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Modeling CNS receptor binding profiles of small molecules

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Resumo

A identificação de novos compostos ativos, passíveis de serem aplicados no tratamento de doenças, é a principal preocupação da indústria farmacêutica, que se foca em encontrar compostos de atuação altamente específica, evitando assim a existência de efeitos secundários. Contudo, este processo nem sempre é fácil, pois tem sido comprovado que muitas moléculas têm como alvo mais do que um recetor. Estas são moléculas promiscuas que ao se ligarem a diferentes recetores podem levar ao surgimento de efeitos inesperados. Este problema recebe o nome de polifarmacologia e muitos estudos têm sido desenvolvidos no seu âmbito.

Na primeira parte deste trabalho, tentou-se estabelecer uma relação entre os perfis de ligação de moléculas a diferentes recetores e a sua relação com a semelhança entre as sequências proteicas dos mesmos. Verificou-se que não existe um padrão constante e que, na maioria dos casos, as moléculas apresentam perfis de ligação diferentes, mesmo para recetores muito semelhantes. Este resultado mostrou que a polifarmacologia é, de facto, um problema complexo e que é necessário investir em diferentes tipos de informação para prever perfis de ligação e evitar o surgimento de efeitos secundários indesejados.

Para prever todos os efeitos resultantes da atuação de uma molécula, é necessário ter um conhecimento prévio acerca das interações entre esta e os recetores, conhecer os tipos de ligações e também as suas forças. Uma forma de obter este conhecimento passa por experiências laboratoriais, no entanto, estes são processos muito dispendiosos e que consomem muito tempo.

Uma forma mais acessível de abordar esta questão foi criando modelos computacionais capazes de prever possíveis interações entre moléculas e recetores com o objetivo de identificar moléculas alvo para a realização dos ensaios experimentais, aumentando assim a probabilidade de sucesso.

Muitos destes modelos computacionais são baseados em métodos de aprendizagem automática, abordagens muito comuns em informática. Estes métodos baseiam-se num processo de aprendizagem de entidades, tendo como fundamento as suas caraterísticas já conhecidas, para criar um modelo capaz de classificar novas entidades. O sucesso destas técnicas tem sido comprovado em

vários contextos da bioinformática e são uma aposta promissora na predição de interações entre moléculas e recetores.

Com este trabalho, pretendeu-se utilizar uma abordagem de aprendizagem automática para desenvolver um modelo de predição de interações entre moléculas e recetores, tendo por base as semelhanças estruturais entre as moléculas e os seus respetivos níveis de atividade, já conhecidos, para recetores de serotonina e dopamina.

O interesse nestas duas famílias de recetores recai no facto de fazerem parte da superfamília de recetores acoplados à proteína G, uma das mais importantes presentes no Sistema Nervoso Central. Para além disso, é conhecido o envolvimento de recetores de serotonina e dopamina em doenças neurológicas, como a doença de Parkinson e o Distúrbio de Défice de Atenção e Hiperatividade. Assim, surge a necessidade de identificar, para estes recetores, moléculas candidatas a serem utilizadas como ponto de partida para o desenvolvimento de novos fármacos, a serem aplicados no tratamento de algumas destas doenças neurológicas.

Como técnica de aprendizagem automática, optou-se pela utilização de um classificador de Naive Bayes, um método de aprendizagem supervisionada baseado no Teorema de Bayes e que tem como pressuposto a independência entre as características que classificam uma entidade.

Para obter a semelhança estrutural entre as moléculas foi utilizado o NAMS (Non-contiguous Atom Matching Structural Similarity), um método que identifica o alinhamento ótimo entre os átomos de duas moléculas tendo em conta, não só os seus perfis topológicos, mas também os próprios átomos e as características das ligações entre os mesmos.

Para a concretização deste trabalho foi obtida informação acerca de moléculas com ligações, já identificadas, a recetores de serotonina e dopamina, tendo estes dados sido recolhidos com base em informação presente no ChEMBL. Adicionalmente, foram também recolhidos os valores de bioatividade de cada molécula para cada recetor, sobre a forma de K_is, as constantes de inibição que quantificam as forças de interação entre as moléculas e os recetores em estudo.

No decorrer deste trabalho, foram construídos três modelos de predição de interações molécula-recetor. Estes incluíram informação relativa a semelhanças estruturais entre moléculas e os seus níveis de bioatividade, perfis de ligação de moléculas para com diferentes recetores e uma combinação de toda a informação anterior.

O primeiro modelo de predição foi construído tendo em conta apenas a informação relativa a semelhanças estruturais entre as moléculas e os seus níveis de atividade. Para isso, foram identificadas, para cada recetor, moléculas kernel, isto é, moléculas muito ativas e estruturalmente distintas das restantes, com as quais as moléculas em teste são comparadas. Tendo por base as suas semelhanças estruturais a cada molécula kernel, as probabilidades de ligação a cada recetor são então calculadas. Apesar deste modelo ter demonstrado resultados promissores durante o processo de validação, uma elevada taxa de falsos negativos mostrou que se trata de um modelo conservador e que deve ser aplicado quando se pretendem resultados mais precisos.

O segundo modelo foi construído de modo a verificar se a informação relativa ao comportamento de ligação de uma molécula para com outros recetores pode ser relevante na predição da sua interação com novos recetores. Para isso, foram tidas em conta apenas as moléculas comuns entre recetores e os seus níveis de bioatividade. Com esta informação, foram construídas duas bases de dados contendo as probabilidades usadas aquando do cálculo das probabilidades de interação entre as moléculas em teste e os recetores. Durante o processo de validação, este modelo evidenciou melhores resultados do que o primeiro modelo. Contudo, estes foram considerados como devidos a uma sobrerrepresentação de moléculas ativas nos dados recolhidos. No entanto, não querendo descartar a informação proveniente de outros recetores, os dois modelos foram integrados para construir o terceiro modelo.

O terceiro modelo, integrando informação relativa a semelhanças estruturais entre moléculas, os seus níveis de bioatividade e informação relativa a outros recetores, foi o que demostrou melhores resultados, atingindo o maior nível de acuidade. Para além disso, foi também o modelo que mostrou um maior equilíbrio entre as proporções de falsos positivos e falsos negativos. Consequentemente, este modelo mostrou ser a melhor opção na identificação de potenciais interações entre um conjunto de moléculas e recetores de serotonina e dopamina.

Numa tentativa de aumentar o desempenho dos modelos propostos, tentou-se identificar, para cada recetor, um valor de probabilidade mais preciso a partir do

qual uma molécula deveria ser classificada como ativa. No entanto, apesar de aumentar a especificidade e precisão dos modelos propostos, este ajustamento não conduziu a um melhor desempenho.

Em conjunto, os resultados obtidos mostraram que o classificador de Naive Bayes é um método passível de ser utilizado na construção de modelos de predição de interações entre moléculas e recetores. Também a ferramenta NAMS demostrou um bom desempenho durante a comparação estrutural de moléculas, o que se tornou evidente pelos resultados obtidos durante o processo de validação dos modelos. Adicionalmente, verificou-se que a utilização da semelhança estrutural entre moléculas em conjunto com os seus níveis de bioatividade é uma abordagem promissora na identificação de moléculas candidatas a validação experimental.

A nível global, verificámos que a integração de informação de diferentes tipos continua a ser a melhor alternativa na previsão de perfis de ligação entre moléculas e recetores. Para além disso, comprovámos, mais uma vez, que os métodos de aprendizagem automática são uma forma eficiente e pouco dispendiosa de selecionar novos compostos candidatos para validação *in vitro*.

Palavras-chave: Polifarmacologia; aprendizagem automática; Classificador de Naive Bayes; Recetores acoplados à proteína G; NAMS

Abstract

Pharmaceutical industry has been focused on finding highly selective single-target drugs. However, different studies have been showing that this is not always possible since many molecules can bind to more than one receptor. These molecules are described as promiscuous compounds and their polypharmacological behavior has been case of many studies.

In the first part of our work, we have investigated the relationship between molecules binding profiles and the sequence similarity of their target receptors. We have found different patterns but no evident relationship was identified since many molecules present different binding patterns for different receptors, even when they are very closed. These results show the level of complexity inherent to pharmacology and the importance of finding new methods to predict molecules binding profiles.

When binding to different receptors, a drug can led to unpredictable side-effects which is a limitation in case of disease treatment.

To avoid side-effects it is import to get knowledge on molecules' binding profiles. With this purpose, different approaches have been developed to predict interactions between molecules and receptors. Many of these approaches rely on the use of machine learning techniques to predict drug-target interactions. These techniques have been widely used in informatics and have already shown their contribute to bioinformatics.

In this work, we have used a machine learning method to predict interactions between molecules and serotonin and dopamine receptors, two of the most important families of receptors present in the Central Nervous System.

To construct our model, we have used the Naïve Bayes classifier, which is a supervised learning method based on applying Bayes' Theorem with the assumption of conditional independence between features.

We have developed three different models that include co-activity data between receptors, molecular similarity and a combination of these two. Despite the three models have presented promising results, the model integrating all the data has shown to be the one with the best performance.

Our results have demonstrated that Naïve Bayes is an efficient method to drug-target interactions prediction. Moreover, it was demonstrated that structural similarity between compounds together with their bioactivity levels is a promising approach to identify candidate molecules for further *in vitro* validation.

Keywords: Polypharmacology; Machine learning; Naïve Bayes classifier; G-protein coupled receptors; NAMS

Table of Contents

List of Figures

List of Tables

1. Introduction

1.1 Polypharmacology: Drug Discovery for the Future

Despite pharmaceutical industry focus on the development of highly selective single-target drugs, it has been proved that many compounds can bind to more than one receptor. This drug promiscuity or polypharmacology has been referred as one of the hottest fields of modern science. Promiscuous compounds can led to undesired effects when binding to different targets and this behavior compromises their viability as potential drugs to therapy of known diseases (Reddy and Zhang, 2013). On the other hand, this represents an opportunity not only to discover new uses for already known compounds but also to increase the efficacy of already known drugs. In fact, efforts have been made to point promiscuous drugs as solution for complex diseases (Haupt *et al.*, 2013).

In order to address this problem, the identification of drug-target interaction networks is an area of intense research. This identification of new drug-target interactions appears as the key to find new targets to old drugs and also new drug candidates for known targets (Cao *et al.*, 2014). Moreover, drug-target interactions are pointed to facilitate the process of drug discovery, drug side-effect prediction and drug repurposing (Ding *et al.*, 2013).

Despite the importance of drug-target interactions discovery, the current knowledge is very limited. For instance, from the 35 million compounds present in the PubChem database, less than 7000 have information relative to target proteins (Ding *et al.*, 1013). However, the increasing amount of available data present in public databases, during the last years, offers the opportunity to explore and integrate the existing knowledge, contributing to the development of more effective and efficient methods to predict drug-target interactions (Kuhn *et al.*, 2008).

In order to face the problems inherent to *in vitro* prediction methods, which are extremely costly and time-consuming, *in silico* approaches have been developed to find potential drug-target interactions that can be validated later through *in vitro* techniques. Docking simulation and machine learning represent two of these major *in silico* approaches (Ding *et al.*, 2013).

With some limitations associated to docking simulation, machine learning techniques have been widely used to construct models able to predict drug-target interactions. With special emphasis on supervised learning techniques, many studies show that machine learning is an efficient alternative to *in vitro* experiments, allowing the identification of potential drug candidates for known receptors (Ding *et al.*, 2013; Mousavian and Masoudi-Nejad, 2014).

The G-protein coupled receptors (GPCRs) have been used as target receptors in many of these studies not only for being one of the receptors families for which there is more available information but also for representing one of the most important families of receptors present in the Central Nervous System (CNS) (Shiraishi *et al.*, 2013). Thus, the identification of potential drugs for these receptors can be a starting point to develop new therapies for neurological diseases like Attention-Deficit Hyperactivity Disorder (ADHD) and Parkinson's disease (Vallone *et al.*, 2000; Fox et al. 2009).

1.2 Objectives

With the present work we expect to contribute with a new machine learning-based method to predict potential drug-target interactions. Our aim is to develop a model integrating information relative to structural similarity between compounds and their bioactivity levels for known targets. For that, we will use a Naïve Bayes classifier as supervised learning method and NAMS to measure molecular similarities. As target receptors we have choosed the Serotonin and Dopamine receptors, two families of GPCRs present in the CNS.

