Articles

Lead Generation Using Pharmacophore Mapping and Three-Dimensional Database Searching: Application to Muscarinic M_3 Receptor Antagonists

David P. Marriott,[†] Iain G. Dougall,[‡] Premji Meghani,[§] Yu-Jiang Liu,[‡] and Darren R. Flower*,[†]

Departments of Physical and Metabolic Sciences, Pharmacology, and Medicinal Chemistry, Astra Charnwood, Bakewell Road, Loughborough, Leicestershire LE11 5RH, U.K.

Received July 10, 1998

By using a pharmacophore model, a geometrical representation of the features necessary for molecules to show a particular biological activity, it is possible to search databases containing the 3D structures of molecules and identify novel compounds which may possess this activity. We describe our experiences of establishing a working 3D database system and its use in rational drug design. By using muscarinic M_3 receptor antagonists as an example, we show that it is possible to identify potent novel lead compounds using this approach. Pharmacophore generation based on the structures of known M_3 receptor antagonists, 3D database searching, and medium-throughput screening were used to identify candidate compounds. Three compounds were chosen to define the pharmacophore: a lung-selective M_3 antagonist patented by Pfizer and two Astra compounds which show affinity at the M_3 receptor. From these, a pharmacophore model was generated, using the program DISCO, and this was used subsequently to search a UNITY 3D database of proprietary compounds; 172 compounds were found to fit the pharmacophore. These compounds were then screened, and 1-[2-(2-(diethylamino)ethoxy)phenyl]-2-phenylethanone (pA_2 6.67) was identified as the best hit, with *N*-[2-(piperidin-1-ylmethyl)cycohexyl]-2-propoxybenzamide (pA_2 4.83) and phenylcarbamic acid 2-(morpholin-4-ylmethyl)cyclohexyl ester (pA_2 5.54) demonstrating lower activity. As well as its potency, 1-[2-(2-(diethylamino)ethoxy)phenyl]-2-phenylethanone is a simple structure with limited similarity to existing M_3 receptor antagonists.

Introduction

The pharmacophore is an important and unifying concept in rational drug design which embodies the notion that molecules are active at a particular receptor because they possess a number of key features (i.e. functional groups) that interact favorably with this receptor and which possess a geometry complementary to it. It is possible to derive pharmacophores in several ways: by analogy to a natural substrate or known ligand, by inference from a series of dissimilar biologically active molecules (the so-called active analogue approach), or by direct analysis of the structure of a target protein.[1] Having derived a pharmacophore model there are, in general, two ways to identify molecules which share its features and may thus elicit a desired biological response. First, there is de novo design which seeks to link the disjoint parts of the pharmacophore together with fragments in order to generate hypothetical structures that are chemically reasonable yet typically wholly novel.[2] The second is "3D database searching", where large databases comprising 3D structures are searched for those that match to a pharmacophoric pattern.[3] One key advantage that 3D database searching has over de novo design is that it allows the ready identification of existing molecules which are either easily available or have a known synthesis. Moreover, with the application of results from graph theory,[4] the celerity of 3D database searching has allowed it to establish itself quickly as a tool in drug design with proven success in practical applications.[5,6]

In this paper we detail our strategy for integrating 3D database searching in rational drug design and describe our experiences of both its implementation and use. To exemplify our work we have sought to identify novel antagonists of the muscarinic M_3 receptor.

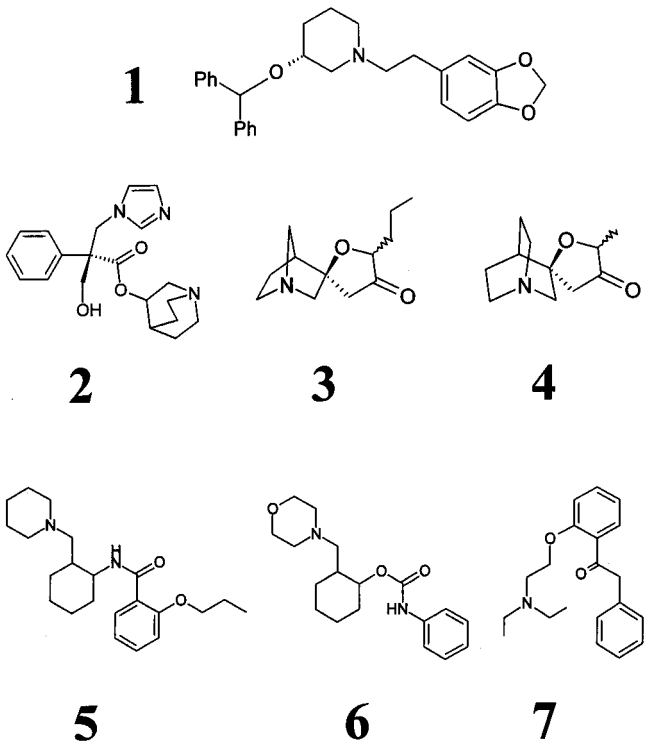
Muscarinic acetylcholine receptors have been shown to exist as 5 different subtypes (m_1−m_5), all of which have been cloned.[7] Four of these (M_1−M_4) have been pharmacologically defined and correlate with m_1−m_4 gene products, respectively.[8] Muscarinic M_3 receptor antagonists have therapeutic potential for the treatment of disorders associated with altered smooth muscle contractility or tone.[9] These include irritable bowel syndrome (IBS), chronic obstructive airways disease (COAD), and urinary incontinence.

