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Physiochemical drug properties associated with in vivo toxicological outcomes

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ABSTRACT

Relationships between physicochemical drug properties and toxicity were inferred from a data set consisting of animal in vivo toleration (IVT) studies on 245 preclinical Pfizer compounds; an increased likelihood of toxic events was found for less polar, more lipophilic compounds. This trend held across a wide range of types of toxicity and across a broad swath of chemical space.

Recent high-profile withdrawals of marketed drugs have highlighted both the importance and the difficulty for the pharmaceutical industry of developing safe drugs. Even prior to marketing, failure of drug candidates due to safety-related concerns represents a major cost to the industry, accounting for approximately 30% of clinical attrition in 2000.[1] The preclinical cost is perhaps even more significant as safety screening is often the final hurdle in the drug discovery pipeline prior to entry into the clinic, and few effective strategies for avoiding toxicity exist to guide medicinal chemistry design programs before that point.[2,3] Structure–toxicity relationships and in silico models for toxicity, where sufficient data exist to build them, are generally either focused on a narrow type of toxicity or are applicable to a small subset of chemical space. For example, predictive models can be built for binding to the hERG channel, which is associated with QT prolongation,[4] and in silico filters have been developed for small numbers of specific chemical substructures associated with toxicity.[5]

The difficulty in developing predictive molecular models for toxicity comes about because of the diversity of mechanisms that give rise to toxic outcomes. These mechanisms can be grouped into four broad classes according to the causative molecular features or activities: (1) the primary pharmacology or mechanism of action of the molecule under study, (2) the secondary pharmacology of the molecule, (3) the presence of a well-defined structural fragment or toxicophore in the molecule, and (4) the overall physicochemical properties of the molecule.[6] From a molecular design perspective, little can be done at the outset of a drug discovery project to address toxicity arising from primary pharmacology. Extensive experimentation is often required to establish a clear link between a mechanism of action and a particular toxicity, and this will usually not have been completed at that point. An example of this is the link between PDE4 inhibition and emesis in animals[7] and humans.[8] Useful information is emerging about the second and third classes of toxicity, including associations between secondary pharmacology[9] or defined structural fragments (toxicophores or structural alerts) and adverse outcomes.[10,11] For these classes of toxicity, and explicitly for the fourth class, it is reasonable to expect that some general physicochemical trends might be found that would be useful in guiding molecular design decisions by medicinal chemistry project teams toward safer regions of chemical space.

Animal in vivo toleration (IVT) studies are utilized in the preclinical drug development process when candidate compounds

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have been synthesized that have desirable levels of potency for the target of interest and acceptable pharmacokinetic parameters. These studies have the advantage of being agnostic with regard to the details of the mechanism of toxicity. With a single study, the likelihood of seeing an adverse event in any particular organ can be ascertained, regardless of whether the toxicity is compound-based or pharmacology-based, either primary or secondary. Since these are usually the first experiments in which a compound will have been tested in an in vivo toxicology setting, little optimization for safety will have been done beyond avoidance of well-known liabilities. Therefore, these experiments provide a unique opportunity to extract unbiased physicochemical property–toxicity associations. Overall physicochemical properties of drugs have been associated with bioavailability, for example with the well-known 'Rule of Five'.[12] However, to our knowledge, little has been done to examine possible correlations between compound physicochemical properties and in vivo adverse outcomes. Toward this end, we collected data from in-house animal toxicity and exposure studies performed on potential drug candidates at Pfizer over the past five years. We assessed the likelihood of observing an adverse outcome in these studies as a function of a broad set of physicochemical properties. Additionally, for the subset of compounds for which we had adequate screening data, we assessed whether promiscuous secondary pharmacology could be linked to a propensity for toxicity.

