Supplementary Information

Development of a Coelenterazine Derivative with Enhanced Superoxide Anion-Triggered Chemiluminescence in Aqueous Solution

José Pedro Silva [1], Patricia González-Berdullas [1], Joaquim C. G. Esteves da Silva [1,2] and Luís Pinto da Silva [1,2,*]

[1] Chemistry Research Unit (CIQUP), Institute of Molecular Sciences (IMS), Faculty of Sciences, University of Porto (FCUP), Rua do Campo Alegre 687, 4169-007 Porto, Portugal; up201705721@edu.fc.up.pt (J.P.S.); patricia.berdullas@fc.up.pt (P.G.-B.); jcsilva@fc.up.pt (J.C.G.E.d.S.)

[2] LACOMEPHI, GreenUPorto, Department of Geosciences, Environment and Territorial Planning, Faculty of Sciences, University of Porto (FCUP), Rua do Campo Alegre 687, 4169-007 Porto, Portugal

* Correspondence: luis.silva@fc.up.pt (L.P.d.S.)

1. General Synthetic Procedures

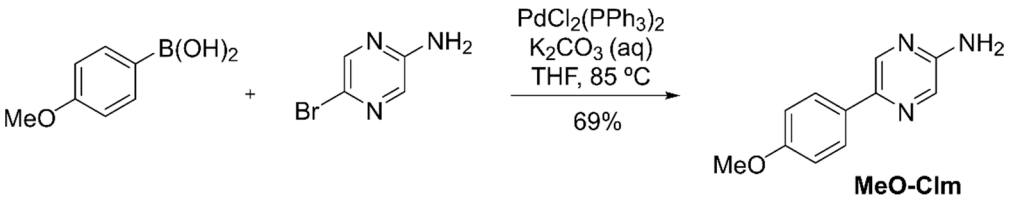
Reagents and solvents were purchased from Merck and used without further purification. All reactions involving oxygen or moisture-sensitive compounds were carried out under a dry nitrogen atmosphere. Ice-water and silicon baths were used for reactions at low and high temperatures, respectively, with all reaction temperatures referring to the external bath. Organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated using a rotary evaporator (Büchi® Rotavapor® R-210, Büchi® B-491 Heating Bath 120V, KNF Neuberger D-79112 Vacuum Pump N 035.1.2 AN.18).

Reactions were monitored by thin-layer chromatography (TLC) using aluminum-backed Merck 60 F_{254} silica gel plates and *n*-hexanes-ethyl acetate solvent systems. After visualization under ultraviolet light at 254 nm and 365 nm, the plates were developed by immersion in a solution containing a mixture of *p*-anisaldehyde (2.5%), acetic acid (1%), and sulfuric acid (3.4%) in 95% ethanol, followed by heating. Solid compounds were mixed with SiO_2, redissolved in DCM, and concentrated under reduced pressure before purification through column chromatography using silica gel (Aldrich, 230–400 mesh) and EtOAc–hexanes mixtures. Compounds were systematically named following IUPAC recommendations with ChemDraw v20.0.0.41 (Perkin-Elmer, Waltham, MA, USA).

NMR spectra were recorded in $CDCl_3$ or MeOH-d_4 solutions on a Bruker NMR spectrometer (Bruker Advance III 400 MHz Ascend, 9.4 Tesla), and chemical shifts are reported on the δ scale (ppm) using the residual solvent signals—δ = 7.26 ppm (^{1}H, s, $CDCl_3$); δ = 77.0 ppm (^{13}C, t, $CDCl_3$) or δ = 3.31 ppm (^{1}H, qu, MeOH-d_4), 4.78 ppm (^{1}H, s, MeOH-d_4); δ = 49.2 ppm (^{13}C, hep, MeOH-d_4)—as internal standards. Coupling constants (*J*) are reported in Hz. FT-MS analyses were performed on an LTQ OrbitrapTM XL hybrid mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) controlled by LTQ Tune Plus and Xcalibur 2.1.0.

bs = broad singlet; ESI = electrospray ionization; EtOAc = ethyl acetate; EtOH = ethanol; hep = heptet; NBS = *N*-Bromosuccinimide; NMR = nuclear magnetic resonance; FT-MS = Fourier transform mass spectrometry; qu = quintet; *rt* = room temperature; s = singlet; t = triplet; THF = tetrahydrofuran; TLC = thin layer chromatography.

