

## 2-(3-Pyridyl)thiazolidine-4-carboxamides. 1. Novel Orally Active Antagonists of Platelet-Activating Factor (PAF)<sup>1)</sup>

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In a search for novel platelet-activating factor (PAF) antagonists, we found that 1-(3-phenylpropyl)-4-[2-(3-pyridyl)thiazolidine-4-carbonyl]piperazine (3x) showed *in vitro* and *in vivo* PAF-antagonistic activities. Introduction of functional groups at the benzylic methylene carbon of 3x afforded some compounds with more potent PAF-antagonistic activity than 3x. Among them 1-(3-methyl-3-phenylbutyl)-4-[2-(3-pyridyl)thiazolidine-4-carbonyl]-piperazine fumarate (3al, YM264) was found to be one of the most potent PAF antagonists.

**Key words** [2-(3-pyridyl)thiazolidine-4-yl]carboxamide; PAF antagonist; structure-activity relationship; YM264

Platelet activating factor (PAF) is an ether-linked phospholipid mediator, which is released from a variety of cell types including neutrophils, eosinophils, macrophages, platelets and endothelial cells.<sup>3)</sup> PAF has a wide spectrum of biological actions, such as induction of platelet aggregation, increase in vascular permeability, bronchoconstriction, systemic hypotension, and cardiac and renal dysfunction.<sup>4-8)</sup> Because of its various actions, PAF has been suggested to play an important role in the pathogenesis of asthma, shock, glomerulonephritis and other diseases.<sup>9)</sup> The development of PAF antagonists therefore may offer a novel approach to the prevention and therapy of these diseases. For this reason, a search for novel PAF antagonists is under way and several compounds including PAF analogues (CV-3988, CV-6209), thienodiazepines (WEB-2086, Y-24180, E-6123) and other compounds have already been reported.<sup>10)</sup> Some of them are under clinical trial.

In a search for potent and orally active PAF antagonists, we tried a chemical file screening approach and found that 2-(3-pyridyl)-2,3-dihydrobenzothiazole (**1**) is almost equipotent to RP48740 (**2**),<sup>11)</sup> a PAF antagonist, in inhibiting PAF-induced platelet aggregation (IC<sub>50</sub> = 15 μM). We were interested in the similarity between **1** and **2** in chemical structure and prepared a variety of aryl-substituted heterocyclic compounds, which we tested for PAF-antagonistic activity. Among them, pyridyl thiazolidinecarboxamide (**3a**) was found to be more potent than **1**. Then we focused our attention on pyridyl thiazolidinecarboxamides **3** and prepared a number of their derivatives for activity testing. In this paper, we describe the synthesis and structure-activity relationship of a novel series of pyridyl thiazolidinecarboxamides as PAF antagonists.

### Synthesis

The 3-pyridylthiazolidinecarboxamides (**3**) were prepared as described below (Chart 2). The 3-pyridylthiazolidinecarboxylic acid (**4**), prepared from 3-pyridylaldehyde and L-cysteine, was treated with various amines in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) (method A), or diphenylphosphoryl azide (DPPA) (method B) to give the amides (**3**). In some cases,

**4** was converted to *tert*-butylcarbamate (**5**) and then coupled with amines to give **6**. Finally, **6** was treated with trifluoroacetic acid (TFA) to furnish the amide (**3**) (method C). Compound **3x** could also be prepared by the coupling of anhydride (**7**) and an amine (method D). The anhydride (**7**) was easily prepared as stable crystals from **5** by the treatment with (COCl)<sub>2</sub> and Et<sub>3</sub>N.

It is well-known that 2,4-disubstituted thiazolidine isomerizes freely between the *cis* and *trans* diastereomers in solution and exists as equilibrium mixture.<sup>12)</sup> NMR spectra revealed that all of the compounds prepared here exist as an about 1 to 1 mixture of *cis* and *trans* isomers in solution. Configurations at C-4 are considered to be *R* because the compounds were derived from L-cysteine.

### Pharmacological Activity and Discussion

The compounds were tested for PAF-antagonistic activity by measuring the inhibition of PAF-induced platelet aggregation using rabbit platelet-rich plasma (PRP).

Compound **3a** antagonized PAF-induced platelet aggregation with an IC<sub>50</sub> of 1.5 μM. Deletion of the alkylsulfide group (**3b**) reduced the activity, while deletion of the ester group (**3c**) retained the activity. Simple alkyl amides (**3d**, **3e**) retained the potency. Anilide (**3f**) and benzylamide (**3g**) derivatives also exhibited PAF-antagonist activity and the introduction of a methoxy group (**3h**) in the phenyl ring of **3g** improved the activity. The introduction of hydrophobic groups such as phenylalkoxy (**3i-m**) and branched alkoxy (**3n**) groups in the phenyl ring of **3g** caused further improvement of the antagonistic

