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Computational prediction of formulation strategies for beyond-rule-of-5 compounds☆

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ABSTRACT

The physicochemical properties of some contemporary drug candidates are moving towards higher molecular weight, and coincidentally also higher lipophilicity in the quest for biological selectivity and specificity. These physicochemical properties move the compounds towards beyond rule-of-5 (B-r-o-5) chemical space and often result in lower water solubility. For such B-r-o-5 compounds non-traditional delivery strategies (i.e. those other than conventional tablet and capsule formulations) typically are required to achieve adequate exposure after oral administration. In this review, we present the current status of computational tools for prediction of intestinal drug absorption, models for prediction of the most suitable formulation strategies for B-r-o-5 compounds and models to obtain an enhanced understanding of the interplay between drug, formulation and physiological environment. In silico models are able to identify the likely molecular basis for low solubility in physiologically relevant fluids such as gastric and intestinal fluids. With this baseline information, a formulation scientist can, at an early stage, evaluate different orally administered, enabling formulation strategies. Recent computational models have emerged that predict glass-forming ability and crystallisation tendency and therefore the potential utility of amorphous solid dispersion formulations. Further, computational models of loading capacity in lipids, and therefore the potential for formulation as a lipid-based formulation, are now available. Whilst such tools are useful for rapid identification of suitable formulation strategies, they do not reveal drug localisation and molecular interaction patterns between drug and excipients. For the latter, Molecular Dynamics simulations provide an insight into the interplay between drug, formulation and intestinal fluid. These different computational approaches are reviewed. Additionally, we analyse the molecular requirements of different targets, since these can provide an early signal that enabling formulation strategies will be required. Based on the analysis we conclude that computational biopharmaceutical profiling can be used to identify where non-conventional gateways, such as prediction of 'formulate-ability' during lead optimisation and early development stages, are important and may ultimately increase the number of orally tractable contemporary targets.

Contents

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1. Introduction

An increasing number of discovery biology frameworks appear to require highly lipophilic drug candidates to attain reasonable potency and selectivity. Such targets include those within lipid metabolic pathways where the natural ligands are lipids [1,2], targets within neurotransmitter pathways where the endogenous agonists are highly lipophilic [3], and anatomical targets where access to them requires lipid transport pathways [4], and/or partitioning into deep intracellular or nuclear targets [5]. Typically, highly lipophilic compounds exhibit poor solubility in water, and it is estimated that 40% to 70% of current drug candidates have sufficiently poor aqueous solubility that complete absorption from the intestine is compromised [6,7]. As oral dosage forms are the most convenient for patients, new medicines usually include oral delivery as part of their target product profile. This is in particular true when the disease is chronic and requires long term, or even life-long, treatment.

Computational tools based on calculations from molecular structure are available to facilitate the rational development of hits, leads and drug candidates with favourable physicochemical profiles for oral absorption [8–11]. These tools assist medicinal chemists in designing molecules with acceptable absorption, distribution, metabolism, elimination and toxicity (ADMET) properties. Nevertheless, the current drug discovery and clinical pipeline is still dominated by small molecules that often have problems with absorption and adequate drug exposure. Indeed, the trend is towards larger molecular weight and more lipophilic drug molecules that are even less well predisposed to good oral absorption. This trend has been attributed to the intrinsic biology of the target [5,12–14], the nature of the chemistry used to develop libraries [12,15,16], and organisational factors such as screening mechanisms, decision gates, experience and historical libraries [16,17].

Lipophilic ligands pose challenges at all ADMET levels. A recent analysis of the attrition rate of drug candidates highlights the need to better understand non-clinical toxicity, i.e. toxicity identified before clinical trials. Of 356 compounds from four major pharmaceutical companies, non-clinical toxicity was responsible for 59% of compound attrition. In contrast, only 2.8% of the pre-clinical attrition of candidate drugs was due to pharmacokinetics (PK), bioavailability and formulation. This number increases, however, after moving into the clinic i.e., Phase I–III clinical studies. Clinical safety is still responsible for the highest fraction of attrition (25%) in Phase I, but PK, bioavailability and formulation account for 17% of the attritions [13]. Failures in obtaining good oral exposure – stemming from poor formulation performance, absorption and other PK related factors – tend to be identified late, with costly termination of the projects as a result.

Targets are broadly evaluated for their 'druggability', a term generally defined as the likelihood of being able to functionally modulate a target through interaction with a small molecule or biological ligand [18–20]. However, methods that more specifically determine whether the target is tractable from the perspective of a (readily) orally delivered drug product are also required but are not currently explicitly available. In this review we present the use of in silico tools for prediction of absorption barriers to orally administered drugs with focus on solubility in intestinal fluids (S) and permeability (P_{app}). For those compounds where solubility is the main limiting factor, we present recent in silico developments for assessment of the molecular basis for this poor solubility; this information can then guide the selection of formulation strategy. We also present a computational approach that combines biopharmaceutical performance of the active pharmaceutical ingredient (API) with its likely 'formulate-ability'. The concept underpinning this approach is that such an analysis may make it possible to identify drug targets for which traditional delivery technologies are likely to result in good absorption. More interestingly, it will flag targets with future needs for non-standard, enabling formulations to provide acceptable exposure after oral administration of e.g., highly lipophilic, poorly water-soluble drug candidates. There is a diverse range of enabling formulation strategies that can be employed; herein we present, in detail, computational approaches to predict i) solid-state transformations to indicate the potential use of the amorphous form to improve drug absorption and ii) solubility in non-aqueous vehicles, to indicate the potential utility of lipid-based formulations (LBFs).

2. Understanding the target biology

To develop target-relevant decision gates to guide lead optimisation (also discussed in Section 7) acknowledgement of the influence of the inherent biology of the target on druggability is increasingly important [5,21]. Several tools that identify target druggability have been proposed [18–20,22]. These are mainly suggested as a means to identify new druggable binding sites, which are typically defined as chemical target regions in which the binding pocket may be occupied by compounds that comply with the rule-of-5. Although this approach may change the target biology used in certain therapeutic areas, and result in the development of new compound libraries with more drug-like properties, other targets will likely remain in the 'non-druggable', beyond rule-of-5 (B-r-o-5), chemical space. This space is typically occupied by highly lipophilic ligands to e.g. intracellular targets such as nuclear receptors including the Farnesoid X Receptor (FXR) and retinoic acid receptor (RAR), or larger, hydrophilic ligands within the chemical space occupied by e.g. peptides, antibiotics and macrocycles. The latter are described in detail by Matsson et al. [23].

The druggability of the target can be identified computationally by defining the pocket with geometric criteria and using molecular descriptors [22]. If the analysis shows the pocket to be a lipophilic non-druggable binding site, this may be a 'stop, or at least a caution' gate way in the target identification process provided that other, more druggable, specific targets are available for the disease area under evaluation. However, this type of analysis can also be used to identify druggable, B-r-o-5 targets, i.e. those that need macrocycles or highly lipophilic ligands to become activated. In a recent paper, a computational analogue of NMR measurements, in which the algorithm is based on the biophysics of binding rather than empirical parametrisation, was successfully identifying druggable and non-druggable targets in the B-r-o-5 space [24].

The following sections focus on absorption enhancement for B-r-o-5 ligands. Ligands in the hydrophilic B-r-o-5 chemical space are typically water soluble but permeability-limited in their absorption; Matsson et al. and Krämer et al. give a state-of-the-art analysis of this chemical domain [23,25]. Our focus is on ligands occupying the highly lipophilic B-r-o-5 chemical space; compounds that tend to be highly permeable but with solubility (or dissolution)-limited absorption. Although the analysis focuses on absorption, it needs to be emphasised that to

discover functional drugs for these inherently lipophilic targets, all ADMET properties need to receive the same level of acknowledgement as pharmacological potency in the early stages of the research programme.

3. Computational models based on quantitative structure property relationships (QSPR)

A number of different computational tools are available to better predict and understand properties involved in drug absorption from the gastrointestinal tract (GIT). Statistical models based on regression analysis of a number of descriptors and the response of interest (e.g. solubility, permeability, interactions with transport proteins) have gained in popularity, partly due to their ease to use and the speed by which the predictions are performed. These multivariate data analysis models are inspired by quantitative structure–activity relationships (QSAR) used in medicinal chemistry to explore potency of ligand series to estimate the activity of new analogues. QSAR as a phenomenon appeared in the early 1970s and typically was based on the correlation between a single property (univariate) and the response in an activity screen. Later more complex models for activity was developed; these were based on several molecular properties (multivariate) to be more predictive. Translating QSAR to prediction of processes importance for e.g. drug absorption and distribution resulted in the term QSPR where the P stands for Property. Mayer and van de Waterbeemd reviewed the early work in the QSAR and QSPR field in 1985 [26]; they already then concluded that quantitative approaches of pharmacokinetics and toxicity are of considerable interest. However, there also a number of risks with relying too much on QSPR approaches, some of which are described here.

A factor significantly influencing the accuracy and applicability domain of the model is the dataset used. The models cannot be better than the quality of the experimental data that is used, and great efforts are put into establishing large enough datasets to allow modelling but at the same time producing experimental data of high quality. The applicability domain is within the chemical space that has been used for training the model. To simplify, the training set used for the model development (i.e. the compounds that have been used to extract descriptors that correlate to the response) can be viewed as the 'standard curve' used for the assessment (prediction); the validity of the model is within this domain. Hence, the model can be used to predict new compounds within this chemical domain and should not be used for compounds sitting outside this space. Typically compounds that are sitting outside the validated chemical space are identified for the user by e.g. flagging that the compound is not described well by the training set. If working within a specific chemical space it is often better to develop local models, i.e. models that are built around a particular ligand series or chemistry, to obtain good predictions of new compounds within this chemical space. If instead the model is supposed to be of general applicability and allow prediction of any new compound, global models based on large, structurally diverse drug-like compounds should be used [27].

