Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Potent Inhibitors of tRNA-Guanine Transglycosylase, an Enzyme Linked to the Pathogenicity of the *Shigella* Bacterium: Charge-Assisted Hydrogen Bonding

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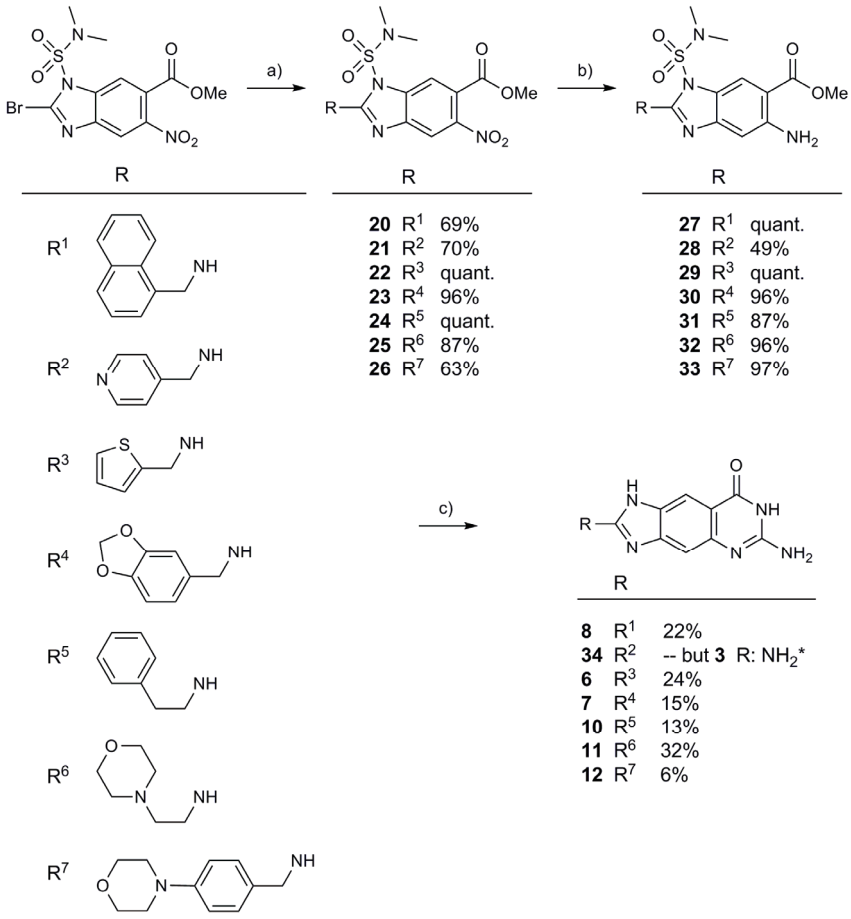
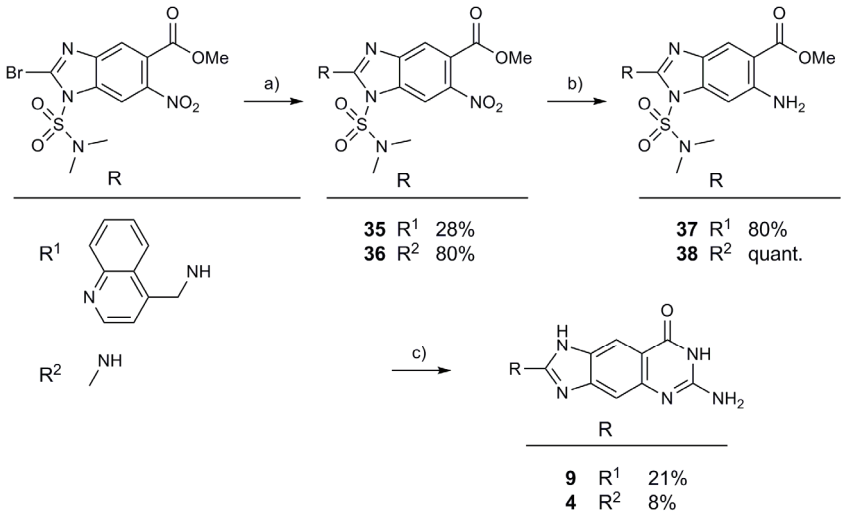
Synthesis Schemes

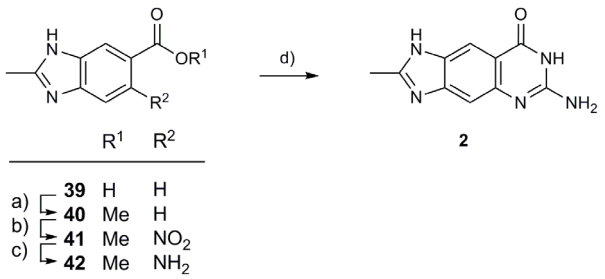


Scheme 1SI. Synthesis of ligands **3, 6-8, 10-12.** a) RNH_2, 25 °C; b) Zn, AcOH, H_2O, 25 °C; c) dimethyl sulfone, chloroformamidinium chloride, 150 °C.

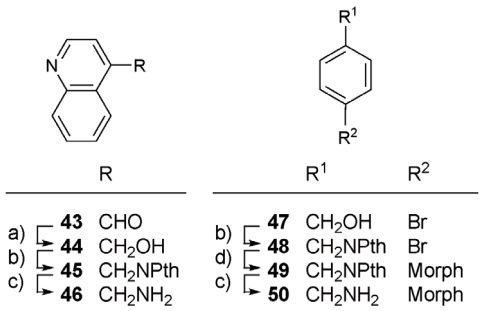
***** During purification of the crude compound **34** by preparative HPLC, the pyridin-4-ylmethanamine moiety was cleaved off due to the acid that was used in the mobile phase, to yield the target molecule **3.**



Scheme 2SI. Synthesis of ligands **4** and **9**. a) RNH_2, 25 °C; b) Zn, AcOH, H_2O, 25 °C; c) dimethyl sulfone, chloroformamidinium chloride, 150 °C.



Scheme 3SI. Synthesis of ligand **2**. a) $SOCl_2$, MeOH, 50 °C, quant.; b) HNO_3/H_2SO_4, 50 °C, 49%; c) Zn, AcOH, H_2O, 25 °C, 90%; d) dimethyl sulfone, chloroformamidinium chloride, 150 °C, 20%.



Scheme 4SI. Synthesis of amines **46** and **50**. a) $NaBH_4$, EtOH, 25 °C, 80%; b) Phthalimide, PPh_3, DIAD, THF, 25 °C, 81% (**45**), 78% (**48**); c) $MeNH_2$, EtOH, 25 °C, quant. (**46**), 45% (**50**); d) $[Pd_2(dba)_3]$, 2-(dicyclohexylphosphino)biphenyl, morpholine, $LiN(TMS)_2$, 65 °C, 22%.

Experimental Protocols

General: Solvents and reagents were purchased reagent-grade and used without further purification. Compounds **14** and **15** are published elsewhere [1]. All reactions were carried out under an Ar atmosphere unless otherwise stated. CH_2Cl_2 and toluene were freshly distilled over CaH_2 and sodium, respectively. All products were dried under high vacuum (10^{-2} Torr) before analytical characterization. TLC: Conducted on glass sheets precoated with 0.2 mm *Merck* silica gel, with 254 nm fluorescent indicator. Compounds were visualized under 254 or 366 nm UV light, or by staining with a $KMnO_4$ solution [$KMnO_4$ (1.5 g), K_2CO_3 (10 g), 5% NaOH (2.5 mL) and H_2O (150 mL)] and subsequent heating. Column chromatography: carried out with *Fluka* silica gel 60 (particle size 40-63 μm, 230-400 mesh), 0-0.3 bar pressure, and distilled technical solvents. Analytical RP-HPLC: performed with a *LaCHrom* system from *Merck-Hitachi* consisting of interface L-7000, pump L-7100, autosampler L-7200, and UV-detector L-7400. Separation was accomplished with a *Merck LiChrospher 100 C18* and a *Knauer Pronto-Sil 120 C18 5.0 μm* column. As the mobile phase, with a flow rate of 1 mL/min, nanopure water, mostly with 0.1% TFA, and MeCN (for HPLC) were used as indicated in the procedures, UV-detection at 254 nm. Preparative RP-HPLC: performed with a *LaCHrom* system from *Merck-Hitachi* consisting of interface L-

7000, pump L-7150, and UV-detector L-7400. Separation was accomplished with a *Merck LiChrosorb C18* and a *Knauer Pronto-Sil 120-5 C18 AQ* column. As the mobile phase, with a flow rate of 10 mL/min, nanopure water, mostly with 0.1% TFA, and MeCN (for HPLC) were used as indicated in the procedures, UV-detection at 254 nm. Melting points (mp): *Büchi-510* apparatus; uncorrected. IR Spectra: *Perkin Elmer Spectrum BX FTIR System* spectrometer (*ATR-unit, Attenuated Total Reflection, Golden Gate*). NMR spectra (^{1}H, ^{13}C): *Varian Gemini-300*, Bruker AMX-400, and *Bruker AMX-500*; spectra were recorded at 25 °C using the solvent peak as an internal reference. Coupling constants (*J*) are given in Hz. The resonance multiplicity is described as s (singlet), br. s (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). *Mass spectra* (MS): performed by the MS service at *Laboratorium für Organische Chemie* of ETH Zürich. EI-MS (*m/z* (%)): *VG Tribid* instrument, 70 eV. HR-EI-MS (*m/z* (%)): *Waters Autospec NT* instrument, 70 eV. HR-ESI-MS (m/z (%)): *Finnigan TSQ 7000* instrument. HR-MALDI-FT-MS (*m/z* (%)): *Ion Spec Ultima 4.7* ion cyclotron resonance mass spectrometer, matrix: 3-HPA (3-hydroxy-picolinic acid). Molecular ions M^{+} reported for salts refer to the neutral compounds. Elemental analyses: performed by the Mikrolabor at the Laboratorium für Organische Chemie, ETH Zürich. The nomenclature was generated with the computer program *ACD/Name* (*ACD/Labs*).

General Procedures

General Procedure A for the Synthesis of an Aromatic Ester. The acid (1.0 eq.) was suspended in dry methanol (5 mL per 1 mmol) and cooled to 0 °C. After slow addition of $SOCl_2$ (5 eq.), the suspension was heated to 50 °C for 16-24 h. The resulting solution was concentrated under reduced pressure, and the residue was dissolved in saturated aqueous $NaHCO_3$ solution (2.5 mL per 1 mmol). The solution was extracted with ethyl acetate (3x 2.5 mL per 1 mmol). After washing the combined organic phases with saturated aqueous NaCl solution (7.5 mL per 1 mmol), drying with Na_2SO_4, and filtration, the solution was concentrated under reduced pressure.

General Procedure B for the Reduction of an Aromatic Nitro Group to an Amine. To the solution of the nitro derivative (1 eq.) in acetic acid/water (25 mL per 1 mmol + 5 mL per 1 mmol), zinc powder (10 eq.) was added portionwise at 25 °C. The mixture was stirred for 15 min–2 h, filtered through a pad of celite, and the solvent evaporated under reduced pressure. The residue was suspended in dichloromethane (25 mL per 1 mmol), filtered through a pad of celite to remove the non-soluble zinc salts, and the solvent evaporated under reduced pressure.

General Procedure C for the Cyclization with Chloroformamidinium Chloride. The anthranilic ester

derivative (1 eq.), chloroformamidinium chloride (1.5-2 eq.), and dimethyl sulfone (50 eq.) were heated to 150 °C for 3 h. When cooled to 25 °C, 25% aqueous NH_3 solution (20 mL per 1 mmol) and water (20 mL per 1 mmol) were added, and the resulting suspension was filtered. The residue was washed with water, methanol, and acetone to yield the crude *lin*-benzoguanine derivative.

General Procedure D for the Aromatic Substitution with an Amine. To **17a** or **17b** (1.0 eq.), neat or as a solution (solvent and amount given in the corresponding procedure), the amine (0.67-25 eq.), neat or as a solution, was added at 0-25 °C. The mixture was stirred at 0-50 °C until all benzimidazole starting material was consumed, as judged by TLC (10 min-24 h). In the case of a resulting suspension, the mixture was filtered, in the case of a solvent used, it was evaporated under reduced pressure. Further modifications are given in the corresponding procedures.

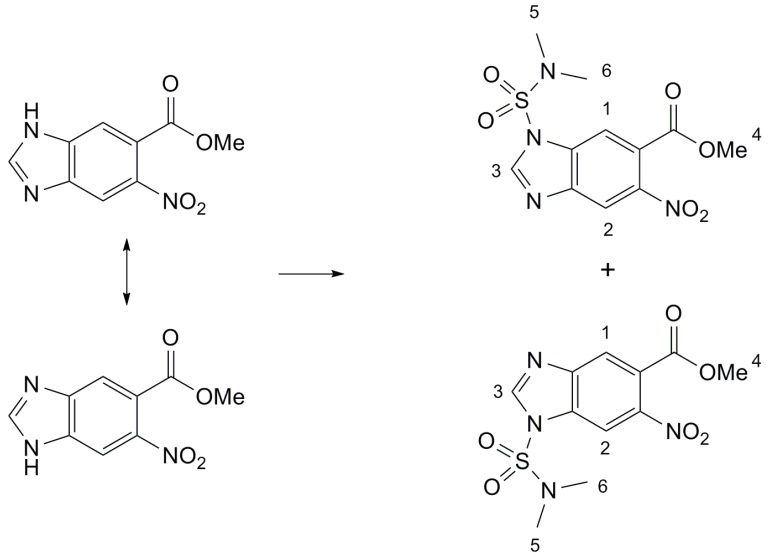
General Procedure E for the Synthesis of a Phthalimide by a Mitsunobu *reaction.* To the solution of the alcohol (1.0 eq.), phthalimide (1.2 eq.), and PPh_3 (1.2 eq.) in THF (5-7 mL per 1 mmol), DIAD (1.2 eq.) was added at 25°C and the reaction was stirred for 17 h. The resulting white suspension was filtered, and the filtrate was concentrated under reduced pressure and dried.

General Procedure F for the Deprotection of a Phthalimide. The phthalimide (1 eq.) was dissolved in 33% NH_2Me in EtOH (6-25 mL per 1 mmol) and stirred at 25° for 15-60 h. The solvent was evaporated under reduced pressure, the resulting residue taken up in 10% aqueous AcOH solution (15 mL per 1 mmol), and washed with CH_2Cl_2 (6 x 10 mL per 1 mmol). The aqueous phase was treated with 1 M aqueous NaOH solution until pH > 12 was reached and extracted with CH_2Cl_2/*i*-PrOH 3:1 (6 x 10 mL per 1 mmol). The combined org. phases were dried with $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and dried.

Synthetic Procedures

Methyl 1-[(dimethylamino)sulfonyl]-5-nitro-1*H*-benzimidazole-6-carboxylate (**16a**) and

Methyl 1-[(dimethylamino)sulfonyl]-6-nitro-1*H*-benzimidazole-5-carboxylate (**16b**)



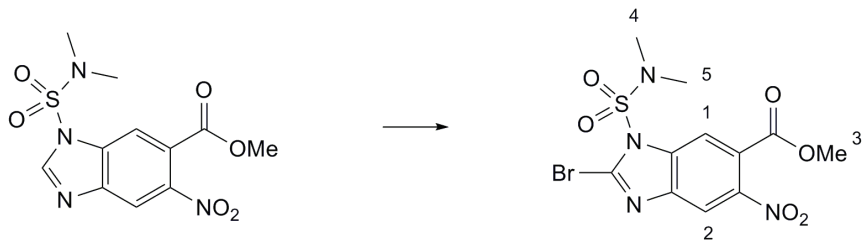
To a suspension of **15** (4.44 g, 20.1 mmol, 1 eq.) in freshly distilled toluene (50 mL), triethylamine (2.23 g, 3.1 mL, 22.1 mmol, 1.1 eq.) and dimethylsulfamoyl chloride (3.17 g, 2.4 mL, 22.1 mmol, 1.1 eq.) were added by syringe. After heating to reflux for 3 h and stirring at 25 °C for 12 h, the resulting solution was filtered, and the liquid evaporated under reduced pressure to yield a mixture of **16a** and **16b** as a beige solid. The regioisomers were partially separated by column chromatography (silica gel; hexane/ethyl acetate 3:1 → ethyl

acetate) to afford **16a** (2.32 g, 7.06 mmol, 35%), **16b** (2.20 g, 6.71 mmol, 33%), and a mixture of the regioisomers (0.69 g, 2.09 mmol, 3:7, 11%). Total yield 79%. Identification of the regioiosomers was carried out by X-ray crystal structure determination of **16b** (see below).

16a: Pale yellow solid. M.p. 132 °C. R_f (ethyl acetate) 0.46. ^{1}H NMR (300 MHz, $CDCl_3$): 8.44 (*s*, 1 H, C(1)H); 8.36 (*s*, 1 H, C(3)H); 8.16 (*s*, 1 H, C(2)H); 3.92 (*s*, 3 H, OC(4)H_3); 2.99 (*s*, 6 H, N(C(5)H_3 and C(6)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.59; 145.80; 145.39; 144.09; 133.55; 124.96; 117.59; 114.39; 53.45; 38.29 (2x). IR: 3110, 1725, 1533, 1452, 1393, 1348, 1301, 1250, 1158, 1049, 968, 898, 780, 729. HR-EI-MS: 328.0471 (52, $[M]^+$; $C_{11}H_{12}N_4O_6S^+$; calc. 328.0478). Anal. calc. for $C_{11}H_{12}N_4O_6S$ (328.31): C 40.24, H 3.68, N 17.07; found C 40.40, H 3.48, N 16.85.

