REVIEW ARTICLE

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Pharmacophore Mapping and Drug Design

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A very important part of drug design is prediction of small molecule binding to a target macromolecule. A reasonable qualitative prediction of binding can be made by specifying the spatial arrangement of a small number of atoms or functional groups. Such arrangement is called pharmacophore. The pharmacophore search finds molecules with different overall chemistries, but which have the functional groups in the correct geometry. A common use of pharmacophores is to search 3D databases for molecules that contain the pharmacophore.

A pharmacophore is the spatial mutual orientation of atoms or groups of atoms assumed to be recognized by and interacted with a receptor or the active site of a receptor. In conjunction with the receptor concept, the notion of a pharmacophore relates directly to the lock-and-key theory proposed by Fischer and Ehrlich around the beginning of this century *(Corpora non agunt nisi fixata)*. In a broad sense, a pharmacophore or pharmacophoric pattern is the set of features a compound must have to elicit a certain biological activity. These features are typically any combination of structural, chemical and physical attributes of a molecular structure. For example, a description of a pharmacophore can be relatively simple and nonspecific such as two lipophilic centers separated by 10-12 Å. On the other side of the spectrum a pharmacophoric description could comprise a three-dimensional (3D) array of specific functional groups and their geometric relation.

A wide range of experimental and theoretical data is routinely used to develop pharmacophoric patterns. This process is generally refereed to as pharmacophore mapping and involves three main aspects: finding the features required for biological activity; determining the molecular conformation required (i.e., the bioactive conformation) and developing a superposition or alignment rule for the series of compounds.

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In drug design, the term pharmacophore refers to a set of features that is common to a series of active molecules. Hydrogen bond donors and acceptors, positively and negatively charged groups and hydrophobic groups are typical features. Such features are referred to as pharmacophoric groups. A 3D pharmacophore specifies the spatial relationships between the groups. These relationships are often expressed as distances or distance ranges but may also include other geometrical measures such as angles and planes. For example a commonly used two-dimensional (2D) pharmacophore for antihistamines contain two aromatic rings and tertiary nitrogen distributed as shown in fig. 1. The objective of a procedure known as pharmacophore mapping is to determine possible 3D pharmacophores for a series of active compounds. Once a pharmacophore has been developed it can then be used to find or suggest other active molecules[1,2].

There are two aspects to consider when calculating 3D pharmacophores. Unless the molecules are all completely rigid, one must take account of their conformational properties. The second aspect is to determine those combinations of pharmacophoric groups that are common to the molecules and can be positioned in a similar orientation in space. More than one pharmacophore may be possible; indeed, some algorithms can generate hundreds of possible pharmacophores which must then be evaluated to determine which best fits the data. It is important to realize

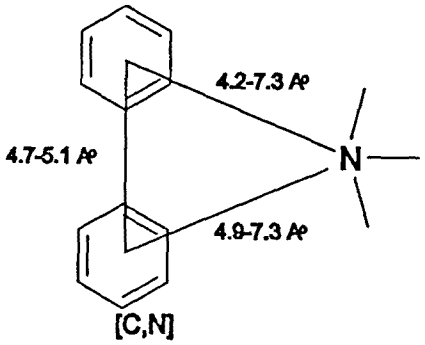

Fig. 1: Antihistamine 3 D pharmacophore.

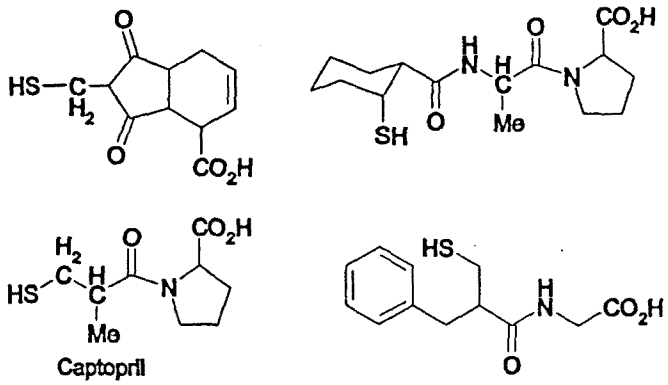

Fig. 2: Four typical ACE inhibitors.

that all of these approaches to finding 3D pharmacophores assume that all the molecules bind in a common manner to the macromolecule[3].

