$ values revealed that the lack of relationship was mainly due to the values of both chlorambucil and domperidone, strong outliers. As a matter of the fact, a reasonable relationship was only observed after the exclusion of the two outliers (equation (2)).

log BB = 0.305 (± 0.037) log P - 0.446 (± 0.115) (2)

n = 19 r^2 = 0.800 s = 0.257

Analogously, data of both chlorambucil and domperidone also affected negatively the relationships between log BB and both log$k_w^{IAM.MG}$ and log $k_w^{IAM.DD2}$; however, their exclusion produced weaker relationships than that with log P^N, for both log$k_w^{IAM.MG}$ (Figure 3A and equation (3)) and log $k_w^{IAM.DD2}$ (Figure 3B and equation (4)).

log BB = 0.478 (± 0.067) log$k_w^{IAM.MG}$ - 0.412 (± 0.128) (3)

n = 19 r^2 = 0.750 s = 0.287

log BB = 0.456 (± 0.069) log$k_w^{IAM.DD2}$ - 0.487 (± 0.147) (4)

n = 19 r^2 = 0.720 s = 0.304

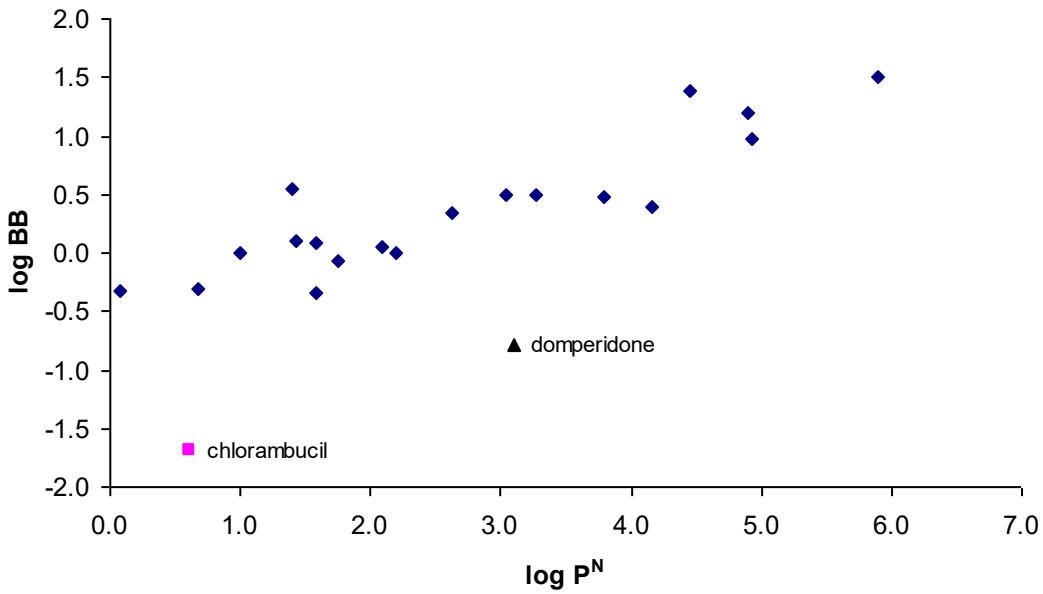
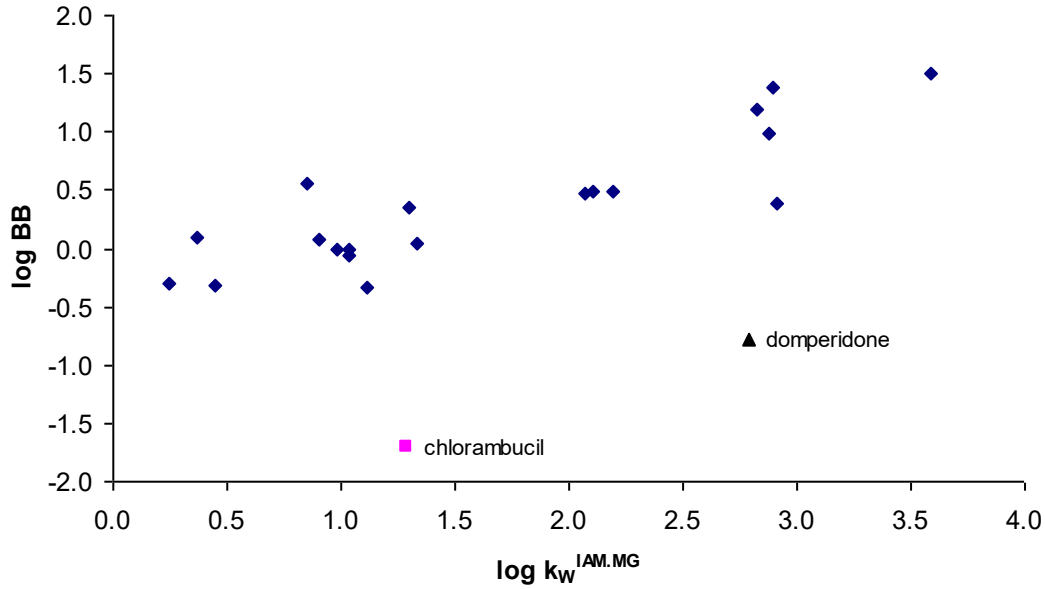


Figure 2. Relationships between log BB and log P^N values for the 21 compounds considered (log $D^{7.4}$ for chlorambucil, hexobarbital, and methohexital).

A



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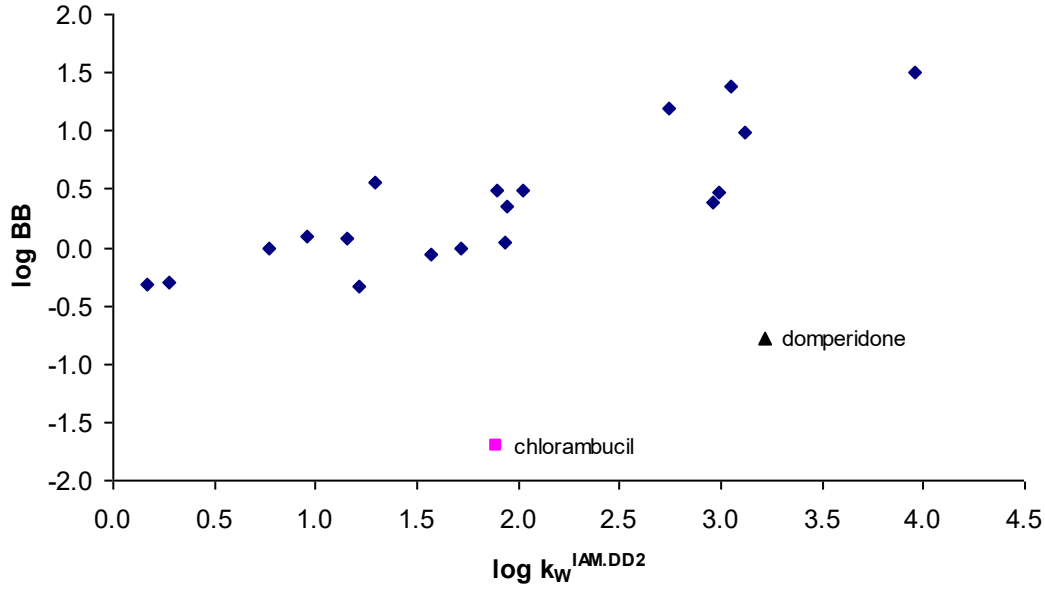


Figure 3. Relationships between log BB and either $\log k_w^{IAM.MG}$ (A) or $\log k_w^{IAM.DD2}$ (B) values for the 21 compounds considered.

In contrast, a linear inverse relationship was observed between log BB and $\Delta/\Delta'\log k_w^{IAM}$ values for all considered compounds (Figure 4), according to the results previously found [Grumetto et al., 2012, 2013]. $\Delta\log k_w^{IAM}$ were used for all compounds but the acids (i.e. hexobarbital and methohexital) and chlorambucil, for which $\Delta'\log k_w^{IAM}$ were used. Although a same trend can be observed with data from the two IAM phases, and also considering that biological data, such as log BB, are affected by a relatively high uncertainty, the relationship with $\Delta/\Delta'\log k_w^{IAM.MG}$ (equation (5)) is not as good as one would like. In contrast, a reasonable relationship is observed between log BB and $\Delta/\Delta'\log k_w^{IAM.DD2}$ (equation (6)).

$$\log BB = -1.195\ (\pm 0.188)\ \Delta/\Delta'\log k_w^{IAM.MG} + 0.636\ (\pm 0.114) \quad (5)$$

$n = 21 \qquad r^2 = 0.681 \qquad s = 0.424$

$$\log BB = -0.931\ (\pm 0.098)\ \Delta/\Delta'\log k_w^{IAM.DD2} + 0.588\ (\pm 0.079) \quad (6)$$

$n = 21 \qquad r^2 = 0.825 \qquad s = 0.314$

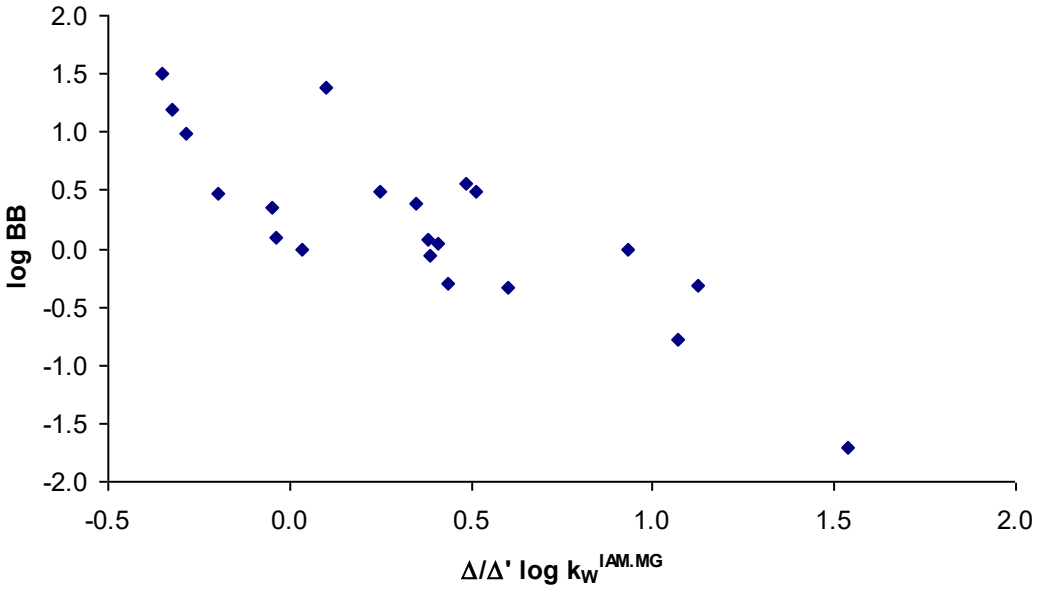
Furthermore, the relationship of equation (6) is improved if the points of codeine and bromperidol are excluded (equation (7)):

$$\log BB = -0.930\ (\pm 0.071)\ \Delta/\Delta'\log k_w^{IAM.DD2} + 0.516\ (\pm 0.058) \quad (7)$$

$n = 19 \qquad r^2 = 0.909 \qquad s = 0.221$

It is difficult to explain why these compounds are weak outliers. A possible reason may be related to particular structural features do not accounted for by the physico-chemical parameters we considered; indeed, in a previous work from our lab two structurally similar compounds, morphine and haloperidol, also behaved as outliers [Grumetto et al., 2012].

A



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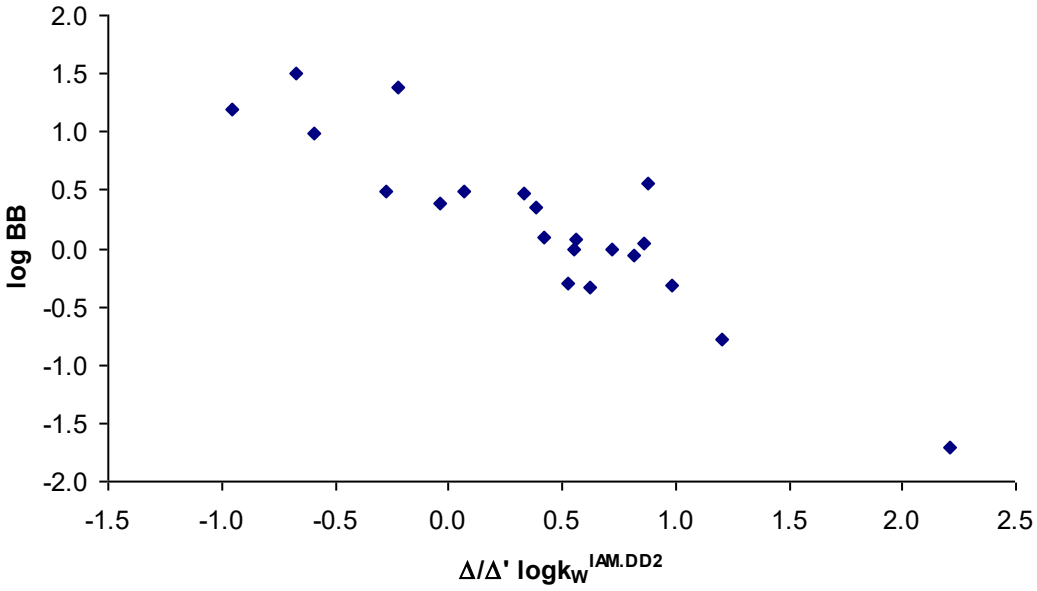


Figure 4. Relationships between log BB and either $\Delta/\Delta' \log k_w^{IAM.MG}$ (A) or $\Delta/\Delta' \log k_w^{IAM.DD2}$ (B) values for the 21 compounds considered.

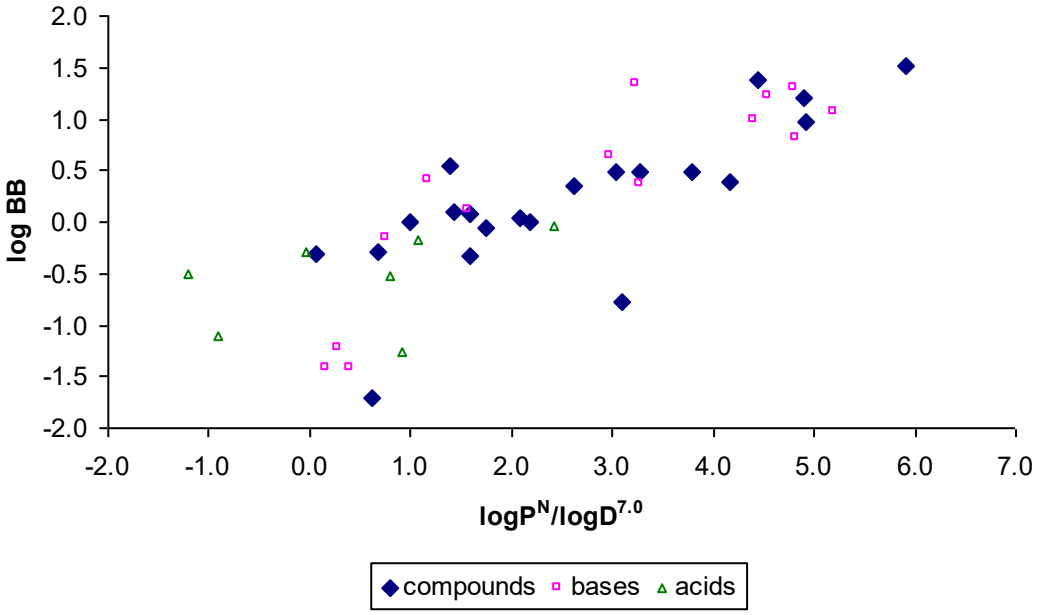


Figure 5. Relationship between log BB and either log P^N or log $D^{7.0}$ values for the 21 considered compounds in comparison with 21 compounds (bases and acids) previously reported (log $D^{7.4}$ for chlorambucil, hexobarbital, and methohexital) [Grumetto et al., 2012, 2013].

The observed relationships were similar to those we already observed for other drugs, i.e. fourteen bases [Grumetto et al., 2012] and seven acids [Grumetto et al., 2013]. Their values of lipophilicity, $logk_w^{IAM}$, $\Delta/\Delta' logk_w^{IAM}$, and log BB are summarized in Table 3. The inclusion of these data in the set of data obtained in this work allowed us to verify the soundness of the relationships above reported on a single set of forty-two compounds.

As can be seen in Figure 5, log BB values related poorly to lipophilicity values in *n*-octanol (log P^N for bases and log $D^{7.0}$ for acids).

Analogously, differently from the results of other authors (Salminen et al., 1997; Ducarme et al., 1998; Reichel and Begley, 1998; Kepczyńska et al., 2000; Pehourcq et al. 2004), log BB values also related poorly with both $logk_w^{IAM.MG}$ and $logk_w^{IAM.DD2}$ values (Figure 6).

In contrast, reasonable relationships between log BB and $\Delta/\Delta' logk_w^{IAM}$ were observed for the whole set of forty-two compounds (Figure 7), clearly indicating that log BB decrease linearly at increasing $\Delta/\Delta' logk_w^{IAM}$values.

Differently from what concluded in our previous work [Grumetto et al., 2012], the enlarged set here considered suggests that the linear dependence log BB *vs* $\Delta/\Delta' logk_w^{IAM}$ can also apply for negative values of $\Delta/\Delta' logk_w^{IAM}$ and both chlorpromazine and haloperidol behave as outliers. Actually, good relationships can be observed after their exclusion (equation (8) and (9)).

$$\log BB = -1.228\ (\pm 0.119)\ \Delta/\Delta' logk_w^{IAM.MG} + 0.671\ (\pm 0.090) \quad (8)$$

$n = 40 \qquad r^2 = 0.738 \qquad s = 0.424$

$$\log BB = -0.975\ (\pm 0.072)\ \Delta/\Delta' logk_w^{IAM.DD2} + 0.650\ (\pm 0.070) \quad (9)$$

$n = 40 \qquad r^2 = 0.826 \qquad s = 0.345$

As already observed in equations (5) and (6), log BB relate better with $\Delta/\Delta' logk_w^{IAM.DD2}$ than with $\Delta/\Delta' logk_w^{IAM.MG}$ values, although the extension of the set to 40 compounds improved the latter relationship.

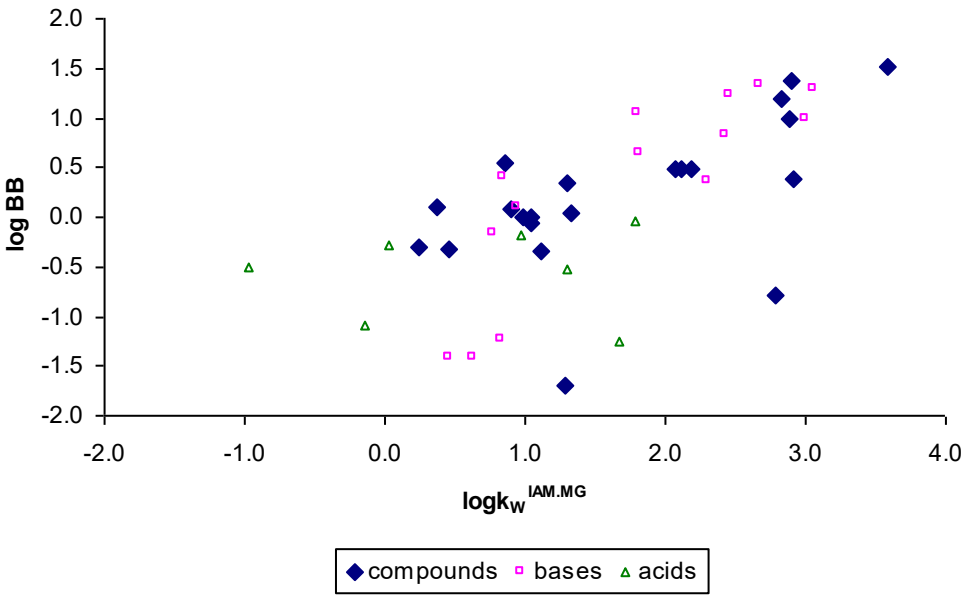
Compound	log P	$\log k_w^{IAM.MC}$	$\log k_w^{IAM.DD2}$	$\Delta/\Delta' \log k_w^{IAM.MC}$	$\Delta/\Delta' \log k_w^{IAM.DD2}$	log BB
Atenolol[a]	0.16	0.458	0.765	1.063	1.499	-1.420
Ranitidine[a]	0.27	0.834	0.812	1.352	1.443	-1.230
Cimetidine[a]	0.40	0.633	1.048	1.048	1.557	-1.420
Morphine[a]	0.76	0.767	1.180	0.897	1.353	-0.160
Nicotine[a]	1.17	0.844	1.184	0.649	0.974	0.401
Clonidine[a]	1.57	0.948	1.316	0.437	0.733	0.110
Propranolol[a]	2.98	1.821	2.480	0.193	0.580	0.640
Haloperidol[a]	3.23	2.670	2.780	0.844	0.646	1.340
Midazolam[a]	3.27	2.302	2.505	0.444	0.334	0.360
Mianserin[a]	4.41	3.003	3.131	0.242	-0.105	0.990
Promazine[a]	4.55	2.462	3.260	-0.410	-0.107	1.230
Imipramine[a]	4.80	3.064	3.008	-0.006	-0.592	1.300
Promethazine[a]	4.81	2.432	3.075	-0.646	-0.535	0.824
Chlorpromazine[a]	5.19	1.799	2.225	-1.579	-1.739	1.060
Acetylsalicylic acid[b]	1.19	-0.965	-0.850	0.717	1.154	-0.500
Salicylic acid[b]	2.26	-0.143	-0.075	1.302	1.649	-1.100
Phenylbutazone[b]	3.16	1.305	1.232	1.405	1.370	-0.520

Phenytoin[b]	2.47	1.787	1.789	0.602	0.412	-0.040
Ibuprofen[b]	3.50	0.972	1.170	0.857	1.054	-0.180
Indomethacin[b]	4.27	1.674	2.080	1.685	2.113	-1.260
Theophylline[b]	-0.02	0.033	0.153	0.797	1.073	-0.290

[a]Ref. Grumetto et al., 2012; [b]Ref. Grumetto et al., 2013;

Table 3. Values of lipophilicity, $\log k_w^{IAM.MG}$, $\log k_w^{IAM.DD2}$, differences between observed and expected logarithms of retention factors on IAM stationary phases ($\Delta/\Delta'\log k_w^{IAM.MG}$ and $\Delta/\Delta'\log k_w^{IAM.DD2}$), and logarithms of the ratio brain/blood concentrations for the bases and the acids reported in previous works.

A



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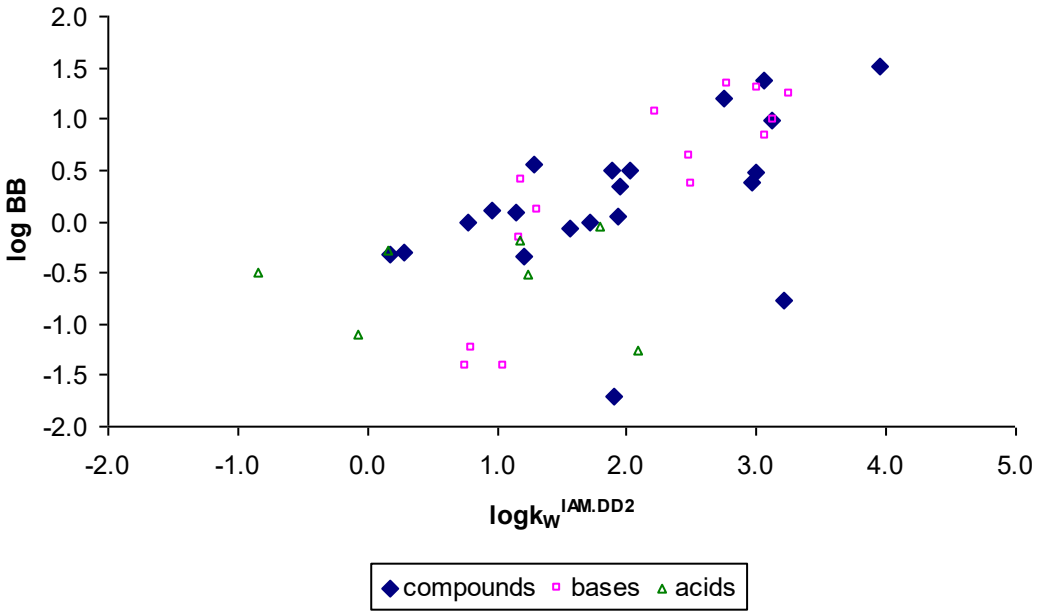
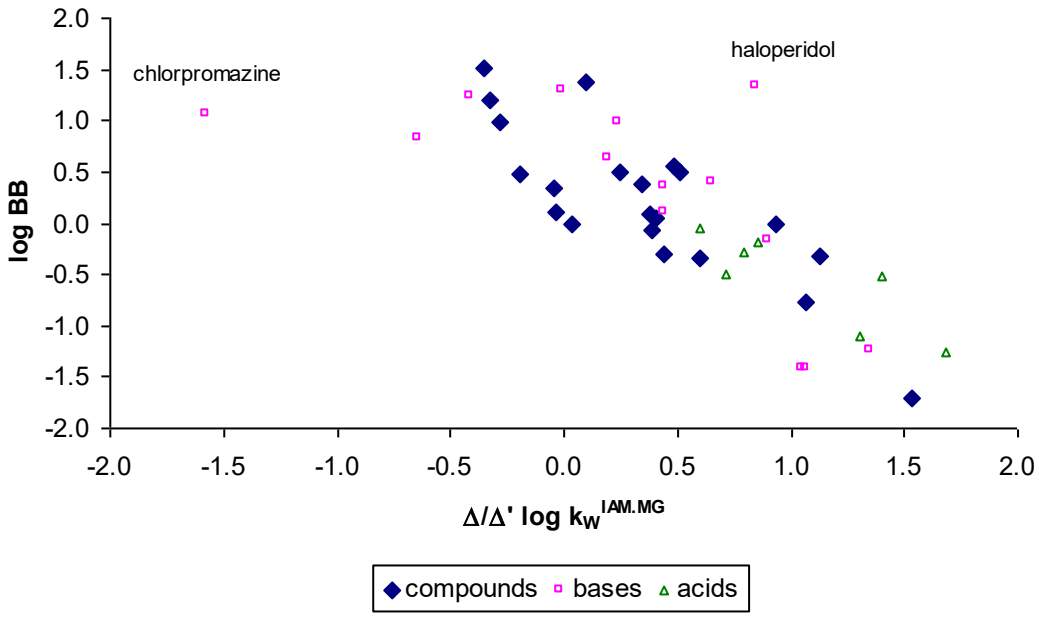


Fig. 6. Relationships between log BB and either $\log k_w^{IAM.MG}$ (A) or $\log k_w^{IAM.DD2}$ (B) values for the 21 considered compounds in comparison with 21 compounds (bases and acids) previously reported [Grumetto et al., 2012, 2013].

A



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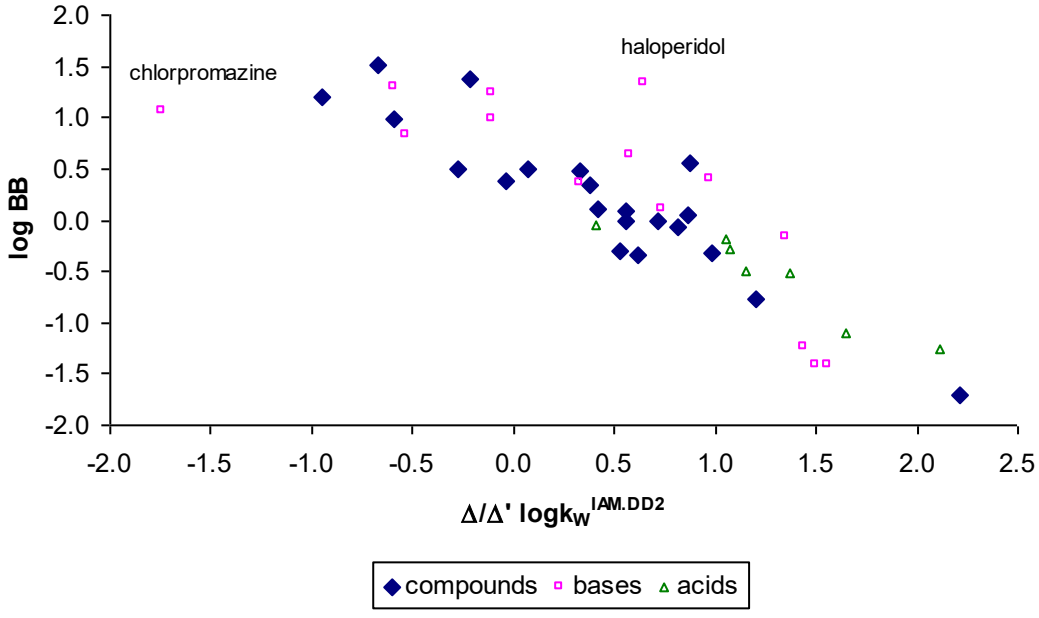

Fig. 7. Relationships between log BB and either $\Delta/\Delta' \log k_w^{IAM.MG}$ (A) or $\Delta/\Delta' \log k_w^{IAM.DD2}$ (B) values for the 21 compounds considered in comparison with 21 compounds (14 bases and 7 acids) previously reported [Grumetto et al., 2012, 2013].

2.4 Conclusion

This study confirms, and partially modifies, the results of a previous study performed by this research group [Grumetto et al., 2012, 2013].

The results of this work confirm that IAM data are descriptive of membrane partition but not of membrane passage; furthermore, we also confirm that log D^{pH} values underestimate partition capability of charged forms in phospholipids, since the correction of lipophilicity on the pH of the medium does not appropriately account for the ionization effects at membrane level.

We propose a simplified model to evaluate membrane passage, based on the use of the parameter $\Delta/\Delta' \log k_w^{IAM}$, obtained by combining interaction data with phospholipids ($\log k_w^{IAM}$) with the "classical" lipophilicity data in *n*-octanol (log P or log D^{pH}). The results of this work, as well as those of previous works performed in our lab [Grumetto et al., 2012, 2013], suggest that it is a meaningful physico-chemical parameter actually representing the electrostatic forces involved in membrane phospholipid interactions.

As to the driving forces for membrane permeation, they seem closer related to the lipophilicity of the neutral form, log P^N, than to both log D^{pH} and $\log k_w^{IAM}$, although some points had to be excluded to observe reasonable relationships.

In contrast, the relationships with $\Delta/\Delta' \log k_w^{IAM}$ were more significant than those with log P^N without excluding any point.

Although the model proposed might appear over-simplified, it can give a reasonably reliable estimate of membrane permeation capability and, being based on the experimental determination of only two physico-chemical parameters, it is suitable for medium-throughput screening studies.

Drugs targeted to Central Nervous System should have low, possibly negative, values of $\Delta/\Delta' \log k_w^{IAM}$, as frequently found for basic compounds; conversely, drugs intended for an only peripheral action should have high values of $\Delta/\Delta' \log k_w^{IAM}$, as usually, but not only, occurs for acidic compounds.

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3.0 RELATIONSHIPS BETWEEN IAM DERIVED PARAMETERS AND JEJUNAL ABSORPTION DATA MEASURED *IN VIVO*

3.1 Introduction

The extent of intestinal absorption of drugs in humans is usually determined by *in vivo* experiments. However, in the last years increasing ethical issues have been raised about *in vivo* pharmacological experiments on both animals and humans, making desirable the development of alternative *in vitro* methods. The methods based on the determination of physico-chemical parameters, albeit ineffective when active transport mechanisms occur, are highly reproducible and at high/medium throughput; furthermore, they may be useful both to predict the oral bioavailability of drug candidates at the early stages of their development and to formulate mechanistic hypotheses useful for drug/prodrug design. Their use could avoid, or at least reduce, *in vivo* experiments, such as those based on the Loc-I-Gut method. The latter yields the effective intestinal permeability values, P_{eff}, which, although actually accounting for the enterocyte apical membrane absorption, were demonstrated strictly related to the extent of human intestinal absorption [Lennernäs 1997].

Passive drug absorption of orally administered drugs is assumed to be related to drug lipophilicity [Liu et al., 2011], expressed as the logarithm of the *n*-octanol/water partition coefficient, log P, when referred to single species (neutral or ionized). Indeed, this parameter appears to describe adequately the partition coefficients of neutral compounds in the membrane, which, according to the Fick's first law (as modified to take into account the existence of a barrier), is a driving force for the membrane passage. However, a large majority of drugs support at least one ionizable function [Comer et al., 2001] and their *n*-octanol lipophilicity depends on their ionization degree. The values corrected for ionization, i.e. log D values, are the weighted average of log P values of the various forms, ionized and neutral, existing in solution as a function of the pH value [Leo et al., 1971] and are always smaller than log P values of the related neutral forms. Membrane passage of ionizable compounds seems to be more realistically described by the so-called "flip-flop" model

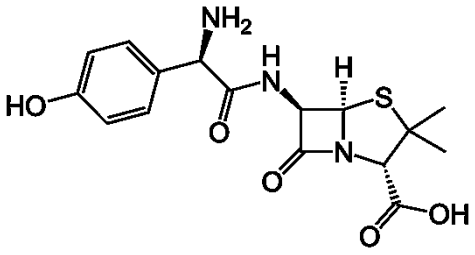
[Gurtovenko and Vattulainen, 2007; Krämer et al., 2009]. According to this model, both neutral and ionized forms, in dynamic equilibrium, are involved in the passage of membrane phospholipid bilayer. Furthermore, comparisons between partition data in phospholipids and log D values demonstrated that the latter are often inadequate at describing the interactions actually occurring between ionizable analytes and membrane phospholipids [Amato et al., 2000; Barbato 2006; Barbato et al., 1996, 1997a, 1997b, 1998, 2004, 2005, 2007].

On the basis of the results achieved on the passage of BBB and on the role played by the polar/electrostatic forces as parameterized by either $\Delta\log k_w^{IAM}$ or $\Delta'\log k_w^{IAM}$ values [Grumetto et al., 2012, 2013, 2014], we decided to investigate about possible relationships between these parameters and intestinal permeation data. In fact, it was suggested that "pure passive membrane diffusion is universal for membranes with different physiological functions and physicochemical properties" [Lennernäs 1997].

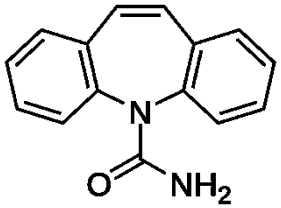
The effective intestinal permeability values, P_{eff}, determined by the Loc-I-Gut method, were assumed as those more closely reflecting the actual *in vivo* intestinal absorption. A thorough review of the literature highlighted that noticeable differences can occur between data from different laboratories. Furthermore, these data can reflect not only absorption values arising from passive mechanism of passage through the enterocyte membrane but also the effects of active transport mechanisms acting as either influx or efflux systems. Obviously, only data arising from passive transport mechanism and not affected by inter-laboratory variability can be taken into account for a study on their possible relationships with physico-chemical parameters.

Based on these limitations, we considered fifteen structurally unrelated molecules, usually orally administered and supposed to be absorbed by mainly passive mechanism at the intestinal level (although an involvement of active transport mechanism was reported for the intestinal absorption of cefalexin, its contribution to the total absorption would play an only minor role [Bretschneider et al., 1999]).

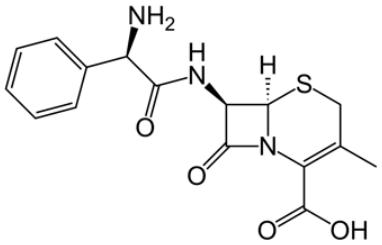
The set consisted of six bases (cimetidine, desipramine, propranolol, ranitidine, terbutaline, verapamil), five acids (fluvastatin, hydrochlorothiazide, isotretinoin, ketoprofen, naproxen), three zwitterions (amoxicillin, cefalexin and piroxicam), and one neutral compound (carbamazepine) (Scheme 1).



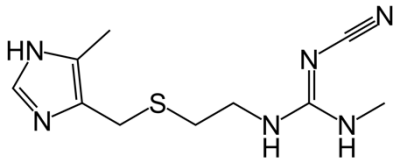
Amoxicillin



Carbamazepine



Cefalexin



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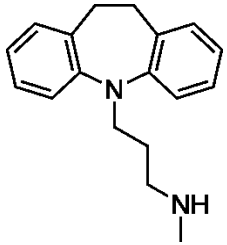
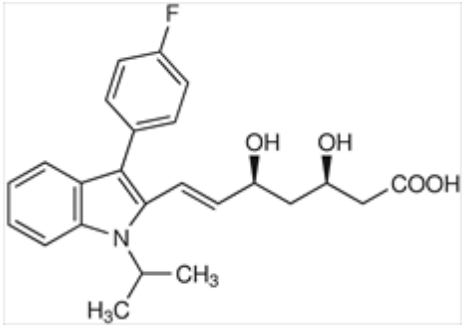
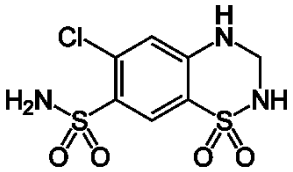


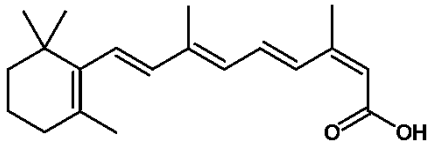
Desipramine



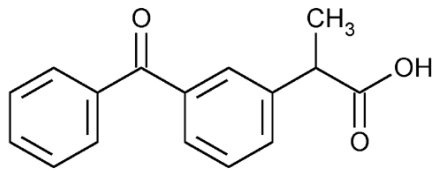
Fluvastatin



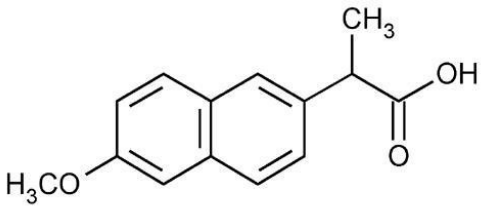
Hydrochlorothiazide



Isotretinoin



Ketoprofen



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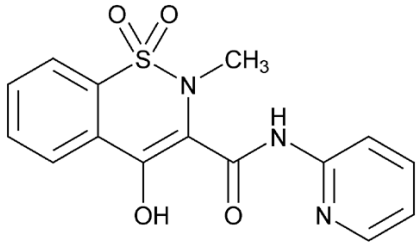
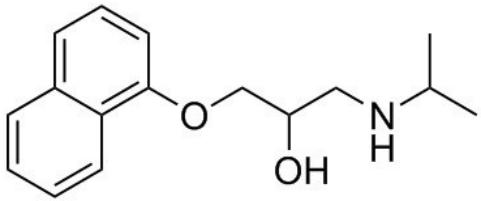
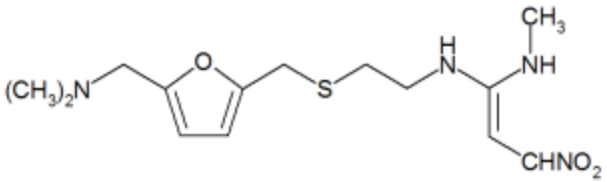


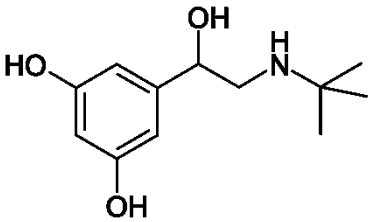
Piroxicam



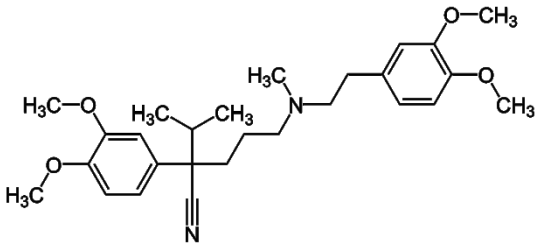
Propranolol



Ranitidine



Terbutaline



Verapamil

Scheme 1. Chemical structures of the compounds considered.

Their phospholipid affinity data were experimentally measured by IAM-HPLC technique on two different phospholipid stationary phases (IAM.PC.MG and IAM.PC.DD2) to reasonably exclude that the data were affected by secondary retention mechanisms.

Comparisons between the scales of *n*-octanol lipophilicity and IAM data were preliminarily performed to highlight similarities and dissimilarities. Finally, possible relationships between jejunal absorption data and either *n*-octanol lipophilicity data or IAM data were investigated.

3.2 Materials and methods

3.2.1 Chromatographic conditions and equipment

The analyses were performed according to the method previously reported in the paragraph 2.2.

3.2.2. Sample preparation

Each analyte was dissolved in the mobile phase or in methanol to *ca*. 10^{-4} M concentration.

3.2.3. Lipophilic and biological activity parameters

log P^N values, i.e. partition coefficients *n*-octanol/aqueous phase of the neutral form of analytes, were from the literature [Avdeef, 2012; Law et al., 2014; La Rotonda et al., 1983; Lombardo et al., 2000; Tsai et al., 1993; Winiwarter et al. 1998].

The *n*-octanol/aqueous buffer at pH 6.5 partition coefficients (log $D^{6.5}$) were calculated according to the following equations:

$$\log D^{6.5} = \log P^N - \log (1 + 10^{6.5 - pKa}) \quad \text{(for acids)}$$

$$\log D^{6.5} = \log P^N - \log (1 + 10^{pKa - 6.5}) \quad \text{(for bases)}$$

with the exception of i) amoxicillin, whose log $D^{6.5}$ value was taken from the literature [Winiwarter et al. 1998], ii) cefalexin and desipramine, whose experimental log $D^{7.4}$ [Avdeef, 2012] were assumed as a reasonable estimate of log $D^{6.5}$ values, and iii) piroxicam and propranolol, whose experimental log $D^{6.07}$ and log $D^{6.7}$, respectively, [Tsai et al., 1993; Barbato et al., 1990] were also assumed as a reasonable estimate of log $D^{6.5}$ values.

pKa values were either calculated by the program ACD/labs (release 12.00) or taken from the literature (Bernhard and Zimmermann, 1984; Grumetto et al., 2012; Khan et al., 2007; Law et al., 2014; Panigrahi et al., 2005; Van de Waterbeemd and Testa, 2008).

Log P_{eff} values, measured at pH 6.5, were from the literature [Lennernäs 2014].

3.2.4 Statistical analysis

Linear regression analysis was performed by a commercially available statistical package (Microsoft Excel 2003) for personal computer observing the requirements of significant regression analysis.

3.3 Results and discussions

3.3.1 Selection of the physicochemical parameters

Ionizable compounds show different lipophilicity values in *n*-octanol at different pH of the aqueous phase (log D values), according to the abundance of the neutral and ionized forms in solution. log D values theoretically calculated by the equations above reported in the section "materials and methods" do not take into account the contribution of the ionized forms to the partition. This implies that the theoretical values are close to the experimental ones only if the contribution of the ionized forms is negligible. Therefore, log D calculated at the pH values at which the fraction of the neutral form is << 1% do not adequately reflect the actual lipophilicity values. For ten of the considered compounds that showed at pH 6.5 an appropriate ionized/neutral form ratio we took into account the calculated log $D^{6.5}$ values. For the bases propranolol and desipramine we considered their experimentally determined log $D^{6.7}$ and log $D^{7.4}$, respectively. Since propranolol is already extensively ionized at pH 6.7 (ionized/neutral form ratio > 500) and, even so more at pH 6.5, its log $D^{6.7}$ is expected to be very close to its log $D^{6.5}$ value. Analogously, log $D^{7.4}$ value for desipramine, showing an ionized/neutral form ratio 1,000 at pH 7.4, is expected to be very close to its log $D^{6.5}$ value. Log $D^{6.5}$ values for the zwitterionic ampholytes amoxicillin, cefalexin, and piroxicam cannot be theoretically calculated by the equations above reported. The value of log $D^{6.5}$ for amoxicillin was taken from the literature [Winiwarter et al. 1998] whereas, to the best of our knowledge, no experimental log $D^{6.5}$ value is reported in the literature for both cefalexin and piroxicam. Since zwitterions behave as "lipophilicity buffers" [Pagliara et al., 1997], we assumed the experimentally determined log $D^{7.4}$ value for cefalexin [Avdeef,

2012] and the experimentally determined log $D^{6.07}$ value for piroxicam [Tsai et al., 1993] as reasonable estimates of their log $D^{6.5}$.

Compound	pK_a	[Ionized] / [unionized] ratio at pH 6.5	log P^N	log $D^{6.5}$	log $k_w^{IAM.MG}$	log $k_w^{IAM.DD2}$
Amoxicillin	2.44[*]/7.14[*]	**	-1.22[a]	-1.70[a]	-0.920	-0.728
Hydrochlorothiazide	7.90[b]	0.04	-0.03[c]	-0.05	0.540	0.977
Ranitidine	8.20[d]	50	0.27[b]	-1.44	1.130	0.860
Cimetidine	6.80[e]	2	0.40[b]	-0.08	1.030	0.783
Cefalexin	3.12[*]/6.84[*]	**	0.65[b]	-1.00[c $]	-0.220	0.021
Terbutaline	10.10[f]	3981	0.90[b]	-2.70	0.662	0.863
Carbamazepine	-	**	2.19[g]	2.19[g]	1.039[h]	1.717[h]
Piroxicam	5.46[i]/1.86[i]	**	3.00[j]	1.20[j §]	1.850[k]	1.767
Ketoprofen	4.45[b]	112	3.12[b]	1.07	1.120[k]	1.360
Propranolol	9.42[b]	832	3.28[c]	0.36[l ‡]	1.821[e]	2.480[e]
Naproxen	4.15[b]	224	3.34[m]	0.99	1.260[k]	1.339
Verapamil	8.92[b]	263	3.79[b]	1.37	2.049	3.085
Fluvastatin	4.30[n]	158	4.17[c]	1.97	2.210	2.843
Isotretinoin	4.76[*]	55	4.20[b]	2.45	2.807	3.704
Desipramine	10.40[b]	7943	4.90[b]	1.38 [c$]	2.826[h]	2.741[h]

* pKa values calculated; ** not reported because either zwitterion or neutral compound; $ log $D^{7.4}$; § log $D^{6.07}$; ‡ log $D^{6.7}$.

a: Winiwarter et al., 1998; *b*: Law et al., 2014; *c*: Avdeef, 2012; *d:* Khan et al., 2005; *e:* Grumetto et al., 2012; *f:* Panigrahi et al., 2005; *g:* Lombardo et al., 2000; *h:* Grumetto et al., 2014; *i:* Bernhard and Zimmermann, 1984; *j:* Tsai et al., 1993; *k:* Barbato et al., 1996; *l:* Barbato et al., 1990; *m:* La Rotonda et al., 1983; *n:* Van de Waterbeemd and Testa, 2008.

Table 1. pKa values, ionization degrees at pH 6.5, and logarithms of lipophilicity values in *n*-octanol and of chromatographic retention factors on IAM phases for the compounds considered.

Table 1 summarizes pKa values, the ratios between ionized and unionized form concentrations at pH 6.5 (calculated by the Henderson-Hasselbalch equation), log P^N, and log $D^{6.5}$ values for the compounds considered, as well as their $\log k_w^{IAM.MG}$ and $\log k_w^{IAM.DD2}$ values, i.e. $\log k_w^{IAM}$ on IAM.PC.MG and IAM.PC.DD2 stationary phases, respectively. As can be seen, the compounds considered span a very large range of log P^N values (- 1.22 – 4.90).

It is worth underlining that using eluents at pH 7.0, to maximize column stability and data reproducibility, does not negatively impact on the significance of the data as measures of membrane interactions occurring at slightly different pH values (e.g. pH 7.4 and pH 6.5, for the BBB passage and the jejunum absorption, respectively). Indeed, it was demonstrated that retention on IAM phases, even for ionizable compounds, is only negligibly affected by variations of the pH of the eluent within the range 5.5 – 7.0 [Amato et al., 2000].

In a first instance, we verified the relationship between IAM retention data determined on the two stationary phases used, i.e. IAM.PC.MG and IAM.PC.DD2. Analogously to that reported in the paragraph 2.3, $\log k_w^{IAM.MG}$ and $\log k_w^{IAM.DD2}$ values for the analytes considered were found strongly collinear (equation (1)) supporting the hypothesis that IAM data from both stationary phases can be assumed as substantially reflecting the interactions between analytes and phospholipids, with secondary interaction mechanisms playing an only minor role.

$$\log k_w^{IAM.DD2} = 1.106\ (\pm 0.104)\ \log k_w^{IAM.MG} + 0.171\ (\pm 0.170) \quad (1)$$

$n = 15$ $r^2 = 0.896$ $s = 0.404$ $F_{1,13} = 112.23$ $F_{1,13}\ \alpha,0.001 = 17.82$

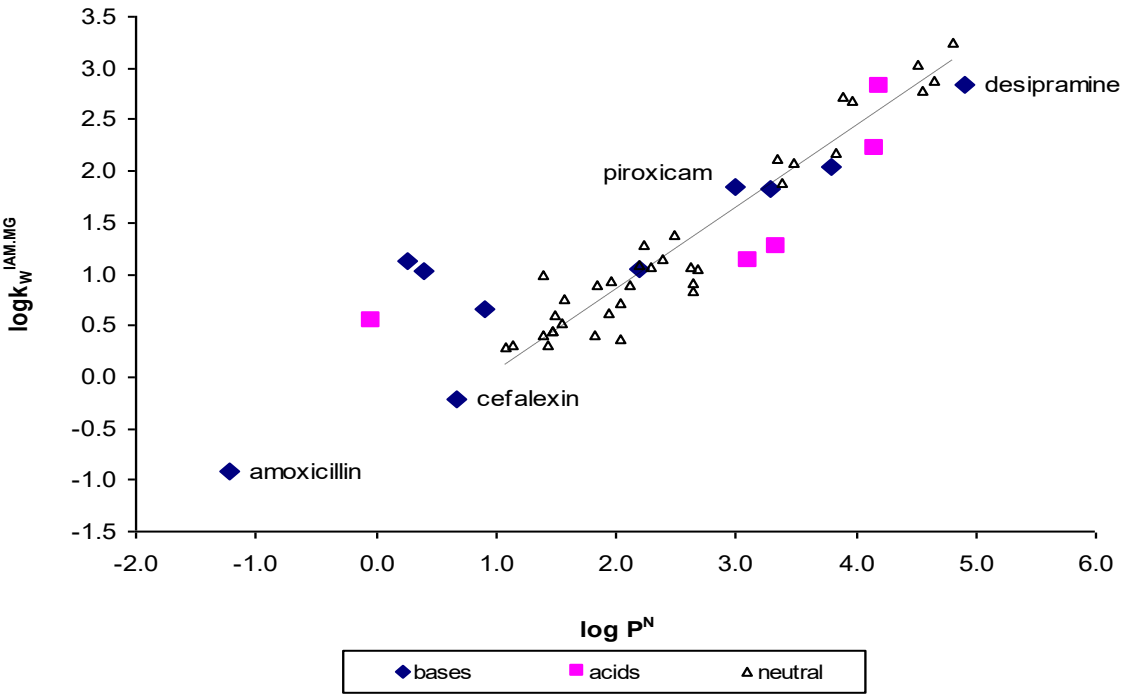
3.3.2. Relationships among the physicochemical parameters

It is worth to remember that the $logk_w^{IAM}$ values of structurally non-related neutral compounds relate unambiguously with *n*-octanol lipophilicity values in the log P range 1.0 – 4.8 (see equations (10) and (11) and in paragraph 1.5).

The plots $logk_w^{IAM}$ *vs.* log P^N of the compounds considered in the present work, superimposed to the plots of the neutral compounds, are reported in Figure 1.

It is interesting to note that not only, as expected, the point relative to carbamazepine, a neutral compound, but also the points relative to the zwitterions amoxicillin, cefalexin, and piroxicam are very close to the line of the neutral compounds; this confirms that phospholipid interaction of zwitterions is related to the *n*-octanol lipophilicity of their neutral form despite of the fact that the latter does not exist as sole form at any pH of the medium [Barbato et al., 2007]. The points of the less lipophilic compounds (log P < 1) are shifted upward with respect to the regression line. However, it should be remembered that at so low lipophilicity values the linearity of the relationship $logk_w^{IAM}$ *vs* log P^N is no longer observed for neutral compounds, too [Taillardat-Bertschinger et al., 2002]. The behavior of poorly lipophilic compounds suggests that polar interactions between analytes and phospholipids (including the electrostatic ones in the case of ionizable compounds) become predominant when lipophilicity falls down. The other points of basic compounds are close to the regression line of neutral compounds, with the exception of desipramine that, as can be seen in Figure 1B, reporting $logk_w^{IAM.DD2}$ *vs.* $logP^N$, lies below the regression line.

A



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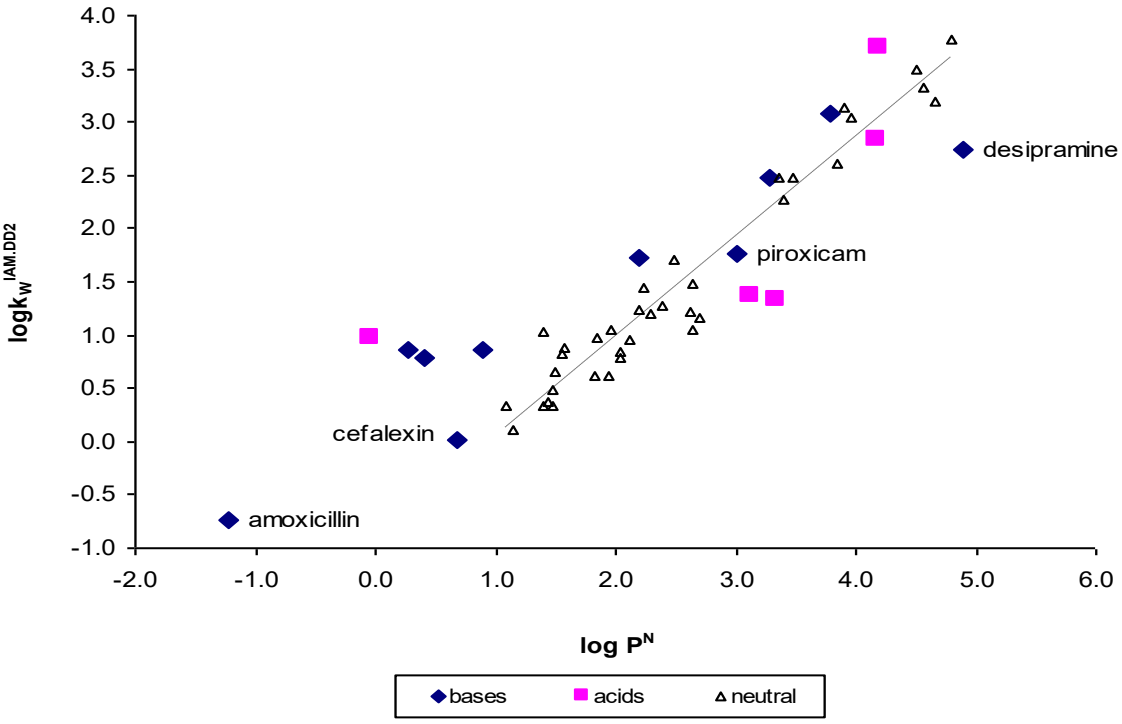


Figure 1. Relationships between either $\log k_w^{IAM.MG}$ (A) or $\log k_w^{IAM.DD2}$ (B) and log P^N values for the fifteen compounds considered in comparison to the plots of 36 neutral compounds [Taillardat-Bertschinger et al., 2002].

Desipramine is the most lipophilic compound in the set considered with a log P^N value of 4.90. Its behavior confirms that strongly lipophilic bases interact with phospholipids weaker than isolipophilic neutral compounds, as already observed on IAM.PC.DD2 phase [Grumetto et al., 2012, 2014].

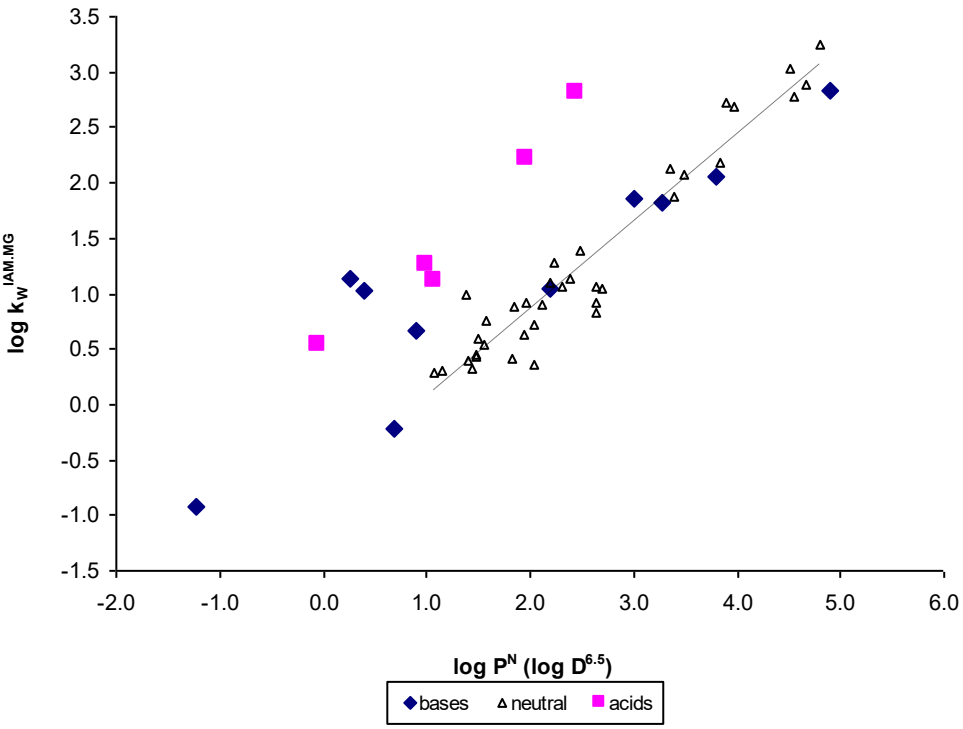
It is worth to remember that the distance of the points from the regression line represents $\Delta\log k_w^{IAM.MG}$ and $\Delta\log k_w^{IAM.DD2}$, for IAM.PC.MG and IAM.PC.DD2 phases, respectively.

Furthermore, as already observed in a previous work of our research group [Grumetto et al., 2013, 2014] as well as in the study on BBB passage above reported (see chapter 2) BBB passage data related with $\Delta\log k_w^{IAM}$ values for only basic compounds whereas for acids they related with $\Delta'\log k_w^{IAM}$ values; the latter are the distances from the regression line of neutral compounds calculated taking into account their log $D^{7.4}$ values, i.e. the lipophilicity actually displayed at the physiological pH of the blood. At jejunum level the compounds considered are in solution at pH 6.5. As can be seen in Figure 2, taking into account the log $D^{6.5}$ values for the acidic compounds considered in the present work, the distances of the points from the regression line of neutral compounds noticeably increase.

Table 2 summarizes $\Delta\log k_w^{IAM.MG}$, $\Delta\log k_w^{IAM.DD2}$, $\Delta'\log k_w^{IAM.MG}$, $\Delta'\log k_w^{IAM.DD2}$, and log P_{eff} values for the compounds considered.

On both IAM phases, a moderate inverse linear relationship was found between $\Delta\log k_w^{IAM}$ values and log P^N values (r^2 = 0.589, $F_{1, 13}$ = 18.65 and r^2 = 0.602, $F_{1, 13}$ = 19.68 for IAM.PC.MG and IAM.PC.DD2, respectively).

A



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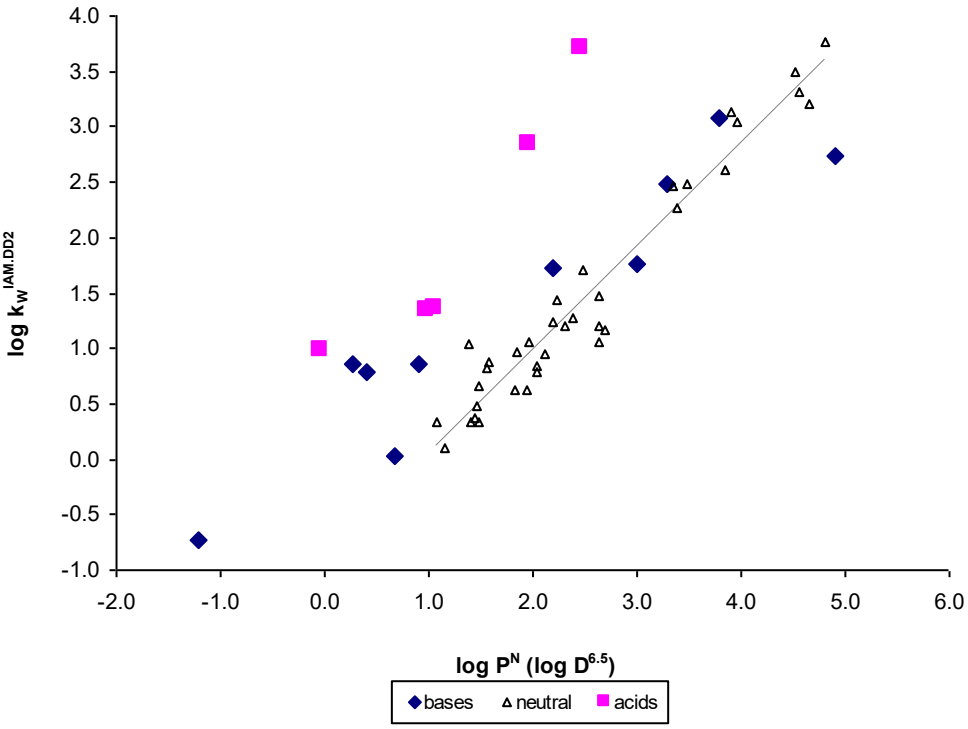


Figure 2. Relationships between either $logk_w^{IAM.MG}$ (A) or $logk_w^{IAM.DD2}$ (B) and the combination of log P^N values for ten compounds (bases, zwitterions, neutral) and log $D^{6.5}$ values for five acids, in comparison to the plots of 36 neutral compounds.

Compound	$\Delta \log k_w^{IAM.MC}$	$\Delta' \log k_w^{IAM.MC}$	$\Delta \log k_w^{IAM.DD2}$	$\Delta' \log k_w^{IAM.DD2}$	$\log P_{eff}$
Amoxicillin	0.778	1.158	1.294	1.743	-4.50
Hydrochlorothiazide	1.296	1.309	1.888	1.904	-5.06
Ranitidine	1.648	3.001	1.491	3.087	-4.57
Cimetidine	1.445	1.823	1.292	1.737	-4.58
Cephalexin	-0.003	1.304	0.297	1.838	-3.81
Terbutaline	0.681	3.532	0.905	4.268	-4.52
Carbamazepine	0.038	0.038	0.555	0.555	-3.37
Piroxicam	0.206	1.632	-0.152	1.529	-3.18
Ketoprofen	-0.619	1.008	-0.671	1.247	-3.06
Propranolol	-0.045	2.268	0.299	3.027	-3.54
Naproxen	-0.653	1.209	-0.898	1.299	-3.07
Verapamil	-0.221	1.697	0.428	2.690	-3.17
Fluvastatin	-0.361	1.384	-0.169	1.889	-3.62
Isotretinoin	0.216	1.600	0.664	2.297	-4.00
Desipramine	-0.319	2.469	-0.953	2.335	-3.35

Table 2. Values of the differences between observed and expected logarithms of retention factors on IAM.PC.MG and IAM.PC.DD2 stationary phases ($\Delta \log k_w^{IAM.MG}$ and $\Delta' \log k_w^{IAM.MG}$, and $\Delta \log k_w^{IAM.DD2}$ and $\Delta' \log k_w^{IAM.DD2}$, respectively) and logarithms of the human effective jejunum permeability values for the compounds considered ($\log P_{eff}$).

3.3.3. Relationships with intestinal absorption data

Since inter-laboratory variability of biological data is generally too high for correlation studies, we only took into account the data, reported in the literature, from a single source [Lennernäs 2014] to obtain a consistent scale of jejunal P_{eff} values. Obviously, we could not take into account the compounds undergoing active transport mechanisms, e.g. L-dopa and amino acids.

Log P_{eff} values, experimentally measured at pH 6.5 at jejunum level [Lennernäs 2014], moderately related linearly with log P^N values (Figure 3A and equation (2)).

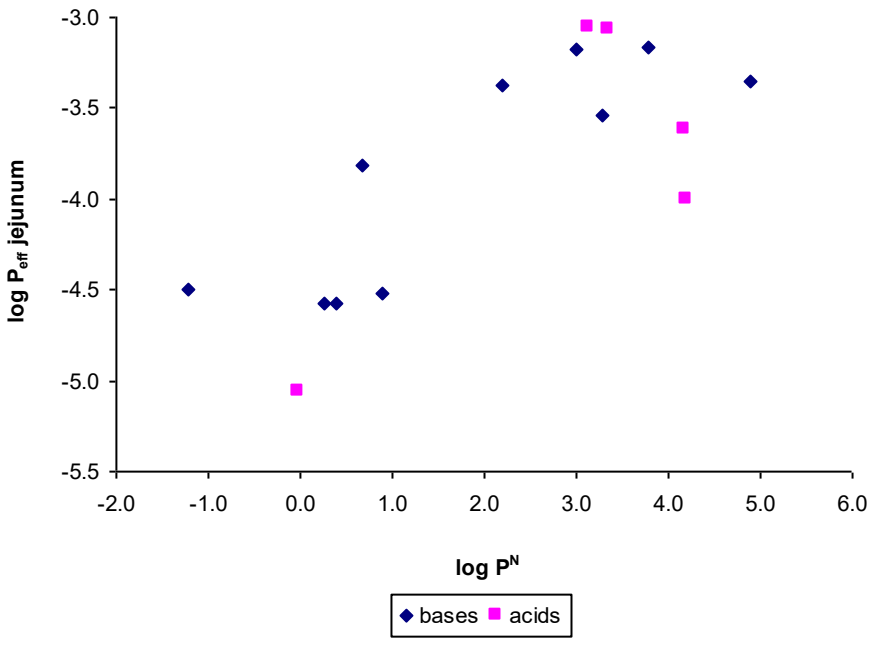
$$\log P_{eff} = 0.273 (\pm 0.062) \log P^N - 4.427 (\pm 0.177) \quad (2)$$

$n = 15$ $r^2 = 0.598$ $s = 0.437$ $F_{1, 13} = 19.32$ $F_{1, 13}\ \alpha,0.001 = 17.82$

Parabolic relationships between log P_{eff} values and log P^N values were not statistically significant (at both α levels 0.001 and 0.01) for both the whole set and a set reduced by the exclusion of acids.

Since the permeability values were measured at pH 6.5 we also verified the possible relationships with log $D^{6.5}$. Both linear and parabolic relationships were not statistically significant (data not shown). However, after the exclusion of two compounds (cimetidine and hydrochlorothiazide) (Figure 3B), a parabolic relationship was significant at α level 0.01 ($r^2 = 0.733$, $F_{2, 10} = 13.72$). Finally, we plotted log P_{eff} values against the combination of log P^N values and, for only acids, log $D^{6.5}$ values. Both linear and parabolic relationships were not statistically significant (data not shown).

A



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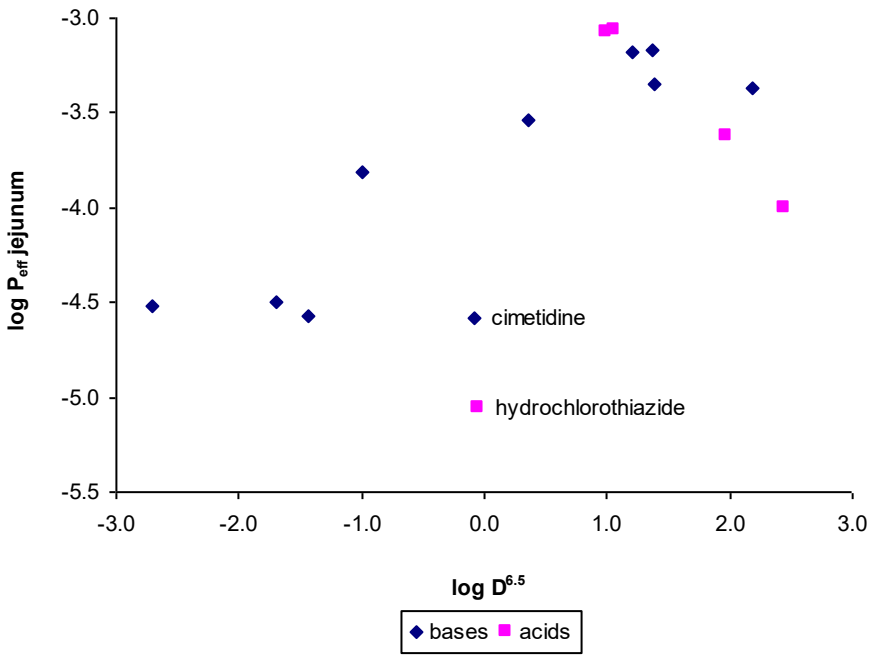


Figure 3. Relationships between log P_{eff} and either log P^N values (A) or log $D^{6.5}$ values for the 15 compounds considered.

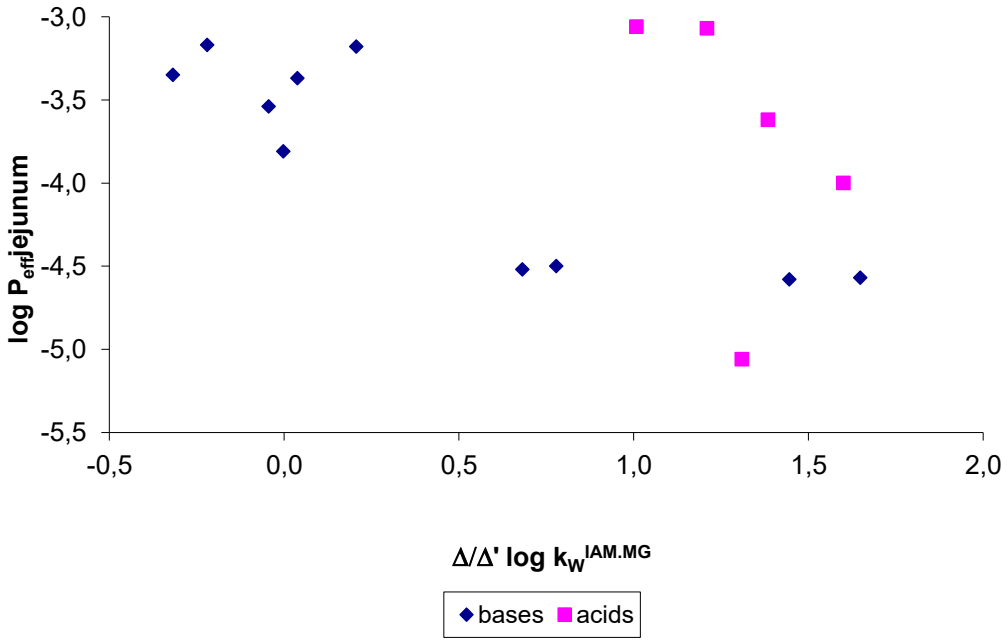
No significant relationship was found between log P_{eff} values and either $logk_w^{IAM.MG}$ or $logk_w^{IAM.DD2}$ values (linear relationship statistics: r^2 = 0.223, $F_{1, 13}$ = 3.72 and r^2 = 0.233, $F_{1, 13}$ = 3.95, for IAM.PC.MG and IAM.PC.DD2, respectively).

The lack of a direct relationship between log P_{eff} and membrane phospholipid interaction data was not surprising since it was already verified in previous studies on the mechanism of BBB passage [Grumetto et al., 2012, 2013, 2014] as well as in the study on BBB passage reported in chapter 2. In all those studies significant relationships with biological data were only found when both $\Delta logk_w^{IAM}$ and $\Delta' logk_w^{IAM}$ values, i.e. $\Delta \log k_w^{IAM}$ values for bases and $\Delta' logk_w^{IAM}$ values for acids, were taken into account. However, in the present study, no relationship is found between log P_{eff} and the analogous combination of $\Delta logk_w^{IAM}$ and $\Delta' logk_w^{IAM}$ values (Figure 4) (r^2 = 0.262, $F_{1, 13}$ = 4.63 and r^2 = 0.238, $F_{1, 13}$ = 4.07, for IAM.PC.MG and IAM.PC.DD2 respectively), i.e. taking into account $\Delta' \log k_w^{IAM}$ values for

acids. Similarly, no relationship is observed with log P_{eff} taking into account $\Delta'\log k_w^{IAM}$ values for all the analytes (data not shown).

In contrast, significant inverse linear relationships are found between log P_{eff} and $\Delta\log k_w^{IAM}$ values taking into account $\Delta\log k_w^{IAM}$ values for all the compounds (Figure 5A and equation (3) for IAM.PC.MG and Figure 5B and equation (4) for IAM.PC.DD2).

A



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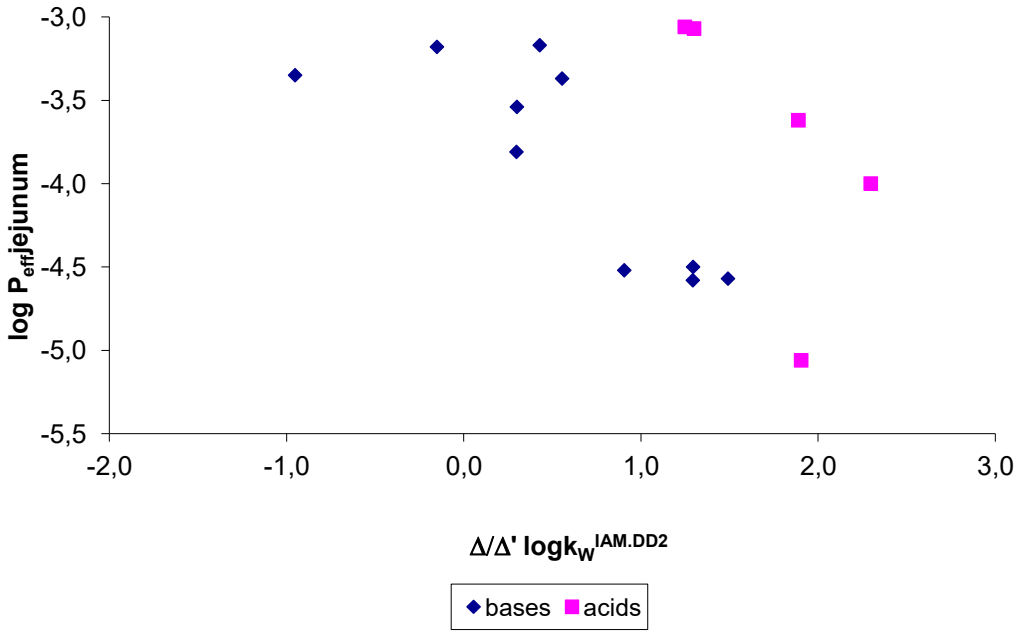
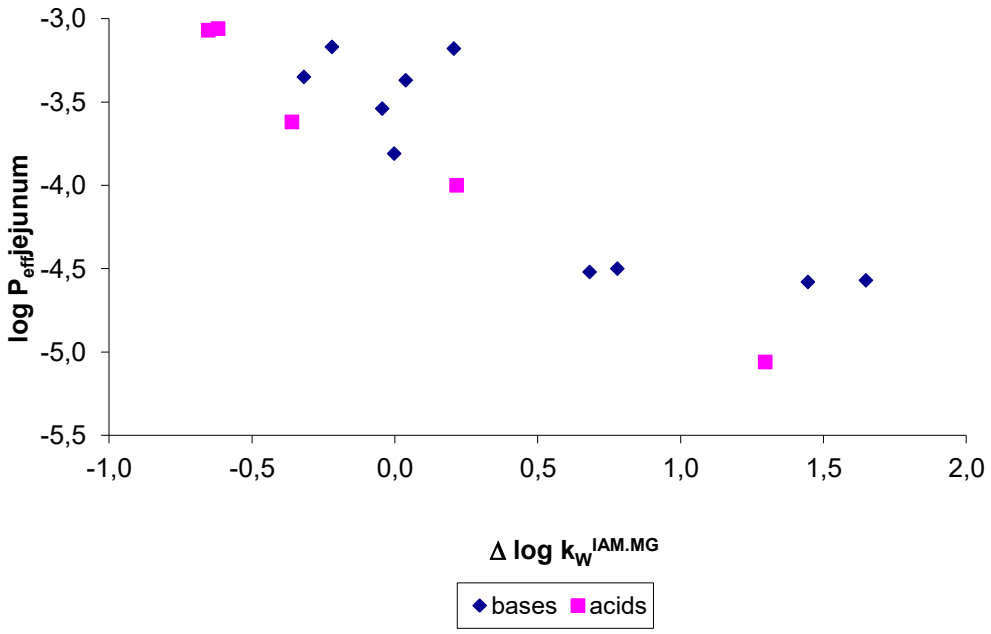


Figure 4. Relationships between log P_{eff} and either $\Delta\log k_w^{IAM.MG}$ ($\Delta'\log k_w^{IAM.MG}$) (A) or $\Delta\log k_w^{IAM.DD2}$ ($\Delta'\log k_w^{IAM.DD2}$) (B) values for the 15 compounds considered. $\Delta'\log k_w^{IAM.MG}$ and $\Delta'\log k_w^{IAM.DD2}$ were considered for only acids.

A



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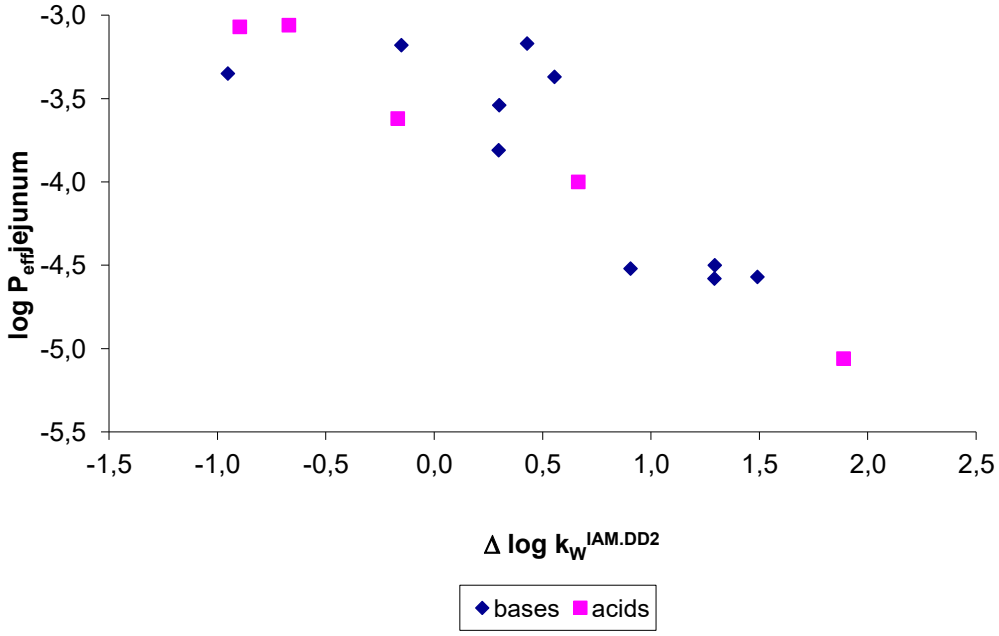

Figure 5. Relationships between log P_{eff} and either $\Delta\log k_w^{IAM.MG}$ (A) or $\Delta\log k_w^{IAM.DD2}$ (B) values for the 15 compounds considered.

$\log P_{eff}$ = -0.807 (± 0.111) $\Delta\log k_w^{IAM.MG}$ - 3.607 (± 0.084) (3)

n = 15 r^2 = 0.803 s = 0.306 $F_{1, 13}$ = 53.00 $F_{1, 13}$ α,0.001 = 17.82

$\log P_{eff}$ = -0.674 (± 0.098) $\Delta\log k_w^{IAM.DD2}$ - 3.545 (± 0.092) (4)

n = 15 r^2 = 0.784 s = 0.321 $F_{1, 13}$ = 47.06 $F_{1, 13}$ α,0.001 = 17.82

Therefore, differently from BBB permeation, jejunum permeability data of not only bases but also acids relate with $\Delta\log k_w^{IAM}$.

As hypothesized by other authors [Lennernäs 1997], these results suggest that jejunum absorption and BBB passage realize by essentially similar mechanisms, being the two barriers similar in their chemical composition. However, polar/electrostatic interactions appear as more effective in hindering BBB passage than jejunal absorption, but for only acidic compounds. As a matter of fact, these forces are quantified by $\Delta\log k_w^{IAM}$ parameter, as for basic compounds, in the relationships with jejunal absorption but had to be quantified by $\Delta'\log k_w^{IAM}$, i.e. magnified, in the relationships with BBB passage.

Based on a so small number of data, it is difficult to rationalize why only acids, but not bases, are affected by polar/electrostatic forces more strongly at level of BBB than at level of jejunal barrier. As a hypothesis to be verified on a larger set of data, it may be suggested that the different behavior observed for acids may be related to different physical phenomena encoded in the two biological parameters considered. Indeed, P_{eff} values account for the rate of disappearance of a drug from the jejunal content whereas log BB are parameters related to the concentrations at the steady-state observed after a given time and also reflect other processes including plasma protein binding and tissue binding [Bickel 2005].

3.4 Conclusions

In this study we found that both log P^N and, even more significantly, $\Delta\log k_w^{IAM}$ values, related with log P_{eff} values, which, in turn, are non-linearly related to the drug fraction absorbed. This suggests that $\Delta\log k_w^{IAM}$ parameter is a suitable measure of the polar/electrostatic interactions occurring *in vivo* at membrane level.

These results are partially in accordance with the previous study on BBB passage, reported in chapter 2, and, once again, are consistent with the “flip-flop” model of membrane passage. Indeed, this model suggests that the charged forms of the analytes are able to interact with the charged head groups of phospholipid bilayers but unable to migrate in

their uncharged inner moieties. This latter step is operated by the neutral forms in dynamic equilibrium. Accordingly, the linear inverse relationships observed between log P_{eff} and $\Delta\log k_w^{IAM}$ values indicate that polar/electrostatic interactions act as "trapping" forces at membrane level, although promoting drug partition. Furthermore, the direct linear relationship found between log P_{eff} and log P^N values may suggest that it is the lipophilicity of the neutral forms to act as a driving force for membrane passage.

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4.0 RELATIONSHIPS BETWEEN POLAR INTERACTIONS DRUG/ PHOSPHOLIPIDS ESTIMATED BY IAM-HPLC AND CULTURED CELL LINE PASSAGE DATA

4.1 Introduction

Cultured cell models, using either Caco-2 (colorectal adenocarcinoma) or MDCK (Madin – Darby canine kidney) epithelial cell lines, represent an effective method to predict jejunal absorption. As suggested by Avdeef "when the cell-based permeability assays are done optimally...the cellular assays can be direct predictors of the human jejunal permeability, as well as human intestinal absorption" [Avdeef 2012a].

Nevertheless, cultured cell based methods are difficult to standardize and often the results from different laboratories are not comparable; furthermore, albeit simpler that the methods *in vivo*, they are expensive and time-consuming. Therefore, the *in vitro* methods based on the measures of physico-chemical properties of the analytes and able to predict at least passive absorption are desirable, also having the advantage of giving rational explanations on the mechanisms of adsorption and, consequently, on the molecular structural features requested to optimize the oral bioavailability.

