Pakistan Journal of Pharmaceutical Sciences
Vol. 11(2) July 1988, pp. 5-12

PLATELET ACTIVATING FACTOR ANTAGONISTS

SYEDA AFROZ, AZMAT ARA* AND M. ARIF**
*Department of Pharmacology, *Department of Pharmaceutics*
***Department of Pharmaceutical Chemistry*
University of Karachi, Karachi

ABSTRACT

Platelet Activating Factor (PAF) is a D-glycerol derived phosopholipid which is a potent endogenous mediator of inflammation. PAF is synthesized and released by a variety of cell types and elicits its biological activity by interacting with specific G-protein coupled receptors found on platelets, neutrophils, and other inflammatory cells. The physiological consequences of the interaction on PAF with its receptor includes an increase in vascular permeability, hypotension, bronchoconstriction, and platelet and neutrophil aggregation. These biological effects are consistent with the concept that PAF is involved in a number of inflammatory diseases such as septic shock and asthma (Arimura A., 1998). Given the potent pathophysiological effects of PAF, a great deal of effort has been focused on the discovery of agents which block the action of PAF at its receptor. Within the past 10 years, a wide range of structures have been identified as PAF antagonists. These include not only PAF analogs, but also antagonists derived form natural product as well as non-lipid synthetic compounds. Several theories have been proposed to unify these diverse, structural classes, but sophisticated molecular models of the receptor have not been widely employed (Braquet P., 1987). The discovery of new PAF antagonists has relied heavily on traditional medicinal chemistry approaches. A number of PAF antagonists have advanced to clinical evaluation. While several *ear*ly compounds demonstrated efficacy in animal models of asthma they have failed to provide benefit for this condition in man. The current generation of potent antagonists are being evaluated as therapies for sepsis, pancreatitis and other disorders (Braquet C., 1991).

INTRODUCTION

Platelet activating factor (PAF) was first described in 1972, and its structure was established seven years later. Over the subsequent fifteen years, extensive research has been devoted to elucidating the role of PAF in human disease, and identifying opportunities for therapeutic intervention through preventing its actions. A number of monographs and review articles describing various aspects of the field have appeared (Dragnet C., 1991). This review focuses on the discovery and optimization of selected structural classes of PAF antagonists, with an emphasis on the drug design process. While we have made an effort to include representative example spanning the diverse collection of structures having binding activity at the PAF receptor, the review is not meant to be comprehensive in the sense of including all known compounds. Rather, we have chosen to focus on efforts to optimize the pharmaceutical properties of selected structures. Following a brief summary of PAF and its role in disease, the review is organized, based on the source of the leads: analogs of PAF, derived form natural products, discovered by screening of

synthetic chemicals, and finally a discussion of structure-based drug design as applied to PAF antagonists (Dyson M.C., 1990).

Platelet Activating Factor Structure, Receptors and Role In Disease:

Structure of PAF

Platelet activating factor is not a unique chemical structure, but rather a collection of related molecules acting at a common cellular receptor. The term PAF refers to a class of glycerol based molecules with a long chain ether linked to C_1, an acetyl at C_2 and phosphocholine at C_3. The nature of the alkyl ether moiety varieties with species and cell type, but the 16 and 18 carbon saturated chains predominate. A twelve carbon chain is required for significant receptor interaction with 16 carbon unit being most active (Godfroid J.J., 1990). It has been shown that the C_2 position of the glycerol backbone must be in the R configuration and capped with only short chain acyl groups. The C_2 non-acylated lyso-PAF the natural degradation product of PAF, is inactive. The C_3 position can accommodate a variety of quarternary ammonium groups appended through a short spacer (Handley D.A., 1990).

Cellular sources and synthesis of PAF:

PAF is synthesized upon appropriate stimulation by a variety of inflammatory cells including neutrophils, platelets, macrophages, monocytes and mast cells. The production of PAF by these myeloid derived cells is consistent with the role of PAF in inflammation. PAF is also synthesized in other cells and tissues. Among these are endothelial cells, skin fibroblasts, gastric mucosa, and kidneys. The role of PAF synthesis in these cells is not well understood.

