Design and synthesis of substituted (1-(benzyl)-1*H*-1,2,3-triazol-4-yl)(piperazin-1-yl)methanone conjugates: Study on their apoptosis inducing ability and tubulin polymerization inhibition

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Experimental Section

Chemistry

Materials and methods

All Chemicals and reagents were purchased from the commercial suppliers Alfa Aesar, Sigma Aldrich and used without further purification. The reaction progress was monitored by Thin layer chromatography (TLC) was performed using pre-coated silica gel 60 F_{254} MERCK. TLC plates were visualized and analysed by exposure to UV light or iodine vapours' and aqueous solution of ninhydrin. Column chromatography was performed with Merck flash silica gel with 60–120 mesh size. Melting points were determined on an Electro thermal melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra for ^{1}H NMR were obtained on Avance 300, 400 and 500 MHz and analyzed using Mestrenova software and the chemical shifts are reported in ppm from tetramethylsilane (0 ppm) or the solvent resonance as the internal standard ($CDCl_3$ 7.26 ppm, DMSO-d_6 2.49 ppm) and for ^{13}C NMR the chemical shifts are reported in ppm from the solvent resonance as the internal standard ($CDCl_3$ 77 ppm, DMSO-d_6 39.3 ppm). Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). HRMS was performed on a Varian ESI- QTOF instrument.

General synthetic procedure for the preparation of compound 2a-e

Compound **2a-e** was synthesized according to the procedures described in the literature [1].

Compound 2a: colourless liquid: 88% yield; HRMS (ESI): *m/z* calcd for $C_8H_{10}BrO$: 200.9912; found: 200.9915 $[M+H]^+$.

Compound 2b: light yellow colour liquid: 90% yield; HRMS (ESI): *m/z* calcd for C_7H_8BrF: 189.9793; found: 189.9793 $[M+2]^+$.

Compound 2c: colourless to light yellow colour liquid: 85% yield; HRMS (ESI): *m/z* calcd for C_7H_9Br: 171.9872; found: 171.9888 $[M+2]^+$.

Compound 2d: colourless solid: 92% yield; mp: 78–82 °C; HRMS (ESI): *m/z* calcd for C_7H_8BrI: 297.8854; found: 297.8854 $[M+2]^+$.

Compound 2e: colourless liquid: 95% yield; HRMS (ESI): *m/z* calcd for $C_{13}H_{12}BrO$: 263.0072; found: 263.0072 $[M+H]^+$.

General synthetic procedure for the preparation of compound 3a-e

Compound **3a-e** was synthesized based on previous synthetic procedures [2].

Compound 3a: 85% yield; HRMS (ESI): *m/z* calcd for $C_8H_{10}N_3O$: 164.0824; found: 164.0824 $[M+H]^+$.

Compound 3b: 88% yield; HRMS (ESI): *m/z* calcd for $C_7H_7FN_3$: 152.0624; found: 152.0624 $[M+H]^+$.

Compound 3c: 84% yield; HRMS (ESI): *m/z* calcd for $C_7H_8N_3$: 134.0708; found: 134.0718 $[M+H]^+$.

Compound 3d: 90% yield; HRMS (ESI): *m/z* calcd for $C_7H_7IN_3$: 259.9674; found: 259.9685 $[M+H]^+$.

Compound 3e: 92% yield; HRMS (ESI): *m/z* calcd for $C_{13}H_{12}N_3O$: 226.0976; found: 226.0980 $[M+H]^+$.

General synthetic procedure for the preparation of compound 5a-e

Compound **5a-e** was synthesized based on previous synthetic procedures [3].

Compound 5a: 82% yield; HRMS (ESI): *m/z* calcd for $C_{13}H_{16}N_3O_3$: 262.1186; found: 262.1192 $[M+H]^+$.

Compound 5b: 85% yield; HRMS (ESI): *m/z* calcd for $C_{12}H_{13}FN_3O_2$: 250.0982; found: 250.0992 $[M+H]^+$.

Compound 5c: 80% yield; HRMS (ESI): *m/z* calcd for $C_{12}H_{14}N_3O_2$: 232.1086; found: 232.1086 $[M+H]^+$.

Compound 5d: 88% yield; HRMS (ESI): *m/z* calcd for $C_{12}H_{13}IN_3O_2$: 358.0048; found: 358.0052 $[M+H]^+$.

Compound 5e: 90% yield; HRMS (ESI): *m/z* calcd for $C_{18}H_{18}N_3O_3$: 324.1348; found: 324.1348 $[M+H]^+$.

General synthetic procedure for the preparation of compound 6a-e

Compound **6a-e** was synthesized based on previous synthetic procedures [3].

Compound 6a: 80% yield; HRMS (ESI): *m/z* calcd for $C_{11}H_{12}N_3O_3$: 234.0874; found: 234.0879 $[M+H]^+$.

Compound 6b: 78% yield; HRMS (ESI): *m/z* calcd for $C_{10}H_9FN_3O_2$: 222.0679; found: 222.0679 $[M+H]^+$.

Compound 6c: 75% yield; HRMS (ESI): *m/z* calcd for $C_{10}H_{10}N_3O_2$: 204.0764; found: 204.0773 $[M+H]^+$.

Compound 6d: 88% yield; HRMS (ESI): *m/z* calcd for $C_{10}H_9IN_3O_2$: 329.9734; found: 329.9739 $[M+H]^+$.

Compound 6e: 85% yield; HRMS (ESI): *m/z* calcd for $C_{16}H_{14}N_3O_3$: 296.1024; found: 296.1035 $[M+H]^+$.

General synthetic procedure for the preparation of compound 9a-f

Substituted phenyl sulfonyl piperazines **9a-f** was synthesized by adding triethylamine (31.5 mmol) slowly to a solution of piperazine in CH_2Cl_2 at 0 °C, and then commercially available benzenesulfonyl chloride (**7a-f**, 11.4 mmol) was added and stirred for 30 min. After the completion of reaction, which was confirmed by TLC (petroleum ether and ethyl acetate (2:1)), the reaction was quenched with water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and concentrated on reduced pressure to give **9a-f** [4].

Compound 9a: White solid: 90% yield; HRMS (ESI): *m/z* calcd for $C_{11}H_{17}N_2O_2S$: 241.1008; found: 241.1011 $[M+H]^+$.

Compound 9b: White solid: 92% yield; HRMS (ESI): *m/z* calcd for $C_{10}H_{14}ClN_2O_2S$: 261.0455; found: 261.0465 $[M+H]^+$.

Compound 9c: Off White solid: 94% yield; HRMS (ESI): *m/z* calcd for $C_{10}H_{14}BrN_2O_2S$: 304.9944; found: 304.9959 $[M+H]^+$.

Compound 9d: White solid: 88% yield; HRMS (ESI): *m/z* calcd for $C_{10}H_{15}N_2O_2S$: 227.0854; found: 227.0854 $[M+H]^+$.

Compound 9e: Off White solid: 95% yield; HRMS (ESI): *m/z* calcd for $C_{14}H_{23}N_2O_2S$: 283.1434; found: 283.1480 $[M+H]^+$.

Compound 9f: White solid: 88% yield; HRMS (ESI): *m/z* calcd for $C_{11}H_{17}N_2O_3S$: 257.0956; found: 257.0960 $[M+H]^+$.

General synthetic procedure for the preparation of Substituted (1-benzyl-1*H*-1,2,3-triazol-4-yl)(4-(phenylsulfonyl)piperazin-1-yl)methanone derivatives (10aa-ef)

To a stirred solution of substituted benzyl-triazole acids (**6a-e,** 1 mmol) in DMF (10 mL), Et_3N (3 mmol), HOBt (1.2 mmol), HBTU (1.2 mmol) and substituted (phenylsulfonyl)piperazine (**9a-f**, 1 mmol) were added at 0 ^oC. Then the resulting mixture was stirred at room temperature for 8 h, reaction was monitored by TLC. After the reaction was complete, diluted with water, the aqueous solution was then extracted twice with ethyl acetate. The extracts were combined and dried with anhydrous Na_2SO_4. Evaporation of the solvent gave crude solids those were purified by silica-gel column chromatography using ethyl acetate-hexane as eluent in increasing polarity to afford the desired pure products **10aa-ef.**

(1-(3-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)(4-tosylpiperazin-1-yl)methanone (10aa)

Off white solid: 85% yield; mp: 128–130 °C; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.94 (s, 1H), 7.63 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 7.9 Hz, 1H), 6.89 (dd, J = 8.3, 2.1 Hz, 1H), 6.85 (d, J = 7.5 Hz, 1H), 6.79 (s, 1H), 5.47 (s, 2H), 4.42 (bs, 2H), 3.82 (bs, 2H), 3.78 (s, 3H), 3.11 (bs, 2H), 3.07 (bs, 2H), 2.42 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$ + DMSO-d_6) δ: 158.86, 158.57, 143.07, 142.48, 134.85, 130.92, 129.10, 128.89, 127.88, 126.65, 119.33, 113.01, 112.95, 54.22, 52.81, 45.57, 44.88, 44.79, 40.69, 20.47; MS (ESI): *m/z* 456 $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{22}H_{26}N_5O_4S$: 456.1700; found: 456.1706 $[M+H]^+$.

