New perspectives in lead generation II: Evaluating molecular diversity

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The identification of biological targets was discussed in part I. The next major hurdle is to discover a compound that selectively interacts with the target. In the second part of a two-part article, the authors focus on the problem of how to define and measure diversity within and between compound libraries in order to optimize molecular diversity for lead generation.

Having identified the biological target, the next major hurdle is to discover a compound which selectively interacts with it. Recent developments in high-capacity screening techniques mean that screening is no longer a rate-determining step in the process of lead generation. The rate of discovery of new leads now depends on the diversity of the compound collections under consideration. The advent of combinatorial chemistry will enable rapid generation of a wide variety of chemical libraries. These, together with synthetic and natural product company compound collections, should provide a good starting point for lead generation. The key question is how to define and measure diversity within and between compound libraries so that existing compound libraries can be enriched with the maximum relevant diversity. *De novo* drug design will also play an increasing role in the lead generation process, and may be particularly powerful when used in conjunction with combinatorial chemistry.

Lead generation will be only as effective as the quality and relevance of the compounds being examined. For example, if the target protein is a zinc metalloproteinase, it is highly unlikely that a lead will be found that does not contain a zinc-binding moiety, irrespective of the number of compounds tested. Thus the enrichment of compound collections, whether of synthetic or natural origin, must use measures of compound diversity to give the maximum chance of finding a lead.

Computer-aided drug design (CADD) for lead generation and optimization routinely uses methods for determining molecular similarity and diversity that take account of the conformational flexibility of the structures involved. Three-dimensional (3D) database searching has proved an effective tool for lead generation, and the importance of allowing for conformational flexibility has often been reported[1–7].

A general strategy for the generation of leads by CADD methods applicable to situations where little or no information is available on the structure of the target enzyme or receptor, or about the interacting ligands through to *de novo* structure generation is shown in Figure 1.

Many means of measuring molecular diversity on the basis of 2D and 3D structural features have been proposed. Cluster analysis of very large databases of structures (such as the SPRESI database of 3.2 million chemical structures; Daylight Chemical Information Systems, Irvine, CA, USA) is now feasible using structural characterizations from topological analysis (for example, Daylight fingerprints[8]). When the goal is a representative set, such clustering methods can be effective, but missing diversity is not readily identified. Methods that partition quantifiable properties can be more suitable for identifying missing diversity and for comparing diversity between libraries. The properties of interest are those that define the shape of a molecule and the properties expressed

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on the surface of the molecule, such as hydrophobicity or hydrogen-bond donor or acceptor status.

