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Fragment screening to predict druggability (ligandability) and lead discovery success

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Target druggability – ligandability

It is estimated that only ~1% of drug discovery projects make it to market industry-wide. There is increasing regulatory pressure for new products to show significant improvement over existing therapies. Despite genomic initiatives, only three new targets are addressed each year with synthetic drugs [1]. Late-stage failure in clinical trials are costly, therefore significant cost savings will be achieved by improving the selection of protein targets and selecting winning projects early on in the process. Thus, pharmaceutical companies face a major challenge today: we need to reduce attrition throughout the drug discovery process to reduce cost and increase success rates while, at the same time, exploiting novel mechanisms for new drugs – to differentiate from competitors.

The term 'druggability' usually refers to the likelihood of finding orally bioavailable small molecules that bind to a particular target in a disease-modifying way [2]. Unless there are other known compounds, either on the market or in clinical trials, acting on a particular target, intrinsic druggability is unknown. It is, therefore, useful to distinguish the ability of a target to bind small molecules from the more complex pharmacokinetic and pharmacodynamic mechanisms included in the term druggability. In recent years, the term druggability has increasingly been adopted to describe the ability of a protein target to bind small molecules with high affinity; however, we believe the term 'ligandability' is more appropriate for this purpose. Although ligandability is a requirement for finding drugs for a particular target, it is not a guarantee that such ligands will make good drugs. In other words, ligandability is a necessary but not sufficient condition for druggability. As we will discuss here, ligandability is, however, relatively straightforward to assess at an early stage in drug discovery. In this article, we will henceforth use the term ligandability.

Fragment screening

Any predictive tool that would help distinguish ligandable from non-ligandable targets clearly would be of great interest to the pharmaceutical industry. A variety of computational methods for predicting ligandability have been described [3]. These methods either require that the 3D structure of the protein target is known, so that an analysis of the binding pocket can be done to predict ligandability, or that there is ligand information available in the public domain that can be used to understand what ligand space is compatible with the target. In their article from 2005, Hajduk and co-workers [4] suggested that experimental fragment-based screening is a good indicator of target ligandability. Fragments are molecules of low complexity, which sample chemical space exponentially more effectively than drug-sized molecules. Different estimates exist of the size of chemical space [5] but even conservative estimates put the number of possible molecules of fragment size (say, <200 Da) some 10 orders of magnitude smaller than drug-sized molecules (<450 Da). To put this number into perspective, this implies that a diverse set of 1000 fragments represents its chemical space about as effectively as would 10 trillion diverse drug-sized molecules. Therefore, fragment screens are ideally suited for assessing whether or not ligands can easily be found for a particular binding site: the high sampling efficiency renders the outcome of the screen less dependent on the compounds being screened, and more closely a function of the characteristics of the target at hand.

In principle, fragment-based ligandability screening can be conducted with any method that reliably detects binding with affinity as weak as low mM values. The sensitivity and reliability of different biophysical techniques applied to fragment screening have been discussed in detail in a recent publication in *Drug Discovery Today* [6]. Within AstraZeneca, historically we have almost exclusively used NMR for fragment screening because of its very high reliability and information content, but other techniques such as SPR (surface plasmon resonance) are gaining in prevalence, particularly when obtaining mg amounts of soluble protein is a challenge. It is of crucial importance that false negative and false positive rates are low if reliable predictions of ligandability are to be obtained. False negatives could be particularly damaging: to classify a target erroneously as being undruggable, only to witness a competitor bring a compound to market, would count as a costly mistake. In this

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TABLE 1

List of 36 targets used in this study, with ligandability score, and outcome of HTS and success of hit-finding by any means

Target	Ligandability score	Successful HTS	Entry into hit-to-lead
Aspartyl protease (BACE1)	medium	no	yes
ATPase	high	no	yes
Cysteine protease (RV3CP)	low	no	no
Cysteine protease	low	no	no
DNA polymerase	low	no	no
GPCR ECD	low	no	no
Interleukin	low	no	no
Ligase	low	no	no
Ligase	medium	yes	yes
Metalloprotease	high	yes	yes
NHR	high	yes	yes
NHR	high	yes	yes
NHR	medium	yes	yes
NHR	medium	no	no
NHR (ERβ)	medium	yes	yes
Nucleoside kinase	high	no	no
Peptidase	low	no	yes
Peroxidase	medium	yes	yes
Phospholipase	high	yes	yes
Phosphotransferase	medium	yes	yes
Primase	low	no	no
Pyrophosphatase	high	no	no
Ser/Thr kinase	medium	yes	yes
Ser/Thr kinase	high	yes	yes
Serine protease	low	no	no
Serine protease	medium	yes	yes
Serine protease	high	yes	yes
Serine protease	high	no	no
Synthetase	low	no	no
Synthetase	medium	no	yes
Synthetase	high	yes	yes
Synthetase	high	yes	yes
Topoisomerase	high	yes	yes
Tyrosine kinase	high	yes	yes
Tyrosine phosphatase (PTP1B)	low	no	yes
Tyrosine phosphatase	low	no	no

context, we will discuss ways to interpret ligandability data that use the available information content more conservatively.