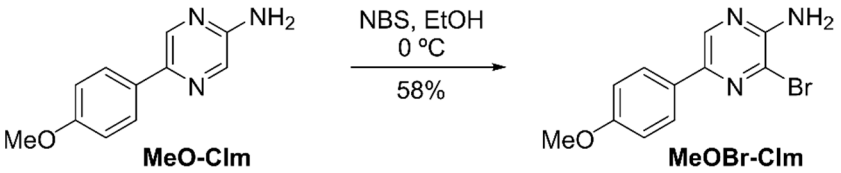
1.1. 5-(4-Methoxyphenyl)pyrazin-2-amine (MeO-Clm)



An aqueous solution of K_2CO_3 (1 M, 7.4542 mmol, 7.5 equiv) was added to a solution of (4-methoxyphenyl)boronic acid (0.151 g, 0.9939 mmol, 1 equiv) and 5-bromopyrazin-2-amine (0.173 g, 0.9939 mmol, 1 equiv) in THF (7.5 mL) and was deoxygenated with N_2. Then, $PdCl_2(PPh_3)_2$ (0.070 g, 0.0994 mmol, 0.10 equiv) was added and the resulting mixture was stirred at 85 °C until no starting material was detected by TLC (1:1 EtOAc-hexanes). The reaction mixture was cooled to *rt* and the aqueous phase was discarded. The combined organic layers were washed with brine, dried, and concentrated under reduced pressure. The resulting solid was purified by column chromatography (SiO_2, Ø 2.5 × 5 cm, 30% EtOAc/hexanes gradient) to give 5-(4-methoxyphenyl)pyrazin-2-amine (MeO-Clm) as a pale-yellow solid (0.138 g, 69 %, R_f = 0.38 (50% EtOAc/hexanes)).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.40–8.37 (d, *J* = 1.5, 1H), 8.04–8.02 (d, *J* = 1.5, 1H), 7.83–7.78 (m, 2H), 7.00–6.94 (m, 2H), 4.58 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 160.0 (C), 152.8 (C), 143.1 (C), 138.5 (CH), 131.6 (CH), 129.8 (C), 127.1 (2 × CH), 114.4 (2 × CH), 55.5 (CH_3). FTMS-ESI (+): m/z: calcd for $[C_{11}H_{12}N_3O]^+$: 202.0980 $[M + H]^+$; found 202.0989 $[C_{11}H_{12}N_3O]^+$.

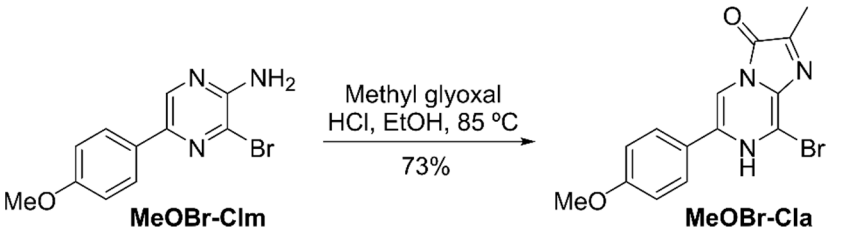
1.2. 3-Bromo-5-(4-methoxyphenyl)pyrazin-2-amine (MeOBr-Clm)



NBS (0.062 g, 0.346 mmol, 1.2 eq) was added to a solution of 3-bromo-5-(4-methoxyphenyl)pyrazin-2-amine (MeO-Clm) (0.058 g, 0.289 mmol, 1 eq) in ethanol (5 mL), which was previously cooled to 0 °C, and stirred at that temperature for 30 min. The reaction mixture was then diluted with EtOAc and washed with brine. The combined organic layers were dried with anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a brown solid, which was purified by column chromatography (SiO_2, Ø 2.5 × 5.5 cm, 25% hexanes-EtOAc gradient) to afford 3-bromo-5-(4-methoxyphenyl)pyrazin-2-amine (MeOBr-Clm) as a yellow solid (0.047 g, 58 %, R_f = 0.71 (50% EtOAc/hex)).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.32 (s, 1H), 7.84–7.76 (m, 2H), 7.03–6.91 (m, 2H), 5.08 (bs, 2H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 160.3 (C), 150.8 (C), 143.4 (C), 137.1 (CH), 128.4 (C), 127.2 (CH), 125.9 (C), 114.4 (CH), 55.5 (CH_3). FTMS-ESI (+): m/z: calcd for $[C_{11}H_{11}BrN_3O]^+$: 280.0085 $[M + H]^+$; found 280.0093 $[C_{11}H_{11}{}^{79}BrN_3O]^+$, 282.0070 $[C_{11}H_{11}{}^{81}B_2N_3O]^+$.

