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6-Amino-2,4-lutidine carboxamides: α-aminoamide derivatives as systemic and topical inflammation inhibitors

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Abstract – The development of new potential anti-inflammatory compounds resulting from the incorporation of α-aminoacid residues into 6-amino-2,4-lutidine afforded (N-protected) aminoamides with interesting inhibitory activity. Out of 28 tested compounds, 10 (**5a**, **5b**, **7d**, **8a**, **8b**, **8d**, **10a**, **11b**, **12a** and **12b**) exerted potent (> 90%) inhibition in the carrageenan foot edema (CFE) rat model after oral administration of 0.4 mmol kg^{-1}. Except for Cbz-glycyl, Cbz-alanyl, Fmoc-valyl and Cbz-alanyl-glycyl derivatives (**5a**, **5b**, **7d** and **11b**), N-deprotection afforded more active compounds. Introduction of a glycyl residue in the previously studied highly active 3-fluorobenzamide **2**, which led to **10a**, maintained potent peripheral edema inhibition but had a detrimental effect in the acute TPA-induced mouse ear-swelling model. Glycylglycinamide **12a**, which had an ID_{50} of 9.0 mg kg^{-1} in the CFE test, appeared to be the most efficient compound tested in this new series of non-carboxylic nonsteroidal anti-inflammatory drugs. Glycinamide **8a**, although less potent in the same assay (14.3 mg kg^{-1}), exerted a significant inhibitory effect in acute and chronic ear-swelling tests after topical application of 3 mg/ear. © Elsevier, Paris

6-amino-2,4-lutidine / α-aminoamide derivatives / systemic and topical inflammation inhibitors

1. Introduction

The therapeutic efficiency of currently available nonsteroidal anti-inflammatory drugs (NSAIDs) is significantly limited by associated gastrointestinal toxicity which causes a higher incidence of morbidity in long-term NSAID users. In previous studies [1-4], we described the synthesis and anti-inflammatory activity of aryl(alkyl)carboxamides issued from 2-amino-4,6-dimethylpyridine. The corresponding benzamide **1** and 3-fluoro derivative **2** exhibited significant inhibition of rat foot pad edema after oral administration: ID_{50} = 35.2 and 12.1 mg kg^{-1}, respectively. Our ongoing interest in this pharmacophore relates to its potential therapeutic benefit. In addition to a significant anti-edematous response, these aryl(alkyl)carboxamides are characterized by their low toxicity (LD_{50}/ID_{50} = 25-50) and lack of notable damage to rat gastrointestinal mucosa [3].

These non-carboxylic NSAIDs do not block the initial step in the biosynthesis of prostaglandins from arachidonic acid, nor do they act by inhibiting the 5-lipoxygenase (5-LO) involved in leukotriene formation [1]. Nevertheless, it has been confirmed that **1** blocks ^{3}H-AA release from cultured mouse peritoneal macrophages [1]. Radioenzymatic determination of in vitro PLA_2 activity in the presence of **1** showed that it had no direct inhibitory effect on the enzyme. More recently, it has been determined that these NSAIDs could act upstream from the phospholipasic level through an inhibitory process involving PLA_2 activation by protein kinase C via MAP kinase. Simultaneous down-regulation of TNFα production by monocytes apparently originates from the same mechanism [5].

The present work focused on the incidence of incorporation of α-aminoacids into the 2-amino-4,6-dimethylpyridine moiety. Numerous oligopeptides have been re-

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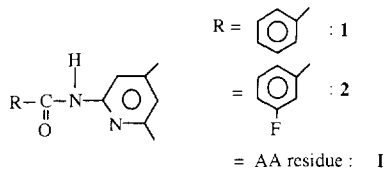
ported to be efficient in the treatment of inflammatory conditions [6-10] or life-threatening inflammation [11].

Moreover, the advantage of incorporating amino acids or oligopeptides into analgesic and antiinflammatory drugs, whether NSAIDs (salicylic and arylalkanoic acid derivatives) or steroidal anti-inflammatory drugs (SAIDs), is well documented [12-15]. Finally, oligopeptides have also been associated with major NSAIDs as anti-ulcerous agents [16].

The fact that N-(4,6-dimethylpyridin-2-yl)alkanecarboxamides also inhibit the inflammatory process [1] prompted us to carry out such pharmacomodulation.



Among the 28 tested new α-aminoamides and dipeptides **I**, glycyl and glycylglycyl derivatives **8a** and **12a** elicited potent inhibitory effects. Therefore, they were selected, along with **5a**, **7d** and **10a**, for evaluation of their topical inhibitory activity in the TPA-induced mouse ear edema test.

Such compounds could be useful in the treatment of psoriatic lesions since it is clearly established that increased levels of prostaglandins and leukotrienes are often found in psoriatic tissues. Clinical results have been obtained with putative 5-LO inhibitors such as benoxaprofen [17] and lonapalene [18], and preclinical results have been reported with the 5-LO inhibitor A64077 [19], hydroxamic acids [20], DuP 654 [21], CGS 8515 [22] and dual (CO and LO) inhibitors such as tepoxalin [23] and FPL 62064 [24].

2. Chemistry

Figure 1 illustrates the procedures used to synthesize compounds **5-12**. In the first step, N^2-Cbz, N^2-Boc or N^2-Fmoc *N*-(4,6,-dimethylpyridin-2-yl)aminoamides **5**, **6** and **7** were obtained under mild conditions by reacting a (*S*)N-protected aminoacid **2**, **3** or **4**, first activated by 1,1'-carbonyldiimidazole, with 2-amino-4,6-dimethylpyridine. After purification by silica gel chromatography, N-Cbz- or Boc-protected aminoamides **5** and **6** were isolated in satisfactory yields (60-80%). Yields were more moderate (45%) in Fmoc subseries **7**. Attempts at improving yields by four alternative procedures (PCl_5, DCC, DCC/HOBT, $PPh_3/XCCl_3$) failed. Catalytic hydrogenation with Pd/C in methanol of the N-Cbz-protected derivatives **5a-h** afforded the corresponding aminoamides **8a-h**. The N-Boc protecting group of **6a** and **6h** was removed by trifluoroacetic acid treatment, and, after neutralization to generate the free amine, the desired amides **8a** and **8h** were obtained in a 65 and 85% yield, respectively. The N^2-3-fluorobenzoyl-aminoamide **10a** was prepared from **8a** by the acid chloride method in the presence of triethylamine. Condensation, via the imidazolide derivative, between the aminoamide **8a** and N-Cbz-glycine and (*S*)-Cbz-alanine afforded dipeptides **11a** and **11b** in fairly good yields (60%). The Cbz-protecting group of these compounds was removed by classical catalytic hydrogenolysis to give peptides **12a** and **12b**.

Protection of the side-chain functions of the hydroxyaminoacids serine **13i** and tyrosine **13j** was successfully performed through direct alkylation of the hydroxy group by treatment with sodium hydride followed by addition of benzyl bromide (*figure 2*). The resulting benzyl ether aminoacids **14i** and **14j** reacted via an imidazolide with 2-amino-4,6-dimethylpyridine to yield N^2-(4,6-dimethylpyridin-2-yl)aminoamides **15i** and **15j**. The benzyl and benzyloxycarbonyl groupings of **15j** were simultaneously cleaved by catalytic hydrogenation, leading to tyrosinamide **16j**.

Tables I, *II* and *III* summarize the experimental and physical data for synthesized compounds.

