Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 1037–1044

BIOORGANIC & MEDICINAL CHEMISTRY

Catechol Derivatives of Aminopyrazine and Cell Protection Against UVB-Induced Mortality

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Received 5 October 2000; accepted 27 November 2000

Abstract—A series of 5-aryl- and 3,5-*bis*-aryl-2-amino-1,4-pyrazine derivatives **4** and **6**, and related imidazolopyrazinones **7**, has been synthesized, the aryl groups of which are catechol and/or phenol substituents. These compounds, tested against human keratinocyte cells stressed by UVB irradiation, showed high antioxidative properties. One compound (**6f**) was more active than EGCG/ECG (green tea extract) in reducing cell mortality.

Introduction

Reactive oxygen species (ROS) are very important mediators of cell injury due to damages they can inflict to biological macromolecules such as membrane lipids, proteins and nucleic acids. ROS are directly or indirectly implicated in the pathogenesis of various disorders such as cardiovascular diseases, reperfusion injury, Alzheimer's and other neurodegenerative diseases, as well as in the ageing process.[1–4] Therefore, a growing interest in the protective role of antioxidants in these pathologies has been observed over the last 10 years. Natural antioxidants[5–7] (vitamin E, vitamin C, β-carotene) and polyphenolic compounds extracted from green tea leaves[8] have beneficial effects in protecting against these diseases by preventing cellular damages induced by ROS.[9–15] Therefore, the search of synthetic antioxidants[16] generally makes use of (poly)phenolic moieties as free radical scavengers[17] incorporated in the active structures.[18–21]

Our interest in the evolutionary origins of marine bioluminescence[22] led us to disclose the antioxidative properties[23–25] of coelenterazine (CLZ) and coelenteramine (CLM) (Scheme 1).

CLZ is the chromophoric ligand[26,27] of aequorin[28] and other calcium-sensitive photoproteins found in marine bioluminescent organisms. Its oxidative metabolism produces coelenteramide (CLA) and CLM, according to the mechanism outlined in Scheme 2.[29] We previously demonstrated that synthetic analogues of the natural CLZ are endowed with excellent antioxidative properties;[30,31] they are able to scavenge a wide variety of ROS such as superoxide anion, singlet oxygen, peroxinitrite, hydroxyl, alkyl and peroxyl radicals. Possessing also chain-breaking properties, CLM produced in situ prolongs the antioxidant action of its mother compound. Such an antioxidant cascade could be of great interest in medicinal chemistry. We therefore examined the possibility of improving the biological activities of aminopyrazine **2** and related imidazolopyrazinone **1** derivatives by the introduction of phenol and catechol groups as the R′ and R″ substituents (Scheme 2). In this paper, we describe the synthesis of the target compounds **4** and **6** (Scheme 3), and their evaluation in a cellular test representative of their antioxidative efficiency.

Results

5-Substituted 2-amino-1,4-pyrazines **4** were readily prepared by Pd-mediated cross-coupling of arylboronic acids to 5-bromo-2-amino-1,4-pyrazine **3**.[32] The Suzuki reaction is usually applied for the construction of functionalized

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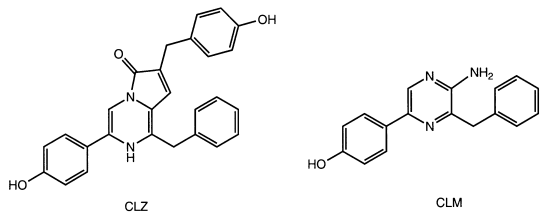
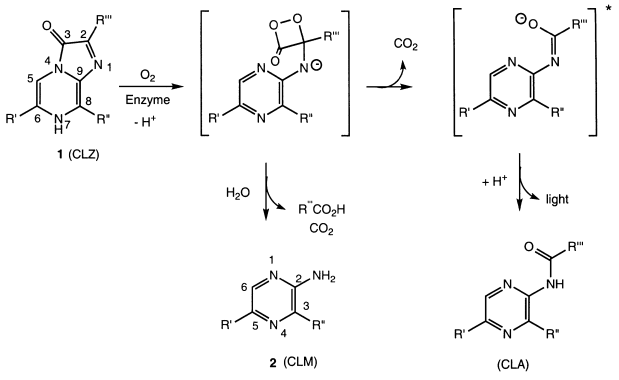
0968-0896/01/$ - see front matter
PII: S0968-0896(00)00321-7

biaryl systems;[33] the experimental conditions are compatible with the presence of protected phenol and catechol moieties.[34] Thus, compounds **4a**,[35] **4b**, **4c** and **4d** were obtained by reacting **3** with 4-methoxy-,3,4-methylenedioxy-,3,4-dimethoxy- and 2,4-dimethoxyphenylboronic acids, respectively, in the presence of [1,4-*bis*(diphenylphosphino) butane]-palladium (II) chloride as the catalyst (Scheme 3). The 3,5-disubstituted aminopyrazines **6** were synthesized from the precursors **4a–b** in two steps, including bromination with *N*-bromonosuccinimide to give the intermediates **5a–b**, followed by the Suzuki coupling reaction as previously conducted. Compounds **6b–6d** were isolated in good yields after column chromatography on silica gel. The symmetrically substituted derivative **6a** was obtained in one step by making a double cross-coupling reaction of 4-methoxyphenyl-boronic acid with 2-amino-3,5-dibromo-1,4-pyrazine **3b**[36] (Scheme 3).



Scheme 1.

