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A Monte Carlo pharmacophore generation procedure: Application to the human PAF receptor*

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SUMMARY

A novel pharmacophore definition procedure is described, which uses a Monte Carlo method to superimpose molecules. Pharmacophore space is searched by a technique similar to high temperature annealing. Subsequent refinement of candidate pharmacophores by energy minimization produces low-energy conformations that may be involved in receptor binding. The method has been applied to compounds that bind to the human platelet-activating factor (PAF) receptor. Alternative binding site models for the PAF receptor are presented and discussed.

INTRODUCTION

Defining a receptor pharmacophore is an important activity in drug design [2]. Various approaches to the problem have been developed, notably the systematic method of Marshall et al. [3,4] and the ensemble distance geometry technique of Sheridan et al. [5]. Such methods aim to superimpose molecules by aligning functional groups assumed to be involved in similar binding roles. An extension of the method is to align sites capable of interacting with functional groups, such that the pharmacophore is defined in terms of site points in the receptor itself [6]. However, the increase in the number of degrees of freedom inherent in this extension can render a systematic solution too computationally intensive. While distance geometry provides a fast approximate solution to such problems [5], its sampling properties are not fully understood. In this paper an alternative strategy is described that employs a Monte Carlo search procedure to produce solutions to the pharmacophore definition problem, using a reduced force-field parameter set in order to lower energy barriers between conformations. Candidate pharmacophores are then refined by

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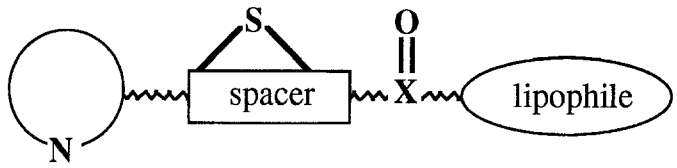


Fig. 1. Schematic representation of heterocyclic nitrogen PAF antagonists.

energy minimization with a full force-field to produce low-energy conformations of molecules overlaid in a common binding mode. The procedure aims to produce both a description of the pharmacophore in terms of the geometry of the binding site, and likely binding conformations of the molecules used to generate the pharmacophore.

The method has been applied to compounds which are known to bind to the human PAF receptor [7,8] with high affinity. PAF is the bioactive phospholipid 1-*O*-hexadecyl/octadecyl-2-acetyl-*sn*-glyceryl-3-phosphoryl choline which is released directly from cell membranes and mediates a range of effects on target cells by binding to a specific G protein-coupled receptor [9,10]. A diverse range of compounds has been identified as PAF antagonists but the manner in which these compounds bind to the PAF receptor is not well understood.

A number of attempts have been made to model the interactions between antagonists and the PAF receptor. A two-zone, lipophilic and hydrophilic, model has been suggested [11,12], and a model in which the receptor was likened to a pair of 'ear muffs' of positive potential [13,14] has been revised to a flexible multipolarized cylinder [15,16]. However, these models and other studies [17,18] do not satisfactorily explain the requirements for high affinity binding to the PAF receptor, since for the most part they are based upon studies of weakly active natural product PAF antagonists. In addition, Schreiber et al. [19] have proposed a PAF receptor binding model in which molecules contain a rigid five-atom network terminating in heteroatoms engaged in push-pull delocalization.

It has been hypothesized by Tilley and co-workers [20] that the PAF receptor binding site comprises, at minimum, a large lipophilic binding pocket which is tolerant of steric bulk, a hydrogen bond donor which can interact with a carbonyl group, and either a π interaction with a pyridine ring or an electrostatic interaction with a pyridine nitrogen lone pair. The current work shows that a consistent binding model may be derived from a diverse set of compounds using most of these assumptions.

At an early stage in the PAF antagonist programme at British Bio-technology [21], two moderately active benzimidazole derivatives, BB-182 and BB-350, were identified, for which the unsubstituted sp^2 nitrogen of the heterocycle was crucial for activity. A number of other PAF antagonists also possess a heterocyclic sp^2 nitrogen such as the 3-pyridyl derivatives RP 59227 [22], YM461 [23a,b] and Ro 24-0238 [24], imidazo[4,5-c]pyridine compounds such as UK-74,505 [25] and hetrazepine derivatives such as WEB 2086 [26]. It was hypothesized that these compounds might bind to the PAF receptor in the manner described by Tilley et al. [20] since in addition to the sp^2 nitrogen these compounds possess some other structural features in common; a carbonyl/sulphonyl moiety, a 'lipophilic' group, and for some, but not all, compounds a sulphur atom. Figure 1 shows a schematic representation of the molecular features proposed to be important for activity. A survey of known PAF antagonists suggests that there is a large group of compounds

that conform to this schematic representation [8]. For these compounds there is only limited variation in the nature of the sp^2 nitrogen heterocycle. The three main types of sp^2 nitrogen heterocycle are triazolo-1,4-diazepines (hetrazepines), pyridines and imidazoles (e.g. benzimidazole and imidazo[4,5-c]pyridine) [8]. The SAR for these PAF antagonists indicates not only the importance of the sp^2 nitrogen atom but also the effect of its spatial orientation on activity. For example, for analogues of the pyridine derivative Ro 24-0238 the 2-pyridyl and 4-pyridyl compounds are less potent than the 3-pyridyl compounds [24]. Similarly, for sp^2 nitrogen heterocyclic PAF antagonists that possess a sulphur atom, its presence and position appears to be important for activity [23,26]. The SAR data for the role of the carbonyl/sulphonyl group is less clear and there are a few examples of sp^2 nitrogen heterocycle PAF antagonists that do not possess a carbonyl/sulphonyl group [8]. There is considerable variation in the nature of the 'lipophilic' group which can vary from substituted aryl moieties to long C_{16} alkyl chains [8]. If the features identified in Fig. 1 are important for receptor interaction one might expect the relative spatial orientation of these groups to be similar. The work presented here is the first attempt to apply conformational analysis to the problem of PAF pharmacophore generation using a structurally varied set of antagonists [1].

METHODS

The stages involved in pharmacophore generation are described below and outlined in Fig. 2. Each stage is encoded in a standard software package.

(a) Molecule building

Molecular models were built using the 3D-editor in SYBYL [27] with dummy atoms representing hydrogen-bonding vectors. In each case the distance from the acceptor atom to the dummy atom was 3 Å. For sp^2 nitrogen acceptors, the vector bisected the nitrogen valence angle. The oxygen to dummy atom vector in carbonyls was co-linear with the carbonyl, and the sulphur to dummy atom vector in sulphonyls bisected the oxygen atoms, the dummy being 3 Å from each oxygen. While the dummy atom on a sulphonyl could be assigned to one or other oxygen, the symmetrical set-up is a convenient approximation and simplifies the pharmacophore generation. However, the limitation of such an approximation should be pointed out, as fixed dummy atoms only allow approach of a hydrogen-bond donor along the dipole axis, and hydrogen bonds accepted by sulphonamides are less directional than those made by carbonyl groups.