Moreover, we expect to infer the evolutionary relationship between the receptors in study and try to relate it with the binding profiles of their common binding molecules by using their measured binding affinities. We also expect to include this information in our prediction model and improve its performance.

1.3 Overview

The present work is subdivided into three independent parts. First, an overview over the biological background and the informatics techniques is provided. They are described concepts relative to the receptors in study and the drug-receptors interactions. The used methodologies in this work are also presented and a reference to previous similar studies is made at the end.

In the second part, all the execution of the work is described with special emphasis on the detailed description of the used techniques and the justification of all choices made. The construction of models is presented in detail with the algorithms implementation detailed step-by-step.

In the third and final part, all the results obtained through the execution of the proposed techniques and different approaches are displayed and discussed. The performance of the developed models is analyzed and general conclusions of the presented work are provided.

2. Concepts and State of The Art

2.1 Drug Interactions with Receptors

Physiological receptors are protein receptors for endogenous regulatory ligands and act as target receptors to many drugs. These receptors have evolved to recognize and respond with great selectivity to specific signaling molecules, which can be primary classified on the basis of their action when coupled with a target receptor as agonists, antagonists, partial agonists or inverse agonists (Goodman *et al*. 2011).

Agonists, Partial Agonists and Inverse Agonists

They are considered as agonistic drugs the ones that once associated with a specific physiological receptor mimic the regulatory effects of the endogenous signaling molecules (**Figure 2.1**). Furthermore, agonistic compounds can also be classified as *primary agonists* or *allosteric* (or *allotropic*) *agonists*. *Primary agonists* represent drugs that bind to the same recognition site as the endogenous agonist, while *allosteric agonists* couple to a different region at the target receptor, the allosteric (or allotopic) site.

Compounds that despite the concentration employed are only partly as effective as agonists are described as partial agonists, while the inverse agonists are represented by compounds that stabilize in an inactive conformation receptors whose constitutive activity is exhibited in the absence of a regulatory ligand (**Figure 2.1**)(Goodman *et al*. 2011).

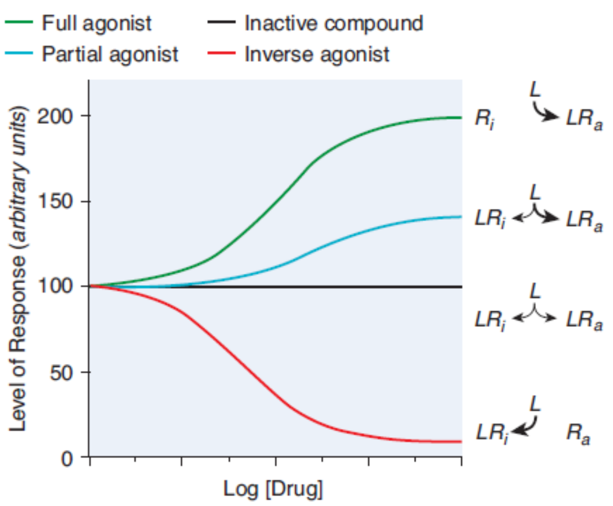


Figure 2.1: Regulation of the activity of a receptor with conformation-selective drugs. The ordinate is the activity of the receptor produced by R_a, the active receptor conformation (e.g., stimulation of adenylyl cyclase by a β adrenergic receptor) while the coordinate represents the concentration of drug L present at the receptor. Taken from Goodman *et al.* (2011).

Antagonists

Drugs that block or reduce the action of an agonist with respect to a specific receptor are considered as antagonistic compounds. These drugs can compete with agonistic molecules for the same overlapping site on the receptor (syntopic interaction) or may interact and bind to different sites. In the second case the compounds are defined as *allosteric antagonists*.

Antagonists can be also combined with agonists to produce a response and this type of interaction is termed *chemical antagonism*. Moreover, agonistic cellular or physiological effects can also be indirectly inhibited by *functional antagonists* (Goodman *et al.* 2011).

2.1.1 Quantifying Binding Activities

In order to better understand a drug-receptor interaction a quantification of molecules' binding affinities is imperative, mostly when trying to find selective compounds for target receptors. Despite the fact that different measures for binding affinities have been developed, some of them remain more used like the half maximal effective concentration (EC50), the half maximal inhibitory concentration (IC50) and the inhibitory constant (K_i).

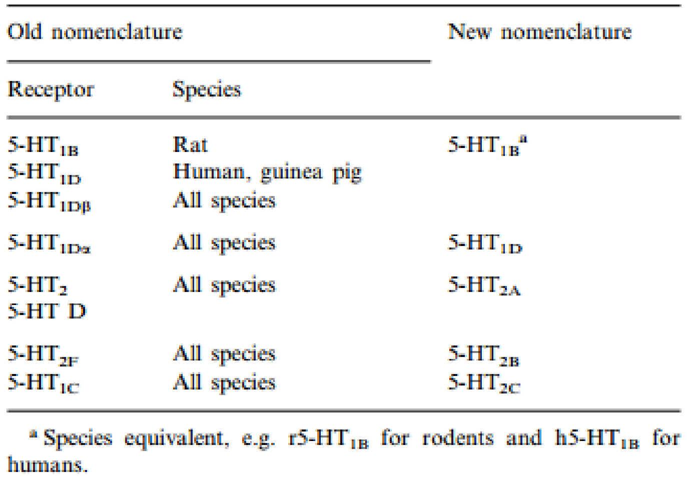
EC50 value represents the molar concentration (M = mol/L) of an agonist that produces half of the maximal possible effect of that agonist, while IC50 value can have different meanings: (1) the molar concentration of an antagonist that reduces the response to an agonist by 50%; (2) the molar concentration of an unlabeled agonist or antagonist that inhibits the binding of a radioligand by 50%; (3) the molar concentration of an inhibitory agonist that reduces a response by 50% of the maximal inhibition that can be attained (Cortés *et al.*, 2001; Neubig *et al.*, 2003).

K_i value is used to quantify a ligand-receptor interaction based on the equilibrium dissociation constant (K) and refers to the equilibrium dissociation constant of a ligand determined in inhibition studies. It is typically determined in a competitive radioligand binding study through the measurement of the inhibition of the binding of a reference radioligand by the competing ligand of interest under equilibrium conditions and is expressed as a molar concentration. Therefore, the smaller the K_i value, the smaller the ligand quantity needed to inhibit the radioligand binding, which means that small values of K_i are associated with higher ligand-receptor binding affinities (Goodman *et al.*, 2011; Neubig *et al.*, 2003).

K_i values are sometimes represented as pK_i values, which are described as the negative logarithm to base 10 of the equilibrium dissociation constant, in this case the K_i value. The use of the pK_i measure instead of the equilibrium constant itself allows an easier comparison of binding affinities given the fact that K_i values often ranges over many orders of magnitude (from 10^{-10} M to >10^{-3} M), while pK_i values mostly range from about 10 to 3. Moreover, from a statistical vision, concentration parameters are generally distributed in a log normal manner. Thus, standard deviations are symmetrical for pK_i values but not for K_i values (Neubig *et al.*, 2003).

2.2 Serotonin Receptors

Serotonin (5-HT) receptors family remains one of the most complex and constantly updated receptors family since new members still being discovered and old ones have been reclassified over time (Barnes and Sharp, 1999). In order to avoid misunderstandings, only the new nomenclature will be considered (**Figure 2.2**).



Old nomenclature		New nomenclature
Receptor	Species	
5-HT_{1B}	Rat	5-HT_{1B}[a]
5-HT_{1D}	Human, guinea pig	
5-$HT_{1D\beta}$	All species	
5-$HT_{1D\alpha}$	All species	5-HT_{1D}
5-HT_2	All species	5-HT_{2A}
5-HT D		
5-HT_{2F}	All species	5-HT_{2B}
5-HT_{1C}	All species	5-HT_{2C}

[a] Species equivalent, e.g. r5-HT_{1B} for rodents and h5-HT_{1B} for humans.

Figure 2.2: Summary of changes in 5-HT receptor nomenclature. Taken from Barnes and Sharp (1999).

Currently, 5-HT receptors are subdivided, according to the NC-IUPHAR subcommittee on 5-HT receptors, into seven distinct classes (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7) comprising a total of 14 distinct receptors in humans (Pauwels, 2003), which are considered to have evolved from a primordial rhodopsin – G-protein-coupled receptor (GPCR) family (**Figure 2.3**) (Barnes and Sharp, 1999). Moreover, splice variants (belonging to 5-HT_3, 5-HT_4, 5-HT_6 and 5-HT_7 classes) and edited isoforms (belonging to 5-HT_{2C} receptor) have also been identified, which makes receptors classification and functional studies even harder (Barnes and Sharp, 1999; Pauwels, 2003; Nichols and Nichols, 2008). In fact, 5-HT receptors classification not only takes into account operational criteria like drug-related characteristics but also information on intracellular signal-transduction and amino acid sequences (Pauwels, 2003).

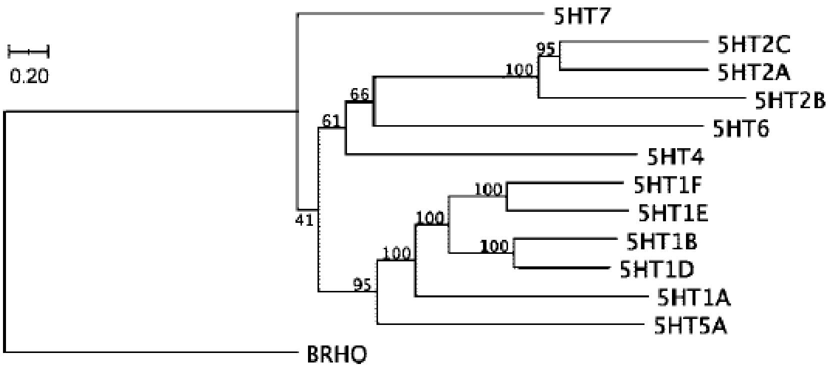


Figure 2.3: Scaled phylogenetic tree comparing all human serotonin receptors with bovine rhodopsin (BRHO) which is considered to be the primordial serotonin receptor. Results of bootstrap analysis with 100 replications are given above the branches. The scale bar corresponds to 0.2 substitutions per position for a unit branch length. The tree was constructed using the most current NIH Entrez sequence for each receptor with CLC Free Workbench software (CLC bio, Cambridge, MA). Taken from Nichols and Nichols (2008).

With the exception of 5-HT$_3$ class, all 5-HT receptors belong to the GPCR superfamily and have a major role in the regulation of adenylyl cyclase (AC) pathway. They can increase or decrease cyclic AMP (cAMP) intracellular levels. Additionally, 5-HT$_2$ class is also involved in the stimulation of phosphoinositide hydrolysis (Barnes and Sharp, 1999; Pauwels, 2003; Nichols and Nichols, 2008). Beyond these main functions, their involvement in other signaling pathways has been well described over time (Barnes and Sharp, 1999; Nichols and Nichols, 2008). With respect to 5-HT$_3$ class, it represents a serotonin-gated cation channel composed by two subunits (5-HT$_{3A}$ and 5-HT$_{3B}$) and its receptors are classified as belonging to the ligand-gated ion channel superfamily (Barnes and Sharp, 1999; Pauwels, 2003; Nichols and Nichols, 2008) (**Table 2.1**).

5-HT receptor class	5-HT$_1$	5-HT$_2$	5-HT$_3$	5-HT$_4$	5-HT$_5$	5-HT$_6$	5-HT$_7$
Subtypes	5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1F}$	5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$	5-HT$_{3A}$, 5-HT$_{3B}$	-	5-HT$_{5A}$	-	-
Major signaling pathways	cAMP	IP$_3$	Ion channel	cAMP	cAMP	cAMP	cAMP

Table 2.1: Different 5-HT receptor subtypes and major signaling pathways. Adapted from Pauwels (2003).

5-HT receptors are widely distributed in the Central Nervous System (CNS) with some of them also present in the periphery. This widespread distribution is closely related with the numerous functions performed by this well-known family of receptors (Barnes and Sharp, 1999; Nichols and Nichols, 2008). The association of 5-HT receptors with fundamental mechanisms related to survival of species like feeding, reproduction and homeostasis has already been described. In addition to that, their role in the regulation of mood states, cognition and memory was also verified. In fact, these findings have led to the study of 5-HT receptors involvement in psychiatric disorders (Barnes and Sharp, 1999; Nichols and Nichols, 2008) and the implication of some serotonin receptors in degenerative disorders has been demonstrated, with some serotonergic dysfunctions showing to be on the basis of several symptoms associated with Parkinson's disease (Fox *et al.*, 2009).