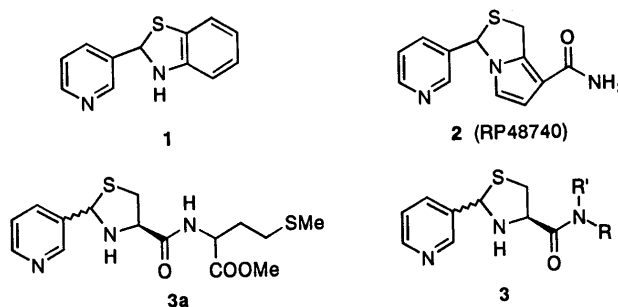
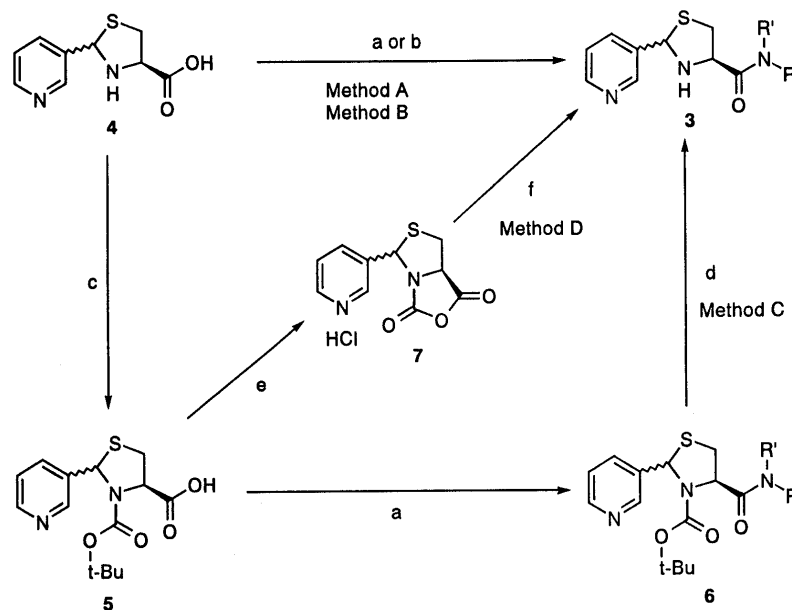


Chart 1

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(a) RNH<sub>2</sub>, DCC, HOBT / THF or DMF; (b) RNH<sub>2</sub>, DPPA, Et<sub>3</sub>N / DMF;  
 (c) (t-BuOCO)<sub>2</sub>O, NaOH / dioxane, H<sub>2</sub>O; (d) TFA; (e) (COCl)<sub>2</sub>, DMF, CH<sub>2</sub>Cl<sub>2</sub>;  
 (f) RNH<sub>2</sub>, DMSO

Chart 2

Table 1. Physicochemical Data and PAF-antagonist Activities for Thiazolidinecarboxamides

Compd. No.	R	R'	Salt	Method	mp (°C)	Formula	Analysis (%)				Platelet aggregation inhibition IC <sub>50</sub> (μM)
							Calcd (Found)				
							C	H	N	S	
3a	CH(COOCH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub> SCH <sub>3</sub>	H	—	C	Oil	C <sub>15</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	50.68 (50.39)	5.95 (5.93)	11.82 (11.60)		1.5
3b	CH <sub>2</sub> COOCH <sub>3</sub>	H	—	C	Oil	C <sub>12</sub> H <sub>15</sub> N <sub>3</sub> O <sub>3</sub> S	50.27 (50.18)	5.48 (5.36)	14.65 (14.31)	11.19	14
3c	(CH <sub>2</sub> ) <sub>3</sub> SCH <sub>3</sub>	H	—	C	Oil	C <sub>13</sub> H <sub>21</sub> Cl <sub>2</sub> N <sub>3</sub> OS <sub>2</sub>	52.55 (52.49)	6.26 (6.44)	14.11 (14.11)	21.62 (21.56)	1.6
3d	(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>	H	—	C	79	C <sub>16</sub> H <sub>25</sub> N <sub>3</sub> OS	62.50 (62.50)	8.20 (8.22)	13.67 (13.56)	10.43 (10.23)	1.5
3e	(CH <sub>2</sub> ) <sub>9</sub> CH <sub>3</sub>	H	—	A	88	C <sub>19</sub> H <sub>31</sub> N <sub>3</sub> OS	65.48 (65.16)	8.68 (8.80)	12.06 (11.91)	9.20 (9.04)	3.9
3f	C <sub>6</sub> H <sub>5</sub>	H	—	A	Oil	C <sub>15</sub> H <sub>15</sub> N <sub>3</sub> OS ·0.2H <sub>2</sub> O	62.35 (62.74)	5.37 (7.30)	14.54 (14.52)	11.10 (11.25)	2.5
3g	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	—	A	Oil	C <sub>16</sub> H <sub>17</sub> N <sub>3</sub> OS ·0.2H <sub>2</sub> O	63.42 (63.55)	5.79 (5.83)	13.87 (13.85)	10.58 (10.34)	3.7
3h	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> -4-(OCH <sub>3</sub> )	H	—	A	103	C <sub>17</sub> H <sub>19</sub> N <sub>3</sub> O <sub>2</sub> S	61.98 (61.99)	5.81 (5.81)	12.76 (12.67)	9.73 (9.94)	0.90
3i	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> -4-[O(CH <sub>2</sub> ) <sub>4</sub> C <sub>6</sub> H <sub>5</sub> ]	H	2HCl	A	135	C <sub>26</sub> H <sub>29</sub> N <sub>3</sub> O <sub>2</sub> S ·0.2H <sub>2</sub> O·2HCl	59.58 (59.58)	6.04 (6.02)	8.02 (7.96)	6.12 (6.23)	0.43
3j	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> -4-(OCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> )	H	—	B	140	C <sub>23</sub> H <sub>23</sub> N <sub>3</sub> O <sub>2</sub> S	68.12 (68.23)	5.72 (6.74)	10.36 (10.31)	7.91 (8.01)	0.38
3k	CH <sub>2</sub> C <sub>6</sub> H <sub>6</sub> -4-[O(CH <sub>2</sub> ) <sub>2</sub> C <sub>6</sub> H <sub>5</sub> ]	H	—	B	116	C <sub>24</sub> H <sub>25</sub> N <sub>3</sub> O <sub>2</sub> S	68.71 (68.56)	6.01 (6.01)	10.02 (9.94)	7.64 (7.63)	0.15
3l	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> -4-[O(CH <sub>2</sub> ) <sub>3</sub> C <sub>6</sub> H <sub>5</sub> ]	H	2HCl	A	116	C <sub>25</sub> H <sub>27</sub> N <sub>3</sub> O <sub>2</sub> S ·2HCl	59.28 (58.95)	5.77 (5.74)	8.30 (8.21)	6.33 (6.36)	0.39
3m	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> -4-[O(CH <sub>2</sub> ) <sub>5</sub> C <sub>6</sub> H <sub>5</sub> ]	H	—	B	83	C <sub>27</sub> H <sub>31</sub> N <sub>3</sub> O <sub>2</sub> S	70.25 (70.33)	6.77 (6.81)	9.10 (9.06)	6.95 (6.84)	0.58
3n	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> -4-[O(CH <sub>2</sub> ) <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> ]	H	2HCl	A	128	C <sub>21</sub> H <sub>27</sub> N <sub>3</sub> O <sub>2</sub> S ·2HCl·0.4H <sub>2</sub> O	54.17 (54.23)	6.45 (6.37)	9.02 (8.96)	6.89 (7.00)	0.24
3o	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	Me	2HCl	A	110	C <sub>17</sub> H <sub>19</sub> N <sub>3</sub> OS ·2HCl·H <sub>2</sub> O	50.50 (50.63)	5.73 (5.60)	10.39 (10.43)	7.93 (7.98)	0.79
WEB-2086											0.12

