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Synthesis of Imidazo[1,2-*a*]pyrazine Derivatives with Uterine-Relaxing, Antibronchospastic, and Cardiac-Stimulating Properties

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A series of imidazo[1,2-*a*]pyrazine derivatives was synthesized by condensation of α-halogenocarbonyl compounds and aminopyrazines. Various compounds resulted from competitive reactions or reagent isomerization and demonstrated in vitro uterine-relaxing and in vivo antibronchospastic activities. On isolated atria, 5-bromoimidazo[1,2-*a*]pyrazine showed positive chronotropic and inotropic properties; the latter was associated with an increase in the cyclic AMP tissue concentration. Potentiation of the isoproterenol positive inotropic effect of 5-bromoimidazo[1,2-*a*]pyrazine and the lack of blockade of the 5-bromoimidazo[1,2-*a*]pyrazine positive inotropic effect by propranolol suggested phosphodiesterase-inhibiting properties.

Several structural analogues of purines have been recently developed as potential chemotherapeutic and pharmacologically active agents.[1-3] Among the deazapurine homologues containing the pyrazine ring, only a few studies on the imidazo[1,2-*a*]pyrazine have been reported.[4-8] Recent work has shown that some compounds exhibit various pharmacological properties,[9,10] such as antiinflammatory[11,12] and β-blocking activities.[9]

The present work described the synthesis of a series of deazapurine derivatives, showing their uterine-relaxing and antibronchospastic properties and analyzing the cardiac properties and the mechanism of action of one compound of the series, the 5-bromoimidazo[1,2-*a*]pyrazine (**14**).

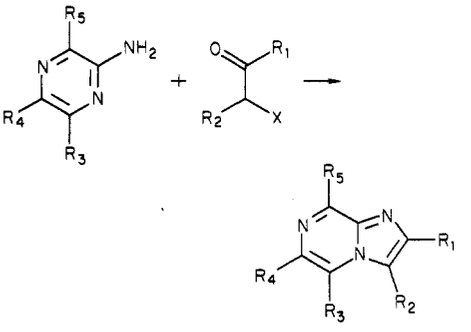
Chemistry. Condensation of α-halogenocarbonyl compounds with aminopyrazine led to some substituted imidazo[1,2-*a*]pyrazines, without the Dimroth-type rearrangement[5] (Scheme I).

In the case of ethyl 2-chloroacetoacetate condensation with aminopyrazine, three reactions occured competitively, giving simultaneously three different compounds: two imidazo[1,2-*a*]pyrazine derivatives **10** and **11** and one substituted 5*H*-pyrrolo[2,3-*b*]pyrazine, **12** (Scheme II). The ^{1}H NMR data of various final products are shown in Table I.

For most compounds (Scheme I) and both compounds **10** and **11**, the condensation resulted from a classic nucleophilic attack of the aminopyrazine endocyclic nitrogen on the 2-position of ethyl 2-chloroacetoacetate, followed by a cyclization between the primary amine function and one of the carbonyl groups (ketone or ester) of the lateral chain. For compound **11**, the chlorine atom in the 2-position can result from the rearrangement with dehydratation of an intermediate lactam–lactim hydrochloride.

Compound **12** resulted from a reaction mechanism similar to the former, in which the primary amine function of aminopyrazine was responsible for the initial nucleophilic attack. The further cyclization has been related to

Scheme I



1, $R_1 = R_2 = R_3 = R_4 = R_5 = H$; X = Cl
2, $R_1 = R_2 = R_3 = H$; $R_4 = R_5 = Br$; X = Cl
3, $R_1 = Me$; $R_2 = R_3 = R_4 = R_5 = H$; X = Cl
4, $R_1 = CH_2Cl$; $R_2 = R_3 = R_4 = R_5 = H$; X = Cl
5, $R_1 = CO_2Et$; $R_2 = R_3 = R_4 = R_5 = H$; X = Br
6, $R_1 = R_2 = R_3 = R_4 = H$; $R_5 = CO_2Et$; X = Cl
7, $R_1 = CO_2Et$; $R_2 = R_3 = H$; $R_4 = R_5 = Br$; X = Br
8, $R_1 = CO_2Me$; $R_2 = R_3 = R_4 = R_5 = H$; X = Br
9, $R_1 = CH_2CO_2Et$; $R_2 = R_3 = H$; $R_4 = R_5 = Br$; X = Cl

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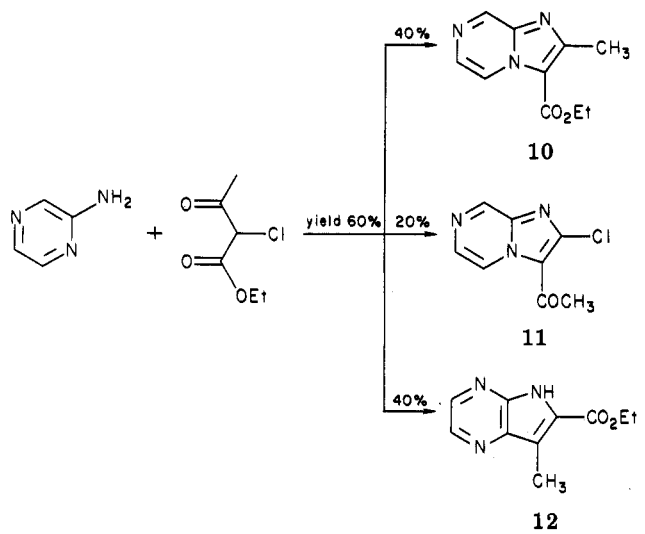
0022-2623/84/1827-0206$01.50/0