PAF is commonly viewed as an intercellular mediator excreted by cells, but some excrete only a portion of the PAF they produce. In fact endothelial cells retain all of the PAF they make (Herbert J.M., 1992). For this reason, it has been suggested that in addition to an autocrine or exocrine role, PAF may function as an intracellular messenger or cell surface bound intercellular messenger (Herbert J.M., 1992).

Platelet activating factor is primarily prepared via the remodeling of pre-existing membrane phosph6lipids (Hirayama Y., 1993). Upon cellular stimulation, the C_2 long chain fatty acids, including arachidonic acid, are removed form these lipids to produce *lyso*- PAF which is acetylated to form PAF. The simultaneous release of arachidonic acid and *lyso*-PAF resulting from a common stimuli implies that leukotrienes, prostaglandin, thromboxanes and other arachidonate derived inflammatory mediators can be released along with PAF. In addition to this remodeling pathway, PAF has also been shown to be synthesized via a *de nova* route beginning with a simple ether phosphoglycerol (Houlihan W.J., 1991). The function of PAF prepared in this manner is not well understood.

PAF receptors:

PAF acts through specific receptors found on numerous cells and tissues. Some of the same cells that produce PAF also possess receptors for it (Miyamoto T., 1985). These include platelets, neutrophils, eosinophils, macrophages, as well as kidney, liver, lung and brain. The K_d for the high affinity binding of PAF to intact cells or membrane preparations is generally between 0.1 and 10

nM.

The PAF receptor has been cloned from several sources including guinea pig lung, human heart, human leukocytes, HL-60 derived granulocytes, (Perico N., 1997) and U937 myeloid cells. The guinea pig and human receptors are each 342 amino acids in length with 83% overall identity. There is also considerable homology to order G-protein linked *receptors,* and hydropathy analysis of the primary sequence shows the presence of the characteristic seven transmembrane domains. As with other G-protein linked receptors, no definite structural information exists to define precisely the nature of the PAF or antagonist binding regions. However, computer models of the receptor structure have been proposed.

Homology screening of several libraries from different cell sources has failed to demonstrate the existence of receptor subtypes. Receptor heterogeneity has been suggested by variations in the rank order of potencies among various antagonists, but this may be the result of different receptor conformation induced by membrane or by differences in the G-protein coupling (Summers J.B., 1995)

Pathophsiologic effects of PAF:

Stimulation of the PAF receptor results in a variety of cellular responses including phosphoinositide turnover, platelet and granulocyte aggregation and degranulation, eosinophils chemotaxis, and respiratory bursts in neutrophils and macrophages. When administered to animals, PAF produces systemic hypotension, bronchoconstriction, pulmonary hypertension, decreased cardiac output, increased vascular permeability, glomerular nephritis and thromboctopenia (Takatani M., 1990). These responses have linked PAF to a multitude of diseases. Among these are asthma, septic shock, ARDS, transplant rejection, reperfusion injury, pancreatitis, and psoriasis.

Not only in PAF capable of producing many of the sings and symptoms of disease, but the lipid mediator has also been detected at elevated levels in various diseased tissues and fluids. For example, several studies have detected PAF in the bronchoaverolar savage fluid of asthmatic subjects, while not detecting it in normal individuals. Elevated levels have also been detected in the blood of septic shock patients (Takatani M., 1990), the bronochaveolar lavage fluid of individuals with ARDS, and the plasma of infants with necrotizing enterocolities. In addition, PAF has been detected in tissues and fluids in animal models of human disease (Tokumura A., 1995).

Given the ability of PAF to initiate many of the signs and symptoms of diseases and in view of the elevated levels of the mediator during disease states, it is logical to pursue the discovery of agents that would modulate the actions of PAF. Indeed more than two decades of research at nearly three dozen major pharmaceutical companies has led to the identification of a vast array of structurally diverse PAF antagonists. The design and characterization of these compounds is the subject of the remainder of this review (Winslow C.M., 1987).

Design Based on the Structure of PAF:

In the years following the discovery of the structure of PAF, extensive studies in a number of laboratories examined the effects of changes in the structure on agonist, and ultimately antagonist activity. These early studies have been well reviewed (Valone F.H., 1982), so only elements rele-

vant to the design of particular antagonists will be presented here. In keeping with our theme of drug design, the primary focus will be on the modification of compounds over time by particular research groups, with the grouping of compounds by structural similarities as a secondary concern (Inarrea P., 1984).