3. Pharmacology

3.1. Effect in carrageenan foot-pad edema

Most of the N-protected aminoamides **5**, **6**, **7** and **10** exhibited significant anti-inflammatory activity in carrageenan-induced rat-paw edema after administration of 0.4 mmol kg^{-1} by the oral route. Steric hindrance at the level of the R residue reduced the level of activity in **5d**, **5e**, **5g**, **5h** and **6h**, or even suppressed it in **5c** and **5f** which incorporated valyl and phenylalanyl residues. The same phenomenon was observed in the *O*-Bn and *N*-Cbz serinamide and tyrosinamide **15i** and **15j**. It seemed likely that this lack of activity was also at least partly related to their too high lipophilicity.

Glycyl and alanyl derivatives **5a** and **5b** were the most potent compounds in the series. A comparison between the level of activity of the protected glycinamides **5a**, **6a** and **7a** indicated decreased inhibition resulting from the replacement of Cbz by Boc and especially Fmoc grouping. The Cbz-methioninamide **5h** also showed a greater effect than the corresponding Boc derivative **6h**.

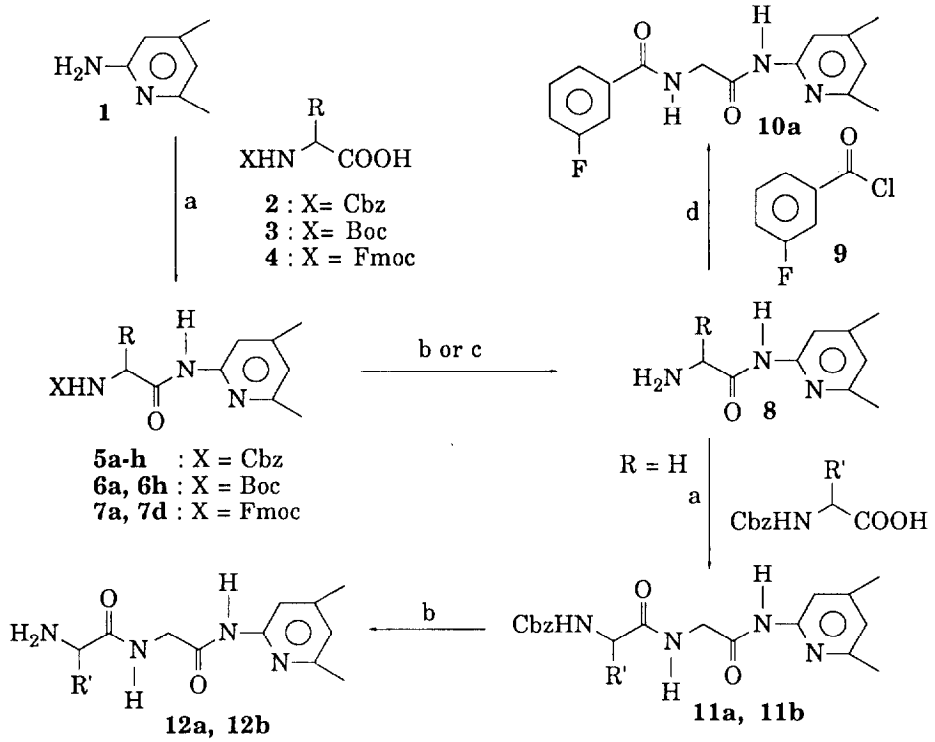


Figure 1. Reagents: (a) CDI, THF, room temperature; (b) Pd/C, MeOH, room temperature in the case of X = Cbz; (c) CF_3COOH, room temperature, Et_3N, in the case of X = Boc; (d) THF, Et_3N, room temperature.

Surprisingly, an opposite effect was observed in the leucyl subseries in which the Fmoc derivative **7d** was more potent than Cbz **5d**. Replacing Cbz in **5a** with the 3-fluorobenzoyl grouping present in the potent benzamide **2** maintained a high level of activity (96.6 and 96.8% for **5a** and **10a**, respectively).

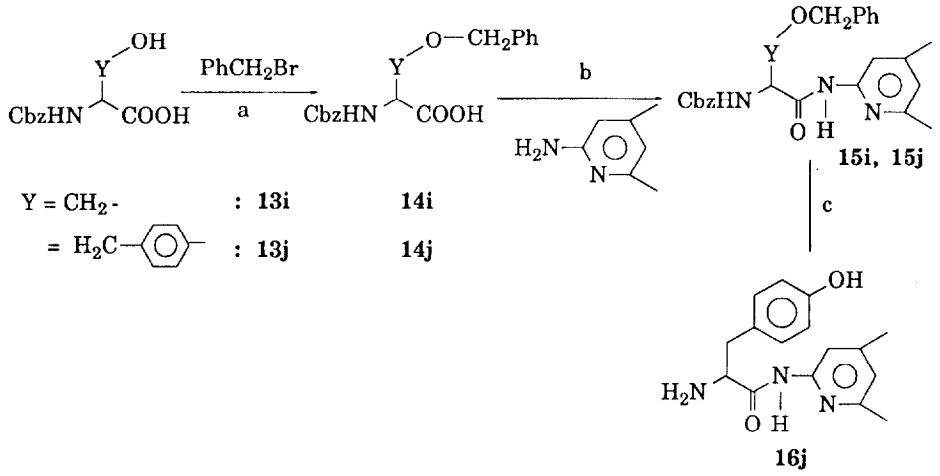
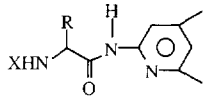
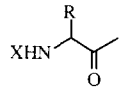
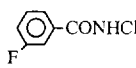


Figure 2. Reagents: (a) NaH, DMF, 0 °C and room temperature; (b) CDI, THF, room temperature; (c) H_2, Pd/C, MeOH, room temperature.

Table I. Physical data and anti-inflammatory activity of N-protected aminoamides.



No.		Formula	Yield (%)	M.p. (°C) solvent[a]	Carrageenan-induced rat-paw edema inhibition %[b]
5a	$CbzNHCH_2CO$–	$C_{17}H_{19}N_3O_3$	77	103	96.6 ± 2.9
5b	$CH_3CH(CbzNH)CO$–	$C_{18}H_{21}N_3O_3$	70	121	94.8 ± 3.2
5c	H_3C, H_3C $CHCH(CbzNH)CO$-	$C_{20}H_{25}N_3O_3$	73	134	NI[c]
5d	H_3C, H_3C $CHCH_2CH(CbzNH)CO$-	$C_{21}H_{27}N_3O_3$	68	114	71.1 ± 9.6
5e	H_3C, H_5C_2 $CHCH(CbzNH)CO$-	$C_{21}H_{27}N_3O_3$	67	84	58.0 ± 7.0
5f	$PhCH_2CH(CbzNH)CO$–	$C_{24}H_{25}N_3O_3$	60	139	NI[c]
5g		$C_{20}H_{22}N_3O_3$	64	165	68.8 ± 8.9
5h		$C_{20}H_{25}N_3O_3S$	70	87	82.9 ± 6.7
6a	$H_3CS(CH_2)_2CH(BocNH)CO$–	$C_{14}H_{21}N_3O_3$	65	120	80.6 ± 5.3
6h	$BocNHCH_2CO$	$C_{17}H_{27}N_3O_3S$	60	97	59.1 ± 9.0
	$H_3CS(CH_2)_2CH(CbzNH)CO$–	$C_{24}H_{23}N_3O_3$	63	186	36.0 ± 7.9
7a	$FmocNHCH_2CO$–	$C_{28}H_{31}N_3O_3$	43.5	97	92.8 ± 4.4
7d	H_3C, H_3C $CHCH_2CH(FmocNH)CO$-				
10a		$C_{16}H_{16}FN_3O_2$	53	161	96.8 ± 3.2
15i	$PhCH_2OCH_2CH(CbzNH)COM$–	$C_{25}H_{27}N_3O_4$	40	112	NI[c]
15j	$PhCH_2O$-  -$CH_2CH(CbzNH)CO$-	$C_{31}H_{31}N_3O_4$	40	122	NI[c]

[a] Diisopropylic ether; [b] after oral administration of 0.4 mmol kg^{-1}; [c] inhibition < 20%.

