RESEARCH ARTICLE

View Article Online
View Journal | View Issue

Designing safer oral drugs†‡

M. C. Wenlock

Cite this: *Med. Chem. Commun.*,
2017, 8, 571Received 20th December 2016,
Accepted 16th January 2017

DOI: 10.1039/c6md00706f

rsc.li/medchemcomm

Introduction

From the perspective of early (small molecule) oral drug discovery, assessing whether a compound has the potential to be a safe and efficacious drug in humans is paramount, and the ability to do this as early as possible would minimise the efforts spent on compounds that are not drug-like. An oral drug can be defined as a compound that achieves *in vivo* plasma concentration (C_p) free levels that are both efficacious and safe for a given oral dosing regimen in humans, and the balancing of a compound's efficacy, safety and pharmacokinetics (PK) can be simplified to the requirement that its PK window is less than or equal to its therapeutic window. In this scenario, a compound's therapeutic window reflects the ratio of its highest C_p level where no observable adverse effects occur (*i.e.*, $C_{p,NOAEL}$) to its required efficacious C_p level (*i.e.*, $C_{p,effective}$), and a compound's PK window reflects its steady state peak-to-trough C_p fluctuation (*i.e.*, $C_{p,max}/C_{p,min}$) (see Fig. 1).

In early drug discovery, there are various ways of estimating a compound's PK window, including methods that employ machine learning techniques.^{1–4} The method proposed

Designing an oral drug such that its estimated dose to humans is both efficacious and safe is challenging. During the early design stage, where *in vitro* or preclinical species *in vivo* safety data are limited, heuristic-based criteria often related to physicochemical properties are used for guidance. The causal relationship between a compound's log P and its human *in vivo* toxicity is considered. With respect to designing efficacious oral drugs that potentially have reduced toxicity liabilities, an alternative heuristic-based criterion is proposed based on the amount of compound in the body at steady state. In humans, a threshold for the amount of compound in the body at steady state of 0.5 mg kg^{-1} is suggested. The criterion is based on the minimum toxic blood–plasma concentration that produces clinically relevant side effects or symptoms in humans for 242 oral drugs. It can be used to estimate a therapeutic window against which a compound's estimated *in vivo* plasma levels for a particular dose size and frequency can be assessed. The relationship between this criterion and acceptable oral dose sizes for different charge types with different *in vivo* plasma clearances is discussed.

by Wenlock⁴ is based on the following assumptions: (i) plateau C_p levels can be estimated using a one-compartment PK model that assumes instantaneous distribution and simulation of a multiple-dose regimen; (ii) dose-independent PK for the dose regimen considered; (iii) efficacy and toxicity responses are directly proportional to free C_p levels; (iv) at plateau, a compound is sub-therapeutic if its free $C_{p,min}$ is less than its free $C_{p,effective}$; (v) at plateau, a compound is toxic if its free $C_{p,max}$ exceeds its free $C_{p,NOAEL}$ (*i.e.*, toxicity is driven by $C_{p,max}$). To estimate a compound's therapeutic

Fig. 1 Therapeutic and PK windows.

InSilicoLynx Ltd, BioHub at Alderley Park, Mereside, Alderley Park, Cheshire,
SK10 4TG, UK. E-mail: mark.wenlock@insilicolynx.com

† In carrying out this work, the author has used computational tools supplied by InSilicoLynx Ltd, of which he is the sole company share owner. InSilicoLynx Ltd is a member of the RSC Enterprise Plus program.

‡ Electronic supplementary information (ESI) available: A file containing the relevant compound data; a file containing the dose simulation data; a document describing the technical details associated to the *in silico* human V_{ss} model. See DOI: 10.1039/c6md00706f

window, its $C_{p, \text{effective}}$ can be initially based on an *in vitro* potency value along with the use of an empirically derived value for free $C_{p, \text{NOAEL}}$. For an unknown compound, a conservative estimated free $C_{p, \text{NOAEL}}$ of 100 nM in humans has been suggested.⁴ This was based on the observation that of 104 drugs with reported human toxicity levels, most displayed no clinically relevant side effects or symptoms at, or below, this level.⁵ The purpose of this study is to consider a larger set of 385 compounds with toxic blood–plasma concentrations extracted from the same data set (compiled by Schulz *et al.*⁵), along with data on human plasma volume of distribution at steady state (V_{ss}), to offer refinements to this heuristic-based free $C_{p, \text{NOAEL}}$ criterion.

