# Development of a Novel Cell-Permeable Protein-Protein Interaction Inhibitor for the Polo-box Domain of Polo-like Kinase 1

[*‡†§ιο]David J. Huggins, [‡]Bryn S. Hardwick, [‡#]Pooja Sharma, [‡]Amy Emery, [§□]Luca Laraia, [§¶]Fengzhi Zhang, [‡Ω]Ana J. Narvaez, [‡]Meredith Roberts-Thomson, [‡]Alex T. Crooks, [¡]Robert G. Boyle, [¡Þ] Richard Boyce, [¡]David W. Walker, [§]Natalia Mateu, [‡Þ]Grahame J. McKenzie, [§]David R. Spring, [*‡]Ashok R.Venkitaraman

Affiliations:

‡Medical Research Council Cancer Cell Unit, Hutchison/MRC Research Centre, University of Cambridge, Hills Road, Cambridge CB2 2XZ, United Kingdom

†TCM Group, Cavendish Laboratory, University of Cambridge, 19 J J Thomson Avenue, Cambridge CB3 0HE, United Kingdom

§Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom

¡Sentinel Oncology Ltd., Cambridge Science Park, Milton Road, Cambridge CB4 0EY, United Kingdom.

Current Addresses:

ιοTri-Institutional Therapeutics Discovery Institute, Belfer Research Building, 413 East 69th Street, New York, NY 10021, USA

#Cancer Research UK-AstraZeneca Antibody Alliance Laboratory The Portway Building, Granta Park, Great Abington, Cambridge, CB21 6GS, United Kingdom

□Technical University of Denmark, Department of Chemistry, Kemitorvet 207, 2800, Kgs. Lyngby, Denmark

¶Zhejiang University of Technology, College of Pharmaceutical Science, Hangzhou, Hangzhou, Zhejiang, CN 310014, China

ÞPhoreMost Ltd., Babraham Research Campus, Cambridge CB22 3AT, United Kingdom.

ΩDiscovery Sciences, Innovative Medicines and Early Development Biotech Unit, AstraZeneca, Darwin Building, Cambridge CB4 0WG, UK

*Corresponding Authors: Ashok R. Venkitaraman, David J. Huggins

Email: arv22@cam.ac.uk, djh210@cam.ac.uk

**Supplementary Tables**

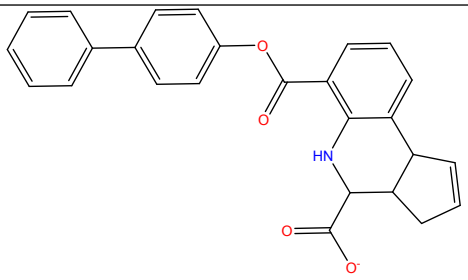
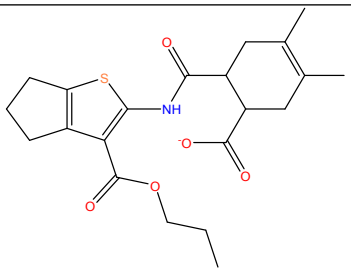
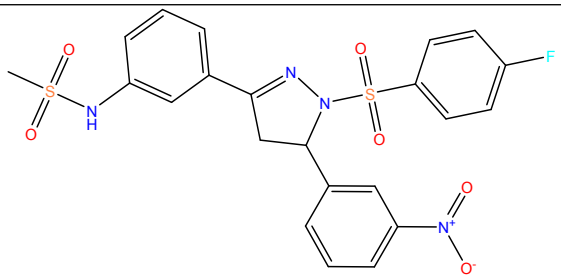
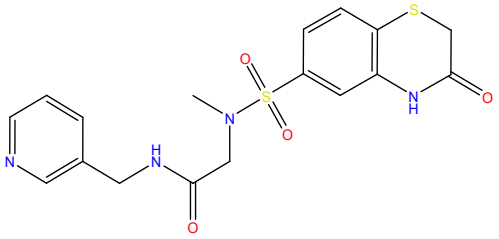
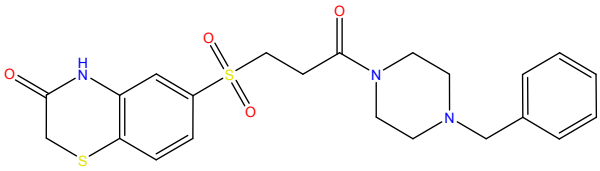
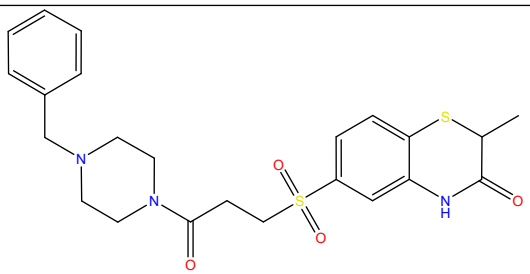
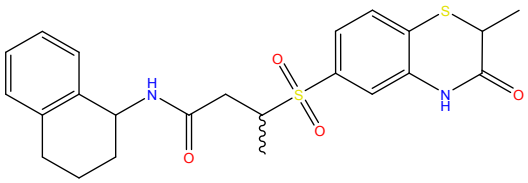
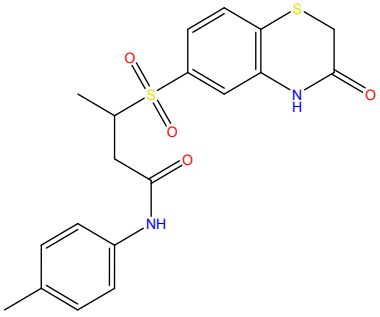
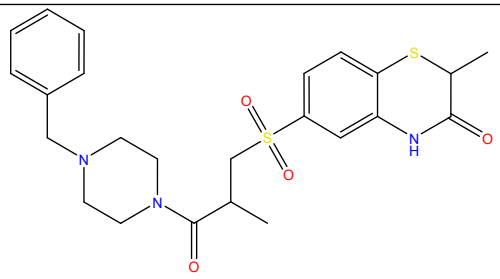
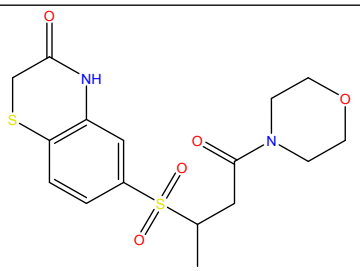
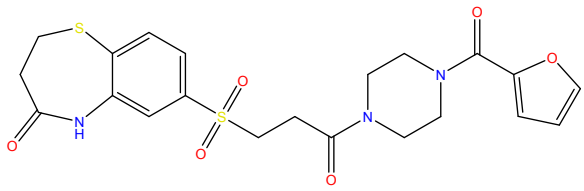
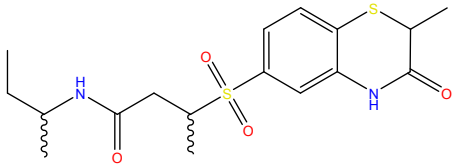
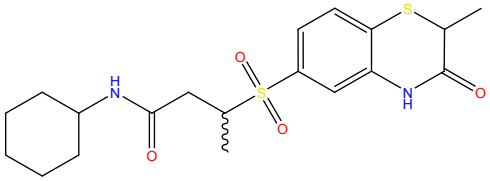
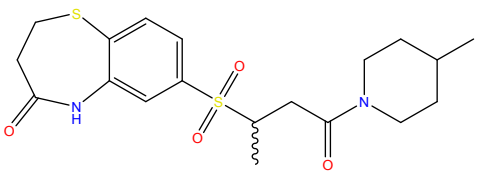
| Compound | Structure | Liability |
|---|---|---|
| **S1** |  | PAINS |
| **S2** |  | PAINS |
| **S3** |  | Fluorescent |