1.3. 8-Bromo-6-(4-methoxyphenyl)-2-methylimidazo[1,2-a]pyrazin-3(7H)-one (MeOBr-Cla)



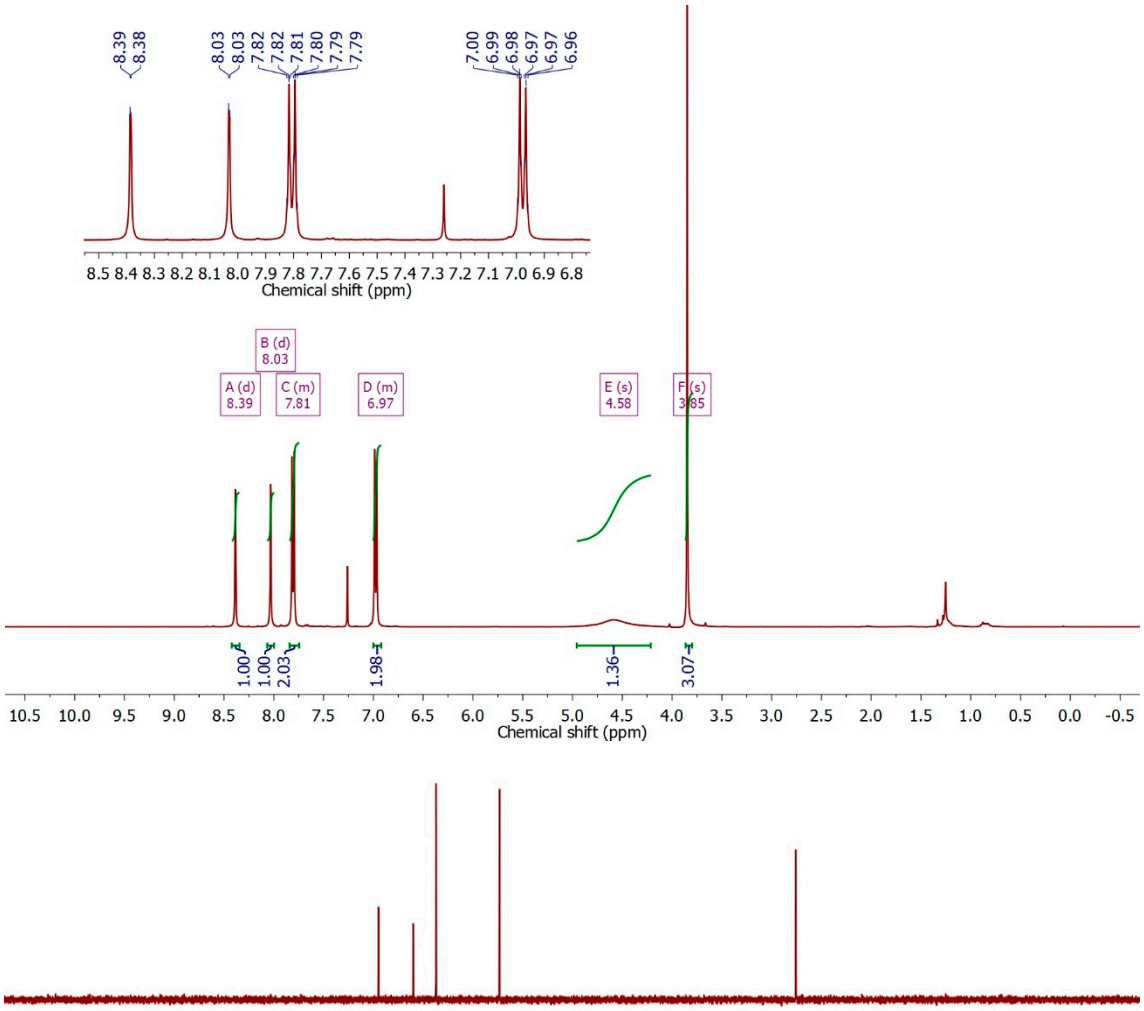
A solution of 3-bromo-5-(4-methoxyphenyl)pyrazin-2-amine (MeOBr-Clm) (0.040 g, 0.1428 mmol, 1 equiv) and methylglyoxal (0.095 mL, 0.2142 mmol, 1.5 equiv) in EtOH (2 mL) was deoxygenated with N_2. Then the resulting mixture was cooled to 0 °C, HCl (0.015 mL, 37%, 0.5141 mmol, 3.6 equiv) was added, and the solution was stirred up to *rt*, and then stirred at 80 °C until no starting material was detected by TLC (1:1 EtOAc-hexanes). The reaction mixture was cooled to *rt* and the resulting solution was concentrated under reduced pressure to give a brown oil, which was redissolved in the minimum amount of EtOAc, precipitated with diethyl ether, and vacuum-dried to afford 8-

bromo-6-(4-methoxyphenyl)-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (MeOBr-Cla) as a yellow solid (0.035 g, 73 %).

