CHEMMEDCHEM

DOI: 10.1002/cmdc.200800411

Pharmacological Promiscuity: Dependence on Compound Properties and Target Specificity in a Set of Recent Roche Compounds

Jens-Uwe Peters,* Patrick Schnider, Patrizio Mattei, and Manfred Kansy[a]

The term "pharmacological promiscuity" describes a compound's pharmacological activity at multiple targets. Pharmacological promiscuity is undesired in typical drug discovery projects, which focus on the "one drug–one target" paradigm. Off-target activity can lead to adverse drug reactions, or can obscure pharmacodynamic effects in animal models. Therefore, advanced lead compounds, pharmacological tool compounds, and drug candidates are usually screened against panels of safety-relevant targets to detect unwanted pharmacological activities. To identify determinants of pharmacological promiscuity, we compared the panel screening outcomes of 213 recent Roche compounds with their molecular properties. Pronounced promiscuity was not observed below a threshold Clog*P* value of 2. For basic compounds, the propensity for weak off-target activity was found to increase with calculated basicities, whereas the potential for strong off-target activity depends more qualitatively on the presence of a positive charge at physiological pH. Compounds originating from projects with an aminergic receptor or transporter as a therapeutic target are particularly prone to promiscuity; the promiscuity of such compounds is mainly caused by their activity at other aminergic targets in the screening panel.

Introduction

Typical drug discovery programs aim to identify drug candidates that are selective for a single target. Interactions with two or several related targets might also be pursued, as in the SNRI class of antidepressants.[1] Multiple activities at a number of relevant targets might contribute to pharmacological activity, as with antipsychotic drugs,[2] or might lead to an improved therapeutic effect, as with kinase-inhibiting anticancer drugs.[3] Although such tailored polypharmacology can be beneficial, unintended polypharmacology needs to be avoided, as it can lead to adverse drug reactions (ADRs). For instance, the off-target agonistic 5-HT_{2B} receptor activity of a metabolite of the anorectic drug fenfluramine (withdrawn in 1997) was linked to irreversible and potentially fatal pulmonary hypertension[4] and the development of heart valve abnormalities.[5] A compilation of selected cardiovascular targets and potential ADRs can be found in a report by researchers at Novartis.[6]

To detect unwanted off-target activities, compounds are often submitted to contract research organizations (such as Cerep, Novascreen, MDS PanLabs) for screening against panels typically composed of 60–150 targets. In-house panel screens[6] and in silico prediction tools[7,8] have been used as less expensive, higher-throughput alternatives to address pharmacological promiscuity. These methods cover only a fraction of the estimated 3000 drugable targets in the human genome;[9] in silico target profiling methods, which can cover greater numbers of targets, have therefore been used to complement in vitro results. Such drug target profiling suggests that drugs interact, on average, with as many as 6.3 target proteins.[10]

Several structural features and molecular parameters have been associated with promiscuity. Indole, furan, and piperazine motifs were found to be over-represented in promiscuous compounds, whereas carboxylic acid groups seem to prevent promiscuity.[8] Several studies found that promiscuity increases with lipophilicity.[8,11–13] Bases are more prone to promiscuity than uncharged compounds and acids.[12] These studies were performed with different datasets, such as the BioPrint® dataset,[12,14] Pfizer HTS and project data,[11] or Novartis safety pharmacology profiling (SPP) data.[8] To investigate which parameters are the most relevant determinants for promiscuity within the chemical space of recent compounds at our organization, F. Hoffmann-La Roche Ltd. (Roche), we performed a similar analysis for Roche compounds that were profiled at Cerep in the years 2004–2007. The results of this analysis are described below.

A special aspect of unwanted polypharmacology is the interaction of compounds with promiscuous targets such as the hERG channel[15] or metabolic enzymes.[16] Furthermore, polypharmacology might contribute to the phenomenon of "frequent hitters",[17] although assay perturbation, micelle formation, or compound aggregation are probably more important.[18] Such issues are addressed differently and are therefore not within the scope of this analysis.

