***In silico* and *in vitro* study towards the rational design of 4,4´-disarylbisthiazoles as a selective α-synucleinopathy biomarker**

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1. **Chemical synthesis**
   1. **General methods**

All commercial reagents and solvents were used without further purification. The building blocks were purchased from commercial suppliers (e.g. Chemspace (Latvia) and Sigma Aldrich (Germany). Semiprep reversed-phase HPLC (SHP) was performed using a Shimadzu system, which comprises 2 LC-20AP quaternary low-pressure gradient pumps, an SPD-M30A photodiode array detector, and a CBM-20A system controller. Reaction completion (for all the synthesis carried out) and quality control for chemical synthesis was carried out with a high-performance analytical HPLC system (Shimadzu) with a Photo Diode Array detector (Shimadzu) using a Chromolith RP18e (4.6 × 100 mm) column. As eluents were used water (0.1% v/v formic acid) and MeCN (0.1% v/v formic acid) with a flow rate of 3 mL/min. LC/MS experiments were carried out with LCMS-2020 ESI (Shimadzu) connected to the above analytical HPLC system. The 1H (500 MHz), and 13C (126 MHz) spectra were recorded on a cryo Bruker 500 MHz spectrometer. Sonication was performed with an Ultrasonic cleaner USC-TH sonicator (VWR, Germany). Evaporation of solvents was done with Büchi Rotavapor® R-100 and lyophilized with a Christ Alpha 1-2 LDplus freeze dryer.

* 1. **General procedure for the synthesis of the 4-aryl/heteroarylthiazole-2-carbothioamide intermediate, b**

To 1.5 mL dimethylformamide (DMF) solution of dithiooxamide (DTO) 180.3 mg (1.5 mmol. equiv.) in a 10 mL vial while stirring was added dropwise the corresponding phenacyl/heteroacylbromide or a 3,4-methylenedioxy(phenacylbromide) (1 mmol) in 500 µL of DMF and left to react overnight at rt (Scheme 1). Deviations from the general procedure are specified.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | | |
| **b** | **W** | **X** | **Y** | **Z** | **R1** | **R2** | **R3** |
| **b3** | C | C | CH | CH | OH | OCH3 | H |
| **b4** | C | C | CH | CH | F | OCH3 | H |
| **b8** | C | CH | CH | N | H | F | H |
| **b10** | C | N | CH | N | - | F | H |
| **b11** | C | CH | N | N | H | OH | - |
| **b12** | C | C | CH | CH | F | OH | H |
| **b13** | C | CH | CH | CH | H | OH | H |
| **b16** | C | C | CH | CH | H | OH | OH |

**Scheme 1. Synthesis of the 4-aryl/heteroarylthiazole-2-carbothioamides intermediates, b**

* + 1. **4-(benzo[d][1,3]dioxol-5-yl)thiazole-2-carbothioamide, b1**

**b1** was synthesized according to the general procedure using 180.3 mg DTO and 243.1 mg of 1-(benzo[d][1,3]dioxol-5-yl)-2-bromoethan-1-one. Semiprep HPLC purification (SHP) with 30% aqueous MeOH solution, 0.1% THF, 0.1% TFA (solvent A) and MeCN, 0.1 % TFA (solvent B) with a flow-rate of 5 mL/min and a gradient A/B: 80:20 to 18:82 in 17 min. **b1** was obtained in 64.7 ± 3.86 %, (n = 5) yield, with ≥99% HPLC purity. [M+1] 265.0. 1H (DMSO-d6) δ 6.07 (s, 2H), 7.00 (d, J = 5.5 Hz, 1H), 7.61 (dd, J = 4.3, 8.3 Hz, 1H), 7.69 (s, 1H), 8.27 (s, 1H), 9.94 (s, 1H), 10.19 (s, 1H). 13C (DMSO-d6) δ 39.02, 39.19, 39.35, 39.52, 39.69, 39.85, 40.02, 101.30, 106.77, 108.55, 120.25, 121.60, 127.98, 147.54, 147.86, 155.27, 167.64, 186.46

* + 1. **4-(3-hydroxy-4-methoxyphenyl)thiazole-2-carbothioamide, b3**

**b3** was synthesized according to the general procedure using 180.3 mg DTO and 245.1 mg of 2-bromo-1-(3-hydroxy-4-methoxyphenyl)ethan-1-one. SHP with 30% aqueous MeOH solution, 0.1% THF, 0.1% TFA (solvent A) and MeCN, 0.1 % TFA (solvent B) with a flow-rate of 5 mL/min and a gradient A/B: 95:5 to 23:77 in 17 min. **b3** was obtained in 75.6 ± 4.37 %, (n = 2) % yield, with ≥99% HPLC purity. [M+1] 267.0. 1H (DMSO-d6) δ 3.82 (s, 3H), 7.00 (d, J = 5.6 Hz, 1H), 7.33 – 7.59 (m, 2H), 8.16 (s, 1H), 9.05 (s, 1H), 9.87 (s, 1H), 10.18 (s, 1H). 13C (DMSO-d6) δ 55.68, 112.05, 113.71, 117.69, 121.19, 126.74, 146.57, 148.18, 155.85, 167.71, 186.62.

* + 1. **4-(3-fluoro-4-methoxyphenyl)thiazole-2-carbothioamide, b4**

**b4** was synthesized according to the general procedure using 180.3 mg DTO and 247.1 mg of 2-bromo-1-(3-fluoro-4-methoxyphenyl)ethan-1-one. SHP with an aqueous mixture of THF and MeOH solution (20%:10%), 0.1% TFA (solvent A) and MeCN, 0.1 % TFA (solvent B) with a flowrate of 5 mL/min and a gradient A/B: 70:30 to 32:68 in 17 min. **b4** was obtained in 60.0 ± 1.04 %, (n = 2), with ≥99% HPLC purity. [M+1] 296.1. 1H (DMSO-d6) δ 3.89 (s, 3H), 7.21 – 7.29 (m, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.98 (d, J = 13.0 Hz, 1H), 8.34 (s, 1H), 9.98 (s, 1H), 10.21 (s, 1H). 13C (DMSO-d6) δ 39.02, 39.19, 39.35, 39.52, 39.69, 39.85, 40.02, 101.30, 106.77, 108.55, 120.25, 121.60, 127.98, 147.54, 147.86, 155.27, 167.64, 186.46.

* + 1. **4-(6-fluoropyridin-3-yl)thiazole-2-carbothioamide, b8**

**b8** was synthesized according to the general procedure using 180.3 mg DTO and 219.0 mg of 2-bromo-1-(6-fluoropyridin-3-yl)ethan-1-one. SHP with an aqueous mixture of THF and methanol solution (0.2%:40%), 0.1% TFA (solvent A) and acetonitrile, 0.1 % TFA (solvent B) with a flow-rate of 5 mL/min and a gradient A/B: 90:10 to 25:75 in 18 min. **b8** was obtained in 88.2%, n =1 yield, with ≥99% HPLC purity. [M+1] 240.0. 1H (DMSO-d6) δ 7.33 (d, J = 8.5 Hz, 1H), 8.54 (d, J = 3.1 Hz, 1H), 8.61 (d, J = 8.7 Hz, 1H), 8.97 (s, 1H), 10.03 (s, 1H), 10.27 (s, 1H). 13C (DMSO-d6) δ 109.69, 124.24, 128.13, 128.16, 139.67, 139.74, 145.50, 145.63, 151.45, 161.86, 163.75, 168.62, 186.24.

* + 1. **4-(2-fluoropyrimidin-4-yl)thiazole-2-carbothioamide, b10**

To 1.0 mL DMF solution of DTO (62.9 mg, 0.5243 mmol) in a 10 mL vial while stirring was added dropwise 2-bromo-1-(2-fluoropyrimidin-5-yl)ethan-1-one (82.3 mg, 0.3495 mmol) in 0.5 mL of DMF. The mixture was left to run over night at rt. SHP was carried out with 30% aqueous MeCN solution, 0.1% TFA (solvent A) and MeCN, 0.1 % TFA (solvent B) with a flow-rate of 5 mL/min and a gradient A/B: 92:08 to 22:78 in 17 min. **b10** was obtained in 32 %, n = 1 yield, with ≥98% HPLC purity. [M+1] 241.2. 1H (DMSO-d6) δ 8.65 (q, J = 2.4 Hz, 1H), 9.44 (s, 1H), 10.07 (s, 1H), 10.31 (s, 1H). 13C (DMSO-d6) δ 125.99, 127.09, 127.13, 148.82, 159.47, 159.57, 161.37, 163.08, 169.47, 186.50.

* + 1. **4-(5-hydroxypyrimidin-2-yl)thiazole-2-carbothioamide, b11**

**b11** was synthesized according to the general procedure using 180.3 mg DTO and 217 mg of 2-bromo-1-(5-hydroxypyrimidin-2-yl)ethan-1-one. SHP with 30% aqueous MeOH solution, 0.1% THF, 0.1% TFA (solvent A) and MeCN, 0.1 % TFA (solvent B) with a flow-rate of 5 mL/min and a gradient A/B: 90:10 to 30:70 in 17 min. **b11** was obtained in 73.3 ± 2.42 %, (n = 2) yield, with ≥99% HPLC purity. [M+1] 239.2. 1H (DMSO-d6) δ 8.43 (d, J = 2.5 Hz, 2H), 8.50 (d, J = 2.5 Hz, 1H), 9.81 (s, 1H), 10.23 (s, 1H), 10.72 (s, 1H). 13C (DMSO-d6) δ 128.73, 144.52, 150.99, 151.66, 155.06, 168.74, 186.67.