Experimental

Estimating human $C_{p, \text{NOAEL}}$

A compound's (total) human $C_{p, \text{NOAEL}}$ is assumed to be the minimum toxic blood–plasma concentration (mg L^{-1}) that produces clinically relevant side effects or symptoms in humans, as reported by Schulz *et al.*⁵ – for the 1023 substances listed, 445 compounds had a value. More accurately, the $C_{p, \text{NOAEL}}$ is the highest C_p level where no adverse effects were observed and the minimum toxic blood–plasma concentration is the lowest C_p level where adverse effects were observed. In theory, any concentration below the minimum toxic blood–plasma concentration could be considered safe, hence these two terms can be considered synonymous.

Molecular data

The molecular structure (*i.e.*, SMILES) for a substance with a human $C_{p, \text{NOAEL}}$ was extracted from the European Bioinformatics Institute's ChEMBL database⁶ by matching the Schulz *et al.*⁵ substance name against the ChEMBL name or synonym name database fields. In total, 415 compounds (93%) with a human $C_{p, \text{NOAEL}}$ value had a SMILES string that could be found within the ChEMBL database and, where necessary, associated salts were removed. The ACD most acidic pK_a and most basic pK_a were retrieved from the ChEMBL database, and these predictions were manually checked against structure to determine a compound's charge type at pH 7.4; a significant acidic pK_a had a value of 7.4 or less and a significant basic pK_a had a value of 7.4 or more. Fifteen compounds were removed for having a molecular weight (Mwt) less than 120 Da (acetaldehyde, acetone, 4-aminopyridine, aniline, benzyl alcohol, butanone, ethanol, ethyl chloride, ethylene glycol, isopropanol, methanol, phenol, piperazine, propylene glycol, thiocyanate) and five more were removed for having a Mwt greater than 850 Da (amphotericin B, sirolimus, suramin, teicoplanin, vancomycin). In addition, five compounds were removed for having a quaternary charge type (diquat, edrophonium, pancuronium, paraquat, thiazinamium) and a further five compounds were removed for having more than two significant pK_a s at pH 7.4 (amikacin, kanamycin, lisinopril, streptomycin, tobramycin). The final data set was composed of 385 compounds with Mwt ranging from 120 to 850 Da and

a charge type definition at pH 7.4 of any of the following: acid (monoacid or diacid), neutral, base (monobase or dibase) or zwitterion. As an estimate of a compound's logarithmic base-10 partition coefficient between an aqueous buffer phase and an octan-1-ol phase (*i.e.*, $\log P$), the ACD $\log P$ prediction was also retrieved for these 385 compounds from the ChEMBL database.

Estimating human $C_{p, \text{effective}}$

A compound's (total) human $C_{p, \text{effective}}$ is assumed to be the minimum therapeutic blood–plasma concentration (mg L^{-1}) in humans, as reported by Schulz *et al.*⁵ These values were assumed to be based on the trough C_p levels at steady state. Of the final data set comprising 385 compounds, 347 were identified with a value.

Oral drug status

Compounds were identified as having been approved as an oral drug by searching the oral database field within the molecule dictionary database table found within the ChEMBL database. In total, 242 of the 385 compounds were identified.

Human V_{ss}

The ChEMBL database was searched for measured human V_{ss} (L kg^{-1}) data and a value found for 189 of the 385 compounds. These values were derived from six publications, and where repeat measurements were found the mean value was reported.^{7–12} For compounds without a measured human V_{ss} , a prediction *via* an *in silico* model was considered acceptable.¹³ Details of how the *in silico* human V_{ss} model was built can be found in the ESI.†

Results and discussion

Log P and toxicity

Log P , whether measured or predicted *via in silico* methods, is a useful gauge of hydrophobicity, and when considering the thermodynamics of a compound's transfer between aqueous and non-aqueous phases, the entropic component associated with the change in free energy plays a significant role (at 298 K).¹⁴ The entropic benefit of removing a hydrophobe from an aqueous environment, in part resulting from the breaking of the ordered hydrogen bond network of the surrounding water molecules, is relevant to understanding how a compound behaves within a complex *in vivo* system. Having simple rules based on log P , as well as other bulk physico-chemical parameters,¹⁵ can offer valuable insight at the design stage, albeit any causality is often argued in broad terms.

The connection between a compound's log P and its propensity to be toxic has been discussed previously. Notably, Leeson and Springthorpe¹⁶ introduced the concept of maximising ligand-lipophilicity efficiency (LLE) to reduce *in vitro* promiscuity; lowering the contribution of hydrophobic binding¹⁷ to the overall free energy associated with a

ligand binding to a specific receptor may reduce its off-target effects. Although a compound's *in vitro* promiscuity does not necessarily imply an *in vivo* adverse drug response, both Hughes *et al.*¹⁸ and Price *et al.*¹⁹ found that the risk of a toxicological outcome in preclinical species at fixed plasma exposure levels (10 μM total drug and 1 μM free drug) was lower for hydrophilic/polar compounds. Wenlock⁴ showed for 104 drugs a decreasing linear dependence on $\log P$ for both total and free human $C_{\text{p,NOAEL}}$ (nM).

