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Short communication

Research on heterocyclic compounds, XLI. 2-Phenylimidazo[1,2-b]pyridazine-3-acetic derivatives: synthesis and anti-inflammatory activity

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Abstract – The synthesis of a group of 2-phenylimidazo[1,2-b]pyridazine-3-acetic esters and acids is described. The structures of the new compounds are supported by ^{1}H-NMR spectra. These compounds were tested in vivo for their anti-inflammatory, analgesic and ulcerogenic activity. All new compounds showed remarkable anti-inflammatory action in the carrageenan rat paw oedema (one third of that for indomethacin) but no significant analgesic activity in the acetic acid writhing test together with negligible ulcerogenic action, and were also found to be lacking inhibitory activity on cyclooxygenase in vitro. © 1999 Éditions scientifiques et médicales Elsevier SAS

imidazo[1,2-b]pyridazines / anti-inflammatory activity / analgesic activity / ulcerogenic activity / cyclooxygenase inhibition

1. Introduction

In the context of our research on the structure-activity relationships and mode of action of bicyclic imidazo-derivatives with anti-inflammatory and analgesic activity, we synthesized a series of 2-phenylimidazo[1,2-b]pyridazine-3-carboxylic acids **1** (*figure 1*) which showed high analgesic activity in the acetic acid writhing test in mice, low or no anti-inflammatory activity in the carrageenan-induced rat paw oedema and low ulcerogenic action on the rat gastric mucosa [1, 2]. We then synthesized three series of analogues, namely 2-methylimidazo[1,2-b]-pyridazine-3-carboxylic acids **2** [3], imidazo[1,2-b]-pyridazine-2-acetic acids **3** [4] and imidazo[1,2-b]-pyridazine-2-carboxylic acids **4** [5].

In comparison with the first series of acids **1**, compounds **2** and **3** showed a lower analgesic activity, whereas the same activity reached the lowest level in the last series (acids **4**). However, the pharmacological profile was the same in all four groups of compounds, since the analgesic activity was always coupled with low or no anti-inflammatory and ulcerogenic action.

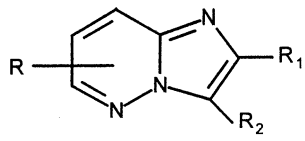

Figure 1. Imidazo[1,2-b]pyridazine acidic derivatives.

In consideration of the fact that the highest analgesic activity was shown by the first type of compounds, i.e. 2-phenylimidazo[1,2-b]pyridazine-3-carboxylic acid **1**, and in order to extend the study of the structure-activity relationships, we have synthesized a new series of 2-phenyl derivatives, with only one structural change, i.e. the replacement of the carboxylic moiety in position 3 by an acetic group.

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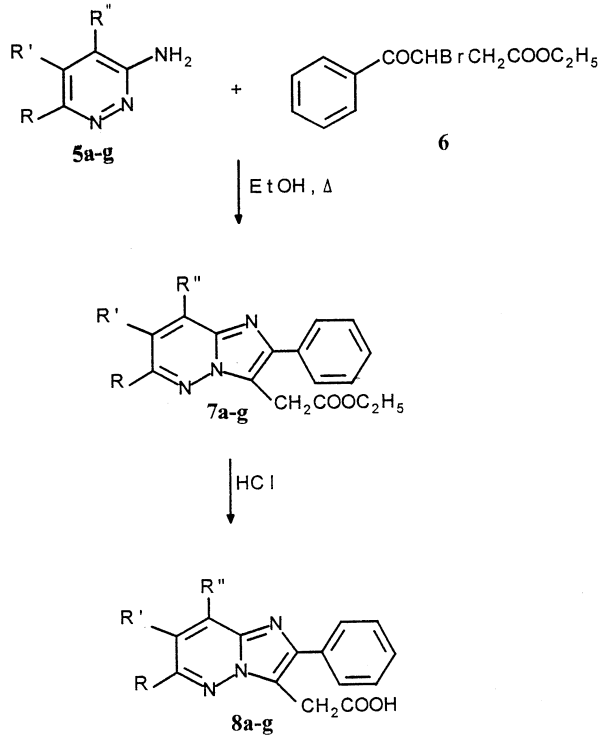

	a	b	c	d	e	f	g
R	H	Cl	OCH_3	H	H	Cl	Cl
R'	H	H	H	CH_3	H	CH_3	H
R"	H	H	H	H	CH_3	H	CH_3

Figure 2. Synthetic method for 2-phenylimidazo[1,2-b]pyridazine-3-acetic derivatives.

