SUPPORTING INFORMATION

Development of Unsymmetrical Dyads as Potent Noncarbohydrate-based Inhibitors against Human β-*N*-Acetyl-D-Hexosaminidase

Peng Guo,† Qi Chen,‡ Tian Liu,‡ Lin Xu,† Qing Yang*,‡, and Xuhong Qian*,†

†Shanghai Key Laboratory of Chemical Biology, Institute of Pesticides and Pharmaceuticals, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

‡ School of Life Science and Biotechnology, Dalian University of Technology, Dalian 116024, China

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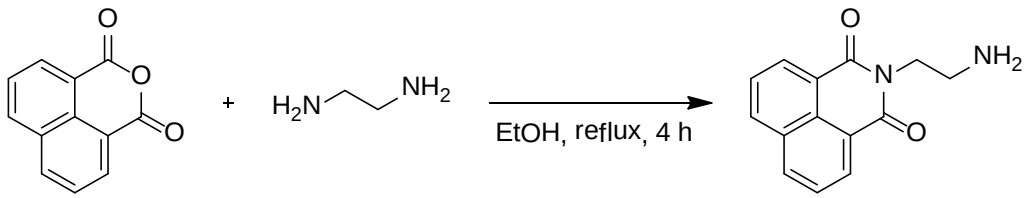
Experimental details and characterization of compounds

General: All chemicals or reagents were purchased from standard commercial supplies and treated with standard methods before use. Solvents were dried in a routine way and redistilled. All melting points (m.p.) were obtained with a Büchi Melting Point B540 and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AM-400 (400 MHz) spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent and TMS as the internal standard. Chemical shifts are reported in δ (parts per million) values. Coupling constants ^{n}J are reported in Hz. High-resolution mass spectra (HRMS) were recorded under electron impact (70 eV) condition using a MicroMass GCT CA 055 instrument. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F254), and spots were visualized with ultraviolet (UV) light. The mixtures were separated by gravity chromatography. Analytical HPLC was performed on a Hewlett-Packard 1100 system chromatograph equipped with photodiode array detector using a Zorbax Bonus-RP 5 μM 250 mm × 4.6 mm column (reverse phase) to detect the purity of the products. The mobile phase was a gradient of 0-100% acetonitrile and 10 mM NH_4OAc/AcOH in water (pH 6.0) at a flow rate of 1.0 mL/min.

2-(2-aminoethyl)-1H-benzo[de]isoquinoline-1,3(2*H*)-dione (1a):



Scheme S1. Synthetic route of compound **1a**

1,2-diaminoethane (0.24 mL, 3.63 mmol) was added to a suspension of 1,8-naphthalic anhydride (240 mg, 1.21 mmol) in 10 mL ethanol. The mixture was refluxed for about 4 h until the completion was detected by TLC. Then the hot reaction mixture filtered, the filtrate was cooled to room temperature and filtered again to get the crude product. The crude product was purified by recrystallization in ethanol to get **1a** (150 mg, 52%) as slightly yellow solid. R_f =0.38 ($CHCl_3$/MeOH, 10:1); mp: 141-142 ℃; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.34 (d, J = 7.6 Hz, 2H), 8.31 (d, J = 8.0 Hz, 2H), 7.75 (dd, J = 8.0, 7.6 Hz, 2H), 4.01 (t, J = 6.8 Hz, 2H), 2.79 (t, J = 6.8 Hz, 2H), 2.62 ppm (br, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.9, 134.4, 131.5, 130.9, 127.7, 127.4, 122.4, 43.2, 40.2 ppm; HRMS-ESI (m/z): calcd for $C_{14}H_{13}N_2O_2$ $[M+H]^+$, 241.0977; found, 241.0978.

HPLC purity: Area% = 99.6.

The compounds **1b-g** were synthesized following the same procedures as **1a**:

2-(3-aminopropyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (1b): White solid. Yield: 61%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.47 (d, J = 7.6 Hz, 2H), 8.43 (d, J = 7.6 Hz, 2H), 7.85 (dd, J = 7.6, 7.6 Hz, 2H), 4.09 (t, J = 6.8 Hz, 2H), 2.60 (t, J = 6.8 Hz, 2H), 1.74 ppm (tt, J = 6.8, 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.9, 134.7, 131.7, 131.1, 127.7, 127.6, 122.4, 39.5, 38.1, 31.5 ppm; calcd for $C_{15}H_{15}N_2O_2$ $[M+H]^+$, 255.1134; found, 255.1130. HPLC purity: Area% = 96.4.

2-(4-aminobutyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (1c): White solid. Yield: 59%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.45 (d, J = 7.6 Hz, 2H), 8.41 (d, J = 8.0 Hz, 2H), 7.84 (dd, J = 8.0, 7.6 Hz, 2H), 4.02 (t, J = 7.2 Hz, 2H), 2.55 (t, J= 7.2 Hz, 2H), 1.68 – 1.60 (m, 2H), 1.44–1.36 ppm (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.8, 134.7, 131.7, 131.2, 127.7, 127.6, 122.4, 41.7, 31.1, 25.6 ppm; HRMS-ESI (m/z): calcd for $C_{16}H_{17}N_2O_2$ $[M+H]^+$, 269.1290; found, 269.1289. HPLC purity: Area% = 96.7.

2-(6-aminohexyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (1d): White solid. Yield: 50%.^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.49 (d, J = 7.6 Hz, 2H), 8.45 (d, J = 8.0 Hz, 2H), 7.87 (dd, J = 8.0, 7.6 Hz, 2H), 4.04 (t, J = 7.2 Hz, 2H), 1.63 (t, J = 6.0 Hz, 2H), 1.41 – 1.26 ppm (m, 6H); HRMS-ESI (m/z): calcd for $C_{18}H_{21}N_2O_2$ $[M+H]^+$, 279.1603; found, 279.1605. HPLC purity: Area% = 95.0.

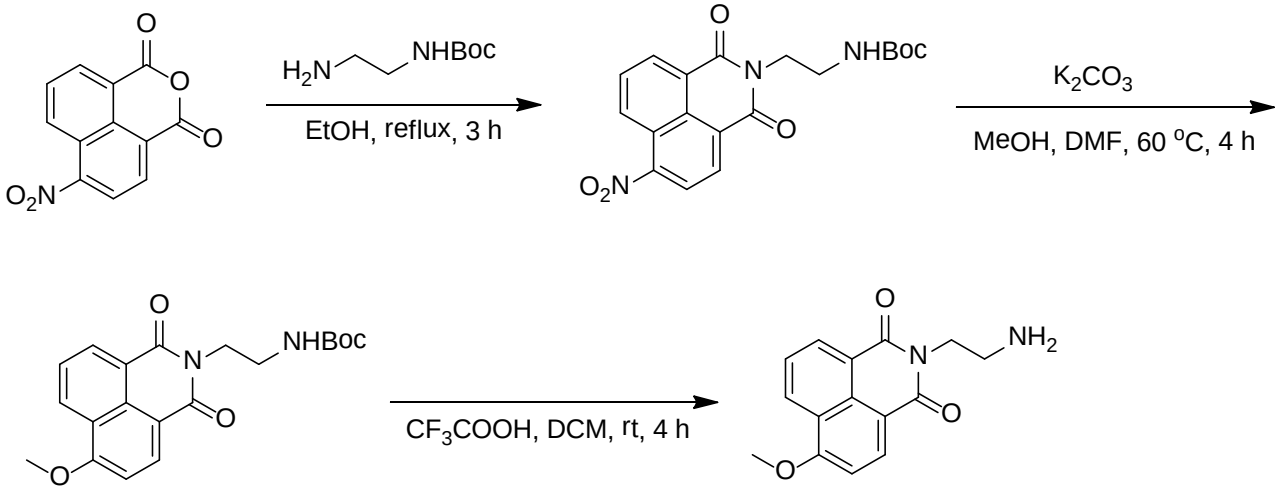
2-(2-hydroxyethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (1e): White solid. Yield: 93%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.46 (d, J = 7.2 Hz, 2H), 8.42 (d, J = 8.0 Hz, 2H), 7.85 (dd, J = 8.0, 7.2 Hz, 2H), 4.79 (t, J = 6.0 Hz, 1H), 4.16 (t, J = 6.4 Hz, 2H), 3.63 ppm (td, J = 6.4, 6.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.0, 134.6, 131.7, 131.1, 127.8, 127.6, 122.6, 58.3, 42.3 ppm; calcd for $C_{14}H_{12}NO_3$ $[M+H]^+$, 242.0817; found, 242.0813. HPLC purity: Area% = 98.9.