^{1}H NMR (400 MHz, MeOD) δ = 8.69 (d, *J* = 5.6, 1H), 8.01 (d, *J* = 8.7, 2H), 7.08 (d, *J* = 8.5, 2H), 3.89 (s, 3H), 2.55 (s, 3H). FTMS-ESI (+): m/z: calcd for $[C_{14}H_{13}BrN_3O_2]^+$: 334.0191 $[M + H]^+$; found 334.0194 $[C_{14}H_{13}{}^{79}Br^{35}N_3O_2]^+$, 336.0172 $[C_{14}H_{13}{}^{79}BrN_3O_2]^+$.

2. Supporting Figures

2.1. NMR Spectra



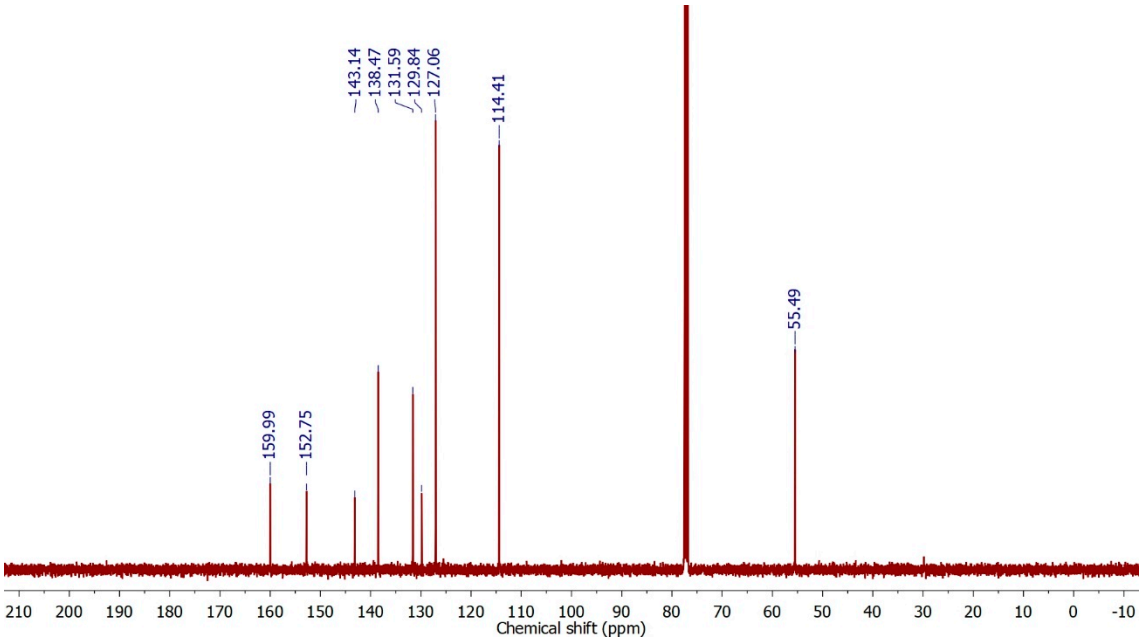
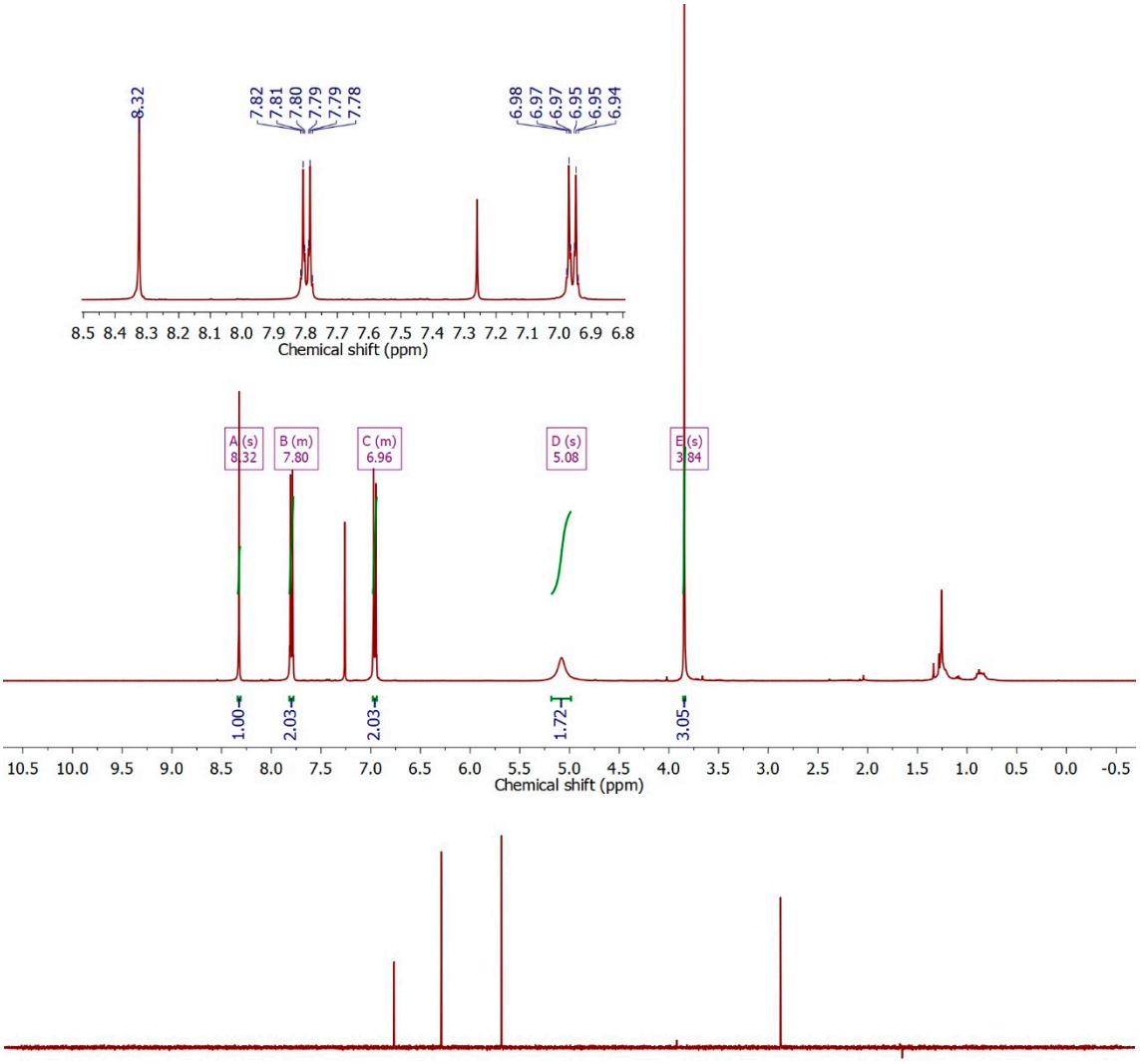