2. Chemistry

The required compounds were prepared using the synthetic method depicted in *figure 2*. The reaction in dry ethanol of 3-aminopyridazines **5a–g** with ethyl 3-benzoyl-3-bromopropionate **6**, prepared ad hoc, afforded the corresponding ethyl 2-phenylimidazo[1,2-b]pyridazine-3-acetates **7a–g**. These esters were converted into the respective acids **8a–g** by acid hydrolysis. Esters **7a**, **7b** and **7c** were prepared by reacting **6** with 3-aminopyridazine **5a**, 3-amino-6-chloropyridazine **5b**, and 3-amino-6-methoxypyridazine **5c**, respectively, prepared ad hoc following the methods described by Wermuth et al. [6] and Steck et al. [7].

The couples of isomers **7d–e** and **7f–g** were obtained with the same method previously employed to obtain the corresponding isomeric couples in the preceding series of imidazo[1,2-b]pyridazines **1–4** [5]. The couple **7f–g** was prepared using as starting material a mixture (nearly 1:1) of 3-amino-6-chloro-5-methylpyridazine (**5f**) and 3-amino-6-chloro-4-methylpyridazine (**5g**): this mixture was obtained from 3,6-dichloro-4-methylpyridazine [8] following the Mori procedure [9]. After reaction with **6**, the products **7f** and **7g** were separated by column chromatography. The above mixture of the amines **5f** and **5g** afforded the corresponding dehalogenated mixture of 3-amino-5-methylpyridazine (**5d**) and 3-amino-4-methylpyridazine (**5e**) by catalytic hydrogenation. The reaction of **6** with the mixture **5d–5e** afforded the esters **7d** and **7e**.

The correct structural assignments to these products were performed by ^{1}H-NMR spectra (*table I*), and are in accordance with the literature data, in particular with the chemical shifts found by Kobe et al. [10] for H-6, H-7, H-8 in the imidazo[1,2-b]pyridazine.

3. Pharmacology

The new esters **7a–g** and acids **8a–g** were tested in vivo using the acetic acid writhing test in mice and carrageenan induced rat paw oedema to study analgesic and anti-inflammatory activity. Higher doses were administered to rats to study the irritative and ulcerogenic action on the mucosa of the stomach and small intestine. Indomethacin was used in all tests as reference drug. These three tests should allow us not only to extend the SAR study to another series of imidazopyridazines, but also to obtain some information about their mechanism of action: they should display a similar level of activity in all three tests if they are inhibitors of prostaglandin biosynthesis. In order to unequivocally resolve this question, some new compounds (the more and less active ones) were also subjected to two different cyclooxygenase activity assays in vitro [11, 12].

4. Results and discussion

The anti-inflammatory activity displayed by the compounds under examination is reported in *table II*. The esters **7c**, **7d** and **7g**, and the acids **8a**, **8c, 8f** and **8g** are the most active compounds with comparable levels of activity, as can be seen on the basis of reported values of ED_{50} (approximately one third of that for indomethacin). The results obtained in the acetic acid writhing test are quite different (*table III*), in fact all compounds show weak or no activity. The gastrointestinal irritative and ulcerogenic action (*table IV*) was almost completely absent in all compounds.

There is no parallelism between the results of all three in vivo tests. This is certainly not the pharmacological

Table I. Yields, m.p. and ^{1}H-NMR spectral data of 2-phenylimidazo[1,2-b]pyridazine-3-acetic derivatives.

