Synthesis, structural characterization and antioxidative properties of aminopyrazine and imidazolopyrazine derivatives

Ingrid Devillers,[a] Bertrand de Wergifosse,[b] Marie-Pierre Bruneau,[a] Bernard Tinant,[c] Jean-Paul Declercq,[c] Roland Touillaux,[c] Jean-François Rees[b] and Jacqueline Marchand-Brynaert*[a]

[a] *Laboratoire de Chimie Organique de Synthèse, Université catholique de Louvain, Bâtiment Lavoisier, Pl. L. Pasteur 1, B-1348 Louvain-la-Neuve, Belgium*
[b] *Laboratoire de Physiologie Animale, Université catholique de Louvain, Bâtiment Carnoy, Pl. Croix du Sud 4, B-1348 Louvain-la-Neuve, Belgium*
[c] *Laboratoire de Chimie Physique et Cristallographie, Université catholique de Louvain, Bâtiment Lavoisier, Pl. L. Pasteur 1, B-1348 Louvain-la-Neuve, Belgium*

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The reaction of 2-aminopyrazine **1** with the glyoxal derivatives **2a**–**c** gave the bicyclic imidazolopyrazinones **3a**,**b** and the monocyclic N-substituted aminopyrazines **4b** and **5c**. Compounds **3** feature unique crystallographic characteristics. Their reactivity towards superoxide anion is about five times the reactivity of Trolox®, while the monocyclic pyrazines are inactive.

Introduction

Coelenterazine (CLZ) is an imidazolopyrazinone derivative isolated from various marine organisms.[1] This active compound functions as the luminescent substrate of enzymes (luciferases); the catalyzed oxidation of coelenterazine with oxygen produces carbon dioxide and coelenteramide (CLD) in an excited state which deactivates by emission of light (Fig. 1).[2]

Recently we demonstrated that coelenterazine could also exercise another function: this molecule showed high antioxidative properties in cells submitted to oxidative stress induced with *tert*-butyl hydroperoxide. Moreover, the ability of coelenterazine to inhibit the oxidation of linoleate induced by azoperoxyl radicals was also pointed out.[3] It is proposed that coelenterazine evolved from this antioxidant function to its present use as a luciferin (luciferase substrate) when marine animals migrated into deeper waters where both oxidative stress and solar irradiance are reduced, thus allowing the chemiluminescent properties of coelenterazine to serve in ecological interactions.[4]

These results[1,4] also suggest that imidazolopyrazinone (IMPZ) derivatives could be considered as new potential leads in medicinal chemistry for the treatment of diseases related to the production of reactive oxygen species (ROS)[5] in excessive concentrations, or in the wrong locations.[6] However, coelenterazine is not readily accessible from natural sources, and its total synthesis,[7] although recently optimized,[8] involves 14 steps. Therefore the question arose of the possibility of using simple, easily accessible imidazolopyrazinone representatives to evaluate the therapeutic potential of this family.

In this paper, we describe the one-step reaction of 2-aminopyrazine **1** with glyoxal derivatives **2**. The resulting condensation products, the bicyclic **3** and monocyclic compounds **4** and **5**, were fully characterized by X-ray diffraction and spectroscopic analyses. Their reactivity towards superoxide anion has been examined.

Results and discussion

Synthesis

The reaction of 2-aminopyrazine **1** with pyruvaldehyde **2a**[9] and phenylglyoxal **2b**[10,11] has been previously investigated; the authors reported the formation of 2-methyl- and 2-phenyl-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-ones **3a** and **3b** respectively. These structures were assigned mainly on the basis of UV spectra analyses.[9] Later, detailed mass spectrometric studies were also performed.[12]

By mixing **1** and **2a** in aqueous ethanol and HCl at room temperature, we recovered orange crystals of **3a** in good yield. A similar reaction conducted with **2b** gave red crystals of **3b** in modest yield, while glyoxal itself did not lead to the formation of the bicyclic product **3c**[13] (Scheme 1, Table 1). Aiming to increase the yields of **3b** and **3c**, we heated the reaction mixtures, but without any success. However, chromatography on silica gel of those crude mixtures allowed us to isolate the less

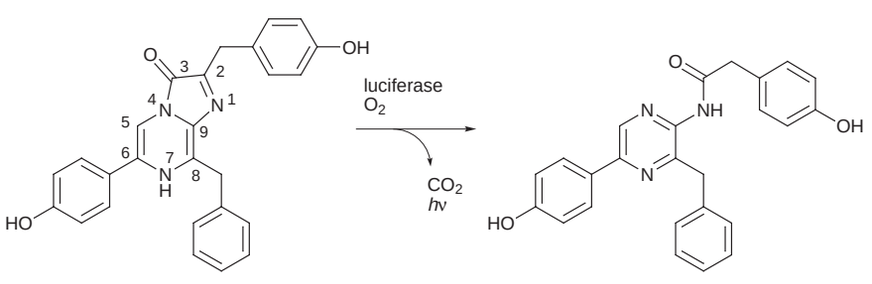


Fig. 1 Coelenterazine (CLZ) and coelenteramide (CLD).

