Morpheus: a conformation-activity relationships and receptor modeling package

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Our molecular modeling software package, MORPHEUS, allows the study of the interactions between biologically active molecules and their receptors. The package is capable of exploring the multidimensional conformational space accessible to each molecule of the data set under study. By specifying distance constraints or hypothetical receptor binding points, the package is able to filter the biologically accessible conformations of each active compound and deduce a three-dimensional model of the binding sites consistent with the properties of the agonists (or antagonists) under scrutiny. The electrostatic potentials in the environment of a putative binding site can also be investigated using the MORPHEUS package. The molecular modeling module CRYS-X, which is written in FORTRAN 77 for IBM PC machines, is capable of building, displaying and manipulating molecules.

Keywords: molecular modeling, drug design, computer-assisted molecular design, conformational analysis, receptor mapping

INTRODUCTION

During the last decade, the design of new biologically active substances using computer graphics and other computational methods has become generally accepted. This has arisen partly because of increased understanding of the mechanisms of drug action and partly because computer technology has advanced to the point where it is feasible to do necessary computational work on a reasonably small machine. The revolution in computer graphics hardware and software has also provided the impetus for commercial development of a number of molecular modeling systems, such as SYBYL (Tripos Associates, St. Louis, MO) and Chem-X (Chemical Design Limited, Oxford, UK).

The MORPHEUS software system has evolved over several years in our laboratory. It was specifically designed to investigate interactions between biologically

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active molecules and their receptors, and to undertake the study of conformation-activity relationships. It is capable of displaying, building and manipulating molecular structures in three dimensions, of superimposing molecules (in color) to find topographical similarities indicative of receptor binding requirements, and of exploring the conformational space of flexible molecules. It has several unique capabilities that result from its implementation of a method of receptor mapping called "the extended molecule method." The package has an additional advantage in that its molecular modeling module, CRYS-X, operates on the IBM PC and similar machines.[1] A comparison of the molecular modeling capabilities of several PC-based molecular modeling programs, including CRYS-X, has recently been published by Sadek and Munro.[2]

OVERVIEW OF THE SOFTWARE PACKAGE

The MORPHEUS system is a suite of programs written in FORTRAN. The relationships between the components of the MORPHEUS software package are illustrated in Figure 1. Several other commercially available software packages (such as AMPAC,[3] PLUTO[4] and CNDO/2[5]) are also used in conjunction with MORPHEUS and are not discussed here.

Molecular graphics

The ability to superimpose the structures of several biologically active molecules is an important requirement for molecular modeling. CRYS-X is the central molecular graphics package in the MORPHEUS system. It is a development of a package of the same name written by Peter Pauling, Mary Lee and Doug Richardson at University College, London. CRYS-X operates on IBM PC computers and near compatibles, as well as most other machines for which FORTRAN is available. Its primary purpose is to facilitate the building, manipulation and display of molecular structure. It accepts typed commands, which are used to build or manipulate chemical structures, and prompts the user, when necessary, for any missing parameters. Experienced users can

