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A Partial Pharmacophore for the Platelet Activating Factor (PAF) Receptor

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Summary: A partial pharmacophore for the platelet activating factor receptor has been generated by a molecular modelling comparison of five heterocyclic sp2 nitrogen PAF antagonists using a Monte Carlo 'Boltzmann Jump' technique. This pharmacophore defines the relative spatial orientation of the plane of the heterocyclic ring, the sp2 nitrogen, a carbonyl/sulphonyl pharmacophoric group and a sulphur atom.

Platelet activating factor (PAF) is the bioactive phospholipid 1-*O*-hexadecyl/octadecyl-2-acetyl-*sn*-glyceryl-3-phosphoryl choline,[1,2] which is released directly from cell membranes and mediates a range of effects on target cells resulting in a variety of physiological responses.[3,4] PAF appears to be involved in many inflammatory disorders including asthma and endotoxin shock[3,4] and PAF receptor antagonists may be of clinical benefit in such diseases. A diverse range of structures has been identified as PAF antagonists,[5] but the manner in which these compounds bind to the PAF receptor is not well understood. The PAF receptor has recently been cloned and sequence analysis indicates that the receptor belongs to the superfamily of G protein-coupled receptors,[6-8] but its three dimensional structure remains to be determined. Molecular modelling studies have been conducted on both PAF agonists and PAF antagonists with a view to generating a model of the PAF receptor.[9-12] A hypothesis for a pharmacophore for the receptor has been made based on the apparent structural homology of a series of PAF antagonists,[10] a two zone, lipophilic and hydrophilic, model has been suggested,[11] and a model in which the receptor was likened to a pair of "Ear Muffs" of positive potential[12] has been revised to a flexible multipolarised cylinder.[13] However, these models do not satisfactorily explain the requirements for high affinity binding to the PAF receptor, since for the most part they are based upon modelling studies of weakly active natural products such as the Ginkgolides and Kadsurenone.

At an early stage in our PAF antagonist programme we identified two benzimidazole derivatives, the sulphonamide BB-182, and the carboxamide BB-350 as moderately active lead compounds (Table).[14] To try to understand how these molecules compared with other PAF antagonists, we undertook a series of comparative molecular modelling studies. In this communication we report the generation of a partial pharmacophore for the PAF receptor using a novel Monte Carlo method to superimpose molecules.

From the synthesis of analogues of BB-182 and BB-350 it was apparent that the unsubstituted sp2 nitrogen of the benzimidazole was required for activity.[15] A survey of other known PAF antagonists reveals that for a number of compounds an unsubstituted sp2 nitrogen is also a crucial requirement for activity. These include imidazo[4,5-c]pyridine compounds such as UK-74,505 (Modipafant),[16] 3-pyridyl derivatives such as RP 59227 (Tulopafant),[17] YM461,[18] and Ro 24-0238,[19] and hetrazepine derivatives such as WEB 2086 (Apafant)[20] (Table 1).

Figure 1: Schematic representation of heterocyclic nitrogen PAF antagonists.

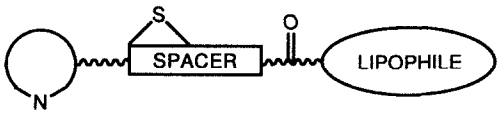
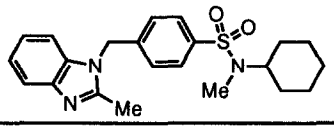
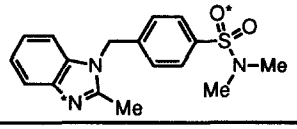
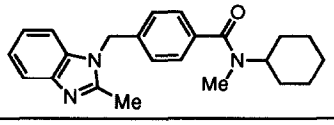
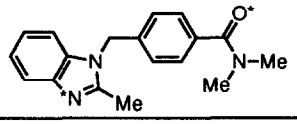
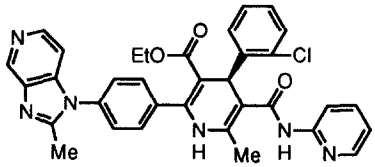
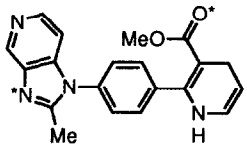
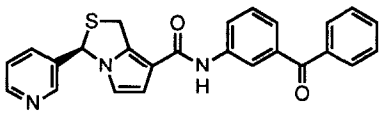
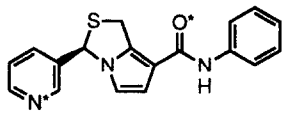
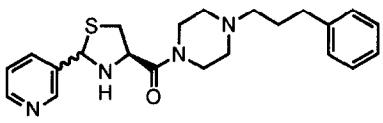
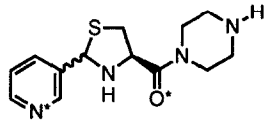
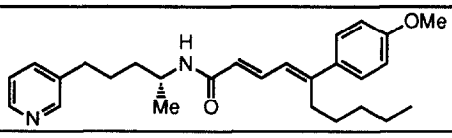
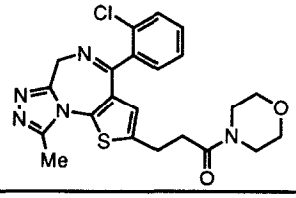
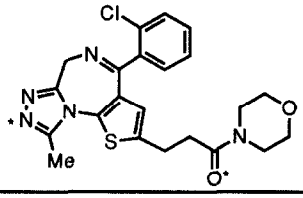