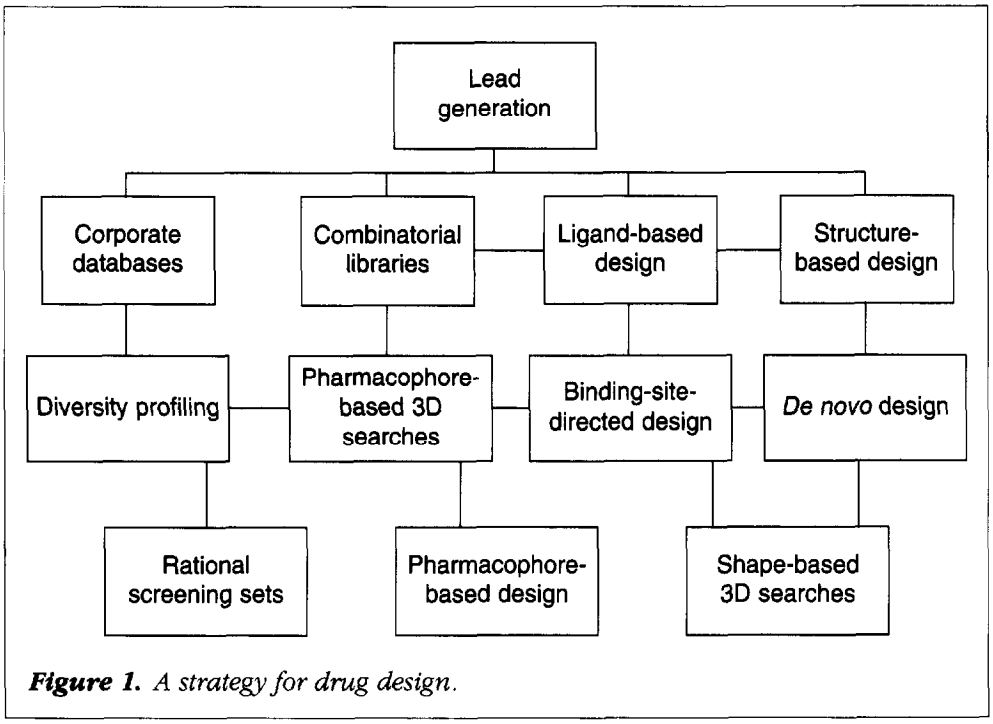


Figure 1. *A strategy for drug design.*

Rational screening set generation

Some biological screening procedures are unsuited to a high-capacity screening approach, and the generation of small rational libraries (up to 10,000 compounds) may be necessary in order to improve the chances of finding a lead. The strategy here is to produce a set of compounds with properties representative of the large library of compounds from which it is derived, so it is very important to be able to define and measure diversity. Methods can be used to ensure a good distribution of properties defined by one or more diversity or similarity metrics, selecting the minimum number of compounds to cover the range of properties of interest. A strategic decision is whether to use clustering or partitioning methods to derive a small set 'representative' of a large compound collection. Cluster analysis can be used to examine what is known about the set and can give representative grouping, but it is difficult to evaluate what is missing or to compare different sets. Partitioning requires a quantifiable property, or combination of properties, and leads to groupings less representative of a particular set, but missing diversity is easily identified and different sets of compounds are readily compared. Examples of the two methods are Jarvis–Patrick clustering of Daylight fingerprints[8] (a type of structural characterization) and partitioning according to molecular and physicochemical properties.

Assessment of molecular similarity or diversity

Many descriptors have been used to evaluate molecular similarity, and these are often used as the basis of approaches for evaluation of molecular diversity. The descriptors can be calculated from 1D, 2D and 3D structures (Box 1). A final diversity metric is likely to use a combination of descriptors. Two approaches used at Rhône-Poulenc Rorer (RPR) to evaluate molecular diversity, using molecular and physicochemical properties and using 3D pharmacophores, are discussed below. Other approaches and implementations have been published, for example those of Chiron[9] and Merck[10].

Molecular and physicochemical properties

On the basis of the concept that enzymes and receptors recognize the shape and associated electronic properties of a molecule, rather than particular atoms, the diverse property-derived (DPD)[6] method was developed to enable selection and profiling of compounds on the basis of their molecular and/or physicochemical properties. Molecular properties not only affect the forces involved in ligand–receptor binding, but are also important in the transport of the drug to its target; this can be important in cell-based biological assays. The method is based on partitioning of six non-correlated molecular and physicochemical property descriptors and covers such properties as hydrophobicity, polarity and/or electronic properties, flexibility, hydrogen-bond acceptor or donor properties, and shape. The descriptors chosen for the DPD method were cLog*P* (Ref. 11) [CLOGP3/Daylight (Ref. 12)], an electrotopological index [normalized sum of the squares of atom-based electrotopological state indices calculated by MOLCONN-X (Ref. 13); Hall Associates Computing, Quincy, MA, USA], a flexibility index (division of kappa shape indices[14], MOLCONN-X), group counts for hydrogen-bond acceptors and donors (GENIE; Daylight Chemical Information Systems, Irvine, CA, USA), and aromatic density [a non-correlated index calculated by division of the number of aromatic rings by the molecular volume (GENIE)]. All of these descriptors could be readily calculated from the SMILES 2D representation of a molecule[15], and no two properties were correlated

Box 1. Examples of measurement of similarity or diversity using one-, two- or three-dimensional structures

One-dimensional structures
Molecular weight

Two-dimensional structures
Topological descriptors and/or structural characterizations (e.g. Daylight fingerprints, MACCS keys)
Flexibility and shape (e.g. Kappa indices)
Molecular and physicochemical property descriptors
Hydrophobicity (e.g. cLog*P*)
Hydrogen-bonding group indices

Three-dimensional structures
Feature–feature distance keys (e.g. Unity, Chem-X/Chem DBS-3D)
Pharmacophores with ≥3 centers (e.g. ChemDiverse, PDQ)
Shape indices
Quantum mechanical descriptors

>0.5 (r^2 maximum 0.25, average r 0.2). The groups were split into two to four partitions (Table 1) to give 576 theoretical combinations ('bins').

