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Most pharmacologically promiscuous compounds share defined molecular properties and characteristic structural motifs; they can be identified at the outset of a drug discovery program.

Can we discover pharmacological promiscuity early in the drug discovery process?

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The term 'pharmacological promiscuity' describes the activity of a single compound against multiple targets. When undesired, promiscuity is a major safety concern that needs to be detected as early as possible in the drug discovery process. The analysis of large datasets reveals that the majority of promiscuous compounds are characterized by recognizable molecular properties and structural motifs, the most important one being a basic center with a pK_a(B) > 6. These compounds interact with a small set of targets such as aminergic GPCRs; some of these targets attract surprisingly high hit rates. In this review, we discuss current trends in the assessment of pharmacological promiscuity and propose strategies to enable early detection and mitigation.

Industrial drug discovery aims at identifying drug candidates with the highest possible chance of completing clinical trials, reaching the market, and establishing themselves as efficacious and well-tolerated, safe medicines. Such drug candidates require a balance of favorable pharmacological, pharmacokinetic and physicochemical properties. The absence of unintended pharmacological promiscuity, that is, the absence of interactions with non-therapeutic 'off-targets', is one important aspect of that balance. Pharmacological promiscuity can lead to adverse drug reactions (ADRs) [1–4] and has been linked to preclinical findings of toxicity [5]. ADRs and animal toxicity account for 30% of all drug candidate termination [6], and the proportion of promiscuous compounds decreases with advancing clinical development [7]. Off-target-activity-related ADRs have been reasons for the injury or death of patients, or poor patient compliance, leading to market withdrawals or competitive disadvantages [1–3].

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Thus, many contemporary drug discovery programs aim to avoid pharmacological promiscuity, even though it has been noted that many successful drugs are not highly selective for a single target [8–10]. It is however increasingly being appreciated that promiscuity across a network of several therapeutic targets may be required for efficacy in polygenic diseases, or when drug resistance plays a role [11–14]. Some recently approved drugs are therefore deliberately designed to be promiscuous across a target family, such as GPCRs (e.g. asenapine [15]), kinases (e.g. sunitinib [16]) or ion channels (e.g. dronedarone [17]) (for pharmacological profiles, see references). In such cases, a 'targeted' promiscuity is desired, whereas promiscuity for non-therapeutic targets with deleterious effects ('antitargets') must be avoided (for a more thorough discussion and case studies, see Refs. [11,18]).

The mainstay of pharmacological promiscuity assessment is the screening against large panels of safety-relevant targets. Due to the high costs and limited throughput, such screening is usually performed only for a small number of proof-of-concept compounds, clinical candidates or other key compounds at an advanced stage of lead optimization. A finding of safety-relevant off-target activities at this point usually requires considerable modifications of an already optimized compound, or can even be a reason for late attrition. It is therefore highly desirable to assess promiscuity earlier in the drug discovery process, for example, during hit selection after an HTS, or in the hit-to-lead phase. At this stage, pharmacological profiles could be a criterion for the prioritization of lead series, or a matter for early optimization.

Studies investigating the dependence of promiscuity on molecular properties [7,19–21] and chemical structure [22–24] have therefore attracted considerable interest in recent years. Insight into property–promiscuity relationships can lead to an early recognition of potentially promiscuous compounds, or can be useful for their optimization. We have recently analyzed the BioPrint® dataset [25] (Box 1) in a collaboration between Cerep Inc. and F. Hoffmann-La Roche Ltd. (Roche), to identify associations between compound properties and promiscuity, and to investigate opportunities for a cost- and time-efficient assessment of early lead compounds. In this review, we discuss our findings and summarize, in parallel, the results reported by previous studies. The discussion focuses on unintended off-target activities encountered in typical safety panel screening; we do not discuss computational approaches to predict off-target activities, promiscuity across narrow target families, or 'DMPK antitargets' (CYPs, CYP inducers and efflux transporters).

BOX 1

Definitions and terms used throughout this review

The BioPrint® dataset (provided by CEREP) is a nearly complete matrix of 2413 drugs and drug-like compounds, screened against 141 safety-relevant targets (ten of them being CYP 450 enzymes, which were omitted in our study). The majority of compounds (~54%) are drugs which are marketed in the US and other countries for a variety of disease areas (cardiovascular and metabolic diseases, infections, CNS disorders [such as psychosis, depression, and other], pain/inflammation, oncology, respiratory diseases, and other diseases). They are complemented by reference compounds (~36%) chosen for diversity and biological activity, and smaller numbers of withdrawn drugs, metabolites, prodrugs, herbal or nutritional actives, veterinary drugs and development compounds [25]. The screening panel contains a diverse range of targets, such as aminergic GPCRs (~19%), peptide GPCRs (~18%), other GPCRs (~9%) ion channels (~12%), proteases (~6%), kinases (~6%), other non-CYP enzymes (~6%), nuclear receptors (~4%), amine transporters (~2%), and other targets (the hERG channel is not part of the BioPrint® dataset).

Pharmacological promiscuity describes the activity of a single compound at multiple pharmacological targets. Within this review, a compound with a hit rate >5% at the BioPrint® panel is classified as 'promiscuous', whereas a compound which hits not more than one target is considered as 'selective'.

The hit rate of a compound is the percentage of targets across the whole BioPrint® panel (excluding CYP 450 enzymes), which are 'hit' by this compound, that is, which bind the compound with a $pIC_{50} > 6$ in a radioligand displacement assay. For instance, a compound with a hit rate of 5.3% has sub-micromolar affinity for 7 (~5.3%) out of the 131 (non-CYP) BioPrint® targets.

In contrast, the target hit rate counts the percentage of hits that are attracted by a single target from a set of compounds. For instance, the 5-HT_{2B} receptor has, in the BioPrint® dataset, a target hit rate of 14%, which means that 327 (~14%) of the 2399 compounds, which were tested at this receptor, bind to it with sub-micromolar affinity.

Ionization constants (cpK_a) were calculated using the commercial prediction tool, MoKa. Ionization states were assigned according to cpK_a values: For instance, a compound with a basic center, and a $cpK_a(B) > 7$ will be predominantly protonated at *p*H 7 (approximately the assay and physiological *p*H), and is therefore classified as 'positively charged'. Similarly, acids with a $cpK_a(A) < 7$ are 'negatively charged', compounds with both, acidic and basic centers, and a $cpK_a(B) > 7$, and a $cpK_a(A) < 7$ are 'zwitterionic'. Neutrals carry no, or an only weakly basic or acidic center, with a $cpK_a(B) < 7$, or a $cpK_a(A) > 7$. Importantly, weakly basic compounds classified as 'neutral' can still form a positively charged fraction at pH 7, which may contribute to the aminergic activity of some neutrals. Compounds carrying a quarternary ammonium ion are classified as 'permanently positively charged compounds' (and are not included in the class of 'positively charged compounds').

Molecular properties associated with pharmacological promiscuity

Lipophilicity is generally recognized as an important determinant of promiscuity, and all published property–promiscuity analyses, irrespective of their dataset, do indeed report that promiscuity increases with lipophilicity. An earlier analysis of the BioPrint® dataset, for example, shows that the median promiscuity increases with increasing *c* log *P* for basic, neutral, and acidic compounds [20]. We found that marked promiscuity (hit rates >5%, for definition see Box 1) is rarely observed for *c* log *P* values below 1 (Fig. 1a) [26]. Similarly, Roche and Pfizer found that maximum promiscuity among their project compounds was observed for a threshold *c* log *P* higher than 2.5–3 [19,21]. These findings add to the crucial importance of lipophilicity as a lead optimization parameter; however, the large variability of the data also suggests that lipophilicity alone is not generally useful to evaluate or to optimize the selectivity of individual compounds, even though this may have been successful in certain cases [27].