* + 1. **4-(3-fluoro-4-hydroxyphenyl)thiazole-2-carbothioamide, b12**

To 1.0 mL DMF solution of DTO (116.0 mg, 0.9655 mmol) in a 10 mL vial while stirring was added dropwise 2-bromo-1-(3-fluoro-4-hydroxyphenol)ethan-1-one (150 mg, 0.6436 mmol) in 1.0 mL of DMF. The mixture was left to run over night at rt. SHP with 30% aqueous MeOH solution, 0.1% THF, 0.1% TFA (solvent A) and MeCN, 0.1 % TFA (solvent B) with a flow-rate of 5 mL/min and a gradient A/B: 90:10 to 22:78 in 16 min. **b12** was obtained in 76.1 ± 2.51%, (n = 3) yield, with ≥99% HPLC purity. [M+1] 255.2. 1H (DMSO-d6) δ 7.01 (t, J = 8.7 Hz, 1H), 7.69 (dd, J = 2.1, 8.4 Hz, 1H), 7.91 (dd, J = 2.1, 12.7 Hz, 1H), 8.25 (s, 1H), 9.94 (s, 1H), 10.15 (s, 1H), 10.19 (s, 1H). 13C (DMSO-d6) δ 114.23, 114.38, 117.92, 117.95, 121.50, 122.62, 122.64, 125.59, 125.64, 145.30, 145.40, 150.25, 152.16, 154.69, 154.71, 167.77, 186.46.

* + 1. **4-(4-hydroxyphenyl)thiazole-2-carbothioamide, b13**

To 0.5 mL DMF solution of DTO (83.84 mg, 0.6975 mmol) in a 10 mL vial while stirring was added dropwise 2-bromo-1-(4-hydroxyphenyl)ethan-1-one (100 mg, 0.4650 mmol) in 0.5 mL of DMF. The mixture was left to run over night at rt. SHP was carried out with 30% aqueous MeOH solution, 0.1% TFA and 0.1% THF (solvent A) and MeCN, 0.1 % TFA (solvent B) with a flow-rate of 5 mL/min and a gradient A/B: 70:30 to 20:80 in 18 min. **b13** was obtained in 72.2 %, (n = 1) yield, with ≥99% HPLC purity. [M+1] 237.1. 1H (DMSO-d6) δ 6.79 – 6.87 (m, 2H), 7.88 (dd, *J* = 2.2, 8.7 Hz, 2H), 8.14 (d, *J* = 2.1 Hz, 1H), 9.70 (s, 1H), 9.86 (s, 1H), 10.16 (s, 1H). 13C (DMSO-d6) δ 115.51, 120.45, 124.91, 127.80, 156.03, 157.94, 167.67, 186.61.

* + 1. **4-(2,4-dihydroxyphenyl)thiazole-2-carbothioamide, b16**

To 1.0 mL DMF solution of DTO (120 mg, 1.5 mmol) in a 10 mL vial while stirring was added dropwise 4-(2,4-dihydroxyphenyl)thiazole-2-carbothioamide (100 mg, 0.4328 mmol) in 0.5 mL of DMF. The mixture was left to run over night at rt. SHP was carried out with 30% aqueous MeOH solution, 0.1% TFA and 0.2% THF (solvent A) and MeCN, 0.1 % TFA (solvent B) with a flow-rate of 5 mL/min and a gradient A/B: 95:05 to 16:84 in 17 min. The ligandwas obtained in 70.6 %, (n = 1) yield, with ≥99% HPLC purity. [M+1] 253.1. 1H (DMSO-d6) δ 6.32 (dd, *J* = 2.3, 8.6 Hz, 1H), 6.41 (d, *J* = 2.2 Hz, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 8.14 (s, 1H), 9.56 (d, *J* = 1.4 Hz, 1H), 9.91 (s, 1H), 10.13 (s, 1H), 10.14 (s, 1H). 13C (DMSO-d6) δ 102.84, 107.08, 111.69, 122.59, 130.19, 153.42, 156.33, 158.64, 166.17, 186.60.

* 1. **General procedure for the synthesis of the asymmetric DABTAs, d**

To 1.0 mL DMF solution of another phenacyl/heteroacylbromide or a 3,4-methylenedioxy (heteroacylbromide) (1.3 mmol equiv.) (Figure 2) in a 10 mL vial while stirring was added the corresponding 4-aryl/heteroarylthiazole-2-carbothioamides intermediates (**b**) in 500 µL of DMF (quantity is specified for each ligand) and stirred overnight. A solid precipitate (the product, **d**) is formed as a result (deviations will be stated). Centrifugations carried during trituration were done at 10°C. All the ligands were dried in vacuo to remove the organic solvents followed by lyophilization in order to remove residual. The reaction schemes are shown below in Scheme 2a-b.



|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **d** | **W** | **X** | **Y** | **Z** | **R1** | **R2** |
| **d1** | C | C | CH | CH | OH | OCH3 |
| **d2** | C | C | CH | CH | F | OCH3 |
| **d5** | C | CH | CH | N | H | Br |
| **d6** | C | CH | CH | N | H | F |
| **d12** | C | CH | N | CH | H | OH |
| **d14** | C | CH | CH | CH | H | OH |
| **d18** | N | C | CH | CH | Cl | - |

**Scheme 2a**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | | | |
| **d** | **W** | **X** | **Y** | **Z** | **R1** | **R2** | **R3** |
| **d3** | C | C | CH | CH | OH | OCH3 | H |
| **d4** | C | C | CH | CH | F | OCH3 | H |
| **d7** | C | CH | CH | N | H | Br | H |
| **d8** | C | CH | CH | N | H | F | H |
| **d9** | C | N | CH | N | - | Cl | H |
| **d10** | C | N | CH | N | - | F | H |
| **d11** | C | CH | N | CH | OH | OH | - |
| **d13** | C | C | CH | CH | F | OH | H |
| **d15** | C | CH | CH | CH | H | OH | H |
| **d16** | C | C | CH | CH | NO2 | Cl | H |
| **d20** | N | C | CH | CH | Cl | - | H |
| **d22** | C | CH | CH | CH | H | OH | OH |

**Scheme 2b**

**Scheme 2. Synthesis of the asymmetric DABTAs, d**

* + 1. **5-(4'-(benzo[d][1,3]dioxol-5-yl)-[2,2'-bithiazol]-4-yl)-2-methoxyphenol, d1**

**d1** was synthesized according to the general procedure (Section 1.3). 193.1 mg of 2-bromo-1-(3-hydroxy-4-methoxyphenyl)ethan-1-one dissolved in 500 µL DMF was added to 160.0 mg (0.6060 mmol) of **b1** dissolved in 500 µL of DMF in a 10 mL glass vial. The mixture was stirred overnight at 50°C. The resulting mixture was transferred into a 10 mL falcon tube, 5 mL of methanol was added, and the mixture was centrifuged at 6000 r.p.m for 10 min. The resulting sediment was then dissolved in DMSO. Both the supernatant and the dissolved sediment in DMSO solution were then purified using semipreparative HPLC. SHP was carried out with 20% aqueous solution of THF, 0.1% TFA (solvent A) and MeCN, 0.1 % TFA (solvent A) with a flowrate of 5 mL/min and a gradient A/B: 40:60 to 14:86 in 18 min. **d1** was obtained as a pale yellow amorphous solid after solvent removal with ≥99% purity. [M+1] 411.1. 1H (DMSO-d6) δ 3.82 (s, 3H), 6.09 (s, 2H), 7.03 (dd, J = 8.3, 10.5 Hz, 2H), 7.43 (dd, J = 22, 8.4 Hz, 1H), 7.48 (d, J = 2.2 Hz, 1H), 7.59 (d, J = 8.0 Hz, 2H), 8.11 (s, 1H), 8.21 (s, 1H), 9.21 (s, 1H). 13C (DMSO-d6) δ 55.65, 101.37, 106.40, 108.69, 112.35, 113.48, 114.94, 115.36, 117.40, 120.30, 126.42, 127.69, 146.71, 147.58, 147.91, 148.20, 155.15, 155.64, 160.03, 160.31.

* + 1. **4-(benzo[d][1,3]dioxol-5-yl)-4'-(3-fluoro-4-methoxyphenyl)-2,2'-bithiazole, d2**

**d2** was synthesized according to the general procedure (Section 1.3). 15.6 mg bromo-1-(3-fluoro-4-methoxyphenyl)ethan-1-one dissolved in 500 µL DMF was added to 20 mg (0.04849 mmol) of **b1** dissolved in 500 µL of DMF solution in a 10 mL vial. The mixture was stirred overnight at 45°C. The resulting mixture was transferred into a 10 mL falcon tube and centrifuged at 6000 r.p.m for 10 min. The resulting sediment was sonicated twice in 5 mL of acetone for 10 min. Each time followed by centrifugation for 10 min (6000 r.p.m) till 99% HPLC purity of **d2**. **d2** was obtained as pale violet amorphous solid after solvent removal. [M+1] 413.1. 1H (DMSO-d6) δ 3.90 (s, 3H), 6.09 (s, 2H), 7.04 (d, J = 8.0 Hz, 1H), 7.29 (t, J = 8.7 Hz, 1H), 7.56 – 7.61 (m, 2H), 7.80 – 7.89 (m, 2H), 8.23 (s, 1H), 8.30 (s, 1H). 13C (DMSO-d6) 2-δ 56.11, 101.37, 106.39, 108.68, 113.67, 114.13, 115.57, 116.14, 120.29, 122.65, 126.57, 127.64, 147.43, 147.91, 150.65, 152.58, 154.17, 155.20, 160.05, 160.43.

