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Novel Agents Combining Platelet Activating Factor (PAF) Receptor Antagonist with Thromboxane Synthase Inhibitor (TxSI)

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Abstract—Compounds **1** or **2** which possess dual-acting PAF antagonist/TxSI in a previous paper were modified and evaluated for the dual-acting activity. It was found that several compounds were potent dual-acting PAF antagonist/TxSI in and ex vivo. 6-(2-Chlorophenyl)-3-[4-[(*E*/*Z*)-6-ethoxycarbonyl-1-(3-pyridyl)-1-hexenyl]phenylmethyl]-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′: 4,5]thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine (**12**) is excellent orally dual-acting PAF antagonist/TxSI.

Platelet activating factor (PAF) is a mediator of inflammation and plays important roles in the pathology of ischemia/thrombosis,[1] septic shock,[2] asthma.[3] Thromboxane A_2 (TxA_2) is a potent vasoconstrictor and platelet aggregating agent, may make an important contribution to the pathogenesis of various circulatory and certain renal disorders.[4–7] Therefore, it has been proposed that novel agents combining PAF antagonist and TxA_2 synthase inhibitor (TxSI) would be more beneficial than either agent alone in the treatment of these disorders. To avoid the complications associated with administration of two separate drugs we have engaged in a program to synthesize agents that possess both the above biological activities in the same molecule.

In a previous paper we have described a series of (*E*/*Z*)-5-[phenyl(3-pyridyl)methyleneaminooxy]pentanoic acid derivatives represented by **1** or **2**, which possess dual-acting PAF antagonist/TxSI has been generated by the approach of linking the known PAF antagonists and TxSIs, that is, the structural hybridization (Fig. 1).[8]

The synthesis of these and related compounds[9] started from the substituted bromobenzene and utilized synthetic sequence previously reported by us.[8] All compounds except for **3**, **7** and **8** in Table 1, and compound **10** in Table 2 were provided by coupling or condensation of the diazepines[10] and the mesylated esters shown in Scheme 1. As shown in Scheme 1, in the case of hydroxyethyl group, the mesylation gave the corresponding ethylene in high yield without the desired mesylate. On the other hand, the mesylated products were easily chlorinated by treatment with brine. Furthermore, interestingly, it appeared that the syntheses of compounds **4** and **5** were accompanied with producing the carbamates.[11] Compound **9** was given using the *N*-bromoacetyl diazepine. Compound **3** was afforded by similar previous methodology using phthalic anhydride.

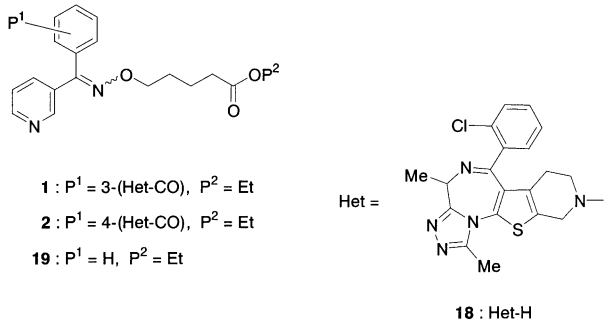

Figure 1.

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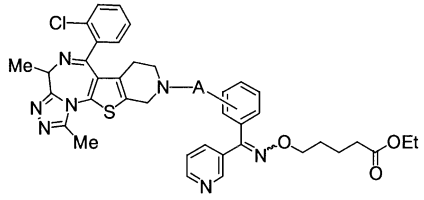
Compounds **7** and **8** were prepared from the diazepines bearing the corresponding ketones synthesized by Wittig olefination, catalytic hydrogenation, and so on of 3-bromobenzaldehyde as shown in Scheme 2. Compounds **11** and **12** in Table 3 were obtained using the corresponding esters by Wittig olefination and esterification as shown in Scheme 3. Compounds **14**–**17** in Table 4 were prepared using the corresponding 5-bromovalerates or amide as shown in Scheme 4.

