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Non-carboxylic antiinflammatory compounds. III. *N*-(4,6-Dimethylpyridin-2-yl)arylcarboxamides and arylthiocarboxamides acting as brain edema inhibitors

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Summary — Pharmacomodulation of the non-carboxylic NSAID *N*-(4,6-dimethylpyridin-2-yl)benzamide **1** led to the synthesis of structurally related furan, thiophene and pyrrole carboxamides **3–14**. The derivatives benzenethiocarboxamides **15–18** and heteroarylthiocarboxamides **19–22** were also prepared by oxygen/sulfur exchange; this reaction was more efficiently carried out by P_4S_{10} than by Lawesson's reagent. The 20 synthesized compounds were evaluated against peripheral edema by a foot-pad carrageenin-induced edema test. Amides **3–5**, **8**, **9**, **11**, **12** and **14** were most active, exhibiting > 90% inhibition after oral administration of 0.8 mmol·kg^{-1}. Two amides, **3** and **5**, were selected for evaluation of their inhibitory activity in PLA_2-induced brain edema and were found to be more potent than dexamethasone after IP administration.

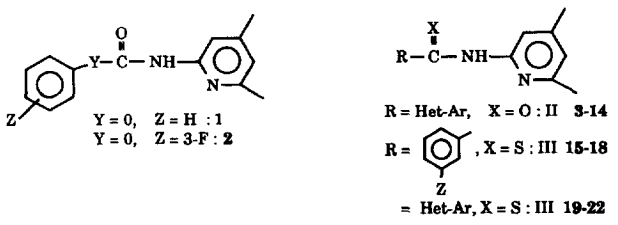
2-amino-4,6-dimethylpyridine / heteroarylcarboxamide / (hetero)arylthiocarboxamide / non-carboxylic NSAID / brain edema inhibitor

Introduction

In previous papers, we described the synthesis and antiinflammatory activity of *N*-(4,6-dimethylpyridin-2-yl)benzamides [1], phenylacetamides and cinnamamides [2]. In the first series, benzamide **1** exhibited high potency, ID_{50} = 35.2 mg·kg^{-1}, and extensive benzene ring (*Z*) pharmacomodulation led to 3-fluorobenzamide **3**, which showed the highest activity, ID_{50} = 12.1 mg·kg^{-1}. Intercalation of an alkyl or vinyl group (Y) between the benzene ring and the amidic function generally produced a detrimental effect, and only the unsubstituted cinnamamide (Y = CH=CH, Z = H) demonstrated a level of activity comparable to **1**. The fact that these 6-amino-2,4-lutidine derivatives, contrary to classical non-steroidal anti-inflammatory drugs (NSAIDs), reduced arachidonic acid production without any blocking activity on cyclooxygenase prompted us to consider further structural modifications of this new pharmacophore.

Pharmacomodulation was based on isosteric bioanalogies [3]. Replacement of the homocycle by heterocycles and the amide function by a thioamide grouping was intended to modify the basicity, lipophilicity and intrinsic volume of the studied molecules. In the furan subseries, the high potency of unsubstituted amide **3** justified the introduction of electron-donating (ED), or, more particularly, electron-withdrawing (EW) substituents, insofar as it was previously observed that an EW character (especially NO_2 and Br fixed at the *meta* position) enhances the activity level, at least in the phenylacetamide series [2].

New amides II and thioamides III (scheme 1) were first experimented in rat peripheral edema, and then two active amides, **3** and **5**, were evaluated for their capacity to inhibit experimental rat brain edema. A preliminary study of their potential therapeutic value was performed by determining the acute toxicity of **5** and the gastric irritation liability of lead compound **1**.



Scheme 1.

Chemistry

The amides (table I) and thioamides (table II) were prepared using the synthetic methods outlined in scheme 2. Activation of acids I by formation of an acyloxyphosphonium salt [4–6] was more easily and efficiently carried out using $BrCCl_3$ *(Method B)* than CCl_4 *(Method A)*. Synthesis of *N*-methylpyrrole-carboxamide **13** by *Method B* gave *N*-methylpyrrole-2-carboxylic anhydride **25** as a by-product in a 28% yield. Preliminary *N*-substitution of indole-2-carboxylic acid was necessary to afford the desired *N*-(4,6-dimethylpyridinyl)-1-methylindole-2-carboxamide **14**. Indeed, only the formation of pyrazino-diindoledione **26** was observed, in a 48% yield, with indole-2-carboxylic acid.

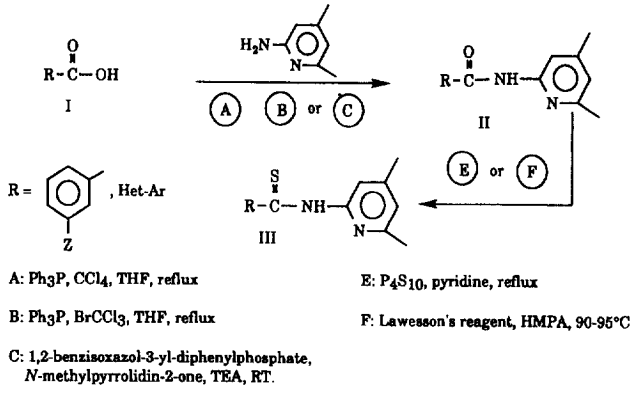
Attempts were made to increase yields by activating furan-2-carboxylic acid through the formation of an anhydride with 1,2-benzisoxazol-3-yldiphenylphosphate [7]. This anhydride reacted sluggishly in acetonitrile, giving a very low yield (10%), which was moderately increased (to 40%) when this solvent was replaced with *N*-methylpyrrolidine-2-one *(Method C)*.

Cyanation of amide 5, performed with NaCN in DMSO at 120°C, gave 5-cyanofuran-2-carboxamide **6** in a 32% yield *(Method D)*.

