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Chemical probes for biological systems

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According to the latest definition in use by the NIH Molecular Libraries Screening Centers Network, a compound to be nominated as a chemical probe should have, on the one hand, an affinity below 100 nM for the primary target and, on the other hand, at least tenfold selectivity against related targets. Taking drugs as the ultimate product of an affinity and selectivity optimization process, it is found that only 14.4% of them would actually qualify as chemical probes under those criteria. Therefore, if chemical probes are expected to give rise to new medicines, strict adherence to the current probe definition might result in many compounds of potential therapeutic interest being overlooked.

Defining a chemical probe

The identification of a small-molecule modulator for each individual function of all human proteins has been proposed as one of the grand challenges for chemical biology in the years to come [1]. Such an ambitious goal can only be achieved through large-scale coordinated initiatives involving chemical and screening centers at a multinational level. Recently, centers from 12 countries have agreed to combine efforts in assembling the European Infrastructure of Open Screening Platforms for Chemical Biology (EU-OPENSCREEN), which is currently halfway through its preparatory phase (2009–2011) [2]. In a much more advanced stage, the US National Institutes of Health (NIH) has completed the pilot phase (2004–2008) of its Molecular Libraries and Imaging initiative comprising ten high-throughput screening centers known as the Molecular Libraries Screening Centers Network (MLSCN) [3,4]. Within those five years, a Molecular Libraries Small Molecule Repository was created and screened against an impressive total of 691 assays, covering 171 targets and 29 phenotypic screens [5]. All the interaction data between small molecules and biochemical assays generated during this period have been deposited and made publicly accessible in PubChem [6].

The focus of these chemical biology initiatives is the identification of probe molecules to be used in basic research of biological systems. In this respect, the screening centers of the MLSCN have already collectively nominated 64 bioactive small molecules as chemical probes (see Glossary), most of which were considered to be of medium to high confidence by a panel of medicinal chemistry and drug discovery experts [5]. However, making a decision on the exact range of values for the physicochemical and pharmacological properties that a small molecule should have to be considered a useful research tool for biology is difficult and, consequently, the definition has evolved naturally over the past few years. According to the latest definition in use by the MLSCN, compounds to be nominated as chemical probes should comply with the following criteria: affinity below 100 nM (pAffinity > 7) for the primary target and at least tenfold selectivity (pSelectivity > 1) against related targets [5]. These potency and selectivity criteria are arguably the optimal ones, but they seek to establish a certain level of confidence that the biological response observed upon use is due to the interaction with the assigned primary target rather than additional, often unsuspected, interactions with multiple other proteins [7].

Even though the aim of chemical biology initiatives is not to deliver clinically useful compounds, it is clearly expected that the chemical probes being identified are optimized by chemists to translate basic research discoveries into therapeutics [3]. With these expectations in mind, it is important to consider the mounting evidence that therapeutic efficacy is better attained via the modulation of multiple proteins rather than through selective interaction with a single target [8–12]. In the context of drug discovery, therefore, current potency and selectivity criteria for the nomination of chemical probes might need to be reassessed, mainly to ensure that compounds with affinity profiles of potential therapeutic interest are not overlooked.

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GLOSSARY

Chemical probe A small molecule with the ability to perturb one or multiple components of a protein system giving rise to a unique biological response. Under a systems perspective, polypharmacology balances the relevance of potency and selectivity when deciding whether a small molecule qualifies as a chemical probe. In this respect, multiple pharmacological profiles might actually converge into the same biological response and, thus, they might all be considered redundant chemical probes; by contrast, similar pharmacological profiles with different relative affinities might result in essentially distinct biological responses.
Single probe [5] A small molecule with affinity $pA_1 > a$ for its primary target and selectivity $pA_1 - pA_i > s$ for any other protein $i \neq 1$, with ideally $pA_{i\neq1} \leq a$. According to the latest definition in use by the MLSCN, a = 7 and s = 1.
Multiple probe A small molecule with affinity $pA_{\{n\}} > a$ for a set of {n} targets and selectivity $\min(pA_{\{n\}}) - pA_i > s$ for any other protein $i \neq \{n\}$, with ideally $pA_{i\neq\{n\}} \leq a$. To be consistent with the current definition of a single probe, the values of the parameters are also a = 7 and s = 1.

Do drugs qualify as chemical probes?

Drugs constitute the privileged minute portion of chemical space that has been thoughtfully optimized to attain therapeutic efficacy and thus, they can be considered the most representative set of small molecules that act as a chemical perturbation on a protein target in the context of a biological system. Accordingly, it would be interesting to explore to what extent currently known drugs fulfil current potency and selectivity criteria for chemical probes.