Our final goal was to develop a dual-acting agent which possesses excellent in and ex vivo activities as a clinically useful drug. According to our previous results, compounds **1** and **2** proved to show potent PAF antagonist activity in orally dosed mouse in vivo test. Therefore, based on this information, chemical modifications of compounds **1** and **2** were started. We initially investigated in vitro SAR concerning the appropriate positions and length for linking the diazepine to the ethyl ester (Table 1). All compounds except for **3** with a substituent at the 2-position of phenyl group showed similar results in both PAF antagonist and TxSI activity. From this result, it was clear that 2-substitution was not desirable. On the other hand, from these results, compounds **1**–**9** indicated more potent PAF antagonist activity than

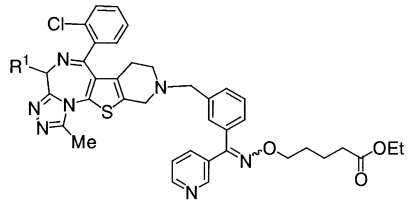
Table 1. In vitro PAF antagonist and TxA_2 synthase inhibitory activities of compounds **1**–**9**, **18** and **19**



Compd	A	Postn	E/Z[a]	IC_{50} (μM)	
				PAF antagonist[b]	TxA_2 synthase[c]
3	CO	2	2:3	0.200	>1.000
1	CO	3	2:3	0.060	0.065
2	CO	4	2:3	0.100	0.072
4	CH_2	3	2:3	0.047	0.059
5	CH_2	4	2:3	0.046	0.080
6	$COCH_2$	4	2:3	0.085	0.069
7	$CO(CH_2)_3$	3	2:3	0.039	0.041
8	$(CH_2)_4$	3	2:3	0.054	0.021
9	$COCH_2OCH_2$	4	2:3	0.060	0.058
18[d]				0.245	>1.000
19[d]			2:3	NT[e]	0.001
(±)-E6123[f]				0.036	>1.000
UK74505[g]				0.029	NT
Ozagrel				NT	0.024
Isbogrel[h]				NT	0.0009

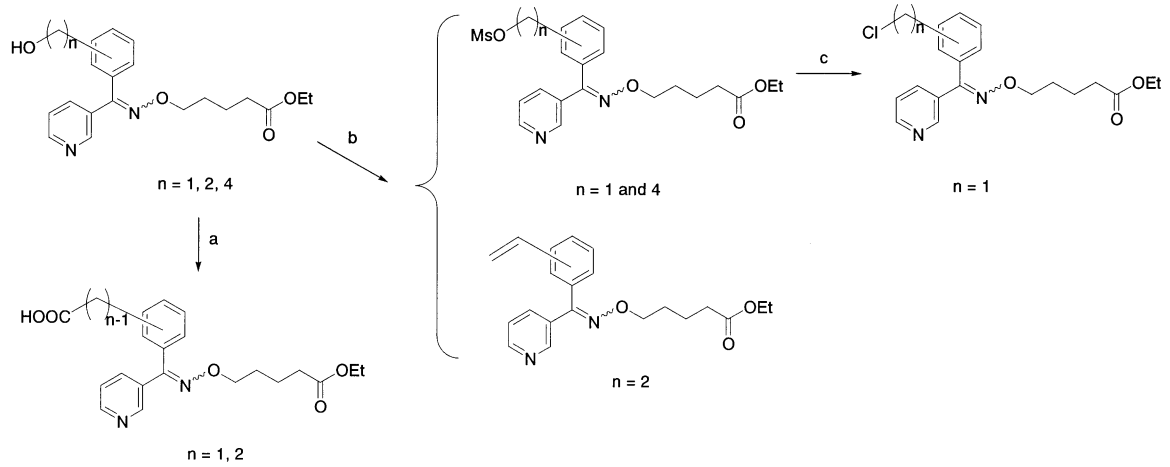
[a]Determined by ^1H NMR spectra.
[b]Inhibition of the PAF-induced platelet aggregation in rabbit platelet rich plasma (PRP). This was performed according to the method of Terashita et al. with slight modification.[13]
[c]Inhibition of TxB_2 production by incubating prostaglandin $H_2(PGH_2)$ with human platelet microsomes. This was performed according to the method of Terashita et al. with slight modification.[12]
[d]See Fig. 1.
[e]NT = not tested.
[f]See ref 10.
[g]See ref 14.
[h]See ref 15.

Table 2. In vitro PAF antagonist and TxA_2 synthase inhibitory activities of compounds **4** and **10**



Compd	R^1	E/Z	IC_{50} (μM)	
			PAF antagonist[a]	TxA_2 synthase[b]
4	Me	2:3	0.047	0.059
10	H	2:3	0.200	0.065

