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Journal of Molecular Graphics and Modelling 23 (2004) 15–21
Journal of Molecular Graphics and Modelling
www.elsevier.com/locate/JMGM

Design of a gene family screening library targeting G-protein coupled receptors

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Received 17 September 2003; received in revised form 15 December 2003; accepted 3 March 2004
Available online 30 April 2004

Abstract

An iterative process for the design of a G-protein coupled receptor (GPCR) gene family screening library has been developed. A key element of this process is the computational generation of pharmacophore descriptors of known GPCR ligands. Subsequent iterative analysis allows prioritization of scaffolds and sub-libraries for inclusion in the library. The final library, which consisted of 13,769 compounds, displayed a 2.6% hit rate when screened against the μ-opioid receptor.

Keywords: Gene family screening library; G-protein coupled receptors; μ-opioid receptor; Pharmacophore; Combinational library design

1. Introduction

As a result of the explosive growth in the availability of genomic information, the number of potential targets for drug discovery is increasing rapidly. At first this may seem like a very favorable development; however, the amount of research that must be done for target prioritization, including assay development, in vivo target validation, generation of gene knock-outs, patent searching, etc., also increases dramatically. Thus there is a clear need to develop efficient strategies that will expedite the drug discovery process.

Along with others, we have focused on groups of targets related by sequence or protein fold—the so-called *gene family* (or *target class*) approach [1,2]. The underlying assumption is that related targets will share similarities in their ligand-binding domains and therefore the ligands will also share certain characteristics. In this respect, the concept of privileged structures has been a very powerful strategy in medicinal chemistry [3]. Obvious advantages to a gene family-based approach include greater efficiency in the development of high throughput screens for related targets, from both the activity and selectivity perspectives, and the opportunity to focus screening efforts on compound libraries that have been designed to hit specific gene families.

What, then, should be the objectives for a gene family screening library, and what is the best way to design one? We felt that the library should lead to enhanced hit rates against targets within a selected gene family and that it should allow rapid follow-up once hits have been identified. Therefore, we chose to assemble a library of small (50–500 compounds) sub-libraries based on scaffolds for which the synthetic and analytical procedures already have been optimized. In order to

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1093-3263/$ – see front matter
doi:10.1016/j.jmgm.2004.03.001

maximize reproducibility and interpretability, the library would contain purified single compounds screened as discretes rather than mixtures. But which sub-libraries to include? Clearly, the novelty and patentability of well-known privileged structures such as benzodiazepines is limited. In addition, some privileged structures would not be amenable to chemistry for rapid optimization. We thought it is advantageous to go beyond the inclusion of privileged scaffolds and side chains, and therefore have used 3D whole molecule descriptors to characterize the target space and compounds that may be selected for inclusion in the library. Three- and four-point pharmacophores have been proven to be computationally efficient descriptors that are able to capture compounds that are chemically different but similar in activity [4,5]. While traditionally a staple of ligand-based design, pharmacophores can easily be combined with 3D structural information from the binding sites of drug targets when such information is available [6]. After screening a gene family library developed in this manner, follow-up libraries based upon active compounds can be generated quickly and efficiently. In addition, by using a so-called informative design strategy to select molecules for the library, the interpretability of screening data is optimized against any experimental outcome [7]. The resulting activity data then can be used to refine a pharmacophore model to be employed in further rounds of compound selection and synthesis for that specific target. An application of informative design to model refinement for a single target has recently been described [8] and thus will not be further covered here.

In this article, we describe a computational procedure for characterizing a gene family target space and a mechanism to sample that space with compounds. We have used a GPCR-targeted gene family library as an example to illustrate the whole process; alternative approaches to drug design for targets in this family have been recently reviewed [9]. Screening of this library against the μ-opioid receptor lead to enhanced hit rates relative to another reported library and to the identification of active scaffolds that can be optimized rapidly. Synthetic and analytical details from this GPCR screening library will be published elsewhere.