We became interested in this area with the reported observations that a number of potent M_3-selective antagonists (such as Zamifenacin (**1**), Chart 1) exhibited further selectivity between M_3 receptors expressed in

* To whom correspondence should be addressed. Current address: Edward Jenner Institute for Vaccine Research, Compton, Berkshire RG20 7NN, U.K. Phone: 01635 577954. Fax: 01635 577901. E-mail: darren.flower@jenner.ac.uk.
[†] Department of Physical and Metabolic Sciences.
[‡] Department of Pharmacology.
[§] Department of Medicinal Chemistry.

10.1021/jm980409n CCC: $18.00
Published on Web 08/05/1999

Chart 1



different tissues of the same species.[9] Exploitation of these findings may provide further therapeutic advantages as drug candidates in any of the associated diseases mentioned above.

For example, Zamifenacin has undergone clinical evaluation by Pfizer for the treatment of IBS. It is a potent M_3-selective antagonist with some 12-fold further selectivity for ileal over tracheal M_3 receptors in the guinea pig. Pfizer has also published patents claiming a number of lung-selective M_3 antagonists for utility in COAD and asthma,[10] one example of which is exemplified by structure **2**.

Muscarinic pharmacology is an exceedingly well-explored field replete with inherent difficulties. To seek alternative novel chemical starting points aimed toward ultimately identifying lung M_3 receptor-selective compounds, we turned to a related program of work concerned with identifying M_1-selective agonists for potential utility in Alzheimer's disease.[11] Among them we discovered a number of compounds, as exemplified by structures **3** and **4**, which displayed additional M_3 antagonism in tissues derived from guinea pig lung and gut.[12] In addition, we aimed to find other novel structures, from our compound bank and commercial chemical suppliers, that would possess M_3 receptor antagonism—but not necessarily tissue selectivity at an early stage—which should be amenable to subsequent optimization of potency and selectivity. Pharmacophore generation based on the structures of known M_3 receptor antagonists, 3D database searching, and medium-throughput screening were used successfully to identify a small set of simple, novel lead compounds.

Experimental Section

Construction of 3D Databases. There are several published accounts of database construction concerned with building databases containing the accumulated compound banks of particular institutions.[13,14] Because the power and efficacy of 3D database searching is dependent on both the size and diversity of the compounds to be searched, we sought to extend the scope of our databases, beyond the limitations and bias of chemical series developed "in-house", by accessing purchasable compounds from the catalogues of chemical suppliers, for example, Maybridge, Aldrich, Specs & Biospecs, Bionet, etc.

Having obtained an initial set of 2D databases in a variety of different formats, we converted the atom and connectivity data present in each source database into a single common format, that of Weininger's SMILES notation, using the program ALTER.[15] As an independent check on the veracity of our conversion algorithm and the quality of the initial information, each file was passed through the program CCT, part of the Daylight software package.[16] This process transformed our arbitrary SMILES strings into so-called "uniquified SMILES", which provides an unambiguous description, or name, unique to a particular structure. An ordered leader-type clustering algorithm—similar to that employed successfully in the construction of nonredundant composite sequence databases[17]—was used to remove redundant data from our final 3D databases. A priority was assigned to the set of initial databases. By working down this ordered list of uniquified SMILES, any duplicated string identical to a higher-priority string, i.e. one above it in the combined list, could be identified and eliminated. The majority of 3D structures corresponding to the resulting SMILES strings were built using CORINA[18] and then imported into the UNITY database system.[19] A 3D database corresponding to each source catalog or databank was created in order to facilitate the administration and logistics of compound recovery for screening.

To have built all databases including redundant information would have wasted time converting very many structures to 3D several times over and wasted considerable space storing duplicate structures. Retaining duplicate structures would also have impinged deleteriously on the efficiency of searches (by searching the same structure many times) and the ergonomics of assessing search output (identifying and deleting duplicate matches).

Pharmacophore Generation. Three series of compounds were chosen to define our M_3 receptor pharmacophore: one series exemplified by lung-selective M_3 antagonist **2** and two series of related but distinct M_1 muscarinic ligands, exemplified by **3** and **4**, respectively, which also show affinity for the M_3 receptor. The structures of the three exemplifying compounds were constructed manually using SYBYL 6.2.[20] The geometries of the fused ring systems were taken from experimental structures found by searching the CSD.[21]

Having defined its rotatable bonds, 100 initial conformations were generated for **2** using the random search facility in SYBYL. By filtering these conformations to the lowest 5 kcal, a final set of 44 conformations was obtained. Compounds **3** and **4** were assumed to be rigid.

Derivation of the pharmacophore model was undertaken using DISCO,[22] although a simpler approach based on flexible fitting might have proved equally effective. Initial standard DISCO features (pharmacophore points) were edited to remove ring centroids. All features were also removed from the imidazole ring in **2**; this group was believed to confer tissue selectivity and was thus not appropriate for inclusion in our more generalized M_3 pharmacophore.

A DISCO run was undertaken using **3** as the reference, 0.5 Å tolerances, and requiring models to have between three and eight matched features. DISCO produced five different pharmacophore models satisfying these constraints. Each of the models showed at least four matched features. Of the different models, visual inspection of the resulting structural superpositions showed two models gave a good structural overlay of the three compounds, while the other models did not match the tertiary nitrogen common to the three molecules. The two remaining models are similar, yet distinct. Each of these two models was used to search our 3D databases and identify compounds for testing in our M_3 screen.

