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Tailored Multi-Target Agents. Applications and Design Considerations

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Abstract: *Background:* In contrast to the one target-one drug paradigm, multi-target agents seem as a promising alternative to manage complex disorders and health conditions linked to drug resistance issues. In fact, many long-standing drugs are in fact unintended multi-functional therapeutics that have emerged from phenotypic screening. The last two decades, however, have witnessed the emergence of tailored multi-target agents, which according to our perspective combine the best aspects of target-based and phenotypic-based drug discovery. *Methods:* We discuss a number of considerations related to the design, screening and computer-aided discovery of multi-targeted drugs, along with overlooked advantages that this type of agents might have in clinical trials. A theoretic example is included to explain the reduced positive predictive value in virtual screening campaigns focused on multi-target agents. *Conclusion*: Multi-target agents present great therapeutic potential for the treatment of complex health conditions and the solution of drug resistance phenomena. However, they are certainly challenging for computer-aided drug discovery approaches. Merged or overlapping pharmacophores should be preferred whenever possible. It is thus suggested perform a careful selection of the combination of pursued targets, preferring target combinations supported by co-evolution or similar biding sites.

Keywords: Tailored multi-target agents, virtual screening, drug design, *in silico* screening, binding efficiency metrics, drug resistance, network pharmacology.

1. INTRODUCTION

There are many good reasons that explain why, during the last two to three decades, target-driven approximations have dominated the field of drug discovery. Target-centered drug discovery was originally linked to the "one drug one target" model, which seeks for exquisitely selective agents capable of modulating a single target linked to a disease state. A target-based drug discovery campaign begins with a hypothesis on the role of a given molecular target on the pathophysiology of disease; then, the effect of drug candidates on the purified target is measured through *in vitro* assays and only at that point the drug progresses to more complex models. The target-based approach is indeed tempting: it allegedly allows the definition of rational drug discovery programs [1,2] and increased screening capacity [1] compared to phenotypic screening, which first looks at the effect of chemical compounds on cells, tissues or even whole organisms. Highly selective agents are less likely to present side-effects due to off-target interactions [1, 3] and also seem to present shorter development timelines [2], possibly due to the complex process of target deconvolution that follows phenotypic-based discovery [4, 5].

While target-based approximations are well suited to Mendelian disorders where the inheritance of a single gene can be linked to the disease [6], they have generally failed to fulfill expectations as treatments of more complex disorders and are frequently cited as one of the possible reasons for the decline in the number of new drugs that reach the clinical practice normalized for the cost of drug discovery and development [1, 3, 4, 6, 7]. Modern pharmacology, in line with systems biology, understands biological systems as robust entities that usually require multiple perturbations to lose functionality [3]; consistently, a disease emerging from multiple factors would also be a robust state. Strikingly, first-in-class small molecule drugs identified through phenotypic screening seem to outnumber those emerging from target-centered projects [7]. Multi-target agents, simultaneously impacting on an array of disease-related molecular targets thus appear as promising approaches to cure complex diseases and infectious disorders (see next section) [8]. Under this new light, renewed interest into phenotypic screening has arisen in the last years [2, 7], since such strategy is prone to the discovery of molecules capable of modifying previously undescribed targets or acting simultaneously on more than one target.

In the debate on phenotypic- versus target-based strategies, tailored multi-target agents can be thus regarded as the middle way. They are an expansion of previous target-centered approaches that incorporates the perspective of network pharmacology. Whereas in phenotypic screening the target/s are, if lucky, defined *a posteriori* and the multiplicity of targets for a hit is unintended (and sometimes goes unnoticed), tailored multi-functional agents are *a priori* conceived to selectively modulate a number of chosen targets of interest, thus heavily relying on computational drug design and data analysis tools and avoiding target deconvolution. Their multi-functional nature imposes however certain particularities when applying computer-guided drug discovery tools. After a brief overview of multi-target drugs applications, this review discusses some of the particular considerations that should be taken into account when screening or designing a multi-target drug.

