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Using the Golden Triangle to optimize clearance and oral absorption

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ABSTRACT

The Golden Triangle is a visualization tool developed from in vitro permeability, in vitro clearance and computational data designed to aid medicinal chemists in achieving metabolically stable, permeable and potent drug candidates. Classifying compounds as permeable and stable and plotting molecular weight (MW) versus octanol:buffer (pH 7.4) distribution coefficients (log *D*) or estimated octanol:buffer (pH 7.4) distribution coefficients (*e*log *D*) reveals useful trends. Analysis of at least two orthogonal trends, such as permeability and clearance, can be extremely effective in balancing and optimizing multiple properties. In addition, molecular weight and log *D* impact potency-efficiency calculations, allowing potency, clearance and permeability to be optimized simultaneously.

The successful design of new drugs requires optimization of many parameters simultaneously. Lipinski[1,2] and others[3] established useful guidelines for achieving acceptable oral exposure as part of the 'rule of 5'. We desired to focus simultaneously on absorption and clearance. Since both absorption and clearance are important in the pharmacokinetics of drug discovery and generally display opposing trends relative to the octanol:buffer (pH 7.4) distribution coefficients (log *D*), focus on these two absorption–distribution-metabolism-excretion (ADME) properties will bring impact and balance to the design of drug candidates. Both log *D* and molecular weight (MW) were chosen based on their impact on a variety of parameters assessing drug-likeness and efficiency (Fig. 1).[3]

Many molecular properties contribute to the permeability[4] and clearance of compounds. Both molecular weight and lipophilicity act as sufficient surrogates for molecular descriptors such as polar surface area (PSA), molecular volume and surface area, the number of rotate-able bonds, heteroatoms, hydrogen bond donors/acceptors, and other descriptors that correlate with permeability and clearance. In fact, a recent publication by Waring suggests that log *D* and molecular weight are the most important factors in determining the permeability of drug candidates.[5] The convenience and simplicity of these two surrogates will inevitably give rise to 'outliers' and the inability of a single descriptor to account for a property should be considered during the interpretation of data.

Polarity and molecular weight also have a significant impact on the survival rates of drugs in development. Recent published findings by Leeson and Springthorpe show that increased lipophilicity correlates with increased promiscuity in Cerep Bioprint results and escalating threat of compound related toxicological attrition.[6] Analyzing animal in vivo toleration (IVT) studies on 245 preclinical

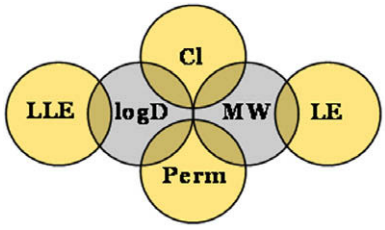

Figure 1. Impact of MW and log *D* on clearance, permeability, ligand efficiency (LE), and ligand-lipophilicity efficiency (LLE).

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Pfizer compounds, Hughes et al. show that poor toxicological outcomes correlate with increased lipophilicity.[7] Other analyses highlight the reduction of molecular mass and lipophilicity as compounds progress through Phase I, II, and III.[7,8]

A comprehensive, diverse set of 47,018 compounds with in vitro permeability, clearance and experimental and estimated octanol:buffer (pH 7.4) distribution coefficient (log *D* and *e*log *D*) measurements was analyzed. Human liver microsome (HLM)[9,10] stability data were used as predictors of clearance, while colorectal adenocarcinoma (Caco-2, pH 7.4/7.4)[11–13] data were used as predictors of overall permeability, both passive and active. Acceptable in vitro permeability and clearance criteria were chosen based on in vitro and in vivo correlation data.[14] Molecular weight was plotted on the *y*-axis while *e*log *D* was plotted along the *x*-axis. A combination of high performance liquid chromatography (HPLC) estimated log *D* (*e*log *D*, pH 7.4) and shake-flask method log *D* (pH 7.4) determinations were used.[15–18] In early stages of target design where log *D* values are unavailable, the calculated octanol:buffer distribution coefficient (*c*log *D*) should be an adequate replacement assuming the model used is a good predictor of experimental values.

Permeation can occur via trans-cellular diffusion, para-cellular diffusion, and transporter-mediated mechanisms. While permeability via para-cellular diffusion occurs mostly at low molecular weight and low lipophilicity, trans-cellular diffusion is a more important contributing factor at drug discovery molecular weights (>200) across a broad range of lipophilicities.[19] Mostly, transporter-mediated mechanism effects are overwhelmed by more general, non-specific influences on membrane permeability (i.e., molecular weight and polarity) when analyzing large sets of data.

