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Synthesis of Substituted Cinnamoyl-tyramine Derivatives and their Platelet Anti-aggregatory Activities

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Substituted cinnamoyl-tyramine derivatives were synthesized by DCC-coupling of substituted cinnamic acid with tyramine or tyramine methyl-1-ether to evaluate PAF-receptor binding antagonistic activities and inhibitory activities on PAF-induced platelet aggregation with interest on structure-activity relations. The results show that 3,4-dimethoxy-cinnamoyl tyramine-amide or its methyl ether have significant PAF-receptor binding antagonistic activity and platelet anti-aggregatory activities.

Key words : Platelet anti-aggregatory activities, PAF-receptor binding antagonistic activity, Substituted cinnamoyl tyramine amides

INTRODUCTION

N-p-Coumaroyltyramine (Matano *et al.*, 1986; Okuyama *et al.*, 1986) and *N-trans*-feruloyltyramine derivatives in *Allium bakeri* Reg. (*Lilliaceae*) were shown to be potent inhibitors against ADP-induced platelet aggregation (Shibata *et al.*, 1986) and also to be potent thromboxane synthesis inhibitor (Sankawa *et al.*, 1984). Recently, PAF and its antagonists were extensively studied since PAF is known to play various pathophysiological roles including platelet aggregation, inflammation, asthma, endotoxic shock and hypotension (Benveniste *et al.*, 1972; Braquet *et al.*, 1987). Based upon above background, we synthesized various substituted-cinnamoyltyramine amide derivatives, which have various substituents as hydroxy or methoxy or both substituents on the aromatic ring of cinnamoyl group in order to evaluate the platelet antiaggregatory activity and PAF-antagonistic activity of the derivatives with interests on structure-activity relationships. The platelet anti-aggregatory activities of the compounds were also tested for the collagen induced or ADP induced model methods of platelet aggregation.

MATERIALS AND METHODS

General procedures for the synthesis of substituted cinnamoyltyramine amide derivatives

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To a solution of 500 mg (2.8 mmol) of substituted cinnamic acid derivatives and 500 mg (3.6 mmol) of tyramine derivatives in 300-320 ml of tetrahydrofuran (THF), the THF-solution (15-20 ml) of 1000 mg (4.8 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC) was added slowly and stirred for 24 hours at 25°C. The reaction mixture was evaporated under reduced pressure at room temperature and then diluted with water (200 ml). The resulting precipitate was extracted with ethyl acetate three times (each 100 ml). The organic layer was washed successively with 5% HCl (100 ml $\times$ 2), 5% $NaHCO_3$ (100 ml $\times$ 2) and brine, and dried over $MgSO_4$. The dried ethyl acetate layer was evaporated under reduced pressure to give solid products. The solid products were recrystallized in the mixed solvent of MeOH and $CHCl_3$.

Preparation of washed rabbit platelet suspension

Six volume of blood was collected from the rabbit heart directly into one volume of ACD solution. The blood was centrifuged at 270 g for 10 min. and the top platelet rich plasma (PRP) was carefully removed. The PPP was centrifuged at 750 g for 10 min. Platelets were washed three times by centrifugation at 900 g 10 min. in tris-tyrode buffer. The final platelet concentration was adjusted to 3×10^8 count/ml.

In vitro PAF receptor binding assay

^{3}H-PAF receptor binding experiments to rabbit platelets were carried out according to the modified

method of Valone (Valone, 1982). The reaction mixture was consisted of 200 μl of washed rabbit platelet suspension, 25 μl of ^{3}H-PAF (0.6 nM, 60,000 dpm) with or without cold PAF (500 fold molar excess) and 25 μl of sample or control solution. The reaction mixture was incubated at room temperature for one hour. The free and bound ligands were separated by filtration using Whatman GF/C glass fiber membranes. The radioactivities of the dried filter membranes were counted on liquid scintillation counter. The difference between total radioactivity counts in the absence and presence of excess cold PAF is defined as specific binding of the radiolabeled ligand. Percentage inhibition of the sample was obtained by the following equation :

$$\% \text{ Inhibition} = \frac{Sc-Ss}{Sc} \times 100 = \frac{(Tc-Nc)-(Ts-Ns)}{Tc-Nc} \times 100$$

*Sc=specific binding of control

Ss=specific binding of sample

Tc=total binding of control, Ts=total binding of sample

Nc=nonspecific binding of control, Ns=nonspecific binding of sample

Platelet Aggregation Test

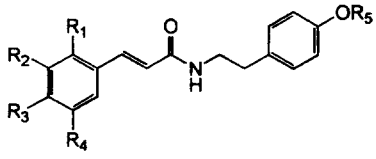
This was carried out by a modification of the method of Born *et al.* (1963).