16b: Pale yellow solid. M.p. 147 °C. R_f (ethyl acetate) 0.39. ^{1}H NMR (300 MHz, $CDCl_3$): 8.45 (*s*, 1 H, C(1)H); 8.39 (*s*, 1 H, C(3)H); 8.15 (*s*, 1 H, C(2)H); 3.93 (*s*, 3 H, OC(4)H_3); 2.98 (*s*, 6 H, N(C(5)H_3 and C(6)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.55; 146.04; 145.79; 145.26; 132.13; 124.42; 122.70; 109.92; 53.40; 38.31 (2x). IR: 3121, 1716, 1538, 1453, 1427, 1394, 1366, 1301, 1256, 1194, 1159, 1109, 979, 949, 902, 730. HR-EI-MS: 328.0474 (26, M^+; $C_{11}H_{12}N_4O_6S^+$; calc. 328.0478). Anal. calc. for $C_{11}H_{12}N_4O_6S$ (328.31): C 40.24, H 3.68, N 17.07; found C 40.28, H 3.74, N 16.80.

Methyl 2-bromo-1-[(dimethylamino)sulfonyl]-5-nitro-1*H*-benzimidazole-6-carboxylate (**17a**)



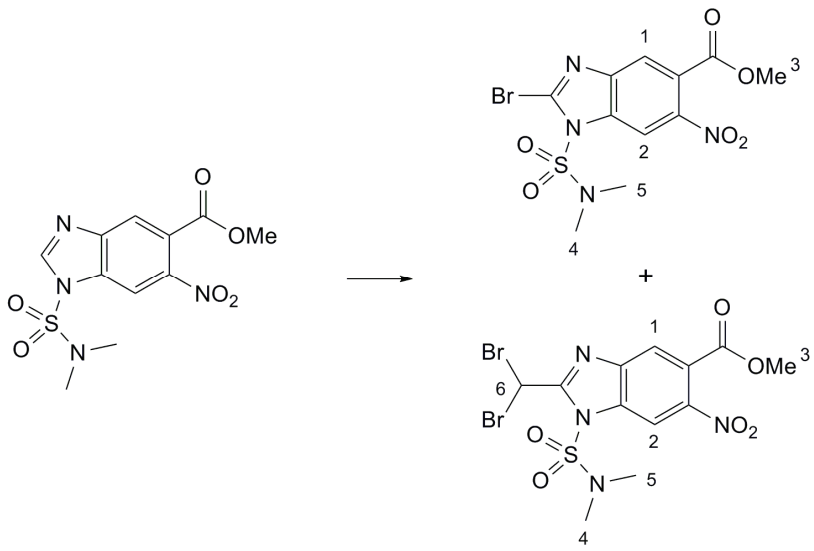
To the cooled solution (-78 °C) of **16a** (1121 mg, 3.42 mmol) in freshly distilled THF (40 mL), a solution of $LiN(TMS)_2$ (~1M in THF, 4.1 mL, 4.1 mmol) was added and the yellow solution stirred for one hour. After the addition of CBr_4 (1244 mg, 3.76 mmol) in freshly distilled THF (40 mL), the mixture was stirred at -78 °C for 1 h and then allowed to warm to 25 °C. The reaction was quenched with saturated aqueous NH_4Cl solution (10 mL) and diluted with water (30 mL). The layers were separated, and the aqueous layer was extracted three times with ethyl acetate (3 x 60 mL), and the combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by column chromatography (silica gel; hexane/ethyl acetate 3:1 → 7:3) to afford **17a** (1049 mg, 2.58 mmol, 75%). Yellow solid.

M.p. 131 °C. R_f (ethyl acetate) 0.63. ^{1}H NMR (300 MHz, $CDCl_3$): 8.27 (*s*, 1 H, C(1)H); 8.23 (*s*, 1 H, C(2)H); 3.92 (*s*, 3 H, OC(3)H_3); 3.08 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.60; 145.24; 142.63; 137.12; 130.97; 124.78; 116.05; 115.71; 53.48; 38.83(2x). IR: 3136, 1727, 1537, 1451,

1432, 1394, 1367, 1295, 1204, 1170, 1115, 1017, 975, 953, 903, 724. HR-EI-MS: 405.9577 (10, M^+; $C_{11}H_{11}BrN_4O_6S^+$; calc. 405.9583). Anal. calc. for $C_{11}BrH_{11}N_4O_6S$ (407.20): C 32.45, H 2.72, N 13.76; found C 32.74, H 2.61, N 13.57.

Methyl 2-bromo-1-[(dimethylamino)sulfonyl]-6-nitro-1*H*-benzimidazole-5-carboxylate (**17b**) and

Methyl 2-(dibromomethyl)-1-[(dimethylamino)sulfonyl]-6-nitro-1*H*-benzimidazole-5-carboxylate (**17c**)



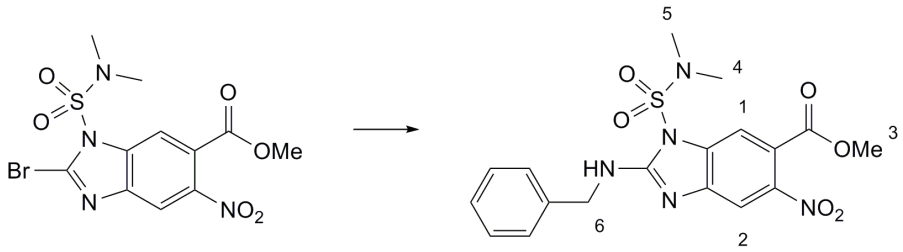
To the cooled solution (-78 °C) of **16b** (2200 mg, 6.71 mmol) in freshly distilled THF (80 mL), a solution of $LiN(TMS)_2$ (~1M in THF, 8.05 mL, 8.05 mmol) was added and the orange solution stirred for 1 h. After addition of CBr_4 (2442 mg, 7.38 mmol) in freshly distilled THF (80 mL), the mixture was stirred at -78 °C for 1 h and then allowed to warm to 25 °C. The reaction was quenched with saturated aqueous NH_4Cl solution (40 mL).

The resulting suspension was diluted with water (60 mL), the layers separated, the aqueous layer extracted three times with ethyl acetate (3x 120 mL), and the combined organic layers dried with $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by column chromatography (silica gel; hexane/ethyl acetate 4:1 $\rightarrow$ 3:1) to afford **17b** (1637 mg, 4.02 mmol, 60%) and **17c** (1039 mg, 2.08 mmol, 31%).

17b: Yellow solid. M.p. 146 °C. R_f (ethyl acetate) 0.62. ^{1}H NMR (300 MHz, $CDCl_3$): 8.54 (*s*, 1 H, C(1)H); 8.04 (*s*, 1 H, C(2)H); 3.95 (*s*, 3 H, OC(3)H_3); 3.09 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.42; 145.60; 143.04; 142.40; 135.18; 124.46; 121.18; 111.11; 53.42; 38.67 (2x). IR: 3121, 2924, 1715, 1537, 1455, 1422, 1395, 1367, 1349, 1302, 1257, 1217, 1198, 1166, 1110, 1006, 980, 950, 902, 892, 766, 730. HR-EI-MS: 405.9579 (2, M^+; $C_{11}H_{11}BrN_4O_6S^+$; calc. 405.9583). Anal. calc. for $C_{11}BrH_{11}N_4O_6S$ (407.20): C 32.45, H 2.72, N 13.76; found C 32.64, H 2.81, N 13.50.

17c: Yellow solid. M.p. 163 °C. R_f (ethyl acetate) 0.67. ^{1}H NMR (300 MHz, $CDCl_3$): 8.45 (*s*, 1 H, C(1)H); 8.20 (*s*, 1 H, C(2)H); 7.36 (*s*, 1 H, C(6)H); 3.96 (*s*, 3 H, OC(3)H_3); 3.07 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.31; 154.86; 146.28; 143.04; 133.33; 125.06; 122.76; 111.37; 53.52; 38.65 (2x); 25.27. IR: 3691, 2989, 2901, 2360, 1723, 1533, 1393, 1353, 1292, 1251, 1161, 1065, 1027, 961, 839, 735, 720, 628. HR-EI-MS: 497.8836 (10, M^+; $C_{12}H_{12}Br_2N_4O_6S^+$; calc. 497.8836).

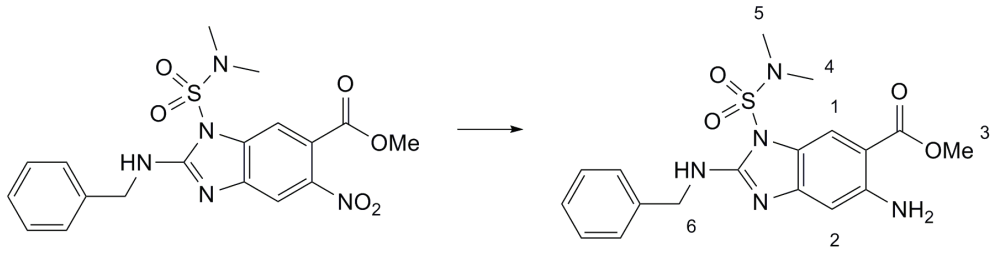
Methyl 2-(benzylamino)-1-[(dimethylamino)sulfonyl]-5-nitro-1*H*-benzimidazole-6-carboxylate (**18**)



General Procedure D started from **17a** (200 mg, 0.49 mmol) and benzylamine (1.34 mL, 1.32 g, 12.3 mmol), 0 °C, 10 min, to yield, after purification by column chromatography (silica gel; hexane/ethyl acetate 3:1 → 1:1), **18** (195 mg, 0.45 mmol, 92%). Yellow solid.

M.p. 113 °C. R_f (ethyl acetate) 0.67. ^{1}H NMR (300 MHz, $CDCl_3$): 7.91 (*s*, 1 H, C(1)H); 7.81 (*s*, 1 H, C(2)H); 7.37-7.30 (*m*, 5 H, C(arom.)H); 6.70 (*t*, *J* = 6, 1 H, NH); 4.74 (*d*, *J* = 6, 2 H, C(6)H_2); 3.89 (*s*, 3 H, OC(3)H_3); 2.92 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.97; 155.26; 146.46; 144.40; 137.00; 133.74; 128.95 (2x); 128.08; 127.70 (2x); 119.75; 113.14; 112.44; 53.14; 47.40; 38.72 (2x). IR: 3413, 1721, 1563, 1525, 1366, 1347, 1297, 1244, 1156, 970, 780, 757. HR-ESI-MS: 433.1057 (17, M^+; $C_{18}H_{19}N_5O_6S^+$; calc. 433.1051).

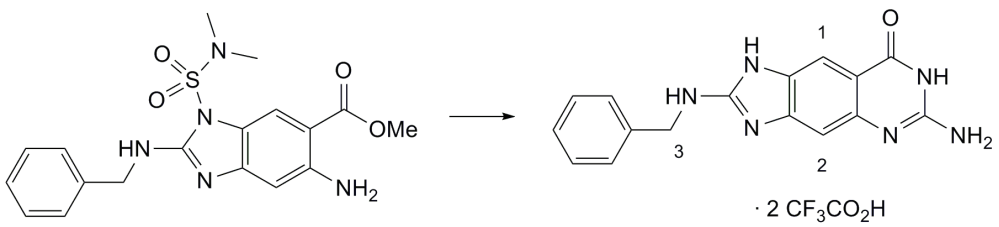
Methyl 5-amino-2-(benzylamino)-1-[(dimethylamino)sulfonyl]-1*H*-benzimidazole-6-carboxylate (**19**)



General Procedure B started from **18** (349 mg, 0.81 mmol), zinc powder (527 mg, 8.05 mmol), and acetic acid/water (20 mL + 4 mL), 20 min, to yield **19** (306 mg, 0.76 mmol, 94%). Light brown solid, sufficiently pure for further use.

M.p. 194 °C. R_f (ethyl acetate) 0.65. ^{1}H NMR (300 MHz, $CDCl_3$): 8.03 (*s*, 1 H, C(1)H); 7.37-7.30 (*m*, 5 H, C(arom.)H); 6.64 (br *s*, 2 H, C(2)H and NH); 4.72 (*d*, J = 5.4, 2 H, C(6)H_2); 3.86 (*s*, 3 H, OC(3)H_3); 2.88 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (125 MHz, $CDCl_3$): 168.52; 155.19; 149.29; 147.95; 137.45; 128.82 (2x); 127.82; 127.65 (2x); 123.70; 114.06; 104.66; 102.77; 51.47; 47.26; 38.74 (2x). IR: 3414, 1721, 1564, 1525, 1347, 1297, 1245, 1156, 970, 726, 700. HR-EI-MS: 403.1310 (23, M^+; $C_{18}H_{21}N_5O_4S^+$; calc. 403.1309).

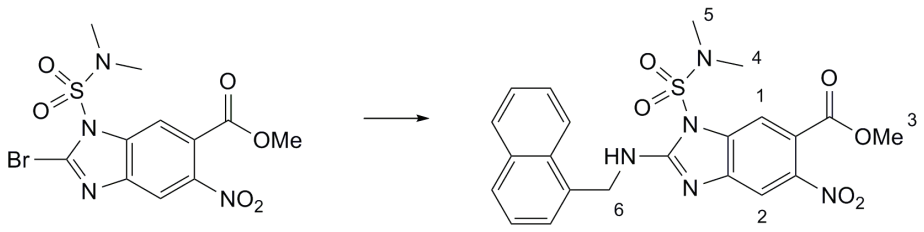
6-Amino-2-(benzylamino)-8-oxo-7,8-dihydro-1*H*-imidazo[4,5-*g*]quinazolin-3-ium bis(trifluoroacetate) (**5**)



General Procedure C started from **19** (306 mg, 0.76 mmol), chloroformamidinium chloride (175 mg, 1.52 mmol), and dimethyl sulfone (3.57 g, 37.95 mmol) to yield the crude product, brown powder (150 mg). Purification of samples (80 mg) for the biological investigation was carried out by preparative HPLC with water (0.1% TFA)/MeCN 95:5 → 0:100 to yield **5** as the bis-TFA salt (44 mg, 82 µmol, 20%). Pale yellow powder.

M.p. >280 °C (dec.). ^{1}H NMR (300 MHz, CD_3OD): 8.00 (br *s*, 1 H, C(1)H); 7.45-7-30 (br *m*, 6 H, C(2)H and C(arom.)H); 4.69 (br *s*, 2 H, $C(3)H_2$). ^{13}C (75 MHz, CD_3OD): 161.17; 153.97; 152.79; 138.18; 137.21; 136.85; 130.09 (2x); 129.94; 129.37; 128.50 (2x); 112.87; 110.31; 100.20; 40.43. IR: 2918, 1620, 1393, 1249, 1127, 1010, 836, 689, 644. HR-MALDI-MS (3-HPA): 307.1296 (100, $[M+H]^+$; $C_{16}H_{15}N_6O^+$; calc. 307.1302).

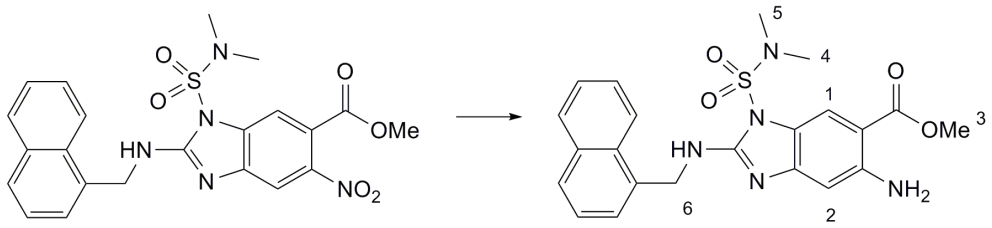
Methyl 1-[(dimethylamino)sulfonyl]-2-[(1-naphthylmethyl)amino]-5-nitro-1*H*-benzimidazole-6-carboxylate (**20**)



General Procedure D started from **17a** (300 mg, 0.74 mmol) and 1-naphthylmethylamine (1.34 mL, 1.32 g, 12.3 mmol), 25 °C, 10 min, to yield, after purification by column chromatography (silica gel; hexane/ethyl acetate 3:1 → 2:1), **20** (220 mg, 0.46 mmol, 69%). Yellow tar.

R_f (ethyl acetate) 0.76. ^{1}H NMR (300 MHz, $CDCl_3$): 8.02-7.99 (*m*, 1 H, C(arom.)H); 7.91-7.82 (*m*, 4 H, C(1)H, C(2)H, and 2 C(arom.)H); 7.54-7.41 (*m*, 4 H, C(arom.)H); 6.64 (*t*, *J* = 6.0, 1 H, NH); 5.17 (*d*, *J* = 5.4, 2 H, C(6)H_2); 3.88 (*s*, 3 H, OC(3)H_3); 2.76 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.87; 154.91; 146.36; 144.36; 133.86; 133.75; 132.15; 131.16; 129.08; 128.96; 126.80; 126.63; 126.05; 125.38; 122.91; 119.65; 113.08; 112.38; 53.09; 45.53; 38.50 (2x). IR: 3048, 1644, 1561, 1510, 1370, 1293, 1262, 1162, 772. HR-MALDI-MS (3-HPA): 484.1277 (100, $[M+H]^+$; $C_{22}H_{22}N_5O_6S^+$; calc. 484.1285).

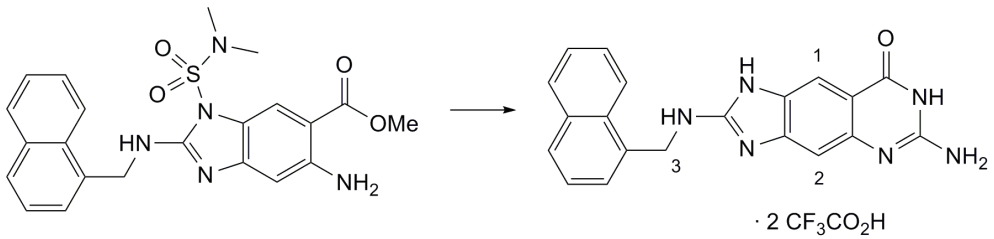
Methyl 5-amino-1-[(dimethylamino)sulfonyl]-2-[(1-naphthylmethyl)amino]-1*H*-benzimidazole-6-carboxylate (**27**)



General Procedure B started from **20** (180 mg, 0.38 mmol), zinc powder (248 mg, 3.79 mmol), and in acetic acid/water (10 mL + 2 mL), 2 h, to yield, after purification by column chromatography (silica gel; hexane/ethyl acetate 5:1 → 7:3), **27** (172 mg, 0.38 mmol, quant.). Yellow solid.

