Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 1059–1065

BIOORGANIC & MEDICINAL CHEMISTRY

New Imidazo[1,2-*a*]pyrazine Derivatives with Bronchodilatory and Cyclic Nucleotide Phosphodiesterase Inhibitory Activities

Olivier Vitse,[a] Florence Laurent,[b] Tristan M. Pocock,[c] Véronique Bénézech,[a] Lahcen Zanik,[a] Keith R. F. Elliott,[c] Guy Subra,[a] Karine Portet,[b] Jacques Bompart,[a] Jean-Pierre Chapat,[a] Roger C. Small,[c] Alain Michel[b] and Pierre-Antoine Bonnet[a,*]

[a]*Pharmacochimie & Biomolécules, Laboratoire de Chimie Organique Pharmaceutique, Faculté de Pharmacie, 15, Av. Ch. Flahault, 34060 Montpellier Cedex 2, France*
[b]*Laboratoire de Pharmacodynamie, Faculté de Pharmacie, Montpellier, France*
[c]*School of Biological Sciences, University of Manchester, Manchester, UK*

Received 28 April 1998; accepted 13 October 1998

Abstract—New imidazo[1,2-*a*]pyrazine derivatives have been synthesized either by direct cyclization from pyrazines or by electrophilic substitutions. The presence of electron donating groups on position 8 greatly enhances the reactivity of the heterocycle towards such reactions on position 3 of the heterocycle. The activities of these derivatives in trachealis muscle relaxation and in inhibiting cyclic nucleotide phosphodiesterase (PDE) isoenzyme types III and IV have been assessed. All compounds demonstrated significantly higher relaxant potency than theophylline. All the derivatives were moderately potent in inhibiting the type IV isoenzyme of PDE but only those with a cyano group on position 2 were potent in inhibiting the type III isoenzyme.

Introduction

SCA 40, a (methylamino)imidazo[1,2-*a*]pyrazine derivative, has been shown to be a potent smooth muscle relaxant agent in vitro as well as in vivo, especially on blood vessels and in the airways.[1–8] In vivo, SCA 40 exhibited potent anti-bronchospastic effects in guinea-pigs[6] and caused marked hypotension in rats.[2] Tested on guinea-pig isolated trachea[1,3,6] or bronchus and on human isolated bronchus[4,5,7] it produced concentration-dependent suppression of spontaneous tone. It also relaxed the same tissues when they were precontracted by different spasmogens. In such tests, pD_2 values for SCA40 lay within the range 8.61–6.85.

The synthesis and preliminary pharmacological evaluations of SCA 40 and various 8-alkoxy and 8-(alkylamino)imidazo[1,2-*a*]pyrazines have been reported previously.[9] The biological activity appeared to closely depend upon the presence of a bromine atom on position 6 and the presence of an amino or alkylamino group on position 8. Therefore, we decided to extend and modify the type of substitutions on positions 2, 3, and 8 of the heterocycle in order to obtain more insight into the structure–activity relationships for this azaindolizinic series.

Chemistry

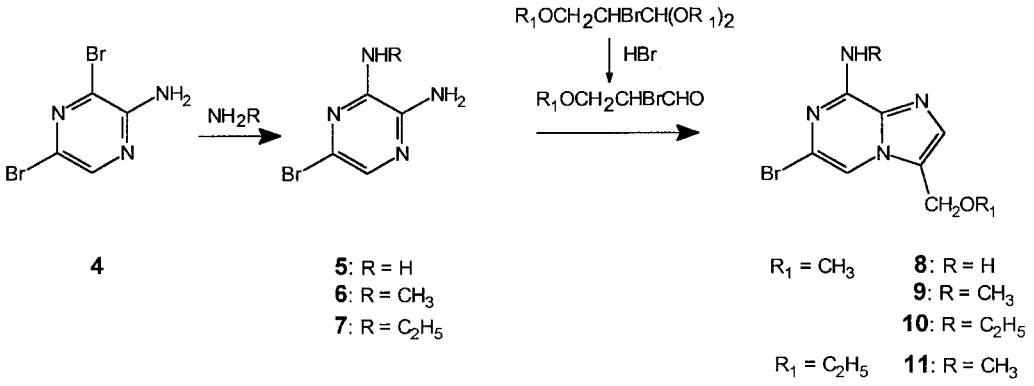
The different routes of synthesis of the imidazo[1,2-*a*]pyrazine series have been the subject of a recent review.[10] The two major synthetic strategies comprise the condensations of either an α-halogenocarbonyl compound with an aminopyrazine or an α-aminoalcohol with a chloropyrazine. In the latter strategy, and after the condensation, the alcohol group is further oxidized to a ketone. This ketone can easily undergo cyclization with the aromatic nitrogen to yield, after dehydration, the imidazopyrazine heterocycle.

Following the first synthetic pathway, some 3-substituted imidazo[1,2-*a*]pyrazines can be obtained by the use of appropriate α-bromocarbonyl derivatives (Scheme 1).

After acidic hydrolysis, the 1,1,3-trimethoxy- and 1,1,3-triethoxy-2-bromopropanes gave direct access to compounds **8–11**, depending on the aminopyrazine used as starting material. It is interesting to note that such condensation did not occur on the 3,5-dibromo-2-amino-

Key words: Imidazo[1,2-*a*]pyrazine; SCA 40; bronchodilation; phosphodiesterase inhibition.
*Corresponding author. Tel.: 33-4-67-54-38-14; fax: 33-4-67-54-38-14; e-mail: pabonnet@pharma.univ-montp1.fr

0968-0896/99/$ - see front matter
PII: S0968-0896(99)00019-X



Scheme 1.

pyrazine, probably as a result of low reactivity of the nitrogen on position 1. When the bromine on position 3 was substituted by an electron donating group such as an amino or an aminoalkyl group, the condensation took place with 30% yields.