On the other hand, it is obvious from Fig. 1a that almost all promiscuous compounds bear a positive charge, that is, they are strong bases that will be protonated at pH 7, or are quarternary ammonium ions. This is in line with an analysis of the Roche project compounds [21] and an earlier analysis of the BioPrint®

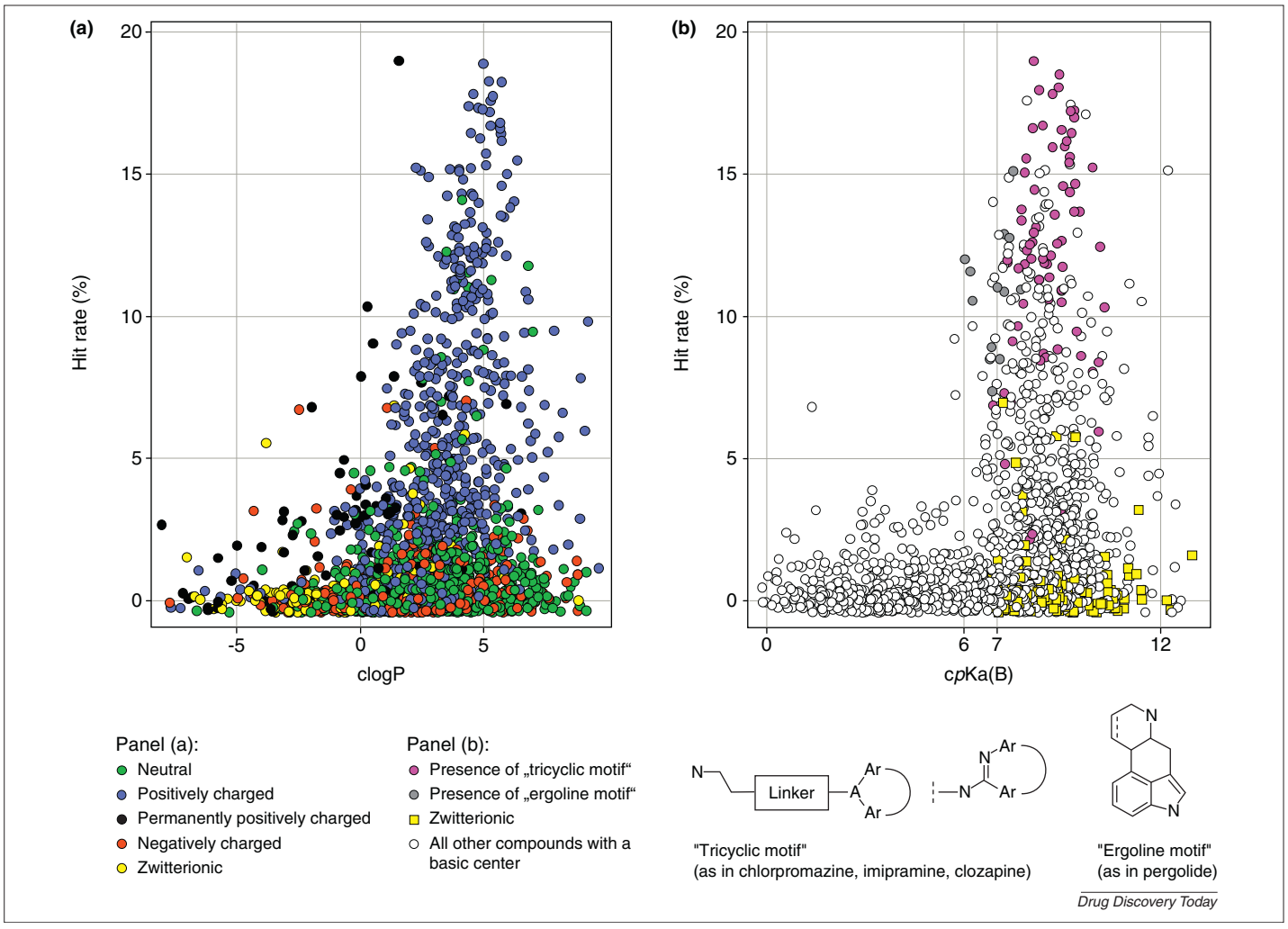

FIGURE 1

Dependence of hit rates on molecular properties and structural motifs. Panel (a): the large majority of promiscuous compounds is positively charged and has a *c* log $P > 1$. Panel (b): the promiscuity of basic compounds increases sharply above a threshold of $cpK_a(B) > 6$, indicating that a positive charge under assay conditions (rather than simply a basic center) is responsible for the increased promiscuity potential. Compounds with certain 'aminergic' motifs (e.g. 'tricyclic motif', 'ergoline motif'; [A = any atom]) are in almost all cases promiscuous, whereas compounds with an additional negative charge (i.e. zwitterions) have a reduced propensity to be promiscuous. Data points are jittered (randomly displaced for small distances) for better visibility.

data [20]. To further investigate this observation, we plotted hit rate versus basicity for all compounds with a basic center (Fig. 1b). Promiscuity sharply increases above a threshold $cpK_a(B)$ of 6, that is, for compounds which are at least partly protonated under assay conditions (and physiological conditions). This indicates that it is indeed a positive charge, rather than merely the presence of a basic center, which leads to an increased promiscuity potential. (It can be observed from Fig. 1b that many compounds in the $cpK_a(B)$ range of 6–7 are promiscuous. Although such compounds are not predicted to be predominantly positively charged, their observed promiscuity can still be attributed to a positive charge, for two reasons: First, a small fraction of these compounds is protonated under assay conditions, and this positively charged fraction may be a cause of promiscuity. Second, the standard error of estimate (SEE) for the cpK_a prediction tool, MoKa, was found to be up to 0.5 log units [depending on the dataset] in a benchmarking study [28]; therefore, some compounds with a cpK_a just below 7 may have an actual $pK_a > 7$, and may thus be predominantly positively charged. Hence, a $cpK_a(B) > 6$ is chosen as a cutoff for the recognition of potentially promiscuous compounds.)

To investigate which other molecular properties may further increase the propensity for promiscuity of positively charged compounds, we binned the compounds of the dataset according to molecular property ranges and ionization states, and calculated average hit rates (as a measure of promiscuity) for each property bin. As may be expected, the promiscuity of positively charged compounds increases with increasing lipophilicity, reaches a maximum at *c* log *P* 5–6, and decreases slightly for very lipophilic compounds (Fig. 2a). The same pattern was observed in a previous analysis of the BioPrint® dataset [20]. The promiscuity decrease for highly lipophilic compounds may be due to insufficient solubility, and thus erroneous assay results (although it has been previously noted that poor solubility does not seem to substantially affect panel screening outcomes [21]). Neutral and negatively charged compounds show a tendency for increased hit rates with increasing lipophilicity, albeit on a much lower level.