Table: Structures and activities of PAF antagonists.

Entry	Name	Structure	Modelled Structure	Activity IC_{50} nM†
1	BB-182			300
2	BB-350			500
3	UK-74,505			13
4	RP 59227			11
5	YM461			2.3‡
6	Ro 24-0238		Not modelled here.	40§
7	WEB 2086			50

† Biological activities for the inhibition of [^{3}H]-PAF receptor binding to washed human platelet membranes,[24] except ‡ (inhibition of [^{3}H]-PAF receptor binding to washed rabbit platelet membranes[18]) and § (inhibition of [^{3}H]-PAF receptor binding to washed dog platelets[19]). * Hydrogen-bond acceptors whose interaction vectors were modelled by attachment of dummy atoms.

From an analysis of the SAR for Ro 24-0238 and its analogues Tilley and co-workers suggested that the PAF receptor comprises, at minimum, a large lipophilic binding pocket that is tolerant of steric bulk, a hydrogen bond donor that can interact with a carbonyl group and either a π interaction with a pyridine or an electrostatic interaction with a pyridine nitrogen lone pair.[19] We hypothesised that the PAF antagonists shown in the Table might be binding to the PAF receptor in a similar manner, since these compounds, besides the sp2 nitrogen atom, possess several common structural features; a carbonyl moiety, a "lipophilic" group and for some but not all compounds a sulphur atom. We presumed that the possible interactions of the receptor with these compounds might be by hydrogen bonds to the sp2 nitrogen, carbonyl moiety and sulphur atom, whilst the "lipophilic" group provides a less specific hydrophobic interaction with the receptor. If this were the case one might expect these pharmacophoric groups to be held in specific relative orientations to each other by an appropriate spacer group as represented schematically in Figure 1.

Our early lead compounds BB-182 and BB-350 are significantly less potent than the other PAF antagonists listed in the Table. We were concerned as to how the relative orientation of the sp2 nitrogen and the carbonyl moieties compared with that of other more active compounds. We decided to compare BB-182 and BB-350 with three potent rigid compounds (UK-74,505, RP 59227 and YM461) and exclude highly flexible compounds such as Ro 24-0238 from the study. To simplify the modelling the "lipophilic" group was not considered and the abbreviated structures shown in the Table were modelled. A model of each of the five compounds was built with a dummy atom representing the hydrogen-bond vector associated with the sp2 nitrogen atom and the carbonyl/sulphonyl group. It is not known whether it is the *cis*- or the *trans*-diastereoisomer that is the active form of YM461, since the two diastereoisomers equilibrate to a 3:2 mixture in aqueous solution.[18] Consequently, the comparison was applied to the five molecules firstly with *trans*-YM461 (series 1) and secondly with *cis*-YM461 (series 2). For each series candidate pharmacophores were generated using a Monte Carlo search procedure using a reduced force-field parameter set that allows barriers between local energy minima to be overcome when searching torsional space. The molecules were treated as an ensemble with strong restraining potentials joining equivalent pharmacophoric sites in the molecules, the hydrogen-bond vectors to the sp2 nitrogen and to the carbonyl/sulphonyl, with a restraint also set for the heterocyclic rings containing the sp2 nitrogens to lie in the same plane. At the outset the molecules were overlaid in random orientations with random torsion angles. Conformational space was searched by allowing the structure of each molecule to be derived from a previous higher energy one by simultaneously perturbing all variable dihedral angles by a random amount and in addition allowing random global rotation and translation of molecules.[21] For each series forty candidate pharmacophores were generated, which were refined using the MULTIFIT facility in Sybyl[22] and the conformational energies relative to the global minima recorded. For each candidate pharmacophore, the energies of the five conformations were summed to give the pharmacophore energy (PE), and the single highest conformation energy (HCE) was examined to see if the PE was due to one molecule. Based on these criteria a single well defined solution was obtained for series 1 and three solutions were obtained for series 2. Comparison of the solutions indicated that the distances between the pharmacophoric groups were within tight ranges, but that 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. This would suggest that the *cis*-isomer is the active form of YM461.