Predicting lead discovery success

To quantify the predictive power of fragment screening in our discovery setting, we analysed data from 36 discovery projects from the period 2001–2008. These were projects where a conventional HTS programme was run, and where we also performed a fragment screen, usually in the context of fragment-based lead generation (FBLG). Typical fragment library sizes were 768–2000 compounds. The composition of the library, which was designed to be generic, has evolved somewhat over the years – the general concepts have been described by Blomberg *et al.* [7]. We have devised a simple ligandability scoring system: low (red), medium (amber), high (green). This score is not solely based on fragment screening hit rates but also takes into account affinities, in some cases estimated, and the diversity of the hits. Simply looking at hit rates in a fragment screen is not particularly reliable because hit rates depend on the detection limit of the assay as well as the threshold definition of a hit. We use the following classification: high ligandability = high hit rate, best affinities $<$0.1 mM, larger diversity; medium ligandability = intermediate hit rate, best affinities 0.1–1.0 mM, some diversity; low ligandability = low hit rate, best affinities $>$1 mM, low diversity of hits. A list of the targets used in this study is shown in Table 1.

After scoring the targets we compared the results with the HTS outcome and the project success in lead discovery. As shown in the graph in Fig. 1, in 100% of cases (12 targets) where the target had been deemed non-ligandable (low ligandability score) in the fragment screen the HTS had indeed failed to yield sufficient high-quality hits to enter a hit-to-lead programme; whereas for targets deemed ligandable (medium–high ligandability score) the HTS had been successful in a majority of cases and the projects had indeed entered hit-to-lead programmes. The overall success rates for the 36 projects was 50%, which agrees well with the overall success rate for AstraZeneca projects for this period, although this particular data set has a strong bias towards soluble targets. At least from the test target set, a low ligandability score is always coupled with HTS failure, which is a remarkable result. On the basis of these data we believe that this approach is a highly useful tool for downgrading target priority for entry into the portfolio. However, even for targets deemed ligandable, the failure rates were significant at one third. There will be various reasons for this, not all of them linked to ligandability (e.g. assay quality, target validation, and the quality and composition of the compound bank). This discrepancy between apparently ligandable targets and project success rates further underscores the need for the more accurate term 'ligandability prediction'. The graph in Fig. 2 illustrates

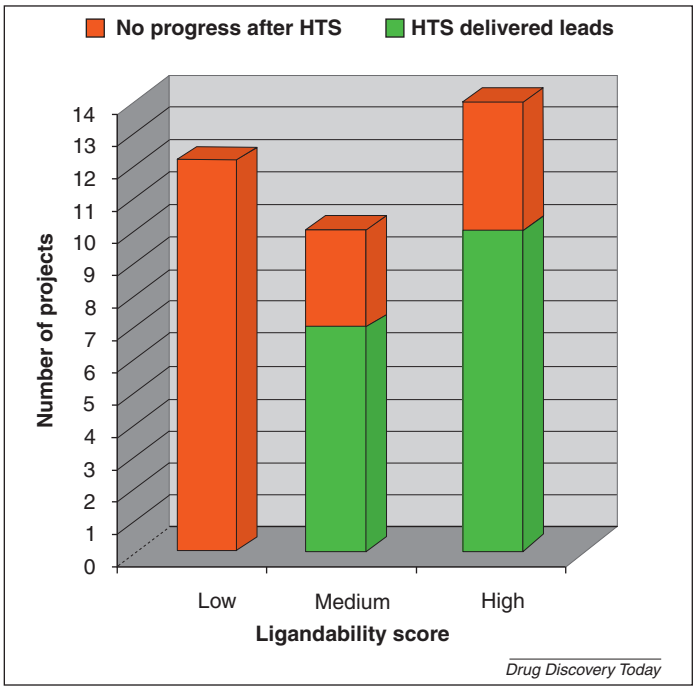

FIGURE 1

Outcome of 36 drug discovery projects, binned according to low, medium and high ligandability score, and colour-coded according to HTS success.

the impact of applying alternative approaches to lead discovery in some projects, such as fast-follower and fragment-based strategies. Interestingly, a few targets in the low ligandability category could be progressed when one or more of these alternative approaches were pursued.