Recently, Palmer and Mitchell provided data that indicated that the (poor) performance of solubility models is not related to the varying data quality of different training sets used, rather it is related to the deficiency in the descriptors used [28]. QSPR models are so called ligand-based models where information extracted from the structure of the compounds is related to the response through a statistical model. A number of theoretical approaches and algorithms are available that can provide such information and typically the information is presented as different molecular descriptors. Some of these are rather easily calculated and interpreted, e.g. the number of double bonds and the number of rotatable bonds and typically the values of these do not vary between different methodological approaches. However, molecular descriptors heavily dependent on conformational changes, e.g. intramolecular hydrogen bonds as described by Matsson et al. [23] and Krämer et al. [25], will be highly dependent on the 3D conformer(s) used for calculations. To reduce this effect dynamic descriptors can be calculated, a strategy that has been used when calculating molecular surface area such as the polar surface area (PSA) [29–31]. The dynamic PSA is calculated according to a Boltzmann distribution where every low energy conformer is weighted for its probability of existence. If instead the static PSA is used, the PSA is calculated on the conformer at the global energy minimum. For both these measures a conformational search has to be performed to obtain the description of the surface area. The calculation of PSA was simplified by Ertl and colleagues by making use of tabulated surface contributions of polar fragments [32]. Through this approach they established the rapidly calculated topological PSA (often abbreviated TPSA) which today is the commonly used PSA descriptor. The TPSA is 2–3 orders of magnitude faster to calculate but still highly correlated to the 3D PSA for molecules with a molecular weight of 100–800 (R of 0.99). However, the TPSA will not allow for identification of importance of conformational changes on the polarity of the compounds and hence, for compounds where conformational flexibility will assist in changing hydrogen bond capacity, the TPSA and dynamic PSA will likely differ significantly. In addition, the importance of which conformer that is used for calculation is greater for larger molecules such as macromolecules [33]. This discussion on PSA serves to illustrate how different approaches and algorithms may produce similar (or dissimilar) values when used to calculate molecular descriptors.

The statistical model that is used will further influence the accuracy of the prediction. The most commonly used methods for prediction of absorption related properties (solubility, permeability, transporter interactions) are different linear and non-linear methods such as partial least squares projection to latent structures (PLS), support vector regression/machines (SVR/SVM), random forest (RF) and artificial neural network (ANN); the interested reader is referred to articles that provide details on these methods and their use for QSPR [34,35]. Sometimes these models are used in combination in so called consensus models to obtain more robust predictions. Common for all the models is that they extract descriptors that are correlated to the response parameter. To certify that the extracted descriptors truly are correlated to the response (and not only a result of e.g. a biased dataset) several different measures are taken and typically resampling measures are used to investigate model robustness and significance of descriptors. Examples of such are the cross-validated R^2 (Q^2), boot strapping and permutation tests. Q^2 is a statistical measure of how well the model performs when subsets of the training set are excluded from the model generation. R^2 and Q^2 are similar if not heavily biased towards particular compounds in the dataset. The significance of the descriptors included in the final model should then be tested by e.g. performing permutation tests (randomisation of the data set) or bootstrapping (resampling the dataset). Finally, the model should be validated with an external test set, i.e. a dataset that has not been included during the model development. If all these measures are good, i.e. the model is proven not heavily weighted by particular compounds and provides good prediction of the external dataset, the model is validated and can be released to use. By using this workflow and taking these precautions the risk for identifying factors that are not truly related to the response are significantly reduced. However, it is important that model developers and users of computational models are aware of the influence of the quality of the training set and descriptors used for the model development, as well as the impact the statistical model has, on the final predictions.

4. Prediction of solubility

4.1. Intrinsic solubility

Intrinsic solubility is the thermodynamic solubility of the drug at a pH where the compound is completely non-ionised. It is determined after equilibrium has been established between the solid (stable polymorph) and the dissolved form. Computational models that predict

this property from molecular structure alone are typically based on multivariate data analysis, e.g. methods such as PLS, ANN, SVR and RF. These methods provide quantitative output. The accuracy of the prediction is around 0.5 up to 1 $\log_{10}$ unit, i.e. a predicted solubility value can be expected to be up to 3–10 times different from that experimentally determined [27,28]. Molecular descriptors that appear to be important for intrinsic solubility are lipophilicity, non-polar surface area, molecular flexibility and aromaticity/π–π interactions [27,36,37].

Thermodynamically, solubility is a result of the ease with which a molecule dissociate from its crystal unit cell and the ease with which it is hydrated once in solution. This is clearly visualised in the General Solubility Equation (GSE). This equation describes the stability of the crystal lattice via the melting point (Tm) and the ease with which a compound is hydrated by the partition coefficient between octanol and water (logP) (Fig. 1) [38]. Tm is commonly used to identify compounds where solubility is limited by solid-state properties. These compounds are sometimes colloquially referred to as "brick dust" molecules to visualise a densely packed, tightly "glued" solid structure [37,39]. In contrast, logP identifies compounds having a solvation-limited solubility. These are sometimes colloquially referred to as "grease ball" molecules because of their unfavourable interactions with bulk water [36,40]. The somewhat lower accuracy of solubility models as compared to other response data such as permeability and lipophilicity has been attributed, at least in part, to the influence of solid state properties on solubility. It is well-known that it is not yet possible to quantitatively predict the strength of the crystal lattice by computational means and hence, the solid-state contribution to the solubility is not captured well by in silico models. However, successful MD simulations of the crystal lattice (without need of experimental input) of organic, small molecules (n-alkylamides) were recently published [41], but crystal structure of molecules that are larger, flexible and with several polar functions (typical drug-like features) has been proven more difficult to predict [42]. When successful, these simulations hold great potential to improve predictions of phenomena dependent on the solid state (e.g. dissolution, solubility, supersaturation), since they are ab initio calculations and hence, can be used to predict the crystal structure of any new drug.

Attempts to predict Tm have been made, and computationally it is possible to predict whether a compound is likely to be low, intermediate or high melting using classification models with cut-off values of e.g. 120 and 180 °C [43]. However, in absolute numbers, melting point cannot be expected to be predicted with accuracy greater than 30 °C for compounds in general [44], and up to 40 °C for drug-like compounds [43]. These inaccuracies should be kept in perspective; APIs that are solids at room temperature typically have Tms in the range of ~60–350 °C (a rather narrow interval in absolute number), with most of them melting between ~100 and 250 °C. A recent study based on in silico solid state perturbation and the 2D molecular structure, presents an interesting computational methodology to study the impact of the solid state on the final solubility [39]. Future development of this method and methodologies based on molecular and quantum mechanics have the potential to contribute to more accurate predictions of crystal lattice properties and their influence on other responses such as dissolution rate and solubility.

4.2. Solubility in intestinal fluids

The intrinsic solubility value is not a physiologically relevant measure for many ionisable compounds; rather it should be seen as a physicochemical fingerprint of the molecule. After oral administration, a drug will be exposed to a wide range in pH. In the fasted state, the pH of the stomach is acidic (pH of 1.7–3.3; median of 2.5) whereas it is neutral or slightly basic (6.5–7.8; median of 6.9) in the distal part of jejunum [45]. The pH thereafter remains at a high level, with an average pH of 8.1 and 7.8 reported for the fasted distal part of the ileum and colon, respectively [46,47]. In addition to the pH-gradient, the secretion of bile in the duodenum results in the generation of lipoidal nanoaggregates, e.g. mixed micelles and vesicles composed of phospholipids and bile salts, that are naturally present in the intestinal fluid. The combination of changes to pH and the presence of nanoaggregates with high solubilising capacity, significantly influences the final solubility of drugs in the intestinal fluid. The solubility therefore differs between individuals and is dependent on food composition and prandial state [48].

As a result, solubility prediction in the intestinal fluids is complex. The simplest strategy is to use in silico models to predict intrinsic solubility and pKa, and then to put these values into the Hendersson–Hasselbalch equation. More recently in silico models that target prediction of solubility in fasted human intestinal fluid (HIF), or in biorelevant dissolution medium mimicking this fluid, have appeared [49]. With chemical information calculated from the molecular structure alone these models can predict the solubility in these complex body fluids. Fagerberg et al. reported root mean square error (RMSE) for predictions

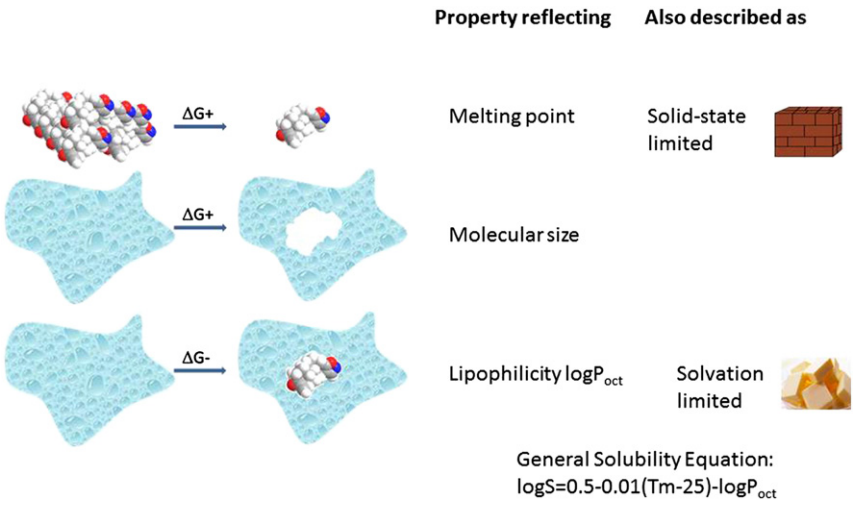

Fig. 1. The thermodynamic principles of dissolution. The drug molecule needs to dissociate from its solid crystal lattice and the solvent (e.g. water) needs to open up a cavity large enough to incorporate the drug molecule. Both these processes demand energy. The molecule can thereafter become hydrated, a process that releases energy. For each of these steps drug properties can be used to indicate which process is likely to limit solubility. For dissociation from the solid state melting point is typically used. Compounds displaying a melting point >200 °C are at greatest risk of solid-state limitations (i.e. intermolecular force) to solubility; these are sometimes referred to as brick dust molecules. Compounds with high lipophilicity are poorly hydrated and compounds with a logD > 3 are at risk for having a solvation limited solubility; such compounds are sometimes referred to as grease balls. The impact of melting point (Tm) and lipophilicity (in form of $\log P_{oct}$) on solubility is captured by the General Solubility Equation established by Yalkowsky and coworkers [38].

in HIF are 0.34 and 0.80 (log_{10} units) for the model training and validation set, respectively. Similar accuracy has been obtained for prediction of drug solubility in fasted state simulated intestinal fluid (FaSSIF). To date, there are two different datasets and models openly available to the scientific community for prediction of drug solubility in FaSSIF. One of these is a recently published PLS model based on 56 compounds used for training the model and 30 compounds for validation [49]. This model had an RMSE of 0.77 (log_{10} units) for the validation set. The other model is based on ANN and is available in the software ADMET Predictor [50]. This model was developed based on 141 compounds and validated using 14 compounds resulting in an RMSE of 0.48 log_{10} units for the validation set.