FINDING AND DERIVING 3D PHARMACOPHORES[4]

Constrained systematic search:

A well-known example is the pharmacophore for angiotensin-converting enzyme (ACE), which is involved in regulating blood pressure. ACE is a zinc metallopeptidase. Four typical ACE inhibitors are shown in fig. 2. Three features are required for activity; a terminal carboxyl group (believed to interact with an arginine residue in the enzyme), an amido carbonyl group (which hydrogen bonds to an amide carbonyl in the enzyme) and a zinc-binding group. The problem is to determine conformations in which the inhibitors can position these three pharmacophoric groups, in the same relative position in space. One of the most widely used methods for tackling this problem is the constrained systematic search method of Dammkoehler *et al.*[5].

To derive an ACE pharmacophore, four points were defined for each molecule. The derivation of these four points for captopril is shown in fig. 3. Two distinct 3D pharmacophores (I, II) were obtained from the search, shown in fig. 4. The constrained search could be performed three orders of magnitude faster than the approach involving a separate systematic search on all the molecules.

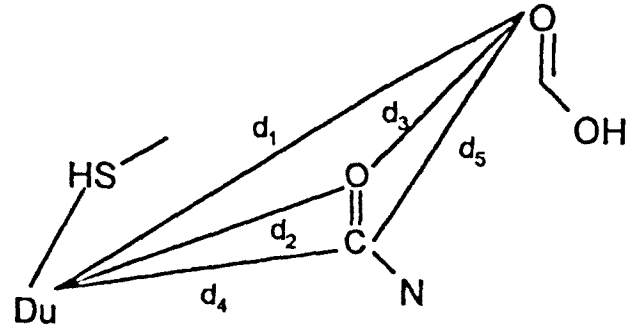

Fig. 3: Four points and five distances define the ACE pharmacophore.

Ensemble distance geometry and ensemble molecular dynamics:

Ensemble distance geometry can be used to simultaneously derive a set of conformations with a previously defined set of pharmacophoric groups overlaid. Ensemble distance geometry uses the same steps as standard distance geometry with the special feature that the conformational spaces of all the molecules are considered simultaneously. This is done using much larger bounds and distance matrices, with dimensions equal to the sum of the atoms in all the molecules. The first application of ensemble

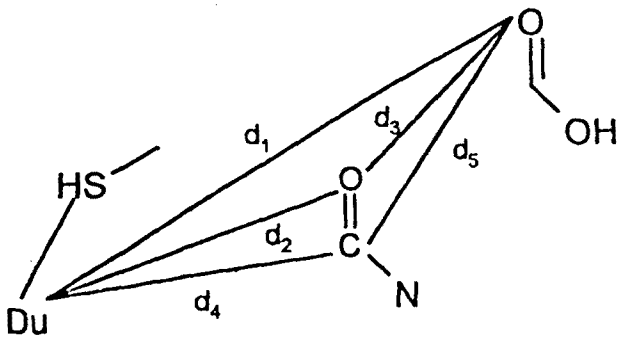

Fig. 4: Two ACE pharmacophores identified by constrained systematic search.

For series I, d_1=8.50-8.785 Å, d_2=4.75-5.250 Å, d_3=3.75-4.000 Å, d_4=5.25-5.500 Å and d_5=4.00-4.250 Å. For series II, d_1=5.75-6.25 Å, d_2=2.75-3.00 Å, d_3=3.75-4.00 Å, d_4=3.25-3.75 Å and d_5=4.00-4.25 Å.

distance geometry was to derive a model of nicotinic pharmacophore using the four nicotinic agonists shown in fig. 5. This pharmacophore can be represented as a triangle. Note that the B-C distance is fixed at the length of the C=O bond. Also note that in (-)-nicotine the centroid of the pyridine ring is defined as one of the pharmacophoric points. The pharmacophore was then tested by determining whether low-energy conformations should be generated for other known nicotinic agonists that were consistent with the distance constraints of the pharmacophore.

Clique detection methods for finding pharmacophores:

When there are many pharmacophoric groups present in the molecule it may be very difficult to identify all possible combinations of the functional groups (there may be thousands of possible pharmacophores). To tackle this problem, clique detection algorithms can be applied to a set of precalculated conformations of the molecules. To understand what we mean by a clique we need to understand some simple elements of graph theory.