To investigate this aspect, a set of 2548 drugs was compiled from four major public resources, namely ChEMBLdb (http://www.ebi.ac.uk/chembldb), PDSP [13], BindingDB [14] and IUPHARdb [15], from which 19 250 drug–target interactions covering 1243 individual targets were extracted (see Supplementary Fig. 1 in the Supplementary Data online). It is worth stressing that these repositories contain mainly drug–target interaction data originally generated in a large variety of laboratories that ultimately reported them in multiple bibliographic sources and thus, heterogeneity and consistency of interaction data is an issue. In this respect, if a drug had different values of the same interaction type for exactly the same target interaction (either within the same database or across databases), an average interaction value was assigned. Four interaction types were considered: pK_i, pK_d, pIC_{50} and pEC_{50}. A systematic analysis of the variations found in compounds with multiple interaction data of the same type for the same target revealed an average standard deviation of approximately 0.5 log units, irrespective of the value range. In the end, a total of 3618 drug entries with consistent interaction data over one or multiple targets were compiled, comprising 1633 entries with consistent pK_i data, 1609 with pIC_{50} data, 331 with pK_d data and 45 with pEC_{50} data, one drug having the possibility of being represented by more than one drug entry of consistent interaction data (Supplementary Fig. 1). This means that, on average, each drug entry is currently defined by approximately five target interactions of consistent type.

Among the 3618 entries, 1365 corresponded to drug entries from 1204 drugs for which only interaction data of some type on a single target were available and thus selectivity criteria could not be applied to them. Therefore, focus was given to the remaining 2253 drug entries from 1662 drugs for which interactions of the same type for multiple targets were available in the public domain. From an interaction type perspective, they include 1083 (48.1%), 1030 (45.7%), 126 (5.6%) and 14 (0.6%) entries composed of consistent pK_i, pIC_{50}, pK_d and pEC_{50} data, respectively; from a target coverage perspective, they contain 572 (25.4%), 856 (38.0%) and 825 (36.6%) entries with consistent interaction data for two, three to five and more than five targets, respectively. For the sake of clarity, note that among these 1662 drugs, 318 also had interaction data of a different type on a single target and thus were included within the set of 1204 drugs with single-target interaction data.

The next step was to filter out all drug entries not having among their interaction data a value of pAffinity > 7 for at least one target. A total of 1293 drug entries from 974 drugs remained, with almost 55% of them (706) being composed entirely of pK_i data. With respect to completeness, they contain 273 (21.1%), 440 (34.0%) and 580 (44.9%) entries with consistent interaction data for two, three to five and more than five targets, respectively. Finally, applying the filter of pSelectivity > 1 between their primary target and any other target for which interaction data are available, one is left with 414 drug entries representative of 367 drugs, with more than 57% of them (237) containing pK_i data only. From a completeness perspective, they contain 153 (37.0%), 156 (37.7%) and 105 (25.4%) entries with consistent interaction data for two, three to five and more than five targets, respectively.

In summary, starting with a total of 2548 drugs for which interaction data were available from public sources, only 1662 drugs (65.2%) contained data for more than one target, of which 974 drugs (38.2%) fulfilled current potency criteria and 367 (14.4%) complied with both potency and selectivity criteria for chemical probes. Most interestingly, it was found that the percentage of drugs that qualify as chemical probes is severely reduced as more information on affinity data is available. As can be observed in Fig. 1, 26.7% of drugs with known affinity for two targets would be nominated as chemical probes, whereas this percentage is reduced to just 12.7% for drugs with known affinity for more than five targets. In addition, imposing pAffinity > 7 for at least one target, 56.0% of drugs with known affinity for two targets would then qualify as chemical probes, whereas this value is drastically reduced to 18.1% for drugs with known affinity for more than five targets. These results emphasize the relevance of data completeness and, thus, the need to perform extensive screenings on multiple targets for chemical probe nomination [7].

A detailed analysis of the 367 drugs fulfilling the current chemical probe criteria for potency and selectivity revealed that they can be classified in two different classes. The largest class is composed of 247 drugs that are characterized by having pAffinity < 7 for any target other than the primary target. Escitalopram is a representative example of this class of drug chemical probes. As illustrated in Fig. 2, this antidepressant has a strong affinity ($pK_i = 8.78$) for the sodium-dependent serotonin transporter and shows high selectivity over the rest of targets for which interaction data are available, the largest affinity among those ($pK_i = 5.91$, for the muscarinic acetylcholine receptor M_1) being clearly below current potency criteria for chemical probes.

