Inverted positioning of DNMT1 inhibitor in the active site of DNMT1 caused by hydrophobicity/hydrophilicity of the terminal structure

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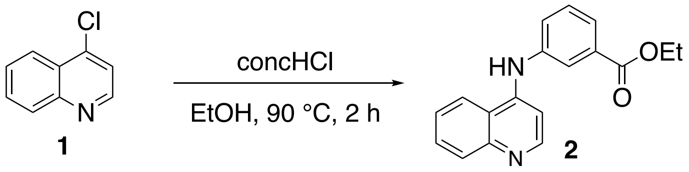
1. Chemical synthesis

General protocol

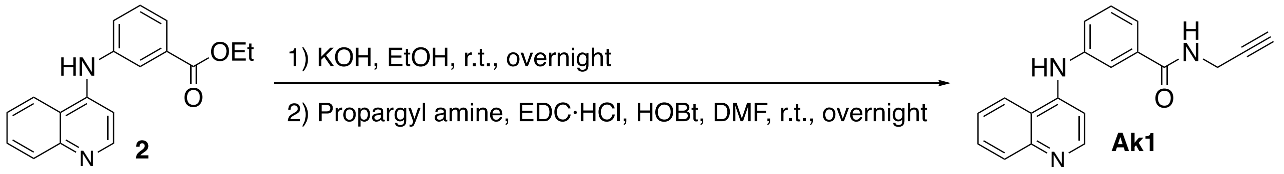
Reagents and solvents were purchased from Wako pure Chemical Industries and Tokyo Chemical Industry. Column chromatography was performed using silica gel supplied by Fuji Silysia Chemical. Proton nuclear magnetic resonance spectra was recorded using a JEOL JNM-AL300 spectrometer in solvent as indicated. Chemical shifts (δ) are reported in parts per million relative to the internal standard tetramethylsilane. ESI-mass spectra were recorded by JEOL JMS-T100CS mass spectrometer. The purification of all tested compounds was performed by HPLC using Japan Analytical Industry LC-9210 with a JAIGEL 1H column (600 mm x φ20 mm, Japan Analytical Industry) (UV detection, λ = 254 nm; flow 1 mL/min; $CHCl_3$).

Synthesis of ethyl 3-(quinolin-4-ylamino)benzoate (2)



To a solution of 4-chloroquinoline (1.01 g, 6.11 mmol) and ethyl 3-aminobenzoate (1.01 mL, 6.70 mmol) in EtOH (30 mL) was added conc HCl (5 drops). The reaction mixture was heated under reflux for 2 h, and filtrated. The residue was washed with water three times to obtain compound **2** (1.63 g, 91%); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.34 (t, *J* = 7.0 Hz 3H), 4.36 (q, *J* = 7.0 Hz, 2H), 6.86 (d, *J* = 7.0 Hz, 1H), 7.73 (t, *J* = 8.0 Hz 1H), 7.81-7.85 (m, 2H), 7.99 (d, *J* = 8.0 Hz, 1H), 8.04-8.07 (m, 2H), 8.16 (d, *J* = 8.0 Hz, 1H), 8.56 (d, *J* = 7.0 Hz, 1H), 8.91 (d, *J* = 8.5 Hz, 1H).

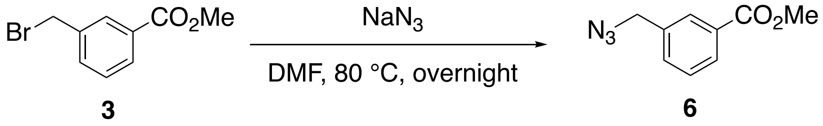
Synthesis of *N*-(prop-2-yn-1-yl)-3-(quinolin-4-ylamino)benzamide (Ak1)



To a solution of compound **2** (574 mg, 1.96 mmol) in EtOH (20 mL) was added a solution of KOH (5.0 mL, 2.0 M in water). The mixture was stirred at r.t. overnight and added conc HCl to adjust to pH 3. The resulting mixture was filtered, and the residue was washed with water three times to obtain desired compound (461 mg, 89%).

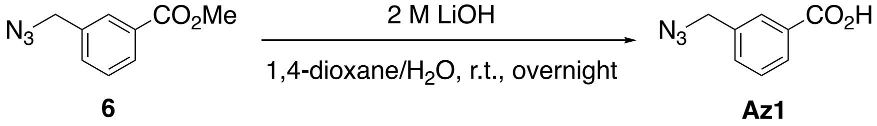
To a solution of the above compound (1.89 g, 6.46 mmol) and propargylamine (0.61 mL, 9.68 mmol) in DMF (25 mL) were added EDC·HCl (1.97 g, 10.3 mmol) and HOBt (1.36 g, 10.1 mmol). The mixture was stirred at r.t. overnight and added saturated NaCl. The resulting mixture was filtered, and the residue was washed with water three times to obtain compound **Ak1** (1.41 g, 72%): ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 3.16 (s, 1H), 4.09 (d, *J* = 6.0 Hz, 2H), 6.86 (d, *J* = 7.0 Hz, 1H), 7.69-7.71 (m, 2H), 7.83 (dd, *J* = 6.5, 9.0 Hz, , 1H), 7.91-7.93 (m, 1H), 7.89 (s, 1H), 8.03-8.10 (m, 2H), 8.55 (d, *J* = 7.0 Hz, 1H), 8.80 (d, *J* = 9.0 Hz, 1H), 9.14 (t, *J* = 6.0 Hz, 1H), 11.00 (s, 1H).