Generally speaking, *N*-deprotection led to more hydrophilic compounds, resulting in a marked increase of activity, especially in valyl and phenylalanyl derivatives **8c** and **8f** whose precursors were inactive. Only the isoleucyl and tyrosyl congeners **8e** and **16j** exhibited inhibition of less than 80%.

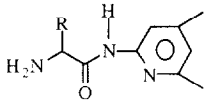
The coupling of glycinamide **8a** with Cbz-glycine exerted a deleterious effect. The activity of **11a** was moderate (44%), but surprisingly activity was maintained in the Cbz-glycylalanyl derivative **11b** (98.5%). Deprotection restored a high level of activity (90%) in **12a**, and that (92%) of **12b** was maintained.

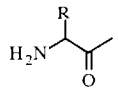
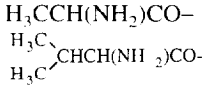
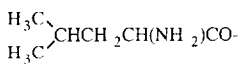
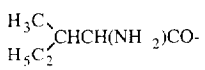
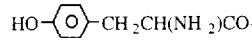
When the potent N-glycinamide **8a** was experimented at a low dose of 0.15 mmol kg^{-1}, the percentage of inhibition remained significant, 1 and 2 h after carrageenan injection (*table IV*). The corresponding glycylglycyl dipeptide **12a** was equipotent to **8a** at the same dose and remained active at a dose of 0.04 mmol kg^{-1}. Their ID_{50} and that of **11b** were 9.0 ± 0.5, 14.3 ± 3.6 and 46.1 ± 11.1 mg kg^{-1}, respectively, 3 h after carrageenan injection.

3.2. Effect in the acute TPA-induced mouse ear-swelling test

Psoriatic skin shares many of the pathologic features of phorbol ester-treated mouse skin, including elevated levels of arachidonic acid metabolism products, epidermal cell proliferation and inflammatory cells [25]. Among the most active *N*-(2,4-lutidinyl)aminoamides, five compounds (**5a**, **7d**, **8a**, **10a** and **12a**) were selected for evaluation of their effect in a model of topical inflammation, the acute TPA-induced mouse ear-swelling test [26]. After topical application of 10 mg/ear, three derivatives exerted a significant reduction in ear thickness: 45, 57 and 64% respectively for **5a**, **7d** and **8a** (*table V*). **8a** re-

Table II. Physical data and anti-inflammatory activity of aminoamides.



No.		Formula	Yield (%)	M.p. (°C) solvent[c]	Carrageenan-induced rat-paw edema inhibition%[d]
8a	H_2N-CH_2CO–	$C_9H_{13}N_3O$	75[a]	105	95.8 ± 3.4
8b	$H_3CCH(NH_2)CO$–	$C_{10}H_{15}N_3O$	38	oil	90.6 ± 1.0
8c		$C_{12}H_{19}N_3O$	61[a]	107	80.3 ± 5.1
8d		$C_{13}H_{21}N_3O$	66[a]	78	94.8 ± 1.9
8e		$C_{13}H_{21}N_3O$	48[a]	81	63.3 ± 7.8
8f	$PhCH_2CH(NH_2)CO$–	$C_{16}H_{19}N_3O$	60[a]	97	80.9 ± 8.5
8g		$C_{12}H_{17}N_3O$	68[a]	oil	86.8 ± 5.4
8h	$H_3CS(CH_2)_2CH(NH_2)CO$–	$C_{12}H_{19}N_3OS$	85[b]	84	86.8 ± 7.1
16j		$C_{16}H_{19}N_3O_2$	34[a]	122	38.7 ± 11.7

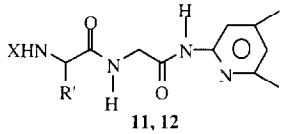
[a] After hydrogenolysis; [b] after acidolysis; [c] diethylic ether; [d] after oral administration of 0.4 mmol kg $^{-1}$.

mained topically effective at 3 mg/ear (57%), and its minimum effective dose was 1mg/ear. Indomethacin, as a reference drug, was also topically effective: 66% inhibition at 2.5 mg/ear (*figure 3*).

3.3. Effect of ***8a*** *in the chronic TPA-induced mouse ear-swelling test*

In the multiple TPA-induced model of chronic inflammation [27], considered to be a relevant model of human psoriasis, **8a** was topically effective on changes in ear thickness from day 7 to day 15 (*table VI*): 18 to 49% inhibition respectively at 3 mg/ear (*figure 4*). The reference compounds, dexamethasone 21-acetate and cyclosporin A, were also topically effective: 29 to 71% respectively at 0.3 mg/ear from day 8 to day 15 and 5 to 58% at 0.1 mg/ear from day 7 to day 15. At day 15, **8a**, dexamethasone 21-acetate and cyclosporin A were also effective

Table III. Physical data and anti-inflammatory activity of dipeptides **11** and **12**.



11, 12

No.	X	R'	Formula	Yield (%)	M.p. (°C) solvent[a]	Carrageenan-induced rat-paw edema inhibition%[b]
11a	Cbz	H	$C_{19}H_{22}N_4O_4$	60	191	43.9 ± 7.2
11b	Cbz	CH_3	$C_{20}H_{24}N_4O_4$	60	113	98.5 ± 1.5
12a	H	H	$C_{11}H_{16}N_4O_2$	62	180 (dec.)	90.0 ± 3.2
12b	H	CH_3	$C_{12}H_{18}N_4O_2$	60	106 (dec.)	91.6 ± 2.7

[a] Diethylic ether; [b] after oral administraton of 0.4 mmol kg^{-1}.

Table IV. Inhibition of rat foot pad edema after oral administration of **8a** and **12a**, measured 1, 2 and 4 h after carrageenan injection.

No.	Dose[a] (mmol kg^{-1})	Inhibition% after		
		1 h	2 h	4 h
8a	0.15	70.9[b]	75.5[b]	38.8
12a	0.15	74.5[b]	79.9[b]	49.7
	0.04	61.3[b]	50.1[b]	26.9

[a] Number of animals: 8; [b] $p < 0.05$, Anova, Newman-Keuls test.

Table V. Effect of **5a**, **7d**, **8a**, **10a** and **12a** in the acute PMA-induced mouse ear-swelling test.

No.	Inhibition% after topical application of		
	10 mg/ear	3 mg/ear	1 mg/ear
5a	45[a]		
7d	57[a]		
8a	64[a]	57[a]	31
10a	27 (NS)		
12a	15 (NS)		
indomethacin		66[a]	

[a] $p < 0.001$; $n = 5$.

Table VI. Inhibition of net swelling of daily ear thickness after multiple topical application of phorbol ester and test compound in groups of 8 animals.

Day	7	8	9	10	11	12	13	14	15
8a	18	37	44	46	49	47	35	49	41
dexamethasone 21-acetate	0	29	54	55	62	66	61	71	67
cyclosporin A	5	30	47	51	58	51	43	47	45

on ear weight (*figure 5*), affording 53, 70 and 53% inhibition at 3 mg/ear, 0.3 mg/ear and 0.1 mg/ear, respectively.