In addition, a set of 120 drugs constitutes another class that share the property of having pAffinity > 7 for one or more secondary targets. Nortriptyline is a representative example of this second class of drug chemical probes. As can be observed in Fig. 2, this second-generation tricyclic antidepressant presents strong affinity ($pK_i = 8.85$) for the sodium-dependent norepinephrine transporter and despite showing more than tenfold selectivity against the rest of targets for which interaction data are available, the

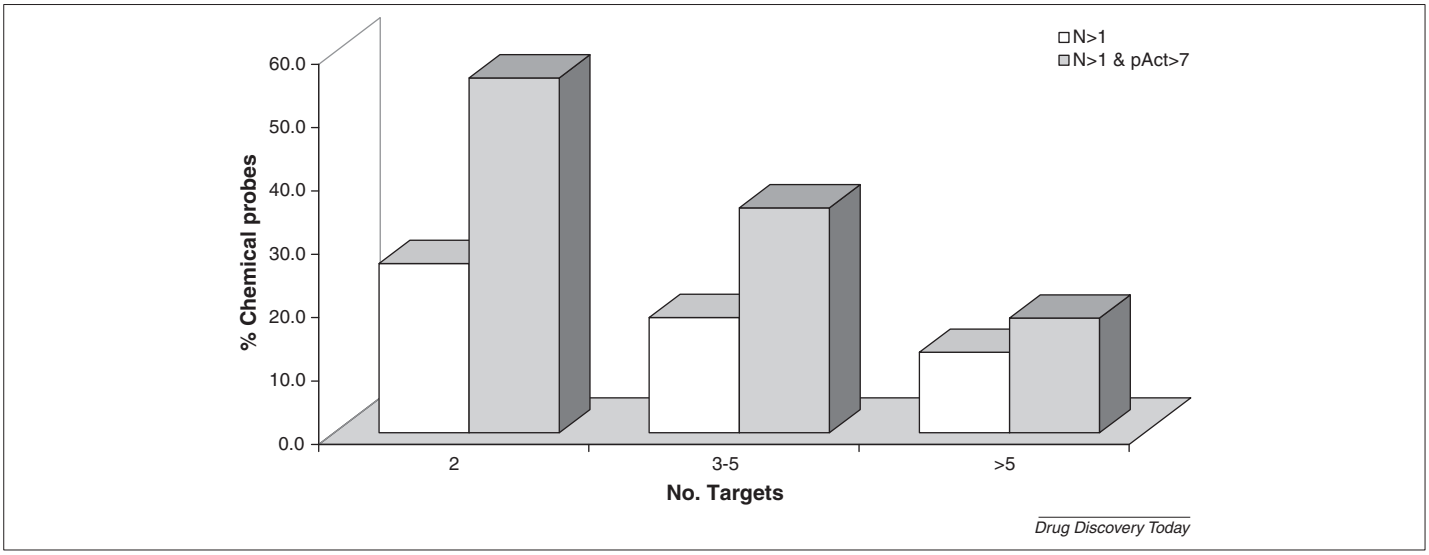

FIGURE 1

Probability of drugs to qualify as chemical probes depending on information known about their target profile (see text for details).

affinities for the sodium-dependent serotonin transporter ($pK_i = 7.71$), serotonin receptor 5-HT_{2A} ($pK_i = 7.59$) and histamine H_1 receptor ($pK_i = 7.22$) are still above the affinity threshold defined currently by chemical probe criteria. This example highlights the fact that besides potency and selectivity criteria, one might need to define additional criteria for the maximum affinity on secondary targets because, in the context of a biological system, those affinities can be highly relevant and ultimately influence the biological response observed.

The distribution of the 414 drug entries qualified as chemical probes in the space defined by current potency and selectivity criteria is presented in Fig. 3. Each circle represents a drug entry and varies with size and color: size is related to the amount of information available for each drug entry (small, medium and large circles mark drug entries with consistent interaction data for two targets, three to five targets and more than five targets, respectively), and color is associated with the major protein families of therapeutic relevance, with red, blue and yellow identifying drug entries for which the primary target is an enzyme, a G-protein-coupled receptor (GPCR) or neither of the two. The dashed line marks the boundary between the two different classes of drug chemical probes defined above: those below the line correspond to the 124 drug entries from 120 drugs that, despite complying with the selectivity criteria, have pAffinity > 7 for a protein other than the primary target.

With respect to the amount of information available, it is observed that whereas 70.5% of the large circles are located within the range of low pSelectivity values between 1 and 2, 83.1% of the circles above a pSelectivity value of 2 correspond to small- and medium-sized circles. These results emphasize again that data completeness and our perception of drug selectivity are somehow related. With respect to protein families, almost half of the drug chemical probes (44.7%) have as their primary target a GPCR protein. However, its distribution across the affinity–selectivity plane does not follow any particular trend. With the caution that data completeness imposes, based on information currently available from public sources one could conclude that the notion that drugs targeting enzymes are more selective than those targeting GPCRs or proteins from other families seems not to apply, and examples of selective drugs from the different families can be found.

Beyond probing a single target

The application of the current potency and selectivity criteria for nominating chemical probes aims to identify small molecules with high affinity for one target and clear selectivity over any other protein. Accordingly, such target-directed chemical probes will be hereafter referred to as 'single probes'. As discussed above, on the basis of currently available public interaction data, a total of 367 drugs can be qualified as single probes, representing only 14.4% of the total number of drugs considered in this study. Close inspection of the remaining 85.6% revealed that 886 drugs (34.8%) have exclusively known interaction data for one target only; 688 drugs (27.0%) have interaction data for more than one target but none with pAffinity > 7; 355 drugs (13.9%) have interaction data for more than one target and pAffinity > 7 for at least one target but could not meet the selectivity criteria; and a final set of 252 drugs (9.9%) have interaction data for more than one target and pAffinity > 7 for more than one target, do not meet the selectivity criteria within any of the targets for which pAffinity > 7, but do meet the selectivity criteria then against any remaining target. This last set of drugs will be referred to as 'multiple probes'.

