From magic bullets to designed multiple ligands

Richard Morphy, Corinne Kay and Zoran Rankovic

Increasingly, it is being recognised that a balanced modulation of several targets can provide a superior therapeutic effect and side effect profile compared to the action of a selective ligand. Rational approaches in which structural features from selective ligands are combined have produced designed multiple ligands that span a wide variety of targets and target classes. A key challenge in the design of multiple ligands is attaining a balanced activity at each target of interest while simultaneously achieving a wider selectivity and a suitable pharmacokinetic profile. An analysis of literature examples reveals trends and insights that might help medicinal chemists discover the next generation of these types of compounds.

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With the introduction of *in vitro* biochemical assays thirty years ago, followed by technological advances in genomics and HTS, the drug discovery paradigm has gradually shifted from a 'black box' approach that relies on animal studies to the reductionist 'one-target-one-disease' philosophy of today. This approach of producing highly selective 'magic bullets' has provided notable successes, such as the selective cyclooxygenase 2 (COX-2) inhibitors that exhibit favourable efficacy and safety profiles. There is now an increasing realisation that modulating a multiplicity of targets can be an asset in the treatment of a range of disorders. Most multiple-action drugs in clinical use today were discovered serendipitously and their mode of action elucidated retrospectively. The deliberate and rational design of ligands that act on specific multiple targets is a more recent trend, as indicated by the substantial increase over the past few years in the number of publications describing such approaches. This increasing trend in research has already been reflected in a number of designed molecules that have reached a late stage of clinical development, for example, Omapatrilat [1], which is a dual angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP) inhibitor, and Netoglitazone, which is a peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ agonist [2].

The study of the history of non-steroidal anti-inflammatory drugs (NSAIDs) reveals an intriguing three-stage evolution that commenced with non-selective agents, such as aspirin (inhibits COX-1 and COX-2), then progressed through a selective stage (COX-2 inhibitors) and, more recently, moved towards designed multiple agents, for example, dual COX-2 and 5-lipoxygenase (5-LOX) inhibitors that might combine even greater efficacy with the reduced side effects of selective COX-2 inhibitors [3]. This evolutionary process has also been observed in other therapeutic areas, notably in psychiatry where several target-selective agents have failed to deliver sufficient efficacy in the clinic.

The atypical antipsychotic drug, Clozapine, displays an extremely complex *in vitro* pharmacology. A number of ligands that are selective for single receptors targeted by the drug were developed, including the dopamine-4 (D_4) receptor and 5-hydroxytryptamine-2a (5-HT_{2a}) receptor antagonists, but these compounds lacked sufficient clinical efficacy [4]. It is now widely accepted that activity at a single receptor is insufficient and recent research has focussed on ligands that have multiple activities, for example, ligands that act at both D_2- and 5-HT_{2a}-receptors [5,6]. The same trend is observed for antidepressants, from non-selective tricyclics (e.g. Amitriptyline), through selective serotonin transporter (SERT) inhibitors, to dual SERT and norepinephrine transporter (NET) inhibitors, which appear to combine a faster onset of action with increased efficacy [7].

For many years, clinicians have treated unresponsive patients by combining therapeutic mechanisms with a cocktail of drugs. Compared with drug combinations, there are several advantages associated with multiple ligands, such

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Table 1. Target combinations for 92 designed multiple ligands

Primary target	Other target(s)
Overlapping pharmacophores	
ACE	NEP (2); TxS (1); and ECE–NEP (2)
AChE	MAO (1)
$\alpha_4\beta_1$	$\alpha_4\beta_7$ (1)
Aromatase	STS (1)
AT_1	AT_2 (1) and ET_A (2)
COX-2	5-LOX (1)
D_2	D_4 (2); 5-HT_3 (1); β_2 (1); 5-HT_{2A} (1); and 5-HT_{1A} (1)
DHFR	TS (1)
FPT	GPT (1)
H_1	H_3 (1); NK_1 (1); and PAF (2)
H_3	HNMT (2)
5-HT_3	5-HT_{1A} (1) and α7-nAChR (1)
MMP	Cathepsin (1) and TACE (3)
μ	δ–κ (2); κ (2); and δ (1)
NEP	APN (1) and ECE (1)
NK_1	NK_2 (7)
Pgp	MRP_1 (1)
PKC	GSK3 (1)
PPAR-α	PPAR-γ (3) and PPAR-γ–PPAR-δ (1)
SERT	NK_1 (1); 5-$HT_{1D/B}$ (1); 5-HT_{1A} (5); α_2 (1); AChE (2); DAT (1); NET (1); and NET–DAT (1)
IIa	VIIa (1)
TxR	LTD_4 (1)
TxS	PAF (2); TxR (2); aromatase (1); and 5-LOX (3)
Conjugated pharmacophores	
ATIII	IIa (2)
A_1	A_3 (1)
APN	NEP (1)
COX	GR (1)
EGFR	DNA (1)
H_2	Gastrin (2)
μ	δ–κ (2)
NO release	Calcium channel (1); COX (1); GR (1); and H_3 (1)
NOS	Antioxidant (1)
PPAR-α	PPAR-γ–PPAR-δ (1)