Table I. ^{1}H NMR Spectra of Imidazo[1,2-*a*]pyrazine and 5*H*-Pyrrolo[2,3-*b*]pyrazine Derivatives[a]

	chemical shifts, ppm						coupling constants, Hz		
compd	H_2	H_3	H_5	H_6	H_8	R	$J_{2,3}$	$J_{5,6}$	$J_{5,8}$
1	7.76	7.66	8.08	7.81	9.07			4.5	1.5
2	7.8	7.8	8.26						
3		7.42	7.98	7.75	8.92	2.45		4.25	1.5
4		7.74	8.05	7.87	9.04	4.77		4.5	1.5
5		8.29	8.18	7.93	9.16	4.47-1.43		4.5	1.5
6	7.81	7.95	8.28	7.99		4.6-1.5		4.5	
7		8.39	8.43			4.47-1.42			
8		8.41	8.29	7.95	9.14	3.98		4.5	1.5
9		7.87	8.2			4.21-1.28-3.96			
10			9.09	8.04	9.06	4.47-1.55-2.74		4.5	
11			8.65	8.15	9.05	2.66		2.5	0.8
12	8.25	8.6				4.45-1.28-3.96	2.75		

[a] Solution in $CDCl_3$, except for the compound 11 ($CDCl_3$ and Me_2SO-d_6).

Scheme II



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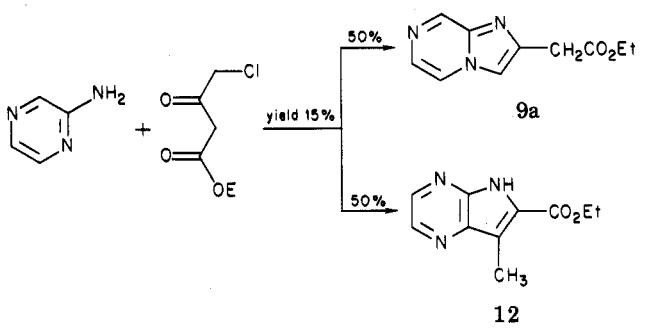
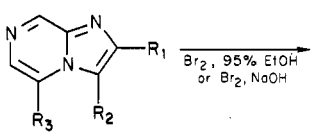
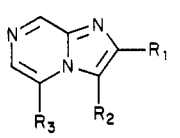


Scheme IV



1, $R_1 = R_2 = R_3 = H$
5, $R_1 = CO_2Et$; $R_2 = R_3 = H$
18, $R_1 = CONH_2$; $R_2 = R_3 = H$



13, $R_1 = H$; $R_2 = Br$; $R_3 = H$
14, $R_1 = H$; $R_2 = H$; $R_3 = Br$
15, $R_1 = H$; $R_2 = R_3 = Br$
16, $R_1 = CO_2Et$; $R_2 = H$; $R_3 = Br$
25, $R_1 = NH_2$; $R_2 = Br$; $R_3 = H$

the reactivity in the ortho position of nuclear pyrazinic amines.[13] Likewise, during the synthesis of 5*H*-pyrrolo-[2,3-*b*]pyrazine derivatives,[14] imidazo[1,2-*a*]pyrazine derivatives appeared simultaneously.

From a structural point of view, the ^{1}H NMR spectra of the synthesized compounds were consistent:[5] the H_5 proton of compound **10** was strongly deshielded because of the proximity of the carboxylate group in the 3-position. Compound **11** had no signals corresponding to imidazolic protons because of an acetyl group in the 3-position and a chlorine atom in the 2-position. The presence of the chlorine atom was confirmed by microanalysis and mass spectrometry studies (see Experimental Section and Table II).

Compound **12** showed two pyrazinic coupled protons: the absence of a strongly deshielded signal (approximately 9 ppm), characteristic of H_8 in imidazo[1,2-*a*]pyrazine derivatives, confirmed this structure. As well, the ^{13}C NMR data demonstrated the presence of four quaternary carbon atoms and only two carbon atoms in the ortho position carrying one proton ($J_{C_6H_6} = J_{C_7H_7} = 184$ Hz; $J_{C_6H_7}$ and $J_{C_7H_6} = 10$ and 12 Hz). This structure was consistent with the molecular weight measured by mass spectrometry.

The use of ethyl 4-chloroacetoacetate as reagent in the former reactions also supplied, though with a small yield, an imidazo[1,2-*a*]pyrazine derivative, **9a**, and compound **12**, resulting from partial reagent isomerization of the chlorine derivative in the 2-position (Scheme III).

The use of the 3,5 dibromoaminopyrazine[15] with ethyl 2-chloroacetoacetate or ethyl 4-chloroacetoacetate resulted only in the compound **9**. There was no formation of the 5*H*-pyrrolo[2,3-*b*]pyrazine derivative, likely because of the blockage of the position of cyclization by a bromine atom

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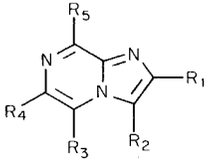
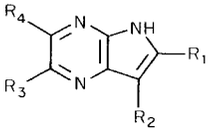
Table II. Synthetic Data and Physical Constants of Some Imidazo[1,2-*a*]pyrazine Derivatives