2-(2-(dimethylamino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (1f): White solid. Yield:

87%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.57 (d, J = 7.2 Hz, 2H), 8.19 (d, J = 7.6 Hz, 2H), 7.74 (dd, J = 7.6, 7.2 Hz, 2H), 4.33 (t, J = 7.2 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H), 2.37 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.2, 133.9, 131.5, 131.2, 128.2, 126.9, 122.6, 57.0, 45.8, 38.1 ppm; HRMS-ESI (m/z): calcd for $C_{16}H_{17}N_2O_2$ $[M+H]^+$, 269.1290; found, 269.1274. HPLC purity: Area% = 99.8.

2-(2-aminoethyl)-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (1g): White solid. Yield: 50%. mp: 151-152 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.53 (d, J = 8.4 Hz, 1H), 8.49 (d, J = 7.6 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 7.96 (dd, J = 8.4, 7.6 Hz, 1H), 4.07 (t, J = 6.8 Hz, 2H), 2.85 (t, J = 6.8 Hz, 2H), 2.51 ppm (br, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.6, 163.5, 132.9, 131.9, 131.8, 131.3, 130.2, 129.4, 129.2, 128.8, 123.4, 122.6, 42.9, 39.9 ppm; calcd for $C_{14}H_{12}N_2O_2Br$ $[M+H]^+$, 319.0082; found, 319.0079. HPLC purity: Area% = 99.3.

2-(2-aminoethyl)-6-methoxy-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (1h):



Scheme S2. Synthetic route of compound **1h**

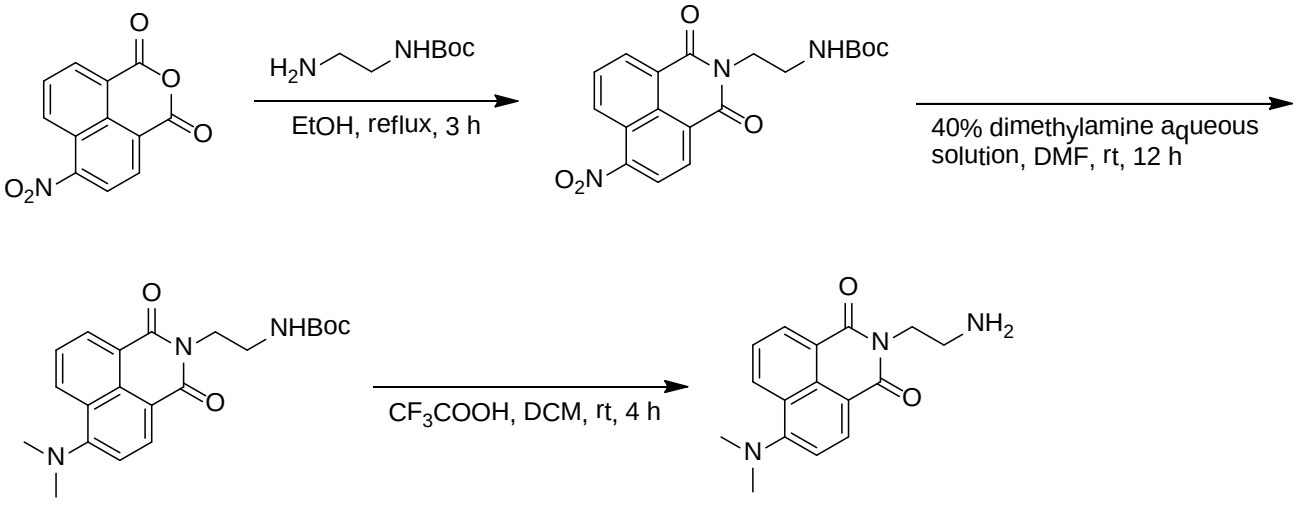
Tert-butyl (2-aminoethyl)carbamate (165 mg, 1.0 mmol) was added to the solution of 4-nitro-1,8-naphthalic anhydride (125 mg, 0.5 mmol) in 10 mL ethanol. The mixture was refluxed for 3 h and the suspended 4-nitro-1,8-naphthalic anhydride dissolved slowly during the process of the reaction. The reaction mixture was cooled to room temperature and removed the solvent by filtration to afford the desired product (200 mg, 100%) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.83 (d, J = 8.0 Hz, 1H), 8.74 (d, J = 8.0 Hz, 1H), 8.70 (d, J = 8.0 Hz, 1H), 8.41 (d, J = 8.0 Hz, 1H), 7.99 (t, J = 8.0 Hz, 1H), 4.93 (br, 1H), 4.38 (t, J = 5.6 Hz, 2H), 3.50–3.60 (m, 2H), 1.25 (s, 9H);

^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.6, 162.8, 156.1, 149.6, 132.5, 129.9, 129.8, 129.3, 129.2, 126.9, 123.8, 123.7, 122.9, 79.3, 40.5, 39.2, 28.2 ppm.

This yellow solid (50 mg, 130 μmol) and potassium carbonate (100 mg, 725 μmol) were added to the solution of 15 mL methanol and 2 mL DMF. The mixture was stirred at 60 ^{o}C for 4 h followed by filtration. The filtrate was evaporated under reduced pressure. The intermediate (30 mg, 62%) was isolated by flash column chromatography on silica gel (eluent, CH_2Cl_2).

2 mL trifluoroacetic acid was added to a solution of the intermediate obtained above (30 mg, 80 μmol) in 10 mL dichloromethane. The mixture was allowed to stir at room temperature for 4 h. After evaporation of the solvent, **1h** (22 mg, 100%) was obtained as light green solid. mp: 205-206 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.54 (dd, J = 8.4, 1.2 Hz, 1H), 8.49 (dd, J = 7.6, 1.2 Hz, 1H), 8.44 (d, J = 8.4 Hz, 1H), 7.95 (br, 2H), 7.82 (dd, J = 8.4, 7.6 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 4.30 (t, J = 6.0 Hz, 2H), 4.14 (s, 3H), 3.16 (t, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.7, 163.9, 160.9, 133.7, 131.4, 129.2, 128.8, 126.8, 123.2, 122.6, 114.9, 106.7, 57.1, 38.1, 37.8 ppm; HRMS-ESI (m/z): calcd for $C_{15}H_{15}N_2O_3$ $[M+H]^+$, 271.1083; found, 271.1092. HPLC purity: Area% = 99.7.

2-(2-aminoethyl)-6-(dimethylamino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (1i):



Scheme S3. Synthetic route of compound **1i**

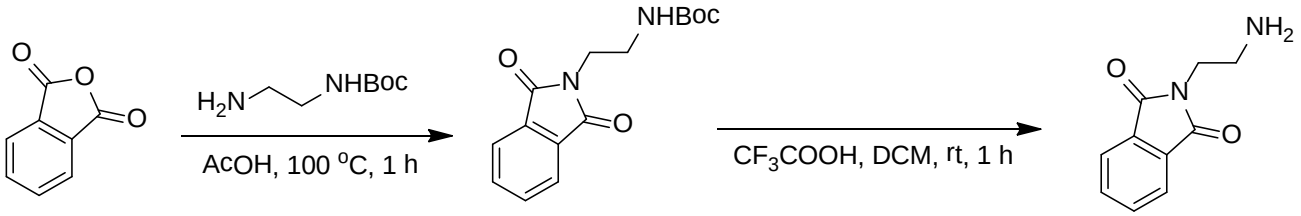
Tert-butyl (2-aminoethyl)carbamate (165 mg, 1.0 mmol) was added to the solution of 4-nitro-1,8-naphthalic anhydride (125 mg, 0.5 mmol) in 10 mL ethanol. The mixture was refluxed for 3 h and the suspended 4-nitro-1,8-naphthalic anhydride dissolved slowly during the process of

the reaction. The reaction mixture was cooled to room temperature and removed the solvent by filtration to afford the desired product (200 mg, 100%) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.83 (d, J = 8.0 Hz, 1H), 8.74 (d, J = 8.0 Hz, 1H), 8.70 (d, J = 8.0 Hz, 1H), 8.41 (d, J = 8.0 Hz, 1H), 7.99 (t, J = 8.0 Hz, 1H), 4.93 (br, 1H), 4.38 (t, J = 5.6 Hz, 2H), 3.50–3.60 (m, 2H), 1.25 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.6, 162.8, 156.1, 149.6, 132.5, 129.9, 129.8, 129.3, 129.2, 126.9, 123.8, 123.7, 122.9, 79.3, 40.5, 39.2, 28.2 ppm.