Synthesis of methyl 3-(azidomethyl)benzoate (6)



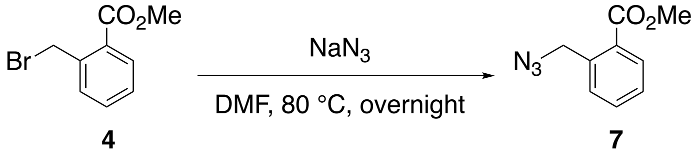
To a solution of Methyl-3-(Azidomethyl)benzoate (821 mg, 3.60 mmol) in DMF (20 mL) was added sodium azide (705 mg, 10.8 mmol). The mixture was stirred at 80 °C overnight and added water. The resulting mixture was extracted with EtOAc three times and washed with brine. The combined extracts were dried over Na_2SO_4 and concentrated to give compound **6** (637 mg, 93%); ^{1}H-NMR (DMSO-*d$_6$*, 500 MHz): δ 3.85 (s, 3H), 4.55 (s, 2H), 7.52 (dd, *J* = 7.5, 8.0 Hz 1H), 7.62 (d, *J* = 7.5 Hz 1H), 7.92(d, *J* = 8.0 Hz, 1H), 7.96 (s, 1H).

Synthesis of 3-(azidomethyl)benzoic acid (Az1)



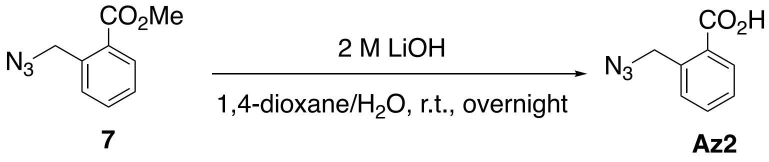
To a solution of compound **6** (637 mg, 3.33 mmol) in 1,4-dioxane (20 mL) was added a solution of LiOH (8 mL, 2 M in water). The mixture was stirred at r.t. overnight and added added conc HCl to adjust to pH 3. The resulting mixture was extracted with EtOAc three times and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain compound **Az1** (582 mg, 99%); ^{1}H-NMR (DMSO-*d$_6$*, 500 MHz): δ 4.57 (s, 2H), 7.55 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.94 (d, 1H, *J* = 7.5 Hz, 1H), 7.96 (s, 1H).

Synthesis of methyl 2-(azidomethyl)benzoate (7)



To a solution of Methyl-2-(Azidomethyl)benzoate (1.22 g, 5.32 mmol) in DMF (10 mL) was added sodium azide (661 mg, 10.2 mmol). The mixture was stirred at 80 °C overnight and added water. The resulting mixture was extracted with EtOAc three times and washed with brine. The combined extracts were dried over Na_2SO_4 and concentrated to give compound **7** (925 mg, 91%); ^{1}H-NMR (DMSO-*d$_6$*, 500 MHz): δ 3.87 (s, 3H), 4.79 (s, 2H), 7.51 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.56 (d, *J* = 7.0 Hz, 1H), 7.66 (dd, *J* = 7.0, 7.5 Hz, 1H), 7.94 (d, *J* = 7.5 Hz, 1H).

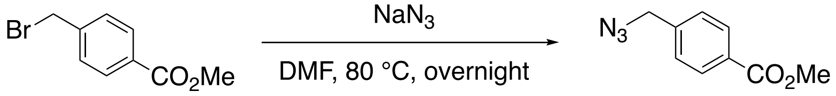
Synthesis of 2-(azidomethyl)benzoic acid (Az2)



To a solution of compound **7** (925 mg, 4.84 mmol) in 1,4-dioxane (20 mL) was added a solution of LiOH (7 mL, 2 M in water). The mixture was stirred at r.t. overnight and added added conc HCl to adjust to pH 3. The resulting mixture was extracted with EtOAc three times and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain compound **Az2** (442 mg, 72%); ^{1}H-NMR

(DMSO-d_6, 500 MHz): δ 4.81 (s, 2H), 7.49 (dd, J = 7.5, 8.0 Hz, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.62 (dd, J = 7.5, 8.0 Hz, 1H), 7.95 (d, J = 7.5 Hz, 1H).

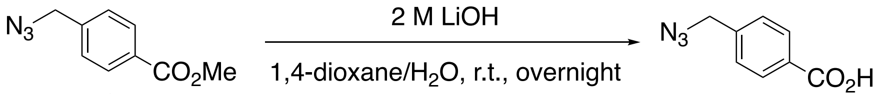
Synthesis of methyl 4-(azidomethyl)benzoate (8)



5 **8**

To a solution of Methyl-4-(Azidomethyl)benzoate (1.08 g, 4.71 mmol) in DMF (30 mL) was added sodium azide (851 mg, 13.1 mmol). The mixture was stirred at 80 °C overnight and added water. The resulting mixture was extracted with EtOAc three times and washed with brine. The combined extracts were dried over Na_2SO_4 and concentrated to give compound **8** (861 mg, 96%); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.88 (s, 3H), 4.60 (s, 2H), 7.52 (d, J = 8.0 Hz, 2H), 8.00 (d, J = 8.0 Hz, 2H)

