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Use of the Øie-Tozer Model in Understanding Mechanisms and Determinants of Drug Distribution

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TITLE RUNNING HEAD Analysis of the Øie-Tozer Model of Drug Distribution

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ABBREVIATIONS: f_{up}, fraction unbound in plasma; f_{ut}, fraction unbound in tissue; GMFE, geometric mean fold error; $R_{E/I}$, extravascular-to-intravascular binding protein ratio; VD, volume of distribution; VD_{ss}, volume of distribution at steady-state.

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ABSTRACT

Volume of distribution (VD) is a key pharmacokinetic property that together with clearance determines the half-life or residence time of drug in the body. It is commonly expressed as steady state volume of distribution VD_{ss} with a physiological basis for its understanding developed by Øie and Tozer (1979). The Øie-Tozer equation uses terms for plasma protein binding (f_{up}), tissue binding (f_{ut}), the extravascular-to-intravascular ratio of albumin as well as constants for the volumes of plasma, extracellular fluid and tissue. We explored this model using a dataset of 553 drugs for which VD_{ss} and plasma protein binding were available in human. Eighteen percent of cases (102 compounds) did not obey the Øie-Tozer model, with the rearranged equation giving an aberrant f_{ut} ($f_{ut} < 0$ or $f_{ut} > 1$), in particular for compounds with $VD_{ss} < 0.6$ $L.kg^{-1}$ and $f_{up} > 0.1$. Further analysis of this group of compounds revealed patterns in physicochemical attributes with a high proportion exemplified by logP less than zero (i.e. very hydrophilic), polar surface area > 150 $Å^2$, and a difference between logP and logD > 2.5. In addition there was a high representation of certain drug classes including anti-infectives as well as neuromuscular blockers and contrast agents. The majority of compounds were also found to have literature evidence implicating active transport processes in their disposition. This analysis provides some important insights for pharmacokinetic optimization in this particular chemical space, as well as in the application of the Øie-Tozer model for predicting volume of distribution in human.

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INTRODUCTION

Volume of distribution is a key pharmacokinetic parameter relating the systemic concentration of drug to the amount in the body. It is generally considered a theoretical rather than physical term, which can be expressed in various forms including VD central, VD at steady-state and VD terminal (Gibaldi et al., 1969). VD central (VD_c) represents the initial dilution volume of the drug, calculated as dose divided by the initial plasma concentration (C_0 extrapolated) following an intravenous dose, and is usually small as equilibration into tissues has yet to occur. VD terminal (VD_z or VD_β) is calculated as clearance divided by the terminal phase rate constant and represents the stage when distribution is complete with redistribution from tissues to plasma being predominant. As such it is heavily dependent on the terminal phase rate constant and characterization of this phase can prove problematic as the limits of bioanalytical quantification are reached. VD steady-state (VD_{ss}) can be thought of as a 'time-averaged' volume lying somewhere between VD_c and VD_z, and when tissue concentrations have reached a maximum. This VD parameter is calculated as the product of dose and area under the first moment curve divided by the square of the area under the curve and is generally considered most useful in assessing potential dosing regimens and expected accumulation in multiple dosing scenarios.

The determinants of volume of distribution tend to include tissue affinity driven by lipophilic and electrostatic interactions with membrane phospholipids as well as pH partition mechanisms into organelles such as lysosomes (Smith, 1997; Van de Waterbeemd et al., 2001; Lombardo et al., 2002; Lombardo et al., 2004; Obach et al., 2008). Plasma protein binding is also important, and it is generally driven by lipophilicity, plus anionic characteristics due to an electrostatic interaction with a basic residue in the most abundant plasma protein, albumin. Another contributing factor which has received much attention recently is the role of active transport processes in the volume of distribution (Grover and Benet, 2009; Shugarts and Benet, 2009). By analyzing literature data on pharmacokinetic interactions at the transporter level, Grover and Benet (2009) were able to show that the greatest impact

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of transporters on VD was once distribution equilibrium had occurred i.e. VD_{ss} and VD_z. In addition, uptake and efflux interactions at the liver generally decreased VD whilst efflux interactions at the kidney generally increased VD.

Transport of a xenobiotic against its concentration gradient, utilizing ATP hydrolysis or facilitated by an opposing endogenous concentration gradient, is an important process in drug disposition. The proteins responsible are expressed in many tissues including but not limited to intestine, liver, kidney and brain. This has become, over the years, an area of major focus and a multitude of active transporters implicated in drug transport have been identified, cloned and recombinantly expressed (Xia et al., 2008). They typically fall into 2 categories: uptake (from luminal/vascular to tissue) e.g. organic anion transporting polypeptide (OATP); and efflux (from tissue to luminal/vascular) e.g. multidrug resistance protein 1 (MDR1) or P-glycoprotein. The most well-known and widely studied transporter is P-gp (MDR1) identified as playing a key role in limiting oral absorption and CNS penetration as well as mediating biliary excretion of substrates, all as a result of high expression at the gut wall, blood brain barrier and hepatocyte sinusoidal/bile canaliculi membranes respectively.

In 1979, Øie and Tozer (Oie and Tozer, 1979) proposed the model for volume of distribution at steady state described by plasma and tissue drug binding, building on the work of Gillette (Gillette, 1976) by including a term for the extravascular-intravascular ratio of non-specific drug binding sites or amount of binding protein. The primary model assumption is that steady-state is reached via purely passive diffusion phenomena and does not account for active transport of drug against concentration gradients.

More recently, this model was shown to be useful in predicting human VD_{ss}, from animal data, by rearrangement of the equation to describe tissue free fraction. Tissue binding is generally considered to be consistent across species as this is typically driven by hydrophobic and electrostatic interactions with common cellular constituents such as membrane phospholipids. By using VD_{ss} and plasma free fraction in preclinical species to generate a ‘species-independent’ tissue free fraction, this figure could be used

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together with human plasma free fraction in the original form of the model to calculate human VD_{ss} (Lombardo et al., 2002; Lombardo et al., 2004). The aim of the present work was to explore the Øie-Tozer model further using a dataset of VD_{ss} and plasma protein binding values in human on 553 compounds. A reasonable proportion of the dataset (18%) did not obey the model giving rise to aberrant f_{ut} values either less than zero or greater than 1. The sensitivity of the model to the exact value of extravascular-intravascular binding protein ratio was also demonstrated. Moreover, on closer analysis, the violating compounds revealed trends in physicochemical properties and therapeutic class, with the majority of them having literature data supporting their action as substrates of various active transporters. Finally, we show that for these types of compounds, the application of the Øie-Tozer equation could lead to erroneous predictions if aberrant f_{ut} values are not observed in animals, and then used for human VD_{ss} prediction.

