Using Chiral Molecules as an Approach to Address Low-Druggability Recognition Sites

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The content of chiral carbon atoms or structural complexity, which is known to correlate well with relevant physicochemical properties of small molecules, represents a promising descriptor that could fill the gap in existing drug discovery between ligand library filtering rules and the corresponding properties of the target's recognition site.

Herein, we present an *in silico* study on the yet unclear underlying correlations between molecular complexity and other more sophisticated physicochemical and biological properties. By analyzing thousands of protein–ligand complexes from DrugBank, we show that increasing molecular complexity of drugs is an approach to addressing particularly low-druggability and polar recognition sites. We also show that biologically relevant protein classes characteristically bind molecules with a certain degree of structural complexity. Three distinct behaviors toward drug recognition are described.

The reported results set the basis for a better understanding of protein–drug recognition, and open the possibility of including target information in the filtering of large ligand libraries for screening. © 2014 Wiley Periodicals, Inc.

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Introduction

Experimental and virtual high-throughput screenings are complementary, powerful, widely used techniques that have dramatically accelerated the identification of new bioactive molecules both in academia and industry.[1,2] The main strength of such methods is the possibility of discovering biologically active molecules from libraries comprising thousands and even millions of compounds.[3,4] However, to increase the value of the screening results it is strongly recommended to use knowledge-based methods for the systematic elimination of potentially reactive, promiscuous, non-bioavailable, toxic, or poorly soluble candidates in the early stages of the discovery process.[5–7] Additionally, the inclusion of structural information extracted either from the biological target under study or from collections of related bioactive and bioinactive molecules can provide a quicker path to promising candidate molecules from arbitrary compilations.[8] These structure- or ligand-based methods, however, require an expert, individual analysis of the binding site of interest and do not allow for direct extrapolation across targets.

Molecular complexity, vaguely described as a principle behind compound promiscuity and binding success rate, has gained increasing attention in the drug discovery field.[9,10] The complexity of a small molecule can be rationalized by evaluating its saturation (shape complexity, $C_{sp3}/[C_{sp2} + C_{sp3}]$) or its total content in chiral carbon atoms (stereochemical or structural complexity, $C_{stereogenic}/C_{total}$).[11] Both formulae have shown utility in the drug discovery field. For instance, Lovering et al.[12] reported that the hybridization of molecules increases during drug development. Notably, they also found that shape complexity correlated well with aqueous solubility. Clemons et al.[11] additionally showed that target specificity increases with structural complexity.

Herein, we present an *in silico* study on the neglected correlations between structural complexity and other more sophisticated physicochemical and biological properties. By analyzing thousands of protein–ligand complexes from DrugBank, we have additionally identified critical correlations between a molecule's complexity and the hydrophobic/hydrophilic character and druggability of its corresponding protein's recognition site. Finally, we have analyzed the ligand complexity demands of biologically relevant protein classes and observed characteristic, clearly differentiated structural complexity requirements among them.

Methods

The steps followed during data collection and analysis are schematically represented in Figure 1.

Selection and processing of small molecules

All small molecules from DrugBank[13] were downloaded (6583 molecules, accessed on January 15, 2014) and processed using the open-source software ChemicalToolBoX (http://ctb.pharmaceutical-bioinformatics.org) to remove counterions and fragmented entities, yielding 6224 unique chemicals. LigPrep v2.8 followed by QikProp v3.8 (Schrödinger, LLC, New York) were subsequently used to compute many physicochemical descriptors and absorption and distribution properties for the compounds. Small molecules with at least one ring system were considered for further analysis (5176 chemicals). Their

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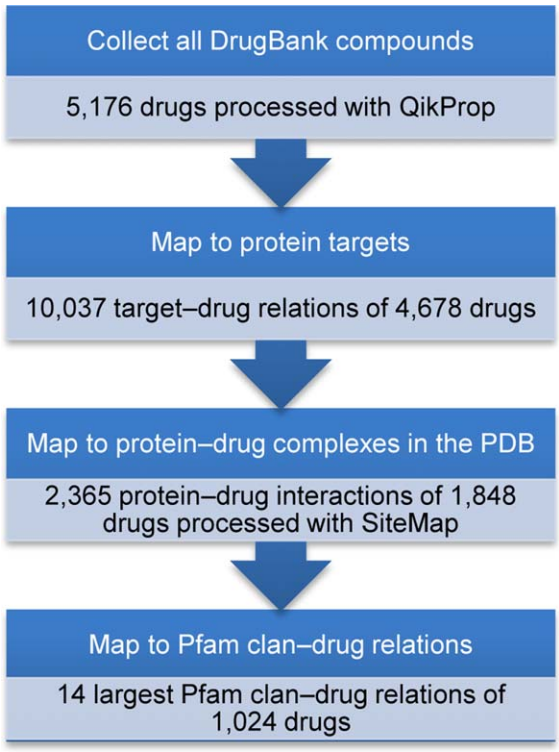