[a] *Dr. J.-U. Peters, Dr. P. Schnider, Dr. P. Mattei, Dr. M. Kansy*
Discovery Chemistry Basel
F. Hoffmann-La Roche Ltd., 4070 Basel (Switzerland)
E-mail: jens-uwe.peters@roche.com

Results and Discussion

Assessment of pharmacological promiscuity

A recent report by AstraZeneca researchers demonstrated a particularly compelling correlation between lipophilicity and promiscuity,[12] and triggered our own investigations. Our analysis included 213 compounds, which had been sent to Cerep for panel screening, and that fulfilled certain selection criteria as described below in the Experimental Section. These compounds were prepared for 62 recent projects from the therapeutic areas listed in Table 1. In analogy to the AstraZeneca paper, we counted for each compound the number of off-targets at which the compound displayed $\geq 30\%$ activity at a concentration of 10 μM. Because the overall number of targets was different across screens, we normalized this count by dividing it by the overall number of targets tested (see Experimental Section). This ratio, expressed in percent, was termed "percentage of off-targets with $\geq 30\%$ activity", and was used as a measure of promiscuity.

Table 1. Origin of compounds from therapeutic areas.

Therapeutic Area	Percentage of Compounds
central nervous system	33%
metabolic diseases	21%
oncology	15%
vascular diseases, lipid disorders	13%
inflammatory diseases	7%
others/not classified	11%

Relationship between lipophilicity, basicity, and promiscuity

Similar to previous studies by others, we investigated the relationship between the promiscuity of our compounds and commonly assessed molecular descriptors, such as Clog *P*, calculated pK_a, and molecular weight (M_r) (see Experimental Section). In plots of promiscuity against Clog *P* (Figure 1 A) or Clog $D_{7.4}$ (data not shown), we found a similar distribution of data points as in the Pfizer[11] and AstraZeneca[12b] studies. Pronounced promiscuity is not observed below a threshold Clog *P* value of 2 (or a threshold Clog $D_{7.4}$ value of 1). Although the number of compounds below this threshold is too small to draw definite conclusions, this corroborates the findings of Pfizer scientists with larger datasets, that Clog $P > 2.5$–3 is observed for the most promiscuous compounds.[11] The only outlier in our dataset, with $>10\%$ off-target hits despite a Clog $P <$ 2, is a peptide ($M_r > 1000$ Da), whereas all other compounds in the dataset are small molecules. A large variation in promiscuity is observed in the range of Clog *P* 2–6, and medium promiscuity is observed for Clog $P > 6$. It is apparent that positively charged compounds are more prone to promiscuity than neutral or negatively charged compounds (Figure 1 A; for dataset composition, compare Table 2). This is in agreement with the observation by AstraZeneca researchers, that bases and quaternary bases in the BioPrint® dataset are notably more promiscuous than acids, neutral compounds, or zwitterions.[12] Interestingly, the likelihood of compounds with a basic center to show promiscuity increases with rising pK_a(B) (Figure 1 B); this trend was observed across projects. A correlation between promiscuity and molecular descriptors unrelated to pK_a and Clog *P* was not found. In particular, no relationship between M_r and promiscuity could be established. Molecular weight can be regarded as an estimate of molecular complexity,[11] which is generally thought to have an impact on polypharmacology.[19] Observations from previous analyses of various datasets with regard to M_r and promiscuity are inconsistent: Whereas compounds with higher M_r were less promiscuous in the Pfizer HTS dataset,[11] higher M_r led to increased promiscuity in the Novartis SPP dataset;[8] a more complex relationship was described in the AstraZeneca analysis of BioPrint® data.[12]

"Weak" versus "strong" off-target activity

For comparability with the AstraZeneca paper, we used a threshold of 30% activity at off-targets at a concentration of 10 μM for our assessment of promiscuity in the analysis described above. An activity of $<50\%$ at 10 μM should, in theory, correspond to $IC_{50} > 10$ μM. Such an activity can be regarded as weak relative to the often low-nanomolar activity of advanced project compounds at their therapeutic targets. Although some targets may cause a physiological response even after weak stimulation or inhibition, or may be more easily accessible depending on the compound's tissue distribution, there is usually a sufficient therapeutic window if the off-target activity of a compound is several orders of magnitude lower than the activity at the therapeutic target. Weak (micromolar) off-target activities are therefore rarely a major concern. Much more relevant are off-target activities of 90% or more at 10 μM, because they often correspond to an IC_{50} value in the nanomolar range and can be regarded as strong. Such strong off-target activities are often unacceptable in pharmacological tool compounds and drug candidates. We therefore decided to repeat our analysis with a threshold of 90% activity at off-targets at a concentration of 10 μM, a threshold which appeared more relevant for Roche projects.