To expand upon these observations, the correlation between the (total) human $C_{\text{p,NOAEL}}$ (nM) and $\log P$ for 385 compounds was considered. Free C_{p} levels are typically derived from measured total C_{p} levels subsequently adjusted for fraction unbound in plasma (f_{u}). As $\log P$ and f_{u} are highly correlated, this could complicate any interpretation of a free C_{p} (*i.e.*, total human $C_{\text{p,NOAEL}}$ multiplied by f_{u}) *versus* $\log P$ correlation.⁴ For the purpose of this section, total C_{p} levels are considered.

Fig. 2 shows the linear relationships for the different charge types between the human $C_{\text{p,NOAEL}}$ (nM) and $\log P$ for the 385 compounds: acidic compounds (45), $\log_{10}(\text{human } C_{\text{p,NOAEL}}) = -0.16 \times \log P + 5.12$, $r^2 = 0.06$, $p > 0.05$; neutral compounds (143), $\log_{10}(\text{human } C_{\text{p,NOAEL}}) = -0.40 \times \log P + 4.69$, $r^2 = 0.29$, $p = 3.08 \times 10^{-12}$; basic compounds (183), $\log_{10}(\text{human } C_{\text{p,NOAEL}}) = -0.11 \times \log P + 3.43$, $r^2 = 0.04$, $p = 6.45 \times 10^{-3}$; zwitterionic compounds (14), $\log_{10}(\text{human } C_{\text{p,NOAEL}}) = -0.01 \times \log P + 4.52$, $r^2 = 0.00$, $p > 0.05$. A significant correlation ($p < 0.05$) was only found for the neutral and basic compounds. Collectively, this plot indicates a trend such that compounds with higher $\log P$ are more likely to have a lower human $C_{\text{p,NOAEL}}$. When considering a 10 μM total C_{p} level, the average $\log P$ of the compounds with a higher human $C_{\text{p,NOAEL}}$ is 1.6, compared to 3.0 for those with a lower human $C_{\text{p,NOAEL}}$ – this latter region also includes 87% of all

the basic compounds. However, basic compounds tend to be more highly distributed within the body. Therefore, to further understand Fig. 2, the extent of distribution with respect to $\log P$ should also be considered.

Fig. 3 shows the linear relationships for the different charge types between the human V_{ss} (L kg^{-1}) and $\log P$ for the 385 compounds: acidic compounds, $\log_{10}(\text{human } V_{\text{ss}}) = 0.05 \times \log P - 0.62$, $r^2 = 0.06$, $p > 0.05$; neutral compounds, $\log_{10}(\text{human } V_{\text{ss}}) = 0.08 \times \log P - 0.03$, $r^2 = 0.17$, $p = 3.94 \times 10^{-7}$; basic compounds, $\log_{10}(\text{human } V_{\text{ss}}) = 0.14 \times \log P + 0.25$, $r^2 = 0.22$, $p = 1.34 \times 10^{-11}$; zwitterionic compounds, $\log_{10}(\text{human } V_{\text{ss}}) = 0.06 \times \log P - 0.14$, $r^2 = 0.09$, $p > 0.05$. A significant correlation ($p < 0.05$) was only found for the neutral and basic compounds – this is similar to what was observed for the linear regressions for these charge types, shown in Fig. 2. It follows that any interpretation of the correlations in Fig. 2, in particular for neutral and basic compounds, needs to account for how $\log P$ affects the extent of distribution within the body.²⁰ It is apparent that $\log P$ can influence multiple PK parameters, such that causality is hard to establish in complex *in vivo* systems.

Volume of distribution (V) is a proportionality term that relates the amount of drug in the body at time t (A_t) and the C_{p} at time t (*i.e.*, $C_{\text{p},t}$), as given by eqn (1):

$$V = \frac{A_t}{C_{\text{p},t}} \quad (1)$$

Rearranging eqn (1) allows A_t to be estimated by multiplying $C_{\text{p},t}$ by V . Hypothetically, if the human $C_{\text{p,NOAEL}}$ values being considered are treated as levels achieved under steady-state conditions, it is possible to estimate what can be described as the maximum safe amount of (absorbed)

Fig. 2 $\log_{10}(\text{human } C_{\text{p,NOAEL}} \text{ (nM)})$ *versus* $\log P$ for 385 compounds. Red stars: acids; green stars: neutrals; blue stars: bases; magenta stars: zwitterions; coloured lines: best-fit linear regression for each charge type; grey dashed line: 10 μM level.