Figure 2 shows the comprehensive data set assessed for in vitro permeability using Caco-2 apical to basolateral (AB) measurements. Compounds passing permeability criteria are colored green, while compounds failing criteria are colored gray. In general, as log *D* rises, the in vitro permeability increases, and then decreases again at higher lipophilicities, displaying a parabolic relationship. Also, as molecular mass decreases within a log *D* bin, the permeability increases. These trends are intuitive since increasing lipophilicity encourages passive diffusion through lipid membranes. Also, molecular weight acts as a surrogate for the size, number of rotate-able bonds, hydrogen bond donor/acceptors, tPSA, and heteroatoms in a molecule, all of which are inversely correlated with permeability. Several highly lipophilic compounds show hints of poor permeability, a possible consequence of membrane 'solvation', where greasy compounds are driven into the lipid membrane and remain there due to less favorable interactions in the aqueous phase. Similar trends to those highlighted in Figure 2 were shown and rationalized by Waring.[5] Interestingly, the sole permeable outlier in the molecular weight 750 and log *D* 1.5 bin belongs to the erythromycin macrolide antibiotic class.

Ionization state also plays a role influencing permeability. Although not addressed here, Gleeson shows the influence of ionization state and calculated partition coefficient (*c*log *P*) measurements on permeability, assessed using an artificial membrane permeability assay.[20] Although the analysis was performed independent of molecular weight, Gleeson shows that, in general, zwitterions are the least permeable, followed by acids, bases and then neutrals.

Although in vitro permeability assessments utilizing Caco-2 models are traditionally concerned with oral absorption, this and subsequent analyses become particularly relevant to cellular potency, especially in cancer or other cells over-expressing P-glycoprotein (P-gp) efflux receptors.[21–24] Permeability and efflux issues can significantly impact oncology cellular IC50s and subsequent dose calculations and efficacy.

Stability trends across molecular mass and log *D* are revealed in Figure 3. Magenta colored compounds pass clearance criteria, while gray-colored compounds fail criteria. In general, as lipophilicity and molecular weight decrease, the in vitro clearance prediction improves. Since more polar compounds are less prone to phase I-type metabolism and increasing molecular mass should correlate with an increase in metabolic 'soft-spots', these trends are rational. It is interesting to note that there are several high molecular weight measured low clearance compounds.

Gleeson describes the contribution of molecular weight, ionization state and *c*log *P* on in vivo clearance.[20] Most notable are differences in log clearance (Cl) between ionization states at *c*log *P* >5, and less pronounced at *c*log *P* 3–5 and *c*log *P* <3. The contribution of molecular weight was analyzed independent of ionization state and *c*log *P*.

Combining both the permeability and clearance plots, and coloring passing compounds blue and failing compounds gray, as shown in Figure 4, reveals several interesting trends: (1) as molecular weight decreases, the lipophilicity range affording both permeable and stable compounds increases; (2) within a log *D* bin,

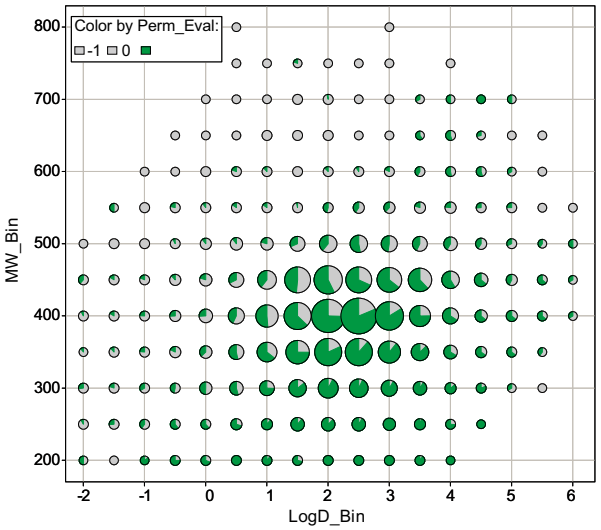


Figure 2. Comprehensive in vitro Caco-2 AB permeability trends across molecular weight and log *D*. 16,227 total records sized by record count.