The potential for strong off-target activity depends on positive charge and compound origin

Promiscuity assessment at this point was based on a threshold of $\geq 90\%$ response for consideration of an off-target as a hit. Using this threshold, the potential for promiscuity was found to be highly dependent on the presence of a positive charge (Figure 1 C,D): essentially all compounds in our dataset that hit $>4\%$ of the targets in their Cerep panels carry a positive charge. The only exception is a compound classified as neutral, and that hits ~10% of its Cerep targets. However, this compound has a pK_a(B) = 6.6, is therefore partly protonated at physiological pH, and thus forms a positively charged fraction. In contrast to the observation that the potential for "weak-ac-

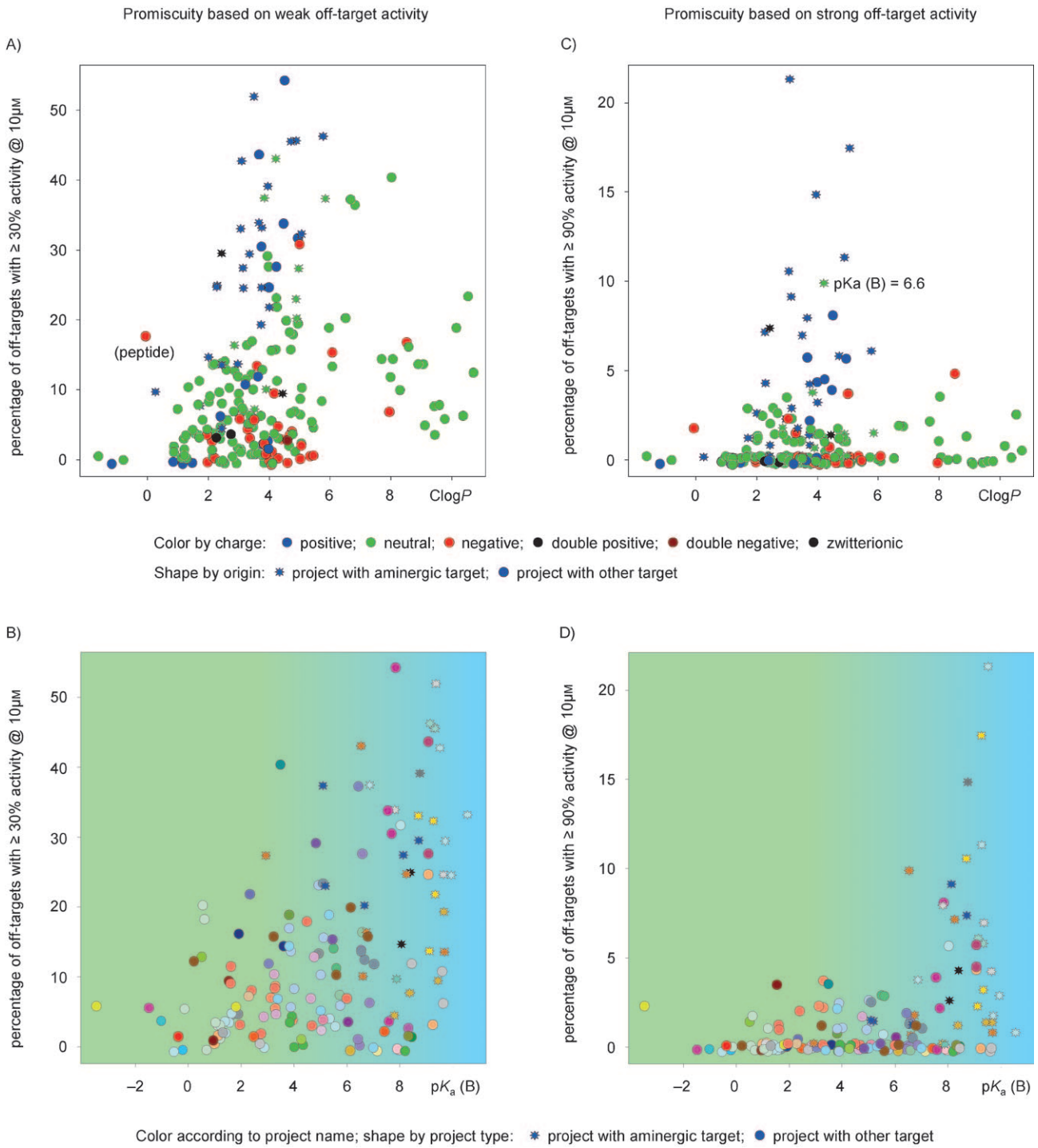

Figure 1. Relationship between Clog*P*, basicity, project type, and pharmacological promiscuity. The potential for weak off-target activity increases A) at a threshold Clog*P* value of 2 and B) correlates with basicity. C, D) A positive charge ($pK_a(B) > 7$) is an important parameter governing strong off-target activity. Compounds from projects targeting an aminergic GPCR or transporter are much more likely to show off-target activities than compounds from projects with other targets. Charge and $pK_a(B)$ were calculated using the commercial prediction tool, MoKa, trained with Roche internal measurement data. Only compounds with a basic center are included in panels B) and D); $pK_a(B) = pK_a$ of protonated base. For illustration, the topmost data point in panel A) represents a compound that elicits an activity of $\geq 30\%$ at $> 50\%$ of all targets in the screening panel.