2. Computational strategy

Our approach is illustrated by the flowchart in Fig. 1. In the first step, we define a source pool set of GPCR ligands that contain characteristics that we wish to approximate with our screening library. These ligands are abstracted in the form of pharmacophore descriptors to generate a GPCR gene family target ''space''. Candidate molecules from virtual libraries are described in the same manner, and we evaluate whether these molecules present pharmacophore descriptors relevant to GPCR ligands—that is, if they help ''cover'' the design space. If so, molecules from these sub-libraries may be selected for synthesis and inclusion in the GPCR gene family screening library. If not, the pharmacophore information can be used to drive new chemistry ideas and virtual libraries to be considered.

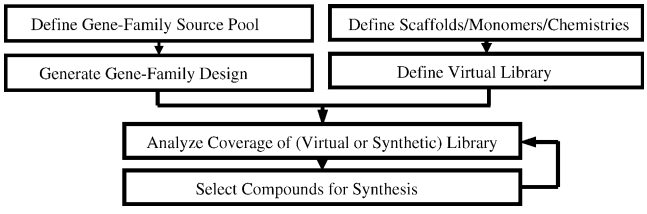

Fig. 1. Iterative design process for the gene family library.

2.1. GPCR gene family design space definition

To define a GPCR ''target space'' for library design and analysis, a collection of known GPCR ligands was obtained, using the MDL Drug Data Report [10] database (version 2000.2) as the primary data source. The MDDR represents a compilation of the scientific and patent literature regarding chemical structure and biological activity of the more than 100,000 compounds listed therein, and has proved a rich data source for a number of studies [11–15]. Following the protocol of Ajay et al. [15] these compounds were filtered to retain molecules likely to have in vivo activity. This process resulted in a set of approximately 18,000 ''drug-like'' compounds. The ''activity class'' field of the database was then queried to obtain a list of all biological activities defined within the database, and this list was pruned to 114 well-defined GPCR keywords.[9] Activity toward a receptor within the GPCR gene family was assumed if a molecule was listed as belonging to any of these classes. At this point, 3321 molecules remained. Finally, the ''rule of five'' suggested by Lipinski et al. [16]. was applied, along with a rotatable bond upper-bound of 14, resulting in 2785 GPCR ligands with ''drug-like'' properties that contain characteristics that we wanted to approximate within our gene family screening library. The distribution of these compounds by activity class and agonist/antagonist annotation is shown in Fig. 2.

Next, the gene family descriptor space based on this ligand source pool was established. From purely geometric constraints, three- and four-point pharmacophore descriptors were generated, each of which includes chemical features selected from positive charge, negative charge, or hydrophobic features (at most two of each), as well as hydrogen bond donor, acceptor, and aromatic ring center features. The representations employed 25 distance bins (non-uniform width) spanning the range of 1.6–24 Å, which resulted in a generic set of 35 million pharmacophores. The conformational model for each ligand was generated using an in-house program, CONAN [17], allowing a maximum of 1000 conformers per stereoisomer, and the presence or absence of particular pharmacophores was recorded in a bit

[9] Supporting information available: one table of GPCR activity class queries.