4. Discussion and conclusion

Among the test compounds, 10 derivatives blocked carrageenan-triggered prostaglandin production effectively, promoting an inflammatory response: Cbz-α-aminoamides **5a** and **5b**, Fmoc-leucinamide **7d**, their N-deprotected derivatives **8a**, **8b** and **8d**, N-(3-fluorobenzoyl)glycinamide **10a**, and the three dipeptides **11b**, **12a** and **12b**. Such non-carboxylic NSAIDs, especially the most potent derivatives **8a** and **12a**, could be safer than classical arylalkanoic acids, which are known to be associated with gastrointestinal intolerance, particu-

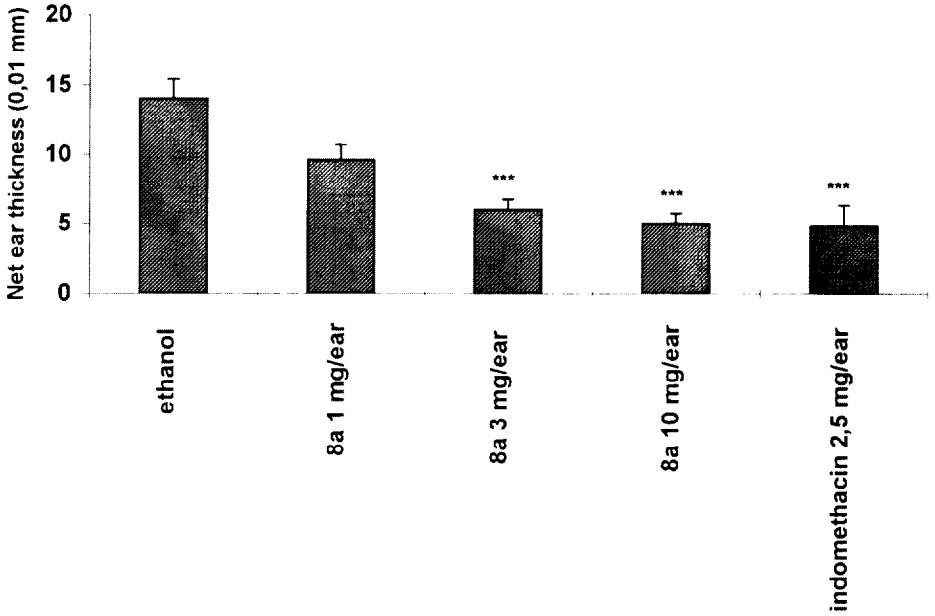


Figure 3. Effect of **8a** in the acute TPA-induced mouse ear-swelling test: reduction in net ear thickness; ***: $p < 0.001$, ANOVA, Newman-Keuls test, treated group versus control group, $n = 5$.

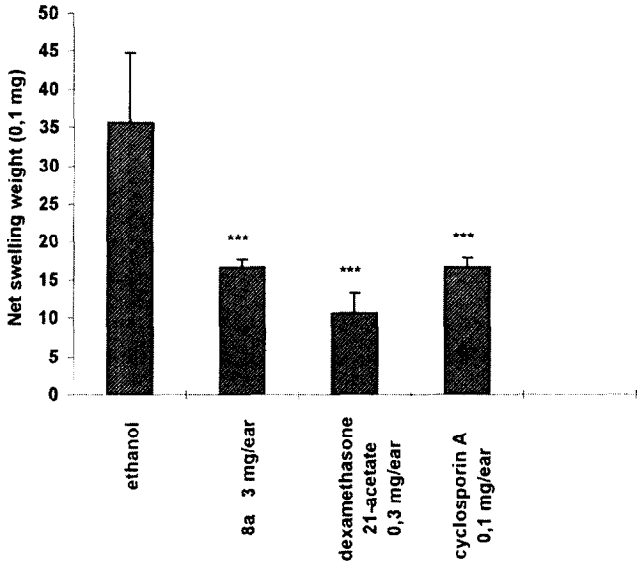


Figure 4. Effect of **8a** in the subchronic TPA-induced mouse ear-swelling test: reduction in net ear weight measured at day 15, $n = 8$; ***: $p < 0.0001$.

larly since the corresponding benzamide **1**, experimented in a rat pylorus ligature assay, proved devoid of ulcerogenous liability [3].

Glycinamide **8a** also exhibited a potent inhibitory effect in topical treatment of ear edema. It is well-established that prostaglandin concentrations are elevated

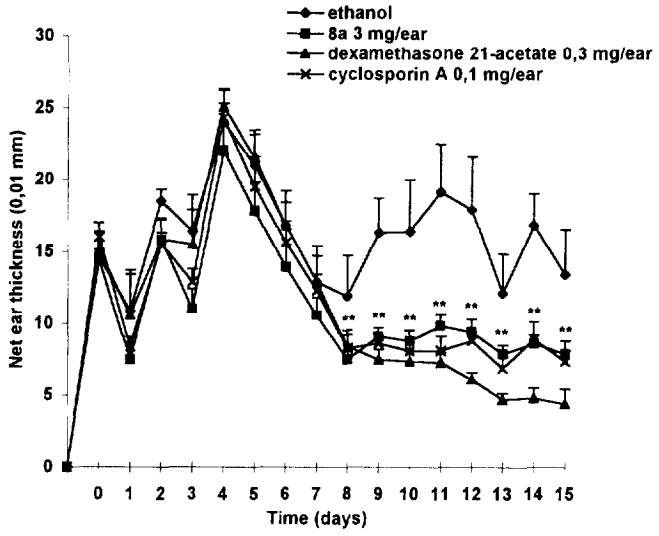


Figure 5. Effect of **8a** in the subchronic TPA-induced mouse ear-swelling test: net daily ear thickness after multiple application; $n = 8$; **: $p < 0.01$.

in psoriatic skin [28]. Moreover, compared to normal human skin, psoriatic lesions possess 7 to 11 times the level of chemoattractants LTB_4 and 12-HETE, so that even non-involved skin of psoriatic patients showed enhanced LO activity [29]. Increased expression of human non-pancreatic phospholipase A_2 was also found in psoriatic tissue [30]. Finally, evidence was found in psoriatic skin of the up-regulation of various cytokines (IL-1, TNFα) that stimulate endothelial cell adhesiveness for lymphocytes [31]. Taken together, these results suggest that our compounds, which interfere with arachidonic acid biosynthesis, through a mechanism of action involving an indirect blockade of PLA_2 [5], could also be potential antipsoriatic agents. Moreover, their ability to inhibit TNFα production could be a major factor in their anti-inflammatory effect.

Until now, varying effects have been observed with 5-LO inhibitors in the topical treatment of psoriasis. lonapalene (RS43179) induced a relevant anti-psoriatic effect [32], whereas MK 886 [33] and R 85355 [34] proved ineffective in treating plaque psoriasis at a dosage inducing significant inhibition of LTB_4 production.

Simultaneous inhibition of prostaglandin, leukotriene and TNFα overexpression in diseased skin after topical treatment with **8a** could induce more appropriate therapeutic responses in psoriasis treatment [35].

The overexpression of adhesion molecules in psoriatic skin prompted us to investigate the interaction of **8a** and analogous derivatives with integrin expression and lymphocytes. Corresponding histopathologic and human polymorphonuclear chemotaxis studies are now in progress.