tivity-based" promiscuity increases gradually with $pK_a(B)$ (Figure 1 B), "strong-activity-based" promiscuity depends more qualitatively on a $pK_a(B) > 7$, that is, on a positive charge (Figure 1 D). An inspection of our dataset revealed that strong off-target activities are particularly common among compounds from projects with aminergic GPCRs or transporters as therapeutic targets: approximately half (49%) of the compounds with such an origin showed strong activity at $\geq 3\%$ of their Cerep targets. The majority of these compounds are positively charged; this raised the question if a compound's origin from

Table 2. Ionization states of compounds in the dataset.

Charge	Percentage of Compounds
positive (single/double charge)	21%
negative (single/double charge)	15%
neutral	63%
zwitterionic	1%

an aminergic project, rather than a positive charge, is the principal determinant for the observed increased potential for promiscuity. However, within the subset of compounds stemming from "non-aminergic" projects, positively charged compounds were also found to be over-represented (55%) among the promiscuous compounds hitting $\geq$3% of their Cerep targets, and under-represented (9%) among the "clean" compounds hitting no off-targets. Thus, a positive charge seems to increase a compound's potential for promiscuity, regardless of origin. On the other hand, it is apparent that compounds that stem from aminergic projects have a particularly pronounced risk of being promiscuous (Figure 1C, D). For instance, the five most promiscuous compounds, hitting $\geq$10% of their Cerep targets, originate from projects with aminergic targets. We concluded that compounds with a positive charge carry a higher risk of promiscuity than neutral or negatively charged compounds, and that this risk is substantially increased if compounds stem from projects with aminergic targets ("promiscuous" defined as eliciting $\geq$90% responses at off-targets at a concentration of 10 μM).

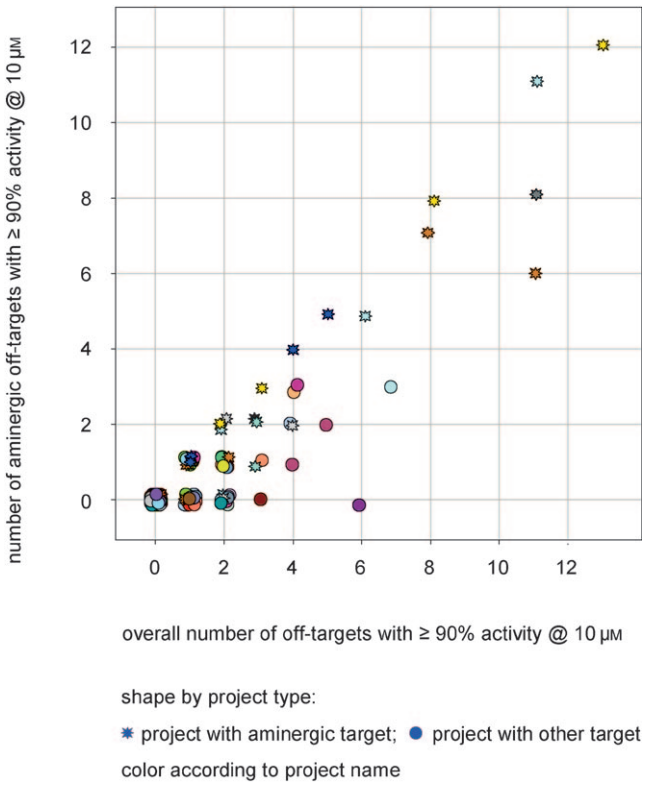

Figure 2. Overt promiscuity of positively charged compounds is mainly due to activity at aminergic targets, especially if the compounds stem from projects targeting an aminergic GPCR or transporter. For instance, the top-right data point represents a compound that hits 13 off-targets in a screening panel, 12 of which are aminergic targets. (Only positively charged compounds are shown.)