An example of a multiple probe drug is trifluprom azine. As illustrated in Fig. 4, this antipsychotic has a strong affinity ($pK_i = 8.68$) for the dopamine D_2 receptor. For trifluprom azine to qualify as a single probe, the affinity with any other target should have a pK_i value smaller than 7.68 (dotted line). However, it also shows high potency ($pK_i = 8.4$) for the serotonin receptor 5-HT_{2A}, well within the selectivity window, and thus this target is added to the list of targets probed by this drug. At this stage, for trifluprom azine to qualify as a multiple probe, the interaction with any remaining target should be less than 7.4 (dashed line). Indeed, among the additional interaction data known at present, the most potent affinity for a target is below that threshold ($pK_i = 7.28$ for the histamine H_1 receptor, HRH1). Therefore, trifluprom azine would be finally nominated as a multiple probe of the probing profile defined by the dopamine D_2 receptor and the serotonin receptor 5-HT_{2A}. Because, on the basis of the information currently available, the number of probing targets in this case would be two, tri-

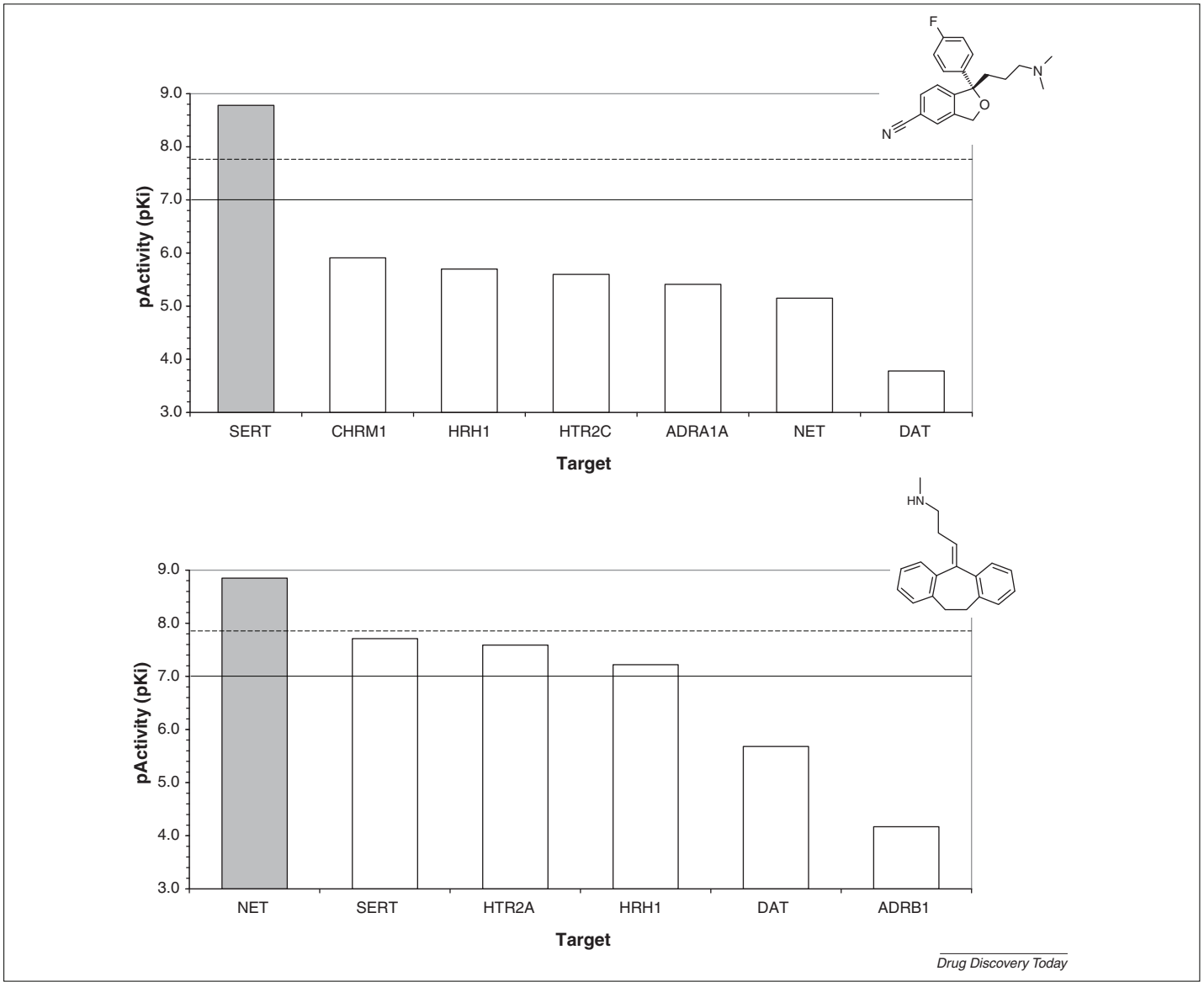

FIGURE 2

Escitalopram (top) and nortriptyline (bottom), two examples of drugs designated as chemical probes of the sodium-dependent serotonin (SERT) and norepinephrine (NET) transporters. Solid and dashed lines mark the affinity and selectivity thresholds, respectively.

flupromazine would be referred to as a multiple probe of level 2. This notwithstanding, probe level designation should be continuously reassessed as more information on the target profile becomes available.

Extending the analysis beyond drugs, our interest turned then to identifying all chemical probes present among all small molecules contained in the four major public resources of interaction data (http://www.ebi.ac.uk/chembldb) [13–15], as a means to assess the current coverage of probing profiles. In total, 34 460 small molecules qualified as chemical probes, of which 27 459 are single probes for 527 targets and 7001 are multiple probes for 959 distinct target profiles. The distribution of the number of target profiles currently covered at each probe level is illustrated in Fig. 5. Contrary to the expected combinatorial explosion of possible