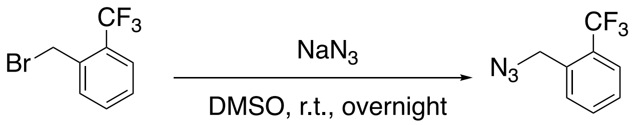
Synthesis of 4-(azidomethyl)benzoic acid (Az3)



8 **Az3**

To a solution of compound **8** (839 mg, 4.39 mmol) in 1,4-dioxane (20 mL) was added a solution of LiOH (8 mL, 2 M in water). The mixture was stirred at r.t. overnight and added added conc HCl to adjust to pH 3. The resulting mixture was extracted with EtOAc three times and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain compound **Az3** (722 mg, 93%); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 4.57 (s, 2H), 7.49 (d, J = 8.0 Hz, 2H), 7.97 (d, J = 8.0 Hz, 1H).

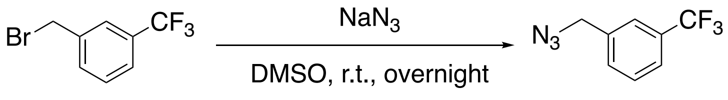
Synthesis of 1-(azidomethyl)-2-(trifluoromethyl)benzene (Az4)



9 **Az4**

To a solution of 2-(trifluoromethyl)benzyl bromide (639 mg, 2.67 mmol) in DMSO (5 mL) was added sodium azide (282 mg, 4.34 mmol). The mixture was stirred at r.t. overnight and added water. The resulting mixture was extracted with EtOAc three times and washed with brine. The combined extracts were dried over Na_2SO_4 and concentrated to give compound **Az4** (284 mg, 53%); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 4.61 (s, 2H), 7.53 (dd, J = 7.0, 7.5 Hz, 1H), 7.65-7.70 (m, 2H), 7.72 (d, J = 8.0 Hz, 1H).

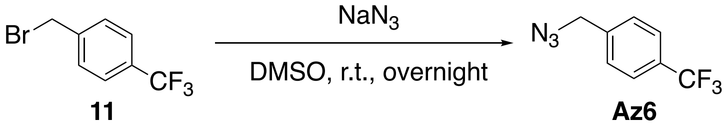
Synthesis of 1-(azidomethyl)-3-(trifluoromethyl)benzene (Az5)



10 **Az5**

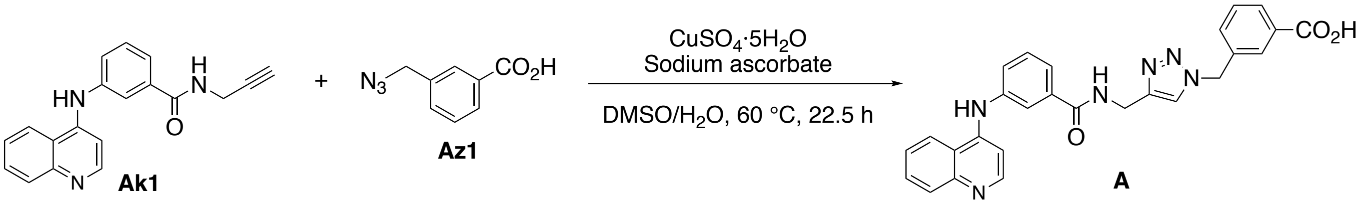
To a solution of 3-(trifluoromethyl)benzyl bromide (239 mg, 1.00 mmol) in DMSO (3 mL) was added sodium azide (149 mg, 4.34 mmol). The mixture was stirred at r.t. overnight and added water. The resulting mixture was extracted with EtOAc three times and washed with brine. The combined extracts were dried over Na_2SO_4 and concentrated to give compound **Az5** (162 mg, 81%); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 4.63 (s, 2H), 7.56 (dd, J = 7.5, 8.0 Hz, 1H), 7.71-7.73 (m, 2H), 7.77 (s, 1H).

Synthesis of 1-(azidomethyl)-4-(trifluoromethyl)benzene (Az6)



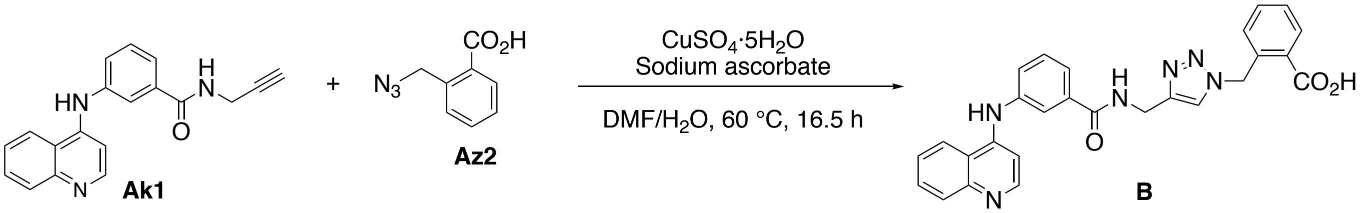
To a solution of 4-(trifluoromethyl)benzyl bromide (807 mg, 3.37 mmol) in DMSO (5 mL) was added sodium azide (273 mg, 4.20 mmol). The mixture was stirred at r.t. overnight and added water. The resulting mixture was extracted with EtOAc three times and washed with brine. The combined extracts were dried over Na_2SO_4 and concentrated to give compound **Az6** (598 mg, 88%); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 4.63 (s, 2H), 7.56 (dd, *J* = 7.5, 8.0 Hz, 1H), 7.71-7.73 (m, 2H), 7.77 (s, 1H).

