SUPPORTING INFORMATION

Multi-substituted 8-Aminoimidazo[1,2-*a*]pyrazines by Groebke-Blackburn-Bienaymé Reaction and Their Hsp90 Inhibitory Activity

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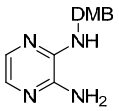
1. Chemistry

1) General.

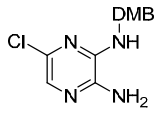
All solvents were dried and purified prior to use: Toluene was distilled from sodium, Et_2O and THF were distilled from potassium, and DCM was distilled from CaH_2. All other commercially available reagents were used as received. Reactions at −78 °C were performed in a dry ice/acetone bath. All moisture sensitive reactions were performed under N_2 (ca. +1.1 bar) in heating-gun (500-600 °C)/vacuum dried glassware sealed with rubber septa. Flash chromatography was performed on silica gel (300-400 mesh ASTM), and monitored by thin layer chromatography (TLC) on HSGF-254 (10-40 μm) TLC plates. NMR data were collected on a *Varian* Mercury-300 High Performance Digital FT-NMR, a *Varian* Mercury-400 High Performance Digital FT-NMR, or a *Bruker* Ultrashield 500 NMR. Spectra from solutions in $CDCl_3$ (δC = 77.0 ppm) are calibrated relative to TMS (δH = 0.00 ppm). HRMS were carried out on a *Thermo Finnigan* MAT-95 spectrometer (for EI), or on a *Waters*, Q-Tof Ultima Global spectrometer (for ESI). Melting points were measured on an uncorrected SGW X-4 micro melting point apparatus. HPLC analysis was performed on a Gilson HPLC system (306 pump, UV/vis-156 Detector, 215 liquid handle) with a YMC-ODS column (4.6 x 50 mm, 5 μm). HPLC conditions: solvent A = H_2O containing 0.1% (v/v) TFA, solvent B = MeCN containing 0.1% (v/v) TFA; flow rate = 2.5 mL/min; Gradient (B%): 0-0.5 min (4% isostatic), 0.5-4.5 min (4% - 95%), 4.5-6.1 min (95% isostatic), 6.1-6.3 min (95% - 4%), 6.3-8.0 min (4% isostatic); peaks were identified at 254 nm and 214 nm. An Elementar Vario EL Cube analyzer was used for elemental analysis.

2) Experimental procedures and characterization of products



Preparation of N^2-(2,4-dimethoxybenzyl)pyrazine-2,3-diamine (2)

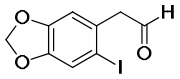
Compound **1** (15 g, 116 mmol), 2,4-dimethoxybenzylamine (52.3 mL, 347 mmol), and DIPEA (30.3 mL, 174 mmol) in NMP (30 mL) were heated at 130 °C in a sealed tube for 24 h. The reaction mixture was diluted with DCM and washed with H_2O, brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was triturated with DCM and 60 – 90 ^oC PE, and filtered to give product **2** (light brown solid, 14.8 g). The filtrate was concentrated and purified by flash column chromatography (SiO_2, DCM : MeOH = 20:1) to yield the second bach of product **2** (9.4 g). The total yield was 80%. Mp = 110-111 °C (from DCM and 60 – 90 ^oC PE). ^1H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 3.2 Hz, 1H, ArH), 7.38 (d, J = 3.2 Hz, 1H, ArH), 7.27 (d, J = 8.2 Hz, 1H, ArH), 6.50 (d, J = 2.4 Hz, 1H, ArH), 6.47 (dd, J = 8.2, 2.4 Hz, 1H, ArH), 4.64 (brs, 1H, NH), 4.53 (d, J = 5.3 Hz, 2H, CH_2), 4.27 (brs, 2H, NH_2), 3.85 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.43 (ArC), 158.70 (ArC), 144.93 (ArC), 143.85 (ArC), 132.38 (ArC), 130.72 (ArC), 129.46 (ArC), 119.14 (ArC), 103.85 (ArC), 98.57 (ArC), 55.35 (OCH_3), 55.30 (OCH_3), 41.03 (CH_2). HRMS (ESI+) calcd for $C_{13}H_{16}N_4O_2H^+$ 261.1352, found 261.1358.



Preparation of 6-chloro-N^2-(2,4-dimethoxybenzyl)pyrazine-2,3-diamine (6)

3-Bromo-5-chloropyrazin-2-amine (440 mg, 2.11 mmol), 2,4-dimethoxybenzylamine (0.64 mL, 4.22 mmol) and DIPEA (0.74 mL, 4.2 mmol) were dissolved in butan-1-ol (5 mL). The reaction mixture was heated at 140 °C in a microwave reactor for 1 h. The mixture was cooled to RT and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, DCM : MeOH = 20:1) to give product **6** (light brown solid, 588 mg, 95% yield). Mp = 170-171°C (from DCM and MeOH). ^1H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H, ArH), 7.28 (d, J = 8.1 Hz, 1H, ArH), 6.48 – 6.44 (m, 2H, ArH), 4.87 (brs, 1H, NH), 4.51 (d, J = 5.0 Hz, 2H, CH_2), 4.27 (brs, 2H, NH_2), 3.83 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.67 (ArC), 158.83 (ArC), 144.30 (ArC), 141.74 (ArC), 136.52 (ArC),

131.28 (ArC), 125.98 (ArC), 118.60 (ArC), 103.93 (ArC), 98.72 (ArC), 55.44 (2×C, OCH_3), 41.28 (CH_2). HRMS (ESI+) calcd for $C_{13}H_{15}ClN_4O_2H^+$ 295.0962, found 295.0956.



Preparation of 2-(6-iodobenzo[*d*][1,3]dioxol-5-yl)acetaldehyde (28)

To a vigorously stirred suspension of silver acetate (697 mg, 4.17 mmol) in AcOH (10 mL) containing 2-(6-iodobenzo[*d*][1,3]dioxol-5-yl)ethanol (720 mg, 2.47 mmol) was added solid I_2 (1.06 g, 4.17 mmol) in small portions such that a red color was maintained. After the I_2 addition was complete, the yellow solid suspension was stirred for 1 h at RT, and then diluted with DCM. The organic layer was washed with satd. aq. $Na_2S_2O_3$, satd. aq. $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was used in the next step without further purification. To the solution of the residue in 30 mL of anhydrous DCM was added Dess-Martin periodinane (1.74 g, 4.11 mmol). The mixture was stirred at RT for 2 h. The reaction was diluted with another 30 mL of DCM. The organic layer was washed with satd. aq. $Na_2S_2O_3$, satd. aq. $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 60 – 90 ^oC PE : EtOAc = 10:1) to give product **28** (white solid, 444 mg, 62% yield). Mp = 64-65 °C (from 60 – 90 ^oC PE and EtOAc). ^1H NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 1H, $CH_2C\underline{H}O$), 7.31 (s, 1H, ArH), 6.75 (s, 1H, ArH), 6.01 (s, 2H, OCH_2O), 3.83 (s, 2H, $C\underline{H_2}CHO$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.53 ($CH_2\underline{C}HO$), 148.81 (ArC), 148.00 (ArC), 129.09 (ArC), 118.83 (ArC), 110.62 (ArC), 101.87 (OCH_2O), 88.85 (ArC), 54.48 ($\underline{C}H_2CHO$). HRMS (EI) m/z calcd for $C_9H_7IO_3$ 289.9440, found 289.9440.

