Scaffold Morphing to Identify Novel DprE1 Inhibitors with Antimycobacterial Activity

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SUPPORTING INFORMATION

Experimental Section:

All anhydrous solvents, reagent grade solvents for chromatography and starting materials were purchased from either Sigma Aldrich Chemical Co. or Fisher Scientific. Water was distilled and purified through a Milli-Q water system (Millipore Corp., Bedford, MA). General methods of purification of compounds involved the use of silica cartridges purchased from Grace Purification systems. The reactions were monitored by TLC on precoated Merck 60 F254 silica gel plates and visualized using UV light (254 nm). All compounds were analyzed for purity by HPLC and characterized by ^{1}H NMR using Bruker 300 MHz NMR and/or Bruker 400 MHz NMR spectrometers. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak in the corresponding spectra; chloroform δ 7.26, methanol δ 3.31, DMSO δ 3.33 and coupling constants (*J*) are reported in hertz (Hz) (where s = singlet, bs = broad singlet, d = doublet, dd = double doublet, bd = broad doublet, ddd = double doublet of doublet, t = triplet, tt – triple triplet, q = quartet, m = multiplet) and analyzed using ACD NMR data processing software. Mass spectra values are reported as *m/z*. All reactions were conducted under Nitrogen unless otherwise noted. Solvents were removed *in vacuo* on a rotary evaporator. All final compounds for biological testing were purified by reverse phase HPLC with >95% purity [Shimadzu HPLC instrument with a Hamilton reversed phase column (HxSil, C18, 3μm, 2.1 mm × 50 mm (H2)). Eluent A: 5% CH_3CN in H_2O, eluent B: 90% CH_3CN in H_2O. A flow rate of 0.2 mL/min was used with UV detection at 254 and 214 nm]

Abbreviations: NMP = N-methyl Pyrrolidine; HCl = hydrochloric acid; DMF = *N*,*N*-dimethylformamide; NaH = sodium hydride. EI = electrospray ionization; HRMS = high resolution mass spectrometry.

N-(2-fluoroethyl)-1-((6-methoxy-5-methylpyrimidin-4-yl)methyl)-1H-benzo[d]imidazole-4-carboxamide (2)

ES+MS m/z: 344

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.28 (s, 3 H) 3.69 - 3.86 (m, 2 H) 3.94 (s, 3 H) 4.54 (t, *J*=4.99 Hz, 1 H) 4.70 (t, *J*=4.90 Hz, 1 H) 5.76 (s, 2 H) 7.34 (t, *J*=7.82 Hz, 1 H) 7.69 (dd, *J*=8.10, 0.94 Hz, 1 H) 7.90 (dd, *J*=7.54, 0.94 Hz, 1 H) 8.41 (s, 1 H) 8.56 (s, 1 H) 10.02 (t, *J*=5.75 Hz, 1 H)

HRMS (M+H) calculated for $C_{17}H_{18}FN_5O_2$: 344.1517, observed: 344.15164.

N-(2-fluoroethyl)-1-((6-methoxy-5-methylpyrimidin-4-yl)methyl)-6-methyl-1H-pyrrolo[3,2-b]pyridine-3-carboxamide (3)

ES+MS m/z: 358

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.24 (s, 3 H) 2.40 (s, 3 H) 3.62 - 3.80 (m, 2 H) 3.93 (s, 3 H) 4.49 (t, *J*=4.99 Hz, 1 H) 4.65 (t, *J*=4.99 Hz, 1 H) 5.64 (s, 2 H) 7.76 (s, 1 H) 8.16 (s, 1 H) 8.32 - 8.43 (m, 2 H) 8.88 (t, *J*=5.75 Hz, 1 H)

HRMS (M+H) calculated for $C_{18}H_{20}FN_5O_2$: 358.16735, observed: 358.16814.

1-((6-(difluoromethoxy)-5-methylpyrimidin-4-yl)methyl)-N-(2-fluoroethyl)-6-methyl-1H-benzo[d]imidazole-4-carboxamide (4)

ES+MS m/z: 394

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.34 (s, 3 H) 2.43(s, 3h) 3.72 (d, *J*=5.09 Hz, 1 H) 3.81 (d, *J*=5.27 Hz, 1 H) 4.53 (t, *J*=4.99 Hz, 1 H) 4.69 (t, *J*=4.99 Hz, 1 H) 5.82 (s, 2 H) 7.50 – 8.04 (m, 3 H) 8.44 (s, 1 H) 8.53 (s, 1 H) 9.96 (t, *J*=5.11 Hz, 1 H)

HRMS (M+H) calculated for $C_{18}H_{18}F_3N_5O_2$: 394.1485, observed: 394.14969.