* + 1. **5-(4'-([1,3]dioxolo[4,5-b]pyridin-6-yl)-[2,2'-bithiazol]-4-yl)-2-methoxyphenol, d3**

**d3** was synthesized according to the general procedure (Section 1.3). 188.7 mg of 1-([1,3]dioxolo[4,5-b]pyridin-6-yl)-2-bromoethan-1-one dissolved in 500 µL DMF acidified with 25 μL of glacial acetic acid was added to 158.4 mg (0.5948 mmol) of **b3** dissolved in 500 µL of DMF acidified with 25 μL of glacial acetic acid in a 10 mL glass vial. The mixture was stirred overnight at 50°C. The resulting mixture was transferred into a 10 mL falcon tube, the mixture was centrifuged at 6000 r.p.m for 10 min. The resulting sediment was sonicated twice in 5 mL of MeOH for 10 min. Each time followed by centrifugation for 10 min yielding **d3** with 99% HPLC purity. The resulting supernatants were combined was purified using semiprep HPLC. SHP was carried out with 30% aqueous MeOH solution, 0.4% THF, 0.1% TFA (solvent A) and MeCN, 0.1 % TFA (solvent A) with a flowrate of 5 mL/min and a gradient A/B: 80:20 to 14:86 in 19 min. Overall, **d3** was obtained with ≥99% HPLC purity as a brownish white amorphous solid after solvent removal. [M+1] 412.1. 1H (DMSO-d6) δ 9.26 (s, 1H), 8.29 (d, J = 10.6 Hz, 2H), 8.10 (s, 1H), 7.80 (s, 1H), 7.46 (s, 1H), 7.42 (d, J = 7.4 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.21 (s, 2H), 3.81 (s, 3H). 13C (DMSO-d6)1-δ 55.72, 100.88, 112.30, 112.40, 113.52, 115.23, 116.65, 117.52, 124.52, 126.45, 136.85, 140.54, 146.77, 148.30, 152.64, 155.75, 158.23, 159.79, 160.95.

* + 1. **6-(4'-(3-fluoro-4-methoxyphenyl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, d4**

**d4** was synthesized according to the general procedure (Section 1.3). 23.6 mg of 1-([1,3]dioxolo[4,5-b]pyridin-6-yl)-2-bromoethan-1-one dissolved in 500 µL DMF acidified with 25 μL of glacial acetic acid was added to 20.0 mg (0.5948 mmol) of **b4** dissolved in 500 µL of DMF acidified with 25 μL of glacial acetic acid in a 10 mL glass vial. The mixture was stirred overnight at 45°C. The resulting mixture was then transferred to a 10 mL falcon tube. The rest followed as described in 1.3.2. **d4** was obtained with ≥99% HPLC purity as a pale yellow amorphous solid after solvent removal. [M+1] 414.1. 1H (DMSO-d6/THF-d8 4:3 vol.) δ 3.92 (s, 3H), 6.22 (s, 2H), 7.25 – 7.33 (m, 1H), 7.82 – 7.89 (m, 3H), 8.29 – 8.38 (m, 3H). 13C (DMSO-d6/THF-d8 4:3 vol.) δ 55.90, 100.84, 112.00, 113.45, 113.61, 113.89, 115.99, 116.43, 122.47, 122.50, 124.52, 126.69, 126.74, 136.96, 140.48, 147.57, 147.65, 150.87, 152.80, 152.85, 154.48, 158.26, 160.27, 160.71.

* + 1. **4-(benzo[d][1,3]dioxol-5-yl)-4'-(6-fluoropyridin-3-yl)-2,2'-bithiazole, d6**

**d6** was synthesized according to the general procedure (Section 1.3). 37.5 mg of bromo-1-(6-fluoropyridin-3-yl)ethan-1-one dissolved in 500 µL DMF was added to 35.0 mg (0.1324 mmol) of **b1** dissolved in 500 µL of DMF solution in a 10 mL vial. The mixture was stirred overnight at 35°C. The resulting mixture was transferred into a 10 mL falcon tube and centrifuged at 6000 r.p.m for 10 min. The resulting sediment was sonicated twice in 5 mL of MeOH for 10 min. Each time followed by centrifugation for 10 min (6000 r.p.m) till 99% HPLC purity of **d6**. **d6** was obtained as an off-white amorphous solid after solvent removal. [M+1] 384.0. 1H (DMSO-d6) δ 6.07 – 6.12 (m, 2H), 7.04 (dd, J = 2.8, 7.9 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 7.59 (dd, J = 2.8, 10.5 Hz, 2H), 8.23 – 8.27 (m, 1H), 8.48 – 8.52 (m, 1H), 8.54 – 8.61 (m, 1H), 8.89 (s, 1H). 13C (DMSO-d6) δ 101.40, 106.40, 108.71, 109.85, 110.15, 115.85, 118.31, 120.32, 127.59, 127.90, 127.94, 139.75, 139.81, 145.23, 145.35, 147.64, 147.93, 151.42, 155.28, 159.80, 161.20, 161.84, 163.73.

* + 1. **6-(4'-(6-fluoropyridin-3-yl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, d8**

**d8** was synthesized according to the general procedure (Section 1.3). 46.4 mg of 1-([1,3]dioxolo[4,5-b]pyridin-6-yl)-2-bromoethan-1-one dissolved in 500 µL DMF acidified with 10 μL of glacial acetic acid was added to 35.0 mg (0.1463 mmol) of **b7** dissolved in 500 µL of DMF acidified with 15 μL of glacial acetic acid in a 10 mL glass vial. The mixture was stirred overnight at rt. The resulting mixture was transferred into a 10 mL falcon tube and centrifuged at 6000 r.p.m for 10 min. The resulting sediment was sonicated twice in 10 mL of 1:1 mixture of acetone and methanol for 5 min. Each time followed by centrifugation for 10 min (6000 r.p.m) till 99% HPLC purity of **d8**. **d8** was obtained as an off-white amorphous solid after solvent removal. [M+1] 385.3. 1H (DMSO-d6/THF-d8 4:3 vol.) δ 6.23 (s, 2H), 7.29 – 7.35 (m, 1H), 7.85 (d, J = 5.9 Hz, 1H), 8.34 – 8.40 (m, 2H), 8.54 (s, 1H), 8.61 (s, 1H), 8.93 (d, J = 3.9 Hz, 1H). 13C (DMSO-d6/THF-d8 4:3 vol.) 1-δ 100.85, 109.59, 109.89, 112.02, 116.75, 118.24, 124.46, 127.97, 136.97, 139.55, 139.62, 140.49, 145.27, 145.40, 151.66, 152.93, 158.29, 160.42, 161.07, 162.03, 163.92.

* + 1. **4-(4'-(2-fluoropyrimidin-5-yl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, d10**

**d10** was synthesized according to the general procedure (Section 1.3). 38.0 mg of 1-([1,3]dioxolo[4,5-b]pyridin-6-yl)-2-bromoethan-1-one dissolved in 200 µL DMF acidified with 10 μL of glacial acetic acid was added to 28.8 mg (0.1199 mmol) of **b10** dissolved 400 µL of DMF acidified with 20 μL of glacial acetic acid in a 10 mL glass vial. The rest followed as described in 1.3.2. **d7** was obtained with ≥99% HPLC purity as a yellowish-white amorphous solid after solvent removal. [M+1] 386.3. 1H (DMSO-d6/THF-d8 4:3 vol.) δ 6.22 (s, 2H), 7.85 (d, J = 6.3 Hz, 1H), 8.38 (d, J = 11.8 Hz, 2H), 8.68 (s, 1H), 9.41 (s, 2H). 13C (DMSO-d6/THF-d8 4:3 vol.) δ 100.87, 112.00, 116.97, 119.75, 124.42, 126.46, 137.01, 140.50, 148.63, 153.03, 158.32, 158.82, 158.91, 160.16, 161.18, 161.68, 162.90.

* + 1. **2-(4'-([1,3]dioxolo[4,5-b]pyridin-6-yl)-[2,2'-bithiazol]-4-yl)pyrimidin-5-ol, d11**

**d11** was synthesized according to the general procedure (Section 1.3). 170.0 mg of 1-([1,3]dioxolo[4,5-b]pyridin-6-yl)-2-bromoethan-1-one in 500 µL DMF acidified with 25 μL of glacial acetic acid was added to 128.0 mg (0.5372 mmol) of **b11** dissolved in 500 µL of DMF acidified with 25 μL in a 10 mL glass vial. The mixture was stirred overnight at 45°C. The resulting mixture was transferred into a 10 mL falcon tube and centrifuged at 6000 r.p.m for 10 min. The resulting sediment was sonicated twice in 10 mL of MeOH for 5 min. Each time followed by centrifugation for 10 min (6000 r.p.m) till 99% HPLC purity of **d11**. **d11**was obtained as brown amorphous solid after solvent removal. [M+1] 384.2. 1H (DMSO-d6) δ 6.23 (s, 2H), 7.84 (s, 1H), 8.33 (s, 2H), 8.47 (s, 3H), 10.74 (s, 1H). 13C (DMSO-d6) δ 100.81, 112.27, 116.90, 122.87, 124.42, 136.83, 140.49, 144.61, 150.96, 151.51, 152.62, 154.94, 158.20, 160.41, 160.84

* + 1. **2-(4'-(benzo[d][1,3]dioxol-5-yl)-[2,2'-bithiazol]-4-yl)pyrimidin-5-ol, d12**

**d12** was synthesized according to the general procedure (Section 1.3). 160.0 mg of 2-bromo-1-(5-hydroxypyrimidin-2-yl)ethan-1-one dissolved in 500 µL DMF was added to 150.0 mg (0.5675 mmol) of **b1** dissolved in 500 µL of DMF in a 10 mL glass vial. The mixture was stirred overnight at 40°C. The rest followed as described in 2.2.2.11. ≥99% HPLC pure **d12** was obtained as a yellowish amorphous solid. [M+1] 383.2. 1H (DMSO-d6) δ 6.09 (s, 2H), 7.04 (d, J = 11.4 Hz, 1H), 7.56 – 7.62 (m, 2H), 8.23 (s, 1H), 8.43 – 8.49 (m, 3H), 10.74 (s, 1H). 13C (DMSO-d6) 2- δ 101.39, 106.43, 108.71, 115.67, 120.32, 122.65, 127.66, 144.60, 147.61, 147.93, 150.94, 151.54, 154.90, 155.18, 160.27, 160.63.