(4-((4-Chlorophenyl)sulfonyl)piperazin-1-yl)(1-(3-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10ab)

Off white solid: 82% yield; mp: 154–156 °C; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.71–7.67 (m, 2H), 7.53–7.49 (m, 2H), 7.32–7.27 (m, 1H), 6.90 (dd, J = 8.1, 2.2 Hz, 1H), 6.85 (d, J = 7.5 Hz, 1H), 6.79 (d, J = 1.9 Hz, 1H), 5.47 (s, 2H), 4.45 (bs, 2H), 3.84 (bs, 2H), 3.78 (s, 3H), 3.13 (d, J = 2.9 Hz, 2H), 3.10 (d, J = 4.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 160.28, 159.69, 144.15, 139.96, 135.07, 133.85, 130.53, 129.71, 129.26, 128.67, 120.63, 114.49, 114.29, 55.46, 54.51, 46.79, 46.04, 45.49, 42.10; MS (ESI): *m/z* 476 $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{21}H_{23}ClN_5O_4S$: 476.1153; found: 476.1157 $[M+H]^+$.

(4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)(1-(3-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10ac)

Off white solid: 84% yield; mp: 160–162 °C; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.68 (d, J = 8.6 Hz, 2H), 7.64–7.58 (m, 2H), 7.30 (d, J = 7.9 Hz, 1H), 6.90 (dd, J = 8.3, 2.0 Hz, 1H), 6.85 (d, J = 7.5 Hz,

1H), 6.79 (d, *J* = 1.8 Hz, 1H), 5.47 (s, 2H), 4.44 (bs, 2H), 3.84 (bs, 2H), 3.78 (s, 3H), 3.13 (d, *J* = 3.4 Hz, 2H), 3.10 (d, *J* = 4.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 160.26, 159.67, 144.12, 135.07, 134.35, 132.67, 130.51, 129.31, 128.67, 128.45, 120.61, 114.47, 114.27, 55.44, 54.49, 46.77, 46.02, 42.07; MS (ESI): *m/z* 522 $[M+2]^+$; HRMS (ESI): *m/z* calcd for $C_{21}H_{23}BrN_5O_4S$: 520.0648; found: 520.0654 $[M+H]^+$.

(1-(3-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)(4-(phenylsulfonyl)piperazin-1-yl)methanone (10ad)

Off white solid: 78% yield; mp: 168–170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.95 (s, 1H), 7.76 (d, *J* = 7.3 Hz, 2H), 7.61 (t, *J* = 7.3 Hz, 1H), 7.54 (t, *J* = 7.5 Hz, 2H), 7.31–7.26 (m, 1H), 6.89 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.84 (d, *J* = 7.6 Hz, 1H), 6.78 (s, 1H), 5.47 (s, 2H), 4.43 (bs, 2H), 3.83 (bs, 2H), 3.78 (s, 3H), 3.13 (d, *J* = 4.4 Hz, 2H), 3.10 (d, *J* = 4.9 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 160.32, 159.74, 144.19, 135.56, 135.14, 133.22, 130.48, 129.33, 128.65, 127.84, 120.60, 114.53, 114.26, 55.43, 54.48, 46.79, 46.11, 46.05, 42.15; MS (ESI): *m/z* 442 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{21}H_{24}N_5O_4S$: 442.15435; found: 442.1542 $[M+H]^+$.

(4-((4-(Tert-butyl)phenyl)sulfonyl)piperazin-1-yl)(1-(3-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10ae)

Off white solid: 80% yield; mp: 166–168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.95 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 7.9 Hz, 1H), 6.89 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.85 (d, *J* = 7.5 Hz, 1H), 6.79 (s, 1H), 5.47 (s, 2H), 4.42 (bs, 2H), 3.84 (bs, 2H), 3.78 (s, 3H), 3.14 (bs, 2H), 3.10 (bs, 2H), 1.34 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 160.31, 159.76, 157.04, 144.25, 135.09, 132.29, 130.51, 128.58, 127.79, 126.35, 120.62, 114.56, 114.25, 55.45, 54.51, 46.80, 46.14, 46.04, 42.16, 35.34, 31.19; MS (ESI): *m/z* 498 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{25}H_{32}N_5O_4S$: 498.2169; found: 498.2173 $[M+H]^+$.

(1-(3-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)(4-((4-methoxyphenyl)sulfonyl)piperazin-1-yl)methanone (10af)

Off white solid: 85% yield; mp: 182–184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.96 (s, 1H), 7.68 (d, *J* = 8.3 Hz, 2H), 7.29 (d, *J* = 7.9 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.89 (d, *J* = 7.9 Hz, 1H), 6.85 (d, *J* = 7.4 Hz, 1H), 6.79 (s, 1H), 5.47 (s, 2H), 4.42 (bs, 2H), 3.86 (s, 3H), 3.83 (bs, 2H), 3.78 (s, 3H), 3.10 (bs, 2H), 3.07 (bs, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 163.36, 160.25, 159.68, 144.18, 135.11, 130.46, 129.98, 128.58, 126.78, 120.58, 114.49, 114.22, 55.74, 55.42, 54.44, 46.80, 46.05, 42.08; MS (ESI): *m/z* 472 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{22}H_{26}N_5O_5S$: 472.1649; found: 472.1646 $[M+H]^+$.

(1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)(4-tosylpiperazin-1-yl)methanone (10ba)

Off white solid: 84% yield; mp: 175–177 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 (s, 1H), 7.62 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 6.2 Hz, 2H), 7.32–7.27 (m, 2H), 7.06 (t, J = 8.5 Hz, 2H), 5.48 (s, 2H), 4.41 (bs, 2H), 3.82 (bs, 2H), 3.11 (bs, 2H), 3.07 (bs, 2H), 2.42 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.37, 161.89, 159.62, 144.27 (d, $J_{C\text{-}F}$ = 15.5 Hz), 132.25, 130.39 (d, $J_{C\text{-}F}$ = 8.4 Hz), 129.96, 129.67, 128.46, 127.85 (d, $J_{C\text{-}F}$ = 11.8 Hz), 116.43 (d, $J_{C\text{-}F}$ = 21.9 Hz), 53.77, 46.79, 46.06, 45.57, 42.13, 21.65; MS (ESI): *m/z* 444 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{21}H_{23}FN_5O_3S$: 444.1500; found: 444.1503 $[M+H]^+$.

(4-((4-Chlorophenyl)sulfonyl)piperazin-1-yl)(1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10bb)

Off white solid: 78% yield; mp: 190–192 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.95 (s, 1H), 7.70–7.67 (m, 2H), 7.52–7.50 (m, 2H), 7.29–7.26 (m, 2H), 7.08–7.04 (m, 2H), 5.48 (s, 2H), 4.44 (s, 2H), 3.84 (s, 2H), 3.13 (d, J = 4.6 Hz, 2H), 3.10 (d, J = 4.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.39, 161.91, 159.60, 144.28, 139.96, 133.86, 130.41 (d, $J_{C\text{-}F}$ = 8.5 Hz), 129.70, 129.25, 128.54, 116.46 (d, $J_{C\text{-}F}$ = 21.9 Hz), 53.80, 46.76, 46.03, 45.48, 42.11; MS (ESI): *m/z* 464 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{20}ClFN_5O_3S$: 464.0953; found: 464.0955 $[M+H]^+$.

