2,6-Diamino-3,5-diaryl-1,4-pyrazine Derivatives as Novel Antioxidants

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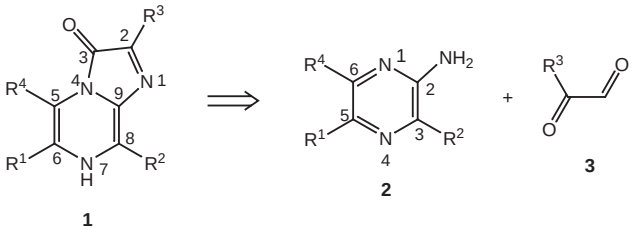
Abstract: The coupling of arylboronic acids with 2,6-diamino-3,5-dibromo-1,4-pyrazine (**6**) gave 2,6-diamino-3,5-diaryl-1,4-pyrazines (**7**). The reaction of **7** with methyl glyoxal in aqueous EtOH-HCl led to the N,N'-disubstituted products **8**, instead of the expected bicyclic imidazolopyrazinones **1**. The 2,6-bis[1-(ethoxycarbonyl)-ethylamino]-3,5-diaryl-1,4-pyrazines **8** are powerful inhibitors of the AAPH-induced linoleate peroxidation.

Key words: 2,6-diamino-3,5-diaryl-1,4-pyrazine, imidazolopyrazinone, antioxidant, coelenterazine analogs



Scheme 1

Recently we became interested in the synthesis of imidazolopyrazinone derivatives **1** (Scheme 1) as potential antioxidants useful in medicinal chemistry for the design of drugs against injuries caused by oxidative stress.[1,2] Coelenterazine (CLZ), a naturally occurring imidazolopyrazinone of marine origin (**1**: R^1 = *p*-HO-Ph; R^2 = $PhCH_2$; R^3 = *p*-HO-$PhCH_2$; R^4 = H), is the luminescent substrate of enzymes (luciferases) producing light in the presence of oxygen.[3] Synthetic derivatives of coelenterazine can be obtained by condensing 2-amino-1,4-pyrazine precursors **2** with α-keto-aldehydes **3** (or the corresponding acetals) in aqueous acidic medium (Scheme 1).[4] Two routes have been exploited for the synthesis of aminopyrazines **2** equipped with natural or non-natural substituents R^1, R^2, and R^4: (i) condensation of 1,2-propanedione-2-oxime derivatives with 2-amino-propionitrile compounds to form the substituted heterocycles;[5] (ii) functionalization of bromo-aminopyrazine derivatives via a Suzuki coupling reaction with boronic acid derivatives.[6] This last method was exemplified in our group for the preparation of a series of 3,5-diaryl-2-amino-1,4-pyrazines **2** (R^4 = H) and the related 6,8-diaryl-imidazolopyrazinones **1** (R^1 and R^2 = aryl; R^4 = H) which reveal to be powerful antioxidants.[1b]

In this context, we examined the possibility of using 2,6-diamino-1,4-pyrazine (**5**) (Scheme 2) as the starting material for the synthesis of C-5 substituted imidazolopyrazinones **1** (R^4 = NH_2) susceptible to be further derivatized, for instance with lipophilic acyl chains (R^4 = NHCO-C_nH_{2n+1}). This led to the discovery of unexpected *N*,*N*'-disubstituted pyrazine derivatives **8**, displaying remarkable antioxidative properties.

2,6-Diaminopyrazine (**5**)[7] (Scheme 2) prepared in two steps from 2,6-dichloropyrazine,[8] was treated with *N*-bromosuccinimide to give 2,6-diamino-3,5-dibromo-1,4-pyrazine (**6**) in moderate overall yield; in our hands, this method was more efficient than the synthesis of Y. C. Tong starting from tetrabromopyrazine.[9] The following step was a double Suzuki coupling reaction[10,11] with phenylboronic acid and 4-methoxyphenylboronic acid to yield respectively the 3,5-diaryl-2,6-diaminopyrazines **7a** and **7b**. We used [1,4-bis(diphenylphosphino)butane]palladium(II) chloride as the catalyst, prepared in situ from bis(benzonitrile)palladium(II) dichloride and 1,4-bis(diphenylphosphino)butane (dppb) in toluene.[6] Deprotection of the methoxy group of **7b** was realized with sodium ethanethiolate in hot DMF,[12] furnishing **7c** in 67% yield. Reaction of **7** with methyl glyoxal **3a** in aqueous HCl–ethanol at 80 °C, led to the double *N*,*N*'-alkylation products **8** (Scheme 2); the expected bicyclic compounds **1** could neither be isolated nor detected from the crude mixtures (NMR and UV analysis).

