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# Research on heterocyclic compounds. XXXVII. Synthesis and antiinflammatory activity of methyl-substituted imidazo[1,2-*a*]pyrazine derivatives

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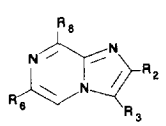
**Summary** — A series of methyl-substituted imidazo[1,2-*a*]pyrazines **8** bearing a carboxylic acid group on the imidazole ring were synthesized. The structures of new compounds were confirmed by $^1$H- and $^{13}$C-NMR spectral data; the correct assignment of carbon resonances was made by means of HETCOR and COLOC experiments. Antiinflammatory, analgesic and ulcerogenic activities in vivo were evaluated and compared with those of antiinflammatory imidazopyrazines (**2** and **3**) and indomethacin. The inhibitory action on cyclooxygenase activity was evaluated in vitro. Compounds **8** were found to be less potent than indomethacin in these assays. SARs are discussed.

**imidazo[1,2-*a*]pyrazine / antiinflammatory activity / analgesic activity / ulcerogenic activity**

## Introduction

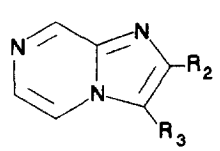
During our research on the relationships between chemical structure and antiinflammatory activity of fused imidazo heterocyclic derivatives and on their mode of action [1], we have prepared and pharmacologically tested a number of imidazo[1,2-*a*]pyrazine derivatives all related to the general formula **1** depicted in figure 1.

We synthesized imidazo[1,2-*a*]pyrazine-2-acetic acids ($R_2$ = $CH_2COOH$, $R_3$ = H) [2, 3], imidazo[1,2-*a*]-pyrazine-2-methyl-3-carboxylic acids ($R_2$ = $CH_3$, $R_3$ = COOH) [4], imidazo[1,2-*a*]pyrazine-2-methyl-3-acetic acids ($R_2$ = $CH_3$, $R_3$ = $CH_2COOH$) [5], imidazo[1,2-*a*]-pyrazine-2-phenyl-3-carboxylic acids ($R_2$ = $C_6H_5$, $R_3$ = COOH) [5, 6] and imidazo[1,2-*a*]pyrazine-2-(*p*-chloro-phenyl)-3-carboxylic acids ($R_2$ = *p*-$ClC_6H_4$, $R_3$ = COOH) [6]. Most of these compounds showed anti-inflammatory and analgesic activity and low or negligible ulcerogenic action at the gastrointestinal level. In the carrageenan rat paw edema test three compounds were found to be the most active (fig 2).




1

**Fig 1.** Structure of compound **1**.




**2:** $R_2$ = $CH_2COOH$, $R_3$ = H
**3:** $R_2$ = COOH, $R_3$ = H
**4:** $R_2$ = $CH_3$, $R_3$ = COOH

**Fig 2.** Structure of compound **2–4**.

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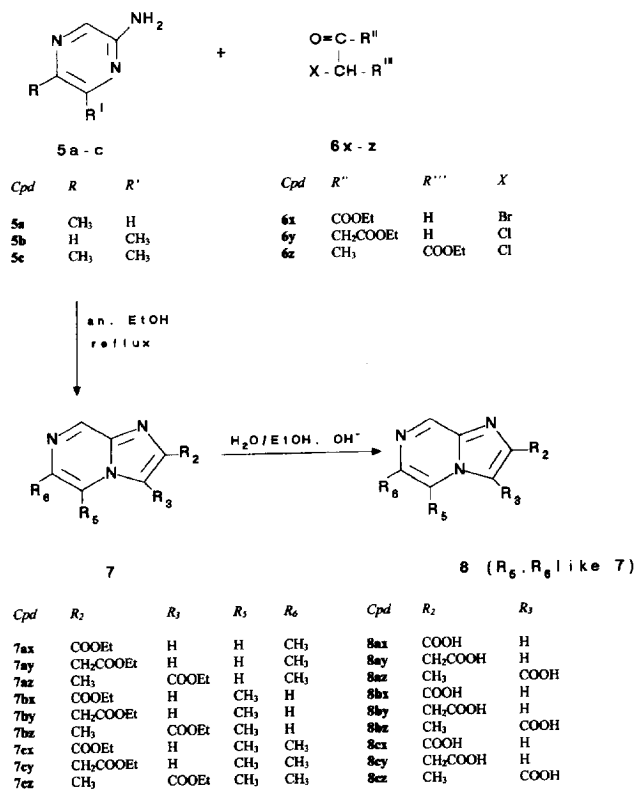
The acetic acid **2** and the carboxylic acid **3** also displayed a good analgesic and antipyretic action [2, 3], whereas the 2-methyl-3-carboxylic acid **4** was essentially an antiinflammatory agent [4]. The presence of substituents such as Br, $OCH_3$ and $OC_2H_5$ on the pyrazine ring as well as a phenyl moiety on the imidazole ring proved to be unfavourable and led to lower activity. However, in other series of analogs such as imidazo[1,2-*a*]pyridines we found a clear relationship between the presence of a methyl group in the 6 position and the pharmacological activity [7]. This finding prompted us to prepare some methyl-substituted imidazopyrazine derivatives in order to explore the possibility of increasing the activity in this series.