2. APPLICATIONS

2.1. Complex Diseases

Understandably, single-target agents usually fail to treat multi-factorial disorders with polygenic origin and/or a strong environmental component, which can be thought as "the high-hanging fruit" in drug discovery. In an ageing and stressful world, CNS drugs are potentially among the most profitable drug treatments. Magic bullets have however been particularly disappointing in the fields of neurology and neuropsychiatry [9] which now look at polyspecific agents and polypharmacology with renewed interest [10-12]. Remarkably, most of the more successful CNS agents are in fact unintentional multi-mechanistic drugs discovered by seren-

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dipity or physiological "black box" models which present a highly complex pharmacology [13-15].

The brain is the most complex organ in the human body; it exerts control over the rest of the organs and it is responsible for higher functions such as language, cognition or memory. Ensuring brain homeostasis is thus particularly relevant to the general well-functioning of the whole organism, which is wonderfully illustrated by the fact that, under normal conditions, the traffic of circulating chemicals and cells into the CNS is strictly controlled by the blood-brain barrier. Accordingly, brain function tends to be compromised whenever exogenous compounds reach the CNS in pharmacologically relevant quantities. It is no surprise that most of the drugs targeting a brain disorder display important side effects (sleep, attention or sensorial perturbations, among others); as already mentioned, they tend to present a certain degree of non-selectivity that is essential to their pharmacological efficacy. However, simultaneously modulating a number of CNS targets without perturbing brain functions can be challenging, especially under the traditional "the more potent the drug, the better" paradigm, which focuses in the development of high affinity drug candidates. Today, it is being increasingly speculated that using low-affinity multi-target ligands to modulate several non-crucial nodes neighboring key nodes might be the best choice to restore a malfunctioning network to a healthy state [15-17]. Note that this implies a double paradigm shift: searching for a) multiple- instead of single-targeted drugs and; b) searching for low affinity instead of high affinity ligands. The later might be particularly true when treatments for chronic conditions are being sought. Memantine (Fig. **1**) constitutes a very good illustrative example of the potential benefits of low-affinity multi-target ligands on CNS disorders [17, 18]. It is currently prescribed for the treatment of moderate to severe Alzheimer's disease and other types of dementia; the drug is recommended when acetylcholinesterase inhibitors are not well-tolerated, showing moderate decrease in clinical deterioration. Unlike high-affinity uncompetitive blocker of the N-methyl-D-aspartate receptors (NMDAr) dizocilpine, which has not reached the market due to severe side reactions including cognitive disruption, psychotic reactions and Olney's lesions, memantine possesses surprising low-affinity binding to NMDARs (in the high nanomolar to low micromolar range), fast on/off kinetics and almost no selectivity among subtype NMDARs [17], being much better tolerated. It also shows uncompetitive antagonist activity on several other receptors, including serotonin 5-HT3 [19], nicotinic [20] and dopamine D2 receptors [21] (in all cases, with a potency similar or slightly higher to that for the NMDA receptors). Recently, Prati et al. have highlighted the balanced potency of one triazinone found by fragment-based approaches on both BACE-1 and GSK-3β (IC50s in the mid-micromolar range) as potential treatments for Alzheimer's, showing that the paradigm shift has started to be felt in the field of tailored multi-target agents [22].

Abundant reviews exist on the current and potential applications of multi-target agents to a number of disorders including Alzheimer's [17, 23] and Parkinson's diseases [24] and mood disorders [25-27], among several others (see [8] and refs therein). It should be noted, however, that many of these tailored multi-modal agents are dual or at most triple-target drugs (see for instance [23, 24, 26, 27]). Is simultaneous modulation of two or three targets enough to control multi-factorial diseases? The existing examples of multi-target therapeutics, found through serendipity or physiologic models, often display a far more complex pharmacology. At the same time, as will be discussed later, each desired selective interaction with a given target imposes its own structural restrictions; the restrictions imposed by different targets might well be mutually exclusive. The more targets pursued the more complex the structure-activity relationships get, especially when unrelated targets are approached.

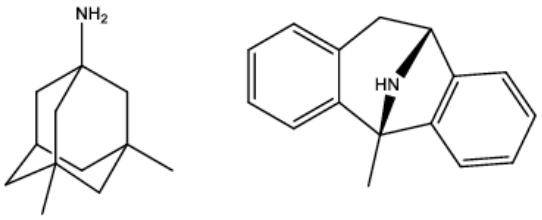

Fig. (1). Memantine (left) exemplifies the potential advantages of low-affinity multiple ligands compared with high affinity ones like dizocilpine (right).

