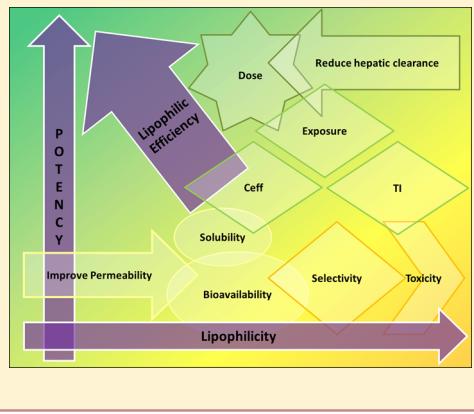


Lipophilic Efficiency as an Important Metric in Drug Design

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ABSTRACT: Lipophilic efficiency (LipE) is an important metric that has been increasingly applied in drug discovery medicinal chemistry lead optimization programs. In this Perspective, using literature drug discovery examples, we discuss the concept of rigorously applying LipE to guide medicinal chemistry lead optimization toward drug candidates with potential for superior *in vivo* efficacy and safety, especially when guided by physiochemical property-based optimization (PPBO). Also highlighted are examples of small structural modifications such as addition of single atoms, small functional groups, and cyclization that produce large increases in LipE. Understanding the factors that may contribute to LipE changes through analysis of ligand–protein crystal structures and using structure-based drug design (SBDD) to increase LipE by design is also discussed. Herein we advocate for use of LipE analysis coupled with PPBO and SBDD as an efficient mechanism for drug design.



INTRODUCTION

Lipophilicity is a measurable and predictable physical property of a molecule that is determined experimentally by quantifying molecule distribution between water and a hydrophobic phase, typically octanol.¹ The parameter $\log D$ is an experimental descriptor of lipophilicity reflecting the distribution of a solute between water and octanol phases at pH = 7.4 (eq 1, Figure 1, left panel), which accounts for physiologically relevant ionization. The lipophilicity of a molecule results from the balance of hydrophobic features, which favor partitioning into a lipophilic phase, and polar/ionic features which favor partitioning into the aqueous phase through energetically favorable solvation by water (eq 2).² The hydrophobicity term describes hydrophobic and dispersion/van der Waals forces, while the polarity term accounts for numerous forces such as hydrogen bonding, orientation, and inductive effects. The ionic interaction term accounts for attractive and repulsive forces due to charge. The lipophilicity of molecules can be varied by design through controlling the balance of hydrophobic features to polar and ionic features. While measured $\log P$ can also be used as description of lipophilicity, it does not consider ionization of groups at different pH and could be misleading in a more biologically relevant context. Calculated $\log D$ or $\log P$ values should be used with caution as they may not predict measured values. Measured values should be checked with calculated values to assess the predictability of models.

$$\log D = \log_{10}([\text{ligand}]_{\text{octanol}} / [\text{ligand}]_{\text{water}}) \quad (1)$$

$$\text{lipophilicity} = \text{hydrophobicity} + \text{polarity} + \text{ionic interactions} \quad (2)$$

Ligand lipophilicity impacts ligand–protein affinity since most druggable target binding sites contain at least one hydrophobic pocket or region and exist in aqueous environments. The hydrophobic effect drives binding of lipophilic ligands to hydrophobic protein binding sites via replacement of interactions between the

protein and solvating waters with more favorable hydrophobic interactions for both ligand and protein.³ Notably, initial observations of compound lipophilicity correlating to pharmacological effect were made in the 1890s,^{4–7} although sophisticated analyses of target binding were not developed until almost 100 years later.^{8,9} Of course, in contrast to distribution of a ligand between hydrophobic and aqueous phases, ligand association with a protein is governed by both bulk ligand lipophilicity and specific, complementary binding site interactions (Figure 1, right panel).¹⁰

Increases in affinity for ligands reversibly binding to a protein, described by the equilibrium constant (K), are driven by increased release of Gibbs free energy (ΔG) upon formation of the bound ligand–protein complex. The relationship between the equilibrium constant, ΔG , enthalpy (ΔH), and entropy (ΔS) changes are described in eq 3. A lipophilic molecule in water has an ordered solvation shell. Upon movement of that molecule from water to a more lipophilic environment, entropy is increased via increasing degrees of freedom for water released from the solvation shell. In addition, increases in lipophilicity of the ligand decrease desolvation enthalpy. Therefore, both of these enthalpic and entropic considerations favor binding of a lipophilic molecule to a protein binding site when the binding site environment is more lipophilic than water.^{11,12}

$$-RT \ln(K) = \Delta G = \Delta H - T\Delta S \quad (3)$$

The importance of lipophilicity has been a popular topic of discussion in the scientific literature since the late 1990s.^{13,14} Many papers discuss the optimal range of lipophilicity to achieve “druglikeness”^{7,15,16} because in addition to binding affinity, lipophilicity has effects on other molecular attributes. Variation of lipophilicity within a series of designed compounds can predictably affect various important properties such as absorption,

Received: January 16, 2018

Published: March 28, 2018

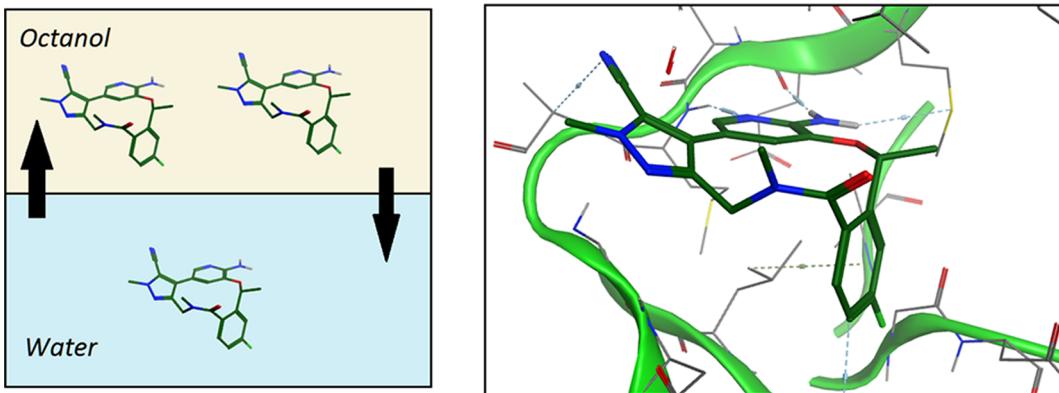


Figure 1. Left panel: Octanol/water partitioning. Right panel: Ligand–protein interactions governed by both lipophilicity-driven binding and binding site environment (compound 37 in ALK-L1196M; 1.7 Å resolution; PDB code 4CLJ).

distribution, metabolism, and excretion (ADME).^{17–19} Both ADME properties and potency affect the dose of a drug required for efficacy (eq 4). Herein we use the term potency interchangeably with C_{eff} which may represent binding, mechanistic, or functional activity in a cell or cell-free environment. The drug dose is proportional to the unbound efficacious concentration (C_{eff_u}), unbound clearance (Cl_u), bioavailability (F), and the dosing interval (τ). Low dose compounds are important from a clinical point of view since higher dose compounds can lead to formulation complications, increased pill burden, reduced patient compliance, and increased risk for idiosyncratic toxicities.^{20–23} Kaplanowitz published that dose *and* lipophilicity are important to consider for idiosyncratic toxicities.²⁰ To achieve lower doses, a high affinity ligand resulting in low efficacious concentrations (C_{eff}) is required. Dose is also influenced by clearance, which is often correlated with lipophilicity within a series of related analogs. In addition to first pass clearance, bioavailability (F) is influenced by absorption which requires acceptable solubility and membrane permeability. Both solubility and permeability are impacted by lipophilicity. As a result, for orally dosed drugs, it can be important to identify an optimal lipophilicity range that renders each of these properties suitable for low oral dosing. A recent paper by Gleeson et al. highlights the traditional overemphasis on high *in vitro* potency which may not be consistent with acceptable ADME.²⁴

$$\text{dose} \propto (C_{eff_u} \times Cl_u) \tau / F \quad (4)$$

Clearance is a major factor affecting drug dose.¹⁷ Detailed analysis of over 45,000 Pfizer compounds revealed a trend of lower lipophilicity associated with reduced *in vitro* clearance,¹⁵ which can be broadly explained by the lower affinity of less lipophilic compounds for the proteins responsible for metabolism, reduced hepatocyte permeability, and other factors. The highest probability for obtaining low clearance was seen for compounds with a $\log D$ of less than 3. Small drug molecules can also be cleared by the kidneys, which is not captured in the aforementioned *in vitro* assays. Studies have shown that renal clearance increases below a $\log D$ of 0.^{7,25} Thus, by avoidance of excessively high metabolic or renal clearance, the lipophilicity range most likely to provide an acceptable level of *in vivo* clearance for a drug is $\log D$ of 0–3. Of course, there are examples of successful oral drugs outside of this range of $\log D$,^{14,26} but the odds of success in achieving acceptable *in vivo* clearance are highest within $\log D$ of 0–3.

Within a series, solubility can be adversely affected by increasing lipophilicity. To achieve a low oral dose, sufficient aqueous drug solubility is required for dissolution prior to crossing lipid membranes. According to a recent analysis by Waring,

a compound is considered highly soluble if it achieves greater than 100 μM concentration in water, which is well above the concentration of most *in vitro* and *in vivo* experiments. By use of the empirical equation for solubility (eq 5), $\log P < 3.25$ provided the best odds for obtaining >100 μM solubility for a typical drug molecule (melting point of 150 °C).^{7,27}

$$\log(\text{Sol}_{aq}) = 0.5 - \log P - 0.01(\text{MP-25}) \quad (5)$$

Lipophilicity strongly influences membrane passive permeability which is required for oral absorption and access of the drug to intracellular compartments and tissue penetration. At average molecular weight of 400 and $\log D$ of 1, over 50% of compounds have good permeability.^{15,28} If the molecular weight is lower, compounds can accommodate lower $\log D$ while maintaining good permeability. Highly lipophilic compounds can be poorly absorbed or unable to fully access intracellular compartments due to trapping in lipophilic cell membranes² and/or through very low solubility as mentioned above.²⁹ At molecular weight of approximately 400, membrane permeability declines with increasing lipophilicity beginning at $\log D = 3.5$.¹⁵ These considerations define a $\log D$ of 1–3.5 for acceptable permeability. Compounds that are transporter substrates can also influence permeability; therefore, concentration of inhibitor, timing, and expression of transporters may override the impact of lipophilicity on permeability.^{30,31}

Designing within a $\log D$ range of 1–3 maximizes the opportunity to combine the above-mentioned ADME properties for an effective oral drug (Figure 2). The upper and lower limits of the

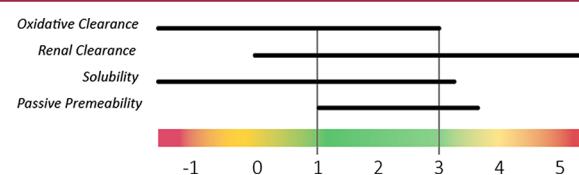


Figure 2. The $\log D$ range of 1–3 as a guide for best chance of achieving overlap of low oxidative clearance, low renal clearance, high solubility, and high passive permeability.