Fig. 3 $\log_{10}(\text{human } V_{\text{ss}} \text{ (L kg}^{-1}\text{)})$ *versus* $\log P$ for 385 compounds. Red stars: acids; green stars: neutrals; blue stars: bases; magenta stars: zwitterions; coloured lines: best-fit linear regression for each charge type.

compound in the human body at steady state ($A_{\text{NOAEL,ss}}$) (mg kg^{-1}) by multiplying $C_{\text{p,NOAEL}}$ (mg L^{-1}) by human V_{ss} (L kg^{-1}). Importantly, two compounds can have the same human $A_{\text{NOAEL,ss}}$ but different human $C_{\text{p,NOAEL}}$ (e.g., an acidic and a basic compound both with an $A_{\text{NOAEL,ss}}$ of 2 mg kg^{-1} and a $C_{\text{p,NOAEL}}$ of 4 and 0.5 mg L^{-1} , respectively, would imply a V_{ss} of 0.5 and 4.0 L kg^{-1} , respectively). Focusing on $A_{\text{NOAEL,ss}}$ removes the complication of the influence of $\log P$ on V_{ss} , and any observed dependence between $A_{\text{NOAEL,ss}}$ and $\log P$ would be more convincing evidence for the influence of $\log P$ on *in vivo* toxicity.

Fig. 4 compares each compound's $A_{\text{NOAEL,ss}}$ to its $\log P$ in an attempt to understand the relationship between a molecule's overall hydrophobicity and the amount of compound that can be in the human body at steady state while producing no clinically relevant side effects or symptoms. It shows the linear relationships for the different charge types between the human $A_{\text{NOAEL,ss}}$ and $\log P$ for the 385 compounds: acidic compounds, $\log_{10}(\text{human } A_{\text{NOAEL,ss}}) = -0.08 \times \log P + 0.86$, $r^2 = 0.02$, $p > 0.05$; neutral compounds, $\log_{10}(\text{human } A_{\text{NOAEL,ss}}) = -0.28 \times \log P + 1.02$, $r^2 = 0.19$, $p = 4.07 \times 10^{-8}$; basic compounds, $\log_{10}(\text{human } A_{\text{NOAEL,ss}}) = 0.07 \times \log P + 0.02$, $r^2 = 0.01$, $p > 0.05$; zwitterionic compounds, $\log_{10}(\text{human } A_{\text{NOAEL,ss}}) = 0.11 \times \log P + 0.74$, $r^2 = 0.04$, $p > 0.05$. A significant correlation was only found for the neutral compounds and is suggestive that higher $\log P$ compounds tend to be toxic at lower body amounts. Conversely, for ionisable compounds, no significant correlation (i.e., $p < 0.05$) is observed. The behaviour of basic compounds in Fig. 3 and 4 would suggest that the linear dependence between human $C_{\text{p,NOAEL}}$ and $\log P$ is likely an artefact of the extent of their distribution.

Similar observations to that seen in Fig. 4 can be seen when considering what could be described as the minimum

Fig. 4 $\log_{10}(\text{human } A_{\text{NOAEL,ss}} \text{ (mg kg}^{-1}\text{)})$ versus $\log P$ for 385 compounds. Red stars: acids; green stars: neutrals; blue stars: bases; magenta stars: zwitterions; coloured lines: best-fit linear regression for each charge type.

therapeutic amount of compound in the human body at steady state ($A_{\text{effective,ss}}$) (mg kg^{-1}), which is calculated by multiplying $C_{\text{p,effective}}$ (mg L^{-1}) by human V_{ss} (L kg^{-1}). Fig. 5 shows the linear relationships, for the different charge types, between the human $A_{\text{effective,ss}}$ and $\log P$ for the 347 compounds: acidic compounds (36), $\log_{10}(\text{human } A_{\text{effective,ss}}) = -0.01 \times \log P - 0.20$, $r^2 = 0.00$, $p > 0.05$; neutral compounds (126), $\log_{10}(\text{human } A_{\text{effective,ss}}) = -0.24 \times \log P - 0.03$, $r^2 = 0.13$, $p = 2.58 \times 10^{-5}$; basic compounds (171), $\log_{10}(\text{human } A_{\text{effective,ss}}) = 0.04 \times \log P - 1.11$, $r^2 = 0.00$, $p > 0.05$; zwitterionic compounds (14), $\log_{10}(\text{human } A_{\text{effective,ss}}) = 0.04 \times \log P - 0.43$, $r^2 = 0.01$, $p > 0.05$. A significant correlation was only found for the neutral compounds and is suggestive that higher $\log P$ compounds are efficacious at lower body amounts. Conversely, for ionisable compounds, no significant correlation (i.e., $p < 0.05$) is observed between $\log P$ and human $A_{\text{effective,ss}}$.