Pronounced promiscuity of positively charged compounds is mainly due to monoaminergic activity

A frequent challenge in projects targeting aminergic GPCRs or transporters is to achieve selectivity over related aminergic targets. This pointed to the possibility that the increased promiscuity potential of compounds from such projects is due to activity at other aminergic targets in the screening panel. Indeed, an inspection of the dataset unveiled that these "aminergic" compounds and, to a lesser extent, positively charged compounds from non-aminergic projects hit a disproportionately high number of aminergic targets in their screening panels: 70% of the targets hit by positively charged compounds were aminergic targets, whereas aminergic targets constitute, on average, only 28% of the screening panels. Some aminergic targets were hit by >5% of the compounds which were tested at that target, with many of the hits being positively charged compounds (Table 3). The dominating influence of aminergic activity on promiscuity might be a peculiarity of our dataset, as psychiatric diseases, which are particularly rich in aminergic targets, have been an area of focus at our company, and 16% of the analyzed compounds originate from aminergic projects. Our dataset may, however, be representative for large research organizations, which focus on similar disease areas, or have aminergic ligands in their screening libraries. Similar results were obtained in the Novartis analysis of SPP data, in which the 5-HT receptors, in particular the 5-HT_{2B} receptor, were found to attract a high hit rate.[6] A major contribution of aminergic activity was also found in in silico target profiling studies, where drugs targeting aminergic GPCRs showed the most promiscuous pharmacological profiles.[20] These findings suggest that cross-screening against a relatively small array of aminergic receptors may be a fast and cost-effective way to identify promiscuity liabilities of positively charged compounds.

Table 3. Targets hit[a] by >5% of the dataset compounds that were tested at that target.

Target	All Ionization States [%][b]	Positively Charged [%][c]
5-HT_{2A} receptor	6.5	25.7
dopamine transporter	6.4	17.9
NE transporter	6.1	26.3
M_1 receptor	6.1	24.3
5-HT_{2B} receptor	5.9	33.3
5-HT_{1A} receptor	5.4	16.7

[a] Showing activity of $\geq$90% at compound concentrations of 10 μM. [b] Percentage of compounds (all ionization states) active at target. [c] Percentage of positively charged compounds active at target.

The influence of poor solubility on panel screening outcomes

Our dataset contains some very lipophilic compounds (Clog $P > 6$), which show a relatively benign polypharmacology profile (Figure 1C). Solubility data[21] were available for 13 of these compounds. With two exceptions, solubilities are $< 10\,\mu M$, the test concentration of the Cerep panel. Poorly soluble compounds might precipitate upon dilution, may therefore appear to be less active or inactive in pharmacological assays, and, as a result, might appear less promiscuous. This raised the question whether we failed to establish a relationship between promiscuity and very high lipophilicity due to erroneous panel results caused by insufficient solubility. To address this question, we compared all compounds for which solubility data were available, with respect to promiscuity. We found that the distribution over the promiscuity scale for poorly soluble compounds and for highly soluble compounds is similar, especially if compounds of the same ionization state are compared (Figure 3).[22,23] This indicates that the panel screening outcomes, at least for the compounds in our dataset, are not substantially affected by poor solubility. Poorly soluble compounds may show reasonable pharmacological responses in in vitro assays because pre-dissolution in DMSO, assay solution components, and a slow rate of precipitation might contribute to sufficient solubility in the assay media.[24]