The numbers in parentheses refer to the total number of examples of each particular combination of activities identified from the literature. Abbreviations: A, adenosine receptor; α_2, α_2-adrenoceptor; $\alpha_4\beta_1$, $\alpha_4\beta_7$, $\alpha_4\beta_1$, $\alpha_4\beta_7$ integrins; ACE, angiotensin-converting enzyme; AChE, acetylcholinesterase; α7-nAChR, α7-nicotinic acetylcholine receptor; APN, aminopeptidase-N; AT, angiotensin receptor; ATIII, antithrombin III; β_2, β_2-adrenoceptor; COX, cyclooxygenase; δ, δ-opioid receptor; D, dopamine receptor; DAT, dopamine transporter; DHFR, dihydrofolate reductase; ECE, endothelin-converting enzyme; EGFR, epidermal growth factor receptor; ET_A, endothelin-A receptor; FPT, farnesyl protein transferase; GPT, geranyl protein transferase; GR, glucocorticoid receptor; GSK3, glycogen synthase kinase-3; H, histamine receptor; HNMT, histamine *N*-methyltransferase; 5-HT, 5-hydroxytryptamine receptor; κ, κ-opioid receptor; 5-LOX, 5-lipoxygenase; LTD_4, leukotriene D_4 receptor; μ, μ-opioid receptor; MAO, monoamine oxidase; MMP, matrix metalloprotease; MRP_1, multidrug resistance protein-1; NEP, neutral endopeptidase; NET, norepinephrine transporter; NK, neurokinin receptor; NO, nitric oxide; NOS, nitric oxide synthase; PAF, platelet-activating factor receptor; Pgp, P-glycoprotein; PKC, protein kinase-C; PPAR, peroxisome proliferator-activated receptor; SERT, serotonin transporter; STS, steroid sulfatase; TACE, tumour necrosis factor-α-converting enzyme; TS, thymidylate synthase; TxR, thromboxane-A_2 receptor; TxS, thromboxane-A_2 synthase.

as the more predictable pharmacokinetic (PK) and pharmacodynamic (PD) relationship that is a consequence of the administration of a single medicine, as well as improved patient compliance.

The key aim of this article is to review the medicinal chemistry behind the rational design of multiple ligands; it is not our intention to assess the merits of single-target versus multiple-target approaches for any given disease. By discussing examples from the recent literature, we hope to challenge the common perceptions that multiple ligands cannot be rationally designed and that they are merely discovered accidentally. To analyse recent trends, a database of 92 published examples was compiled (Table 1). The database is intended to be representative rather than exhaustive, with the examples spanning 58 different target combinations and covering all the key target superfamilies. A common approach is to take a primary target that is well-validated for a given disease and add secondary activities to enhance efficacy and to reduce side effects. As a consequence of the difficulties encountered with the tracking of relevant articles, our literature analysis could be incomplete. Numerous terms are currently used to describe ligands that have multiple activities: the words dual, binary, bivalent, dimeric, mixed, triple or balanced are used in conjunction with numerous suffixes, for example, ligand, inhibitor, agonist, antagonist, conjugate or blocker. To improve communication and awareness of this emerging field within the drug discovery community, the authors propose using the term 'designed multiple' (DM) ligands as a generic phrase to describe compounds that are rationally designed to modulate multiple targets of relevance to a disease, with the overall goal of enhancing efficacy and/or improving safety. According to this definition, compounds that demonstrate significant activity at other targets, which are irrelevant to the disease, are

regarded as non-selective rather than designed compounds.

The molecular starting point for a multiple-ligand project is generated using one of two distinct approaches – either rational design by a combination of pharmacophores or the screening of compound libraries or known drugs.

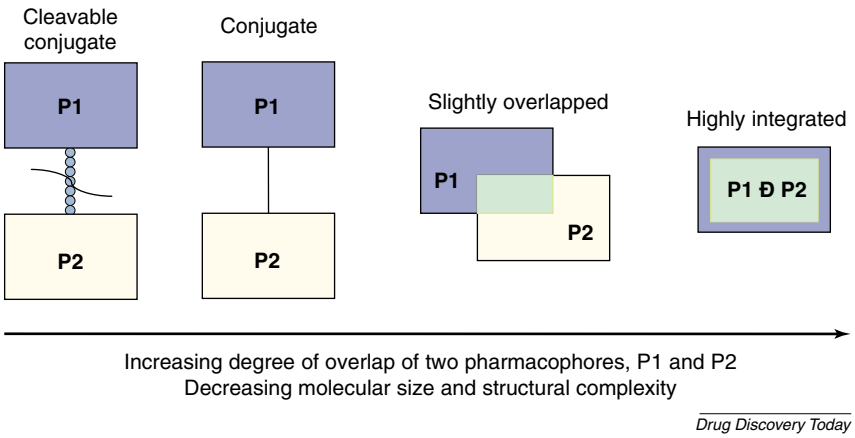


Figure 1. The degree of pharmacophore overlap varies significantly among 'designed multiple' ligands. There is a continuum from conjugates where the pharmacophores are well separated by a linker group to ligands where the pharmacophores are highly intermingled. Abbreviation: P, pharmacophore.

Pharmacophore combination approach

The methodical combination of pharmacophores from selective ligands is currently the predominant technique used for the generation of multiple ligands. The pharmacophores are joined together by a cleavable or non-cleavable linker (termed 'conjugates') or, more commonly, they are overlapped by taking advantage of structural commonalities ('overlapping pharmacophores') (Figure 1). The degree of pharmacophoric overlap forms a continuum, with high-molecular weight (MW) conjugates at one extreme and simpler molecules with highly integrated pharmacophores at the other. To integrate the pharmacophores, structural motifs that occur in both selective ligands are overlapped. Frequently, these 'consensus substructures' are hydrophobic or basic ring systems. A detailed knowledge of the structure–activity relationship (SAR) of the functionalities surrounding the motif to be overlapped is invaluable.

Cleavable conjugates

The majority of reported examples of cleavable conjugates contain an ester linker that is cleaved by plasma esterases to release two individual drugs that then act independently. Although the PK–PD relationship could become complex after cleavage of the linker, at the time of administration cleavable conjugates are a single medicine, which is one potential advantage that this approach has over drug cocktails. Several examples of cleavable conjugates contain a nitric oxide (NO)-releasing functionality that is linked to a known drug. For example, NO-Aspirin **(1)** (NCX4016) [8] and an Ibuprofen derivative **(2)** [9] are currently under investigation as anti-inflammatory agents (Figure 2). A disulfide-based cleavable conjugate **(3)** was reported to be analgesic via release of inhibitors of aminopeptidase-N (APN) and NEP [10].