ideal lipophilicity are bounded by oxidative clearance and permeability, respectively. It is not implied that all drug design should adhere to these restrictions since oral drugs exist with $\log D$ outside this window. Compounds within this lipophilicity range simply have increased probability of achieving acceptable overlapping ADME properties required for good *in vivo* performance.⁷

Given the importance of lipophilicity in both ligand association with proteins and the ADME characteristics underpinning dose,

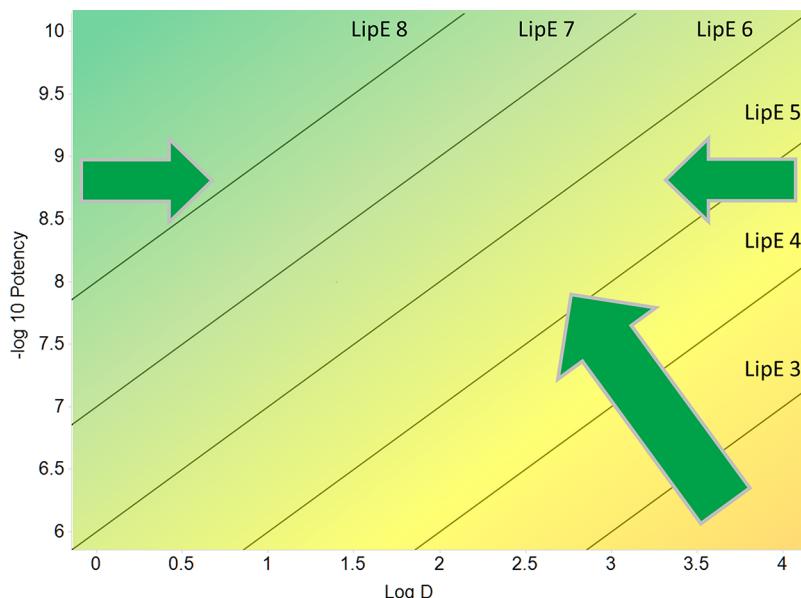


Figure 3. Optimal log D range, biochemical potency, and efficiency for drug design. Color denotes improving LipE from orange to yellow to green. Arrows represent improving LipE and moderating log D for overall ADME improvement.

experimental lipophilicity measurements have been a cornerstone of drug design and assessment. Varying the lipophilicity of analogs to improve ADME properties of lead compounds is a powerful strategy as lipophilicity can be predicted computationally and then measured experimentally once the analogs have been synthesized.³² In subsequent design cycles, the accuracy of the computational models predicting lipophilicity can be re-evaluated with the experimental results to inform future designs.

As previously stated, a paramount goal of drug discovery is to achieve low dose and dosing frequency. Therefore, it is most efficient to vary lipophilicity to control ADME properties of designed analogs in parallel with optimization of potency. For example, an often successful tactic to improve metabolic stability is reduction of log D. However, analogs with lower clearance due to decreased lipophilicity are of lower interest if accompanied by a reduction in potency. Given the role of lipophilicity in binding of ligands to many drug target proteins described above, it is not surprising that reduction of lipophilicity is often coupled with reduced potency. This illustrates the critical need to monitor potency as it relates to lipophilicity. Identifying structural changes within a lead series that maintain or improve potency while reducing lipophilicity is key to successful optimization in a drug discovery program in order to achieve optimal potency and clearance that translate to a low dose (see eq 4). This knowledge enables the optimization of potency and ADME properties in parallel that lead to low dose drug candidates.

Lipophilic efficiency (LipE), also known as lipophilic ligand efficiency (LLE), is a metric that normalizes the potency for the lipophilicity of the molecule by subtracting the log D of the molecule from the negative log₁₀ of the potency (M) against the desired protein target.^{33–35} The potency can be represented by early measures, such as biochemical cell-free assays, or by more functionally relevant cell-based assays (eq 6). Although not considered here, kinetics of binding directly can impact drug efficacy and safety.^{36,37}

$$\text{LipE} = \text{LLE} = -\log(\text{potency}) - \log D \quad (6)$$

Although discussion will be restricted to application of LipE in drug discovery optimization programs, the concept of LipE has

been used in concert with ligand efficiency (LE) and fit quality (FQ), which correct binding affinity by molecular size, and with lipophilicity corrected ligand efficiency (LELP) which normalizes binding affinity by molecular size *and* lipophilicity.^{38–41} The following will explain the basis for rigorously applying LipE to guide medicinal chemistry lead optimization progress toward drug candidates with potential for superior *in vivo* efficacy and safety, independent of administration route. Shultz recently published work correlating LipE with enthalpy-driven binding and concluded that LipE should be prioritized over other metrics based on the importance of enthalpic optimization.⁴² Shultz further concluded that since increased binding enthalpy is desired at the earliest stages of hit and lead generation, it is recommended that LipE be utilized throughout the entire drug discovery process.

For LipE tracking in optimization programs, a very important tool for analysis that captures three key molecule attributes (log D, potency, and LipE) in a simple visualization is the LipE plot that shows log D on the x-axis and $-\log_{10}(\text{potency})$ on the y-axis (Figure 3). For many drug discovery optimization programs, log D will span approximately 4 log units (0–4) and potency will span approximately 4 log units (1 μM to 100 pM). The ranges of log D and $-\log(\text{potency})$ coincidentally and elegantly coincide for simple analysis of lipophilic efficiency, which is defined by a 45° trajectory if the x and y axes of the LipE plot are equally scaled. Compounds that have the same LipE are positioned on the same 45° line (a LipE line). Generally speaking the goal of a LipE based optimization is to improve LipE while staying in an appropriate log D range for overlap of potency and ADME properties. Historically, potency alone has been used as the most important attribute to judge progress in a drug discovery lead optimization program which can lead to poor ADME.²⁴ When evaluating two compounds based on potency alone, a number of factors may cause differences in binding. The net change in potency is a combination of effects based on structural changes to the ligand, and it can be difficult to determine the impact of the various contributing factors. A LipE analysis reveals the lipophilicity component of binding and makes it easier to hypothesize how a specific ligand structural change impacted binding to the

protein. More specifically, we can examine whether an increase in potency could be simply the result of increased lipophilicity. For any compound that has increased potency but equivalent LipE to the reference ligand, it is possible that the increase in lipophilicity alone is responsible for the increased potency, although other factors associated with the specific structural change cannot be ruled out. Increases in LipE for the new ligand suggest that potency improvements are beyond lipophilicity increases alone. By examining series of compounds in a LipE plot format rather than in a potency-only format, medicinal chemists are able to immediately identify the compounds that are worthy of further optimization because they have the highest LipE rather than just the best potency. Examination of structure–potency relationships in the LipE plot format also enables the rapid identification of special structural features within the series of interest, such as those associated with an increase in LipE relative to close structural neighbors.

The ultimate goal of efficiency-based optimization is to identify highly potent, sufficiently selective molecules at a $\log D$ that provides good pharmacokinetic properties and low dose. Therefore, while molecules with increased lipophilic efficiencies are generally highly desirable, ADME properties as predictors of absorption and dose should be closely monitored. The concept of LipMetE is similar to LipE in that it describes metabolic stability relative to lipophilicity.⁴³ A more efficient compound will have a lower clearance per unit of $\log D$ than a less efficient

compound. In particular it is advantageous to avoid structural motifs that are metabolic soft spots and lead to high clearance even in analogs with low $\log D$ (<3); that is, avoid structural motifs that impart a poor LipMetE. Parallel LipMetE and LipE optimization strategies have been effectively used in drug design, although they are out of the scope of this Perspective.

There is an additional advantage to scrutinizing lipophilicity and LipE in drug discovery optimization programs regardless of administration route (Figure 4). In addition to optimizing ADME properties and potency, delivering clinical candidates with sufficient selectivity against off-targets and thus potential for safety is important for successful drug discovery.⁴⁴ Off-targets can include proteins that may reduce efficacy, pose a safety/toxicity liability, and/or have detrimental effects on ADME. Many studies have associated high lipophilicity with both increased likelihood of promiscuous binding to proteins and polypharmacology as well as in vivo adverse safety outcomes.^{14,45–49} Drug discovery optimization programs that use lipophilicity-driven ADME optimization design strategies coupled with knowledge gained from tracking LipE as a key metric can improve the chances of achieving selectivity over off-targets by producing clinical candidates that do not have high lipophilicity. A strategy aimed at avoiding high lipophilicity candidates alone will not guarantee improved selectivity because the ability to optimize lead molecules for selectivity also depends on the overall similarity of the desired and undesired protein ligand binding sites. Optimization of selectivity is best achieved when coupled with knowledge gained from examining ligand–protein structures for desired and undesired protein targets to produce clinical candidates that are optimized for low oral dose and selectivity for the desired protein target. This maximizes therapeutic benefit to patients. Drug discovery is complex, and while lipophilic efficiency is fundamentally important, clinical candidates are often not the highest LipE compounds within a series as they must overlap many properties. It is this strategy of combining both the LipE and lipophilicity-driven ADME optimization strategies (physiochemical property based optimization, PPBO) with structure based design (SBDD) that we will exemplify in this Perspective.

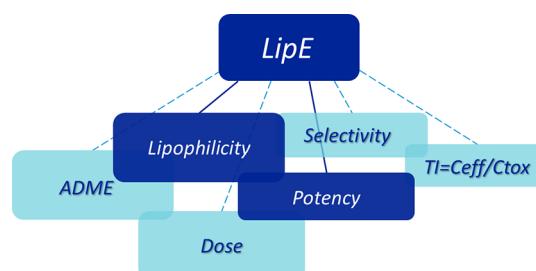


Figure 4. Lipophilicity and potency, components of LipE, affect all determining factors of dose.

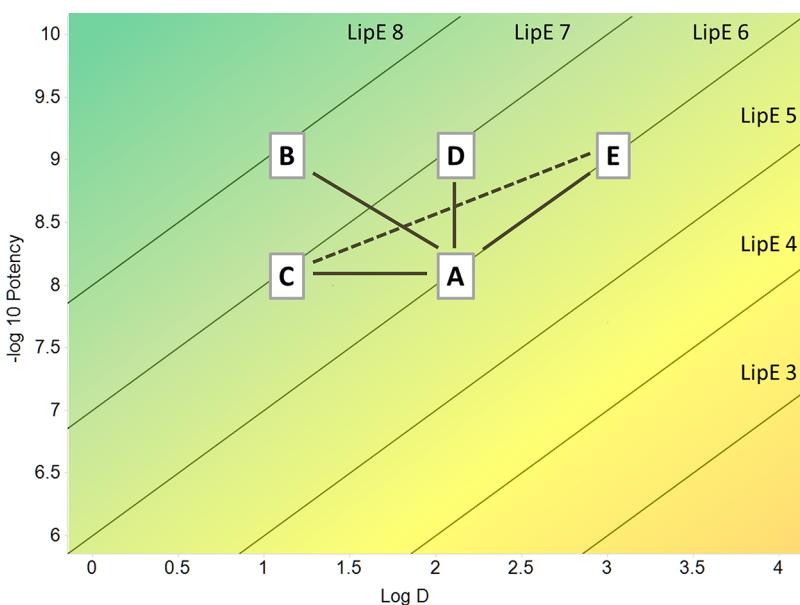


Figure 5. Plot of lipophilic efficiency changes. Color denotes improving LipE from orange to yellow to green. Dotted line represents loss in potency but gain in LipE from compound E to compound C.