Pharmacology. 1. Guinea Pig Isolated Trachea. The

muscarinic M_3 receptor activity of compounds was assessed in the guinea pig isolated trachea.[23] Male albino Dunkin−Hartley guinea pigs (300−400 g) were killed by cervical dislocation, and the whole trachea was removed. After clearing the adherent connective tissue, the trachea was cut into six segments, each three cartilage bands wide, and then suspended in 10-mL organ baths containing Krebs solution of the following composition (mM): NaCl 117.56, KCl 5.36, NaH_2PO_4 1.15, $MgSO_4$ 1.18, glucose 11.10, $NaHCO_3$ 25.00, and $CaCl_2$ 2.55. This was maintained at 37 °C and continually gassed with 5% CO_2 in O_2. Indomethacin (2.8 μM) was included in the Krebs solution to prevent development of smooth muscle tone due to the synthesis of cyclooxygenase products. The tracheal rings were suspended between two tungsten wire hooks, one attached to an Ormed Beam isometric force transducer and the other to a stationery support in the organ bath. Changes in isometric force were recorded on 2-channel Advance Bryans flat-bed reorders.

2. Experimental Protocols. At the beginning of each experiment a force of 1.0 g was applied to the tissues, and this was re-instated over a 60-min equilibration period during which time the tissues were washed (replacement of bath fluid) twice. The following standard compounds were used: carbachol chloride (Sigma Chemical Co.), indomethacin (Sigma Chemical Co.). Indomethacin was dissolved in 10% w/v Na_2CO_3. All other compounds were dissolved in and diluted in distilled water.

3. Initial Screening. At the end of the equilibration period, tissues were contracted with 1 μM carbachol (a near maximally effective concentration). On reaching a stable response, test compounds were added at a concentration of 0.1 mM. Compounds that reversed the carbachol-induced contraction by greater than 50% were subsequently tested at 0.01 mM and 1 μM, in the same manner. This procedure was used to identify compounds ("hits") worthy of further investigation.

4. Testing of Hits Identified in Initial Screen. At the end of the equilibration period, an agonist concentration−effect (E/[A]) curve was generated in each tissue by cumulative additions of carbachol, at 0.5 log unit increments. Tissues were then washed and allowed to recover. Subsequently, the test compound (**5**, **6**, **7**, or vehicle) was added, and following a 60-min incubation period, a second carbachol E/[A] curve was generated. Contractile responses were expressed as percentages of the first curve maximum.

5. Data Analysis. Carbachol E/[A] curves obtained in the absence (control) and presence of the test compounds were fitted to the following form of the Hill equation:[24]

$$E = \frac{\alpha[A]^{n_H}}{[A]^{n_H} + [A]^{n_H}_{50}} \quad (1)$$

in which α, $[A]_{50}$, and n_H are the asymptote, location, and slope parameters, respectively. $[A]_{50}$ values were assumed to be log-normally distributed and estimated as $p[A]_{50}$ ($-\log [A]_{50}$) values.

Antagonist affinity estimations were made by calculating concentration−ratios (r) from the $[A]_{50}$ estimates for each pair of curves (control and in the presence of antagonist, B) and fitting these values to eq 2:[25]

$$\log(r - 1) = \log [B] + pA_2 \quad (2)$$

The affinities (pA_2 values) of the test compounds were estimated using a single concentration of **7** (3 μM), **6** (30 μM), or **5** (100 μM) in each of four separate experiments. Results are expressed as mean values ±SE. In the case of **7**, several additional concentrations (1 μM (n = 1), 10 μM (n = 3), and 30 μM (n = 2)) were employed across the four experiments, allowing a Schild analysis[24] to be carried out.

Results

We have sought to identify novel antagonists of the muscarinic M_3 receptor, our initial aim being the discovery of potent, but not necessarily tissue-selective, M_3 antagonists amenable to subsequent optimization. Pharmacophore generation based on the structures of known M_3 antagonists, 3D database searching, and medium-throughput screening were used to identify candidate compounds. Our pharmacophore model was derived from three series of compounds: a series exemplified by the Pfizer M_3 antagonist **2** and two structurally analogous series of compounds, exemplified by **3** and **4**, respectively, exhibiting affinity for the M_1 receptors but also showing significant M_3 potency.

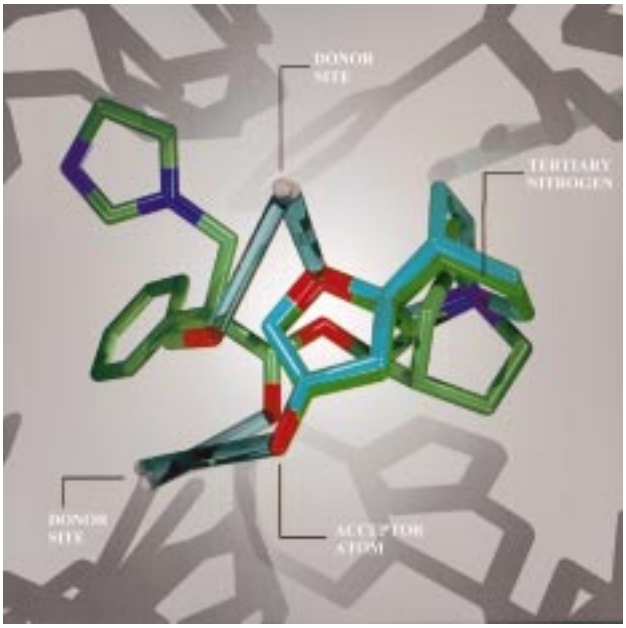


Figure 1. 3D overlay of compounds **2**, **3**, and **4** showing the superimposition of the four features common to pharmacophore models 1 and 2. The four features involved (a tertiary nitrogen, a hydrogen bond acceptor atom, and two separate hydrogen bond donor atoms) are labeled in the figure.