1-((6-(dimethylamino)-5-methylpyrimidin-4-yl)methyl)-N-(2-fluoroethyl)-6-methyl-1H-benzo[d]imidazole-4-carboxamide (5)

ES+MS m/z: 371

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.27 - 2.35 (m, 3 H) 2.40 - 2.47 (m, 3 H) 2.96 (s, 6 H) 3.31 (s, 1 H) 3.68 - 3.85 (m, 2 H) 4.53 (t, *J*=4.90 Hz, 1 H) 4.69 (t, *J*=4.90 Hz, 1 H) 5.61 (s, 2 H) 7.49 (s, 1 H) 7.73 (s, 1 H) 8.22 (s, 1 H) 8.43 (s, 1 H) 9.99 (t, *J*=5.65 Hz, 1 H)

HRMS (M+H) calculated for $C_{19}H_{23}FN_6O$: 371.19898, observed: 371.19908.

N-(2,2-difluoroethyl)-1-((6-(dimethylamino)-5-methylpyrimidin-4-yl)methyl)-6-methyl-1H-benzo[d]imidazole-4-carboxamide (6)

ES+MS m/z: 389

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.31 (s, 3 H) 2.44 (s, 3 H) 2.97 (s, 6 H) 3.92 (tdd, 2 H) 5.62 (s, 2 H) 6.05-6.42 (tt, 8 Hz, 1 H) 7.52 (s, 1 H) 7.74 (s, 1 H) 8.22 (s, 1 H) 8.45 (s, 1 H) 10.03 (t, *J*=5.93 Hz, 1 H)

HRMS (M+H) calculated for $C_{19}H_{22}F_2N_6O$: 389.18956, observed: 389.19021.

N-(2-fluoroethyl)-6-methoxy-1-((6-methoxy-5-methylpyrimidin-4-yl)methyl)-1H-benzo[d]imidazole-4-carboxamide (7)

ES+MS m/z: 374

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.26 (s, 3 H) 3.70 - 3.84 (m, 5 H) 3.94 (s, 3 H) 4.53 (t, *J*=4.90 Hz, 1 H) 4.69 (t, *J*=4.99 Hz, 1 H) 5.71(s, 2 H) 7.30 (d, *J*=2.45 Hz, 1 H) 7.47 (d, *J*=2.45 Hz, 1 H) 8.40 (s, 1 H) 8.43 (s, 1 H) 9.98 (t, *J*=5.11 Hz, 1 H).

HRMS (M+H) calculated for $C_{18}H_{20}FN_5O_3$: 374.16226, observed: 374.1628.

N-(2,2-difluoroethyl)-1-((6-(difluoromethoxy)-5-methylpyrimidin-4-yl)methyl)-6-methoxy-1H-benzo[d]imidazole-4-carboxamide (8)

ES+MS m/z: 428

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.34 (s, 3 H) 3.79 (s, 3 H) 3.82 - 4.04 (m, 2 H) 5.82 (s, 2 H) 6.06-6.44 (tt, 1 H) 7.35 (d, *J*=2.45 Hz, 1 H) 7.49 (d, *J*=2.45 Hz, 1 H) 7.57- 8.04 (s, 1 H) 8.41 (s, 1 H) 8.54 (s, 1 H) 10.02 (t, *J*=6.03 Hz, 1 H)

HRMS (M+H) calculated for $C_{18}H_{17}F_4N_5O_3$: 428.134, observed: 428.1348.

1-((6-(dimethylamino)-5-methylpyrimidin-4-yl)methyl)-N-(2-fluoroethyl)-6-methoxy-1H-benzo[d]imidazole-4-carboxamide (9)

ES+MS m/z: 387

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.31 (s, 3 H) 2.96 (s, 6 H) 3.70 - 3.86 (m, 5 H) 4.53 (t, *J*=4.99 Hz, 1 H) 4.69 (t, *J*=4.90 Hz, 1 H) 5.60 (s, 2 H) 7.29 (d, *J*=2.45 Hz, 1 H) 7.48 (d, *J*=2.45 Hz, 1 H) 8.25 (s, 1 H) 8.37 (s, 1 H) 9.99 (t, *J*=5.65 Hz, 1 H).

