Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 10, pp. 1091-1096, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00173-5

Synthesis and Bioactivities of Heterocyclic Lipids as PAF Antagonists. 1

S. K. Chung,[a]* S. H. Ban[b], S. H. Kim[b], B.E. Kim[b] and S. H. Woo[b]*

Department of Chemistry, Pohang University of Science and Technology [a]
and Research Institute of Industrial Science and Technology [b] Pohang 790-784, Korea

James B. Summers and Richard G. Conway

Immunnosciences Research Area, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois 60064, USA

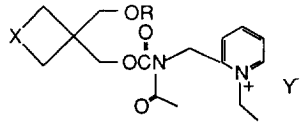
Abstract: Conformationally constrained analogues of platelet activating factor incorporating various combinations of a lipophile and a pyridine-like heterocycle via hydrogen bond acceptors such as ether and/or carbamate linked to a suitable core group such as 1,1-bis(hydroxymethyl)cycloalkane and 3,3-bis(hydroxymethyl)-oxetane, -thietane and -azetidine skeletons have been synthesized, and are shown to be powerful and selective PAF receptor antagonists.

Platelet activating factor (PAF) is a class of bioactive phospholipids such as 1-O-hexadecyl- and octadecyl-2-acetyl-*sn*-glyceryl-3-phosphoryl choline, which are released directly from cell membranes and mediate a wide range of effects on target cells resulting in a variety of significant physiological responses.[1-3] PAF appears to be involved at least in part in many immunological, inflammatory and vascular disorders including asthma, endotoxic shock and disseminated intravascular coagulation (DIC). Thus, PAF antagonists may be of potential clinical utility in such diseases.[3,4]

The PAF receptor has been cloned and is shown by the sequence analysis to belong to the superfamily of G-protein coupled receptor, although its three dimensional structure remains to be determined.[5-7] A wide variety of structural analogues of PAF have been studied in order to examine the structural requirements for agonist and antagonist activity of the PAF receptor. For the agonist activity, the following structural requirements are known to be essential: 1) the natural R configuration at the C2 position with a relatively small substituent size, 2) the ether functionality with a hydrophobic moiety at the C1 position, and 3) the phosphate and the ammonium head group with the optimal two carbon chain length in between.[2] A fairly diverse range of structural types has been identified for the PAF antagonism. However, their exact binding interactions with the PAF receptor have not been established. The known chemical structures of PAF antagonists generally fall into four categories: 1) acyclic PAF analogues such as CV 3988, Ono 6240 and SRI 63119, 2) constrained versions of PAF analogues such as SRI 63072 and BN 52111, 3) synthetic compounds structurally unrelated to PAF, e.g. apafant (WEB 2086) and triazolam, and 4) natural products, e.g. ginkgolides, kadsurenone and gliotoxins.[8]

There has been a number of attempts to define the pharmacophoric patterns of the PAF receptor on the basis of the structures of known PAF antagonists with the aid of molecular modelling methods.[9-13] For example, Godfroid, et al. have proposed a bipolarized cylinder (10-12 Å diameter) like pharmacophore model for the receptor on the basis of the 3-D electrostatic potential calculations of selected PAF antagonists including ginkgolides, synthetic furanoid neolignan (L-652731), and triazolothienobenzo-

Table 1.

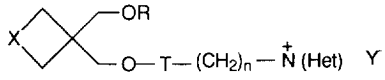


Comp.	X	R	Y	Ki[a] (nM)	IC_{50}[b](nM)
1	—	$CONHC_{18}H_{37}$	I	13.0	394
2	CH_2	$CONHC_{18}H_{37}$	I	6.6	118
3	$(CH_2)_2$	$CONHC_{18}H_{37}$	I	4.2	573
4	$(CH_2)_3$	$CONHC_{18}H_{37}$	I	56.0	286
5	$(CH_2)_4$	$CONHC_{18}H_{37}$	I	110.0	365
6	$(CH_2)_2$	$CONHC_{16}H_{33}$	I	1.2	350
7	O	$CONHC_{18}H_{37}$	I	22.6	123
8	O	$CONHC_{18}H_{37}$	Cl	20.0	158
9	O	$CONHC_{16}H_{37}$	I	95.0	93
10	O	$C_{16}H_{33}$	I	110.0	219
11	N-Ac	$CONHC_{16}H_{33}$	I	278.0	188
CV-6209				7.2	55
Gingkolide B					418

a) Standard PAF receptor assay [15]using New Zealand rabbit platelet membranes prepared and stored frozen in Tris/$MgCl_2$/EDTA(TME buffer, pH 7.0)/ 0.25M Sucrose.

b) Standard assay[16] with a chronolog aggregometer using New Zealand rabbit blood platelets in citrate buffer.