The PLS models published by Fagerberg et al. were based on structurally diverse datasets. For these datasets, molecular size and aromaticity were found to be two of the most important limiting molecular descriptors for solubility in aspirated and simulated intestinal fluids [49]. The datasets used were biased towards lipophilic compounds since these are known to be significantly solubilised in the colloidal structures of the intestinal fluid and therefore merit experimental determination in e.g. aspirated human intestinal fluid. The $logD_{pH\ 6.5}$ of the compounds used to train the HIF and FaSSIF models was in the range of 0.3–6.0 and hence, hydrophilic compounds were not included in the analysis. The size factor was interpreted as reflecting the fact that larger molecules require larger cavities in the solvent to hydrate the molecule. However, it is well-known that as molecular size increases, lipophilicity also usually increases, and hence, size descriptors may also indirectly carry information from other variables, such as lipophilicity, and therefore include their impact on solubility. Similarly, aromaticity carries information about lipophilicity, but is also important in determining the likelihood of formation of a stable and densely packed crystal lattice [37,51,52]. For example, interconnected aromatic rings in a structure usually raise the Tm. Once such highly interconnected structures are in solution, the aromatic features cannot be shielded from the water by folding. This is unlike the situation for highly flexible, aliphatic structures which can fold to minimise the surface area exposed to water. The models also showed that the potential to form hydrogen bonds is positive for solubility. This is intuitive since functional groups that can form hydrogen bonds also facilitate direct interaction with the water molecules. Another, recent analysis of solubility data measured in HIF and FaSSIF revealed that compounds with logD > 3 should be evaluated for intestinal solubility using a biorelevant dissolution medium with lipids present [53]. Above this value, the solubility in FaSSIF or fed state simulated intestinal fluid (FeSSIF) is often 10 to 100-fold higher than that measured in a pure buffer without phospholipids and bile salt. However, even at lower logD values, there can be a 2 to 4-fold higher solubility in these media compared to pure buffers.

5. Molecular determinants of solubility: can you see a grease ball (or brick dust) coming?

The molecular features determining solid-state limited or solvation-limited solubility have been studied by using a range of poorly soluble compounds [36,37,40,54]. Solvation-limited molecules, i.e. “grease balls”, are relatively large, flexible and lipophilic (logD > 3) [36]; many of them are on the border of, or inside, the B-r-o-5 chemical space. An analysis of dosage forms employed for such compounds reveals the requirement for excipients that facilitate dissolution and solubilisation (e.g. disintegrants, suspending agents, wetting agents, solubilisers) in order to develop well-functioning oral dosage forms [36]. Hence, poorly soluble, solvation-limited, compounds are possible to bring to the market, albeit after extensive formulation development. Other studies have focused on solid-state limited compounds. According to the GSE, these compounds are usually found in the lower lipophilicity range (logP-values<2) (since higher log P compounds are likely to be solvation limited). Indeed, based on a dataset of compounds with low lipophilicity (logP of ~2) but up to 1000-fold differences in solubility, strong correlations were observed between solubility and Tm and enthalpy of fusion (H_{fus}) (R of 0.84 for both properties) [37,55]. Further analysis of these compounds showed that, in agreement with previous Tm analyses, solid-state limited compounds were often small molecules with a large degree of planarity (therefore favouring molecular packing). Compounds with reasonable solubility in this data set had flexible side chains and therefore greater configurational entropy. Hence, the molecular determinants of “grease ball” and “brick dust” molecules can be clearly differentiated. The latter are smaller, rigid and typically positioned inside the rule-of-5 chemical space, whereas “grease balls” are larger, flexible and lipophilic and often on the border of, or inside, the B-r-o-5 chemical space. Intermediates of these extremes are also present; these are highly lipophilic, highly aromatic structures hindered in their solubility by both their solid state properties and poor hydration. Examples of such structures are ligands of the RAR (e.g. acitretin with a logP of 5.6 and Tm of 221 °C) and ligands of hormone receptors such as the thyroid hormone receptors (e.g. levothyroxine with a logP of 4.6 and Tm of 235 °C) [56].

Whilst such structures may be highly potent in biochemical screens, they are extremely challenging molecules to translate to functional medicines. High lipophilicity as a result of aromatic structures such as benzene rings, not only reduces solubility, but is also known to increase metabolic vulnerability. Further it increases the likelihood of non-specific hydrophobic interactions with off-target receptors (receptor promiscuity) and therefore the potential for toxicity [57–59]. Such structures are difficult to formulate since several approaches must be used in concert to overcome both solid-state and solvation limitations. Nonetheless, the two examples above, acitretin and levothyroxine, have been successfully developed as rather conventional oral dosage forms mainly taking use of disintegrants. Levothyroxine has a dose in the lower μg range and hence, the required solubility (for complete dissolution) in the intestinal fluid for this compound is only 0.8 μM — most likely explaining the relatively straightforward formulation. In contrast, according to the Swedish Physician's Desk Reference acitretin has a 500-fold higher dose than levothyroxine and might therefore be expected to provide a much greater challenge. The product labelling for acitretin suggests that it should be taken with a meal since the absorption is doubled in the presence of food or milk. Milk is a natural emulsion of fat with a complex digestion pattern and forms solubilising lipodal nanoaggregates of different liquid crystalline forms during lipolysis [60]. In the case of acitretin food is providing the additional solubilisation capacity required to support absorption. The bioavailability of acitretin is 60% under fed conditions, when high amounts of naturally available lipids are present, but still it is highly variable (36–95%) [61].

6. Prediction of permeability

The permeability of compounds is positively related to lipophilicity and negatively related to hydrogen bond capacity and molecular size [29,30,62]. Hence, membrane permeability, resulting from passive diffusion across the lipoidal membrane, is typically high and absorption is not permeability-limited for compounds belonging to the lipophilic, B-r-o-5 chemical space. In contrast, highly polar and larger molecules commonly exhibit permeability-limited absorption. The reader is referred to the recent review on macromolecules by Matsson et al. for further details [23]. For lipophilic drugs, whilst intrinsic membrane permeability is often high, other cellular mechanisms may play a role in the transport rate and the ease with which highly lipophilic compounds cross cells and, e.g., are absorbed from the intestine. Entrapment in the membrane, non-specific binding to intracellular proteins, as well as specific binding to membrane-bound transport proteins responsible for efflux, may become significant determinants of the rate and extent of the cellular transport [63–65].

The complexity of cellular transport is not considered in traditional in silico models of cell permeability. Instead datasets obtained from

cell-based models (e.g. Caco-2, MDCK, 2/4/A1 cell lines) and methods designed to delineate the passive diffusion component are used for development of computational models [29,33,66]. Other factors of importance for absorption, such as active transport and inhibition thereof, are treated in separate computational models [67]. The permeability values obtained from cell-based models have been shown to correlate with the effective permeability (P_{eff}) in humans [68,69]. These cell-based assays allow rapid determination of permeability at a relatively low cost and are therefore used as surrogates for in vivo determinations. The availability of in vitro cell-lines and development of high throughput permeability methods, make it feasible to screen enough compounds that the resulting datasets are of sufficient size to extract chemical information of importance for cell permeability. In addition to the cell-based permeability datasets, P_{eff} data from human perfusion studies have been used for permeability modelling. An update of available P_{eff} data of chemically structurally diverse compounds is provided by Dahlgren et al. [70]

Similar computational model strategies and multivariate tools (i.e. PLS, ANN, SVR, RF) are used for prediction of permeability as for solubility. A number of models are commercially available, see e.g. the review by Norinder and Bergstrom [34]. The accuracy of in silico models is 0.39–1.43 $\log_{10}$ units for the validation test sets [71]. In silico models to predict P_{eff} have also been developed; these typically have similar accuracy as the cell-based models [50,72]. However, it should be noted that the P_{eff} models are usually evaluated using fewer test compounds (generally $n<10$), since the available dataset is small.

7. Computational biopharmaceutical profiling

A tool for computational biopharmaceutical profiling (CBP) was recently developed in-house to explore the 'required' lipophilicity for particular targets. The hypothesis was that such a tool could be used to provide an early signal of the need of an enabling formulation strategy for particular biologies (Box 1). The CBP is based on in silico models for predictions of solubility in FaSSIF and FeSSIF, and P_{eff} (Box 2). From 1620 discovery compounds in the patent literature it was possible to extract the molecular requirements of ligands for 32 different targets. In general, the ligands investigated were clustered in the oral drug-like chemical space, but in the region where poorly soluble, solvation limited compounds are positioned, i.e. the compounds examined were largely poorly water-soluble compounds resulting from poor hydration [36]. In the following sections the use of CBP to prospectively indicate the need for enabling formulations is described.