Conjugated pharmacophores

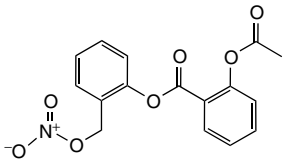
Buijsman *et al.* [11] designed a conjugate **(4)** in which a thrombin inhibitor is linked to a pentasaccharide inhibitor of antithrombin III-mediated Factor Xa (Figure 3). The structure of the co-crystallised thrombin inhibitor was used to identify a 'tolerant position' for the attachment of the linker that would not affect potency. The conjugate **(4)** provided a stronger and longer-lasting antithrombotic effect compared with a cocktail of the pentasaccharide and thrombin inhibitor. In addition, homodimeric opioid conjugates ('bivalent ligands') have been developed to improve potency and selectivity compared to simple monomers [12]. The bivalent ligand first undergoes univalent binding to one protein of a receptor homodimer, which produces an increase in positive entropy that leads to more facile association of the second pharmacophore to the other protein of the homodimer.

Overlapping pharmacophores

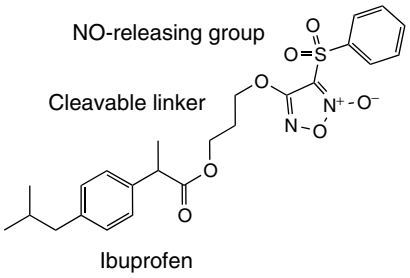
In their quest for a more efficacious antihypertensive agent, Murugesan *et al.* [13] developed a DM ligand for the angiotensin-1 (AT_1) receptor and the endothelin-A (ET_A) receptor. The design approach is based on the observation that the selective AT_1 receptor **(5)** and ET_A receptor **(6)** antagonists share a biaryl core (Figure 2). Further research identified that both the AT_1 receptor and ET_A receptor can accommodate an acylsulfonamide moiety at the 2-position of the biaryl and an imidazolinone group at the 4′-position. From this initial lead **(7)**, the introduction of a 2′-substituent produced **(8)**, which was found to have a balanced activity at the AT_1- and ET_A-receptors.

In other examples, the gap between monoamine and peptide G-protein-coupled receptors (GPCRs) has been successfully bridged. For example, a DM ligand for histamine-1 (H_1) and neurokinin-1 (NK_1) receptors has been synthesised

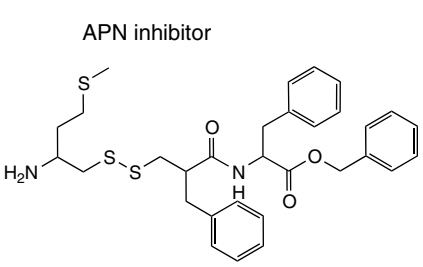
Cleavable conjugates



(1) NO-Aspirin (NCX4016)

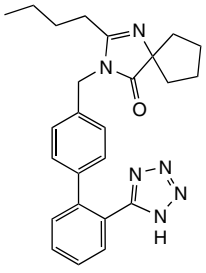


(2)

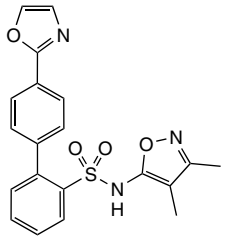


(3) NEP inhibitor

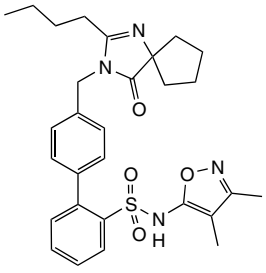
DM ligands for peptide GPCRs



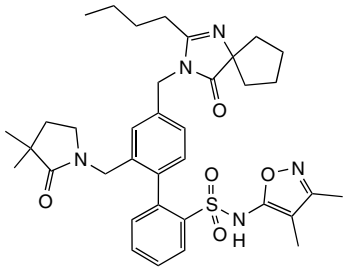
(5) Irbesartan
AT_1 K_i = 0.8 nM
ET_A K_i >10.0 μM



(6) AT_1 K_i >10.0 μM
ET_A K_i = 1.4 nM

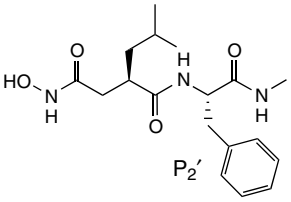


(7) AT_1 K_i = 4.7 nM
ET_A K_i = 39.0 nM

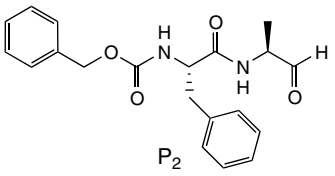


(8) AT_1 K_i = 6 nM
ET_A K_i = 1.9 nM
AT_1 and ET_B K_i >1 μM

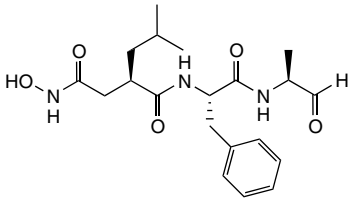
DM ligands for enzyme targets



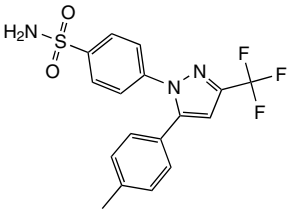
(9) IC_{50} = 3 nM (MMP-1)
IC_{50} = 1000 nM (Cat L)



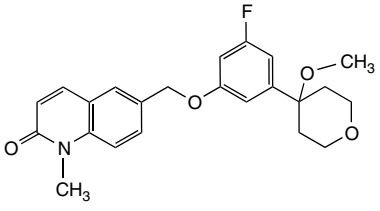
(10) IC_{50} = 1000 nM (MMP-1)
IC_{50} = 3 nM (Cat L)