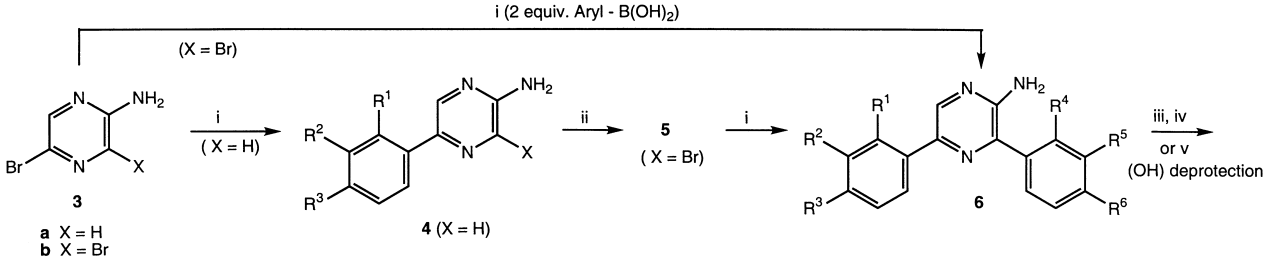


Scheme 2.

Deprotection of the methoxy-aryl functions was realized by nucleophilic displacement with ethanethiolate in hot dimethylformamide.[37] This method applied to compounds **4a** and **6a** gave, respectively, the phenol-free derivatives **4e**[38] and **6e** (Scheme 3). The methylene-catechol and dimethoxy-aryl groups were more efficiently deprotected in acidic medium, by using aluminium trichloride (or tribromide) in the presence of ethanethiol.[39] Thus, the precursors **4c**, **4d**, **6c** and **6d** furnished, respectively, the catechol- and polyphenol-free compounds **4f**, **4g**, **6f** and **6g** (Scheme 3).

Three representative imidazolopyrazinones were prepared by reacting deprotected aminopyrazines with methylglyoxal in a mixture of aqueous HCl and ethanol, according to a previously described procedure[30,40] (Scheme 4).

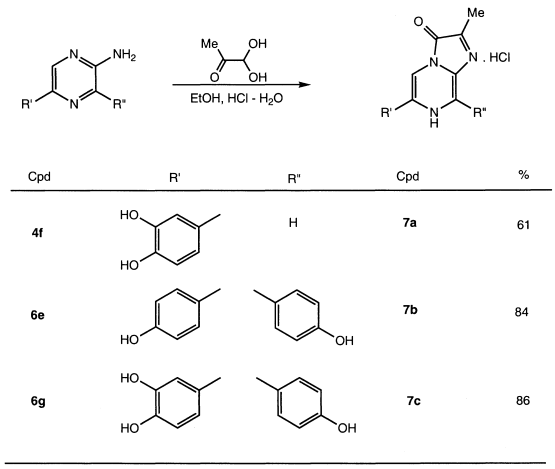
The OH-protected derivatives **4a–d**, **5a–b** and **6a–d** could be purified, as usual, by column-chromatography. On the other hand, the OH-free compounds **4e–g**, **6e–g** and **7a–c** were unstable under the normal chromatographic conditions, most probably due to their sensitivity towards oxidation. They were roughly purified by several washings and isolated in the form of hydrates and/or hydrochlorides, as ascertained by the elemental analysis. All the derivatives were properly characterized by their ^{1}H and ^{13}C NMR spectral data (see Experimental).



	R^1	R^2	R^3	%
a	H	H	OMe	75
b	H	O–CH_2–O		68
c	H	OMe	OMe	70
d	OMe	H	OMe	51
e	H	H	OH	80
f	H	OH	OH	29
g	OH	H	OH	51

	R^1	R^2	R^3	R^4	R^5	R^6	%
a	H	H	OMe	H	H	OMe	69
b	H	H	OMe	H	O–CH_2–O		92
c	H	H	OMe	H	OMe	OMe	98
d	H	O–CH_2–O		H	H	OMe	86
e	H	H	OH	H	H	OH	80
f	H	H	OH	H	OH	OH	71
g	H	OH	OH	H	H	OH	93

Scheme 3. (i) Aryl-$B(OH)_2$, $PdCl_2$ (dppb) catal., toulene, reflux, 24 h; (ii) NBS, H_2O, DMSO, 20 °C, 4 h; (iii) EtSNa, DMF, 100 °C, 24 h; (iv) EtSH, $AlCl_3$, 20 °C, 24 h; (v) EtSH, $AlBr_3$, 20 °C, 24 h.



Scheme 4.

Solar UV radiations (UVB: 290–320 nm; UVA: 320–380 nm) interact with several biomolecules to generate free radicals producing cellular damages. In particular, UVB radiations affect drastically the survival of keratinocyte cells.[41,42] Therefore, the protective effect of the synthesized aminopyrazines and imidazolopyrazinones has been evaluated on human keratinocytes submitted to UVB irradiation. Cellular death could be determined by measuring the amount of lactate dehydrogenase (LDH) released into the cell culture supernatant.[43,44] The irradiation of HaCaT cells at 200 mJ/cm^2 induced the release of 20% of the total cellular LDH after 24 h, comparatively to the non-irradiated cells (control: 0% LDH release). Compounds **4c**, **4f**, **4g**, **6f** and **7a** were tested at 50 μM and compared to catechol. With the exception of **4c** (and catechol), the compounds were non-toxic for the cells (Fig. 1; 0 mJ/cm^2). The UVB-induced mortality (Fig. 1; 200 mJ/cm^2) decreased when compounds **4c**, **4f** and **4g** were added in the culture medium. The protection is significantly reduced when the catechol function of **4f** (38% of protection) is methylated (**4c**; 17% of protection). Compound **7a**, the imidazolopyrazinone corresponding to the catechol derivative **4f**, showed a high protection (72%), while the *bis*-aryl aminopyrazine **6f** confered a total protection (100%) against UVB damages (Fig. 1). Compound **6g** behaved similarly (results not shown). Catechol used as reference in our test slightly increased UVB toxicity.

