Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

The identification of highly efficacious functionalised tetrahydrocyclopenta[c]pyrroles as inhibitors of HBV viral replication through modulation of HBV capsid assembly

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Supporting Information

Table of Contents

1. Synthetic Procedures S2

2. ^{1}H and ^{13}C NMR Spectra S27

3. HPLC and Chiral SFC Chromatograms S37

4. HepDE19 Cellular activity assay and cell viability assessment S58

5. *In vitro* Microsomal stability determination S60

6. Solubility determination S61

7. Mouse pharmacokinetic studies S62

8. HBV Hydrodynamic injection mouse studies S63

9. Cp Y132A crystallographic studies S64

1. General Experimental Methods:

All commercially available reagents and solvents were purchased from commercial sources and used without further purification unless otherwise noted.

^{1}H NMR spectra were obtained on Bruker Biospin (400 MHz) or Agilent 400-MR (400 MHz) spectrometer at ambient temperature. Chemical shifts were measured in parts per million or ppm (δ), referenced to tetramethylsilane (TMS) as the internal standard or to the residual solvent peak ($CDCl_3$:7.26 ppm; DMSO-d_6:2.50 ppm). Multiplicities were indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m, (multiplet), dd (doublet of doublets). Coupling constants were reported in Hertz (Hz).

^{13}C NMR spectra were obtained on Bruker AVIII (126 MHz) spectrometer at ambient temperature. Chemical shifts were measured in parts per million or ppm (δ), referenced to the residual solvent peak (DMSO-d_6:39.52 ppm). Multiplicities were indicated as singlets unless noted as follows: d (doublet). Coupling constants were reported in Hertz (Hz).

Low resolution mass spectra (LRMS) were recorded using the electrospray ionization (ESI) method, using a Waters Acquity UPLC coupled to a Waters SQ Detector.

High-resolution mass spectrometry (HRMS) data were recorded on a Waters Xevo G2-X2 QTof instrument in ESI+ ionization mode.

Products were purified by flash chromatography using pre-packed Reveleris® silica gel columns on a Grace automated chromatography system.

All compounds screened in the biological assays were determined to be ≥95% pure by LCMS, HPLC,and chiral SFC analysis.

Preparative chiral SFC separations were performed using a Waters SFC 80 supercritical fluid chromatography purification system using the specified columns and mobile phases.

Analytical chiral SFC analyses were performed using a Waters Investigator supercritical fluid chromatography system using the specified columns and mobile phases.

LCMS Method A: Waters Acquity UPLC system employing a Waters Acquity UPLC BEH C18, 1.7 μm, 50 x 2.1 mm column with an aqueous component of 0.05% formic acid in

water and an organic component of 0.05% formic acid in acetonitrile. Solvent events: 0-0.4 min, isocratic 3% (0.05% formic acid in acetonitrile); 0.4-2.5 min, linear gradient of 3-98% of (0.05% formic acid in acetonitrile); 2.5-3.4 min, isocratic 98% (0.05% formic acid in acetonitrile); 3.4-3.5 min, linear gradient of 98-3% of (0.05% formic acid in acetonitrile); 3.5-4 min, isocratic 5% (0.05% formic acid in acetonitrile); Flow rate = 0.6 mL/min. PDA 210.0 to 400.0 nm; Column temperature 35 °C.

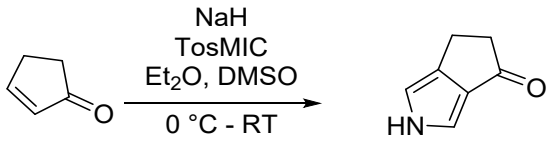
HPLC Method A: Waters 2695/2998 system employing a Waters Xbridge C18, 3.5 µm, 150 x 4.6 mm column with an aqueous component of 10 mM ammonium acetate in water and an organic component of acetonitrile. Solvent events: 0-1 min, isocratic 5% acetonitrile; 1-12 min, linear gradient of 5-95% of acetonitrile; 12-15 min, isocratic 95% acetonitrile; 15-17 min, linear gradient of 95-5% of acetonitrile; 17-20 min, isocratic 5% acetonitrile; Flow rate = 1.0 mL/min. PDA 210.0 to 400.0 nm; Column temperature 35 °C.

HPLC Method B: Waters Acquity UPLC system employing a Waters Acquity BEH C18, 1.7 µm, 50 x 2.1 mm column with an aqueous component of 0.1% trifluoroacetic acid in water and an organic component of acetonitrile. Solvent events: 0-0.5 min, isocratic 5% acetonitrile; 0.5-5 min, linear gradient of 5-95% of acetonitrile; 5-6.5 min, isocratic 95% acetonitrile; 6.5-6.6 min, linear gradient of 95-5% of acetonitrile; 6.6-8 min, isocratic 5% acetonitrile. Flow rate = 0.5 mL/min. PDA 210.0 to 400.0 nm; Column temperature 35 °C.

HPLC Method C: Waters Acquity UPLC system employing a Waters Acquity BEH C18, 1.7 µm, 50 x 2.1 mm column with an aqueous component of 0.1% formic acid in water and an organic component of acetonitrile. Solvent events: 0-0.5 min, isocratic 5% acetonitrile; 0.5-5 min, linear gradient of 5-95% of acetonitrile; 5-6.5 min, isocratic 95% acetonitrile; 6.5-6.6 min, linear gradient of 95-5% of acetonitrile; 6.6-8 min, isocratic 5% acetonitrile. Flow rate = 0.5 mL/min. PDA 210.0 to 500.0 nm; Column temperature 35 °C.

Synthesis & Characterization of Intermediates 2-9 and *i-v* and Compounds 10-18

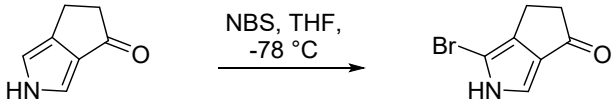
Preparation of 5,6-dihydrocyclopenta[c]pyrrol-4(2*H*)-one (**2**):



1 **2**

To 3.9 g (97.5 mmol, 1.6 eq.) of a 60% dispersion of sodium hydride in mineral oil in 80 mL of 1:1 (*v/v*) diethyl ether:dimethyl sulfoxide at 0 °C under a nitrogen atmosphere was added drop-wise a solution of 5.0 g (60.9 mmol, 1.0 eq.) of cyclopent-2-en-1-one (**1**) and 13.0 g (66.6 mmol, 1.1 eq.) of tosylmethyl isocyanide (TosMIC) in 80 mL of 1:1 (*v/v*) diethyl ether:dimethyl sulfoxide. The reaction mixture was stirred at room temperature for 16 h and then quenched by the addition of 100 mL of sat. ammonium chloride solution. The mixture was extracted with 3 x 100 mL of ethyl acetate and the combined organic extracts were washed with 100 mL of water, 100 mL of brine, dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with a linear gradient of 30-50% ethyl acetate/petroleum ether) to provide 5,6-dihydrocyclopenta[c]pyrrol-4(2*H*)-one (2.5 g, 20.6 mmol, 34%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.67 (bs, 1H), 7.14-7.16 (m, 1H), 6.63-6.64 (m, 1H), 2.76-2.81 (m, 2H), 2.70–2.73 (m, 2H); LRMS (ESI+): *m/z* found 122.2, $[M+H]^+$.

Preparation of 1-bromo-5,6-dihydrocyclopenta[c]pyrrol-4(2*H*)-one (**3**):

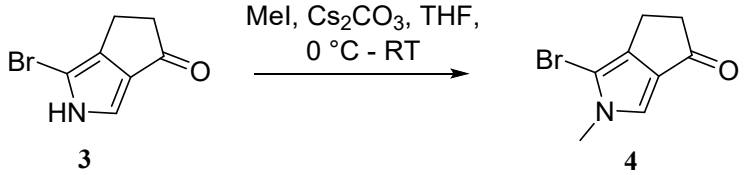


2 **3**

To a solution of 2.0 g (16.5 mmol, 1.0 eq.) of 5,6-dihydrocyclopenta[c]pyrrol-4(2*H*)-one (**2**) in 70 mL of THF at -78 °C under a nitrogen atmosphere was added drop-wise a solution of 2.9 g (16.5 mmol, 1.0 eq.) of *N*-bromosuccinimide in 10 mL of THF and the mixture was stirred at -78 °C for 2 h. The mixture was allowed to warm to room temperature and diluted with 70 mL of water. The mixture was then and extracted with 3 x 50 mL of ethyl acetate and the combined organic extracts were washed with 50 mL of water, 50 mL of brine, dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with a linear gradient of 5-20% ethyl acetate/petroleum ether) to provide 1-bromo-5,6-dihydrocyclopenta[c]pyrrol-4(2*H*)-one (1.5 g, 7.5 mmol, 45%) as an off-white solid. ^{1}H NMR

(400 MHz, $CDCl_3$): δ 9.00 (br s, 1H), 7.14-7.15 (m, 1H), 2.88-2.92 (m, 2H), 2.80-2.84 (m, 2H). The above reaction was performed on multiple batches with consistent results.

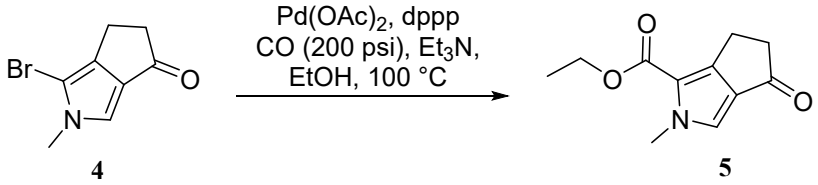
Preparation of 1-bromo-2-methyl-5,6-dihydrocyclopenta[c]pyrrol-4(2*H*)-one (4):



3 **4**

To a solution of 10.0 g (50.0 mmol, 1.0 eq.) of 1-bromo-5,6-dihydrocyclopenta[c]pyrrol-4(2*H*)-one (**3**) in 150 mL of THF at 0 °C was added 32.7 g (100.4 mmol, 2.0 eq.) of caesium carbonate and 10.6 g (74.6 mmol, 1.5 eq.) of methyl iodide. The mixture was then allowed to warm to room temperature and stirred for 16 h. The mixture was diluted with 200 mL of water and extracted with 3 x 300 mL of ethyl acetate. The combined organic extracts were washed with 200 mL of water, 200 mL of brine, dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue was purified by trituration with 100 mL of 1:1 (*v/v*) diethyl ether:*n*-pentane and dried under high vacuum to provide 1-bromo-2-methyl-5,6-dihydrocyclopenta[c]pyrrol-4(2*H*)-one (8.1 g, 37.8 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (s, 1H), 3.67 (s, 3H), 2.76-2.85 (m, 4H); LRMS (ESI+): *m/z* found 214.3/216.3 $[M+H]^+$.

Preparation of ethyl 2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxylate (5):

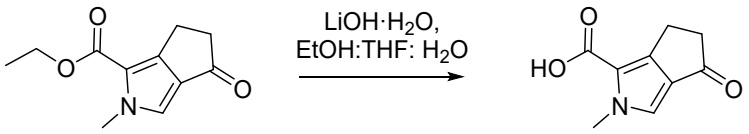


4 **5**

To a solution of 3.0 g (14.0 mmol, 1.0 eq.) of 1-bromo-2-methyl-5,6-dihydrocyclopenta[c] pyrrol-4(2*H*)-one (**4**) in 30 mL of ethanol was added 9.2 mL (66.0 mmol, 4.7 eq.) of triethylamine. The mixture was degassed with argon for 15 min and 0.3 g (1.33 mmol, 0.1 eq.) of palladium(II)acetate was added followed by and 0.81 g (1.96 mmol, 0.15 eq.) of 1,3-bis(diphenylphosphino)propane (dppp). The mixture was then stirred at 100 °C under 200 psi of carbon monoxide gas for 16 h. The mixture was allowed to cool to room temperature, filtered

through CELITE® and the pad was washed with 20 mL of ethanol. The solvent was removed *in vacuo* and the residue was resuspended in 100 mL of water. The mixture was extracted with 3 x 80 mL of ethyl acetate and the combined organic extracts were washed with 50 mL of water, 50 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with a linear gradient of 10-25% ethyl acetate/petroleum ether) to provide ethyl 2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxylate (2.1 g, 10.1 mmol, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10 (s, 1H), 4.31 (q, 2H), 4.00 (s, 3H), 3.06-3.10 (m, 2H), 2.82-2.85 (m, 2H), 1.37 (t, 3H).

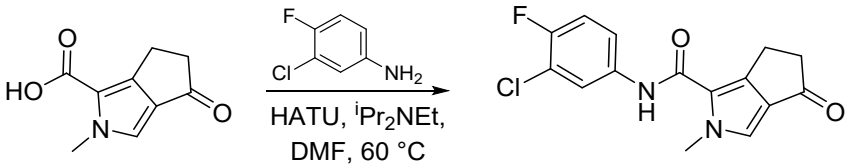
Preparation of 2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxylic acid (6):



5 **6**

To a solution of 5.5 g (26.5 mmol, 1.0 eq.) of ethyl 2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c] pyrrole-1-carboxylate (**5**) in 100 mL of 2:2:1 (*v/v/v*) ethanol:THF:water was added 2.5 g (59.5 mmol, 2.3 eq.) of lithium hydroxide monohydrate and the mixture was stirred at room temperature for 16 h. The organics were removed *in vacuo* and the residue was acidified using 5 M aq. HCl to pH ~3. The precipitated solid was isolated by vacuum filtration, washed with 30 mL of *n*-pentane and then dried under high vacuum to provide 2-methyl-4-oxo-2,4,5,6-tetrahydro cyclopenta[c]pyrrole-1-carboxylic acid (4.5 g, 25.1 mmol, 95%). LRMS (ESI+): *m/z* found 180.4 $[M+H]^+$.

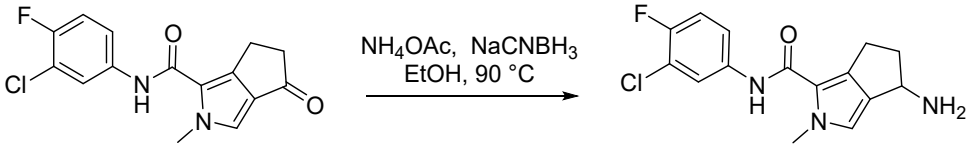
Preparation of *N*-(3-chloro-4-fluorophenyl)-2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**7**):



6 **7**

To a solution of 4.5 g (25.1 mmol, 1.0 eq.) of 2-methyl-4-oxo-2,4,5,6-tetrahydro cyclopenta[c]pyrrole-1-carboxylic acid (**6**) in 35 mL of DMF at 0 °C was added 9.7 g (75.4 mmol, 3.0 eq.) of *N,N*-diisopropylethylamine followed by 14.3 g (37.6 mmol, 1.5 eq.) of hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) and 5.4 g (37.0 mmol, 1.5 eq.) of 3-chloro-4-fluoro aniline, and the mixture was heated at 60 °C for 16 h. The mixture was allowed to cool to room temperature and diluted with 100 mL of ice-cold water. The resulting precipitate was isolated by vacuum filtration, dried under vacuum and purified by trituration with *n*-pentane and with diethyl ether to provide *N*-(3-chloro-4-fluorophenyl)-2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (5.6 g, 18.5 mmol, 74 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76-7.73 (m, 1H), 7.36-7.32 (m, 1H), 7.22 (br s, 1H), 7.16 (s, 1H), 7.13 (t, 1H), 4.06 (s, 3H), 3.17 (t, 2H), 2.98-2.95 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 197.85, 159.31, 153.21 (d, J = 242.7 Hz), 144.15, 136.22 (d, J = 3.0 Hz), 125.55, 123.22, 121.34, 120.57, 120.24 (d, J = 6.8 Hz), 119.04 (d, J = 18.2 Hz), 116.81 (d, J = 21.8 Hz), 41.27, 37.00, 20.38; LCMS (ESI-): *m/z* found 305.3/307.3 $[M-H]^-$, RT = 1.85 min (Method A); HPLC: 10.1 min (Method A). HRMS (ESI-TOF) m/z calculated for $C_{15}H_{12}ClFN_6O_2H^+$: 307.0655, found 307.0666 (Δ 3.58 ppm).

Preparation of 4-amino-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta [c]pyrrole-1-carboxamide (8):

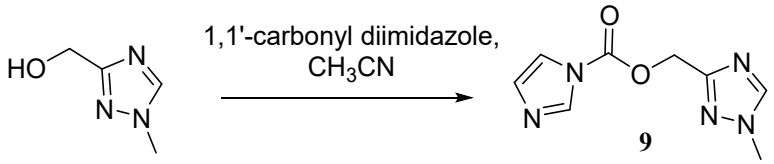


7 **8**

To a solution of 0.25 g (0.82 mmol, 1.0 eq.) of *N*-(3-chloro-4-fluorophenyl)-2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**7**) in 10 mL of ethanol was added 1.03 g (13.36 mmol, 16.3 eq.) of ammonium acetate and 0.13 g (2.07 mmol, 2.5 eq.) of sodium cyanoborohydride and the mixture was subjected to microwave irradiation maintaining a reaction temperature of 90 °C for 2 h. The solvent was removed *in vacuo* and the residue was resuspended in 30 mL of ethyl acetate. The mixture was washed 20 mL of water, 20 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with a linear gradient of 15-20% ethyl acetate/hexanes to provide

4-amino-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6- tetrahydrocyclopenta[c]pyrrole-1-carboxamide (0.20 g, 0.65 mmol, 79%). The above reaction was performed on multiple batches with consistent results. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.30 (bs, 1H), 7.91-7.94 (m, 1H), 7.55-7.60 (m, 1H), 7.36 (t, 1H), 6.75 (s, 1H), 4.14-4.19 (m, 1H), 3.77 (s, 3H), 2.90-2.98 (m, 1H), 2.71-2.79 (m, 1H), 2.49-2.56 (m, 1H), 1.85-1.91 (m, 1H); LCMS (ES-): *m/z* found 306.2/308.2 $[M-H]^-$, RT = 1.46 min (Method A).

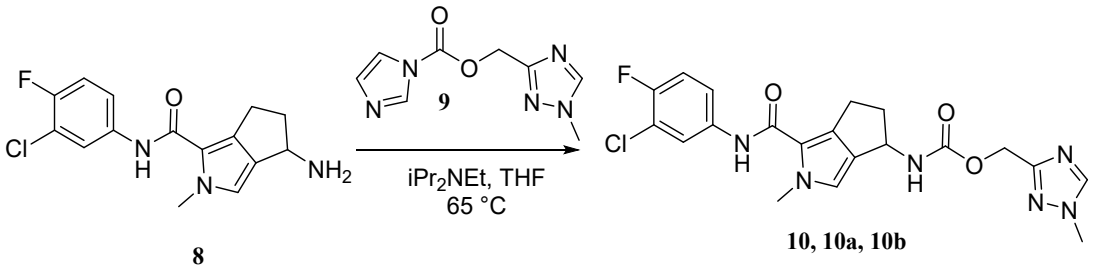
Preparation of (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl 1*H*-imidazole-1-carboxylate (9):



To a solution of 0.5 g (4.42 mmol, 1.0 eq.) of (1-methyl-1*H*-1,2,4-triazol-3-yl)methanol in 20 mL of acetonitrile was added 1.07 g (6.63 mmol, 1.5 eq.) of 1,1'-carbonyl diimidazole and the mixture was stirred at room temperature for 2 h. Volatiles were removed *in vacuo* and the residue was resuspended in 20 mL of water. The mixture was extracted with 3 x 50 mL of 5 % methanol in methylene chloride. The combined organic extracts were washed with 50 mL of sat. sodium carbonate solution, followed by 50 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo* to provide 0.77 g of (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl 1*H*-imidazole-1-carboxylate.

General Procedure A:

Preparation of (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (10, 10a, 10b):



To a solution of 0.55 g (1.79 mmol, 1.0 eq.) of 4-amino-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8**) in 6 mL of THF at 0 °C under a nitrogen atmosphere was added 0.67 g (5.18 mmol, 2.9 eq.) of *N,N*-diisopropylethylamine followed by 0.56 g (2.68 mmol, 1.5 eq.) of (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl 1*H*-imidazole-1-carboxylate (**9**). The reaction mixture was then stirred at 65 °C for 16 h. The mixture was allowed to cool to room temperature and diluted with 100 mL of water. The mixture was extracted with 3 x 100 mL of ethyl acetate and the combined organic extracts were washed with 80 mL of water, 80 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with a linear gradient of 0-4 % methanol in methylene chloride) to provide racemic (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**10**, 0.55 g, 1.23 mmol, 68 %). The enantiomers were subsequently separated by SFC (Waters SFC-080. Method isocratic, Mobile phase MeOH: CO_2 – 40:60. Column: Chiralcel OJ-H (30 x 250 mm), 5 µm, flow rate: 70 g/min.

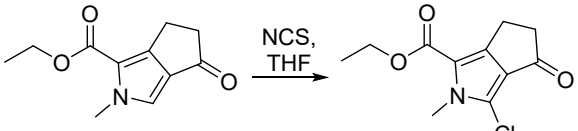
The first eluting isomer was isolated as (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (R)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**10a**). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.37 (s, 1H), 8.43 (s, 1H), 7.96 – 7.90 (m, 1H), 7.62 – 7.56 (m, 2H), 7.37 (t, J = 9.1 Hz, 1H), 6.81 (s, 1H), 4.99 (s, 2H), 4.84 – 4.78 (m, 1H), 3.84 (s, 3H), 3.77 (s, 3H), 2.96 – 2.90 (m, 1H), 2.85 – 2.78 (m, 1H), 2.61 – 2.56 (m, 1H, overlapping with DMSO-d_6), 2.12 – 2.08 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.05, 159.32, 155.39, 152.96 (d, J = 242.3 Hz), 145.26, 136.61 (d, J = 2.9 Hz), 134.23, 129.33, 121.61, 121.08, 120.01 (d, J = 6.9 Hz), 118.94 (d, J = 18.2 Hz), 118.13, 116.70 (d, J = 21.6 Hz), 58.59, 50.16, 37.82, 36.15, 35.76, 24.28; HRMS (ESI-TOF) m/z calculated for $C_{20}H_{20}ClFN_6O_3H^+$: 447.1353, found 447.1337 (Δ -3.58 ppm); LCMS (ESI+): *m/z* found 447.3/449.2 $[M+H]^+$, RT = 1.83 min, 96.5 % (Method A); HPLC: RT = 9.86 min, 98.6 % (Method A); Chiral analytical SFC: RT = 2.13 min, 99.9 %, Column: Chiralcel OJ-H 4.6 x 250 mm, 5 µm, 40% of methanol, Flow rate: 3.0 g/min.

The second eluting isomer was isolated as (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-

yl)carbamate (**10b**). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.35 (s, 1H), 8.42 (s, 1H), 7.93 (dd, J = 6.9, 2.6 Hz, 1H), 7.62 – 7.53 (m, 2H), 7.36 (t, J = 9.1 Hz, 1H), 6.81 (s, 1H), 5.05 – 4.93 (m, 2H), 4.84 – 4.78 (m, 1H), 3.84 (s, 3H), 3.77 (s, 3H), 3.00 – 2.88 (m, 1H), 2.85 – 2.77 (m, 1H), 2.60 – 2.55 (m, 1H, overlapping with DMSO-d_6), 2.15 – 2.05 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.03, 159.30, 155.37, 152.93 (d, J = 242.2 Hz), 145.24, 136.60 (d, J = 3.1 Hz), 134.20, 129.31, 121.59, 121.05, 119.99 (d, J = 6.7 Hz), 118.91 (d, J = 18.2 Hz), 118.11, 116.68 (d, J = 21.5 Hz), 58.57, 50.14, 37.80, 36.13, 35.75, 24.26; HRMS (ESI-TOF) m/z calculated for $C_{20}H_{20}ClFN_6O_3H^+$: 447.1353, found 447.1337 (Δ -3.58 ppm); LCMS (ESI+): *m/z* found 447.1/449.1 $[M+H]^+$, RT = 1.93 min, 95.3% (Method A); HPLC: RT = 9.87 min, 96.8% (Method A); Chiral analytical SFC: RT = 5.23 min, 99.6%, Column: Chiralcel OJ-H 4.6 x 250 mm, 5 μm, 40% of methanol, Flow rate: 3.0 g/min.

Preparation of (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (S)-(3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (11)

Preparation of ethyl 3-chloro-2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxylate (5a):

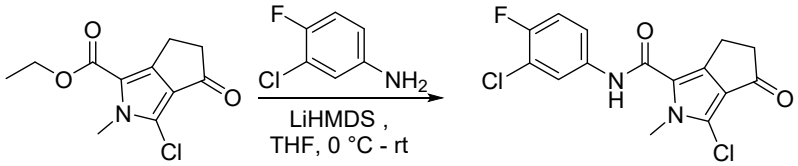


5 **5a**

To a solution of 2.5 g (12.1 mmol, 1.0 eq.) of ethyl 2-methyl-4-oxo-2,4,5,6-tetrahydro cyclopenta[c]pyrrole-1-carboxylate (**5**) in 25 mL of anhydrous THF was added 2.0 g (15.0 mmol, 1.2 eq.) of *N*-chlorosuccinimide and the mixture was stirred at room temperature for 16 h. The mixture was then diluted with 100 mL of ice-cold water and extracted with 2 x 100 mL of ethyl acetate. The combined organic extracts were washed with 100 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with linear gradient of 10 - 30% ethyl acetate in petroleum ether) to provide ethyl 3-chloro-2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxylate

(**5a**, 2.5 g, 10.3 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.31 (q, 2H), 3.96 (s, 3H), 3.04-3.07 (m, 2H), 2.83-2.86 (m, 2H), 1.36 (t, 3H); LRMS (ESI+): *m/z* found 242.0/244.1 $[M+H]^+$.

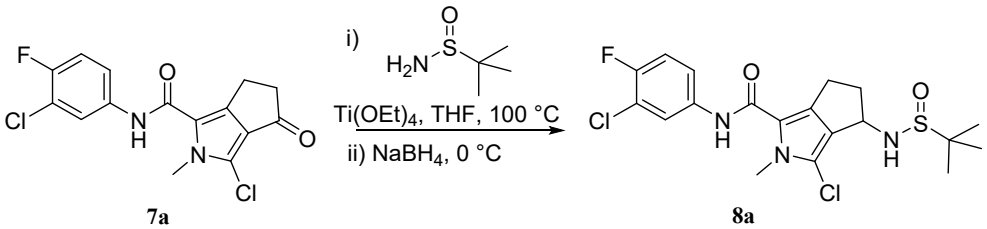
Preparation of 3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta [c]pyrrole-1-carboxamide (7a):



5a **7a**

To a solution of 1.0 g (4.14 mmol, 1.0 eq.) of ethyl 3-chloro-2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxylate (**5a**) in 10 mL of anhydrous THF at 0 °C under a nitrogen atmosphere was added 0.9 g (6.18 mmol, 1.5 eq.) of 3-chloro-4-fluoroaniline followed by 25 mL (25.0 mmol, 6 eq.) of a 1 M solution of lithium bis(trimethylsilyl)amide in THF. The mixture was allowed to warm to room temperature stirred at for 2 h. The reaction was quenched with 50 mL of saturated ammonium chloride solution and extracted with 3 x 50 mL of ethyl acetate. The combined organic extracts were washed with 100 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with linear gradient of 30-80% ethyl acetate in petroleum ether) to provide 3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-4-oxo-2,4,5,6-tetrahydro cyclopenta[c]pyrrole-1-carboxamide (**7a**, 0.8 g, 2.35 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71-7.73 (m, 1H), 7.31-7.35 (m, 1H), 7.11-7.17 (m, 2H), 4.01 (s, 3H), 3.12-3.16 (m, 2H), 2.96-2.98 (m, 2H); LRMS (ESI+): *m/z* found 341.0/343.0 $[M+H]^+$.

