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Fragment based drug discovery

Ligand efficiency as a guide in fragment hit selection and optimization

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Fragment-based screening (FBS) has become an established approach for hit identification. Starting points identified by FBS, are small fragments that require substantial modification to become leads. As fragments are different from classical hits a process tailored for fragment evolution is required. Scores for ligand efficiency have been proposed as guides for this process. Here we review how these have been applied to guide the selection and optimization of fragment hits.

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Introduction

A common approach for hit identification is high-throughput screening (HTS) [1]. In HTS a large number of compounds ($\sim 10^6$) are screened to assess biological activity against a target. Nevertheless, considering the theoretically large chemical space of drug-like compounds [2], the probability of finding hits is relatively low [3]. This has led to the development of alternative approaches such as FBS and fragment-based drug discovery (FBDD) [4–15]. Advantages of FBS are (a) a more efficient sampling due to the smaller chemical space of fragment-sized compounds [16,17] and (b) a higher probability of fragments possessing good complementarity with the target [18]. Both aspects are likely to be the cause for the higher hit rates which are typically observed for FBS in comparison to HTS [10]. However, fragment hits have lower affinities towards the target. As a consequence, more effort has to be spent on optimization to obtain lead compounds with an acceptable affinity. Strategies have been proposed to guide and evaluate this process. These strategies aim at the efficient optimization of fragments while maintaining their generally good physicochemical properties. In this review we discuss various efficiency indices and how to best leverage them in FBDD projects.

Considerations during hit selection and optimization

Traditionally, affinity is the first aspect considered for hit selection and optimization. However, affinity alone can be misleading as it is often found to be linked with molecular size. Thus a focus on affinity leads to a bias towards a selection of bigger compounds. In addition, optimization of affinity during subsequent stages of drug discovery typically leads to a further increase in molecular weight (MW) [19]. Moreover, affinity is often optimized through the introduction of lipophilic groups, as these contribute favourably to the hydrophobic effect without the need for specific interactions with the target. This contrasts with polar groups, which need to establish very good interactions with the target to compensate the desolvation penalty. For this reason, polar groups are often used to improve solubility rather than affinity [20]. This phenomenon is reflected in the general trend towards generation of not only bigger but also more lipophilic compounds during the hit optimization process [19].

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Table 1. Comparison of fragment-like and drug-like compounds

Type of compound	Fragment-like	Drug-like
Rule	Rule-of-Three* [34]	Rule-of-Five [21]
Thresholds		
MW	<300	≤500
c log P	≤3	≤5
H-bond donors	≤3	≤5
H-bond acceptors	≤3	≤10
Typical values		
pIC_{50}	4.4**	8
HA	~15**	38
Ligand efficiencies		
LE	0.38	0.29
FQ	0.55	0.81

*The authors suggest number of rotatable bonds ≤3 and polar surface area ≤60 as additional useful criteria.
**Median values taken from the literature examples (see supplementary material).

Ultimately, affinity for the target is not the only aspect that has to be considered during drug discovery. To enter later stages of drug development, a compound needs to have suitable physicochemical properties. Lipinski *et al.* [21] studied the properties of oral drugs that managed to enter clinical Phase II. The study resulted in the 'Rule-of-Five' stating that poor absorption or permeation is more likely if more than one of the relevant parameters (see Table 1) are outside the range typically observed for drug-like compounds.

The 'Rule-of-Five' has had a strong influence on the drug discovery process. Good physicochemical properties help to reduce the attrition rate at later stages towards to market [22]. A recent study showed that almost all ADMET parameters deteriorate with either increasing MW and/or log *P* [23]. These studies emphasize the importance for selecting appropriate hits and monitoring MW and lipophilicity in addition to affinity during hit optimization.

Ligand efficiency scores

Ligand efficiency

To escape the affinity-biased selection and optimization towards larger ligands the focus should be directed towards the generation of compounds that use their atoms most efficiently. To estimate the efficiency of compounds, Hopkins *et al.* [24] recommended to assess binding affinity in relation to the number of heavy atoms in a molecule and introduced the term ligand efficiency (LE)

$$\mathrm{LE} = -\frac{\Delta G}{\mathrm{HA}}$$

where $-\Delta G$ is the free energy of binding and HA is the number of non-hydrogen atoms of the ligand. Instead of considering the affinity of the whole compound, the average affinity contribution per atom is taken into account. This provides a way to compare the affinity of molecules corrected for their size. Abad-Zapatero and Metz [25] introduced the binding efficiency index (BEI) defined as $\mathrm{BEI} = p\mathrm{IC}_{50}/\mathrm{MW}$ as an alternative metric. Prioritizing hits according to their LE allows also smaller low affinity compounds to be attractive for further optimization.