General procedure A for the reactions in Table 1, entries 1 and 3:

To a solution of compound **2** (70 mg, 1 equiv) in MeOH (5 mL) was added 2-phenylacetaldehyde (1.2 equiv), 1-isocyanobutane (1.2 equiv), and $Yb(OTf)_3$ (0.1 equiv) or TfOH (0.1 equiv). The mixture was stirred at 70 °C for 5 h. The reaction mixture was cooled to RT and concentrated in vacuo. EtOAc was

added and the organic phase was washed with H_2O, brine and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure and dissolved in DCM (5 mL). TFA (1 mL, 12.98 mmol) was added dropwise and the reaction mixture was stirred at 50 °C for 1 h. The reaction mixture was cooled to RT and concentrated in vacuo. The residue was diluted with EtOAc and satd. aq. $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, DCM : MeOH = 20:1) to give compound **5**.

General procedure B for the reactions in Table 1, entries 2 and 5:

3-Chloropyrazin-2-amine **1** (100 mg, 1 equiv) or compound **2** (70 mg, 1 equiv) was dissolved in CH_3CN and 2-phenylacetaldehyde (1.2 equiv) was added under nitrogen atmosphere. The reaction was refluxed for 2 h. The solvent was removed under vacuum and the residue was taken up with toluene and evaporated. A solution of TMSCl (1.2 equiv) in DCM was added and the reaction was stirred at RT for 30 min under nitrogen atmosphere. Then, a solution of the 1-isocyanobutane (1.2 equiv) in CH_3CN was added and the reaction was stirred at 100 °C for 12 h. No desired product could be detected by TLC.

General procedure C for the reactions in Table 1, entries 4, 6 and 7:

To a suspension of compound **1** (100 mg, 1 equiv) in MeOH (3 mL) was added 2-phenylacetaldehyde (1.2 equiv), 1-isocyanobutane (1.2 equiv), and TfOH or $Sc(OTf)_3$ or $Yb(OTf)_3$ (0.1 equiv). The mixture was stirred at 100 °C for 12 h. The reaction mixture was cooled to RT and concentrated in vacuo. EtOAc was added and the organic phase was washed with H_2O, brine and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure and dissolved in dioxane (2 mL) in a sealed tube. Ammonium hydroxide (5 mL) was added. The reaction was stirred at 120 °C for 24 h. EtOAc was

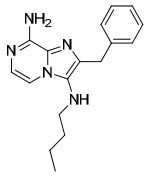
added and the organic phase was washed with H_2O, brine, and dried over Na_2SO_4. The crude material was purified by flash chromatography (SiO_2, DCM : MeOH = 20:1) to give compound **5**.

General procedure D for the reactions in Scheme 2 and Table 2, products 17-21, 29-35:

To a solution of pyrazines (1 equiv) in MeOH (5 mL) was added isocyanides (1.2 equiv), aldehydes (1.2 equiv) and $Yb(OTf)_3$ (0.1 equiv). The mixture was stirred at 70 °C for 5 h, cooled to RT, and concentrated in vacuo. EtOAc was added and the organic phase was separated and washed with H_2O, brine, and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure and dissolved in DCM (5 mL). TFA (1 mL, 12.98 mmol) was added dropwise and the reaction mixture was stirred at 50 °C for 1 h. The reaction mixture was cooled to RT and concentrated in vacuo. The residue was diluted with EtOAc and satd. aq. $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, DCM : MeOH = 20:1) to give compounds **17-21**, and **29-35**.

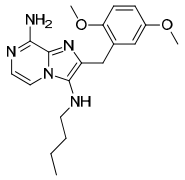
General procedure E for the reactions in Scheme 2, products 22-26:

A mixture of pyrazines (1 equiv), isocyanides (1.2 equiv), aldehydes (1.2 equiv) and $Yb(OTf)_3$ (0.1 equiv) in MeOH (5 mL) were heated at 70 °C for 5 h. The reaction mixture was cooled to RT and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 60 – 90 ^oC PE : EtOAc = 2:1) to give compounds **22-26**.



Preparation of 2-benzyl-N^3-butylimidazo[1,2-*a*]pyrazine-3,8-diamine (5)

From compound **2** (70 mg, 0.27 mmol, 1 equiv), 2-phenylacetaldehyde **4** (1.2 equiv), 1-isocyanobutane **3** (1.2 equiv), and $Yb(OTf)_3$ (0.1 equiv), according to general procedure A, compound **5** (light brown solid, 60 mg, 75%) was obtained. Mp = 72-73 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.19 (m, 7H, ArH), 6.07 (brs, 2H, NH_2), 4.14 (s, 2H, $PhCH_2$), 2.84 (t, *J* = 7.2 Hz, 2H, $C\underline{H_2}(CH_2)_2CH_3$), 2.72 (brs, 1H, NH), 1.45 – 1.29 (m, 4H, $CH_2(C\underline{H_2})_2CH_3$), 0.89 (t, *J* = 7.2 Hz, 3H, $CH_2(CH_2)_2C\underline{H_3}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.36 (ArC), 139.26 (ArC), 135.99 (ArC), 129.75 (ArC), 128.61 (2×C, ArC), 128.57 (2×C, ArC), 127.94 (ArC), 126.85 (ArC), 126.42 (ArC), 107.82 (ArC), 48.52 ($\underline{C}H_2(CH_2)_2CH_3$), 33.90, 32.76, 20.05 ($CH_2(\underline{C}H_2)_2CH_3$), 13.89 ($CH_2(CH_2)_2\underline{C}H_3$). HRMS (ESI+) calcd for $C_{17}H_{21}N_5H^+$ 296.1870, found 296.1869.