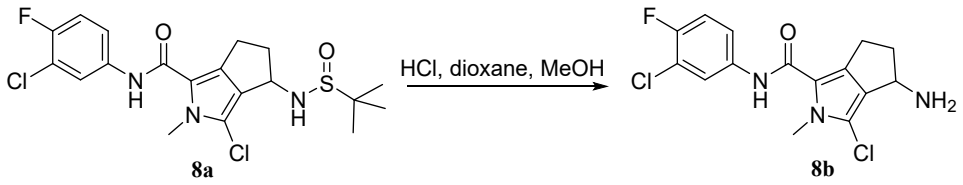
Preparation of 4-((*tert*-butylsulfinyl)amino)-3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydro cyclopenta[c]pyrrole-1-carboxamide (**8a**):



7a **8a**

To a solution of 0.6 g (1.76 mmol, 1.0 eq.) of 3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-4-oxo-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**7a**) in 8 mL of THF was added 0.43 g (3.52 mmol, 2.0 eq.) of (±)-2-methylpropane-2-sulfinamide followed by 2.4 g (10.52 mmol, 6.0 eq.) of titanium tetraethoxide at room temperature. The reaction vessel was sealed and the mixture was heated at 100 °C for 48 h. The mixture was cooled to 0 °C and 0.27 g (7.14 mmol, 4.1 eq.) of sodium borohydride was added. The mixture was then stirred at 0 °C for 4 h. The mixture was diluted with 100 mL of ice-cold water and extracted with 3 x 100 mL of ethyl acetate. The combined organic extracts were washed with 100 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with linear gradient of 20-70% ethyl acetate in petroleum ether) to provide 4-((*tert*-butylsulfinyl)amino)-3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8a**, 0.55 g, 1.23 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72-7.74 (m, 1H), 7.31-7.33 (m, 2H), 7.08-7.12 (m, 1H), 4.83-4.87 (m, 1H), 4.30 (m, 1H), 3.92 (s, 3H), 3.14-3.20 (m, 1H), 2.72-2.90 (m, 2H), 2.49-2.54 (m, 1H), 1.21 (s, 9H); LRMS (ESI+): *m/z* found 446.3/448.3 $[M+H]^+$.

Preparation of 4-amino-3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (8b):

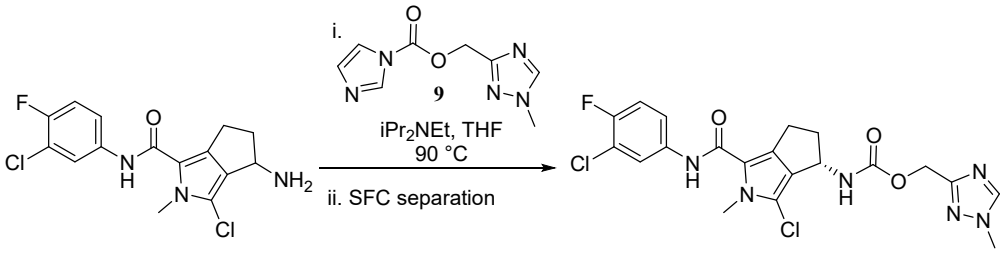


8a **8b**

To a solution of 0.55 g (1.23 mmol, 1.0 eq.) of 4-((*tert*-butylsulfinyl)amino)-3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide in 5 mL of methanol at 0 °C was added 1.8 mL of a 4 M solution of HCl in 1,4-dioxane. The resulting mixture was allowed to warm to room temperature and stirred for 2 h. The solvent was removed *in vacuo* and the residue was redissolved in 10 mL of water and 10 mL of saturated sodium bicarbonate solution and stirred for 20 min. The resulting precipitate was collected by filtration and dried under high vacuum to provide 4-amino-3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8b**, 0.35 g, 1.02 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70-7.73 (m, 1H), 7.29-7.32 (m, 1H), 7.13-7.20

(m, 1H), 7.08-7.12 (m, 1H), 4.42-4.43 (m, 1H), 3.91 (s, 3H), 3.04-3.08 (m, 1H), 2.81-2.86 (m, 2H), 2.08-2.14 (m, 1H), 1.50 (bs, 2H).

Preparation of (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (S)-(3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (11):



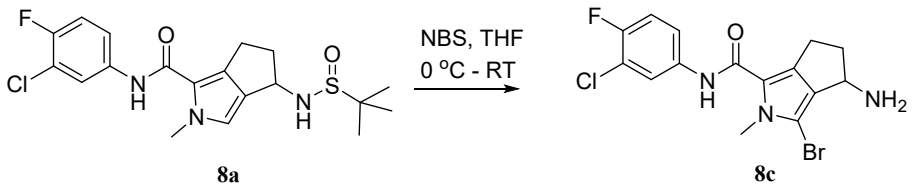
To a solution of 0.35 g (1.02 mmol, 1.0 eq.) of 4-amino-3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8b**) in 6 mL of anhydrous THF at 0 °C under inert atmosphere was added 0.39 g (3.06 mmol, 3.0 eq.) of *N,N*-diisopropylethylamine followed by 0.30 g (1.45 mmol, 1.4 eq.) of (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl 1*H*-imidazole-1-carboxylate (**9**). The reaction vessel was sealed and the mixture was heated at 90 °C for 16 h. The mixture was allowed to cool to room temperature, diluted with 100 mL of water and extracted with 3 x 100 mL of ethyl acetate. The combined organic extracts were washed with 80 mL of water, 60 mL of brine, dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with a linear gradient of 0-5% methanol in methylene chloride) to provide racemic (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl(3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (0.35 g, 0.72 mmol, 71%). The enantiomers were subsequently separated by SFC (Waters SFC-080). Method: isocratic, Mobile phase MeOH: CO_2 – 35:65. Column: Chiralpak IA (30 x 250 mm), 5 µm, flow rate: 100 g/min.

The first eluting enantiomer was isolated as (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (S)-(3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (s, 1H), 8.43 (s, 1H), 7.92 (dd, J = 6.8, 2.6 Hz, 1H), 7.66 – 7.54 (m, 2H), 7.38 (t, J = 9.1 Hz, 1H), 5.06 – 4.93 (m, 3H), 3.84 (s, 3H), 3.71 (s, 3H), 3.01 – 2.89 (m, 1H), 2.89 – 2.77 (m, 1H), 2.65 – 2.58 (m, 1H, overlapping with DMSO-d_6), 2.14 – 2.01 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ

159.34, 159.25, 155.23, 153.10 (d, J = 242.4 Hz), 145.26, 136.36 (d, J = 3.1 Hz), 134.11, 125.70, 121.24, 120.16 (d, J = 6.9 Hz), 118.98 (d, J = 18.5 Hz), 118.88, 116.77 (d, J = 21.7 Hz), 114.74, 58.72, 49.90, 38.25, 35.76, 32.50, 24.41; HRMS (ESI-TOF) m/z calculated for $C_{20}H_{19}Cl_2FN_6O_3H^+$: 481.0963, found 481.0952 (Δ -2.29 ppm); LCMS (ESI+): *m/z* found 481.2/483.3 $[M+H]^+$, RT = 2.35 min, 98.7% (Method A); HPLC: RT = 10.39 min, 97.2% (Method A); Chiral analytical SFC: RT = 7.51 min, 99.8%, Column: Chiralpak IA 4.6 x 250 mm, 5 µm, 35% of methanol, Flow rate: 3.0 g/min.

Preparation of (1-methyl-1H-1,2,4-triazol-3-yl)methyl (S)-(3-bromo-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**12**):

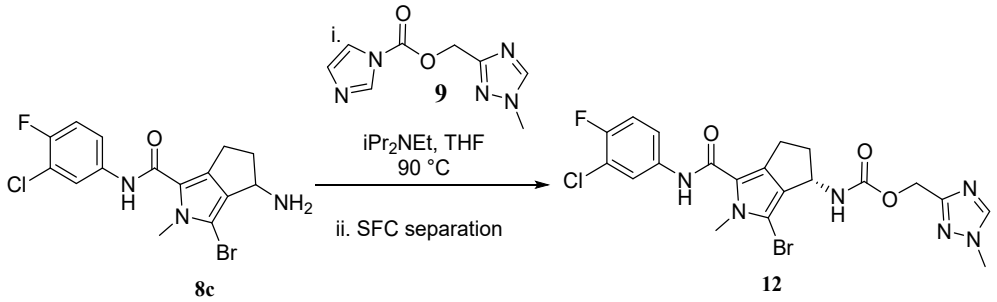
Preparation of 4-amino-3-bromo-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta [c]pyrrole-1-carboxamide (8c):



8a **8c**

To a solution of 0.5 g (1.20 mmol, 1.0 eq.) of ethyl 4-((*tert*-butylsulfinyl)amino)-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8a**) in 15 mL of THF at 0 °C under a nitrogen atmosphere was added 0.24 g (1.33 mmol, 1.1 eq.) of *N*-bromosuccinimide. The mixture was allowed to warm to room temperature and stirred for 2 h. The solvent was removed *in vacuo* and the residue was dissolved in 10 mL of water and 10 mL of saturated $NaHCO_3$ solution and stirred at room temperature for 10 min. The resulting precipitate was collected by filtration and dried under high vacuum to provide of 4-amino-3-bromo-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8c**, 0.25 g, 0.65 mmol, 54%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.53 (s, 1H), 7.92 (dd, 1H), 7.55-7.60 (m, 1H), 7.34-7.40 (m, 1H), 4.14-4.18 (m, 1H), 3.72 (s, 3H), 3.02-2.92 (m, 2H), 3.02-2.92 (m, 2H), 2.81-2.73 (m, 1H), 1.93-1.90 (m, 1H).

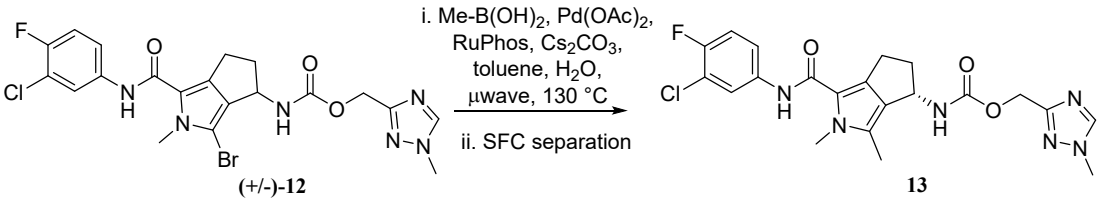
Preparation of (1-methyl-1H-1,2,4-triazol-3-yl)methyl (S)-(3-bromo-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (12):



(1-Methyl-1*H*-1,2,4-triazol-3-yl)methyl (3-bromo-1-((3-chloro-4-fluorophenyl) carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate was synthesized in a similar manner as described above (General Procedure A) from 4-amino-3-bromo-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8c**) and (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl 1*H*-imidazole-1-carboxylate (**9**). The enantiomers were subsequently separated by SFC (Waters SFC-080). Method isocratic, Mobile phase MeOH: CO_2 – 20:80. Column: Chiralcel OJ-H (21 x 250 mm), 5 µm, flow rate: 60 g/min.

The second eluting enantiomer was isolated as (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (S)-(3-bromo-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**12**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.43 (s, 1H), 7.92 (dd, J = 6.8, 2.5 Hz, 1H), 7.63 – 7.54 (m, 2H), 7.38 (t, J = 9.1 Hz, 1H), 5.08 – 4.87 (m, 3H), 3.84 (s, 3H), 3.72 (s, 3H), 3.01 – 2.78 (m, 2H), 2.71 – 2.55 (m, 1H, overlapping with DMSO-d_6), 2.11 – 2.03 (m, 1H); ^{13}C NMR (126 MHz, DMSO-*d6*) δ 159.36, 159.23, 155.23, 153.10 (d, J = 242.7 Hz), 145.25, 136.35 (d, J = 3.1 Hz), 134.58, 128.97, 121.22, 120.77, 120.14 (d, J = 6.8 Hz), 118.98 (d, J = 18.3 Hz), 116.77 (d, J = 21.8 Hz), 102.81, 58.73, 50.23, 38.24, 35.75, 34.04, 24.37; HRMS (ESI-TOF) m/z calculated for $C_{20}H_{19}BrClFN_6O_3H^+$: 525.0477, found 525.0430 (Δ -3.23 ppm); LCMS (ESI+): *m/z* found 525.3/527.3/529.3 $[M+H]^+$, RT = 2.37 min, 95.0% (Method A); HPLC: RT = 10.48 min, 96.0% (Method A); Chiral analytical SFC: RT = 5.05 min, 99.9%, Column: CHIRALCEL OJ-H 4.6 x 250 mm, 5 µm, 40% of methanol, Flow rate: 3.0 g/min

Preparation of (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2,3-dimethyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (13)

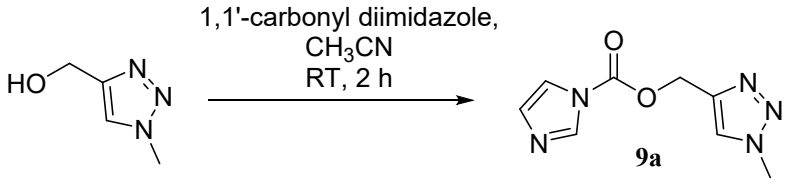


A solution of 120 mg (0.23 mmol, 1.0 eq.) of racemic (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (3-bromo-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate in 2 mL of toluene in a microwave vial was degassed with argon gas for 10 min. To the degassed solution was added 68 mg (1.14 mmol, 5.0 eq.) of methyl boronic acid followed by 3 mg (0.011 mmol, 0.05 eq.) of palladium (II) acetate, 10.6 mg (0.022 mmol, 0.1 eq.) of 2-dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl and 220 mg (0.68 mmol, 3.0 eq.) of caesium carbonate. The mixture was degassed with argon for a further 5 min and then subjected to microwave irradiation maintaining a reaction temperature of 130 °C for 30 min. The mixture was allowed to cool to room temperature, diluted with 20 mL of water and extracted with 3 x 50 mL of ethyl acetate. The combined organic extracts were washed with 50 mL of water, 40 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The residue was purified by semi-preparative HPLC to provide racemic (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2,3-dimethyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (15 mg, 0.032 mmol, 14%). LCMS (ESI+): *m/z* found 461.1/463.0 $[M+H]^+$, RT = 1.88 min (Method A). The enantiomers were subsequently separated by preparative SFC (Waters SFC-080). Method: isocratic, Mobile phase MeOH: CO_2 – 25:75. Column: Chiralpak AD (21 x 250 mm, 5 μm), flow rate: 60 g/min.

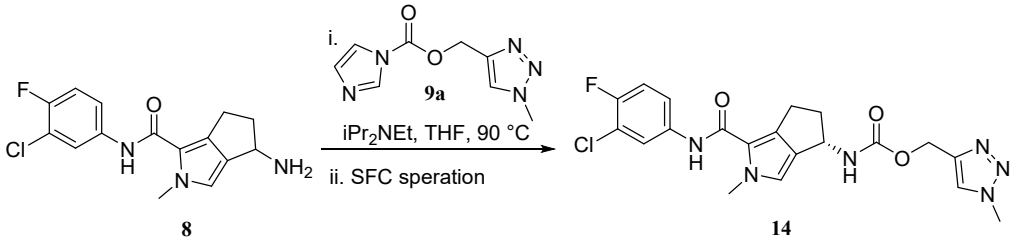
The first eluting enantiomer was isolated as (1-methyl-1*H*-1,2,4-triazol-3-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2,3-dimethyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**13**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.41 (s, 1H), 7.92 (dd, J = 6.9, 2.6 Hz, 1H), 7.61 – 7.48 (m, 2H), 7.34 (t, J = 9.1 Hz, 1H), 5.00 (s, 2H), 4.91 – 4.85 (m, 1H), 3.84 (s, 3H), 3.66 (s, 3H), 2.98 – 2.86 (m, 1H), 2.82 – 2.72 (m, 1H), 2.66 – 2.53 (m, 1H, overlapping with DMSO-d_6), 2.14 (s, 3H), 2.13 – 2.01 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.10, 159.36, 155.37, 152.79 (d, J = 242.0 Hz), 145.22, 136.82 (d, J = 3.0 Hz), 133.63, 128.52, 127.36,

120.87, 119.83 (d, *J* = 6.8 Hz), 118.87 (d, *J* = 18.3 Hz), 117.22, 116.66 (d, *J* = 21.6 Hz), 58.62, 49.92, 38.19, 35.74, 32.09, 24.20, 10.50; HRMS (ESI-TOF) m/z calculated for $C_{21}H_{22}ClFN_6O_3H^+$: 461.1510, found 461.1497 (Δ -2.82 ppm); LCMS (ESI+): *m/z* found 461.1/463.0 $[M+H]^+$, RT = 1.88 min, 99.9% (Method A); HPLC: RT = 10.10 min, 99.5% (Method A); Chiral analytical SFC: RT = 3.05 min, 99.2%, Column: Chiralcel AD-3 4.6 x 250 mm, 5 µm, 40% of methanol, Flow rate: 3.0 g/min.

Preparation of (1-methyl-1*H*-1,2,3-triazol-4-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (14)



To a solution of 400 mg (3.53 mmol, 1.0 eq.) of (1-methyl-1*H*-1,2,3-triazol-4-yl)methanol in 10 mL of acetonitrile was added 860 mg (5.31 mmol, 1.5 eq.) of 1,1'-carbonyl diimidazole at room temperature and the reaction mixture was stirred at room temperature for 2 h. The reaction solution was concentrated under reduced pressure and the residue was diluted with 20 mL of water and extracted with 3 x 100 mL of ethyl acetate. The combined organic extracts were washed with 30 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo* to provide 600 mg of (1-methyl-1*H*-1,2,3-triazol-4-yl)methyl 1*H*-imidazole-1-carboxylate (**9a**) as an off-white solid.



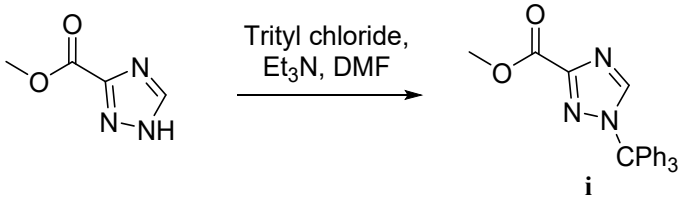
(1-Methyl-1*H*-1,2,3-triazol-4-yl)methyl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate was synthesized in a similar manner as described above (General Procedure A) from 4-amino-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8**) and (1-methyl-1*H*-1,2,3-

triazol-4-yl)methyl 1*H*-imidazole-1-carboxylate (**9a**). The enantiomers were subsequently separated by SFC (Waters SFC-080). Method: isocratic, Mobile phase MeOH: CO_2 - 45:55. Column: Chiralpak IA (30 x 250 mm), 5 µm, flow rate: 100 g/min.

The first eluting enantiomer was isolated as (1-methyl-1*H*-1,2,3-triazol-4-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**14**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 8.06 (s, 1H), 7.92 (dd, J = 6.8, 2.6 Hz, 1H), 7.61 – 7.49 (m, 2H), 7.36 (t, J = 9.1 Hz, 1H), 6.80 (s, 1H), 5.05 (s, 2H), 4.89 – 4.72 (m, 1H), 4.04 (s, 3H), 3.77 (s, 3H), 2.98 – 2.88 (m, 1H), 2.84 – 2.73 (m, 1H), 2.68 – 2.53 (m, 1H, overlapping with DMSO-d_6), 2.15 – 2.05 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.03, 155.57, 152.94 (d, J = 242.2 Hz), 142.78, 136.60 (d, J = 3.2 Hz), 134.21, 129.30, 125.40, 121.59, 121.07, 120.01 (d, J = 6.7 Hz), 118.92 (d, J = 18.3 Hz), 118.13, 116.70 (d, J = 21.5 Hz), 56.83, 50.10, 37.84, 36.21, 36.14, 24.25; HRMS (ESI-TOF) m/z calculated for $C_{20}H_{20}ClFN_6O_3H^+$: 447.1353, found 447.1359 (Δ 1.35 ppm); LCMS (ESI+): *m/z* found 447.5/449.5 $[M+H]^+$, RT = 2.26 min, 95.5% (Method A); HPLC: RT = 3.12 min, 98.0% (Method B); Chiral analytical SFC: RT: 3.83 min, 98.7%, Column: Chiralpak IA 4.6 x 250 mm, 5 µm, 40 % of methanol, Flow rate: 4.0 g/min.

Preparation of (1*H*-1,2,4-triazol-3-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**15**):

Preparation of methyl 1-trityl-1*H*-1,2,4-triazole-3-carboxylate (i):

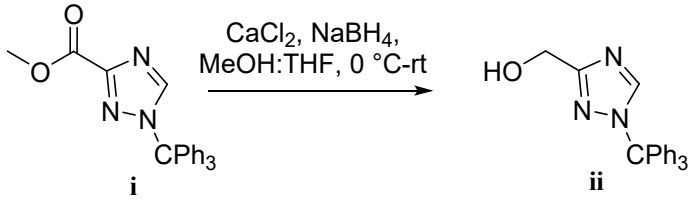


i

To a solution of 2.0 g (15.7 mmol, 1.0 eq.) of methyl 1*H*-1,2,4-triazole-3-carboxylate in 10 mL of DMF at 0 °C under a nitrogen atmosphere was added 6.5 mL (47.2 mmol 3.0 eq.) of triethylamine followed by 6.58 g (23.6 mmol, 1.5 eq.) of trityl chloride. The mixture was allowed to warm to room temperature and stirred for 16 h. The mixture was then diluted with 50 mL of ice-cold water and stirred for a further 30 min. The precipitated solid was collected by filtration, washed with 50 mL of water, 50 mL of diethyl ether and dried under vacuum to

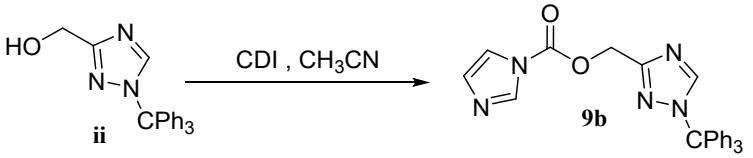
provide 4.0 g (10.8 mmol, 69%) of methyl 1-trityl-1*H*-1,2,4-triazole-3-carboxylate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.29-7.38 (m, 9H), 7.10-7.14 (m, 6H), 3.96 (s, 3H).

Preparation of (1-trityl-1*H*-1,2,4-triazol-3-yl)methanol (ii):



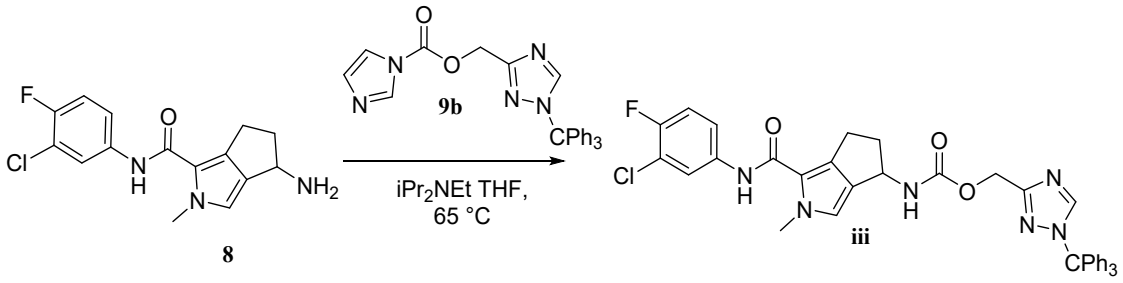
To a solution of 4.0 g (10.8 mmol, 1.0 eq.) of methyl 1-trityl-1*H*-1,2,4-triazole-3-carboxylate (**i**) in 45 mL of THF at 0 °C under a nitrogen atmosphere was added 2.6 g (23.4 mmol, 2.2 eq.) of calcium (II) chloride followed by 1.8 g (47.58 mmol, 4.4 eq.) of sodium borohydride and the dropwise addition of 9 mL of methanol. The mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was diluted with 50 mL of ice-cold water and extracted with 3 x 75 mL of ethyl acetate. The combined organic extracts were washed with 80 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo* to provide (1-trityl-1*H*-1,2,4-triazol-3-yl)methanol (3.6 g, 10.54 mmol, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.03 (s, 1H), 7.38-7.43 (m, 9H), 7.03-7.09 (m, 6H), 5.31 (t, 1H), 4.44 (d, 2H).

Preparation of (1-trityl-1*H*-1,2,4-triazol-3-yl)methyl 1*H*-imidazole-1-carboxylate (9b):



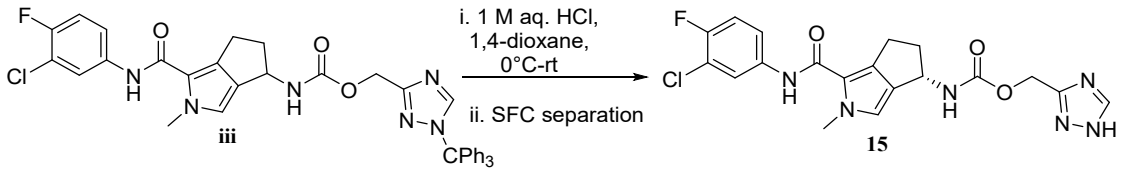
To a solution of 1.0 g (2.93 mmol, 1.0 eq.) of (1-trityl-1*H*-1,2,4-triazol-3-yl)methanol (**ii**) in 10 mL of acetonitrile was added 0.71 g (4.39 mmol, 1.5 eq.) of 1,1'-carbonyldiimidazole and the mixture was stirred at room temperature for 2 h. The solvent was removed *in vacuo* and the residue was redissolved in 20 mL of ethyl acetate. The organic solution was washed with 40 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo* to provide 1.1 g of (1-trityl-1*H*-1,2,4-triazol-3-yl)methyl 1*H*-imidazole-1-carboxylate. LRMS (ESI+): *m/z* found 436.4 $[M+H]^+$.