HRMS (M+H) calculated for $C_{19}H_{23}FN_6O_2$: 387.19389, observed: 387.19438.

N-(2,2-difluoroethyl)-1-((6-(dimethylamino)-5-methylpyrimidin-4-yl)methyl)-6-methoxy-1H-benzo[d]imidazole-4-carboxamide (10)

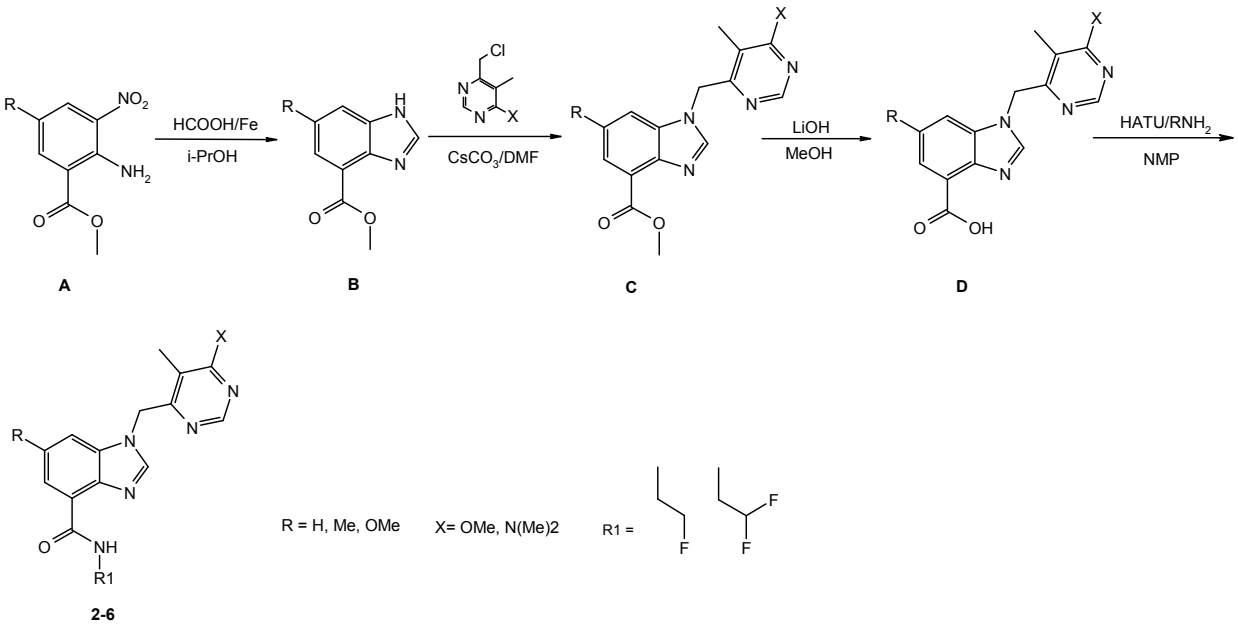
ES+MS m/z: 405

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.31 (s, 3 H) 2.97 (s, 6 H) 3.8 (s, 3 H) 3.92 (tdd, 2 H) 5.61 (s, 2 H) 6.05-6.43 (tt, *J*=3.58 Hz, 1 H) 7.32 (d, *J*=2.45 Hz, 1 H) 7.48 (d, *J*=2.45 Hz, 1 H) 8.24 (s, 1 H) 8.39 (s, 1 H) 10.03 (t, *J*=5.93 Hz, 1 H).

HRMS (M+H) calculated for $C_{19}H_{22}F_2N_6O_2$: 405.18447, observed: 405.18462.