Sulfur/oxygen exchange of amides was carried out with phosphorus pentasulfide. Among the different anhydrous solvents experimented (pyridine, 3-methylpyridine, dioxane, HMPA, 1,3-dimethylimidazolidin-2-one, 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1*H*)-pyrimidinone) only pyridine [8] afforded satisfactory results. The best yields, at least with the studied *N*-(2-dimethylpyridinyl)arylcarboxamides, were obtained by addition of the reagent to the gently heated (100°C) solution of amide in pyridine, followed by 75 min reflux (115°C). Prolongation of the reaction time resulted in the formation of increased gummy by-products. Yields ranged between 45 and 75%, except for bromo derivative **20** which gave only a 16% yield which was not increased when the reaction was carried out in the presence of $NaHCO_3$ [9] in refluxing pyridine or toluene. Attempts to improve the yield of **20** by recourse to Lawesson's reagent [10–14] in refluxing toluene or HMPA at 90–95°C failed and only thioamides **15–17** could be isolated in very moderate yields (10–20%).

Minor by-products IV, corresponding to metadithiophosphonic derivatives (liable to exert antiinflammatory activity), were isolated during purification of thioamides **15** and **21**. The structural assignment of these orange-yellow compounds (**23** and **24**, respectively) was ascertained by IR, ^1H, ^{31}P-NMR and MS techniques (table III). The marked deshielding of pyridinyl H^3, H^5 and α,γ-CH_3 (when compared to their values in 6-amino-2,4-lutidine) argued in favor of a quaternized structure, with the cationic pyridinium site being equilibrated by the anionic sulfide site in IV. Further structural evidence of a betaine [1,4,2]-diazaphospholo[1,5-*a*]pyridinium sulfide structure IV was obtained from X-ray study [15].

The characteristic B, C, D and G infrared bands [16] of the thioamidic system were observed in the studied arylthiocarboxamides **15–22**. The most salient feature of the ^1H-NMR spectra of amides and thioamides was the marked deshielding of the H^3 signal (compared to the H^5 signal) of the pyridine nucleus, induced by the paramagnetic effect of the (thio)carbonyl group: $\Delta\delta \sim 1.2$ ppm and ~ 1.85 ppm for amides and thioamides, respectively. In the thiobenzamide series, the N-H peak appeared as a discrete broad singlet, likely due to rapid quadrupole-induced relaxation. IR and NMR data of amides I and thioamides II are shown in tables IV and V.



Scheme 2. Synthetic methods for amides II and thioamides III.

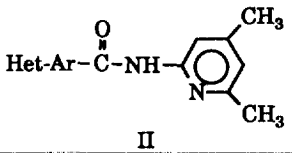
Pharmacological study and discussion

Inhibition of peripheral edema

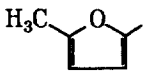
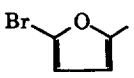
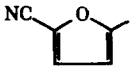
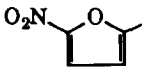
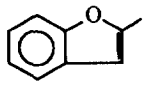
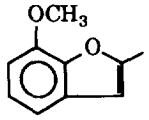
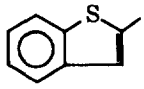
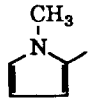
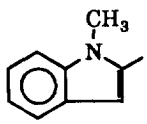
The newly synthesized amides and thioamides were experimented as potential antiinflammatory molecules by measuring the inhibition of carrageenin-induced rat-paw edema after oral administration of 0.8 mmol·kg^{-1}. All tested heteroarylcarboxamides exerted an anti-edematous effect; compounds **3–5**, **8**, **9**, **11**, **12** and **14** demonstrated the highest potency (≥ 90% inhibition).

The presence of an EW group at the *meta* position in the furan ring exerted a varied effect: a marked decrease with cyano and nitro groups (**6** and **7**) and a slight increase with a bromo atom (**5**). Practically the same level of activity was obtained by an ED group like methyl (**4**). Benzene ring fusion to the furan

Table I. Physicochemical data and anti-edematous effect of heteroarylcarboxamides **3–14**.

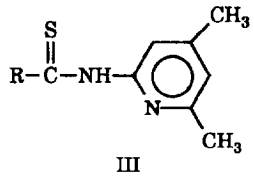


II

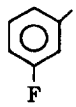
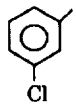
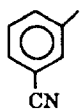
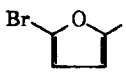
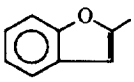
N°	Het-Ar	Formula M_r	Method yield (%)	F°C (diisopr. ether)	Carragenin-induced rat-paw edema [a] Inhibition % [b] m ± Sm
3		$C_{12}H_{12}N_2O_2$ 216.24	A: 60 B: 61 C: 40	99-100	90.2 ± 4.8
4		$C_{13}H_{14}N_2O_2$ 230.37	B: 28	100-101	94.7 ± 3.7
5		$C_{12}H_{11}BrN_2O_2$ 295.14	A: 55 B: 51	128-129	97.3 ± 5.9
6		$C_{13}H_{11}N_3O_2$ 241.36	D: 32	97	78.7 ± 4.3
7		$C_{12}H_{11}N_3O_4$ 261.24	B: 60	178-179	60.7 ± 10.4
8		$C_{16}H_{14}N_2O_2$ 266.30	B: 55	129-130	91.6 ± 4.1
9		$C_{17}H_{16}N_2O_3$ 296.33	B: 46	155-157	91.7 ± 5.8
10		$C_{12}H_{12}N_2SO$ 232.24	A: 62 B: 65	152-153	80.8 ± 5.6
11		$C_{12}H_{12}N_2SO$ 232.24	B:47	129	100
12		$C_{16}H_{14}N_2SO$ 282.37	B: 64	131	97.9 ± 4.7
13		$C_{13}H_{15}N_3O$ 229.28	B: 28	131	50.2 ± 6.0
14		$C_{17}H_{17}N_3O$ 279.34	B: 42	117-118	94.8 ± 7.7

[a]Dose: 0.8 mmol·kg^{-1} po; [b]number of animals for each group = 5.

