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In vitro and in vivo studies of 6,8-(diaryl)imidazo[1,2-*a*]pyrazin-3(7*H*)-ones as new antioxidants

Frederic De Wael [a], Paul Jeanjot [a,✠], Cédric Moens [a,b], Tony Verbeuren [c], Alex Cordi [c], Eliete Bouskela [d], Jean-François Rees [b], Jacqueline Marchand-Brynaert [a,*]

[a] *Unité de Chimie Organique et Médicinale, Université catholique de Louvain, Bâtiment Lavoisier, Place Louis Pasteur 1, B-1348 Louvain-la-Neuve, Belgium*
[b] *Institut des Sciences de la vie, Université catholique de Louvain, Bâtiment Carnoy, Place Croix du Sud 5, B-1348 Louvain-la-Neuve, Belgium*
[c] *Institut de Recherches Servier, Rue des Moulineaux 11, F-92150 Suresnes, France*
[d] *Laboratorio de Pesquisas em Microcirculaçao, Universidade do estado do Rio de Janeiro, Rua Sao Francisco Xavier 524, 20550-013 Rio de Janeiro, Brazil*

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ABSTRACT

A series of 5-aryl and 3,5-diaryl-2-amino-1,4-pyrazines and the derived imidazopyrazinones has been synthesized to study the chemical oxidative degradation of the bicyclic systems in vitro. Imidazopyrazinones mainly degraded following two independent pathways producing their precursors, namely aminopyrazines, and the corresponding amidopyrazines, respectively. Despite the fact that there is no influence of the substituent of the 3-aryl group on the ratio of the products aminopyrazine/amidopyrazine, diarylimidazopyrazinones and diarylaminopyrazines are good antioxidants in vivo. They protected against microvascular damages in ischemia/reperfusion with similar efficiencies.

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in aerobic organisms as part of the normal physiological and metabolic processes. They are very important mediators of cell injury or death due to the damages they can inflict if they are produced in excessive concentrations or in wrong locations. The damages that ROS/RNS cause, essentially on biological macromolecules (membrane lipids, proteins, nucleic acids, ...), are directly or indirectly implicated in the pathogenesis of various disorders such as cardiovascular diseases, reperfusion injury, Alzheimer's and other neurodegenerative diseases, cancer development and progression, inflammation as well as in the aging process.[1–9]

Therefore the interest for the protective role of antioxidants in medicine has been growing over the last 15 years. Antioxidants are considered as potential drugs due to their ability to reduce or inhibit the free radical reactions initiated by ROS/RNS. Currently available radical scavengers are structurally related to natural antioxidants (vitamin A/β-carotene, vitamin C, vitamin E, green tea extracts, flavonoids, ...) and to industrial compounds such as highly hindered *ortho*-substituted phenols.[10–14]

Coelenterazine **1a** (Scheme 1) and more generally imidazo[1,2-*a*]pyrazin-3(7*H*)-ones (imidazopyrazinones) are bioluminescent substrates of luciferases,[15–17] naturally designed to react with oxygen in light-producing reactions. Numerous studies have been devoted to the oxidation mechanism of coelenterazine and related derivatives **1**, essentially in the context of colored light production via an efficient biochemical process and the development of analytical tools in biochemistry.[18–23] A few years ago, our research group considered coelenterazine and other imidazopyrazinones **1** as potential leads in the discovery of novel antioxidants for therapeutic use.[24] We showed that imidazopyrazinones have good antioxidant properties as they are highly reactive with radical anion superoxide, lipid radicals, nitrofurantoin-derived radicals and peroxynitrite; LDL protection by compounds **1** has been further demonstrated in vitro. Finally, cellular protection against oxidative stress and UV-irradiation damages has been observed, in the presence of compounds **1**, on various cell lines (human keratinocytes, rat hepatocytes, rat neuronal cells, fish erythrocytes).[24] During these previous studies, we found that the chemical precursors of imidazopyrazinones synthesis, namely the 2-amino-1,4-pyrazine derivatives **3**, are also endowed with remarkable antioxidant properties providing R^3 is a *para*-phenol (or a catechol) moiety.[25,26]

The main metabolite of luciferase-catalyzed oxidation of coelenterazine (**1a**) is coelenteramide (**2a**) which is initially produced in the excited state; this species emits light during decay (Scheme 1).[27,28] Similarly, numerous synthetic analogs **1** display

* Corresponding author. Tel.: +32 10 472740; fax: +32 10 474168.
E-mail address: jacqueline.marchand@uclouvain.be (J. Marchand-Brynaert).
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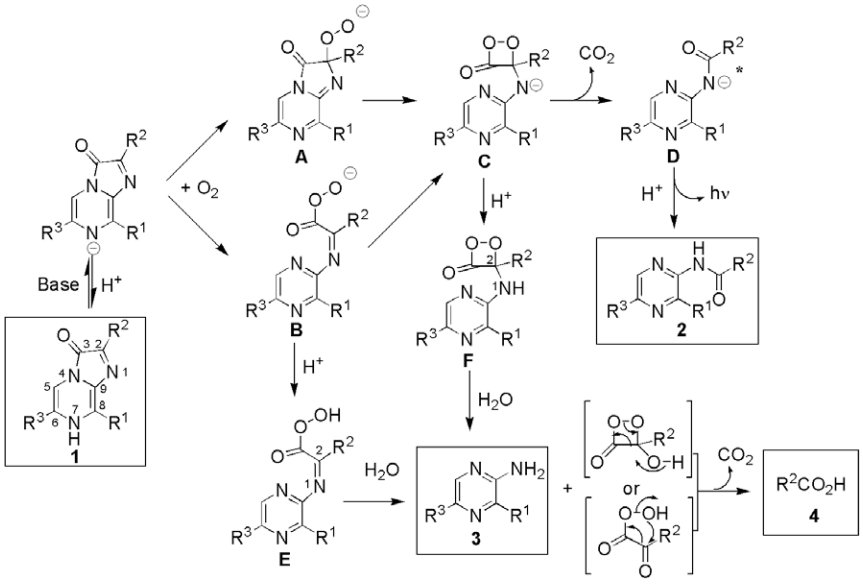


1a, **2a** : R^1 = CH_2Ph; R^2 = CH_2-4-OH-Ph; R^3 = 4-OH-Ph (coelenterazine, coelenteramide)
1b, **2b** : R^1 = H; R^2 = Ph; R^3 = 4-OH-Ph (model compound)
3a : R^1 = CH_2Ph; R^3 = 4-OH-Ph (coelenteramine); **3b** : R^1 = H; R^3 = 4-OH-Ph
3c : R^1 = CH_2Ph; R^3 = 4-OMe-Ph (internal standard for HPLC study)

Scheme 1. Oxidative degradation of imidazopyrazinones **1**.

bioluminescence and chemiluminescence properties under enzyme (luciferase) processing or chemical oxidation conditions.[15] The so-produced amides **2**, formally derived from aminopyrazines **3**, were however inactive in antioxidant tests.[24,25]

In the course of chemical and air oxidation studies of coelenterazine (**1a**), we observed the formation of coelenteramine (**3a**), in quite significant amounts, together with the expected coelenteramide (**2a**). This interesting observation was general: the oxidative degradation of imidazopyrazinones **1** led always to both amides **2** and aminopyrazines **3**. Thus besides the luminescence pathway of coelenterazine (**1**) decomposition giving an inactive compound (**2**), another route can take place which produces in situ a daughter-antioxidant (**3**) (Scheme 1).

