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Fragments, network biology and designing multiple ligands

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Modulating multiple protein targets simultaneously can be beneficial for treating complex diseases. The redundancy that exists within biological networks means that modulating single proteins might not be sufficient to produce the desired efficacy while, at the same time, minimizing adverse effects. Designing multi-target drugs can be challenging for medicinal chemists, with current lead-discovery strategies often producing large, complex molecules with low ligand efficiency and poor oral bioavailability. Paradoxically, analyses of the relationship between the selectivity of biologically active compounds and their molecular size suggest that promiscuous compounds should typically be smaller than target-selective compounds. A fragment-based approach to multi-target drug discovery could lead to a new generation of compounds with improved physicochemical and pharmacokinetic properties.

Introduction

Drug discovery at the start of the 21st century is largely based upon a 'one molecule – one target – one disease' philosophy, but there is a growing recognition that molecules that modulate multiple targets simultaneously (i.e. multiple ligands) can be beneficial for treating a range of diseases [1,2]. The existence of inherent redundancy and robustness in many biological networks and pathways means that inhibiting a single target might fall short of producing the desired therapeutic effect [3,4]. Some researchers have proposed that the remaining 'low-hanging fruits' among disease-relevant targets, which exhibit one-to-one mapping between genotype and pathological phenotype, are likely to be rare compared with pathologies that originate in the dysfunction of a dynamic network of interacting proteins [5]. Hence, targeting disease-associated network states rather than individual proteins alone will undoubtedly be an enduring trend for future generations of drug discoverers.

Current approaches and challenges

Although many currently marketed drugs act via multiple targets, the discovery of their multiple mechanism of action was usually serendipitous and retrospective. The premeditated design of compounds with a predefined multi-target profile [i.e. 'designed' multiple ligands (DMLs)] is a recent development and presents several important new challenges to medicinal chemists [6,7]. Combining a desirable biological profile with druglike physicochemical properties is a particularly difficult aspect of working with multiple ligands [8]. This difficulty is illustrated by the trend for the current generation of DMLs to be larger and more lipophilic than marketed drugs [9] or preclinical compounds [10,11] (Figure 1a,b). DMLs are also less efficient than a broad set of preclinical compounds in terms of their binding energy per unit of molecular weight (MW) or lipophilicity (cLogP) (Figure 1c,d).

This complexity, which has potentially detrimental consequences for oral bioavailability, is, at least in part, a consequence of the current strategies through which DMLs are discovered [10]. The framework combination strategy generates dual ligands by integrating structural elements from two selective ligands [10] (Figure 2a). If the starting compounds are large or only a low degree of integration of the selective ligand frameworks is possible, multiple ligands with high MW and low ligand efficiency will be obtained. Framework combination can be a successful strategy for producing dual ligands with good oral bioavailability, as illustrated by the discovery of the antipsychotic drug, ziprasidone [12]; but in such cases the selective ligands are small and/or the frameworks are well integrated. An alternative lead-discovery approach is the screening of corporate compound libraries.