## Chemistry

A series of methyl-substituted imidazo[1,2-*a*]pyrazines bearing an acidic function on the imidazole ring were prepared using the synthetic method usually employed for fused imidazole systems; in the present case (fig 3) a methyl-substituted 2-aminopyrazine (**5a–c**) was refluxed in anhydrous ethanol with either ethyl bromopyruvate **6x**, ethyl 4-chloroacetoacetate **6y**, or ethyl 2-chloroacetoacetate **6z** to obtain the ethyl esters. These esters were converted to the corresponding acids **8** by alkaline hydrolysis.




| Cpd | R | R' |
|---|---|---|
| 5a | $CH_3$ | H |
| 5b | H | $CH_3$ |
| 5c | $CH_3$ | $CH_3$ |

| Cpd | R'' | R''' | X |
|---|---|---|---|
| 6x | COOEt | H | Br |
| 6y | $CH_2COOEt$ | H | Cl |
| 6z | $CH_3$ | COOEt | Cl |

| Cpd | $R_2$ | $R_3$ | $R_5$ | $R_6$ |
|---|---|---|---|---|
| 7ax | COOEt | H | H | $CH_3$ |
| 7ay | $CH_2COOEt$ | H | H | $CH_3$ |
| 7az | $CH_3$ | COOEt | H | $CH_3$ |
| 7bx | COOEt | H | $CH_3$ | H |
| 7by | $CH_2COOEt$ | H | $CH_3$ | H |
| 7bz | $CH_3$ | COOEt | $CH_3$ | H |
| 7cx | COOEt | H | $CH_3$ | $CH_3$ |
| 7cy | $CH_2COOEt$ | H | $CH_3$ | $CH_3$ |
| 7cz | $CH_3$ | COOEt | $CH_3$ | $CH_3$ |

| Cpd | $R_2$ | $R_3$ |
|---|---|---|
| 8ax | COOH | H |
| 8ay | $CH_2COOH$ | H |
| 8az | $CH_3$ | COOH |
| 8bx | COOH | H |
| 8by | $CH_2COOH$ | H |
| 8bz | $CH_3$ | COOH |
| 8cx | COOH | H |
| 8cy | $CH_2COOH$ | H |
| 8cz | $CH_3$ | COOH |

**Fig 3.** Synthesis of compounds **8**.

The haloketoesters **6x–z** are commercially available, whereas the amines **5a–c** were prepared ad hoc by modifying literature methods. At first we adopted the method described by Pitrè and Boveri [8], which offered the possibility of obtaining both 2-amino-5-methylpyrazine **5a** and 2-amino-5,6-dimethylpyrazine **5c** through a multistep procedure which started from aminoacetonitrile hydrochloride and led to aminoacetamidine. This product was converted without difficulty into **5c** by the final reaction with 2,3-butanedione. According to Pitrè and Boveri, the replacement of this diketone with methylglioxal should provide **5a**, but in our hands the reaction afforded a mixture of 2-amino-5-methylpyrazine **5a** and 2-amino-6-methylpyrazine **5b**.

The notable difficulties encountered in the separation of this isomeric mixture and the unsatisfactory yields, particularly of **5b**, persuaded us to employ more specific methods to separately obtain the monomethyl amines. Therefore, 2-amino-5-methylpyrazine **5a** was prepared on the basis of the method of Weijlard et al [9] with some modifications, starting from the commercially available 5-methylpyrazine-2-carboxylic acid. This compound was converted into the carboxamide (via the methyl ester), which gave the required amine by Hofmann reaction with potassium hypobromite.

The isomer **5b**, namely 2-amino-6-methylpyrazine, was prepared by a method which resulted from the combination of two different sources [9, 10]. The cyclocondensation of 5,6-diamino-2,4-dihydroxypyrimidine with methylglyoxal in acidic aqueous solution gave a precipitate of 2,4-dihydroxy-7-methylpyrimido-[4,5-*b*]pyrazine **9**. This product was hydrolyzed to 2-amino-6-methylpyrazine-3-carboxylic acid **10** which was then converted into the required amine **5b** by decarboxylation at 205 °C in tetralin (fig 4).

