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Short communication

Imidazo[1,2-*a*]quinoxalines: synthesis and cyclic nucleotide phosphodiesterase inhibitory activity

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Abstract – A group of imidazo[1,2-*a*]quinoxalines have been synthesised from quinoxaline by condensation of an appropriate haloester or intramolecular cyclisation of a keto moiety on an intracyclic nitrogen atom. The reactivity of the heterocycle was explored through diverse reactions such as electrophilic substitution, lithiation and halogen–metal exchange to give access to a new series of derivatives. Confirmation of their structure was mainly performed by NMR, after careful assignment of the signals in comparison to previous attributions made on the parent imidazo[1,2-*a*]quinoxaline and discussion of available data in the literature. The cyclic nucleotide phosphodiesterase inhibitor activity of some of these derivatives has been assessed on isoenzymes type III and type lV. Compound **15**, 4-(methylamino)imidazo[1,2-*a*]quinoxaline-2-carbonitrile, exhibited potent relaxant activity on smooth muscle, with a potency similar to the one measured with SCA 40, its structural analogue in the imidazo[1,2-*a*]pyrazine series. © 2001 Éditions scientifiques et médicales Elsevier SAS

imidazo[1,2-*a*]quinoxaline / lithiation / halogen–metal exchange / phosphodiesterase inhibition / smooth muscle relaxant activity

1. Introduction

SCA40, 6-bromo-8-(methylamino)imidazo[1,2-*a*]-pyrazine-2-carbonitrile (*figure 1*), has been shown to be a potent smooth muscle relaxant agent in vitro as well as in vivo [1–9].

Structure–activity relationships studies in the imidazo[1,2-*a*]pyrazine series have demonstrated that substitution on position 2, 3 and 8 have an influence on PDE III and/or IV inhibitory activity and, then, on smooth muscle relaxant activity [10].

It was then decided to study a new series of compounds, with an imidazo[1,2-*a*]pyrazine structural basic frame condensed with a phenyl on the 6–7 face, i.e. imidazo[1,2-*a*]quinoxaline derivatives.

Therefore, substitutions on positions 1, 2, 4 (equivalent positions 2, 3, 8 in the imidazo[1,2-*a*]-pyrazinic series) were modified in this imidazo[1,2-

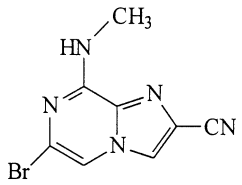

Figure 1. SCA 40.

Abbreviations: PDE III, phosphodiesterase type III; PDE IV, phosphodiesterase type IV; cAMP, cyclic adenosine monophosphate; DME, ethylene glycol dimethyl ether; DMF, dimethylformamide; *n*-Buli, *n*-butyllithium; DMSO, dimethylsulfoxide.

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a]quinoxalinic series in order to study their influence on PDE III and/or IV inhibitory activity as well as on smooth muscle relaxation.

2. Chemistry

The 4-substituted imidazo[1,2-*a*]quinoxalines are synthesised by an intramolecular cyclisation reaction on an aromatic position. This cyclisation may occur in either of two reactions leading to the formation of either a quinoxaline [11, 12] or an imidazole ring.

In this study, we chose to build the imidazolic ring starting from the quinoxaline. The classical Tschitschibabin condensation of an α-halogeno carbonyl compound with 2-amino-3-chloroquinoxaline **2** led to the formation of the 4-chloroimidazo[1,2-*a*]-quinoxaline **3**. Such strategy allows the access to derivatives with some variety of substitutions on the two-position of the heterocycle (*figures 2 and 3*). Hydrogenation of **3** using Pd/C as catalyst led to the imidazo[1,2-*a*]quinoxaline **8** for a basic NMR study.

In another strategy, the condensation of an aminoalcohol with 2,3-dichloroquinoxaline **1** [11]

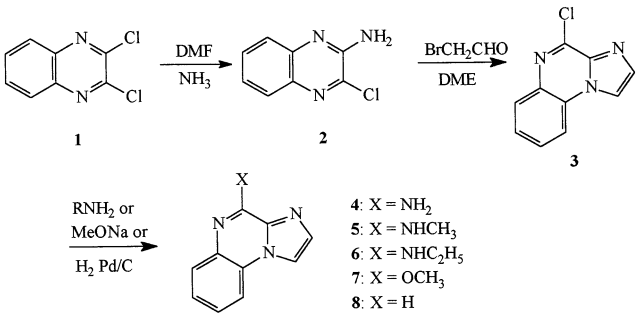


Figure 2. Synthesis of **2**–**8**.

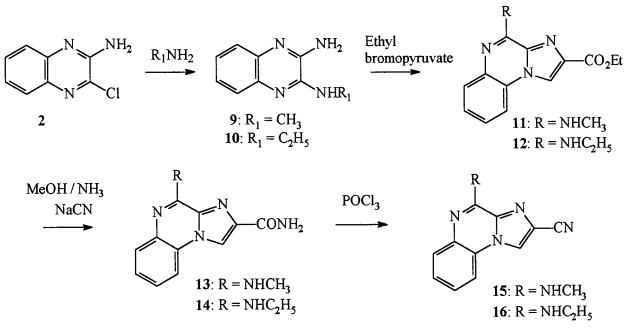


Figure 3. Synthesis of **9**–**16**.

(*figure 4*) was followed by oxidation of the hydroxyl function of the intermediate alcohol **17** using the sulfur trioxide trimethylamine complex as the oxidant. The carbonyl compound **18** was then cyclised in a trifluoroacetic anhydride/trifluoroacetic acid medium to give the 4-chloro-1-methylimidazo[1,2-*a*]quinoxaline **19**.