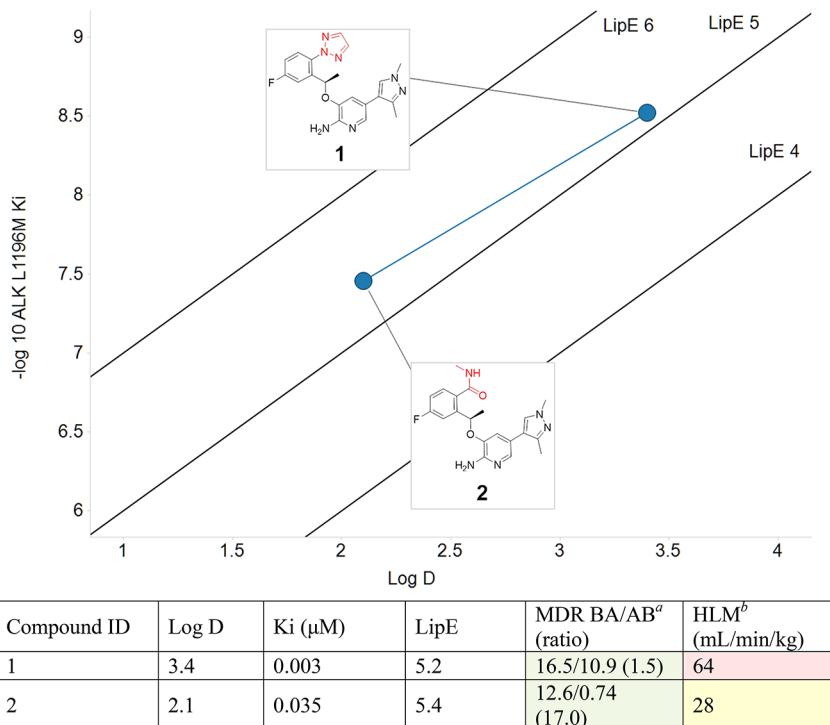


Figure 6. Isoefficient ALK inhibitors. ^aMDCK-MDR1 BA/AB efflux at 2 μM substrate concentration and pH 7.4. ^b $\text{Cl}_{\text{int,app}}$ refers to the total intrinsic clearance obtained from scaling in vitro half-lives in human liver microsomes.

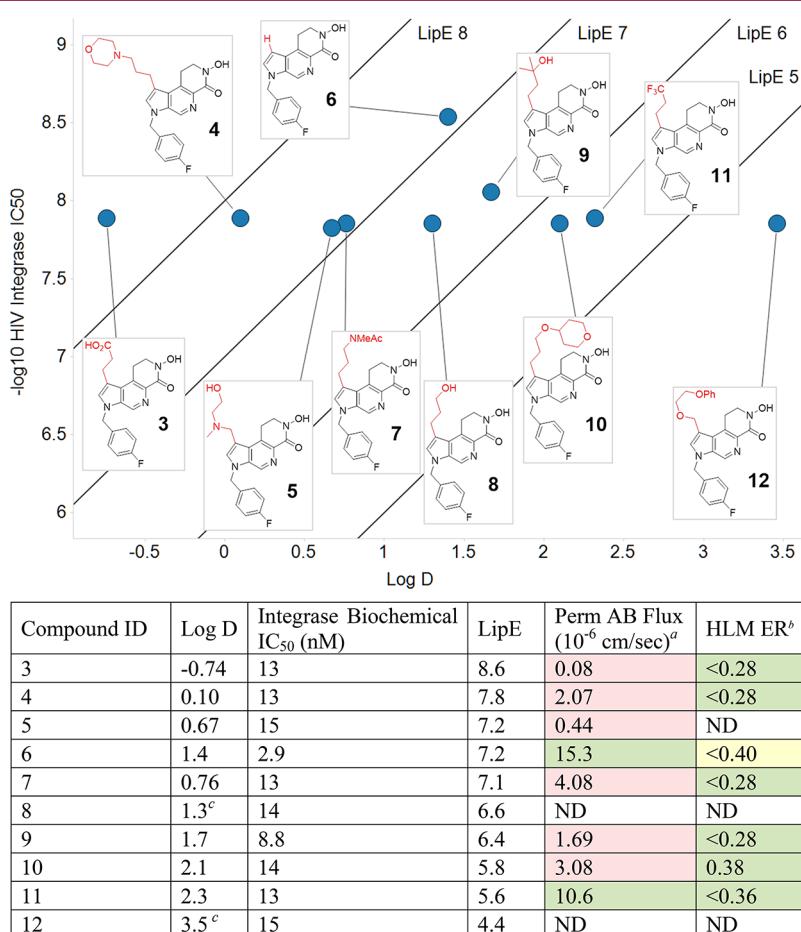


Figure 7. Comparison of solvent-front polarity on potency and LipE in a cell-free assay. ^aPermeability assessed using Caco-2 flux assay. ^bExtraction ratio in human liver microsomes. ^cInternal data.

■ INTERPRETATION OF LIPOPHILIC EFFICIENCY CHANGES

Figure 5 shows examples of arbitrary potency and lipophilicity combinations associated with different lipophilic efficiencies. Compounds A and E represent an iso-LipE change of potency since these two compounds have the same LipE. Compounds A and C represent an isopotent change of LipE. Lipophilic efficiency increased for compound C relative to compound A because of decreased lipophilicity with unchanged potency. Compounds A and D illustrate an isolipophilic change of both LipE and potency. Potency increased while lipophilicity remained the same resulting in increased LipE. Compound A and compound B reflect a LipE change that arises from a change in potency *and* lipophilicity. Finally, compound E to compound C illustrate an example of an efficiency increase with potency and log *D* decrease. Importantly, potency-centric comparisons of compounds such as these may not reveal potentially valuable compounds: in terms of potency alone, compounds B, D, and E are indistinguishable. From the LipE plot compound D and especially compound B are favored relative to E, with equal potency achieved at 1 and 2 log *D* units lower than for E. There is more potential to optimize starting from D and B, using design strategies that increase log *D* (for example, to increase potency, permeability, or selectivity relative to an undesired off-target) *within the preferred log D space of 1–3*. There is the potential that a modification of compound B that produces a new compound with one unit higher log *D*, even if

it is just an iso-LipE change, would provide a compound that was 10-fold more potent than compound D and at the center (a sweet spot) of the favored log *D* range. Herein we discuss published examples of these LipE changes in the context of structure based drug design and physiochemical properties based optimizations.

■ ISO-LIPE COMPOUNDS

Multiple potency and lipophilicity combinations can result in the same LipE. Therefore, in isolation, knowing only the LipE of a molecule is not informative. It is important to analyze sets of compounds in the LipE plot format to gain knowledge useful for future optimization-oriented designs. For example, compound 1 had an ALK-L1196M *K_i* value of 2.6 nM with a measured log *D* of 3.4, resulting in a lipophilic efficiency of 5.2 (Figure 6).⁵⁰ Compound 2, while 13-fold less potent with an ALK-L1196M *K_i* value of 35 nM, had a log *D* of 2.1 resulting in a similar lipophilic efficiency of 5.4. If considering potency alone, compound 1 would appear significantly better than compound 2. These two compounds are isoeficient and comparably attractive for follow-up. Furthermore, given that 1 had a log *D* of 3.4 which is at the high end of what is optimal for ADME properties, compound 2 could be a better starting place despite the lower potency. In fact, compound 1 had a higher clearance relative to compound 2 while both had good permeability. This illustrates the importance of interpreting LipE changes in the context of lipophilicity. Equally

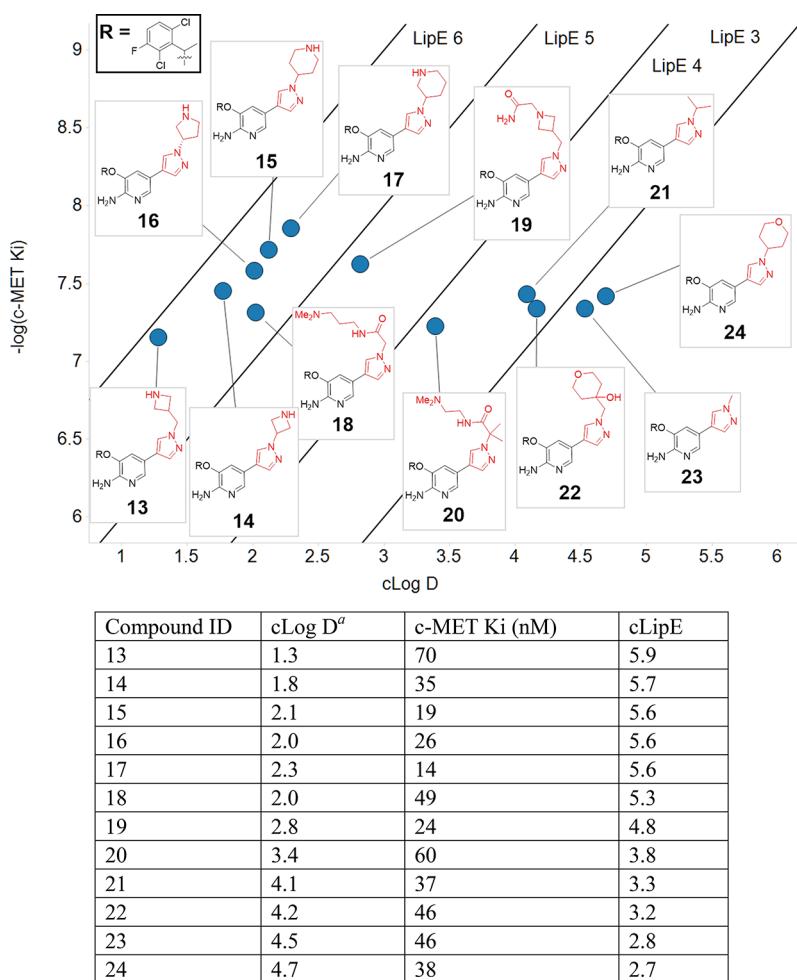


Figure 8. Example of variation of solvent-exposed substituent significantly varying log *D* and LipE over a narrow range of potency (LipE based on calculated cLogD figures, hence cLipE). ^acLogD calculated with ACD Labs version 12.1.

important to lead compound selection are the specific optimization strategies and tactics, as multiple lead compounds can lead to next generation analogs with overall improved profile. It is commonly reported in the literature that specific structural changes induce a large change in potency without recognition of the change in lipophilicity and the consequent LipE change. As illustrated by compounds **1** and **2**, a 10-fold increase in potency that required a 1.3 unit increase in $\log D$ is significantly less valuable to an optimization program than a structural change that delivered a

10-fold increase in potency with no increase (or even a decrease) in $\log D$. An exception to this arises if the former results in a significant improvement on another program goal (e.g., selectivity vs a key off-target).^{51–54}

■ ISOPOTENT EFFICIENCY CHANGES

In contrast to the above example, two compounds with the same potency can have different lipophilic efficiencies. Without considering lipophilicity, they would appear similar, but the