**Table S1** - Examples of primary hits removed due to liabilities. A number of PAINS scaffolds, such as those in compounds **S1** (fused tetrahydroquinolines) and **S2** (2-amino-3-carbonyl-thiophenes), appeared in more than one primary hit but with markedly different substituents.

| Supplier ID | Structure | % inhibition at 50 μM |
|---|---|---|
| E465-0291 |  | < 10% |
| E511-1115 |  | < 10% |
| E511-1355 |  | < 10% |
| E699-2445 |  | < 10% |
| E699-2484 |  | < 10% |

| | | |
|---|---|---|
| E912-0521 |  | < 10% |
| K906-4425 |  | < 10% |
| L705-0165 |  | < 10% |
| E699-2344 |  | < 10% |
| E699-2356 |  | < 10% |
| L705-0464 |  | < 10% |

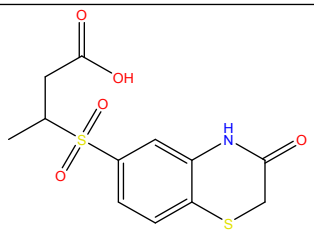
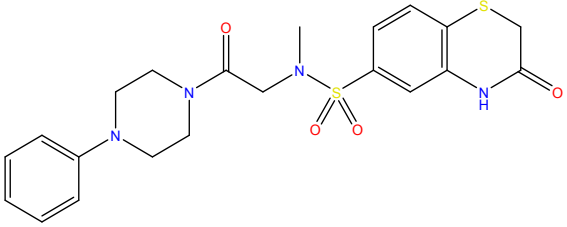
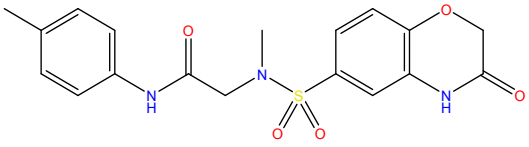
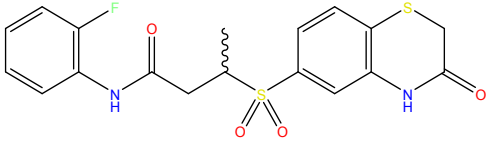
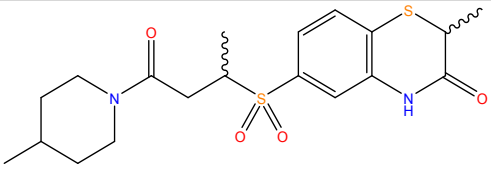
| | | |
|---|---|---|
| BB01-1504 |  | < 10% |
| E465-0345 |  | <10% |
| G805-1078 |  | <10% |
| E699-2373 |  | <10% |
| E699-3202 |  | <10% |

**Table S2:** FP results for a set of compound **1** analogues tested at 50µM. All $IC_{50}$ values were measured by FP and values represent the mean of two independent experiments.

**Supplementary Figures**

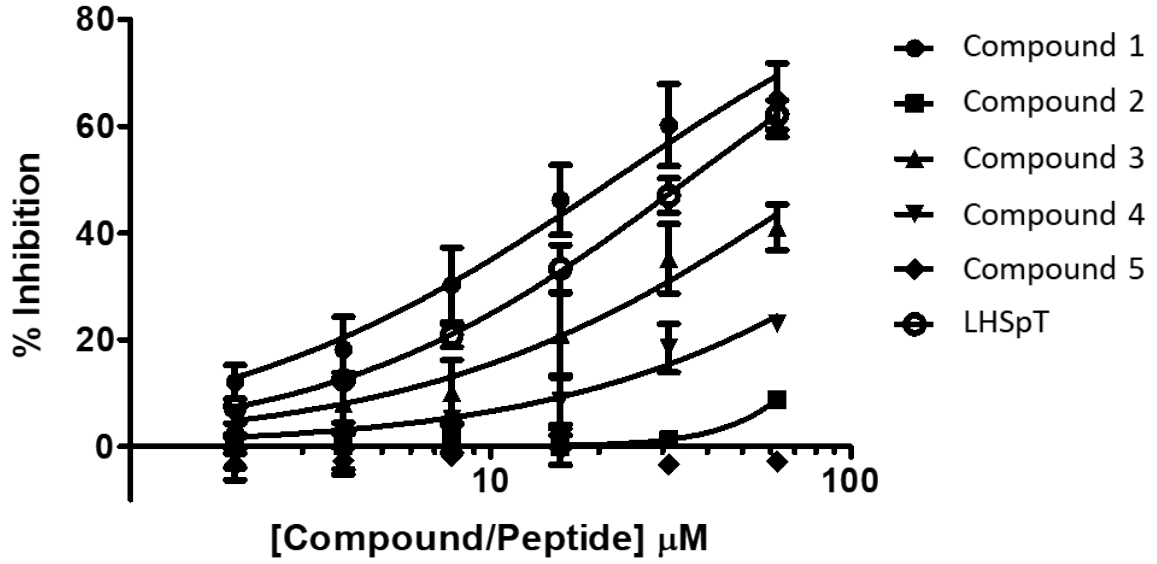



**Figure S1: FP assay curves for the primary hit compound 1 and related analogues.** See Table 1 for structures and quantitation. Peptide LHSpT was included as a positive control and showed 58.5% (± 4.7) inhibition at 50μM. All points are the mean of three independent experiments and error bars indicate SD.