Table II. Physicohemical data and anti-edematous effect of arylthiacarboxamides **15–18** and heteroarylthiocarboxamides **19–22**.



III

No	R	Formula M_r	Method yield (%)	m.p. solvent	Carragenin-induced rat-paw edema[d] Inhibition % [e] m ± Sm
15[a]		$C_{14}H_{14}N_2S$ 242.35	E: 60 F: 19	115-116 b	22.3 ± 8.5
16[a]		$C_{14}H_{13}FN_2S$ 260.33	E: 47 F: 14	99 b	86.9 ± 5.7
17[a]		$C_{14}H_{13}ClN_2S$ 276.79	E: 73 F: 12	63-64 b	NI
18[a]		$C_{15}H_{13}N_3S$ 267.36	E: 45	137-138 b	60.8 ± 10.1
19		$C_{12}H_{12}N_2OS$ 232.31	E: 55	95 b	74.4 ± 4.5
20		$C_{12}H_{11}BrN_2OS$ 311.21	E: 16	93-94 b	20.4 ± 9.2
21		$C_{12}H_{12}N_2S_2$ 248.37	E: 52	125 b	84.4 ± 5.9
22		$C_{16}H_{14}N_2OS$ 282.37	E: 66	123 c	61.5 ± 7.5

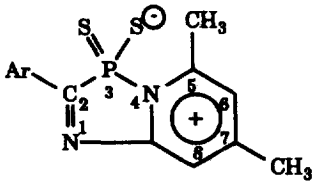
[a]Amides have been described previously [1]; [b]diisopropyl ether; [c]EtOH; [d]dose: 0.8 mmol·kg^{-1} po; [e]NI: no inhibition; number of animals for each group = 5.

moiety did not modify the inhibition percentage (**3** → **8**), but a marked increase of activity was observed in the thiophene and especially the pyrrole subseries as a result of such a structural modification (**10** → **12** and **13** → **14**).

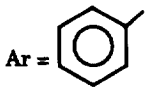
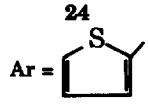
Transformation into thioamides did not notably change the level of activity in compounds **16**, **18** and **21** (by comparison with the corresponding amides), but led more generally to a decrease in potency or even a loss of ability to inhibit edema (compound **17**). Lastly, integration of the pharmacophore into bicyclic betaine derivatives **23** and **24** caused the same dramatic loss of activity (inhibition < 20%).

Althoug tentative correlations were made between different physicochemical parameters (basicity (pK_a), lipophilicity (log P) and intrinsic volume (V_x)) and antiinflammatory activity (I%), no satisfactory single correlation was obtained. Multiple correlation analysis

Table III. Physicochemical and spectral data for compounds **23** and **24**.



IV

Compound	method : yield % m.p. °C; solvent	IR(KBr) cm^{-1}	^{1}H-NMR (DMSO-d6) δ ppm	^{31}P-NMR ($CDCl_3$) δ ppm (32.44 MHz)	MS
23 Ar =  $C_{14}H_{13}N_2PS_2$ 304.36	E : 10 255 dec.; CH_2Cl_2	1620 (νC=N) 720 (νP=S)	2.60 (s, 3H, 7-CH_3); 3.38 (s, 3H,5-CH_3); 7.17 (s, 1H, H^6); 7.52 (s, 1H, H^8); 7.55-7.65 (m, 3 arom. H); 8.60-8.63 (m, 2 arom. H).	109.11 s	304 (11, M^+) 240 (100, M^+-2S) 106 (77, $C_7H_8N^+$)
24 Ar =  $C_{12}H_{11}N_2PS_3$ 310.40	E : 4 245 dec.; CH_2Cl_2	1620 (νC=N) 710 (νP=S)	2.58 (s, 3H, 7-CH_3); 3.34 (s, 3H, 5-CH_3); 7.12 (s, 1H, H^6); 7.46 (s, 1H, H^8); 7.32 (dd, 1H, $H^{4'}$, $J_{H4'H5'}$=3.9 Hz, $J_{H4'H3'}$=4.5 Hz) ;7.83 (dd, 1H, $H^{5'}$, $J_{H5'H4'}$ = 3.9Hz, $J_{H5'H3'}$=1Hz);8.55 (dd, 1H, $H^{3'}$, $J_{H3'H4'}$=4.5H, $J_{H3'H5'}$=1Hz).		310 (15, M^+) 246 (100, M^+-2S) 106 (59, $C_7H_8N^+$)

of the data showed that pK_a had no significant influence on activity level. Finally, a satisfactory relationship taking into account log P and V_x was obtained for six compounds (**3–5**, **7**, **11** and **13**):

$$\log I = 3.5 + 0.23 \log P - 11.21 \frac{V_x}{100} \quad (r = 0.975)$$

This suggests that, at a constant volume, activity is increased by higher lipophilicity and *vice versa*. Additional in-depth results, including values for previously described molecules as well as technical details, will be presented in a forthcoming paper.

The ID_{50} value of one of the most active compounds, 5-bromofuran-2-carboxamide **5**, was determined by linear regression after oral administration of four different doses to rats. It was higher than that of 3-fluorobenzamide **2**, 30.95 mg·kg^{-1} (I% = 32.225 D + 122.657; lack of fit: P = 0.001, NS) and 12.1 mg·kg^{-1} respectively, but slightly lower than that of **1** (35.2 mg·kg^{-1}).