The yellow solid (50 mg, 130 μmol) was added to 40% dimethylamine aqueous solution 10 mL, and then 2 mL DMF was added to dissolve the material. The mixture was stirred at room temperature for 12 h, and then the solvent was evaporated under reduced pressure. The intermediate (30 mg, 82%) was isolated by flash column chromatography on silica gel (eluent, CH_2Cl_2/CH_3OH 40:1).

2 mL trifluoroacetic acid was added to a solution of the intermediate obtained above (30 mg, 80 μmol) in 10 mL dichloromethane. The mixture was allowed to stir at room temperature for 4 h. After evaporation of the solvent, **1i** (22 mg, 100%) was obtained as orange solid. mp: 172-174 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.53 (d, J = 8.4 Hz, 1H), 8.46 (d, J = 7.6 Hz, 1H), 8.34 (d, J = 8.0 Hz, 1H), 7.99 (br, 2H), 7.75 (dd, J = 8.4, 7.6 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 4.30 (t, J = 6.0 Hz, 2H), 3.15 (t, J = 6.0 Hz, 2H), 3.11 (s, 3H), 3.09 ppm (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.8, 164.0, 157.2, 132.7, 132.1, 131.0, 130.3, 125.4, 124.6, 123.0, 113.9, 113.3, 44.9, 38.2, 37.7 ppm; HRMS-ESI (m/z): calcd for $C_{16}H_{18}N_3O_2$ $[M+H]^+$, 284.1399; found, 284.1399. HPLC purity: Area% = 94.9.

2-(2-aminoethyl)isoindoline-1,3-dione (1j):



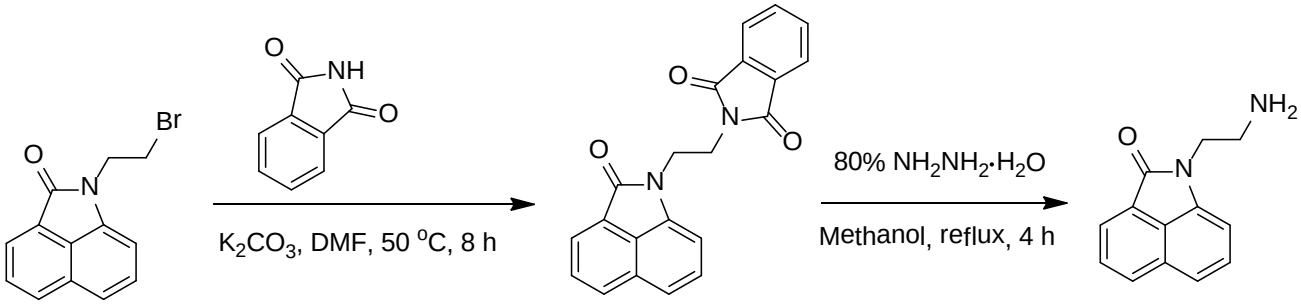
Scheme S4. Synthetic route of compound **1j**

tert-butyl (2-aminoethyl)carbamate (160 mg, 1.0 mmol) was added to a solution of isobenzofuran-1,3-dione (150 mg, 1.0 mmol) in 5 mL acetic acid. The mixture was stirred for 1 h at 100 ^{o}C. After being cooled to room temperature, it was treated with water (30 mL) and extracted with dichloromethane (20 mL) three times. The organic layer was washed with water, dried over

Na_2SO_4, and concentrated *in vacuo*. The intermediate (white solid, 200 mg, 69%) was isolated by flash column chromatography on silica gel.

6 mL trifluoroacetic acid was added to a solution of the intermediate obtained above (200 mg, 0.70 mmol) in 15 mL dichloromethane. The mixture was allowed to stir at room temperature for 1 h. After evaporation to remove the solvent and trifluoroacetic acid, **1j** (60 mg, 90%) was obtained as white solid. mp: 205-206 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.08 (br, 2H), 7.89-7.85 (m, 4H), 3.86 (t, J = 5.6 Hz, 2H), 3.19-3.06 ppm (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.4, 134.8, 132.4, 123.5, 37.9, 35.8 ppm; HRMS-ESI (m/z): calcd for $C_{10}H_{11}N_2O_2$ $[M+H]^+$, 191.0821; found, 191.0816. HPLC purity: Area% = 97.8.

1-(2-aminoethyl)benzo[*cd*]indol-2(1*H*)-one (1k):



Scheme S5. Synthetic route of compound **1k**

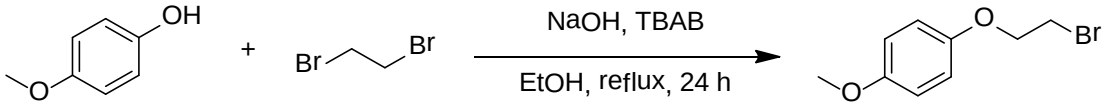
potassium carbonate (50 mg, 0.36 mmol) was added to a solution of 1-(2-bromoethyl)benzo[*cd*]indol-2(1*H*)-one (100 mg, 0.36 mmol, obtained by the described synthetic route in the literature[1]) and isoindoline-1,3-dione (54 mg, 0.36 mmol) in 5 mL DMF. The mixture was stirred at 50 ^{o}C for 8 h and then concentrated in vacuo. The residue was dissolved in dichloromethane and washed with water. The organic layer was washed with water, dried over Na_2SO_4, and concentrated in vacuo to give a residue, which was purified by silica gel column chromatography to give the intermediate 2-(2-(2-oxobenzo[*cd*]indol-1(2*H*)-yl)ethyl)isoindoline-1,3-dione (110 mg, 89%) as yellow solid.

80% hydrazinium hydroxide 5 mL was added to a solution of intermediate obtained above (100 mg, 0.29 mmol) in 5 mL methanol. The mixture was stirred at reflux until the completion of reaction was detected by TLC. After being cooled to room temperature, it was treated with water (50 mL) and extracted with dichloromethane (50 mL) three times. The organic layer was washed with water, dried

over Na_2SO_4, and concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography using CH_2Cl_2/CH_3OH (15:1) to give **1k** (50 mg, 80%) as yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.08 (d, *J* = 6.8 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.73 (dd, *J* = 8.0, 6.8 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.48 (dd, *J* = 8.4, 6.8 Hz, 1H), 6.98 (d, *J* = 6.8 Hz, 1H), 4.02 (t, *J* = 6.0 Hz. 2H), 3.13 (t, *J* = 6.0 Hz, 2H), 1.47 ppm (br, 2H); HRMS-ESI (m/z): calcd for $C_{13}H_{13}N_2O$ $[M+H]^+$, 213.1028; found, 213.1023. HPLC purity: Area% = 95.8.

General procedure for the synthesis of intermediates 2a-m:

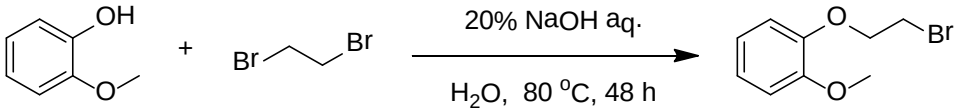
Method A. 1-(2-bromoethoxy)-4-methoxybenzene (2a):



Scheme S6. Synthetic route of compound **2a**

The mixture of 4-methoxyphenol (2 g, 16.1 mmol), sodium hydroxide (650 mg, 16.1 mmol), tetrabutyl ammonium bromide (520 mg, 1.6 mmol) and alcohol (20 mL) was cooled to 30 ^oC after it was heated and refluxed for 1 h. 1,2-dibromoethane (5 mL, 48.3 mmol) was added, heated and refluxed for 24 h, and it was condensed, extracted with ethyl acetate. The organic phase was washed with water, dried over Na_2SO_4, and concentrated in vacuo to give a residue, which was purified by silica gel column chromatography using AcOEt/PE (1:10) to give 2a (1.5 g, 40%) as white solid. ^1H NMR (400 MHz, $CDCl_3$): δ = 6.92–6.82 (m, 4H), 4.25 (t, *J* = 6.4 Hz, 2H), 3.79 (s, 3H), 3.63 ppm (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.4, 152.2, 116.1, 114.8, 68.8, 55.7 29.5 ppm; GCMS: m/z 232.0, 230.0 (M^+), 123.1, 109.0, 95.1, 80.1.