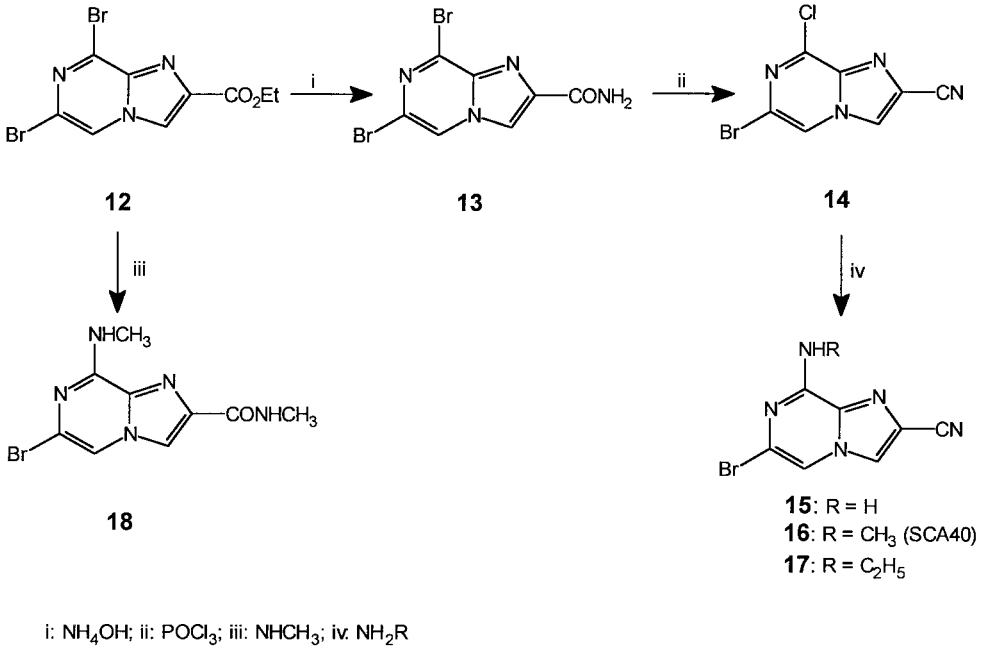
SCA40, 6-bromo-8-(methylamino)imidazo[1,2-*a*]pyrazine-2-carbonitrile **16** and the amino and ethylamino derivatives **15** and **17** have been obtained from the ester **12** in three steps (Scheme 2).

Amide **13** was dehydrated with phosphorus oxychloride to give the carbonitrile function on position 2. During this step, elimination/addition of the bromine on position 8 by chlorine occurred, with no incidence on the bromine atom on position 6. Such replacement is due to the general halogen scrambling that occurs in reactions of haloaminopyrazines[11] and 8-haloimidazo[1,2-*a*]pyrazines.[12] The last step was the regioselective nucleophilic substitution of the chlorine on position 8 by the appropriate amine. Interestingly, nucleophilic attack by methylamine of the 8-bromine atom of compound **12** led to concomitant transformation of the ester function on position 2 to yield amide **18** (Scheme 2).

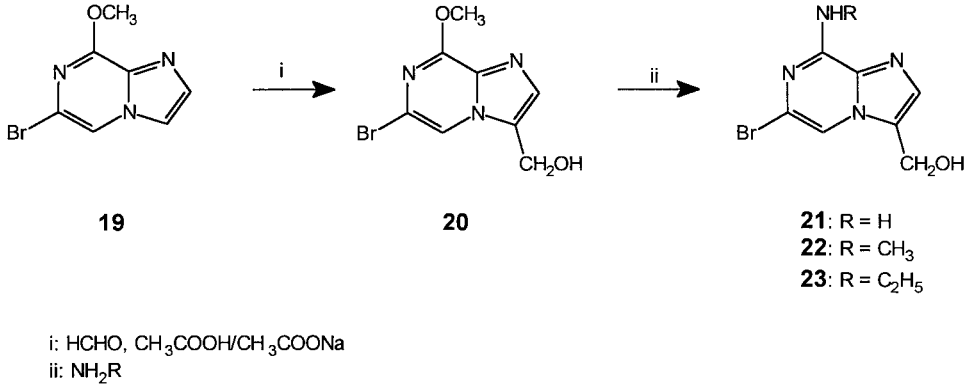
Electrophilic substitution of the unsubstituted imidazo[1,2-*a*]pyrazine has always presented many difficulties.[13,14] The introduction of electron-donating groups such as alkoxy or alkylamino enhances the nucleophilic character of position 3 and favors electrophilic substitution on this position. Studies of the electronic distributions and molecular electrostatic potential isodensity surfaces have confirmed that position 3 is the most reactive position towards electrophiles.[14,15]

Various electrophilic substitution reactions were performed in this series.

Hydroxymethylation of **19** by formaldehyde in an acetic acid/sodium acetate medium led to **20** in 45% yield[9] (Scheme 3). The methoxy derivative **20** was further transformed into amino- and (alkylamino)imidazo[1,2-



Scheme 2.