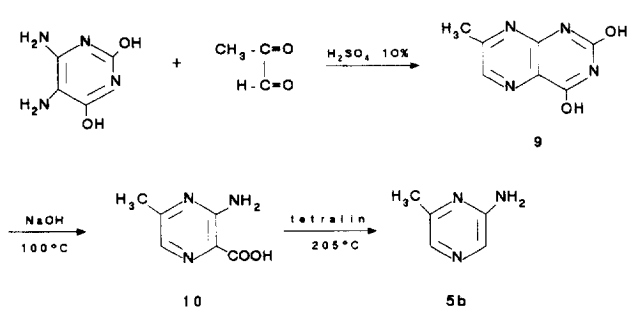



**Fig 4.** Synthesis of compound **5b**.

The structures assigned to all new imidazo[1,2-*a*]-pyrazine derivatives are consistent with their $^1$H- and $^{13}$C-NMR spectra reported in tables I and II. In order to unequivocally assign all carbon resonances, we accomplished the assignment of the carbon resonances of compound **7cy**, namely ethyl 5,6-dimethyl-imidazo[1,2-*a*]pyrazine-2-acetate, by means of ($^1$H, $^{13}$C) heteronuclear shift correlation spectroscopy via $J_1$ (HETCOR) and via $J_2$ and $J_3$ (COLOC). Data from the HETCOR experiment allowed us to assign the CH, $CH_2$ and $CH_3$ carbon resonances using those of the directly bonded protons (table III). The chemical shifts of quaternary carbons were obtained by the analysis of the COLOC data. In this experiment it was possible to observe cross-peaks between protons and carbons through two or three bonds (fig 5). These correlations (reported in table IV) allowed us to complete the unequivocal assignment of the carbon resonances of compound **7cy** and consequently also those of the other compounds, as detailed in table II.

## Pharmacology

All new acids **8** were tested in vivo in order to evaluate their pharmacological activity.

The carrageenan rat paw edema and the acetic acid writhing test in mice were employed to assess anti-inflammatory and analgesic activity, respectively. Each compound was administered orally by gavage at a dosage level of 40 mg/kg. In the case of significant activity, lower and/or higher doses were then administered and the related values of $ED_{50}$ were calculated, when possible. The irritative and ulcerogenic action on the mucosa of stomach and small intestine was evaluated in rats using doses of 100 mg/kg.

These three tests were selected in order to obtain information on the mode of action of the acids **8**. In fact, if they are able to inhibit the prostaglandin biosynthesis, they should show a similar level of potency in all three tests. The most active and some less active compounds were also investigated by means of a cyclooxygenase activity assay in vitro, in order to get unequivocal information about this question. For the same reason, we included in all tests not only indomethacin as reference drug, but also imidazo[1,2-*a*]-pyrazine-2-acetic acid **2** and imidazo[1,2-*a*]pyrazine-2-carboxylic acid **3**, which are the most active imidazopyrazine derivatives obtained by us to date.

## Results and discussion

The experimental results obtained in the pharmacological tests in vivo are reported in tables V–VII.

Results reported in table V clearly show that all new acids of the **8** type display significant antiinflammatory activity. The most active members of this

**Table I.** $^1$H-NMR data for ethyl esters **7** and acids **8**.

| *Compound* | *Chemical shift (δ)*[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | *H-3(s)* | *H-5(s)* | *H-6(s)* | *H-8(s)* | *-$CH_2$COO(s)* | *Ethyl (q, t)* | *Methyl groups (s)* |
| **7ax** | 7.79 | 8.07 | – | 9.01 | – | 4.37, 1.34 | 2.43 |
| **7ay** | 7.51 | 7.72 | – | 8.80 | 3.75 | 4.10, 1.20 | 2.45 |
| **7az** | – | 8.83 | – | 8.86 | – | 4.30, 1.30 | 2.45, 2.60 |
| **7bx** | 7.70 | – | 8.10 | 8.98 | – | 4.35, 1.35 | 2.50 |
| **7by** | 7.63 | – | 7.71 | 8.93 | 3.94 | 4.22, 1.26 | 2.58 |
| **7bz** | – | – | 7.75 | 8.90 | – | 4.40, 1.40 | 2.65[b], 2.62[b] |
| **7cx** | 7.89 | – | – | 8.75 | – | 4.20, 1.20 | 2.35[b], 2.31[b] |
| **7cy** | 7.53 | – | – | 8.80 | 3.89 | 4.15, 1.23 | 2.50 (6H) |
| **7cz** | – | – | – | 8.85 | – | 4.40, 1.30 | 2.55[b], 2.53[b], 2.62[b] |
| **8ax** | 8.45 | 8.55 | – | 9.02 | – | – | 2.60 |
| **8ay** | 8.05 | 8.42 | – | 8.98 | 3.96 | – | 2.58 |
| **8az** | – | 8.90 | – | 9.10 | – | – | 2.58[b], 2.60[b] |
| **8bx** | 8.32 | – | 8.60 | 9.00 | – | – | 2.50 |
| **8by** | 8.25 | – | 8.40 | 9.30 | 4.17 | – | 2.18 |
| **8bz** | – | – | 7.90 | 8.91 | – | – | 2.70[b], 2.75[b] |
| **8cx** | 8.60 | – | – | 9.00 | – | – | 2.40, 2.50 |
| **8cy** | 8.10 | – | – | 9.00 | 3.90 | – | 2.40, 2.50 |
| **8cz** | – | – | – | 8.70 | – | – | 2.50[b], 2.55[b], 2.68[b] |

[a]Spectra of esters **7** were recorded in $CDCl_3$ solution, spectra of acids **8** were recorded in $CD_3OD$ solution; [b]assignment uncertain.

