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Research on heterocyclic compounds. Part XXXVI. Imidazo[1,2-*a*]pyrimidine-2-acetic derivatives: synthesis and antiinflammatory activity

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imidazo[1,2-*a*]pyrimidines / antiinflammatory activity / analgesic activity / ulcerogenic activity / cyclooxygenase inhibition

Introduction

In the context of our research on the structure–activity relationships and mode of action of bicyclic imidazo derivatives with antiinflammatory/analgesic activity [1], all related to the structural model **1** (fig 1), we synthesized a series of imidazo[1,2-*a*]pyrimidine-2-carboxylic acids, esters and amides (fig 2) which showed significant antiinflammatory activity in the carrageenan-induced rat paw edema and remarkable analgesic action in the acetic acid writhing test in mice, accompanied by a not insignificant ulcerogenic and hyperaemic action on rat gastric mucosa. All compounds were tested in vitro by means of the isolation and determination of PGE_2: they showed no inhibitory action on cyclooxygenase activity [2]. Consequently we decided to synthesize the series of imidazo[1,2-*a*]pyrimidine-2-acetic analogues bearing the same substituents on the pyrimidine ring in order to explore the influence exerted by homologation on pharmacological activity.

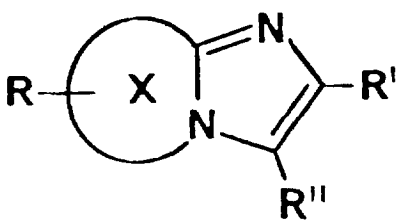

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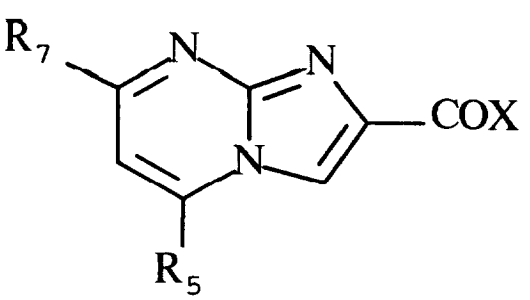
Fig 1. General structural model.

Chemistry

The required compounds were prepared using a synthetic method closely related to the general procedure normally employed to obtain bicyclic imidazo derivatives [1]. In the present case (fig 3) the starting 2-aminopyrimidine **3** was refluxed with ethyl 4-chloro-



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X = OEt, OH, NH_2

R_5, R_7 = Cl, CH_3, OMe

Fig 2. Imidazo[1,2-*a*]pyrimidine-2-carboxylic derivatives.

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acetoacetate **4** in methanolic solution to obtain the corresponding ethyl imidazo[1,2-*a*]pyrimidine-2-acetate **5**. This product underwent alkaline hydrolysis to afford the acetic acid **6** and ammonolysis to yield the carboxamide **7**. In this manner, starting from 2-amino-4-chloro-6-methylpyrimidine **3a** [3], we obtained a mixture of the ethyl ester **5a** and the methyl ester **8** (fig 4). In the case of 2-amino-4,6-dimethoxypyrimidine **3c** [3], the reaction afforded only the required ethyl ester **5c** (fig 5). On the contrary, 2-amino-4-methyl-6-methoxypyrimidine **3b** [4] was found to be almost incapable of reacting with **4**, so that the ester **5b** was obtained in very low yield. Consequently the methoxylation of **5a** in chloroform solution was employed in order to prepare the methyl ester **9** in good yield (fig 6, *Method A*). It should be noted that the same reaction carried out without chloroform directly afforded 5-methoxy-7-methyl-imidazo[1,2-*a*]pyrimidine-2-acetic acid **6b** (fig 6, *Method D*).

Both the acids **6a**, **6c** and the amides **7a–c** were prepared by refluxing the corresponding esters with 30% aqueous ammonia: the latter products were obtained by maintaining the solution at 100 °C and the former at 150–160 °C (figs 5, 6).