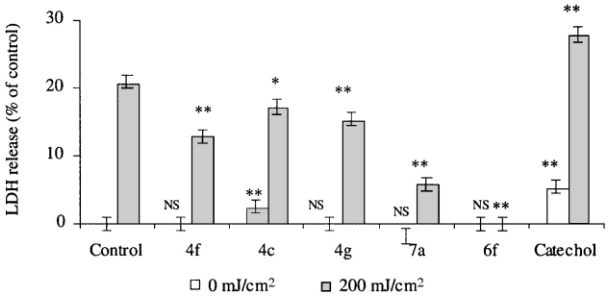


Figure 1. Effects of aminopyrazine derivatives compared to catechol on UVB-induced cytotoxicity. HaCaT cells were pre-treated with 50 μM compounds and then exposed to UVB (200 mJ/cm^2). The cells were incubated with a further 24 h before determining LDH released. Data are expressed as means + SD of six experiments. NS, non significant; $^{**}p < 0.01$, $^{*}p < 0.05$: treated cells irradiated and non-irradiated were compared to non-treated cells (control) irradiated and non-irradiated, respectively.

As the catechol-phenol derivative **6f** was the most efficient compound, it was compared to green tea extract (Hauser, 77.6%) containing mainly EGCG ((−)-epigallocatechin gallate) and ECG ((−)-epicatechin gallate) whose protective effect against UVB damages is well established.[45–47] Both **6f** and EGCG/ECG prevented LDH-release in a dose-dependent manner (Fig. 2). At lower concentrations, **6f** was more efficient than EGCG/ECG; indeed, at 7.8×10^{-7} M, the protective effects of **6f** and green tea were respectively of 35 and 3%. For both compounds, maximum protection was observed at 6.25×10^{-6} M (Table 1). The *bis*-phenolic derivatives **6e** and **7b** protected less efficiently the keratinocyte cells; this was particularly visible when using higher concentrations (Fig. 2, Table 1). The differences between aminopyrazine (**6e**) and imidazolopyrazinone (**7b**) appeared non significant.

Conclusion

The lethal damage induced by UVB irradiation is partly mediated by reactive oxygen species (ROS)-caused oxidative injury.[48,49] Consequently, the protective effect of

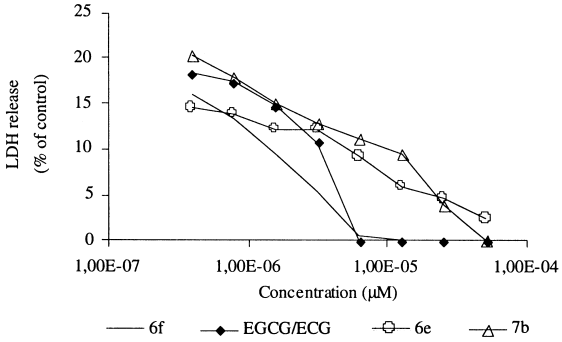


Figure 2. Dose effects of the compounds **6f**, **6e**, **7b**, and EGCG/ECG on UVB-induced cytotoxicity measured by LDH released (as described in Fig. 1).

Table 1. Percentage of protection by tested compounds against UVB-induced cytotoxicity[a,b]

Concn. (M)	Green tea	6f	6e	7b
3.9×10^{-7}	0.0 ^{NS}	21.6**	24.4*	1.0^{NS}
7.8×10^{-7}	3.5 ^{NS}	34.7**	27.7**	3.0^{NS}
1.56×10^{-6}	12.2 ^{NS}	53.5**	36.4**	27.4*
3.12×10^{-6}	40.3**	73.9**	36.2**	36.9**
6.25×10^{-6}	99.7**	97.8**	52.1**	45.4**
1.25×10^{-5}	100.0**	100.0**	69.0**	53.4**
2.5×10^{-5}	100.0**	100.0**	75.8**	80.9**
5×10^{-5}	100.0**	100.0**	87.7**	100.0**

[a]Results described in Figure 2 were expressed as the quotient of LDH released by HaCaT cells irradiated and treated with the compounds at different concentrations divided by LDH released by irradiated but non-treated cells.

[b]NS: non significant, $^{**}p < 0.01$, $^{*}p < 0.05$: treated cells irradiated were compared to non-treated cells (control).

tested compounds towards the mortality of cells submitted to UVB, gives a direct measurement of the antioxidative potential of these compounds. Our results suggest that the catechol motif, and the phenol motif in a lesser manner, in conjugative interaction with the 2-amino-1,4-pyrazine nucleus confer strong antioxidative properties. Indeed, at low concentrations, compound **6f** was more active than EGCG/ECG extracted from green tea. This structural framework is now considered in our laboratory for the development of new lead-compounds that could be useful in cosmetic and therapeutic fields. Since the monocyclic derivatives (**4**, **6**) were as active as the corresponding bicyclic ones (**7**), the previous compounds will be particularly studied and functionalized at the NH_2 position in view to optimalize both their activity and their lipophilic/hydrophilic balance.

Experimental

Synthesis

Solvents were dried prior to use. Reagents (Aldrich or Acros) were used as purchased. Melting points (uncorrected) were determined on an electrothermal apparatus. ^{1}H (200 or 300 MHz) and ^{13}C (50 or 75 MHz) NMR spectra were recorded on Varian Gemini 200 and 300 spectrometers. Chemical shifts are reported as δ values downfield from tetramethyl silane (TMS). The mass spectra (FAB or EI modes) were obtained on a Finnigan MAT TSQ-70 instrument. Thin-layer chromatography (TLC) were carried out using silica gel 60 F_{254} plates (0.2 mm, Merck) and the spots were visualized by UV (254 nm). Column chromatographies were performed on Merck silica gel 60 (70–230 mesh). Elemental analyses were obtained at the University College of London.