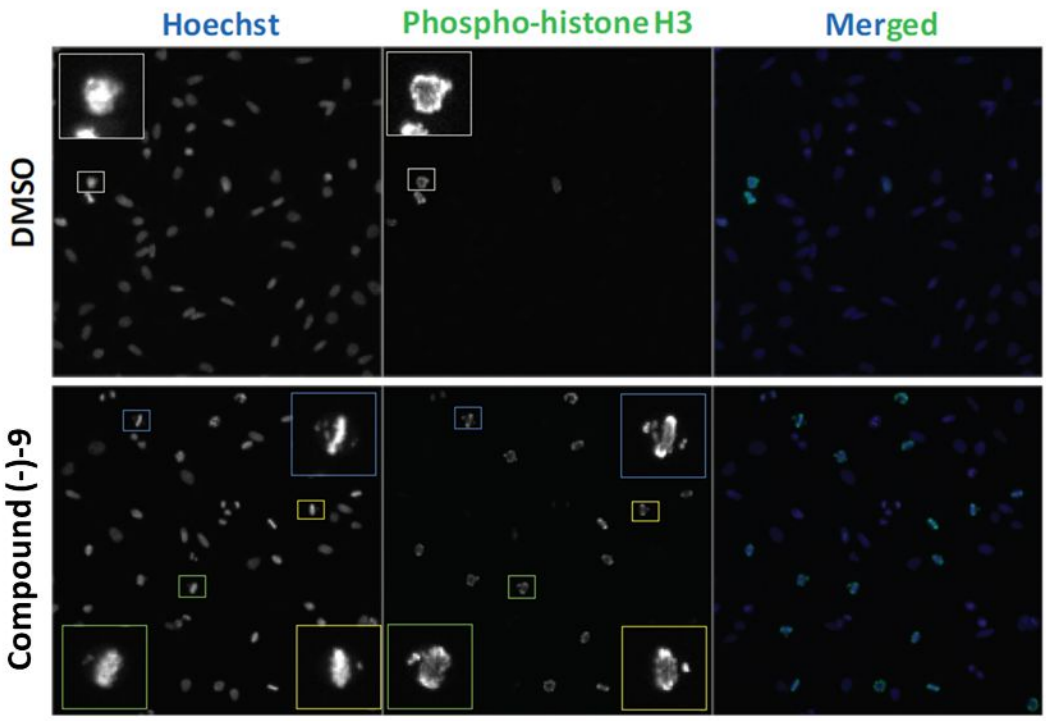



**Figure S2: Treatment with Compound (-)-9 causes mitotic arrest.** Representative images used for mitotic index determination (see Fig5B) from Cellomics ArrayScan with 20X Planfluor objective x 0.4 NA. Cells were stained with Hoechst and phospho-histone H3 (shown in the merged image in blue and green respectively). Insets magnified digitally to show chromosome congression in Compound **(-)-9** treated cells.

## Supplementary Methods

*Compound Purchase*

All analogues were purchased from the supplier ChemDiv at greater than 90% purity.

*Chemical Synthesis*

The general procedure I for amide coupling is shown in Figure S3 and is described below.

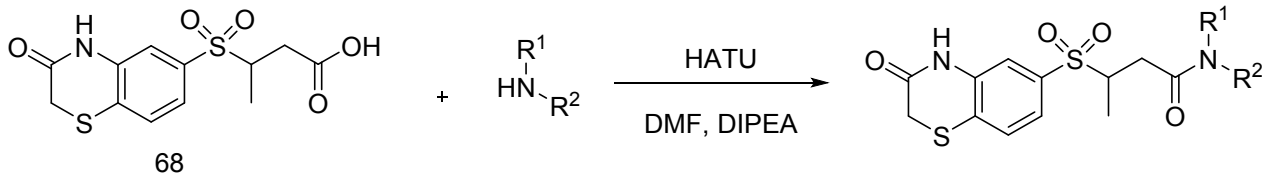
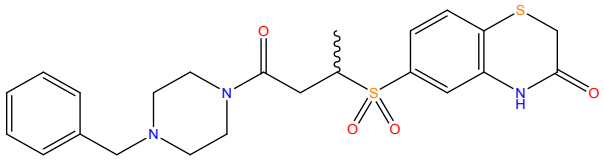



**Figure S3** - The general procedure I for amide coupling

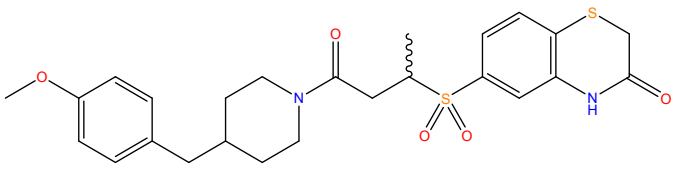
To a solution of Intermediate-68 (100 mg, 1.0 eq., 0.317 mmol) in DMF (1.5 mL) was added HATU (180 mg, 1.5 eq., 0.475 mmol) at 0 °C and reaction was stirred for 30 min at 0 °C. Then was added amine (1.1 eq., 0.348 mmol) at 0 °C followed by drop wise addition of DIPEA (0.16 mL, 3.0 eq., 0.951 mmol) and resulting mixture was stirred at same temperature for 30 min. After completion of the reaction, the mixture was diluted with water (25 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with brine solution (50 mL), dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to afford impure product. The impure product was triturated with pentane to yield pure product.

Compound **6**



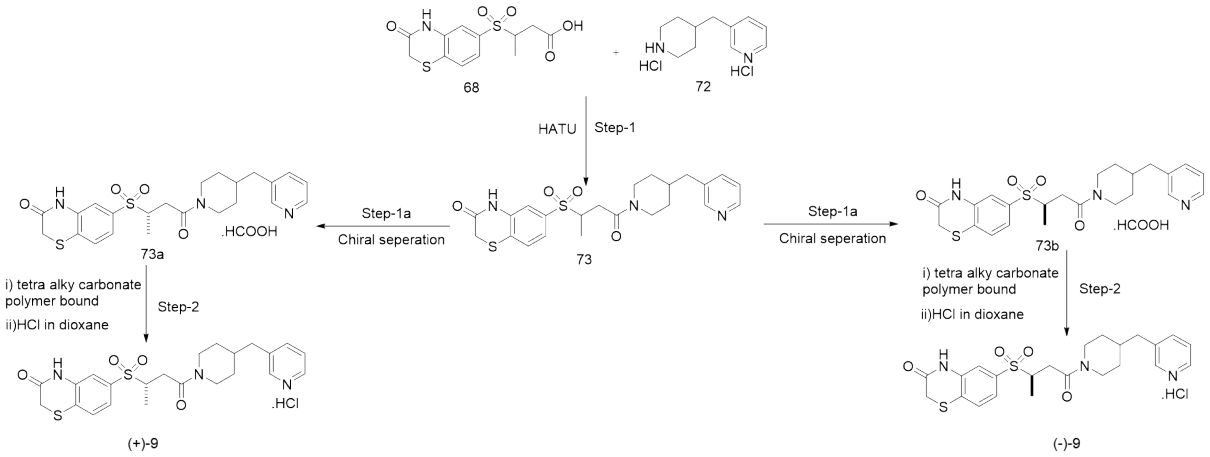
Prepared according to the general procedure I. 1H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 8.56 (ddd, J = 4.9, 1.8, 0.9 Hz, 1H), 8.01 (s, 1H), 7.66 (td, J = 7.6, 1.8 Hz, 1H), 7.49 (t, J = 1.2 Hz, 1H), 7.46 (d, J = 1.1 Hz, 2H), 7.37 (dt, J = 7.9, 1.1 Hz, 1H), 7.18 (ddd, J = 7.5, 4.9, 1.2 Hz, 1H), 3.76 (ddd, J = 9.8, 6.7, 3.2 Hz, 1H), 3.67 (s, 2H), 3.61 (q, J = 4.5 Hz, 2H), 3.52 – 3.45 (m, 4H), 3.04 (dd, J = 16.1, 3.3 Hz, 1H), 2.49 (dt, J = 16.1, 5.8 Hz, 5H), 1.28 (d, J = 6.7 Hz, 3H).