**Table II.** $^{13}$C-NMR data for ethyl esters **7**.

| *Compound* | *Chemical shifts (δ)*[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *C-2* | *C-3* | *C-5* | *C-6* | *C-8* | *C-8a* | *CO* | *$CH_2CO$* | *Ethyl* | *Methyl groups* |
| **7ax** | 139.5 | 115.5 | 116.8 | 138.4 | 144.6 | 139.2 | 162.4 | – | 61.5, 14.5 | 20.5 |
| **7ay** | 141.8 | 111.6 | 115.5 | 138.0 | 142.0 | 139.0 | 170.2 | 35.0 | 61.0, 14.0 | 20.6 |
| **7az** | 153.0 | 113.2 | 117.3 | 140.4 | 141.2 | 140.0 | 160.8 | – | 60.7, 14.2 | 21.0 |
| **7bx** | 140.3 | 114.6 | 128.3 | 128.8 | 142.9 | 138.3 | 162.5 | – | 61.4, 14.2 | 15.4 |
| **7by** | 142.3 | 109.3 | 127.7 | 128.0 | 140.7 | 140.4 | 170.3 | 35.2 | 61.1, 14.1 | 15.6 |
| **7bz** | 152.3 | 115.7 | 131.0 | 131.6 | 141.7 | 140.3 | 160.4 | – | 61.4, 14.2 | 18.7, 16.0 |
| **7cx** | 140.0 | 114.6 | 124.4 | 136.0 | 141.7 | 138.4 | 162.8 | – | 61.5, 14.3 | 14.6, 20.0 |
| **7cy** | 142.0 | 109.3 | 124.0 | 134.8 | 139.1 | 138.8 | 170.5 | 35.2 | 61.1, 14.1 | 14.6, 19.9 |
| **7cz** | 152.5 | 115.6 | 127.6 | 141.5 | 138.7 | 138.3 | 160.8 | – | 61.3, 14.3 | 16.0, 17.7, 21.0 |

[a]Chemical shifts of quaternary carbon atoms are written in italics; all spectra were recorded in $CDCl_3$ solution.

**Table III.** $^1$H- and $^{13}$C-NMR data for **7cy** obtained in an HETCOR experiment ($CDCl_3$, 500 MHz; see fig 5).

| *Position* | $\delta_H$ | $\delta_C$ |
|---|---|---|
| 3 | 7.53 | 109.3 |
| 8 | 8.80 | 139.1 |
| Ethyl $CH_2$ | 4.15 | 61.1 |
| Acetic $CH_2$ | 3.89 | 35.2 |
| 5-$CH_3$ | 2.50 | 14.6 |
| 6-$CH_3$ | 2.50 | 19.9 |
| Ethyl $CH_3$ | 1.23 | 14.1 |

**Table IV.** $^1$H- and $^{13}$C-NMR selected data of **7cy** obtained in an COLOC experiment ($CDCl_3$, 500 MHz; see fig 5).

| *Position* | $\delta_H$ | *COLOC* | $\delta_C$ |
|---|---|---|---|
| H-3 | 7.53 | C-2 | 142.0 |
| | | C-8a | 138.8 |
| H-8 | 8.80 | C-8a | 138.8 |
| | | C-6 | 134.8 |
| Acetic $CH_2$ | 3.89 | C=O | 170.5 |
| | | C-2 | 142.0 |
| | | C-3 | 109.3 |
| 5-$CH_3$ | 2.50 | C-5 | 124.0 |
| 6-$CH_3$ | 2.50 | C-6 | 134.8 |

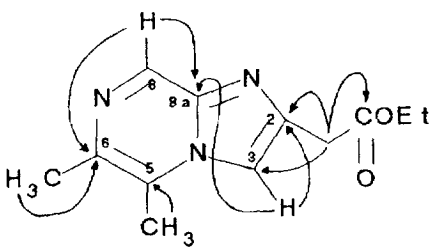



**Fig 5.** Interactions causing cross-peaks in the COLOC experiment on compound **7cy**.

series are **8cy** and **8cz**, namely 5,6-dimethylimidazo-[1,2-*a*]pyrazine-2-acetic acid and 2,5,6-trimethyl-imidazo[1,2-*a*]pyrazine-3-carboxylic acid, respectively. However, the presence of two methyl groups in positions 5 and 6 is not important per se, as evidenced by **8cx**, which is one of the less active products. Also, no clear relationship exists between the substitution pattern on the imidazole ring and the antiinflammatory activity. However, all the acids **8** are notably less active than the unsubstituted acids **2** and **3** (fig 2), whose activity is 0.5–0.6 times indomethacin.