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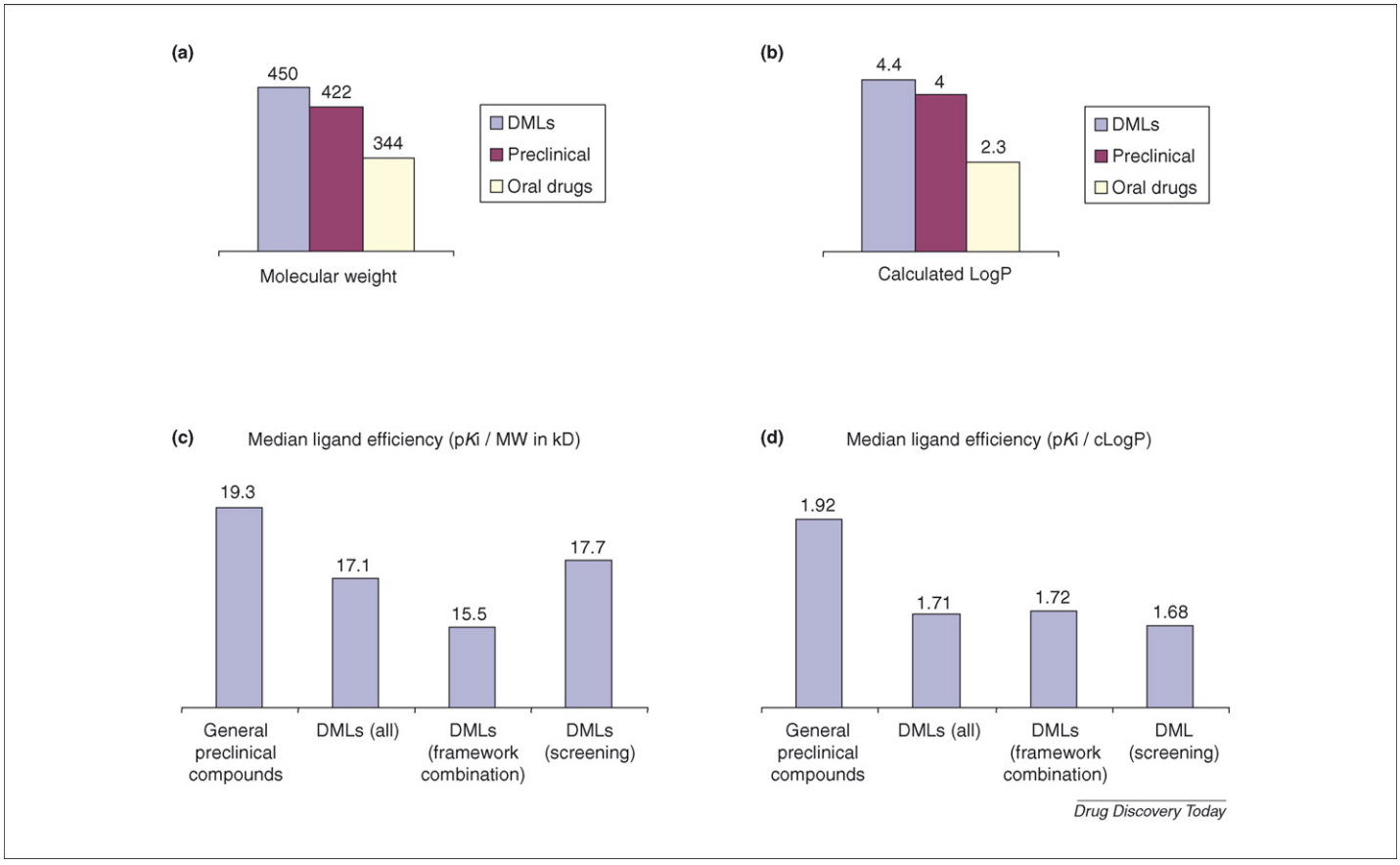

FIGURE 1

Property values for oral drugs, preclinical compounds and multiple ligands. The median physicochemical property values for designed multiple ligands (DMLs) are higher than those for oral drugs [9] or a broad set of preclinical compounds from Organon's SCOPE database [10]: **(a)** median molecular weights (MW); **(b)** median cLogP values. The number of H-bond acceptors and rotatable bonds is also much higher (data not shown). Measures of the median ligand efficiency of DMLs are typically lower than those for preclinical compounds: **(c)** efficiency based upon molecular size (p*K*i/MW in kDa); **(d)** efficiency based upon lipophilicity (p*K*i/cLogP).

Although the screening-derived DMLs reported in the literature tend to have higher ligand efficiency than those derived via framework combination, they are still usually less efficient than preclinical compounds as a whole (Figure 1c,d). Many of the reported examples of DMLs are inefficient in their binding because they contain groups that are only important for one of the targets and are merely tolerated by the other targets [10] (Figure 2a). In view of these drawbacks, new lead-discovery approaches are required so that this area fulfils its potential to deliver new medicines.