Of the defined 'bins', 86% (494) could be filled by compounds from the RPR corporate compound repository, and a small diverse screening set was produced by selecting approximately three compounds from each bin. Use of this property-derived rational screening set has produced hits, for example a diaminopyrimidine compound active in the low-density lipoprotein (LDL) upregulator screen (see *3D database searching* below), similar to the compounds identified from the 3D database searches.

Combinatorial library design

The most important aspect of the design of combinatorial libraries is to ensure maximum diversity within and between libraries before they are produced. Combinatorial libraries range in extent from hundreds of compounds to more than one million. The larger libraries usually comprise mixtures of compounds, and deconvolution is necessary in order to find the 'active hit'. This can be time-consuming, and there may be great difficulty in identifying the active compounds. The strategy for the future is, therefore, to produce monomeric libraries of diverse structures (100–10,000) appropriate to the biological target(s) under consideration. Small combinatorial libraries used to optimize leads also require careful design in their diversity in order to predict further, more potent, compounds from the biological data generated.

A strategic decision in the design and profiling of the diversity of combinatorial libraries is whether to look at the building blocks (core scaffold plus reactants) or the final structures. Although choosing a diverse set of reactants and/or substituents can be useful, the ability to analyze the diversity of the final structures is essential. If a focused library has been developed to probe a certain receptor area, then the two choices may coalesce. The choice of metric(s) used to

Table 1. Descriptors, methods and divisions used for the diverse property-derived method for selection and profiling of compounds on molecular and physicochemical criteria

PROPERTY	Hydrophobicity	Polarity and electronic status	Hydrogen-bonding donor/acceptor status		Flexibility – Shape	
DESCRIPTOR	cLog*P*	Electrotopological index	No. of acceptors	No. of donors	Flexibility index	Aromatic density
METHOD	CLOGP3	MOLCONN-X normalized sum of squares	GENIE		MOLCONN-X kappa1/kappa2 shape indices	GENIE number of rings per Å^3 x 10^3
DIVISIONS	4	3	2	2	3	4
DIVISION VALUES	<1.5 1.5–4.0 >4.0 N+	<10 10–20 >20	0–2 ≥3	0–1 ≥2	<3.5 3.5–6.5 >6.5	0 0.01–3.5 3.5–5.5 >5.5

evaluate diversity is very important. Some results from the use of 2D and 3D metrics on complete structures from compound databases and combinatorial libraries, with consideration of the effect of conformational flexibility on 3D features, are presented below.

The effectiveness of lead generation using pharmacophore-based searching of 3D databases (including the conformational flexibility of the structures), as described below, indicates that 3D pharmacophores are essential components of a diversity profiling method.

3D pharmacophoric diversity

Enzymes and receptors recognize shape and electronic properties rather than particular atoms of structures. Diversity of 2D structures may result in diversity of such properties, but as a design and profiling approach, this lacks a theoretical basis. Although approaches such as DPD consider overall molecular properties, they cannot be used for consideration of 3D aspects. Given that a pharmacophore is a necessary condition for binding to a biological receptor, it can be considered to represent important aspects of bioactive shape and electronic properties. A two-center pharmacophore, such as a database feature–feature distance key, does not adequately represent such shape, whereas a four-center pharmacophore can be chiral and represent polyhedral shapes of properties in a molecule. Three-center pharmacophores represent slices through molecular shapes, and were chosen initially as a probe for 3D diversity; the computational benefits of such a choice enable more variation of the properties of the center to be probed.