Finally, it should be underlined that many of the tailored multi-target agents reported so far do not fit the low-affinity ligand paradigm, that is, many researchers are still guided by the perhaps outdated "the more potent, the better" model, with drug discovery campaigns being often focused on those drug candidates that display the highest activity towards the c targets.

2.2. Drug Resistance

It has long been noted that there exists a relationship between the multi-target nature of successful antimicrobials and their lowered potential for target-based resistance [28]. Drugs that interact with a single gene product are susceptible to single step development of high level resistance, while those that bind multiple gene products are less likely to present high level resistance. Apart from the applications in the field of anti-infective agents, network pharmacology might be a valid alternative to treat non-infectious conditions characterized by high incidence of the multi-drug resistance phenomena, such as epilepsy [12, 15], where pre-clinical studies suggest that combining drug with different modes of actions may enhance seizure protection [29, 30] and some of the most useful drugs in clinical practice are those with broad-spectrum activity [31]. Naturally, multi-modal drugs do not pose a particular therapeutic advantage when the drug resistance phenomenon is of pharmacokinetic nature (e.g. up-regulation of efflux transporters or biotransformation systems, decreased cell permeability, gain-of-function polymorphic variants of drug metabolic enzymes). But they might indeed be useful for other resistance mechanisms such as target modifications and compensatory responses.

As previously insinuated, the best multi-modal strategy is highly dependent on both the therapeutic objective and the mechanisms underlying the drug resistance phenomenon. A multi-target approach could involve vertical (or serial) targeting, in which targets belong to the same metabolic pathway, or parallel targeting, in which members of different pathways are modulated [8, 32]. The first approach might be valid for drug resistance based on target modifications; the second could be more relevant to approach resistance based on compensatory responses [33]. As long as the host does not present homologs to the targeted proteins, high-affinity candidates and targeting of hubs (highly connected nodes in a biochemical pathway or network) are valid approaches for the development of anti-infective drugs. Conversely, when the therapeutic goal is to restore an altered pathway to normal functioning, low-affinity candidates and avoiding key nodes might be preferred to minimize safety issues.

2.3. Pharmacological Profiling

Pharmacological profiling involves the *in vitro* and/or *in vivo* screening of a drug candidate against a broad range of targets (e.g. metabolic enzymes, transporters, ion channels) or systems that are different from the intended therapeutic target [34, 35], in order to identify secondary functions/off-target effects of the drug. If one gives it some thought, uncovering off-target interactions is nothing but exposing the multi-target nature of a drug. In the era of systematic drug repurposing and drug rescue (i.e. finding novel therapeutic

indications for existing drugs, including approved, discontinued and shelved drugs and also drugs in the pipeline), pharmacological profiling gains a completely new meaning. It not only involves selecting drug candidates with reduced toxic potential and providing clues on which effects should be carefully monitored during clinical trials: it may also serve to identify new useful therapeutic indications of an investigational drug, as envisioned by Williams many years back [35].

Interestingly, early detection of secondary effects at the discovery or preclinical stages can enhance the probability for a drug to gain approval. It is well known that pre-definition of study design (including endpoints and statistical analyses) is much necessary to avoid bias in the analysis of the study outcome. Pharmacological profiling can help deciding between a superiority or non-inferiority trial, and whether a composite endpoint (particularly useful for drugs that can benefit the patients in several ways) may be appropriate. When using a composite endpoint it is usually primary; ideally, the components of the composite variable should be similarly important [36, 37]. The non-inferiority trials are a viable alternative when a new treatment might be equivalent to the standard therapy in terms of efficacy but preferable owing to other reasons (e.g. safety, convenience, or others) [38, 39]. Today, *in silico* pharmacological profiling could be a valuable tool to decide, at the early stage of drug development, which candidates should advance to further studies, aiding in the detection of additional functionalities which could pose an advantage over established treatments (e.g. ability of the new drug to treat a common co-morbid condition besides the primary therapeutic goal) [8].