1-11, 13-28



12

compd	R_1	R_2	R_3	R_4	R_5	solvent	chromatography	yield, %	mp, °C	formula[a]
1[c]	H	H	H	H	H	DMF	basic alumina/CH_2Cl_2	50	92	$C_6H_5N_3$
2[d]	H	H	H	Br	Br	DMF	silica/CH_2Cl_2	45	166	$C_6H_3N_3Br_2$
3[e]	Me	H	H	H	H	DMC		15	89	$C_7H_7N_3$
4[f]	CH_2Cl	H	H	H	H	EtOH	neutral alumina/CH_2Cl_2	45	156	$C_7H_6N_3Cl$
5[g]	CO_2Et	H	H	H	H	ether + HMPT	EtOH[b]	30	179	$C_9H_9N_3O_2$
6	H	H	H	H	CO_2Et	ether + HMPT	neutral alumina/CH_2Cl_2	10	179	$C_9H_9N_3O_2$
7[d]	CO_2Et	H	H	Br	Br	DMC	silica/CH_2Cl_2	40	182	$C_9H_7N_3O_2Br_2$
8	CO_2Me	H	H	H	H	DMF	silica/CH_2Cl_2 + MeOH	10	220	$C_8H_7N_3O_2$
9[g]	CH_2CO_2Et	H	H	Br	Br	DMC	neutral alumina/CH_2Cl_2	42	100	$C_{10}H_9N_3O_2Br_2$
10	Me	CO_2Et	H	H	H	EtOH	neutral alumina/CH_2Cl_2	25	146	$C_{10}H_{11}N_3O_2$
11	Cl	COMe	H	H	H	EtOH	neutral alumina/CH_2Cl_2	10	174	$C_8H_6N_3OCl$
12	CO_2Et	Me	H	H		EtOH	neutral alumina/CH_2Cl_2	25	210	$C_{10}H_{11}N_3O_2$
13[h]	H	Br	H	H	H	EtOH	silica/ether	5	196	$C_6H_4N_3Br$
14	H	H	Br	H	H	EtOH	silica/ether	81	136	$C_6H_4N_3Br$
15[i]	H	Br	Br	H	H	EtOH	silica/ether	5	150	$C_6H_3N_3Br_2$
16	CO_2Et	H	Br	H	H	EtOH	neutral alumina/CH_2Cl_2	67	157	$C_9H_8N_3O_2Br$
17	CH_2OH	H	H	H	H	H_2O	alumina/CH_2Cl_2 + MeOH	45	139	$C_7H_7N_3O$
18[g]	$CONH_2$	H	H	H	H	$HONH_4$		86	>260	$C_7H_6N_4O$
19	$CONH_2$	H	H	Br	Br	$HONH_4$		25	>260	$C_7H_4N_4OBr_2$
20	$CONH_2$	H	Br	H	H	$HONH_4$		45	>260	$C_7H_5N_4OBr$
21	CN	H	H	H	H	$POCl_3$	alumina/CH_2Cl_2	78	220	$C_7H_4N_4$
22	CN	H	H	Br	Br	$POCl_3$	alumina/CH_2Cl_2	55	210	$C_7H_2N_4Br_2$
23	CN	H	Br	H	H	$POCl_3$	alumina/CH_2Cl_2	55	200	$C_7H_3N_4Br$
24	CO_2Et	Cl	Cl	Cl	Cl	PCl_5	silica/CH_2Cl_2	10	158	$C_9H_5N_3O_2Cl_4$
25	NH_2	Br	H	H	H	NaOBr/NaOH	alumina/CH_2Cl_2 + MeOH	30	220	$C_6H_5N_4Br$
26	$CONHNH_2$	H	H	H	H	EtOH	EtOH[b]	70	>260	$C_7H_7N_5O$
27	$C(=NNH_2)NH_2$	H	H	H	H	EtOH	EtOH[b]	80	>260	$C_7H_8N_6$
28[g]	COOH	H	H	H	H	NaOH/HCl		90	>260	$C_7H_5N_3O_2$

[a] Elemental analyses for C, H, N, and Br or Cl were within ±0.4% of calculated values for all compounds. [b] Recrystallization in EtOH. [c] See ref 6 and 7, mp 83-85 °C. [d] See ref 8, mp 165-166 °C. [e] See ref 5, mp 89 °C. [f] See ref 11, mp >110 °C dec. [g] See ref 12, mp 84-84 °C. [h] See ref 7, mp 194-196 °C. [i] See ref 8, mp 150-151 °C.

Table III. Uterine-Relaxing and Antibronchospastic Activities of Imidazo[1,2-*a*]pyrazine Derivatives and Theophylline

compd	uterine-relaxing act.: ED_{50},[a] mmol	antibronchospastic act.: ED_{50},[a] μmol/kg
1	1.60 ± 0.16 (4)[c]	154 ± 12 (4)
2	0.24 ± 0.06 (7)[c]	43 ± 4 (4)
3	0.75 ± 0.04 (4)	>50[b]
4	1.4 ± 0.38 (6)	>72[b]
5	1.11 ± 0.07 (4)	78 ± 6 (4)
7	0.15 ± 0.01 (4)[c]	>16[b]
8	>2.4[b]	>34[b]
9	0.096 ± 0.001 (10)	21 ± 1.6 (8)[c]
10	0.152 ± 0.007 (13)[c]	19 ± 1.4 (7)[c]
11	0.88 ± 0.05 (4)	87 ± 11 (5)
12	0.102 ± 0.007 (13)[c]	24 ± 2 (7)
14	0.29 ± 0.02 (7)[c]	18 ± 2 (7)[c]
16	1.02 ± 0.05 (5)	52 ± 4 (4)
17	2.7 ± 0.25 (4)	>200[b]
18	1.5 ± 0.05 (4)	>56[b]
19	0.33 ± 0.02 (4)[c]	>18[b]
20	0.41 ± 0.04 (4)[c]	38 ± 4 (4)
21	2.38 ± 0.19 (6)	>167[b]
22	0.09 ± 0.015 (9)[c]	16 ± 2 (5)[c]
23	0.78 ± 0.12 (5)	>15[b]
25	0.34 ± 0.2 (4)[c]	40 ± 4 (5)
28	1.72 ± 0.15 (4)[c]	>20[b]
theophylline	0.9 ± 0.04 (9)	43 ± 6.6 (18)

[a] Results are given as the mean plus or minus SEM; numbers in parentheses indicate the number of determinations. [b] Highest concentration or dose tested. [c] Statistically different ($p < 0.05$) from theophylline.

in the 3-position on the pyrazine ring.