General procedure for the preparation of compounds 4a–d

A mixture of *bis* (benzonitrile) palladium (II) dichloride (0.05 equiv) and 1,4-*bis* (diphenylphosphino) butane (dppb) (0.06 equiv) in dry toluene (2 mL/mmol **3a**) was stirred at 20 °C, under argon atmosphere for 30 min, until a creamy orange slurry of [1,4-*bis*(diphenylphosphino)butane]-palladium (**II**) chloride was formed. Then, 2-amino-5-bromo-1,4-pyrazine **3a** (1 equiv), arylboronic acid (1.1 equiv), ethanol (0.43 mL/mmol **3a**), 1 M aqueous sodium carbonate (1 equiv) and toluene (2 mL/mmol **3a**) were added to the preformed catalyst. The mixture was refluxed for 24 h under stirring. After cooling, water (5 mL/mmol **3a**) and ethyl acetate (6.7 mL/mmol **3a**) were added. The aqueous phase was separated and extracted twice with ethyl acetate. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered over Celite, and concentrated in vacum. The crude coupling product **4** was purified by column-chromatography on silica gel.

2-Amino-5-(4′-methoxyphenyl)-1,4-pyrazine (4a). 4a was obtained from 220 mg of Pd (II), 249.1 mg of dppb, 2 g of **3a** (11.5 mmol), 1.84 g of *p*-methoxyphenylboronic acid, and 11.5 mL of 1 M sodium carbonate. Chromatography gave 1.8 g (78%) of pure **4a** as a red solid (R_f 0.18; ethyl acetate/cyclohexane, 3:5): mp 169.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.42 (d, 1H, J = 1.5 Hz, H-3), 8.05 (d, 1H, J = 1.5 Hz, H-6), 7.84 (d, 2H, J = 8.9 Hz), 7.01 (d, 2H, J = 8.9 Hz), 4.65 (s, 2H, NH_2), 3.88 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 159.9, 152.7 (C-2), 143.1 (C-6), 138.4 (C-5), 131.4 (C-3), 129.8, 126.9, 114.3, 55.3; EIMS m/z 201 (M^+), 186, 158. Anal. calcd for $C_{11}H_{11}N_3O$: C, 65.66; H, 5.51; N, 20.88. Found: C, 65.02; H, 5.45; N, 20.64.

2-Amino-5-(3′,4′-methylenedioxyphenyl)-1,4-pyrazine (4b). 4b was obtained from 83 mg of Pd (II), 110 mg of dppb, 750 mg of **3a** (4.31 mmol), 787 mg of 3,4-methylenedioxyphenylboronic acid, and 4.3 mL of 1 M sodium carbonate. Chromatography gave 634 mg (68%) of pure **4b** as a pale brown solid (R_f 0.19; ethyl acetate/cyclohexane, 3:5): mp 159.8 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, 1H, J = 1.5 Hz, H-3), 8.02 (d, 1H, J = 1.5 Hz, H-6), 7.37 (dd, 1H, J = 1.8 and 7.9 Hz), 7.34 (d, 1H, J = 1.8 Hz), 6.88 (d, 1H, J = 7.9 Hz), 6.0 (s, 2H), 4.75 (s, 2H, NH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.9 (C-2), 148.5, 148.0, 138.8 (C-6), 138.6 (C-5), 131.5 (C-3), 128.5, 119.6, 108.8, 106.5, 101.4; EIMS m/z 215 (M^+). Anal. calcd for $C_{11}H_9N_3O_2$: C, 61.35; H, 4.18; N, 19.51. Found: C, 61.08; H, 4.16; N, 19.21.

2-Amino-5-(3′,4′-dimethoxyphenyl)-1,4-pyrazine (4c). 4c was obtained from 48 mg of Pd (II), 64 mg of dppb, 435 mg of **3a** (2.5 mmol), 500 mg of 3,4-dimethoxyphenylboronic acid, and 2.5 mL of 1 M sodium carbonate. Chromatography gave 404 mg (70%) of pure **4c** as a pale brown solid (R_f 0.11; ethyl acetate/cyclohexane, 1:1) : mp 191 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (d, 1H, J = 1.5 Hz, H-3), 8.04 (d, 1H, J = 1.5 Hz, H-6), 7.49 (d, 1H, J = 2.1 Hz), 7.39 (dd, 1H, J = 2.1 and 8.3 Hz), 6.94 (d, 1H, J = 8.3 Hz), 4.62 (s, 2H, NH_2), 3.98 (s, 3H), 3.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.9 (C-2), 149.6, 149.5, 143.0 (C-5), 138.7 (C-6), 131.5 (C-3), 130.7, 118.1, 111.4, 109.0, 56.2; EIMS m/z 231 (M^+), 216. Anal. calcd for $C_{12}H_{13}N_3O_2$: C, 62.32; H, 5.66; N, 18.17. Found : C, 61.68; H, 5.69; N, 17.42.

2-Amino-5-(2′,4′-dimethoxyphenyl)-1,4-pyrazine (4d). 4d was obtained from 96 mg of Pd (II), 128 mg of dppb, 869 mg of **3a** (5 mmol), 1 g of 2,4-dimethoxyphenylboronic acid, and 5.3 mL of 1 M sodium carbonate. Chromatography gave 590 mg (51%) of pure **4d** as an orange solid (R_f 0.15; ethyl acetate-cyclohexane, 1:1): mp 122.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, 1H, J = 1.5 Hz, H-3), 8.05 (d, 1H, J = 1.5 Hz, H-6), 7.68 (d, 1H, J = 8.5 Hz), 6.60 (dd, 1H, J = 2.4 and 8.5 Hz), 6.55 (d, 1H, J = 2.4 Hz), 4.63 (s, 2H, NH_2), 3.84 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.1, 157.9, 152.4 (C-2), 142.9 (C-6), 141.4 (C-5), 131.5 (C-3), 131.1, 119.5, 105.3, 99.0, 55.7, 55.6; EIMS m/z 231 (M^+), 214. Anal. calcd for $C_{12}H_{13}N_3O_2$: C, 62.32; H, 5.66; N, 18.17. Found: C, 62.09; H, 5.67; N, 18.87.