(4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)(1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10bc)

Off white solid: 80% yield; mp: 200–202 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.95 (s, 1H), 7.69–7.66 (m, 2H), 7.62–7.60 (m, 2H), 7.29–7.26 (m, 2H), 7.08–7.04 (m, 2H), 5.48 (s, 2H), 4.43 (s, 2H), 3.84 (s, 2H), 3.12 (d, J = 4.5 Hz, 2H), 3.09 (d, J = 4.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.40, 161.93, 159.60, 144.30, 134.46, 132.69, 130.41 (d, $J_{C\text{-}F}$ = 8.4 Hz), 129.61, 129.32, 128.50 (d, $J_{C\text{-}F}$ = 7.7 Hz), 116.46 (d, $J_{C\text{-}F}$ = 21.9 Hz), 53.80, 46.76, 46.04, 42.12; MS (ESI): *m/z* 510 $[M+2]^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{20}BrFN_5O_3S$: 508.0448; found: 510.0435 $[M+2]^+$.

(1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)(4-(phenylsulfonyl)piperazin-1-yl)methanone (10bd)

Off white solid: 76% yield; mp: 176–178 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.94 (s, 1H), 7.75 (dd, J = 5.2, 3.3 Hz, 2H), 7.64–7.59 (m, 1H), 7.53 (dd, J = 10.4, 4.7 Hz, 2H), 7.29–7.26 (m, 1H), 7.25 (s, 1H), 7.08–7.03 (m, 2H), 5.48 (s, 2H), 4.42 (s, 2H), 3.83 (s, 2H), 3.13 (d, J = 4.6 Hz, 2H), 3.10 (d, J = 5.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.39, 161.91, 159.63, 144.34, 135.41, 133.26, 130.39 (d, $J_{C\text{-}F}$ = 8.4 Hz), 129.35, 128.49, 127.85, 116.44 (d, $J_{C\text{-}F}$ = 21.9 Hz), 53.78, 46.78, 46.05, 45.57, 42.15; MS (ESI): *m/z* 430 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{21}FN_5O_3S$: 430.1343; found: 430.1342 $[M+H]^+$.

(4-((4-(Tert-butyl)phenyl)sulfonyl)piperazin-1-yl)(1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10be)

Off white solid: 84% yield; mp: 232–234 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 (s, 1H), 7.68–7.64 (m, 2H), 7.54–7.51 (m, 2H), 7.27 (dd, J = 4.9, 1.7 Hz, 1H), 7.25 (s, 1H), 7.05 (dd, J = 9.5, 7.6 Hz, 2H), 5.48 (bs, 2H), 4.41 (bs, 2H), 3.83 (bs, 2H), 3.13 (bs, 2H), 3.10 (d, J = 4.5 Hz, 2H), 1.33 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 163.14 (d, $J_{C\text{-}F}$ = 248.9 Hz), 159.66, 157.06, 144.36, 132.26, 130.39 (d, $J_{C\text{-}F}$ = 8.4 Hz), 129.64, 128.47, 127.78, 126.34, 116.43 (d, $J_{C\text{-}F}$ = 21.9 Hz), 53.78, 46.76, 46.12, 46.02, 42.16, 35.32, 31.17; MS (ESI): *m/z* 486 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{24}H_{29}FN_5O_3S$: 486.1969; found: 486.1971 $[M+H]^+$.

(1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)(4-((4-methoxyphenyl)sulfonyl)piperazin-1-yl)methanone (10bf)

Off white solid: 82% yield; mp: 198–200 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 (s, 1H), 7.70–7.66 (m, 2H), 7.29–7.26 (m, 2H), 7.09–7.03 (m, 2H), 7.00–6.96 (m, 2H), 5.48 (s, 2H), 4.41 (s, 2H), 3.86 (s, 3H), 3.85–3.80 (m, 2H), 3.10 (d, J = 4.8 Hz, 2H), 3.07 (d, J = 4.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.89 (d, $J_{C\text{-}F}$ = 99.6 Hz), 161.91, 159.64, 144.35, 130.40 (d, $J_{C\text{-}F}$ = 8.4 Hz), 130.02, 129.68, 128.49, 126.82, 116.44 (d, $J_{C\text{-}F}$ = 21.9 Hz), 114.52, 55.78, 53.78, 46.81, 46.07, 42.14; MS (ESI): *m/z* 460 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{21}H_{23}FN_5O_4S$: 460.1449; found: 460.1453 $[M+H]^+$.

(1-Benzyl-1*H*-1,2,3-triazol-4-yl)(4-tosylpiperazin-1-yl)methanone (10ca)

Off white solid: 85% yield; mp: 194–196 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 (s, 1H), 7.63 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 8.3 Hz, 1H), 7.37 (d, J = 2.5 Hz, 2H), 7.32 (d, J = 7.9 Hz, 2H), 7.28 (d, J = 3.5 Hz, 2H), 5.51 (s, 2H), 4.42 (s, 2H), 3.82 (s, 2H), 3.11 (d, J = 4.8 Hz, 2H), 3.08 (s, 2H), 2.42 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.71, 144.22, 144.18, 133.71, 132.25, 129.96, 129.40, 128.55, 128.48, 127.91, 127.80, 54.56, 46.81, 46.07, 45.58, 42.12, 21.66; MS (ESI): *m/z* 426 $[M+H]^+$; HRMS (ESI): *m/z* calcd for$C_{21}H_{24}N_5O_3S$: 426.15944; found: 426.1618 $[M+H]^+$.

(1-Benzyl-1*H*-1,2,3-triazol-4-yl)(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)methanone (10cb)

Off white solid: 86% yield; mp: 182–184 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.95 (s, 1H), 7.71–7.68 (m, 2H), 7.53–7.49 (m, 2H), 7.38–7.38 (m, 1H), 7.37 (t, J = 3.1 Hz, 2H), 7.29–7.27 (m, 2H), 5.51 (s, 2H), 4.44 (s, 2H), 3.84 (s, 2H), 3.13 (d, J = 4.0 Hz, 2H), 3.10 (d, J = 4.4 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.70, 144.17, 139.96, 133.88, 133.68, 129.70, 129.42, 129.30, 129.26, 128.64, 128.49, 54.59, 46.79,

46.04, 45.49, 42.11; MS (ESI): *m/z* 446 [M + H]$^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{21}ClN_5O_3S$: 446.1048; found: 446.1051 [M+H]$^+$.

(1-Benzyl-1*H*-1,2,3-triazol-4-yl)(4-((4-bromophenyl)sulfonyl)piperazin-1-yl)methanone (10cc)

Off white solid: 84% yield; mp: 191–193 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 (s, 1H), 7.69–7.65 (m, 2H), 7.63–7.58 (m, 2H), 7.38 (d, *J* = 2.0 Hz, 1H), 7.37 (d, *J* = 2.6 Hz, 2H), 7.27 (dd, *J* = 5.9, 2.1 Hz, 2H), 5.51 (s, 2H), 4.44 (bs, 2H), 3.84 (d, *J* = 4.4 Hz, 2H), 3.17–3.11 (m, 2H), 3.09 (d, *J* = 4.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.69, 144.16, 134.42, 133.69, 132.68, 129.42, 129.32, 128.64, 128.48, 54.58, 46.78, 46.04, 42.10; MS (ESI): *m/z* 492 [M+2]$^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{21}BrN_5O_3S$: 490.0543; found: 490.0545 [M+H]$^+$.

(1-Benzyl-1*H*-1,2,3-triazol-4-yl)(4-(phenylsulfonyl)piperazin-1-yl)methanone (10cd)

Off white solid: 78% yield; mp: 148–150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.93 (s, 1H), 7.77–7.74 (m, 2H), 7.72 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.61 (tt, *J* = 2.6, 1.7 Hz, 1H), 7.54 (dd, *J* = 6.4, 1.3 Hz, 2H), 7.38–7.37 (m, 1H), 7.36 (dd, *J* = 4.1, 2.1 Hz, 2H), 7.28–7.26 (m, 1H), 5.50 (s, 2H), 4.43 (bs, 2H), 3.83 (bs, 2H), 3.13 (d, *J* = 4.8 Hz, 2H), 3.10 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 159.07, 143.04, 134.51, 132.74, 128.78, 128.50, 128.24, 127.68, 127.06, 77.59, 77.33, 53.45, 46.05, 45.33, 44.78, 41.22; MS (ESI): *m/z* 412 [M+H]$^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{22}N_5O_3S$: 412.1437; found: 412.1437 [M+H]$^+$.