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METHODS

This analysis utilized a database of human intravenous pharmacokinetic parameters recently published (Obach et al., 2008). Within this 670 compound dataset, there were 553 compounds for which human plasma protein binding data were available. Data on these 553 compounds formed the basis for this work.

The physiological model for volume of distribution at steady state (VD_{ss}) as defined by Øie and Tozer (1979) is as follows:

$$VD_{ss} = V_P(1 + R_{E/I}) + f_{up}V_P(V_E/V_P - R_{E/I}) + \frac{V_R f_{up}}{f_{ut}}$$

where f_{up} is the fraction unbound in plasma, f_{ut} is the fraction unbound in tissues, $R_{E/I}$ is the extravascular:intravascular ratio of binding proteins (usually 1.4 for albumin). V_P, V_E and V_R refer to the volumes of plasma, extracellular fluid and remainder fluid with values of 0.0436, 0.151 and 0.38 $L.kg^{-1}$ respectively in human. This equation was rearranged to express f_{ut} in terms of VD_{ss} and f_{up} as follows:

$$f_{ut} = \frac{V_R f_{up}}{\left[VD_{ss} - V_P - (f_{up}V_E)\right] - \left[(1 - f_{up})R_{E/I}V_P\right]}$$

The sensitivity of the $R_{E/I}$ index on generating an aberrant f_{ut} value for 102 compounds was assessed by varying this parameter from 0.1 to 2.5 in 0.1 unit increments with all volume terms (V_P, V_E, V_R) held constant.

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Physicochemical descriptors were calculated using Volsurf+ (version 1.0, Molecular Discovery Ltd., UK) and included lipophilicity parameters (logP, $logD_{7.4}$) as well as polar surface area (PSA) and molecular weight. Literature searches for compound related information pertaining to substrates of active transport processes were undertaken using SciFinder 2007.

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RESULTS

The rearranged Øie-Tozer equation expressing f_{ut} in terms of VD_{ss} and f_{up} gave aberrant results for 102 of 553 compounds, where f_{ut} was less than zero or greater than 1. The 102 outlier compounds all had VD_{ss} of less than 0.6 $L.kg^{-1}$, and as illustrated in Figure 1, fall into 2 classes with respect to their plasma protein binding; 12 compounds were highly plasma protein bound with $f_{up} < 0.1$ and VD_{ss} less than 0.2 $L.kg^{-1}$, whilst the remaining 90 compounds had $f_{up} > 0.1$ with a large proportion showing no binding to plasma proteins ($f_{up} = 1$).

Based on this finding, simulations were performed across a range of VD_{ss} and f_{up} values as shown in Figure 2. From this analysis it is clear that for high VD_{ss} (1 and 3 $L.kg^{-1}$) there are no model violation occurrences across the range of f_{up}. However, in the two low VD_{ss} situations (0.3 and 0.5 $L.kg^{-1}$) there are 2 separate threshold values of f_{up} which lead to aberrant f_{ut} values ($f_{up} > 0.3$ and > 0.85 respectively). In the case of very low VD_{ss} (0.1 $L.kg^{-1}$), generally accepted as the distributional volume of albumin, the model fails across the entire range of f_{up}.

In light of the number of model violations (102 out of 553) the sensitivity of the extravascular-to-intravascular ratio of drug binding protein ($R_{E/I}$) parameter was explored. Typically this is set at 1.4 based on the distribution of albumin. For the 102 outlier compounds, simulations were performed varying $R_{E/I}$ from 0.1 (very low extravascular distribution of plasma proteins) to 2.5 (extremely high extravascular distribution of plasma proteins) with 2 separate classes based on extent of plasma protein binding (Figure 3). Incremental increases in $R_{E/I}$ from 1.4 to 2.5 had no impact for either group on the proportion of compounds generating f_{ut} values between 0 and 1. However, a change was observed when $R_{E/I}$ was lowered, with low f_{up} (< 0.1) compounds showing a much higher sensitivity to changes in this parameter. For example, if $R_{E/I}$ is lowered to unity, a substantial number of highly bound compounds (40%) generate f_{ut} values that fall within the acceptable range whilst there is a negligible change for the low binding compounds.

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The physicochemical properties of the 90 outlier compounds, where VD_{ss} is less than 0.6 $L.kg^{-1}$ and f_{up} is greater than 0.1, were investigated and are summarized in Figure 4. Trends were observed with lipophilicity expressed as clogP, polar surface area (PSA), molecular weight and extent of ionization (expressed as the difference between clogP and clogD) relative to the whole dataset of 553 compounds. From these plots it is clear the majority of the aberrant compounds have very low lipophilicity with clogP typically less than zero; 46% of the compounds with clogP less than zero give rise to an aberrant f_{ut}. High polarity is also an important contributing factor with 50% of the compounds with PSA > 150 $Å^2$ also leading to Øie-Tozer model violations. In addition, there is a trend with the degree of ionization where 34% of highly ionized compounds (defined as a difference between logP and logD greater than 2.5 log units) produce aberrant f_{ut} values. The trend with MW is less clear although there are a greater proportion of outlier compounds with high MW in excess of 400.

Possible explanations for the behavior of the 90 outlier compounds with low VD_{ss} and high f_{up} were investigated further. Table 1 shows a considerable number of these compounds have been shown to be substrates for various human active transport proteins.

Furthermore, in order to determine the predictive accuracy of the approach on the 102 violating compounds, calculations of human VD_{ss} were made using measured f_{up} and 3 hypothetical f_{ut} values within the normal range (0.1, 0.5 and 0.9), as might normally be obtained from the rearranged Øie-Tozer equation with preclinical data. The summary statistics are displayed in Table 2.

