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2-Phenylimidazo[1,2-*a*]pyridine-3-carboxylic Acid Derivatives: Synthesis and Antiinflammatory Activity

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***Key Words**: Imidazo[1,2-a]pyridines; antiinflammatory activity; analgesic activity; ulcerogenic activity.*

Summary

A series of 2-phenylimidazo[1,2-*a*]pyridine-3-carboxylic esters, acids, and amides were synthesized and pharmacologically tested in order to evaluate their antiinflammatory and analgesic activity and their ulcerogenic action on the gastro-intestinal tract. The most active member of this series of compounds was found to be 6-methyl-2-phenylimidazo[1,2-*a*]pyridine-3-carboxylic acid (**5c**).

Introduction

Our research on chemical and pharmacological properties of heterocyclic compounds was originally based on the assumption that molecules derived from the general structural model **1** (Figure 1) could have antiinflammatory activity, since this model was derived from the map proposed by Gund and Shen[1] for the active site of cyclooxygenase: a compound related to the structure **1** should have the minimal structural features which are required to act as a cyclooxygenase inhibitor.

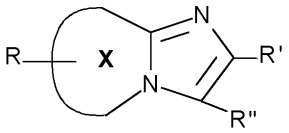

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Figure 1. General structural model (X = 5- or 6-membered ring; R′ or R″ = acidic function; R = substituents).

Many compounds related to **1** showed antiinflammatory and/or analgesic activity[2], but the peculiar features of their pharmacological profile (e.g. no parallelism between antiinflammatory and analgesic activities, low or negligible ulcerogenic action on the rat gastric mucosa), and the lack of inhibitory activity *in vitro* on the prostaglandin (PG) biosynthesis of some compounds with high antiinflammatory activity *in vivo*[3] seem to indicate that the inhibition of PG biosynthesis plays a secondary role at the most in their mode of action.

In this context, particularly interesting results were obtained on studying the structure-activity relationships in the series of imidazo[1,2-*a*]pyridine acidic derivatives (Figure 2).

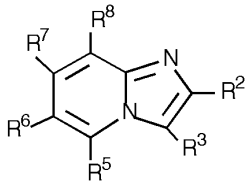

Figure 2. Imidazo[1,2-*a*]pyridines.

We previously synthesized and pharmacologically tested a number of imidazo[1,2-*a*]pyridine-2-carboxylic (R^2 = COOH) and 2-acetic (R^2 = CH_2COOH) acids, 2-methyl-3-carboxylic (R^2 = CH_3, R^3 = COOH), and 2-methyl-3-acetic (R^2 = CH_3, R^3 = CH_2COOH) acids[4,5]. In this series of compounds, the most active one was found to be 6-methylimidazo[1,2-*a*]pyridine-2-carboxylic acid. Antiinflammatory activity decreased or disappeared when the methyl group was eliminated or shifted to another position, and also when the carboxylic moiety was replaced by an acetic acid group. In order to improve the activity, we synthesized a number of 2-phenylimidazo[1,2-*a*]pyridine-3-carboxylic acids[6] of which the 6-methyl derivative **5c** showed high antiinflammatory activity[7]. Since the introduction of phenyl or *p*-chlorophenyl as a lipophilic moiety also increased the pharmacological activity in other series of imidazo-derivatives, e.g. imidazo[1,2-*b*]pyridazines[8] and imidazo[1,2-*a*]pyrazines[9], we decided to synthesize a number of 2-phenylimidazo[1,2-*a*]pyridine-3-carboxylic esters, acids, and amides in order to acquire a deeper knowledge of structure-activity relationships in this series of compounds and possibly of their mechanism of action.

Chemistry

The required compounds were prepared by means of the synthetic method depicted in Figure 3: this method is closely similar to the procedure normally used by us to obtain many series of bicyclic imidazo-derivatives with a bridgehead nitrogen atom, such as those cited in the Introduction[2–5].