(1-Benzyl-1*H*-1,2,3-triazol-4-yl)(4-((4-(tert-butyl)phenyl)sulfonyl)piperazin-1-yl)methanone (10ce)

Off white solid: 85% yield; mp: 186–188 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 (s, 1H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.38 (s, 1H), 7.36 (d, *J* = 2.5 Hz, 2H), 7.29–7.25 (m, 2H), 5.51 (s, 2H), 4.42 (bs, 2H), 3.83 (bs, 2H), 3.13 (d, *J* = 4.5 Hz, 2H), 3.10 (d, *J* = 4.9 Hz, 2H), 1.34 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.76, 157.05, 144.26, 133.71, 132.28, 129.41, 129.28, 128.55, 128.48, 127.79, 126.34, 54.57, 46.79, 46.14, 46.03, 42.16, 35.33, 31.18; MS (ESI): *m/z* 468 [M+H]$^+$; HRMS (ESI): *m/z*: calcd for $C_{24}H_{30}N_5O_3S$: 468.2063; found: 468.2068 [M+H]$^+$.

(1-Benzyl-1*H*-1,2,3-triazol-4-yl)(4-((4-methoxyphenyl)sulfonyl)piperazin-1-yl)methanone (10cf)

Off white solid: 88% yield; mp: 188–190 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.94 (s, 1H), 7.68 (d, *J* = 8.3 Hz, 2H), 7.37 (d, *J* = 0.8 Hz, 1H), 7.36 (d, *J* = 1.6 Hz, 2H), 7.28–7.25 (m, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 5.50 (s, 2H), 4.41 (bs, 2H), 3.86 (s, 3H), 3.82 (bs, 2H), 3.10 (bs, 2H), 3.06 (bs, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.38, 159.71, 144.23, 133.73, 130.01, 129.40, 129.26, 128.56, 128.47, 126.82, 114.51, 55.77, 54.55, 46.82, 46.07, 42.11; MS (ESI): *m/z* 442 [M+H]$^+$; HRMS (ESI): *m/z* calcd for $C_{21}H_{24}N_5O_4S$: 442.1543; found: 442.1545 [M+H]$^+$.

(1-(4-Iodobenzyl)-1*H*-1,2,3-triazol-4-yl)(4-tosylpiperazin-1-yl)methanone (10da)

Off white solid: 84% yield; mp: 194–196 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.95 (s, 1H), 7.72–7.69 (m, 2H), 7.63 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 7.9 Hz, 2H), 7.01 (d, J = 8.3 Hz, 2H), 5.44 (s, 2H), 4.40 (s, 2H), 3.82 (s, 2H), 3.10 (d, J = 4.7 Hz, 2H), 3.08 (s, 2H), 2.42 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.58, 144.42, 144.20, 138.55, 133.42, 132.32, 130.15, 129.97, 128.55, 127.92, 127.80, 95.14, 53.95, 46.80, 46.08, 45.59, 42.16, 21.67; MS (ESI): *m/z* 552 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{21}H_{23}IN_5O_3S$: 552.0560; found: 552.0565 $[M+H]^+$.

(4-((4-Chlorophenyl)sulfonyl)piperazin-1-yl)(1-(4-iodobenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10db)

Off white solid: 82% yield; mp: 190–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.72 (s, 1H), 7.71–7.66 (m, 2H), 7.53–7.49 (m, 1H), 7.01 (d, J = 8.3 Hz, 2H), 5.45 (s, 2H), 4.43 (s, 2H), 3.84 (s, 2H), 3.12 (s, 2H), 3.10 (d, J = 5.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.56, 144.36, 139.98, 138.57, 133.94, 133.38, 130.16, 129.71, 129.26, 128.63, 95.17, 53.98, 46.77, 46.05, 45.50, 42.14; MS (ESI): *m/z* 594 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{20}ClIN_5O_3S$: 572.0014; found: 572.0021 $[M+H]^+$.

(4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)(1-(4-iodobenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10dc)

Off white solid: 86% yield; mp: 201–203 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.71 (d, J = 8.3 Hz, 2H), 7.69–7.66 (m, 2H), 7.63–7.59 (m, 2H), 7.01 (d, J = 8.3 Hz, 2H), 5.45 (s, 2H), 4.43 (s, 2H), 3.84 (s, 2H), 3.12 (d, J = 4.5 Hz, 2H), 3.10 (d, J = 4.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.56, 144.35, 138.56, 134.46, 133.38, 132.70, 130.16, 129.32, 128.64, 128.48, 95.17, 53.97, 46.76, 46.04, 42.13; MS (ESI): *m/z* 618 $[M+2]^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{20}BrIN_5O_3S$: 615.9509; found: 617.9509 $[M+H]^+$.

(1-(4-Iodobenzyl)-1*H*-1,2,3-triazol-4-yl)(4-(phenylsulfonyl)piperazin-1-yl)methanone (10dd)

Off white solid: 80% yield; mp: 176–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 (s, 1H), 7.77–7.73 (m, 2H), 7.70 (d, J = 8.3 Hz, 2H), 7.61 (ddd, J = 6.5, 3.8, 1.3 Hz, 1H), 7.57–7.51 (m, 2H), 7.01 (d, J = 8.3 Hz, 2H), 5.44 (s, 2H), 4.42 (s, 2H), 3.83 (s, 2H), 3.13 (d, J = 5.0 Hz, 2H), 3.10 (d, J = 5.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.58, 144.40, 138.55, 135.42, 133.40, 133.28, 130.15, 129.37, 128.59, 127.86, 95.15, 53.96, 46.79, 46.06, 42.17; MS (ESI): *m/z* 538 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{21}IN_5O_3S$: 538.0404; found: 538.0409 $[M+H]^+$.

(4-((4-(Tert-butyl)phenyl)sulfonyl)piperazin-1-yl)(1-(4-iodobenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10de)

Off white solid: 84% yield; mp: 214–216 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 (s, 1H), 7.70 (d, J = 8.3 Hz, 2H), 7.66 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 8.3 Hz, 2H), 5.44 (s, 2H), 4.41 (s, 2H), 3.83 (s, 2H), 3.12 (d, J = 4.8 Hz, 2H), 3.10 (d, J = 4.4 Hz, 2H), 1.34 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 159.63, 157.09, 144.47, 138.58, 133.40, 132.31, 130.16, 128.56, 127.81, 126.37, 95.17, 53.98, 46.78, 46.16, 46.04, 42.20, 35.36, 31.20; MS (ESI): *m/z* 594 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{24}H_{29}IN_5O_3S$: 594.10303; found: 594.1036 $[M+H]^+$.

(1-(4-Iodobenzyl)-1*H*-1,2,3-triazol-4-yl)(4-((4-methoxyphenyl)sulfonyl)piperazin-1-yl)methanone (10df)

Off white solid: 82% yield; mp: 224–226 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 (s, 1H), 7.72 (s, 1H), 7.70 (d, J = 2.5 Hz, 2H), 7.67 (d, J = 2.0 Hz, 1H), 7.02 (s, 1H), 7.00 (s, 2H), 6.98 (d, J = 1.9 Hz, 1H), 5.45 (s, 2H), 4.41 (bs, 2H), 3.86 (s, 3H), 3.83 (bs, 2H), 3.10 (bs, 2H), 3.07 (d, J = 5.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.40, 159.57, 144.42, 138.54, 133.42, 130.15, 130.02, 128.56, 126.83, 114.52, 95.14, 55.79, 53.95, 46.81, 46.07, 42.15; MS (ESI): *m/z* 568 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{21}H_{23}IN_5O_4S$: 568.0509; found: 568.0522 $[M+H]^+$.

(1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)(4-tosylpiperazin-1-yl)methanone (10ea)

Off white solid: 84% yield; mp: 134–136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.63 (d, J = 8.2 Hz, 2H), 7.60 (d, J = 8.2 Hz, 1H), 7.34 (d, J = 5.7 Hz, 2H), 7.33–7.30 (m, 2H), 7.13 (t, J = 7.4 Hz, 1H), 6.99 (d, J = 7.7 Hz, 2H), 6.97–6.94 (m, 2H), 6.90 (s, 1H), 5.47 (s, 2H), 4.41 (bs, 2H), 3.83 (bs, 2H), 3.12 (bs, 2H), 3.08 (bs, 2H), 2.42 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.68, 158.38, 156.39, 144.29, 144.19, 135.56, 132.27, 130.77, 130.07, 129.98, 128.61, 127.93, 124.10, 122.76, 119.49, 118.94, 118.35, 54.20, 46.82, 46.09, 45.59, 42.15, 21.67; MS (ESI): *m/z* 518 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{27}H_{28}N_5O_4S$: 518.1856; found: 518.1864 $[M+H]^+$.