M.p. 158 °C. R_f (ethyl acetate) 0.68. ^{1}H NMR (300 MHz, $CDCl_3$): 8.05-8.02 (*m*, 2 H, C(arom.)H and C(1)H); 7.90-7.82 (*m*, 2 H, C(arom.)H); 7.54-7.42 (*m*, 4 H, C(arom.)H); 6.69 (*s*, 1 H, C(2)H); 6.58 (*t*, *J* = 6.0, 1 H, NH); 5.76 (br *s*, 2 H, NH_2); 5.16 (*d*, *J* = 5.1, 2 H, C(6)H_2); 3.86 (*s*, 3 H, OC(3)H_3); 2.76 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 168.46; 154.81; 149.29; 147.99; 133.80; 132.60; 131.24; 128.80 (2x); 126.57; 126.46; 125.93; 125.34; 123.66; 123.16; 113.99; 104.48; 102.69; 51.40; 45.36; 38.55 (2x). IR: 3400, 1691, 1594, 1565, 1461, 1376, 1256, 1200, 1151, 1042, 971, 841, 790, 720. HR-MALDI-MS (3-HPA): 454.1537 (58, $[M+H]^+$; $C_{22}H_{24}N_5O_4S^+$; calc. 454.1544). Anal. calc. for $C_{22}H_{23}N_5O_4S$ (453.51): C 58.26, H 5.11, N 15.44; found C 58.20, H 5.25, N 15.35.

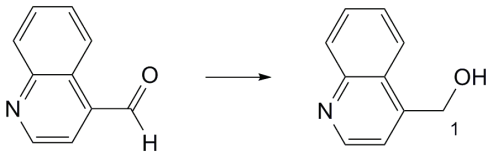
6-Amino-2-[(1-naphthylmethyl)amino]-8-oxo-7,8-dihydro-1*H*-imidazo[4,5-*g*]quinazolin-3-ium bis(trifluoroacetate) (**8**)



General Procedure C started from **27** (143 mg, 0.36 mmol), chloroformamidinium chloride (82 mg, 0.71 mmol), and dimethyl sulfone (1.67 g, 17.74 mmol) to yield the crude product, fawn powder (105 mg). Purification of samples (80 mg) for the biological investigation was carried out by preparative HPLC with water (0.1% TFA)/MeCN 95:5 → 0:100 to yield **8** as the bis-TFA salt (35 mg, 60 µmol, 22%). Pale yellow powder.

M.p. >230 °C (dec.). ^{1}H NMR (500 MHz, CD_3OD): 8.07 (*d*, *J* = 8.5, 1 H, C(arom.)H); 7.95-7.93 (*m*, 2 H, C(1)H and C(arom.)H); 7.90-7.88 (*d*, *J* = 8.5, 1 H, C(arom.)H); 7.61-7.45 (*m*, 3 H, C(arom.)H); 7.49 (*t*, *J* = 7.8, 1 H, C(arom.)H); 7.31 (*s*, 1 H, C(2)H); 5.14 (*s*, 2 H, $C(3)H_2$). ^{13}C NMR (125 MHz, CD_3OD): 162.42; 155.42; 152.93; 140.73; 138.49; 135.47; 132.57; 132.50; 131.28; 130.10; 130.00; 127.88; 127.30; 126.54; 126.44; 123.96; 112.37; 109.80; 101.08; 46.03. IR: 3080, 1651, 1525, 1461, 1369, 1296, 1186, 1130, 1025, 966, 836, 792, 770, 721. HR-MALDI-MS (3-HPA): 357.1452 (100, $[M+H]^+$; $C_{20}H_{17}N_6O^+$; calc. 357.1458).

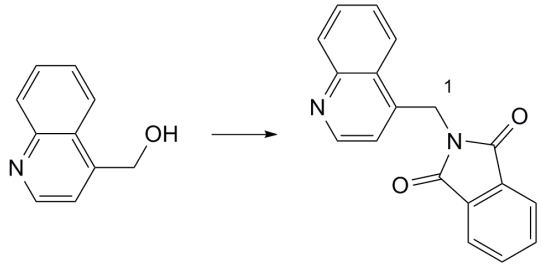
Quinolin-4-ylmethanol (**44**)



$NaBH_4$ (227 mg, 6.0 mmol) was added in small portions to a suspension of quinoline-4-carbaldehyde (**43**, 785 mg, 5.0 mmol) in ethanol (10 mL) at 25 °C. After stirring for 1 h, all starting material was dissolved and Et_2O (25 mL) was added to the colorless solution. The organic phase was washed with water (2 x 25 mL), and this aqueous phase was extracted with Et_2O (2 x 50 mL). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure to yield **44** (638 mg, 4.0 mmol, 80%). White solid, sufficiently pure for further use.

M.p. 97 °C ([2]: 99-100 °C). ^{1}H NMR (300 MHz, $CDCl_3$): 8.91 (*d*, *J* = 4.2, 1 H, C(arom.)H); 8.14 (*d*, *J* = 8.7, 1 H, C(arom.)H); 7.97 (*d*, *J* = 8.7, 1 H, C(arom.)H); 7.76-7.71 (*m*, 1 H, C(arom.)H); 7.61-7.55 (*m*, 2 H, C(arom.)H); 5.25 (*d*, *J* = 4.2, 2 H, C(1)H_2). HR-EI-MS: 159.0680 (100, M^+; $C_{10}H_9NO^+$; calc. 159.0680).

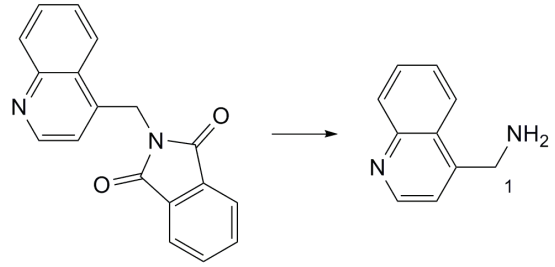
2-(Quinolin-4-ylmethyl)-1*H*-isoindole-1,3(2*H*)-dione (**45**)



General Procedure E started from **44** (638 mg, 4.0 mmol), phthalimide (882 mg, 6.0 mmol), PPh_3 (1572 mg, 6.0 mmol), THF (20 mL), and DIAD (1.2 mL, 1.2 g, 6.0 mmol) to yield **45** (933 mg, 3.2 mmol, 81%). White solid, sufficiently pure for further use.

M.p. 208 °C. R_f (ethyl acetate) 0.47. ^{1}H NMR (500 MHz, $CDCl_3$): 8.85 (*d*, *J* = 4.5, 1 H, C(arom.)H); 8.29 (*dd*, *J* = 8.4, 0.8, 1 H, C(arom.)H); 8.14 (*d*, *J* = 7.7, 1 H, C(arom.)H); 7.89, 7.75 (*AA'MM'*, 4 H, C(arom.)H); 7.76-7.73 (*m*, 1 H, C(arom.)H); 7.66-7.63 (*m*, 1 H, C(arom.)H); 7.37 (*d*, *J* = 4.4, 1 H, C(arom.)H); 5.34 (*d*, *J* = 0.6, 2 H, C(1)H_2). ^{13}C NMR (125 MHz, $CDCl_3$): 167.92 (2x); 150.15; 148.43; 140.88; 134.35 (2x); 131.94 (2x); 130.36; 129.48; 127.12; 126.26; 123.65 (2x); 123.14; 120.50; 38.38. IR: 3029, 1771, 1704, 1598, 1421, 1391, 1330, 1109, 947, 829, 758, 715. HR-EI-MS: 288.0894 (10, M^+; $C_{18}H_{12}N_2O_2^+$; calc. 288.0899).

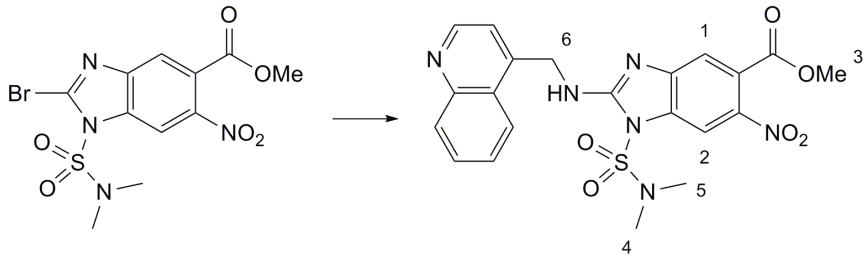
1-Quinolin-4-ylmethanamine (Lepidylamine, **46**) [3]



General Procedure F started from **45** (1.77 g, 6.14 mmol) and 33% NH_2Me in EtOH (62 mL), 15 h, to yield lepidylamine (**46**, 970 mg, 6.14 mmol, quant.). Purple tar, sufficiently pure for further use.

^{1}H NMR (300 MHz, $CDCl_3$): 8.84 (*d*, *J* = 4.5, 1 H, C(arom.)H); 8.10 (*dd*, *J* = 8.6, 0.8, 1 H, C(arom.)H); 7.96 (*dd*, *J* = 8.4, 0.9, 1 H, C(arom.)H); 7.68 (*dt*, *J* = 7.8, 0.9, 1 H, C(arom.)H); 7.56-7.50 (*m*, 1 H, C(arom.)H); 7.41 (*d*, *J* = 4.5, 1 H, C(arom.)H); 4.32 (*d*, *J* = 1.2, 2 H, C(1)H_2). HR-EI-MS: 158.0835 (35, M^+; $C_{10}H_{10}N_2^+$; calc. 158.0844).

Methyl 1-[(dimethylamino)sulfonyl]-6-nitro-2-[(quinolin-4-ylmethyl)amino]-1*H*-benzimidazole-5-carboxylate (**35**)

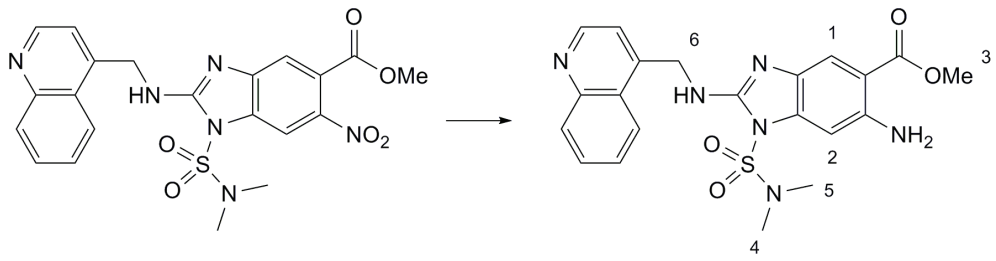


General Procedure D started from **17b** (300 mg, 0.74 mmol) in dry DME (1.5 mL) and lepidylamine (**46**, 163 mg, 1.03 mmol) in

dry DME (3.5 mL), at 25 °C, 10 h, to yield, after filtration and purification by column chromatography (silica gel; hexane/ethyl acetate 4:1 → ethyl acetate (1% TEA)), **35** (100 mg, 0.21 mmol, 28%). Yellow solid.

M.p. 192 °C. R_f (ethyl acetate) 0.32. ^{1}H NMR (300 MHz, $CDCl_3$): 8.91 (*d*, *J* = 4.5, 1 H, C(arom.)H); 8.25 (*s*, 1 H, C(1)H); 8.17 (*d*, *J* = 8.4, 1 H, C(arom.)H); 8.04 (*d*, *J* = 8.4, 1 H, C(arom.)H); 7.77 (*dt*, *J* = 7.7, 1.4, 1 H, C(arom.)H); 7.64-7.58 (*m*, 1 H, C(arom.)H); 7.57 (*s*, 1 H, C(2)H); 7.41 (*d*, *J* = 4.5, 1 H, C(arom.)H); 6.87 (*t*, *J* = 5.7, 1 H, NH); 5.27 (*d*, *J* = 6.0, 2 H, C(6)H_2); 3.94 (*s*, 3 H, OC(3)H_3); 2.89 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (125 MHz, $CDCl_3$): 166.78; 155.43; 150.34; 148.48; 145.85; 141.94; 141.41; 132.15; 130.65; 129.69; 127.26; 126.39; 126.20; 122.62; 119.80; 117.02; 109.01; 53.31; 44.21; 38.67 (2x). IR: 2922, 1693, 1525, 1435, 1331, 1299, 1247, 1018, 865, 781, 759, 712. HR-EI-MS: 484.1160 (22, $[M+H]^+$; $C_{21}H_{20}N_6O_6S^+$; calc. 484.1165). Anal. calc. for $C_{21}H_{20}N_6O_6S$ (484.49): C 52.06, H 4.16, N 17.35; found C 52.13, H 4.39, N 17.12.

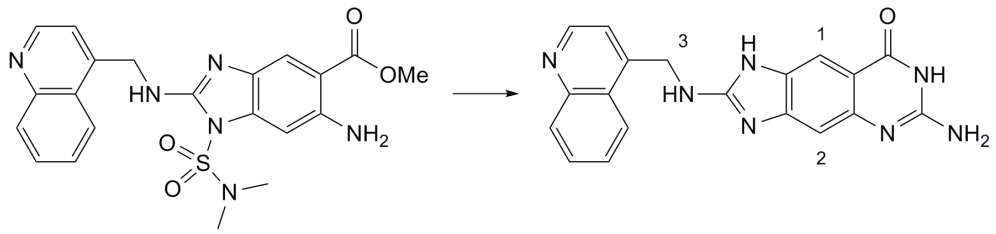
Methyl 6-amino-1-[(dimethylamino)sulfonyl]-2-[(quinolin-4-ylmethyl)amino]-1*H*-benzimidazole-5-carboxylate (**37**)



General Procedure B started from **35** (93 mg, 0.19 mmol), zinc powder (126 mg, 1.92 mmol), and acetic acid/water (5 mL + 1 mL), 1 h, to yield, after purification by column chromatography (silica gel; CH_2Cl_2 (1% TEA)), **37** (69 mg, 0.15 mmol, 80%). Pale yellow solid.

M.p. >180 °C (dec.). R_f (ethyl acetate) 0.29. ^{1}H NMR (300 MHz, $CDCl_3$): 8.89 (*d*, *J* = 4.5, 1 H, C(arom.)H); 8.15 (*d*, *J* = 7.8, 1 H, C(arom.)H); 8.06 (*d*, *J* = 8.4, 1 H, C(arom.)H); 7.93 (*s*, 1 H, C(1)H); 7.77-7.71 (*m*, 1 H, C(arom.)H); 7.61-7.55 (*m*, 1 H, C(arom.)H); 7.42 (*d*, *J* = 4.5, 1 H, C(arom.)H); 6.93 (*s*, 1 H, C(2)H); 6.42 (*t*, *J* = 5.9, 1 H, NH); 5.69 (br *s*, 2 H, NH_2); 5.27 (*d*, *J* = 5.7, 2 H, C(6)H_2); 3.86 (*s*, 3 H, OC(3)H_3); 2.86 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 168.38; 151.25; 150.19; 148.12; 146.75; 142.79; 137.03; 132.71; 130.24; 129.34; 126.82; 126.24; 122.79; 119.40; 118.77; 108.10; 99.48; 51.67; 44.10; 38.71 (2x). IR: 3447, 2951, 1660, 1627, 1595, 1551, 1463, 1375, 1274, 1239, 1188, 1165, 1048, 962, 789. HR-MALDI-MS (3-HPA): 455.1488 (100, $[M+H]^+$; $C_{21}H_{23}N_6O_4S^+$; calc. 455.1496).

6-Amino-2-[(quinolin-4-ylmethyl)amino]-1,7-dihydro-8*H*-imidazo[4,5-*g*]quinazolin-8-one (**9**)

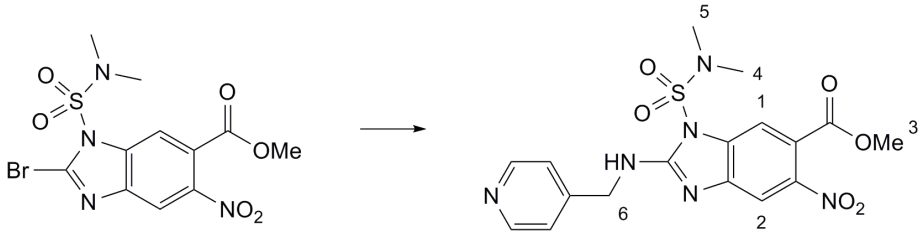


General Procedure C started from **37** (30 mg, 0.07 mmol), chloroformamidinium chloride (15 mg, 0.13 mmol), and dimethyl sulfone (311 mg, 3.30 mmol) to yield the crude product, orange powder (38 mg). Purification by column chromatography on LiChroprep®NH_2 with MeOH/MeCN 1:1 afforded the target compound contaminated with LiChroprep®NH_2. Suspending the residue in DMF and filtration improved the purity, but did not yield the pure compound. Only preparative HPLC with water/MeCN (95:5 → 0:100, 30 mg) finally yielded **9** (4 mg, 0.01 mmol, 21%). Pale yellow powder.

M.p. >250 °C (dec.). ^{1}H NMR (300 MHz, CD_3OD): 8.79 (*d*, *J* = 4.8, 1 H, C(arom.)H); 8.23 (*d*, *J* =7.8, 1 H, C(arom.)H); 8.08 (*d*, *J* = 8.1, 1 H, C(arom.)H); 7.85-7.79 (*m*, 2 H, C(arom.)H and C(1)H); 7.72-67 (*m*, 1 H, C(arom.)H); 7.56 (*d*, *J* = 4.5, 1 H, C(arom.)H); 7.05 (*s*, 1 H, C(2)H); 5.20 (br *s*, 2 H, C(3)H_2). ^{13}C NMR (125 MHz, CD_3OD): 159.66; 154.37; 151.13; 148.61; 147.29; 143.76; 136.18; 136.12; 130.98; 129.85; 128.28; 127.93; 124.47; 119.78; 111.82; 108.17; 107.79; 103.63; 44.20.

IR: 3410, 2931, 1597, 1435, 1385, 1328, 1291, 1259, 1140, 1053, 1011, 936, 748, 723. HR-MALDI-MS (3-HPA): 358.1411 (100, $[M+H]^+$; $C_{19}H_{16}N_7O^+$; calc. 358.1411).