The analgesic activity (table VI) is lower for all compounds, even if in this case the methyl substitution seems to have a positive influence on such pharmacological action. The ulcerogenic action (table VII) appears significant but unrelated to the substitution pattern. Moreover, the most potent antiinflammatory compounds **2** and **3** are much less active than acids **8** as analgesic and ulcerogenic agents.

Compounds **2** and **3** were then investigated in vitro for their cyclooxygenase inhibiting activity, together with **8ay** and **8cy** (which showed significant anti-

**Table V.** Carrageenan rat paw edema: antiinflammatory activity of acids **8**.

| *Compound* | *Dose (mg/kg po)* | *Percentage edema inhibition relative to control at* | | | | *$ED_{50}$ (mg/kg) (fiducial limits)* | |
|---|---|---|---|---|---|---|---|
| | | *1st hour* | *2nd hour* | *3rd hour* | *4th hour* | *3rd hour* | *4th hour* |
| **2** | 5 | –19 | –16 | –12 | –11 | 11.07 | 10.80 |
| | 7.5 | –32 | –34 | –28 | –32 | (4.49–27.30) | (5.54–21.06) |
| | 10 | –58 | –70 | –74 | –68 | | |
| | 20 | –64 | –75 | –78 | –82 | | |
| | 40 | –71 | –79 | –82 | –86 | | |
| **3** | 5 | –6 | –7 | –6 | –6 | 14.20 | 12.60 |
| | 7.5 | –35 | –25 | –20 | –25 | (6.80–29.40) | (5.70–27.60) |
| | 10 | –52 | –66 | –54 | –63 | | |
| | 20 | –61 | –73 | –76 | –70 | | |
| | 40 | –68 | –77 | –80 | –84 | | |
| **8ax** | 20 | –36 | –36 | –36 | –36 | – | 51.80 |
| | 40 | –46 | –46 | –44 | –50 | | (32.00–83.10) |
| | 80 | 0 | –25 | –35 | –54 | | |
| **8ay** | 20 | –17 | –18 | –24 | –27 | 48.30 | 49.40 |
| | 40 | –15 | –24 | –45 | –52 | (39.50–59.10) | (38.02–64.30) |
| | 80 | –57 | –54 | –65 | –59 | | |
| **8az** | 20 | –28 | –44 | –44 | –52 | – | – |
| | 40 | –66 | –60 | –57 | –50 | | |
| | 80 | –35 | –51 | –50 | –50 | | |
| **8bx** | 20 | – 3 | –20 | –25 | –30 | – | – |
| | 40 | –25 | –33 | –52 | –58 | | |
| | 80 | –68 | –67 | –50 | –41 | | |
| **8by** | 20 | 0 | 0 | –18 | –25 | – | 54.90 |
| | 40 | –33 | –33 | –43 | –50 | | (41.70–72.30) |
| | 80 | – 9 | –31 | –40 | –55 | | |
| **8bz** | 40 | –20 | –33 | –28 | –28 | – | – |
| **8cx** | 40 | –33 | –40 | –28 | –25 | – | – |
| **8cy** | 20 | –35 | –33 | –37 | –41 | 40.07 | 29.60 |
| | 40 | –25 | –54 | –52 | –58 | (27.90–57.50) | (21.80–40.10) |
| | 80 | –50 | –50 | –61 | –69 | | |
| **8cz** | 10 | 0 | – 8 | –16 | –23 | 33.10 | 28.10 |
| | 20 | – 3 | –20 | –25 | –30 | (26.70–40.40) | (10.80–48.40) |
| | 40 | –39 | –63 | –61 | –66 | | |
| Indomethacin | 5 | – 3 | –40 | –37 | –35 | 7.00 | 6.72 |
| | 7.5 | –16 | –33 | –49 | –55 | (4.50–10.81) | (4.83–8.73) |
| | 10 | –39 | –55 | –67 | –79 | | |

inflammatory action; see table V), **8bx** (significant activity, but lack of dose-dependency) and **8cx** (low activity). The cyclooxygenase assay was carried out by measuring the rate of conversion of [1-$^{14}$C]arachidonic acid to $PGE_2$ in the microsomal fraction of mucosa preparations of rabbit distal colon, after incubation with test compound [6]. In this test (table VIII), compounds **2** and **3** displayed a minimal inhibitory activity (≈ 20%), whereas indomethacin showed high activity (90%) at the same concentration (10 μM).