This unusual result in the field of heterocyclic synthesis could be explained as follows: under our experimental conditions, methyl glyoxal would be transformed into the corresponding diethyl α-keto-acetal **3b**, reacting on **2** preferentially with the ketone function to give a Schiff base **9a** in equilibrium with its enamine tautomer **9b** (Scheme 3).[2] This intermediate could stabilize either by cyclization into **1**, or by hydrolysis into **8**, depending on the nature of the R^4 substituent. The C-6 amino-substituted intermediates **9** (R^4 = NH_3^+; alkyl-NH_2^+) conducted exclusively to the monocyclic products **8**, most probably for steric and electronic reasons, while the C-6 unsubstituted intermediates **9** (R^4 = H) evolved mainly to the formation

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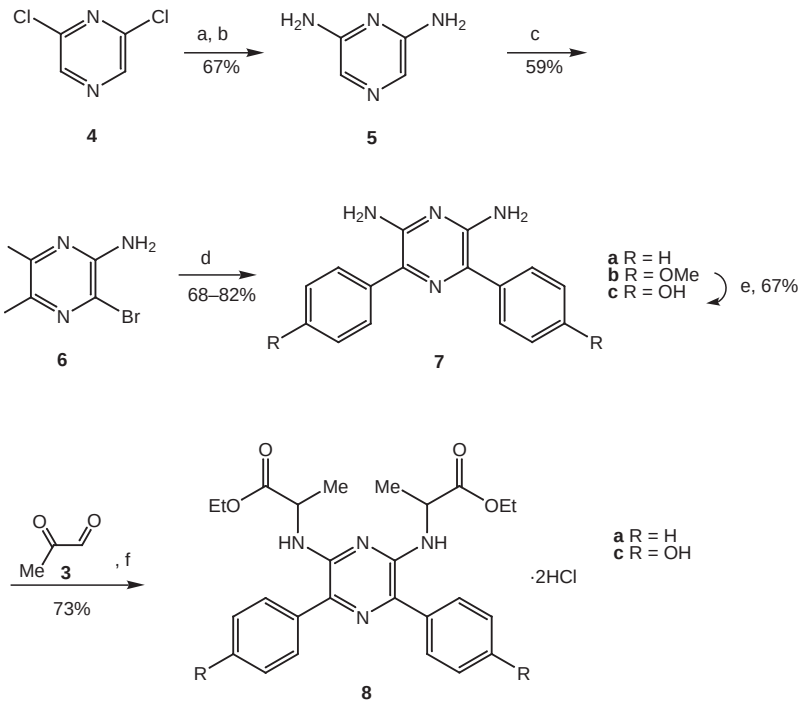


Scheme 2 a) NaN_3, DMSO, 65 °C; b) H_2, Pd–C 10%, DME, NH_4OH; c) NBS, DMSO, H_2O, 20 °C; d) R–Ph–$B(OH)_2$, $PdCl_2$ (dppb) cat., toluene, reflux, 24 h; e) EtSNa, DMF, 100 °C, 24 h; f) EtOH, 37% HCl, 80 °C, 4 h.

of bicyclic products **1**.[2,4] Indeed, in a control experiment, starting from 2-amino-3,5-dibromo-1,4-pyrazine (**10**)[13] (Scheme 4), we prepared, in two steps, the corresponding 3,5-bis(*p*-hydroxyphenyl) aminopyrazine **11c** which was further reacted with methyl glyoxal to furnish the imidazolopyrazinone **12c**.