activity. The tertiary amide (**3o**) was about four times more potent than the corresponding secondary amide (**3g**). From these results, it was revealed that hydrophobic groups were favorable as *N*-substituents for PAF-antagonistic activity (Table 1).

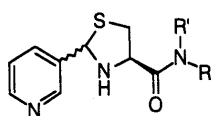
Based on these results, we focused on cyclic amine amides as a hydrophobic moiety and tertiary amides. The results are shown in Table 2. Pyrrolidine (**3p**) and morpholine (**3r**) amides showed weaker activity than **3a**. Piperidine (**3q**) and 4-carboethoxypiperazine (**3s**) amides retained the activity. Methylpiperazine (**3t**) showed reduced activity while phenyl piperazine (**3u**) was almost as potent as **3s**. Furthermore, the introduction of more hydrophobic groups such as phenylalkyl (**3v—3z**) and long chain alkyl (**3aa—3ae**) groups at the 4-position of the

piperazine ring of **3u** improved the antagonistic activity. Among these compounds, **3x**, **3z**, **3ac** and **3ad** exhibited strong PAF-antagonist activities. On the other hand, 4-substituted piperidides (**3af**, **3ag**) were much less potent than the corresponding piperazines (**3w**, **3x**) and the *S*-isomer of **3x** (*S*-**3x**: prepared from D-cysteine) showed less activity (70% inhibition at 100  $\mu$ M). The piperidyl amide (**3ah**) also showed weak antagonistic activity.

Based on these results, benzyl amides (**3l**, **3n**) and piperazyl amides (**3x**, **3z**, **3ac**, **3ad**) were selected for further evaluation. The results are shown in Table 3.

In the *in vitro* study, most of the compounds inhibited PAF-induced aggregation of human platelets. It is interesting that **3z** was ten times less potent than **3x** in human platelets, though they were almost equipotent in rabbit

Table 2. Physicochemical Data and PAF-Antagonist Activities for Thiazolidinecarboxamides