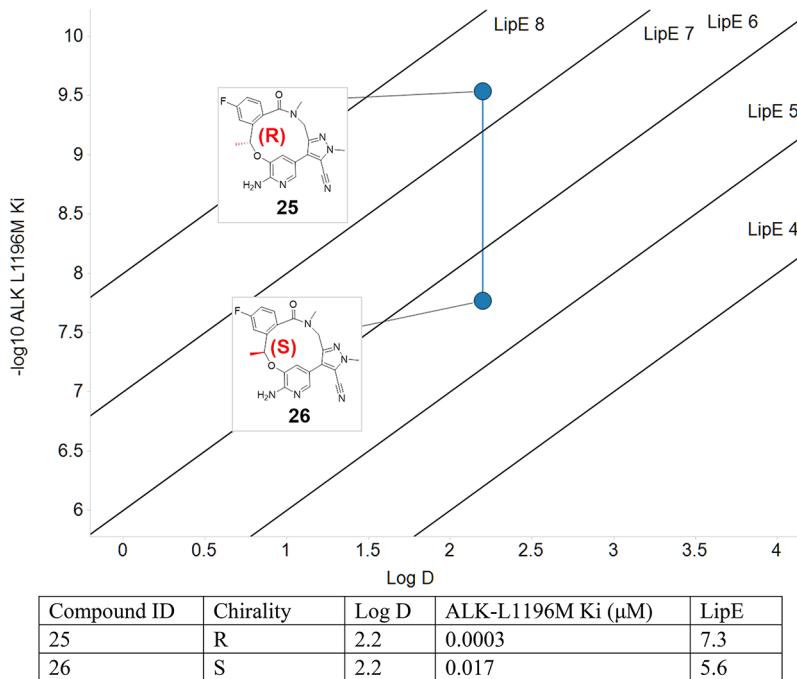


Figure 9. Chirality based LipE analysis of ALK inhibitors.

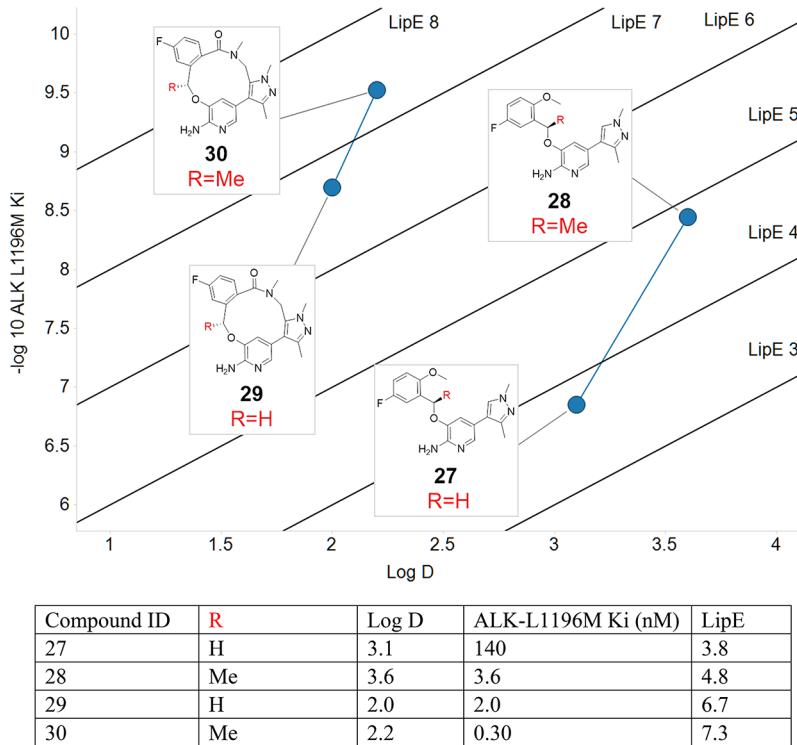


Figure 10. Methyl effect on LipE in the context of ALK biochemical activity.

compound with lower log D will have a higher lipophilic efficiency. This is an isopotent efficiency change. Isopotent efficiency differences vary in origin but can commonly result from introduction of ligand polarity in solvent-exposed regions of the binding pocket. This results in the reduction of lipophilicity without adversely affecting potency since there is no effect on the factors driving the affinity of the ligand for the protein. Increasing bound ligand polarity in solvent-exposed protein regions avoids desolvation penalties arising from ligand–protein binding. An example of this effect is illustrated by the potency of HIV-1 integrase inhibitors 3–12 in a cell-free assay, in which decreases in lipophilicity are seen while potency is maintained, which resulted in a LipE increase (Figure 7).⁵⁵ On the basis of a model of HIV-1 integrase,⁵⁶ the R-group modifications were made in solvent-exposed regions. These changes delivered an increase in lipophilic efficiency that was unrelated to the identity of the binding site. In this case, we hypothesize that increased LipE would not be expected to impart greater selectivity for the target over other homologous proteins. This example illustrates that

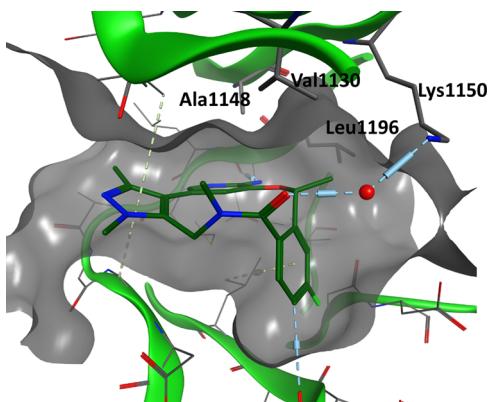


Figure 11. Cocrystal structure of 30 with ALK (1.8 Å resolution; PDB code 4CMU).

interpretation of changes in LipE is most effective when accompanied by ligand–protein complex structural understanding for the comparator ligands. Also important to note was the shift in ADME properties across the compounds within this series related to log D . All compounds were within a few fold of each other in terms of potency, despite log D ranging from −1 to 4. Only two analogs had moderate to high in vitro permeability (6 and 11). Of these compounds, 6 had significantly higher LipE, making it a valuable lead. Without a LipE-based analysis, this compound would be lost in the subset of equipotent compounds.

A previous report of c-Met inhibitors highlighted another example of isopotent LipE changes (Figure 8).⁵⁷ In this case, extending polar substituents toward solvent via substitution on the *N*-pyrazole moiety allowed for tuning of lipophilicity to achieve both the desired potency and optimal physiochemical properties. This observation was supported by ligand–protein crystal structures. Across all cLogD values (1–5), potency varied by less than 6-fold, as structural modifications were limited to the

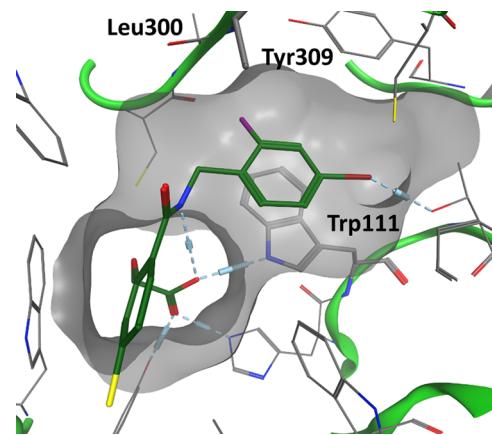
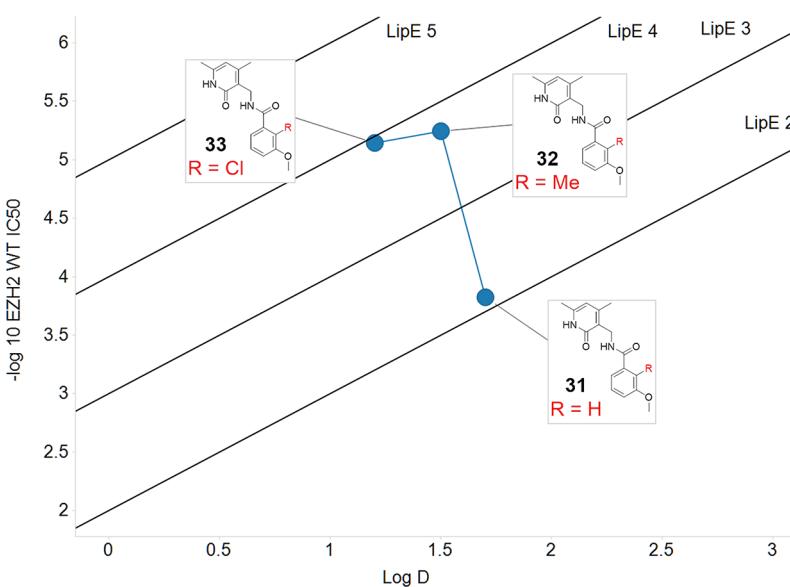


Figure 13. Aldose reductase inhibitor with fluorine LipE winner (1.5 Å resolution; PDB code 2IKI).



Compound ID	R	Log D	EZH2 biochemical IC ₅₀ (μM)	LipE
31	H	1.7	>150	<2.1
32	Me	1.5	5.7	3.7
33	Cl	1.2	7.2	4.0

Figure 12. Methyl LipE effect in the context of EZH2 activity.

solvent-exposed region of the binding pocket where desolvation penalties and meaningful protein/ligand interactions were minimized. These analogs ranged from cLipE 3 to cLipE 6. Modifying functionality in the solvent-exposed region of the binding pocket to modulate lipophilicity is a common strategy for maintaining potency and manipulating ADME properties.⁵⁸

■ ISOLIPOPHILIC LIP E CHANGES

Invariant lipophilicity coupled with potency changes across a series of analogs results in isolipophilic changes in LipE.

A common isolipophilic difference in efficiency occurs with pairs of enantiomers, although they are not restricted to such examples (Figure 9).⁵⁰ Analysis of isolipophilic LipE changes do not distinguish from a potency-centric analysis because the log D is the same.

Simultaneously improving potency and decreasing log D results in the largest increases in lipophilic efficiency. A number of examples are highlighted in the next section. These efficiency increases are typically indicative of increased affinity of a ligand for its target protein through enhanced protein/ligand

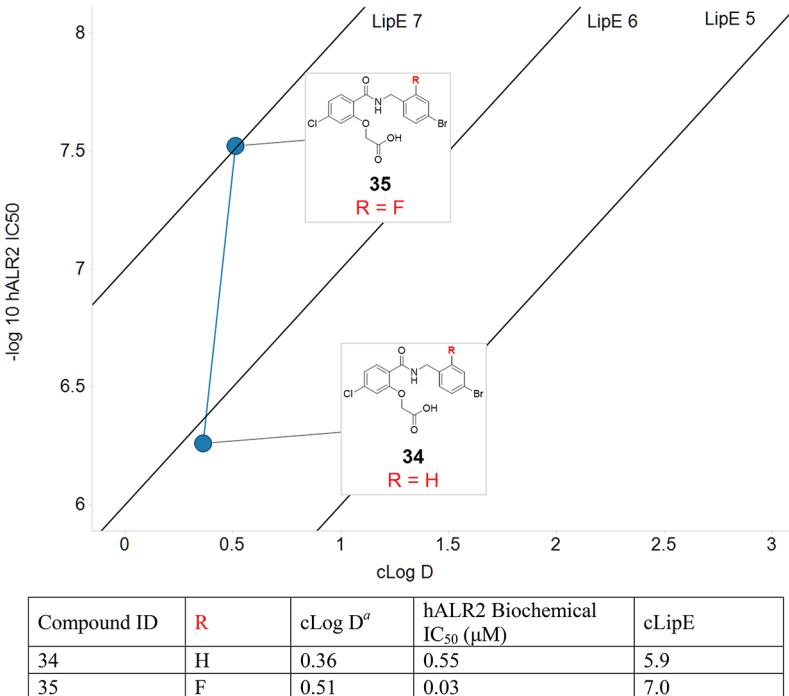


Figure 14. Aldose reductase inhibitor LipE increase caused by addition of a fluorine atom. ^acLogD calculated with ACD Labs version 12.1.