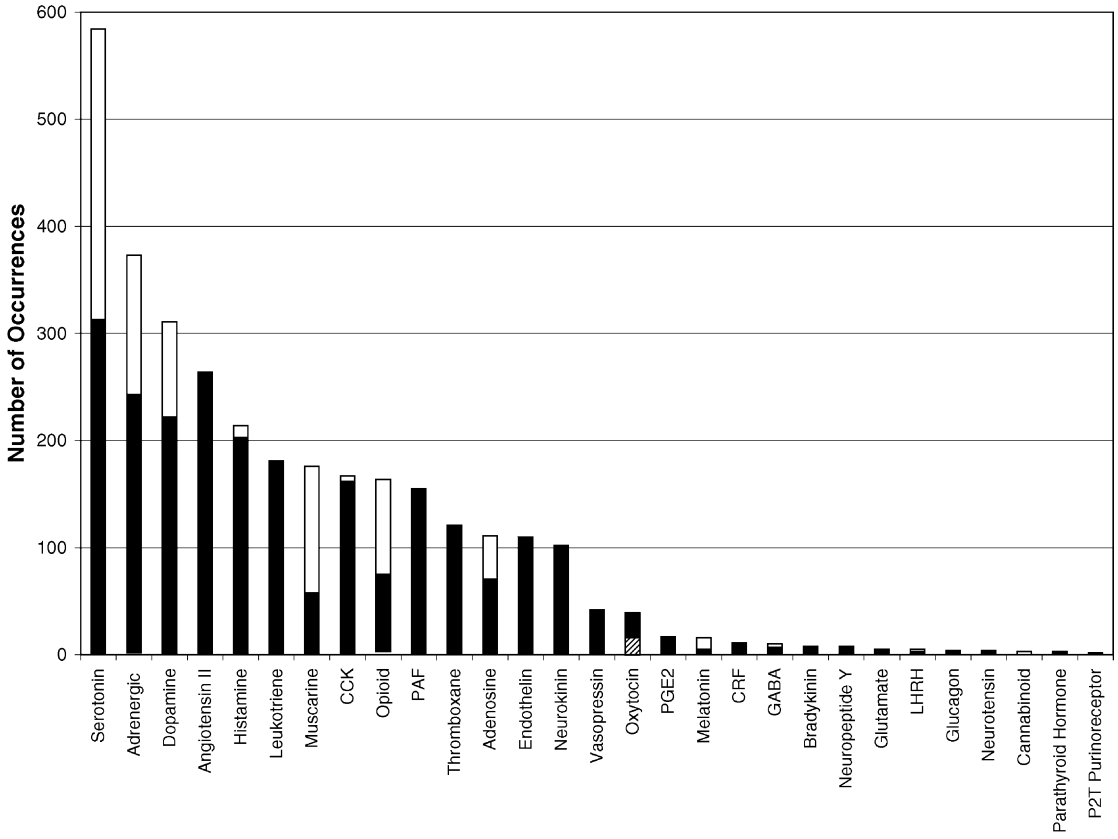

Fig. 2. A histogram illustrating the distribution of GPCR activity classes (grouped by query). The fraction designated with agonist or antagonist activity toward a receptor in the class is indicated within each bar: agonist = white; antagonist = black; other = striped. Molecules may be mapped to more than one activity class.

string (molecular signature). With full conformational sampling and the distance-binning approach employed, the pharmacophores contained in only a few molecules were of less interest. With this in mind, we chose to retain only pharmacophores contained in more than 10 molecules and, by this measure, ''GPCR drug space'' contains 1,121,763 pharmacophores. If we had increased the threshold and required, for example, 50 or more molecules from the ligand set to contain a pharmacophore before that descriptor was retained, we might have focused our space on trivial pharmacophores that appear in many molecules of any type, unrelated to GPCR biological activity. Thus, we chose to err on the side of a broad GPCR gene family target space.

Note that the process of using a subset of GPCR activity class labels from the MDDR database was expedient but not fully representative of all known GPCRs. For numerous chemokine receptors of pharmaceutical interest [18], for example, CCR3 and CCR5, there were no ''activity class'' keywords. A search of the biological ''action'' field of the MDDR (version 2002.1) for such chemokine ligands and their patent analogs, followed by filtering with the ''rule of five'', resulted in a set of 151 ligands not included in our original gene family target collection. These molecules contained 137,042 pharmacophores, of which all but 308 (i.e., 99.7%) were contained in the GPCR gene family target space. This point provides validation that the design space as currently defined may supply pharmacophores for orphan GPCR targets as well.

2.2. Compound selection

Candidate chemistries, scaffolds, and monomers were largely obtained by a thorough analysis of the literature and through internal chemistry projects. Virtual libraries for these chemistries were created, analyzed, and evaluated for incorporation into the screening library as the project progressed. In each case, a collection of compounds was prioritized for inclusion if its pharmacophore signature, generated in the same manner as the design space above, including the threshold of descriptors sampled by more than 10 molecules, had significant overlap with the GPCR target space (logical AND of the bit strings). The significance of the overlap was judged in conjunction with other factors such as the synthetic accessibility of the candidate library and its size.