As reported in chapter 3, jejunal absorption values for 15 structurally non-related basic, acidic, ampholytic, and neutral drugs, measured *in vivo* by the Loc-I-Gut technique (log P_{eff}) related with $\Delta\log k_w^{IAM}$ values by a highly significant linear inverse relationship [Grumetto et al., 2015].

For structurally non-related neutral analytes with a zero value of polar surface area (PSA), phospholipid affinity indexes ($\log k_w^{IAM}$) linearly relate unambiguously to the *n*-octanol lipophilicity values ($\log P^N$) by a single relationship. In contrast, for both ionizable and, in a much smaller extent, neutral analytes with positive PSA values, $\log k_w^{IAM}$ and $\log P^N$ values are not collinear and $\Delta\log k_w^{IAM}$ are the differences between the values experimentally observed and the values expected for neutral compounds, with zero PSA, having the same $\log P^N$.

The relationships previously found between $\Delta\log k_w^{IAM}$ and biological data suggested that this component plays a pivotal role in the passage of cellular barriers acting as "trapping" forces at intestinal barrier level, despite they can contribute positively to the total interaction observed.

As already mentioned, this hypothesis would be consistent with the "flip-flop" model [Gurtovenko and Vattulainen, 2007; Krämer et al., 2009], according to which the global interaction forces drug/membrane are expressed by the $\log k_w^{IAM}$, the lipophilicity component of the neutral forms, $\log P^N$ represents the driving-force for permeation and the polar/electrostatic component, $\Delta\log k_W^{IAM}$ represents a "trapping" force. These interaction forces modulate membrane permeation and, in the case of ionizable analytes, arise from the dynamic equilibriums between neutral and ionized forms at membrane microenvironment level.

The relationships found between $\Delta\log k_w^{IAM}$ and absorption values at jejunal level, P_{eff}, were supported by a relatively small set of compounds. Indeed, only the compounds supposed to be absorbed by mainly passive mechanism and, to reasonably reduce the uncertainty of biological data, whose P_{eff} values were determined in a single laboratory could be taken into account.

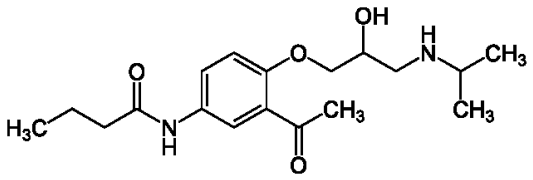
Large sets of data on the passage through cellular lines are available in the literature. They are directly related to intestinal absorption data and can offer an interesting possibility to verify the soundness of $\Delta\log k_w^{IAM}$ parameters at describing the intestinal absorption potential of drugs. Therefore, we aimed at verifying whether these data related with $\Delta\log k_w^{IAM}$ parameters to validate the proposed model.

Therefore, we took into account two sets of structurally non-related basic, acidic, ampholytic, and neutral drugs. The first one consisted of 38 compounds whose Caco-2 permeation data, $\log P_{app}$, were reported in the literature [Camenisch et al., 1998; Yazdanian et al., 1998]; the second one consisted of 47 compounds (including 27 compounds also considered in the first set) whose Caco-2/MDCK permeation data were corrected to express the sole transcellular intrinsic permeability of the drugs, $\log P_0^{Caco\text{-}2/MDCK}$ [Avdeef 2012b].

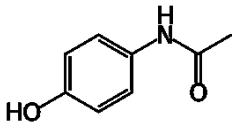
It is reported in the literature that the *in vitro* apparent permeability values, $\log P_{app}$, i.e. the crude permeation data measured on cultured cell lines, can be separated into four contributions [Ho et al., 2000]: i) aqueous boundary layer (represented by the accessible intestinal surface area - P_{ABL}), ii) filter-determined permeability related to the polycarbonate porous support of the cultured cells (P_f), transcellular permeability (P_C), and paracellular permeability (P_{para}), according to the following expression:

$$\frac{1}{P_{app}} = \frac{1}{P_{ABL}} + \frac{1}{P_f} + \frac{1}{P_C + P_{para}}$$

These contributions are different between *in vitro* and *in vivo* systems. Therefore, it has been reported that log P_{app} values cannot be directly equated to the corresponding human *in situ* log P_{eff} values, since a normalization for such differences is required [Avdeef 2012b]. In contrast, since for most drugs it is the transcellular passage to play the major role in the intestinal absorption, log $P_0^{Caco\text{-}2/MDCK}$ values can be assumed as reasonably good estimates of drug *in vivo* absorption potential, although they do not encode P_{ABL} and P_{para} contributions. The whole set of 58 compounds consisted of thirty-two bases, thirteen acids, six zwitterions, and seven neutral compounds (Scheme 1).



Acebutolol



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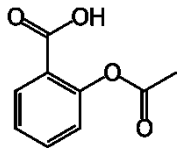

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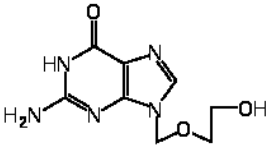
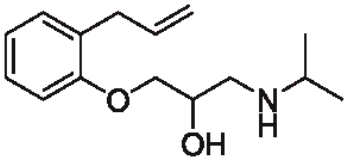
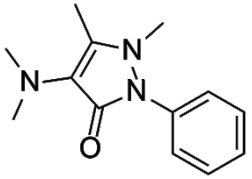


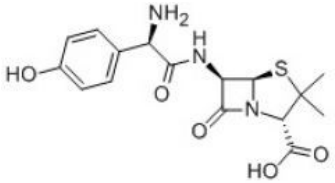
Acyclovir



Alprenolol



Aminopyrine



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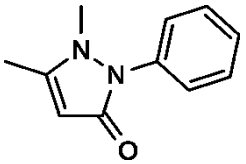
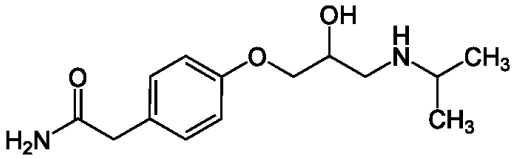


Antipyrine



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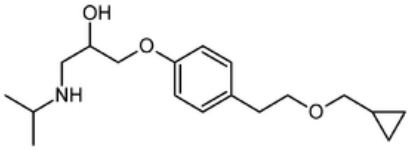
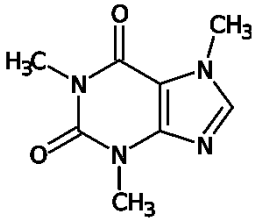
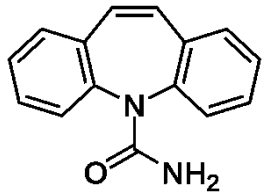


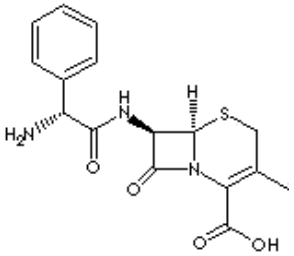
Betaxolol



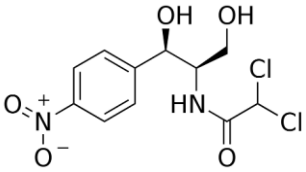
Caffeine



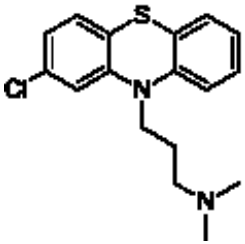
Carbamazepine



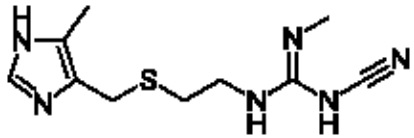
Cefalexin



Chloramphenicol



Chlorpromazine



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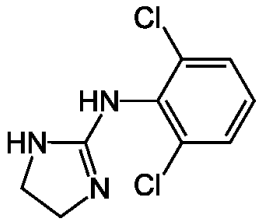

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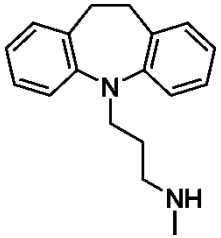
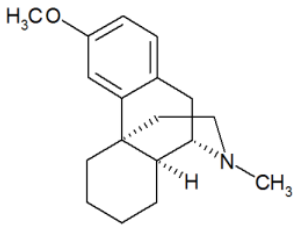
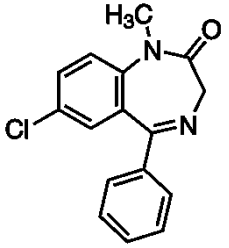


Desipramine



Dextrometorphan



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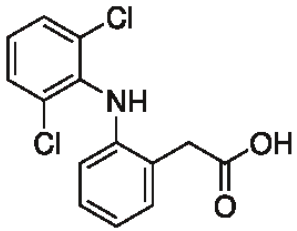

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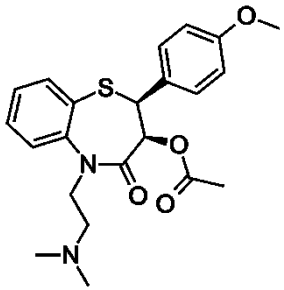

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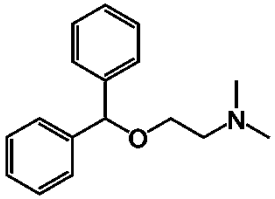

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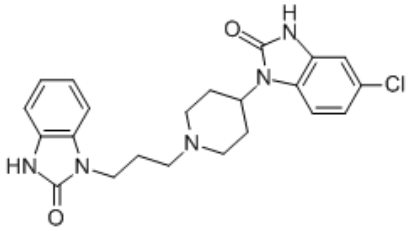

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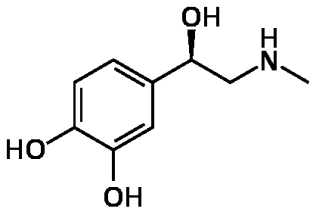

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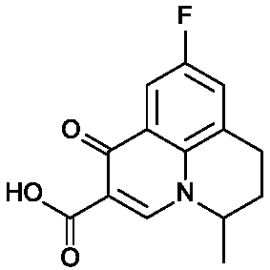

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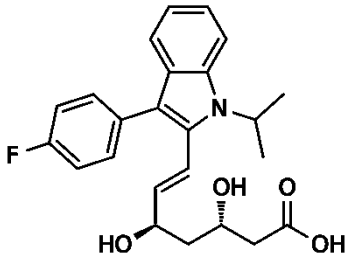

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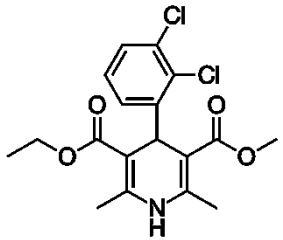

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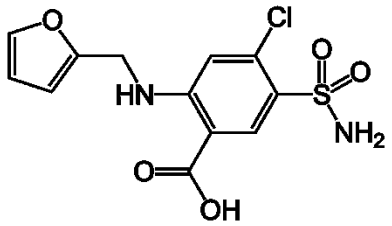

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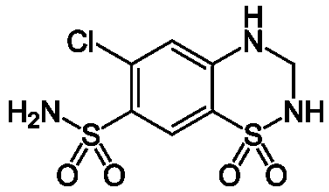

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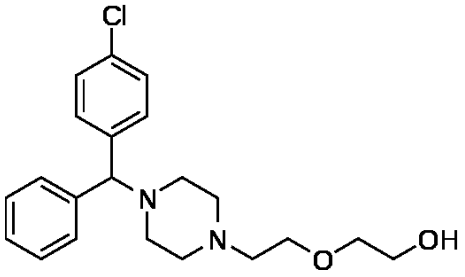

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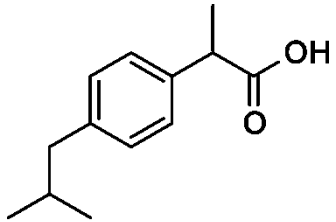

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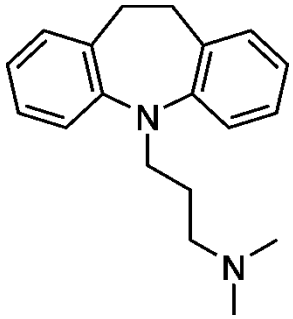

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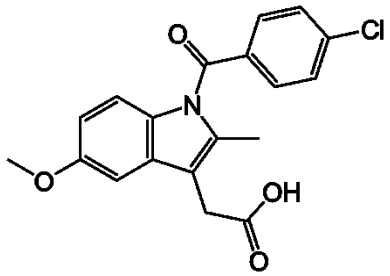

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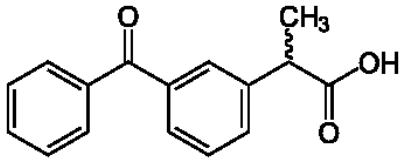

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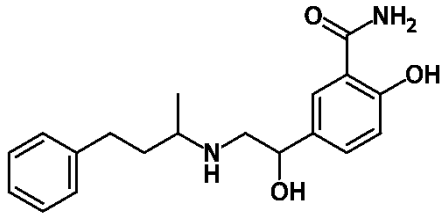

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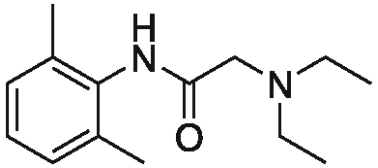

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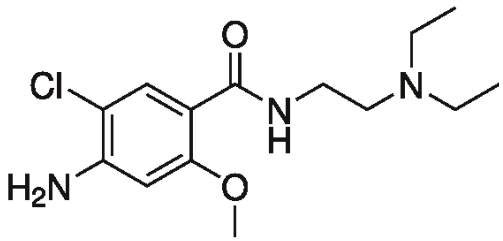

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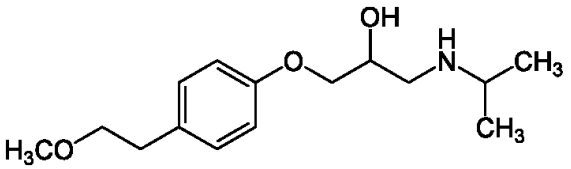

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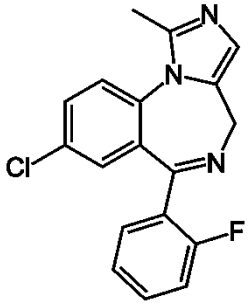

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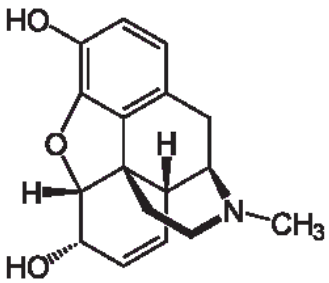

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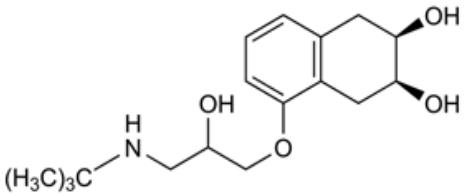

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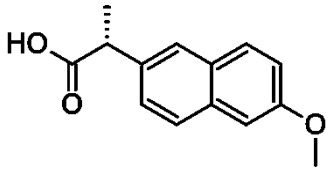

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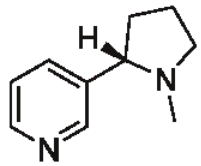

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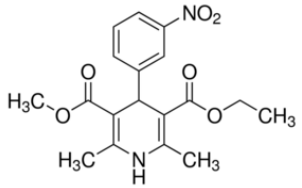

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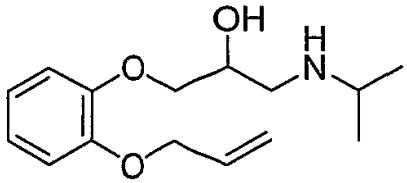

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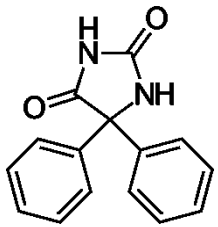

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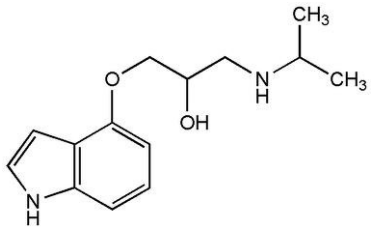

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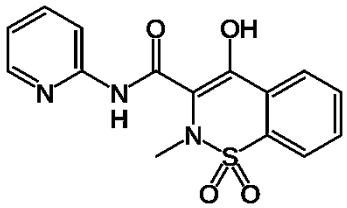

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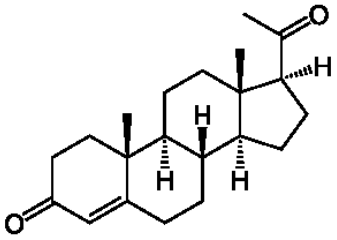

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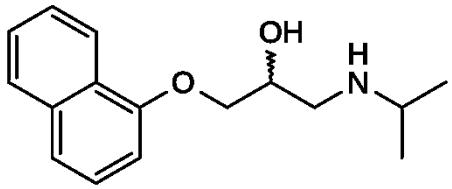

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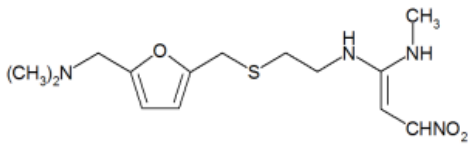

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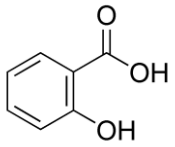

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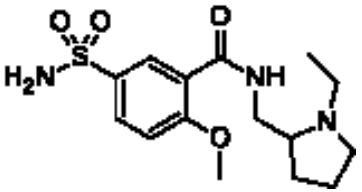

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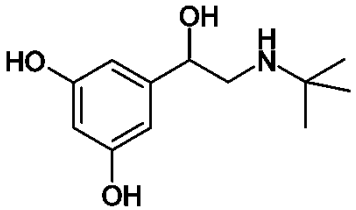

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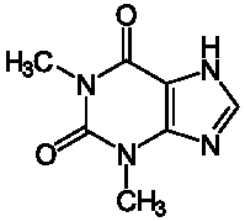

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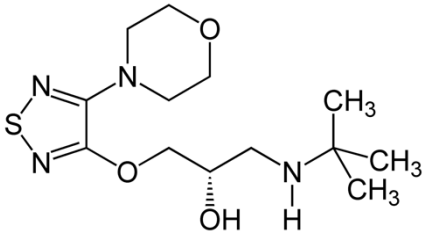
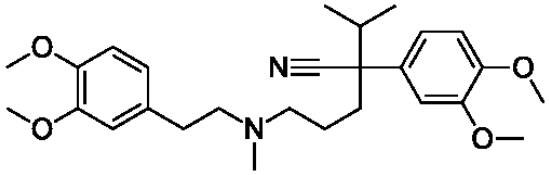


Timolol



Verapamil

Scheme 1. Chemical structures of the compounds investigated in the present study.

Their phospholipid affinity, expressed as $logk_w^{IAM}$, was experimentally measured by the IAM-HPLC technique performed on two different phospholipid stationary phases (IAM.PC.MG and IAM.PC.DD2) and the respective $\Delta logk_w^{IAM}$ values were calculated.

Besides $\Delta logk_w^{IAM}$, various physico-chemical parameters, such as i) *n*-octanol lipophilicity of the neutral forms, log P^N, ii) *n*-octanol lipophilicity of the mixtures of neutral and ionized forms at pH 7.4, log $D^{7.4}$, iii) phospholipid affinity indexes, $logk_w^{IAM}$, and iv) the differences between the experimental $logk_w^{IAM}$ values and those expected for neutral isolipophilic compounds, but calculated taking into account the log $D^{7.4}$ values of the analytes, $\Delta' logk_w^{IAM}$, were also considered and their possible relationships with permeation data were investigated.

4.2 Materials and methods

4.2.1 Chromatographic conditions and equipment

The analyses were performed according to the method previously reported in paragraph 2.2.

The analytical HPLC columns were a IAM.PC.MG (4.6 mm x 150 mm; Regis Chemical Company, Morton Grove, IL) and a IAM.PC.DD2 (4.6 mm x 100 mm; Regis Chemical Company, Morton Grove, IL).

Only one IAM.PC.MG and only one IAM.PC.DD2 column was used throughout the present study. To avoid that the experimental measurements were affected by retention changes due to column aging, the retention times of five test compounds (amlodipine, p-nitroaniline, toluene, isradipine, and flurbiprofen) were weekly checked . No correction was done to the experimental retention values since no retention value of test compounds changed more than 4% during the study.

4.2.2. Sample preparation

Each analyte was dissolved in the mobile phase or in methanol to *ca*. 10^{-4} M concentration.

4.2.3. Lipophilic and biological activity parameters

log P^N values, i.e. partition coefficients *n*-octanol/aqueous phase of the neutral form of analytes, were from the literature, either reported by the clog P database (Clog P for Windows version 2.0, Biobyte Corp., Claremont, CA) or from other literature sources [Avdeef, 2012c; Barbato et al., 1990; Barbato et al, 1996; Barbato et al., 1997a; Barbato et

al., 1998; La Rotonda et al., 1983; Lombardo et al., 2000; Seydel and Wiese, 2002; Tsai et al., 1993; Wishart et al., 2006].

The *n*-octanol/aqueous buffer at pH 7.4 partition coefficients (log $D^{7.4}$) were calculated according to the following equations:

$\log D^{7.4} = \log P^N - \log (1 + 10^{7.4 - pKa})$ (for acids)

$\log D^{7.4} = \log P^N - \log (1 + 10^{pKa - 7.4})$ (for bases)

with the exception of i) acebutolol, aciclovir, amoxicillin, atenolol, cefalexin, furosemide, hydrochlorothiazide, ibuprofen, labetalol, metoprolol, morphine, nadolol, naproxen, oxprenolol, pindolol, sulpiride and timolol, whose values were taken from the literature [Avdeef, 1996; Avdeef, 2012c; Barbato et al., 1990; Kerns et al., 2005; Sugano et al., 2010; Winiwarter et al., 1998], and ii), piroxicam whose experimental log $D^{6.07}$ was assumed as a reasonable estimate of its log $D^{7.4}$ value [Tsai et al., 1993].

Caco-2 log P_{app} values, measured at pH 7.4, were from the literature [Camenisch et al., 1998; Yazdanian et al., 1998]. Caco-2 permeation data corrected to extract the sole transcellular component of cellular passage, log $P_0^{Caco\text{-}2/MDCK}$, were also taken from the literature [Avdeef 2012b].

4.2.4 Statistical analysis

Linear regression analysis was performed by a commercially available statistical package (Microsoft Excel 2003) for personal computer observing the requirements of significant regression analysis. PSA was calculated by the software VEGA 3.0.5 for Windows-based PCs [Pedretti et al., 2004].

4.3 Results and discussions

Table 1 summarizes pKa values, the percentages of the ionized forms at pH 7.4 (calculated by the Henderson-Hasselbalch equation), log P^N, and log $D^{7.4}$ values for the compounds considered, as well as their $logk_w^{IAM.MG}$ and $logk_w^{IAM.DD2}$ values, i.e. $logk_w^{IAM}$ on IAM.PC.MG and IAM.PC.DD2 stationary phases, respectively.

Table 2 summarizes the values of $\Delta logk_w^{IAM}$, $\Delta' logk_w^{IAM}$, Caco-2 permeability data ($logP_{app}$), and log $P_0^{Caco\text{-}2/MDCK}$ for the whole set of analytes.

$logk_w^{IAM}$ values can be assumed as direct measures of the interactions between analytes and phospholipids. Indeed, possible secondary interactions between the analytes and the residual groups from end-capping of the propylamino-silica core can be reasonably

excluded because, for the whole set of 58 compounds, the $\log k_w^{IAM}$ values measured on IAM.PC.MG (supporting residual hydroxy groups) and those measured on IAM.PC.DD2 (supporting C10 and C3 alkyl chains) were found strongly collinear (Figure 1 and equation (1)), as several times previously verified for other sets of compounds.

$$\log k_W^{IAM.DD2} = 1.015\ (\pm\ 0.039)\ \log k_W^{IAM.MG} + 0.292\ (\pm\ 0.061) \qquad (1)$$

$n = 58$ $\quad r^2 = 0.924$ $\quad s = 0.299$ $\quad F_{1,56} = 675.62$ $\quad F_{1,56}\ \alpha,0.001 = 12.22$

series	Compound	pKa	% ionized at pH 7.4	log P^{N}	log $D^{7.4}$	log $k_w^{IAM.MG}$	log $k_w^{IAM.DD2}$	Chemical character
1, 2	Acebutolol	9.67	99.47	1.81[a]	-0.27[a]	1.761[b]	1.409[b]	B
2	Acetaminophen	*	*	0.34[c]	0.34	0.126[d]	0.280[d]	N
1, 2	Acetylsalicylic acid	3.50	99.99	1.19[e]	-1.20	-0.965[d]	-0.850[d]	A
2	Acyclovir	2.55	0.00	-1.80[c]	-1.81[c]	-0.530	-0.728	B
1, 2	Alprenolol	9.60	99.37	3.10[f]	0.50	1.530[g]	2.260[b]	B
1	Aminopyrine	4.50	0.13	1.00[e]	0.99	0.536	0.573	B
1, 2	Amoxicillin	2.44/7.14	*	-1.22[c]	-1.70[h]	-0.920[i]	-0.728[i]	B/A
1,2	Antipyrine	0.65	0	0.56[c]	0.38	0.599	0.393	B
1, 2	Atenolol	9.60	99.37	0.14[a]	-1.61[a]	-0.005[j]	0.554[j]	B
1	Betaxolol	9.40	99.01	2.81[e]	0.42	1.155[j]	1.838[j]	B
1, 2	Caffeine	0.52	0	-0.07[n]	-0.07	0.128	0.116	B
2	Carbamazepine	*	*	2.19[k]	2.19	1.039[l]	1.717[l]	N
2	Cefalexin	3.12/6.84	*	0.65[c]	-1.10[m]	-0.220[i]	0.021[i]	B/A
2	Chloramphenicol	*	*	1.14[e]	1.14	0.567	1.346	N
1	Chlorpromazine	9.41	99.03	5.19[n]	2.89	1.799[o]	2.225[o]	B
1, 2	Cimetidine	6.80	34.42	0.40[e]	0.19	0.633[o]	1.048[o]	B
1, 2	Clonidine	8.02	80.65	1.57[n]	0.25	0.948[o]	1.316[o]	B
1, 2	Desipramine	10.40	99.90	4.90[e]	1.38	2.826[l]	2.741[l]	B
2	Dextrometorphan	9.13	98.17	3.60[e]	1.86	1.578	2.579	B
1, 2	Diazepam	3.40	0.01	2.99[p]	2.99	1.731	2.198[d]	B

2	Diclofenac	4.18	99.94	4.51[e]	1.30	2.430[q]	2.850	A
1, 2	Diltiazem	8.94	97.20	3.41[n]	2.02	2.121	2.780	B
2	Diphenhydramine	8.76	95.82	3.18[c]	1.80	2.219	2.170	B
2	Domperidone	9.00	97.55	3.90[e]	2.29	2.790[l]	3.213[l]	B
1	Epinephrine	9.16	98.29	-0.68[n]	-2.59	-0.098	0.250	B
2	Flumequine	5.70	98.00	1.72[c]	0.65	0.800[r]	1.183[r]	A
2	Fluvastatin	4.56	99.86	4.17[c]	1.14	2.210[i]	2.843[i]	A
1	Felodipine	*	*	4.80[s]	4.80	2.980[s]	3.470[j]	N
1	Furosemide	3.04	100	2.29[t]	-0.24[c]	0.780	0.920	A
2	Hydrochlorothiazide	8.95	2.74	-0.03[c]	-0.18[c]	0.540[i]	0.977[i]	A
2	Hydroxyzine	6.62	14.23	3.55[c]	3.48	2.908[l]	2.965[l]	B
2	Ibuprofen	4.41	99.90	4.13[c]	1.44[c]	0.972[d]	1.170[d]	A
1, 2	Imipramine	9.49	99.19	4.80[e]	2.30	3.064[o]	3.008[o]	B
2	Indomethacin	3.96	99.96	4.27[e]	0.68	2.390[s]	2.080[d]	A
2	Ketoprofen	4.23	99.93	3.16[c]	-0.01	1.120[q]	1.360	A
1, 2	Labetalol	8.21/9.3	*	2.85[a]	1.09	1.439[j]	2.017[j]	B/A
1	Lidocaine	7.90	75.97	2.48[f]	1.53	0.750[f]	1.094	B
2	Metoclopramide	9.08	97.95	2.72[c]	1.031	1.199	1.902	B
1, 2	Metoprolol	9.43	99.08	1.95[a]	-0.26[a]	0.642[j]	1.099[j]	B
2	Midazolam	*	*	3.12[c]	3.12	2.302[o]	2.505[o]	N
2	Morphine	9.48/9.25	*	0.89[c]	-0.07[u]	0.767[o]	1.180[o]	B/A
1	Nadolol	9.40	99.01	0.93[a]	-1.30[a]	0.401[j]	1.005[j]	B
2	Naproxen	4.15	99.94	3.24[c]	0.09[c]	1.260[q]	1.339[i]	A

1, 2	Nicotine	8.00	79.92	1.17[e]	0.13	0.844[o]	1.184[o]	B
1	Nitrendipine	*	*	4.15[s]	4.15	2.270[s]	3.040[s]	N
1	Oxprenolol	9.50	99.21	2.16[a]	0.13[a]	0.936[j]	1.455[j]	B
1, 2	Phenytoin	8.28	11.65	2.47[e]	2.42	1.787[d]	1.789[d]	A
1, 2	Pindolol	9.70	99.50	1.80[a]	-0.10[a]	0.902[j]	1.302[j]	B
1, 2	Piroxicam	5.46/1.86	*	3.00[v]	1.20	1.850[q]	1.767[i]	B/A
1	Progesterone	*	*	3.87[n]	3.87	2.769[d]	3.317[d]	N
1, 2	Propranolol	9.50	99.21	3.28[a]	0.48	1.821[o]	2.480[o]	B
1, 2	Ranitidine	8.36	90.12	0.27[a]	-1.15	0.834[o]	0.812[o]	B
1, 2	Salicylic acid	2.97	100	2.27[w]	-0.90	-0.143[d]	-0.075[d]	A
1, 2	Sulpiride	9.98/8.97	*	1.11[n]	-1.15[x]	1.175	1.512	B/A
1, 2	Terbutaline	10.10	99.80	0.90[e]	-2.70	0.662[i]	0.863[i]	B
1, 2	Theophylline	8.60	5.94	-0.02[e]	-0.04	-0.130	0.100	A
1, 2	Timolol	8.80	96.17	1.98[a]	-0.52[a]	0.610[j]	1.058[j]	B
1, 2	Verapamil	8.90	96.93	3.79[e]	1.88	2.892	3.085[i]	B

Series 1: compounds for relationships with CACO-2 permeation data;

Series 2: compounds for relationships with CACO-2/MDCK data corrected to represent only transcellular passage.

A = acid; B = base; N = neutral; B/A = ampholyte. For the ampholytes the two pKa values reported refer to the acidic and basic functions, respectively.

[*] not reported because either zwitterions or neutral compound.

a: Barbato et al., 1990
b: Barbato et al., 2009
c: Avdeef, 2012c
d: Grumetto et al., 2013
e: Wishart et al., 2006
f: Barbato et al., 1997a
g: Barbato et al., 2004
h: Winiwarter et al., 1998
i: Grumetto et al., 2015
j: Barbato et al., 2005
k: Lombardo et al., 2000
l: Grumetto et al., 2014
m: Sugano et al., 2010
n: clog P, Biobyte Corp
o: Grumetto et al., 2012
p: Seydel and Wiese, 2002
q: Barbato et al., 1997b
r: Barbato et al., 2007
s: Barbato et al., 1996
t: Barbato et al., 1998
u: Avdeef, 1996
v: Tsai et al., 1993
w: La Rotonda et al., 1983
x: Kerns et al., 2005

Table 1. pKa values, ionization percentages at pH 7.4, logarithms of lipophilicity values in *n*-octanol and of chromatographic retention factors on IAM stationary phases for the compounds considered.

series	compound	$\Delta\log k_w^{IAM.MG}$	$\Delta\log k_w^{IAM.DD2}$	$\Delta'\log k_w^{IAM.MG}$	$\Delta'\log k_w^{IAM.DD2}$	$\log P_{app}$ Caco-2	$\log P_{0}$ Caco-2/MDCK
1, 2	Acebutolol	1.191	0.839	2.968	3.001	-5.83	-4.19
2	Acetaminophen	0.812	1.238	0.812	1.238	--	-4.34
1, 2	Acetylsalicylic acid	-1.005	-0.775	1.036	1.708	-5.06	-1.53
2	Acyclovir	1.983	2.453	1.983	2.453	--	-5.87
1, 2	Alprenolol	-0.141	0.350	2.079	3.052	-4.62	-2.23
1	Aminopyrine	0.658	0.845	0.667	0.855	-4.44	--
1, 2	Amoxicillin	1.098	1.851	1.508	2.349	-6.10	-5.70
2	Antipyrine	1.097	1.122	1.250	1.309	-4.55	-4.05
1, 2	Atenolol	0.851	1.720	2.346	3.538	-6.44	-4.34
1	Betaxolol	-0.269	0.229	1.772	2.713	-4.52	--
1, 2	Caffeine	1.164	1.500	1.164	1.500	-4.41	-4.14
2	Carbamazepine	0.145	0.753	0.145	0.753	--	-3.69
2	Cefalexin	0.201	0.657	1.695	2.475	--	-6.03
2	Chloramphenicol	0.569	1.473	0.569	1.473	--	-4.47
1	Chlorpromazine	-1.657	-1.856	0.307	0.533	-4.70	--
1, 2	Cimetidine	1.267	1.943	1.447	2.162	-5.89	-6.06
1, 2	Clonidine	0.583	0.996	1.711	2.367	-4.59	-3.91
1, 2	Desipramine	-0.383	-1.039	2.623	2.618	-4.64	-1.67
2	Dextrometorphan	-0.520	0.150	0.966	1.957	--	-2.60
1, 2	Diazepam	0.154	0.402	0.154	0.402	-4.32	-4.20
2	Diclofenac	-0.446	-0.525	2.296	2.810	--	-1.07
1, 2	Diltiazem	0.185	0.548	1.372	1.992	-4.38	-3.12

2	Diphenydramine	0.479	0.177	1.658	1.611	--	-3.12
2	Domperidone	0.435	0.472	1.810	2.145	--	-4.46
1	Epinephrine	1.459	2.268	3.090	4.252	-6.02	--
2	Flumequine	0.307	0.707	1.221	1.819	--	-2.47
2	Fluvastatin	-0.375	-0.179	2.212	2.970	--	-1.33
1	Felodipine	-0.143	-0.206	-0.143	-0.206	-4.64	--
1	Furosemide	-0.200	-0.148	1.961	2.480	-6.51	--
2	Hydrochlorothiazide	1.542	2.319	1.670	2.475	--	-6.32
2	Hydroxyzine	0.852	0.588	0.912	0.660	--	-4.13
2	Ibuprofen	-1.579	-1.810	0.718	0.985	--	-0.53
1, 2	Imipramine	-0.058	-0.668	2.077	1.929	-4.85	-1.82
2	Indomethacin	-0.281	-1.046	2.785	2.684	--	-0.81
2	Ketoprofen	-0.603	-0.612	2.105	2.681	--	-1.23
1, 2	Labetalol	-0.019	0.367	1.484	2.195	-5.03	-4.27
1	Lidocaine	-0.392	-0.172	0.419	0.815	-4.21	--
2	Metoclopramide	-0.148	0.387	1.295	2.142	--	-2.54
1, 2	Metoprolol	-0.047	0.384	1.840	2.680	-4.59	-1.85
2	Midazolam	0.614	0.574	0.614	0.574	--	-3.44
2	Morphine	0.983	1.566	1.803	2.564	--	-4.55
1	Nadolol	0.583	1.350	2.487	3.667	-5.41	--
2	Naproxen	-0.531	-0.716	2.159	2.556	--	-0.95
1, 2	Nicotine	0.821	1.279	1.709	2.360	-4.71	-3.62
1	Nitrendipine	-0.298	0.039	-0.298	0.039	-4.77	--
1	Oxprenolol	0.067	0.522	1.801	2.631	-4.68	--
1, 2	Phenytoin	0.654	0.534	0.696	0.586	-4.57	-4.16
1, 2	Pindolol	0.341	0.743	1.963	2.717	-4.78	-2.22

1, 2	Piroxicam	0.264	-0.039	1.801	1.831	-4.45	-2.01
1	Progesterone	0.440	0.607	0.440	0.607	-4.37	--
1, 2	Propranolol	-0.004	0.383	2.387	3.292	-4.58	-1.54
1, 2	Ranitidine	1.579	1.842	2.792	3.318	-6.31	-5.27
1, 2	Salicylic acid	-1.106	-1.123	1.602	2.171	-4.79	-0.43
1, 2	Sulpiride	1.203	1.670	3.133	4.018	-6.16	-4.16
1, 2	Terbutaline	0.869	1.239	3.944	4.979	-6.38	-5.23
1, 2	Theophylline	0.863	1.432	0.880	1.453	-4.35	-4.17
1, 2	Timolol	-0.105	0.312	2.030	2.909	-4.85	-2.42
1, 2	Verapamil	0.631	0.458	2.262	2.443	-4.58	-2.18

Series 1: compounds for relationships with Caco-2 permeation data;

Series 2: compounds for relationships with Caco-2/MDCK data corrected to represent only transcellular passage.

Table 2. Values of the differences between observed and expected logarithms of retention factors on IAM.PC.MG and IAM.PC.DD2 stationary phases ($\Delta\log k_w^{IAM.MG}$ and $\Delta'\log k_w^{IAM.MG}$, $\Delta\log k_w^{IAM.DD2}$ and $\Delta'\log k_w^{IAM.DD2}$, respectively), of logarithms of Caco-2 permeation data ($\log P_{app}$) and of corrected permeation data on Caco-2/MDCK expressing the transcellular intrinsic permeability ($\log P_0^{Caco\text{-}2/MDCK}$).

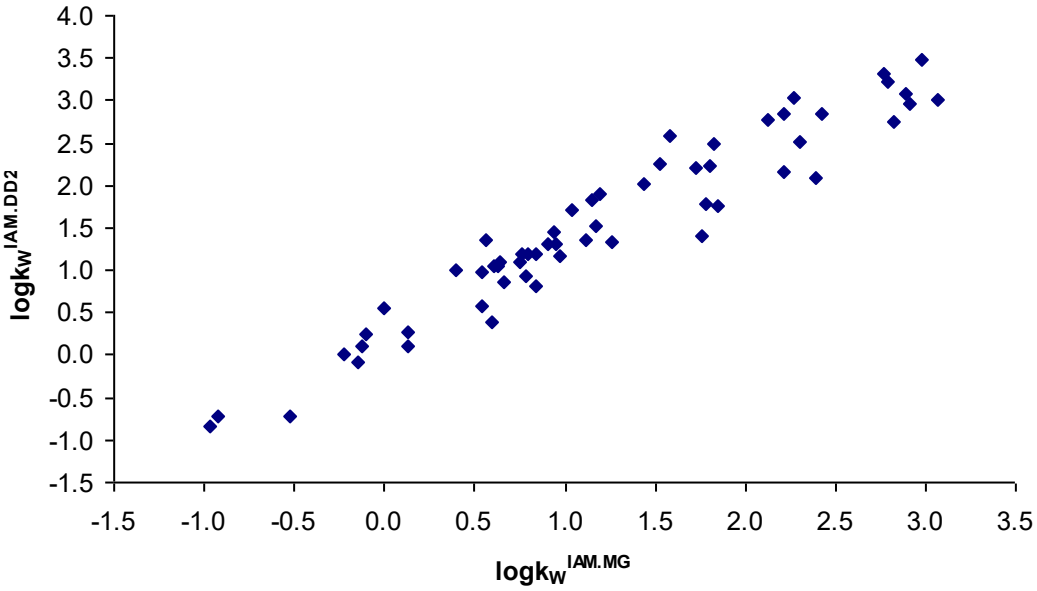


Figure 1. Relationship between $logk_w^{IAM.DD2}$ and $logk_w^{IAM.MG}$ for the 58 compounds considered.

It is worth mentioning that, even for ionisable compounds, retention on IAM phases, is only negligibly affected by variations of the pH of the eluent within the range 5.5 – 7.0 [Amato et al., 2000]. Therefore, despite IAM-HPLC data were determined using eluents at pH 7.0, to maximize column stability and data reproducibility, they are suitable for correlative studies with measures of membrane interactions occurring at slightly different pH values.

In this study the calculation of $\Delta\log k_w^{IAM}$ was based on the fact that the $\log k_w^{IAM}$ values of structurally non-related neutral compounds having PSA = 0 relate unambiguously with *n*-octanol lipophilicity values. Equations (2) and (3) were generated taking into account 17 neutral compounds with PSA = 0 whose log P^N values span the range 1.15 – 4.80. The values of log P^N, $\log k_w^{IAM.MG}$ and $\log k_w^{IAM.DD2}$ are summarized in Table 3.

$$\log k_w^{IAM.MG} = 0.854\ (\pm 0.047)\ \log P - 0.976\ (\pm 0.156) \qquad (2)$$

$n = 17$ $\quad r^2 = 0.957$ $\quad s = 0.214$ $\quad F_{1,15} = 331{,}35$ $\quad F_{1,15}\ \alpha{,}0.001 = 16.59$

$$\log k_w^{IAM.DD2} = 1.039\ (\pm 0.051)\ \log P - 1.311\ (\pm 0.169) \qquad (3)$$

$n = 17$ $\quad r^2 = 0.965$ $\quad s = 0.232$ $\quad F_{1,15} = 417.54$ $\quad F_{1,15}\ \alpha{,}0.001 = 16.59$

It is interesting to underline that these equations do not appreciably differ from those previously considered in the other studies reported in this thesis, which were based on 36 data points, taken from the literature [Taillardat-Bertschinger et al., 2002], also including neutral analytes with positive PSA values.

compound	log P^N	$logk_w^{IAM.MG}$	$logk_w^{IAM.DD2}$
dichloromethane	1.15	0.309	0.107
1,2-dichloroethane	1.48	0.444	0.337
chloroform	1.94	0.625	0.620
benzene	2.05	0.620	0.720
tetrachloroethane	2.39	1.140	1.278
carbon tetrachloride	2.63	1.062	1.209
1-chlorobutane	2.64	0.922	1.053
toluene	2.69	1.041	1.169
naphthalene	3.35	2.122	2.471
n-pentane	3.39	1.877	2.276
1,3-dichlorobenzene	3.48	2.077	2.475
mesitylene	3.84	2.174	2.609
biphenyl	3.90	2.723	3.137
1,2,4,5-tetrachlorobenzene	4.51	3.028	3.497
pentamethylbenzene	4.56	2.771	3.323
heptane	4.66	2.882	3.197
bibenzyl	4.80	3.243	3.766

Table 3. Lipophilicity values in *n*-octanol (log P^N) and logarithms of retention factors on IAM stationary phases for the 17 neutral compounds with PSA = 0 considered.

4.3.1 Relationships among the physico-chemical parameters for series 1 compounds

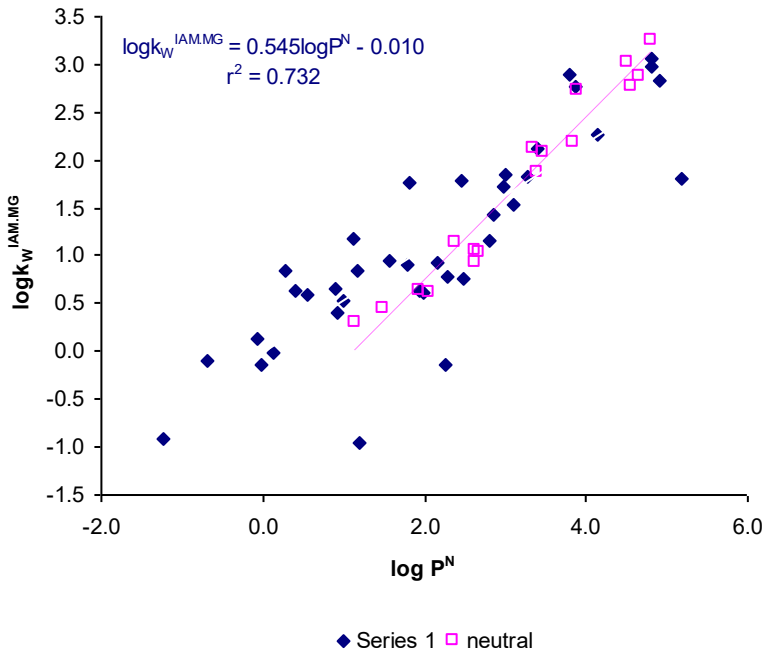
Series 1 of the present study consists of 38 compounds whose Caco-2 permeability data ($logP_{app}$), experimentally determined, were reported in the literature [Camenish et al., 1998; Yazdanian et al., 1998].

Both $logk_w^{IAM.MG}$ and $logk_w^{IAM.DD2}$ of the compounds of series 1 moderately related linearly with log P^N and log $D^{7.4}$ values (r^2 values spanning from 0.609 to 0.740). Figure 2 shows the plots $logk_w^{IAM}$ *vs.* log P^N of the compounds of "series 1" superimposed to the plots of the 17 neutral compounds used to generate the equations (2) and (3); as can be seen, the points in the graphs are quite scattered (*s* values spanning from 0.548 to 0.565).

As previously reported, the distances of the points from the regression line of the neutral compounds were expressed as $\Delta logk_w^{IAM.MG}$ and $\Delta logk_w^{IAM.DD2}$, for IAM.PC.MG and IAM.PC.DD2 phases, respectively. Therefore, $\Delta logk_w^{IAM.MG}$ are the differences between the experimentally measured $logk_w^{IAM.MG}$ values and the values calculated from log P^N by equation (2) whereas $\Delta logk_w^{IAM.DD2}$ are the differences between the experimentally measured $logk_w^{IAM.DD2}$ values and the values calculated from log P^N by equation (3).

The plots of $logk_w^{IAM}$ *vs.* log $D^{7.4}$ of the compounds of "series 1", superimposed to the plots of the neutral compounds, are reported in Figure 3. With respect to the plots in Figure 2, most points are shifted to the left of the graph and more scattered; however, a linear relationship is again apparent.

A



B

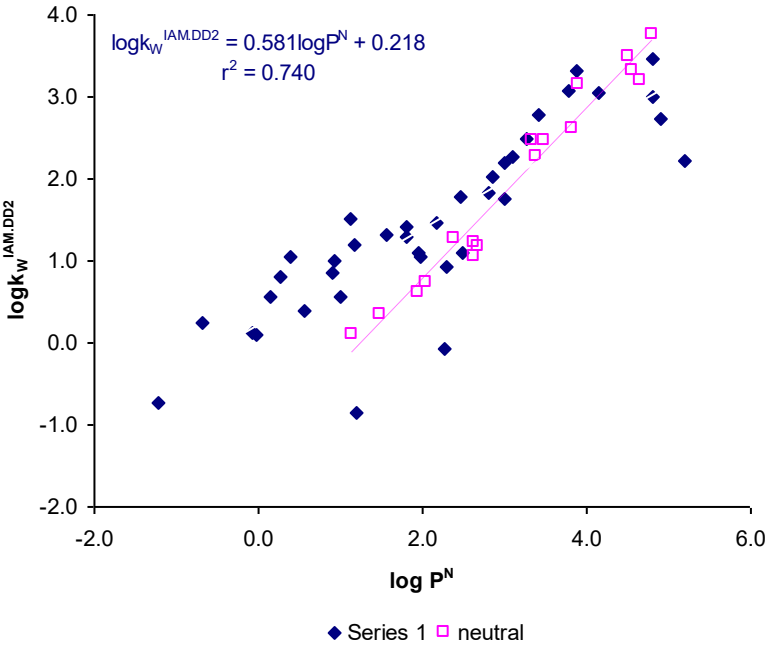
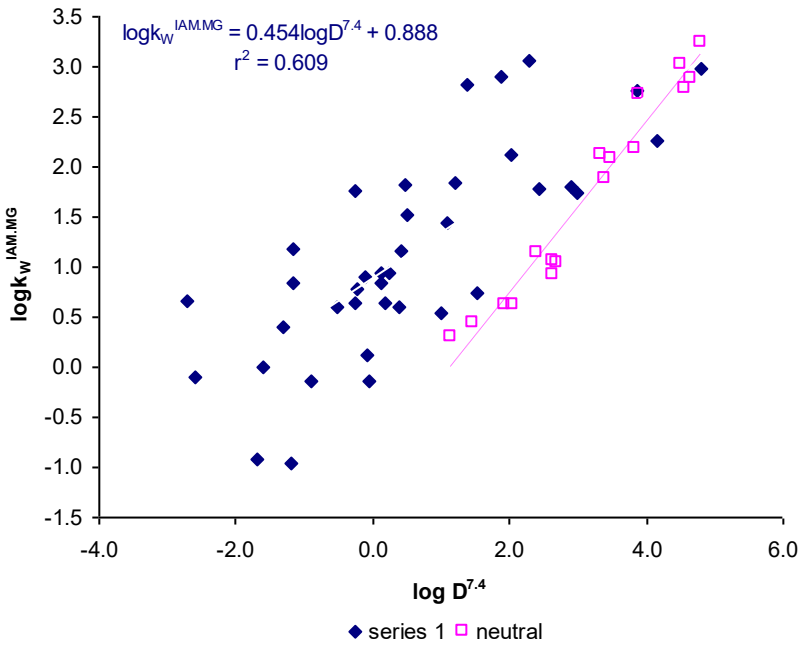


Figure 2. Relationships between either $logk_w^{IAM.MG}$ (A) or $logk_w^{IAM.DD2}$ (B) and log P^N values for the 38 compounds of "series 1" in comparison to the plots of 17 neutral compounds.

A



B

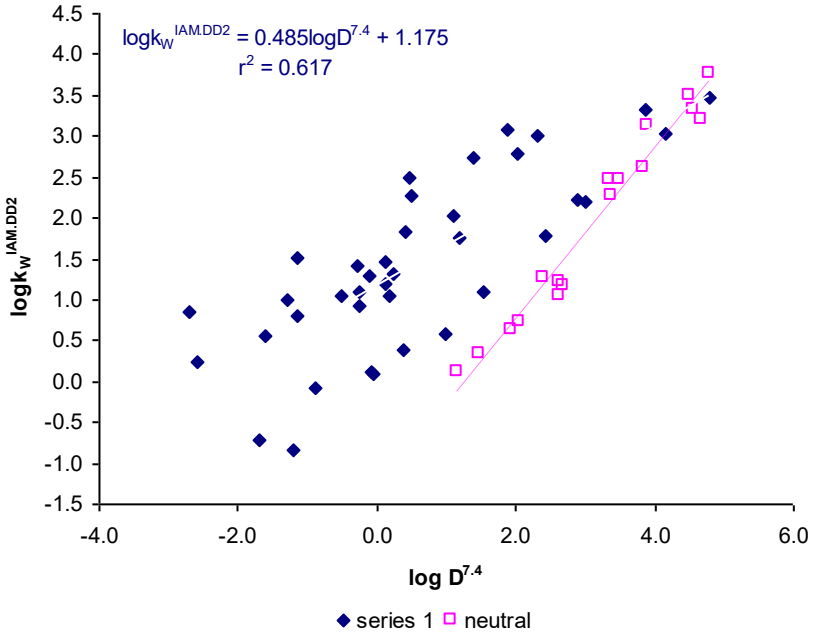


Figure 3. Relationships between either $logk_w^{IAM.MG}$ (A) or $logk_w^{IAM.DD2}$ (B) and log $D^{7.4}$ values for the 38 compounds of "series 1" in comparison to the plots of 17 neutral compounds.

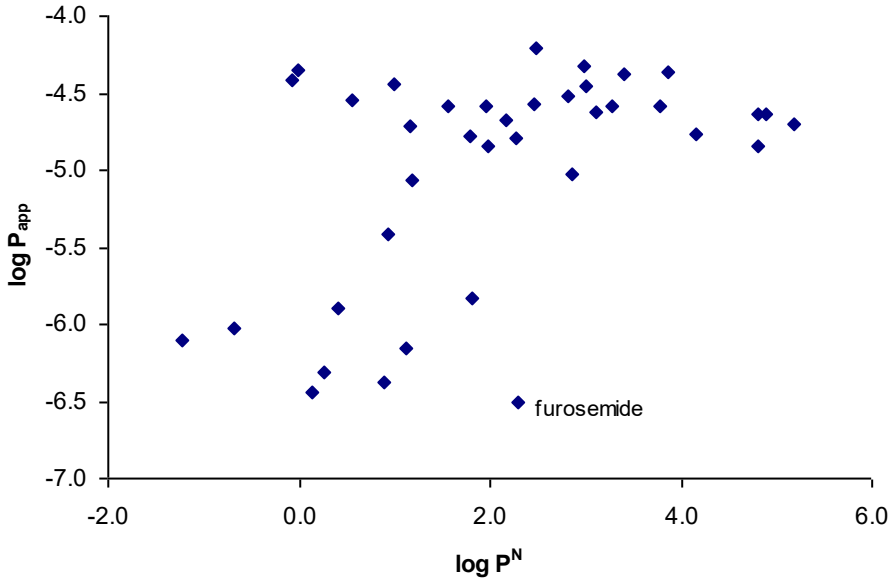
The distances from the regression line of the neutral compounds calculated taking into account the log $D^{7.4}$ values of the analytes, i.e. their lipophilicity at the experimental pH of the Caco-2 passage measures [Camenish et al., 1998; Yazdanian et al., 1998], are $\Delta'\log k_w^{IAM}$ values. These values were also taken into account since it was reported that, for acidic compounds, they related better than $\Delta\log k_w^{IAM}$ values with data of passage through the Blood-Brain Barrier as previously reported in chapter 3.

4.3.2 Relationships between physicochemical parameters and Caco-2 passage data (log P_{app}) for "series 1" compounds.

log P_{app} values relate with both log P^N and log $D^{7.4}$ values according to a parabolic trend (Figure 4). However, the relationship with log $D^{7.4}$ values was more significant (n = 37, r^2 = 0.608, s = 0.433, $F_{2, 34}$ = 26.43) than that with log P^N values (n = 37, r^2 = 0.351, s = 0.558, $F_{2, 34}$ = 9.21). Furosemide behaved as an outlier and was excluded from these and the next relationships. Actually, this drug was reported to be a substrate of a saturable active transport system [Flanagan et al., 2002].

The relationships between log P_{app} and $\Delta\log k_w^{IAM}$ values are shown in Figure 5. As can be seen, differently from the relationships previously observed between jejunal absorption data and $\Delta\log k_w^{IAM}$ values, log P_{app} linearly decrease at increasing $\Delta\log k_w^{IAM}$ values, but only for the analytes with positive $\Delta\log k_w^{IAM}$ values, whereas they are almost constant for the analytes with negative $\Delta\log k_w^{IAM}$ values; furthermore, the points are quite scattered.

A



B

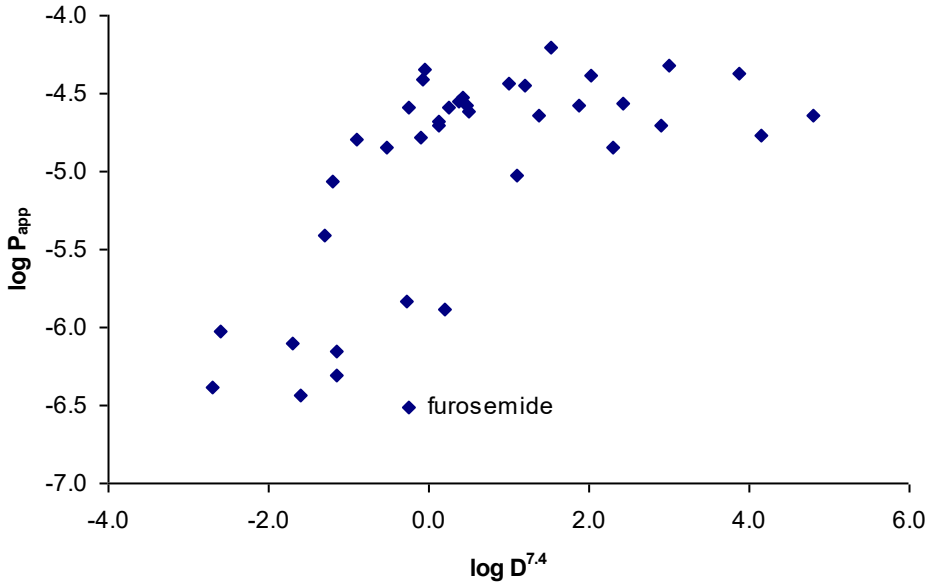
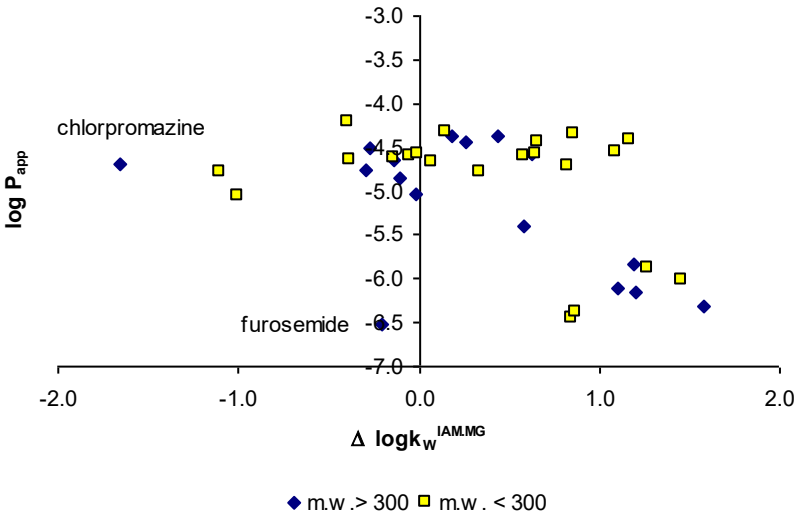


Figure 4. Relationships between log P_{app} and either log P^N values (A) or log $D^{7.4}$ values (B) for the 38 compounds of "series 1".

A



B

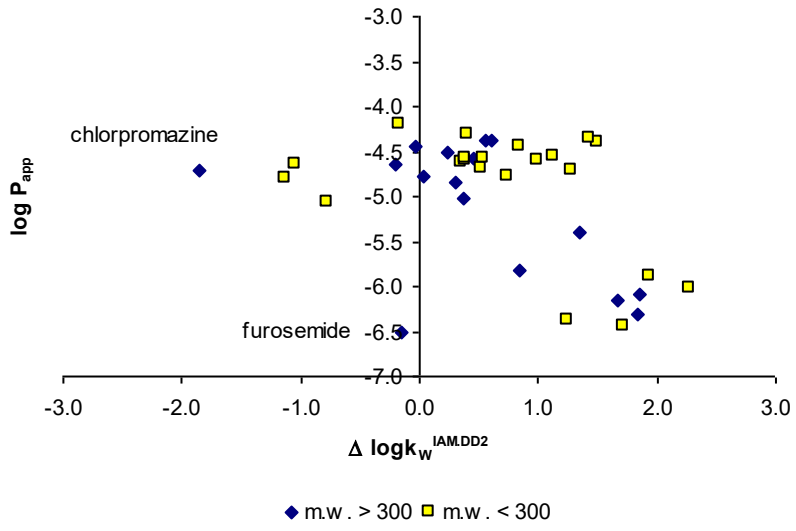


Figure 5. Relationships between log P_{app} and either $\Delta\log k_w^{IAM.MG}$ (A) or $\Delta\log k_w^{IAM.DD2}$ (B) values for the 38 compounds of "series 1".

A possible explanation may consist in the fact that Caco-2 passage data are differently affected by aqueous boundary layer (P_{ABL}), filter (P_f), transcellular (P_C), and paracellular (P_{para}) contributions with respect to the jejunal absorption data in vivo [Avdeef 2012b].

Since a recent study [Tam et al. 2010] suggested that paracellular diffusion can be considered a minor transport route *in vivo* for drug molecules heavier than m.w. 300 Da, the whole set was split in two subsets, the first one including the analytes with m.w. > 300 Da and the second one the analytes with m.w. < 300 Da. Actually, as can be seen in Figure 5, taking only into account the points of the subset with m.w. > 300 a quite good inverse linear relationship becomes apparent between log P_{app} and $\Delta\log k_w^{IAM}$ values, with the exception of chlorpromazine and, as already mentioned above, furosemide.

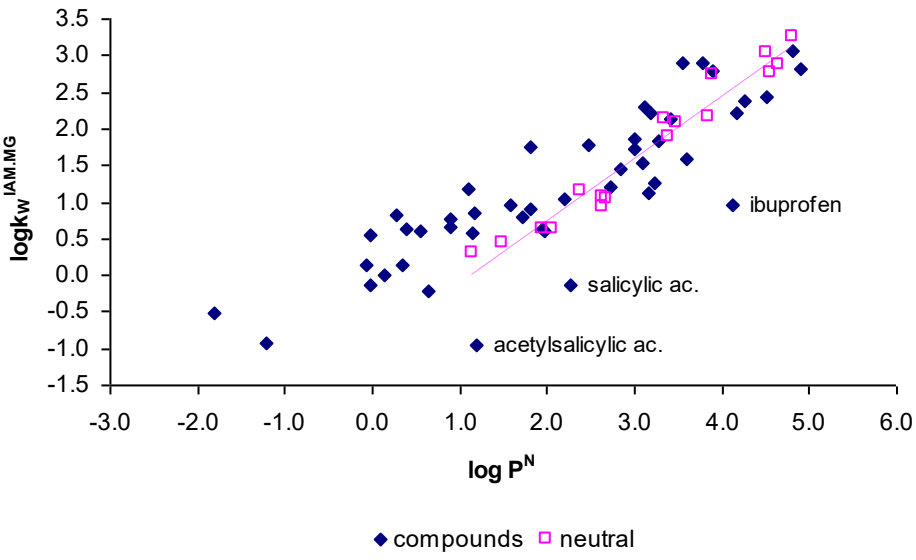
4.3.3 Relationships among the physico-chemical parameters for "series 2" compounds

A database of Caco-2/MDCK permeability determinations of about 200 drugs, corrected for the effects of the ABL and paracellular permeability (based on nearly 700 published individual measurements), and claimed as expressing the transcellular intrinsic permeability of the drugs ($\log P_0^{Caco\text{-}2/MDCK}$), is reported in the literature [Avdeef 2012b]. The values for 47

compounds, here reported as "series 2", were taken into account to investigate about possible relationships with phospholipid affinity indexes.

The plots of $logk_w^{IAM}$ *vs* log P^N (Figure 6) show that the phospholipid affinity is quite close to that found for isolipophilic neutral compounds for the compounds with log $P^N > 1$, whereas it is higher for the compounds with log $P^N < 1$. As already reported in the literature [Barbato et al., 1997b], three acid compounds, i.e. salicylic acid, acetylsalicylic acid and ibuprofen, showed a phospholipid affinity ($logk_w^{IAM}$) much lower than that expected for neutral isolipophilic compounds.

A



B

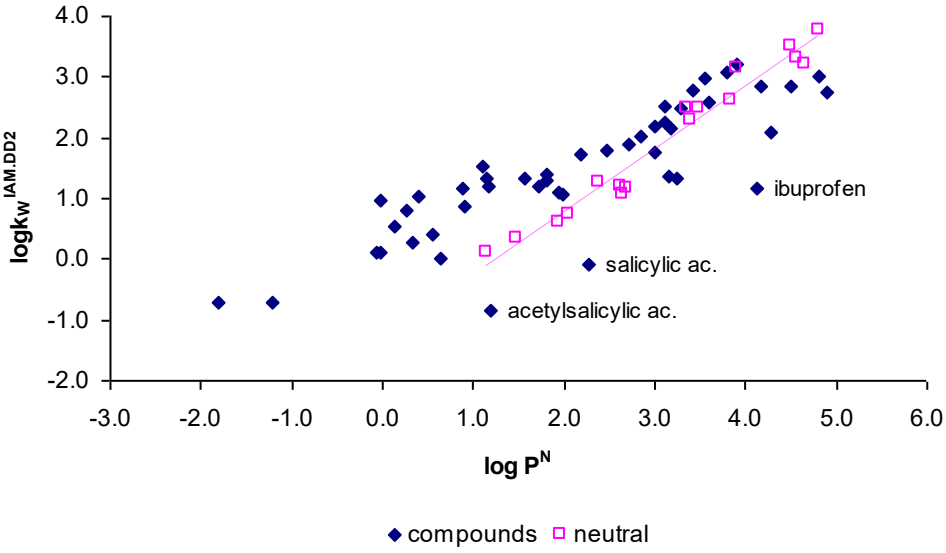


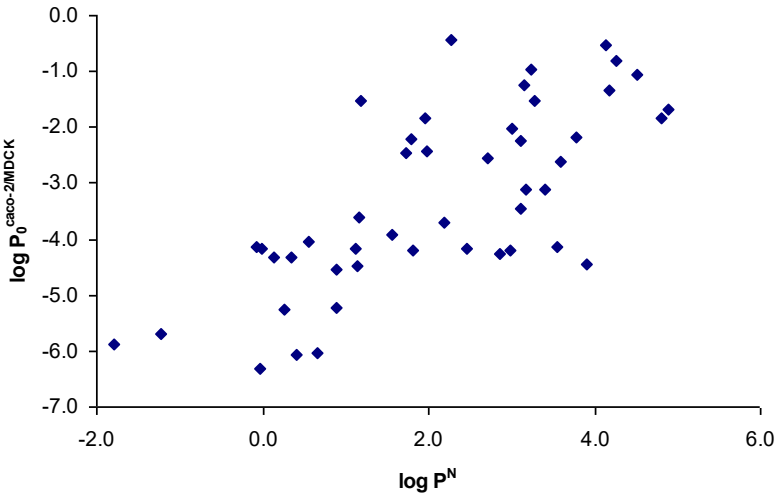
Figure 6. Relationships between either $logk_w^{IAM.MG}$ (A) or $logk_w^{IAM.DD2}$ (B) and log P^N values for the 47 compounds of "series 2" in comparison to the plots of 17 neutral compounds.

4.3.4 Relationships between physicochemical parameters and corrected Caco-2/MDCK passage data (log $P_0^{Caco\text{-}2/MDCK}$) for "series 2" compounds.

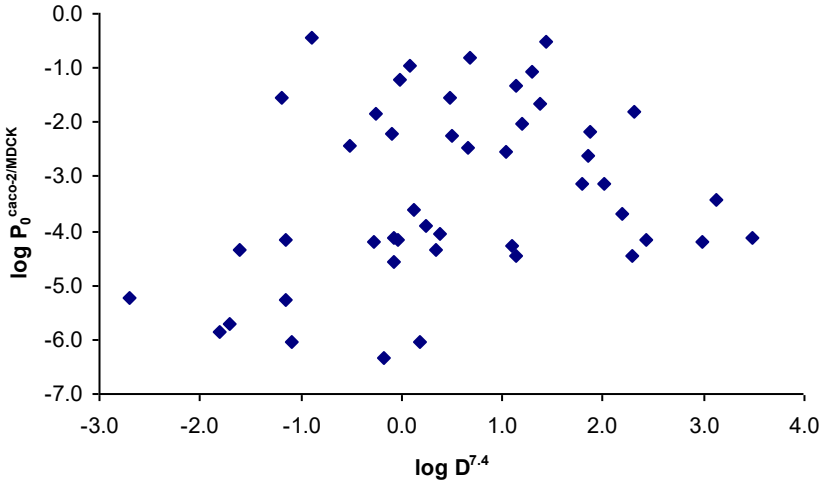
The relationships between log $P_0^{Caco\text{-}2/MDCK}$ values and *n*-octanol lipophilicity parameters are shown in Figure 7 in which they are plotted against i) log P^N (Figure 7A), ii) log $D^{7.4}$ (Figure 7B), and iii) a combination of log P^N and (for only acids) log $D^{7.4}$ values (Figure 7C). As can be seen in Figure 7A, log $P_0^{Caco\text{-}2/MDCK}$ values increase at increasing log P^N values according to a moderately significant linear relationship (r^2 = 0.520; s = 1.130; $F_{1, 45}$ = 48.76; $F_{1, 45}$ α,0.001 = 12.39). The relationships between log $P_0^{Caco\text{-}2/MDCK}$ and either log $D^{7.4}$ (Figure 7B) or log P^N(log $D^{7.4}$) values (Figure 7C), although showing a similar trend, are less significant as it is apparent in the respective plots, in which the data points are much more scattered.

Furthermore, similar trends were also observed in the relationships between log $P_0^{Caco\text{-}2/MDCK}$ and phospholipid affinity indexes, i.e. $logk_w^{IAM.MG}$ and $logk_w^{IAM.DD2}$ (Figure 8). It is interesting to note that, apart from the high scattering of the points, two points strongly deviated from an imaginary regression line, being shifted in the left upper corner of the graph. These data points represent salicylic acid and acetylsalicylic acid, whose permeability was strongly underestimated by IAM parameters, as well as by the other lipophilicity parameters (Figure 7).

A



B



C

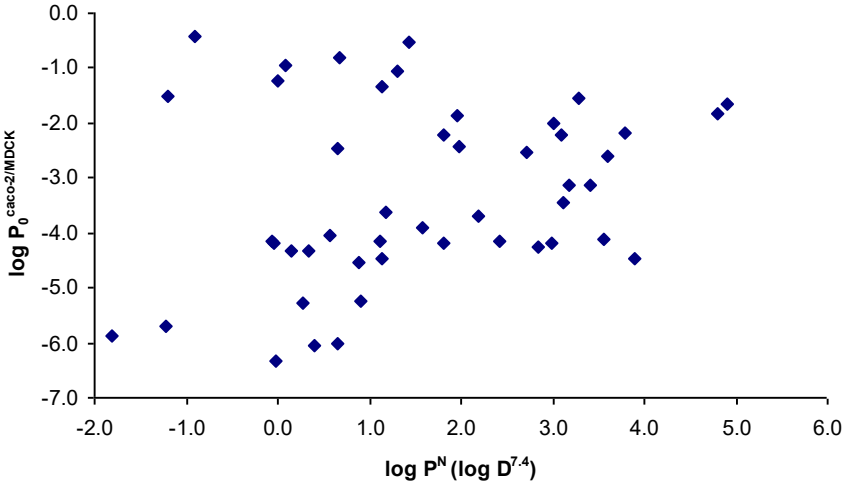
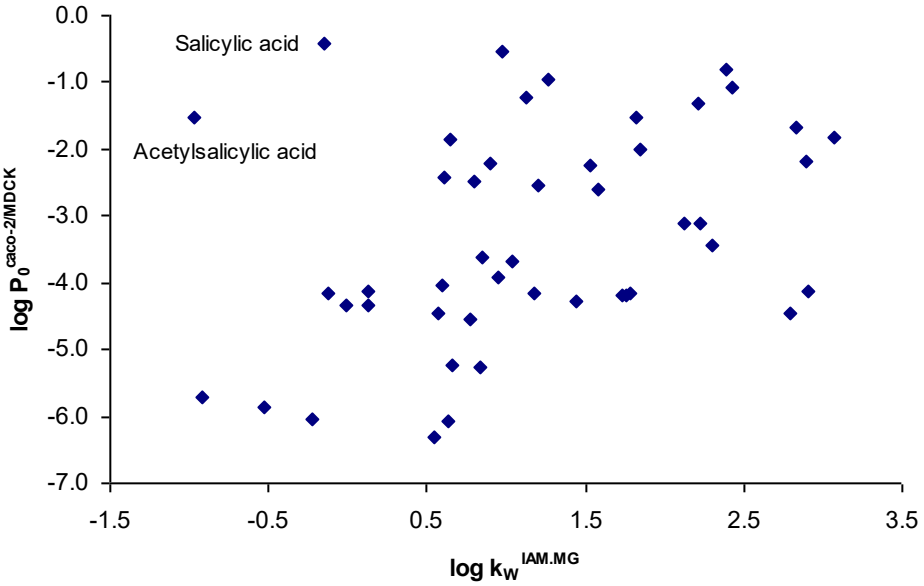


Figure 7. Relationships between log $P_0^{Caco\text{-}2/MDCK}$ and i) log P^N values (A), ii) log $D^{7.4}$ (B), iii) log P^N and (for only acids) log $D^{7.4}$ values for the 47 compounds of "series 2".

A



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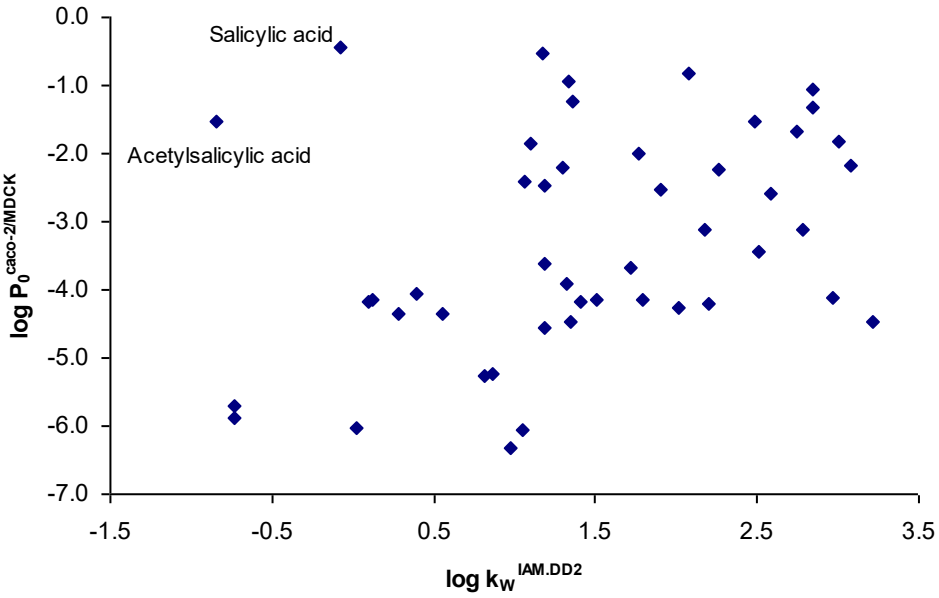


Figure 8. Relationships between log $P_0^{Caco-2/MDCK}$ and either $logk_w^{IAM.MG}$ (A) or $logk_w^{IAM.DD2}$ (B) values for the 47 compounds of "series 2".

Acetylsalicylic acid and salicylic acid were already recognized as outliers in a relationship between percentages of oral absorption and $\log k_w^{IAM}$ indexes reported in a recent work [Tsopelas et al., 2015b]; the authors hypothesized that they deviated from the regression due to their low m.w. allowing a permeation through the paracellular route. However, $\log P_0^{Caco\text{-}2/MDCK}$ values do not account for paracellular passage and the poor relationships found with $\log k_w^{IAM}$ values suggest that the latter, as well as *n*-octanol lipophilicity parameters, are inadequate at describing the membrane passage of these benzoic acid derivatives.

In contrast, highly significant inverse linear relationships are found between $\log P_0^{Caco\text{-}2/MDCK}$ and both $\Delta\log k_w^{IAM.MG}$ and $\Delta\log k_w^{IAM.DD2}$ values. As can be seen in Figure 9, permeation values decrease at increasing $\Delta\log k_w^{IAM}$ values with only one point, i.e. that referring to cefalexin, quite far from the imaginary regression line including the other 46 points. After the exclusion of cefalexin from the regression, the relationships are expressed by the equations (4) and (5), for IAM.PC.MG and IAM.PC.DD2 phases, respectively.

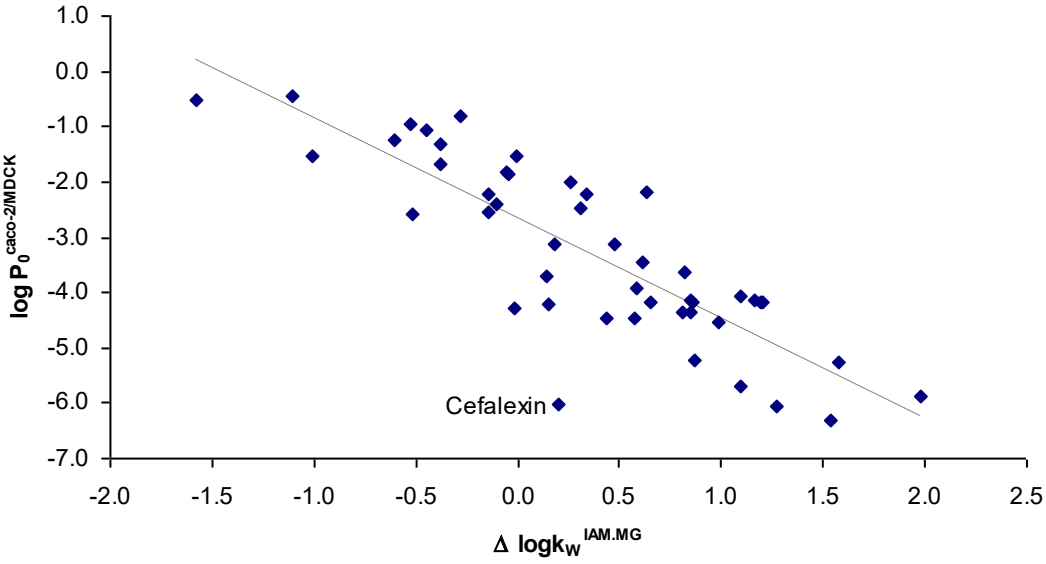
$$\log P_0^{Caco\text{-}2/MDCK} = -1.833\ (\pm 0.153)\ \Delta \log k_W^{IAM.MG} - 2.581\ (\pm 0.126) \quad (4)$$

$n = 46$ $r^2 = 0.765$ $s = 0.774$ $F_{1,44} = 143.31$ $F_{1,44}\ \alpha,0.001 = 12.39$

$$\log P_0^{Caco\text{-}2/MDCK} = -1.456\ (\pm 0.108)\ \Delta \log k_W^{IAM.DD2} - 2.396\ (\pm 0.120) \quad (5)$$

$n = 46$ $r^2 = 0.806$ $s = 0.702$ $F_{1,44} = 183.17$ $F_{1,44}\ \alpha,0.001 = 12.39$

A



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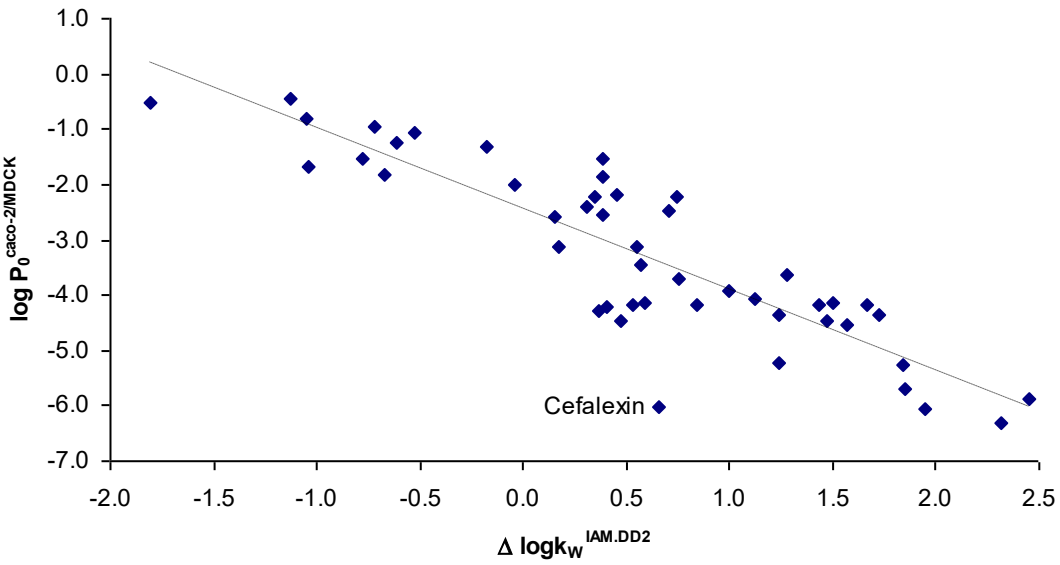

Figure 9. Relationships between log $P_0^{Caco\text{-}2/MDCK}$ and either $\Delta\log k_w^{IAM.MG}$ (A) or $\Delta\log k_w^{IAM,DD2}$ (B) values for the 47 compounds of "series 2".

A possible explanation for the fact that cefalexin deviates from an imaginary regression line may be that it is a PEPT-1 enzyme substrate [Sugano et al., 2010]. However, the contribution of this active transport mechanism to the total absorption should play an only minor role [Bretschneider et al., 1999] and, in fact, cefalexin was not recognized as an outlier when we studied the relationships between $\Delta\log k_w^{IAM}$ parameters and jejunum absorption values, P_{eff} , measured by the Loc-I-Gut technique [Grumetto et al., 2015] (see chapter 3). On the other hand, amoxicillin, another analyte reported as PEPT-1 substrate, was found as an outlier neither in the relationships between $\Delta\log k_w^{IAM}$ and P_{eff} values [Grumetto et al., 2015] (see chapter 3) nor in the present study. It is interesting to note that $\log P_0^{Caco\text{-}2/MDCK}$ value of cefalexin (-6.03) is smaller than that of amoxicillin (-5.70) whereas its log P_{eff} *in vivo* is higher (-3.81 *vs.* -4.50). Therefore, it is reasonable to suppose that $\log P_0^{Caco\text{-}2/MDCK}$ reported for cefalexin underestimates its actual intestinal passage potential. Analogously, $\Delta\log k_W^{IAM}$ of both cimetidine, an OCT-1 and OCT-2 enzyme substrate, and verapamil, a P-gp substrate, relates significantly with both log P_{eff} (see chapter 3) and log $P_0^{Caco\text{-}2/MDCK}$ values. For verapamil it has been suggested that the efflux mechanism is eclipsed by the high passive transcellular diffusion [Sugano et al., 2010].

The relationships between log $P_0^{Caco\text{-}2/MDCK}$ and $\Delta'\log k_w^{IAM}$ values (or the combination of $\Delta\log k_w^{IAM}$ and, for only acids, $\Delta'\log k_w^{IAM}$ values) were much less significant (data not shown).

4.4 Conclusion

The present study confirms the soundness of $\Delta\log k_w^{IAM}$ parameters in the prediction of the intestinal absorption of drugs.

The data of passage through Caco-2 cultured cell lines for 38 structurally unrelated compounds moderately related to lipophilicity values measured at pH 7.4 ($\log D^{7.4}$), according to a parabolic pattern, but poorly related with $\Delta\log k_w^{IAM}$ values. However, it has been reported that Caco-2 passage data also encode secondary passage mechanisms, which participate in a different extent to the jejunal absorption *in vivo*; therefore, $\log P_{app}$ values cannot be directly equated to the corresponding human *in situ* $\log P_{eff}$ values, since a normalization for such differences is required [Avdeef 2012b]. As a matter of fact, highly significant inverse linear relationships are observed between $\Delta\log k_w^{IAM}$ measured on both IAM.PC.MG and IAM.PC.DD2 stationary phases and $\log P_0^{Caco\text{-}2/MDCK}$ values for 47 structurally unrelated compounds, i.e. cultured cell line passage data expressing transcellular intrinsic permeability, corrected for the effects of the ABL and paracellular permeability. $\log P_0^{Caco\text{-}2/MDCK}$ values poorly relate with lipophilicity values in *n*-octanol. Furthermore, in partial contrast to other studies previously reported in the literature [Kotecha et al., 2007; Kotecha et al., 2008; Tsopelas et al., 2015b], they relate poorly with the affinity data with phospholipids, $\log k_w^{IAM}$, too.