Besides the preceding condensations, a series of transformations of the derivatives **1**, **4**, **5**, and **7** led to the derivatives **13** to **28** (Table II). The monobromo derivatives in the 3- or 5-position (**13** or **14**) were respectively prepared from the structure **1** by the action of bromine in 95% ethanol (Scheme IV). A small amount (5%) of the 3,5-dibromo (**15**) derivative also appeared. The ester **5**, deactivated in the 3-position by the ethyl carboxylate group, reacted only on the 5-position (**16**) with bromine in 95% ethanol. These different bromination positions were demonstrated by NMR spectra, in reference to previous work concerning compounds **13**[7] and **15**[8], and by comparison with a corresponding chlorine derivative of compound **14**, the structure being well-known.[8]

The 2-amino-3-bromoimidazo[1,2-*a*]pyrazine **25** resulted from Hoffman's reaction on the amide **18**, with decarbonylization and, at the same time, bromination at the 3-position. The tetrachloro compound **24** was prepared by the action of phosphorus pentachloride on the heterocycle **5**. The transformations of the ester function on the 2-position (**5**) led to the acid **28**, the amides **18–20**, and the corresponding nitriles **21–23** with good yields. The hydrazine **26** and the amidrazone **27** resulted from hydrazine action on the ester **5** and nitrile **21**.

Synthetic data and physical constants for all products prepared in this study are listed in Table II. Several compounds could not be tested: **6** was too unstable, **13** and **15**, previously described,[7,8] were formed in too small a quantity with the method used, and compounds **24**, **26**, and **27** were too insoluble.

Pharmacology. Imidazo[1,2-*a*]pyrazine derivatives were tested for their capacity to relax smooth muscles on two experimental models: the isolated rat uterus and the artificially respired, anesthetized guinea pig. ED_{50}'s were determined for the inhibition of spontaneous uterine contraction and for inhibition of the bronchospasm induced by intravenous (iv) doses of histamine. Theophylline was tested for comparison. Results are presented in Table III. .

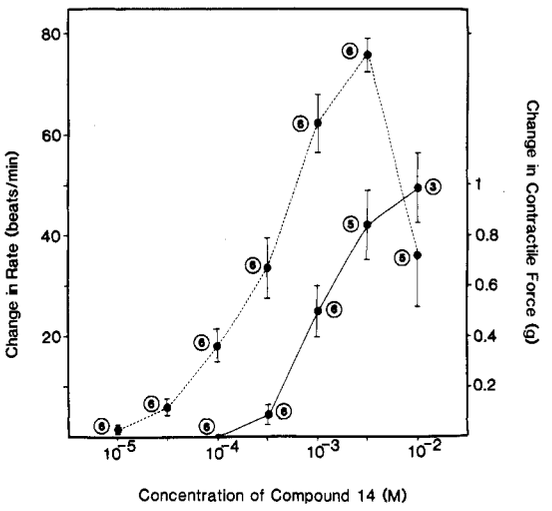


Figure 1. The effect of cumulative doses of **14** on cardiac contractile force (●–●) and rate (●---●). Each point represents the mean plus or minus SEM, and the numbers in parentheses represent the number of determinations.

All tested compounds, except **8**, demonstrated uterine-relaxing activity. Most of the compounds tested also demonstrated an antibronchospasmic activity, although the maximum amount of liquid that could be injected intravenously (3 mL/kg) to the guinea pig limited the doses tested for some, and, therefore, the ED_{50}'s could not be determined. Monobromation seemed to enhance the activity of the compounds (compare **1** and **14**, **18** and **20**, and **21** and **23**). A dibromination generally led to the most active compounds.

In both experimental models, the activity of theophylline was significantly lower than the activity of some compounds of the series, such as **9**, **10**, **14**, and **22**.

In order to perform a more extensive study of the cardiac properties and the mechanism of action of the new structure, we had to choose a compound characterized by good solubility (10^{-2} M) and good stability and one that could easily be prepared in sufficient quantity. The most active compound having these characteristics was **14**.

The cardiac properties of **14** were studied by using isolated guinea pig atria.[16] The left atrium, electrically paced (1.6 Hz), was used to measure the effects of drugs on tension, while the right atrium was used to measure the effects of drugs on rate. Cyclic AMP was measured on left atria frozen with clamps that had been previously cooled in liquid nitrogen.

Cumulative dose–response curves (CDR) on tension and rate are shown in Figure 1. Compound **14** demonstrated inotropic ($ED_{50} \simeq 10^{-3}$ M) and chronotropic ($ED_{50} \simeq 3.7 \times 10^{-4}$ M) properties. Chronotropic activity was maximum at 3×10^{-3} M; at 10^{-2} M, the activity decreased. At 10^{-2} M, the inotropic activity was still maximum. It was not possible to test higher doses because of the limited solubility of **14** (10^{-2} M).

The time course of tension changes and cyclic AMP content following 3×10^{-3} M **14** is shown in Figure 2. The tension increased immediately, was maximum after 60 s, and remained elevated. Cyclic AMP followed a similar time course; however, the maximum cyclic AMP concentration was reached later (90 s). Cyclic AMP, measured 90 s after different concentrations of **14**, increased in a

(16) Tenner, T. E.; McNeill, J. H. *Can. J. Physiol. Pharmacol.* **1978**, *56*, 926–33.

Table IV. Cyclic AMP Concentrations in Rat Left Atria[a]

control (a)	14, 10^{-3} M (b)	14, 3×10^{-3} M (c)	14, 10^{-2} M (d)	propranolol, 10^{-7} M (e)	propranolol, 10^{-7} M, + 14, 3×10^{-3} M (f)
479.3 ± 31.2 (10)	853.5 ± 97.1 (6)	1392[b] ± 53.3 (6)	1800 ± 175 (5)	432.2 ± 36.5 (6)	992.2[b] ± 126.4 (6)

[a] Cyclic AMP concentration (femtomoles per milligram of tissue) measured in the absence of any drug (a), in the presence of different concentrations of 14 (b–d), and in the presence of propranolol (10^{-7} M) (e), and propranolol (10^{-7} M) plus 14 (3×10^{-3} M) (f). b–d = atria were frozen 1.5 min after 14; e = atria were frozen 15 min after the addition of propranolol; f = propranolol was added to the bath 15 min before the addition of 14. Then atria were frozen 1.5 min after 14. [b] c and f: significantly different ($p < 0.02$). Numbers in parentheses indicate the number of determinations.