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DISCUSSION

The analysis presented has demonstrated a number of outlying scenarios for the Øie-Tozer model when VD_{ss} is low (< 0.6 $L.kg^{-1}$). Twelve of the compounds giving rise to aberrant f_{ut} values have f_{up} < 0.1, likely due to variation at the upper end of the plasma protein binding range, where accurate experimental determination of very low free fractions can become analytically challenging. In addition, the model is not applicable for compounds with VD_{ss} of 0.1 $L.kg^{-1}$ or lower: as f_{up} tends to zero, VD_{ss} approximates to 0.105 $L.kg^{-1}$. There is some sensitivity to the exact value of $R_{E/I}$ used for highly plasma bound compounds as might be expected; altering the extravascular-to-intravascular distributional ratio of binding protein has a more significant impact on the f_{ut} as the distribution of these compounds is primarily driven by affinity for plasma proteins such as albumin. In cases of high plasma protein binding, an $R_{E/I}$ value of 1.4 may not be appropriate and so the model should be applied with caution. Alternatively, information on the particular plasma proteins involved in drug binding may allow the $R_{E/I}$ to be tailored to compound-specific predictive application.

The remaining 90 compounds were explored further with respect to physicochemical attributes and possible pharmacokinetic explanations for the model violations. The Øie-Tozer model has a number of assumptions which can aid understanding in cases where aberrant f_{ut} values are obtained. The assumptions are: (1) drug distribution is driven by non-specific binding between blood and tissue, (2) rapid equilibration occurs between blood and tissue, (3) drug distributes uniformly within each organ or tissue, (4) there is no contribution from active transport processes, and (5) distributional processes are non-saturating. In this regard, we have shown a substantial number of the 90 violating compounds have literature evidence supporting their action as substrates of human active transport proteins (Table 1). The transporters cited are typically of an efflux nature, limiting distribution into those tissues expressing the transport protein and consequently contributing toward limiting VD_{ss} to the volume of

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extracellular fluid (0.6 $L.kg^{-1}$) or lower. This is not to say that all active transport substrates violate the Øie-Tozer model e.g. the HIV protease inhibitors and angiotensin receptor antagonists are known to be actively transported and obey the Øie-Tozer model. In addition, it is not the case that low VD_{ss} is the sole driver for failures of the equation. Of the 216 compounds with VD_{ss} < 0.6 $L.kg^{-1}$, 114 obey the model whilst 102 compounds do not. Furthermore, it is not possible to substantiate the involvement of active transport as the exclusive determinant for whether or not model violations will be observed. There are obviously many other considerations including affinity for the transport protein, the magnitude of local, unbound concentrations, and dose size. These complicating factors make it difficult to assess the sensitivity of the model in this respect. For example, some compounds may give rise to back-calculated f_{ut} values that are realistic (i.e. between 0 and 1) but which remain inaccurate due to one or more model assumptions e.g. many of the statins (OATP1B1 substrates) are low VD_{ss}, high f_{up} compounds which in principle obey the model. Nevertheless, in cases of low VD_{ss} and high plasma free fraction, this analysis implicates the involvement of active transporters limiting tissue partitioning.

It has been observed that tissue-to-plasma partition ratios are elevated in certain tissues in mdr1a knockout mice relative to wildtype, albeit not for every compound studied and in all tissues (Lee et al., 2009). An analysis of published reports evidencing changes in distribution volume, in animals and human, mediated by drug-drug interactions, genetic polymorphism or gene knockout, showed some similar trends; uptake interactions at the liver tended to cause a decrease in VD_{ss}. However, the efflux interactions at the liver did not trend in the opposing direction which could be a consequence of assessing interactions in a tissue generally considered part of the central compartment (Grover and Benet, 2009). This work highlights the potential complexity of drug distribution; a paradigm shift from simple passive diffusion phenomena to more intricate, active mechanisms including efflux, uptake and intracellular sequestration. In addition, Dobson et al. (Dobson and Kell, 2008) concluded that active transport processes are fundamental in determining drug disposition being likely more common than

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usually assumed, citing the mounting evidence in the literature on specific drug examples as well as observations that drugs can concentrate in specific tissues.

It is clear that a large proportion of the 90 compounds are beta-lactam antibiotics. Interestingly, this class of compounds was intentionally excluded from the Rule-of-5 analysis since these natural products (and close derivatives) are highly likely to have 'evolved' transport protein interactions (Lipinski et al., 1997; Ganesan, 2008). In addition, there are examples in the literature of this class of compounds accumulating in specific tissues by various mechanisms, a further model assumption violation. The beta-lactam antibiotics have been shown to accumulate in the choroid plexus of rat via an active carrier-mediated transport process (Nohjoh et al., 1989). The aminoglycosides listed in Table 1, including amikacin, gentamicin, isepamicin, kanamycin, netilmicin and tobramycin, have been shown to selectively accumulate in renal cortex leading to renal injury. This selective accumulation is thought to be mediated by megalin, a large endocytic receptor abundantly expressed in renal proximal tubules (Tod et al., 2000; Nagai, 2006). Furthermore, a similar mechanism by which these compounds accumulate in sensory hair cells leading to ototoxicity has also been identified (Hashino et al., 1997). Following receptor-mediated endocytosis, the compounds are transported by vesicular trafficking into lysosomes. Accumulation of drug leads to lysosomal disruption and rupture, with subsequent hair cell degeneration. Compare this active mechanism of lysosomal and mitochondrial trapping to that generally regarded for basic drugs where a passive, pH partition mechanism is implicated (Okumura et al., 1989; Daniel and Wojcikowski, 1997). Contrast agents are also well represented including a number of iodinated compounds such as iohexol as well as the gadolinium-containing compound, gadoversetamide. These agents are typically limited to the intravascular and extracellular fluid spaces and are renally cleared by glomerular filtration, in line with their intended clinical imaging applications. However, cytochemistry studies with the diagnostic indicator dye, fluorescein, have demonstrated active transport-driven renal accumulation in mitochondria (Masereeuw et al., 1994).