Figure S1. H NMR, DEPT, and ^{13}C NMR spectra of 5-(4-methoxyphenyl)pyrazin-2-amine (MeO-Clm). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.40–8.37 (d, J = 1.5, 1H), 8.04 – 8.02 (d, J = 1.5, 1H), 7.83–7.78 (m, 2H), 7.00–6.94 (m, 2H), 4.58 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 160.0 (C), 152.8 (C), 143.1 (C), 138.5 (CH), 131.6 (CH), 129.8 (C), 127.1 (2xCH), 114.4 (2 × CH), 55.5 (CH_3).



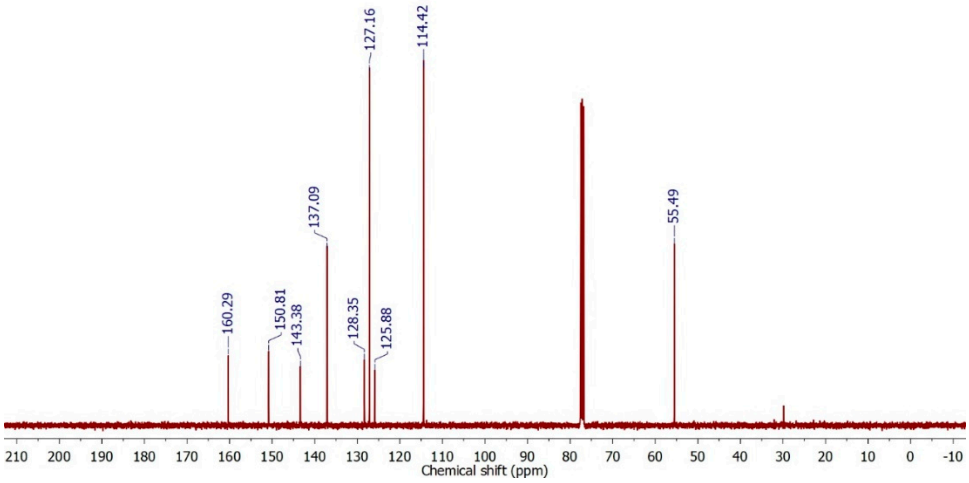


Figure S2. amine (MeOBr-Clm). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.32 (s, 1H), 7.84–7.76 (m, 2H), 7.03–6.91 (m, 2H), 5.08 (bs8i, 2H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 160.3 (C), 150.8 (C), 143.4 (C), 137.1 (CH), 128.4 (C), 127.2 (CH), 125.9 (C), 114.4 (CH), 55.5 (CH_3).