This confirms the general hypothesis that fragment-based methods afford a higher chance of success than HTS against more challenging targets, at least in part because of the more optimal chemical space sampling of fragment screening. Indeed, the overall project success rate for the medium–high ligandability targets was an impressive 83%.

From a target class perspective our data reflect what is basically well known. Kinases and nuclear receptors are generally druggable, and there are numerous drugs on the market. Proteases as a target class are more challenging, whereas bacterial enzymes have historically tended to be difficult targets. In terms of specific targets used in our study, estrogen receptor beta (ERβ) represents a target known to be druggable with a wealth of known antagonists and drugs against breast cancer on the market. At the opposite end of the spectrum is protein tyrosine phosphatase 1B (PTP1B), long considered a promising target for the treatment of diabetes, which is scored as having low ligandability. This target illustrates the difference between ligandability and druggability well, because an internal fragment-based approach did result in potent inhibitors that, however, lacked the potential for further development [8]. Indeed, considerable efforts have been expended on PTP1B across the industry, yet no drug is on the market [9,10]. Another example of an apparently non-druggable target, at least by non-covalent inhibitors, is rhinoviral 3C protease (RV3CP), which failed, in our hands, after extensive hit finding efforts lasting some two years [11]. β-Secretase (BACE1) represents an interesting target at the interface between low and medium ligandability. After failing at HTS, a fragment-based approach was finally successful [12], illustrating that wider lead discovery strategies are justified for high-value targets. It is clear that many considerations, in addition to ligandability, such as target validation and potential market value, are of crucial importance. Notwithstanding this, we have little hesitation in drawing the general inference that a portfolio comprising a large number of targets with low predicted ligandability would carry a high risk of expensive failure.

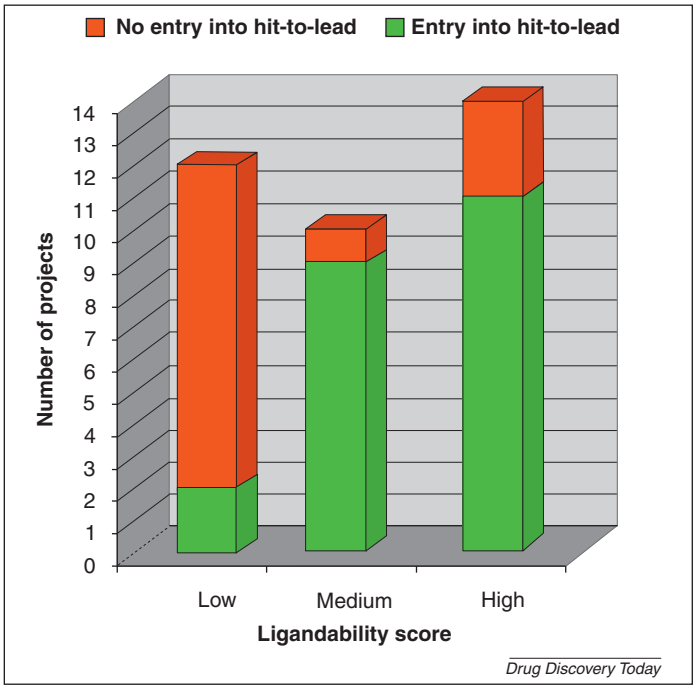

FIGURE 2

Outcome of 36 drug discovery projects, binned according to low, medium and high ligandability score, and colour coded based on entry into hit-to-lead programmes.

Recommendations

We believe our data show clearly how fragment-based ligandability screening can be used in a truly predictive fashion and could be a powerful tool in reducing target attrition by filtering out non-ligandable targets before entry into the portfolio. Alternatively, a low ligandability score coupled with very strong target validation through compelling clinical disease linkage might indicate the need to apply multiple, parallel hit-finding approaches to increase the chances of success in 'must-win' areas. For example, one could decide to run the HTS with an extra, orthogonal (e.g. cell-based or affinity-

based) assay, or complement with FBLG or virtual screening approaches.