Compd. No.	NRR'	Salt	Method	mp (°C)	Formula	Analysis (%)				Platelet aggregation inhibition IC <sub>50</sub> ( $\mu$ M)
						Calcd		Found		
						C	H	N	S	
<b>3p</b>	1-pyrrolidinyl	2HCl	A	136	C <sub>13</sub> H <sub>17</sub> N <sub>3</sub> OS·2HCl	44.80	6.19	11.04	8.42	21
					·H <sub>2</sub> O·0.3dioxane	(44.62)	(5.95)	(10.89)	(8.65)	
<b>3q</b>	1-piperidinyl	2HCl	A	172	C <sub>14</sub> H <sub>19</sub> N <sub>3</sub> OS·2HCl	47.27	6.12	11.81	9.01	3.0
					·0.3H <sub>2</sub> O	(47.36)	(6.03)	(11.75)	(9.01)	
<b>3r</b>	4-morpholinyl	2HCl	A	143	C <sub>13</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub> S·2HCl	43.65	5.52	11.75	8.96	14
					·0.3H <sub>2</sub> O	(43.79)	(5.22)	(11.66)	(9.09)	
<b>3s</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> NCOOEt	2HCl	A	Oil	C <sub>16</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub> S·2HCl	45.39	5.71	13.23		1.1
						(45.12)	(5.52)	(13.02)		
<b>3t</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> NMe	3HCl	C	182	C <sub>14</sub> H <sub>20</sub> N <sub>4</sub> OS·3HCl	40.93	5.89	13.64	7.81	7.7
					·0.5H <sub>2</sub> O	(41.02)	(5.89)	(13.55)	(7.61)	
<b>3u</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> NPh	3HCl	A	169	C <sub>19</sub> H <sub>22</sub> N <sub>4</sub> OS·3HCl	48.63	5.50	11.94	6.83	0.77
					·0.3H <sub>2</sub> O	(48.73)	(5.67)	(11.71)	(6.70)	
<b>3v</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> NCH <sub>2</sub> Ph	3HCl	A	165	C <sub>20</sub> H <sub>24</sub> N <sub>4</sub> OS·3HCl	49.34	5.80	11.51	6.59	0.45
					·0.5H <sub>2</sub> O	(49.29)	(5.87)	(11.46)	(6.83)	
<b>3w</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>2</sub> Ph	3HCl	A	155	C <sub>21</sub> H <sub>25</sub> N <sub>4</sub> OS·3HCl	50.64	5.83	11.25	6.44	0.22
					·0.4H <sub>2</sub> O	(50.74)	(6.11)	(11.21)	(6.44)	
<b>3x</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>3</sub> Ph	Fumarate	A, D	175	C <sub>26</sub> H <sub>32</sub> N <sub>4</sub> O <sub>5</sub> S	60.92	6.29	10.62	6.26	0.071
						(60.62)	(6.25)	(10.79)	(6.17)	
( <i>S</i> )- <b>3x</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>3</sub> Ph	Fumarate	A	191	C <sub>26</sub> H <sub>32</sub> N <sub>4</sub> O <sub>5</sub> S	60.92	6.29	10.62	6.26	70% inhibn. at 100 $\mu$ M
						(60.68)	(6.15)	(10.86)	(6.13)	
<b>3y</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>4</sub> Ph	3HCl	A	157	C <sub>25</sub> H <sub>29</sub> N <sub>4</sub> OS·3HCl	51.45	6.38	10.43	5.97	0.12
					·H <sub>2</sub> O	(51.32)	(6.41)	(10.43)	(5.98)	
<b>3z</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>5</sub> Ph	3HCl	A	136	C <sub>24</sub> H <sub>32</sub> N <sub>4</sub> OS·3HCl	52.22	6.76	10.15	5.81	0.072
					·H <sub>2</sub> O	(51.98)	(6.71)	(10.12)	(5.93)	
<b>3aa</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>	Fumarate	A	128	C <sub>23</sub> H <sub>34</sub> N <sub>4</sub> O <sub>5</sub> S	57.72	7.16	11.71	6.70	0.26
						(57.60)	(7.22)	(11.61)	(6.60)	
<b>3ab</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>	Fumarate	A	153	C <sub>24</sub> H <sub>36</sub> N <sub>4</sub> O <sub>5</sub> S	58.51	7.37	11.37	6.51	0.11
						(58.46)	(7.38)	(11.28)	(6.63)	
<b>3ac</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub>	Fumarate	A	135	C <sub>25</sub> H <sub>38</sub> N <sub>4</sub> O <sub>5</sub> S	59.27	7.56	11.06	6.33	0.067
						(59.01)	(7.66)	(10.95)	(6.27)	
<b>3ad</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>9</sub> CH <sub>3</sub>	3HCl	A	170	C <sub>23</sub> H <sub>37</sub> N <sub>4</sub> OS·3HCl	50.59	7.94	10.26	5.87	0.028
					·H <sub>2</sub> O	(50.50)	(7.81)	(10.22)	(6.07)	
<b>3ae</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>2</sub> ) <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	3HCl	A	153	C <sub>18</sub> H <sub>28</sub> N <sub>4</sub> OS·3HCl	44.26	7.10	11.47	6.56	0.16
					·1.7H <sub>2</sub> O	(44.28)	(6.97)	(11.47)	(6.74)	
<b>3af</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> CH(CH <sub>2</sub> ) <sub>2</sub> Ph	2HCl	A	117	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> OS·2HCl	56.36	6.58	8.96	6.84	900
					·0.8H <sub>2</sub> O	(56.27)	(6.55)	(8.92)	(6.94)	
<b>3ag</b>	N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> CH(CH <sub>2</sub> ) <sub>3</sub> Ph	2HCl	A	112	C <sub>23</sub> H <sub>29</sub> N <sub>3</sub> OS·2HCl	58.07	6.74	8.83	6.74	1.9
					·0.4H <sub>2</sub> O	(58.03)	(6.64)	(8.80)	(6.81)	
<b>3ah</b>	NHCH(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> NCH <sub>2</sub> Ph	—	A	134	C <sub>21</sub> H <sub>26</sub> N <sub>4</sub> OS	65.94	6.85	14.65	8.38	29
						(65.69)	(6.83)	(14.46)	(8.43)	
	WEB-2086									0.12