**Table VI.** Acetic acid writhing test in mice: analgesic activity of acids **8**.

| *Compound* | *Dose (mg/kg po)* | *Percentage decrease of writhes in 25 min after treatment relative to control* | *$ED_{50}$ (mg/kg) (fiducial limits)* |
|---|---|---|---|
| **2** | 40 | –29.5 | – |
| **3** | 40 | –20.1 | – |
| **8ax** | 40 | –9.8 | – |
| **8ay** | 40 | –26.8 | 65.21 |
| | 60 | –49.0 | (57.31–74.44) |
| | 80 | –58.0 | |
| **8az** | 40 | –36.8 | 66.45 |
| | 60 | –48.0 | (50.11–88.10) |
| | 80 | –54.0 | |
| **8bx** | 40 | –12.2 | – |
| **8by** | 40 | –15.0 | – |
| **8bz** | 40 | –43.0 | 53.15 |
| | 60 | –53.0 | (41.11–68.73) |
| | 80 | –60.0 | |
| **8cx** | 40 | –4.1 | – |
| **8cy** | 40 | –21.6 | – |
| **8cz** | 40 | –35.9 | 81.57 |
| | 60 | –41.0 | (60.81–101.0) |
| | 80 | –51.0 | |
| Indomethacin | 5 | –56.0 | 4.43 |
| | 7.5 | –66.0 | (2.73–7.22) |
| | 10 | –81.0 | |

The four compounds of the type **8** are completely devoid of activity, despite of their antiinflammatory action.

On the basis of these experimental results, which are similar to those repeatedly obtained with several series of other imidazo derivatives, it seems logical to conclude that the in vivo activity of the acids **8** is supported by multiple mechanisms of action, among which only the inhibition of prostaglandin biosynthesis is of marginal importance.

## Experimental protocols

*Chemistry*

Precoated silica-gel Merck 60 F254 plates were used for thin layer chromatography; detection of components was made by UV light and/or treatment with iodine vapours. Preparative chromatographic separations were performed in columns packed with silica-gel (Farmitalia Carlo Erba RS, ∅ mm 0.05–0.20). Melting points were determined by a Kofler hot stage microscope and are uncorrected. Elemental analyses indicated by the symbols of the elements were found to be within ±0.4% of calculated values. The $^1H$ and $^{13}C$ measurements and experiments were performed on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer.

*2-Amino-5-methylpyrazine* ***5a***
Prepared following the method of Wejlard et al [9]. $^1H$-NMR ($CDCl_3$): δ 7.96 (1H, s, H-6), 7.76 (1H, s, H-3), 2.37 (3H, s, 5-$CH_3$). $^{13}C$-NMR ($CDCl_3$): δ 154.3 (C-2), 151.0 (C-5), 134.0 (CH-6), 128.0 (CH-3), 20.0 (5-$CH_3$).

*2-Amino-5,6-dimethylpyrazine* ***5c***
Prepared following the method of Pitrè and Boveri [8]. $^1H$-NMR ($CDCl_3$): δ 7.79 (1H, s, H-3), 2.36, 2.29 (two s, 3H each, 5- and 6-$CH_3$). $^{13}C$-NMR ($CDCl_3$): δ 152.0 (C-2), 149.0 (C-5), 140.0 (C-6), 128.1 (CH-3), 21.5, 20.5 (5- and 6-$CH_3$).

**Table VII.** Gastrointestinal lesions in rats.

| *Compounds* | *Dose (mg/kg po)* | *Remarks at the 6th hour after treatment* | |
|---|---|---|---|
| | | *percentage of animals with hyperaemia* | *percentage of animals with ulcers* |
| **2** | 250 | 100 | 20 |
| **3** | 250 | 20 | 20 |
| **8ax** | 100 | 60 | 40 |
| **8ay** | 100 | 40 | 20 |
| **8az** | 100 | 60 | 30 |
| **8bx** | 100 | 50 | 50 |
| **8by** | 100 | 60 | 30 |
| **8bz** | 100 | 20 | 10 |
| **8cx** | 100 | 50 | 20 |
| **8cy** | 100 | 40 | 40 |
| **8cz** | 100 | 40 | 40 |
| Indomethacin | 5 | 100 | 60 |

**Table VIII.** Cyclooxygenase activity assay: inhibitory action of some selected acidic derivatives.

| *Compound*[a] | *Percentage inhibition* |
|---|---|
| **2** | 19.2 |
| **3** | 21.1 |
| **8ay** | 4.7 |
| **8bx** | 3.6 |
| **8cx** | 4.8 |
| **8cy** | ni |
| Indomethacin | 90.0 |

[a]A concentration of 10 μM was used for all compounds; ni: no inhibition.