(b) Pharmacophore generation

Candidate pharmacophores were generated a Monte Carlo procedure known as 'Boltzmann jump'. The molecules were treated as an ensemble with strong restraining potentials joining equivalent pharmacophoric sites in the molecules. The Boltzmann jump method [28], is a modified Monte Carlo procedure designed to search conformational space. Each structure is derived from the previous one by simultaneously perturbing all variable dihedral angles by a random amount within a user-defined window. In addition, global rotation and translation of all molecules is performed at random within a given window. The simultaneous perturbation of all variables is in contrast to the generally accepted procedure of altering a single randomly selected variable but was found to be satisfactory for the current application. If the new structure is lower

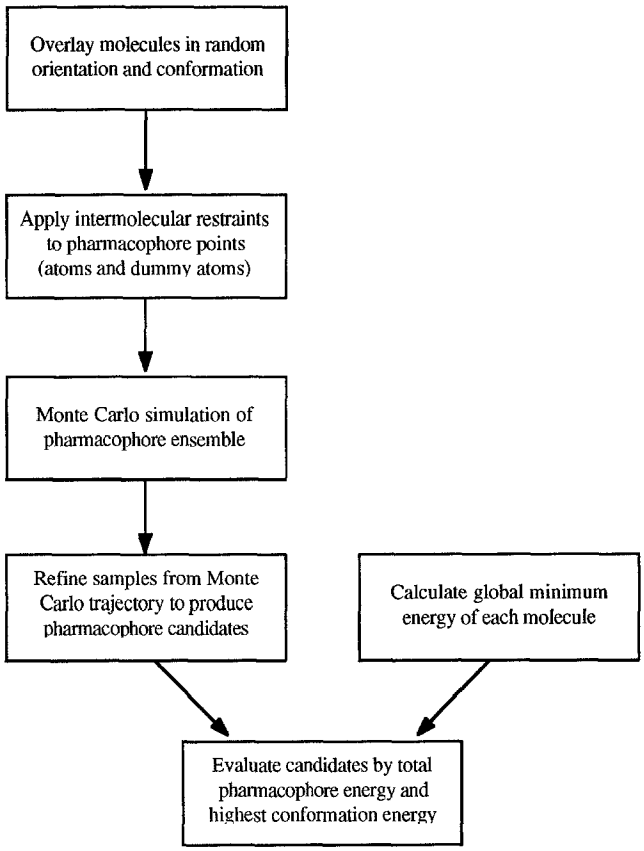


Fig. 2. The pharmacophore generation procedure.

in energy than its precursor it is used to generate the next structure and the procedure continues. If the energy of the new conformation is higher than that of its precursor by ΔE, it is accepted with a probability equal to the Boltzmann factor $e^{\Delta E/RT}$, where T is the temperature and R is the gas constant [29]. The method is designed to allow barriers between local energy minima to be overcome in a search of torsional conformation space. The method, as implemented in the QUANTA software [28], also allows energy minimization to be performed whenever the current structure is sufficiently different from the last minimized one. The minimization criterion is a root mean squared difference in all torsion angles, defined by the user. The energy function evaluated by the Boltzmann jump procedure consisted of the pharmacophoric restraints (force constant 20 kcal/mol/Å^2) and a limited number of terms designed to maintain approximately standard bond lengths and angles and to prevent gross distortion of planar groups such as amides and aromatic rings. In practice this was achieved by removing all the van der Waals' interactions (both general and 1–4) and torsional terms associated with rotatable bonds from the CHARMm parameter set version 21A [30]. Of course the resulting parameter set bears little relation to CHARMm. The geometry of the hydrogen-bonding vectors was kept constant throughout the procedure.

At the outset, molecules were overlaid in random orientations with random torsion angles, and

eight trajectories of Monte Carlo simulation were calculated. The final structure from each trajectory was used as the starting point for the next. The recipe for the eight steps is shown in Table 1, with each step generating 100 structures. In the first four steps there was no energy minimization and the trajectories were completed when 100 structures had been accepted. In steps five and six a few steps of minimization were applied to each of the 100 accepted structures. In the final two steps minimization was performed only when the RMS torsion angle criterion was satisfied and these trajectories produced 100 minimized structures. Consequently the latter trajectories were considerably longer than the earlier ones, requiring as many as 40 accepted structures to produce a single minimized one. Every tenth minimized structure from the eighth trajectory was chosen as a candidate pharmacophore for further refinement. The eight steps were designed to converge to a solution of the pharmacophore definition problem, and then to search pharmacophore space. Hence, early steps were run at a low 'pseudo-temperature' of 5000 K enabling low-energy structures to be generated and pharmacophore groups to come together, and later steps combine higher temperature with minimization. The final two steps might be designated the 'equilibration' and 'production' phases. The temperature of each trajectory was designed to keep the numbers of accepted and rejected structures approximately equal (in fact the theoretical maximum efficiency in Metropolis procedures is reached when the ratio of accepted to rejected moves is 0.5). However, it should be stressed that the recipe for the Monte Carlo runs was not optimized. Overall the method is similar to simulated annealing, a technique that has been applied to a variety of conformational searching problems [31–33], although the approach described here differs in identifying a range of solutions rather than the global energy minimum.

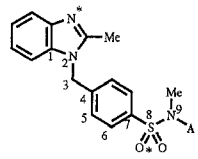
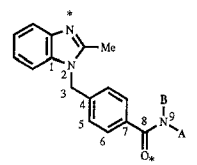
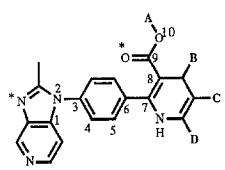
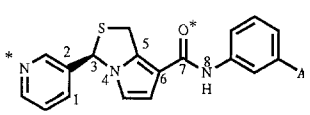
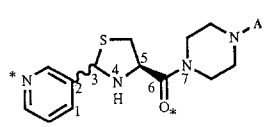
(c) Refinement of candidate pharmacophores

Candidate pharmacophores were refined using the Multifit procedure [34] implemented in the SYBYL software, version 5.3 [27], using default settings unless otherwise stated. The method allows restraining potentials to be applied between equivalent pharmacophoric site points in superimposed molecules. Such restraints are the only intermolecular contributions to the energy of the system and the whole system is minimized as an ensemble. The full Tripos force-field [35] was used in this step, without electrostatics and with harmonic restraining potentials with a force constant of 20 kcal/mol/Å^2. The minimization was performed with the Powell conjugate gradient optimization method [36] to a convergence of 5×10^{-4} kcal/mol.