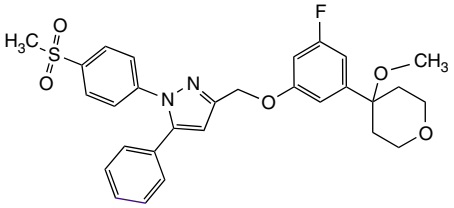
(11) IC_{50} = 25 nM (MMP-1)
IC_{50} = 25 nM (Cat L)



(12) Celecoxib



(13) ZD2138



(14) IC_{50} = 50 nM (COX-2)
IC_{50} = 3 nM (5-LOX)
IC_{50} >10 μM (COX-1)

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Figure 2. Cleavable conjugates and 'designed multiple' ligands for GPCR and enzyme targets. Abbreviations: APN, aminopeptidase-N; AT, angiotensin receptor; CAT-L, cathepsin L; COX, cyclooxygenase; DM, designed multiple; ET, endothelin receptor; GPCR, G-protein-coupled receptor; MMP, matrix metalloproteases; NEP, neutral endopeptidase; NO, nitric oxide.

[14]. DM ligands for H_1- and platelet-activating factor (PAF)-receptors have also been reported [15,16].

In addition, several DM ligands for zinc-dependent metalloproteases have recently been described, including triple inhibitors of endothelin converting enzyme (ECE), ACE and NEP [17], and dual inhibitors of matrix metalloproteases (MMPs) and tumour necrosis factor-α converting enzyme (TACE) [18,19]. The commonality between the P_2' and P_2 requirements for MMP-13 and cathepsin L inhibitors (both phenylalanine) permitted the combination of 'warhead' groups from **(9)** and **(10)** to provide a dual metallo- and cysteine-protease inhibitor **(11)** [20].

Synthesis of a compound incorporating functional groups from Celecoxib **(12)** and the 5-LOX inhibitor ZD23138 **(13)** generated an orally active dual COX-2 and 5-LOX inhibitor **(14)** [3]. Modelling studies suggest that the aryltetrahydropyran group interacts positively with several residues in the active site, rather than solely being tolerated by COX-2, which indicates that the pharmacophores are well-integrated in this DM ligand.

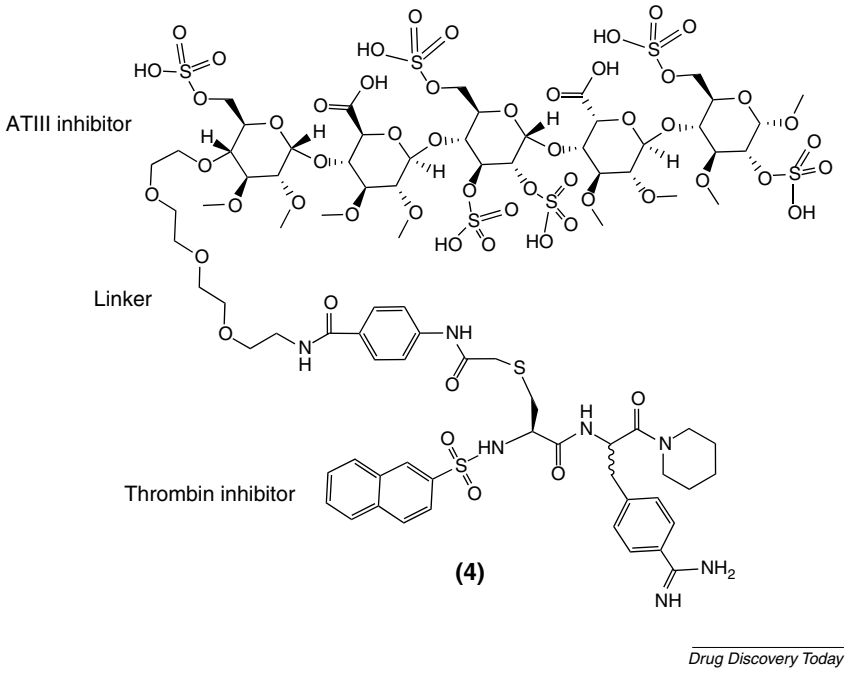

Figure 3. A high molecular weight conjugate in which a direct thrombin inhibitor is linked to a pentasaccharide fragment via a polyethylene glycol linker. Dual inhibition of thrombin and ATIII-mediated factor Xa produces potent antithrombotic activity. Abbreviation: AT, antithrombin.

Several examples show that it is even possible to cross the barriers between seemingly unrelated receptor superfamilies while still accommodating both pharmacophores within a single molecule. Kogen *et al.* [21] tackled an unusual enzyme-transporter combination in their design of a dual acetylcholinesterase (AChE) and SERT inhibitor for the treatment of Alzheimer's disease. A pharmacophoric model of the active site of AChE showed that Rivastigmine **(15)** (Figure 4) has three elements of the proposed pharmacophore, but crucially lacked a fourth hydrophobic site. By adding the lipophilic phenoxyethyl motif from Fluoxetine **(16)**, a balanced, but weak, dual inhibitor **(17)** was obtained. Conformational constraint produced **(18)**, which exhibited enhanced inhibitory potency against AChE and SERT targets. This work represents one of the rare examples published to date that uses biostructural information to guide the combination of pharmacophores.

Addition of an aryl sulfonamide group, which is present in the thromboxane-A_2 receptor (TxR)-selective antagonist **(19)**, transformed a selective thromboxane-A_2 synthase (TxS) inhibitor **(20)** into a DM ligand **(21)** [22]. In another example [23], the aim was to incorporate PAF receptor antagonism into the selective TxS inhibitor, Ridogrel **(22)**. The tolerance of a range of substituents on the phenyl ring led to this site being chosen as the site of attachment for the PAF antagonist E6123 **(23)**. The pharmacophores are bolted together to generate **(24)**, with an overlap of just one carbon atom.