* + 1. **4-(4'-([1,3]dioxolo[4,5-b]pyridin-6-yl)-[2,2'-bithiazol]-4-yl)-2-fluorophenol, d13**

**d13** was synthesized according to the general procedure (Section 1.3). 125.0 mg of 1-([1,3]dioxolo[4,5-b]pyridin-6-yl)-2-bromoethan-1-one dissolved in 500 µL DMF acidified with 25 μL of glacial acetic acid was added to 100.0 mg (0.3932 mmol) of **b12** dissolved in 500 µL of DMF acidified with 100 μL glacial acetic acid in a 10 mL glass vial. The mixture was stirred overnight at rt. The rest followed as described in 2.2.2.11. ≥99% HPLC pure **d13** was obtained as an off-white solid. [M+1] 400.3. 1H (DMSO-d6) δ 6.22 (s, 2H), 7.05 (t, J = 8.8 Hz, 1H), 7.68 (dd, J = 2.0, 8.4 Hz, 1H), 7.75 – 7.84 (m, 2H), 8.22 (s, 1H), 8.31 (d, J = 1.9 Hz, 2H), 10.20 (s, 1H). 13C (DMSO-d6) δ 100.81, 112.22, 113.86, 114.01, 115.59, 116.74, 118.10, 118.13, 122.68, 122.71, 124.42, 125.21, 125.26, 136.80, 140.47, 145.37, 145.47, 150.21, 152.12, 152.61, 154.63, 154.65, 158.17, 160.04, 160.71.

* + 1. **4-(4'-(benzo[d][1,3]dioxol-5-yl)-[2,2'-bithiazol]-4-yl)phenol, d14**

**d14** was synthesized according to the general procedure (Section 1.3). 87.5 mg of 2-bromo-1-(4-hydroxyphenyl)ethan-1-one dissolved in 500 µL DMF acidified with 50 μL was added to 75.4 mg (0.2853 mmol) of **b1** dissolved in 500 µL of DMF in a 10 mL glass vial. The mixture was stirred overnight at 40°C. The resulting mixture was transferred into a 10 mL falcon tube and centrifuged at 6000 r.p.m for 10 min.after which it was transferred to a 10 mL falcon tube and Milli-Q® water was added to the mixture until a 10 mL mixture which resulted in the precipitation of **d14**. The mixture was left in the -20°C fridge for 20 min, then was centrifuged at 15°C for 10 min. The rest followed as described in 2.2.2.11. ≥99% HPLC pure **d14** was obtained as a white solid. [M+1] 381.2. 1H (DMSO-d6) δ 6.09 (s, 2H), 6.87 (d, J = 8.7 Hz, 2H), 7.01 – 7.07 (m, 1H), 7.59 (d, J = 7.8 Hz, 2H), 7.85 (d, J = 8.7 Hz, 2H), 8.10 (s, 1H), 8.22 (s, 1H), 9.74 (s, 1H). 13C (DMSO-d6) δ 101.39, 106.41, 108.71, 114.17, 115.37, 115.66, 120.30, 124.64, 127.67, 127.71, 147.60, 147.93, 155.15, 155.87, 157.94, 160.06, 160.36, 162.35.

* + 1. **4-(4'-([1,3]dioxolo[4,5-b]pyridin-6-yl)-[2,2'-bithiazol]-4-yl)phenol, d15**

**d15** was synthesized according to the general procedure (Section 1.3). 47.1 mg of 1-([1,3]dioxolo[4,5-b]pyridin-6-yl)-2-bromoethan-1-one dissolved in 500 µL DMF acidified with 100 μL of glacial acetic acid was added to 30.4 mg (0.1286 mmol) of **b13** dissolved in 300 µL of DMF acidified with 100 μL glacial acetic acid in a 10 mL glass vial. Additional 50 μL glacial acetic acid was added to the mixture. The mixture was stirred overnight at rt. The resulting mixture was transferred into a 10 mL falcon tube and centrifuged at 6000 r.p.m for 10 min. The rest followed as described in 2.2.2.11. ≥99% HPLC pure **d15** was obtained as an off-white amorphous solid. [M+1] 381.2. 1H (DMSO-d6) δ 6.22 (s, 2H), 6.87 (dd, *J* = 2.8, 8.6 Hz, 2H), 7.80 – 7.87 (m, 3H), 8.11 (d, *J* = 2.7 Hz, 1H), 8.31 (t, *J* = 3.5 Hz, 2H), 9.76 (s, 1H). 13C (DMSO-d6) δ 100.84, 112.26, 114.42, 115.70, 116.63, 124.49, 124.62, 127.70, 136.82, 140.51, 152.60, 155.93, 157.98, 158.20, 159.85, 160.95.

* + 1. **4-(4'-([1,3]dioxolo[4,5-b]pyridin-6-yl)-[2,2'-bithiazol]-4-yl)benzene-1,3-diol, d22**

**d22** was synthesized according to the general procedure. 54.2 mg of 1-([1,3]dioxolo[4,5-b]pyridin-6-yl)-2-bromoethan-1-one dissolved in 500 µL DMF acidified with 100 μL of glacial acetic acid was added to 40 mg (0.1585 mmol) of **b16** dissolved in 500 µL of DMF acidified with 100 μL glacial acetic acid in a 10 mL glass vial. The reaction was left to run overnight at 45°C. The resulting mixture was transferred into a 10 mL falcon tube and centrifuged at 6000 r.p.m for 10 min. The rest followed as described in 2.2.2.11. ≥99% HPLC pure **d22** was obtained as an orange-yellow amorphous solid. [M+1] 398.2. 1H (DMSO-d6) δ 6.22 (s, 2H), 6.38 (dd, J = 2.3, 8.5 Hz, 1H), 6.45 (d, J = 2.3 Hz, 1H), 7.82 (d, J = 1.9 Hz, 1H), 7.91 (d, J = 8.5 Hz, 1H), 8.12 (s, 1H), 8.27 – 8.35 (m, 2H), 9.59 (s, 1H), 10.31 (s, 1H). 13C (DMSO-d6) δ 100.77, 102.91, 107.20, 111.52, 112.21, 116.41, 124.48, 129.77, 136.77, 140.44, 152.56, 152.91, 156.31, 158.13, 158.18, 158.63, 161.00.

* 1. **Synthesis of the fluoroethylated and PEGylated DABTAs from the phenol and pyrimidinol DABTAs**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | | | | | |
| **f** | **Z** | **Y** | **R1** | **R2** | **R3** | **R4** | **R5** | **R6** | **LG** |
| **f4** | N | N | H | OH | - | - | H | O(CH2)2F | Br |
| **f5** | CH | N | H | OH | - | - | H | O(CH2)2F | Tos |
| **f6** | CH | N | H | OH | - | - | H | O(CH2)2O(CH2)2F | Br |
| **f7** | CH | N | H | OH | - | - | H | O(CH2)2(O(CH2)2)2F | Br |
| **f8** | N | N | H | OH | - | - | H | O(CH2)2O(CH2)2F | Br |
| **f9** | N | CH | OH | OCH3 | H | H | O(CH2)2O(CH2)2F | OCH3 | Br |
| **f10** | N | CH | F | OH | H | H | F | O(CH2)2(O(CH2)2)2F | Br |
| **f11** | CH | CH | H | OH | H | H | H | O(CH2)2F | Tos |
| **f12** | N | CH | F | OH | H | H | H | O(CH2)2(O(CH2)2)2F | Br |
| **f13** | N | N | H | OH | - | - | H | O(CH2)2(O(CH2)2)2F | Br |
| **f14** | CH | CH | OH | OCH3 | H | H | O(CH2)2(O(CH2)2)2F | OCH3 | Br |
| **f15** | CH | N | H | OH | - | - | H | OCH3 | Tos |
| **f16** | N | N | H | OH | - | - | H | OCH3 | Tos |
| **f17** | N | CH | H | OH | H | H | OCH3 | OCH3 | I |
| **f18** | N | CH | OH | OCH3 | H | OCH3 | H | OCH3 | I |
| **f19** | N | CH | H | OH | OH | H | H | OCH3 | I |
| **f20** | N | CH | H | OH | OH | O(CH2)2O(CH2)2F | H | O(CH2)2O(CH2)2F | Br |
| LG: leaving group, Tos: tosylate | | | | | | | | | | |

**Scheme 3. Synthesis of the Standard ligands f4 to f20**

* + 1. **6-(4'-(5-(2-fluoroethoxy)pyrimidin-2-yl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f4**

K2CO3 (7.2 mg, 0.0521 mmol, 2.0 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 10 mg (0.0261 mmol) of **d11** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 14.1 µL (0.04937 mmol, 2.1 mol. equiv) of TBAH (1M) was added and the mixture was stirred at rt until complete dissolution of the suspension. Subsequently, 2.5 µL (0.0339 mmol, 1.3 mol. equiv.) of 1-bromo-2-fluoroethane was added to the mixture and it was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d11**. 10 µL of 1N NaOH was added to the mixture and it was then transferred to a 10 mL falcon tube. 10 mL of Milli-Q® water was added to the mixture, and it was left for 10 min at 4°C. 1 mL of MeOH was added to the mixture and it was centrifuged at 6000 r.p.m for 10 min. Iterative washing of the sediments was carried out using 5 mL of MeOH, with 2 min sonication followed by centrifugation for 10 min (6000 r.p.m) yielding 99% HPLC purity. MeOH was completely evaporated in vacuo and **f4** was obtained as a white amorphous solid. [M+1] 430.3. 1H (DMSO-d6) δ 4.52 (d, J = 29.9 Hz, 2H), 4.82 (d, J = 48.5 Hz, 2H), 6.23 (s, 2H), 7.81 – 7.90 (m, 1H), 8.31 – 8.37 (m, 2H), 8.48 – 8.59 (m, 1H), 8.67 – 8.78 (m, 2H). 13C (DMSO-d6) δ 68.05, 68.20, 81.34, 82.67, 100.81, 112.26, 116.97, 123.84, 124.40, 136.83, 140.48, 144.37, 151.44, 152.65, 152.86, 154.53, 158.20, 160.60, 160.75.