Table 3. PAF-Antagonist Activities of Selected Thiazolidinecarboxamides

Compound No.	Platelet aggregation inhibition IC <sub>50</sub> (μM) <sup>a)</sup>		ED <sub>50</sub> (mg/kg <i>p.o.</i> )		
	Rabbit	Human	Hemoconcentration in rats <sup>b)</sup>	Vascular permeability in G. P. skin <sup>c)</sup>	PAF-induced death in mice <sup>d)</sup>
<b>3l</b>	0.39	2.2	8.4	(8%) <sup>e)</sup>	12.5
<b>3n</b>	0.24	0.10	7.5	12.9	2.0
<b>3x</b>	0.071	0.11	0.22	2.1	0.33
<b>3z</b>	0.072	1.0	1.1	4.6	3.8
<b>3ac</b>	0.067	<sup>f)</sup>	0.83	7.6	4.5
<b>3ad</b>	0.028	0.16	3.0	13.5	2.1
WEB-2086	0.12	0.11	4.2	0.37	0.57

a) Concentration needed to inhibit PAF (200–400 nM)-induced platelet aggregation in human PRP by 50%. b) Compounds were orally administered 1 h prior to PAF (1.65 μg/kg *i.v.*). c) Compounds were orally administered 1 h prior to PAF (5.5 ng/site *i.d.*). d) Compounds were orally administered 1 h prior to PAF (12.5 μg/kg *i.v.*). e) Inhibition percent at 30 mg/kg *p.o.* f) Not determined.

platelets and have very similar structures.

Next, the compounds were tested for their *in vivo* activity to inhibit PAF-induced death in mice, hemoconcentration in rats and the increase in vascular permeability in guinea-pigs after oral administration. In general, piperazides exhibited better oral activities than benzyl amides. Among the piperazides, aralkyl piperazides (**3x**, **3z**) were more potent than alkyl piperazides (**3ac**, **3ad**). Above all, **3x** showed the best oral activities. This compound was nineteen times and two times more potent than WEB-2086 in rats and mice, respectively, but five times less potent in guinea-pigs. We selected **3x** as a candidate for further evaluation.

Compound **3x** inhibited [<sup>3</sup>H]PAF binding to rabbit platelet membranes with a p*K*<sub>i</sub> value of 8.90. In an *in vivo* study, **3x** inhibited the bronchoconstriction induced by PAF (ED<sub>50</sub> = 1.2 mg/kg *p.o.*) in anesthetized guinea-pigs. The compound at doses above 3 mg/kg *p.o.* significantly inhibited antigen-induced anaphylactic asthma in conscious guinea-pigs.<sup>13)</sup>

Metabolic study of **3x** revealed that it is metabolized to give piperazide (**8**) and unsaturated ketone (**9**) in guinea pigs after oral administration.<sup>14)</sup> The metabolic pathway appears to be as shown in Chart 3. Compound **3x** was oxidized at the benzyl position to give **3am** and then retro-Michael type degradation gave **8** and **9**. Such degradation might lead to a short duration of action, which could be a drawback in clinical use. Thus, to prevent benzylic oxidation, we introduced some substituents at the benzylic position of **3x** and tested these derivatives for PAF-antagonistic activity (Table 4).

The methyl derivative (**3ai**) was almost as potent as **3x**. The introduction of a methoxy (**3aj**) or a phenyl (**3ak**) group reduced the activity. The dimethyl derivative (**3al**) was twice as potent as **3x**. The oxo derivative (**3am**) was also a potent antagonist. We selected **3ai**, **3al** and **3am** for further evaluation. The results are shown in Table 5.

Compounds **3al** and **3am** inhibited human and rabbit platelet aggregation at almost the same concentrations. In the *in vivo* studies, all of the compounds potently inhibited PAF-induced death in mice, hemoconcentration in rats and vascular permeability in guinea-pigs after oral administration. Among them, **3al** (YM264) exhibited the best oral activities. YM264 showed ED<sub>50</sub> values of 0.30,

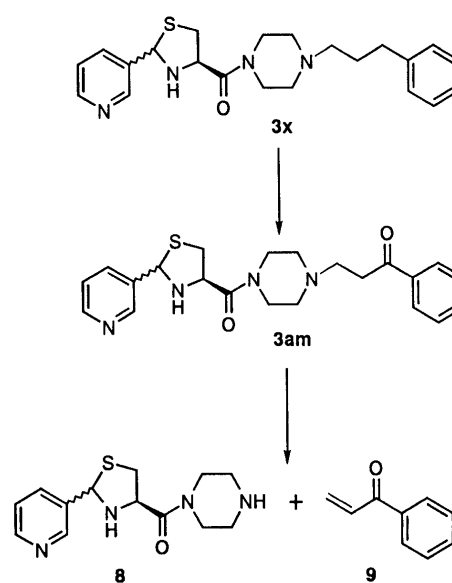


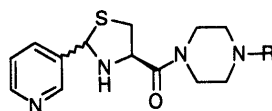
Chart 3

0.49 and 0.19 mg/kg *p.o.* in rats, guinea-pigs and mice respectively. These results show that YM264 is a potent and orally active PAF antagonist. The data also suggest that there is little species difference in the anti-PAF action of YM264.