A comparison of the estimated therapeutic windows (human $A_{\text{NOAEL,ss}}$ (mg kg^{-1})/human $A_{\text{effective,ss}}$ (mg kg^{-1})) against $\log P$ shows no significant linear relationship (i.e., $p < 0.05$) for neutrals, bases and zwitterions, and only a weakly significant relationship for acids. These charge-type trends are shown in Fig. 6 for the 347 compounds: acidic compounds (36) $\log_{10}(\text{human } A_{\text{NOAEL,ss}}/\text{human } A_{\text{effective,ss}}) = 0.09 \times \log P + 0.77$, $r^2 = 0.12$, $p = 3.71 \times 10^{-2}$; neutral compounds (126), $\log_{10}(\text{human } A_{\text{NOAEL,ss}}/\text{human } A_{\text{effective,ss}}) = 0.01 \times \log P + 1.02$, $r^2 = 0.00$, $p > 0.05$; basic compounds (171), $\log_{10}(\text{human } A_{\text{NOAEL,ss}}/\text{human } A_{\text{effective,ss}}) = 0.03 \times \log P + 1.11$, $r^2 = 0.01$, $p > 0.05$; zwitterionic compounds (14), $\log_{10}(\text{human } A_{\text{NOAEL,ss}}/\text{human } A_{\text{effective,ss}}) = 0.07 \times \log P + 1.18$, $r^2 = 0.09$,

Fig. 5 $\log_{10}(\text{human } A_{\text{effective,ss}} \text{ (mg kg}^{-1}\text{)})$ versus $\log P$ for 347 compounds. Red stars: acids; green stars: neutrals; blue stars: bases; magenta stars: zwitterions; coloured lines: best-fit linear regression for each charge type.

§ An identical plot to that shown in Fig. 6 occurs if the ratio of human $C_{\text{p,NOAEL}}$ to human $C_{\text{p,effective}}$ is used. This also applies to free levels.

$p > 0.05$. The observation for acidic compounds seems contradictory when the trend in Fig. 4 and 5 is considered, but the trend in Fig. 4 is weighted by two very high $\log P$ compounds that do not appear in Fig. 5 or Fig. 6, as they do not have an $A_{\text{effective,ss}}$ value. For basic and zwitterionic compounds, the lack of any significant linear dependency (*i.e.*, $p < 0.05$) with $\log P$ is consistent with the lack of any dependency between $\log P$ and human $A_{\text{NOAEL,ss}}$ and human $A_{\text{effective,ss}}$, respectively. However, for neutral compounds, where significant (*i.e.*, $p < 0.05$) $\log P$ dependencies are observed for both human $A_{\text{NOAEL,ss}}$ and human $A_{\text{effective,ss}}$, there is no significant dependency between $\log P$ and the therapeutic window. In other words, the two separate dependencies seem to have been effectively cancelled out. The observed trend for neutral compounds in Fig. 4 supports the view that higher $\log P$ compounds are toxic at lower body amounts. This may be due to higher $\log P$ compounds having a greater capacity to form significant hydrophobic interactions with secondary targets, leading to toxicity. However, the observed trend for neutral compounds in Fig. 5 supports the view that efficacy is achieved using smaller amounts of a higher $\log P$ compound; this may be due to higher $\log P$ compounds being more potent with respect to their primary target.

The risk of promiscuity, which leads to toxicity, may increase proportionally with $\log P$, but this seems to be overcome by maintaining selectivity to the primary target, as is presumably the case for the neutral compounds in Fig. 6 – a view consistent with those of Leeson and Springthorpe¹⁶ and Gleeson *et al.*²¹ This might suggest that contribution of non-specific hydrophobic binding to a compound's affinity with its primary target is to be minimised, but contributions from hydrophobic interactions in specific molecular regions and orientations may be beneficial. Therefore, any

drug-design criteria based on $\log P$ may need to be contextualised against what constitutes appropriate and inappropriate use of hydrophobic binding.²² Furthermore, although Fig. 2 indicates a trend suggesting that compounds with higher $\log P$ tend to be toxic at lower C_p levels, this must be rationalised against the possibility of a similar trend between $\log P$ and efficacy. In this scenario, modulating a compound's $\log P$ would have no net effect on the size of its therapeutic window.