Three-dimensional distances (as used, for example, in 3D database feature–feature distance keys) have been used as a metric of diversity, but have not been shown to be much more useful than are 2D metrics. They do not reflect the additional information regarding properties and shape that is available from 3D analyses. 3D pharmacophores with three or more centers and other shape descriptors do begin to exploit true 3D information, but conformational flexibility needs to be taken into account to ensure an increase in yield over that achievable with 2D diversity metrics. Two methods have recently been developed and customized around ChemDBS 3D databases[16]: Pharmacophore-Derived Queries (PDQ, RPR in-house) and ChemDiverse (ChemDBS-3D and ChemDiverse are included in the Chem-X modelling package; Chemical Design, Chipping Norton, UK). Both methods are based on the identification of three-center pharmacophores in 3D structures, and take advantage of the powerful and customizable center perception and conformational sampling (including systematic) methods available in the Chem-X/ChemDBS-3D system.

Pharmacophore-profiling methods

Pharmacophore-Derived Queries method

The PDQ method is based on the analysis of a 3D database of compounds by means of a large number of 3D queries. These are defined and searched automatically, and can use all the features associated with normal 3D searches. Queries of the ChemDBS-3D database can, therefore, be clearly defined using specific atom types. Initial studies used a selection of three centers from the following six possible types of pharmacophore:

- General O,N hydrogen-bond donors
- General O,N hydrogen-bond acceptors
- Basic nitrogen
- Acidic centers
- Aromatic ring centers
- Hydrophobic groups

These were assigned using a customized parameterization file and database in Chem-X. Six distance ranges were then searched (2–4.5 Å; 4.5–7 Å; 7–10 Å; 10–14 Å; 14–19 Å; 19–24 Å), giving, with the 56 three-point combinations of the six types of pharmacophore, a total of 5,916 non-degenerate queries. The theoretical number of 12,096 combinations is reduced by symmetry and triangle inequality (the third distance must be less than or equal to the sum of the other two distances). The method is relatively slow if conformational flexibility is to be taken into account, because conformational sampling is necessary for each query. The 3D distance keys can, however, reduce the set of possible compounds. If it is necessary to identify only the presence (diversity) of pharmacophores in a library, then only those not found during a search of the stored conformations need be sought with conformational regeneration. Search results are obtained on a compound-by-compound basis, and thus a pharmacophore key for each structure is readily produced. This enables further 3D analysis (for example, clustering studies or as a rapid means of pharmacophore identification after screening) and identifies promiscuous compounds that match a large number of pharmacophores.

ChemDiverse method

The ChemDiverse 3D pharmacophore method is based on the perception, at search time, of a very large number of

pharmacophores. These are created internally using a set of distance ranges and choosing three-center combinations of pharmacophore types. The default distance ranges are currently those used to define the 3D distance keys (30 ranges: 1.7–3.0 Å in 0.1 Å steps; 3.0–8.0 Å in 0.5 Å steps; 8–15 Å in 1 Å steps) and the three-center combinations are chosen from four to seven generic center types. These are defined using a customizable parameterization database file; the first four are usually:

- General O,N hydrogen-bond donors
- General O,N hydrogen-bond acceptors
- Positively charged and/or basic nitrogens
- Aromatic ring centers and/or hydrophobic groups

This gives a combination of 160,844 three-center pharmacophores with valid geometry (from the combination of the 20 three-point combinations of the four pharmacophore types and 30 distance ranges). A greater number of pharmacophore types can now be used, giving increased pharmacophoric resolution. Extension to seven types enables separate consideration of up to three new environments; these are normally:

- Hydrophobic groups
- Acidic centers
- Basic nitrogen

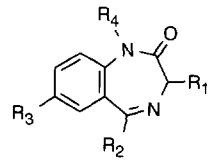
This gives a total of 848,925 valid three-center pharmacophores (from the combination of 83 three-center pharmacophores and 30 distances). These pharmacophore triangles are sought during one analysis with conformational regeneration, a bit (in memory) being set for the presence of a pharmacophore in any of the conformations. The analyses are relatively fast, running at greater than 1,000 compounds per hour with the corporate database on a Silicon Graphics machine (R4400; 200 Mhz); only one complete search of the database is required.