RESULTS AND DISCUSSION

1) Twenty three compounds of substituted cinnamoyl tyramine-amide derivatives having various combination of hydroxy or methoxy substitutents on aromatic rings of cinnamoyl group were synthesized by same synthetic procedure using different starting materials.

Their chemical structures were identified by typical

Table I. Newly synthesized cinnamoyl tyramine derivatives



No.	R_1	R_2	R_3	R_4	R_5	Crystal(solvent)	m.p.(°C)
1	-H	-OH	-OH	-H	-H	amorphous	217-219
2	-OH	-H	-H	-H	-H	amorphous	190-192
3	-H	-H	-OH	-H	-H	amorphous	245-247
4	-H	-H	-OH	-H	$-CH_3$	plate(MeOH)	188-190
5	-OH	-H	-H	-H	$-CH_3$	amorphous	114-116
6	-H	-OH	-OH	-H	$-CH_3$	needle (MeOH)	161-163
7	$-OCH_3$	-H	-H	-H	-H	prism (MeOH)	140-142
8	-H	$-OCH_3$	-H	-H	-H	amorphous	114-116
9	-H	-H	$-OCH_3$	-H	-H	needle (EtOH)	145-147
10	$-OCH_3$	-H	-H	-H	$-CH_3$	needle (MeOH)	108-110
11	-H	$-OCH_3$	-H	-H	$-CH_3$	needle (MeOH)	86-88
12	-H	-H	$-OCH_3$	-H	$-CH_3$	needle (MeOH)	137-139
13	-H	$-OCH_2O-$		-H	-H	prism (MeOH)	162-164
14	-H	$-OCH_3$	-OH	-H	-H	needle (MeOH)	122-124
15	-H	$-OCH_3$	-H	$-OCH_3$	-H	amorphous	156-158
16	-H	$-OCH_3$	-H	$-OCH_3$	$-CH_3$	needle (MeOH)	109-111
17	-H	-OH	-H	-H	$-CH_3$	plate (EtOH)	129-130
18	-H	$-OCH_3$	$-OCH_3$	-H	-H	amorphous	146-150
19	-H	$-OCH_3$	$-OCH_3$	-H	$-CH_3$	needle (EtOH)	120-122
20	$-OCH_3$	-H	-H	$-OCH_3$	-H	amorphous	146-149
21	$-OCH_3$	-H	-H	$-OCH_3$	$-CH_3$	needle (MeOH)	125-128
22	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	-H	prism (MeOH)	160-163
23	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-CH_3$	needle (MeOH)	90-92

