Brief Articles

A Rapid Computational Method for Lead Evolution: Description and Application to α_1-Adrenergic Antagonists

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The high failure rate of drugs in the development phase requires a strategy to reduce risks by generating lead candidates from different chemical classes. We describe a new three-dimensional computational approach for lead evolution, based on multiple pharmacophore hypotheses. Using full conformational models for both active and inactive compounds, a large number of pharmacophore hypotheses are analyzed to select the set or "ensemble" of hypotheses that, when combined, is most able to discriminate between active and inactive molecules. The ensemble hypothesis is then used to search virtual chemical libraries to identify compounds for synthesis. This method is very rapid, allowing very large virtual libraries on the order of a million compounds to be filtered efficiently. In applying this method to α_1-adrenergic receptor ligands, we have demonstrated lead evolution from heterocyclic α_1-adrenergic receptor ligands to highly dissimilar active N-substituted glycine compounds. Our results also show that the active N-substituted glycines are part of our smaller filtered library and thus could have been identified by synthesizing only a portion of the N-substituted glycine library.

Introduction

One of the main problems facing drug discovery and development efforts today is how to efficiently move from one chemical class to another while maintaining the desired activity. The high failure rate of drugs in the development phase can be attributed to a significant degree to problems with pharmacokinetics and safety,[1] both of which are to some extent coupled to the chemical class of the candidate. Thus success in moving compounds from discovery to the clinic requires a strategy to reduce risks by generating lead candidates from different chemical classes.

This process, which we will refer to as "lead evolution", is critical when a series of compounds is found to have insurmountable development problems (i.e. absorption, distribution, metabolism, and/or toxicity related problems). Lead evolution capability would also be very useful if a chemical series cannot be patented or even when a new discovery program is initiated from an existing program. Three-dimensional (3D) database docking can be used as a lead evolution strategy,[2,3] when a high-resolution target structure is available. An obvious limitation is that structures for the majority of pharmacologically interesting receptors are unknown, in particular in the early phases of a project. In addition, the flexibility of the target structure is usually not considered in docking studies, which can lead to erroneous results. In cases where the 3D structure of a target receptor is unknown, the structural features required for biological activity must be inferred by comparing small molecules. Traditional lead evolution efforts that generate this data, such as assaying of large corporate databases or diverse combinatorial libraries, are both resource and time intensive. These burdens can be significantly reduced through the use of computational filters that are both scaffold-independent and effective discriminators of activity. This report contains the description and application of an innovative and rapid computationally driven procedure to evolve lead compounds from existing activity data that meets both these criteria.

Most computational approaches to lead evolution, such as similarity searches or diversity analyses, rely on chemical connectivities or two-dimensional (2D) descriptors.[4] 2D topological methods can be limited by their emphasis on bond connectivity. This is illustrated by a study from Zheng and co-workers[5] who applied the Focus-2D method to searching a combinatorial N-substituted glycine (NSG) virtual library. Using a NSG as a lead compound they identified all of the monomers required for active NSG molecules. However, when morphine was used as the lead, they identified only two of the five building blocks found in the active NSGs. For whole molecules, the challenge of lead evolution is even greater than for molecular fragments. Some successes have been reported with 3D database searches using a single 3D structure of a ligand as a query.[6−15] Ensemble four-point pharmacophore-based methods seem better