The alkaline hydrolysis of the ethyl ester **5a** with KOH in hydroethanolic solution afforded 5-ethoxy-7-methylimidazo[1,2-*a*]pyrimidine-2-acetic acid **6d** instead of the expected acid **6a** (fig 6, *Method E*). The ethyl ester **5c**, when reacted under the same conditions, yielded 5-ethoxy-7-methoxyimidazo[1,2-*a*]pyrimidine-2-acetic acid **6e** (fig 5, *Method D*).

The correct structural assignments of these products were carried out via ^{1}H- and ^{13}C-NMR spectra (see

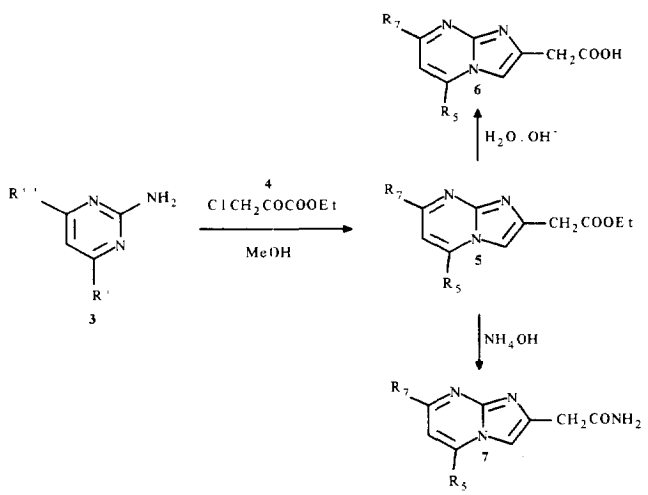

Fig 3. General synthetic scheme.

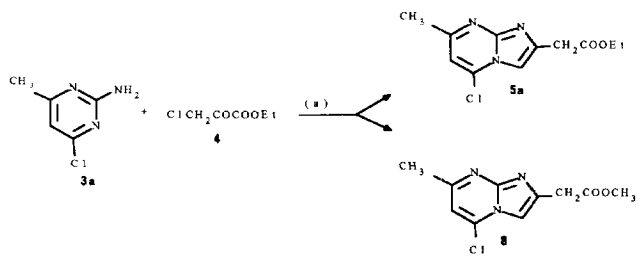

Fig 4. Synthesis of **5a**: (a) MeOH, reflux (ext temp 100 °C), **5a** (yield 13%), **8** (yield 9%).

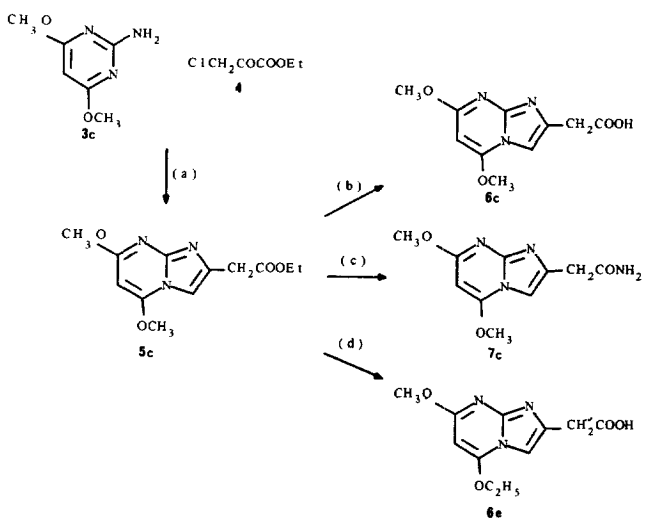

Fig 5. Synthesis and derivatives of **5c**: (a) MeOH, reflux (ext temp 160 °C), 24 h, 18%; (b) NH_4OH 30%, MeOH, reflux (ext temp 150–160 °C), 2 h, 55%; (c) NH_4OH 30%, MeOH, reflux 6 h, 67%; (d) KOH 2 N, EtOH, reflux 1 h, 63%.

