

Part I
Introduction to Lead Generation

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Introduction: Learnings from the Past – Characteristics of Successful Leads

Mike Hann

Contemporary nodding sages in drug discovery will often be heard to say “Tut, tut, if I wanted to get there, I wouldn’t start from here.” Such comments are based on their experience (aka insights from hindsight!) where failure of compounds in late lead optimization, preclinical, or clinical work can all too often be associated with poor chemical and physicochemical properties of the chemical series being pursued. It is, of course, one of the basic truisms of science that where we start an optimization process will likely have profound influences on where it ends up!

If this is true then why does so much of medicinal chemistry, and hence drug discovery, still suffer from a lack of awareness of these facts? After all they can save enormous amounts of time and money that are spent on taking forward compounds that fall outside of “drug-like space” until they predictably failed.

Is it (1) because people still do not believe in a drug-like space and thus ignore the fact that compounds invariably get bigger and more lipophilic as lead optimization progresses in the search for potency? Or is it (2) because they believe they will be exceptional in their skills and that this will allow their project to be equally exceptional and succeed outside of received or accepted wisdom? Or is it (3) that they just cannot find a good starting point that will deliver or, possibly, they have not tried hard enough to find such a starting point? Or is it (4) that such a poor choice of target that finding a small molecule to effectively interact with it is nigh impossible? All or any of these can be crucial in determining what course a project takes, but one of the biggest confounding issues is that although it can be argued (see below) that a drug-like space exists, there are many good drugs that fall outside of this drug-like space. Thus that paradoxical saying “the exception that proves the rule” is all too often used to justify continuing. The effect of this is to allow reason (2) to be actively beckoning teams away from sticking to the drug-like space mantra. Only if you have exhaustively tried and failed to find success in the drug-like space should you feel you have permission to go beyond it and then you will most definitely need not only all your skills but probably also a large slice of luck! Note that if we all choose to back the low odds scenario, that is, (2), all of the time, then we are indeed guaranteeing a poor return on investment.

So what is drug-like space? This has somehow erroneously become associated with Chris Lipinski's rule of five (Ro5) that actually refers to the probability that a compound will be orally bioavailable in humans [1]. The Ro5 states that if a compound has more than one violation of the following criteria – greater than 5 hydrogen bond donors (defined as the total number of hydrogens directly bonded to O or N), greater than 10 hydrogen bond acceptors (defined by all N or O atoms in the molecule), a molecular weight greater than 500, an octanol–water partition coefficient $\text{Log}P$ greater than 5 – then it will unlikely be orally bioavailable. Thus, the Ro5 only refers to one aspect (the oral adsorption) of the overall adsorption, distribution, metabolism, excretion, and toxicology (ADMET) profile of a compound. Clearly this, coupled with essential target engagement, is critical to the likelihood of it being an acceptable and efficacious drug. This conflation of Ro5 with drug space is probably due to the fact that most drug discovery projects do aspire to having oral bioavailability but while this may be desirable it is not sufficient. To truly define a drug-like space, we need guidance on parameters such as solubility, permeability, dose, toxicity, metabolism, and so on. Over the past 5–10 years, many analyses of large data sets from pharma companies have been published. A selection of the resulting “rules of thumb” about the preferred drug-like space are summarized in Table 1.1¹⁾. The use of such cutoff-based rules has often been criticized as being too black and white and, as a consequence, other more subtle ways of doing data fusion have been introduced (e.g., the quantitative estimate of drug-likeness (QED) by Hopkins and colleagues that uses weighted desirability functions [2]).

The prevalence of lipophilicity in these rules indicates how important it is to pay particular attention to this property. The term “molecular obesity” was introduced as a way to anthropomorphize the impact and danger of too much lipophilicity in compounds in development by analogy to the dangers of medical obesity [12]. The causative reasons why there is a tendency to allow lipophilicity to increase were also analyzed in this paper and Table 1.2 lists a number of the more obvious ones.

At the end of the day, it is often the required human dose that defines whether a drug is successful. Dosage determinants can be broadly divided into two key components – first, how much drug gets to the site of action and second how tightly does the drug bind to its target thus eliciting the desired effect. This balance between potency and availability is elegantly expressed in the drug efficiency index (DEI) developed by scientists at GSK in Verona [16]. Drug efficiency (DE) itself is defined as the fraction of administered dose that becomes available as the biophase concentration. The derived term DEI is then defined as the affinity $\text{p}K_i$ (log of affinity constant) added to $\log_{10}\text{DE}$. Thus, if the drug efficiency is less than 1%, it contributes a negative number when the logarithm is taken and rapidly detracts from the intrinsic potency of a compound. Another

1) If you've ever tried using your thumb to measure something, then you will be only too well aware how imprecise yet somehow reliable and convenient it is as a measure in the absence of something more precise!