5. Experimental protocols

5.1. Chemistry

Melting points, as determined in open capillary tubes with a Tottoli-Büchi apparatus, are uncorrected. Infrared spectra were obtained on a Beckman IR 4230 spectrophotometer using KBr pellets or an NaCl disk. The ^{1}H-NMR spectra were recorded on a Bruker AC-250 instrument (250 MHz); chemical shifts are expressed in δ units (ppm) using the solvent signal as reference. Microanalyses for C, H, N were performed using a Perkin Elmer C,H,N 240 apparatus. Analyses, indicated by the element symbols, were within ± 0.4% of theoretical values.

*5.1.1. General procedure for the preparation of N^2-protected aminoamides **5**, **6**, **7**, **11** and **15***

*N^2-Benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl) glycinamide **5a***: Cbz-glycine (2.09 g, 10 mmol) was dissolved in dry THF (20 mL), and carbonyldiimidazole (1.94 g, 12 mmol) was added. The mixture was stirred at room temperature for 1 h. 2-amino-4,6-

dimethylpyridine (1.22 g, 10 mmol) was then added, and the mixture was stirred overnight at room temperature. The solvent was evaporated and the residue chromatographed on silica gel (diethyl ether) to yield 2.41 g of pure N^2-benzyloxycarbonyl-*N*-(4,6-dimethylpyridin-2-yl) glycinamide as a white solid: IR (KBr) 3220, 3090, 1720, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.32 (s, 3H, 4-CH_3), 2.40 (s, 3H, 6-CH_3), 4.06 (d, J = 5.3 Hz, 2H, CH_2), 5.17 (s, 2H, OCH_2), 5.57 (m, 1H, N*H*CH_2), 6.76 (s, 1H, pyr. H-5), 7.36 (m, 5H, Ph-H), 7.82 (s, 1H, pyr. H-3), 8.34 (m, 1H, NH amide). Anal. $C_{17}H_{19}N_3O_3$ C,H,N.

The following compounds were prepared according to an analogous procedure from the corresponding N-protected (Cbz, Boc or Fmoc) aminoacid:

N^2-Benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl)alaninamide ***5b***: White solid. IR (KBr) 3300, 3100, 1720, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.45 (d, J = 7.0 Hz, 3H, CH_3), 2.30 (s, 3H, 4-CH_3), 2.38 (s, 3H, 6-CH_3), 4.52 (m, 1H, C*H*NH), 5.10 (d, J = 12.2 Hz, 1H, OC*H*H), 5.18 (d, J = 12.2 Hz, 1H, OCH*H*), 5.85 (d, J = 7.4 Hz, N*H*CH), 6.73 (s, 1H, pyr. H-5), 7.32 (m, 5H, Ph-H), 7.85 (s, 1H, pyr. H-3), 8.87 (m, 1H, NH amide). Anal. $C_{18}H_{21}N_3O_3$ C,H,N.

N^2-Benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl)valinamide ***5c***: White solid. IR (KBr) 3250, 1710, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.94 (d, J = 6.8 Hz, 3H, CH_3), 1.01 (d, J = 6.8 Hz, 3H, CH_3), 2.19 (sept., J = 6.8 Hz, 1H, CH), 2.27 (s, 3H, 4-CH_3), 2.31 (s, 3H, 6-CH_3), 4.25 (m, 1H, C*H*NH), 5.10 (d, J = 12.2 Hz, 1H, OC*H*H), 5.18 (d, J = 12.2 Hz, 1H, OCH*H*), 5.50 (m, 1H, N*H*CH), 6.74 (s, 1H, pyr. H-5), 7.35 (m, 5H, Ph-H), 7.86 (s, 1H, pyr. H-3), 8.43 (m, 1H, NH amide). Anal. $C_{20}H_{25}N_3O_3$ C,H,N.

N^2-Benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl)leucinamide ***5d***: White solid. IR (KBr) 3300, 3120, 1715, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.82 (d, J = 5.6 Hz, 3H, CH_3), 0.86 (d, J = 6.0 Hz, 3H, CH_3), 1.62 (m, 3H, CH_2 and CH), 2.27 (s, 3H, 4-CH_3), 2.37 (s, 3H, 6-CH_3), 4.52 (m, 1H, C*H*NH), 5.04 (d, J = 12.3 Hz, 1H, OC*H*H), 5.16 (d, J = 12.3 Hz, 1H, OCH*H*), 5.98 (m, 1H, N*H*CH), 6.70 (s, 1H, pyr. H-5), 7.28 (m, 5H, Ph-H), 7.86 (s, 1H, pyr. H-3), 9.38 (m, 1H, NH amide). Anal. $C_{21}H_{27}N_3O_3$ C,H,N.

N^2-Benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl)isoleucinamide ***5e***: White solid. IR (KBr) 3270, 1715, 1665 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, J = 7.5 Hz, 3H, CH_3), 0.97 (d, J = 6.8 Hz, 3H, CH_3), 1.20 (m, 1H, C*H*HCH_3), 1.55 (m, CH*H*CH_3), 1.96 (m, 1H, CH), 2.29 (s, 3H, 4-CH_3), 2.37 (s, 3H, 6-CH_3), 4.34 (m, 1H, C*H*NH), 5.09 (d, J = 12.3 Hz, 1H, OC*H*H), 5.18 (d, J = 12.3 Hz, 1H, OCH*H*), 5.66 (m, 1H, N*H*CH), 6.73 (s, 1H, pyr. H-5), 7.33 (m, 5H, Ph-H), 7.87 (s, 1H, pyr. H-3), 8.61 (m, 1H, NH amide). Anal. $C_{21}H_{27}N_3O_3$ C,H,N.

N^2-Benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl)phenylalaninamide ***5f***: White solid. IR (KBr) 3230, 3080, 1720, 1685 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.32 (s, 3H, 4-CH_3), 2.40 (s, 3H, 6-CH_3), 3.13(dd, J = 13.9 Hz and 9.1 Hz, 1H, C*H*HPh), 3.22 (dd, J = 13.9 Hz and 6.1 Hz, 1H, CH*H*Ph), 4.61 (m, 1H, CH), 5.10 (d, J = 12.3 Hz, 1H, OC*H*H), 5.15 (d, J = 12.3 Hz, 1H, OCH*H*), 5.48 (m, 1H, N*H*CH), 6.77 (s, 1H, pyr. H-5), 7.30 (m, 10H, Ph-H), 7.88 (s, 1H, pyr. H-3), 8.60 (m, 1H, NH amide). Anal. $C_{24}H_{25}N_3O_3$ C,H,N.

N^2-Benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl)prolinamide ***5g***: White solid. IR (KBr) 3280, 1710, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.93 (m, 4H, Prol.CH_2), 2.30 (s, 3H, 4-CH_3), 2.39 (s, 3H, 6-CH_3), 3.61 (m, 2H, Prol.CH_2), 4.48 (m, 1H, Prol.CH), 5.19 (m, 2H, OCH_2), 6.73 (s, 1H, pyr. H-5), 7.35 (m, 5H, Ph-H), 7.86 (s, 1H, pyr. H-3), 8.92 (m, 1H, NH amide). Anal. $C_{20}H_{23}N_3O_3$ C,H,N.

N^2-Benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl)methioninamide ***5h***: White solid. IR (KBr) 3216, 1720, 1673 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.00 (m, 1H, 1H, CHC*H*H), 2.09 (s, 3H, SCH_3), 2.18 (m, 1H, 1H, CHCH*H*), 2.31 (s, 3H, 4-CH_3), 2.40 (s, 3H, 6-CH_3), 2.61 (m, 2H, CH_2S), 4.54 (m, 1H, CH), 5.12 (d, J = 12.2 Hz, 1H, OC*H*H), 5.17 (d, J = 12.2 Hz, 1H, OCH*H*), 5.67 (d, J = 7.9 Hz, 1H, N*H*CH), 6.75 (s, 1H, pyr. H-5), 7.35 (m, 5H, Ph-H), 7.83 (s, 1H, pyr. H-3), 8.55 (m, 1H, NH amide). Anal. $C_{20}H_{25}N_3O_3S$ C,H,N.