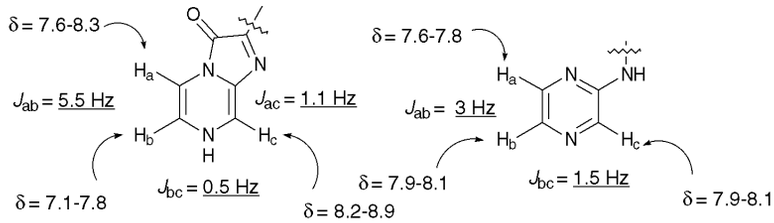
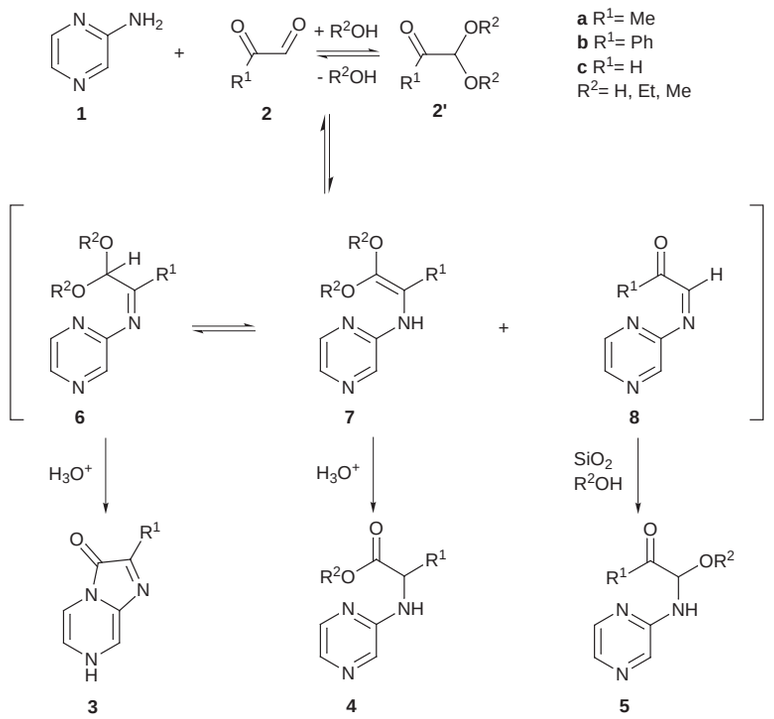


Fig. 2 ^{1}H NMR characteristics.



Scheme 1 Synthesis of aminopyrazine derivatives.

Table 1 Yields of isolated products

			Yield (%)		
3–5	R^1	R^2	**3**	**4**	**5**
a	Me	—	75[a]	—	—
b	Ph	Me	20[b]	—	25[c]
c	H	Et	—	5[d]	—

[a] Crystallized as hydrochloride. [b] Crystallized as hydrate. [c] Isolated by column chromatography on silica-gel (MeOH–CH_2Cl_2, 4:96). [d] Isolated by column chromatography on silica-gel (MeOH–CH_2Cl_2, 2:98).

polar monocyclic side-products **5b** and **4c**,[13] in modest and low yields respectively (Scheme 1, Table 1), which furnished information concerning the reactivity of glyoxal derivatives towards 2-aminopyrazine.

Under our experimental conditions (EtOH, H_2O, HCl), the glyoxals **2** should be present mainly as hydrates, hemiacetals or acetals **2′**. Therefore, the alkyl ketone function of **2a** (R^1 = Me) could be more electrophilic than the masked aldehyde function; this led to the formation of the intermediate Schiff base **6a** (most probably in equilibrium with the corresponding amino alcohol) which cyclized into the imidazolopyrazinone **3a**. In the case of **2b** (R^1 = Ph), the aryl ketone function appeared less reactive, and both intermediates **6b** and **8b** (most probably in equilibrium with the corresponding alcohols, and with the starting materials) could be formed, resulting from the nucleophilic addition of the amine function of **1** onto the ketone carbonyl and the masked aldehyde, respectively. This last intermediate was accidentally stabilized by addition of methanol, under the chromatographic conditions, to form compound **5b**. On the other hand, the intermediates (**6c** or **8c**, since R^1 = H) formed with **1** and the dialdehyde **2c**, which did not cyclize, could generate the ethyl 2-aminoacetate derivative **4c** *via* the enamine tautomer **7c** (Scheme 1).