3. COMPUTATIONAL APPROACHES TO MULTI-TARGET DRUG DISCOVERY

There are two basic ways to approach the computer-guided search of multi-target therapeutics: virtual screening and computer-guided drug design [40]. In a certain way, both are intrinsically related to the pharmacophore (from the greek, "medicine bearer") concept, that is, the idea that there exists a set of molecular features that a ligand should gather in order to bind a given molecular target in a given binding site. These essential features are common to all ligands that interact with the same target in the same way. The pharmacophore notion is clearly related to the key and lock analogy [8] as long as one bears in mind that, while keys are rigid, ligands can be flexible and "adapt" to the target features (and the other way around). Apart from the pharmacophoric features, there are secondary features that, if present or absent, may lead to activity gain or activity loss.

Virtual screening supposes using computational models (e.g. QSAR models, pharmacophoric hypotheses) to rank chemical compounds from digital libraries or databases according to their likelihood of presenting one or more desired activity/ies [41]. Although it is possible to screen libraries of theoretical, not-yet-existing compounds [42], it is far more common to submit collections of existing chemicals to *in silico* screening procedures. Conversely, computer-aided drug design implies using computational models to generate, in a rational manner, novel active compounds. Particular considerations should be taken into account when using either methodology to discover new multi-target agents.

3.1. Virtual Screening for Multi-Target Agents. The Importance of Sensitivity

A general condition when performing a virtual screening campaign is that inactive compounds will be greatly overrepresented in the screened library. This is to be expected: if looking for a compound which specifically binds to a molecular target, the very definition of specific (*exerting a distinctive influence; sharing or being those properties of something that allow it to be referred to a particular category*) makes it more probable not finding the relevant molecular features that determine the binding event than finding them. The hit rate has actually been estimated to range between only 0.01 and 0.14% [43].

When working with modeling techniques that provide continuous or ordinal output/score, it is possible to optimize the score threshold that will be used to discriminate between hits and non-hits (predicted active and inactive compounds). Such optimization intends to find an adequate balance between the model's specificity (Sp) and sensitivity (Se). What is, however, and adequate balance? As clearly explained in the seminal work from Triballeau *et al*. [44] the choice of such balance is not a statistical matter but a context-dependent decision. Specificity represents the true positives (TP) rate; sensitivity, the true negative (TN) rate. The unachievable perfect model would display a Sp and a Se equal to 1, representing a perfect classifier. Unfortunately, Sp and Se evolve in opposite senses. If abundant funding exists, the user may choose to relax Sp in favor of Se, in order to retain as much active scaffolds as possible. On the contrary, if funding is limited, as it usually is, the user may prefer to prioritize Sp over Se, so to minimize the number of false positives (compounds moved to *in vitro* or *in vivo* testing that will fail to display the pursued activity). Triballeau et al. proposed Receiving Operating Characteristic (ROC) curves as a graphical tool to analyze the balance between Sp and Se (Fig. **2**); additionally, the area under the ROC (AUROC) can be used to statistically compare the performance of a screening procedure with either random classification or other screening approaches. Later, other metrics have been proposed to address some of the limitations of the AUROC, in particular, the "early recognition" problem [45-47].

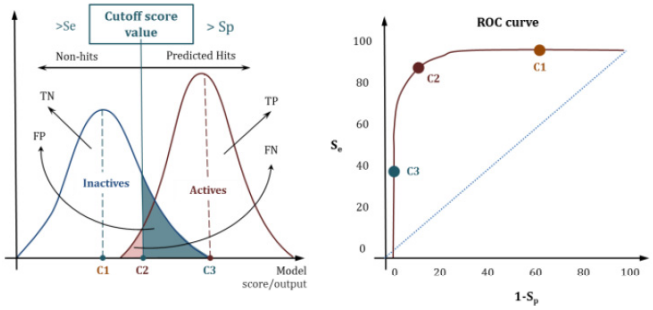

Fig. (2). Schematic representation of ROC curve construction.