Table 2.



Comp.	X	R	T	n	N(Het)	Y	Ki (nM)	IC_{50}(nM)
12	$(CH_2)_2$	$CONHC_{18}H_{37}$	CO	4	pyridine	Br	112.0	1,450
13	$(CH_2)_2$	$CONHC_{18}H_{37}$	CO	4	thiazole	Br	139.0	1,140
14	$(CH_2)_2$	$CONHC_{18}H_{37}$	CO	4	quinoline	Br	9.4	95
15	$(CH_2)_2$	$CONHC_{18}H_{37}$	PO_3	2	pyridine	—	>1000	1,270
16	$(CH_2)_2$	$CONHC_{18}H_{37}$	PO_3	2	thiazole	—	>1000	10,840
17	O	$CONHC_{18}H_{37}$	CO	5	pyridine	Br	188.0	684
18	O	$CONHC_{18}H_{37}$	CO	5	thiazole	Br	30.5	382
19	O	$C_{16}H_{33}$	CO	5	pyridine	Br	>1000	1,466
20	O	$C_{16}H_{33}$	CO	5	thiazole	Br	53.0	1,256
21	S	$CONHC_{18}H_{37}$	CO	5	pyridine	Br	20.0	228
22	S	$CONHC_{18}H_{37}$	CO	5	thiazole	Br	28.0	270
23	S	$CONHC_{18}H_{37}$	CO	5	quinoline	Br	30.0	57
24	S	$C_{16}H_{33}$	CO	5	quinoline	Br	596.0	658
CV-6209							7.2	55
Gingkolide B								418

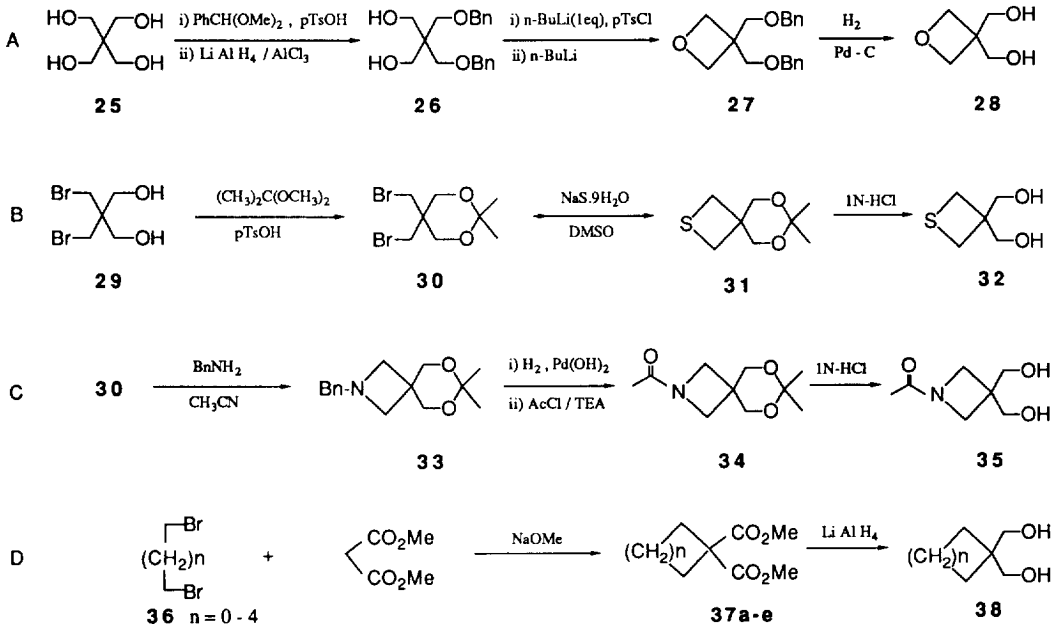
diazepine (WEB 2086).[11] More recently, they suggested the presence of a second shorter (6-7 Å diameter) ear-muffs domain for the receptor based on their studies with antagonists having the 1,4-bis(3', 4', 5'-trimthoxybenzoyl)piperazine structure.[13] On the other hand, Whittaker et al. studied five heterocyclic sp^2 nitrogen containing PAF antagonists such as UK-74505, RP 59227, YM 461, and WEB 2086 using a Monte Carlo Boltzmann Jump technique, and proposed a pharmacophore which could be defined by the relative spatial orientation of the plane of the heterocyclic ring, the sp^2 nitrogen, a carbonyl/sulfonyl group and a sulfur atom.[12]