DISCO produced five different pharmacophore models. Of these different models, visual inspection of the resulting structural superpositions showed two models which gave a good structural overlay of the three compounds, while the remaining models did not match the tertiary nitrogen common to the three molecules. Figure 1 shows the structural overlay corresponding to model 1. The two acceptable models are similar, yet distinct (see Figure 2), and represent significantly different conformations, or rather binding modes, accessible to the three compounds. Each of these two models was used separately to search a UNITY 3D database.

The first model gave 176 hits from the Astra compound bank. The second model gave 173 hits; 172 compounds were common to the two sets, reflecting the similarity in the pharmacophores. Of the total 177 compounds identified by our search, only 172 compounds were, co-incidentally, for reasons of compound availability, solubility, etc., found to be available for screening in our M_3 receptor assay. Three compounds from our chemical bank were found to demonstrate significant activity (see Figure 3). **7** (1-[2-(2-(diethylamino)ethoxy)phenyl]-2-phenylethanone; $pA_2 = 6.67 \pm 0.07$ (n = 4)) was identified as the most active, with two other compounds, **6** (phenylcarbamic acid 2-(morpholin-4-ylmethyl)cyclohexyl ester; $pA_2 = 5.54 \pm 0.06$ (n = 4)) and **5** (*N*-[2-(piperidin-1-ylmethyl)cycohexyl]-2-propoxy-

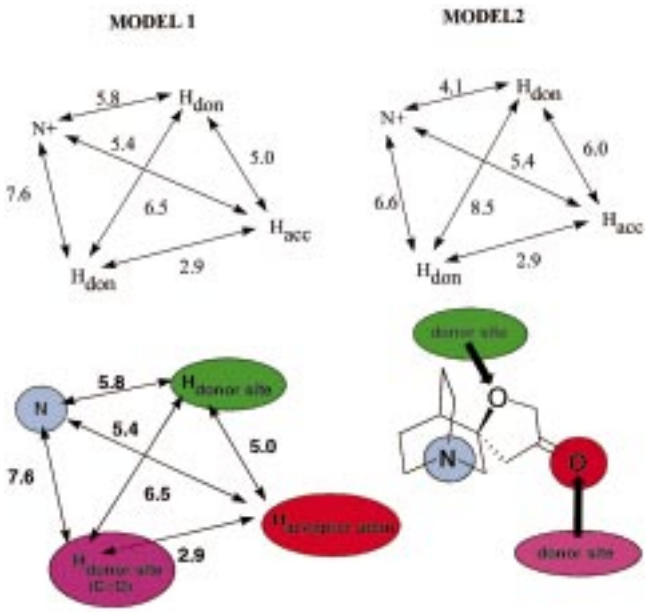


Figure 2. (Top) DISCO-generated pharmacophore models used to search databases. The two pharmacophore models used in database searching are shown in terms of the explicit distances between the four features used: N^+, tertiary nitrogen; H_{don}, hydrogen bond donor; H_{acc}, hydrogen bond acceptor. Tolerances of ±0.3 Å were applied to each distance. (Bottom) Schematic diagram showing how the features of pharmacophore model 1 map onto the functional groups in compound **4**.

benzamide; $pA_2 = 4.83 \pm 0.07$ ($n = 4$)), demonstrating lower but still significant activity. In addition, a Schild analysis carried out with **7** confirmed that this compound behaves as a simple competitive antagonist ($pK_B = 6.69 \pm 0.04$; 9 degrees of freedom) (data not shown).

Compound **7** was aligned with **4**. The matching conformation of **7** was retrieved from the UNITY database search, appropriate extension points were added, and the two structures were superposed (see Figure 4). This conformation of **7** clearly satisfies all the pharmacophore distances.

As well as its potency, **7** is a simple structure with little similarity to existing M_3 receptor antagonists, and its physical properties, such as molecular weight and log *D*, are within the range expected of good drugs. Moreover, compounds such as **5, 6**, and **7** should be amenable to further optimization in order to improve M_3 antagonist potency. Simple, straightforward structures such as these should allow optimization of potency, or selectivity, by combinatorial chemistry or parallel synthesis, for example, by formation of the amide bond in **5** or carbamate bond in **6** by classical carboxylic acid−amine coupling methods or isocyanate−alcohol condensation methods, respectively. The wide range of carboxylic acid and isocyanate libraries that are available to us would make this approach particularly attractive and would also ensure that a diverse set of compounds was synthesized. Replacement of the oxygen atom for a nitrogen atom in the carbamate moiety of structure **6,** such that a central urea moiety is generated, would lead to further combinatorial opportunities, with various amine libraries open to exploration.

Design modifications would attempt to seek, identify, and implant those features which may be responsible for aiding tissue selectivity in the Pfizer lung-selective compound (**2**) into our newly identified M_3 receptor antagonists. It would be interesting, for example, to explore substitution of the benzyl ring in **7**. Another obvious feature would be placement of the imidazole moiety onto the pharmacophore of these structures in various positions based on their overlay with structure **2**. This would allow us to explore further the role of this, and possibly other functional groups, in conferring tissue selectivity.

Again incorporation of the imidazole moiety in various positions onto any newly identified more potent M_3 receptor antagonists from combinatorial chemical methods could be used to add or enhance tissue selectivity by considering its structural overlay onto compound **2**.

Discussion

The potential power of 3D database searching is demonstrated here by our identification of novel antagonists of the muscarinic M_3 receptor. A pharmacophore was generated, using the program DISCO, from the structures of known M_3 antagonists. The 172 candidate compounds identified by 3D database searching were screened using a medium-throughput assay. Three compounds (**5**, **6**, **7**), with little similarity to existing M_3 antagonists, were found to exhibit potency in our screen; they constitute quality lead structures ripe for optimization. The hit rate for leads from this study is thus several orders of magnitude greater than from high-throughput screening, indicating the efficiency of pharmacophore-driven 3D database searching as a tool for lead identification.

