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CMI-206: A POTENT DUAL PLATELET ACTIVATING FACTOR ANTAGONIST AND 5-LIPOXYGENASE INHIBITOR

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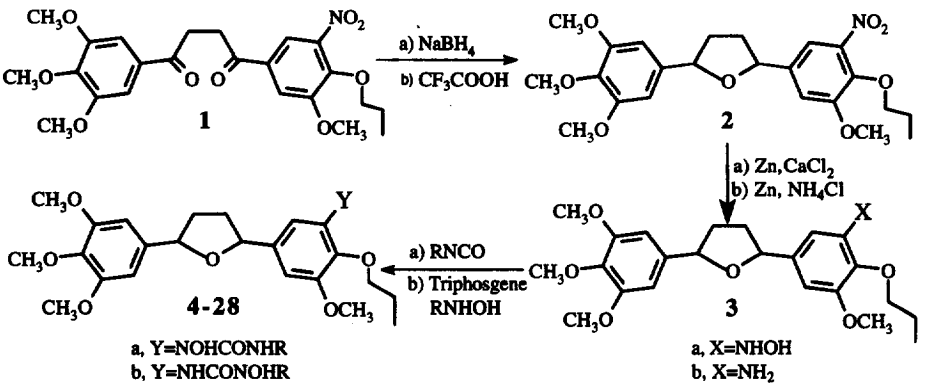
Abstract: CMI-206, *trans*-2[3-methoxy-4-propoxy-5(N'-butyl-N'-hydroxyureidyl)phenyl]-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran, was synthesized and was found to antagonize PAF receptor binding and inhibit 5-lipoxygenase activity both *in vitro* and *in vivo*.

Introduction: Platelet activating factor (PAF) is a potent inflammatory phospholipid mediator found in a wide variety of cells.[1] Its involvement in many human diseases has stimulated a substantial research effort to identify its receptor antagonists.[2] Arachidonic acid (AA) is oxidized by the enzyme 5-lipoxygenase (5-LO) to a variety of leukotrienes.[3] They are, like PAF, potent local mediators, playing a major role in inflammatory and allergic responses.

Since both PAF and leukotrienes are released simultaneously from leukocytes and upon cellular activation, act synergistically in many biological models, a single compound which effectively inhibits the actions of both PAF and leukotrienes may offer certain therapeutic advantages in terms of efficacy and pharmacodynamics over agents which inhibit either mediator alone.[5] The 2,5-diaryl tetrahydrofuran class of compounds are extensively studied PAF receptor antagonists.[6] On the other hand, hydroxamic acids and hydroxyureas are the most potent 5-lipoxygenase inhibitors known.[7] This article describes the design, synthesis and biological activity of a series of substituted diaryl tetrahydrofurans where one of the aromatic hydrogens has been replaced by hydroxyureidyl moiety to determine if these types of compounds show dual activities.[8,9]

Synthesis: A general synthetic route is shown in **Scheme 1**. The 1,4-diketone (**1**) was synthesized in five steps from 3,4,5-trimethoxyacetophenone and 5-nitrovanillin in 42% yield following the procedure of Biftu et al.[10] Reduction of the diketone (**1**) with $NaBH_4$ in CH_3OH and THF gave the 1,4-diol which was cyclized with 5% TFA in $CHCl_3$ at 0°C to give an equilibrium mixture of cis and trans isomers of 2,5-diaryl tetrahydrofuran (**2**) in 70% yield. The geometrical isomers (**2**) (almost 1:1) were separated by column chromatography using silica gel with hexane-ethyl acetate as the eluant. Reduction of the nitro group of (**2**) with Zn and $CaCl_2$ in EtOH-H_2O gave the aniline (**3b**).[11] Alternatively, reduction with Zn and NH_4Cl in THF-H_2O gave the corresponding hydroxylamine (**3a**).[12] The aniline (**3b**) was treated with the corresponding hydroxylamine, triphosgene and triethylamine to give the normal hydroxyurea (Where the hydroxyl group is at the nitrogen bearing the small alkyl group, compounds **4-23, Table 1**).[13]