Let us use the previous information to shed some light into some particular difficulties of virtual screening when searching for multi-target drug candidates. To this purpose, we will discuss a hypothetic example. Assume we are using two models (model 1 and model 2) to search for dual drugs acting on two molecular targets of interest (molecular target 1 and molecular target 2, respectively). The models will be applied in a serial manner to screen a 100,000 compound database. For the sake of simplicity, let us say that both models have, for a particular score threshold, a Sp of 0.98 and a Se of 0.90. Note that the balance between Sp and Se is thus, in both cases, very good. Let us be generous and assume that the yield of actives for the database is about 1% for both molecular targets (quite a generous proportion compared with the 0.01 to 0.14% estimation). In other words, in the hypothetic dataset there are 1,000 ligands for molecular target number 1 and 1,000 ligands for molecular target number 2. Assume that being a ligand for target 1 does not increase or decrease the chance of being a ligand for target 2. Since we are looking for dual ligands, we are interested in the intersection between these two 1,000 compound subsets. As we said, we will work serially: we will apply model 1 to the 100,000 compound database, and we will then apply model 2 to identify dual ligands among the resulting compounds from the first screening step. Let us now translate this into raw numbers.

In relation to molecular target 1, there are 1,000 active compounds and 99,000 inactive compounds in the database. Now, from the 1,000 active compounds, 900 will be identified by model 1 (Se=0.90). From the 99,000 inactive compounds in relation to molecular target 1, two in 100 compounds would be misclassified as positives (Sp=0.98, false positive rate = 0.02). In other words, 1,980 compounds from those 99,000 will be labeled as positives. In total, we will have 900 actives and 1,980 inactive as predicted hits (2,880 in total). The Positive Predictive Value, i.e. the activity probability of one of these selected candidates can be computed through the following expression [44]:

$$PPV = (Se * Ya) / [Se * Ya + (1\text{-}Sp)(1 - Ya)] \quad (1)$$

Ya being the yield of actives for the database. If we replace and solve the equation, PPV is 0.3125. In other words, one in every three assayed predicted hits will be actually active on target 1. Note that the yield of actives cannot be known *a priori* in a real virtual screening application; thus, the true PPV is ignored before conducting the screening.

Now let us apply model 2 (which is absolutely independent from model 1) to the 2,880 compound subset resulting from application of model 1. Only around 29 compounds in this set (1%) are ligands for molecular target 2; to them, we should add 57 false positives selected by model 2. Among the resulting 83 hit compounds for model 2, approximately one in three (26) is a real ligand for molecular target 2 (note that as we are assuming that being a ligand for target 1 is independent from being a ligand for target 2, and both models share Sp and Se balance, PPV1 is identical to PPV2). About 8 hits will be dual ligands; the overall PPV (the probability that a hit emerging from the serial application of the individual models will be experimentally active against both targets) corresponds to the product of the PPV associated to each individual model:

$$Overall\ PPV = PPV_1 \times PPV_2 \quad (2)$$

While in our example, PPV_1 and PPV_2 are circumstantially identical, this will not be the general situation.

The preceding analysis is illustrated in Fig. (**3**). Note how small that number is considering we started from 100,000 compounds from which 1,000 were ligands for target 1 and 1,000 for target 2. The number could be higher if target 1 and target 2 have co-evolved and share ligand specificity (resulting in partially or totally overlapping pharmacophores); on the contrary, the number could be lower if the correspondent pharmacophores are mutually exclusive.

From the previous analysis it is no surprise that virtual screening for multiple-target drugs could be disappointing. Note that in the example we have only considered *dual* ligands; the scenario could be even worse for triple ligands or further, since the general expression for overall PPV:

$$Overall\ PPV = \prod_{i=1}^{n} PPV_i \quad (3)$$

where n represents the number of molecular targets approached in a serial manner in the VS campaign. Remember that the balance between Sp and Se is hardly as good as the ones in the example (which correspond to area under the ROC curves very similar to that of the ideal ROC curve, i.e. 1).

At the expense of increasing the false positive rate, an alternative to improve this situation is to relax Sp in favor of Se (the opposite of the more common balance of these two parameters) [48]; such strategy, of course, results in an increment of experiment-related costs (diminished active enrichment). Alternatively, choosing the pursued targets on the basis of previous evidence on shared ligands could be a good advice to improve the likelihood of success. This frustrating perspective is however compensated by the vast and exponentially expanding chemical universe: the Chemical Abstract Service today holds more than 100,000,000 entries; on the basis of the Pubchem entries, more than half of these accessible chemicals correspond to small drug –like molecules.