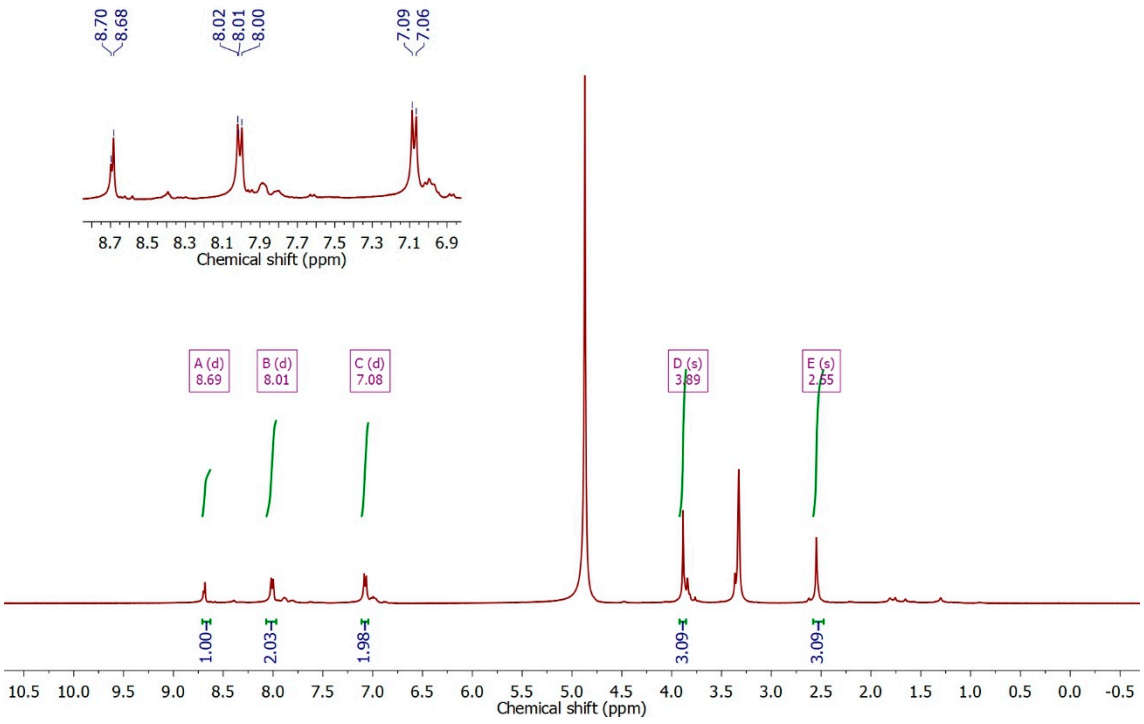


Figure S3. H NMR spectrum of 8-bromo-6-(4-methoxyphenyl)-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (MeOBr-Cla). ^{1}H NMR (400 MHz, MeOD) δ = 8.69 (d, *J* = 5.6, 1H), 8.01 (d, *J* = 8.7, 2H), 7.08 (d, *J* = 8.5, 2H), 3.89 (s, 3H), 2.55 (s, 3H).