*2,4-Dihydroxy-7-methylpyrimido[4,5-b]pyrazine **9***
A mixture of 25 g (0.176 mol) of 5,6-diamino-2,4-dihydroxypyrimidine sulfate and 700 mL water was added with 50 mL of 40% aqueous solution of methylglyoxal followed by 30 mL of 10% sulfuric acid up to pH 2. The mixture was boiled, evaporated to half volume, cooled to 2 °C, adjusted to pH 9 with aqueous NaOH and then left standing overnight. The product precipitated and was filtered off, washed with water and dried (yield 19 g, 60%).

*2-Amino-6-methylpyrazine-3-carboxylic acid **10***
The product **9** obtained as described above (19 g, 0.1 mol) was vigorously refluxed with a solution of 16 g of NaOH in 100 mL water for 20 h. The solution was then left standing at room temperature for 8 h and adjusted to pH 2.5 with dilute HCl. After standing overnight, the precipitate was filtered off, washed with water and dried (yield 4.7 g, 30%).

*2-Amino-6-methylpyrazine **5b***
A mixture of 15 g (0.1 mol) of **10** and 250 mL anhydrous tetralin was refluxed for 30 min. The solution was then cooled and repeatedly extracted with small portions of 10% aqueous HCl. The extracts were combined, washed with petroleum ether and basified with 20% aqueous NaOH. The alkaline solution was extracted many times with diethyl ether to obtain 8 g of the amine **5b** (yield 73%), mp 124–125 °C. $^1$H-NMR ($CDCl_3$) : δ 7.78, 7.79 (two s, 1H each, H-3 and H-5), 2.35 (3H, s, 6-$CH_3$). $^{13}$C-NMR ($CDCl_3$): δ 152.3 (C-2), 142.6 (C-6), 140.8 (CH-5), 131.2 (CH-3), 21.0 (6-$CH_3$).

*Ethyl esters **7***
Equimolar amounts of a 2-aminopyrazine (**5a–c**) and a haloketoester (**6x–z**) were refluxed in anhydrous ethanol for at least 5 h. The solvent was then removed under reduced pressure. The residue was treated with a saturated solution of $NaHCO_3$ and then extracted three times with chloroform. The organic extracts were combined, washed with water, dried over anhydrous sodium sulphate, concentrated in vacuo up to a small volume and then chromatographed on a silica-gel column eluting with chloroform. The product obtained was recrystallized from *n*-hexane.

Using this general procedure the following compounds were obtained.

*Ethyl 6-methylimidazo[1,2-a]pyrazine-2-carboxylate* ***7ax***. Mp 142 °C, yield 31%. Anal $C_{10}H_{11}N_3O_2$ (C, H, N).

*Ethyl 6-methylimidazo[1,2-a]pyrazine-2-acetate* ***7ay***. Mp 60–62 °C, yield 23%. Anal $C_{11}H_{13}N_3O_2$ (C, H, N).

*Ethyl 2,6-dimethylimidazo[1,2-a]pyrazine-3-carboxylate* ***7az***. Mp 95 °C, yield 18%. Anal $C_{11}H_{13}N_3O_2$ (C, N, H).

*Ethyl 5-methylimidazo[1,2-a]pyrazine-2-carboxylate* ***7bx***. Mp 147 °C, yield 29%. Anal $C_{10}H_{11}N_3O_2$ (C, H, N).

*Ethyl 5-methylimidazo[1,2-a]pyrazine-2-acetate* ***7by***. Mp 85 °C, yield 19%. Anal $C_{11}H_{13}N_3O_2$ (C, H, N).

*Ethyl 2,5-dimethylimidazo[1,2-a]pyrazine-3-carboxylate* ***7bz***. Mp 110 °C, yield 17%. Anal $C_{11}H_{13}N_3O_2$ (C, N, H).

*Ethyl 5,6-dimethylimidazo[1,2-a]pyrazine-2-carboxylate* ***7cx***. Mp 141–143 °C, yield 20%. Anal $C_{11}H_{13}N_3O_3$ (C, N, H).

*Ethyl 5,6-dimethylimidazo[1,2-a]pyrazine-2-acetate* ***7cy***. Reaction time 16 h. Mp 76–77 °C, yield 15%. Anal $C_{12}H_{15}N_3O_2$ (C, N, H).

*Ethyl 2,5,6-trimethylimidazo[1,2-a]pyrazine-3-carboxylate* ***7cz***. Reaction time 8 h. Mp 100 °C, yield 17%. Anal $C_{12}H_{15}N_3O_2$ (C, N, H).

*Acids* ***8***

These acids were obtained by alkaline hydrolysis of the corresponding esters in aqueous/alcoholic NaOH solution (2 N, ethanol/water 7:3), stirring the mixture for 1 h at 60 °C. The solution was then partially evaporated in vacuo to remove ethanol and the resulting concentrated aqueous solution was adjusted to pH 3.5 with 2 N HCl. The precipitate was collected, washed with water and recrystallized from ethanol. The yield of the reaction was always about 80%.