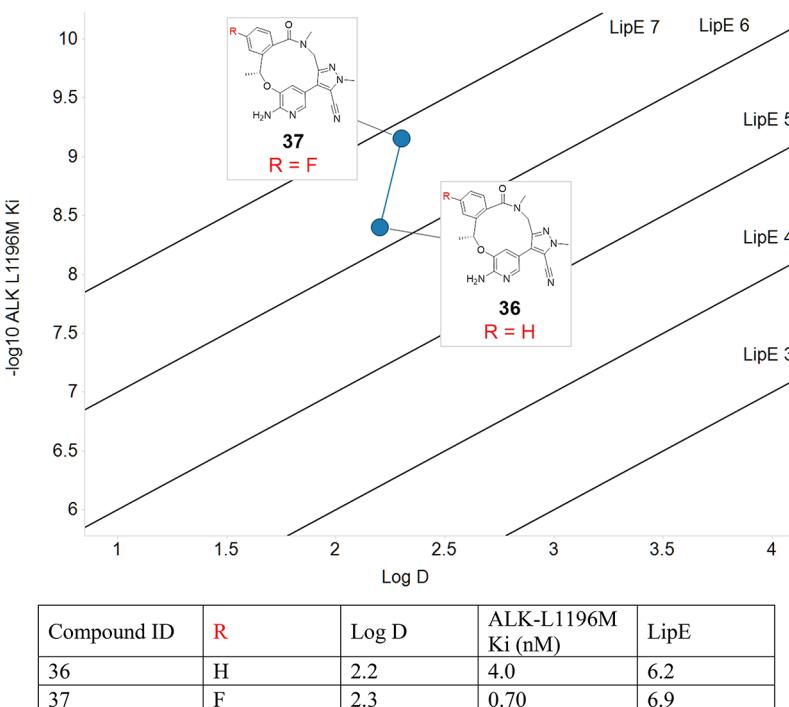


Figure 15. Hydrogen/fluorine ALK inhibitor matched pairs and impact on LipE.

interactions, as opposed to the improvement in lipophilic efficiency via introduction of polarity in solvent exposed regions, as discussed above.

MATCHED MOLECULAR PAIRS: LARGE LIPOPHILIC EFFICIENCY CHANGES

When appropriately placed, small structural changes have generated significant increases in LipE. We refer to the functional groups that increase lipophilic efficiency as LipE winners. The beneficial effects of methyl groups on binding have been noted for decades.^{59,60} The addition of a single methyl group that increases LipE has been colloquially termed a “magic methyl”.^{61–65} The change in lipophilicity due to addition of a methyl group depends on the specific structural context and can vary widely but typically provides a mean increase of 0.5 units.⁶⁴ If a 0.5 unit increase in log D enhances potency of the new analogs in line with the increase in lipophilicity, the potency increase would be 3-fold ($\log_{10}(3) = 0.48$). Beyond such 3-fold changes, potency increases on addition of a methyl group cannot solely be due to the

hydrophobic effect. Significant potency improvement by introduction of a methyl group can be due to a combination of ligand strain minimization and increased van der Waals forces between ligand and protein when the new methyl group optimally occupies a lipophilic pocket on the target protein.⁶⁶

A recent example of a methyl LipE winner was published by Johnson and Richardson et al. in the context of small molecule inhibition of ALK-L1196M (Figure 10).⁵⁰ Acyclic aminopyridine **27** had a lipophilic efficiency of 3.8. The methyl analog **28** increased the LipE by one unit to 4.8. In the macrocyclic series, **29** had a LipE of 6.7 and methylated macrocycle **30** had a LipE of 7.3, with an increase in LipE of 0.6 units. Both examples suggested potency increases beyond that due to the hydrophobic effect.

A cocrystal structure of compound **30** in ALK was obtained. The chiral methyl group filled a lipophilic pocket created by the conserved lysine (Lys1150), the gatekeeper leucine (Leu1196), and G-loop residues (Val1130 and Ala1148) (Figure 11).

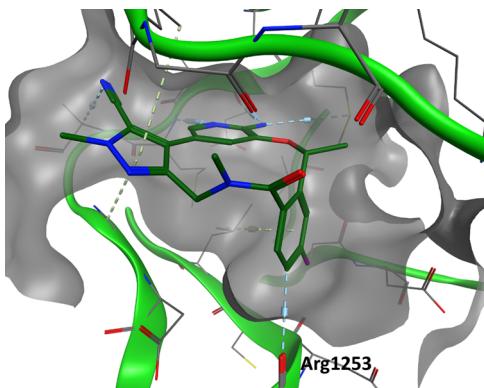


Figure 16. Cocrystal structure of **37** in ALK-L1196M (1.7 Å resolution; PDB code 4CLJ).

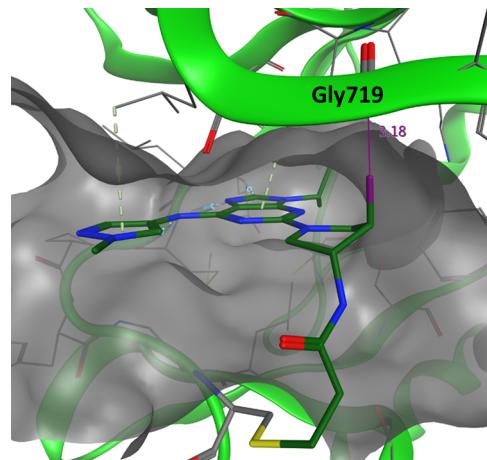


Figure 18. Cocrystal structure of **39** in EGFR-L858R/T790M mutant (1.5 Å resolution; PDB code 5UG8).

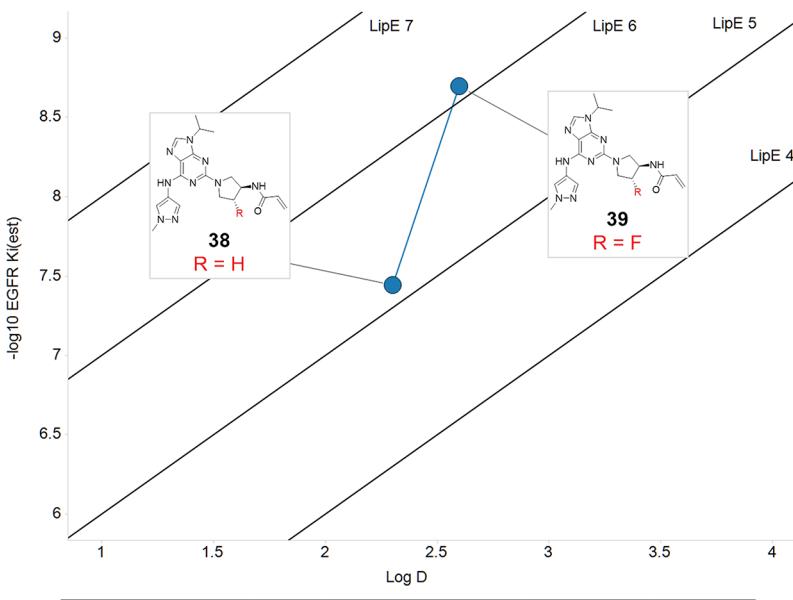


Figure 17. Hydrogen/fluorine EGFR inhibitor matched pairs and impact on LipE.

Another example of a methyl group LipE winner was exhibited by EZH2 inhibitors 31, 32, and 33 (Figure 12).⁶⁷ The analog with R = H (31) had an IC₅₀ of >150 μ M with a log D of 1.7 and a corresponding LipE of <2.1. Addition of an *ortho*-methyl group (32) improved the IC₅₀ to 5.7 μ M and reduced the log D to 1.5. The reduction in log D is thought to result from twisting the amide out of plane from the aryl system and thus increasing the water solvation of the system. The conformation bias by addition of methyl or chloro group also favors the bound conformation (approximately 50° dihedral angle Ph–CO bond). This analysis was performed using available small molecule X-ray structures in the Cambridge Structural Database (CSD) since no EZH2 cocrystal structure was available at the time. The combined effect of improved potency and reduction in lipophilicity resulted in over a 1.6 increase in LipE (LipE = 3.7). Similarly, addition of an *ortho*-chloro group (33, chlorine LipE winner) resulted in an IC₅₀ of 7.2 μ M with a log D of 1.2 and a corresponding LipE of 4.0.

Addition of a fluorine atom is also a commonly observed LipE winner. Van Zandt and co-workers described an example of a fluorine LipE winner that achieved lipophilic efficiency improvement through filling of a lipophilic pocket in aldose reductase created by residues with lipophilic side chains (Ala299, Leu300, Tyr309, Phe311) and enhancement of a critical π – π interaction with a tryptophan residue (Trp111) (Figure 13).^{68,69} The des-fluoro analog (34) had a biochemical IC₅₀ of 550 nM, whereas the fluorinated compound (35) had an IC₅₀ of 30 nM, representing an 18-fold improvement in potency. Since the typical calculated log D increase for addition of a fluorine atom to a phenyl ring is approximately 0.15 units,⁷⁰ this change is estimated to correspond to a 1.1 unit increase in lipophilic efficiency (Figure 14). Several reviews have described the use of fluorine in drug discovery.^{71–73}

In the context of ALK-L1196M inhibitors identified via structure based drug design, a fluorine LipE winner was observed when introduced on the phenyl ring (Figure 15). This structural change drove a 0.7 unit increase in LipE. In this case, the LipE

increase was due to a small (0.1 log D units) increase in lipophilicity in concert with an almost 6-fold improvement in potency.

In this example, the fluorine atom not only filled a lipophilic pocket, but it also increased the polarization of adjacent hydrogen atoms.¹⁰ This allowed for a strengthened polarized C–H hydrogen bond to a backbone carbonyl group (Arg1253) in the binding pocket (Figure 16). This dual role of the fluorine atom increased potency and lipophilic efficiency.

Another example of a large increase in LipE driven by introduction of a fluorine atom is shown in Figure 17. EGFR inhibitor 38 had an estimated K_i of 36 nM.^{74,75} Introduction of a *trans*-fluorine (39) improved potency to 2 nM while only increasing log D by 0.3 units. This resulted in a one unit increase in lipophilic efficiency. Although these compounds are unique in their ability to irreversibly bind EGFR, because binding affinity was considered, the LipE interpretation was appropriate.