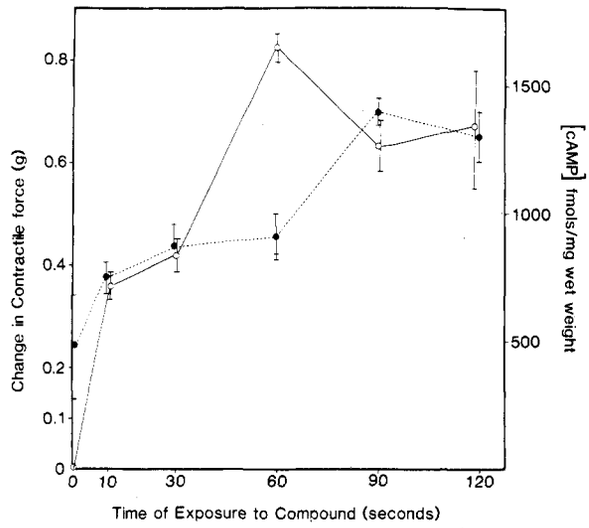


Figure 2. Time–response effect of 14 (3×10^{-3} M) on cardiac contractile force (●–●) and cyclic AMP (●---●) measured in left atria. Each point represents the mean plus or minus SEM of six determinations.

dose-dependent manner (Table IV).

In order to determine if β-adrenergic stimulation was involved in the effects of **14**, we tested **14** (3×10^{-3} M) on tension and on cyclic AMP, measured at 90 s in the presence and absence of the β-blocking agent, propranolol. Propranolol was used at a dose (10^{-7} M) too small to alter the basal tension but able to block the inotropic effect of released norepinephrine.[17] The maximum increase in tension developed after 3×10^{-3} M 14 was not significantly different in the absence of propranolol (0.775 ± 0.118 g, six experiments) nor in its presence (0.596 ± 0.108 g, six experiments). The cyclic AMP concentration was not significantly altered by propranolol (10^{-7} M) alone, but the increase following 3×10^{-3} M 14 was found to be lower in the presence of propranolol (10^{-7} M) than in its absence (Table IV).

Cumulative dose–response curves of the β-adrenergic drug isoproterenol were performed on left atria in the absence and then in the presence of **14** (10^{-4} M) (see experimental Design of Cardiac Studies under Experimental Section). The ED_{50}'s were, respectively, 4.13×10^{-8} and 2.3×10^{-8} M (both geometric means of six values). A comparison using the Student's t test on paired values showed a significant decrease of the ED_{50}'s (mean of the differences of the log values of ED_{50}'s: 0.256 ± 0.0687, six experiments, $p < 0.02$).

In summary, the imidazo[1,2-*a*]pyrazine derivatives have demonstrated relaxing properties on isolated rat uterus and antibronchospasmic activities on anesthetized guinea pigs. These activities were generally improved by the bromination of the molecule, and some compounds of the series were found to be more potent than theophylline. Compound **14** demonstrated positive chronotropic and inotropic properties, the latter associated with a dose-dependent increase in cyclic AMP. The activity of compound **14** was not blocked by a β-blocking agent, propranolol, and **14** potentiated the inotropic activity of the β-adrenergic drug, isoproterenol.

The pharmacological activities suggest a mechanism of action in relation with phosphodiesterase-inhibiting properties. This hypothesis has recently been confirmed by Francoise et al.,[18] who demonstrated the in vitro thymocyte phosphodiesterase inhibiting properties of several compounds described above. For example, when a phosphodiesterase preparation with an activity of 4 nmol mg^{-1} min^{-1} (cyclic AMP concentration 1.14 μM) was used, compound **14** (0.2 mM) inhibited the enzyme activity by 18%, compared to a 35% inhibition by the same concentration of theophylline.

However, some discrepancies have been found with **14** between the inotropic effect and cyclic AMP: (a) after 3×10^{-3} M **14**, the inotropic effect was maximum at 1 min, and after the cyclic AMP concentration, at 1.5 min. (b) Propranolol (10^{-7} M) did not significantly decrease the maximum inotropic effect of 3×10^{-3} M 14 but did significantly alter the cyclic AMP concentration measured at 1.15 min.

Such discrepancies between pharmacological activity and cyclic AMP concentration have been found for known PDE inhibitors, such as theophylline,[19] indicating that its mechanism of action is not only due to its PDE-inhibiting properties. This is probably also the case for the imidazo[1,2-*a*]pyrazine derivatives.

Experimental Section

Chemical Synthesis. Melting points were taken with a Kofler hotbench. IR spectra were recorded as KBr disks on a Beckman Acculab 2 or Leitz spectrophotometer. NMR spectra were determined on a Brucker WP80CW of a Varian A60, EM390, or HA100 spectrometer, with Me_4Si as internal standard. The TLC data were obtained with a silica gel 60 F 254 or neutral alumina 60 F 254 E (Merck) plate. The microanalyses, performed in the CNRS Laboratory for C, H, N, and Cl, are given in Table II. Mass spectra were obtained on a JEOL JMS D100 spectrometer and a coupled whole LKB 2091.

Imidazo[1,2-*a*]pyrazines (1–12). The appropriate α-halogenocarbonyl compound (0.1 mol) was added to aminopyrazine (9.5 g, 0.1 mol) or to a substituted aminopyrazine dissolved in the minimum quantity of cooled solvent. The solution was then heated at reflux for 3 h and stirred at room temperature for 15 h. The hydrochloride precipitate was collected, washed with ether, dissolved in absolute ethanol, and heated at reflux for 1 h. After the mixture had been cooled (and concentrated in vacuo if necessary), the precipitate was collected, washed with ether, and dissolved in a 10% $NaHCO_3$ solution, and the aqueous basic solution was extracted with CH_2Cl_2. The combined extracts were dried ($CaCl_2$) and filtered, and the CH_2Cl_2 was then removed in vacuo. The residual product was crystallized or purified by

(17) Laher, I.; McNeill, J. H. *Can. J. Physiol. Pharmacol.* **1980**, *58*, 1114–1116.