All in vivo toleration (IVT) studies that were performed in rats or dogs at Pfizer over five years (2002–2006) and for which corresponding summary pharmacokinetic exposure data (C_{max} and AUC) could be found were included in this analysis. These studies typically involved dose escalation over 3 or more dose levels and had a duration of 4 days or longer. No distinction was made regarding the sex of the animals assessed in the studies; roughly 60% involved males only, 30% both, and 10% female only. All routes of administration were included although most compounds were orally dosed (98%). The list of organs evaluated, histopathology endpoints, and signs and symptoms monitored differed across studies depending upon the program needs at the time; however, a common core group of evaluations was available for most compounds studied. A database of 245 compounds was built containing the compound structures, the measured and calculated properties, and the primary pharmacology characteristics of each compound. In cases where the primary pharmacology of the compound had a well-known correlate with a particular adverse in vivo outcome, these compounds were included in the data set but the particular outcomes known to be linked to the primary pharmacology of the compound in question were not included as part of the analysis.

For the results of this analysis to be broadly applicable, it is imperative that the set of compounds be diverse and representative of pharmacological chemical space. Figure 1 shows the molecular property distribution of the IVT compound data set (colored in red) compared to that for a selection of compounds (colored in blue) taken from a diverse subset of the Pfizer file chosen for its coverage of chemical space. While the property distributions of these two sets of compounds are clearly not identical, there is good overall coverage of MW–C log *P*–total polar surface area (TPSA) property space by the IVT compounds. Approximately 50% of the compounds are basic, 40% are neutral, and 10% are acidic.

Given the exposure data and the toxicity determination for each dose in a given IVT study, the intrinsic toxicity of the compound was determined by assessing toxicity at a specific exposure threshold. Within the data set it was interesting to see a close, linear relationship between C_{max} and AUC so C_{max} was selected as the single parameter to represent exposure in the subsequent analysis. Since we have data for only a discrete number of doses for each study, we make the assumption that a toxic outcome at a given exposure

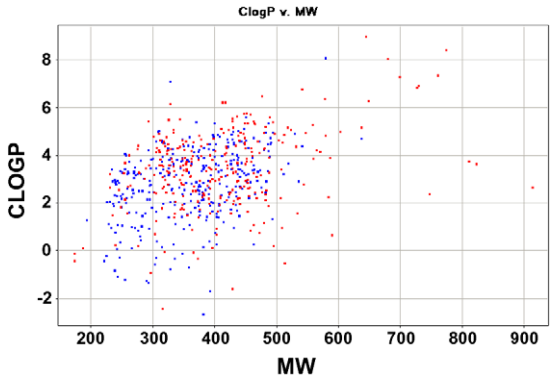

Figure 1. The comparison of the ivt data set with the distribution from a diverse subset selected from the Pfizer file.

implies that the same toxicity would be observed at any higher exposure, and conversely that any clean exposure implies that no toxicity would be found at any lower exposure level. In this way, the data from an IVT study can be used to label each point in the exposure spectrum as toxic, clean, or uncertain, Figure 2.

The particular exposure threshold for this study was then selected to give a balance of toxic and clean classifications. If the threshold is set too high more compounds will be found with significant findings and vice versa. Scanning through different values for the exposure threshold, we selected 10 μM C_{max} (total drug) as a pragmatic endpoint, Figure 3. Only about 20% of the compounds are classified as uncertain using this threshold.

The analysis was performed in parallel using both free-drug and total-drug exposure. The free-drug levels were calculated using plasma protein binding (PPB) measurements from a separate in vitro binding assay. The free-drug data set was smaller due to lack of historical data and unavailability of material for 28% of the compounds. Applying the approach described above to determine the appropriate exposure threshold results in a value of 1 μM free-drug. This 1 μM threshold is consistent with the pharmacological profile of many drug candidates. If a typical compound has 10 nM affinity (K_i) for its primary target, assuming a 3× K_i free-drug level for >75% receptor occupancy (for antagonist pharmacology),[13] then approximately 30 nM free-drug concentration would be required for efficacy. A compound clean at 1 μM free-drug concentration would exceed a 30-fold in vivo safety margin, often considered to be adequate.