YM264 inhibited [<sup>3</sup>H]PAF binding to rabbit platelet membrane with a p*K*<sub>i</sub> value of 8.85. The p*A*<sub>2</sub> values for YM264 against PAF-induced aggregation in human, rabbit and guinea-pig platelets were 8.68, 8.33 and 8.14, respectively. YM264, at 10<sup>-4</sup> M, did not exhibit any inhibitory effect on rabbit or human platelet aggregation induced by ADP, collagen, arachidonic acid and epinephrine.<sup>15)</sup> These results indicate that YM264 is a potent and selective antagonist of PAF.

In the *in vivo* tests, YM264 inhibited the PAF-induced hypotension in rats with an ED<sub>50</sub> value of 0.005 mg/kg *i.v.* The inhibitory effect of YM264, after 1 and 3 mg/kg *p.o.* persisted for more than 6 h in the rat hemoconcentration model, indicating that the action of YM264 is relatively long-lasting.<sup>15)</sup> In an animal model of asthma, the compound at doses above 0.3 mg/kg *p.o.* significantly inhibited antigen-induced anaphylactic asthma in conscious guinea pigs.<sup>1)</sup> YM264 also showed beneficial ef-

Table 4. Physicochemical Data and PAF-antagonist Activities for Thiazolidinecarboxamides



Compd. No.	R	Salt	Method	mp (°C)	Formula	Analysis (%)				Platelet aggregation inhibition IC <sub>50</sub> (μM)
						Calcd (Found)				
						C	H	N	S	
<b>3ai</b>	CH <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> )Ph	Fumarate	A	166—168	C <sub>27</sub> H <sub>34</sub> N <sub>4</sub> O <sub>5</sub> S	61.58 (61.21)	6.51 (6.45)	10.64 (10.58)	6.09 (6.42)	0.064
<b>3aj</b>	CH <sub>2</sub> CH <sub>2</sub> CH(OCH <sub>3</sub> )Ph	Fumarate	A	129—132	C <sub>27</sub> H <sub>34</sub> N <sub>4</sub> O <sub>6</sub> S	60.41 (60.01)	6.52 (6.39)	10.06 (9.92)	5.76 (5.89)	0.31
<b>3ak</b>	CH <sub>2</sub> CH <sub>2</sub> CH(Ph)Ph	Fumarate	A	169—172	C <sub>32</sub> H <sub>36</sub> N <sub>4</sub> O <sub>5</sub> S	65.29 (65.55)	6.16 (6.10)	9.52 (9.44)	5.45 (5.70)	0.32
<b>3al</b>	CH <sub>2</sub> CH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> Ph	Fumarate	A	172—173	C <sub>28</sub> H <sub>36</sub> N <sub>4</sub> O <sub>5</sub> S	62.20 (61.92)	6.71 (6.67)	10.36 (10.17)	5.93 (6.09)	0.030
<b>3am</b>	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> COPh	3HCl	A	145	C <sub>23</sub> H <sub>27</sub> N <sub>4</sub> O <sub>2</sub> S ·3HCl·1.5H <sub>2</sub> O	49.25 (49.40)	6.11 (5.97)	9.99 (9.79)	5.72 (5.92)	0.034
WEB-2086										0.12

Table 5. PAF-antagonist Activities of Selected Thiazolidinecarboxamides

Compound No.	Platelet aggregation inhibition IC <sub>50</sub> (μM) <sup>a)</sup>		ED <sub>50</sub> (mg/kg <i>p.o.</i> )		
	Rabbit	Human	Hemoconcentration in rats <sup>b)</sup>	Vascular permeability in G. P. skin <sup>c)</sup>	PAF-induced death in mice <sup>d)</sup>
<b>3ai</b>	0.064	<sup>e)</sup>	0.14	1.1	0.73
<b>3al</b>	0.030	0.030	0.30	0.49	0.19
<b>3am</b>	0.034	0.027	0.19	9.0	0.4
WEB-2086	0.12	0.11	4.2	0.37	0.57

a) Concentration needed to inhibit PAF (200—400 nM)-induced platelet aggregation in human PRP by 50%. b) Compounds were orally administered 1 h prior to PAF (1.65 μg/kg *i.v.*). c) Compounds were orally administered 1 h prior to PAF (5.5 ng/site *i.d.*). d) Compounds were orally administered 1 h prior to PAF (12.5 μg/kg *i.v.*). Number in parentheses is the number of determinations. e) Not determined.

fects in some models of glomerulonephritis. For example, YM264 inhibited proteinuria and improved hypercholesterolemia and hypoalbuminemia in puromycin aminonucleoside-induced nephropathy in rats.<sup>16)</sup>

In summary, we developed a new series of PAF antagonists, 2-(3-pyridyl)thiazolidine-4-carboxamides. In particular, **3al** (YM264) was shown to be a potent and orally active antagonist. Further studies on these compounds are in progress.