(18) Francoise, M. A.; Bonnafous, J. C.; Levallois, C.; Mani, J. C.; Sablayrolles, C.; Chapat, J. P., unpublished results.

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chromatography (Table II) if necessary. The ^1H NMR data are noted in Table I: mass spectrum, *m/e* for **4**, 167 (M^+); for **7**, 347, 351 (M^+); for **10**, 205 (M^+); for **11**, 195, 197 (M^+); ^{13}C NMR δ 153 (dd, C_8), 147, 141, 116 (3 quaternary C, C_{8a}, C_3 and C_2), 132 (ddd, C_6), 117 (dd, C_5), 162 (q, CO, J_{COCH_3} = 6.5 Hz), 23 ppm (q, CH_3, J = 126 Hz) ($J_{C_8H_8}$ = 195 Hz, $J_{C_8H_6}$ = 10 Hz, $J_{C_6H_6}$ = 191 Hz, $J_{C_6H_5}$ = 14 Hz, $J_{C_6H_8}$ = 6 Hz, $J_{C_5H_5}$ = 192 Hz, $J_{C_5H_6}$ = 13 Hz); for **12**, 205 (M^+); ^{13}C NMR δ 150.3, 141.13, 139.4, and 103.9 ppm (four quaternary C), 139.8 and 136.2 ppm (C_6 or C_7, $J_{C_5H_6} = J_{C_7H_7}$ = 184 Hz, $J_{C_6H_7}$ and $J_{C_7H_6}$ = 10 and 12 Hz), 164.4 (CO), 60.3 (CH_2), 15 and 14 (CH_3, CH_3CH_2).

3-Bromo-, 5-Bromo-, and 3,5-Dibromoimidazo[1,2-*a*]pyrazines (13–15). To a stirred solution of imidazo[1,2-*a*]pyrazine (0.01 mol) in 50 mL of 95% ethanol was added dropwise a solution of Br_2 (0.02 mol) in 10 mL of cooled ethanol. The solution was then heated at slight reflux for 1 h and then cooled. The hydrobromide precipitate was collected and then dissolved in a 10% NaOH solution. After extraction by CH_2Cl_2, the product was purified by chromatography on neutral silica/dry ether. **13**: yield 5%; NMR δ 7.84 (s, H_2), 8.19 (s, H_5 and H_6), 9.15 (s, H_8); see ref 7. **14**: yield 81%; NMR δ 7.92 (s, H_2, H_3), 8.07 (s, H_6), 9.1 (s, H_8). **15**: yield 5%; NMR δ 7.76 (s, H_2), 7.97 (s, H_6), 8.96 (s, H_8); see ref 8.

Ethyl 5-Bromoimidazo[1,2-*a*]pyrazine-2-carboxylate (16). The product was prepared as described previously for **14** and **15**, using ethyl imidazo[1,2-*a*]pyrazine-2-carboxylate instead of imidazo[1,2-*a*]pyrazine: yield 67%; NMR δ 8.15 (s, H_3), 8.46 (s, H_6), 9.16 (s, H_8); IR $\nu_{C=O}$ 1710 cm^{-1}.

2-(Hydroxymethyl)imidazo[1,2-*a*]pyrazine (17). A solution of 2-(chloromethyl)imidazo[1,2-*a*]pyrazine (**4**; 0.167 g, 0.001 mol) in 10 mL of 10% aqueous Na_2CO_3 was heated at reflux for 1 h. After extraction with CH_2Cl_2, the product was purified by chromatography on neutral alumina/CH_2Cl_2 + 2% MeOH: yield 46%; NMR δ 4.94 (s, CH_2), 7.72 (s, H_3), 7.87 (d, H_6), 8.07 (d, H_5), 9.03 (s, H_8) (J_{56} = 4.5 Hz); IR $\nu_{OH_{ass.}}$ 3280 cm^{-1}.

Preparation of Amides 18–20. A mixture of ester (0.01 mol) and 25 mL of a concentrated NH_4OH solution was heated at reflux for 1 h. After the mixture was cooled, the amide precipitate was collected and air-dried: yield 80–85%. **18**: IR $\nu_{C=O}$ 1680 cm^{-1}. **19**: IR $\nu_{C=O}$ 1650 cm^{-1}. **20**: IR $\nu_{C=O}$ 1670 cm^{-1}.

Preparation of Nitriles 21–23. A mixture of 1 g of amide and 9 mL of $POCl_3$ was heated at reflux for 1 h. After distillation of 6 mL of $POCl_3$, the residue was poured carefully onto ice. After the destruction of excess $POCl_3$, the mixture was alkalinized, and the nitrile was extracted with CH_2Cl_2. The crude nitrile was purified by chromatography on neutral alumina/CH_2Cl_2: yield 50–80%. **21**: ^1H NMR (Me_2SO-d_6) δ 8.81 (s, H_3), 8.06 (d, H_6), 8.68 (q, H_5) (J_{56} = 4.5 Hz, J_{58} = 1.5 Hz); IR $\nu_{C\equiv N}$ 2250 cm^{-1}. **22**: ^1H NMR δ 8.74 (s, H_3), 8.85 (s, H_5); IR $\nu_{C\equiv N}$ 2230 cm^{-1}. **23**: IR $\nu_{C\equiv N}$ 2235 cm^{-1}.

Ethyl 3,5,6,8-Tetrachloroimidazo[1,2-*a*]pyrazine-2-carboxylate (24). Ethyl imidazo[1,2-*a*]pyrazine-2-carboxylate (**5**; 1 g, 0.00525 mol) and phosphorus pentachloride (11 g, 0.05 mol) were placed in a sealed steel reaction bomb and heated at 230 °C for 4 h. To the cooled bomb were then added 200 g of ice and 200 mL of chloroform. After the phosphorus pentachloride had been decomposed, the mixture was collected and boiled on a steam bath for 10 min. The chloroform was collected and filtered, and the $CHCl_3$ was removed in vacuo. The residual product was purified by chromatography (Table II): ^1H NMR δ 4.52 (q, CH_2), 1.45 (t, CH_3); IR $\nu_{C=O}$ 1720 cm^{-1}; mass spectrum, *m/e* (relative intensity) 327 (M^+), 329 (135), 331 (54), 333 (13.7).

