Considerations for Target Selection in CNS Drug Discovery Programs

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The Druggable Genome

Currently approved drugs mediate their therapeutic effects via a relatively small subpopulation of the druggable genome. Contemporary estimates suggest that approximately 3000 druggable genes exist, yet current drugs act on only ~400 defined unique molecular targets (Rask-Andersen et al., 2011). These statistics suggest that the vast majority of the druggable genome is, as yet, unexploited by pharmacotherapy. The current repertoire of drugged targets is dominated by cell surface receptors, transporters, and soluble enzymes (Fig. 1). The drugged members within these target families are considered by many to represent the "low-hanging fruit" within the druggable genome, implying that the next generation of targets will be substantially more challenging.

The concept of small-molecule druggability has evolved from an analysis of the overlap between druggable biological target space and oral drug-like chemical space. The convergence of these two concepts requires first that the target play a role in human disease and second that it has the potential to be modulated in a selective manner by a small molecule that has oral, drug-like physicochemical properties. A target's druggability is usually estimated by classifying it with known gene families that have previously been successfully targeted with drugs. However, this approach has some inherent limitations, so for targets of known structure, researchers have used a mathematical model that uses structural information about a target's binding site in order to estimate a maximal achievable affinity for a drug-like molecule (Cheng et al., 2007).

The concept of oral drug-likeness has its origins in the mid 1990s and initially centered on the relationship between compound properties and solubility and permeability. The Lipinski Rule of Five (Lipinski, 2004) provided guidelines for oral drug-likeness based on lipophilicity, as defined by the logarithm of the octanol/water partition coefficient ($\log P \leqslant 5$), hydrogen bond donor count ($HBD \leqslant 5$), hydrogen bond acceptor count ($HBA \leqslant 10$), and molecular weight ($MW \leqslant 500\,Da$). Further analyses of druggable chemical space have revealed additional relationships between compound properties and other absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties that must be satisfied for a successful drug candidate (Meanwell, 2011). Favorable ADMET properties and biological target potency generally show a diametrically opposed relationship with respect to MW and logP. There is also a growing body of evidence that compound promiscuity on average increases with increasing MW and logP. This characteristic suggests that the level of potency sought in a candidate molecule must be carefully balanced with physical properties, ADMET characteristics, and promiscuity — especially for CNS targets (Gleeson et al., 2011).

The CNS Druggable Genome

Despite recent advances in technology demonstrating the delivery of antibodies across the blood–brain barrier (BBB) in animal models (Atwal et al., 2011), drugged CNS targets remain exclusively within the domain of small-molecule therapeutics. The nonfenestrated endothelium of brain capillaries that constitutes the BBB physically limits the passive permeability of compounds and presents a metabolic barrier to CNS entry in the form of metabolizing enzymes and transport proteins. A further refinement of the concept of drug-likeness is therefore required for compounds intended for CNS targets in order to account for the limitations imposed by BBB permeability. The fact that CNS-accessible chemical space represents a subset of oral drug-like space can be illustrated by plotting simple physicochemical descriptors such as MW, polar surface area (PSA), and calculated LogP (cLogP) of drugs marketed for CNS indications against those intended for peripheral targets (Fig. 2). For example, there is greater latitude for higher PSA in drugs that do not require entry into the CNS. The same is true of MW. The greater stringency around CNS compound properties translates into a
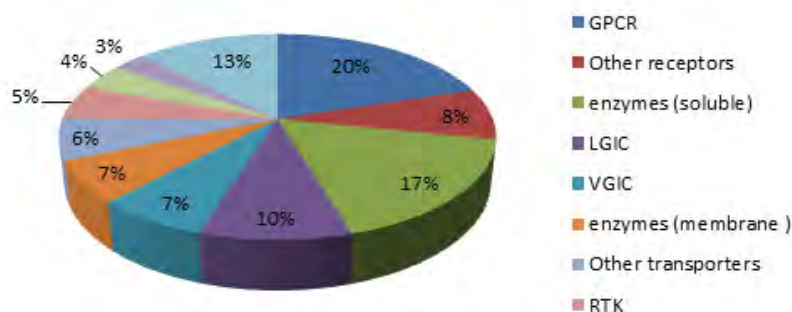
Figure 1. Percent distribution of the targets of approved therapeutics by target class (Rask-Andersen et al., 2011). LGIC, Ligand-gated ion channel; NR, nuclear receptor; RTK, receptor tyrosine kinase; SLC, solute carrier class; VGIC, Voltage-gated ion channel.
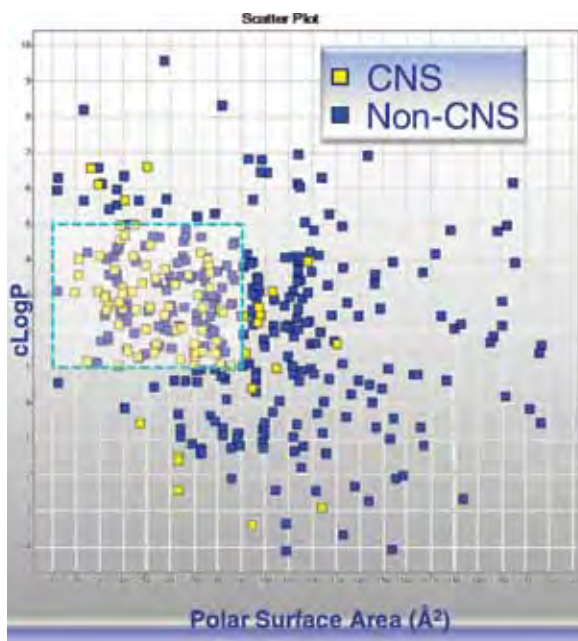
Figure 2. PSA and cLogP values of oral drugs in the MDL Drug Data Report (MDDR) database.