All the isolated compounds were characterized in solution by the usual techniques (NMR and UV fluorescence, see Experimental). The typical ^{1}H NMR features of imidazolopyrazine derivatives (bicyclic compounds **3a**, **3b**) *versus* aminopyrazine derivatives (monocyclic compounds **4c**, **5b**) are the coupling constant values of the pyrazinyl protons: we found J_{ab} = 5.5 and 3.0 Hz, and J_{bc} = 0.5 Hz and 1.5 Hz, respectively. The order of deshielding of the aromatic protons was H_b (δ 7.1–7.8), H_a (δ 7.6–8.3) and H_c (δ 8.2–8.9) for the bicyclic structures, and H_a (δ 7.6–7.8), H_b + H_c (δ 7.9–8.1) for the monocyclic structures (Fig. 2). The ^{13}C NMR spectra of the fully aromatic bicyclic structures **3a**,**b** were characterized by an important shielding of all the pyrazinyl carbon atoms as compared to the monocyclic pyrazines (Fig. 3). Moreover, the C-3 carbonyl carbon atom appeared at δ 143–150;[14] this important shielding could result from a significant contribution of the enol tautomeric form **3′** in solution (Scheme 2). The naturally occurring preluciferins are in fact enolic derivatives of CLZ stabilized as the sulfate salt[15] or the 1-β-D-glucopyranosyl uronic salt.[16] Interestingly, in the crystal structures, the 2-methyl derivative **3a** is stabilized in its enol form **3′a**, while the 2-phenyl compound **3b** appears in the ketone form.

X-Ray diffraction analyses

Single crystal X-ray diffraction was used to determine the molecular conformation of **3a**, **3b** and **5b** in the solid state. As

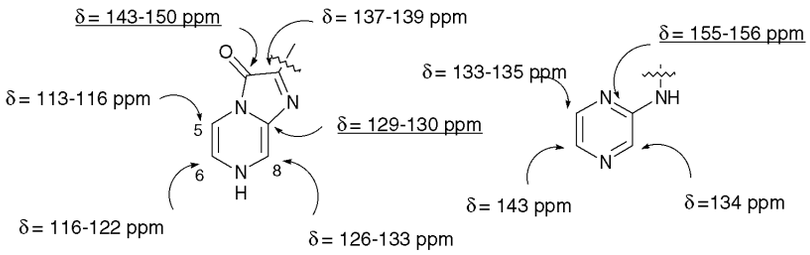
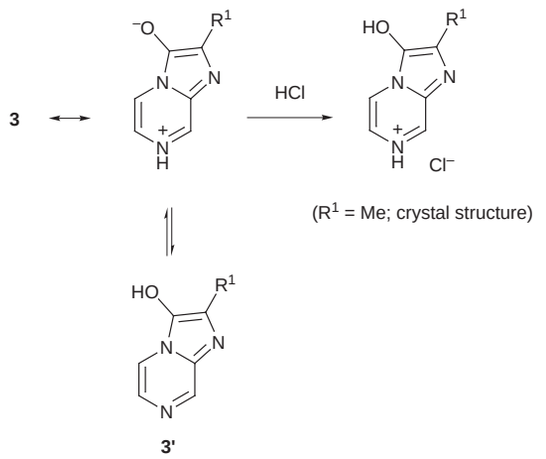


Fig. 3 ^{13}C NMR characteristics.



Scheme 2 Enolization of imidazolopyrazines **3**.

shown on the views of the molecules (Fig. 4),[17] **3a** and **3b** are bicyclic imidazolopyrazines while **5b** is a monocyclic aminopyrazine.

The structures of **3a** and **3b** merit some comments as, to the best of our knowledge, there are no other imidazolopyrazines for which the X-ray structure has been discussed.[18] The 2-methyl derivative **3a** was stabilized in its enol form by addition of HCl and a water molecule was co-crystallized. The 2-phenyl derivative was stabilized in the ketone form by hydrogen bonding with two water molecules.

Excepting the hydrogens of the methyl group and the Cl^- anion, all the atoms of molecule **3a** are in the same plane. The largest deviation from the best mean plane through all the bicycle atoms is 0.040 Å for C11. Even the water oxygen (O11) lies in this plane (its deviation from the plane is 0.044 Å). Molecule **3b** is also essentially planar. The maximum deviation from the imidazolopyrazine plane is 0.020 Å for N7 and the dihedral angle between this plane and the phenyl ring is only 2°. In this case, the two co-crystallized water molecules are clearly out of the bicyclic plane.

The bond lengths in the bicyclic skeleton of **3a** and **3b** are compared in Table 2. The values clearly indicate that N4 does not participate to the conjugated system which extends from N–H to C=O through C8, C9, N1, C2 and C3. Indeed, for both molecules, in this part of the skeleton, the same lengths for single and double bonds are observed. From one molecule to the other, the distances are very similar, except in the carbonyl region. In the enol form **3a**, the C2–C3 and C3–N4 bonds are shorter and C=O significantly longer than for the carbonyl form **3b**. The geometry of the protonated carbonyl compares very well to that reported for the sulfuric acid *N*,*N*′-(*p*-phenylene)-dibenzamide complex (C=O 1.293 Å and 1.301 Å)[19] or caprylolactam hydrochloride (C=O 1.303 Å).[20] More interesting is the observation that this protonated carbonyl is strongly hydrogen-bonded to a water molecule. Indeed, only one such interaction is found in the Cambridge Structural Data Base;[18] it has been observed in the structure of an aminocarboxylate complex of titanium.[21] In that structure, the bond lengths of C=O (1.278 *vs*. 1.313 Å in **3a**) and C=OH$^+\cdots$O$_w$ (1.64 *vs*. 1.59 Å in **3a**) indicate a less conjugated system.