profiles upon increasing the number of targets, current coverage of probing profiles decreases rapidly as the probing level increases. Thus, while multiple probes for 439 probing profiles of two targets could be identified, only 61 probing profiles of five targets are currently covered. This emphasizes the traditional focus towards generating highly potent and selective compounds for a single target rather than pluripotent compounds over combinations of multiple targets.

In addition, probing profiles were assigned to major protein families on the basis of their constituent targets. In the case that all probing targets are enzymes or GPCRs, the probing profile is assigned to enzymes or GPCRs, respectively. All other probing profiles contain probing targets that belong to any of the other major therapeutic families (e.g. ion channels or nuclear receptors) or are simply a combination of targets from different families. As can be observed, enzyme probing profiles seem to be more populated than GPCR and other probing profiles at probe levels from 1 to 5, whereas probing profiles containing combinations of targets belonging to different families seem to be the most common option for probing profiles composed of more than five targets. For example, representative single probes (probe level 1,

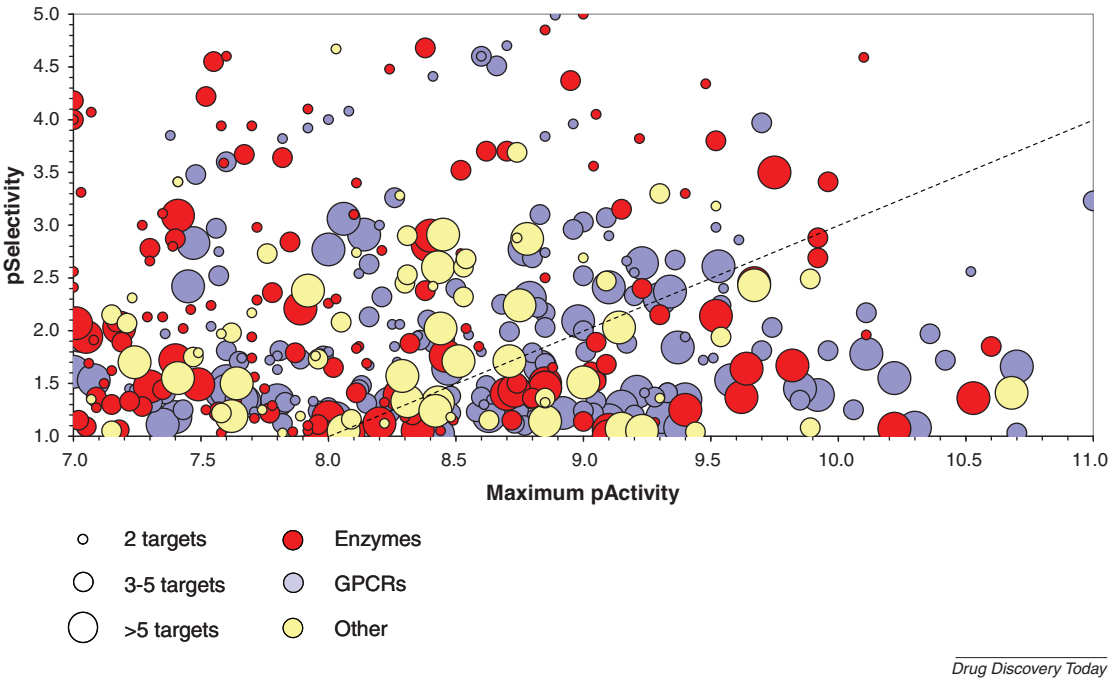

FIGURE 3

Distribution of chemical probes in the space defined by current potency and selectivity criteria. Size of circles denotes degree of information available. Color of circles relates to the major protein family associated with the primary target. The dashed line marks the boundary between the two classes of chemical probes (see text for details).

such as the ones in Fig. 2) were identified for 295 enzymes, 131 GPCRs and 101 other proteins, whereas representative level 2 multiple probes (probe level 2, such as the one in Fig. 4) were identified for 208 combinations of two enzymes, 136 combinations of two GPCRs and 95 combinations of any two targets belonging to different families. Again, it should be stressed that the high degree of incompleteness of public interaction data [7] means the present conclusions should be taken with caution; improving data completeness might promote some small molecules to chemical probes but could disqualify others.