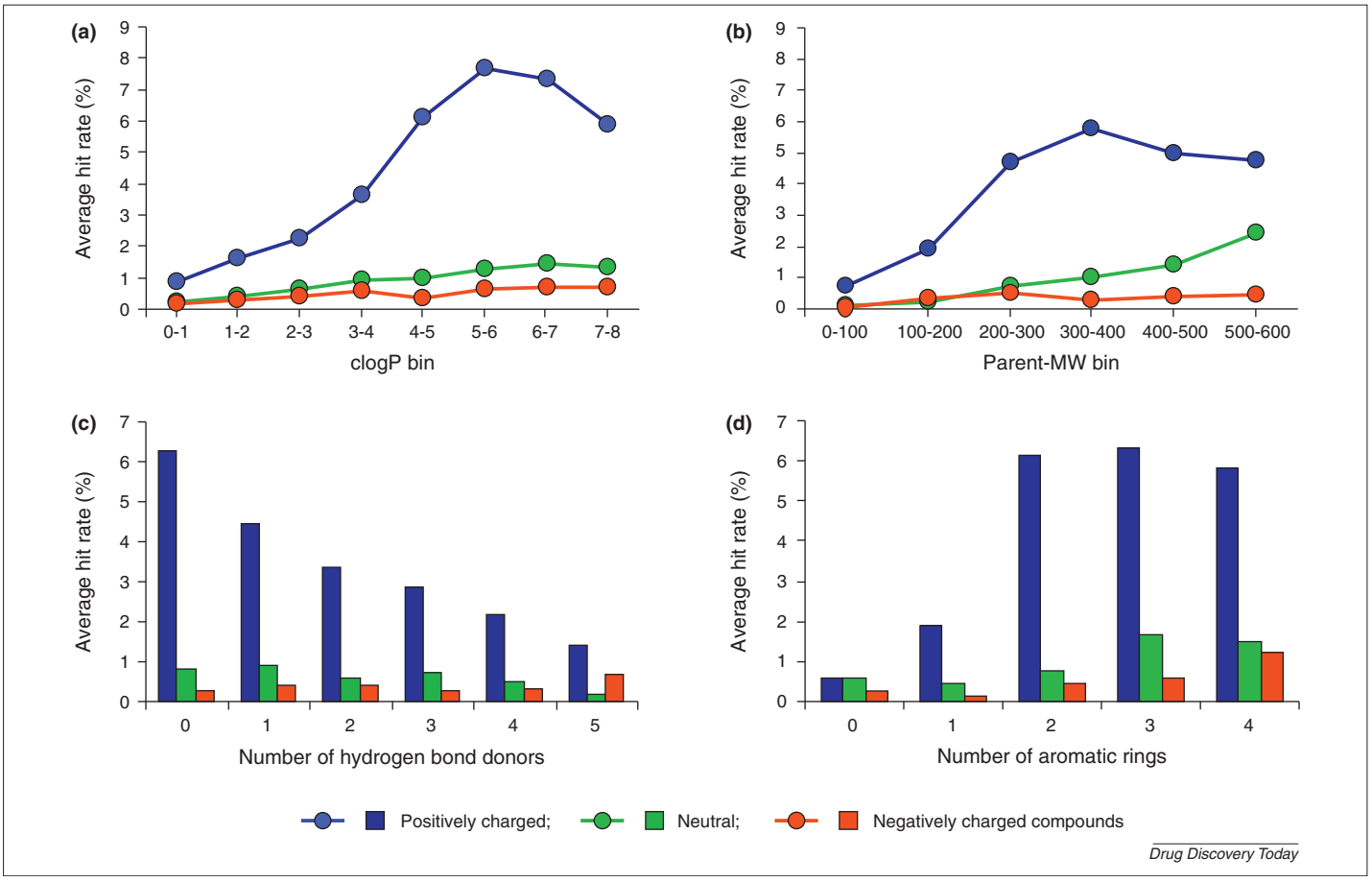

FIGURE 2

Dependence of hit rates on ionization states and molecular properties. Among positively charged compounds, hit rates increase with increasing lipophilicity (up to *c* log *P* bin 5–6), and a decreasing count of hydrogen bond donors (Panels a and c). Positively charged compounds with more than one aromatic ring are particularly liable for promiscuity (Panel d); hit rates are similar across the drug-like MW range (MW 200–600, Panel b). Trends for neutral and negatively charged compounds are less pronounced, with low average hit rates across the data ranges (shown are averaged hit rates of binned data; for illustration, the topmost data point in Panel A represents all positively charged compounds with a *c* log *P* between 5 and 6, which have an average hit rate of 7.6%).

Based on theoretical considerations, it is commonly assumed that molecular weight (MW) is an important determinant for promiscuity. For instance, MW can be considered as a surrogate for molecular complexity, and, as systems become more complex, the chances of a randomly chosen ligand–protein interaction should decrease [29,30]. Surprisingly, analyses of different datasets have not given consistent results: within the Pfizer HTS data, smaller MW led to increasing promiscuity, whereas within the Novartis safety profiling data and the Roche HTS data of drug-like compounds, the average MW was higher for promiscuous compounds than for selective ones. Within the BioPrint® dataset, positively charged compounds have, on average, similar hit rates across the drug-like MW range of 200–600 (Fig. 2b). A similar plateau was observed in a previous analysis of BioPrint® data for basic compounds [20]. In our analysis, the hit rate for neutral compounds increases with increasing MW.

Also, the promiscuity of positively charged compounds in the BioPrint® dataset increases with a decreasing count of hydrogen bond donors (HBD, Fig. 2c), and hydrogen bond acceptors (HBA, not shown), whereas the influence of HBD and HBA appeared to be insignificant in an analysis of Novartis safety profiling data [7], and in the Roche project and HTS data [21,31] (across all ionization states). Perhaps practically most useful, it was found that positively charged compounds with two or more aromatic rings tend to be promiscuous, whereas positively charged compounds with no or only one aromatic ring are, on average, rather selective (Fig. 2d). Lipophilicity usually correlates roughly with the number of aromatic rings, and correlates inversely with HBD and HBA, and the observed trends may reflect these interconnections. In our analysis, we did not find convincingly clear correlations between promiscuity and other molecular descriptors, such as polar surface area (PSA) or number of rotatable bonds. Promiscuity did however show a strong correlation with lower topological PSA in a set of Pfizer project data, in particular for compounds with a high *c* log *P* [5]. Promiscuous compounds within sets of Novartis safety profiling data [7], and Roche HTS data [31], contained above-average numbers of rotatable bonds.

Frequently hit targets

To investigate which targets are most frequently hit, especially by compounds with molecular properties that predispose them to promiscuity, we sorted the BioPrint® targets into main target classes and determined their average target hit rates (Table 1). Aminergic GPCRs attract by far the highest hit rate (5.6% overall),

TABLE 1

Average target hit rates for main target classes

Average hit rates (%)	Aminergic GPCRs (27)	Peptide GPCRs[a] (22)	Ion channels (17)	Proteases (9)	Kinases (9)	Nuclear receptors (5)
Total	**5.6**	0.52	**1.3**	0.2	0.5	**1.7**
Positively charged compounds	**15**	0.63	**2.8**	0.04	0.2	0.1
Permanently positively charged compounds	**7.5**	0.46	**1.9**	0.4	0	0
Neutrals	**1.1**	0.55	0.7	0.2	0.3	**3.9**
Negatively charged compounds	0.056	0.18	0.06	0.1	0.6	0.3
Zwitterions	0.89	0.15	0.15	0.9	0.9	0
Positively charged compounds with ≥2 arom. rings	**22**	0.98	**4.2**	0.08	0.4	0.2
Positively charged compounds with a *c* log *P* > 3	**21**	0.76	**4.7**	0.06	0.3	0.2

Among the main target classes, aminergic GPCRs attract the highest hit rates. For illustration, each compound of the BioPrint® dataset has, on average, a 5.6% chance of hitting any one of the 27 aminergic GPCRs in the panel (row 1, column 1). This increases to a 15% chance, if the compound carries a positive charge, and to >20%, if the positively charged compound has in addition two or more aromatic rings, or a *c* log *P* > 3 (rows 2/7/8, column 1). A similar trend, albeit with lower target hit rates, is observed for ion channels. Peptide GPCRs, proteases and kinases feature low hit rates. A significant proportion of steroids in the dataset may explain the high hit rates at nuclear receptors. Not covered are transporters of biogenic amines, and opioid receptors, which also attract high hit rates from positively charged compounds. High target hit rates are marked in bold.

[a] Without opioid receptors.

with an average target hit rate of 15% from positively charged compounds. The target hit rate at aminergic GPCRs increases to more than 20% for positively charged compounds, which are also lipophilic (*c* log *P* > 3) or have two or more aromatic rings. Transporters of biogenic amines, and the σ and the opioid receptors are not covered in Table 1, but also attract high hit rates, especially from positively charged compounds.

A closer inspection revealed that some individual aminergic targets exhibit surprisingly high hit rates (Fig. 3): for instance, the 5-HT_{2B} receptor interacts with more than one third (36%) of all positively charged compounds, and with even more than half of all positively charged compounds with 2 or more aromatic rings (52%), or with a *c* log *P* > 3 (54%). Other serotonergic receptors, as well as the adrenergic α-receptors and some dopaminergic receptors, attract similarly high hit rates in the 20–30% range from positively charged compounds. Analogous results were obtained from Novartis [32,33] and the Roche safety profiling data [21], where serotonergic GPCRs, and in particular the 5-HT_{2B} receptor, had very high hit rates.