Glycerol derivatives:

The first reported specific antagonist of PAF was CV-3988, discovered by researchers at Takeda. The compound was initially prepared as part of a program to discover novel phospholipids anti-tumor agents and antimicrobials (Kloprogge E., 1984). When the structure of PAF was re-ported, these compounds *were* tested for PAF agoinst and antagonist activity. The structure of CV-3988 introduces three changes from PAF itself: the alkyl chain at CI of glycerol backbone is linked through a carbamate rather than an ether, the metabolically unstable acetoxy substituent at C2 is re-placed with a methyl ether, and a thiazolim slat replaces the trimethyl ammonium head group attached at C3. This compound inhibited [^{3}1-I] PAF binding to washed human platelets with an IC50 of 160 nM. As might be expected for a very early compound, CV-3988 displayed a number of short comings as a pharmaceutical agent. In particular, it was found to have poor oral activity (Wade P.J., 1986) and displayed some agonist properties at higher concentrations. Slight hemolysis was also observed at the injection site in clinical trials. Several of these undesirable properties were postulated to be related to the phospholipids structure of the molecule (Hwang S.B., 1983)

Glycerol replacements:

Other researchers have introduced more extensive changes in an effort to obtain compounds with better overall properties than those obtained with direct PAF analogs.

The Sandoz Research Institute undertook an active program pursuing both cyclic and acylic analogs of PAF. As might be expected, some of the initial glycerol-containing compounds, as exemplified by SDZ 63-119. A number of cyclic analogs were studied which attempted to constrain or mimic the glycerol backbone. The most studied compounds to arise from this effort were SDS 63-072, which introduced a spirofused tetreahydrofuran moiety at C_2 of the glycerol unit, along, with SDZ 63-441 and SDZ 63-675, which replaced the glycerol backbone with a 2,5-cis- substituted tetrahydrofuran. These compounds all exhibit modest IC_{50} values in the range of 300-400 nM versus human platelet binding, and displayed limited duration of activity in vivo (Valone F.H., 1983). These studies, along with other contemporary efforts to prepare cyclic PAF analogs have been reviewed. The Sandoz workers also explored ways of minimizing the phosopholipied nature of their compounds in an effort to increase their duration of action *in vivo,* ultimately arriving at the acylic compound SDZ 64-619, which is similar in structure to CV-6209. The Sandoz compound differs at the glycerol C_3 substituent, having a methane sulfonyl on the carbamate linkage as op-posed to an acetyl, and a methyl pyridinium, rather than ethyl pyridinium head group. The compound has an IC_{50} of 50nM against PAF binding to human platelets, and displayed activity and duration similar to CV-6209 (Hwang S.B., 1987).

DESIGN BASED ON NATURAL PRODUCTS

Ginkgolides:

Some of the most widely studied compounds with PAF antagonist activity are obtained from the leaves of tree *Ginkgo bilboa.* Ginkgo extracts are known to have been used as medicinal agents for more than 5,000 years and are still used today in some parts of the world. Interestingly, they have been employed for the treatment of diseases thought to be PAP mediated, including asthma and other inflammatory conditions (Domingo M.T., 1988).

The PAF antagonist activity of the Ginkgo extracts have been attributed to a series of terpenoid natural products referred to as ginkgolides A, B, C and J, BN 52063 is a 1:2:1 mixture of A, B, C. The most potent member of the class, ginkgolide B (BN 52021), is a modest inhibitor of PAF binding to rabbit platelet membranes with an IC_{5o} of 250 nM. Ginkgolides C and J (BN 5202 and BN 52024) which place hydroxyl groups in the vicinity of the lipophilic *t-butyl* moiety display only weak receptor interaction (7,100 nM and 54,000 nM, respectively). Ginkgolide A (BN 52020) is intermediate in activity (IC_{50} = 700 nM) (Doty M., 1988).