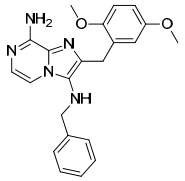


Preparation of N^3-butyl-2-(2,5-dimethoxybenzyl)imidazo[1,2-*a*]pyrazine-3,8-diamine (17)

From compound **2** (70 mg, 0.27 mmol, 1 equiv), 1-isocyanobutane **3** (1.2 equiv), and 2-(2,5-dimethoxyphenyl)acetaldehyde **12** [1] (1.2 equiv), according to general procedure D, compound **17** (brown oil, 50 mg, 52%) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, *J* = 4.7 Hz, 1H, ArH), 7.24 (d, *J* = 4.7 Hz, 1H, ArH), 6.87 (d, *J* = 3.0 Hz, 1H, ArH), 6.81 (d, *J* = 8.8 Hz, 1H, ArH), 6.73 (dd, *J* = 8.8, 3.0 Hz, 1H, ArH), 5.80 (brs, 2H, NH_2), 4.06 (s, 2H, $PhCH_2$), 3.83 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.37 (brs, 1H, NH), 2.91-2.81 (m, 2H, $C\underline{H_2}(CH_2)_2CH_3$), 1.55-1.35 (m, 4H, $CH_2(C\underline{H_2})_2CH_3$), 0.93 (t, *J* = 7.2 Hz, 3H, $CH_2(CH_2)_2C\underline{H_3}$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.72 (ArC), 150.94 (ArC), 149.16 (ArC), 134.90 (ArC), 130.06 (ArC), 128.79 (ArC), 127.73 (ArC), 126.71 (ArC), 116.61 (ArC), 111.87 (ArC), 111.32 (ArC), 107.98 (ArC), 56.06 (OCH_3), 55.68 (OCH_3), 48.48 ($\underline{C}H_2(CH_2)_2CH_3$),

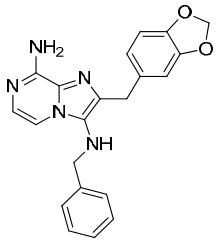
[1] J. M. Beierlein, K. M. Frey, D. B. Bolstad, P. M. Pelphrey, T. M. Joska, A. E. Smith, N. D. Priestley, D. L. Wright, A. C. Anderson, *J. Med. Chem.,* 2008, **51**, 7532-7540.

32.81, 28.02, 20.15 ($CH_2(\underline{C}H_2)_2CH_3$), 13.96 ($CH_2(CH_2)_2\underline{C}H_3$). HRMS (ESI+) calcd for $C_{19}H_{25}N_5O_2H^+$ 356.2087, found 356.2087.



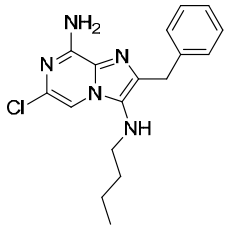
Preparation of N^3-benzyl-2-(2,5-dimethoxybenzyl)imidazo[1,2-*a*]pyrazine-3,8-diamine (18)

From compound **2** (70 mg, 0.27 mmol, 1 equiv), benzyl isocyanide **9** (1.2 equiv), 2-(2,5-dimethoxyphenyl)acetaldehyde **12** [1] (1.2 equiv), according to general procedure D, compound **18** (brown oil, 56 mg, 53%) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.29 (m, 5H, ArH), 7.27 (s, 1H, ArH), 7.19 (d, J = 4.8 Hz, 1H, ArH), 6.89 – 6.85 (m, 1H, ArH), 6.76 (d, J = 8.9 Hz, 1H, ArH), 6.71 (dd, J = 8.9, 2.9 Hz, 1H, ArH), 6.22 (brs, 2H, NH_2), 4.01 (s, 2H, NHC$\underline{H}_2$), 3.88 (s, 2H, $PhCH_2$), 3.73 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), benzylamine-NH was not observed. ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.72 (ArC), 150.81 (ArC), 149.04 (ArC), 138.95 (ArC), 136.01 (ArC), 129.56 (ArC), 128.70 (2×C, ArC), 128.55 (ArC), 128.14 (2×C, ArC), 127.70 (ArC), 127.54 (ArC), 125.86 (ArC), 116.68 (ArC), 111.98 (ArC), 111.42 (ArC), 107.82 (ArC), 55.98 (OCH_3), 55.68 (OCH_3), 52.81 ($NHCH_2$), 27.91 ($PhCH_2$). HRMS (ESI+) calcd for $C_{22}H_{23}N_5O_2H^+$ 390.1930, found 390.1923.



Preparation of 2-((benzo[*d*][1,3]dioxol-5-yl)methyl)-N^3-benzylimidazo[1,2-*a*]pyrazine-3,8-diamine (19)

From compound **2** (120 mg, 0.46 mmol, 1 equiv), benzyl isocyanide **9** (1.2 equiv), and 2-(benzo[*d*][1,3]dioxol-5-yl)acetaldehyde **13** [2] (1.2 equiv), according to general procedure D, compound **19** (brown solid, 107 mg, 62%) was obtained. Mp = 124-125 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.31 (m, 4H, ArH), 7.27-7.24 (m, 1H, ArH), 7.24-7.19 (m, 2H, ArH), 6.72 (d, *J* = 7.9 Hz, 1H, ArH), 6.68 (s, 1H, ArH), 6.64 (dd, *J* = 7.9, 1.6 Hz, 1H, ArH), 5.91 (s, 2H, OCH_2O), 5.76 (brs, 2H, NH_2), 4.00 (d, *J* = 6.2 Hz, 2H, $NHCH_2$), 3.86 (s, 2H, $PhCH_2$), 3.09 (t, *J* = 6.2 Hz, 1H, NH CH_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.27 (ArC), 147.80 (ArC), 146.09 (ArC), 138.92 (ArC), 136.96 (ArC), 132.98 (ArC), 128.73 (2×C, ArC), 128.66 (ArC), 128.21 (2×C, ArC), 128.13 (ArC), 127.76 (ArC), 127.34 (ArC), 121.31 (ArC), 109.12 (ArC), 108.24 (ArC), 107.89 (ArC), 100.91 (OCH_2O), 52.84 ($NHCH_2$), 33.37 ($PhCH_2$). HRMS (ESI+) calcd for $C_{21}H_{19}N_5O_2H^+$ 374.1612, found 374.1601.

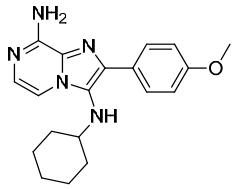


Preparation of 2-benzyl-*N*^{3}-butyl-6-chloroimidazo[1,2-*a*]pyrazine-3,8-diamine (20)

From compound **6** (50 mg, 0.17 mmol, 1 equiv), 1-isocyanobutane **3** (1.2 equiv), 2-phenylacetaldehyde **4** (1.2 equiv), according to general procedure D, compound **20** (brown oil, 47 mg, 83%) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H, ArH), 7.34 – 7.29 (m, 2H, ArH), 7.27 – 7.21 (m, 3H, ArH), 6.13 (brs, 2H, NH_2), 4.12 (s, 2H, $PhCH_2$), 2.83 (t, *J* = 7.1 Hz, 2H, $CH_2(CH_2)_2CH_3$), 1.46-1.29 (m, 4H, $CH_2(CH_2)_2CH_3$), 0.90 (t, *J* = 7.2 Hz, 3H, $CH_2(CH_2)_2CH_3$), butylamine-NH was not observed. ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.51 (ArC), 138.41 (ArC), 136.49 (ArC), 132.46 (ArC), 129.68 (ArC), 128.18 (2×C, ArC), 128.04 (2×C, ArC), 126.05 (ArC), 125.99 (ArC), 103.89 (ArC), 47.94 ($CH_2(CH_2)_2CH_3$),

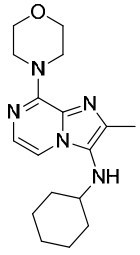
[2] Q. Liu, E. M. Ferreira, B. M. Stoltz, *J. Org. Chem.,* 2007, **72**, 7352-7358.