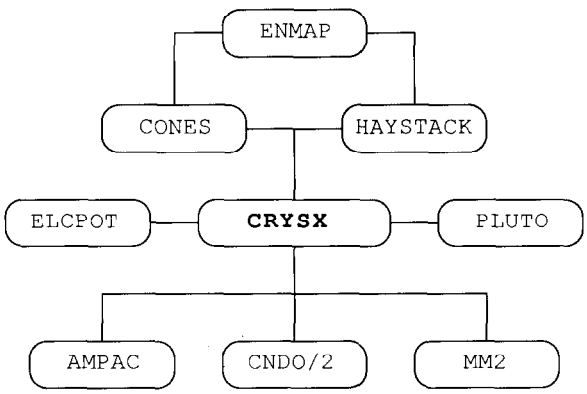


Figure 1. Block diagram of the MORPHEUS software package

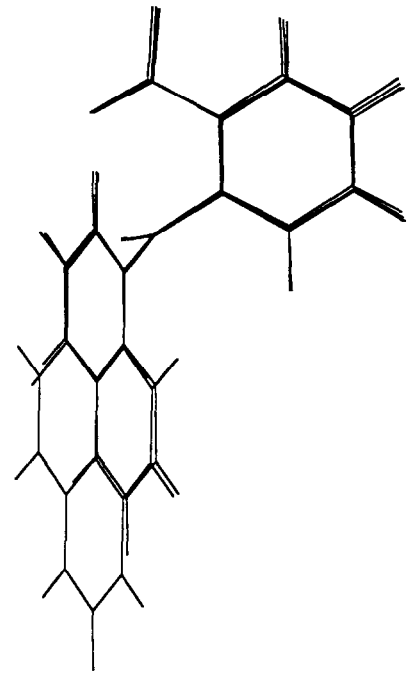


Figure 2. Superimposition of phytotropins[20] using the CRYS-X module in MORPHEUS

abbreviate commands to the first two or three characters. Because the purpose of this paper is to discuss the unique features of this system, we will not discuss the specific command set applicable to this program in detail here.

Molecular structural input to CRYS-X is either derived from Cartesian or reduced (crystal structure) coordinates obtained from the literature or by application of the molecule building capabilities of CRYS-X. These capabilities allow molecular structures to be built up either atom by atom or by the joining together of molecular fragments from a fragment library. Display options include the ability to rotate around any arbitrary axis, display lateral and red-green stereo views, color atoms according to atom type, automatically display multiple bonds and automatically display a complex molecule in the most informative view (involving minimum atom overlap).

Using an empirical force field, CRYS-X can calculate an approximate barrier to rotation around any flexible torsion angle. It can also superimpose selected atoms in a target molecule onto specified guide points on another, thus achieving docking of the two molecules, as Figure 2 illustrates.

Conformational energy barrier and docking calculations assume rigid rotation or translation of molecules or molecular fragments.

Electrostatic potentials

The three-dimensional (3D), or topographical, properties of molecules are not sufficient to determine all of the likely interactions of molecules with receptors. Electronic properties are very important in recognition of the molecule by a receptor, orientation of the molecule in the

vicinity of the receptor and binding of the molecule, in its biologically active conformation, to the receptor.[6] The molecular electrostatic potential (MEP) is a parameter that has been used extensively to describe the important electronic interactions of molecules and receptors. Quantum mechanical procedures have been used widely to obtain molecular charge distributions for a variety of drugs (e.g., Franke[7]). However, the interaction between these drugs and their receptors or active sites is too complex for rigorous quantum mechanical calculations to be effectively tackled using contemporary computers. Less rigorous empirical methods have to be employed using quantum mechanical calculations on molecular fragments as starting points. ELCPOT is such a program; it is used to gain semiquantitative information on drug receptor recognition mechanisms. It uses the average spherical atomic orbital approximation with complete neglect of differential overlap to determine the electrostatic potential surrounding a molecule.[8] The potential is given by

$$V_p = \sum_A \frac{Z_{A'}}{R_{AB}} - \sum_A \rho_A \int \frac{\varphi_{SA'}}{R_{AB}} \, dv$$

where $Z_{A'}$ is the effective valence nulear charge on atomic center A and ρ_A and φ_{SA} are the total valence electron density and averaged spherical atomic orbital on center A, respectively.