Synthesis of 3-((4-((3-(quinolin-4-ylamino)benzamido)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)benzoic acid (A)



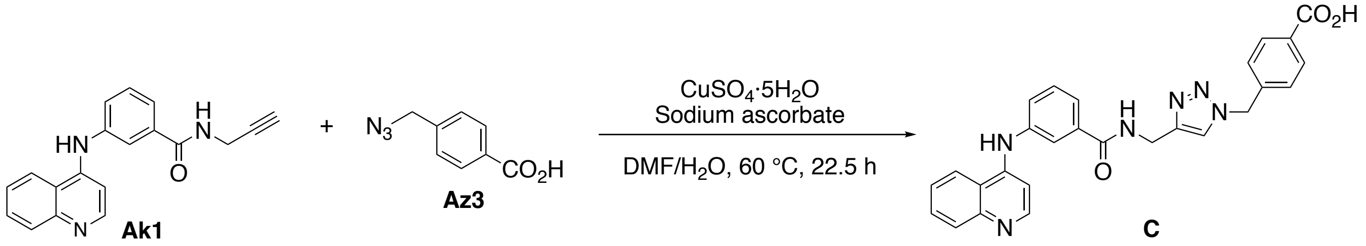
To a solution of compound **Ak1** (214 mg, 0.771 mmol) and compound **Az1** (133 mg, 0.751 mmol) in DMSO and H_2O (7 mL, DMSO:H_2O = 2:1) were added $CuSO_4 \cdot 5H_2O$ (85.2 mg, 0.341 mmol) and sodium ascorbate (122 mg, 0.616 mmol). The mixture was stirred at 60 °C for 22.5 h and added EtOAc and water. The resulting mixture was filtered. The filtrate was extracted with EtOAc twice. The combined extracts were concentrated and purified by chromatography on silica gel ($CHCl_3$/MeOH) to give compound **A** (127 mg, 64%);^{1}H-NMR (DMSO-d_6, 500 MHz) δ 4.67(s, 2H), 5.68(s, 2H), 6.93(d, *J* = 7.0Hz, 1H), 7.48(q, *J* = 8.0 Hz, 1H), 7.57(d, *J* = 7.5 Hz, 1H), 7.67-7.70(m, 2H), 7.82(dd, *J* = 7.5, 8.5 Hz, 1H), 7.91(d, *J* = 6.5 Hz, 1H), 7.94-7.99(m, 4H), 8.05(dd, *J* = 7.0, 7.5 Hz, 1H), 8.10(s, 1H), 8.39(d, *J* = 7.5 Hz, 1H), 8.58(d, *J* = 8.0 Hz, 1H); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 35.4, 52.8, 120.2, 121.8, 122.7, 123.1, 123.8, 125.6, 125.7, 128.9, 129.4, 129.5, 130.0, 130.3, 132.5, 132.7, 135.9, 137.0, 141.1, 145.9, 148.5, 150.6, 166.4, 167.8; HRMS(ESI) calcd for $C_{27}H_{23}N_6O_3$ 479.1826 [M+], found 479.1827.

Synthesis of 2-((4-((3-(quinolin-4-ylamino)benzamido)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)benzoic acid (B)



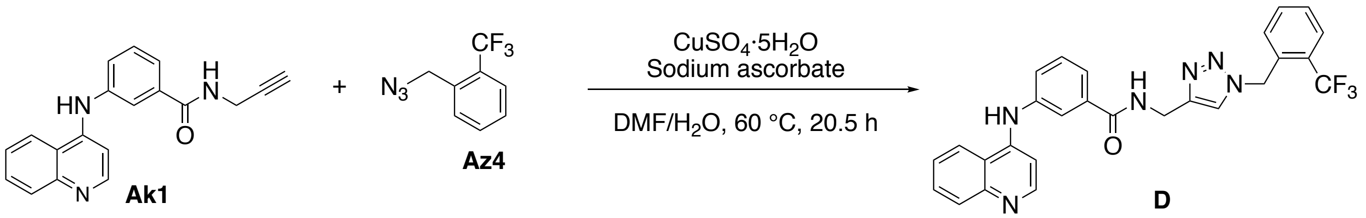
To a solution of compound **Ak1** (154 mg, 0.512 mmol) and compound **Az2** (100 mg, 0.566 mmol) in DMF and H_2O (7.5 mL, DMF:H_2O = 2:1) were added $CuSO_4 \cdot 5H_2O$ (62.2 mg, 0.249 mmol) and sodium ascorbate (111 mg, 0.632 mmol). The mixture was stirred at 60 °C for 16.5 h and concentrated. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH) to give compound **B** (130 mg, 53%): ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 4.54(d, *J* = 3.3 Hz, 2H), 5.94(s, 2H), 6.90-7.00(m, 2H), 7.45(t, *J* = 7.5 Hz, 1H), 7.54(t, *J* = 7.5 Hz, 1H), 7.58-7.60(m, 2H), 7.70(dd, *J* = 7.5, 8.0 Hz, 1H), 7.79(dd, *J* = 3.0, 4.5 Hz, 1H), 7.91-7.96(m, 4H), 8.60(s, 1H), 9.12(t, *J* = 5.5 Hz, 1H); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 35.4, 51.5, 123.3, 124.2, 124.9, 125.1, 126.7, 127.2, 128.8, 129.6, 130.3, 131.3, 132.4, 132.9, 136.2, 137.4, 139.5, 145.5, 151.9, 166.0; HRMS(ESI) calcd for $C_{27}H_{23}N_6O_3$ 479.1826 [M+], found 479.1827.