Towards a complete probing chemome

The analysis presented above on current coverage of probing profiles points to the fact that,

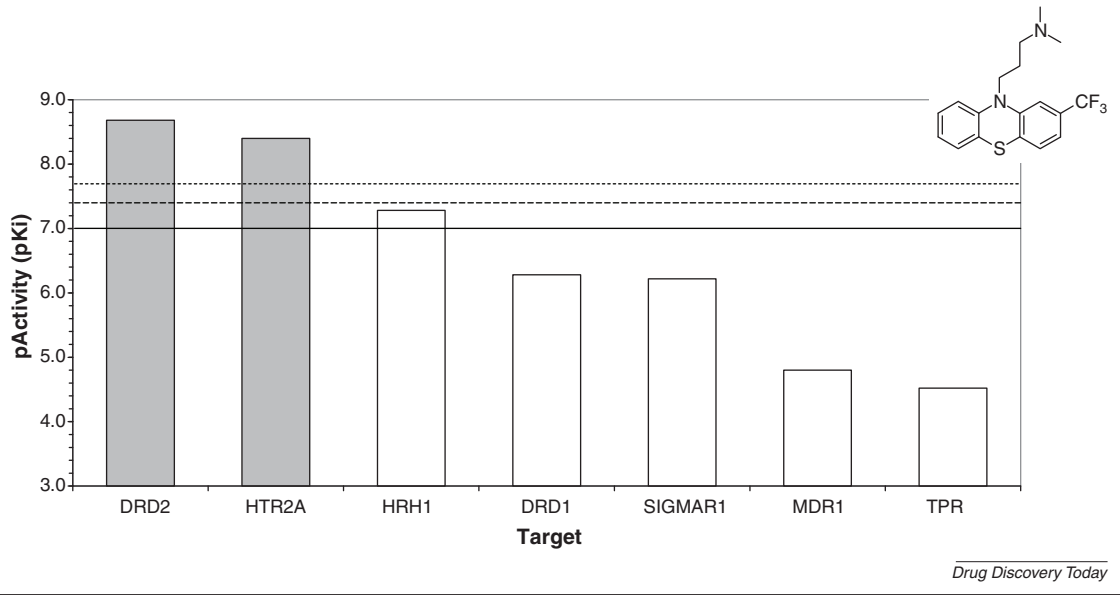

FIGURE 4

Trifluprom azine, an example of a drug designated as a level 2 multiple probe of the target profile defined by the dopamine D_2 (DRD2) and serotonin 5-HT_{2A} (HTR2A) receptors. Solid line marks the affinity threshold, and dotted and dashed lines mark the selectivity thresholds relative to DRD2 and 5-HT2A, respectively.