The two center nuclear attraction integrals have been derived for first, second and third row elements using Roothaan's formula[9] and Slater's effective nuclear charges,[10]

$$[a|1S] = (\zeta/\rho)\{1 - [1+\rho]e^{-2\rho}\}$$

$$[a|2S] = (\zeta/\rho)\{1 - [1 + 4/3\rho + 2/3\rho^2]e^{-2\rho}\}$$

$$[a|3S] = (\zeta/\rho)\{1 - [1 + 3/2\rho + \rho^2 + 1/3\rho^3]e^{-2\rho}\}$$

$$[a|5S] = (\zeta/\rho)\{1 - [1 + 5/3\rho + 4/3\rho^2 + 2/3\rho^3 + 2/9\rho^4 + 2/45\rho^5]e^{-2\rho}\}$$

$$[a|7S] = (\zeta/\rho)\{1 - [1 + 7/4\rho + 3/2\rho^2 + 5/6\rho^3 + 1/3\rho^4 + 1/10\rho^5 + 1/45\rho^6 + 1/315\rho^7]e^{-2\rho}\}$$

where $\rho = \zeta R$ and ζ is the Slater orbital exponent.

Previous applications of this approximation to semiempirical wave functions have shown it to reproduce important features of *ab initio* calculations.[11] The MEP is essentially the energy resulting from a proton being "rolled around" a molecule. The fields can indicate electrostatic potential barriers between docking partners and can provide clues to the line of attack and binding orientation simply by inspecting the positions of potential well and barriers.[12] An example of a molecular electrostatic potential map derived by ELCPOT is given in Figure 3.

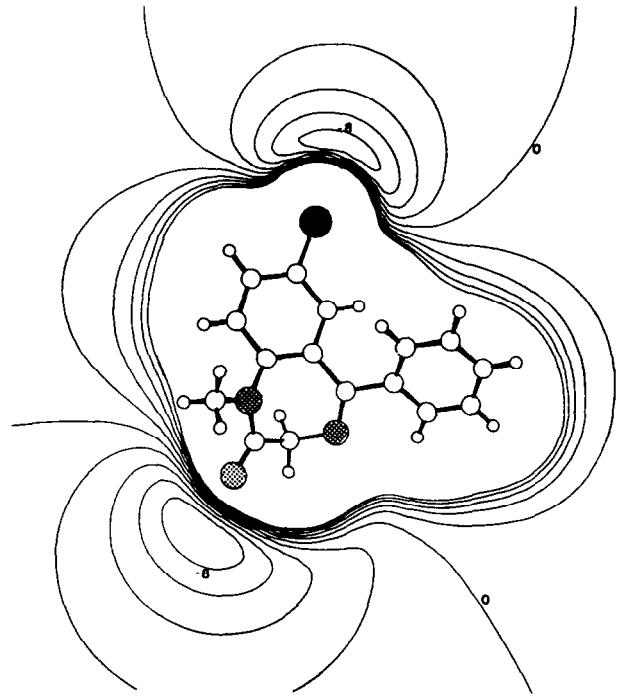


Figure 3. An ELCPOT electrostatic potential map of diazepam showing two electronegative regions around the chloride and carbonyl moieties. The contour interval is 2 kcal/mole. Numbers indicate depth of wells in kcal/mole

Conformational calculations

Most drug molecules are inherently flexible and consequently may adopt a number of conformations at physiological temperatures. Molecular graphics-based SAR and receptor modeling studies must take account of all low energy conformations accessible to the molecules under study in order to determine which one is biologically active. CONES is a conformational analysis package based on the COMOL program.[13] CONES calculates the energy of a molecule as a function of up to three independently variable torsion angles. The energy is calculated as a sum of two components comprising the nonbonded (van der Waals) component, E_{nb}, and an electrostatic component, E_{el}.

The nonbonded term is calculated using the mixed Buckingham/Lennard-Jones potential.[14]

$$E_{nb} = \sum_{i \neq j} \frac{a_{ij}\exp(-b_{ij}r_{ij})}{r_{ij}^{d_{ij}}} - \frac{c_{ij}}{r_{ij}^6}$$

where r_{ij} is the distance between atoms i and j and a, b, c and d are parameters.

The electrostatic term E_{el} is simply the Coulomb energy arising from the interaction of all the partial charges in the molecule.

$$E_{el} = \sum_{i \neq j} 331.0 \frac{q_i q_j}{\varepsilon r_{ij}}$$

where q_i and q_j are the partial charges and ε is the dielectric constant (with a value assumed to be between 2 and 5 in a receptor environment). The factor of 331.0

yields energies in kcal/mole when q_i and q_j are units of electron charge and r_{ij} is in Å.