Toxicity tests

The acute toxicity of amide **5** was determined by oral administration of increasing doses to rats. Its LD_{50} value, calculated according to the method of Lichtfield and Wilcoxon [17], was markedly higher than that of **2**, 1830 mg·kg^{-1} (1407–2380) and 280 mg·kg^{-1} (189–414), respectively. Thus, amide **5** exhibited a more satisfactory therapeutic index (LD_{50}/ID_{50}) than fluorobenzamide **2** or even benzamide **1** (59, 23 and 52, respectively).

As the therapeutic potential of NSAIDs in chronic inflammatory processes is often burdened by a harmful effect on gastrointestinal mucosa, the ulcerogenic effect of **1** was evaluated by the rat pylorus ligature technique [18]. Its ulceration index, calculated by the Lwoff method [19], after intraduodenal administration of 200 mg·kg^{-1}, was practically identical with that obtained in control rats: 61.5 and 63.2. This value should be compared with that (300) obtained with phenylbutazone after administration of 100 mg·kg^{-1}.

Table IV. Spectral data for heteroarylcarboxamides and **3–14**.

Compound	IR (KBr) cm^{-1} νNH, νCO, δNH, comb NH/CN	^{1}H-NMR ($CDCl_3$) δ ppm
3	3400; 1665; 1530; 1280.	2.34 (s, 3H, 4-CH_3); 2.42 (s, 3H, 6-CH_3); 6,55 (dd, 1H, H^4, J_{H4H3} = 3.5Hz, J_{H4H5} = 1.6Hz); 6.76 (s,1H, pyr. H^5); 7.25 (dd, 1H, H^3, J_{H3H4} = 3.5Hz, J_{H3H5} = 0.8Hz); 7.50 (dd, 1H, H^5, J_{H5H4}=1.6Hz, J_{H5H3}=0.8 Hz); 7.99 (s, 1H, pyr. H^3); 8.74 (s, 1H, NH).
4	3400; 1675; 1550; 1275.	2.33 (s, 3H, pyr. 4-CH_3); 2,43 (s, 3H, pyr. 6-CH_3); 2.36 (s, 3H, 5-CH_3); 6.15 (d, 1H, H^4, J_{H4H3} = 3.0Hz); 6.75 (s, 1H, pyr. H^5); 7.15 (d, 1H, H^3, J_{H3H4} = 3.0Hz); 7.99 (s, 1H, pyr. H^3); 8.63 (s, 1H, NH).
5	3410; 1690; 1530; 1280.	2.34 (s,3H, 4-CH_3); 2.44 (s, 3H, 6-CH_3); 6.49 (d, 1H, H^4, J_{H4H3} = 3.0 Hz); 6.77 (s, 1H, pyr. H^5); 7.21 (d, 1H, H^3, J_{H3H4} = 3.0Hz); 7.95 (s, 1H, pyr. H^3); 8.63 (s, 1H, NH).
6	3100; 2220 (νC=N); 1685; 1550; 1285.	2.36 (s, 3H, 4-CH_3); 2.45 (s, 3H, 6-CH_3); 6.82 (s, 1H, pyr. H^5); 7.22 (d, 1H, H^4, J_{H4H3} = 3.7 Hz); 7.31 (d, 1H, H^3, J_{H3H4} = 3.7Hz); 7.91 (s, 1H, pyr. H^3); 8.68 (s, 1H, NH).
7	3210; 1685; 1555; 1280.	2.36 (s, 3H, 4-CH_3); 2.44 (s, 3H, 6-CH_3); 6.82 (s, 1H, pyr. H^5); 7.41 (s, 2H, H^3 and H^4); 7.91 (s, 1H, pyr. H^3); 8.91 (s, 1H, NH).
8	3400; 1680; 1525; 1275.	2.36 (s, 3H, 4-CH_3); 2.46 (s, 3H, 6-CH_3); 6.79 (s, 1H, pyr. H^5); 7.22-7.73 (m, 5 arom. H); 8.04 (s, 1H, pyr. H^3); 8.97 (s, 1H, NH).
9	3400; 1675; 1535; 1280.	2.37 (s, 3H, 4-CH_3); 2.48 (s, 3H, 6-CH_3); 4.03 (s, 3H, OCH_3); 6.80 (s, 1H, pyr. H^5); 6.92 (dd, 1H, H^5, J_{H5H4} = 6.0Hz, J_{H5H6} = 3.0Hz); 7.24 (d, 1H, H^4, J_{H4H5} = 6.0Hz); 7.26 (d, 1H, H^6, J_{H6H5} = 3.0Hz); 7.61 (s, 1H, H^3); 8.06 (s, 1H, pyr. H^3); 9.15 (s, 1H, NH).
10	3400; 1655; 1555; 1275.	2.33 (s, 6H, 4-CH_3 and 6-CH_3); 6.73 (s, 1H, pyr. H^5); 7.06 (dd, 1H, H^4, J_{H4H3} = 3.5Hz, J_{H4H5} = 4.9Hz); 7.52 (dd, 1H, H^5, J_{H5H4} = 4.9Hz, J_{H5H3} = 0.92Hz); 7.64 (dd, 1H, H^3, J_{H3H4} = 3.5Hz, J_{H3H5}=0.92Hz); 7.99 (s, 1H, pyr. H^3); 9.17 (s, 1H, NH).
11	3240; 1660; 1560; 1270.	2.35 (s, 3H, 4-CH_3); 2.38 (s, 3H, 6-CH_3); 6.75 (s, 1H, pyr. H^5); 7.39 (d, 1H, H^4, J_{H4H5} = 3.0Hz); 7.54 (d, 1H, H^5, J_{H5H4} = 3.0Hz); 8.02 (s, 2H, H^2 and pyr. H^3); 8.69 (s, 1H, NH).
12	3415; 1675; 1540; 1280.	2.36 (s, 3H, 4-CH_3); 2.44 (s, 3H, 6-CH_3); 6.79 (s, 1H, pyr. H^5); 7.40-7.92 (m, 4 arom. H); 7.94 (s, 1H, H^3); 8.02 (s, 1H, pyr. H^3); 8,72 (s, 1H, NH).
13	3230; 1660; 1545; 1260.	2.33 (s, 3H, 4-CH_3); 2.41 (s, 3H, 6-CH_3); 3.99 (s, 3H, NCH_3); 6.71 (s, 1H, pyr. H^5); 6,13 (dd, 1H, H^4, J_{H4H3} = 3.9Hz, J_{H4H5} = 2.8Hz); 6.79 (dd, 1H, H^5, J_{H5H4} = 2.8Hz, J_{H5H3} = 1.7Hz); 6.82 (dd, 1H, H^3, J_{H3H4} = 3.9Hz, J_{H3H5} = 1.7Hz); 7.93 (s, 1H, pyr. H^3); 8.41 (s, 1H, NH).
14	3420; 1665; 1520; 1260.	2.35 (s, 3H, 4-CH_3); 2,43 (s, 3H, 6-CH_3); 4.10 (s, 3H, NCH_3); 6.76 (s, 1H, pyr. H^5); 7.11-7.40 (m, 4 arom. H); 7.67 (s, 1H, H^3); 7.99 (s, 1H, pyr. H^3); 8.64 (s, 1H, NH).