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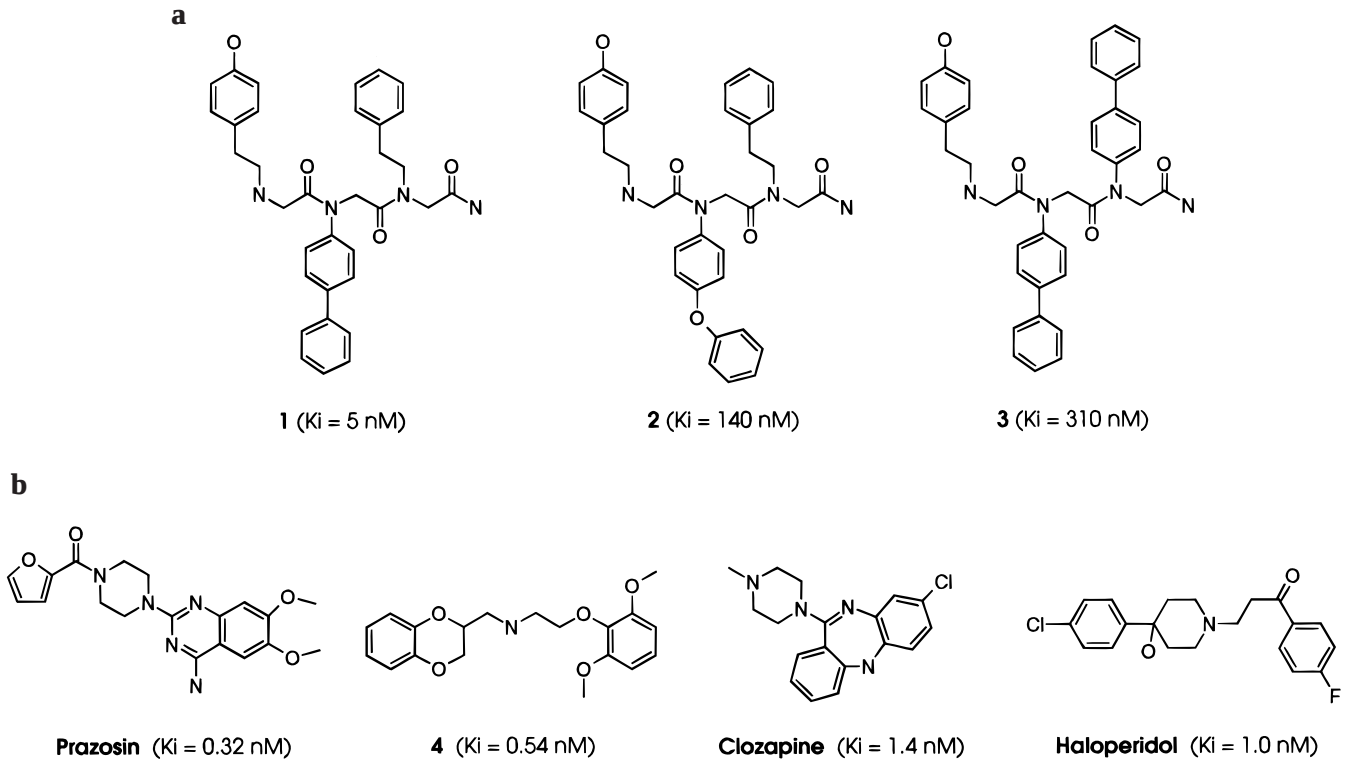

Figure 1. (a) Three NSG trimers[49−51] identified as α_1-adrenergic receptor ligands by Zuckerman et al.[22] through synthesis and screening of a diverse ~4000-member NSG library. **1** is also known to be an α_1-adrenergic receptor antagonist. (b) Sample α_1-adrenergic receptor ligands from the literature with associated activity values.[33,44,52] Using Daylight fingerprints,[48] the highest pairwise Tanimoto similarity between the active NSGs and any of the 105 literature molecules was <0.45 and the average pairwise Tanimoto similarity was 0.27.

suited for lead evolution since they are general enough to be scaffold-independent, spanning different chemical classes, and they allow for inclusion of many conformations.

Pharmacophore-based screening has been used successfully to identify novel classes of ligands.[7−13,16,17] Our method[18,19] differs from these in at least two significant ways. First we use an ensemble of 100−1000 pharmacophores, and second, we include the inactive compounds in the generation of the model. In a typical application of these methods, only a single pharmacophore or a very small number of pharmacophores derived from a set of known "actives" are considered or as in the work of Mason and Cheney[16] pairwise comparisons of an active and an inactive molecule were performed. Information from inactives is generally not used in a systematic way for model development, with some exceptions, e.g. recent reports on the application of pharmacophores to QSAR and 3D database queries.[8,20,21] In this report, we describe a method that uses 3D pharmacophore descriptors to generate a filter to assist lead evolution efforts.