[1)] Part 39: S. Laneri, A. Sacchi, M. Gallitelli, F. Arena, E. Luraschi, E. Abignente, W. Filippelli, F. Rossi, *Eur. J. Med. Chem.* **1998**, *33*, 163–170.

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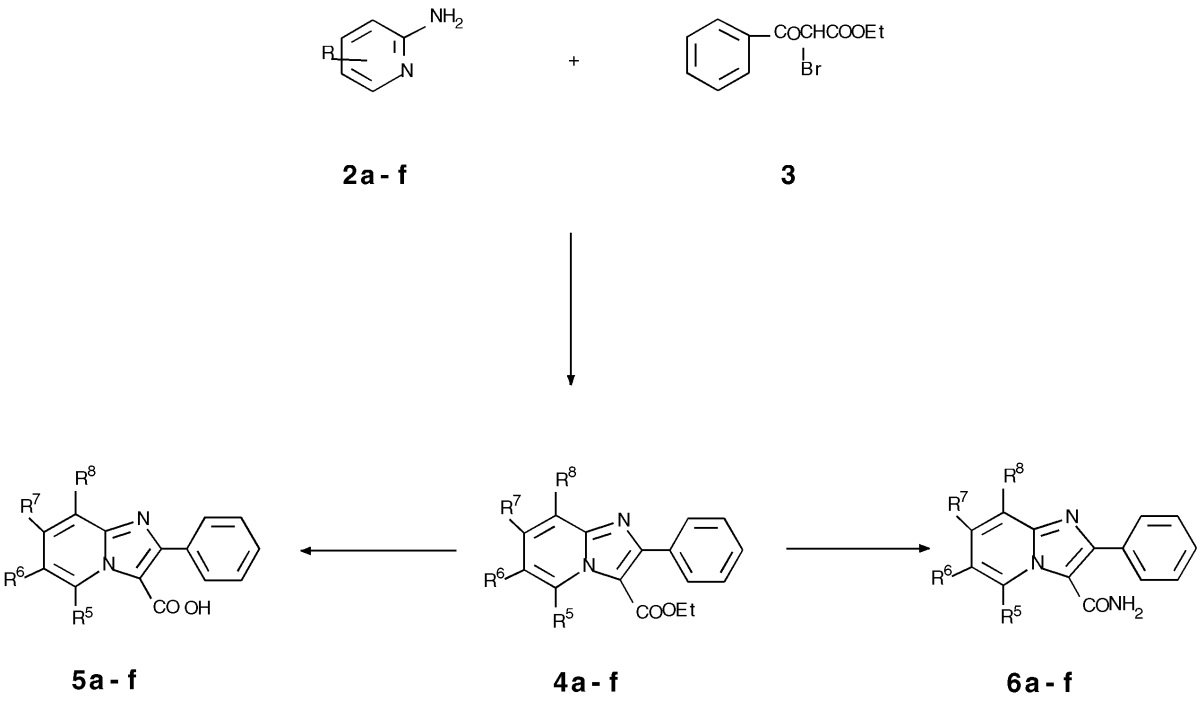

	R^5	R^6	R^7	R^8
a	H	H	H	H
b	Me	H	H	H
c	H	Me	H	H
d	H	H	Me	H
e	H	H	H	Me
f	H	Cl	H	H

Figure 3. Synthetic methods for ethyl esters **4a-f**, acids **5a–f**, and amides **6a–f.**

2-Aminopyridines (**2a–f**) were refluxed with an equimolar amount of ethyl 2-bromo-3-oxo-3-phenylpropionate **3** in ethanolic solution to afford the corresponding ethyl ester **4**. The esters **4a–f** were converted into the respective carboxylic acids (**5a–f**) by alkaline hydrolysis, whereas the corresponding carboxamides (**6a–f**) were prepared by ammonolysis.

All new compounds showed ^{1}H and ^{13}C-NMR spectra coherent with the structures assigned and in good accordance with available literature spectral data on 2-phenylimidazo[1,2-*a*]pyridines[6].