Aiming to optimize this unique antioxidant system acting in cascade (**3** prolonging the activity of its mother-compound **1**), we studied the outcome of the five-membered ring R^2 substituent and the R^1 substituents effect on the **2**:**3** product ratios formed when processing imidazopyrazinones **1** under aerobic conditions in the laboratory. Here we report the HPLC analysis of a model reaction (R^1 = H, R^2 = Ph), the synthesis of novel derivatives **1** (R^2 = Me) with R^1 being various *para*-substituted phenyl groups, their in vitro oxidative decomposition pathways and their in vivo biological activity in the 'hamster cheek pouch' assay.

2. Results and discussion

2.1. Possible pathways involved in imidazopyrazinone oxidation (Scheme 1)

Results from the literature suggest that bioluminescence and chemiluminescence of imidazopyrazinones (**1**) might involve a similar mechanism for the production of amides (**2**) and light.[15] However, the formation of aminopyrazines (**3**) is occasionally mentioned.[29,30] The main questions concern the outcome of the imidazole moiety (which bears the substituent R^2) from precursors **1** and the production of **2** and **3** via parallel routes or via consecutive reactions (i.e., formation of **2** and subsequent transformation into **3**).

Although the intermediates in the luminescence reactions of imidazopyrazinones (**1**) have not yet been firmly established, it is generally accepted that the emission of light originates from the thermal decomposition of a dioxetanone species. The anion of **1** reacts (more easily than the neutral substrate) with triplet molecular oxygen to give the anion peroxide intermediates **A** or **B**. Both can conduct to the key-dioxetanone intermediate **C** which decomposition, with the loss of carbon dioxide, generates a singlet-excited state of amidopyrazine anion **D**. Amide **2** is then produced via protonation of **D** and emission of light.[15] Hydrolysis of **2** into aminopyrazine **3** is not likely under the smooth conditions of air oxidation (protic or aprotic polar solvent, added with a weak organic base or an aqueous buffer). Therefore, we hypothesized that aminopyrazine **3** could be formed via a parallel route to the luminescence pathway, involving the hydrolysis of the protonated intermediates **B** or **C**, namely **E** and **F**, by water nucleophilic attack on C2 followed by C2-N1 bond cleavage, the aminopyrazinyl moiety being a good leaving group. Such a mechanism would simultaneously produce the acid **4** bearing the R^2 substituent. The possible occurrence of this alternative route of oxidative decomposition has been examined on a simple imidazopyrazinone **1** with a view to easily identify and quantify the three products **2**, **3** and **4**[31] by using HPLC as analytical method.

2.2. HPLC study of a model reaction

Imidazopyrazinone **1b** (R^1= H, R^2= Ph, R^3= 4-OH-Ph) was selected as model compound for the study of chemical oxidative degradation, indeed: (i) **1b** features the minimum pharmacophoric pattern for antioxidant activity[26] and is readily accessible; (ii) the reference products **2b** and **3b** are also easily prepared; (iii) the acid **4** that should be produced is benzoic acid, a commercial reference visible in HPLC with UV detection; (iv) a structurally related molecule, stable in oxidative conditions (because the phenol moiety is protected), namely aminopyrazine **3c**, can be used as internal standard (Scheme 1).

The synthesis of **1b**, **3b** and **3c** was performed as previously described.[32–35] Amide **2b** was independently prepared from 2-ami-

no-5-[*p*-(*t*-butyldimethylsilyloxy)-phenyl]-1,4-pyrazine **3d** (see Scheme 2),[36] by benzoylation (benzoyl chloride, pyridine, reflux) followed by deprotection of the phenol moiety (Bu_4NF, THF, 20 °C). For HPLC analysis all compounds and benzoic acid (**4**) were dissolved in acetonitrile (ACN)–EtOH (96:4, v/v) at 8×10^{-5} M final concentration. We used a C18 column and a gradient of ACN–H_2O (see Section 4) for the elution; benzoic acid is visible at 220 nm, while the other components are more conveniently detected at 280 nm. The following retention times were recorded: **4**, R_T = 2.1 min; **3b**, R_T = 14 min; **1b**, R_T = 16 min; **3c**, R_T = 17.8 min; **2b**, R_T = 19.5 min.

A 1:1 mixture of imidazopyrazinone **1b** and standard **3c** (8×10^{-5} M) in 96:4 ACN–EtOH solution was placed in the dark, at 37 °C and controlled by HPLC, each hour, during 18 h. Air-oxidation was a very slow process[37]: 80% of **1b** is still present and only 20% has been transformed into amide **2b** (<5%), aminopyrazine **3b** (9%) and other unidentified derivatives. In the presence of azo-bis-isobutyronitrile (AIBN, 4×10^{-3} M), oxidation of **1b** at 37 °C was complete within 18 h, leading to **2b** (26%) and **3b** (44%) as the main products (Fig. 1). Concentration evolution as a function of time, depicted in Figure 1, shows clearly the simultaneous appearance of **2b** and **3b**; amide **2b** is not the precursor of aminopyrazine **3b** (this has been also controlled with pure **2b** placed in the same experimental conditions).

Air-oxidation of **1b** (8×10^{-5} M) was further examined in a micellar solution of linoleic acid (1.6×10^{-4} M, phosphate buffer pH 7.4 ACN–EtOH; 85:12.5:2.5, v/v/v). In this case, the disappearance of **1b** was rapid (less than 6 h), and the concomitant formation of amide **2b** and amine **3b** was observed as above. The final ratio of oxidation products **2b**/**3b** was 66%/30%. Benzoic acid was detected in similar amount as **3b** (**4**: 27%, Fig. 2). Addition of AIBN (4×10^{-3} M) did not change dramatically the reaction profile: **1b** is totally oxidized within 5 h, at 37 °C in the dark as previously, to furnish **2b**, **3b** and **4** in, respectively, 57%, 40% and 33%. Differences were visible after several hours, when monitoring the products stability during one day: **2b** (amide) and **4** (benzoic acid) are stable in both conditions; **3b** (aminopyrazine) is stable in the absence of AIBN, but is gradually consumed in its presence. This observation is consistent with the capacity of aminopyrazine derivatives to inhibit lipid peroxidation.[38]

This HPLC study of in vitro air-oxidation of imidazopyrazinone **1b** has pointed out the occurrence of two independent routes of degradation (Scheme 1), leading respectively to amidopyrazine **2b** and aminopyrazine **3b** accompanied by a carboxylic acid **4** bearing the R^2 substituent. Depending on the experimental conditions, the reaction was (very) slow ($\geqslant$18 h) or rapid ($\leqslant$5 h), and the corresponding product ratios **2b**/**3b** were in favor of aminopyrazine **3b** (26/44) or amidopyrazine **2b** (66/30). This is in agreement with previous reports showing that chemiluminescence efficiency of **1** is

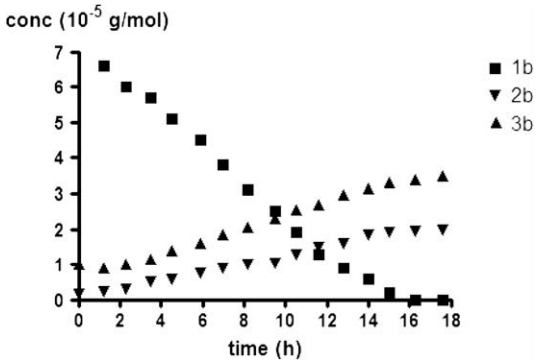


Figure 1. Evolution of concentrations during air-oxidation of imidazopyrazinone **1b** at 37 °C in the presence of AIBN in large excess.