Structurally, the fluorine atom reinforced the diaxial conformation of the pyrrolidine substituents, filled a lipophilic pocket formed by G-loop residues and backbone, and additionally may have benefited from lone pair interaction with empty

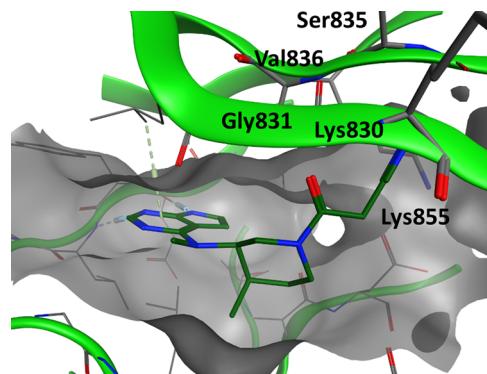
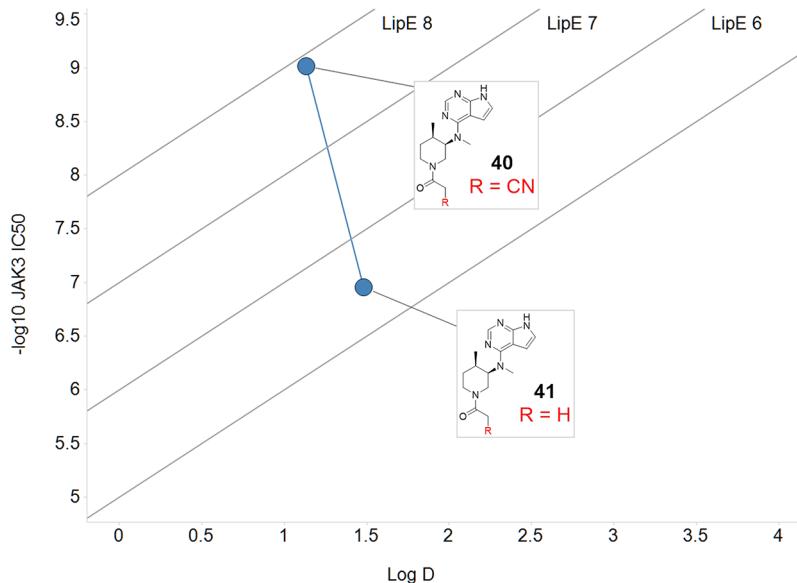


Figure 20. Cocrystal structure of tofacitinib (**40**) with JAK3 (2.0 Å resolution; PDB code 3LXK).



Compound ID	R	Log D	JAK3 Biochemical IC ₅₀ (nM)	LipE
40	CN	1.1	1.0	7.9
41	H	1.5	110	5.5

Figure 19. Nitrile LipE winner impact on LipE for JAK inhibitors.

orbitals of the amide carbonyl of Gly719 (Figure 18). This was corroborated by the close contact between the fluorine and carbon atom.

Yet another example of a small structural change that can produce a large increase in lipophilic efficiency is the introduction of a nitrile group. Unlike the addition of a methyl group or a fluorine atom, introduction of a nitrile typically decreases $\log D$ (unless it has the effect of reducing the pK_a of an adjacent cationic charged group). Therefore, if this structural change maintains

or improves potency, lipophilic efficiency improves as well. Tofacitinib (40) is a clinically marketed JAK inhibitor that contains a nitrile with a pronounced effect on LipE (Figure 19).⁷⁶ Amide 41 had a LipE of 5.5 while tofacitinib (40), which only differed from 41 by the addition of a nitrile group, had a LipE of 7.9. This dramatic 2.4 unit increase in LipE was an additive effect due to a 0.4 unit decrease in $\log D$ and 116-fold enhancement in biochemical potency.

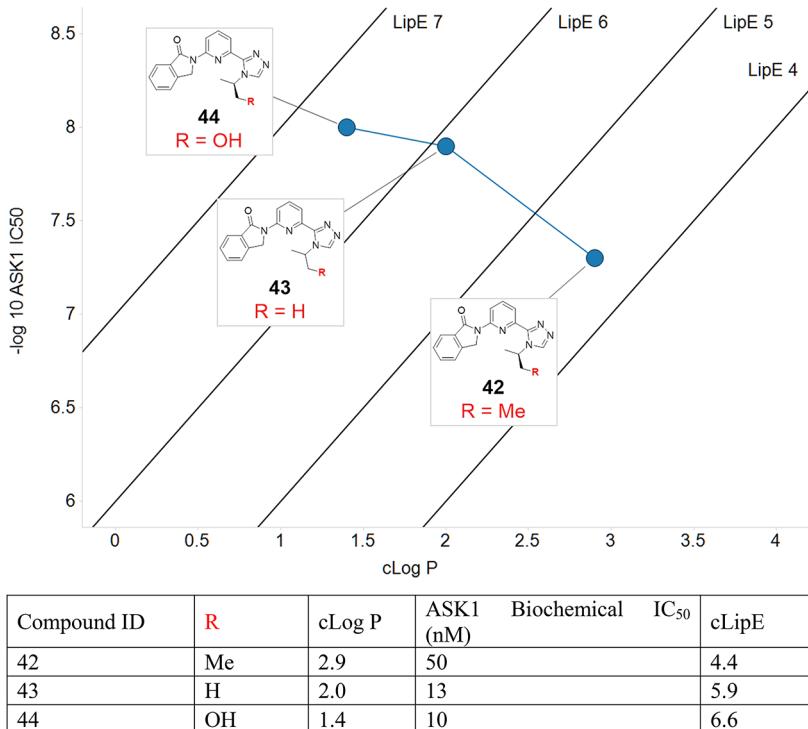


Figure 21. LipE of ASK1 inhibitions.

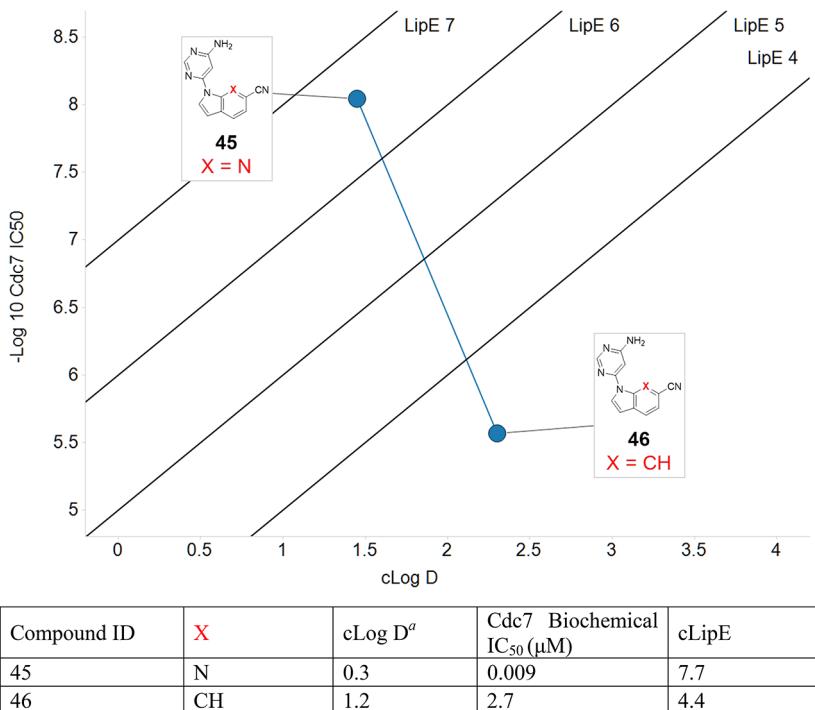


Figure 22. Potency shift resulting from nitrogen atom incorporation. ^acLogD calculated with ACD Labs version 12.1.

A cocrystal structure of tofacitinib (**40**) in JAK3 showed the nitrile filling a channel created by the G-loop (Val836, Ser835,

Gly834, Gly831, Lys830) and the conserved lysine (Lys855), with complementary electrostatic interactions (Figure 20).⁷⁷

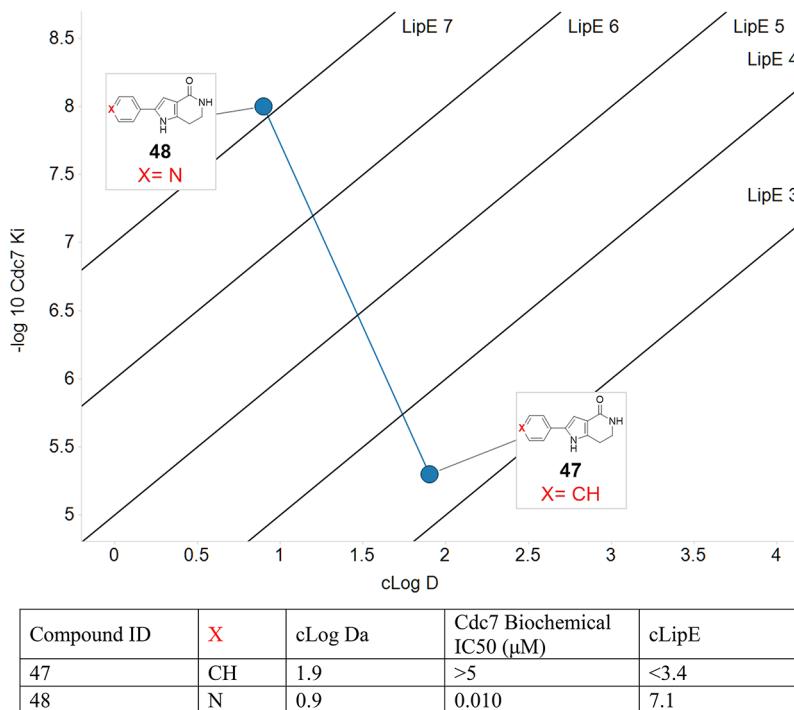


Figure 23. Potency shift resulting from nitrogen atom incorporation. ^acLogD calculated with ACD Labs version 12.1.