Preparation of (1-trityl-1*H*-1,2,4-triazol-3-yl)methyl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (iii):



(1-Trityl-1*H*-1,2,4-triazol-3-yl)methyl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate was synthesized according to General Procedure A from 4-amino-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta [c]pyrrole-1-carboxamide (**8**) and (1-trityl-1*H*-1,2,4-triazol-3-yl)methyl 1*H*-imidazole-1-carboxylate (**9b**). LRMS (ESI+): *m/z* found 675.4/677.4 $[M+H]^+$.

Preparation of (1*H*-1,2,4-triazol-3-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (15):

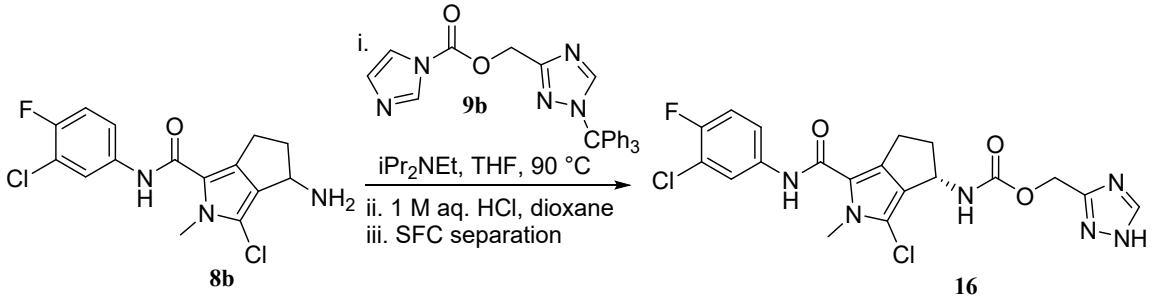


To a solution of 0.6 g (0.89 mmol, 1.0 eq.) of (1-trityl-1*H*-1,2,4-triazol-3-yl)methyl (1-((3-chloro-4-fluorophenyl) carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**iii**) in 6 mL of 1,4-dioxane at 0 °C was added 1 mL of a 1 M aqueous solution of HCl drop-wise. The mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was then basified with saturated sodium bicarbonate solution and extracted with 3 x 40 mL of ethyl acetate. The combined organic extracts were washed with 40 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluting with linear gradient of 0-5% methanol in methylene chloride) to provide racemic (1*H*-1,2,4-triazol-3-yl)methyl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (0.17 g, 0.39 mmol, 44%). The enantiomers were subsequently separated by SFC (Waters SFC-080). Method: isocratic, Mobile

phase MeOH: CO_2 - 30:70. Column: CHIRALPAK IA (30 x 250 mm), 5 µm, flow rate: 90 g/min.

The first eluting enantiomer was isolated as (1*H*-1,2,4-triazol-3-yl)methyl (S)-((1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**15**). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.36 (s, 1H), 8.33 (s, 1H), 7.93 (dd, J = 7.0, 2.6 Hz, 1H), 7.63 – 7.53 (m, 2H), 7.36 (t, J = 9.1 Hz, 1H), 6.81 (s, 1H), 5.04 (s, 2H), 4.84 – 4.78 (m, 1H), 3.77 (s, 3H), 2.98 – 2.88 (m, 1H), 2.85 – 2.77 (m, 1H), 2.67 – 2.53 (m, 1H, overlapping with DMSO-d_6), 2.14 – 2.08 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.04, 156.76, 155.40, 152.94 (d, J = 242.2 Hz), 146.49, 136.61 (d, J = 3.1 Hz), 134.22, 129.30, 121.60, 121.06, 119.99 (d, J = 6.7 Hz), 118.92 (d, J = 18.2 Hz), 118.14, 116.69 (d, J = 21.6 Hz), 58.18, 50.15, 37.83, 36.14, 24.27; HRMS (ESI-TOF) m/z calculated for $C_{19}H_{18}ClFN_6O_3H^+$: 433.1197, found 433.1178 (Δ -4.39 ppm); LCMS (ESI+): *m/z* found 433.2/435.2 $[M+H]^+$, RT = 1.76 min, 99.2% (Method A); HPLC: RT = 2.87 min, 97.7% (Method B); Chiral analytical SFC: RT = 3.06 min, 99.5%, Column: Chiralpak IA 4.6 x 250 mm, 5 µm, 40% of (0.5% diethylamine in ethanol), Flow rate: 2.4 g/min.

Preparation of (1*H*-1,2,4-triazol-3-yl)methyl (S)-(3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (16):



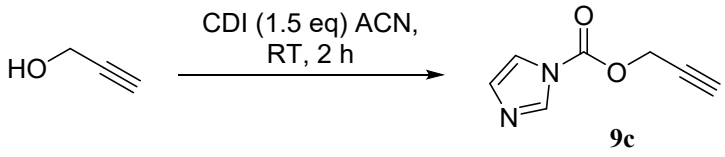
(1*H*-1,2,4-Triazol-3-yl)methyl (3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate was synthesized in a similar manner as described above according to General Procedure A from 4-amino-3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8b**) and (1-trityl-1*H*-1,2,4-triazol-3-yl)methyl 1*H*-imidazole-1-carboxylate (**9b**), followed by acid mediated detritylation. The enantiomers were subsequently separated by SFC (Waters SFC-080).

Method: isocratic, Mobile phase MeOH: CO_2 - 20:80. Column: Chiralcel OJ-H (30 x 250 mm), 5 µm, flow rate: 70 g/min.

The second eluting enantiomer was isolated (1*H*-1,2,4-triazol-3-yl)methyl (S)-(3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**16**). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.01 (s, 1H), 9.56 (s, 1H), 8.40 (s, 1H), 7.92 (dd, J = 6.9, 2.6 Hz, 1H), 7.65 (d, J = 8.5 Hz, 1H), 7.62 – 7.54 (m, 1H), 7.38 (t, J = 9.1 Hz, 1H), 5.12 – 5.00 (m, 2H), 5.00 – 4.91 (m, 1H), 3.71 (s, 3H), 3.01 – 2.89 (m, 1H), 2.89 – 2.78 (m, 1H), 2.66 – 2.57 (m, 1H, overlapping with DMSO-d_6), 2.15 – 2.03 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.32, 156.71, 155.18, 153.09 (d, J = 242.7 Hz), 146.16, 136.34 (d, J = 3.1 Hz), 134.09, 125.67, 121.24, 120.15 (d, J = 6.7 Hz), 118.97 (d, J = 18.1 Hz), 118.88, 116.73 (d, J = 21.7 Hz), 114.74, 58.08, 49.90, 38.22, 32.48, 24.39; HRMS (ESI-TOF) m/z calculated for $C_{19}H_{17}Cl_2FN_6O_3H^+$: 467.0807, found 467.0821 (Δ 3.00 ppm); LCMS (ESI+): *m/z* found 467.2/469.2 $[M+H]^+$, RT = 2.25 min, 99.0% (Method A); HPLC: RT = 3.16 min, 99.0% (Method B); Chiral analytical SFC: RT: 4.02 min, 99.8%, Column: Chiralpak OJ-H 4.6 x 250 mm, 5 µm, 30% of methanol, Flow rate: 3.0 g/min.

Preparation (1*H*-1,2,3-triazol-4-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (17):

Preparation of prop-2-yn-1-yl 1*H*-imidazole-1-carboxylate (9c)

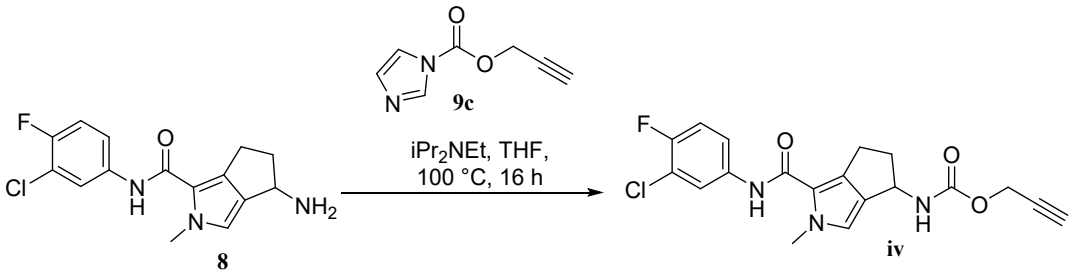


9c

To a solution of 1.0 g (17.84 mmol, 1.0 eq.) of prop-2-yn-1-ol in 10 mL of acetonitrile was added 4.4 g (27.10 mmol, 1.5 eq.) of 1,1′-carbonyldiimidazole and the reaction mixture was stirred at room temperature for 2 h. The solvent was removed *in vacuo* and the residue was resuspended in 40 mL of water and extracted with 3 x 50 mL of ethyl acetate. The combined organic extracts were washed with 50 mL of brine, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo* to provide 1.6 g of crude prop-2-yn-1-yl 1H-imidazole-1-carboxylate (**2**) as a

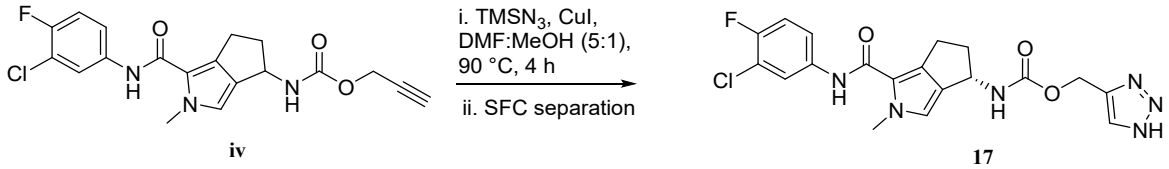
pale yellow liquid, which was taken to the next step without further purification. LCMS (ESI+): *m/z* found 151.1 $[M+H]^+$.

Preparation of prop-2-yn-1-yl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (iv):



To a stirred solution of 0.55 g (1.79 mmol, 1.0 eq.) of 4-amino-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8**) in 5.5 mL of THF, was added 1.5 mL (8.61 mmol, 4.8 eq.) of *N*,*N*-diisopropylethylamine followed by 0.54 g (3.58 mmol, 2.0 eq.) of prop-2-yn-1-yl 1*H*-imidazole-1-carboxylate (**9c**) at room temperature under inert atmosphere. The mixture was heated to 100 ˚C for 16 h. The reaction was allowed to cool to room temperature and quenched with 25 mL ice-cold water. The resultant mixture was extracted with 3 x 50 mL of ethyl acetate and the combined organic extracts were washed with 50 mL of water, 50 mL of brine 50 mL, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The reaction was repeated and the crude product from both batches combined and purified by flash chromatography (SiO_2, eluting with linear gradient of 10-20 % ethyl acetate in petroleum ether) to provide 0.76 g (1.95 mmol, 54%) of prop-2-yn-1-yl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**iv**) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.36 (s, 1H), 7.95-7.91 (m, 1H), 7.66 (br d, 1H), 7.61-7.55 (m, 1H), 7.37 (t, 1H), 6.81 (s, 1H), 4.82-4.77 (m, 1H), 4.64 (s, 2H), 3.77 (s, 3H), 3.48 (s, 1H), 2.99-2.90 (m, 1H), 2.87-2.78 (m, 1H), 2.63-2.54 (m, 1H), 2.13-2.04 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.03, 154.91, 152.92 (d, J = 242.3 Hz), 136.65 (d, J = 2.9 Hz), 134.22, 129.09, 121.54, 121.05, 120.00 (d, J = 6.7 Hz), 118.91 (d, J = 18.4 Hz), 118.19, 116.70 (d, J = 21.5 Hz), 79.45, 77.04, 51.31, 50.16, 37.90, 36.15, 24.26; HRMS (ESI-TOF) m/z calculated for $C_{19}H_{17}ClFN_6O_3H^+$: 390.1015, found 390.1025 (Δ 2.58 ppm);

Preparation (1*H*-1,2,3-triazol-4-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (17):



iv **17**

To a solution of 0.19 g (0.49 mmol, 1.0 eq.) of prop-2-yn-1-yl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**iv**) in 3 mL of 5:1 (*v/v*) DMF:methanol in a sealed tube was added 9.1 mg (0.048 mmol, 0.1 eq.) of copper(I) iodide followed by 0.63 mL (6.30 mmol, 12.9 eq.) of azidotrimethylsilane at room temperature. The reaction tube was sealed and heated at 90 °C for 4 h. The mixture was allowed to cool to room temperature and diluted with 10 mL of ice-cold water. The precipitated pale green solid was collected by filtration and dried under vacuum. The solid was then treated with 50 mL of 20% methanol in methylene chloride at room temperature for 20 min, filtered and the filtrate was concentrated under reduced pressure to afford the crude product. The reaction was repeated in triplicate and the combined crude product from all four batches was purified by flash chromatography (SiO_2, eluting with linear gradient of 5-20 % methanol in methylene chloride) to provide 200 mg (0.46 mmol, 24%) of (1*H*-1,2,3-triazol-4-yl)methyl (1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**17**) as an off-white solid.

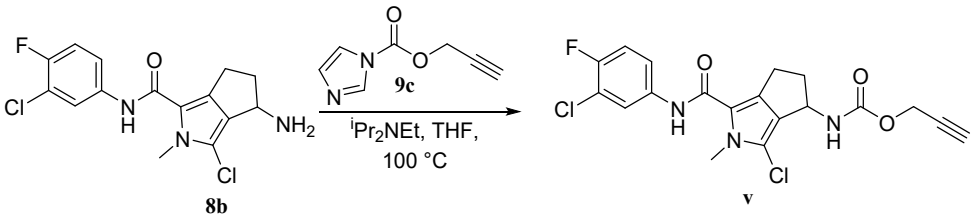
The enantiomers were subsequently separated by SFC (Waters SFC-080). Method: isocratic, Mobile phase MeOH: CO_2 - 35:65. Column: Chiralcel OJ-H (30 x 250 mm, 5 µm), flow rate: 70 g/min.

The second eluting enantiomer was isolated as (1*H*-1,2,3-triazol-4-yl)methyl (S)-(1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**17**) ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.35 (s, 1H), 7.92 (dd, J = 6.9, 2.6 Hz, 1H), 7.76 (s, 1H), 7.61 – 7.53 (m, 2H), 7.36 (t, J = 9.1 Hz, 1H), 6.80 (s, 1H), 5.10 (s, 2H), 4.84 – 4.78 (m, 1H), 3.76 (s, 3H), 2.99 – 2.88 (m, 1H), 2.85 – 2.77 (m, 1H), 2.68 – 2.55 (m, 1H, overlapping with DMSO-d_6), 2.15 – 2.06 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.02, 155.54, 152.93 (d, J = 242.3 Hz), 141.67, 136.60 (d, J = 3.0 Hz), 134.20, 129.79, 129.30, 121.57, 121.04, 119.98 (d, J = 6.6 Hz), 118.91 (d, J = 18.2 Hz), 118.12, 116.69 (d, J = 21.6 Hz), 56.61, 50.10, 37.83, 36.13, 24.25; HRMS (ESI-TOF) m/z calculated for $C_{19}H_{18}ClFN_6O_3H^+$: 433.1197, found

433.1199 (Δ 0.46 ppm); LCMS (ESI+): *m/z* found 433.5/435.4 $[M+H]^+$, RT = 1.87 min, 95.1% (Method A); HPLC: RT = 3.06 min, 96.4% (Method B); Chiral analytical SFC: RT: 5.30 min, 99.7%, Column: Chiralpak OJ-3 4.6 x 250 mm, 5 µm, 30% of methanol, Flow rate: 3.0 g/min.

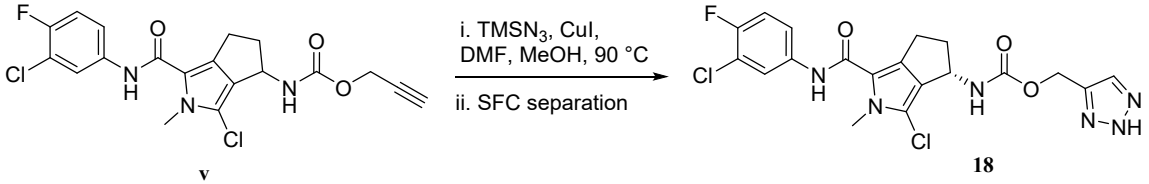
Preparation of (2*H*-1,2,3-triazol-4-yl)methyl (3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (18)

Prop-2-yn-1-yl (3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (v):



Prop-2-yn-1-yl (3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate was synthesized in a similar manner as described above (General Procedure A) from 4-amino-3-chloro-*N*-(3-chloro-4-fluorophenyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboxamide (**8b**) and prop-2-yn-1-yl 1*H*-imidazole-1-carboxylate (**9c**). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.56 (s, 1H), 7.91-7.94 (m, 1H), 7.71 (d, 1H), 7.56-7.61 (m, 1H), 7.38 (m, 1H), 4.91-4.95 (m, 1H), 4.64 (s, 2H), 3.71 (s, 3H), 3.48 (t, 1H), 2.92-2.98 (m, 1H), 2.81-2.88 (m, 1H), 2.56-2.67 (m, 1H), 2.04-2.12 (m, 1H); LRMS (ESI+): *m/z* found 424.0 $[M+H]^+$.

(2*H*-1,2,3-Triazol-4-yl)methyl (S)-(3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (18)

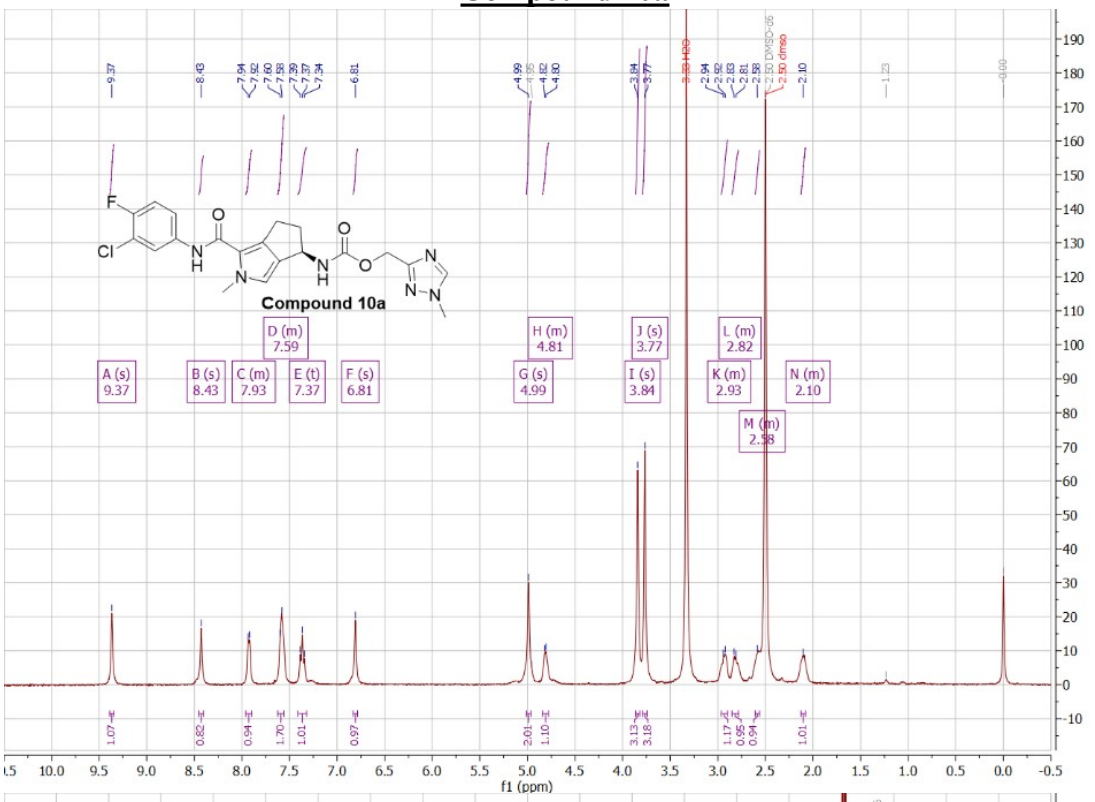


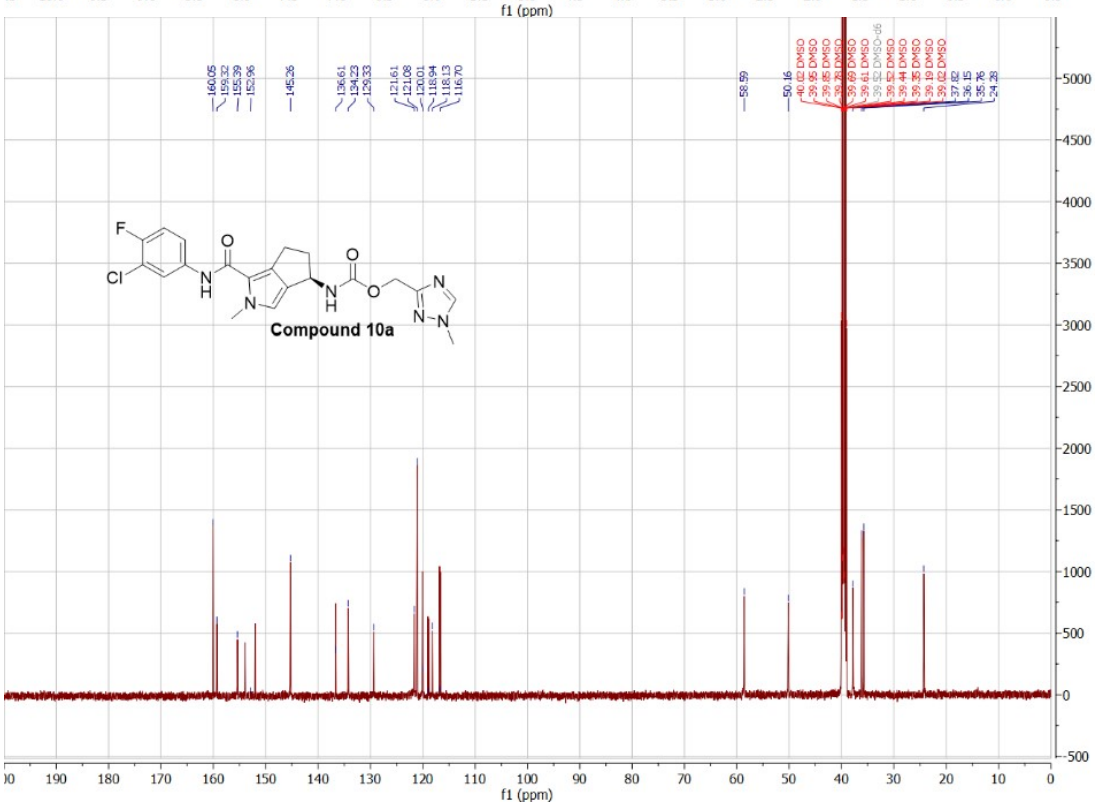
To a solution of 0.50 g (1.18 mmol, 1.0 eq.) of prop-2-yn-1-yl (3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**v**) in 10 mL of 4:1 (*v/v*) DMF:methanol was added 22 mg (0.12 mmol, 0.1 eq.) of copper(I)iodide followed by 1.36 g (11.8 mmol, 10.0 eq.) of trimethylsilyl azide and the mixture was heated at 90 °C for 5 h. The mixture was then allowed to cool to room temperature, diluted with 15 mL of water and the resulting solids collected by filtration and dried under high vacuum. The solids were then triturated with 80 mL of 10 % methanol in methylene chloride and filtered. The crude product was purified by reverse phase preparative HPLC (Column: XBRIDGE C18 (250 x 19 mm), 5 µm, to provide racemic (2*H*-1,2,3-triazol-4-yl)methyl (3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (75 mg, 0.16 mmol, 14%). LRMS (ESI+): *m/z* found 467.2/469.2 $[M+H]^+$. The enantiomers were subsequently separated by SFC (Waters SFC-080). Method isocratic, Mobile phase MeOH: CO_2 – 30:70. Column: Chiralcel OJ-H (21 x 250 mm), 5 µm, flow rate: 70 g/min.

The second eluting enantiomer was isolated as (2*H*-1,2,3-triazol-4-yl)methyl (S)-(3-chloro-1-((3-chloro-4-fluorophenyl)carbamoyl)-2-methyl-2,4,5,6-tetrahydrocyclopenta[c]pyrrol-4-yl)carbamate (**18**). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.55 (s, 1H), 7.92 (dd, J = 6.9, 2.6 Hz, 1H), 7.78 (s, 1H), 7.62 – 7.54 (m, 2H), 7.42 – 7.33 (m, 1H), 5.10 (s, 2H), 4.99 – 4.93 (m, 1H), 3.70 (s, 3H), 3.00 – 2.89 (m, 1H), 2.89 – 2.77 (m, 1H), 2.63 – 2.58 (m, 1H, overlapping with DMSO-d_6), 2.13 – 2.00 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.32, 155.39, 153.09 (d, J = 242.7 Hz), 141.64, 136.34 (d, J = 3.0 Hz), 134.06, 129.83, 125.73, 121.24, 120.16 (d, J = 7.0 Hz), 119.04, 118.89, 116.75 (d, J = 21.6 Hz), 114.66, 56.72, 49.84, 38.19, 32.48, 24.38; HRMS (ESI-TOF) m/z calculated for $C_{19}H_{17}ClFN_6O_3H^+$: 467.0807, found 467.0822 (Δ 3.21 ppm); LCMS (ESI+): *m/z* found 467.2/469.2 $[M+H]^+$, RT = 2.33 min, 96.4% (Method A); HPLC: RT = 3.45 min, 96.2% (Method C); Chiral analytical SFC: RT = 4.62 min, 99.6%,Column: Chiralpak OJ-H 4.6 x 250mm, 5 µm, 45% of methanol, Flow rate: 3.0 g/min.