Synthesis of 4-((4-((3-(quinolin-4-ylamino)benzamido)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)benzoic acid (C)



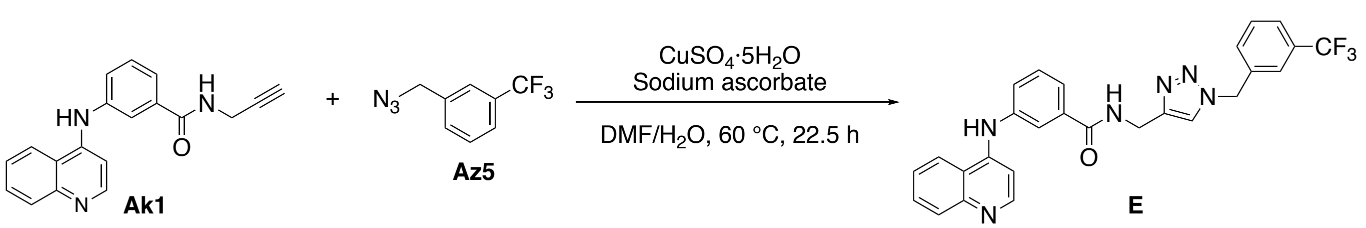
To a solution of compound **Ak1** (203 mg, 0.675 mmol) and compound **Az3** (129 mg, 0.731 mmol) in DMF and H_2O (7.5 mL, DMF:H_2O = 2:1) were added $CuSO_4 \cdot 5H_2O$ (185 mg, 0.741 mmol) and sodium ascorbate (275 mg, 1.56 mmol). The mixture was stirred at 60 °C for 22.5 h and concentrated. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH) to give compound **C** (261 mg, 81%): ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 4.54(d, *J* = 6.0 Hz, 1H), 5.67(s, 1H), 6.88(d, *J* = 6.5Hz,1H), 7.39(d, *J* = 9.0Hz, 1H), 7.64-7.66(m, 2H), 7.78(dd, *J* = 6.0, 9.0 Hz, ,1H), 7.87(d, *J* = 5.0 Hz, 1H), 7.93(d, *J* = 9.0 Hz, 2H), 7.95(s, 1H), 7.98-8.01(m, 2H), 8.10(s, 1H), 8.54(d, *J* = 6.5Hz, 1H), 8.65(d, *J* = 8.5 Hz, 1H), 9.15(t, *J* = 6.0 Hz, 1H);^{13}C-NMR (DMSO-d_6, 125 MHz) δ 35.5, 52.8, 123.2, 124.0, 124.9, 126.9, 127.1, 128.6, 130.4, 136.2, 139.6, 141.3, 166.1; HRMS(ESI) calcd for $C_{27}H_{23}N_6O_3$ 479.1826 [M+], found 479.1827.

Synthesis of 3-(quinolin-4-ylamino)-*N*-((1-(2-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazol-4-yl)methyl)benzamide (D)



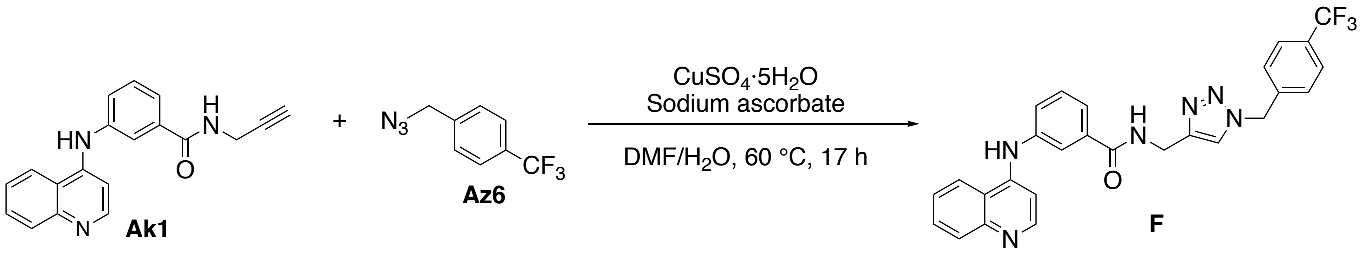
To a solution of compound **Ak1** (207 mg, 0.686 mmol) and compound **Az4** (147 mg, 0.731 mmol) in DMF and H_2O (10 mL, DMF:H_2O = 2:1) were added $CuSO_4 \cdot 5H_2O$ (178 mg, 0.713 mmol) and sodium ascorbate (241 mg, 1.37 mmol). The mixture was stirred at 60 °C for 20.5 h and concentrated. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH) to give compound **D** (236 mg, 69%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 4.66(d, *J* = 5.5 Hz, 2H), 5.66(s, 2H), 6.68(d, *J* = 5.5Hz, 1H), 7.11(d, *J* = 7.5 Hz, 1H), 7.31(dd, *J* = 7.5, 9.0 Hz, 1H), 7.39-7.47(m, 4H), 7.59-7.67(m, 4H), 7.83(s, 1H, 7.97(d, *J* = 9.0 Hz, 1H), 8.23(d, *J* = 5.0 Hz, 1H), 8.30(s, 1H), 8.46(d, *J* = 9.0 Hz, 1H); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 34.9, 49.5, 122.5, 123.1, 124.1, 125.1, 125.5, 126.2, 126.4, 126.6, 128.9, 129.6, 130.1, 133.2, 133.9, 135.5, 140.7, 165.8; HRMS(ESI) calcd for $C_{27}H_{22}F_3N_6O$ 503.1802 [M+], found 503.1803.