Although both the PDQ and ChemDiverse methods are currently based on identifying three-center pharmacophores, the methods work in very different ways. Explicit atom types (for example *N*-basic and acidic) and queries are used in the PDQ method. In contrast, in ChemDiverse, a limited number of generic center types (for example donor and acceptor) and generic queries are used. Another important difference is that in the PDQ method, the molecules are required to fit on to the pharmacophore (within a defined tolerance), as in normal 3D database searching, whereas in ChemDiverse, only the distance criteria need be met, although currently with small distance tolerances. The latter distance-only method can allow structures to be hits where the pharmacophoric shape is over-distorted, particularly when larger distance tolerances are used, and the consequences of potential false positives using this method have recently been shown[17]. The pharmacophoric resolution possible is, however, potentially far greater in the ChemDiverse method, enabling profiling of up to almost one million pharmacophores with conformational sampling, compared with 6,000 for the PDQ method, with much slower search times with conformational sampling. By using individual queries, as in PDQ, more information and post-processing is possible at the compound level.

Pharmacophore volume [proportion (%) of the structure involved in the pharmacophore being analyzed] and pharmacophore accessibility (for example, checking that the lone pairs are not pointing within the triangle defining the pharmacophore) are also very important, and approaches to accommodate these are under development in the PDQ method, and are becoming available in ChemDiverse. The methods of pharmacophoric analysis can be considered as partitioning, rather than clustering, and missing pharmacophoric diversity is readily identified as all valid distance range combinations are searched.

Example of library diversity measurement

An expanded version of the benzodiazepine combinatorial library proposed by De Witt and coworkers[18] was analyzed using the PDQ and ChemDiverse methods. The structures (1,232) were built into a ChemDBS-3D database using CONCORD[19] (Tripos, St Louis, MO, USA) to generate the 3D structures and the RPR customized center perception



R_1 – Gly, Ala, Val, Phe, Trp, Asp, Asn, Glu, Gln, Thr, Lys
R_2 – Ph, 4-MeOPh, cyclohexyl, 2-thienyl, 4-NO_2Ph, 4-NH_2Ph
R_3 – H, Cl, NO_2, OMe, NH_2
R_4 – H, Me, benzyl, *i*-Bu

Figure 2. *Benzodiazepine combinatorial library used for pharmacophore analysis.*

parameterization. The composition of the library is shown in Figure 2; the library was essentially a combination of 11 (R_1) × 6 (R_2) × 5 (R_3) × 4 (R_4) substituents, with removal of 88 combinations incompatible with the chemical synthesis proposed.

Analysis of the database by the PDQ method found that 23% of the queries (1,366 from 5,916) were matches, all compounds matching at least one pharmacophore-derived query. Of the 56 three-point pharmacophore class combinations possible, 12 were not covered; these mainly involved basic groups. Using the parameterization described above for pharmacophores chosen from four center types with ChemDiverse, 7.6% of the possible queries were found in the library (12,186 from 160,844 queries).