Diversity on position 1 was also obtained through a reactivity study of the tricyclic heterocycle, either under electrophilic substitution, lithiation or halogen-metal exchange conditions [13]. These studies were preferably performed on 4-methoxy- and not on 4-chloroimidazo[1,2-*a*]quinoxaline in order first to increase the reactivity of the aromatic position towards electrophilic reagents and second, to avoid secondary possible reactions on position 4 such as nucleophilic substitution or halogen–metal exchange.

Hydroxymethylation was performed using the classical electrophilic substitution already described for azaindolizines [14] and which have given interesting results in some of these series known to be quite reluctant to electrophilic substitution (32 and 41% yield on imidazo[1,2-*a*]pyrimidine and imidazo[1,2-*a*]pyrazine, respectively). Following the same procedure, 1-(hydroxymethyl)-4-methoxyimidazo[1,2-*a*]-quinoxaline **22** was obtained by direct reaction of formaldehyde on **7** (*figure 5*) but with a very poor yield of 5%. The formylation reaction was studied under Vilsmeier–Haack conditions [15] but, unfortunately, no substitution occurred, with only degradation products being obtained.

Since, as it is quite usual in these polyazaheterocycles, electrophilic substitutions were difficult to achieve, we were then interested in lithiation and halogen–metal exchange reactions. A detailed study of the reactivity of the heterocycle towards such reactions is available elsewhere [13]. We then applied already determined optimal conditions for the lithiation of 4-methoxyimidazo[1,2-*a*]quinoxaline (2 equiv. of *n*-BuLi at −75°C, followed by the addition of 10 equiv. of the electrophile at −75°C) [13] to obtain various derivatives with substitutions on position 1 as indicated in *figure 5*. The formylation, which was shown to be impossible through Vilsmeier–Haack conditions, was realised after lithiation and subsequent quenching with DMF [15]. The formylated compound **23** was so obtained with a 60% yield. Alkyl halides were also tested as alternative electrophilic reagents in the quenching of intermediate lithiated derivatives [16]. However, no reaction occurred with

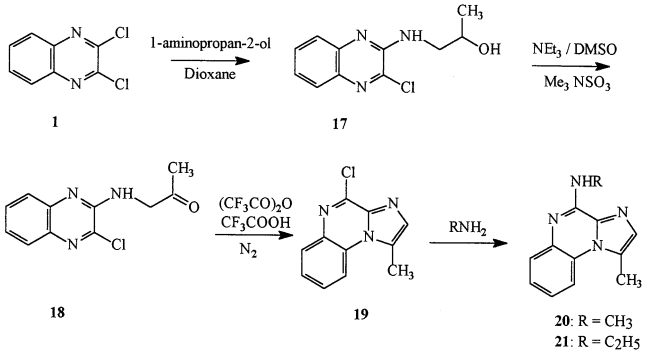


Figure 4. Synthesis of **17**–**21**.

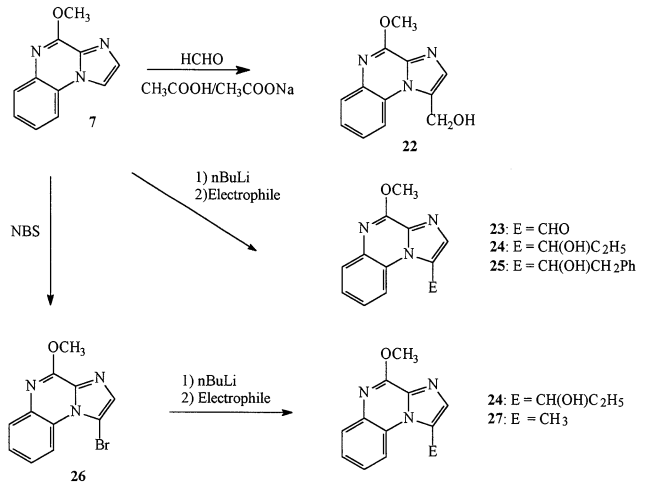


Figure 5. Synthesis of **22**–**27**.

bromoalkanes and, even when the quenching reaction was performed at increasing temperature (−75°C to room temperature), only starting material was recovered.

The halogen–metal exchange reaction was also applied to 1-bromo-4-methoxyimidazo[1,2-*a*]quinoxaline **26**, obtained upon *N*-bromosuccinimide bromination in dichloromethane of 4-methoxyimidazo[1,2-*a*]quinoxaline [17]. The exchange was performed through previously defined optimal lithiation conditions [13], and with methyl iodide used as the electrophile, 4-methoxy-1-methylimidazo[1,2-*a*]quinoxaline **27** was obtained with a 91% yield.

2.1. NMR study

^{1}H- and ^{13}C-NMR were used to unambiguously assign the position of substitution on the heterocycle following above described reactivity studies and, in a more general concern, the exact chemical structure of the newly synthesised imidazo[1,2-*a*]quinoxaline derivatives. Our first attempt was to base our assignments on data generated by Cobb and Cheeseman in 1985 [18] for diverse two-ring and three-ring heterocycles, and in particular for imidazo[1,2-*a*]quinoxaline (*table I*). In their ^{1}H-NMR study, which was performed in $CDCl_3$, H-6 (8.05 ppm) was more deshielded than H-9 (7.83 ppm). From our carbon–proton correlation study, performed in DMSO-d_6, we observed, for the same imidazo[1,2-*a*]quinoxaline and in contradiction with the above data, a correlation between C-6 at 129.8 ppm and the shielded proton at 8.06 ppm and, by the way, another correlation between C-9 at 116 ppm and the deshielded proton at 8.38 ppm.