The symmetrically *N*,*N*'-disubstituted compounds **8** are original products. However, related *N*-monosubstituted 2-aminopyrazine compounds have already been mentioned in the literature; they were prepared by reaction of 2-aminopyrazines with α-keto-acids in the presence of hydrogen and palladium on charcoal (reductive amination).[14,15]

Compounds **7**, **8**, and **11**, bearing phenyl and *p*-methoxyphenyl substituents could be easily purified by column chromatography. On the other hand, compounds **7c**, **8c**, **11c**, and **12c**, bearing *p*-hydroxyphenyl substituents (unprotected phenols) were unstable under the usual chromatographic conditions, most probably due to their sensitivity towards oxidation. They were roughly purified by washing, and isolated as the hydrates and/or hydrochlorides, according to the elemental analysis. All the derivatives were properly characterized by their ^1H and ^{13}C NMR data (see experimental section).

The ability of the synthesized compounds to inhibit lipid peroxidation has been tested on the AAPH-induced oxidation of linoleate.[16] A micellar solution of linoleic acid (1.6 mM) is incubated at 37 °C with AAPH [2 mM, 2,2'-azobis(2-amidinopropane) dihydrochloride] as the free radical generator, in a microplate-based spectrophotometer. The production of conjugated dienes by the peroxidation of linoleate is monitored continuously at 234 nm. Antioxidants (added at 5 mM) can at once delay the onset of the oxidation process and reduce the rate of oxidation. The results are pictured in Figure 1. All the 3,5-diarylaminopyrazines behaved as excellent antioxidants, but the best compound clearly was **8c**. Moreover, comparatively to vitamin E (α-tocopherol)[17–19] and Trolox^R,[2] considered as good references in the domain, on the one hand, and the bicyclic derivative imidazolopyrazinone **12c**,[1b] on the other hand, the monocyclic compound **8c** was still the more active one (Figure 2).

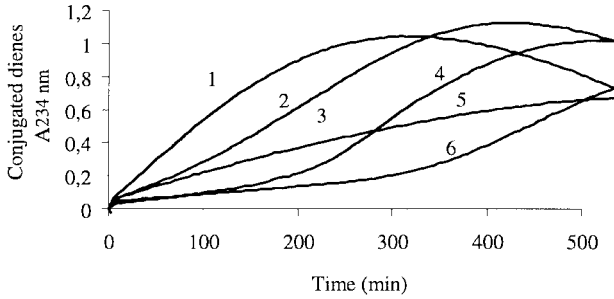
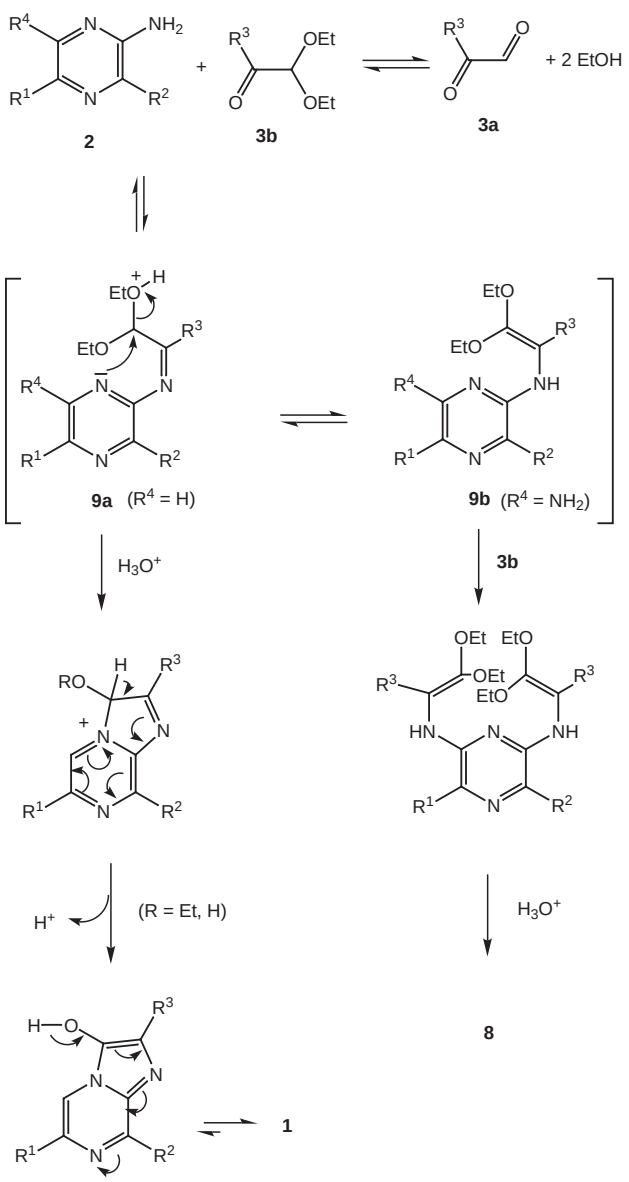