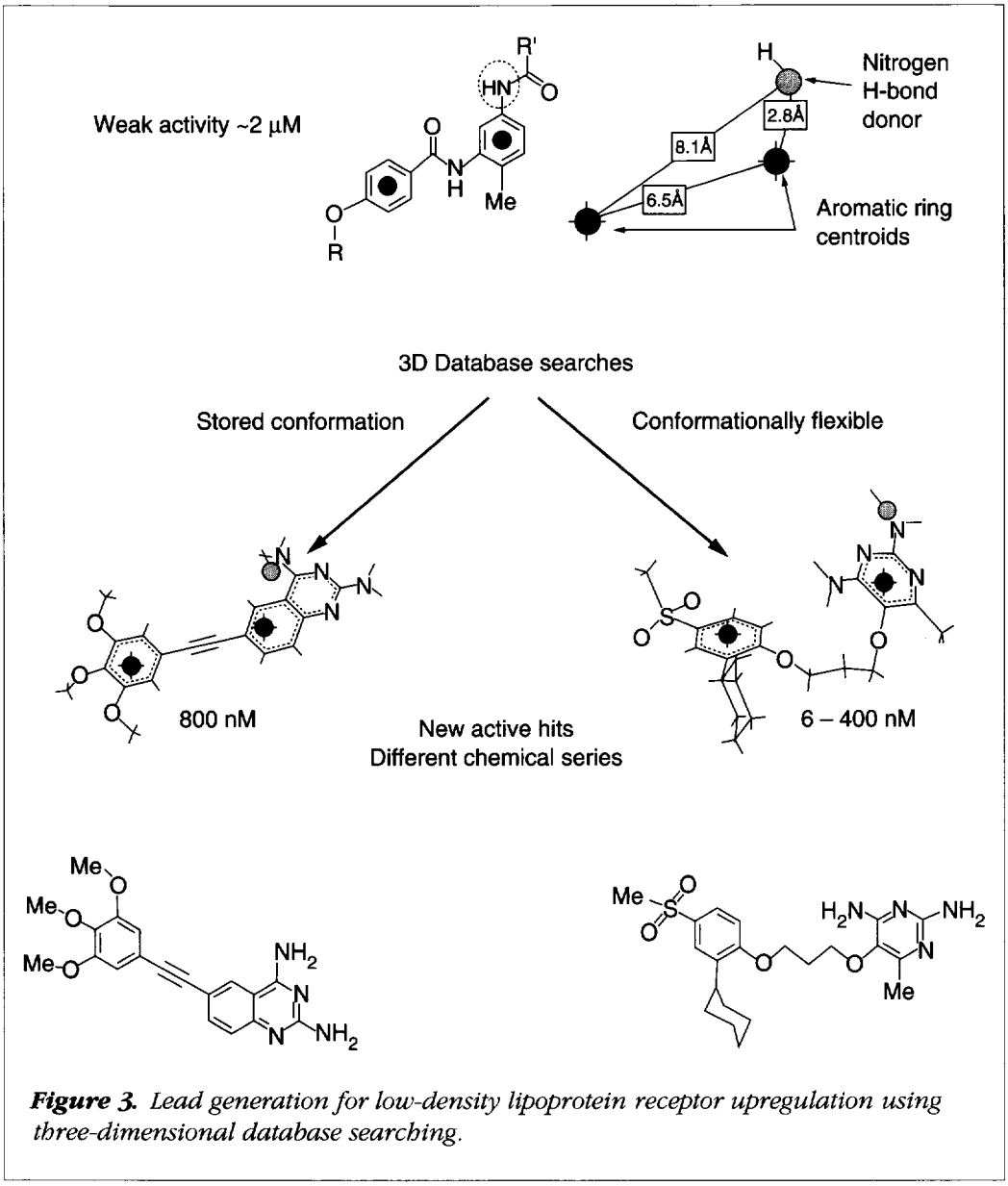


Figure 3. *Lead generation for low-density lipoprotein receptor upregulation using three-dimensional database searching.*

Example of diverse subset identification

Another powerful use of pharmacophore-profiling methods is for identification of the minimum subset of a database that represents most of the possible pharmacophores. For example, with the benzodiazepine library of 1,232 structures described above, 12,186 pharmacophores were found with ChemDiverse. If the library is reanalyzed, rejecting structures with more than 90% overlap of their pharmacophores with the sum of those already searched, it is possible to identify 93 compounds that cover 11,013 pharmacophores. Thus, 7% of the structures can cover 90.4% of the possible pharmacophoric diversity of the library. By reanalysis with 95% overlap rejection, it is possible to increase the coverage further, such that 9.6% of the library represents 96.1% of the total exhibited pharmacophores. The method is search-order dependent, these results were from a set presorted by their group and distance key diversity; almost twice as many compounds (13%) were needed to cover 90.3% of the pharmacophores in an unsorted set. A final subset selection might include diversity of other properties, but this example illustrates an approach dealing with pharmacophoric diversity. By use of the pharmacophore key of an existing library or library subset as the starting point, this method can be used for ready identification of those compounds that add to existing diversity.