Figure 2: Stereoview of pharmacophore solution constructed from abbreviated structures of BB-182, BB-350, UK-74,505, RP 59227 and *cis*-YM461.

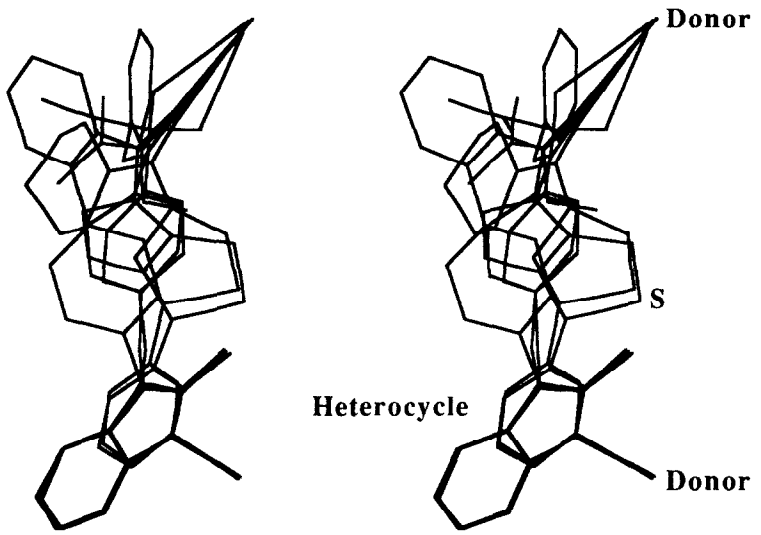
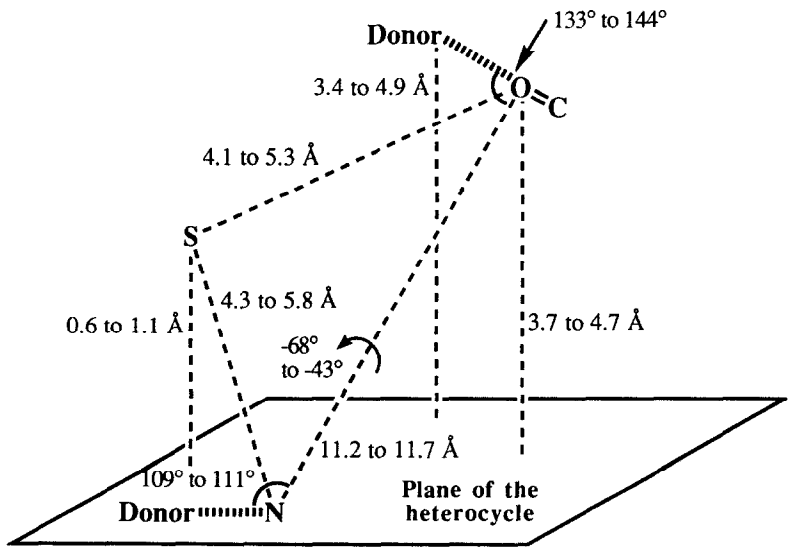


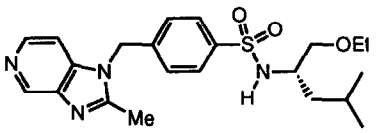
Figure 3: Pharmacophore map defining geometric relationships of the plane of the heterocycle, a hydrogen bond to the heterocyclic sp2 nitrogen, a hydrogen bond to the carbonyl/sulphonyl group and the position of the sulphur atom.