Synthesis of 3-(quinolin-4-ylamino)-*N*-((1-(3-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazol-4-yl)methyl)benzamide (E)



To a solution of compound **Ak1** (207 mg, 0.686 mmol) and compound **Az5** (147 mg, 0.731 mmol) in DMF and H_2O (10 mL, DMF:H_2O = 2:1) were added $CuSO_4 \cdot 5H_2O$ (178 mg, 0.713 mmol) and sodium ascorbate (241 mg, 1.37 mmol). The mixture was stirred at 60 °C for 22.5 h and concentrated. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH) to give compound **E** (236 mg, 69%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 4.51(d, *J* = 3.5 Hz, 2H), 5.680(s, 2H), 7.48-7.51(m, 4H), 7.60-7.65(m, 4H), 7.69-7.72(m, 3H), 7.87(s, 1H), 7.99(s, 1H), 8.12(s, 1H), 9.06(s, 1H),9.14(s, 1H); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 35.5, 52.6, 122.1, 123.4, 125.2, 125.4, 125.6, 126.0, 126.5, 129.7, 130.0, 130.1, 130.4, 132.7, 136.0, 138.0, 140.9, 166.2; HRMS(ESI) calcd for $C_{27}H_{22}F_3N_6O$ 503.1802 [M+], found 503.1801.

Synthesis of 3-(quinolin-4-ylamino)-*N*-((1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazol-4-yl)methyl)benzamide (F)



To a solution of compound **Ak1** (204 mg, 0.677 mmol) and compound **Az6** (147 mg, 0.731 mmol) in DMF and H_2O (10 mL, DMF:H_2O = 2:1) were added $CuSO_4\cdot5H_2O$ (91.5 mg, 0.366 mmol) and sodium ascorbate (134 mg, 0.761 mmol). The mixture was stirred at 60 °C for 17 h and concentrated. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH) to give compound **F** (190 mg, 56%); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 4.52(d, *J* = 5.5 Hz, 2H), 5.69(s, 2H), 7.49-7.53(m, 5H), 7.66(d, *J* = 7.0 Hz, 1H), 7.75(d, *J* = 8.0 Hz, 2H), 7.89(s, 1H), 8.11(s, 1H), 9.07(s, 1H), 9.20(s, 1H); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 35.5, 52.9, 121.2, 121.3, 122.9, 123.5, 125.1, 125.7, 126.2, 127.8, 128.8, 129.0, 129.3, 129.5, 130.4, 136.2, 141.2, 141.7, 166.1; HRMS(ESI) calcd for $C_{27}H_{22}F_3N_6O$ 503.1802 [M+], found 503.1803.