TABLE 1
BOLTZMANN JUMP PARAMETERS IN THE EIGHT STEPS TO PHARMACOPHORE GENERATION

Trajectory number	Angle window (degrees)	Rigid rotation window (degrees)	Rigid translation window (Å)	Temperature (K)	Angle RMS limit (degrees)	Steps of minimization
1	30.0	30.0	4.0	5000	0.0	0
2	10.0	10.0	1.0	5000	0.0	0
3	5.0	5.0	0.5	5000	0.0	0
4	3.0	3.0	0.2	5000	0.0	0
5	3.0	3.0	0.2	5000	0.0	5
6	3.0	3.0	0.2	10000	0.0	25
7	3.0	3.0	0.0	20000	5.0	100
8	3.0	3.0	0.0	20000	5.0	100

TABLE 2
ACTIVITIES OF THE FIVE COMPOUNDS USED TO GENERATE THE PAF RECEPTOR PHARMACOPHORE

Compound name	IC_{50}[a] (nM)	Ref. no.	Actual structure	Modelled structure
BB-182	300	21	A = cyclo-C_6H_{11} 	A = CH_3
BB-350	500	21	A = cyclo-C_6H_{11} B = C_2H_5 	A = CH_3 B = CH_3
UK-74,505	13	25	A = C_2H_5 B = 2-Cl-Ph C = CONH(pyridin-2-yl) D = Me 	A = CH_3 B = H C = H D = H
RP 59227	11	22	A = COPh 	A = H
YM461	2.3	23	A = $(CH_2)_3Ph$ 	A = H

Atoms marked with an asterisk are the hydrogen-bond acceptors whose dipole vectors are used in the pharmacophore definition. The atom numbers are those used to define the dihedral angles listed in Table 5.

[a] The assay used in each case is inhibition of [^{3}H]PAF receptor binding to washed human platelet membranes [40] with the exception of YM461 where the assay is for the inhibition of [^{3}H]PAF receptor binding to washed rabbit platelet membranes [23].

The ability of the Tripos force-field to represent an aromatic sulphonamide, found in one of the compounds studied, was given some consideration as there are no explicit terms for the S-N bond. It was found that the 1–4 van der Waals contributions to the total energy were sufficient to reproduce the favoured conformation in which the S-N bond is in a plane normal to the aromatic ring. However, other conformations which are less common in nature are found to be energy minima, notably that in which the plane of the ring bisects the sulphonamide oxygens.

(d) Determination of global energy minima

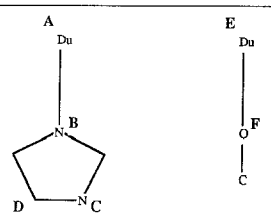
The conformational searching technique of Saunders [37] was used to locate global energy minima for each molecule. While Saunders' original method operates in Cartesian coordinate space, the method implemented in SYBYL [27] called 'Random Search' uses torsional variables as described by Chang et al. [38]. However, the principle is the same and the two approaches have been shown to be nearly equivalent in their efficiency of finding minima [39]. The search of conformational space was considered complete when each local minimum energy conformation had been visited six times. Conformations with an all-atom RMS fit of less than 0.2 Å were considered the same, and energy minimization was performed with the Tripos force-field [35] and a Powell minimizer [36] to a convergence of 5×10^{-4} kcal/mol. All conformation energies are relative to the global minimum value found by this procedure.

RESULTS AND DISCUSSION

(a) Structure–activity relationship for the PAF receptor

Table 2 shows five molecules with their PAF receptor binding activities. In each case there is an aromatic ring containing a potential hydrogen-bond accepting nitrogen and a carbonyl or sulphonyl group capable of accepting a hydrogen-bond. While such a pattern is not observed in all known PAF antagonists, the motif seems to be essential for activity in the five molecules [see also Ref. 20]. The stereochemistry of all the compounds is known, with the exception of YM461 for which the active form is one of two diastereoisomers which equilibrate to a 3:2 mixture in aqueous solution [23]. Consequently the pharmacophore generation procedure was applied to the five molecules, first with the *trans*-YM461 (series 1) and then with *cis*-YM461 (series 2).

TABLE 3
THE DISTANCES USED IN THE PHARMACOPHORE DEFINITION



Distance:	d1	d2	d3	d4	d5	d6	d7	d8
Atoms:	A-E	A-F	B-E	B-F	C-E	C-F	D-E	D-F

Representative distances are shown for BB-350, between the pairs of atoms shown. The symbol 'Du' represents a dummy atom.

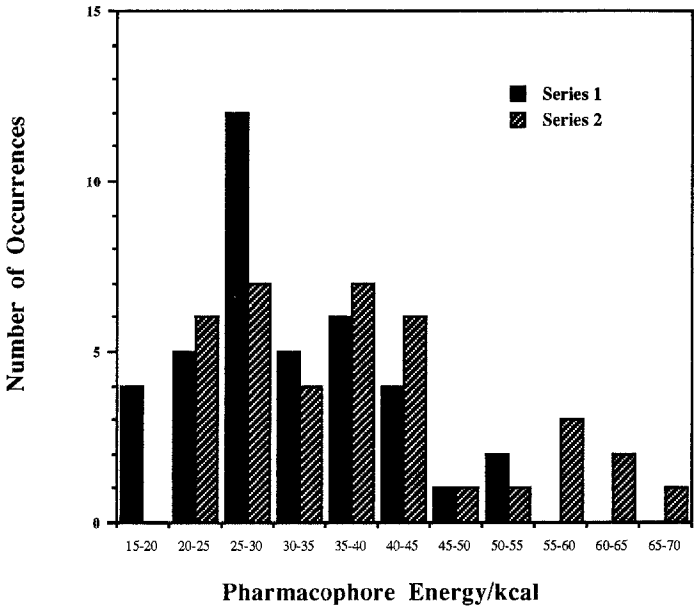


Fig. 3. The distribution of pharmacophore energy for the 40 candidate pharmacophores from each series.

(b) Pharmacophore generation

A model of each of the five compounds was built with a dummy atom representing the hydrogen-bond vector associated with the aromatic nitrogen atom and the carbonyl/sulphonyl group. The six atoms shown in Table 3 (labelled A to F) were restrained with intermolecular

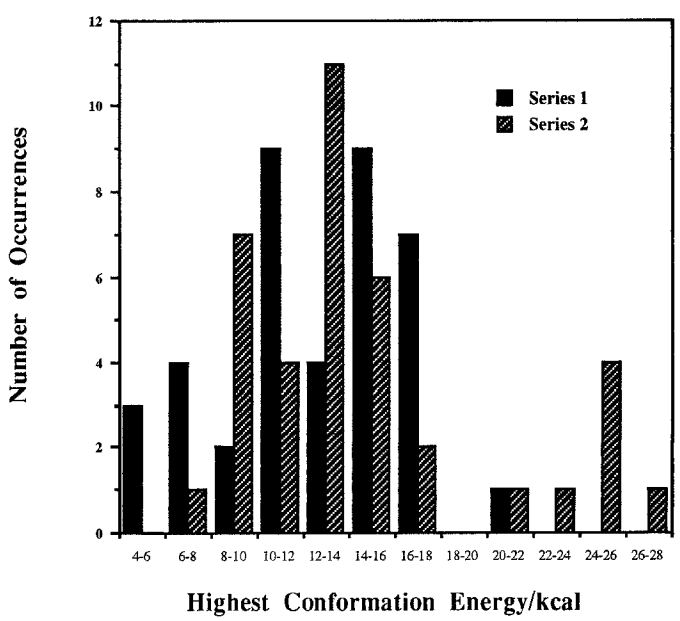


Fig. 4. The distribution of highest conformation energy of the 40 candidate pharmacophores from each series.