These results are in a complete agreement with the results of our previous study [Grumetto et al., 2015] on the relationships between jejunal absorption data measured in vivo and $\Delta\log k_W^{IAM}$ values (see chapter 3). From a mechanistic point of view, they confirm that the polar/electrostatic forces occurring between drugs and phospholipids, $\Delta\log k_w^{IAM}$, play a major role in the passage through biomembranes. Furthermore, these data, easier to achieve and much more reproducible than crude Caco-2 passage data, demonstrated to be more effective than the latter at describing the *in vivo* intestinal absorption if it occurs by only passive mechanism through the transcellular route.

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5.0 RELATIONSHIPS BETWEEN IAM DERIVED PARAMETERS AND BBB PERMEATION DATA MEASURED IN SITU AND IN *PAMPA-BBB* SYSTEM

5.1 Introduction

In the previous studies reported in chapter 1 and 2, we found that, according to the "flip-flop" model, BBB permeation was inversely related to both $\Delta\log k_w^{IAM}$ and $\Delta'\log k_w^{IAM}$ values. We took into account twenty-one drugs whose BBB permeation capability was reported in the literature as log BB values, i.e. the ratios between brain and blood concentrations [Platts et al., 2001] and assembled in a single set the data achieved in that work (21 compounds) [Grumetto et al., 2015] and the analogous data reported in previous works [Grumetto et al., 2012, 2013] such as to obtain a set of 42 compounds that were used to support statistically the proposed model.

The enlarged set considered suggested that log BB inversely relates to $\Delta/\Delta'\log k_w^{IAM}$. Both chlorpromazine and haloperidol behaved as outliers and good relationships were only observed after their exclusion (equations (1) and (2)).

$$\log BB = -1.228\ (\pm 0.119)\ \Delta/\Delta'\log k_w^{IAM.MG} + 0.671\ (\pm 0.090) \quad (1)$$

$n = 40 \qquad r^2 = 0.738 \qquad s = 0.424$

$$\log BB = -0.975\ (\pm 0.072)\ \Delta/\Delta'\log k_w^{IAM.DD2} + 0.650\ (\pm 0.070) \quad (2)$$

$n = 40 \qquad r^2 = 0.826 \qquad s = 0.345$

However, in order to validate the proposed model, it would be desirable to test it on further analytes. The main problem, often occurring in designing Quantitative Structure-Activity Relationship (QSAR) studies, is the lack of a suitable number of biological activity data to take into account. Indeed, it should be remembered that biological activity data are usually affected by a high degree of uncertainty, which can negatively affect the statistical validation of the relationships. Again, the inter-laboratory variations of biological data are often too high to allow the assembling of data from different laboratories in a single data set.

Avdeef reported a study about the possible prediction of *in situ* measured BBB passive permeation data by the use of *in vitro* passage data achieved by the so called "PAMPA-BBB" technique [Avdeef, 2012]. In this study, BBB passive permeability was expressed as the values of $P_0^{\text{in situ}}$, i.e. *in situ* brain perfusion permeability values (on rat and mouse) selected from studies which used some sort of carrier-mediated transport inhibition (e.g. GF120918, PSC833, cyclosporin A, self-inhibition at high concentrations, mdr1a(–/–)/mrp1(– /–)/brcp - knockout mouse model), allowing the assumption of *in situ* data as free of efflux effects. A total of 197 values were selected. It is important to underline that $P_0^{\text{in situ}}$ values refer to the permeability of the neutral form of the analytes and represent the "intrinsic permeability" regardless any effect given by ionization.

The *in vitro* data were those achieved by PAMPA-BBB technique and expressed as $P_0^{\text{PAMPA-BBB}}$ values. PAMPA-BBB technique was firstly described by Dagenais [Dagenais et al., 2009], who employed a PAMPA membrane made of 20% w/v lecithin dissolved in dodecane, however, the data considered by Avdeef were those achieved by an improved model proposed by Tsinman [Tsinman et al., 2011]. It consists of a new PAMPA–BBB formulation based on 10% w/v porcine brain lipid extract (PBLE), using a fivefold higher lipid concentration in a more viscous alkane solvent than dodecane and with thinner membranes.

Good relationships were found between log $P_0^{\text{in situ}}$ and log $P_0^{\text{PAMPA-BBB}}$ for 197 compounds, but only after they were divided in four predominant-charge groups (positive, negative, neutral, and zwitterionic). Furthermore, an Abraham solvation descriptor had to be added as a second term in the equations.

Abraham solvation descriptors are:

α : H - bond acidity,

β : H - bond basicity,

π : polarity/polarizability due to solute–solvent interactions between bond dipoles and induced dipoles,

R ($dm^3\ mol^{-1}$ /10): excess molar refraction, which models dispersion force interaction arising from π and *n* electrons of the solute, and

Vx : McGowan molar volume ($dm^3\ mol^{-1}$ /100) of the solute.

The better equations were:

For bases (positively charged)

$$\log P_0^{\text{in situ}} = -0.01 + 0.94 \log P_0^{\text{PAMPA-BBB}} - 0.64\ \alpha \qquad (3)$$

$n = 85$ $r^2 = 0.86$ $s = 0.46$ $F = 253$

For acids (negatively charged)

$\log P_0^{\text{in situ}} = 2.54 + 1.11 \log P_0^{\text{PAMPA-BBB}} - 0.65 (\alpha + \beta)$ (4)

$n = 28$ $r^2 = 0.61$ $s = 0.56$ $F = 20$

For neutral compounds

$\log P_0^{\text{in situ}} = -0.40 + 0.63 \log P_0^{\text{PAMPA-BBB}} - 0.44 (\alpha + \beta)$ (5)

$n = 79$ $r^2 = 0.88$ $s = 0.33$ $F = 255$

For ampholytes

$\log P_0^{\text{in situ}} = -4.81 + 0.73 (\alpha - \beta)$ (6)

$n = 8$ $r^2 = 0.86$ $s = 0.22$ $F = 38$

It is important to note that, according to equation (6), *in situ* permeability of ampholytes did not depend on $\log P_0^{\text{PAMPA-BBB}}$ values and therefore, the authors concluded that BBB passage of the ampholytes seemed not depending at all on lipophilicity.

In the present study we selected $\log P_0^{\text{PAMPA-BBB}}$ and $\log P_0^{\text{in situ}}$ values for 37 and 39 analytes, respectively, reported in the work above mentioned (Table 1 and Table 2) including L-Dopa, which is know to cross BBB *in vivo* by active transport mechanism. Thirty-tree compounds belonged to both groups. The set considered for $P_0^{\text{PAMPA-BBB}}$ values includes 24 bases, 7 acids, 3 ampholytes, and 3 neutral compounds. The set considered for $P_0^{\text{in situ}}$ values includes 25 bases, 6 acids, 4 ampholytes, and 4 neutral compounds.

Their phospholipid affinity, expressed as $\log k_w^{\text{IAM}}$, was experimentally measured by the IAM-HPLC technique performed on two different phospholipid stationary phases (IAM.PC.MG and IAM.PC.DD2) and the respective $\Delta\log k_w^{\text{IAM}}$ and $\Delta'\log k_w^{\text{IAM}}$ values were calculated.

Compound	log P	log $D^{7.4}$	log $k_w^{IAM.MG}$	log $k_w^{IAM.DD2}$	Δlog $k_w^{IAM.MG}$	Δlog$k_w^{IAM.DD2}$	log $P_0^{in\ situ}$
Aminopyrine	1.00	0.99	0.536	0.573	0.658	0.845	-3.30
Amitriptyline	4.92	2.80	2.881	3.122	-0.345	-0.679	-1.48
Antipyrine	0.56	0.56	0.599	0.393	1.097	1.122	-3.98
Buspirone	2.63	2.39	1.742	1.986	0.472	0.564	-2.53
Caffeine	-0.07	-0.07	0.128	0.116	1.164	1.500	-3.85
Carbamazepine	2.19	2.19	1.039	1.717	0.145	0.753	-3.74
Chlorambucil	3.41	0.61	1.288	1.897	-0.648	-0.335	-0.80
Cimetidine	0.40	0.19	0.633	1.048	1.267	1.943	-5.92
Codeine	1.39	0.22	0.855	1.290	0.644	1.157	-3.80
Diazepam	2.99	2.99	1.731	2.198	0.154	0.402	-3.35
Diltiazem	3.41	2.02	2.121	2.780	0.185	0.548	-2.81
Diphenydramine	3.18	1.80	2.219	2.170	0.479	0.177	-1.90
Domperidone	3.90	2.29	2.790	3.213	0.435	0.472	-4.45
Doxorubicin	1.97	-0.33	2.223	1.764	1.517	1.028	-5.55
Fluoxetine	4.50	2.28	3.181	3.522	0.314	0.158	-1.11
Fluphenazine	4.36	4.33	3.588	3.957	0.841	0.738	-3.35
Hydrocortisone	1.61	1.61	1.550	1.660	1.151	1.298	-5.85
Lidocaine	2.48	1.53	1.112	1.650	-0.030	0.384	-3.24
Loratadine	4.80	4.80	3.354	3.623	0.231	-0.053	-3.48
Methadone	3.93	2.26	2.646	2.828	0.266	0.056	-2.02

Metoclopramide	2.72	1.03	1.199	1.902	-0.148	0.387	-2.86
Morphine	0.89	-0.07	0.767	1.180	0.983	1.566	-5.43
Nicotinammide	-0.37	-0.37	0.351	-0.179	1.643	1.516	-4.88
Progesterone	3.87	3.87	2.769	3.317	0.440	0.607	-3.74
Propranolol	3.28	0.48	1.821	2.480	-0.004	0.383	-1.26
Pyrilamine	3.27	1.80	2.109	1.893	0.292	-0.194	-2.04
Quinine	3.44	2.19	2.313	2.810	0.351	0.547	-3.45
Risperidone	3.04	1.66	2.189	2.028	0.569	0.180	-2.94
Temazepam	2.19	2.19	2.190	1.697	1.296	0.733	-3.35
Thiourea	-1.08	-1.08	-0.817	-1.081	1.081	1.352	-5.45
Verapamil	3.79	1.88	2.892	3.085	0.631	0.458	-2.19
Chlorpromazine	5.19	2.89	1.799	2.225	-1.657	-1.856	-1.33

Compound	log P	log $D^{7.4}$	log $k_w^{IAM.MG}$	log $k_w^{IAM.DD2}$	Δlog $k_w^{IAM.MG}$	Δlog$k_w^{IAM.DD2}$	log $P_0^{in\ situ}$	Δ'log $k_w^{IAM.MG}$	Δ'log$k_w^{IAM.DD2}$
flurbiprofen	4.16	0.91	1.870	1.950	-0.707	-1.061	-0.58	2.069	2.316
Indomethacin	4.27	0.68	2.390	2.080	-0.281	-1.046	-1.06	2.785	2.684
Naproxen	3.24	0.09	1.260	1.339	-0.531	-0.716	-0.77	2.159	2.556
Phenytoin	2.47	2.42	1.787	1.789	0.654	0.534	-4.09	0.696	0.586
Theophylline	-0.02	-0.04	-0.130	0.100	0.863	1.432	-5.09	0.880	1.453
Ibuprofen	4.13	1.44	0.972	1.170	-1.579	-1.810	-1.22	0.718	0.985
L-Dopa	-2.39	-3.67	-0.342	-0.720	2.675	3.074	-3.89		

Table 1. Log P, log $D^{7.4}$ values, logarithms of the chromatographic retention coefficients measured on IAM.PC.MG (log $k_w^{IAM.MG}$) and IAM.PC.DD2 (log $k_w^{IAM.DD2}$) stationary phases, $\Delta\log k_w^{IAM.MG}$, $\Delta\log k_w^{IAM.DD2}$ values and $\Delta'\log k_w^{IAM.MG}$, $\Delta'\log k_w^{IAM.DD2}$ values (for acids only) and *in situ* permeation values (log $P_0^{in\ situ}$) for the 39 compounds considered.

Compound	log P	log $D^{7.4}$	log $k_w^{IAM.MG}$	log $k_w^{IAM.DD2}$	$\Delta\log k_w^{IAM.MG}$	$\Delta\log k_w^{IAM.DD2}$	$\Delta'\log k_w^{IAM.MG}$	$\Delta'\log k_w^{IAM.DD2}$	log $P_0^{PAMPA-BBB}$
Amitriptyline	4.92	2.80	2.881	3.122	-0.345	-0.679			-1.27
Antipyrine	0.56	0.56	0.599	0.393	1.097	1.122			-6.14
Buspirone	2.63	2.39	1.742	1.986	0.472	0.564			-3.85
Caffeine	-0.07	-0.07	0.128	0.116	1.164	1.500			-5.92
Carbamazepine	2.19	2.19	1.039	1.717	0.145	0.753			-4.54
Cimetidine	0.40	0.19	0.633	1.048	1.267	1.943			-6.40
Codeine	1.39	0.22	0.855	1.290	0.644	1.157			-3.68
Diazepam	2.99	2.99	1.731	2.198	0.154	0.402			-3.83
Diltiazem	3.41	2.02	2.121	2.780	0.185	0.548			-3.18
Diphenydramine	3.18	1.80	2.219	2.170	0.479	0.177			-2.64
Domperidone	3.90	2.29	2.790	3.213	0.435	0.472			-3.36
Doxorubicin	1.97	-0.33	2.223	1.764	1.517	1.028			-4.23
Fluoxetine	4.50	2.28	3.181	3.522	0.314	0.158			-1.39
Fluphenazine	4.36	4.33	3.588	3.957	0.841	0.738			-2.36
Hydrocortisone	1.61	1.61	1.550	1.660	1.151	1.298			-5.17
Lidocaine	2.48	1.53	1.112	1.650	-0.030	0.384			-3.65
Methadone	3.93	2.26	2.646	2.828	0.266	0.056			-2.18

Metoclopramide	2.72	1.03	1.199	1.902	-0.148	0.387			-1.11
Morphine	0.89	-0.07	0.767	1.180	0.983	1.566			-4.47
Progesterone	3.87	3.87	2.769	3.317	0.440	0.607			-3.58
Propranolol	3.28	0.48	1.821	2.480	-0.004	0.383			-1.93
Pyrilamine	3.27	1.80	2.109	1.893	0.292	-0.194			-2.63
Quinine	3.44	2.19	2.313	2.810	0.351	0.547			-2.99
Risperidone	3.04	1.66	2.189	2.028	0.569	0.180			-4.00
Verapamil	3.79	1.88	2.892	3.085	0.631	0.458			-2.03
flurbiprofen	4.16	0.91	1.870	1.950	-0.707	-1.061	2.069	2.316	-2.35
Indomethacin	4.27	0.68	2.390	2.080	-0.281	-1.046	2.785	2.684	-2.67
Naproxen	3.24	0.09	1.260	1.339	-0.531	-0.716	2.159	2.556	-2.63
Phenytoin	2.47	2.42	1.787	1.789	0.654	0.534	0.697	0.585	-4.34
Theophylline	-0.02	-0.04	-0.130	0.100	0.863	1.432	0.880	1.452	-6.41
Ibuprofen	4.13	1.44	0.972	1.170	-1.579	-1.810	0.718	0.985	-2.64
Chlorpromazine	5.19	2.89	1.799	2.225	-1.657	-1.856			-1.46
Fluvastatin acido	4.30		2.210	2.843	-0.486	-0.314			-3.56
Haloperidol	4.30		2.670	2.780	-0.026	-0.377			-2.06
Hydroxyzine	3.55		2.908	2.965	0.852	0.588			-3.72
Theobromine	-0.78		-0.156	-0.088	1.486	2.033			-8.00

Table 2. Log P, log $D^{7.4}$ values, logarithms of the chromatographic retention coefficients measured on IAM.PC.MG (log $k_w^{IAM.MG}$) and IAM.PC.DD2 (log $k_w^{IAM.DD2}$) stationary phases, $\Delta\log k_w^{IAM.MG}$, $\Delta\log k_w^{IAM.DD2}$ values and $\Delta'\log k_w^{IAM.MG}$, $\Delta'\log k_w^{IAM.DD2}$ values (for acids only) and in vitro permeation values (log $P_0^{PAMPA\text{-}BBB}$) for the 37 compounds considered.

The calculation of $\Delta\log k_w^{IAM}$ and $\Delta'\log k_w^{IAM}$ was based on the fact that the $\log k_w^{IAM}$ values of structurally non-related neutral compounds having PSA = 0 relate unambiguously with *n*-octanol lipophilicity values. The relation equations are discussed in chapter 4.3 (Equations (2) and (3)); they are based on 17 data points whose values of log P^N, $\log k_w^{IAM.MG}$ and $\log k_w^{IAM.DD2}$ are summarized in Table 3 of chapter 4. For reader's convenience the equations are reported below:

$\log k_w^{IAM.MG} = 0.854\ (\pm 0.047) \log P - 0.976\ (\pm 0.156)$

$n = 17$ $r^2 = 0.957$ $s = 0.214$ $F_{1,15} = 331{,}35$ $F_{1,15}\ \alpha,0.001 = 16.59$

$\log k_w^{IAM.DD2} = 1.039\ (\pm 0.051) \log P - 1.311\ (\pm 0.169)$

$n = 17$ $r^2 = 0.965$ $s = 0.232$ $F_{1,15} = 417.54$ $F_{1,15}\ \alpha,0.001 = 16.59$

We determined the values of $\Delta\log k_w^{IAM.MG}$, $\Delta'\log k_w^{IAM.MG}$, $\Delta\log k_w^{IAM.DD2}$, and $\Delta'\log k_w^{IAM.DD2}$ according to the procedure described in the previous chapters.

In a first step $P_0^{in\ situ}$ values were related to $P_0^{PAMPA\text{-}BBB}$ values. Then, the relationships between both $P_0^{PAMPA\text{-}BBB}$ and $P_0^{in\ situ}$ values and $\Delta\log k_w^{IAM}$(or $\Delta'\log k_w^{IAM}$) were investigated.

5.2 Materials and methods

All samples were obtained from commercial source. All chemicals were of HPLC grade and used without further purification.

5.2.1 Chromatographic system

LC-10AD liquid chromatographic apparatus (Shimadzu Corporation, Kyoto, Japan); SPD-10AV UV detector (Shimadzu), set at λ of maximum absorbance for each compound; 7725 Rheodyne injection valve (fitted with a 20 µl loop).

Data processing: Cromatoplus software for personal computer (Shimadzu).

Analytical HPLC columns:

- IAM.PC.MG (4.6 mm x 150 mm; 12 µm, 300Å; Regis Chemical Company, Morton Grove, IL);
- IAM.PC.DD2 (4.6 mm x 100 mm, 10 µm, 300Å; Regis Chemical Company, Morton Grove, IL).

5.2.2 Chromatographic conditions

The analyses were performed at room temperature with 0.1 M phosphate buffer at pH 7.0 in mixture with acetonitrile at various percentages. The flow rate was selected according to retention time of each analyte (1.0, 2.0, and 3.0 mL/min).

Sample preparation: each analyte was dissolved in the mobile phase or in methanol to *ca*. 10^{-4} M concentration. Chromatographic retention data are reported as log k (the logarithm of the retention factor), calculated by the expression: $\log k = \log [(t_r - t_0)/t_0]$ where t_r and t_0 are the retention times of the drug and a non-retained compound (acetone), respectively. Direct measurements of log k values in fully aqueous mobile phases ($\log k_w^{IAM}$) were only possible for the compounds eluting within 20 min, whereas for the solutes requiring the addition of acetonitrile in the eluent, the $\log k_w^{IAM}$ values were calculated by an extrapolation method [Braumann et al., 1983]: log k values were determined at four different mobile phase compositions varying for the acetonitrile percentages (φ) (from 10 to 30% v/v) and the intercept values of the linear relationships between log k and φ values, found for all compounds in the range of eluent composition examined ($r^2 \geq 0.99$), were assumed as $\log k_w^{IAM}$ values.

All reported log k values are the average of at least three measurements; for each log k value the 95% confidence interval associated with each value never exceeded 0.04. To avoid that the experimental measurements were affected by retention changes due to column ageing, the retention times of five test compounds (amlodipine, p-nitroaniline, toluene, isradipine, and ketoprofen) were checked weekly. No correction was done to the experimental retention values since no retention value of test compounds changed more than 4% during the study

Lipophilic parameters:

log P^N values, i.e. partition coefficients *n*-octanol/aqueous phase of the neutral form of analytes, were either from the literature or calculated (clog P) by the program ClogP for Windows version 2.0 (Biobyte Corp., Claremont, CA). The *n*-octanol/aqueous buffer at pH 7.4 distribution coefficients (log $D^{7.4}$) were taken from the literature or calculated according to the following equations:

$\log D^{7.4} = \log P - \log (1 + 10^{7.4 - pKa})$ (for acids)

$\log D^{7.4} = \log P - \log (1 + 10^{pKa - 7.4})$ (for bases)

5.2.3 Statistical analysis

Linear regression analysis was performed by a commercially available statistical package for personal computer observing the requirements of significant regression analysis.

5.3 Results and discussions

Log P, log $D^{7.4}$ values, logarithms of the chromatographic retention coefficients measured on IAM.PC.MG (log $k_w^{IAM.MG}$) and IAM.PC.DD2 (log $k_w^{IAM.DD2}$) stationary phases, $\Delta\log k_w^{IAM.MG}$, $\Delta\log k_w^{IAM.DD2}$ values and $\Delta'\log k_w^{IAM.MG}$, $\Delta'\log k_w^{IAM.DD2}$ values (for acids only) and *in vitro* permeation values (log $P_0^{PAMPA-BBB}$) for the 37 compounds considered are summarized in Table 1.

Log P, log $D^{7.4}$ values, logarithms of the chromatographic retention coefficients measured on IAM.PC.MG (log $k_w^{IAM.MG}$) and IAM.PC.DD2 (log $k_w^{IAM.DD2}$) stationary phases, $\Delta\log k_w^{IAM.MG}$, $\Delta\log k_w^{IAM.DD2}$ values and $\Delta'\log k_w^{IAM.MG}$, $\Delta'\log k_w^{IAM.DD2}$ values (for acids only) and *in situ* permeation values (log $P_0^{in\ situ}$) for the 39 compounds considered are summarized in Table 2. A highly significant relationship was found between log $P_0^{PAMPA-BBB}$ and log P^N values (equation (7) and Figure 1).

$$\log P_0^{PAMPA-BBB} = 0.939\ (\pm 0.085)\ \log P^N - 6.210\ (\pm 0.276) \quad (7)$$

$n = 36$ $r^2 = 0.782$ $s = 0.765$ $F_{1,34} = 121.63$ $F_{1,34}\ \alpha,0.001 = 12.90$

In contrast, log $P_0^{PAMPA-BBB}$ values related quite poorly with phospholipid affinity data, $\log k_w^{IAM.MG}$ and $\log\log k_w^{IAM.DD2}$, as well as with $\Delta\log k_w^{IAM.MG}$ and $\Delta\log k_w^{IAM.DD2}$ (equations (8), (9), (10), and (11), respectively).

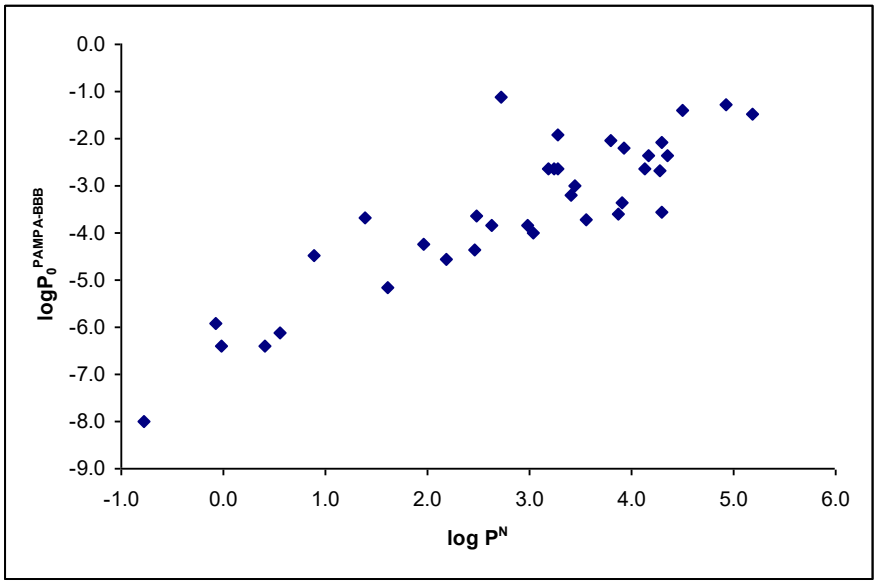

Figure 1. Relationship between log $P_0^{PAMPA-BBB}$ and log P^N values.

$$\log P_0^{\text{PAMPA-BBB}} = 1.193\ (\pm 0.210)\log k_w^{\text{IAM.MG}} - 5.654\ (\pm 0.425) \quad (8)$$

$n = 36 \quad r^2 = 0.487 \quad s = 1.172 \quad F_{1,34} = 32.29 \quad F_{1,34}\ \alpha,0.001 = 12.90$

$$\log P_0^{\text{PAMPA-BBB}} = 1.192\ (\pm 0.192)\ \log k_w^{\text{IAM.DD2}} - 5.936\ (\pm 0.433) \quad (9)$$

$n = 36 \quad r^2 = 0.531 \quad s = 1.120 \quad F_{1,34} = 38.56 \quad F_{1,34}\ \alpha,0.001 = 12.90$

$$\log P_0^{\text{PAMPA-BBB}} = -1.460\ (\pm 0.282)\ \Delta\log k_w^{\text{IAM.MG}} - 3.045\ (\pm 0.223) \quad (10)$$

$n = 36 \quad r^2 = 0.441 \quad s = 1.223 \quad F_{1,34} = 26.86 \quad F_{1,34}\ \alpha,0.001 = 12.90$

$$\log P_0^{\text{PAMPA-BBB}} = -1.283\ (\pm 0.204)\ \Delta\log k_w^{\text{IAM.DD2}} - 3.048\ (\pm 0.200) \quad (11)$$

$n = 36 \quad r^2 = 0.536 \quad s = 1.114 \quad F_{1,34} = 39.40 \quad F_{1,34}\ \alpha,0.001 = 12.90$

The above reported relationships suggest that PAMPA-BBB data substantially reflect the *n*-octanol lipophilicity of the analytes, log P^N. In contrast, phospholipophilicity indexes are ineffective to describe them.After the exclusion of L-Dopa, known to be transported by active influx mechanism, the log $P_0^{\text{in situ}}$ values for 32 compounds moderately related linearly to log $P_0^{\text{PAMPA-BBB}}$ values (Figure 2 and equation (12)).

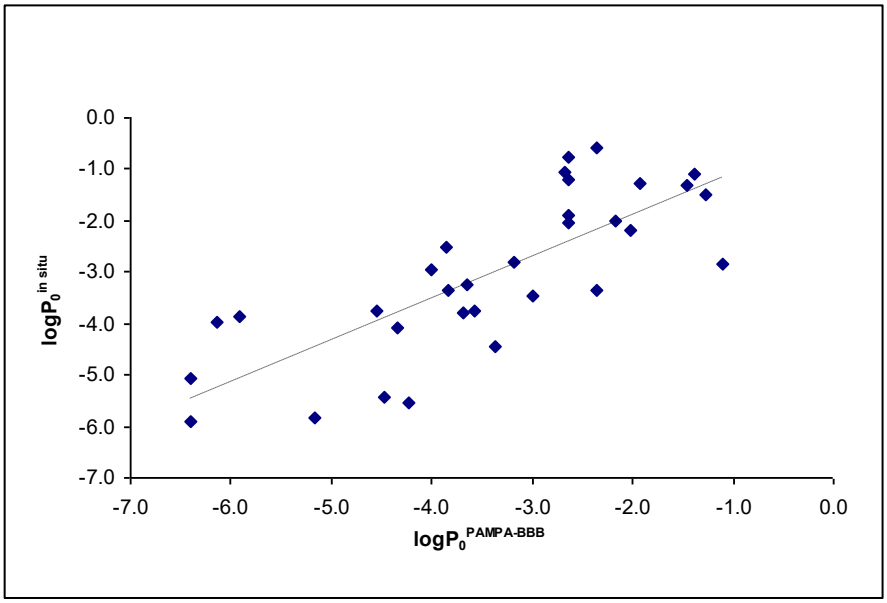

Figure 2. Relationship between log $P_0^{\text{in situ}}$ and log $P_0^{\text{PAMPA-BBB}}$ values.

$$\log P_0^{\text{in situ}} = 0.809\ (\pm 0.120)\ \log P_0^{\text{PAMPA-BBB}} - 0.274\ (\pm 0.443) \quad (12)$$

$n = 32 \quad r^2 = 0.604 \quad s = 0.989 \quad F_{1,30} = 45.74 \quad F_{1,30}\ \alpha,0.001 = 13.29$

Although log $P_0^{PAMPA\text{-}BBB}$ relate quite well to log P^N values, the latter were less effective to describe in situ permeability; as a matter of fact, log $P_0^{in\ situ}$ relate poorly to the log P^N values (Figure 3 and equation (13))

$$\log P_0^{in\ situ} = 0.692\ (\pm 0.112)\ \log P^N - 4.970\ (\pm 0.347) \quad (13)$$

$n = 38$ $r^2 = 0.517$ $s = 1.089$ $F_{1,36} = 38.57$ $F_{1,36}\ \alpha,0.001 = 12.61$

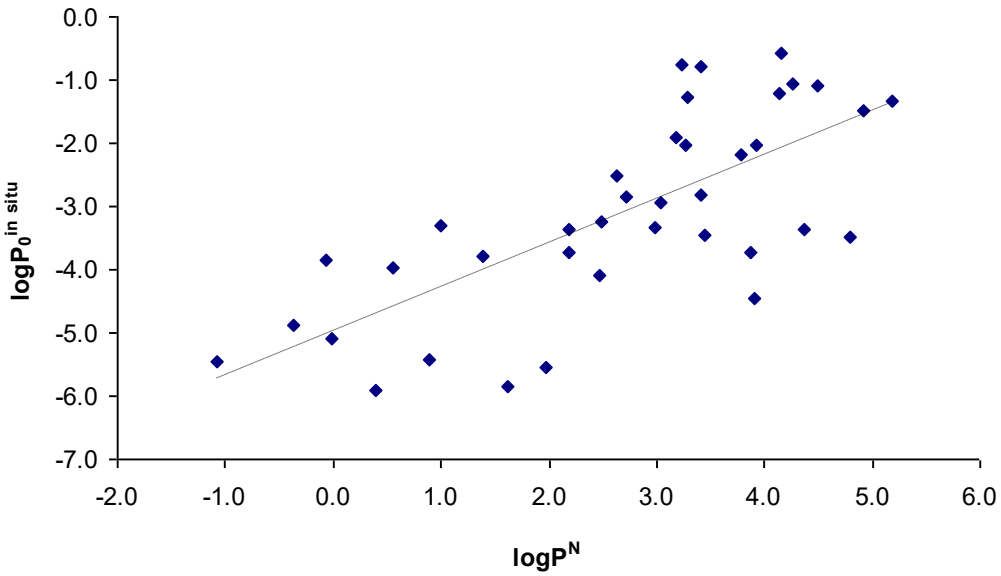

Figure 3. Relationship between log $P_0^{in\ situ}$ and log P^N values.

Similarly, the relationships between log $P_0^{in\ situ}$ and either $logk_w^{IAM.MG}$ or $logk_w^{IAM.DD2}$ values were not significant (data not shown).

In contrast, significant relationships were found between log $P_0^{in\ situ}$ and both $\Delta logk_w^{IAM.MG}$ and $\Delta logk_w^{IAM.DD2}$, but only after the exclusion of ibuprofen and chlorpromazine which behaved as outliers (Figure 4 and equations (14) and (15)). It is interesting to note that chlorpromazine already behaved as an outlier in the previous work (see chapter 2) where its log BB value was related to $\Delta logk_w^{IAM}$.

$$\log P_0^{in\ situ} = -2.077\ (\pm 0.258)\ \Delta logk_w^{IAM.MG} - 2.225\ (\pm 0.195) \quad (14)$$

$n = 36$ $r^2 = 0.656$ $s = 0.907$ $F_{1,34} = 53.34$ $F_{1,34}\ \alpha,0.001 = 12.90$

$$\log P_0^{in\ situ} = -1.818\ (\pm 0.176)\ \Delta logk_w^{IAM.DD2} - 2.266\ (\pm 0.157) \quad (15)$$

$n = 36$ $r^2 = 0.757$ $s = 0.762$ $F_{1,34} = 105.98$ $F_{1,34}\ \alpha,0.001 = 12.90$

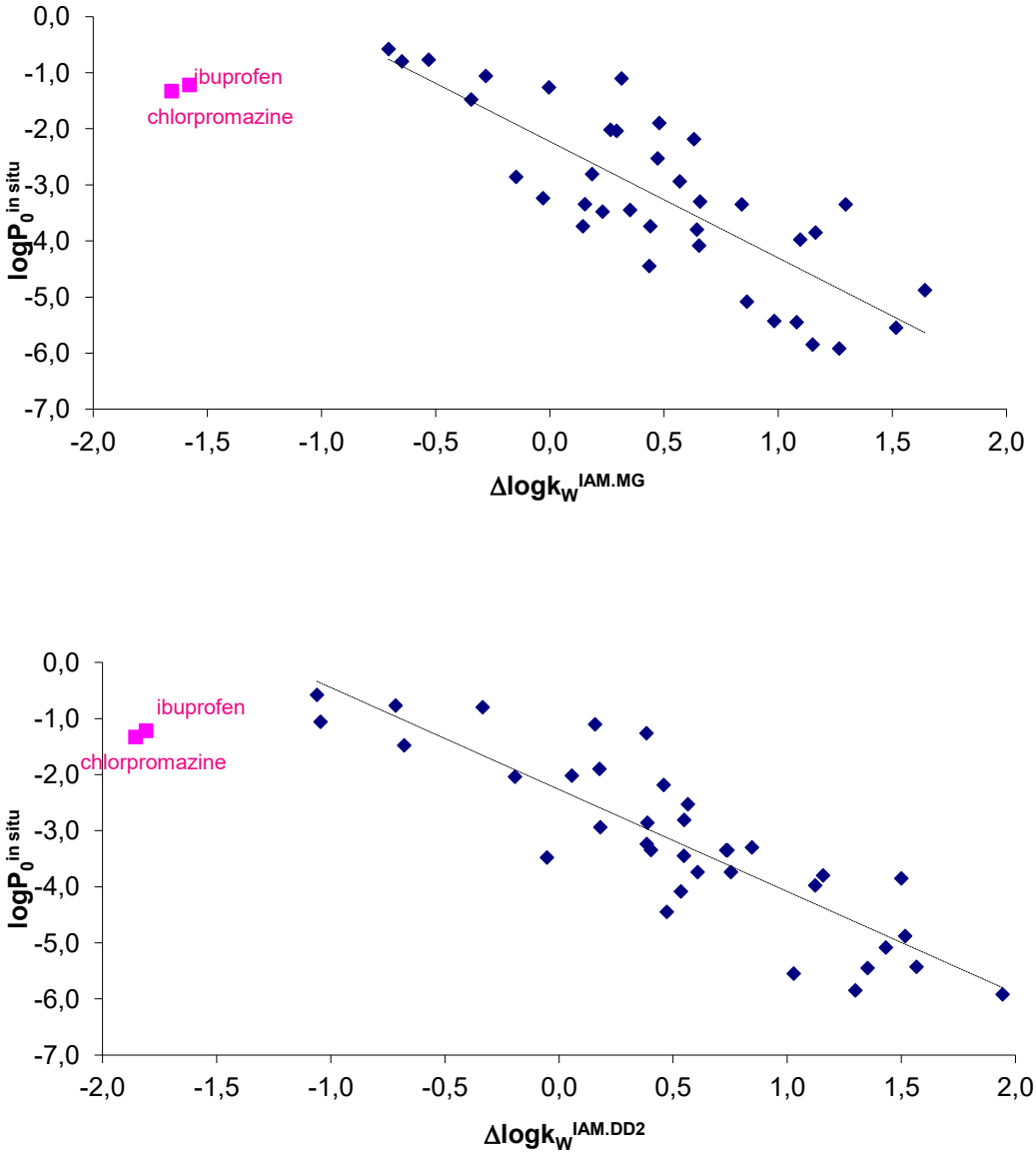

Figure 4. Relationships between log $P_0^{\text{in situ}}$ and $\Delta\log k_w^{\text{IAM}}$ values.

By replacing $\Delta\log k_w^{\text{IAM}}$ values with $\Delta'\log k_w^{\text{IAM}}$ values, for acids, the relationships with log $P_0^{\text{in situ}}$ were not significant (data not shown). These results confirm the soundness of $\Delta\log k_w^{\text{IAM}}$ at predicting BBB passage. However, the fact that $\Delta\log k_w^{\text{IAM}}$ values, and not $\Delta'\log k_w^{\text{IAM}}$, must be used for also acidic analytes may appear in contrast with the results of our previous study where the BBB passage was parameterized as log BB.

However, it should be underlined that log $P_0^{\text{in situ}}$ values express the "*intrinsic permeability*" of the analytes, regardless their ionization degree. This implies that the $P_0^{\text{in situ}}$ values of acids, which are extensively ionized at the physiological pH 7.4, greatly overestimate their

actual capability to cross the BBB since they refer to the neutral forms and express their "intrinsic" tendency to cross the barrier.

5.4 Conclusion

The present study appears as a further validation of the results previously obtained in log BB prediction. As already mentioned, in partial disagreement with our previous results, the BBB passage of acidic compounds in this study was better described by $\Delta\log k_w^{IAM}$ values, and not by $\Delta'\log k_w^{IAM}$. However this discrepancy may be related to the fact that $P_0^{in\ situ}$ values accounts for the partitioning of the neutral forms regardless the relative abundance of the species at the physiological pH.

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6.0 PREDICTION OF DRUG PHOSPHOLIPID AFFINITY BY PHYSICO-CHEMICAL PARAMETERS CALCULATED *IN SILICO*

6.1 Introduction

Drug affinity for membrane phospholipids, so-called phospholipophilicity, is experimentally estimated by various techniques, including both partition measures in phospholipid vesicles (liposomes) and chromatographic measures performed by HPLC on phospholipid-like stationary phases (IAM-HPLC). Although IAM-HPLC data arise from interactions with a monolayer of phospholipids, whereas liposome partition occurs on a phospholipid bilayer that more closely mimics biological membrane bilayers, IAM-HPLC technique has become more and more popular because of its superior reproducibility, speediness and ease of use. Drug/phospholipid interaction data achieved by this technique are expressed as the logarithms of chromatographic retention coefficients (log k), and generally referred to 100% aqueous phase ($\log k_w^{IAM}$).

Traditionally, membrane passage is assumed as dependent on drug lipophilicity expressed as partition coefficient between *n*-octanol/aqueous phase, which is expressed as log P (when referred to a single species) or log D^{pH} when referred to a mixture of neutral and ionized forms existing at a given pH of the solution.

As already reported, it has been demonstrated [Taillardat-Bertschinger et al., 2002] that, in the log P range 1.0 – 4.8, log k_w^{IAM} values relate unambiguously with *n*-octanol/water log P values of structurally non-related neutral compounds and such relationships are even stronger when neutral analytes, having PSA equal to zero, are considered (see equations (2) and (3) reported in chapter 4.3, as well as Table 3 of capter 4). In contrast, numerous experimental works demonstrated that log k_w^{IAM} scale is distinctive from both log P and log D scales when ionizable drugs are taken into account. This discrepancy can be attributed to the different intermolecular interactions occuring between electrically charged species and an electrically charged/anisotropic phase, such as phospholipid layers in IAM, with respect to a neutral/isotropic phase, such as *n*-octanol.

In these cases, i.e. when the scales are not collinear, the scale of $logk_w^{IAM}$ values was frequently found to mimic the drug/membrane interactions actually occurring *in vivo* more closely than lipophilicity in *n*-octanol [Barbato et al., 2006].

In our recent studies, reported in chapter 2 and 3, we found that, according to the *flip-flop* model [Gurtovenko and Vattulainen, 2007; Krämer et al., 2009], the passage of biological barriers inversely related to the polar/electrostatic component of interaction drug/membrane, accounting for the interaction of charged species at phospholipid charged outer surfaces.

These interaction forces were expressed as $\Delta logk_w^{IAM}$ or $\Delta' logk_w^{IAM}$, resulting from the combination of the total interaction drug/phosholipids, i.e. log k_w^{IAM} values, with the lipophilic/hydrophobic interaction component, i.e. either log P or log D values. While the values of both log P and log D can be easily calculated *in silico* with rather good approximation, the values of log k_w^{IAM} for ionizable analytes are still not predictable and must be experimentally determined. Therefore, the prediction of barrier passage based on $\Delta logk_w^{IAM}/\Delta' logk_w^{IAM}$ values cannot be considered a high-throughput method and cannot be applied for hypothetical molecules without them being actually synthesized.

The possibility to calculate *in silico* the $logk_w^{IAM}$ values for ionizable molecules could lead to a high-throughput method aimed at calculating $\Delta logk_w^{IAM}/\Delta' logk_w^{IAM}$ values for a screening of new drug/prodrug candidates according to their capability to cross either the BBB or the intestinal barrier at the early stages of their development. Furthermore, the assessment of the physico-chemical and topological properties governing phospholipid interactions of ionized species can contribute to elucidate the mechanisms involved in drug/membrane interactions.

In the present work, we took into account 205 and 161 analytes, whose $logk_w^{IAM.MG}$ and $logk_w^{IAM.DD2}$ values, respectively, had been previously determined experimentally by the research group headed by Prof. Francesco Barbato from 1996 to present. A QSPR (Quantitative Structure Properties Relationships) study was performed and various models were obtained including four independent variables, i.e. physico-chemical and topological properties calculated by software. For each training set, the regression coefficients were calculated to evaluate the test set in terms of standard deviation of errors (*SE*), angular coefficient, intercept, Amemiya predictive criterion (*PC*), statistical value of Fisher F of the regression (*F*) and r^2 of the trend line of the chart of the predicted vs. experimental activities.

6.1 Materials and methods

$logk_w^{IAM}$ values had been experimentally determined by us on two different IAM stationary phases, i.e. IAM.PC.MG ($logk_w^{IAM.MG}$) and IAM.PC.DD2($logk_w^{IAM.DD2}$), and reported in the literature [Amato et al., 2000; Barbato et al., 1996; Barbato et al., 1997a; Barbato et al., 1998; Barbato et al., 1997b; Barbato et al., 2004; Barbato et al., 2005; Barbato et al., 2007; Barbato et al., 2009; Barbato et al., 2011; Grumetto et al., 2012; Grumetto et al., 2013; Quaglia et al., 2005].

The three-dimensional structures of the considered molecules were downloaded from PubChem [Bolton et al., 2008] and they where considered in both neutral, and ionized Gasteiger – Marsili [Gasteiger and Marsili, 1980]. Atom charges were applied to perform the next molecular mechanics calculations. For ampholytes, the distribution at the experimental pH (7.0), was calculated by the software MarvinSketch 16.2.15.0 2016 for Mac OS X [ChemAxon, 2016]. An extensive conformational analysis was carried out by using the Boltzmann Jump method implemented in AMMP software [Harrison, 1993] and the so obtained lowest energy conformation was further optimized by performing a semi-empirical calculation with Mopac 2012 program [Steward James, 2012] (keywords: PM7 PRECISE MMOK). Physico-chemical and topological properties (Virtual logP [Gaillard et al., 1994], lipole [Pedretti et al., 2002a], volume, polar surface area, surface accessible to the solvent, gyration radius, ovality, mass, number of atoms, angles, dihedrals, etc) were calculated by VEGA ZZ software [Pedretti et al., 2002b] and finally, all molecules were inserted into a Microsoft Access database. The QSPR models were obtained by the automatic stepwise approach implemented in "Automatic linear regression" script of VEGA ZZ software, calculating regression models, including from 1 to 4 independent variables. The predictive strength of the best equation was evaluated not only by leave-one-out (LOO) cross validation, but also by splitting randomly the whole dataset in 20 pairs of training and test sets. For each training set, the regression coefficients were calculated to evaluate the test set in terms of standard deviation of errors, angular coefficient, intercept and r^2 of the trend line of the chart of the predicted vs. experimental activities. This validation was performed automatically by "Model validator" script also included in VEGA ZZ.

6.2 Results and discussions

The dataset consisted of 205 and 161 analytes whose log $k_w^{IAM.MG}$ and log $k_w^{IAM.DD2}$ values, were determined respectively. As already mentioned, the values from the same research group were taken in an attempt to minimize the inter-laboratory variability. Such values along with the pKa values are reported in Table 1.

Analyte	pKa	log $k_w^{IAM.MG}$	log $k_w^{IAM.DD2}$
1,2,4,5-tetrachlorobenzene	-	3.028	3.497
1,2 -dichloroethane	-	0.444	0.337
1,3-dichlorobenzene	-	2.077	2.475
1-chloro butane	-	1.275	1.435
1-chloro-2-nitrobenzene	-	0.922	1.053
1-hexanol	-	0.727	0.833
1-naphthylamine	3.93	1.460	
1-nitrobutane	-	0.44	0.476
1-pentanol	-	0.399	0.331
2-aminobiphenyl	3.82	1.860	
2-chloroaniline	2.57	0.840	
2-methyl-2 butanol	-	0.190	-0.082
2-phenylethyl acetate	-	1.063	1.207
2-phenylethylamine	9.82	0.230	
3-chloro phenol	-	1.381	1.702
4-chlorobenzylalcohol	-	0.927	1.057
4-methylbenzylamine	9.41	0.310	
4-nitroaniline	-	0.983	
acebutolol	9.67	1.761	1.409
acetonitrile	-	0.052	-0.695
acetophenone	-	0.763	0.88
acetylsalicylic acid	3.50	-0.950	-0.850
acridine	5.58	2.030	
alprazolam	2.37	1.330	1.935
alprenolol	9.60	1.779	2.26
aminophenazone	4.50	0.980	0.771
amitriptyline	9.18	2.881	3.122
amlodipine	9.10	2.590	
amoxicillin	-	-0.920	-0.728
aniline	4.63	0.12	
anisole	-	0.895	0.954
atenolol	9.43	-0.005	0.765
benzene	-	0.620	0.720
benzyl cyanide	-	0.291	0.335
benzylalcohol	-	0.010	3.037
benzylamine	9.31	2.686	
benzylbenzoate	-	0.533	0.820
benzylmethylketon	-	0.317	0.375

betahistine	10.10	0.244	0.279
betaxolol	9.40	1.155	1.838
bibenzyl	-	3.243	3.766
biperidene	-	3.187	3.354
biphenyl	-	2.723	3.137
bromazepam	-	1.234	1.503
bromperidol	8.04	2.893	3.053
bupivacaine	8.10	1.450	
buprenorphine	-	2.485	3.190
butylacetate	-	0.414	0.622
caffeine	0.52	0.185	0.680
carbamazepine	-	1.039	1.717
carbamazepine epoxide	-	1.118	1.213
carbon tetrachloride	-	1.062	1.209
cephalexin	-	-0.220	0.021
chlorambucil	4.82	1.288	1.897
chloroform	-	0.625	0.62
chlorpromazine	9.41	1.799	2.225
cimetidine	6.80	0.633	1.048
cinoxacin	-	-0.538	-0.301
ciprofloxacin	-	0.786	1.341
clobazam	-	1.296	1.946
clonidine	8.02	0.948	1.316
clorazepate	-	0.884	0.750
codeine	8.21	0.855	1.29
cotinine	4.79	0.450	0.167
delorazepam	-	2.471	2.441
desipramine	10.40	2.826	2.741
dextromethorphan	-	1.578	2.579
diazepam	3.40	2.314	2.198
dichloromethane	-	0.309	0.107
diclofenac	4.50	2.430	
diethylether	-	0.248	-0.065
diflunisal	3.00	2.330	
diltiazem	7.50	2.121	2.780
diphenhydramine	9.10	2.219	2.170
dipropyl ether	-	0.358	0.784
domperidone	7.90	2.790	3.213
epinephrine	8.59	-0.098	0.250

ethanol	-	0.040	-0.623
ethylacetate	-	-0.346	-0.253
ethylbenzoate	-	0.829	1.481
etidocaine	7.70	1.550	
felodipine	-	2.980	3.470
fenbufen	4.50	1.660	
flufenamic acid	3.90	2.860	
flumazenil	0.86	1.137	1.389
flumequine	-	0.800	1.183
fluphenazine	7.90	3.588	3.957
flurazepam	-	2.392	2.532
flurbiprofen	4.60	2.020	
fluvastatin	-	2.210	2.843
furosemide	-	0.780	0.920
GEA 968	7.70	0.380	
granisetron	-	1.417	1.979
griseofulvin	-	1.975	
haloperidol	8.04	2.670	2.780
heptane	-	2.882	3.197
hexobarbital	8.20	0.855	1.290
hydrochlorothiazide	7.90	0.540	0.977
hydrocortisone	-	1.503	
hydroxyzine	7.82	2.908	2.965
ibuprofen	5.20	0.972	1.170
imipramine	9.49	3.065	3.008
indomethacin	4.50	2.390	2.080
indoprofen	4.60	1.170	
isosorbide dinitrate	-	-0.146	
isotretinoin	-	2.807	3.704
isradipine	-	2.130	2.48
ketamine	6.46	1.002	1.339
ketoprofen	4.60	1.120	1.360
labetalol	-	1.439	2.017
lacidipine	-	3.520	4.000
levosulpiride	-	1.175	2.780
lidocaine	7.90	0.750	1.139
loratadine	-	3.354	3.623
lorazepam	-	2.422	2.293
mefenamic acid	4.20	2.460	

mepivacaine	7.60	0.770	
mepyramine	8.85	2.109	1.893
mesitylene	-	2.174	2.609
metadone	-	2.646	2.828
methohexital	8.73	1.039	1.569
methylacetate	-	-0.618	-0.657
methylsulfoxide	-	-1.011	-1.092
metoclopramide	-	1.199	1.902
metoprolol	9.70	0.642	1.099
mianserin	8.26	3.003	3.131
midazolam	6.03	2.302	2.505
morphine	8.25	0.767	1.180
N.N-dimethylaniline	5.15	0.930	
N.N-dimethyl-p-toluidine	5.33	1.200	
nadolol	9.40	0.401	1.005
nalidixic acid	8.60	0.158	0.657
naphtalene	-	2.122	2.471
naproxen	4.15	1.260	1.339
nebivolol	8.65	2.537	2.746
N-ethylaniline	5.11	0.780	
nicardipine	6.50	3.140	
nicotinamide	3.54	0.351	-0.179
nicotine	8.00	0.844	1.184
nifedipine	-	1.740	2.030
nimodipine	-	2.350	3.060
nisoldipine	-	2.630	3.260
nitrendipine	-	2.270	3.040
nitrobenzene	-	0.888	0.965
N-methylbenzylamine	9.58	0.130	
N-methylnaphthalen-1-amine	9.30	1.090	
N-methylphenethylamine	10.15	0.330	
norfloxacin	-	0.808	
N-pentane	-	1.877	2.276
N-propanol	-	0.085	-0.420
ofloxacin	-	0.836	
ondansetron	7.40	1.633	2.308
oxazepam	-	2.189	2.163
oxolinic acid	6.90	0.798	0.992

oxprenolol	9.50	0.936	1.455
paracetamol	9.50	0.126	0.280
pentamethylbenzene	-	2.771	3.323
phenazone	0.65	0.599	0.729
phenobarbital	7.30	0.546	0.853
phenol	-	0.592	0.653
phenylbutazone	4.70	1.305	1.232
phenylpropanolamine	9.40	0.313	0.579
phenytoin	8.28	1.787	1.789
physostigmine	8.32	0.902	1.151
pindolol	9.70	0.902	1.302
pipemidic acid	-	-0.066	0.484
piromidic acid	-	0.756	1.167
piroxicam	1.86	1.850	1.767
p-nitroaniline	1.10	0.931	
prilocaine	7.80	0.620	
procaine	9.00	0.390	
progesterone	-	2.769	3.317
promazine	9.43	2.462	3.260
promethazine	8.98	2.432	3.075
propionitrile	-	0.103	-0.347
propiophenone	-	1.091	1.232
propofol	-	2.073	2.991
propranolol	9.50	1.821	2.480
p-toluidine	5.08	0.530	
pyridine	5.23	-0.010	
ranitidine	8.36	0.834	0.812
risperidone	8.76	2.189	2.028
rufloxacin	-	0.777	1.346
salicylic acid	2.97	0.126	-0.075
sotalol	9.10	0.117	0.692
sulindac	4.50	1.800	
temazepam	-	2.190	1.697
terbutaline	-	0.662	0.863
tert- butyl alcohol	-	0.097	0.370
tetracaine	8.50	1.750	
tetrachloro ethane	-	1.140	1.278
tetrahydrofurane	-	0.145	-0.178
theobromine	9.90	-0.156	-0.088

theophylline	8.60	0.033	0.153
thiopental	7.40	1.238	1.328
timolol	8.80	0.610	1.058
tocainide	7.80	0.530	
tolfenamic acid	4.20	2.750	
tolmetin	3.50	1.130	
toluene	-	1.030	1.210
tramadol	8.30	0.893	1.347
trimecaine	7.40	1.210	
tropisetron	-	1.778	2.531
verapamil	8.90	2.892	3.085
W 36017	7.40	0.490	

Table 1. pKa, log $k_w^{IAM.MG}$ and log $k_w^{IAM.DD2}$ values for the compounds considered.

6.2.1 Static properties in log $k_w^{IAM.MG}$ modeling

At first the static properties, i.e. 1) angles, 2) charges, 3) dihedrals, 4) dipole, 5) EZ bonds, 6) gyration radius, 7) H-bond acceptor, 8) H-bond donor, 9) heavy atoms, 10) impropers, 11) lipole, 12) monoisotopic mass, 13) molecular mass, 14) number of atoms, 15) number of bonds, 16) number of chiral atoms, 17) number of flexible torsions, 18) number of rings, 19) number of torsions, 20) ovality, 21) polar surface area, 22) surface, 23) surface accessible to the solvent, 24) surface diameter, 25) VirtualLog P, 26) volume, 27) volume accessible to the solvent, 28) volume diameter for the neutral forms were derived. A Microsoft Access database was generated including calculation sheets for each compound to allow regression analysis by VEGA software. The properties are shown in Table 2. The best models (equations (1) and (2)), developed by taking into account the electrically neutral forms for the whole set of 205 compounds, were based on the following 4 properties: VirtualLogP, number of heavy atoms, number of flexible torsions and ovality.

$$\log k_w^{IAM.MG} = 0.4528 + 0.5326\ VirtualLogP + 0.0867\ HeavyAtoms - 1.1191\ Ovality - 0.0525\ FlexTorsions \quad (1)$$

n= 205 r^2 = 0.74 q^2 = 0.71 SE= 0.512 F= 143.98 $F\alpha$ 0.001= 19.98 PC=54.185

Best optimized model (n-1):

$$\log k_w^{IAM.MG} = 0.4788 + 0.5397\ VirtualLogP + 0.0890\ HeavyAtoms - 1.1592\ Ovality - 0.0571\ FlexTorsions \quad (2)$$

n= 204 r^2 = 0.75 SE= 0.505 F= 149.61 $F\alpha$ 0.001= 19.98 PC=52.468 ExRow: alprazolam

Analyte	Angles	Atoms	Bonds	Charge	ChiralAtms	Dipole	EzBnds	FlexTorsions	Gyrrad
1,2,4,5-tetrachlorobenzene	18	12	12	0	0	0.001	0	0	2.721
1,2 -dichloroethane	12	8	7	0	0	2.035	0	0	1.664
1,3-dichlorobenzene	18	12	12	0	0	1.195	0	0	2.337
1-chloro butane	24	14	13	0	0	1.339	0	1	2.106
1-chloro-2-nitrobenzene	21	14	14	0	0	3.217	0	0	2.185
1-hexanol	37	21	20	0	0	1.701	0	4	2.666
1-Naphthylamine	33	20	21	0	0	0.252	0	0	2.227
1-nitrobutane	27	16	15	0	0	2.642	0	2	2.205
1-pentanol	31	18	17	0	0	1.702	0	3	2.300
2-Aminobiphenyl	39	24	25	0	0	0.249	0	1	2.638
2-Chloroaniline	21	14	14	0	0	1.330	0	0	2.007
2-methyl-2 butanol	31	18	17	0	0	1.712	0	0	1.709
2-phenylethyl acetate	40	24	24	0	0	1.815	0	4	2.729
2-Phenylethylamine	33	20	20	0	0	0.742	0	2	2.388
3-chloro phenol	19	13	13	0	0	0.657	0	0	2.145
4-chlorobenzylalcohol	25	16	16	0	0	1.192	0	1	2.486
4-Methylbenzylamine	33	20	20	0	0	0.891	0	1	2.292
4-nitroaniline	24	16	16	0	0	2.285	0	0	2.318
Acebutolol	92	52	52	0	1	5.862	0	10	4.237
Acetonitrile	7	6	5	0	0	2.280	0	0	1.182
Acetophenone	27	17	17	0	0	2.477	0	1	2.160
Acetylsalicylic acid	32	21	21	0	0	1.094	0	3	2.449
Acridine	40	23	25	0	0	1.300	0	0	2.722
Alprazolam	63	35	38	0	0	2.243	0	1	3.449

Alprenolol	71	41	41	0	1	2.381	0	8	3.269
Aminophenazone	60	34	35	0	0	2.522	1	2	3.028
Amitriptyline	81	44	46	0	0	0.900	0	3	3.324
Amlodipine	93	53	54	0	1	1.934	2	10	3.802
Amoxicillin	81	44	46	0	4	1.999	0	4	3.618
Aniline	21	14	14	0	0	0.243	0	0	1.748
Anisole	25	16	16	0	0	1.698	0	1	2.010
Atenolol	71	41	41	0	1	5.004	0	8	3.865
Benzene	18	12	12	0	0	0.000	0	0	1.516
Benzyl cyanide	25	16	16	0	0	2.244	0	1	2.236
Benzylalcohol	25	16	16	0	0	1.648	0	1	2.016
Benzylamine	27	17	17	0	0	0.866	0	1	2.030
Benzylbenzoate	46	28	29	0	0	0.648	0	4	3.541
Benzylmethylketon	33	20	20	0	0	2.355	0	2	2.632
Betahistine	37	22	22	0	0	1.183	0	3	2.697
Betaxolol	96	51	52	0	1	4.083	0	11	3.876
Bibenzyl	48	28	29	0	0	0.004	0	3	3.377
Biperidene	106	52	55	0	4	2.384	1	2	3.709
Biphenyl	36	22	23	0	0	0.000	0	1	2.644
Bromazepam	50	29	31	0	0	1.827	0	1	3.407
Bromperidol	91	49	51	0	0	3.142	0	6	6.163
Bupivacaine	93	49	50	0	1	3.032	0	5	3.622
Buprenorphine	163	75	81	0	7	4.882	0	3	3.949
Butylacetate	34	20	19	0	0	1.414	0	4	2.695
Caffeine	43	24	25	0	0	1.457	0	0	2.481
Carbamazepine	51	30	32	0	0	2.313	1	1	2.813

Carbamazepine epoxide	58	31	34	0	2	4.058	1	1	2.785
Carbon tetrachloride	6	5	4	0	0	0.000	0	0	1.730
Cephalexin	74	41	43	0	3	2.532	1	4	3.722
Chlorambucil	67	38	38	0	0	1.686	0	7	4.325
Chloroform	6	5	4	0	0	1.184	0	0	1.621
Chlorpromazine	73	40	42	0	0	1.310	0	4	3.399
Cimetidine	55	33	33	0	0	1.931	0	7	3.840
Cinoxacin	52	29	31	0	0	2.498	0	2	3.275
Ciprofloxacin	82	42	45	0	0	3.542	1	3	3.895
Clobazam	60	34	36	0	0	2.793	0	1	3.260
Clonidine	40	23	24	0	0	0.389	0	2	2.743
Clorazepate	56	33	35	0	1	2.756	0	2	3.539
Codeine	90	43	47	0	5	4.793	1	1	3.007
Cotinine	46	25	26	0	1	3.246	0	1	2.586
Delorazepam	52	30	32	0	0	2.192	0	1	3.220
Desipramine	78	42	44	0	0	1.018	0	4	3.339
Dextromethorphan	94	45	48	0	3	1.865	0	1	3.037
Diazepam	58	33	35	0	0	1.786	0	1	3.329
Dichloromethane	6	5	4	0	0	1.450	0	0	1.419
Diclofenac	49	30	31	0	0	1.421	0	4	3.135
Diethylether	25	15	14	0	0	1.725	0	2	1.853
Diflunisal	41	26	27	0	0	2.176	0	2	3.470
Diltiazem	99	55	57	0	2	1.687	0	7	3.917
Diphenhydramine	70	40	41	0	0	2.257	0	6	3.437
Dipropyl ether	37	21	20	0	0	1.735	0	4	2.575
Domperidone	105	54	58	0	0	3.867	0	5	5.287

Epinephrine	42	26	26	0	1	3.487	0	3	2.805
Ethanol	13	9	8	0	0	1.698	0	0	1.193
Ethylacetate	22	14	13	0	0	0.795	0	2	1.881
Ethylbenzoate	34	21	21	0	0	0.718	0	3	2.664
Etidocaine	87	48	48	0	1	2.248	0	7	3.456
Felodipine	77	44	45	0	1	2.953	2	6	3.435
Fenbufen	55	33	34	0	0	1.948	0	5	4.315
Flufenamic Acid	49	30	31	0	0	1.995	0	3	3.471
Flumazenil	65	36	38	0	0	2.106	0	3	3.658
Flumequine	58	31	33	0	1	3.936	1	1	3.137
Fluphenazine	107	56	59	0	0	2.908	0	6	5.037
Flurazepam	91	50	52	0	0	3.900	0	6	3.937
Flurbiprofen	52	31	32	0	1	2.702	0	1	3.532
Fluvastatin	99	56	58	0	2	2.453	1	8	4.013
Furosemide	53	32	33	0	0	5.050	0	5	3.900
GEA 968	81	46	46	0	0	5.653	0	7	4.383
Granisetron	94	47	50	0	2	2.337	0	2	4.078
Griseofulvin	76	41	43	0	2	4.980	1	3	3.456
Haloperidol	91	49	51	0	0	3.250	0	6	5.783
Heptane	42	23	22	0	0	0.003	0	4	2.686
Hexobarbital	63	33	34	0	1	0.660	1	1	2.795
Hydrochlorothiazide	45	25	26	0	0	9.149	0	1	3.094
Hydrocortisone	117	56	59	0	7	3.847	1	2	3.964
Hydroxyzine	98	53	55	0	1	1.557	0	8	4.975
Ibuprofen	58	33	33	0	1	1.362	0	1	3.203
Imipramine	84	45	47	0	0	1.025	0	4	3.419

Indomethacin	71	41	43	0	0	1.030	0	4	4.102
Indoprofen	64	36	38	0	1	2.195	0	1	4.121
Isosorbide dinitrate	46	24	25	0	4	2.451	0	2	2.989
Isotretinoin	88	50	50	0	0	1.440	5	5	4.918
Isradipine	86	48	50	0	1	2.016	2	6	3.296
Ketamine	60	32	33	0	1	4.382	0	2	2.634
Ketoprofen	55	33	34	0	1	2.252	0	2	3.289
Labetalol	83	48	49	0	2	3.458	0	8	3.498
Lacidipine	117	66	67	0	0	5.505	3	10	4.055
Levosulpiride	85	46	47	0	1	5.519	0	6	4.279
Lidocaine	69	39	39	0	0	3.040	0	5	3.343
Loratadine	95	50	53	0	0	3.658	0	2	4.280
Lorazepam	53	31	33	0	1	2.432	0	1	3.296
Mefenamic Acid	55	33	34	0	0	1.360	0	3	3.268
Mepivacaine	75	40	41	0	1	3.114	0	2	3.307
Mepyramine	77	44	45	0	0	1.220	0	7	3.836
Mesitylene	36	21	21	0	0	0.148	0	0	2.189
Metadone	90	50	51	0	1	2.904	0	7	3.229
Methohexital	65	37	37	0	2	2.213	0	3	3.043
Methylacetate	16	11	10	0	0	1.401	0	1	1.574
Methylsulfoxide	15	10	9	0	0	8.663	0	0	1.319
Metoclopramide	73	42	42	0	0	3.458	0	7	4.092
Metoprolol	78	44	44	0	1	4.068	0	9	3.592
Mianserin	78	40	43	0	1	0.595	0	0	3.125
Midazolam	128	71	72	0	1	2.170	0	12	4.637
Morphine	84	40	44	0	5	4.288	1	0	2.879

N,N-Dimethylaniline	33	20	20	0	0	0.129	0	1	2.156
N,N-Dimethyl-p-toluidine	39	23	23	0	0	0.051	0	1	2.422
Nadolol	91	49	50	0	3	4.115	0	5	3.482
Nalidixic acid	50	29	30	0	0	2.168	1	2	3.042
Naphtalene	30	18	19	0	0	0.000	0	0	2.112
Naproxen	53	31	32	0	1	1.292	0	1	3.442
Nebivolol	103	54	57	0	4	3.211	0	6	5.148
N-Ethylaniline	33	20	20	0	0	0.210	0	2	2.369
Nicardipine	113	64	66	0	1	3.791	2	10	3.884
Nicotinamide	22	15	15	0	0	0.980	0	1	2.115
Nicotine	49	26	27	0	1	2.211	0	1	2.499
Nifedipine	74	43	44	0	0	2.801	2	5	3.172
Nimodipine	99	56	57	0	1	2.381	2	9	3.619
Nisoldipine	92	52	53	0	1	2.388	2	6	3.472
Nitrendipine	80	46	47	0	1	2.326	2	6	3.459
Nitrobenzene	21	14	14	0	0	2.527	0	0	2.055
N-Methylbenzylamine	33	20	20	0	0	0.928	0	2	2.362
N-methylnaphthalen-1-amine	39	23	24	0	0	0.315	0	1	2.436
N-Methylphenethylamine	39	23	23	0	0	0.797	0	3	2.720
Norfloxacin	76	41	43	0	0	3.348	1	3	3.874
N-pentane	30	17	16	0	0	0.003	0	2	1.973
N-propanol	19	12	11	0	0	1.702	0	1	1.568
Ofloxacin	89	46	49	0	1	3.839	1	2	3.967
Ondansetron	79	41	44	0	1	3.820	0	2	3.527
Oxazepam	53	31	33	0	1	2.696	0	1	3.354
Oxolinic acid	54	30	32	0	0	2.830	1	2	3.302

Oxprenolol	72	42	42	0	1	2.147	0	9	3.236
Paracetamol	31	20	20	0	0	2.919	0	1	2.642
Pentamethylbenzene	48	27	27	0	0	0.093	0	0	2.381
Phenazone	45	26	27	0	0	2.657	1	1	2.644
Phenobarbital	51	29	30	0	0	0.705	0	2	2.729
Phenol	19	13	13	0	0	1.637	0	0	1.752
Phenylbutazone	78	43	45	0	0	0.860	0	5	3.362
Phenylpropanolamine	40	24	24	0	2	2.079	0	2	2.445
Phenytoin	54	31	33	0	0	1.905	0	2	2.933
Physostigmine	79	41	43	0	2	1.508	0	2	3.576
Pindolol	68	38	39	0	1	2.600	0	6	3.257
Pipemidic acid	72	39	41	0	0	1.933	1	3	3.849
Piromidic acid	69	37	39	0	0	2.327	1	3	3.682
Piroxicam	62	36	38	0	0	3.569	1	2	3.715
P-Nitroaniline	24	16	16	0	0	2.285	0	0	2.318
Prilocaine	63	36	36	0	1	3.214	0	5	3.297
Procaine	64	37	37	0	0	2.643	0	7	3.814
Progesterone	114	53	56	0	6	1.609	1	1	3.782
Promazine	73	40	42	0	0	1.162	0	4	3.048
Promethazine	73	40	42	0	1	1.359	0	3	3.171
Propionitrile	13	9	8	0	0	2.298	0	0	1.493
Propiophenone	33	20	20	0	0	2.484	0	2	2.433
Propofol	55	31	31	0	0	1.547	0	0	2.707
Propranolol	71	40	41	0	1	2.339	0	6	3.366
P-toluidine	27	17	17	0	0	0.356	0	0	2.019
Pyridine	16	11	11	0	0	1.336	0	0	1.483

Ranitidine	74	43	43	0	0	3.638	1	9	4.164
Risperidone	114	57	61	0	0	3.621	1	4	5.736
Rufloxacin	83	43	46	0	0	4.016	1	2	3.942
Salicylic acid	23	16	16	0	0	2.909	0	1	2.182
Sotalol	67	38	38	0	1	5.645	0	6	4.033
Sulindac	73	42	44	0	1	6.890	2	4	4.353
Temazepam	59	34	36	0	1	2.918	0	1	3.382
Terbutaline	60	35	35	0	1	2.607	0	3	3.487
Tert- butyl alcohol	25	15	14	0	0	1.708	0	0	1.493
Tetracaine	76	43	43	0	0	2.791	0	9	4.703
Tetrachloro ethane	12	8	7	0	0	1.436	0	0	1.949
Tetrahydrofurane	25	13	13	0	0	2.035	0	0	1.367
Theobromine	37	21	22	0	0	1.879	0	0	2.384
Theophylline	37	21	22	0	0	1.318	0	0	2.358
Thiopental	63	34	34	0	1	0.503	0	2	2.957
Timolol	84	45	46	0	1	2.393	0	6	3.515
Tocainide	51	30	30	0	1	3.092	0	2	2.777
Tolfenamic acid	49	30	31	0	0	2.090	0	3	3.461
Tolmetin	58	34	35	0	0	2.993	0	4	3.735
Toluene	24	15	15	0	0	0.122	0	0	1.781
Tramadol	83	44	45	0	2	2.697	0	4	3.231
Trimecaine	75	42	42	0	0	3.026	0	5	3.543
Tropisetron	82	41	44	0	2	0.571	0	3	3.982
Verapamil	128	71	72	0	1	2.170	0	12	4.637
W 36017	57	33	33	0	0	3.165	0	3	3.047

Table 2A. Number of angles, atoms, bonds, charge, chiral atoms, dipole, E-Z bonds, flexible torsions and gyrrad for the whole dataset of the analytes assumed as neutral.

Name	HbAcc	HbDon	HeavyAtoms	Impropers	Lipole	Mass	MassMI	Ovality	Psa	Rings
1,2,4,5-tetrachlorobenzene	0	0	10	0	0.000	215.892	213.891	1.351	0.000	1
1,2 -dichloroethane	0	0	4	0	0.451	98.959	97.969	1.228	0.000	0
1,3-dichlorobenzene	0	0	8	0	0.720	147.002	145.969	1.285	0.000	1
1-chloro butane	0	0	5	0	0.304	92.567	92.039	1.332	0.000	0
1-chloro-2-nitrobenzene	2	0	10	3	2.301	157.555	156.993	1.287	39.048	1
1-hexanol	1	1	7	0	4.537	102.175	102.105	1.450	23.720	0
1-Naphthylamine	0	2	11	3	3.223	143.185	143.074	1.306	25.477	2
1-nitrobutane	3	0	7	3	3.335	103.120	103.063	1.344	41.722	0
1-pentanol	1	1	6	0	3.910	88.148	88.089	1.384	23.183	0
2-Aminobiphenyl	0	2	13	3	2.681	169.222	169.089	1.388	25.477	2
2-Chloroaniline	0	2	8	3	2.574	127.572	127.019	1.262	26.147	1
2-methyl-2 butanol	1	1	6	0	1.859	88.148	88.089	1.365	22.019	0
2-phenylethyl acetate	2	0	12	3	3.689	164.201	164.084	1.462	28.316	1
2-Phenylethylamine	1	2	9	3	4.104	121.180	121.089	1.409	28.345	1
3-chloro phenol	1	1	8	0	3.604	128.556	128.003	1.252	22.648	1
4-chlorobenzylalcohol	1	1	9	0	4.198	142.583	142.019	1.334	23.632	1
4-Methylbenzylamine	1	2	9	3	3.920	121.180	121.089	1.393	28.419	1
4-nitroaniline	2	2	10	6	1.521	138.124	138.043	1.293	66.784	1
Acebutolol	5	3	24	12	2.165	336.426	336.205	1.803	89.640	1
Acetonitrile	0	0	3	0	2.284	41.052	41.027	1.130	18.823	0
Acetophenone	1	0	9	3	2.481	120.149	120.058	1.291	17.563	1
Acetylsalicylic acid	4	1	13	6	1.562	180.157	180.042	1.414	64.673	1
Acridine	1	0	14	0	1.463	179.217	179.074	1.372	10.623	3
Alprazolam	3	0	22	3	2.690	308.765	308.083	1.550	38.391	4
Alprenolol	3	2	18	9	2.949	249.349	249.173	1.707	45.115	1

Aminophenazone	1	0	17	18	1.056	231.294	231.137	1.579	27.656	2
Amitriptyline	1	0	21	9	2.280	277.403	277.183	1.641	4.845	3
Amlodipine	6	3	28	24	1.425	408.876	408.145	1.831	104.557	2
Amoxicillin	6	5	25	18	2.713	365.404	365.105	1.718	165.880	3
Aniline	0	2	7	3	2.953	93.126	93.058	1.225	27.041	1
Anisole	1	0	8	0	2.039	108.138	108.058	1.273	11.291	1
Atenolol	4	4	19	9	1.954	266.336	266.163	1.713	91.279	1
Benzene	0	0	6	0	0.000	78.112	78.047	1.192	0.000	1
Benzyl cyanide	0	0	9	0	3.918	117.148	117.058	1.313	18.636	1
Benzylalcohol	1	1	8	0	3.254	108.138	108.058	1.317	23.453	1
Benzylamine	1	2	8	3	3.276	107.153	107.074	1.337	29.053	1
Benzylbenzoate	2	0	16	3	2.381	212.244	212.084	1.499	26.345	2
Benzylmethylketon	1	0	10	3	4.521	134.175	134.073	1.405	17.743	1
Betahistine	2	1	10	3	1.921	136.194	136.100	1.423	25.820	1
Betaxolol	4	2	22	3	1.960	307.428	307.215	1.821	59.091	2
Bibenzyl	0	0	14	0	0.000	182.261	182.110	1.504	0.000	2
Biperidene	2	1	23	9	1.056	311.461	311.225	1.676	23.479	4
Biphenyl	0	0	12	0	0.000	154.208	154.078	1.358	0.000	2
Bromazepam	3	1	19	9	2.881	316.153	315.001	1.501	50.198	3
Bromperidol	3	1	26	6	0.957	420.315	419.090	1.739	39.866	3
Bupivacaine	2	1	21	9	1.178	288.428	288.220	1.731	33.276	2
Buprenorphine	5	2	34	3	1.397	467.640	467.304	1.810	61.771	7
Butylacetate	2	1	8	3	3.352	116.158	116.084	1.436	40.323	0
Caffeine	3	0	14	12	0.293	194.191	194.080	1.430	54.408	2
Carbamazepine	1	2	18	15	3.044	236.269	236.095	1.442	44.516	3
Carbamazepine epoxide	2	2	19	9	2.483	252.268	252.090	1.471	57.360	4

Carbon tetrachloride	0	0	5	0	0.000	153.823	151.875	1.241	0.000	0
Cephalexin	5	4	24	24	2.006	347.389	347.094	1.681	139.796	3
Chlorambucil	2	1	19	6	4.531	304.212	303.079	1.707	44.557	1
Chloroform	0	0	4	0	0.456	119.378	117.914	1.191	0.000	0
Chlorpromazine	1	0	21	6	2.066	318.864	318.096	1.646	30.317	3
Cimetidine	2	3	17	9	2.282	252.339	252.116	1.638	101.577	1
Cinoxacin	6	1	19	12	1.686	262.218	262.059	1.512	94.510	3
Ciprofloxacin	4	2	24	21	2.275	331.342	331.133	1.654	79.938	4
Clobazam	2	0	21	12	3.156	300.740	300.067	1.561	39.090	3
Clonidine	1	2	14	9	2.219	230.094	229.017	1.501	41.987	2
Clorazepate	4	2	22	12	3.666	314.723	314.046	1.573	79.775	3
Codeine	4	1	22	9	1.633	299.364	299.152	1.561	47.083	5
Cotinine	2	0	13	6	1.718	176.215	176.095	1.439	32.071	2
Delorazepam	2	1	20	9	3.353	305.159	304.017	1.536	42.804	3
Desipramine	1	1	20	6	2.727	266.381	266.178	1.640	18.291	3
Dextromethorphan	2	0	20	3	2.116	271.397	271.194	1.594	15.763	4
Diazepam	2	0	20	9	2.904	284.740	284.072	1.566	31.368	3
Dichloromethane	0	0	3	0	0.450	84.933	83.953	1.163	0.000	0
Diclofenac	2	2	19	6	3.353	296.149	295.017	1.573	49.126	2
Diethylether	1	0	5	0	0.294	74.122	74.073	1.329	10.574	0
Diflunisal	3	2	18	3	5.064	250.198	250.044	1.483	59.232	2
Diltiazem	5	0	29	12	1.512	414.518	414.161	1.827	84.625	3
Diphenhydramine	2	0	19	3	2.758	255.355	255.162	1.660	15.089	2
Dipropyl ether	1	0	7	0	0.494	102.175	102.105	1.441	10.574	0
Domperidone	3	2	30	21	2.269	425.911	425.162	1.790	75.981	5
Epinephrine	4	4	13	3	0.932	183.204	183.090	1.489	83.115	1

Ethanol	1	1	3	0	1.924	46.068	46.042	1.190	23.541	0
Ethylacetate	2	0	6	3	1.902	88.105	88.052	1.320	27.600	0
Ethylbenzoate	2	0	11	3	1.703	150.175	150.068	1.400	26.704	1
Etidocaine	2	1	20	9	0.798	276.417	276.220	1.737	31.011	1
Felodipine	4	1	25	21	1.621	384.254	383.069	1.742	66.691	2
Fenbufen	3	1	19	6	4.625	254.281	254.094	1.594	56.676	2
Flufenamic Acid	2	2	20	6	3.869	281.230	281.066	1.546	49.957	2
Flumazenil	4	0	22	9	1.379	303.288	303.102	1.595	61.799	3
Flumequine	3	1	19	15	3.099	261.248	261.080	1.502	58.866	3
Fluphenazine	3	1	30	9	4.680	437.522	437.175	1.801	57.914	4
Flurazepam	3	0	27	12	1.878	387.878	387.151	1.752	33.798	3
Flurbiprofen	2	1	18	3	4.399	244.261	244.090	1.559	39.941	2
Fluvastatin	4	3	30	9	3.326	411.466	411.185	1.833	86.725	3
Furosemide	6	4	21	9	3.120	330.744	330.008	1.607	127.354	2
GEA 968	3	2	21	15	2.090	291.389	291.195	1.747	60.758	1
Granisetron	3	1	23	9	0.520	312.409	312.195	1.662	48.817	4
Griseofulvin	6	0	24	12	2.445	352.766	352.071	1.699	80.290	3
Haloperidol	3	1	26	6	0.739	375.864	375.140	1.740	39.694	3
Heptane	0	0	7	0	0.006	100.202	100.125	1.469	0.000	0
Hexobarbital	3	1	17	21	1.180	236.267	236.116	1.541	68.332	2
Hydrochlorothiazide	6	4	17	9	2.171	297.739	296.965	1.519	141.531	2
Hydrocortisone	5	3	26	12	1.641	362.460	362.209	1.683	96.350	4
Hydroxyzine	4	1	26	6	3.732	374.904	374.176	1.773	42.391	3
Ibuprofen	2	1	15	3	3.667	206.281	206.131	1.583	40.120	1
Imipramine	1	0	21	6	2.297	280.407	280.194	1.670	7.454	3
Indomethacin	4	1	25	6	2.647	357.788	357.077	1.699	71.195	3

Indoprofen	3	1	21	9	1.062	281.306	281.105	1.602	59.537	3
Isosorbide dinitrate	10	0	16	6	0.061	236.136	236.028	1.495	129.000	2
Isotretinoin	2	1	22	33	3.045	300.435	300.209	1.774	39.224	1
Isradipine	7	1	27	21	1.229	371.387	371.148	1.739	103.147	3
Ketamine	2	1	16	6	2.016	237.725	237.092	1.496	30.277	2
Ketoprofen	3	1	19	6	0.495	254.281	254.094	1.559	56.720	2
Labetalol	4	5	24	9	2.517	328.406	328.179	1.771	98.458	2
Lacidipine	6	1	33	30	1.801	455.543	455.231	1.935	91.954	2
Levosulpiride	6	3	23	12	2.035	341.426	341.141	1.730	108.421	2
Lidocaine	2	1	17	9	1.635	234.337	234.173	1.662	32.910	1
Loratadine	3	0	27	12	2.095	382.883	382.145	1.721	40.473	4
Lorazepam	3	2	21	9	4.278	321.158	320.012	1.546	63.414	3
Mefenamic Acid	2	2	18	6	3.457	241.285	241.110	1.532	48.132	2
Mepivacaine	2	1	18	9	1.808	246.348	246.173	1.622	33.947	2
Mepyramine	3	0	21	6	1.939	285.384	285.184	1.716	28.622	2
Mesitylene	0	0	9	0	0.013	120.192	120.094	1.390	0.000	1
Metadone	2	0	23	6	1.708	309.445	309.209	1.720	18.947	2
Methohexital	3	1	19	21	1.883	262.304	262.132	1.618	66.458	1
Methylacetate	2	1	5	3	1.470	74.079	74.037	1.241	39.762	0
Methylsulfoxide	1	0	4	3	1.153	78.133	78.014	1.236	33.001	0
Metoclopramide	3	3	20	12	0.488	299.796	299.140	1.723	67.504	1
Metoprolol	4	2	19	3	1.703	267.364	267.183	1.743	56.712	1
Mianserin	1	0	20	6	2.657	264.365	264.163	1.570	8.386	4
Midazolam	5	0	33	3	1.877	454.602	454.283	1.994	67.541	2
Morphine	4	2	21	9	1.622	285.338	285.137	1.504	58.014	5
N,N-Dimethylaniline	0	0	9	3	1.380	121.180	121.089	1.348	3.355	1

N,N-Dimethyl-p-toluidine	0	0	10	3	2.023	135.206	135.105	1.411	3.727	1
Nadolol	5	4	22	3	0.383	309.401	309.194	1.720	86.831	2
Nalidixic acid	4	1	17	15	2.908	232.235	232.085	1.525	69.370	2
Naphtalene	0	0	10	0	0.000	128.171	128.063	1.298	0.000	2
Naproxen	3	1	17	3	2.364	230.259	230.094	1.542	51.702	2
Nebivolol	5	3	29	3	1.818	405.435	405.175	1.785	79.261	4
N-Ethylaniline	0	1	9	3	1.187	121.180	121.089	1.368	13.596	1
Nicardipine	7	1	35	27	0.762	479.525	479.206	1.925	111.876	3
Nicotinamide	2	2	9	6	2.190	122.125	122.048	1.260	53.111	1
Nicotine	2	0	12	3	0.869	162.232	162.116	1.433	15.282	2
Nifedipine	6	1	25	24	0.973	346.335	346.117	1.691	102.261	2
Nimodipine	7	1	30	24	0.821	418.440	418.174	1.854	116.342	2
Nisoldipine	6	1	28	24	1.759	388.414	388.163	1.782	102.042	2
Nitrendipine	6	1	26	24	1.279	360.361	360.132	1.741	107.433	2
Nitrobenzene	2	0	9	3	2.277	123.109	123.032	1.241	39.743	1
N-Methylbenzylamine	1	1	9	3	2.214	121.180	121.089	1.367	16.167	1
N-methylnaphthalen-1-amine	0	1	12	3	2.421	157.212	157.089	1.355	13.038	2
N-Methylphenethylamine	1	1	10	3	3.124	135.206	135.105	1.432	16.130	1
Norfloxacin	4	2	23	21	2.221	319.331	319.133	1.643	78.460	3
N-pentane	0	0	5	0	0.007	72.149	72.094	1.362	0.000	0
N-propanol	1	1	4	0	2.635	60.095	60.058	1.243	23.497	0
Ofloxacin	5	1	26	21	0.802	361.368	361.144	1.686	78.438	4
Ondansetron	2	0	22	3	1.511	293.363	293.153	1.633	34.723	4
Oxazepam	3	2	20	9	4.242	286.713	286.051	1.522	63.167	3
Oxolinic acid	5	1	19	15	1.888	261.230	261.064	1.501	82.283	3
Oxprenolol	4	2	19	9	2.371	265.348	265.168	1.721	57.488	1

Paracetamol	2	2	11	6	1.590	151.163	151.063	1.394	54.207	1
Pentamethylbenzene	0	0	11	0	0.007	148.245	148.125	1.451	0.000	1
Phenazone	1	0	14	15	1.860	188.226	188.095	1.478	24.824	2
Phenobarbital	3	2	17	15	1.444	232.235	232.085	1.481	81.506	2
Phenol	1	1	7	0	2.996	94.111	94.042	1.203	22.580	1
Phenylbutazone	2	0	23	12	1.684	308.374	308.153	1.699	42.775	3
Phenylpropanolamine	2	3	11	3	3.578	151.206	151.100	1.426	48.598	1
Phenytoin	2	2	19	12	2.652	252.268	252.090	1.505	65.254	3
Physostigmine	3	1	20	12	0.740	275.346	275.163	1.645	49.878	3
Pindolol	3	3	18	3	2.942	248.321	248.153	1.662	63.356	2
Pipemidic acid	6	2	22	21	2.032	303.317	303.133	1.635	97.173	3
Piromidic acid	5	1	21	18	3.637	288.302	288.122	1.630	82.534	3
Piroxicam	5	2	23	15	0.351	331.346	331.063	1.589	101.706	3
P-Nitroaniline	2	2	10	6	1.521	138.124	138.043	1.293	66.784	1
Prilocaine	2	2	16	9	2.192	220.311	220.158	1.628	43.561	1
Procaine	3	2	17	9	0.803	236.310	236.153	1.659	59.285	1
Progesterone	2	0	23	12	1.116	314.462	314.225	1.669	34.768	4
Promazine	1	0	20	6	1.773	284.419	284.135	1.615	29.684	3
Promethazine	1	0	20	6	2.490	284.419	284.135	1.600	30.009	3
Propionitrile	0	0	4	0	2.770	55.079	55.042	1.180	18.450	0
Propiophenone	1	0	10	3	1.804	134.175	134.073	1.371	16.488	1
Propofol	1	1	13	0	2.200	178.271	178.136	1.540	21.526	1
Propranolol	3	2	19	3	3.278	259.343	259.157	1.664	45.741	2
P-toluidine	0	2	8	3	3.657	107.153	107.074	1.303	26.818	1
Pyridine	1	0	6	0	1.646	79.100	79.042	1.156	10.809	1
Ranitidine	4	2	21	18	1.379	314.404	314.141	1.754	100.735	1

Risperidone	5	0	30	18	2.093	410.485	410.212	1.799	58.550	5
Rufloxacin	4	1	25	21	0.855	363.407	363.105	1.664	88.200	4
Salicylic acid	3	2	10	3	2.591	138.121	138.032	1.299	61.466	1
Sotalol	4	3	18	6	1.965	272.364	272.120	1.673	91.657	1
Sulindac	3	1	25	18	1.557	356.411	356.088	1.701	72.663	3
Temazepam	3	1	21	9	3.686	300.740	300.067	1.568	51.217	3
Terbutaline	4	4	16	3	2.075	225.284	225.137	1.604	81.731	1
Tert- butyl alcohol	1	1	5	0	1.873	74.122	74.073	1.299	22.489	0
Tetracaine	3	1	19	9	3.846	264.363	264.184	1.737	46.667	1
Tetrachloro ethane	0	0	6	0	0.432	167.849	165.891	1.291	0.000	0
Tetrahydrofurane	1	0	5	0	1.568	72.106	72.058	1.207	11.112	1
Theobromine	3	1	13	12	0.536	180.164	180.065	1.397	67.976	2
Theophylline	3	1	13	12	0.203	180.164	180.065	1.389	67.548	2
Thiopental	2	2	16	15	1.594	242.338	242.109	1.567	88.009	1
Timolol	6	2	21	6	1.598	316.420	316.157	1.731	107.664	2
Tocainide	2	3	14	9	3.577	192.258	192.126	1.530	57.298	1
Tolfenamic acid	2	2	18	6	3.622	261.704	261.056	1.513	47.669	2
Tolmetin	3	1	19	6	2.249	257.285	257.105	1.599	59.604	2
Toluene	0	0	7	0	0.017	92.138	92.063	1.254	0.000	1
Tramadol	3	1	19	3	1.450	263.375	263.189	1.654	35.320	2
Trimecaine	2	1	18	9	2.141	248.364	248.189	1.692	33.634	1
Tropisetron	3	1	21	6	1.851	284.353	284.153	1.606	46.432	4
Verapamil	5	0	33	3	1.877	454.602	454.283	1.994	67.541	2
W 36017	2	1	15	9	2.340	206.284	206.142	1.586	34.089	1

Table 2B. Number of H-bond acceptor group, H-bond donor group, of heavy atoms, of impropers, lipole, mass, monoisotopic mass, ovality, polar surface area (Psa) and number of rings for the whole dataset of the analytes assumed as neutral.