5-HT receptors wide distribution in association with an extensive variety of functions and implications in degenerative disorders has made this GPCR family one of the most important classes of therapeutic targets (Nichols and Nichols, 2008). In fact, the search for selective agonists and antagonist has been a priority, since differentiation between related receptors is not an easy process (Barnes and Sharp, 1999; Nichols and Nichols, 2008). For instance, when comparing the affinity values (pK_i) of various ligands for 5-HT$_2$ receptor subtypes we verify that despite some selective compounds have been identified as selective for one receptor subtype, the differences in pK_i values for the remaining receptors are not as high as we expected (Barnes and Sharp, 1999).

2.3 Dopamine Receptors

Dopamine receptors (DA-Rs) are among the most well-known receptors present in the Central Nervous System (CNS). Their involvement in several signaling pathways is already known and their association with some neurological diseases like Parkinson's disease and Attention-Deficit Hyperactivity Disorder (ADHD) has been already verified (Vallone *et al*, 2000).

Although their main location in the CNS, some receptors are also found in the periphery, with their presence being described in kidney and heart (**Table 2.2**) (Civelli *et al.*, 1993; Lachowiczl and Sibley, 1997; Vallone *et al.*, 2000).

DA-Rs are classified as seven transmembrane domain (7TM) proteins and they belong to the G-protein coupled receptors superfamily. DA-Rs interaction with heterotrimeric G proteins leads to the activation of adenylyl cyclase (AC) that is responsible for increasing or decreasing cyclic AMP (cAMP) intracellular levels. Moreover, DA-Rs are also involved in the activation of additional pathways like stimulation and inhibition of Ca^{2+} and K^+ currents and modulation of arachidonic acid (AA) synthesis (Civelli *et al.*, 1993; Vallone *et al*, 2000).

Five different DA-Rs were discovered and subdivided, based on their structural, pharmacological and functional similarities, into two distinct subfamilies: the D_1-like subfamily and the D_2-like subfamily. The main reason which led to this initial separation was the different role played by each receptor in the regulation of the cAMP pathway. While D_1-like subtype receptors are positive regulators of cAMP levels, D_2-like subtype receptors are responsible for the inhibition of adenylyl cyclase and subsequently for decreasing cAMP intracellular levels.

The D_1-like subfamily includes the D_1 and D_5 receptors, while the D_2-like subfamily comprises the D_2, D_3 and D_4 receptors (**Table 2.2**) (Civelli *et al.*, 1993; Lachowiczl and Sibley, 1997; Vallone *et al.*, 2000).

Receptor subfamily	D_1 – like		D_2 – like		
Receptor subtype	D_1	D_5	D_2	D_3	D_4
Amino acids	446	477	414/443 *	400	387
Introns in ORF	No	No	Yes	Yes	Yes
Effector pathways	↑ cAMP	↑ cAMP	↓ cAMP ↑↓ K^+ channel ↓ Ca^{2+} channel	↑↓ K^+ channel ↓ Ca^{2+} channel	↓ cAMP
Distribution	Caudate putamen Nucleus accumbens	Hippocampus Kidney	Caudate putamen Nucleus accumbens	Nucleus accumbens Hypothalamus	Frontal cortex Heart

Table 2.2: Properties of cloned dopamine receptor subtypes. Adapted from Lachowicz and Sibley (1997).

Despite dopaminergic ligands being easily distinguished between D_1-like ligands and D_2-like ligands, their discrimination as selective ligands to the different subfamilies members is a more complex process. For instance, when comparing binding affinities of several compounds for D_1 and D_5 receptors we verify that differences in the affinity values are minimal and in some cases even inexistent. A more heterogeneous scenery is found when comparing compounds affinities for each of the D_2-like subfamily members, with some compounds showing evident differences in their affinity values for different receptors, while for other compounds the identification of a target receptor is a harder process (Vallone *et al.*, 2000).

2.4 Data Mining and Machine Learning

Data mining has emerged to deal with huge datasets, extracting from them patterns and relationships (Hand *et al.*, 2001). In this field, machine learning algorithms are being used to discover knowledge from this large amount of information. These methods are based on the construction of algorithms that can learn from and data and make predictions on it. They can be classified as supervised or unsupervised learning methods. Supervised approaches assume that training examples are labeled by class labels, while unsupervised ones use unclassified examples (Mitchell, 1997). Machine learning has been of great importance in bioinformatics field, especially the supervised learning approaches. They have been used in genomics, proteomics, microarrays, systems biology, evolution and text mining (Larrañaga *et al.*, 2006). Although some of these techniques have become more popular than others, like artificial neural networks, support vector machines, Markov models, decision trees and random forests (Jensen and Bateman, 2011), other approaches have been also used with good results, like the Bayesian classifiers (Chinnasamy *et al.*, 2005; Wang *et al.*, 2007).

2.4.1 Naïve Bayes Classifier

Naïve Bayes Classifier is a supervised machine learning method based on applying Bayes' Theorem with the assumption of conditional independence between features (**Figure 2.4**). It is the simplest Bayesian classifier and it is applied as a conditional probability model to assign class labels to problem instances. For that, the model involves a learning step in which the conditional probabilities are estimated, counting the frequency of various data combinations within the training set. After this training step in a supervised learning setting, the model is then used to classify problem instances. Each instance is described by a combination of attribute values. When a new sample is presented, its attributes are used to classify it by obtaining the product of the probabilities for the individual features (Mitchel, 1997; Hand *et al.*, 2001).

Naïve Bayes is distinguished from other supervised learning methods by its assumption of independence between features. However, it has proved its usefulness as machine learning method in bioinformatics and chemoinformatics studies, performing well when predicting coupling between transmembrane domain receptors and G-proteins (Cao *et al.*, 2003) and also in the prediction of heterodimeric protein complexes (Maruyama, 2013).

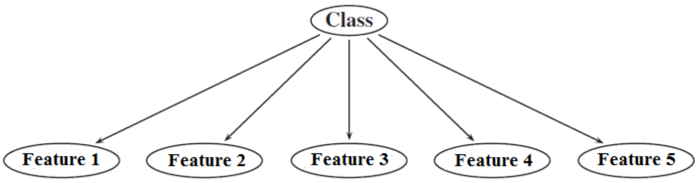


Figure 2.4: Structure of a Naïve Bayes Model.

2.5 Molecular Similarity

Similarity between molecules can be obtained based on chemical similarity, pharmacological similarity, therapeutic similarity and drug-drug interaction similarity (Mousavian and Masoudi-Nejad, 2014).

Chemical-based similarity takes into account the size of the shared substructures between two molecules and use that information to obtain a similarity measure, which can be done using 2D or 3D representations of the compounds (Nettles, 2006). Pharmacological-based similarity is computed by encoding each molecule in a binary vector, in which each element represents a pharmacological keyword. After, a correlation coefficient between vectors is calculated and the obtained value represents the similarity score (Yamanishi *et al.*, 2010). Alternatively, pharmacological-based similarity can be obtained by comparing the number of shared side effects between compounds (Kim *et al.*, 2013). The therapeutic-based similarity uses a hierarchical drug classification system, the Anatomical Therapeutical Chemical (ATC), to classify each drug with an ATC term and measures the similarity between compounds based on their corresponding ATC terms (Skrbo *et al.*, 2004). At last, the drug-drug interaction-based similarity uses a drug-drug interaction network to obtain the similarity between two drugs, calculating the shortest distance between them or obtaining it from the direct interaction between the two compounds (Kim *et al.*, 2013).

In addition to these similarity methods, other studies have used similarity based on features to compare different compounds. For that, each drug-target pair is represented as a vector of descriptors. In these methods, a set of features represent different properties of drugs and targets and allow drug-target interactions (DTIs) prediction by using the most discriminative descriptors (Cao *et al.*, 2012). Each drug, target or drug-target pair is characterized as a fixed-length vector representing the set of chosen features and this information is then used to infer DTIs in the genome scale (Mousavian and Masoudi-Nejad, 2014). Fingerprints are among the most used descriptors with the 2D structure of compounds being converted in a vector of bits by encoding (hashing) the presence or absence of a defined list of molecular substructures (Kuhn *et al.*, 2008).

2.5.1 NAMS

Non-contiguous Atom Matching Structural Similarity (NAMS) is a method used to obtain the structural similarity between molecules through atom alignment. In this method, an annotated molecular graph is created and an optimal atom alignment using pairwise matching algorithms is used to obtain the similarity measure between two molecules by comparing the bonding profiles of their atoms. For that, they are taken into account not only the topological profiles of molecules but also their atoms and bonds characteristics. Despite of a higher computational cost, this method has showed good results when distinguishing either different or very similar compounds, performing better than other most used similarity methods like the Fingerprint-based ones (Teixeira and Falcão, 2013).

2.6 Related Work

Target-based methods and ligand-based methods are two distinct *in silico* approaches that have been widely used in drug-target interaction (DTI) prediction studies. Target-based methods or docking simulation relies on receptors conformations and molecules chemical structures matches to predict DTIs. For that, a knowledge of target proteins' three dimensional conformations is required. However, this is not always possible once that little information about crystallized receptors is available. For instance, only a few number of GPCRs have already their structural conformation totally described (Ballesteros and Palczewski, 2001;

Klabunde and Hessler, 2002). Moreover, this type of simulation has shown not only to be time-consuming but also to require a lot of computational resources (Ding *et al.*, 2013). Despite docking strategies be powerful tools resulting in strong DTI predictions their applicability in a large-scale context is compromised by the existence of such limitations.

Contrariwise, ligand-based methods apply learning-based methods to DTI prediction by comparing a new ligand to known ligands of a target receptor. Contrarily to docking simulation, these approaches do not need a previous knowledge on proteins' three-dimensional (3D) structures to predict new interactions and can be applied even when we do not have information about the structural conformation of target receptors. On the other hand, ligand-based methods can be compromised by small numbers of known ligands once that it will result in inaccurate predictions (Mousavian and Masoudi-Nejad, 2014).

In this context, learning-based methods can be categorized into supervised and semi-supervised methods, with supervised learning methods subdivided into similarity-based methods and feature-based methods (Mousavian and Masoudi-Nejad, 2014). Supervised methods infer a model based on labeled training data and use it to infer true labels for unknown instances. For that, both known and unknown interactions between drugs and targets are considered as positive and negative samples, respectively (Cao *et al.*, 2014). However, the selection of unknown interactions as negative samples is taken as an inherent limitation since one does not know which of them represent in fact true interactions (Ding *et al.*, 2013). In order to face this problem, semi-supervised methods have been applied by using a small number of labeled data in conjunction with numerous unlabeled data (Mousavian and Masoudi-Nejad, 2014).

The underlying idea of learning-based methods is that similar drugs are likely to interact with similar receptors and DTIs prediction can be performed by using similarities among drugs, target receptors or both.

Many studies have been integrating different types of information in order to construct more robust DTIs prediction models. A method using a support vector machine algorithm based on chemical-based similarity for ligands and sequence-based similarity for proteins has been proposed by Jacob *et al.* (Jacob and Vert, 2008). A different approach adopted by Yamanishi *et al.* uses target sequences, drug chemical structures and the topology of a drug-target network not only to identify possible drug-target interactions but also to investigate which features become more relevant in DTIs prediction studies. For that, the authors have used a new method, the bipartite graph learning method (Yamanishi *et al.*, 2008). In another study, Yamanishi *et al.* proposed the use of an algorithm based on distance learning to investigate the relationship between the chemical space, the pharmacological space and the topology of drug-target network. The authors have showed that chemical structure similarity is less correlated with DTIs than pharmacological similarity (Yamanishi *et al.*, 2010). Another study using a kernel-based method and the information on therapeutic and pharmacological data have been proposed by Wang *et al.* (Wang *et al.*, 2011). More recently, a new approach integrating functional data with chemical data, genomic data, pharmacological data, and the topology of interaction networks has been developed by Yang *et al.*. In this study the authors have presented a probabilistic graphical model to more accurately predict missing interactions between drugs and targets (Yang *et al.*, 2014).

3. Material and Methods

3.1 Tools

The data relative to receptors' binding molecules and their bioactivity values were obtained by using the Python v.2.7.6 programming language through the use of *math*, *bioservices* and *numpy* libraries (Cokelaer *et al.*, 2013; van der Walt *et al.*, 2011).