Screening approaches

Although the screening of compound collections or known drugs has provided useful starting points, this appears to be a less common approach thus far than pharmacophore combination. This could potentially be due to a lower probability of the screening of compounds delivering suitable combinations of activities, or is perhaps due to the logistical complications of conducting multiple screens.

A random screen by Ryckmans *et al.* [24] produced a multiple ligand that had a surprising combination of activities at the NK_1 receptor and SERT and has potential as an antidepressant (Figure 5). Although the initial hit **(25)** had only modest activity, optimisation of each aromatic moiety in turn provided a more potent compound that had a balanced activity at the NK_1 receptor and SERT **(26)**.

In addition, researchers [25] have identified DM ligands for the AT_1- and ET_A-receptors from a focussed screen of compounds from an AT_1 programme. As discussed previously, a

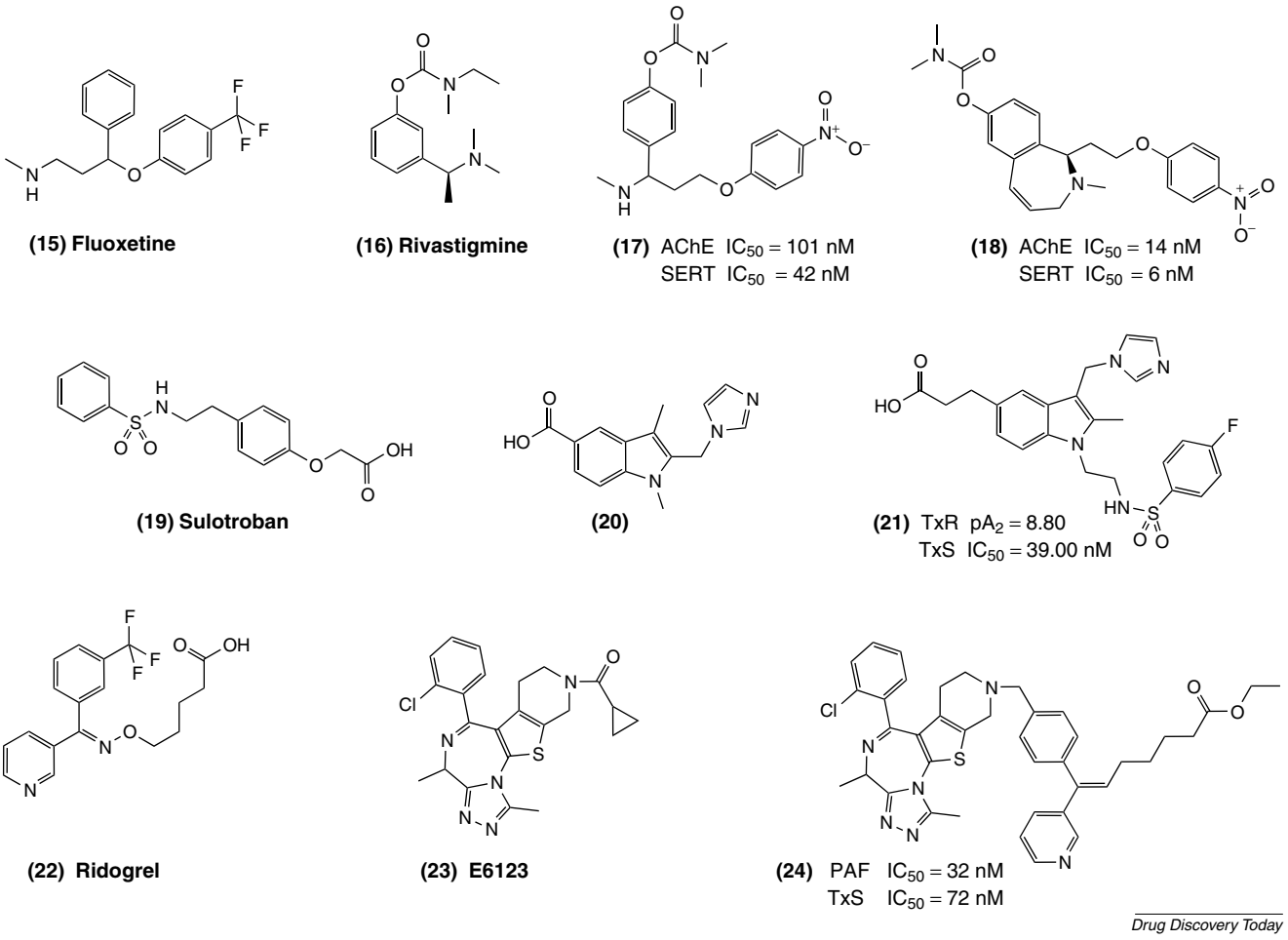

Figure 4. Designed multiple ligands can span target superfamilies. Enzyme and transporter activity was observed for **(18)** and enzyme and GPCR activity for **(21)** and **(24)**. Abbreviations: AChE, acetylcholinesterase; PAF, platelet-activating factor; SERT, serotonin transporter; TxR, thromboxane-A_2 receptor; TxS, thromboxane-A_2 synthase.

ligand with the same profile **(8)** (Figure 2) was identified by a pharmacophore combination approach, which indicates the complementarity of the rational and screening approaches to lead generation. Information derived from structure- and ligand-based modelling [26] has been used to design a library of lipophilic carboxylic acids for the identification of PPAR-γ and PPAR-δ dual agonists **(27)**.

Unexpected multiple activities can sometimes be found by revisiting well-known marketed drugs [27]. The 5-HT_3 antagonist Tropisetron **(28)** was also found to act at the α_7-adrenoceptor (α_7), which led to the discovery of a novel quinuclidine analogue **(29)** [28]. Celecoxib **(12)** was recently reported to potently inhibit carbonic anhydrase, as well as COX-2 [29]. Crystal structures of Celecoxib bound to carbonic anhydrase and COX-2 showed that, despite the different biochemical mechanism of action of these enzymes, the topography and physicochemical surface properties of the binding pockets were similar for the two enzymes. An algorithm, Cavbase, was developed to predict such cross-reactivity between unrelated proteins by searching for overlap in surface-exposed physicochemical features. By looking for similar cavities, such computational methods have great potential to help in the identification of target combinations for which DM projects are most feasible.