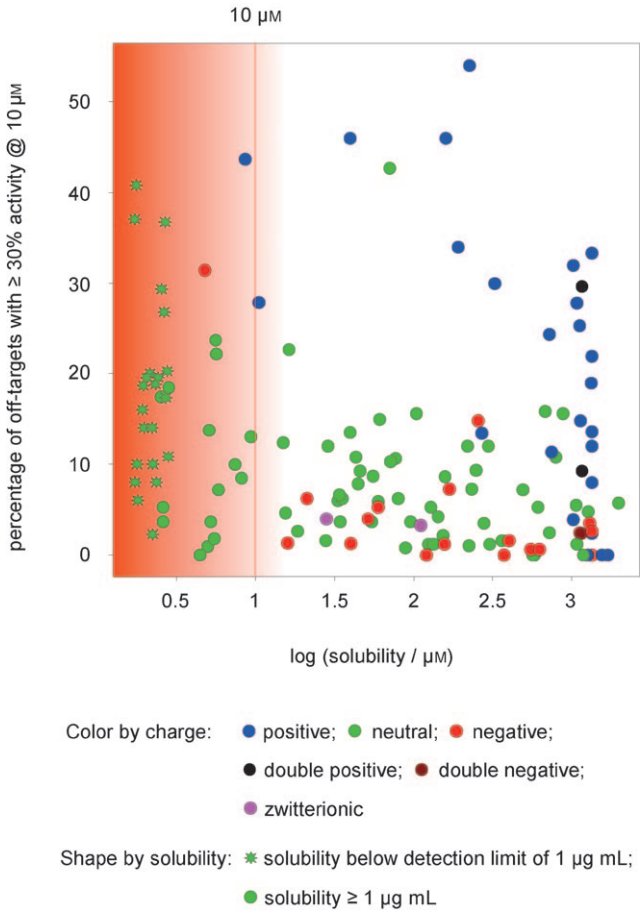

Figure 3. Poorly soluble and highly soluble compounds are similarly distributed over the promiscuity scale, indicating that solubility below the test concentration (red area) does not substantially compromise the validity of a panel screen.

Conclusions

There has long been awareness in the drug discovery community that molecular parameters such as lipophilicity, basicity, ionization state, molecular weight, and complexity as well as the presence of certain structural motifs have an influence on pharmacological promiscuity. Several recent studies have provided a data basis for this tacit knowledge. We analyzed the panel screens of 213 recent Roche compounds in a similar study to identify the principal determinants of pharmacological promiscuity within the Roche chemical space. In our assessment of promiscuity based on "weak" off-target activities in a panel screen, we found that the promiscuity potential of the compounds in our dataset sharply increases at a threshold Clog $P \approx 2$, and that the promiscuity potential of basic compounds increases gradually with their calculated pK_a(B) values. When promiscuity was assessed on the basis of "strong" off-target activities, we found a positive charge to be a main determinant of promiscuity potential for the compounds in our data set. In particular, positively charged compounds originating from projects with an aminergic target were found to have a high risk of being promiscuous. The pharmacological promiscuity of positively charged compounds is mainly due to activity at aminergic off-targets in the screening panel. This might suggest that the screening of positively charged compounds at a representative set of aminergic targets is a time- and cost-effective way to recognize promiscuous compounds or compound series early in the drug discovery process.

Experimental Section

Details on data collection and analysis

Selection criteria: This analysis includes the panel screens of 213 Roche compounds that were profiled at Cerep in the years 2004–2007. Given the high cost of such screens, it is inferred that these compounds are druglike and are of high value for Roche projects: advanced lead compounds or reference compounds, for example. Only screens performed at a compound concentration of $10\,\mu M$ were included. The screening panels contained 80 targets on average; screenings with fewer than 20 targets were omitted. An overview of the target type composition of the panel screens is given in Table 4.

"Percentage of off-targets with $\geq$30% activity": This value was calculated for each screen by counting the number of targets that showed activity above a threshold of $\geq$30%. Targets that were measured several times were counted only once; project target(s) were not counted. This count was regarded as the number of off-target hits. "percentage of off-targets with $\geq$30% activity" was then calculated by Equation 1, and served as a measure of promiscuity based on "weak" off-target activity:

$$\frac{\text{number of off-target hits}}{\text{overall number of panel targets}} \times 100\,\% \qquad (1)$$

Table 4. Distribution of target types in the data set.

Target Type	Percentage of Targets
aminergic GPCRs	24.3%
aminergic transporters	3.9%
non-aminergic GPCRs	39.9%
ion channels	15.0%
enzymes	11.1%
nuclear receptors	2.2%
others	3.5%

"Percentage of off-targets with ≥90% activity": This value was calculated as above, using a threshold of ≥90%. The value served as a measure of promiscuity based on "strong" off-target activity.

Molecular properties data collection: The data types collected are listed in Table 5. Measured physicochemical properties were not available for all compounds. Physicochemical properties were typically determined with the same compound batch that was also submitted for panel screening; only if data from the same batch were not available, the measured properties of a different compound batch were used.