The progress of the screening library was evaluated in three stages as the synthesis proceeded on a number of scaffolds. With each iteration, the number of the gene family target pharmacophores ''covered'' by the screening library was evaluated. Thus, at any stage of the process, it was known which pharmacophores had been explored by the library, and how much of the gene family target space

remained to be covered, i.e., where there were ‘‘holes’’. The characteristics of the remaining pharmacophores could be analyzed to give direction to synthetic efforts, and the chemical property profiles of the gene family target ligands and the screening library were easily compared.

An example of the process, following stage three of synthesis, is given. At this point, 85% of the GPCR gene family target space had been covered and approximately 173,000 pharmacophores remained to be sampled (at the requisite depth). To generate ideas for accessible, target-directed chemistries for exploration, 43 virtual libraries were searched. Random samples of 500 molecules from each library were selected and their pharmacophore signature generated as described above. These sub-libraries were then ranked based on their coverage, at a depth of at least 10 molecules, of the remaining gene family target space. The top-ranking chemistries were considered for inclusion in the screening library. Informative design, as implemented in the program Luddite [19], was used to select matrices of molecules from the virtual library that optimally sampled the remaining pharmacophore space. As has been described in detail, molecules were selected to sample the pharmacophore space in a manner that provides information regarding the activity-relevant pharmacophores when the molecules are screened [6,7,19].

3. Results and discussion

The intent of our library design strategy was to provide answers to questions such as ‘‘How large a library do we need to insure the pharmacophores of GPCR-active molecules are represented?’’, ‘‘What types of molecules are missing?’’, and then ‘‘What should we make next?’’ through iterative library analysis and synthesis. We did not seek to derive a GPCR family activity model but rather to select molecules for the library that sampled the pharmacophores of GPCR-active molecules and allowed these pharmacophore hypotheses to be tested experimentally as the library is screened against each new GPCR target. Activity models for specific targets of interest could then be built using this data [5,8]. An intrinsic advantage of the simple three- and four-point pharmacophores employed here is that they are readily interpretable descriptors that suggest both chemical feature types and their location requirements, in three dimensions, for planning additional compound synthesis or selection. For gene families in which protein structures are known, pharmacophores derived from binding sites have been used to augment or replace ligand-derived pharmacophores as a design space (M.L. Lamb, R.V. Stanton, E.K. Bradley, unpublished data). Alternative approaches recently described that employ 2D molecular fragment [20] or more abstract BCUT [13,21] descriptors lack these features, and interpretability may be further obscured through the use of neural network models built using such descriptors [13,20]. Of course, these models may still be useful for selecting a required number of potentially active compounds from a collection or virtual library.

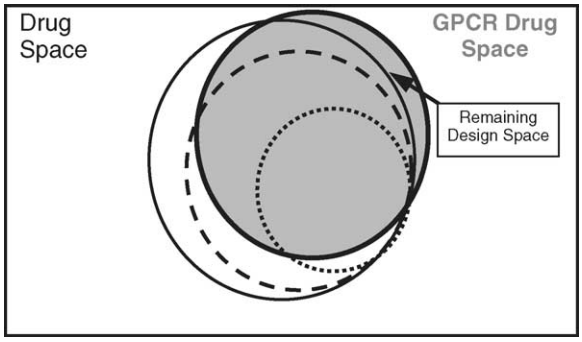

Fig. 3. Venn diagram description of coverage of the GPCR gene family target space (GPCR drug space, grey filled circle, 1.12 million pharmacophores) at each stage of library synthesis: stage 1, the dotted circle; stage 2, the dashed circle, and stage 3, the solid circle. The rectangle surrounding the diagram represents the 3.4 million pharmacophores of ‘‘drug space’’ that arise from drug-like molecules in the MDDR described in the same fashion.