Figure 1 Inhibition of AAPH-induced peroxidation of linoleic acid by aminopyrazines. Measure of the absorbance at 234 nm as a function of time. The compounds were tested at 5 ∝M. 1 = AAPH (control); 2 = **7a**; 3 = **8a**; 4 = **7c**; 5 = **11c**; 6 = **8c**

Solvents were dried prior to use. Reagents (Aldrich or Acros) were used as purchased. Melting points (uncorrected) were determined on an Electrothermal apparatus. ^1H (200 MHz or 300 MHz) and ^{13}C (50 MHz or 75 MHz) NMR spectra were recorded on Varian Gemini 200 and 300 spectrometers. Chemical shifts are reported as δ values downfield from TMS. The mass spectra (FAB or EI modes)



Scheme 3

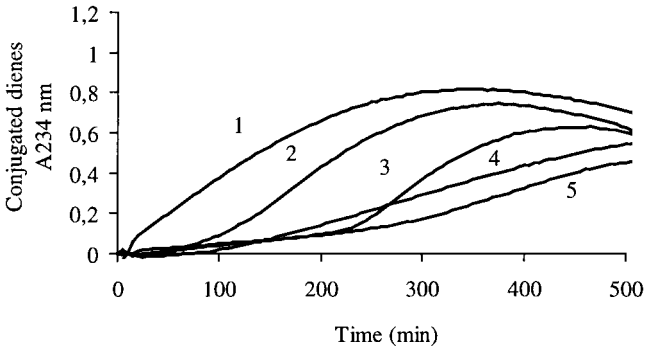


Figure 2 Inhibition of AAPH-induced peroxidation of linoleic acid by compound **8c** comparatively to compound **12c**, and references. Measure of the absorbance at 234 nm as a function of time. The compounds were tested at 5 µM. 1 = AAPH (control); 2 = TroloxR; 3 = Vitamin E; 4 = **12c**; 5 = **8c**

were obtained on a Finnigan MAT TSQ–70 instrument. TLC were carried out using silica gel 60 F_{254} plates (0.2 nm, Merck) and the spots were visualized using UV light (254 nm). Column chromatography was performed on Merck silical gel 60 (70–230 mesh). Elemental analyses were obtained at the University College of London.

2,6-Diamino-3,5-dibromo-1,4-pyrazine (5)

To a solution of 2,6-diamino-1,4-pyrazine[8] (966 mg, 8.78 mmol) in DMSO (30 mL) and H_2O (1.22 mL, 5.56 equiv) was added *N*-bromosuccinimide in small portions (3.44 g, 2.2 equiv). The mixture was stirred for 4 h at 20 °C, then diluted with H_2O (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic phases were washed with 5% aq Na_2CO_3 (2 × 150 mL) and brine (2 × 100 mL), then dried over $MgSO_4$ and concentrated in vacuo. Recrystallization from $CHCl_3$ gave **5** (1.63 g, 69% yield) as a brown solid.

Mp 183.7° C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.29 (s, NH_2).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 151.5 (C-2), 150.1 (C-3).

MS (EI, 70 eV): *m/z* = 270 (M + 2), 268 (M), 266 (M – 2).

Anal. Calcd for $C_4H_4Br_2N_4$: C, 17.93; H, 1.50; N, 20.91. Found: C, 18.10; H, 1.46; N, 20.70.