N^2-Benzyloxycarbonyl-O-benzyl-N-(4,6-dimethylpyridin-2-yl) serinamide ***15i***: To a stirred solution of *N*-benzyloxycarbonyl-L-serine (2.40 g, 10 mmol) in dry DMF (50 mL) was added sodium hydride (60% in dispersion in oil, 1 g, 24 mmol). When no more gas evolved, benzyl bromide (1.25 mL, 10 mmol) was added, and the mixture was stirred at room temperature for 6 h. The solvent was removed in vacuo and the residue dissolved in water (50 mL) and extracted with ether. The aqueous layer was acidified to pH 3.5 with 1M HCl and then extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the resulting colorless oil corresponding to the *N*-benzyloxycarbonyl-*O*-benzyl-L-serine **14i** [36] was condensed without further purification with 2-amino-4,6-dimethylpyridine, after imidazolide (CDI) formation, according to the general procedure used for compound **5a**. N^2-Benzyloxycarbonyl-*O*-benzyl-*N*-(4,6-dimethylpyridin-2-yl) serinamide was obtained as a white solid after purification by column chromatography (diethyl ether). IR (KBr) 3280, 1710, 1670 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.30 (s, 3H, 4-CH_3), 2.37 (s, 3H, 6-CH_3), 4.0 (dd, J = 3.44 and 12.0 Hz, 1H, C*H*H), 4.51 (dd, J = 11.8 and 9.2 Hz, 1H, CH*H*), 5.08 (s, 2H, OCH_2), 5.39 (m, 1H, CH), 6.75 (s, 1H, H-5), 7.40 (m, 10H, Ph-H), 7.70 (d, J = 8.40 Hz, 1H, NH), 7.86 (s, 1H, H-3), 8.30 (s, 1H, NH amide). Anal. $C_{25}H_{27}N_3O_4$ C,H,N.

N^2-Benzyloxycarbonyl-O-benzyl-N-(4,6-dimethylpyridin-2-yl) tyrosinamide ***15j***: *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine **14j** was prepared similarly to *N*-benzyloxycarbonyl-*O*-benzyl serine [33]. After extraction with ether, the aqueous layer was acidified to pH = 3.5.

The precipitate corresponding to **14j** was filtered and condensed without further purification with CDI and 2-amino-4,6-dimethylpyridine. Compound **15j** was then obtained as a white solid after column chromatography (diethyl ether). IR 3280, 1710, 1670 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.32 (s, 3H, 4-CH_3), 2.37 (s, 3H, 6-CH_3), 3.15 (m, 2H, CH_2), 4.58 (m, 1H, CH), 5.00 (s, 2H, OCH_2), 5.06 (d, J = 12.3 Hz, 1H, OC*H*H), 5.15 (s, J = 12.2 Hz, 1H, OCH*H*), 5.40 (d, J = 8.3 Hz, 1H, NH), 6.74 (s, 1H, H-5), 6.86 (d, J = 8.6 Hz, 2H, Tyr-H), 7.08 (d, J = 8.5 Hz, 2H, Tyr-H), 7.35 (m, 5H, Ph-H), 7.86 (s, 1H, H-3), 8.47 (s, 1H, NH amide). Anal. $C_{31}H_{31}N_3O_4$ C,H,N.

*N^2-Benzyloxycarbonyl*N*-(4,6-dimethylpyridin-2-yl)glycyl-glycinamide* ***11a***: White solid. IR 3400, 3280, 1720, 1700, 1660 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.29 (s, 3H, 4-CH_3), 2.38 (s,

3H, 6-CH_3), 3.72 (d, J = 6.10 Hz, 2H, CH_2), 3.97 (d, J = 6.5 Hz, 2H, CH_2), 5.08 (s, 2H, OCH_2), 6.84 (s, 1H, H-5), 7.39 (m, 5H, Ph-H), 7.55 (t, J = 6.0 Hz, 1H, NH), 7.75 (s, 1H, H-3), 8.19 (t, J = 5.5 Hz, 1H, NH), 10.35 (s, 1H NH amide). Anal. $C_{19}H_{22}N_4O_4$ C,H,N.

N^2-BenzyloxycarbonylN-(4,6-dimethylpyridin-2-yl)alanylglycinamide ***11b***: White solid. IR 3350, 3280, 1720, 1700, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 1.28 (d, J = 7.1 Hz, 3H, CH_3), 2.29 (s, 3H, 4-CH_3), 2.37 (s, 3H, 6-CH_3), 3.90 (dd, J = 17.1 Hz and 5.6 Hz, 1H, C*H*HNH), 3.97 (dd, J = 17.1 Hz and 5.9 Hz, 1H, CH*H*NH), 4.13 (quint., J = 7.3 Hz, CH), 5.05 (d, J = 12.7 Hz, 1H, OC*H*H), 5.10 (d, J = 12.7 Hz, 1H, OCH*H*), 6.84 (s, 1H, H-5), 7.40 (m, 5H, Ph-H), 7.55 (d, J = 7.8 Hz, N*H*CH), 7.75 (s, 1H, H-3), 8.20 (m, 1H, N*H*CH_2), 10.32 (s, 1H, NH amide). Anal. $C_{20}H_{24}N_4O_4$ C,H,N.

N^2-tertbutoxycarbonyl-N-(4,6-dimethylpyridin-2-yl)glycinamide ***6a***: White solid; IR (KBr) 3230, 3100, 1710, 1685 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.48 (s, 9H, CH_3), 2.34 (s, 3H, 4-CH_3), 2.44 (s, 3H, 6-CH_3), 3.99 (d, J = 5.6 Hz, 2H, CH_2), 5.24 (m, 1H, N*H*CH_2), 6.78 (s, 1H, pyr. H-5), 7.90 (s, 1H, pyr. H-3), 8.87 (m, 1H, NH amide). Anal. $C_{14}H_{21}N_3O_3$ C,H,N.

N^2-tertbutoxycarbonyl-N-(4,6-dimethylpyridin-2-yl)methioninamide ***6h***: White solid. IR (KBr) 3230, 1720, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.46 (s, 9H, CH_3), 1.97 (m, 1H, 1H, CHC*H*H), 2.12 (s, 3H, SCH_3), 2.22 (m, 1H, CHCH*H*), 2.32 (s, 3H, 4-CH_3), 2.41 (s, 3H, 6-CH_3), 2.60 (m, 2H, SCH_2), 4.43 (m, 1H, CH), 5.23 (m, 1H, N*H*CH), 6.76 (s, 1H, pyr. H-5), 7.84 (s, 1H, pyr. H-3), 8.41 (m, 1H, NH amide). Anal. $C_{17}H_{27}N_3O_3S$ C, H, N.

N^2-Fluorenylmethyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl)glycinamide ***7a***: White solid. IR (KBr) 3240, 3100, 1710, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.20 (s, 3H, 4-CH_3), 2.38 (s, 3H, 6-CH_3), 3.87 (d, J = 6.1 Hz, 2H, CH_2), 4.34 (m, 3H, OCH_2 and CH), 6.84 (s, 1H, H-5), 7.40 (m, 4H, Ph-H), 7.46 (t, J = 6,1 Hz, 1H, NH), 7.75 (m, 3H, H-3 and Ph-H), 7.93 (m, 2H, Ph-H), 10.35 (s, 1H, NH amide). Anal. $C_{24}H_{23}N_3O_3$ C,H,N.