Like many words used in science, as in life generally, "pharamacophore" has many meanings. Some use it to describe somewhat vague models of the environment within a ligand binding site. We take it to be something more specific and, possibly, more useful: an ensemble of interactive functional groups with a defined geometry. Although the medicinal chemistry literature is littered with pharmacophore definitions, we know of few generally useful compendia. For example, Jakes et al.[26] list 10 rather old pharmacophoric patterns, but even the most perfunctory examination of the literature shows this to be only a small proportion of all definitions. Most pharmacophores tend to be fairly simple two-, three-, or four-point (i.e. functional group) pharmacophores, although some incorporate more elaborate features such as best planes and regions of excluded volume. Figure 5 collects together a number of literature pharmacophores. These examples help illustrate the different flavors of pharmacophoric pattern. Certain of these examples, such as the 5-HT_3 pharmacophore, are dominated by a common substructure, with a concomitant effect on the performance of searches. Overspecifying a pharmacophoric pattern through the use of restrictive substructure criteria will limit the overall diversity and novelty of hits. In an ideal pharmacophore, the generality of functional groups does not restrict structural classes while the pharmacophore geometry supplies discriminating power to the search.

The principal advantage of 3D database methods over de novo design is the ability to identify extant molecules which can be obtained ready-made or synthesized by a validated method. This is clearly rather more efficient and economic than attempting the synthesis of specula-

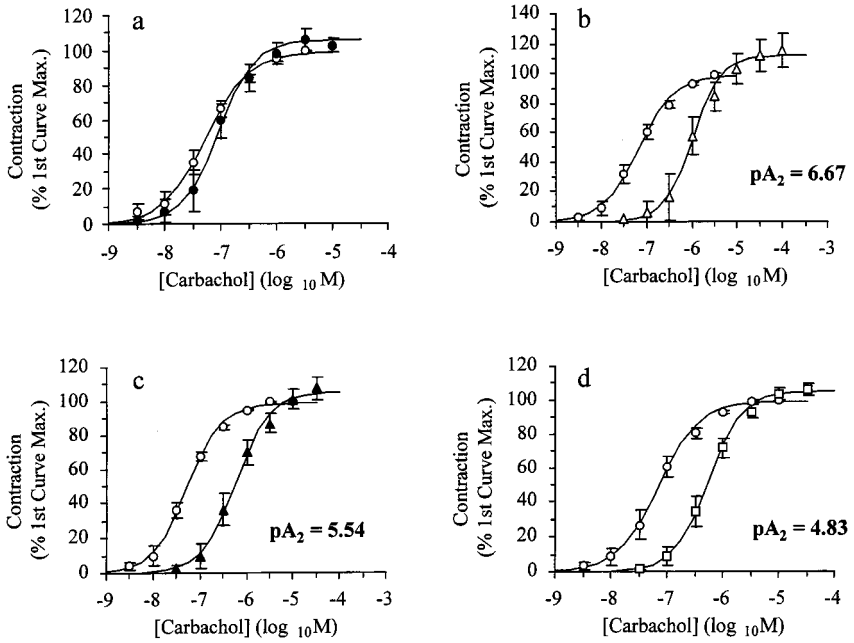


Figure 3. Antagonism of carbachol-induced contractions in guinea pig isolated trachea by **7**, **6**, and **5**: (a) paired carbachol control *E*/[*A*] curves, first curve (○) and second curve (●); (b) *E*/[*A*] curves to carbachol in the absence (○) and presence of 3 μM **7** (△); (c) *E*/[*A*] curves to carbachol in the absence (○) and presence of 30 μM **6** (▲); *E*/[*A*] curves to carbachol in the absence (○) and presence of 100 μM **5** (□). The data shown are the mean of four experiments in all cases, with the vertical lines indicating the SE. The lines drawn through the data points are the result of fitting them to eq 1.

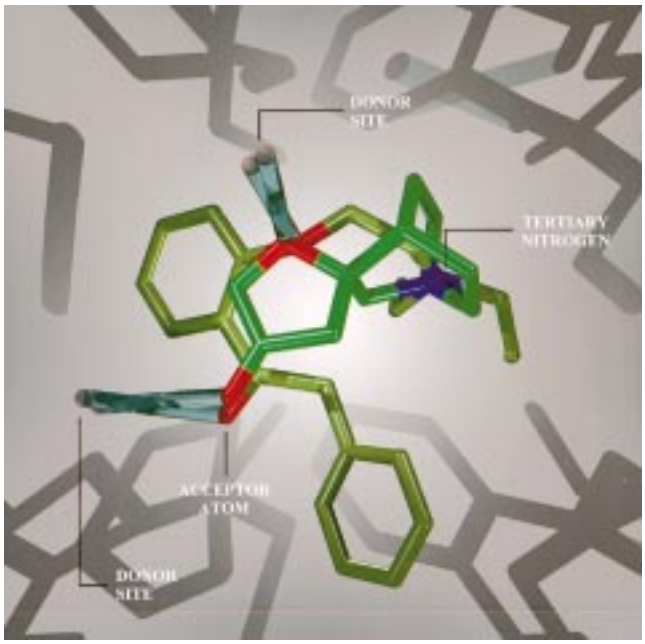


Figure 4. Structural overlay of compound **7** onto compound **4**. A 3D overlay of compound **7**, our most potent hit, onto the structure of one of the compounds used to define the pharmacophore. Note how the four features of the pharmacophore are superimposed in this overlay.

tive novel molecules. It approximates to a guided screening process whereby a set of molecules is identified for biological testing which are believed to be good candidates for activity. This contrasts with true random screening where no assumptions are made about structures to be tested and potentially large numbers of compounds are screened indiscriminately. This may be appropriate in the case of high-throughput screens, but in circumstances where these are not available it is necessary to prioritize compounds to be tested. In such cases, we might wish to design focused libraries or complement this by selecting from extant compounds using pharmacophore methods.