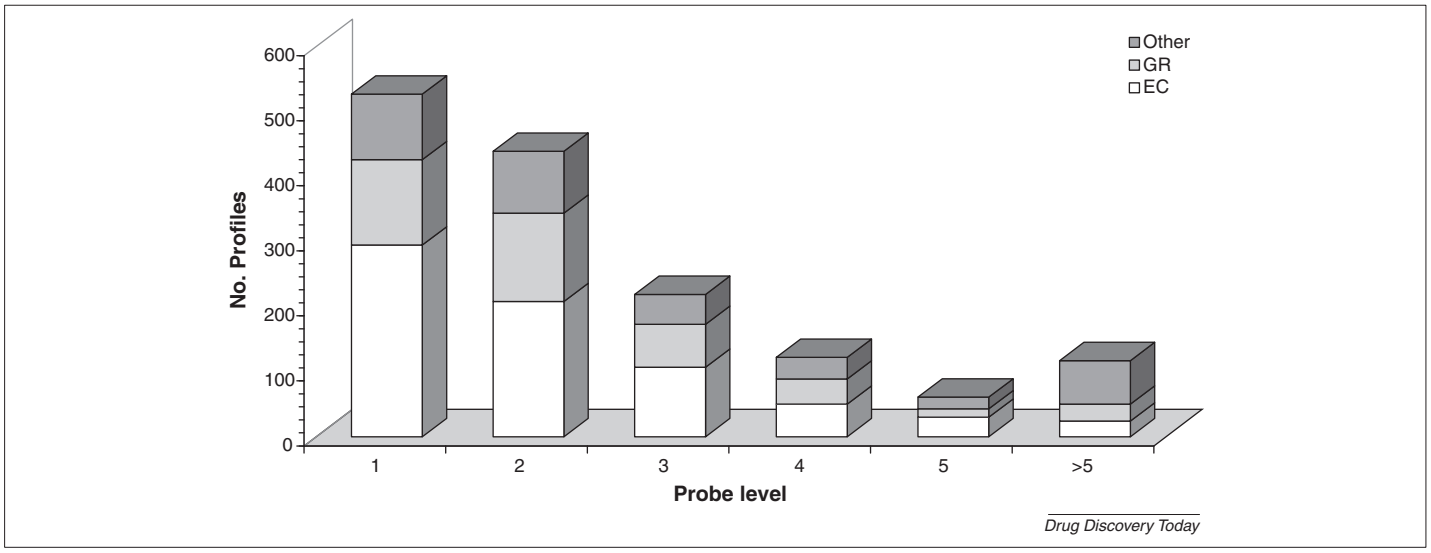

FIGURE 5

Number of target profiles covered by chemical probes identified in public databases. For any given chemical probe for which a profile of interaction data is known for $N > 1$ targets, a probe level of $n < N$ indicates that there are at least n targets for which the compound has pAffinity > 7.0 and that these n targets show pSelectivity > 1.0 over the remaining N–n targets in the profile.