N^2-Fluorenylmethyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl)leucinamide ***7d***: White solid. IR (KBr) 3220, 1720, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.94 (m, 6H, $2CH_3$), 1.70 (m, 3H, CH_2 and CH), 2.20 (s, 3H, 4-CH_3), 2.41 (s, 3H, 6-CH_3), 4.21 (m, 1H, CH), 4.42 (m, 3H, OCH_2 and C*H*NH), 5.42 (m, 1H, NH), 6.75 (s, 1H, H-5), 7.30 (m, 4H, Ph-H), 7.57 (m, 2H, Ph-H), 7.77 (m, 2H, Ph-H), 7.86 (s, 1H, H-3), 8.55 (s, 1H, NH amide). Anal. $C_{28}H_{31}N_3O_3$ C,H,N.

5.1.2. General procedure for the preparation of aminoamides ***8a-g*** *and* ***16j***

N^2-(4,6-dimethylpyridin-2-yl)glycinamide ***8a***: A mixture of N^2-benzyloxycarbonyl-*N*-(4,6-dimethylpyridin-2-yl)glycinamide **5a** (3.14 g, 10 mmol), 5% Pd/C (2 g, 1 mmol) and 80 mL of methanol was hydrogenated at room temperature and at atmospheric pressure until no more hydrogen was consumed. Filtration of Pd/C, evaporation of the solvent and recrystallization from dichoromethane and diisopropylic ether yielded 1.35 g of **8a** as a white solid: IR (KBr) 3380, 3280, 3240, 1665 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.20 (m, 2H, NH_2), 2.32 (s, 3H, 4-CH_3), 2.42 (s, 3H, 6-CH_3), 3.50 (s, 2H, CH_2), 6.72 (s, 1H, pyr. H-5), 7.87 (s, 1H, pyr. H-3), 9.64 (m, 1H, NH amide). Anal. $C_9H_{13}N_3O$ C,H,N.

The following compounds were prepared in the same manner as **8a** from the corresponding N^2-benzyloxycarbonyl-*N*-(4,6-dimethylpyridin-2-yl) carboxamides:

N-(4,6-Dimethylpyridin-2-yl)alaninamide ***8b***: Yellow oil. IR (NaCl) 3300, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.40 (d, J = 7.0 Hz, 3H, CH_3), 2.20 (m, 2H, NH_2), 2.28 (s, 3H, 4-CH_3), 2.39 (s, 3H, 6-CH_3), 3.60 (q, J = 7.0 Hz, 1H, CH), 6.70 (s, 1H, pyr. H-5), 7.86 (s, 1H, pyr. H-3), 9.80 (m, 1H, NH amide). Anal. $C_{10}H_{15}N_3O$ C,H,N.

N-(4,6-Dimethylpyridin-2-yl)valinamide ***8c***: Pale oil. IR (NaCl) 3380, 3260, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.86 (d, J = 6.9 Hz, 3H, CH_3), 1.01 (d, J = 6.9 Hz, 3H, CH_3), 1.80 (m, 2H, NH_2), 2.28 (s, 3H, 4-CH_3), 2.38 (m, 4H, 6-CH_3 and CH), 3.41 (d, J = 3.8 Hz, 1H, CH), 6.70 (s, 1H, pyr. H-5), 7.89 (s, 1H, pyr. H-3), 8.72 (m, 1H, NH amide). Anal. $C_{12}H_{19}N_3O$ C,H,N.

N-(4,6-Dimethylpyridin-2-yl)leucinamide ***8d***: White solid. IR (KBr) 3360, 3240, 1665 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.96 (d, J = 6.0 Hz, 3H, CH_3), 0.98 (d, J = 6.2 Hz, 3H, CH_3), 1.40 (m, 1H, CH), 1.61 (m, 2H, NH_2), 1.79 (m, 2H, CH_2), 2.31 (s, 3H, 4-CH_3), 2.41 (s, 3H, 6-CH_3), 3.51 (dd, J = 9.7 and 3.7 Hz, $CHNH_2$), 6.78 (s, 1H, pyr. H-5), 7.90 (s, 1H, pyr. H-3), 9.72 (m, 1H, NH amide). Anal. $C_{13}H_{21}N_3O$ C,H,N.

N-(4,6-Dimethylpyridin-2-yl)isoleucinamide ***8e***: White solid. IR (KBr) 3360, 3300, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.91 (t, J = 7.3 Hz, 3H, CH_3), 1.02 (d, J = 7.0 Hz, 3H, CH_3), 1.14 (m, 1H, C*H*HCH_3), 1.40 (m, 1H, CH*H*CH_3), 1.80 (m, 2H, NH_2), 2.11 (m, 1H, CH_3C*H*), 2.31 (s, 3H, 4-CH_3), 2.41 (s, 3H, 6-CH_3), 3.40 (d, J = 3.7 Hz, 1H, CH), 6.73 (s, 1H, pyr. H-5), 7.93 (s, 1H, pyr. H-3), 9.80 (m, 1H, NH amide). Anal $C_{13}H_{21}N_3O$ C,H,N.

N-(4,6-Dimethylpyridin-2-yl)phenylalaninamide ***8f***: White solid. IR (KBr) 3350, 3240, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.22 (m, 2H, NH_2), 2.32 (s, 3H, 4-CH_3), 2.40 (s, 3H, 6-CH_3), 2.76 (dd, J = 13.7 and 9.8 Hz, 1H, C*H*HPh), 3.42 (dd, J = 13.7 and 3.8 Hz, 1H, CH*H*Ph), 3.72 (dd, J = 9.8 and 3.8 Hz, CH), 6.74 (s, 1H, pyr. H-5), 7.26 (m, 5H, Ph-H), 7.88 (s, 1H, pyr. H-3), 9.80 (m, 1H, NH amide). Anal. $C_{16}H_{19}N_3O$ C,H,N.

N-(4,6-Dimethylpyridin-2-yl)prolinamide ***8g***: Oil. IR (NaCl) 3275, 1665 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.66 (m, 2H, Prol.3-CH_2), 1.93 (m, 1H, Prol.4-C*H*H), 2.12 (m, 1H, Prol.4-CH*H*), 2.21 (s, 3H, 4-CH_3), 2.31 (s, 3H, 6-CH_3), 2.95 (m, 2H, Prol.2-CH_2), 3.78 (dd, J = 9.2 and 5.2 Hz, 1H, Prol.CH), 6.99 (s, 1H, pyr. H-5), 7.81 (s, 1H, pyr. H-3), 9.99 (m, 1H, NH amide). Anal. $C_{12}H_{17}N_3O$ C,H,N.

N-(4,6-Dimethylpyridin-2-yl)tyrosinamide ***16j***: White solid. IR (KBr) 3390, 3280, 1676 cm^{-1}, ^{1}H-NMR (DMSO-d_6) δ 2.30 (s, 3H, 4-CH_3), 2.37 (s, 3H, 6-CH_3), 2.62 (dd, J = 13.6 and 8.2 Hz, 1H, C*H*H), 2.95 (dd, J = 13.6 and 3.7 Hz, 1H, CH*H*), 3.21 (m, 2H, NH_2), 3.63 (m, 1H, CH), 6.70 (d, J =.6.9 Hz, 2H, Tyr.H), 6.84 (s, 1H, H-5), 7.07 (d, J = 7.1 Hz, 2H, Tyr.H), 7.82 (s, 1H, H-3), 9.20 (s, 1H, NH amide), 10.20 (m, 1H, OH). Anal. $C_{16}H_{19}N_3O_2$ C,H,N.