As a demonstration of our lead evolution procedure, we will describe the generation of a discriminating model composed of an ensemble or set of pharmacophores (hypotheses). This model was derived from 105 published heterocyclic α_1-adrenergic receptor ligands encompassing multiple chemical classes. We then used the ensemble model as a computational filter to search a diverse combinatorial library of NSGs that was synthesized and screened for α_1-adrenergic receptor binding activity by Zuckerman et al.[22] We show that the application of this multiple pharmacophore filter to the ~4000-compound virtual library effectively enriches our final designed synthetic library, by filtering out most of the inactives while retaining the actives, including a 5 nM α_1-adrenergic antagonist (**1**, Figure 1a).

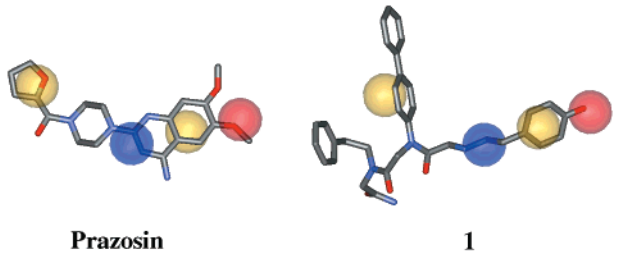

Figure 2. Four-point pharmacophore mapped onto conformations of **1** and prazosin (the features of the pharmacophores are color-coded: yellow, aromatic ring; blue, positive charge; red, hydrogen-bond acceptor). Although the 2D similarity between the molecules is low (Tanimoto similarity between Daylight fingerprints is 0.26), this pharmacophore shows how their similarity can be revealed by the 3D descriptors.

Computational Models

In the construction of the aforementioned computational filter, we used pharmacophore-based 3D whole molecule descriptors. Pharmacophore descriptions of molecules and their application to virtual library searching and design have been described elsewhere[20,23,24] and will only be summarized here. The major component of our 3D descriptors is the "four-point pharmacophore" which consists of four chemical features and the six interfeature distances (Figure 2). The features are selected to reflect the interactions important to ligand binding and activity. These routinely include hydrogen-bond acceptors and donors, hydrophobes, negative and positive charges, and aromatic groups.[25] We reduce the potential number of pairwise feature distances to a specific set of distance bins (e.g.

interfeature distances between 3.5 and 5 Å map to a single distance bin). Thus the "pharmacophore space" (all possible combinations of two, three, and four features) is predetermined by interfeature distance bins and the specific set of features. Similar to Mason and Cheney,[16] we use a "molecular signature", a bit string where the presence or absence of each of the two-, three-, or four-point pharmacophores is recorded. For the α_1-adrenergic receptor data, the predetermined set of all possible pharmacophores was also subject to the biological constraint that each pharmacophore must contain at least one positive charge feature. This resulted in a signature length of ~9 million bits. A molecular signature is created for each molecule by generating a full conformational model, followed by mapping the presence or absence of all possible two-, three-, and four-point pharmacophores that are present in a molecule's conformers into a single bit string.

We generate the conformational model for each compound using an in-house program CONAN (CONformational ANalysis by intersection) described in more detail elsewhere.[26−28] Briefly, CONAN is similar to a deterministic search algorithm in that the final list of low-energy conformations lies on a predefined torsion grid. It is distinct because it is a fragment-based algorithm designed to take advantage of the nature of combinatorial libraries. Each molecule is decomposed into overlapping fragments, and the conformations of these fragments are analyzed separately and stored in a database, permitting their reuse. The conformational models of the fragments are used to construct conformations for the entire molecule through the intersection of the overlapping substructures. This intersection process quickly generates a complete description of the low-energy space of a molecule.

To select the subset of all pharmacophores that is best able to differentiate between active and inactive molecules, the signatures of all compounds (active and inactive) are systematically analyzed in the context of their associated activity data. We considered each of the 9 million pharmacophores to be a separate hypothesis potentially describing/determining activity. Each pharmacophore hypothesis is ranked for its usefulness on the basis of its ability to discriminate between actives and inactives across the entire data set. The ranking criterion is "information content":[29]

$$I = [q \log_2(q/p) + (1 - q) \log_2((1 - q)/(1 - p))] \qquad (1)$$

where I is information content, q is the fraction of active molecules that contain the pharmacophore, and p is the fraction of inactive molecules that contain the pharmacophore. Note that the information content is a function of both active and inactive molecules.The hypotheses with the greatest information content together define the "ensemble hypothesis" (model). It should be noted that eq 1 makes the implicit assumption that the hypotheses are uncorrelated. Because this is not true, our analysis may lead to ensembles that contain correlated bits. We have investigated the impact of this assumption by clustering hypotheses to remove the correlation among them and then ranking and selecting the most informative subset. This procedure resulted in smaller ensembles but qualitatively identical models.[30] Additionally, for those hypotheses with high but not perfect correlation, our ensemble model is flexible enough to capture the "fuzziness" of the set of active compounds. In practice we find that ensemble sizes of up to 1000 pharmacophores can effectively be used to filter virtual library searches.