The most peculiar feature of the ^{1}H-NMR spectra of these new compounds is the chemical shift of H-5, which is strongly deshielded by the presence of the -COOR group in the 3-position, with consequent large downfield shift of the signal of this proton (δ 9.2–9.5 for esters **4** in $CDCl_3$, δ 10.2–10.5 for acids **5** in CF_3COOD).This deshielding was already observed for similar compounds[10, 11] by us and was first observed and studied by Hand and Paudler[12] in a series of imidazo[1,2-*a*]pyridines: this large downfield shift of the H-5 signal appears when the 3-position bears a substituent -XY containing an electron-rich atom (Y) two bonds removed from C-3 (like COOEt).

The ^{13}C-NMR spectrum of the ethyl ester **4a** is reported in the experimental part as an example.

Results and Discussion

The *in vivo* antiinflammatory, analgesic, and ulcerogenic activities of the esters **4a,c–f**, the acids **5a,c–f**, and the amides **6a,c–f** were determined, using indomethacin (IMA) as the reference drug. Antiinflammatory activity was measured by means of the carrageenan-induced rat paw edema: these results are listed in Table 1. The most active compound was 6-methyl-2-phenylimidazo[1,2-*a*]pyridine-3-carboxylic acid **5c**, which showed ED50 values sharply lower in comparison with IMA. The corresponding ethyl ester **4c** was much less active and amide **6c** was inactive. A fairly good activity was displayed by both the ester **4d** and the amide **6a**, whereas the remaining compounds showed moderate antiinflammatory action.

Analgesic activity was determined using the acetic acid writhing test in mice and the results obtained are summarized in Table 2. In this assay, too, the acid **5c** afforded the best result, but its activity level was clearly not comparable with that obtained in the edema test. The corresponding ethyl ester **4c** was less active and the amide **6c** almost inactive. Lower or negligible analgesic activity was shown by the other compounds.

The gastrointestinal irritative and ulcerogenic action (Table 3) was negligible in the series of esters **4**, a little higher in

Table 1. Carrageenan rat paw edema: antiinflammatory activity of esters **4**, acids **5**, and amides **6**.

Compound	Dose mg/kg po	% Edema inhibition relative to control at the 1st h	2nd h	3rd h	4th h	ED50, mg/kg (fiducial limits) 3rd h	4th h
4a	20	–11	–12	–11	–12	–	–
	40	–32	–26	–19	–33		
	60	–82	–69	–50	–46		
4c	20	–19	–20	–19	–20	–	–
	40	–40	–34	–29	–27		
	60	–59	–51	–46	–42		
4d	10	–27	–17	–12	–8	–	37.2
	20	–33	–25	–20	–17		(31.4–44.0)
	40	–82	–69	–63	–58		
4e	40	–59	–34	–19	–18	–	–
4f	20	0	0	0	–17	–	–
	40	–19	–20	–19	–33		
	60	–40	–34	–47	–41		
5a	25	–11	–12	–11	–12	–	–
	40	–32	–26	–19	–17		
	50	–46	–40	–42	–49		
5c	1.25	–40	–38	–41	–40	2.2	2.4
	2.5	–48	–50	–53	–52	(1.5–3.3)	(1.4–3.35)
	5	–59	–60	–63	–66		
	25	–70	–71	–73	–73		
	50	–82	–83	–86	–80		
5d	40	–16	–33	–25	–20	–	–
5e	40	–3	–18	–25	–30	–	–
5f	40	–9	–20	–18	–23	–	–
6a	10	–10	–10	–10	–10	34.5	29.8
	20	–17	–20	–25	–33	(28.8–41.4)	(25.2–35.2)
	40	–29	–51	–58	–63		
6c	40	0	–15	–9	–3	–	–
6d	20	–11	–12	–11	–12	–	–
	40	–32	–26	–19	–17		
	60	–33	–67	–43	–38		
6e	20	–19	–20	–19	–20	–	–
	40	–24	–26	–19	–17		
	60	–32	–45	–56	–58		
6f	40	0	–18	–29	–38	–	–
IMA	5	–3	–40	–37	–35	7.0	6.5
	7.5	–16	–33	–49	–55	(6.1–8.1)	(5.9–7.1)
	10	–39	–55	–67	–79		

the group of acids **5** and rather remarkable in the series of amides **6**, except **6c**.