Compounds[1]	yield %	m.p. °C	^{1}H-NMR in ppm[2] δ: H-6	H-7	H-8	$J_{6,7}$	$J_{6,8}$ Hz	$J_{7,8}$	Ph	CH_2 (s)	Ethyl	Substituents
7a	32	103–105	8.50 (dd)	7.20 (dd)	7.85 (dd)	4.5	2.0	10	7.70 (m) 7.60 (m)	4.10	4.00 (q) 1.00 (t)	
7b	20	108–110		7.00 (d)	7.90 (d)			10	7.70 (m) 7.40 (m)	4.10	4.05 (q) 1.20 (t)	
7c	40	104–105		6.65 (d)	8.70 (d)			10	8.60 (m) 8.40 (m)	4.10	4.05 (q) 1.10 (t)	6-OCH_3: 3.90 (s)
7d	40	110–112	8.25 (d)		7.95 (d)		2.4		7.50 (m) 7.40 (m)	4.10	4.08 (q) 1.20 (t)	7-CH_3: 2.60 (s)
7e	40	121–123	8.15 (d)	7.19 (d)		4.3			7.60 (m) 7.50 (m)	4.10	4.05 (q) 1.30 (t)	8-CH_3: 2.80 (s)
7f	30	113–115			7.70 (s)				7.65 (m) 7.50 (m)	4.20	4.10 (q) 1.30 (t)	7-CH_3: 2.50 (s)
7g	30	125–127		6.85 (s)					7.80 (m) 7.50 (m) 7.30 (m)	4.20	4.10 (q) 1.30 (t)	8-CH_3: 2.70 (s)
8a	55	> 200	8.49 (dd)	7.20 (dd)	7.80 (dd)	4.2	2.0	10	7.60 (m) 7.55 (m)	4.05		
8b	55	> 200		7.21 (d)	7.95 (d)			10	7.90 (m) 7.68 (m)	4.10		
8c	50	> 200		6.75 (d)	8.77 (d)			10	8.67 (m) 8.42 (m)	4.10		6-OCH_3: 3.90 (s)
8d	55	> 200	8.35 (d)		8.04 (d)		2.3		7.60 (m) 7.45 (m)	4.10		7-CH_3: 2.65 (s)
8e	60	> 200	8.25 (d)	7.20 (d)		4.3			7.65 (m) 7.50 (m)	4.10		8-CH_3: 2.55 (s)
8f	60	> 200			7.75 (s)				7.90 (m) 7.50 (m)	4.10		7-CH_3: 2.55 (s)
8g	60	> 200		6.80 (s)					7.82 (m) 7.45 (m)	4.20		8-CH_3: 2.68

[1]All compounds were analysed for C, H, N (also for Cl when present): found values were within ± 0.4% compared with theoretical values.
[2]Solvents: $CDCl_3$ for **7a**–**g**, CD_3OD for **8a**–**g**.

Table II. Anti-inflammatory activity by the carrageenan rat paw oedema test.

Compound	Dose mg/kg p.o.	% Oedema inhibition relative to control at the: 1st h	2nd h	3rd h	4th h	ED_{50}, mg/kg (fiducial limits) 3rd h	4th h
7a	40	–17	–26	–29	–44	–	–
7b	40	–7	–14	–19	–21	–	–
7c	20	–62	–53	–34	–37	35.1	28.9
	40	–17	–26	–47	–59	(29.4–41.8)	(24.0–34.8)
	80	–52	–72	–80	–87		
7d	20	–28	–14	–24	–42	36.5	–
	40	–63	–56	–53	–51	(32.1–41.7)	
	80	–62	–78	–84	–90		
7e	40	–17	–26	–47	–44	–	–
7f	40	0	–20	–43	–44	–	–
7g	20	–5	–17	–20	–37	–	33.2
	40	–27	–34	–37	–51		(27.0–40.7)
	80	–57	–75	–82	–77		
8a	10	–30	–31	–22	–32	–	21.4
	20	–47	–42	–33	–47		(16.7–27.5)
	40	–53	–68	–73	–66		
8b	40	–14	–31	–33	–24	–	–
8c	10	–30	–20	–22	–24	31.4	27.8
	20	–58	–42	–41	–34	(24.5–40.1)	(22.6–34.2)
	40	–83	–66	–55	–64		
8d	40	–67	–51	–43	–25	–	–
8e	40	–33	–51	–43	–38	–	–
8f	10	–22	–33	–33	–22	17.8	19.8
	20	–66	–65	–51	–55	(14.7–21.5)	(17.0–23.1)
	40	–86	–81	–76	–75		
8g	10	–22	–28	–22	–25	21.9	23.0
	20	–58	–42	–41	–34	(18.9–25.4)	(1.6–33.7)
	40	–86	–72	–76	–75		
IMA	5	–3	–40	–38	–35	7.0	6.7
	7.5	–16	–33	–49	–55	(4.5–10.8)	(4.8–8.7)
	10	–39	–55	–67	–79		