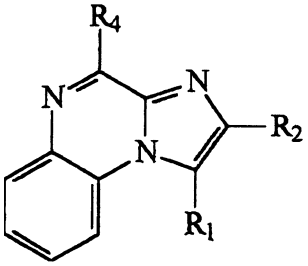
In order to confirm all carbon and proton NMR assignments, we carried out a n.O.e. study. In this study, irradiation of H-1 at 8.81 ppm showed correlations with two protons at 7.85 and 8.38 ppm. The proton at 8.38 ppm also exhibited correlations with two protons at 8.81 and 7.75 ppm. On the other hand, the proton at 8.06

Table I. Proton and carbon-13 chemical shifts (ppm) for the imidazo[1,2-*a*]quinoxaline.

δ [a] ($CDCl_3$)	δ [b] ($CDCl_3$)	δ [b] (DMSO-d_6)	Proton assignment	δ [b] (DMSO-d_6)	Carbon-13 assignment
9.057	9.11	9.095	H-4	143.9	C-4
8.063	8.84	8.81	H-1	134.2	C-2
8.051	8.06	8.06	H-6	129.8	C-6
7.828	8.40	8.38	H-9	129.0	C-8
7.759	7.86	7.85	H-2	126.4	C-7
7.592	7.76	7.75	H-8	116.0	C-9
7.526	7.64	7.64	H-7	114.1	C-1

[a] Data and assignments from Cobb and Cheeseman [18].
[b] Our study.

Table II. Imidazo[1,2-*a*]quinoxalines.



Compound	R_1	R_2	R_4	M.p. (°C)	Formula
3	H	H	Cl	192	$C_{10}H_6N_3Cl$
4	H	H	NH_2	200	$C_{10}H_8N_4$
5	H	H	$NHCH_3$	178	$C_{11}H_{10}N_4$
6	H	H	NHC_2H_5	106	$C_{12}H_{12}N_4$
7	H	H	OCH_3	153	$C_{11}H_9N_3O$
8	H	H	H	122	$C_{10}H_7N_3$
11	H	CO_2Et	$NHCH_3$	203	$C_{14}H_{14}N_4O_2$
12	H	CO_2Et	NHC_2H_5	194	$C_{15}H_{16}N_4O_2$
13	H	$CONH_2$	$NHCH_3$	>250	$C_{12}H_{11}N_5O$
14	H	$CONH_2$	NHC_2H_5	>250	$C_{13}H_{13}N_5O$
15	H	CN	$NHCH_3$	220	$C_{12}H_9N_5$
16	H	CN	NHC_2H_5	180	$C_{13}H_{11}N_5$
19	CH_3	H	Cl	182	$C_{11}H_8N_3Cl$
20	CH_3	H	$NHCH_3$	186–187	$C_{12}H_{12}N_4$
21	CH_3	H	NHC_2H_5	139	$C_{13}H_{14}N_4$
22	CH_2OH	H	OCH_3	184	$C_{12}H_{11}N_3O_2$
23	CHO	H	OCH_3	210	$C_{12}H_9N_3O_2$
24	$CH(OH)–C_2H_5$	H	OCH_3	167	$C_{14}H_{15}N_3O_2$
25	$CH(OH)–CH_2Ph$	H	OCH_3	202	$C_{19}H_{17}N_3O_2$
26	Br	H	OCH_3	146	$C_{11}H_8N_3OBr$
27	CH_3	H	OCH_3	165	$C_{12}H_{11}N_3O$

ppm exhibited a correlation with only one proton at 7.64 ppm.

These results led us to assign with absolute certainty the deshielded peak at 8.38 ppm to H-9, which correlates with H-1 (8.81 ppm) and H-8 (7.75 ppm), and the shielded peak at 8.06 ppm to H-6 which correlates only with H-7 (7.64 ppm). Such study reveals that Cobb and Cheeseman had confused the NMR assignment of the protons H-6 and H-9, the proton H-9 being actually more deshielded than the proton H-6.

All synthesised imidazo[1,2-*a*]quinoxaline derivatives are described in *table II*. Their ^{1}H-NMR assignments are listed in the experimental part.

3. Biological results and discussion

The inhibitory activity of type III and/or IV isoenzymes of cyclic nucleotide phosphodiesterase was measured in vitro. The results are summarised in *table III*. All tested compounds exhibited relative potency as inhibitors of isoenzyme type IV, their activities being similar or higher to that of the selective inhibitor Ro 20-1724 (*table III*). Most of them presented a very low PDE III inhibitory activity as compared to the reference compound SKF 94120 (*table III*). Only compound **15**, with the cyano group on position 2, showed moderate inhibitory activity against isoenzyme type III. **15** exhibited an unique profile since it appeared to be PDE III and IV inhibitor, without selectivity. These results could be compared to those previously obtained in a imidazo[1,2-*a*]pyrazine series [10]. It has been published that these compounds exhibited PDE IV activity higher than the PDE III activity with the exception of two compounds, with a cyano group on position 2,

which showed potent inhibitory activity against isoenzyme type III. It is interesting to note that the only compound exhibiting potent PDE III inhibitory activity exhibits a large structural analogy (cyano and aminomethyl groups on equivalent positions) with SCA40 which was shown, in the imidazo[1,2-*a*]pyrazine series, to be the more potent PDE III inhibitor [10]. We can also note that the presence, in the imidazoquinoxaline series, of a supplementary phenyl on face e of the parent imidazo[1,2-*a*]pyrazinic heterocycle induced a dramatic loss of inhibitory activity against PDE III and a one to half a log decrease against PDE IV in comparison with the imidazopyrazine series.