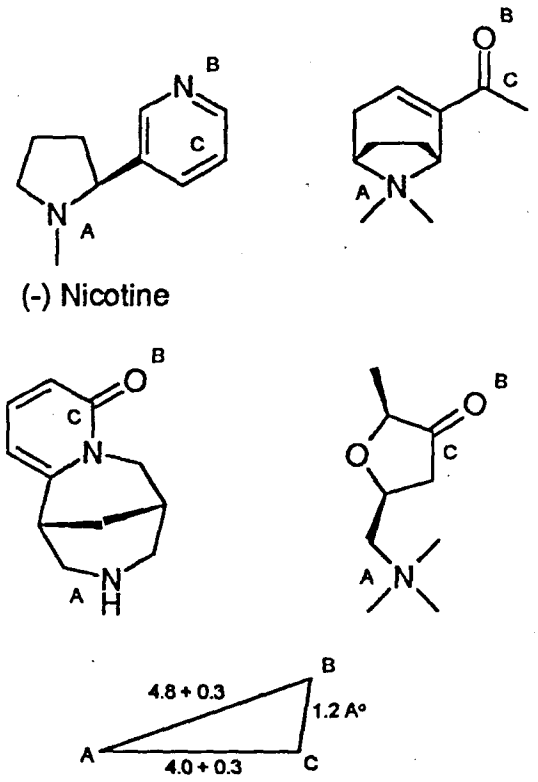

Fig. 5: Four molecules used to derive the nicotinic pharmacophore by distance geometry and the pharmacophore obtained.

A clique is defined as a maximal completely connected subgraph. In the clique detection approach, the first step is to generate a family of low energy conformations for the molecules. The molecule with the smallest number of conformations is used as the starting point, with each of its conformations being used in turn as the reference structure. Each conformation of every other molecule is then compared with the reference conformations and the cliques identified. The cliques for each molecule are obtained by combining the results for each of its conformations. Those cliques that are common to at least one conformation from each molecule can then be combined to give a possible 3D pharmacophore for the entire set.

Incorporating geometric features in a 3D pharmacophore:

The features used to define a 3D pharmacophore are most easily derived from the positions of specific atoms within each molecule. It may be more appropriate to consider locations around the molecule where the receptor might position its functional groups. This is especially useful for hydrogen bond donors and acceptors; two ligands may be able to hydrogen bond to the same protein atom with the ligand atoms being in a completely different location in the binding site. The 3D pharmacophore may also contain features that are designed to mimic the presence of the receptor. These are commonly represented as exclusion spheres, which indicate locations within the 3D pharmacophore where no part of a ligand is permitted to be positioned.

COMPUTATIONAL METHODS FOR PHARMACOPHORE MAPPING[6]

The experimental medicinal chemistry lays the foundation for pharmacophore-mapping exercises. Some of the methods focus on developing alignment rules (usually for pairs of molecules), whereas others treat the generation of bioactive conformations more heavily. Some of the more sophisticated approaches simultaneously consider both of these aspects. The methods rely on well-known molecular-modelling techniques, as well as more recently exploited tools, such as genetic algorithms and clique detection.

Alignment or bioactive conformation methods:

Steric and Electrostatic ALignment Method (SEAL) method uses steric and electrostatic features along with exhaustive searching to compare all possible orientations[7]. The method considers how these features affect ligand binding, without knowing the structure of the protein. Three

assumptions are made (which are common to pharmacophore-mapping techniques); all compounds in the set studied bind to the same site and groups in the protein, the three dimensional structure of the protein binding site is similar for each ligand, therefore, the site can be held fixed and several representative, low-energy conformations of each ligand can be used.

Electrostatic features are represented by partial atomic charges. The molecular volume and the van der Waals radii represent the steric or shape features. The heart of the method is the scoring function, which measures the degree of similarity between two superimposed molecules. The seal work has been extended and modified by the inclusion of terms in the alignment function for atomic hydrophobicites and refractivites, which treat lipophilicity and polarizability.