The parameters *a*, *b*, *c* and *d*, used for the nonbonded term, are those of Giglio[15] supplemented with those derived by the method of Kollman *et al.*[16] and values calculated from the second virial coefficient.[17] The parameters are tabulated in Table 1.

CONES has been shown to give good estimates of the conformational preferences of molecules in previous calculations.[18–20] CONES will print out a series of potential energy surfaces representing the conformational energy as a function of two specified torsion angles, at selected values of a third torsion angle.

CONFORMATION–ACTIVITY RELATIONSHIPS AND RECEPTOR MAPPING

Several receptor mapping techniques have been reported in the literature. The minimal topological difference (MTD) analysis of Simon *et al.*[21] derives a receptor model containing only information on steric accessibility but says nothing about the chemical nature of the parts of the site. Marshall's "active analogue" approach[22,23] determines the relative positions of putative binding groups in a series of active compounds and infers the topographical properties of the receptor from these. In

Table 1. Parameters for nonbonded potential function

A Parameter

	H	C	N	O	Cl/S	F	P
H	6600	44800	52100	42000	40500	9361	78250
C	44800	301200	340000	278700	255400	100700	628300
N	52100	340000	387000	316200	288600	64520	506500
O	42000	278700	316200	259000	239200	55710	361500
Cl/S	40500	255400	288600	239200	251600	296700	1537000
F	9361	100700	64520	55710	296700	36520	290400
P	78250	628300	506500	361500	1537000	290400	1707000

B Parameter

	H	C	N	O	Cl/S	F	P
H	4.080	2.040	2.040	2.040	3.851	0.0	0.0
C	2.040	0.0	0.0	0.0	1.811	0.0	0.0
N	2.040	0.0	0.0	0.0	1.811	0.0	0.0
O	2.040	0.0	0.0	0.0	1.811	0.0	0.0
Cl/S	3.851	1.811	1.811	1.811	3.475	0.0	0.0
F	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P	0.0	0.0	0.0	0.0	0.0	0.0	0.0

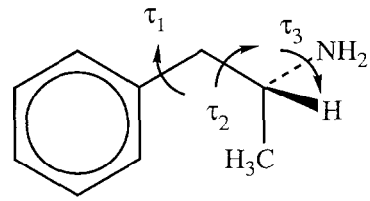
C Parameter

	H	C	N	O	Cl/S	F	P
H	49.2	125.0	132.0	132.7	265.2	79.8	264.8
C	125.0	327.2	340.0	342.0	684.0	223.2	742.6
N	132.0	340.0	354.0	356.0	711.5	218.7	712.6
O	132.7	342.0	356.0	358.5	715.5	221.8	738.7
Cl/S	265.2	684.0	711.5	715.5	1430.0	457.6	1512.0
F	79.8	223.2	218.7	221.8	457.6	134.6	446.5
P	264.8	742.6	712.6	738.7	1512.0	446.5	1473.0

D Parameter

	H	C	N	O	Cl/S	F	P
H	0	6	6	6	0	12	12
C	6	12	12	12	6	12	12
N	6	12	12	12	6	12	12
O	6	12	12	12	6	12	12
Cl/S	0	6	6	6	0	12	12
F	12	12	12	12	12	12	12
P	12	12	12	12	12	12	12

the "distance geometry" approach of Crippen,[24] binding sites of a receptor are considered to be a collection of ligand points that may be empty (accessible) or filled (inaccessible). We have developed two methods for modeling receptors: distance mapping using accessible conformations and the "extended molecule" method, which has a more flexible approach to the design of receptor models.



Structure 1

Distance mapping using accessible conformations

CONES can fit a conformationally flexible molecule to a set of fixed (target) points representing a model of a hypothetical receptor. A least-squares fit of guides (atoms within the molecule presumed to be binding to the receptor, or points of extended molecular structure (*vide infra*)) to the targets for each value of independent torsion angle of the flexible molecule is calculated and plotted in a similar fashion to that commonly used for energy surfaces.