N-(4,6-Dimethylpyridin-2-yl)glycylglycinamide ***12a***: White solid. IR 3340, 3300, 3250, 1660, 1650 cm^{-1}; ^{1}H-NMR (DMSO-

d_6) δ 2.28 (s, 3H, 4-CH_3), 2.38 (s, 3H, 6-CH_3), 2.99 (m, 2H, NH_2), 3.25 (s, 2H, C*H*2NH_2), 3.99 (m, 2H, C*H*2NH), 6.84 (s, 1H, H-5), 7.74 (s, 1H, H-3), 8.30 (m, 1H NH), 10.40 (s, 1H, NH amide). Anal. $C_{11}H_{16}N_3O_2$ C,H,N.

N-(4,6-Dimethylpyridin-2-yl)alanylglycinamide **12b**: White solid. IR 3350, 3200, 3080, 1690, 1660 cm $^{-1}$; ^{1}H-NMR (DMSO-d_6) δ 1.20 (d, *J* = 9.2 Hz, 3H, CH_3), 2.29 (s, 3H, 4-CH_3), 2.38 (s, 3H, 6-CH_3), 3.40 (m, 2H, NH_2), 3.96 (m, 3H, CH_2 and CH), 6.84 (s, 1H, H-5), 7.74 (s, 1H, H-3), 8.25 (m, 1H, NH), 10.38 (s, 1H, NH amide). Anal. $C_{12}H_{18}N_3O_2$ C,H,N.

Deprotection of N-Boc aminoamide 5h. N-(4,6-Dimethylpyridin-2-yl)methioninamide **8h**: N^2-tertbutoxycarbonyl-*N*-(4,6-dimethylpyridin-2-yl)methioninamide **6h** (3.63 g, 10 mmol) was dissolved in trifluoroacetic acid and left to stand at room temperature for 1 h. The solution was then evaporated to dryness. The resulting oil was dissolved in dichloromethane, and triethylamine was added to the mixture which was washed with water. The organic layer was dried ($MgSO_4$), filtered, and concentrated to give the crude product as a white solid. IR (KBr) 3400, 3240, 1670 cm $^{-1}$; ^{1}H-NMR ($CDCl_3$) δ 1.85 (m, 3H, 1H CHC*H*H and NH_2), 2.12 (s, 3H, SCH_3), 2.23 (m, 1H, CHCH*H*), 2.26 (s, 3H, 4-CH_3), 2.41 (s, 3H, 6-CH_3), 2.66 (m, 2H, SCH_2), 3.64 (dd, *J* = 8.3 and 4.4 Hz, CH), 6.74 (s, 1H, pyr. H-5), 7.89 (s, 1H, pyr. H-3), 9.73 (m, 1H, NH amide). Anal. $C_{12}H_{19}N_3OS$ C,H,N.

N^2-Acylation of glycinamide **8a**; *N^2-(3-Fluoro)benzoyl-N-(4,6-dimethylpyridin-2-yl)glycinamide* **10a**: A solution of 3-fluorobenzoyl chloride (1.5 g, 10 mmol) in dry ether (15 mL) was added to a stirred solution of *N*-(4,6-dimethylpyridin-2-yl) glycinamide **8a** (1.8 g, 10 mmol) and triethylamine (1.3 mL, 10 mmol) in dry ether (50 mL). The mixture was then stirred at room temperature for 3 h. The triethylamine hydrochloride was filtered and the solvent evaporated. Recrystallisation from diisopropylic ether provided the pure product as a wite solid. IR (KBr) 3300, 1680, 1600, 1530 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.29 (s, 3H, 4-CH_3), 2.39 (s, 3H, 6-CH_3), 4.13 (d, *J* = 5.8 Hz, 2H, CH_2), 6.85 (s, 1H, pyr. H-5), 7.60 (m, 5H, pyr. H-3 and Ph-H), 8.96 (m, 1H, N*H*CH_2), 10.48 (m, 1H, NH amide). Anal. $C_{16}H_{16}FN_3O_2$ C,H,N.

5.2. *Pharmacology*

5.2.1. *Carrageenan-induced rat-paw edema*

The inhibitory activity of the studied molecules on carrageenan-induced rat-paw edema was determined according to the method of Winter et al. [37], with slight modifications. The drugs were orally administered 1 h before injection of 0.05 mL of a 1% suspension of carrageenan in saline into the subcutaneous tissues of one hind paw. The other hind paw was injected identically with 0.05 mL of a saline solution. Since the hydration state of animals can modify the intensity of swelling, rats were fasted 24 h before the experiment, and water (1.5 mL/100 g body weight) was orally administered twice (20 h and 4 h) before injections. The volumes of both hind paws of control and treated animals were measured with a plethysmograph 3 h after injection. Rats were kept in the same experimental conditions.

The inhibition percentage of the inflammatory reaction was determined for each animal by comparison with controls and calculated with the following formula:

$$I(\%) = (1 - dt / dc) \times 100$$

where d*t* is the difference in paw volume in the drug-treated group and d*c* the difference in paw volume in the control group. Data are expressed as mean ± SE.

5.2.2. *Acute and subchronic phorbol ester-induced mouse ear-swelling test*

Male or female Charles River-derived ICR mice (20-24 g) were purchased from the Animal Resources Center (College of Medecine, National Taiwan University) and housed (10 mice per cage) in a light-controlled (12-h light/day) and temperature-controlled (23 ± 1 °C) environment. The animals were kept in plastic boxes on sawdust with ad libitum access to tap water and laboratory chow (Taiwan Co. Sugar Products).

In the acute mouse ear-swelling test [26], 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Sigma) (5 μL in 20 μL ethanol:water in an 8:2 ratio) was applied topically in a single dose to the inner and outer surfaces of the right ear of mice. The mice were randomly divided into five groups: vehicle; 1, 3 and 10 mg per ear of **8a** and reference compounds, indomethacin at 3mg/ear. The appropriate doses of **8a** were dissolved in 95% ethanol (vol/vol) and applied topically on the right ear (2 × 20 μL at 5-min intervals in absolute alcohol), 30 min before TPA application. The intact group of the left ears of the **8a** treated groups received the vehicle alone. Ear thickness (mm) as an index of inflammation was measured after 6 h in 5 mice per group using a Dyer model micrometer gauge (Dyer Co. Inc., Lancaster, PA, USA).

In the subchronic mouse ear-swelling test [27], TPA (1 μg in 20 μL of 95% ethanol per ear) was applied topically on days 0, 2, 4, 7, 11 and 14. The mice were randomly divided at day 6 into four groups: vehicle (ethanol), **8a** at 3 mg per ear and reference compounds, dexamethasone 21-acetate at 0.3 mg per ear and cyclosporin A at 0.1 mg per ear. **8a**, reference compounds and vehicle were applied topically 30 min before and after TPA application on days 7, 8, 9, 10, 11, 12, 13, 14, with only a single application on day 15. On day 15, the animals were sacrificed by cervical dislocation, after ear thickness measurement. Uniform pieces of ear were punched out (5.2 mm diameter punch) and individually weighed. The ear thickness (mm) of a group of 8 animals was measured using a Dyer model micrometer gauge at TPA pretreatment on day 0, and then daily after treatment. Ear weight (mg) was also assessed on day 15.

5.2.3. *Statistical analysis*

The Newmann-Keuls test (ANOVA) was used to compare data in studies involving topical application of ethanol or compound in the acute and subchronic phorbol ester-induced mouse ear-swelling tests.

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