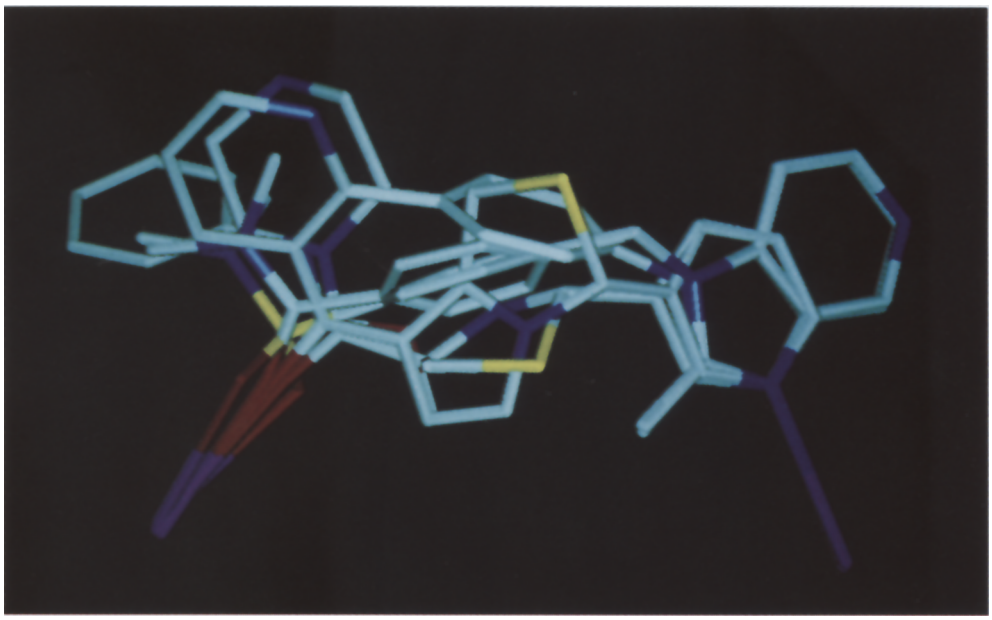


Fig. 5. Pharmacophore solution number 1 from series 1, containing BB-182, BB-350, UK-74,505, RP 59227 and *trans*-YM461. The dummy atoms representing hydrogen-bond donors in the receptor are in magenta.

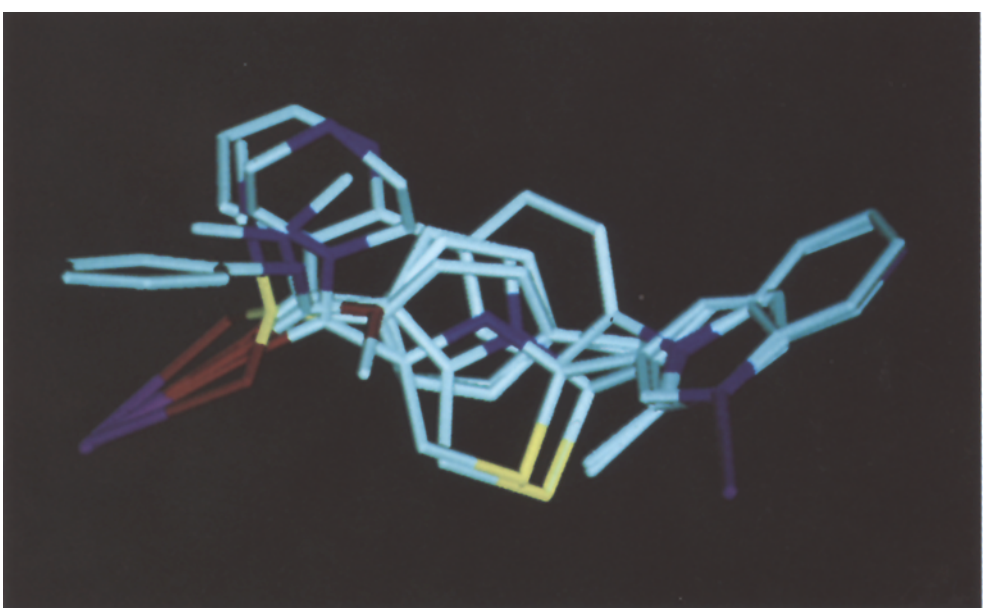


Fig. 6. Pharmacophore solution number 1 from series 2, containing BB-182, BB-350, UK-74,505, RP 59227 and *cis*-YM461. The dummy atoms representing hydrogen-bond donors in the receptor are in magenta.

potentials in the way described in the Methods section. Atom D was included to ensure that the heterocycles were overlaid in the same plane. It was found that using only atoms A, B and C, which are nearly colinear, was insufficient. Although it is not necessary to include distances to atom D (d7 and d8) in the final description of the pharmacophore, they have been included for completeness. While there was no force-field term to ensure gross steric overlap, the application of restraints to both real and dummy atoms ensured that the molecules were overlaid in a sensible manner. The Boltzmann jump procedure was then applied four times to both series 1 and 2, and 40 candidate pharmacophores generated for each. These were refined using Multifit and the conformational energies relative to the global minima recorded. For each candidate pharmacophore, two measures were used to estimate the quality of the solution. First, the pharmacophore energy (PE), the sum of the five conformation energies, gives an overall guide to the quality of the pharmacophore, while the single highest conformation energy (HCE) highlights cases where most of the pharmacophore energy is due to one molecule. These measures are plotted as histograms in Figs. 3 and 4. Both graphs show that the solutions for series 1 are of higher quality than those for series 2. However, given the tight constraint imposed on solutions (i.e. that the hydrogen-bond vectors should be exactly superimposable), the differences are acceptable. For each series the best seven pharmacophores were analysed in more detail. The best pharmacophores (the ones with the smallest HCE) from series 1 and 2 are shown in Figs. 5 and 6.