Table III. Analgesic activity by the acetic acid writhing test in mice.

Compound	Dose mg/kg p.o.	% Decrease of mean no. of writhes in 25 min after treatment relative to control
7a	40	–22.5
7b	40	–4.5
7c	40	–34.4
7d	40	–8.8
7e	40	–32.3
7f	40	–4.0
7g	40	–25.1
8a	40	–17.2
8b	40	–8.9
8c	40	–26.5
8d	40	–10.8
8e	40	–9.3
8f	40	–29.6
8g	40	–15.8
IMA	5	–51.2

profile expected from compounds acting as inhibitors of the prostaglandin biosynthesis. In order to investigate this aspect of the question, our compounds were tested in vitro for their cyclooxygenase-inhibiting activity.

The most active compounds **7c**, **7d**, **8c**, **8f** and **8g** were tested for their cyclooxygenase-inhibiting activity by measuring the rate of conversion of [1-^{14}C]arachidonic acid into PGE_2 in the microsomal fraction of mucosa preparation of rabbit distal colon after incubation with test compounds, following the method previously reported [13].

All compounds were found to be devoid of inhibitory activity, i.e. 0.7–9.0% relative to control, compared with 90–92% of indomethacin at the same concentration (10 μM).

Table IV. Incidence of gastrointestinal lesions in rats.

Compound	Dose mg/kg p.o.	Remarks at 6 h after treatment: % animal with:	
		hyperaemia	ulcers
7a	100	20	0
7b	100	10	0
7c	100	30	0
7f	100	30	0
7g	100	20	0
8a	100	30	0
8b	100	40	0
8c	100	20	0
8f	100	30	0
8g	100	30	0
IMA	5	80	50

The second test was carried out by measuring the rate of conversion of exogenous arachidonic acid into PGE_2 in the rat medullary and cortical kidney microsomes. Inhibition of microsomal PGE_2 production was measured by radioimmunoassay as reported previously [13]. This test confirmed the above results: no compound showed significant activity (< 12% relative to control), except indomethacin (90–94%) at the same concentration (10 μM).

Therefore in the present case the remarkable anti-inflammatory activity shown by these compounds was found to be independent of the cyclooxygenase inhibition. It should be noted that we recently came to the same conclusion for a group of imidazo[1,2-a]pyrimidine analogues [14].

From the point of view of our studies on structure-activity relationships of imidazo[1,2-b]pyridazine acidic derivatives **1–4** (*figure 1*), the most significant finding is that the 2-phenylimidazo[1,2-b]pyridazine-3-acetic derivatives (esters **7** and acids **8**) showed weak analgesic activity and proved to be completely lacking ulcerogenic action, whereas showed a significant anti-inflammatory activity. The pharmacological profile resulting from the above data for these 2-phenylimidazo[1,2-b]pyridazine-3-acetic derivatives is different from that previously observed for **1–4** series in which the analgesic activity was clearly prevailing over the anti-inflammatory action, so further investigation will be necessary in order to explain the activity of these imidazo-derivatives, which is probably due to the multiple mechanism of action differently influenced by the structural changes.