A significantly different technique for aligning sets of flexible molecules has been reported by Perkins and Dean[8]. An important feature of this strategy is the use of simulated annealing and cluster analysis to find a small set of very different conformers to represent the conformational space of each molecule in the set. All pairs of conformers are aligned by matching randomly selected sets of atoms and minimizing a difference distance metric (simulated-annealing techniques are also used here). Matches are then analyzed in three ways- (i) clustering of all matched pairs to find representative conformers, (ii) matching of each representative conformation with each molecule as a reference, and (iii) determination of the best set of conformations independent of any reference molecule.

Pharmacophore mapping attempts to find features important for receptor binding. Therefore researchers are developing alignment techniques that focus on deriving and comparing potential receptor-binding sites from ligands, rather then just using the atoms of the ligands. A good example of this is the AUTOFIT program developed by Itai and colleagues[9,10].

HipHop is an another alignment method embodied in a computer program. A wide range of structural features can be aligned, including hydrogen bond donors and acceptors, several classes of hydrophobic regions and user-defined regions. The method is applicable to very flexible molecules and does not require the designation of a reference compound. In addition, HipHop can identify subsets of matching pharmacophoric points, rather than requiring that all regions match.

Pharmacophore map generation and validation methods:

One of the first reported methods for pharmacophore or receptor mapping, which used structurally diverse active and inactive compounds, is known as the active analogue approach[11,12]. This approach begins by using observed structure-activity relations to propose functional groups important for bioactivity. For example, in a series of 28 potent angiotensin converting enzyme (ACE) inhibitors, three requirements for binding were a terminal carboxyl group, a carbonyl oxygen (preferably in an amide group), and a good zinc-binding group (such as a carboxylic or hydroxamic acid)[11]. An initial pharmacophore map was constructed by evaluating the geometric relation of these proposed pharmacophoric points in several potent rigid analogues. A conformational search for each of the 28 compounds, using the systematic search facility in the molecular-modelling program SYBYL, was carried out to find the conformer that best matched the map. A related approach, used in identifying a pharmacophore for a series of cholesterol biosynthesis inhibitors, has been reported[13]. This led to the selection of the following types of proposed pharmacophoric points: a point for the lactonic moiety, two lipophilic, one bulky, and one polar. An example of this involving a series of active and inactive cannabinoids was reported[14]. The approach here is to attempt to determine the receptor essential volume (REV) (i.e., that region of space occupied by the atoms of inactive analogs that is not occupied by atoms of active analogs).

Several newer approaches to pharmacophore mapping incorporate the notion of calculating and treating potential receptor interaction sites as one of the features central to their technique. One such example is a program named DISCO (distance comparisons) developed by Martin and coworkers[15]. DISCO simultaneously determines bioactive conformations and superposition rules (i.e., active ligands) for the biological target of interest. An important feature of DISCO is the calculation of hydrogen bond accepting and bond donating sites. These points are automatically computed from atoms of interest in the compound and they represent putative sites of interaction with the biological target. For example, a carbonyl group would have two hydrogen bond-donating sites, at a distance of 2.9 A° from the oxygen atom. These represent possible hydrogen-bond interaction sites with a hydrogen bond donating group in a protein. Finally, the user selects a reference compound (usually the one with the fewest conformations).

Each conformation of all compounds (excluding the

reference) is compared with the reference compound to find sets of pharmacophoric points that are common to at least one conformation of each compound. This is done by comparing corresponding interpoint distances of the reference with those in every other conformation. From this, a clique detection algorithm[15,16] finds the largest set (cliques) of common distances. Compared distances are considered to be within the same range if they differ by no more than a user-specified tolerance value. Finally, the cliques that meet any user-defined criteria, such as required number and type of pharmacophoric points, are output as proposed pharmacophore maps. Two examples are; a set of dopaminergic agonists[17], and a diverse collection of benzodiazepine agonists[18].

An additional pharmacophore-mapping strategy that has been integrated in a commercially available molecular-modelling program is available in the Chem-X program. The approach follows a familiar theme. First, a conformational analysis is performed to identify low-energy conformers for the data set. The next step is automatic assignment of potential pharmacophoric points, which are hydrogen bond acceptors, hydrogen bond donors, positively charged atoms, and aromatic ring centroids. During this process, distances between pairs of these points (distance keys) and data on the number, types, and interpoint connectivity are stored (formula keys). Searching the distance and formula keys for all possible three-or four point pharmacophore identifies proposed pharmacophores. Checking that at least one conformation of each compound matches the pharmacophore must then validate these pharmacophores.