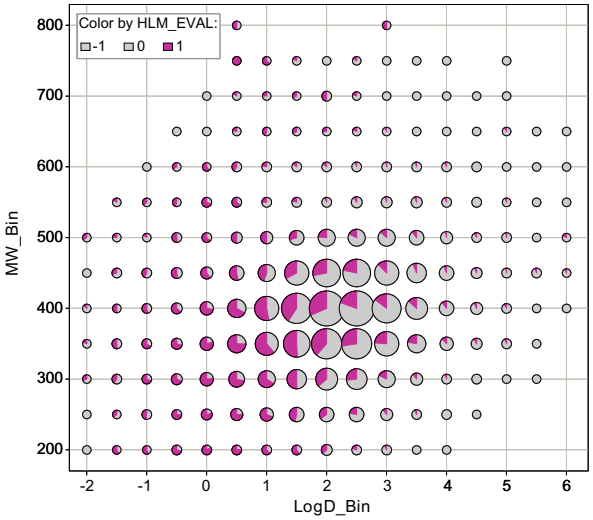


Figure 3. Comprehensive in vitro HLM clearance trends across molecular weight and log *D*. 47,018 total records sized by record count.

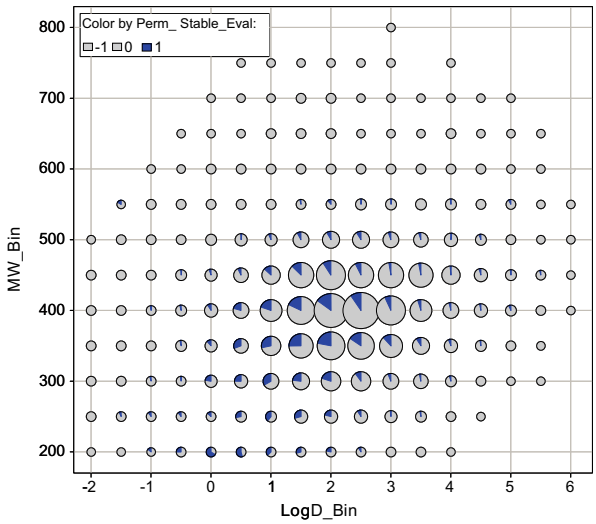


Figure 4. Comprehensive combined in vitro clearance and in vitro permeability trends across molecular weight and log *D*. 16,090 total records sized by record count.

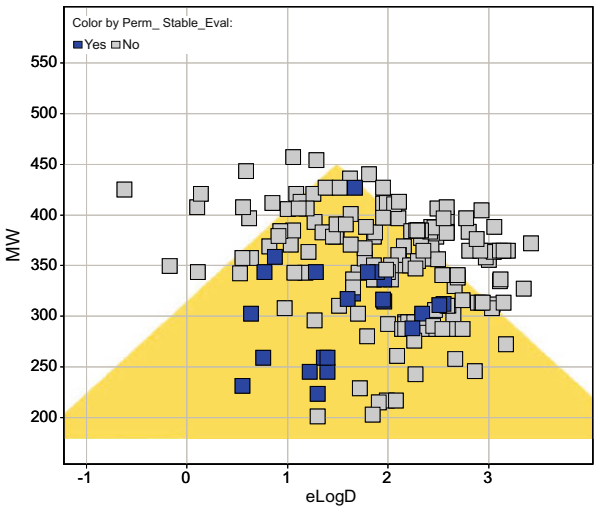


Figure 5. Series A: combined in vitro permeability and clearance trends across MW and log *D*.

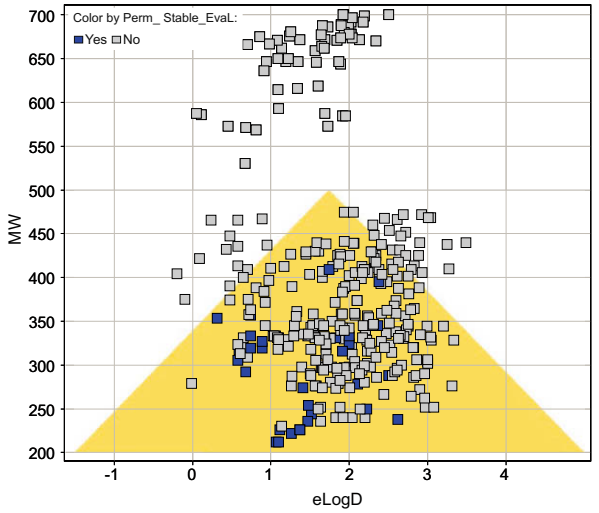


Figure 6. Series B: combined in vitro permeability and clearance trends across MW and log *D*.