Discussion

Balancing of the activities

Balancing the activities of the lead compound is required such that each target is modulated to an appropriate degree *in vivo* at similar plasma or brain concentrations. In most examples, the aim has been to obtain *in vitro* activities that are within an order of magnitude of each other, presumably in an attempt to achieve similar levels of receptor occupancy *in vivo*. DM ligands with large differences in *in vitro* affinity could only act as multiple ligands *in vivo* at high doses. For example, the dual SERT and NET blocker,

Venlafaxine, is now regarded by some as more of a selective serotonin reuptake inhibitor, because the NET blockade does not occur until considerably higher doses of compound are used [7]. Newer drugs with a more balanced *in vitro* profile, such as Duloxetine [30], are now being evaluated as antidepressants. However, on occasion a larger difference in the *in vitro* activities might be preferred, for example, in cases where a different level of receptor occupancy for each target is associated with a desired pharmacological effect. Factors such as the distribution of the compound and receptor and/or enzyme densities in different tissues will also influence the optimal balance of *in vitro* activities. Feedback from the clinic is extremely useful in optimising the *in vitro* activity ratio for second-generation compounds. It has been postulated that the unique antipsychotic profile of Clozapine might be the result of a precise ratio of D_2- and D_4- receptor affinities; attempts have been made to reproduce this ratio in a DM ligand [31].

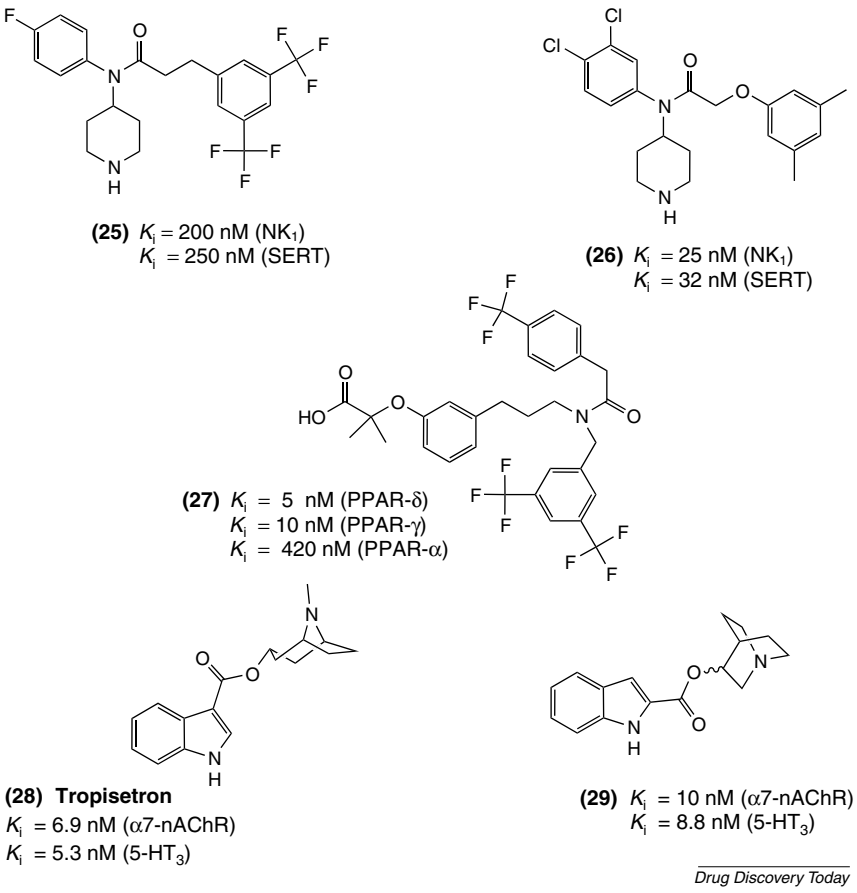

Figure 5. 'Designed multiple' ligands can be identified from the screening of random libraries [e.g. **(26)**], focussed libraries [e.g. **(27)**] or known drugs [e.g. **(29)**]. Abbreviations: 5-HT_3, 5-hydroxytryptamine-3 receptor; α7-nAChR, α7-nicotinic acetylcholine receptor; NK_1, neurokinin-1 receptor; PPAR, peroxisome proliferator-activated receptor; SERT, serotonin transporter.

Often the SAR does not transfer directly from the selective ligands to the DM ligand. It pays to keep an open mind while balancing the activities and not to be overly constrained by the selective-ligand SARs. For example, balanced TxS and TxR inhibition [22] for compound **(21)** was only achieved after extending the carboxylic acid and transposing the imidazole and methyl substituents on the indole core. The synthesis of libraries that include a somewhat more diverse range of compounds can be a useful strategy for accommodating these subtle shifts in the SAR. Frequently, the desired profile can be difficult to attain because of the stringent SAR requirements for one of the activities. In such cases, it is wise to prioritise hits and leads for which an activity, which is known to be difficult to modulate, is already at or close to its desired value. This also applies if knowledge of the SAR and pharmacophore requirements for one of the target activities is significantly less than that of the second target activity.