Another example of a pharmacophore generation program incorporated into commercial molecular-modeling software is the program APEX-3D. Similar to other approaches to pharmacophore mapping, APEX-3D attempts to find sets of features common to low-energy conformations of each compound. These features (pharmacophoric points) can be ring centers, hydrophobic regions, and hydrogen-bonding sites. The program can evaluate two-dimensional (2D; topological) or three-dimensional (3D; topographical) relations between these points in generating proposed pharmacophoric maps. Actually, the authors of APEX-3D have adopted the term biophore for pharmacophore, to denote pharmacological and toxicological activity. Generation of biophores involves determining low-energy, representative conformers for each compound; calculation of descriptors for potential biophoric atoms and searching (usually a clique-detection algorithm) for maximal common 2D or 3D arrangements of biophoric centers. These arrangements or patterns are potential biopnores, which are then evaluated for their statistical, quantitative correlation with biological activity.

A pharmacophore-mapping approach similar in concept to APEX-3D is a program called Catalyst[19,20]. The method uses potential pharmacophoric sites, such as hydrogen bond donors and acceptors, hydrophobic, positive and negative groups, along with a conformational model and biological activity for each compound, to develop a quantitative pharmacophoric map or hypothesis.

Another pharmacophore-mapping strategy using entities related to protein interaction sites is the hypothetical active site lattice (HASL) methodology[21,22]. In this approach, a molecule is represented by a series of three-dimensional points in a lattice. The lattice is a three-dimensional grid of regularly spaced (typical spacing is 3.0 or 2.0 A°) points, into which each molecule is placed. Grid points that lie within the van der Waals radius of the molecule are used to characterize the molecule. Each of these grid points has two values: an atom type of electron-rich, electron-poor, or electron neutral and partial-binding value.

Less Conventional Methods:

Monte Carlo search procedure followed by energy minimization to generate proposed pharmacophore maps[23,24]. This involves generating potential pharmacophoric sites for each compound and then using a Monte Carlo technique to simultaneously search conformational space and orientation space (how the molecules are superimposed). In the search, pharmacophoric sites of the same types are given strong restraining potentials which ensures that only equivalent pharmacophoric sites will be superimposed in the generated pharmacophoric maps. In the Monte Carlo approach used, conformations and orientations are generated at random from a previous structure in an iterative process. Finally, pharmacophores generated by Monte Carlo are refined by energy minimization of all compounds in the map simultaneously, using the Multifit program[25] in SYBYL.

Genetic algorithms (GA)[26], an optimization technique that mimics natural evolution, have been applied to several problems in chemistry including 3D-QSAR and pharmacophore mapping[27-29]. Briefly genetic algorithms work by generating random populations of solution to a problem, scoring the relative quality of the solutions, and carrying forward the most-fit solutions or analogues (generated through mutation and crossover) of other solutions to

iteratively generate (and finally converge on) new, more-fit solutions. A GA approach was used to simultaneously vary conformations and orientations, for many generations, until the resulting pharmacophore maps were of the desired fitness. The fitness of generated maps was gauged by a combination of several measures; amount of deviation in corresponding pharmacophore point distances in different compounds, degree of shape similarity, overlap volume, similarity of charge distribution and steric accessibility of the conformations of each compound.

A quite different approach to pharmacophore investigation involving the concept of a hypermolecule, has been reported[30]. This method hypothesizes that the binding potency of a series of related active site inhibitors could be explained using specific regions of each compound and that these regions can be determined by combining the common portion and structural variations present in each compound into one structure, known as a hypermolecule. One data set studied was a collection of 127 *ortho*-, *meta*-, and *para*-substituted phenyl-*N*-methylcarbamate inhibitors of acetylcholinesterase (AChE). The atom positions in the hypermolecule were characterized using several classes of descriptors to evaluate lipophilic interactions, polarizabilty, electronegativity, sterics and hydrogen bonding.