3.1. GPCR library construction and analysis

The progress of GPCR library construction relative to the gene family pharmacophore design space is illustrated schematically with a Venn diagram in Fig. 3. In the first stage of library synthesis, 2515 molecules were made. There were 505,710 pharmacophores sampled by more than 10 of these molecules, of which 456,326 pharmacophores were in common with the GPCR target pharmacophore space. This resulted in 41% coverage of the target space with the first round of synthesis. This suggested that a library of less than 6500 molecules might be sufficient to explore GPCR space at this depth of coverage. When the size of the library reached 5272 compounds, the computed coverage was 77% (862,960 pharmacophores in common, of 1,064,607 presented by the library). The next round of synthesis included routes that allowed the library to double again in size to 10,943 molecules; however, the level of coverage achieved was only 85% (1,551,389 pharmacophores, 948,912 in common). One reason for the smaller increase in coverage of the target space was a reliance in some sub-libraries on a well-defined set of ‘‘road-tested’’ monomers, whose performance under the reaction conditions routinely provided good yields. The effect on coverage of this synthetic strategy provided an opportunity to balance the future synthetic efforts with the need to cover new pharmacophore space.

Beyond pharmacophore coverage, one molecular property profile that was monitored closely throughout this process was the number of positively charged features contained in library molecules (Fig. 4). Conventional wisdom that ligands (and libraries) for G-protein coupled receptors should contain a positive charge ignores targets such as corticotropin-releasing factor (CRF) and endothelin receptors. In fact, even targets with known preferences for positively charged ligands, such as the μ-opioid receptor, do not absolutely require a positive charge [22]. Approximately

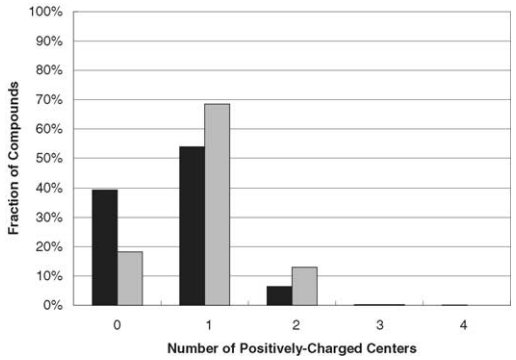

Fig. 4. The distribution of positively charged chemical features within the GPCR gene family space-defining ligands (black bars) and the designed screening library (stage 3 of synthesis, grey bars).

25% of the GPCR design space pharmacophores contained a positive-charge feature, and while the library contains numerous positively charged ligands, there was a concerted effort to keep in mind the preferences of these other targets. We evaluated additional molecular property profiles for both the GPCR source pool ligands and the screening library, given the debate in the literature regarding the desirability of screening ''lead-like'' or ''drug-like'' libraries with respect to the ease of optimizing any hits [23–25]. For example, at stage three of the library, 29% of the library consisted of ''lead-like'' [23] molecules with molecular weight less than 350 amu; 31% of the original GPCR ''drug-like'' ligands were below this threshold. The molecules in the screening library were also ''lead-like'' with respect to their calculated log *P* profile, with 34% of the molecules having a Daylight [26] *c* log *P* value between 1 and 3. Only 25% of the GPCR ''drug-like'' compounds fell into this range.

With only 85% of the target space covered at this stage, however, 172,851 pharmacophores remained to be explored by library molecules. The chemical feature combinations (independent of distance constraints) among the remaining pharmacophores were analyzed, and the 25 most frequently occurring combinations are shown in Fig. 5. The majority of the remaining pharmacophores contained a negative-charge feature that had not yet been adequately explored. This fact itself gave direction to additional chemical efforts, as bioisosteres for carboxylic acids and other negatively charged features were incorporated into scaffold ideas and monomer lists. Of course, including the appropriate chemical features was necessary but not sufficient to cover the ''holes'' in the target space; the features had to be placed at appropriate distances within the molecules for the associated pharmacophores to be covered.