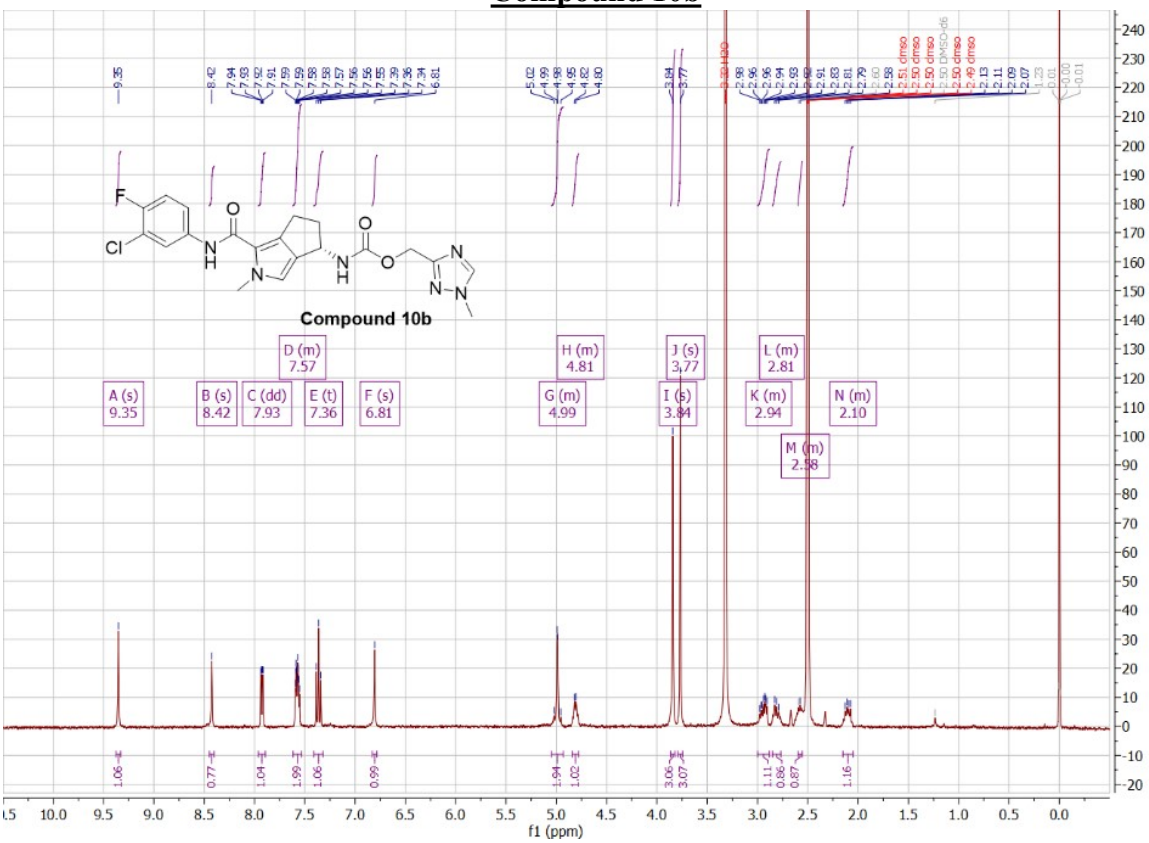
2. ^{1}H and ^{13}C NMR spectra for key compounds

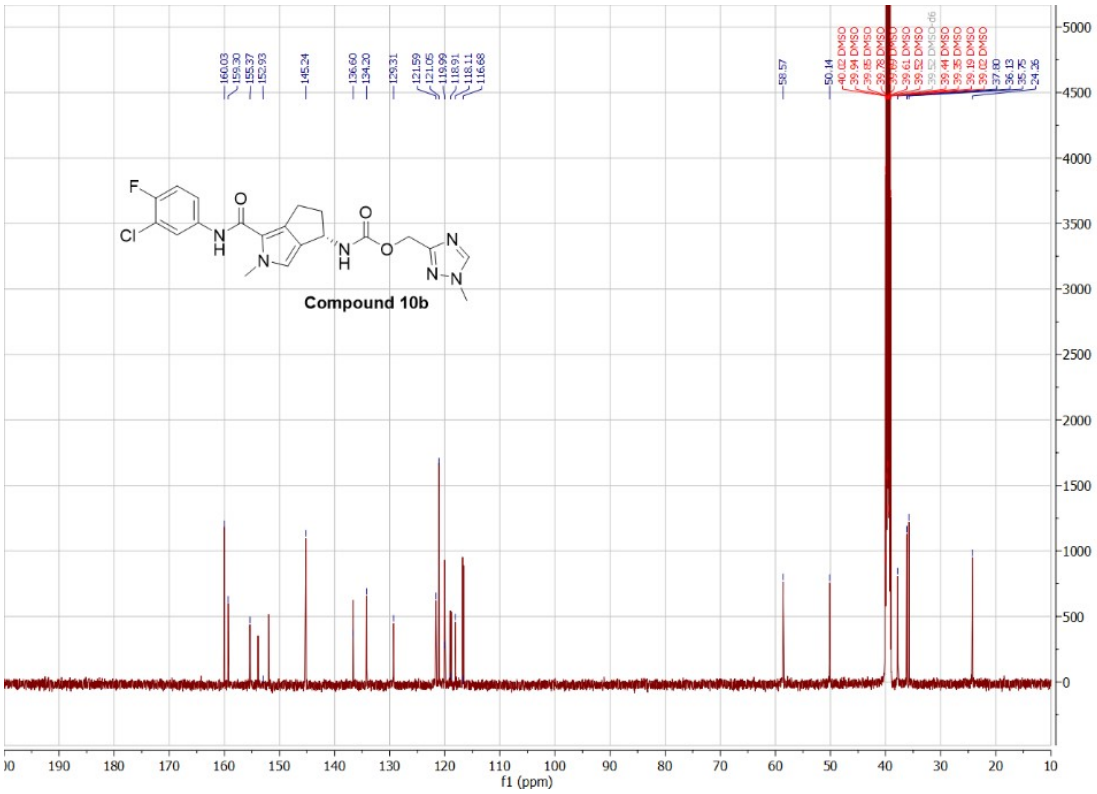
Compound 10a



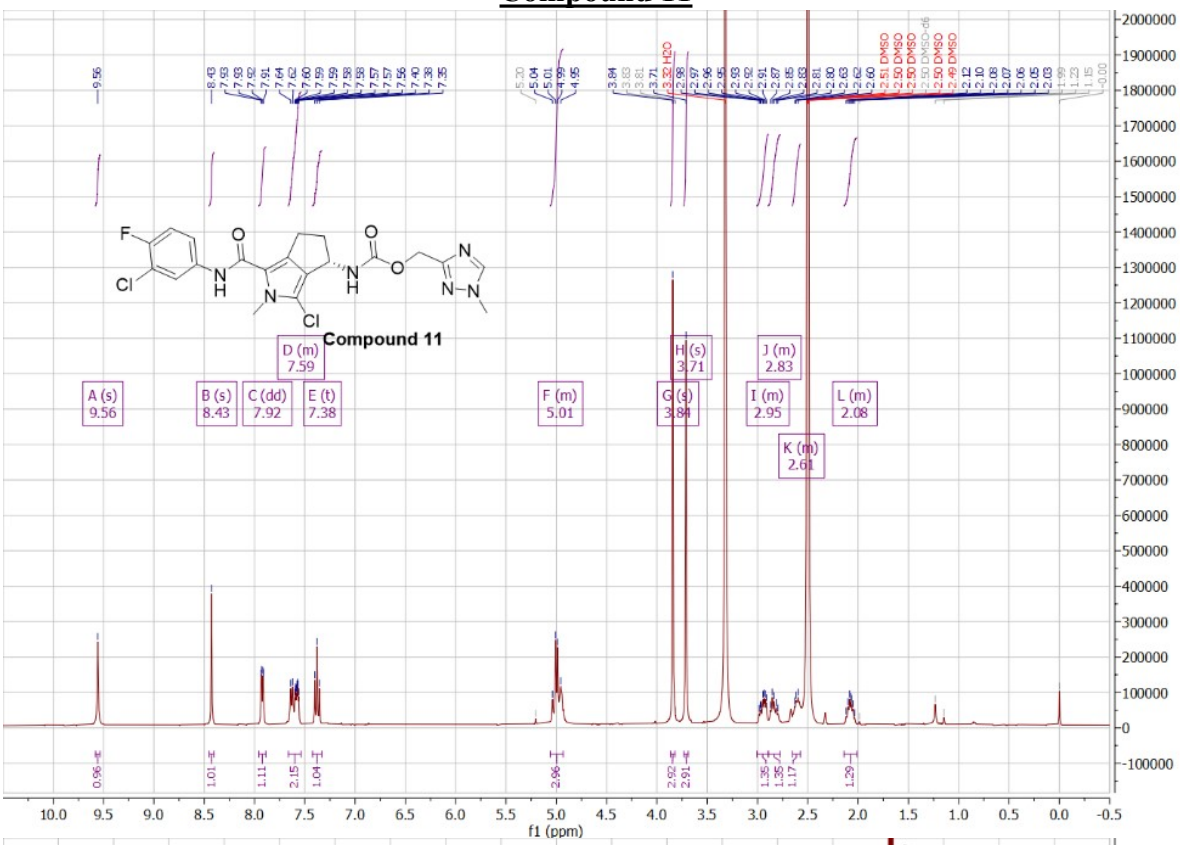


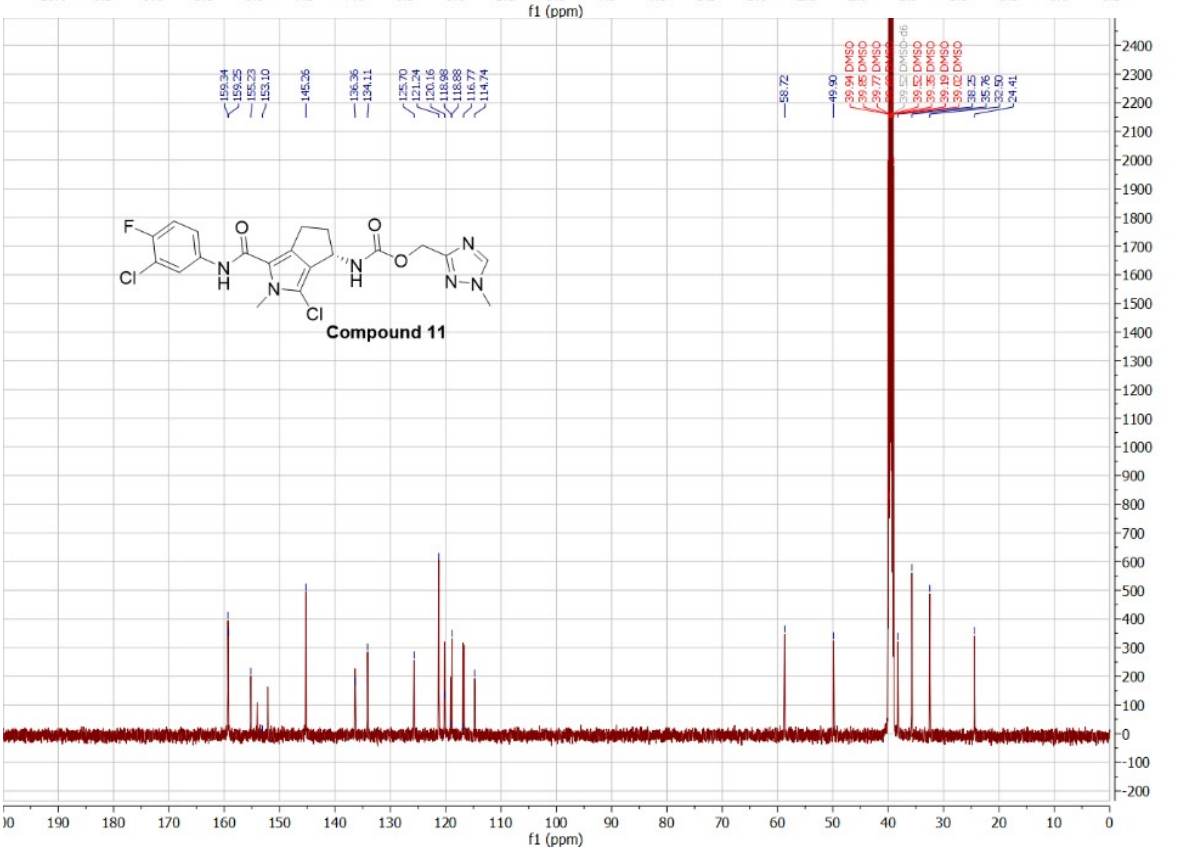
Compound 10b



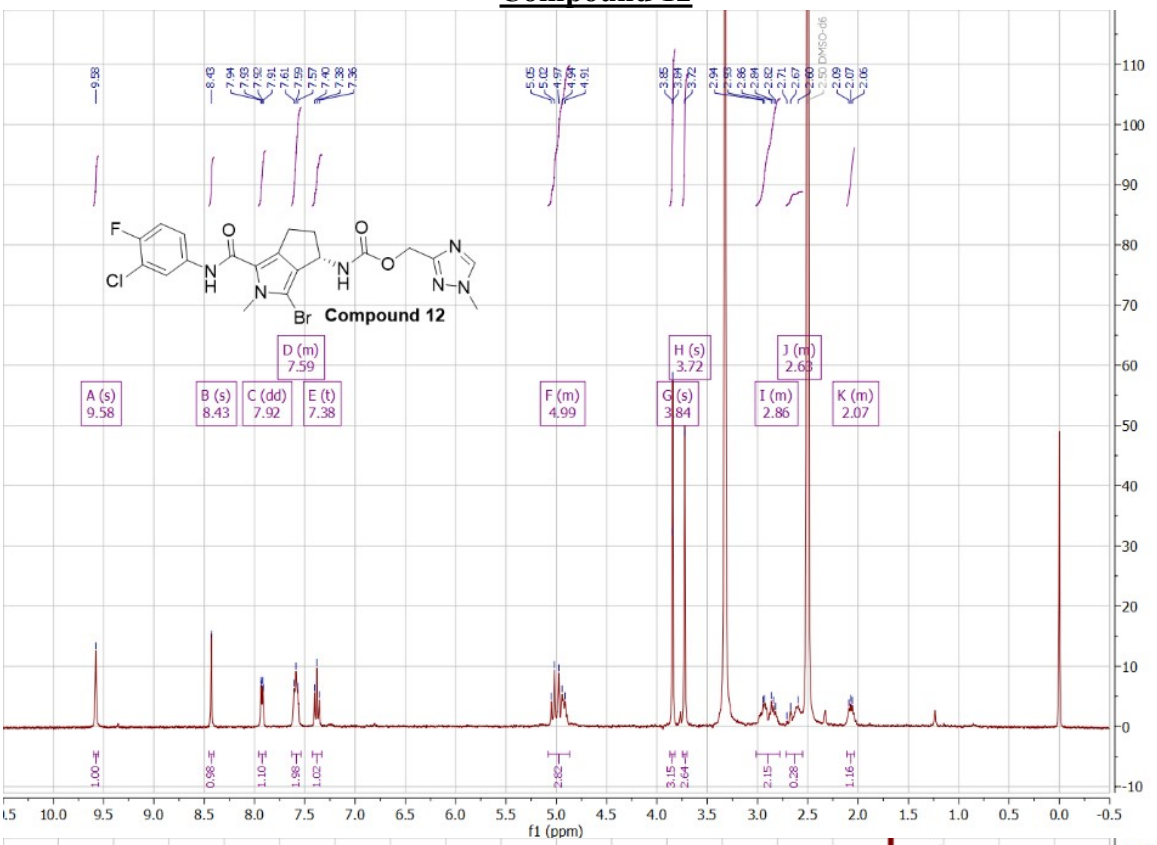


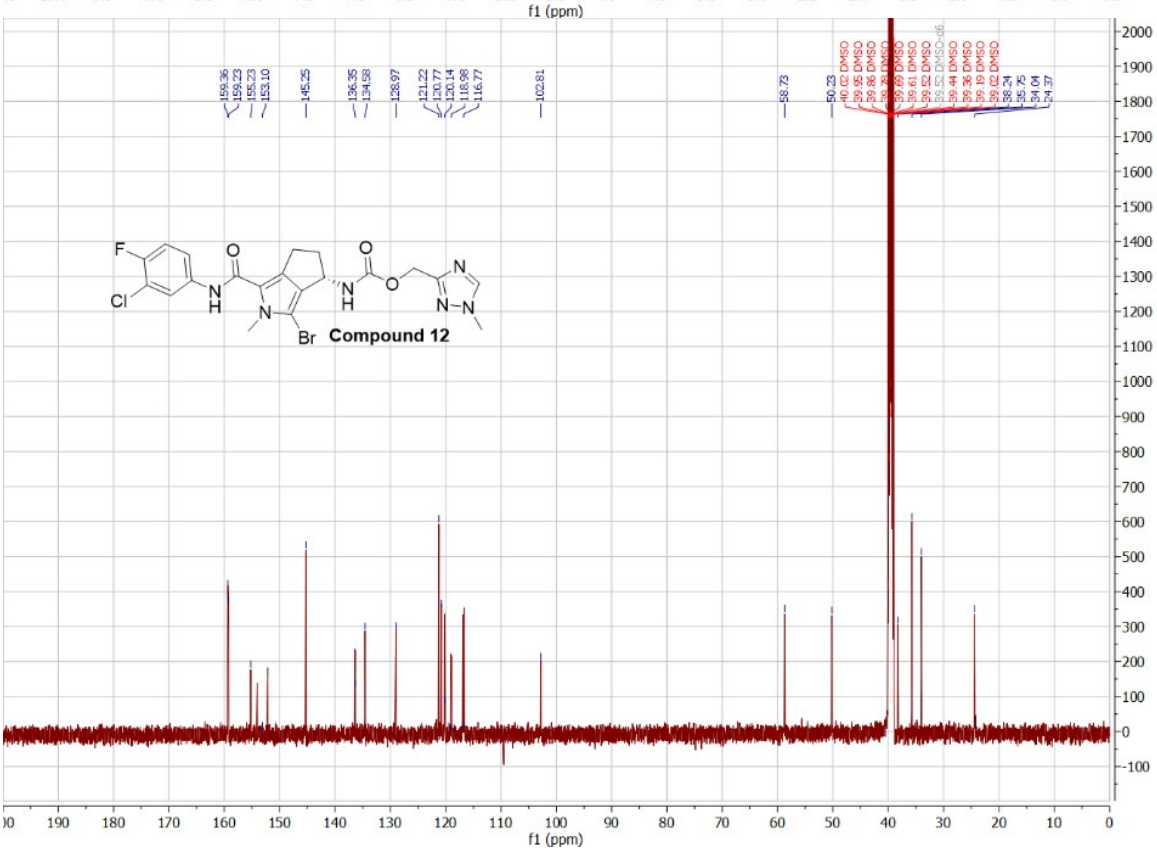
Compound 11


Compound 11
A (s) 9.56
B (s) 8.43
C (dd) 7.92
D (m) 7.59
E (t) 7.38
F (m) 5.01
G (s) 3.84
H (s) 3.71
I (m) 2.95
J (m) 2.83
K (m) 2.61
L (m) 2.08
f1 (ppm)

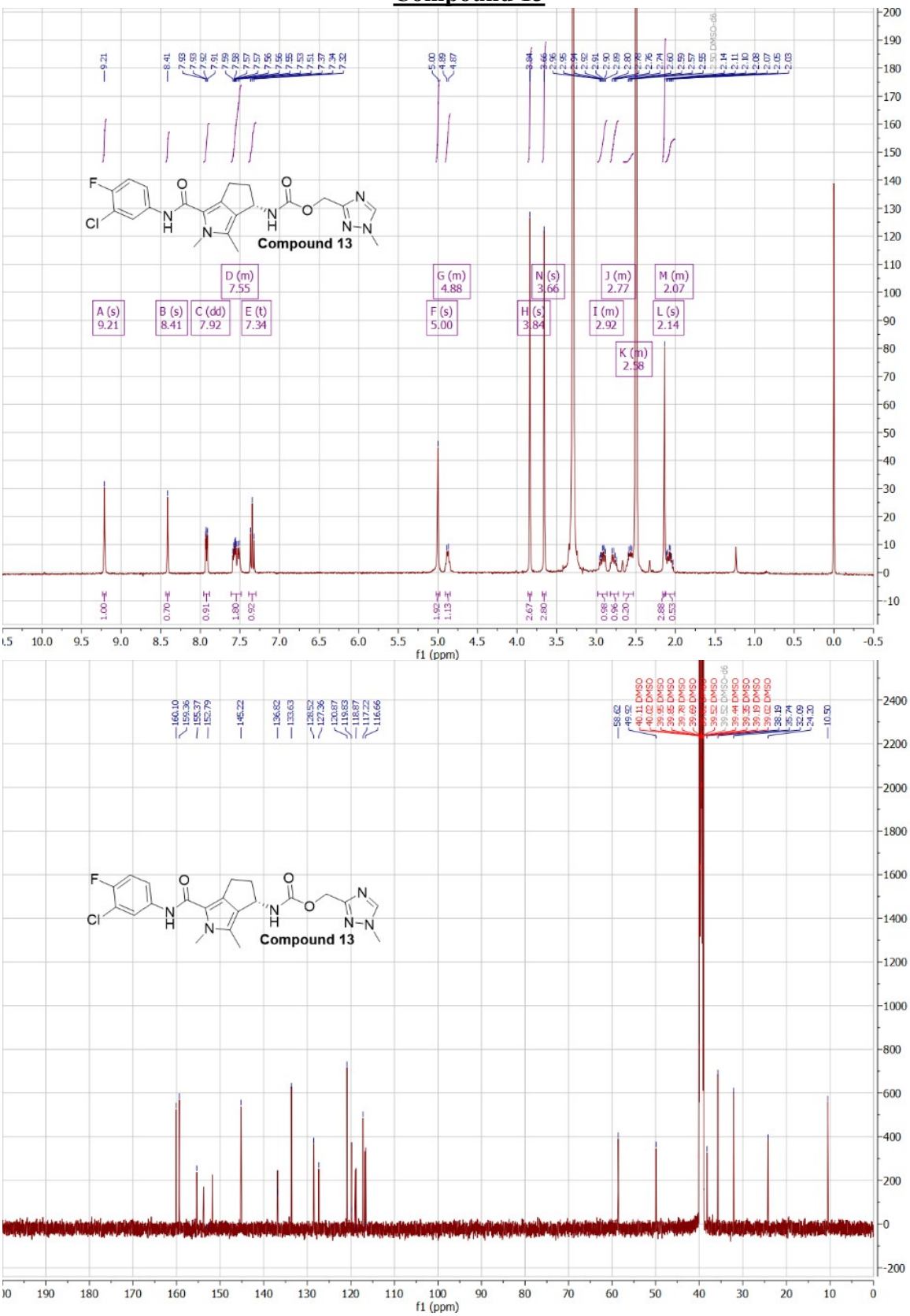

Compound 11
f1 (ppm)

Compound 12

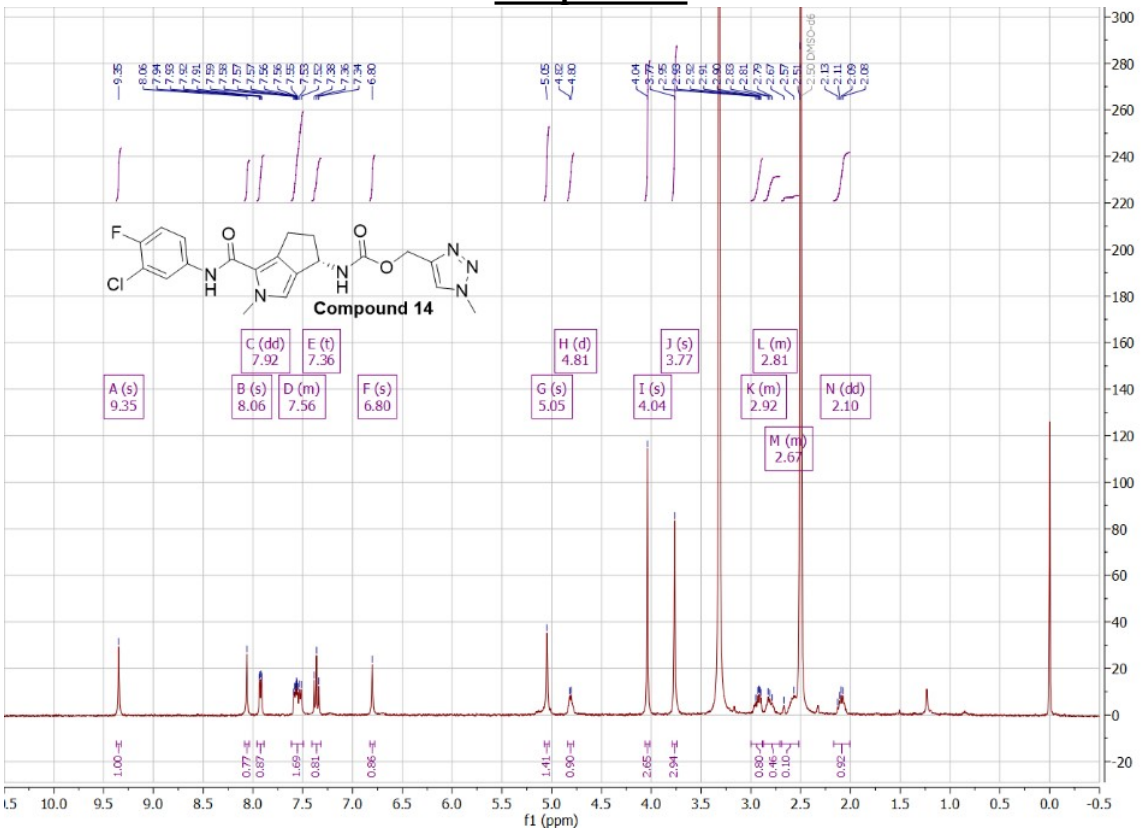

Compound 12
A (s) 9.58
B (s) 8.43
C (dd) 7.92
D (m) 7.59
E (t) 7.38
F (m) 4.99
G (s) 3.84
H (s) 3.72
I (m) 2.86
J (m) 2.63
K (m) 2.07
f1 (ppm)

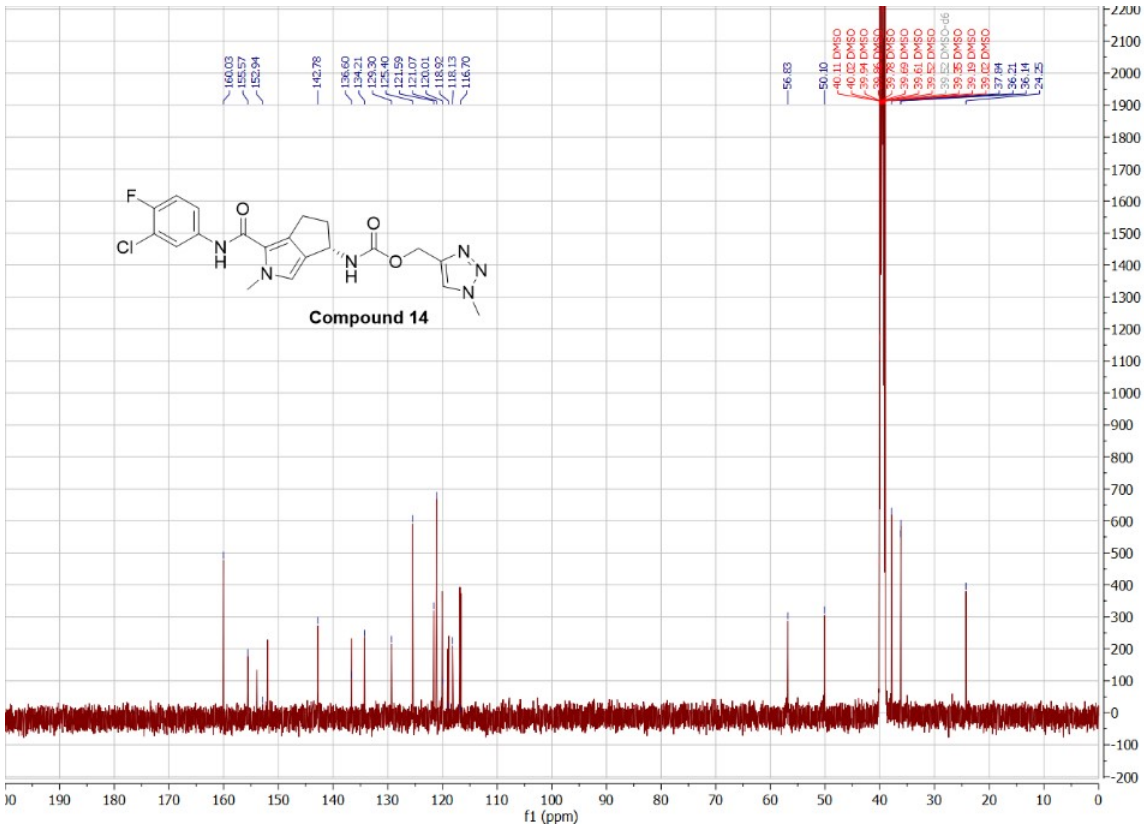

Compound 12
f1 (ppm)