Figure 1. Workflow applied during data collection and processing. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

structural complexity ($C_{stereogenic}/C_{total}$)[11] was then calculated using an in-house Python script based on Open Babel.[14]

Structural processing of protein–drug interactions

Biological targets for the selected 5176 compounds were assigned using DrugBank,[13] yielding 10037 non-redundant gene–drug interactions of 4678 drugs. The chemical structures were mapped to Ligand-Expo[15] and to their corresponding co-crystallized protein data bank (PDB) code, yielding 3880 structures of small molecules in complex with their respective protein target(s). Each co-crystallized drug–protein interaction was processed as follows using an in-house Python program: initially, the ligand was extracted from the PDB file using PyMOL (Schrödinger, LLC); all water molecules present in the crystal structure were removed and the complete chains in contact (6.0 Å) with the ligand of interest were processed with the protein preparation wizard (Schrödinger, LLC) to subsequently fix missing side chains, protonate the system at physiological pH using Epik v2.6, optimize hydrogen-bond networks by means of PROPKA,[16] and perform an energy minimization of hydrogen atoms. The resulting structure was processed using SiteMap v2.9 (Schrödinger, LLC) to characterize the ligand binding site, and the data were collected for further inspection. Resulting structures in which the minimum distance between the centroids or atoms of the ligand of interest and the identified binding site was larger than 4.0 Å were removed based on SiteMap distance parameters (i.e., "*refdist* $>$ 4.0 OR *refmin* $>$ 4.0 OR *refavg* $>$ 4.0 OR *sitemin* $>$ 4.0"), eventually yielding 2365 interactions with structural information, which corresponded to 1848 unique ligands. The median value of each binding site property was taken for ligands with data on multiple targets.

Treatment of biological targets

Protein chains present in the 2365 drug–target interactions processed with SiteMap were assigned to a Pfam clan identifier,[17] yielding 2247 non-redundant drug–Pfam clan relations. Analyses were performed on Pfam clans comprising at least 3 UniProt IDs (Supporting Information Table S1) and that could be linked to a minimum of 30 drugs, leading to 14 clans and 1024 drug–Pfam clan relations.

Statistical analysis

Statistical significance of the two-tail pairwise difference between grouped population distributions was assessed by means of the Wilcoxon Rank Sum test with the Holm correction for multiple testing, as implemented in the statistical package R v3.0.[18] Statistical significance is denoted along the manuscript with * for p-value ≤ 0.05, ** for p-value ≤ 0.01, and *** for p-value ≤ 0.001.

Results

Physicochemical properties of small molecules correlate with their structural complexity

We collected 5176 small molecules from the DrugBank database,[13] from which their structural complexity ($C_{stereogenic}/C_{total}$)[11] and physicochemical properties were computed (Methods). Chemicals traditionally used in drug discovery, that is, drug-like compounds, are of relatively low structural complexity.[11] Accordingly, ligands in the DrugBank database are biased toward simplicity (i.e., we have identified thousands of very simple molecules and only a few tens presenting high complexity). However, representatives covering a wide range of structural complexities are comprised. Examples of drugs included in the study with diverse content in chiral carbon atoms and corresponding maximum number of stereoisomers are shown in Figure 2.

The distribution of several physicochemical properties of compounds grouped by their degree of structural complexity is included as Figure 3. Molecules with a high degree of structural complexity gather physicochemical properties different from those of most known drugs. The polar behavior of the molecules can be particularly characterized by their content in stereogenic carbon atoms. Molecules with a complexity above 40% present a markedly hydrophilic nature, as indicated by higher values of hydrogen-bond acceptor (HBA) and donor (HBD) groups, and octanol/water partition coefficient (log $P_{o/w}$). For these properties, their threshold in the ubiquitously used "Rule of Five" (ro5) for suitable solubility and permeability[7] is shown. The distributions show that molecules with high degree of complexity mainly present a number of HBA and HBD groups above their respective ro5 threshold (HBA $>$ 10 and HBD $>$ 5). This finding can be