Box 1
Dataset used for computational biopharmaceutical profiling.

The molecular requirements of the targets were analysed based on reviews of ligand patents [73,138–165]. The therapeutic areas covered were: addiction, allergy, arthritis, bacterial and viral infections, cancer, cardiovascular diseases, diabetes, epilepsy, inflammation, liver diseases, CNS-related diseases (anxiety, Alzheimer's disease, cognition impairment, depression, Parkinson's disease and schizophrenia), immunological disorders, metabolic syndrome (lipid metabolism, carbohydrate metabolism, obesity), pain, pulmonary diseases and stroke. The final data set consisted of 1620 compounds and for these ligands data were available to perform target-specific analysis for: adenosine receptors A1 to 3 (A1–3), anaplastic lymphoma kinase (ALK), bacterial topoisomerase (BT) 2 and 4, cannabinoid receptors (CB) 1 and 2, cholesteryl ester transfer protein (CETP), farnesoid X receptor (FXR), FMS-like tyrosine kinase 3 (FLT3), $GABA_B$, glucocorticoid receptor (GR), histamine 3 receptor (H3R), HIV chemokine receptors (CCR5, CXCR4), HIV-1 non-nucleoside reverse transcriptase (NNRT), 17β-hydroxysteroid dehydrogenases (17β-HSD3), 5-hydroxytryptamine subtype 2c (5-HT2), 5-hydroxytryptamine subtype 6 (5-HT6), melatonin receptors (MT) 1 and 2, neurokinin receptors (NK) 1 and 3, platelet derived growth factor (PDGF), prostaglandin D2 receptor (CRTH2), protease activated receptor 1 (PAR), retinoic acid receptor (RAR), γ-secretase (Aβ42), soluble epoxide hydrolase (sEH), type T calcium channels (TTCC and Cav3.1) and viral envelope glycoprotein 120 (gp120).

Box 2
Development of the computational biopharmaceutical profiling (CBP) tool to assess absorption and signal need for enabling formulations.

Molecular descriptors and multivariate data analysis: All molecular structures were produced as sdfs in ChemDraw Ultra 11.0 (Cambridge Soft, UK) and submitted for calculation of molecular descriptors by the software DragonX 1.4 (Talete, Italy). Physiologically relevant properties were predicted with ADMET Predictor (Simulations Plus, CA), e.g. permeability, pH-adjusted lipophilicity and solubility (pH 6.5.and 7.4), and solubility in fasted state simulated gastric fluid (FaSSGF) and fasted and fed state intestinal fluids (FaSSIF and FeSSIF, respectively). These analyses were performed at the compound level and the target level, the latter being used to identify the importance of target biology on the biopharmaceutical profile of the ligands.
A modified biopharmaceutics classification system (BCS) applicable to the drug discovery setting: The solubility criterion used was the maximum concentration (solubility) that could be achieved in the intestinal fluid (pH 6.5) rather than the likelihood of complete dissolution of a dose at the pH interval 1–6.8 which is the criterion used by e.g., the European Medical Agency (EMA) and the World Health Organization (WHO). These adjustments were performed to make the framework applicable to the discovery setting where the dose is not yet established and to give higher priority to the intestinal compartment where the majority of the absorption takes place. In addition, intermediate classes were introduced to provide a more transparent system with regard to the analysis of formulation dependence during the early development stages. Another reason for including an intermediate class was to increase the reliability of the predictions of high and low permeability/solubility by having this class as separator. The following cut-off values were applied: High effective permeability (P_{eff}) $> 1.0 \times 10^{-4}$ cm/s, Low $P_{eff} < 0.2 \times 10^{-4}$ cm/s, and Intermediate permeability in between these values. High solubility $>$ 200 μM, Low solubility $<$ 50 μM, Intermediate solubility in between these values. For an 'average' oral drug with a molecular weight of 350, and an available dissolution volume of 250 ml (based on the volume of a glass of water), this corresponds to doses in the lower milligrame scale. The 50 μM solubility value, in conjunction with a 250 ml dissolution volume, suggests that a dose of 4.4 mg can be dissolved. Assuming a 200 μM solubility limit a dose of up to 17.5 mg may be soluble. Hence, these cut-off values do not exaggerate the impact that solubility will have on the absorption, rather the reverse.

7.1. Lipophilicity of targets

Whilst the properties of the complete dataset were generally consistent with previous reports, the drug candidates to different target biologies had significantly different lipophilicity profiles (Fig. 2). Human targets with ligands having logPs of 2–3 (typically suggested to be ideal for good absorption) included adenosine receptors A1 to 3 (A1–3), HIV chemokine receptor (CXCR4), 5-hydroxytryptamine subtype 2c (5-HT2), and melatonin receptors (MT) 1 and 2. Of all the ligands

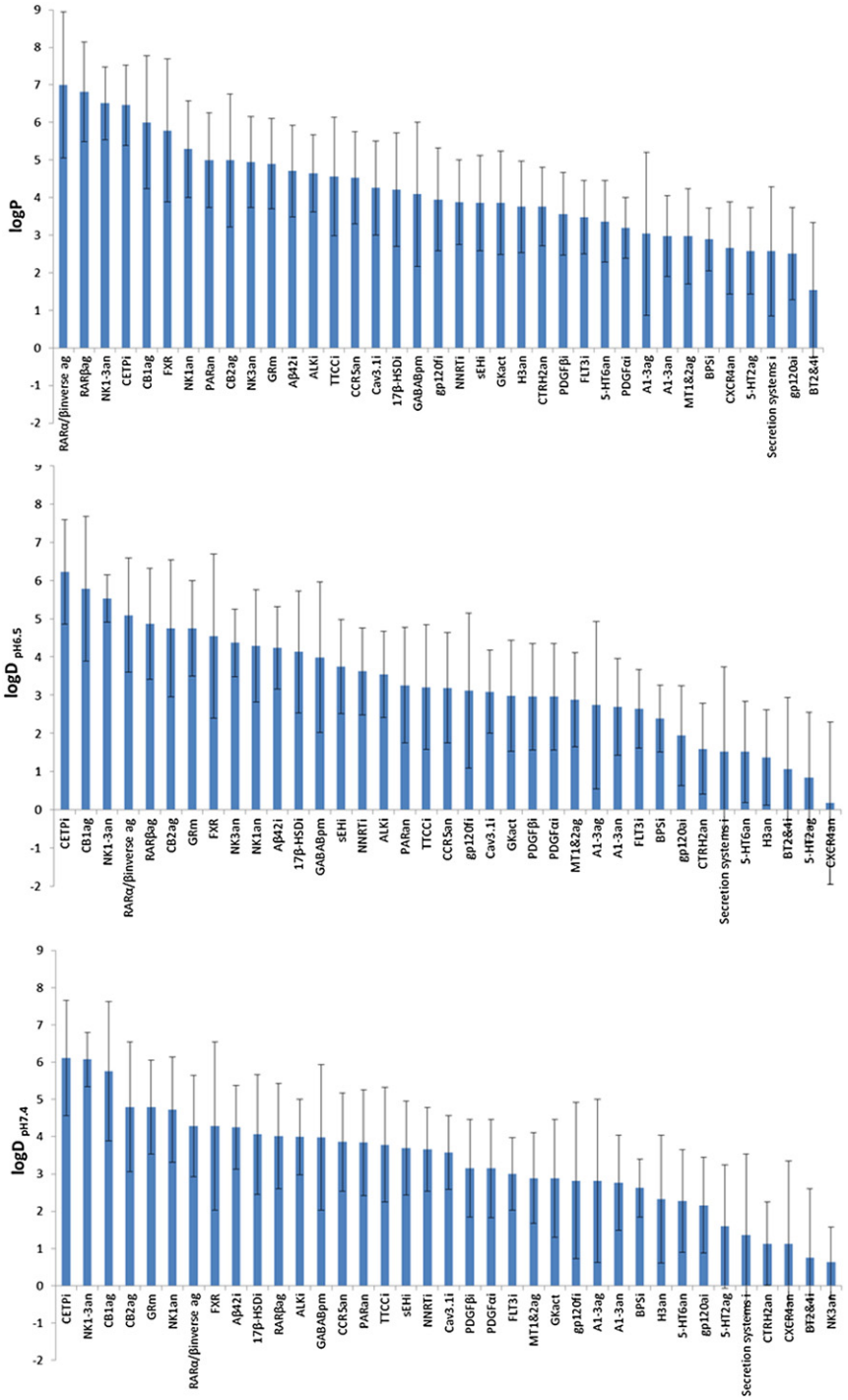

Fig. 2. Target specific lipophilicity profiles obtained through calculation of the partition coefficient between octanol and water for ligand series for each receptor. Upper panel: Profile based on logP, i.e. the partition coefficient determined in pH-adjusted water such that only the neutral species is present. Mid panel: Profile calculated at pH 6.5 to predict the lipophilicity ($logD_{pH\ 6.5}$) in the small intestine. Lower panel: Profile calculated at pH 7.4 to predict the lipophilicity ($logD_{pH\ 7.4}$) in the systemic circulation. Abbreviations of targets are provided in Box 1. For function the following abbreviations are used: Agonist (ag), inhibitor (i), antagonist (an), modulator (m).

analysed, only the ligand series for bacterial topoisomerase 2 and 4 had a mean logP of less than 2. Several targets (e.g., glucocorticoid receptor (GR), cannabinoid (CB) 2, 17β-hydroxysteroid dehydrogenases (17β-HSD3), γ-secretase (Aβ42), anaplastic lymphoma kinase (ALK)) had ligands in close proximity to the rule-of-5 cut off for lipophilicity (Fig. 2). Perhaps most importantly, the analysis reveals that for some targets the compound libraries had mean logP ≥ 5, i.e. the mean value of ligands to each of these targets is outside of traditional r-o-5 space with respect to lipophilicity. These targets were: CB 1, cholesteryl ester transfer protein (CETP), farnesoid X receptor (FXR), neurokinin receptors (NK) 1 to 3, protease activated receptor (PAR) 1, and RAR α and β.