Compound 13

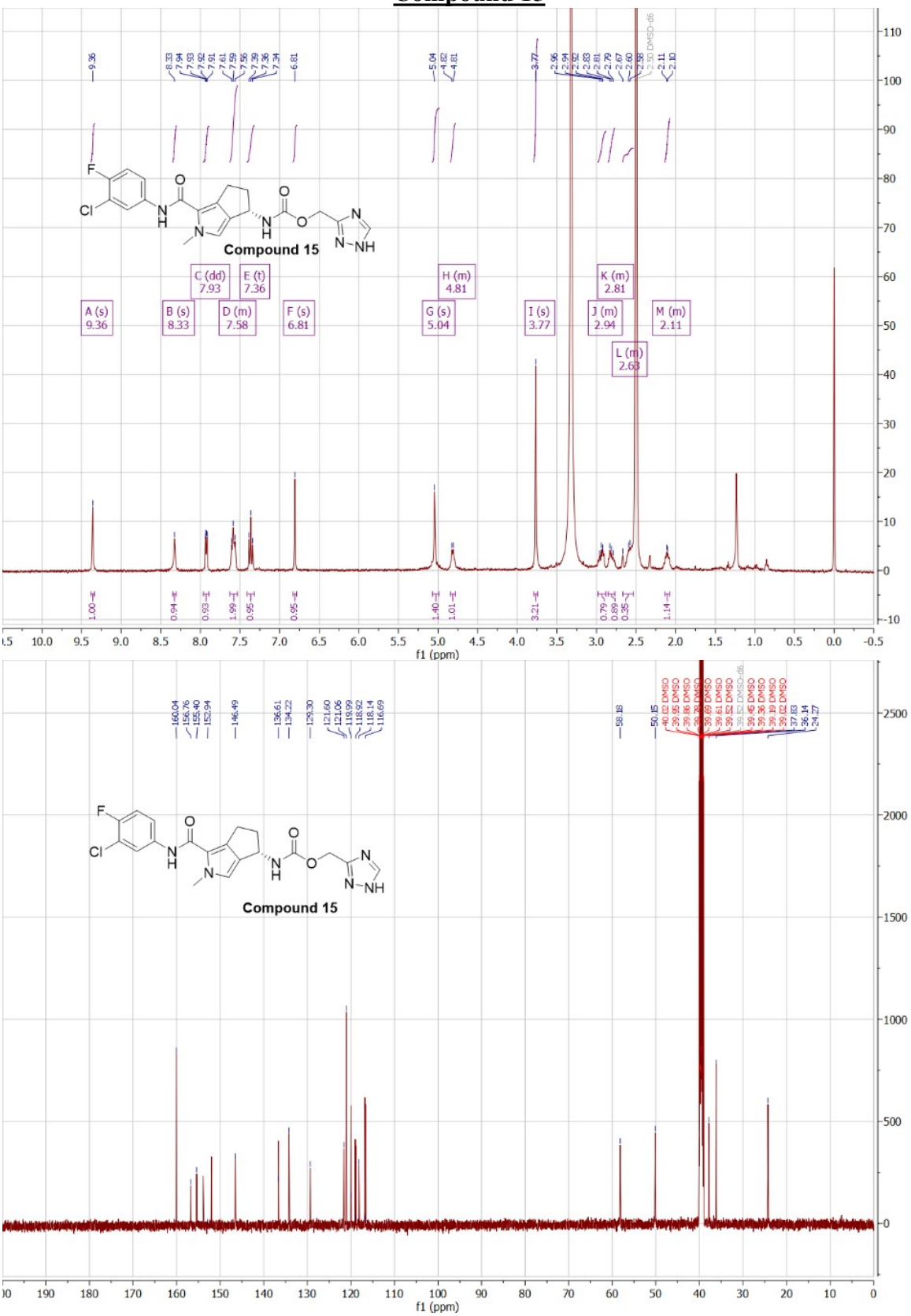

Compound 13
A (s) 9.21
B (s) 8.41
C (dd) 7.92
D (m) 7.55
E (t) 7.34
F (s) 5.00
G (m) 4.88
H (s) 3.84
N (s) 3.66
I (m) 2.92
J (m) 2.77
K (m) 2.58
L (s) 2.14
M (m) 2.07
f1 (ppm)
Compound 13
f1 (ppm)

Compound 14

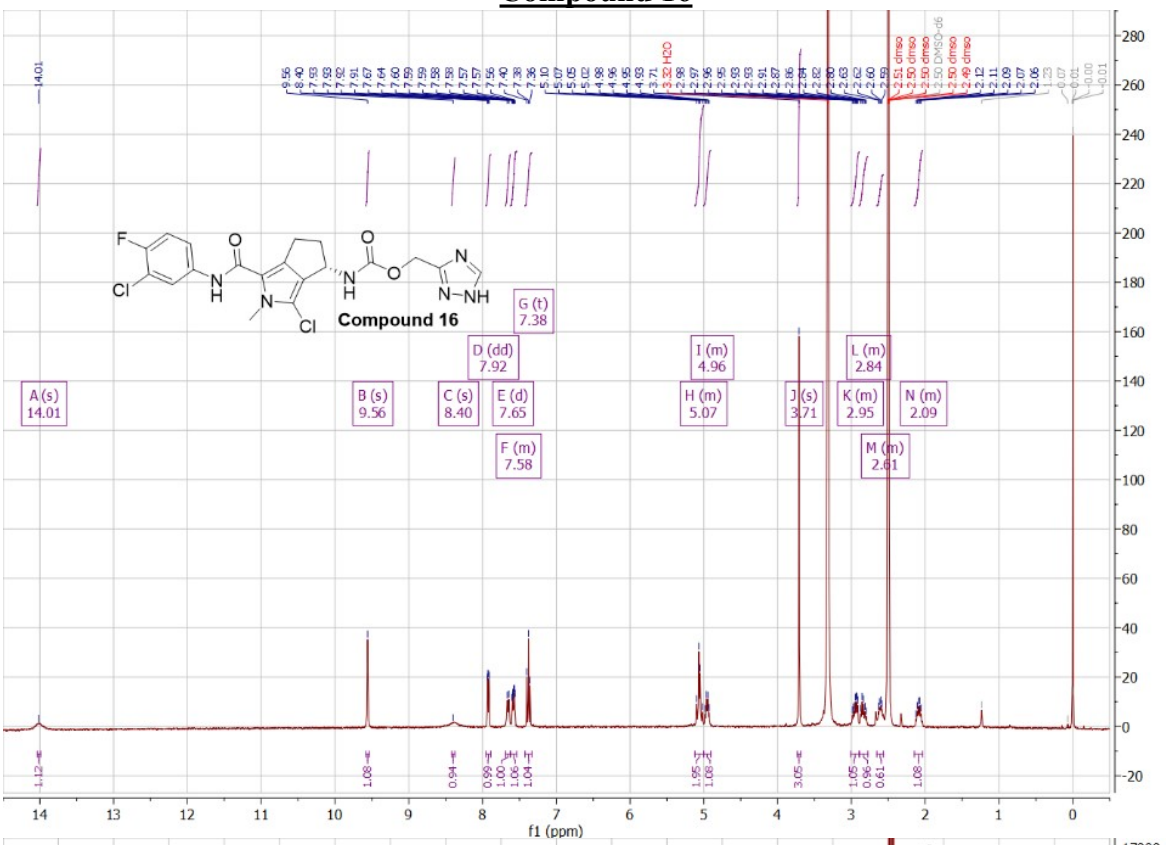

Compound 14
A (s) 9.35
B (s) 8.06
C (dd) 7.92
D (m) 7.56
E (t) 7.36
F (s) 6.80
G (s) 5.05
H (d) 4.81
I (s) 4.04
J (s) 3.77
K (m) 2.92
L (m) 2.81
M (m) 2.67
N (dd) 2.10
f1 (ppm)

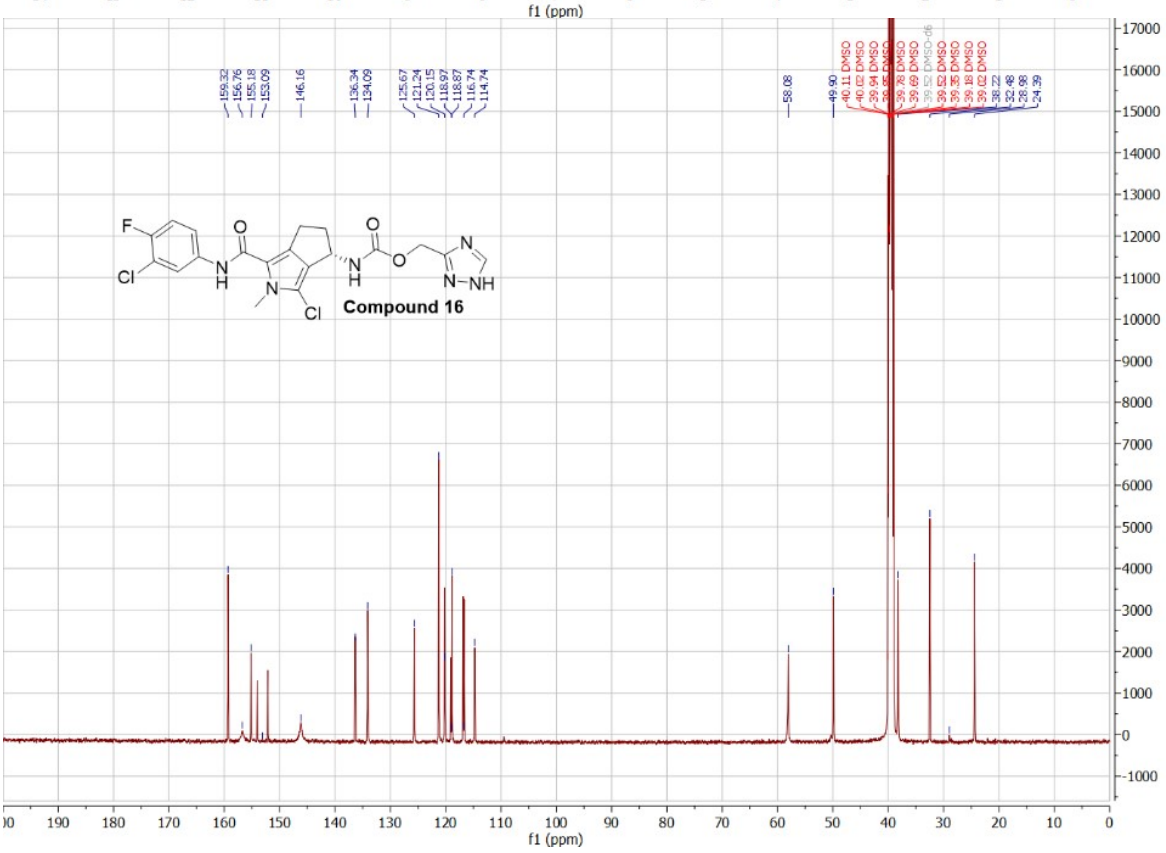

Compound 14
f1 (ppm)

Compound 15

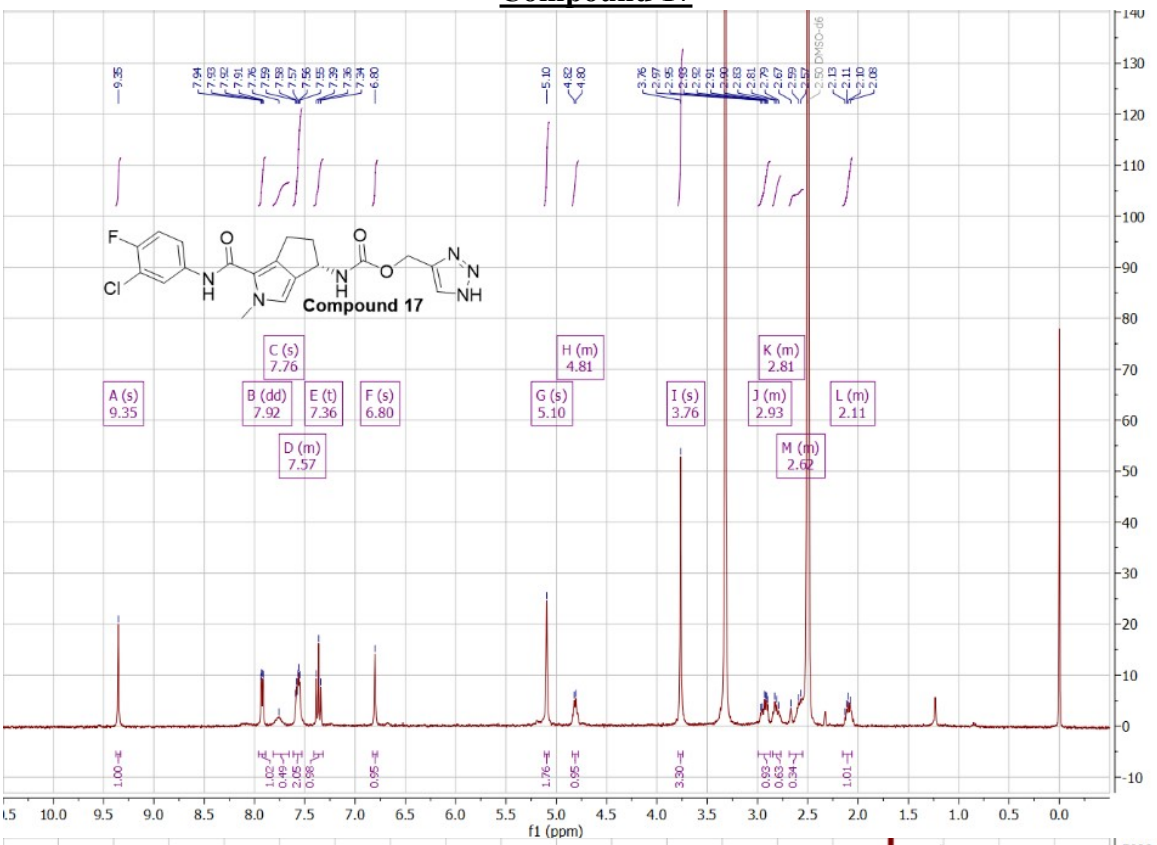

Compound 15
A (s) 9.36
B (s) 8.33
C (dd) 7.93
D (m) 7.58
E (t) 7.36
F (s) 6.81
G (s) 5.04
H (m) 4.81
I (s) 3.77
J (m) 2.94
K (m) 2.81
L (m) 2.63
M (m) 2.11
f1 (ppm)
Compound 15
f1 (ppm)

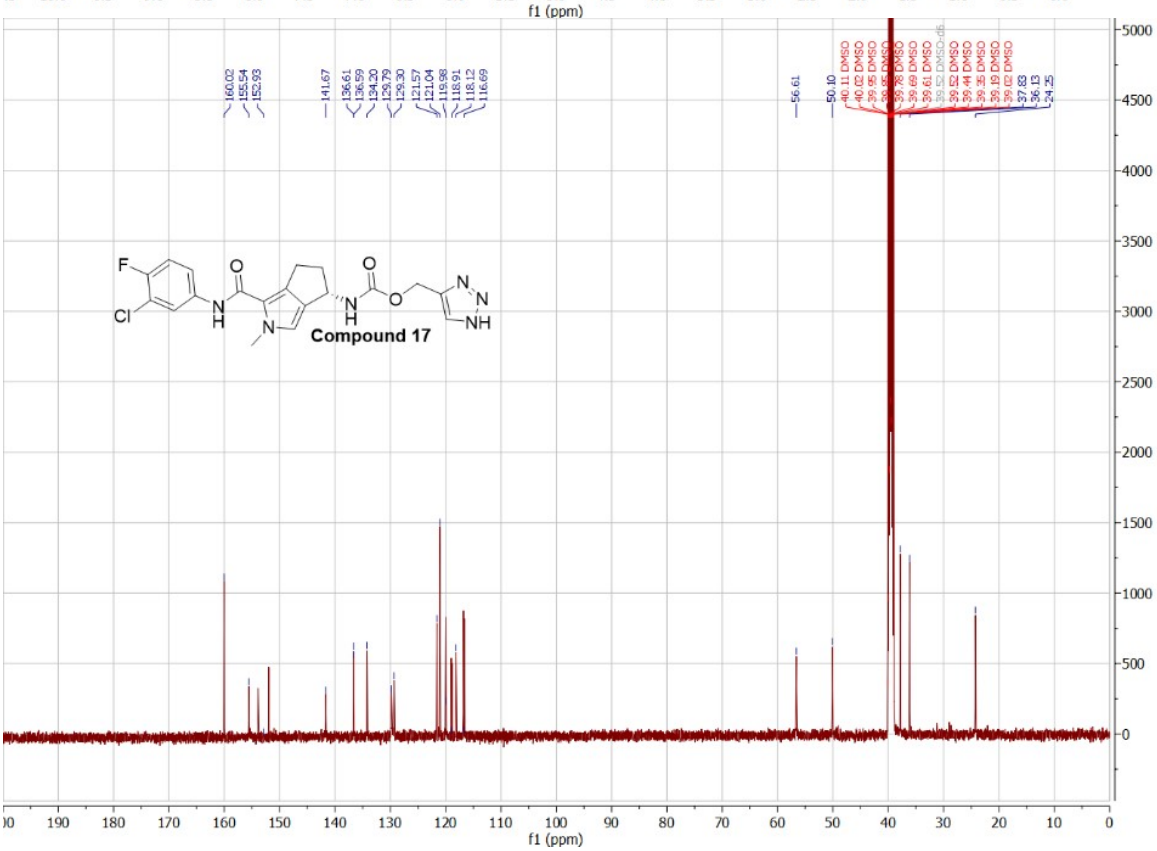
Compound 16


Compound 16
A (s) 14.01
B (s) 9.56
C (s) 8.40
D (dd) 7.92
E (d) 7.65
F (m) 7.58
G (t) 7.38
H (m) 5.07
I (m) 4.96
J (s) 3.71
K (m) 2.95
L (m) 2.84
M (m) 2.61
N (m) 2.09
f1 (ppm)

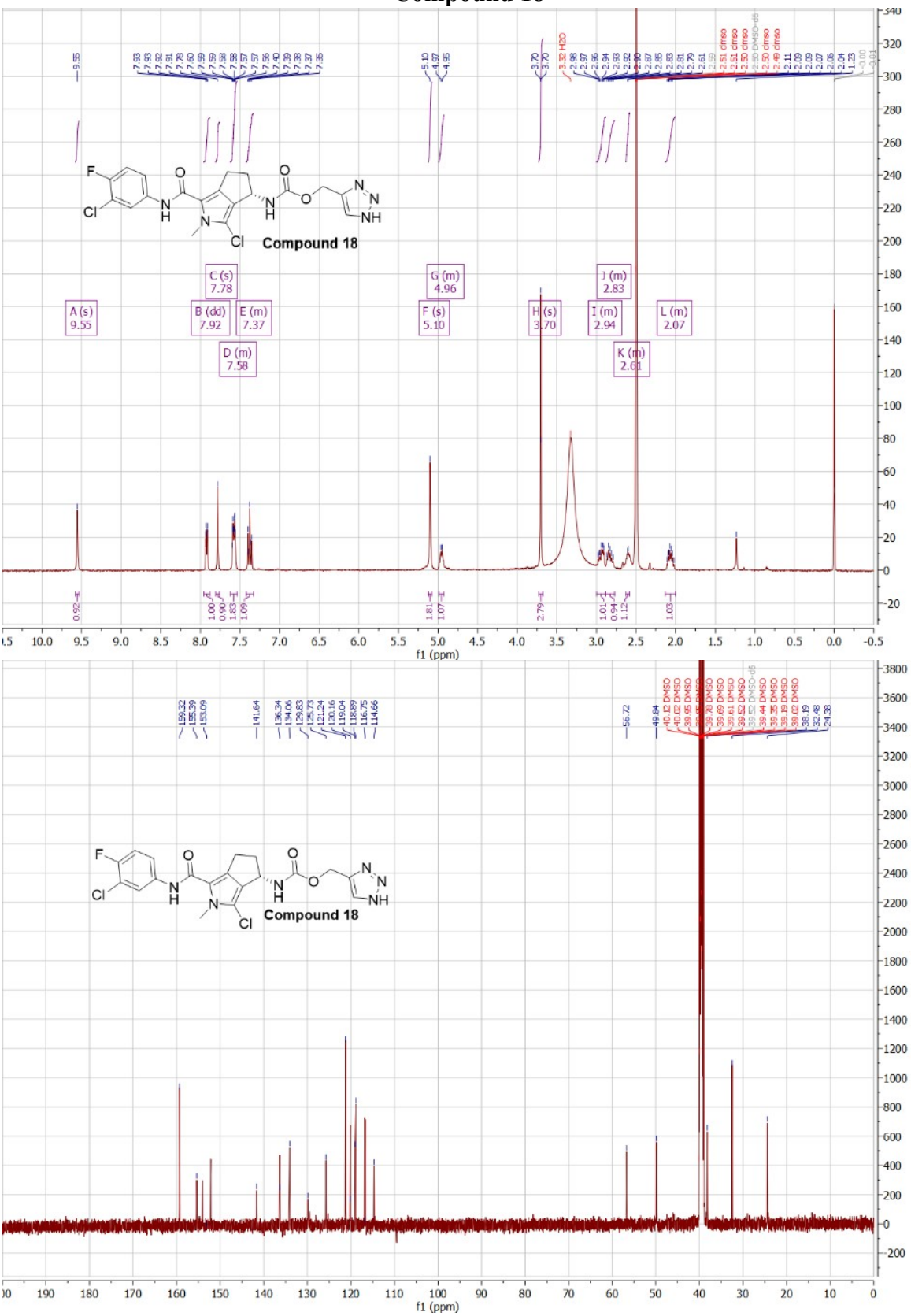

Compound 16
f1 (ppm)

Compound 17


Compound 17
A (s) 9.35
B (dd) 7.92
C (s) 7.76
D (m) 7.57
E (t) 7.36
F (s) 6.80
G (s) 5.10
H (m) 4.81
I (s) 3.76
J (m) 2.93
K (m) 2.81
L (m) 2.11
M (m) 2.62
f1 (ppm)


Compound 17
f1 (ppm)

Compound 18

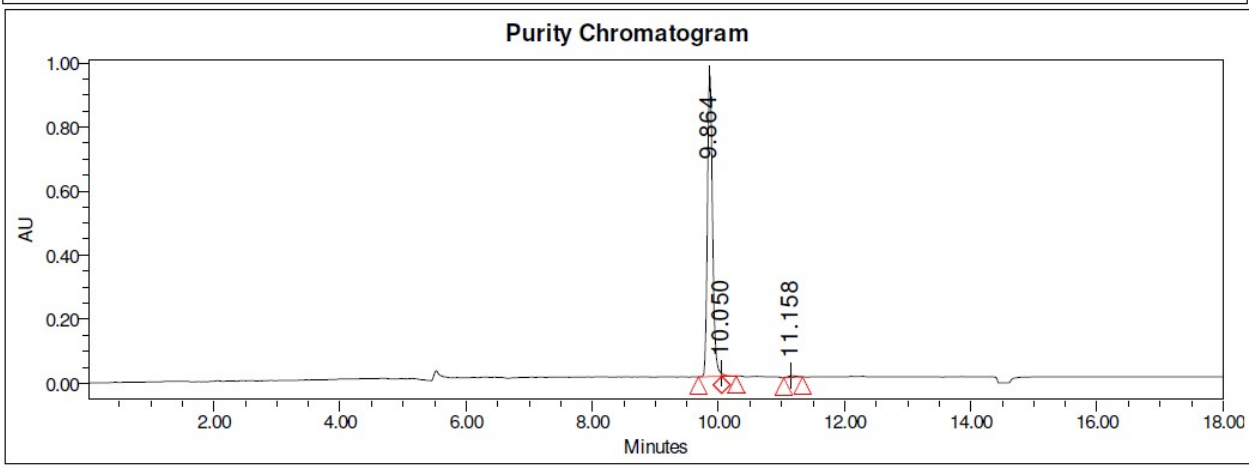

Compound 18
A (s) 9.55
B (dd) 7.92
C (s) 7.78
D (m) 7.58
E (m) 7.37
F (s) 5.10
G (m) 4.96
H (s) 3.70
I (m) 2.94
J (m) 2.83
K (m) 2.61
L (m) 2.07
f1 (ppm)
Compound 18
f1 (ppm)

3. HPLC chromatograms of key compounds

Compound 10A

HPLC Method Conditions:

Column: X-Bridge C18 (150mmX4.6mm, 3.5µm)
Mobile Phase: B: 10 mM Ammonium acetate in Water , A: 100% Acetonitrile
Gradient(T%/A) : 0/5, 1.0/5,12/95,15/95, 17/5,20.0/5
Flow Rate : 1.0ml/min
Diluent : ACN+H2O