To test the validity of the binding models produced by the pharmacophore generation procedure, another PAF antagonist WEB 2086 (Entry 7 of the Table) was fitted to each of the four pharmacophore solutions. Using the SEARCH program in Sybyl[22] low energy conformations of WEB 2086 were found that fitted the geometric constraints of only one pharmacophore from series 2. In this solution the sulphur atom of WEB 2086 resides close to those of RP 59227 and *cis*-YM461. This pharmacophore is shown in a stereoview of the molecules from which it was constructed in Figure 2 and as a map in Figure 3.

The binding site illustrated in Figure 3 at best represents a partial pharmacophore for the PAF receptor. The lipophilic region described by Tilley *et al.*[19] has not been characterised and is the subject of further studies. The partial pharmacophore does not account for the binding of PAF, structurally related antagonists and other important classes of antagonists such as the 2,5-diaryltetrahydrofurans (e.g. Merck MK-287),[23] also it is not easily related to the multipolarised cylinder model of the PAF receptor.[13] However, this partial pharmacophore does explain how potent PAF antagonists that possess heterocyclic sp2 nitrogen atoms interact with the PAF receptor.

A possible criticism of our partial pharmacophore is that two of the compounds used in its generation, our early leads BB-182 and BB-350, are only moderately active. We concluded that the relative spatial orientation of the sp2 nitrogen and carbonyl pharmacophoric groups of BB-182 and BB-350 was not significantly different to that of UK-74,505, RP 59227, YM461 and WEB 2086. Therefore, we decided to concentrate our medicinal chemistry programme on the optimisation of the heterocycle and the "lipophilic" moiety. This led to our recently identifying BB-823 a PAF antagonist with picomolar activity (IC_{50} 15 pM for inhibition of [^{3}H]-PAF bonding to washed human platelet membranes).[24] Clearly BB-823 satisfies our partial pharmacophore as it corresponds to the abbreviated modelled structure shown in Entry 1 of the Table.



BB-823

The novel Monte Carlo method that has been used here for the superimposition of molecules[21] should have general applicability to pharmacophore definition. As such it represents an alternative method to Marshall's systematic method[25] and the distance geometry technique of Sheridan.[26]