2-Amino-3-bromoimidazo[1,2-*a*]pyrazine (25). After 1.3 g (0.008 mol) of bromine was dissolved in 1.9 g of sodium hydroxyde (0.0475 mol) and 10.25 g of ice–water, 1 g (0.006 mol) of imidazo[1,2-*a*]pyrazine-2-carboxamide was added. The solution was warmed, with stirring for 1 h. The next day, the precipitate was collected and dried. The product dissolved in water gave an alkaline solution, which evolved carbon dioxide on acidification with by 10% HCl. After alkalinization, the product was extracted with CH_2Cl_2 and purified by chromatography on neutral alumina (CH_2Cl_2 and 5% MeOH) (Table II): ^1H NMR (Me_2SO-d_6) δ 8.59 (d, H_8), 8.10 (dd, H_5), 7.9 (d, H_6) (J_{56} = 4.5 Hz, J_{58} = 1.5 Hz); mass spectrum, *m/e* 212 (M^+), 214.

Preparation of Hydrazide 26 and Amidrazone 27. To a stirred solution in ethanol of ester or cyanoimidazo[1,2-*a*]pyrazine (0.01 mol) was slowly added a solution 98% NH_2NH_2 (0.02 mol), and the mixture was heated at reflux for 1 h. After the mixture was cooled, the precipitate was collected. Careful evaporation of the solvent increased the yield. The crude product was recrystallized from ethanol. **26**: IR $\nu_{C=O}$ 1680 cm^{-1}. **27**: IR $\nu_{C=N}$ = 1650 cm^{-1}; mass spectrum, *m/e* 176 (M^+).

Imidazo[1,2-*a*]pyrazine-2-carboxylic Acid (28). A solution of ester **5** (1.91 g 0.01 mol) in 20 mL of 10% aqueous Na_2CO_3 was heated at reflux for $^1/_2$ h, after dissolution. The solution was extracted with CH_2Cl_2 to eliminate the unreacted ester. Then the solution was acidified with 6 N HCl up to formation of the precipitate. The compound was collected and dried: IR ν_{CO} 1700 cm^{-1}.

Pharmacology. Rat Uterus Preparation.[20] Female rats (150 to 180 g) were decapited 24 h after intraperitoneal injection of stilboestrol (0.1 mg/kg), and the external third of the uterine horn was dissected out and mounted vertically in a tissue chamber (37 °C) containing oxygenated De Jalon solution with the following composition (mM): NaCl (153.8), KCl (5.6), $CaCl_2$ (2.16), $NaHCO_3$ (1.8), dextrose (5.5).

One end of the tissue was attached to a fixed pin and the other end to a level fitted with a frontal writing point. The load on the level was about 0.5 g, and the magnification was about 5-fold. Spontaneous uterine contractions were recorded on a kymograph. The preparation was left for 30 min, and the liquid was changed 3 times before any administration of drug. Drugs were added directly to the bath after dissolution in De Jalon solution, and their activities were measured as the dose required to reduce the amplitude of the spontaneous contractions by 50% (ED_{50}). The results presented in Table III are means plus or minus the SEM of 4 to 10 determinations.

Bronchospasm Induced in Anesthetized Guinea Pigs. Guinea pigs of either sex weighing between 400 and 600 g were anesthetized (ethyl carbamate, 1.20 g/kg, ip), and the jugular vein was cannulated to allow intravenous administration of drugs. The trachea was cannulated after tracheotomy, and the animal was artificially respired with a Palmer pump delivering a constant air flow (1 mL/100 g × 60/min). A tube was connected from the trachea cannula to a Marey capsule fitted to a lever in order to record the variation of the air excess at each insufflation on a kymograph. Bronchospasms were induced by intravenous injections of histamine. The dose of histamine (8 to 12 μg/kg) was determined for each animal and induced a variation double that which was induced in the basal condition and which gave three identical responses at 10-min intervals. The tested compound, dissolved in saline, was injected and was followed, 30 s later, by another histamine administration. The ED_{50} of the tested compound was represented as the dose that reduced by 50% the air excess induced by histamine. The results presented in Table III are means plus or minus the SEM of 4 to 18 determinations.

Preparation of Atria.[16] Guinea pigs of either sex weighing between 300 and 500 g were killed by a blow on the head, and the heart was rapidly removed and placed in a beaker containing Chenoweth–Koelle solution (CKS) bubbled with 95% O_2 and 5% CO_2 of the following composition (mM): NaCl (120), KCl (5.63), $CaCl_2$ (2.0), dextrose (9.7), $MgCl_2$ (2.0), $NaHCO_3$ (26.0).

Right and left atria were removed from the heart and mounted as described previously.[16] Right atria were allowed to beat spontaneously, while left atria were paced at a frequency of 1.6 Hz, with a duration of 5 ms and a voltage twice the threshold by means of a Grass stimulator. Responses were recorded on a Grass polygraph. The tissues were allowed to equilibrate for 45 min after the basal tension had been adjusted to 1 g and were washed every 15 min. Drugs were added directly to the bath. Right atria were used to measure the effects of drugs on rate, and left atria were used to measure the effects of drugs on tension. Drug effects were measured as differences in developed tension or rate from basal activity.

Cyclic AMP Measurements. After the addition of drugs, the left atria were frozen with clamps that had been previously

(20) Staff of the Department of Pharmacology, University of Edinburgh, "Pharmacological Experiments on Isolated Preparations"; E. & S. Livingstone: Edinburgh and London, 1968, pp 92–93.

cooled in liquid nitrogen. Control atria, without any drug added, were frozen under the same experimental conditions. The atria thus obtained were stored at -80 °C until analyzed for cyclic AMP. Cyclic AMP was measured by radioimmuno assay by using a cyclic AMP kit obtained from Becton Dickinson.