In cases where there is pre-existing chemical equity described in literature or patents before the start of a drug discovery project, the chemical tractability of the protein target is routinely assessed based on the properties of these ligands. Interestingly, the ligandability scores described here correlate well with the initial ligand-based chemical tractability assessments carried out for those targets when such information was available at the outset (in-house data, not shown). However, as more novel targets and target classes are pursued, chemical tractability assessments of this type will not be feasible or are likely to be relatively unreliable if based on endogenous ligands, natural products or peptidic ligands, for example. Nor are computational pocket analyses likely to be very predictive of lead generation success except in cases where there are obviously well-defined and comparatively rigid cavities present. Intrinsic target flexibility could confound the identification of potential inducible binding pockets from analyses based on static structures. Furthermore, these computational analyses are not available for targets for which no crystal structure has been determined. For such novel targets there is little prior ligand knowledge, which means that all such targets will be, by default, deemed of high chemical risk regardless of whether they are actually ligandable or not. We suggest that fragment-based ligandability screening should be a significant factor in deciding whether or not to pursue these targets.

Concluding remarks

At AstraZeneca we are now applying this method in several different ways, particularly in early target assessment. Where it is technically feasible, novel targets with unknown or low chemical tractability are subjected to ligandability screening before project launch. Such high-risk targets are then only progressed if the outcome of the ligandability screen is favourable; in comparison, targets scoring low on ligandability are considered of low priority for entry to the portfolio unless also possessing very compelling clinical disease linkage and evidence for a desirable phenotypic effect from target modulation. Previously intractable targets, for instance targets that have failed conventional HTS approaches, are screened for ligandability to assess their further potential. In several cases such targets have been deemed ligandable and alternative lead generation strategies have been pursued. Ligandability screening is also used in selecting promising new targets from a panel of potential candidates. These could comprise targets with equally strong biological or clinical hypotheses, or from a novel target class; in this sort of case the targets with the best ligandability score would tend to carry the lowest chemical risk and are likely to be privileged for entry into the portfolio. One caveat to be considered is that the form of a target (isolated domain versus full-length, apo versus substrate/cofactor-bound, whether natively post-translationally modified, etc.) could have an influence on ligandability and this should always be considered when selecting the experimental system to be used and in the resulting decisions on target priority. The outcome of the fragment ligandability screen gives early access to ligand information in projects, and intriguingly could provide an early 'fingerprint' of the physicochemical property space likely to be occupied by more elaborated, lead-like compounds that will emerge later on in the hit-to-lead stage. It also provides a natural proof-of-concept for alternative fragment-based lead generation strategies.

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References

1 Betz, U.A. (2005) How many genomic targets can a portfolio afford? *Drug Discov. Today* 10, 1057–1063
2 Hopkins, A.L. and Groom, C.R. (2002) The druggable genome. *Nat. Rev. Drug Discov.* 1, 727–730
3 Egner, U. and Hillig, R.C. (2008) A structural biology view of target drugability. *Exp. Opin. Drug Discov.* 3, 391–400
4 Hajduk, P.J. *et al.* (2005) Predicting protein druggability. *Drug Discov. Today* 10, 1675–1682
5 Medina-Franco, J.L. *et al.* (2008) Visualization of the chemical space in Drug Discovery. *Curr. Comput. – Aided Drug Design* 4, 322–333
6 Dalvit, C. (2009) NMR methods in fragment screening: theory and a comparison with other biophysical techniques. *Drug Discov. Today* 14, 1051–1057
7 Blomberg, N. *et al.* (2009) Design of compound libraries for fragment screening. *J. Comput. Aided Mol. Des.* 23, 513–525
8 Black, E. *et al.* (2005) Structure-based design of protein tyrosine phosphatase-1B inhibitors. *Bioorg. Med. Chem. Lett.* 15, 2503–2507
9 Combs, A.P. (2010) Recent advances in the discovery of competitive protein tyrosine phosphatase 1B inhibitors for the treatment of diabetes, obesity and cancer. *J. Med. Chem.* 53, 2333–2344
10 Scott, L. *et al.* (2010) Targeting protein tyrosine phosphatases for anticancer drug discovery. *Curr. Pharm. Des.* 16, 1843–1862
11 Baxter, A. *et al.* (2010) Non-covalent inhibitors of rhinovirus 3C protease. *Bioorg. Med. Chem. Lett.* 21, 777–780
12 Edwards, P.D. *et al.* (2007) Application of fragment-based lead generation to the discovery of novel, cyclic amidine beta-secretase inhibitors with nanomolar potency, cellular activity, and high ligand efficiency. *J. Med. Chem.* 50, 5912–5925

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