Group efficiency

Verdonk and Rees [26] introduced group efficiency (GE) as a metric to compare the quality of added groups. It is defined as

$$\mathrm{GE} = -\frac{\Delta\Delta G}{\Delta\mathrm{HA}}$$

$$\Delta\Delta G = \Delta G(\mathrm{B}) - \Delta\mathrm{DG}(\mathrm{A})$$

$$\Delta\mathrm{HA} = \mathrm{HA}(\mathrm{B}) - \mathrm{HA}(\mathrm{A})$$

where the affinity gained by molecule B, through the introduction of additional non-hydrogen atoms ΔHA to molecule A, is expressed as the difference of the free energies of binding ($-\Delta\Delta G$). The group efficiency describes the average affinity gain contributed by each atom of an added group. Only the addition of groups with the same (or a better) group efficiency, compared to the LE of the initial molecule A, will allow to maintain (or increase) the LE during compound optimization.

Fit quality

Reynolds *et al.* systematically investigated the size-dependence of LE [27]. In their study, the binding affinity data and corresponding LEs taken from the BindingDB [28] were plotted against the number on non-hydrogen atoms. Altogether, over 8000 ligands for 28 targets were considered. The result of their study is that the maximum observed ligand efficiency decreases with molecular size. The authors conclude that LE cannot be evaluated independent of the molecular size. To enable a size-independent comparison of ligands they derived a scaling function (LE_Scale) by fitting an exponential function to the maximal ligand efficiency values observed for a given HA count. Dividing the actual observed ligand efficiency by the calculated maximal achievable ligand efficiency (the scaling function) results in a scaled ligand efficiency called fit quality (FQ):

$$\mathrm{FQ} = \frac{\mathrm{LE}}{\mathrm{LE\_Scale}}$$

Different ways to obtain the scaling function are published [29–31]. Nevertheless, independent of how the scaling function is derived, FQ values near one indicate near optimal ligand binding.

Ligand-lipophilicity efficiency

LE and corresponding FQ are useful for optimizing affinity with respect to molecular size. However, to achieve optimal

ADMET properties molecular size and lipophilicity are important factors to consider. If lipophilicity is too high, the likelihood of a compound to bind to multiple targets increases [32]. To facilitate optimization of affinity with respect to lipophilicity, Leeson and Springthorpe [32] defined the ligand-lipophilicity efficiency (LLE):

$$LLE = pIC_{50} - c\log P$$

High LLE favours compounds that gain a lot of their affinity through directed interactions thus making the interaction with the receptor more specific.

While one can say that LLE describes how efficient a ligand exploits its lipophilicity, no explicit measure of molecular size is used. Therefore, a lipophilicity corrected LE is needed to enable optimization of affinity without the extensive use of lipophilic nonspecific interactions. Keseru and Makara [19] proposed not only LELP = log *P*/LE as monitoring function to achieve that goal, but also other ways to combine molecular size and lipophilicity into a single efficiency measurement are being discussed [33].

Application of ligand efficiency scores to FBDD

Clinical candidates are generally preferred to be 'Rule-of-Five' compliant with a special focus on lipophilicity. To achieve this, the starting restrictions for fragments should be obviously stronger. Congreve *et al.* [34] studied fragment hits that could be successfully optimized into potent leads, and noticed they have particular physicochemical properties. These properties and congruent thresholds are summarized as the 'Rule-of-Three' (see Table 1). To stay within these thresholds has been suggested as a criterion for fragment library design [34].

For fragment hit selection, LE has become a widely accepted metric. In general, it is best to start with a fragment that shows a high LE because in most cases LE decreases during optimization. Looking at the examples in the literature [5–8,14] and following the evolution of LE, there are fewer examples where LE could be maintained or even increased. In the majority of examples (~70%, see suppl. material), LE decreases during fragment optimization (by either fragment linking or growth). Therefore, starting with a highly efficient fragment hit makes it easier to optimize the fragment into a drug-like compound.

In general, an orally available clinical candidate possesses a potency of better than 10 nM and, if 'Rule-of-Five'-compliant, a maximal molecular weight of 500 Da (which equals, on average, 38 HA). This means that a LE of at least 0.29 kcal mol^{-1} HA^{-1} needs to be maintained during hit optimization. Screening only 'Rule-of-Three'-compliant fragments ensures the identification of good starting points for optimization if an affinity in the range of 1 μM can be achieved. For a fragment hit with less than 300 Da (that equals on average 23HA) this would result in a LE of at least 0.36 kcal mol^{-1} HA^{-1}. Looking at examples from the literature (see suppl. material) such a high affinity cannot always be achieved (the median pIC_{50} is 4.35), but because the average fragment hit is also smaller than 23 HA (median number of HA is 15), the median LE of fragment hits considered here is 0.38 kcal mol^{-1} HA^{-1}. Therefore, some loss of LE during optimization is acceptable in most cases.