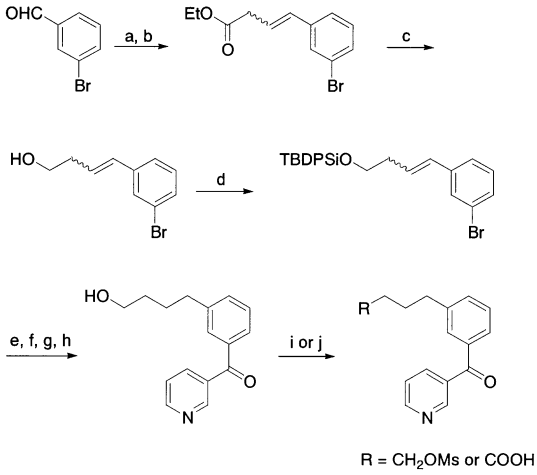
[a]Inhibition of the PAF-induced platelet aggregation in rabbit platelet rich plasma (PRP). This was performed according to the method of Terashita et al. with slight modification.[13]
[b]Inhibition of TxB_2 production by incubating prostaglandin $H_2(PGH_2)$ with human platelet microsomes. This was performed according to the method of Terashita et al. with slight modification.[12]



Scheme 1. Reagents and conditions: (a) ($n=1$) $KMnO_4$, 90% aq acetone, 54–65%; ($n=2$) Jones reagent, −20 °C, 39%; (b) MsCl, Et_3N, CH_2Cl_2; (c) brine, $CHCl_3$, 77% quant.

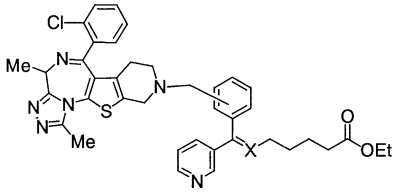
compound **18** without having N-substitution, but weaker TxSI activity than compound **19** without the substitution of phenyl moiety.

Next, we modified a part of the diazepine and the ester, respectively. We considered that a balance of both activities was not kept by a large modification, since the diazepine and the ester have already been optimized, respectively. Thus, replacement of methyl with hydrogen atom indicated weaker PAF antagonist activity, while TxSI activity was retained (Table 2).



Scheme 2. Reagents and conditions: (a) $Br^{-}Ph_3P^{+}(CH_2)_2COOH$, KO^{t}Bu, THF; (b) $SOCl_2$, EtOH, quant. (2 steps); (c) LAH, THF, 75%; (d) TBDPSiCl, imidazole, DMF, quant.; (e) nicotinaldehyde, ^{n}BuLi, THF, −78 °C, 62%; (f) H_2, 10%Pd-C, AcOH, MeOH, 65%; (g) MnO_2, CH_2Cl_2, reflux, 86%; (h) TBAF, THF, quant; (i) MsCl, Et_3N, CH_2Cl_2, quant; (j) $KMnO_4$, H_2O, 60 °C, 55%.

Table 3. In vitro PAF antagonist and TxA_2 synthase inhibitory activities of compounds **4, 5, 11** and **12**

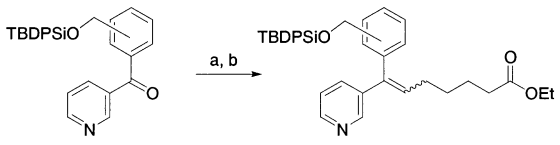


Compd	X	Position	E/Z[a]	IC_{50} (μM)	
				PAF antagonist[b]	TxA_2 synthase[c]
4	NO	3	2:3	0.047	0.059
5	NO	4	2:3	0.046	0.080
11	CH	3	1:1	0.039	0.068
12	CH	4	1:2	0.032	0.072

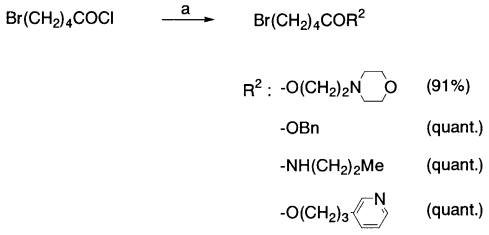
[a]Determined by ^{1}H NMR spectra.
[b]Inhibition of the PAF-induced platelet aggregation in rabbit platelet rich plasma (PRP). This was performed according to the method of Terashita et al. with slight modification.[13]
[c]Inhibition of TxB_2 production by incubating prostaglandin H_2(PGH_2) with human platelet microsomes. This was performed according to the method of Terashita et al. with slight modification.[12]

Tables 3 and 4 summarize the in vitro activity for the modified esters. Changing oxime moiety to methine moiety makes little difference to activity. However, the modification of ethyl group of ester affected activities. Namely, replacement of ethoxy moiety with other substitutions showed weaker PAF antagonist activity, in particular, hydroxyl was remarkable. On the contrary, only hydroxyl displayed more potent TxSI activity.