Method B. 1-(2-bromoethoxy)-2-methoxybenzene (2c):

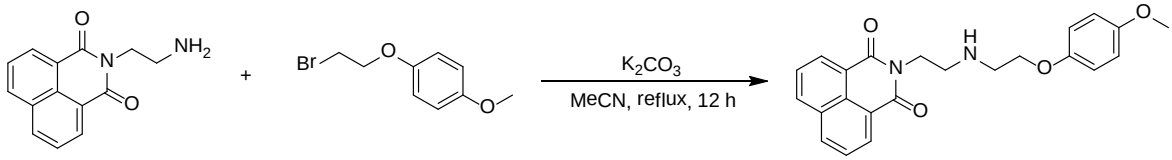


Scheme S7. Synthetic route of compound **2c**

2-methoxyphenol (5 g, 40.3 mmol), water (24 mL), 1,2-dibromoethane (7 mL, 80.6 mmol) and 20% NaOH aq. (3 g, 12 mL) were heated together for 48 h at 80 ^oC temperature. Organic phase was separated; aqueous phase was shaken with dichloromethane. The combined organic phase was washed with 10% NaOH aq. And water and then solvent was evaporated. Oily residue was

chromatographed on silica gel using AcOEt/PE (1:10) to give 2c (6.7 g, 72%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.06-6.88 (m, 4H), 4.35 (t, J = 6.8 Hz, 2H), 3.89 (s, 3H), 3.67 ppm (t, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.1, 147.6, 122.5, 121.0, 115.3, 112.5, 69.4, 56.1, 29.1 ppm; GCMS: m/z 232.0, 230.0 (M^+), 151.1, 137.1, 123.1, 109.0, 95.1, 77.1.

2-(2-((2-(4-methoxyphenoxy)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3a):



Scheme S8. Synthetic route of compound **3a**

Potassium carbonate (530 mg, 3.75 mmol) was added to a solution of 1a (450 mg, 1.87 mmol) and 2a (430 mg, 1.87 mmol) in 20 mL acetonitrile. The mixture was stirred at reflux until the completion of reaction was detected by TLC and then the undissolved substance was removed by filtration. The filtrate was concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography using CH_2Cl_2/CH_3OH (30:1) to give 3a (400 mg, 54%) as yellowish solid. R_f =0.43 ($CHCl_3$/MeOH, 30:1); mp: 107-109 ℃; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.61 (d, J = 7.6 Hz, 2 H), 8.22 (d, J = 8.0 Hz, 2 H), 7.77 (dd, J = 8.0, 7.6 Hz, 2 H), 6.86 - 6.76 (m, 4 H), 4.40 (t, J = 6.4 Hz, 2 H), 4.03 (t, J = 5.2 Hz, 2 H), 3.77 (s, 3 H), 3.16 – 3.06 (m, 4 H), 1.97 ppm (br, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.0, 153.7, 152.9, 133.7, 131.3, 127.8, 126.7, 122.3, 115.4, 114.5, 68.0, 55.6, 48.6, 47.4, 39.9 ppm; HRMS-ESI (m/z): calcd for $C_{23}H_{23}N_2O_4$ $[M+H]^+$, 391.1658; found, 391.1656. HPLC purity: Area% = 97.1.

The compounds **3b-m** were synthesized following the same procedures as **3a**:

2-(2-((2-(3-methoxyphenoxy)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3b): yellowish solid. Yield: 65%. mp: 79-80 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.58 (d, J = 7.2 Hz, 2H), 8.19 (d, J = 7.6 Hz, 2H), 7.74 (dd, J = 7.6, 7.2 Hz, 2H), 7.13 (t, J = 8.0 Hz, 1H), 6.52-6.42 (m, 3H), 4.37 (t, J = 6.4 Hz, 2H), 4.05 (t, J = 5.2 Hz, 2H), 3.77 (s, 3H), 3.14-3.06 (m, 4H), 1.93 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 160.7, 160.1, 133.9, 131.6, 131.2, 129.8, 128.2, 126.9, 122.6, 106.6, 106.5, 100.9, 67.3, 55.2, 48.5, 47.4, 39.9 ppm; HRMS-ESI (m/z): calcd for $C_{23}H_{23}N_2O_4$

$[M+H]^+$, 391.1658; found, 391.1654. HPLC purity: Area% = 96.0.

2-(2-((2-(2-methoxyphenoxy)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3c): yellowish solid. Yield: 67%. mp: 116-118 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.56 (d, *J* = 7.6 Hz, 2H), 8.17 (d, *J* = 8.0 Hz, 2H), 7.72 (dd, *J* = 8.0, 7.6 Hz, 2H), 6.88 (m, 4H), 4.36 (t, *J* = 6.8 Hz, 2H), 4.12 (t, *J* = 5.2 Hz, 2H), 3.81 (s, 3H), 3.13 (t, *J* = 5.2 Hz, 2H), 3.08 (t, *J* = 6.8 Hz, 2H), 1.89 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.3, 149.7, 148.4, 133.9, 131.5, 131.2, 128.1, 128.9, 122.6, 121.4, 120.8, 114.1, 111.9, 68.8, 56.8, 48.5, 47.4, 40.0 ppm; HRMS-ESI (m/z): calcd for $C_{23}H_{23}N_2O_4$ $[M+H]^+$, 391.1658; found, 391.1653. HPLC purity: Area% = 99.8.

2-(2-((2-((4-methoxyphenyl)thio)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3d): yellow solid. Yield: 55%. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.61 (d, *J* = 7.2 Hz, 2H), 8.22 (d, *J* = 8.4 Hz, 2H), 7.77 (dd, *J* = 8.4, 7.2 Hz, 2H), 7.35 (d, *J* = 8.8 Hz, 2H), 6.81 (d, *J* = 8.8 Hz, 2H), 4.34 (t, *J* = 6.4 Hz, 2H), 3.79 (s, 3H), 3.01 (t, *J* = 6.4 Hz, 2H), 2.97 (t, *J* = 6.0 Hz, 2H), 2.88 (t, *J* = 6.0 Hz, 2H), 1.85 ppm (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.2, 158.9, 133.8, 133.3, 131.4, 131.1, 128.0, 126.8, 125.8, 122.5, 114.5, 55.3, 48.0, 47.1, 40.0, 36.0 ppm; HRMS-ESI (m/z): calcd for $C_{23}H_{23}N_2O_3S$ $[M+H]^+$, 407.1429; found, 407.1429. HPLC purity: Area% = 95.0.

2-(2-((3-(4-methoxyphenoxy)propyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3e): yellowish solid. Yield: 72%. mp: 110-111 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.59 (d, *J* = 7.6 Hz, 2H), 8.21 (d, *J* = 8.0 Hz, 2H), 6.78 (m, 4H), 4.38 (t, *J* = 6.4 Hz, 2H), 3.97 (t, *J* = 6.4 Hz, 2H), 3.77 (s, 3H), 3.05 (t, *J* = 6.4 Hz, 2H), 2.90 (t, *J* = 6.4 Hz, 2H), 1.96 (tt, *J* = 6.4, 6.4 Hz, 2H), 1.75 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 153.6, 153.1, 134.0, 131.6, 131.3, 128.2, 126.9, 122.6, 115.3, 114.5, 67.0, 55.7, 47.7, 46.7, 40.0, 29.7 ppm; HRMS-ESI (m/z): calcd for $C_{24}H_{25}N_2O_4$ $[M+H]^+$, 405.1814; found, 405.1812. HPLC purity: Area% = 97.1.

2-(2-((3-(3-methoxyphenoxy)propyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3f): yellowish solid. Yield: 70%. mp: 85-86 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.57 (d, *J* = 7.6 Hz, 2H), 8.19 (d, *J* = 8.4 Hz, 2H), 7.73 (dd, *J* = 8.4, 7.6 Hz, 2H), 7.12 (dd, *J* = 8.0, 7.6 Hz, 1H), 6.50-6.42 (m, 3H), 4.36 (t, *J* = 6.4 Hz, 2H), 4.00 (t, *J* = 6.4 Hz, 2H), 3.76 (s, 3H), 3.05 (t, *J* = 6.4 Hz, 2H), 2.90

(t, *J* = 6.8 Hz, 2H), 1.96 (dt, *J* = 6.8, 6.4 Hz, 2H), 1.54 ppm (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 160.8, 160.2, 133.9, 131.6, 131.2, 129.7, 128.2, 126.9, 122.6, 106.6, 106.3, 100.9, 66.3, 55.2, 47.7, 46.6, 40.0, 29.7 ppm; HRMS-ESI (m/z): calcd for $C_{24}H_{25}N_2O_4$ $[M+H]^+$, 405.1814; found, 405.1818. HPLC purity: Area% = 95.2.