Figure 3. *a*, P-gp ER properties of 4125 compounds bucketed by PSA. *b*, Average PSA of CNS drugs, all marketed oral drugs (MKT), and kinase inhibitor drugs (KID). Chico et al. (2009), their Figure 4, adapted with permission.

lower likelihood of simultaneously achieving good BBB permeability and potent activity for targets with large and/or polar binding sites.

Retrospective analysis of marketed drugs has revealed that a very low prevalence of P-glycoprotein (P-gp) efflux, coupled with moderately to highly passive permeability, are two key properties that distinguish drugs that engage targets in the CNS from those that act predominantly in the periphery. The efflux properties that differentiate CNS drugs have been quantified using *in vitro* assays for human and rodent P-gp (Doan et al., 2002; Feng et al., 2008). Efflux properties have also been revealed *in vivo* by comparing brain drug exposures in P-gp (*mdr1a/b*[−/−]) knock-out mice with those observed in wild-type mice (Doran et al., 2005). Despite the lore that correlates compound lipophilicity with brain exposure, LogP is not correlated with brain unbound drug levels, which are the true determinants of pharmacologically relevant exposure. In fact, molecular descriptors related to hydrogen bonding (PSA, HBD, HBA) dominate the relationship with the steady-state unbound brain-to-plasma concentration ratio ($K_{p,uu,brain}$) (Fridén et al., 2009). This finding likely results from the additive effect that PSA, HBD, and HBA have on simultaneously reducing passive permeability while increasing the probability of interactions with efflux transporters.
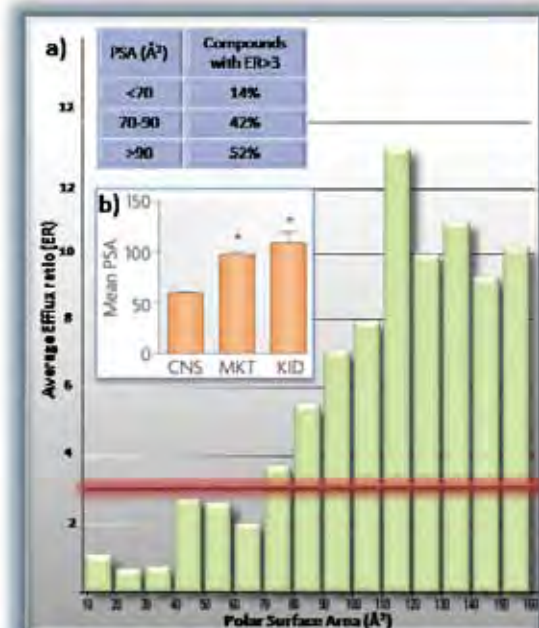
Figure 3*a* shows the relationship between PSA and average P-gp efflux ratio (ER) of 4125 compounds tested in LLC-PK cells transfected with human multidrug resistance 1 (MDR1) cDNA (P-gp). Compounds with ER >3 are considered to be P-gp substrates. Figure 3*b* illustrates the average PSA of CNS drugs, all marketed oral drugs (MKT), and kinase inhibitor drugs (KID) (Chico et al., 2009). The high PSA generally required for potent ATP-competitive kinase inhibition explains the generally poor CNS penetration of this class of compounds.

Approved CNS drugs are dominated by compounds that directly engage biogenic amine receptors or indirectly modulate their activity by inhibiting neurotransmitter metabolism or uptake. This circumstance owes much to capacity for achieving high affinity at such targets with low-molecular-weight compounds (high ligand efficiency) that have relatively low PSA and is aided by a traditionally low stringency for biological target selectivity.