This is illustrated in the example (a) in Fig. 1: the LE score of the initial fragment hit is quite high (0.59). Although, LE decreases during fragment growth, the final potent compound (IC_{50} = 3 nM) reaches a LE score of 0.42. This is still a high value which is significantly above the suggested value of about 0.3.

Another possible scenario is illustrated by example (b): The LE score can be maintained throughout the optimization process. This is achieved through the introduction of groups that have GEs comparable to LE of the starting compound. As long as the initial fragment hit has a LE score > 0.3, also this strategy can lead to potent drug-like compounds.

Example (c) shows one of the rare cases where LE is increased during fragment growth. Although the LE of the starting fragment is below 0.3, it was possible to significantly improve the affinity by introducing an additional group to finally reach a potent drug-like compound.

Revisiting example (a) illustrates the usefulness of FQ (in addition to LE) for fragment optimization. Although LE is decreasing, FQ is maintained, indicating that fragment optimization is on the right track. In general, the goal should be to either maintain or increase FQ during fragment assembly to reach a near optimal affinity for the final compound.

LLE provides a way to evaluate the affinity of a compound with respect to its lipophilicity. The challenge is to increase potency without increasing lipophilicity at the same time. As lipophilicity is the major factor for promiscuity of compounds, LLE optimized compounds should be more selective. It is suggested to target a LLE in a range of 5–7 or even higher [32].

In example (a) LLE is increased during optimization. The final compound reaches a LLE of 7.3 which is even above the suggested range of 5–7. In combination with the acceptable LE of 0.42 this indicates that this compound was successfully optimized. Comparing the *c* log *P* values of the compounds reveals that lipophilicity was kept fairly constant during fragment growth. This means that affinity is mainly gained by the introduction of groups making specific directed interactions.

In the other two examples, (b) and (c) LLE is increasing during optimization but none of the compounds reaches a LLE above 5. In these cases the gain of affinity is accompanied by an increase of lipophilicity. In this respect, optimization was not as optimal as in the first example.