(4-((4-Chlorophenyl)sulfonyl)piperazin-1-yl)(1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10eb)

Off white solid: 83% yield; mp: 154–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (s, 1H), 7.71-7.66 (m, 2H), 7.53–7.49 (m, 2H), 7.38–7.32 (m, 2H), 7.30 (d, J = 8.0 Hz, 1H), 7.17–7.11 (m, 1H), 7.00 (t, J = 1.6 Hz, 1H), 6.99–6.96 (m, 2H), 6.95 (d, J = 2.4 Hz, 1H), 6.92–6.89 (m, 1H), 5.47 (s, 2H), 4.44 (bs, 2H), 3.84 (bs, 2H), 3.12 (bs, 2H), 3.10 (d, J = 5.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.66, 158.37, 156.38, 144.22, 139.96, 135.54, 133.89, 130.77, 130.07, 129.71, 129.26, 128.69, 124.10, 122.77, 119.47, 118.96, 118.37, 54.21, 46.78, 46.05, 45.49, 42.12; MS (ESI): *m/z* 540 $[M+2]^+$; HRMS (ESI): *m/z* calcd for $C_{26}H_{25}ClN_5O_4S$: 538.1310; found: 538.1318 $[M+H]^+$.

(4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)(1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10ec)

Off white solid: 85% yield; mp: 158–160 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.98 (s, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.61 (d, J = 8.1 Hz, 2H), 7.36 (d, J = 7.8 Hz, 2H), 7.32 (d, J = 11.3 Hz, 1H), 7.14 (t, J = 7.4 Hz, 1H), 6.99 (d, J = 8.0 Hz, 3H), 6.96 (d, J = 7.9 Hz, 1H), 6.91 (s, 1H), 5.47 (s, 2H), 4.44 (bs, 2H), 3.85 (bs, 2H), 3.14 (bs, 2H), 3.10 (bs, 2H);^{13}C NMR (126 MHz, $CDCl_3$) δ: 159.57, 158.31, 156.32, 144.15, 135.45, 134.53, 132.58, 130.66, 129.94, 129.21, 128.61, 128.33, 123.97, 122.64, 119.36, 118.87, 118.27, 54.13, 46.67, 45.95, 42.04, 31.06; MS (ESI): *m/z* 584 $[M+2]^+$; HRMS (ESI): *m/z* calcd for $C_{26}H_{25}BrN_5O_4S$: 582.0805; found: 582.0812 $[M+H]^+$.

(1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)(4-(phenylsulfonyl)piperazin-1-yl)methanone (10ed)

Off white solid: 75% yield; mp: 164–166 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.75 (dd, J = 5.3, 3.3 Hz, 2H), 7.61 (ddd, J = 8.7, 4.2, 1.6 Hz, 1H), 7.55 (dd, J = 6.5, 1.3 Hz, 2H), 7.37–7.32 (m, 2H), 7.30 (d, J = 8.0 Hz, 1H), 7.15–7.11 (m, 1H), 7.00–6.97 (m, 2H), 6.96 (dd, J = 8.2, 2.3 Hz, 2H), 6.90 (t, J = 2.0 Hz, 1H), 5.46 (s, 2H), 4.42 (s, 2H), 3.84 (d, J = 4.6 Hz, 2H), 3.16–3.12 (m, 2H), 3.11 (d, J = 4.1 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.70, 158.39, 156.39, 144.28, 135.55, 135.39, 133.29, 130.78, 130.08, 129.38, 128.64, 127.88, 124.11, 122.76, 119.50, 118.96, 118.36, 54.22, 46.82, 46.11, 45.58, 42.17; MS (ESI): *m/z* 504 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{26}H_{26}N_5O_4S$: 504.1700; found: 504.1704 $[M+H]^+$.

(4-((4-(Tert-butyl)phenyl)sulfonyl)piperazin-1-yl)(1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10ee)

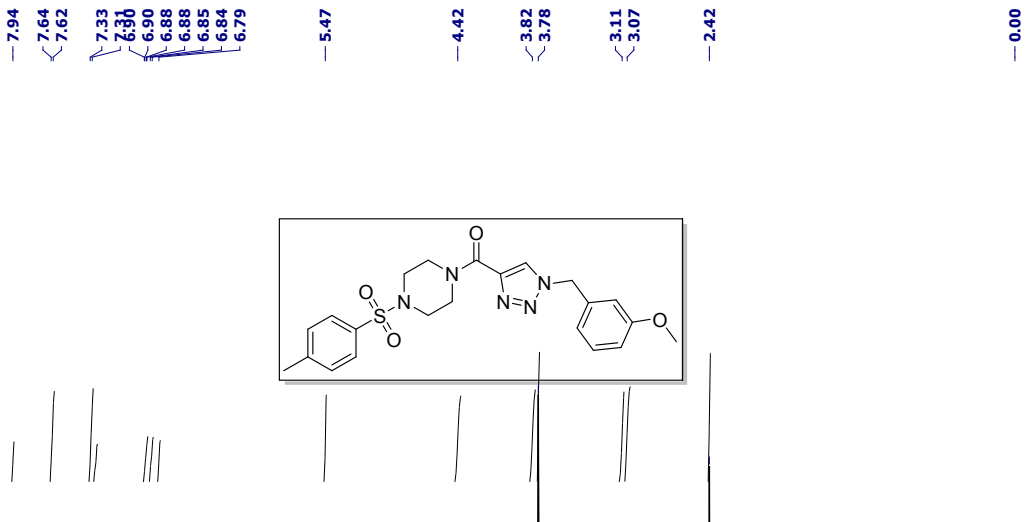
Off white solid: 88% yield; mp: 152–154 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.36–7.31 (m, 2H), 7.30 (d, J = 7.9 Hz, 1H), 7.13 (t, J = 7.4 Hz, 1H), 7.00–6.97 (m, 2H), 6.97–6.94 (m, 2H), 6.90 (s, 1H), 5.47 (s, 2H), 4.42 (bs, 2H), 3.84 (bs, 2H), 3.14 (bs, 2H), 3.11 (bs, 2H), 1.34 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 159.78, 158.46, 157.09, 156.47, 144.37, 135.61, 132.52, 130.79, 130.08, 128.64, 127.83, 126.36, 124.11, 122.77, 119.52, 118.99, 118.39, 54.25, 46.82, 46.20, 46.07, 42.23, 35.35, 31.20; MS (ESI): *m/z* 560 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{30}H_{34}N_5O_4S$: 560.2326; found: 560.2334 $[M+H]^+$.