Methyl 1-[(dimethylamino)sulfonyl]-5-nitro-2-[(pyridin-4-ylmethyl)amino]-1*H*-benzimidazole-6-carboxylate (**21**)

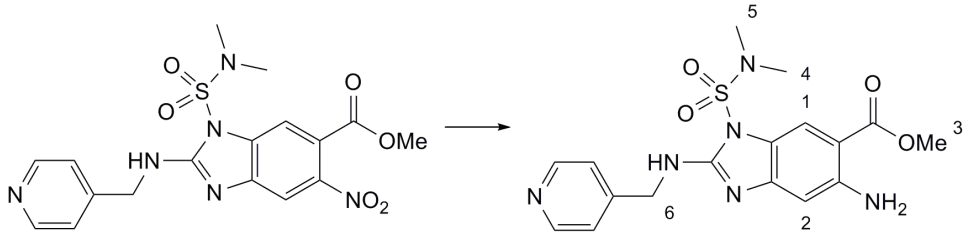


General Procedure D started from **17a** (100 mg, 0.25 mmol) in dry DME (2.5 mL) and 4-(aminomethyl)pyridine (41 mg, 26 µL, 38 mmol), 25 °C, 15 h, to yield, after filtration and purification by column chromatography (silica gel; hexane/ethyl acetate 7:3 → ethyl acetate), **21** (72 mg, 0.17 mmol, 70%). Yellow solid.

M.p. 163 °C. R_f (CH_2Cl_2/MeOH 10:1) 0.61. ^{1}H NMR (300 MHz, $CDCl_3$): 8.57 (*dd*, *J* = 4.5, 1.8, 2 H, C(arom.)H); 7.91 (*s*, 1 H, C(1)H); 7.79 (*s*, 1 H, C(2)H); 7.27 (*dd*, *J* = 4.4, 1.4, 2 H, C(arom.)H); 6.87 (*t*, *J* = 6.2, 1 H, NH); 4.77 (*d*, *J* = 6.0, 2 H, $C(6)H_2$); 3.88 (*s*, 3 H, $OC(3)H_3$); 2.97 (*s*, 6 H, $N(C(4)H_3$ and $C(5)H_3$)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.81; 155.12; 150.23 (2x); 146.38; 146.21; 143.97; 133.69; 122.07 (2x); 119.98; 113.13; 112.64; 53.15; 45.98; 38.69 (2x). IR: 3423, 2970, 1710, 1599, 1569, 1536, 1376, 1292, 1254, 1214, 1152, 1114, 1000, 973,

894, 749, 730. HR-MALDI-MS (3-HPA): 435.1085 (100, $[M+H]^+$; $C_{17}H_{19}N_6O_6S^+$; calc. 435.1081). Anal. calc. for $C_{17}H_{18}N_6O_6S$ (434.43): C 47.00, H 4.18, N 19.34; found C 47.20, H 4.27, N 19.12.

Methyl 5-amino-1-[(dimethylamino)sulfonyl]-2-[(pyridin-4-ylmethyl)amino]-1*H*-benzimidazole-6-carboxylate (**28**)

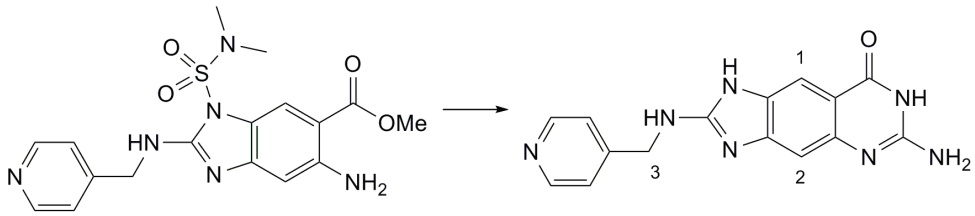


General Procedure B started from **21** (240 mg, 0.55 mmol), zinc powder (361 mg, 5.52 mmol), and in acetic acid/water (15 mL + 3 mL), 30 min, to yield, after purification by column chromatography (silica gel; CH_2Cl_2 (1-2% MeOH)), **28** (109 mg, 0.27 mmol, 49%). Pale yellow solid.

M.p. 198 °C. R_f (CH_2Cl_2/MeOH 10:1) 0.53. ^{1}H NMR (300 MHz, $CDCl_3$): 8.58 (*d*, *J* = 6.0, 2 H, C(arom.)H); 8.02 (*s*, 1 H, C(1)H); 7.28 (*d*, *J* = 6.0, 2 H, C(arom.)H); 6.80 (*t*, *J* = 6.2, 1 H, NH); 6.60 (*s*, 1 H, C(2)H); 4.76 (*d*, *J* = 6.3, 2 H, C(6)H_2); 3.86 (*s*, 3 H, OC(3)H_3); 2.92 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 168.45; 154.98; 149.88 (2x); 149.30; 147.54; 147.03; 123.59; 122.15 (2x); 114.09; 104.90; 102.91; 51.56; 45.81; 38.76 (2x). IR: 3419, 1687, 1596, 1571, 1457, 1410, 1363, 1251, 1230, 1205, 1148, 999, 967751, 726. HR-

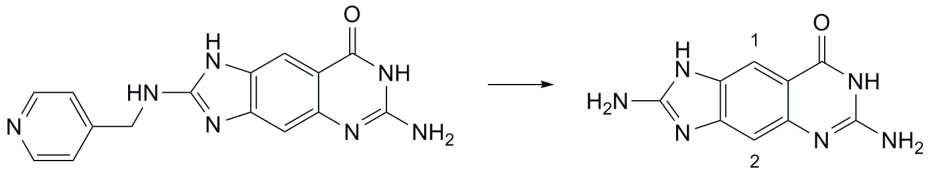
MALDI-MS (3-HPA): 405.1334 (47, [*M*+H]$^+$; $C_{17}H_{21}N_6O_4S^+$; calc. 405.1340). Anal. calc. for $C_{17}H_{20}N_4O_6S$ (404.45): C 50.49, H 4.98, N 20.78; found C 50.31, H 5.23, N 20.53.

6-Amino-2-[(pyridin-4-ylmethyl)amino]-1,7-dihydro-8*H*-imidazo[4,5-*g*]quinazolin-8-one (**34**)



General Procedure C started from **28** (109 mg, 0.27 mmol), chloroformamidinium chloride (15 mg, 0.13 mmol), and dimethyl sulfone (311 mg, 3.30 mmol) to yield crude **34** (38 mg) as an orange powder. HR-MALDI-MS (3-HPA): 308.1259 (100, [*M*+H]$^+$; $C_{15}H_{14}N_7O^+$; calc. 308.1254). Purification by preparative HPLC with water (0.1% TFA)/MeCN 95:5 → 0:100 led to cleavage of the pyridin-4-ylmethanamine moiety and therefore to the target molecule **3**.

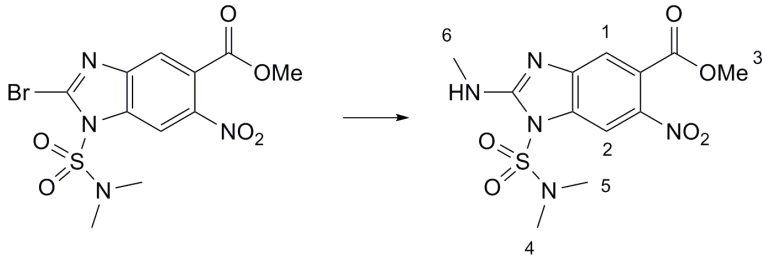
2,6-diamino-1,7-dihydro-8*H*-imidazo[4,5-*G*] quinazolin-8-one (2-Amino-*lin*-benzoguanine, **3**)



During purification of **34** (30 mg), for the biological investigation by preparative HPLC with water (0.1% TFA)/MeCN 95:5 → 0:100 the 4-(aminomethyl)pyridine moiety was cleaved off to yield crude **3** as a brown powder (27 mg). Purification by column chromatography on LiChroprep®NH_2 with MeOH/MeCN 1:1 afforded the target compound contaminated with LiChroprep®NH_2. Suspending the residue in DMF and filtration improved the purity, but did not yield the pure compound. Only preparative HPLC with water/MeCN (95:5 → 0:100, 30 mg) finally yielded 2-amino-*lin*-benzoguanine (**3**) (5 mg, 0.02 mmol). Off-white powder.

M.p. >290 °C (dec.). ^{1}H NMR (300 MHz, CD_3OD): 7.76 (*d*, *J* = 0.6, 1 H, C(1)H); 7.01 (*d*, *J* = 0.6, 1 H, C(2)H). ^{13}C NMR (125 MHz, CD_3OD): 161.34; 154.80; 152.95; 137.91; 137.53; 129.75; 113.03; 110.39; 100.41. IR: 3356, 2924, 2853, 1684, 1603, 1208, 1185, 1136, 802, 724. HR-MALDI-MS (3-HPA): 217.0832 (100, $[M+H]^+$; $C_9H_9N_6O^+$; calc. 217.0832).

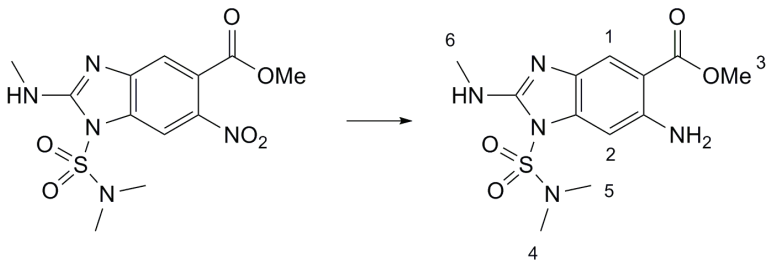
Methyl 1-[(dimethylamino)sulfonyl]-2-(methylamino)-6-nitro-1*H*-benzimidazole-5-carboxylate (**36**)



17b (350 mg, 0.86 mmol) was suspended in ethanolic $MeNH_2$ solution (2 mL, 33%, ~8M) and stirred at 25 °C for 10 min. The resulting red suspension was filtered, the residue washed with cold ethanol (5 mL) and dried under reduced pressure to yield pure **36** (124 mg, 0.35 mmol, 40%). The orange filtrate was evaporated under reduced pressure to yield a red oil that was subjected to column chromatography (silica gel; hexane/ethyl acetate 4:1 → ethyl acetate (1% TEA)) to afford more of the desired compound (121 mg, 0.34 mmol, 40%). Total yield: 80%. Pale yellow powder.

M.p. 146 °C. R_f (CH_2Cl_2/MeOH 10:1) 0.77. ^{1}H NMR (300 MHz, $CDCl_3$): 8.22 (*s*, 1 H, C(1)H); 7.52 (*s*, 1 H, C(2)H); 6.45 (*d*, *J* = 4.8, 1 H, NH); 3.93 (*s*, 3 H, OC(3)H_3); 3.18 (*d*, *J* = 5.1, 3 H, C(6)H_3); 2.97 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 166.80; 156.43; 146.17; 140.54; 131.92; 126.14; 116.17; 108.71; 53.28; 38.67 (2x); 30.11. IR: 3409, 2930, 1729, 1633, 1580, 1516, 1434, 1385, 1296, 1243, 1164, 1143, 1053, 1011, 965, 891, 745, 723. HR-MALDI-MS (3-HPA): 358.0807 (100, $[M+H]^+$; $C_{12}H_{16}N_5O_6S^+$; calc. 358.0816).

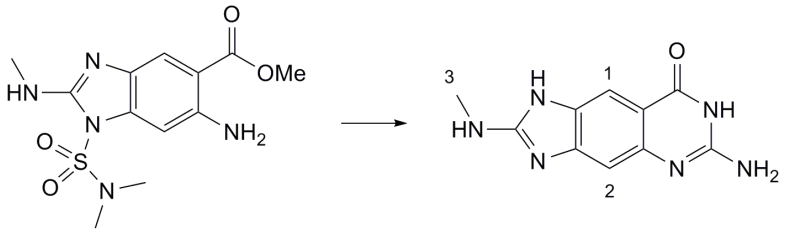
Methyl 6-amino-1-[(dimethylamino)sulfonyl]-2-(methylamino)-1*H*-benzimidazole-5-carboxylate (**38**)



General Procedure B started from **36** (124 mg, 0.35 mmol), zinc powder (227 mg, 3.47 mmol), and acetic acid/water (10 mL + 2 mL), 20 min, to yield, after purification by column chromatography (silica gel; CH_2Cl_2 (1% TEA)), **38** (113 mg, 0.35 mmol, quant.). Pale yellow solid.

M.p. 196 °C. R_f (CH_2Cl_2/MeOH 10:1) 0.65. ^{1}H NMR (300 MHz, $CDCl_3$): 7.90 (*s*, 1 H, C(1)H); 6.91 (*s*, 1 H, C(2)H); 5.95 (*d*, *J* = 4.8, 1 H, NH); 3.86 (*s*, 3 H, OC(3)H_3); 3.08 (*d*, *J* = 5.1, 3 H, C(6)H_3); 2.91 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 168.62; 152.88; 146.57; 136.98; 132.94; 118.35; 108.09; 99.62; 51.58; 38.71 (2x); 29.91. IR: 3438, 2953, 1686, 1642, 1582, 1459, 1363, 1293, 1205, 1159, 1132, 1016, 961, 847, 787, 753, 715. HR-MALDI-MS (3-HPA): 327.0991 (100, M^+; $C_{12}H_{17}N_5O_4S^+$; calc. 327.1001). Anal. calc. for $C_{12}H_{17}N_5O_4S$ (327.36): C 44.03, H 5.23, N 21.39; found C 44.23, H 5.32, N 21.38.

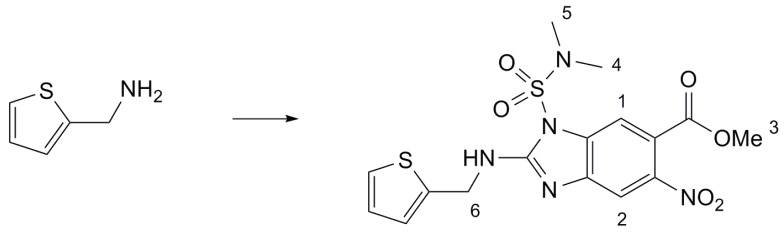
6-Amino-2-(methylamino)-1,7-dihydro-8*H*-imidazo[4,5-*g*]quinazolin-8-one (**4**)



General Procedure C started from **38** (183 mg, 0.56 mmol), chloroformamidinium chloride (129 mg, 1.12 mmol), and dimethyl sulfone (2.63 g, 27.95 mmol) to yield crude **4**, obtained as a pale brown powder (80 mg). Purification by column chromatography on LiChroprep®NH_2 with MeOH/MeCN 1:1 afforded the target compound contaminated with LiChroprep®NH_2. Suspending the residue in DMF and filtration improved the purity, but did not yield the pure compound. Only preparative HPLC with water/MeCN (95:5 → 0:100, 30 mg) finally yielded **4** (4 mg, 0.01 mmol, 8%). Pale yellow powder.

M.p. >250 °C (dec.). ^{1}H NMR (300 MHz, CD_3OD): 7.79 (*s*, 1 H, C(1)H); 7.03 (*s*, 1 H, C(2)H); 3.00 (*s*, 3 H, C(3)H_3). ^{13}C NMR (125 MHz, CD_3OD): 169.27; 159.91; 147.16; 146.88; 136.19; 132.18; 112.75; 107.40; 103.78; 29.51. IR: 3329, 3140, 1623, 1527, 1456, 1417, 1367, 1293, 880, 779. HR-MALDI-MS (3-HPA): 231.0987 (100, $[M+H]^+$; $C_{10}H_{11}N_6O^+$; calc. 231.0989).

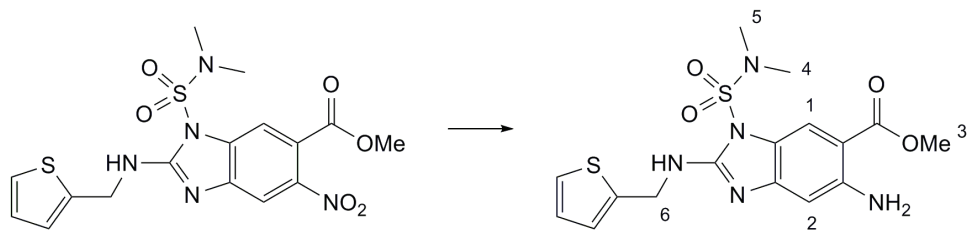
Methyl 1-[(dimethylamino)sulfonyl]-5-nitro-2-[(2-thienylmethyl)amino]-1*H*-benzimidazole-6-carboxylate (**22**)



General Procedure D started from **17a** (368 mg, 0.90 mmol) in ethyl acetate (4 mL) and 1-(2-thienyl)methanamine (512 mg, 4.52 mmol), 25 °C, 1 h, to yield, after filtration and purification by column chromatography (silica gel; hexane/ethyl acetate 4:1 → ethyl acetate), **22** (397 mg, 0.90 mmol, quant.). Yellow solid.

M.p. 103 °C. R_f (ethyl acetate) 0.73. ^{1}H NMR (300 MHz, $CDCl_3$): 7.91 (*s*, 1 H, C(1)H); 7.81 (*s*, 1 H, C(2)H); 7.22 (*dd*, *J* = 5.4, 1.2, 1 H, C(arom.)H); 7.78 (*dd*, *J* = 3.3, 1.2, 1 H, C(arom.)H); 6.95 (*dd*, *J* = 5.4, 3.3, 1 H, C(arom.)H), 6.71 (*t*, *J* = 5.7, 1 H, NH); 4.91 (*d*, *J* = 5.1, 2 H, C(6)H_2); 3.87 (*s*, 3 H, OC(3)H_3); 2.90 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (125 MHz, $CDCl_3$): 165.65; 154.56; 146.12 143.97; 139.32; 133.63; 126.84; 126.50; 125.44; 119.61; 113.01; 112.35; 53.12; 42.02; 38.69 (2x). IR: 2985, 1706, 1577, 1469, 1422, 1390, 1329, 1303, 1241, 1159, 1109, 947, 829, 747, 712. HR-MALDI-MS (3-HPA): 440.0701 (100, $[M+H]^+$; $C_{16}H_{18}N_5O_6S_2^+$; calc. 440.0693).