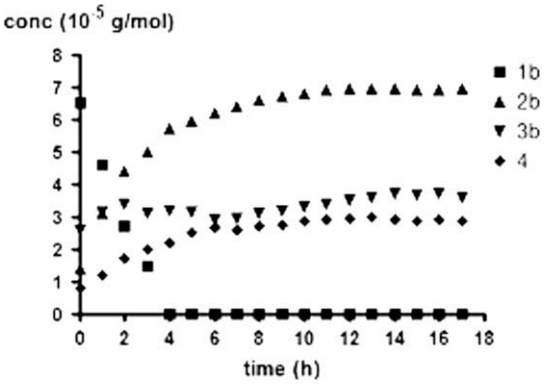
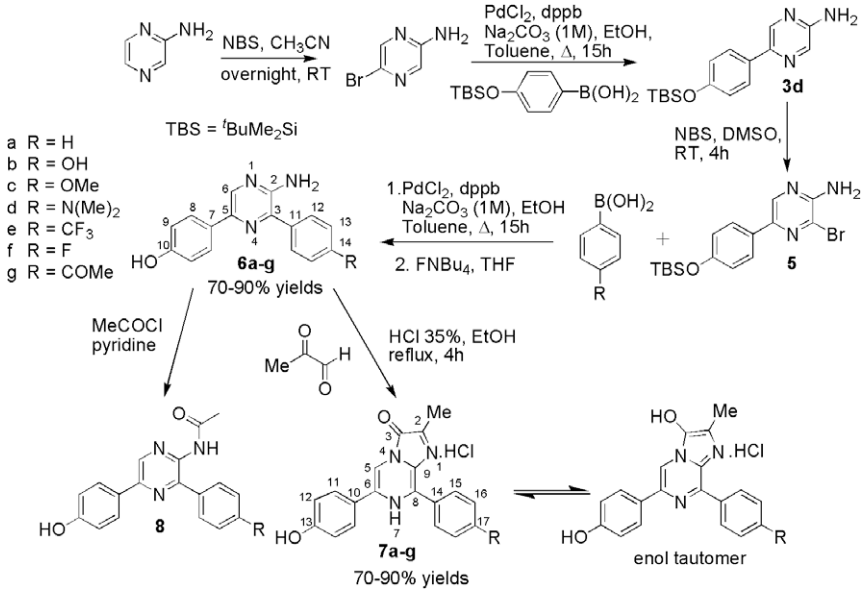


Figure 2. Evolution of concentrations during air-oxidation of imidazopyrazinone **1b** at 37 °C in the presence of linoleic acid micelles.



Scheme 2. Synthesis of aminopyrazines **6** and imidazopyrazinones **7**.

strongly enhanced in micelle solutions of surfactants or in the presence of cyclodextrins mimicking the hydrophobic active site of luciferase.[39–41]

2.3. Synthesis of novel imidazopyrazinones (Scheme 2)

We had already established[24,25] that the antioxidant properties of imidazopyrazinones **1** is linked to the hetero-bicyclic core itself and not so much to the decorating substituents. On the other hand, the activity of 2-aminopyrazines **3** is mainly due to their *para*-substituent (phenol/catechol moiety), the *ortho*-substituent appearing less crucial. Accordingly, we decided to prepare a series of novel imidazopyrazinones **7** (Scheme 2) derived from 2-amino-3,5-diaryl-1,4-pyrazines **6** (introduction of R^1 substituents with different electronic effects) and methyl glyoxal (introduction of the smallest R^2 substituent), in view to possibly promote the route of oxidative degradation leading to the 'cascade effect', namely the production of aminopyrazines.

We exploited a strategy set up by our group for the preparation of unsymmetrical 2-amino-3,5-diaryl-1,4-pyrazines, based on two successive Suzuki coupling reactions.[35] TBS protected 2-amino-3-bromo-5-[4-(*t*-butyl-dimethyl-silyloxy)phenyl]-1,4-pyrazine (**5**) was obtained by monobromination in position 5 of commercially available 2-amino-1,4-pyrazine, then Suzuki coupling to introduce the protected *p*-phenol moiety in position 5 giving **3d** and finally bromination of position 3. Reaction of 3,5-dibromo-2-amino-1,4-pyrazine with one equivalent of *p*-(*t*-butyl-dimethyl-silyloxy)phenyl boronic acid in the presence of Pd-catalyst furnished **5** in low yield (mixture of mono- and di-substituted products and starting material).[42]

The target-compounds **7** were readily obtained from **5** after Suzuki coupling in position 3 with the required *para*-substituted phenylboronic acids, deprotection of *t*-butyldimethylsilyl ether and condensation with methylglyoxal[40] in a mixture of ethanol and aqueous HCl at reflux. With this sequence of reactions, we had in hands seven couples of 3,5-disubstituted-2-aminopyrazines **6** and imidazopyrazinones **7** of which five were not previously described (i.e., couples **c**, **d**, **e**, **f**, **g**).[34,38]

Compounds **6** and **7** were characterized as usual (see Section 4). Tables 1 and 2 summarize typical NMR values, showing only small differences between the series of compounds **6** and **7**, respectively, that could be attributed to the electron-donating/withdrawing character of the R substituents, as well as to a solvent effect when the spectra are recorded in different solvents. Carbon atoms 2 and 3 of compounds **7** could not be attributed precisely due to the lack of correlation with ^{1}H and the occurrence of enol tautomeric forms (see Scheme 2): C-3 is highly downfield shifted comparatively to 'normal' carbonyl-type carbons.

2.4. Air-oxidation of imidazopyrazinones 7

The effect of R substituents on the degradation pathways of imidazopyrazinones **7a–g** has been examined by HPLC. Common experimental conditions were set up (aerated solution of dichloromethane (DCM) and wet isopropanol (*i*PrOH) containing diethylamine (DEA) as base for accelerating the oxidation), taking into account both the solubility and the chromatographic resolution of all the members **a–g**, for the three series of compounds, namely the precursors **7**, and the oxidation products **8** (amides) and **6** (amines) (Scheme 2), the concomitantly formed acetic acid (**4** with R^2 = Me) being not detectable by HPLC.[43] The reference acetamides **8** were obtained according to Jeanjot et al.[36] The selected internal standard, stable under air-oxidation conditions, was 3,5-diphenyl-2-amino-1,4-pyrazine.[42]

For HPLC analysis, all compounds (**7**, **6** and **8**) were dissolved in CH_2Cl_2/isopropanol (90:10, v/v) with 0.1% diethylamine (DEA), at 10^{-3} M final concentration. We used laboratory packed silica columns and different types of isocratic elution (CH_2Cl_2/isopropanol/DEA, **A** 90:10:0.1, **B** 95:5:0.1 or **C** 96:4:0.1). All compounds were detected at 280 nm. The retention times (in min) of the products are given in Table 3.

Calibration curves were established to further quantify the amounts of products formed by aerobic oxidation of the various precursors **7**. A typical experiment is described below: a mixture of imidazopyrazinone **7a** (10^{-3} M) and internal standard (5×10^{-4} M) in 90:10:0.1 CH_2Cl_2/isopropanol/DEA solution was introduced in a hermetic vial and placed under white light (to accelerate the oxidation) at 23 °C. After 19 h, **7a** was totally decomposed (disappearance of **7a** on TLC) and transformed into **6a** (66%), **8a** (15%) and other unidentified derivatives. Both the internal standard and **8a** were stable in those conditions, but **6a** slowly degraded with time. The concentration of **6a** diminished of about ~50% after another 24 h. The instability under oxidative conditions of aminopyrazines was already observed with **3b** (see above).

All the results of imidazopyrazinones (**7**) oxidative degradation are collected in Table 3. This HPLC study is illustrated with a typical chromatogram shown in Figure 3. The series of 6,8-diarylimidazo[1,2-*a*]pyrazin-3(7*H*)-ones produced aminopyrazines (**6**) as major products and the corresponding amides (**8**) as minor products. The experimental **6**:**8** ratios were within 3.0 and 4.8; no correlation with the electronic effect of R substituents could be drawn.