Scheme 3.

a]pyrazines **21–23**. The 6-bromo-3-(hydroxymethyl)-8-(methylamino)imidazo[1,2-*a*]pyrazine-2-carbonitrile **24** was also obtained from **16** following the same procedure. Interestingly, our various attempts at direct chloromethylation on **19** were unsuccessful.

Vilsmeier–Haack formylation of the 6-bromo-8-(methylamino)imidazo[1,2-*a*]pyrazine with dimethylformamide and phosphorus oxybromide gave the 3-formyl derivative **25**. Phosphorus oxybromide was used instead of the classical Vilsmeier–Haack reagent phosphorus oxychloride to avoid the elimination/addition of the bromine atom on position 6 by chlorine.

Nitration of **2** and **3** using nitric acid (d = 1.38) in 98.2% sulfuric acid at room temperature gave compounds **26** and **27** in 50% yields. Such reaction conditions led to a separable mixture of unchanged material and an imidazo[1,2-*a*]pyrazine derivative that was mononitrated on position 3.

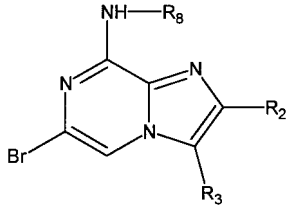
All newly synthesized imidazo[1,2-*a*]pyrazine derivatives are described in Table 1.

Pharmacological Results and Discussion

All compounds were tested in vitro. The smooth muscle relaxant activity of all compounds was evaluated on guinea-pig trachea. The results are summarized in Table 2.

All test drugs, except **27**, produced concentration-dependent and full inhibition of tracheal contraction

Table 1. Imidazo[1,2-*a*]pyrazines



Compound	R_2	R_3	R_8	mp, °C	Formula
1	H	H	H	209–210	$C_6H_5N_4Br$
2	H	H	CH_3	161–162	$C_7H_7N_4Br$
3	H	H	C_2H_5	172–174	$C_8H_9N_4Br$
8	H	CH_2OCH_3	H	180–182	$C_8H_9N_4OBr$
9	H	CH_2OCH_3	CH_3	221–222	$C_8H_{11}N_4OBr$
10	H	CH_2OCH_3	C_2H_5	225–227	$C_{10}H_{13}N_4OBr$
11	H	$CH_2OC_2H_5$	CH_3	133–135	$C_{10}H_{13}N_4OBr$
15	CN	H	H	216–218	$C_7H_4N_5Br$
16 (SCA40)	CN	H	CH_3	218–220	$C_8H_6N_5Br$
17	CN	H	C_2H_5	223–225	$C_9H_8N_5Br$
18	$CONHCH_3$	H	CH_3	215–217	$C_9H_{10}N_5OBr$
21	H	CH_2OH	H	195–197	$C_7H_7N_4OBr$
22	H	CH_2OH	CH_3	183–185	$C_8H_9N_4OBr$
23	H	CH_2OH	C_2H_5	183–185	$C_9H_{11}N_4OBr$
24	CN	CH_2OH	CH_3	175–177	$C_9H_8N_5OBr$
25	H	CHO	CH_3	213–215	$C_8H_7N_4OBr$
26	H	NO_2	CH_3	231–233	$C_7H_6N_5O_2Br$
27	H	NO_2	C_2H_5	223–225	$C_8H_8N_5O_2Br$

Table 2. Activity of the imidazo[1,2-*a*]pyrazine derivatives as relaxants of guinea-pig isolated trachea

Compound	*n*	$-\log EC_{50}$	$-\log EC_{50}$ (in the presence of IbTx 180 nM)	Relaxation (% maximum)
Theophylline	6	4.84 ± 0.10	N.D.	91.4 ± 2.9
Milrinone	6	5.29 ± 0.15	4.59 ± 0.11	94.2 ± 1.5
1	4	5.00 ± 0.10	4.00 ± 0.22	100.0 ± 0.0
2	10	4.87 ± 0.12	4.57 ± 0.17	100.0 ± 0.0
3	4	5.55 ± 0.11	4.13 ± 0.15	100.0 ± 0.0
8	4	5.50 ± 0.05	4.42 ± 0.12	100.0 ± 0.0
9	8	5.42 ± 0.10	4.56 ± 0.07	100.0 ± 0.0
10	9	5.22 ± 0.10	4.70 ± 0.10	97.7 ± 0.9
11	10	5.53 ± 0.18	4.94 ± 0.28	100.0 ± 0.0
15	4	5.94 ± 0.08	4.91 ± 0.19	100.0 ± 0.0
16 (SCA40)	8	6.06 ± 0.14	5.18 ± 0.26	100.0 ± 0.0
17	6	5.70 ± 0.10	5.52 ± 0.11	100.0 ± 0.0
18	4	5.75 ± 0.07	4.67 ± 0.02	100.0 ± 0.0
21	5	4.85 ± 0.16	4.46 ± 0.08	100.0 ± 0.0
22	8	5.51 ± 0.05	4.64 ± 0.07	100.0 ± 0.0
23	5	4.95 ± 0.09	4.43 ± 0.08	94.8 ± 1.4
24	5	4.98 ± 0.13	4.34 ± 0.04	100.0 ± 0.0
25	4	4.98 ± 0.15	N.D.	100.0 ± 0.0
26	4	5.01 ± 0.06	N.D.	91.8 ± 4.9
27	4	4.55 ± 0.13	N.D.	82.8 ± 5.6