2.2. FT-MS Spectra

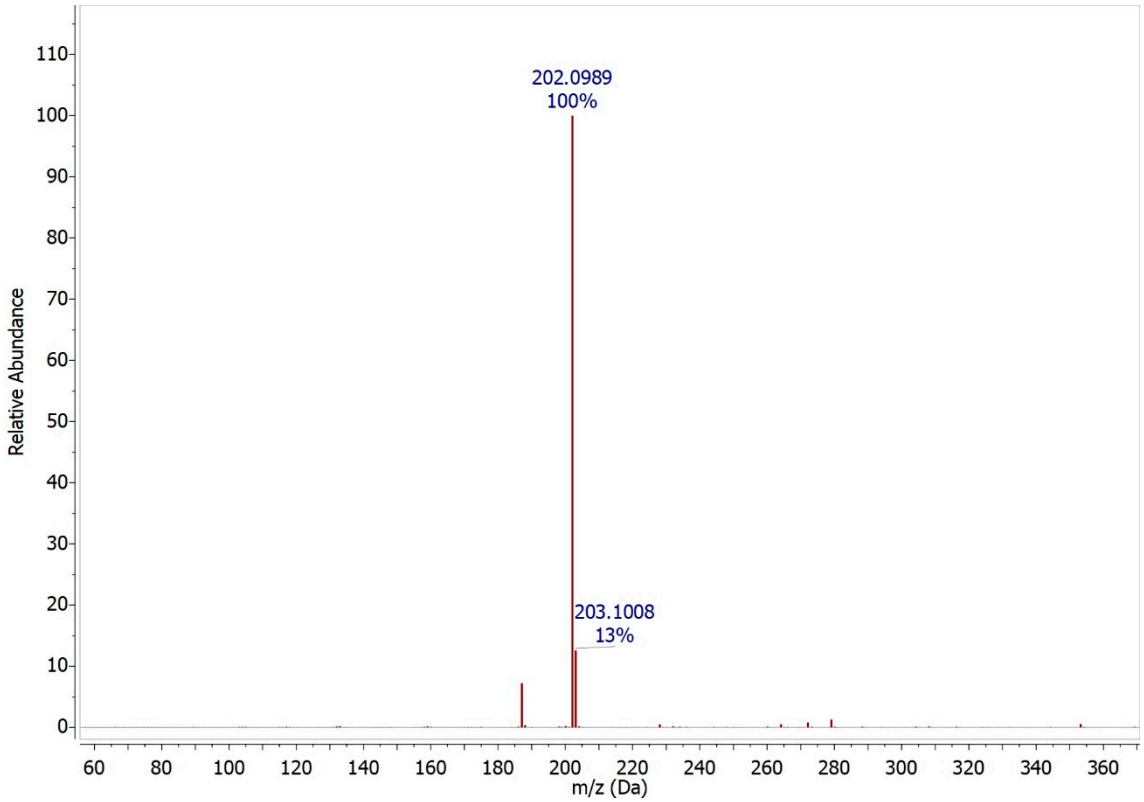


Figure S4. FT-MS spectrum of 5-(4-methoxyphenyl)pyrazin-2-amine (MeO-Clm). FTMS-ESI (+): m/z: calcd for $[C_{11}H_{12}N_3O]^+$: 202.0980 $[M + H]^+$; found 202.0989 $[C_{11}H_{12}N_3O]^+$.