The distance maps and conformational energy surfaces produced by CONES may be plotted as contour maps by ENMAP, a modification of the contouring program KONTOR.[25] These plots are similar to those of Ramachandran *et al.*[26] By comparing the minima on the distance maps and the corresponding potential energy maps, it is possible to obtain useful information on the binding requirements of receptors and enzyme active sites. For example, the conformational search over the three variable torsion angles τ_1, τ_2, τ_3 of the (S)-(+)-amphetamine, as seen in Structure 1, indicated a large number of low energy conformations.[27]

This number was substantially reduced by simultaneous fitting of the amphetamine to the common CNS receptor model[28,29] and by comparison of the corresponding potential energy and distance maps. Only one conformation ($\tau_1 = 110°$, $\tau_2 = 180°$, $\tau_3 = -60°$) showed no overlap with the receptor essential volume and corresponded to both a conformational energy minimum and near-optimal fit to a common drug–receptor model, as Figure 4 illustrates.

The conformation denoted by the asterisk corresponds to that of the benzobicyclo(2.2.2)octanes, known active rigid analogues of amphetamine.[28]

The extended molecule method

A common assumption of QSAR or receptor modeling studies is that equivalent atoms in each of the various ligands binding at a given receptor should all occupy approximately the same points in space. In the case of the group of dihydrofolate reductase substrates and inhibitors illustrated in Figure 5, for example, it is generally assumed that the common 2,4–diamino ring in each