* + 1. **4-(benzo[d][1,3]dioxol-5-yl)-4'-(5-(2-fluoroethoxy)pyrimidin-2-yl)-2,2'-bithiazole, f5**

K2CO3 (10.8 mg, 0.0784 mmol, 1.5 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 20 mg (0.0523 mmol) of **d12** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 19.2 µL (0.03654 mmol, 1.4 mol. equiv) of TBAH (1M) was added to mixture followed by 13.4 μL (0.07844 mmol, 1.5 mol. equiv.) of 2-fluoroethyl 4-methylbenzenesulfonate.The mixture was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d12**. Further workup follows as described in 2.2.3.1. **f5** was obtained as a white waxy amorphous solid. [M+1] 429.3. 1H (DMSO-d6) δ 4.48 (t, J = 3.6 Hz, 1H), 4.54 (t, J = 3.5 Hz, 1H), 4.77 (t, J = 5.0 Hz, 1H), 4.87 (t, J = 3.3 Hz, 1H), 6.09 (s, 2H), 7.04 (dd, J = 3.2, 8.2 Hz, 1H), 7.59 (dd, J = 3.1, 8.3 Hz, 2H), 8.24 (d, J = 3.3 Hz, 1H), 8.54 (s, 1H), 8.71 (s, 2H). 13C (DMSO-d6) δ 68.05, 68.20, 81.35, 82.67, 101.39, 106.43, 108.70, 115.73, 120.33, 123.61, 127.64, 144.35, 147.62, 147.92, 151.43, 152.89, 154.50, 155.21, 160.18, 160.82.

* + 1. **4-(benzo[d][1,3]dioxol-5-yl)-4'-(5-(2-(2-fluoroethoxy)ethoxy)pyrimidin-2-yl)-2,2'-bithiazole, f6**

K2CO3 (10.8 mg, 0.0784 mmol, 1.5 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 20 mg (0.0523 mmol) of **d12** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 19.2 µL (0.03654 mmol, 1.4 mol. equiv) of TBAH (1M) was added to mixture followed by 7.8 µL (0.0362 mmol, 1.5 mol. equiv.) of 1-bromo-2-(2-fluoroethoxy)ethane. The mixture was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d12**. Further workup follows as described in 2.2.3.1. **f6** was obtained as a white amorphous solid. [M+1] 473.3. 1H (DMSO-d6) δ 3.71 (t, J = 3.7 Hz, 1H), 3.75 – 3.80 (m, 1H), 3.82 – 3.87 (m, 2H), 4.34 – 4.40 (m, 2H), 4.49 – 4.54 (m, 1H), 4.61 (t, J = 3.6 Hz, 1H), 6.10 (s, 2H), 7.04 (d, J = 15.6 Hz, 1H), 7.56 – 7.61 (m, 2H), 8.24 (s, 1H), 8.53 (s, 1H), 8.70 (s, 2H). 13C (DMSO-d6) δ 68.23, 68.81, 69.76, 69.91, 82.41, 83.72, 101.40, 106.43, 108.72, 115.74, 120.34, 123.48, 127.65, 144.30, 147.63, 147.93, 151.71, 152.68, 154.57, 155.22, 160.20, 160.80.

* + 1. **4-(benzo[d][1,3]dioxol-5-yl)-4'-(5-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)pyrimidin-2-yl)-2,2'-bithiazole, f7**

K2CO3 (10.8 mg, 0.0784 mmol, 1.5 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 20 mg (0.0523 mmol) of **d12** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 19.2 µL (0.03654 mmol, 1.4 mol. equiv) of TBAH (1M) was added to mixture followed by 12.0 µL (0.0784 mmol, 2.0 mol. equiv.) of 1-bromo-2-(2-(2-fluoroethoxy)ethoxy)ethane. The mixture was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d12**. Further workup follows as described in 2.2.3.1. **f7** was obtained as a yellowish-white amorphous solid. [M+1] 517.3. 1H (DMSO-d6) δ 3.56 – 3.67 (m, 5H), 3.67 – 3.71 (m, 1H), 3.79 – 3.84 (m, 2H), 4.33 – 4.38 (m, 2H), 4.44 – 4.50 (m, 1H), 4.54 – 4.59 (m, 1H), 6.10 (s, 2H), 7.01 – 7.07 (m, 1H), 7.59 (d, J = 7.7 Hz, 2H), 8.24 (s, 1H), 8.53 (s, 1H), 8.69 (s, 2H). 13C (DMSO-d6) δ 30.75, 68.25, 68.82, 69.66, 69.81, 69.85, 69.95, 82.43, 83.75, 101.41, 106.44, 108.72, 115.74, 120.33, 123.46, 127.65, 144.28, 147.63, 147.94, 151.73, 152.65, 154.58, 155.21, 160.21, 160.79.

* + 1. **6-(4'-(5-(2-(2-fluoroethoxy)ethoxy)pyrimidin-2-yl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f8**

K2CO3 (7.2 mg, 0.0521 mmol, 2.0 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 10 mg (0.0261 mmol) of **d11** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 14.1 µL (0.04937 mmol, 2.1 mol. equiv) of TBAH (1M) was added and the mixture was stirred at rt until complete dissolution of the suspension. Subsequently, 6.0 µL (0.05218 mmol, 2 mol. equiv.) of 1-bromo-2-(2-fluoroethoxy)ethane was added to the mixture and it was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d11**. Further workup follows as described in 2.2.3.1. **f8** was obtained as an off-white amorphous solid. [M+1] 474.3. 1H (DMSO-d6) δ 3.69 – 3.74 (m, 1H), 3.75 – 3.80 (m, 1H), 3.82 – 3.88 (m, 2H), 4.35 – 4.41 (m, 2H), 4.49 – 4.54 (m, 1H), 4.58 – 4.64 (m, 1H), 6.23 (s, 2H), 7.85 (d, J = 1.9 Hz, 1H), 8.31 – 8.37 (m, 2H), 8.56 (s, 1H), 8.70 (s, 2H). 13C (DMSO-d6) δ 68.24, 68.82, 69.76, 69.91, 82.42, 83.74, 100.84, 112.29, 116.99, 123.73, 124.43, 136.85, 140.51, 144.32, 151.74, 152.66, 154.61, 158.22, 160.59, 160.79.

* + 1. **6-(4'-(3-(2-(2-fluoroethoxy)ethoxy)-4-methoxyphenyl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f9**

K2CO3 (10.1 mg, 0.0729 mmol, 2 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 15 mg (0.0364 mmol) of **d3** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 5 min at 80°C. Subsequently, 22.9 µL (0.1017 mmol, 3 mol. equiv) of TBAH (1M) was added to the mixture followed by 10 μL (0.1001 mmol, 2 mol. equiv.) of 1-bromo-2-(2-fluoroethoxy)ethane. The mixture was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d3**. Further workup follows as described in 2.2.3.1. **f9** was obtained as an off-white amorphous solid. [M+1] 502.2. 1H (DMSO-d6) δ 3.70 – 3.76 (m, 1H), 3.76 – 3.86 (m, 6H), 4.18 – 4.24 (m, 2H), 4.50 – 4.55 (m, 1H), 4.59 – 4.65 (m, 1H), 6.22 (s, 2H), 7.08 (d, J = 8.4 Hz, 1H), 7.57 – 7.64 (m, 2H), 7.82 (d, J = 1.9 Hz, 1H), 8.27 (s, 1H), 8.32 (d, J = 1.5 Hz, 2H). 13C (DMSO-d6) δ 55.60, 68.04, 69.05, 69.78, 69.93, 82.49, 83.80, 100.81, 111.19, 112.18, 112.22, 115.51, 116.66, 119.32, 124.44, 126.24, 136.80, 140.48, 148.10, 149.52, 152.59, 155.60, 158.17, 159.90, 160.80.