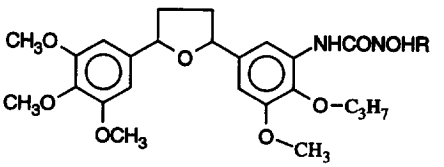
Scheme 1



The hydroxylamine (**3a**) was treated with commercial isocyanates at room temperature to give the reverse hydroxyurea (where the hydroxyl group is at the nitrogen bearing the aryl group, compounds **27-28, Table 2**) in almost quantitative yield. Compounds (**24-26, Table 2**) were prepared from the aniline (**3b**) by treating with acetic anhydride and reducing the resulting amide with LAH in boiling THF, followed by addition of hydroxylamine and triphosgene.

Results and Discussion: Merck's PAF antagonist, MK 287 has been studied quite extensively for its *i.v.* and oral activity.[14] We thought it would be relatively easy to introduce dual activity in this type of molecule by replacing the sulfonyl group with a variety of substituted hydroxyureidyl groups as shown in **Table 1** and **2**. For anti-PAF activity the trans isomer was always more potent, however, isomer preference has not been been determined for 5-LO inhibition. Further introduction of hydroxyurea might change the conformation of the molecule to give anti-PAF activity irrespective of the geometry of the isomer. So both the cis and trans isomers of the diaryl tetrahydrofuran type of compounds (**Table 1**) were synthesized. Initial attempts were made to find the optimum R (among short chain aliphatic and aromatic hydrocarbons) group of the ureidyl group. All biological assays (both *in vitro* and *in vivo*) were done as if there was no interaction between 5-LO inhibition and PAF antagonism. *In vitro* dual activities were determined by a PAF receptor binding assay[10] and a 5-LO inhibition assay using rat basophilic leukemiac (RBL) cell extracts.[7] There is practically no difference between cis and trans isomers in inhibiting 5-LO within the same R group. In contrast, there is about ten fold difference between cis and trans isomers in antagonizing PAF. Compounds (both cis and trans isomers) containing a medium size alkyl R are the most active in both RBL and PAF (compound **5-10, Table 1**) assays. After optimizing R (**Table 1**) attention was given to the R_1 function (**Table 2**), which was changed from hydrogen

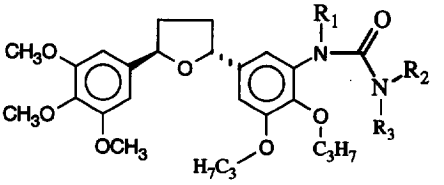
Table 1. *In vitro* Biological Properties of Diaryl tetrtahydrofuran



No.	R	isomer	[a]5-LO	[b]PAF Recep
			IC_{50} (μM)	IC_{50} (nM)
4	H	trans	NT	30
5	Me	trans	0.63	17
6	Et	trans	0.15	8
7	*n*-Pr	trans	10.00	NT
8	*i*-Pr	trans	3.00	58
9	*n*-Bu	trans	0.42	33
10	*sec*-Bu	trans	0.60	25
11	*tert*-Bu	trans	2.90	26
12	cyclohexyl	trans	NT	278
13	Bn	trans	2.24	423
14	H	cis	NT	300
15	Me	cis	NT	514
16	Et	cis	3.00	383
17	*n*-Pr	cis	10.00	NT
18	*i*-Pr	cis	2.83	858
19	*n*-Bu	cis	0.50	1313
20	*sec*-Bu	cis	0.45	1606
21	*tert*-Bu	cis	NT	456
22	cyclohexyl	cis	2.54	1000
23	Bn	cis	8.00	585
[c]Zileuton			1.25	
[d]MK-287				10

[a]The 5-LO inhibitory activity was measured by the conversion of ^{14}C-AA to leukotrienes and other biologically active molecules using thin layer chromatography in RBL-2H3 cells; [b]PAF receptor antagonistic activity was determined by the inhibition of ^{3}H-PAF specific receptor binding to human platelet membranes. The IC_{50} value was defined as the concentration of the inhibitor to obtain 50% inhibition.[c]obtained from Abbott Research laboratories, our in house data. [d]obtained from Merck Research Laboratories, our in house data. NT, not tested.