General procedure for the preparation of compounds 5a–b. To a solution of 2-amino-5-aryl-1,4-pyrazine **4** (1 equiv) and water (2.78 equiv) in DMSO (2 mL/mmol **4**) was added, in small portions, *N*-bromosuccinimide (NBS, 1.1 equiv). The mixture was stirred for 4 h at 20 °C, then

diluted with water (10 mL/mmol **4**) and extracted three times with ehtyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuum. The crude product **5** was purified by column-chromatography on silica gel.

2-Amino-3-bromo-5-(4′-methoxyphenyl)-1,4-pyrazine (5a). 5a was obtained from 1.7 g of **4a** (8.45 mmol), 0.423 mL of water, and 1.65 g of NBS. Chromatography gave 2.26 g (80%) of pure **5a** as a yellow solid (R_f 0.51; ethyl acetate/cyclohexane, 3:5): mp 167.9 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.32 (s, 1H, H-6), 7.79 (d, 2H, J = 8.8 Hz), 6.95 (d, 2H, J = 8.8 Hz), 5.09 (s, 2H, NH_2), 3.83 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 160.1, 150.7 (C-2), 143.2 (C-5), 137.0 (C-6), 129.5, 127.0, 125.7 (C-3), 114.2, 55.3; EIMS m/z 281 (M^+), 279 (M^+), 266, 264. Anal. calcd for $C_{11}H_{10}BrN_3O$: C, 47.16; H, 3.59; N, 15.0. Found: C, 46.84; H, 3.47; N, 14.62.

2-Amino-3-bromo-5-(3′,4′-methylenedioxyphenyl)-1,4-pyrazine (5b). 5b was obtained from 600 mg (2.78 mmol) of **4b**, 0.140 mL of water, and 546 mg of NBS. Chromatography gave 784 mg (96%) of pure **5b** as a pale brown solid (R_f 0.39; ethyl acetate/cyclohexane, 3:5): mp 157 °C; ^{1}H NMR (200 MHz, acetone-d_6) δ 8.48 (s, 1H, H-6), 7.49 (dd, 1H, J = 1.7 and 8.1 Hz), 7.44 (d, 1H, J = 1.7 Hz), 6.92 (d, 1H, J = 8.1 Hz), 6.16 (s, 2H, NH_2), 6.05 (s, 2H); ^{13}C NMR (50 MHz, acetone-d_6) δ 153.1 (C-2), 149.5, 149.0, 143.2 (C-5), 138.6 (C-6), 131.3, 125.6 (C-3), 120.1, 109.4, 106.5, 102.4; EIMS m/z 295 (M^+), 293 (M^+). Anal. calcd for $C_{11}H_8BrN_3O$: C, 45.88; H, 2.72; N, 14.28; Br, 27.17. Found: C, 45.93; H, 2.82; N, 13.88; Br, 26.97.

General procedure for the preparation of compounds 6b–d (unsymmetrical substitution)

The procedure described for compounds **4a**–**d** was applied, using preformed palladium catalyst (0.05 equiv), 2-amino-3-bromo-5 aryl-1,4-pyrazine **5a**–**b** (1 equiv), arylboronic acid (1.1 equiv), and 1 M aqueous sodium carbonate (1 equiv) in ethanol (0.43 mL/mmol **5**) and toluene (4 mL/mmol **5**).

2-Amino-3-(3′,4′-methylenedioxyphenyl)-5-(4′-methoxyphenyl)-1,4-pyrazine (6b). 6b was obtained from 32 mg of Pd (II), 42 mg of dppb, 440 mg (1.66 mmol) of **5a**, 303 mg of 3,4-methylenedioxyphenylboronic acid, and 1.7 mL of 1M sodium carbonate. Chromatography gave 462 mg (92%) of pure 6b as a yellow solid (R_f 0.25; ethyl acetate/cyclohexane, 1:1): mp 82.2 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H, H-6), 7.88 (d, 2H, J = 8.8 Hz), 7.34 (d, 1H, J = 1.7 Hz), 7.32 (m, 1H), 6.97 (d, 2H, J = 8.8 Hz), 6.92 (d, 1H, J = 7.7 Hz), 6.02 (s, 2H), 4.85 (s, 2H, NH_2), 3.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.0, 150.4 (C-2), 148.5, 143.0 (C-5), 139.3 (C-3), 136.6 (C-6), 131.6, 129.9, 127.1, 122.2, 114.3, 109.3, 108.8, 101.6, 55.5; EIMS m/z 321 (M^+). Anal. calcd for $C_{18}H_{15}N_3O_3 + 0.1\ H_2O$: C, 66.91; H, 4.71; N, 13.01. Found: C, 66.86; H, 4.61; N, 12.88.

2-Amino-3-(3′,4′-dimethoxyphenyl)-5-(4′-methoxyphenyl)-1,4-pyrazine (6c). 6c was obtained from 41 mg of Pd (II), 55 mg of dppb, 600 mg (2.14 mmol) of **5a**, 429 mg of 3,4-dimethoxyphenylboronic acid, and 2.2 mL of 1 M sodium carbonate. Chromatography gave 720 mg (98%) of pure **6c** as a beige solid (R_f 0.26; ethyl acetate/cyclohexane, 1:1): mp 152.7 °C (recrystallization from cold ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H, H-6), 7.90 (d, 2H, J = 8.6 Hz), 7.41 (dd, 1H, J = 1.9 and 8.2 Hz), 6.37 (d, 1H, J = 1.9 Hz), 6.99 (d, 2H, J = 8.6 Hz), 6.97 (d, 1H, J = 8.2 Hz), 4.82 (s, 2H, NH_2), 3.95 (s, 3H), 3.94 (s, 3H), 3.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.9, 150.6 (C-2), 149.9, 149.6, 143.0 (C-5), 139.5 (C-3), 136.8 (C-6), 130.3, 130.0, 127.2, 120.8, 114.3, 111.9, 111.3, 56.2, 55.5; EIMS m/z 337 (M^+). Anal. calcd for $C_{19}H_{19}N_3O_3 + 0.2\ H_2O$: C, 66.94; H, 5.69; N, 12.33. Found: C, 66.93; H, 5.64; N, 12.09.