These methodologies could also be used to measure diversity and to suggest compounds required to increase the diversity in existing collections. The PDQ and ChemDiverse 3D pharmacophore-profiling methods enable either evaluation of

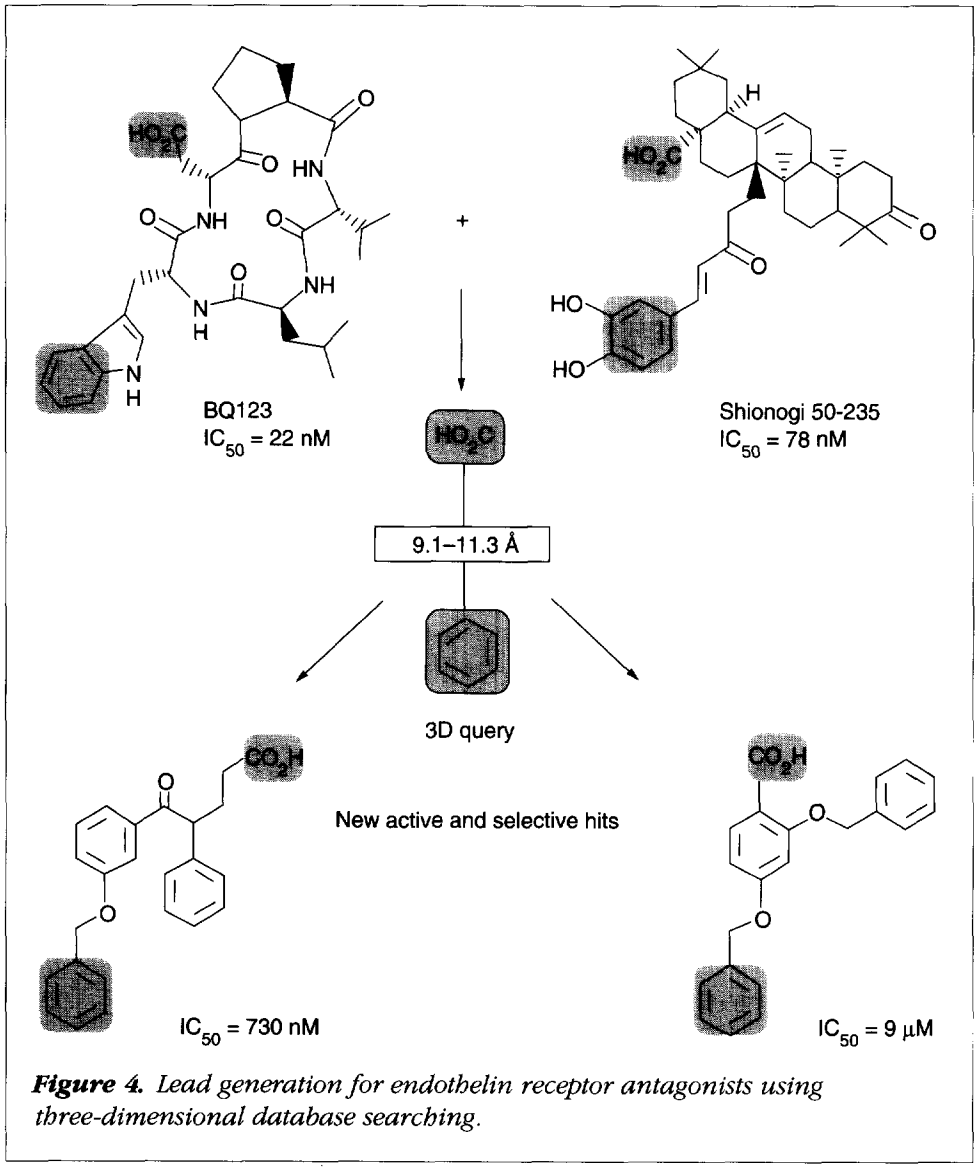


Figure 4. *Lead generation for endothelin receptor antagonists using three-dimensional database searching.*

the diversity within a library, as described above, or the identification of compounds that add to existing diversity. Compounds that exhibit a desired pharmacophore can be identified directly after PDQ analysis or by 3D database searching.