2,6-Diamino-3,5-diphenyl-1,4-pyrazine (7a)

A mixture of bis(benzonitrile)palladium (II) dichloride (72 mg, 0.1 equiv) and 1,4-bis(diphenylphosphino)butane (96 mg, 0.12 equiv) in anhyd toluene (8 mL) 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. 2,6-Diamino-3,5-dibromo-1,4-pyrazine (**5**) (500 mg, 1.87 mmol, 1 equiv), phenylboronic acid (475 mg, 2.1 equiv) in anhyd toluene (8 mL), EtOH (1.6 mL) and aq Na_2CO_3 (1 M, 3.7 mL, 2 equiv) were added to the preformed catalyst. The mixture was stirred under reflux for 24 h. After addition of H_2O (75 mL), the mixture was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with brine (2 × 50 mL), filtered over celite and concentrated in vacuo. Purification by column chromatography on silica gel gave pure **7a** (332 mg, 68% yield) as a yellow solid.

Mp 157–158 °C; R_f 0.23 (EtOAc–cyclohexane, 3:5).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.70 (dd, *J* = 8.0, 1.5 Hz, 4H), 7.40 (m, 4H), 7.31 (dd, *J* = 7.3, 1.5 Hz, 2H), 4.65 (s, 4H, NH_2).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 149.9 (C-2), 138.2, 129.8 (C-3), 128.9, 128.3, 127.8.

MS (EI, 70 eV): *m/z* = 262 (M).

Anal. Calcd for $C_{16}H_{14}N_4.0.2\ H_2O$: C, 72.26; H, 5.41; N, 21.07. Found: C, 72.31; H, 5.33; N, 20.93.

2,6-Diamino-3,5-di(*p*-methoxyphenyl)-1,4-pyrazine (7b)

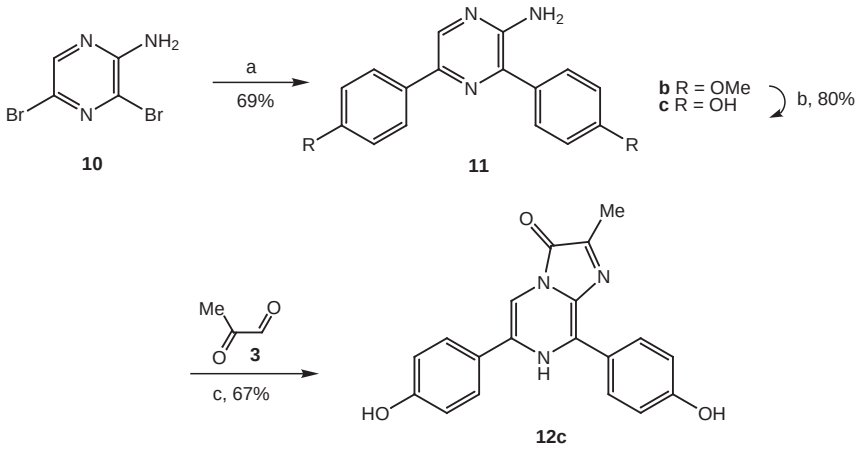
7b was prepared as described for **7a**, by using 98.6 mg (0.1 equiv) of bis(benzonitrile)palladium(II) dichloride, 131.5 mg (0.12 equiv) of 1,4-bis(diphenylphosphino)butane, 689 mg (2.57 mmol, 1 equiv) of 2,6-diamino-3,5-dibromopyrazine (**5**), 860 mg (2.2 equiv) of 4-methoxyphenylboronic acid, 2.28 mL of EtOH, 5.2 mL (2 equiv) of 1 M aq Na_2CO_3, and 2 × 10 mL of toluene. Column chromatography on silica gel gave 678 mg (82% yield) of **7b** as a yellow solid.

Mp 152.3 °C; R_f 0.11 (EtOAc–cyclohexane, 3:5).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.65 (d, *J* = 8.8 Hz, 4H), 6.98 (d, *J* = 8.8 Hz, 4H), 4.54 (s, 4H, NH_2), 3.84 (s, 6H, OCH_3).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 159.3, 149.4 (C-2), 130.7, 129.8 (C-3), 129.6, 114.4, 55.6.

MS (EI, 70 eV): *m/z* = 322 (M), 307 (M-CH_3).



Scheme 4 a) R–Ph–B(OH)2, PdCl2 (dppb) cat., toluene, reflux, 24 h; b) EtSNa, DMF, 100 °C, 24 h; c) EtOH, 37 % HCl, 80 °C, 4 h.