All the analyses were performed using R v.3.2.1 programming language with the R studio as main interface. Moreover, all the graphics and tables present in this study were designed by using the available R tools.

3.2 Data Collection and Processing

All the data used in this study was collected from ChEMBL and Uniprot databases. First, a search for all the Serotonin and Dopamine receptors from human (*Homo sapiens*) was carried out and only those present at ChEMBL and for which binding molecules affinities information, in the form of inhibitory constant (K_i), was available were included in the dataset. In total, we ended with a dataset with 14 different serotonin receptors and 5 different dopamine receptors. For 5-HT$_{3B}$ serotonin receptor only 4 molecules binding affinities records for human were collected. Due to its lack of binding molecules information it was considered as part of the dataset but it was excluded from some posterior analysis.

In order to handle with outliers, a correction step was applied to records, from different studies, showing different binding affinity levels for the same receptor-molecule pair. For each case the following process was performed: (i) the mean and standard deviation for the registered values were calculated; (ii) all the values above or under the mean plus two times the standard deviation were excluded from the records; (iii) a new mean was calculated using only the remaining values and it was used as the final receptor-molecule pair binding affinity value. In total, we ended with 22690 receptor-molecule records corresponding to 11341 different molecules.

After, all the K_i values were converted into scaled pK_i (spK_i) values according to the following rules:

$$\text{If } K_i >= 10000, \text{sp}K_i = 0$$

$$\text{If } 10000 > K_i > 1, \text{sp}K_i = \frac{4 - \log_{10}(K_i)}{4}$$

$$\text{If } 1 \geq K_i, \text{sp}K_i = 1$$

(1)

where K_i is the inhibitory constant and spK_i represents the scaled pK_i.

3.3 Evolutionary Analysis

In total, 14 protein sequences of distinct serotonin receptors (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1F}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_{3A}$, 5-HT$_{3B}$, 5-HT4, 5-HT$_{5A}$, 5-HT6 and 5-HT7) belonging to 7 different subfamilies and 5 different ones of dopamine receptors (D$_1$, D$_2$, D$_3$, D$_4$ and D$_5$) belonging to the 2 existing subfamilies were collected from Uniprot in FASTA format. After, a ClustalW multiple alignment was carried out between all the protein sequences. With the obtained alignment a phylogenetic Neighbor-Joining Phylogenetic Tree was constructed using *MEGA* version 6 (Tamura *et al.*, 2013).

A sequence identity matrix was obtained using the same 19 protein sequences previously collected. For this purpose, the *seqinr* R package was used to read the ClustalW multiple alignment in FASTA format, comparing pairs of sequences and calculating their sequence identity value, scaled between 0 (no sequence identity) and 1 (total sequence identity)(Charif and Lobry, 2007). For a better visualization of the obtained results, a colour matrix integrating all the information was generated.

3.4 Molecular Similarity

The NAMS Webtool was used to obtain the structural similarities between the 11341 unique molecules based on the structural/topological relationships of their atoms. As way to reduce the required computational time of this process only molecules with less than 50% of difference between their molecular weights were compared since we expect that molecules with great differences in their molecular weight may not to be sufficiently similar to become relevant in our study.

3.5 The Probabilistic Model

The 11341 unique molecules were subdivided into validation set, with 2000 molecules, and training set, comprising the remaining 9341 molecules. Validation molecules were randomly selected from the set of all molecules and their records were removed from the receptors' original data files. Probabilistic models were constructed and trained with the training set molecules.

3.5.1 Receptors Database

Taken into account only the information relative to shared binding molecules between receptors and their respective bioactivity levels we have constructed a database storing the conditional probabilities of a certain molecule bind to a receptor given its active behavior towards other receptors. These probabilities were obtained for each pair of receptors by calculating the proportion between the number of active molecules for the first receptor which are also active for the second receptor and the total number of active molecules for the second receptor.

A second database was constructed to store the same probabilities but considering a non-active behavior for the second receptor. For that, the probabilities were calculated as the proportion between the number of active molecules for the first receptor which are non-actives for the second receptor and the total number of non-active molecules for the second receptor.

3.5.2 Kernels Selection

For each receptor a group of molecules from the training set was selected to represent kernel molecules due its discriminant role among all the molecules. Thus, kernels are expected to represent structurally distinct active molecules for a given receptor. The selection was made for each receptor according to the following process:

1. The molecules with spK_i value under 0.1 are discarded;
2. The molecule with the highest spK_i value is selected as the first kernel;
3. Structural similarities between the selected kernel and all the other molecules are computed by NAMS;
4. Molecules with similarities above 0.6 to the selected kernel are discarded;
5. The molecule with the highest spK_i value among the remaining molecules is selected as the new kernel;
6. Return to point 3 and end when there are no more molecules.

In total, they were selected 807 distinct kernel molecules with some of them being selected as kernel for more than one receptor. The D_2 dopamine receptor registered the highest number of kernels with 143 selected molecules. Otherwise, 5-HT_{1E}, 5-HT_{1F} and 5-HT_{3B} serotonin receptors showed the lowest numbers of kernels with only 5, 4, and 3 molecules being selected, respectively.

3.5.3 Kernels Database

All the binding molecules for each receptor were compared with the selected kernels for the respective receptors and they were grouped in a database based on their spK_i values for the receptor and their similarity to the kernels. For that, molecules were subdivided into actives, with spK_i $>$ 0.1, and less actives, with spK_i $<=$ 0.1, and they were classified according to their similarity to each kernel as S1, if they showed less than 60% of similarity, S2, if they showed between 60% and 80%

of similarity, or S3, if they had more than 80% of similarity to the kernel. This information was stored in a database as counts, which are on the basis of the probabilities used later during the classification process using the Naïve Bayes classifier.

3.5.4 Algorithm Implementation

We have developed three different models in order to predict drug-target interactions. The first model uses obtained information on structural similarities between molecules and their binding affinities to receptors. The second model is based on co-activity data between receptors and the third model was constructed as a combination of the first two models.

3.5.4.1 *Molecular Similarity vs Bioactivity Levels*

The first approach takes advantage on the information from structural similarity between molecules and their bioactivity values for the target receptors to compute the binding probability between a molecule and a receptor. The following process is applied to each molecule in order to predict its behavior with respect to the considered receptors:

1. The structural similarity between the molecule in test and all kernels is computed by NAMS;
2. Kernels with more than 50% of similarity to the test molecule are selected;
3. The selected kernels are grouped according to their respective receptors. They are selected for test the receptors for which at least 3 kernels were selected;
4. For each receptor under test, molecule binding probability is calculated based on a Naïve Bayes method using the structural similarities to the selected kernels as features:

I. For each kernel, the level of similarity to the test molecule is classified as S1, S2 or S3 (similarity under 60%, between 60% and 80% or more than 80%, respectively);

II. The conditional probability of the test molecule having the specified level of similarity to the kernel given an active behavior is calculated using the counts stored in the kernels database:

$$P(S_k = S_l \,/\, A) = \frac{\textit{number of active molecules to R with } S_k = S_l}{\textit{Total of active molecules to R}} \tag{2}$$

where S_l is the level of similarity to the kernel S_k, A represents an active behavior and R is the receptor under test.

III. The same process is applied to obtain the conditional probability given an non-active behavior:

$$P(S_k = S_l \,/\, NA) = \frac{\textit{number of non-active molecules to R with } S_k = S_l}{\textit{Total of non-active molecules to R}} \tag{3}$$

where S_l is the level of similarity to the kernel S_k, NA represents an non-active behavior and R is the receptor under test.

IV. The conditional probabilities for each kernel given an active behavior are multiplied as independent events to obtain the probability of the test molecule being active for the given receptor:

$$P(A) = \prod_{i=1}^{n} P(S_k = S_l \,/\, A) \tag{4}$$

where $P(A)$ is the probability of the test molecule being active and n represents the number of kernels for which they were obtained the conditional probabilities in equation 2.

V. The conditional probabilities given a non-active behavior are also multiplied to achieve the probability of the test molecule being non-active for the same receptor:

$$P(NA) = \prod_{i=1}^{n} P(S_k = S_l \,/\, NA) \tag{5}$$

where *P(NA)* is the probability of the test molecule being non-active and *n* represents the number of kernels for which they were obtained the conditional probabilities in equation 3.

VI. In order to adjust the final probabilities both are individually divided by the total of their sum.

3.5.4.2 *Integrating Receptors Information*

In an attempt to improve the model based uniquely on molecules similarity and bioactivity levels, the information relative to shared receptors was also integrated according to the following procedure:

1. For each molecule, they are selected the receptors for which there is previous knowledge about the molecule binding behavior. This information is obtained by consulting real data.
2. For each receptor in test during the classification process, the information relative to other receptors is included. Depending on the molecule behavior, one of the following proceedings is applied:

I. If the molecule is active for other receptor, the conditional probability of the molecule being active or non-active to the receptor in test given its active behavior for other receptor is extracted from the receptors database:

$$P(A_{R1}) = \prod_{i=1}^{a} P(A_{R1}/A_{R2}) \tag{6}$$

$$P(NA_{R1}) = \prod_{i=1}^{a} P(NA_{R1}/A_{R2})$$

(7)

where $P(A_{R1})$ and $P(NA_{R1})$ represent, respectively, the probability of the test molecule being active and non-active to a receptor *R1* as the product of the conditional probabilities of being active and non-active for *R1* given being active for other receptor *R2*. *a* represents the number of receptors for which it is known the test molecule is active.

II. If the molecule is non-active for other receptor, the same proceeding is applied but this time extracting the probability of the molecule to be active or non-active to the receptor in test given its non-active behavior for other receptor:

$$P(A_{R1}) = \prod_{i=1}^{na} P(A_{R1}/NA_{R2})$$

(8)

$$P(NA_{R1}) = \prod_{i=1}^{na} P(NA_{R1}/NA_{R2})$$

(9)

where $P(A_{R1})$ and $P(NA_{R1})$ represent, respectively, the probability of the test molecule being active and non-active to a receptor *R1* as the product of the conditional probabilities of being active and non-active for *R1* given being non-active for other receptor *R2*. *na* represents the number of receptors for which it is known the test molecule is non-active.

3. In the end, the final probabilities of the test molecule to be active or non-active to a certain receptor are given by multiplying the probabilities obtained through the kernel molecules (eqs 4 and 5) by the probabilities based on the molecule binding behavior with other receptors (eqs 6, 7, 8 and 9):

$$P(A_{R1}) = \prod_{i=1}^{n} P(S_k = S_l \,/\, A_{R1}) \times \prod_{i=1}^{a} P(A_{R1}/A_{R2}) \times \prod_{i=1}^{na} P(A_{R1}/NA_{R2}) \tag{10}$$

$$P(NA_{R1}) = \prod_{j=1}^{n} P(S_k = S_l \,/\, NA_{R1}) \times \prod_{j=1}^{a} P(NA_{R1}/A_{R2}) \times \prod_{j=1}^{na} P(NA_{R1}/NA_{R2}) \tag{11}$$

where $P(A_{R1})$ and $P(NA_{R1})$ are the probabilities of the test molecule being active and non-active for a receptor *R1* as the product of equations 4, 6, and 8 and 5, 7, and 9, respectively.

4. In order to adjust the final probabilities both are individually divided by the total of their sum.

3.5.4.3 *Receptors Role during Classification Process*

To better understand the impact of including information on known receptors during classification process, a new approach comprising only this kind of information was developed. For each test molecule, they were used only the conditional probabilities from the receptors database, depending on the molecule behavior with respect to other receptors, as previously described (eqs 6 – 9). Subsequently, the final probabilities of one molecule to be active or non-active to a receptor in test are obtained by multiplying only these probabilities as independent events.

3.5.5 Classification Process

Initially, it was defined a threshold of 50% to classify a molecule as active or non-active for a given receptor, which means that if a molecule has a final probability of being active above 0.5 it can be considered as active for the receptor under study. In opposition, a probability under 0.5 allows its classification as non-active.

Afterward, using the training set classification results, they were calculated the False Positive and False Negative rates, for each receptor, which were plotted as a function of the used classification thresholds. The used thresholds varied between 0% and 100% with an increment of 1%. Subsequently, it was selected, for each receptor, the threshold value for which the number of False Positives and Negatives were smaller and it was used as a new classification threshold.

3.6 Validation

Validation process was performed by classifying the 2000 molecules from the validation set. The classified molecules for each receptor were grouped and classified as True Positives (TP), True Negatives (TN), False Positives (FP) or False Negatives (FN) according to the real data.