Table 2. *In vitro* Biological Properties of Diaryl Tetrahydrofuran



No.	R_1	R_2	R_3	5-LO IC_{50} (μM)	PAF Recep IC_{50} (nM)
24	Et	OH	Me	3.07	68
25	Et	OH	Et	1.34	17
26	Et	OH	*n*-Pr	NT	56
27	OH	H	Et	NT	59
28	OH	H	*n*-Pr	3.00	91
Zileuton				1.25	
MK-287					10

NT, not tested

to either ethyl or hydroxyl (compounds **24-28, Table 2**), but that didn't improve activity. When we determined that some of these compounds exhibit dual activity, further biological (*in vitro* and *in vivo*) characterization (human whole blood LTB_4[7] and PAF-induced hemoconcentration, PAF

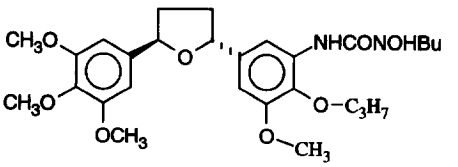
Table 3. Biological Data of Selected Compounds.

No.	[a]HWB(% inhibition) at 3 μM	[b]PAF HTc (% inhibition) 3 mg/kg
6	23	58
9	64	31
10	57	NT
MK-287		97
Zileuton	75	

[a]This assay was done by measuring the production of LTB_4 in A23187-activated human whole blood by the enzyme immunoassay. [b]PAF HTc in mice was evaluated by giving test compounds i.v. 15 minutes prior to PAF challenge. Hemoconcentration was calculated from plasma volume differences between treated and untreated animals. NT, not tested.

HTc in mice[15]) was performed on selected compounds (**Table 3**). Both compounds **6** and **9** (**Table 3**) reasonably antagonize PAF in the PAF HTc assay.Only compound **9** (**Table 1**,CMI-206) was extensively studied in both *in vivo* PAF antagonism and 5-LO inhibition assays. The results are presented in **Table 4**. These initial data are encouraging and warrant further biological, pharmacological and toxicological studies. More relevant data will be published in the near future.We have shown that it is possible to design and synthesize a small, low molecular weight compound that simultaneously antagonizes PAF and inhibits leukotriene biosynthesis.

Table 4. Biological Data of CMI-206



RBL IC_{50} (nM)	HWB IC_{50} (μM)	[a]ex vivo LTB_4 % inh 5 mg/kg	PAF recep IC_{50} (nM)	PAF HTc %inh 3 mg/kg	[b]AA Edema %inh 3 mg/kg
424	1.68±0.05	67±3	33	31±1.4	60±0.79

[a]Anti-coagulated blood samples were collected after intravenous administration of test compounds from female CD rats, the whole blood was stimulated with A23187 and the concentrations of LTB_4 was determined by an enzyme immunoassay.[16]
[b]AA-induced ear edema in mice was determined by topically applying AA to the ears of mice. Mice were sacrificed 1 hour later, and the ear punch biopsies were weighed. Edema was calculated from the weight differences between treated and untreated ears.[17] The test compounds were given i.v. 15 minutes prior to AA.

References:

(1) Page, C. P.; Asthma: Basic Mechanisms and Clinical Management; Academic Press Ltd.: London, 1988, 283-304.

(2) Hwang, S-B. Immunopharmacology of lipid mediators., Cuningham, F. Ed. Academic press Ltd.: London, 1994, 297-360.

(3) Samuelsson, B. *Science* **1983**, *220*, 568.

(4) Ford-Hutchinson, A. W. *Fed Proc.* **1985**, *44*, 25.