Table 2 Selected bond lengths for **3a** and **3b**

	Bond length/Å	
	3a	**3b**
N1–C2	1.342(4)	1.344(3)
N1–C9	1.342(4)	1.345(3)
C2–C3	1.407(4)	1.444(4)
C2–C11	1.488(4)	1.461(3)
C3–O10	1.313(4)	1.259(3)
C3–N4	1.340(4)	1.366(3)
N4–C5	1.388(4)	1.392(3)
N4–C9	1.397(4)	1.402(3)
C5–C6	1.328(5)	1.333(4)
C6–N7	1.369(4)	1.374(3)
N7–C8	1.336(4)	1.345(3)
C8–C9	1.365(5)	1.352(3)

Table 3 Hydrogen bonds in **3a** and **3b**

	D$\cdots$A/Å	H$\cdots$A/Å	D–H$\cdots$A (°)
Compound **3a**			
O13–H13A$\cdots$Cl12[a]	3.150(2)	2.29(1)	164(1)
O13–H13B$\cdots$N1[b]	2.804(4)	2.00(2)	166(1)
O10–H10$\cdots$O13[c]	2.490(4)	1.59(2)	164(1)
N7–H7$\cdots$Cl12[d]	3.003(2)	2.09(1)	169(1)
Compound **3b**			
N7–H7$\cdots$O18[e]	2.726(4)	1.77(2)	171(1)
O17–H17A$\cdots$N1[c]	2.836(4)	1.96(2)	155(1)
O17–H17B$\cdots$O10[f]	2.839(4)	2.01(2)	160(1)
O18–H18A$\cdots$O17[g]	2.857(4)	1.93(2)	168(1)
O18–H18B$\cdots$O10[c]	2.792(4)	1.91(2)	176(1)

[a] $1-x, -y, -1-z$. [b] $1+x, y, z$. [c] x, y, z. [d] $-x, -y, -z$. [e] $x, y, z-1$. [f] $1-x, 2-y, 1-z$. [g] $-x, 2-y, 1-z$.

The hydrogen bonds are listed in Table 3. For **3a**, the water molecule is involved in three bonds: besides being an acceptor for the protonated carbonyl, it acts also as donor to the Cl^- anion and the N–H of symmetry-related molecules. A strong N–H$\cdots$$Cl^-$ interaction[22] completes the network so that the planar molecules are stacked almost parallel to the *ac* plane (Fig. 5). For **3b**, each of the two water molecules participates in three H-bonds, one of which being a bridge between themselves. They assure the cohesion between the layers of planar imidazopyrazines along the *c* axis.

Reactivity towards superoxide anion

Recently, coelenterazine analogs were proposed as chemiluminescent probes for the detection of superoxide anion in biological systems;[23] all the tested compounds were characterized by the presence of a *p*-methoxyphenyl or *p*-hydroxyphenyl substituent in the 6-position (see Fig. 1).

Using the hypoxanthine–xanthine oxidase system as the source of superoxide anion,[24] we tested the reactivity of the 6-unsubstituted imidazolopyrazinones (IMPZ) **3** and the monocyclic aminopyrazine derivatives **4**–**5**, compared to the natural CLZ [2-(4-hydroxybenzyl)-6-(4-hydroxyphenyl)-8-