Analogs of the ginkgolides derived from synthetic modifications have revealed additional structure activity features. The hydroxyl at R^1 can be convened to a methyl or ethyl ether without loss in activity, but loss of the hydroxyl at R^3 is deleterious to receptor binding. Similarly opening or reduction of the lactone rings C, E, or F results in loss of activity (Domingo M. T., 1988).

DIARYL TETRAHYDORFURANS AND RELATED MOLECULES

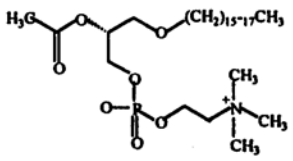
Lingnan natural products:

The natural products screening program at Merck which was responsible of the discovery of kadsurenone also lead to the identification of a series of lignan natural products as PAF antagonists (Lambrecht G., 1986). Although the natural products themselves were only weak antagonists (e.g. veragunesin : IC_{50}= 1,100 nM, saucemeol: IC_{50} = 4, 500 nM, rabbit platelet membrane binding, this screening formed the foundation for a long line of diaryl tetrahydrofuran containing antagonists from Merck.

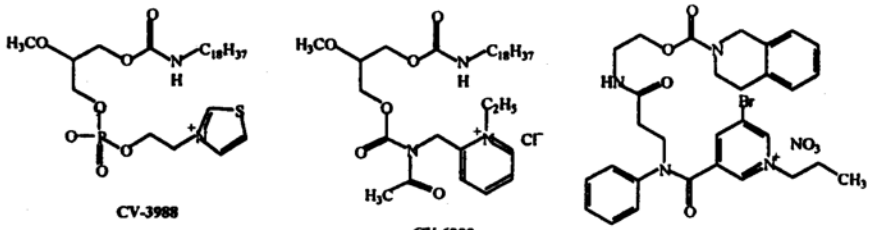
Examination of most of the stereoisomers of these lignans as well as several close analogs revealed that the 3,4-dimethyl substituent caused steric interactions that decreased potency. Removal of these substituent increased binding affinity by nearly 10 fold. Unlike kadsurenone, the addition of a third methoxy substituent on each of the acyl substituent yielded anther 20 fold increase in potency. The resulting 6is-trimethoxphenyl tetrahydrofuran, L-652,731, has been extensively evaluated.

Other natural product PAF antagonists:

Natural product screening programs have lead to the discovery of several other series of PAF antagonists. One of the most active programs has occurred at Fujisawa where researchers have identified several natural products which block the actions of PAF (Voelkci N.E. 1982). For example, FR90452, isolated from culture broth of *Streptomcyes phaeofaciens No.7739,* was the basis of

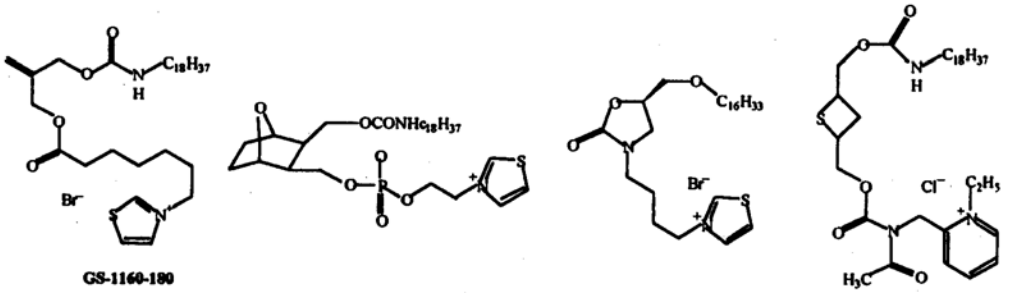


Structure of Platelet Activiting Factor



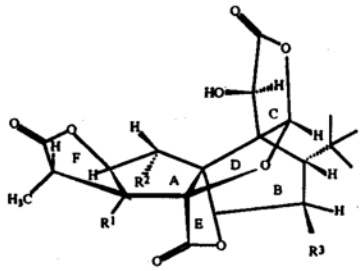
CV-3988 CV-6209 ICV-309

PAF analog antagonists from Takeda.

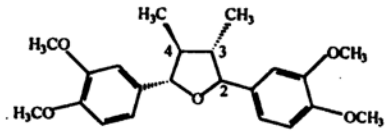


GS-1160-180

Miscellaneous antagonists based on the structure of PAF.