Although none of these pharmacophoric models can satisfactorily explain the high affinity binding of all known antagonists, it seems quite certain that the PAF receptor contains a large lipophilic binding pocket that is tolerant of considerable steric bulk, a hydrogen bond donor that can interact with either a carbonyl group or an oxygen atom, and a group capable of either electrostatic or hydrophobic interactions with a pyridine-like moiety. Based on the structures of known PAF antagonists and the various proposed partial pharmacophoric fragments, a potent PAF antagonist is expected to have an appropriate spatial arrangement of a lipophile linked up to a pyridine-like heterocycle via a hydrogen bond acceptor (e.g. carbonyl, ether, amide, carbamate etc.), and a suitable spacer or core group as its minimal requirements.[12,13] Thus, we have carried out syntheses of a number of compounds incorporating various combinations of these structures based on conformationally constrained PAF skeletons such as simple 1,1-bis(hydroxymethyl)cycloalkanes and 3,3-bis(hydroxymethyl)-oxetane, -thietane and -azetidine, and wish to describe herein the synthesis and the biological activities of these heterocyclic lipid compounds **1-24** (Table 1 and 2).

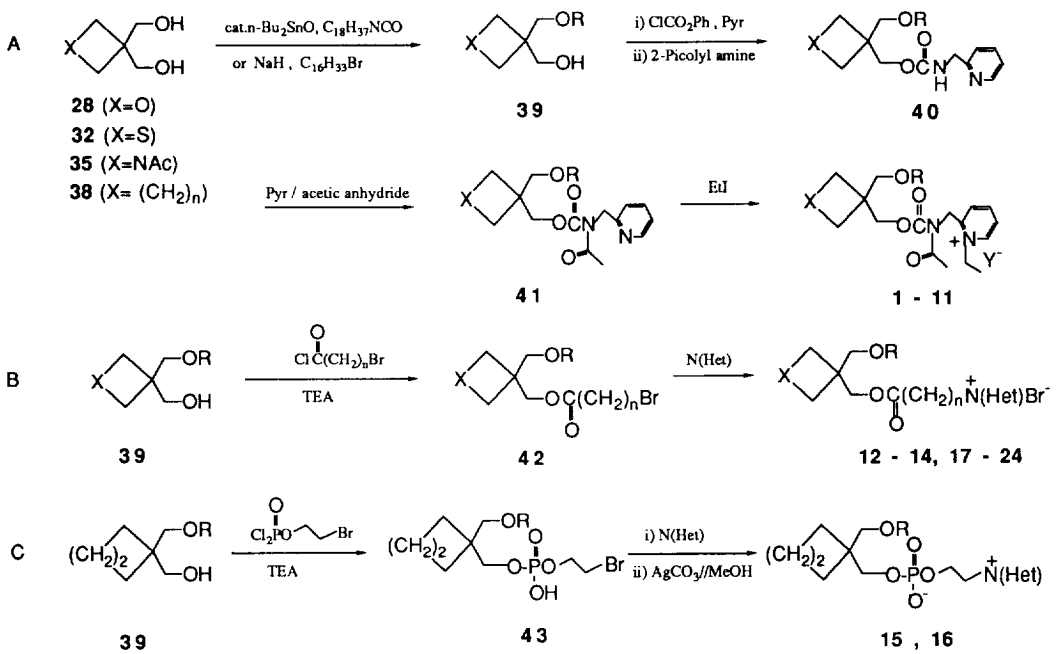
All target compounds (**1-24**) were prepared from the corresponding diols **28, 32, 35,** and **38** (Schemes). Pentaerythritol (**25**) was converted to **26** in 77 % yield by a reaction with benzaldehyde methyl acetal in the presence of p-TsOH, and a subsequent reduction with $LiAlH_4$ and $AlCl_3$. The mono-tosylation of **26** with one equiv. of n-BuLi and p-toluenesulfonyl chloride, followed by treatment with n-BuLi provided the oxetane ring (**27**) in 74 % yield. Reductive removal of the benzyl protecting group in **27** gave the required 3,3-bis(hydroxymethyl)-oxetane intermediate (**28**) (Scheme 1A). The structural elaborations of the diols (**28, 32, 35,** and **38**) to the target compounds (**1-11**) have been carried out as illustrated for structure **10**. The necessary monoalkylation and monocarbamoylation of the diol **28** were efficiently performed by reaction with NaH