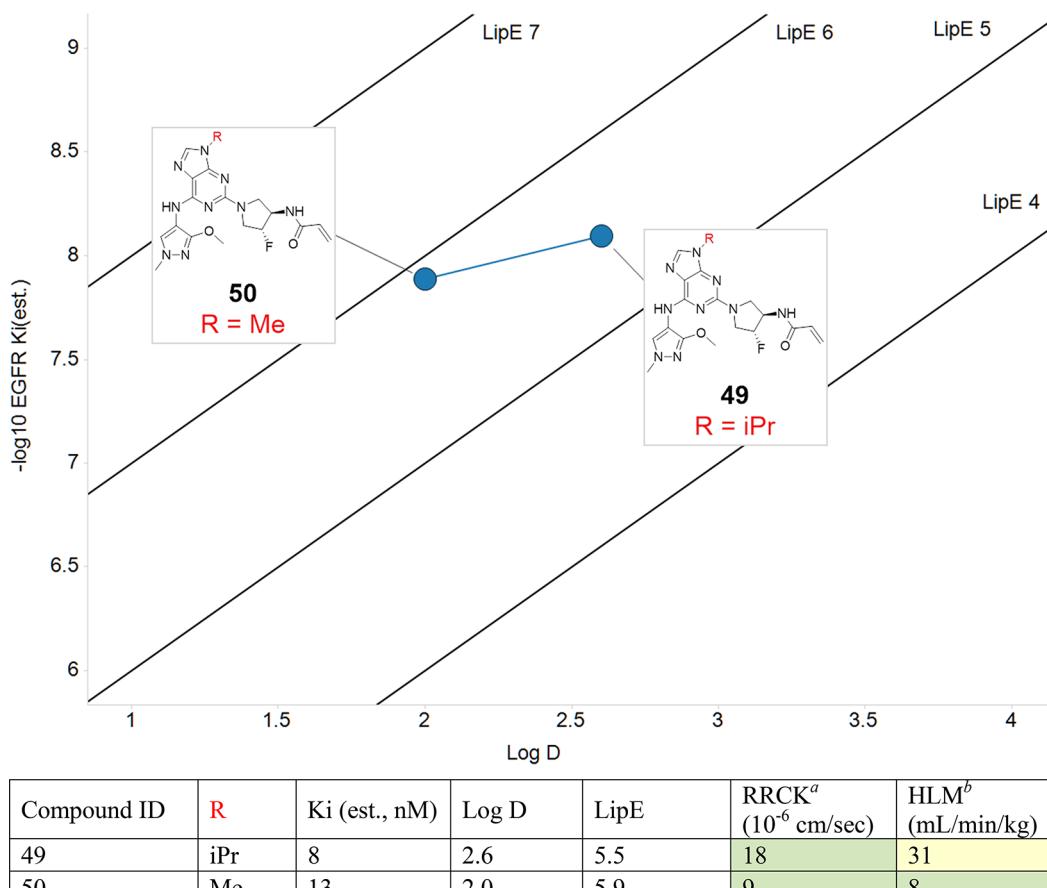


Figure 24. Truncation of EGFR inhibitors to improve HLM-mediated clearance. ^aRRCK: permeability as measured using low-efflux MDCK-LE cell line. ^bHLM: human liver microsomal clearance.

Lanier and co-workers recently published an example of an efficiency based optimization of ASK1 inhibitors.⁷⁸ They showed that incorporation of an alcohol group provided a small improvement in biochemical potency coupled with a large decrease in lipophilicity to increase the LipE of the resulting analog (42 and 43 to 44, Figure 21). The alcohol group extended into a polar area, deep in the active site, toward asparagine and serine residues forming a productive interaction in this region. The isopropyl and *n*-butyl functionalities on 42 and 43 may partially desolvate these polar residues, resulting in decreased LipE relative to the alcohol analog (44).

In addition to the above-described structural changes, the colloquially termed “necessary nitrogen atom” is a commonly cited example of a structural modification that can have a large impact on potency and lipophilicity.⁷⁹ A 2013 publication from Allen et al. reported incorporation of a nitrogen atom that improved biochemical potency against Cdc7 by 300-fold (Figure 22).^{79,80} The nitrogen-containing analog (45) had a 0.9 unit lower calculated cLogD than the all-carbon analog (46) which resulted in a 3.3 unit increase in lipophilic efficiency. Incorporation of the nitrogen atom was reported to change the torsional profile of the biaryl system and allowed a low energy planar conformation. These calculations, coupled with the dramatic difference in potency and efficiency, enabled determination of the preferred ligand binding conformation in the absence of a crystal structure.

Another example of a nitrogen LipE winner in the context of Cdc7 inhibitors was reported in 2008 by Vanotti and co-workers.⁸¹ There was a greater than 500-fold improvement in biochemical potency from the phenyl analog (47) to the pyridyl analog (48) in addition to a reduction in log D, which resulted in a dramatic 3.7 unit higher lipophilic efficiency for compound 48 (Figure 23).

Although no crystal structure was available, analysis of a Cdc7 homology model indicated that the large difference in potency and efficiency was likely due to a favorable interaction between the pyridine nitrogen of 48 and a backbone N–H. The phenyl

analog (47) cannot compensate for the high desolvation penalty of the backbone N–H due to unfavorable phenyl C–H/backbone N–H interaction, reconciling the dramatic loss in potency for 47.

■ TRUNCATION OF INEFFICIENT FEATURES AND LIOPHILIC EFFICIENCY

In addition to the above summarized examples of incorporation of small functional groups and one atom changes to improve LipE and modulate physiochemical properties, truncation of inefficient features can be a valuable strategy for improvement of efficiency and ADME properties as well. In the context of EGFR inhibitors, compound 49 was highly potent with a biochemical potency of 8 nM but moderate HLM-mediated clearance ($31 \mu\text{L min}^{-1} \text{mg}^{-1}$) (Figure 24).⁷⁵ Truncation of the *N*-isopropyl group to an *N*-methyl group (50) maintained potency while decreasing log D by 0.6 units. This resulted in a 0.4 unit increase in LipE and is an example of efficiency increase with potency and log D decrease. Importantly, this change also produced a large reduction in HLM-mediated clearance (to $8 \mu\text{L min}^{-1} \text{mg}^{-1}$) while maintaining

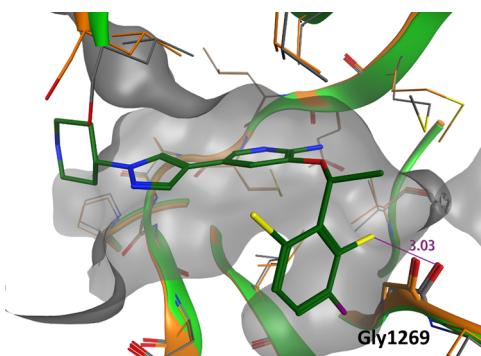


Figure 26. Cocrystal structure of 51 with ALK-L1196M (green/gray; 1.7 Å resolution; PDB code 2YFX); Apo crystal structure of ALK-L1196M (orange; 1.9 Å resolution; PDB code 2YHV).

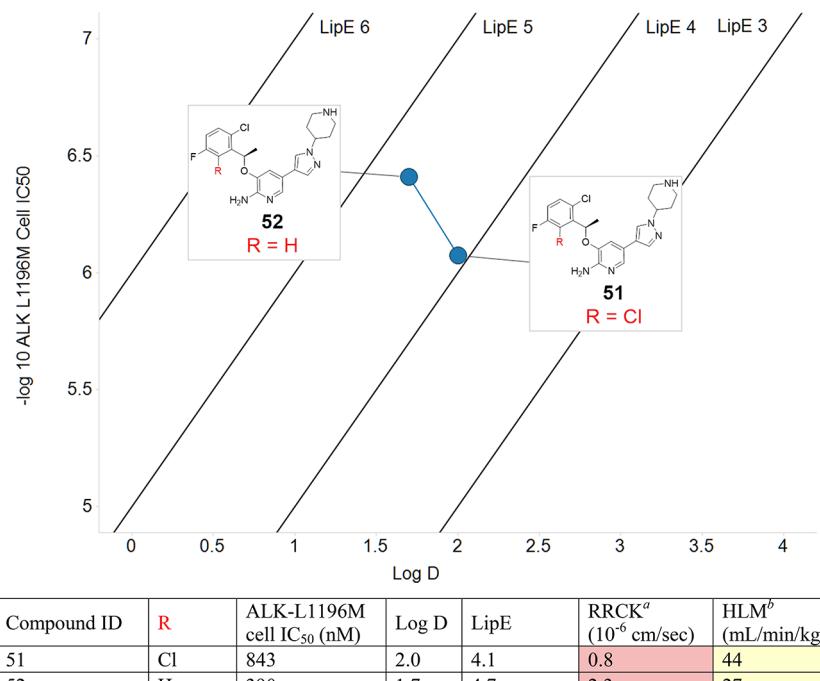


Figure 25. Truncation of ALK inhibitors to improve HLM-mediated clearance. ^aRRCK cells with low transporter activity were isolated from Madin–Darby kidney cells and were used to estimate intrinsic absorptive permeability. ^bHLM: human liver microsomal clearance.

acceptable permeability. Figure 18 (above) shows a cocrystal structure of a related analog in EGFR.

Johnson and co-workers reduced molecular weight and improved LipE in the search for a second generation ALK inhibitor, exemplified by **51** and **52** (Figure 25).¹⁰ Truncation of the aryl chlorine improved potency by approximately 2-fold while decreasing log D by 0.3 units. This resulted in a 0.6 unit increase in LipE and improved HLM-mediated clearance and permeability.

The des-chloro analog (**52**) was designed based on analysis of the cocrystal structure ALK-L1196M with compound **51**

(green/gray, Figure 26). The chlorine atom was removed to allow the Gly1269 backbone carbonyl to relax toward the inhibitor, better resembling the apo conformation and improving the binding affinity for ALK (orange, Figure 26).

CYCLIZATION/MACROCYCLIZATION AND LIOPHILIC EFFICIENCY

Decreasing ligand strain in the bound conformation is a common strategy for increasing lipophilic efficiency by maximizing enthalpic

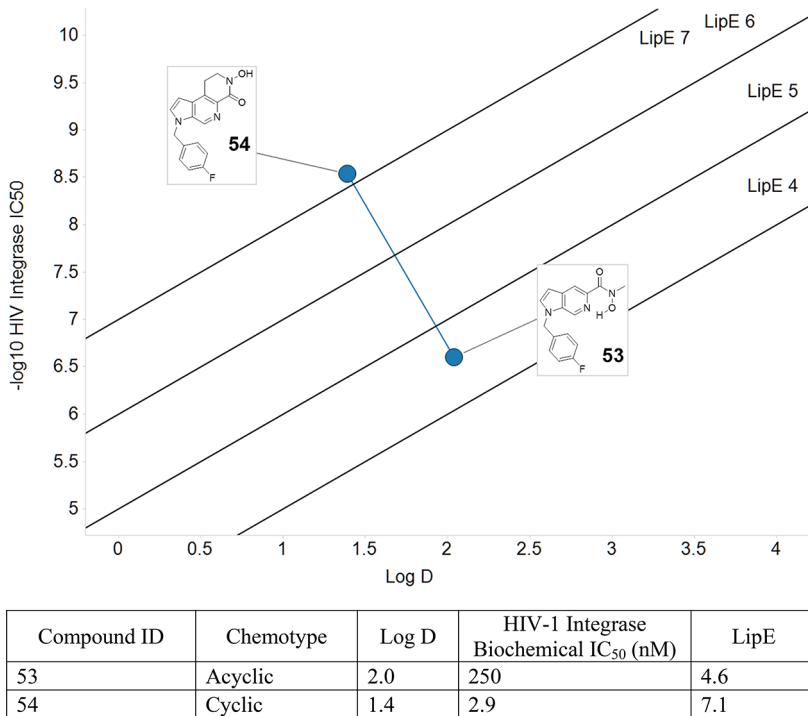


Figure 27. Improvement of LipE by reduction of ligand strain.