To generate chemistry ideas, random samples of 500 molecules from additional virtual libraries were evaluated for coverage of the remaining space. These libraries ranged in size from hundreds to tens of millions of molecules and represented chemistry for which experimental protocols were available in-house. The top five sub-libraries covered from 12,000 to 28,000 pharmacophores in the remaining GPCR gene family target space. Further examination revealed that one of these virtual library scaffolds was similar to substructures of 72 GPCR source pool ligands,

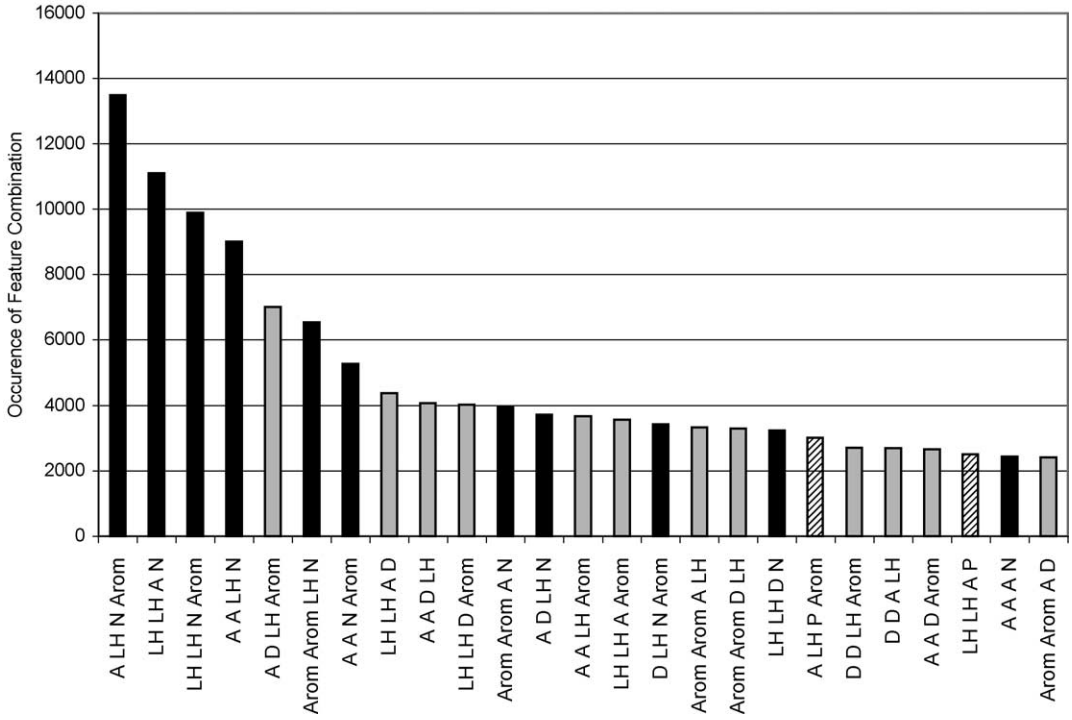

Fig. 5. The 25 most populated chemical feature combinations in the remaining GPCR gene family target space. A, hydrogen bond acceptor; D, hydrogen bond donor; P, positive charge; N, negative charge; Arom, aromatic ring center; LH, hydrophobic feature. Feature combinations containing negatively charged features are highlighted with black bars, while those that contain a positive charge have striped bars.

while there were four GPCR ligands similar to molecules from another. Optimization of synthetic matrices around these two scaffolds that informatively sampled the remaining space resulted in the selection of individual sub-libraries (ca. 500 molecules each) that covered 27,000 to 63,000 of the remaining pharmacophores. Matrix variations that reduced the number of chemical intermediates to be purified were utilized for synthesis. Thus, this gene family design protocol was flexible enough to incorporate medicinal chemistry expertise, capitalize on existing synthetic protocols, adapt to synthetic constraints, and provide metrics of the progress of library construction.