By means of this procedure the following products were obtained.

*6-Methylimidazo[1,2-a]pyrazine-2-carboxylic acid* ***8ax***. Mp 239 °C. Anal $C_8H_7N_3O_2$ (C, H, N).

*6-Methylimidazo[1,2-a]pyrazine-2-acetic acid* ***8ay***. Mp 195–197 °C. Anal $C_9H_9N_3O_2$ (C, H, N).

*2,6-Dimethylimidazo[1,2-a]pyrazine-3-carboxylic acid* ***8az***. Mp 218 °C dec. Anal $C_9H_9N_3O_2$ (C, N, H).

*5-Methylimidazo[1,2-a]pyrazine-2-carboxylic acid* ***8bx***. Mp >230 °C dec. Anal $C_8H_7N_3O_2$ (C, H, N).

*5-Methylimidazo[1,2-a]pyrazine-2-acetic acid* ***8by***. Mp 182 °C. Anal $C_9H_9N_3O_2$ (C, H, N).

*2,5-Dimethylimidazo[1,2-a]pyrazine-3-carboxylic acid* ***8bz***. Mp >230 °C dec. Anal $C_9H_9N_3O_2$ (C, N, H).

*5,6-Dimethylimidazo[1,2-a]pyrazine-2-carboxylic acid* ***8cx***. Mp 218–220 °C. Anal $C_9H_9N_3O_2$ (C, N, H).

*5,6-Dimethylimidazo[1,2-a]pyrazine-2-acetic acid* ***8cy***. Mp 160–162 °C. Anal $C_{10}H_{11}N_3O_2$ (C, N, H).

*2,5,6-Trimethylimidazo[1,2-a]pyrazine-3-carboxylic acid* ***8cz***. Mp >230 °C dec. Anal $C_{10}H_{11}N_3O_2$ (C, N, H).

*Pharmacology*

Test compounds were administered orally by gavage in 1% methylcellulose suspension, using first a dose of 40 mg/kg and then, if a significant activity was found, lower and/or higher doses in order to study dose-dependence of antiinfiammatory and analgesic activity.

Gastric ulcerogenic action was studied in rats treated orally with higher doses (100 or 200 mg/kg).

Indomethacin was included in all tests for comparison purposes.

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

The following experimental procedures were employed.

*Antiinflammatory activity*

Paw edema inhibition test [11] was used on rats. Groups of five rats of both sexes (body weight 120–160 g), pregnant females excluded, were given a dose of a test compound. Thirty minutes later 0.2 mL of 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 a 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 the control group (five rats treated with carrageenan, but not treated with test compounds) at the same time intervals and percent inhibition values were calculated. Experimental results are listed in table V.

*Analgesic activity*

Acetic acid writhing test [12] was used on mice. Groups of five mice (body weight 20–25 g) of both sexes, pregnant females excluded, were given a dose of a test compound. Thirty minutes later 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 percent decrease compared with the control group (five mice not treated with test compounds) were calculated. Experimental results are listed in table VI.

*Ulcerogenic action*

Groups of five rats (body weight 200–220 g) of both sexes, pregnant females excluded, fasted for 24 h, were treated with an oral dose of a test compound, except the control group. All animals were killed 6 h after dosing and their stomachs and small intestines were macroscopically examined to assess the incidence of hyperaemia and ulcers. Experimental results are listed in table VII.

*Cyclooxygenase activity assay [13]*

This test was carried out in vitro 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 [15]. Colonic mucosa (≈ 2–3 g), stripped as previously described [15], was minced and homogenized in Potter homogenizer in 3 vol of Tris buffer 0.1 M, pH 8.0. The homogenate was centrifuged for 30 min at 10 000 *g*. The resulting supernatant was centrifuged for 1 h at 100 000 *g*. The precipitate was suspended in Tris buffer 0.1 M, pH 8.0, and recentrifuged for 1 h at 100 000 *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 Tris-HCl, pH 8.0, containing 2 mM reduced glutathione, 5 mM L-tryptophan, 1 μM hematin. The substrate, 20 μM arachidonic acid with tracer amounts of [1-$^{14}$C]arachidonic acid (≈ 220 000 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 an $N_2$ stream and radiolabelled arachidonic acid was separated from radiolabelled $PGE_2$ by RP-HPLC as previously described [15]. HPLC analysis was performed on a Hitachi spectrophotometer (Model 100-40) equipped with a flow cell; the sample was injected on an Ultrasphere column (Beckman) ODS 5 mm, 4.6 mm x 25 cm, with 2 nmol unlabelled $PGE_2$ as an internal standard. 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 co-eluted with authentic $PGE_2$ was quantified by liquid scintillation spectrometry.

Test samples were compared to paired control incubations.

The percentage of inhibition was calculated as follows:

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

The results obtained are reported in table VIII.

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