Very little variation in the results was seen for different organ groupings; there was an increase in the relative importance of measures of basicity for both kidney and lung. The most significant descriptors using a total-drug-based threshold were measures of polarity, led by TPSA. When a free-drug-based threshold was used,

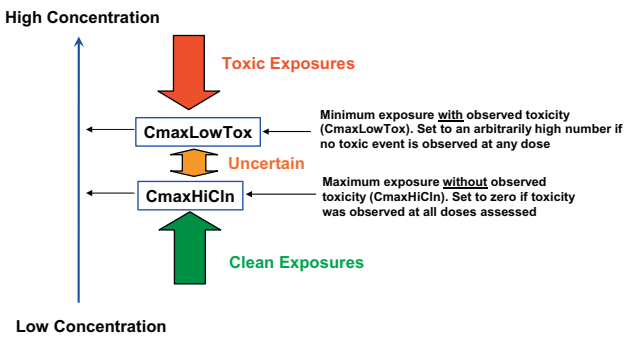

Figure 2. Definition of CmaxLowTox and CmaxHiCln.

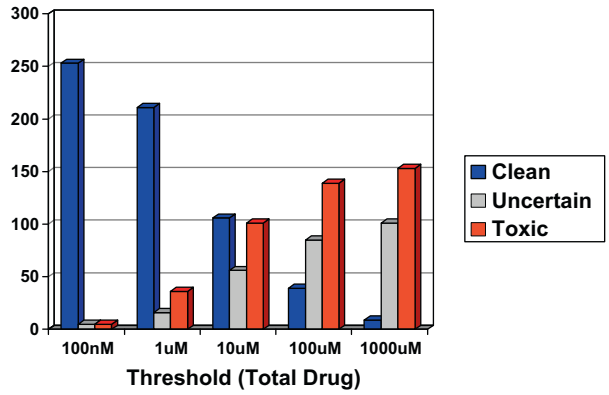

Figure 3. Drug toxicity classification as a function of total-drug exposure.

the measures of polarity were still significant, but plasma protein binding and *C*log*P* rose to the top. Plasma protein binding (PPB) was not significantly associated with toxicity at all when assessed at 10 μM total drug, and *C*log*P* was relatively weakly associated. Care must be taken in interpreting this observation, however, since PPB is involved in the calculation of the free-drug level from the total-drug level. In fact, it can be shown that the strong association of toxicity with PPB at 1 μM free-drug is a direct consequence of the *lack* of an association at 10 μM total-drug exposure. The free-drug level is calculated as $C_{max}(\text{free}) = C_{max}(\text{total})^{*}$ FU, where FU is the fraction unbound or (100 − %PPB)/100. The toxicity classification based on free-drug exposure will be the same as for total-drug exposure for compounds with 90% PPB, at which point the 10 μM total-drug threshold maps precisely to the 1 μM free-drug threshold. For compounds with >90% PPB, the 1 μM free-drug threshold is equivalent to a total-drug exposure above 10 μM, at which the likelihood of a compound being classed as toxic is increased. Therefore, highly protein-bound compounds (>90% PPB), which are equally likely to be toxic as not at 10 μM total drug C_{max}, will appear to have a higher incidence of toxicity when assessed at 1 μM free-drug C_{max}. The reverse holds for compounds with <90% protein binding. Together, this increase in incidence of toxicity for high-PPB compounds and decrease for low-PPB compounds generates a strong association of protein binding with toxicity when assessed at a particular free-drug level. Properties linked with plasma protein binding, such as *C*log*P* and Log*D*, will also appear to be more strongly associated with toxicity based on free drug than on total drug.

TPSA[14] gave the strongest, most consistent correlation of compound physicochemical descriptors to incidence of adverse outcomes across both free- or total-drug analyses. An increased incidence of adverse toxicological outcomes in compounds with low polar surface area is consistent with the ability of these compounds to cross biological membranes and distribute widely into tissue compartments. Indeed, other measures of membrane permeability such as in silico predicted blood brain barrier penetration also correlated with incidence of adverse outcome in this data set (data not shown). Since toxicity in this analysis is assessed at a uniform exposure level of 10 μM total drug, this trend with molecular polarity is not a proxy for general bioavailability. Similarly, we found no significant association between volume of distribution and toxicity, indicating that variation in propensity to distribute into tissues is not the primary underlying reason for the polarity–toxicity trend.