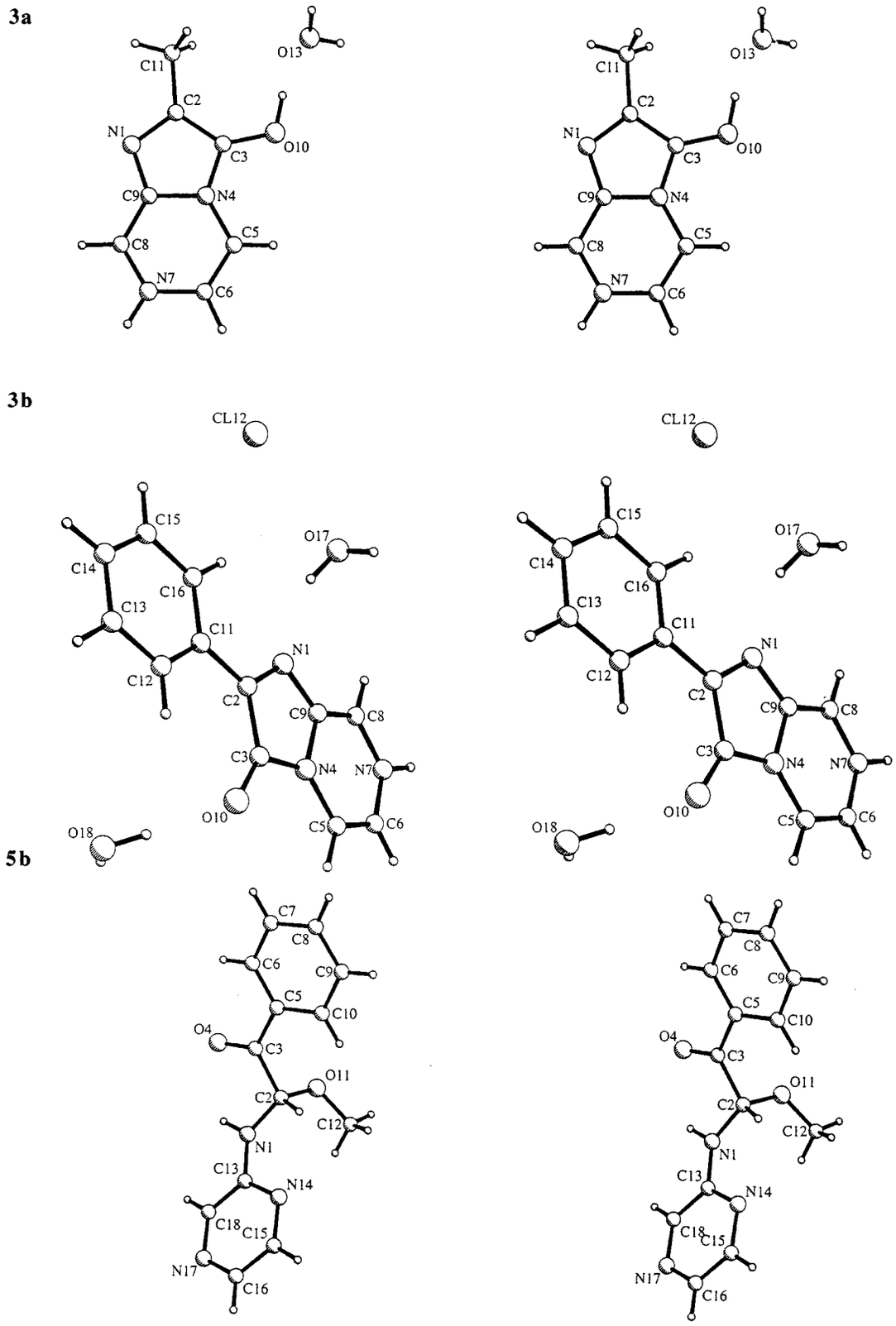


Fig. 4 Stereotopic view of **3a**, **3b** and **5b**.[17]

benzyl-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one, see Fig. 1], the corresponding 6-(4-methoxyphenyl) derivative (see Table 4) and Trolox®, a hydrosoluble derivative of vitamin E.[25]

The rate constants (k_1) of the reaction of CLZ and 6-(4-methoxyphenyl)-CLZ[26] with superoxide anion were determined. The method makes use of the light emission produced by the reaction. The diminution of light intensity is measured when the reaction is conducted in the presence of a competitor whose rate constant k_2 with superoxide anion is known. The diminution of light intensity can be directly correlated with the ratios of CLZ and the competing substance. Similarly, the reaction with superoxide anion in the presence of various amounts of Trolox® as competitor (known k_2 value of 1.7×10^4 M^{-1} s^{-1})[25] allowed the calculation of the rate constants of CLZ and 6-(4-methoxyphenyl)-CLZ; we found similar values of (10–12) $\times 10^4$ M^{-1} s^{-1}, showing that the natural imidazolopyrazine derivatives are about ten times more active than the reference (Table 4, entries 1 and 2).

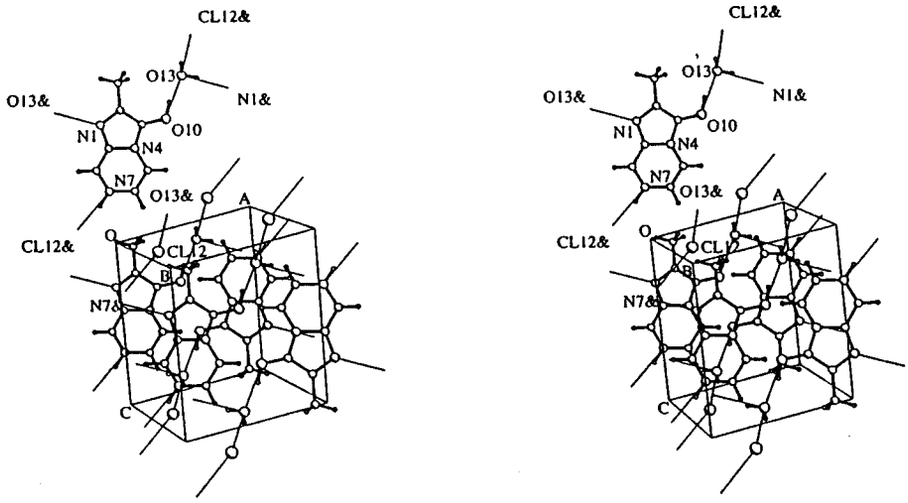
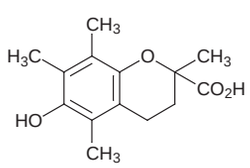


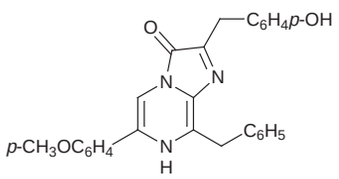
Fig. 5 Molecular stacking of **3a**.