2. Biological analysis

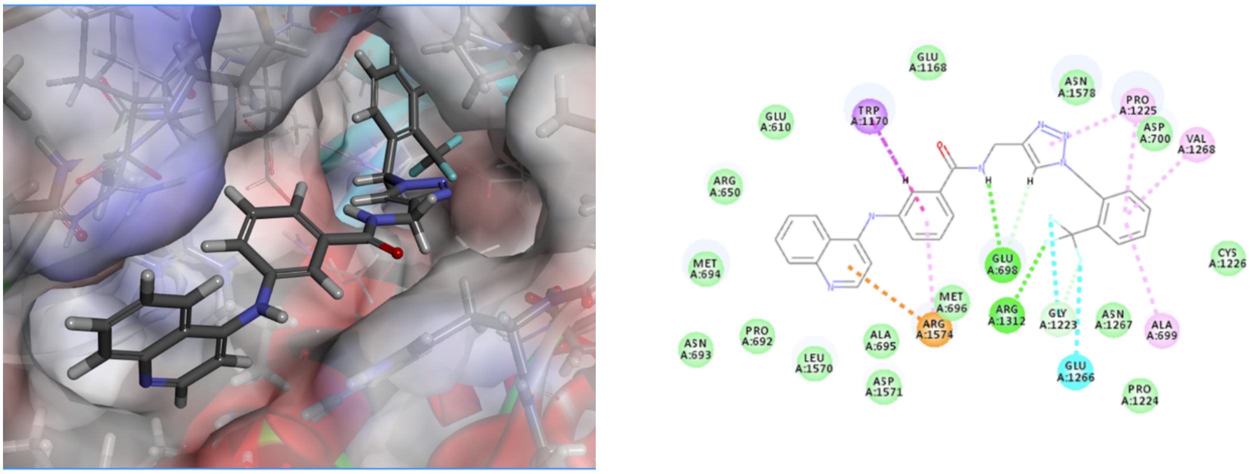
DNMT1 inhibition assay

These assays were performed using DNMT1 Direct Activity Assay Kit (BPS, #52050L) according to the supplyer's instruction. Assays were conducted in 96 well white plates precoated with DNA substrate. Test compounds (10% DMSO in DNMT1 assay buffer, 5 μL/well), DNMT1 solution (10 ng/μL in DNMT1 assay buffer, 20 μL/well), S-adenosylmethionine solution (400 μM in DNMT1 assay buffer, 5 μL/well), DNMT1 assay buffer (7.5 μL/well), and H_2O (12.5 μL/well) were incubated for 1 h at 37 °C. In the washout experiment, the supernatant was removed and washed with TBST buffer. After blocking with blocking buffer, every well was probed with anti-5-methylcytosine antibody (1:400 dilution) in blocking buffer and incubated 1 h. The proved well were washed with blocking buffer and added secondary HRP-labeled antibody (1:1000 dilution) in blocking buffer and incubated 30 min. After removing the supernatant, HRP chemiluminescence was added to every well. The chemiluminescence of the wells was measured with 2104 Envison Multimode Plate Reader (Perkin Elmer). The % inhibition was calculated from the chemiluminescence readings.

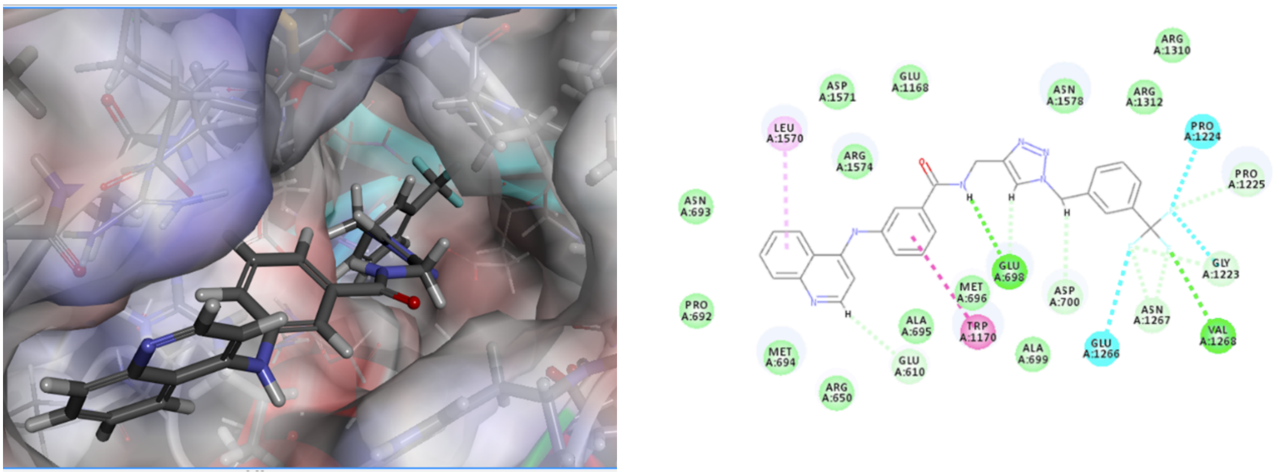
3. Molecular modeling

Binding simulation

The X-ray structure of DNMT1 (PDB code 3SWR) was retrieved from PDB database and was prepared using Discovery Studio 2020 client by removing ligands and water molecules. Ligands structures of test compound were prepared using Discovery Studio 2020 client by minimizing the energy. Water molecules in the active pocket of DNMT1 were incorporated into the docking calculations. Protein and ligands were docked by Discovery Studio (Search space: (X, Y, Z) = (-5.80, -6.94, 40.32) with radius 21.1). Compound **C**, **D**, **E** and **F** were docked into the active site of the prepared protein as described above. The favorable binding poses, which allowed the ligand conformation to be flexibly explored while holding the protein as a rigid structure was determined by the standard precision mode of Glide. The docking results analysis was based on two dimensions and three dimensions visualization.



(A) Compound **D** **(B)** Compound **D**



(C) Compound **E** **(D)** Compound **E**

Figure **SI** (**A**), (**C**). View of conformation of compound **D** and **E** docked in the DNMT1 catalytic core. (**B**), (**D**) View of schematic diagram of compound **D** and **E** docked in the DNMT1 catalytic core.