Table 1
Typical ^{1}H and ^{13}C NMR data (δ) of **6** (atom numbering of Scheme 2)

Entry	6-H	2-C	3-C	5-C	6-C
a[c]	8.46	152.3	139.2	149.2	139.4
b[a]	8.35	151.1	138.0	140.3	135.8
c[a]	8.40	152.5	143.9	143.8	138.5
d[b]	8.20	152.4	141.8	143.6	136.1
e[a]	8.49	152.4	141.8	140.6	137.8
f[b]	8.31	152.5	139.9	143.8	137.8
g[a]	8.38	152.5	143.9	143.8	138.5

[a] Spectra recorded in DMSO-d_6.
[b] Spectra recorded in methanol-d_4.
[c] Spectra recorded in acetone-d_6.

Table 2
Typical ^{1}H and ^{13}C NMR data (δ) of **7** (atom numbering of Scheme 2)

Entry	5-H	2-Me	2-C	3-C	5-C	6-C	8-C	9-C
a[a]	8.74	2.09	125.2	125.5	109.6	133.8	144.1	136.8
b[a]	8.67	2.46	123.9	124.7	107.9	126.4	143.9	136.9
c[b]	8.40	2.54	Not visible	139.0	109.2	142.0	145.5	126.X
d[b]	8.48	2.56	122.8	139.3	109.7	141.5	144.7	126.8
e[b]	8.55	2.54	119.8	138.8	110.1	143.5	144.5	126.5
f[b]	8.50	2.54	116.8	135.6	106.6	140.2	142.0	123.4
g[a]	8.48	2.42	nd	nd	nd	nd	nd	nd

[a,b] See Table 1; nd: not determined.

Table 3
HPLC results–products ratio at the end of the oxidation

Entry	6		8		6:8	Elution mode
	t_R	%	t_R	%		
a	6.2	66	9.3	15	4.4	A
b	7.4	77	10.7	16	4.8	A
c	15.5	71	31.1	21	3.4	C
d	12.3	68	20.2	18	3.8	B
e	11.7	78	19.0	16	4.8	B
f	13.5	76	21.7	17	4.5	B
g	6.8	67	10.4	22	3.0	A

t_R internal standard = 3.6, 6.1, 7.8 min elution mode A, B, C, respectively.

2.5. In vivo activity

The protective effect of compounds **6** and **7** against microvascular damages in ischemia/reperfusion was assayed in vivo. The evaluation was realized with the experimental model of 'hamster cheek pouch'[44] allowing quantitative studies of macromolecular permeability by direct observation on microscope. Fluorescent-labeled dextran was injected intravenously and changes in the number of microvascular leaky sites were measured after local ischemia/reperfusion. Animals were treated by gavage with the tested compounds at 3 mg/kg, 30 min before anesthesia. Results are given in percentages of inhibition of leaky sites, determined 30 min after the start of reperfusion. In this test, Apocynin (a flavonoid derivative used as reference) gave about 40% inhibition at 3 mg/kg.

Results collected in Table 4 showed that all compounds provided a moderate to good protection against the increase of microvascular permeability due to ischemia/reperfusion. However, the mother-antioxidants **7** were not systematically more active than the corresponding daughter-antioxidant **6**: in two cases, **7** was more potent than **6** (**b**, R = OH; **c**, R = OMe), in two other cases, **7** was less potent than **6** (**d**, R = NMe_2; **f**, R = F) and in two last cases, **7** and **6** were almost equally active (**a**, R = H; **e**, R = CF_3). Disappointingly, the 'cascade effect', disclosed by the HPLC studies under laboratory oxidation conditions, is not a relevant guide principle for optimizing the biological activity of imidazopyrazinones. Indeed, the in vivo test takes into account not only the intrinsic antioxidant activity of the couples **7**/**6**, but also the oral bioavailability, metabolic stability and distribution of the tested compounds in the specific tissue where the efficacy is measured.

Table 4
Results of 'hamster cheek pouch' assay

6	% Inhibition 3 mg/kg	**7**	% Inhibition 3 mg/kg
a	18	a	12
b	29	b	40
c	10	c	44
d	50	d	21
e	14	e	17
f	42	f	34

3. Conclusion

Amongst the various strategies of drug discovery, we became interested in the 'non-natural natural products' approach which, after about 25 years of 'combinatorial chemistry technologies', is nowadays reconsidered as very promising by distinguished scientists in the field.[45]

Coelenterazine (a marine luciferine, **1a**) was our natural 'lead' compound for the discovery of potential antioxidants, due to its intrinsic capacity of quenching oxygen and ROS/RNS. Two pharmacophores were identified from our previous studies, namely the bicyclic core of **1a** (imidazo[1,2-*a*]pyrazin-3(7*H*)-one) and 2-amino-5-(*p*-hydroxyphenyl)-1,4-pyrazine (**3b**). In this paper, we have experimentally proved that an aminopyrazine is one of the oxidation products of the imidazopyrazinone, formed via a parallel route to the luminescence process. However, the so-called 'cascade effect' (i.e., a mother-antioxidant decomposes into a daughter-antioxidant), could not be optimized by varying the electronic proper-

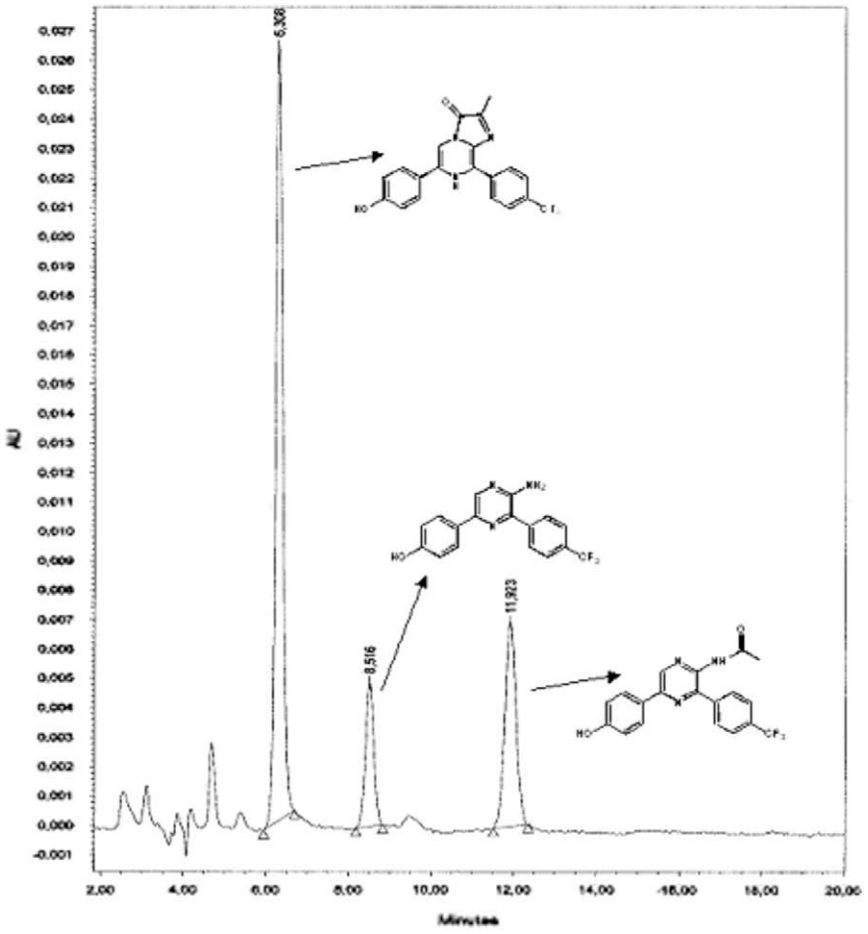


Figure 3. HPLC analysis of oxidative degradation of imidazopyrazinone **7e** after 4 h with elution mode A.

ties of the C8 substituent of **1** (≡C3 substituent in **3**); our idea was to improve the leaving group capacity of the aminopyrazinyl moiety by increasing the electron-withdrawing character of R^1 (see Scheme 1). Nevertheless, in protic media, the series of non-natural imidazopyrazinones **7a–g** furnished always the corresponding aminopyrazines **6a–g** as the major products of aerobic degradation.