Notwithstanding the differences in performance between different software implementations of 3D database searching, a single pharmacophore is unlikely to recover all compounds known to be active against a particular receptor. This is especially true for antagonists and enzyme inhibitors which can bind in a number of different ways to block agonist or substrate binding. Each structurally distinct class may make its own individual subset of interactions within the total available within a binding site. Single compounds may also bind to more than one subsite or in several different binding modes. Given the more stringent requirements of receptor activation, agonists may exhibit less diversity in binding. Thus to span the structural diversity and different binding modes exhibited by antagonists and other ligands, many pharmacophores may be required to characterize fully the structural requirements of a given receptor or pharmacological activity.

Although 3D database searching is a directed approach, there is always a need to test a reasonable number of molecules which fit a pharmacophore model. Although a particular compound may fit the pharmacophore, reflecting receptor complementarity, its activity is not guaranteed. It may possess unfavorable physical properties, overall lipophilicity for example. Likewise, it may penetrate excluded volumes within the receptor or introduce the pairing of like charges. For hits, a range of activities is obtained, including unexpected enhancements as advantageous additional interactions are made with the receptor. 3D database searching will ideally identify compounds with properties outside that of the set of molecules used to define the pharmacophore. This allows for the identification of novel chemical structures

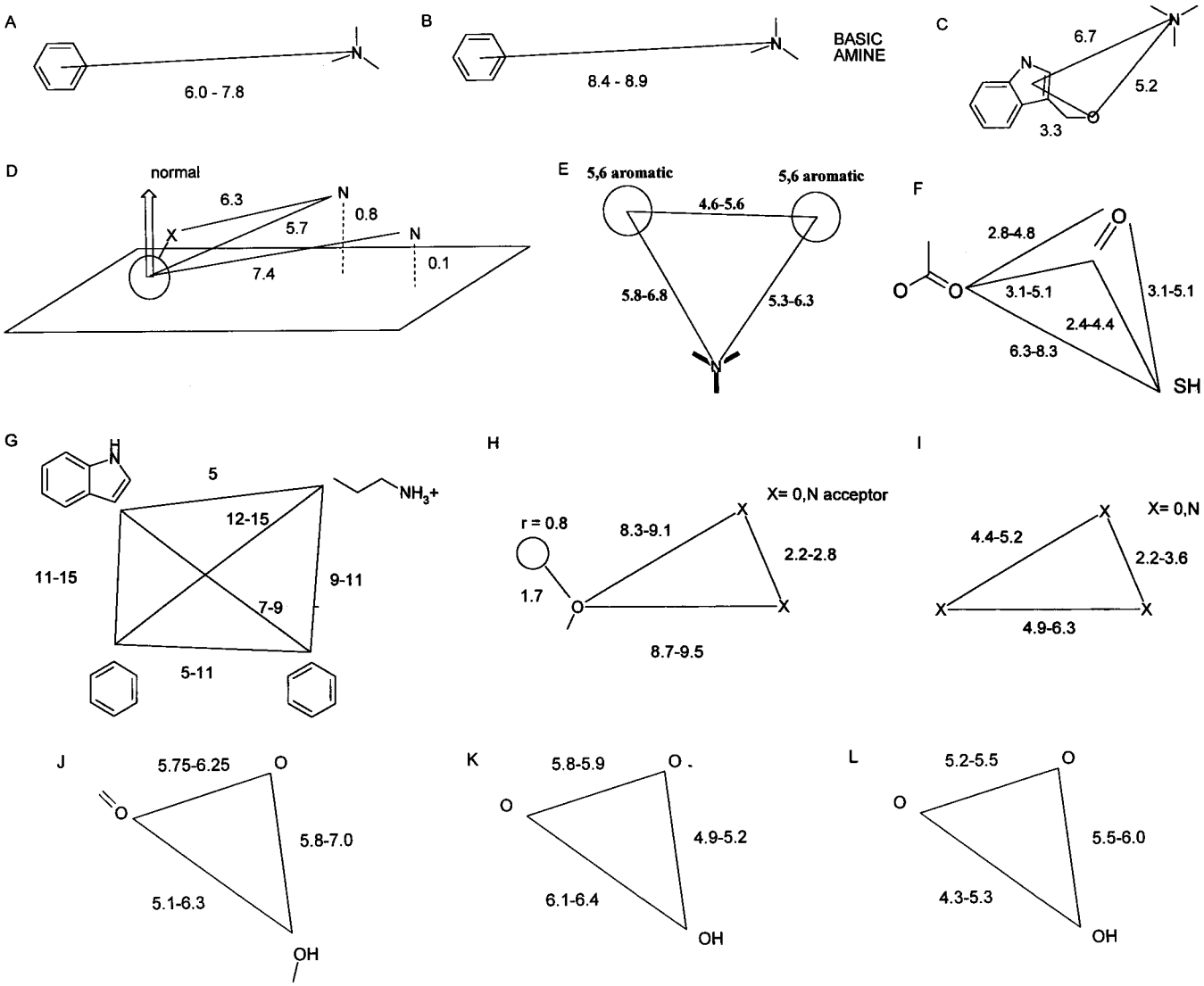


Figure 5. Compendium of pharmacophores. A set of pharmacophores extracted from the literature which illustrates the different flavors of pharmacophore, highlighting both the commonalties between such search patterns, as well as the diversity possible in the specification of structural queries. Overspecifying a pharmacophoric pattern through the use of restrictive substructure criteria will limit the overall diversity and novelty of hits. In an ideal pharmacophore, functional groups do not restrict structural classes, while the geometry of the pharmacophore gives discriminating power to the search: (A) $5HT_3$ antagonists,[27] (B) $5HT_3$ antagonists,[28] (C) $5HT_3$ antagonists,[29] (D) $5HT_4$ antagonists,[30] (E) H1 antagonists,[31] (F) ACE inhibitors,[32] (G) somatastatin antagonists,[33] (H) HIV-1 integrase,[34] (I) HIV-1 integrase,[35] (J) protein kinase C inhibitors,[36] (K) protein kinase C inhibitors (DAG pharmacophore),[37] and (L) protein kinase C inhibitors (phorbol pharmacophore).[38]

and molecular features leading to both increased and decreased activity.

Conclusion

The power of pharmacophore methods lies in their ability to suggest molecules potentially possessing a desired biological activity but which have unexpected chemical structures. The main advantage of 3D database searching over other pharmacophore-based methods is that they are capable of identifying extant molecules which can be obtained from a corporate compound bank, bought from a chemical supplier, or synthesized using an established protocol.

Assuming a consistent definition of what constitutes a hit, different high-throughput screening campaigns give different overall hit rates: sometimes these can be very low—only a few hits out of many hundreds of thousands of compounds tested. Alternatively, hit rates can be raised artificially, but still only weak compounds are found. Pharmacophore searching on the other hand, while not guaranteeing to find actives, is, as we have shown, potentially more efficient. It does, of course, presuppose knowledge of molecules displaying the desired activity. In the absence of such information, one's only recourse is to high-throughput screening. Similarly, a sensible and intelligent choice of pharmacophore model will have a profound effect on both the number and type of molecule identified in database searches.

It is our experience, exemplified here by our M_3 result, that it is possible to identify readily obtainable molecules that fit pharmacophore patterns using existing 3D database technology and that this approach has an important role to play alongside other techniques in lead discovery, particularly where resources are limited.

References

(1) Goodford, P. J. A computational procedure for determining energetically favourable binding sites on biologically important macromolecules. *J. Med. Chem.* **1985**, *28*, 849−857.