SELECTED PHARMACOPHORE-MAPPING STUDIES

Kovar and co-workers[31] reported a pharmacophore map for 5-HT-reuptake inhibitors, using the active analogue approach, electrostatic potential and molecular volume. The study involves the investigation of 25 active and 7 inactive structurally diverse 5-HT-reuptake inhibitors. The active analogue approach was used to generate a pharmacophore map for the active compounds. A low-energy conformation of one of the most potent and somewhat rigid compounds of this set was used as the reference compound. Representative low-energy conformations of the active compounds were searched to find those that met the distance constraints of the reference. A map including the foregoing points and at least one conformation of each active compound was generated. From the overlay of all the active compounds onto the map, two additional pharmacophore regions were proposed, an electronegative group and a second aromatic ring. With each active compound superimposed on the map, further analyses, such as volume and molecular electrostatic potential comparisons, can be performed. Superposition of inactive compounds onto the pharmacophore map can also provide useful information. For example, inactive compounds often occupy regions of the map that are outside the union volume of all active compounds. In this study, the authors described examples of inactive compounds that illustrate these points. Finally, one method to help validate a pharmacophore model is an attempt to fit potent compounds not used to build the model. In this study, the bioactive conformation of the potent 5-HT-reuptake-inhibitor citalopram was generated by finding a low energy conformation that matched the pharmacophore map.

Development of pharmacophore models, followed by experimental verification, is a powerful combination to aid in design of biologically active compounds. This point is illustrated in design of biologically active compounds.

CURRENT DIRECTIONS

A common use of pharmacophores is to search 3D databases for molecules that contain the pharmacophore. In the best cases not only does this result in the discovery of new active molecules without the need for testing large numbers of compounds, but at can also give a structurally diverse set of active molecules[32,33].

Most of the recent work in the area of pharmacophore mapping involves the use of pharmacophore maps in related molecular-modelling applications, such as database searching, 3D-QSAR, and *de novo* compound design. Pharmacophoric patterns can often be translated into queries for 3D database searching, as illustrated by the work of Sheridan and co-workers[34]. Two specific examples of using pharmacophoric pattern searching can be found in reports involving endothelin antagonists[35] and nootropic agents[36]. Pharmacophore maps are often used to develop 3D-QSAR in series of biologically active compounds. In fact, 3D-QSAR generation methods have been partly used to validate pharmacophoric hypothesis[37].

An important area in computer-aided molecular modeling is the development of methods for *de novo* compound design[38]. Several approaches in this field can use pharmacophore models as starting points for structure design. For example, in the program NEW LEAD, key fragments from bioactive conformations are joined with spacers to generate new structures to fit the model[39]. In the program SPROUT, templates, such as five- and six-member rings and acyclic fragments are joined such that atoms in the resulting structure correspond to atoms in a pharmacophore model[40]. Finally, a program called ChemNovel has been used to generate a structurally diverse set of compounds to fit a pharmacophore model for 5-HT_3 receptor antagonists.