2-(2-((3-((4-methoxyphenyl)thio)propyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3g): yellowish solid. Yield: 65%. mp: 97-98 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.56 (d, *J* = 7.6 Hz, 2H), 8.18 (d, *J* = 8.0 Hz, 2H), 7.72 (dd, *J* = 8.0, 7.6 Hz, 2H), 7.30 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 8.8 Hz, 2H), 4.31 (t, *J* = 6.4 Hz, 2H), 3.77 (s, 3H), 2.98 (t, *J* = 6.4 Hz, 2H), 2.84 (t, 7.2 Hz, 2H), 2.78 (t, *J* = 6.8 Hz, 2H), 1.74 (dt, *J* = 7.2, 6.8 Hz, 2H), 1.35 ppm (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.3, 158.7, 133.9, 133.0, 131.5, 131.2, 128.1, 126.9, 126.6, 122.6, 114.5, 55.3, 48.3, 47.7 40.0, 33.5, 29.7 ppm; HRMS-ESI (m/z): calcd for $C_{24}H_{25}N_2O_3S$ $[M+H]^+$, 421.1586; found, 421.1583. HPLC purity: Area% = 95.1.

2-(2-((2-(3,4-dimethoxyphenoxy)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3h): yellow solid. Yield: 45%. mp: 119-121 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (d, *J* = 7.6 Hz, 2H), 8.20 (d, *J* = 8.0 Hz, 2H), 7.74 (dd, *J* = 8.0, 7.6 Hz, 2H), 6.73 (d, *J* = 8.8 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 6.37 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.40 (t, *J* = 6.4 Hz, 2H), 4.04 (t, *J* = 5.2 Hz, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.15 – 3.08 (m, 4H), 2.39 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 153.5, 149.8, 143.6, 133.9, 131.6, 131.2, 128.2, 126.9, 122.6, 111.9, 103.9, 101.0, 67.8, 56.5, 55.8, 48.5, 47.4, 39.9 ppm; HRMS-ESI (m/z): calcd for $C_{24}H_{25}N_2O_5$ $[M+H]^+$, 421.1763; found, 421.1760. HPLC purity: Area% = 96.7.

2-(2-((2-(benzo[*d*][1,3]dioxol-5-yloxy)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3i): yellow solid. Yield: 44%. mp: 119-120 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.61 (d, *J* = 7.6 Hz, 2H), 8.22 (d, *J* = 8.0 Hz, 2H), 7.77 (dd, *J* = 8.0, 7.6 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 1H), 6.46 (d, *J* = 1.2 Hz, 1H), 6.29 (dd, *J* = 8.4, 1.2 Hz, 1H), 5.91 (s, 2H), 4.39 (t, *J* = 6.4 Hz, 2H), 3.99 (t, *J* = 5.2 Hz, 2H), 3.18-3.00 (m, 4H), 1.92 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 154.3, 148.1, 141.6, 134.0, 131.6, 131.3, 128.2, 126.9, 122.6, 107.8, 105.6, 101.1, 98.1, 68.2, 48.5, 47.4 39.9 ppm; HRMS-ESI (m/z): calcd for $C_{23}H_{21}N_2O_5$ $[M+H]^+$, 405.1450; found, 405.1455. HPLC purity: Area%

= 99.4.

2-(2-((2-(4-(trifluoromethoxy)phenoxy)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3j): yellowish solid. Yield: 50%. mp: 93-94 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.62 (d, *J* = 7.6 Hz, 2H), 8.23 (d, *J* = 8.4 Hz, 2H), 7.77 (dd, *J* = 8.4, 7.6 Hz, 2H), 7.10 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 4.40 (t, *J* = 6.4 Hz, 2H), 4.05 (t, *J* = 5.2 Hz, 2H), 3.15 – 3.05 (m, 4H), 1.90 ppm (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 157.4, 142.7, 134.0, 131.6, 131.3, 128.2, 126.9, 122.6, 122.3, 121.1 (q, *J* = 254 Hz), 115.3, 67.9, 48.3, 47.4, 39.9 ppm; HRMS-ESI (m/z): calcd for $C_{23}H_{20}N_2O_4F_3$ $[M+H]^+$, 445.1375; found, 445.1375. HPLC purity: Area% = 98.8.

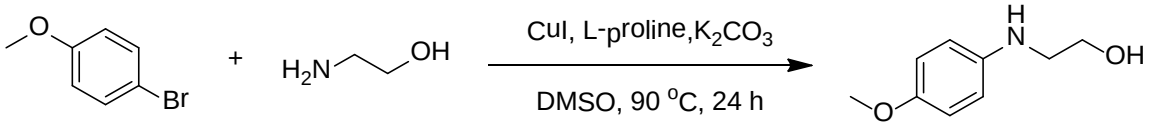
Methyl 4-(2-((2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethyl)amino)ethoxy) benzoate (3k): white solid. Yield: 53%. mp: 107-109 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.61 (d, *J* = 8.0 Hz, 2H), 8.22 (d, *J* = 8.4 Hz, 2H), 7.93 (d, *J* = 7.2 Hz, 2H), 7.75 (dd, *J* = 8.4, 8.0 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 4.39 (t, *J* = 6.4 Hz, 2H), 4.12 (t, *J* = 5.2 Hz, 2H), 3.89 (s, 3H), 3.15 – 3.08 (m, 4H), 1.94 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.8, 164.4, 162.6, 134.0, 131.6, 131.5, 131.3, 128.2, 126.9, 122.6, 114.1, 67.5, 51.8, 48.2, 47.4, 39.8 ppm; HRMS-ESI (m/z): calcd for $C_{24}H_{23}N_2O_4$ $[M+H]^+$, 419.1607; found, 419.1607. HPLC purity: Area% = 98.5.

2-(2-((2-(4-chlorophenoxy)ethyl)amino)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3l): yellowish solid. Yield: 65%. mp: 106-107 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.58 (d, *J* = 7.2 Hz, 2H), 8.20 (d, *J* = 8.4 Hz, 2H), 7.74 (dd, *J* = 8.4, 7.2 Hz, 2H), 7.16 (d, *J* = 8.8 Hz, 2H), 6.77 (d, *J* = 8.8 Hz, 2H), 4.37 (t, *J* = 6.4 Hz, 2H), 4.01 (t, *J* = 5.2 Hz, 2H), 3.11 – 3.05 (m, 4H), 1.77 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 157.5, 134.0, 131.6, 131.2, 129.2, 128.2, 126.9, 125.5, 122.6, 115.7, 67.7, 48.3, 47.4, 39.9 ppm; HRMS-ESI (m/z): calcd for $C_{22}H_{10}N_2O_3Cl$ $[M+H]^+$, 395.1162; found, 395.1154. HPLC purity: Area% = 97.5.

2-(2-((2-(4-nitrophenoxy)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3m): yellowish solid. Yield: 60%. mp: 158-160 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (d, *J* = 7.6 Hz, 2H), 8.22 (d, *J* = 8.4 Hz, 2H), 8.11 (d, *J* = 9.2 Hz, 2H), 7.76 (dd, *J* = 8.4, 7.6 Hz, 2H), 6.89 (d, *J* = 9.2 Hz, 2H), 4.38 (t, *J* = 6.4 Hz, 2H), 4.13 (t, *J* = 5.2 Hz, 2H), 3.16 – 3.08 (m, 4H), 1.69 ppm (s, 1H); ^{13}C

NMR (100 MHz, $CDCl_3$): δ = 164.4, 163.9, 141.4, 134.0, 131.6, 131.3, 128.2, 126.9, 125.8, 122.5, 114.4, 68.4, 48.0, 47.4, 39.9 ppm; HRMS-ESI (m/z): calcd for $C_{22}H_{20}N_3O_5$ $[M+H]^+$, 406.1403; found, 406.1404. HPLC purity: Area% = 96.5.