Such results show that in the series of imidazo[1,2-*a*]pyridines under examination a clear structure-activity relationship is readily distinguishable: the contemporary presence of a methyl group in the 6-position and a phenyl moiety in the 2-position largely increased the antiinflammatory activity, leading to a compound (**5c**) more potent than indomethacin in the edema test. In this connection, it must be noted that the corresponding unsubstituted 2-carboxylic acid was found to be completely devoid of pharmacological activity[4].

In regard to analgesic action, similar results were obtained, but the potency of the most active compound **5c** is sharply lower in comparison with its antiinflammatory activity.

The ulcerogenic action of compounds **4**, **5**, and **6**, summarized in Table 3 shows an absolute lack of parallelism with the antiinflammatory and analgesic data. Such an observation is not surprising for us: in fact, we have observed this pharmacological profile in almost all series of derivatives of fused imidazole systems synthesized until now. In regard to the most active compound, namely **5c**, it should be noted that the antiinflammatory activity is higher by far than the analgesic one, whereas the ulcerogenic action is practically absent.

This is certainly not the pharmacological profile expected from a compound hypothetically acting as inhibitor of the prostaglandin biosynthesis.

In order to investigate this aspect of the problem, some compounds were tested *in vitro* for their cyclooxygenase-inhibiting activity as detailed in the experimental part. We

Table 2. Acetic acid writhing test in mice: analgesic activity of compounds **4**, **5**, and **6**.

Compound	Dose mg/kg po	% Decrease of writhes relative to control	ED50, mg/kg (fiducial limits)
4a	40	–3.6	–
4c	20	–22.2	49.9
	40	–45.3	(41.0–60.7)
	60	–54.4	
4d	20	–27.8	46.7
	40	–41.3	(37.7–58.0)
	60	–59.9	
4e	40	29.3	–
4f	40	23.0	–
5a	40	–16.1	–
5c	5	–20.0	39.1
	25	–33.0	(28.6–53.3)
	40	–51.5	
	50	–60.0	
5d	40	–34.3	–
5e	40	–18.0	–
5f	40	–27.1	–
6a	40	–27.5	–
6c	40	–6.0	–
6d	40	–30.4	–
6e	40	–25.1	–
6f	40	–8.4	–
IMA	2.5	–24.0	4.7
	5	–56.0	(4.1–5.3)
	7.5	–66.0	
	10	–81.0	

selected for this test three compounds which showed pharmacological activity *in vivo* (**4d**, **5c**, **6a**) and two inactive compounds (**4a**, **5a**): all five compounds showed very low inhibitory activity, i.e. <10% relative to control, compared with 90% of indomethacin at the same concentration (10 μM).

It is logical to argue from these results that the *in vivo* activity is independent from the cyclooxygenase inhibition and therefore must be supported by different mechanisms of action.

Acknowledgments

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Experimental

Chemistry

The course of reactions and the purity of products were controlled by TLC using precoated silica gel plates (Merck 60 F254). Detection of components was made by UV light and/or treatment with iodine vapors. Preparative separations were performed in columns containing Merck 60 silica gel (70–230 mesh ASTM). Melting points were determined with a Kofler hot stage microscope and are uncorrected.

Table 3. Gastrointestinal lesions in rats.

Compound (100 mg/kg po.)	Observations at the 6th hour after treatment: % animals with	
	hyperaemia	ulcers
4a	0	0
4c	40	20
4d	20	10
4e	0	0
4f	10	0
5a	100	0
5c	100	0
5d	0	0
5e	40	40
5f	30	20
6a	80	40
6c	10	0
6d	40	20
6e	40	30
6f	20	20
IMA[a)]	80	50

[a)] 5 mg/kg po.