Inhibitory activity against isoenzyme type IV depends strongly on the nature of the substitution on position 1. The methyl, hydroxypropyl or bromine groups on position 1 favoured potent inhibitory activity against this isoenzyme. Compound **26**, with a bromine substituent, appears to be the most potent PDE IV selective inhibitor.

The relaxant activity of guinea-pig isolated trachea was measured for two characteristic compounds: **15**, unselective PDE III and IV inhibitor and structural analogue to SCA40 in the imidazo[1,2-*a*]pyrazine series, and **26**, the more potent PDE IV inhibitor of the series (*table IV*). These two compounds exhibited a higher or quite similar activity compared to milrinone, a selective PDE III inhibitor. The most potent derivative was shown to be compound **15** with a pD_2 of 6.02 ± 0.10, similar to the value measured in the same conditions for SCA40. So, like in the imidazo[1,2-*a*]pyrazine series [10], the potent relaxant activity of these compounds is related in a complex way to its simultaneous inhibition of PDE isoenzymes III and IV.

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

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
5	<3.0	4.89 ± 0.12
7	<3.0	4.79 ± 0.21
11	<3.0	4.83 ± 0.21
15	5.22 ± 0.07	5.08 ± 0.23
20	<3.0	5.50 ± 0.05
22	<3.0	4.70 ± 0.15
23	<3.0	4.88 ± 0.18
24	<3.0	5.45 ± 0.15
25	<3.0	4.65 ± 0.05
26	4.29 ± 0.09	6.19 ± 0.09

[a] Data indicate mean ($\pm$S.E. mean) values of $-\log IC_{50}$ for each compound.

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

Compound	*n*	$-\log EC_{50}$
Milrinone	6	5.29 ± 0.15
Ro 20-1724	6	3.50 ± 0.23
SCA 40	10	6.06 ± 0.14
15	10	6.02 ± 0.10
26	10	4.60 ± 0.03

[a] Data are mean ($\pm$S.E. mean) values ($n = 6$ or 10) of pD_2 ($-\log EC_{50}$).

4. Experimental

4.1. Chemistry

All melting points were determined using a Köfler hot plate melting point apparatus and are uncorrected. 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 or 200 spectrometer. Elemental analysis were performed by the Microanalytical Center (Montpellier, France). Analyses indicated by the symbols of the elements or functions were within $\pm 0.4\%$ of the theorical values.

4.1.1. 2-Amino-3-chloroquinoxaline (2)

A mixture of 2,3-dichloroquinoxaline **1** (9.2 g, 46.2 mmol) in 150 mL of ammonia solution in DMF was stirred at 0°C for 1 h. The mixture was filtered and concentrated in vacuo. The residue was chromatographed on a silica gel column (eluent: methanol–dichloromethane: 5/95) to give **2** (7.5 g, 90%); m.p. 139°C; ^{1}H-NMR ($CDC1_3$) δ (100 MHz): 7.61 (m, 4H, $H_5+H_6+H_7+H_8$), 5.51 (br s, 2H, NH_2). Anal. ($C_8N_3H_6Cl$) C, H, N.

4.1.2. 4-Chloroimidazo[1,2-a]quinoxaline (3)

A mixture of bromoacetaldehyde diethyl acetal (18.7 g, 95 mmol), concentrated hydrobromic acid (2.8 mL), and water (11.3 mL) was heated under reflux for 1 h. The mixture was then cooled, diethylether was added, and the layers were separated. The organic phase was dried and poured into a solution of **2** (7 g, 39 mmol) in

DME (7.5 mL). The reaction mixture was stirred at room temperature for 2 days and the precipitate was separated by filtration. A mixture of this precipitate in dry ethanol (75 mL) was heated under reflux for 2 h. The solvent was removed in vacuo and the residue was purified by chromatography on silica (eluent: methanol–dichloromethane: 5/95) to give **3** (3.89 g, 49%); ^{1}H-NMR (DMSO-d_6) δ (200 MHz): 9.05 (d, 1H, H_1), 8.51 (dd, 1H, H_1), 8.1 (dd, 1H, H_6), 7.99 (d, 1H, H_2), 7.89 (td, 1H, H_8), 7.76 (td, 1H, H_7). Anal. ($C_{10}N_3H_6Cl$) C, H, N.

*4.1.3. 4-Aminoimidazo[1,2-a]quinoxaline (**4**)*

A mixture of **3** (2.03 g, 100 mmol) in 30% ammonia solution (20 mL) was heated at 100°C for 4 h in a 125 mL autoclave. The mixture was then cooled at room temperature and extracted with dichloromethane. The organic phase was dried over anhydrous sodium sulfate. After evaporation in vacuo, the residue was chromatographed on silica gel (eluent: dichloromethane–methanol: 90/10) to give **4** (0.47 g, 80%); ^{1}H-NMR (DMSO-d_6) δ (200 MHz): 8.71 (d, 1H, H_1,), 8.20 (dd, 1H, H_9), 7.73 (d, 1H, H_2), 7.65 (dd, 1H, H_6), 7.5 (td, 1H, H8), 7.39 (td, 1H, H_7), 7.3 (br s, 2H, NH_2). Anal. ($C_{10}N_4H_8$) C, H, N.