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As well as model assumptions concerning no active transport processes and uniform tissue distribution there is also a requirement that distributional characteristics are non-saturating. This could explain cases such as cefazolin where plasma protein binding has been shown to be non-linear in rat (Tsuji et al., 1983). The free fraction in rat serum at 10, 100 and 200 $\mu g.mL^{-1}$ was measured as 11, 20 and 41 % respectively. This marked change in plasma protein binding would likely shift the distributional behavior in tissues.

The Øie-Tozer model has been effectively applied to the prediction of human VD_{ss} for basic and neutral compounds (Obach et al., 1997; Lombardo et al., 2002; Lombardo et al., 2004). In this approach the rearranged Øie-Tozer equation is used together with VD_{ss} and f_{up} in preclinical species in order to calculate f_{ut}. This value is considered species-independent since tissue binding tends to be determined by the extent of interaction with phospholipid membranes. This f_{ut} together with experimental human f_{up} measurements can be put into the standard form of the Øie-Tozer model to generate VD_{ss} for human (Obach et al., 1997). In order to determine the predictive accuracy of the approach on the 102 violating compounds, calculations of VD_{ss} were made using measured f_{up} and 3 hypothetical f_{ut} values within the normal range (0.1, 0.5 and 0.9), as might normally be obtained from the rearranged Øie-Tozer equation with preclinical data. From the summary statistics displayed in Table 2, it is clear that when apparently normal f_{ut} values from preclinical species are used, large errors in prediction can be observed; assuming passive diffusion-mediated distribution leads, in the vast majority of cases, to over-prediction of human VD_{ss}. Even when the more likely scenario of low tissue binding is applied (f_{ut} 0.9), the percentage of predictions with less than 2-fold error is lower than 60%, with a maximum error of 9-fold in this test set. The errors observed could be further exacerbated by the high proportion of actively transported drugs within the 102 compound set, especially given the known species differences in transporter expression and activity.

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Multiple linear regression approaches have also been applied to the calculation of f_{ut} using 2 experimentally determined physicochemical properties, ElogD and fraction ionized at pH 7.4 (Lombardo et al., 2002; Lombardo et al., 2004). With the exception of the borderline example, metronidazole, where a slightly different plasma free fraction was reported, there are no violating compounds present in these original reports. However, in recent work where the ElogD approach was modified to an HPLC-IAM measurement, a number of acidic compounds were utilized and, despite some of them giving rise to aberrant f_{ut} values ($\log f_{ut} > 0$), they were included in the subsequent model building (Sui et al., 2009). The present investigation highlights the importance of careful application of the Øie-Tozer model and the need to be aware of the potential for aberrant f_{ut} values. In the same manner, and as shown in this work, the generation of an aberrant f_{ut} parameter from preclinical data, although not an all-encompassing diagnostic, can give some useful insights into potential disposition properties of novel chemical entities, implicating active transport, selective tissue accumulation or non-linear, non-uniform distributional behavior. The judicious application of the Øie-Tozer model to predictions of human VD_{ss} for novel compounds is also noteworthy, in cases where the physicochemical property profile or drug class overlaps with that demonstrated in this analysis. Further work in the field of drug transporters will help elucidate the nature of drug distributional behavior and provide further insights to aid drug design.

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FIGURE LEGENDS

Figure 1. Plot of volume of distribution at steady state against log plasma free fraction for the 102 compounds which gave rise to an aberrant tissue free fraction using the rearranged Øie-Tozer equation. Plasma free fraction less than 0.1 (open circles) and greater than 0.1 (closed circles).

Figure 2. Simulations of the Øie-Tozer model for hypothetical compounds with VD_{ss} of 0.1, 0.3, 0.5, 1 and 3 $L.kg^{-1}$ with varying f_{up}, illustrating model violations and the scenarios where f_{ut} becomes aberrant (grey shading).

Figure 3. Sensitivity analysis of the plasma protein extravascular-to-intravascular ratio ($R_{E/I}$) for compounds with high ($f_{up} > 0.1$, closed symbols) and low ($f_{up} < 0.1$, open symbols) plasma free fraction. All compound dependent variables and physiological terms remained constant whilst $R_{E/I}$ was varied from 0.1 to 2.5.

Figure 4. Trends in physicochemical properties observed between compounds with f_{ut} within normal range ($0< f_{ut} <1$; black) and compounds with aberrant f_{ut} (closed circle data-points in Figure 1. i.e. $0 > f_{ut}$ or $f_{ut} >1$; hashed). A, clogP; B, polar surface area (in $Å^2$); C, molecular weight; D, extent of ionization described by the difference between clogP and clogD at pH 7. Numbers above each bar represent % of compounds with aberrant f_{ut} within each range.

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Table 1. Compounds which do not obey the Øie-Tozer model together with designated therapeutic activity and literature evidence for interaction with active transporters.

Drug	Therapeutic Class	Transporter/s or active mechanism implicated [1]	Reference
Acetylsalicylic acid	NSAID	OAT1	(Apiwattanakul et al., 1999)
Adefovir	anti-viral	MRP4, OAT1, OAT3, Secretory renal CL	(Imaoka et al., 2007; Uwai et al., 2007; Varma et al., 2009)
Amikacin	anti-bacterial	Megalin	(Tod et al., 2000; Nagai, 2006)
Aminohexanoic acid, 6-	hematologic agent		
Amoxicillin	anti-bacterial	PEPT1, PEPT2, OAT1, secretory renal CL	(Hill et al., 2002; Li et al., 2006; Varma et al., 2009)
Ampicillin	anti-bacterial	MRP4, NPT1 (mouse), PEPT1	(Yabuuchi et al., 1998; Uchida et al., 2007; Dobson and Kell, 2008)
Atracurium	neuromuscular blocker		
Azlocillin	anti-bacterial	Secretory renal CL	(Varma et al., 2009)
Aztreonam	anti-bacterial	Secretory renal CL	(Varma et al., 2009)