Elemental analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. ^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker WM-250 spectrometer or a Bruker AMX-500 instrument equipped with a Bruker X-32 computer. Chemical shift values are reported in δ units (ppm) relative to TMS used as internal standard.

*Ethyl 2-phenylimidazo[1,2-a]pyridine-3-carboxylates **4a–f***

General procedure: a solution of equimolar amounts of a 2-aminopyridine **2** and ethyl 2-bromo-3-oxo-3-phenylpropionate (**3)** in anhydrous ethanol was refluxed for 4–6 h. The solvent was then removed under reduced pressure, the residue was treated with $NaHCO_3$ saturated solution and extracted with chloroform. The organic extract was washed with water, dried on Na_2SO_4, concentrated *in vacuo* up to small volume and then chromatographed on a silica gel column, eluting with *n*-hexane/diethyl ether mixtures with decreasing percentage of *n*-hexane. The products so obtained were recrystallized from *n*-hexane.

*Ethyl 2-phenylimidazo[1,2-a]pyridine-3-carboxylate **4a** (R_5, R_6, R_7, R_8 = H)*

Prepared from 2-aminopyridine following a method previously described [6].

^{13}C-NMR ($CDCl_3$): 160.97 (C=O), 153.47 (C-2), 146.98 (C-8a), 134.43 (C-1'), 130.15 (CH-2', 6'), 128.58, 128.20, 127.80 (CH-4', CH-5, CH-7), 127.46 (CH-3', 5'), 117.33 (CH-8), 113.95 (CH-6), 111.50 (C-3), 60.35 (CH_2), 13.91 (CH_3).

*Ethyl 5-methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxylate **4b** (R_5 = CH_3, R_6, R_7, R_8 = H)*

Prepared from 2-amino-6-methylpyridine. Oil, yield 5%. Anal. ($C_{17}H_{16}N_2O_2$) C, H, N. NMR ($CDCl_3$): 7.68 (m, 2H) and 7.40 (m, 3H) (phenyl protons), 7.50 (d, 1H, 8-H), 7.19 (dd, 1H, 7-H), 6.85 (d, 1H, 6-H), 4.30 (q, 2H, CH_2), 2.48 (s, 3H, 5-CH_3), 1.15 (t, 3H, CH_3). $J_{7,8}$ = 9.32 Hz, $J_{6,7}$ = 7.05 Hz.

*Ethyl 6-methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxylate **4c** (R_6 = CH_3, R_5, R_7, R_8 = H)*

Prepared from 2-amino-5-methylpyridine according to ref. [6].

Ethyl 7-methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxylate ***4d*** *(R_7 = CH_3, R_5, R_6, R_8 = H)*

Prepared from 2-amino-4-methylpyridine. Mp 85 °C, 27%. Anal. ($C_{17}H_{16}N_2O_2$) C, H, N. NMR ($CDCl_3$): 9.22 (d, 1H, 5-H), 7.70 (m, 2H) and 7.37 (m, 3H) (phenyl protons), 7.44 (s, 1H, 8-H), 6.81 (d, 1H, 6-H), 4.23 (q, 2H, CH_2), 2.41 (s, 3H, 7-CH_3) 1.16 (t, 3H, CH_3). $J_{5,6}$ = 7.1 Hz.

Ethyl 8-methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxylate ***4e*** *(R_8 = CH_3, R_5, R_6, R_7 = H)*

Prepared from 2-amino-3-methylpyridine. Mp 75 °C, 22%. Anal. ($C_{17}H_{16}N_2O_2$) C, H, N. NMR ($CDCl_3$): 9.22 (dd, 1H, 5-H), 7.69 (m, 2H) and 7.36 (m, 3H) (phenyl protons), 7.18 (t, 1H, 7-H), 6.89 (t, 1H, 6-H), 4.22 (q, 2H, CH_2), 2.63 (s, 3H, 8-CH_3) 1.13 (t, 3H, CH_3). $J_{5,6}$ = $J_{6,7}$ = 6.95 Hz, $J_{5,7}$ = 1.62 Hz.