We have further confirmed the interest of both families **7** and **6** in drug discovery by a representative in vivo animal assay, namely the protection against ischemia/reperfusion injury. Here again, no particular benefit due to the 'antioxidant cascade', nor substituent effect, could be demonstrated; the most active compounds were imidazopyrazinones **7b**, **7c** and aminopyrazines **6d**, **6f**, respectively, bearing different R^1 substituents.

The oxidative release of benzoic acid (see Scheme 1, **4** with R^2 = Ph) we demonstrated by HPLC studies, suggests that imidazopyrazinone derivatives could be developed not only for their intrinsic antioxidant properties, but also as carriers to deliver acidic drugs on their site of action. This novel type of potential dual-action drug or pro-drug is currently under investigation.[46]

4. Experimental

4.1. Chemistry: general methods

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian Gemini-300 spectrometer. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a BRUCKER AM-500 spectrometer. ^{19}F (282 MHz) NMR spectra were recorded on a BRUCKER AVANCE-300 spectrometer. The attributions were established by selective decoupling experiments. Chemical shifts are reported as δ values (in ppm) downfield from TMS for ^{1}H and ^{13}C or from $CFCl_3$ for ^{19}F. The mass spectra (FAB or APCI modes) were obtained using a Finnigan-MAT TSQ-700 instrument. Elemental analyses were obtained at the University College of London (UK). HRMS analysis by the ESI method was performed at the University of Mons-Hainaut (Belgium). UV spectra were recorded on a UV–vis–NIR Varian-Cary spectrophotometer (λ given in nm). IR spectra were determined using a FT Biorad FTS 135 apparatus. Thin-layer chromatography was carried out on Silica Gel 60 plates F_{254} (Merck, 0.2 mm thick); product visualization was effected with UV light (λ = 254 nm). For flash chromatography we used Merck Silica Gel 60 of 230–400 mesh ASTM. Melting points (uncorrected) were determined on an Electrothermal apparatus.

Anhydrous solvents used were of P.A. quality grade and freshly distilled [(CH_2Cl_2 and NEt_3 from CaH_2) and (THF, ether, benzene and toluene from Na and benzophenone)]. All reagents were obtained from Aldrich-Fluka, Acros or ROCC.

HPLC analysis of **1b** and degradation products was carried out on Thermoseparation system (Thermoquest, Belgium). The system consisted of a P2000 binary pump plus a temperature-controlled AS1000 autosampler and a UV3000 UV–vis detector. All analyses were performed on a 250 × 4.6 mm 5 μm C18 column (Alltech) equipped with a precolumn cartridge, maintained at a temperature of 35 °C. The volume of full-loop injections is 10 μL. Solutions are made with solvents of HPLC quality grade for organic solutions and millipore water for aqueous ones.

HPLC system used for the analysis of **7a–g** and degradation products consisted of a Beckman 126P pump connected to Beckman 168 PDA detector (chromatograms were extracted at λ = 280 nm). The HPLC column length was 250 mm × 4.6 mm packed in the laboratory with Silica 60, 15–40 μm, irregular. Organic solutions were made with solvents of HPLC quality grade without drying.

Compounds **6a**, **6b**, **7a** and **7b** were obtained according to Refs. 34 and 36.

4.2. General procedure A for the synthesis of 3,5-diaryl-2-aminopyrazines

1,4-Bis(diphenylphosphino)butane (6 mol %) was added to a suspension of bis(benzonitrile)dichloro palladium (5 mol %) in dry toluene (3 mL/mmol) and was stirred for 30 min under argon atmosphere at room temperature. 2-Amino-3-bromo-5-(*p*-*t*butyldimethylsilyloxyphenyl)-1,4-pyrazine (1 equiv), arylboronic acid (1.2 equiv), ethanol (0.4 mL/mmol), 1 M Na_2CO_3 (1 equiv) and toluene (3 mL/mmol) were added successively and the mixture was heated to reflux overnight. The mixture was cooled to room temperature, diluted with water and extracted with ethyl acetate (3×). The organic layers were combined, washed with brine and dried over $MgSO_4$, filtered and concentrated under vacuum.

The residue was dissolved in THF (10 mL/mmol) and tetrabutylammonium fluoride (1 M in THF, 2.5 equiv) was added dropwise at 0 °C. The mixture is stirred 10 min at 0 °C and another 3 h at room temperature. Water (1 mL/mmol) was added and the mixture was extracted with ethyl acetate (1 mL/mmol, 4×). The organic layers were combined, washed with brine and dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography.

4.2.1. 2-Amino-3-(4-methoxyphenyl)-5-(4-hydroxyphenyl)-1,4-pyrazine (6c)

Compound **6c** was prepared according to the general procedure A using 1,4-Bis(diphenylphosphino)butane (33 mg, 0.077 mmol, 6 mol %), bis(benzonitrile)dichloro palladium (23 mg, 0.059 mmol, 5 mol %), dry toluene (2 × 20 mL), **5** (425 mg, 1.11 mmol, 1 equiv), corresponding arylboronic acid (204 mg, 1.34 mmol, 1.2 equiv), ethanol (350 μL), 1 M Na_2CO_3 (1.1 mL, 1 equiv), THF (6 mL) and tetrabutylammonium fluoride (2.8 mL, 2.5 equiv); Column chromatography using ether/cyclohexane: 3/1; 249 mg of **6c** as pale yellow solid; 85% yield; R_f 0.6 (ether/ethyl acetate: 6/4); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.84 (s, 3H, 15-H), 6.06 (br s, 2H, NH_2), 6.82 (d, $^3J_{9,8}$ = 8.7 Hz, 2H, 9-H), 7.07 (d, $^3J_{13,12}$ = 8.7 Hz, 2H, 13-H), 7.75 (d, $^3J_{8,9}$ = 8.7 Hz, 2H, 8-H), 7.80 (d, $^3J_{12,13}$ = 8.7 Hz, 2H, 12-H), 8.40 (s, 1H, 6-H), 9.6 (br s, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.8 (16-C), 116.6 (9-C), 128.0 (8-C), 129.8 (12-C), 129.9 (13-C), 130.1 (7-C), 138.3 (14-C), 138.5 (6-C), 139.2 (11-C), 143.8 (5-C), 143.9 (3-C), 152.5 (2-C), 159.1 (10-C); MS (FAB$^+$) $C_{17}H_{15}N_3O_2$ (M_W = 293.33) *m/z* 294 ($(M+H)^+$), 278 ($(M-CH_3)^+$); Anal. Calcd for $C_{17}H_{15}N_3O_2{\cdot}0.3H_2O$ (298.52): C, 68.77; H, 5.22; N, 14.16. Found: C, 68.85; H, 5.41; N, 13.52. UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 365 (0.93), 327 (0.50), 285 (2.5), 251 (1.8); mp 222–224 °C.