Table V. Spectral data for arylthiocarboxamides and heteroarylthiocarboxamides **15–22**.

Compound	IR(KBr) cm^{-1} Bands B, C, D, and G [a]	^{1}H-NMR ($CDCl_3$) δ ppm
15	1530, 1330, 1025, 780	2.40 (s, 3H, 4-CH_3); 2.44 (s, 3H, 6-CH_3); 6.87 (s, 1H, pyr. H^5); 7.45 (m, 3H, H^3, $H^4$and H^5); 7.85 (m, 2H, H^2 and H^6) 8.80 (s, 1H, pyr. H^3); 10.0-10.5 (bs, 1H, NH).
16	1530, 1330, 1160, 780	2.39 (s, 3H, 4-CH_3); 2.41 (s, 3H, 6-CH_3); 6.86 (s, 1H, pyr. H^5); 7.13-7.67 (m, 4 arom. H); 8.60 (s, 1H, pyr. H^3); 10.0-10.5 (bs, 1H, NH).
17	1530, 1340, 1200, 710	2.40 (s, 3H, 4-CH_3); 2.46 (s, 3H, 6-CH_3); 6.87 (s, 1H, pyr. H^5); 7.23-7.87 (m, 4 arom. H); 8.62 (s, 1H, pyr. H^3); 10.0-10.5 (bs, 1H, NH).
18	1595, 1320, 1140, 720	2.41 (s, 3H, 4-CH_3); 2.46 (s, 3H, 6-CH_3); 6.88 (s, 1H, pyr. H^5); 7.30-7.48 (m, 4 arom. H); 8.27 (s, 1H, pyr. H^3); 10.0-10.5 (bs, 1H, NH).
19	1530, 1345, 1040, 760	2.38 (s, 3H, 4-CH_3); 2.48 (s, 3H, 6-CH_3); 6.52 (dd, 1H, H^4, J_{H4H3} = 3.6 Hz, J_{H4H5} = 1.8 Hz); 6.86 (s, 1H, pyr. H^5); 7.46 (dd, 1H, H^3, J_{H3H4} = 3.6Hz, J_{H3H5} = 0.7Hz); 7.51 (dd, 1H, H^5, J_{H5H4} = 1.8 Hz, J_{H5H3} = 0.7 Hz); 8.81 (s, 1H, pyr. H^3); 10.01 (s, 1H, NH).
20	1560, 1360, 1020, 800	2.38 (s, 3H, 4-CH_3); 2.47 (s, 3H, 6-CH_3); 6.48 (d, 1H, H^4, J_{H4H3} = 3.6 Hz); 6.87 (s, 1H, pyr. H^5); 7.50 (d, 1H, H^3, J_{H3H4} = 3.6Hz); 8.76 (s, 1H, pyr. H^3); 9.90 (s, 1H, NH).
21	1560, 1330, 1050, 715	2.38 (s, 3H, 4-CH_3); 2.41 (s, 3H, 6-CH_3); 6.81 (s, 1H, pyr. H^5); 7.06 (dd, 1H, H^4, J_{H4H3} = 4.5 Hz, $J_{H^4H^5}$ = 3.5 Hz); 7.50 (dd, 1H, H^3, J_{H3H4} = 4.5Hz, J_{H3H5} = 1Hz); 7.62 (dd, 1H, H^5, J_{H5H4} = 3.0Hz, J_{H5H3} = 1.0Hz); 8.36 (s, 1H, pyr. H^3); 10.77 (s, 1H, NH).
22	1530, 1350, 1120, 750	2.38 (s, 3H, 4-CH_3); 2.49 (s, 3H, 6-CH_3); 6.87 (s, 1H, pyr. H^5); 7.35-7.51 (m, 5 arom. H); 8.88 (s, 1H, pyr. H^3); 10.25 (s, 1H, NH).

[a] See ref [15].

Thus, these non-carboxylic NSAIDs could be particularly attractive for the development of new, safer antiinflammatory agents. These promising preliminary results justify further toxicity studies, which will be reported in a future manuscript by the same authors.

Inhibition of experimental brain edema

Among antiinflammatory drugs, only glucocorticoids are effective in the treatment of brain edema, although their therapeutic usefulness is limited by frequent and marked side effects. Two amides, **3** and **5**, were selected to evaluate inhibition of bain edema because of their high activity in peripheral edema. The assay was carried out using a rat PLA_2-induced brain edema model [20]. As eicosanoid production has been clearly implicated in the development of experimental brain edema [21, 22], the decrease in edema was determined by measuring the PGE_2 concentration in drug-treated and untreated lesioned rats after IP administration of **3**, **5** and dexamethasone.