Ethyl 6-chloro-2-phenylimidazo[1,2-a]pyridine-3-carboxylate ***4f*** *(R_6 = Cl, R_5, R_7, R_8 = H)*

Prepared from 2-amino-5-chloropyridine. Mp 120 °C, 21%. Anal. ($C_{16}H_{13}ClN_2O_2$) C, H, Cl, N. NMR ($CDCl_3$): 9.55 (d, 1H, 5-H), 7.96(d, 1H, 8-H), 7.65 (m, 2H) and 7.43 (m, 3H) (phenyl protons), 7.53 (dd, 1H, 7-H), 4.28 (q, 2H, CH_2), 1.16 (t, 3H, CH_3). $J_{7,8}$ = 9.46 Hz, $J_{5,7}$ = 1.84 Hz.

2-Phenylimidazo[1,2-a]pyridine-3-carboxylic acids ***5a,c–f***

General procedure. A mixture of 10 mmol of each ethyl ester, 50 ml of 10% NaOH and 60 ml of ethanol was refluxed for 1 h. Ethanol was then removed under reduced pressure, the residual aqueous solution was adjusted to pH 4–5 with HCl to obtain the precipitation of the acid which was then recrystallized from methanol.

2-Phenylimidazo[1,2-a]pyridine-3-carboxylic acid ***5a****:*

Prepared from **4a** according to ref. [6].

6-Methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxylic acid ***5c***

Prepared from **4c** according to ref. [6].

7-Methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxylic acid ***5d***

Prepared from **4d**. Mp 143°C, yield 54%. Anal. ($C_{15}H_{12}N_2O_2$) C, H, N. NMR (CF_3COOD): 10.17 (d, 1H, 5-H), 8.41 (s, 1H, 8-H), 8.25 (d, 1H, 6-H), 8.30 (m, 2H) and 8.16 (m, 3H) (phenyl protons), 3.29 (s, 3H, 7-CH_3). $J_{5,6}$ = 6.65 Hz.

8-Methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxylic acid ***5e***

Prepared from **4e**. Mp 145°C, 55%. Anal. ($C_{15}H_{12}N_2O_2$) C, H, N. NMR (CF_3COOD): 10.26 (dd, 1H, 5-H), 8.63 (t, 1H, 7-H), 8.22 (t, 1H, 6-H), 8.34 (m, 2H) and 8.28 (m, 3H) (phenyl protons), 3.40 (s, 3H, 8-CH_3). $J_{5,6}$ = $J_{6,7}$ = 7.0 Hz, $J_{5,7}$ = 1.8 Hz.

6-Chloro-2-phenylimidazo[1,2-a]pyridine-3-carboxylic acid ***5f***

Prepared from **4f**. Mp 120°C, 55%. Anal. ($C_{14}H_9ClN_2O_2$) C, H, Cl, N. NMR (CF_3COOD): 10.49 (d, 1H, 5-H), 8.71 (d, 1H, 8-H), 8.26 (dd, 1H, 7-H), 8.52 (m, 2H) and 8.37 (m, 3H) (phenyl protons), $J_{7,8}$ = 9.38 Hz, $J_{5,7}$ = 1.88 Hz.

2-Phenylimidazo[1,2-a]pyridine-3-carboxamides ***6a,c–f***

General procedure: 10 mmol of the starting ethyl ester **4** was dissolved in 50 ml of methanol, added with 50 ml of 32% aqueous ammonia and heated in a closed vessel under a pressure of 400 psi for 24 h. After cooling, the solution was evaporated in *vacuo*. The residue was dissolved in a small volume of chloroform and chromatographed on silica gel with *n*-hexane/diethyl ether mixtures. The product was then recrystallized from methanol.