Analyte	Sas	Sav	Sdiam	Surface	Torsions	Vdiam	VirtualLogP	Volume
1,2,4,5-tetrachlorobenzene	339.068	497.136	7.542	178.705	6	6.490	5.471	143.103
1,2 -dichloroethane	233.344	305.700	5.761	104.280	1	5.199	1.995	73.599
1,3-dichlorobenzene	289.864	409.540	6.821	146.177	6	6.017	3.766	114.083
1-chloro butane	282.837	383.603	6.528	133.863	2	5.657	2.926	94.774
1-chloro-2-nitrobenzene	293.357	421.938	6.962	152.254	7	6.135	3.409	120.925
1-hexanol	336.936	476.368	7.418	172.874	5	6.161	2.493	122.440
1-Naphthylamine	318.338	471.535	7.346	169.541	12	6.428	2.139	139.082
1-nitrobutane	297.846	410.190	6.710	141.449	3	5.787	2.372	101.499
1-pentanol	303.155	417.441	6.883	148.853	4	5.851	1.982	104.881
2-Aminobiphenyl	381.088	573.543	8.047	203.444	14	6.831	2.827	166.876
2-Chloroaniline	286.895	402.690	6.642	138.582	7	5.911	1.898	108.157
2-methyl-2 butanol	272.901	387.763	6.867	148.135	0	5.877	1.419	106.298
2-phenylethyl acetate	387.608	581.301	8.193	210.906	10	6.777	2.464	162.994
2-Phenylethylamine	325.330	470.396	7.483	175.927	9	6.304	1.631	131.167
3-chloro phenol	281.257	393.474	6.561	135.250	7	5.864	2.349	105.578
4-chlorobenzylalcohol	314.148	449.687	7.138	160.065	8	6.180	2.021	123.591
4-Methylbenzylamine	334.303	478.098	7.444	174.097	8	6.308	1.750	131.406
4-nitroaniline	299.201	426.160	6.849	147.379	8	6.022	1.690	114.364
Acebutolol	628.987	1056.390	11.532	417.796	18	8.587	2.489	331.573
Acetonitrile	189.979	225.452	4.730	70.297	0	4.451	0.453	46.165
Acetophenone	303.809	434.254	6.924	150.614	7	6.093	1.407	118.452
Acetylsalicylic acid	366.645	545.633	7.983	200.213	10	6.714	1.209	158.444
Acridine	364.759	548.793	8.039	203.048	16	6.864	3.763	169.365
Alprazolam	524.471	844.911	9.880	306.655	23	7.937	3.815	261.769
Alprenolol	501.907	823.323	10.360	337.180	16	7.930	3.404	261.133

Aminophenazone	469.880	742.408	9.455	280.828	13	7.525	1.531	223.113
Amitriptyline	536.034	891.070	10.480	345.057	21	8.181	3.977	286.662
Amlodipine	642.016	1119.587	12.020	453.907	23	8.884	2.022	367.094
Amoxicillin	553.543	948.012	11.039	382.825	22	8.422	-1.182	312.797
Aniline	261.555	358.708	6.216	121.394	7	5.615	1.137	92.710
Anisole	282.913	397.347	6.686	140.418	7	5.926	2.188	108.959
Atenolol	526.045	850.636	10.399	339.712	16	7.945	1.351	262.552
Benzene	238.870	321.014	5.951	111.247	6	5.450	2.136	84.761
Benzyl cyanide	316.263	444.275	6.965	152.385	8	6.078	1.977	117.558
Benzylalcohol	286.366	403.429	6.843	147.107	8	5.962	1.325	110.960
Benzylamine	297.084	418.722	6.957	152.035	8	6.016	1.203	114.004
Benzylbenzoate	453.976	686.551	8.882	247.834	16	7.254	3.197	199.839
Benzylmethylketon	343.545	497.411	7.623	182.562	9	6.431	2.235	139.249
Betahistine	360.361	521.195	7.716	187.021	9	6.468	1.023	141.691
Betaxolol	607.994	1041.480	11.398	408.148	21	8.448	3.508	315.639
Bibenzyl	434.540	661.026	8.751	240.598	15	7.135	4.527	190.167
Biperidene	589.294	988.409	10.976	378.465	22	8.479	3.227	319.182
Biphenyl	354.685	527.046	7.759	189.150	13	6.658	3.564	154.549
Bromazepam	477.100	745.741	9.284	270.782	19	7.578	1.988	227.888
Bromperidol	650.724	1075.771	11.500	415.502	25	8.720	3.926	347.153
Bupivacaine	568.819	958.634	10.988	379.304	18	8.353	4.487	305.124
Buprenorphine	686.412	1270.715	12.869	520.269	32	9.566	4.249	458.298
Butylacetate	335.005	473.734	7.427	173.281	5	6.197	2.211	124.618
Caffeine	376.477	573.756	8.137	208.022	10	6.805	-0.226	164.995
Carbamazepine	426.195	677.095	8.843	245.662	19	7.364	2.141	209.112
Carbamazepine epoxide	439.091	702.241	9.108	260.631	21	7.509	1.392	221.680

Carbon tetrachloride	247.840	334.073	6.089	116.494	0	5.467	2.161	85.565
Cephalexin	569.786	939.376	10.722	361.144	22	8.270	-0.196	296.181
Chlorambucil	552.137	880.957	10.480	345.039	16	8.021	4.481	270.165
Chloroform	226.795	294.540	5.591	98.213	0	5.124	2.384	70.424
Chlorpromazine	528.141	888.982	10.506	346.739	20	8.188	5.257	287.393
Cimetidine	517.315	804.739	9.755	298.934	14	7.622	0.487	231.829
Cinoxacin	459.429	715.309	9.094	259.812	18	7.396	-0.860	211.822
Ciprofloxacin	550.661	911.519	10.589	352.264	24	8.233	0.929	292.209
Clobazam	504.634	824.067	9.813	302.530	19	7.855	2.853	253.804
Clonidine	414.840	626.734	8.625	233.698	13	7.041	2.515	182.751
Clorazepate	517.258	827.664	9.897	307.714	21	7.891	2.127	257.243
Codeine	496.723	839.244	10.093	320.010	24	8.077	1.303	275.892
Cotinine	374.993	571.209	8.199	211.183	12	6.834	0.773	167.129
Delorazepam	486.159	781.072	9.634	291.569	19	7.774	3.135	245.965
Desipramine	525.569	856.343	10.292	332.776	21	8.037	3.396	271.813
Dextromethorphan	505.824	850.023	10.195	326.553	21	8.076	3.712	275.795
Diazepam	493.368	783.481	9.766	299.633	19	7.805	2.474	248.944
Dichloromethane	203.406	252.972	5.149	83.293	0	4.775	1.511	57.010
Diclofenac	475.438	769.295	9.654	292.811	17	7.697	4.390	238.760
Diethylether	282.603	374.991	6.360	127.082	2	5.517	1.666	87.917
Diflunisal	434.503	667.481	8.882	247.834	16	7.293	3.167	203.101
Diltiazem	699.328	1191.370	12.103	460.172	25	8.955	3.704	375.963
Diphenhydramine	530.984	855.802	10.248	329.917	18	7.955	3.545	263.558
Dipropyl ether	344.205	478.414	7.400	172.027	4	6.165	2.689	122.661
Domperidone	687.106	1157.061	11.915	445.995	31	8.905	2.469	369.720
Epinephrine	397.881	600.003	8.439	223.755	12	6.916	-0.242	173.215

Ethanol	202.585	249.582	5.130	82.693	1	4.704	0.392	54.485
Ethylacetate	277.992	371.953	6.395	128.471	2	5.566	1.182	90.304
Ethylbenzoate	355.006	516.655	7.746	188.501	9	6.546	2.218	146.857
Etidocaine	535.382	910.628	10.929	375.257	14	8.292	4.281	298.540
Felodipine	579.794	992.879	11.260	398.336	18	8.532	3.798	325.194
Fenbufen	507.905	793.508	9.693	295.174	18	7.677	2.632	236.944
Flufenamic Acid	471.459	740.095	9.418	278.640	16	7.574	3.864	227.485
Flumazenil	521.286	836.252	9.966	312.045	19	7.891	2.494	257.307
Flumequine	443.539	704.969	9.210	266.478	17	7.514	1.221	222.109
Fluphenazine	724.740	1206.980	12.105	460.303	29	9.018	4.311	384.061
Flurazepam	644.044	1081.240	11.572	420.672	24	8.743	3.593	349.879
Flurbiprofen	477.927	747.052	9.393	277.176	14	7.522	3.876	222.836
Fluvastatin	637.792	1133.169	12.195	467.190	28	9.007	3.505	382.564
Furosemide	524.527	830.298	9.912	308.645	18	7.820	2.214	250.396
GEA 968	598.362	967.435	10.932	375.414	15	8.271	2.922	296.307
Granisetron	580.568	955.881	10.705	360.003	23	8.303	2.270	299.723
Griseofulvin	553.771	924.759	10.786	365.457	19	8.275	2.579	296.656
Haloperidol	642.888	1063.298	11.453	412.095	25	8.682	3.718	342.706
Heptane	355.619	505.527	7.616	182.245	4	6.284	4.378	129.944
Hexobarbital	435.250	700.383	9.269	269.887	13	7.466	1.537	217.860
Hydrochlorothiazide	427.153	671.520	9.047	257.116	13	7.341	-0.124	207.103
Hydrocortisone	566.319	999.508	11.335	403.624	25	8.738	0.020	349.305
Hydroxyzine	674.079	1120.613	11.669	427.789	27	8.764	2.881	352.477
Ibuprofen	444.487	702.035	9.343	274.233	8	7.425	3.275	214.353
Imipramine	556.111	916.788	10.592	352.456	21	8.197	4.102	288.423
Indomethacin	596.099	974.884	10.905	373.586	22	8.366	4.226	306.605

Indoprofen	524.300	831.793	9.960	311.677	18	7.871	1.974	255.281
Isosorbide dinitrate	388.181	593.259	8.481	225.953	13	6.936	0.274	174.717
Isotretinoin	632.997	1034.947	11.285	400.070	16	8.473	3.164	318.495
Isradipine	557.847	986.468	11.319	402.534	22	8.583	3.174	331.076
Ketamine	431.511	700.033	9.215	266.744	14	7.535	3.366	223.983
Ketoprofen	483.402	769.522	9.573	287.932	15	7.667	2.240	235.964
Labetalol	568.656	983.147	11.331	403.359	23	8.514	1.588	323.138
Lacidipine	709.027	1288.342	13.099	539.074	23	9.418	3.536	437.389
Levosulpiride	595.146	979.867	10.984	378.998	19	8.350	1.120	304.878
Lidocaine	487.869	795.872	10.042	316.794	12	7.790	2.913	247.485
Loratadine	646.566	1086.045	11.417	409.490	27	8.703	5.411	345.160
Lorazepam	506.532	813.816	9.723	296.968	20	7.820	2.466	250.417
Mefenamic Acid	474.051	746.581	9.369	275.760	16	7.569	3.711	227.028
Mepivacaine	487.021	801.421	9.987	313.341	15	7.842	2.969	252.552
Mepyramine	583.099	946.007	10.741	362.449	19	8.199	3.509	288.552
Mesitylene	330.491	478.400	7.508	177.091	6	6.369	3.359	135.268
Metadone	558.553	975.645	11.156	391.003	19	8.506	4.224	322.191
Methohexital	494.232	806.815	9.953	311.234	11	7.826	2.921	250.972
Methylacetate	235.127	306.768	5.771	104.628	2	5.180	0.760	72.765
Methylsulfoxide	232.052	302.312	5.760	104.231	0	5.180	-0.656	72.792
Metoclopramide	562.683	914.262	10.609	353.602	15	8.083	1.955	276.505
Metoprolol	548.905	895.452	10.699	359.594	16	8.103	2.760	278.612
Mianserin	495.791	814.730	9.984	313.148	22	7.968	2.054	264.854
Midazolam	786.785	1394.667	13.538	575.795	25	9.588	4.414	461.535
Morphine	459.793	775.245	9.691	295.045	24	7.903	0.776	258.460
N,N-Dimethylaniline	322.036	463.937	7.296	167.253	7	6.284	2.952	129.933

N,N-Dimethyl-p-toluidine	355.154	520.383	7.747	188.528	7	6.521	3.492	145.207
Nadolol	543.248	930.603	10.955	377.055	19	8.353	1.772	305.191
Nalidixic acid	440.660	684.286	9.059	257.818	14	7.335	0.864	206.633
Naphtalene	304.181	442.172	7.183	162.078	11	6.305	3.107	131.266
Naproxen	464.347	721.721	9.211	266.540	13	7.417	3.135	213.620
Nebivolol	690.973	1142.616	11.786	436.434	30	8.822	3.535	359.516
N-Ethylaniline	322.649	464.609	7.337	169.115	8	6.274	2.746	129.299
Nicardipine	681.867	1265.657	13.098	538.936	29	9.440	2.860	440.416
Nicotinamide	285.164	399.912	6.646	138.753	8	5.919	-0.178	108.598
Nicotine	369.407	562.042	8.148	208.573	12	6.807	1.488	165.126
Nifedipine	541.248	920.834	10.874	371.470	18	8.362	2.503	306.116
Nimodipine	630.865	1128.337	12.286	474.210	22	9.023	3.634	384.661
Nisoldipine	607.505	1066.060	11.701	430.150	19	8.766	3.398	352.671
Nitrendipine	592.077	1005.095	11.195	393.731	19	8.486	3.234	319.920
Nitrobenzene	277.564	388.778	6.500	132.752	7	5.836	2.663	104.081
N-Methylbenzylamine	334.584	479.518	7.373	170.792	8	6.306	1.417	131.273
N-methylnaphthalen-1-amine	354.538	528.486	7.767	189.526	12	6.672	2.954	155.504
N-Methylphenethylamine	361.668	527.601	7.813	191.763	9	6.529	1.802	145.746
Norfloxacin	546.259	887.328	10.435	342.094	21	8.141	1.038	282.536
N-pentane	288.885	393.596	6.649	138.869	2	5.698	3.328	96.850
N-propanol	236.945	308.452	5.716	102.654	2	5.128	0.966	70.603
Ofloxacin	574.024	958.319	10.997	379.922	24	8.469	-0.386	318.083
Ondansetron	524.262	867.088	10.320	334.575	22	8.075	2.708	275.692
Oxazepam	491.667	777.945	9.456	280.896	20	7.664	1.924	235.710
Oxolinic acid	449.230	707.890	9.111	260.787	18	7.437	-0.123	215.339
Oxprenolol	516.292	861.378	10.521	347.755	17	8.020	3.094	270.053

Paracetamol	351.943	510.234	7.593	181.118	9	6.431	1.166	139.239
Pentamethylbenzene	366.534	558.541	8.275	215.100	6	6.869	4.193	169.671
Phenazone	402.350	609.769	8.513	227.691	12	7.003	1.267	179.861
Phenobarbital	413.526	655.278	8.885	248.016	14	7.301	1.464	203.810
Phenol	252.338	344.550	6.115	117.493	7	5.576	1.587	90.775
Phenylbutazone	556.565	921.282	10.749	362.997	22	8.246	2.606	293.631
Phenylpropanolamine	358.770	539.791	7.974	199.745	10	6.677	0.668	155.850
Phenytoin	466.554	734.585	9.224	267.299	19	7.519	2.389	222.596
Physostigmine	524.030	846.519	10.205	327.176	17	7.958	1.794	263.833
Pindolol	476.886	778.145	9.981	312.942	17	7.742	2.765	242.952
Pipemidic acid	543.941	877.371	10.212	327.652	21	7.986	-0.021	266.700
Piromidic acid	523.997	843.431	10.049	317.267	20	7.870	1.252	255.252
Piroxicam	539.356	870.094	10.101	320.533	21	8.012	1.538	269.281
P-Nitroaniline	299.201	426.160	6.849	147.379	8	6.022	1.690	114.364
Prilocaine	498.126	775.485	9.676	294.131	12	7.584	2.710	228.422
Procaine	511.019	804.766	9.912	308.625	14	7.695	1.550	238.565
Progesterone	545.801	956.214	11.004	380.400	21	8.518	2.863	323.648
Promazine	486.068	821.801	10.210	327.503	20	8.034	4.377	271.500
Promethazine	505.098	840.821	10.177	325.369	19	8.044	4.188	272.558
Propionitrile	222.842	280.160	5.307	88.475	1	4.886	0.978	61.081
Propiophenone	330.562	483.040	7.500	176.719	8	6.405	1.877	137.590
Propofol	409.092	640.123	9.011	255.084	7	7.262	3.689	200.488
Propranolol	502.395	826.250	10.207	327.324	18	7.913	3.350	259.463
P-toluidine	295.583	420.383	6.772	144.090	7	5.933	1.713	109.327
Pyridine	237.209	313.736	5.692	101.800	6	5.295	1.216	77.712
Ranitidine	604.714	967.288	10.860	370.523	16	8.199	2.125	288.640

Risperidone	693.891	1159.206	11.999	452.331	31	8.947	3.187	374.975
Rufloxacin	571.755	948.658	10.778	364.973	24	8.357	-0.426	305.570
Salicylic acid	305.497	434.788	6.962	152.260	9	6.109	0.922	119.384
Sotalol	523.315	827.837	10.146	323.423	13	7.844	1.740	252.700
Sulindac	602.524	983.953	10.981	378.846	22	8.420	1.105	312.607
Temazepam	517.802	833.083	9.858	305.284	20	7.872	1.887	255.407
Terbutaline	464.060	727.977	9.558	286.990	12	7.547	2.154	225.084
Tert- butyl alcohol	251.506	345.029	6.263	123.223	0	5.495	1.004	86.862
Tetracaine	586.378	926.603	10.589	352.237	15	8.033	3.429	271.442
Tetrachloro ethane	272.457	382.912	6.590	136.434	0	5.799	2.499	102.103
Tetrahydrofurane	233.841	310.641	5.791	105.372	5	5.271	1.082	76.662
Theobromine	349.591	520.553	7.783	190.289	10	6.585	-0.734	149.480
Theophylline	350.772	520.876	7.756	188.968	10	6.581	-0.282	149.238
Thiopental	439.711	708.843	9.445	280.242	8	7.544	2.523	224.827
Timolol	529.549	898.190	10.859	370.449	18	8.253	2.061	294.303
Tocainide	419.236	657.869	8.892	248.378	10	7.188	1.537	194.477
Tolfenamic acid	467.485	736.157	9.307	272.128	16	7.566	3.945	226.771
Tolmetin	494.628	775.034	9.742	298.185	16	7.704	2.083	239.408
Toluene	280.434	388.130	6.414	129.236	6	5.727	2.784	98.337
Tramadol	526.749	870.264	10.344	336.162	17	8.043	2.716	272.423
Trimecaine	520.682	849.338	10.336	335.615	12	7.945	3.491	262.602
Tropisetron	539.699	866.466	10.102	320.622	22	7.971	3.236	265.156
Verapamil	786.785	1394.667	13.538	575.795	25	9.588	4.414	461.535
W 36017	448.665	707.574	9.301	271.793	10	7.386	2.241	210.996

Table 2C. Surface accessible to solvent, volume accessible to solvent, superficial diameter, surface, number of torsions, volume diameter, VirtualLogP and volume for the whole dataset of the analytes assumed as neutral.

Since these models were generated by taking into account the neutral properties of the analytes, VirtualLog P values can be assumed as reasonable estimates of log P^N values. It is interesting to note how in these models, the number of HeavyAtoms, but not the molecular mass, is included. According to this highly predictive model, the retention on IAM.PC.MG stationary phase would be enhanced for highly lipophilic analytes and hindered for flexible molecules.

Taking into account ionization, when applicable, for the same set of 205 compounds, the best models (equations (3) and (4)) were based on the following properties: gyration radius, VirtualLog P, number of atoms, number of torsions. The molecular properties of the analytes assumed, when applicable, as entirely charged are reported in Table 3.

$$\log k_w^{IAM.MG} = -0.6165 + 0.0416\ \text{Torsions} + 0.0179\ \text{Atoms} + 0.1212\ \text{Gyrrad} + 0.3219\ \text{VirtualLogP} \quad (3)$$

n= 205 r^2 = 0.69 q^2 = 0.67 SE= 0.562 F= 110.52 $F\alpha$ 0.001= 19.98 PC=65.459

Best optimized model (n-1):

$$\log k_w^{IAM.MG} = -0.6154 + 0.0455\ \text{Torsions} + 0.0165\ \text{Atoms} + 0.1174\ \text{Gyrrad} + 0.3270\ \text{VirtualLogP} \quad (4)$$

n= 204 r^2 = 0.70 SE= 0.556 F= 114.38 $F\alpha$ 0.001= 19.98 PC=63.711 ExRow: alprazolam

Taking into account ionization markedly worsened the relationships. This evidence may be attributed to the electric shielding effects of the charged phospholipid heads that could, in part, act by neutralizing the electric charges supported on ionizable analytes. In this case, the number of torsions but not of flexible torsions, as reported in equations (1) and (2), are found as directly related to phospholipophilicity, as measured on IAM.PC.MG stationary phase. It should be also pointed out that Gyrrad is a parameter, in a large extent, related to molecule flexibility.

According to analyte's pKa, a weighted average of the properties of neutral and charged forms at pH 7.0 (i.e. the experimental pH of log $k_w^{IAM.MG}$ determinations) was performed. The results are reported in Table 4. The best models (equations (5) and (6)) for the relationships with log $k_w^{IAM.MG}$ were based on the following four properties: VirtuallogP, number of heavy atoms, gyration radius and flexible torsions.

Analyte	Angles	Atoms	Bonds	Charge	ChiralAtms	Dipole	EzBnds	FlexTorsions	Gyrrad
1,2,4,5-tetrachlorobenzene	18	12	12	0	0	0.001	0	0	2.721
1,2 -dichloroethane	12	8	7	0	0	2.035	0	0	1.664
1,3-dichlorobenzene	18	12	12	0	0	1.195	0	0	2.337
1-chloro butane	24	14	13	0	0	1.339	0	1	2.106
1-chloro-2-nitrobenzene	21	14	14	0	0	3.217	0	0	2.185
1-hexanol	37	21	20	0	0	1.701	0	4	2.666
1-Naphthylamine	33	20	21	0	0	0.252	0	0	2.227
1-nitrobutane	27	16	15	0	0	2.642	0	2	2.205
1-pentanol	31	18	17	0	0	1.702	0	3	2.300
2-Aminobiphenyl	39	24	25	0	0	0.249	0	1	2.638
2-Chloroaniline	21	14	14	0	0	1.330	0	0	2.007
2-methyl-2 butanol	31	18	17	0	0	1.712	0	0	1.709
2-phenylethyl acetate	40	24	24	0	0	1.815	0	4	2.729
2-Phenylethylamine	36	21	21	1	0	15.444	0	2	2.415
3-chloro phenol	19	13	13	0	0	0.657	0	0	2.145
4-chlorobenzylalcohol	25	16	16	0	0	1.192	0	1	2.486
4-Methylbenzylamine	36	21	21	1	0	14.741	0	1	2.308
4-nitroaniline	24	16	16	0	0	2.285	0	0	2.318
Acebutolol	95	53	53	1	1	17.487	0	10	4.530
Acetonitrile	7	6	5	0	0	2.280	0	0	1.182
Acetophenone	27	17	17	0	0	2.477	0	1	2.160
Acetylsalicylic acid	31	20	20	-1	0	14.762	0	2	2.426
Acridine	40	23	25	0	0	1.300	0	0	2.722

Alprazolam	63	35	38	0	0	2.243	0	1	3.449
Alprenolol	74	42	42	1	1	10.395	0	8	3.298
Aminophenazone	63	35	36	1	1	8.375	1	2	3.040
Amitriptyline	84	45	47	1	0	15.943	0	2	3.328
Amlodipine	96	54	55	1	1	31.701	2	10	3.807
Amoxicillin	83	44	46	0	4	43.972	0	3	3.578
Aniline	21	14	14	0	0	0.243	0	0	1.748
Anisole	25	16	16	0	0	1.695	0	1	2.010
Atenolol	74	42	42	1	1	14.610	0	8	4.123
Benzene	18	12	12	0	0	0.000	0	0	1.516
Benzyl cyanide	25	16	16	0	0	2.244	0	1	2.236
Benzylalcohol	25	16	16	0	0	1.648	0	1	2.016
Benzylamine	30	18	18	1	0	11.791	0	1	2.047
Benzylbenzoate	46	28	29	0	0	0.648	0	4	3.541
Benzylmethylketon	33	20	20	0	0	2.355	0	2	2.632
Betahistine	40	23	23	1	0	12.152	0	3	2.709
Betaxolol	99	52	53	1	1	11.144	0	11	3.747
Bibenzyl	48	28	29	0	0	0.004	0	3	3.377
Biperidene	109	53	56	1	4	12.358	1	2	3.716
Biphenyl	36	22	23	0	0	0.000	0	1	2.644
Bromazepam	50	29	31	0	0	1.827	0	1	3.407
Bromperidol	94	50	52	1	0	7.044	0	6	6.176
Bupivacaine	96	50	51	1	2	10.110	0	5	3.696
Buprenorphine	166	76	82	1	8	17.166	0	3	3.972

Butylacetate	34	20	19	0	0	1.414	0	4	2.695
Caffeine	43	24	25	0	0	1.457	0	0	2.481
Carbamazepine	51	30	32	0	0	2.313	1	1	2.813
Carbamazepine epoxide	58	31	34	0	2	4.058	1	1	2.785
Carbon tetrachloride	6	5	4	0	0	0.000	0	0	1.730
Cephalexin	76	41	43	0	3	35.574	1	3	3.594
Chlorambucil	66	37	37	-1	0	29.672	0	6	4.308
Chloroform	6	5	4	0	0	1.184	0	0	1.621
Chlorpromazine	76	41	43	1	0	12.622	0	3	3.384
Cimetidine	58	34	34	1	0	12.257	0	7	3.887
Cinoxacin	51	28	30	-1	0	26.644	0	1	3.257
Ciprofloxacin	84	42	45	0	0	55.873	1	2	3.865
Clobazam	60	34	36	0	0	2.793	0	1	3.260
Clonidine	43	24	25	1	0	12.663	0	2	2.762
Clorazepate	55	32	34	-1	1	25.216	0	1	3.522
Codeine	93	44	48	1	6	18.627	1	1	3.008
Cotinine	46	25	26	0	1	3.246	0	1	2.586
Delorazepam	52	30	32	0	0	2.192	0	1	3.220
Desipramine	81	43	45	1	0	19.559	0	4	3.362
Dextromethorphan	97	46	49	1	4	15.242	0	1	3.039
Diazepam	58	33	35	0	0	1.786	0	1	3.329
Dichloromethane	6	5	4	0	0	1.450	0	0	1.419
Diclofenac	48	29	30	-1	0	20.407	0	3	3.117
Diethylether	25	15	14	0	0	1.725	0	2	1.853

Diflunisal	40	25	26	-1	0	21.340	0	1	3.460
Diltiazem	102	56	58	1	2	20.626	0	6	3.921
Diphenhydramine	73	41	42	1	0	16.104	0	5	3.408
Dipropyl ether	37	21	20	0	0	1.735	0	4	2.575
Domperidone	108	55	59	1	0	6.821	0	5	5.437
Epinephrine	45	27	27	1	1	12.308	0	3	2.810
Ethanol	13	9	8	0	0	1.698	0	0	1.193
Ethylacetate	22	14	13	0	0	0.795	0	2	1.881
Ethylbenzoate	34	21	21	0	0	0.718	0	3	2.664
Etidocaine	90	49	49	1	2	9.262	0	4	3.473
Felodipine	77	44	45	0	1	2.953	2	6	3.435
Fenbufen	54	32	33	-1	0	33.659	0	4	4.273
Flufenamic Acid	48	29	30	-1	0	19.835	0	2	3.449
Flumazenil	65	36	38	0	0	2.106	0	3	3.658
Flumequine	57	30	32	-1	1	25.798	1	0	3.116
Fluphenazine	113	58	61	2	0	32.097	0	6	5.077
Flurazepam	94	51	53	1	0	14.763	0	3	3.905
Flurbiprofen	51	30	31	-1	1	24.156	0	1	3.501
Fluvastatin	98	55	57	-1	2	27.992	1	7	4.007
Furosemide	52	31	32	-1	0	15.609	0	4	3.869
GEA 968	84	47	47	1	0	20.934	0	4	4.394
Granisetron	97	48	51	1	2	17.710	0	2	4.074
Griseofulvin	76	41	43	0	2	4.980	1	3	3.456
Haloperidol	94	50	52	1	0	6.912	0	6	5.795

Heptane	42	23	22	0	0	0.003	0	4	2.686
Hexobarbital	61	32	33	-1	1	11.382	1	1	2.788
Hydrochlorothiazide	45	25	26	0	0	9.149	0	1	3.094
Hydrocortisone	117	56	59	0	7	3.847	1	2	3.964
Hydroxyzine	104	55	57	2	1	6.404	0	8	4.987
Ibuprofen	57	32	32	-1	1	22.834	0	1	3.176
Imipramine	87	46	48	1	0	18.477	0	3	3.445
Indomethacin	70	40	42	-1	0	25.080	0	3	4.086
Indoprofen	63	35	37	-1	1	28.507	0	1	4.062
Isosorbide dinitrate	46	24	25	0	4	2.451	0	2	2.989
Isotretinoin	87	49	49	-1	0	38.005	5	4	4.888
Isradipine	86	48	50	0	1	2.039	2	6	3.299
Ketamine	63	33	34	1	1	8.247	0	2	2.638
Ketoprofen	54	32	33	-1	1	15.678	0	2	3.259
Labetalol	86	49	50	1	2	15.162	0	8	3.532
Lacidipine	117	66	67	0	0	5.505	3	10	4.055
Levosulpiride	88	47	48	1	2	19.045	0	6	4.261
Lidocaine	72	40	40	1	0	12.536	0	2	3.369
Loratadine	95	50	53	0	0	3.658	0	2	4.280
Lorazepam	53	31	33	0	1	2.432	0	1	3.296
Mefenamic Acid	54	32	33	-1	0	21.536	0	2	3.245
Mepivacaine	78	41	42	1	2	12.985	0	2	3.298
Mepyramine	80	45	46	1	0	20.867	0	6	3.853
Mesitylene	36	21	21	0	0	0.147	0	0	2.189

Metadone	93	51	52	1	1	12.758	0	6	3.249
Methohexital	63	36	36	-1	2	11.083	0	3	3.072
Methylacetate	16	11	10	0	0	1.401	0	1	1.574
Methylsulfoxide	15	10	9	0	0	8.663	0	0	1.319
Metoclopramide	76	43	43	1	0	15.861	0	4	4.153
Metoprolol	81	45	45	1	1	10.074	0	9	3.509
Mianserin	81	41	44	1	2	14.770	0	0	3.126
Midazolam	65	36	39	0	0	1.998	0	1	3.490
Morphine	87	41	45	1	6	17.073	1	0	2.880
N,N-Dimethylaniline	33	20	20	0	0	0.129	0	1	2.156
N,N-Dimethyl-p-toluidine	39	23	23	0	0	0.051	0	1	2.422
Nadolol	94	50	51	1	3	8.303	0	5	3.552
Nalidixic acid	49	28	29	-1	0	25.984	1	1	3.019
Naphtalene	30	18	19	0	0	0.000	0	0	2.112
Naproxen	52	30	31	-1	1	21.689	0	1	3.410
Nebivolol	106	55	58	1	4	1.173	0	6	5.065
N-Ethylaniline	33	20	20	0	0	0.210	0	2	2.369
Nicardipine	116	65	67	1	2	14.909	2	8	3.840
Nicotinamide	22	15	15	0	0	0.980	0	1	2.115
Nicotine	52	27	28	1	2	7.497	0	1	2.518
Nifedipine	74	43	44	0	0	2.795	2	5	3.172
Nimodipine	99	56	57	0	1	2.381	2	9	3.619
Nisoldipine	92	52	53	0	1	2.387	2	6	3.466
Nitrendipine	80	46	47	0	1	2.318	2	6	3.448

Nitrobenzene	21	14	14	0	0	2.527	0	0	2.055
N-Methylbenzylamine	36	21	21	1	0	9.381	0	2	2.376
N-methylnaphthalen-1-amine	39	23	24	0	0	0.315	0	1	2.436
N-Methylphenethylamine	42	24	24	1	0	13.130	0	3	2.737
Norfloxacin	78	41	43	0	0	55.011	1	2	3.831
N-pentane	30	17	16	0	0	0.003	0	2	1.973
N-propanol	19	12	11	0	0	1.702	0	1	1.568
Ofloxacin	91	46	49	0	1	53.522	1	1	3.854
Ondansetron	79	41	44	0	1	3.821	0	2	3.526
Oxazepam	53	31	33	0	1	2.696	0	1	3.354
Oxolinic acid	53	29	31	-1	0	25.883	1	1	3.284
Oxprenolol	75	43	43	1	1	8.441	0	9	3.158
Paracetamol	31	20	20	0	0	2.912	0	1	2.642
Pentamethylbenzene	48	27	27	0	0	0.093	0	0	2.381
Phenazone	48	27	28	1	1	8.270	1	1	2.666
Phenobarbital	47	27	28	-2	0	17.408	0	2	2.725
Phenol	19	13	13	0	0	1.637	0	0	1.752
Phenylbutazone	78	43	45	0	0	0.860	0	5	3.362
Phenylpropanolamine	43	25	25	1	2	13.495	0	2	2.461
Phenytoin	52	30	32	-1	0	13.669	0	2	2.930
Physostigmine	82	42	44	1	3	15.253	0	2	3.577
Pindolol	71	39	40	1	1	9.511	0	6	3.259
Pipemidic acid	74	39	41	0	0	55.831	1	2	3.832
Piromidic acid	68	36	38	-1	0	31.789	1	2	3.661

Piroxicam	61	35	37	-1	0	2.133	0	2	3.699
P-Nitroaniline	24	16	16	0	0	2.285	0	0	2.318
Prilocaine	66	37	37	1	1	12.464	0	5	3.382
Procaine	67	38	38	1	0	13.863	0	4	3.877
Progesterone	114	53	56	0	6	1.609	1	1	3.782
Promazine	76	41	43	1	0	11.864	0	3	3.016
Promethazine	76	41	43	1	1	14.974	0	2	3.169
Propionitrile	13	9	8	0	0	2.298	0	0	1.493
Propiophenone	33	20	20	0	0	2.484	0	2	2.433
Propofol	55	31	31	0	0	1.547	0	0	2.707
Propranolol	74	41	42	1	1	11.147	0	6	3.373
P-toluidine	27	17	17	0	0	0.356	0	0	2.019
Pyridine	16	11	11	0	0	1.336	0	0	1.483
Ranitidine	77	44	44	1	0	21.601	1	8	4.151
Risperidone	117	58	62	1	0	3.326	1	4	5.741
Rufloxacin	85	43	46	0	0	55.579	1	1	3.912
Salicylic acid	22	15	15	-1	0	15.483	0	0	2.148
Sotalol	70	39	39	1	1	18.065	0	6	4.048
Sulindac	72	41	43	-1	1	22.536	2	3	4.328
Temazepam	59	34	36	0	1	2.918	0	1	3.382
Terbutaline	63	36	36	1	1	8.662	0	3	3.497
Tert- butyl alcohol	25	15	14	0	0	1.708	0	0	1.493
Tetracaine	79	44	44	1	0	29.446	0	8	4.759
Tetrachloro ethane	12	8	7	0	0	1.436	0	0	1.949

Tetrahydrofurane	25	13	13	0	0	2.035	0	0	1.367
Theobromine	35	20	21	-1	0	11.751	0	0	2.378
Theophylline	37	21	22	0	0	1.318	0	0	2.358
Thiopental	59	32	32	-2	1	29.187	0	2	2.965
Timolol	87	46	47	1	1	13.875	0	6	3.540
Tocainide	54	31	31	1	1	17.547	0	2	2.788
Tolfenamic acid	48	29	30	-1	0	19.551	0	2	3.439
Tolmetin	57	33	34	-1	0	27.777	0	3	3.712
Toluene	24	15	15	0	0	0.122	0	0	1.781
Tramadol	86	45	46	1	2	12.229	0	3	3.221
Trimecaine	78	43	43	1	0	14.788	0	2	3.570
Tropisetron	85	42	45	1	2	18.206	0	3	3.990
Verapamil	131	72	73	1	2	18.586	0	10	4.547
W 36017	60	34	34	1	0	15.643	0	2	3.057

Table 3A. Number of angles, atoms, bonds, charge, chiral atoms, dipole, E-Z bonds, flexible torsions and gyrrad for the whole dataset of the analytes assumed as ionized.

Analyte	**HbAcc**	**HbDon**	**HeavyAtoms**	**Impropers**	**Lipole**	**Mass**	**MassMI**	**Ovality**	**Psa**	**Rings**
1,2,4,5-tetrachlorobenzene	0	0	10	0	0.000	215.892	213.891	1.351	0.000	1
1,2 -dichloroethane	0	0	4	0	0.451	98.959	97.969	1.228	0.000	0
1,3-dichlorobenzene	0	0	8	0	0.720	147.002	145.969	1.285	0.000	1
1-chloro butane	0	0	5	0	0.304	92.567	92.039	1.332	0.000	0
1-chloro-2-nitrobenzene	2	0	10	3	2.301	157.555	156.993	1.287	39.048	1
1-hexanol	1	1	7	0	4.537	102.175	102.105	1.450	23.720	0

1-Naphthylamine	0	2	11	3	3.223	143.185	143.074	1.306	25.477	2
1-nitrobutane	3	0	7	3	3.335	103.120	103.063	1.344	41.722	0
1-pentanol	1	1	6	0	3.910	88.148	88.089	1.384	23.183	0
2-Aminobiphenyl	0	2	13	3	2.681	169.222	169.089	1.388	25.477	2
2-Chloroaniline	0	2	8	3	2.574	127.572	127.019	1.262	26.147	1
2-methyl-2 butanol	1	1	6	0	1.859	88.148	88.089	1.365	22.019	0
2-phenylethyl acetate	2	0	12	3	3.689	164.201	164.084	1.462	28.316	1
2-Phenylethylamine	0	3	9	0	4.090	122.188	122.097	1.428	32.808	1
3-chloro phenol	1	1	8	0	3.604	128.556	128.003	1.252	22.648	1
4-chlorobenzylalcohol	1	1	9	0	4.198	142.583	142.019	1.334	23.632	1
4-Methylbenzylamine	0	3	9	0	3.934	122.188	122.097	1.409	33.367	1
4-nitroaniline	2	2	10	6	1.521	138.124	138.043	1.293	66.784	1
Acebutolol	4	4	24	9	3.447	337.434	337.213	1.821	93.730	1
Acetonitrile	0	0	3	0	2.284	41.052	41.027	1.130	18.823	0
Acetophenone	1	0	9	3	2.481	120.149	120.058	1.291	17.563	1
Acetylsalicylic acid	4	0	13	6	2.104	179.150	179.034	1.401	59.859	1
Acridine	1	0	14	0	1.463	179.217	179.074	1.372	10.623	3
Alprazolam	3	0	22	3	2.690	308.765	308.083	1.550	38.391	4
Alprenolol	2	3	18	6	2.702	250.357	250.181	1.708	49.978	1
Aminophenazone	1	1	17	15	0.655	232.302	232.145	1.572	32.618	2
Amitriptyline	0	1	21	6	3.119	278.411	278.191	1.638	9.011	3
Amlodipine	5	4	28	21	3.558	409.884	409.153	1.842	110.810	2
Amoxicillin	5	5	25	15	2.963	365.404	365.105	1.706	163.078	3
Aniline	0	2	7	3	2.953	93.126	93.058	1.225	27.041	1

Anisole	1	0	8	0	2.040	108.138	108.058	1.273	11.291	1
Atenolol	3	5	19	6	1.295	267.344	267.171	1.708	95.970	1
Benzene	0	0	6	0	0.000	78.112	78.047	1.192	0.000	1
Benzyl cyanide	0	0	9	0	3.918	117.148	117.058	1.313	18.636	1
Benzylalcohol	1	1	8	0	3.254	108.138	108.058	1.317	23.453	1
Benzylamine	0	3	8	0	3.287	108.161	108.081	1.352	33.740	1
Benzylbenzoate	2	0	16	3	2.381	212.244	212.084	1.499	26.345	2
Benzylmethylketon	1	0	10	3	4.521	134.175	134.073	1.405	17.743	1
Betahistine	1	2	10	0	2.679	137.202	137.108	1.451	31.066	1
Betaxolol	3	3	22	0	2.696	308.436	308.223	1.815	61.671	2
Bibenzyl	0	0	14	0	0.000	182.261	182.110	1.504	0.000	2
Biperidene	1	2	23	6	1.296	312.469	312.233	1.687	27.644	4
Biphenyl	0	0	12	0	0.000	154.208	154.078	1.358	0.000	2
Bromazepam	3	1	19	9	2.881	316.153	315.001	1.501	50.198	3
Bromperidol	2	2	26	3	0.696	421.323	420.097	1.752	45.026	3
Bupivacaine	1	2	21	6	1.601	289.436	289.228	1.730	37.314	2
Buprenorphine	4	3	34	0	0.736	468.648	468.311	1.804	64.878	7
Butylacetate	2	1	8	3	3.352	116.158	116.084	1.436	40.323	0
Caffeine	3	0	14	12	0.293	194.191	194.080	1.430	54.408	2
Carbamazepine	1	2	18	15	3.044	236.269	236.095	1.442	44.516	3
Carbamazepine epoxide	2	2	19	9	2.483	252.268	252.090	1.471	57.360	4
Carbon tetrachloride	0	0	5	0	0.000	153.823	151.875	1.241	0.000	0
Cephalexin	4	4	24	21	2.682	347.389	347.094	1.678	139.657	3
Chlorambucil	2	0	19	6	5.633	303.204	302.072	1.694	38.668	1

Chloroform	0	0	4	0	0.456	119.378	117.914	1.191	0.000	0
Chlorpromazine	0	1	21	3	2.821	319.872	319.104	1.643	32.947	3
Cimetidine	2	4	17	6	2.399	253.347	253.124	1.665	106.711	1
Cinoxacin	6	0	19	12	3.008	261.210	261.051	1.500	90.711	3
Ciprofloxacin	3	2	24	18	0.606	331.342	331.133	1.662	77.475	4
Clobazam	2	0	21	12	3.156	300.740	300.067	1.561	39.090	3
Clonidine	1	3	14	6	3.094	231.102	230.025	1.473	45.593	2
Clorazepate	4	1	22	12	4.689	313.715	313.038	1.562	75.432	3
Codeine	3	2	22	6	2.107	300.372	300.160	1.569	51.831	5
Cotinine	2	0	13	6	1.718	176.215	176.095	1.439	32.071	2
Delorazepam	2	1	20	9	3.353	305.159	304.017	1.536	42.804	3
Desipramine	0	2	20	3	3.847	267.389	267.186	1.636	22.941	3
Dextromethorphan	1	1	20	0	2.912	272.405	272.201	1.601	20.376	4
Diazepam	2	0	20	9	2.904	284.740	284.072	1.566	31.368	3
Dichloromethane	0	0	3	0	0.450	84.933	83.953	1.163	0.000	0
Diclofenac	2	1	19	6	4.141	295.141	294.009	1.555	43.118	2
Diethylether	1	0	5	0	0.294	74.122	74.073	1.329	10.574	0
Diflunisal	3	1	18	3	5.496	249.190	249.036	1.468	52.920	2
Diltiazem	4	1	29	9	2.650	415.526	415.169	1.822	87.524	3
Diphenhydramine	1	1	19	0	3.388	256.363	256.170	1.662	17.793	2
Dipropyl ether	1	0	7	0	0.494	102.175	102.105	1.441	10.574	0
Domperidone	2	3	30	18	2.199	426.919	426.170	1.799	79.258	5
Epinephrine	3	5	13	0	1.452	184.212	184.097	1.495	85.893	1
Ethanol	1	1	3	0	1.924	46.068	46.042	1.190	23.541	0

Ethylacetate	2	0	6	3	1.902	88.105	88.052	1.320	27.600	0
Ethylbenzoate	2	0	11	3	1.703	150.175	150.068	1.400	26.704	1
Etidocaine	1	2	20	6	1.216	277.425	277.228	1.747	37.682	1
Felodipine	4	1	25	21	1.621	384.254	383.069	1.742	66.691	2
Fenbufen	3	0	19	6	5.992	253.273	253.087	1.550	51.615	2
Flufenamic Acid	2	1	20	6	4.841	280.222	280.059	1.524	43.576	2
Flumazenil	4	0	22	9	1.379	303.288	303.102	1.595	61.799	3
Flumequine	3	0	19	15	4.250	260.240	260.072	1.490	54.052	3
Fluphenazine	1	3	30	3	4.752	439.537	439.191	1.806	68.036	4
Flurazepam	2	1	27	9	2.280	388.886	388.159	1.764	39.001	3
Flurbiprofen	2	0	18	3	5.516	243.253	243.082	1.548	35.127	2
Fluvastatin	4	2	30	9	3.641	410.458	410.177	1.809	80.839	3
Furosemide	6	3	21	9	3.595	329.736	329.000	1.617	123.281	2
GEA 968	2	3	21	12	2.981	292.397	292.203	1.743	64.661	1
Granisetron	2	2	23	6	2.683	313.417	313.203	1.659	52.714	4
Griseofulvin	6	0	24	12	2.445	352.766	352.071	1.699	80.290	3
Haloperidol	2	2	26	3	0.419	376.872	376.148	1.753	44.264	3
Heptane	0	0	7	0	0.006	100.202	100.125	1.469	0.000	0
Hexobarbital	4	0	17	18	1.773	235.259	235.108	1.517	65.248	2
Hydrochlorothiazide	6	4	17	9	2.171	297.739	296.965	1.519	141.531	2
Hydrocortisone	5	3	26	12	1.641	362.460	362.209	1.683	96.350	4
Hydroxyzine	2	3	26	0	2.446	376.920	376.192	1.784	51.800	3
Ibuprofen	2	0	15	3	4.811	205.273	205.123	1.574	34.768	1
Imipramine	0	1	21	3	3.481	281.415	281.202	1.669	11.397	3

Indomethacin	4	0	25	6	3.698	356.780	356.069	1.681	66.597	3
Indoprofen	3	0	21	9	2.291	280.298	280.097	1.595	55.127	3
Isosorbide dinitrate	10	0	16	6	0.061	236.136	236.028	1.495	129.000	2
Isotretinoin	2	0	22	33	4.727	299.427	299.201	1.763	34.768	1
Isradipine	7	1	27	21	1.224	371.387	371.148	1.739	103.147	3
Ketamine	1	2	16	3	2.214	238.733	238.100	1.496	34.392	2
Ketoprofen	3	0	19	6	1.366	253.273	253.087	1.542	51.973	2
Labetalol	3	6	24	6	2.356	329.413	329.187	1.763	103.405	2
Lacidipine	6	1	33	30	1.801	455.543	455.231	1.934	91.954	2
Levosulpiride	5	4	23	9	1.137	342.434	342.149	1.737	114.097	2
Lidocaine	1	2	17	6	2.134	235.345	235.181	1.653	36.881	1
Loratadine	3	0	27	12	2.095	382.883	382.145	1.721	40.473	4
Lorazepam	3	2	21	9	4.278	321.158	320.012	1.545	63.302	3
Mefenamic Acid	2	1	18	6	4.495	240.277	240.102	1.518	42.496	2
Mepivacaine	1	2	18	6	2.532	247.356	247.181	1.617	36.979	2
Mepyramine	2	1	21	3	3.630	286.392	286.192	1.743	34.204	2
Mesitylene	0	0	9	0	0.013	120.192	120.094	1.390	0.000	1
Metadone	1	1	23	3	2.609	310.453	310.217	1.708	21.750	2
Methohexital	4	0	19	18	2.100	261.296	261.124	1.615	61.814	1
Methylacetate	2	1	5	3	1.470	74.079	74.037	1.241	39.762	0
Methylsulfoxide	1	0	4	3	1.153	78.133	78.014	1.236	33.001	0
Metoclopramide	2	4	20	9	1.560	300.804	300.148	1.717	72.482	1
Metoprolol	3	3	19	0	2.618	268.372	268.191	1.749	62.188	1
Mianserin	0	1	20	3	2.985	265.373	265.171	1.570	12.887	4

Midazolam	2	0	23	3	2.128	325.767	325.078	1.570	24.973	4
Morphine	3	3	21	6	1.443	286.346	286.144	1.510	62.217	5
N,N-Dimethylaniline	0	0	9	3	1.380	121.180	121.089	1.348	3.355	1
N,N-Dimethyl-p-toluidine	0	0	10	3	2.023	135.206	135.105	1.411	3.727	1
Nadolol	4	5	22	0	1.138	310.409	310.202	1.741	94.403	2
Nalidixic acid	4	0	17	15	4.173	231.227	231.077	1.508	64.316	2
Naphtalene	0	0	10	0	0.000	128.171	128.063	1.298	0.000	2
Naproxen	3	0	17	3	3.845	229.251	229.087	1.523	46.059	2
Nebivolol	4	4	29	0	1.787	406.443	406.183	1.795	81.015	4
N-Ethylaniline	0	1	9	3	1.187	121.180	121.089	1.368	13.596	1
Nicardipine	6	2	35	24	0.983	480.533	480.214	1.916	114.525	3
Nicotinamide	2	2	9	6	2.190	122.125	122.048	1.260	53.111	1
Nicotine	1	1	12	0	1.295	163.240	163.124	1.449	20.342	2
Nifedipine	6	1	25	24	0.973	346.335	346.117	1.692	102.261	2
Nimodipine	7	1	30	24	0.821	418.440	418.174	1.854	116.342	2
Nisoldipine	6	1	28	24	1.745	388.414	388.163	1.772	101.677	2
Nitrendipine	6	1	26	24	1.300	360.361	360.132	1.748	107.508	2
Nitrobenzene	2	0	9	3	2.277	123.109	123.032	1.241	39.743	1
N-Methylbenzylamine	0	2	9	0	2.348	122.188	122.097	1.385	20.406	1
N-methylnaphthalen-1-amine	0	1	12	3	2.421	157.212	157.089	1.355	13.038	2
N-Methylphenethylamine	0	2	10	0	3.191	136.214	136.113	1.457	20.704	1
Norfloxacin	3	2	23	18	0.871	319.331	319.133	1.644	78.305	3
N-pentane	0	0	5	0	0.007	72.149	72.094	1.362	0.000	0
N-propanol	1	1	4	0	2.635	60.095	60.058	1.243	23.497	0

Ofloxacin	4	1	26	18	0.710	361.368	361.144	1.681	76.512	4
Ondansetron	2	0	22	3	1.510	293.363	293.153	1.633	34.909	4
Oxazepam	3	2	20	9	4.242	286.713	286.051	1.522	63.167	3
Oxolinic acid	5	0	19	15	3.196	260.222	260.056	1.500	77.522	3
Oxprenolol	3	3	19	6	2.236	266.356	266.176	1.719	59.921	1
Paracetamol	2	2	11	6	1.588	151.163	151.063	1.370	53.588	1
Pentamethylbenzene	0	0	11	0	0.007	148.245	148.125	1.451	0.000	1
Phenazone	1	1	14	12	1.439	189.234	189.103	1.478	30.488	2
Phenobarbital	5	0	17	9	2.814	230.219	230.069	1.479	75.770	2
Phenol	1	1	7	0	2.996	94.111	94.042	1.203	22.580	1
Phenylbutazone	2	0	23	12	1.684	308.374	308.153	1.699	42.775	3
Phenylpropanolamine	1	4	11	0	4.116	152.214	152.108	1.445	53.754	1
Phenytoin	3	1	19	9	3.544	251.260	251.082	1.487	61.320	3
Physostigmine	2	2	20	9	1.917	276.354	276.171	1.648	53.999	3
Pindolol	2	4	18	0	2.350	249.329	249.160	1.654	68.549	2
Pipemidic acid	5	2	22	18	0.857	303.317	303.133	1.627	96.121	3
Piromidic acid	5	0	21	18	4.918	287.294	287.114	1.612	77.182	3
Piroxicam	5	1	23	15	0.508	330.339	330.055	1.599	103.105	3
P-Nitroaniline	2	2	10	6	1.521	138.124	138.043	1.293	66.784	1
Prilocaine	1	3	16	6	2.530	221.319	221.165	1.627	48.262	1
Procaine	2	3	17	6	0.759	237.318	237.160	1.665	64.129	1
Progesterone	2	0	23	12	1.116	314.462	314.225	1.669	34.768	4
Promazine	0	1	20	3	2.416	285.427	285.143	1.621	33.654	3
Promethazine	0	1	20	3	3.372	285.427	285.143	1.599	34.035	3

Propionitrile	0	0	4	0	2.770	55.079	55.042	1.180	18.450	0
Propiophenone	1	0	10	3	1.804	134.175	134.073	1.371	16.488	1
Propofol	1	1	13	0	2.200	178.271	178.136	1.540	21.526	1
Propranolol	2	3	19	0	3.031	260.351	260.165	1.675	50.360	2
P-toluidine	0	2	8	3	3.657	107.153	107.074	1.303	26.818	1
Pyridine	1	0	6	0	1.646	79.100	79.042	1.156	10.809	1
Ranitidine	3	3	21	15	2.046	315.412	315.149	1.768	104.609	1
Risperidone	4	1	30	15	1.319	411.492	411.220	1.801	63.432	5
Rufloxacin	3	1	25	18	0.723	363.407	363.105	1.670	87.922	4
Salicylic acid	3	1	10	3	3.483	137.113	137.024	1.270	52.852	1
Sotalol	3	4	18	3	1.485	273.372	273.127	1.690	97.821	1
Sulindac	3	0	25	18	0.832	355.403	355.080	1.688	68.128	3
Temazepam	3	1	21	9	3.686	300.740	300.067	1.568	51.217	3
Terbutaline	3	5	16	0	1.138	226.292	226.144	1.621	87.355	1
Tert- butyl alcohol	1	1	5	0	1.873	74.122	74.073	1.299	22.489	0
Tetracaine	2	2	19	6	5.452	265.371	265.192	1.742	50.877	1
Tetrachloro ethane	0	0	6	0	0.432	167.849	165.891	1.291	0.000	0
Tetrahydrofurane	1	0	5	0	1.568	72.106	72.058	1.207	11.112	1
Theobromine	4	0	13	9	1.662	179.156	179.057	1.375	64.027	2
Theophylline	3	1	13	12	0.203	180.164	180.065	1.389	67.548	2
Thiopental	4	0	16	9	3.143	240.322	240.093	1.542	83.833	1
Timolol	5	3	21	3	1.059	317.428	317.165	1.721	112.701	2
Tocainide	1	4	14	6	4.278	193.266	193.134	1.535	60.656	1
Tolfenamic acid	2	1	18	6	4.668	260.696	260.048	1.505	42.824	2

Tolmetin	3	0	19	6	3.595	256.277	256.097	1.575	54.977	2
Toluene	0	0	7	0	0.017	92.138	92.063	1.254	0.000	1
Tramadol	2	2	19	0	2.416	264.383	264.196	1.663	39.321	2
Trimecaine	1	2	18	6	2.654	249.372	249.197	1.677	36.539	1
Tropisetron	2	2	21	3	3.641	285.361	285.160	1.610	50.754	4
Verapamil	4	1	33	0	2.703	455.610	455.291	2.001	72.399	2
W 36017	1	2	15	6	3.055	207.292	207.150	1.577	37.568	1

Table 3B. Number of H-bond acceptor group, H-bond donor group, of heavy atoms, of impropers, lipole, mass, monoisotopic mass, ovality, polar surface area (Psa) and number of rings for the whole dataset of the analytes assumed as ionized.

Analyte	**Sas**	**Sav**	**Sdiam**	**Surface**	**Torsions**	**Vdiam**	**VirtualLogP**	**Volume**
1,2,4,5-tetrachlorobenzene	339.068	497.136	7.542	178.705	6	6.490	5.471	143.103
1,2 -dichloroethane	233.344	305.700	5.761	104.280	1	5.199	1.995	73.599
1,3-dichlorobenzene	289.864	409.540	6.821	146.177	6	6.017	3.766	114.083
1-chloro butane	282.837	383.603	6.528	133.863	2	5.657	2.926	94.774
1-chloro-2-nitrobenzene	293.357	421.938	6.962	152.254	7	6.135	3.409	120.925
1-hexanol	336.936	476.368	7.418	172.874	5	6.161	2.493	122.440
1-Naphthylamine	318.338	471.535	7.346	169.541	12	6.428	2.139	139.082
1-nitrobutane	297.846	410.190	6.710	141.449	3	5.787	2.372	101.499
1-pentanol	303.155	417.441	6.883	148.853	4	5.851	1.982	104.881
2-Aminobiphenyl	381.088	573.543	8.047	203.444	14	6.831	2.827	166.876
2-Chloroaniline	286.895	402.690	6.642	138.582	7	5.911	1.898	108.157

2-methyl-2 butanol	272.901	387.763	6.867	148.135	0	5.877	1.419	106.298
2-phenylethyl acetate	387.608	581.301	8.193	210.906	10	6.777	2.464	162.994
2-Phenylethylamine	330.033	480.844	7.594	181.173	8	6.354	-2.148	134.321
3-chloro phenol	281.257	393.474	6.561	135.250	7	5.864	2.349	105.578
4-chlorobenzylalcohol	314.148	449.687	7.138	160.065	8	6.180	2.021	123.591
4-Methylbenzylamine	340.603	490.510	7.578	180.390	7	6.384	-1.888	136.216
4-nitroaniline	299.201	426.160	6.849	147.379	8	6.022	1.690	114.364
Acebutolol	646.643	1068.662	11.645	426.025	18	8.631	-0.273	336.601
Acetonitrile	189.979	225.452	4.730	70.297	0	4.451	0.453	46.165
Acetophenone	303.809	434.254	6.924	150.614	7	6.093	1.407	118.452
Acetylsalicylic acid	358.674	533.703	7.880	195.062	9	6.657	0.434	154.492
Acridine	364.759	548.793	8.039	203.048	16	6.864	3.763	169.365
Alprazolam	524.471	844.911	9.880	306.655	23	7.937	3.815	261.769
Alprenolol	495.301	826.712	10.378	338.348	16	7.941	0.490	262.226
Aminophenazone	450.380	725.825	9.502	283.658	13	7.579	-1.164	227.967
Amitriptyline	540.231	903.432	10.533	348.552	20	8.230	0.897	291.923
Amlodipine	642.402	1120.882	12.086	458.891	22	8.904	-1.519	369.597
Amoxicillin	549.631	942.855	10.998	380.024	20	8.420	-4.657	312.510
Aniline	261.555	358.708	6.216	121.394	7	5.615	1.137	92.710
Anisole	282.692	396.614	6.691	140.642	7	5.929	2.196	109.144
Atenolol	553.405	884.944	10.421	341.155	16	7.974	-1.629	265.443
Benzene	238.870	321.014	5.951	111.247	6	5.450	2.136	84.761
Benzyl cyanide	316.263	444.275	6.965	152.385	8	6.078	1.977	117.558
Benzylalcohol	286.366	403.427	6.843	147.107	8	5.962	1.322	110.959

Benzylamine	305.166	432.604	7.066	156.833	7	6.076	-2.434	117.430
Benzylbenzoate	453.976	686.552	8.882	247.834	16	7.254	3.197	199.839
Benzylmethylketon	343.545	497.410	7.623	182.562	9	6.431	2.235	139.249
Betahistine	363.509	529.045	7.889	195.518	9	6.549	-2.188	147.100
Betaxolol	590.686	1031.389	11.478	413.872	21	8.520	0.644	323.860
Bibenzyl	434.540	661.026	8.751	240.598	15	7.135	4.527	190.167
Biperidene	587.862	991.545	11.073	385.208	22	8.524	0.789	324.318
Biphenyl	354.685	527.046	7.759	189.150	13	6.658	3.564	154.549
Bromazepam	477.100	745.741	9.284	270.782	19	7.578	1.988	227.888
Bromperidol	665.028	1099.435	11.510	416.194	25	8.695	1.900	344.203
Bupivacaine	564.817	947.704	10.998	379.985	18	8.361	2.280	305.997
Buprenorphine	685.281	1269.927	12.849	518.677	32	9.566	1.933	458.407
Butylacetate	335.005	473.734	7.427	173.281	5	6.197	2.211	124.618
Caffeine	376.477	573.757	8.137	208.022	10	6.805	-0.226	164.995
Carbamazepine	426.195	677.096	8.843	245.662	19	7.364	2.141	209.112
Carbamazepine epoxide	439.091	702.241	9.108	260.631	21	7.509	1.392	221.680
Carbon tetrachloride	247.840	334.073	6.089	116.494	0	5.467	2.161	85.565
Cephalexin	569.801	945.637	10.720	361.005	20	8.275	-3.566	296.640
Chlorambucil	541.706	863.759	10.408	340.285	15	7.996	3.598	267.696
Chloroform	226.795	294.540	5.591	98.213	0	5.124	2.384	70.424
Chlorpromazine	525.969	888.755	10.542	349.139	19	8.225	2.075	291.319
Cimetidine	526.735	812.183	9.890	307.312	14	7.665	-2.079	235.792
Cinoxacin	450.751	699.112	9.021	255.677	17	7.365	-1.476	209.175
Ciprofloxacin	552.006	910.387	10.582	351.811	23	8.208	-3.242	289.503

Clobazam	504.634	824.067	9.813	302.530	19	7.855	2.853	253.804
Clonidine	412.076	632.292	8.572	230.820	13	7.063	-0.739	184.472
Clorazepate	507.292	811.181	9.827	303.370	20	7.862	1.431	254.445
Codeine	499.032	845.969	10.160	324.309	24	8.113	-1.239	279.566
Cotinine	374.993	571.211	8.199	211.183	12	6.834	0.773	167.129
Delorazepam	486.159	781.072	9.634	291.569	19	7.774	3.135	245.965
Desipramine	536.422	876.670	10.324	334.853	21	8.071	0.329	275.248
Dextromethorphan	506.204	854.987	10.243	329.597	21	8.094	1.210	277.686
Diazepam	493.368	783.481	9.766	299.633	19	7.805	2.474	248.944
Dichloromethane	203.406	252.972	5.149	83.293	0	4.775	1.511	57.010
Diclofenac	471.627	760.966	9.577	288.161	16	7.680	3.436	237.153
Diethylether	282.603	374.991	6.360	127.082	2	5.517	1.666	87.917
Diflunisal	426.664	659.320	8.780	242.195	15	7.247	2.291	199.280
Diltiazem	700.361	1193.812	12.120	461.499	24	8.979	0.558	379.046
Diphenhydramine	534.493	864.510	10.310	333.961	17	7.997	0.465	267.800
Dipropyl ether	344.205	478.414	7.400	172.027	4	6.165	2.689	122.661
Domperidone	694.305	1162.525	11.993	451.862	31	8.941	0.397	374.257
Epinephrine	402.175	611.427	8.481	225.968	12	6.937	-3.020	174.798
Ethanol	202.585	249.582	5.130	82.693	1	4.704	0.392	54.485
Ethylacetate	277.992	371.953	6.395	128.471	2	5.566	1.182	90.304
Ethylbenzoate	355.006	516.655	7.746	188.501	9	6.546	2.218	146.857
Etidocaine	542.778	921.754	10.971	378.120	11	8.301	2.171	299.478
Felodipine	579.794	992.879	11.260	398.336	18	8.532	3.798	325.194
Fenbufen	504.734	783.849	9.498	283.385	17	7.629	1.995	232.443

Flufenamic Acid	467.505	732.348	9.304	271.979	15	7.538	2.995	224.248
Flumazenil	521.286	836.252	9.966	312.045	19	7.891	2.494	257.307
Flumequine	438.980	697.068	9.101	260.210	16	7.455	0.532	216.908
Fluphenazine	717.441	1211.536	12.198	467.406	29	9.077	-0.141	391.604
Flurazepam	648.105	1088.303	11.686	429.056	21	8.799	1.313	356.732
Flurbiprofen	468.767	731.245	9.301	271.802	13	7.475	3.051	218.718
Fluvastatin	643.714	1139.946	12.067	457.491	27	8.971	2.806	378.082
Furosemide	514.086	813.100	9.919	309.067	17	7.799	1.407	248.423
GEA 968	604.243	977.249	10.933	375.504	12	8.281	0.459	297.316
Granisetron	574.774	952.356	10.744	362.669	23	8.343	-0.269	304.046
Griseofulvin	553.771	924.759	10.786	365.457	19	8.275	2.579	296.656
Haloperidol	663.512	1090.429	11.473	413.513	25	8.665	1.714	340.689
Heptane	355.619	505.527	7.616	182.245	4	6.284	4.378	129.944
Hexobarbital	434.801	696.856	9.120	261.317	13	7.404	0.924	212.536
Hydrochlorothiazide	427.153	671.520	9.047	257.116	13	7.341	-0.124	207.103
Hydrocortisone	566.319	999.508	11.335	403.624	25	8.738	0.020	349.305
Hydroxyzine	672.635	1127.028	11.804	437.753	27	8.838	-1.206	361.450
Ibuprofen	438.142	691.722	9.257	269.217	7	7.379	2.462	210.407
Imipramine	547.655	910.178	10.619	354.269	20	8.219	0.864	290.667
Indomethacin	585.365	957.310	10.813	367.310	21	8.340	3.311	303.769
Indoprofen	524.147	829.170	9.872	306.146	17	7.815	1.164	249.950
Isosorbide dinitrate	388.181	593.259	8.481	225.953	13	6.936	0.274	174.717
Isotretinoin	625.738	1025.719	11.238	396.736	15	8.462	2.412	317.312
Isradipine	557.849	987.774	11.321	402.647	22	8.586	3.197	331.382

Ketamine	439.338	713.883	9.244	268.438	14	7.558	0.347	226.039
Ketoprofen	473.908	753.061	9.468	281.618	14	7.625	1.407	232.120
Labetalol	572.259	985.422	11.262	398.443	23	8.481	-0.819	319.378
Lacidipine	709.027	1288.341	13.097	538.850	23	9.418	3.536	437.417
Levosulpiride	590.735	984.153	11.032	382.317	19	8.371	-1.344	307.149
Lidocaine	499.459	811.128	10.009	314.722	9	7.786	0.530	247.133
Loratadine	647.091	1086.849	11.417	409.490	27	8.703	5.411	345.160
Lorazepam	506.532	813.819	9.721	296.857	20	7.819	2.466	250.339
Mefenamic Acid	471.650	740.803	9.278	270.461	15	7.530	2.920	223.544
Mepivacaine	495.905	815.381	9.984	313.132	15	7.851	0.633	253.393
Mepyramine	594.197	968.003	10.871	371.265	18	8.233	0.458	292.247
Mesitylene	330.491	478.404	7.510	177.203	6	6.371	3.351	135.383
Metadone	560.735	968.845	11.172	392.130	18	8.549	1.676	327.162
Methohexital	490.222	800.469	9.908	308.383	11	7.795	2.295	248.008
Methylacetate	235.127	306.768	5.771	104.628	2	5.180	0.760	72.765
Methylsulfoxide	232.052	302.312	5.760	104.231	0	5.180	-0.656	72.792
Metoclopramide	570.275	914.797	10.686	358.725	12	8.155	-0.552	284.017
Metoprolol	529.879	890.961	10.786	365.515	16	8.156	-0.114	284.071
Mianserin	498.724	824.776	9.984	313.170	22	7.967	0.361	264.785
Midazolam	527.747	855.859	10.079	319.165	23	8.045	4.523	272.596
Morphine	454.242	770.010	9.731	297.455	24	7.918	-1.719	259.930
N,N-Dimethylaniline	322.036	463.937	7.296	167.253	7	6.284	2.952	129.933
N,N-Dimethyl-p-toluidine	355.154	520.383	7.747	188.528	7	6.521	3.492	145.207
Nadolol	534.369	923.480	11.115	388.095	19	8.423	-0.626	312.933

Nalidixic acid	432.639	669.996	8.944	251.308	13	7.283	0.262	202.232
Naphtalene	304.181	442.172	7.183	162.078	11	6.305	3.107	131.266
Naproxen	455.568	707.516	9.113	260.898	12	7.383	2.280	210.746
Nebivolol	680.521	1140.879	11.855	441.488	30	8.848	1.035	362.707
N-Ethylaniline	322.649	464.609	7.337	169.115	8	6.274	2.746	129.299
Nicardipine	664.948	1243.297	13.084	537.774	27	9.452	1.480	442.207
Nicotinamide	285.164	399.912	6.646	138.753	8	5.919	-0.178	108.598
Nicotine	376.885	575.714	8.248	213.745	12	6.853	-1.173	168.548
Nifedipine	540.861	919.836	10.882	372.029	18	8.365	2.492	306.440
Nimodipine	630.865	1128.337	12.286	474.210	22	9.023	3.634	384.661
Nisoldipine	608.167	1068.745	11.673	428.106	19	8.770	3.355	353.155
Nitrendipine	586.777	996.046	11.233	396.382	19	8.496	3.200	321.104
Nitrobenzene	277.564	388.778	6.500	132.752	7	5.836	2.663	104.081
N-Methylbenzylamine	336.707	486.840	7.459	174.807	8	6.338	-1.537	133.279
N-methylnaphthalen-1-amine	354.538	528.486	7.767	189.526	12	6.672	2.954	155.504
N-Methylphenethylamine	371.094	544.116	7.937	197.906	9	6.575	-1.344	148.858
Norfloxacin	541.491	883.612	10.381	338.586	20	8.097	-3.087	277.969
N-pentane	288.885	393.596	6.649	138.869	2	5.698	3.328	96.850
N-propanol	236.945	308.452	5.716	102.654	2	5.128	0.966	70.603
Ofloxacin	583.947	974.090	10.924	374.913	23	8.425	-2.716	313.161
Ondansetron	525.229	867.637	10.318	334.426	22	8.073	2.710	275.493
Oxazepam	491.667	777.945	9.456	280.896	20	7.664	1.924	235.710
Oxolinic acid	446.935	700.186	9.077	258.826	17	7.412	-0.782	213.214
Oxprenolol	515.085	861.113	10.597	352.767	17	8.081	0.380	276.345

Paracetamol	342.070	496.272	7.556	179.379	9	6.456	1.129	140.900
Pentamethylbenzene	366.534	558.541	8.275	215.100	6	6.869	4.193	169.671
Phenazone	403.565	618.810	8.544	229.327	12	7.028	-1.875	181.783
Phenobarbital	415.635	653.444	8.838	245.418	14	7.269	0.249	201.066
Phenol	252.338	344.550	6.115	117.493	7	5.576	1.587	90.775
Phenylbutazone	556.565	921.282	10.749	362.997	22	8.246	2.606	293.631
Phenylpropanolamine	365.224	550.409	8.065	204.343	9	6.710	-2.170	158.200
Phenytoin	443.774	703.196	9.146	262.808	19	7.501	1.572	221.000
Physostigmine	527.057	854.066	10.246	329.838	17	7.981	-0.711	266.197
Pindolol	483.141	791.474	9.987	313.317	17	7.766	-0.075	245.251
Pipemidic acid	541.258	873.675	10.173	325.143	20	7.977	-4.084	265.756
Piromidic acid	510.756	821.845	9.937	310.234	19	7.826	0.675	250.987
Piroxicam	516.885	846.771	10.132	322.494	20	8.011	0.765	269.231
P-Nitroaniline	299.201	426.160	6.849	147.379	8	6.022	1.690	114.364
Prilocaine	498.151	786.563	9.711	296.249	12	7.612	0.165	230.936
Procaine	512.580	802.835	10.025	315.710	11	7.769	-1.083	245.502
Progesterone	545.801	956.214	11.004	380.400	21	8.518	2.863	323.648
Promazine	502.560	844.079	10.300	333.264	19	8.089	1.538	277.161
Promethazine	501.245	837.817	10.196	326.593	18	8.064	1.322	274.549
Propionitrile	222.842	280.160	5.307	88.475	1	4.886	0.978	61.081
Propiophenone	330.562	483.040	7.500	176.719	8	6.405	1.877	137.590
Propofol	409.092	640.123	9.011	255.084	7	7.262	3.689	200.488
Propranolol	500.271	827.525	10.321	334.631	18	7.974	0.267	265.478
P-toluidine	295.583	420.383	6.772	144.090	7	5.933	1.713	109.327

Pyridine	237.209	313.736	5.692	101.800	6	5.295	1.216	77.712
Ranitidine	600.043	967.905	10.930	375.288	15	8.221	-1.255	290.906
Risperidone	693.053	1162.631	12.007	452.951	31	8.947	0.924	375.005
Rufloxacin	560.142	942.428	10.775	364.747	23	8.339	-2.844	303.644
Salicylic acid	286.060	410.041	6.820	146.112	8	6.052	0.024	116.088
Sotalol	532.452	854.836	10.230	328.798	13	7.869	-1.687	255.153
Sulindac	598.457	976.902	10.912	374.087	21	8.400	0.333	310.348
Temazepam	517.802	833.083	9.858	305.284	20	7.872	1.887	255.407
Terbutaline	484.837	766.886	9.658	293.059	12	7.586	-0.850	228.550
Tert- butyl alcohol	251.506	345.029	6.263	123.223	0	5.495	1.004	86.862
Tetracaine	590.813	933.302	10.642	355.771	14	8.062	0.145	274.373
Tetrachloro ethane	272.457	382.912	6.590	136.434	0	5.799	2.499	102.103
Tetrahydrofurane	233.841	310.641	5.791	105.372	5	5.271	1.082	76.662
Theobromine	340.793	505.668	7.669	184.773	10	6.539	-1.382	146.410
Theophylline	350.772	520.876	7.756	188.968	10	6.581	-0.282	149.238
Thiopental	445.132	707.634	9.311	272.374	8	7.498	1.245	220.734
Timolol	544.405	925.386	10.839	369.100	18	8.263	-0.509	295.401
Tocainide	423.201	666.100	8.932	250.614	9	7.209	-1.347	196.194
Tolfenamic acid	460.341	723.574	9.222	267.171	15	7.517	3.127	222.397
Tolmetin	488.692	764.870	9.618	290.645	15	7.665	1.259	235.809
Toluene	280.434	388.130	6.414	129.236	6	5.727	2.784	98.337
Tramadol	527.639	875.901	10.401	339.830	16	8.065	-0.137	274.694
Trimecaine	523.316	853.579	10.315	334.274	9	7.966	1.012	264.645
Tropisetron	540.077	871.842	10.133	322.587	22	7.987	0.376	266.764

Verapamil	781.240	1389.892	13.579	579.312	23	9.599	2.591	463.155
W 36017	450.304	717.340	9.340	274.040	9	7.438	-0.797	215.457

Table 3C. Surface accessible to solvent, volume accessible to solvent, superficial diameter, surface, number of torsions, volume diameter, VirtualLogP and volume for the whole dataset of the analytes assumed as ionized.