Data are mean (± s.e. mean) values ($n = 6$–8) of pD_2 ($-\log EC_{50}$) and percentage maximum relaxation.

induced by carbachol (1 μM). Most of the imidazo[1,2-*a*]pyrazine compounds demonstrated significantly higher relaxant potency compared with theophylline, a non-selective PDE inhibitor. Some compounds (**3**, **8**, **9**, **10**, **11**, **15**, **SCA40**, **17**, **18**, and **22**) exhibit similar or higher activity than milrinone, a selective PDE III inhibitor. The most potent derivative of the series remained SCA 40 with a pD_2 of 6.06 ± 0.14. Since the relaxant activity of SCA40 was previously found to be inhibited by iberiotoxin (IbTx),[16] an agent known to block the large-conductance Ca^{++}-dependent K^+-channel (BK_{Ca} channel),[17] we tested whether this toxin would antagonise our novel derivatives. Table 2 shows that the toxin antagonised all tested imidazo[1,2-*a*]pyrazine derivatives. However it caused no significant modification to the maximum responses of these agents (data not shown). The tracheal relaxant action of SCA 40 was subsequently shown not to depend upon the opening of the BK_{Ca} channel because the antagonism provided by inhibitory toxins was found to be only functional.[3] Furthermore, SCA 40 did not exhibit any BK_{Ca} channel opening properties on diverse ion flux and electrophysiological experiments.[8,18,19]

In fact the relaxant activity of imidazo[1,2-*a*]pyrazines appears more likely to depend upon their inhibition of type III and/or IV isoenzymes of cyclic nucleotide phosphodiesterase. Such inhibitory activity was measured for eight derivatives of the series (Table 3).

Most tested compounds proved to be PDE III and IV inhibitors. All tested compounds exhibit moderate potency as inhibitors of isoenzyme type IV, the most potent being compound **9** with a methoxymethyl group on position 3. Inhibitory activity against PDE isoenzyme type III depends strongly on the nature of the substitution on position 2 or 3. Only compounds **15** and SCA40, compound **16**, with the cyano group on position 2 show potent inhibitory activity against this isoenzyme while all the other compounds behave as weak inhibitors exhibiting more potent PDE IV versus PDE III inhibitory activity. SCA40, with the methylamino substitution on position 8, exhibits an unique profile since it appears to be the most selective PDE III inhibitor. Electron withdrawing groups such as formyl or nitro on position 3 lead to compounds that are inactive against PDE III. For compounds **2**, **9**, **15**, **SCA40**, **18**, **22**, and **26** we obtained not only pD_2 values for relaxation of guinea pig isolated trachea but also IC_{50} values against PDE isoenzymes III and IV. Analysis of these data failed to reveal a significant correlation between pD_2 and the IC_{50} against PDE III (correlation coefficient 0.7048; $p = 0.0509$) or between pD_2 and the IC_{50} against PDE IV (correlation coefficient 0.286; $p = 0.4922$). This does not necessarily argue against the role of PDE inhibition in mediating the tracheal relaxant activity of these compounds for the activity of both PDE III and PDE IV is known[20,21] to modulate the mechanical tone of guinea-pig trachea. It may be that the relaxant potency of these compounds is related in a complex way to their simultaneous inhibition of PDE

Table 3. Phosphodiesterase inhibitory activity of the imidazo[1,2-*a*]pyrazine derivatives

Compound	$-\log IC_{50}$ PDE III	$-\log IC_{50}$ PDE IV
SKF 94120	5.14 ± 0.06	< 3.0
Ro 20-1724	3.83 ± 0.13	4.63 ± 0.20
2	4.25 ± 0.24	5.26 ± 0.13
9	4.30 ± 0.07	6.43 ± 0.18
15	6.05*	5.83 ± 0.03
16 (SCA40)	7.34 ± 0.06	5.55 ± 0.07
18	3.85 ± 0.11	4.98 ± 0.15
22	4.25 ± 0.08	5.60 ± 0.07
25	< 4.0	5.56 ± 0.06
26	4.01 ± 0.24	4.92 ± 0.21

*Mean of two measurements; standard error non-calculable.
Data indicate mean (± s.e. mean) values of $-\log IC_{50}$ for each compound.

isoenzymes III and IV. In conclusion, all imidazo[1,2-*a*]pyrazine derivatives tested are PDE IV inhibitors of moderate potency whereas the presence of a cyano group on position 2 specifically leads to PDE III inhibitors of high potency, such as SCA40.

Experimental

Chemistry

All melting points are uncorrected and were determined using a Köfler hot plate melting point apparatus. Purification was checked by thin-layer chromatography on silica gel (230–240 mesh from Merck). ^{1}H NMR spectra were recorded using a Brucker AC 100 spectrometer. Elemental analyses were performed by the Microanalytical Centre (Montpellier, France).

Compounds **1–4**, **6**, **9**, **11**, **12**, **SCA40**, **19**, **20** and **22** were synthesized as previously reported.[9,22]

5-Bromo-2,3-diaminopyrazine (5). A mixture of 5 g (20 mmol) of 2-amino-3,5-dibromopyrazine **4** in 50 mL of a 30% NH_4OH solution was heated at 130 °C for 5 h in a 125 mL autoclave. Extraction with methylene chloride followed by chromatography on a silica gel column eluted with 5% methanol–methylene chloride afforded 2.8 g (15.2 mmol, 80%) of **5**. ^{1}H NMR (DMSO-d_6): δ 7.17 (s, 1H), 6.39 (br s, 2H), 6.08 (br s, 2H). Anal. calcd for $C_4H_5N_4Br$: C, 25.42; H, 2.67; N, 29.64. Found: C, 25.33; H, 2.70; N, 29.72.