Compound **8**



Prepared according to the general procedure I. 1H NMR (400 MHz, DMSO-d6) δ 10.89 – 10.81 (m, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.44 (dd, J = 6.3, 1.4 Hz, 2H), 7.13 – 7.04 (m, 2H), 6.84 (dd, J = 8.7, 2.1 Hz, 2H), 4.26 (d, J = 12.6 Hz, 1H), 3.72 (d, J = 1.2 Hz, 4H), 3.69 – 3.61 (m, 1H), 3.59 (d, J = 4.0 Hz, 2H), 2.89 – 2.75 (m, 2H), 2.43 (d, J = 7.0 Hz, 4H), 1.74 – 1.47 (m, 3H), 1.18 (dd, J = 6.9, 1.6 Hz, 3H), 1.11 – 0.83 (m, 2H).

**Compounds (-)-9 and (+)-9**

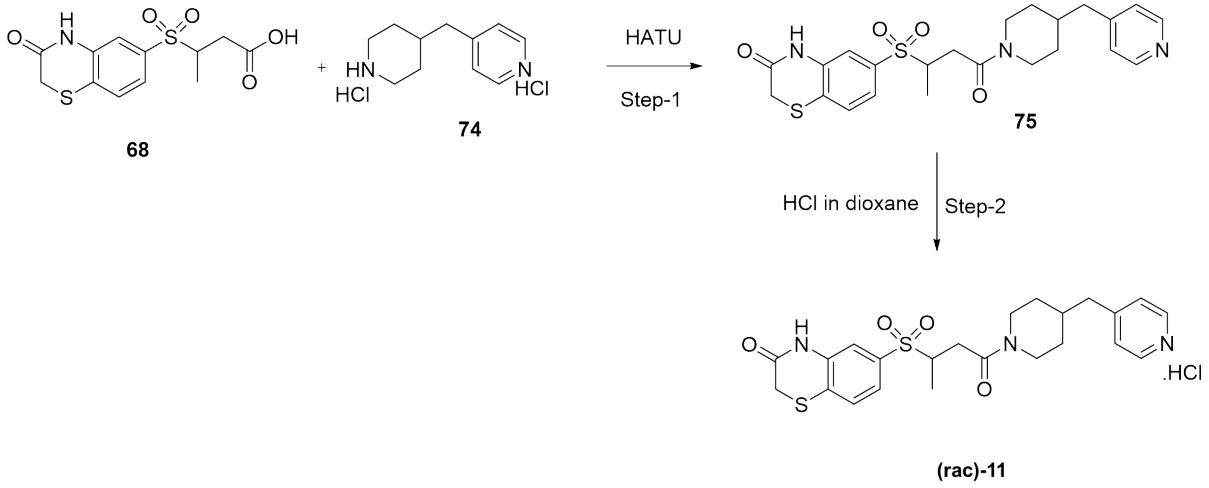



To a solution of Intermediate-68 (100 mg, 1.0 eq., 0.317 mmol) in DMF (1.5 mL) was added HATU (180 mg, 1.5 eq., 0.475 mmol) at 0 °C and reaction was stirred for 30 minutes at 0 °C. Then was added Intermediate-72 (63 mg, 1.1 eq., 0.348 mmol) at 0°C followed by drop wise addition of DIPEA (0.16 mL, 3.0 eq., 0.951 mmol) and resulting mixture was stirred at same temperature for 30 minutes. After completion of the reaction, the mixture was diluted with water (25 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with brine solution (50 mL), dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to afford impure product. The impure product was triturated with pentane to give 110 mg (73%) of pure product. The Intermediate-73 (110 mg) was submitted to Chiral prep HPLC for the separation both enantiomers. The isomer that comes out first from the column was considered as Int-73b and used to prepare **(-)-9** and whereas the second fraction was considered as Int-73a and used to prepare **(+)-9**. To a solution of Intermediate-73b (30 mg, 1.0 eq., 0.063 mmol) in DCM (2 mL) and methanol (0.5 ml) was added tetra alkyl ammonium carbonate polymer bound (40 mg) and stirred at room temperature for 4 h until the pH of the solution became basic. After 4 h, the mixture was filtered, washed with DCM and concentrated. The obtained free base was dissolved in dioxane (1ml) and 4N HCl in dioxane (0.3 mL)

was added at 10°C followed by stirred the reaction mass for 16 h at room temperature. After completion of reaction, reaction mixture was concentrated, triturated with pentane to give 30 mg (98%) of pure product **(-)-9**. 1H NMR (400 MHz, DMSO): 10.88 (d, 1H), 8.78 (s, 2H), 8.35 (d, 1H), 7.95 (m, 1H), 7.63 (t, 1H), 7.44 (t, 2H), 4.27 (d, 1H), 3.77 (d, 1H), 3.60 (s, 3H), 2.92 (b, 1H), 2.86 (bm, 1H), 2.71 (d, 2H), 2.45 (m, 2H), 1.84 (m, 1H), 1.55 (t, 2H), 1.18 (t, 3H), 0.98 (bt, 1H). LCMS: 473.8 (M+H).

To a solution of Intermediate-73a (30 mg, 1.0 eq., 0.063 mmol) in DCM (2 mL) and methanol (0.5 ml) was added tetra alky ammonium carbonate polymer bound (40 mg) and stirred at room temperature for 4h till the pH of the solution became basic. After 4 h, RM was filtered, washed with DCM and concentrated. The obtained free base was dissolved in dioxane (1ml) and 4N HCl in dioxane (0.3 mL) was added at 10 °C followed by stirred the reaction mass for 16 h at room temperature. After completion of reaction, reaction mixture was concentrated, triturated with pentane to give 31 mg (quant.) of pure product **(+)-9** . $[\alpha]^{25}_{D}$ = +27.4 (c 0.2, MeOH). All other data was consistent with **(-)-9**.