Experimental protocols) on the basis of experimental evidence similar to that already discussed in the case of the preceding series of carboxylic analogues [2]. Only in the case of compound **6e** it was necessary to obtain further experimental data in order to assign the correct structure. The structure of **6e** was established by a nOe difference experiment which showed the ethoxylic group in position 5 and the methoxylic group in position 7. In fact by irradiation at 3H, a positive nOe effect was observed for the methyl group

of $-OC_2H_5$, whereas the methoxyl group was completely unaffected. On the other hand, irradiation of the ethoxylic methyl group produced a positive nOe effect for 3H and 6H, whereas irradiation of the methoxyl group caused a positive nOe effect only for 6H. These positive nOe effects showed inequivocally that **6e** had $-OCH_3$ in position 7 and $-OC_2H_5$ in position 5.

Pharmacology

The new acetic esters (**5a**, **5c**, **8** and **9**), acids (**6a–e**) and amides (**7a–c**) were tested in vivo to evaluate their pharmacological activity. Carrageenan-induced rat paw edema [5] was used to study antiinflammatory activity, whereas analgesic activity was assessed via the acetic acid writhing test in mice [6]. 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 tests were selected to provide information on the mode of action of these compounds. In fact, they should display a similar level of activity in all three tests if they act as inhibitors of prostaglandin biosynthesis. In order to unequivocally resolve this question, some new compounds were also subjected to two different cyclooxygenase activity assays in vitro [7, 8].

The experimental procedures have already been described in detail in a previous paper [2].

Results and discussion

The anti-inflammatory activity displayed by the acetic derivatives under examination is reported in table I. All compounds showed more or less anti-inflammatory action, although only the ethyl ester **5a** and acids **6c**, **6d** and **6e** displayed a good level of activity toward rat paw edema induced by carrageenan. The most potent compound was 5-ethoxy-7-methylimidazo[1,2-*a*]pyrimidine-2-acetic acid **6d** (~ 0.4 x indomethacin), whereas the activity level of the other three compounds was within 0.25–0.166 x indomethacin.

If one compares the results listed in table I with those reported in table II (analgesic activity in the acetic acid writhing test), it is possible to observe a clear parallel: in fact, the same four above-cited compounds showed the best activity. Compound **6d** was again the most potent, but the analgesic action of **6c** was closely similar (~ 0.125 x indomethacin); **5a** and **6e** were less active (~ 0.083 x indomethacin). It should be noted that the parallel between anti-inflammatory and analgesic activity is qualitative but not quantitative, since the latter activity was markedly lower compared to the former.

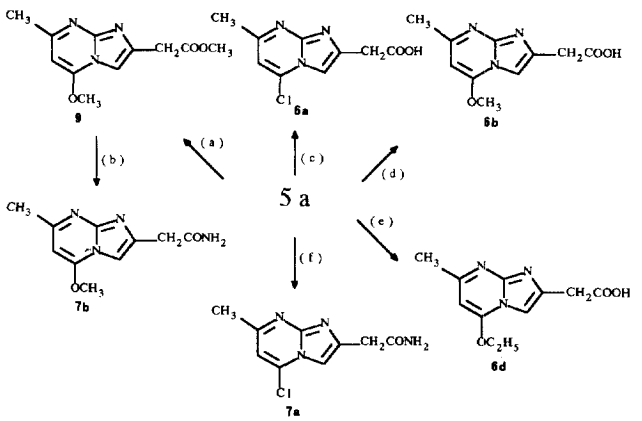

Fig 6. Derivatives of **5a**: (a) MeONa, MeOH, $CHCl_3$, reflux 2 h, 86%; (b) NH_4OH 30%, MeOH, reflux 6 h, 22%; (c) NH_4OH 30%, MeOH, reflux (ext temp 150–160 °C), 2 h, 56%; (d) MeONa, MeOH, reflux 4 h, 62%; (e) KOH 2 N, EtOH, reflux 1 h, 44%; (f) NH_4OH 30%, MeOH, reflux 6 h, 45%.

The ulcerogenic activity (table III) was also in accordance with the preceding tests: such activity was not negligible for all compounds, and in this case the most significant ulcerogenic and irritative action on the gastrointestinal mucosa was also displayed by the same four compounds.