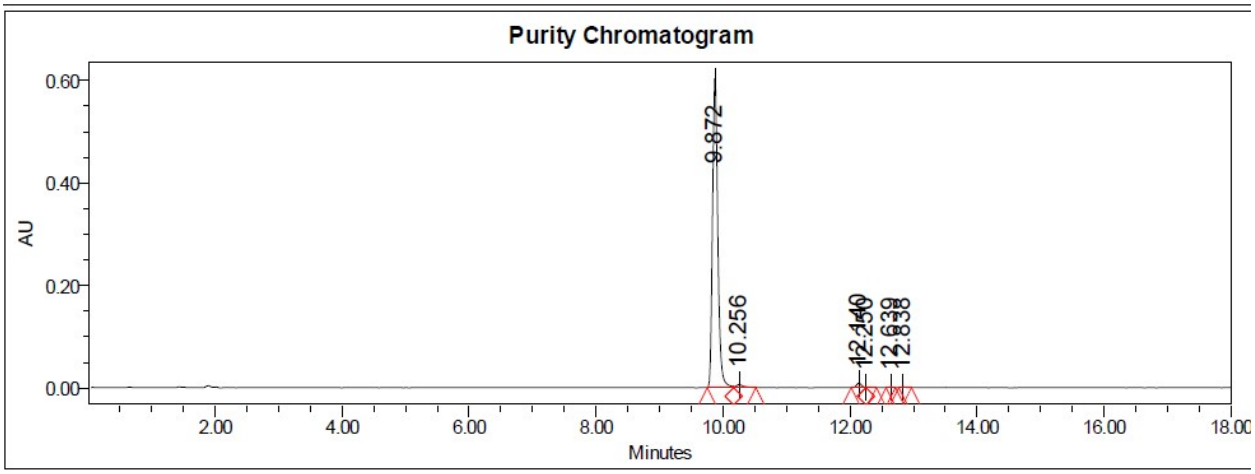
Peak Results

	RT	Area	% Area
1	9.864	5280079	98.64
2	10.050	28585	0.53
3	11.158	43981	0.82

Compound 10B

HPLC Method Conditions:

Column: X-Bridge C18 (150mmX4.6mm, 3.5µm)
Mobile Phase: B: 10 mM Ammonium acetate in Water , A: 100% Acetonitrile
Gradient(T%/A) : 0/5, 1.0/5,12/95,15/95, 17/5,20.0/5
Flow Rate : 1.0ml/min
Diluent : ACN+H2O



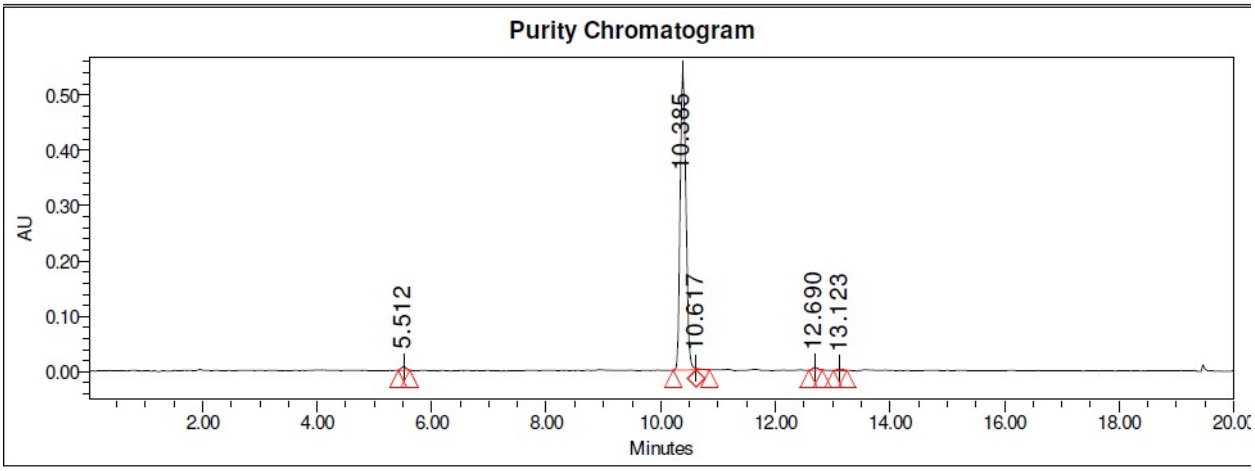
Peak Results

	RT	Area	% Area
1	9.872	3468477	96.80
2	10.256	49248	1.37
3	12.140	50456	1.41
4	12.250	4005	0.11
5	12.639	4313	0.12
6	12.838	6507	0.18

Compound 11

HPLC Method Conditions:

Column: X-Bridge C18 (150mmX4.6mm, 3.5µm)
Mobile Phase: A: 10 mM Ammonium acetate in Water ,D: 100% Acetonitrile
Gradient(T%/D) : 0/5, 1.0/5,12/95,15/95, 17/5,20.0/5
Flow Rate : 1.0ml/min
Diluent : ACN: H2O



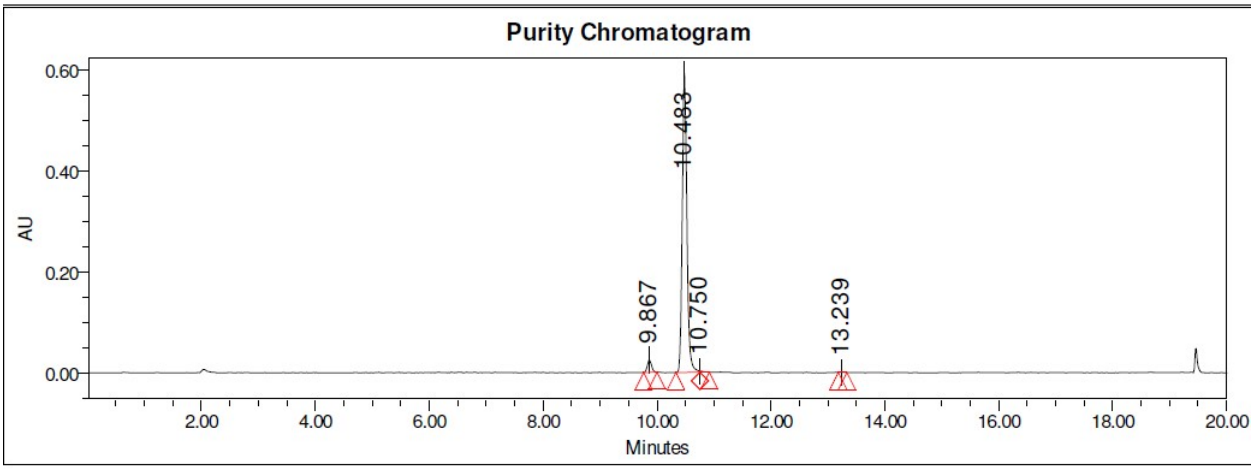
Peak Results

	RT	Area	% Area
1	5.512	37556	0.96
2	10.385	3808463	97.23
3	10.617	16683	0.43
4	12.690	34502	0.88
5	13.123	19560	0.50

Compound 12

HPLC Method Conditions:

Column: X-Bridge C18 (150mmX4.6mm, 3.5µm)
Mobile Phase: A: 10 mM Ammonium acetate in Water ,D: 100% Acetonitrile
Gradient(T%/A) : 0/5, 1.0/5,12/95,15/95, 17/5,20.0/5
Flow Rate : 1.0ml/min
Diluent : ACN: H2O:DMSO



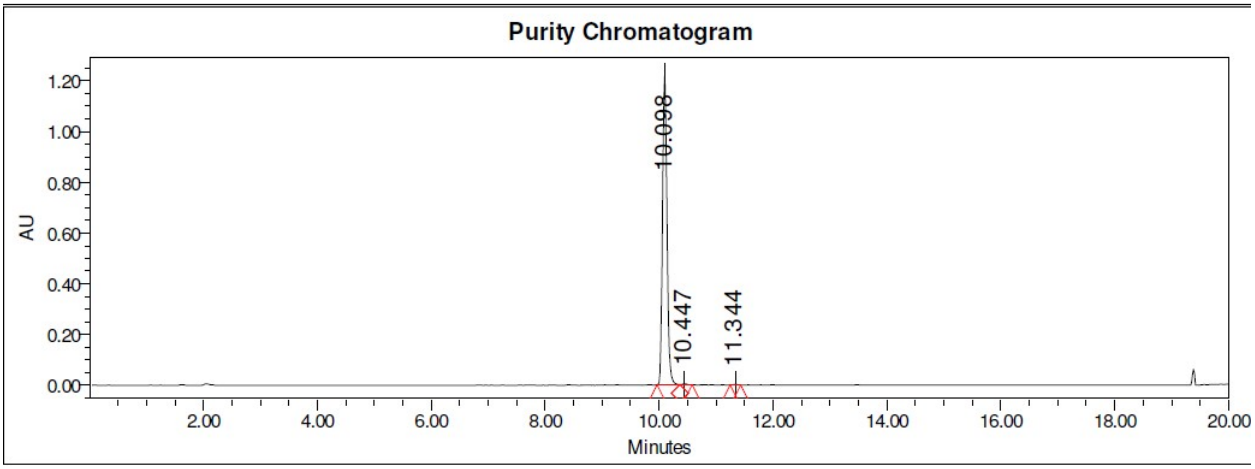
Peak Results

	RT	Area	% Area
1	9.867	127182	3.62
2	10.483	3369302	95.97
3	10.750	10439	0.30
4	13.239	3685	0.10

Compound 13

HPLC Method Conditions:

Column: XBridge C18 (150mmX4.6mm, 3.5µm)
Mobile Phase: A: 10 mM Ammonium acetate in Water ,D: 100% Acetonitrile
Gradient(T%/D) : 0/5, 1.0/5,12/95,15/95, 17/5,20.0/5
Flow Rate : 1.0ml/min
Diluent : ACN: H2O



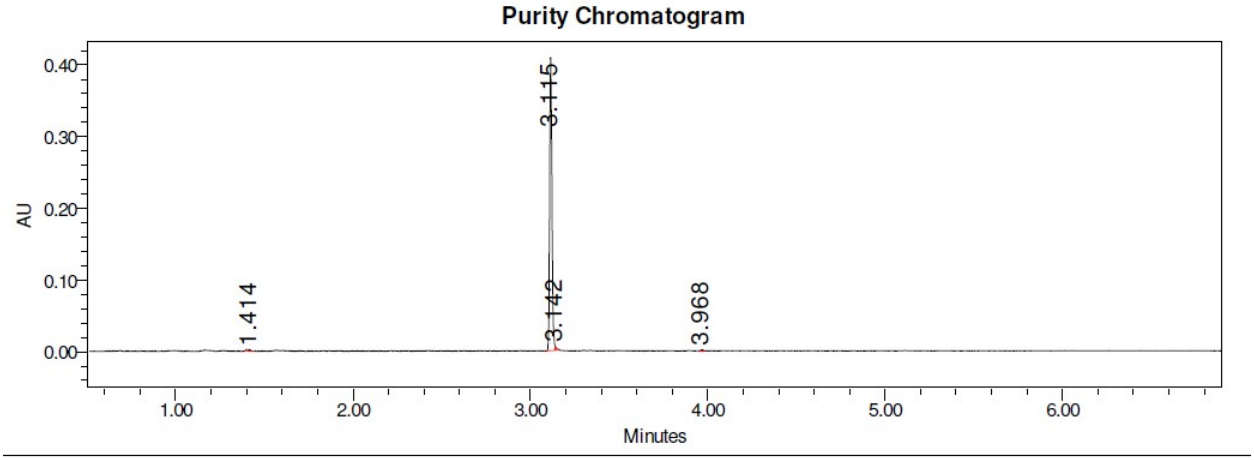
Peak Results

	RT	Area	% Area
1	10.098	6625225	99.48
2	10.447	23109	0.35
3	11.344	1179	0.18

Compound 14

UPLC Method Conditions:

Column: Acquity BEH C18 (50mmX2.1 mm, 1.7 µm)
Mobile Phase: A : 0.1% TFA in Water B: 100% Acetonitrile
Gradient(T/%B) : 0/5, 0.5/5, 5.0/95, 6.50/95, 6.60/5, 8.0/5.
Flow Rate :0.5 ml/min
Diluent : ACN:H20



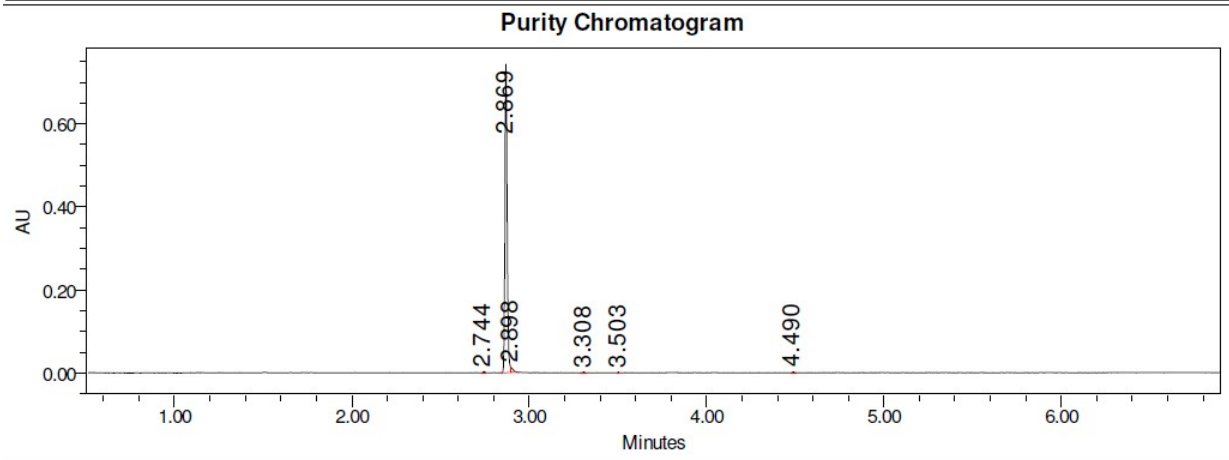
Peak Results

	RT	Area	% Area
1	1.414	3441	0.91
2	3.115	370578	97.99
3	3.142	2594	0.69
4	3.968	1554	0.41

Compound 15

UPLC Method Conditions:

Column: Acquity BEH C18 (50mmX2.1 mm, 1.7 µm)
Mobile Phase: A : 0.1% TFA in Water B: 100% Acetonitrile
Gradient(T/%B) : 0/5, 0.5/5, 5.0/95, 6.50/95, 6.60/5, 8.0/5.
Flow Rate :0.5 ml/min
Diluent : ACN:H20



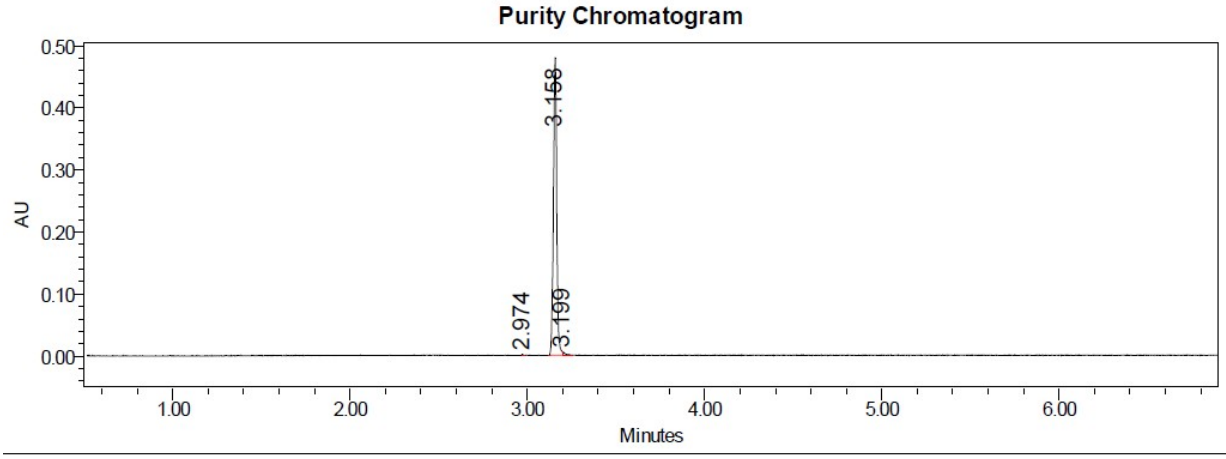
Peak Results

	RT	Area	% Area
1	2.744	2437	0.37
2	2.869	648427	97.72
3	2.898	9080	1.37
4	3.308	1491	0.22
5	3.503	452	0.07
6	4.490	1701	0.26

Compound 16

UPLC Method Conditions:

Column: Acquity BEH C18 (50mmX2.1 mm, 1.7 µm)
Mobile Phase: A : 0.1% TFA in Water B: 100% Acetonitrile
Gradient(T/%B) : 0/5, 0.5/5, 5.0/95, 6.50/95, 6.60/5, 8.0/5.
Flow Rate :0.5 ml/min
Diluent : ACN:H2O



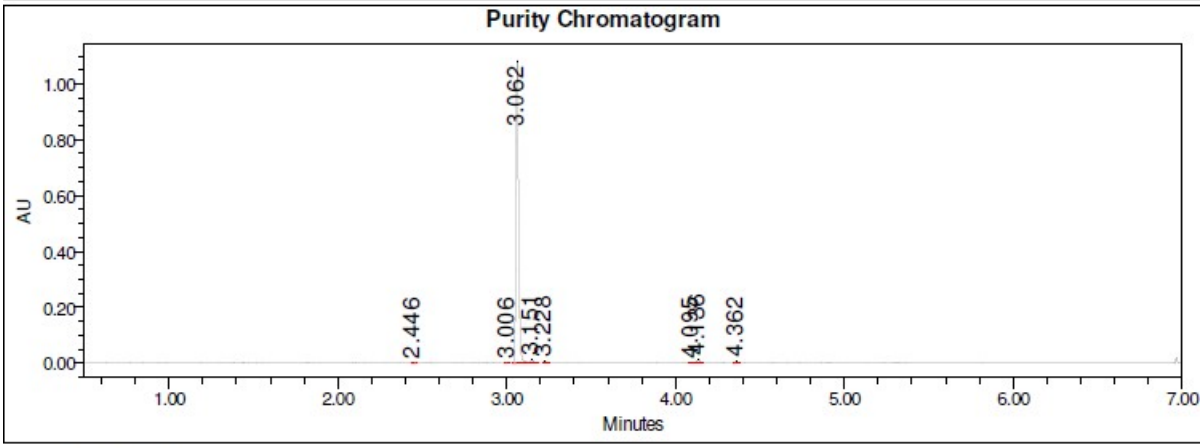
Peak Results

	RT	Area	% Area
1	2.974	667	0.11
2	3.158	614525	99.01
3	3.199	5509	0.89

Compound 17

UPLC Method Conditions:

Column: Acquity BEH C18 (50mmX2.1 mm, 1.7 µm)
Mobile Phase: A : 0.1% TFA in Water B: 100% Acetonitrile
Gradient(T/%B) : 0/5, 0.5/5, 5.0/95, 6.50/95, 6.60/5, 8.0/5.
Flow Rate :0.5 ml/min
Diluent :ACN:H2O



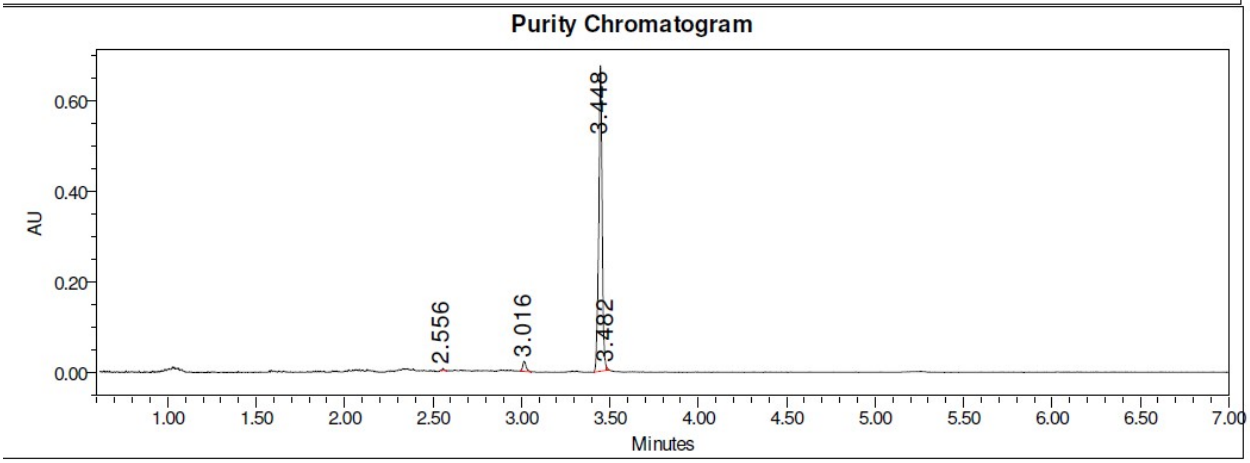
Peak Results

	RT	Area	% Area
1	2.446	1088	0.09
2	3.006	1150	0.10
3	3.062	1158204	96.38
4	3.151	11079	0.92
5	3.228	5486	0.46
6	4.095	2493	0.21
7	4.136	17045	1.42
8	4.362	5179	0.43

Compound 18

UPLC Method Conditions:

Column: Acquity BEH C18 (50mmX2.1 mm, 1.7 µm)
Mobile Phase: A : 0.1% FA in Water B: 100% Acetonitrile
Gradient(T/%B) : 0/5, 0.5/5, 5.0/95, 6.50/95, 6.60/5, 8.0/5.
Flow Rate :0.5 ml/min
Diluent : ACN:H2O

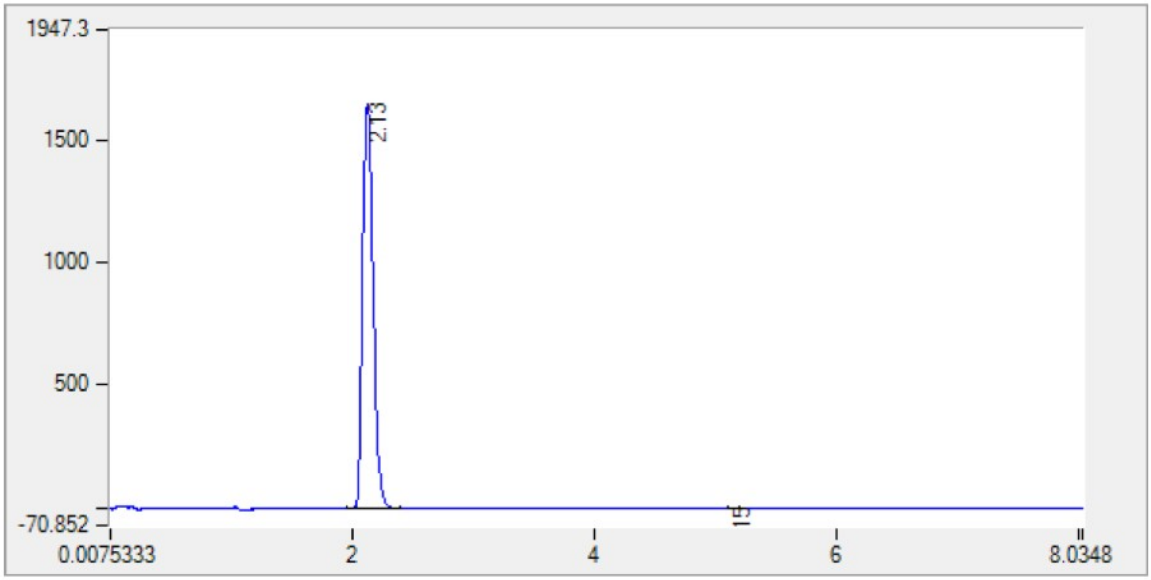


Peak Results

	RT	Area	% Area
1	2.556	2590	0.27
2	3.016	29619	3.14
3	3.448	906865	96.24
4	3.482	3208	0.34