**Compound (rac)-11**

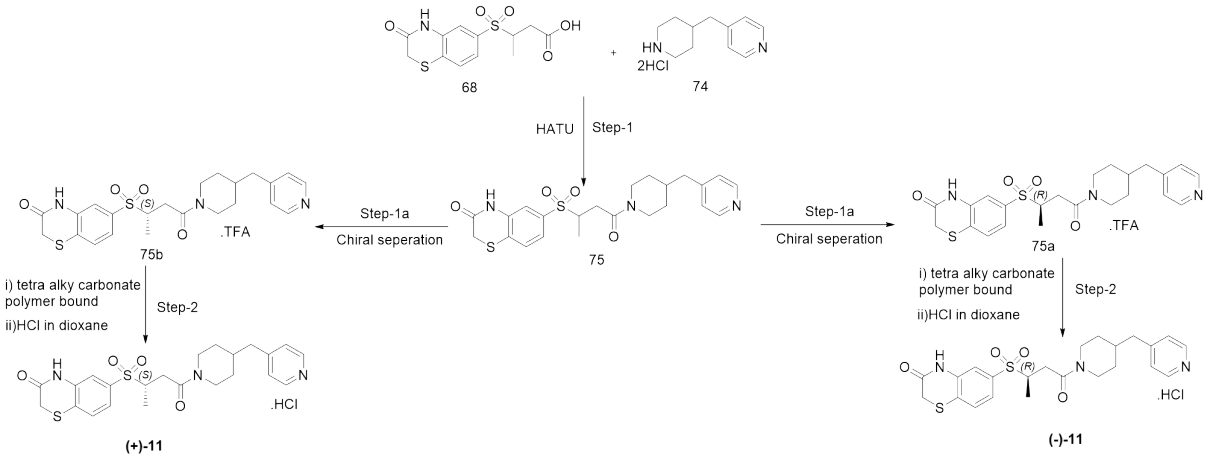



68 74 75 (rac)-11

To a solution of Intermediate-68 (40 mg, 1.0 eq., 0.127 mmol) in DMF (0.5 mL) was added HATU (75 mg, 1.5 eq., 0.19 mmol) at 0 °C and the reaction was stirred for 30 min at 0 °C. Then was added Intermediate-74 (32 mg, 1.1 eq., 0.139 mmol) at 0°C followed by drop wise addition of DIPEA (0.08 mL, 3.0 eq., 0.38 mmol). The resulting mixture was stirred at same temperature for 30 minutes. After completion of the reaction, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed brine solution (25 mL), dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to afford impure product. This was triturated with pentane to give 42 mg (70%) of pure product. To a solution of Intermediate-

75 (42 mg, 1.0 eq., 0.085 mmol) in dioxane (1 mL) was added 4N HCl in dioxane (0.3 mL) at 10 °C and reaction mass was stirred for 16 h at room temperature. After completion of reaction, reaction mixture was concentrated, triturated with pentane to give 35 mg (77%) of pure product. 1H NMR (400 MHz, DMSO): 10.92 (d, 1H), 8.81 (d, 2H), 7.87 (d, 2H), 7.63 (m, 1H), 7.43 (d, 2H), 4.26 (d, 1H), 3.77 (d, 1H), 3.61 (t, 3H), 2.93 (t, 1H), 2.86 (d, 3H), 2.46 (merged with DMSO, 2H), 1.93 (m, 1H), 1.55 (t, 2H), 1.17 (t, 3H), 1.11 (bm, 1H), 1.00 (bt, 1H). LCMS: 473.8 (M+H)

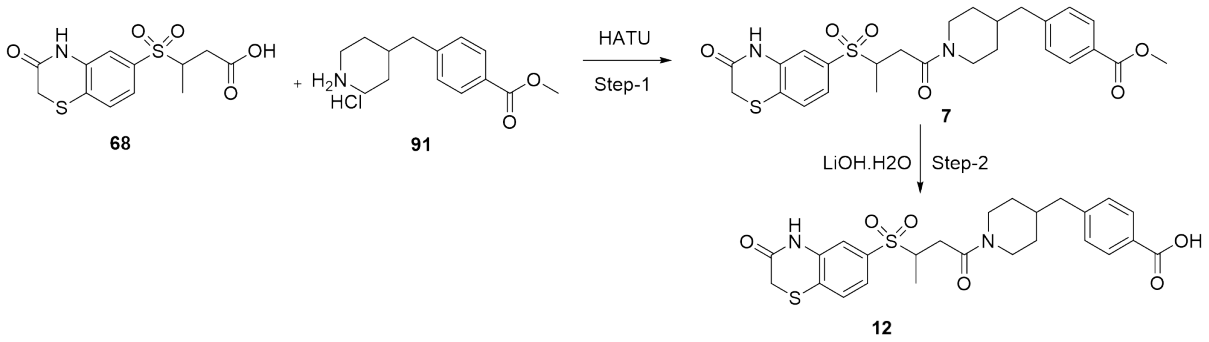
**Compounds (-)-11 and (+)-11**




To a solution of Intermediate-68 (150 mg, 1.0 eq., 0.475 mmol) in DMF (3 mL) was added HATU (540 mg, 1.5 eq., 1.42 mmol) at 0 °C and reaction mass was stirred for 30 min at 0°C. Then was added Intermediate-74 (130 mg, 1.1 eq., 0.523 mmol) at 0 °C followed by drop wise addition of DIPEA (0.4 mL, 3.0 eq., 2.37 mmol) and the resulting mixture was stirred at same temperature for 3 h. After completion of the reaction, the mixture was diluted with cold water and the resulting precipitate was filtered and dried to obtain 130 mg (58%) of pure product. The Intermediate-75 (130 mg) was submitted to Chiral prep HPLC for the separation of R & S isomers. The isomer that comes out first from the column was considered as Int-75a and used to prepare **(-)-11** whereas the second fraction was considered as Int-75b and used to prepare **(+)-11**. To a solution of Intermediate-75a (40 mg, 1.0 eq., 0.084 mmol) in DCM (1 mL) and methanol (1 drop) was added tetra alkyl ammonium carbonate polymer bound (40 mg) and stirred at room temperature for 2 h until the pH of the solution became basic. After 2 h, RM was filtered, washed with DCM and concentrated. The obtained free base was dissolved in dioxane (1 mL) and 4N HCl in dioxane (0.3 mL) was added at 10°C followed by stirring at room temperature for 16 h. After completion of the reaction, the mixture was concentrated, triturated with pentane to give 35 mg (98%) of pure product **(-)-11**. $[\alpha]^{25}_{D}$ = -17.8 (c 0.2, MeOH). All other data consistent with (rac)-**11**.

To a solution of Intermediate-75b (45 mg, 1.0 eq., 0.095 mmol) in DCM (1 mL) and methanol (1 drop) was added tetra alky ammonium carbonate polymer bound (45 mg) and stirred at room temperature for 2 h till the pH of the solution became basic. After 2 h, RM was filtered, washed with DCM and concentrated. The obtained free base was dissolved in dioxane (1 mL) and 4N HCl in dioxane (0.3 mL) was added at 10°C followed by stirring at room temperature for 16 h. After completion of reaction, reaction mass was concentrated, triturated with pentane to give 31 mg (99%) of pure product **(+)-11**. $[\alpha]^{25}_{D}$ = +32.4 (*c* 0.2, MeOH). All other data consistent with (rac)-**11**.