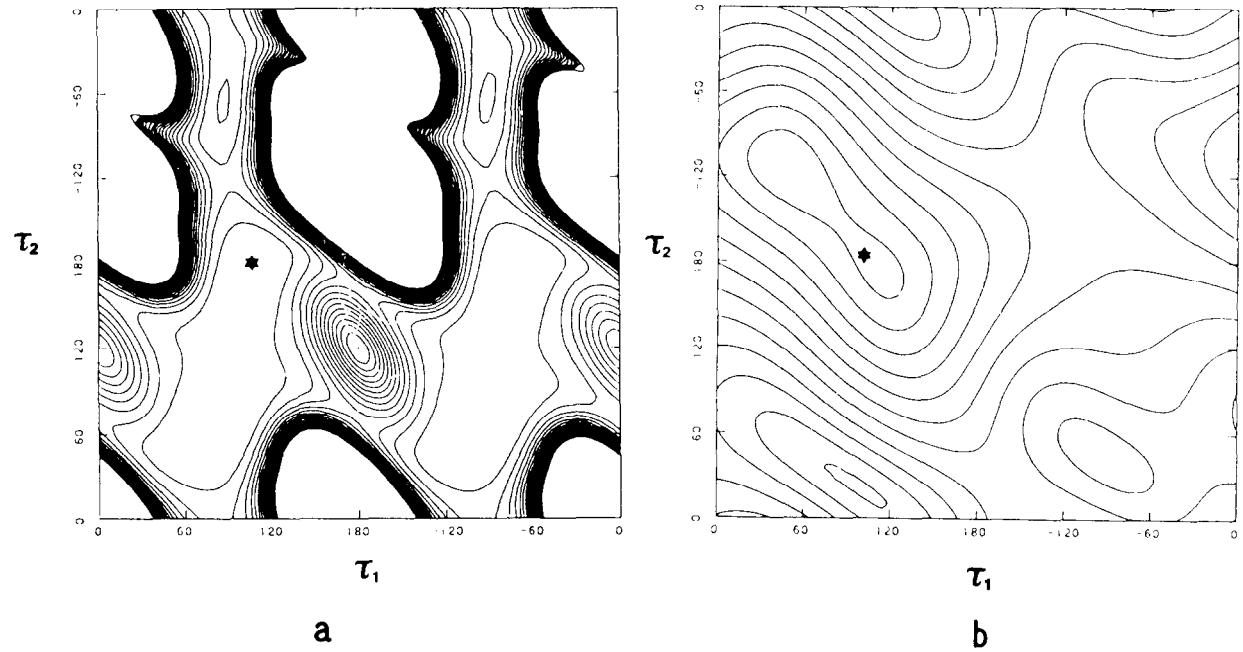


Figure 4. Plots of (a) potential energy and (b) distance between receptor points vs. torsion angles τ_1 and τ_2 for (S)-(+)-amphetamine (Structure 1) with τ_3 fixed at $-60°$ produced by CONES and ENMAP. The combination of lowest energy and best fit of the four points of the common CNS receptor model[28,29] to the corresponding points of (S)-(+)-amphetamine occurs at $\tau_1 = 110°$ and $\tau_2 = 180°$, shown as an asterisk. Figure kindly reproduced from Lloyd and Andrews[27]

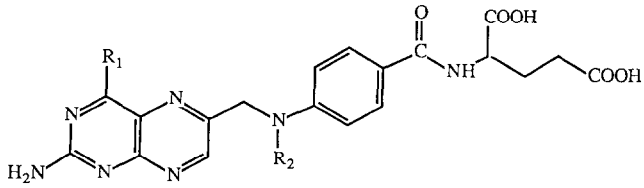


Figure 5. Inhibitors and substrates of dihydrofolate reductase: (a) for methotrexate (MTX), $R_1 = NH_2$, $R_2 = CH_3$; (b) for aminopterin (APT), $R_1 = NH_2$, $R_2 = H$; (c) for folate, $R_1 = OH$, $R_2 = H$; (d) for dihydrofolate (DHF), ring 7,8-dihydro, $R_1 = OH$, $R_2 = H$; (e) for tetrahydrofolate (THF), ring 5,6,7,8-tetrahydro, $R_1 = OH$, $R_2 = H$

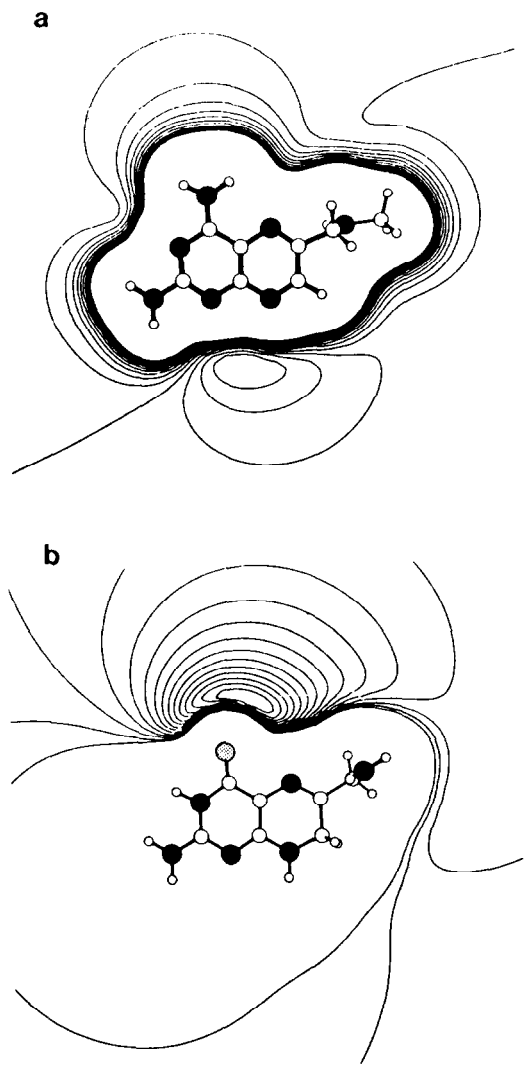


Figure 6. Electrostatic potential maps (ELCPOT) of (a) methotrexate (MTX), (b) dihydrofolate (DHF). The global minima are −8 and −26.7 kcal/mole, respectively, and the contour interval is 2 kcal/mole. For clarity, the benzoyl glutamate region has been removed from each structure

of the inhibitors would take up a similar position and orientation in the active site.