Endogenous ligands and target families

For 80 out of the 92 DM ligands, the individual endogenous ligands of the two targets are either identical or highly similar (e.g. another monoamine), and 76 involve targets from the same superfamily. Where the superfamily is different, the endogenous ligand is almost always similar and in most cases is identical (often a monoamine, such as 5-HT, or an eicosanoid such as thromboxane-A_2). In part, this trend is the result of a relationship between the endogenous ligand and a disease (e.g. serotonin and depression). Nonetheless, it is probable that a relationship also exists between the feasibility of multiple ligand design and the similarity of the targets, which can be measured in terms of either the endogenous ligand or their phylogenetic relationship. This is consistent with the common experience of medicinal chemists that achieving multiple activities for closely related targets can be straightforward, but achieving the required selectivity over other closely related targets is the real challenge. In some examples, the desired dual profile was achieved but related side activities could not be removed [32]. Notably, few publications discuss the

Table 2. Average physicochemical properties for oral drugs and designed multiple ligands

	MW	cLogP	PSA	H-Accs	H-Dons	Rotatable bonds
Oral drugs						
Non-CNS	348.0	2.5	64.0	4.6	2.0	5.8
CNS	304.0	3.0	37.0	2.9	1.1	3.9
DM ligands						
Overlapping DM	465.0	4.5	60.1	4.6	1.5	7.6
Monoamine GPCR	411.0	4.2	45.8	3.8	1.2	6.1
Peptide GPCR	583.0	5.7	61.5	5.3	0.8	8.6

Abbreviations: cLogP, calculated LogP; CNS, central nervous system; DM, designed multiple; GPCR, G-protein-coupled receptor; H-Accs, hydrogen bond acceptors, H-Dons, hydrogen bond donors; MW, molecular weight; PSA, polar surface area.

key issue of wider selectivity, so it is frequently difficult to judge whether true selectivity has been achieved. It cannot be categorically stated that all 92 known examples of DM ligands are strictly DM ligands (according to our definition) and some might be more non-selective than was intended by the authors. In one noteworthy example [33], dual agonism at D_2- and β_2-adrenoceptor (β_2) receptors was first achieved and then undesired activity at the α_1 receptor was eliminated.

Of the 92 DM ligands known, only two examples involve targets from different superfamilies that also have dissimilar endogenous ligands – TxS and PAF [23], and SERT and NK_1 [24]. This rarity is probably related to the difficulty of achieving multiple activities in a compact compound for highly dissimilar targets. The only realistic option could be conjugation of the pharmacophores through exploitation of tolerant positions in each component. For example, conjugation was the method adopted to combine the hydrophobic gastrin pharmacophore with the hydrophilic histamine-2 (H_2)-receptor pharmacophore [34].

Importantly, there are a few examples that show that it is possible to achieve the desired multiple activities, even for seemingly unrelated targets, while simultaneously obtaining selectivity over much more closely related targets. Notable examples are the AT_1- and ET_A-receptor dual ligand **(8)** (exhibits selectivity for these receptors over the AT_2- and ET_B-receptors), the AChE and SERT inhibitor **(18)** (exhibits selectivity over butyrylcholinesterase and NET) and the COX-2 and 5-LOX ligand **(14)** (exhibits selectivity over COX-1).

As yet, few examples of rationally designed multiple ligands have been reported for ion channels and kinases. Historically, it has proven difficult to identify truly selective ligands for members of these superfamilies and the rational design of DM ligands usually relies upon knowledge of the SAR from selective ligands. One exception is a dual inhibitor of protein kinase-C-β (PKC-β) and glycogen synthase kinase-3β (GSK-3β), which was found to be selective over a panel of other kinases [35].

Physicochemical and pharmacokinetic profiles

It might be expected that DM ligands would be larger and more complex than single target ligands. Indeed, the comparison of the MWs and other physicochemical properties of the 92 DM ligands with the same properties of known drugs highlights the considerably larger size and complexity of DM ligands (Table 2). Because larger and more flexible molecules have been associated with poorer PK profiles, optimising the pharmacokinetics of the lead compound while retaining a balanced target profile is frequently the most challenging aspect of a DM project [36,37]. Attempting to optimise too many parameters during lead optimisation will often end in failure, and thus there is an acute need for high quality DM leads. If the original selective ligands already suffer physicochemical liabilities, these are likely to be intensified in the resulting DM ligand. Thus, where possible, 'lead-like' templates are highly desirable [38].

The investigation of DM ligand metabolism is not only key for achieving good oral activity and duration of action, but is also important for determining the activity profile of any metabolites. Even if a metabolite is structurally similar to the parent molecule, its multiple profile could be significantly different. Usually, it is preferred that metabolites either share the activity ratio of the parent molecule or are inactive. There are several published examples where the multiple activities reside in different enantiomers, which necessitates the use of a racemate [39–41]. Over time, differences in metabolism between the two enantiomers could change the activity ratio. Ideally, all activities should reside in a single enantiomer.

Given that oral administration is usually desired, small and simple DM ligands in which the pharmacophores are highly merged are preferred. An analysis of the MW of DM

ligands based on the superfamilies that they target indicates that DM ligands for monoamine GPCRs have some of the most 'drug-like' properties (Table 2). This is consistent with the expectation that pharmacophores would be easier to integrate for more closely related targets, thereby producing a smaller molecule. On average, MW is much higher for multiple ligands that target peptide GPCRs. In part, this reflects a higher MW for the initial templates but also suggests that integrating peptide GPCR pharmacophores while minimising MW will often prove difficult. In the case of the DM ligand that is selective for the AT_1- and ET_A-receptor [13], a MW increase from 429 to 660 was required (relative to the selective AT_1 ligand). Nonetheless, this dual ligand was one of several high MW ligands that had good oral activity (rat oral bioavailability = 38%), which suggests that the desired PK profile could still be achievable. Other large DM ligands with good oral activity are an ACE and TxS inhibitor (MW 746) [42] and the TxS and PAF ligand **(24)** (MW 705) [22].

For highly dissimilar targets, DM ligands tend to be larger and more predisposed to a poorer PK profile. However, for intravenously administered peripherally-acting drugs, the use of conjugates is certainly a valid approach, as illustrated by conjugate **(4)** (Figure 3) that has a MW of 2351 [11].