(2) Gillet, V. J.; Newell, W.; Mata, P.; Myatt, G.; Sike, S.; Zsoldos, Z.; Johnson, A. P. SPROUT: recent developments in the de novo design of molecules. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 207−217.
(3) Willett, P. A review of 3-dimensional chemical-structure retrieval-systems. *J. Chemom.* **1992**, *6*, 289−305.
(4) Brint, A. T.; Willett, P. Pharmacophoric pattern matching in files of 3D chemical structures: comparison of geometric searching algorithms. *J. Mol. Graph.* **1987**, *5*, 49−56.
(5) Martin, Y. C. 3D Database searching in Drug Design. *J. Med. Chem.* **1992**, *35*, 2145−2154.
(6) Manallack, D. T. Getting That Hit − 3d Database Searching In Drug Discovery. *Drug Discov. Today* **1996**, *1*, 231−238.
(7) Buckley, M. J.; Bonner, T. I.; Buckley, C. M.; Braun, M. R. Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. *Mol. Pharmacol.* **1989**, *35*, 469−476.
(8) Caulfield, M. P. Muscarinic receptors − characterization, coupling and function. *Pharmacol. Ther.* **1993**, *58*, 319−379.
(9) Wallis, R. M. Preclinical and clinical-pharmacology of selective muscarinic m(3) receptor antagonists. *Life Sci.* **1995**, *56*, 861−868.
(10) Patent WO9306098-A1, WO9204346-A.
(11) Wu, E. S.; Griffith, R. C.; Loch, J. T.; Kover, A.; Murray, R. J.; Mullen, G. B.; Blosser, J. C.; Machulskis, A. C.; McCreedy, S. A. In vitro muscarinic activity of spiromuscarones and related analogues. *J. Med. Chem.* **1995**, *38*, 1558−1570.
(12) Unpublished results.
(13) Rusinko, A.; Sheridan, R. P.; Nilakantan, R.; Haraki, K. S.; Bauman, N.; Venkataraghavan, R. Using CONCORD to Construct a Large Database of Three-Dimensional Coordinates from Connection Tables. *J. Chem. Inf. Comput. Sci.* **1989**, *29*, 251−255.
(14) Hendrickson, M. A.; Nicklaus, M. C.; Milne, G. W. A.; Zaharevitz, D. CONCORD and CAMBRIDGE: Comparison of Computer-Generated Chemical Structures with X-ray Crystallographic Data. *J. Chem. Inf. Comput. Sci.* **1993**, *33*, 155−163.
(15) Flower, D. R. ALTER: Eclectic management of molecular structure data. *J. Mol. Graph. Mod.* **1997**, *15*, 161−169.
(16) James, C. A.; Weininger, D. *Daylight Theory Manual;* Daylight Chemical Information Systems, Inc., 1995.
(17) Bleasby, A. J.; Wootton, J. C. Construction of validated, nonredundant composite protein sequence databases. *Protein Eng.* **1990**, *3*, 153−159.
(18) Gasteiger, J.; Rudolph, C.; Sadowski, J. Automatic generation of 3D-atomic coordinates for organic molecules. *Tetrahedron Comput. Methods* **1990**, *3*, 537−547.
(19) UNITY 2.4; Tripos Inc., 1699 South Hanley Rd, St. Louis, MO, 63144.
(20) SYBYL 6.2; Tripos Inc., 1699 South Hanley Rd, St. Louis, MO, 63144.
(21) Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwright, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rodgers, J. R.; Watson, D. G. The Cambridge crystal data centre: computer-based search, retrieval, analysis, and display of information. *Acta Crystallogr.* **1979**, *B35*, 2331−2339.
(22) Martin, Y.; Bures, M.; Dahaner, E.; DeLazzer, J.; Lico, I.; Pavlik, P. A Fast Approach to Pharmacophore Mapping and its Application to Dopaminergic and Benzodiazepine Agonists. *J. Comput.-Aided Mol. Des.* **1993**, *7*, 83−102.
(23) Leff, P.; Dougall, I. G.; Harper, D. Estimation of partial agonist affinity by interaction with a full agonist: a direct operational model-fitting approach. *Br. J. Pharmacol.* **1993**, *110*, 239−244.