(c) Series 1 pharmacophore

Table 4 lists the pharmacophore distances (d1–d8 as defined in Table 3) for the best seven pharmacophores from series 1. The distances for the seven solutions are within very small ranges, the greatest range being 0.7 Å for d1. Thus, the pharmacophore is very well defined in distance space. The HCEs are in the range 5–8 kcal/mol and the PEs are in the range 15–23 kcal/mol. The conformations of BB-182 from each of the seven solutions are shown superimposed in Fig. 7. Figure 8 contains a schematic representation of the PAF receptor binding site, showing the geometric relationships between the carbonyl hydrogen-bond vector and the heterocycle. The ranges of values in Fig. 8 reflect the variation in the seven pharmacophores. Clearly the seven candidate pharmacophores represent a single well-defined solution, each of the five compounds being found in only one conformation. Note that these solutions were taken from two of the four Monte Carlo trajectories, implying that the results are reproducable. The torsion angles of each conformation from each pharmacophore solution are listed in Table 5. The T3 angles in BB-182 are in the range 70–105°, close to the commonly observed crystal structure value for sulphonamides of around 90° [41]. T4 for BB-182 is the torsion angle about the CH_2-Ph-SO_2 linear unit and is the angle between the plane of the aromatic heterocycle and the sulphonamide. T4 is listed because a change in T2 may be compensated by a change in T3, without affecting T4 or the pharmacophore. The same is true for the T2 and T3 angles of BB-350.

(d) Series 2 pharmacophore

The seven best pharmacophores from series 2 are listed in Table 4. The pharmacophore distances (d1–d8) are within tight ranges, although the third solution seems to have values most different from the other solutions. The greatest range for a single distance is 1.5 Å for d1. Hence the pharmacophore is well defined in distance-space. Indeed, the pharmacophores for series 1 and 2 are virtually identical in distance space. The quality of the series 2 pharmacophores is lower

TABLE 4
THE BEST CANDIDATE PHARMACOPHORES FROM SERIES 1 AND 2

No.	HCE	PE	Pharmacophore distances (Å)							
	(kcal/mol)		d1	d2	d3	d4	d5	d6	d7	d8
Series 1										
1	5.0	18.0	10.7	9.5	9.9	8.0	9.4	7.0	10.5	8.0
2	5.2	19.3	10.9	9.7	9.9	8.0	9.4	7.0	10.2	7.9
3	5.3	15.5	10.9	9.6	10.1	8.0	9.5	7.1	10.5	8.0
4	6.1	20.4	10.3	9.2	9.6	7.7	9.3	6.9	10.0	7.8
5	6.4	19.8	11.0	9.7	10.1	8.1	9.5	7.0	10.3	7.9
6	7.5	22.6	10.6	9.5	9.8	7.9	9.4	7.0	10.2	7.9
7	7.6	21.7	10.6	9.5	9.8	7.9	9.4	7.0	10.3	8.0
Series 2										
1	6.1	21.0	11.6	9.4	10.5	8.0	9.6	7.0	10.8	8.1
2	8.4	28.8	11.9	9.6	10.4	8.0	9.2	6.9	10.3	8.1
3	9.1	27.8	10.4	9.5	9.7	8.0	9.2	7.0	10.4	8.1
4	9.5	22.9	11.7	9.4	10.6	8.0	9.7	7.1	10.9	8.2
5	9.6	24.1	11.2	9.4	10.2	8.0	9.5	7.0	10.7	8.1
6	9.7	21.0	11.3	9.5	10.3	8.0	9.5	7.0	10.7	8.1
7	9.7	24.6	11.7	9.5	10.6	8.0	9.6	7.0	10.8	8.1

Seven pharmacophores are listed for each series, with the highest conformation energy (HCE), pharmacophore energy (PE) and pharmacophore distances. The distances are defined in Table 3.

than those of series 1 with HCEs in the range 6–10 kcal/mol and PEs from 21–29 kcal/mol. As before, given the tight constraint on solutions, these ranges are acceptable. However, analysis of the conformations contained in the seven candidate pharmacophores reveals that there are essentially three sets of solutions. Solutions 1, 4, 5, 6 and 7 form one group, with 2 and 3 being unique. The conformations of BB-182 from solutions 1, 2 and 3 are shown in Fig. 9. It is evident that, despite the distances between pharmacophoric points being similar, there are three different pharmacophores represented by the three solutions. Each of the five molecules adopts a unique conformation for a given solution. Figure 10 contains a schematic representation of the PAF receptor binding site deduced from the first solution, showing the geometric relationships between the carbonyl hydrogen-bond vector and the heterocycle. Importantly, solutions 1 and 2 came from the same trajectory, showing the ability of the method to cross barriers between solutions. Note how the ring sulphur atoms of RP 59227 and *cis*-YM461 lie very close together in Fig. 6. This is also observed in the other two solutions, but not for series 1 (see Fig. 5) in which the sulphurs are on opposite sides of the pharmacophore.

(e) Verification of the pharmacophore solutions

In order to test the validity of the binding models produced by the pharmacophore generation procedure, four more PAF antagonists, WEB 2086, Ro 24-0238, **I** [42] and **II** [43] from structural classes distinct from those used to generate the pharmacophores (Table 6), were fitted to the four pharmacophore solutions. The Ro 24-0238 structure was truncated for purposes of modelling to

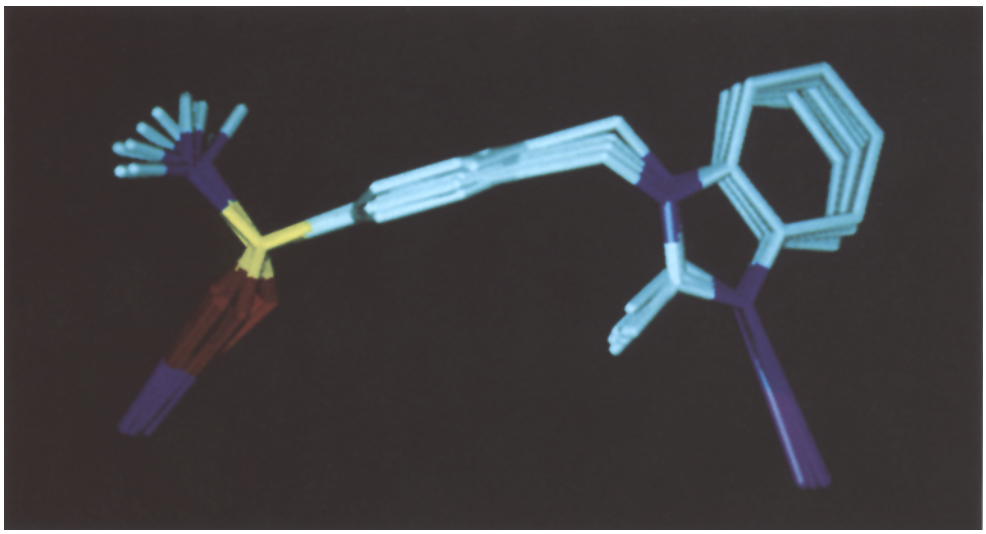


Fig. 7. The conformations of BB-182 from the best seven pharmacophore solutions from series 1. The dummy atoms representing hydrogen-bond donors in the receptor are in magenta.