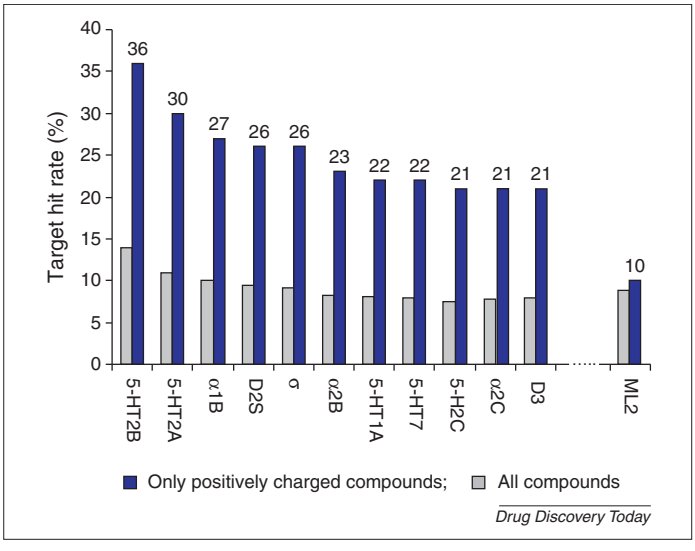

FIGURE 3

Target hit rates of the most frequently hit targets. Some aminergic GPCRs and the sigma receptor attract very high hit rates from positively charged compounds. For instance, 36% of the positively charged compounds (and 14% of all compounds) bind to (hit) the 5-HT_{2B} receptor with sub-micromolar affinity. The ML_2 receptor (far right) is the only receptor in the BioPrint® panel with a high target hit rate, and no preference for positively charged ligands.

The high promiscuity of positively charged, aromatic and lipophilic compounds across aminergic and opioid GPCRs may be explained by the similarity of the binding sites of these receptors: In all of these GPCRs, a highly conserved aspartate residue on TM3 serves as a counter-ion for the ligand's positively charged center, whereas other conserved regions of the binding sites retain a predominantly hydrophobic and aromatic character [34].

The somewhat increased hit rates of ion channels (Table 1) are mainly due to high hit rates of two individual sodium and calcium channels. The hit rates attracted by nuclear receptors, especially from neutral compounds, can be traced back to the presence of a significant portion (2.6%) of steroids in the BioPrint® dataset, and may thus be a peculiarity of the dataset. The melatonin receptor type 2 (ML_2) is the only target with a high hit rate, but no preference for positively charged ligands. Peptide GPCRs, proteases, and, somewhat surprisingly, kinases attract low hit rates of less than 1% from compounds of all ionization states. This last observation suggests that randomly chosen compounds rarely pick up kinase activity, despite the frequently observed promiscuity of kinase inhibitors. However, the 9 kinases in the panel attract a high average target hit rate of ~19% from the 41 compounds in the dataset, which bind to at least one other kinase.

The dominant source of promiscuity in the BioPrint® dataset is clearly the affinity of positively charged compounds for targets with a preference for positively charged ligands: affinity measurements (pIC_{50} determinations) of positively charged compounds on 'frequently hit' targets (aminergic GPCRs, amine transporters, opioid and σ-receptors, and the sodium channel site 2) represent only 12% of all data points in the BioPrint® dataset, but they account for 70% of the hits. It may be argued that this dominance

of 'aminergic' interaction is an artifact of the BioPrint® dataset (19% of the BioPrint® panel targets are aminergic GPCRs), but any typical safety screening panel will contain a similar numbers of aminergic targets.

Aminergic activities also play an important role in the promiscuity of clinically used drugs. Drug-target network analyses have shown that aminergic receptors, such as the histamine H_1, the cholinergic M_1, the α_{1A} adrenergic and the dopamine D_2 receptor, bind the highest number of FDA-approved drugs [35]. Drugs targeting aminergic GPCRs showed the most promiscuous pharmacological profiles in an *in silico* profiling study [36]. In an analysis of cardiovascular targets and ligands, aminergic GPCRs displayed the highest degree of cross-pharmacology, with the serotonin 5-HT_{1A} and 5-HT_{2A}, dopamine D_3 and α_{1D} adrenergic receptors sharing a particularly high number of common ligands with other targets [37].

Structural motifs and promiscuity

It has long been recognized that basic, secondary or tertiary amines, which are connected by a two to five atom linker to an aromatic ring, form the prototypical pharmacophore of many GPCR and ion channel ligands [38,39]. Such motifs are also particularly liable to be promiscuous: 79% of all positively charged compounds with a hit rate higher than 5% contain such a motif in our BioPrint® dataset. Some specific motifs, such as the 'tricyclic motif' and the 'ergoline' motif (Fig. 1) form the core motifs of several polypharmacological drugs and lead almost invariably to pharmacological promiscuity. Similarly, indole and piperazine motifs, which are found in many (promiscuous) aminergic ligands, were over-represented among the promiscuous compounds of the Novartis safety profiling data [7]. Within the Roche safety profiling data, compounds from projects with an aminergic therapeutic target are more promiscuous than others [21], which again suggests that the presence of 'aminergic motifs' contributes to promiscuity.

Privileged structures such as spiropiperidines, benzodiazepines, or dihydropyridines have been exploited in the design of GPCR and ion channel ligands, and may confer some degree of cross-pharmacology [40–42].

A separate chemogenomical space is covered by the hinge-region-binding kinase inhibitors [43,44]. Such compounds show often promiscuity across many kinases, with little or no activity at other targets. Also, specific functional motifs frequently lead to promiscuity across smaller target families, such as metalloproteases [45,46], or carbonic anhydrases [47].

Some structural motifs may also reduce promiscuity liabilities. For instance, an additional negative charge in the presence of a positive charge, that is, a zwitterionic structure, seems to prevent the promiscuity associated with positively charged compounds in most cases (BioPrint® dataset, Fig. 1b): only 2% of the zwitterionic compounds are promiscuous (with hit rates of >5%), as compared with 32% of the positively charged compounds. Ninety percent of the zwitterions are selective (hit not more than one target), compared to only 34% of the positively charged compounds. Thus the promiscuity of zwitterionic compounds is comparable to that of neutral compounds with 2% being promiscuous and 81% being selective. Similarly, the carboxylic acid function was identified as a key selectivity feature which 'virtually ruled out' promiscuity in Novartis project compounds [7].

Implications for drug design

A basic center with a $cpK_a(B) > 6$ is the most important determinant of promiscuity in typical safety panels. The necessity of any basic center must therefore be carefully considered, though it may prove necessary or desirable in many cases: throughout the history of drug discovery, more oral drugs with a basic center have been brought to the market than neutral or acidic ones [48]. A positive charge may be part of the pharmacophore, for example, if the therapeutic target is an aminergic GPCR. It should be noted, however, that neutral ligands with a reduced liability for polypharmacology may be found for such targets, as exemplified by neutral antagonists of the serotonin 5-HT_{2C} [49] and 5-HT_6 receptors [50]. In other cases, a positive charge may not be avoidable (e.g. basically all DPP-IV inhibitors carry a positive charge [51]), or a positive charge may be desired for other reasons, for example, to improve a compound's physicochemical and pharmacokinetic properties (e.g. solubility, brain penetration). In such cases, promiscuity liability can be reduced by having not more than one aromatic ring or, perhaps less practicably, by substantial reductions of lipophilicity, or the introduction of multiple hydrogen bond donors and acceptors. Adding an additional negative charge, that is, the generation of a zwitterion, may be a particularly effective method for the reduction of 'aminergic' promiscuity, if this is compatible with activity at the therapeutic target. The withdrawal of the antihistamine, terfenadine, and its replacement by fexofenadine [52] may illustrate this point: the positively