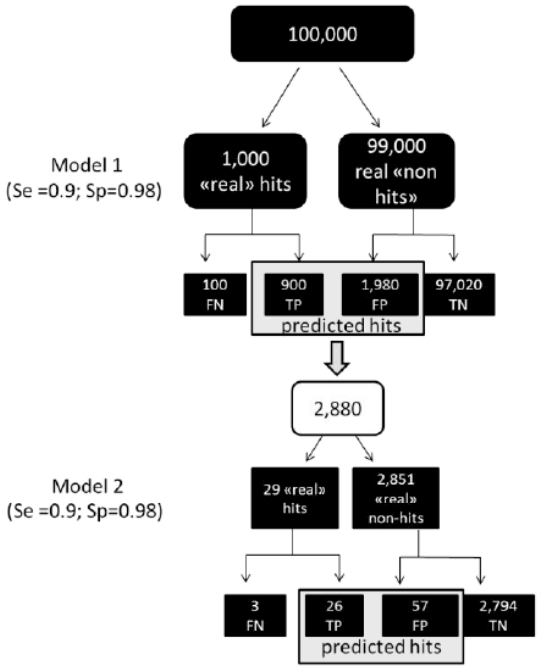

Fig. (3). Schematic representation of the hypothetic virtual screening campaign described in section 3.1. Only around 8 dual hits will be selected at the end of the protocol; only 1 in 6 predicted hits will be active. Note that rather generous model performances and yields of actives have been hypothesized. Real situations could be even worse.

This theoretic analysis on the intrinsic limitations for the applications of VS to select dual (or further multi-target agents) that result in a very limited number of actual multi-target hits is reflected in real VS campaigns for multi-target agents. For instance, in their search for more efficacious anti-inflammatory agents, Moser et al. recourse to pharmacophore-based virtual screening of Asinex merged-fragment database for dual 5-lipoxygenase/soluble epoxide hydrolase inhibitors [49]. From 36 predicted dual inhibitors, only one (that is, a PPV for dual agents of only 2.8%) confirmed dual action experimentally. Similarly, Ruggeri et al. implemented a pharmacophore- and docking-based search to retrieve dual inhibitors against *P. falciparum* alanyl aminopeptidase, *Pf*A-M1, and leucyl aminopeptidase, *Pf*A-M17 from 18 million compounds from ZINC database [50]. Despite the broad substrate specificity of *Pf*A-M1 and its partially shared substrate specificity with *Pf*A-M17 (which increase the probability of finding common inhibitors) only two among the 12 (16.6%) experimentally assayed hits confirmed dual activity.

3.2. Designing Multi-Target Agents. Binding Efficiency, Entropy, Bioavailability

The design of multi-targets agents is based in the combination of pharmacophores from single-target ligands (that is, a fragment-based approach). The distinct pharmacophores might be linked together through stable or cleavable links [51]. This category of multi-target agents corresponds to what Sturn *et al*. have called *bianchor ligands*, which use different sets of atoms to interact with the target proteins (note that pairs of targets sharing ligands were considered) [52]. When using stable links to join distinctive pharmacophores, the designer should carefully watch that if the result-

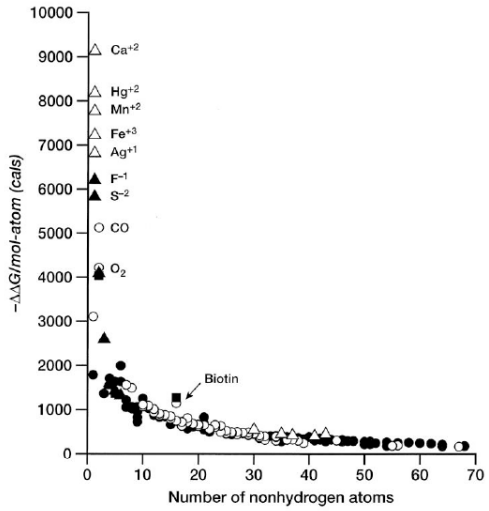

Fig. (4). Free energy of binding per atom for ligands and enzyme inhibitors versus the number of non-hydrogen atoms in the ligand. Note that the binding energy per atom tens to decrease for larger drugs. Reproduced with permission from**.** D. Kuntz et al. PNAS 1999; 96: 9997-10002. Copyright (1999) National Academy of Sciences, U.S.A.

ing molecule does not violate drug-likeness criteria, e.g Lipinski rules, Veber rules, etc. Naturally, the chance of violating drug-likeness rules and compromising bioavailability increases as the number of distinct anchors, and thus molecular targets, rises. In addition to the potential bioavailability issues of this type of multi-ligand, one should also consider the associated binding efficiency metrics.