Table 1.1 Drug-like space guidance on physchem properties.

Drug-like property considered	Guidance	Reference
Lipinski/Pfizer Ro5 for Oral bioavailability	Violating 2 or more of MW < 500, LogP < 5, HBD < 5, and HBA < 10 results in poor oral bioavailability	[1]
AZ receptor promiscuity	Maximize LLE = pIC ₅₀ – logP to reduce promiscuity	[3]
Pfizer 3/75 rule for toxicity	Keep cLogP < 3 and PSA > 75 to minimize toxicity	[4]
GSK 4/400 rule for general ADMET	Keep cLogP < 4 and MW < 400 for generally favorable ADMET properties	[5]
AZ permeability model	On average larger “small molecules” need more lipophilicity to penetrate membranes	[6]
GSK PFI model for general drug-like properties (including solubility)	Favored space from Property Forecast Index (PFI) when PFI = mChrom-LogD7.4 + number of aromatic rings < 6. But note that permeability max in PFI = 6–8	[7]
Pfizer dosage guidance for reducing toxicity	Keep predicted human efficacious dosage of <250 nM (total drug) and <40 nM (free drug)	[8]
GSK Developability Classification System based on permeability, dose, and solubility	Compounds with DCS classification of I, IIa, or III are much easier to develop	[9]
Drug-like property reviews	General overviews	[10,11]

useful way of thinking about drug efficiency is in terms of the amount of a dose that is being wasted; for instance, if a drug has a DE of 0.1%, then it means that 99.9% of a dose is never used at the target to elicit the required pharmacology!

Low dosage is not only good from the point of helping reduce the cost of goods but is also one of the only known predictors for low incidence of

Table 1.2 Reasons for lipophilicity increases in discovery projects.

Reason for logP increase	How to mitigate	Reference
Potency is most easily attained by lipophilic interactions that are nondirectional	Ensure maximum potency through polar (enthalpic) interactions is achieved	[13]
Permeability sweet spot is often found by indiscriminate use of lipophilicity – particularly for larger molecules	Use of LLE to control lipophilicity-driven membrane effects	[14]
Organic synthesis favors purification of lipophilic compounds	Design synthesis, work-up, and purification schemas that can cope with more polar molecules	[15]

idiosyncratic toxicity [17]. Thus, it is generally considered very unlikely that idiosyncratic drug reactions will occur at a total dose of 10 mg per day.

Another perspective on this can be gained from a study by Pfizer scientists on the survival of CNS active compounds as they progress through the development process, which suggests that a predicted human efficacious concentration of ≤ 250 nM (total drug) and ≤ 40 nM (free drug) was a good concentration to aim for [8]. It is interesting to note that 250 nM of a 500 Da molecule is equivalent to 10 mg dissolved in 80 l, (i.e., the approximate size of an average adult), and while we are not all water and certainly not homogeneous, it is interesting how these two widely different approaches to gaining insights into a good drug dosage converge on the same number. It is also worth remembering that the median affinity for small-molecule drugs at their actual target, where known, is around 20 nM, which is again consistent with the sorts of concentrations discussed here [18]. So, potency is a good thing but not in isolation of good pharmacokinetic properties. The challenge for medicinal chemistry is to optimize the two in parallel, which can be difficult when potency is easily driven by lipophilic interactions. Furthermore, potency is often the quickest experimental measurement to be returned to a project team and thus all too often overinfluences the next round of medicinal chemistry.

Another aspect of dose that is often misunderstood is the influence of the solubility of a drug, which is often far too low (especially for lipophilic molecules) to allow sufficient drug to be absorbed in the time that it passes through the GI tract. A useful way to represent this is via the developability classification system (DCS) that explores the interplay between permeability, dose, and solubility [9]. Poor-solubility compounds (i.e., those with dose/solubility ratios greater than 1000) require more liquid than the GI tract has available, meaning that the only less soluble compounds that can be sufficiently absorbed are those with very high permeability (this is class IIa in the DCS system). The fact that “brick dust” is a common term in the medicinal chemist’s lexicon is testament to the prevalence of this challenge.