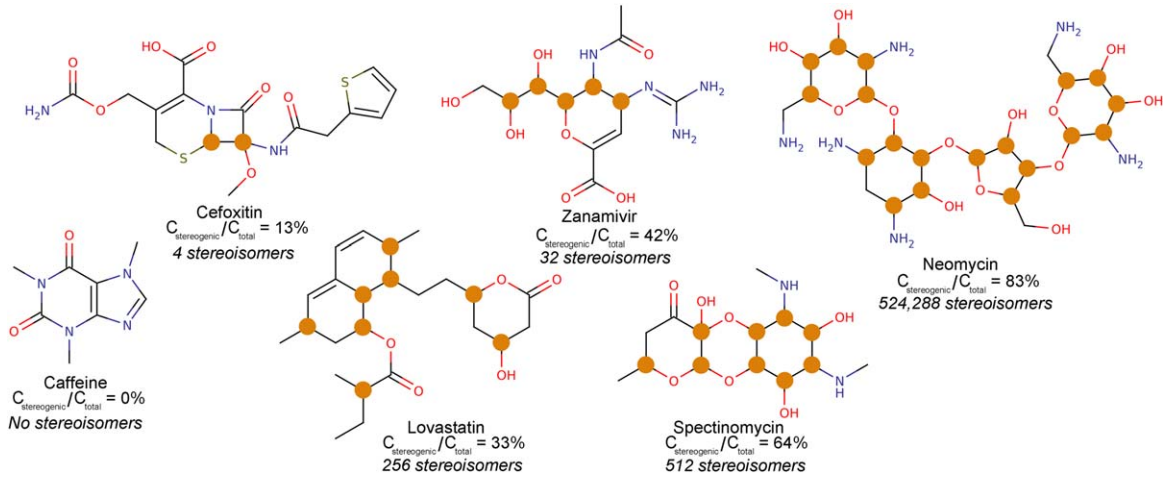

Figure 2. Examples of drugs included in the study. Stereogenic carbon atoms are highlighted in orange. From left to right, molecules present an increasing structural complexity. The maximum number of possible stereoisomers per compound is indicated. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

rationalized taking into account the demands in diverse, richly substituted carbon atoms of molecules with complex nature (Fig. 2).

Lovering et al.[12] could show that increasing saturation is a valid approach to improving clinical success and, specifically, aqueous solubility. Our results indicate that increasing chirality improves aqueous solubility as well, in accordance with the observed increase in hydrophilicity but not with the ro5 assumptions, suggesting that the increase in polar groups above the ro5 thresholds can be compensated with an