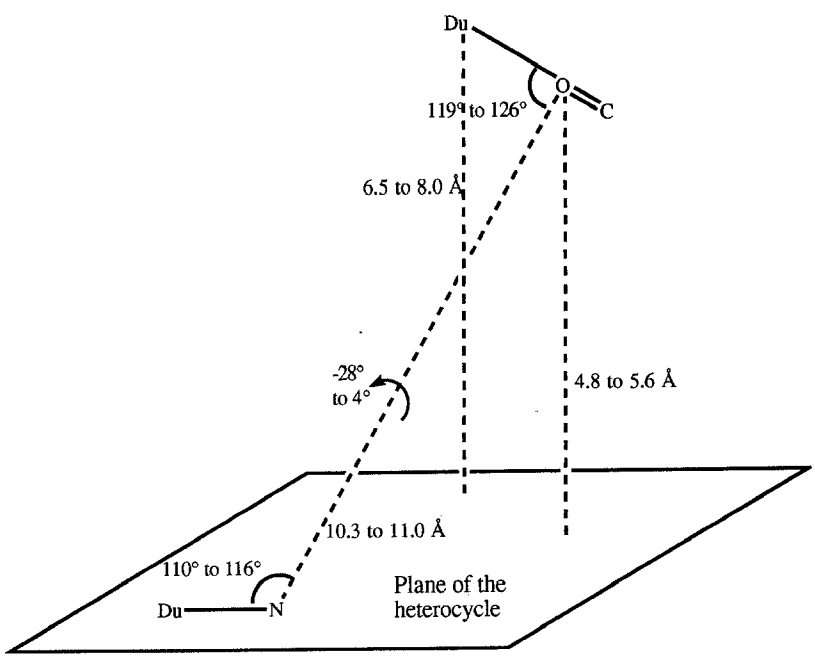


Fig. 8. Schematic representation of the PAF receptor binding site, corresponding to the pharmacophore from series 1. Distances to sulphonyl oxygens were averaged to give the N–O and plane–O distances.

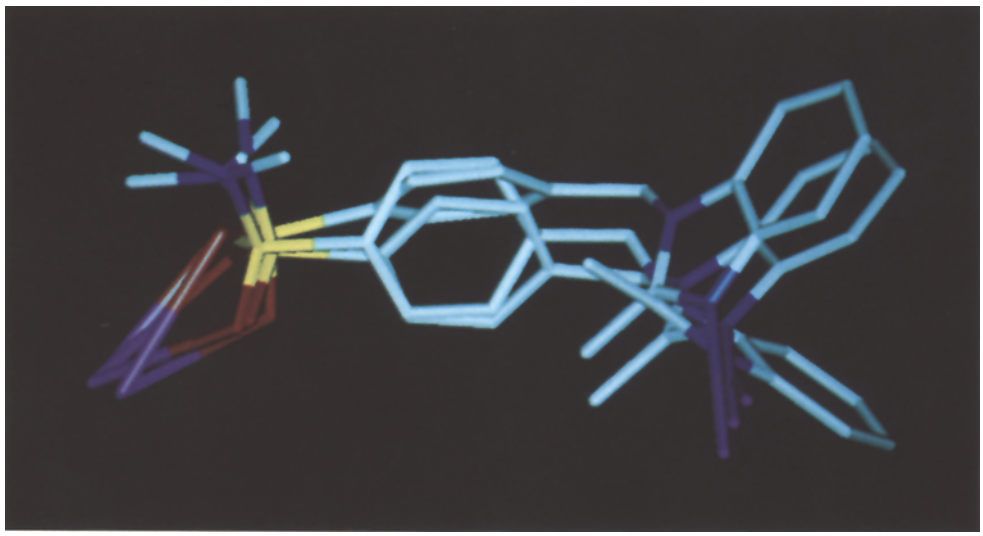


Fig. 9. The conformations of BB-182 from pharmacophore solutions 1, 2 and 3 from series 2. The dummy atoms representing hydrogen-bond donors in the receptor are in magenta.

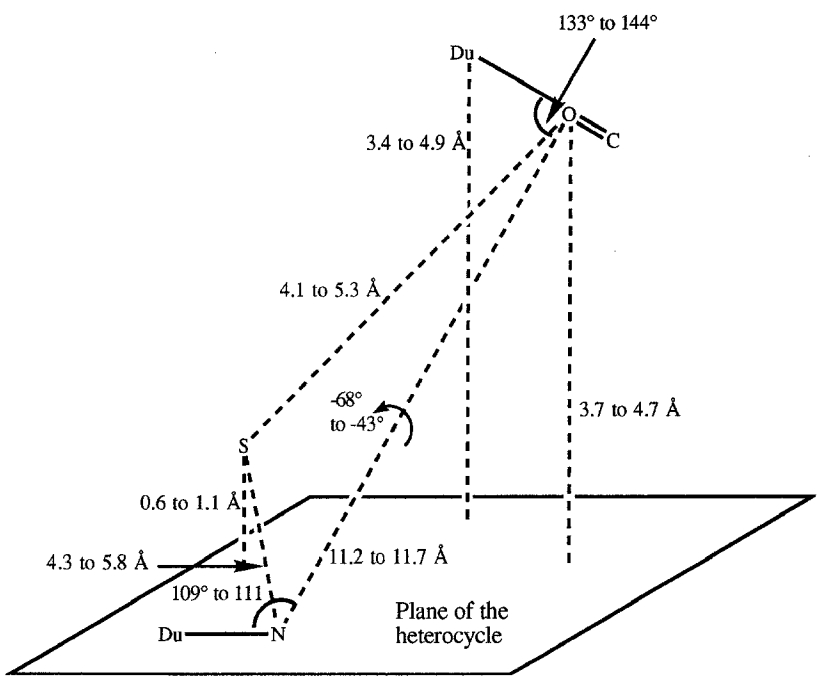


Fig. 10. Schematic representation of the PAF receptor binding site, corresponding to the first pharmacophore solution from series 2. Distances to sulphonyl oxygens were averaged to give the S–O, N–O and plane–O distances.

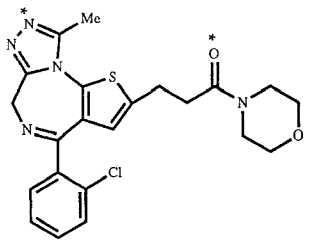
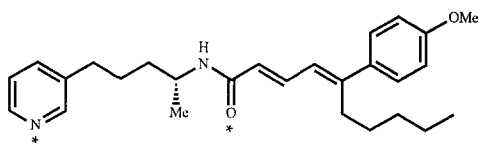
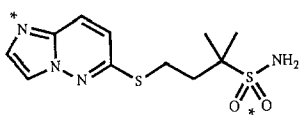
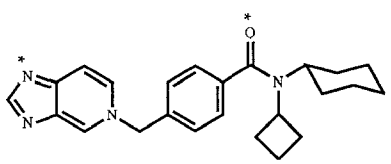
TABLE 5
DIHEDRAL ANGLES OF MOLECULES COMPRISING THE REFINED CANDIDATE PHARMACOPHORES DESCRIBED IN TABLE 4