2-Amino-5-bromo-3-(ethylamino)pyrazine (7). This agent was prepared, in an analogous manner, from 2-amino-3,5-dibromopyrazine **4** and a 70% ethylamine aqueous solution. Elution with 5% methanol–methylene chloride yielded 3.5 g (16 mmol, 84%) of **7**. ^{1}H NMR ($CDCl_3$): δ 7.34 (s, 1H), 4.59 (br t, 1H), 4.47 (br s, 2H), 3.39 (q, 2H), 1.18 (t, 3H). Anal. calcd for $C_6H_9N_4Br$: C, 33.20; H, 4.18; N, 25.81. Found: C, 33.42; H, 4.15; N, 25.70.

8-Amino-6-bromo-3-(methoxymethyl)imidazo[1,2-*a*]pyrazine (8). A mixture of 3 g (14 mmol) freshly distilled 2-bromo-1,1,3-trimethoxypropane,[23] concentrated hydrobromic acid (1 mL), and water (10 mL) was heated under reflux for 1 h. The mixture was then cooled. Diethylether was added and the layers were separated. The organic phase which contained the 2-bromo-3-methoxypropanal was dried and poured into a solution of 2 g (10 mmol) of 5-bromo-2,3-diaminopyrazine **5** in dry DMF (2 mL). The reaction mixture was stirred at room temperature for 2 days. Upon evaporation of the solvent, 100 mL of dry ethanol were added to the oily residue. The resulting solution was heated under reflux for 2 h. The solvent was evaporated in vacuo and the residue was dissolved in water (10 mL), neutralized with sodium carbonate and extracted with methylene chloride. The organic phase was dried and evaporated. Chromatography on a silica gel column eluted with 2% methanol–methylene chloride furnished, besides 0.9 g (4.8 mmol) of unreacted pyrazine **5**, a small amount of compound **21** and 0.7 g (2.73 mmol, 26%) of **8**. ^{1}H NMR ($CDCl_3$): δ 7.69 (s, 1H), 7.45 (s, 1H), 5.87 (br s, 2H), 4.64 (s, 2H), 3.33 (s, 3H). Anal. calcd for $C_8H_9N_4OBr$: C, 37.38; H, 3.53; N, 21.79. Found: C, 37.55; H, 3.68; N, 21.84.

6-Bromo-8-(ethylamino)-3-(methoxymethyl)imidazo[1,2-*a*]pyrazine (10). Compound **10** was prepared, in an analogous manner, from 2-amino-5-bromo-3-(ethylamino)-pyrazine **7**. Elution with 2% methanol–methylene chloride yielded 0.8 g (2.8 mmol, 30%) of **10** and 0.6 g of unreacted product. No detectable trace of **23** was observed. ^{1}H NMR ($CDCl_3$): δ 7.57 (s, 1H), 7.37 (s, 1H), 6.00 (br t, 1H), 4.62 (s, 2H), 3.74–3.47 (m, 2 H, J = 8.0 Hz), 3.32 (s, 3H), 1.29 (t, 3H, J = 8.0 Hz). Anal. calcd for $C_{10}H_{13}N_4OBr$: C, 42.12; H, 4.60; N, 19.65. Found: C, 41.97; H, 4.43; N, 19.84.

6,8-Dibromoimidazo[1,2-*a*]pyrazine-2-carboxamide (13). A mixture of 1 g (2.85 mmol) of **12** in 500 mL of a 30% NH_4OH solution was stirred in the dark for 4 h at room temperature. Filtration using a Büchner funnel afforded crude amide **13** in 53% yield. ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H), 7.91 (s, 1H), 5.57 (s, 2H). Anal. calcd for $C_7H_4N_4OBr_2$: C, 26.28; H, 1.26; N, 17.51. Found: C, 26.43; H, 1.22; N, 17.65.

6-Bromo-8-chloroimidazo[1,2-*a*]pyrazine-2-carbonitrile (14). A mixture of 1.65 g (5.15 mmol) of crude **13** in 16.5 mL of phosphorus oxychloride was heated under reflux for 1 h. After cooling, the mixture was poured onto ice. After alkalinization with Na_2CO_3 and extraction with methylene chloride, the organic layer was dried and evaporated. The residue was purified by chromatography on a silica gel column eluted with methylene chloride to give the desired compound in 87% yield. ^{1}H NMR ($CDCl_3$): δ 8.26 (s, 1H), 8.16 (s, 1H). Anal. calcd for $C_7H_2N_4ClBr$: C, 32.65; H, 0.78; N, 21.76. Found: C, 32.56; H, 0.79; N, 21.84.

8-Amino-6-bromoimidazo[1,2-*a*]pyrazine-2-carbonitrile (15). A mixture of 1.3 g (4.8 mmol) of **14** in 10 mL of a 30% NH_4OH solution was stirred for 5 h at room temperature. Extraction with methylene chloride followed by chromatography on a silica gel column eluted with methylene chloride afforded 0.9 g (3.6 mmol, 75%) of **15**. ^{1}H NMR ($CDCl_3$): δ 7.78 (s, 1H), 7.69 (s, 1H), 6.54 (br s, 2H). Anal. calcd for $C_7H_4N_5Br$: C, 35.32; H, 1.69; N, 29.42. Found: C, 35.19; H, 1.70; N, 29.13.

