Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 10, pp. 1097-1102, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00174-3

Synthesis and Bioactivities of Heterocyclic Lipids as PAF Antagonists. 2

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Abstract: Conformationally constrained analogues of platelet activating factor incorporating a lipophile and a pyridine-like heterocycle linked to core groups such as 1,1-bis(hydroxymethyl)cyclobutane and 2,4-bis(hydroxymethyl)-oxetane, -thietane and -azetidine skeletons via hydrogen bond acceptors such as ether and/or carbamate have been synthesized, and their *in vitro* and *in vivo* bioactivitites have indicated potent and selective PAF antagonism.

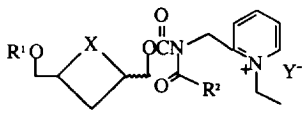
Platelet activating factor (PAF) is a class of highly potent phospholipid mediator of inflammation,[1-3] and appears to be involved in many immunological, inflammatory and vascular disorders including asthma, endotoxic shock and disseminated intravascular coagulation (DIC). PAF antagonists, therefore, may be of potential clinical utilities in such diseases.[3,4] The PAF receptor are found in a variety of cell types. The cloned receptor 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 with regard to the structural requirements for the agonist and antagonist activities of the PAF receptor, and fairly diverse structural types have been identified for the PAF antagonism.[8] However, their exact binding interactions with the PAF receptor have not been established. There also have 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 techniques.[9-13] Although none of the proposed 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 these results, we have designed a number PAF antagonists incorporating various combinations of a lipophile and a pyridine-like heterocyclic on conformationally constrained PAF skeletons such as 1,1-bis(dihydroxymethyl)cycloalkanes and 3,3-bis(dihydroxymethyl)-oxetane, -thietane and -azetidine.[14] Now, we wish to report the synthesis and the biological activities of PAF antagonists analogously incorporating a lipophile and a heterocycle on the core groups such as 1,3-bis(dihydroxymethy)cyclobutane, and 2,4-bis(dihydroxymethyl)-oxetane, -thietane, and -azetidine, i.e. compounds **1-15** (Table 1).

All target compounds (**1-15**) were synthesized from the corresponding diols **19**, **21**, **24**, and

Table 1.



Comp.	Stereochem.	X	R^1	R^2	Y	Ki (nM)	IC_{50} (nM)
1.	*trans*	CH_2	$CONHC_{18}H_{37}$	CH_3	I	4.3	56
2.	*cis*	O	"	CH_3	I	9.2	97
3.	*trans*	O	"	CH_3	I	5.7	57
4.	*cis*	O	"	CH_3	Cl	15.0	101
5.	*cis*	O	$C_{16}H_{33}$	CH_3	I	21.2	95
6.	*trans*	O	"	CH_3	I	14.7	57
7.	*cis*	S	$CONHC_{18}H_{37}$	CH_3	Cl	9.8	61
8.	*trans*	S	"	CH_3	Cl	4.3	57
9.	*trans*	S	$CONHC_{16}H_{33}$	CH_3	Cl	9.8	21
10.	*trans*	S	$C_{16}H_{33}$	CH_3	Cl	20.0	52
11.	*trans*	N-Ac	$CONHC_{18}H_{37}$	CH_3	Cl	35.0	29
12.	*trans*	N-COPh	"	CH_3	Cl	104.0	138
13.	*trans*	S	$CONHC_{18}H_{37}$		Cl	34.0	84
14.	*trans*	S	$CONHC_{16}H_{33}$		Cl	26.0	69
15.	*trans*	S	$C_{16}H_{33}$		Cl	99.0	119
CV-6209						7.2	55
Gingkolide B						-	418

Table 2. Effect of PAF Antagonists on PAF-induced Bronchoconstriction in Guinea pigs.

Compound	plt aggregation IC_{50} (nM)	dose (mg/Kg)	absolute BC (%)	inhibition rate of BC (%)[a]
Control			92± 2.3	
CV6209	55	0.01	12± 6.2	87
1	56	0.01	12± 3.5	87
3	57	0.01	7 ± 3.2	92
9	21	0.01	7 ± 3.2	92
11	29	0.01	5 ± 0.9	95