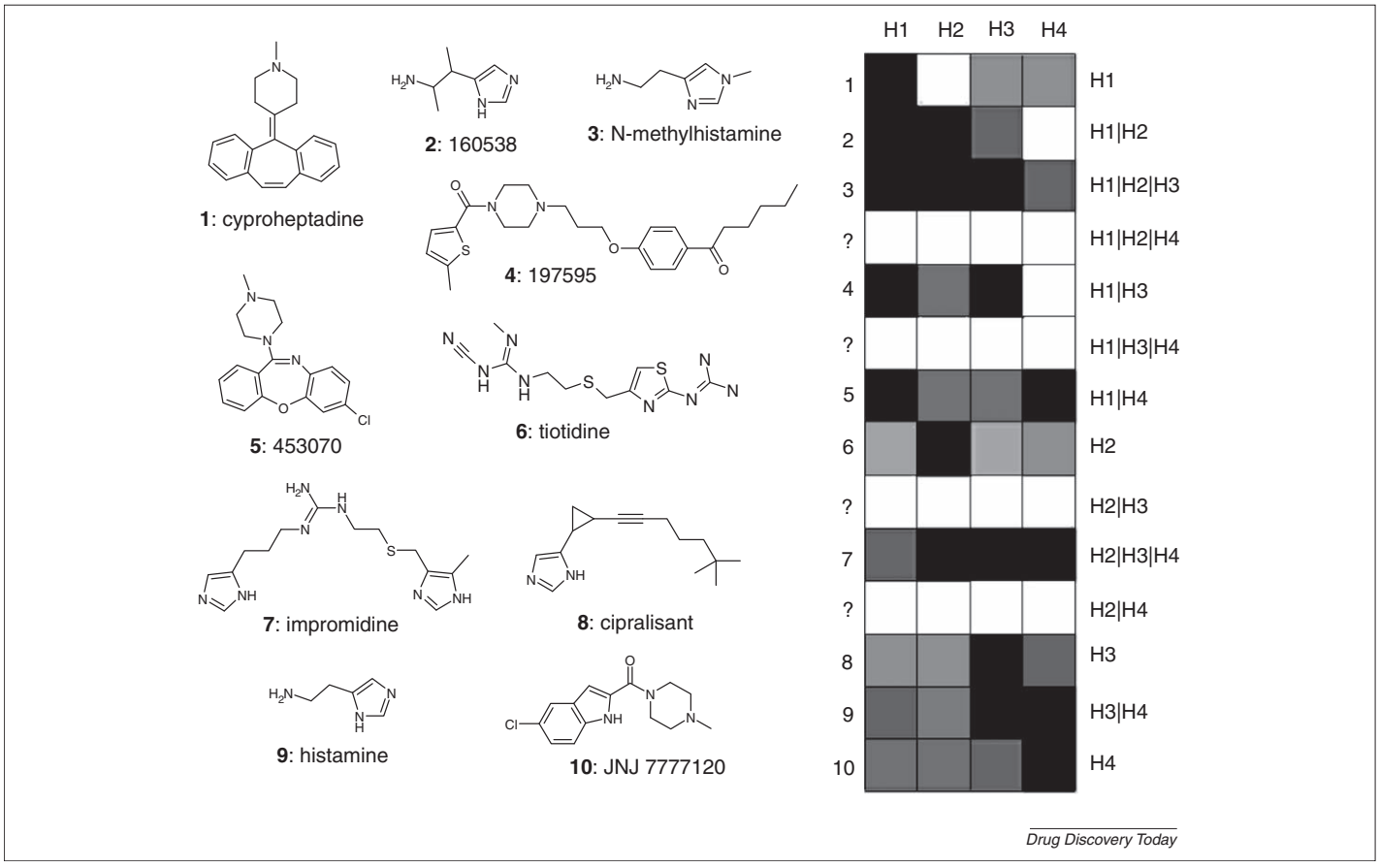

FIGURE 6

Current level of coverage for a complete chemical probing of the family of histamine receptors. Gray gradation towards black reflects increased potency; white cells indicate current lack of information. Labels on the right indicate all possible combinations of probing profiles. Structures of molecules representative of each probing profile are given on the left. Molecules are identified either by their short name or by their ChEMBLdb identifier (compounds 2, 4 and 5) (http://www.ebi.ac.uk/chembldb). The four current probing gaps are indicated with question marks.

beyond chemical probes selective for single targets, small molecules exist that probe multiple targets in a selective manner. This observation establishes the basis for the systematic probing of biological targets at a systems level. The coordination of this rather ambitious challenge could be addressed effectively by focusing on achieving complete probing of segments of biological systems, such as all members of a protein family or all proteins of a biochemical pathway. As an illustrative example, Fig. 6 contains the current probing status of the histamine receptor family.

Histamine receptors are a class of GPCRs composed of four members, namely H1, H2, H3 and H4 [16]. Accounting for all possible target combinations, complete affinity probing of this entire class would require the identification of 14 chemical probes: four single probes, having high affinity for each of the individual members and selectivity over the rest, and ten multiple probes, covering all probing profiles that can be generated from targeting several receptors at a time. By exploring the current information contents of the major public repositories of interaction data, small molecules covering ten out of the fourteen probing profiles could be identified, five drugs being among them. The structures and affinity profiles of these molecules are collected in Fig. 6. In the interaction map provided, each row corresponds to a different combination of probing targets, and the potency of small molecules for each receptor is reflected by a color gradation: black indicates high potency, light gray indicates weak potency and white indicates interaction data for which no information is currently available in the public domain.

In all cases for which a representative compound is provided, drugs were given priority to other small molecules that could fit the potency and selectivity criteria for probing a given profile. Among them, cyproheptadine, tiotidine, ciproxifan and JNJ 7777120 [17] were selected as the single probe representatives of the H1, H2, H3 and H4 probing profiles, respectively. In addition, N-methylhistamine and impromidine were found to fit the potency and selectivity criteria as multiple probes for the probing profiles defined by the H1, H2 and H3 receptors and the H2, H3 and H4 receptors, respectively. It should be stressed that all molecules selected as chemical probes of the respective target profiles were identified on the basis of their affinities for the histamine receptors. Some of them were found to have gaps of interaction data for all four receptors (such as probes 1, 2 and 4 for the interaction on H2, H4 and H4, respectively) and others might have affinities for proteins other than histamine receptors. The main purpose of the current selection is to illustrate that it is conceptually feasible to generate a complete set of small molecules that cover all possible profile combinations arising from a given set of targets representative of a particular segment of a biological system, that being constituting members of a protein family or biochemical pathway. In fact, it was recently shown that the application of *in silico* target screening to an academic chemical library enabled the identification of novel antagonists for all four members of the adenosine receptor family [18]. In this respect, the development of novel methodologies for the *in silico* target screening of molecules [19–21] is expected to have a considerable impact on assembling a complete probing chemome.

Concluding remarks

Biological systems are implicitly robust, and selectively acting on one particular target might not be the most efficacious way of modulating or interfering with that system [22]. Indeed, recent evidence indicates that most drugs attain their *in vivo* efficacy through the modulation of multiple targets, rather than selective interaction on a single target. In addition, drugs represent the ultimate product of a long optimization process in which potency and selectivity, among other pharmacokinetic and pharmacodynamic properties, are improved. Any chemical point less advanced in this process will, in principle, show less optimal potency and selectivity criteria. Accordingly, if only 14.4% of drugs qualify as chemical probes, a much lower percentage is expected for the starting chemical points of drug discovery projects; therefore, many compounds with therapeutic potential coming out of screening campaigns might be completely overlooked [23]. Outside this drug discovery focus, however, it should be clarified that the proposed NIH criteria for chemical probe discovery are not representative for the current probe qualification in the field of chemical biology, in which small molecules that could be considered suboptimal chemical probes under those criteria are, often, still taken to gain useful biological information [24,25].

Although in recent years drug discovery has gradually shifted away from the 'one chemical, one protein' paradigm, strict adherence to the NIH definition of a probe might encourage future chemical biology efforts to remain very much anchored in it. The conceptual change from probing a single biological target to probing an intrinsically robust biological system requires adjusting current probe criteria within each particular context to minimize the risk of missing many low-affinity, poorly selective small molecules that could well be of potential therapeutic interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.drudis.2010.11.004.

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