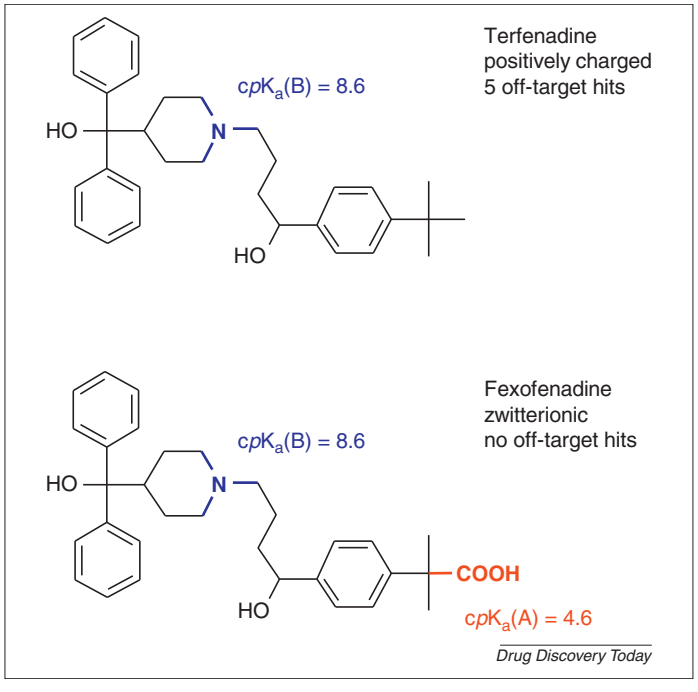

FIGURE 4

Comparison of the antihistamines, fexofenadine and terfenadine. A high propensity for promiscuity is observed for positively charged compounds, but not for zwitterions. Fexofenadine and terfenadine are a point in case: The zwitterionic fexofenadine hits no off-targets in the BioPrint® panel, whereas the positively charged terfenadine hits 5 off-targets with an $IC_{50} < 1$ μM (5-HT_{2B} receptor, Ca^{2+} channel [L-DHP site], Ca^{2+} channel [L-verapamil site], dopamine transporter, Na^+ channel site 2) (both compounds also 'hit' their therapeutic target, the H_1 receptor. Terfenadine was withdrawn due to QT-interval prolongation in 1997).

charged terfenadine hits five off-targets in the BioPrint® panel with sub-micromolar affinity, whereas the zwitterionic fexofenadine hits no off-targets (Fig. 4). Also, numerous ACE inhibitors and quinolone antibiotics demonstrate that successful drugs can be zwitterionic [53].

Excessive lipophilicity increases should be avoided during lead optimization to prevent promiscuity, as well as for numerous other reasons [54,55]. However it should be considered, that strong improvements of ligand affinity for many therapeutic targets are frequently accompanied by modest lipophilicity increases [56,57], depending on the target family, for example, average increases of 0.3 log units for GPCR ligands [58]. Hence, such carefully optimized compounds may be more selective, despite an increased lipophilicity, because the achieved improvement of therapeutic activity compensates for the increased liability for off-target activities.

Is an early recognition of pharmacological promiscuity possible?

As outlined above, relatively few targets with a preference for positively charged ligands are the dominant source of promiscuity in the BioPrint® dataset. This is also true for the Novartis and the Roche safety profiling datasets. It seems therefore reasonable to focus on these targets in the assessment of early compounds, especially of early compounds with a basic center.

We investigated whether the screening against a few representative targets is useful to identify promiscuous compounds, a strategy that is already applied in the field of kinase inhibition [59]. Muscarinic ligands, for example, are rarely selective for one receptor subtype and often bind to all five subtypes, M_1–M_5 [33]. To reduce cost and workload, it seems possible to screen early compounds at only one of these receptors. Empirically, we found that the affinity for certain frequently hit targets is somewhat predictive for overall promiscuity across the whole BioPrint® panel: for instance, 70% of the compounds hitting the 5-HT_{2B} receptor were promiscuous across the whole panel, whereas only 0.64% of the compounds with no measurable affinity ($pIC_{50} < 3.5$) for the 5-HT_{2B} receptor were promiscuous. A few diverse and predictive targets may therefore be combined in a small panel for a time- and cost-efficient screening of early compounds.

Among the 36 most frequently hit targets listed in Fig. 5, we calculated the sensitivity of each assay for any of the other assays, and selected six assays on the basis of sensitivity profile, assay hit rate, diversity, and potential relevance for safety (Fig. 5, heat map header). Together, these six assays form a panel with at least one receptor having >50% sensitivity for any one of the other frequently hit receptors (except ML_2, I_1). The diagram in Fig. 5 demonstrates that compounds which hit none of these receptors are also non-promiscuous across the whole dataset, whereas almost all compounds which hit three or more receptors are promiscuous. It has to be noted, of course, that compounds which hit three targets in the small panel have to hit only four additional targets in the remainder of the BioPrint® panel to reach a hit rate >5%, and to be therefore classified as 'promiscuous'. Nevertheless, the predictivity of such a small panel seems useful. The six targets in Fig. 5 were chosen as an example to demonstrate the validity of the concept, but it is likely that similar panels of targets are equally suited to identify many of the promiscuous compounds at an early stage (especially compounds with a basic center), as long as these targets are frequently hit and reasonably diverse. In practice, the choice of assays can therefore also be based on pragmatic considerations, such as the in-house availability of assays. Pharmacophore-based computational models of the α_1, the 5-HT_{2A}, and the D_2 receptor have been published [60,61], and *in silico* pharmacophore screens may be considered as an alternative to 'wet' pharmacology. Also, the clearly recognizable aminergic motifs in many of the promiscuous compounds in the BioPrint® panel suggest that such compounds can be flagged by computational tools; similar tools have been described [22,62].

Emerging trends in safety panel screening

Small panels of representative, frequently hit targets may be valuable tools for the prioritization of early compounds and series, but they cannot be a substitute to a more comprehensive characterization of increasingly advanced compounds.

Currently, safety screening is based on large and costly panels, which have grown historically based on the availability of targets and assays with an assumed relevance for safety; for current recommendations on exploratory safety pharmacology, see Ref. [63]. Smaller screening panels would be necessary to increase compound throughput; such panels should focus on targets with a high predictivity for adverse events, and omit targets of lesser relevance. For instance, numerous Roche compounds have been screened against panels containing the 5-HT_{5A} receptor, even though activity at this receptor could not be clearly associated with any adverse behavioral or physiological responses [64], and 5-HT_{5A} receptor knockout animals show only minor alterations compared to wild type subjects [65]. Moreover, the 5-HT_{5A} receptor is expressed mainly in only some brain areas, and the relevance of 5-HT_{5A} receptor activity for the safety of compounds with low brain penetration is therefore questionable.

Novartis scientists have very convincingly advocated the use of smaller screening panels [33,66], which are designed to be predictive of toxicity and ADRs, rather than to establish complete selectivity (which appears elusive when considering the estimated number of ~3000 druggable targets in the human genome [67]). These 20–100 target panels are tailored for the different stages of drug discovery, and focus on targets which are clearly associated with ADRs, with functional or biochemical readouts from specific targets. The reason for focusing on functional assays is two fold. First, it was found that agonists were not detected in a number of radioligand displacement assays, when the radioligand is an antagonist. In our analysis of the BioPrint® dataset, we also found that the endogenous aminergic neurotransmitters, serotonin, acetylcholine, epinephrine, norepinephrine, and histamine, did not show any measurable affinity for some of their respective receptors (5-HT_{4E}, β_3, H_1 receptors), or only very weak affinity ($IC_{50} > 30$ μM; M_1, M_3, H_2 receptors). This insensitivity of displacement assays may be well-known among pharmacologists, but its consequences for safety screening may have not been always fully appreciated. A second reason for a focus on functional assays is that, for a number of receptors, only agonistic activity is associated with ADRs, whereas antagonistic activity is not known to elicit an adverse response. Antagonists, often accounting for the majority of hits in displacement assays, may therefore be unnecessarily flagged or deprioritized. For instance, agonistic activity at the 5-HT_{2B} receptor