The results (table VI) show that the three non-steroidal compounds had a marked anti-edematous activity on experimental brain edema, which was higher than that observed with dexamethasone. It was previously observed [1] that benzamide **1** could reduce eicosanoid production despite its lack of direct inhibitory activity on cyclooxygenase, 5-lipoxygenase and phospholipase A_2. On the basis of these results, it is feasible to assume that new structurally related

heteroarylcarboxamides act by a similar mechanism, which is now under investigation. Recent experiments, especially those carried out with compound **5**, suggest that these new heteroarylcarboxamides perturb PKC activity (Lang *et al*, submitted for publication). Their mechanism of action would be partially related to an inhibitory effect on proteins located upstream from phospholipase A_2 on the pathway leading to activation of this enzyme by phosphorylation [23].

In conclusion, we have described the synthesis of some novel *N*-(4,6-dimethylpyridin-2-yl)heteroarylcarboxamides and a number of these possessed promising *in vivo* antiinflammatory activity. Furthermore two representative edema inhibitors, **3** and **5**, demonstrated good activity in brain edema. Confirmation of the original mechanism of action of these lead structures and their improved safety profile, when compared with standard antiinflammatory agents, is now under study and will be published in due course.

Experimental protocols

Chemistry

Melting points were determined on a Tottoli–Büchi apparatus, without correction. The structures of the described products were confirmed by IR, ^{1}H-NMR and microanalytical data. IR spectra were run with KBr pellets on a Beckman IR 4230 infrared spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker AC 250 spectrometer (250 MHz), $CDCl_3$ as solvent; chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (Me_4Si, 0.00 ppm) as the internal standard and coupling constants in hertz. The ^{31}P-NMR spectrum of **23** was recorded on a Jeol JNM-FX 200 spectrometer (32.44 MHz), $CDCl_3$ as solvent; chemical shifts (δ) are reported in ppm from phosphoric acid (H_3PO_4, 0.00 ppm) as an external standard. Structural assignment of compounds **23–26** was completed by mass spectroscopy, using a Hewlett-Packard HP 5989 A spectrometer operating in electron ionization (EI) mode (70 eV). Samples were introduced by a direct inlet probe at the minimum temperature providing adequate vapor pressure; source temperature: 220°C Microanalyses were performed on a Perkin-Elmer CHN 240 apparatus. Analyses indicated by the symbols of the elements were within ± 0.40% of theoretical values. TLC was performed on precoated silica gel aluminium plates (0.2 mm, GF254, E Merck). Spots were located by UV detection.

Sodium sulfate was always used as the drying agent. Products were routinely purified by short columns of silica gel (silica gel 60, 70-230 mesh, E Merck), with CH_2Cl_2 as an eluent. Commercially available (Aldrich, Janssen) acids were used, except for 5-methylfuran-2-carboxylic acid, which was prepared by $KMnO_4$ or Ag_2O oxidation of the corresponding aldehyde in 26 and 35% yields respectively; mp = 109–110°C (diisopropyl ether), lit [24]: 119°C. IR (KBr) cm^{-1}: 3180–2520 (νOH), 1680 (νC=O), 905 (δOH). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.35 (s, 3H, CH_3); 6.30 (d, 1H, J = 3.3 Hz, H^4), 7.12 (d, 1H, H^3); 12.84 (s, 1H, COOH).

The following experimental procedures are illustrative of the general ones used to afford amides and thioamides.

Method A. N-(4,6-Dimethylpyridin-2yl)furan-2-carboxamide 3
A mixture of triphenylphosphine (7 g, 26.77 mmol) and tetrachloromethane (5.17 ml, 53.54 mmol) in THF (70 ml) was refluxed for 30 min and cooled at 5°C, after which furan-2-carboxylic acid (3 g, 26.77 mmol) was added. The mixture was stirred for 10 min before 2-amino-4,6-dimethylpyridine (6.54 g, 53.54 mmol) was added. The solution was again refluxed for 1 h and cooled to room temperature. The precipitate was removed by filtration, and filtrate concentrated by evaporation under reduced pressure. The residue, purified by column chromatography, gave 3.46 g of pure product. Yield: 60%; mp: 98–100°C (diisopropyl ether). IR (KBr) cm^{-1}: 3340 (νNH); 1675 (νC=O); 1570 (δNH); 1280 (combined NH/CN). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.34 (s, 3H, 4-CH_3); 2.42 (s, 3H, 6-CH_3); 6.55 (dd, 1H, H^4, $J_{H^4H^3}$ = 3.5 Hz, $J_{H^4H^5}$ = 1.6 Hz); 6.76 (s, 1H, pyr H^5); 7.25 (dd, 1H, H^3, $J_{H^3H^4}$ = 1.6 Hz, $J_{H^3H^5}$ = 0.8 Hz); 7.50 (dd, 1H, H^5, $J_{H^5H^4}$ = 1.6 Hz, $J_{H^5H^3}$ = 0.8 Hz); 7.99 (s, 1H, pyr H^3); 8.74 (s, 1H, NH). Anal $C_{12}H_{12}N_2O_2$ (C, H, N).

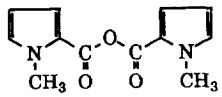
Method B. N-(4,6-Dimethylpyridin-2-yl)thiophene-2-carboxamide 10
A mixture of triphenylphosphine (8.19 g, 31.21 mmol), thiophene-2-carboxylic acid (4.00 g, 31.21 mmol), bromotrichloromethane (6.15 ml, 62.42 mmol) and 2-amino-4,6-dimethylpyridine (7.62 g, 31.21 mmol) was refluxed under stirring for 34 h and then cooled to room temperature. The mixture was filtered under vacuum, and the filtrate was concentrated under reduced pressure to give a brown oil. After purification by column chromatography, this residue gave 4.68 g of pure carboxamide **10**. Yield: 65%; mp: 152–153°C (diisopropyl ether). IR (KEr) cm^{-1}: 3200 (νNH); 1660 (νC=O); 1560 (δNH); 1275 (combined NH/CN). ^{1}H-NMR ($CDCl_3$) δ ppm:

Table VI. Influence of drug treatment on the brain PGE_2 tissue concentration.