4.2.2. 2-Amino-3-(4-(*N*,*N*)-dimethylaminophenyl)-5-(4-hydroxyphenyl)-1,4-pyrazine (6d)

Compound **6d** was prepared according to the general procedure A using 1,4-bis(diphenylphosphino)butane (27 mg, 0.062 mmol, 6 mol %), bis(benzonitrile)dichloro palladium (20 mg, 0.051 mmol, 5 mol %), dry toluene (2 × 10 mL), **5** (290 mg, 1.04 mmol, 1 equiv), corresponding arylboronic acid (198 mg, 1.2 mmol, 1.16 equiv), ethanol (400 μL), 1 M Na_2CO_3 (1 mL, 1 equiv), THF (6 mL) and tetrabutylammonium fluoride (2.8 mL, 2.5 equiv); column chromatography using cyclohexane/ethyl acetate 5/3; 261 mg of **6d** as yellow green solid; 82% yield; R_f 0.31 (cyclohexane/ethyl acetate: 5/4); ^{1}H NMR (500 MHz, CD_3OD) δ 3.02 (s, 6H, 15-H), 6.85 (d, $^3J_{9,8}$ = 8.8 Hz, 2H, 9-H), 6.89 (d, $^3J_{13,12}$ = 8.8 Hz, 2H, 13-H), 7.68 (d, $^3J_{12,13}$ = 8.8 Hz, 2H, 12-H), 7.76 (d, $^3J_{8,9}$ = 8.8 Hz, 2H, 8-H), 8.20 (s, 1H, 6-H). ^{13}C NMR (125 MHz, CD_3OD) δ 40.6 (15-C), 113.4 (9-C), 116.5 (13-C), 126.3 (11-C), 128.1 (8-C), 130.1 (7-C), 130.3 (12-C), 136.1 (6-C), 141.8 (3-C), 143.6 (5-C), 152.4 (2-C), 152.6 (14-C), 158.9 (10-C); MS (FAB$^+$) $C_{18}H_{18}N_4O$ (M_W = 306.37) *m/z* 307.3 ($(M+H)^+$). Anal. Calcd for $C_{18}H_{18}N_4O$: C, 70.57; H, 5.92; N, 18.29.

Found: C, 70.60; H, 6.08; N, 17.95. UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 378 (1.3), 287 (2.06), 267 (2.15); mp 186–188 °C.

4.2.3. 2-Amino-3-(4-trifluoromethylphenyl)-5-(4-hydroxyphenyl)-1,4-pyrazine (6e)

Compound **6e** was prepared according to the general procedure A using 1,4-bis(diphenylphosphino)butane (55 mg, 0.13 mmol, 6 mol %), bis(benzonitrile)dichloro palladium (39 mg, 0.10 mmol, 5 mol %), dry toluene (2×10 mL), **5** (645 mg, 1.7 mmol, 1 equiv), corresponding arylboronic acid (410 mg, 2.16 mmol, 1.27 equiv), ethanol (700 μL), 1 M Na_2CO_3 (1.7 mL, 1 equiv), THF (30 mL) and tetrabutylammonium fluoride (4.3 mL, 2.5 equiv); column chromatography using cyclohexane/ethyl acetate 5/4; 512 mg of **6e** as yellow solid; 91% yield; R_f 0.30 (cyclohexane/ethyl acetate: 5/4); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.29 (br s, 2H, NH_2), 6.81 (d, $^3J_{9,8}$ = 8.7 Hz, 2H, 9-H), 7.80 (d, $^3J_{8,9}$ = 8.7 Hz, 2H, 8-H), 7.84 (d, $^3J_{13,12}$ = 8.1 Hz, 2H, 13-H), 8.01 (d, $^3J_{12,13}$ = 8.1 Hz, 2H, 12-H), 8.49 (s, 1H, 6-H), 9.56 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 115.5 (9-C), 124.27 (q, $^1J_{15,F}$ = 272 Hz, 15-C), 125.42 (q, $^3J_{13,F}$ = 3.1 Hz, 13-C) 126.4 (8-C), 127.7 (7-C), 128.5 (d, $^2J_{14,F}$ = 32 Hz, 14-C), 129.0 (12-C), 135.6 (11-C), 137.8 (6-C), 140.6 (5-C), 141.8 (3-C), 151.4 (2-C), 157.4 (10-C); MS (FAB$^+$) $C_{17}H_{12}F_3N_3O$ (M_W = 331.29) *m/z* 332.0 ((M+H)$^+$); Anal. Calcd for $C_{17}H_{12}F_3N_3O{\cdot}0.4H_2O$ (338.50): C, 60.32; H, 3.81; N, 12.41. Found: C, 60.54; H, 3.74; N, 12.01. UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 371 (0.91), 292 (2.18); mp > 250 °C decomposition.

4.2.4. 2-Amino-3-(4-fluorophenyl)-5-(4-hydroxyphenyl)-1,4-pyrazine (6f)

Compound **6f** was prepared according to the general procedure A using 1,4-bis(diphenylphosphino)butane (49 mg, 0.116 mmol, 6 mol %), bis(benzonitrile)dichloro palladium (36 mg, 0.093 mmol, 5 mol %), dry toluene (2×10 mL), **5** (719 mg, 1.89 mmol, 1 equiv), corresponding arylboronic acid (310 mg, 2.21 mmol, 1.2 equiv), ethanol (800 μL), 1 M Na_2CO_3 (1.9 mL, 1 equiv), THF (15 mL) and tetrabutylammonium fluoride (2.6 mL, 2.5 equiv); Column chromatography using cyclohexane/ethyl acetate 4/5; 367 mg of **6f** as yellow solid; 69% yield; R_f 0.32 (cyclohexane/ethyl acetate: 5/4); ^{1}H NMR (500 MHz, CD_3OD) δ 4.62 (br s, 2H, NH_2), 6.85 (d, $^3J_{9,8}$ = 9 Hz, 2H, 9-H), 7.24 (t, $^3J_{13,12}$ = $^3J_{13,F}$ = 8.8 Hz, 2H, 13-H), 7.76 (d, $^3J_{8,9}$ = 9 Hz, 2H, 8-H), 7.82 (dd, $^3J_{12,13}$ = 8.8 Hz and $^3J_{12,F}$ = 5.2 Hz, 2H, 12-H), 8.31 (s, 1H, 6-H). ^{13}C NMR (125 MHz, CD_3OD) δ 116.6 (9-C), 116.7 (d, $^2J_{13,F}$ = 23.6 Hz, 13-C), 128.4 (8-C), 129.8 (7-C), 131.7 (d, $^3J_{12,F}$ = 8.4 Hz, 12-C), 135.2 (11-C), 137.8 (6-C), 139.9 (3-C), 143.8 (5-C), 152.5 (2-C), 159.0 (10-C), 164.46 (d, $^1J_{14\text{-}F}$ = 247 Hz, 14-C); MS (FAB$^+$) $C_{16}H_{12}FN_3O$ (M_W = 281.28) *m/z* 282.3 ((M+H)$^+$); Anal. Calcd for $C_{16}H_{12}FN_3O{\cdot}0.1H_2O$ (283.08): C, 67.89; H, 4.34; N, 14.84. Found: C, 68.01; H, 4.63; N, 14.39. UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 365 (0.79), 329 (0.51), 287 (2.3); mp 232–234 °C.