The α_1-adrenergic ensemble hypothesis was developed from 105 structurally diverse α_1-adrenergic receptor ligands from the literature, including 43 actives that ranged in activity from 0.1 to 5 nM, and 62 inactives.[31−46] Representative active compounds are shown in Figure 1b. The most informative hypotheses were then selected as an "ensemble" as described above. The ensemble was further analyzed by plotting the fraction of the compounds (active and inactive) verses the fraction of the ensemble matched. The graph, shown in Figure 3, demonstrates that it is possible to identify an ensemble model that will select (match) a large fraction of the active data set and eliminate (not match) a large portion of the inactive data. The final informative ensemble contained 500 hypotheses.

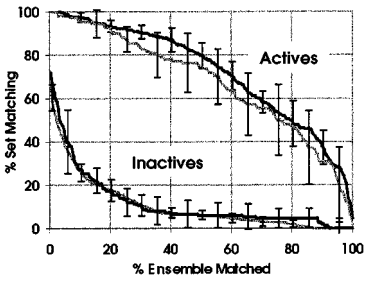

Figure 3. Ensemble hypothesis performance graph for the α_1-adrenergic literature compounds using pharmacophore hypotheses. The "actives" were defined as compounds with K_i < 5 nM and "inactives" as K_i > 5 μM. This resulted in an active set of 43 compounds and an inactive set of 62 compounds. For training and cross-validation the data was split into two sets. A random subset of 75% of both active and inactive compounds was selected for use as the training set, and the remaining 25% were withheld as a test set. Using the training set, the information content (eq 1) of each pharmacophore hypothesis was calculated, and the 500 pharmacophores with the highest information content were retained as the ensemble model. This ensemble model was cross-validated by scoring the holdout data (test) compounds against the derived ensemble and comparing the performance of the ensemble on the training and test sets. The ensemble model is evaluated by plotting the fraction of molecules (active and inactive) verses the fraction of the ensemble matched. The cross-validation process (random selection of training set, ranking by information content, selection of the ensemble, and checking test set performance) was repeated 10 times for the data set. The graph shows the average results of the 10 cross-validation trials with error bars indicating the standard deviation of the trials. The training active and training inactive data sets are shown as solid black lines. The active and inactive data used for testing are gray lines.

Once an ensemble model is developed that has such discriminating behavior, criteria for virtual library search/screening are determined. The point along the *X*-axis of Figure 3 (i.e. the fraction of the ensemble matched) that provides the greatest separation between the active and inactive compounds is chosen as a threshold for the virtual filter. When applied to a pool of compounds, a large fraction of the actives passes the filter, while a large fraction of the inactives is excluded. All compounds that meet or exceed the threshold are considered as synthetic candidates. The threshold for the α_1-adrenergic ensemble was set at 40% of the ensemble matched, which filters out >90% of the inactive compounds while retaining 80% of the actives.

Results

We applied this computational filter to a virtual library of 3924 NSGs, compounds chemically very different from the heterocyclic literature data set. This library of dimer and trimer NSGs was designed and synthesized as a diverse set of compounds with a subset of side chains resembling known 7-transmembrane G-protein-coupled receptor ligands.[22] It was screened for α_1-adrenergic receptor binding affinity by Zuckerman et al.[22] Three α_1-adrenergic receptor ligands with K_i's < 500 nM were identified (Figure 1a), including an α_1-adrenergic receptor antagonist, **1**.