33.32, 32.24, 19.52 ($CH_2(\underline{C}H_2)_2CH_3$), 13.37 ($CH_2(CH_2)_2\underline{C}H_3$). HRMS (ESI+) calcd for $C_{17}H_{20}ClN_5H^+$ 330.1485, found 330.1477.



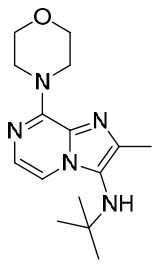
Preparation of N^3-cyclohexyl-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyrazine-3,8-diamine (21)

From compound **2** (70 mg, 0.27 mmol, 1 equiv), cyclohexyl isocyanide **10** (1.2 equiv), 4-methoxybenzaldehyde **14** (1.2 equiv), according to general procedure D, compound **21** (gray solid, 60 mg, 66%) was obtained. Mp = 185-186 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.5 Hz, 2H, ArH), 7.45 (d, *J* = 4.6 Hz, 1H, ArH), 7.29 (d, *J* = 4.6 Hz, 1H, ArH), 7.01 (d, *J* = 8.5 Hz, 2H, ArH), 5.73 (brs, 2H, NH_2), 3.88 (s, 3H, OCH_3), 3.10 (d, *J* = 5.9 Hz, 1H, NH), 3.01-2.92(m, 1H, cyclohexyl-CH), 1.87 – 1.79 (m, 2H, cyclohexyl-CH_2), 1.74 – 1.66 (m, 2H, cyclohexyl-CH_2), 1.61 – 1.55 (m, 1H, cyclohexyl-CH_2),1.25-1.13 (m, 5H, cyclohexyl-CH_2). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.10 (ArC), 149.40 (ArC), 136.25 (ArC), 128.38 (ArC), 128.32 (2×C, ArC), 127.21 (ArC), 126.92 (ArC), 126.52 (ArC), 114.08 (2×C, ArC), 108.21 (ArC), 56.94, 55.31, 34.23 (2×C, cyclohexyl-CH_2), 25.66 (cyclohexyl-CH_2), 24.83 (2×C, cyclohexyl-CH_2). HRMS (ESI+) calcd for $C_{19}H_{23}N_5OH^+$ 338.1981, found 338.1982.



Preparation of *N*-cyclohexyl-2-methyl-8-morpholinoimidazo[1,2-*a*]pyrazin-3-amine (22)

From compound **7** [3] (50 mg, 0.28 mmol, 1 equiv), cyclohexyl isocyanide **10** (1.2 equiv), and acetaldehyde **15** (5 equiv), according to general procedure E (except 5 equiv of aldehyde was used), compound **22** (light brown oil, 70 mg, 79%) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 4.4 Hz, 1H, ArH), 7.32 (d, *J* = 4.4 Hz, 1H, ArH), 4.18 (t, *J* = 4.7 Hz, 4H, morpholine-C$\underline{H_2}$OC$\underline{H_2}$), 3.86 (t, *J* = 4.7 Hz, 4H, morpholine-C$\underline{H_2}$NC$\underline{H_2}$), 2.90-2.80 (m, 1H, NH), 2.76-2.65 (m, 1H, cyclohexyl-CH), 2.35 (s, 3H, CH_3), 1.89-1.81 (m, 2H, cyclohexyl-CH_2), 1.78-1.71 (m, 2H, cyclohexyl-CH_2), 1.64-1.56 (m, 1H, cyclohexyl-CH_2), 1.24-1.15 (m, 5H, cyclohexyl-CH_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.09 (ArC), 133.61 (ArC), 129.53 (ArC), 126.70 (ArC), 126.64 (ArC), 108.33 (ArC), 67.09 (2×C, morpholine-$\underline{C}H_2O\underline{C}H_2$), 56.96 (cyclohexyl-CH), 46.86 (2×C, morpholine-$\underline{C}H_2N\underline{C}H_2$), 34.29 (2×C, cyclohexyl-CH_2), 25.73 (cyclohexyl-CH_2), 24.86 (2×C, cyclohexyl-CH_2), 13.04 (CH_3). HRMS (ESI+) calcd for $C_{17}H_{25}N_5OH^+$ 316.2137, found 316.2139.

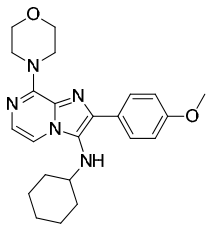


Preparation of *N*-(*tert*-butyl)-2-methyl-8-morpholinoimidazo[1,2-*a*]pyrazin-3-amine (23)

From compound **7** (50 mg, 0.28 mmol, 1 equiv), *tert*-butyl isocyanide **11** (1.2 equiv), and acetaldehyde **15** (5 equiv), according to general procedure E (except 5 equiv of aldehyde was used), compound **23** (white solid, 77 mg, 95%) was obtained. Mp = 85-86 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59-7.55 (m, 1H, ArH), 7.36-7.32 (m, 1H, ArH), 4.20 (t, *J* = 4.6 Hz, 4H, morpholine-C$\underline{H_2}$OC$\underline{H_2}$), 3.90 (t, *J* = 4.6 Hz, 4H, morpholine- C$\underline{H_2}$NC$\underline{H_2}$), 2.40 (s, 3H, CH_3), 1.20 (s, 9H, $C(CH_3)_3$), tert-butylamine-NH was not observed. ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.64 (ArC), 135.44 (ArC),

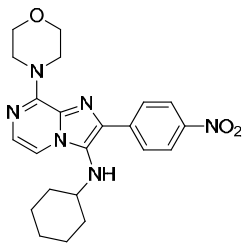
[3] J. M. Bartolome-Nebreda, *et al.* WO2011110545.

129.71 (ArC), 125.86 (ArC), 124.99 (ArC), 108.78 (ArC), 66.57 (2×C, morpholine-$\underline{C}H_2O\underline{C}H_2$), 55.44 ($\underline{C}(CH_3)_3$), 46.43 (2×C, morpholine-$\underline{C}H_2N\underline{C}H_2$), 29.87 (3×C, $C(\underline{C}H_3)_3$), 13.48 (CH_3). HRMS (ESI+) calcd for $C_{15}H_{23}N_5OH^+$ 290.1981, found 290.1989.