4.2.5. 2-Amino-3-(4-acetylphenyl)-5-(4-hydroxyphenyl)-1,4-pyrazine (6g)

Compound **6g** was prepared according to the general procedure A using 1,4-bis(diphenylphosphino)butane (21 mg, 0.048 mmol, 6 mol %), bis(benzonitrile)dichloro palladium (15 mg, 0.039 mmol, 5 mol %), dry toluene (2×15 mL), **5** (275 mg, 0.72 mmol, 1 equiv), corresponding arylboronic acid (126 mg, 0.77 mmol, 1.05 equiv), ethanol (300 μL), 1 M Na_2CO_3 (0.75 mL, 1 equiv), THF (13 mL) and tetrabutylammonium fluoride (1.8 mL, 2.5 equiv); Column chromatography using cyclohexane/ethyl acetate: 2/3; 165 mg of **6g** as yellow solid; 78% yield; R_f 0.3 (cyclohexane/ethyl acetate: 2/3); ^{1}H NMR (500 MHz, CD_3OD) δ 2.67 (s, 3H, 16-H), 4.6 (br s, 2H, NH_2), 6.86 (d, $^3J_{9,8}$ = 8.7 Hz, 2H, 9-H), 7.79 (d, $^3J_{8-9}$ = 8.7 Hz, 2H, 8-H), 7.97 (d, $^3J_{12,13}$ = 8.7 Hz, 2H, 12-H), 8.15 (d, $^3J_{13,12}$ = 8.7 Hz, 2H, 13-H), 8.38 (s, 1H, 6-H). ^{13}C NMR (125 MHz, CD_3OD) δ 26.8 (16-C), 116.6 (9-C), 128.0 (8-C), 129.8 (12-C), 129.9 (13-C), 130.1 (7-C), 138.3 (14-C), 138.5 (6-C), 139.2 (11-C), 143.8 (5-C), 143.9 (3-C), 152.5 (2-C), 159.1 (10-C); MS (FAB$^+$) $C_{18}H_{15}N_3O_2$ (M_W = 305.34) *m/z* 306.4 ((M+H)$^+$), 186 ((M−C_8H_7O)$^+$), 119 ((C_8H_7O)$^+$); Anal. Calcd for $C_{18}H_{15}N_3O_2{\cdot}0.3H_2O$ (312.57): C, 69.57; H, 5.07; N, 13.52. Found: C, 69.64; H, 4.94; N, 13.20. UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 377 (0.83), 290 (1.75), 272 (1.9), 259 (1,9); mp 223–225 °C.

4.3. General procedure B for the condensation of 3,5-diaryl-2-aminopyrazines (6) with methylglyoxal

Methylglyoxal (40% w/v aqueous solution, 2 equiv) and HCl 37% (3.6 equiv) were added to a solution of **6** (1 equiv) in ethanol (3 mL/mmol) under argon atmosphere. The mixture was heated at 80 °C for 4 h. After cooling, the mixture was evaporated to dryness, the residue was washed with ether and ethyl acetate. Compounds **7** were precipitated from cold methanol.

4.3.1. 2-Methyl-6-(4-hydroxyphenyl)-8-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (7c)

Compound **7c** was prepared according to the general procedure B using methylglyoxal (115 μL, 2 equiv), HCl 37% (110 μL, 3.6 equiv), **6c** (100 mg, 0.34 mmol, 1 equiv), ethanol (10 mL); 96 mg of **7c** as orange yellow solid; 81% yield; ^{1}H NMR (300 MHz, CD_3OD) δ 2.54 (s, 3H, 10-H), 3.94 (s, 3H, 19-H), 6.89 (d, $^3J_{18,17}$ = 8.7 Hz, 2H, 13-H), 7.19 (d, $^3J_{13,12}$ = 9.0 Hz, 2H, 17-H), 7.86 (d, $^3J_{17,18}$ = 8.7 Hz, 2H, 12-H), 8.04 (d, $^3J_{12,13}$ = 9.0 Hz, 2H, 16-H), 8.40 (s, 1H, 5-H). ^{13}C NMR (75 MHz, CD_3OD) δ 9.6 (10-C), 56.1 (19-C), 109.2 (5-C), 115.7 (17-C), 116.8 (13-C), not observed (2-C or 3-C), 126.2, 126.4, 126.6 (15-C, 11-C, 9-C), 129.4 (12-C), 132.0 (16-C), 139.0 (2-C or 3-C), 142.0 (6-C), 145.5 (8-C), 160.7 (14-C), 164.2 (18-C); MS (FAB$^+$) $C_{20}H_{17}N_3O_3$ (M_W = 347.38) *m/z* 348.2 ((M+H)$^+$); HRMS for $C_{20}H_{18}N_3O_3$: Calcd 348.1348, found 348.1348; UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 441 (0.40), 351 (0.65), 307 (1.25), 274 (1.58); mp > 300 °C, decomposition.

4.3.2. 2-Methyl-6-(4-hydroxyphenyl)-8-(4-(*N*,*N*)-dimethylaminophenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (7d)

Compound **7d** was prepared according to the general procedure B using methylglyoxal (90 μL, 2 equiv), HCl 37% (90 μL, 4.2 equiv), **6d** (80 mg, 0.26 mmol, 1 equiv), ethanol (10 mL); 66 mg of **7d** as yellow solid; 70% yield; ^{1}H NMR (300 MHz, CD_3OD) δ 2.56 (s, 3H, 10-H), 3.35 (s, 6H, 19-H), 6.93 (d, $^3J_{18,17}$ = 8.4 Hz, 2H, 13-H), 7.58 (d, $^3J_{13,12}$ = 9.0 Hz, 2H, 17-H), 7.89 (d, $^3J_{17-18}$ = 8.4 Hz, 2H, 12-H), 8.21 (d, $^3J_{12,13}$ = 9.0 Hz, 2H, 16-H), 8.48 (s, 1H, 5-H). ^{13}C NMR (125 MHz, CD_3OD) δ 9.9 (10-C), 44.2 (19-C), 109.7 (5-C), 116.9 (13-C), 118.6 (17-C), 122.8 (2-C or 3-C), 126.1 (11-C), 126.8 (9-C), 128.7 (15-C), 129.6 (12-C), 132.4 (16-C), 139.3 (2-C or 3-C), 141.5 (6-C), 144.7 (8-C), 146.8 (18-C), 160.8 (14-C); MS (APCI) $C_{21}H_{19}N_4O_2$ (M_W = 360.42) *m/z* 361.4 ((M+H)$^+$); HRMS for $C_{21}H_{21}N_4O_2$: calculated 361.1665, found 361.1660; UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 453 (1.08), 394 (1.62), 260 (2.27); mp > 300 °C, decomposition.

4.3.3. 2-Methyl-6-(4-hydroxyphenyl)-8-(4-trifluoromethylphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (7e)

Compound **7e** was prepared according to the general procedure B using methylglyoxal (280 μL, 2.2 equiv), HCl 37% (250 μL, 3.6 equiv), **6e** (282 mg, 0.84 mmol, 1 equiv), ethanol (15 mL); 325 mg of **7e** as pale yellow solid; 92% yield; ^{1}H NMR (300 MHz, CD_3OD) δ 2.54 (s, 3H, 10-H), 6.85 (d, $^3J_{18,17}$ = 8.4 Hz, 2H, 13-H), 7.88 (d, $^3J_{17,18}$ = 8.4 Hz, 2H, 12-H), 7.96 (d, $^3J_{13,12}$ = 8.1 Hz, 2H, 17-H), 8.20 (d, $^3J_{12,13}$ = 7.9 Hz, 2H, 16-H), 8.55 (s, 1H, 5-H). ^{13}C NMR

(125 MHz, CD_3OD) δ 9.2 (10-C), 110.1 (5-C), 116.8 (13-C), 119.8 (2-C or 3-C), 126.5 (9-C), 126.7 (11-C), 127.1 (d, $^3J_{C-F}$ = 3.6 Hz, 17-C), 129.1 (12-C), 130.8 (16-C), 133.9 (d, $^2J_{C-F}$ = 32.8 Hz, 18-C), 138.4 (15-C), 138.8 (2-C or 3-C), 143.5 (6-C), 144.5 (8-C), 160.7 (14-C). ^{19}F NMR (282 MHz, CD_3OD) δ −62.7 (19-F); MS (APCI) $C_{20}H_{14}F_3N_3O_2$ (M_W = 385.35) *m/z* 386.3 ($(M+H)^+$); Anal. Calcd for $C_{20}H_{14}F_3N_3O_2{\cdot}HCl{\cdot}1.5H_2O$ (448.84): C, 53.52; H, 3.71; N, 9.36. Found: C, 53.40; H, 3.78; N, 9.24. UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 450 (0.38), 353 (0.47), 307 (1.21), 270 (2.26); mp > 300 °C, decomposition.