Correlations: All parameters listed in Table 5 were plotted against "percentage of off-targets with ≥30% activity" and "percentage of off-targets with ≥90% activity", using Spotfire® Decision Site® 8.2. Data points were jittered (displaced randomly for small distances) for better visibility of overlapping data points. Parameters not mentioned in the text were not found to correlate with promiscuity. For correlations of promiscuity with basicity and lipophilicity, calculated values (Clog P/Clog $D_{7.4}$ for lipophilicity, pK_a values predicted by MoKa) were used, because the use of measured data would have led to the selection of biased datasets: very high lipophilicities are not routinely measured, and pK_a values are only determined for compounds with basic or acidic centers, with a bias for compounds in which charge or high basicities are properties to be optimized. For consistency, the predicted pK_a values were also used for the determination of ionization states: acidic compounds with pK_a < 7 were regarded as negatively charged; basic compounds with pK_a(B) > 7 were regarded as positively charged; compounds with a basic and an acidic center, pK_a < 7, and pK_a(B) > 7 were regarded as zwitterions; and all other compounds (compounds with no basic/acidic centers, acids with pK_a > 7, bases with pK_a(B) < 7) were classified as neutral.

Table 5. Assessed molecular properties.

In silico Properties	Measured Physicochemical Properties
M_r	solubility (LYSA)[21]
number of heavy atoms	log $D_{7.4}$[28]
number of H-bond donors (HBD)	permeability (PAMPA)[29]
number of H-bond acceptors (HBA)	PAMPA: % compound in donor compartment
number of rotatable bonds	PAMPA: % compound in acceptor compartment
number of rings	PAMPA: % compound in membrane
number of aromatic rings	pK_a[30]
number of non-aromatic rings	
polar surface area	
Clog P	
amphiphilicity (CAFCA)[25]	
predicted pK_a (MoKa)[26]	
ionization state	
Clog $D_{7.4}$[27]	

Acknowledgements

We are grateful to Dr. Holger Fischer and Dr. Wolfgang Guba for helpful discussions and for the calculation of in silico parameters.

Keywords: drug discovery · molecular properties · polypharmacology · promiscuity · safety pharmacology

[1] M. Bauer, H.-J. Möller, E. Schneider, *Expert Opin. Pharmacother.* **2006**, *7*, 421–427.
[2] B. L. Roth, D. J. Sheffler, W. K. Kroeze, *Nat. Rev. Drug Discovery* **2004**, *3*, 353–359.
[3] T. Hampton, *JAMA J. Am. Med. Assoc.* **2004**, *292*, 419–422.
[4] a) L. Abenhaim, Y. Moride, F. Brenot, S. Rich, J. Benichou, X. Kurz, T. Higenbottam, C. Oakley, E. Wouters, M. Aubier, G. Simonneau, B. Bégaud, *N. Engl. J. Med.* **1996**, *335*, 609–616; b) E. J. Mark, E. D. Patalas, H. T. Chang, R. J. Evans, S. C. Kessler, *N. Engl. J. Med.* **1997**, *337*, 602–606; c) W. Ni, M. W. Li, K. Thakali, D. Gregory, S. W. Watts, *J. Pharmacol. Exp. Ther.* **2004**, *309*, 845–852.
[5] H. M. Connolly, J. L. Crary, M. D. McGoon, D. D. Hensrud, B. S. Edwards, W. D. Edwards, H. V. Schaff, *N. Engl. J. Med.* **1997**, *337*, 581–588.
[6] S. Whitebread, J. Hamon, D. Bojanic, L. Urban, *Drug Discovery Today* **2005**, *10*, 1421–1433.
[7] A. Bender, J. Scheiber, M. Glick, J. W. Davies, K. Azzaoui, J. Hamon, L. Urban, S. Whitebread, J. L. Jenkins, *ChemMedChem* **2007**, *2*, 861–873.
[8] K. Azzaoui, J. Hamon, B. Faller, S. Whitebread, E. Jacoby, A. Bender, J. L. Jenkins, L. Urban, *ChemMedChem* **2007**, *2*, 874–880.
[9] A. L. Hopkins, C. R. Groom, *Nat. Rev. Drug Discovery* **2002**, *1*, 727–730.
[10] J. Mestres, E. Gregori-Puigjané, S. Valverde, R. V. Solé, *Nat. Biotechnol.* **2008**, *26*, 983–984.
[11] A. Hopkins, J. S. Mason, J. P. Overington, *Curr. Opin. Struct. Biol.* **2006**, *16*, 127–136.
[12] a) P. D. Leeson, B. Springthorpe, *Nat. Rev. Drug Discovery* **2007**, *6*, 881–890; b) http://www.nature.com/nrd/journal/v6/n11/extref/nrd2445-s3.pdf (Supplementary Information, Box S3).
[13] G. A. Whitlock, P. V. Fish, M. J. Fray, A. Stobie, F. Wakenhut, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2896–2899.
[14] C. M. Krejsa, D. Horvath, S. L. Rogalski, J. E. Penzotti, B. Mao, F. Barbosa, J. C. Migeon, *Curr. Opin. Drug Discovery Dev.* **2003**, *6*, 470–480.
[15] a) O. Roche, G. Trube, J. Zuegge, P. Pflimlin, A. Alanine, G. Schneider, *ChemBioChem* **2002**, *3*, 455–459; b) M. C. Sanguinetti, M. Tristan-Firouzi, *Nat. Chem. Biol.* **2006**, *2*, 463–469; c) P. Hoffmann, B. Warner, *J. Pharmacol. Toxicol. Methods* **2006**, *53*, 87–105; d) W. S. Redfern, L. Carlsson, A. S. Davis, W. G. Lynch, I. MacKenzie, S. Palethorpe, P. K. Siegl, I. Strang, A. T. Sullivan, R. Wallis, A. J. Camm, T. G. Hammond, *Cardiovasc. Res.* **2003**, *58*, 32–45.
[16] a) R. J. Riley, K. Grime, *Drug Discovery Today Technol.* **2004**, *1*, 365–372; b) J. M. Hutzler, D. M. Messing, L. C. Wienkers, *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 51–58; c) J. H. Lin, A. Y. H. Lu, *Clin. Pharmacokinet.* **1998**, *35*, 361–390.
[17] O. Roche, P. Schneider, J. Zuegge, W. Guba, M. Kansy, A. Alanine, K. Bleicher, F. Danel, E. M. Gutknecht, M. Rogers-Evans, W. Neidhart, H. Stalder, M. Dillon, E. Sjogren, N. Fotouhi, P. Gillespie, R. Goodnow, W. Harris, P. Jones, M. Taniguchi, S. Tsujii, W. von der Saal, G. Zimmermann, G. Schneider, *J. Med. Chem.* **2002**, *45*, 137–142.
[18] a) S. L. McGovern, B. K. Shoichet, *J. Med. Chem.* **2003**, *46*, 2895–2907; b) S. L. McGovern, E. Caselli, N. Grigorieff, B. K. Shoichet, *J. Med. Chem.* **2002**, *45*, 1712–1722.