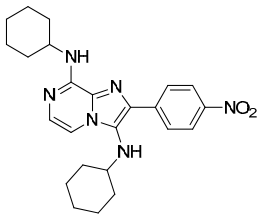
Preparation of *N*-cyclohexyl-2-(4-methoxyphenyl)-8-morpholinoimidazo[1,2-*a*]pyrazin-3-amine (24)

From compound **7** (50 mg, 0.28 mmol, 1 equiv), cyclohexyl isocyanide **10** (1.2 equiv), and 4-methoxybenzaldehyde **14** (1.2 equiv), according to general procedure E, compound **24** (gray solid, 93 mg, 81%) was obtained. Mp = 118-119 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 2H, ArH), 7.48 (d, *J* = 4.5 Hz, 1H, ArH), 7.37 (d, *J* = 4.5 Hz, 1H, ArH), 7.01 (d, *J* = 8.3 Hz, 2H, ArH), 4.33 (t, *J* = 4.9 Hz, 4H, morpholine-$C\underline{H_2}OC\underline{H_2}$), 3.91 (t, *J* = 4.9 Hz, 4H, morpholine-$C\underline{H_2}NC\underline{H_2}$), 3.88 (s, 3H, OCH_3), 3.02-2.92(m, 1H, cyclohexyl-CH), 1.87-1.80 (m, 2H, cyclohexyl-CH_2), 1.74-1.67 (m, 2H, cyclohexyl-CH_2), 1.62-1.55 (m, 1H, cyclohexyl-CH_2), 1.30-1.14 (m, 5H, cyclohexyl-CH_2), cyclohexylamine-NH was not observed. ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.53 (ArC), 148.75 (ArC), 134.43 (ArC), 129.34 (ArC), 127.66 (2×C, ArC), 126.37 (ArC), 126.31 (ArC), 125.36 (ArC), 113.49 (2×C, ArC), 107.62 (ArC), 66.66 (2×C, morpholine-$\underline{C}H_2O\underline{C}H_2$), 56.35, 54.80, 46.41 (2×C, morpholine-$\underline{C}H_2N\underline{C}H_2$), 33.73 (2×C, cyclohexyl-CH_2), 25.18 (cyclohexyl-CH_2), 24.34(2×C, cyclohexyl-CH_2). HRMS (ESI+) calcd for $C_{23}H_{29}N_5O_2H^+$ 408.2400, found 408.2409.



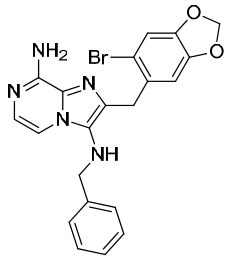
Preparation of *N*-cyclohexyl-8-morpholino-2-(4-nitrophenyl)imidazo[1,2-*a*]pyrazin-3-amine (25)

From compound **7** (50 mg, 0.28 mmol, 1 equiv), cyclohexyl isocyanide **10** (1.2 equiv), and 4-nitrobenzaldehyde **16** (1.2 equiv), according to general procedure E, compound **25** (light yellow solid, 97 mg, 82%) was obtained. Mp = 190-191 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (d, *J* = 8.6 Hz, 2H, ArH), 8.30 (d, *J* = 8.6 Hz, 2H, ArH), 7.78 (d, *J* = 4.6 Hz, 1H, ArH), 7.38 (d, *J* = 4.6 Hz, 1H, ArH), 5.13 (d, *J* = 7.4 Hz, 1H, NH), 4.30 – 4.17 (m, 4H, morpholine-C$\underline{H_2}$OC$\underline{H_2}$), 3.83 – 3.69 (m, 4H, morpholine- C$\underline{H_2}$NC$\underline{H_2}$), 2.88-2.73 (m, 1H, cyclohexyl-CH), 1.79–1.70 (m, 2H, cyclohexyl-CH_2), 1.67–1.58 (m, 2H, cyclohexyl-CH_2), 1.52–1.45 (m, 1H, cyclohexyl-CH_2), 1.35–1.22 (m, 2H, cyclohexyl-CH_2) ,1.13–1.02 (m, 3H, cyclohexyl-CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 149.14 (ArC), 146.11 (ArC), 141.15 (ArC), 130.49 (ArC), 130.46 (ArC), 129.98 (ArC), 127.62 (ArC), 127.08(2×C, ArC), 124.31(2×C, ArC), 108.84 (ArC), 66.69 (2×C, morpholine-$\underline{C}H_2O\underline{C}H_2$), 57.55 (cyclohexyl-CH), 46.83 (2×C, morpholine-$\underline{C}H_2N\underline{C}H_2$), 34.02 (2×C, cyclohexyl-CH_2), 25.75 (cyclohexyl-CH_2), 25.09 (2×C, cyclohexyl-CH_2). HRMS (ESI+) calcd for $C_{22}H_{26}N_6O_3H^+$ 423.2145, found 423.2148.



Preparation of N^3, N^8-dicyclohexyl-2-(4-nitrophenyl)imidazo[1,2-*a*]pyrazine-3,8-diamine (26)

From compound **8** [4] (50 mg, 0.26 mmol, 1 equiv), cyclohexyl isocyanide **10** (1.2 equiv), and 4-nitrobenzaldehyde **16** (1.2 equiv), according to general procedure E, compound **26** (light brown solid, 96 mg, 85%) was obtained. Mp = 176-177 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35-8.28 (m, 4H, ArH), 7.37 (d, *J* = 4.7 Hz, 1H, ArH), 7.28 (d, *J* = 4.7 Hz, 1H, ArH), 6.06 – 5.92 (m, 1H, NH), 4.20-4.05 (m, 1H, NH), 3.13 – 2.93 (m, 2H, cyclohexyl-CH), 2.23 – 2.09 (m, 2H, cyclohexyl-CH_2), 1.90 – 1.79 (m, 4H, cyclohexyl-CH_2), 1.77-1.67 (m, 3H, cyclohexyl-CH_2), 1.65-1.58 (m, 1H, cyclohexyl-CH_2), 1.56 – 1.35 (m, 4H, cyclohexyl-CH_2), 1.31 – 1.15 (m, 6H, cyclohexyl-CH_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ 148.50 (ArC), 146.41 (ArC), 140.81 (ArC), 132.88 (ArC), 129.77 (ArC), 128.85 (ArC), 128.70 (ArC), 126.97 (2×C, ArC), 123.91(2×C, ArC), 105.97 (ArC), 57.31 (cyclohexyl-CH), 49.21 (cyclohexyl-CH), 34.35 (2×C, cyclohexyl-CH_2), 33.27 (2×C, cyclohexyl-CH_2), 25.73 (cyclohexyl-CH_2), 25.57 (cyclohexyl-CH_2), 25.03 (2×C, cyclohexyl-CH_2), 24.85 (2×C, cyclohexyl-CH_2). HRMS (ESI+) calcd for $C_{24}H_{30}N_6O_2H^+$ 435.2508, found 435.2509.