3.7 Results Analysis

The validation results were analyzed by receptor through a confusion matrix. Moreover, Precision-Recall and ROC curves were also plotted for information on the quality of the model for each receptor. Both curves were obtained by varying the threshold used during the classification process.

For the Precision-Recall curve the values of Precision and Recall (or Sensitivity) were calculated as $\frac{TP}{TP+FP}$ and $\frac{TP}{TP+FN}$, respectively. For the ROC curve they were used the False Negative rate, $\frac{FN}{FN+TP}$, and the False Positive rate, $\frac{FP}{FP+TN}$ (Hand *et al.*, 2001).

In order to classify models performances they were also used specificity, $\frac{TN}{TN+FP}$, and accuracy, $\frac{TP+TN}{TP+TN+FP+FN}$, values (Powers, 2011).

4. Results and Discussion

4.1 Data Analysis

For the 19 Serotonin and Dopamine receptors, it was collected a total of 22690 receptor-molecule interactions records, corresponding to 11341 different molecules (see Appendix). D_2 receptor registered the highest number of measured K_is with information for a total of 4634 molecules. In opposition, 5-HT_{1E}, 5-HT_{1F} and 5-HT_{3B} serotonin receptors recorded the smallest numbers of records with only 86, 99 and 4 molecules' binding affinities being collected (**Figure 4.1**). Little information for the 5-HT_{3B} serotonin receptor was expected once that it represents only a 5-HT_3 receptor subunit (Nichols and Nichols, 2008). For this reason, it was excluded from the majority of the analysis.

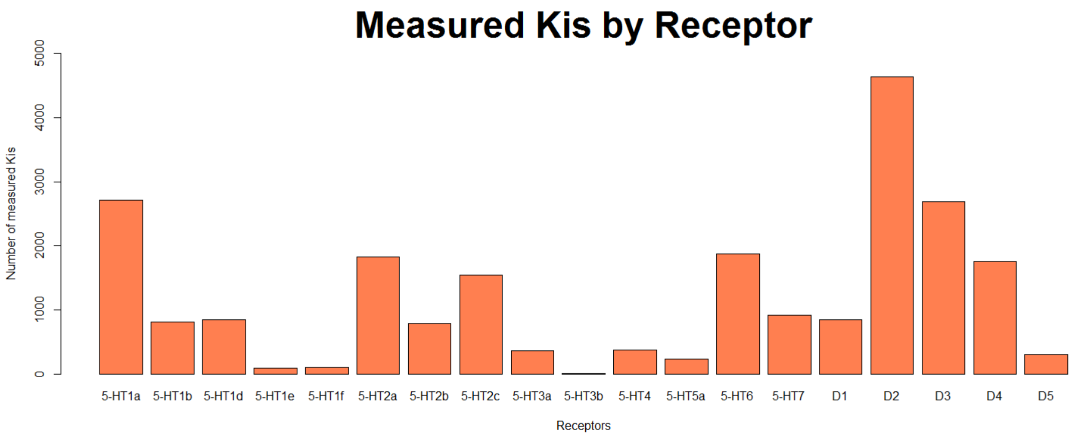


Figure 4.1: Number of molecular binding affinities measured by receptor. Data collected from ChEMBL database.

Through the observation of K_i values distribution, we have identified the presence of 4 peaks (around 1, 1000, 5000 and 10000 values) (**Figure 4.2**). We believe this occurrence is due to different criteria used during competitive radioligand binding studies. The interest in a specific molecule can vary with its binding affinity value once that smaller affinity values correspond to more active compounds. Therefore, when considering a threshold K_i value to distinguish between relevant and not so relevant compounds, different criteria can be used by different groups and during different studies. For this reason, we think these peak values correspond to 4 different thresholds. In fact, this can be a problem since we cannot know which values correspond to real K_i values and which ones are underestimates of the real values.

In this study, we have considered a threshold of $K_i = 5000$, whenever it was required. With respect to spK_i values, we have choosed a value of 0.1 ($K_i = 3981$). We consider these values a threshold to distinguish between active and non-active compounds.

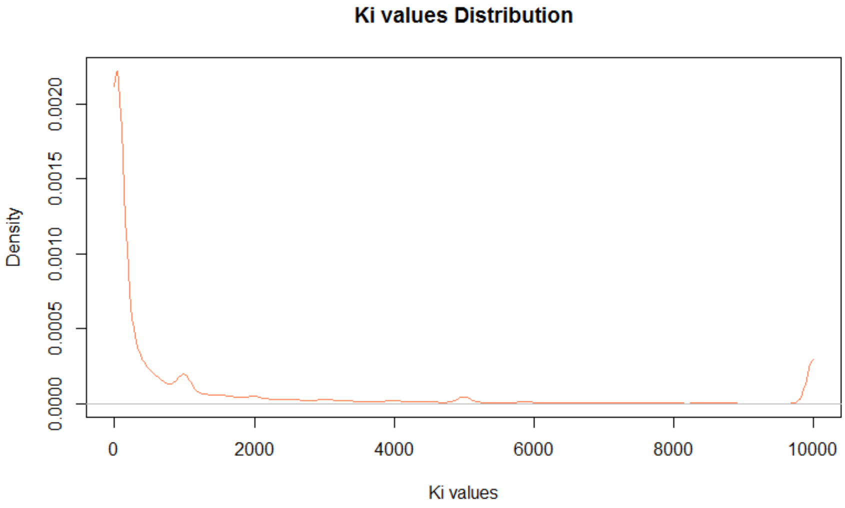


Figure 4.2: K_i values distribution.

Considering the same graphic, we can also observe a much higher quantity of K_i values under 1000, which indicates an overrepresentation of active molecules in our records. This occurrence is not a surprise once that our data comes from real studies and most of them tend to test molecules for which the suspicion of activity exists, either by being related to other active molecules or because they have already been tested for other similar receptors.

This overrepresentation can also be verified when observing the mean K_i and spK_i values by receptor (**Figures 4.3 and 4.4**). With the exception of the 5-HT_{1E} serotonin receptor, all receptors have a mean K_i value under 5000 and a spK_i value above 0.1. This overrepresentation is taken into account during the models implementation and posterior validation.

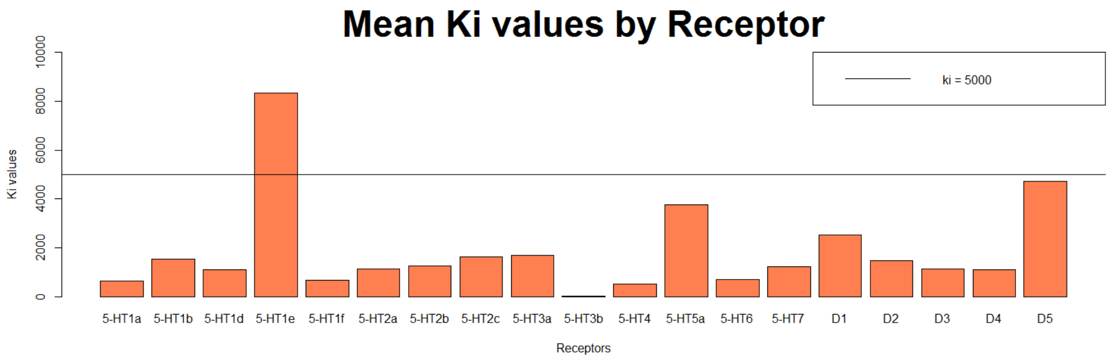


Figure 4.3: Mean K_i values by receptor.

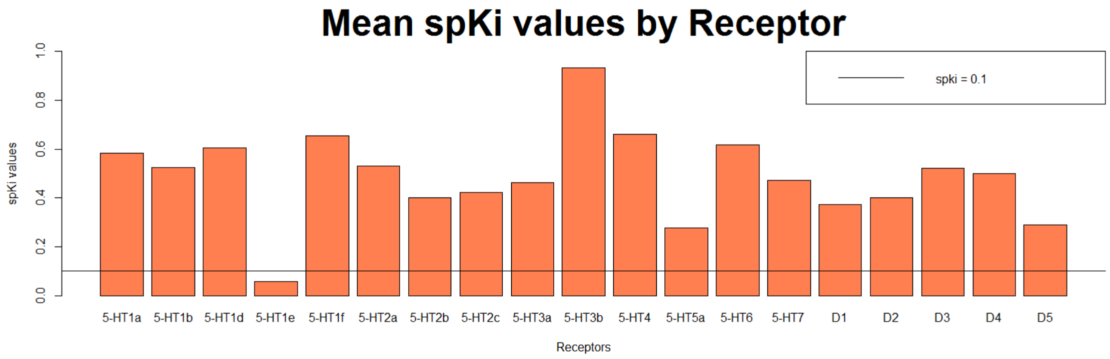


Figure 4.4: Mean spK_i values by receptor.

When analyzing the structural similarity values obtained through a pairwise comparison for all molecules present in our dataset, we verify that the majority of our similarity values are around 0.4914, which means that a large part of our compounds have about 50% of structural similarity to other compounds (**Figure 4.5**). In order to confirm the accuracy of these data, we have observed the distribution of 4950 structural similarity values obtained through the comparison of 100 molecules randomly selected from ChEMBL. What we found in this case was the majority of similarity values distributed around 40% (**Figure 4.5**).

We consider that the presence of a higher number of structurally similar molecules in our dataset is associated with the overrepresentation of active molecules. Following the assumption that similar compounds can share similar binding behaviors, we believe that a part of the many active molecules present in our dataset correspond to structurally similar compounds. This result highlights once more the presence of a bias in our data.

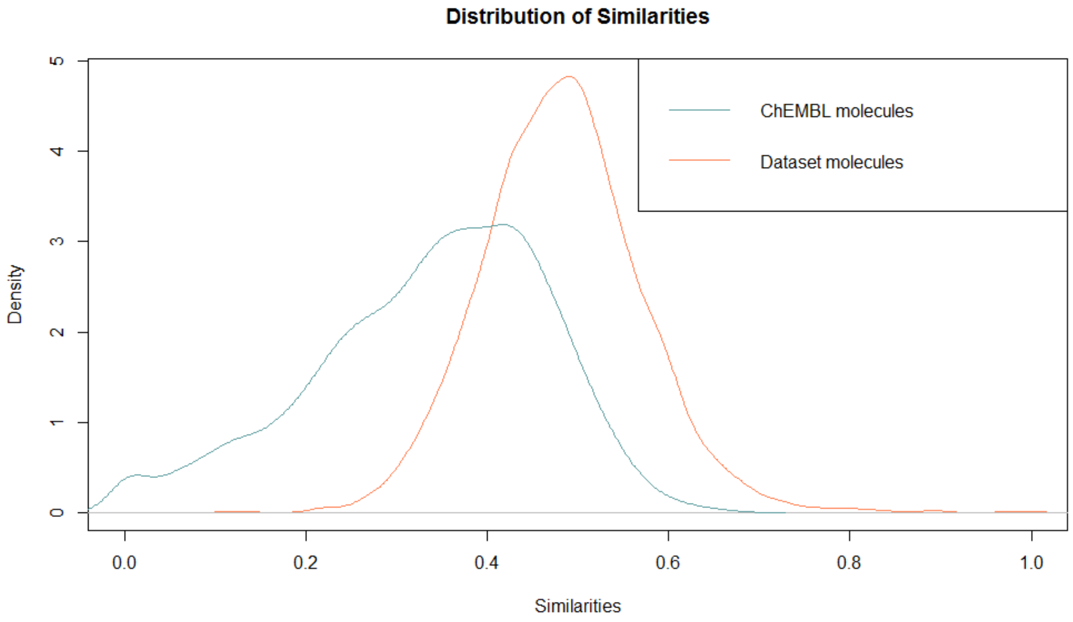


Figure 4.5: Comparison between the distributions of 5000 structural similarity values obtained through the comparison of dataset molecules and 4950 structural similarity values obtained through the comparison of 100 molecules randomly selected from ChEMBL database. Structural similarity values were obtained by using NAMS.

4.2 Evolutionary Analysis

A Neighbor-Joining phylogenetic tree and a sequence identity matrix allow to infer relationships between the serotonin and dopamine receptors under study. At first sight we can see that receptors are grouped by subfamily, as expected. However, they are notable the similarities of sequence between receptors not only from different subfamilies but also from distinct receptor families (**Figure 4.6, A and B**). Three pairs of receptors (5-HT$_{1D}$ and 5-HT$_{1B}$, 5-HT$_{2C}$ and D$_1$, and D$_3$ and D$_4$) were selected in order to infer a relation between their sequences similarity and the affinity values of their common binding molecules.