For each of the virtual compounds, conformational analysis was performed with CONAN,[26] features were assigned, and pharmacophore signatures were generated. The library was then filtered using the ensemble

hypothesis and threshold described above. Of the 3924 compounds in the library, 16% passed and all three active molecules from the synthetic library (**1**−**3**) were contained in our set of "hypothesis-matching" compounds. This is an "enriched" pool (3 actives/639 molecules) relative to the larger synthetic library (3 actives/3924 molecules) undertaken by Zuckermann and co-workers.[22]

The next step is to produce a synthetic reagent matrix typical of combinatorial chemistry that results in the synthesis of the maximum number of hypothesis-matching compounds. We produced an optimally dense reagent matrix for 160 compounds (approximately two 96-well plates) using the "cut-down" approach described by Stanton and co-workers.[47] Both **1** and **2** appeared in this final matrix design. Had this been a real-life example, and this library design was carried forward to chemical synthesis, our library of 160 NSG compounds would have yielded two-thirds of the active NSG trimers found by Zuckerman et al.[22] Additionally, incorporation of the inactive compounds from the library in the analysis of subsequent rounds of ensemble derivation would yield a more refined filter. Thus, in a single round of synthesis our method would have produced promising new lead compounds and data that could be used to optimize both the computational model and the compound activity.

Discussion

The ultimate objective of lead evolution strategies is to enhance the number and chemical diversity of development candidates for particular biological targets. This enhances the chance for success in moving compounds from discovery to the clinic. As stated at the outset, various computational methods can and have been used to tackle the problem of lead evolution. In this paper, we have used a novel method to demonstrate lead evolution from heterocyclic compounds to NSGs. Our starting point was a small set of diverse α_1-adrenergic receptor ligands gathered from literature sources.[31−46] It should be noted that our model was built using activity data from assays where α_1-adrenergic receptor binding inhibition is not subtype selective. Thus we do not expect to obtain subtype selectivity in our model. With a model built using these data, we searched a virtual library of NSGs[22] and found that our procedure would have proposed a synthetic library enriched for activity. From the approximately ∼4000-compound pool, 639 compounds passed our 3D filter, including all three active NSGs. Moreover, in a subsequent step, the compound matrix proposed for synthesis was also enriched for activity in that our library design contained two of these three NSGs.

This type of lead evolution is possible for a number of reasons. Primarily, our method is truly scaffold-independent. Many methods are based on 2D topological descriptors, and while some are quite sophisticated, these methods are limited in their ability to jump chemical classes.[5] Other 2D techniques, such as those relying on Tanimoto similarities of Daylight fingerprints,[48] would not have identified these NSGs because the average similarity between them and the active literature compounds is only 0.27. The method presented here, which is based on 3D feature distributions, does not face these same limitations (Figure 2). It should be noted that 3D pharmacophore methods have been described before and pairwise comparisons of pharmacophore signatures of active and inactive molecules have been used for analyzing similarity or selectivity.[16,29] However, our method differs from previous 3D methods by integrating three important features: we have included an ensemble of pharmacophores, we have considered both active and inactive compounds, and we have included a full ensemble of conformations for each molecule.

While we have demonstrated this method on a rather small example, both the speed and efficiency make it ideally suited for analyzing high-throughput qualitative screens. Using only 2D information as input, we are able to generate 3D pharmacophore signatures for each compound, including a full conformational analysis, on average in just over a second each on a 400-MHz Pentium II processor. This allows us to routinely filter very large virtual libraries (i.e. up to 10^6) very quickly, thereby increasing the likelihood of finding suitable synthetic candidates. Additionally, because we incorporate both active and inactive compounds into our process, we are able to converge rapidly toward an informative ensemble of hypotheses. This approach, when used in an iterative fashion alongside rapid synthesis and screening, makes a lead evolution process more efficient, increasing the chances for finding multiple clinical candidates covering chemically diverse scaffolds.

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(49) **1** is [*N*-[2-(4-hydroxyphenyl)ethyl]glycyl][*N*-(4-biphenylyl)glycyl]-*N*-(2-phenylethyl)glycinamide.
(50) **2** is [*N*-[2-(4-hydroxyphenyl)ethyl]glycyl][*N*-(4-phenoxyphenyl)-glycyl]-*N*-(2-phenylethyl)glycinamide.
(51) **3** is [*N*-[2-(4-hydroxyphenyl)ethyl]glycyl][*N*-(4-biphenylyl)glycyl]-*N*-(2- biphenyl)glycinamide.
(52) **4** is 2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxan.

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