[19] M. M. Hann, A. R. Leach, G. Harper, *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 856–864.
[20] E. Gregori-Puigjané, J. Mestres, *Comb. Chem. High Throughput Screening* **2008**, *11*, 669–676.
[21] Solubility measurements were performed according to an in-house-developed method to assess solubility from a 10 mM DMSO stock solution. This method is similar to the classical thermodynamic shake-flask solubility assay, with the only difference that DMSO is removed at the beginning by an additional lyophilization step. Therefore, this assay is known as a lyophilized solubility assay (LYSA).
[22] The comparison of positively charged compounds with neutral compounds might be misleading, as the former are intrinsically more soluble, and at the same time more promiscuous.
[23] Neutral insoluble compounds tend to be even more promiscuous than neutral soluble compounds; this might be explained by the correlation of solubility and lipophilicity, and the connection between lipophilicity and promiscuity.
[24] L. Di, E. H. Kerns, *Drug Discovery Today* **2006**, *11*, 446–451.
[25] H. Fischer, M. Kansy, D. Bur, *Chimia* **2000**, *54*, 640–645.
[26] MoKa is a commercially available tool trained with Roche internal measurement data: F. Milletti, L. Storchi, G. Sforna, G. Cruciani, Gabriele, *J. Chem. Inf. Model.* **2007**, *47*, 2172–2181.
[27] Calculations were performed according to: a) M. Kansy, H. Fischer, H. van de Waterbeemd, *Trends in QSAR and Molecular Modeling 94*, (Ed.: F. Sanz), Prous, Barcelona **1995**; b) H. van de Waterbeemd, M. Kansy, B. Wagner, H. Fischer, *Lipophilicity in Drug Action and Toxicology*, (Eds.: V. Pliška, B. Testa, H. van de Waterbeemd), VCH, Weinheim **1996**.
[28] Measurements were performed according to an in-house-developed miniaturized method to measure the shake-flask octanol–water partition coefficient at pH 7.4 (log *D*).
[29] Measurements were performed according to: M. Kansy, F. Senner, K. J. Gubernator, *J. Med. Chem.* **1998**, *41*, 1007–1010.
[30] Measurements were performed according to: R. I. Allen, K. J. Box, J. Comer, C. Peake, K. Y. Tam, *J. Pharm. Biomed. Anal.* **1998**, *17*, 699–712.

Received: November 28, 2008
Revised: February 3, 2009
Published online on March 5, 2009