5. Experimental protocols

5.1. Chemistry

Thin layer chromatography by precoated silica gel plates (Merck 60 F254) was used to control the course of reactions and purity of products: all compounds were designated as pure when they showed a single spot after elution with chloroform/methanol mixture (95:5); detection of components was made by UV light and/or treatment with iodine vapors. Preparative separations were performed in columns packed with silica gel from Farmitalia Carlo Erba (RS, ϕ mm 0.05:0.20). Melting points were determined with a Kofler hot stage microscope and are uncorrected. Elemental analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values. The ^{1}H-NMR spectra were recorded using a Bruker AMX- 500 spectrometer equipped with a Bruker X-32 computer; chemical shift values are reported

in δ units (ppm) relative to tetramethylsilane used as internal standard.

5.1.1. Ethyl 3-benzoyl-3-bromopropionate **6**

A solution of 3-benzoylpropionic acid (19.6 g, 0.1 mol) in 100 mL of dry ethanol with concentrated H_2SO_4 (5 mL) was refluxed for 7 h. After cooling, ethanol was removed in vacuo, and the residue dissolved in diethyl ether and extracted with $NaHCO_3$ saturated solution. The organic extract was washed with water, dried on Na_2SO_4 and evaporated in vacuo to obtain ethyl 3-benzoylpropionate as an oil in 75% yield.

A solution of ethyl 3-benzoylpropionate in 100 mL of diethyl ether was added slowly with an equimolar amount of bromine at 0 °C. The solution was stirred at room temperature for 1 h. The organic solution was washed three times with $NaHCO_3$ saturated solution, dried on Na_2SO_4 and evaporated in vacuo to obtain the required compound **6** as an oil in 65% yield.

5.1.2. Ethyl-2-phenylimidazo[1,2-b]pyridazine-3-acetates **7a** *and* **7c–g**

General procedure: a mixture of the starting 3-aminopyridazine and ethyl 3-benzoyl-3-bromopropionate **6** (molar ratio 1:1.5) in ligroine was refluxed for 3 h. After cooling, the mixture was filtered and the filtrate was extracted with 10% aqueous HCl. The aqueous layer was separated and adjusted to pH 7–8 with $NaHCO_3$ to obtain the precipitation of the ester which was then recrystallized from n-hexane. This procedure allowed us to obtain the esters **7a** and **7c**.

In the case of the isomeric mixtures **7d–e** and **7f–g**, obtained starting from the mixtures of the isomeric amines **5d–e** and **5f–g**, respectively, the crude product precipitated was subjected to column chromatographic separation, eluting with n-hexane/diethyl ether mixtures with increasing percentage of ether. The single products obtained from the columns were then recrystallized from n-hexane.

5.1.3. Ethyl 6-chloro-2-phenylimidazo[1,2-b]pyridazine-3-acetate, **7b**

A mixture of a 3-amino-6-chloropyridazine **5b** and ethyl 3-benzoyl-3-bromopropionate **6** (molar ratio 1:1.5) in anhydrous ethanol was refluxed for 10 h. After cooling, ethanol was removed in vacuo, and the residue treated with $NaHCO_3$ saturated solution and extracted with $CHCl_3$. The organic extract was washed with water, dried on Na_2SO_4 and evaporated in vacuo to obtain the required product **7b**, which was recrystallized from n-hexane.

5.1.4. 2-Phenylimidazo[1,2-b]pyridazine-3-acetic acids **8a–g**

General procedure: a mixture of 10 mmol of each ethyl ester and 40 mL of 10% aqueous HCl was refluxed for 2–3 h. After cooling, the solution was adjusted to pH 4–5 with $NaHCO_3$ to obtain the precipitation of the acid which was recrystallized from ethanol.

5.2. Pharmacology

As regards the experiments carried out in vivo, test compounds were administered orally by gavage in 1% methylcellulose suspension. In the oedema and writhing test each compound was first tested at 40 mg/kg. If a significant activity was observed, lower and/or higher doses were administered in order both to study the dose-dependence of the pharmacological activity and to calculate ED_{50} values, when 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 II–IV*).

5.2.1. Anti-inflammatory activity

The paw oedema inhibition test [15] 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 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 a 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 II*.

5.2.2. Analgesic activity

Acetic acid writhing test [16] 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 III*.

5.2.3. 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 intestine were macroscopically examined to assess the incidence of hyperaemia and ulcers. The experimental results are listed in *table IV*.

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