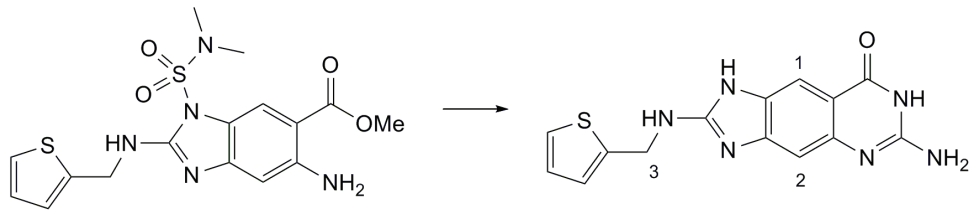
Methyl 5-amino-1-[(dimethylamino)sulfonyl]-2-[(2-thienylmethyl)amino]-1*H*-benzimidazole-6-carboxylate (**29**)



General Procedure B started from **22** (119 mg, 0.27 mmol), zinc powder (177 mg, 2.71 mmol), and acetic acid/water (7.5 mL + 1.5 mL), 15 min, to yield **29** (111 mg, 0.27 mmol, quant.). Pale brown solid, sufficiently pure for further use.

M.p. 195 °C. R_f (ethyl acetate) 0.65. ^{1}H NMR (300 MHz, $CDCl_3$): 8.03 (*s*, 1 H, C(1)H); 7.23 (*dd*, *J* = 5.0, 1.4, 1 H, C(arom.)H); 7.06-7.05 (*m*, 1 H, C(arom.)H); 6.96 (*dd*, *J* = 5.0, 3.5, 1 H, C(arom.)H); 6.65-6.20 (*m*, 2 H, C(2)H and NH); 4.89 (*d*, *J* = 5.4, 2 H, C(6)H_2); 3.85 (*s*, 3 H, OC(3)H_3); 2.87 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 168.31; 154.43; 149.04, 147.48; 139.79; 126.81; 126.27; 125.32; 123.57; 114.02; 104.71; 102.76; 51.54; 41.99; 38.80 (2x). IR: 3379, 2945, 1689, 1639, 1584, 1459, 1384, 1288, 1254, 1205, 1157, 967, 712. HR-MALDI-MS (3-HPA): 410.0943 (100, $[M+H]^+$; $C_{16}H_{20}N_5O_4S_2^+$; calc. 410.0950).

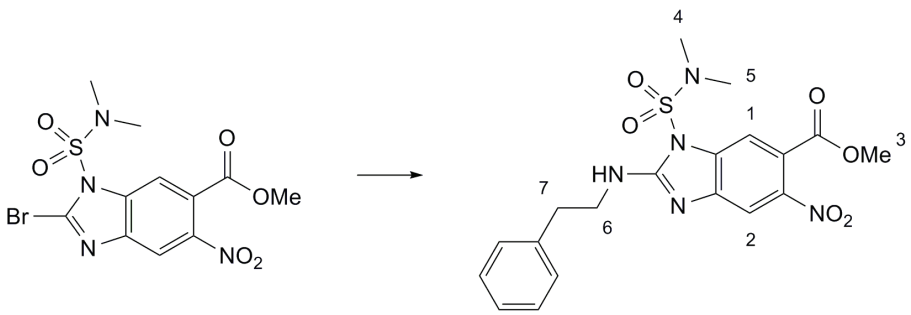
6-Amino-2-[(2-thienylmethyl)amino]-1,7-dihydro-8*H*-imidazo[4,5-*g*]quinazolin-8-one (**6**)



General Procedure C started from **29** (169 mg, 0.43 mmol), chloroformamidinium chloride (95 mg, 0.83 mmol), and dimethyl sulfone (1.94 g, 20.6 mmol) to yield the crude product, brown powder (118 mg). Purification by column chromatography on LiChroprep®NH_2 with methanol afforded the target compound contaminated with LiChroprep®NH_2. Suspending the residue in DMF and filtration improved the purity, but did not yield the pure compound. Only preparative HPLC with water/MeCN (95:5 → 0:100, 30 mg) finally yielded the pure *lin*-benzoguanine **6** (8 mg, 0.03 mmol, 24%). Pale yellow powder.

M.p. >250 °C (dec.). ^{1}H NMR (300 MHz, CD_3OD): 7.80 (*s*, 1 H, C(1)H); 7.29 (*dd*, *J* = 5.1, 1.2, 1 H, C(arom.)H); 7.08 (*dd*, *J* = 3.6, 1.2, 1 H, C(arom.)H); 7.07 (*s*, 1 H, C(2)H); 6.96 (*dd*, *J* = 5.3, 3.5, 1 H, C(arom.)H); 4.78 (*d*, *J* = 0.9, 2 H, C(3)H_2). ^{13}C NMR (125 MHz, CD_3OD/TFA 95:5): 159.88; 152.06; 151.07; 138.76; 136.68; 135.80; 128.58; 128.06; 127.96; 127.18; 111.80; 109.63; 99.56; 41.84. IR: 2920, 1610, 1568, 1515, 1446, 1361, 1285, 1226, 1074, 848, 785, 694. HR-MALDI-MS (3-HPA): 313.0867 (100, $[M+H]^+$; $C_{14}H_{13}N_6OS^+$; calc. 313.0866).

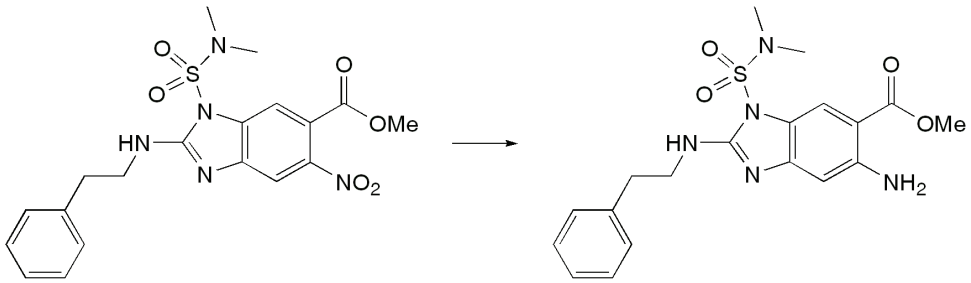
Methyl 1-[(dimethylamino)sulfonyl]-5-nitro-2-[(2-phenylethyl)amino]-1*H*-benzimidazole-6-carboxylate (**24**)



General Procedure D started from **17a** (950 mg, 2.33 mmol) in ethyl acetate (30 mL) and phenylethylamine (7.06 g, 58.3 mmol, 7.34 mL), 0 °C, 10 min, then hexane (150 mL) was added, and the solution was cooled to 4 °C for 16 h for crystallization. Filtration, washing with hexane, and purification by column chromatography (silica gel; hexane/ethyl acetate 3:1) yielded **24** (1.02 g, 2.29 mmol, quant.). Yellow solid.

M.p. 150 °C. R_f (ethyl acetate) 0.69. ^{1}H NMR (300 MHz, $CDCl_3$): 7.88 (*s*, 1 H, C(1)H), 7.81 (*s*, 1 H, C(2)H); 7.35-7.17 (*m*, 5 H, C(arom.)H); 6.35 (*t*, *J* = 5.4, 1 H, NH); 3.89 (*s*, 3 H, OC(3)H_3); 3.85 (*q*, *J* = 7.1, 2 H, C(6)H_2); 3.04 (*t*, *J* = 7.0, 2 H, C(7)H_2); 2.75 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.81; 154.99; 146.24; 144.33; 137.93; 133.55; 128.70 (2x); 128.59 (2x); 126.75; 119.49; 112.94; 112.18; 53.18; 44.33; 38.59 (2x); 34.95. IR: 3416, 3020, 2961, 2904, 1721, 1601, 1570, 1537, 1383, 1289, 1258, 1196, 1157, 1105, 1037, 963, 799, 745, 723, 698. HR-MALDI-MS (3-HPA): 448.1282 (100, $[M+H]^+$; $C_{19}H_{21}N_5O_6S^+$; calc. 448.1285).

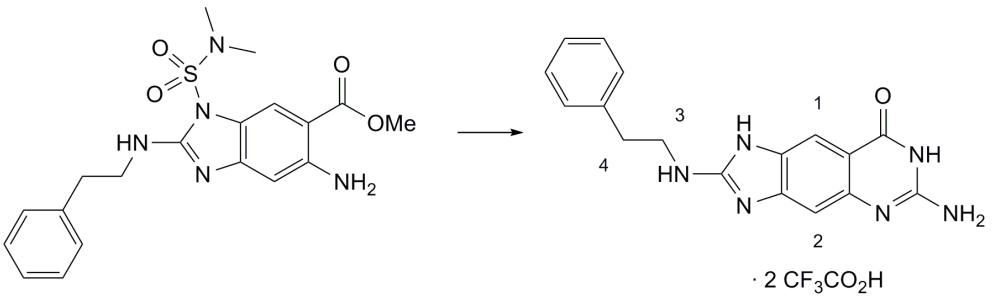
Methyl 5-amino-1-[(dimethylamino)sulfonyl]-2-[(2-phenylethyl)amino]-1*H*-benzimidazole-6-carboxylate (**31**)



General Procedure B started from **24** (1.03 g, 2.29 mmol), zinc powder (1.50 g, 22.9 mmol), and acetic acid/water (60 mL + 12 mL), 20 min, to yield, after purification by column chromatography (silica gel; hexane/ethyl acetate 3:1 → 1:1), **31** (830 mg, 1.99 mmol, 87%). Yellow solid.

M.p. 123 °C. R_f (ethyl acetate) 0.65. ^{1}H NMR (300 MHz, CD_3OD): 7.98 (*d*, *J* = 0.6, 1 H, C(1)H); 7.32-7.16 (*m*, 5 H, C(arom.)H); 6.64 (*d*, *J* = 0.6, 1 H, C(2)H); 3.83 (*s*, 3 H, OC(3)H_3); 3.74 (*t*, *J* = 7.3, 2 H, C(6)H_2); 3.00 (*t*, *J* = 7.3, 2 H, C(7)H_2); 2.72 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 168.35; 154.88; 149.11; 147.95; 138.20; 128.59 (2x); 128.55 (2x); 126.55; 123.50; 113.81; 104.30; 102.49; 51.46; 44.17; 38.62; 35.13. IR: 3486, 3409, 3378, 2953, 1683, 1586, 1455, 1433, 1371, 1255, 1236, 1187, 1152, 1106, 1030, 963, 834, 787, 718, 669. HR-MALDI-MS (3-HPA): 418.1529 (100, $[M+H]^+$; $C_{19}H_{23}N_4O_5S^+$; calc. 418.1544).

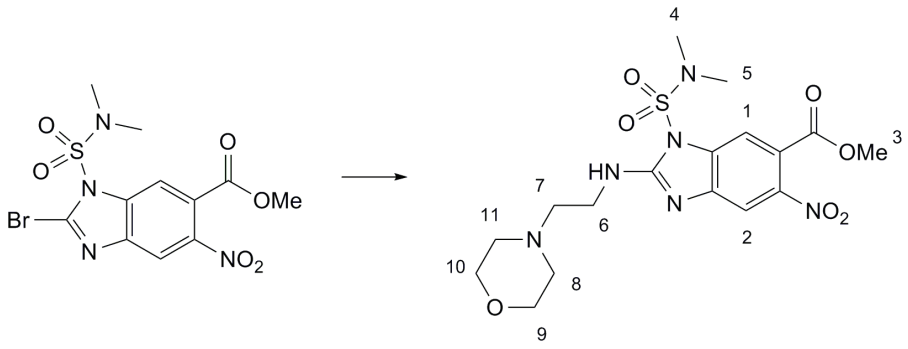
6-Amino-2-[(2-phenylethyl)amino]-8-oxo-7,8-dihydro-1*H*-imidazo[4,5-*g*]quinazolin-3-ium bis(trifluoroacetate) (**10**)



General Procedure C started from **31** (775 mg, 1.86 mmol), chloroformamidinium chloride (427 mg, 3.71 mmol), and dimethyl sulfone (8.74 g, 92.8 mmol) to yield the crude product, pale brown powder (497 mg). Preparative HPLC (50 mg) with water (0.1% TFA)/MeCN 95:5 → 0:100 yielded **10** as the bis-TFA salt (13.5 mg, 25 µmol, 13%). Pale yellow powder. Ion exchange to yield the bis-HCl salt was achieved using Amberlite® IRA-402 from Merck.

M.p. >250 °C (dec.). ^{1}H NMR (300 MHz, D_2O): 7.89 (*d*, *J* = 0.6, 1 H, C(1)H); 7.31-7.29 (*m*, 4 H, C(arom.)H); 7.23 (*d*, *J* = 0.6, 1 H, C(2)H); 7.20-7.16 (*m*, 1 H, C(arom.)H); 3.74 (*t*, *J* = 6.6, 2 H, C(3)H_2); 3.01 (*t*, *J* = 6.6, 2 H, C(4)H_2). ^{13}C NMR (125 MHz, CD_3OD/TFA 95:5): 173.57; 153.92; 152.93; 138.07; 137.96; 137.19; 129.93; 129.74 (2x); 129.69 (2x); 127.90; 112.64; 110.08; 99.99; 45.86; 36.04. IR: 3676, 2989, 2973, 2901, 2363, 2341, 1616, 1569, 1507, 1448, 1373, 1285, 1227, 1075, 1066, 1052, 891, 840, 783, 749, 696. HR-MALDI-MS (3-HPA): 321.1454 (100, $[M+H]^+$; $C_{17}H_{17}N_6O^+$; calc. 321.1458).

Methyl 1-[(dimethylamino)sulfonyl]-2-[(2-morpholin-4-ylethyl)amino]-5-nitro-1*H*-benzimidazole-6-carboxylate (**25**)

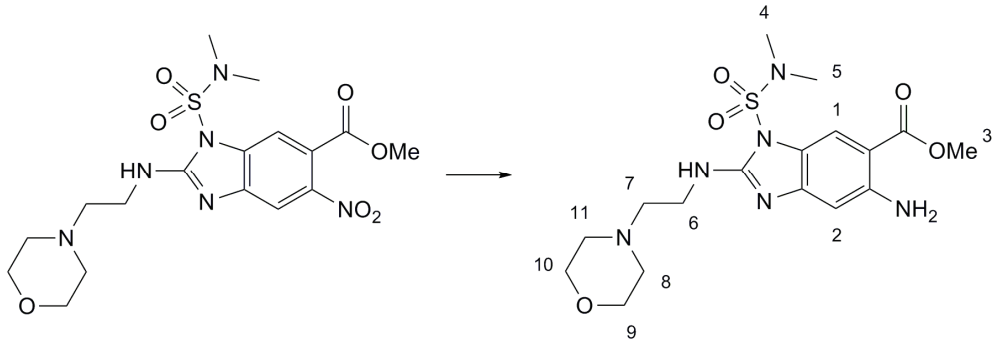


General Procedure D started from **17a** (650 mg, 1.60 mmol) in ethyl acetate (20 mL) and aminoethylmorpholine (5.21 g, 40 mmol, 5.26 mL), 0 °C, 10 min, then the solvent was evaporated under reduced pressure, hexane (150 mL) added and the solution cooled to 4 °C for 16 h for crystallization. Filtration, washing with hexane, and purification by column chromatography (silica gel; CH_2Cl_2/MeOH 99:1) yielded **25** (637 mg, 1.40 mmol, 87%). Lemon-yellow solid.

M.p. 145 °C. R_f (ethyl acetate) 0.55. ^{1}H NMR (300 MHz, $CDCl_3$): 7.91 (*s*, 1 H, C(1)H), 7.78 (*s*, 1 H, C(2)H); 7.11 (*t*, *J* = 4.5, 1 H, NH); 3.89 (*s*, 3 H, OC(3)H_3); 3.72 (*t*, *J* = 7.9, 4 H, C(9)H_2 and C(10)H_2); 3.62 (*q*, *J* = 5.7, 2 H, C(6)H_2); 2.96 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)); 2.67 (*t*, *J* = 6.2, 2 H, C(7)H_2); 2.52 (*t*, *J* = 4.7, 4 H, C(8)H_2 and C(11)H_2). ^{13}C NMR (75 MHz, $CDCl_3$): 165.52; 154.98; 145.90; 144.22; 133.61; 118.99; 112.66; 111.70; 66.76 (2x); 55.86; 52.96 (2x); 52.91; 39.25; 38.57 (2x). IR: 3410, 2955, 2841, 1737, 1631, 1571, 1530, 1448, 1383, 1348, 1291, 1263, 1206, 1158, 1117, 1054, 1021, 975,

895, 857, 784, 721, 665, 613. HR-MALDI-MS (3-HPA): 457.1493 (100, $[M+H]^+$; $C_{17}H_{24}N_6O_7S^+$; calc. 457.1500). Anal. calc. for $C_{17}H_{24}N_6O_7S$ (457.15): C 44.73, H 5.30, N 18.41; found C 44.69, H 5.32, N 18.24.

Methyl 5-amino-1-[(dimethylamino)sulfonyl]-2-[(2-morpholin-4-ylethyl)amino]-1*H*-benzimidazole-6-carboxylate (**32**)

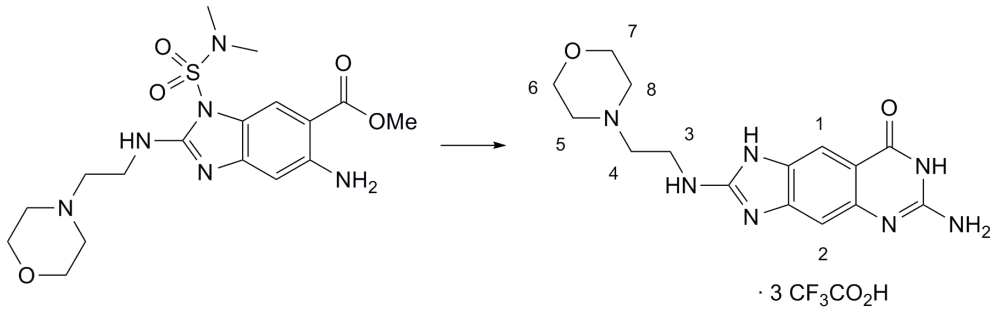


General Procedure B started from **25** (560 mg, 1.23 mmol), zinc powder (803 mg, 12.3 mmol), and acetic acid/water (30 mL + 6 mL), 20 min, to yield, after purification by column chromatography (silica gel; CH_2Cl_2/MeOH 99:1 → 97:3), **32** (502 mg, 1.18 mmol, 96%). Orange solid.