Table 5 summarizes in vivo PAF antagonist and ex vivo TxA_2 synthase inhibitory activities. All of compounds

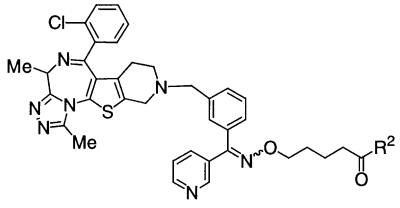


Scheme 3. Reagents and conditions: (a) $Br^{-}Ph_3P^{+}(CH_2)_5COOH$, NaH, ^{t}BuOH, 56–87%; (b) conc.H_2SO_4, EtOH, 91%.



Scheme 4. Reagents and conditions: (a) alcohols or amine, pyridine–CH_2Cl_2.

Table 4. In vitro PAF antagonist and TxA_2 synthase inhibitory activities of compounds **2** and **13–17**



Compd	R^2	E/Z[a]	IC_{50} (μM)	
			PAF antagonist[b]	TxA_2 synthase[c]
4	OEt	2:3	0.047	0.059
13	OH	2:3	0.980	0.024
14	$O(CH_2)_2$-4-Morpholinyl	2:3	0.081	0.151
15	OCH_2Ph	2:3	0.098	0.465
16	$NH(CH_2)_2Me$	2:3	0.071	0.540
17	$O(CH_2)_3$-3-Pyridyl	2:3	0.065	>1.000

[a]Determined by ^{1}H NMR spectra.
[b]Inhibition of the PAF-induced platelet aggregation in rabbit platelet rich plasma (PRP). This was performed according to the method of Terashita et al. with slight modification.[13]
[c]Inhibition of TxB_2 production by incubating prostaglandin H_2(PGH_2) with human platelet microsomes. This was performed according to the method of Terashita et al. with slight modification.[12]

Table 5. In vivo PAF antagonist and ex vivo TxA_2 synthase inhibitory activities of compounds **1**–**19**

Compd	ED_{50} (mg/kg iv)		ED_{50} (mg/kg po)	
	PAF antagonist[a]	TxA_2 synthase[b]	PAF antagonist[a]	TxA_2 synthase[b]
3	2.4	>1.0	>10.0	NT[c]
1	0.47	>1.0	2.3	NT
2	0.2	>1.0	0.5	NT
4	0.2	0.1	>10.0	>10.0
5	1.35	1.0	>10.0	>10.0
6	4.2	>1.0	>10.0	NT
7	1.05	>1.0	>10.0	NT
8	0.38	0.53	>10.0	6.9
9	4.2	>1.0	>10.0	NT
10	0.8	0.1	>10.0	>10.0
11	0.60	0.71	>10.0	3.2
12	0.55	1.0	1.6	5.2
13	0.47	1.0	>10.0	10.0
14	0.54	0.77	>10.0	8.9
15	0.54	>1.0	>10.0	NT
16	0.54	>1.0	>10.0	NT
17	0.54	>1.0	>10.0	NT
18	>5.0	NT	NT	NT
19	NT	0.01	NT	>0.1
(±)-E6123	0.02	NT	0.025	NT
UK74505	0.1	NT	1.55	NT
Ozagrel	NT	0.02	NT	0.1
Isbogrel	NT	0.01	NT	0.05

[a]Activity in vivo was demonstrated by the ability to protect mice from the lethal effects of an injection of PAF. The ED_{50} values represent the dose reduced mortality by 50%. This was performed according to the method of Cooper et al. with slight modification.[14]
[b]Inhibition of serum TxB_2 production in the rats. This was performed according to the method of Terashita et al. with slight modification.[12]
[c]NT = not tested.

1–**19** were tested in and ex vivo after intravenous administration. PAF antagonist activity was assessed in the mouse, using a PAF-induced death assay.[14] As the results, the ED_{50} values for all compounds except for **18** and **19** were in the range 0.2–4.2 mg/kg. On the other hand, TxSI activity was assessed by ex vivo inhibition of serum TxB_2 production in the rat.[12] As the results, the ED_{50} values for compounds **4**–**14** except for **6**, **7** and **9** were in the range 0.1–1.0 mg/kg, and compound **19** was 0.01 mg/kg.

Furthermore, these representative compounds were tested in or ex vivo after oral administration by the same methodology as after intravenous administration. As the results, compound **12** showed 1.6 and 5.2 mg/kg in both PAF antagonist and TxSI activity, respectively.

In conclusion, we have found that several compounds indicate good dual PAF antagonist/TxSI activity by intravenous dosing, and that a compound (**12**) indicate excellent dual activity by oral dosing. These compounds appeared to be not parted in the diazepine and the ester in vivo. Further progress in this work will be the subject of future reports.

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