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Biapenem	anti-infective		
Busulphan	immunosuppressant/anti-neoplastic		
Carbenicillin	anti-bacterial	Secretory renal CL	(Varma et al., 2009)
Carboplatin	anti-neoplastic	ATP7A, ATP7B	(Safaei, 2006)
Carumonam	anti-bacterial		
Cefadroxil	anti-bacterial	PEPT1, OAT1, OAT3, OAT4, secretory renal CL	(Bretschneider et al., 1999; Takeda et al., 2002; Varma et al., 2009)
Cefamandole	anti-bacterial	OATs, secretory renal CL	(Rizwan and Burckhardt, 2007; Varma et al., 2009)
Cefatrizine	anti-bacterial	Secretory renal CL	(Varma et al., 2009)
Cefazolin	anti-bacterial	OAT1, OAT3, OAT4, MRP4, secretory renal CL	(Takeda et al., 2002; Ci et al., 2007; Varma et al., 2009)
Cefepime	anti-bacterial	OCTN2	(Ganapathy et al., 2000)
Cefetamet	anti-bacterial		
Cefixime	anti-bacterial	PEPT1, PEPT2, NPT1	(Ganapathy et al.,

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		(mouse)	1997; Yabuuchi et al., 1998)
Cefmetazole	anti-bacterial	MRP4	(Uchida et al., 2007)
Cefodizime	anti-bacterial	Unidentified transporter in isolated rat choroid plexus	(Nohjoh et al., 1989)
Ceforanide	anti-bacterial		
Cefotaxime	anti-bacterial	OAT1, OAT3, OAT4, secretory renal CL	(Takeda et al., 2002; Varma et al., 2009)
Cefotetan	anti-bacterial		
Cefoxitin	anti-bacterial		
Cefpirome	anti-bacterial		
Cefprozil	anti-bacterial		
Ceftazidime	anti-bacterial	MRP4	(Uchida et al., 2007)
Ceftizoxime	anti-bacterial	MRP4, NPT1 (mouse)	(Yabuuchi et al., 1998; Ci et al., 2007)
Ceftobiprole	anti-bacterial		
Cefuroxime	anti-bacterial	Secretory renal CL	(Varma et al., 2009)
Cephalexin	anti-bacterial	MATE1, secretory renal CL	(Varma et al., 2009) Tanahara et al., 2007;

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Cephaloridine	anti-bacterial	OAT1, OAT3, OAT4, OCTN2, NPT1 (mouse)	(Yabuuchi et al., 1998; Ganapathy et al., 2000; Takeda et al., 2002)
Cephalothin	anti-bacterial	OAT1, OAT3, OAT4, NPT1 (mouse)	(Yabuuchi et al., 1998; Takeda et al., 2002)
Cephapirin	anti-bacterial		
Cephradine	anti-bacterial	MATE1, OAT1, NPT1 (mouse)	(Yabuuchi et al., 1998; Rizwan and Burckhardt, 2007; Tanihara et al., 2007)
Cidofovir	anti-viral	MRP4, OAT1, OAT3, secretory renal CL	(Imaoka et al., 2007; Uwai et al., 2007; Varma et al., 2009)
Cilastatin	protease inhibitor	OAT3	(Takeda et al., 2001)
Cisatracurium	neuromuscular blocker		
Clavulanic Acid	anti-bacterial		
Dalfopristin	anti-bacterial		
Dexrazoxane	immunosuppressant/anti-neoplastic		
Diatrizoic acid (Amidotrizoate)	contrast agent		

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Doxacurium	neuromuscular blocker		
Doxifluridine	immunosuppressant/anti-neoplastic	CNT1, secretory renal CL	(Larrayoz et al., 2004; Varma et al., 2009)
Enalaprilat	anti-hypertensive	MRP2, secretory renal CL	(Liu et al., 2006; Varma et al., 2009)
Eptifibatide	hematologic agent		
Ertapenem	anti-bacterial		
Fluorescein	contrast agent	OAT3, MRP2, active transport-driven accumulation in mitochondria	(Masereeuw et al., 1994; Hawkins et al., 2007)
Fluorouracil, 5-	immunosuppressant/anti-neoplastic	AQP9, OAT2	(Tsukaguchi et al., 1998; Rizwan and Burckhardt, 2007)
Foscarnet	anti-viral	MCT1 (rat), NPT1 (mouse)	(Yabuuchi et al., 1998; Tamai et al., 1999; Kido et al., 2000)
Fosfomycin	anti-bacterial		
Gadoversetamide	contrast agent		
Gentamicin	anti-bacterial	Megalin	(Tod et al., 2000; Nagai, 2006)

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Hydroxyurea	anti-neoplastic	OATP2, MDR1	(Dogruel et al., 2003)
Imipenem	anti-bacterial		
Iohexol	contrast agent		
Iopamidol	contrast agent		
Iopromide	contrast agent		
Iothalamic acid (Iothalamate)	contrast agent		
Isepamicin	anti-bacterial	Megalin	(Tod et al., 2000; Nagai, 2006)
Kanamycin	anti-bacterial	Megalin	(Tod et al., 2000; Nagai, 2006)
Lamifiban	hematologic agent		
Melagatran	hematologic agent		
Meropenem	anti-bacterial	OAT1, OAT3, secretory renal CL	(Shibayama et al., 2007; Varma et al., 2009)
Metocurine	neuromuscular blocker		
Metrizoate	contrast agent	Secretory renal CL	(Varma et al., 2009)
Metronidazole	anti-protozoal/anti-bacterial		

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Mezlocillin	anti-bacterial	Secretory renal CL	(Varma et al., 2009)
Miglitol	hypoglycemic agent		
Mivacurium (cis/cis)	neuromuscular blocker		
Moxalactam	anti-bacterial		
Netilmicin	anti-bacterial	Megalin	(Tod et al., 2000; Nagai, 2006)
Oseltamivir acid	anti-viral	Secretory renal CL, MDR1, PEPT1	(Morimoto et al., 2008; Ogihara et al., 2009; Varma et al., 2009)
Penicillin G	anti-bacterial	MRP4, OATPs, PEPT1, PEPT2, NPT1, OAT1, OAT3, secretory renal CL	(Uchino et al., 2000; Varma et al., 2009)
Piperacillin	anti-bacterial	MRP4, secretory renal CL	(Uchida et al., 2007; Varma et al., 2009)
Pipecuronium	neuromuscular blocker		
Probenecid	uricosuric agent/anti-bacterial adjunct	OAT1, MCT6	(Murakami et al., 2005; Rizwan and Burckhardt, 2007)
Quinaprilat	anti-hypertensive	OAT3, secretory renal CL	(Varma et al., 2009; Yuan et al., 2009)
Rocuronium	neuromuscular blocker	OATP1A2	(Dobson and Kell,