Anal. Calcd for $C_{18}H_{18}N_4O_2$: C, 67.10; H, 5.60; N, 17.40. Found: C, 66.94; H, 5.45; N, 17.21.

2,6-Diamino-3,5-di(*p*-hydroxyphenyl)-1,4-pyrazine (7c)
A stirred solution of pyrazine **7b** (360 mg, 1.12 mmol, 1 equiv) and sodium ethanethiolate (790 mg, 8 equiv) in DMF (6 mL) was heated under argon atmosphere at 100 °C for 24 h. EtOAc (30 mL) and aq NH_4Cl (25 mL, saturated solution) were added. The aqueous phase was extracted with EtOAc (4 × 25 mL). The combined organic layers were washed with brine (2 × 25 mL), dried over $MgSO_4$ and concentrated in vacuo. Recrystallization from $CHCl_3$ gave **7c** (monohydrate, 236 mg, 67% yield) as a red solid.

Mp 88–90 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.45 (s, 2H, OH), 7.47 (d, *J* = 7.5 Hz, 4H), 6.82 (d, *J* = 7.5 Hz, 4H), 5.49 (s, 4H, NH_2).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 156.3, 149.5 (C-2), 129.4, 128.9, 127.1 (C-3), 115.1.

MS (EI, 70 eV): *m/z* = 294 (M).

Anal. Calcd for $C_{16}H_{16}N_4O_3$ (312.3): C, 61.48; H, 5.12; N, 17.93. Found: C, 61.13; H, 5.12; N, 17.71.

2,6-Bis(1-ethoxycarbonylethylamino)-3,5-diphenyl-1,4-pyrazine (8a)
A mixture of 2,6-diaminopyrazine (**7a**) (150 mg, 0.57 mmol, 1 equiv), methyl glyoxal (132 mL, 40% solution in H_2O, 2.2 equiv) and 37% aq HCl (171 mL, 3.6 equiv) in EtOH (5 mL) was heated under argon atmosphere at 80 °C during 4 h. After concentration in vacuo, the crude solid was washed with Et_2O to afford **8a** (hydrochloride monohydrate, 79 mg, 27% yield) as a yellow solid.

Mp 133–136 °C (dec.).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.43 (d, *J* = 8.6 Hz, 4H), 6.95 (m, 6H), 4.55 (q, *J* = 7.1 Hz, 2H), 4.08 (q, *J* = 6.9 Hz, 4H), 1.38 (d, *J* = 7.1 Hz, 6H), 1.20 (t, *J* = 6.9 Hz, 6H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.0, 151.0 (C-2), 136.5, 129.0, 128.5, 127.5 (C-3), 127.0, 60.6, 59.5, 16.7, 13.6.

MS (FAB$^+$): *m/z* = 462 (M + 1).

Anal. Calcd for $C_{26}H_{30}N_4O_4$•1.1HCl. 0.8 H_2O (517.03): C, 60.39; H, 6.32; Cl, 7.75; N, 10.83. Found: C, 60.00; H, 6.62; Cl, 7.30; N, 10.95.

2,6-Bis(1-ethoxycarbonylethylamino)-3,5-bis(*p*-hydroxyphenyl)-1,4-pyrazine (8c)
8c was prepared as described for **8a**, by using 2,6-diaminopyrazine **7c** (165 mg, 0.58 mmol, 1 equiv), methyl glyoxal (0.2 mL, 40% in H_2O, 2.2 equiv), 37% aq HCl (0.17 mL, 3.6 equiv), and EtOH (4.5 mL). Compound **8c** (dihydrochloride, 231 mg, 73% yield) is a red solid.

Mp 174–176 °C (dec.).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.45 (d, *J* = 8.6 Hz, 4H), 6.94 (d, *J* = 8.6 Hz, 4H), 4.51 (q, *J* = 7.3 Hz, 2H), 4.02 (q, *J* = 7.1 Hz, 4H), 1.32 (d, *J* = 7.3 Hz, 6H), 1.18 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.9, 158.2, 149.7 (C-2), 132.3, 130.5, 129.6 (C-3), 115.7, 60.6, 59.5, 17.0, 14.0.

MS (FAB$^+$): *m/z* = 495 (M + 1).