These observations highlight ambiguities associated with $\log P$ -based criteria for safety. Nonetheless, striving for low $\log P$ compounds within drug discovery is important, arguably for the simple reason of improved aqueous solubility. The C_p (and tissue) levels for an orally dosed compound will be determined by the dose size and frequency, along with absorption, distribution and elimination properties. Provided that dose-independent PK exist, levels can be adjusted by changing the dose regimen. It is well established that a major cause of dose dependency is a compound's aqueous solubility, which can be shown to be inversely correlated to $\log P$.²³ Therefore, low- $\log P$ compounds tend to have better aqueous solubility, permitting more of an oral dose to be available for absorption.

$A_{\text{NOAEL,ss}}$ guidance

Heuristic approaches are often developed by the medicinal chemistry community to facilitate drug design. Lipinski *et al.*'s rule of five¹⁵ is a notable example, providing oral drug-likeness guidance resulting from the collective assessment of the magnitude of certain molecular properties in a set of 2245 drug-like molecules. Such approaches rely on the premise that molecularly similar compounds behave similarly. With respect to a compound's potential to cause toxicity in humans, a simple heuristic approach is to determine a maximum safe amount that can be tolerated within the body through collective assessment of known compounds. As the goal of this study is to develop a safety criterion for oral drugs, it is reasonable that such an analysis should focus on the 242 oral drugs identified from the 385 compounds with $C_{p,\text{NOAEL}}$ data.

Fig. 7a shows a frequency bar graph for the binned human $C_{p,\text{NOAEL}}$ (nM) data, with bases and acids broadly grouped at either end of the binned C_p scale. This plot is simply another representation of a subset of the $C_{p,\text{NOAEL}}$ data plotted in Fig. 2, and the distinct charge-type groups partly reflect the extent of distribution associated with these different classes, as indicated in Fig. 3. Human $A_{\text{NOAEL,ss}}$ (mg kg^{-1}) can be calculated by multiplying $C_{p,\text{NOAEL}}$ (mg L^{-1}) by human V_{ss} (L kg^{-1}) – Fig. 7b shows the associated binned-frequency bar graph. The plot shows that the different charge-type classes are less separated, with the 1 to 10 mg kg^{-1} range being most populated – 41% of the 242 oral drugs considered can be safely tolerated within the body at steady-state amounts ranging from 1 to 10 mg kg^{-1} . From the perspective of early drug discovery, it is prudent to minimise the risk of toxicity when designing compounds. Therefore, a

Fig. 6 $\text{Log}_{10}(\text{human } A_{\text{NOAEL,ss}} (\text{mg kg}^{-1}) / A_{\text{effective,ss}} (\text{mg kg}^{-1}))$ versus $\log P$ for 347 compounds. Red stars: acids; green stars: neutrals; blue stars: bases; magenta stars: zwitterions; coloured lines: best-fit linear regression for each charge type.

Fig. 7 Frequency bar graphs for the binned human $C_{p,NOAEL}$ (nM) (a) and human $A_{NOAEL,ss}$ (mg kg^{-1}) (b) for 242 oral drugs. Red bars: acids; green bars: neutrals; blue bars: bases; magenta bars: zwitterions.

cut-off above which the majority of known oral drugs (*i.e.*, 80% or 90%) appear to be safe could represent a helpful threshold. For the 242 oral drugs considered, 0.5 mg kg^{-1} is a reasonable threshold, corresponding to an 80% cut-off. Such a threshold, which would apply to all four charge types, implies that there is an 80% chance that a compound molecularly similar to the 242 oral drugs considered would be safe at steady-state levels of 0.5 mg kg^{-1} . With respect to a (steady state) human $C_{p,NOAEL}$ criterion (nM) for an unknown compound, this can be calculated by dividing the human $A_{NOAEL,ss}$ (mg kg^{-1}) criterion by human V_{ss} (L kg^{-1}), predicted *via in silico* methods^{3,4,13} (followed by unit conversion from mg L^{-1} to nM). Wenlock⁴ originally proposed a 100 nM (steady state) free human $C_{p,NOAEL}$ criterion, but this study indicates that a compound's (steady state) human $C_{p,NOAEL}$ criterion is partly dependent on its human V_{ss} . By way of illustration, the corresponding (steady state) free human $C_{p,NOAEL}$ criterion for each charge type (using some

typical values for oral drugs taken from Wenlock⁴) is shown in Table 1 (estimated for a compound with a Mwt of 400 Da). The 0.5 mg kg^{-1} human $A_{NOAEL,ss}$ criterion represents an assumed safe amount of absorbed compound that can be present in the body at steady state; this is not the same as the dose size that may be given during an oral dosing regimen.