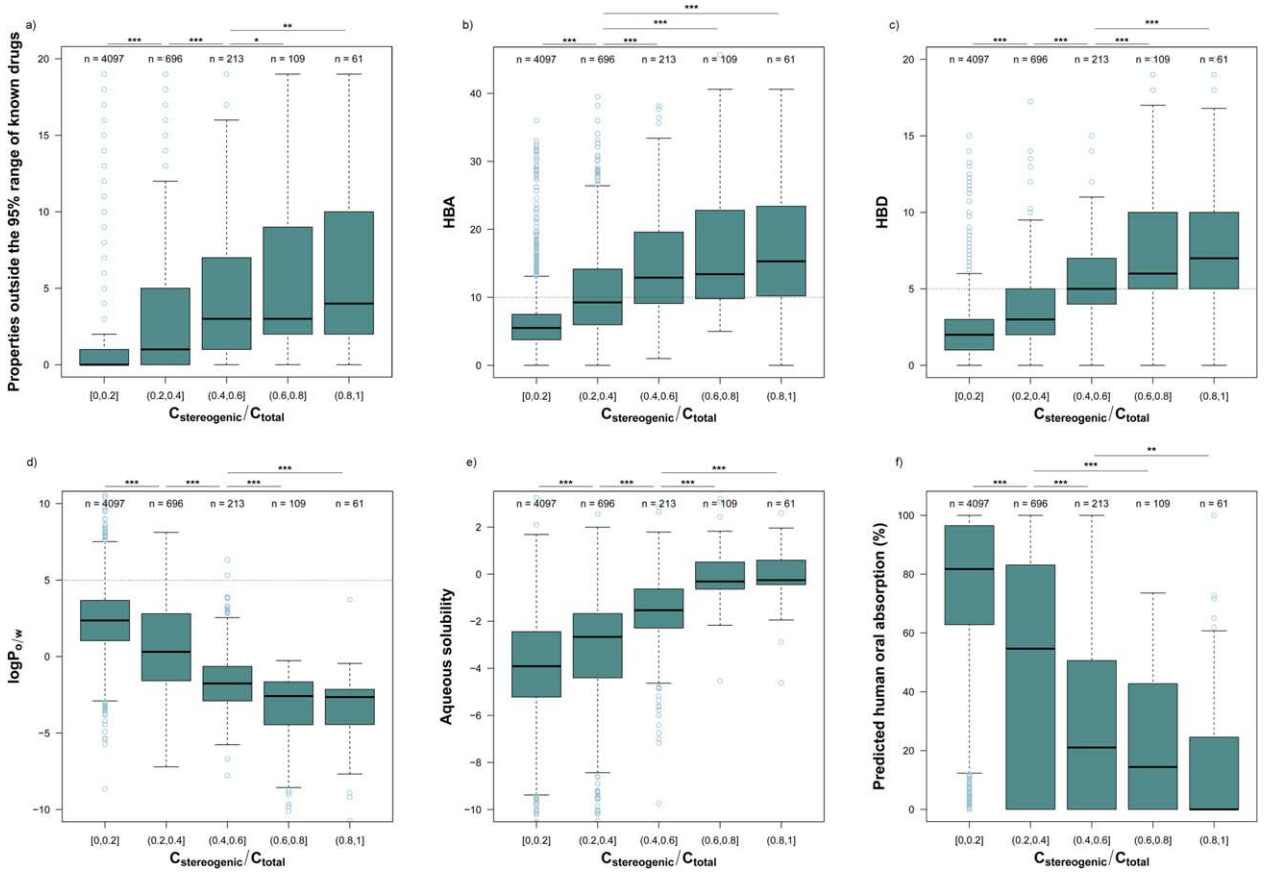

Figure 3. Boxplot distribution of physicochemical and absorption properties of DrugBank compounds grouped by degree of structural complexity. In b), c), and d), a horizontal dotted line indicates the corresponding threshold value within the ro5 (HBA $\leq$ 10, HBD $\leq$ 5, and log Po/w $\leq$ 5).[7] In a), b), c), d), and e), observations beyond the range of the plotted ordinate axis have been omitted in the representation for the sake of clarity. The number of observations per group, *n*, and the closest statistically significant different pair are indicated (Methods for details). HBA and HBD values are averages taken over a number of configurations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