Back in 1999, an empirical study by Juntz *et al*. showed that, across a wide variety of small molecule-macromolecule complexes, maximal contributions to binding free-energy per ligand non-hydrogen atom are similar to −1.5 kcal/mol; they also noticed a significant trend to smaller contribution per atom as the molecular mass of the ligand increases (Fig. **4**) [53]. Interestingly, while during lead optimization the compound molecular weight usually increases, the average molecular weight for drugs in clinical development declines in each subsequent stage towards approval [54]. Apparently, normalizing the potency using the number of heavy atoms in the ligand or its molecular weight can be useful to assess the druggability of leads and targets. On the other hand, several studies suggest that ligand promiscuity is inversely related to molecular weight [52, 55, 56], with a trend to bind a higher number of targets for those compounds with molecular weight below 200 g/mol. All in all, there seems to exist an optimal ratio between potency and molecular size that provides and adequate balance between promiscuity and other pharmaceutically relevant features. In the case of the bianchor agents, one might speculate that, since only a fraction of the molecule takes part in the interaction between the ligand and each molecular target, efficiency metrics will tend to be low. Cleavable links are a possible solution to this issue: once inside the body, the link will be cleaved to release each individual anchor/pharmacophore. However, this approach will compromise some of the advantages of multi-target agents over drug combinations (in particular, simplified pharmacokinetics).

Multi-target agents in which the distinct pharmacophores are overlapped in a single moiety of reasonably low size may solve the binding efficiency and bioavailability issues of bianchor agents. Among these, the class that Sturn et al. named *flexible ligands* includes ligands which can adopt different conformations in the binding sites of the different targeted proteins; the same set of heavy atoms locates in the binding sites, but different atoms are involved in direct interactions with the targets [52]. It should be warned, though, that an excessive flexibility could conspire against the binding free energy owing to the entropy cost associated to the loss of conformational freedom. Some druglikeness rules also preclude the presence of a large number (e.g. > 10) of rotatable bonds. In a third category Sturn et al considered a class that they labeled as "difficult to rationalize", which consists in ligands that display a high overlay between the heavy atoms of the ligands located in the two binding sites and also share at least some of the atoms involved in direct interactions with each target. Though this situation may be expected for closely related targets with similarities at the binding sites, the authors signaled a subset of compounds that they named "super-promiscuous", which could bind to non-homologous targets and shared however some of the atoms involved in direct interactions with each of them. Remarkably, these superpromiscuous ligands tend to present low or high complexity.

Direct correlations have also been found between promiscuity and calculated logP [57, 58]. Bases and quaternary bases are markedly more promiscuous than acids, neutral compounds or zwitterions [58]. The molecular topology can also influence promiscuity: the number of rings and the fraction of molecular framework (fMF) have shown to be directly correlated with promiscuity at least for large fMF values [57, 58].

CONCLUSION

Multi-target agents present great therapeutic potential for the treatment of complex health conditions and the solution of drug resistance phenomena. However, they are certainly challenging for computer-aided drug discovery approaches. Possible multi-target designs include multi-anchor drugs (a different part of the molecules interacts with each of the targeted binding sites, separate pharmacophores) and partially or completely merged pharmacophores. The first could give rise to limited bioavailability and low binding efficiency. Overlapping or pharmacophores could be favored by including a certain degree of flexibility; excessive flexibility, however, can impact negatively on binding free energy due to unfavorable entropic contribution and it can also compromise drug bioavailability. Low complex and low molecular weight candidates, as wells as high complex ones, should be avoided owing to increase probability of promiscuity.

The designer should always contemplate the possibility that two selected targets could be mutually exclusive (incompatible pharmacophores) precluding the applicability of the merged pharmacophore approach. It is recommended to perform a careful, rational selection of the combination of pursued targets, preferring target combinations supported by co-evolution or similar biding sites.

SUPPLEMENTARY MATERIAL

Supportive/Supplementary material intended for publication must be numbered and referred to in the manuscript but should not be a part of the submitted paper. List all Supportive/Supplementary Material and include a brief caption line for each file describing its contents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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