Table 4 Reactivity towards superoxide anion

	Compound	$k/10^{-4}$ M^{-1} s^{-1}
1	CLZ	12 ± 0.1
2	6-(4-MeOC$_6$H$_4$)-CLZ	9.6 ± 0.1
3	**3a**	6.0 ± 0.1
4	**3b**	8.4 ± 0.6
5	**1**	≤0.5
6	**4c**	≤0.8
7	**5b**	≤1.7
8	Trolox®	1.7



Trolox®



6-(4-MeOC$_6$H$_4$)-CLZ

The evaluation of the poorly or non-luminescent substrates was realized in the presence of 6-(4-methoxyphenyl)-CLZ as luminescent competitor, after its rate constant had been determined following the above method. We found rate constant values of (6–8) × 10^4 M^{-1} s^{-1} for the bicyclic compounds **3** (Table 4, entries 3 and 4), and no measurable activity for the monocyclic compounds **1**, **4** and **5** (Table 4, entries 5, 6 and 7). Thus, the simple imidazolopyrazines **3** are about five times more active than the reference (Trolox®).

Conclusion

The condensation mechanism of 2-aminopyrazine **1** with simple glyoxal derivatives **2** has been clarified concerning the occurrence of side-reactions which diminish the yields of bicyclic imidazolopyrazinones **3**. These 6,8-unsubstituted analogs of coelenterazine (CLZ) have been fully characterized in solution and in the solid state; their crystallographic structures display unique features with particular hydrogen bonding networks.

Interestingly, compounds **3** are reactive against superoxide anion in the same way as the natural CLZ derivatives, while the corresponding monocyclic aminopyrazines **4**–**5** are not active at all. Thus, the imidazolopyrazine moiety is clearly responsible for the antioxidative properties and, in particular, an aromatic substituent at the 6-position appears not absolutely necessary. This observation opens the route towards the search for new leads in medicinal chemistry.

Experimental

Synthesis

The reagents (analytical grade) were purchased from Acros and Aldrich. The solvents were distilled before use. Thin-layer chromatography was carried out on silica gel 60 plates F254 (Merck, 0.2 mm thick); product visualization was effected with UV light. For flash chromatography we used Merck silica gel 60 of 230–400 mesh ASTM.

The NMR spectra were recorded on a Bruker AM-500 spectrometer; the attributions were established by selective decoupling experiments. The UV spectra were taken on a UV-VIS-NIR Varian Cary spectrophotometer. The fluorescence spectra were obtained with a SLM AMINCO instrument. The mass spectra were recorded on a Finnigan-MAT TSQ-70 instrument for the electronic impact (EI) measurements and on a Xenon ION TECH 8KV for the Fast Atom Bombardment (FAB) experiments. The IR spectra were obtained using a Nicolet 205 FT-IR instrument. Microanalyses were performed at the Christopher Ingold Laboratories, University College, London (UK).

2-Methyl-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one 3a

To a solution of 2-aminopyrazine **1** (1 g, 10.52 mmol) in ethanol (22 ml) and 37% aqueous HCl (3.1 ml) was added methylglyoxal **2a** (40 wt% solution in water, 2.84 g, 15.77 mmol, 1.5 equiv.). The mixture was stirred for 18 h at 20 °C. After addition of methanol, the solution was placed in the refrigerator (−20 °C) for 2 days; the product **3a** crystallized. Filtration of the first crop, concentration of the filtrate and crystallization from methanol (2 times) gave a total yield of 75% (1.45 g) of **3a** (orange crystals) as a hydrochloride salt (Found: C, 45.09; H, 4.48; N, 21.70. $C_7H_7N_3O$·HCl (185.45) requires C, 45.29; H, 4.31; N, 22.65%); R_f (SiO_2, acetone–MeOH 70:30) = 0.33; δ_H (500 MHz, CD_3OD) 2.56 (3 H, s), 7.81 (1 H, d, *J* 5.4, H-6), 8.31 (1 H, dd, *J* 5.4 and 1.0, H-5), 8.92 (1 H, d, *J* 1.0, H-8); δ_H (DMSO-d_6) 2.46 (3 H, s), 7.65 (1 H, d, *J* 5.4, H-6), 8.20 (1 H, d, *J* 5.4 Hz, H-5), 8.88 (1 H, s, H-8); δ_C (125 MHz, CD_3OD) 12.27, 116.13 (C-5), 122.0 (C-6), 130.29 (C-9), 133.0 (C-8), 136.41 (C-2), 143.36 (C-3); δ_C (DMSO-d_6) 12.89, 114.41 (C-5), 119.15 (C-6), 128.84 (C-9), 130.15 (C-8), 137.37 (C-2), 142.96 (C-3); *m*/*z* (FAB$^+$) 150 (100%), 149 (55), 122 (39), 81 (14); λ_{max}(MeOH, 10^{-5} M)/nm 258, 316, 418; Fluorescence $\lambda_{excitation}$ (MeOH, 10^{-5} M)/nm 418; $\lambda_{max\ emission}$ 522.