#### Experimental

Melting points were taken on a Yanaco MP-3 melting point apparatus and are uncorrected. <sup>1</sup>H-NMR were taken with a JEOL FX-90Q, FX-100 or GSX-400 spectrometer with tetramethylsilane as an internal standard. Mass spectra were determined on an MS-5970 or JEOL JMS-DX300 mass spectrometer. Column chromatography was carried out on silica gel (Wakogel C-200).

Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were used without further purification. In general, organic extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> or MgSO<sub>4</sub> and the solvent was evaporated under reduced pressure. All nonaqueous reactions were performed in dry glassware under an atmosphere of dry Ar.

**N-tert-Butoxycarbonyl-2-(3-pyridyl)thiazolidine-4-carboxylic Acid (5)** Di-*tert*-butyl dicarbonate (2.4 g, 11 mmol) and 10 ml of 1 N aqueous NaOH were added to a mixture of 2.1 g (10 mmol) of 2-(3-pyridyl)thiazolidine-4-carboxylic acid (**4**), 20 ml of water and 40 ml of dioxane at 4 °C, and the mixture was stirred at room temperature for 30 min, then concentrated. The residue was taken up in 30 ml of water, the pH was adjusted to 2 by the addition of 0.5 M aqueous citric acid, and the mixture was extracted with ethyl acetate. The extract was washed with

water, dried and concentrated, and the residue was recrystallized from ethyl acetate to give 1.0 g (3.2 mmol, 32%) of **5**, mp 167—169 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.12 (s, 5.4H), 1.36 (s, 3.6H), 3.05—3.28 (m, 1H), 3.48—3.54 (m, 1H), 4.55—4.64 (m, 0.4H), 4.72—4.81 (m, 0.6H), 6.01 (s, 0.6H), 6.18 (s, 0.4H), 7.33—7.42 (m, 1H), 7.98—8.12 (m, 1H), 8.41—8.51 (m, 1H), 8.73—8.84 (m, 1H), 13.12 (br, 1H); chemical ionization (CI) mass spectrometry *m/z* 311 (M<sup>+</sup> + H); *Anal.* Calcd for C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S: C, 54.18; H, 5.85; N, 9.03; S, 10.33. Found: C, 54.17; H, 5.76; N, 9.06; S, 10.32.

**1,3-Dioxo-5-(3-pyridyl)thiazolidino[3,4-*c*]oxazolidine Hydrochloride (7)** (COCl)<sub>2</sub> (0.21 ml) was added to a mixture of **5** (500 mg, 1.6 mmol), dimethylformamide (DMF) (1 drop) and CH<sub>2</sub>Cl<sub>2</sub> (5 ml) at -78 °C and the mixture was stirred at room temperature for 12 h. The crystals were collected by filtration to give 280 mg (1.0 mmol, 64%) of **7**: mp 170 °C; electron impact (EI) mass spectra *m/z* 236 (M - HCl<sup>+</sup>); *Anal.* Calcd for C<sub>10</sub>H<sub>9</sub>ClN<sub>2</sub>O<sub>3</sub>S: C, 44.04; H, 3.33; N, 10.27; S, 13.00. Found: C, 43.94; H, 3.37; N, 10.24; S, 13.30.

**Method A. N-Decyl-2-(3-pyridyl)thiazolidine-4-carboxamide (3e)** A mixture of 500 mg (2.4 mmol) of **4**, 370 mg (2.4 mmol) of decylamine, 500 mg (2.4 mmol) of DCC and 500 mg (3.7 mmol) of HOBT in 25 ml of tetrahydrofuran (THF) was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate, and the insoluble material was removed by the filtration. The filtrate was washed with aqueous NaHCO<sub>3</sub> and a saturated aqueous solution of NaCl, dried and concentrated. The purification of the residue by silica gel column chromatography (eluent; ethyl acetate:methanol=10:1) gave 690 mg (2.0 mmol, 84%) of **3e**: mp 88 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.76—1.95 (m, 3H), 2.10—2.70 (m, 16H), 3.17—3.48 (m, 3H), 3.60—3.76 (m, 1H), 4.22—4.36 (m, 1H), 5.42 (s, 0.9H), 5.62 (s, 0.1H), 7.25—7.40 (m, 1H), 7.77—7.91 (m, 1H), 8.59 (dd, *J*=5.0, 2.0 Hz, 1H), 8.74 (d, *J*=5.0 Hz, 1H); mass spectrum (EI) *m/z* 349 (M<sup>+</sup> + H).

**Method B. N-(4-Benzyloxybenzyl)-2-(3-pyridyl)thiazolidine-4-carbox-**

**amide (3j)** A solution of 210 mg (0.99 mmol) of **4** and triethylamine (280  $\mu$ l, 2.0 mmol) in 4 ml of DMF was cooled on ice and a solution of DPPA (270 mg, 0.98 mmol) in 2 ml of DMF and a solution of 220 mg (0.88 mmol) of 4-benzyloxybenzylamine hydrochloride (220 mg, 0.88 mmol) in 3 ml of DMF were added to it. The mixture was stirred at room temperature for 12 h. After dilution with ethyl acetate, the reaction mixture was washed with a saturated aqueous solution of NaHCO<sub>3</sub>, water and a saturated aqueous solution of NaCl, dried and concentrated. The residue was recrystallized from 2-propanol to give 160 mg (0.39 mmol, 44%) of **3j**: mp 139–140 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 3.30–3.50 (m, 1H), 3.65–3.83 (m, 1H), 4.23–4.25 (m, 3H), 5.06 (s, 2H), 5.32 (s, 0.6H), 5.44 (s, 0.4H), 6.86–7.02 (m, 2H), 7.11–7.46 (m, 8H), 7.72–7.84 (m, 1H), 8.56 (dd,  $J=5.0, 2.0$  Hz, 1H), 8.67 (d,  $J=2.0$  Hz, 1H); mass spectrum (FAB)  $m/z$  406(M<sup>+</sup> + H).