Table II. ^{1}H-NMR spectral data(ppm from TMS) of several compounds

No.	solvent	δ=ppm, *J* in Hz
1	$CDCl_3$+DMSO-d_6	7.45 (d, 1H, *J*=15.6), 7.05 (m, 3H, *J*=7.3), 6.78-6.95 (m, 4H), 6.28 (d, 1H, *J*=15.6), 3.52 (t, 2H, *J*=6.8), 2.78 (t, 2H, *J*=7.0)
2	$CDCl_3$+DMSO-d_6	7.83 (d, 1H, *J*=16.0), 7.40 (m, 1H), 7.28 (m, 1H), 7.14 (d, 2H, *J*=19.3), 6.82 (d, 1H, *J*=8.4) 6.78 (m, 3H), 6.95 (d, 1H, *J*=15.7), 3.52 (t, 2H, *J*=6.7), 2.79 (t, 2H, *J*=7.4)
3	$CDCl_3$+DMSO-d_6	7.49 (d, 1H, *J*=15.6), 7.35 (d, 2H, *J*=8.3), 7.02 (d, 2H, *J*=8.2), 6.80 (m, 2H), 6.78 (d, 2H, *J*= 8.2), 6.40 (d, 1H, *J*=15.7), 3.43 (t, 2H, *J*=15.7), 2.74 (t, 2H, *J*=6.7)
4	MeOH-d_4	7.34-7.39 (m, 3H), 7.09 (d, 2H, *J*=8.5), 6.77 (m, 2H), 6.71 (d, 2H, *J*=8.5), 6.30 (d, 1H, *J*=15.7), 3.78 (s, 3H), 3.39 (t, 2H, *J*=7.6), 2.71 (t, 2H, *J*=7.5)
5	MeOH-d_4	7.72 (d, 1H, *J*=15.6), 7.35 (m, 2H), 7.10 (d, 2H, *J*=6.5), 6.76 (m, 2H), 6.74 (d, 2H, *J*=5.1), 6.60 (d, 1H, *J*=16.0), 3.72 (s, 3H), 3.45 (t, 2H, J=7.0), 2.70 (t, 2H, *J*=7.3)
6	MeOH-d_4	7.32 (d, 1H, *J*=15.6), 7.08 (d, 2H, *J*=8.4), 6.69 (d, 2H, *J*=8.1), 6.65-6.94 (m, 3H), 6.28 (d, 1H, *J* =15.6), 3.70 (s, 3H), 3.38 (t, 2H, *J*=7.2), 2.71 (t, 2H, *J*=7.2)
7	$CDCl_3$+DMSO-d_6	7.86 (d, 1H, *J*=15.8), 7.27-7.49 (m, 2H), 7.05 (d, 2H, *J*=8.0), 6.89-6.97 (m, 2H), 6.79 (d, 2H, *J*= 7.9), 6.55 (d, 1H, *J*=15.6), 3.87 (s, 3H), 3.54 (t, 2H, *J*=6.3), 2.78 (t, 2H, *J*=6.7)
8	$CDCl_3$+DMSO-d_6	7.53 (d, 1H, *J*=15.6), 7.30 (m, 1H), 7.03-7.10 (m ,2H), 7.07 (d, 2H, *J*=7.0), 6.88 (m, 1H), 6.78 (d, 2H, *J*=7.8), 6.50 (d, 1H, *J*=15.7), 3.83 (s, 3H), 3.52 (t, 2H, *J*=5.2), 2.78 (t, 2H, *J*=6.0)
9	$CDCl_3$	7.74 (d, 1H, *J*=15.6), 7.43 (d, 2H, *J*=8.7), 7.07 (d, 2H, *J*=8.3), 6.88 (d, 2H, *J*=8.7), 6.81 (d, 2H, *J*=8.3), 6.19 (d, 1H, *J*=15.6), 3.82 (s, 3H), 3.61 (t, 2H, *J*=6.8), 2.81 (t, 2H, *J*=6.8)
10	$CDCl_3$	7.87 (d, 1H, *J*=15.8), 7.43-7.48 (m, 1H), 7.27-7.36 (m, 1H), 7.15 (d, 2H, *J*=8.5), 6.93-6.97 (m, 2H), 6.87 (d, 2H, *J*=8.4), 6.45 (d, 1H, *J*=15.8), 3.87 (s, 3H), 3.80 (s, 3H), 3.62 (t, 2H, *J*=6.7), 2.83 (t, 2H, *J*=6.8)
11	$CDCl_3$	7.58 (d, 1H, *J*=15.3), 6.89-7.27 (m, 8H), 6.30 (d, 1H, *J*=15.8), 3.80 (s, 6H), 3.62 (t, 2H, *J*=6.6), 2.83 (t, 2H, *J*=6.8)
12	$CDCl_3$	7.58 (d, 1H, *J*=15.6), 7.44 (d, 2H, *J*=8.6), 7.15 (d, 2H, *J*=8.4), 6.88 (m, 4 *J*=15.5), 3.83 (s, 3H), 3.80 (s, 3H), 3.62 (t, 2H, *J*=6.4), 2.83 (t, 2H, *J*=6.8)