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			2008)
Streptomycin	anti-bacterial		
Sulbactam	anti-bacterial	MRP4, secretory renal CL	(Uchida et al., 2007; Varma et al., 2009)
Sulbenicillin	anti-bacterial	Secretory renal CL	(Varma et al., 2009)
Sulfadiazine	anti-protozoal/anti-bacterial	Active uptake in leukocytes	(Climax et al., 1986)
Ticarcillin	anti-bacterial	Secretory renal CL	(Varma et al., 2009)
Tobramycin	anti-bacterial	MDR1, megalin	(Banerjee et al., 2000; Tod et al., 2000; Nagai, 2006)
Tomopenem	anti-bacterial		
Zanamivir	anti-viral		

[1] Compounds classified as exhibiting net secretory renal CL based on threshold defined by Varma et al. (2009) where $CLr > 1.2 \bullet f_{up} \bullet GFR$.

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Table 2. Summary statistics on the performance of the Øie-Tozer model on the 102 outlier compounds applying various hypothetical estimates of f_{ut} (0.1, 0.5 and 0.9) as may usually be obtained from preclinical species.

Statistic	**f_{ut} 0.1**	**f_{ut} 0.5**	**f_{ut} 0.9**
GMFE	9.7	2.7	1.9
% within 2-fold	3	46	57
Maximum fold error	55	13	9
% > 2-fold over-predicted	97	50	43
% > 2-fold under-predicted	0	4	0