and alkyl bromide in DMF, and alkylisocyanate in the presence of a catalytic amount of nBu_2SnO, respectively. A series of successive reactions on **39** with i) phenyl chloroformate and pyridine, ii) picolylamine, iii) acetic anhydride in pyridine, and iv) ethyl iodide provided the target compound **10** in good yield (Scheme 2A). The transformations of structure **39** to the target compounds (**12-14** and **17-24**) have been done as shown for compound **12**. Successive treatments of **39** ($X=CH_2CH_2$, $R=CONHC_{18}H_{37}$) with i) triethylamine and 5-bromopentanoyl chloride in THF, and ii) pyridine at 100°C gave **12** in good yield (Scheme 2B). In the analogous manner, successive reactions of **39** ($X=CH_2CH_2$, $R=CONHC_{18}H_{37}$) with i) 2-bromoethyl phosphorodichloridate and triethylamine, ii) pyridine at 80°C, and iii) $AgCO_3$ in methanol gave **15** (Scheme 2C).[14]

The other requisite alcohols (**32**, **35** and **38**) were prepared as follows. The reaction of 2,2-Bis(bromomethyl)1,3-propanediol (**29**) with dimethoxypropane and pTsOH in THF gave the dibromoketal (**30**), which upon treatment with sodium sulfide in DMSO was converted to the protected form

Scheme 1



Scheme 2



of 1,1-bis(hydroxymethyl)-thietane (**31**) in 87 % yield. Compound **31** could be readily hydrolyzed in 1N HCl to 3,3-bis(hydroxymethyl)-thietane (**32**) (Scheme 1B). On the other hand, the dibromoketal (**30**) was reacted with benzylamine in acetonitrile at reflux temperature to give in 43 % yield the cyclized product (**33**). The benzyl protecting group in compound **33** was reductively removed, and the amine was acetylated to give compound **34**, which was hydrolyzed in 1N-HCl to give 3,3-bis(hydroxymethyl)-azetidine (**35**) (Scheme 1C). The preparations of a series of 1,1-bis(dihydroxymethyl)-cycloalkanes were carried out as shown in Scheme 1D, albeit in variable yields depending on the ring size. Mixtures of dimethyl malonate, a dibromoalkane and sodium methoxide in methanol were refluxed for several hours to provide in 10-70 % yields the cyclic diester products (**37**), which were reduced to the corresponding alcohols **38** with $LiAlH_4$.