13	$CDCl_3$+DMSO-d_6	7.50 (d, 1H, *J*=15.5), 7.21 (m, 1H), 6.96-7.06 (m, 3H), 6.75-6.82 (m, 3H), 6.37 (d, 1H, *J*=15.6), 6.01 (s, 2H), 3.49 (t, 2H, *J*=6.4), 2.77 (t, 2H, *J*=6.8)
14	$CDCl_3$+DMSO-d_6	7.39 (d, 1H, *J*=14.3), 6.88-6.97 (m, 4H), 6.57-6.79 (m, 3H), 6.51-6.54 (m, 1H), 6.21 (d, 1H, *J*= 15.6), 3.80 (s, 3H), 3.44 (t, 2H, *J*=6.6), 2.68 (t, 2H, *J*=7.34)
15	$CDCl_3$+DMSO-d_6	7.48 (d, 1H, *J*=15.5), 7.04 (d, 2H, *J*=8.2), 6.82 (d, 2H, *J*=8.2), 6.65 (s, 2H), 6.48 (d, 1H, *J*=15.7), 6.45 (s, 1H), 3.81 (s, 6H), 3.55 (t, 2H, *J*=6.1), 2.79 (t, 2H, *J*=6.3)
16	$CDCl_3$+DMSO-d_6	7.52 (d, 1H, *J*=15.7), 7.15 (d, 2H, *J*=8.2), 6.86 (d, 2H, *J*=8.5), 6.63 (s, 2H), 6.46 (s, 1H), 6.33 (d, 1H, *J*=15.6), 3.81 (s, 9H), 3.61 (t, 2H, J=6.5), 2.83 (t, 2H, *J*=6.9)
17	$CDCl_3$+DMSO-d_6	7.39 (d, 1H, *J*=16.5), 7.03 (d, 2H, *J*=8.6), 7.01-7.09 (m, 1H), 6.83-6.86 (m, 2H), 6.72 (d, 2H, *J*=8.5), 6.70-6.74 (m, 1H), 6.27 (d, 1H, *J*=15.6), 3.67 (s, 3H), 3.43 (q, 2H, *J*=7.0), 2.70 (t, 2H, *J*=7.0)
18	DMSO-d_6	7.33 (d, 1H, *J*=15.7), 7.00 (d, 2H, *J*=8.4), 6.94-7.13 (m, 3H), 6.67 (d, 2H, *J*=8.2), 6.48 (d, 1H, *J* =15.7), 3.78 (s, 3H), 3.77 (s, 3H), 3.27 (t, 2H, *J*=7.21), 2.63 (t, 2H, *J*=7.34)
19	$CDCl_3$	7.56 (d, 1H, *J*=15.5), 7.01-7.18 (m, 4H), 6.90-6.83 (m, 3H), 6.19 (d, 1H, *J*=15.5), 3.91 (s, 6H), 3.63 (t, 2H, *J*=6.7), 3.58 (s, 3H), 2.83 (t, 2H, J=6.8)
20	$CDCl_3$	7.61 (d, 1H, *J*=15.9), 6.89-7.04 (m, 5H), 6.67 (d, 2H, *J*=8.1), 6.63 (d, 1H, *J*=15.7), 3.83 (s, 3H), 3.72 (s, 3H), 3.28 (t, 2H, *J*=7.2), 2.64 (t, 2H, J=6.8)
21	$CDCl_3$	7.83 (d, 1H, *J*=15.8), 7.15 (d, 2H, *J*=8.5), 7.00 (m, 1H), 6.87 (d, 2H, *J*=8.2), 6.85-6.89 (m, 2H), 6.43 (d, 1H, *J*=15.8), 3.82 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 3.62 (t, 2H, *J*=6.60), 2.83 (t, 2H, *J* =6.8)
22	DMSO-d_6	7.49 (d, 1H, *J*=16.0), 7.26 (d, 1H, *J*=8.6), 7.01 (d, 2H, *J*=8.1), 6.87 (d, 1H, *J*=8.2), 6.67 (d, 2H, *J*=7.8), 6.53 (d, 1H, *J*=16.0), 3.85 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 3.33 (t, 2H, *J*= 6.2), 2.63 (t, 2H, J=6.2)
23	$CDCl_3$	7.75 (d, 1H, *J*=15.7), 7.24 (d, 1H, *J*=9.7), 7.16 (d, 2H, *J*=8.5), 6.87 (d, 2H, *J*=8.4), 6.67 (d, 1H, *J*=8.7), 6.35 (d, 1H, *J*=15.9), 3.89 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.62 (t, 2H, *J*= 6.7), 2.83 (t, 2H, *J*=6.8)