Comparison of the pharmacological results just described for the acetic derivatives with the analogous data previously reported for similar carboxylic derivatives [2] clearly reveals that the substitution of the carboxylic moiety by the acetic moiety did not improve the antiinflammatory or in particular the analgesic activity, which on the contrary were lowered.

Nevertheless, the substantial parallel observed in the in vivo tests suggests that the activity showed by this series of acetic derivatives could be due to the inhibition of prostaglandin biosynthesis. In order to investigate this mechanistic aspect, the four most active compounds (**5a**, **6c**, **6d**, **6e**) and the less active **5c** and **7c** were tested for their cyclooxygenase-inhibiting activity and found to be completely lacking in inhibitory activity. This was not surprising, since the same result had already been obtained with the carboxylic derivatives [2]. Also in the present case, the conclusion was that the in vivo activity was independent of cyclooxygenase inhibition and must take place via different mechanisms of action.

Experimental protocols

Chemistry

Thin–layer chromatography utilizing precoated silica gel plates (Merck 60 F254) was used to assess reactions and purity of

Table I. Antiinflammatory activity: carrageenan rat paw edema.

Compound	*Dose (mg/kg po)*	*% Edema inhibition relative to control at (h)*				*ED_{50}, mg/kg (fiducial limits; at h)*	
		1st	*2nd*	*3rd*	*4th*	*3rd*	*4th*
5a	10	–8	–24	–20	–30	36.6	27.4
	20	–20	–35	–29	–32	(30.9–43.4)	(23.5–32.0)
	40	–50	–50	–57	–70		
8	40	–78	–31	–21	–17	–	–
9	40	–81	–63	–36	–55	–	–
5c	40	–67	–50	–43	–39	–	–
6a	40	–39	–39	–36	–55	–	–
6b	40	–78	–53	–41	–44	–	–
6c	10	ni[1]	–26	–17	–14	41.9	39.9
	20	–12	–12	–27	–37	(21.7–69.9)	(31.8–49.9)
	40	–44	–44	–52	–67		
6d	10	–32	–50	–40	–43	17.4	16.1
	20	–28	–28	–56	–48	(11.3–26.9)	(12.0–21.5)
	40	–54	–54	–60	–72		
6e	10	–8	–24	–29	–32	42.9	40.6
	20	–12	–47	–41	–49	(27.7–66.5)	(23.6–70.1)
	40	–39	–39	–48	–51		
7a	40	–39	–39	–36	–33	–	–
7b	40	–81	–63	–36	–33	–	–
7c	40	–50	–16	–14	–9	–	–
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		

[1]ni: no inhibition.

products: all compounds were designated as pure when they showed a single spot after elution with a mixture of chloroform/methanol (95:5); detection of components was made by UV light and/or treatment with iodine vapor. Preparative separation was performed in columns packed with silica gel (Farmitalia Carlo Erba; RS, Ø mm 0.05:0.20). Melting points were determined with a Kofler hot stage microscope and were uncorrected. Elemental analyses indicated by symbols of the elements were within ± 0.4% of theoretical values.

The ^{1}H- and ^{13}C-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 as internal standard.