For the CETP ligand dataset [73] high lipophilicity was also accompanied by high molecular weight, resulting in 91% of the ligands being in the B-r-o-5 chemical space (i.e. two properties outside of r-o-5 limits). However, it should be noted that this subset is small and based on patented exemplar structures rather than all discovered CETP ligands, and there are evidence of that also less lipophilic CETP ligands can be active

[74]. In a recent proof-of-concept study it was shown that (marginally) less lipophilic ligands of CETP can be highly potent. Trieselmann et al. synthesised a 1,1′-spiro-substituted hexahydrofuroquinoline derivative (denoted cpd 26 in their paper) with an IC_{50} of 18 nM and calculated logP of 4.6 [75]. The inhibition was highly related to the stereochemistry and one enantiomer of cpd 26 had an IC_{50} of 576 nM. The authors did not provide information on permeability, solubility or fraction absorbed but animal studies show that the compound is as efficient as anacetrapib albeit with a shorter half-life. These data suggest that it is possible to reduce lipophilicity to some extent using focused structure–activity relationships; however, the CETP binding pocket is lipophilic and the resulting ligands still sit within the highly lipophilic chemical space.

In addition to analyses centred on logP, a similar approach has been undertaken for pH-dependent lipophilicity, logD, at the pH of the small intestine (pH 6.5) and blood (pH 7.4). Whilst ligands to CETP, CB1 and NK 1 to 3 were highly lipophilic (logD > 5) also at these pH-values, RAR α and β had a $logD_{pH\ 6.5} > 5$ but a $logD_{pH\ 7.4} < 5$ as a consequence of increased ionisation of acidic functional groups at higher pH. The mean lipophilicity of ligands to CB2 and GR was just below the suggested lipophilicity cut off of 5 at pH 7.4 and 6.5 (Fig. 2). In most cases, however, the explored targets had highly lipophilic ligands even after taking into consideration the effect of physiological pH on the ionisation of the drug molecules.

For druggable targets where most ligands sit in the B-r-o-5 chemical space, other gateways might usefully be applied for progression, rather than those used for targets where more ligands within drug-like, rule-of-5 chemical space are possible. In particular, lipophilic APIs often show poor absorption, increased volume of distribution, increased metabolic liability and increased toxicity. The relationship between permeability and metabolic liability has allowed the development of a modification of the biopharmaceutics classification system (BCS) [76,77], that uses metabolism instead of permeability to classify compounds — the biopharmaceutics drug disposition classification system (BDDCS). Both BCS and BDDCS use the same solubility criterion to classify compounds. The combined analysis provided by the BDDCS and BCS is valuable to better understand drug absorption, bioavailability and systemic disposition of these lipophilic compounds. Whilst increased metabolic liability is likely with increasing lipophilicity, the need for lipophilic fragments to activate some targets makes this an inevitable complication for such targets. Judicious choice of chemistries that maintain lipophilicity whilst reducing or redirecting metabolic pathways to those with lower practical disadvantage will be crucial for the success of such ligand libraries [78]. A further complication of lipophilic drug candidates is their increased potential for toxicity [79]. The lipophilicity efficiency (LipE) measure captures the intrinsic potency of a ligand at a particular drug target, corrected for lipophilicity. LipE can be used to identify molecules where the balance of potency versus lipophilicity is maximised, thereby minimising downstream risk of toxicity and metabolic liability [16,74,80].

7.2. Biopharmaceutical profiling of ligands may be used to signal food effects

The aqueous solubility of highly lipophilic, large and flexible molecules, increases significantly when bile salt concentrations rise and when lipids are present. This reflects drug solubilisation within the lipid aggregates formed by the bile components and e.g. lipid digestion products [40,53,81]. Biorelevant dissolution media containing lipids naturally present in the small intestine are therefore better predictors of the solubility in vivo than assessments in pure buffers [49,82,83]. The computational approach was used to assess the potential impact of the fasted and fed state on solubility and to evaluate how that would influence absorption. This was undertaken to investigate whether the target-specific compound libraries identified as having high inherent lipophilicity were also enriched with poorly soluble compounds where solubility was significantly enhanced in the fed state — properties that are common for "grease balls". Our hypothesis was that predictions of large increases in solubility in FeSSIF would signal the likelihood of a positive food effect and also the likelihood that e.g. a lipid-based formulation would be able to efficiently deliver the compound via the oral route.

The CBP used in silico models to predict the solubility in the fasted and fed states of gastric and intestinal fluids (Fig. 3 and Box 2) [50,84]. The intestinal fluids of both states contain bile salts and phospholipids, but with approximately five times higher concentration in the post-prandial state. For solid state limited compounds (i.e. "brick dust") it is expected that the solubility in fasted and fed state would be relatively similar since the amount of lipids is not decisive for the solubility obtained [53]. Of all ligands, 40% had a predicted solubility in FaSSIF of less than 50 μM and this proportion decreased to 16% under fed state conditions. Interestingly, some targets had a significantly larger proportion of poorly soluble ligands than others. Indeed, for the 'lipophilic targets' most of the ligands were poorly soluble in the fasted state, a number that dropped significantly in the fed state. The impact of changes in the GIT pH on solubility is also evident and exemplified by the acidic RAR modulators which had the lowest solubility under gastric conditions (pH 1.6) where the compounds are in the unionised state.

Predictions of human jejunal effective permeability (P_{eff}) suggest that 80% of the dataset have high intestinal permeability ($P_{eff} > 1.0 \times 10^{-4}$ cm/s), and hence, that >95% of the dose is expected to be absorbed after oral administration provided solubility limitations can be overcome [85]. Only 7% of the total dataset were predicted to have low permeability ($P_{eff} < 0.2 \times 10^{-4}$ cm/s). Compound series in which molecules with poor permeability were enriched were those targeting infections such as the macrolides and quinolones, where 45 and 47%, respectively, were predicted to have poor permeability. These ligand series were the least lipophilic with $logD_{pH\ 7.4}$ of 0.7 and 2.6 for quinolones and macrolides, respectively. They also had increased capacity to interact with water through hydrogen bonds, as indicated by the large average TPSA (surface area of nitrogen, oxygen and hydrogen bound to these heteroatoms) [32] of 127 and 186 $Å^2$ for quinolones and macrolides, respectively. These values should be compared to the other targets, where a majority of the ligands (85%) had TPSA<100 $Å^2$. A large TPSA will also, as discussed in Section 6, reduce passive diffusion through the cellular membranes. In two different datasets it has been observed that when the TPSA is >140$Å^2$ the fraction absorbed from the intestine can be expected to be <10% [30,86].

The CBP is based on a variant of the BCS [76], herein adjusted to match analyses performed in the drug discovery setting (Box 2). The BCS is traditionally used to signal the need for bioequivalence studies

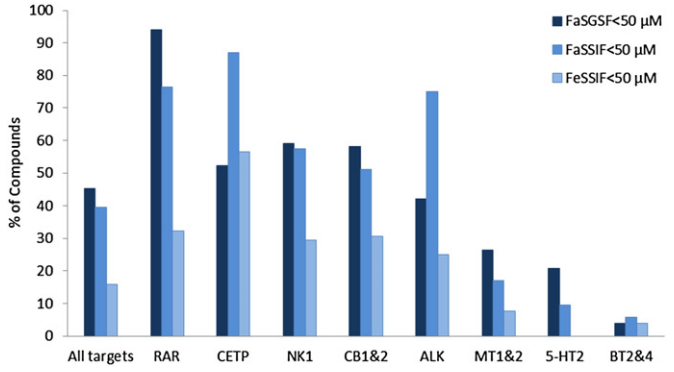

Fig. 3. pH-dependent solubility and solubilisation of exemplar ligands to a range of receptor classes. Solubility was predicted in fasted state gastric simulated intestinal fluid (FaSGSF; pH 1.6), fasted state simulated intestinal fluid (FaSSIF; pH 6.5) and fed state simulated intestinal fluid (FeSSIF; pH 5.8). The fraction of compounds with a solubility <50 μM is presented, a value that for a model compound with molecular weight of 350 Da corresponds to a concentration of 17 μg/ml. Abbreviations of targets are provided in Box 1.

during life cycle management and consists of a schedule of four drug classes defined on the basis of solubility and permeability properties. BCS class 1 compounds have high solubility and high permeability; they are expected to have high absorption regardless of the physiological conditions and/or the dosage form administered. BCS class 2 compounds have low solubility/high permeability and are expected to show solubility and/or dissolution limited absorption, and therefore the amount that is absorbed becomes highly dependent on the formulation. BCS class 3 and 4 compounds have high solubility/low permeability and low solubility/low permeability, respectively. The applicability of the BCS is limited in the discovery setting since it relies on the knowledge of the oral dose to classify solubility. In the discovery setting this information is usually not available and the CBP was therefore based on predicted solubility values without dose adjustment (for more information, see Box 2).