Crystallographic studies in several laboratories provide general support for this assumption.[30] In view of the close structural relationship between one of these inhibitors, methotrexate, and the substrate for the enzyme, dihydrofolate, one might also quite reasonably assume that the binding mode of these two molecules at the active site would also be similar. In fact, the two molecules bind with their heterocyclic rings inverted relative to each other. In this way the complementary, molecular electrostatic potentials of the molecules and the receptor, rather than the atoms of the molecules themselves, are superimposed in space.[31] This is illustrated in Figure 6, which shows that the corresponding electrostatic potential minima of the two molecules lie on opposite sides of the pteridine ring. On ring rotation of one of the molecules, the two minima coincide and define the likely location for a receptor group capable of interacting with both chemical species.

The extended molecule approach is based on the concept that this problem of alternative binding conformations can be overcome by treating the receptor as an extension of the drug molecule, with the "pharmacophore" then being developed primarily by superimposition of receptor points. For this purpose, the positions of the receptor points are defined in terms of internal coordinates (bond lengths, bond angles, torsion angles) in the same way that one normally defines the positions and conformations of functional groups in the drug.

Consider, for example, the extended molecular structure for a simple amphetamine (Structure 1) depicted in Figure 7.

Since stacked aromatic rings are separated by an average of 3.5 Å (although larger distances clearly occur when the rings deviate significantly from planarity), one can define the receptor site for a phenyl ring by placing hydrophobic receptor points R_1 and R_2 at a given distance, usually 3.5 Å above or below the center of the ring. This vertical axis totally defines the position and

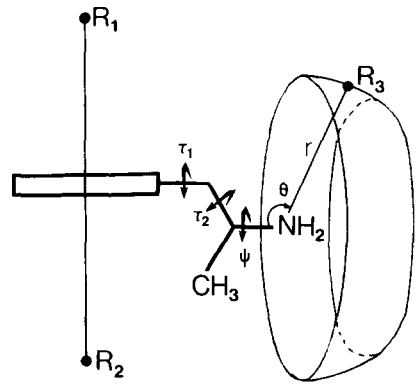


Figure 7. Extended molecular description of amphetamine. The phenyl ring and its associated receptor points are defined by R_1 and R_2, and the nitrogen-receptor interaction by N and R_3. The bowl-shaped surface delineates the range of positions at which the nitrogen receptor point may be found, as ψ and θ are varied

orientation of the phenyl ring (without specifying its point of attachment) as well as the position and orientation of possible receptor groups above and/or below the plane of the ring. Note that there is no need to specify which side of the phenyl ring, if not both, is occupied by the receptor.

The nitrogen receptor point, R_3, is more complex, being governed by two conformational variables (θ and ψ) in addition to the N–R_3 bond distance and the two intramolecular conformational variables (τ_1 and τ_2). Assuming for the purpose of this example that the nitrogen atom is sp^3 hybridized and that its interaction with the receptor point is via a hydrogen bond, the N–R_3 bond length can be defined by an average N . . . H . . . X hydrogen bond distance of approximately 2.8 Å. A bond angle θ is also somewhat restricted, although data on the deviation from linearity of N . . . H . . . O bonds[32,33] suggest that a range of at least 90° to 120° is appropriate for this variable. Finally, in the absence of bulky substituents attached to the nitrogen, the torsion angle ψ may reasonably be expected to fall anywhere from 0° to 360°. Combination of these two variables thus leads to the possibility of R_3 falling anywhere on a bowl-shaped surface like that illustrated in Figure 7, where the position and orientation of this surface is itself further dependent on the two internal conformation variables, τ_1 and τ_2.