The relationship between molecular size and promiscuity

The success of efforts to improve the druglikeness of multiple ligands will be dependent upon the fundamental relationship between the structural complexity of ligands and their likelihood of possessing multiple activities. Hann *et al.* [13] found that the probability of ligands binding to a given protein target falls dramatically as those ligands become more complex because the chance of a mismatch with the molecular recognition features of a protein-binding site increases dramatically. With multiple proteins, the differences in the binding sites will inevitably cause the probability of a ligand binding to multiple sites to fall even more sharply as molecular complexity increases. A recent paper from Hopkins *et al.* [14] showed that there was a correlation between the mean MW of compounds and the total number of targets that those compounds were found to show at least minimal activity against (IC_{50} <10 μM). This suggests that more-promiscuous compounds tend to be smaller than highly target-selective ones. Data from Organon's SCOPE database [10], which comprises a broad set of preclinical compounds, show an increase in the selectivity of ligands for a single target as molecular size increases, supporting the conclusion that promiscuous compounds are smaller than more-selective ones (Figure 3). The fact that the relationship between MW and promiscuity is pronounced, even for this limited dataset, is somewhat surprising given the fact that medicinal chemists will select, as starting points, compounds that are smaller (i.e. more druglike) and more selective. It is, therefore, predicted that the relationship that would be observed for a set of compounds that have been subject to little or no pre-selection by medicinal chemists, such as actives from a HTS campaign, would be higher. It might also be expected that the trend would be higher if the secondary targets were less closely related to the primary target. This is in accordance with the pronounced trend between mean MW and the total number of targets hit (R^2 = 0.93), reported by Hopkins *et al.* [14].

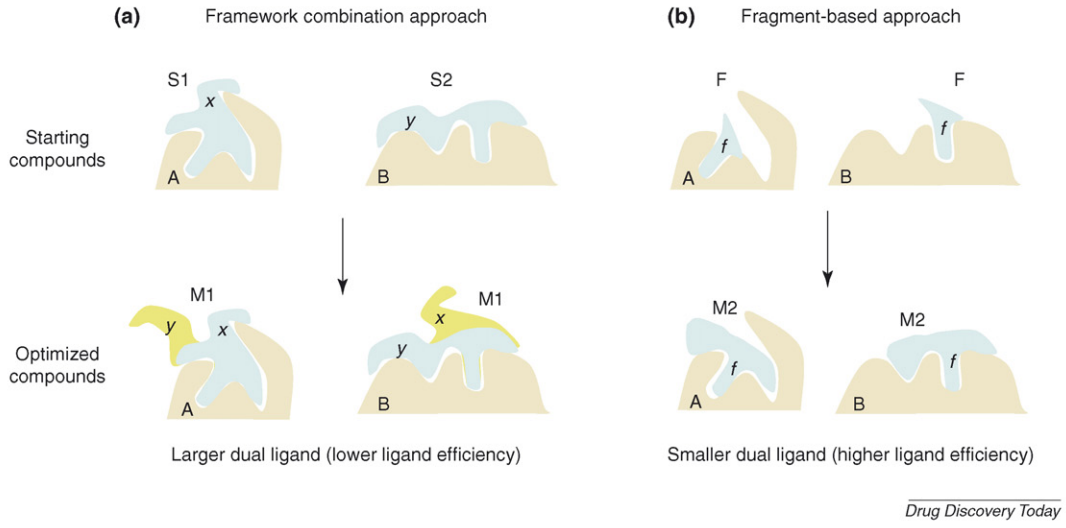

FIGURE 2

Alternative approaches to the design of multiple ligands. (a) In the framework combination approach, knowledge of the SARs for target-selective ligands is used to integrate the frameworks of the two selective molecules, S1 and S2, retaining the groups that are essential for binding each target, such that both activities are incorporated into a single molecular entity M1.The degree of merger of the frameworks that is possible will often be slight if there are few similarities between S1 and S2. This tends to give larger molecules M1 with poorer ligand efficiency because some regions contribute considerably to the binding energy for only one of the targets, and are merely tolerated by the other target (these tolerated regions *y* and *x* in M1 are shown in yellow for targets A and B). **(b)** In an alternative fragment-based approach, an initial screen would be conducted to identify a fragment (F) that is capable of binding to both proteins A and B. Fragment F could then be optimized to give a ligand M2 that fits targets A and B with higher ligand efficiency than M1 because the tolerated regions are absent.