Estimating the dose size and frequency that leads to peak amounts at the plateau equivalent to this human $A_{NOAEL,ss}$ level requires appropriate modelling based on a compound's PK parameters. The approach used by Wenlock⁴ simulates the plateau C_p levels using a multiple-dose regimen, a one-compartment PK model and assumptions as described above. Fig. 8 illustrates how this human $A_{NOAEL,ss}$ criterion can be used to guide drug design. The plot in Fig. 8 summarises the results of simulations for once-daily dosing over a 14-day period by plotting the estimated maximum safe human dose that achieves a maximum human $A_{NOAEL,ss}$ of 0.5 mg kg^{-1} , increasing with respect to human *in vivo* plasma clearance (Cl). Table 1 lists the representative values for human V_{ss} and human f_u for compounds of different charge type; in all cases, the Mwt was set to 400 Da, human oral bioavailability (F) was set to a fixed value of 50% and the human absorption rate constant (k_a) was set to $1.40 \times 10^{-4} \text{ s}^{-1}$.²⁴ An arbitrary (steady state) free human $C_{p,effective}$ requirement of 10 nM was assumed. Points represented with an open circle indicate a potential maximum safe human dose size that also results in a (steady state) free human $C_{p,min}$ greater than 10 nM across the simulated multiple-dosing regimen – in other words they represent safe and therapeutic doses – and the other doses (*i.e.*, those without open circles) are safe but sub-therapeutic doses. The observed pattern for the open-circle points arises because as Cl increases, so too does the peak-to-trough C_p fluctuation (*i.e.*, $C_{p,max}/C_{p,min}$). It is worth noting that the human $A_{NOAEL,ss}$ criterion of 0.5 mg kg^{-1} is based on the minimum toxic blood-plasma concentration (mg L^{-1}) for 242 oral drugs that produces clinically relevant side effects or symptoms in humans, but no distinction is made regarding the mechanism for the observed toxicity. It has been proposed that a 50 mg daily dose is less likely to be associated with non-selective metabolite toxicity.²⁵ Some of the simulated potential maximum safe human dose sizes are less than this value (points represented as diamonds) but others exceed it (points represented as crosses). With respect to the latter, the sub-therapeutic doses can be ignored, but for those that are deemed to be therapeutic there is a clear disconnect

Table 1 Typical human $C_{p,NOAEL}$ (steady state) criteria for different charge types, assuming a Mwt of 400 Da

Charge type	V_{ss} (L kg^{-1})	f_u	Human $C_{p,NOAEL}$ (nM) (steady state)	Free human $C_{p,NOAEL}$ (nM)(steady state)
Monoacid	0.3	0.04	4167	167
Neutral	1.2	0.23	1042	240
Monobase	3.7	0.24	338	81
Zwitterion	0.9	0.63	1389	875

Fig. 8 Potential maximum safe human dose (mg) versus human CL ($\text{mL min}^{-1} \text{kg}^{-1}$). Red points: acids; green points: neutrals; blue points: bases; magenta points: zwitterions; circles: compound's estimated (steady state) free human $C_{p,\min}$ greater than 10 nM; diamonds: potential maximum safe human dose less than 50 mg; crosses: potential maximum safe human dose greater than 50 mg. Simulations run according to the approach described by Wenlock⁴ (one-compartment PK model, once-daily dosing for 14 days) using the details in Table 1. In all cases the Mwt was set to 400 Da, and the human F and human k_a were set to 50% and $1.40 \times 10^{-4} \text{ s}^{-1}$, respectively.

with previous safety guidance and such hypothetical doses may warrant further investigation.

Conclusions

Within early drug design, in the absence of *in vitro* or preclinical species *in vivo* safety data, this study provides an alternative heuristic-based criterion to physicochemical properties to mitigate against potential human toxicity. This criterion corresponds to a reasonable risk threshold, restricting the amount of compound in the body at steady state to 0.5 mg kg^{-1} . This criterion does not make a distinction between the types of toxicity seen, rather it is based on the minimum toxic blood–plasma concentration that produces clinically relevant side effects or symptoms in humans for 242 oral drugs. The criterion may underestimate the $C_{p,\text{NOAEL}}$ for an unknown compound, and for compounds that are distinct from the 242 oral drugs it may not be applicable. However, this criterion assumes that toxicity is related to C_p levels, and is intended for use with PK methods that simulate such levels based on dose size and frequency, providing an alternative to bulk physicochemical property criteria which can oversimplify the *in vivo* behaviour of a compound.