6-Bromo-8-(ethylamino)imidazo[1,2-*a*]pyrazine-2-carbonitrile (17). was prepared in an analogous manner from **14** and a 70% ethylamine aqueous solution. Elution with methylene chloride yielded 0.8 g (3.1 mmol, 84 %) of **17**. ^{1}H NMR ($CDCl_3$): δ 7.62 (s, 1 H), 7.43 (s, 1 H), 6.11 (br s, 1 H), 3.54 (q, 2 H), 1.23 (t, 3 H). Anal. calcd for $C_9H_8N_5Br$: C, 40.62; H, 3.03; N, 26.32. Found: C, 40.86; H, 3.12; N, 26.01.

6-Bromo-8-(methylamino)imidazo[1,2-*a*]pyrazine-2*N*-methylcarboxamide (18). A mixture of ester **12** (1 g, 2.9 mmol) and 10 mL of aqueous methylamine (40%) was stirred in the dark for 4 h at room temperature. After extraction with methylene chloride, the organic phase was dried and evaporated. The residue was purified by

chromatography on a silica gel column eluted with methylene chloride to give 0.7 g (2.4 mmol, 82%) of the desired compound. ^{1}H NMR ($CDCl_3$): δ 7.92 (s, 1H), 7.51 (s, 1H), 7.16 (br m, 1H), 6.07 (br m, 1H), 3.12 (d, 3H, J = 5.0 Hz), 2.95 (d, 3 H, J = 5.0 Hz). Anal. calcd for $C_9H_{10}N_5OBr$: C, 38.05; H, 3.55; N, 24.65. Found: C, 37.96; H, 3.60; N, 24.74.

8-Amino-6-bromo-3-(hydroxymethyl)imidazo[1,2-*a*]pyrazine (21). A mixture of 1 g of 6-bromo-3-(hydroxymethyl)-8-methoxyimidazo[1,2-*a*]pyrazine **20**[9] (3.8 mmol) in 100 mL of a 30% NH_4OH solution was heated to 120 °C for 2 h in a 250 mL autoclave. Extraction with methylene chloride followed by chromatography on a silica gel column eluted with 5% methanol-methylene chloride afforded 0.24 g (1 mmol, 25%) of the desired compound **21** and 0.5 g (1.9 mmol) of unreacted product. ^{1}H NMR ($CDCl_3$): δ 7.67 (s, 1H), 7.47 (s, 1H), 5.64 (br s, 2H), 4.91 (d, 2H, J = 2.0 Hz). Anal. calcd for $C_7H_7N_4OBr$: C, 34.59; H, 2.90; N, 23.05. Found: C, 34.48; H, 3.00; N, 22.97.

6-Bromo-8-(ethylamino)-3-(hydroxymethyl)imidazo[1,2-*a*]pyrazine (23). This agent was prepared in an analogous manner with a 70% ethylamine aqueous solution. Elution with 5% methanol–methylene chloride yielded 0.5 g (1.9 mmol, 50%) of **23**. ^{1}H NMR ($CDCl_3$): δ 7.62 (s, 1H), 7.29 (s, 1H), 5.99 (br s, 1H), 4.82 (s, 2H), 3.58–3.50 (m, 2H, J_1 = 6.0 Hz, J_2 = 7.0 Hz), 1.25 (t, 3H, J = 7.0 Hz). Anal. calcd for $C_9H_{11}N_4OBr$: C, 39.87; H, 4.09; N, 20.67. Found: C, 39.88; H, 3.99; N, 20.84.

6-Bromo-3-(hydroxymethyl)-8-(methylamino)imidazo[1,2-*a*]pyrazine-2-carbonitrile (24). A mixture of 6-bromo-8-(methylamino)imidazo[1,2-*a*]pyrazine-2-carbonitrile **16** (1 g, 4 mmol), sodium acetate (1.4 g, 17 mmol), acetic acid (1 mL, 17 mmol), and 10 mL (129 mmol) of a 37% solution of formaldehyde in water was heated in an autoclave at 120 °C for 2 h. On cooling, 30 mL of water was added to the reaction mixture. After alkalinization with Na_2CO_3 and extraction with methylene chloride, the organic phase was dried and evaporated. The residue was purified by chromatography on a silica gel column eluted with 2% methanol–methylene chloride to give 0.4 g (1.4 mmol, 35%) of the desired compound. ^{1}H NMR ($CDCl_3$): δ 8.19 (s, 1H), 6.23 (br m, 1H), 5.00 (d, 2H, J = 6.0 Hz), 3.14 (d, 3H, J = 5.0 Hz). Anal. calcd for $C_9H_8N_5OBr$: C, 38.32; H, 2.86; N, 24.83. Found: C, 38.41; H, 2.84; N, 25.00.

6-Bromo-3-formyl-8-(methylamino)imidazo[1,2-*a*]pyrazine (25). Phosphorus oxy-bromide (7.5 g, 26 mmol) was added dropwise, with stirrring, to cooled (0 °C), dry dimethylformamide (10 mL). 0.5 g (2.2 mmol) of compound **2** in 15 mL of dry dimethylformamide was then slowly added to the stirred mixture. After being allowed to warm to 120 °C, the mixture was stirred for 2 h at 85 °C. After cooling on ice, 20 mL of 35% HCl were added and the cooled solution was then basified with an aqueous solution of sodium hydroxide (2 M), extracted with dichloromethane, dried and evaporated. The resulting oil was washed with diethylether. Chromatography on silica gel, eluted with 2% methanol–methylene chloride gave 0.14 g (0.55 mmol, 25%) of **25** and 0.2 g (0.88 mmol) of unreacted product. ^{1}H NMR ($CDCl_3$): δ 10.28 (s, 1H), 8.65 (s, 1H), 8.08 (s, 1H), 6.27 (br m, 1H), 3.15 (d, 3H, J = 5.0 Hz). Anal. calcd for $C_8H_7N_4OBr$: C, 37.67; H, 2.77; N, 21.96. Found: C, 37.67; H, 2.84; N, 22.01.