2-((4-methoxyphenyl)amino)ethanol (4a):



Scheme S9. Synthetic route of compound **4a**

To a mixture of 1-bromo-4-methoxybenzene (5 g, 26.7 mmol), CuI (510 mg, 2.7 mmol), L-proline (615 mg, 5.4 mmol) and K_2CO_3 (7.4 g, 53.5 mmol) in a 50 mL flask, were added 2-aminoethanol (4.8 mL, 80.2 mmol) and DMSO (20 mL). After the reaction mixture was degassed and then introduced under Ar atmosphere with magnetic stirring, it was heated at 80 °C until 1-bromo-4-methoxybenzene disappeared. The mixture was diluted with water (100 mL) and then extracted with EtOAc (100 mL) three times. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give **4a** (3.5 g, 78%) as lemon yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.77 (d, *J* = 8.8 Hz, 2H), 6.57 (d, *J* = 8.8 Hz, 2H), 3.83 (s, 2H), 3.72 (s, 3H), 3.70 (t, *J* = 5.2 Hz, 2H), 3.13 ppm (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.1, 142.0, 114.6, 114.5, 60.6, 55.4, 46.8 ppm; GCMS: m/z 167.1 (M^+), 136.1, 121.1, 108.1, 93.1, 77.1.

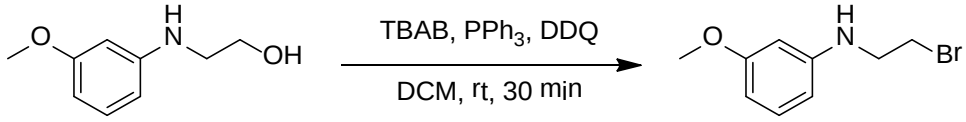
The compounds **4b-c** were synthesized following the same procedures as **4a**:

2-((3-methoxyphenyl)amino)ethanol (4b): light yellow oil. Yield: 89%. ^{1}H NMR (400 MHz, $CDCl_3$): δ =7.09 (d, *J* = 8.0 Hz, 1H), 6.31 (dd, *J* = 8.0, 0.8 Hz, 1H), 6.26 (dd, *J* = 8.0, 0.8 Hz, 1H), 6.2 (s, 1H), 3.76 (m, 5H), 3.37 (br, 2H), 3.23 ppm (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.6, 149.4, 129.9, 106.2, 102.7, 99.1, 60.9, 55.0, 45.9 ppm; GCMS: m/z 167.1 (M^+), 136.1, 121.1, 108.0, 92.0, 77.0.

2-((2,4-dimethoxyphenyl)amino)ethanol (4c): yellow oil. Yield: 90%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.50 (d, *J* = 8.4 Hz, 1H), 6.43 (d, *J* = 2.0 Hz, 1H), 6.36 (dd, *J* = 8.4, 2.0 Hz, 1H), 3.99 (br, 2H), 3.72 (m, 8H), 3.13 ppm (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.8, 147.9, 131.7,

110.6, 103.4, 98.6, 60.5, 55.2, 54.9, 46.3 ppm; GCMS: m/z 197.1 (M^+), 179.1, 166.1, 151.0, 136.0, 122.9, 107.0, 95.0, 78.0.

***N*-(2-bromoethyl)-3-methoxyaniline (5b):**



Scheme S10. Synthetic route of compound **5b**

To a stirred solution of DDQ (486 mg, 2.1 mmol) and PPh_3 (562 mg, 2.1 mmol) in CH_2Cl_2 was added (*n*-butyl)$_4$NBr (690 mg, 2.1 mmol) at room temperature. **4b** (240 mg, 1.4 mmol) was then added to this mixture, which immediately turned the yellow color of the reaction mixture to deep red. The mixture was stirred at room temperature for 30 min. The solvent was then removed under vacuum. The residue was purified by column chromatography on silica gel using AcOEt/PE (1:2) to give the corresponding bromide product **5b** (270 mg, 82%) as pale red oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.07 (t, *J* = 8.0 Hz, 1H), 6.30 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.23 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.17 (s, 1H), 4.35 (s, 1H), 3.73 (s, 3H), 3.47 ppm (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.7, 147.9, 130.0, 106.1, 103.2, 99.2, 54.9, 45.2, 31.7 ppm; GCMS: m/z 231.0, 229.0 (M^+), 149.1, 136.1, 121.1, 108.1, 92.1, 77.1.

The compounds **5a** and **5c** were synthesized following the same procedures as **5b**:

***N*-(2-bromoethyl)-4-methoxyaniline (5a):** yellow oil. Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.79 (d, *J* = 8.8 Hz, 2H), 6.70 (d, *J* = 8.8 Hz, 2H), 5.20 (br, 1H), 3.74 (s, 3H), 3.53 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.3, 139.3, 115.7, 114.9, 55.6, 47.0, 31.5 ppm; GCMS: m/z 231.0, 229.0 (M^+), 216.0, 149.1, 136.1, 121.1, 107.0, 92.1, 77.1.

***N*-(2-bromoethyl)-2,4-dimethoxyaniline (5c):** brown oil. Yield: 44%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.56 (d, *J* = 8.8 Hz, 1H), 6.48 (d, *J* = 2.0 Hz, 1H), 6.41 (dd, *J* = 8.8, 2.0 Hz, 1H), 4.63 (br, 1H), 3.83 (s, 3H), 3.76 (s, 3H), 3.54 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.5, 148.2, 130.4, 110.7, 103.6, 99.2, 55.5, 55.4, 45.9, 31.7 ppm; GCMS: m/z 261.0, 259.0 (M^+), 2440, 217.9, 179.0, 166.1, 151.1, 136.1, 122.0, 107.0, 95.0, 79.0.

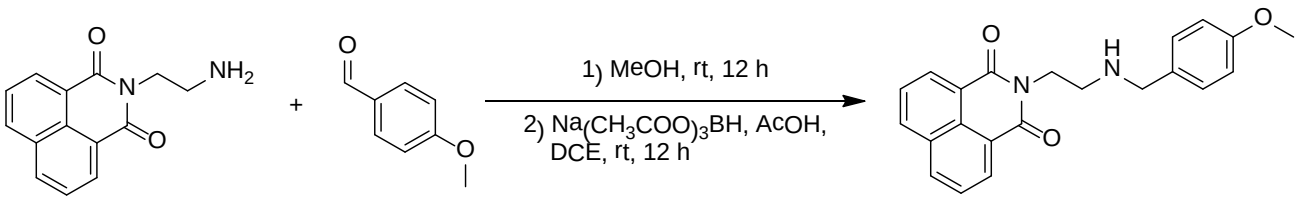
The compounds **6a-c** were synthesized following the same procedures as **3a**:

2-(2-((2-((4-methoxyphenyl)amino)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (6a): red solid. Yield: 55%. mp: 100-101 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.58 (d, *J* = 7.2 Hz, 2H), 8.20 (d, *J* = 7.6 Hz, 2H), 7.74 (dd, *J* = 7.6, 7.2 Hz, 2H), 6.69 (d, *J* = 9.2 Hz, 2H), 6.50 (d, *J* = 9.2 Hz, 2H), 4.41 (t, *J* = 6.4 Hz, 2H), 3.72 (s, 3H), 3.20 (t, *J* = 6.0 Hz, 2H), 3.13 (t, *J* = 6.4 Hz, 2H), 3.04 ppm (t, *J* = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.5, 152.0, 142.6, 134.1, 134.0, 131.6, 131.4, 131.3, 128.2, 126.9, 126.8, 122.5, 119.3, 114.8, 114.2, 113.9, 55.8, 48.1, 47.2, 43.8, 39.2 ppm; HRMS-ESI (m/z): calcd for $C_{23}H_{24}N_3O_3$ $[M+H]^+$, 290.1818; found, 290.1819. HPLC purity: Area% = 96.9.

2-(2-((2-((3-methoxyphenyl)amino)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (6b): yellow solid. Yield: 70%. mp: 148-149 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.60 (dd, *J* = 7.6, 0.8 Hz, 2H), 8.21 (dd, *J* = 8.4, 0.8 Hz, 2H), 7.75 (dd, *J* = 8.4, 7.6 Hz, 2H), 7.01 (dd, *J* = 8.4, 8.0 Hz, 1H), 6.23 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.14 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.11 (dd, *J* = 2.0, 1.6 Hz, 1H), 4.36 (t, *J* = 6.4 Hz, 2H), 4.22 (br, 1H), 3.75 (s, 3H), 3.17 (m, 2H), 3.06 (t, *J* = 6.4 Hz, 2H), 2.98 (t, *J* = 5.6 Hz, 2H), 1.46 ppm (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.5, 160.7, 150.0, 134.0, 131.6, 131.3, 129.8, 128.2, 126.9, 122.6, 105.9, 102.4, 98.6, 55.0, 48.1, 47.4, 43.5, 39.9 ppm; HRMS-ESI (m/z): calcd for $C_{23}H_{24}N_3O_3$ $[M+H]^+$, 290.1818; found, 290.1819. HPLC purity: Area% = 98.1.