2-Phenyl-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one 3b

To a solution of 2-aminopyrazine **1** (1 g, 10.52 mmol) in ethanol (5 ml) and 37% aqueous HCl (1.5 ml) was added phenylglyoxal monohydrate **2b** (1.4 g, 9.21 mmol, 0.87 equiv.)

Table 5 Data collection and refinement parameters

	3a	3b	5b
Formula	$C_7H_7N_3O{\cdot}HCl{\cdot}H_2O$	$C_{12}H_9N_3O{\cdot}2H_2O$	$C_{13}H_{13}N_3O$
M_r	203.63	247.25	243.26
System	triclinic	triclinic	monoclinic
Space group	$P\bar{1}$	$P\bar{1}$	$P2_1/a$
a/Å	7.352(3)	6.832(2)	12.022(4)
b/Å	7.357(2)	9.076(6)	8.616(2)
c/Å	8.938(2)	9.679(3)	13.244(4)
α (°)	87.39(2)	87.86(4)	90
β (°)	100.03(2)	101.21(3)	114.22(3)
γ (°)	72.66(2)	88.92(4)	90
V/Å^3	452.0(2)	588.1(5)	1251.1(6)
D_x/g cm^{-3}	1.50	1.40	1.29
Z	2	2	4
λ/Å	0.71069	1.54178	0.71069
Cell parameters from reflections	22	15	30
2θ range	5–20	5–50	5–20
$F(000)$	212	260	512
μ/mm^{-1}	0.393	0.856	0.090
Crystal size/mm	0.04 × 0.16 × 0.56	0.04 × 0.15 × 0.20	0.12 × 0.20 × 0.20
$2\theta_{max}$ (°) for data collection	55	135	47
Range of hkl	$0 \le h \le 8$ $-8 \le k \le 9$ $-11 \le l \le 11$	$0 \le h \le 8$ $-10 \le k \le 10$ $-11 \le l \le 11$	$0 \le h \le 13$ $0 \le k \le 8$ $-14 \le l \le 14$
Standard reflections	−1 −1 −1	1 0 −3	−1 −1 2
No. of unique reflections	2095	2126	1858
No. of observed reflections [$I \ge 2\sigma(I)$]	1278	1529	749
U for H atoms/Å^2	0.090 (common)	0.060–0.12	0.117 (common)
No. of parameters	140	216	170
Extinction coefficient	—	0.014	—
R	0.057	0.055	0.068
ωR	0.120	0.143	0.105
x[a]	0.0641	0.1022	0.042
S	0.945	0.983	0.848
Δ/σ	0.001(1)	0.000(1)	0.002(1)
$\Delta\rho$ (max, min)/e Å^{-3}	0.29, −0.27	0.25, −0.26	0.14, −0.14

[a] $w = 1/[\sigma^2(F_o^2) + xP^2]$, where $P = (F_o^2 + 2F_c^2)/3$.

dissolved in ethanol (4 ml). The mixture was stirred for 3 h under argon atmosphere, then left for 15 h at 20 °C. The red precipitate was filtered off, washed with water (10 ml) and ethyl acetate (3 × 10 ml) to give 20% (420 mg) of **3b** as a hydrate (Found: C, 62.98; H, 4.09; N, 18.58. $C_{12}H_9N_3O{\cdot}H_2O$ (229) requires C, 62.88; H, 4.80; N, 18.34%); R_f (SiO_2, CH_2Cl_2–MeOH 90:10) = 0.48; δ_H (500 MHz, DMSO-d_6) 7.04 (1 H, d, J 5.6, H-6), 7.33 (1 H, t, J 7.5), 7.44 (2 H, dd, J 7.5 and 7.8), 7.63 (1 H, d, J 5.6, H-5), 8.25 (1 H, s, H-8), 8.43 (2 H, d, J 7.8); δ_C (125 MHz, DMSO-d_6) 112.49 (C-5), 115.48 (C-6), 125.74 (C-8), 126.16, 128.35, 128.45, 129.06 (C-9), 133.34, 139.40 (C-2), 149.97 (C-3); m/z (FAB^+) 212 (23%), 184 (85), 81 (100); λ_{max}(MeOH, 10^{-5} M)/nm 225, 283, 441.5; Fluorescence $\lambda_{excitation}$(MeOH, 10^{-5} M)/nm 441; $\lambda_{max\ emission}$ 523.