The industry shift from phenotypic screening and polypharmacology to single-targeted therapeutics has been particularly hard on CNS drug discovery (Swinney and Anthony, 2011). The orthosteric binding sites among many biogenic amine G-protein coupled receptor (GPCR) family members are

highly conserved. Thus, the quest for subtype-selective ligands for even these low-hanging targets has required reliance upon additional interactions in nonconserved binding regions, thus driving up the molecular weight of compounds. The surge of interest in allosteric receptor modulators is a direct response to the challenge of attaining selectivity within the confines of CNS-accessible chemical space (Conn et al., 2009). The origins of low success rates at each stage of drug discovery are multifactorial, but the limited structural space available to achieve target engagement in the brain is a major contributing element to the high attrition in CNS programs. Despite the large industry-wide investment in new targets, only four new CNS drugs that act by previously unexploited mechanisms have been launched in the past decade in the United States (Table 1).

Table 1. CNS Drugs launched during 2000–2010 that act on new targets.

Drug	Target	Gene family	Launch	Indication
Aprepitant	NK1	GPCR	2003	CINV
Ramelteon	MT1/MT2	GPCR	2005	Insomnia
Varenicline	α4β2	LGIC	2006	Nicotine addiction
Fingolimod	S1P1	GPCR	2010	Multiple sclerosis

CINV, Chemotherapy-induced nausea and vomiting; GPCR, G-protein coupled receptor; LGIC, ligand-gated ion channel.

Simple physicochemical guidelines and computational models can be helpful in altering the probability of encountering P-gp efflux; however, it is abundantly evident that compounds with identical physicochemical properties can have vastly different efflux properties. As with any small molecule–protein interaction, the composition and presentation of functionality on a molecule play a critical role in the recognition process. However, unlike the typical small molecule–drug target interaction, P-gp is a highly permissive protein recognizing a wide diversity of substrates. Biological targets with larger, more polar binding sites (such as those that have peptides, amino acids, and nucleotides as ligands or substrates) are disadvantaged when it comes to achieving sufficient target affinity within physicochemical space that is favorable for CNS entry. However, this limitation does not mean that reconciling potency, selectivity, and CNS entry for such targets is impossible; it is simply more demanding.

The aspartyl protease β-amyloid cleaving enzyme-1 (BACE1) is a good example. BACE is a hotly pursued target in the quest for a disease-modifying therapy for Alzheimer's disease (Varghese, 2010). Reconciling the structural requirements for potency and protease selectivity with the need for low metabolic clearance, adequate passive permeability, and avoidance of P-gp efflux in individual compounds has proven extremely challenging for those that have tackled BACE inhibitor optimization. It is worthwhile contrasting the timeline involved in advancing HIV protease inhibitors from target discovery to drug launch with the time that has elapsed since the first disclosure of BACE as a molecular target. HIV protease was first disclosed in 1988 and, remarkably, within seven short years, saquinavir had received FDA approval and was quickly followed by several other entries. In contrast, BACE was disclosed in 1999 and only recently have the first inhibitors begun to enter clinical development, still many years from potential FDA approval. Although many factors distinguish HIV infection from Alzheimer's disease, a major differentiator is the absolute requirement that BACE inhibitors enter the CNS. The requirement for CNS penetration imparts additional confinements around available structural and physicochemical space, reducing the scope of options for solving potency, selectivity, and pharmacokinetic deficiencies.

Numerous other examples highlight successful efforts to engineer CNS target coverage while maintaining biological target activity, selectivity, and in many cases, favorable pharmacokinetic properties for targets with large and/or polar binding sites. These include bradykinin, orexin, opioid peptide GPCRs, GABA, glutamate and glycine receptors, and several phosphodiesterases and kinases.

Target Selection and Validation

It has been said that a drug target's relevance to disease is often inferred with strong belief but fragile evidence. It can also be argued that, since the complete sequencing of the human genome, the key task in biomedical research is no longer target identification but rather target validation. However, given the additional limitations around CNS-accessible chemical space, assessment of potential targets for the probability of achieving CNS druggability should be conducted early in the target selection process. Druggability should then be weighed against other evidence in support of or against the target. Target validation is a continuum whereby an increasing

body of data is amassed that culminates in positive efficacy in humans. The initial target selection and subsequent validation path may include evidence from the expression pattern of the target, human and animal genetics, validation in animal disease models (preferably across multiple species), and observations with model compounds (based on human or animal data). The target selection and validation path should also include consideration of a human biomarker, preferably a functional endpoint or surrogate endpoint.

In recent years, there has been a number of high-profile CNS drugs subject to black box label warnings, others subject to outright withdrawal, and FDA rejections of several CNS drug candidates. In many cases, mechanism-related side effects have resulted in an unfavorable risk-to-benefit ratio for the patient. Therefore, in addition to establishing target validation, assessing the likelihood and nature of mechanism-related side effects and the ability to empirically measure and defuse these risks early in the drug discovery process are important. Pursuing targets that have selective expression patterns (preferably limited to the dysfunctional circuit or cell type of interest) would confer the advantage of lowering the probability of mechanism-related side effects.

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