Experimental Design of Cardiac Studies. Cumulative dose–response (CDR) curves were performed in order to measure the effects of compound **14** on force and rate.

Propranolol (10^{-7} M) was first injected and gave no significant alteration of basal tensions (mean of the differences between tension values before and after propranolol = -0.06 ± 0.03; six experiments, $p > 0.05$). The same dose of propranolol had previously blocked the inotropic effect of histamine (10^{-2} M) on the same experimental model.[17] The effect of histamine under these circumstances was entirely due to the release of norepinephrine. Compound **14** (3×10^{-3} M) was injected after propranolol; tension and cyclic AMP were measured 1.5 min after the administration of compound **14**.

For the determination of isoprenaline potentiation by **14**, the following procedure was undertaken in order to correct the shift to the right of isoprenaline CDR curves, commonly observed when CDR curves were successively obtained for each atrium, and the first one was discarded. The second was used as a control for the isoprenaline ED_{50} in the absence of **14**. The third one was performed in the presence of **14**. For each set of six experiments, the same series of three CDR curves was done on one atrium without **14**, which allowed for the calculation, for each dose of isoprenaline, of the percentage of decrease of the effect between the second and the third CDR. These percentages were used to correct the effect of each dose of the second isoprenaline CDR curve for the remaining five atria. The corrected curves were used to calculate the control ED_{50}'s of each CDR curve in the presence of **14**.

Calculation of Results and Statistics. All ED_{50}'s have been calculated by interpolation of dose-response curves. Numbers are given as the mean plus or minus the standard error of the mean (SEM). Comparisons were done using the Student's t test for unpaired or paired data; $p \leq 0.05$ was considered significant.

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Synthesis and Antiinflammatory Activity of [(Cycloalkylmethyl)phenyl]acetic Acids and Related Compounds

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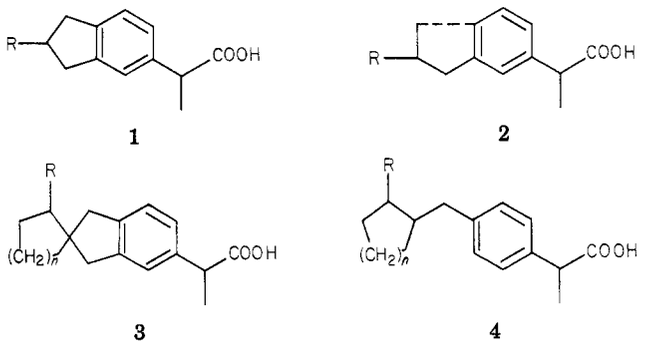
[(Cycloalkylmethyl)phenyl]acetic acid derivatives and related compounds were synthesized to test their antiinflammatory and analgesic activities. Some of the compounds in this series were found to have good activity in the carrageenan edema test. Among them, sodium 2-[4-[(2-oxocyclopentyl)methyl]phenyl]propionate dihydrate (**15**) and 2-[4-[(2-oxocyclohexylidene)methyl]phenyl]propionic acid (**13b**) showed potent analgesic and antiadjuvant arthritis activities with excellent antipyretic properties.

In recent years, numerous arylacetic and propionic acid derivatives have been synthesized in the search for nonsteroidal antiinflammatory agents.[1]

In a previous paper,[2] we reported the synthesis and antiinflammatory activity of indanylacetic acid derivatives **1**, among which 2-(2-isopropyl-5-indanyl)propionic acid [R = $CH(CH_3)_2$] exhibited particularly potent antiinflammatory activity. The indanylacetic acid derivatives **1** can be related to the structure of ibuprofen (**2**, R = CH_3) by drawing a dotted line between the isobutyl group and the benzene ring (Chart I). In the continuation and extension of our synthetic studies of nonsteroidal antiinflammatory agents, we have synthesized the spiro indanylacetic acid derivatives **3**. These compounds **3**,[3] however, showed only weak antiinflammatory activity. We, therefore, carried out the synthesis of a number of phenylacetic and propionic acid derivatives **4** having the cycloalkylmethyl group at the paraposition of phenylacetic acid.

Chemistry. [(Cycloalkylmethyl)phenyl]acetic and propionic acid derivatives **8** were synthesized by the two basic routes shown in Scheme I. In the first procedure [A], the compounds were prepared as follows: Treatment of ethyl [*p*-(chloromethyl)phenyl]acetate (**5**)[4] with ethyl 2-oxocycloalkanecarboxylate **6** gave the condensed products **7**. Hydrolysis of **7**, followed by decarboxylation, afforded the [4-[(2-oxocycloalkyl)methyl]phenyl]acetic acid derivatives **8**. The other synthetic method [B] utilized the morpholino enamine of cycloalkanone as starting material.

Chart I



Reaction of **5** with enamine **9**, followed by hydrolysis with HCl, gave **8**. The methylene compound **10** was obtained by Clemmensen reduction of **8b**. The 2-oxo derivatives **8** were converted to the oximes **11** by reaction with hydroxylamine.

[†] Chemical Research Laboratories.
[‡] Biological Research Laboratories.

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(2) N. Yoshida, A. Terada, K. Tomita, K. Wachi, S. Naruto, S. Tanaka, H. Miyazawa, K. Tanaka, E. Misaka, Y. Iizuka, Y. Umemuro, T. Kojima, and R. Tachikawa, *Annu. Rep. Sankyo Res. Lab.*, **32**, 65 (1980).
(3) Unpublished data from our laboratories. For example, the ID_{50} value for 2-[spiro(cyclopentane-1,2′-indan)-5′-yl]propionic acid was 85 mg/kg in the carrageenan edema test.
(4) J. W. Baker and J. A. L. Brieux, *J. Chem. Soc.*, 404 (1956).

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