2-Amino-3-(4′-methoxyphenyl)-5-(3′,4′-methylenedioxyphenyl)-1,4-pyrazine (6d). 6d was obtained from 49 mg of Pd (II), 65 mg of dppb, 747 mg of **5b** (2.54 mmol), 425 mg of 4-methoxyphenylboronic acid, and 2.6 mL of 1 M sodium carbonate. Chromatography gave 702 mg (86%) of pure **6d** as a yellow solid (R_f 0.20; ethyl acetate/cyclohexane, 3:5): mp 181.2 °C; ^{1}H NMR (200 MHz, acetone-d_6) δ 8.42 (s, 1H, H-6), 7.82 (d, 2H, J = 8.9 Hz), 7.56 (dd, 1H, J = 1.8 and 8.7 Hz), 7.53 (d, 1H, J = 1.8 Hz), 7.07 (d, 2H, J = 8.9 Hz), 6.92 (d, 1H, J = 8.7 Hz), 6.03 (s, 2H), 5.63 (s, 2H, NH_2), 3.88 (s, 3H); ^{13}C NMR (50 MHz, acetone-d_6) δ 161.2, 152.6 (C-2), 149.4, 148.6, 142.2 (C-5), 137.7 (C-6), 133.1 (C-3), 131.3, 130.7, 127.8, 119.9, 115.1, 109.3, 106.7, 102.3, 55.8; EIMS m/z 321 (M^+). Anal. calcd for $C_{18}H_{15}N_3O_3 + 0.2\ H_2O$: C, 66.54; H, 4.74; N, 12.95. Found: C, 66.56; H, 4.98; N, 13.32.

General procedure for the preparation of compound 6a (symmetrical substitution). The procedure described for compounds **4a**–**d** was applied, using preformed palladium catalyst (0.1 equiv), 2-amino-3,5-dibromo-1,4-pyrazine **3b** (1 equiv), arylboronic acid (2.1 equiv), and 1 M aqueous sodium carbonate (2 equiv), in ethanol (0.86 mL/mmol **3b**) and toluene (8 mL/mmol **3b**).

2-Amino-3,5-bis(4′-methoxyphenyl)-1,4-pyrazine (6a). 6a was obtained from 306 mg of Pd (II), 408 mg of dppb, 2.02 g of **3b** (8 mmol), 2.55 g of 4-methoxyphenylboronic acid, and 16 mL of 1 M sodium carbonate. Chromatography gave 1.7 g (69%) of pure **6a** as a yellow solid (R_f 0.21; ethyl acetate/cyclohexane, 3:5): mp 136.6 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (s, 1H, H-6), 7.90 (d, 2H, J = 8.7 Hz), 7.75 (d, 2H, J = 8.7 Hz), 7.06 (d, 2H, J = 8.7 Hz), 6.98 (d, 2H, J = 8.7 Hz), 6.11 (s, 2H, NH_2), 3.81 (s, 3H), 3.78 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.5, 159.0, 151.4 (C-2), 139.8 (C-5), 137.7 (C-3), 136.4 (C-6), 129.9, 129.6, 129.5, 126.2, 114.0, 55.2, 55.1; EIMS m/z 307 (M^+). Anal. calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.57; N, 13.67. Found: C, 70.16; H, 5.56; N, 13.53.

General procedure for the deprotection of 4-methoxyphenyl group (method A)

A stirred solution of **4** (or **6**) (1 equiv), sodium ethanethiolate (4 equiv or 8 equiv) in DMF (2 mL/mmol **4** or 4 mL/mmol **6**) was heated, under argon atmosphere, at 100 °C for 24 h. Ethyl acetate (25 mL/mmol) and

aqueous ammonium chloride (20 mL/mmol; saturated solution) were added. The aqueous phase was extracted four times with ethyl acetate. The combined organic layers were washed twice with brine, dried over $MgSO_4$ and concentrated in vacuum. The crude solid was washed with ethyl acetate/ether (1:1).

2-Amino-5-(4′-hydroxyphenyl)-1,4-pyrazine (4e). 4e was obtained from 1.01 g of **4a** (5.02 mmol) and 1.69 g of EtSNa, as a yellow solid (750 mg, 80%): ^{1}H NMR (200 MHz, CD_3OD) δ 8.15 (d, 1H, J = 1.2 Hz, H-6), 7.71 (d, 1H, J = 1.2 Hz, H-3), 7.54 (d, 2H, J = 8.7 Hz), 6.73 (d, 2H, J = 8.7 Hz); ^{13}C NMR (50 MHz, CD_3OD) δ 158.9, 155.5 (C-2), 142.9 (C-5), 139.1 (C-6), 132.8 (C-3), 129.8, 127.9, 116.6; EIMS m/z 187 (M^+), 160 (M-HCN). Anal. calcd for $C_{10}H_9N_3O + 0.1\ H_2O$: C, 63.55; H, 4.87. Found: C, 63.33; H, 5.03.