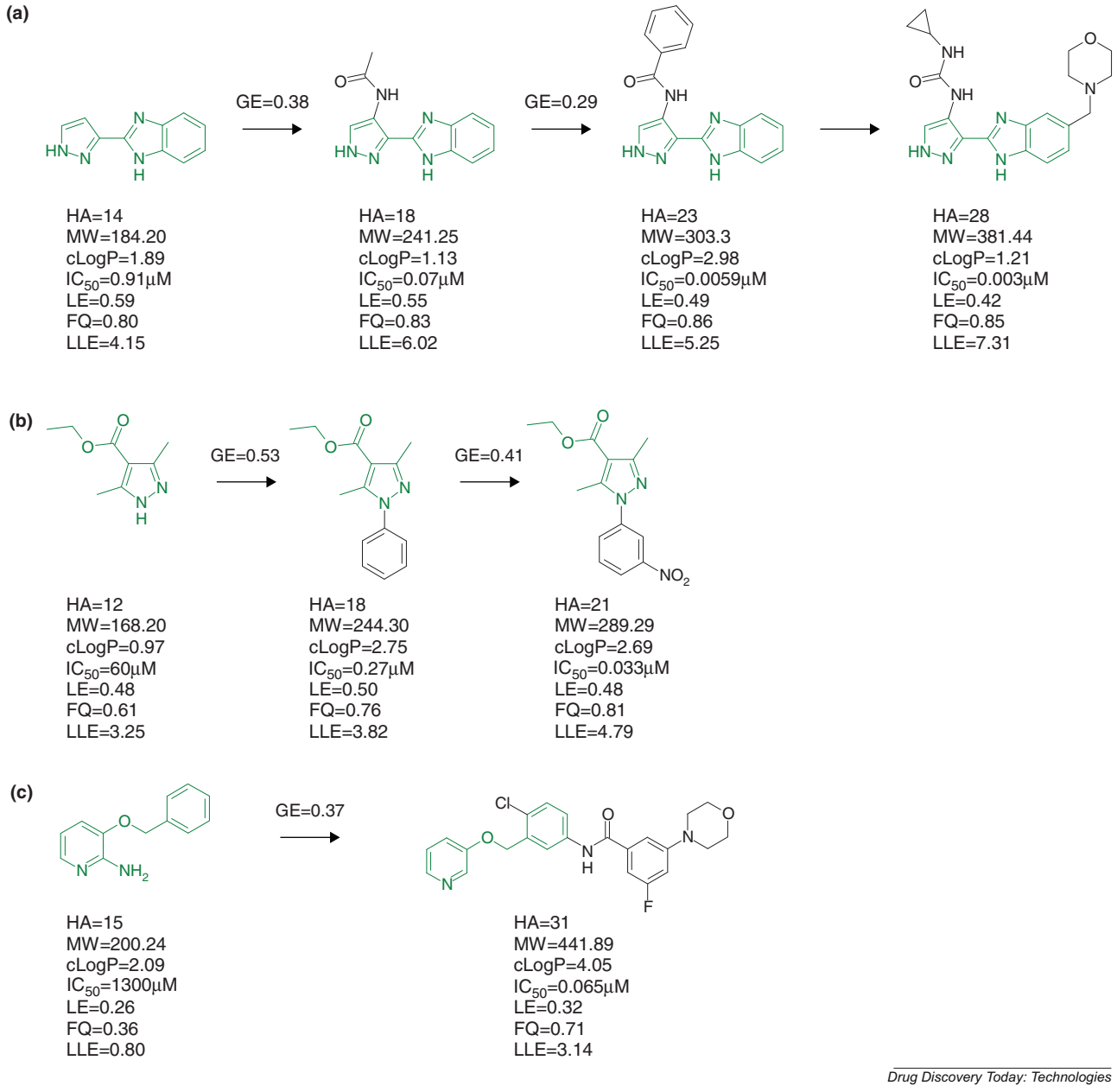

Figure 1. Examples of successful fragment-based drug discovery campaigns (a) Aurora [38], (b) PDE4B [39], and (c) p38α [40,41].

Conclusions

FBDD, as illustrated in Table 1, typically starts with a 'Rule-of-Three'-compliant fragment and ideally ends up with a potent 'Rule-of-Five'-compliant candidate compound. Colleagues from Astex proposed that an efficient fragment growth is one where LE can be maintained [4]. This goal is further supported by a study which concluded that a linear increase of binding affinity with molecular mass is possible [35]. If maintenance of LE at an acceptable level (LE $\geq$ 0.3) can be achieved, FQ scores will rise during fragment growth and the affinity of the final compound will approach a near optimal affinity.

At the same time, retrospective studies show that in most cases LE scores decrease during fragment assembly [5,8]. Still, an acceptable affinity of the final compound can be reached if FQ can be maintained at a high level (FQ $\geq$ 0.8). Therefore, Bembenek *et al.* [36] suggested that, unlike LE alone, the FQ score can be used as a measure of efficiency across the entire optimization process from initial fragment hit to optimized clinical candidate.

Another mentionable guide to maintain the good physicochemical properties of fragment hits is to consider LLE during FBDD. Lipinski states in the 'Rule-of-Five' that the *c* log *P*, which is used to calculate the LLE, should be smaller

Table 2. Summary of ligand efficiency scores to be considered during FBDD

Parameter	Definition	Focus during fragment hit selection leads to	Recommended range for fragment hits	Aim during fragment optimization
Ligand efficiency	LE = $-\Delta DG/HA$	Bias towards smaller compounds	LE $\geq$ 0.3	Try to maintain (decrease acceptable for starting fragments with LE $\gg$ 0.3)
Fit quality	FQ = LE/LE_Scale	Size independent selection of efficient compounds	FQ $\geq$ 0.8	Maintain at high level or increase to FQ $\sim$ 1
Ligand-lipophilicity efficiency	LLE = $p\mathrm{IC}_{50} - \log P$	Selection of more specific compounds	LLE $\geq$ 3	Maintain at high level or increase to LLE $>$ 5–7

than 5. However, a 10 nM compound with an acceptable *c* log *P* of 5 will have a LLE of 3. This is much smaller than the suggested range of 5–7 [32]. To achieve a LLE in this range, the *c* log *P* must be smaller than 3. This is in agreement with a recent study which showed that there is an increased risk of adverse outcome with *c* log $P > 3$ [37].

Table 2 summarizes the ligand efficiency scores that should be considered during FBDD. Both LE and FQ have been very helpful in guiding the selection and optimization of fragment hits. In addition, LLE is expected to become increasingly popular to ensure an increase of affinity more than lipophilicity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ddtec.2010.11.003.

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