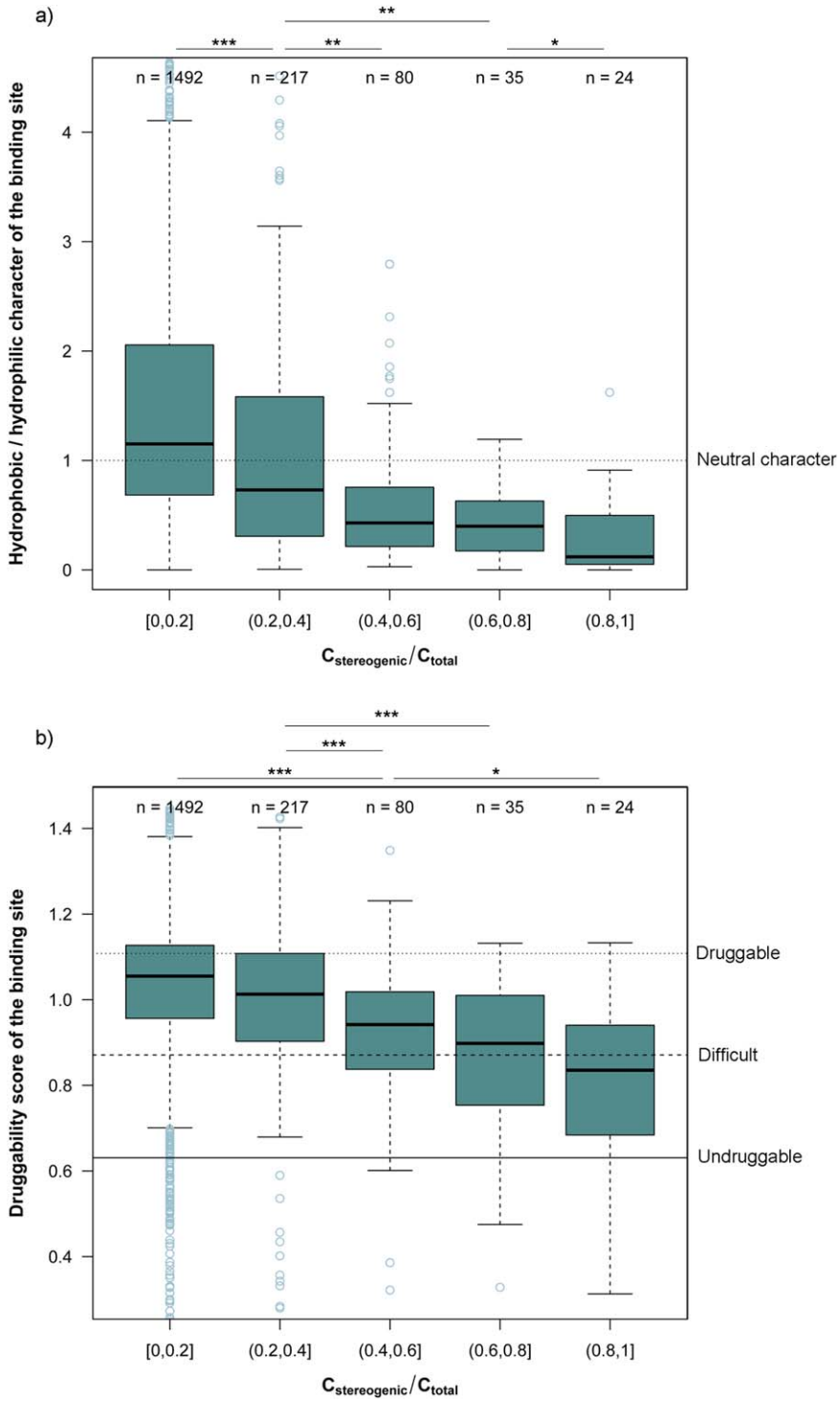

Figure 4. DrugBank compounds grouped by degree of structural complexity and related to a) hydrophobicity and b) druggability of their binding sites. In b), average values for druggable (1.108), difficult (0.871), and undruggable (0.631) targets are indicated.[21] Observations beyond the range of the plotted ordinate axis have been omitted in the representation for the sake of clarity. The number of observations per group, *n*, and the closest statistically significant different pair are indicated (Methods for details). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

increase in the total content of stereogenic carbon atoms to achieve proper aqueous solubility values.

We additionally predicted absorption and distribution properties for those molecules (Methods). The predicted human oral absorption, based on a quantitative multiple linear regression model, drops rapidly with increasing molecular complexity. It is worth noting that most small molecules with chirality content above 40% present an oral absorption of less than 50%. This observation is in agreement with the ro5 for highly polar molecules.

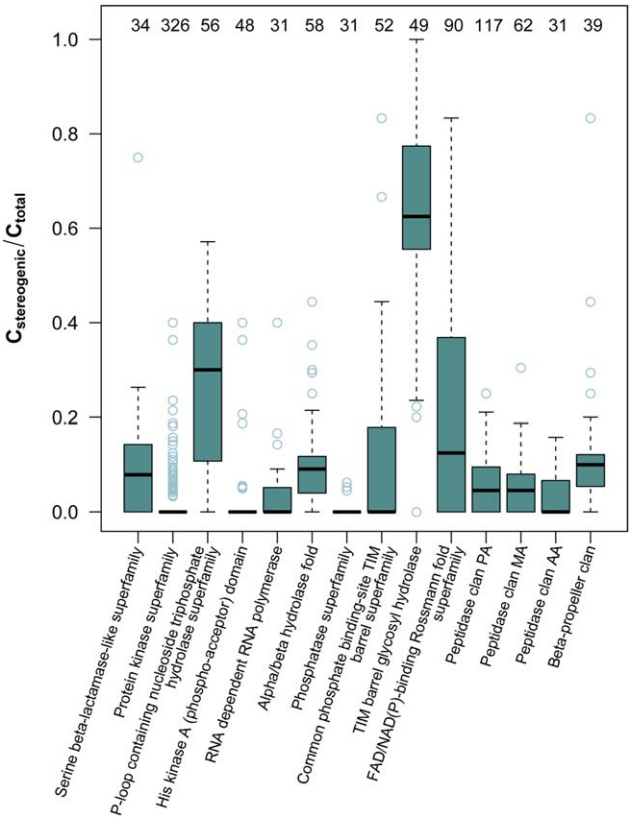

Figure 5. Boxplot distribution of the degree of structural complexity in the largest Pfam clans. The number of observations per group is indicated on the top (Methods for details). The statistically significant different pairs are identified in the Supporting Information (Table S2). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Physicochemical properties and druggability of recognition sites correlate with the structural complexity of their small molecule partner(s)