2-Amino-3,5-bis(4′-hydroxyphenyl)-1,4-pyrazine (6e). 6e was obtained from 1.6 g of **6a** (5.21 mmol) and 3.5 g of EtSNa, as a yellow solid (1.28 g, 88%): mp 251.1 °C (recrystallization from chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.64 (s, 2H, OH), 8.35 (s, 1H, H-6), 7.79 (d, 2H, J = 8.7 Hz), 7.64 (d, 2H, J = 8.5 Hz), 6.88 (d, 2H, J = 8.7 Hz), 6.82 (d, 2H, J = 8.5 Hz), 5.99 (s, 2H, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.8, 157.3, 151.1 (C-2), 140.3 (C-5), 138.1 (C-3), 135.8 (C-6), 128.4, 128.2, 129.5, 126.3, 115.5, 115.4; EIMS m/z 279 (M^+). Anal. calcd for $C_{16}H_{13}N_3O_2$. 0.5 H_2O: C, 66.60; H, 5.86. Found: C, 66.85; H, 5.56.

General procedure for the deprotection of dimethoxyphenyl and methylenedioxyphenyl groups (method B)

To a stirred solution of $AlCl_3$ or $AlBr_3$ (4 equiv/ether function) in ethanethiol (5–10 mL/mmol of **4** or **6**), cooled at 0 °C (ice-water bath), was added protected **4** or **6** in one portion. The mixture was stirred for 18 h at 20 °C, then poured into water, acidified with 1 N HCl and extracted three times with ethyl acetate. The organic layer was washed with brine and 5% sodium carbonate. The aqueous phases were extracted twice with ethyl acetate, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuum.

2-Amino-5-(3′4′-dihydroxyphenyl)-1,4-pyrazine (4f). 4f was obtained from 700 mg of **4c** (3.03 mmol), 3.23 g of $AlCl_3$ and 20 mL of EtSH. Crystallization from chloroform gave pure **4f** (190 mg, 29%) as a red solid: mp 136 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.08 (s, 2H, OH), 8.39 (d, 1H, J = 1.3 Hz, H-6), 7.97 (d, 1H, J = 1.3 Hz, H-3), 7.41 (d, 1H, J = 2 Hz), 7.25 (dd, 1H, J = 2 and 8.2 Hz), 6.85 (d, 1H, J = 8.2 Hz), 6.43 (s, 2H, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.2 (C-2), 145.4, 145.1, 139.8 (C-5), 137.7 (C-6), 131.0 (C-3), 128.7, 116.0, 115.8, 112.4; FAB MS m/z 204 ($M + H^+$). Anal. calcd for $C_{10}H_9N_3O_2 + H_2O$: C, 54.25; H, 4.97. Found: C, 54.11; H, 4.90.

2-Amino-5-(2′4′-dihydroxyphenyl)-1,4-pyrazine (4g). 4g was obtained from 300 mg of **4d** (1.3 mmol), 1.38 g of $AlCl_3$ and 5 mL of EtSH. Crystallization from chloroform gave pure **4g** (134 mg, 51%) as a red solid: mp 108.2 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.05 (s, 1H, OH), 9.52 (s, 1H, OH), 8.59 (s, 1H, H-6), 7.79 (s, 1H, H-3), 7.63 (d, 1H, J = 8.5 Hz), 6.3 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.5, 157.8, 153.7 (C-2), 139.9 (C-5), 139.3 (C-6), 127.1 (C-3), 126.6, 111.2, 107.2, 103.4; EIMS m/z 203 (M^+). Anal. calcd for $C_{10}H_9N_3O_2$: C, 59.05; H, 4.43. Found: C, 58.94; H, 4.38.

2-Amino-3-(3′,4′-dihydroxyphenyl)-5-(4-hydroxyphenyl)-1,4-pyrazine (6f). 6f was obtained from 486 mg of **6c** (1.44 mmol), 2.3 g of $AlCl_3$ and 10 mL of EtSH. Crystallization from chloroform gave pure **6f** (275 mg, 65%, monohydrate) as a red solid: mp 135.8 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.50 (s, OH), 8.34 (s, 1H, H-6), 7.79 (d, 1H, J = 7.4 Hz), 7.11 (d, 1H, J = 7.4 Hz), 6.81 (m, 5H), 5.96 (s, 2H, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.2, 151.1 (C-2), 146.0, 145.3, 140.2 (C-5), 138.1 (C-3), 135.6 (C-6), 128.8, 128.2, 126.3, 119.3, 115.7, 115.5; EIMS m/z 295 (M^+), 278 ($M-NH_3^+$). Anal. calcd for $C_{16}H_{13}N_3O_3 + H_2O$: C, 60.24; H, 4.89. Found: C, 60.34; H, 5.21.

2-Amino-3-(4′-hydroxyphenyl)-5-(3′,4′-dihydroxyphenyl)-1,4-pyrazine (6g). 6g was obtained from 100 mg of **6d** (0.31 mmol), 498 mg of $AlBr_3$ and 4 mL of EtSH. Crystallization from chloroform gave pure **6g** (86 mg, 93%, dihydrate) as a green solid: mp 147.8 °C; ^{1}H NMR (200 MHz, acetone-d_6) δ 8.60 (s, 3H, OH), 8.33 (s, 1H, H-6), 7.73 (d, 2H, J = 8.7 Hz), 7.57 (d, 1H, J = 2.1 Hz), 7.39 (dd, 1H, J = 2.1 and 8.3 Hz), 7.0 (d, 2H, J = 8.7 Hz), 6.9 (d, 1H, J = 8.3 Hz), 5.20 (s, 2H, NH_2); ^{13}C NMR (50 MHz, acetone-d_6) δ 159.2, 151.2 (C-2), 146.5, 146.3, 142.6 (C-5), 136.4 (C-3), 134.1 (C-6), 130.8, 130.3, 129.7, 118.1, 116.6, 116.5, 113.6; EIMS m/z 295 (M^+), 278 ($M-NH_3^+$). Anal. calcd for $C_{16}H_{13}N_3O_3 + 2\ H_2O$: C, 57.96; H, 5.13. Found: C, 58.46; H, 5.23.