Ginkgolides A, B, C, J.



Veraguensin 2-*R*
Saucerneol 2-*S*

Lignan PAF antagonists

a series of diketopiperazine containing synthetic antagonist (Braquet P., 1987). FR900452, which blocks PAF induced platelet aggregation with an ICSO of 370 nM, was the basis for the simplified compound FR76600, which is about equipotent with the original natural product. FR76600 blocks platelet aggregation with an $_{IC5O}$ of 440 nM and inhibits PAF-induced hypotension in the rat with an EDSO of 4.2 mg/kg, i.v. (Braquet P., 1987).

REFERENCES

Arimura, A., Asahuima, F. and Harada, M. (1992). Antiasihmatic activity of a nove thromoboxane Az antagonist. *Int. Arch. Allergy Immunol.* **98**(3): 239-246.

Braquet, P. (1987). The ginkgolides : potent platelet activating factor antagonists isolated form *Ginkgo biloba L. Drugs of the Future.* **12**: 643-699.

Braquet, C., Etienne, A., Touvay, C., Pignol, B., Mencia-Huerta, J: M. and Braquet, P. (1991). Aminoacylates and aminocarbamates of 2-substituted 4-hydroxymethl 1,3-dioxolans:Structure activity relationships and biological activity as PAF antagonists. In CRC Handbook of PAF and PAF Antagonists (Braquet, P., ed.) CRC Press, Boca Raton, pp.119-133.

Braquet, P. and Godfroid,J.J. (1987). Conformational properties of the PAF receptor in platelets based on structure-activity studies. In: Platelet Activating Factor (Synder, F., ed.). Plenum Press, New York.

Doly, M., Braquet, P., Bonhomme, B. and Meyniel, G. (1988). Presence of specific PAF-acether receptors inside the retina and their electrophysiological involvement (abstract). *Clin. Exp. Physiol. Pharmacol.* Suppl. **13**: 15.

Domingo, M.T., van Delst, G.L., Nerbey, M.L.G., Van Haerinjen, N.G., Chabrier, P.E. and Braquet, P. (1988). Specific binding of PAF in iris and ciliary body in pigmented rabbit eye. In: Proceedings of the British Pharmacological Society, 6-8 January, C 106.

Domingo, M.T., Spinnewyn, B., Chabrier, P.E. and Braquet, P. (1988). Presence of specific binding sites for platelet- activating factor (PAF) in gerbil brain. *Biochem, Bioyphys. Res. Comm.* **151:** 730-736.

Dyson, M.C., Bellan J.A., Minkes, R.K. and Wagmann, M.J. (1990). Influence of BN0730 on bronchostrictor. *J. Pharmacol. Exp. Ther.* **255**(3): 1320-1327.

Godfroid, J. J. and Dive, G. (1990). PAF receptor agonists: Structure-activity relationships. In ; Platelet Activating Factor in Endotoxin and Immune Diseases (Handley, D.A., Houlihan,W.J., Saunders, R.N. and Tomeshch, J.C., eds.). Marcel Dekker, New York, pp.15.

Handely, D.A., Houlihan, W.J., Saunders, R.N. and Tomesh, J.C. (1990). Platelet-Activating Factor in Endotoxin and Immune Diseases. Marcel Dekker, New York.

Herbert J.M. (1992). Characterization of specific binding sites of platelet activating factor. *Biochem. J.* **284** (Pt. 1): 201-206.

Hirayama, Y., Lei, Y.H. and Barnes, P.J. (1993). Effects of two novel tachykinin antagonist. *Br. J. Pharmacol.* **108**(3): 844-851.

Houlihan, W.J. (1991). Structure activity relationship in 5 aryal-2, dihydorimidazal [2, 1-a] isoquinolines PAF antagonists. In: CRC Handbook of PAF and PAF Antagonists (Braquet, P., ed.). CRC Press, Boca Ration, FL, pp.221-228.