As the properties of a small molecule and its target site have a critical impact on the drug discovery process,[19,20] they can be rationally exploited to increase the discovery rate. We collected the physicochemical and druggability properties of 2365 protein recognition sites from the PDB in complex with DrugBank compounds (Methods). Distributions of the hydrophobic/hydrophilic character and the druggability score of the binding sites in terms of the grouped degree of structural complexity of their corresponding ligand(s) is reported in Figure 4. Molecules with low content of stereogenic carbons ($<$ 20%) are of hydrophobic nature. Accordingly, they address mainly hydrophobic sites in a very significant manner. Conversely, molecules with more than 40% chiral carbon atoms are polar and are recognized by hydrophilic binding sites. We have further analyzed the druggability of protein sites. It has been reported that the screening of underexplored regions of the chemical space is fundamental when modulating "undruggable" targets.[22] Druggable sites (identified with higher druggability scores) favor the interaction with achiral molecules, and, *vice versa* lower druggability scores correlate with more complex molecules.

Protein classes bind molecules with a certain degree of structural complexity

We classified 2365 protein recognition sites into Pfam clans (Methods), which encompass Pfam families arising from a single evolutionary origin,[17] and studied the structural complexity of their corresponding small molecule partners (Fig. 5 and Supporting Information Tables S1 and S2). Among the largest 14 clans, which include relevant drug discovery superfamilies, significant differences exist in terms of the corresponding drugs' chiral content. Most clans preferably bind simple small molecules (structural complexity below 20%): for example, kinases and phosphatases are specifically targeted by achiral compounds, as well as peptidases and β-lactamases, which attract simple small molecules. Other clans preferably interact with medium complexity molecules. That is the case of the nucleoside triphosphate hydrolase and the FAD/NAD(P)-binding Rossmann fold superfamilies. Remarkably, the glycosyl hydrolase superfamily binds molecules comprising more than 60% stereogenic carbon atoms.

Discussion

Increasing complexity as an approach to address low-druggability recognition sites

Rules of thumb exist to preselect potential drug-like and fragment-like molecules from large datasets.[7,23] Those rules, however, do not incorporate any advanced prior knowledge regarding the biological target of interest. There have been several attempts to identify common features of binding sites addressed by specific compound classes.[24] This has been particularly the case in fragment-based drug discovery (FBDD).[25,26] The general principle behind the higher hit rate of this technique in comparison to traditional small molecule screening is that lower MW compounds have higher chances of promiscuous binding, due to their lower possibilities of undesirable interactions with a protein site's features. Chen and Hubbard presented a thorough analysis of FBDD campaigns. By means of SiteMap (Schrödinger, LCC), they could show that the hit rate of FBDD experiments was strongly dependent on the physicochemical properties of a protein's recognition site and its druggability.[25]

In the light of the described correlations between physicochemical properties of small molecules and their content in chiral carbon atoms, we have shown that a molecule's degree of structural complexity is imprinted in the surrounding surface of the corresponding protein's recognition site (Fig. 4). Hydrophobic, druggable binding sites recognize achiral or poorly stereogenic molecules and, *vice versa* polar, undruggable binding sites recognize richly stereogenic molecules. These findings have extraordinary impact on the drug discovery field. Here, the content in chiral carbons is exhibited as a powerful rationale for the preselection of candidate molecules for screening based on the druggability or hydrophobic/hydrophilic character of the target site of interest. In contrast to other physicochemical descriptors, such as log $P_{o/w}$ or polar surface area, the structural complexity is an objective, easy-to-