**Method C. [2-(3-Pyridyl)thiazolidine-4-carbonyl]-L-methionine Methyl Ester Dihydrochloride (3a)** To a solution of 600 mg (1.9 mmol) of **5** in 10 ml of THF, 390 mg (2.0 mmol) of L-methionine methyl ester hydrochloride, 390 mg (2.9 mmol) of HOBT, 190 mg (1.9 mmol) of *N*-methylmorpholine and 440 mg (2.1 mmol) of DCC were added below 4 °C. The mixture was stirred at 4 °C for 1 h. The precipitate was removed by the filtration and the filtrate was concentrated. The residue was taken up in 50 ml of ethyl acetate, the insoluble material was removed by the filtration and the filtrate was washed with 0.5 M aqueous citric acid, water, 5% aqueous NaHCO<sub>3</sub> and water, dried and concentrated to give 440 mg of [3-*tert*-butoxycarbonyl-2-(3-pyridyl)thiazolidine-4-carbonyl]-L-methionine methyl ester. This compound was treated with 5 ml of TFA with ice-water cooling, and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated. Ethyl acetate was added to the residue, and the mixture was again concentrated. The residue was purified by silica gel column chromatography (eluent; CHCl<sub>3</sub>:methanol=10:1) to give 249 mg (0.70 mmol, 36%) of **3a**: <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.88–2.27 (m, 2H), 2.06 (s, 1.2H), 2.11 (s, 1.8H), 2.40–2.60 (m, 2H), 3.31–3.45 (m, 1H), 3.64–6.79 (m, 1H), 3.77 (s, 3H), 4.04–4.07 (m, 0.4H), 3.36–3.38 (m, 0.6H), 4.67–4.74 (m, 1H), 5.48 (s, 0.6H), 5.65 (s, 0.4H), 7.30–7.34 (m, 1H), 7.76–7.91 (m, 1H), 8.56–8.59 (m, 1H), 8.74–8.76 (m, 1H); mass spectrum (EI)  $m/z$  355(M<sup>+</sup>).

**Method D. 1-(3-Phenylpropyl)-4-[2-(3-pyridyl)thiazolidine-4-carbonyl]-piperazine (3x)** A solution of 1-(3-phenylpropyl)piperazine (40 mg, 0.20 mmol) in dimethyl sulfoxide (DMSO) (0.5 ml) was added to a solution of **7** (50 mg, 0.18 mmol) in DMSO (1 ml) at room temperature. The mixture was stirred at room temperature for 2 h, diluted with ethyl acetate, washed with a saturated aqueous solution of NaHCO<sub>3</sub>, water and a saturated aqueous solution of NaCl, dried and concentrated to give 70 mg (0.17 mmol, 94%) of **3x**. The physical properties of this compound were identical with those of **3x** prepared by method A.

**PAF-Induced Platelet Aggregation** Rabbit and human PRP were obtained by centrifugation at 270  $\times g$  for 10 min. The platelet concentrations were adjusted with platelet-poor plasma to 5  $\times 10^8$  cells/ml for rabbit and to 3  $\times 10^8$  cells/ml for human. Platelet aggregation was measured by the method of Born<sup>17)</sup> using a Hema Tracer VI (Niko Bioscience, Tokyo, Japan). Various concentrations of the test compounds were added to the PRP 2 min prior to the addition of PAF (10 nM for rabbit and 200–400 nM for human). All experiments were carried out within 4 h following blood collection to avoid a decrease in the sensitivity of the platelets to the aggregating agent.

**PAF-Induced Death in Mice** ICR mice, 6 weeks old, were injected with 12.5  $\mu$ g/kg PAF and the mortality was estimated 24 h after the administration of PAF. The test compounds were administered orally 1 h prior to PAF.

**PAF-Induced Hemoconcentration** Wistar rats, weighing 200 to 336 g, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the right carotid artery was cannulated for blood collection. Hemoconcentration was determined by measuring the changes of hematocrit values before and 10 min after the intravenous injection of 1.65  $\mu$ g/kg PAF. The test compounds were administered orally at 1 h before the PAF injection.

**PAF-Induced Increase in Vascular Permeability** The test compounds

were administered orally to Hartley guinea-pigs, weighing 290 to 355 g, 1 h prior to the dorsal intradermal injection of 5.5 ng PAF. At 2 min prior to PAF injection, 1 ml of saline containing 1.8% Evans blue was injected intravenously. The animals were then sacrificed 40 min after the injection of PAF. The leaked dye at the site of PAF or vehicle injection was extracted according to the method of Harada *et al.*<sup>18)</sup> and the absorbance was measured at 620 nm.

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