Analyte	**Angles**	**Atoms**	**Bonds**	**Charge**	**ChiralAtms**	**Dipole**	**EzBnds**	**FlexTorsions**	**Gyrrad**
1,2 -dichloroethane	12.000	8.000	7.000	0.000	0.000	2.035	0.000	0.000	1.664
1,2,4,5-tetrachlorobenzene	18.000	12.000	12.000	0.000	0.000	0.001	0.000	0.000	2.721
1,3-dichlorobenzene	18.000	12.000	12.000	0.000	0.000	1.195	0.000	0.000	2.337
1-chloro butane	24.000	14.000	13.000	0.000	0.000	1.339	0.000	1.000	2.106
1-chloro-2-nitrobenzene	21.000	14.000	14.000	0.000	0.000	3.217	0.000	0.000	2.185
1-hexanol	37.000	21.000	20.000	0.000	0.000	1.701	0.000	4.000	2.666
1-Naphthylamine	33.000	20.000	21.000	0.000	0.000	0.252	0.000	0.000	2.227
1-nitrobutane	27.000	16.000	15.000	0.000	0.000	2.642	0.000	2.000	2.205
1-pentanol	31.000	18.000	17.000	0.000	0.000	1.702	0.000	3.000	2.300
2-Aminobiphenyl	39.000	24.000	25.000	0.000	0.000	0.249	0.000	1.000	2.638
2-Chloroaniline	21.000	14.000	14.000	0.000	0.000	1.330	0.000	0.000	2.007
2-methyl-2 butanol	31.000	18.000	17.000	0.000	0.000	1.712	0.000	0.000	1.709
2-phenylethyl acetate	40.000	24.000	24.000	0.000	0.000	1.815	0.000	4.000	2.729
2-Phenylethylamine	33.005	20.002	20.002	0.002	0.000	0.764	0.000	2.000	2.388
3-chloro phenol	19.000	13.000	13.000	0.000	0.000	0.657	0.000	0.000	2.145
4-chlorobenzylalcohol	25.000	16.000	16.000	0.000	0.000	1.192	0.000	1.000	2.486
4-Methylbenzylamine	33.012	20.004	20.004	0.004	0.000	0.945	0.000	1.000	2.292

4-nitroaniline	24.000	16.000	16.000	0.000	0.000	2.285	0.000	0.000	2.318
Acebutolol	92.006	52.002	52.002	0.002	1.000	5.887	0.000	10.000	4.238
Acetonitrile	7.000	6.000	5.000	0.000	0.000	2.280	0.000	0.000	1.182
Acetophenone	27.000	17.000	17.000	0.000	0.000	2.477	0.000	1.000	2.160
Acetylsalicylic acid	31.000	20.000	20.000	-1.000	0.000	14.758	0.000	2.000	2.426
Acridine	40.000	23.000	25.000	0.000	0.000	1.300	0.000	0.000	2.722
Alprazolam	63.000	35.000	38.000	0.000	0.000	2.243	0.000	1.000	3.449
Alprenolol	71.008	41.003	41.003	0.003	1.000	2.401	0.000	8.000	3.269
Aminophenazone	62.991	34.997	35.997	0.997	0.997	8.356	1.000	2.000	3.040
Amitriptyline	81.020	44.007	46.007	0.007	0.000	0.999	0.000	2.993	3.324
Amlodipine	93.024	53.008	54.008	0.008	1.000	2.168	2.000	10.000	3.802
Amoxicillin	81.879	43.626	45.626	-0.374	4.000	37.028	0.000	3.000	3.551
Aniline	21.000	14.000	14.000	0.000	0.000	0.243	0.000	0.000	1.748
Anisole	25.000	16.000	16.000	0.000	0.000	1.698	0.000	1.000	2.010
Atenolol	71.011	41.004	41.004	0.004	1.000	5.040	0.000	8.000	3.866
Benzene	18.000	12.000	12.000	0.000	0.000	0.000	0.000	0.000	1.516
Benzyl cyanide	25.000	16.000	16.000	0.000	0.000	2.244	0.000	1.000	2.236
Benzylalcohol	25.000	16.000	16.000	0.000	0.000	1.648	0.000	1.000	2.016
Benzylamine	27.015	17.005	17.005	0.005	0.000	0.919	0.000	1.000	2.030
Benzylbenzoate	46.000	28.000	29.000	0.000	0.000	0.648	0.000	4.000	3.541
Benzylmethylketon	33.000	20.000	20.000	0.000	0.000	2.355	0.000	2.000	2.632
Betahistine	37.002	22.001	22.001	0.001	0.000	1.192	0.000	3.000	2.697
Betaxolol	96.012	51.004	52.004	0.004	1.000	4.111	0.000	11.000	3.875
Bibenzyl	48.000	28.000	29.000	0.000	0.000	0.004	0.000	3.000	3.377

Biperidene	106.000	52.000	55.000	0.000	4.000	2.384	1.000	2.000	3.709
Biphenyl	36.000	22.000	23.000	0.000	0.000	0.000	0.000	1.000	2.644
Bromazepam	50.000	29.000	31.000	0.000	0.000	1.827	0.000	1.000	3.407
Bromperidol	91.251	49.084	51.084	0.084	0.000	3.468	0.000	6.000	6.164
Bupivacaine	93.221	49.074	50.074	0.074	1.074	3.553	0.000	5.000	3.628
Buprenorphine	163.140	75.047	81.047	0.047	7.047	5.455	0.000	3.000	3.950
Butylacetate	34.000	20.000	19.000	0.000	0.000	1.414	0.000	4.000	2.695
Caffeine	43.000	24.000	25.000	0.000	0.000	1.457	0.000	0.000	2.481
Carbamazepine	51.000	30.000	32.000	0.000	0.000	2.313	1.000	1.000	2.813
Carbamazepine epoxide	58.000	31.000	34.000	0.000	2.000	4.058	1.000	1.000	2.785
Carbon tetrachloride	6.000	5.000	4.000	0.000	0.000	0.000	0.000	0.000	1.730
Cefalexin	74.886	40.629	42.629	-0.371	3.000	33.966	1.000	3.000	3.666
Chlorambucil	66.007	37.007	37.007	-0.993	0.000	29.489	0.000	6.007	4.308
Chloroform	6.000	5.000	4.000	0.000	0.000	1.184	0.000	0.000	1.621
Chlorpromazine	73.012	40.004	42.004	0.004	0.000	1.353	0.000	3.996	3.398
Cimetidine	56.839	33.613	33.613	0.613	0.000	8.262	0.000	7.000	3.869
Cinnoxacin	51.000	28.000	30.000	-1.000	0.000	26.657	0.000	1.000	3.256
Ciprofloxacin	83.992	42.033	45.033	0.033	0.000	53.790	1.000	2.054	3.869
Clobazam	60.000	34.000	36.000	0.000	0.000	2.793	0.000	1.000	3.260
Clonidine	40.262	23.087	24.087	0.087	0.000	1.459	0.000	2.000	2.745
Clorazepate	55.000	32.000	34.000	-1.000	1.000	25.212	0.000	1.000	3.522
Codeine	90.174	43.058	47.058	0.058	5.058	5.597	1.000	1.000	3.007
Cotinine	46.000	25.000	26.000	0.000	1.000	3.246	0.000	1.000	2.586
Delorazepam	52.000	30.000	32.000	0.000	0.000	2.192	0.000	1.000	3.220

Desipramine	78.001	42.000	44.000	0.000	0.000	1.025	0.000	4.000	3.339
Dextromethorphan	94.143	45.048	48.048	0.048	3.048	2.503	0.000	1.000	3.037
Diazepam	58.000	33.000	35.000	0.000	0.000	1.786	0.000	1.000	3.329
Dichloromethane	6.000	5.000	4.000	0.000	0.000	1.450	0.000	0.000	1.419
Diclofenac	48.003	29.003	30.003	-0.997	0.000	20.347	0.000	3.003	3.117
Diethylether	25.000	15.000	14.000	0.000	0.000	1.725	0.000	2.000	1.853
Diflunisal	40.000	25.000	26.000	-1.000	0.000	21.338	0.000	1.000	3.460
Diltiazem	99.721	55.240	57.240	0.240	2.000	6.237	0.000	6.760	3.918
Diphenhydramine	70.024	40.008	41.008	0.008	0.000	2.366	0.000	5.992	3.437
Dipropyl ether	37.000	21.000	20.000	0.000	0.000	1.735	0.000	4.000	2.575
Domperidone	105.335	54.112	58.112	0.112	0.000	4.197	0.000	5.000	5.304
Epinephrine	42.075	26.025	26.025	0.025	1.000	3.708	0.000	3.000	2.805
Ethanol	13.000	9.000	8.000	0.000	0.000	1.698	0.000	0.000	1.193
Ethylacetate	22.000	14.000	13.000	0.000	0.000	0.795	0.000	2.000	1.881
Ethylbenzoate	34.000	21.000	21.000	0.000	0.000	0.718	0.000	3.000	2.664
Etidocaine	87.499	48.166	48.166	0.166	1.166	3.414	0.000	6.501	3.459
Felodipine	77.000	44.000	45.000	0.000	1.000	2.953	2.000	6.000	3.435
Fenbufen	54.003	32.003	33.003	-0.997	0.000	33.559	0.000	4.003	4.273
Flufenamic Acid	48.001	29.001	30.001	-0.999	0.000	19.820	0.000	2.001	3.449
Flumazenil	65.000	36.000	38.000	0.000	0.000	2.106	0.000	3.000	3.658
Flumequine	57.092	30.092	32.092	-0.908	1.000	23.788	1.000	0.092	3.118
Fluphenazine	107.671	56.224	59.224	0.224	0.000	6.172	0.000	6.000	5.042
Flurazepam	91.000	50.000	52.000	0.000	0.000	3.900	0.000	6.000	3.937
Flurbiprofen	51.004	30.004	31.004	-0.996	1.000	24.071	0.000	1.000	3.501

Fluvastatin	98.002	55.002	57.002	-0.998	2.000	27.940	1.000	7.002	4.007
Furosemide	52.000	31.000	32.000	-1.000	0.000	15.606	0.000	4.000	3.869
GEA 968	81.499	46.166	46.166	0.166	0.000	8.195	0.000	6.501	4.385
Granisetron	94.012	47.004	50.004	0.004	2.000	2.398	0.000	2.000	4.078
Griseofulvin	76.000	41.000	43.000	0.000	2.000	4.980	1.000	3.000	3.456
Haloperidol	91.251	49.084	51.084	0.084	0.000	3.556	0.000	6.000	5.784
Heptane	42.000	23.000	22.000	0.000	0.000	0.003	0.000	4.000	2.686
Hexobarbital	62.881	32.941	33.941	-0.059	1.000	1.296	1.000	1.000	2.795
Hydrochlorothiazide	45.000	25.000	26.000	0.000	0.000	9.149	0.000	1.000	3.094
Hydrocortisone	117.000	56.000	59.000	0.000	7.000	3.847	1.000	2.000	3.964
Hydroxyzine	98.789	53.263	55.263	0.263	1.000	2.194	0.000	8.000	4.977
Ibuprofen	57.016	32.016	32.016	-0.984	1.000	22.499	0.000	1.000	3.176
Imipramine	84.010	45.003	47.003	0.003	0.000	1.081	0.000	3.997	3.419
Indomethacin	70.003	40.003	42.003	-0.997	0.000	25.004	0.000	3.003	4.086
Indoprofen	63.004	35.004	37.004	-0.996	1.000	28.402	0.000	1.000	4.062
Isosorbide dinitrate	46.000	24.000	25.000	0.000	4.000	2.451	0.000	2.000	2.989
Isotretinoin	87.006	49.006	49.006	-0.994	0.000	37.781	5.000	4.006	4.889
Isradipine	86.000	48.000	50.000	0.000	1.000	2.016	2.000	6.000	3.296
Ketamine	62.328	32.776	33.776	0.776	1.000	7.382	0.000	2.000	2.637
Ketoprofen	54.004	32.004	33.004	-0.996	1.000	15.625	0.000	2.000	3.260
Labetalol	85.920	48.920	49.920	0.920	2.000	14.569	0.240	8.000	3.637
Lacidipine	117.000	66.000	67.000	0.000	0.000	5.505	3.000	10.000	4.055
Levosulpiride	87.884	46.961	47.961	0.961	1.961	16.988	0.000	6.000	4.255
Lidocaine	69.335	39.112	39.112	0.112	0.000	4.102	0.000	4.665	3.346

Loratadine	95.000	50.000	53.000	0.000	0.000	3.658	0.000	2.000	4.280
Lorazepam	53.000	31.000	33.000	0.000	1.000	2.432	0.000	1.000	3.296
Mefenamic Acid	54.002	32.002	33.002	-0.998	0.000	21.504	0.000	2.002	3.245
Mepivacaine	75.602	40.201	41.201	0.201	1.201	5.096	0.000	2.000	3.305
Mepyramine	77.042	44.014	45.014	0.014	0.000	1.494	0.000	6.986	3.836
Mesitylene	36.000	21.000	21.000	0.000	0.000	0.148	0.000	0.000	2.189
Metadone	90.034	50.011	51.011	0.011	1.000	3.016	0.000	6.989	3.230
Methohexital	64.963	36.982	36.982	-0.018	2.000	2.375	0.000	3.000	3.044
Methylacetate	16.000	11.000	10.000	0.000	0.000	1.401	0.000	1.000	1.574
Methylsulfoxide	15.000	10.000	9.000	0.000	0.000	8.663	0.000	0.000	1.319
Metoclopramide	73.016	42.005	42.005	0.005	0.000	3.524	0.000	6.984	4.092
Metoprolol	78.006	44.002	44.002	0.002	1.000	4.080	0.000	9.000	3.592
Mianserin	78.156	40.052	43.052	0.052	1.052	1.333	0.000	0.000	3.125
Midazolam	71.097	39.387	42.194	0.000	0.097	2.014	0.000	2.065	3.601
Morphine	84.160	40.053	44.053	0.053	5.053	4.969	1.000	0.000	2.879
N,N-Dimethylaniline	33.000	20.000	20.000	0.000	0.000	0.129	0.000	1.000	2.156
N,N-Dimethyl-p-toluidine	39.000	23.000	23.000	0.000	0.000	0.051	0.000	1.000	2.422
Nadolol	91.012	49.004	50.004	0.004	3.000	4.132	0.000	5.000	3.482
Nalidixic acid	49.077	28.077	29.077	-0.923	0.000	24.153	1.000	1.077	3.021
Naphtalene	30.000	18.000	19.000	0.000	0.000	0.000	0.000	0.000	2.112
Naproxen	52.001	30.001	31.001	-0.999	1.000	21.660	0.000	1.000	3.410
Nebivolol	103.066	54.022	57.022	0.022	4.000	3.167	0.000	6.000	5.147
N-Ethylaniline	33.000	20.000	20.000	0.000	0.000	0.210	0.000	2.000	2.369
Nicardipine	115.279	64.760	66.760	0.760	1.760	12.238	2.000	8.481	3.851

Nicotinamide	22.000	15.000	15.000	0.000	0.000	0.980	0.000	1.000	2.115
Nicotine	49.273	26.091	27.091	0.091	1.091	2.692	0.000	1.000	2.501
Nifedipine	74.000	43.000	44.000	0.000	0.000	2.801	2.000	5.000	3.172
Nimodipine	99.000	56.000	57.000	0.000	1.000	2.381	2.000	9.000	3.619
Nisoldipine	92.000	52.000	53.000	0.000	1.000	2.388	2.000	6.000	3.472
Nitrendipine	80.000	46.000	47.000	0.000	1.000	2.326	2.000	6.000	3.459
Nitrobenzene	21.000	14.000	14.000	0.000	0.000	2.527	0.000	0.000	2.055
Norfloxacin	77.994	41.035	43.035	0.035	0.000	52.694	1.000	2.056	3.830
Ofloxacin	88.361	45.144	48.144	-0.856	1.000	34.857	1.000	1.036	3.931
N-Methylbenzylamine	33.008	20.003	20.003	0.003	0.000	0.950	0.000	2.000	2.362
N-methylnaphthalen-1-amine	39.000	23.000	24.000	0.000	0.000	0.315	0.000	1.000	2.436
N-Methylphenethylamine	39.002	23.001	23.001	0.001	0.000	0.805	0.000	3.000	2.720
N-pentane	30.000	17.000	16.000	0.000	0.000	0.003	0.000	2.000	1.973
N-propanol	19.000	12.000	11.000	0.000	0.000	1.702	0.000	1.000	1.568
Ondansetron	79.000	41.000	44.000	0.000	1.000	3.821	0.000	2.000	3.527
Oxazepam	53.000	31.000	33.000	0.000	1.000	2.696	0.000	1.000	3.354
Oxolinic acid	53.443	29.443	31.443	-0.557	0.000	15.678	1.000	1.443	3.292
Oxprenolol	72.009	42.003	42.003	0.003	1.000	2.167	0.000	9.000	3.236
Paracetamol	31.000	20.000	20.000	0.000	0.000	2.919	0.000	1.000	2.642
Pentamethylbenzene	48.000	27.000	27.000	0.000	0.000	0.093	0.000	0.000	2.381
Phenazone	48.000	27.000	28.000	1.000	1.000	8.270	1.000	1.000	2.666
Phenobarbital	49.665	28.332	29.332	-0.668	0.000	6.282	0.000	2.000	2.728
Phenol	19.000	13.000	13.000	0.000	0.000	1.637	0.000	0.000	1.752
Phenylbutazone	78.000	43.000	45.000	0.000	0.000	0.860	0.000	5.000	3.362

Phenylpropanolamine	40.012	24.004	24.004	0.004	2.000	2.124	0.000	2.000	2.445
Phenytoin	53.900	30.950	32.950	-0.050	0.000	2.491	0.000	2.000	2.933
Physostigmine	79.137	41.046	43.046	0.046	2.046	2.136	0.000	2.000	3.576
Pindolol	68.006	38.002	39.002	0.002	1.000	2.613	0.000	6.000	3.257
Pipemidic acid	73.942	38.994	40.994	-0.006	0.000	54.619	1.000	2.019	3.832
Piromidic acid	68.039	36.039	38.039	-0.961	0.000	30.625	1.000	2.039	3.662
Piroxicam	61.000	35.000	37.000	-1.000	0.000	2.426	0.000	2.000	3.678
P-Nitroaniline	24.000	16.000	16.000	0.000	0.000	2.285	0.000	0.000	2.318
Prilocaine	63.410	36.137	36.137	0.137	1.000	4.480	0.000	5.000	3.309
Procaine	64.030	37.010	37.010	0.010	0.000	2.754	0.000	6.970	3.814
Progesterone	114.000	53.000	56.000	0.000	6.000	1.609	1.000	1.000	3.782
Promazine	73.011	40.004	42.004	0.004	0.000	1.202	0.000	3.996	3.048
Promethazine	73.031	40.010	42.010	0.010	1.000	1.501	0.000	2.990	3.171
Propionitrile	13.000	9.000	8.000	0.000	0.000	2.298	0.000	0.000	1.493
Propiophenone	33.000	20.000	20.000	0.000	0.000	2.484	0.000	2.000	2.433
Propofol	55.000	31.000	31.000	0.000	0.000	1.547	0.000	0.000	2.707
Propranolol	71.009	40.003	41.003	0.003	1.000	2.366	0.000	6.000	3.366
P-toluidine	27.000	17.000	17.000	0.000	0.000	0.356	0.000	0.000	2.019
Pyridine	16.000	11.000	11.000	0.000	0.000	1.336	0.000	0.000	1.483
Ranitidine	74.125	43.042	43.042	0.042	0.000	4.390	1.000	8.958	4.163
Risperidone	114.051	57.017	61.017	0.017	0.000	3.616	1.000	4.000	5.736
Rufloxacin	82.261	42.109	45.109	-0.891	0.000	35.473	1.000	1.032	3.918
Salicylic acid	22.000	15.000	15.000	-1.000	0.000	15.482	0.000	0.000	2.148
Sotalol	67.024	38.008	38.008	0.008	1.000	5.743	0.000	6.000	4.033

Sulindac	72.003	41.003	43.003	-0.997	1.000	22.487	2.000	3.003	4.328
Temazepam	59.000	34.000	36.000	0.000	1.000	2.918	0.000	1.000	3.382
Terbutaline	60.000	35.000	35.000	0.000	1.000	2.607	0.000	3.000	3.487
Tert- butyl alcohol	25.000	15.000	14.000	0.000	0.000	1.708	0.000	0.000	1.493
Tetracaine	76.092	43.031	43.031	0.031	0.000	3.609	0.000	8.969	4.704
Tetrachloro ethane	12.000	8.000	7.000	0.000	0.000	1.436	0.000	0.000	1.949
Tetrahydrofurane	25.000	13.000	13.000	0.000	0.000	2.035	0.000	0.000	1.367
Theobromine	36.997	20.999	21.999	-0.001	0.000	1.892	0.000	0.000	2.384
Theophylline	37.000	21.000	22.000	0.000	0.000	1.318	0.000	0.000	2.358
Thiopental	61.861	33.431	33.431	-0.569	1.000	8.671	0.000	2.000	2.959
Timolol	84.047	45.016	46.016	0.016	1.000	2.572	0.000	6.000	3.516
Tocainide	51.410	30.137	30.137	0.137	1.000	5.069	0.000	2.000	2.779
Tolfenamic acid	48.002	29.002	30.002	-0.998	0.000	19.524	0.000	2.002	3.439
Tolmetin	57.000	33.000	34.000	-1.000	0.000	27.769	0.000	3.000	3.712
Toluene	24.000	15.000	15.000	0.000	0.000	0.122	0.000	0.000	1.781
Tramadol	83.143	44.048	45.048	0.048	2.000	3.152	0.000	3.952	3.230
Trimecaine	75.854	42.285	42.285	0.285	0.000	6.375	0.000	4.146	3.551
Tropisetron	82.037	41.012	44.012	0.012	2.000	0.790	0.000	3.000	3.982
Verapamil	128.037	71.012	72.012	0.012	1.012	2.374	0.000	11.975	4.635
W 36017	57.854	33.285	33.285	0.285	0.000	6.718	0.000	2.715	3.050

Table 4A. Weighted average at pH 7.0, according to each analyte's pKa, of number of angles, atoms, bonds, charge, chiral atoms, dipole, E-Z bonds, flexible torsions and gyrrad for the whole dataset.

Analyte	HbAcc	HbDon	HeavyAtoms	Impropers	Lipole	Mass	MassMI	Ovality	Psa	Rings
1,2 -dichloroethane	0.000	0.000	4.000	0.000	0.451	98.959	97.969	1.228	0.000	0.000
1,2,4,5-tetrachlorobenzene	0.000	0.000	10.000	0.000	0.000	215.892	213.891	1.351	0.000	1.000
1,3-dichlorobenzene	0.000	0.000	8.000	0.000	0.720	147.002	145.969	1.285	0.000	1.000
1-chloro butane	0.000	0.000	5.000	0.000	0.304	92.567	92.039	1.332	0.000	0.000
1-chloro-2-nitrobenzene	2.000	0.000	10.000	3.000	2.301	157.555	156.993	1.287	39.048	1.000
1-hexanol	1.000	1.000	7.000	0.000	4.537	102.175	102.105	1.450	23.720	0.000
1-Naphthylamine	0.000	2.000	11.000	3.000	3.223	143.185	143.074	1.306	25.477	2.000
1-nitrobutane	3.000	0.000	7.000	3.000	3.335	103.120	103.063	1.344	41.722	0.000
1-pentanol	1.000	1.000	6.000	0.000	3.910	88.148	88.089	1.384	23.183	0.000
2-Aminobiphenyl	0.000	2.000	13.000	3.000	2.681	169.222	169.089	1.388	25.477	2.000
2-Chloroaniline	0.000	2.000	8.000	3.000	2.574	127.572	127.019	1.262	26.147	1.000
2-methyl-2 butanol	1.000	1.000	6.000	0.000	1.859	88.148	88.089	1.365	22.019	0.000
2-phenylethyl acetate	2.000	0.000	12.000	3.000	3.689	164.201	164.084	1.462	28.316	1.000
2-Phenylethylamine	0.998	2.002	9.000	2.995	4.104	121.181	121.091	1.409	28.352	1.000
3-chloro phenol	1.000	1.000	8.000	0.000	3.604	128.556	128.003	1.252	22.648	1.000
4-chlorobenzylalcohol	1.000	1.000	9.000	0.000	4.198	142.583	142.019	1.334	23.632	1.000
4-Methylbenzylamine	0.996	2.004	9.000	2.988	3.920	121.184	121.093	1.393	28.438	1.000
4-nitroaniline	2.000	2.000	10.000	6.000	1.521	138.124	138.043	1.293	66.784	1.000
Acebutolol	4.998	3.002	24.000	11.994	2.167	336.428	336.207	1.803	89.649	1.000
Acetonitrile	0.000	0.000	3.000	0.000	2.284	41.052	41.027	1.130	18.823	0.000
Acetophenone	1.000	0.000	9.000	3.000	2.481	120.149	120.058	1.291	17.563	1.000
Acetylsalicylic acid	4.000	0.000	13.000	6.000	2.104	179.150	179.035	1.401	59.860	1.000
Acridine	1.000	0.000	14.000	0.000	1.463	179.217	179.074	1.372	10.623	3.000

Alprazolam	3.000	0.000	22.000	3.000	2.690	308.765	308.083	1.550	38.391	4.000
Alprenolol	2.997	2.003	18.000	8.992	2.948	249.351	249.175	1.707	45.127	1.000
Aminophenazone	1.000	0.997	17.000	15.009	0.656	232.298	232.142	1.572	32.602	2.000
Amitriptyline	0.993	0.007	21.000	8.980	2.286	277.410	277.190	1.641	4.873	3.000
Amlodipine	5.992	3.008	28.000	23.976	1.442	408.884	408.153	1.831	104.606	2.000
Amoxicillin	5.374	4.626	25.000	16.121	3.015	365.028	364.728	1.712	162.547	3.000
Aniline	0.000	2.000	7.000	3.000	2.953	93.126	93.058	1.225	27.041	1.000
Anisole	1.000	0.000	8.000	0.000	2.039	108.138	108.058	1.273	11.291	1.000
Atenolol	3.996	4.004	19.000	8.989	1.951	266.340	266.167	1.713	91.296	1.000
Benzene	0.000	0.000	6.000	0.000	0.000	78.112	78.047	1.192	0.000	1.000
Benzyl cyanide	0.000	0.000	9.000	0.000	3.918	117.148	117.058	1.313	18.636	1.000
Benzylalcohol	1.000	1.000	8.000	0.000	3.254	108.138	108.058	1.317	23.453	1.000
Benzylamine	0.995	2.005	8.000	2.985	3.276	107.158	107.078	1.337	29.076	1.000
Benzylbenzoate	2.000	0.000	16.000	3.000	2.381	212.244	212.084	1.499	26.345	2.000
Benzylmethylketon	1.000	0.000	10.000	3.000	4.521	134.175	134.073	1.405	17.743	1.000
Betahistine	1.999	1.001	10.000	2.998	1.921	136.195	136.101	1.423	25.825	1.000
Betaxolol	3.996	2.004	22.000	2.988	1.963	307.432	307.219	1.821	59.101	2.000
Bibenzyl	0.000	0.000	14.000	0.000	0.000	182.261	182.110	1.504	0.000	2.000
Biperidene	2.000	1.000	23.000	9.000	1.056	311.461	311.225	1.676	23.479	4.000
Biphenyl	0.000	0.000	12.000	0.000	0.000	154.208	154.078	1.358	0.000	2.000
Bromazepam	3.000	1.000	19.000	9.000	2.881	316.153	315.001	1.501	50.198	3.000
Bromperidol	2.916	1.084	26.000	5.749	0.935	420.399	419.174	1.741	40.298	3.000
Bupivacaine	1.926	1.074	21.000	8.779	1.209	288.502	288.294	1.731	33.573	2.000
Buprenorphine	4.953	2.047	34.000	2.860	1.366	467.687	467.351	1.810	61.916	7.000

Butylacetate	2.000	1.000	8.000	3.000	3.352	116.158	116.084	1.436	40.323	0.000
Caffeine	3.000	0.000	14.000	12.000	0.293	194.191	194.080	1.430	54.408	2.000
Carbamazepine	1.000	2.000	18.000	15.000	3.044	236.269	236.095	1.442	44.516	3.000
Carbamazepine epoxide	2.000	2.000	19.000	9.000	2.483	252.268	252.090	1.471	57.360	4.000
Carbon tetrachloride	0.000	0.000	5.000	0.000	0.000	153.823	151.875	1.241	0.000	0.000
Cefalexin	4.372	3.629	24.000	22.115	2.495	347.015	346.720	1.677	137.693	3.000
Chlorambucil	2.000	0.007	19.000	6.000	5.626	303.211	302.078	1.694	38.706	1.000
Chloroform	0.000	0.000	4.000	0.000	0.456	119.378	117.914	1.191	0.000	0.000
Chlorpromazine	0.996	0.004	21.000	5.988	2.069	318.868	318.100	1.646	30.327	3.000
Cimetidine	2.000	3.613	17.000	7.161	2.354	252.957	252.734	1.655	104.725	1.000
Cinnoxacin	6.000	0.000	19.000	12.000	3.011	261.210	261.051	1.493	89.973	3.000
Ciprofloxacin	3.021	2.033	24.000	18.062	0.695	331.375	331.167	1.659	77.877	4.000
Clobazam	2.000	0.000	21.000	12.000	3.156	300.740	300.067	1.561	39.090	3.000
Clonidine	1.000	2.087	14.000	8.738	2.295	230.182	229.105	1.498	42.301	2.000
Clorazepate	4.000	1.000	22.000	12.000	4.689	313.715	313.038	1.562	75.433	3.000
Codeine	3.942	1.058	22.000	8.826	1.661	299.423	299.211	1.562	47.359	5.000
Cotinine	2.000	0.000	13.000	6.000	1.718	176.215	176.095	1.439	32.071	2.000
Delorazepam	2.000	1.000	20.000	9.000	3.353	305.159	304.017	1.536	42.804	3.000
Desipramine	1.000	1.000	20.000	5.999	2.727	266.381	266.179	1.640	18.293	3.000
Dextromethorphan	1.952	0.048	20.000	2.857	2.154	271.445	271.242	1.594	15.984	4.000
Diazepam	2.000	0.000	20.000	9.000	2.904	284.740	284.072	1.566	31.368	3.000
Dichloromethane	0.000	0.000	3.000	0.000	0.450	84.933	83.953	1.163	0.000	0.000
Diclofenac	2.000	1.003	19.000	6.000	4.138	295.144	294.012	1.555	43.137	2.000
Diethylether	1.000	0.000	5.000	0.000	0.294	74.122	74.073	1.329	10.574	0.000

Diflunisal	3.000	1.000	18.000	3.000	5.496	249.190	249.036	1.468	52.920	2.000
Diltiazem	4.760	0.240	29.000	11.279	1.785	414.760	414.403	1.826	85.321	3.000
Diphenhydramine	1.992	0.008	19.000	2.976	2.763	255.363	255.170	1.660	15.111	2.000
Dipropyl ether	1.000	0.000	7.000	0.000	0.494	102.175	102.105	1.441	10.574	0.000
Domperidone	2.888	2.112	30.000	20.665	2.261	426.024	425.275	1.791	76.348	5.000
Epinephrine	3.975	4.025	13.000	2.925	0.945	183.230	183.115	1.489	83.185	1.000
Ethanol	1.000	1.000	3.000	0.000	1.924	46.068	46.042	1.190	23.541	0.000
Ethylacetate	2.000	0.000	6.000	3.000	1.902	88.105	88.052	1.320	27.600	0.000
Ethylbenzoate	2.000	0.000	11.000	3.000	1.703	150.175	150.068	1.400	26.704	1.000
Etidocaine	1.834	1.166	20.000	8.501	0.868	276.585	276.388	1.739	32.120	1.000
Felodipine	4.000	1.000	25.000	21.000	1.621	384.254	383.069	1.742	66.691	2.000
Fenbufen	3.000	0.003	19.000	6.000	5.988	253.276	253.090	1.550	51.631	2.000
Flufenamic Acid	2.000	1.001	20.000	6.000	4.841	280.223	280.059	1.524	43.581	2.000
Flumazenil	4.000	0.000	22.000	9.000	1.379	303.288	303.102	1.595	61.799	3.000
Flumequine	3.000	0.092	19.000	15.000	4.143	260.333	260.165	1.493	54.500	3.000
Fluphenazine	2.776	1.224	30.000	8.329	4.688	437.747	437.400	1.802	59.046	4.000
Flurazepam	3.000	0.000	27.000	12.000	1.878	387.878	387.151	1.752	33.798	3.000
Flurbiprofen	2.000	0.004	18.000	3.000	5.512	243.257	243.086	1.548	35.146	2.000
Fluvastatin	4.000	2.002	30.000	9.000	3.641	410.460	410.179	1.809	80.851	3.000
Furosemide	6.000	3.000	21.000	9.000	3.595	329.737	329.000	1.617	123.282	2.000
GEA 968	2.834	2.166	21.000	14.501	2.238	291.556	291.362	1.746	61.407	1.000
Granisetron	2.996	1.004	23.000	8.988	0.528	312.413	312.199	1.662	48.832	4.000
Griseofulvin	6.000	0.000	24.000	12.000	2.445	352.766	352.071	1.699	80.290	3.000
Haloperidol	2.916	1.084	26.000	5.749	0.712	375.948	375.224	1.741	40.076	3.000

Heptane	0.000	0.000	7.000	0.000	0.006	100.202	100.125	1.469	0.000	0.000
Hexobarbital	3.059	0.941	17.000	20.822	1.215	236.207	236.056	1.540	68.149	2.000
Hydrochlorothiazide	6.000	4.000	17.000	9.000	2.171	297.739	296.965	1.519	141.531	2.000
Hydrocortisone	5.000	3.000	26.000	12.000	1.641	362.460	362.209	1.683	96.350	4.000
Hydroxyzine	3.737	1.263	26.000	5.211	3.563	375.169	374.441	1.774	43.628	3.000
Ibuprofen	2.000	0.016	15.000	3.000	4.793	205.289	205.139	1.574	34.852	1.000
Imipramine	0.997	0.003	21.000	5.990	2.300	280.411	280.197	1.670	7.467	3.000
Indomethacin	4.000	0.003	25.000	6.000	3.694	356.783	356.072	1.681	66.612	3.000
Indoprofen	3.000	0.004	21.000	9.000	2.287	280.302	280.101	1.595	55.145	3.000
Isosorbide dinitrate	10.000	0.000	16.000	6.000	0.061	236.136	236.028	1.495	129.000	2.000
Isotretinoin	2.000	0.006	22.000	33.000	4.716	299.433	299.207	1.763	34.796	1.000
Isradipine	7.000	1.000	27.000	21.000	1.229	371.387	371.148	1.739	103.147	3.000
Ketamine	1.224	1.776	16.000	3.672	2.170	238.508	237.874	1.496	33.471	2.000
Ketoprofen	3.000	0.004	19.000	6.000	1.362	253.277	253.090	1.542	51.992	2.000
Labetalol	3.000	5.920	24.000	7.443	2.308	329.333	329.106	1.751	102.249	2.000
Lacidipine	6.000	1.000	33.000	30.000	1.801	455.543	455.231	1.935	91.954	2.000
Levosulpiride	5.039	3.961	23.000	9.116	1.127	342.395	342.110	1.734	112.754	2.000
Lidocaine	1.888	1.112	17.000	8.665	1.691	234.450	234.286	1.661	33.354	1.000
Loratadine	3.000	0.000	27.000	12.000	2.095	382.883	382.145	1.721	40.473	4.000
Lorazepam	3.000	2.000	21.000	9.000	4.278	321.158	320.012	1.546	63.414	3.000
Mefenamic Acid	2.000	1.002	18.000	6.000	4.493	240.279	240.104	1.518	42.505	2.000
Mepivacaine	1.799	1.201	18.000	8.398	1.953	246.550	246.376	1.621	34.556	2.000
Mepyramine	2.986	0.014	21.000	5.958	1.962	285.398	285.198	1.717	28.700	2.000
Mesitylene	0.000	0.000	9.000	0.000	0.013	120.192	120.094	1.390	0.000	1.000

Metadone	1.989	0.011	23.000	5.966	1.718	309.457	309.221	1.720	18.978	2.000
Methohexital	3.018	0.982	19.000	20.945	1.887	262.286	262.113	1.617	66.373	1.000
Methylacetate	2.000	1.000	5.000	3.000	1.470	74.079	74.037	1.241	39.762	0.000
Methylsulfoxide	1.000	0.000	4.000	3.000	1.153	78.133	78.014	1.236	33.001	0.000
Metoclopramide	2.995	3.005	20.000	11.984	0.493	299.802	299.145	1.723	67.531	1.000
Metoprolol	3.998	2.002	19.000	2.994	1.705	267.366	267.185	1.743	56.723	1.000
Mianserin	0.948	0.052	20.000	5.844	2.674	264.417	264.215	1.570	8.621	4.000
Midazolam	2.290	0.000	23.968	3.000	2.104	338.236	337.583	1.611	29.092	3.806
Morphine	3.947	2.053	21.000	8.840	1.613	285.391	285.190	1.504	58.238	5.000
N,N-Dimethylaniline	0.000	0.000	9.000	3.000	1.380	121.180	121.089	1.348	3.355	1.000
N,N-Dimethyl-p-toluidine	0.000	0.000	10.000	3.000	2.023	135.206	135.105	1.411	3.727	1.000
Nadolol	4.996	4.004	22.000	2.988	0.386	309.405	309.198	1.720	86.861	2.000
Nalidixic acid	4.000	0.077	17.000	15.000	4.074	231.304	231.154	1.506	64.531	2.000
Naphtalene	0.000	0.000	10.000	0.000	0.000	128.171	128.063	1.298	0.000	2.000
Naproxen	3.000	0.001	17.000	3.000	3.843	229.253	229.088	1.523	46.067	2.000
Nebivolol	4.978	3.022	29.000	2.934	1.817	405.457	405.197	1.785	79.300	4.000
N-Ethylaniline	0.000	1.000	9.000	3.000	1.187	121.180	121.089	1.368	13.596	1.000
Nicardipine	6.240	1.760	35.000	24.721	0.930	480.291	479.971	1.918	113.888	3.000
Nicotinamide	2.000	2.000	9.000	6.000	2.190	122.125	122.048	1.260	53.111	1.000
Nicotine	1.909	0.091	12.000	2.727	0.908	162.323	162.207	1.434	15.742	2.000
Nifedipine	6.000	1.000	25.000	24.000	0.973	346.335	346.117	1.691	102.261	2.000
Nimodipine	7.000	1.000	30.000	24.000	0.821	418.440	418.174	1.854	116.342	2.000
Nisoldipine	6.000	1.000	28.000	24.000	1.759	388.414	388.163	1.782	102.042	2.000
Nitrendipine	6.000	1.000	26.000	24.000	1.279	360.361	360.132	1.741	107.433	2.000

Nitrobenzene	2.000	0.000	9.000	3.000	2.277	123.109	123.032	1.241	39.743	1.000
Norfloxacin	3.021	2.035	23.000	18.062	0.975	319.366	319.168	1.648	77.483	3.000
Ofloxacin	4.892	0.144	26.000	20.675	1.839	360.505	360.281	1.673	74.209	4.000
N-Methylbenzylamine	0.997	1.003	9.000	2.992	2.214	121.182	121.092	1.367	16.178	1.000
N-methylnaphthalen-1-amine	0.000	1.000	12.000	3.000	2.421	157.212	157.089	1.355	13.038	2.000
N-Methylphenethylamine	0.999	1.001	10.000	2.998	3.124	135.207	135.106	1.432	16.133	1.000
N-pentane	0.000	0.000	5.000	0.000	0.007	72.149	72.094	1.362	0.000	0.000
N-propanol	1.000	1.000	4.000	0.000	2.635	60.095	60.058	1.243	23.497	0.000
Ondansetron	2.000	0.000	22.000	3.000	1.510	293.363	293.153	1.633	34.776	4.000
Oxazepam	3.000	2.000	20.000	9.000	4.242	286.713	286.051	1.522	63.167	3.000
Oxolinic acid	5.000	0.443	19.000	15.000	2.617	260.668	260.502	1.500	79.630	3.000
Oxprenolol	3.997	2.003	19.000	8.991	2.371	265.351	265.171	1.721	57.495	1.000
Paracetamol	2.000	2.000	11.000	6.000	1.590	151.163	151.063	1.394	54.205	1.000
Pentamethylbenzene	0.000	0.000	11.000	0.000	0.007	148.245	148.125	1.451	0.000	1.000
Phenazone	1.000	1.000	14.000	12.000	1.439	189.234	189.103	1.478	30.488	2.000
Phenobarbital	3.668	1.332	17.000	12.997	1.901	231.562	231.412	1.480	79.591	2.000
Phenol	1.000	1.000	7.000	0.000	2.996	94.111	94.042	1.203	22.580	1.000
Phenylbutazone	2.000	0.000	23.000	12.000	1.684	308.374	308.153	1.699	42.775	3.000
Phenylpropanolamine	1.996	3.004	11.000	2.988	3.580	151.210	151.104	1.426	48.618	1.000
Phenytoin	2.050	1.950	19.000	11.850	2.697	252.218	252.040	1.504	65.058	3.000
Physostigmine	2.954	1.046	20.000	11.863	0.794	275.392	275.209	1.645	50.066	3.000
Pindolol	2.998	3.002	18.000	2.994	2.941	248.323	248.155	1.662	63.366	2.000
Pipemidic acid	5.026	1.994	22.000	18.077	0.931	303.310	303.127	1.630	96.773	3.000
Piromidic acid	5.000	0.039	21.000	18.000	4.865	287.333	287.154	1.612	77.571	3.000

Piroxicam	5.000	1.000	23.000	15.000	0.565	330.339	330.055	1.602	103.648	3.000
P-Nitroaniline	2.000	2.000	10.000	6.000	1.521	138.124	138.043	1.293	66.784	1.000
Prilocaine	1.863	2.137	16.000	8.590	2.238	220.449	220.295	1.628	44.204	1.000
Procaine	2.990	2.010	17.000	8.970	0.803	236.320	236.162	1.659	59.333	1.000
Progesterone	2.000	0.000	23.000	12.000	1.116	314.462	314.225	1.669	34.768	4.000
Promazine	0.996	0.004	20.000	5.989	1.775	284.423	284.138	1.615	29.699	3.000
Promethazine	0.990	0.010	20.000	5.969	2.499	284.430	284.145	1.600	30.051	3.000
Propionitrile	0.000	0.000	4.000	0.000	2.770	55.079	55.042	1.180	18.450	0.000
Propiophenone	1.000	0.000	10.000	3.000	1.804	134.175	134.073	1.371	16.488	1.000
Propofol	1.000	1.000	13.000	0.000	2.200	178.271	178.136	1.540	21.526	1.000
Propranolol	2.997	2.003	19.000	2.991	3.277	259.347	259.160	1.664	45.756	2.000
P-toluidine	0.000	2.000	8.000	3.000	3.657	107.153	107.074	1.303	26.818	1.000
Pyridine	1.000	0.000	6.000	0.000	1.646	79.100	79.042	1.156	10.809	1.000
Ranitidine	3.958	2.042	21.000	17.875	1.407	314.446	314.183	1.755	100.897	1.000
Risperidone	4.983	0.017	30.000	17.949	2.080	410.502	410.229	1.799	58.633	5.000
Rufloxacin	3.924	0.109	25.000	20.771	1.982	362.508	362.207	1.660	84.127	4.000
Salicylic acid	3.000	1.000	10.000	3.000	3.482	137.113	137.024	1.270	52.853	1.000
Sotalol	3.992	3.008	18.000	5.976	1.961	272.372	272.127	1.673	91.706	1.000
Sulindac	3.000	0.003	25.000	18.000	0.834	355.406	355.084	1.688	68.143	3.000
Temazepam	3.000	1.000	21.000	9.000	3.686	300.740	300.067	1.568	51.217	3.000
Terbutaline	4.000	4.000	16.000	3.000	2.075	225.284	225.137	1.604	81.732	1.000
Tert- butyl alcohol	1.000	1.000	5.000	0.000	1.873	74.122	74.073	1.299	22.489	0.000
Tetracaine	2.969	1.031	19.000	8.908	3.896	264.394	264.215	1.738	46.796	1.000
Tetrachloro ethane	0.000	0.000	6.000	0.000	0.432	167.849	165.891	1.291	0.000	0.000

Tetrahydrofurane	1.000	0.000	5.000	0.000	1.568	72.106	72.058	1.207	11.112	1.000
Theobromine	3.001	0.999	13.000	11.996	0.538	180.163	180.063	1.397	67.971	2.000
Theophylline	3.000	1.000	13.000	12.000	0.203	180.164	180.065	1.389	67.548	2.000
Thiopental	2.569	1.431	16.000	13.292	2.035	241.764	241.535	1.560	86.820	1.000
Timolol	5.984	2.016	21.000	5.953	1.590	316.435	316.173	1.731	107.743	2.000
Tocainide	1.863	3.137	14.000	8.590	3.673	192.395	192.264	1.531	57.757	1.000
Tolfenamic acid	2.000	1.002	18.000	6.000	4.666	260.697	260.049	1.505	42.832	2.000
Tolmetin	3.000	0.000	19.000	6.000	3.594	256.277	256.098	1.575	54.978	2.000
Toluene	0.000	0.000	7.000	0.000	0.017	92.138	92.063	1.254	0.000	1.000
Tramadol	2.952	1.048	19.000	2.857	1.496	263.423	263.237	1.655	35.511	2.000
Trimecaine	1.715	1.285	18.000	8.146	2.287	248.651	248.476	1.688	34.461	1.000
Tropisetron	2.988	1.012	21.000	5.963	1.874	284.365	284.165	1.606	46.486	4.000
Verapamil	4.988	0.012	33.000	2.963	1.887	454.614	454.296	1.994	67.601	2.000
W 36017	1.715	1.285	15.000	8.146	2.543	206.571	206.429	1.583	35.079	1.000

Table 4B. Weighted average at pH 7.0, according to each analyte's pKa, of number of H-bond acceptor group, H-bond donor group, of heavy atoms, of impropers, lipole, mass, monoisotopic mass, ovality, polar surface area (Psa) and number of rings for the whole dataset.

Analyte	**Sas**	**Sav**	**Sdiam**	**Surface**	**Torsions**	**Vdiam**	**VirtualLogP**	**Volume**
1,2 -dichloroethane	233.344	305.700	5.761	104.280	1.000	5.199	1.995	73.599
1,2,4,5-tetrachlorobenzene	339.068	497.136	7.542	178.705	6.000	6.490	5.471	143.103
1,3-dichlorobenzene	289.864	409.540	6.821	146.177	6.000	6.017	3.766	114.083
1-chloro butane	282.837	383.603	6.528	133.863	2.000	5.657	2.926	94.774

1-chloro-2-nitrobenzene	293.357	421.938	6.962	152.254	7.000	6.135	3.409	120.925
1-hexanol	336.936	476.368	7.418	172.874	5.000	6.161	2.493	122.440
1-Naphthylamine	318.338	471.535	7.346	169.541	12.000	6.428	2.139	139.082
1-nitrobutane	297.846	410.190	6.710	141.449	3.000	5.787	2.372	101.499
1-pentanol	303.155	417.441	6.883	148.853	4.000	5.851	1.982	104.881
2-Aminobiphenyl	381.088	573.543	8.047	203.444	14.000	6.831	2.827	166.876
2-Chloroaniline	286.895	402.690	6.642	138.582	7.000	5.911	1.898	108.157
2-methyl-2 butanol	272.901	387.763	6.867	148.135	0.000	5.877	1.419	106.298
2-phenylethyl acetate	387.608	581.301	8.193	210.906	10.000	6.777	2.464	162.994
2-Phenylethylamine	325.337	470.412	7.483	175.935	8.998	6.304	1.625	131.172
3-chloro phenol	281.257	393.474	6.561	135.250	7.000	5.864	2.349	105.578
4-chlorobenzylalcohol	314.148	449.687	7.138	160.065	8.000	6.180	2.021	123.591
4-Methylbenzylamine	334.327	478.146	7.445	174.122	7.996	6.308	1.736	131.425
4-nitroaniline	299.201	426.160	6.849	147.379	8.000	6.022	1.690	114.364
Acebutolol	629.024	1056.416	11.532	417.814	18.000	8.587	2.483	331.583
Acetonitrile	189.979	225.452	4.730	70.297	0.000	4.451	0.453	46.165
Acetophenone	303.809	434.254	6.924	150.614	7.000	6.093	1.407	118.452
Acetylsalicylic acid	358.676	533.707	7.880	195.064	9.000	6.657	0.434	154.494
Acridine	364.759	548.793	8.039	203.048	16.000	6.864	3.763	169.365
Alprazolam	524.471	844.911	9.880	306.655	23.000	7.937	3.815	261.769
Alprenolol	501.890	823.331	10.360	337.183	16.000	7.930	3.397	261.136
Aminophenazone	450.442	725.877	9.502	283.649	13.000	7.579	-1.155	227.952
Amitriptyline	536.062	891.151	10.481	345.080	20.993	8.181	3.956	286.696
Amlodipine	642.019	1119.597	12.021	453.946	22.992	8.884	1.994	367.114

Amoxicillin	549.552	944.305	10.987	379.215	20.374	8.398	-3.624	310.083
Aniline	261.555	358.708	6.216	121.394	7.000	5.615	1.137	92.710
Anisole	282.913	397.347	6.686	140.418	7.000	5.926	2.188	108.959
Atenolol	526.146	850.762	10.399	339.717	16.000	7.945	1.340	262.563
Benzene	238.870	321.014	5.951	111.247	6.000	5.450	2.136	84.761
Benzyl cyanide	316.263	444.275	6.965	152.385	8.000	6.078	1.977	117.558
Benzylalcohol	286.366	403.429	6.843	147.107	8.000	5.962	1.325	110.960
Benzylamine	297.123	418.789	6.957	152.058	7.995	6.016	1.185	114.021
Benzylbenzoate	453.976	686.551	8.882	247.834	16.000	7.254	3.197	199.839
Benzylmethylketon	343.545	497.411	7.623	182.562	9.000	6.431	2.235	139.249
Betahistine	360.364	521.201	7.716	187.028	9.000	6.468	1.021	141.696
Betaxolol	607.925	1041.440	11.398	408.170	21.000	8.448	3.497	315.671
Bibenzyl	434.540	661.026	8.751	240.598	15.000	7.135	4.527	190.167
Biperidene	589.294	988.409	10.976	378.465	22.000	8.479	3.227	319.182
Biphenyl	354.685	527.046	7.759	189.150	13.000	6.658	3.564	154.549
Bromazepam	477.100	745.741	9.284	270.782	19.000	7.578	1.988	227.888
Bromperidol	651.919	1077.749	11.501	415.560	25.000	8.718	3.757	346.907
Bupivacaine	568.524	957.830	10.989	379.354	18.000	8.353	4.325	305.189
Buprenorphine	686.359	1270.678	12.868	520.195	32.000	9.566	4.140	458.303
Butylacetate	335.005	473.734	7.427	173.281	5.000	6.197	2.211	124.618
Caffeine	376.477	573.756	8.137	208.022	10.000	6.805	-0.226	164.995
Carbamazepine	426.195	677.095	8.843	245.662	19.000	7.364	2.141	209.112
Carbamazepine epoxide	439.091	702.241	9.108	260.631	21.000	7.509	1.392	221.680
Carbon tetrachloride	247.840	334.073	6.089	116.494	0.000	5.467	2.161	85.565

Cefalexin	567.415	936.987	10.702	359.811	20.372	8.263	-2.582	295.409
Chlorambucil	541.774	863.871	10.408	340.316	15.007	7.996	3.604	267.713
Chloroform	226.795	294.540	5.591	98.213	0.000	5.124	2.384	70.424
Chlorpromazine	528.133	888.981	10.506	346.749	19.996	8.188	5.245	287.409
Cimetidine	523.091	809.303	9.838	304.071	14.000	7.648	-1.086	234.259
Cinnoxacin	453.396	700.554	8.992	254.042	17.000	7.359	-1.599	208.694
Ciprofloxacin	550.119	907.574	10.579	351.600	23.054	8.212	-3.081	290.003
Clobazam	504.634	824.067	9.813	302.530	19.000	7.855	2.853	253.804
Clonidine	414.599	627.218	8.620	233.447	13.000	7.043	2.231	182.901
Clorazepate	507.294	811.185	9.827	303.371	20.000	7.862	1.432	254.446
Codeine	496.857	839.635	10.097	320.259	24.000	8.079	1.155	276.106
Cotinine	374.993	571.211	8.199	211.183	12.000	6.834	0.773	167.129
Delorazepam	486.159	781.072	9.634	291.569	19.000	7.774	3.135	245.965
Desipramine	525.573	856.351	10.292	332.776	21.000	8.037	3.394	271.815
Dextromethorphan	505.842	850.260	10.198	326.698	21.000	8.077	3.593	275.885
Diazepam	493.368	783.481	9.766	299.633	19.000	7.805	2.474	248.944
Dichloromethane	203.406	252.972	5.149	83.293	0.000	4.775	1.511	57.010
Diclofenac	471.639	760.992	9.578	288.176	16.003	7.680	3.439	237.158
Diethylether	282.603	374.991	6.360	127.082	2.000	5.517	1.666	87.917
Diflunisal	426.665	659.321	8.780	242.196	15.000	7.247	2.291	199.280
Diltiazem	699.576	1191.957	12.107	460.491	24.760	8.961	2.948	376.704
Diphenhydramine	531.012	855.870	10.248	329.948	17.992	7.955	3.520	263.591
Dipropyl ether	344.205	478.414	7.400	172.027	4.000	6.165	2.689	122.661
Domperidone	687.911	1157.672	11.924	446.651	31.000	8.909	2.237	370.227

Epinephrine	397.988	600.289	8.440	223.810	12.000	6.917	-0.311	173.255
Ethanol	202.585	249.582	5.130	82.693	1.000	4.704	0.392	54.485
Ethylacetate	277.992	371.953	6.395	128.471	2.000	5.566	1.182	90.304
Ethylbenzoate	355.006	516.655	7.746	188.501	9.000	6.546	2.218	146.857
Etidocaine	536.612	912.479	10.936	375.733	13.501	8.294	3.930	298.696
Felodipine	579.794	992.879	11.260	398.336	18.000	8.532	3.798	325.194
Fenbufen	504.744	783.880	9.498	283.422	17.003	7.629	1.997	232.458
Flufenamic Acid	467.508	732.355	9.305	271.984	15.001	7.538	2.996	224.251
Flumazenil	521.286	836.252	9.966	312.045	19.000	7.891	2.494	257.307
Flumequine	441.235	699.386	9.123	261.483	16.092	7.465	0.593	217.861
Fluphenazine	723.924	1207.489	12.115	461.098	29.000	9.025	3.814	384.904
Flurazepam	644.044	1081.240	11.572	420.672	24.000	8.743	3.593	349.879
Flurbiprofen	468.804	731.308	9.302	271.823	13.004	7.475	3.054	218.734
Fluvastatin	643.702	1139.932	12.068	457.511	27.002	8.972	2.808	378.091
Furosemide	514.089	813.105	9.919	309.067	17.000	7.799	1.407	248.424
GEA 968	599.340	969.067	10.932	375.429	14.501	8.273	2.513	296.474
Granisetron	580.545	955.867	10.705	360.013	23.000	8.303	2.260	299.740
Griseofulvin	553.771	924.759	10.786	365.457	19.000	8.275	2.579	296.656
Haloperidol	644.612	1065.566	11.455	412.213	25.000	8.681	3.550	342.537
Heptane	355.619	505.527	7.616	182.245	4.000	6.284	4.378	129.944
Hexobarbital	435.223	700.174	9.260	269.379	13.000	7.462	1.501	217.544
Hydrochlorothiazide	427.153	671.520	9.047	257.116	13.000	7.341	-0.124	207.103
Hydrocortisone	566.319	999.508	11.335	403.624	25.000	8.738	0.020	349.305
Hydroxyzine	673.889	1121.456	11.687	429.099	27.000	8.774	2.344	353.657

Ibuprofen	438.241	691.883	9.258	269.296	7.016	7.380	2.475	210.468
Imipramine	556.084	916.767	10.592	352.462	20.997	8.197	4.092	288.430
Indomethacin	585.399	957.365	10.813	367.330	21.003	8.340	3.314	303.778
Indoprofen	524.148	829.180	9.872	306.168	17.004	7.816	1.167	249.971
Isosorbide dinitrate	388.181	593.259	8.481	225.953	13.000	6.936	0.274	174.717
Isotretinoin	625.782	1025.776	11.238	396.756	15.006	8.463	2.416	317.320
Isradipine	557.847	986.468	11.319	402.534	22.000	8.583	3.174	331.076
Ketamine	437.586	710.782	9.237	268.059	14.000	7.553	1.023	225.579
Ketoprofen	473.946	753.126	9.468	281.643	14.004	7.625	1.410	232.135
Labetalol	579.683	988.597	11.226	395.940	22.920	8.485	-0.777	319.839
Lacidipine	709.027	1288.342	13.099	539.074	23.000	9.418	3.536	437.389
Levosulpiride	592.470	985.276	11.036	382.627	19.000	8.381	-1.224	308.229
Lidocaine	489.165	797.578	10.038	316.562	11.665	7.789	2.646	247.445
Loratadine	647.089	1086.846	11.417	409.490	27.000	8.703	5.411	345.160
Lorazepam	506.532	813.816	9.723	296.968	20.000	7.820	2.466	250.417
Mefenamic Acid	471.654	740.812	9.279	270.469	15.002	7.530	2.921	223.549
Mepivacaine	488.804	804.223	9.986	313.299	15.000	7.844	2.500	252.720
Mepyramine	583.254	946.314	10.743	362.572	18.986	8.199	3.467	288.603
Mesitylene	330.491	478.400	7.508	177.091	6.000	6.369	3.359	135.268
Metadone	558.578	975.568	11.156	391.016	18.989	8.506	4.195	322.247
Methohexital	494.159	806.699	9.952	311.182	11.000	7.825	2.909	250.918
Methylacetate	235.127	306.768	5.771	104.628	2.000	5.180	0.760	72.765
Methylsulfoxide	232.052	302.312	5.760	104.231	0.000	5.180	-0.656	72.792
Metoclopramide	562.723	914.265	10.610	353.630	14.984	8.083	1.941	276.545

Metoprolol	548.867	895.443	10.699	359.605	16.000	8.104	2.754	278.623
Mianserin	495.943	815.253	9.984	313.149	22.000	7.968	1.966	264.851
Midazolam	552.817	908.006	10.414	344.002	23.194	8.194	4.512	290.882
Morphine	459.498	774.966	9.693	295.173	24.000	7.904	0.643	258.538
N,N-Dimethylaniline	322.036	463.937	7.296	167.253	7.000	6.284	2.952	129.933
N,N-Dimethyl-p-toluidine	355.154	520.383	7.747	188.528	7.000	6.521	3.492	145.207
Nadolol	543.213	930.574	10.956	377.099	19.000	8.354	1.763	305.222
Nalidixic acid	433.911	673.417	8.936	250.893	13.077	7.282	0.256	202.217
Naphtalene	304.181	442.172	7.183	162.078	11.000	6.305	3.107	131.266
Naproxen	455.580	707.536	9.113	260.906	12.001	7.383	2.281	210.750
Nebivolol	690.744	1142.578	11.788	436.545	30.000	8.823	3.481	359.586
N-Ethylaniline	322.649	464.609	7.337	169.115	8.000	6.274	2.746	129.299
Nicardipine	669.013	1248.669	13.087	538.053	27.481	9.449	1.812	441.777
Nicotinamide	285.164	399.912	6.646	138.753	8.000	5.919	-0.178	108.598
Nicotine	370.087	563.285	8.157	209.043	12.000	6.811	1.246	165.437
Nifedipine	541.248	920.834	10.874	371.470	18.000	8.362	2.503	306.116
Nimodipine	630.865	1128.337	12.286	474.210	22.000	9.023	3.634	384.661
Nisoldipine	607.505	1066.060	11.701	430.150	19.000	8.766	3.398	352.671
Nitrendipine	592.077	1005.095	11.195	393.731	19.000	8.486	3.234	319.920
Nitrobenzene	277.564	388.778	6.500	132.752	7.000	5.836	2.663	104.081
Norfloxacin	544.060	888.513	10.414	340.681	20.056	8.112	-2.997	279.536
Ofloxacin	574.661	959.992	10.904	373.537	23.036	8.431	-1.255	313.742
N-Methylbenzylamine	334.589	479.537	7.373	170.802	8.000	6.306	1.409	131.278
N-methylnaphthalen-1-amine	354.538	528.486	7.767	189.526	12.000	6.672	2.954	155.504

N-Methylphenethylamine	361.674	527.612	7.813	191.768	9.000	6.529	1.800	145.748
N-pentane	288.885	393.596	6.649	138.869	2.000	5.698	3.328	96.850
N-propanol	236.945	308.452	5.716	102.654	2.000	5.128	0.966	70.603
Ondansetron	524.537	867.244	10.319	334.533	22.000	8.074	2.709	275.635
Oxazepam	491.667	777.945	9.456	280.896	20.000	7.664	1.924	235.710
Oxolinic acid	447.951	703.596	9.092	259.694	17.443	7.423	-0.490	214.154
Oxprenolol	516.288	861.377	10.521	347.771	17.000	8.020	3.085	270.073
Paracetamol	351.912	510.190	7.593	181.112	9.000	6.431	1.165	139.244
Pentamethylbenzene	366.534	558.541	8.275	215.100	6.000	6.869	4.193	169.671
Phenazone	403.565	618.810	8.544	229.326	12.000	7.028	-1.875	181.783
Phenobarbital	414.230	654.666	8.870	247.149	14.000	7.290	1.058	202.894
Phenol	252.338	344.550	6.115	117.493	7.000	5.576	1.587	90.775
Phenylbutazone	556.565	921.282	10.749	362.997	22.000	8.246	2.606	293.631
Phenylpropanolamine	358.795	539.833	7.974	199.764	9.996	6.677	0.656	155.859
Phenytoin	465.418	733.020	9.220	267.075	19.000	7.518	2.349	222.517
Physostigmine	524.168	846.864	10.207	327.297	17.000	7.959	1.680	263.941
Pindolol	476.898	778.171	9.981	312.943	17.000	7.742	2.759	242.956
Pipemidic acid	539.932	872.281	10.192	326.362	20.019	7.983	-3.989	266.359
Piromidic acid	511.676	822.972	9.937	310.210	19.039	7.828	0.698	251.117
Piroxicam	521.121	851.853	10.124	322.024	20.000	8.000	0.793	268.052
P-Nitroaniline	299.201	426.160	6.849	147.379	8.000	6.022	1.690	114.364
Prilocaine	498.130	777.000	9.681	294.421	12.000	7.588	2.362	228.766
Procaine	511.034	804.747	9.913	308.696	13.970	7.696	1.524	238.634
Progesterone	545.801	956.214	11.004	380.400	21.000	8.518	2.863	323.648

Promazine	486.129	821.883	10.210	327.524	19.996	8.034	4.366	271.521
Promethazine	505.058	840.790	10.177	325.382	18.990	8.044	4.158	272.579
Propionitrile	222.842	280.160	5.307	88.475	1.000	4.886	0.978	61.081
Propiophenone	330.562	483.040	7.500	176.719	8.000	6.405	1.877	137.590
Propofol	409.092	640.123	9.011	255.084	7.000	7.262	3.689	200.488
Propranolol	502.389	826.254	10.208	327.347	18.000	7.914	3.341	259.482
P-toluidine	295.583	420.383	6.772	144.090	7.000	5.933	1.713	109.327
Pyridine	237.209	313.736	5.692	101.800	6.000	5.295	1.216	77.712
Ranitidine	604.518	967.313	10.863	370.722	15.958	8.200	1.984	288.734
Risperidone	693.876	1159.265	11.999	452.341	31.000	8.947	3.148	374.976
Rufloxacin	563.552	941.534	10.732	361.832	23.032	8.329	-1.201	302.576
Salicylic acid	286.061	410.043	6.820	146.113	8.000	6.052	0.025	116.089
Sotalol	523.387	828.050	10.147	323.465	13.000	7.844	1.713	252.719
Sulindac	598.470	976.924	10.912	374.102	21.003	8.400	0.335	310.355
Temazepam	517.802	833.083	9.858	305.284	20.000	7.872	1.887	255.407
Terbutaline	464.062	727.981	9.558	286.990	12.000	7.547	2.154	225.084
Tert- butyl alcohol	251.506	345.029	6.263	123.223	0.000	5.495	1.004	86.862
Tetracaine	586.514	926.808	10.590	352.346	14.969	8.034	3.328	271.532
Tetrachloro ethane	272.457	382.912	6.590	136.434	0.000	5.799	2.499	102.103
Tetrahydrofurane	233.841	310.641	5.791	105.372	5.000	5.271	1.082	76.662
Theobromine	349.579	520.535	7.783	190.282	10.000	6.585	-0.735	149.476
Theophylline	350.772	520.876	7.756	188.968	10.000	6.581	-0.282	149.238
Thiopental	441.255	708.499	9.407	278.002	8.000	7.531	2.159	223.661
Timolol	529.781	898.614	10.859	370.428	18.000	8.253	2.021	294.320

Tocainide	419.778	658.995	8.897	248.684	9.863	7.191	1.142	194.711
Tolfenamic acid	460.352	723.594	9.222	267.178	15.002	7.517	3.128	222.403
Tolmetin	488.694	764.873	9.619	290.647	15.000	7.665	1.260	235.811
Toluene	280.434	388.130	6.414	129.236	6.000	5.727	2.784	98.337
Tramadol	526.791	870.533	10.347	336.337	16.952	8.044	2.580	272.531
Trimecaine	521.432	850.545	10.330	335.233	11.146	7.951	2.785	263.184
Tropisetron	539.704	866.533	10.103	320.647	22.000	7.971	3.201	265.175
Verapamil	786.716	1394.608	13.539	575.839	24.975	9.588	4.391	461.555
W 36017	449.132	710.355	9.312	272.432	9.715	7.401	1.376	212.266

Table 4C. Weighted average at pH 7.0, according to each analyte's pKa, of surface accessible to solvent, volume accessible to solvent, superficial diameter, surface, number of torsions, volume diameter, VirtualLogP and volume for the whole dataset.

$\log k_w^{IAM.MG}$ = -0.8749 + 0.4477 VirtualLogP + 0.0660 HeavyAtoms + 0.1152 Gyrrad - 0.0863 FlexTorsions (5)

n= 205 r^2 = 0.71 q^2 = 0.69 *SE*= 0.536 *F*= 116.45 *F*α 0.001= 19.98 *PC*=55.442

Best optimized model (n-1):

$\log k_w^{IAM.MG}$ = -0.8913 + 0.4547 VirtualLogP + 0.0678 HeavyAtoms + 0.1133 Gyrrad - 0.0915 FlexTorsions (6)

n= 204 r^2 = 0.72 *SE*= 0.529 *F*= 120.97 *F*α 0.001= 19.98 *PC*=63.711 ExRow: alprazolam

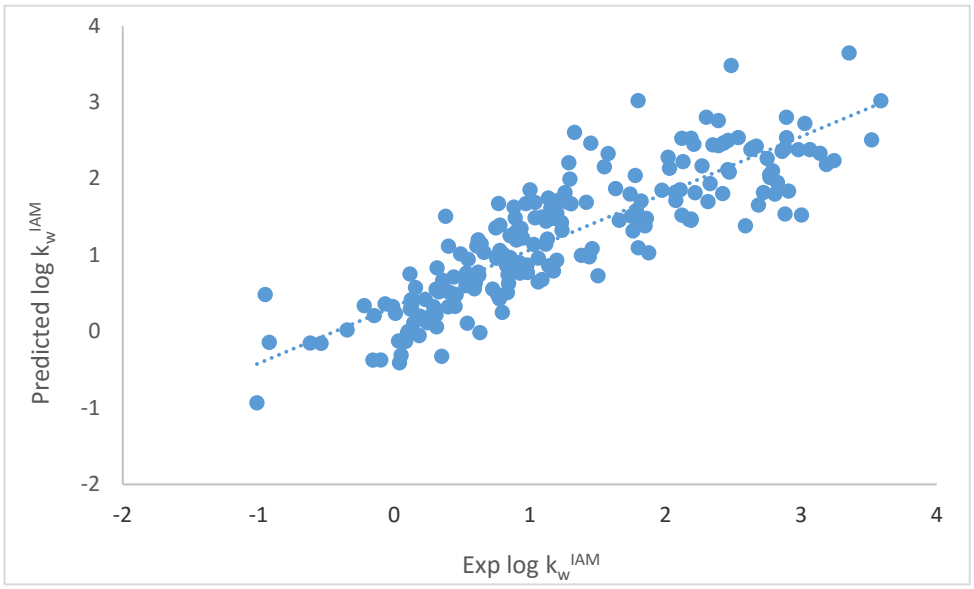
Performing the weighted average of the properties according to the pKa of the ionizable analytes and to the microspecies distribution of the ampholytes improved the relationships in comparison with those obtained starting from the properties of the analytes assumed as completely ionized (see equations (4) and (5)). However, the relationships observed are weaker than those obtained starting from the properties of the analytes assumed as neutral. Indeed, accordingly, some authors [Barbato et al. 2006] reported how, for basic compounds, the retention of analytes on phospholipid stationary phases was more dependent on log P^N than on log D^{pH} values. The six above proposed models are shown in Figure 1, Figure 2 and Figure 3.

6.2.2 Static properties in log $k_w^{IAM.DD2}$ modeling

In an attempt to predict log $k_w^{IAM.DD2}$, 161 analytes were taken into account. Taking into account all the compounds, both ionizable and not ionizable, in their neutral form, the best models (equations (7) and (8)) for the relationships with log $k_w^{IAM.DD2}$ were based on the following four properties: VirtualLog P, volume diameter, number of flexible torsions, EZ bonds. The best optimized model was based on the same properties. It is interesting to note that, taking into account the neutral properties of the analytes, phospholipophilicity, as measured on both IAM stationary phases, appears as dependent on VirtualLog P that is again an estimation of *n*-octanol/water log P^N values and on the flexibility of the molecules, as expressed by FlexTorsions parameter.

This is easy to verify by comparing equations (7) and (8) with equations (1) and (2).

A



B

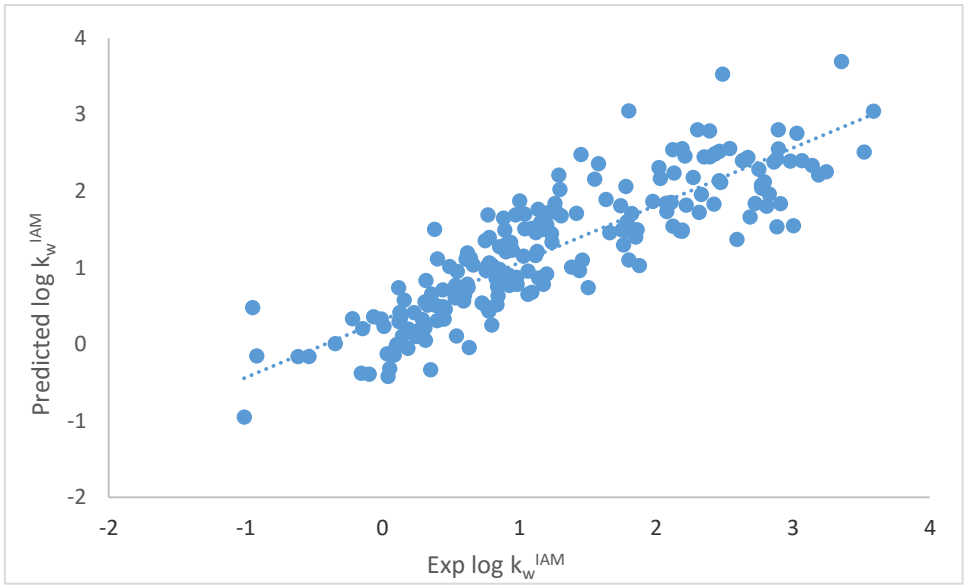
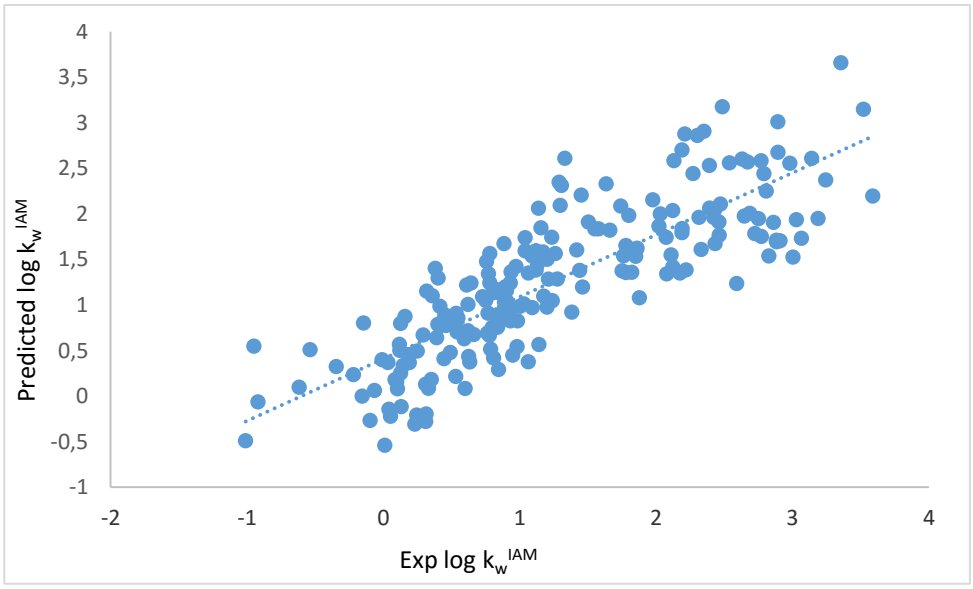


Figure 1. Plots experimental vs predicted log $k_w^{IAM.MG}$ for the analytes, considered as neutral, taken into account before (A) and after (B) optimization.

A



B

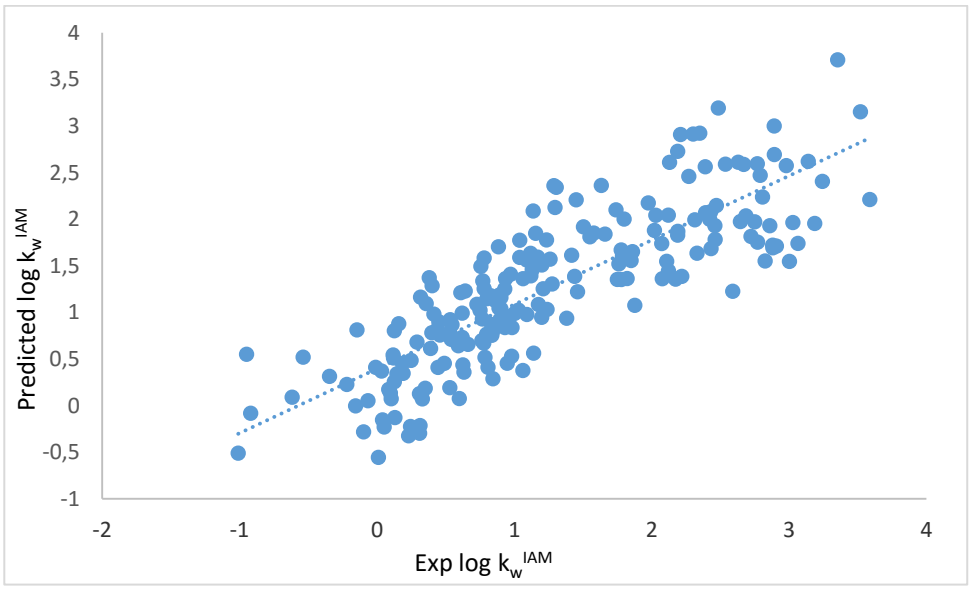
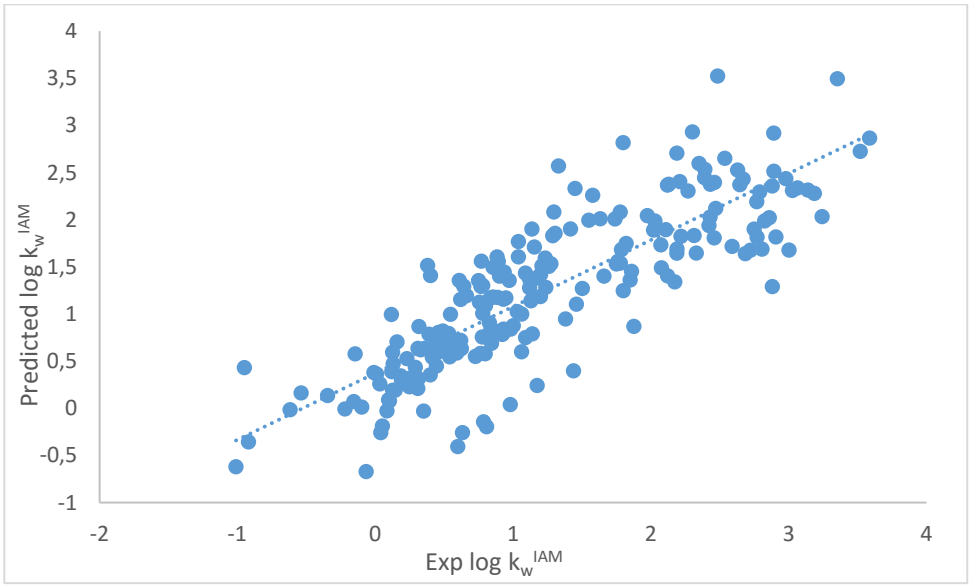


Figure 2. Plots experimental vs predicted log $k_w^{IAM.MG}$ for the analytes, considered as ionized, taken into account before (A) and after (B) optimization.

A



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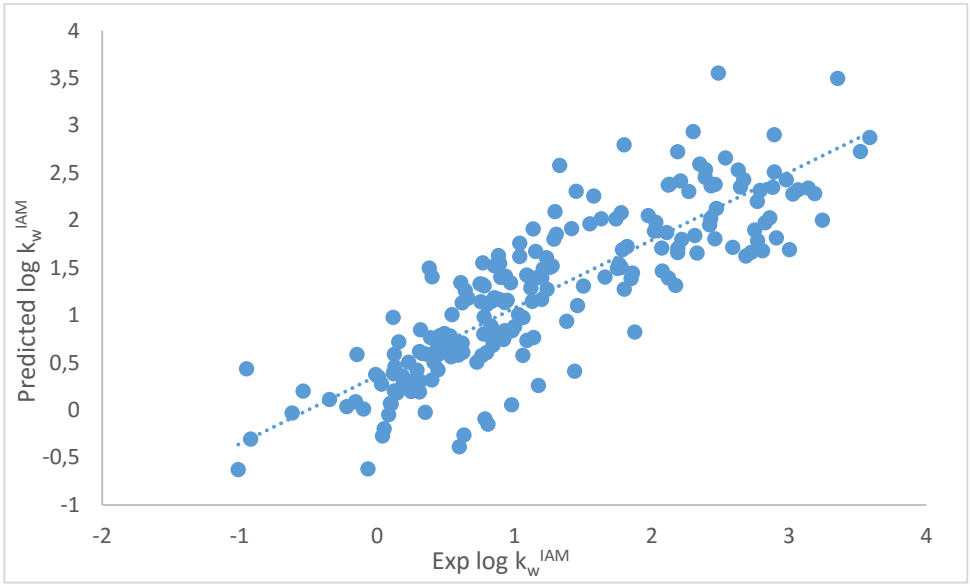


Figure 3. Plots experimental vs predicted log $k_w^{IAM.MG}$ for the weighted average of the properties of the analytes taken into account before (A) and after (B) optimization.

$\log k_w^{IAM.DD2} = -2.8933 + 0.5769\ VirtualLogP + 0.4485\ Vdiam - 0.0890\ FlexTorsions + 0.2714\ EzBnds$ (7)

$n= 161 \quad r^2 = 0.76 \quad q^2 = 0.75 \quad SE= 0.569 \quad F= 126.32 \quad F\alpha\ 0.001= 25.44 \quad PC=52.450$

Best optimized model (n-1):

$\log k_w^{IAM.DD2} = -3.0320 + 0.5814\ VirtualLogP + 0.4642\ Vdiam - 0.0901\ FlexTorsions + 0.2727\ EzBnds$ (8)

$n= 160\ r^2 = 0.79 \quad SE= 0.531 \quad F= 148.89 \quad F\alpha\ 0.001= 25.44 \quad PC=45.437$ ExRow: Benzylalcohol

Taking into account ionization, when applicable, for the same set of 161 compounds, the best models (equations (9) and (10)) were based on the following four properties: volume diameter, virtual logP, number of flexible torsions, charges. The best optimized models (r^2 = 0.76) were based on the same properties.

$\log k_w^{IAM.DD2} = -3.9260 + 0.7013\ Vdiam + 0.3820\ VirtualLogP - 0.0835\ FlexTorsions + 0.5117\ Charge$ (9)

$n= 161 \quad r^2 = 0.74 \quad q^2 = 0.72 \quad SE= 0.597 \quad F= 111.40 \quad F\alpha\ 0.001= 25.44 \quad PC=57.653$

Best optimized model (n-1):

$\log k_w^{IAM.DD2} = -4.0569 + 0.7174\ Vdiam + 0.3822\ VirtualLogP - 0.0843\ FlexTorsions + 0.5107\ Charge$ (10)

$n= 160\ r^2 = 0.76 \quad SE= 0.567 \quad F= 125.84 \quad F\alpha\ 0.001= 25.44 \quad PC=51.801$ ExRow: Benzylalcohol

The electrical charge appears in equations (10) and (9), suggesting that IAM partitioning of drugs would be enhanced for basic compounds and hindered for the acidic ones. The behavior suggested by such equations supports the experimental evidence [Grumetto et al., 2012; Grumetto et al., 2013]. According to analyte's pKa, a weighted average of the properties of neutral and charged forms at pH 7.0 (i.e. the experimental pH of $\log k_w^{IAM}$ determinations) was performed. The best models (equations (11) and (12)) were based on the following four properties: Virtual LogP, volume diameter, EZ bonds, H-bond acceptor.

$\log k_w^{IAM.DD2} = -0.8384 + 0.4009\ VirtualLogP + 0.0069\ Surface + 0.0083\ Rings - 0.1205\ FlexTorsions$ (11)

n= 161 r^2 = 0.70 q^2 = 0.68 SE= 0.639 F= 93.49 $F\alpha$ 0.001= 25.44 PC=66.444

Best optimized model (n-1):

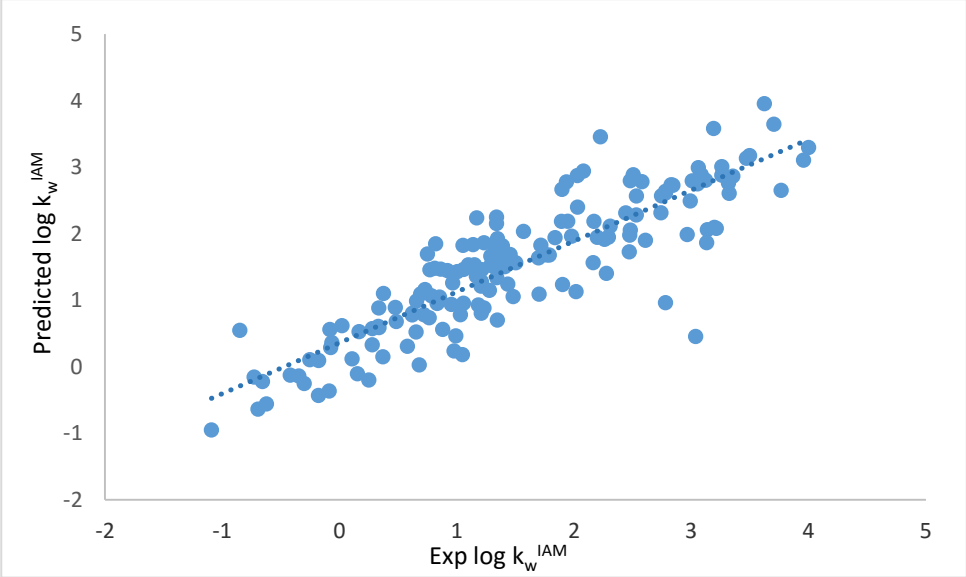
$$\log k_w^{IAM.DD2} = -0.9111 + 0.4023\ VirtualLogP + 0.0072\ Surface - 0.0015\ Rings - 0.1264\ FlexTorsions \quad (12)$$

n= 160 r^2 = 0.73 SE= 0.610 F= 105.14 $F\alpha$ 0.001= 25.44 PC=60.163 ExRow: Benzylalcohol

In equations (11) and (12), VirtualLog P parameter represents an approximation of log $D^{7.0}$ as it arises from the weighted average of the VirtualLogPs of the neutral (log P^N) and ionized (log P^I) forms. Surface parameter refers to the superficial area of the molecules. According to those equations, retention on IAM stationary phases would be enhanced for molecules having high apparent lipophilicity and superficial area.

The plots experimental vs predicted log $k_w^{IAM.DD2}$ are displayed in Figure 4, 5 and 6.

A



B

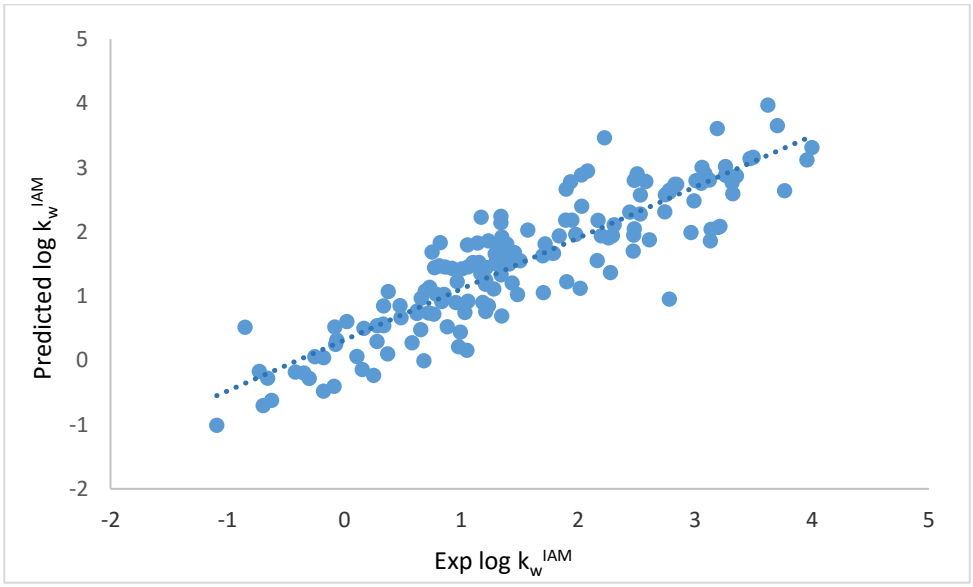
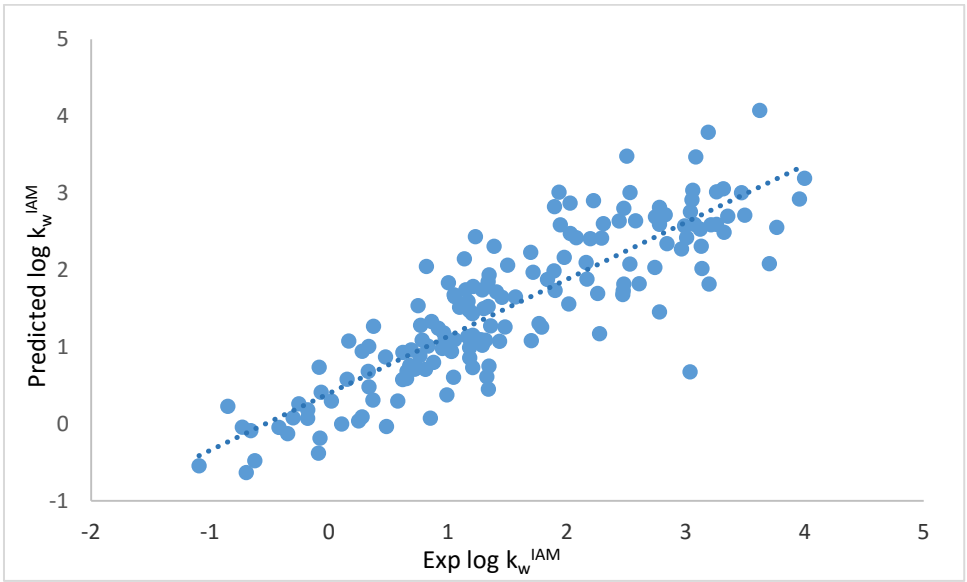


Figure 4. Plots experimental vs predicted log $k_w^{IAM.DD2}$ for the analytes, considered as neutral, taken into account before (A) and after (B) optimization.