3.2. Screening of the GPCR library

In the end, a GPCR-targeted library comprising 13,769 molecules was screened first against the μ-opioid receptor. Of the molecules in this library, 357 exhibited activity against the μ-opioid receptor, with activity defined as greater than 50% inhibition at a concentration of 10 μM (average, $n = 2$). This translated to a hit rate of 2.6%. In contrast, a conventional diverse, drug-like library of 10,560 molecules reported by Poulain et al. [27] resulted in a hit rate of only 1.7% under apparently analogous conditions. Compounds with an average percent inhibition of at least 70% in the primary screen were followed up with 11-point IC_{50} measurements. An activity of better than 10 μM was found for 149 molecules. Ten scaffolds exhibited active compounds (Fig. 6), with the majority of the active molecules synthesized on scaffold 1, for example, contained in the known compound spiroxatrine.

At the time the gene family target space was defined, entries in the MDDR database designated spiroxatrine as an antagonist at the dopamine D2 receptor with further activity against the serotonin 5HT1A receptor. Spiroxatrine, or a compound presenting similar pharmacophores, might be expected to exhibit activity against other members of the GPCR family. Indeed, subsequent editions of the MDDR database designated spiroxatrine as having activity against the μ-opioid receptor. The additional biological activity for spiroxatrine-like molecules would thus have been "discovered" through this strategy. The pharmacophores

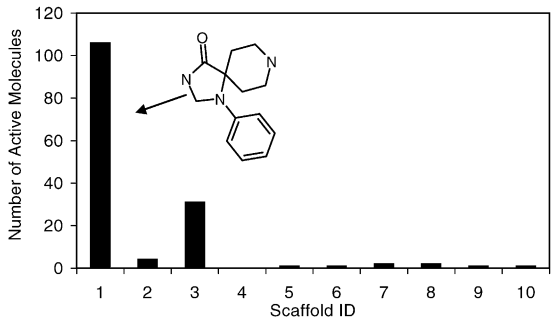

Fig. 6. Number of active molecules (hits) with an IC_{50} of less than 10 μM against the μ-opioid receptor plotted as a function of chemical scaffold assignment.

sampled in the gene family target space, when processed using the data from the full library—molecules active and inactive with respect to the μ-opioid receptor—can readily yield computational models for target-specific library design [5,8]. These models, in addition to the 10 opioid receptor-active scaffolds and their synthetic protocols, represent an excellent starting point for initiating a drug discovery program focused on developing therapeutic molecules active against the μ-opioid receptor, where this receptor a novel target. This library was screened against additional targets, outside the amine-containing ligand subclass of GPCR receptors, as well. In one case, for a difficult orphan receptor, no hits from the initial inhibition assay were confirmed in follow-up IC_{50} determinations. For another target, however, a number of interesting hits were found, including molecules that did not contain the expected pharmacophore for this receptor. Of course, screening against many more G-protein coupled receptors would provide additional validation for this approach.

4. Conclusion

We have described a process for the design of gene family-targeted libraries, incorporating pharmacophores from known ligands across a gene family and an algorithm that selects molecules for the library based on their potential to provide information useful for refining a pharmacophore model after screening against a particular target. When applied to the GPCR gene family, an iteratively designed screening library of ∼14,000 compounds covered more than 85% of the space defined by a set of carefully selected GPCR ligands. Screening of this library against the μ-opioid receptor gave encouraging results (hit rate of 2.6% in the single-point inhibition assay). In addition, we have preliminary evidence that the GPCR library is able to pick up new chemotypes for known targets. The results described herein suggest that gene family-targeted libraries designed in this manner would provide an effective strategy to tackle multiple related targets simultaneously.

Acknowledgement

The authors wish to thank P. Myers, D. Rogers, D. Spellmeyer, P. Parry, S. Harris, R. Hewitt, E. Erb, E. Evensen, W. Shirley, T.-S. Chou, D. Comer, F. Hassman, X. He, L. Hwang, S. Ma, J. MaCall, V. Thai, D. Kassel, J. Isbell, T. Quinn, and T. Wang for their contributions to this project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2004.03.001.

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