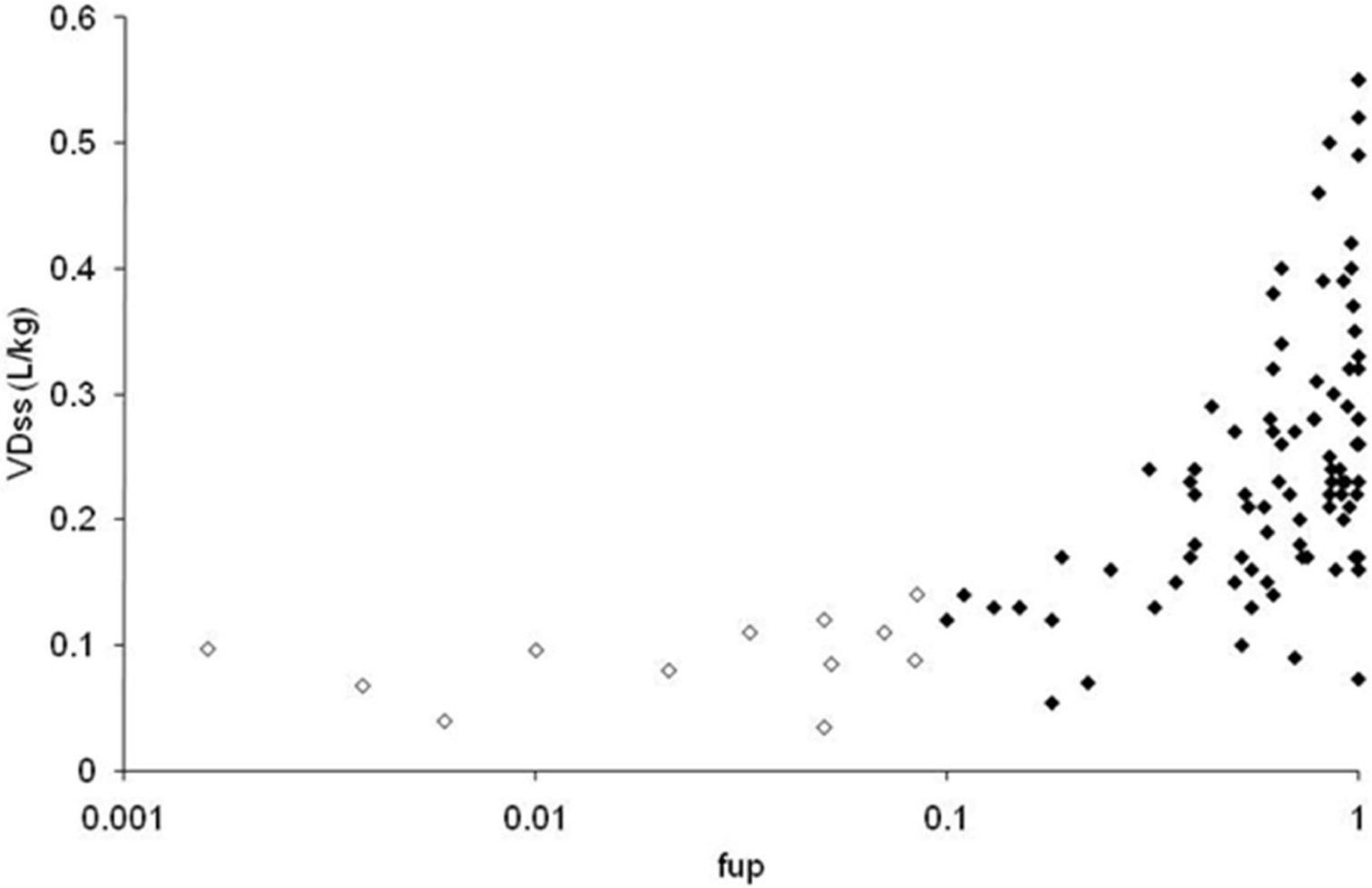


Figure 1

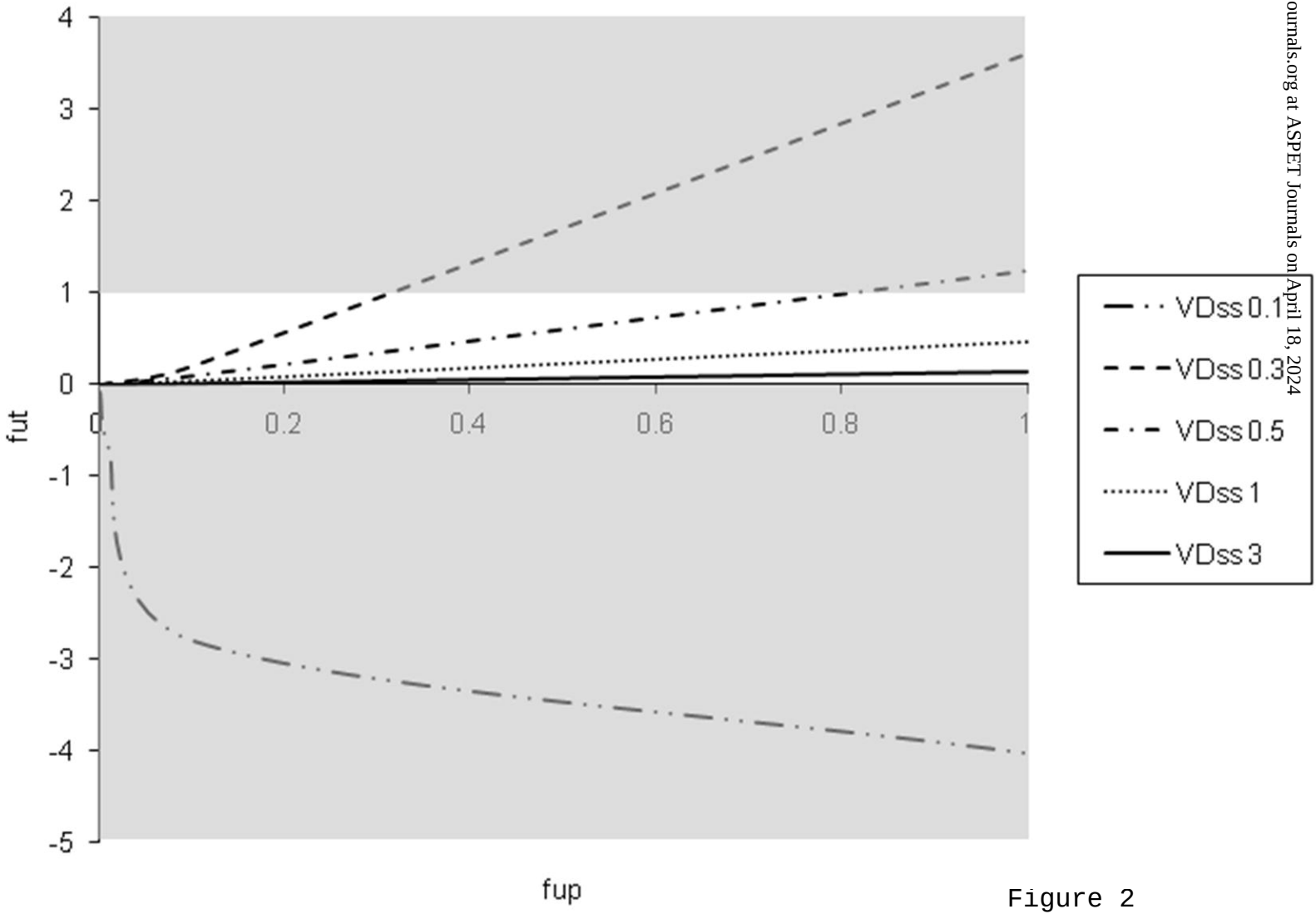


Figure 2

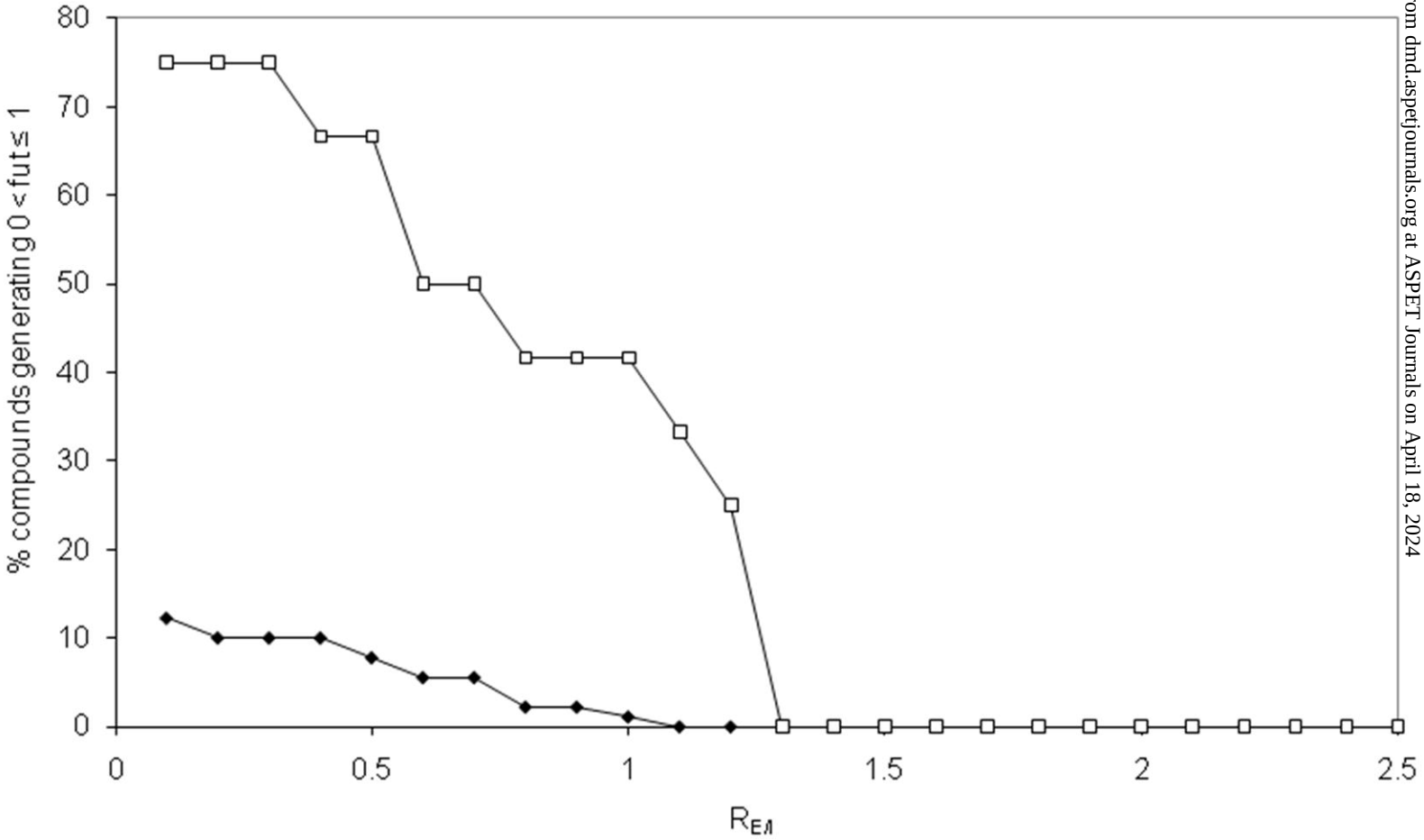
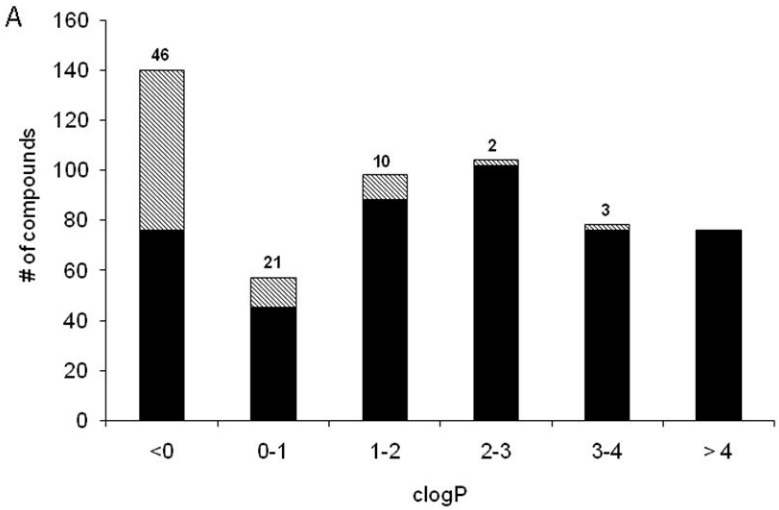