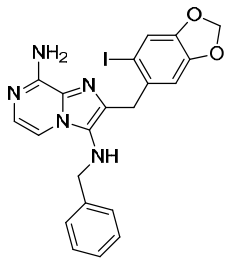


Preparation of N^3-benzyl-2-((6-bromobenzo[*d*][1,3]dioxol-5-yl)methyl)imidazo[1,2-*a*]pyrazine-3,8-diamine (29)

From compound **2** (120 mg, 0.46 mmol, 1 equiv), benzyl isocyanide **9** (1.2 equiv), and 2-(6-bromobenzo[*d*][1,3]dioxol-5-yl)acetaldehyde **27** [2] (1.2 equiv), according to general procedure D, compound **29** (orange solid, 131 mg, 63%) was obtained. Mp = 135-136 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.29 (m, 4H, ArH), 7.28 – 7.23 (m, 3H, ArH), 7.00 (s, 1H, ArH),

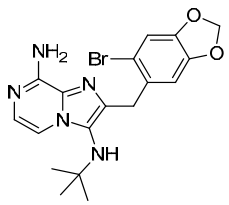
[4] B. Delouvrié, H. Germain, C. S. Harris, M. Lamorlette, H. Lebraud, H. T. H. Nguyen, A. Noisier, G. Ouvry, *Tetrahedron Lett*., 2012, **53**, 5380-5384.

6.71 (s, 1H, ArH), 5.92 (s, 2H, OCH_2O), 5.65 (brs, 2H, NH_2), 4.03 (d, J = 6.4 Hz, 2H, $NHC\underline{H_2}$), 3.95 (s, 2H, $PhCH_2$), 3.33 (t, J = 6.4 Hz, 1H, $N\underline{H}CH_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.27 (ArC), 147.47 (ArC), 147.00 (ArC), 138.82 (ArC), 135.45 (ArC), 131.71 (ArC), 128.83 (ArC), 128.70 (2×C, ArC), 128.44 (ArC), 128.21 (2×C, ArC), 127.73 (ArC), 127.52 (ArC), 114.10 (ArC), 112.44 (ArC), 110.40 (ArC), 108.01 (ArC), 101.67 (OCH_2O), 52.92 ($NHCH_2$), 33.39 ($PhCH_2$). HRMS (ESI+) calcd for $C_{21}H_{18}BrN_5O_2H^+$ 452.0717, found 452.0705.



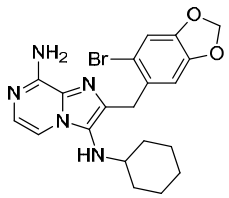
Preparation of N^3-benzyl-2-((6-iodobenzo[*d*][1,3]dioxol-5-yl)methyl)imidazo[1,2-*a*]pyrazine-3,8-diamine (30)

From compound **2** (120 mg, 0.46 mmol, 1 equiv), benzyl isocyanide **9** (1.2 equiv), and 2-(6-iodobenzo[*d*][1,3]dioxol-5-yl)acetaldehyde **28** (1.2 equiv), according to general procedure D, compound **30** (gray solid, 113 mg, 49%) was obtained. Mp = 154-155 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.29 (m, 4H, ArH), 7.28 – 7.23 (m, 4H, ArH), 6.71 (s, 1H, ArH), 5.92 (s, 2H, OCH_2O), 5.64 (brs, 2H, NH_2), 4.03 (d, J = 6.4 Hz, 2H, $NHC\underline{H_2}$), 3.95 (s, 2H, $PhCH_2$), 3.32 (t, J = 6.4 Hz, 1H, $N\underline{H}CH_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.20 (ArC), 148.56 (ArC), 147.11 (ArC), 138.78 (ArC), 135.72 (ArC), 135.21 (ArC), 128.94 (ArC), 128.71 (2×C, ArC), 128.44 (ArC), 128.24 (2×C, ArC), 127.74 (ArC), 127.37 (ArC), 118.34 (ArC), 110.01 (ArC), 108.05 (ArC), 101.61 (OCH_2O), 87.85 (ArC), 52.95 ($NHCH_2$), 38.53 ($PhCH_2$). HRMS (ESI+) calcd for $C_{21}H_{18}IN_5O_2H^+$ 500.0578, found 500.0567.



Preparation of 2-((6-bromobenzo[*d*][1,3]dioxol-5-yl)methyl)-N^3-(*tert*-butyl)imidazo[1,2-*a*]pyrazine-3,8-diamine (31)

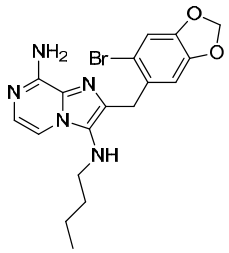
From compound **2** (70 mg, 0.27 mmol, 1 equiv), *tert*-butyl isocyanide **11** (1.2 equiv), 2-(6-bromobenzo[*d*][1,3]dioxol-5-yl)acetaldehyde **27** [2] (1.2 equiv), according to general procedure D, compound **31** (gray solid, 54 mg, 48%) was obtained. Mp = 163-164 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 4.7 Hz, 1H, ArH), 7.26 (d, *J* = 4.7 Hz, 1H, ArH), 7.00 (s, 1H, ArH), 6.82 (s, 1H, ArH), 5.94 (s, 2H, OCH_2O), 5.72 (brs, 2H, NH_2), 4.13 (s, 2H, $PhCH_2$), 2.90 (brs, 1H, NH), 1.21 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.61 (ArC), 146.95 (ArC), 146.44 (ArC), 137.72 (ArC), 131.47 (ArC), 128.75 (ArC), 126.82 (ArC), 126.15 (ArC), 113.71 (ArC), 111.97 (ArC), 110.03 (ArC), 108.80 (ArC), 101.19 (OCH_2O), 55.30 ($\underline{C}(CH_3)_3$), 33.18 ($PhCH_2$), 29.92 (3×C, $C(\underline{C}H_3)_3$). HRMS (ESI+) calcd for $C_{18}H_{20}BrN_5O_2H^+$ 418.0873, found 418.0870.



Preparation of 2-((6-bromobenzo[*d*][1,3]dioxol-5-yl)methyl)-N^3-cyclohexylimidazo[1,2-*a*]pyrazine-3,8-diamine (32)

From compound **2** (70 mg, 0.27 mmol, 1 equiv), cyclohexyl isocyanide **10** (1.2 equiv), and 2-(6-bromobenzo[*d*][1,3]dioxol-5-yl)acetaldehyde **27** [2] (1.2 equiv), according to general procedure D, compound **32** (gray solid, 68 mg, 57%) was obtained. Mp = 180-181 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 4.6 Hz, 1H, ArH), 7.28 (d, *J* = 4.6 Hz, 1H, ArH), 7.02 (s, 1H,