Ethyl 5-chloro-7-methylimidazo[1,2-a]pyrimidine-2-acetate ***5a***

A solution of 2-amino-4-chloro-6-methylpyrimidine **3a** (4 g, 0.028 mol) in 100 mL of methanol was added with 5.8 mL (6.9 g, 0.042 mol) of ethyl 4-chloroacetoacetate **4** and then stirred in an oil bath mantained at 100 °C for 18 h. After cooling, the solution was evaporated under reduced pressure to dryness and the residue was treated with $NaHCO_3$ saturated aqueous solution. This alkaline mixture was extracted three times with chlorofom, then the organic extracts were combined, dried on Na_2SO_4, concentrated in vacuo to a small volume and chromatographed in a silica gel column, eluting with diethyl ether/petroleum ether (4:1). By means of this procedure 0.9 g of the required **5a** was obtained (yield 13%); mp = 143–145 °C (from *n*-hexane). TLC R_f = 0.78, anal $C_{11}H_{12}ClN_3O_2$ (C, H, Cl, N). ^{1}H-NMR ($CDCl_3$): δ 7.51 (s, 1H, H3), 6.73 (s, 1H, H6), 4.21 (q, 2H, ethyl CH_2), 3.91 (s, 2H, acetic CH_2), 2.63 (s, 3H, CH_3-7), 1.30 (t, 3H, ethyl CH_3). ^{13}C-NMR ($CDCl_3$): δ 170.45 (C=O), 150.75 (C7), 146.52 (C8a), 144.67 (C5), 142.14 (C2), 108.88 (CH3), 106.71 (CH6), 61.15 (ethyl CH_2), 35.27 (acetic CH_2), 18.26 (CH_3-7), 14.18 (ethyl CH_3).

The above chromatographic procedure allowed us to isolate also 0.6 g (yield 9%) of methyl 5-chloro-7-methylimidazo[1,2-*a*]-pyrimidine-2-acetate **8**; mp = 101–103 °C (from *n*-hexane). TLC R_f = 0.72, anal $C_{10}H_{10}ClN_3O_2$ (C, H, Cl, N). ^{1}H-NMR ($CDCl_3$): δ 7.51 (s, 1H, H3), 6.74 (s, 1H, H6), 3.92 (s, 2H, acetic CH_2), 3.76 (s, 3H, ester CH_3), 2.64 (s, 3H, CH_3-7). ^{13}C-NMR ($CDCl_3$): δ 170.79 (C=O), 150.79 (C7), 147.05 (C8a), 144.67 (C5), 141.50 (C2), 108.87 (CH3), 106.67 (CH6), 52.14 (ester CH_3), 34.92 (acetic CH_2), 18.20 (CH_3-7).

Table II. Analgesic activity: acetic acid writhing test in mice.

Compound	*Dose (mg/kg po)*	*% Decrease in mean no of writhes 25 min after treatment rel to control*	*ED50, mg/kg (fiducial limits)*
5a	20	–28	52.8
	40	–48	(39.5–70.6)
	80	–57	
8	40	–5	–
9	40	–36	–
5c	40	–13	–
6a	40	–24	–
6b	40	–36	–
6c	10	–15	36.2
	20	–32	(28.4–46.2)
	40	–53	
6d	10	–23	34.6
	20	–34	(28.4–46.2)
	40	–55	
6e	20	–14	54.1
	40	–50	(28.1–77.6)
	80	–59	
7a	40	–27	–
7b	40	–21	
7c	40	–2	–
IMA	5	–56	4.4
	7.5	–66	(2.7–7.2)
	10	–81	

Methyl 5-methoxy-7-methylimidazo[1,2-a]pyrimidine-2-acetate ***9***

A solution of ethyl 5-chloro-7-methylimidazo[1,2-*a*]pyrimidine-2-acetate **5a** (1 g, 0.004 mol) in 50 mL chloroform was added with CH_3ONa (0.1 g Na and 4 mL MeOH) and then stirred and refluxed for 2 h. After cooling the solution was filtered, concentrated in vacuo to a small volume and chromatographed in a silica gel column eluting with diethylether. By means of this procedure 0.8 g of the required **9** was obtained (yield 86%); mp = 80–82 °C, anal $C_{11}H_{13}N_3O_3$ (C, H, N). ^{1}H-NMR ($CDCl_3$): δ 7.28 (s, 1H, H3), 6.23 (s, 1H, H6), 4.01 (s, 3H, OCH_3-5), 3.84 (s, 2H, acetic CH_2), 3.76 (s, 3H, ester CH_3), 2.53 (s, 3H, CH_3-7). ^{13}C-NMR ($CDCl_3$): δ 171.32 (C=O), 163.20 (C7), 148.50 (C8a), 144.60 (C5), 138.96 (C2), 105.36 (CH3), 99.48 (CH6), 53.98 (OCH_3-5) 52.13 (ester CH_3), 35.04 (acetic CH_2), 18.16 (CH_3-7).