(5) (a) Shen, T.Y.; Hwang, S.-B.; Doebber, T.W; Robbins, J. C. Platele Activating Factor and Related Lipid Mediators, Plenum Publishers, New York, 1987, 153-190. (b) O'Donnel, M.; Welton, A. Therapeutic Approaches to Inflammatory Diseases. Lewis. A. J.; Doherty, N. S.; Ackerman, N. R. Eds. Elsevoir: New York, 1989, 147-193. (c) Shen, T. Y. Lipids, **1991**, *26*, 1154. (d) Goldstein, D. M.; Shen, T. Y. *Med. Chem. Res.* **1992**, *2* , 443, ibid, **1992**, *2*, 451. (e) Marfat, A.; Chambers, R. J.; Cheng, J. B.; Damon, D. B., Deleunt, J. C.; Eggler, J. F.; Watson, J. W. 7th International Conference, Inflammation Research Association, White Haven, September 25-29, 1994. (f) McDonald, P. P.; McColl, S. R.; Braquet, P.; Borgreat, P. *Br. J. Pharmacol,* **1994**, *111*, 852. (g) Demonchaux, P.; Lenoir, P.; Augert, G.; Dupassieux, P. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2383.

(6) Thompson, A. S.; Tschaen, D. M.; Simpson, P.; Mcswine, D. J.; Reamer, R. A.; Verhoeven, T. R.; Shinkai, I. *J. Org. Chem.* **1992**, *57*, 7044.

(7) a) Carter, G. W.; Young, P. R.; Albert D. H.; Bouska, J.; Bell, R. L.; Summers, J. R.; Brooks, D. W. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 929. b) Jackson, W. P.; Islip, P. J.; Kneen, G.; Pugh, A.; Wates, P. J. *J. Med. Chem.* **1988**, *31*, 499.

(8) Cai, X.; Hussoin, M. S.; Killian, D. B.; Scannell, R.; Yaeger, D.; Eckman, J.; Hwang, S-B.; Garahan, L-L.; Qian, C.; Yeh, G.; Ip, S. 207th American Chemical Society Meeting, San Diego, CA, March 13-17, 1994: MEDI 123

(9) Hussoin, M. S.; Cai, X.; Scannell, R.; Yaeger, D.; Killian, D. B.; Eckman, J.; Hwang, S. B.; Garahan, L-L.; Qian, C.; Yeh, G.; Ip, S. 208 th American Chemical Society Meeting, Washington, DC, August 21-25, 1994: MEDI 126.

(10) Girotra, N.N.; Biftu, T.; Ponpipom, M.M.; Acton, J.J.; Alberts, A. W.; Bach, T.N.; Ball, R.G.; Bugianeasi, R.L.; Parsons, W.H.; Chabala, J.C.; Davies, P.; Doebber, T.W.; Doherty, J.; Graham, D.W.; Hwang, S.-B.; Kuo, C.H.; Lam, M.-H.; Luell, S.; MacIntyre, D.E.; Meurer, R.; Roberts, C.D.; Sahoo, S.P.; Wu, M.S.; *J. Med. Chem.* **1992**, *35*, 3474.

(11) Kamm, O.; Marvel, C. S. *Org. Syn. Coll. 1*, **1941**, 445.

(12) Kuhn, W. E.; Fieser, L. F.; Walker, J. T., *Org. Syn. Coll. 2*, **1943**, 447.

(13) Eckert, H.; Forster, B. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 894.

(14) Sahoo, S. P.; Graham, D. W.; Acton, J. J.; Biftu, T.; Bugianeasi, 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.; *Bioor. Med. Chem. Lett.* **1991**, *1*, 327.

(15) Inarrea, P.; Alonso, F.; Sanchez-Crespo, M. *Immunopharmacology*, **1983**, *6*, 7.

(16) Young, J. M.; Wagner, B. M.; Spires, D. A. *J. Invest. Dermatol,* **1983**, *80*, 48.

(17) Opas, E. E.; Bonney, R. J.; Humes, J. L. *J. Invest. Dermatol.* **1985**, *84*, 253.

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