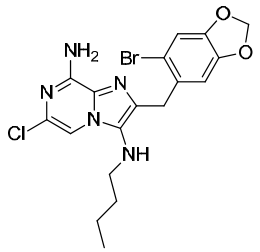
ArH), 6.78 (s, 1H, ArH), 5.93 (s, 2H, OCH_2O), 5.65 (brs, 2H, NH_2), 4.13 (s, 2H, $PhCH_2$), 2.90 (d, J = 6.3 Hz, 1H, NH), 2.86-2.76 (m, 1H, cyclohexyl-CH), 1.86-1.77 (m, 2H, cyclohexyl-CH_2), 1.77-1.70 (m, 2H, cyclohexyl-CH_2), 1.65-1.57 (m, 1H, cyclohexyl-CH_2),1.25 – 1.14 (m, 5H, cyclohexyl-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.69 (ArC), 147.04 (ArC), 146.51 (ArC), 135.42 (ArC), 131.39 (ArC), 128.10 (ArC), 127.98 (ArC), 126.61 (ArC), 113.63 (ArC), 111.95 (ArC), 109.93 (ArC), 107.97 (ArC), 101.21 (OCH_2O), 56.92 (cyclohexyl-CH), 33.76 (2×C, cyclohexyl-CH_2), 32.97 ($PhCH_2$), 25.22 (cyclohexyl-CH_2), 24.46 (2×C, cyclohexyl-CH_2). HRMS (ESI+) calcd for $C_{20}H_{22}BrN_5O_2H^+$ 444.1030, found 444.1024.



Preparation of 2-((6-bromobenzo[*d*][1,3]dioxol-5-yl)methyl)-N^3-butylimidazo[1,2-*a*]pyrazine-3,8-diamine (33)

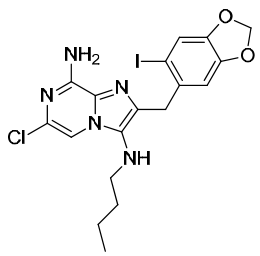
From compound **2** (70 mg, 0.27 mmol, 1 equiv), 1-isocyanobutane **3** (1.2 equiv), and 2-(6-bromobenzo[*d*][1,3]dioxol-5-yl)acetaldehyde **27** [2] (1.2 equiv), according to general procedure D, compound **33** (light yellow solid, 60 mg, 53%) was obtained. Mp = 139-140 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 4.7 Hz, 1H, ArH), 7.28 (d, J = 4.7 Hz, 1H, ArH), 7.03 (s, 1H, ArH), 6.77 (s, 1H, ArH), 5.94 (s, 2H, OCH_2O), 5.72 (brs, 2H, NH_2), 4.14 (s, 2H, $PhCH_2$), 2.99-2.93 (m, 1H, NH), 2.93 – 2.85 (m, 2H, $C\underline{H_2}(CH_2)_2CH_3$), 1.54-1.32 (m, 4H, $CH_2(C\underline{H_2})_2CH_3$), 0.92 (t, J = 7.2 Hz, 3H, $CH_2(CH_2)_2C\underline{H_3}$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.17 (ArC), 147.53 (ArC), 147.03 (ArC), 134.69 (ArC), 131.78 (ArC), 129.91 (ArC), 128.13 (ArC), 127.02 (ArC), 114.08 (ArC), 112.44 (ArC), 110.37 (ArC), 108.07 (ArC), 101.70 (OCH_2O), 48.66 ($\underline{C}H_2(CH_2)_2CH_3$), 33.54, 32.79, 20.12

($CH_2(\underline{C}H_2)_2CH_3$), 13.93 ($CH_2(CH_2)_2\underline{C}H_3$). HRMS (ESI+) calcd for $C_{18}H_{20}BrN_5O_2H^+$ 418.0873, found 418.0880.



Preparation of 2-((6-bromobenzo[*d*][1,3]dioxol-5-yl)methyl)-N^3-butyl-6-chloroimidazo[1,2-*a*]pyrazine-3,8-diamine (34)

From compound **6** (50 mg, 0.17 mmol, 1 equiv), 1-isocyanobutane **3** (1.2 equiv), and 2-(6-bromobenzo[*d*][1,3]dioxol-5-yl)acetaldehyde **27** [2] (1.2 equiv), according to general procedure D, compound **34** (white solid, 61 mg, 79%) was obtained. Mp = 195-196 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (s, 1H, ArH), 7.24 (brs, 2H, NH_2), 7.21 (s, 1H, ArH), 6.63 (s, 1H, ArH), 6.01 (s, 2H, OCH_2O), 4.72 (t, J = 6.3 Hz, 1H, NH), 4.00 (s, 2H, $PhCH_2$), 2.90-2.80 (m, 2H, $C\underline{H}_2(CH_2)_2CH_3$), 1.40 – 1.33 (m, 2H, $CH_2(C\underline{H}_2)_2CH_3$), 1.30 – 1.25 (m, 2H, $CH_2(C\underline{H}_2)_2CH_3$), 0.81 (t, J = 7.2 Hz, 3H, $CH_2(CH_2)_2C\underline{H}_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 149.06 (ArC), 147.49 (ArC), 147.01 (ArC), 133.36 (ArC), 132.76 (ArC), 132.70 (ArC), 131.76 (ArC), 126.46 (ArC), 114.00 (ArC), 112.48 (ArC), 110.52 (ArC), 103.47 (ArC), 102.22 (OCH_2O), 47.79 ($\underline{C}H_2(CH_2)_2CH_3$), 33.14, 32.72, 19.99 ($CH_2(\underline{C}H_2)_2CH_3$), 14.22 ($CH_2(CH_2)_2\underline{C}H_3$). HRMS (ESI+) calcd for $C_{18}H_{19}BrClN_5O_2H^+$ 452.0483, found 452.0492.



Preparation of N^3-butyl-6-chloro-2-((6-iodobenzo[*d*][1,3]dioxol-5-yl)methyl)imidazo[1,2-*a*]pyrazine-3,8-diamine (35)

From compound **6** (50 mg, 0.17 mmol, 1 equiv), 1-isocyanobutane **3** (1.2 equiv), and 2-(6-iodobenzo[*d*][1,3]dioxol-5-yl)acetaldehyde **28** (1.2 equiv), according to general procedure D, compound **35** (gray solid, 68 mg, 80%) was obtained. Mp = 216-217 °C (from DCM and MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (s, 1H, ArH), 7.38 (s, 1H, ArH), 7.24 (brs, 2H, NH_2), 6.58 (s, 1H, ArH), 5.99 (s, 2H, OCH_2O), 4.70 (t, J = 6.3 Hz, 1H, NH), 3.97 (s, 2H, $PhCH_2$), 2.90-2.77 (m, 2H, $C\underline{H_2}(CH_2)_2CH_3$), 1.42-1.22 (m, 4H, $CH_2(C\underline{H_2})_2CH_3$), 0.81 (t, J = 7.2 Hz, 3H, $CH_2(CH_2)_2C\underline{H_3}$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 149.07 (ArC), 148.36 (ArC), 147.06 (ArC), 136.17 (ArC), 133.61 (ArC), 132.70 (ArC), 131.83 (ArC), 126.45 (ArC), 118.20 (ArC), 110.04 (ArC), 103.45 (ArC), 102.05 (OCH_2O), 88.93 (ArC), 47.81 ($\underline{C}H_2(CH_2)_2CH_3$), 38.29, 32.74, 20.01($CH_2(\underline{C}H_2)_2CH_3$), 14.25 ($CH_2(CH_2)_2\underline{C}H_3$). HRMS (ESI+) calcd for $C_{18}H_{19}ClIN_5O_2H^+$ 500.0345, found 500.0340.