Table **SI**. (**A**) DFT calculation of interactions between test compound and amino acid residues of DNMT1 (compound **C** and **D**)

Name	**Ligand binding pocket**	**H-bonds (donor - acceptor)**	**Other interaction (donor - acceptor)**
Compound **C**	GLU698, GLU1168, TRP1170, LEU1570, ARG1574	TRP1170: HE1 - ;Compound C: N25 (2.62 Å) ARG1574: HH12 - ;Compound C: O35 (2.67 Å) Compound C: H56 - ;GLU698: OE2 (2.22 Å)	ARG1574: NH1 - ;Compound C: O36 (Attractive Charge, 3.69 Å) Compound C: N7 - ;GLU1168: OE2 (Attractive Charge, 5.11 Å) Compound C: H41 - ;GLU1168: OE1 (Carbon Hydrogen Bond, 2.52 Å) Compound C - ;LEU1570 (Carbon Hydrogen Bond, 5.16 Å)
Compound **D**	GLU698, ALA699, TRP1170, GLY1223, PRO1225, GLU1266, VAL1268, ARG1312, ARG1574	ARG1312: HH11 - ;Compound D: F36 (2.89 Å) ARG1312: HH12 - ;Compound D: F36 (2.95 Å) Compound D: H49 - ;GLU698: OE2 (2.41 Å)	GLY1223: HA1 - ;Compound D: F35 (Carbon Hydrogen Bond, 2.88 Å) Compound D: H52 - ;GLU698: OE2 (Carbon Hydrogen Bond, 2.50 Å) GLY1223: O - ;Compound D: F35 (Halogen (Fluorine), 3.44 Å) GLY1223: O - ;Compound D: F37 (Halogen (Fluorine), 3.36 Å) GLU1266: OE1 - ;Compound D: F35 (Halogen (Fluorine), 3.35 Å) GLU1266: O - ;Compound D: F35 (Halogen (Fluorine), 3.67 Å) Compound G: N25 - ;Compound D: F37 (intramolecular Halogen (Fluorine), 3.35 Å) ARG1574: NH1 - ;Compound D (π-Cation, 4.98 Å) Compound D: H45 - ;TRP1170: NH1 (π-Sigma, 2.76 Å) TRP1170 - ;Compound D (π-π T-shaped, 5.17 Å) TRP1170 - ;Compound D (π-π T-shaped, 5.21 Å) Compound D - ;ARG1574 (π-Alkyl, 5.33Å) Compound D - ;ARG1574 (π-Alkyl, 4.86Å) Compound D - ;PRO1225 (π-Alkyl, 5.01Å) Compound D - ;ALA699 (π-Alkyl, 5.24Å) Compound D - ;PRO1225 (π-Alkyl, 4.88Å) Compound D - ;VAL1268 (π-Alkyl, 5.48Å)

Table **SI**. (**B**) DFT calculation of interactions between test compound and amino acid residues of DNMT1 (compound **E** and **F**)

Name	**Ligand binding pocket**	**H-bonds (donor - acceptor)**	**Other interaction (donor - acceptor)**
Compound **E**	GLU610, GLU698, ASP700, TRP1170, GLY1223, PRO1224, PRO1225, GLU1266, ASN1267, VAL1268, LEU1570	VAL1268: HN - ;Compound E: F36 (2.84 Å) Compound E: H49 - ;GLU698: OE2 (2.64 Å)	GLY1223: HA1 - ;Compound E: F37 (Carbon Hydrogen Bond, 2.72 Å) PRO1225: HA - ;Compound E: F35 (Carbon Hydrogen Bond, 2.69 Å) ASN1267: HA - ;Compound E: F36 (Carbon Hydrogen Bond, 2.50 Å) ASN1267: HA - ;Compound E: F37 (Carbon Hydrogen Bond, 2.44 Å) Compound E: H42 - ;GLU610: OE2 (Carbon Hydrogen Bond, 2.64 Å) Compound E: H52 - ;GLU698: OE2 (Carbon Hydrogen Bond, 2.58 Å) Compound E: H53 - ;ASP700: OD2 (Carbon Hydrogen Bond, 2.82 Å) GLY1223: O - ;Compound E: F35 (Halogen (Fluorine), 2.89 Å) GLY1223: O - ;Compound E: F37 (Halogen (Fluorine), 3.35 Å) PRO1224: O - ;Compound E: F35 (Halogen (Fluorine), 2.84 Å) GLU1266: OE1 - ;Compound E: F37 (Halogen (Fluorine), 3.33 Å) GLU1266: O - ;Compound E: F37 (Halogen (Fluorine), 3.17 Å) ASN1267: OD1 - ;Compound E: F36 (Halogen (Fluorine), 2.83 Å) TRP1170 - ;Compound E (π-π T-shaped, 5.37 Å) TRP1170 - ;Compound E (π-π T-shaped, 5.40 Å) Compound E - ;LEU1570 (π-Alkyl, 5.43Å)
Compound **F**	GLU610, ARG650, MET694, ALA695, MET696, GLU698, PRO1225, ARG1312, ASN1578	MET696: HN - ;Compound F: O19 (2.02 Å) ARG1312: HH12 - ;Compound F: F36 (2.81 Å) Compound F: H44 - ;MET694: O (2.05 Å)	ALA695: HA - ;Compound F: O19 (Carbon Hydrogen Bond, 2.93 Å) Compound F: H50 - ;GLU698: OE2 (Carbon Hydrogen Bond, 2.86 Å) ASN1578: O - ;Compound F: F36 (Halogen (Fluorine), 3.18 Å) ASN1578: O - ;Compound F: F37 (Halogen (Fluorine), 2.89 Å) ARG650: NH1 - ;Compound F (π-Cation, 3.72 Å) GLU610: OE2 - ;Compound F (π-Anion, 3.95 Å) GLU698: OE2 - ;Compound F (π-Anion, 3.04 Å) Compound F - ;PRO1225 (π-Alkyl, 5.21Å)