4.3.4. 2-Methyl-6-(4-hydroxyphenyl)-8-(4-fluorophenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (7f)

Compound **7f** was prepared according to the general procedure B using methylglyoxal (190 μL, 2.5 equiv), HCl 37% (190 μL, 4.7 equiv), **6f** (137 mg, 0.49 mmol, 1 equiv), ethanol (10 mL); 133 mg of **7f** as yellow solid; 73% yield; ^1H NMR (300 MHz, CD_3OD) δ 2.54 (s, 3H, 10-H), 6.86 (d, $^3J_{17,16}$ = 8.8 Hz, 2H, 13-H), 7.41 (t, $^3J_{13,12} = {}^3J_{13-F}$ = 8.7 Hz, 2H, 17-H), 7.88 (d, $^3J_{16,17}$ = 8.7 Hz, 2H, 12-H), 8.09 (dd, $^3J_{12,13}$ = 8.9 Hz and $^4J_{12-F}$ = 5.3, 2H, 16-H), 8.50 (s, 1H, 5-H). ^{13}C NMR (75 MHz, CD_3OD) δ 6.21 (10-C), 106.6 (5-C), 113.7 (13-C), 114.3 (d, $^2J_{C-F}$ = 22.2 Hz, 17-C), 116.8 (2-C or 3-C), 123.4 (9-C), 123.7 (11-C), 126.1 (12-C), 127.9 (15-C), 129.6 (d, $^3J_{C-F}$ = 9.0 Hz, 16-C), 135.6 (2-C or 3-C), 140.2 (6-C), 142.0 (8-C), 157.6 (14-C), 163.2 (d, $^1J_{C-F}$ = 249 Hz, 18-C). ^{19}F NMR (282 MHz, CD_3OD) δ −108.4 (18-F); MS (APCI) $C_{19}H_{14}FN_3O_2$ (M.W. = 335.34) *m/z* 336.3 ($(M+H)^+$); Anal. Calcd for $C_{19}H_{14}FN_3O_2{\cdot}HCl{\cdot}H_2O$ (389.82): C, 58.54; H, 4.40; N, 10.78. Found: C, 58.70; H, 4.51; N, 10.57. UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 444 (0.45), 352 (0.44), 306 (1.21), 268 (2.13); mp > 300 °C, decomposition.

4.3.5. 2-Methyl-6-(4-hydroxyphenyl)-8-(4-acetylphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (7g)

Compound **7g** was prepared according to the general procedure B using methylglyoxal (61 μL, 2.2 equiv), HCl 37% (80 μL, 3.6 equiv), **6g** (81 mg, 0.27 mmol, 1 equiv), ethanol (5 mL); 91 mg of **7g** as red clay solid; 85% yield; ^1H NMR (300 MHz, DMSO-d_6) δ 2.42 (s, 3H, 10-H), 2.67 (s, 3H, 20-H), 6.89 (d, $^3J_{19,18}$ = 8.7 Hz, 2H, 13-H), 7.99 (d, $^3J_{18,19}$ = 8.7 Hz, 2H, 12-H), 8.15 (d, $^3J_{12,13}$ = 8.4 Hz, 2H, 16-H), 8.48 (s, 1H, 5-H), 8.75 (d, $^3J_{13,12}$ = 8.4 Hz, 2H, 17-H); MS (FAB^+) $C_{21}H_{17}N_3O_3$ (M_W = 359.39) *m/z* 360.1 ($(M+H)^+$); Anal. Calcd for $C_{21}H_{17}N_3O_3{\cdot}HCl{\cdot}0.25H_2O$ (400.36): C, 63.0; H, 4.62; N, 10.49. Found: C, 63.03; H, 4.65; N, 10.31. UV–vis (MeOH, 5×10^{-5} M, λ_{max} (ε)): 458 (0.28), 362 (0.65), 312 (1.5), 273 (2.7); mp > 300 °C, decomposition.

4.4. Biological assays: permeability in the hamster cheek pouch submitted to ischemia/reperfusion (I/R)

Male Syrian hamsters (Mesocricetus auratus, Engle Labs Farmersburg, Indianapolis, USA), 7–10 weeks old, weighing 90–130 g, were used for these studies. Anesthesia was induced by an intraperitoneal injection of 0.1–0.2 ml of sodium pentobarbital (Sanofi, France, 60 mg/ml) and maintained with α-chloralose (100 mg/kg) administered through the femoral vein. The femoral artery was cannulated for measurement of arterial blood pressure. Throughout surgery and the subsequent experiment, the temperature of the animals was kept at 37.5 °C with a heating pad controlled by a rectal thermistor. A tracheal tube was inserted to facilitate spontaneous breathing. The hamster was placed on a microscope stage. The cheek pouch was gently averted and pinned with 4–5 needles into a circular well filled with silicone rubber to give a flat bottom layer thus avoiding stretching of the tissue but preventing shrinkage. In this position, the pouch was submerged in a superfusion solution that continuously flushed the pool of the microscope stage. The superfusion solution was a *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-supported HCO_3^--buffered saline solution (composition in mM: NaCl 110.0, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, $NaHCO_3$ 18.0, Hepes 15.39 and Hepes Na^+-salt 14.61) whose temperature was maintained at 36.5 °C and the superfusion rate was 6 ml/min. The pH was set to 7.40 by bubbling the solutions continuously with 5% CO_2 in 95% N_2. Thirty min after the completion of the preparative procedure, fluorescein isothiocyanate (FITC)-dextran, molecular weight 150,000 Da (Sigma), with a degree of substitution of 2 FITC molecules per 1000 glucose molecules in the polysaccharide chain was given in a dose of 25 mg/100 g body weight as an intravenous injection of 5% solution in 0.9% saline. Observations were made with a Leitz Ortholux microscope with X3.5 objective and X10 oculars. The light source was a 100 W mercury DC lamp (Irem model EL-XH5 P/L). The specific light filters used for observations in fluorescent light (Leitz BG12, BG38, GG455 and KP490) were positioned between the light source and the condenser to give a light for optimal excitation at 490 nm of FITC-dextran. A barrier filter (K530) was placed between the objective and the eyepieces. Observations of the number of leakage sites (=leaks) were made by scanning manually the total observation area twice at X35 magnification. The fluorescent spots formed at leakage sites could be traced when they reached a certain minimal size and fluorescent intensity. Each site was classified as a leakage site when its diameter was larger than 100 μm. The number of leakage sites is reported per square centimeter. All hamsters with the prepared area showing spontaneous nonfading leaks or more than 10 fading leaks during the first 30 min control period, after FITC-dextran was given, were discarded. A group of three animals were treated by gavage with 0.2 ml of a solution containing the compound at 3 mg/kg while another group of three animals received 0.2 ml of the solvent. Each animal was treated with the compound or the solvent 30 min before anesthesia. The preparations were used to investigate the effect of local ischemia, which was obtained by a cuff, made of thin latex tubing, mounted around the neck of the everted pouch where it leaves the mouth of the hamster. The cuff can be placed without any visible interference with local blood flow. The intratubular pressure of the cuff can be rapidly increased by air compression using a syringe, and also be rapidly decreased at evacuation; an intratubular of 200–220 mmHg resulted in a complete arrest of the microvascular flow within a few seconds. Throughout the 30-min occlusion period, minor adjustments of blood movement could be observed in the larger blood vessels. At evacuation, an immediate increase in blood flow occurred and the blood flow eventually returned to the pre-occlusion values. The maximal number of leaky sites per cm^2 was determined 30 min after the start of reperfusion. The mean of the leaky sites was calculated for the solvent and the treated groups of animals and the percentage of inhibition was calculated as follow: $[1 - (\text{mean treated})/(\text{mean control})] \times 100$.

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