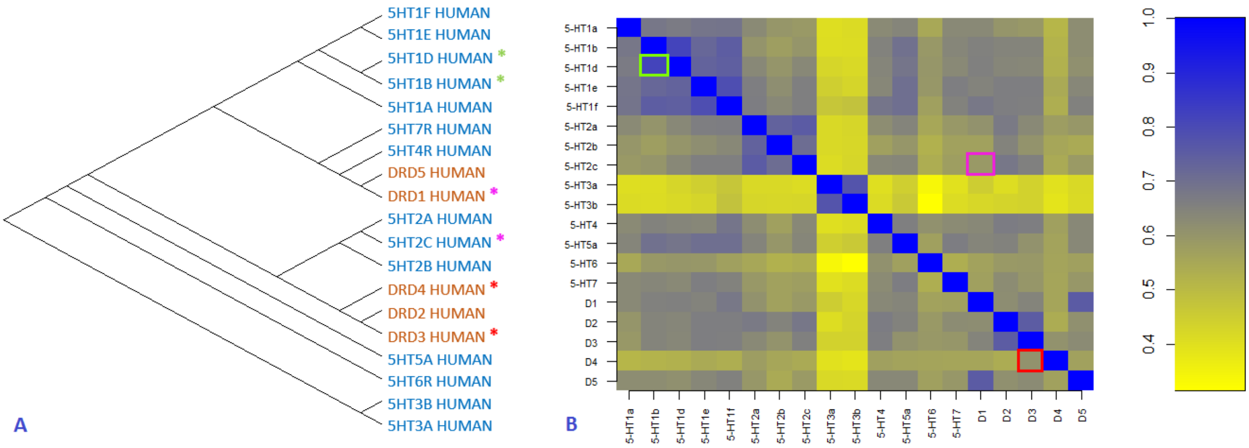


Figure 4.6. A) Neighbor-Joining phylogenetic tree for Serotonin and Dopamine receptors. B) Sequence identity matrix for Serotonin and Dopamine receptors, where yellow (0.0) means "0% of sequence identity" and blue (1.0) means "100% of sequence identity". The green, pink and red colours identify three pairs of analyzed receptors.

Are molecular' binding affinities related with sequence similarity between receptors?

When using a Spearman coefficient to compare binding profiles for the common molecules between pairs of receptors, we have found different patterns. For instance, some pairs have shown a high value of correlation between their molecules binding patterns e.g. (D1 and D5, $\rho = 0.8637$), while others have revealed almost a total independence between their molecules behaviors e.g. (D3 and D4, $\rho = 0.1558$). Considering the first case, we can think the high spearman coefficient value is related with the fact of both receptors belong to the same subfamily. This can be true. However, when analyzing the second case, despite both receptors belong to the same subfamily, an almost independent behavior was found between their molecules' binding profiles. These results show that molecules not always have similar binding behaviors even when considering evolutionary closed receptors. This conclusion is not a surprise since molecules have evolved to be receptor-specific.

Even so, we have tried to relate the levels of similarity between molecules' binding behaviors and their receptors' protein sequences. As result, we have found three distinct cases, corresponding to the three pairs of receptors previously identified (**Figure 4.6**).

For 5-HT$_{1B}$ and 5-HT$_{1D}$ serotonin receptors, we have found a strong relation between their molecules' binding affinities and their sequences similarity (**Figure 4.7, A1**). This would be considered as a normal case since it represents evolutionary closed receptors. When analyzing the results for the 5-HT2c and D1 receptors we have identified a strong relation between their molecules' binding behaviors, which do not have association with the level of similarity between receptors' protein sequences (**Figure 4.7, A2**). At last, for the two closely related receptors, D3 and D4, no relevant relation was found between their molecules' binding profiles, which have already been noticed before (**Figure 4.7, A3**).

Together, these results show that, contrary to what would be desired, no significant relationship exists between proteins sequences similarity and molecular binding patterns.

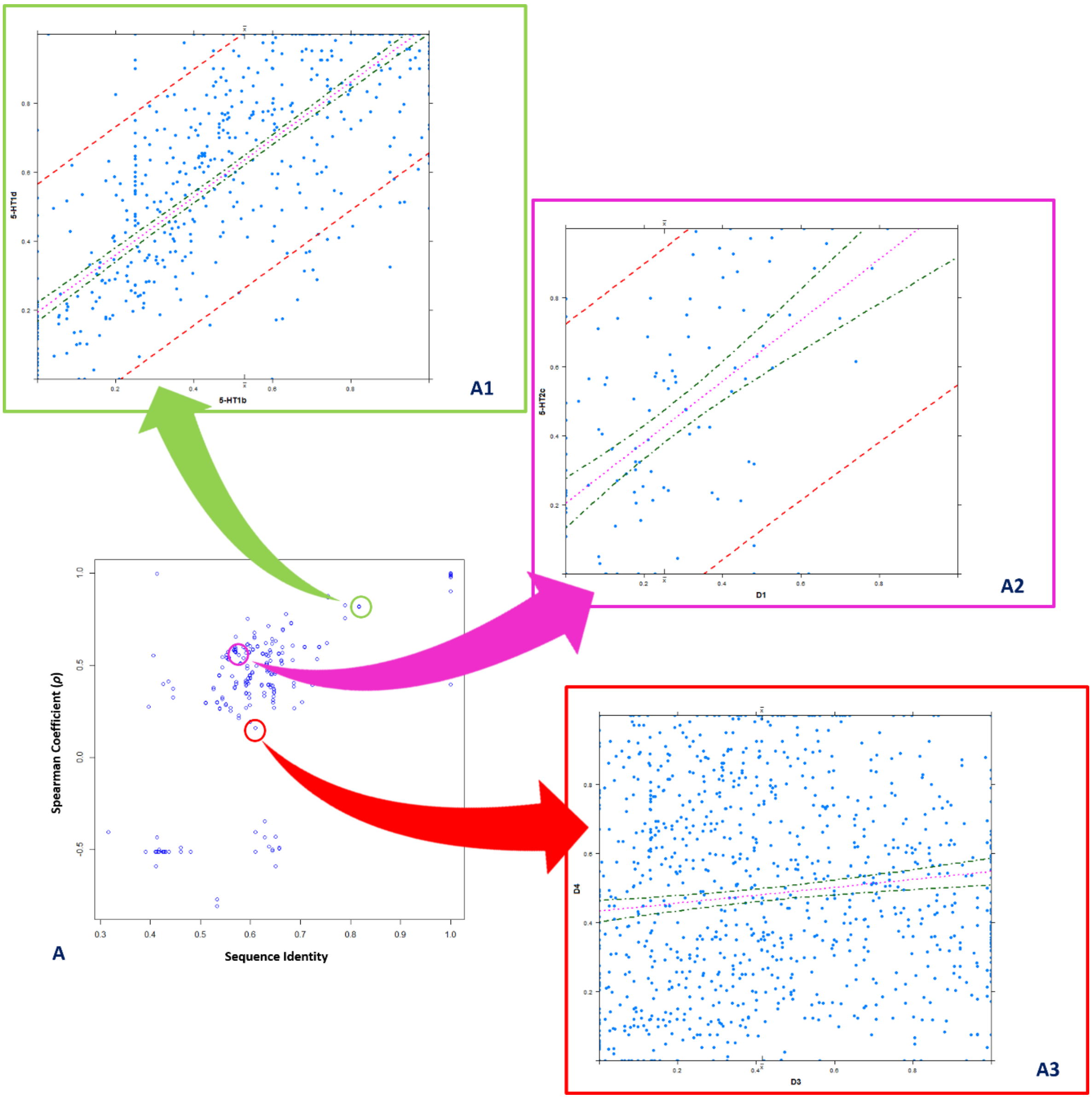


Figure 4.7: A) Relation between Sequence Identity and Spearman Coefficient (ρ) for molecules' binding affinities from each pair of pairwise compared receptors. Only the significant Spearman coefficient values were plotted. The different colours refer to the receptors pairs identified in Figure 4.6. A1) Biactivity values (spK_i) representation for the same binding molecules from 5-HT1b and 5-HT1d. A2) Biactivity values (spK_i) representation for the same binding molecules from 5-HT2c and D1. A3) Biactivity values (spK_i) representation for the same binding molecules from D3 and D4. Pink dotted lines: fitted line. Green dotted bounds: confidence interval. Red dotted bounds: Prediction interval.

4.3 Kernels Selection

A total of 807 distinct molecules were selected due their discriminant nature as kernel molecules (see Material and Methods, section 3.5.2). From these, 308 were selected as kernel molecules for more than one receptor, which means they have high binding affinities for more than one receptor. Possibly, this happens as consequence of similar receptors or as a result of a low binding specificity of these molecules. The D_2 dopamine receptor has registered the highest number of kernels with 143 selected molecules, which makes sense since it is the receptor with the highest number of records. Otherwise, 5-HT_{1E}, 5-HT_{1F} and 5-HT_{3B} serotonin receptors have shown the lowest numbers of kernels with only 5, 4, and 3 molecules being selected, respectively (**Figure 4.8**).

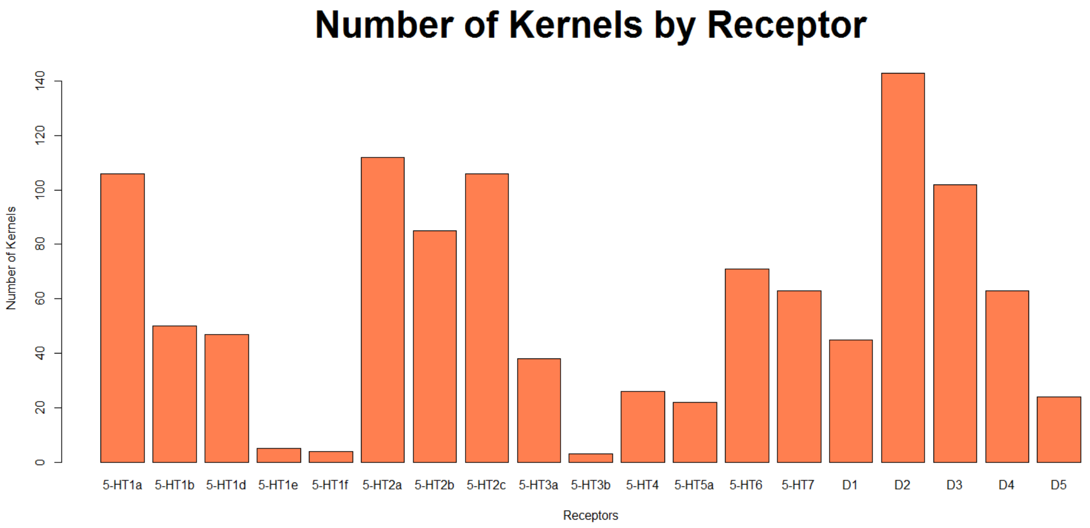


Figure 4.8: Number of selected kernel molecules by receptor.

Analyzing the distribution of the similarity values between kernels and also their bioactivity values for the respective receptors, we verify that the majority of the selected kernels have values of similarity between them around 0.43 and their K_i values are near 59.35. These results confirm that our selection of kernels fulfills the criterion of being distinct active molecules for each receptor (**Figures 4.9 and 4.10**).

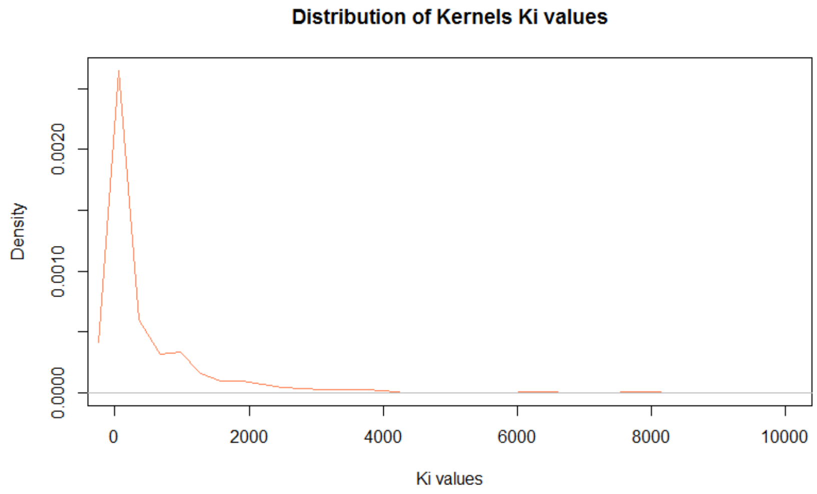


Figure 4.9: Distribution of kernel molecules *K*i values. The *K*i values were obtained for the respective receptors.