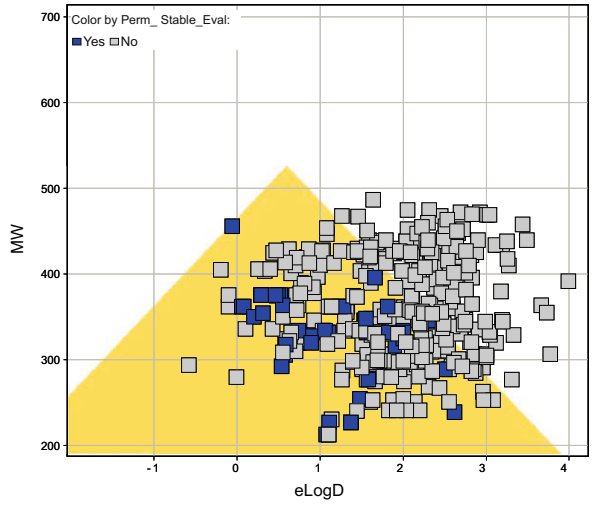


Figure 7. Series C: combined in vitro permeability and clearance trends across MW and log *D*.

the relative number of permeable and stable compounds increases as molecular mass declines; (3) Within a log *D* range of 1.0–2.0, the opportunity for permeable and metabolically stable compounds increases; (4) all trends combined lead to the observation that the polarity and molecular weight of in vitro permeable and low clearance compounds are concentrated within an area with a base-line from log *D* = −2.0 to log *D* = 5.0 at MW = 200 and an apex at log *D* = 1.0–2.0 and MW = 450. These trends lead to a triangular shaped area known as the Golden Triangle and molecules within this area that are low clearance and permeable are said to obey the Golden Triangle Rule. While log *D* positively correlates with permeability at a given molecular mass, log *D* is negatively correlated with the in vitro clearance, giving rise to a balanced maximal effect near log *D* 1.0–2.0. The strong influence of polarity and molecular weight on permeability and in vitro metabolic stability give rise to the observed improvements in both clearance and absorption inside the Golden Triangle. In the center of the Golden Triangle (log *D* 1.5, MW 350), 25% of the compounds pass permeability and clearance criteria, although percentages will vary according to limits imposed defining stable and permeable compounds. Compare this 25% 'success' rate in the heart of the Golden Triangle with the 3% 'success' rate at molecular weight 450 at both log *D* 0 and 3.0 and the odds of preparing an in vitro low clearance and permeable compound diminishes by roughly eightfold. Beyond a molecular weight of 450, there is a precipitous lowering of likelihood that in vitro low clearance and permeable compounds exist. Despite the center of the Golden Triangle being the optimum space for superior clearance and oral absorption, there are molecules that do not meet one or both criteria. Possible reasons for this might be the presence of metabolic 'soft-spots' negatively impacting clearance and/or an increase in the number of hydrogen bond donors, acceptors, heteroatoms, rotate-able bonds, etc. relative to passing compounds in the same region contributing to poor permeability. Ionization state may also be a contributing factor.[20]

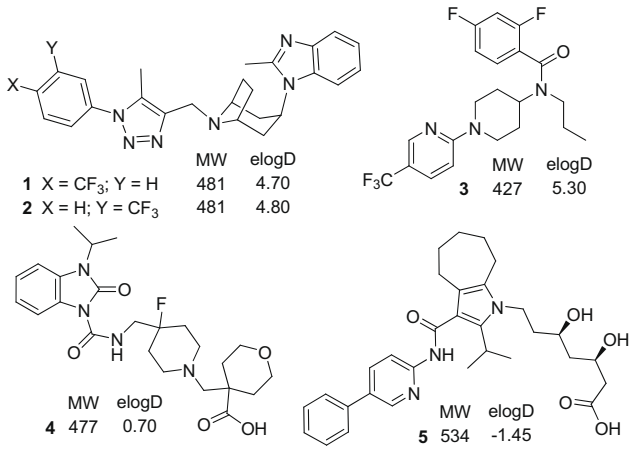
To further assess the predictive power of this molecular space defining the Golden Triangle, we evaluated several structurally distinct series. Although the Golden Triangle boundaries shift slightly from series to series (Figs. 5–7) the general previously described trends persist and can inform analogue design. Several programs representing a single chemotype were chosen that demonstrate the concept of the Golden Triangle. Figure 5 shows a set of program targets mostly lying within the boundaries of the Golden Triangle,

with a few nearly compliant outliers. Even with subtle property changes outside in vitro permeable and stable chemical space, no passing outliers exist. In general, the more lipophilic compounds fail for clearance reasons, while the more polar compounds fail for permeability reasons.