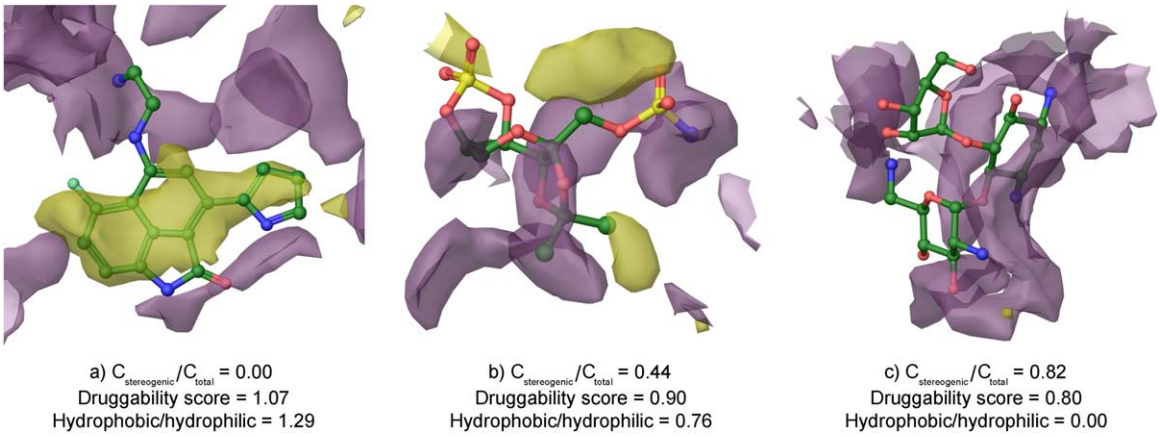

Figure 6. Examples of complexity-driven drug–target interactions. Yellow and purple volumes indicate, respectively, regions of the protein surface with hydrophobic and hydrophilic demands (Methods). The drugs' structural complexity and the hydrophobic/hydrophilic character of each binding site and its druggability score, computed using SiteMap (Schrödinger, LCC) are indicated. a) Structure of human cyclin-dependent kinase 2 (CDK2) in complex with a trisubstituted naphthostyril inhibitor (PDB ID: 1P2A; DrugBank ID: DB07163). b) Structure of human carbonic anhydrase II in complex with an anticonvulsant sulfamate derivative (PDB ID: 1EOU; DrugBank ID: DB02894). c) Structure of aminoglycoside 2′-*N*-acetyltransferase from *Mycobacterium tuberculosis* in complex with the antibiotic ribostamycin (PDB ID: 1M4G; DrugBank ID: DB03615).

compute descriptor, making it specifically useful for the inexpensive filtering of large compound collections to generate focused ligand libraries. Moreover, the molecules shown in Figure 2 suggest that the structural complexity is independent of a compound's size. We have studied the correlation of the content of chiral carbons and the volume of either the small molecule or its target binding site (Supporting Information Fig. S3). Remarkably no trend exists, indicating that the filtering of compound libraries in terms of structural complexity could be complementary to other rules that incorporate molecular size, for example, Rule of Three and ro5.[7,23]

Moreover, increasing the structural complexity of small molecules is an approach to addressing particularly low-druggability targets (Fig. 4). This is in agreement with Dandapani and Marcaurelle,[22] who reported that the screening of underexplored regions of the chemical space is fundamental when targeting "undruggable" proteins. Notably, molecules traditionally used in drug discovery are of low structural complexity, indicating that the currently neglected chiral chemical space might comprise very promising drug candidates capable of modulating low-druggability proteins. The binding site's polar requirements of some representative molecules with varied degree of structural complexity are depicted in Figure 6. A clear trend of decreasing hydrophobicity can be observed for more complex molecules (shrinking yellow volumes). The achiral, mainly hydrophobic kinase inhibitor shown is attracted to an equally hydrophobic, planar, druggable recognition site. The higher demands of polar substrates at the recognition site of the human carbonic anhydrase II are, as expected, preferably satisfied with a more complex agent (Fig. 6b). Finally, mostly polar, low-druggability cavities impose geometric constraints that can be only satisfied by engaging with drugs featuring specific, localized, richly decorated polar substitutions at many chiral centers. Complex natural products, such as the glycoside antibiotic ribostamycin (Fig. 6c), are specifically and uniquely fitted to address those binding sites.

Structural complexity as an approach to rationalizing evolutionarily drug–protein class recognition phenomena

We have shown that recognition sites interact preferably with small molecules that fulfill specific complexity demands (Figs. 4 and 6). Thus, binding sites with similar physicochemical properties, especially druggability score and hydrophobic/hydrophilic character, are expected to interact with compounds of similar degree of complexity. The classification of DrugBank targets into Pfam clans (Methods) indicates that protein classes markedly recognize molecules with a certain degree of structural complexity (Results, Fig. 5 and Supporting Information Table S1). Representative Pfam clan members in complex with small molecules are shown in Figure 7. Kinase and phosphatase superfamilies exclusively interact with very simple ligands. Kinases have been traditionally targeted with achiral molecules. Nonetheless, increasing chirality has been suggested as an approach to widening the catalog of kinase inhibitors.[27] Our findings show that the historical success of achiral or relatively simple molecules to address kinases is not accidental but a result of their hydrophobic, druggable recognition site. Inversely, we have identified Pfam families that preferably bind highly complex molecules. This is the case of enzymes acting on glycosyl moieties, such as the TIM barrel glycosyl hydrolase superfamily. Finally, we have also discovered Pfam families with an ambivalent character: they bind small molecules presenting a wide range of structural complexities. The case of the NADP-binding Rossmann fold superfamily is shown in Figure 7c. Not only can this family bind molecules with diverse degrees of complexity but also they are recognized by a similar region of the enzyme. A superposition of the human prostaglandin reductase 2 and the estradiol-17-β-dehydrogenase 1 shows that the Rossmann fold region is significantly conserved. Yet, the substrate recognition site has evolved divergently to accommodate both types of molecules and no conservation of the secondary structure exists.