Ethyl 5,7-dimethoxyimidazo[1,2-a]pyrimidine-2-acetate ***5c***

A solution of 2-amino-4,6-dimethoxypyrimidine **3c** (4.3 g, 0.028 mol) in 100 mL methanol was added with 5.8 mL (6.9 g, 0.042 mol) of **4** and then stirred in an oil bath at 160 °C for 24 h. After cooling the solution was worked up as described for **5a** to obtain 1.25 g of the required **5c** (yield 18%); mp = 132–134 °C (from *n*-hexane), anal $C_{12}H_{15}N_3O_4$ (C, H, N,). ^{1}H-NMR ($CDCl_3$): δ 7.39 (s, 1H, H3), 5.70 (s, 1H, H6), 4.24 (q, 2H, CH_2), 4.10 (s, 3H, OCH_3-7), 4.05 (s, 3H, OCH_3-5), 3.82 (s, 2H, acetic CH_2), 1.33 (t, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ 170.80 (C=O), 165.14 (C7),156.11 (C5),148.51 (C8a), 138.36 (C2), 103.92 (CH3), 77.01 (CH6), 60.93 (ethyl CH_2), 56.69 (OCH_3-7), 54.31 (OCH_3-5), 35.12 (acetic CH_2), 14.23 (ethyl CH_3).

5-Chloro-7-methylimidazo[1,2-a]pyrimidine-2-acetic acid ***6a***

The ethyl ester **5a** (1 g, 0.004 mol) was dissolved in the minimum necessary amount of methanol and then added with a large excess of 30% ammonium hydroxide solution. The solution was refluxed vigorously for 2 h. After cooling the solution was extracted three times with chloroform, and the aqueous solution was acidified with 10% aqueous HCl up to pH = 4. The precipitate was filtered and recrystallized from methanol to obtain 0.6 g (yield 56%) of **6a**, mp > 200 °C dec, anal $C_9H_8ClN_3O_2$ (C, H, N, Cl). ^{1}H-NMR (CD_3OD): δ 7.48 (s, 1H, H3), 6.47 (s, 1H, H6), 3.71 (s, 2H, acetic CH_2), 2.57 (s, 3H, CH_3-7).

Table III. Incidence of gastrointestinal lesions in rats.

Compounds	*Dose (mg/kg po)*	*% Animals 6 h after treatment with:*	
		Hyperaemia	*Ulcers*
5a	100	60	50
8	100	30	10
9	100	50	30
5c	100	40	20
6a	100	50	30
6b	100	50	40
6c	100	60	50
6d	100	70	60
6e	100	60	50
7a	100	40	30
7b	100	30	20
7c	100	20	10
IMA	5	100	40
	7.5	100	80

5-Methoxy-7-methylimidazo[1,2-a]pyrimidine-2-acetic acid ***6b***
A suspension of 1.1 g (0.0043 mol) of ester **5a** and 0.0087 mol of MeONa (0.2 g Na and 8 mL MeOH), was stirred and refluxed for 4 h. The suspension was filtered and the solution was evaporated under reduced pressure to dryness, and the residue recrystallized from ethanol to obtain 0.6 g (yield 63%) of **6b**, mp > 200 °C dec, anal $C_{10}H_{11}N_3O_3$ (C, H, N,). ^{1}H-NMR (CD_3OD): δ 7.94 (s, 1H, H3), 6.97 (s, 1H, H6), 4.16 (s, 3H, OCH_3-5), 4.05 (s, 2H, acetic CH_2), 2.78 (s, 3H, CH_3-7).

5,7-Dimethoxyimidazo[1,2-a]pyrimidine-2-acetic acid ***6c***
Starting from 1 g (0.0038 mol) of the ester **5c**, the same procedure as that described for **6a** afforded 0.5 g (yield 56%) of **6c**, mp > 200 °C dec, anal $C_{10}H_{11}N_3O_4$ (C, H, N,). ^{1}H-NMR (CD_3OD): δ 7.80 (s, 1H, H3), 6.55 (s, 1H, H6), 4.30 (s, 3H, OCH_3-7), 4.15 (s, 3H, OCH_3-5), 3.88 (s, 2H, acetic CH_2).