M.p. 144 °C. R_f (ethyl acetate) 0.49. ^{1}H NMR (300 MHz, $CDCl_3$): 8.02 (*s*, 1 H, C(1)H); 7.02 (br *s*, 1 H, NH); 6.60 (*s*, 1 H, C(2)H); 3.86 (*s*, 3 H, OC(3)H_3); 3.73 (*t*, *J* = 5.0, 4 H, C(9)H_2 and C(10)H_2); 3.61 (*q*, *J* = 5.6, 2 H, C(6)H_2); 2.92 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)); 2.66 (*t*, *J* = 6.2, 2 H, C(7)H_2); 2.52 (*t*, *J* = 4.7, 4 H, C(8)H_2 and C(11)H_2). ^{13}C NMR (75 MHz, $CDCl_3$): 168.30; 154.94; 149.16; 147.73; 123.42; 113.64; 104.01;

102.22; 66.60; 56.03 (2x); 52.88 (2x); 51.20; 39.02; 38.51 (2x). IR: 3462, 3391, 3353, 2952, 2895, 2814, 1676, 1580, 1455, 1376, 1355, 1298, 1275, 1235, 1184, 1145, 1112, 1046, 967, 915, 891, 834, 787, 763, 719, 669. HR-MALDI-MS (3-HPA): 427.1763 (100, $[M+H]^+$; $C_{17}H_{26}N_6O_5S^+$; calc. 427.1758).

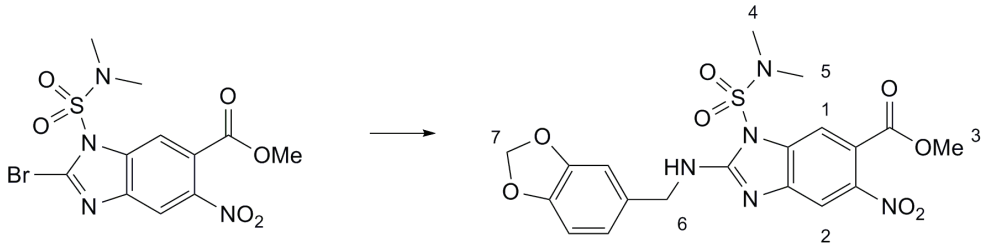
6-Amino-2-[(2-morpholin-4-ylethyl)amino]-8-oxo-7,8-dihydro-1*H*-imidazo[4,5-*g*]quinazolin-3-ium tris(trifluoroacetate) (**11**)



General Procedure C started from **32** (350 mg, 0.82 mmol), chloroformamidinium chloride (189 mg, 1.64 mmol), and dimethyl sulfone (3.86 g, 41.0 mmol), but the product was soluble in diluted NH_3 solution, therefore the liquid was evaporated at 80 °C, and dimethyl sulfone was removed under HV at 90 °C to give the crude product, pale brown powder (1409 mg). Purification of samples (550 mg) for the biological investigation was carried out by preparative HPLC (550 mg) with water (0.1% TFA)/MeCN 95:5 → 0:100 to yield **11** as the tris-TFA salt (69.1 mg, 0.10 mmol, 32%). White powder. Ion exchange to yield the tris-HCl salt was achieved using Amberlite® IRA-402 from Merck.

M.p. >250 °C (dec.). ^{1}H NMR (300 MHz, CD_3OD): 7.91 (*s*, 1 H, C(1)H); 7.26 (*s*, 1 H, C(2)H); 3.99 (*t*, *J* = 4.8, 4 H, C(6)H_2 and C(7)H_2); 3.84 (*t*, *J* = 5.7, 2 H, C(3)H_2), 3.43-3.38 (*m*, 6 H, C(4)H_2, C(5)H_2 and C(8)H_2). ^{13}C NMR (125 MHz, CD_3OD/TFA 95:5): 161.61; 152.38; 150.63; 136.60; 135.53; 128.46; 111.50; 110.00; 99.57; 63.86 (2x); 55.12; 52.43 (2x); 37.65. IR: 3679, 2989, 2972, 2901, 2360, 2344, 1682, 1465, 1437, 1394, 1191, 1129, 1079, 1066, 1052, 798, 835, 722. HR-MALDI-MS (3-HPA): 330.1673 (100, $[M+H]^+$; $C_{15}H_{19}N_7O_2^+$; calc. 330.1673).

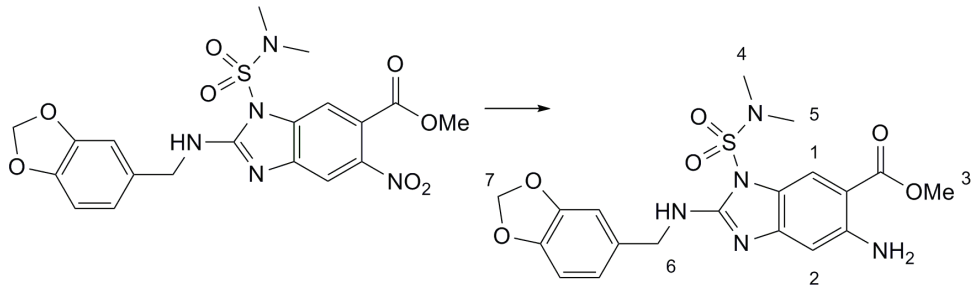
Methyl 2-[(1,3-benzodioxol-4-ylmethyl)amino]-1-[(dimethylamino)sulfonyl]-5-nitro-1*H*-benzimidazole-6-carboxylate (**23**)



General Procedure D started from **17a** (650 mg, 1.60 mmol) in ethyl acetate (20 mL) and piperonylamine (4.84 g, 32.0 mmol, 3.98 mL), 0 °C, 10 min, then hexane (150 mL) was added and the solution cooled to 4 °C for 16 h for crystallization. Filtration, washing with hexane, and purification by column chromatography (silica gel; heptane/ethyl acetate 4:1 → 2:1) yielded **23** (730 mg, 1.53 mmol, 96%). Lemon-yellow solid.

M.p. 176 °C. R_f (ethyl acetate) 0.59. ^{1}H NMR (300 MHz, $CDCl_3$): 7.91 (*s*, 1 H, C(1)H), 7.83 (*s*, 1 H, C(2)H); 6.86–6.77 (*m*, 3 H, C(arom.)H); 6.61 (*t*, *J* = 5.7, 1 H, NH); 5.96 (*s*, 2 H, C(7)H_2); 4.63 (*d*, *J* = 5.9, 2 H, C(6)H_2); 3.90 (*s*, 3 H, OC(3)H_3); 2.93 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.77; 154.98; 147.92; 147.28; 146.29; 144.2; 133.59; 130.65; 121.19; 119.63; 113.04; 112.35; 108.44; 108.23; 101.16; 53.23; 47.30; 38.81 (2x). IR: 3409, 2959, 1724, 1604, 1567, 1531, 1491, 1427, 1380, 1290, 1262, 1239, 1202, 1155, 1104, 1026, 995, 965, 933, 911, 869, 836, 806, 782, 748, 675. HR-MALDI-MS (3-HPA): 478.1020 (100, [*M*+H]$^+$; $C_{19}H_{19}N_5O_8S^+$; calc. 478.1027).

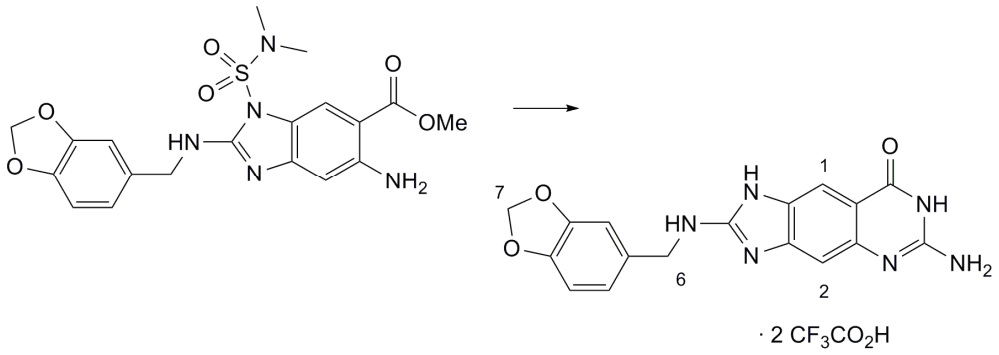
Methyl 5-amino-2-[(1,3-benzodioxol-4-ylmethyl)amino]-1-[(dimethylamino)sulfonyl]-1*H*-benzimidazole-6-carboxylate (**30**)



General Procedure B started from **23** (534 mg, 1.12 mmol), zinc powder (713 mg, 11.2 mmol), and acetic acid/water (60 mL + 12 mL), 20 min, to yield, after purification by column chromatography (silica gel; heptane/ethyl acetate 3:1 → 2:1), **30** (478 mg, 1.07 mmol, 96%). Faint orange solid.

M.p. 134 °C. R_f (ethyl acetate) 0.53. ^{1}H NMR (300 MHz, $CDCl_3$): 8.02 (*s*, 1 H, C(1)H); 6.86–6.76 (*m*, 3 H, C(arom.)H); 6.63 (*s*, 1 H, C(2)H); 6.56 (*t*, *J* = 5.9, 1 H, NH); 5.96 (*s*, 2 H, C(7)H_2); 5.72 (br *s*, 2 H, NH_2); 4.62 (*d*, *J* = 5.6, 2 H, C(6)H_2); 3.86 (*s*, 3 H, OC(3)H_3); 2.88 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 168.53; 155.07; 149.30; 148.02; 147.95; 147.22; 131.26; 123.66; 121.16; 114.01; 108.40; 108.30; 104.54; 102.71; 101.11; 51.48; 47.06; 38.75 (2x). IR: 3458, 3402, 3319, 2921, 1689, 1580, 1489, 1458, 1375, 1236, 1190, 1157, 1096, 1031, 965, 925, 804, 750, 718, 645. HR-MALDI-MS (3-HPA): 448.1280 (100, $[M+H]^+$; $C_{19}H_{21}N_5O_6S^+$; calc. 448.1285).

6-Amino-2-[(1,3-benzodioxol-4-ylmethyl)amino]-8-oxo-7,8-dihydro-1*H*-imidazo[4,5-*g*]quinazolin-3-ium bis(trifluoroacetate) (**7**)

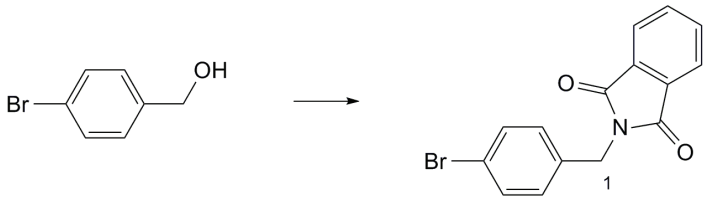


General Procedure C started from **31** (327 mg, 0.73 mmol), chloroformamidinium chloride (168 mg, 1.46 mmol), and dimethyl sulfone (3.44 g, 36.5 mmol), but the product was soluble in diluted NH_3 solution, therefore the liquid was evaporated at 80

°C, and dimethyl sulfone was removed under HV at 90 °C to give the crude product, pale brown powder (409 mg). Purification of samples (100 mg) for the biological investigation was carried out by preparative HPLC with water (0.1% TFA)/MeCN 95:5 → 0:100 yielded **7** as the bis-TFA salt (15.8 mg, 27 µmol, 15%). White powder.

M.p. >250 °C (dec.). ^{1}H NMR (300 MHz, D_2O): 7.95 (*s*, 1 H, C(1)H); 7.29 (*s*, 1 H, C(2)H); 6.88–6.83 (*m*, 3 H, C(arom.)H); 5.90 (*s*, 2 H, C(7)H_2); 4.53 (br *s*, 2 H, C(6)H_2). ^{13}C NMR (125 MHz, CD_3OD): 160.88; 153.63; 152.77; 149.77; 149.27; 137.88; 137.06; 130.32; 129.82; 122.25; 113.01; 110.43; 109.50; 109.03; 102.74; 100.17; 47.86. IR: 3691, 3399, 2989, 2973, 2902, 2363, 1692, 1652, 1485, 1458, 1447, 1374, 1257, 1234, 1102, 1078, 1035, 1012, 930, 882, 843, 775, 748, 668. HR-MALDI-MS (3-HPA): 351.1195 (100, $[M+H]^+$; $C_{17}H_{14}N_6O_3^+$; calc. 351.1200).

2-(4-Bromobenzyl)-1*H*-isoindole-1,3(2*H*)-dione (**48**)

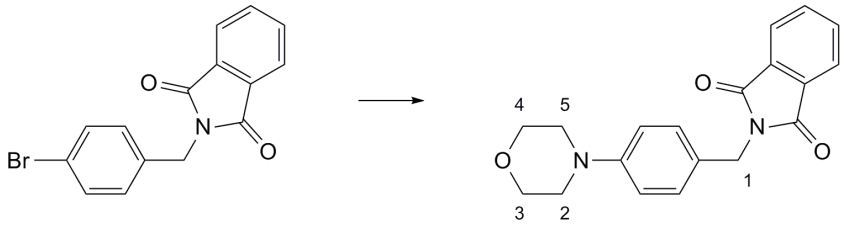


General Procedure E started from (4-bromophenyl)methanol (**47**, 1.87 g, 10 mmol), phthalimide (1.77 g, 12 mmol) and PPh_3 (3.15 g, 12 mmol), THF (70 mL) and DIAD (2.43 g, 12 mmol), 72 h, to yield, after purification by column chromatography (silica

gel; heptane/ethyl acetate 6:1 → 3:1), **48** (2.48 mg, 7.84 mmol, 78%). White powder.

M.p. 128 °C ([4]: 133 °C). ^{1}H NMR (300 MHz, $CDCl_3$): 7.85, 7.72 (*m*, AA`MM`, 4 H, C(arom.)H); 7.44 (*dt*, *J* = 8.7, 2.1, 2 H, C(arom.)H); 7.31 (*dt*, *J* = 8.7, 2.3, 2 H, C(arom.)H); 4.79 (*s*, 2 H, C(1)H_2). HR-EI-MS: 314.9888 (65, M^+; $C_{15}H_{10}BrNO_2^+$; calc. 314.9889).

2-(4-Morpholin-4-ylbenzyl)-1*H*-isoindole-1,3(2*H*)-dione (**49**)

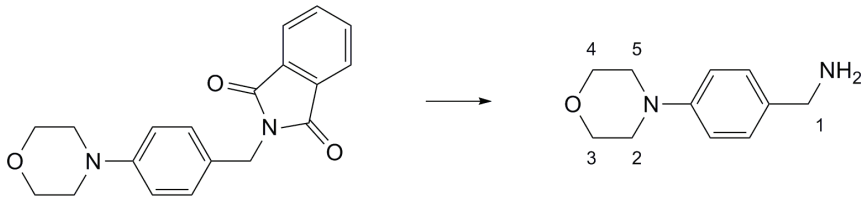


48 (1.50 g, 4.75 mmol), [$Pd_2(dba)_3$] (43.4 mg, 47.0 µmol) and biphenyl-2-yldicyclohexylphosphine (39.9 mg, 0.114 mmol) were placed in an oven-dried Schlenk tube under argon. Morpholine (496 mg, 5.69 mmol) and $LiN(TMS)_2$ (1M in THF, 10.4 mL) were added, giving a deep wine-red color. The tube was sealed and the reaction stirred and heated to 65 °C for 24 h. Then it was cooled to 25 °C, 1M HCl (0.5 mL) was added, and stirring at 25 °C was continued for 5 min. After neutralization with aqueous saturated $NaHCO_3$ solution, the mixture was diluted and extracted with ethyl acetate (4x 10 mL). The combined organic phases were dried over $MgSO_4$, and ethyl acetate was removed under reduced pressure to yield, after purification by column

chromatography (silica gel; heptane/ethyl acetate 3:1), **49** (311 mg, 0.965 mmol, 22%). Yellow solid.

M.p. 180 °C. R_f (ethyl acetate) 0.75. ^{1}H NMR (300 MHz, $CDCl_3$): 7.83, 7.69 (*m*, AA`MM`, 4 H, C(arom.)H); 7.37 (*dt*, *J* = 8.7, 1.4, 2 H, C(arom.)H); 6.84 (*dt*, *J* = 9.3, 2.3, 2 H, C(arom.)H); 4.77 (*s*, 2 H, C(1)H_2); 3.83 (*t*, *J* = 5.0, 4 H, C(3)H_2 and C(4)H_2); 3.12 (*t*, *J* = 5.0, 4 H, C(2)H_2 and C(5)H_2). ^{13}C NMR (75 MHz, $CDCl_3$): 168.09; 150.84; 133.88 (2x); 132.17; 129.87 (2x); 127.76; 123.24 (2x); 115.55 (2x); 66.82 (2x); 49.10 (2x); 41.04. IR: 2975-2841 (5x), 1764, 1712, 1614, 1521, 1428, 1393, 1329, 1264, 1243, 1215, 1184, 1121, 1085, 929, 858, 801, 743, 714, 609. HR-MALDI-MS (3-HPA): (3-HPA): 323.1386 (100, $[M+H]^+$; $C_{19}H_{18}N_2O_3^+$; calc. 323.1390).