Another descriptor that showed a consistent link with adverse outcomes was *C*log*P*. In the case shown, higher *C*log*P* compounds have a greater incidence of adverse outcomes relative to lower *C*log*P* compounds. Recent work from Gleeson et al. indicates a strong correlation between *C*log*P* and rat or human plasma protein binding in a large data set of diverse compound structures from the GSK corporate file.[15] Therefore, one might expect a correlation between *C*log*P* and plasma protein binding in our data set, which was indeed the case. Having found a few physicochemical properties to be associated with toxicity, given that these properties were not independent of each other, we sought to determine whether one or more of these properties were dominant over the others or whether they displayed any coordinated relationship in their association with toxicity. Since we were dealing with a very small set of properties, we took a manual approach to the problem, and one particular pattern emerged. Table 1 displays the toxicity odds (ratio of toxic to non-toxic compounds) for analyses based on total-drug and free-drug exposure when compounds are separated into four categories based on high/low TPSA/*C*log*P*. We set high/low thresholds for *C*log*P* and TPSA using simple round-number near-median cutoffs of 3.0 and 75 A^2, respectively. In both the free-drug and total-drug cases, it is clear that the dominant trend involves compounds for which both risk factors are present. A mild increase in risk may be associated with compounds having one risk factor but not the other, though the trend is weak and inconsistent across the two different analysis approaches. When both risk factors are present, a very clear, consistent trend emerges. Compounds with low-*C*log*P*/high-TPSA are approximately 2.5 times more likely to be clean as to be toxic, whereas precisely the reverse holds when both risk factors are present: high-*C*log*P*/low-TPSA compounds are approximately 2.5 times more likely to be toxic as to be clean, representing an odds ratio of greater than 6.

Table 1
Observed odds for toxicity versus *C*log*P*/TPSA

Toxicity	Total-drug		Free-drug	
	TPSA > 75	TPSA < 75	TPSA > 75	TPSA < 75
*C*log*P* < 3	0.39 (57)	1.08 (27)	0.38 (44)	0.5 (27)
*C*log*P* > 3	0.41 (38)	*2.4 (85)*	0.81 (29)	*2.59 (61)*

One hypothesis is that the combination of high-*C*log*P* with low-TPSA increases the likelihood of promiscuous binding to off-target pharmacology. To investigate this hypothesis, we acquired profiling data for compounds that had been submitted to the CEREP Bioprint™ panel. This is a set of biological assays for a wide range of targets including a variety of target classes (GPCRs, enzymes, ion channels, etc.) and assay types (agonist, antagonist). A full matrix of assay data was found for 108 compounds by 48 assays. Promiscuity was defined by whether a compound demonstrated >50% activity at 10 μM in three or more assays out of the set of 48. This measure of promiscuity demonstrated a strong correlation with higher *C*log*P* and lower TPSA, and it was highly predictive of toxicity. Furthermore, the variation of promiscuity in *C*log*P*–TPSA space was very similar to that observed for toxicity, Table 2, The strongest effect was again seen only when both risk factors were present, and in this case a 25-fold shift in odds was observed (though this is based on a relatively small sample size). This finding supports a plausible biological rationale for the physicochemical-property/toxicity trends presented here. This agrees with recent work suggesting a link between promiscuity (using a similar defi-

Table 2
Observed odds for promiscuity versus *C*log*P*/TPSA

Promiscuity	TPSA > 75	TPSA < 75
*C*log*P* < 3	0.25 (25)	0.80 (18)
*C*log*P* > 3	0.44 (13)	*6.25 (29)*

nition as here) and attrition, though different physicochemical property trends were found in that analysis.[16]

This analysis is an ongoing work and with further data we will report further findings in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.071.

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