SFC chromatograms of key compounds

Compound 10A

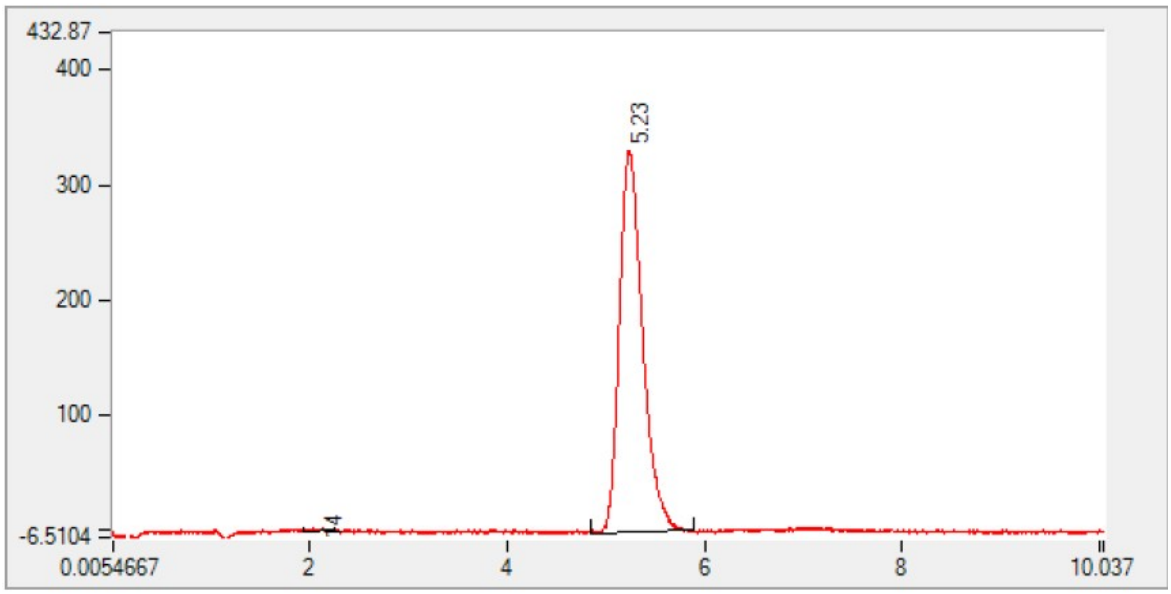


214 nm

Results UV1			
Sr.No.	RT	Area	Area %
1	2.13	9897.149	99.952
2	5.15	4.773	0.048

Comp

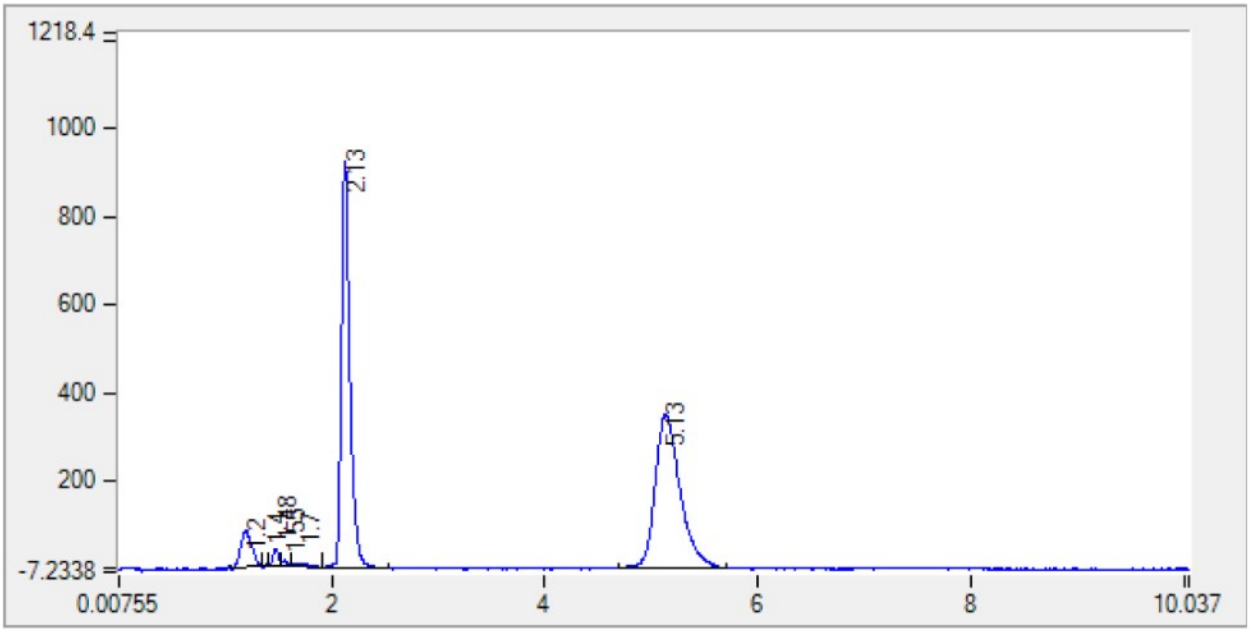
ound 10B



280 nm

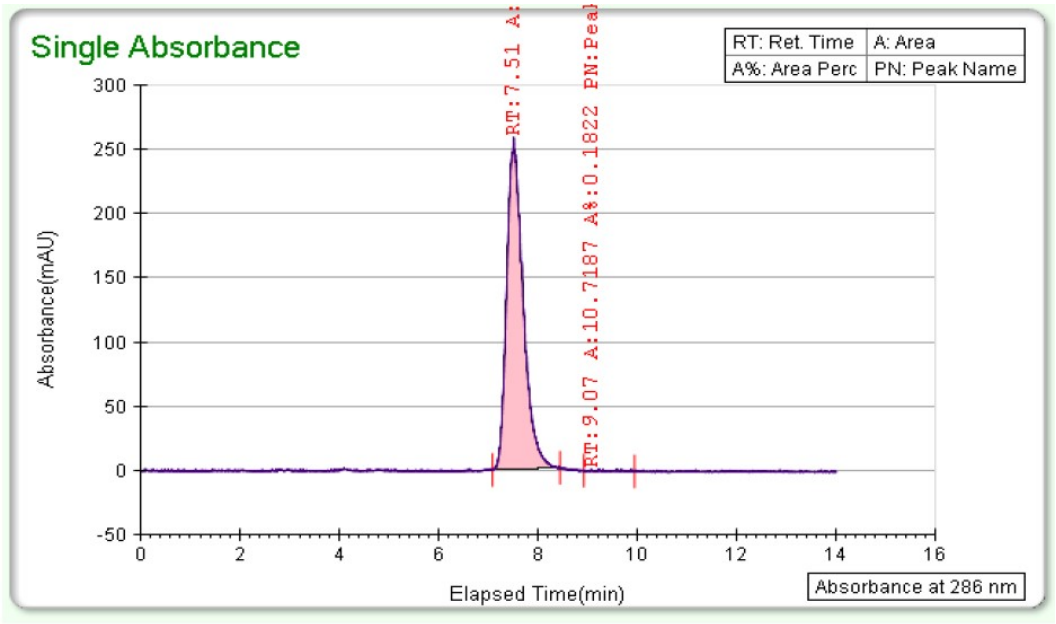
Results UV3			
Sr.No.	RT	Area	Area %
1	2.14	19.088	0.354
2	5.23	5373.851	99.646

Compound 10-Racemate



Results UV1			
Sr.No.	RT	Area	Area %
1	1.20	558.979	4.688
2	1.40	9.470	0.079
3	1.48	163.829	1.374
4	1.55	61.998	0.520
5	1.70	97.440	0.817
6	2.13	5289.774	44.367
7	5.13	5741.352	48.154

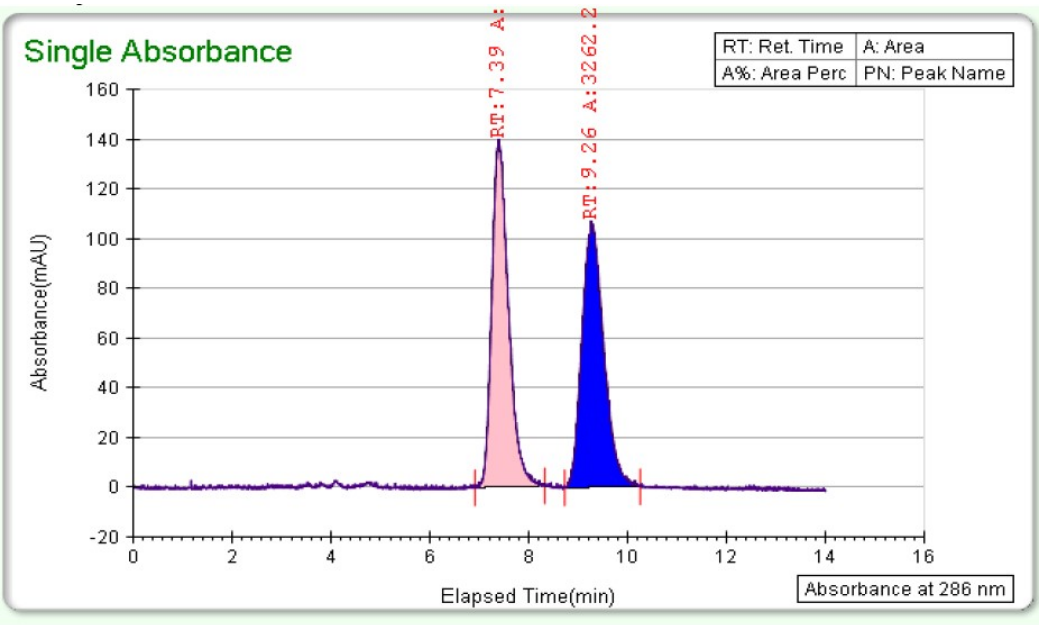
Compound 11



Peak Info

Number	Area %	Area	RT (min)
1	99.8178	5870.6959	7.51
2	0.1822	10.7187	9.07

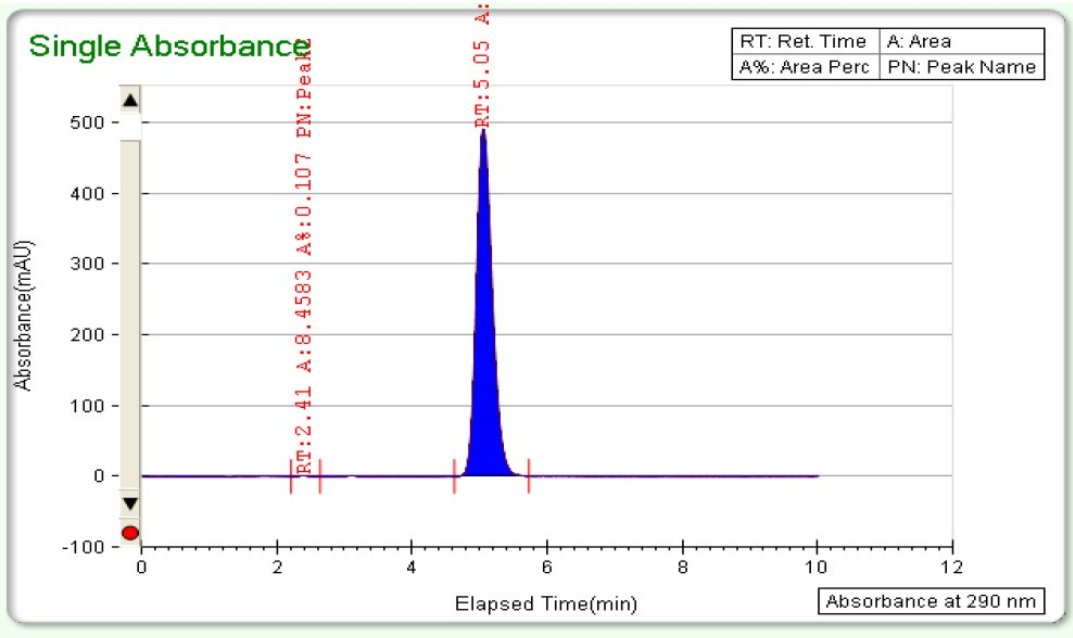
Compound 11-Racemate



Peak Info

Number	Area %	Area	RT (min)
1	49.9955	3261.6235	7.39
2	50.0045	3262.2163	9.26

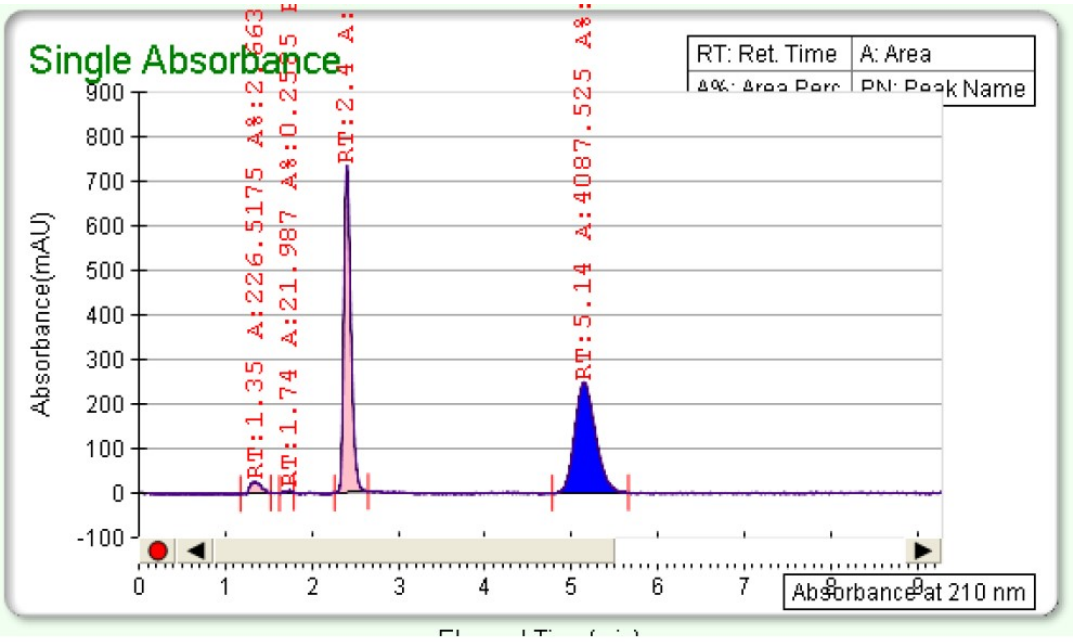
Compound 12



Peak Info

Number	Area %	Area	RT (min)
1	0.107	8.4583	2.41
2	99.893	7893.9038	5.05

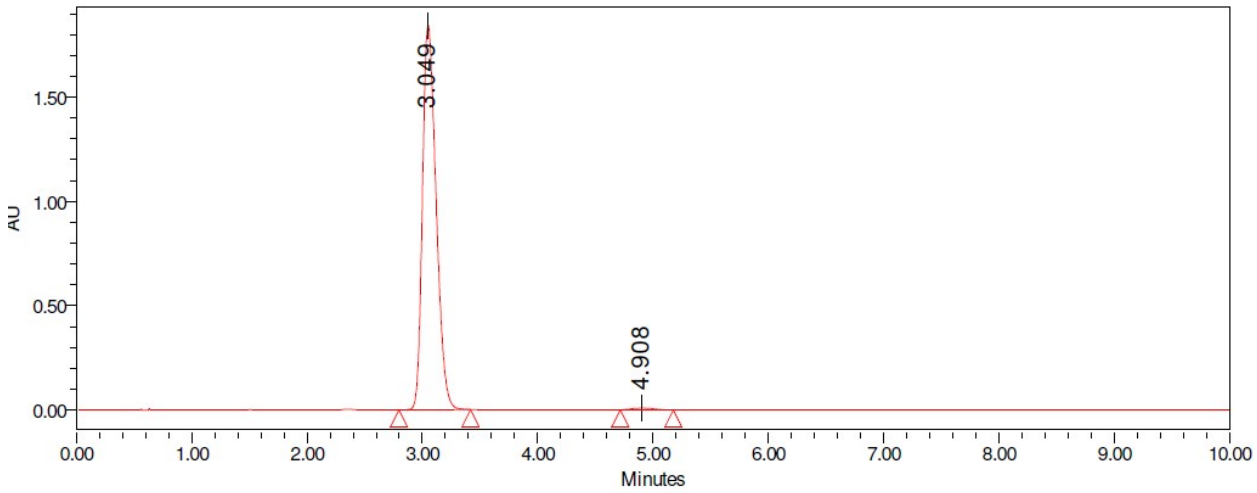
Compound 12-Racemate



Peak Info

Number	Area %	Area	RT (min)
1	2.663	226.5175	1.35
2	0.2585	21.987	1.74
3	49.0252	4170.197	2.4
4	48.0533	4087.525	5.14

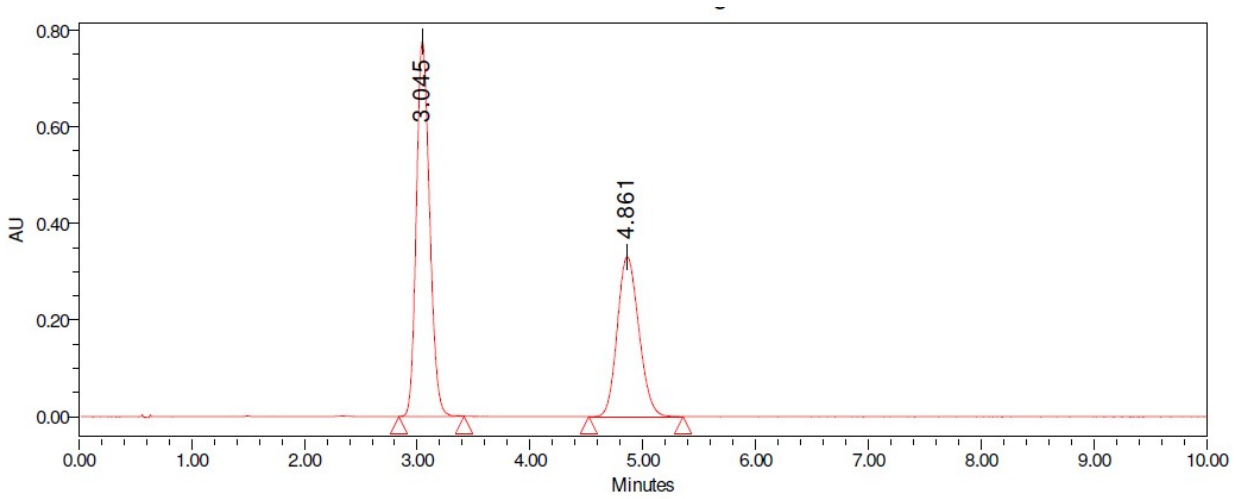
Compound 13



Peak Results

	Name	RT	Area	% Area
1		3.05	15172797	99.24
2		4.91	116909	0.76

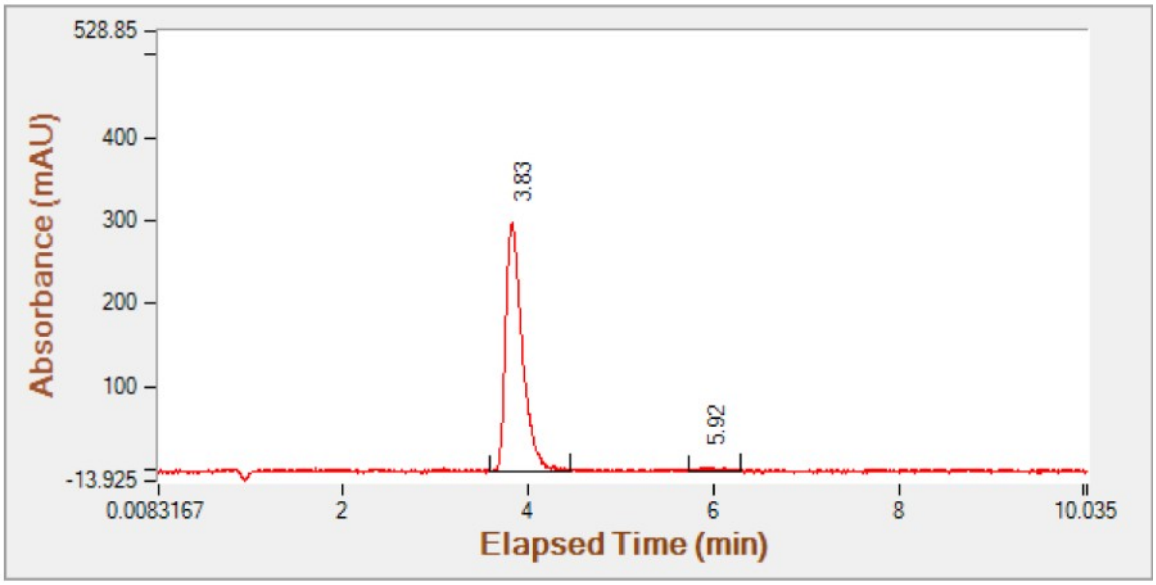
Compound 13-Racemate



Peak Results

	Name	RT	Area	% Area
1		3.05	6339891	59.01
2		4.86	4404235	40.99

Compound 14

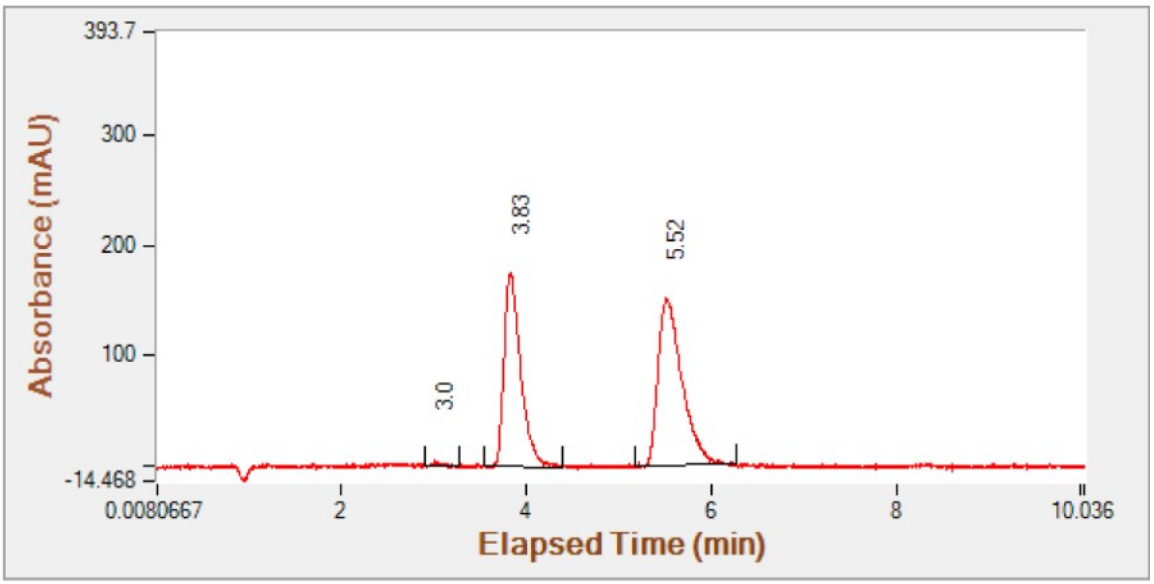


280 nm

Results UV3-N°1			
Sr.No.	RT	Area	Area %
1	3.83	3742.443	98.672
2	5.92	50.349	1.328

Comp

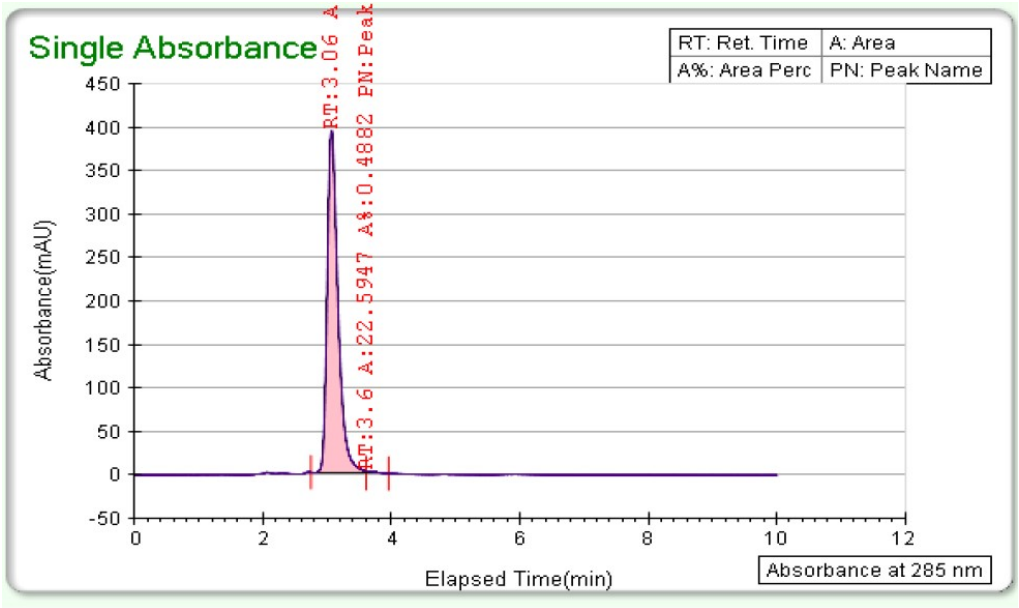
ound 14-Racemate



280 nm

Results UV3-N°1			
Sr.No.	RT	Area	Area %
1	3.00	30.510	0.613
2	3.83	2192.028	44.040
3	5.52	2754.816	55.347

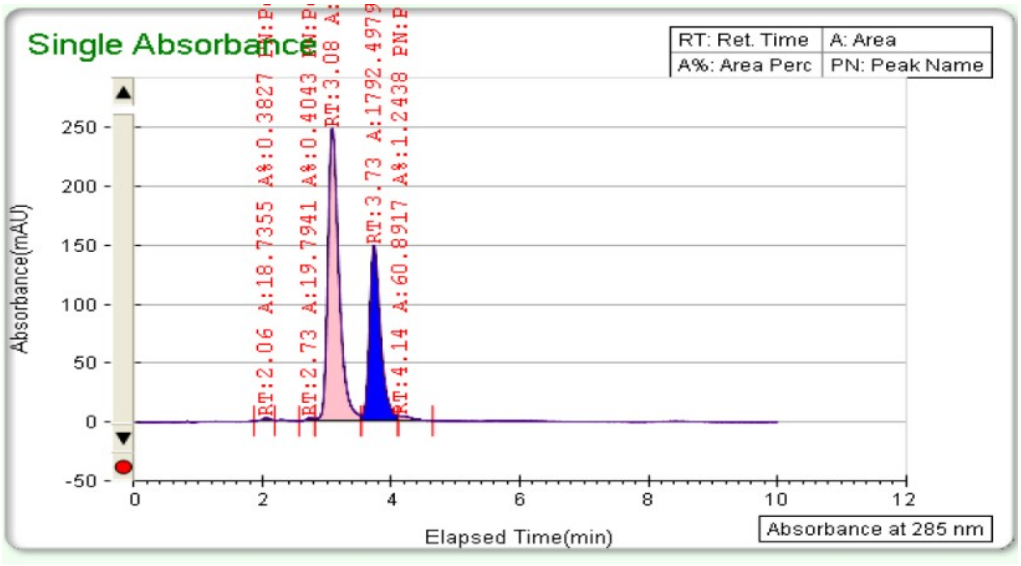
Compound 15



Peak Info

Number	Area %	Area	RT (min)
1	99.5118	4605.7283	3.06
2	0.4882	22.5947	3.6

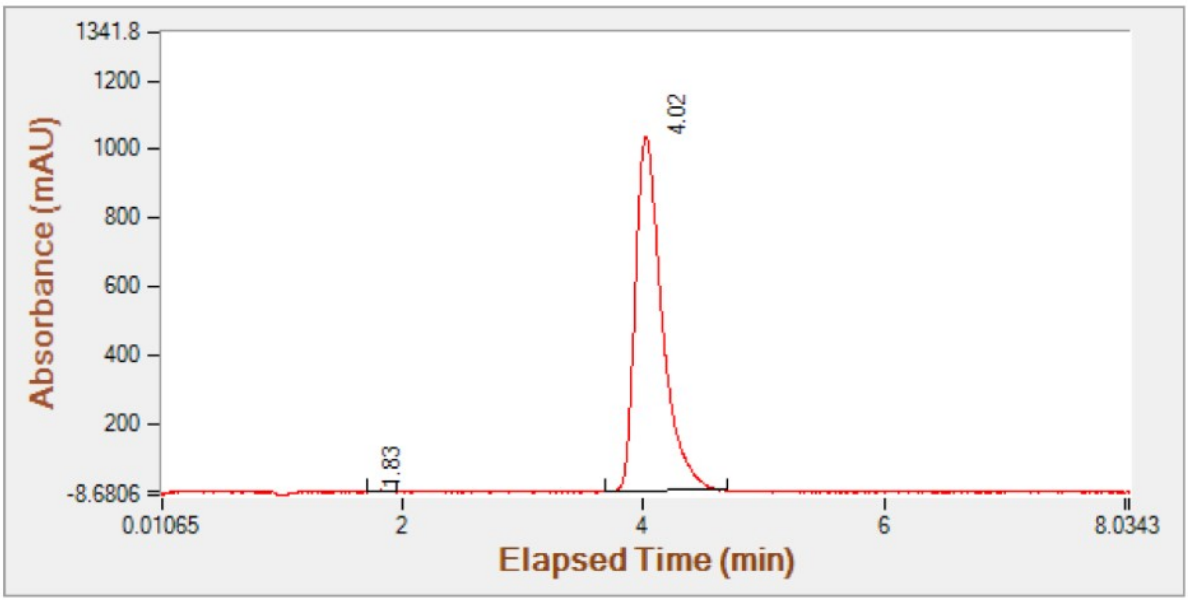
Compound 15-Racemate



Peak Info

Number	Area %	Area	RT (min)
1	0.3827	18.7355	2.06
2	0.4043	19.7941	2.73
3	61.3541	3003.6108	3.08
4	36.615	1792.4979	3.73
5	1.2438	60.8917	4.14