Synthesis of key intermediates and final compounds

Scheme 1



General Procedures

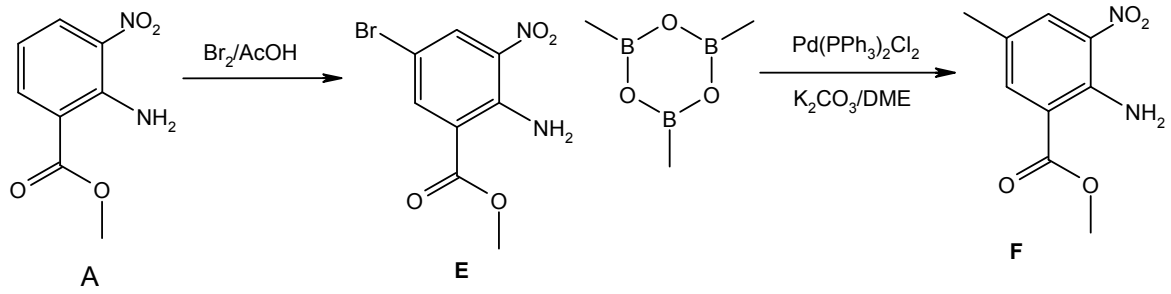
Intermediate B: Methyl 2-amino-3-nitrobenzoate **A** (1 mmol), ammonium chloride (15 mmol) and iron (10 mmol) were taken in iPrOH (5 ml) and formic acid (5 mL). The reaction mixture was stirred at 80 °C for 2h. The reaction mixture was diluted with 2-PrOH (20 mL) and filtered to remove insoluble material. The filtrate was concentrated to dryness, and the resulting residue partitioned between CH_2Cl_2 (50 mL) and (10 mL) sat. aq $NaHCO_3$. The aqueous layer was extracted with additional CH_2Cl_2 (5 × 30 mL). The combined organic extracts were dried over sodium sulphate, filtered, and concentrated to yield the pure solid of Intermediate **B.**

Intermediate C: A solution of Intermediate **B** (1 mmol) in DMF (5 mL) and cesium carbonate (3 mmol) was added. The mixture was stirred for 10 min at rt and then 4-(chloromethyl)-X-5-methylpyrimidine (1.5 mmol) was added followed by addition of sodium iodide (1.5 mmol). The resulting mixture was stirred at rt for 5h. The reaction mixture was diluted with ethyl acetate (25 mL) and washed with water. The organic layer was dried over sodium sulphate, filtered and concentrated to give residue. The crude was taken in minimum amount of methanol and excess of water to precipitate solid. Solid was filtered and dried under high vacuum to afford a solid of Intermediate **C**.

Intermediate D: To a solution of Intermediate **C** (1 mmol) in MeOH (5 mL), 5M solution of lithium hydroxide was added. The mixture was heated to 60^{O}C for 2h. The reaction mixture was cooled and concentrated to get residue which was acidified carefully with 2N HCL to precipitate solid. Solid was filtered and dried under high vacuum to afford a solid of Intermediate **D**.

Final compounds 2-6: A solution of Intermediate **D** (1 mmol) in NMP (2 mL) and HATU (1.2 mmol) was added. The mixture was stirred for 10 min at rt. Amine (1.2 mmol) and triethyl amine (2.5 mmol) were added and stirred for 1h at rt. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was dried over sodium sulphate and concentrated to give gum. Crude compound was purified by flash chromatography eluting with 0-10% MeOH:DCM. The pure fractions were concentrated to afford final compounds **2-6**.