A



B

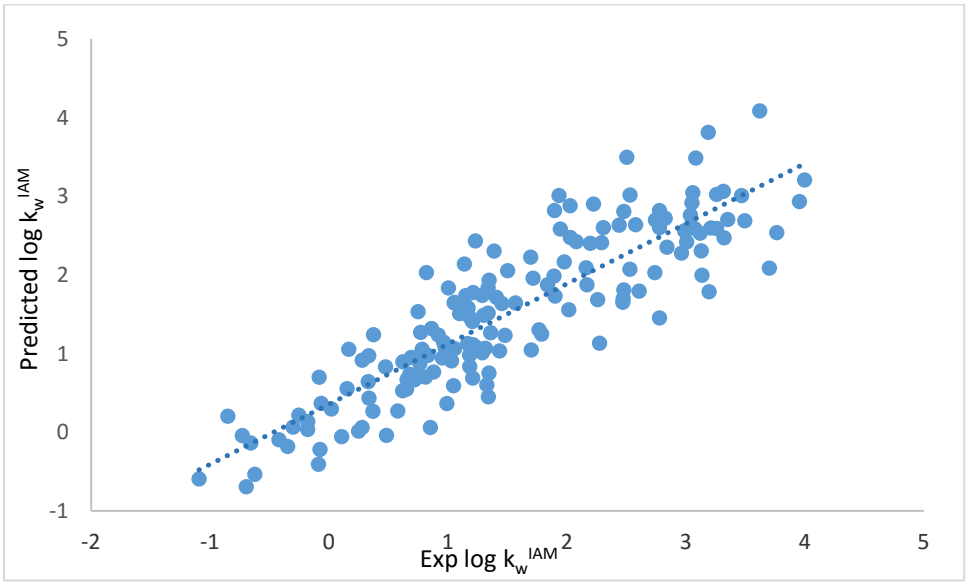
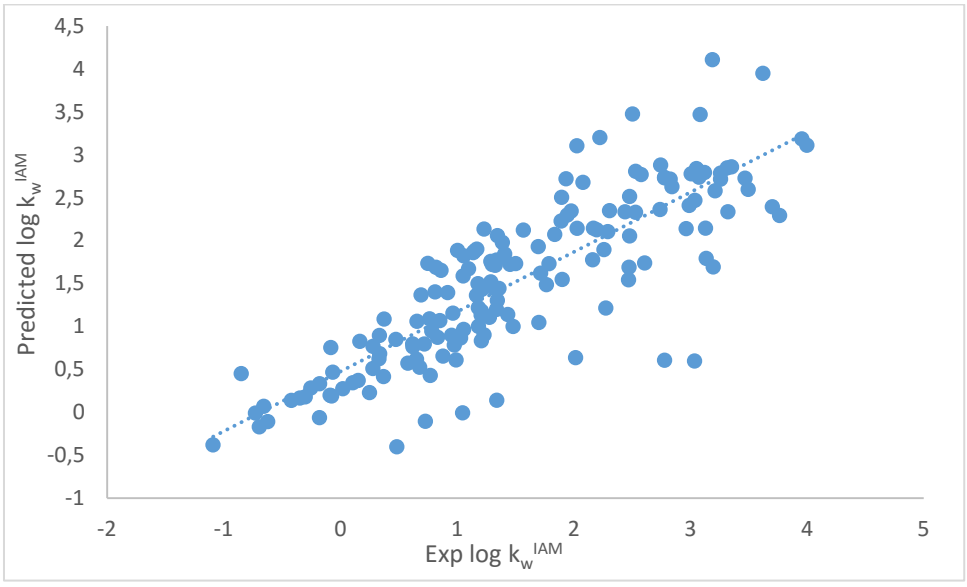


Figure 5. Plots experimental vs predicted log $k_w^{IAM.DD2}$ for the analytes, considered as ionized, taken into account before (A) and after (B) optimization.

A



B

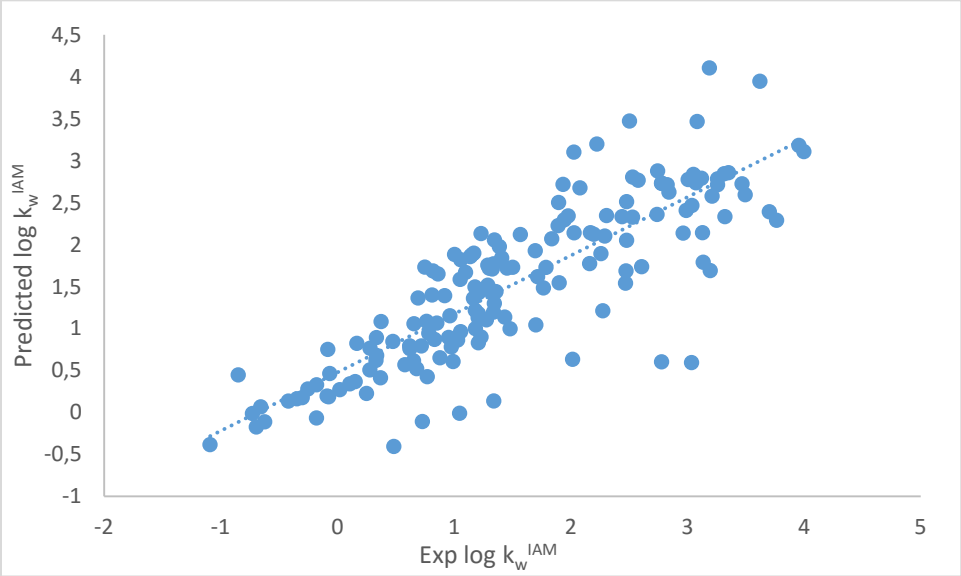


Figure 6. Plots experimental vs predicted log $k_w^{IAM.DD2}$ for the weighted average of the properties of the analytes taken into account before (A) and after (B) optimization.

6.2.3 Conformational properties in $logk_w^{IAM.MG}$ modeling

An extensive conformational analysis was performed and the derived properties were added to the models in an attempt to maximize their predictive strength. The three-dimensional structures of the considered molecules were downloaded from PubChem and were considered in both neutral and ionized Gasteiger – Marsili [Gasteiger and Marsili, 1980] forms. Atom charges were applied to perform the next molecular mechanic calculations.

Taking into account all the compounds, both ionizable and not ionizable, in their electrically neutral form, the best models (equations (13) and (14)) were based on the following 4 properties: VirtuallogP, number of heavy atoms, standard deviations of volume values, and standard deviations of superficial area values.

$$\log k_w^{IAM.MG} = -0.8791 + 0.5390\ VirtualLogP + 0.0751\ HeavyAtoms - 0.2572\ Volume\ ds + 0.0185\ AS\ ds \quad (13)$$

n= 205 r^2 = 0.75 q^2 = 0.71 SE= 0.507 F= 146.60 $F\alpha$ 0.001= 19.98 PC=52.850

Best optimized model (n-1):

$$\log k_w^{IAM.MG} = -0.9021 + 0.5470\ VirtualLogP + 0.0773\ HeavyAtoms - 0.2710\ Volume\ ds + 0.0182\ AS\ ds \quad (14)$$

n= 204 r^2 = 0.75 SE= 0.499 F= 153.11 $F\alpha$ 0.001= 19.98 PC=50.970 ExRow: alprazolam

The standard deviation of a measure is an estimation of how far each measure is from the average value; in general, these values are higher for molecules supporting many flexible torsions as their rotation generates many more conformers. Therefore, the fact that standard deviation values are reported in equations (13) and (14) supports the important role that molecular flexibility plays in IAM retention. Taking into account ionization, when applicable, for the same set of compounds, the best models (equations (15) and (16)) were based on the following properties: number of torsions, number of atoms, minimum values of superficial diameter (DS Min) and VirtualLogP.

$$\log k_w^{IAM.MG} = -0.0969 + 0.0513\ Torsions + 0.0284\ Atoms - 0.0714\ DS\ Min + 0.3280\ VirtualLogP \quad (15)$$

n= 205 r^2 = 0.69 q^2 = 0.67 SE= 0.562 F= 110.63 $F\alpha$ 0.001= 19.98 PC=65.418

Best optimized model (n-1):

$\log k_w^{IAM.MG}$ = -0.0993 + 0.0551 Torsions + 0.0269 Atoms - 0.0717 DS Min + 0.3332 VirtualLogP

(16)

n= 204 r^2 = 0.70 *SE*= 0.556 *F*= 114.61 *F*α 0.001= 19.98 *PC*=63.624 ExRow: alprazolam

Looking at equations (15) and (16) reveals that, in this specific case, the conformational search did not benefit the relationships as one would have liked, as only one descriptor (DS Min), in these equations, is generated by it. According to analyte's pKa, a weighted average of the properties of neutral and charged forms at pH 7.0 (i.e. the experimental pH of log $k_w^{IAM.MG}$ determinations) was performed. The best models (equations (17) and (18)) were based on the following properties: Volume range, VirtualLog P, number of heavy atoms, and maximum values of superficial diameter.

$\log k_w^{IAM.MG}$ = -0.3244 + 0.4177 VirtualLogP + 0.0978 HeavyAtoms - 0.0747 DS Max - 0.0249 Volume Range (17)

n= 205 r^2 = 0.71 q^2 = 0.69 *SE*= 0.543 *F*= 121.18 *F*α 0.001= 19.98 *PC*=60.699

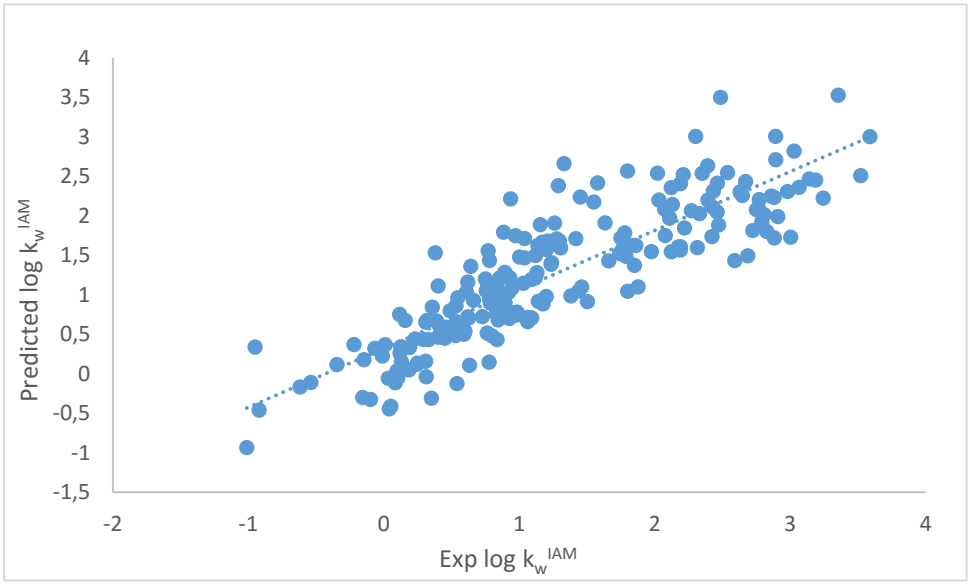
Best optimized model (n-1):

$\log k_w^{IAM.MG}$ = -0.6730 + 0.4265 VirtualLogP + 0.0985 HeavyAtoms - 0.0013 AS Max - 0.0267 Volume Range (18)

n= 204 r^2 = 0.72 *SE*= 0.536 *F*= 125.77 *F*α 0.001= 19.98 *PC*=58.911 ExRow: alprazolam

Such relationships, albeit fairly good, are less significant than those obtained starting from the neutral properties of the analytes. This is an interesting point as it highlights how, for ionizable analytes, the apparent lipophilicity (log D), measured or, as in this case, calculated at the experimental pH, albeit predicting the retentive behavior on ODS stationary phases, fails when it comes to describing molecular interaction involved in IAM retention. The plots experimental *vs* predicted $\log k_w^{IAM.MG}$ are shown in Figure 7, 8 and 9.

A



B

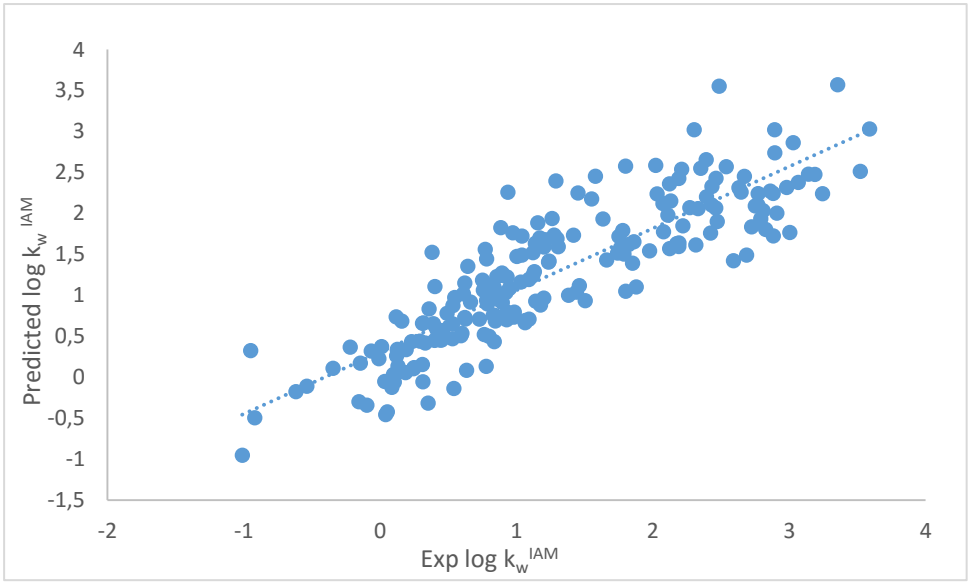
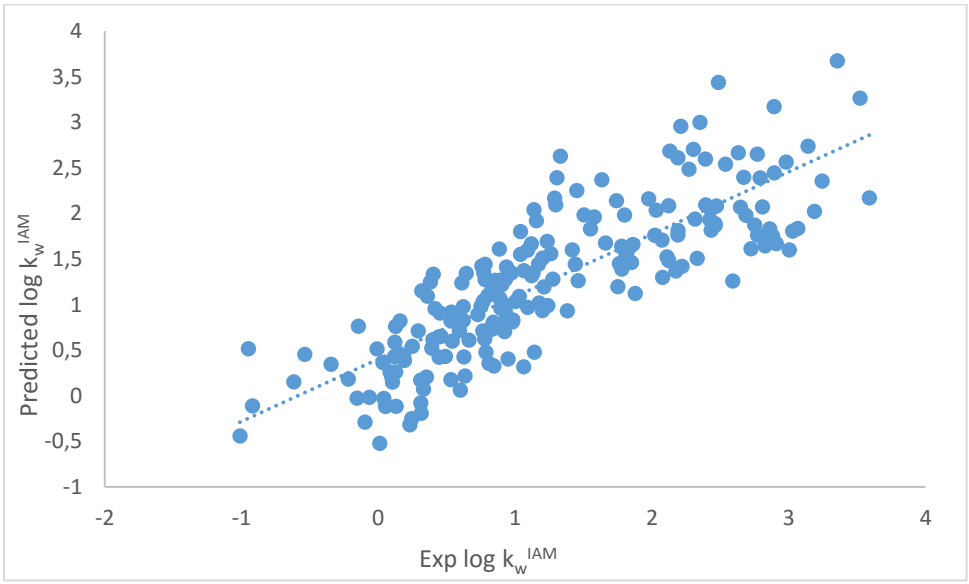


Figure 7. Plots experimental vs predicted $\log k_w^{IAM.MG}$ for the combination of static and conformational properties of the analytes, considered as neutral, taken into account before (A) and after (B) optimization.

A



B

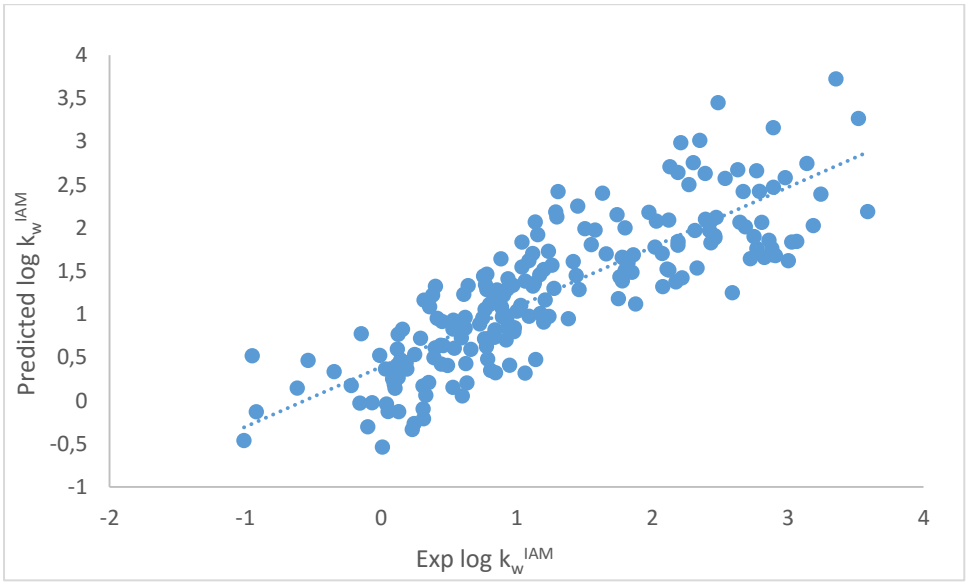
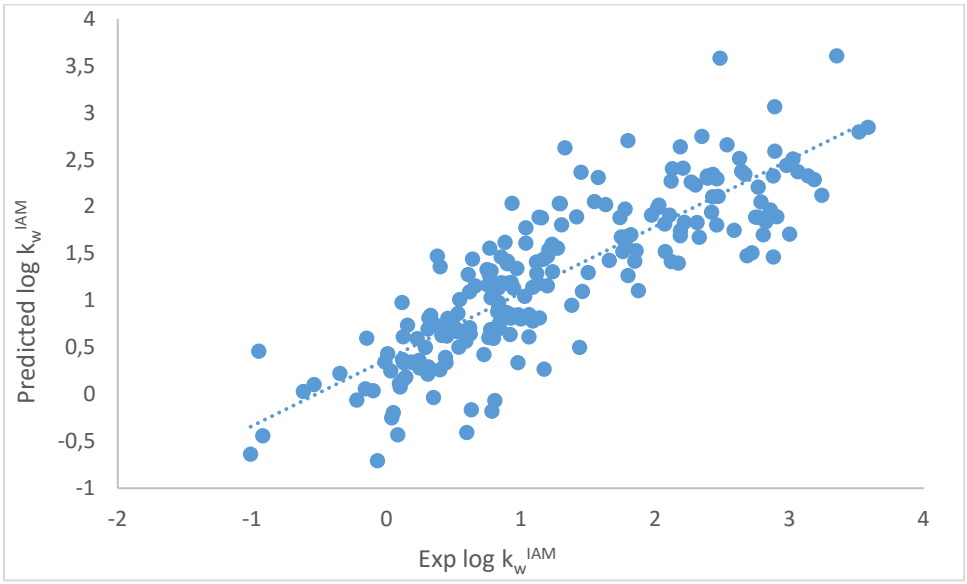


Figure 8. Plots experimental vs predicted $\log k_w^{IAM.MG}$ for the combination of static and conformational properties of the analytes, considered as ionized, taken into account before (A) and after (B) optimization.

A



B

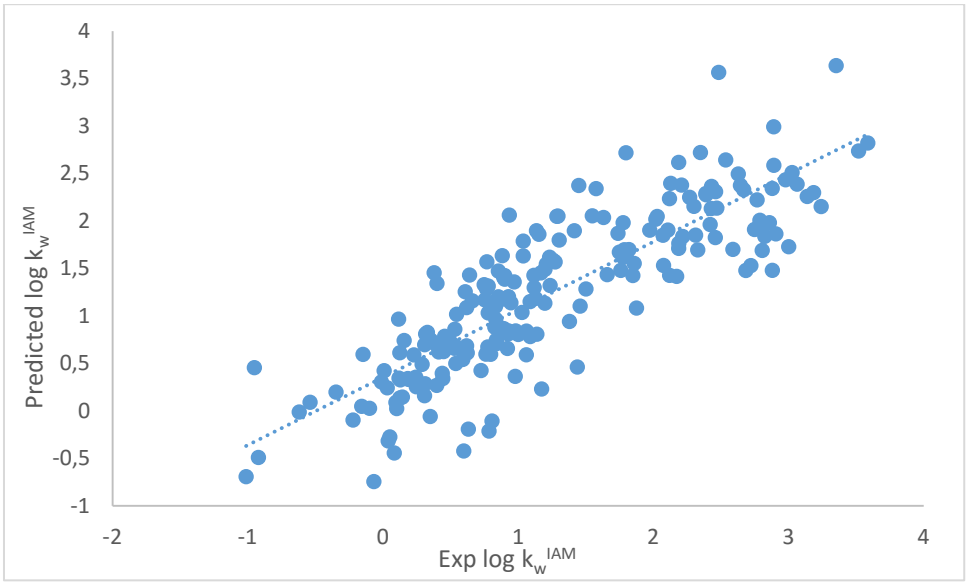


Figure 9. Plots experimental vs predicted log$k_w^{IAM.MG}$ for the weighted average at the experimental pH (7.0) of static and conformational properties of the analytes, taken into account before (A) and after (B) optimization.

6.2.4 Conformational properties in $\log k_w^{IAM.DD2}$ modeling

Taking into account all the compounds both ionizable and non-ionizable, in their electrically neutral form, the best models (equations (19) and (20)) were based on the following properties: VirtualLog P, minimum values of Volume, minimum values of dipolar momentum and Ovality range.

$\log k_w^{IAM.DD2}$ = -0.5668 + 0.5134 VirtualLogP + 0.0070 Volume Min - 0.1455 MD Min - 3.9855 Ovality Range (19)

n= 161 r^2 = 0.77 q^2 = 0.75 SE= 0.566 F= 127.96 $F\alpha$ 0.001= 25.44 PC=51.933

Best optimized model (n-1):

$\log k_w^{IAM.DD2}$ = -0.6323 + 0.5177 VirtualLogP + 0.0071 Volume Min - 0.1406 MD Min - 3.8387 Ovality Range (20)

n= 160 r^2 = 0.79 SE= 0.534 F= 146.91 $F\alpha$ 0.001= 25.44 PC=45.921 ExRow: Benzylalcohol

In QSAR studies, ovality refers to a measure of how the shape of a molecule approaches a sphere (at one extreme) or a cigar shape (at the other). Taking into account ionization, when applicable, for the same set of compounds, the best models (equations (21) and (22)) were based on the following properties: number of bonds, gyration radius, VirtualLogP and the range values of Volume.

$\log k_w^{IAM.DD2}$ = -0.8238 + 0.0482 Bonds + 0.1884 Gyrrad + 0.3340 VirtualLogP - 0.0332 Volume Range (21)

n= 161 r^2 = 0.68 q^2 = 0.65 SE= 0.667 F= 82.60 $F\alpha$ 0.001= 25.44 PC=72.381

Best optimized model (n-1):

$\log k_w^{IAM.DD2}$ = -0.8761 + 0.0483 Bonds + 0.1962 Gyrrad + 0.3338 VirtualLogP - 0.0320 Volume Range (22)

n= 160 r^2 = 0.70 SE= 0.643 F= 90.47 $F\alpha$ 0.001= 25.44 PC=66.979 ExRow: Benzylalcohol

The Volume range was calculated from the difference between the maximum and minimum values of molecular volumes as given by conformational search. According to analyte's pKa and to the microspecies distribution for amphoteric drugs, a weighted

average of the properties of neutral and charged forms at pH 7.0 (i.e. the experimental pH of log $k_w^{IAM.DD2}$ determinations) was performed. The best models (equations (23) and (24)) were based on the following properties: VirtualLogP, surface, number of rings amd number of flexible torsions. It should be pointed out that, in this case, all the properties are static, therefore the conformational analyses did not play an appreciable role in maximizing the predictive strength of the regression.

$$\log k_w^{IAM.DD2} = -0.8384 + 0.4009\ \text{VirtualLogP} + 0.0069\ \text{Surface} + 0.0083\ \text{Rings} - 0.1205\ \text{FlexTorsions} \quad (23)$$

n= 161 r^2 = 0.70 q^2 = 0.68 SE= 0.639 F= 93.49 $F\alpha$ 0.001= 25.44 PC=66.444

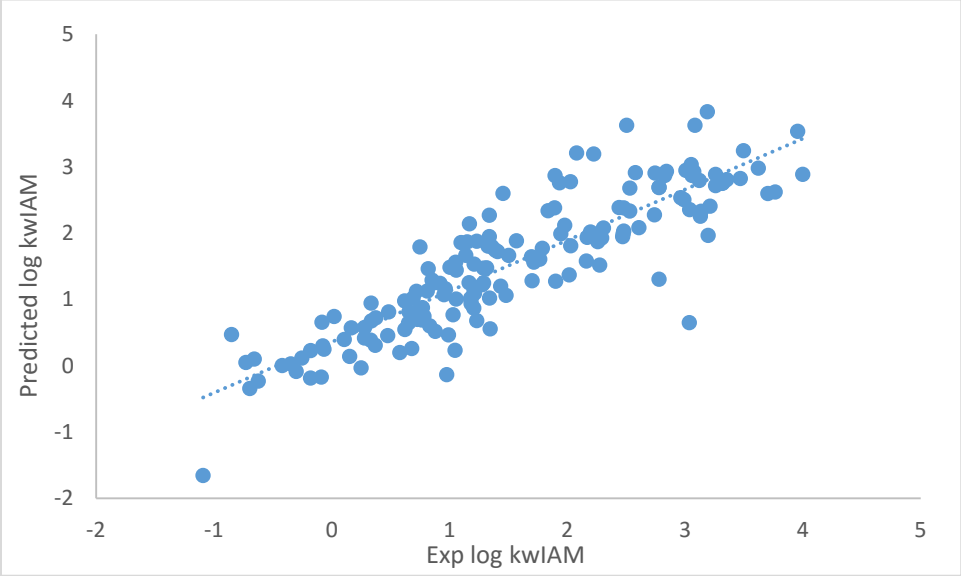
Best optimized model (n-1):

$$\log k_w^{IAM.DD2} = -0.9111 + 0.4023\ \text{VirtualLogP} + 0.0072\ \text{Surface} - 0.0015\ \text{Rings} - 0.1264\ \text{FlexTorsions} \quad (24)$$

n= 160 r^2 = 0.73 SE= 0.610 F= 105.14 $F\alpha$ 0.001= 25.44 PC=60.163 ExRow: Benzylalcohol

Experimental vs predicted log $k_w^{IAM.DD2}$ plots are shown in Figure 10,11 and 12.

A



B

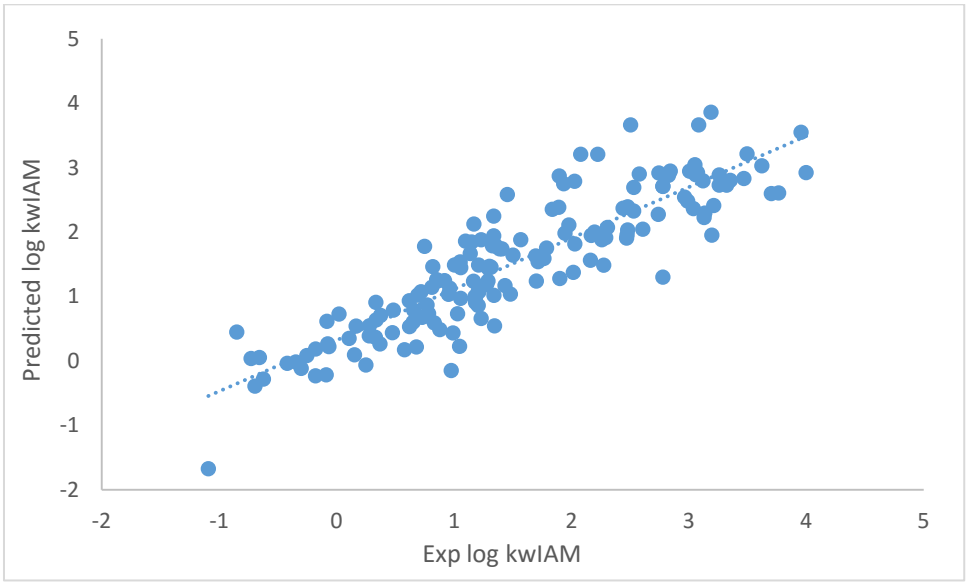
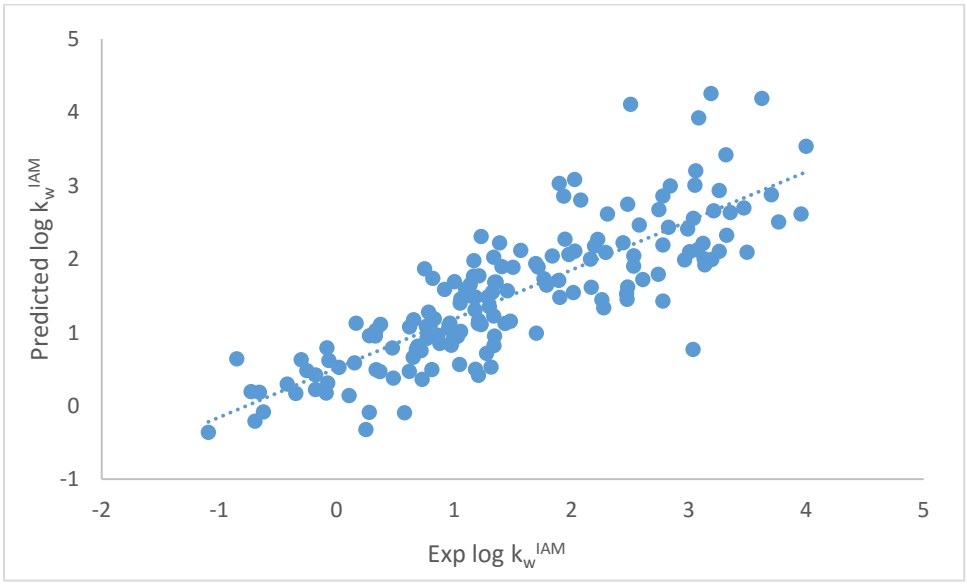


Figure 10. Plots experimental vs predicted log $k_w^{IAM.DD2}$ for the combination of static and conformational properties of the analytes, considered as neutral, taken into account before (A) and after (B) optimization.

A



B

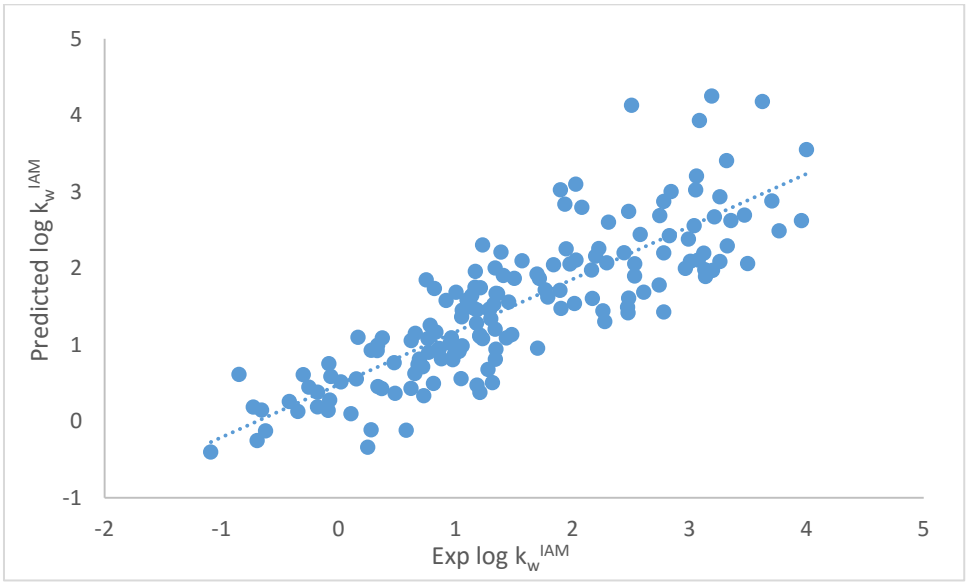
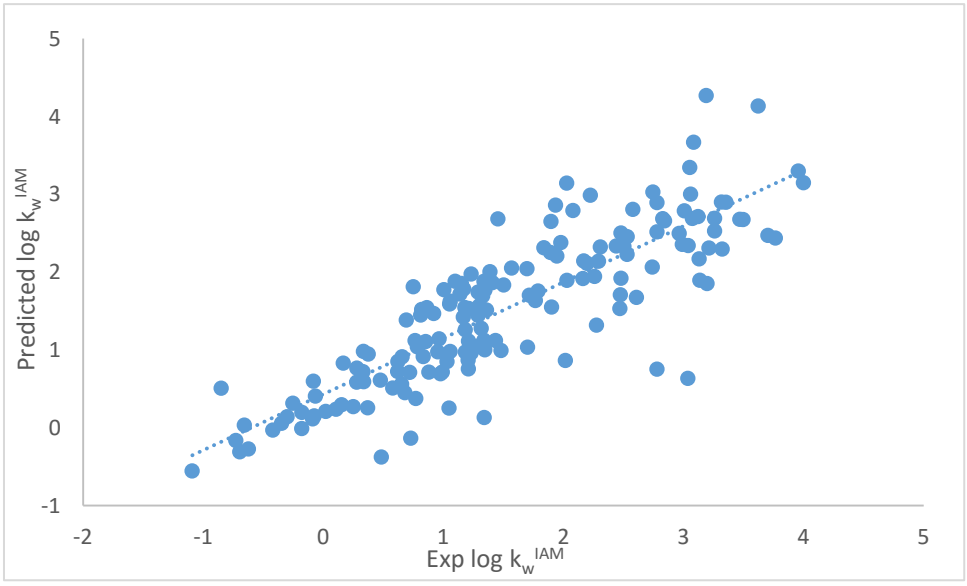


Figure 11. Plots experimental vs predicted log $k_w^{IAM.DD2}$ for the combination of static and conformational properties of the analytes, considered as ionized, taken into account before (A) and after (B) optimization.

A



B

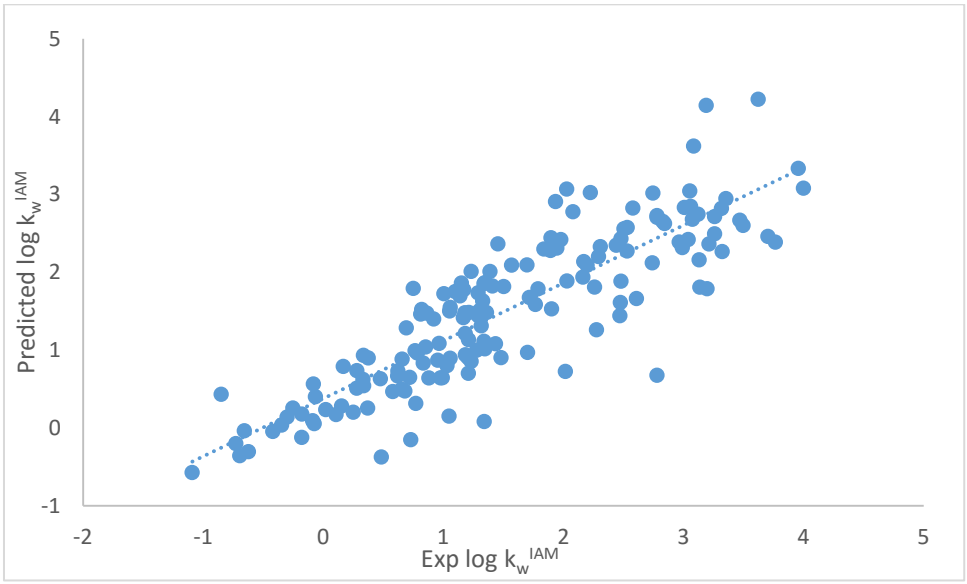


Figure 12. Plots experimental vs predicted log $k_w^{IAM.DD2}$ for the weighted average at the experimental pH (7.0) of static and conformational properties of the analytes, taken into account before (A) and after (B) optimization.

6.3 Conclusion

Highly significant relationships were obtained in the prediction of drug phospholipophilicity, as measured on both phosphatidylcholine-based stationary phases. This approach contributed also to gain further knowledge of the molecular mechanisms involved in drug phospholipid interactions. Furthermore, the ability of predicting, with a high degree of accuracy, the phospholipid affinity indexes of new drugs/prodrugs will also allow a rapid approximation of $\Delta\log k_w^{IAM}$ values such as to provide an ultra-high throughput screening method oriented at a preliminary intestinal absorption/BBB passage potential assessment of new leads.

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7.0 *IN VITRO* AND *IN SILICO* INDEXES IN MODELING THE BLOOD-BRAIN BARRIER PARTITIONING OF DRUGS: AN IAM/MLC-HPLC STUDY

7.1 Introduction

Pharmaceutical drug development is still a highly inefficient process: over one fourth of the failures in drug candidate development occurs due to unsatisfactory pharmacokinetic properties [Lesko, 2000], mainly regarding absorption, metabolism and toxicity and the attrition rates for Central Nervous System (CNS) active drugs are even higher [Kola et al., 2004]. In fact, before reaching the blood circulation, a drug diffuses through the biological barriers that separate the circulating blood from the interstitial fluid that surrounds the tissues. For orally administered drugs, this barrier is the intestinal epithelium whereas the passage of drugs designed to act at the CNS level is further regulated by the Blood-Brain barrier (BBB). The BBB is one of the most complex and extensively studied biological barriers, and its function is to preserve mammalian brain integrity against possible injurious substances. It is made of endothelial cells, narrowly adherent one to the other to form *tight* junctions that restrict the passage of solutes [Van Bree et al., 1992; Keaney and Campbell, 2015]. Indeed, drug transport is strongly limited by this peculiar biological structure to pure passive *transcellular* diffusion of drugs. In fact, the *paracellular* route, i.e. the passage of actives through the gaps between each endothelial cell, is completely hindered. Apart from active transport mechanisms, whose occurrence is difficult to predict on a solely chemical structure basis, drugs can therefore cross the BBB only by the passive *transcellular* route.
Plenty of *in vivo*, *ex vivo*, *in vitro* methods are available for measuring the BBB partitioning of analytes. Historically, one of the most used and reputed method is the determination of log BB values [Bickel, 2005], where log BB is defined as:

$$\log BB = \log \frac{C_{Brain}}{C_{Blood}}$$

in which C_{Brain} is the concentration that the analyte realizes in the brain tissues, and C_{Blood} is the concentration that the analyte realizes in the blood. However, this method involves the use of animal models, usually rodents, and does not provide any mechanistic information about the nature of the passage and it is time-consuming and raises ethical issues.

Methods based on the employment of cultured cell lines can also be effective, however, astrocytes cell cultures are often difficult to grow and recreating an *in vitro* environment similar to the *in vivo* BBB can be challenging even for the most experienced scientists. Caco-2 model based methods may also be an alternative, however, apart from the structural dissimilarities with the other cell cultures [Lundquist et al., 2002], they are difficult to standardize complicating comparison of the data determined in different laboratories.

In silico methods, generally based on the calculation of physico-chemical parameters, yield various advantages. They are much faster to perform, they allow to screen large libraries of compounds (even solutes not synthetized yet) and to assist in the elucidation of the molecular mechanisms involved in membrane permeation. However, they also suffer from several limitations including the aspect that they are unable to take into account all phenomena actually occurring *in vivo* [Ekins et al., 2007].

In vitro methods based on the use of biomimetic stationary phases coupled with high performance liquid chromatography (HPLC) are often used to surrogate BBB permeation data [Grumetto et al., 2014]. The main advantages are that they are much more reproducible and easier to perform and, albeit conceptually simple, they can be incidentally able to provide an in-depth understanding of the mechanisms involved in membrane barrier passage. Such biomimetic stationary phases include, for instance, Immobilized Artificial Membrane (IAM). IAM stationary phases are based on phosphatidylcholine, which is the major component of biological membranes, and the determination of chromatographic retention coefficients of the analytes on such stationary phases are assumed as direct measures of their phospholipophilicity [Barbato et al., 2004], i.e. the affinity that the analytes have for phospholipids. In addition, other chromatographic indexes, whose drug BBB-penetration predictivity has been demonstrated [De Vrieze et al., 2015; Verzele et al., 2012], include those achieved by the Micellar Liquid Chromatography (MLC) technique. This technique is a based on the addition of surfactants to an aqueous mobile phase at concentrations higher than their critical micelle concentrations (CMC) [Berthod and Garcia-Alvarez-Coque, 2000].

In the present work, 79 structurally non-related analytes have been taken into account and their chromatographic retention coefficients, measured by IAM-LC, and MLC employing

sodium dodecyl sulfate (SDS) as surfactant, were determined. Such indexes have subsequently been used for the development of BBB-passage predictive statistic models using partial least squares (PLS) automatic regression along with physico-chemical parameters, calculated *in silico*. Such hybrid approach is aimed at combining the speediness in the achievement of computational chemistry derived physico-chemical parameters with the improved predictivity of the *in vitro* methods. Beside, the chromatographic conditions have been carefully studied and optimized to obtain the indexes in a relatively short time such as to meet the demands of pharmaceutical companies in look for BBB-passage potential-oriented high throughput screening methods. Furthermore, their being based on physico-chemical parameters offers an insight into the molecular mechanisms actually taking place in membrane diffusion of drugs.

7.2 Materials and methods

7.2.1 Chemicals

MLC and IAM experiments were performed on an Agilent Zorbax SB-C18 Rapid Resolution (3.5 µm, 50 mm x 2.1 mm; Santa Clara, CA, USA) and Regis IAM Fast Mini Screening (10 µm, 10 mm × 3.0 mm; Morton Grove, IL, USA) columns, respectively. The solutes were obtained from commercial source.

7.2.2 Apparatus

7.2.2.1 MLC-HPLC

MLC chromatographic analysis was performed on an Alliance, Waters 2690 chromatograph (Milford, MA, USA) with a quaternary pump and an automatic injector. A Waters 2487 dual-wavelength absorbance ultraviolet detector was used. The detection wavelength was set at the maximum absorbance of each analyte and was always in the range between 210 and 300 nm. Data acquisition and processing were performed using a PeakSimple Chromatography Data System (model 202) and PeakSimple software (SRI Instruments, Torrance, CA, USA). For MLC experiments, analysis was performed at 37 °C, the flow rate was 1.0 mL $min^{-1}$and the injection volume was 20 µL.

7.2.2.2. IAM-HPLC

IAM based chromatographic analysis was performed on an Agilent Capillary 1200 system (Santa Clara, CA, USA). The system included a capillary pump, a micro vacuum degasser and

an automatic injector. An Agilent 1200 Series variable wavelength detector was used and set at the maximum absorbance wavelength of each analyte. The IAM-HPLC experiments were carried out at room temperature (20 ± 2 °C), the flow rate was 300 μL $min^{-1}$and the injection volume was 1 μL.

7.2.3 Mobile phase and sample preparation

MLC mobile phases were composed of aqueous solutions of 0.05 mol L^{-1} sodium dodecyl sulfate (SDS) (Acros). Water (18.2 MΩ/cm) was purified and deionized in house via a Milli-Q plus instrument from Millipore (Bedford, New Hampshire, USA). pH was adjusted with pH 7.4 phosphate buffer, prepared with 0.05 mol L^{-1} disodium hydrogen phosphate (Sigma–Aldrich) and potassium dihydrogen phosphate (Sigma–Aldrich). To reproduce the osmotic pressure of biological fluids, NaCl (9.20 g L^{-1}) (Sigma–Aldrich) was added to the micellar mobile phase. IAM mobile phases consisted of a solution 70/30 v/v Dulbecco's phosphate-buffered saline (DPBS) / methanol (HPLC-grade; Biosolve, Valkenswaard, The Netherlands). DPBS was composed of 2.7 mmol L^{-1} KCl, 1.5 mmol L^{-1} potassium dihydrogen phosphate, 137.0 mmol L^{-1} NaCl, and 8.1 mmol L^{-1} disodium hydrogen phosphate (Sigma–Aldrich). Such solution had a pH value of 7.40 ± 0.05, and no pH adjustment was performed. Indeed, different mobile phases and elution programs were tested starting from 100% aqueous phase; however, the latter condition did not allow the elution of the most lipophilic bases in a reasonable amount of time. All mobile phases were vacuum-filtered through 0.20 μm nylon membranes (Grace, Lokeren, Belgium) before use. Stock solutions of all drugs were prepared by dissolving 10 mg in 1 mL of methanol except for quinidine and theobromine, for which stock concentrations of 1 mg mL^{-1} and 200 μg mL^{-1}, respectively, were used, caffeine and theophylline, which were dissolved in water (10 mg mL−1), domperidone, which was dissolved in dimethyl sulfoxide (10 mg mL^{-1}) and chlorpromazine, which was dissolved in acetonitrile. Stock solutions were stored at 4 °C, except for atenolol, zidovudine, chlorambucil and rifampicin, which were stored at −20 °C. Working solutions were freshly prepared at the beginning of each day by dilution of the stock solutions to 50 μg mL^{-1} with mobile phase for all the analytes, except for valproic acid and halothane that were diluted to 250 μg mL^{-1}.

7.2.4 Data sources

Log BB values were taken from the literature [Abraham et al., 1994; Abraham et al., 2006; Avdeef, A., 2012a; Björkman, 2002; Katritzky et al., 2006; Mente & Lombardo, 2005; Platts et al., 2001]. pKa values were obtained from the literature [Avdeef, A., 2012b] except for

amobarbital, donepezil, fluphenazine, hydroxyzine, ketorolac, paroxetine and ropinirole, whose values were calculated by the software Marvin Sketch 15.1 for Mac OS X [ChemAxon, Budapest, HU].

7.2.5 Software

7.2.5.1 Molecular modeling

Molecular modeling was performed by the software Vega ZZ 3.0.5 for Windows-based PCs [Pedretti et al., 2004]. The starting three-dimensional structures of the considered molecules were downloaded from PubChem database [Kim et al., 2015] and they were considered in both neutral and ionized form. Gasteiger – Marsili [Gasteiger and Marsili, 1980] atom charges were applied to perform the next molecular mechanics calculations. An extensive conformational analysis was carried out *in vacuum* by using the Boltzmann Jump method implemented in AMMP software [Harrison, 1993] and the obtained lowest energy conformation was further optimized by performing a semi-empirical calculation with Mopac 2012 program [Stewart Computational Chemistry] (keywords: PM7 PRECISE MMOK). A cluster analysis has been performed in order to select the most populated conformation states. Physico-chemical and topological properties (Virtual logP [Gaillard et al., 1994], lipole [Pedretti et al., 2002], volume, polar surface area, surface accessible to the solvent, gyration radius, ovality, mass, number of atoms, angles, dihedrals, etc) were calculated by VEGA ZZ software and finally, all molecules were inserted into a Microsoft Access database.

The QSPR models were obtained by the automatic stepwise approach implemented in "Automatic linear regression" script of VEGA ZZ software, calculating regression models, including from 1 to 4 independent variables. The predictive strength of the best equation was evaluated not only by leave-one-out cross validation, but also by splitting randomly the whole dataset in 20 pairs of training and test sets. For each training set, the regression coefficients were calculated to evaluate the test set in terms of standard deviation of errors, angular coefficient, intercept and r^2 of the trend line of the chart of the predicted vs. experimental activities. This validation was performed automatically by "Model validator" script also included in VEGA ZZ.

7.2.5.2 Molecular docking

Molecular docking calculations were carried out using Autodock Vina software [Trott and Olson, 2010]. High resolution (3.4 Å) p-glycoprotein (P-gp) crystallographic structure (PDB

code: 4Q9H) was downloaded from Protein Data Bank (PDB) Database. Gasteiger partial charges were calculated on ligand atoms. Polar hydrogens were added to P-gp and Gesteiger [Gasteiger and Marsili, 1980] partial charges were calculated using Autodock Tools [Morris et al., 2009]. Simulation boxes were centered on the ligands in the structures of P-gp-ligand complexes (PDB codes: 4Q9I, 4Q9J, 4Q9K, 4Q9L) as reported in the literature [Szewczyk et al., 2015]. The simulation boxes were adjusted to accommodate the ligand in each complex and the sizes were between 26x26x26 Å and 30x26x30 Å. An exhaustiveness option of 24 (maximal accuracy) was used in each docking calculation.

7.2.6 Processing

The chromatographic retention coefficients of each analytes were calculated by using the following expression:

$$k = \frac{t_r - t_0}{t_0}$$

in which t_r is the retention time of the compound of interest and t_0 the retention time of an unretained compounds (acetone). Three different sets of properties were generated. At first, all the analytes were considered as uncharged (having full charge equal to 0), subsequently analytes having acidic or basic functions were considered ionized and zwitterions were considered with both the acidic and basic functions in their charged forms. Eventually, a weighted average of the static properties at pH 7.4 according to the pKa of each analyte was performed; for zwitterions, the relative abundance of each microspecies (neutral species, zwitterion, anion and cation) in solution at the physiological pH (7.4) was calculated by the software Marvin Sketch 15.1 for Mac OS X [ChemAxon, Budapest, HU]. This approach was also extended to the conformational analysis performed *in vacuum*, yielding three different sets of conformational properties (conformational properties of the neutral forms of the analytes, conformational properties of the ionized forms of the analytes, and average of the conformational properties at pH 7.4 according to the pKa of each analytes and the calculated microspecies distribution for zwitterions). For each of the properties taken into account (Molecular lipophilicity potential (MLP) [Gaillard et al., 1994], lipole [Pedretti et al., 2002], volume, polar surface area, superficial area, gyration radius, ovality, volume diameter, dipolar moment, etc), minimum and maximum value, average, range and standard deviation for each population of conformers were calculated and incorporated in the statistical models.

7.3 Results and discussion

The IAM-LC and MLC chromatographic retention coefficients as well as the pKa and the log BB values are presented in Table 1. In MLC the highest retained compound (triprolidine) eluted within 33 minutes, whereas in IAM-LC the maximum run time was 37 minutes (fluphenazine). The log BB values span a very large range (from -2.00 to +1.51).

Analyte	pKa	log k_w^{SDS}	log $k_{30\%MeOH}^{IAM}$	log BB
2-(Methylamino)pyridine	-	1.611	-0.164	-0.30[a]
2,2,2-trifluoroethyl vinyl ether	-	0.929	-0.142	0.13[a]
2,6-diisopropylphenol	-	1.688	1.097	0.91[b]
Acetaminophen	9.69	-0.092	-0.204	-1.00[b]
Acetylsalicylic acid	3.50	-0.301	-0.274	-1.30[b]
Aminopyrine	5.03	1.486	-0.206	0.00[b]
Amitriptyline	9.17	2.230	1.606	1.30[b]
Amobarbital	7.48/11.15*	1.208	0.059	0.04[b]
Antipyrine	1.44	1.059	-0.277	-0.10[b]
Atenolol	9.19	1.156	-0.162	-1.00[b]
Benzene	-	1.202	0.036	0.37[c]
Betahistine	7.84	0.125	-0.193	-0.30[d]
Caffeine	0.60	0.910	-0.284	-0.06[b]
Carbamazepine	-	1.191	0.210	0.00[b]
Celecoxib	9.38	1.461	1.613	0.10[d]
Chlorambucil	4.60	0.787	0.308	-1.70[b]
Chlorpromazine	9.50	2.169	2.038	1.36[d]
Cimetidine	7.01	1.003	-0.177	-1.42[b]
Citalopram	9.22	1.832	1.005	0.48[d]
Clonidine	8.08	1.436	0.171	0.11[b]
Clozapine	7.90	1.784	1.529	0.60[d]
Cotinine	-	1.424	-0.260	-0.32[b]
Cyclobenzaprine	8.47	2.092	1.607	1.08[d]
Desipramine	10.28	2.144	1.536	1.20[b]
Diclofenac	3.99	0.602	0.024	-1.70[d]
Diphenhydramine	8.86	2.077	0.858	1.20[d]
Domperidone	9.68	1.937	1.562	-0.78[b]
Donepezil	8.54*	1.968	0.858	0.89[e]
Eserine	8.17	1.656	0.030	0.08[b]
Ethosuximide	9.27	0.545	-0.228	0.04[d]
Ethylbenzene	-	1.588	0.600	0.26[c]

Fluphenazine	7.84/2.08*	2.207	2.066	1.51[b]
Haloperidol	8.29	2.366	1.483	1.34[e]
Halothane	-	1.215	0.152	0.35[c]
Hexobarbital	8.20	1.284	-0.008	0.10[b]
Hydroxyzine	7.52/1.58*	2.038	1.337	0.90[d]
Ibuprofen	4.24	0.626	0.090	-0.18[b]
Imipramine	9.52	2.190	1.452	1.30[b]
Indomethacin	4.13	0.647	-0.257	-1.26[b]
Ketorolac	3.84	-0.097	-0.500	-2.00[d]
Lamotrigine	5.36	1.316	-0.006	0.48[f]
Levofloxacin	8.59/5.89*	1.388	-0.099	-0.70[d]
Metanol	-	0.000	-0.447	0.02[f]
Metoclopramide	9.71	1.610	0.346	0.08[d]
Metoprolol	9.56	1.771	0.198	1.15[e]
Mianserin	6.92	2.152	1.456	0.99[b]
Naproxen	4.14	0.153	-0.090	-1.70[d]
Nicotine	8.11	1.969	-0.139	0.40[c]
Nitrofurantoin	7.05	-0.074	-0.447	-2.00[d]
Norfloxacin	8.50/6.25*	1.332	-0.062	-1.00[d]
Nortriptyline	10.13	2.169	1.639	1.04[d]
Olanzapine	7.80	1.825	0.843	0.80[d]
Omeprazole	9.33/4.31*	1.591	-0.229	-0.82[b]
Oxazepam	-	1.420	0.707	0.61[b]
Paroxetine	9.77	2.104	1.796	0.48[d]
Pentobarbital	8.18	1.243	0.103	0.12[b]
Phenylbutazone	4.34	0.996	0.273	-0.52[b]
Phenytoin	8.28	1.311	0.382	-0.04[b]
Pindolol	9.54	0.811	0.312	0.30[d]
Primidone	-	0.710	-0.152	-0.07[d]
Promazine	9.36	2.030	1.643	1.23[c]
Promethazine	9.00	2.040	1.613	1.30[g]
Propranolol	9.16	2.028	0.992	0.85[d]
Quinidine	8.56	2.245	0.982	0.33[e]

Ranitidine	8.33	1.233	-0.239	-1.23[b]
Rifampicin	1.70	1.900	0.990	-1.52[d]
Ropinirole	10.17	1.685	0.326	0.25[b]
Salicylic acid	2.82	-0.280	-0.302	-1.10[b]
Theobromine	-	0.347	-0.284	-0.28[b]
Theophylline	-	0.447	-0.218	-0.29[b]
Toluene	-	1.459	0.330	0.37[c]
Tramadol	9.41	1.692	0.256	0.70[d]
Trazodone	7.30	2.223	0.780	0.30[d]
Triprolidine	8.64	2.493	0.789	0.78[d]
Valproic acid	4.54	0.001	-0.279	-0.84[b]
Venlafaxine	9.67	1.900	0.429	0.48[d]
Verapamil	8.68	2.271	1.169	-0.52[b]
Zidovudine	9.40	0.271	-0.264	-1.00[c]
Zolmitriptan	9.55	0.974	-0.159	-1.40[d]

* calculated by Marvin Sketch 15.1 software

REFERENCES

a: [Abraham et al., 1994]

b: [Katritzky et al., 2006]

c: [Platts et al., 2001]

d: [Avdeef, A., 2012a]

e: [Mente & Lombardo, 2005]

f: [Abraham et al., 2006]

g: [Björkman 2002]

Table 1. pKa values, log k_w^{SDS}, log $k_{30\%MeOH}^{IAM}$ indexes and log BB values for the analytes taken into account.

ANALYTE	P-GP 1 Min	P-GP 1 Max	P-GP 2 Min	P-GP 2 Max	P-GP 3 Min	P-GP 3 Max	P-GP 4 Min	P-GP 4 Max
2-(Methylamino)pyridine	-4.1	-3.5	-4.1	-3.9	-4.7	-3.9	-4.1	-3.7
2,2,2-trifluoroethyl vinyl ether	-4.1	-3.4	-4.3	-3.9	-4.4	-3.6	-4.3	-3.7
2,6-diisopropylphenol	-5.8	-5.3	-5.9	-5.7	-6.2	-5.1	-5.9	-5.7
Acetaminophen	-5.1	-4.5	-5.1	-4.6	-5.8	-4.8	-5.1	-4.7
Acetylsalicylic acid	-5.3	-5.1	-5.6	-5.2	-6.3	-5.6	-5.6	-5.1
Aminopyrine	-5.8	-5.0	-5.8	-5.4	-6.5	-5.2	-5.9	-5.5
Amitriptyline	-9.2	-6.8	-9.3	-7.0	-7.4	-5.9	-9.2	-7.2
Amobarbital	-6.1	-5.4	-6.1	-5.6	-5.6	-4.9	-6.1	-5.6
Antipyrine	-5.7	-5.3	-6.0	-5.5	-6.2	-5.3	-6.0	-5.4
Atenolol	-5.7	-5.2	-6.0	-5.5	-5.7	-5.3	-6.2	-5.6
Benzene	-4.3	-3.8	-4.3	-3.7	-4.4	-4.0	-4.3	-4.2
Betahistine	-4.4	-4.1	-4.4	-4.0	-4.7	-4.1	-4.5	-4.1
Caffeine	-4.9	-4.7	-5.3	-4.8	-6.0	-5.6	-5.4	-4.8
Carbamazepine	-9.0	-6.6	-9.0	-6.6	-7.8	-6.5	-9.0	-7.0
Celecoxib	-8.7	-7.7	-8.5	-7.5	-7.3	-6.1	-8.5	-7.4
Chlorambucil	-5.5	-4.9	-5.6	-5.3	-5.5	-5.0	-5.6	-5.5
Chlorpromazine	-6.8	-5.7	-7.3	-6.4	-5.8	-5.0	-7.2	-6.3
Cimetidine	-5.2	-4.7	-5.4	-4.8	-5.5	-4.9	-5.4	-4.8
Citalopram	-7.3	-6.3	-7.3	-6.6	-6.4	-6.0	-7.4	-6.5

Clonidine	-5.7	-5.1	-5.7	-5.3	-5.8	-5.0	-5.7	-5.3
Clozapine	-7.9	-6.7	-7.9	-7.2	-7.4	-5.9	-7.9	-7.0
Cotinine	-5.2	-4.7	-5.8	-5.2	-6.1	-5.3	-5.8	-5.2
Cyclobenzaprine	-8.6	-6.4	-8.6	-6.6	-7.7	-6.5	-8.7	-6.8
Desipramine	-7.7	-6.2	-7.7	-6.3	-6.5	-5.9	-7.7	-6.3
Diclofenac	-7.0	-6.1	-7.0	-6.5	-6.4	-5.9	-7.0	-6.5
Diphenhydramine	-7.2	-6.5	-7.2	-6.6	-5.9	-5.5	-7.2	-6.6
Domperidone	-7.9	-7.2	-9.4	-8.3	-7.9	-6.8	-9.5	-8.3
Donepezil	-8.0	-7.3	-8.4	-7.7	-8.1	-7.3	-8.5	-7.7
Eserine	-6.6	-5.8	-6.6	-6.0	-6.2	-5.5	-6.6	-6.1
Ethosuximide	-5.1	-4.2	-5.1	-4.3	-5.5	-4.4	-4.8	-4.4
Ethylbenzene	-5.5	4.7	-5.4	-4.7	-5.3	-4.5	-5.4	-4.6
Fluphenazine	-7.9	-7.0	-7.7	-7.1	-6.7	-5.8	-7.8	-7.1
Haloperidol	-7.6	-7.2	-8.7	-8.0	-7.3	-6.8	-8.7	-7.8
Halothane	-4.2	-3.6	-4.5	-3.9	-4.5	-3.9	-4.4	-3.9
Hexobarbital	-6.3	-6.1	-7.0	-6.1	-6.2	-5.6	-7.0	-6.0
Hydroxyzine	-7.1	-6.3	-7.2	-6.6	-6.1	-5.5	-6.9	-6.7
Ibuprofen	-6.6	-5.5	-6.4	-5.9	-6.7	-5.1	-6.6	-6.0
Imipramine	-7.9	-6.1	-8.0	-6.4	-6.8	-5.9	-8.0	-6.4
Indomethacin	-7.0	-6.0	-7.2	-6.2	-6.8	-5.7	-7.2	-6.3
Ketorolac	-6.9	-6.4	-7.1	-6.4	-7.4	-6.0	-7.2	-6.5

Lamotrigine	-5.8	-5.2	-6.2	-5.7	-5.7	-5.3	-6.2	-5.8
Levofloxacin	-6.5	-6.1	-6.6	-6.3	-7.3	-5.7	-6.6	-6.4
Metanol	-2.0	-1.5	-2.0	-1.6	-1.8	-1.5	-2.0	-1.6
Metoclopramide	-5.6	-5.2	-5.7	-5.5	-5.9	-5.0	-5.7	-5.2
Metoprolol	-5.5	-5.2	-5.7	-5.3	-5.6	-5.2	-5.6	-5.1
Mianserin	-9.0	-6.9	-9.1	-7.3	-7.5	-6.4	-9.1	-7.0
Naproxen	-6.5	-5.8	-6.5	-6.2	-7.0	-6.3	-6.5	-6.2
Nicotine	-5.2	-4.6	-5.4	-4.9	-6.1	-4.9	-5.4	-4.9
Nitrofurantoin	-6.1	-5.5	-6.4	-5.7	-6.3	-5.4	-6.4	-5.7
Norfloxacin	-6.8	-5.8	-6.8	-6.2	-6.9	-5.8	-6.7	-6.1
Nortriptyline	-8.7	-6.7	-9.0	-7.0	-7.2	-5.6	-8.7	-6.7
Olanzapine	-8.4	-6.1	-8.4	-6.6	-6.9	-5.5	-8.4	-6.6
Omeprazole	-6.5	-6.2	-6.9	-6.6	-6.8	-6.0	-7.5	-7.0
Oxazepam	-8.9	-7.4	-8.9	-7.8	-7.9	-6.3	-8.9	-7.8
Paroxetine	-7.4	-7.1	-8.0	-7.6	-6.8	-6.2	-7.6	-7.2
Pentobarbital	-5.4	-5.0	-5.4	-5.2	-5.4	-4.9	-5.4	-5.2
Phenylbutazone	-7.1	-6.5	-7.1	-6.6	-6.4	-6.0	-7.2	-6.5
Phenytoin	-8.5	-7.3	-8.5	-7.6	-6.9	-6.4	-8.4	-7.5
Pindolol	-5.9	-5.5	-6.2	-5.8	-6.4	-5.8	-6.2	-5.8
Primidone	-6.5	-5.9	-6.5	-6.1	-6.1	-5.5	-6.6	-6.1
Promazine	-7.0	-5.6	-7.0	-6.0	-6.2	-5.5	-7.0	-5.8

Promethazine	-7.3	-6.0	-7.1	-6.2	-6.4	-5.6	-7.3	-6.3
Propranolol	-6.9	-6.5	-6.9	-6.4	-6.7	-6.0	-7.0	-6.3
Quinidine	-7.4	-6.7	-7.6	-6.9	-6.7	-5.7	-8.1	-6.9
Ranitidine	-5.6	-4.7	-4.9	-4.6	-4.8	-4.4	-4.9	-4.5
Rifampicin	-7.1	-6.5	-6.8	-6.5	-6.7	-6.0	-6.8	-6.2
Ropinirole	-6.8	-6.1	-6.3	-6.0	-7.0	-5.9	-6.3	-6.1
Salicylic acid	-5.2	-4.6	-5.2	-4.7	-5.4	-5.0	-5.2	-4.7
Theobromine	-4.9	-4.5	-5.3	-4.9	-5.8	-5.5	-5.3	-4.8
Theophylline	-5.1	-4.5	-5.2	-4.8	-6.0	-5.6	-5.3	-4.8
Toluene	-4.9	-4.4	-4.9	-4.3	-4.9	-4.6	-4.9	-4.4
Tramadol	-6.2	-5.6	-6.6	-6.0	-6.2	-5.2	-6.6	-5.9
Trazodone	-7.6	-6.6	-8.1	-7.5	-7.9	-6.9	-8.2	-7.5
Triprolidine	-8.4	-7.6	-8.2	-7.5	-6.7	-6.2	-7.7	-7.0
Valproic acid	-4.7	-4.0	-4.5	-4.2	-5.2	-4.0	-4.6	-4.2
Venlafaxine	-6.5	-5.8	-6.7	-6.1	-6.0	-5.3	-6.7	-6.0
Verapamil	-6.3	-5.8	-7.7	-6.9	-6.7	-6.4	-7.1	-6.8
Zidovudine	-6.4	-5.8	-7.0	-5.9	-6.7	-5.6	-6.9	-5.8
Zolmitriptan	-6.2	-5.8	-6.8	-6.4	-6.8	-5.7	-6.9	-6.6

Table 2.0. Minimum and maximum values, expressed in kcal mol^{-1}, of the affinities that each analyte has for the first four (from 1 to 4) discrete binding sites located on the P-gp.

ANALYTE	P-GP 5 Min	P-GP 5 Max	P-GP 6 Min	P-GP 6 Max	P-GP 7 Min	P-GP 7 Max	P-GP 8 Min	P-GP 8 Max
2-(Methylamino)pyridine	-4.1	-3.8	-4.0	-3.5	-4.1	-3.8	-4.1	-3.8

2,2,2-trifluoroethyl vinyl ether	-4.0	-3.5	-4.1	-3.6	-4.1	-3.6	-4.1	-3.5
2,6-diisopropylphenol	-5.9	-5.5	-5.7	-5.6	-5.9	-5.7	-5.7	-5.6
Acetaminophen	-5.1	-4.6	-5.1	-4.6	-5.0	-4.6	-5.0	-4.6
Acetylsalicylic acid	-5.2	-4.8	-5.3	-5.0	-5.3	-5.0	-5.6	-5.1
Aminopyrine	-5.8	-5.4	-5.8	-5.2	-5.8	-5.4	-5.8	-5.4
Amitriptyline	-9.1	-7.2	-8.8	-6.8	-9.1	-7.2	-9.1	-6.9
Amobarbital	-6.1	-5.5	-6.1	-5.6	-6.1	-5.6	-6.1	-5.4
Antipyrine	-5.7	-5.3	-6.0	-5.3	-6.0	-5.4	-6.0	-5.4
Atenolol	-6.2	-5.6	-5.9	-5.5	-6.2	5.7	-5.9	-5.5
Benzene	-4.3	-3.8	-4.3	-4.0	-4.3	-4.0	-4.3	-3.7
Betahistine	-4.6	-4.0	-4.4	-3.9	-4.2	-4.1	-4.5	-4.0
Caffeine	-4.9	-4.6	-4.9	-4.5	-4.9	-4.5	-5.3	-4.6
Carbamazepine	-9.0	-6.9	-9.0	6.6	-9.0	-6.9	-9.0	-6.6
Celecoxib	-8.7	-7.7	-8.5	-7.5	-8.5	-7.4	-8.5	-7.5
Chlorambucil	-5.1	-4.9	-5.5	-5.2	-5.4	-5.1	-5.6	-5.1
Chlorpromazine	-6.8	-6.2	-6.7	-6.0	-6.8	-6.1	-6.7	-6.1
Cimetidine	-5.2	-4.8	-5.6	-5.0	-5.5	-4.8	-5.4	-4.8
Citalopram	-7.3	-6.4	-7.4	-6.5	-7.5	-6.8	-7.5	-6.6
Clonidine	-5.7	-5.1	-5.7	-5.1	-5.7	-5.1	-5.7	-5.3
Clozapine	-7.9	-6.9	-7.9	-7.2	-7.9	-7.3	-7.9	-7.3

Cotinine	-5.3	-4.7	-5.8	-4.9	-5.8	-5.0	-5.8	-5.1
Cyclobenzaprine	-8.5	-6.6	-8.4	-6.4	-8.8	-6.7	-8.5	-6.7
Desipramine	-7.7	-6.2	-7.7	-6.2	-7.7	-6.3	-7.7	-6.3
Diclofenac	-7.0	-6.2	-7.0	-6.2	-7.0	-6.5	-7.0	-6.5
Diphenhydramine	-7.2	-6.7	-7.2	-6.7	-7.2	-6.6	-7.3	-6.8
Domperidone	-8.8	-8.1	-9.4	-8.2	-9.4	-8.3	-9.4	-8.3
Donepezil	-8.0	-7.2	-8.5	-7.7	-8.5	-7.6	-8.4	-7.6
Eserine	-6.6	-6.0	-6.6	-5.9	-6.6	-5.9	-6.6	-6.1
Ethosuximide	-5.1	-4.2	-5.1	-4.2	-5.1	-4.3	-4.8	-4.4
Ethylbenzene	-5.4	-4.5	-5.4	-4.6	-5.4	-4.7	-5.4	-4.6
Fluphenazine	-7.9	-7.1	-7.8	-6.9	-7.9	-7.1	-7.6	-7.1
Haloperidol	-8.1	-7.4	-8.6	-7.8	-8.6	7.9	-8.6	-7.9
Halothane	-4.4	-3.7	-4.5	-3.9	-4.5	-3.8	-4.3	-3.9
Hexobarbital	-6.2	-5.6	-6.0	-5.7	-7.0	-6.0	-7.0	-6.0
Hydroxyzine	-6.9	-6.4	-6.9	-6.5	-7.2	-6.4	-7.1	-6.5
Ibuprofen	-6.5	-5.5	-6.3	-5.7	-6.4	-5.9	-6.4	-5.7
Imipramine	-7.9	-6.4	-8.0	-6.4	-8.0	-6.5	-8.0	-6.5
Indomethacin	-7.0	-6.2	-7.2	-6.3	-7.3	-6.3	-7.3	-6.7
Ketorolac	-6.6	-6.3	-6.9	-6.3	-7.1	-6.4	-7.1	-6.4
Lamotrigine	-5.8	-5.3	-6.1	-5.6	-6.2	-5.6	-6.2	-5.8
Levofloxacin	-6.6	-6.1	-6.5	-6.0	-6.6	-6.3	-6.9	-6.6

Metanol	-2.0	-1.6	-2.0	-1.5	-2.0	-1.5	-2.0	-1.5
Metoclopramide	-5.6	-5.0	-5.6	-5.1	-5.6	-5.4	-5.6	-5.1
Metoprolol	-5.7	-5.2	-5.6	-5.1	-5.7	-5.1	-5.6	-5.3
Mianserin	-9.1	-7.1	-9.1	-6.7	-9.1	-7.0	-9.1	-7.1
Naproxen	-6.4	-5.9	-6.5	-6.1	-6.6	-6.2	-6.6	-6.0
Nicotine	-5.2	-4.9	-5.4	-5.0	-5.4	-4.9	-5.4	-4.9
Nitrofurantoin	-6.1	-5.5	-6.1	-5.6	-6.1	-5.7	-6.5	-5.7
Norfloxacin	-6.8	-5.8	-6.8	-5.8	-6.8	-6.1	-6.8	-6.0
Nortriptyline	-8.6	-6.8	-8.8	-7.0	-9.1	-7.0	-8.8	-7.0
Olanzapine	-8.4	-6.4	-8.4	-6.2	-8.4	-6.7	-8.4	-6.5
Omeprazole	-7.0	-6.6	-6.9	-6.5	-6.9	-6.5	-7.4	-6.9
Oxazepam	-8.9	-7.6	-8.9	-7.4	-8.9	-7.7	-8.9	-7.6
Paroxetine	-8.0	-7.7	-8.0	-7.7	-7.9	-7.6	-8.0	-7.6
Pentobarbital	-5.5	-5.2	-5.4	-5.0	-5.4	-5.0	-5.4	-5.2
Phenylbutazone	-7.1	-6.5	-7.1	-6.5	-7.1	-6.5	-7.1	-6.5
Phenytoin	-8.3	-7.5	-8.5	-7.5	-8.5	-7.7	-8.5	-7.6
Pindolol	-5.8	-5.4	-6.2	-5.6	-6.2	-5.5	-6.1	-5.7
Primidone	-5.7	-5.3	-6.5	-6.0	-6.6	-6.1	-6.5	-5.9
Promazine	-7.0	-5.9	-7.0	-5.6	-7.0	-6.0	-7.0	-5.9
Promethazine	-7.2	-6.3	-7.1	-5.9	-7.1	-6.1	-7.1	-6.2
Propranolol	-6.7	-6.2	-6.7	-6.2	-7.0	-6.4	-7.0	-6.3

Quinidine	-7.6	-6.1	-8.1	-6.7	-7.6	-6.9	-8.1	-7.0
Ranitidine	-5.0	-4.7	-5.3	-4.8	-5.2	-4.8	-5.1	-4.8
Rifampicin	-7.1	-6.5	-7.0	-6.2	-7.1	-6.3	-7.4	-6.8
Ropinirole	-6.2	-5.8	-6.3	-6.0	-6.3	-6.1	-5.8	-5.3
Salicylic acid	-5.2	-4.7	-5.2	-4.7	-5.2	-4.7	-5.2	-4.7
Theobromine	-5.0	-4.6	-5.1	-4.6	-5.1	-4.7	-5.3	-4.7
Theophylline	-5.1	-4.4	-5.2	-4.5	-5.2	-4.5	-5.2	-4.8
Toluene	-4.9	-4.4	-4.9	-4.2	-4.9	-4.5	-4.9	-4.3
Tramadol	-6.2	-5.7	-6.5	-6.0	-6.6	-6.0	-6.6	-6.0
Trazodone	-7.8	-7.2	-8.1	-7.3	-8.0	-7.7	-7.9	-7.5
Triprolidine	-8.0	-7.4	-8.4	-7.9	-8.2	-7.5	-8.4	-7.6
Valproic acid	-4.4	-4.1	-4.6	-4.2	-4.5	-4.1	-4.5	-4.2
Venlafaxine	-6.3	-5.7	-6.7	-5.9	-6.7	-6.1	-6.7	-6.1
Verapamil	-7.4	-6.6	-7.4	-6.7	-7.3	-6.8	-7.2	-6.9
Zidovudine	-6.2	-5.4	-6.4	-5.7	-6.1	-5.5	-6.9	-5.8
Zolmitriptan	-6.4	-5.9	-6.8	-5.9	-6.8	-6.3	-6.5	-6.2

Table 2.1. Minimum and maximum values, expressed as kcal mol^{-1}, of the affinities that each analyte has for the second four (from 5 to 8) discrete binding sites located on the P-gp.

The P-gp affinities, expressed in kcal mol^{-1}, of the drugs considered are listed in Table 2. They were incorporated in each of the following steps in an attempt to model even the BBB passage of analytes undergoing P-gp effux mechanisms.

7.3.1 MLC Indexes in log BB prediction

MLC indexes were used in an attempt to develop BBB passage potential predicting models along with either static or conformational properties. At first, all the analytes were assumed as neutral, even the ones supporting one or more ionizable functions. The equations along with the statistical validation are reported in Table 3. In the equations hereby reported, r^2 is the multiple regression coefficient, q^2 is the r^2 validated by Leave-One-Out (LOO) Optimization, *SE* is the error standard deviation, *F* represents the Fischer regression statistic value, *PC* is the Amemiya predictive criterion and ExRow is the analyte excluded in order to maximize the predictive strength of the statistic model. The respective plots Experimental versus Predicted log BB values are shown in Figure 1. Their static physico-chemical descriptors are listed in Table 4. If not differently indicated, every regression was developed by employing four different independent variables (MLC indexes + three other physico-chemical descriptors). Surprisingly, even if over two thirds of the analytes support one or more ionizable functions, fairly good relationship, as the one expressed by equations (1) and (2), are obtained even not taking into account the presence of electric charges.

MOLECULAR DESCRIPTORS	r^2	q^2	*SE*	*F*	*PC*	r^2 (n-1)	*SE* (n-1)	*F* (n-1)	*PC* (n-1)	EX-ROW	EQUATIONS	EQ No
STATIC												
NEUTRAL PROPERTIES	0.69	0.64	0.518	41.10	21.442	0.70	0.509	43.53	20.396	-	log BB = -0.3294 + 0.8126 log k_w^{SDS} - 0.0156 Psa - 0.0614 VirtualLogP + 0.1409 HbDon	1
										2-(Methylamino) pyridine	log BB = -0.2770 + 0.8326 log k_w^{SDS} - 0.0163 Psa - 0.0790 VirtualLogP + 0.1524 HbDon	2
IONIZED PROPERTIES	0.68	0.63	0.528	38.83	22.291	0.70	0.512	42.52	20.675	-	log BB = -0.4123 + 0.7120 log k_w^{SDS} - 0.0089 Psa + 0.0960 Charge - 0.0187 Impropers	3
										Verapamil	log BB = -0.4708 + 0.7548 log k_w^{SDS} - 0.0078 Psa + 0.0846 Charge - 0.0241 Impropers	4
WEIGHTED AVERAGE	0.71	0.68	0.498	46.01	19.810	0.73	0.484	49.72	18.483	-	log BB = -0.3136 + 0.6610 log k_w^{SDS} - 0.0085 Psa - 0.0188 Dipole + 0.2539 Charge	5
										Verapamil	log BB = -0.3807 + 0.7023 log k_w^{SDS} - 0.0079 Psa - 0.0200 Dipole + 0.2184 Charge	6
STATIC + CONFORMATIONAL												

NEUTRAL PROPERTIES	0.69	0.64	0.516	41.69	21.233	0.71	0.506	44.18	20.182	-	log BB = -0.3561 + 0.8177 log k_w^{SDS} - 0.0150 PSA Max + 0.1436 HbDon - 0.0515 MLP Max	7
										2-(Methylamino) pyridine	log BB = -0.3055 + 0.8384 log k_w^{SDS} - 0.0156 PSA Max + 0.1538 HbDon - 0.0684 MLP Max	8
IONIZED PROPERTIES	0.68	0.63	0.526	39.36	22.085	0.71	0.511	42.72	20.605	-	log BB = -0.4637 + 0.7477 log k_w^{SDS} + 0.1035 Charge - 0.0070 PSA Max - 0.0236 Impropers	9
										Verapamil	log BB = -0.4772 + 0.7556 log k_w^{SDS} - 0.0079 PSA Average + 0.0830 Charge - 0.0233 Impropers	10
WEIGHTED AVERAGE	0.72	0.69	0.487	48.73	19.175	0.74	0.774	52.30	17.972	-	log BB = -0.2193 + 0.6223 log k_w^{SDS} - 0.0094 PSA Average - 0.0198 Dipole + 0.2993 Charge	11
										Verapamil	log BB = -0.2783 + 0.6596 log k_w^{SDS} - 0.0088 PSA Average - 0.0208 Dipole + 0.2669 Charge	12

Table 3. Statical validation of the models developed employing log k_w^{SDS} values of the dataset (n=79) along with three other physico-chemical descriptors.

Analyte	Angles	Atoms	Bonds	Charge	ChiralAtms	Dipole	EzBnds	FlexTorsions	Gyrrad
2-(Methylamino)pyridine	25	16	16	0	0	1.152	0	1	1.985
2,2,2-trifluoroethyl vinyl ether	19	13	12	0	0	3.473	0	2	2.109
2,6-diisopropylphenol	55	31	31	0	0	1.546	0	0	2.707
Acetaminophen	31	20	20	0	0	2.919	0	1	2.641
Acetylsalicylic acid	32	21	21	0	0	1.099	0	3	2.449
Aminopyrine	60	34	35	0	0	2.517	1	2	3.028
Amitriptyline	81	44	46	0	0	0.900	0	3	3.324
Amobarbital	63	34	34	0	0	0.836	0	3	2.850
Antipyrine	45	26	27	0	0	2.657	1	1	2.644
Atenolol	71	41	41	0	1	5.000	0	8	3.872
Benzene	18	12	12	0	0	0.000	0	0	1.516
Betahistine	37	22	22	0	0	1.184	0	3	2.697
Caffeine	43	24	25	0	0	1.457	0	0	2.481
Carbamazepine	51	30	32	0	0	2.311	1	1	2.812
Celecoxib	70	40	42	0	0	4.557	0	3	4.246
Chlorambucil	67	38	38	0	0	1.732	0	7	4.281
Chlorpromazine	73	40	42	0	0	1.317	0	4	3.398
Cimetidine	55	33	33	0	0	1.963	0	7	3.838
Citalopram	83	45	47	0	1	2.407	0	5	4.010

Clonidine	40	23	24	0	0	0.385	0	2	2.743
Clozapine	79	42	45	0	0	1.965	0	1	3.837
Cotinine	46	25	26	0	1	3.247	0	1	2.585
Cyclobenzaprine	75	42	44	0	0	0.890	0	4	3.314
Desipramine	78	42	44	0	0	1.019	0	4	3.338
Diclofenac	49	30	31	0	0	1.415	0	4	3.134
Diphenhydramine	70	40	41	0	0	2.244	0	6	3.437
Domperidone	105	54	58	0	0	3.861	0	5	5.287
Donepezil	110	57	60	0	1	3.268	0	6	5.440
Eserine	79	41	43	0	2	1.502	0	2	3.575
Ethosuximide	39	21	21	0	1	2.432	0	1	2.142
Ethylbenzene	30	18	18	0	0	0.123	0	1	2.039
Fluphenazine	107	56	59	0	0	2.920	0	6	5.040
Haloperidol	91	49	51	0	0	3.257	0	6	5.784
Halothane	12	8	7	0	1	1.718	0	0	1.868
Hexobarbital	63	33	34	0	1	0.657	1	1	2.795
Hydroxyzine	98	53	55	0	1	1.551	0	8	4.974
Ibuprofen	58	33	33	0	1	1.359	0	1	3.203
Imipramine	84	45	47	0	0	1.026	0	4	3.419
Indomethacin	71	41	43	0	0	1.015	0	4	4.100
Ketorolac	58	32	34	0	1	2.451	0	3	3.311

Lamotrigine	36	23	24	0	0	1.805	0	1	3.086
Levofloxacin	89	46	49	0	1	3.949	1	2	3.947
Metanol	7	6	5	0	0	1.653	0	0	0.854
Metoclopramide	73	42	42	0	0	3.451	0	7	4.093
Metoprolol	78	44	44	0	1	4.067	0	9	3.593
Mianserin	78	40	43	0	1	0.588	0	0	3.126
Naproxen	53	31	32	0	1	1.295	0	1	3.442
Nicotine	49	26	27	0	1	2.211	0	1	2.498
Nitrofurantoin	38	23	24	0	0	0.761	0	2	3.784
Norfloxacin	76	41	43	0	0	3.345	1	3	3.874
Nortriptyline	75	41	43	0	0	0.826	0	3	3.465
Olanzapine	80	42	45	0	0	2.062	0	1	3.595
Omeprazole	76	43	45	0	1	8.545	0	5	4.670
Oxazepam	53	31	33	0	1	2.698	0	1	3.355
Paroxetine	84	44	47	0	2	0.819	0	4	3.809
Pentobarbital	63	34	34	0	1	0.940	0	2	2.842
Phenylbutazone	78	43	45	0	0	0.851	0	5	3.361
Phenytoin	54	31	33	0	0	1.909	0	2	2.932
Pindolol	68	38	39	0	1	2.598	0	6	3.257
Primidone	54	30	31	0	0	2.879	0	2	2.600
Promazine	73	40	42	0	0	1.160	0	4	3.048

Promethazine	73	40	42	0	1	1.354	0	3	3.167
Propranolol	71	40	41	0	1	2.319	0	6	3.370
Quinidine	93	48	51	0	4	1.383	0	4	3.551
Ranitidine	74	43	43	0	0	3.634	1	9	4.163
Rifampicin	217	117	121	0	9	3.311	4	5	4.920
Ropinirole	81	43	44	0	0	2.360	0	7	3.783
Salicylic acid	23	16	16	0	0	2.379	0	1	2.178
Theobromine	37	21	22	0	0	1.879	0	0	2.384
Theophylline	37	21	22	0	0	1.318	0	0	2.358
Toluene	24	15	15	0	0	0.120	0	0	1.781
Tramadol	83	44	45	0	2	2.669	0	4	3.232
Trazodone	91	48	51	0	0	1.081	2	5	4.904
Triprolidine	79	43	45	0	0	1.848	1	4	3.560
Valproic acid	46	26	25	0	0	1.449	0	2	2.597
Venlafaxine	89	47	48	0	1	2.996	0	2	3.434
Verapamil	128	71	72	0	1	2.214	0	12	4.619
Zidovudine	61	34	35	0	3	2.832	1	3	3.273
Zolmitriptan	79	42	44	0	1	3.714	0	5	3.836

Table 4A. Angles, atoms, bonds, chiral atoms (ChiralAtms), dipole, E-Z bonds (EzBnds), flexible torsions (FlexTorsions), gyration radius (Gyrrad) of the analytes, assumed as neutral, taken into account.