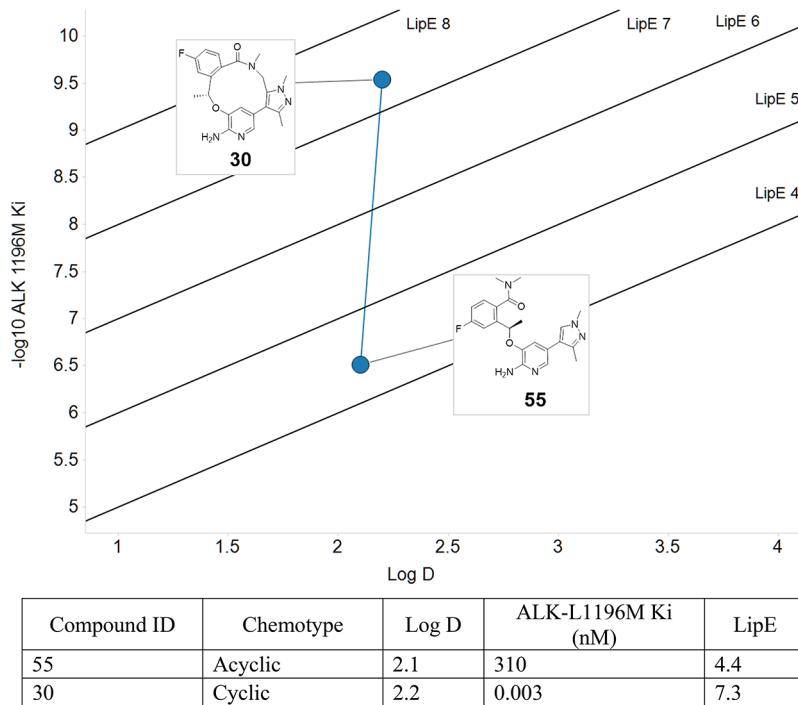


Figure 28. Improvement of LipE by reduction of ligand strain.

contributions to binding. A tactic that can be used to achieve this is cyclization, but if cyclization provides an undesired conformation relative to binding site requirements, this incurs a loss of potency and efficiency. In theory, cyclization can also increase binding affinity through reduction of entropic penalties. There is a common misperception that cyclization of ligands improves affinity through reducing conformational entropy losses on binding to a protein.⁸² Cyclization using a lipophilic linker is often pursued to improve potency, although many published examples do not analyze LipE so it is initially unclear if potency improvements are due to reduction of strain or simply lipophilicity-driven binding.^{83,84}

A lipophilic efficiency analysis was reported for cyclization to improve potency in the context of HIV-1 integrase inhibition.⁵⁵ Hydroxamate **53** inhibited HIV-1 integrase with a biochemical IC₅₀ value of 250 nM and a LipE of 4.6 (Figure 27). It was hypothesized that there was significant ligand strain in the bound conformation for this compound, whereas the low energy conformation of the corresponding lactam (**54**) mimics the ideal binding conformation of the hydroxamate and thus would not incur ligand strain. Lactam **54** was less lipophilic due to lack of the

intramolecular hydrogen bond between the acid and the pyridine nitrogen and an additive dipole effect with increased solvation needs of the carbonyl and azole nitrogen atoms to reduce the repulsion energy of the adjacent lone pairs.⁸⁵ The decrease in log D coupled with almost 100-fold improvement in potency resulted in a dramatic increase in LipE by 2.5 units. We refer to this as a LipE winner via cyclization. Interestingly, the bound conformation of hydroxamate **54** was thought to produce 6 kcal of strain according to modeling. On the basis that -1.36 kcal/mol corresponds to 10-fold increase in potency, relieving the ligand strain should account for a 44-fold enhancement in activity. Given that the improvement in potency is 86-fold, there are potentially other contributors to the potency increase apart from stabilizing the bound conformation.

A second example of a LipE winner via cyclization is illustrated by a matched pair of ALK inhibitors. Cyclization to form the 12-membered amide dramatically improved potency with log D unchanged, resulting in a LipE improvement of 3 units (Figure 28).⁵⁰

The cocrystal structures of both analogs were revealing. Internal clash in the acyclic series caused rotation of the amide to an unproductive conformation for beneficial interactions with the G-loop (His1124) and conserved lysine (Lys1150) of ALK (**55**, Figure 29, left panel). On the other hand, the macrocyclic amide was able to form productive water-mediated interactions to the G-loop (His1124) and conserved lysine (Lys1150) (**30**, Figure 29, right panel). The cyclization had minimal impact on the low energy conformation of the ligand and was also able to maintain other important interactions such as a polarized C–H hydrogen bond to Arg1253 at the bottom of the pocket. If strategically designed, cyclization can greatly improve both potency and efficiency. These examples are most elegant when they result from small structural changes that enhance protein/ligand interactions and/or minimize ligand strain, while only minimally impacting log D.

Kung and co-workers utilized cyclization as a tactic to improve potency and efficiency as well (Figure 30). Acyclic EZH2

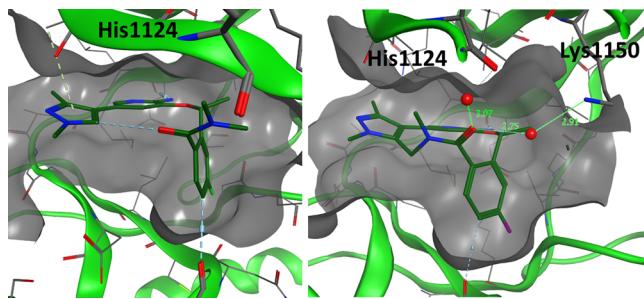
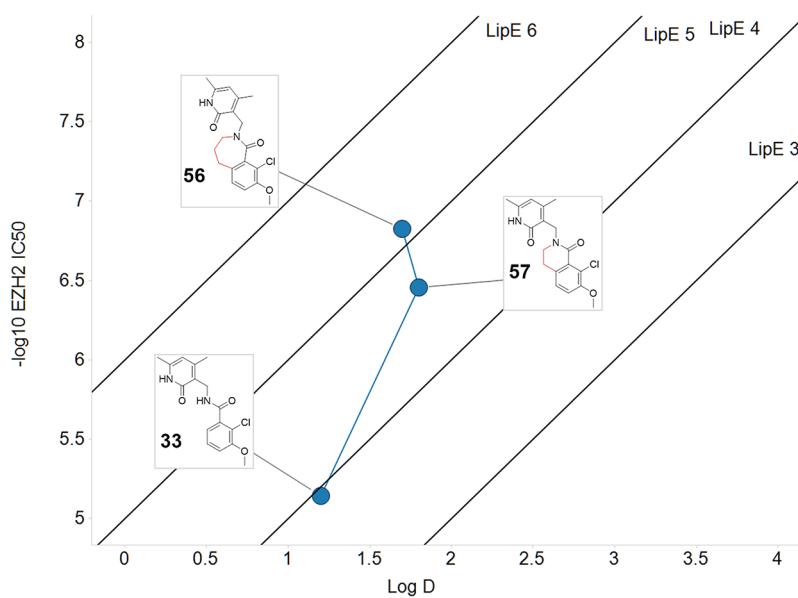


Figure 29. Left panel: Co-xtl of acyclic amide **55** (2.3 Å resolution; PDB code 5KZ0). Right panel: Co-xtl of macrocyclic **30** amide (1.8 Å resolution; PDB code 4CMU).



Compound ID	EZH2 IC ₅₀ (μM)	Log D	LipE	HLM ^a (μL/min/mg protein)
33	7.2	1.2	4.0	8
56	0.15	1.7	5.2	82
57	0.35	1.8	4.7	35

Figure 30. Cyclization of EZH2 inhibitors: impact on potency, efficiency, and ADME properties. ^aHLM: human liver microsomal clearance.

inhibitor 33 had an IC_{50} of 7.2 μM .⁶⁷ Cyclization to the seven-membered lactam (**56**) or the six-membered lactam (**57**) improved potency by 48-fold and 21-fold respectively, relative to the acyclic analog (**33**). This resulted in a 1.2 unit increase in LipE for the seven-membered ring (**56**) and a 0.7 unit increase for the six-membered ring (**57**). On the basis of these results, it was inferred that cyclization stabilized the ideal aryl–carbonyl torsion angle for binding.

CONCLUSIONS

While potency is a component of lipophilic efficiency, it is important to weigh both potency and lipophilicity together when selecting lead compounds for drug design. Selection between two compounds based on potency alone may provide an entirely different trajectory for the project than when potency and lipophilicity are considered in concert. In addition, the context of lipophilicity and its impact on ADME properties should not be underestimated. The optimization of LipE through modifications that move lead molecules toward acceptable log D ranges based on desired ADME properties is an effective way to design small molecule inhibitors with overlapping potency, selectivity, and ADME properties for reduced risk of attrition in clinical trials. The use of LipE-based optimization with structure based drug design helps to identify specific structural changes that can have a large effect on compound efficiency against a target of interest. The examples presented, and many others published in the literature, exhibit how small structural changes can have a large impact on potency and efficiency. This trend promotes a philosophy to think critically about small, deliberate changes as part of the drug design process.

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Notes

The authors declare the following competing financial interest(s): The authors are employees and shareholders of Pfizer.

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Rebecca A. Gallego completed her Ph.D. in 2013 with Prof. Richmond Sarpong at the University of California at Berkeley. Her graduate work

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Martin P. Edwards, Ph.D., is VP and Head of Oncology Medicinal Chemistry at Pfizer's R&D site in La Jolla, CA. Martin is an accomplished drug discovery leader who is closely involved with medicinal chemistry practice and drug discovery innovation on Pfizer drug discovery projects. Martin is the originator of the drug design concept “lipophilic efficiency” and its use in drug discovery. Chemists working under Martin's leadership have advanced numerous compounds to the clinic, most notably maraviroc, crizotinib and lorlatinib. Martin completed Ph.D. studies at Imperial College in Professor Steve Ley's group and postdoctoral research in The Stork Group at Columbia University. He is a member of the American Chemical Society and an inventor or author of around 40 patents, patent applications, peer-reviewed papers, and invited book chapters.

ACKNOWLEDGMENTS

The authors acknowledge Mark E. Flanagan for discussion of the structure based drug design of tofacitinib (**40**). In addition, the authors acknowledge Klaus Dress for translation of refs **4** and **5** and Vincent N. G. Lindsay for translation of ref **6**, as well as Jennifer Lafontaine for helpful discussions. Finally, we thank Robert Hoffman for design of Figure 1 and Mehran Jalaie for design of Figures 1, 11, 13, 16, 18, 20, 26, and 29.

ABBREVIATIONS USED

ACD Labs, advanced chemistry development labs; ADME, absorption, distribution, metabolism, excretion; Ala, alanine; ALK, anaplastic lymphoma kinase; Arg, arginine; ASK1, apoptosis signal-regulating kinase 1; Cdc7, cell division cycle 7; c-Met, met proto-oncogene; EGFR, epidermal growth factor receptor; ER, extraction ratio; EZH2, enhancer of zeste homolog 2; Gly, glycine; His, histidine; HIV-1, human immunodeficiency virus; HLM, human liver microsome; Leu, leucine; JAK3, Janus kinase 3; LipE, lipophilic efficiency; LipMetE, lipophilic metabolic efficiency; Lys, lysine; MDCK, Madin–Darby canine kidney cell line; MDR, multidrug resistance; PPBO, physiochemical property-based optimization; SAR, structure–activity relationship; SBDD, structure based drug design; Ser, serine; Trp, tryptophan; Tyr, tyrosine; Val, valine

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