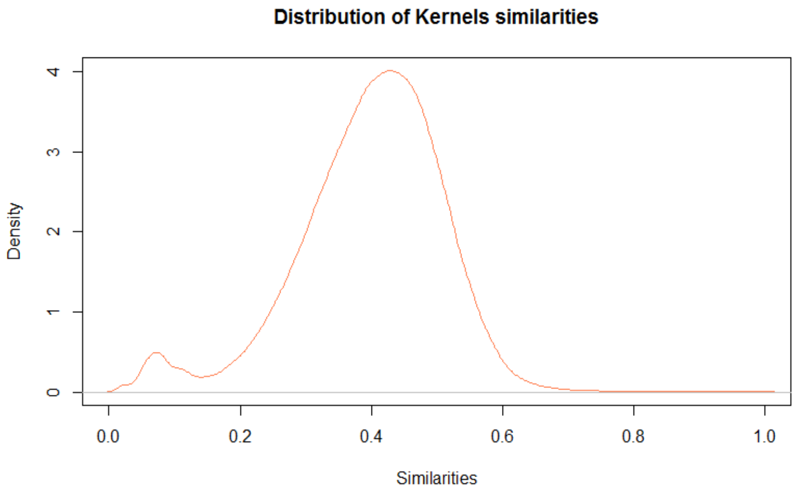


Figure 4.10: Distribution of structural similarities between kernels. The structural similarities were obtained by using NAMS.

4.4 Validation Results

After classification of the 2000 molecules from the validation set, they were only considered for validation analysis the receptors for which they were classified at least 15 molecules. Due this criterion, they were excluded from validation analysis of Similarity vs Bioactivity levels (SvsBl) model the 5-HT$_{1E}$, 5-HT$_{1F}$, 5-HT$_{3A}$, 5-HT$_{3B}$, 5-HT$_{4}$, and 5-HT$_{5A}$ serotonin receptors.

The classified molecules for each receptor were grouped as True Positives (TP), True Negatives (TN), False Positives (FP) or False Negatives (FN) according to real information.

4.4.1 Models Performances

First model, considering only molecules' structural similarities and bioactivity levels (SvsBl model), has shown a mean percentage of correctly classified molecules (TP + TN) around 80.59%.

5-HT_{1D} serotonin receptor has recorded the highest number of correctly classified molecules, with the model obtaining an accuracy of 92%. In contrast, the smallest number of true predictions was recorded by 5-HT_{2C} serotonin receptor, for which the model has registered an accuracy value of 62.22% (**Figure 4.11**). Taking into account the results for all receptors, the model has registered an overall accuracy of 77.37%. For the sensitivity (hit rate or recall), specificity (true negative rate) and precision (positive predictive value), they were obtained values of 78.93%, 62.76% and 95.21%, respectively (**Table 4.1**).

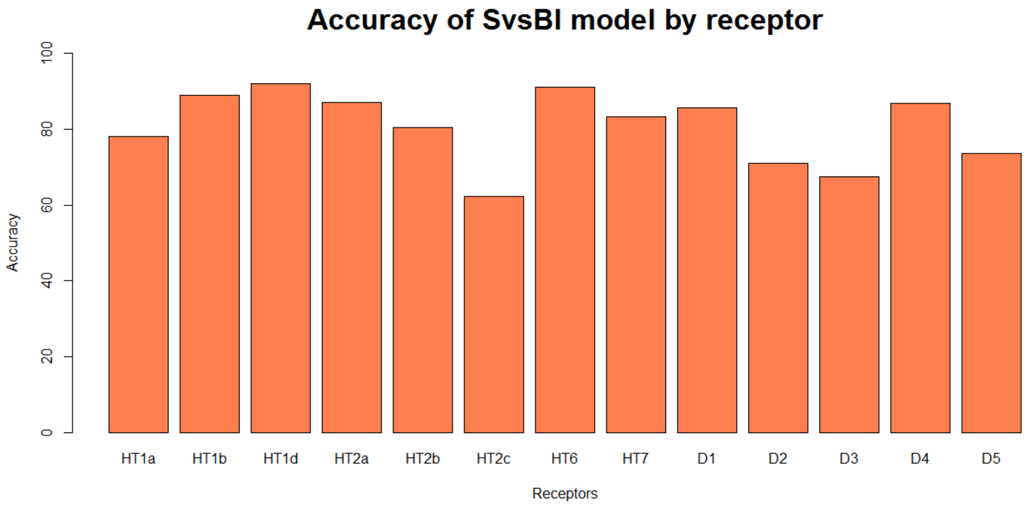


Figure 4.11: Accuracy of Similarity vs Bioactivity levels (SvsBl) model for each receptor.

Considering the model including only the information relative to shared receptors (R model), the mean percentage of correctly classified molecules was around 87.17%, 6.58% more than when using only the information relative to molecular similarity and bioactivity levels.

The highest level of accuracy was obtained for the 5-HT$_{1B}$ serotonin receptor (93.10%), while the D$_5$ dopamine receptor has registered the smallest number of correctly classified molecules (80.43%) (**Figure 4.12**).

At overall level, R model has obtained an accuracy of 89.05%, 11.68% more than SvsBl model. Its values of sensitivity, specificity and precision were 98.45%, 31.4% and 89.8%, respectively (**Table 4.1**).

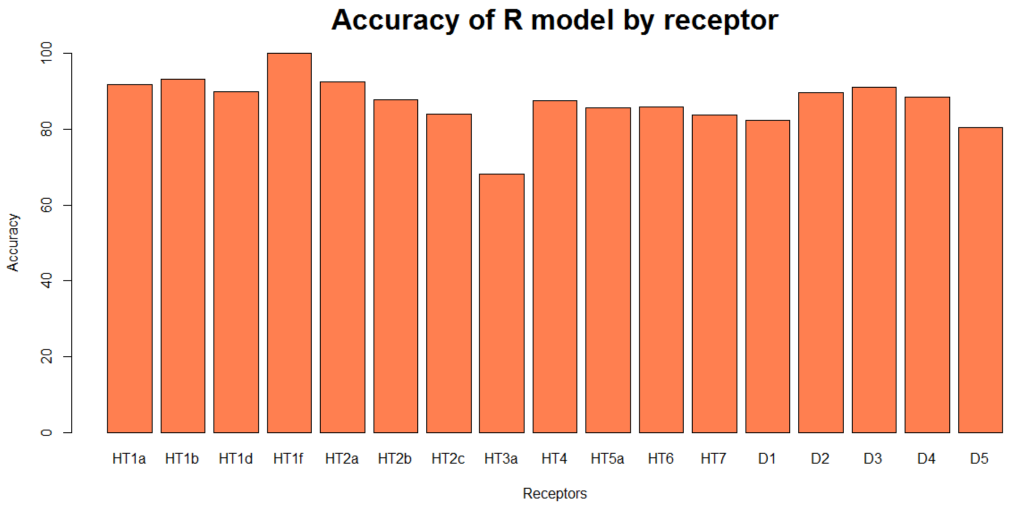


Figure 4.12: Accuracy of Receptors (R) model for each receptor.

After integrating information relative to structural similarity between molecules, bioactivity levels and shared receptors (SvsBl+R model), the mean percentage of correctly classified molecules was around 90.39%.

The receptor for which this model has registered the highest accuracy level was the 5-HT$_6$ serotonin receptor with 97.44% of the molecules correctly classified. For the D$_5$ dopamine receptor it was registered the smallest number of correctly classified molecules (84.21%), which also happened when using only the information relative to shared receptors (**Figure 4.13**).

Analyzing the overall performance after integrate both SvsBl and R models, it was achieved a global accuracy level of 90.36%, while sensitivity, specificity and precision registered values of 95.54%, 41.84% and 93.90%, respectively (**Table 4.1**).

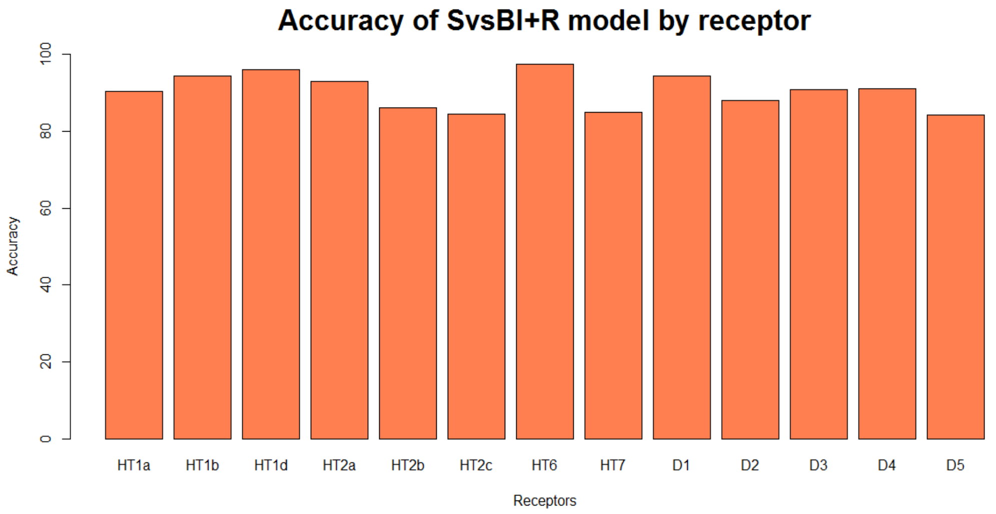


Figure 4.13: Accuracy of Similarity vs Bioactivity levels plus Receptors (SvsBl+R) model for each receptor.

4.4.2 Comparing Models Performances

Comparing the performances of the three models by receptor, we can conclude that Similarity vs Bioactivity levels plus Receptors (SvsBl+R) model has achieved the best results, with accuracy levels being increased for almost all the receptors. The 5-HT$_{1A}$ and 5-HT$_{2B}$ serotonin receptors and the D$_2$ and D$_3$ dopamine receptors were the exception, with Receptors (R) model achieving higher accuracy values for these cases. At global level, Similarity vs Bioactivity levels (SvsBl) model has obtained the worst performance, overcoming R model results for only 3 receptors (5-HT$_{1D}$, 5-HT$_6$ and D$_1$) (**Figure 4.14**).

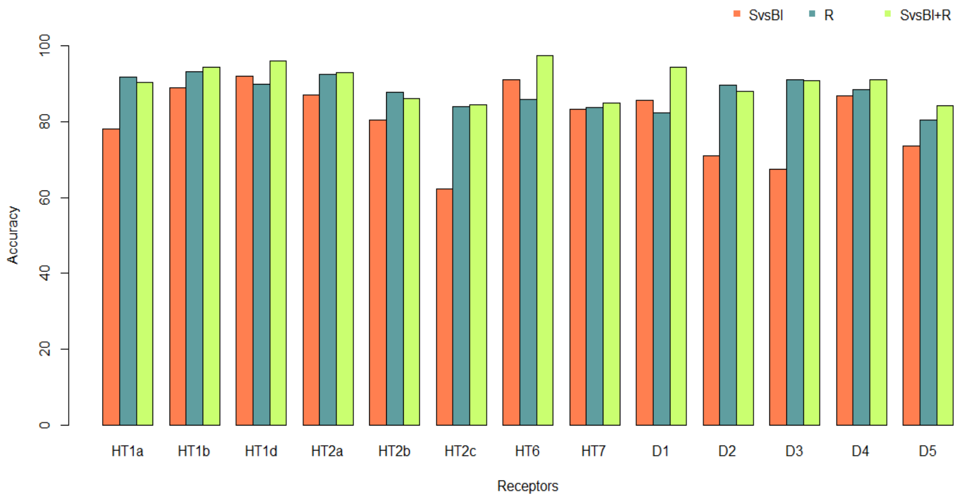


Figure 4.14: Comparison of accuracy levels for each receptor and applied model. SvsBl: Similarity vs Bioactivity levels model; R: Receptors model; SvsBl+R: Similarity vs Bioactivity levels plus Receptors model.