2-(2-((2-((2,4-dimethoxyphenyl)amino)ethyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (6c): red solid. Yield: 68%. mp: 96-97 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (d, *J* = 7.2 Hz, 2H), 8.21 (d, *J* = 7.6 Hz, 2H), 7.75 (dd, *J* = 7.6, 7.2 Hz, 2H), 6.52 (d, *J* = 8.4 Hz, 1H), 6.40-6.35 (m, 2H), 4.37 (t, *J* = 6.8 Hz, 2H), 3.75 (s, 3H), 3.68 (s, 3H), 3.20 (t, *J* = 6.0 Hz, 2H), 3.07 (t, *J* = 6.8 Hz, 2H), 3.00 ppm (t, *J* = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 151.8, 148.1, 133.9, 132.8, 131.6, 131.2, 128.2, 126.9, 122.7, 110.3, 103.8, 99.2, 55.8, 55.3, 48.6, 47.5, 44.2, 40.0 ppm; HRMS-ESI (m/z): calcd for $C_{24}H_{26}N_3O_4$ $[M+H]^+$, 420.1923; found, 420.1922. HPLC purity: Area% = 99.5.

2-(2-((4-methoxybenzyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (7a):



Scheme S11. Synthetic route of compound **7a**

The mixture of **1a** (500 mg, 2.08 mmol) and 4-methoxybenzaldehyde (283 mg, 2.08 mmol) in 20 mL methanol was stirred at room temperature until the completion of reaction was detected by TLC. The white solid in the reaction mixture was filtered and dried over infrared oven.

Sodium triacetoxyborohydride (535 mg, 2.50 mmol) was added to a solution of the intermediate obtained above and AcOH (0.12 mL, 2.08 mmol) in 20 mL 1,2-dichloroethane. The mixture was stirred at room temperature overnight and then treated with water (50 mL) and extracted with dichloromethane. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography using CH_2Cl_2/CH_3OH (30:1) to give **7a** (380 mg, 53% for two steps) as gray solid. R_f =0.43 ($CHCl_3$/MeOH, 30:1); mp: 142-143 ℃; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (d, J = 7.2 Hz, 2H), 8.22 (d, J = 7.6 Hz, 2H), 7.76 (dd, J = 7.6, 7.2 Hz, 2H), 7.26 (d, J = 8.8 Hz, 2H), 6.80 (d, J = 8.8 Hz, 2H), 3.39 (t, J = 6.4 Hz, 2H), 3.85 (s, 2H), 3.76 (s, 3H), 3.06 (t, J = 6.4 Hz, 2H), 1.49 ppm (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 158.5, 133.9, 132.6, 131.6, 131.2, 129.3, 128.2, 126.9, 122.7, 113.7, 55.2, 52.9, 47.0, 40.0 ppm; HRMS-ESI (m/z): calcd for $C_{22}H_{21}N_2O_3$ $[M+H]^+$, 361.1552; found, 361.1551. HPLC purity: Area% = 99.2.

The compounds **7b-c** were synthesized following the same procedures as **7a**:

2-(2-((3-methoxybenzyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (7b): white solid. Yield: 65% for two steps. mp: 110-112 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.58 (d, J = 7.2 Hz, 2H), 8.20 (d, J = 8.0 Hz, 2H), 7.74(dd, J = 8.0, 7.2 Hz, 2H), 7.17 (t, J = 8.0 Hz, 1H), 7.91-6.86 (m, 2H), 6.76-6.71 (m, 1H), 4.38 (t, J = 6.4 Hz, 2H), 3.85 (s, 2H), 3.75 (s, 3H), 3.05 (t, J = 6.4 Hz, 2H), 1.65 ppm (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 159.7, 142.1, 133.9, 131.6, 131.2, 129.3, 128.2, 126.9, 122.7, 120.4, 113.3, 112.6, 55.1, 53.5, 47.1, 40.0 ppm; HRMS-ESI (m/z): calcd for $C_{22}H_{21}N_2O_3$ $[M+H]^+$, 361.1552; found, 361.1554. HPLC purity: Area% = 96.4.

2-(2-((3,4-dimethoxybenzyl)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (7c): white solid. Yield: 62% for two steps. mp: 142-143 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (d, *J* = 7.2 Hz, 2H), 8.21 (d, *J* = 8.0 Hz, 2H), 7.75 (dd, *J* = 8.0, 7.2 Hz, 2H), 6.88 (d, *J* = 1.6 Hz, 1H), 6.83 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 4.38 (t, *J* = 6.4 Hz, 2H), 3.83 (s, 3H), 3.81 (s, 2H), 3.80 (s, 3H), 3.04 (t, *J* = 6.4 Hz, 2H), 1.76 ppm (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.4, 148.9, 147.9, 133.9, 133.0, 131.6, 131.2, 128.2, 126.9, 122.6, 120.2, 111.4, 110.9, 55.9, 55.7, 53.2, 46.9, 39.9 ppm; HRMS-ESI (m/z): calcd for $C_{23}H_{23}N_2O_4$ $[M+H]^+$, 391.1658; found, 391.1656. HPLC purity: Area% = 99.2.

Enzymatic assay

All naphthalimide derivatives were dissolved in 20% ethanol to the concentration of 0.1 mM. The inhibitory activity of naphthalimide derivatives for human β-*N*-acetyl-D-hexosaminidase (Sigma-Aldrich, a mixture of hHex A and hHex B) was measured with 4-Methylumbelliferyl *N*-acetyl-β-D-glucosaminide (4MU-β-GlcNAc, Sigma-Aldrich) as substrate at 37 ℃ in 60 μl reaction system. Firstly, human β-*N*-acetyl-D-hexosaminidase was pre-incubated with different amount of inhibitors in Britton-Robison buffer (pH 4.5) for 10 min. Then two sets of enzymatic reactions were initiated by adding 4MU-β-GlcNAc to the final concentration of 8 and 16 μM, respectively. The reactions were terminated by adding 60 μL of 0.1 M Glycine and the fluorescence of produced 4MU was measured by a spectrofluorometer using excitation and emission wavelengths of 360 and 405 nm, respectively. The inhibition constants (K_i) were calculated by linear regression of data in Dixon plots.

Table S1. Evaluation datas of compounds **1a-k**, **3a-m**, **6a-c**, **7a-c** against human β-*N*-acetyl-D-hexosaminidases A/B.

Compd	K_i	Compd	K_i	Compd	K_i
1a	2.09±0.21	**3a**	1.04±0.04	**3l**	Nd
1b	29.08±3.97	**3b**	0.93±0.03	**3m**	Nd
1c	61.74±5.97	**3c**	1.35±0.08	**6a**	1.08±0.19
1d	Nd	**3d**	1.13±0.33	**6b**	Nd
1e	30.87±4.22	**3e**	1.23±0.08	**6c**	4.63±0.06
1f	2.81±0.47	**3f**	4.39±0.02	**7a**	0.63±0.01
1g	Nd	**3g**	Nd	**7b**	6.22±0.75
1h	Nd	**3h**	0.69±0.59	**7c**	4.99±1.23
1i	Nd	**3i**	3.00±1.08	**M-31850**	3.22±0.86
1j	Nd	**3j**	Nd		
1k	Nd	**3k**	4.20±0.22		

The inhibition constants (K_i [μM]) were measured with 4MU-β-GlcNAc substrate and calculated by linear regression of data in Dixon plots; Nd: The value of K_i was not determined because of the low activity (less than 50% inhibition at 50 μM). For comparison, the K_i of **M-31850** was also determined with the same condition.