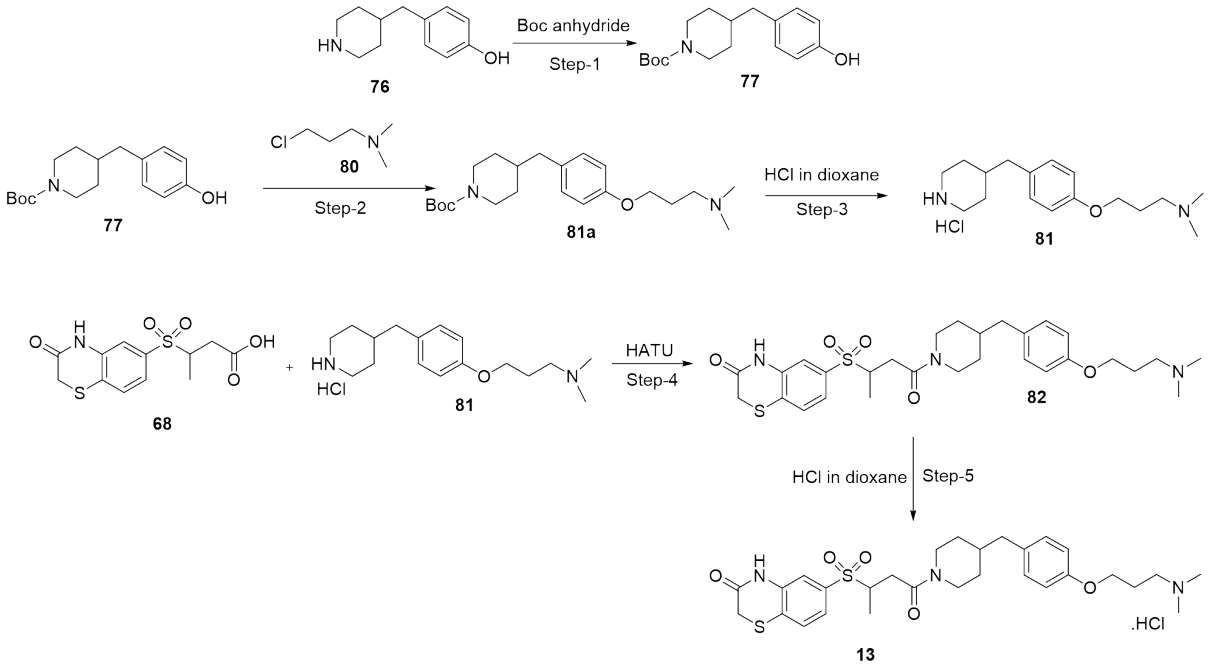
**Compound 12**




To a solution of Intermediate-68 (65 mg, 1.0 eq., 0.2 mmol) in DMF (1 mL) was added HATU (0.117 g, 1.5 eq., 0.3 mmol) at 0 °C and the reaction was stirred for 30 min at the same temperature. To this was added Intermediate-91 (61 mg, 1.1 eq., 0.22 mmol) followed by drop wise addition of DIPEA (0.1 mL, 3.0 eq., 0.3 mmol) at 0 °C and the resulting mixture was stirred at the same temperature for 30 minutes. After completion of the reaction, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (2 x 30 mL). The combined organic layer was washed with brine solution (30 mL), dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to give crude product. The crude product was purified by column chromatography using silica gel of 60-120 mesh size and product was eluted in 1.5% methanol in DCM to give 0.109 g (quant.) of pure product **7**. To a solution of **7** (0.109 g, 1.0 eq., 0.2 mmol) in THF (2 mL) and water (2 mL) was added lithium hydroxide monohydrate (17 mg, 2.0 eq., 0.41 mmol) at room temperature and reaction was stirred at same temperature for 2 h. After completion of the reaction, the mixture was concentrated; the residue was diluted with water (5 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was discarded and the aqueous layer was acidified by dilute HCl. The acidified aqueous layer was extracted with ethyl acetate (2 x 30 mL). The combined organic layers were dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to give crude product. The crude product was purified by preparative HPLC using 0.1% formic acid in water, methanol and ACN to give 25 mg (24%) of pure product. 1H NMR (400 MHz, DMSO): 10.90 (bs, 1H), 7.86 (d, 2H), 7.62 (d, 1H), 7.44 (d, 2H), 7.25 (s, 2H), 4.28 (d, 1H), 3.77 (d, 1H), 3.60 (s, 2H), 3.51 (s, 2H), 2.87 (m, 1H),

2.78 (m, 1H), 1.77 (m, 1H), 1.55 (s, 2H), 1.66 (m, 3H), 1.10 (m, 1H), 0.94 (m, 2H), 0.85 (m, 1H); LCMS: 514.88 (M+H).

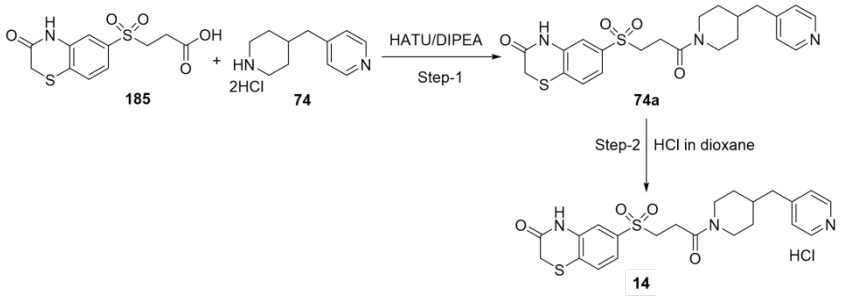
**Compound 13**




To a solution of Intermediate-76 (1 g, 1.0 eq., 4.4 mmol) in dioxane (20 mL) and water (20 mL) was added drop wise boc-anhydride (1.05 g, 1.1 eq., 4.8 mmol) and sodium bicarbonate (3.7 g, 10 eq., 44 mmol) at rt and the reaction mixture was stirred at same temperature for 3 h. After 3 h, the reaction was diluted with water (50 mL) and extracted with ethyl acetate (3 X 50 mL). Combined organics were dried over anhydrous sodium sulphate and volatiles were removed under vacuum followed by trituration with hexanes to give 0.65 g (43%) of pure product. To a solution of Intermediate-77 (0.27 g, 1.0 eq., 0.92 mmol) and Intermediate-80 (0.23 g, 2.0 eq., 1.85 mmol) in DMF (10 mL) was added $Cs_2CO_3$ (0.9 g, 3.0 eq., 2.78 mmol) and KI (catalytic amount) at rt followed by stirring at 80°C for 6 h. After completion of the reaction, the mixture was diluted with water (30 mL) and extracted with ethyl acetate (3 X 25 mL). Combined organics were dried over anhydrous sodium sulphate and volatiles were removed under vacuum. The crude product was purified by column chromatography using neutral silica gel of 60-120 mesh size and 5% MeOH was used as gradient in DCM for elution of product (80 mg, 23%). To a solution of Intermediate-81a (80 mg, 1.0 eq., 0.21 mmol) in dioxane (1 mL) was added 4N HCl in dioxane. (0.3 ml) at 10°C and reaction mixture was stirred at RT for 16 h under $N_2$ atmosphere. After 16 h, reaction mixture was concentrated and triturated with pentane to give 60 mg (90%) of pure product. To a solution of Intermediate-68 (40 mg, 1.0 eq., 0.127 mmol) in DMF (0.5 mL) was added HATU (75 mg, 1.5 eq., 0.19 mmol) at 0°C and the reaction was stirred for 30 minutes at same temperature. Then was added Intermediate-81 (60 mg, 1.2 eq., 0.152 mmol) at 0