2-Phenylimidazo[1,2-a]pyridine-3-carboxamide ***6a***

Prepared from **4a**. Mp 130°C, yield 68%. Anal. ($C_{14}H_{11}N_3O$) C, H, N. NMR ($CDCl_3$): 8.18 (d, 1H, 5-H), 7.72 (d, 1H, 8-H), 7.39 (t, 1H, 7-H), 6.85 (t, 1H, 6-H), 8.01 (m, 2H) and 7.49 (m, 3H) (phenyl protons), 7.92 (bs, 1H) and 7.24 (bs, 1H) ($CONH_2$). $J_{5,6}$ = 6.7 Hz, $J_{7,8}$ = 9 Hz, $J_{5,7}$ = 1.5 Hz.

6-Methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxamide ***6c***

Prepared from **4c**. Mp 150°C, 44%. Anal. ($C_{15}H_{13}N_3O$) C, H, N. NMR (CD_3OD): 8.17 (d, 1H, 5-H), 7.32 (d, 1H, 8-H), 7.16 (dd, 1H, 7-H), 7.87 (m, 2H) and 7.41 (m, 3H) (phenyl protons), 8.05 (bs, 1H) and 7.44 (bs, 1H) ($CONH_2$), 2.31 (s, 3H, 6-CH3). $J_{7,8}$ = 9 Hz, $J_{5,7}$ = 1.4 Hz.

7-Methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxamide ***6d***

Prepared from **4d**. Mp 140°C, 56%. Anal. ($C_{15}H_{13}N_3O$) C, H, N. NMR ($CDCl_3$): 7.94 (d, 1H, 5-H), 7.72 (s, 1H, 8-H), 6.57 (d, 1H, 6-H), 7.87 (m, 3H, two phenyl protons and one amide proton), 7.39 (bs, 1H, one amide proton), 7.36 (m, 3H, phenyl protons), 2.34 (s, 3H, 7-CH_3). $J_{5,6}$ = 6.83 Hz.

8-Methyl-2-phenylimidazo[1,2-a]pyridine-3-carboxamide ***6e***

Prepared from **4e**. Mp 130°C, 44%. Anal. ($C_{15}H_{13}N_3O$) C, H, N. NMR (CD_3OD): 8.25 (dd, 1H, 5-H), 7.35 (t, 1H, 7-H), 6.80 (t, 1H, 6-H), 7.94 (m, 2H) and 7.45 (m, 3H) (phenyl protons), 8.12 (bs, 1H) and 7.09 (bs, 1H) ($CONH_2$), 2.62 (s, 3H, 8-CH3). $J_{5,6}$ = $J_{6,7}$ = 7 Hz, $J_{5,7}$ = 1.6 Hz.

6-Chloro-2-phenylimidazo[1,2-a]pyridine-3-carboxamide ***6f***

Prepared from **4f**. Mp 195°C, 55%. Anal. ($C_{14}H_{10}ClN_3O$) C, H, Cl, N. NMR ($CDCl_3$): 10.48 (d, 1H, 5-H), 8.81 (m, 2H, 8-H and one amide proton), 8.70 (dd, 1H, 7-H), 8.37 (m, 2H) and 8.24 (m, 3H) (phenyl protons), 8.34 (bs, one amide proton). $J_{5,7}$ = 1.7 Hz.

Pharmacology

In regard to the experiments carried out *in vivo*, test compounds were administered orally by gavage in 1% methylcellulose suspension at different dose levels. In the edema and writhing tests, each compound was first tested at 40 mg/kg. If a significant activity was observed, lower and/or higher doses were then administered in order both to study dose-dependence of antiinflammatory and analgesic activity and to calculate ED_{50} values, if possible. Gastric ulcerogenic action was studied in rats which were treated orally with higher doses (100 mg/kg). Indomethacin was included in all tests for comparison purposes (IMA in Tables 1–3).

A cyclooxygenase activity assay was carried out *in vitro* on rabbit colonic microsomes. Microsomal fractions were incubated with test compounds or IMA at the same concentration (10 μM).

The following experimental procedures were employed.