Compound	*IP dose (μmol·kg^{-1})*	*PGE_2 concentration (pg•mg^{-1} of proteins)*	*Number of animals*
1	12.5	79.2 ± 14.3	14
3	12.5	42.9 ± 3.0	13
5	12.5	35.7 ± 2.3	10
Dexamethasone	26	89.8 ± 11.9	11
Lesioned rats		293.9 ± 12.2	11
Control rats		21.1 ± 1.8	16

2.33 (s, 6H, 4-CH_3 and 6-CH_3); 6.73 (s, 1H, pyr H^5); 7.06 (dd, 1H, H^4, $J_{H^4H^5}$ = 4.9 Hz; $J_{H^3H^4}$ = 3.5 Hz); 7.53 (dd, 1H, H^5, $J_{H^5H^4}$ = 4.9 Hz, $J_{H^5H^3}$ = 0.92 Hz); 7.64 (s, 1H, H^3, $J_{H^3H^4}$ = 3.5 Hz, $J_{H^3H^5}$ = 0.92 Hz); 7.99 (s, 1H, pyr H^3); 9.17 (s, 1H, NH). Anal $C_{12}H_{12}N_2SO$ (C, H, N).

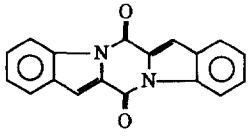
N-Methylpyrrole-2-carboxylic anhydride **25** was simultaneously obtained when the amine was condensed with the corresponding acid by *Method B*.



$C_{12}H_{12}N_2O_3$: 232.24

Yield: 28%; mp: 70°C (diisopropyl ether). IR (KBr) cm^{-1}: 1760 (ν_{as}C-O); 1700 (νC=O); 1035 (νC-O-C). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.97 (s, 6H, $2NCH_3$); 6.16 (dd, 2H, H^4 and $H^{4'}$, $J_{H^4H^3}$ = 4.1 Hz, $J_{H^4H^5}$ = 2.5 Hz); 6.92 (dd, 2H, H^5 and $H^{5'}$, $J_{H^5H^4}$ = 2.5 Hz, $J_{H^5H^3}$ = 1.9 Hz); 7.09 (dd, 2H, H^3 and $H^{3'}$, $J_{H^3H^4}$ = 4.1 Hz, $J_{H^3H^5}$ = 1.9 Hz). MS: 232 (22, $M^{+\cdot}$); 108 (100, $C_6H_6NO^+$).

6*H*,13*H*-Pyrazino[1,2-*a*:4,5-*a'*]diindole-6-13-dione **26** was the only product isolated when an attempt was made to condense 2-amino-4,6-dimethylpyridine with indole-2-carboxylic acid by *Method B*.



$C_{18}H_{10}N_2O_2$: 286.29

Yield: 48%; mp: 306–309°C (CH_2Cl_2). IR (KBr) cm^{-1}: 1695, 1680 (νC=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.44–8.52 (m, 10H). MS: 286 (100, $M^{+\cdot}$), 258 (12, $M^{+\cdot}$ – CO), 143 (43, $C_9H_5NO^+$), 115 (52, $C_8H_5N^+$).

Method C. N-(4,6-Dimethylpyridin-2-yl)furan-2-carboxamide ***3***

Furan-2-carboxylic acid (0.56 g, 4.96 mmol) was dissolved in *N*-methylpyrrolidin-2-one (5 ml). TEA (0.8 ml, 4.96 mmol) and 1,2-benzisoxazol-3-yl diphenylphosphate (2 g, 4.96 mmol) were added. The solution was stirred for 2 h at room temperature and then poured into a 1% water solution of $NaHCO_3$ (30 ml). This mixture was extracted by dichloromethane (3 × 30 ml). The organic layer was dried and concentrated under vacuum. The residue, after purification by column chromatography, gave 0.43 g of pure product. Yield: 40%; mp 98–100°C (diisopropyl ether). IR, ^{1}H-NMR: see *Method A*.

Method D. N-(4,6-Dimethylpyridin-2-yl)-5-cyanofuran-2-carboxamide ***6***

N-(4,6-Dimethylpyridin-2-yl)-5-bromofuran-2-carboxamide **5** (6.63 g, 22.46 mmol) was dissolved in DMSO (30 ml). NaCN (1.13 g, 23 mmol) was added, and the mixture was then stirred at 120°C for 3 h. After cooling to room temperature, water (50 ml) was added, and the mixture was extracted with dichloromethane (3 × 80 ml). The organic layers were collected, dried and evaporated under vacuum. The residue was purified by column chromatography to give 1.74 g of pure product. Yield: 32%; mp: 97°C (diisopropyl ether). IR (KBr) cm^{-1}: 3100 (νNH); 2220 (νC≡N); 1685 (νC=O); 1550 (δNH); 1285 (combined NH/CN). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.36 (s, 3H, 4-CH_3); 2.45 (s, 3H, 6-CH_3); 6.82 (s, 1H, pyr H^5); 7.22 (d, 1H, H^4, $J_{H^3H^4}$ = 3.7 Hz), 7.31 (d, 1H, H^3, $J_{H^4H^3}$ = 3.7 Hz); 7.91 (s, 1H, pyr H^3); 8.68 (s, 1H, NH). Anal $C_{13}H_{10}N_3O_2$ (C, H, N).