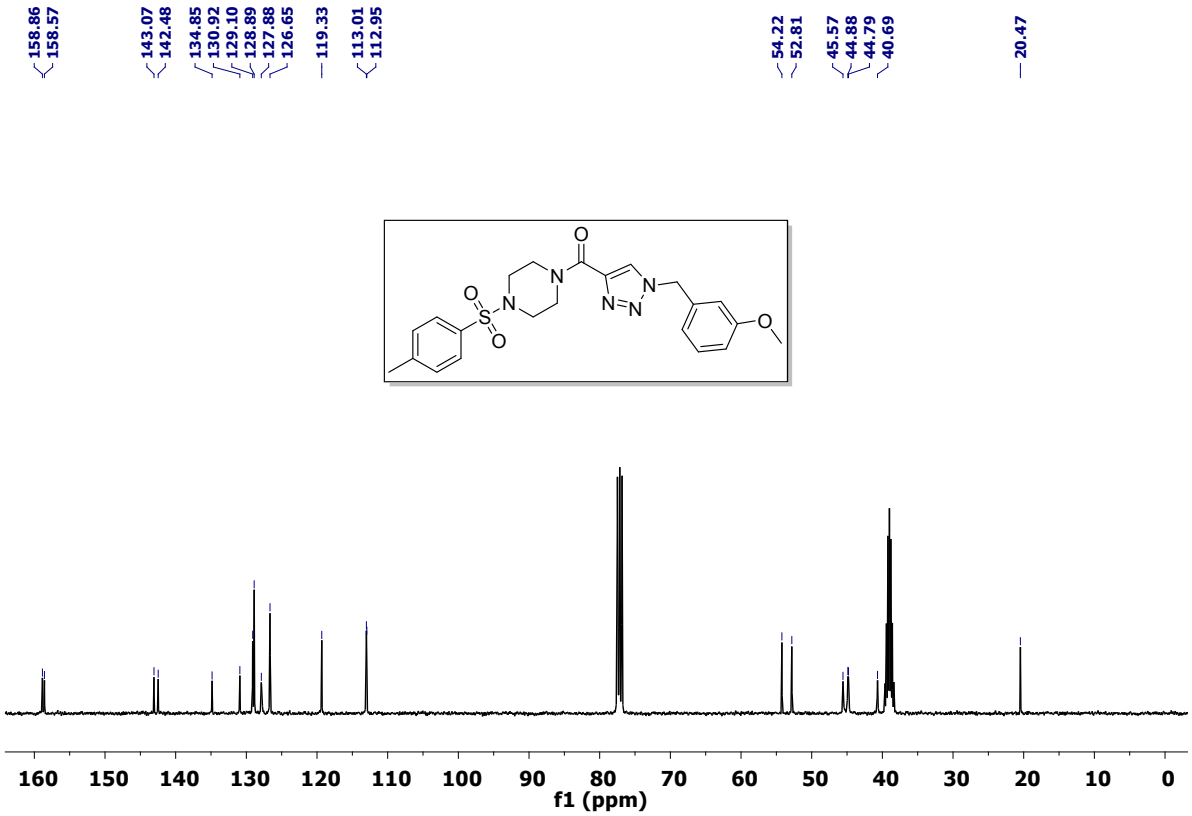
(4-((4-Methoxyphenyl)sulfonyl)piperazin-1-yl)(1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methanone (10ef)

Off white solid: 90% yield; mp: 132–134 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.69 (d, J = 8.5 Hz, 2H), 7.34 (dd, J = 13.3, 5.7 Hz, 2H), 7.30 (d, J = 7.9 Hz, 1H), 7.14 (t, J = 7.2 Hz, 1H), 7.00 (s, 2H),

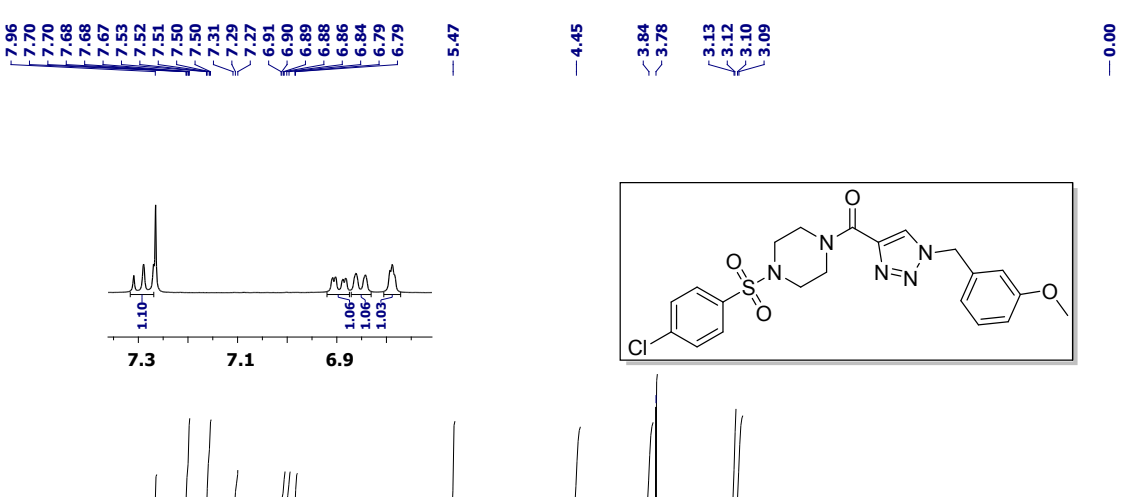
6.98 (s, 2H), 6.96 (d, J = 8.0 Hz, 2H), 6.90 (s, 1H), 5.47 (s, 2H), 4.41 (bs, 2H), 3.86 (s, 3H), 3.83 (bs, 2H), 3.11 (bs, 2H), 3.07 (bs, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.41, 159.69, 158.41, 156.41, 144.33, 135.58, 130.78, 130.08, 130.04, 128.61, 126.88, 124.11, 122.77, 119.50, 118.96, 118.37, 114.54, 55.79, 54.22, 46.85, 46.10, 42.16; MS (ESI): *m/z* 534 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{27}H_{28}N_5O_5S$: 534.1805; found: 534.1813 $[M+H]^+$.

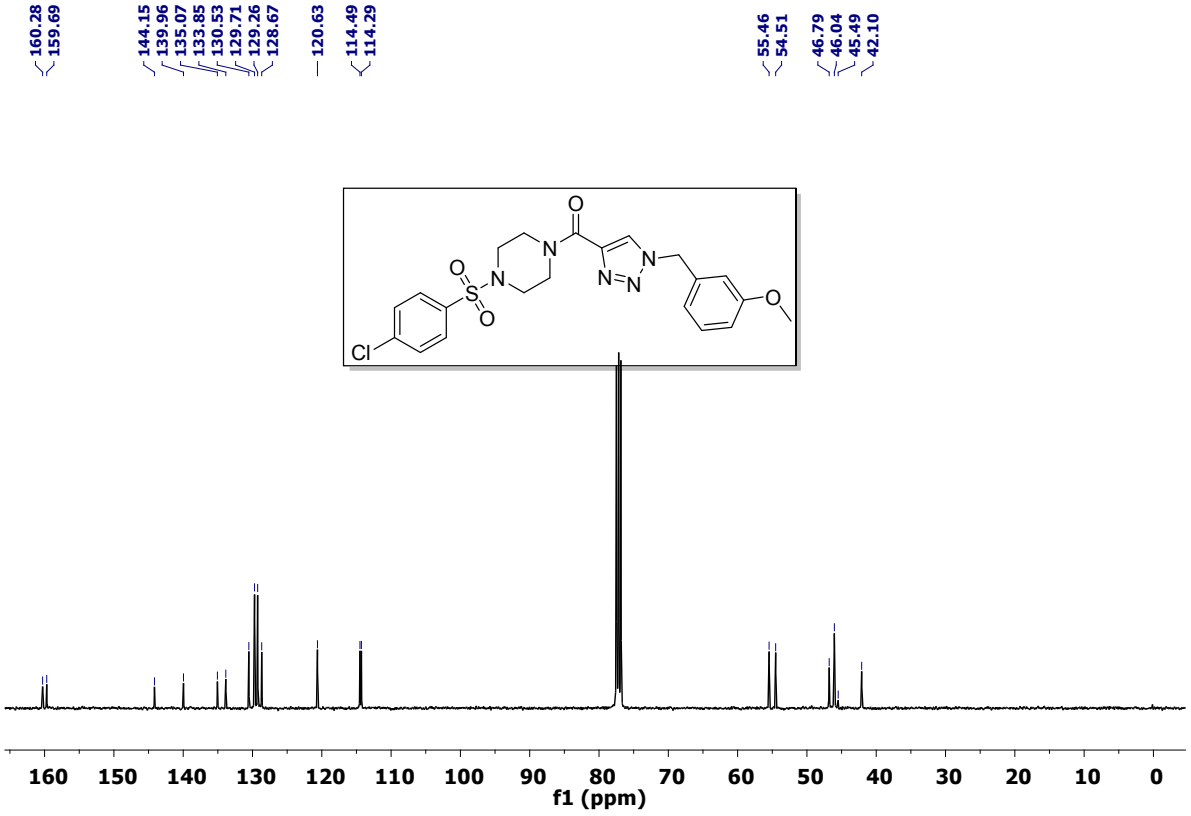
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10aa**