2. Hsp90α fluorescence polarization (FP) competition assay[5]

Hsp90α expression and purification

The sequence-verified cDNA (NCBI reference sequence: NM_005348.3) encoding the human full-length Hsp90α protein was inserted into the pFastBac-HTb plasmid (Invitrogen, USA). Recombinant bacmid was generated by transposition in *E. Coli*, and transfected into SF9 insert cells using Cellfectin II reagent (Cat no. 10362100, Invitrogen, USA). Large-scale protein production was performed in SF9 insert cell cultures infected with high-titer Hsp90α recombinant baculovirus. Cells were harvested by centrifugation after 96 h post infection and stored at -80 °C until use. The cell pellet was resuspended in lysis buffer (50 mM Tris, pH 7.5, 500 mM NaCl, 10% glycerol and 20 mM imidazole), and protein was

[5] J. Kim, S. Felts, L. Llauger, H. He, H. Huezo, N. Rosen, G. Chiosis, *J. Biomol. Screen.,* 2004, **9**, 375-381.

eluted with the same buffer containing 250 mM imidazole and concentrated to 1 mg/mL. Protein preparations were divided to small aliquots, flash frozen in liquid nitrogen, and stored at -70 °C.

FP assay

Based on a test set of Hsp90α inhibitors competing with fluorescent geldanamycin (GM-BODIPY) for binding to Hsp90α, the assays were performed on a multi-mode microplate reader (Synergy4, Bio-Tek, Company, USA). All experiments were conducted in 384-well black flat-bottomed polystyrene plates (Corning no. CLS3575) in a total volume of 40 uL for each well. All inhibitors and GM-BODIPY were prepared in DMSO and diluted with the assay buffer (20 mM Hepes, pH 7.3, 50 mM KCl, 5 mM $MgCl_2$, 20 mM Na_2MoO_4, 0.01% NP40, 0.1 mg/mL BSA and 2 mM DTT). For each assay, background wells (buffer only), negative controls (GM-BODIPY only), positive controls (GM-BODIPY in the presence of Hsp90α), and test wells (inhibitors and GM-BODIPY in the presence of Hsp90α) were included on each assay plate. And the assay plate was incubated at 4 °C for 16 h. The FP values were measured at RT with excitation wavelength at 485 nm and emission wavelength at 535 nm. All experimental data were analyzed using the Origin 7.5 software (OriginLab, USA).

3. HSP90 (residues 9–236) expression, purification and crystallography[6]

Protein purification and crystallization

A cDNA fragment encoding the N-terminal domain of human Hsp90 (residues 9–236, Hsp90 N), was cloned into a pET28 vector. The recombinant plasmid pET28-Hsp90 N was transferred into *E. coli* strain BL21 (DE3) for over expression (Invitrogen, Carlsbad, USA). Bacterial cultures were grown in LB medium at 37 °C to an OD600 of 0.6 – 0.8, and 0.2 mM isopropyl-*beta*-D-thiogalactopyranoside was then added for further growth at 30 °C for another 5 h. The harvested cells were resuspended in

[6] D. E. Scott, A. G. Coyne, S. A. Hudson, C. Abell, *Biochemistry* 2012, **51**, 4990-5003.

lysis buffer (20 mM Tris/300 mM NaCl/5 mM 2-mercaptoethanol/10% glycerol, pH 7.5) and sonicated. The resulted suspension was centrifuged and the supernatant was passed through a nickel-bead column (GE Healthcare, Piscataway, USA). The column was then washed with 20 mM imidazole/300 mM NaCl/20 mM Tris/5 mM 2-mercaptoethanol/10% glycerol, pH 7.5 (wash buffer), until no further protein waseluted. The recombinant Hsp90 N was then eluted with 100 mM imidazole/300 mM NaCl/20 mM Tris/5 mM 2-mercaptoethanol/10% glycerol, pH 7.5 (elution buffer). The eluted proteins were concentrated (Millipore, Billerica, USA) and then injected into a 120 mL Hiload Superdex 75 column (GE Healthcare) from which Hsp90 N was eluted with 20 mM Tris/300 mM NaCl/5 mM ß-mercaptoethanol/10% glycerol, pH 7.5. The fractions containing Hsp90 N were collected and concentrated to 20 mg/mL for crystallization. The purity of protein was assessed by SDS-PAGE to be 95%.

Crystal of the Hsp90 N-**29** complex (PDB code: 4R3M) was obtained by the hanging-drop vapor diffusion method in a 24-well plate. Compound **29** was added in portions to a final concentration of 2 mM, to a protein solution (20 mg/mL). Then 1 μL of the protein−ligand solution was mixed with 1 μL of reservoir solution consisting of 20%-25% (w/v) PEG 2000 monomethyl ether/200 mM magnesium chloride/100 mM sodium cacodylate, pH 6.5. The plate was incubated at 4 °C, and crystals appeared within 3-5 days. The crystals were quickly transferred to a cryo solution consisting of 25% (w/v) PEG 2000 monomethyl ether/200 mM magnesiumchloride/100 mM sodium cacodylate, pH 6.5, 25% (v/v) glycerol, and then flash frozen in liquid nitrogen.

Data collection, structure determination, and refinement

All data sets were collected at 100 K on beamline BL17U1 at the Shanghai Synchrotron RadiationFacility (SSRF, Shanghai, China) and processed with the HKL2000 software package. The structure was solved by molecular replacement, using the PHENIX. The search model used for the crystal of the apo-form was the previously reported structure of the Hsp90-ATP (PDB code 3T0Z), and

the structure was refined using PHENIX. With the aid of the program Coot, compounds, water molecules, and others were fitted into the initial Fo–Fc map. The complete statistics, as well as the quality of the solved structures, were shown in table S1.

Table S1, X-Ray data collection, statistic, and PDB code for the structure of human Hsp90 N bound to compound **29**.

PDB code	4R3M
Space group	*I*222
Wavelength (Å)	0.9795
a, *b*, *c*, (Å)	66.58, 90.93, 98.89
α, β, γ, (º)	90.00, 90.00, 90.00
Total reflections	237930
Unique reflections	28123
Resolution (Å)	33.47-1.80(1.83-1.80)
R_{merge} (%)	5.5 (40.5)
Mean I/σ (I)	50.4 (8.2)
Completeness (%)	99.9 (100.0)
Redundancy	8.5 (8.6)
R_{work} / R_{free} (%)	19.7/23.3
Mean temperature factor (Å^2)	27.5

Bond lengths (Å)	0.011
Bond angles (º)	1.33

Values in parentheses are for the last shell.

The end