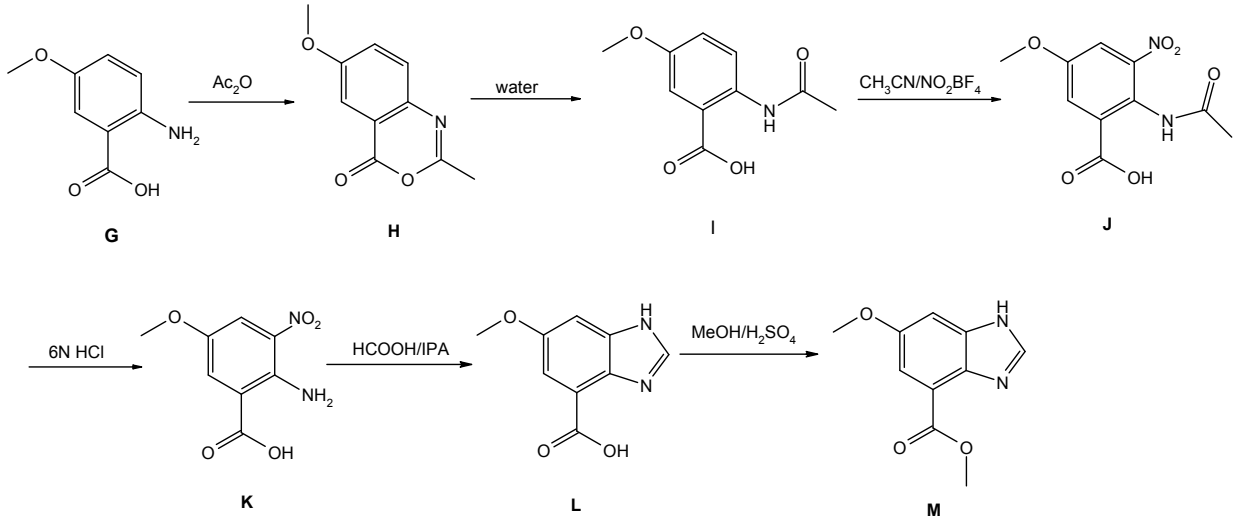
Scheme 2



Methyl 2-amino-5-bromo-3-nitrobenzoate (E): To a solution of methyl methyl 2-amino-3-nitrobenzoate (2 g, 10.20 mmol) in 20 mL of acetic acid, a solution of bromine (0.525 mL, 10.20 mmol) in 20mL of acetic acid was added drop wise over 5 minutes. The mixture was stirred at room temperature for 30 minutes and poured into 100 grams of ice. The precipitated yellow solid was collected by suction filtration and dried to afford as a yellow solid 2.65 g of compound **E** (90 %). ES+MS m/z: 273.

Methyl 2-amino-5-methyl-3-nitrobenzoate (F): A solution of compound **E** (2 g, 7.27 mmol) in 1,4-dioxane (80ml), potassium carbonate (3.01 g, 21.81 mmol), bis(triphenylphosphine)palladium(II) chloride (0.510 g, 0.73 mmol) and 2,4,6-trimethyl-1,3,5,2,4,6-trioxatriborinane (1.369 g, 10.91 mmol) were added. The resulting mixture was stirred at 110°C for O/N. The reaction mixture was concentrated to dryness to get solid. The solid was taken in ethyl acetate (200 mL) and washed with water. The organic layer was dried over sodium sulphate to get gum. The crude was purified by flash chromatography eluting with 0-50% EtOAc:Hexane. The pure fractions were concentrated to afford a pale yellow color solid 1.250 g of compound **F** (80 %). ES+MS m/z: 211.

Scheme 3



6-Methoxy-2-methyl-4H-benzo[d][1,3]oxazin-4-one (H): 2-amino-5-methoxybenzoic acid (5 g, 29.91 mmol) and acetic anhydride (56.4 ml, 598.22 mmol) were taken and stirred at rt for O/N. The reaction mixture was concentrated to give solid 5.72 g of compound **H** (90 %). ES+MS m/z:192 (M-1).

.**2-Acetamido-5-methoxybenzoic acid (I):** A suspension compound **H** (5.72 g, 29.92 mmol) in water (150 mL) was heated to 85 ^{o}C for 3h. After 3h heating, LCMS indicated complete conversion to the product. The reaction mixture was cooled to room temperature and filtered the solid. The solid was washed with water thorougly and dried under high vacuum to yield as a pale pink color solid 5.51 g of compound **I** (80 %). ES+MS m/z: 208.26 (M-1).

2-Acetamido-5-methoxy-3-nitrobenzoic acid (J): Nitronium tetrafluoroborate (3.49 g, 26.29 mmol) was dissolved in acetonitrile (30 mL) was added drop wise to an ice cold solution of compound **I** (5.5 g, 26.29 mmol) in acetonitrile (70 mL), the reaction mixture was stirred for 30 min and then poured into ice to precipitate solid. The solid was collected by filtration and dried to give as a pale yellow solid 5.68 g of compound **J** (80 %). ES+MS m/z:253 (M-1).

2-Amino-5-methoxy-3-nitrobenzoic acid (K): A solution of compound **J** (5.68 g, 22.34 mmol) in MeOH (50 mL), and then 6N HCl (100 mL, 3.93 mmol) was added and the mixture was heated to 85 ^{o}C for O/N. The reaction mixture was concentrated to dryness to get orange colored solid 4.74 g of compound **K** (90 %). ES+MS m/z:213 (M+1).