1-(4-morpholin-4-ylphenyl)methanamine (**50**)

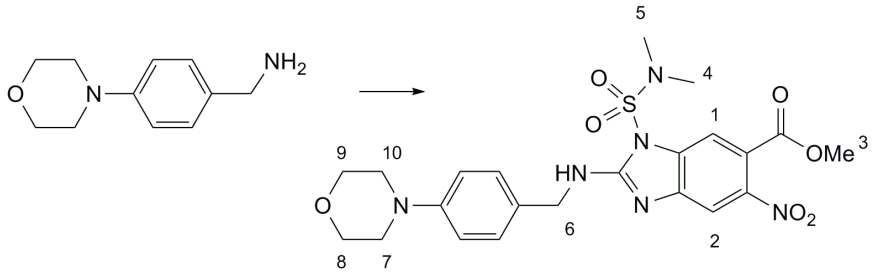


General Procedure F started from **49** (300 mg, 0.93 mmol) and 33% $MeNH_2$ in EtOH (15 mL), 16 h, to yield **50** (80 mg, 0.42 mmol, 45%). Colorless solid, sufficiently pure for further use.

M.p. 98 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.22 (*dt*, *J* = 9.0, 2.4, 2 H, C(arom.)H); 6.88 (*dt*, *J* = 9.0, 2.4, 2 H, C(arom.)H); 3.87-3.83 (*m*, 4 H, C(3)H_2 and C(4)H_2); 3.79 (*s*, 2 H, C(1)H_2); 3.14-3.11 (*m*, 4 H, C(2)H_2 and C(5)H_2); 2.95 (br *s*, 2 H, NH_2). HR-

MALDI-MS (3-HPA): 193.1335 (19, $[M+H]^+$; $C_{11}H_{16}N_2O^+$; calc. 193.1335).

Methyl 1-[(dimethylamino)sulfonyl]-2-[(4-morpholin-4-ylbenzyl)amino]-5-nitro-1*H*-benzimidazole-6-carboxylate (**26**)

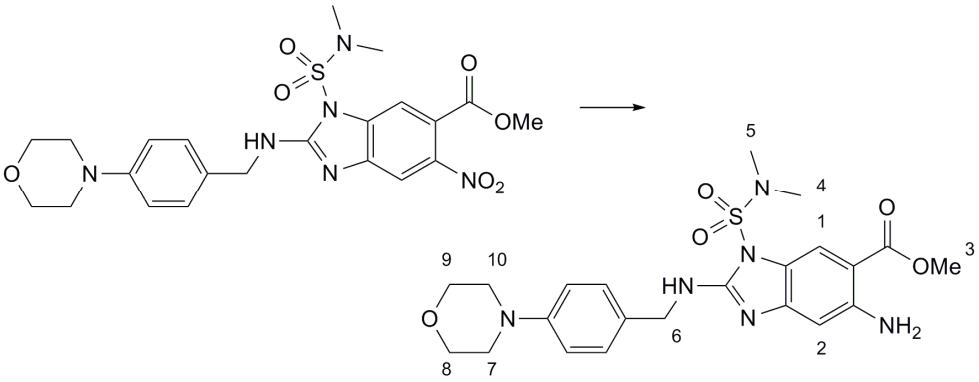


General Procedure D started from **17a** (318 mg, 0.78 mmol) in chloroform (10 mL), **50** (100 mg, 0.52 mmol), and TEA (105 mg, 1.04 mmol) in chloroform (30 mL), 0 °C, 16 h, to yield, after purification by column chromatography (silica gel; heptane/ethyl acetate 4:1), **26** (155 mg, 0.299 mmol, 63%). Yellow solid.

M.p. 190 °C. R_f (ethyl acetate) 0.44. ^{1}H NMR (300 MHz, $CDCl_3$): 7.92 (*s*, 1 H, C(1)H); 7.83 (*s*, 1 H, C(2)H); 7.27 (*d*, *J* = 8.7, 2 H, C(arom.)H); 6.90 (*d*, *J* = 9.0, 2 H, C(arom.)H); 6.59 (*t*, *J* = 6.0, 1 H, NH); 4.65 (*d*, *J* = 6.0, 2 H, C(6)H_2); 3.90 (*s*, 3 H, OC(3)H_3); 3.86 (*t*, *J* = 5.0, 4 H, C(8)H_2 and C(9)H_2); 3.16 (*t*, *J* = 5.0, 4 H, C(7)H_2 and C(10)H_2); 2.92 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 165.97; 155.21; 151.06; 146.45; 144.48; 133.71; 128.91 (2x); 128.01; 119.61; 115.76 (2x); 113.09; 112.33; 66.78 (2x); 53.15; 49.05 (2x); 46.95; 38.72 (2x). IR: 3414, 2954, 2852, 1731, 1630, 1601, 1561, 1535, 1445, 1378, 1347, 1290, 1243, 1211, 1151,

1125, 1090, 1058, 1024, 973, 924, 890, 808, 764, 734, 657, 619. HR-MALDI-MS (3-HPA): 519.1647 (17, $[M+H]^+$; $C_{22}H_{26}N_6O_7S^+$; calc. 519.1656).

Methyl 5-amino-1-[(dimethylamino)sulfonyl]-2-[(4-morpholin-4-ylbenzyl)amino]-1*H*-benzimidazole-6-carboxylate (**33**)

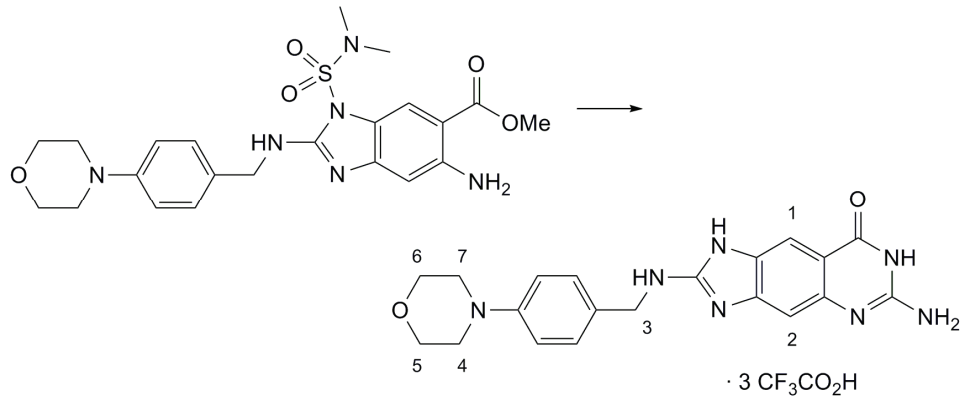


General Procedure B started from **26** (133 mg, 0.26 mmol), zinc powder (167 mg, 2.56 mmol), and acetic acid/water (7.5 mL + 1.5 mL), 20 min, to yield, after purification by column chromatography (silica gel; heptane/ethyl acetate 3:1), **33** (121 mg, 0.248 mmol, 97%). Orange solid.

M.p. 190 °C. R_f (ethyl acetate) 0.38. ^{1}H NMR (300 MHz, $CDCl_3$): 8.02 (*s*, 1 H, C(1)H); 7.28 (*d*, *J* = 9.3, 2 H, C(arom)H); 6.89 (*dt*, *J* = 9.0, 2.0, 2 H, C(arom.)H); 6.64 (*s*, 1 H, C(2)H); 6.54 (*t*, *J* = 5.4, 1 H, NH); 5.72 (br *s*, 2 H, NH_2); 4.63 (*d*, *J* = 5.6, 2 H, C(6)H_2); 3.86 (*s*, 3 H, OC(3)H_3); 3.86 (*t*, *J* = 5.0, 4 H, C(8)H_2 and C(9)H_2); 3.15 (*t*, *J* = 5.1, 4 H, C(7)H_2 and C(10)H_2); 2.86 (*s*, 6 H, N(C(4)H_3 and C(5)H_3)). ^{13}C NMR (75 MHz, $CDCl_3$): 168.53; 155.11; 150.88; 149.29; 148.10; 128.82 (2x);

128.55; 123.64; 115.75 (2x); 113.97; 104.42; 102.62; 66.77 (2x); 51.43; 49.12 (2x); 46.75; 38.73 (2x). IR: 3491, 3414, 3380, 2953, 1690, 1576, 1519, 1455, 1360, 1243, 1181, 1151, 1123, 1076, 1031, 959, 923, 849, 820, 788, 720, 710, 619. HR-MALDI-MS (3-HPA): 489.1910 (46, $[M+H]^+$; $C_{22}H_{28}N_6O_5S^+$; calc. 489.1915).

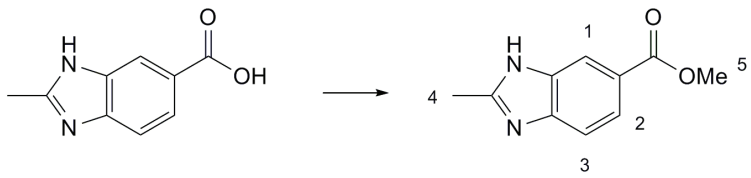
6-Amino-2-[(4-morpholin-4-ylbenzyl)amino]-8-oxo-7,8-dihydro-1*H*-imidazo[4,5-*g*]quinazolin-3-ium tris(trifluoroacetate) (**12**)



General Procedure C started from **33** (110 mg, 0.23 mmol), chloroformamidinium chloride (51.8 mg, 0.45 mmol), and dimethyl sulfone (1.06 g, 11.3 mmol), but the product was soluble in diluted NH_3 solution, therefore the liquid was evaporated at 80 °C, and dimethyl sulfone was removed under HV at 90 °C to give the crude product, purple solid (250 mg). Purification by preparative HPLC (100 mg) with water (0.1% TFA)/MeCN 95:5 → 0:100 yielded **12** as the tris-TFA salt (3.8 mg, 5 µmol, 6%). White powder.

M.p. >250 °C (dec.). ^{1}H NMR (300 MHz, D_2O): 8.00 (*s*, 1 H, C(1)H); 7.59-7.55 (*m*, 4 H, C(arom.)H); 7.34 (*s*, 1 H, C(2)H); 4.85 (*s*, 2 H, C(3)H_2); 4.08 (*t*, *J* = 4.8, 4 H, C(5)H_2 and C(6)H_2); 3.65-3.62 (*m*, 4 H, C(4)H_2 and C(7)H_2). ^{13}C NMR (125 MHz, CD_3OD): 163.43; 155.20; 153.44; 153.18; 131.06 (2x); 130.74; 129.79; 117.17; 116.88 (2x); 114.58; 113.16; 109.63; 102.02; 67.93 (2x); 50.84 (2x); 42.24. IR: 3676, 2989, 2972, 2901, 2362, 2341, 1676, 1643, 1559, 1473, 1458, 1407, 1394, 1287, 1257, 1230, 1103, 1075, 1066, 1052, 763, 669. HR-MALDI-MS (3-HPA): 392.1824 (81, $[M+H]^+$; $C_{20}H_{21}N_7O_2^+$; calc. 392.1830).

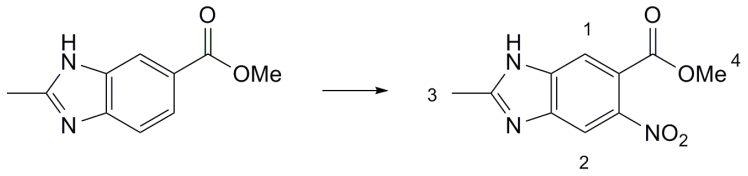
Methyl 2-methyl-1*H*-benzimidazole-5-carboxylate (**40**)



General Procedure A started from 1*H*-benzimidazole-5-carboxylic acid (**39**, 1.76 g, 10.0 mmol), $SOCl_2$ (5.95 g, 3.65 mL, 50 mmol) and dry methanol (50 mL), 16 h, to yield **40** (1.90 g, 10.0 mmol, quant.). Silvery powder, sufficiently pure for further use.

M.p. 170 °C. ^{1}HNMR (300 MHz, $(CD_3)_2SO$): 8.26 (*d*, *J* = 0.9, 1 H, C(1)H); 8.06 (*dd*, *J* = 8.7, 1.2, 1 H, C(2)H); 7.85 (*d*, *J* = 8.4, 1 H, C(3)H); 3.89 (*s*, 3 H, OC(5)H_3); 2.81 (*s*, 3 H, C(4)H_3). HR-ESI-MS: 190.0739 (66, M^+; $C_{10}H_{10}N_2O_2^+$; calc. 190.0742).

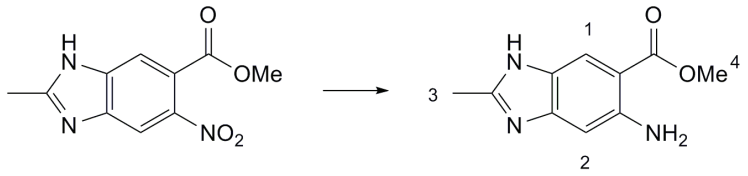
Methyl 2-methyl-5-nitro-1*H*-benzimidazole-6-carboxylate (**41**).



65% HNO_3 (3 mL) was cooled to 0 °C, and conc. H_2SO_4 (3 mL) was added carefully. This colorless liquid was added to **40** (1.70 g, 8.93 mmol) which was also cooled to 0 °C. This yellow suspension was allowed to warm to 25 °C and then heated to 50 °C for 16 h. Pouring the orange reaction mixture onto ice yielded, after addition of a few drops aqueous NaOH (1M), a white precipitate that was filtered off and washed with water. The residue was dissolved in water/ethyl acetate (5 mL + 5 mL), extracted with ethyl acetate (3 x 10 mL), and the combined organic layers dried with $MgSO_4$, and concentrated under reduced pressure to yield **41** (1.04 g, 4.42 mmol, 49%). Pale yellow foam, sufficiently pure for further use.

M.p. >230 °C (dec.). ^{1}H NMR (300 MHz, $CDCl_3$): 8.01 (*s*, 1 H, C(1)H); 7.76 (*s*, C(2)H); 3.84 (*s*, 3 H, OC(4)H_3); 2.68 (*s*, 3 H, C(3)H_3). ^{13}C (75 MHz, $CDCl_3$): 166.95; 157.75; 143.28; 140.49; 139.29; 121.98; 115.48; 111.76; 53.35; 15.21. IR: 2960, 1726, 1629, 1531, 1337, 1315, 1098, 982, 891, 829, 774, 750. HR-EI-MS: 235.0590 (46, M^+; $C_{10}H_9N_3O_4^+$; calc. 235.0593).

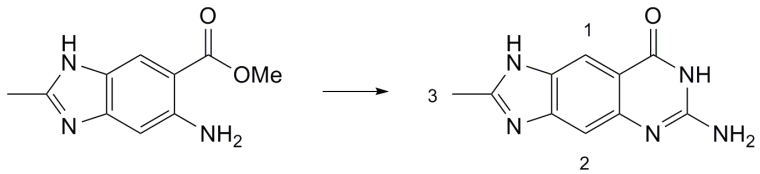
Methyl 5-amino-2-methyl-1*H*-benzimidazole-6-carboxylate (**42**)



General Procedure B started from **41** (1.00 g, 4.26 mmol), zinc powder (2.78 g, 42.6 mmol), and acetic acid/water (125 mL + 25 mL), 1 h, to yield **42** (0.79 g, 3.85 mmol, 90%). Light yellow foam, sufficiently pure for further use.

M.p. 158 °C. ^{1}H NMR (300 MHz, $(CD_3)_2SO$): 7.88 (*s*, 1 H, C(1)H); 6.73 (*s*, C(2)H); 6.34 (br *s*, NH_2); 3.78 (*s*, 3 H, OC(4)H_3); 2.41 (*s*, 3 H, C(3)H_3). ^{13}C NMR (75 MHz, $CDCl_3$): 168.13; 152.84; 147.31; 142.99; 132.98; 117.81; 105.45; 51.14; 14.56. IR: 3369, 2922, 2845, 2694, 1695, 1650, 1588, 1435, 1411, 1398, 1296, 1244, 1194, 1075, 1021, 889, 807, 783, 663. HR-ESI-MS: 206.0925 (100, $[M+H]^+$; $C_{10}H_{12}N_3O_2^+$; calc. 206.0930).

6-Amino-2-methyl-1,7-dihydro-8*H*-imidazo[4,5-g]quinazolin-8-one (2-Methyl-*lin*-benzoguanine, **2**)



General Procedure C started from **42** (790 mg, 3.85 mmol), chloroformamidinium chloride (665 mg, 5.78 mmol), and dimethyl sulfone (9.00 g, 96.0 mmol) to yield crude 2-methyl-*lin*-benzoguanine (457 mg), brown powder. Purification of samples for the biological investigation was carried out by

preparative HPLC with water/MeCN 95:5 → 0:100 to yield **2** (11 mg, 0.05 mmol, 20%). White powder.

M.p. >280 °C (dec.). ^{1}H NMR (300 MHz, CD_3OD): 8.17 (*d*, *J* = 0.6, 1 H, C(1)H); 7.29 (*d*, *J* = 0.6, 1 H, C(2)H); 2.58 (*s*, 3 H, C(3)H_3). ^{13}C NMR (125 MHz, CD_3OD/$(CD_3)_2SO$ 95:5): 157.28 (2x); 154.13; 144.43; 137.71; 131.23; 123.22; 113.20; 104.92; 14.78. IR: 3110, 1645, 1609, 1571, 1435, 1360, 1293, 1015, 783, 611. HR-ESI-MS: 216.0884 (100, $[M+H]^+$; $C_{10}H_{10}N_5O^+$; calc. 216.0885).

Crystal structure of 16b (see Figure 3SI)

Crystal data at 223 K for $C_{11}H_{12}N_4O_6S$, M_r = 328.303, triclinic, space group $P\bar{1}$, D_x = 1.553 Mg m^{-3}, Z = 2, a = 6.5663 (2)Å, b = 10.0354 (3)Å, c = 11.4088 (4)Å, α = 74.2398 (14)°, β = 77.1872 (14)°, γ = 81.373 (2)°, V = 702.28 (4)$Å^3$. Bruker-Nonius Kappa-CCD diffractometer, MoK_α radiation, λ = 0.71073, μ = 0.268 mm^{-1}. Light-yellow cube-like crystal of **16b** with linear dimensions of ca. 0.46× 0.26 × 0.16 mm. Numbers of measured and unique reflections are 5431 and 3207, respectively (R_{int} = 0.023). The structure was solved by direct methods [5] and refined by full-matrix least-squares analysis (SHELXL-97)[6] using an isotropic extinction correction. All non H-atoms were refined anisotropically; H-atoms were refined isotropically, whereby H-positions are based on stereochemical considerations. Final $R(F)$ = 0.0477 $wR(F^2)$ = 0.1428 for 248 parameters and 3207 reflections with $I > 2\sigma(I)$ and 0.998° < θ < 27.485°.