Fragment-based approach

Given that promiscuous molecules often have low MWs, a fragment-based approach to the discovery of designed multiple ligands should be considered as an alternative to more-conventional approaches. Fragment-sized molecules, typically MW <250 Da, are often efficient in terms of their binding energy per heavy atom [15,16]. In the case of multiple ligands, screening of a library of fragments could yield a basic core scaffold that is capable of binding, at least with minimal affinity, to both the targets (Figure 2b). A library of analogues with additional functionality could then be made, guided whenever possible by biostructural information from either X-ray crystallography or NMR studies,

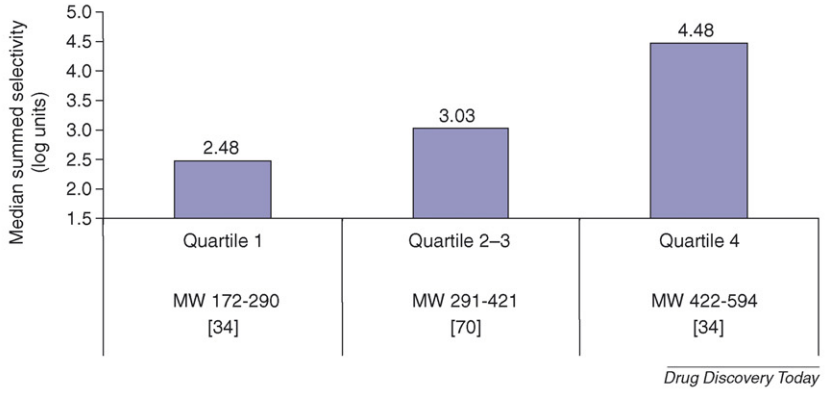

FIGURE 3

The relationship between molecular size and promiscuity. Organon's 'SCOPE' database describes 1860 diverse medicinal chemistry, non-DML, optimizations, each comprising a starting compound, an optimized compound and associated biological data (for more information see Ref. [10]). From this set, p*Ki* or pIC_{50} data were available for three phylogenetically related targets for 138 compounds with a molecular weight (MW) <600 Da. A measure of promiscuity is the sum of the two selectivity values, in log units, for the primary target over the two secondary targets. High summed selectivity corresponds to low promiscuity. To examine the relationship between molecular size and promiscuity, the compounds were divided into four quartiles based upon the MW. Quartiles two and three were combined to give a MW range that was similar to quartiles one and four. Sample sizes are shown in square brackets. As MW increases, the median summed selectivity for the 138 starting compounds increases and therefore promiscuity decreases [10]. The three median summed selectivity values are considerably different from each other according to the non-parametric Mann-Whitney rank test of statistical significance ($p < 0.05$).

with the goal of increasing the affinity at both targets and obtaining the desired balance of activities. The availability of biostructural information is likely to be invaluable given the challenges of designing-in activity at two or more targets. A range of target families of interest in drug discovery, such as kinases and proteases, will be most amenable to this type of fragment-based approach to multiple-ligand discovery. Given the lack of sufficiently high-resolution biostructural information for most membrane receptors, such as G-protein-coupled receptors (GPCRs), the application of a fragment-based approach, although not impossible, will be challenging at the present time. This situation will almost certainly change in future given the current efforts to obtain high-resolution structures for such targets [17].

Optimizing fragments with weak multiple activities into druglike DMLs that possess potent and appropriately balanced affinities will be most easily achieved for targets sharing a conserved binding site [10]. As binding sites become more dissimilar, it will become increasingly difficult to improve and adequately balance the affinities at the levels (i.e. low nanomolar) that are normally associated with acceptable *in vivo* efficacy and safety for target-selective agents. This might call into question the added value of a fragment-based approach for many target combinations because improving the affinity of a fragment to this level for two or more targets simultaneously might frequently prove to be difficult, if not impossible. However, there exists a tantalizing possibility in the field of multiple ligands that might reduce the need for the high-affinity binding that is characteristically required for target-selective agents. Where synergy exists between two or more targets, it is conceivable that multiple ligands with only modest activity at one or more of the targets might still produce superior *in vivo* effects, in terms of efficacy and safety, compared with higher-affinity target-selective compounds [18].