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REFERENCES

1. Goelender, V.E. and Vorpagel, E.R., In; Kubinyi, H, Eds., 3D QSAR in Drug Design, Escom, Ledien, 1993, 137.
2. Goodford, P.J., **J. Med. Chem.**, 1985, 28, 849.
3. Balbes, L.M., Mascarella, S.W. and Boyd, D.B., **Rev. Comput. Chem.**, 1994, 5, 337.
4. Leach, A.R., Eds., Molecular Modelling, Principles and Applications, Longman, Essex, England, 1996, 544.
5. Dammkoehler, R.A., Karasek, S.F., Shands, E.F.B. and Marshall, G.R., **J. Comput. Aid. Molec. Design**, 1989, 3, 3.
6. Bures, M.G., In; Charifson, P.S., Eds., Practical Application of Computer-Aided Drug Design, Marcel Dekker, Inc., New York, 1997, 39.
7. Kearsley, S.K. and Smith, G.M., **Tetrahedran Comput. Methodol.**, 1990, 3, 615.
8. Perkins, T.D.J. and Dean, P.M., **J. Comput. Aid. Molec. Design**, 1993, 7, 325.
9. Kato, Y., Inoue, A., Yamada, M., Tomioka, N. and Itai, A **J. Comput. Aid. Molec. Design**, 1992, 6, 475.
10. Itai, A., Inoue, A., Tomioka, N., Inoue, A. and Kato, Y., In; Kubinyi, H, Eds., 3D QSAR in Drug Design, ESCOM, Ledien, 1993, 200.
11. Mayer, D., Naylor, C.B., Motoc, I. and Marshall, G.R., **J. Comput. Aid. Molec. Design**, 1987, 1, 3.
12. Sufrin, J.R., Dunn, D.A. and Marshall, G.R., **Mol. Pharmacol.**, 1981, 19, 307.
13. Cosentino, U., Moro, G., Pitea, D., Scolastico, S., Todeschini, R. and Scolastico, C., **J. Comput. Aid. Molec. Design**, 1992, 6, 47.
14. Reggio, P.H., Panu, A.M. and Miles, S., **J. Med. Chem.**, 1993, 36, 1761.
15. Martin, Y.C., Bures, M.G., Danaher, E.A., DeLazzer, J., Lico, I. and Pavlik, P.A., **J. Comput. Aid. Molec. Design**, 1993, 7, 83.
16. Waszkowycz, B., Clark, D.E., Frenkek, D., Li, J., Murray, C.W., Robson, B. and Westhead, D.R., **J Med. Chem.**, 1994, 37, 3994.
17. Froimowitz, M. and Cody, V., **J Med. Chem.**, 1993, 36, 2219.
18. Wong, G., Koehler, K.F., Skolnick, P., Gu, Z.Q., Ananthan, S., Schonholzer, P., Hunkeler, W., Zhang, W. and Cook, J.M., **J. Med. Chem.**, 1993, 36, 1820.
19. Smellie, A., Teig, S.L. and Towbin, P.L., **J. Comp. Chem.**, 1995, 16, 171.
20. Smellie, A., Kahn, S.D. and Teig, S.L., **J. Chem. Infs. Comput. Sci.**, 1995, 35, 295.
21. Doweyko, A., **J. Med. Chem.**, 1994, 37, 1769.
22. Saxena, A.K., Saxena, M., Chi, H. and Wiese, M., **Med. Chem. Res.**, 1993, 3, 201.
23. Hodgkin, E.E., Miller, A. and Whittaker, M., **J. Comput. Aid. Molec. Design**, 1993, 7, 515.
24. Hodgkin, E.E., Miller, A. and Whittaker, M., **Bioorg. Med. Chem. Lett.**, 1992, 2, 597.
25. Whitten, J.P., Harrison, B.L., Weintraub, J.R. and McDonald, I., **J. Med. Chem.**, 1992, 35, 1509.
26. Holland, J.H., **Sci. Amer.**, 1992, 267, 66.
27. Walters, D.E. and Hinds, R.M., **J. Med. Chem.**, 1994, 37, 2527.
28. Payne, A.W.R. and Glen, R.C., **J. Mol. Graphics**, 1993, 11, 74.
29. Jones, G., Willett, P. and Glen, R.C., **J. Comput. Aid. Molec. Design**, 1995, 9, 532.
30. Magee, P.S., **Quant. Struct-Act. Relat.**, 1990, 9, 202.
31. Rupp, A., Kovar, K.A., Beuerle, G., Rug, C. and Folkers, G., **Pharm. Acta Helv.**, 1994, 68, 235.
32. Gund, P., **Prog. Mol. Subcell. Biol.**, 1977, 11, 117.
33. Esaki, T., **Chem. Pharm. Bull.**, 1982, 30, 3657.
34. Sheridan, R.P., Rusinko, III, A., Nilakantan, R. and Venkataraghavan, R., **Proc. Natl. Acad. Sci.**, 1994, 34, 197.
35. Chan, M.F., Okuun, I., Stavros, F.L., Hwang, E., Wolff, M.E. and Balaji, V.N., **Biochem. Biophys. Res. Commun.**, 1994, 201, 228.
36. Takahasi, Y., Akagi, T. and Sasaki, S., **Tetrahedron Comput. Methodol.**, 1990, 3, 27.
37. Prendergast, K., Adams, K., Greenlee, W.J., Nachbar, R.B., Patchett, A. and Underwood, D.J., **J. Comput. Aid. Molec. Design**, 1994, 8, 491.
38. Lewis, R.A. and Leach, A.R., **J. Comput. Aid. Molec. Design**, 1994, 8, 467.
39. Tschinke, V. and Cohen, N.C., **J. Med. Chem.**, 1993, 36, 3863.
40. Gillet, V.J., Newell, W., Mata, P., Myatt, G., Sandor, S., Zsoldos, Z. and Johnson, A..P, **J. Chem. Inform. Comput. Sci.**, 1994, 34, 207.