Compound 16

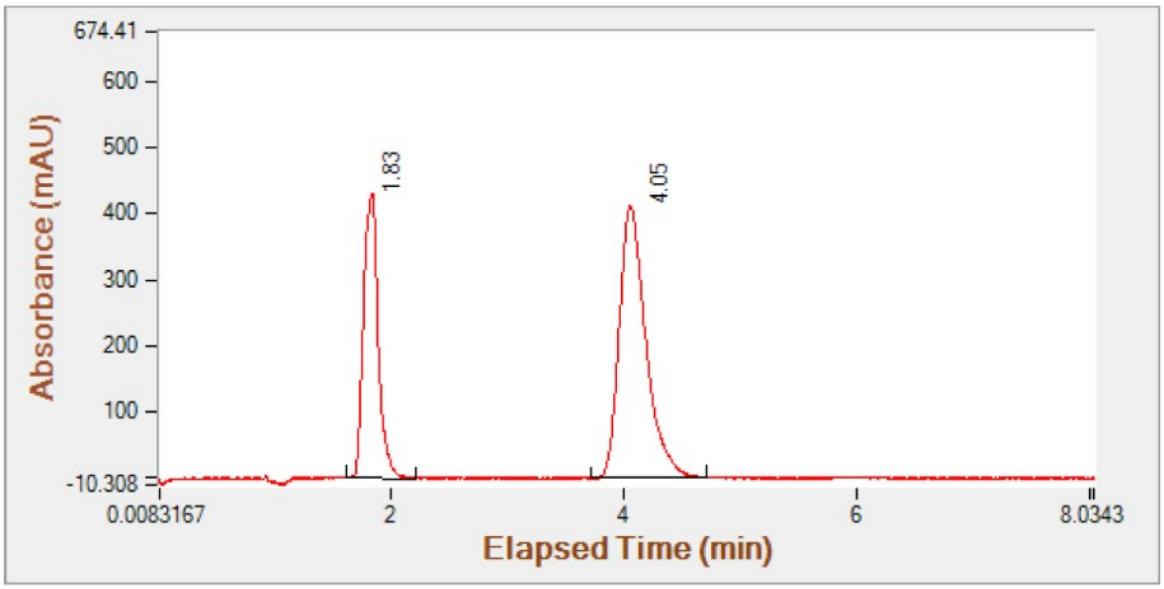


280 nm

Results UV3			
Sr.No.	RT	Area	Area %
1	1.83	28.953	0.181
2	4.02	15975.174	99.819

Co

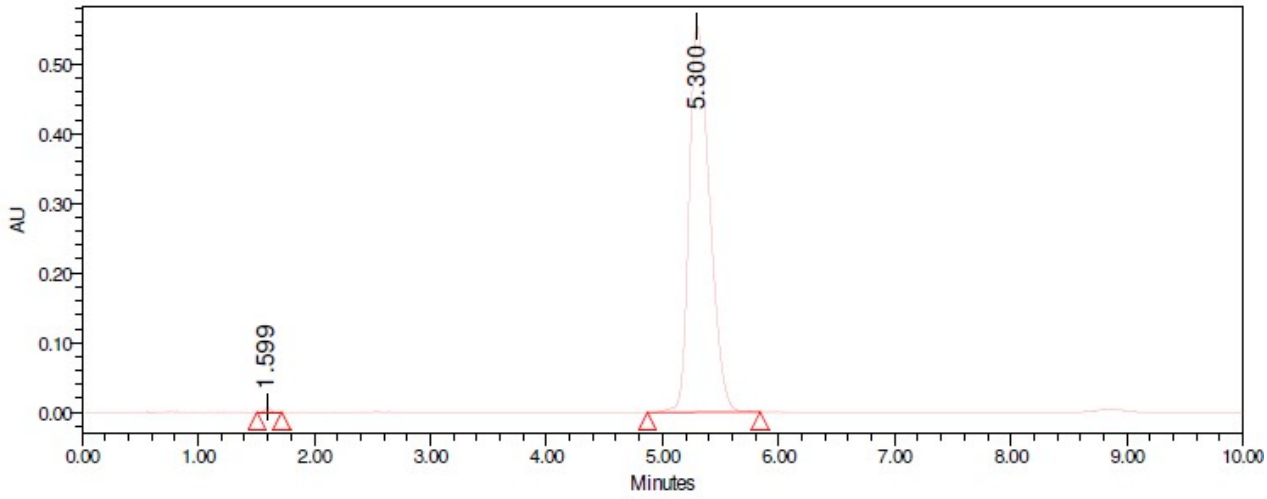
mpound 16-Racemate



280 nm

Results UV3			
Sr.No.	RT	Area	Area %
1	1.83	3789.643	36.710
2	4.05	6533.466	63.290

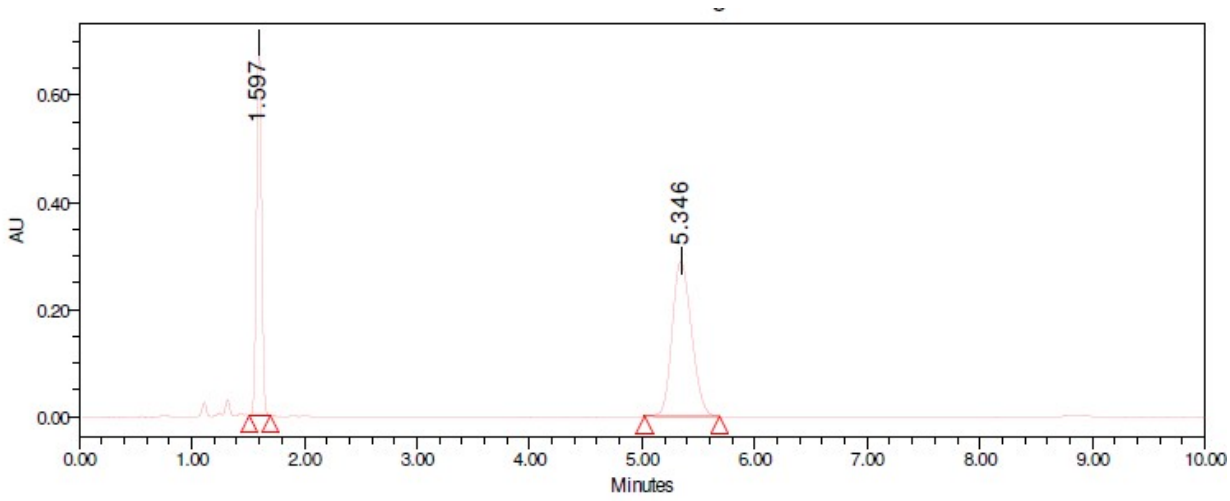
Compound 17



Peak Results

	Name	RT	Area	% Area
1		1.60	20035	0.30
2		5.30	6756563	99.70

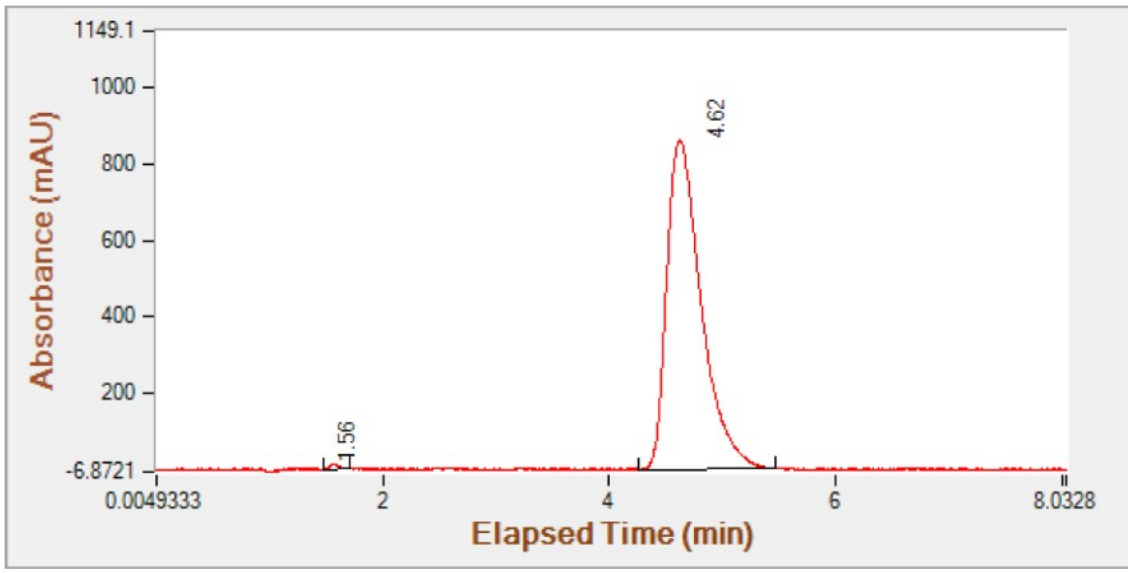
Compound 17-Racemate



Peak Results

	Name	RT	Area	% Area
1		1.60	2112725	38.11
2		5.35	3431437	61.89

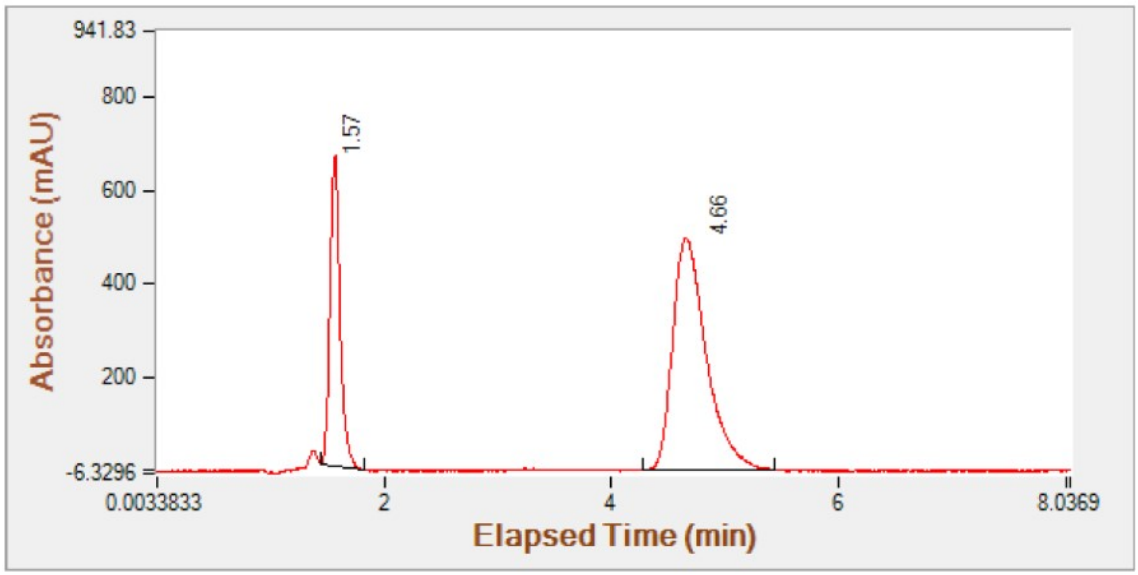
Compound 18



280 nm

Results UV3			
Sr.No.	RT	Area	Area %
1	1.56	74.810	0.410
2	4.62	18192.090	99.590

Compound 18-Racemate



280 nm

Results UV3			
Sr.No.	RT	Area	Area %
1	1.57	4096.336	28.732
2	4.66	10160.640	71.268

4. HepDE19 assay with bDNA quantitation of HBV rcDNA and cell viability assessment

The HepDE19 cell culture system is a HepG2 (human hepatoma)-derived cell line (sourced under a license agreement from Drexel University, Philadelphia, PA and the Blumberg Institute, Doylestown, PA) that supports HBV (genotype D, serotype ayw) DNA replication and cccDNA formation under the control of a CMV Tet-off promoter system (1). HepDE19 cells were plated in 96-well collagen-coated tissue-culture treated microtiter plates (50,000 cells/well) in DMEM/F12 medium supplemented with 10% fetal bovine serum + 1% penicillin-streptomycin with tetracycline (1 μg/mL) and incubated in a humidified incubator at 37 ˚C and 5% CO_2 overnight. On the next day, the cells were switched to fresh medium without tetracycline and incubated for 4 h at 37 °C and 5% CO_2. The cells were switched to fresh Tet-free medium containing compounds at concentrations starting at 10 μM and a serial, ½ log, 8-point, titration series in duplicate. The plates also included wells of untreated (DMSO) cells that served as controls. The final DMSO concentration in the assay was 0.5%. The plates were incubated for 7 days in a humidified incubator at 37 °C and 5% CO_2. The level of rcDNA present in the cells was measured using a Quantigene 2.0 bDNA assay kit (Affymetrix, Santa Clara, CA) with HBV specific custom probe set. The effect of compounds on cell viability was assessed on replicate plates, with HepDE19 cells plated at a lower cell density of 5,000 cells/well and measuring intracellular ATP content using Cell Titer Glo® reagent (CTG; Promega, Madison, WI) as per manufacturer's instructions, following a 4-day incubation with compounds. The plates were read using a Victor luminescence plate reader (PerkinElmer Model 1420 Multilabel counter) and the relative luminescence units (RLU) data generated from each well was calculated as % inhibition of the untreated (DMSO) control wells and analyzed using the XL-Fit module (IDBS, Boston, MA) in Microsoft Excel to determine EC_{50} (bDNA, Supplementary Tables 1 and 2) as well as CC_{50} (CTG) values using a 4-parameter curve fitting algorithm. The mean EC_{50} and CC_{50} values were calculated from at least 2 separate experiments.

Supplementary Table 1. HepDE19 EC_{50} ± S.D. of the desired (S)-enantiomers

Compound	Hep DE19 EC_{50} ± S.D. (nM)
10b	52 ± 23
11	13 ± 4
12	12 ± 2
13	114 ± 40
14	52 ± 11
15	50 ± 19
16	11 ± 1
17	57 ± 15
18	14 ± 5

Supplementary Table 2. HepDE19 EC_{50} ± S.D. of the corresponding (R)-enantiomers

Compound	Hep DE19 EC_{50} ± S.D. (nM)
10a ((R)-enantiomer of 10b)	3,450 ± 1,560
(R)-enantiomer of 11	310 ± 28
(R)-enantiomer of 12	3,070 ± 740
(R)-enantiomer of 13	3,300 ± 510
(R)-enantiomer of 14	6,130 ± 1,040
(R)-enantiomer of 15	970 ± 3
(R)-enantiomer of 16	1,870 ± 380
(R)-enantiomer of 17	4,170 ± 1,140
(R)-enantiomer of 18	1,420 ± 920

1. H. Guo, D. Jiang, T. Zhou, A. Cuconati, T. M. Block and J. T. Guo, *J Virol*, 2007, **81**, 12472-12484.

5. *In vitro* Microsomal Stability Determination

0.3 μM of test compounds (from 90 μM acetonitrile stocks) were incubated with 0.5 mg ml^{-1} human or mouse liver microsomes (BioIVT LLC, NY, USA) in 100 mM potassium phosphate buffer pH 7.4 and 1 mM NADPH at 37 °C.

Disappearance of the test compounds was monitored over 60 minutes with sampling at timepoints: 0, 5, 10, 20, 30 and 60 minutes. The samples were quenched with 3 volumes of acetonitrile. The 0 min samples were collected and quenched with acetonitrile prior to the addition of NADPH. Samples were centrifuged at 1,500 RCF and the supernatant transferred to a new container and analyzed by LC-MS/MS. Analysis was performed using an ABSciex 5500 QTRAP coupled to a Shimadzu Nexera X2 HPLC system. HPLC chromatography was performed using an ACE Super C18 3 μm 2 × 50 mm HPLC column (Advanced Chromatography Technologies Ltd.), with a linear gradient of 10% acetonitrile: 90% (0.1% formic acid in water) to 95% acetonitrile: 5% (0.1% formic acid in water) at a flow rate of 0.5 mL min^{-1} over 3 min.

Sciex Analyst software was utilized to analyze the acquired LC-MS/MS data. Setting the compound signal in the 0 min timepoint sample as 100%, the residual percentages of the remaining time course samples were plotted. From the natural logarithmic plot of the compounds remaining over time, the slope derived from linear range points was used to indicate the elimination rate constant (k) for the compound's metabolic decrease. This was used to calculate the in vitro $T_{1/2}$ calculation according to the following equation:

$$T_{1/2} = \ln(2)/k$$

Following, the compound intrinsic clearance CL_{int} (uL/min/kg) was calculated using the following equation:

$$CL_{int} = (0.693/ t_{1/2})/ (0.5 \text{ mg ml}^{-1} \textit{ microsome concentration})$$

6. Solubility Determination

10 mM DMSO stock solutions of the test compounds were prepared. Aliquots of each DMSO stock solution were transferred to a 96 well plate, then each well was mixed with an aliquot of PBS, pH 7.4 (Gibco™) to a final compound concentration of 200 µM and 2% DMSO. The plate was then sealed and shaken for 18 to 24 h at room temperature (22 °C). The buffer solutions were transferred to a 96-well MultiScreen$_{HTS}$-PCF Filter Plate (MilliporeSigma™), then stacked on top of an empty 96-well collection plate. The stack was centrifuged at 1500 RCF to force the solutions through the filter plate into the collection plate below. A corresponding standards plate was prepared by diluting an aliquot of the DMSO stock solution with DMSO and generating a standard curve spanning 4 µM to 200 µM.

The filtrate samples in the collection plate, and the corresponding standards plate was analyzed by HPLC-UV-MS. All HPLC analyses were carried out using an ACQUITY I-UPLC I-Class System (Waters, Ltd.). An ACQUITY PDA eλ detector (Waters, Ltd.) was used for UV detection, and MS detection was carried out using a Xevo G2-XS QTof (Waters, Ltd.). HPLC chromatography was performed using an Acquity UPLC BEH C18 2.1 x 50 mm, 1.7 µm column (Waters, Ltd.), with a linear gradient of 5% acetonitrile: 95% (0.1% formic acid in water) to 95% acetonitrile: 5% (0.1% formic acid in water) at a flow rate of 0.7 mL min^{-1} over 4 min.

UV and MS data were collected for each sample, then analyzed by TargetLynx™ (Waters, Ltd.). Quantification of the test compounds was done by interpolating the UV chromatographic peak against the respective DMSO compound standard curve. The corresponding MS trace was used to verify mass identity of the tested compound, to ensure the proper UV peak was used for quantification.

7. Pharmacokinetic analysis

All animal experiments were performed at WuXi AppTec (Cranbury, NJ). Female CD-1 mice were acquired from Hilltop Labs (Scottdale, PA). For dosing, test compounds were weighed out and formulated in cosolvent solution consisting of 40% PEG400/ 5% EtOH/ 55% H_2O. Formulations were dosed at 2.0 mg kg^{-1} and 10 mg kg^{-1} for IV and PO administration, respectively. Blood samples were serial collected at 5 min (IV only), 15 min (PO only), 30 min, 2 h, 4 h, 8 h, 12 h, and 24 h after administration of test compounds. Blood was collected into K_2EDTA containing tubes and then centrifuged at 16,000 RCF for 5 min to obtain plasma and frozen at -80 °C until analysis.

For LC-MS/MS analysis and quantification, alongside the plasma samples for each test compound, plasma calibration standards were also prepared by spiking blank mouse plasma with DMSO stocks of said test compound (spanning 0.5 ng/mL to 8000 ng/mL). Compound analytes were extracted from the samples with a one-step protein precipitation method. To each sample 20 volumes of acetonitrile was added. The samples were shaken for 30 minutes at room temperature, then centrifuged at 1500 RCF. The supernatant was transferred to a new container and analyzed by LC-MS/MS. Analysis was performed using an ABSciex 5500 QTRAP coupled to a Shimadzu Nexera X2 HPLC system. HPLC chromatography was performed using an ACE SuperC18 3 μm 2 × 50 mm HPLC column (Advanced Chromatography Technologies Ltd.), with a linear gradient of 10% acetonitrile: 90% (0.1% formic acid in water) to 95% acetonitrile: 5% (0.1% formic acid in water) at a flow rate of 0.5 mL min^{-1} over 3 min.

Sciex Analyst software was utilized to analyze the acquired LC-MS/MS data. Quantitative determination of test compounds in the plasma samples was done by interpolating the analyte signal against that of the plasma calibration standard samples. From the obtained plasma concentration-time curves, the pharmacokinetic parameters for each compound were determined by standard non-compartmental analysis using Phoenix WinNonlin software (Certara L.P.). Calculated pharmacokinetic parameters included area under the plasma concentration-time curve from time 0 extrapolated to infinite time ($AUC_{0-\infty}$), maximum concentration (C_{max}), steady state half-life ($T_{1/2}$) and plasma clearance (CL_{obs}).

8. HBV Hydrodynamic Injection Mouse Studies

All animal-related procedures were conducted according to written operating procedures, in accordance with Canadian Council on Animal Care (CCAC) Guidelines on Good Animal Practices or the In-Life Group Standard Operating Procedures of Inotiv (St. Louis, Missouri, USA). Protocols were approved by Arbutus' Institutional Animal Care and Use Committee (IACUC) or the St. Louis University Animal Care and Use Committee. A total of two independent studies were conducted with 5 or 6 animals per group. Prior to the start of treatment, 10 μg of a plasmid encoding a 1.3-fold-overlength copy of an HBV genotype D sequence (Genbank accession no. V01460, constructed as described previously[2] was administered to six week-old female NOD.CB17- *Prkdc*^{scid}/J mice (The Jackson Laboratory) *via* hydrodynamic injection (HDI; a rapid injection of 1.6 mL into the tail vein within 5 seconds). Seven days after HDI injection, starting on day 0, compound **10b**, compound **15** or compound **17** were administered *via* oral gavage at 3, 10 or 30 mg/kg twice daily (compound **10b**); 3, 10 or 30 mg/kg once daily (compound **17**); 10 mg/kg once daily (compound **15**) or 30 mg/kg once daily (compound **10b**). Animals were administered vehicle once or twice daily *via* oral gavage as a negative control. Blood was collected on days 0 (predose) and 7 for serum for HBV DNA analysis. Total extracted nucleic acid was measured using a quantitative PCR assay with primer/probe sequences described previously.[3]

2. L. G. Guidotti, B. Matzke, H. Schaller and F. V. Chisari, *J Virol*, 1995, **69**, 6158-6169.
3. T. Tanaka, K. Inoue, Y. Hayashi, A. Abe, K. Tsukiyama-Kohara, H. Nuriya, Y. Aoki, R. Kawaguchi, K. Kubota, M. Yoshiba, M. Koike, S. Tanaka and M. Kohara, *J Med Virol*, 2004, **72**, 223-229.

9. Cp Y132A Crystallographic studies

Hepatitis B virus genotype D subtype adyw was modified to consist of the amino acid residues 1-149, with a Y132A mutation, and a TEV (Tobacco Etch Virus) protease cleavage sequence at the C-terminal (remaining after TEV cleavage of a 6xHis-tag). This mutant HBV core protein was expressed from a pEMB30 plasmid in Escherichia coli BL21 (DE3) cells. The protein sample was then purified to homogeneity in a final buffer of 50 mM Tris pH 9.0, 2 mM DTT, purification tags were cleaved, and the proteases and tags were removed in the final samples. To crystalize the apo HBV capsid mutant, large format sitting drops were set up with 2 µL protein at 10 mg/mL and 1 µL reservoir solution against a 300 µL reservoir containing 10% PEG 3350, 10 to 14% (+/-)-2-methyl-2,4-pentanediol (MPD), 8 to 10% isopropanol, and 100 mM ammonium citrate/citric acid (pH 7.0) and were incubated at 16 °C. Large hexagonal crystals grew in two to three days.

The structure of compound **10b** in complex with the mutant HBV core protein was obtained *via* soaking experiment. The soaking solution containing 10% PEG 3350, 14% MPD, 9% isopropanol, and 100 mM ammonium citrate/citric acid (pH 6.5) was prepared. Compound **10b** was diluted from the DMSO stock solutions to 1 mM final concentration in the soaking solution. HBV capsid protein (1-149) Y132A crystals were harvested and transferred to 3 µL sitting drops of soaking solution with compound **10b** and incubated for 60 hours at 16 °C. The soaked crystals were directly mounted in nylon loops and plunged into liquid nitrogen. The frozen crystals were then transported to the European Synchrotron Radiation Facility (ESRF) in Grenoble, France, where complete data sets were collected. The X-ray diffraction data were indexed, integrated, and scaled using XDS.[4] The X-ray diffraction of the selected crystals was identified as having a monoclinic Bravais lattice symmetry, with a space group of C2. The co-structures were solved by molecular replacement (MR) using the CCP4 program Phaser[5] with the initial search model derived from a publicly available structure (PDB ID: 5E0I) stripped of all ligands and water molecules. The model was optimized using Coot[6] and refined with REFMAC5.[7] The structure has been deposited with the RCSB protein data bank as entry code 7S76. The final crystal data statistics are compiled in Supplementary Table 3.

Supplementary Table 3. Data Collection and Refinement Statistics

	HBV capsid Y132A/Compound 10b Complex
Data Collection	
X-ray beam wavelength	0.97625 Å
Space Group	C2
Cell Dimensions a, b, c (Å) a, b, g (˚)	 152.24, 88.31, 103.71 90, 104, 90
Resolution (Å)	50.00 – 2.50 (2.65 – 2.50)*
Measured reflections	153,267 (24,290)
Unique reflections	46,072 (7,351)*
R_{sym} (%)	14.3 (130.7)*
I/s	5.4 (1.1)*
Completeness (%)	99.4 (98.9)*
Redundancy	3.3 (3.3)*
Mn (I) half-set correlation CC(1/2)	99.0 (74.2)*
Protein:compound complexes per asymmetric unit	6
Refinement	
Resolution (Å)	2.50
Refinement range (Å)	50.00 – 2.50
R_{cryst} (%)	26.96
R_{free} (%)	29.75
Average B-factors	A, B, C, D, E, F
Main chain atoms Side chain atoms Ligand	74.18, 75.85, 73.88, 74.92, 70.95, 66.21 74.99, 75.67, 74.40, 75.51, 70.45, 67.96 90.20, 86.63, 90.76, 92.02, 90.37, 91.88
R. M. S. D.§ Bond Length (Å) Bond Angles (˚)	 0.006 1.661
Ramachandran plot (%) Preferred Allowed Outliers	 89.05 8.50 2.45

*Highest resolution shell of 2.65 – 2.50 Å

§Root-mean square deviation from target geometry.

4. W. Kabsch, *Acta Crystallogr D Biol Crystallogr*, 2010, **66**, 125-132.
5. A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni and R. J. Read, *J Appl Crystallogr*, 2007, **40**, 658-674.
6. P. Emsley and K. Cowtan, *Acta Crystallogr D Biol Crystallogr*, 2004, **60**, 2126-2132.
7. A. A. Vagin, R. A. Steiner, A. A. Lebedev, L. Potterton, S. McNicholas, F. Long and G. N. Murshudov, *Acta Crystallogr D Biol Crystallogr*, 2004, **60**, 2184-2195.