Hwang, S.B., Lee, C.S.C., Cheath, M.1. and Shen, T.N. (1983). Specific receptor sites for 1-0-acetyl-*sn*-glycero-3- phosphocholine (platelet activating factor) on rabbit platelet and guinea pig smooth muscle membranes. *Biochemist*ry. **22**: 4756- 4763.

Hwang, S.B., Lam, M.H. and Shen, T.Y. (1985). Specific binding sites for platelet activatingfactor in human lung tissues. *Biochem. Biophys. Res. Commun.* **128**: 972-979.

Innarrea, P., Gomez-Cambronero, J., Nieto, M. and Sanches Grespo, M. (1984). Characteristics of the binding of platelet-activating factor to platelets of different animal species. *Fur. J Phar-*

macol. **105**: 309-15.

Klaoprogge, E. and Akkerman, M.N. (1984). Binding Kinetics of PAF-acteher (I-0-alkyl-2-ace tyl-sn-glycero-3-phosphocholine) to intact human platets. *Biochem.J.* **223**: 901-909.

Lambrecht, G. Pamham, M.J. (1986). Kadsurenone distinguishes between different plateletactivating factor receptor subtypes on macrophages and polymorphonuclear leukocytes. *Br. J. Pharmacol.* **87**: 287-289.

Miyamoto, T., Ohno, H., Yano T., Okada, T., Hamanaka, N. and Kawasaki, A. (1985). ONO6240: A potent antagoinst of PAF. In: Advances in Prostaglandin, Leukotriene Research (Hyaishi,O. and Yamamoto, S., eds.). Raven Press, New York, Vol. 15, pp.719-720.

Perico, N., Lapiski, R. and Konopka, K. (1997). Platelet activating factor mediates angiotensin II-induced proteinuria. *J. Am. Soc. Nephrol.* **8**(9): 1391-1398.

Summers, J.B. and Albert, D.H. (1995). Platelet activating factor antagonists. In: Advances in Pharmacology (August, J.T., Anders, M.W,, Murad, F. and Coyle, J., eds.). Academic Press, New York, Vol.32, pp.67-168.

Takatani, M. and Tsushima, S. (1991). Structure activity relationships in CV-3988 and CV-6209 PAF antagonists series. In: CRC Handbook of PAF and PAF Antagonists (Braquet, P., ed.). CRC Press, Boca Raton, pp.97-118.

Takatani, M., Maezaki, N., Imura, Y., Terashita, Z. I., Nishikwas, K. and Tsushima, S. (1990). Platelet activating (PAF) antagonists: development of a highly potent PAF antagoinst, TCV-309. In: Advances in Prostaglandin, Thromboxanc, Leukotriene Research (Samuelsson, B., ed.). Raven Press, Ltd. New York, Vol.21, pp.943-946.

Tokumura, A. and Tsukatani, H. (1995). Platelet activating factor DAF-like phospholipids. *Biosci. Biotechnol.* Biochem. **59**(8): 1389-1393.

Valone, F.H., Coles, E., Reinhold, V.R. and Goetzl, E.J. (1982). Specific binding of phospholipid platelet-activating factor by human plateles. *J. Immunol* **129**: 1637-1641.

Valone, F.H. and Goetzl, E.L (1983). Specific binding by human polymorphonuclear leukocytes of the immunological mediator 1-0-hexadecyl/octadecyl-2-acetyl-su-glyeero-3-phosphorlcholine *Immunolo*gy. **48**: 141-149.

Voelkei, N.F., Worthen, S., Reeves, J.T., Henson, P.M. and Murphy, R.C. (1982). Non-immunological production of leukotrienes induced by platelet-activating factor. *Science.* 286-288.

Wade, P.J., Lunt, D.O., Lad, N., Tuffio, D.P. and McCullagh, K.G. (1986). Effect of calcium and calcium antagonists on [^{3}]- PAP- acether binding to washed human platelets. *Thromb. Res.* **41**: 251-262.

Winslow, C.M., Anderson, R.C., D'Aries, F.J., Frisch, G.E., DeLillo, A.K., Lee, M.L. and Saunders, R.N. (1987). Toward understanding the mechanism of action of PAF receptor antagonists. *In*: New Horizons in Platelet Activating Factor Research (Winslow, C.M. and Lee, M.L., eds.). Wiley.