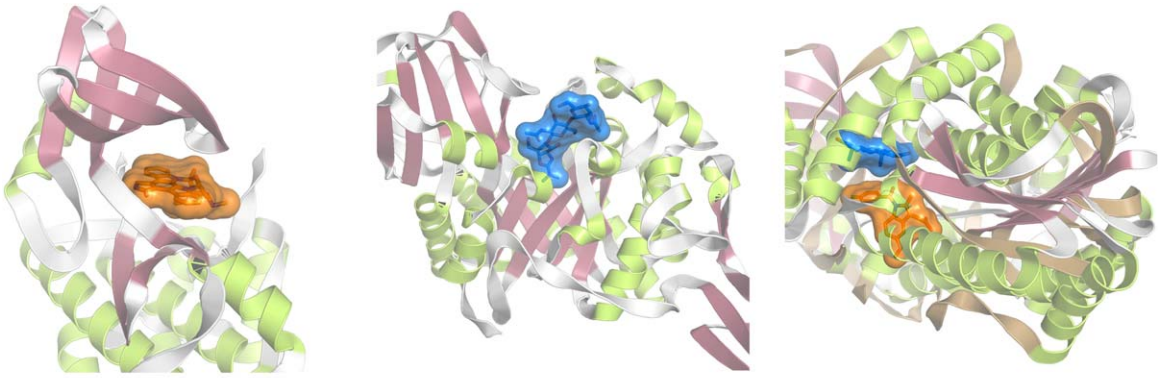

Figure 7. Diverse complexity demands identified among the largest Pfam clans. Secondary structure elements are colored in lemon and raspberry for α-helixes and β-sheets. Small molecules are colored according to their degree of complexity: simple ligands in orange and complex compounds in blue. a) Structure of human CDK2 in complex with the potent kinase inhibitor staurosporine (PDB ID: 1AQ1; DrugBank ID: DB02010). b) Structure of chitinase A in complex with the potent antifungal agent allosamidin (PDB ID: 1X6N; DrugBank ID: DB04628). c) Superposition of the human prostaglandin reductase 2 (highlighted with brown loops) in complex with the anti-inflammatory agent indomethacin, in orange (PDB ID: 2ZB8; DrugBank ID: DB00328), and the human estradiol-17-β-dehydrogenase 1 in complex with androstendione, in blue (PDB ID: 1QYW; DrugBank ID: DB01561).

As the structural complexity of a small molecule is only compatible with specific protein classes and recognition sites, the reported complexity-driven selectivity between protein clans can be additionally used a powerful tool for target class identification. Furthermore, from an evolutionary point of view, as members of a given Pfam clan share a common ancestor,[17] the reported behavior in structural complexity recognition among clans suggests an underlying conservation during evolution.

Conclusions

We have collected thousands of molecules from DrugBank, grouped them by their degree of structural complexity, and have shown that this descriptor correlates well with several medicinal chemistry relevant physicochemical properties, including their polar behavior and aqueous solubility (Fig. 3).

We have further analyzed the correspondence of drug properties, represented by the structural complexity as unifying descriptor, and properties at the protein's recognition site surface. Remarkably, hydrophobic, druggable binding sites recognize achiral or poorly stereogenic molecules, and, *vice versa* polar, undruggable binding sites recognize richly chiral compounds (Figs. 4 and 6). These findings have particular impact on the drug discovery field. On the one hand, the computationally undemanding structural complexity descriptor is exhibited as a powerful rationale to include target information in the preselection of candidate molecules for screening, which can be complementary to other filtering rules. On the other hand, increasing structural complexity is identified here as an approach to addressing particularly difficult or low-druggability recognition sites.

Finally, we have shown that protein classes preferably bind molecules with a certain degree of structural complexity (Figs. 5 and 7). Three different ligand recognition patterns are described: clans which preferably interact with achiral or very simple molecules; clans which preferably bind richly stereogenic substrates; and also ambivalent clans, which recognize both simple and complex molecules. These findings can be exploited in the design of modulators of specific protein classes, in the target identification of small molecules, and in the rationalization of protein–ligand interactions from an evolutionary point of view, in terms of conservation of structural complexity demands.

Keywords: molecular recognition · structural complexity · library preparation · drug design · druggability · chirality

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Additional Supporting Information may be found in the online version of this article.

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