*4.1.4. 4-(Methylamino)imidazo[1,2-a]quinoxaline (**5**)*

A 40% aqueous methylamine solution (2.55 mL, 29.7 mmol) was added to a solution of **3** (1 g, 4.9 mmol) in absolute ethanol (25 mL). The resulting solution was stirred at room temperature for 24 h. Ethanol was removed in vacuo. The residue was redissolved in dichloromethane (80 mL), washed with 5% sodium hydrogen carbonate (50 mL) and water (50 mL), dried and concentrated to give the crude product. Purification by column chromatography (eluent: ethyl acetate–hexane: 30/70) gave the compound **5** (0.81 g, 83%); ^{1}H-NMR (DMSO-d_6) δ (200 MHz): 8.68 (d, 1H, H_1), 8.18 (dd, 1H, H_9), 7.7 (dd, 1H, H_6), 7.7 (d, 1H, H_2), 7.5 (td, 1H, H8), 7.41 (td, 1H, H_7), 7.63 (br q, 1H, NH), 3.13 (d, 3H, CH_3). Anal. ($C_{11}N_4H_{10}$) C, H, N.

*4.1.5. 4-(Ethylamino)imidazo[1,2-a]quinoxaline (**6**)*

It was prepared in an analogous manner of **5** from 4-chloroimidazo[1,2-*a*]quinoxaline **3** and a 70% aqueous ethylamine solution. Chromatography (eluent: methanol–dichloromethane: 10/90) yielded the compound **6** (0.93 g, 89%); ^{1}H-NMR (DMSO-d_6) δ (200 MHz): 8.67 (d, 1H, H_1), 8.17 (dd, 1H, H_9), 7.7 (d, 1H, H_2), 7.67 (dd, 1H, H_6), 7.48 (td, 1H, H_8), 7.36 (td, 1H, H_7), 7.78 (br t, 1H, NH), 3.69 (m, 2H, CH_2), 1.34 (t, 3H, CH_3). Anal. ($C_{12}N_4H_{12}$) C, H, N.

*4.1.6. 4-Methoxyimidazo[1,2-a]quinoxaline (**7**)*

A methanolic solution of sodium methylate was prepared from sodium (0.23 g, 10 mmol) in dry methanol (70 mL). **3** (1 g, 4.9 mmol) was added and the resulting solution was heated under reflux for 2 h and stirred overnight at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residue was redissolved in dichloromethane (150 mL), washed with sodium chlorure (100 mL), water (100 mL), dried and concentrated to give the crude product. Purification by column chromatography (eluent: dichloromethane–methanol: 98/2) gave the compound **7** (0.93 g, 95%); ^{1}H-NMR ($CDC1_3$) δ (200 MHz): 8.77 (d, 1H, H_1), 8.27 (dd, 1H, H_9), 7.81 (dd, 1H, H_6), 7.74 (d, 1H, H_2), 7.56 (m, 2H, H_7+H_8), 4.15 (s, 3H, OCH_3). Anal. ($C_{11}N_3H_9O$) C, H, N.

*4.1.7. Imidazo[1,2-a]quinoxaline (**8**)*

A mixture of **3** (1 g, 4.9 mmol) solubilised in anhydrous methanol was hydrogenated in presence of 10% Pd/C at air-pressure. The mixture was filtrated on celite. The filtrate was evaporated, then the residue was solubilised in minimum methanol and precipited by ether addition. The precipitate was filtrated, solubilised in 15% sodium hydroxide solution and extracted by dichloromethane. The organic phase was dried over calcium chloride and evaporated to give **8** (0.81 g, 98%); ^{1}H-NMR ($CDCl_3$) δ (200 MHz): 9.11 (s, 1H, H_4), 8.84 (d, 1H, H_1), 8.40 (dd, 1H, H_9), 8.06 (dd, 1H, H_6), 7.86 (d, 1H, H_2), 7.76 (ddd, 1H, H_8), 7.64 (ddd, 1H, H_7). Anal. ($C_{10}N_3H_7$) C, H, N.

*4.1.8. 2-Amino-3-(methylamino)quinoxaline (**9**)*

A mixture of **2** (10.4 g, 58 mmol) in a 40% aqueous methylamine solution (36 mL, 419 mmol) was stirred at room temperature overnight. Extraction with dichloromethane and chromatography on a silica gel column (eluent: methanol–methylene chloride: 5/95) provided the desired compound **9** (5.16 g, 51%); ^{1}H-NMR (DMSO) δ (100MHz): 7.45 (m, 4H, $H_5+H_6+H_7+H_8$), 6.95 (m, 1H, NH), 6.64 (s, 2H, NH_2), 3.00 (d, 3H, CH_3). Anal. ($C_9N_4H_{10}$) C, H, N.

*4.1.9. 2-Amino-3-(ethylamino)quinoxaline (**10**)*

It was prepared in an analogous manner of **9** from **2** and a 70% aqueous ethylamine solution. Chromatography (eluent: methanol–methylene chloride: 10/90) pro-

vided the desired compound **10** (6.86 g, 63%); ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 7.47 (m, 4H, $H_5+H_6+H_7+H_8$), 4.59 (br m, 3H, $NH+NH_2$), 3.57 (m, 2H, CH_2), 1.31 (t, 3H, CH_3). Anal. ($C_{10}N_4H_{12}$) C, H, N.