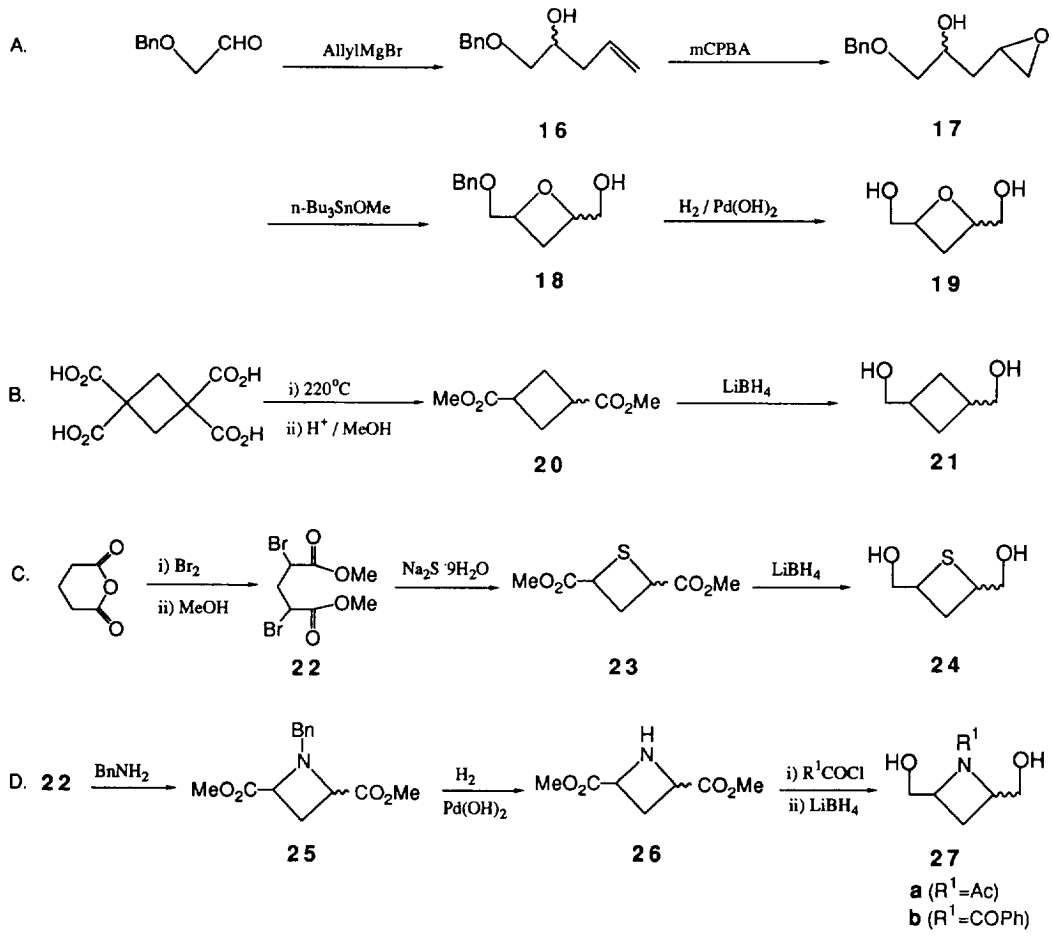
a. Inhibition rate (%) = (1-B/A) x 100; where A is the average bronchoconstriction in the group of untreated animals, and B is the average bronchoconstriction in the group of animal treated with the test compound.

27 (Scheme 1). The α-benzyloxyacetaldehyde, readily prepared from glycerol, was treated with allymagnesium bromide to give **16**. Epoxidation of **16** provided **17**, which was successively treated with i) tri-n-butyltin methoxide at 120-220° C,[15] and ii) H_2 over palladium hydroxide to provide the desired oxetane derivative (**19**) as a geometric mixture in moderate overall yield (Scheme 1A). The preparation of 1,3-bis(hydroxymethyl)cyclobutane (**21**) was accomplished from cyclobutane tetracarboxylate by a high temperature decarboxylation at 220 °C, followed by esterification with methanolic H_2SO_4 to give a *cis* and *trans* mixture of methyl 1,3-cyclobutanedicarboxylate (**20**), which was then reduced with $LiBH_4$ to give **21** (Scheme 1B). The required 2,4-bis(hydroxymethyl)-thietane (**24**) was prepared as shown in Scheme 1C. A mixture of glutaric anhydride and Br_2 was heated at 100° C, and then with methanol at room temperature to give the dibromo diester (**22**) in good yield. The reaction of **22** with sodium sulfide in methanol at -30° C gave in moderate yield the cyclized product (**23**). The *cis* and *trans* isomers were separated at this stage and were individually reduced with $LiBH_4$ to the bis(hydroxymethyl)thietane (**24**).[16] On the other hand, the bis(hydroxymethyl)azetidine derivatives (**27**) were prepared according to Scheme 1D. A solution of the dibromo diester (**22**) and benzylamine in acetonitrile was heated at 80° C to give in good yield a *cis* and *trans* mixture of azetidine dicarboxylate (**25**), which were separated on silica gel. The individual isomer of **25** was reductively treated to give **26**, which was then acylated and reduced to provide the required diol (**27**). The necessary structural elaborations from diols(**19**, **21**, **24**, and **27**) to the target compounds(**1-15**) are shown in Scheme 2. The diol **19** was monoalkylated or monocarbamoylated in good yield to compound **28** by reacting with NaH and alkyl bromide, or alkylisocyanate. The remaining alcohol functionality of **28** was activated by reacting with phenyl chloroformate, and the carbonate product was reacted with 2-picolylamine at reflux to give compound **29** in excellent yield. At this stage, the separation of the *cis* and *trans* isomers could be effected by silica gel chromatography. Each individual isomer of compound **29** was successively treated with acetic anhydride (or ortho-methoxybenzoyl chloride) in pyridine, and ethyl iodide at 80° C to yield the target compound. [17]

The *in vitro* PAF antagonisms of the compound **1-15** 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. [18, 19] The values are listed in Table 1. It is evident 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. From the *in vitro* activity data in Table 1, a number of trends are noteworthy. 1) The present series of compounds are similar to or slightly more potent than the 3,3-bis(hydroxymethy)- series in their in vitro activities.[14] 2) The nature of the X moiety (CH_2, O, S) in the core structure does not appear to have a dramatic effect except that the nitrogen compounds do have somewhat lower activities. 3) The *trans* isomers consistently show activities about twice as potent as the *cis* isomers. 4) The carbamate linkage between the lipophile and the core (R^1 substituents) generally shows better activities than the ether linkage. 5) The compounds with R^2=methyl display higher activities than the ones with R^2=aryl. 6) The nature of the counter ion, i.e. Y^- appears to have some minor effect on the activities.