°C followed by drop wise addition of DIPEA (0.1 mL, 5.0 eq., 0.63 mmol) and resulting mixture was stirred at same temperature for 30 minutes. After completion of reaction, it was diluted with water (10 mL) and extracted with ethyl acetate (2 x 15 mL). The combined organic layers were washed with brine solution (20 mL), dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to afford crude product. The crude product was purified by flash column chromatography using neutral silica gel of 60-120 mesh size and 2-3% MeOH was used as gradient in DCM for elution of product (30 mg) with 41.22% yield of pure product. To a solution of Intermediate-82 (30 mg, 1.0 eq., 0.052 mmol) in dioxane (0.8 mL) was added 4N HCl in dioxane (0.2 mL) at 10°C and reaction mass was stirred for 16 h at room temperature. After completion of reaction, the mixture was concentrated, triturated with pentane to give 28 mg (88%) of pure product. 1H NMR (400 MHz, DMSO): 10.87 (d, 1H), 7.62 (d, 1H), 7.43 (m, 2H), 7.08 (d, 2H), 6.85 (d, 2H), 4.25 (d, 1H), 4.01 (s, 2H), 3.74 (d, 1H), 3.62 (d, 2H), 3.57 (s, 1H), 3.15 (bs, 2H), 2.90 (bt, 1H), 2.77 (s, 6H), 2.43 (d, 4H), 2.08 (s, 2H), 1.67 (s, 1H), 1.52 (bm, 2H), 1.27 (s, 2H), 1.17 (d, 3H), 1.02 (t, 1H). LCMS: 571.8 (M+H)

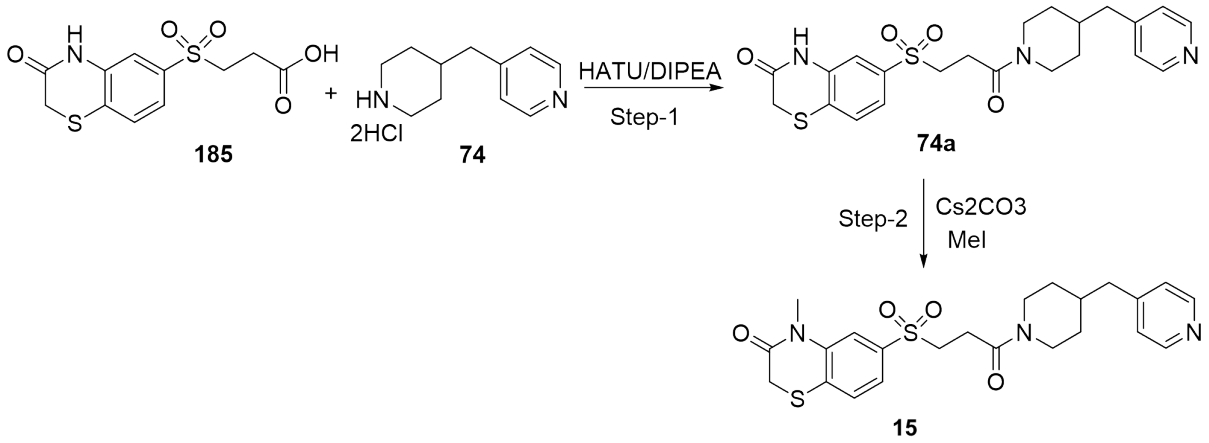
**Compound 14**




To a solution of Intermediate-185 (72 mg, 1.0 eq., 0.24 mmol) in DMF (1 mL) was added HATU (0.137 g, 1.5 eq., 0.36 mmol) at 0°C and the reaction was stirred for 30 minutes at same temperature. Then was added Intermediate-74 (60 mg, 1.0 eq., 0.24 mmol) at 0°C followed by dropwise addition of DIPEA (0.24 mL, 6.0 eq., 1.44 mmol) and the resulting reaction was stirred at the same temperature for 1 h. After completion of the reaction, the mixture was diluted with water (20 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organic layer was washed with brine solution, dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to afford crude product. This was purified by column chromatography using silica gel of 60-120 mesh size and product was eluted in 0.5 % methanol in DCM to give 80 mg (87%) of pure product. To a solution of Intermediate-74a (40 mg, 1.0 eq., 0.087 mmol) in dioxane (1 mL) was added 4N HCl in dioxane (0.2 mL) at 10°C and reaction mass was stirred for 16 h at room temperature. After completion of reaction, reaction mass was concentrated, triturated with pentane to give 40 mg (93%) of pure product. 1H