General procedure for the preparation of compounds 7

A mixture of 2-amino-1,4-pyrazine (1 equiv), methyl glyoxal (40% w/v aqueous solution, 1.5 equiv) and 37% aqueous HCl (3.6 equiv) in ethanol (10 mL/mmol of pyrazine) was heated, under argon atmosphere, at 80 °C for 4 h. After concentration in vacuum, the crude solid was successively washed with ethyl acetate and ether.

2-Methyl-6-(3′,4′-dihydroxyphenyl)-3,7-dihydroimidazolo [1,2-*a*] pyrazin-3-one (7a). 7a was obtained from 53 mg of **4f** (0.26 mmol), 60 μL of methyl glyoxal and 78 μL of 37% HCl in 1.3 mL of ethanol. A red solid was recovered (50 mg, 61%), corresponding to the monohydrate hydrochloride of **7a**: mp 162.6 ° (dec.); FAB MS m/z 256 ($M + 1$). Anal. calcd for $C_{14}H_{14}ClN_3O_4 + 0.5\ H_2O$: C, 48.67; H, 4.68; Cl, 11.07; N, 13.10. Found: C, 48.92; H, 5.01; Cl, 10.59; N, 13.29.

2-Methyl-6,8-bis(4′-hydroxyphenyl)-3,7-dihydroimidazolo [1,2-*a*] pyrazin-3-one (7b). 7b was obtained from 660 mg of **6e** (2.15 mmol), 0.5 mL of methyl glyoxal and 0.62 mL of 37% HCl in 20 mL of ethanol. A red solid was recovered (731 mg, 84%), corresponding to the dihydrate hydrochloride of **7b**: mp 168.3 °C; ^{1}H NMR

(300 MHz, DMSO-d_6) δ 8.67 (s, 1H, H-5), 8.01 (m, 2H), 7.05 (d, 2H, $J=8.9$ Hz), 6.93 (d, 2H, $J=8.4$ Hz), 2.46 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.6, 158.8, 143.9 (C-8), 137.0 (C-9), 131.0, 129.7, 126.4 (C-6), 127.9 (C-16), 125.2, 124.7 (C-3), 123.9 (C-2), 115.8, 115.7, 107.9 (C-5), 9.7; FAB MS m/z 334 ($M+H^+$), 306, 291. Anal. calcd for $C_{19}H_{20}ClN_3O_3+0.5\ H_2O$: C, 55.00; H, 5.06; Cl, 8.56; N, 10.13. Found: C, 55.36; H, 5.08; Cl, 9.34; N, 9.80.

2-Methyl-6-(3′,4′-dihydroxyphenyl)-8-(4′-hydroxyphenyl)-3,7-dihydroimidazolo[1,2-a]pyrazin-3-one (7c). **7c** was obtained from 75 mg of **6g** (0.25 mmol), 60 μL of methyl glyoxal and 75 μL of 37% HCl in 2 mL of ethanol. A red solid was recovered (126 mg, 86%), corresponding to the dihydrate dihydrochloride of **7c**: mp 250 °C (dec.); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.52 (s, 1H, H-5), 8.09 (d, 2H, $J=8.7$ Hz), 7.58 (d, 1H, $J=1.9$ Hz), 7.45 (dd, 1H, $J=1.9$ and 8.3 Hz), 7.05 (d, 2H, $J=8.7$ Hz), 8.90 (d, 1H, $J=8.3$ Hz), 2.40 (s, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 160.5, 147.0, 154.7 (C-8, C-9), 131.1, 130.9, 125.9 (C-3), 125.3 (C-2), 124.2 (C-6), 117.9, 116.0, 115.7, 114.0, 107.7 (C-5), 18.5; FAB MS m/z 350 ($M+1$). Anal. calcd for $C_{19}H_{21}Cl_2N_3O_6$. $CHCl_3$: C, 41.56; H, 3.81; Cl, 30.73; N, 7.27. Found: C, 42.0; H, 3.77; Cl, 30.41; N, 6.14.

Biological evaluation

Human non-tumoral HaCaT cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% (v/v) heat-inactivated fetal calf serum (Gibco) in a humidified atmosphere with 5% (v/v) CO_2 at 37 °C. For all experiments, 2.5×10^4 cells/well were seeded in a 96-well plate and grown for 2 days to near confluency. Just prior to irradiation, cells were washed twice with PBS (phosphate buffer saline) and then pre-incubated with tested compounds in PBS for 30 min. Cells were irradiated in 50 μL PBS with 200 mJ/cm^2 using a BIOSUN System (RMX-3W, Vilbert et Lourmat, France) equipped with two 312 nm T-20M tubes. Immediately after the irradiation, cells were incubated for 24 h in fresh medium (DMEM without phenol red) containing the compounds. Supernatants were then collected and cells were lysed in 150 μL of 1% Triton X-100. The LDH content of the supernatants and the cell lysates was spectrophotometrically measured by the disappearance of NADH during the LDH-catalysed conversion of pyruvate to lactate using a cytotoxicity detection kit (Roche). LDH release was expressed as the quotient of LDH in the supernatant divided by total LDH (LDH in supernatant + LDH within cells). Data shown are expressed as the mean + SD of 6 experiments. Significant differences ($p<0.01$ and $p<0.05$) are determined by one-way ANOVA analysis.

Acknowledgements

This work was supported by the Fonds National de la Recherche Scientifique (F.N.R.S., Belgium) and the Walloon Government (convention no 9713664). J.-F.R. and J.M.-B. are senior research associates of the F.N.R.S.

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