Table III. Inhibitory effects on the PAF receptor binding to rabbit platelet (Concentration of compounds: 20 μg/ml)

Compound No.	% inhibition	Compound No.	% inhibition	Compound No.	% inhibition
1	25	**11**	5	**21**	50
2	70	**12**	-	**22**	12
3	63	**13**	-	**23**	19
4	32	**14**	-		
5	33	**15**	-		
6	42	**16**	-		
7	-	**17**	58		
8	40	**18**	61		
9	5	**10**	71		
10	-	**20**	49		

Table IV. Inhibitory effects on collagen and ADP induced platelet aggregation (Concentration of compounds (50 μg/ml), aspirin (50 μg/ml), adenosine (10 μg/ml), ADP (20 μM), collagen (5 μg/ml))

Compound No.	% inhibition		Compound No.	% inhibition	
	collagen	ADP		collagen	ADP
1	-	33.4	**16**	15.4	7.0
2	-	25.4	**17**	15.4	2.4
3	-	32.6	**18**	0.0	2.4
4	-	-	**19**	0.0	19.6
5	-	-	**20**	20.0	7.0
6	-	-	**21**	20.0	11.8
7	6.2	13.8	**22**	7.7	24.4
8	12.1	1.6	**23**	7.7	29.1
9	14.3	18.4			
10	7.5	10.9			
11	14.3	19.3			
12	16.5	2.5			
13	7.7	13.3			
14	27.7	19.6	Aspirin	60.7	
15		21.3	Adenosin		38.5

NMR spectra of the compounds. All proton peaks were assigned for the given structures as shown in Table II.

2) PAF-receptor binding antagonistic activity data and the inhibitory data on the PAF-induced platelet aggregation of the synthetic substances were shown in Table III and Table V. As shown in Table III, PAF-receptor binding antagonistic activities were appreciably potent in the dimethoxy compounds as 3,4-dimethoxycinnamoyl tyramine-amide and its methyl ether (Comp. 18 & 19), and 2,5-dimethoxycinnamoyl derivatives (Comp. 20 & 21) and some monohydroxy substituted derivatives as Compound 2, 3, and 17. PAF induced platelet anti-aggregatory activities support this tendency in the dimethoxyl substituted derivatives only with some exceptions in the compound 8, 14, and 22 which shows moderate activities as shown in Table V. IC_{50} values of compounds 18, 19, and 20 were calculated to be in the range of 10^{-5} M level.

3) Platelet antiaggregatory activities of the compounds were tested for ADP or collagen induced aggregation. As shown in Table IV, all compounds showed no appreciably inhibitory activities.

Table V. Inhibitory effects on PAF induced platelet aggregation (Concentration of PAF : 10 nM)

Compound	Concn. (μg/ml)	% inhibition	Compound	Concn. (μg/ml)	% inhibition	IC_{50} (M)
1	60	-				
2	60	-	**18**	60	91.5	4.7×10^{-5}
3	60	-		30	83.5	
4	60	-		15	38.4	
5	60	-		7.5	23.7	
6	60	-	**19**	60	100.0	4.7×10^{-5}
7	60	11.6		30	67.4	
8	60	45.4		15	36.8	
9	60	1.7		7.5	29.9	
10	60	12.4	**20**	60	93.1	9.7×10^{-5}
11	60	13.3		30	24.5	
12	60	8.8		15	13.2	
13	60	13.8	**pinusolide**	2.0	83.4	2.6×10^{-6}
14	60	54.0		1.0	54.1	
15	60	3.7		1.5	23.1	
16	60	31.0				
17	60	27.7				
21	60	52.6				
22	30	28.8				
23	30	39.3				

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