Several high molecular weight (650–700 g/mol) compounds generated by a single internal program are worth comment (Fig. 6). Closer analysis of these relatively large compounds reveals that the entire cohort failed due to poor permeability predictions, while fewer failed for clearance prediction reasons. Finally, Figure 7 shows a mostly lipophilic chemotype outside of the Golden Triangle failing to achieve a permeable, low clearance status, mainly as a consequence of poor in vitro clearance.

With a larger data set spanning many programs and series of compounds, passing outliers begin to appear that do not obey the Golden Triangle Rule (Fig. 4). Although there are relatively few passing outliers, analysis of these efficient compounds provide insights into designing more permeable and metabolically stable compounds at any log *D* or molecular size. Generally speaking, lower log *D*/higher molecular weight molecules fail due to low permeability while higher log *D*/higher MW compounds fail due to elevated in vitro clearance. Scheme 1 highlights three examples (**1–3**) of higher log *D*/higher molecular mass low clearance and permeable outliers taken from Figure 4. There are a few possible explanations for the presence of these passing outsiders. Halogen-containing outliers may cause molecular weight to over-estimate the actual size of the compounds, thus improving in vitro clearance in that region of the plot. In these cases, heavy atom count (HACNT), molecular volume, or total surface area may provide a better estimate of the actual size of the compounds. Halogens may also impart stability by blocking metabolic 'soft-spots'. In addition, several outliers (not shown) in this region inhibit cytochrome P450 (CYP) isoforms, reducing their anticipated clearance values.

Scheme 1 also highlights two examples (**4** and **5**) of lower log *D*/higher molecular weight passing outliers taken from Figure 4. Again, the molecular mass may over-estimate the size of the molecule due to the elevated atomic weights of sulfur, chlorine, and fluorine. It is also interesting to note that nearly all of the outliers in this region contain acidic groups where the polarity of the resulting anion is ameliorated through intramolecular hydrogen bonding or zwitterion formation. The intramolecular hydrogen bonding of the carboxylate via a six- or seven-membered ring not only masks the polarity of the carboxylate during absorption, but also rigidifies the molecules, decreasing the flexibility and increasing the overall permeability.



Scheme 1. Higher MW Golden Triangle passing outliers with low in vitro clearance and high in vitro permeability.

The Golden Triangle can be used as a tool to impact the design of new targets by achieving higher probability drug-like space. Unfortunately, attempts to improve the clearance of highly lipophilic, large, and potent leads by simply reducing the lipophilicity, often leads into less potent and less permeable property space. Additionally, the introduction of polar functionality to reduce log *D* usually adds molecular weight, moving designs further from the center of the Golden Triangle. The highest probability of success in maximizing potency, stability and permeability is realized by moving the design properties into the center of the Golden Triangle, which may involve maintaining or lowering molecular weight through innovative new target designs. For example, reducing lipophilicity of a series by replacing lipophilic carbon atoms for nitrogen atoms can reduce log *D* with minimal impact on molecular mass. In addition, the Golden Triangle can be a valuable tool for the rational and successful design of permeable and low clearance compounds that are both inside and outside the boundaries of the Golden Triangle, employing previously described techniques, such as improving permeability through intramolecular hydrogen-bonding strategies or optimizing clearance using halogenation strategies.

Since log *D* and molecular weight descriptors are intimately coupled to both ligand efficiency (LE)[25] and ligand-lipophilicity efficiency (LLE)[6], the Golden Triangle can also be effective for visualizing ligand efficiency, ligand-lipophilicity efficiency, and in vitro clearance and absorption. Design from the most ligand and lipophilic efficient lead into the center of the Golden Triangle should provide maximum potency, stability and permeability. In addition, a recent published concept, metabolism-lipophilicity efficiency (MLE)[26], can also be used in conjunction with the Golden Triangle since it relates the lipophilicity and in vitro clearance of groups or molecules, providing a lipophilic-independent clearance efficiency.

While many different molecular descriptors correlate with clearance and permeability, molecular weight and log *D* are strong drivers and useful surrogates. In addition, molecular weight and log *D* are coupled to the calculation of LE and LLE, and easy to obtain making the Golden Triangle a valuable tool in the medicinal chemistry toolbox. Further analyses, including ionization state effects on in vitro clearance and permeability, are ongoing.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.045.

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