Analyte	HbAcc	HbDon	HeavyAtoms	Impropers	Lipole	Mass	MassMI	Ovality	Psa
2-(Methylamino)pyridine	1	1	8	3	0.903	108.141	108.069	1.294	24.777
2,2,2-trifluoroethyl vinyl ether	1	0	8	6	1.104	126.077	126.029	1.334	10.574
2,6-diisopropylphenol	1	1	13	0	2.200	178.271	178.136	1.537	21.637
Acetaminophen	2	2	11	6	1.587	151.163	151.063	1.364	54.319
Acetylsalicylic acid	4	1	13	6	1.561	180.157	180.042	1.414	64.785
Aminopyrine	1	0	17	18	1.055	231.294	231.137	1.579	27.469
Amitriptyline	1	0	21	9	2.281	277.403	277.183	1.642	4.845
Amobarbital	3	2	16	15	1.852	226.272	226.132	1.582	80.654
Antipyrine	1	0	14	15	1.861	188.226	188.095	1.463	24.638
Atenolol	4	4	19	9	1.959	266.336	266.163	1.705	90.862
Benzene	0	0	6	0	0.000	78.112	78.047	1.192	0.000
Betahistine	2	1	10	3	1.921	136.194	136.100	1.423	25.820
Caffeine	3	0	14	12	0.293	194.191	194.080	1.430	54.408
Carbamazepine	1	2	18	15	3.033	236.269	236.095	1.448	44.702
Celecoxib	4	2	26	3	3.162	381.372	381.076	1.699	85.567
Chlorambucil	2	1	19	6	4.466	304.212	303.079	1.711	44.356
Chlorpromazine	1	0	21	6	2.063	318.864	318.096	1.647	30.317
Cimetidine	2	3	17	9	2.272	252.339	252.116	1.634	101.502
Citalopram	2	0	24	3	1.831	324.392	324.164	1.722	34.091
Clonidine	1	2	14	9	2.220	230.094	229.017	1.501	41.987

Clozapine	2	1	23	12	2.806	326.823	326.130	1.655	30.444
Cotinine	2	0	13	6	1.718	176.215	176.095	1.440	31.705
Cyclobenzaprine	1	0	21	6	3.327	275.388	275.167	1.613	5.405
Desipramine	1	1	20	6	2.729	266.381	266.178	1.639	18.180
Diclofenac	2	2	19	6	3.351	296.149	295.017	1.576	49.126
Diphenhydramine	2	0	19	3	2.752	255.355	255.162	1.660	14.903
Domperidone	3	2	30	21	2.269	425.911	425.162	1.793	76.354
Donepezil	4	0	28	6	0.977	379.492	379.215	1.812	45.714
Eserine	3	1	20	12	0.741	275.346	275.163	1.647	50.057
Ethosuximide	2	1	10	9	1.792	141.168	141.079	1.399	50.899
Ethylbenzene	0	0	8	0	0.019	106.165	106.078	1.351	0.000
Fluphenazine	3	1	30	9	4.685	437.522	437.175	1.804	58.093
Haloperidol	3	1	26	6	0.737	375.864	375.140	1.737	39.515
Halothane	0	0	7	0	0.393	197.382	195.890	1.290	0.000
Hexobarbital	3	1	17	21	1.177	236.267	236.116	1.545	68.400
Hydroxyzine	4	1	26	6	3.733	374.904	374.176	1.778	42.614
Ibuprofen	2	1	15	3	3.665	206.281	206.131	1.581	39.941
Imipramine	1	0	21	6	2.298	280.407	280.194	1.669	7.454
Indomethacin	4	1	25	6	2.647	357.788	357.077	1.699	70.874
Ketorolac	3	1	19	6	1.648	255.269	255.090	1.546	60.255
Lamotrigine	3	4	16	6	4.497	256.091	255.008	1.469	87.480

Levofloxacin	5	1	26	21	0.782	361.368	361.144	1.695	78.170
Metanol	1	1	2	0	1.438	32.042	32.026	1.120	23.429
Metoclopramide	3	3	20	12	0.485	299.796	299.140	1.723	67.535
Metoprolol	4	2	19	3	1.702	267.364	267.183	1.743	56.354
Mianserin	1	0	20	6	2.659	264.365	264.163	1.572	8.386
Naproxen	3	1	17	3	2.364	230.259	230.094	1.542	51.411
Nicotine	2	0	12	3	0.867	162.232	162.116	1.430	15.468
Nitrofurantoin	6	1	17	18	2.843	238.157	238.034	1.489	117.074
Norfloxacin	4	2	23	21	2.221	319.331	319.133	1.641	78.102
Nortriptyline	1	1	20	9	3.403	263.377	263.167	1.619	16.614
Olanzapine	2	1	22	12	2.805	312.433	312.141	1.633	52.449
Omeprazole	5	1	24	3	1.180	345.416	345.115	1.724	89.545
Oxazepam	3	2	20	9	4.240	286.713	286.051	1.522	63.346
Paroxetine	4	1	24	3	2.095	329.365	329.143	1.674	50.665
Pentobarbital	3	2	16	15	2.002	226.272	226.132	1.564	78.161
Phenylbutazone	2	0	23	12	1.678	308.374	308.153	1.698	42.596
Phenytoin	2	2	19	12	2.659	252.268	252.090	1.501	65.254
Pindolol	3	3	18	3	2.943	248.321	248.153	1.661	63.244
Primidone	2	2	16	12	2.187	218.252	218.106	1.477	61.411
Promazine	1	0	20	6	1.773	284.419	284.135	1.614	29.498
Promethazine	1	0	20	6	2.477	284.419	284.135	1.604	29.823

Propranolol	3	2	19	3	3.275	259.343	259.157	1.656	45.562
Quinidine	4	1	24	9	1.303	324.417	324.184	1.668	49.293
Ranitidine	4	2	21	18	1.383	314.404	314.141	1.750	100.362
Rifampicin	15	6	59	39	0.746	822.940	822.405	2.172	208.882
Ropinirole	2	1	19	9	1.573	260.375	260.189	1.711	37.704
Salicylic acid	3	2	10	3	2.589	138.121	138.032	1.278	58.962
Theobromine	3	1	13	12	0.536	180.164	180.065	1.397	67.976
Theophylline	3	1	13	12	0.202	180.164	180.065	1.389	67.548
Toluene	0	0	7	0	0.017	92.138	92.063	1.242	0.000
Tramadol	3	1	19	3	1.447	263.375	263.189	1.657	35.506
Trazodone	3	0	26	30	4.141	371.864	371.151	1.751	45.310
Triprolidine	2	0	21	9	2.004	278.391	278.178	1.660	15.095
Valproic acid	2	1	10	3	2.035	144.211	144.115	1.511	40.590
Venlafaxine	3	1	20	3	1.279	277.402	277.204	1.701	36.808
Verapamil	5	0	33	3	1.885	454.602	454.283	1.995	68.078
Zidovudine	7	4	19	21	0.766	269.257	269.112	1.581	132.551
Zolmitriptan	3	2	21	9	2.306	287.357	287.163	1.691	65.848

Table 4B. Hydrogen bond acceptor (HbAcc), hydrogen bond donor (HbDon) groups, heavy atoms (HeavyAtoms), impropers, lipole, mass, monoisotopic mass (MassMI), ovality and polar surface area (Psa) of the analytes, assumed as neutral, taken into account.

Analyte	Rings	Sas	Sav	Sdiam	Surface	Torsions	Vdiam	VirtualLogP	Volume
2-(Methylamino)pyridine	1	289.355	403.799	6.730	142.272	7	5.915	1.132	108.346
2,2,2-trifluoroethyl vinyl ether	0	281.133	385.340	6.591	136.488	3	5.706	2.463	97.271
2,6-diisopropylphenol	1	408.043	638.038	9.005	254.748	7	7.262	3.678	200.560
Acetaminophen	1	340.442	492.948	7.532	178.206	9	6.449	1.110	140.455
Acetylsalicylic acid	1	367.831	547.381	7.981	200.100	10	6.712	1.216	158.302
Aminopyrine	2	470.183	746.141	9.465	281.425	13	7.532	1.534	223.768
Amitriptyline	3	536.034	891.474	10.482	345.169	21	8.180	3.978	286.574
Amobarbital	1	453.694	725.207	9.360	275.239	9	7.442	2.057	215.808
Antipyrine	2	402.875	608.755	8.491	226.501	12	7.020	1.252	181.106
Atenolol	1	523.202	846.813	10.399	339.746	16	7.963	1.367	264.407
Benzene	1	238.870	321.014	5.951	111.247	6	5.450	2.136	84.761
Betahistine	1	360.361	521.191	7.716	187.021	9	6.468	1.023	141.691
Caffeine	2	376.477	573.739	8.137	208.022	10	6.805	-0.221	164.992
Carbamazepine	3	429.993	681.449	8.870	247.193	19	7.371	2.213	209.707
Celecoxib	3	596.617	969.787	10.815	367.429	21	8.297	3.332	299.113
Chlorambucil	1	553.350	889.523	10.486	345.416	16	8.017	4.572	269.776
Chlorpromazine	3	527.092	887.965	10.513	347.188	20	8.192	5.213	287.865
Cimetidine	1	519.800	807.406	9.722	296.955	14	7.605	0.518	230.294
Citalopram	3	609.203	994.650	11.004	380.416	22	8.387	4.638	308.865
Clonidine	2	414.840	626.731	8.627	233.810	13	7.042	2.515	182.844

Clozapine	4	556.876	919.619	10.572	351.144	24	8.217	2.812	290.496
Cotinine	2	373.419	568.966	8.198	211.153	12	6.832	0.765	166.954
Cyclobenzaprine	3	532.688	875.792	10.268	331.222	21	8.085	4.569	276.750
Desipramine	3	524.823	854.355	10.278	331.880	21	8.029	3.375	271.038
Diclofenac	2	477.847	772.973	9.671	293.819	17	7.704	4.409	239.377
Diphenhydramine	2	532.558	856.809	10.243	329.619	18	7.950	3.554	263.086
Domperidone	5	686.581	1156.548	11.932	447.263	31	8.910	2.451	370.367
Donepezil	4	696.772	1162.015	11.984	451.212	28	8.904	3.801	369.581
Eserine	3	523.285	845.378	10.213	327.692	17	7.958	1.785	263.886
Ethosuximide	1	318.760	472.032	7.538	178.507	6	6.373	1.137	135.551
Ethylbenzene	1	308.252	437.569	7.096	158.200	7	6.105	3.057	119.126
Fluphenazine	4	724.716	1208.129	12.122	461.603	29	9.024	4.317	384.788
Haloperidol	3	643.634	1064.307	11.455	412.250	25	8.692	3.734	343.829
Halothane	0	263.272	361.821	6.402	128.760	0	5.637	3.051	93.803
Hexobarbital	2	433.456	696.555	9.278	270.404	13	7.465	1.520	217.780
Hydroxyzine	3	672.064	1114.955	11.710	430.814	27	8.782	2.839	354.603
Ibuprofen	1	444.709	702.479	9.332	273.606	8	7.421	3.270	214.012
Imipramine	3	556.111	916.720	10.582	351.783	21	8.190	4.124	287.631
Indomethacin	3	597.090	974.846	10.900	373.265	22	8.363	4.223	306.289
Ketorolac	3	461.479	731.908	9.442	280.059	19	7.594	1.357	229.276
Lamotrigine	2	418.765	644.208	8.711	238.371	15	7.187	1.716	194.377

Levofloxacin	4	585.524	975.783	10.988	379.320	24	8.439	-0.463	314.734
Metanol	0	164.494	189.022	4.390	60.556	0	4.148	-0.170	37.377
Metoclopramide	1	566.660	917.280	10.620	354.305	15	8.090	1.930	277.242
Metoprolol	1	549.650	896.369	10.680	358.338	16	8.090	2.749	277.257
Mianserin	4	493.693	810.758	9.979	312.813	22	7.957	2.039	263.826
Naproxen	2	464.347	721.729	9.204	266.138	13	7.413	3.118	213.283
Nicotine	2	374.928	569.945	8.125	207.414	12	6.795	1.493	164.277
Nitrofurantoin	2	420.609	625.820	8.505	227.229	14	6.970	2.116	177.269
Norfloxacin	3	544.989	884.681	10.428	341.623	21	8.141	1.021	282.510
Nortriptyline	3	536.408	868.413	10.211	327.545	21	8.024	3.305	270.542
Olanzapine	4	544.288	889.042	10.464	344.010	23	8.190	3.414	287.602
Omeprazole	3	611.329	987.734	10.943	376.205	21	8.333	2.933	303.016
Oxazepam	3	492.579	778.883	9.451	280.627	20	7.662	1.922	235.534
Paroxetine	4	557.195	921.494	10.741	362.411	26	8.302	2.988	299.585
Pentobarbital	1	436.383	697.132	9.329	273.421	8	7.461	2.099	217.436
Phenylbutazone	3	551.926	914.454	10.745	362.707	22	8.245	2.619	293.506
Phenytoin	3	470.281	739.146	9.207	266.290	19	7.516	2.408	222.282
Pindolol	2	476.886	778.156	9.979	312.830	17	7.742	2.765	242.974
Primidone	2	407.707	648.780	8.876	247.483	14	7.303	0.767	203.964
Promazine	3	486.289	821.618	10.204	327.092	20	8.032	4.375	271.290
Promethazine	3	508.997	847.824	10.177	325.405	19	8.036	4.132	271.739

Propranolol	2	499.938	823.867	10.189	326.136	18	7.917	3.312	259.781
Quinidine	4	576.881	963.915	10.844	369.454	26	8.397	2.704	309.962
Ranitidine	1	605.982	972.698	10.832	368.581	16	8.189	2.140	287.540
rifampicin	5	973.306	1992.701	16.721	878.330	42	11.347	1.983	764.919
Ropinirole	2	556.463	897.392	10.474	344.620	17	8.007	3.377	268.774
Salicylic acid	1	290.597	417.046	6.902	149.644	9	6.106	0.875	119.183
Theobromine	2	350.336	521.999	7.783	190.289	10	6.585	-0.734	149.480
Theophylline	2	350.772	520.871	7.756	188.968	10	6.581	-0.282	149.237
Toluene	1	280.434	388.126	6.375	127.666	6	5.720	2.762	97.977
Tramadol	2	533.843	877.357	10.363	337.359	17	8.049	2.749	273.044
Trazodone	4	633.018	1055.908	11.393	407.802	27	8.610	2.490	334.245
Triprolidine	3	572.884	923.980	10.502	346.481	22	8.150	3.395	283.477
Valproic acid	0	382.111	574.566	8.251	213.902	3	6.712	2.931	158.355
Venlafaxine	2	534.002	888.088	10.760	363.706	15	8.250	3.243	293.976
Verapamil	2	781.708	1386.797	13.547	576.557	25	9.592	4.407	462.028
Zidovudine	2	476.690	753.473	9.523	284.909	17	7.574	-1.590	227.470
Zolmitriptan	3	560.203	896.297	10.486	345.421	20	8.064	2.682	274.548

Table 4C. Rings, solvent accessible surface (Sas), solvent accessible volume (Sav), superficial diameter (Sdiam), surface, torsions, volume diameter (Vdiam), VirtualLogP and Volume of the analytes, assumed as neutral, taken into account.

Analyte	Angles	Atoms	Bonds	Charge	ChiralAtms	Dipole	EzBnds	FlexTorsions	Gyrrad
2-(Methylamino)pyridine	25	16	16	0	0	1.152	0	1	1.985

2,2,2-trifluoroethyl vinyl ether	19	13	12	0	0	3.473	0	2	2.109
2,6-diisopropylphenol	55	31	31	0	0	1.546	0	0	2.707
Acetaminophen	31	20	20	0	0	2.919	0	1	2.641
Acetylsalicylic acid	31	20	20	-1	0	14.784	0	2	2.427
Aminopyrine	63	35	36	1	1	8.368	1	2	3.039
Amitriptyline	84	45	47	1	0	15.944	0	2	3.328
Amobarbital	59	32	32	-2	0	23.780	0	3	2.848
Antipyrine	48	27	28	1	1	8.279	1	1	2.668
Atenolol	74	42	42	1	1	14.592	0	8	4.117
Benzene	18	12	12	0	0	0.000	0	0	1.516
Betahistine	40	23	23	1	0	12.148	0	3	2.708
Caffeine	43	24	25	0	0	1.457	0	0	2.481
Carbamazepine	51	30	32	0	0	2.311	1	1	2.812
Celecoxib	68	39	41	-1	0	21.649	0	3	4.231
Chlorambucil	66	37	37	-1	0	29.703	0	6	4.313
Chlorpromazine	76	41	43	1	0	12.718	0	3	3.390
Cimetidine	58	34	34	1	0	12.200	0	7	3.923
Citalopram	86	46	48	1	1	21.105	0	4	4.017
Clonidine	43	24	25	1	0	12.666	0	2	2.763
Clozapine	85	44	47	2	0	24.546	0	1	3.859
Cotinine	46	25	26	0	1	3.247	0	1	2.585

Cyclobenzaprine	78	43	45	1	0	15.073	0	3	3.312
Desipramine	81	43	45	1	0	19.575	0	4	3.363
Diclofenac	48	29	30	-1	0	20.401	0	3	3.116
Diphenhydramine	73	41	42	1	0	15.981	0	5	3.402
Domperidone	108	55	59	1	0	6.783	0	5	5.443
Donepezil	113	58	61	1	1	20.330	0	6	5.458
Eserine	82	42	44	1	3	15.257	0	2	3.578
Ethosuximide	37	20	20	-1	1	13.310	0	1	2.147
Ethylbenzene	30	18	18	0	0	0.123	0	1	2.039
Fluphenazine	113	58	61	2	0	32.128	0	6	5.080
Haloperidol	94	50	52	1	0	6.820	0	6	5.795
Halothane	12	8	7	0	1	1.718	0	0	1.868
Hexobarbital	63	33	34	0	1	0.657	1	1	2.795
Hydroxyzine	104	55	57	2	1	6.424	0	8	4.988
Ibuprofen	57	32	32	-1	1	22.840	0	1	3.176
Imipramine	87	46	48	1	0	18.476	0	3	3.444
Indomethacin	70	40	42	-1	0	25.184	0	3	4.087
Ketorolac	57	31	33	-1	1	22.362	0	2	3.287
Lamotrigine	36	23	24	0	0	1.805	0	1	3.086
Levofloxacin	91	46	49	0	1	53.838	1	1	3.870
Metanol	7	6	5	0	0	1.653	0	0	0.854

Metoclopramide	76	43	43	1	0	15.910	0	4	4.147
Metoprolol	81	45	45	1	1	10.327	0	9	3.537
Mianserin	81	41	44	1	2	14.774	0	0	3.127
Naproxen	52	30	31	-1	1	21.702	0	1	3.411
Nicotine	52	27	28	1	2	7.496	0	1	2.518
Nitrofurantoin	36	22	23	-1	0	6.501	0	2	3.776
Norfloxacin	78	41	43	0	0	54.848	1	2	3.828
Nortriptyline	78	42	44	1	0	21.254	0	3	3.478
Olanzapine	86	44	47	2	0	24.627	0	1	3.653
Omeprazole	76	43	45	0	1	8.545	0	5	4.670
Oxazepam	53	31	33	0	1	2.698	0	1	3.355
Paroxetine	87	45	48	1	2	21.543	0	4	3.837
Pentobarbital	59	32	32	-2	1	25.146	0	2	2.843
Phenylbutazone	78	43	45	0	0	0.851	0	5	3.361
Phenytoin	52	30	32	-1	0	13.674	0	2	2.931
Pindolol	71	39	40	1	1	9.502	0	6	3.258
Primidone	54	30	31	0	0	2.879	0	2	2.600
Promazine	76	41	43	1	0	11.864	0	3	3.016
Promethazine	76	41	43	1	1	14.946	0	2	3.167
Propranolol	74	41	42	1	1	11.153	0	6	3.377
Quinidine	96	49	52	1	5	7.614	0	4	3.547

Ranitidine	80	45	45	2	0	10.601	1	8	4.690
Rifampicin	223	119	123	2	9	61.626	4	5	5.051
Ropinirole	84	44	45	1	0	9.374	0	4	3.807
Salicylic acid	22	15	15	-1	0	15.480	0	0	2.148
Theobromine	37	21	22	0	0	1.879	0	0	2.384
Theophylline	37	21	22	0	0	1.318	0	0	2.358
Toluene	24	15	15	0	0	0.120	0	0	1.781
Tramadol	86	45	46	1	2	12.245	0	3	3.221
Trazodone	94	49	52	1	0	3.339	2	5	4.829
Triprolidine	82	44	46	1	0	12.002	1	4	3.548
Valproic acid	45	25	24	-1	0	13.674	0	2	2.577
Venlafaxine	92	48	49	1	1	12.752	0	1	3.444
Verapamil	131	72	73	1	2	18.843	0	10	4.505
Zidovudine	62	34	35	0	3	23.396	1	3	3.127
Zolmitriptan	82	43	45	1	1	19.055	0	4	3.817

Table 5A. Angles, atoms, bonds, charge, chiral atoms (ChiralAtms), dipole, E-Z Bonds (EzBnds), flexible torsions (FlexTorsions), gyration radius (Gyrrad) of the analytes, assumed as ionized, taken into account.

Analyte	HbAcc	HbDon	HeavyAtoms	Impropers	Lipole	Mass	MassMI	Ovality	Psa
2-(Methylamino)pyridine	1	1	8	3	0.903	108.141	108.069	1.294	24.777
2,2,2-trifluoroethyl vinyl ether	1	0	8	6	1.104	126.077	126.029	1.334	10.574

2,6-diisopropylphenol	1	1	13	0	2.200	178.271	178.136	1.537	21.637
Acetaminophen	2	2	11	6	1.587	151.163	151.063	1.364	54.319
Acetylsalicylic acid	4	0	13	6	2.106	179.150	179.034	1.404	60.038
Aminopyrine	1	1	17	15	0.654	232.302	232.145	1.584	32.618
Amitriptyline	0	1	21	6	3.119	278.411	278.191	1.638	9.011
Amobarbital	5	0	16	9	3.191	224.256	224.116	1.541	74.158
Antipyrine	1	1	14	12	1.441	189.234	189.103	1.468	30.316
Atenolol	3	5	19	6	1.296	267.344	267.171	1.705	95.374
Benzene	0	0	6	0	0.000	78.112	78.047	1.192	0.000
Betahistine	1	2	10	0	2.679	137.202	137.108	1.454	31.066
Caffeine	3	0	14	12	0.293	194.191	194.080	1.430	54.408
Carbamazepine	1	2	18	15	3.033	236.269	236.095	1.448	44.702
Celecoxib	4	1	26	0	2.615	380.364	380.068	1.686	83.499
Chlorambucil	2	0	19	6	5.645	303.204	302.072	1.690	38.488
Chlorpromazine	0	1	21	3	2.845	319.872	319.104	1.643	33.347
Cimetidine	2	4	17	6	2.434	253.347	253.124	1.653	107.670
Citalopram	1	1	24	0	3.405	325.400	325.172	1.721	38.189
Clonidine	1	3	14	6	3.096	231.102	230.025	1.473	45.667
Clozapine	1	3	23	6	2.934	328.839	328.146	1.649	42.204
Cotinine	2	0	13	6	1.718	176.215	176.095	1.440	31.705
Cyclobenzaprine	0	1	21	3	3.445	276.395	276.175	1.620	9.496

Desipramine	0	2	20	3	3.850	267.389	267.186	1.637	22.866
Diclofenac	2	1	19	6	4.140	295.141	294.009	1.555	43.125
Diphenhydramine	1	1	19	0	3.364	256.363	256.170	1.651	17.502
Domperidone	2	3	30	18	2.196	426.919	426.170	1.799	79.556
Donepezil	3	1	28	3	1.911	380.500	380.223	1.815	49.708
Eserine	2	2	20	9	1.921	276.354	276.171	1.658	54.916
Ethosuximide	3	0	10	6	2.784	140.160	140.071	1.387	47.971
Ethylbenzene	0	0	8	0	0.019	106.165	106.078	1.351	0.000
Fluphenazine	1	3	30	3	4.758	439.537	439.191	1.808	68.220
Haloperidol	2	2	26	3	0.420	376.872	376.148	1.753	44.325
Halothane	0	0	7	0	0.393	197.382	195.890	1.290	0.000
Hexobarbital	3	1	17	21	1.177	236.267	236.116	1.545	68.400
Hydroxyzine	2	3	26	0	2.446	376.920	376.192	1.789	51.807
Ibuprofen	2	0	15	3	4.812	205.273	205.123	1.574	34.768
Imipramine	0	1	21	3	3.481	281.415	281.202	1.673	11.397
Indomethacin	4	0	25	6	3.706	356.780	356.069	1.688	66.232
Ketorolac	3	0	19	6	2.565	254.261	254.082	1.539	55.478
Lamotrigine	3	4	16	6	4.497	256.091	255.008	1.469	87.480
Levofloxacin	4	1	26	18	0.726	361.368	361.144	1.679	75.424
Metanol	1	1	2	0	1.438	32.042	32.026	1.120	23.429
Metoclopramide	2	4	20	9	1.546	300.804	300.148	1.715	72.766

Metoprolol	3	3	19	0	2.657	268.372	268.191	1.756	62.859
Mianserin	0	1	20	3	2.986	265.373	265.171	1.574	12.887
Naproxen	3	0	17	3	3.846	229.251	229.087	1.523	45.880
Nicotine	1	1	12	0	1.298	163.240	163.124	1.447	20.342
Nitrofurantoin	7	0	17	15	4.129	237.149	237.026	1.483	115.907
Norfloxacin	3	2	23	18	0.903	319.331	319.133	1.649	77.797
Nortriptyline	0	2	20	6	4.379	264.385	264.175	1.627	21.450
Olanzapine	1	3	22	6	2.986	314.448	314.157	1.642	63.883
Omeprazole	5	1	24	3	1.180	345.416	345.115	1.724	89.545
Oxazepam	3	2	20	9	4.240	286.713	286.051	1.522	63.346
Paroxetine	3	2	24	0	3.685	330.373	330.151	1.672	54.844
Pentobarbital	5	0	16	9	3.485	224.256	224.116	1.535	72.552
Phenylbutazone	2	0	23	12	1.678	308.374	308.153	1.698	42.596
Phenytoin	3	1	19	9	3.543	251.260	251.082	1.487	61.260
Pindolol	2	4	18	0	2.349	249.329	249.160	1.654	68.369
Primidone	2	2	16	12	2.187	218.252	218.106	1.477	61.411
Promazine	0	1	20	3	2.415	285.427	285.143	1.624	33.654
Promethazine	0	1	20	3	3.365	285.427	285.143	1.605	34.222
Propranolol	2	3	19	0	3.036	260.351	260.165	1.672	50.173
Quinidine	3	2	24	6	1.517	325.425	325.192	1.678	51.936
Ranitidine	3	4	21	12	1.859	316.420	316.157	1.777	112.643

Rifampicin	13	8	59	33	3.249	824.956	824.421	2.202	215.085
Ropinirole	1	2	19	6	0.630	261.383	261.197	1.699	41.839
Salicylic acid	3	1	10	3	3.482	137.113	137.024	1.265	52.561
Theobromine	3	1	13	12	0.536	180.164	180.065	1.397	67.976
Theophylline	3	1	13	12	0.202	180.164	180.065	1.389	67.548
Toluene	0	0	7	0	0.017	92.138	92.063	1.242	0.000
Tramadol	2	2	19	0	2.416	264.383	264.196	1.673	39.694
Trazodone	2	1	26	27	2.968	372.872	372.159	1.739	46.993
Triprolidine	1	1	21	6	2.410	279.399	279.186	1.670	18.851
Valproic acid	2	0	10	3	2.524	143.204	143.107	1.499	35.485
Venlafaxine	2	2	20	0	2.408	278.410	278.212	1.678	38.514
Verapamil	4	1	33	0	2.716	455.610	455.291	2.003	72.646
Zidovudine	7	4	19	15	3.591	269.257	269.112	1.593	138.505
Zolmitriptan	2	3	21	6	3.035	288.365	288.171	1.692	69.492

Table 5B. Hydrogen bond acceptor (HbAcc), hydrogen bond donor (HbDon) groups, heavy atoms (HeavyAtoms), impropers, lipole, mass, monoisotopic mass (MassMI), ovality, polar surface area (Psa) of the analytes, assumed as ionized, taken into account.

Analyte	Rings	Sas	Sav	Sdiam	Surface	Torsions	Vdiam	VirtualLogP	Volume
2-(Methylamino)pyridine	1	289.355	403.799	6.730	142.272	7	5.915	1.132	108.346
2,2,2-trifluoroethyl vinyl ether	0	281.133	385.340	6.591	136.488	3	5.706	2.463	97.271

2,6-diisopropylphenol	1	408.043	638.038	9.005	254.748	7	7.262	3.678	200.560
Acetaminophen	1	340.442	492.948	7.532	178.206	9	6.449	1.110	140.455
Acetylsalicylic acid	1	357.404	532.456	7.899	196.026	9	6.666	0.442	155.108
Aminopyrine	2	450.435	724.619	9.560	287.134	13	7.597	-1.207	229.576
Amitriptyline	3	537.692	898.043	10.525	347.992	20	8.223	0.925	291.154
Amobarbital	1	434.268	690.033	9.187	265.159	9	7.400	0.696	212.212
Antipyrine	2	402.656	617.291	8.518	227.922	12	7.030	-1.809	181.924
Atenolol	1	551.832	884.346	10.389	339.103	16	7.956	-1.612	263.656
Benzene	1	238.870	321.014	5.951	111.247	6	5.450	2.136	84.761
Betahistine	1	362.985	529.101	7.905	196.302	9	6.556	-2.191	147.535
Caffeine	2	376.477	573.739	8.137	208.022	10	6.805	-0.221	164.992
Carbamazepine	3	429.993	681.449	8.870	247.193	19	7.371	2.213	209.707
Celecoxib	3	593.004	959.333	10.778	364.910	21	8.300	3.584	299.374
Chlorambucil	1	544.245	868.062	10.394	339.420	15	7.995	3.640	267.565
Chlorpromazine	3	524.362	883.669	10.523	347.859	19	8.209	2.104	289.648
Cimetidine	1	526.144	804.899	9.877	306.479	14	7.683	-2.113	237.435
Citalopram	3	609.675	994.465	11.023	381.713	21	8.403	1.071	310.717
Clonidine	2	413.264	632.674	8.583	231.454	13	7.071	-0.768	185.141
Clozapine	4	573.067	944.312	10.631	355.055	24	8.280	-1.911	297.205
Cotinine	2	373.419	568.966	8.198	211.153	12	6.832	0.765	166.954
Cyclobenzaprine	3	531.575	879.701	10.323	334.755	20	8.111	1.288	279.417

Desipramine	3	535.152	873.807	10.323	334.778	21	8.069	0.336	275.045
Diclofenac	2	470.881	760.403	9.581	288.391	16	7.684	3.470	237.542
Diphenhydramine	2	536.950	870.292	10.289	332.548	17	8.006	0.542	268.724
Domperidone	5	698.276	1166.446	12.012	453.281	31	8.955	0.330	375.946
Donepezil	4	692.427	1161.992	12.059	456.884	28	8.951	1.366	375.520
Eserine	3	528.245	853.732	10.271	331.427	17	7.977	-0.711	265.807
Ethosuximide	1	320.814	473.599	7.459	174.794	6	6.334	0.480	133.077
Ethylbenzene	1	308.252	437.569	7.096	158.200	7	6.105	3.057	119.126
Fluphenazine	4	716.384	1211.534	12.199	467.535	29	9.073	-0.100	391.082
Haloperidol	3	661.343	1086.934	11.485	414.412	25	8.673	1.719	341.646
Halothane	0	263.272	361.821	6.402	128.760	0	5.637	3.051	93.803
Hexobarbital	2	433.456	696.555	9.278	270.404	13	7.465	1.520	217.780
Hydroxyzine	3	674.513	1125.962	11.804	437.760	27	8.825	-1.187	359.887
Ibuprofen	1	438.887	692.970	9.261	269.441	7	7.381	2.462	210.522
Imipramine	3	549.809	916.609	10.618	354.156	20	8.210	0.869	289.704
Indomethacin	3	587.380	959.392	10.832	368.623	21	8.338	3.342	303.539
Ketorolac	3	457.831	724.896	9.370	275.844	18	7.554	0.534	225.661
Lamotrigine	2	418.765	644.208	8.711	238.371	15	7.187	1.716	194.377
Levofloxacin	4	579.271	972.492	10.902	373.377	23	8.414	-2.794	311.869
Metanol	0	164.494	189.022	4.390	60.556	0	4.148	-0.170	37.377
Metoclopramide	1	559.914	903.882	10.643	355.876	12	8.127	-0.505	281.026

Metoprolol	1	533.964	897.429	10.791	365.852	16	8.143	-0.155	282.757
Mianserin	4	498.641	823.986	9.990	313.503	22	7.964	0.357	264.439
Naproxen	2	455.789	708.588	9.106	260.494	12	7.380	2.274	210.427
Nicotine	2	377.480	576.367	8.251	213.857	12	6.859	-1.169	168.968
Nitrofurantoin	2	421.320	624.697	8.487	226.287	14	6.969	1.512	177.182
Norfloxacin	3	542.149	886.049	10.402	339.928	20	8.099	-3.119	278.185
Nortriptyline	3	539.625	874.632	10.251	330.139	21	8.037	0.122	271.785
Olanzapine	4	570.483	928.558	10.534	348.611	23	8.220	-1.358	290.788
Omeprazole	3	611.329	987.734	10.943	376.205	21	8.333	2.933	303.016
Oxazepam	3	492.579	778.883	9.451	280.627	20	7.662	1.922	235.534
Paroxetine	4	556.745	926.342	10.734	361.938	26	8.301	-0.386	299.537
Pentobarbital	1	433.109	691.010	9.171	264.232	8	7.402	0.820	212.340
Phenylbutazone	3	551.926	914.454	10.745	362.707	22	8.245	2.619	293.506
Phenytoin	3	445.194	705.333	9.149	262.971	19	7.502	1.579	221.063
Pindolol	2	481.871	789.136	9.984	313.137	17	7.763	-0.076	244.927
Primidone	2	407.707	648.780	8.876	247.483	14	7.303	0.767	203.964
Promazine	3	498.377	838.893	10.300	333.264	19	8.082	1.548	276.451
Promethazine	3	501.990	838.895	10.234	329.020	18	8.077	1.328	275.926
Propranolol	2	506.866	834.829	10.304	333.549	18	7.969	0.418	264.999
Quinidine	4	570.309	956.778	10.899	373.213	26	8.413	0.595	311.783
Ranitidine	1	617.732	979.993	11.025	381.868	15	8.271	-4.306	296.255

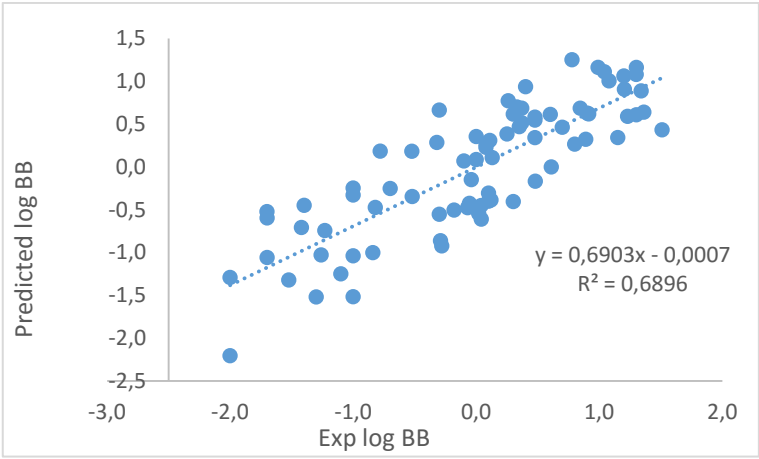
Rifampicin	5	1005.318	2037.298	16.850	891.933	42	11.355	-2.364	766.610
Ropinirole	2	558.158	900.298	10.460	343.712	14	8.023	0.804	270.450
Salicylic acid	1	283.246	406.819	6.784	144.589	8	6.033	0.023	114.957
Theobromine	2	350.336	521.999	7.783	190.289	10	6.585	-0.734	149.480
Theophylline	2	350.772	520.871	7.756	188.968	10	6.581	-0.282	149.237
Toluene	1	280.434	388.126	6.375	127.666	6	5.720	2.762	97.977
Tramadol	2	527.473	875.759	10.430	341.770	16	8.064	-0.117	274.584
Trazodone	4	622.239	1038.826	11.352	404.878	27	8.608	0.184	333.927
Triprolidine	3	568.045	923.213	10.569	350.907	22	8.177	0.953	286.307
Valproic acid	0	375.100	560.628	8.157	209.019	2	6.661	2.100	154.757
Venlafaxine	2	542.811	901.719	10.687	358.801	14	8.249	0.447	293.908
Verapamil	2	772.727	1377.260	13.581	579.448	23	9.595	2.574	462.585
Zidovudine	2	468.090	745.897	9.618	290.638	16	7.621	-5.581	231.737
Zolmitriptan	3	560.461	901.128	10.502	346.487	19	8.074	-0.626	275.550

Table 5C. Rings, solvent accessible surface (Sas), solvent accessible volume (Sav), superficial diameter (Sdiam), surface, torsions, volume diameter (Vdiam), VirtualLogP and volume of the analytes, assumed as ionized, taken into account.

This may be attributed to the fact that, although the molecular mechanisms involved in MLC are multiple and complex, the occurrence of analyte/micelles electrostatic interactions plays a pivotal role in the global retention and appears reasonable to assume that such interactions are encoded in MLC indexes. It should be also highlighted that, in this specific cases, being VirtualLogP values calculated starting from the analytes assumed as neutral they can be reasonably assumed as estimates of their log P^N values. Subsequently, the analytes supporting extensively ionizable functions (i.e. carboxy groups, for acids primary, secondary and tertiary amines for bases) were assumed as completely charged, regardless the relative abundance of the charged species at the physiological pH. Their properties are shown in Table 5. The respective plots Experimental versus Predicted log BB values are shown in Figure 2.

Taking into account the ionizable analytes assumed entirely as charged slightly worsened the relationships (equations (3) and (4)). It should be pointed out that Verapamil, the analyte excluded to maximize the predictive strength of the statistic model is a well-known P-gp substrate [Eyal et al., 2009]. P-gp is an ATP-dependent efflux pump, with broad substrate specificity pumping many foreign substances out of cells [Szewczyk et al., 2015].

A



B

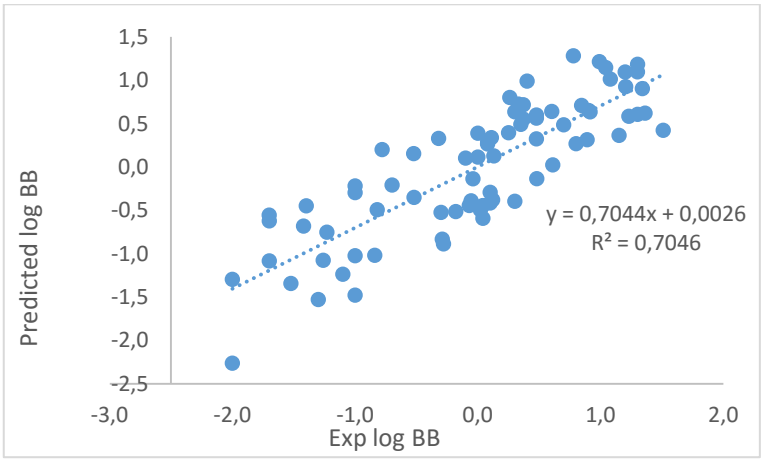
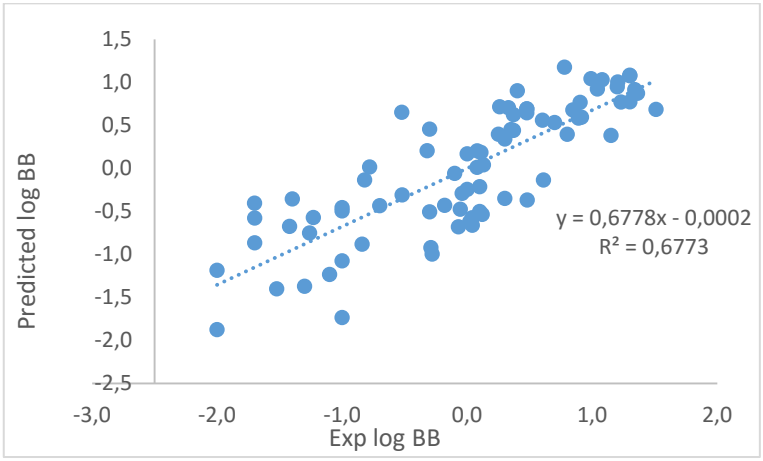


Figure 1. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log k_w^{SDS} and three other static properties of the analytes assumed as neutral before (A) and after (B) optimization.

A



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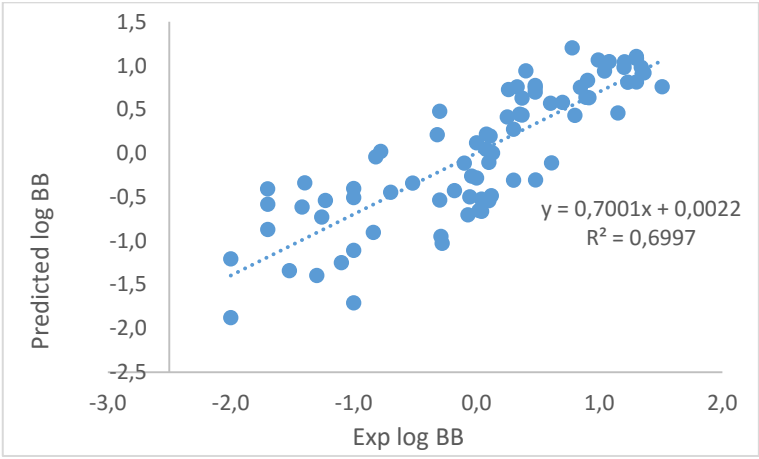


Figure 2. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log k_w^{SDS} and three other static properties of the analytes assumed as completely charged before (A) and after (B) optimization.

Although it is widely expressed in the intestinal epithelium, liver cells and proximal tubule of the kidney, P-gp is also localized in the capillary endothelial cells composing the BBB and is responsible, for some classes of actives, of multi-drug resistance. Eventually, a weighted average of the static properties al physiological pH (7.4), according to the pKa of each compound, was performed. For zwitterions, the static properties were calculated for each microspecies possibly present at pH 7.4 and their relative abundances, calculated by the software Marvin Sketch 15.1 for Mac OS X [ChemAxon, Budapest, HU], were also used to perform the weighted averages. This approach was adapted in an attempt to mirror more closely what actually occurs *in vivo*. The weighed average of the static properties are shown in Table 6. Performing the weighted average of the properties benefited noticeably the relationships as described by equations (5) and (6). It is also interesting to note how, according to the above reported relationships, the BBB penetration of drugs will be enhanced for MLC highly retained compounds, hindered by the occurrence of drug/membrane polar (Psa)/ electrostatic (Dipole) interactions, and also favored for bases (Charge). The respective plots Experimental versus Predicted log BB values are shown in Figure 3. However, by taking into account the analytes assumed as static, the properties are derived considering them in their minimum energy conformations, i.e. after minimization. Indeed, several authors [Vistoli et al., 2009] reported that such conformations are not always the ones actually involved in membrane barrier passage.

Analyte	Angles	Atoms	Bonds	Charge	ChiralAtms	Dipole	EzBnds	FlexTorsions	Gyrrad
2-(Methylamino)pyridine	25.000	16.000	16.000	0.000	0.000	1.152	0	1.000	1.985
2,2,2-trifluoroethyl vinyl ether	19.000	13.000	12.000	0.000	0.000	3.473	0	2.000	2.109
2,6-diisopropylphenol	55.000	31.000	31.000	0.000	0.000	1.546	0	0.000	2.707
Acetaminophen	31.000	20.000	20.000	0.000	0.000	2.919	0	1.000	2.641
Acetylsalicylic acid	31.000	20.000	20.000	-1.000	0.000	14.782	0	2.000	2.427
Aminopyrine	62.987	34.996	35.996	0.996	0.996	8.343	1	2.000	3.039
Amitriptyline	81.050	44.017	46.017	0.017	0.000	1.151	0	2.983	3.324
Amobarbital	61.935	33.467	33.467	-0.533	0.000	6.948	0	3.000	2.850
Antipyrine	48.000	27.000	28.000	1.000	1.000	8.279	1	1.000	2.668
Atenolol	71.048	41.016	41.016	0.016	1.000	5.153	0	8.000	3.876
Benzene	18.000	12.000	12.000	0.000	0.000	0.000	0	0.000	1.516
Betahistine	37.799	22.266	22.266	0.266	0.000	4.104	0	3.000	2.700
Caffeine	43.000	24.000	25.000	0.000	0.000	1.457	0	0.000	2.481
Carbamazepine	51.000	30.000	32.000	0.000	0.000	2.311	1	1.000	2.812
Celecoxib	69.979	39.990	41.990	-0.010	0.000	4.734	0	3.000	4.246
Chlorambucil	66.002	37.002	37.002	-0.998	0.000	29.659	0	6.002	4.313
Chlorpromazine	73.024	40.008	42.008	0.008	0.000	1.407	0	3.992	3.398
Cimetidine	57.132	33.711	33.711	0.711	0.000	9.237	0	7.000	3.898
Citalopram	83.045	45.015	47.015	0.015	1.000	2.685	0	4.985	4.010
Clonidine	40.518	23.173	24.173	0.173	0.000	2.508	0	2.000	2.747

Clozapine	80.442	42.481	45.481	0.481	0.000	7.390	0	1.000	3.842
Cotinine	46.000	25.000	26.000	0.000	1.000	3.247	0	1.000	2.585
Cyclobenzaprine	75.235	42.078	44.078	0.078	0.000	2.002	0	3.922	3.313
Desipramine	78.004	42.001	44.001	0.001	0.000	1.043	0	4.000	3.338
Diclofenac	48.000	29.000	30.000	-1.000	0.000	20.394	0	3.000	3.116
Diphenhydramine	70.101	40.034	41.034	0.034	0.000	2.704	0	5.966	3.435
Domperidone	105.016	54.005	58.005	0.005	0.000	3.876	0	5.000	5.288
Donepezil	110.203	57.068	60.068	0.068	1.000	4.420	0	6.000	5.441
Eserine	79.436	41.145	43.145	0.145	2.145	3.499	0	2.000	3.575
Ethosuximide	38.973	20.987	20.987	-0.013	1.000	2.576	0	1.000	2.142
Ethylbenzene	30.000	18.000	18.000	0.000	0.000	0.123	0	1.000	2.039
Fluphenazine	109.919	56.973	59.973	0.973	0.000	19.451	0	6.000	5.033
Haloperidol	91.342	49.114	51.114	0.114	0.000	3.663	0	6.000	5.785
Halothane	12.000	8.000	7.000	0.000	1.000	1.718	0	0.000	1.868
Hexobarbital	63.000	33.000	34.000	0.000	1.000	0.657	1	1.000	2.795
Hydroxyzine	99.580	53.527	55.527	0.527	1.000	5.392	0	8.000	4.972
Ibuprofen	57.001	32.001	32.001	-0.999	1.000	22.825	0	1.000	3.176
Imipramine	84.023	45.008	47.008	0.008	0.000	1.158	0	3.992	3.419
Indomethacin	70.001	40.001	42.001	-0.999	0.000	25.171	0	3.001	4.087
Ketorolac	57.000	31.000	33.000	-1.000	1.000	22.356	0	2.000	3.287
Lamotrigine	36.000	23.000	24.000	0.000	0.000	1.805	0	1.000	3.086
Levofloxacin	88.153	45.061	48.061	-0.939	1.000	34.253	1	1.015	3.928

Metanol	7.000	6.000	5.000	0.000	0.000	1.653	0	0.000	0.854
Metoclopramide	73.015	42.005	42.005	0.005	0.000	3.512	0	6.985	4.093
Metoprolol	78.021	44.007	44.007	0.007	1.000	4.110	0	9.000	3.593
Mianserin	80.254	40.751	43.751	0.751	1.751	11.245	0	0.000	3.127
Naproxen	52.001	30.001	31.001	-0.999	1.000	21.691	0	1.000	3.411
Nicotine	49.490	26.163	27.163	0.163	1.163	3.074	0	1.000	2.502
Nitrofurantoin	36.618	22.309	23.309	-0.691	0.000	4.729	0	2.000	3.778
Norfloxacin	77.872	40.973	42.973	-0.027	0.000	52.846	1	2.023	3.827
Nortriptyline	75.006	41.002	43.002	0.002	0.000	0.864	0	3.000	3.465
Olanzapine	81.708	42.569	45.569	0.569	0.000	8.487	0	1.000	3.612
Omeprazole	76.000	43.000	45.000	0.000	1.000	8.545	0	5.000	4.670
Oxazepam	53.000	31.000	33.000	0.000	1.000	2.698	0	1.000	3.355
Paroxetine	84.013	44.004	47.004	0.004	2.000	0.907	0	4.000	3.809
Pentobarbital	62.431	33.715	33.715	-0.285	1.000	4.385	0	2.000	2.842
Phenylbutazone	78.000	43.000	45.000	0.000	0.000	0.851	0	5.000	3.361
Phenytoin	53.767	30.884	32.884	-0.116	0.000	3.279	0	2.000	2.932
Pindolol	68.022	38.007	39.007	0.007	1.000	2.647	0	6.000	3.257
Primidone	54.000	30.000	31.000	0.000	0.000	2.879	0	2.000	2.600
Promazine	73.033	40.011	42.011	0.011	0.000	1.276	0	3.989	3.047
Promethazine	73.074	40.025	42.025	0.025	1.000	1.687	0	2.975	3.167
Propranolol	71.051	40.017	41.017	0.017	1.000	2.470	0	6.000	3.371
Quinidine	93.194	48.065	51.065	0.065	4.065	1.786	0	4.000	3.550

Ranitidine	74.631	43.210	43.210	0.210	0.000	4.366	1	8.895	4.218
Rifampicin	223.000	119.000	123.000	2.000	9.000	61.626	4	5.000	5.051
Ropinirole	81.005	43.002	44.002	0.002	0.000	2.372	0	6.995	3.783
Salicylic acid	22.000	15.000	15.000	-1.000	0.000	15.480	0	0.000	2.148
Theobromine	37.000	21.000	22.000	0.000	0.000	1.879	0	0.000	2.384
Theophylline	37.000	21.000	22.000	0.000	0.000	1.318	0	0.000	2.358
Toluene	24.000	15.000	15.000	0.000	0.000	0.120	0	0.000	1.781
Tramadol	83.029	44.010	45.010	0.010	2.000	2.761	0	3.990	3.232
Trazodone	92.672	48.557	51.557	0.557	0.000	2.339	2	5.000	4.862
Triprolidine	79.163	43.054	45.054	0.054	0.000	2.401	1	4.000	3.559
Valproic acid	45.001	25.001	24.001	-0.999	0.000	13.657	0	2.000	2.577
Venlafaxine	89.016	47.005	48.005	0.005	1.000	3.048	0	1.995	3.434
Verapamil	128.150	71.050	72.050	0.050	1.050	3.043	0	11.900	4.613
Zidovudine	61.010	34.000	35.000	0.000	3.000	3.035	1	3.000	3.272
Zolmitriptan	79.021	42.007	44.007	0.007	1.000	3.822	0	4.993	3.836

Table 6A. Weighted average of angles, atoms, bonds, charge, chiral atoms (ChiralAtms), dipole, E-Z bonds, flexible torsions (FlexTorsions), gyration radius (Gyrrad) of the analytes at pH 7.4 performed according to the pKa of each analyte.

Analyte	HbAcc	HbDon	HeavyAtoms	Impropers	Lipole	Mass	MassMI	Ovality	Psa
2-(Methylamino)pyridine	1.000	1.000	8	3.000	0.903	108.141	108.069	1.294	24.777
2,2,2-trifluoroethyl vinyl ether	1.000	0.000	8	6.000	1.104	126.077	126.029	1.334	10.574
2,6-diisopropylphenol	1.000	1.000	13	0.000	2.200	178.271	178.136	1.537	21.637

Acetaminophen	2.000	2.000	11	6.000	1.587	151.163	151.063	1.364	54.319
Acetylsalicylic acid	4.000	0.000	13	6.000	2.106	179.150	179.035	1.404	60.039
Aminopyrine	1.000	0.996	17	15.013	0.656	232.297	232.141	1.584	32.596
Amitriptyline	0.983	0.017	21	8.950	2.295	277.420	277.200	1.642	4.915
Amobarbital	3.533	1.467	16	13.402	2.209	225.735	225.595	1.571	78.924
Antipyrine	1.000	1.000	14	12.000	1.441	189.234	189.103	1.468	30.316
Atenolol	3.984	4.016	19	8.952	1.948	266.352	266.179	1.705	90.934
Benzene	0.000	0.000	6	0.000	0.000	78.112	78.047	1.192	0.000
Betahistine	1.734	1.266	10	2.201	2.123	136.463	136.369	1.431	27.218
Caffeine	3.000	0.000	14	12.000	0.293	194.191	194.080	1.430	54.408
Carbamazepine	1.000	2.000	18	15.000	3.033	236.269	236.095	1.448	44.702
Celecoxib	4.000	1.990	26	2.969	3.156	381.362	381.065	1.699	85.545
Chlorambucil	2.000	0.002	19	6.000	5.643	303.206	302.073	1.690	38.498
Chlorpromazine	0.992	0.008	21	5.976	2.069	318.872	318.104	1.647	30.341
Cimetidine	2.000	3.711	17	6.868	2.387	253.055	252.832	1.647	105.885
Citalopram	1.985	0.015	24	2.955	1.854	324.407	324.179	1.722	34.152
Clonidine	1.000	2.173	14	8.482	2.371	230.268	229.191	1.496	42.623
Clozapine	1.760	1.481	23	10.558	2.837	327.308	326.614	1.654	33.269
Cotinine	2.000	0.000	13	6.000	1.718	176.215	176.095	1.440	31.705
Cyclobenzaprine	0.922	0.078	21	5.765	3.336	275.467	275.246	1.613	5.725
Desipramine	0.999	1.001	20	5.996	2.730	266.382	266.180	1.639	18.186
Diclofenac	2.000	1.000	19	6.000	4.140	295.141	294.009	1.555	43.127

Diphenhydramine	1.966	0.034	19	2.899	2.773	255.389	255.196	1.660	14.990
Domperidone	2.995	2.005	30	20.984	2.268	425.917	425.167	1.793	76.371
Donepezil	3.932	0.068	28	5.797	1.040	379.560	379.283	1.812	45.984
Eserine	2.855	1.145	20	11.564	0.912	275.492	275.310	1.649	50.762
Ethosuximide	2.013	0.987	10	8.960	1.805	141.154	141.066	1.399	50.860
Ethylbenzene	0.000	0.000	8	0.000	0.019	106.165	106.078	1.351	0.000
Fluphenazine	2.027	1.973	30	6.081	4.959	438.502	438.155	2.225	80.339
Haloperidol	2.886	1.114	26	5.658	0.701	375.979	375.255	1.739	40.064
Halothane	0.000	0.000	7	0.000	0.393	197.382	195.890	1.290	0.000
Hexobarbital	3.000	1.000	17	21.000	1.177	236.267	236.116	1.545	68.400
Hydroxyzine	3.473	1.527	26	4.420	3.388	375.435	374.707	1.944	71.642
Ibuprofen	2.000	0.001	15	3.000	4.811	205.274	205.123	1.574	34.772
Imipramine	0.992	0.008	21	5.977	2.306	280.415	280.201	1.669	7.484
Indomethacin	4.000	0.001	25	6.000	3.705	356.780	356.070	1.688	66.234
Ketorolac	3.000	0.000	19	6.000	2.565	254.261	254.082	1.539	55.480
Lamotrigine	3.000	4.000	16	6.000	4.497	256.091	255.008	1.469	87.480
Levofloxacin	4.954	0.061	26	20.862	1.933	360.421	360.197	2.278	102.797
Metanol	1.000	1.000	2	0.000	1.438	32.042	32.026	1.120	23.429
Metoclopramide	2.995	3.005	20	11.985	0.490	299.801	299.145	1.723	67.560
Metoprolol	3.993	2.007	19	2.979	1.709	267.371	267.190	1.743	56.399
Mianserin	0.249	0.751	20	3.746	2.904	265.122	264.920	1.573	11.767
Naproxen	3.000	0.001	17	3.000	3.846	229.252	229.087	1.523	45.883

Nicotine	1.837	0.163	12	2.510	0.937	162.396	162.280	1.433	16.263
Nitrofurantoin	6.691	0.309	17	15.926	3.732	237.460	237.337	1.485	116.267
Norfloxacin	3.050	1.973	23	18.151	1.083	319.303	319.106	2.063	78.719
Nortriptyline	0.998	1.002	20	8.994	3.405	263.379	263.169	1.619	16.623
Olanzapine	1.715	1.569	22	10.292	2.857	313.007	312.715	1.635	55.705
Omeprazole	5.000	1.000	24	3.000	1.180	345.416	345.115	1.724	89.545
Oxazepam	3.000	2.000	20	9.000	4.240	286.713	286.051	1.522	63.346
Paroxetine	3.996	1.004	24	2.987	2.102	329.370	329.147	1.674	50.683
Pentobarbital	3.285	1.715	16	14.146	2.213	225.985	225.845	1.560	77.363
Phenylbutazone	2.000	0.000	23	12.000	1.678	308.374	308.153	1.698	42.596
Phenytoin	2.116	1.884	19	11.651	2.762	252.151	251.973	1.499	64.789
Pindolol	2.993	3.007	18	2.978	2.938	248.328	248.160	1.661	63.281
Primidone	2.000	2.000	16	12.000	2.187	218.252	218.106	1.477	61.411
Promazine	0.989	0.011	20	5.967	1.780	284.430	284.146	1.614	29.543
Promethazine	0.975	0.025	20	5.926	2.499	284.444	284.159	1.604	29.930
Propranolol	2.983	2.017	19	2.949	3.271	259.361	259.174	1.657	45.641
Quinidine	3.935	1.065	24	8.806	1.317	324.482	324.249	1.669	49.464
Ranitidine	3.895	2.210	21	17.369	1.433	314.616	314.353	1.752	101.653
Rifampicin	13.000	8.000	59	33.000	3.249	824.956	824.421	2.202	215.085
Ropinirole	1.998	1.002	19	8.995	1.572	260.376	260.191	1.711	37.711
Salicylic acid	3.000	1.000	10	3.000	3.482	137.113	137.024	1.265	52.561
Theobromine	3.000	1.000	13	12.000	0.536	180.164	180.065	1.397	67.976

Theophylline	3.000	1.000	13	12.000	0.202	180.164	180.065	1.389	67.548
Toluene	0.000	0.000	7	0.000	0.017	92.138	92.063	1.242	0.000
Tramadol	2.990	1.010	19	2.971	1.457	263.385	263.198	1.658	35.547
Trazodone	2.443	0.557	26	28.328	3.488	372.426	371.713	1.744	46.248
Triprolidine	1.946	0.054	21	8.837	2.026	278.446	278.233	1.661	15.300
Valproic acid	2.000	0.001	10	3.000	2.523	143.205	143.109	1.499	35.492
Venlafaxine	2.995	1.005	20	2.984	1.285	277.407	277.210	1.701	36.817
Verapamil	4.950	0.050	33	2.850	1.927	454.652	454.333	1.995	68.306
Zidovudine	7.000	4.000	19	20.941	0.794	269.257	269.112	1.581	132.610
Zolmitriptan	2.993	2.007	21	8.979	2.311	287.364	287.170	1.691	65.874

Table 6B. Weighted average of hydrogen bond acceptor (HbAcc), hydrogen bond donor (HbDon) groups, heavy atoms (HeavyAtoms), impropers, lipole, mass, monoisotopic (MassMI), ovality, polar surface area (Psa) of the analytes at pH 7.4 performed according to the pKa of each analyte.

Analyte	Rings	Sas	Sav	Sdiam	Surface	Torsions	Vdiam	VirtualLogP	Volume
2-(Methylamino)pyridine	1	289.355	403.799	6.730	142.272	7.000	5.915	1.132	108.346
2,2,2-trifluoroethyl vinyl ether	0	281.133	385.340	6.591	136.488	3.000	5.706	2.463	97.271
2,6-diisopropylphenol	1	408.043	638.038	9.005	254.748	7.000	7.262	3.678	200.560
Acetaminophen	1	340.442	492.948	7.532	178.206	9.000	6.449	1.110	140.455
Acetylsalicylic acid	1	357.405	532.458	7.899	196.026	9.000	6.666	0.442	155.108
Aminopyrine	2	450.519	724.710	9.560	287.110	13.000	7.597	-1.196	229.551
Amitriptyline	3	536.062	891.584	10.483	345.216	20.983	8.181	3.927	286.650

Amobarbital	1	448.519	715.838	9.314	272.554	9.000	7.431	1.695	214.850
Antipyrine	2	402.656	617.290	8.518	227.922	12.000	7.030	-1.809	181.924
Atenolol	1	523.658	847.412	10.399	339.735	16.000	7.963	1.320	264.395
Benzene	1	238.870	321.014	5.951	111.247	6.000	5.450	2.136	84.761
Betahistine	1	361.060	523.298	7.766	189.493	9.000	6.492	0.167	143.248
Caffeine	2	376.477	573.739	8.137	208.022	10.000	6.805	-0.221	164.992
Carbamazepine	3	429.993	681.449	8.870	247.193	19.000	7.371	2.213	209.707
Celecoxib	3	596.579	969.679	10.814	367.403	21.000	8.297	3.334	299.116
Chlorambucil	1	544.260	868.096	10.394	339.429	15.002	7.995	3.641	267.569
Chlorpromazine	3	527.071	887.931	10.513	347.193	19.992	8.192	5.189	287.879
Cimetidine	1	524.307	805.625	9.832	303.722	14.000	7.660	-1.352	235.368
Citalopram	3	609.210	994.648	11.004	380.435	21.985	8.387	4.585	308.892
Clonidine	2	414.567	627.758	8.619	233.403	13.000	7.047	1.947	183.241
Clozapine	4	560.766	925.551	10.586	352.084	24.000	8.232	1.677	292.108
Cotinine	2	373.419	568.966	8.198	211.153	12.000	6.832	0.765	166.954
Cyclobenzaprine	3	532.601	876.098	10.272	331.499	20.922	8.087	4.311	276.959
Desipramine	3	524.837	854.381	10.278	331.884	21.000	8.029	3.371	271.043
Diclofenac	2	470.884	760.408	9.581	288.393	16.000	7.684	3.470	237.543
Diphenhydramine	2	532.705	857.261	10.245	329.717	17.966	7.952	3.453	263.275
Domperidone	5	686.642	1156.600	11.932	447.295	31.000	8.910	2.440	370.396
Donepezil	4	696.479	1162.013	11.989	451.595	28.000	8.907	3.636	369.982
Eserine	3	524.005	846.591	10.222	328.234	17.000	7.961	1.423	264.165

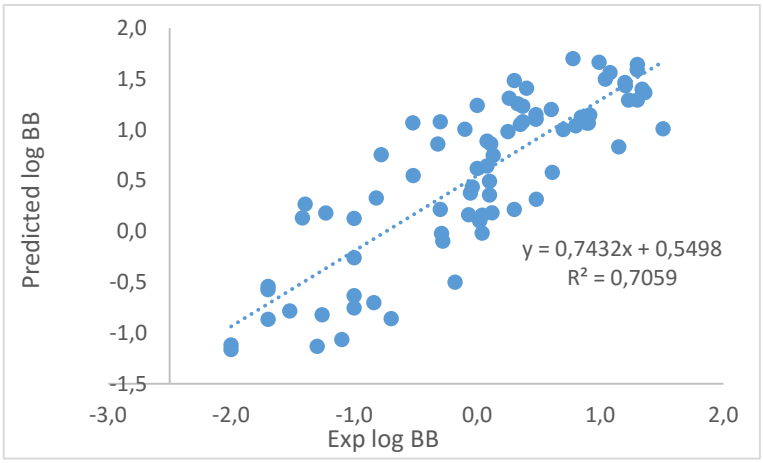
Ethosuximide	1	318.787	472.053	7.537	178.457	6.000	6.373	1.128	135.518
Ethylbenzene	1	308.252	437.569	7.096	158.200	7.000	6.105	3.057	119.126
Fluphenazine	4	1483.542	1779.530	14.442	655.594	29.000	9.682	2.141	475.416
Haloperidol	3	645.655	1066.889	11.459	412.497	25.000	8.690	3.504	343.580
Halothane	0	263.272	361.821	6.402	128.760	0.000	5.637	3.051	93.803
Hexobarbital	2	433.456	696.555	9.278	270.404	13.000	7.465	1.520	217.780
Hydroxyzine	3	981.385	1447.520	12.716	508.088	27.000	9.121	1.720	397.441
Ibuprofen	1	438.891	692.977	9.261	269.444	7.001	7.381	2.463	210.525
Imipramine	3	556.064	916.719	10.582	351.801	20.992	8.190	4.099	287.647
Indomethacin	3	587.385	959.400	10.832	368.625	21.001	8.338	3.343	303.540
Ketorolac	3	457.832	724.898	9.370	275.845	18.000	7.554	0.534	225.662
Lamotrigine	2	418.765	644.208	8.711	238.371	15.000	7.187	1.716	194.377
Levofloxacin	4	1232.474	1542.564	13.765	595.326	23.015	9.121	-1.206	397.283
Metanol	0	164.494	189.022	4.390	60.556	0.000	4.148	-0.170	37.377
Metoclopramide	1	566.628	917.215	10.620	354.312	14.985	8.090	1.919	277.261
Metoprolol	1	549.542	896.376	10.681	358.389	16.000	8.091	2.729	277.294
Mianserin	4	497.410	820.695	9.987	313.331	22.000	7.962	0.775	264.286
Naproxen	2	455.794	708.596	9.106	260.497	12.001	7.380	2.274	210.428
Nicotine	2	375.345	570.993	8.146	208.465	12.000	6.806	1.059	165.042
Nitrofurantoin	2	421.101	625.044	8.492	226.578	14.000	6.969	1.698	177.208
Norfloxacin	3	916.426	1133.867	12.472	488.657	20.023	8.684	-2.780	342.985
Nortriptyline	3	536.414	868.424	10.211	327.550	21.000	8.024	3.299	270.544

Olanzapine	4	551.747	900.294	10.484	345.320	23.000	8.198	2.055	288.509
Omeprazole	3	611.329	987.734	10.943	376.205	21.000	8.333	2.933	303.016
Oxazepam	3	492.579	778.883	9.451	280.627	20.000	7.662	1.922	235.534
Paroxetine	4	557.193	921.515	10.741	362.409	26.000	8.302	2.974	299.584
Pentobarbital	1	435.917	696.260	9.307	272.113	8.000	7.452	1.917	216.711
Phenylbutazone	3	551.926	914.454	10.745	362.707	22.000	8.245	2.619	293.506
Phenytoin	3	467.359	735.208	9.200	265.903	19.000	7.514	2.312	222.140
Pindolol	2	476.922	778.235	9.979	312.832	17.000	7.742	2.744	242.988
Primidone	2	407.707	648.780	8.876	247.483	14.000	7.303	0.767	203.964
Promazine	3	486.420	821.805	10.205	327.159	19.989	8.032	4.345	271.346
Promethazine	3	508.825	847.605	10.179	325.494	18.975	8.037	4.063	271.841
Propranolol	2	500.056	824.054	10.191	326.263	18.000	7.917	3.263	259.870
Quinidine	4	576.456	963.453	10.848	369.697	26.000	8.398	2.568	310.079
Ranitidine	1	607.217	973.465	10.852	369.978	15.895	8.198	1.462	288.456
Rifampicin	5	1005.318	2037.298	16.850	891.933	42.000	11.355	-2.364	766.610
Ropinirole	2	556.466	897.397	10.474	344.618	16.995	8.007	3.373	268.776
Salicylic acid	1	283.246	406.820	6.784	144.589	8.000	6.033	0.023	114.957
Theobromine	2	350.336	521.999	7.783	190.289	10.000	6.585	-0.734	149.480
Theophylline	2	350.772	520.871	7.756	188.968	10.000	6.581	-0.282	149.237
Toluene	1	280.434	388.126	6.375	127.666	6.000	5.720	2.762	97.977
Tramadol	2	533.782	877.342	10.363	337.402	16.990	8.049	2.722	273.059
Trazodone	4	627.010	1046.388	11.371	406.173	27.000	8.609	1.205	334.068

Triprolidine	3	572.621	923.938	10.505	346.722	22.000	8.152	3.263	283.631
Valproic acid	0	375.110	560.647	8.157	209.026	2.001	6.661	2.101	154.762
Venlafaxine	2	534.049	888.161	10.759	363.680	14.995	8.250	3.228	293.976
Verapamil	2	781.260	1386.321	13.549	576.701	24.900	9.592	4.316	462.056
Zidovudine	2	476.605	753.398	9.524	284.966	16.990	7.574	-1.630	227.512
Zolmitriptan	3	560.204	896.331	10.486	345.428	19.993	8.064	2.659	274.555

Table 6C. Weighted average of rings, solvent accessible surface (Sas), solvent accessible volume (Sav), solvent accessible diameter (Sdiam), surface, torsions, volume diameter (Vdiam), VirtualLogP and volume of the analytes at pH 7.4 performed according to the pKa of each analyte.

A



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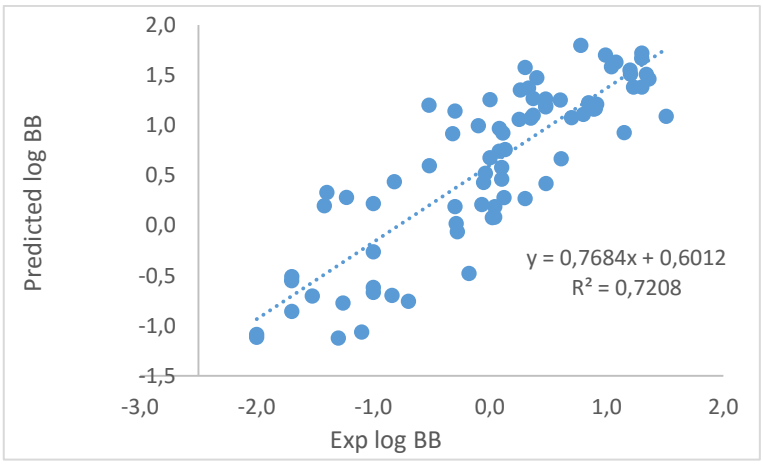
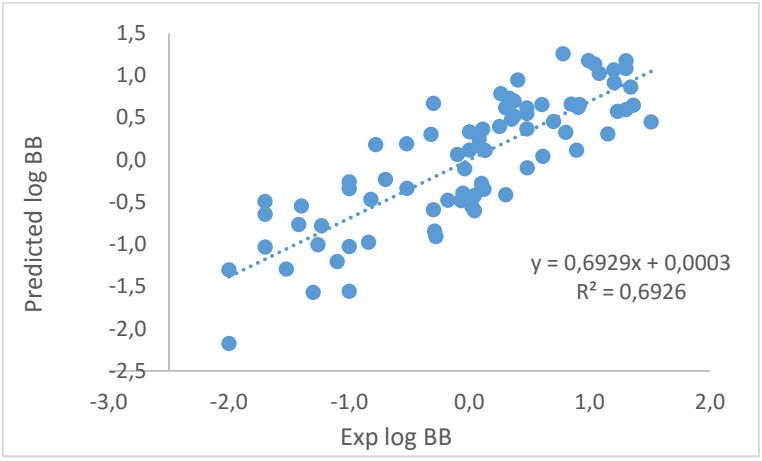


Figure 3. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log k_w^{SDS} and three other descriptors arising from the weighted average of the static properties, according to the respective pKa values, of the analytes at the experimental and physiological pH (7.4) before (A) and after (B) optimization.

Therefore, a conformational analysis was carried out for each analyte included in the data set by using the *Boltzmann Jump* method that generates at random 1000 possible conformations by exploring the conformational space of the rotatable dihedral angles. The conformational analysis was first performed on the analytes assumed as neutral, then of the analytes assumed as completely charged and finally a weighted average of the properties at the experimental pH 7.4, according to the pKa of each analyte, was performed. In the following models the conformational properties were considered along with the static ones in an attempt to improve the predictive strength of the models. The respective plots Experimental versus Predicted log BB values are shown in figure 4. As it is evident from the graphs, not an appreciable improvement was observed after the conformational analysis, even if, in the best optimized model, the regression (7) and (8) resulted slightly more predictive than the one developed starting from only static properties (equations (1) and (2)). Subsequently, the conformational analysis was performed taking into account the ionizable analytes assumed as completely charged (equations (9) and (10)). It is interesting to point out how, among the properties employed for the statistic method development, only one, the charge, depends noticeably on ionization. The respective plots Experimental versus Predicted log BB values are shown in Figure 5. Finally, starting from the conformational properties calculated, a weighted average at pH 7.4, according to the pKa of each compound, was performed. The respective plots Experimental versus Predicted log BB values are shown in Figure 6. Such relationships (equations (11) and (12)) are similar to the ones described by equations (5) and (6), but employing the average of the PSA of all the randomly generated conformers slightly improved the relationships.

A



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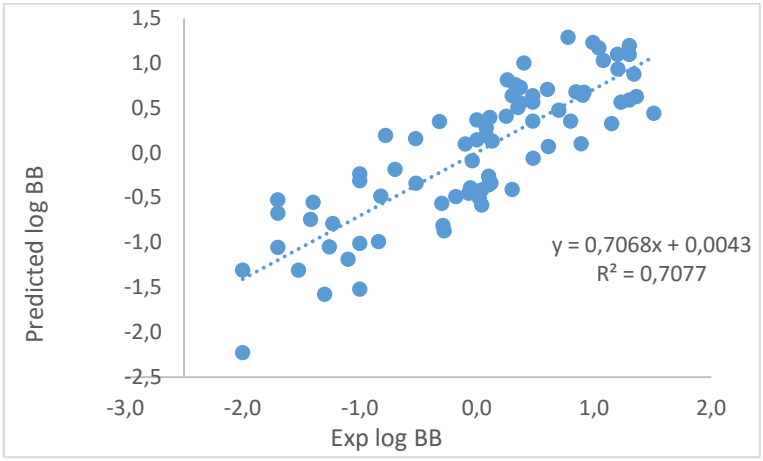
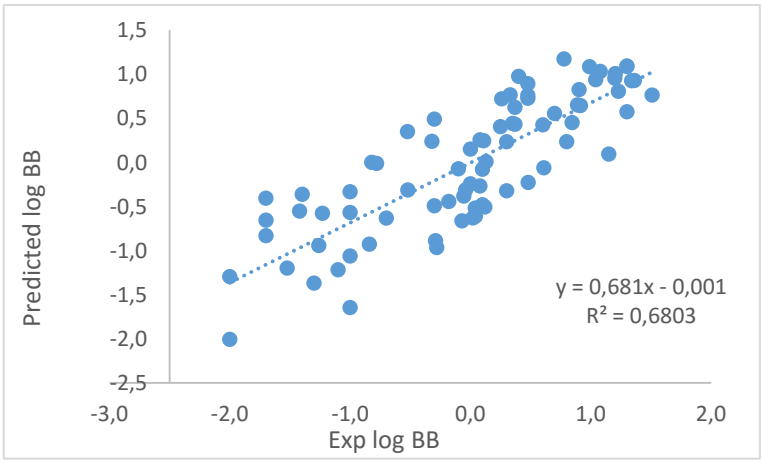


Figure 4. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log k_w^{SDS} and three other static and conformational properties of the analytes assumed as neutral before (A) and after (B) optimization.

A



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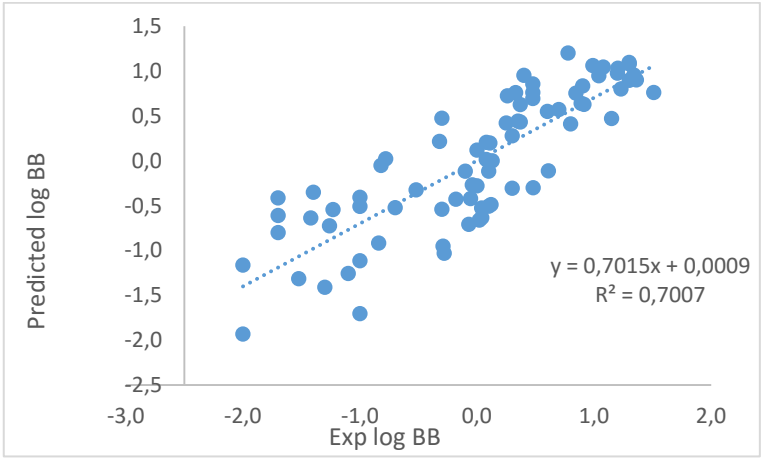
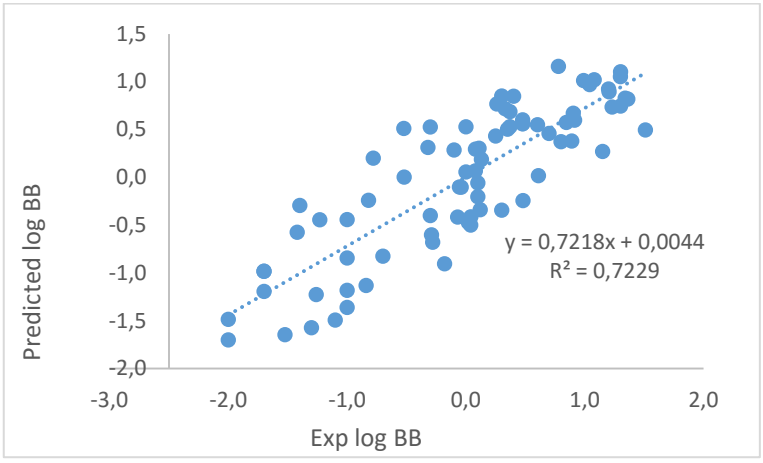


Figure 5. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log k_w^{SDS} and three other static and conformational properties of the analytes assumed as completely charged before (A) and after (B) optimization.

A



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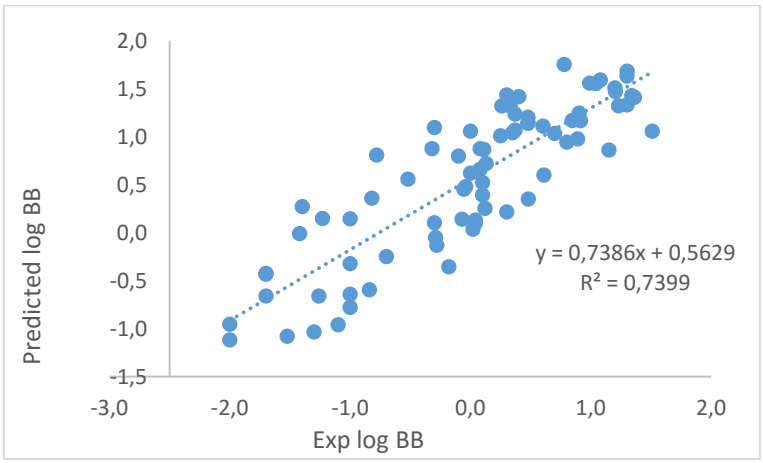


Figure 6. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log k_w^{SDS} and three other descriptors arising from the weighted average of the static and conformational properties, according to the respective pKa values, of the analytes at the experimental and physiological pH (7.4) before (A) and after (B) optimization.

7.3.2 IAM indexes in log BB prediction

The same approach was extended to the IAM indexes. The equations along with the statistical validation coefficient are reported in Table 7. Indeed, taking into account either the properties of the analytes assumed as neutral (equations (13) and (14)) or those of the analytes assumed as completely charged (equations (15) and (16)) resulted in a BBB

passage predictive strength comparable to that obtained by using MLC idexes. Such conclusions are supported by the similar correlation coefficients obtained. It is interesting to note how domperidone, the compound excluded in first best optimized model described by equation (14), is a well-known substrate of the P-gp [Eyal et al., 2009], and is pumped out of cells by such efflux system despite its high biomembrane passive diffusion. Performing the weighted average of the static properties resulted the winning strategy also for this set of experimental measure. Actually, the relationships (equations (17) and (18)) are even better that those obtained by using MLC indexes. This is not surprising, since the IAM stationary phase consists of analogues of phosphatidylcholine, the most abundant phospholipid expressed in the capillary endothelium acting as a barrier between the blood and the cerebrospinal fluids (CSF), thus representing an ideal biomimetic system. Conversely, this kind of SDS based MLC, albeit incidentally able to mirror the drug/membrane interactions involved *in vivo* thanks to the peculiar amphiphilic features of the anionic micelles, have the drawbacks arising from the different chemical structure of SDS in comparison with membrane phospholipids.