*4.1.10. Ethyl-4-(methylamino)imidazo[1,2-a]-quinoxaline-2-carboxylate (**11**)*

2-Amino-3-(methylamino)quinoxaline (**9**) (5 g, 28.7 mmol) was dissolved in DME (30 mL) and DMF (20 mL). A solution of ethyl-3-bromopyruvate (3.64 mL, 28.7 mmol) in DME (6 mL) was added dropwise and the resulting solution was heated at 70°C for 2 h. The precipitate obtained was separated by filtration, dissolved in dry ethanol and heated under reflux for 1 h. The filtrate was evaporated to dryness in vacuo, the residue was dissolved in dry ethanol and heated under reflux for 1 h. Upon cooling, the two ethanolic solutions gave a precipitate. After filtration and chromatography on a silica gel column (eluent: methanol–dichloromethane: 2/98) yielded **11** (2.72 g, 35%); ^{1}H-NMR (DMSO-d_6) δ (200 MHz): 9.29 (s, 1H, H_1), 8.27 (dd, 1H, H_9), 8.07 (br q, 1H, NH), 7.61 (dd, 1H, H_6), 7.44 (td, 1H, H_8), 7.28 (td, 1H, H_7), 4.37 (q, 2H, CH_2), 3.01 (d, 3H, CH_3), 1.35 (t, 3H, CH_3). Anal. ($C_{14}N_4H_{14}O_2$) C, H, N.

*4.1.11. Ethyl-4-(ethylamino)imidazo[1,2-a]-quinoxaline-2-carboxyIate (**12**)*

It was prepared in an analogous manner of **11** from 2-amino-3-(ethylamino)quinoxaline (**10**). Chromatography (eluent: methanol–dichloromethane: 2/98) yielded **12** (2.77 g, 34%); ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 8.52 (s, 1H, H_1), 7.66 (m, 2H, H_9+H_6), 7.42 (m, 2H, H_8+H_7), 6.29 (br t, 1H, NH), 4.47 (q, 2H, CH_2), 3.73 (q, 2H, CH_2), 1.43 (t, 3H, CH_3), 1.33 (t, 3H, CH_3). Anal. ($C_{15}N_4H_{16}O_2$) C, H, N.

*4.1.12. 4-(Methylamino)imidazo[1,2-a]-quinoxaline-2-carboxamide (**13**)*

A mixture of **11** (1 g, 3.7 mmol) in ammonia solution in methanol (500 mL) and sodium cyanide (0.1 g, 2 mmol) was stirred at 0°C for 16 h. The resulting solution was filtered and concentrated in vacuo. The residue was purified by recrystallisation from methanol to give **13** (0.47 g, 53%). Anal. ($C_{12}N_5H_{11}O$) C, H, N.

*4.1.13. 4-(Ethylamino)imidazo[1,2-a]quinoxaline-2-carboxamide (**14**)*

It was prepared in an analogous manner of **13** from ethyl-4-(ethylamino)imidazo[1,2-*a*]quinoxaline-2-carboxylate (**12**). Recrystallisation from methanol afforded **14** (0.52 g, 56%). Anal. ($C_{13}N_5H_{13}O$) C, H, N.

*4.1.14. 4-(Methylamino)imidazo[1,2-a]quinoxaline-2-carbonitrile (**15**)*

A mixture of **13** (0.7 g, 2.9 mmol) in phosphorus oxychloride (25 mL) was heated under reflux for 1 h 30. After distillation of phosphorus oxychloride (23 mL), the residue was poured carefully onto ice. After the destruction of excess phosphorus oxychloride, the mixture was alkalinised with sodium carbonate, and the nitrile was extracted with dichloromethane. The solvent was removed in vacuo and the residue purified by chromatography on a silica gel column (eluent: dichloromethane–methanol: 95/5) to give **15** (0.56 g, 87%); ^{1}H-NMR ($CDCl_3$) δ (200 MHz): 9.51 (s, 1H, H_1), 8.13 (br q, 1H, NH), 8.10 (dd, 1H, H_9), 7.63 (dd, 1H, H_6), 7.48 (td, 1H, H_8), 7.34 (td, 1H, H_7), 3.02 (d, 3H, CH_3). Anal. ($C_{12}N_5H_9$) C, H, N.

*4.1.15. 4-(Ethylamino)imidazo[1,2-a]quinoxaline-2-carbonitrile (**16**)*

It was prepared in an analogous manner of **15** from 4-(ethylamino)imidazo[1,2-*a*]quinoxaline-2-carboxamide (**14**). Chromatography (eluent: dichloromethane–methanol: 95/5) yielded to **16** (0.58 g, 85%); ^{1}H-NMR ($CDCl_3$) δ (200 MHz): 9.51 (s, 1H, H_1), 8.13 (br t, 1H, NH), 8.11 (dd, 1H, H_9), 7.60 (dd, 1H, H_6), 7.47 (td, 1H, H_8), 7.33 (td, 1H, H_7), 3.55 (q, 2H, CH_2), 1.24 (t, 3H, CH_3). Anal. ($C_{13}N_5H_{11}$) C, H, N.

*4.1.16. 2-(2-hydroxypropylamino)-3-chloroquinoxaline (**17**)*

1-Aminopropan-2-ol (11.6 mL, 150.75 mmol) was added to a solution of 2,3-dichloroquinoxaline (**1**) (12 g, 60.3 mmol) in dioxane (60 mL). The resulting solution was heated under reflux for 7 h. Dioxane was removed in vacuo. The residue was redissolved in dichloromethane (100 mL), washed with 10% sodium carbonate (60 mL), water (60 mL), dried and concentrated to give the product **17** (9 g, 63%); m.p. 104°C; ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 7.53 (m, 4H, $H_5+H_6+H_7+H_8$), 5.99 (br t, 1H, NH), 4.17 (br d, 1H, OH), 3.73 (m, 1H, CH), 3.48 (dd, 2H, CH_2), 1.28 (d, 3H, CH_3). Anal. ($C_{11}N_3H_{12}OCl$) C, H, N.