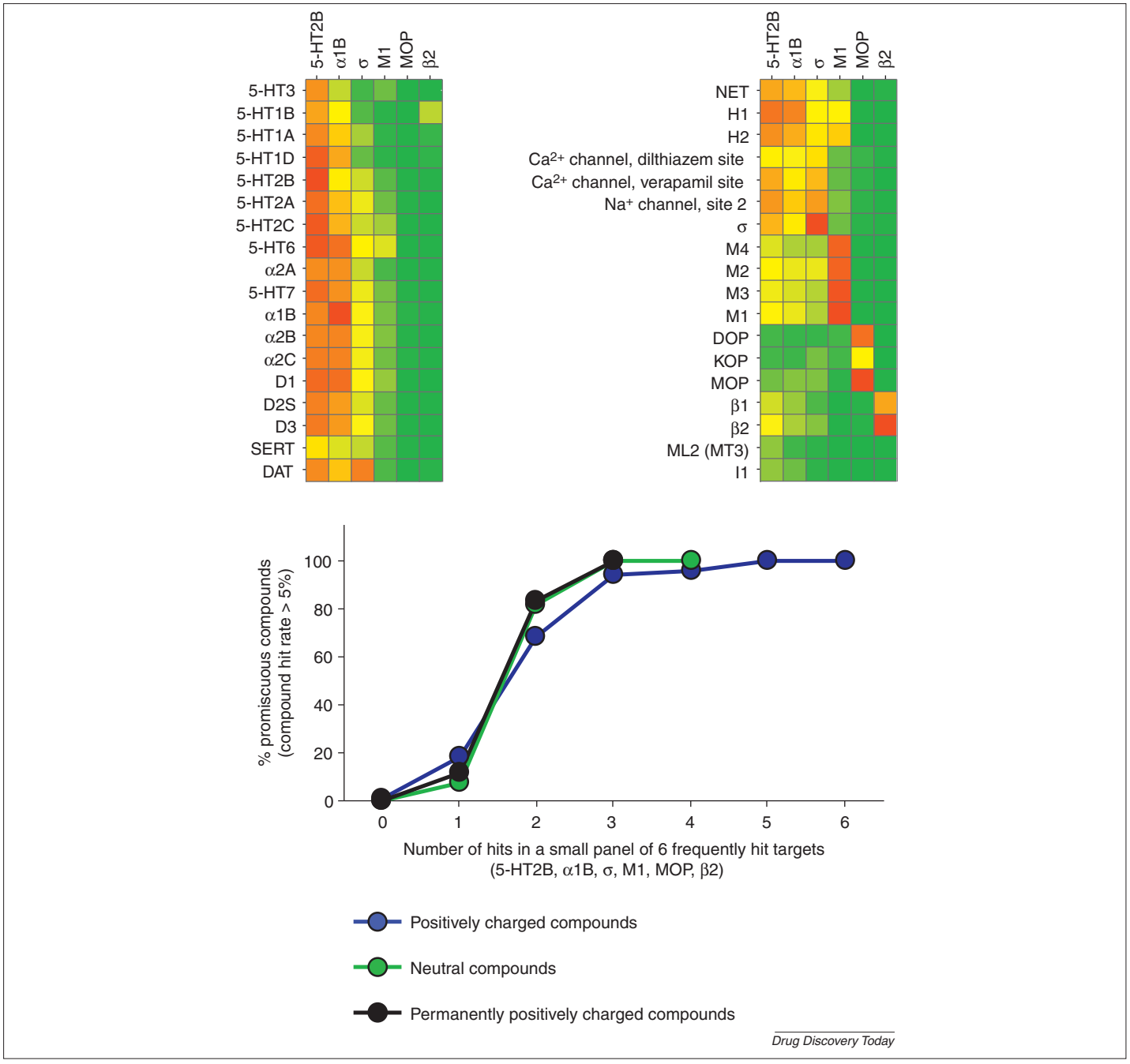

FIGURE 5

Is a small panel of representative, frequently hit targets useful for an early identification of promiscuous compounds? The heat maps show the sensitivity of six selected assays (header line) for each of the most frequently hit targets (columns left of heat maps). Taken together, these six selected receptors appear predictive for the majority of the frequently hit receptors (except ML_2, I_1 receptors). Lower diagram: among the neutral and (permanently) positively charged compounds, compounds which hit more than two of the representative six receptors are almost always promiscuous across the whole BioPrint® panel, whereas all compounds which hit none of the six receptors have low hit rates (<5%). Heat map colors code the sensitivity of assays A (six columns) for assays B (36 rows) (number of compounds hitting A and B/number of compounds hitting B; pure red = 100%, pure yellow = 50%, pure green = 0). The targets are grouped according to the number of shared ligands: Each assay was described by a vector of pIC_{50} values of all the compounds measured against the assay. The assays were clustered using hierarchical clustering (complete linkage, similarity metric: Pearson's correlation coefficient).

has been clearly linked to serious ADRs, such as potentially fatal pulmonary hypertension and permanent heart valve injuries [68–71], whereas antagonistic activity appears to have no or only benign consequences, and several marketed drugs have antagonistic (off-target) activity at the 5-HT_{2B} receptor [33,72]. Antagonists were responsible for 90% of the hits in a 5-HT_{2B} receptor radioligand displacement assay in an analysis of Novartis project data [33]. If it is additionally considered, that the 5-HT_{2B} receptor radioligand displacement assay attracted the highest number of hits in the Novartis safety screening panel (as well as in the BioPrint® and Roche safety profiling data), then a focus on a functional readout detecting 5-HT_{2B} receptor agonists seems reasonable.

Conclusion and outlook

- The activity of positively charged ligands at aminergic GPCRs and transporters, opioid receptors, and certain ion channels is the dominant source of promiscuity in typical safety panel screens. Although the occurrence of promiscuity among aminergic ligands and targets may be widely recognized, the high prevalence of promiscuity among basic compounds, and the high hit rates attracted by some aminergic targets are surprising. It is highly desirable to identify this frequently encountered 'aminergic'-type of promiscuity already in the initial stages of the drug discovery process, when it can be used as a criterion for the selection of lead series, or when it may be addressed by early optimization efforts. Compounds which are predisposed to such promiscuity can be recognized on the basis of molecular properties and structural motifs, and should be screened against a small panel of representative targets, which have been frequently hit by similar compounds. Pharmacophore-based *in silico* screening may also be explored as an alternative to *in vitro* screening.
- Radioligand displacement assays have been the work-horse of safety panels, however, functional assays are currently being considered as primary screens for certain GPCRs and ion channels. GPCR agonists are not always reliably detected in displacement assays, especially when the radioligand is an antagonist. Electrophysiological readouts tend to give more reliable and reproducible results for ion channel blockers than displacement assays.
- As compounds advance through the optimization process, they will warrant an increasingly extensive pharmacological profiling. The current process of screening only a few individual compounds in costly, low-throughput screening panels with a large number of targets is unsatisfactory, because systematic off-target activities are discovered often too late to be effectively addressed. Smaller screening panels, which are tailored for the different stages of drug discovery, or for specific types of compounds and projects, will identify systematic promiscuity issues at an earlier stage, when promiscuity can be addressed with an appropriate optimization strategy. These smaller panels only involve assays with a high relevance for safety, avoid the redundancy of similar targets, and allow therefore for a higher throughput of compounds. *In vitro* profiling may be preceded or supported by computational methods to predict off-target activities. The goal of this profiling will not be the demonstration of high selectivity, but rather the recognition of off-target activities which are highly predictive of adverse events and toxicity. In this context, it must be emphasized that an increasing number of researchers feels that complete selectivity should not be a stringent criterion for the selection of development candidates (as long as off-target *in vitro* activities do not manifest themselves in adverse reactions *in vivo*). This notion is supported by the value of numerous established, yet non-selective drugs, which frequently have unique pharmacological profiles and off-label uses [14].
- The ultimate goal of *in vitro* profiling should be the prediction of adverse events and toxicity. Many ADRs could be linked to specific targets (for an extensive compilation of target-ADR relationships, see Refs. [33,66]). In many other cases, adverse events will be triggered by the interplay between several targets [73]. For instance, dual inhibitory activity at the serotonin transporter and monoamine oxidase [74], or at the norepinephrine transporter and the α_2-autoreceptor [75], may lead to an accumulation of toxic levels of neurotransmitters. The weight gain commonly associated with 5-HT_{2C} receptor antagonism is only observed with compounds having additional activities at other targets, but not with more selective 5-HT_{2C} antagonists [75–77]. On the other hand, multiple off-target activities may also prevent adverse events that are associated with selective activities at certain targets: the selective COX-2 inhibitor rofecoxib (withdrawn 2004) increases the risk of cardiovascular events [78], whereas the dual COX-1/2 inhibition of numerous marketed NSAIDs does not. M_2 receptor antagonism, in the absence of M_3 receptor antagonism, has been considered as a mechanism for life-threatening bronchospasms during treatment with rapacuronium (withdrawn 2001), whereas dual M_2/M_3 receptor antagonism is encountered in several marketed drugs [79]. Analyses of the pharmacological spectra of compounds (the 'pharmacological fingerprint') [25,80–82], possibly in combination with other readouts [83–86], may prove useful to recognize these more complex links between *in vitro* pharmacology and ADRs in the future, and to predict potential ADRs of candidate compounds (e.g. by fingerprint comparison). To this end, larger collections of high-quality data with extended pharmacological information (more assays), metabolic profiles, and *in vivo* effects are urgently needed. This may call for intensified collaborations between partners having large amounts of profiling data generated under standardized conditions. The emergence of numerous commercial and public databases, where chemical structures are annotated with pharmacological data and ADRs (for an overview on such databases, see Ref. [87]) provides another opportunity for further investigations.