Antiinflammatory Activity

The paw edema inhibition test [13] was used on rats. Groups of 5 rats of both sexes (body weight 150–200 g), pregnant females excluded, were given a dose of a test compound. After 30 min 0.2 ml 1% carrageenan suspension in 0.9 % NaCl solution was injected subcutaneously into the plantar aponeurosis of the hind paw and the paw volume was measured by the water plethysmometer Socrel and then measured again 1, 2, 3, and 4 h later. The mean increase of paw volume at each time interval was compared with that of control group (5 rats treated with carrageenan, but not treated with test compounds) at the same time intervals and percentage inhibition values were calculated. The experimental results are listed in Table 1.

Analgesic Activity

Acetic acid writhing test [14]was used on mice. Groups of 5 mice of both sexes (body weight 20–25 g), pregnant females excluded, were given a dose of a test compound. After 30 min the animals were injected intraperitoneally with 0.25 ml/mouse of 0.5 % acetic acid solution and writhes were counted during the following 25 min. The mean number of writhes for each experimental group and percentage decrease compared with the control group (5 mice not treated with test compounds) were calculated. The experimental results are listed in Table 2.

Ulcerogenic Action

Groups of 10 rats of both sexes (body weight 200–220 g), pregnant females excluded, fasted for 24 h, were given an oral dose of a test compound, except the control group. All animals were sacrificed 6 h after dosing and their stomachs and small intestines were macroscopically examined to assess the incidence of hyperaemia and ulcers. The experimental results are listed in Table 3.

Cyclooxygenase Activity Assay in Vitro

Rabbit distal colon cyclooxygenase inhibition. This test[15] was carried out on the microsomal fraction of mucosal preparations of rabbit distal colon. The preparation of colonic microsomes was based on the method of Hassid and Dunn[16]. Following this method, colonic mucosa (2–3 g), stripped as previously described [17], was minced and homogenized in a Potter homogenizer in 3 vol of Tris buffer 0.1 M, pH 8.0. The homogenate was centrifuged for 30 min at 10000 *g*. The resulting supernatant was centrifuged for 1h at 100000 *g*.The precipitate was suspended in Tris buffer 0.1 M, pH 8.0, and centrifuged again for 1 h at 100000 *g*. The microsomal pellet was used immediately for enzyme assay. Cyclooxygenase activity was assayed by measuring the rate of conversion of arachidonic acid to PGE_2. Microsomal fractions (50 µl) were incubated with test agents for 5 min at 37°C in 30 µl of Tris-HCl, pH 8.0, containing 2 mM reduced glutathione, 5mM L-tryptophan, and 1 µM hematin. The substrate, 20 µM arachidonic acid with tracer amounts of [1-^{14}C]arachidonic acid (220000 cpm), was then added and the reaction proceeded for 3 min at 37°C. The reaction was stopped by the addition of 0.2 ml of ethyl ether/methanol/citric acid 0.2 M (30:4:1), which was precooled at –25°C. PGE_2 was extracted twice into the same mixture. The solvent was evaporated under a N_2 stream and radiolabelled arachidonic acid was separated from radiolabelled PGE_2 by RP-HPLC as previously described [17]. Briefly, HPLC analysis was performed on a Hitachi spectrophotometer (Model 100-40) equipped with a flow cell; the sample was injected on a Ultrasphere column (Beckman) ODS 5 mm, 4.6 mm × 25 cm, with 2 nmol unlabelled PGE_2 as an internal standard. The PG chromatographic profile was obtained by isocratic elution with 150 mM H_3PO_4 in water, pH 3.5, containing 30% acetonitrile, at flow rate of 1 ml/min monitoring the UV absorption at 214 nm. Radioactivity that coeluted with authentic PGE_2 was quantified by liquid scintillation spectrometry. Test samples were compared with paired control incubations. The percentage of inhibition was calculated as follows:

$$[(\text{cpm control} - \text{cpm test})/(\text{cpm control})] \times 100$$

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