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References and Notes

1. Demopoulos, C. A.; Pinckard, R. N.; Hanahan, D. J. *J. Biol. Chem.* **1979**, 254, 9355.
2. Benveniste, J.; Tence, M.; Bidault, J.; Boullet, C.; Varence, P.; Polonsky, J. *C. R. Seances Acad. Sci., Ser. D* **1979**, 289, 1037.
3. Braquet, P.; Touqui, L.; Shen, T. Y.; Vargaftig, B. B. *Pharmacol. Rev.* **1987**, 39, 97.
4. Koltai, M.; Hosford, D.; Guinot, P.; Esanu, A.; Braquet, P. *Drugs* **1991**, 42, 9; *Idem, Ibid*, 174.
5. Cooper, K.; Parry, M. J. *Ann. Reports Med. Chem.* **1989**, 24, 81.
6. Honda, Z.-I.; Nakamura, M.; Miki, I.; Minami, M.; Watanabe, T.; Seyama, Y.; Okada, H.; Toh, H.; Ito, K.; Miyamoto, T.; Shimizu, T. *Nature* **1991**, 349, 342.
7. Ye, R. D.; Prossnitz, E. R.; Zou, A.; Cochrane, C. G. *Biochem. Biophys. Res. Commun.* **1991**, 180, 105.
8. Nakamura, M.; Honda, Z.-I.; Izumi, T.; Sakanaka, C.; Mutoh, H.; Minami, M.; Bito, H.; Seyama, Y.; Matsumoto, T.; Noma, M.; Shimizu, T. *J. Biol. Chem.* **1991**, 266, 20400.
9. Dupont, L.; Germain, G.; Dideberg, O. *Pharmacol. Res. Commun.* **1986**, 18, Suppl., 25.
10. Schreiber, S. L.; Porco, Jr., J. A.; Hawley, R. C.; Desmaele, D. *New Methods in Drug Research;* Makriyannis, A., Ed.; J. R. Prous Science Publishers: S.A., 1989; Vol 3, pp. 13-26.
11. Croziet, F.; Langlois, M. H.; Dubost, J. P.; Braquet, P.; Audry, E.; Dallet, Ph.; Colleter, J. C. *J. Mol. Graphics* **1990**, 8, 153; Dubost, J. P.; Langlois, M. H.; Audry, E.; Braquet, P.; Colleter, J. C.; Croizet, F., Dallet, Ph. *CRC Handbook of PAF and PAF Antagonists*; Braquet, P., Ed.; CRC Press, Boca Raton, FL., 1991; pp. 261-267.
12. Dive, G.; Godfroid, J.-J.; Lamotte-Brasseur, J.; Batt, J.-P.; Heymans, F.; Dupont, L.; Braquet, P. *J. Lipid Med.* **1989**, 1, 201; Lamotte-Brasseur, J.; Dive, G.; Lamouri, A.; Heymans, F.; Godfroid, J.-J. *Biochim. Biophys. Acta* **1991**, 1085, 91.
13. Batt, J.-P.; Lamouri, A.; Tavet, F.; Heymans, F.; Dive, G.; Godfroid, J.-J. *J. Lipid Med.* **1991**, 4, 343; Godfroid, J.-J.; Dive, G.; Lamotte-Brasseur, J.; Batt, J.-P.; Heymans, F. *Lipids* **1991**, 26, 1162; Lamotte-Brasseur, J.; Heymans, F.; Dive, G.; Lamouri, A.; Batt, J.-P.; Redeuilh, C.; Hosford, D.; Braquet, P.; Godfroid, J.-J. *Lipids* **1991**, 26, 1167.
14. Whittaker, M.; Floyd, C. D.; Davidson, A. H.; Dickens, J. P. International Pat. Appl. No. WO 90/09997.
15. Details of the SAR for BB-182, BB-350 and analogues will be reported in full elsewhere.
16. Alabaster, V. A.; Keir, R. F.; Parry, M. J.; de Souza, R. N. *Agents and Actions Suppl.* **1991**, 34, 221.
17. Lavé, D. *CRC Handbook of PAF and PAF Antagonists*; Braquet, P., Ed.; CRC Press, Boca Raton, FL., 1991; pp. 203-220.
18. Yamada, T.; Saito, M.; Mase, T.; Hara, H.; Nagaoka, H.; Murase, K.; Tomioka, K. *Lipids* **1991**, 26, 1179.
19. Tilley, J. W.; Clader, J. W.; Zawoiski, S.; Wirkus, M.; LeMahieu, R. A.; O'Donnell, M.; Crowley, H.; Welton, A. F. J. Med. Chem. 1989, 32, 1814; Guthrie, R. W.; Kaplan, G. L.; Mennona, F. A.; Tilley, J. W.; Kierstead, R. W.; Mullin, J. G.; LeMahieu, R. A.; Zawoiski, S.; O'Donnell, M.; Crowley, H.; Yaremko, B.; Welton, A. F.; J. Med. Chem. 1989, 32, 1820; Tilley, J. W.; O'Donnell, M. *CRC Handbook of PAF and PAF Antagonists*; Braquet, P., Ed.; CRC Press, Boca Raton, FL., 1991; pp. 229-258.
20. Weber, K. H.; Heuer, H. O. *Med. Res. Rev.* **1989**, 9, 181; Casals-Stenzel, J. *Lipids* **1991**, 26, 1157.
21. Full details of this procedure will be published elsewhere.
22. Tripos Associates, Inc., 1699 South Hanley Road, Suite 303, St Louis, MO 63144, USA.
23. Sahoo, S.P.; Graham, D. W.; Acton, J.; Biftu, T.; Bugianesi, R. L.; Girotra, N. N.; Kuo, C. -H.; Ponpipom, M. M.; Doebber, T. W.; Wu, M. S.; Hwang, S. -B.; Lam, M. -H.; MacIntyre, D. E.; Bach, T. J.; Luell, S.; Meurer, R.; Davies, P.; Alberts A. W.; Chabala, J. C. *Bioorg. Med. Chem. Lett.* **1991**, 1, 327.
24. Beauchamp, C. L.: Bowles, S. A.; Cackett, K.; Christodoulou, M.; Galloway, W. A.; Longstaff, D. S.; McGuinness, G. P.; Miller, A.; Timmis, D. J.; Whittaker, M.; Wood, L. M. In preparation.
25. Mayer, D.; Naylor, C. B.; Motoc, I.; Marshall, G. R. *J. Comput.-Aided Mol. Design* **1987**, 1, 3.
26. Sheridan, R. P.; Nilakantan, R.; Dixon, J. S.; Venkataraghavan, R. *J. Med. Chem.* **1986**, 29, 899.