*4.1.17. 2-(2-Oxopropylamino)-3-chloroquinoxaline (**18**)*

Sulfur trioxyde trimethylamine complex (25 g, 180 mmol) was added slowly to a solution of **17** (9 g, 37.9 mmol) in triethylamine (60 mL) and DMSO (60 mL).

The resulting solution was stirred for 16 h at room temperature. The mixture was then poured carefully onto ice and extracted with dichloromethane. The solvent was removed in vacuo and the resulting brown oil purified by chromatography on a silica gel column (eluent: dichloromethane–ether: 50/50) to give **18** (6.4 g, 72%); m.p. 154°C; ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 7.58 (m, 4H, $H_5+H_6+H_7+H_8$), 6.42 (br t, 1H, NH), 4.46 (d, 2H, CH_2), 2.33 (s, 3H, CH_3). Anal. ($C_{11}N_3H_{10}OC1$) C, H, N.

*4.1.18. 4-Chloro-1-methylimidazo[1,2-a]quinoxaline (**19**)*

18 (6.35 g, 27 mmol) were dissolved in trifluoroacetic acid (19 mL) under nitrogen. Then, trifluoroacetic anhydride (10 mL) were added slowly. The resulting solution was stirred for 5 h at room temperature under nitrogen. After evaporation in vacuo, the residue was redissolved in dichloromethane (50 mL), washed with 10% sodium hydrogen carbonate (30 mL), water (30 mL), dried and concentrated. Chromatography on a silica gel column (eluent: dichloromethane–ether: 80/20) provided the desired compound **19** (2.35 g, 40%); ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 8.14 (dd, 1H, H_9), 7.97 (dd, 1H, H_6), 7.73 (td, 1H, H_8), 7.65 (td, 1H, H_7), 7.64 (s, 1H, H_2), 2.94 (s, 3H, CH_3). Anal. ($C_{11}N_3H_8Cl$) C, H, N.

*4.1.19. 1-Methyl-4-(methylamino)imidazo[1,2-a]-quinoxaline (**20**)*

A mixture of **19** (1 g, 4.6 mmol) in 40% aqueous methylamine solution (2.37 mL, 27.6 mmol) was stirred at room temperature overnight. Extraction with dichloromethane and chromatography on a silica gel column (eluent dichloromethane–ether: 50/50) provided the desired compound **20** (0.8 g, 82%); ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 8.16 (dd, 1H, H_9), 7.70 (br q, 1H, NH), 7.62 (dd, 1H, H_6), 7.39 (td, 1H, H_8), 7.33 (s, 1H, H_2), 7.25 (td, 1H, H_7), 3.02 (d, 3H, CH_3), 2.84 (s, 3H, CH_3). Anal. ($C_{12}N_4H_{12}$) C, H, N.

*4.1.20. 4-(Ethylamino)-1-methylimidazo[1,2-a]-quinoxaIine (**21**)*

It was prepared in an analogous manner of **20** from 4-chloro-1-methylimidazo[1,2-*a*]quinoxaline **19** and a 70% aqueous ethylamine solution. Chromatography (eluent: ether–dichloromethane: 20/80) provided the desired compound **21** (0.71 g, 68%); ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 8.15 (dd, 1H, H_9), 7.60 (dd, 1H, H_6), 7.54 (br t, 1H, NH), 7.39 (td, 1H, H_8), 7.33 (s, 1H, H_2), 7.25 (td, 1H, H_7), 3.57 (m, 2H, CH_2), 2.85 (s, 3H, CH_3), 1.20 (d, 3H, CH_3). Anal. ($C_{13}N_4H_{14}$) C, H, N.

*4.1.21. 1-(Hydroxymethyl)-4-methoxyimidazo[1,2-a]-quinoxaline (**22**)*

A mixture of **7** (2 g, 10 mmol), sodium acetate (32.6 g, 397 mmol), acetic acid (25.2 mL, 441 mmol), and 37% solution of formaldehyde (225.8 mL, 8130 mmol) in water was heated in an autoclave at 120°C for 2.5 h. On cooling, water (120 mL) was added to the reaction mixture. After alkalinisation with sodium carbonate and extraction with dichloromethane, the organic phase was dried and evaporated. The residue was chromatographed on a silica gel column (eluent: methanol–dichloromethane: 4/96) to give the unreacted product **7** (1.05 g). Further chromatography (eluent: methanol–dichloromethane: 10/90) afforded **22** (0.115 g, 5%); ^{1}H-NMR (DMSO-d_6) δ (100 MHz): 8.42 (m, 1H, H_9), 7.80 (m, 1H, H_6), 7.61 (s, 1H, H_2), 7.54 (m, 2H, H_7+H_8), 5.68 (t, 1H, OH), 5.01 (d, 2H, CH_2), 4.14 (s, 3H, O-CH_3). Anal. ($C_{12}N_3H_{11}O_2$) C, H, N.