5-Ethoxy-7-methylimidazo[1,2-a]pyrimidine-2-acetic acid ***6d***
A solution of 1 g (0.004 mol) of ester **5a** in 30 mL ethanol and 7.5 mL 2 N KOH solution was refluxed for 1 h. After cooling, the ethanol was evaporated and the residual aqueous solution was acidified with 10% aqueous HCl up to pH = 4. The precipitate was filtered and crystallized from ethanol to obtain 0.4 g (yield 45%) of **6d**, mp > 200 °C dec, anal $C_{11}H_{13}N_3O_3$ (C, H, N). ^{1}H-NMR (CD_3OD): δ 7.89 (s, 1H, H3), 6.91 (s, 1H, H6), 4.60 (q, 2H, ethoxyl CH_2), 3.94 (s, 2H, acetic CH_2), 2.75 (s, 3H, CH_3-7), 1.48 (t, 3H, ethoxyl CH_3).

5-Ethoxy-7-methoxyimidazo[1,2-a]pyrimidine-2-acetic acid ***6e***
Starting from 1 g (0.0038 mol) of ester **5c**, the same procedure as that described for **6d** afforded 0.6 g (yield 63%) of **6e**, mp > 200 °C dec, anal $C_{11}H_{13}N_3O_4$ (C, H, N). ^{1}H-NMR (CD_3OD): δ 7.68 (s, 1H, H3), 6.38 (s, 1H, H6), 4.55 (q, 2H, ethoxyl CH_2), 4.10 (s, 3H, OCH_3-7). 3.88 (s, 2H, acetic CH_2), 1.59 (t, 3H, ethoxyl CH_3).

5-Chloro-7-methylimidazo[1,2-a]pyrimidine-2-acetamide ***7a***
The ethyl ester **5a** (1 g, 0.004 mol) was dissolved in the minimum necessary amount of methanol and then a large excess of 30% ammonium hydroxide solution was added. The solution was gently stirred in an oil bath mantained at 100 °C for 6 h. The required product **7a** precipitated after cooling: the precipitate was collected, washed with water, dried and recrystallized from ethanol to obtain 0.4 g (yield 45%) of **7a**, mp > 200 °C, anal $C_9H_9ClN_4O$ (C, H, N, Cl). ^{1}H-NMR (DMSO–d_6): δ 7.83 (s, 1H, H3), 7.21 (s, 1H, H6), 7.51 and 7.09 (two s, 2H), 3.67 (s, 2H, acetic CH_2), 2.74 (s, 3H, CH_3-7).

5-Methoxy-7-methylimidazo[1,2-a]pyrimidine-2-acetamide ***7b***
Starting from 1 g (0.0042 mol) of methyl ester **9** the same procedure as that described for **7a** afforded 0.2 g (yield 22%) of **7b**, mp > 200 °C, anal $C_{10}H_{12}N_4O_2$ (C, H, N). ^{1}H-NMR (DMSO–d_6): δ 7.62 (s, 1H, H3), 7.54 and 7.15 (two s, 2H), 6.65 (s, 1H, H6), 4.05 (s, 3H, OCH_3-5), 3.61 (s, 2H, acetic CH_2), 2.68 (s, 3H, CH_3-7).

5,7-Dimethoxyimidazo[1,2-a]pyrimidine-2-acetamide ***7c***
Starting from 1 g (0.0038 mol) of the ethyl ester **5c**, the same procedure as that described for **7a** afforded 0.6 g (yield 67%) of **7c**, mp > 200 °C, anal $C_{10}H_{12}N_4O_3$ (C, H, N). ^{1}H-NMR (DMSO–d_6): δ 7.52 and 7.13 (two s), 7.51 (s, 1H, H3), 6.26 (s, 1H, H6), 4.27 (s, 3H, OCH_3-7), 4.06 (s, 3H, OCH_3-5), 3.58 (s, 2H, acetic CH_2).

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