(24) Jenkinson, D. H.; Barnard, E. A.; Hoyer, D.; Humphrey, P. P. A.; Leff, P.; Shankley, N. P. International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. IX. Recommendations on Terms and Symbols in Quantitative Pharmacology. *Pharmacol. Rev.* **1995**, *47*, 255−266.
(25) Schild, H. O. pA_x and competitive drug antagonism. *Br. J. Pharmacol.* **1949**, *4*, 277−280.
(26) Jakes, S. E.; Watts, N.; Willett, P.; Bawden, D.; Fisher, J. D. Pharmacophoric pattern matching in files of 3D chemical structures: evaluation of search performance. *J. Mol. Graph.* **1987**, *5*, 41−48.
(27) Schmidt, A. W.; Peroutka, S. J. Three-dimensional steric molecular modeling of the 5-hydroxytryptamine3 receptor pharmacophore. *Mol. Pharmacol.* **1989**, *36*, 505−511.
(28) Swain, C. J.; Baker, R.; Kneen, C.; Moseley, J.; Saunders, J.; Seward, E. M.; Stevenson, G.; Beer, M.; Stanton, J.; Watling, K. Novel 5-HT3 antagonists. Indole oxadiazoles. *J. Med. Chem.* **1991**, *34*, 140−151.
(29) Hibert, M. F.; Hoffman, R.; Miller, R. C.; Carr, A. A. Conformation-Activity Relationship Study of 5-HT3 Receptor Antagonists and a definition of a Model for this Receptor Site. *J. Med. Chem.* **1990**, *33*, 1594−1600.
(30) Buchheit, K. H.; Gamse, R.; Giger, R.; Hoyer, D.; Klein, F.; Kloppner, E.; Pfannkuche, H. J.; Mattes, H. The serotonin 5-HT4 receptor. 1. Design of a new class of agonists and receptor map of the agonist recognition site. *J. Med. Chem.* **1995**, *38*, 2326−2330.
(31) Borea, P. A.; Bertolasi, V.; Gilli, G. Crystallographic and conformational studies on Histamine H1-receptor Antagonists. *Arzneim.-Forsch./Drug Res.* **1986**, *36*, 895−899.
(32) Mayer, D.; Naylor, C. B.; Motoc, I.; Marshall, G. R. A unique geometry of the active site of angiotensin-converting enzyme consistent with structure−activity studies. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 3−16.
(33) Melacini, G.; Zhu, Q.; Osapay, G.; Goodman, M. A refined model for the somatostatin pharmacophore: conformational analysis of lanthionine-sandostatin analogues. *J. Med. Chem.* **1997**, *40*, 2252−2258.
(34) Nicklaus, M. C.; Neamati, N.; Hong, H.; Mazumder, A.; Sunder, S.; Chen, J.; Milne, G. W.; Pommier, Y. HIV-1 integrase pharmacophore: discovery of inhibitors through three-dimensional database searching. *J. Med. Chem.* **1997**, *40*, 920−929.
(35) Hong, H.; Neamati, N.; Wang, S.; Nicklaus, M. C.; Mazumder, A.; Zhao, H.; Burke, T. R., Jr.; Pommier, Y.; Milne, G. W. Discovery of HIV-1 integrase inhibitors by pharmacophore searching. *J. Med. Chem.* **1997**, *40*, 930−936.
(36) Wang, S.; Zaharevitz, D. W.; Sharma, R.; Marquez, V. E.; Lewin, N. E.; Du, L.; Blumberg, P. M.; Milne, G. W. The discovery of novel, structurally diverse protein kinase C agonists through computer 3D-database pharmacophore search. Molecular modeling studies. *J. Med. Chem.* **1994**, *37*, 4479−4489.
(37) Lee, J.; Wang, S.; Milne, G. W.; Sharma, R.; Lewin, N. E.; Blumberg, P. M.; Marquez, V. E. Conformationally constrained analogues of diacylglycerol. 11. Ultrapotent protein kinase C ligands based on a chiral 5-disubstituted tetrahydro-2-furanone template. *J. Med. Chem.* **1996**, *39*, 29−35.
(38) Wang, S.; Milne, G. W.; Nicklaus, M. C.; Marquez, V. E.; Lee, J.; Blumberg, P. M. Protein kinase C Modeling of the binding site and prediction of binding constants. *J. Med. Chem.* **1994**, *37*, 1326−1338.

JM980409N