Conflict of interest

Jacques Migeon and Fabien Tillier are employees of Cerep Inc., which commercializes the BioPrint® dataset. The other authors declare no conflicts of interest.

References

1 Schuster, D. *et al.* (2008) Why drugs fail – a study on side effects in new chemical entities. In *Methods and Principles in Medicinal Chemistry 38 (Antitargets: Prediction and Prevention of Drug Side Effects)* (Vaz, R.J. and Klabunde, T., eds), pp. 3–22, Wiley VCH

2 Car, B.D. (2012) The relevance of off-target polypharmacology. In *Polypharmacology in Drug Discovery* (Peters, J.-U., ed.), pp. 9–14, John Wiley and Sons, Inc.

3 Smith, D.A. and Schmid, E.F. (2006) Drug withdrawals and the lessons within. *Curr. Opin. Drug Discov. Devel.* 9, 38–46

4 Bond, C.A. and Raehl, C.L. (2006) Adverse drug reactions in United States hospitals. *Pharmacotherapy* 26, 601–608

5 Hughes, J.D. *et al.* (2008) Physicochemical drug properties associated with in vivo toxicological outcomes. *Bioorg. Med. Chem. Lett.* 18, 4872–4875

6 Kola, I. and Landis, J. (2004) Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* 3, 711–715

7 Azzaoui, K. *et al.* (2007) Modeling promiscuity based on in vitro safety pharmacology profiling data. *ChemMedChem* 2, 874–880
8 Abbenante, G. *et al.* (2008) 'Clean' or 'Dirty' – just how selective do drugs need to be? *Aust. J. Chem.* 61, 654–660
9 Mestres, J. *et al.* (2009) The topology of drug-target interaction networks: implicit dependence on drug properties and target families. *Mol. Biosyst.* 5, 1051–1057
10 Keiser, M.J. *et al.* (2010) The chemical basis of pharmacology. *Biochemistry* 49, 10267–10276
11 Hopkins, A.L. (2008) Network pharmacology: the next paradigm in drug discovery. *Nat. Chem. Biol.* 4, 682–690
12 Roth, B.L. *et al.* (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discov.* 3, 353–359
13 Morphy, R. (2010) Selectively nonselective kinase inhibition: striking the right balance. *J. Med. Chem.* 53, 1413–1437
14 Mencher, S.K. and Wang, L.G. (2005) Promiscuous drugs compared to selective drugs (promiscuity can be a virtue). *BMC Clin. Pharmacol.* 5, 1–7
15 Minassian, A. and Young, J.W. (2010) Evaluation of the clinical efficacy of asenapine in schizophrenia. *Expert Opin. Pharmacother.* 11, 2107–2115
16 Faivre, S. *et al.* (2007) Molecular basis for sunitinib efficacy and future clinical development. *Nat. Rev. Drug Discov.* 6, 734–745
17 Schweizer, P.A. *et al.* (2011) Dronedarone: current evidence for its safety and efficacy in the management of atrial fibrillation. *Drug Des. Dev. Ther.* 5, 27–39
18 Peters, J.-U., ed. (2012) *Polypharmacology in Drug Discovery*, John Wiley and Sons, Inc.
19 Hopkins, A.L. *et al.* (2006) Can we rationally design promiscuous drugs? *Curr. Opin. Struct. Biol.* 16, 127–136
20 Leeson, P.D. and Springthorpe, B. (2007) The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* 6, 881–890
21 Peters, J.-U. *et al.* (2009) Pharmacological promiscuity: dependence on compound properties and target specificity in a set of recent Roche compounds. *ChemMedChem* 4, 680–686
22 Bender, A. *et al.* (2007) Analysis of pharmacology data and the prediction of adverse drug reactions and off-target effects from chemical structure. *ChemMedChem* 2, 861–873
23 Yang, Y. *et al.* (2010) Investigation of the relationship between topology and selectivity for drug-like molecules. *J. Med. Chem.* 53, 7709–7714
24 Park, K. *et al.* (2009) Predicting the multi-modal binding propensity of small molecules: towards an understanding of drug promiscuity. *Mol. Biosyst.* 5, 844–853
25 Krejsa, C.M. *et al.* (2003) Predicting ADME properties and side effects: the BioPrint approach. *Curr. Opin. Drug Discov. Devel.* 6, 470–480
26 *c* log *P* Values were Calculated Using *c* log *P* v4.94, Daylight Chemical Information Systems, 2008.
27 Whitlock, G.A. *et al.* (2008) Pyridyl-phenyl ether monoamine reuptake inhibitors: impact of lipophilicity on dual SNRI pharmacology and off-target promiscuity. *Bioorg. Med. Chem. Lett.* 18, 2896–2899
28 Miletti, F. *et al.* (2010) Extending pK_a prediction accuracy: high-throughput pK_a measurements to understand pK_a modulation of new chemical series. *Eur. J. Med. Chem.* 45, 4270–4279
29 Hann, M.M. *et al.* (2001) Molecular complexity and its impact on the probability of finding leads for drug discovery. *J. Chem. Inf. Comp. Sci.* 41, 856–864
30 Leach, A.R. and Hann, M.M. (2011) Molecular complexity and fragment-based drug discovery: ten years on. *Curr. Opin. Chem. Biol.* 15, 489–496
31 Peters, J.-U. (2012) Pharmacological promiscuity and molecular properties. In *Polypharmacology in Drug Discovery* (Peters, J.-U., ed.), pp. 47–62, John Wiley and Sons, Inc.
32 Whitebread, S. *et al.* (2005) In vitro safety pharmacology profiling: an essential tool for successful drug development. *Drug Discov. Today* 10, 1421–1433
33 Hamon, J. *et al.* (2009) In vitro safety pharmacology profiling: what else beyond hERG? *Fut. Med. Chem.* 1, 645–665
34 Strader, C.D. *et al.* (1988) Conserved aspartic acid residues 79 and 113 of the β-adrenergic receptor have different roles in receptor function. *Biol. Chem.* 263, 10267–10271
35 Yildirim, M.A. *et al.* (2007) Drug-target network. *Nat. Biotechnol.* 25, 1119–1126
36 Gregori-Puigjane, E. and Mestres, J. (2008) A ligand-based approach to mining the chemogenomic space of drugs. *Comb. Chem. High Throughput Screen.* 11, 669–676
37 Cases, M. and Mestres, J. (2009) A chemogenomic approach to drug discovery: focus on cardiovascular diseases. *Drug Discov. Today* 14, 479–485
38 LaBella, F.S. (1991) Molecular basis for binding promiscuity of antagonist drugs. *Biochem. Pharmacol.* 42 (Suppl.), S1–S8
39 Andrews, P.R. and Lloyd, E.J. (1982) Molecular conformation and biological activity of central nervous system active drugs. *Med. Res. Rev.* 2, 355–393
40 Duarte, C.D. *et al.* (2007) Privileged structures: a useful concept for the rational design of new lead drug candidates. *Mini-Rev. Med. Chem.* 7, 1108–1119
41 Bondensgaard, K. *et al.* (2004) Recognition of privileged structures by G-protein coupled receptors. *J. Med. Chem.* 47, 888–899
42 van der Horst, E. *et al.* (2009) Substructure mining of GPCR ligands reveals activity-class specific functional groups in an unbiased manner. *J. Chem. Inf. Model.* 49, 348–360
43 Aronov, A.M. *et al.* (2008) Kinase-likeness and kinase-privileged fragments: toward virtual polypharmacology. *J. Med. Chem.* 51, 1214–1222
44 Kirchner, S. (2012) Kinases as antitargets in genotoxicity. In *Polypharmacology in Drug Discovery* (Peters, J.-U., ed.), pp. 63–82, John Wiley and Sons, Inc.
45 Dive, V. *et al.* (2009) Inhibition of zinc metallopeptidases in cardiovascular disease – from unity to trinity, or duality? *Curr. Pharm. Des.* 15, 3606–3621
46 Daull, P. *et al.* (2007) Towards triple vasopeptidase inhibitors for the treatment of cardiovascular diseases. *J. Cardiovasc. Pharmacol.* 50, 247–256
47 Supuran, C.T. (2012) Carbonic anhydrases: off-targets, add-on activities, or emerging novel targets? In *Polypharmacology in Drug Discovery* (Peters, J.-U., ed.), pp. 457–489, John Wiley and Sons, Inc.
48 Leeson, P.D. *et al.* (2010) Impact of ion class and time on oral drug molecular properties. *MedChemComm* 2, 91–105
49 Bromidge, S.M. *et al.* (2000) 1-[2-[(Heteroaryloxy)heteroaryl]carbamoyl]indolines: novel and selective 5-HT_{2C} receptor inverse agonists with potential as antidepressant/anxiolytic agents. *Bioorg. Med. Chem. Lett.* 10, 1863–1866
50 Alcalde, E. *et al.* (2009) Indene-based frameworks targeting the 5-HT6 serotonin receptor: ring constraint in indenylsulfonamides using cyclic amines and structurally abbreviated counterparts. *Bioorg. Med. Chem.* 17, 7387–7397
51 Pei, Z. (2008) From the bench to the bedside: dipeptidyl peptidase IV inhibitors, a new class of oral antihyperglycemic agents. *Curr. Opin. Drug Discov. Dev.* 11, 512–532
52 Meeves, S.G. and Appajosyula, S. (2003) Efficacy and safety profile of fexofenadine HCl: a unique therapeutic option in H1-receptor antagonist treatment. *J. Allergy Clin. Immunol.* 112 (Suppl.), S69–S77
53 Sneader, W. (2005) *Drug Discovery – A History*. John Wiley and Sons Inc.
54 Waring, M.J. (2010) Lipophilicity in drug discovery. *Expert Opin. Drug Discov.* 5, 235–248
55 Meanwell, N.A. (2011) Improving drug candidates by design: a focus on physicochemical properties as a means of improving compound disposition and safety. *Chem. Res. Toxicol.* 24, 1420–1456
56 Van de Waterbeemd, H. *et al.* (2001) Lipophilicity in PK design: methyl, ethyl, futile. *J. Comput. Aided Mol. Des.* 15, 273–286
57 Gleeson, M.P. *et al.* (2011) Probing the links between in vitro potency, ADMET and physicochemical parameters. *Nat. Rev. Drug Discov.* 10, 197–208
58 Morphy, R. (2006) The influence of target family and functional activity on the physicochemical properties of pre-clinical compounds. *J. Med. Chem.* 49, 2969–2978
59 Brandt, P. *et al.* (2009) Small kinase assay panels can provide a measure of selectivity. *Bioorg. Med. Chem. Lett.* 19, 5861–5863
60 Klabunde, T. and Evers, A. (2005) GPCR antitarget modeling: pharmacophore models for biogenic amine binding GPCRs to avoid GPCR-mediated side effects. *ChemBioChem* 6, 876–889
61 Klabunde, T. and Evers, A. (2008) GPCR antitarget modeling: pharmacophore models to avoid GPCR-mediated side effects. In *Methods and Principles in Medicinal Chemistry 38 (Antitargets: Prediction and Prevention of Drug Side Effects)* (Vaz, R.J. and Klabunde, T., eds), pp. 127–140, Wiley VCH
62 Keiser, M.J. *et al.* (2009) Predicting new molecular targets for known drugs. *Nature* 462, 175–181
63 Cavero, I. (2009) Exploratory safety pharmacology: a new safety paradigm to de-risk drug candidates prior to selection for regulatory science investigations. *Expert Opin. Drug Saf.* 8, 627–647
64 Volk, B. *et al.* (2010) Medicinal chemistry of 5-HT5A receptor ligands: a receptor subtype with unique therapeutical potential. *Curr. Top. Med. Chem.* 10, 554–578
65 Grailhe, R. *et al.* (1999) Increased exploratory activity and altered response to LSD in mice lacking the 5-HT5A receptor. *Neuron* 22, 581–591
66 Urban, L. *et al.* (2012) Screening for safety-relevant off-target activities. In *Polypharmacology in Drug Discovery* (Peters, J.-U., ed.), pp. 15–46, John Wiley and Sons, Inc.
67 Hopkins, A.L. and Groom, C.R. (2002) The druggable genome. *Nat. Rev. Drug Discov.* 1, 727–730
68 Setola, V. and Roth, B.L. (2005) Screening the receptorome reveals molecular targets responsible for drug-induced side effects: focus on 'fen-phen'. *Expert Opin. Drug Metabol. Toxicol.* 1, 377–387
69 Setola, V. and Roth, B.L. (2008) The emergence of serotonin 5-HT2B receptors as drug antitargets. In *Methods and Principles in Medicinal Chemistry 38 (Antitargets: Prediction and Prevention of Drug Side Effects)* (Vaz, R.J. and Klabunde, T., eds), pp. 143–151, Wiley VCH