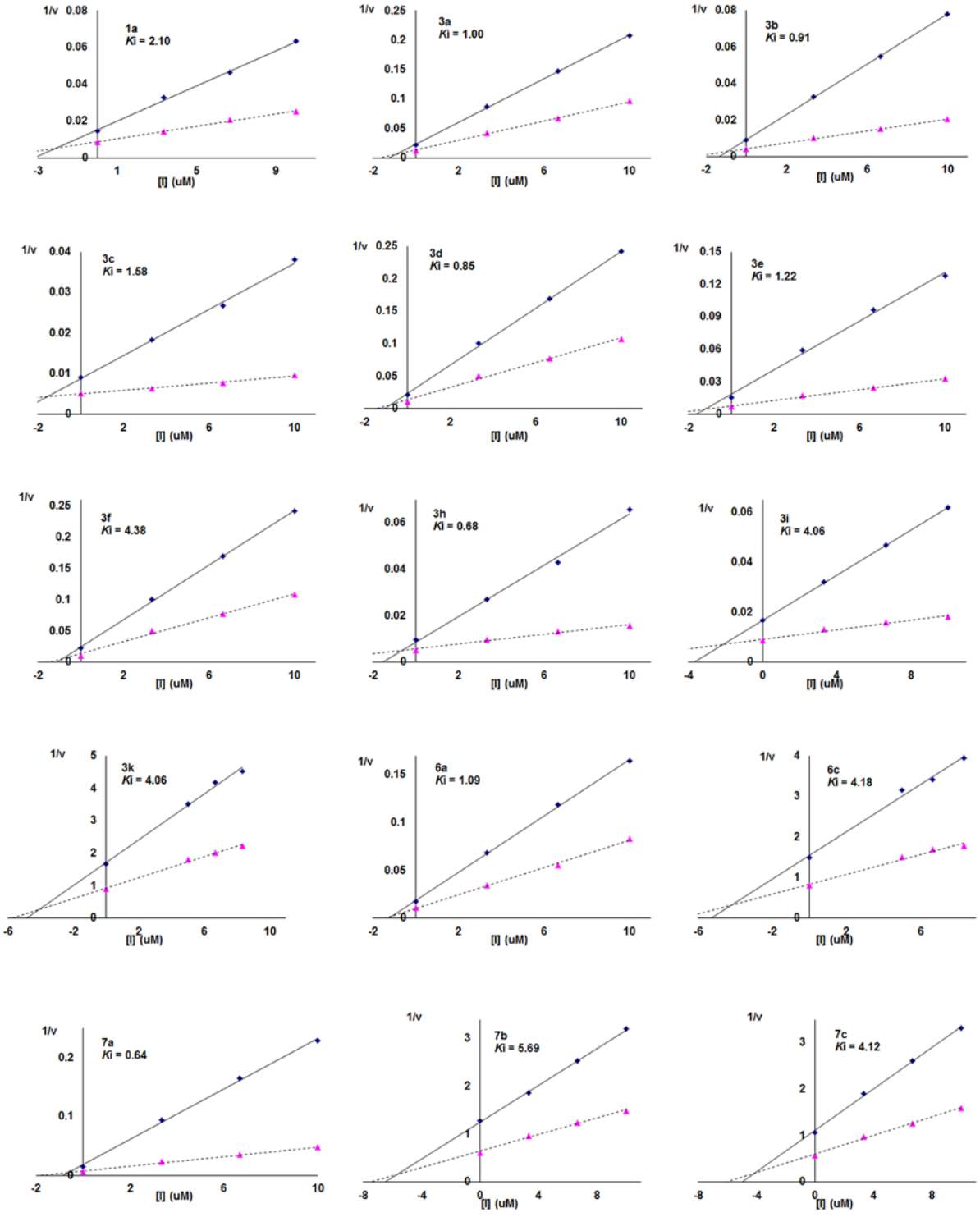


Figure S1. Representative Dixon plots for active compounds against human β-N-acetyl-D-hexosaminidases A/B. The reciprocal velocity is plotted against the inhibitor concentration. The trendlines drawn for each substrate concentration intersect in a single point above the x-axis, indicating competitive inhibition. One out of three experiments is shown as a representative Figure.

Molecular docking

The crystal structures of Hex B from human (PDB ID: 3LMY) was used as 3D model for docking studies. The newest AutoDock 4.2 program was applied to dock the compounds into the binding site of the enzyme.[2] The Gasteiger charges were used for compounds and the active torsions were assigned with the aid of the AutoDockTools-1.5.4 program. The geometric centers of the binding sites were chosen as the grid center, the grid size was set to 50×50×50, and the used grid space was the default value of 0.375 Å. In the docking process, the Lamarkian genetic algorithm (LGA) was applied for conformational searching of the binding complexes. To achieve optimal docking results, the population size and the maximum number of evaluations of the Lamarkian genetic algorithm was set as 150 and 25000000 respectively. The newest adopted Auto-Dock scoring function was used to assess the interaction energies that resulted from probing the compound with Hex B. Cluster analysis was performed on a set of 50 candidates for the docked complex structures, and the best one was selected according to the interaction energy and complementarity in the binding pocket inspected visually.

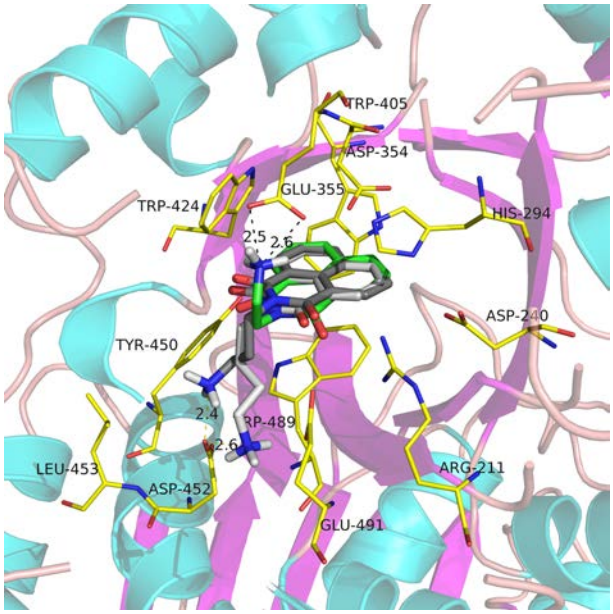


Figure S2. Computational molecular models of **1a**, **1b** and **1c** againstβ chain of hHex (PDB code, 3LMY) in the active site as shown in cartoon (hHex) and sticks (**1a**, **1b** and **1c**). Key residues around the active site are shown as lines including carbon (yellow), nitrogen (blue), and oxygen (red) atoms. The carbon atoms of **1a**, **1b** and **1c** are colored in green, gray and white, respectively. The hydrogen bonds are labelled as dash. The unit of hydrogen bonds is Å. The molecular model was created using the software PyMOL.

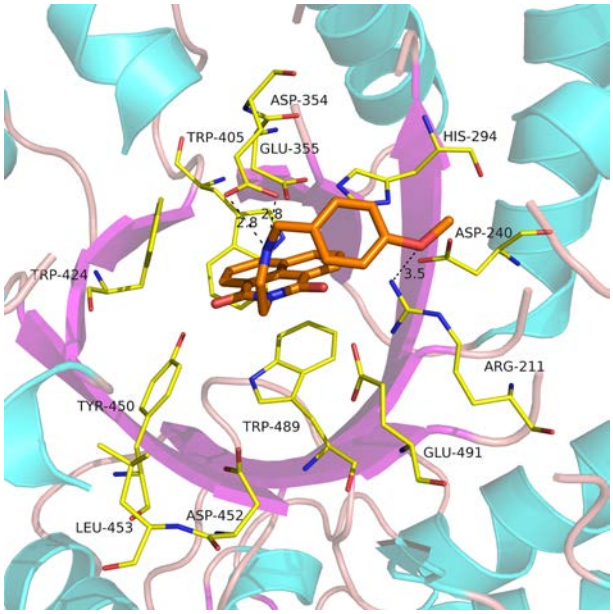


Figure S3. Computational molecular models of **7a** againstβ chain of hHex (PDB code, 3LMY) in the active site as shown in cartoon (hHex) and sticks (**7a**). Key residues around the active site are shown as lines including carbon (yellow), nitrogen (blue), and oxygen (red) atoms. The carbon atoms of **7a** are colored in orange. The hydrogen bonds are labelled as black dash. The unit of hydrogen bonds is Å. The molecular model was created using the software PyMOL.

Figure S3 presents a computational modelling analysis of β chain of hHex in complex with the inhibitor **7a**. The NH of the alkylamine linker still binds with the E355. The methoxyl group of the benzene ring may form a hydrogen bond with the R211. However, this result can not give a perfect explanation to the inhibitory activity difference between the para- and ortho- or meso-methoxybenzyl groups.

References:

[1]. H. Yin, Y. Xu, X. Qian, *Bioorganic & Medicinal Chemistry* **2007**, *15*, 1356-1362.

[2]. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Am. Chem. Soc.* **1998**, 120, 1639–1662.