* + 1. **6-(4'-(3-fluoro-4-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)phenyl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f10**

K2CO3 (10.4 mg, 0.07525 mmol, 1.5 mol. equiv.) was added to 800 µL of DMF which contained a suspension of 20 mg (0.0500 mmol) of **d13** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 5 min at 80°C. Subsequently, 14.2 µL (0.05010 mmol, 2 mol. equiv) of TBAH (1M) was added to the mixture followed by 7.8 µL (0.0362 mmol, 1.5 mol. equiv.) of 1-bromo-2-(2-fluoroethoxy)ethane was dissolved in 200 µL of DMF. The mixture was then heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d13**. Further workup follows as described in 2.2.3.1. **f10** was obtained as an off-white amorphous solid. [M+1] 473.3. 1H (DMSO-d6) δ 3.71 (t, J = 3.7 Hz, 1H), 3.75 – 3.80 (m, 1H), 3.82 – 3.87 (m, 2H), 4.34 – 4.40 (m, 2H), 4.49 – 4.54 (m, 1H), 4.61 (t, J = 3.6 Hz, 1H), 6.10 (s, 2H), 7.04 (d, J = 15.6 Hz, 1H), 7.56 – 7.61 (m, 2H), 8.24 (s, 1H), 8.53 (s, 1H), 8.70 (s, 2H). 13C (DMSO-d6) δ 68.23, 68.81, 69.76, 69.91, 82.41, 83.72, 101.40, 106.43, 108.72, 115.74, 120.34, 123.48, 127.65, 144.30, 147.63, 147.93, 151.71, 152.68, 154.57, 155.22, 160.20, 160.80.

* + 1. **4-(benzo[d][1,3]dioxol-5-yl)-4'-(4-(2-fluoroethoxy)phenyl)-2,2'-bithiazole, f11**

K2CO3 (10.9 mg, 0.07887 mmol, 1.5 mol. equiv.) was added to 800 µL of DMF which contained a suspension of 20 mg (0.05257 mmol) of **d14** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 5 min at 80°C. Subsequently, 19.3 µL (0.07360 mmol, 1.5 mol. equiv) of TBAH (1M) was added to the mixture followed by 16.7 μL (0.0592 mmol, 1.5 mol. equiv.) of 2-fluoroethyl 4-methylbenzenesulfonate. The mixture was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d14**. Further workup follows as described in 2.2.3.1. **f11** was obtained as an off-white solid after lyophilization. [M+1] 427.2. 1H (DMSO-d6) δ 4.24 – 4.29 (m, 1H), 4.30 – 4.35 (m, 1H), 4.70 – 4.75 (m, 1H), 4.80 – 4.85 (m, 1H), 6.09 (s, 2H), 7.04 (d, J = 7.9 Hz, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.59 (d, J = 8.2 Hz, 2H), 7.97 (d, J = 8.8 Hz, 2H), 8.22 (d, J = 4.5 Hz, 2H). 13C (DMSO-d6) δ 67.09, 67.24, 81.53, 82.86, 101.40, 106.41, 108.71, 114.87, 115.17, 115.46, 120.31, 126.48, 127.65, 127.70, 147.60, 147.93, 155.18, 155.30, 158.46, 160.27.

* + 1. **6-(4'-(4-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)phenyl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f12**

K2CO3 (5.4 mg, 0.03907 mmol, 1.5 mol. equiv.) was added to 400 µL of DMF which contained a suspension of 10 mg (0.02622 mmol) of **d15** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 5 min at 80°C. Subsequently, 13.7 µL (0.0524 mmol, 2 mol. equiv) of TBAH (1M) was added to the mixture followed by 7.99 μL (0.0526 mmol, 2 mol. equiv.) of 1-bromo-2-(2-(2-fluoroethoxy)ethoxy)ethane. The mixture was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d15**. Further workup follows as described in 2.2.3.1. **f12** was obtained as an off-white solid after lyophilization. [M+1] 515.2. 1H (DMSO-d6) δ 3.56 – 3.61 (m, 2H), 3.61 – 3.66 (m, 3H), 3.66 – 3.72 (m, 1H), 3.75 – 3.81 (m, 2H), 4.13 – 4.18 (m, 2H), 4.45 – 4.50 (m, 1H), 4.54 – 4.60 (m, 1H), 6.23 (s, 2H), 7.03 – 7.10 (m, 2H), 7.83 (d, J = 1.8 Hz, 1H), 7.92 – 7.99 (m, 2H), 8.22 (s, 1H), 8.32 (d, J = 2.4 Hz, 2H). 13C (DMSO-d6) δ 67.24, 68.97, 69.67, 69.82, 69.87, 69.97, 82.43, 83.75, 100.82, 112.24, 114.83, 115.25, 116.69, 124.45, 126.13, 127.61, 136.81, 140.49, 152.62, 155.43, 158.18, 158.82, 160.00, 160.85.

* + 1. **6-(4'-(5-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)pyrimidin-2-yl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f13**

K2CO3 (5.41 mg, 0.02608 mmol, 1.5 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 10 mg (0.02608 mmol) of **d11** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 14.1 µL (0.04937 mmol, 2.1 mol. equiv) of TBAH (1M) was added and the mixture was stirred at rt until complete dissolution of the suspension7.95 µL (0.0522 mmol, 2.0 mol. equiv.) of 1-bromo-2-(2-(2-fluoroethoxy)ethoxy)ethane was added to the mixture and it was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d11**. 10 mL of Milli-Q® water was added to the mixture, and it was left for 10 min at 4°C. 1 mL of MeOH was added to the mixture and it was centrifuged at 6000 r.p.m for 10 min. Iterative washing of the precipitate was carried out using 5 mL of MeOH and acetone (5:1) with 1.7% glacial acetic acid was added to the resulting sediment with 2 min sonication followed by centrifugation for 10 min (6000 r.p.m) at 10°C yielding 99% HPLC purity. MeOH was completely evaporated in vacuo and **f13** was obtained as an off-white solid after lyophilization. [M+1] 518.2. 1H (DMSO-d6) δ 3.58 – 3.64 (m, 5H), 3.66 – 3.71 (m, 1H), 3.79 – 3.84 (m, 2H), 4.33 – 4.39 (m, 2H), 4.44 – 4.50 (m, 1H), 4.54 – 4.59 (m, 1H), 6.23 (s, 2H), 7.84 (d, *J* = 1.8 Hz, 1H), 8.33 (d, *J* = 1.9 Hz, 1H), 8.34 (s, 1H), 8.55 (s, 1H), 8.69 (s, 2H). 13C (DMSO-d6) δ 68.27, 68.83, 69.67, 69.82, 69.87, 69.96, 82.44, 83.76, 100.84, 112.29, 116.99, 123.71, 124.43, 136.85, 140.51, 144.31, 151.76, 152.63, 152.66, 154.62, 158.22, 160.59, 160.79.

* + 1. **4-(benzo[d][1,3]dioxol-5-yl)-4'-(3-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-4-methoxyphenyl)-2,2'-bithiazole, f14**

K2CO3 (6.74 mg, 0.0487 mmol, 2.0 mol. equiv.) was added to 500 µL of DMF which contained a suspension of 10 mg (0.02436 mmol) of **d1** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 12.5 µL (0.04871 mmol, 2.0 mol. equiv) of TBAH (1M) was added to the mixture followed by 7.42 µL (0.04873 mmol, 2.0 mol. equiv.) of 1-bromo-2-(2-(2-fluoroethoxy)ethoxy)ethane. The mixture was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d1**. Further workup follows as described in 2.2.3.1. **f14** was obtained as an off-white solid after lyophilization. [M+1] 545.2. 1H (DMSO-d6) δ 3.60 (dt, *J* = 2.0, 6.0 Hz, 2H), 3.61 – 3.67 (m, 3H), 3.67 – 3.72 (m, 1H), 3.77 – 3.84 (m, 5H), 4.17 – 4.23 (m, 2H), 4.43 – 4.49 (m, 1H), 4.52 – 4.59 (m, 1H), 6.10 (s, 2H), 7.01 – 7.06 (m, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 7.56 – 7.65 (m, 4H), 8.24 (d, *J* = 11.8 Hz, 2H). 13C (DMSO-d6) δ 55.59, 68.01, 69.05, 69.68, 69.83, 69.90, 69.98, 82.44, 83.76, 101.41, 106.41, 108.73, 111.13, 112.16, 115.30, 115.45, 119.27, 120.30, 126.28, 127.70, 147.61, 147.94, 148.14, 149.48, 155.17, 155.58, 160.15, 160.25.

* + 1. **4-(benzo[d][1,3]dioxol-5-yl)-4'-(5-methoxypyrimidin-2-yl)-2,2'-bithiazole, f15**

K2CO3 (8.13 mg, 0.0588 mmol, 1.5 mol. equiv.) was added to 500 µL of DMF which contained a suspension of 15 mg (0.0262 mmol) of **d12** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 9.6 µL (0.03654 mmol, 1.4 mol. equiv) of TBAH (1M) was added to mixture followed by 7.1 µL (0.04704 mmol, 1.2 mol. equiv.) of methyltosylate. The mixture was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d12**. Further workup follows as described in 2.2.3.1. **f15** was obtained as an off-white solid after lyophilization. [M+1] 397.2. 1H (DMSO-d6) δ 3.99 (s, 3H), 6.10 (s, 2H), 7.02 – 7.08 (m, 1H), 7.57 – 7.63 (m, 2H), 8.25 (s, 1H), 8.54 (s, 1H), 8.68 (s, 2H). 13C (DMSO-d6) δ 56.29, 101.39, 106.42, 108.71, 115.72, 120.32, 123.44, 127.64, 143.85, 147.62, 147.93, 152.30, 152.65, 154.58, 155.20, 160.19, 160.79.