Having explored some of the key aspects and challenges of drug-like space (and other chapters go into this in far greater detail), it is time to turn to the issue of lead-likeness. The term lead-like was first used in 1999 by Simon Teague and his colleagues at AZ in a publication entitled “The design of leadlike combinatorial libraries” [19]. This was an era where combinatorial chemistry was being used to feed high-throughput screens with novel compounds on the premise that ever-increasing numbers of compounds could be screened. The assumption being that large library synthesis would enable such diversity to be assessed that drug discovery could be industrialized and thus increase productivity [20]. The AZ authors were among the first to recognize that the lead optimization journey between a lead compound (i.e., the starting point) and the final candidate compound invariably involves an increase in mass. This occurs as potency is built by making new or better interactions than were in the lead. They proposed that there is a “great

deal of precedent to suggest that libraries consisting of molecules with MW 100–350 and $\text{clog}P$ 1–3.0 are greatly superior to those comprising druglike compounds.” In the following year, both Hann, Leach, and Harper at GSK and Oprea *et al.* at AZ published compilations of property differences between historical drugs and what were considered their lead-like starting points [21,22]. In the publication by Hann, Leach, and Harper, a theoretical analysis of why this increase might occur was also presented based on the concept of molecular complexity [21]. Thus, less complex compounds are easier to find because when there are less features in the ligand there are less bad interactions possible that can abrogate binding. The downside is that less complex starting points will likely need more sensitive assays to detect them. Soon afterward, the concept of ligand efficiency was proposed by Hopkins, Groom, and Alex at Pfizer, which spawned a whole raft of other indices to help medicinal chemists find the most efficient molecules in terms of mass, $\text{log}P$, polar surface area, and so on [23]. Much has been written and reviewed about the pros and cons of using such metrics or indices [24]. Suffice it to say here that one of the major pros has been the heightened awareness of the importance of good leads and the dangers of property inflation in lead optimization. Another consideration that has recently emerged is the importance of ensuring that enthalpic interactions are maximized early in lead optimization to reduce the overreliance on entropic binding for potency (often connected with lipophilic interactions) [25]. Also in this review, seven guidelines for medicinal chemists were suggested to help ensure that appropriate efforts were focussed on finding the balance between many conflicting demands. Thus, it is vitally important that in optimization, a good lean and efficient lead is not squandered by inappropriate addition of, for example, lipophilicity. These guidelines are repeated here as they give a succinct summary of the diversity of issues to be considered.

- 1) Consider the chemical tractability (ligandability) of the target, and if it is poor then investigate different mechanisms of action or different pathways.
- 2) Select multiple, low-complexity polar starting points with high binding enthalpy, and optimize enthalpically toward the lead compound.
- 3) Select appropriate metrics for multidimensional optimization; use ligand efficiency and lipophilic efficiency metrics in hit-to-lead optimization and change to more complex metrics emphasizing dosage to support lead optimization.
- 4) Evaluate available chemistries when entering extensive optimization; prepare what you designed and really want rather than what you can readily synthesize; design, synthesize, and use proprietary building blocks rather than depend on chemistry catalogs.
- 5) Do not be afraid to revert to a series of lower potency if it has better physicochemical properties. Extensive optimization of a scaffold that is not

amenable to achieving a desirable balance of potency and ADME (absorption, distribution, metabolism, and excretion) properties is likely to be a waste of time and resources.

- 6) Stay focused on the “sweet spot” and committed to deliver high-quality compounds, but remain open-minded to the many ways this can be achieved.
- 7) Resist timelines that compromise compound quality.