The CBP predicted that compound series directed to targets with ligands with average lipophilicity (logP 2–3) would be mainly positioned in BCS class 1 region, where solubility and permeability are high, consistent with complete absorption (Fig. 4). Exemplar targets predicted to be compatible with BCS Class 1 ligands include 5-hydroxytryptamine subtype 2c (5-HT2) and melatonin receptors (MT) 1 and 2, with 70% and 51% of molecules, respectively, predicted to belong to this class. Indeed, MT1 agonists in the series investigated here have shown almost complete absorption (>84%) [87,88]; however oral bioavailability is typically limited by extensive first pass hepatic metabolism. Ligands to the non-lipophilic targets (e.g. the quinolone-based antibiotics) are mainly permeability-limited with 77% of the compounds predicted to have permeability-limited absorption (classical BCS Class 3). Although it proved difficult to find permeability data for recently discovered quinolone-based antibiotics, reference quinolones such as ciprofloxacin, levofloxacin and DK-507a (a predecessor to analogues used as exemplar structures) are known to have poor membrane permeability [51]. For the non-lipophilic targets, the impact of the post prandial state on drug solubilisation was minor and hence, the biopharmaceutical profile of ligands to these targets was similar for the solubility predictions of the pre- and post-prandial states. In contrast, ligands of 'lipophilic targets' showed significantly different biopharmaceutical profiles. Two such targets are shown in Fig. 5. A majority of the compounds directed towards these targets are BCS class 2-like compounds and for most of them, absorption is predicted to be solubility-limited. For these targets, and many others, the solubility increased in the fed condition. As examples, the fraction of BCS class 2 RAR ligands and γ-secretase inhibitors was reduced from 0.74 to 0.50 in the fasted state to 0.31 and 0.10 in the fed state, respectively. Hence, the positive solubilising effect of naturally available lipids pushed many of these compounds to become BCS class 1-like.

7.3. Biopharmaceutical profiling of ligands signals need for enabling formulations

The CBP facilitates early identification of targets for which non-traditional decision gateways might be required during drug discovery, and for which non-traditional formulation approaches are likely demanded for successful clinical development. The CBP was used to analyse the requirements of the target rather than the properties of the drug molecule. This approach provided a holistic view of differences in the required physicochemical properties of drug candidates across a range of different target biologies. Targets where a majority of the ligands fall within the lower, right quadrant of the CBP profile (see the graphs presented in Figs. 4 and 5) are likely to require atypical development strategies since current evidence suggests that the identification of water soluble drug candidates, that retain sufficient potency, is unlikely. The fed state CBP also provides information on compounds where solubility is likely to increase when higher concentrations of gastrointestinal amphiphiles (e.g., bile salts and phospholipids) are present and hence signals that solubilising formulations such as lipid-based formulations [89], are likely to result in increased, more reproducible and more reliable absorption. This is consistent, for example, with recently published studies showing enhanced exposure of inhibitors of the lipophilic target CETP after administration in lipid-based formulations [90,91]. Further evidence of the utility and accuracy of the CBP comes from a retrospective analysis of formulations used for different targets. For example, a number of conventional tablets are used for delivery of marketed for 5-HT2c modulators (e.g., marketed products of

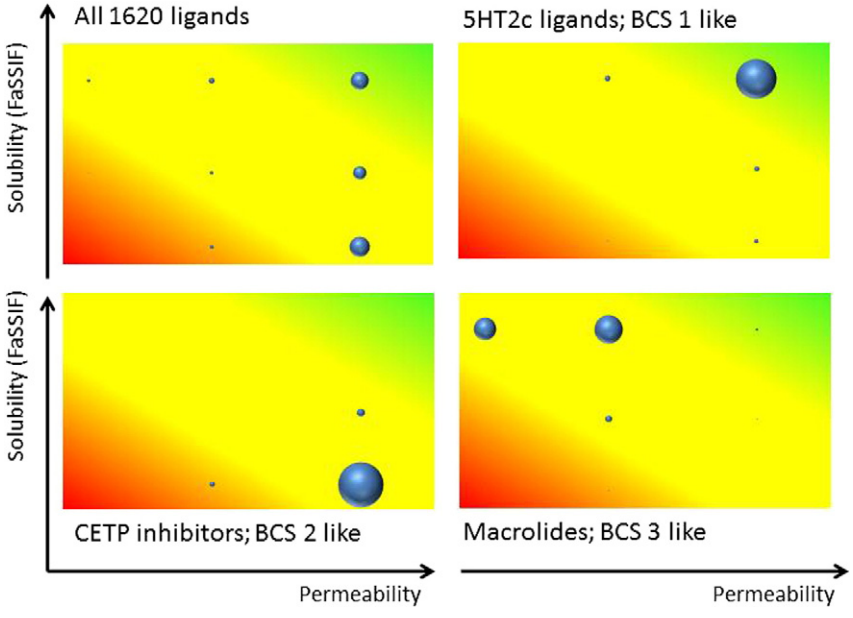

Fig. 4. Computational biopharmaceutical profiling (CBP). The position of the ligand series in a discovery directed variant of the BCS (Box 2). Compounds were classified into a 3 × 3 matrix of solubility versus permeability. The cut-off values used for solubility were: poorly soluble <50 μM; highly soluble >200 μM; intermediate in between these values. The following values were used for permeability: high permeability > 1.0×10^{-4} cm/s; low permeability 0.2×10^{-4} cm/s; intermediate in between these values. The larger the circle the larger the fraction of compounds belonging to this part of the CBP. BCS class 1-like compounds are positioned in the upper right (green) corner, BCS class 2-like compounds in the lower right (yellow) corner, BCS 3-like in the upper left (yellow) corner and BCS 4-like in the lower left (red) corner. The upper left panel shows the distribution of all 1620 ligands without identifying the target profiles. The other panels show that ligands for 5HT2c are mostly BCS class 1, CETP inhibitors are almost entirely BCS Class 2 and macrolides are largely BCS class 3.

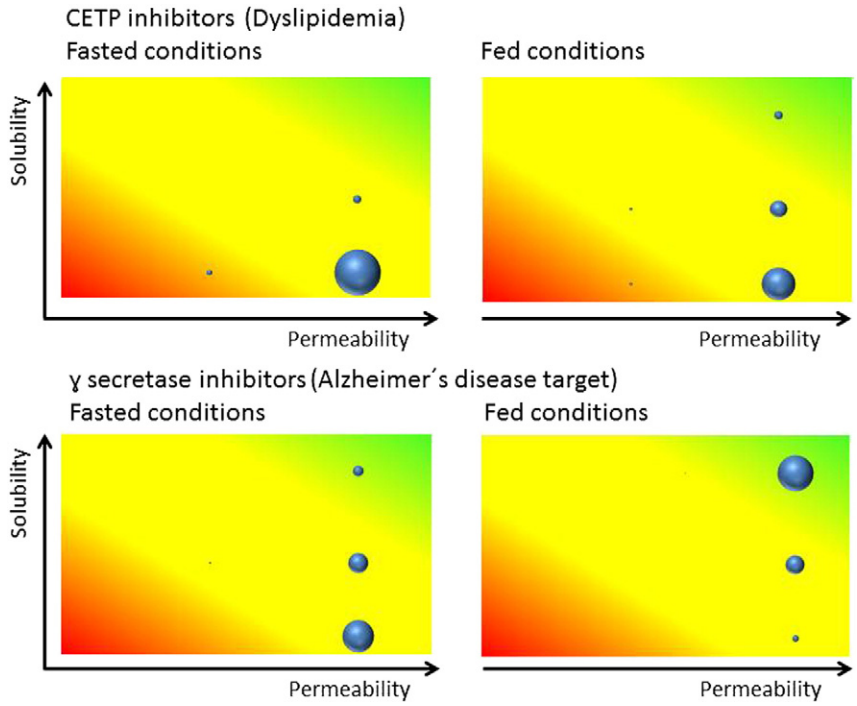

Fig. 5. Impact of lipids present in fed state intestinal content on apparent BCS classification based on computational biopharmaceutical profiling. The position of the ligand series in a discovery directed variant of the BCS (Box 2). Compounds were classified into a 3 × 3 matrix of solubility versus permeability. The cut-off values used for solubility were: poorly soluble <50 μM; highly soluble >200 μM; intermediate in between these values. The following values were used for permeability: high permeability > 1.0×10^{-4} cm/s; low permeability 0.2×10^{-4} cm/s; intermediate in between these values. The larger the circle, the larger the fraction of compounds belonging to this part of the CBP. BCS class 1-like compounds are positioned in the upper right (green) corner, BCS class 2-like compounds in the lower right (yellow) corner, BCS 3-like in the upper left (yellow) corner and BCS 4-like in the lower left (red) corner. For both CETP inhibitors (top panels) and γ-secretase inhibitors (bottom panels) the majority of ligands are expected to be highly permeable but have low solubility in fasted intestinal fluid (left hand panels). In contrast in both cases, solubility in fed intestinal fluids is significantly higher — i.e. a shift towards the green quadrant. This suggests that enabling formulation such as lipid-based formulations may be beneficial for drug delivery for these receptor classes.

aripiprazole and tramadol); these modulators were forecasted by the CBP to belong to the BCS Class 1 group and therefore not in need of an enabling formulation. In contrast, the γ-secretase inhibitors, identified as being BCS Class 2-like with improved solubility in FeSSIF, have been explored in animal models with cosolvents and surfactants (e.g. 80% PEG400 [92] and mixtures of 10% dimethylformamide and 30% propylene glycol [93]). Thus the CBP had accurately signalled the need for an enabling formulation, e.g. lipid-based, in order to achieve acceptable plasma concentrations.

There are at least two ramifications of the CBP. First, it provides data to support a re-examination of the typical pharmaceutical decision-gates for compound progression for some targets (for example relaxing the desire for drugs to have logPs of less than 5). Second, it can signal a likely requirement for a solubility enhancing formulation early in the development cycle. This is an important distinction. Lead optimisation strategies that are re-focused on the identification of drug candidates with physicochemical properties optimised for a particular formulation approach will be quite different to those that adopt traditional lead optimisation strategies that attempt to promote aqueous solubility. For traditional drug-like targets, decision gateways based on historical progression criteria (e.g., molecular weight, number of rotatable bonds, PSA) provide a useful early indicator of the ADMET profile. In contrast, the CBP suggests that rigid application of these criteria is unlikely to be successful for targets with ligands located in the B-r-o-5 and the lower right corner of the graphs displayed in Figs. 4 and 5 (classical BCS class 2). It is also worth noting that the application of rules such as the r-o-5 seems to have driven the discovery process towards the borders of the decision gates, with the end result of the lead optimisation process being compounds with molecular properties that are just slightly below the cut-off values given for these gateways [16]. In the dataset explored herein, ~7–10% of all the molecules had molecular properties 'just below' the margin of the rule-of-5 (e.g., 9 % had molecular weights of 470–500 Da and 7% had a logP of 4.7–5). Whilst these compounds are indeed 'drug-like' based on rigid application of decision gateways, such 'close to B-r-o-5 space' compounds are likely to remain challenging to develop.