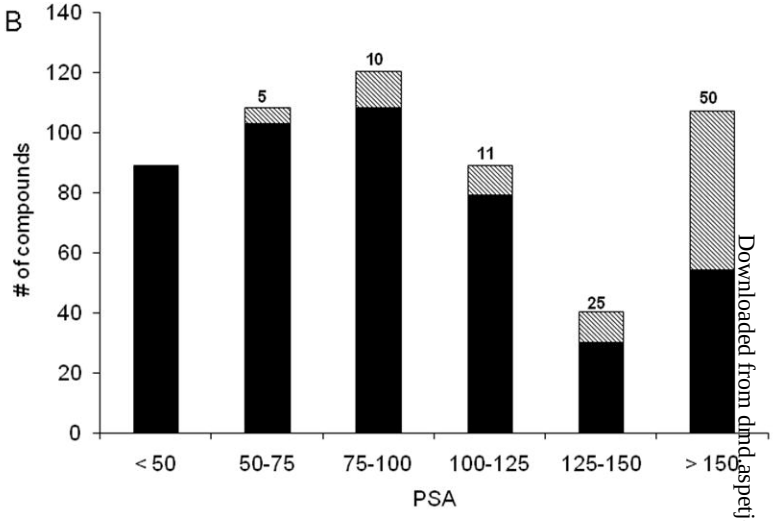
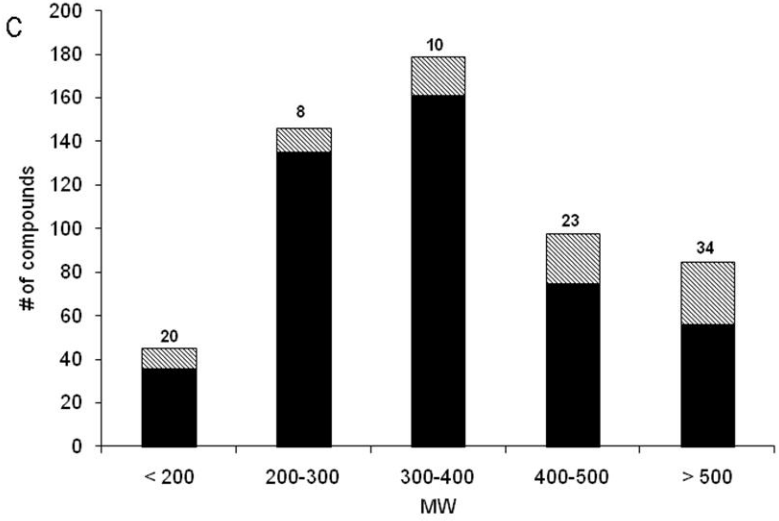


Figure 3







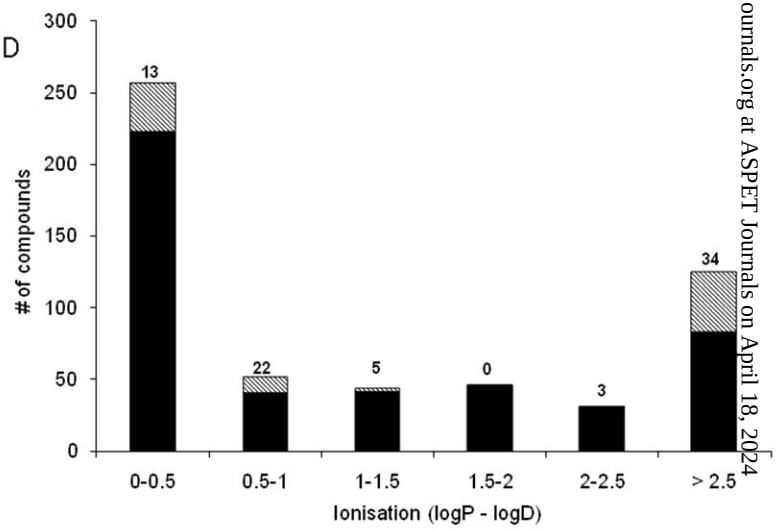


Figure 4