Fragment-based drug discovery (FBDD) has emerged as a key strategy for finding optimal starting points for medicinal chemistry projects. FBDD’s *raison d’être* is to find the smallest effective starting point that can be found to enable structure-based design. Typical fragments (as epitomized by Congreve *et al.*’s rule of three: MW <300, LogP <3, HBD <3, HBA <3, and rot bonds <3) are by definition definitely lead-like [26]. Other methods of finding leads can also produce good starting points with room for optimization. High-throughput screening HTS has been the main stay of hit identification over the past two decades and continues to provide quality leads, especially when the HTS collection being used has been built with more lead-like compounds [27]. Sometimes, there is sufficient knowledge about the target protein structure and ligand interactions with it (e.g., kinases) that a knowledge-based *in silico* method may be used to preselect compounds for consideration in a low or medium throughput assay. This method can also be highly effective though of course, if the knowledge used for the selection was dubious then the hit rate will very low! Another powerful lead generation method is the use of DNA-encoded libraries technology (ELT), wherein billions of compounds are synthesized using split–mix combinatorial chemistry methods [28]. Each molecule is tagged with a unique DNA bar code that can be decoded (after affinity selection of compounds) to provide the recipe for the compound’s synthesis that is then repeated off DNA. At GSK, we have been in a position to use these approaches against a wide range of targets and have been able to compare the mean properties of the leads we have found and these are summarized in Table 1.3. Not surprisingly fragments give the best lead molecules (as defined by ligand efficiency and lipophilicity), but the method is not always applicable. When we have used different methods against the same

Table 1.3 Mean physchem property values of leads from different lead identification methods at GSK.

Mean value	HTS	Encoded libraries technology	Fragments	Knowledge based
cLogP	3.8	4.4	2.2	4.4
Ligand efficiency	0.35	0.31	0.4	0.33
Molecular weight	385	480	325	455

target we have often found the techniques to be very synergistic in that, for instance, a fragment may be recognizable in a hit from HTS or ELT, and the larger compounds from the latter methods can then suggest where it is feasible to build matter onto the fragment.

In conclusion to this introductory chapter, it is appropriate to point out that the compounds that do eventually make it as successful drugs are often those that the inventors have worked exceptionally hard to focus on finding quality molecules. They will do this while implicitly accepting the compromise that often has to be made in the conflicting needs of finding quality molecules, for example, aqueous solubility versus membrane permeability, ligand efficiency versus target potency, and ultimately efficacious *and* safe! These tensions are well illustrated by the type of contour plots that Paul Leeson has championed for displaying the properties of all the compounds published (often in the patent literature) against a target protein and then highlighting where the actual effective drugs against that target are in the property space [24]. Time and again, it is apparent that a vast number of compounds are made with clearly poor property balance. By contrast, the successful drugs are almost outliers clinging to the edge of the distribution of the bulk of poor-quality compounds but nearest to good drug space in their own properties. Figure 1.1 shows this most persuasively for a number of CCR5 inhibitors and the sweet spot occupied by Maraviroc.

The challenge to everyone interested in making drug discovery a sustainable activity is to get to the sweet spot as economically as possible – that is, without making thousands of compounds! The other chapters in this book set out to give

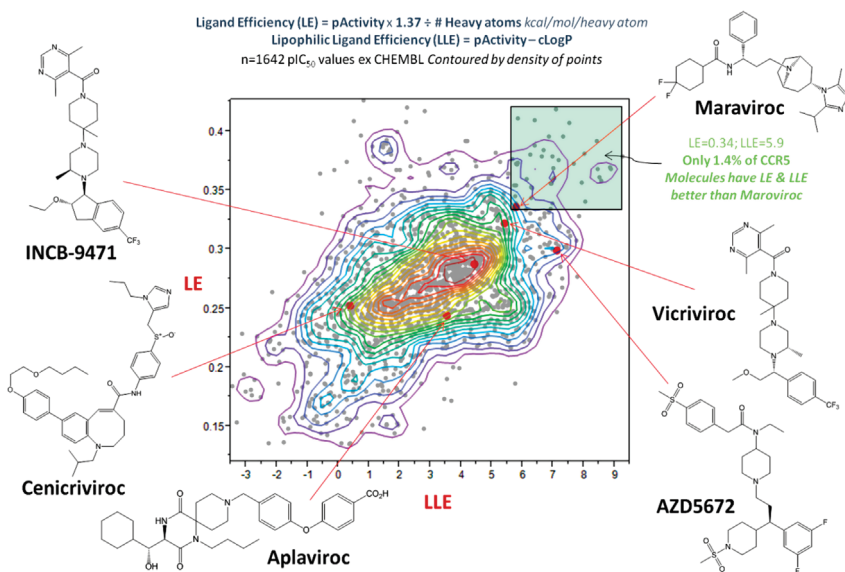


Figure 1.1 Distribution in LE/LLE space of a range of CCR5 antagonists (drawn with data provided by Paul Leeson).

further guidance on how to do this, but remember, where you start really does have a profound influence on where you will finish!

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