6-Bromo-8-(methylamino)-3-nitroimidazo[1,2-*a*]pyrazine (26). Compound **2** (2.2 mmol) was dissolved in cold (−15 °C) 98.2% H_2SO_4 (3.5 mL) and HNO_3 (0.35 mL, d = 1.38) was added dropwise with stirring. The solution was left to stand for 1 h at 0 °C and 2 h at room temperature. Then, the resulting mixture was poured onto ice, neutralized with Na_2CO_3 and extracted with CH_2Cl_2. After drying with Na_2SO_4, the extracts were evaporated and the solid residue was purified by column chromatography on silica gel eluted with methylene chloride. Yield 65%. ^{1}H NMR ($CDCl_3$): δ 8.45 (s, 1H), 7.57 (s, 1H), 3.11 (d, 3H, J = 5.0 Hz). Anal. calcd for $C_7H_6N_5O_2Br$: C, 30.90; H, 2.22; N, 25.74. Found: C, 31.01; H, 2.31; N, 25.77.

6-Bromo-8-(ethylamino)-3-nitroimidazo[1,2-*a*]pyrazine (27). This derivative was prepared, in an analogous manner, from **3** (yield 55%). ^{1}H NMR ($CDCl_3$): δ 8.43 (s, 1H), 7.56 (s, 1H), 3.75 (m, 2H, J = 7.0 Hz), 1.34 (t, 3H, J = 7.0 Hz). Anal. calcd for $C_8H_8N_5O_2Br$: C, 33.59; H, 2.82; N, 24.48. Found: C, 33.64; H, 2.94; N, 24.53.

Pharmacology: guinea pig isolated trachea preparation

Adult male Dunkin–Hartley guinea pigs (Iffa Credo, France), weighing 400–500 g, were killed by a blow to the head. Tracheae were excised and cleaned of adhering adipose and connective tissue. The contractility of tracheal segments (four tracheal rings in all cases) was measured by adapting the method previously described.[24] Tissue segments were suspended in Krebs' solution maintained at 37 °C and gassed with a mixture of 95% O_2, 5% CO_2. All tissues were connected to an isometric force-displacement transducer under a basal tension of 0.5 g. The force-displacement transducer provided input to a Physiograph Narco Bio-system. The bronchoconstrictor agent (1 μM carbachol) induced a contraction which reached a plateau within 5 min. Cumulative log–concentration response curves were constructed for each test compound, relaxation being measured as the percentage reduction in carbachol-induced contraction. Relaxant potency of each compound was expressed as the negative log EC_{50} (pD_2), where EC_{50} is the concentration producing 50% inhibition of the contraction. The EC_{50} values were calculated by linear regression analysis applied to the linear portion of each concentration–response curve. The results presented in Table 2 are means ± SEM of six to eight determinations.

Biochemistry: measurement of inhibitory activity against cyclic nucleotide phosphodiesterase (PDE) isoenzymes

Isoenzymes of cyclic nucleotide PDE were isolated from guinea pig cardiac ventricles (type III) and bovine trachealis (type IV) as previously described.[25] The iden-

tities of the isoenzymes were confirmed by their sensitivities to SKF 94120 and Ro 20-1724, respective PDE III and PDE IV selective inhibitors (Table 3), and cyclic GMP (inhibitor of PDE III but not PDE IV). The isoenzymes used for kinetic analysis were at least 90% pure based on their sensitivities to the above agents.

The activity of isoenzymes III and IV was measured essentially by the method of Thompson and Appleman[26] as modified by Rutten et al.[27] Assays of enzyme activity were performed in a final volume of 100 μL comprising 25 μL of the isoenzyme solution, 50 μL of assay buffer and 25 μL of twice-distilled water or PDE inhibitor (imidazo[1,2-*a*]pyrazine derivative) solution. The assay buffer (pH 8.0) contained 0.2 μCi [^{3}H]-cAMP and yielded final concentrations of 1 μM cAMP, 40 mM Tris–HCl, 2.5 mM $MgCl_2$ and 3.75 mM β-mercaptoethanol in the reaction mixture. 10 mM stock solutions of imidazo[1,2-*a*]pyrazine derivatives were prepared in ethanol. Dilutions from these stock solutions were prepared using twice-distilled water. In tests of enzyme inhibition, the reaction mixture contained concentrations of imidazo[1,2-*a*]pyrazine derivatives in the range of 10 nM–100 μM. The reagents were mixed on ice and the reaction was initiated by transferring the mixture to a water bath at 37 °C. Following 30 min incubation, the reaction was stopped by transferring the reaction tubes to a bath of boiling water for 3 min. After cooling on ice, 20 μL of a 1 mg.mL^{-1} solution of *Ophiophagus hannah* venom was added to each tube and the mixture was incubated at 37 °C for 10 min. Unreacted [^{3}H]-cAMP was removed by the addition of 400 μL of a 1 in 3 suspension of Dowex resin (1×8–400) and incubation on ice for 30 min. Each tube was then centrifuged (10,000 *g*) for 2 min and 200 μL of the supernatant was removed for liquid scintillation counting. Less than 10% of the tritiated cAMP was hydrolysed in any assay.