8. In silico prediction to support selection of formulation strategy: amorphisation vs lipid-based formulations

Selection of an appropriate enabling formulation strategy is still based mainly on results obtained from experimental screening assays [94–96]. Indeed, the properties of the API are often largely ignored when choosing the most appropriate strategy and a large number of standard formulations are simply tested in a somewhat technology-agnostic fashion. To some extent the selection of which formulation strategy to pursue seems to be based on previous experience, i.e., if a formulator has a background with cyclodextrins or lipid-based formulations (LBFs) then these excipients are likely amongst the first to be explored whereas another formulator with experience of amorphisation may use this as a first 'go-to' strategy. There is significant potential therefore, to better inform these decisions if knowledge-based computational tools can be developed to forecast ideal formulation strategies based on the molecular structure of the drug. In the sections below the first steps towards computational tools that allow prediction of the molecular properties that predispose to the utility of amorphisation, as a means to address solid-state limited solubility, and LBFs, as a means to address solvation-limited compounds, are described. The in silico methods currently available for assessment of 'formulate-ability', i.e. models predicting which enabling formulation to match with a particular API, typically apply multivariate data analyses and Molecular Dynamics (MD) simulations. Performance of formulations can be

evaluated in silico with PK software such as SimCyp, GastroPlus and STELLA and examples of recent computer-guided LBF performance analyses are provided by O'Shea et al. [97], Fei et al. [98] and Zheng et al. [99]. It should be noted that often a certain amount of in vitro data is needed as input for these simulations to be accurate and that entirely in silico approaches remain out of reach.

8.1. Amorphous formulations

Amorphous formulations are used to diminish the impact of the solid-state on dissolution and solubility. In the amorphous state no long range order remains and the intermolecular interactions in the solid form are therefore weaker than those in the crystalline form. A number of process technologies can be used to produce the amorphous material, the most common being rapid evaporation of solvents (spray-drying), freeze-drying, increased temperature (melt extrusion, melt quenching), anti-solvent precipitation and mechanical activation [100]. The idea behind using amorphous material for drug delivery is that the amorphous solubility is typically much higher than the crystalline solubility and hence, a supersaturated solution is easier to obtain from the amorphous form. This increase in concentration is then directly related to an increase in flux across the intestinal wall since in a supersaturated solution the molecules exist as monomers [101]. Amorphous materials and supersaturated solutions are both unstable; polymers are therefore used in the formulations to improve product stability and the stability of the supersaturated solutions formed on dissolution by hindering nucleation and crystal growth. As soon as crystal growth starts, the solubility enhancing effect is lost since the material then re-attains the properties of the crystalline material.

The rate of nucleation and crystal growth in the formulation is affected by the dynamics, i.e., molecular mobility, of the amorphous phase. The transformation from the amorphous to the crystalline form is driven by thermodynamics; the crystalline form is the energetically favourable and hence, more stable form. However, the nucleation and crystallisation are results of kinetic barriers that have to be overcome, and different conditions may therefore result in different times for nucleation (induction time). A detailed analysis on the thermodynamic and kinetic aspects of nucleation and crystal growth is provided by Taylor and Zhang [102]. The glass stability is often determined by measurement of the rate at which the amorphous material is transformed into its crystalline counterpart and the glass transition temperature (T_g) has been used to indicate glass stability. However, there are also reports of alterations in amorphous state properties and stability as a function of changes to production methods without any significant alterations in T_g [103,104]. On the other hand, T_g has been used to define suitable storage temperatures and is able to identify drugs for which amorphous formulations are unlikely to be stabilised [105]. Computational tools that can predict T_g are therefore warranted, and recently the first attempts to achieve in silico prediction of T_g were published [106]. The resulting SVR model predicted the test set ($n = 24$) with an RMSE of 18.7 K. In other words, the T_g could be predicted with greater accuracy than Tm (discussed in Section 4.1). The important molecular descriptors for T_g were related to the number of hydrogen bonds, the number of ring structures and the maximum sigma Fukui index. Whilst hydrogen bonds and number of rings are easily understandable the sigma Fukui index may be a more difficult descriptor to understand. Hydrogen bonds have been shown to be a positive factor for good glass-forming ability [107], whereas ring structures have been related, amongst others, to the ease by which a compound can order itself in a dense packing structure in the crystal [52,108]. The sigma Fukui index provides information on local reactivity of sigma electrons in the molecule, and can be further decomposed into nucleophilicity and electrophilicity of the molecule [109].

Glass-formation, i.e., the ability of an API to exist in its amorphous state (using relatively standardised production technologies and assessment of amorphous content at room temperature) is related to hydrogen bonding capacity, molecular volume, and number of rotatable bonds [110–112]. The usefulness of these and other calculated molecular properties to predict glass-forming ability and glass stability has been explored [52,113–115]. The first attempt to predict the glass-forming ability was based on a dataset of 32 compounds. This dataset showed that molecular descriptors related to aromaticity, symmetry, distribution of electronegative atoms, branching of the carbon skeleton and molecular size could be used to accurately sort compounds into glass-formers and non-glass-formers [52]. The properties reflect, to some extent, the configurational entropy (branching of carbon skeleton and molecular size). This property favours glass formation. When a large number of conformers interact with each other, the chances increase that such molecules "lock in" solid states other than the stable polymorph. Aromaticity (described by the number of benzene rings) and symmetry also impose directionality in the interaction between molecules; these two factors hinder glass formation. These aromatic features will drive solid state interactions through strong van der Waals interactions as a result of π–π stacking and therefore favour crystallinity. Symmetry, similarly, creates handles between the molecules in their interaction and gives direction to how compounds interact and form intermolecular bonds, again favouring crystallinity. Finally, well distributed electronegative atoms appear to be favourable for glass-forming ability. This may, in part, be because some of these atoms (in particular, nitrogens and oxygens) form hydrogen bonds, an established factor in glass formation. If these atoms are well distributed over the molecule, the molecule has several anchor points for hydrogen bond formation. Based on larger datasets ($n = 50$ and 131) it is evident that molecular weight (Mw) can be used to provide an early indication of glass-forming ability [113,114]. As a rule of thumb, good glass-formers have a $Mw > 300$ whereas compounds with $Mw < 200$ remain in their crystalline form, regardless of production technology. For compounds with Mw between 200 and 300, a molecular descriptor reflecting both the size and shape of the molecule has been shown to differentiate glass-formers and non-glass-formers (crystalline material) [95].

Whilst Mw and molecular descriptors that carry information regarding both size and shape can indicate compounds that can be readily transformed into the amorphous state, they do not provide information of the stability of the amorphous material. In an attempt to identify the properties that dictate stability, a dataset of 77 compounds containing both stable and non-stable glass-formers, was used to extract the molecular properties that correlate with stability (or instability) [113]. For this dataset two descriptors were predictive for stability; the number of hydrogen bond acceptors and the absolute values of Hückel pi atomic charges for C atoms. In another recent study, hydrogen bond capacity and size were the two most important calculated descriptors [116]. These predictions seem to accurately capture important molecular properties for glass stability since the number of hydrogen bond acceptors has also been identified in laboratory experiments on amorphous stability [117]. It should be noted that whilst Mw alone describes glass-forming ability with 86% accuracy, amorphous stability is somewhat less accurately predicted (training and validation datasets were 78% and 69% correctly classified, respectively) [113]. Hence, stability seems more difficult to predict than glass-forming ability. This is expected since the glass-forming ability only identifies whether the material can form a glass or not, i.e. the potential to "lock in" a solid state structure other than that obtained in a crystalline material with long range order. In contrast, stability models also have to capture the mobility of the amorphous form. Most importantly, the computational models have contributed to the molecular understanding of amorphous materials (Fig. 6).

However, the API has to be formulated with stabilising excipients to achieve the positive effects of amorphisation and improve drug delivery. Theoretical analyses of e.g. polymer–drug interaction, miscibility of excipients and solvents have historically been performed making use of the Flory Huggins equation or the Hansen Solubility Parameters

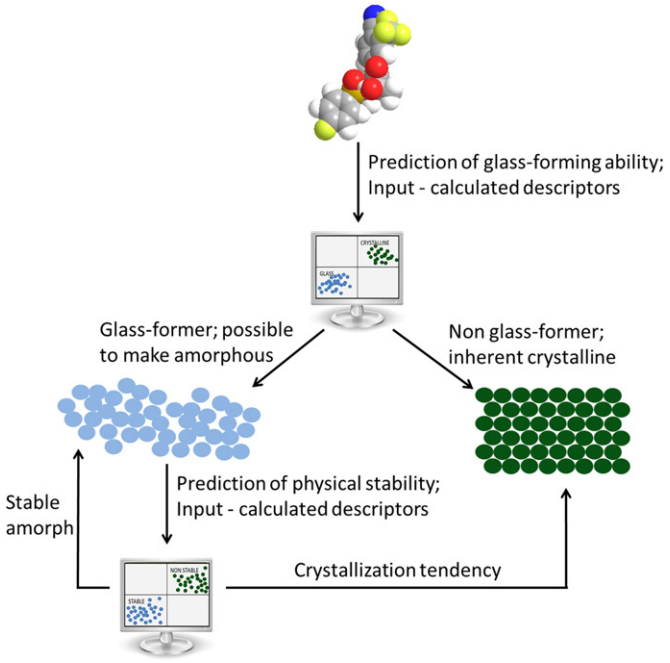

Fig. 6. Computational prediction of glass-forming ability and physical stability from molecular structure of the drug. Computational models obtained using multivariate data analysis are accurately sorting compounds into glass-formers and non-glass-formers based on molecular descriptors as only input [52,113,114]. Typical properties of importance for amorphisation are molecular size and intermolecular hydrogen bond capacity, both of which are positive for the production of an amorphous material. Rigidity, aromaticity and number of benzene rings are strongly related to the formation of a stable crystal lattice; these properties drive crystallisation. Glass-formers can thereafter be further studied with regard to their tendency to crystallise in the solid form and computational models are available for the prediction of physical (dry) stability [113,114]. To our knowledge no computational models exist that can predict stability of amorphous materials exposed to water. This property is of utmost importance for compound performance after oral administration and therefore, computational models predicting this property are actively sought. Current strategies to arrive at such models are making use of MD simulations (see e.g. [120–123]).