SvsBl model has registered the lowest sensitivity value due to a higher quantity of False Negatives (FN), when compared with the other models. However, it has also achieved the highest values of specificity and precision, showing to be the model with the highest proportion of correctly identified negatives and also with the largest proportion of positives that correspond to true observations (**Table 4.1**). Based on these results, the SvsBl model appears to be the most conservative one.

The highest sensitivity value (98.45%) was registered by the R model, which means it had the best performance in identifying True Positives (TP) from all the true observations. This result is associated with a decreasing in the number of False Negatives (FN). However, a classic trade-off is observed since the reduction in the number of FN was accompanied by an increasing in the number of False Positives (FP). This increase in the number of FP has resulted in the smallest values of specificity and precision for R model (**Table 4.1**). This higher number of FP can be associated with the bias present in our dataset since the overrepresentation of active molecules for each receptor may have led to the overestimation of the conditional probabilities on the basis of this model.

The SvsBl+R model registered the highest accuracy level (90.36%), followed by R model (89.05%) and SvsBl model (77.37%). Results show that the joint model obtained intermediate values of sensitivity, specificity and precision when compared with the individual ones. These values can be explained by the contribution of both SvsBl and R models to the final results (**Table 4.1**). The number of FN is reduced due to integration of information relative to shared receptors, while the decrease in the number of FP is associated with the integration of information on molecules' structural similarities and their bioactivity levels.

	Accuracy	Sensitivity	Specificity	Precision
SvsBl	77.37%	78.93%	62.76%	95.21%
R	89.05%	98.45%	31.4%	89.8%
SvsBl + R	90.36%	95.54%	41.84%	93.90%

Table 4.1: Overall values of accuracy, sensitivity, specificity and precision for the proposed models. SvsBl: Similarity vs Bioactivity levels model; R: Receptors model; SvsBl+R: Similarity vs Bioactivity levels plus Receptors model.

At overall level, SvsBl+R model seems to be the most accurate model. Moreover, it appears to attenuate the trade-off between FP and FN, which improve its reliability as prediction model. This model would be the best option to scan a set of molecules and find potential candidates to be further validated *in vitro*. The SvsBl model had more precise results and despite it produces a higher quantity of FN, it can be used as a conservative model to, for example, confirm new molecule-receptor interactions. R model must be used carefully due to the possible bias in the estimation of its conditional probabilities.

4.4.3 Impact of Classification Threshold in Models Performance

We have decided to investigate whether classification threshold influences the performance of the models and in which way it can vary by receptor. We expect more specific receptors to have higher threshold values since the probability of a molecule bind to them is lower, and vice-versa. With this purpose, training set molecules were classified by the three presented models. For each receptor and model, the optimum classification threshold was selected as the value for which the false positive and negative rates values were smaller (**Table 4.2**).

	5-HT1A	5-HT1B	5-HT1D	5-HT2A	5-HT2B	5-HT2C	5-HT6	5-HT7	D1	D2	D3	D4	D5
SvsBl	0.55	0.82	0.9	0.64	0.64	0.15	0.99	0.73	0.54	0.58	0.4	0.95	0.84
R	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SvsBl+R	0.99	0.99	0.99	0.99	0.99	0.94	0.99	0.99	0.93	0.98	0.99	0.99	0.31

Table 4.2: Classification thresholds obtained for each receptor-model pair, through the classification of training set molecules.

We verified that for all the three models the results were worst after using the calculated classification thresholds, instead of the previously used 0.5 threshold. With a few exceptions, all the accuracy levels have stayed under the ones obtained when using the 0.5 value as classification threshold (**Figure 4.15**).

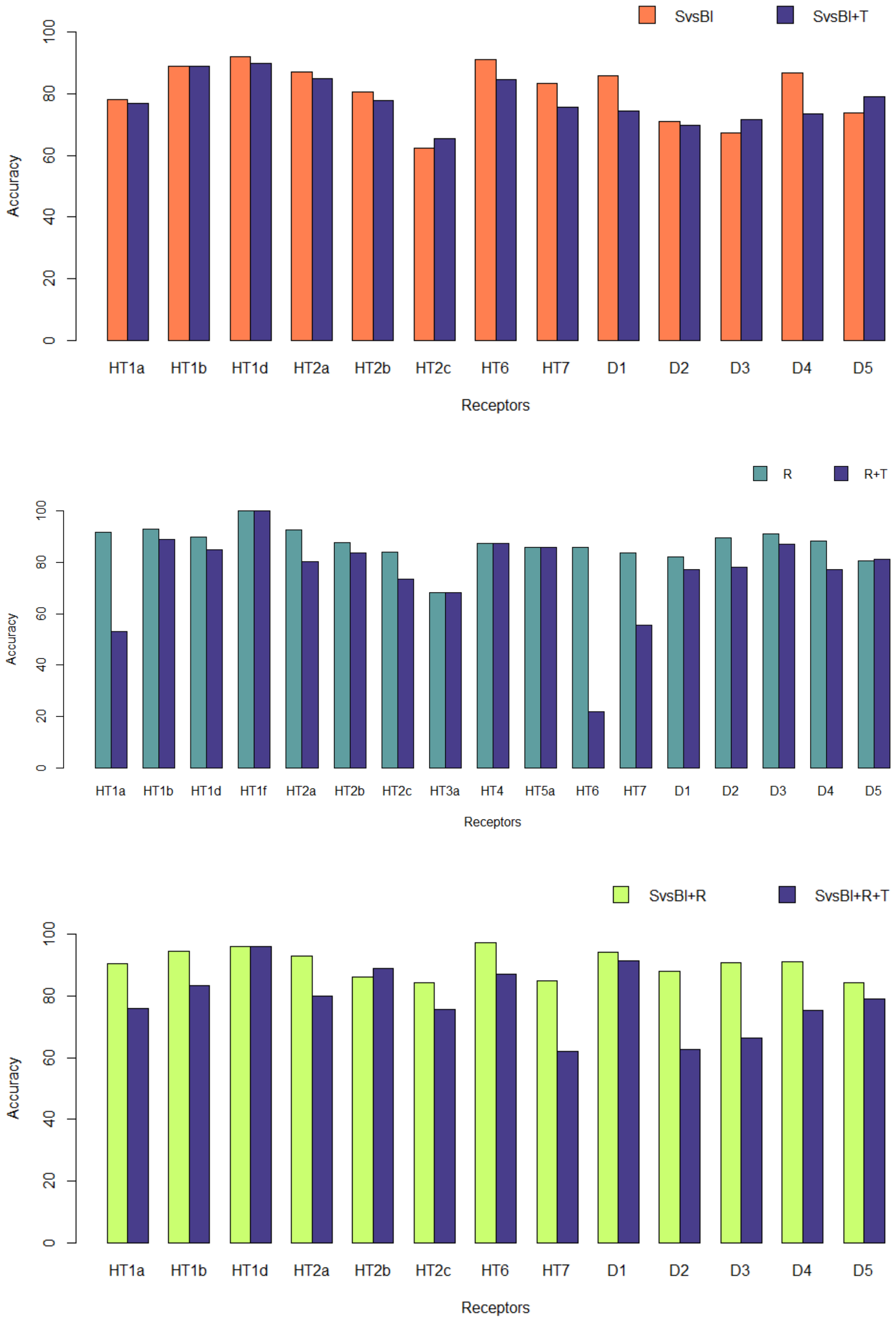


Figure 4.15: Comparison between accuracy levels for each receptor and model before and after applying the classification thresholds. SvsBl: Similarity vs Bioactivity levels model; R: Receptors model; SvsBl+R: Similarity vs Bioactivity levels plus Receptors model. SvsBl + T: Similarity vs Bioactivity levels model plus thresholds; R +

T: Receptors model plus thresholds; SvsBl+R+T: Similarity vs Bioactivity levels plus Receptors model plus thresholds.

At overall level, accuracy levels for the three models have decreased with the variation of the classification thresholds. However, it was registered an increase in the specificity values (**Table 4.3**). This can be justified by the decreasing in the number of FP and, subsequently, by the increasing in the number of TN. A slightly increase in the values of precision was also verified, once more due to decreasing in the number of FP (**Table 4.3**). In fact, these results make sense once that after applying adjusted classification thresholds, we expect to increase the specificity of our models.

	Accuracy	Sensitivity	Specificity	Precision
SvsBl + T	75.06%	75.56%	70.41%	95.99%
R + T	72.05%	76.56%	41.52%	89.86%
SvsBl + R + T	72.70%	72.84%	71.43%	95.98%

Table 4.3: Overall values of accuracy, sensitivity, specificity and precision for the proposed models when adjusted the classification thresholds. SvsBl+T: Similarity vs Bioactivity levels model plus thresholds; R+T: Receptors model plus thresholds; SvsBl+R+T: Similarity vs Bioactivity levels plus Receptors model plus thresholds.

At global level, prediction results were not much worse after applying the classification thresholdss. However, comparing with results when using the 0.5 threshold value, we can verify that the performance of our models is diminished by the adjustment of the classification thresholds.

5. Conclusions and Future Perspectives

In this work, we have proposed three drug-target interactions prediction models. First model integrates information relative to structural similarity between molecules and their binding affinities for known serotonin and dopamine receptors. Despite it has shown promising results during validation process, its greatest amount of false negatives makes it a more conservative model, indicated to validate specific drug-target interactions. It must be also referred that molecules with low values of structural similarity to the selected kernels cannot be classified using this model, which limits its spectrum of action in molecular universe.

The second proposed model was constructed based on the information relative to common molecules between receptors and their binding profiles. Individually, this model achieved better results than the first one. However, a bias in our dataset due to overrepresentation of active molecules was identified and we suspect it is the origin of the good results presented by this model. Moreover, this model can only be applied to identify binding profiles of molecules for which experimentally verified data with respect to other receptors exists. Although this model must be used carefully, we believe this information can have a relevant role in the prediction of drug-target interactions. For this reason, we have integrated the information from the two initial models to construct a third model.

Our third model, integrating all the information, has achieved the best results during validation process. It has shown not only to be the most accurate model but also the one with the best proportion between false positives and false negatives. We believe this model would be the best option to scan a set of molecules and find potential candidates to *in vitro* validation. Nonetheless, it must be remembered this model also share the limitations associated to the individual models, which reduces its applicability to molecular universe.

To increase the performance of our models, we have tried to identify the best classification threshold for each receptor. Better specificity and precision values for the three models were obtained with the variation of the classification thresholds. However, at overall level, the performance of our models was diminished by this variations, showing that the process of identification of classification thresholds needs some adjustments.

We believe that to improve our models performance, we should be able to use a dataset without bias. In fact, a more realistic scenario would be obtained with the overrepresentation of non-active molecules for each receptor.

With the present work we show that Naïve Bayes classifier can be a good option to construct models able to predict new drug-target interactions. At the same time, it is demonstrated that structural similarity between molecules together with bioactivity values can be enough to predict new interactions.

Our evolutionary analysis show that different molecular binding patterns not always are associated with similarity between receptors' protein sequences. However, some patterns have emerged and further studies must be conducted in order to identify the main factors behind these binding profiles.

Together, our results show that despite polypharmacology complexity, the integration of heterogeneous data and the use of machine learning approaches are inexpensive and reliable solutions for drug discovery.

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Appendix

Summary of collected data for each receptor.

Serotonin Receptors (*Homo sapiens*)			
Receptor	**ChEMBL Accession Number**	**Uniprot Accession Number**	**Total Binding Molecules**
5-HT1a	CHEMBL214	P08908	2715
5-HT1b	CHEMBL1898	P28222	811
5-HT1d	CHEMBL1983	P28221	840
5-HT1e	CHEMBL2182	P28566	86
5-HT1f	CHEMBL1805	P30939	99
5-HT2a	CHEMBL224	P28223	1823
5-HT2b	CHEMBL1833	P41595	783
5-HT2c	CHEMBL225	P28335	1546
5-HT3a	CHEMBL1899	P46098	357
5-HT3b	CHEMBL3895	O95264	4
5-HT4	CHEMBL1875	Q13639	368
5-HT5a	CHEMBL3426	P47898	234
5-HT6	CHEMBL3371	P50406	1870
5-HT7	CHEMBL3155	P34969	918
Dopamine Receptors (*Homo sapiens*)			
Receptor	**ChEMBL Accession Number**	**Uniprot Accession Number**	**Total Binding Molecules**
D1	CHEMBL2056	P21728	851
D2	CHEMBL217	P14416	4634
D3	CHEMBL234	P35462	2691
D4	CHEMBL219	P21917	1756
D5	CHEMBL1850	P21918	304