The search for an optimal pharmacophore is then carried out by a systematic conformational search over all possible combinations of torsion angles to find those conformations of the series of molecules that allow a common placement of the receptor points alone, or of the receptor points and selected drug atoms. The obvious disadvantage of this method is the rapid increase in computational overhead because the number of conformational variables (and thus the size of the conformational space) needed to be considered in the systematic search increases. This problem is reduced by using conformational and distance filters in the program HAYSTACK. HAYSTACK steps through the conformations in a systematic manner and can be used either to fit molecules into a defined receptor or to use the conformational properties of active molecules to define a receptor.

When fitting a molecule to a defined receptor, interreceptor distances are calculated for each conformation and compared with constraints. The root mean square (RMS) of the sum of the distances between measured and desired distances for conformations that pass the constraints is recorded for each conformation, and a record of the minimum and maximum RMS, with the corresponding conformation, is retained. The minimum and maximum for each of the interreceptor distances are similarly recorded. The conformations that pass this distance filter (which may be all of them) are subjected to a test based on van der Waals radii. Those conformations having nonbonded atoms separated by less than the sum of their van der Waals radii are discarded as their energies are likely to be too high. At this stage, conformations with the best RMS values can be recorded and displayed using graphics options or subjected to a nonbonded energy filter[14,15] which further reduces the number of eligible conformations for each compound. The program requires the specification of constraints in the form of distances between receptor points and allowed variations in them. In effect, these distances, and the tolerances placed on them, represent the "flexible receptor," a more natural approach than the fixed, hypothetical receptor concept incorporated in distance mapping using accessible conformations.

This process can also be used to derive a receptor geometry from the conformational properties of molecules that bind to it. Thus, starting from the most rigid compound in the series and bypassing the distance filter (by having liberal constraints on interreceptor distances), we obtain a list of the smallest and largest separations for each interreceptor distance, which is used for an initial estimate of constraints. These constraints are used to filter the conformations of the next most flexible compound, and the best conformations fitting within these are constraints found. Consideration of further, more flexible, active compounds results in a gradual narrowing of the constraints that are consistent with all analogues considered. The narrow constraints finally define a set of interreceptor distances corresponding to the optimum pharmacophore. All molecules having accessible conformations and interreceptor distances which fall within these constraints will be superimposable on the pharmacophore.

This methodology was successfully applied[34] to the problem of finding common structural features of some atypical antidepressants (mianserin) and typical tricyclics such as imipramine. An example of the (S)-(+)-amphetamine (Structure 1) can serve for the direct comparison with the CONES receptor fitting method. By applying the constraints (derived from the common receptor model[27]) on the R_3–R_1, R_3–R_2, N–R_2 and N–R_1 distances (see Figure 7), the number of eligible conformations of this flexible molecule was again narrowed down to the conformation with $\tau_1 = 110°$, $T_2 = 180°$ and $\tau_3 = -60°$.[35]

CONCLUSIONS

The MORPHEUS modeling package provides two distinct, but complementary, methods of deducing the 3D binding requirements of a putative receptor. It has advantages over template forcing or distance geometry approaches in that the whole conformational space accessible to the molecules studied may be considered. Consequently, all conformations that are consistent with the putative receptor are disclosed.

The philosophy of the "extended molecule" method is similar to that of Marshall[21,22] but has the advantage of superimpositions (and thus a pharmacophore model) being derived in terms of flexible receptor points, thus allowing the drug's binding groups to approach these receptor points from a nonfixed direction.

The molecular graphics module CRYS-X provides useful molecular modeling capabilities for the IBM PC and similar personal computers. The software is available from the Royal Australian Chemical Institute[1] or by application to the authors.

REFERENCES

1 CRYS-X is supplied without any copy protection and with full source code in order to encourage further developments of the program. CRYS-X will operate successfully on almost any MS-DOS machine running FORTRAN 77 and having CGA or EGA graphics capability. For information, contact Division of Medicinal and Agricultural Chemistry, Royal Australian Chemical Institute, 191 Royal Pde. Parkville 3052, Victoria, Australia

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