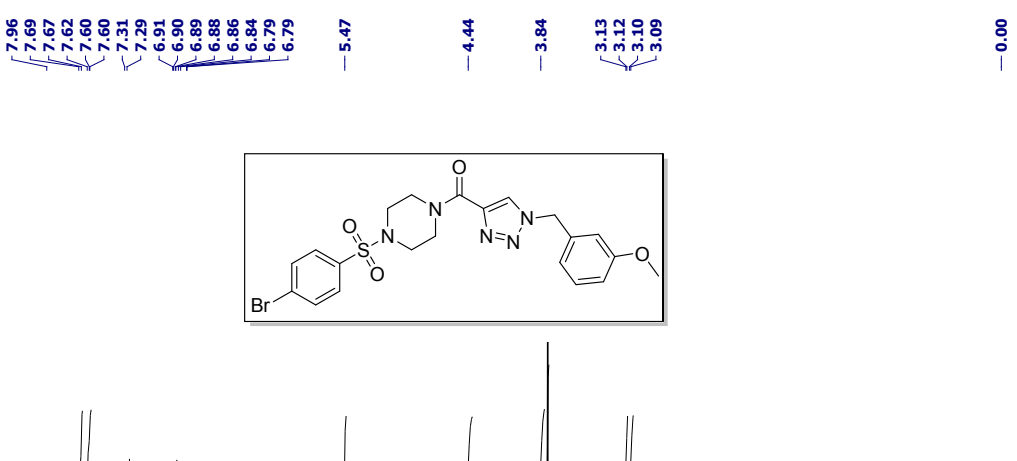


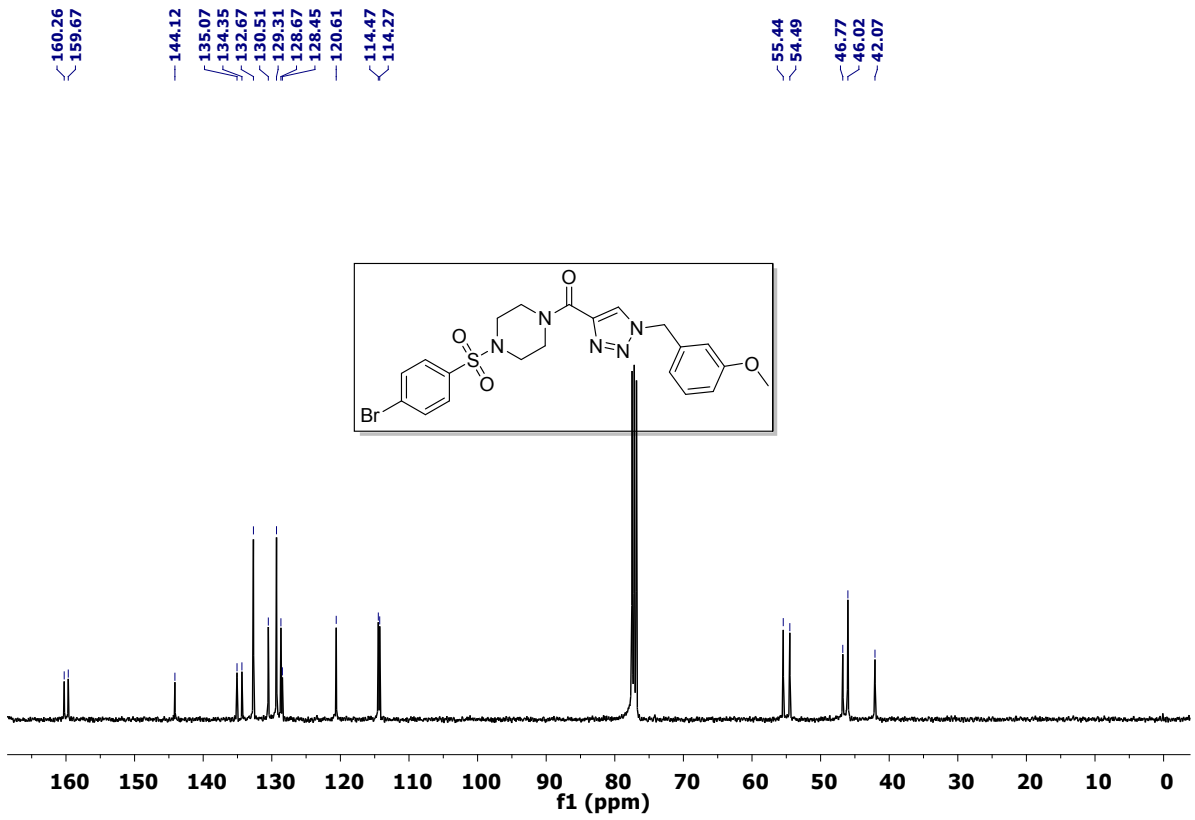
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ab**



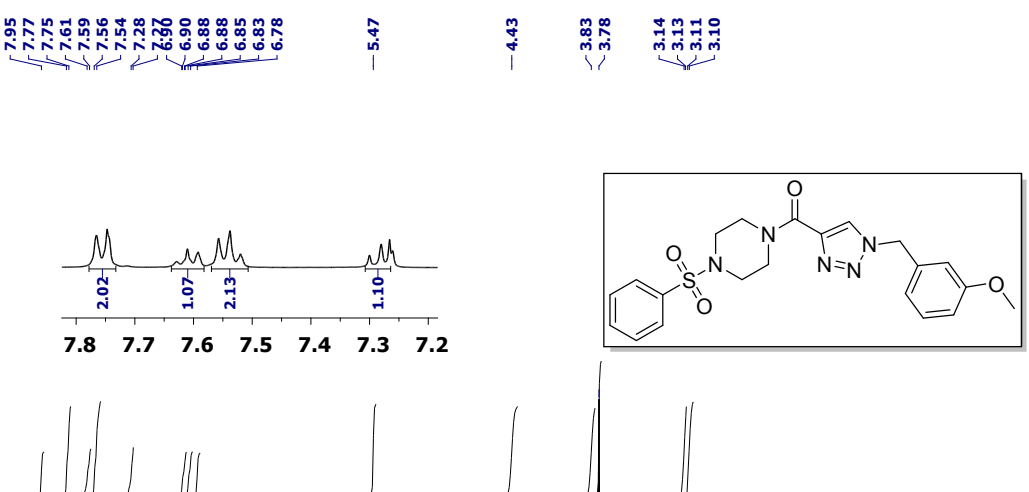


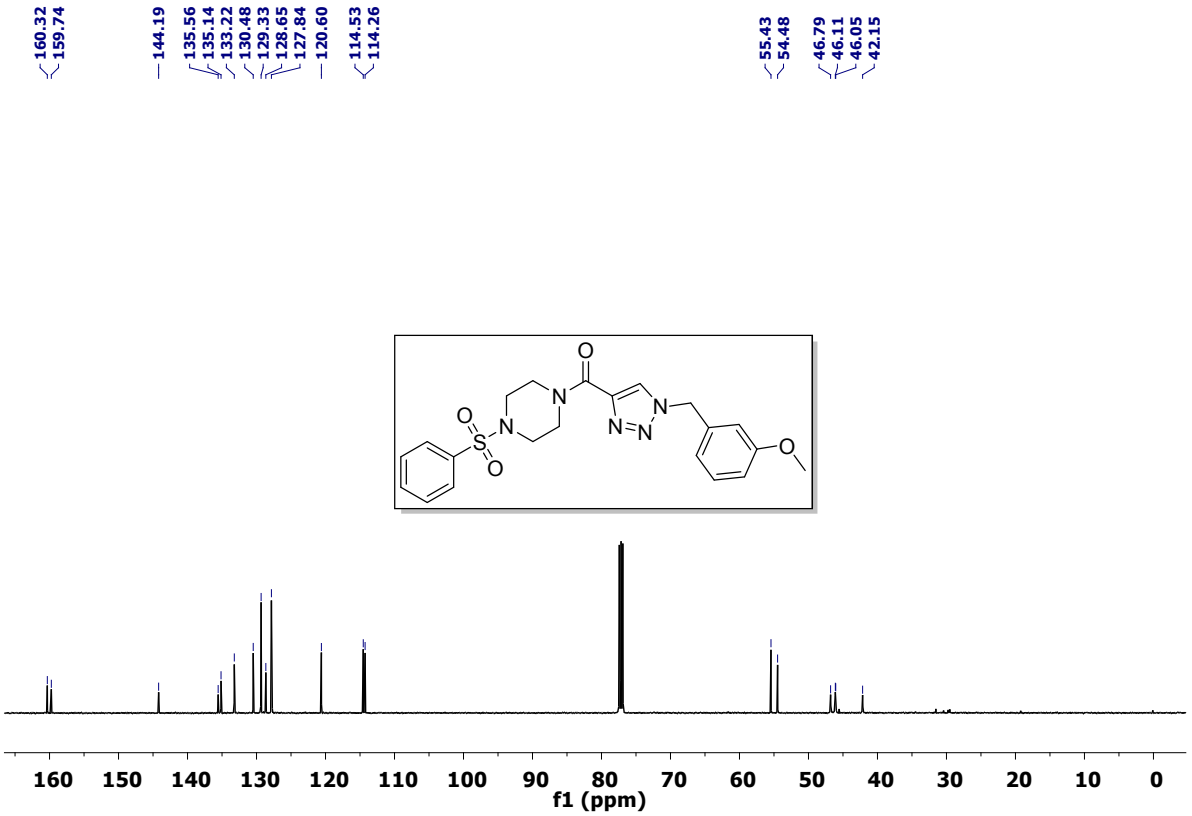
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ac**