Method E. N-(4,6-Dimethylpyridin-2-yl)furan-2-thiocarboxamide ***19***

N-(4,6-Dimethylpyridin-2-yl)furan-2-carboxamide (6.5 g, 30.1 mmol) was dissolved in anhydrous pyridine (60 ml), and the solution was then heated to 100-110°C. P_4S_{10} (10.22 g, 23 mmol) was added, and the mixture was refluxed for 75 min. It was then poured into 300 ml of ice-water and stirred vigorously for 15–20 min. The precipitate was separated by filtration and dried. This crude product, purified by column chromatography, gave 3.85 g of pure yellow product. Yield: 55%; mp: 95°C (diisopropyl ether). IR (KBr) cm^{-1}: 1530 (ν_{as}NCS); 1345 (combined NC/CS); 1040 (ν_sNCS). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.38 (s, 3H, 4-CH_3); 2.46 (s, 3H, 6-CH_3); 6.52 (dd, 1H, H^4, $J_{H^4H^3}$ = 3.6 Hz, $J_{H^4H^5}$ = 1.8 Hz); 6.86 (s, 1H, pyr H^5); 7.46 (dd, 1H, H^3, $J_{H^3H^4}$ = 3.6 Hz, $J_{H^3H^5}$ = 0.7 Hz); 7.51 (dd, 1H, H^5, $J_{H^5H^4}$ = 1.8 Hz, $J_{H^5H^3}$ = 0.7 Hz); 8.81 (s, 1H, pyr H^3); 10.01 (s, 1H, NH). Anal $C_{12}H_{12}N_2OS$ (C, H, N).

Method F. N-(4,6-Dimethylpyridin-2-yl)benzenethiocarboxamide ***15***

N-(4,6-Dimethylpyridin-2-yl)benzenecarboxamide (1 g, 4.42 mmol) was dissolved in HMPA (10 ml) and heated to 90–95°C. Lawesson's reagent (3.6 g, 8.84 mmol) was added, and the mixture was stirred for 10 h. The dark solution obtained was poured into 100 ml of cold water (0–5°C), and the mixture was then stirred for 20 min. The precipitate obtained was washed with water (3 × 100 ml), dried and purified by column chromatography, affording 0.2 g of pure product. Yield: 19%; mp 115–116°C (diisopropyl ether). IR (KBr) cm^{-1}: 1530 (ν_{as}NCS); 1330 (combined NC/CS); 1025 (ν_sNCS). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.40 (s, 3H, 4-CH_3); 2.44 (s, 3H, 6-CH_3); 6.87 (s, 1H, pyr H^5); 7.45 (m, 3H, H^3, H^4 and H^5); 7.85 (m, 2H, H^2 and H^6); 8.80 (s, 1H, pyr H^3); N*H*: not observed. Anal $C_{14}H_{14}N_2S$ (C, H, N).

Pharmacology

These studies were carried out on adult male Wistar CF rats weighing 180–220 g.

Carrageenin-induced rat-paw edema

The inhibitory activity of the studied molecules on carrageenin-induced rat-paw edema was determined according to the method of Winter *et al* [25], with slight modification. The drugs were orally administered 1 h before injection of 0.05 ml of a 1% suspension of carrageenin 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. 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 by the formula:

$$I\% = \left(1 - \frac{dt}{dc}\right) \times 100$$

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

Ulcerogenic activity

Rats were divided into groups of 10 animals and fasted for 24 h prior to drug administration, receiving water *ad libitum*. After pylorus ligature [18], compound **1** was administered intraduodenally to an experimental group at a dose of 200 mg/kg in the form of an aqueous suspension in gum acacia. One group was used as a control, and another received the standard drug phenylbutazone at a dose of 100 mg/kg. The animals were sacrificed 18 h after drug or gum acacia treatment. Their stomachs were removed, opened up along the greater curvature and examined for the presence of gastric ulcers. An index based on the following formula was calculated using the Lwoff method [19]:

$$\frac{\text{sum of scores} \times \text{percentage of animals with ulcers}}{\text{number of animals}}$$

Experimental brain edema

Studies of PGE_2 brain levels were carried out as follows. Rats were housed in the animal room under artificial lighting between 6 am and 9 pm, with an ambient temperature of 21–23°C. They received food and water *ad libitum*. Injections and samplings were performed under ketamine anaesthesia (200 mg/kg intramuscularly) after placement of the animals in a stereotaxic apparatus. Our experiments used a brain edema model, developed in our laboratory, which was obtained by a phospholipase A_2 (PLA_2 from *Naja naja* venom, Sigma, USA) intracerebral injection. After perforation of the skull in the right hemisphere, 4 IU of PLA_2 (dissolved in 100 mM Tris, 1 mM $CaCl_2$, pH 7.4) was injected into the frontal cortex brain structure, according to the following stereotaxic coordinates: A = + 2 mm, L = + 2 mm, H = − 3.5 mm, with respect to the bregma [26]. Animals were kept for 24 h after the operation. Four groups of animals were used: control rats, lesioned rats, rats treated with dexamethasone (26 μmol/kg intraperitoneally) (Merck, Sharp & Dohme Laboratories, France) and rats treated with the studied compounds (12.5 μmol/kg intraperitoneally). Drugs were injected only once (5 h before PLA_2 injection).

Brain sampling was done under liquid nitrogen, 24 h after PLA_2 injection. Brain tissue (50–100 mg) was homogenized with 3 ml of acetone and centrifuged at 1200 *g* (20 min at 4°C). After evaporation of supernatants under nitrogen, residues were resuspended in phosphate-buffered saline (0.1 mol/l, pH 7.4, containing 1 mg/ml of gelatine). Aliquots were used for determination of PGE_2 by radioimmunoassay. It was verified that the drugs used did not interfere with the radioimmunoassay.

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