Anal. Calcd for $C_{26}H_{30}N_4O_6$•1.7HCl•0.5H_2O (565.51): C, 55.22; H, 5.78; Cl, 10.66; N, 9.91. Found: C, 55.16; H, 5.62; Cl, 11.02; N, 9.93.

2-Amino-3,5-bis(*p*-methoxyphenyl)-1,4-pyrazine (11b)
11b was prepared as described for **7a**, by using bis(benzonitrile)palladium(II) dichloride (306.3 mg, 0.1 equiv), 1,4-bis(diphenylphosphino)butane (408.3 mg, 0.12 equiv), 2-amino-3,5-dibromopyrazine (**10**)[13] (2.02 g, 8 mmol, 1 equiv), 4-methoxyphenylboronic acid (2.55 g, 2.1 equiv), 1 M aq Na_2CO_3 (16 mL, 2 equiv), EtOH (6.8 mL), and toluene (2 × 20 mL). Column chromatography on silica gel gave 1.6 g (69% yield) of **11b** as a yellow solid.

Mp 136.6 °C; R_f 0.21 (EtOAc–cyclohexane, 3:5).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.42 (s, 1H, H-6), 7.90 (d, *J* = 8.7 Hz, 2H), 7.75 (d, *J* = 8.7 Hz, 2H), 7.06 (d, *J* = 8.7 Hz, 2H), 6.98 (d, *J* = 8.7 Hz, 2H), 6.11 (s, 2H, NH_2), 3.78 (s, 3H), 3.81 (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 (br), 55.2, 52.1.

MS (EI, 70 eV): *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.

2-Amino-3,5-bis(*p*-hydroxyphenyl)-1,4-pyrazine (11c)
11c was prepared as described for **7c**, by using 2-amino-3,5-bis(*p*–methoxyphenyl)pyrazine (**11b**) (1.6 g, 5.21 mmol, 1 equiv) and so-

dium ethanethiolate (3.5 g, 8 equiv) in DMF (25 mL). The crude solid was washed with EtOAc–Et_2O (1:1, v/v) to give **11c** (1.28 g, 80% yield) as a yellow solid (hygroscopic material).

Mp 250–252°C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.64 (s, 2H, OH), 8.35 (s, 1H, H-6), 7.79 (d, *J* = 8.6 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.4 Hz, 2H), 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), 138.0 (C-3), 135.8 (C-6), 129.5, 128.4, 128.2, 126.3, 115.5, 115.4.

MS (EI, 70 eV): *m/z* = 279 (M).

Anal. Calcd for $C_{16}H_{13}N_3O_2 \cdot H_2O$ (297.29): C, 64.63; H, 5.04; N, 14.13. Found: C, 64.96; H, 5.30; N, 13.91.

2-Methyl-6,8-bis(*p*-hydroxyphenyl)-3,7-dihydroimidazolo[1,2-a]pyrazin-3-one (12c)

A mixture of **11b** (600 mg, 2.15 mmol, 1 equiv), methyl glyoxal (0.5 mL, 40% in H_2O, 1.5 equiv), and 37% aq HCl (0.62 mL, 3.6 equiv) in EtOH (20 mL) was heated under argon atmosphere at 80°C during 4 h. After concentration in vacuo, the solid residue was washed with EtOAc–EtOH (1:1, v/v) to afford **12c** (hydrochloride monohydrate, 731 mg, 67% yield) as a red solid.

Mp 167–169°C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.67 (s, 1H, H-5), 8.01 (m, 4H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.93 (d, *J* = 8.4 Hz, 2H), 2.46 (s, 3H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 160.6, 158.8, 143.9 (C-8), 136.9 (C-9), 130.9, 129.7, 127.9, 126.4 (C-6), 125.2, 124.7 (C-3), 123.9 (C-2), 115.7, 115.6, 107.9 (C-5), 9.7.

MS (FAB$^+$): *m/z* = 334 (M + 1), 306, 291.

Anal. Calcd for $C_{19}H_{15}N_3O_3 \cdot 1.1HCl \cdot 1.3H_2O$ (396.83): C, 57.45 H, 4.75; Cl, 9.85; N, 10.50. Found: C, 57.64; H, 5.26; Cl, 9.57; N, 9.93.

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