* + 1. **5-(4'-(5-methoxypyrimidin-2-yl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f16**

K2CO3 (5.41 mg, 0.02608 mmol, 1.5 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 10 mg (0.02608 mmol) of **d11** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 14.1 µL (0.04937 mmol, 2.1 mol. equiv) of TBAH (1M) was added and the mixture was stirred at rt until complete dissolution of the suspension 5.1 µL (0.0339 mmol, 1.3 mol. equiv.) of methyltosylate was added to the mixture and it was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d11**. Further workup follows as described in 2.2.3.1. **f16**was obtained as a brown waxy amorphous solid after lyophilization. [M+1] 398.3. 1H (DMSO-d6) δ 4.01 (s, 3H), 6.22 (s, 2H), 7.85 (d, *J* = 3.8 Hz, 1H), 8.33 – 8.43 (m, 2H), 8.53 (t, *J* = 2.9 Hz, 1H), 8.68 (t, *J* = 3.0 Hz, 2H). 13C (DMSO-d6) δ 56.44, 100.03, 111.31, 117.52, 120.71, 123.64, 140.92, 142.11, 142.22, 148.29, 150.03, 151.62, 153.87, 153.89, 160.40, 161.89.

* + 1. **6-(4'-(4-methoxyphenyl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f17**

K2CO3 (5.4 mg, 0.03933 mmol, 1.5 mol. equiv.) was added to 500 µL of DMF which contained a suspension of 10 mg (0.02622 mmol) of **d15** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 5 min at 80°C. Subsequently, 13.7 µL (0.0524 mmol, 2 mol. equiv) of TBAH (1M) was added to the mixture followed by 4.9 μL (0.07864 mmol, 2 mol. equiv.) of methyliodide. The mixture was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d15**.Further workup follows as described in 2.2.3.1. **f17** was obtained as an off-white solid after lyophilization. [M+1] 396.3. 1H (DMSO-d6) δ 3.82 (s, 3H), 6.23 (s, 2H), 7.03 – 7.09 (m, 2H), 7.84 (d, *J* = 1.9 Hz, 1H), 7.94 – 7.99 (m, 2H), 8.22 (s, 1H), 8.33 (d, *J* = 2.6 Hz, 2H). 13C (DMSO-d6) δ 55.28, 100.83, 112.25, 114.34, 115.26, 116.70, 118.19, 124.46, 126.09, 127.61, 136.81, 140.50, 152.62, 155.47, 158.20, 159.60, 160.01, 160.86.

* + 1. **6-(4'-(3,4-dimethoxyphenyl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f18**

K2CO3 (5.1 mg, 0.03645 mmol, 1.5 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 10 mg (0.02430 mmol) of **d3** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 5 min at 80°C. Subsequently, 12.7 µL (0.04860 mmol, 2 mol. equiv) of TBAH (1M) was added to the mixture followed by 4.5 μL (0.07290 mmol, 2 mol. equiv.) of methyiodide. The mixture was heated while stirring at 80°C. The reaction was stopped 1 hour when no further decrease in **d3** was observed. The reaction mixture (suspension) was transferred to 10 mL falcon tube and centrifuged. Iterative washing of the resulting sediment was carried out first with 2 mL of DMF with 5 μL of TBAH (1M), then with 2 mL of only DMF and finally with 5 mL of acetone. The sediment was sonicated with the above solvents for 5 min followed by centrifugation for 10 min (6000 r.p.m) yielding 99% HPLC purity. Acetone was completely evaporated in vacuo and **f18** was obtained as an off-white amorphous solid. [M+1] 426.2. 1H (DMSO-d6) δ 3.71 (s, 1H), 3.83 (s, 4H), 6.23 (s, 3H), 7.07 (d, *J* = 8.3 Hz, 1H), 7.60 (d, *J* = 2.0 Hz, 1H), 7.84 (d, *J* = 1.9 Hz, 1H), 8.28 (s, 1H), 8.32 – 8.37 (m, 3H).

* + 1. **6-(4'-(2,4-dimethoxyphenyl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f19**

K2CO3 (9.9 mg, 0.07171 mmol, 3.0 mol. equiv.) was added to 500 µL of DMF solution of **d22** (9.5 mg, 0.0239 mmol) in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 5 min at 80°C. Subsequently, 18.8 µL (0.07168 mmol, 3.0 mol. equiv) of TBAH (1M) was added, followed by the addition of 6.0 µL (0.0956 mmol, 4 mol. equiv.) of methyliodide was added to the mixture and it was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d22**. Further workup follows as described in 2.2.3.1. **f22** was obtained as a dark brown amorphous solid. [M+1] 426.2. 1H (DMSO-d6) δ 3.84 (s, 3H), 3.95 (s, 3H), 6.23 (s, 2H), 6.67 – 6.74 (m, 2H), 7.83 (d, *J* = 2.0 Hz, 1H), 8.09 – 8.17 (m, 2H), 8.29 – 8.34 (m, 2H). 13C (DMSO-d6) δ 55.41, 55.73, 98.66, 100.82, 105.61, 112.24, 114.79, 116.54, 118.28, 124.49, 130.21, 136.79, 140.49, 151.52, 152.58, 157.88, 158.17, 158.32, 160.68, 161.06.

* + 1. **6-(4'-(2,4-bis(2-(2-fluoroethoxy)ethoxy)phenyl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, f20**

K2CO3 (7.0 mg, 0.05065 mmol, 2.0 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 10 mg (0.0216 mmol) of **d22** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 13.8 µL (0.05283 mmol, 2.1 mol. equiv) of TBAH (1M) was added and the mixture was stirred at rt until complete dissolution of the suspension. Followed by the addition of 12.2 µL (0.1006 mmol, 4 mol. equiv.) of 1-bromo-2-(2-fluoroethoxy)ethane was added to the mixture and it was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d22**. Further workup follows as described in 2.2.3.1. **f20** was obtained as a dark brown amorphous solid. [M+1] 426.2. 1H (DMSO-d6) δ 3.68 – 3.86 (m, 6H), 3.89 – 3.97 (m, 2H), 4.17 – 4.23 (m, 2H), 4.25 – 4.35 (m, 2H), 4.47 – 4.59 (m, 2H), 4.59 – 4.68 (m, 2H), 6.23 (s, 2H), 6.68 – 6.81 (m, 2H), 7.83 (d, *J* = 1.9 Hz, 1H), 8.14 (d, *J* = 8.7 Hz, 1H), 8.26 – 8.34 (m, 3H). 13C (DMSO-d6) δ 67.33, 67.51, 68.77, 68.98, 69.63, 69.77, 69.92, 82.47, 83.79, 100.01, 100.81, 106.49, 112.24, 115.08, 116.53, 118.19, 124.49, 130.15, 136.79, 140.49, 151.55, 152.58, 157.00, 158.17, 158.22, 159.73, 161.07.

* + 1. **6-(4'-(5-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)pyrimidin-2-yl)-[2,2'-bithiazol]-4-yl)-[1,3]dioxolo[4,5-b]pyridine, h13**

K2CO3 (5.41 mg, 0.02608 mmol, 1.5 mol. equiv.) was added to 1000 µL of DMF which contained a suspension of 10 mg (0.02608 mmol) of **d11** in a 10 mL glass vial. The mixture was heated while stirring with a magnetic stirrer for 10 min at 80°C. Subsequently, 14.1 µL (0.04937 mmol, 2.1 mol. equiv) of TBAH (1M) was added and the mixture was stirred at rt until complete dissolution of the suspension. 9.5 µL (0.0522 mmol, 2.0 mol. equiv.) of 1,2-bis(2-iodoethoxy)ethane was added to the mixture and it was heated while stirring at 80°C. The reaction was stopped after the complete consumption of **d11**. Further workup follows as described in 2.2.3.2.1. **h13** was obtained as an off-white solid after lyophilization. [M+1] 426.2. 1H (DMSO-d6) δ 3.33 (d, *J* = 6.4 Hz, 2H), 3.58 – 3.70 (m, 6H), 3.78 – 3.86 (m, 2H), 4.33 – 4.40 (m, 2H), 6.23 (s, 2H), 7.85 (d, *J* = 1.8 Hz, 1H), 8.31 – 8.37 (m, 2H), 8.55 (s, 1H), 8.70 (d, *J* = 2.6 Hz, 2H). 13C (DMSO-d6) δ 5.51, 68.28, 68.85, 69.34, 69.96, 71.02, 100.83, 112.28, 116.98, 123.70, 124.42, 136.84, 140.50, 144.32, 151.76, 152.61, 152.65, 154.62, 158.21, 160.57, 160.78.

1. **Competition binding assays with [3H]DCVJ**
   1. **Alpha-synuclein fibrils**

|  |  |  |  |
| --- | --- | --- | --- |
| **d2** |  | **d4** |  |
| **d6** |  | **d8** | Shape  Description automatically generated with low confidence |
| **d10** |  | **f4** |  |
| **f5** |  | **f6** |  |
| **f7** | **BU-10-FTET aSN** | **f9** | **BU-7-MethoFDET aSN** |
| **f10** | **BU-7-FOFTET aSN** | | |

**Figure 1. α-Syn displacement binding curves of the some DABTAsagainst [3H]DCVJ**

* 1. **Beta-amyloid and tau fibrils**

|  |  |  |  |
| --- | --- | --- | --- |
| **d2** |  | **d4** |  |
| **d6** |  | **d8** | Shape  Description automatically generated with medium confidence |
| **d10** |  | **f4** |  |
| **f5** |  | **f6** |  |

**Figure 2. β-amyloid and tau fibrils displacement binding curves of the some DABTAsagainst [3H]DCVJ**

1. **Competition binding assays with [3H]PIB**
   1. **Alpha-synuclein fibrils**

|  |  |  |  |
| --- | --- | --- | --- |
| **d2** | BY-374-19F aSN | **d4** | BU-7-18F aSN |
| **d6** |  | **d8** | **BU-6-19F aSN** |
| **d10** | BU-8-19F aSN | **f4** | BU-9-FET aSN |
| **f5** | **BU-10-FET aSN** | **f6** | **BU-10-FDET aSN** |
| **f7** | **BU-10-FTET aSN** | **f8** |  |
| **f9** | **BU-7-MethoFDET aSN** | **f10** | **BU-7-FOFTET aSN** |
| **f11** |  | **f12** |  |
| **f13** | **BU-9-TFET aSN** | **f14** |  |
| **f15** |  | **f17** |  |
| **f18** |  | **f19** |  |
| **f20** |  | **h13** | BU-9-TeI aSN |