[118,119]. More recently MD simulations have been explored for its potential use in such analysis. MD simulations study the physical movements of atoms and molecules, and dependent on the resolution scale applied, can be used to study movements (folding, diffusion, aggregation etc.) of large molecules in complex, multicomponent systems. MD simulations are well-established in biophysics, e.g. for studies of cellular membranes and proteins, and has recently been introduced as a tool to better understand formulation and dosage form design. In the field of amorphous drug formulations MD has been used to investigate molecular interactions between the API and stabilising polymers typically used in amorphous solid dispersion (ASD) [120–123]. Xiang and Anderson have published a series of papers in which they explore molecular interactions in relatively complex systems. Recently, they explored indomethacin together with the commonly used polymer HPMCAS to investigate the interactions between such ASDs and water. The simulations were performed in the presence of water (0.7–13.2 %w/w) to better understand processes occurring as a result of water being adsorbed by the polymer during storage or processes involved during release to a water-based environment. The MD simulations were used to calculate the density and the T_g and both these were in good agreement with experimental data. At a macroscopic level it could also be observed that the water molecules were isolated at low water content but formed clusters or strands at high concentrations. The water acted as plasticisers resulting in increasing polymer mobility and water diffusivity at higher water content. The movement of the water in the polymer was 'hopping' at low concentrations whereas at higher concentrations water molecules were found to move fast within the water clusters but slowly diffused through the polymer matrix [122]. Xiang and Anderson have performed similar studies for other pharmaceutical polymers, and have been successful in simulating the density, water sorption isotherm and T_g of poly(lactide) (PLA) as well as molecular interaction and miscibility between indomethacin and polyvinylpyrrolidone (PVP) [121,124]. These studies are examples of ongoing computational efforts to explore complex processes that take place in formulations.

To summarise, it has during the last years become evident that computational simulation methodologies are available that may inform us on formulation performance of amorphous products during storage and during dissolution and release in vivo. In the not too distant future, decisions on whether or not to pursue amorphous formulations for a particular API may therefore be computationally-informed at several levels. We foresee the following scenario: a rule-based system based on easily and rapidly calculated molecular properties to determine whether the API is a glass-former and if so, whether it will suffer from stability issues. This is followed by MD simulations of the API together with a number of polymers using different mechanisms for stabilisation, and lead polymers are identified in silico. Initial experimental trials of ASD formulations may thereafter be attempted with drugs that have been proven suitable for the technology and with the excipients that are most likely to promote physical stability and maintain supersaturation once the dosage form is dissolved.

8.2. Lipid-based formulations

LBFs are mixtures of lipids, surfactants and/or cosolvents, and are commonly employed to enhance the oral bioavailability of poorly water-soluble lipophilic drugs. This is achieved firstly by overcoming traditional solid–liquid dissolution limitations to absorption (since the drug is typically predissolved in the formulation) and secondly by providing lipids and surfactants that boost the solubilisation capacity of the GIT. For a more detailed review of LBF development, performance and utility, the reader is referred to Feeney et al. in this theme issue [125]. Although LBF suspensions are possible and have been commercialised, lipid solution formulations are preferred since they simplify manufacture and accurate capsule filing, and often lead to less variable in vivo performance. An important condition for successful use of an LBF is therefore that the drug dose can be adequately dissolved in the lipid system used [7,126]. In silico prediction of lipid solubility is therefore a key goal to enhance the speed and utility of LBF development.

Recent studies of lipid systems have used the log-linear model [127,128], or related models [129–131] to estimate drug solubility in complex lipid mixtures. The log-linear model is based on studies of mixtures of water and cosolvents. This methodology, and its variants, requires experimental solubility measurements and hence, a large amount of compound needs to be synthesised. The experiments are also time consuming, relatively labour intensive and only of medium through-put. Computational prediction of drug solubility in commonly used excipients, or in the LBF itself, would therefore be beneficial and can be expected to increase the throughput and lower the costs of LBF development. Multivariate data analysis was recently employed to develop models that could predict drug solubility in single excipients [56,132]. The dataset consisted of ~40 structurally diverse compounds determined in nine key LBF excipients. This dataset indicates that computationally predicted solubility values obtained from PLS models of each excipient can successfully predict loading capacity of complex LBFs (Fig. 7). To arrive at the final loading in the more complex LBF, the predicted solubility in each excipient is summed after correcting for the weight fraction of each excipient [132]. The molecular descriptors found to be detrimental to good solubility in glyceride lipids are the numbers of nitrogens and conjugated double bonds [56]. The latter partly reflects rigidity and aromaticity. Common features for compounds that have high lipid solubility are that they are neutral or

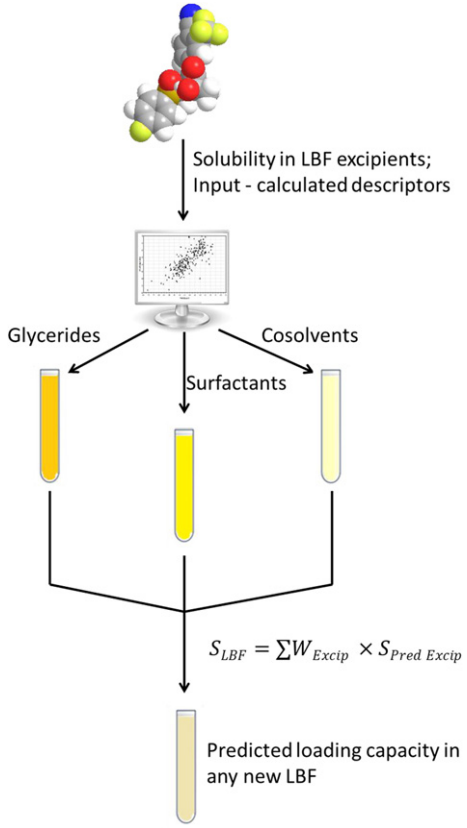

Fig. 7. Computational prediction of drug loading in lipid-based formulations (LBFs). Recently the first attempts to predict solubility in excipients commonly used in LBFs have been published [56,132]. Whilst it has proven possible to predict solubility in glycerides from molecular structure alone, knowledge of the melting point and entropy of fusion improve these models. More importantly, they are required to produce accurate models for ethoxylated surfactants and cosolvents. Important properties driving solubility in lipids are low polar surface area and low melting point. Non-protolytes and weak bases seem to be better solvated by the excipients than acidic compounds. When solubility values have been predicted in the excipients ($S_{Pred\ Excip}$), the loading capacity in any new LBF can be calculated by summing the contribution of each excipient (solubility corrected for the weight fraction, W_{Excip}). Hence, the potential utility of a LBF strategy (at least in terms of potential drug dose) for a particular compound can be assessed early in development using computational approaches alone.

basic, elongated with a certain degree of molecular flexibility, have low PSA and a Tm<150 °C [132].

MD simulations have also been used to study internal structure of LBFs upon dilution in water, largely to better understand the solvation capacity of these formulations when administered with a glass of water [7,133–137]. In a recent study the liquid phase behaviour of sodium oleate, sodium laurate and water was explored to better understand colloidal interactions [133]. The complete ternary phase diagram was reproduced in silico and the simulations identified the three phases (micellar, hexagonal, lamellar) found experimentally. This study shows that MD simulations of systems including naturally available lipids and excipients found within LBFs are feasible and produce accurate structures. A remaining challenge is to perform these simulations in solvents mimicking the fasted and fed intestinal fluids, in which naturally available surfactants (bile salts) and phospholipids influence the formation of nanoaggregates. The intestinal fluid is also dynamic and its solvation capacity changes in response to dilution, digestion, and absorption. When computational simulations can capture such dynamic changes, the intra- and interindividual variability in solvation capacity and the performance of the LBFs may be possible to predict computationally.

9. Conclusion

This work highlights the potential utility of computer-based methods to identify likely formulation options in early drug development. These methods can also be applied to bridge the drug discovery and early development settings and are applicable at several different levels. These include early identification of the likelihood of solubility-limited absorption and potential food effects, as well as a better understanding of the molecular determinants of low solubility. Further they can be used to indicate prospective formulation-dependence of drugs aimed at particular drug targets. Finally, computational methods have the potential to identify the most appropriate formulation approach based on the molecular properties of the drug. These computational approaches will accelerate knowledge-based decision making for drug delivery and formulation science and speed dose form optimisation for a particular API. The computational biopharmaceutical profiling tool enables the influence of target biology on potential drug delivery strategies to be assessed very early in the discovery cycle. This information will help guide project teams during the early target identification and lead optimisation process. This type of computational analysis only requires information calculated from the molecular structure of the ligands and therefore has the potential to provide a virtual signal of the need for adoption of enabling formulation strategies. Early recognition of this need has the potential to influence adoption of non-traditional (but possibly more appropriate) decision pathways as the project progresses. This in turn is of importance for successful development of the highly lipophilic ligands for B-r-o-5 targets that are the current focus of many discovery programmes.

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