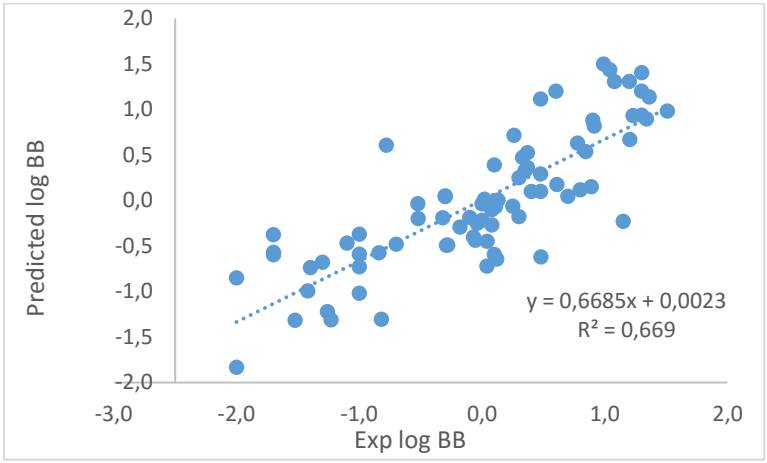
The r^2 observed in equation (18) is high, however it is still noteworthy to mention that domperidone behaves again as a strong *outlier*, being the analyte excluded to maximize the predictive strength in the best optimized model. Furthermore, the physico-chemical descriptors reported in equation (18) are the same as the ones in equation (3), supporting again the concept according to which the polar (Psa) /electrostatic (Dipole) interaction component plays a relevant role in hindering the BBB penetration of drugs. Again, bases seem to be favored in BBB entering and this is also consistent with the clinical experience. In fact, bases polar and extensively protonated at pH 7.4 such as amphetamine and methamphetamine are known to have a CNS activity but it is much harder to recall similar cases for polar acids.

MOLECULAR DESCRIPTORS	r^2	q^2	*SE*	*F*	*PC*	r^2 (n-1)	*SE* (n-1)	*F* (n-1)	*PC* (n-1)	EX-ROW	EQUATIONS	EQ No
STATIC												
NEUTRAL PROPERTIES	0.67	0.62	0.536	37.17	21.442	0.70	0.511	42.52	20.562	-	log BB = 0.6376 + 0.8850 log $k_{30\%MeOH}^{IAM}$ - 0.0158 Psa - 0.1487 VirtualLogP + 0.1037 HbDon	13
										Domperidone	log BB = 0.6470 + 0.9607 log $k_{30\%MeOH}^{IAM}$ - 0.0153 Psa - 0.1698 VirtualLogP + 0.1064 HbDon	14
IONIZED PROPERTIES	0.65	0.59	0.550	34.43	24.143	0.68	0.531	38.36	22.213	-	log BB = 0.2663 + 0.5897 log $k_{30\%MeOH}^{IAM}$ + 0.0475 HbAcc - 0.0140 Psa + 0.2526 Charge	15
										Lamotrigine	log BB = 0.2816 + 0.5943 log $k_{30\%MeOH}^{IAM}$ + 0.0824 HbAcc - 0.0164 Psa + 0.2784 Charge	16
WEIGHTED AVERAGE	0.72	0.68	0.491	47.86	19.256	0.75	0.468	54.04	17.286	-	log BB = 0.2816 + 0.5943 log $k_{30\%MeOH}^{IAM}$ + 0.0824 HbAcc - 0.0164 Psa + 0.2784 Charge	17
										Domperidone	log BB = 0.3066 + 0.6599 log $k_{30\%MeOH}^{IAM}$ - 0.0082 Psa - 0.0217 Dipole + 0.4183 Charge	18
STATIC +												

CONFORMATIONAL												
NEUTRAL PROPERTIES	0.67	0.62	0.536	37.14	22.963	0.70	0.510	42.58	20.541	-	log BB = 0.6370 + 0.8848 log $k_{30\%MeOH}^{IAM}$ - 0.0158 Psa - 0.1486 VirtualLogP + 0.1035 HbDon	19
										Domperidone	log BB = 0.6322 + 0.9570 log $k_{30\%MeOH}^{IAM}$ - 0.0153 PSA Min - 0.1734 VirtualLogP + 0.0909 HbDon	20
IONIZED PROPERTIES	0.65	0.59	0.548	34.70	24.020	0.68	0.524	38.81	21.655	-	log BB = 0.2413 + 0.6017 log $k_{30\%MeOH}^{IAM}$ - 0.0052 PSA Min + 0.2324 Charge - 0.0063 PSA Max	21
										Metoprolol	log BB = 0.2336 + 0.6390 log $k_{30\%MeOH}^{IAM}$ - 0.0020 PSA Min + 0.2206 Charge - 0.0088 PSA Max	22
WEIGHTED AVERAGE	0.73	0.70	0.477	51.48	18.435	0.76	0.455	57.97	16.550	-	log BB = 0.3678 + 0.5975 log $k_{30\%MeOH}^{IAM}$ - 0.0096 PSA Average - 0.0210 Dipole + 0.4719 Charge	23
										Domperidone	log BB = 0.3265 + 0.6493 log $k_{30\%MeOH}^{IAM}$ - 0.0087 PSA Average - 0.0224 Dipole + 0.4354 Charge	24

Table 7. Statical validation of the models developed employing log $k_{30\%MeOH}^{IAM}$ values of the dataset (n=79) along with three other physico-chemical descriptors.

A



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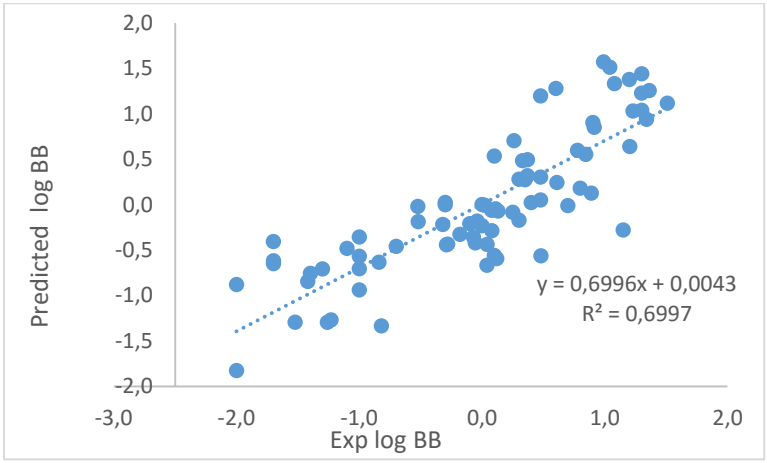
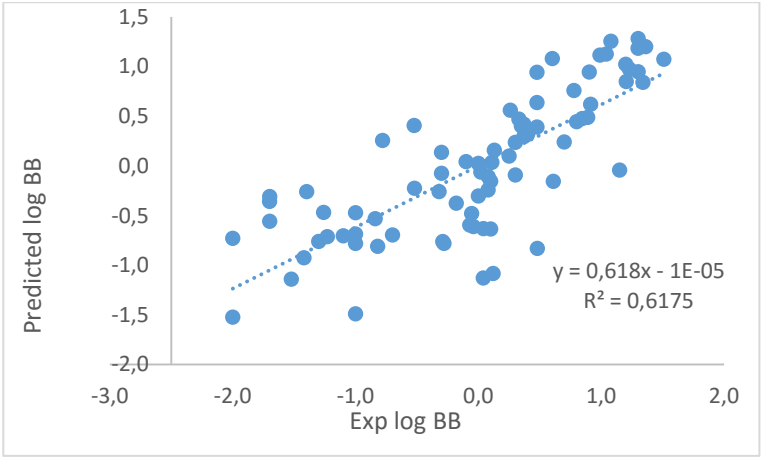


Figure 7. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log $k_{30\%MeOH}^{IAM}$ and three other static properties of the analytes assumed as neutral before (A) and after (B) optimization.

A



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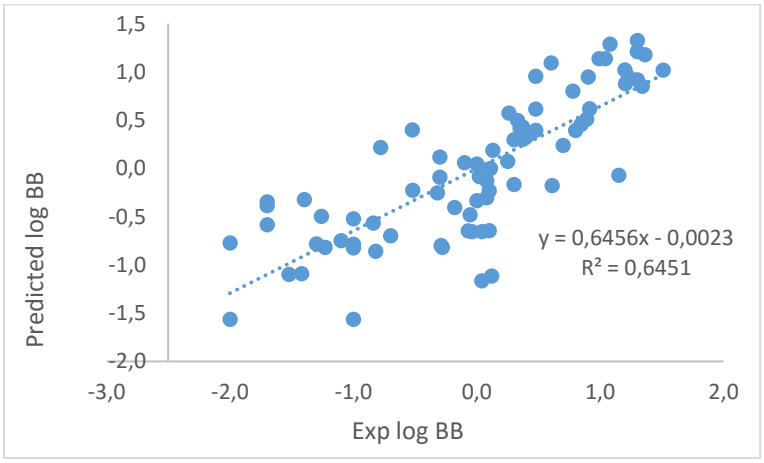
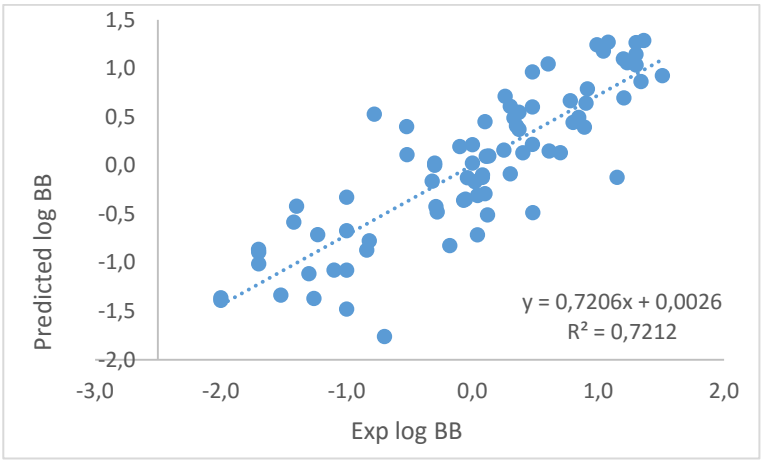


Figure 8. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log $k_{30\%MeOH}^{IAM}$ and three other static properties of the analytes assumed as completely charged before (A) and after (B) optimization.

A



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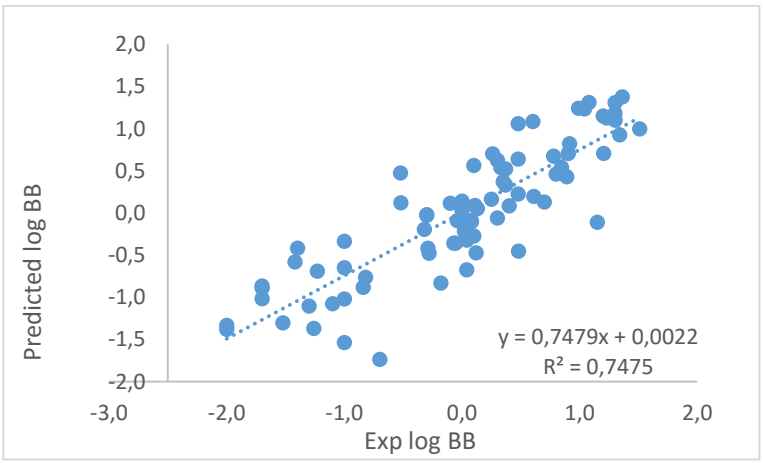
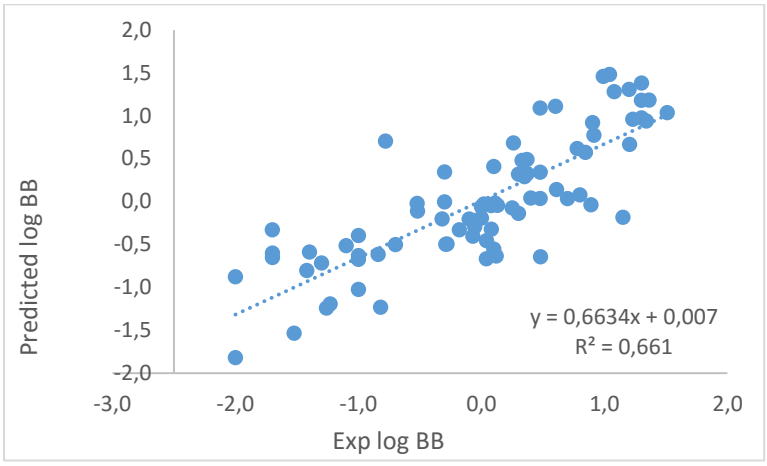


Figure 9. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log $k_{30\%MeOH}^{IAM}$ and three other descriptors arising from the weighted average of the static properties, according to the respective pKa values, of the analytes at the experimental and physiological pH (7.4) before (A) and after (B) optimization.

A



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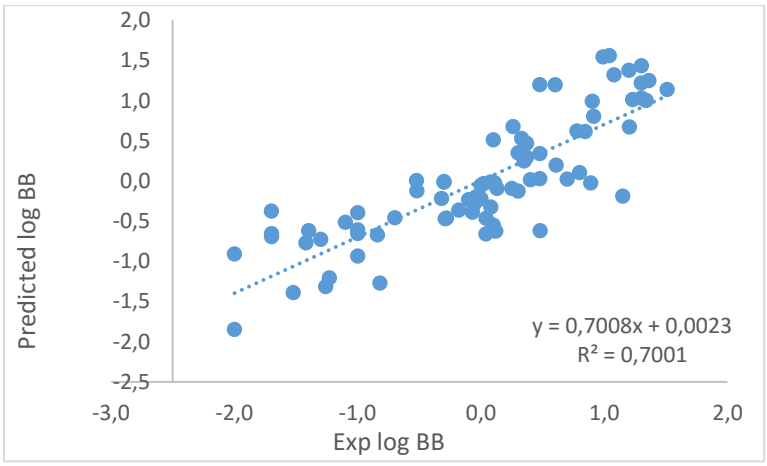
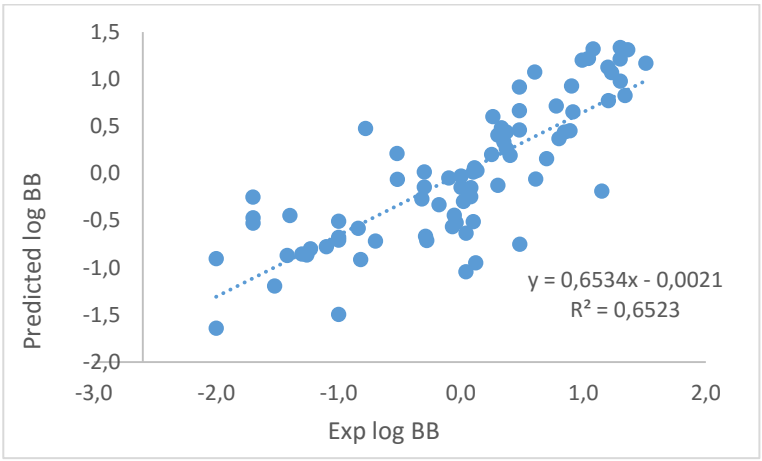


Figure 10. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log $k_{30\%MeOH}^{IAM}$ and three other static and conformational properties of the analytes assumed as neutral before (A) and after (B) optimization.

The conformational analyses of the analytes assumed as neutral (equation (19) and equation (20)) and ionized (equation (21) and (22)) did not benefit the relationships. This is also evident from the fact that conformational properties do not appear at all in equation (19) and equation (20), being Psa, VirtualLogP and HbDon all static properties. Surprisingly, the weighted average of the conformational properties, along with the static descriptor,

markedly improved the relationships. A 0.76 r^2, achieved on a set as large as 79 analytes, employing only four descriptors suggests that the model (equations (23) and (24)) is robust and reliable.

A



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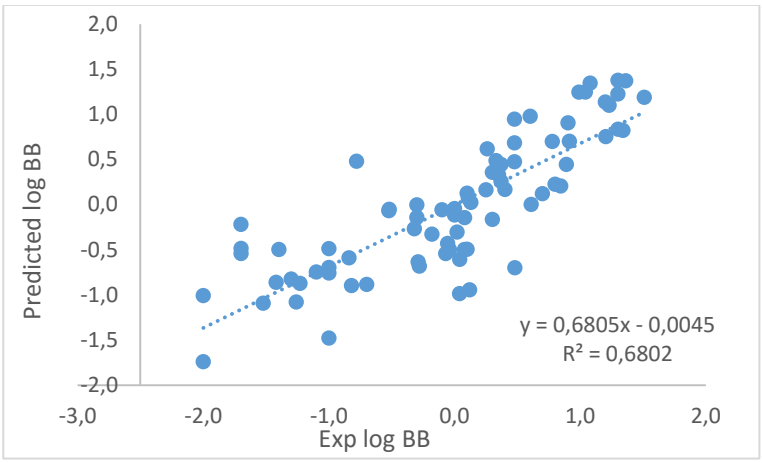
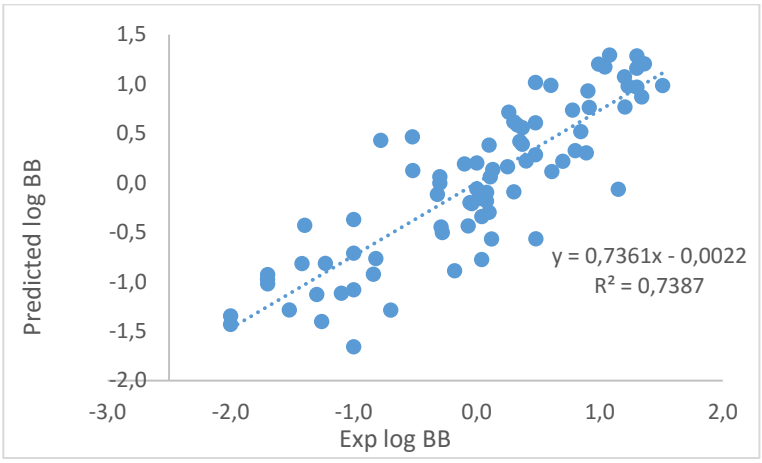


Figure 11. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log $k_{30\%MeOH}^{IAM}$ and three other static and conformational properties of the analytes assumed as completely charged first (A) and before (B) optimization.

A



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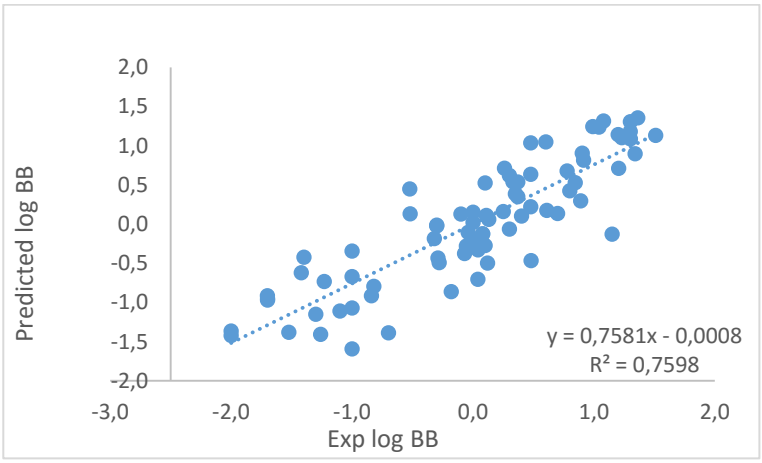


Figure 12. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log $k_{30\%MeOH}^{IAM}$ and three other descriptors arising from the weighted average of the static and conformational properties, according to the respective pKa values, of the analytes at the experimental and physiological pH (7.4) before (A) and after (B) optimization.

7.3.3 IAM+ MLC indexes in log BB prediction

In the present study, the MLC and IAM indexes were, in a first instance, considered separately. However, the evident differences in the elution order observed support a rather diverse selectivity of the two techniques. For this reason, the development of the BBB entering potential statistic models was also performed by taking into account both the chromatographic indexes at the same time, along with three other molecular descriptors (five independent variables in total). This strategy resulted in a markedly improved predictive strength (equations (25) and (26)), as reported in Figure 13.

log BB = -0.0576 + 0.4834 log k_w^{SDS} + 0.4761 log $k_{30\%MeOH}^{IAM}$ - 0.0080 PSA Average - 0.0261 Dipole - 0.1023 MLP Max (25)

r^2 = 0.76 q^2 = 0.72 *SE* = 0.459 *F* = 46.28 $F_{5,79}$ α,0.001 = 16.20 *PC* = 16.990

Best optimized model (n-1):

log BB = -0.0248 + 0.4506 log k_w^{SDS} + 0.5545 log $k_{30\%MeOH}^{IAM}$ - 0.0077 PSA Average - 0.0282 Dipole - 0.1141 VirtualLogP (26)

r^2 = 0.79 *SE* = 0.429 *F* = 54.55 $F_{4,78}$ α,0.001 = 16.41 *PC* = 14.673 ExRow : Domperidone

These relationships may suggest that the molecular mechanism involved in IAM-LC and MLC are different but play both a relevant role in BBB diffusion of drugs.

7.3.4 P-gp affinities in log BB prediction

As already mentioned, each analyte present in the dataset was docked to each discrete binding site on the P-gp (Figure 14) and the binding affinities were incorporated in the development of BBB passage predictive statistic models. From the relationships above reported, P-gp affinities do not seem to have an appreciable role in BBB. This is not entirely true because the statistic model development was carried out using only four independent variables, thus leading the software to select only the four most relevant descriptors, among which P-gp affinities are not included. Indeed, when five independent variables are set in the statistic method development, the P-gp are used by the software to build up the models (Figure 15). As an example, the equations (27) and (28), generated by MLC indexes and four static properties of the analytes, assumed as neutral, are hereby reported.

Log BB = -0.5176 + 0.5900 log k_w^{SDS} - 0.0089 Psa - 0.0194 Dipole + 0.2884 Charge - 0.0501 P-GP 1 Min (27)

r^2 = 0.72 q^2 = 0.67 *SE* = 0.498 *F* = 36.96 *PC* = 20.062

Best optimized model (n-1):

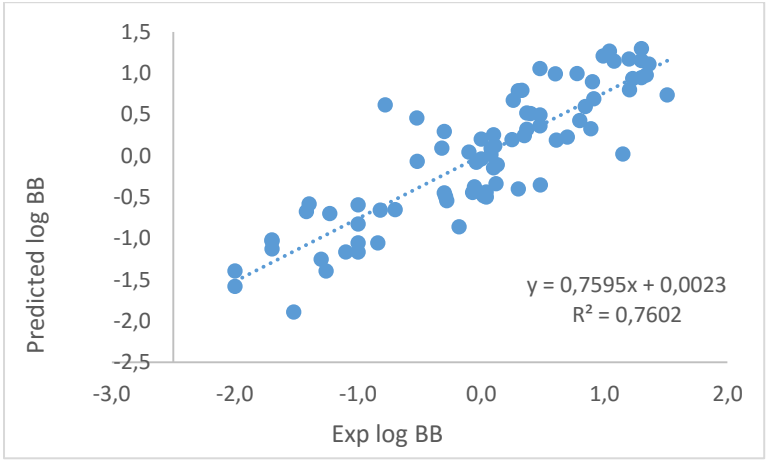
log BB = -0.5092 + 0.6548 log k_w^{SDS} - 0.0081 Psa - 0.0203 Dipole + 0.2422 Charge - 0.0323 P-GP 1 Min (28)

r^2 = 0.73 *SE* = 0.486 *F* = 39.53 *PC* = 18.860 ExRow : Domperidone

This is not surprising because among the considered analytes, the only ones known from the literature to be substrates of P-gp are cimetidine, domperidone, ranitidine, rifampicin,

quinidine and verapamil, [Eyal et al., 2009] and they represent less than 5% of the dataset. Indeed, the compounds considered were selected in the attempt to mirror as accurately and completey as possible the marketed drugs, in terms of diverse chemical nature, molecular volume, CNS activity and molecular lipophilicity. Since the active transport realizes only for a minority of drugs, being the drug uptake prevalently driven by passive transcellular diffusion, the limited predictivity of the P-gp molecular affinity may be dataset related. It is clear that this approach has various limitations, the most evident one being the aspect that the receptor flexibility is not taken into account. The main reason behind it is the large number of degrees of freedom that have to be considered in this kind of calculations, thus requiring remarkable computation power. However, neglecting the receptor flexibility coul lead to poor docking results in terms of binding pose prediction in real-world settings Therefore, these results must be seen as a preliminary attempt to gain new insights and model the active efflux of drugs pumped out of cells by the P-gp, being neither exhaustive nor complete. Other experiments have to be performed and docking conditions further calibrated in order to validate the proposed model.

A



B

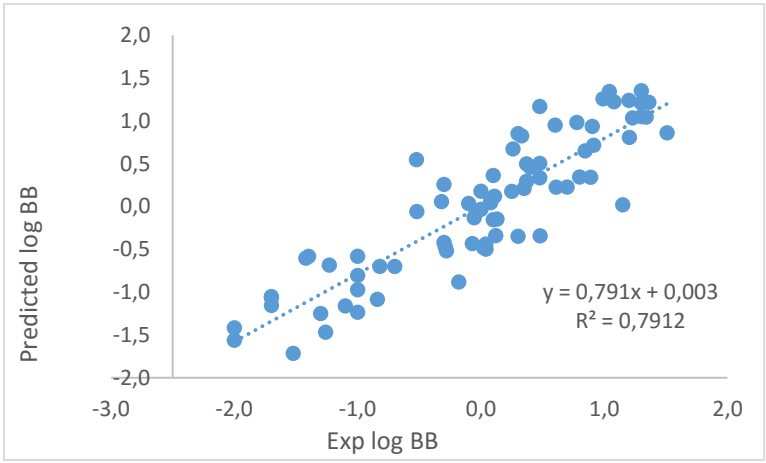


Figure 13. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log k_w^{SDS}, log $k_{30\%MeOH}^{IAM}$ and the weighted average, according to the pKa of each analyte, of three static and conformational properties before (A) and after (B) optimization.

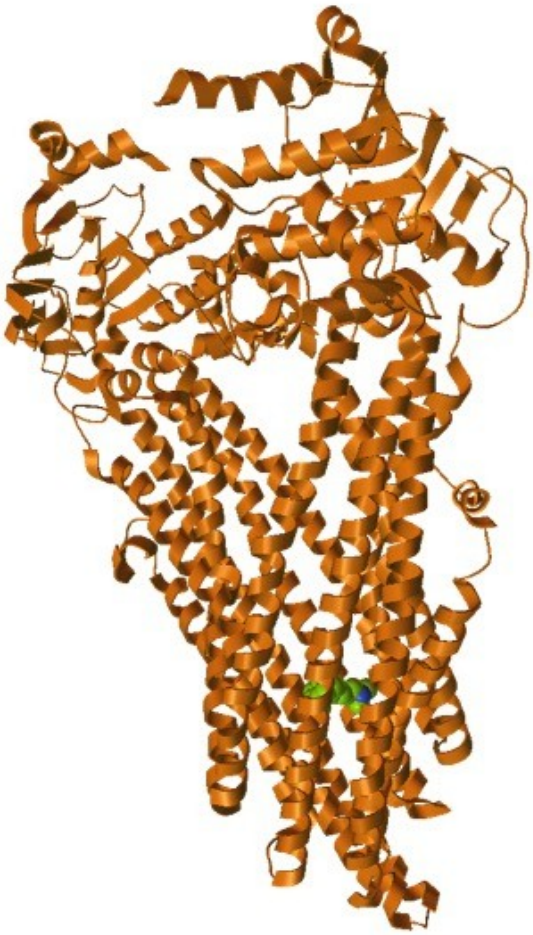
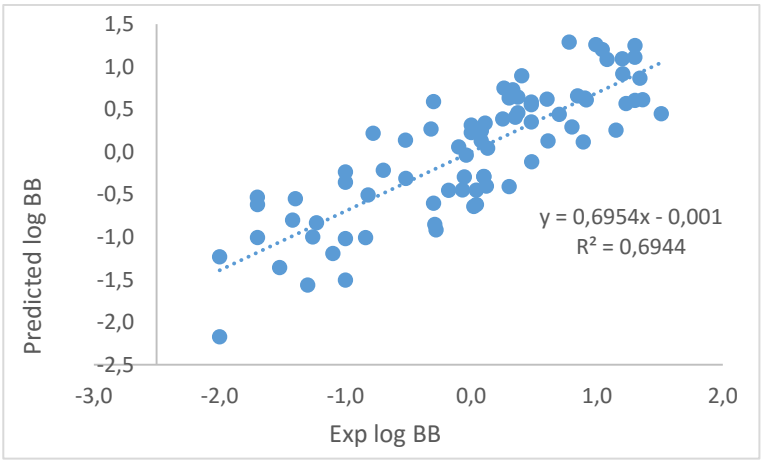


Figure 14. A ligand (amitriptyline, displayed in green) docked into one of the binding sites located on the P-gp.

A



B

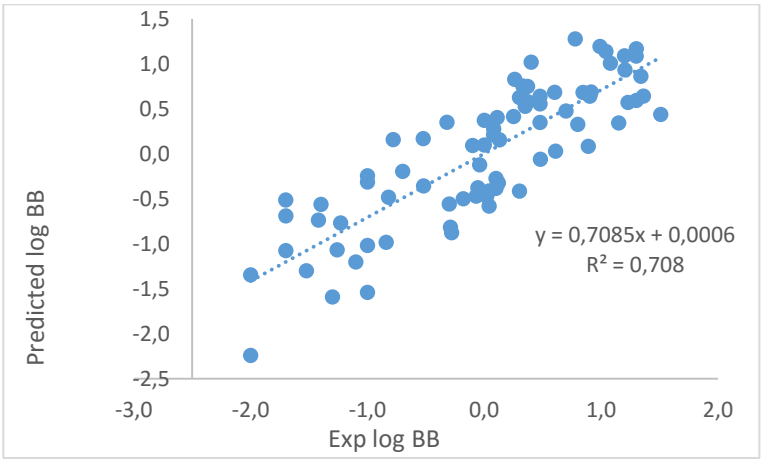


Figure 15. Relationships between Experimental and Predicted log BB values for the statistic models developed for by employing log k_w^{SDS} and four other descriptors (three static properties of the analytes assumed as neutral and P-gp affinities), before (A) and after (B) optimization.

7.4 Conclusion

The proposed method proved effective at developing highly significant (r^2 up to 0.79) BBB entering potential oriented statistical methods and offered new interesting insight into BBB penetration of drugs. It is also suitable for pharmaceutical companies in the search for accurate BBB penetration oriented screening methods as the chromatographic conditions were carefully studied to obtain the indexes in a relatively short time such as to meet their demands. The molecular modeling performed was simple, easy-to-perform and can be configured to run automatically in case of batch analyses. It is also interesting to point out that the chromatographic indexes (MLC and IAM) were always included in the best statistical models and this occurrence confirms that the information encoded in such measures cannot be surrogated by other *in silico* descriptors. Furthermore, as the method is rather cheap and relies on basic HPLC equipment, it offers potential for broad scale application.

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8.0 A HIGH THROUGHPUT IAM-HPLC/MS METHOD FOR A BLOOD BRAIN BARRIER PENETRATION ORIENTED SCREENING OF DRUGS

8.1 Introduction

Nowadays the emerging of combinatorial chemistry and/or organic parallel synthesis offers to medicinal chemists the valuable opportunity of producing hundreds of new compounds at a very fast rate [López-Vallejo et al., 2011]. Such huge amount of new chemical entities (NCEs) has subsequently to be screened according to their ADME properties in the early states of pharmaceutical drug development (drug discovery and drug development pipeline is shown in Figure 1). This occurrence, at the same time, also rose the need of handling accurate screening methods fast enough to process the samples in a rapid and reliable way. For most of the compounds, the critical issue is their membrane barrier passage, i.e. the rate and the extent at which they cross biological membranes [Liu et al., 2011]. This feature has been for long assumed as dependent on the analyte *n*-octanol/water lipophilicity, expressed as log P, classically determined via *shake-flask* method [Hansch and Clayton, 1973]. Apart from being tedious and time-consuming, this method accurately reflects, at a certain extent, the membrane partitioning of only neutral compounds but greatly underestimates that of the analytes supporting one or more electrical charges. The octadecylsilyl (ODS) based liquid chromatography provides for sure a much faster and more reproducible screening method, but has the several drawbacks, being the interactions analyte/stationary phase mainly driven by the analyte molecular hydrophobicity [Rutkowska et al., 2012]. This discrepancy is due to the fact that neither *n*-octanol nor ODS support electrical charges as membrane phospholipids do. For sure, cell-based assays reproduce more closely the asset of fluid membrane bilayers, offering a more realistic model [Abott et al., 2008]. However, the cellular assays, albeit predictive, are lengthy and require cell-culturing skills. In addition, the contribution of the aqueous boundary layer (ABL) and of the polycarbonate filter supporting the cell monolayers as well as the different leakiness of such hydrodynamic biophysical systems might often alter the permeabilit complicating the comparison among data achieved in different laboratories or employing slightly different methodology [Avdeef, 2012a]. Immobilized artificial membrane liquid

chromatography (IAM-LC) [Pidgeon et al., 1995] combines the advantages arising from a more realistic biomimetic system to the increased reproducibility and robustness of liquid chromatography. In the present work, 78 analytes were taken into account and their chromatographic retention coefficients, measured on a IAM stationary phase, were determined. In an attempt to dramatically speed up the technique, from one hand the chromatographic conditions were carefully studied and optimized to obtain the chromatographic retention coefficients in a relatively short time, from the other hand, the LC system was coupled to an Atmospheric Pressure Ionization (API) Electrospray (ESI) Time-of-flight (TOF) Mass spectrometer so as to make the most of the high selectivity given by m/z ratio. Unfortunately, the phosphate buffered saline (PBS) eluents, routinely employed in this kind of determination because able to mirror more closely the physiological microenvironment, are not compatible with MS detection since they may rapidly contaminate the ionization source, thus seriously suppressing sample signals. Therefore, mass-friendly ammonium acetate based eluents were employed for the determination of phospholipid affinity indexes in a first instance using ultraviolet (UV) detection and subsequently by applying a MS-TOF detection to the proposed analytical method. Such strategy led to a dramatically shortening of the analysis times achieved by analyzing the compounds of interest simultaneously in a mixture, markedly improving the throughput of the technique. This approach brings the potential of appealing pharmaceutical companies in search of ultra-high throughput screening method oriented at a fast and reliable assessment of drugs' pharmacokinetic properties. The significance of the phospholipid affinity indexes was also evaluated in terms of BBB penetration predictivity. In fact, statistical models aimed at surrogating BBB penetration data were developed by partial least squares (PLS) regression employing these indexes along with other physico-chemical descriptors, calculated *in silico*. The latters indeed offer interesting advantages: they are fast to perform and easy to set up. Furthermore, they can be incidentally able to offer new interesting insights into drug BBB passage as well as innovative synthetic strategies enhancing the BBB permeability of new drug/prodrug.

In addition, molecular dynamics (MD) experiments were carried out in water boxes offering the opportunity of viewing the dynamic evolution of the system as well and deriving the properties of the most populated conformational states of the analytes of interest. Furthermore, the role played by the different buffered solutions, employed as eluents on (*a*) the chromatographic retention coefficients and the elution order of the analytes (selectivity of the analysis) and (*b*) the BBB passage predictivity of the indexes, were studied. This study was performed to find out whether using ammonium acetate based

buffers rather than the conventional PBS yielded some lacks in the predictive strength of the models, and if so, if this was a reasonable price to pay to the higher selectivity and the increased throughput of the technique.

Figure 1. Drug discovery and drug development pipeline.

8.2 Materials and methods

8.2.1 Chemicals

IAM experiments were performed on Regis IAM Fast Mini Screening (10 µm, 10 mm × 3.0 mm; Morton Grove, IL, USA) column. The solutes were obtained from commercial source.

8.2.2 IAM-HPLC/UV

IAM chromatographic analysis was performed on an Agilent Capillary 1200 system (Santa Clara, CA, USA). The system included a capillary pump, a micro vacuum degasser and an automatic injector. An Agilent 1200 Series variable wavelength detector was used and set at the maximum absorbance wavelength of each analyte. The IAM-HPLC experiments were carried out at room temperature (20 ± 2 °C), the flow rate was 300 µL min^{-1} and the injection volume was 1 µL.

8.2.3 IAM-HPLC/MS

For the MS analyses, an Agilent HPLC 1290 system consisting of a binary pump (Agilent Technologies, Waldbronn, Germany), a diode array detector (DAD) with a micro flow cell (volume: 1 mL, path length: 10 mm) and a 6230 time-of-flight mass spectrometer (TOF-MS) equipped with a Jetstream Electrospray Ionization source (ESI) was employed. The system was operated with the Agilent Masshunter software for instrument control and data

acquisition. UV absorbance values were measured at 254, 230, 210, 290 and 300 nm with a data acquisition rate of 20 Hz. The injection volume was 1.0 µL and all experiments were conducted at a temperature of 20 °C. TOF-MS detection was performed in the positive ionization mode for basic and neutral compounds and in negative ionization mode for acidic compounds. Mass ranges from 90 to 900 amu were scanned.

8.2.4 Mobile phase and sample preparation

IAM mobile phases consisted of a solution 70/30 v/v Ammonium acetate buffer / methanol (HPLC-grade; Biosolve, Valkenswaard, The Netherlands). Water (18.2 MΩ/cm) was purified and deionized in house via a Milli-Q plus instrument from Millipore (Bedford, New Hampshire, USA). The pH was adjusted with ammonia until the aqueous solution had a pH value of 7.40 ± 0.05. The mobile phase was vacuum-filtered through 0.20 µm nylon membranes (Grace, Lokeren, Belgium) before use. Stock solutions of all drugs were prepared by dissolving 10 mg in 1 mL of methanol except for quinidine and theobromine, for which stock concentrations of 1 mg mL^{-1} and 200 µg mL^{-1}, respectively, were used, caffeine and theophylline, which were dissolved in water (10 mg mL−1), domperidone, which was dissolved in dimethyl sulfoxide (10 mg mL^{-1}) and chlorpromazine, which was dissolved in acetonitrile. Stock solutions were stored at 4 °C, except for atenolol, zidovudine, chlorambucil and rifampicin, which were stored at −20 °C. Working solutions were freshly prepared at the beginning of each day by dilution of the stock solutions to 50 µg mL^{-1} with mobile phase for all the analytes, except for valproic acid and halothane that were diluted to 250 µg mL^{-1}.

8.2.5 Data sources

Log BB values were taken from the literature [Abraham et al., 1994; Abraham et al., 2006; Avdeef, A., 2012b; Björkman, 2002; Katritzky et al., 2006; Mente & Lombardo, 2005; Platts et al., 2001]. pKa values were taken from the literature [Avdeef, A., 2012c] except for amobarbital, donepezil, fluphenazine, hydroxyzine, ketorolac, paroxetine and ropinirole, whose values were calculated by the software Marvin Sketch 15.1 for Mac OS X [ChemAxon, Budapest, HU].

8.2.6 Software

8.2.6.1 Molecular modelling

Molecular modelling was performed by the software Vega ZZ 3.0.5 for Windows-based PCs [Pedretti et al., 2004]. The three-dimensional structures of the considered molecules were

downloaded from PubChem database [Kim et al., 2015] and they were considered in both neutral and ionized form. Gasteiger – Marsili [Gasteiger and Marsili, 1980] atom charges were applied to perform the next molecular mechanics calculations. An extensive conformational analysis was carried out *in vacuum* by using the *Boltzmann Jump* method implemented in AMMP software [Harrison, 1993] and the so obtained lowest energy conformation was further optimized by performing a semi-empirical calculation with Mopac 2012 program [Stewart Computational Chemistry] (keywords: PM7 PRECISE MMOK). A cluster analysis was performed in order to select the most populated conformation states. Physico-chemical and topological properties (Virtual logP [Gaillard et al., 1994], lipole [Pedretti et al., 2002], volume, polar surface area, surface accessible to the solvent, gyration radius, ovality, mass, number of atoms, angles, dihedrals, etc) were calculated by VEGA ZZ software and finally, all molecules were inserted into a Microsoft Access database. Additional parameters, including fragment counts, and other topological and geometrical descriptors were calculated by E-Dragon software [VCCLAB; Tetko et al., 2005]. The QSPR models were obtained by the automatic stepwise approach implemented in "Automatic linear regression" script of VEGA ZZ software, calculating PLS based regression models, including from 1 to 4 independent variables. The predictivity strength of the best equation was evaluated not only by leave-one-out (LOO) cross validation, but also by splitting randomly the whole dataset in 20 pairs of training and test sets. For each training set, the regression coefficients were calculated to evaluate the test set in terms of standard deviation of errors, angular coefficient, intercept and r^2 of the trend line of the chart of the predicted vs. experimental activities. This validation was performed automatically by "Model validator" script also included in VEGA ZZ.

8.2.6.2 Molecular dynamics

Molecular dynamics experiments were carried out in 25x25x25 Å cubic water simulation boxes. The solvent cluster was subsequently optimized for the relative position of the solvent molecules, to eliminate any high-energy interaction. The molecular dynamics simulations of each molecule, in every microspecies possibly present at pH 7.4, were carried out for 2 nanoseconds. All simulations had the following characteristics: minimizations with the conjugate gradients algorithm, convergence limit (rms) 0.01, maximal number of iterations 5000; molecular dynamics with constant temperature 300 ± 10 K, integration of Newton's equation each 1 fs according to Verlet's algorithm, calculation of initial atomic velocities according to Boltzmann's equation, and frame stored each 1000 iterations (1.0 ps). The molecular dynamics were carried out in three phases: initial period

of heating from 0 to 300 K over 3000 iterations (3 ps, i.e., 1 K/10 iterations), equilibration period 300 ps with recalculation of atomic velocities during this period each 0.1 ps, and the production phase of simulation of 2.0 ns. Only the frames memorized during this third phase were considered in the conformational analyses. The simulation was carried out using NAMD 2.10 [Phillips et al., 2005] implemented on a Mac OS X dual-core machine. The atom types were assigned using force field CHARMM v27 and the atomic charges according the Gasteiger–Marsili method. The trajectories obtained were analyzed using VEGA software.

8.2.7 Processing

The chromatographic retention coefficients of each analytes were calculated by using the following expression:

$$k = \frac{t_r - t_0}{t_0}$$

In which t_r is the retention time of the compound of interest and t_0 the retention time of an unretained compounds (acetone). Three different sets of properties were generated. At first, all the analytes were considered as uncharged (having full charge equal to 0), subsequently analytes having acidic o basic functions were instead considered ionized and zwitterions were considered with both the acidic and basic functions in their charged forms. Eventually, a weighted average of the static properties at pH 7.4 according to the pKa of each analyte was performed; for zwitterions, the relative abundance of each microspecies (neutral species, zwitterion, anion and cation) in solution at the physiological pH (7.4) was calculated by the software Marvin Sketch 15.1 for Mac OS X [ChemAxon, Budapest, HU]. This approach was also extended to the conformational analysis performed *in vacuum*, yielding three different sets of conformational properties (conformational properties of the neutral forms of the analytes, conformational properties of the ionized forms of the analytes, and average of the conformational properties at pH 7.4 according to the pKa of each analytes and the calculated microspecies distribution for zwitterions). For each of the properties taken into account (Molecular lipophilicity potential (MLP) [Gaillard et al., 1994], lipole [Pedretti et al., 2002], volume, polar surface area, superficial area, gyration radius, ovality, volume diameter, dipolar moment, etc), minimum and maximum value, average, range and standard deviation for each population of conformers were calculated and incorporated in the statistic models.

8.3 Results and discussions

8.3.1 BBB penetration predictive strength of the models

The logarithms of chromatographic retention coefficients on the IAM stationary phase, as well as pKa and log BB values are shown in Table 1. At first the predictive strength of the models was evaluated considering six different sets of properties, as reported in Materials and Methods to assess the role of ionization in BBB passage of analytes. Subsequently among the 1600 descriptors calculated by E-Dragon software, the 99 most predictive were selected and incorporated in the statistic models.

For the sake of brevity, only the most predictive models will be here presented. Equations (1) and (2) describe the models developed by taking into account the weighted average, at pH 7.4, of the conformational and static properties of the analytes (the latter reported in Table 6A, 6B and 6C of chapter 7) according to the experimental pKa of each analyte. Experimental vs predicted log BB data are shown in Figure 2.

Analyte	pKa	log $k_{30\%MeOH}^{IAM}$	log BB
2-(Methylamino)pyridine	-	-0.003	-0.30[a]
2,2,2-trifluoroethyl vinyl ether	-	1.078	0.13[a]
2,6-diisopropylphenol	-	0.038	0.91[b]
Acetaminophen	9.69	-0.259	-1.00[b]
Acetylsalicylic acid	3.50	-0.396	-1.30[b]
Aminopyrine	5.03	-0.225	0.00[b]
Amitriptyline	9.17	1.922	1.30[b]
Amobarbital	7.48/11.15*	0.189	0.04[b]
Antipyrine	1.44	-0.266	-0.10[b]
Atenolol	9.19	0.074	-1.00[b]
Benzene	-	0.108	0.37[c]
Betahistine	7.84	0.038	-0.30[d]
Caffeine	0.60	-0.328	-0.06[b]
Carbamazepine	-	0.235	0.00[b]
Celecoxib	9.38	1.534	0.10[d]
Chlorambucil	4.60	0.309	-1.70[b]
Chlorpromazine	9.50	2.403	1.36[d]
Cimetidine	7.01	0.003	-1.42[b]
Citalopram	9.22	1.389	0.48[d]
Clonidine	8.08	0.476	0.11[b]
Clozapine	7.90	1.684	0.60[d]
Cotinine	-	-0.316	-0.32[b]
Cyclobenzaprine	8.47	1.968	1.08[d]
Desipramine	10.28	1.804	1.20[b]
Diclofenac	3.99	0.383	-1.70[d]
Diphenhydramine	8.86	1.168	1.20[d]
Domperidone	9.68	1.719	-0.78[b]
Donepezil	8.54*	1.106	0.89[e]
Eserine	8.17	0.330	0.08[b]
Ethosuximide	9.27	-0.282	0.04[d]
Ethylbenzene	-	0.544	0.26[c]

Fluphenazine	7.84/2.08*	2.547	1.51[b]
Haloperidol	8.29	1.742	1.34[e]
Halothane	-	0.277	0.35[c]
Hexobarbital	8.20	0.044	0.10[b]
Hydroxyzine	7.52/1.58*	1.636	0.90[d]
Ibuprofen	4.24	0.061	-0.18[b]
Imipramine	9.52	1.710	1.30[b]
Indomethacin	4.13	0.370	-1.26[b]
Ketorolac	3.84	-0.308	-2.00[d]
Lamotrigine	5.36	0.213	0.48[f]
Levofloxacin	8.59/5.89*	0.581	-0.70[d]
Metanol	-	-0.311	0.02[f]
Metoclopramide	9.71	0.666	0.08[d]
Metoprolol	9.56	0.505	1.15[e]
Mianserin	6.92	1.553	0.99[b]
Naproxen	4.14	-0.096	-1.70[d]
Nicotine	8.11	0.006	0.40[c]
Nitrofurantoin	7.05	-0.282	-2.00[d]
Norfloxacin	8.50/6.25*	0.871	-1.00[d]
Nortriptyline	10.13	1.923	1.04[d]
Olanzapine	7.80	1.091	0.80[d]
Omeprazole	9.33/4.31*	0.286	-0.82[b]
Oxazepam	-	0.637	0.61[b]
Paroxetine	9.77	2.211	0.48[d]
Pentobarbital	8.18	0.210	0.12[b]
Phenylbutazone	4.34	-0.040	-0.52[b]
Phenytoin	8.28	0.408	-0.04[b]
Pindolol	9.54	0.653	0.30[d]
Primidone	-	-0.174	-0.07[d]
Promazine	9.36	1.969	1.23[c]
Promethazine	9.00	1.796	1.30[g]
Propranolol	9.16	1.406	0.85[d]
Quinidine	8.56	1.197	0.33[e]

Ranitidine	8.33	0.095	-1.23[b]
Rifampicin	1.70	0.866	-1.52[d]
Ropinirole	10.17	0.345	0.25[b]
Salicylic acid	2.82	-0.368	-1.10[b]
Theobromine	-	-0.330	-0.28[b]
Theophylline	-	-0.298	-0.29[b]
Toluene	-	0.311	0.37[c]
Tramadol	9.41	0.485	0.70[d]
Trazodone	7.30	0.856	0.30[d]
Triprolidine	8.64	1.031	0.78[d]
Valproic acid	4.54	-0.321	-0.84[b]
Venlafaxine	9.67	0.800	0.48[d]
Verapamil	8.68	1.459	-0.52[b]
Zidovudine	9.40	-0.262	-1.00[c]
Zolmitriptan	9.55	0.384	-1.40[d]

* calculated by Marvin Sketch 15.1 software

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a: [Abraham et al.. 1994]

b: [Katritzky et al.. 2006]

c: [Platts et al.. 2001]

d: [Avdeef. A.. 2012a]

e: [Mente & Lombardo. 2005]

f: [Abraham et al.. 2006]

g: [Björkman 2002]

Table 1. pKa values, log $k_{30\%MeOH}^{IAM}$ and log BB values for the analytes taken into account.

$$\log BB = 0.3144 + 0.5456 \log k_{30\%MeOH}^{IAM} - 0.0092\ PSA\ Max - 0.0209\ Dipole + 0.4887\ Charge \quad (1)$$

$r^2 = 0.73$ $q^2 = 0.70$ $SE = 0.480$ $F = 50.78$ $F_{4,79}\ \alpha,0.001 = 25.92$ $PC = 18.610$

Best optimized model (n-1):

$$\log BB = 0.2798 + 0.5832 \log k_{30\%MeOH}^{IAM} - 0.0084\ PSA\ Max - 0.0226\ Dipole + 0.4624\ Charge \quad (2)$$

$r^2 = 0.75$ $SE = 0.460$ $F = 56.42$ $F_{4,78}\ \alpha,0.001 = 26.26$ $PC = 16.892$ ExRow : Domperidone

In this and in the following equations r^2 is the multiple regression coefficient, q^2 is the r^2 validated by Leave-One-Out (LOO) optimization, *SE* is the error standard deviation of the estimate, *F* represents the Fischer regression statistic value, *PC* is the Amemiya predictive criterion and ExRow is the analyte excluded in order to maximize the predictive strength of the statistic model. According to the above reported equations, the penetration of the BBB would be high for extremely IAM retained analytes but also hindered for very polar compounds. The positive sign of the "Charge" term suggests that bases would be favored in BBB partitioning; furthermore, the compounds having high electric dipole would pass the barrier less efficiently. It is also very interesting to notice that domperidone, the analyte excluded from the regression in order to maximize the predictive strength of the models, is a very well known substrate of p-glycoprotein efflux system [Eyal et al., 2009]. It should be underlined that the occurrence of this kind of membrane transport is hardly predictable being based on the recognition of specific molecular structure motifs.

Subsequently, a pool of 99 molecular descriptors, selected among those calculated by E-Dragon software [VCCLAB; Tetko et al., 2005], was used in an attempt to (*a*) improve further the predictive strength of the models (*b*) offer new insight into the mechanisms involved in BBB passive penetration of drugs. The most predictive models are described by equations (3) and (4).

$$\log BB = 0.3599 + 0.3216 \log k_{30\%MeOH}^{IAM} - 0.0124\ PSA\ Max - 1.1013\ nRCOOH + 0.1962\ Mor06m \quad (3)$$

$r^2 = 0.76$ $q^2 = 0.72$ $SE = 0.455$ $F = 58.68$ $F_{4,79}\ \alpha,0.001 = 25.92$ $PC = 16.712$

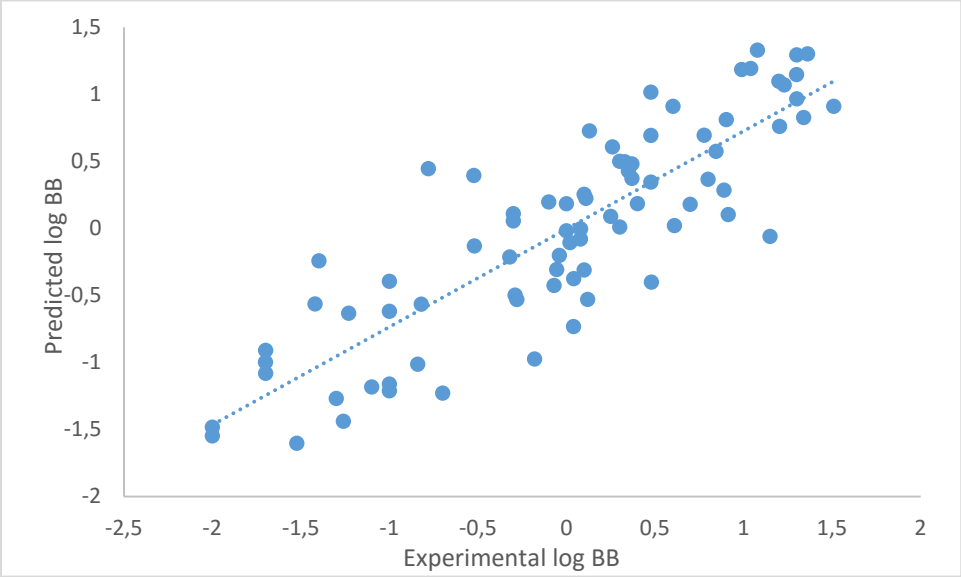
Best optimized model (n-1):

log BB = 0.3468 + 0.3192 log $k_{30\%MeOH}^{IAM}$ - 0.0126 PSA Max - 1.0825 nRCOOH + 0.2010 Mor06m

(4) r^2 = 0.78 *SE* = 0.434 *F* = 64.65 $F_{4,78}$ α,0.001 = 26.26 *PC* = 15.050 ExRow : Metoprolol

In this model, nRCOOH is the number of aliphatic carboxylic acid functions, Mor06m is a 3D-MORSE descriptor corresponding to signal 6. Coherently with the previous model, the BBB penetration of drugs seems not favored for acidic and polar compounds. These models are shown in Figure 3.

A



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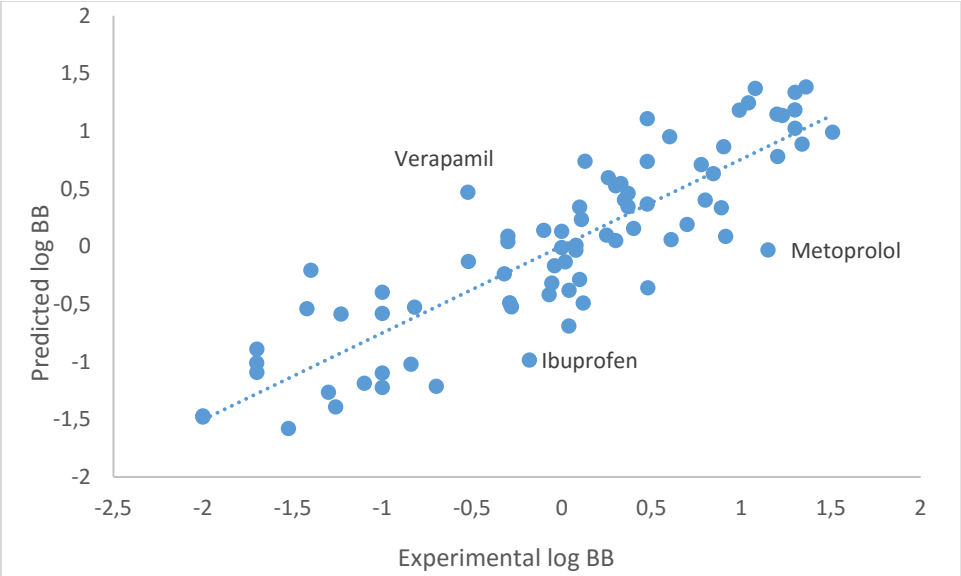
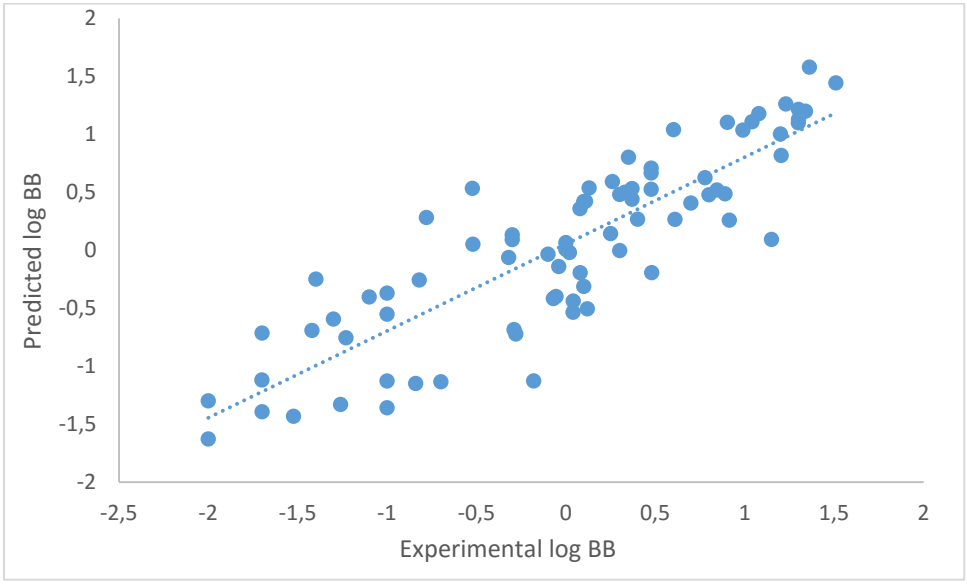


Figure 2. Experimental vs Predicted log BB values considering the weighted average at the physiological pH of the static and conformational properties of the analytes taken into account, before (A) and after (B) optimization.

A



B

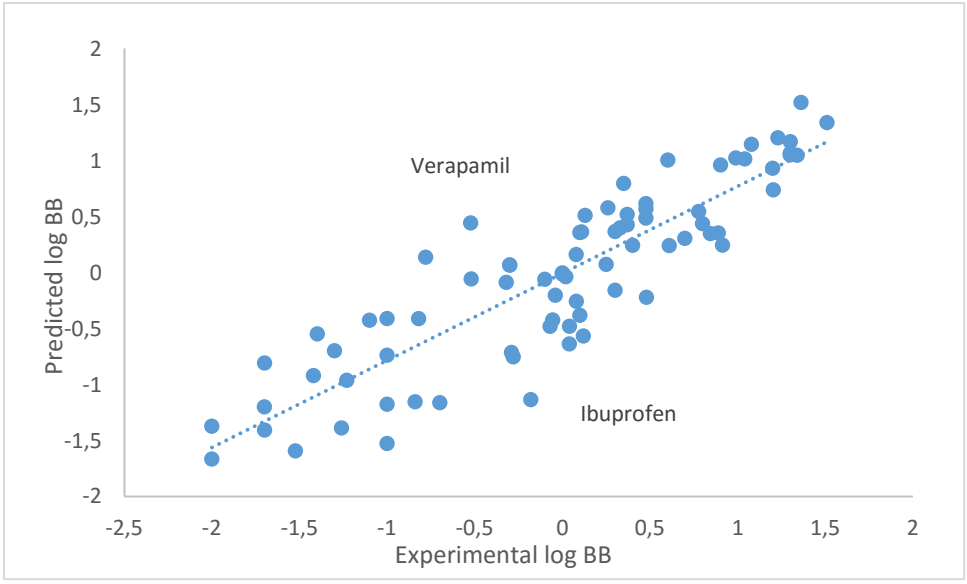


Figure 3. Experimental vs Predicted log BB values considering the static and conformational properties of the analytes, considered as neutral, taken into account, before (A) and after (B) optimization.

8.3.2 Throughput of the technique

The higher selectivity of the MS technique, given by m/z, allowed to analyze up to 8 compounds simultaneously in a mixture. The proposed method was transferred to an HPLC coupled to an Agilent 6230 time-of-flight mass spectrometer (TOF-MS) equipped with a Jetstream Electrospray Ionization source (ESI). MS chromatograms and UV chromatograms are reported in Figure 4 and Figure 5, respectively. As already mentioned, the chromatographic conditions were optimized to obtain the retention coefficients in a relatively short time. When comparing the traditional method [De Vrieze et al., 2013] with the new DPBS (see chapter 7) and MS based methods (here presented), a time gain of a factor of 18 and 100, could be obtained, respectively.

Performing the proposed method allowed to analyze the whole dataset (79 compounds) in less than 10 hours so as to meet the demands of pharmaceutical companies in look for high throughput screening methods. The amount of times required by applying the three different methods is compared in Figure 6.

8.3.3 Phosphate Buffer Saline vs ammonium acetate buffer in BBB passage predictivity

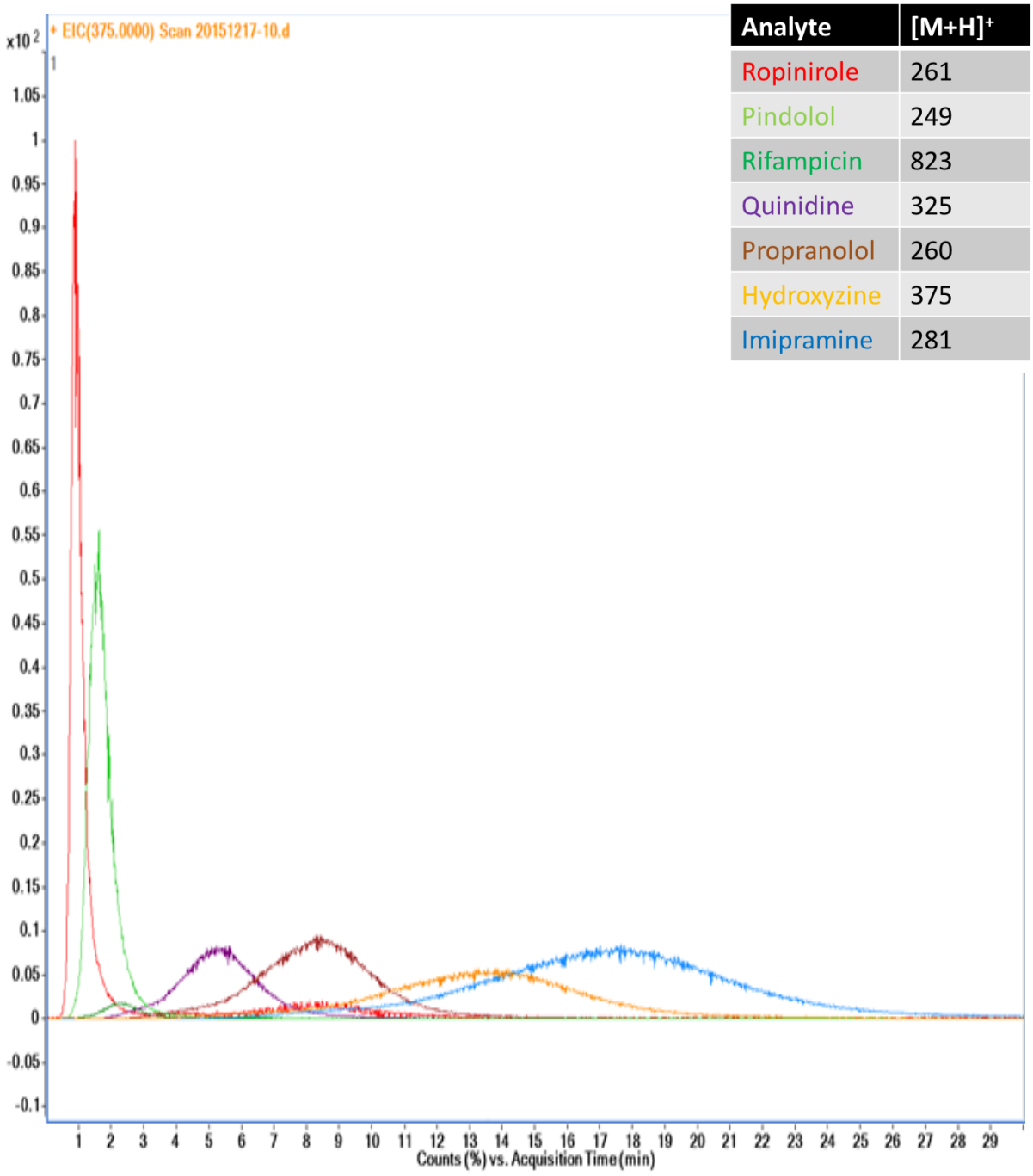
The same simple modelling was performed to compare the predictivity of IAM indexes determined employing two different buffers, i.e. PBS and ammonium acetate based buffers. As it evident from the graphs, reported in Figure 7, using Dulbecco's PBS (DPBS) improves the correlations only negligibly.

8.4 Conclusions

The proposed analytical method was 100 times faster than the one traditionally employed for these determinations [De Vrieze et al., 2013], but likewise predictive; in fact, the r^2 (up to 0.80) values observed confirmed the high predictive strength of the statistical models.

Furthermore, the analytical method proved to be powerful, fast, efficient and suitable for pharmaceutical companies in look for ultra-high throughput drug BBB passage oriented screening methods.

A



Analyte	$[M+H]^+$
Ropinirole	261
Pindolol	249
Rifampicin	823
Quinidine	325
Propranolol	260
Hydroxyzine	375
Imipramine	281

B

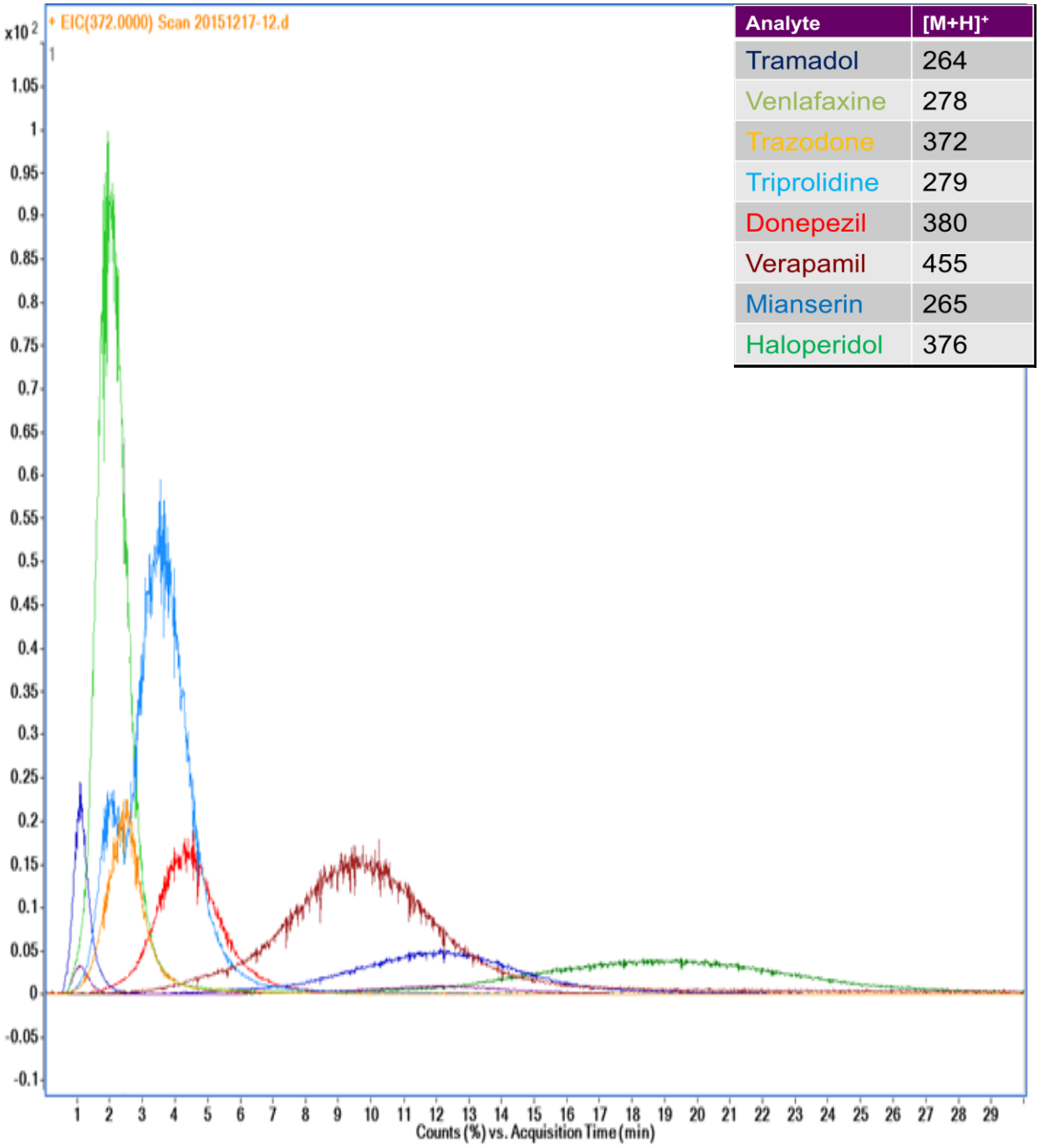
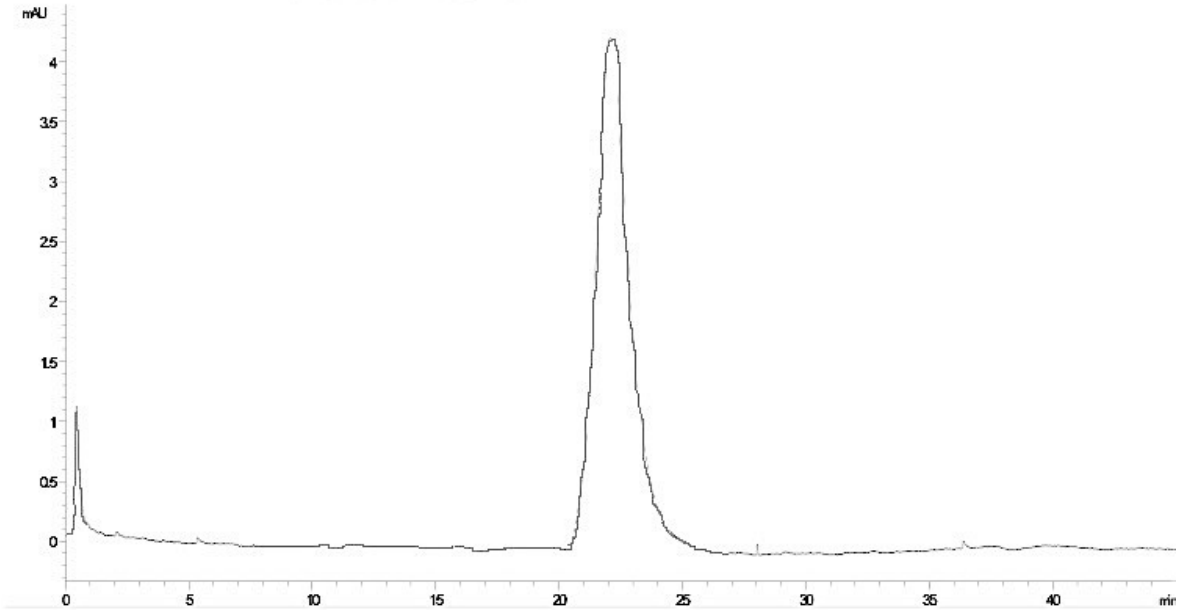


Figure 4. MS Chromatograms of a mixture of (A) Tramadol, Venlafaxine, Trazodone, Triprolidine, Donepezil, Verapamil, Mianserin and Haloperidol and of (B) Ropinirole, Pindolol, Rifampicin, Quinidine, Propranolol, Hydroxyzine and Imipramine. The chromatographic conditions are reported in Materials and Methods section.

A



B

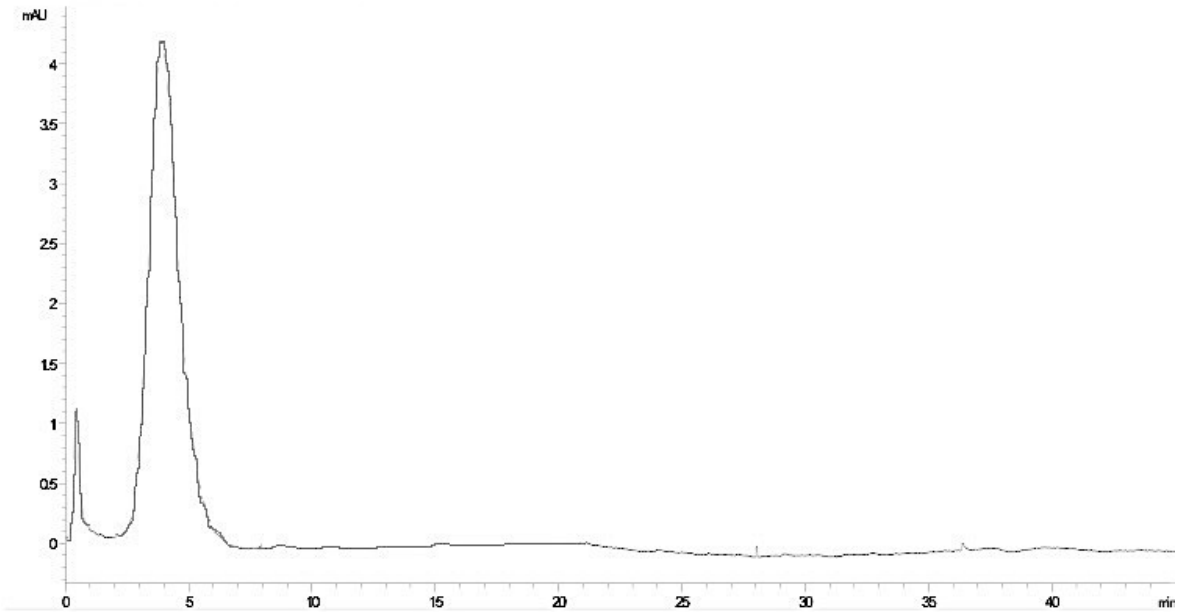


Figure 5. UV Chromatograms of a 50 ppm solution of (A) cyclobenzaprine and (B) diclofenac. The chromatographic conditions are reported in Materials and Methods section.

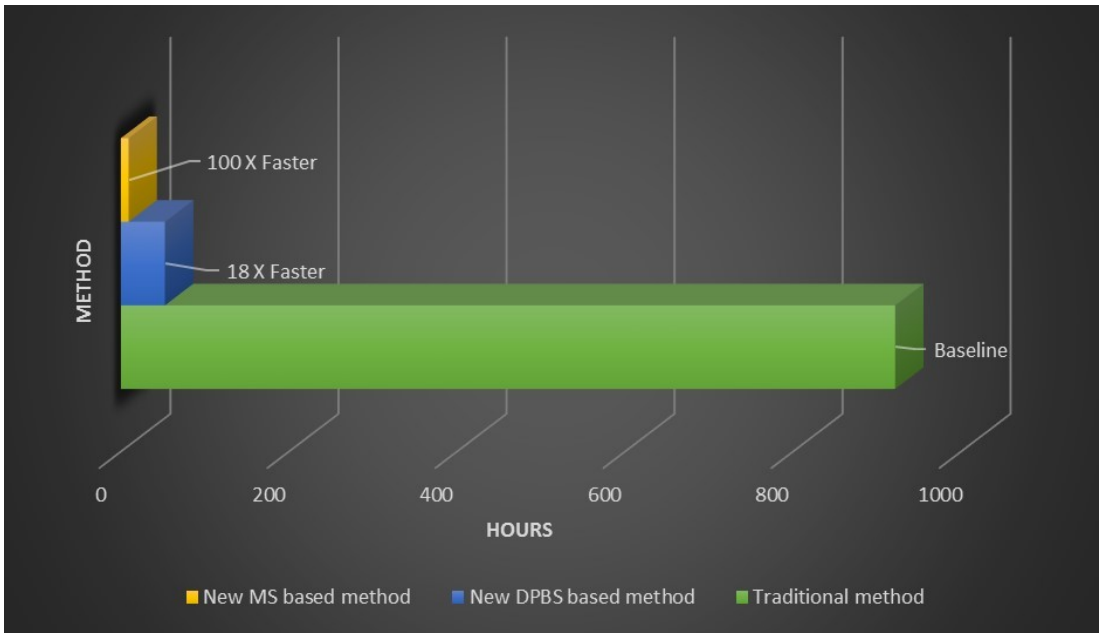
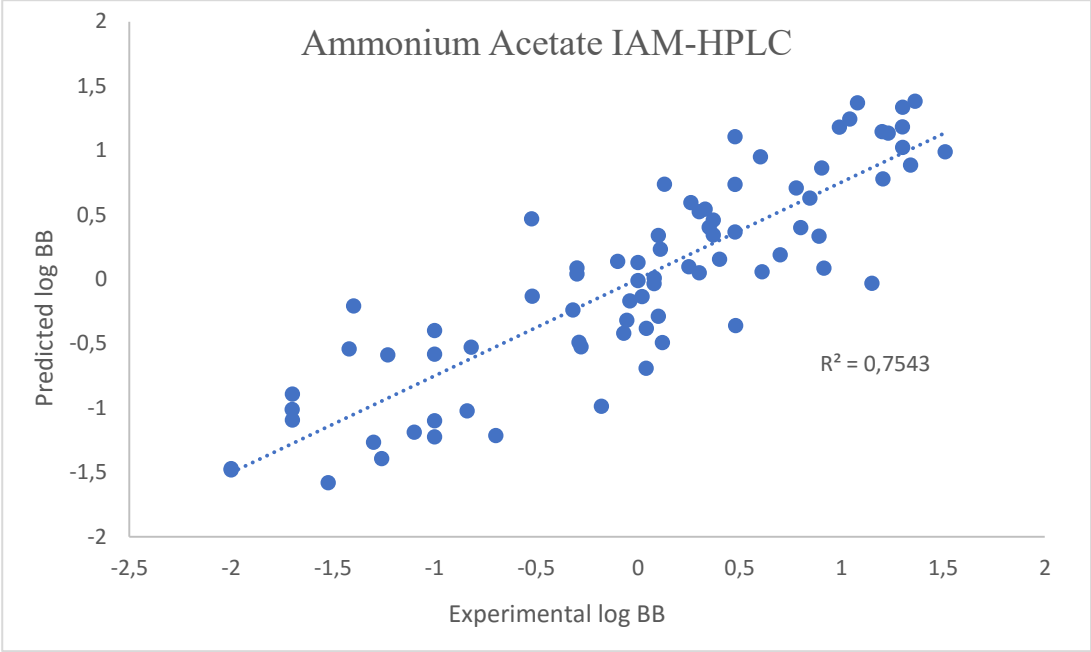


Figure 6. Graph showing the amount of time required for processing the number of analytes taken into account in the present study employing three different methods (the traditional method is reported in [De Vrieze et al., 2013], the New DPBS based method in chapter 7 and New MS based method in the present chapter).

A



B

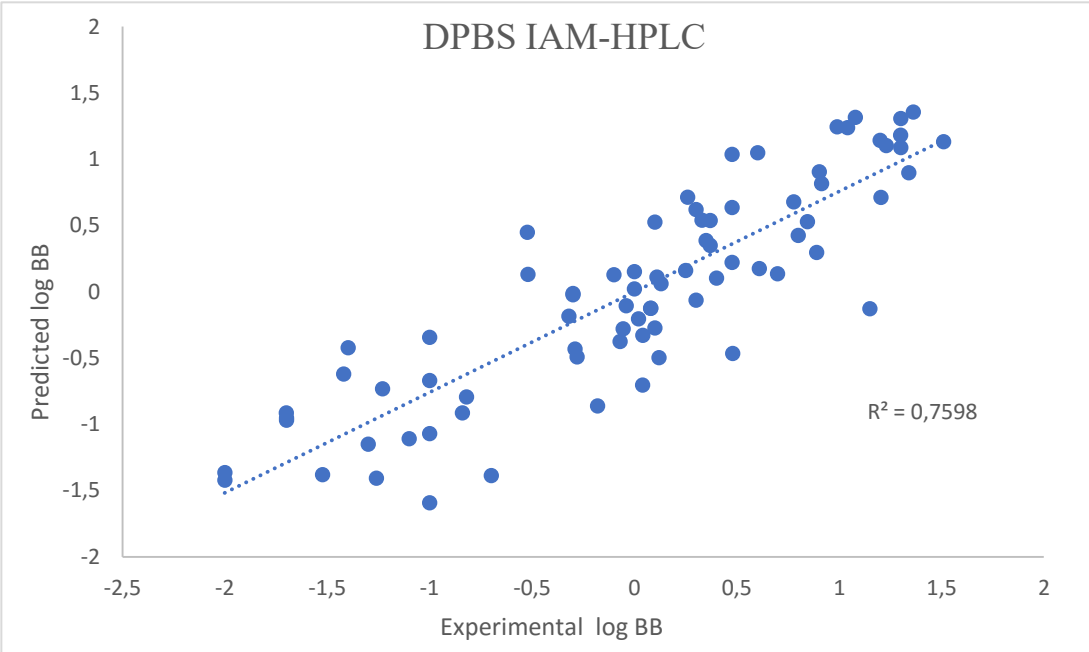


Figure 7. Plots Experimental vs predicted log BB values for statistic models based on (A) Ammonium Acetate buffers and (B) PBS buffers.

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9.0 GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

Phospholipophilicity, measured on either liposomes or IAM stationary phases, has often been regarded as one of the most suitable way of describing the molecular interactions actually occurring between drugs and biomembranes.

However, both phospholipophilicity and *n*-octanol/water lipophilicity data, albeit effective at describing drug interaction with membranes, often fail at adequately describing permeation through them. The research group I worked with recently proposed the use of $\Delta\log k_w^{IAM}$ parameter, arising from the combination of the measures by these two systems, for describing the passage of drugs through the Blood-Brain Barrier. In the studies reported in this Ph.D. thesis not only the effectiveness of $\Delta\log k_w^{IAM}$ in predicting BBB passage was confirmed on a larger set of compounds (42 compounds) but it was proved also effective at describing drug intestinal absorption (62 compounds). Indeed, significant inverse linear relationships were found between $\Delta\log k_w^{IAM}$ and the biological data of drug permeation through these barriers, suggesting that the "flip-flop" model could describe membrane passage more adequately than Fick's law. Conceptually, the basic idea inspiring $\Delta\log k_w^{IAM}$ parameter was similar to that inspiring $\Delta\log$ P. However, whereas the latter parameterizes the H-bond donor capability of the analyte, the former was proved as accounting for the excess of the polar/electrostatic interaction forces occurring between drug and membrane phospholipids. It was found playing a pivotal role in describing the transcellular passive diffusion, mainly that of ionizable drugs, suggesting the structural features allowing an optimal penetration of BBB and/or intestinal barriers.

Furthermore, a study on the possible *in silico* prediction of $\Delta\log k_w^{IAM}$ values was performed resulting in the formulation of two mathematical models. This allows to better understand the molecular mechanisms governing the retention on IAM stationary phases; furthermore, it also allows the prediction of $\log k_w^{IAM}$ even for not yet synthesized drugs/prodrugs contributing to the rational design of new chemical entities oriented to the optimization of their pharmacokinetics.

Finally, many efforts have been put into the optimization of the analytical methodology to measure $\log k_w^{IAM}$, in an attempt to appeal the pharmaceutical companies and meet their demands. The employment of miniaturized LC systems, that can provide shorter analysis times and savings of mobile phase volumes, was investigated. Furthermore, an interesting

approach consisted in the coupling of the LC to MS-TOF detector. The higher selectivity of m/z ratios allowed to analyze the compounds of interest simultaneously in mixtures thus reducing remarkably the analysis time.

Based on these results, it would be interesting, in a near future, to verify whether new, and possibly more accurate, information may be gained by the use of stationary phases functionalized with phospholipids different than phosphatidylcholine (phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine). This is because, although phosphatidylcholine is by far the most abundant phospholipid present in cellular membranes, in some body districts, such as the Blood-Brain Barrier, other phospholipids, such as phosphatidylserine and phosphatidylethanolamine, are also common [Avdeef, 2012]. Furthermore, the application in this research field of the recent "Phase Optimized Liquid Chromatography" (POPLC) [Kuehnle et al., 2008] could allow the development of an LC set up consisting of column segments supporting the different phospholipids (and maybe cholesterol).

The availability of monolithic stationary phases functionalized with immobilized liposomes [Moravcová et al., 2013] may also offer new insight into drug membrane permeation. Such supports, in fact, consist of phospholipid fluid bilayers (rather than the phospholipid monolayer supported on "conventional" IAM phases) thus matching the greater structural similarity of liposomes to cell membranes to the higher reproducibility of LC techniques.

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