The test compound concentration producing 50% inhibition of PDE activity (IC_{50} value) was determined for each compound by use of a nonlinear regression curve fitting program.

The results presented in Table 3 are means ± SEM of at least four experiments.

References

1. Laurent, F.; Michel, A.; Bonnet, P. A.; Chapat, J. P.; Boucard, M. *Br. J. Pharmacol.* **1993**, *108*, 622.
2. Michel, A.; Laurent, F.; Bompart, J.; Hadj-Kaddour, K.; Chapat, J. P.; Boucard, M.; Bonnet, P. A. *Br. J. Pharmacol.* **1993**, *110*, 1031.
3. Cook, S. J.; Archer, K.; Martin, A.; Buchheit, K. H.; Fozard, J. R.; Müller, T.; Miller, A. J.; Elliott, K. R. F.; Foster, R. W.; Small, R. C. *Br. J. Pharmacol.* **1995**, *114*, 143.
4. Naline, E.; Cui, Y. Y.; Michel, A.; Bonnet, P. A.; Bakdach, H.; Advenier, C. *Fundam. Clin. Pharmacol.* **1996**, *10*, 368.
5. Cortijo, J.; Villargrasa, V.; Navarette, C.; Sanz, C.; Berto, L.; Michel, A.; Bonnet, P. A.; Morcillo, E. J. *Br. J. Pharmacol.* **1996**, *119*, 99.
6. Buchheit, K. H.; Hofmann, A.; Pfannkuche, H. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997**, *355*, 217.
7. Müller-Schweinitzer, E.; Fozard, J. R. *Br. J. Pharmacol.* **1997**, *120*, 1241.
8. Pocock, T.; Laurent, F.; Isaac, L. M.; Chiu, P.; Elliott, K. R. F.; Foster, R. W.; Michel, A.; Bonnet, P. A.; Small, R. C. *Eur. J. Pharmacol.* **1997**, *334*, 75.
9. Bonnet, P. A.; Michel, A.; Laurent, F.; Sablayrolles, C.; Rechencq, E.; Mani, J. C.; Boucard, M.; Chapat, J. P. *J. Med. Chem.* **1992**, *35*, 3353.
10. Basiuk, V. A. *Russ. Chem. Rev.* **1997**, *66*, 187.
11. Lumma Jr, W. C.; Randall, W. C., Cresson, E. L.; Huff, J. R.; Hartam, R. D.; Lyon, T. F. *J. Med. Chem.* **1983**, *26*, 357.
12. Meurer, L. C.; Tolman, R. L.; Chapin, E. W.; Saperstein, R.; Vicario, P. P.; Zrada, M. M.; MacCoss, M. *J. Med. Chem.* **1992**, *35*, 3845.
13. Teulade, J. C.; Bonnet, P. A.; Rieu, J. N.; Viols, H.; Chapat, J. P.; Grassy, G.; Carpy, A. *J. Chem. Res. (M)* **1986**, 202.
14. Vitse, O.; Bonnet, P. A.; Bompart, J.; Viols, H.; Subra, G.; Chapat, J. P.; Grassy, G. J. *Heterocyclic Chem.* **1997**, *34*, 701.
15. Arriau, J.; Chalvet, O.; Dargelos, A.; Maury, G. *J. Heterocyclic Chem.* **1974**, *11*, 1013.
16. Laurent, F.; Michel, A.; Bonnet, P. A., Bompart, J.; Chapat, J. P., Boucard, M. *C. R. Soc. Biol.* **1993**, *187*, 526.
17. Giangiacomo, M. M.; Garcia, M. L.; McManus, O. B. *Biochemistry* **1992**, *31*, 6719.
18. MacMillan, S.; Sheridan, R. D.; Chilvers, E. R.; Patmore, L. *Br. J. Pharmacol.* **1993**, *116*, 1656.
19. Fagni, L. et al. unpublished results.
20. Harris, A. L.; Connell, M . J.; Ferguson, E. W.; Wallace, A. M.; Gordon, R. J.; Pagani, E. D.; Silver, P. J. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 199.
21. Berry, J. L.; Elliott, K. R. F.; Foster, R. W.; Small, R. C. *Br. J. Pharmacol.* **1991**, *104*, 206P.
22. Sablayrolles, C.; Cros, G.; Milhavet, J. C.; Rechencq, E.; Chapat, J. P.; Boucard, M.; Serrano, J. J.; McNeill, J. H. *J. Med. Chem.* **1984**, *27*, 206.
23. Castro, C. E., *US Patent*, 3,259,641, **1966**.
24. Hooker, C. S.; Calkins, P. Y.; Fleish, J. H. *Blood Vessels* **1971**, *14*, 1.
25. Elliott, K. R. F.; Berry, J. L.; Bate, A. J.; Foster, R. W.; Small, R. C. *J. Enzyme Inhibition* **1991**, *4*, 245.
26. Thompson, W. J.; Appleman, M. M. *Biochemistry* **1971**, *10*, 311.
27. Rutten, B. M.; Schoot, W. J.; Depont, J. *J. Biochem. Biophys. Acta.* **1973**, *315*, 378.