3D database searching

The power of this method for generating new leads has recently been reviewed[20]. The many uses of 3D databases in drug design have made this method an important CADD tool, and commercial software systems that offer approaches to the fundamental problem of allowing for the conformational flexibility of the structures are now readily available [for example, ChemDBS-3D, UNITY (Tripos, St Louis, MO, USA) and MACCS/3D and ISIS/3D (MDL Information Systems, San Leandro, CA, USA)]. The different systems deal with the conformational sampling problem in different ways, either generating conformations during the searches (systematic, random or torsional forcing to the defined query) or storing a 'diverse' set of conformers. The ChemDBS-3D system appears to be very effective because it enables customizable automatic perception of atom environments (for example basic, acidic or hydrophobic environments) and powerful conformational sampling methods.

Examples of the power of this method are given below. The examples are taken from the LDL receptor upregulator and endothelin antagonist screening programs at RPR.

Example: LDL receptor upregulator

One of the cardiovascular targets at RPR was to find an LDL receptor upregulator[21], and the goal from screening was to identify compounds that reduce blood LDL concentrations. Compounds with low activity (EC_{50}: 1.7 μM), based on a dibenzamide structure, had been identified, but more potent compounds proved elusive. From the common features of active compounds, a three-point 3D pharmacophore model was derived (Figure 3), and 3D searches of the corporate databases using this query in ChemDBS-3D yielded two series of potential leads. One of the new active leads was a diaminoquinazoline (EC_{50}: 0.8 μM), identified after a 3D search of the stored CONCORD-generated conformation (a single conformation search). When conformational flexibility was allowed in the search, by use of rule-based conformer regeneration in ChemDBS-3D, a second more potent diaminopyrimidine series was discovered. From this series, compounds already existing in the corporate registry were identified with activities of about 6 nM (Figure 3); an extended CONCORD conformation clearly would not fit the query, showing the importance

of allowing for conformational flexibility in the 3D searches.

Example: endothelin receptor antagonist

An endothelin receptor antagonist program also illustrated another example of the power of 3D pharmacophoric searches for the discovery of new leads[22]. The goal was to identify selective endothelin (ET_A subtype) receptor antagonists for cardiovascular therapy. The assay was part of a general new leads screening program from which no leads of interest (IC_{50} of less than 10 μM) had been found following screening of approximately 4,000 general compounds.

When a pharmacophoric model derived from molecular modeling studies on two potent selective antagonists, a cyclic pentapeptide and a terpenoid, was used to perform 3D database searching to generate a directed screening set, the yield of hits was much improved. The 3D query used was a common distance identified between acidic and aromatic groups present in both active structures (Figure 4). The 3D searches identified 700 compounds of potential interest from the corporate databases; screening of this directed set of compounds identified ten new leads, active at concentrations below 10 μM, with selective ET_A activity. The most potent compound (IC_{50} ~ 500 nM) was identified only when the 3D search allowed for conformational flexibility. The 3D search strategy and some example hits are shown in Figure 4. A subsequent medicinal chemistry program has turned these leads into very potent endothelin receptor antagonists and has developed improved 3D models for predicting activity. Screening of a further 400 compounds related to the 3D hits by their 2D structural characteristics, but not matching the pharmacophore model, failed to yield further hits, indicating that the activity was a consequence of the correct 3D spatial disposition of important groups, rather than the chemical family itself.

Conclusion

The combination of genomics and molecular biology for the discovery of targets relevant to disease pathologies, together with the emerging technologies of diverse combinatorial chemistry and drug design will lead, in the next few years, to a powerful arsenal of drugs for the treatment of many major diseases. New methods for measuring 3D diversity and the use of the related 3D database searching techniques have yielded interesting results in preliminary studies. Such methods can be used to measure the pharmacophoric diversity of combinatorial and other libraries, and enable the identification of diverse subsets. The techniques can be applied to library design and to the partitioning and clustering of compound databases. These new approaches should facilitate the discovery of new leads from which new drug candidates can be developed within a time-frame not previously considered possible.

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