*4.1.22. 1-Formyl-4-methoxyimidazo[1,2-a]quinoxaline (**23**)*

n-BuLi (2.5 mL, 4 mmol) was added dropwise to a solution of **7** (0.4 g, 2 mmol) in dry THF (15 mL) under nitrogen at −75°C. The resulting solution was stirred for 1 h at −75°C. Freshly distilled DMF (1.55 mL, 20 mmol) was then added and the resulting solution was stirred at −75°C for further 15 min before hydrolysis (−75°C) by water. The precipitate obtained was separated by filtration, washed with water and ether. The crude product was chromatographed on a silica gel column (eluent: methanol–methylene chloride: 2/98) to give **23** (0.27 g, 60%); ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 9.98 (s, 1H, CHO), 9.39 (m, 1H, H_9), 8.38 (s, 1H, H_2), 7.87 (m, 1H, H_6), 7.58 (m, 2H, H_7+H_8), 4.26 (s, 3H, OCH_3). Anal. ($C_{12}N_3H_9O_2$) C, H, N.

*4.1.23. 1-(1-Hydroxypropyl)-4-methoxyimidazo[1,2-a]-quinoxaline (**24**)*

n-BuLi (5 mL, 8 mmol) was added dropwise to a solution of **7** (0.8 g, 4 mmol) or **26** (1.11 g, 4 mmol) in dry tetrahydrofuran (16 mL) under nitrogen at −75°C. The resulting solution was stirred for 1 h at −75°C. Propanal (2.88 mL, 40 mmol) was then added and the resulting solution was stirred at −75°C for further 1 h before hydrolysis (−75°C) by tetrahydrofuran–ethanol–35% HCl (1/1/1). The reaction mixture was then washed with saturated aqueous sodium hydrogen carbonate solution, extracted with dichloromethane, dried and the solvent was removed in vacuo. The residue was purified by chromatography on a silica gel column (eluent:

methanol–methylene chloride: 1/99) to give **24** (0.95 g, 92%); ^{1}H-NMR (DMSO-d_6) δ (100 MHz): 8.63 (m, 1H, H_9), 7.88 (m, 1H, H_6), 7.73 (s, 1H, H_2), 7.61 (m, 2H, H_7+H_8), 5.78 (d, 1H, OH), 5.16 (m, 1H, CH), 4.20 (s, 3H, OCH_3), 2.09 (m, 2H, CH_2), 1.10 (t, 3H, CH_3). Anal. ($C_{14}N_3H_{15}O_2$) C, H, N.

*4.1.24. 1-(1-hydroxy-2-phenylethyl)-4-methoxyimidazo-[1,2-a]quinoxaline (**25**)*

It was prepared in an analogous manner of **24** from **7** and phenylacetaldehyde. Chromatography (eluent: dichloromethane) yielded **25** (0.64 g, 50%); ^{1}H-NMR (DMSO-d_6) δ (100 MHz): 8.52 (m, 1H, H_9), 7.75 (m, 1H, H_6), 7.74 (s, 1H, H_2), 7.50 (m, 2H, H_7+H_8), 7.23 (m, 5H, H_{Ar}), 5.88 (d, 1H, OH), 5.42 (m, 2H, CH+CH), 4.09 (s, 3H, OCH_3). Anal. ($C_{19}N_3H_{17}O_2$) C, H, N.

*4.1.25. 1-Bromo-4-methoxyimidazo[1,2-a]quinoxaline (**26**)*

A solution of **7** (0.5 g, 2.5 mmol) and *N*-bromosuccinimide (0.44 g, 2.5 mmol) in $CHCl_3$ (25 mL) was heated under reflux for 2h. The resulting green solution was cooled, washed with 5% sodium hydrogen carbonate (15 mL), dried and concentrated. The residue was purified by recrystallisation from chloroform to give **26** (0.64 g, 91%); ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 9.04 (m, 1H, H_9), 7.81 (m, 1H, H_6), 7.75 (s, 1H, H_2), 7.50 (m, 2H, H_7+H_8), 4.20 (s, 3H, OCH_3). Anal. ($C_{11}N_3H_8OBr$) C, H, N.

*4.1.26. 4-Methoxy-1-methylimidazo[1,2-a]quinoxaline (**27**)*

n-BuLi (5 mL, 8 mmol) was added dropwise to a solution of **26** (1.11 g, 4 mmol) in dry tetrahydrofuran (16 mL) under nitrogen at -75°C. The resulting solution was stirred for 1 h at −75°C. Methyl iodide (2.49 mL, 40 mmol) was then added and the resulting solution was stirred at −75°C for further 1 h before hydrolysis (−75°C) by tetrahydrofuran–ethanol–35% HCl (1/1/1). The reaction mixture was then washed with saturated aqueous sodium hydrogenocarbonate solution, extracted with dichloromethane, dried and the solvent was removed in vacuo. The residue was purified by chromatography on a silica gel column (eluent: methanol–methylene chloride: 1/99) to give **27** (0.77 g, 91%); ^{1}H-NMR ($CDCl_3$) δ (100 MHz): 7.92 (m, 1H, H_9), 7.65 (m, 1H, H_6), 7.27 (m, 3H, $H_2+H_7+H_8$), 4.14 (s, 3H, OCH_3), 2.77 (s, 3H, CH_3). Anal. ($C_{12}N_3H_{11}O$) C, H, N.

4.2. Biochemistry and pharmacology

4.2.1. 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 [19]. The identities of the isoenzymes were confirmed by their sensitivities to SKF 94120 and Ro 20-1724, respective PDE III and PDE IV selective inhibitors (*table III*), 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 [20] as modified by Rutten et al. [21]. 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-mercapto–ethanol 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 non-linear regression curve fitting program.

The results presented in *table III* are means±S.E.M. of at least four experiments.

4.2.2. 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 [22]. 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 IV* are means±S.E.M. of six or ten determinations.

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