Ethyl 2-(pyrazin-2-ylamino)acetate 4c

A mixture of 2-aminopyrazine **1** (0.5 g, 5.26 mmol), glyoxal **2c** (40 wt% solution in water, 0.9 ml, 6.2 mmol, 1.18 equiv.) and 37% aqueous HCl (0.175 ml) in ethanol (80 ml) and water (7 ml) was heated (under argon atmosphere) at 80 °C during 3 h. Concentration under vacuum and flash chromatography on silica gel (CH_2Cl_2–MeOH 98:2) gave **4** as beige crystals (50 mg, 5% yield); R_f (SiO_2, acetone) = 0.74; ν_{max} (KBr)/cm^{-1} 1724; δ_H (500 MHz, CD_3OD) 1.24 (3 H, t, J 7.1), 4.09 (2 H, s, H-8), 4.16 (2 H, q, J 7.1), 7.68 (1 H, d, J 3.0, H-6), 7.94 (1 H, dd, J 3.0 and 1.5, H-5), 7.96 (1 H, d, J 1.5, H-3); δ_C (125 MHz, CD_3OD) 14.42, 43.47 (C-8), 62.11, 132.66 (C-6), 134.46 (C-3), 142.94 (C-5), 156.45 (C-2), 172.64 (C-9); m/z (EI) 181 (42%), 135 (49), 108 (100), 103 (27), 79 (44); λ_{max}(MeOH, 10^{-5} M)/nm 240, 324; Fluorescence $\lambda_{excitation}$(MeOH, 10^{-5} M)/nm 324; $\lambda_{max\ emission}$ 389.

2-Methoxy-2-(pyrazin-2-ylamino)acetophenone 5b

A mixture of 2-aminopyrazine **1** (1 g, 10.52 mmol), phenylglyoxal monohydrate **2b** (2.3 g, 15.13 mmol, 1.5 equiv.) and 37% aqueous HCl (0.35 ml) in ethanol (150 ml) and water (15 ml) was heated at 60 °C during 7 h. Concentration under vacuum and flash chromatography on silica gel (CH_2Cl_2–MeOH 96:4) gave impure **5** (1.48 g). A second chromatographic separation (CH_2Cl_2–MeOH 99.5:0.5) furnished 25% of pure **5** (550 mg); R_f (SiO_2, CH_2Cl_2–MeOH 95:5) = 0.60; δ_H (500 MHz, CD_3OD) 3.37 (3 H, s), 6.64 (1 H, s, H-8), 7.49 (2 H, t, J 7.3), 7.61 (1 H, dd, J 7.3 and 1.3), 7.84 (1 H, d, J 3.0, H-6), 8.07 (2 H, dd, J 7.3 and 1.3), 8.08 (1 H, dd, J 3.0 and 1.5, H-5), 8.10 (1 H, d, J 1.5, H-3); δ_C (125 MHz, CD_3OD) 54.53, 81.78 (C-8), 129.76, 130.12, 134.51 (C-3), 134.68 (C-6), 134.98, 135.81, 143.08 (C-5), 155.31 (C-2), 194.81 (C-9); m/z (FAB^+) 244 (54%), 212 (100), 138 (72), 105 (30), 81 (10); λ_{max}(MeOH, 10^{-5} M)/nm 241, 318; Fluorescence $\lambda_{excitation}$(MeOH, 10^{-5} M)/nm 318; $\lambda_{max\ emission}$ 383.

X-Ray diffraction analysis

Crystal data, data collection and refinement parameters are summarized in Table 5. The measurements were performed with a Huber four-circle diffractometer using graphite monochromated Cu- or Mo-Kα radiation. For all data collections, one standard reflection was monitored every 50 measurements, no significant deviation was observed. The three structures were solved by direct methods with SHELXS86[27] and refined anisotropically for non-hydrogen atoms using F^2 values with SHELXL93.[28] For **3a** and **3b**, all the H atoms were localized by Fourrier difference, except for methyl C11, which were calculated. For **5b**, the positions of the H atoms were calculated with

AFIX. The H atoms were included in the refinement with an isotropic temperature factor. The poor quality of the crystals of **5b** explains the low ratio of observed to measured reflections. Scattering factors were taken from ref. 29.†

Reaction with superoxide anion

Hypoxanthine (HX), xanthine oxidase (XOD) from buttermilk, Trolox® and albumine were purchased from Sigma. All solutions were made in 50 mM Tris·HCl buffer (pH 7.8) at 25 °C. The XOD solution contains a final 0.1 mg ml^{-1} concentration of albumine. The superoxide anion is enzymatically generated by the reaction of hypoxanthine (HX) with xanthine oxidase (XOD). When HX (500 μM) is added to Tris·HCl buffer containing XOD (5 U l^{-1}) and a chemiluminescent compound such as 6-methoxyphenyl-CLZ (5 μM), a strong chemiluminescence is instantaneously recorded with a luminometer (LB96P, Berthold). The addition of a non-luminescent compound able to react with superoxide anion will reduce the intensity of the luminescence according to its concentration and rate constant k_c (the chemiluminescence intensity is measured for 10 min). By increasing the (competitor)/(IMPZ) ratio, $I°/I_X$, where $I°$ and I_X are respectively the intensities measured in the absence and in the presence of a competitor, will increase. Plotting (competitor)/(IMPZ) against $I°/I_X$ values allows the determination of k_c/k_{IMPZ} from the slope of the linear relationship linking these parameters. Each concentration of the competitor is repeated six times (concentration ratio of 0 to 20 *versus* CLZ).

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