CCDC-648493 (**7**) contains the supplementary crystallographic data (excluding structure factors) for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax:+44(1223)-336-033; e-mail: deposit@ccdc.cam.ac.uk), or via www.ccdc.cam.ac.uk/ data_request/cif.

Trapping experiment

To distinguish pure competitive from non-competitive inhibitors, an initial trapping experiment, consisting on the formation of a covalent adduct between tRNA and TGT, followed by SDS-PAGE was performed with each inhibitor. [1] 5 μM *Z. mobilis* TGT, 100 μM *E. coli* $tRNA^{Tyr}$, and 1 mM of the respective inhibitor (dissolved in Me_2SO) in 10 μL of 100 mM HEPES buffer, pH 7.3, 20 mM $MgCl_2$, and 5 mM dithiothreitol (DTT) were incubated for 1 h at 25°. A total of 10 μL SDS loading buffer was added and incubated for another 1 h at 25°. 10 μL of each sample were loaded onto a 15% SDS gel. After electrophoresis, gels were stained with Coomassie blue.

Inhibition constants for pure competitive inhibition

The inhibition assay was performed using 150 nM TGT, 20 μM guanine/[8-^{3}H]-guanine, and tRNA at two concentrations (1 μM and 1.5 μM). [1] Six reaction mixtures for each tRNA concentration were prepared. To five of them, inhibitor dissolved in Me_2SO (5% final volume) at variable concentrations was added. Initial velocities for the reaction mixtures were determined, and K_i determination was performed using *Dixon* plots. As V_{max} determination is only possible with limited accuracy for independent measurements, the modified equation (1) published by Grädler *et al.* [7] was used to calculate K_i. Linear regression of data points derived from this equation

with *GraFit* [8] resulted in a straight line with the slope $1/K_i$.

$$\frac{v_0}{v_i} \cdot \frac{K_m + [S]}{K_m} = \frac{1}{K_i} \cdot [I] + \left(\frac{[S]}{K_m} + 1 \right) \quad (1)$$

Defined in equation 1 are v_0 initial velocity at given [S] concentration in the absence of inhibitor, v_i initial velocity at given [S] concentration in the presence of inhibitor, [S] tRNA concentration, [I] inhibitor concentration, K_m Michaelis-Menten constant of tRNA, K_i competitive inhibition constant.

TGT Crystal Data

A) Growing of crystals

TGT crystals suitable for ligand soaking were produced in a two-step procedure. Droplets were prepared by mixing 2 µL of concentrated protein solution (14 mg/mL TGT in high-salt-buffer) with 2 µL reservoir solution of the seeding buffer (S-buffer). Micro-crystals were grown at 273 K using the hanging-drop, vapour diffusion method in the presence of 1 mL of reservoir solution of the respective seeding buffer. Micro-crystals of 0.05 mm^3 grew within two weeks.

S-buffer pH 5.5 100 mM morpholino ethylsulfonate (MES), pH 5.5, 1 mM DTT, 8 % (w/v) PEG 8.000, 10 % (v/v) Me_2SO

Subsequently macro-seeding was performed under similar conditions. Again droplets were prepared by mixing 2 µL of concentrated protein solution with 2 µL reservoir solution of

the macro-seeding buffer (MS/CC-buffer). One micro-crystal was transferred into this solution. Single crystals with a size of approximately 0.7 x 0.7 x 0.2 mm^3 grow within two to four weeks per droplet. Small sized compounds were dissolved in Me_2SO and added to the droplet to a final concentration of 2 mM to allow soaking. Crystals were soaked at 293 K for one day.

MS/CC-buffer pH 5.5 100 mM MES, pH 5.5, 1 mM DTT, 5 % (w/v) PEG 8.000, 10 % (v/v) Me_2SO

Cocrystallization was performed under similar conditions. Droplets were prepared by mixing 2 µL of concentrated protein solution with 2 µL reservoir solution of the co-crystallization buffer (MS/CC-buffer). Additionally, small sized compounds were dissolved in Me_2SO and immediately added to the droplet to a final concentration of 2 mM. One micro-crystal was transferred into this solution. Single crystals with a size of approximately 0.7 x 0.7 x 0.2 mm^3 grow within two to four weeks per droplet.

B) Data collection

For data collection, crystals were cryoprotected using glycerol; 4 µL of the crystallization droplet were well mixed with 2.2 µL of glycerol resulting in a 35 % glycerol solution. The soaked crystal was transferred for 10 seconds into this solution and subsequently flash-frozen in liquid N_2. Data sets were collected at cryo conditions (100 K) with CuK_α radiation.

(λ = 1.5418 Å) using a Rigaku RU-300 rotating-anode generator at 50 kV and 90 mA equipped with either focusing mirrors (MSC, USA) and a R-AXIS IV + + image-plate system or with Xenocs focusing optics and a R-AXIS IV detector. The tested crystals exhibit monoclinic symmetry in space group *C*2 containing one monomer per asymmetric unit with Matthews coefficients of 2.3 - 2.4. All data processing and scaling were performed using the HKL2000 package [9].

C) Structure determination and refinement

For *C*2 crystals grown at pH 5.5 or coordinates of the apo TGT crystal structure grown at a pH of 5.5, (PDB-code: 1P0D) were directly applied for initial rigid-body refinement of the protein molecule followed by repeated cycles of conjugate gradient energy minimization, simulated annealing and *B*-factor refinement using the CNS program package [10]. Refinement at the later stages for all structures was performed with SHELXL [6]. Here, up to 20 cycles of conjugate gradient minimization were performed with default restraints on bonding geometry and *B*-values: 5 % of all data were used for R_{free} calculation. Amino acid side-chains were fit to σA-weighted 2 |*F*o| - |*F*c| and |*F*o| - |*F*c| electron density maps using O [11]. Water and glycerol molecules as well as the ligand were located in the difference electron density and added to the model for further refinement cylces. During the last refinement cycles, riding H-atoms were introduced for the protein residues (not for

ligand) without using additional parameters. All final models were validated using PROCHECK [12].

D) Crystal data TGT · 8 (pH 5.5)

A. *Data collection and processing*

No. crystals used	1
Wavelength (Å)	1.5418
Space group	*C*2
Unit cell parameters	
a (Å)	89.87
b (Å)	64.97
c (Å)	70.94
® (deg.)	93.20

B. *Diffraction data*

Resolution range (Å)	20–1.95 (2.00–1.95)
Unique reflections	29,427
R(I)sym (%)	8.2 (43.5)
Completeness (%)	98.8 (97.8)
Redundancy	2.4 (2.4)
I/σ(I)	10.9 (2.0)

C. *Refinement*

Program used for refinement	SHELXL
Resolution range (Å)	10–1.95
Reflections used in refinement	27,447/1,336
Final *R* values	
R_{free} (%)	25.9

R_{work} (%)	19.8
No. of atoms (non-hydrogen)	
Protein atoms	2,796
Water molecules	187
Ligand atoms	27
RMSD, angle (deg.)	2.0
RMSD, bond (Å)	0.006
Ramachandran plot	
most favored regions (%)	94.4
additionally allowed regions (%)	5.3
generously allowed regions (%)	0.3
Mean *B*-factors (Å^2)	
Protein atoms	28.2
Water molecules	32.4
Ligand atoms	59.5(40.6; 86.9)

pK_a Determination and Data Analysis

A) pK_a Determination by photometric titration [13]

The pK_a values (reproducibility: ±0.05 units) were determined by photometric titrations using D-PAS combined with *GLpK_a from Sirius Analytical Instruments Ltd.* D-PAS uses a deuterium UV source coupled with a diode array detector to provide multi-wavelength spectral data as a function of p*H*. In the applied photometric method, a solution of the sample is titrated over a p*H* range in which it passes from being fully protonated to fully deprotonated. The p*K*a values were calculated from the changes in UV absorbance of the samples as a function of p*H*. Absorbance changes with p*H* because the sample converts from an ionized species to an unionized species with different UV absorption. General titration procedure: An exact amount of the sample is placed directly into the vial. The instrument adds 0.15 M KCl (20 cm^3) as background electrolyte. It then checks the p*H* of the sample solution and adds 0.5 M HCl to bring the p*H* down to the initial p*H* 2. It then titrates with standardized base solution (0.5 M KOH), and multi-wavelength UV spectra were collected after each p*H* adjustment. The titration is running under Ar atmosphere to minimize absorption of atmospheric CO_2 because of the pK_a values of carbonate. The data set is downloaded to the computer, and D-PAS calculation uses Principal Component Analysis and matrix algebra to separate signal from noise and to resolve the data

into absorption of each species vs. wavelength and distribution of species vs. p*H*.

Table 1SI. pK_a Values measured by photometric titrations

Compound	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}	pK_{a5}
1	3.3	5.2	10.1	>12	-
2	3.8	5.4	10.1	>12	-
3	4.5	6.3	10.3	>12	-
4	4.4	6.2	10.2	>12	-
6	4.3	5.8	10.3	>12	-
8	4.2	5.9	10.4	>12	-
11	3.8	5.3	6.7	10.3	>12

The assignment of the individual pK_a values is made by comparison with already investigated molecular fragments such as *N*-methylmorpholine [14], 2-methyl- and 2-aminobenzimidazole [15], and 2-(benzylamino)benzimidazole [16]. From these literature values, *N*-methylmorpholine having a pK_a of 7.2, which is 0.3 units lower than the value of 2-aminobenzimidazole (7.5), it could be concluded, that in molecule **11**, where both entities are present, the morpholine pK_a gets shifted down to 5.3 and is therefore assigned pK_{a2}, whereas the pK_a of the 2-aminoimidazole fragment is increased

to 6.9. Protonation at this moiety is also favored at the active site of TGT due to the fact, that only this species is able to engage charge-assisted H-bonding.

The ascending trend of the pK_{a2} values, the deprotonation of the imidazolium species, due to increasing electron donating character of the substituent, from the unsubstituted benzimidazole (5.5), to the 2-methyl (6.3), to the 2-benzylamino (6.9), and to the 2-amino derivative (7.5) is clearly reproduced in the basicity of the inhibitor series: **1** (5.2) < **2** (5.4) < **6** (5.8) < **8** (5.9) < **3** (6.3). These findings are in agreement with the slightly lower pK_{a2} of **4** (6.2) compared to **3**. For the *lin*-benzoguanine structures, when compared to the corresponding benzimidazoles, a lowering of 0.9 -1.2 pK_a units due to formal attachment of the isocytosine moiety is observed; only for the transition from benzimidazole to *lin*-benzoguanine itself, the decrease of pK_{a2} is less pronounced (0.3).

The ΔpK_a difference between the first and the second pK_a value remains almost constant within the whole series (1.5-1.9), indicating that substituents also affect the first deprotonation of the isocytosine moiety. The pK_{a3} values stays almost constant throughout the entire set (10.1-10.4), suggesting that substitution has no effect on the second deprotonation of the isocytosine moiety.

For all these compounds, an additional pK_a value above 12 and therefore out of the measurement range was observed; this

value can be assigned to the deprotonation of the imidazole moiety in agreement with the findings by *Morgenthaler et al.* [17].

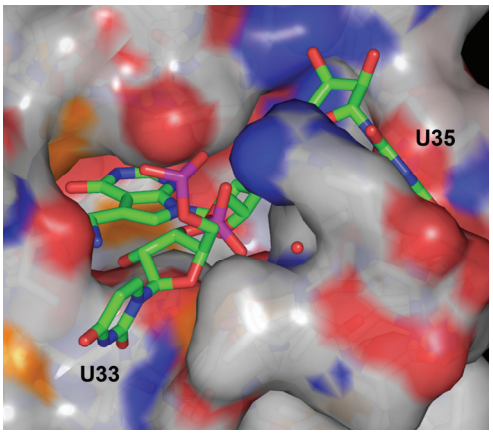
High-throughput log*D* screening

The applied high-throughput (HT) method for the determination of the distribution coefficient HT-log*D* is based on microplate technique and derived from the conventional 'shake flask' method. The compound is distributed between H_2O buffered at a specific p*H* and 1-octanol. The distribution coefficient is then calculated from the difference in concentration in the aqueous phase before and after partitioning and the ratio of the two phases. The "one phase-analysed" experiment starts with a pure Me_2SO solution of the lead molecule of interest which is dispensed in aqueous buffer with *c* (compound) = 0.5 mM. A part of this solution is then analysed by measuring the UV absorption. The obtained optical density (reference) is equal to the concentration of the substance before partitioning.

An exact amount of 1-octanol is added and the mixture incubated by quiet shaking (2 h). The emulsion is allowed to stand overnight to be sure that the partition equilibrium is reached. The next day, the layers need thorough centrifugation at 3000 rpm for 10 min and the concentration of the substance in the aqueous phase is determined again by measuring the UV absorption. The procedure above is carried

out at four different octanol/water ratios, two with a large volume of octanol for hydrophilic compounds (log$D$ < 1) and two with a low volume of octanol for the lipophilic compounds (log$D$ > 1).

a)



b)

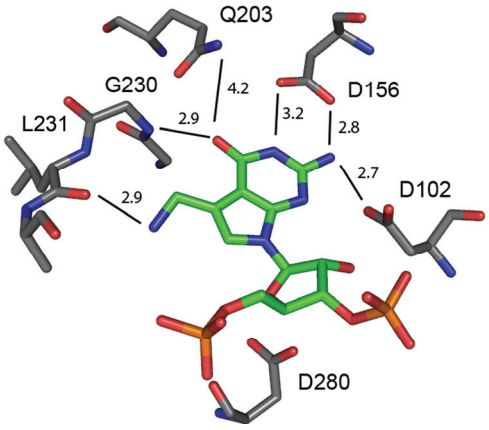


Figure 1SI. a) Binding mode of the modified tRNA-fragment U33-preQ$_1$-U35 in the active site of TGT. PDB-code: 1Q2S [18] resolution 3.2 Å. Color code: ligand skeleton: green; C: grey; O: red; N: blue; S: yellow; P: pink. b) H-Bonding network, distances between heavy atoms shown in Å. Color code: ligand skeleton: green; C: grey; O: red; N: blue; S: yellow, P: orange.

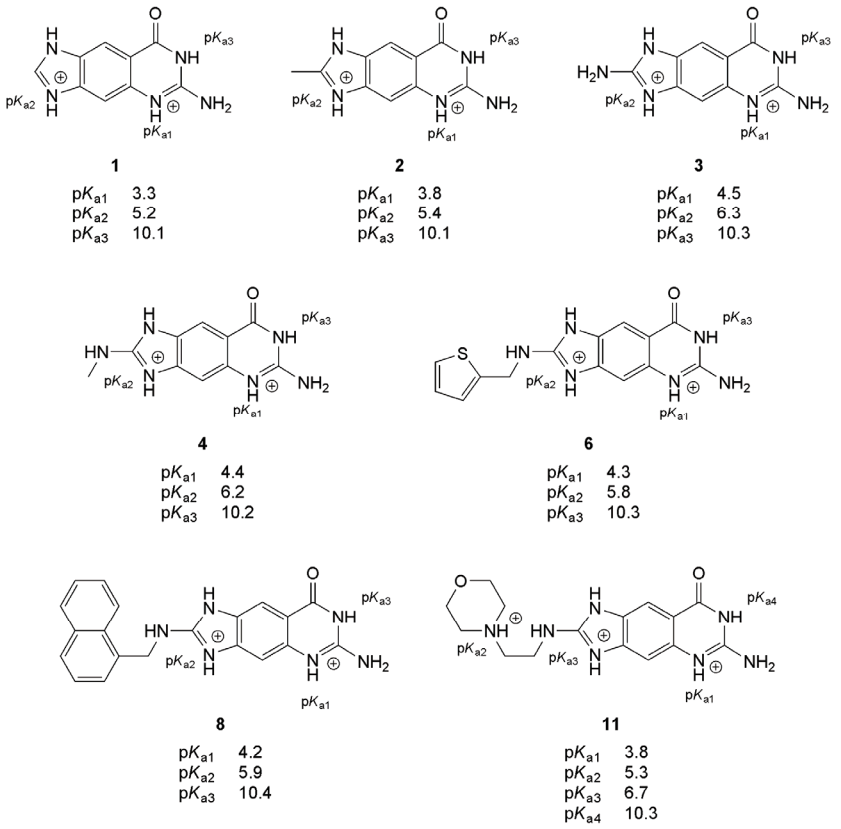


Figure 2SI. pK_a Values of *lin*-benzoguanines (shown in the fully protonated state at p*H* 2), the influence of the different substituents in evidence.

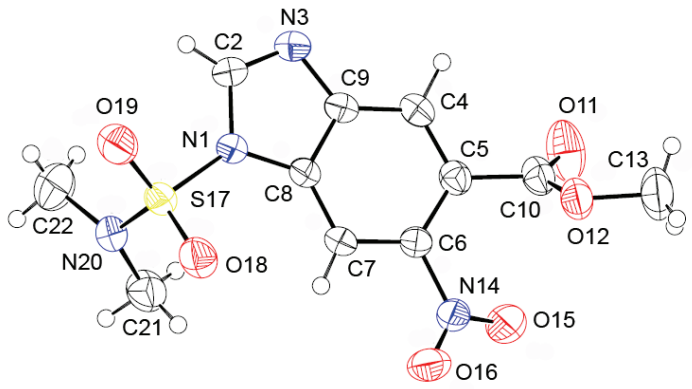


Figure 3SI. ORTEP representation of **16b** with vibrational ellipsoids obtained at 223 K and shown at the 30% probability level. Color code: C: black; O: red; N: blue; S: yellow.

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