No.	BB-182				BB-350			UK-74,505			RP 59227		YM461	
	T1	T2	T3	T4	T1	T2	T3	T1	T2	T3	T1	T2	T1	T2
Series 1														
1	122	132	105	−121	125	154	61	41	84	−12	121	3	99	−33
2	113	128	89	−141	92	−156	−19	40	71	−6	110	−6	85	−33
3	118	139	100	−120	116	110	129	39	85	−13	119	10	98	−29
4	118	120	81	−157	122	137	44	40	76	3	114	−15	90	−41
5	119	126	103	−130	117	112	103	40	80	−6	114	1	92	−36
6	115	130	89	−139	87	−146	−15	39	78	−7	114	−9	90	−34
7	120	138	70	−153	93	−149	−14	40	82	−12	121	1	98	−33
Series 2														
1	−131	51	16	67	−127	47	30	151	60	31	−90	111	−93	−16
2	151	124	−30	95	153	118	−35	97	44	46	−148	158	159	58
3	−138	33	122	159	−137	64	53	133	66	−16	−90	−140	−107	59
4	−141	58	−3	57	−132	57	−24	150	55	33	−94	125	−96	−15
5	−129	49	36	84	−127	47	30	152	67	31	−81	133	−92	−12
6	−127	51	20	70	−121	48	30	154	62	31	−85	146	−91	−13
7	−130	57	5	63	−125	62	−22	151	62	31	−85	123	−89	−13

Dihedral angles are in degrees and are defined according to the numbering in Table 2, as follows:
BB-182: T1 = (1,2,3,4), T2 = (2,3,4,5), T3 = (6,7,8,9), T4 = (2,3,8,9);
BB-350: T1 = (1,2,3,4), T2 = (2,3,4,5), T3 = (6,7,8,9);
UK-74,505: T1 = (1,2,3,4), T2 = (5,6,7,8), T3 = (7,8,9,10);
RP 59227: T1 = (1,2,3,4), T2 = (5,6,7,8);
YM461: T1 = (1,2,3,4), T2 = (4,5,6,7).

exclude the flexible hydrophobic tail. The triazole sp^2 nitrogen at the 8-position of the hetrazepine nucleus of WEB 2086 was chosen as the hydrogen-bond acceptor, since the SAR [26] for the methyl group at the adjacent 9-position is similar to that observed for the heterocyclic methyl group of both UK-74,505 [25] and BB-182 [21]. However, it should be noted that there are data from the SAR for imidazole analogues of WEB 2086 to suggest that it is the triazole sp^2 nitrogen at the 7-position of the hetrazepine nucleus that provides the key interaction [26,44]. Conformational searching was performed using the SEARCH program in SYBYL [27] with distance constraints corresponding to the pharmacophore distances in Table 4. Low-energy conformations (within 5 kcal/mol of the global energy minimum) were found for each of the molecules corresponding to pharmacophore 1 of series 2, but not to the two other pharmacophores from series 2 or that from series 1. In addition the sulphur atoms of WEB 2086 and **I** occupy a similar region of space to those in RP 59227 and *cis*-YM461. The proximity of the sulphurs is emphasized in Fig. 10, in which the distance ranges to sulphur were measured from the conformations of the four molecules, and in Fig. 11 which shows the four sulphur-containing molecules superimposed. It has been shown for the hetrazepine PAF antagonist *Brotizolam*, which is structurally related to

TABLE 6
THE FOUR COMPOUNDS USED TO VERIFY THE PAF RECEPTOR PHARMACOPHORE SOLUTIONS

Compound name	IC_{50}[a] (nM)	Ref. no.
WEB 2086	50 	26
Ro-24-0238	40 	24
I (Takeda)	not known 	42
II (Searle)	20 	43

Atoms marked with an asterisk are the hydrogen-bond acceptors whose dipole vectors are used to fit the molecules to the pharmacophore solutions.

[a] The IC_{50} values are for inhibition of PAF-binding to human platelet membranes, except for Ro 24-0238 (washed dog platelets) [24].

WEB 2086, that the thienyl sulphur atom is an important requirement for activity [26]. Furthermore, Yamata et al. have prepared the pyrollidine analogue of YM461 and report that this compound, in which the sulphur atom is replaced by a methylene group, is 35-fold less potent in vitro [23].

A number of constrained analogues of WEB 2086 are known to be potent PAF antagonists [8],

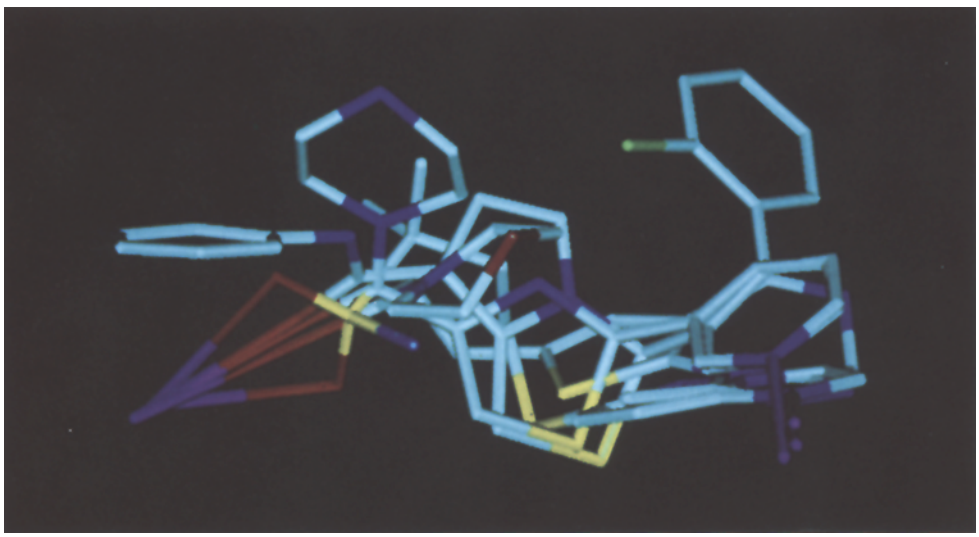


Fig. 11. WEB 2086 and **I** superimposed on the conformations of RP 59227 and *cis*-YM461 from pharmacophore solution 1 of series 2, showing the proximity of the sulphur atoms (in yellow). The dummy atoms representing hydrogen-bond donors in the receptor are in magenta.

for example WEB 2170, Y-24180, E-6123, BN 50739 and Ro 24-4736 (structures not shown). These compounds did not fit any of the pharmacophore candidates, raising the possibility that the WEB 2086 fit is coincidental. Subsequent to the preliminary communication on this work, Weber et al. [44] reported a pharmacophore for the PAF receptor which is related to that presented here. The Weber pharmacophore was generated by overlaying sp^2 nitrogen atoms and carbonyl/sulphonyl groups of a variety of PAF antagonists including WEB 2086 and BB-823 which is a potent PAF antagonist derived from BB-182 (Fig. 12) [40]. However, the Weber group chose to overlay the pyridyl sp^2 nitrogen of BB-823, rather than the imidazo sp^2 nitrogen, on the triazole sp^2 nitrogen at the 7-position of WEB 2086. While the Weber pharmacophore appears to accomodate