6-methoxy-1H-benzo[d]imidazole-4-carboxylic acid (L): Compound **K** (4.74 g, 22.34 mmol), iron (18.72 g, 335.13 mmol) (crude) and ammonium chloride (23.90 g, 446.83 mmol) were taken in iPrOH (240 mL) and formic acid (240 mL). The reaction mixture was stirred at 80 °C for 2h. The reaction mixture was diluted with 2-PrOH (300 mL) and filtered to remove insoluble materials. The filtrate was concentrated to dryness to give brown color solid 4.29 g of compound **L** (90%). The crude was used for next step. ES+MS m/z: 193 (M+1).

Methyl 6-methoxy-1H-benzo[d]imidazole-4-carboxylate (M): Compound **L** (4.29 g, 22.32 mmol) was taken in MeOH (200 mL), cooled to 0 ^{o}C and sulfuric acid (23.80 mL, 446.48 mmol) was added slowly and the reaction mixture was refluxed for O/N. After O/N reflux, the reaction mixture was cooled to rt and concentrated to get thick solution which was acidified with sat. sodium bicarbonate. Then the aqueous layer was extracted with DCM. The organic layer was dried over sodium sulphate and concentrated to give a solid 2.85 g of compound **M** (60 %). ES+MS m/z: 207 (M+1).

Biological assays

Biological assay protocols for MIC determination, cytotoxicity, mutant generation, resistance frequency, whole genome sequencing, DprE1 construct & protein purification, DprE1 enzyme assay & IC_{50} measurement, pharmacokinetics (PK) of benzimidazole compound, *in vivo* efficacy study, solubility assay, plasma protein binding assay, metabolic stability assay (microsomal CL and hepatocyte CL), logD, and hERG assay were used as described before.[3]

Alternate Binding Modes: The binding mode of compound **2** is shown in Figure S1A which is similar to that of compound **3** shown in the main manuscript. Figure S1B shows the other possible modes of binding for the series. The two rotatable bonds for the terminal pyrimidine group leads to other potential binding modes with different orientations of methoxy pyrimidine (shown in peach and yellow, S1B). Additionally, the missing loop res 316-322 in the Mtb DprE1 crystal structure used for modeling (pdb ID: 4WK5) is in close proximity to the pyrimidine unit.

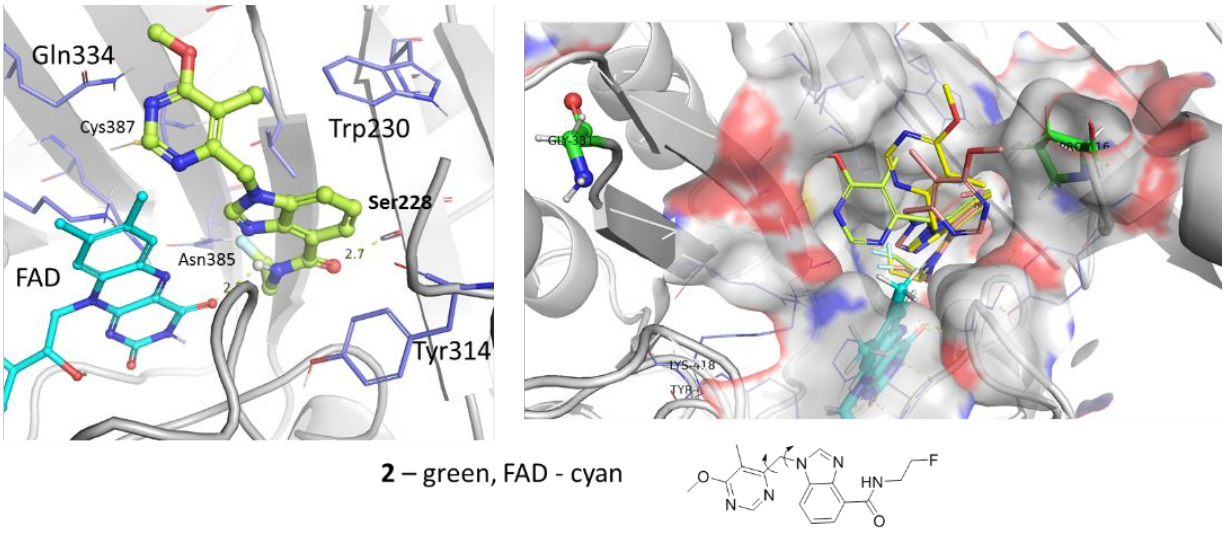


Figure S1. Alternate possible binding modes of BI (**2**) in the DprE1 active site.