70 Ni, W. *et al.* (2004) The fenfluramine metabolite (+)-norfenfluramine is vasoactive. *J. Pharmacol. Exp. Ther.* 309, 845–852
71 Roth, B.L. (2007) Drugs and valvular heart disease. *N. Engl. J. Med.* 356, 6–9
72 Kvernmo, T. *et al.* (2008) Receptor-binding and pharmacokinetic properties of dopaminergic agonists. *Curr. Top. Med. Chem.* 8, 1049–1067
73 Garcia-Serna, R. and Mestres, J. (2010) Anticipating drug side effects by comparative pharmacology. *Expert Opin. Drug Metab. Toxicol.* 6, 1253–1263
74 Sun-Edelstein, C. *et al.* (2008) Drug-induced serotonin syndrome: a review. *Expert Opin. Drug Saf.* 7, 587–596
75 Millan, M.J. (2009) Dual- and triple-acting agents for treating core and co-morbid symptoms of major depression: novel concepts, new drugs. *Neurotherapeutics* 6, 53–77
76 Monck, N.J.T. and Kennett, G.A. (2008) 5-HT2C ligands: recent progress. *Prog. Med. Chem.* 46, 281–390
77 Millan, M.J. (2006) Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol. Ther.* 110, 135–370
78 Khanna, D. *et al.* (2005) COX-2 controversy: where are we and where do we go from here? *Inflammopharmacology* 13, 395–402
79 Jooste, E. *et al.* (2004) Rapacuronium preferentially antagonizes the function of M2 versus M3 muscarinic receptors in guinea pig airway smooth muscle. *Anesthesiology* 102, 117–124
80 Mason, J.S. *et al.* (2008) Use of broad biological profiling as a relevant descriptor to describe and differentiate compounds: structure – in vitro (pharmacology-ADME) – in vivo (safety) relationships. In *Methods and Principles in Medicinal Chemistry 38 (Antitargets: Prediction and Prevention of Drug Side Effects)* (Vaz, R.J. and Klabunde, T., eds), pp. 143–151, Wiley VCH
81 Migeon, J. (2012) Prediction of side effects based on fingerprint profiling and data mining. In *Polypharmacology in Drug Discovery* (Peters, J.-U., ed.), pp. 111–132, John Wiley and Sons, Inc.
82 Fliri, A.F. *et al.* (2005) Biospectra analysis: model proteome characterizations for linking molecular structure and biological response. *J. Med. Chem.* 48, 6918–6925
83 Andersen, M.E. *et al.* (2010) The future of toxicity testing. *J. Toxicol. Environ. Health B* 13, 163–196
84 Merlot, C. (2010) Computational toxicology – a tool for early safety evaluation. *Drug Discov. Today* 15, 16–22
85 Berg, E.L. *et al.* (2010) Chemical target and pathway toxicity mechanisms defined in primary human cell systems. *J. Pharmacol. Toxicol. Methods* 61, 3–15
86 Merino, A. *et al.* (2010) Drug profiling: knowing where it hits. *Drug Discov. Today* 15, 749–756
87 Briansó, F. *et al.* (2011) Cross-pharmacology analysis of g protein-coupled receptors. *Curr. Top. Med. Chem.* 11, 1956–1963