Acknowledgements

In carrying out this work, the author used computational tools supplied by InSilicoLynx Ltd, of which he is the sole company share owner.

Notes and references

- 1 D. F. McGinnity, J. Collington, R. P. Austin and R. J. Riley, *Curr. Drug Metab.*, 2007, **8**, 463–479.
- 2 K. H. Grime, P. Barton and D. F. McGinnity, *Mol. Pharmaceutics*, 2013, **10**, 1191–1206.
- 3 K. M. Page, *Mol. Pharmaceutics*, 2016, **13**, 609–620.
- 4 M. C. Wenlock, *Med. Chem. Commun.*, 2016, **7**, 706–719.
- 5 M. Schulz, S. Iwersen-Bergmann, H. Andresen and A. Schmoldt, *Crit. Care*, 2012, **16**, R136.
- 6 European Bioinformatics Institute's ChEMBL database, <https://www.ebi.ac.uk/chembl/>, (accessed July 2015); A. P. Bento, A. Gaulton, A. Hersey, L. J. Bellis, J. Chambers, M. Davies, F. A. Kruger, Y. Light, L. Mak, S. McGlinchey, M. Nowotka, G. Papadatos, R. Santos and J. P. Overington, *Nucleic Acids Res.*, 2014, **42**, 1083–1090.
- 7 F. Lombardo, R. S. Obach, M. Y. Shalaeva and F. Gao, *J. Med. Chem.*, 2002, **45**, 2867–2876.
- 8 F. Lombardo, R. S. Obach, M. Y. Shalaeva and F. Gao, *J. Med. Chem.*, 2004, **47**, 1242–1250.
- 9 C. A. Evans, L. J. Jolivet, R. Nagilla and K. W. Ward, *Drug Metab. Dispos.*, 2006, **34**, 1255–1265.
- 10 R. S. Obach, F. Lombardo and N. J. Waters, *Drug Metab. Dispos.*, 2008, **36**, 1385–1405.
- 11 X. Sui, J. Sun, H. Li, Y. Wang, J. Liu, X. Liu, W. Zhang, L. Chen and Z. He, *Eur. J. Med. Chem.*, 2009, **44**, 4455–4460.
- 12 M. V. S. Varma, R. S. Obach, C. Rotter, H. R. Miller, G. Chang, S. J. Steyn, A. El-Kattan and M. D. Troutman, *J. Med. Chem.*, 2010, **53**, 1098–1108.
- 13 M. P. Gleeson, N. J. Waters, S. W. Paine and A. M. Davis, *J. Med. Chem.*, 2006, **49**, 1953–1963.
- 14 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 1971, **71**, 525–616.
- 15 C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeny, *Adv. Drug Delivery Rev.*, 1997, **23**, 3–25.
- 16 P. D. Leeson and B. Springthorpe, *Nat. Rev. Drug Discovery*, 2007, **6**, 881–890.
- 17 D. H. Williams, M. S. Searle, J. P. Mackay, U. Gerhard and R. A. Maplestone, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 1172–1178.
- 18 J. D. Hughes, J. Blagg, D. A. Price, S. Bailey, G. A. DeCrescenzo, R. V. Devraj, E. Ellsworth, Y. M. Fobian, M. E. Gibbs, R. W. Gilles, N. Greene, E. Huang, T. Krieger-Burke, J. Loesel, T. Wager, L. Whiteley and Y. Zhang, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 4872–4875.
- 19 D. A. Price, J. Blagg, L. Jones, N. Greene and T. Wager, *Expert Opin. Drug Metab. Toxicol.*, 2009, **5**, 921–931.
- 20 P. Barton, A. M. Davis, D. J. McCarthy and P. J. H. Webborn, *J. Pharm. Sci.*, 1997, **86**, 1034–1039.
- 21 M. P. Gleeson, A. Hersey, D. Montanari and J. Overington, *Nat. Rev. Drug Discovery*, 2011, **10**, 197–208.
- 22 M. M. Hann, *Med. Chem. Commun.*, 2011, **2**, 349–355.
- 23 M. C. Wenlock and P. Barton, *Mol. Pharmaceutics*, 2013, **10**, 1224–1235.
- 24 M. C. Wenlock, *Med. Chem. Commun.*, 2016, **7**, 1995–2002.
- 25 D. A. Smith and R. S. Obach, *Drug Metab. Dispos.*, 2005, **33**, 1409–1417.