The *in vitro* PAF antagonisms of the compound **1-24** were measured by their abilities to displace [^{3}H]-PAF from its receptor in rabbit platelet membranes (Ki) as well as to inhibit the PAF-induced aggregation of rabbit platelets (IC_{50}) according to the well-established methods. [15,16] The values are listed in Table 1 and 2. It is quite clear that the two *in vitro* antagonism data show a reasonable degree of parallelism, and that a number of the synthetic compounds are as potent as or more potent than the reference standards used, i.e. CV-6209 and ginkgolide-B. From the *in vitro* activity data in Table 1 and 2, a number of trends are discernible. 1) The nature of the X moiety in the ring skeleton makes a small difference, since all of them show excellent antagonism. However, thietane derivatives appear to be somewhat superior to others in some cases. 2) The nature of the anion associated with the ammonium salt causes only a minor variation in potency. 3) The nature of the R substituents, i.e. ether or carbamate side chain makes little difference, although in some cases the carbamate series appears to be a little better than the ether series. 4) The carbocyclic ring size makes interesting variations in potency; the cyclobutane and cyclopentane derivatives seem to be the best in terms with the Ki values. 5) The structural environment in the vicinity of the N atom, i.e. the nature of the heterocyclic moiety causes a wide variation in potency : quinoline> thiazole≈ pyridine. Quarternization of the pyridine nitrogen in compounds **1-11** appears to be essential for the activity since the neutral synthetic intermediates show much lower activities (data not shown). Although some of these compounds are already more potent than CV-6209, further optimization of these structures in terms of potency and bioavailability is in progress. [17]

ACKNOWLEDGEMENT. We wish to thank Dr. P. U. Park (Sunkyong Industry) for the collaborations involving the *in vitro* (IC_{50}) bioassays.

REFERENCES AND NOTES

1. Braquet, P.; Touqui, L.; Shen, T. Y.; Vargaftig, B. B. *Pharmacol. Rev.*, **1987**, *39*, 97.
2. Venuti, M. C. *Compre. Med. Chem.*, **1990**, *3*, 715.
3. Kotai, M; Hosford, D.; Guinot, P.; Esanu, A.; Braquet, P. *Drugs*, **1991**, *42*, 9; idem, ibid., **1991**, *42*, 174.
4. Braquet, P.; Paubert-Braquet, M.; Koltai, M.; Bourgain, R.; Bussolino, F.; Hosford, D. *Trends Pharmacol.*, **1989**, *10*, 23.
5. Honda, Z. I.; Nakamura,M.; Miki, I.; Manami, M.; Watanabe, T.; Seyama, Y.;. Okada, H.; Toh, H.; Ito, K.; Miyamoto, T.; Shimizu, T. *Nature*, **1991**, *349*, 342.

6. 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.
7. Ye, R. D.; Prossnitz, E. R.; Zou, A.; Cochrane, C. G. *Biochem. Biophys. Res. Comm.*, **1991**, *180*, 105.
8. Cooper, K.; Parry, M. J. *Ann. Rep. Med. Chem.*, **1989**, *24*, 31.
9. Schreiber, S. L.; Porco, J. A.; Hawley, R. C.; Desmaele, D. in "*New Methods in Drug Research*", Ed. A. Makriyannis, J. R. Prous, Spain, **1989**.
10. Braquet, P.; Godfroid, J. J. *Trends Pharmacol.*, **1986**, *7*, 397.
11. Godfroid, J. J.; Dive, G.; Lamotte-Brasseur, J.; Batt, J. P.; Heymans, F. *Lipids*, **1991**, *26*, 1162 and 1167.
12. Hodgkin, E. E.; Miller, A.; Whittaker, M. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 597.
13. Lamouri, A.; Heymans, F.; Tavet, F.; Dive, G.; Batt, J. P.; Blavet, N.; Braquet, P.; Godfroid, J. J. *J. Med. Chem.*, **1993**, *36*, 990.
14. All new compounds have been fully characterized by satisfactory spectral and elemental analyses.
15. Guinn, D. E.; Summers, J. B.; Heyman, H. R.; Conway, R. G.;Rhein, D. A.; Albert, D. H.; Magoc, T.; Carter, G. W. *J. Med. Chem.*, **1992**, *35*, 2055.
16. Born, F. V. R. *Nature*, **1962**, *194*, 927.
17. Chung, S. K.; Ban, S. H.; Kim, S. H.; Kim. B. E.; Woo, S. H.; Summers, J. B.; Conway, R. G., *Bioorg. Med. Chem. Lett.*, following paper in this issue.

(*Received in Japan* 23 *March* 1995; *accepted* 10 *April* 1995)