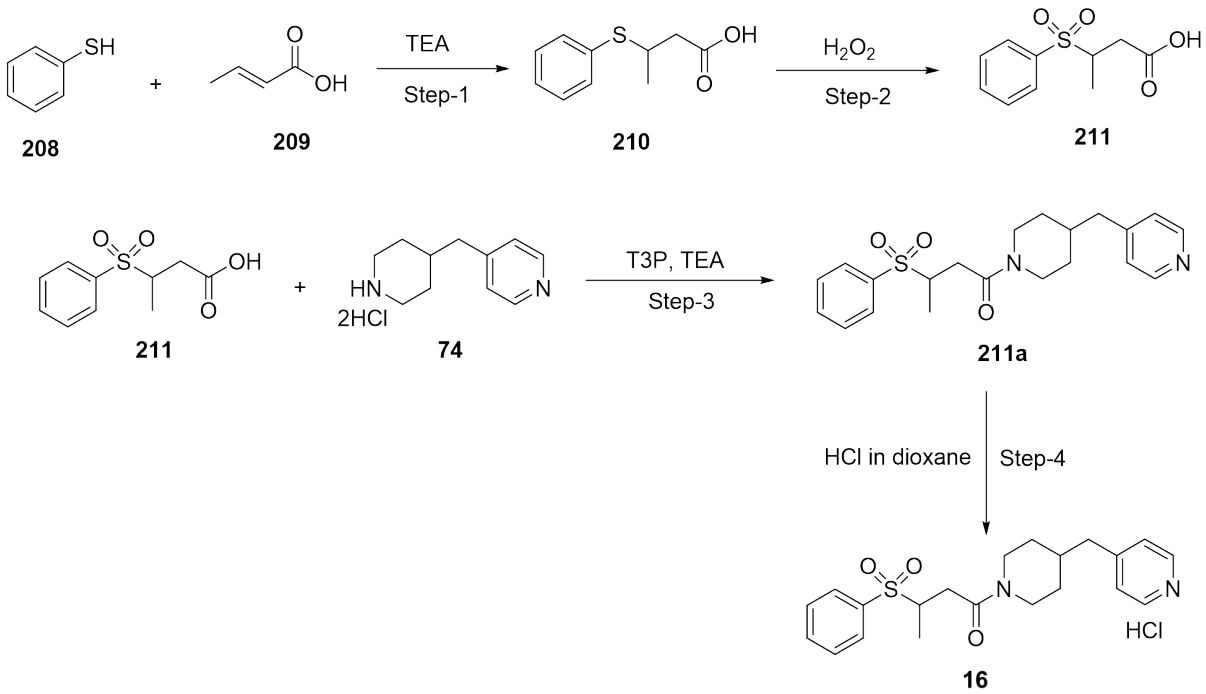
NMR (400 MHz, DMSO): 10.91 (s, 1H), 8.81 (s, 2H), 7.86 (d, 2H), 7.62 (d, 1H), 7.45 (m, 2H), 4.26 (d, 1H), 3.75 (d, 1H), 3.49 (s, 2H), 2.92 (m, 1H), 2.80 (d, 2H), 2.67 (m, 2H), 2.42 (m, 2H), 1.91 (s, 1H), 1.53 (m, 2H), 1.28 (m, 2H), 1.16 (m, 1H), 0.97 (m, 1H). LCMS: 459.97 (M+H).

**Compound 15**




To a solution of Intermediate-185 (0.084 g, 1.0 eq., 0.28 mmol) in DMF (1 mL) was added HATU (0.16 g, 1.5 eq., 0.42 mmol) at 0 °C and reaction mixture was stirred for 30 minutes at same temperature. To this was added Intermediate-74 (0.070 g, 1.0 eq., 0.28 mmol) and DIPEA (0.28 mL, 6.0 eq., 1.68 mmol) at 0 °C and the resulting mixture was stirred at same temperature for 1 h. After completion of the reaction, the mixture was diluted with water (50 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organic layer was washed with brine, dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to give crude product. The crude product was purified by column chromatography using silica gel of 60-120 mesh size and product was eluted in 0.5% methanol in DCM to give 70 mg (55%) of product. To a solution of Intermediate-74a (0.07 g, 1.0 eq., 0.15 mmol) in DMF (1 mL), $Cs_2CO_3$ (0.05 g, 1.0 eq., 0.15 mmol) was added at 0°C and reaction mixture was stirred for 30 minutes at same temperature. After 30 min, methyl iodide (0.022 g, 1.0 eq., 0.15 mmol) was added at same temperature and the reaction mixture was stirred for 6 h at room temperature. After completion of the reaction, the mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layer was dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to give crude product. The crude product was purified by preparative HPLC in 0.1 % formic acid water and acetonitrile to give 8 mg (11%) of product. 1H NMR (400 MHz, DMSO): 8.72 (bs, 2H), 7.57 (m, 3H), 7.39 (m, 2H), 4.56 (d, 1H), 3.86 (m, 1H), 3.04 (m, 1H), 2.88 (m, 2H), 2.69 (m, 2H), 2.51 (m, 1H), 2.13 (m, 2H), 1.91 (m, 3H), 1.19 (m, 6H), 0.90 (m, 1H). LCMS: 473.88 (M+H).

**Compound 16**




Intermediate 211 was prepared using a previously reported procedure.[1] To a solution of Intermediate-208 (5.5 g, 1 eq., 0.05 mol) in THF (8 mL) was added TEA (7.7 mL, 1.1 eq., 0.55 mol) and cooled to 0 °C. To this, a solution of Intermediate-209 (4.3 g, 1 eq., 0.05 mol) in THF (5 mL) was added drop wise at 0 °C and the reaction mass was stirred for 1 h at the same temperature followed by stirring at room temperature for 16 h. After completion of the reaction, the reaction was diluted with 5% HCl solution (40 mL) and extracted with ethyl acetate (2 X 30 mL). The combined organic layers was dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to afford (3.5 g) with 35.67 % yield of pure product. To a solution of Intermediate-210 (3.5 g, 1 eq., 0.018 mol) in acetic acid (7 mL) was added $H_2O_2$ (30% solution, 15 mL) at room temperature and stirred at same temperature for 16 h. After 16 h, acetic acid (3.5 mL) and $H_2O_2$ (30% solution, 7 mL) was added and the reaction was stirred at 80°C for 4 h. After completion of the reaction, the mixture was diluted with water (30 mL) and extracted with DCM (2 X 30 mL). The combined organic layer was washed with water, dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to afford 1.7 g (42%) of pure product. To a solution of Intermediate-74 (0.1 g, 1 eq., 0.4 mmol) and Intermediate-211 (0.1 g, 1.1 eq., 0.44 mmol) in DCM (5 mL) was added TEA (0.3 mL, 5 eq., 2 mmol) at 0°C and the reaction mass was stirred for 10 minutes at same temperature. To this, T3P (50% solution in ethyl acetate) (0.46 mL, 1.5 eq., 0.6 mmol) was added drop wise at 0°C and stirred at same temperature for 1 h. After completion of reaction, reaction mass was diluted with water (15 mL) and extracted with DCM (2 X 30 mL). The combined organic layer was dried over anhydrous sodium sulphate and the volatiles were removed under vacuum to afford crude product. The crude product was purified by column chromatography using silica gel of 60-120 mesh size and product was eluted in 3-5 % methanol in DCM to give 80 mg (47%) of pure product. To a solution

of Intermediate-211a (80 mg, 1 eq., 0.2 mmol) in dioxane (1 mL) was added 4N HCl in dioxane (0.6 mL) at 10°C and reaction mass was stirred at room temperature for 8 h. After completion of reaction, reaction mass was concentrated and triturated with pentane to give 65 mg (74%)of pure product. 1H NMR (400 MHz, DMSO): 8.81 (s, 2H), 7.89 (m, 4H), 7.78 (m, 1H), 7.77 (m, 2H), 4.27 (m, 1H), 3.79 (d, 1H), 3.68 (m, 2H), 2.95 (m, 1H), 2.84 (m, 1H), 2.83 (m, 2H), 2.44 (m, 2H), 1.92 (m, 1H), 1.54 (m, 2H), 1.14 (m, 3H), 1.00 (m, 1H). LCMS: 386.99 (M+H).

**References**

1. "A structure–taste study of arylsulfonyl(cyclo)alkanecarboxylic acids" Lysiak, V., A. Ratajczak, A. Mencel, K. Jarzembek, and J. Polanski. Bioorg. Med. Chem., 2005. **13**(3): p. 671-675.