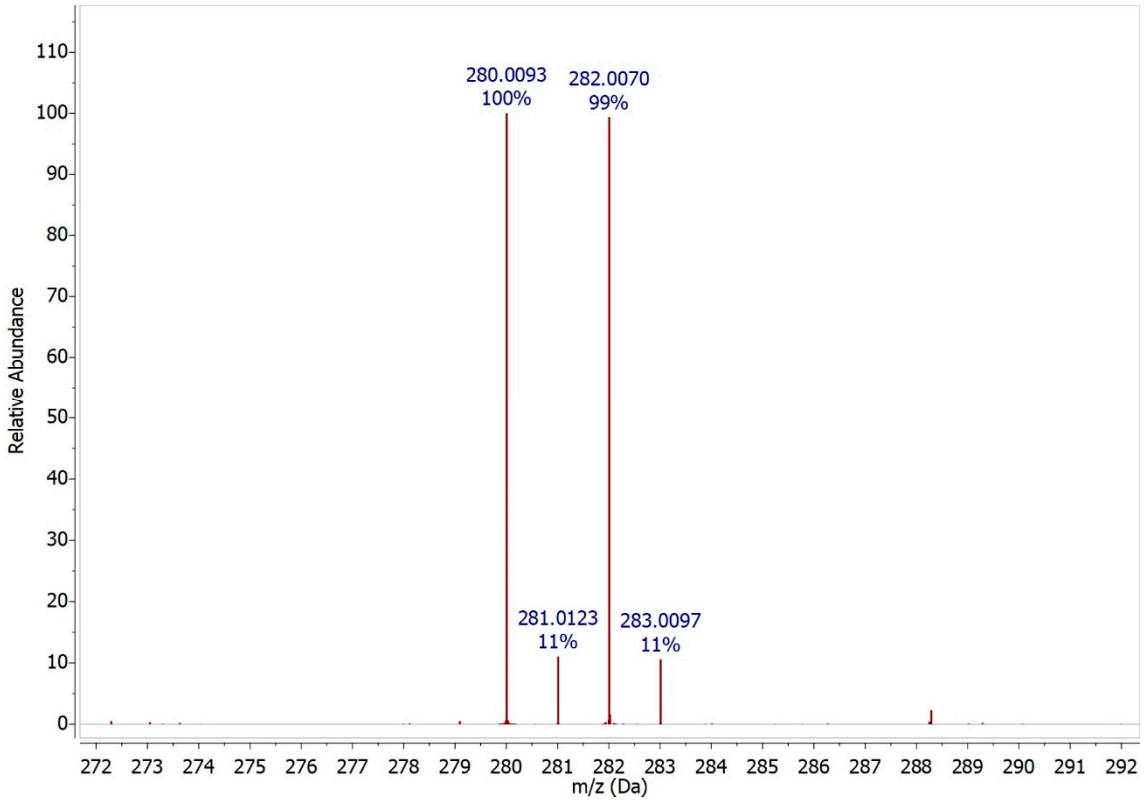


Figure S5. FT-MS spectrum of 3-bromo-5-(4-methoxyphenyl)pyrazin-2-amine (MeOBr-Clm). FTMS-ESI (+): m/z: calcd for $[C_{11}H_{11}BrN_3O]^+$: 280.0085 $[M + H]^+$; found 280.0093 $[C_{11}H_{11}{}^{79}BrN_3O]^+$, 282.0070 $[C_{11}H_{11}{}^{81}B_2N_3O]^+$.

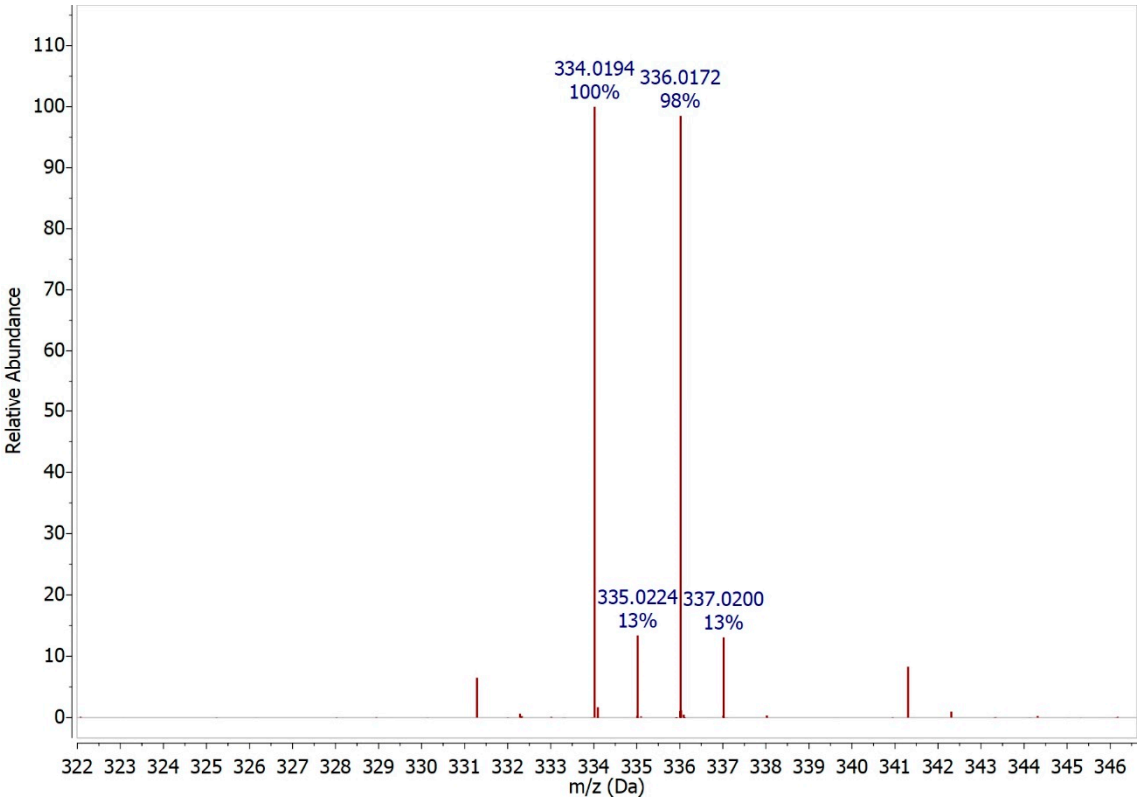


Figure S6. FT-MS spectrum of 8-bromo-6-(4-methoxyphenyl)-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (MeOBr-Cla). FTMS-ESI (+): m/z: calcd for $[C_{14}H_{13}BrN_3O_2]^+$: 334.0191 $[M + H]^+$; found 334.0194 $[C_{14}H_{13}{}^{79}Br^{35}N_3O_2]^+$, 336.0172 $[C_{14}H_{13}{}^{79}BrN_3O_2]^+$.