Several compounds have been selected on the basis of their *in vitro* activities and structural type, and their *in vivo* activities studied. The data in Table 2 show that these compounds are powerful and selective antagonists inhibiting the PAF-induced bronchoconstriction, but not the antigen-induced bronchoconstriction (data not shown). The effects of the antagonists on the PAF-induced hypotension, the

Scheme 1



Scheme 2

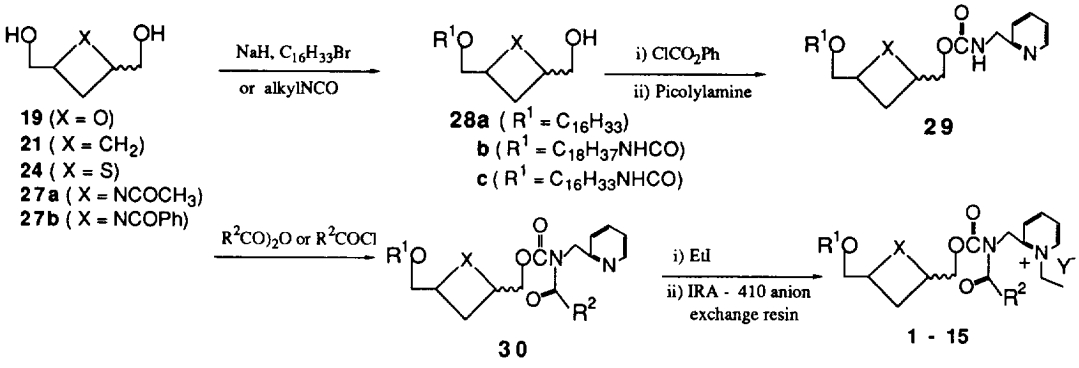


Table 3. Effect of PAF antagonists on the PAF-induced hypotension in SD rats

Compounds	ED50 i.p. (mg/kg)	AUC 100(mg/kg)
1	0.58	1.5
3	0.28	0.7
9	0.16	0.5
11	0.33	0.6
CV-6209	0.35	1.0

ED50 (dose producing 50% inhibition) and AUC100 (dose required to maintain 100% inhibition for 1h) were determined by linear regression analysis. PAF was given by the iv route 10 min after the antagonist administration.

Table 4. Effect of PAF antagonists on the endotoxin-induced hemoconcentration in ICR mice

compounds	Change of hemoconcentration (% change)
Endotoxin	24.0 ± 11.8(100)
1 + E	5.9 ± 5.0 (25)
3 + E	12.7 ± 10.4 (53)
9 + E	12.7 ± 5.9 (53)
11+ E	13.1 ± 10.7 (55)
CV-6209	11.5 ± 8.0 (48)

Overnight-fasted conscious mice were dosed i.p. with each antagonist (1 mg/Kg) 10 min before the endotoxin administration given by tail vein injection (55 mg/kg dose which is equivalent to LD_{80} value). The post-retroorbital blood sample from the endotoxin-treated mice (10 mice per test compound) was obtained 30 min after the endotoxin administration. Hemoconcentration was assessed by centrifugation followed by measurement.

Table 5. Effect of PAF antagonists (0.3 mg/kg) on the survival of ICR mice(15 mice per experiment) exposed to endotoxin (55 mg/kg, ip) over 66 hr period.

Compounds	Time(hrs)						
	1	2	18	24	42	48	66
Control	90	85	60	50	20	10	5
1	100	100	80	73	40	33	27
3	100	100	93	73	53	53	47
9	100	100	80	73	60	60	60
11	100	100	73	73	53	53	53
CV-6209	100	100	93	87	60	47	47

endotoxin-induced hemoconcentration, and the survival ratio of ICR mice exposed to endotoxin are shown in Table 3-5, respectively. From these data, it may be concluded that most of these compounds, especially **9**, are powerful and selective PAF antagonists effective *in vitro* and *in vivo*, and deserve to be seriously evaluated as potential drugs against endotoxic shock syndrome. Molecular modeling studies probing the pharmacophoric pattern of these antagonist will be reported in due course.

ACKNOWLEDGEMENT. We wish to thank Dr. P. U. Park (Sunkyong Industry) for the collaborations involving the *in vitro* (IC_{50}) and the bronchoconstriction bioassays, and Professor N. Kim (Pharmacy School, Seoul National University) for the *in vivo* animal studies.

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