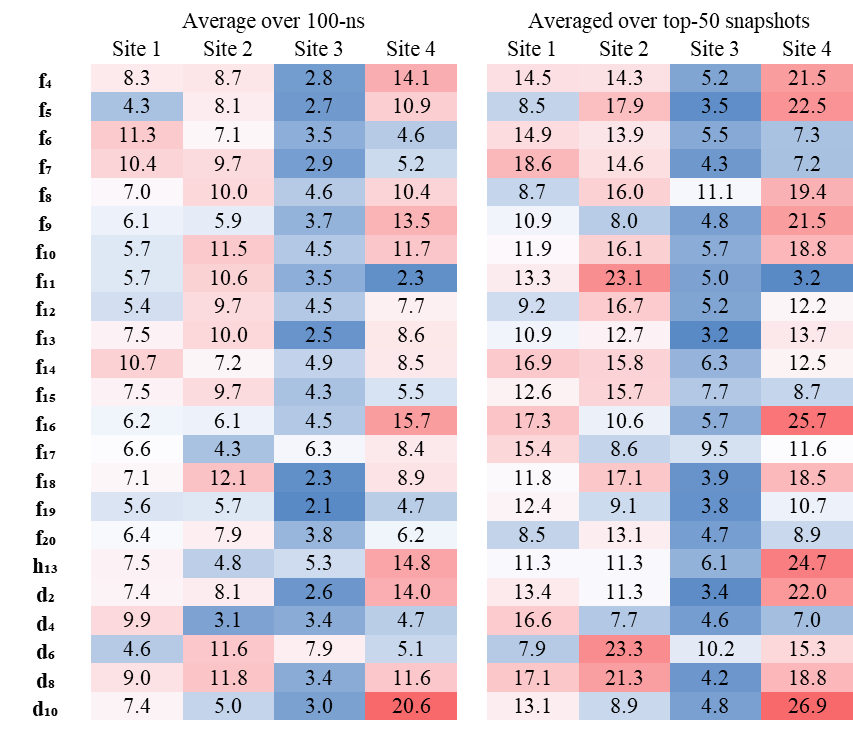
**Figure 3. α-Syn displacement binding curves of the all the DABTAsagainst [3H]PIB**

* 1. **Beta-amyloid and tau fibrils**

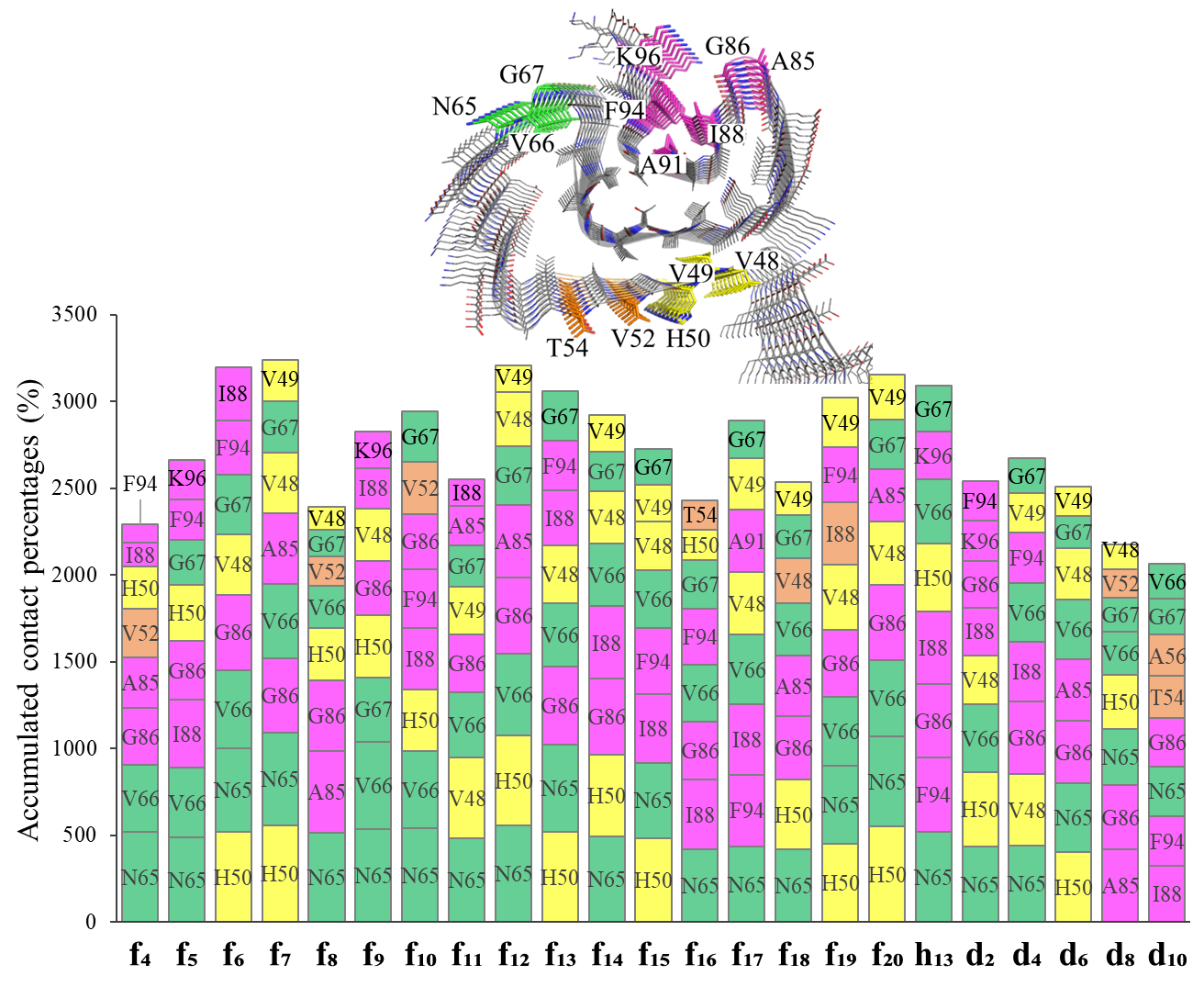
|  |  |  |  |
| --- | --- | --- | --- |
| **d2** | **BY-374-19F Ab Tau** | **d4** | **BU-7-18F Ab Tau** |
| **d6** | **BU-1-19F Ab Tau** | **d8** | **BU-6-19F Ab Tau** |
| **d10** | **BU-8-19F Ab Tau** | **f4** | **BU-9-FET Ab Tau** |
| **f5** | **BU-10-FET  Ab Tau** | **f6** | **BU-10-FDET Ab Tau** |
| **f7** | BU-10-FTET aBeta | **f9** | BU-7-MethoFDET aBeta |
| **f10** | BU-7-FOFTET aBeta | **f11** |  |
| **f12** |  | **f13** | BU-9-TFET abeta |
| **f14** |  | **f15** |  |
| **f17** |  | **f19** |  |
| **f20** |  | | |

**Figure 4. β-amyloid and tau fibrils displacement binding curves all the DABTAsagainst [3H]PIB**

1. **In silico studies**



**Figure 5. Root mean square deviations (RMSDs) of ligand (non-hydrogen atoms) in the 23x4 trajectories**



**Figure 6. Top-8 most contacted surface residues for each compound on α-Syn protofibril throughout 69 (23 compounds \* 3 initial surface sites) 100-ns MD simulations.**

* 1. **Molecular docking and binding free energy calculations**

We adopted the binding sites detected from our previous study with the labels of sites 1-4 [1], in which site-3 is the inner cavity site. The 9-chain protofibril, as it is in the PDB structure, was used and prepared as descripted in previous work [1]. The Glide module in Schrödinger Suite (version 2021-4) was used with the grid center setting to the geometrical center of the residues on the fifth chain of α-Syn protofibril (PDB: 2N0A). To define the grid centers for docking, the geometrical center of the key fifth-chain’s residues was adopted for each site, i.e. G67, G68 and K97 for site-1, G86, I88, F94 and K96 for site-2, E61, T72, G73, A53, V55, T59 and E61 for site-3, and K45, V48 and H50 for site-4. The grid size is assigned to 20 Å.

The docked complexes were subjected to the Prime module implemented in Schrödinger Suite (version 2021-4) for MM/GBSA (molecular mechanics, the generalized Born model, and solvent accessibility) binding free energy calculations. The residues within 8 Å of ligand atoms were energy-minimized by the OPLS4 force field in a VSGB (variable dielectric surface generalized Born) continuum solvation model.

* 1. **Molecular dynamics simulations**

Molecular dynamics (MD) simulations were carried out for the 92 complexes (23 ligands on each of the 4 sites). 100-ns MD simulation was performed for each complex using the Desmond package (version 2021-4) with the similar parameterization of settings to our previous work [2]. Briefly, the OPLS4 force field was used for all the atoms in a solvation box with 10 Å buffering area to the protofibril, which is filled by numbers of sodium/chloride ions (for charge neutralization and a salt concentration of 0.15 M). The Nose-Hoover thermostat and Martyna-Bobias-Klein barostat models were used to maintain the simulations in the NPT ensemble at 300K and 1 atm. Visual Molecular Dynamics (VMD, version 1.9.4a57) and the simulation interaction diagram scripts (implemented in Schrödinger Suite) were used to analyze the trajectories of each system.

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