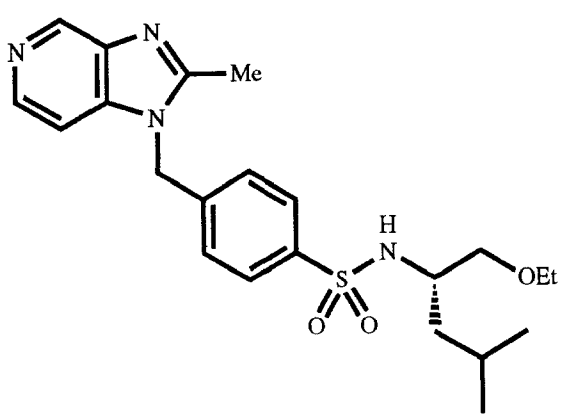


Fig. 12. BB-823, a picomolar PAF antagonist.

the more rigid analogues of WEB 2086, the SAR for BB-823 and its analogues suggests that the imidazo nitrogen provides the key interaction. It is possible that the distance ranges in the pharmacophores presented in this paper could be relaxed to reflect the flexibility of the receptor and include the more rigid WEB 2086 analogues.

CONCLUSION

A novel pharmacophore generation procedure, employing an established Monte Carlo conformational searching algorithm, was successfully applied to a pharmacophore definition problem. The approach treats the pharmacophore as an ensemble of molecules and searches the solution space, constraining the molecules to remain in a given pharmacophoric relationship. Solutions produced by the method are composed of molecules in low-energy conformations. The key to successful sampling of conformational space, under the constraints imposed by the pharmacophore, is to remove internal barriers to rotation of single bonds. It has been shown that the same low-energy solutions are found by different Monte Carlo trajectories, and that a single trajectory may visit more than one solution, implying that the results are reproducable and that the method is exploring pharmacophore space. Improvements to the procedure might be made by optimizing the recipe for performing the Monte Carlo search, in particular by investigating whether pharmacophore candidates can be sampled earlier in the trajectory and still give rise to low-energy pharmacophores. Such a modification might also improve the sampling efficiency of the algorithm. The performance of the approach relative to other pharmacophore generation procedures has not been assessed, however, drawbacks are apparent. The Monte Carlo technique is time-consuming, particularly in comparison with the ensemble distance geometry approach [5], and its sampling properties need to be investigated further, which puts it at a disadvantage to the systematic search method [3,4].

The procedure has been used to derive a binding site model of the human PAF receptor. Constraints were applied that fulfill most of the binding requirements suggested by Tilley et al. [20], namely that PAF antagonists recognize hydrogen-bond donors to a carbonyl group and a heterocyclic aromatic nitrogen, with the aromatic ring in a given plane. Five molecules, representative of highly active series of PAF antagonists, were sufficient to produce well-defined geometric solutions satisfying the proposed criteria for binding, with all molecules in low-energy conformations. The stereochemistry of one of the compounds, YM461, is unknown and pharmacophores were generated with this molecule in both the *trans*- and *cis*-forms (designated series 1 and series 2). The distances between site points in the pharmacophores generated were very similar for the *trans*- and *cis*-series. However, it was shown that distances alone do not provide a unique description of a pharmacophore, as there are three different non-superimposable solutions for the *cis*-series. This gives the procedure a distinct advantage over the systematic search approach [3,4] which works only in intramolecular distance space. In the three solutions from series 2 the ring sulphur atoms of RP 59227 and *cis*-YM461 are very close whereas in the series 1 solution the sulphur atoms are on opposite sides of the pharmacophore. Since the sulphur atom plays a role in receptor binding [23,26] this would suggest that the *cis*-isomer is the active form of YM461. In the *cis*-isomer of YM461 the stereochemistry at the carbon to which the 3-pyridyl is attached is R. This is identical to that of RP 59227 and to that of the more potent enantiomers of a series of 2-(3-pyridyl)thiazolidin-4-ones [45]. Note, however, that the binding site models derived here

represent a partial picture of the PAF pharmacophore. The 'lipophilic' region described by Tilley et al. [20] has not been characterized and is the subject of further studies [46]. It is possible that the 'lipophilic' region is the same part of the PAF receptor that normally accomodates the C_{16}/C_{18} side chain of PAF itself. In support of this idea is the observation that the hetrazepine PAF antagonist BN 50726, which possesses a C_{16} alkyl chain as a 'lipophilic' group, is a potent PAF antagonist [8]. Four important PAF antagonists of different chemical structure to those used to derive the pharmacophore models, WEB 2086, Ro 24-0238, a Takeda compound (**I**) and a Searle compound (**II**), were all shown to fit one (and only one) of the solutions. It was found that four of the seven molecules known to fit this pharmacophore contained sulphur atoms in close proximity. Thus not only is one of the pharmacophore models more successful at explaining binding of PAF antagonists but it suggests an additional binding interaction with the receptor. However, the modelling does not rule out the possibility that multiple binding modes exist for the PAF receptor.

It has not been possible to account for the binding of PAF and its analogues [7] and some important classes of antagonists, for example the gingkolides [7] and Merck's tetrahydrofuran compounds [47], or to establish a relationship between this work and the pharmacophore models of Godfroid [13–16,48]. Recently a classification of PAF antagonists into four categories has been proposed [8], according to the key structural features that they contain: (i) quaternary nitrogen compounds; (ii) heterocyclic sp^2 nitrogen compounds; (iii) diaryl compounds; (iv) miscellaneous compounds. All the molecules used to generate or verify the pharmacophore model discussed in this paper are in the second group. The models produced by Godfroid's group were developed predominantly around the SAR of compounds in the third and fourth categories, although an attempt to include PAF-like molecules from the first category was made [48]. Clearly, diverse families of ligands may give rise to different binding solutions. A more complete model of the PAF receptor may have to await detailed understanding of the 3D structure of the receptor.

A possible criticism of the pharmacophores presented here is that two compounds used in its generation, the early leads BB-182 and BB-350, are only moderately active. However, the discovery that these compounds could adopt a similar orientation of the sp^2 nitrogen and carbonyl/sulphonyl pharmacophoric groups to the other more potent compounds suggested that optimization of the heterocycle and the lipophilic moiety would lead to more active compounds. The recent identification of BB-823 [40], a PAF antagonist with picomolar activity (IC_{50} 0.015 nM) which is shown in Fig. 12, confirms this. BB-823 satisfies the pharmacophores described here, having a structural core in common with BB-182.

Coordinates of all the molecular models discussed in the paper are available from the corresponding author (E.E.H.) by sending a Macintosh 3.5" disk.

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