The effect of ligands with modest activity on biological networks

Our current understanding of how individual proteins interact within biological pathways and networks is at a nascent stage but, without doubt, as our knowledge grows, this area will have a pivotal role in influencing future strategies for discovering multiple ligands [5]. Although many networks are typically characterized by their robustness and resistance to change, they can be vulnerable to multiple hits at sensitive nodes. The modelling of network behaviour by Csermely *et al.* [19] has indicated that the partial inhibition of several targets can be more efficient than complete inhibition of a single target. Using network models of antimicrobial drugs, Csermely *et al.* [19] showed that multi-target attacks can be particularly effective if widely distributed across an entire network, rather than being concentrated around a single target. Although this type of network-based approach involving a multiplicity of interactions was relevant to antimicrobial agents, for other diseases more-specific network models will be needed to examine how effective weak binders might be in areas such as central nervous system disorders. Experimental studies designed to investigate the properties of networks, such as the characterization of molecular complexity within the synaptic proteome, will provide useful information for further study of a range of disorders [20].

A potential disadvantage of employing small molecules with modest multi-target activity might be the likelihood of low selectivity over other closely related targets that are associated with side-effects (i.e. off-target effects). This problem might be circumvented where a highly synergistic relationship exists between the desired targets, enabling a lower dose of compound to be used that does not cause the undesired target to be modulated to a large extent at therapeutic doses. The use of weakly active ligands might also help circumvent mechanism-based side-effects caused by high-potency target-selective agents. If the highly synergistic relationship of the targets is restricted to certain pathological pathways, but the individual targets are also present in healthy tissues, the adverse effects of the drug in healthy tissues might be mitigated because the activity of the drug on either target in isolation will be lower.

There are many reported examples of synergistic interactions in preclinical models where the efficacy of a single-target agent is enhanced by addition of a low-level secondary activity. For example, in pain models the activity of morphine is enhanced by a low dose of a cannabinoid [21] and the activity of gabapentin is enhanced by a low dose of a neurokinin-1 receptor antagonist [22]. Synergy has also been observed in humans, between drugs given as low-dose combination therapies, which results in higher efficacy and/or reduced side-effects compared with higher-dose monotherapies [23]. For example, there is a synergistic interaction between calcium-channel blockers and angiotensin-receptor antagonists for the treatment of hypertension [24]. Similarly, there is evidence that low doses of atypical antipsychotics, such as olanzapine and risperidone, can improve the antidepressant efficacy of selective serotonin reuptake inhibitors (SSRIs), such us fluoxetine, in the treatment of refractory patients [25]. Neuroimaging studies indicate that the dopamine D2 receptor occupancy associated with this low-dose augmentation effect is lower than that required for antipsychotic efficacy [26,27].

The scenario of weakly active low MW compounds producing profound pharmacological effects is illustrated by memantine, a drug for Alzheimer's disease. Memantine is a small, low-affinity, non-selective *N*-methyl-D-aspartic acid (NMDA) receptor blocker [MW 179 Da; affinity (IC_{50}) ~1 μM] that has been reported to cause less side-effects than higher-affinity agents [28]. Interestingly, memantine is a noncompetitive channel blocker, and in some circumstances weak-binding multi-target drugs might provide a better balance of efficacy and safety if their mechanisms of action are allosteric [18,19].

Concluding remarks

Given the current embryonic state of the art, considerable challenges exist for medicinal chemists on the road to discovering druglike multiple ligands. To facilitate the lead-optimization process, the existing approaches for lead discovery need to evolve and mature and fundamentally new strategies need to emerge. For example, medicinal chemists might address DML design at the level of the underlying pharmacophores, rather than simply merging the predefined molecular frameworks found in selective ligands. New screening-based strategies also need to emerge to address the suboptimal physicochemical properties that are frequently encountered with the current generation of multiple ligands. The screening of fragment libraries might offer an attractive opportunity for discovering lead compounds with the desired multi-target activity and improved ligand efficiency. The

attractiveness of such an approach will depend upon the availability of biostructural information to guide the optimization process and deliver high potency at multiple targets. For some target combinations, especially if the binding sites are dissimilar, it will be more difficult to optimize potency and retain druglikeness and high ligand efficiency. However, multi-target compounds with surprisingly weak activity can, in some cases, still exhibit profound *in vivo* effects by virtue of exploiting synergistic connections between network components. Given the increasing interest in systems biology, the targeting of network states rather than individual proteins, allied with a fragment-based approach to multiple ligand discovery, could represent a powerful paradigm for deriving multi-target drugs with improved efficacy and reduced adverse effects compared with target-selective agents.

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