Trajectories to DM ligands

The prevalence in the literature of 'designing in' new activities, that is the synthesis of DM ligands from selective ligands, indicates that this approach is certainly more popular, and probably more feasible, than 'designing out' activities from non-selective ligands. Either of the selective ligands can be used as the initial template. For example, Dickinson *et al.* [22] and Sakurai *et al.* [43] used TxS and TxR templates, respectively, to produce dual TxS and TxR inhibitors. The conversion of single or dual ligands to triple ligands is less common. For example, a δ-opioid ligand was transformed into a δ-, μ- and κ-opioid ligand [44] and an ECE, ACE and NEP inhibitor has been produced [45]. Furthermore, a NET and dopamine transporter (DAT) inhibitor was converted into a SERT, NET and DAT inhibitor [46]. Given the promiscuous nature of many 'old' but efficacious drugs, such as Clozapine, a 'designing out' strategy would be an attractive method of reducing adverse side effects. However, of the 92 DM ligands, there are only two examples where such a strategy has been implemented successfully (triple ligand to dual ligand) [31,47].

Functional activity

Achieving the desired balance of binding affinities does not on its own guarantee a successful project. Optimising functional activities can, in some cases, add further complexity to the task. Within a series of ET_A- and ET_B-receptor antagonists [48], the functional activity of the antagonists did not mirror affinity, with some compounds displaying a similar affinity at the two targets but much

Box 1. Ten aspirations for designing multiple ligands

Given the challenges that are associated with the discovery of 'designed multiple' (DM) ligands that have an optimised *in vitro* and *in vivo* profile, an assessment of project feasibility at an early stage is crucial. The following aspirations could be used to assess the probability of success for any given DM project:

(1) Similar targets: The feasibility of gaining multiple activities should be higher if the targets belong to the same superfamily and/or have a common endogenous ligand. However, because selectivity could then become a crucial issue, the number of undesired closely related targets should be low.

(2) Multiple lead generation approaches: To increase the probability of discovering high-quality leads, both pharmacophore combination and screening approaches should be considered.

(3) Clear *in vitro* and *in vivo* relationship: An understanding of the relationship between the ratio of *in vitro* activities, *in vivo* activity and the clinical profile will be invaluable.

(4) 'Designing in' activities: This approach is more common and probably easier than 'designing out' activities (a single ligand to dual ligand trajectory is the most common).

(5) The SAR should indicate the presence of 'tolerant regions' and 'consensus substructures' that could be overlapped. Consider prioritising DM hits or leads for which the 'most difficult to optimise' or 'least understood' activity (be it desired or undesired) is closest to its optimised value.

(6) Good physicochemical and pharmacokinetic properties for template molecules: Optimising multiple biological and physicochemical parameters during lead optimisation presents a major challenge.

(7) Common functional activities: Ligands having the same functional activity for each target are most common (particularly dual antagonism).

(8) Metabolites should have the same *in vitro* profile as the parent compound or be inactive.

(9) Ligand and protein models. To aid the design process, consider whether pharmacophore models can be built and/or whether biostructural information is available for each target.

(10) A single enantiomer should be responsible for all desired activities: Enantiomers could potentially have different pharmacokinetic properties. Thus, racemates could be more analogous to drug cocktails than single drugs.

weaker functional antagonism at the ET_A receptor. The literature analysis suggests that the probability of success is greatest if the same functional activity is required for each target. The most common profile by far is dual antagonism or inhibition. There are a few examples of multiple agonists, such as D_2- and β_2-agonists [33], triple opioid agonists [44] and triple PPAR agonists [49]. Exploration of the database of DM ligands indicates that there are no multiple agonists where either the endogenous ligand or the target superfamily is dissimilar, which suggests that this would be a difficult undertaking. One unusual example combines μ-opioid agonism with δ-opioid antagonism [50].

Privileged structures and targeted libraries

DM ligands, by their nature, contain substructural features and pharmacophores that are relevant to multiple targets and they are therefore likely to provide useful information for the construction of screening libraries. The number of multiple ligands that 'cross-over' target superfamilies strongly suggests that there is substantial overlap in the chemistry space for the different target superfamilies. Rather than being islands in chemical space, the target superfamilies share significant commonalities in terms of molecular recognition that facilitate the discovery of DM ligands. Pharmacophores and 'privileged' structures that span superfamilies should form a good basis for a general purpose screening library. A number of the 92 DM ligands contain privileged substructures. It is tempting to speculate that choosing a starting template that contains a privileged substructure, such as biaryl [13,44,51–53], will facilitate the rational design of a ligand that is capable of binding to two or more targets.

Conclusion and future trends

The increasing number of publications that describe DM ligands could suggest an ongoing re-evaluation of the 'one-disease-one-drug' paradigm that has dominated thinking in the pharmaceutical industry for the past few decades. Although the likelihood of switching back to an animal-centric approach is minimal, it is now widely recognised that high-specificity for a single target might not always deliver the required efficacy versus side effect profile. Perhaps we are entering an exciting new phase in the history of medicinal chemistry that requires a fundamental shift away from a 'one-compound-one-target' mind-set. If so, real challenges lie ahead for the medicinal chemist, notably the balancing of *in vitro* and *in vivo* activities in concert with optimising the PK and safety profiles. DM projects can be resource intensive in terms of both chemistry and biology and so a careful evaluation of the pharmacological rationale and the feasibility of the chemical plans should be carried out (Box 1). The discovery and validation of novel target combinations will be a crucial factor for success, and exciting new strategies are emerging [54]. Although many of the examples of today of 'mixing and matching' substructures and pharmacophores are intriguing, they will undoubtedly come to be seen as simplistic and should be viewed as just the beginning of the long road ahead. There is huge potential for a greater use of biostructural information and pharmacophore modelling to facilitate the 'designing in' of desired multiple activities and, just as importantly, the 'designing out' of undesired side activities.

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