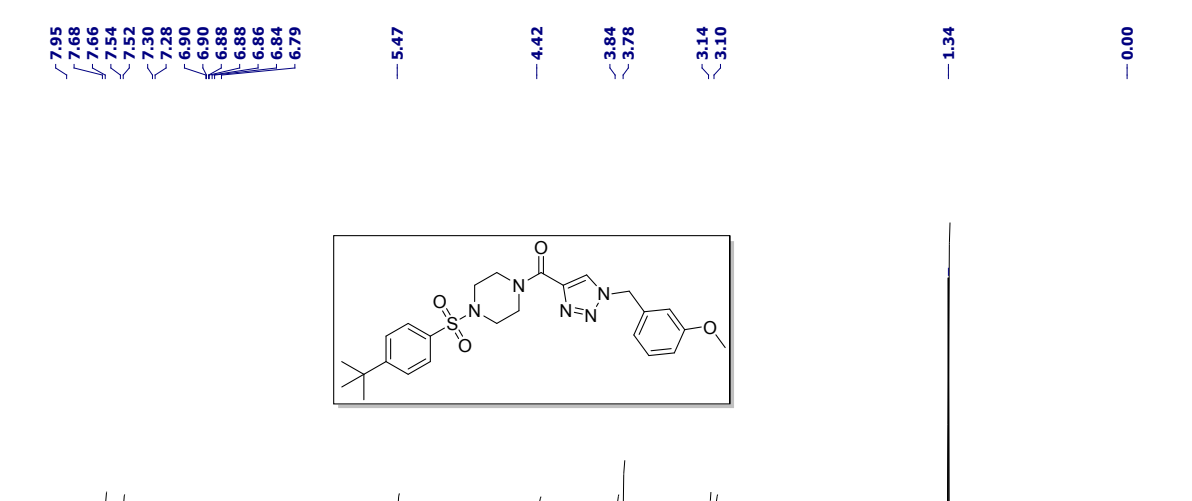


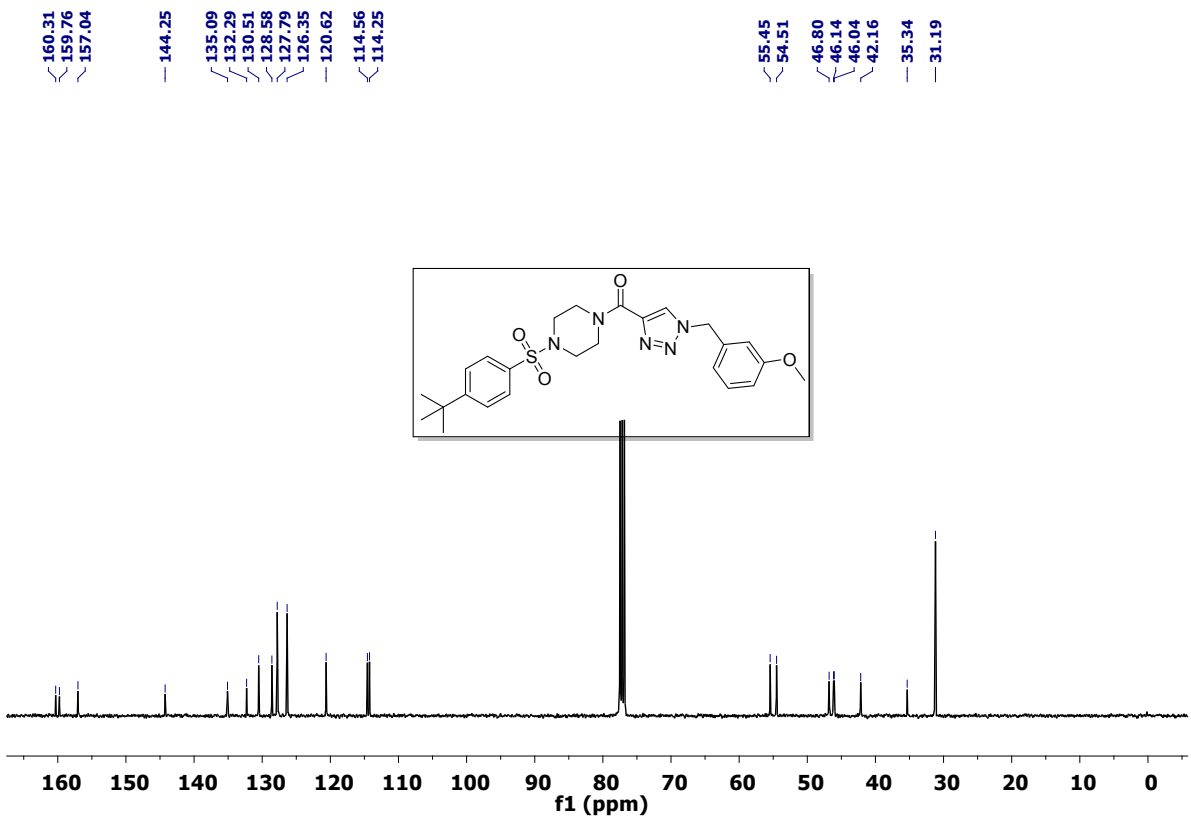
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ad**



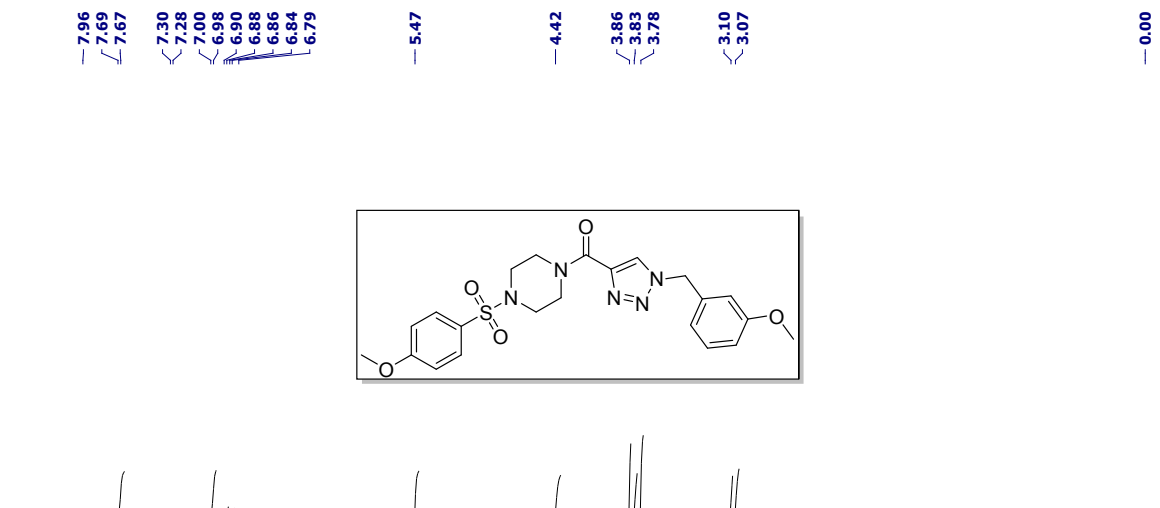


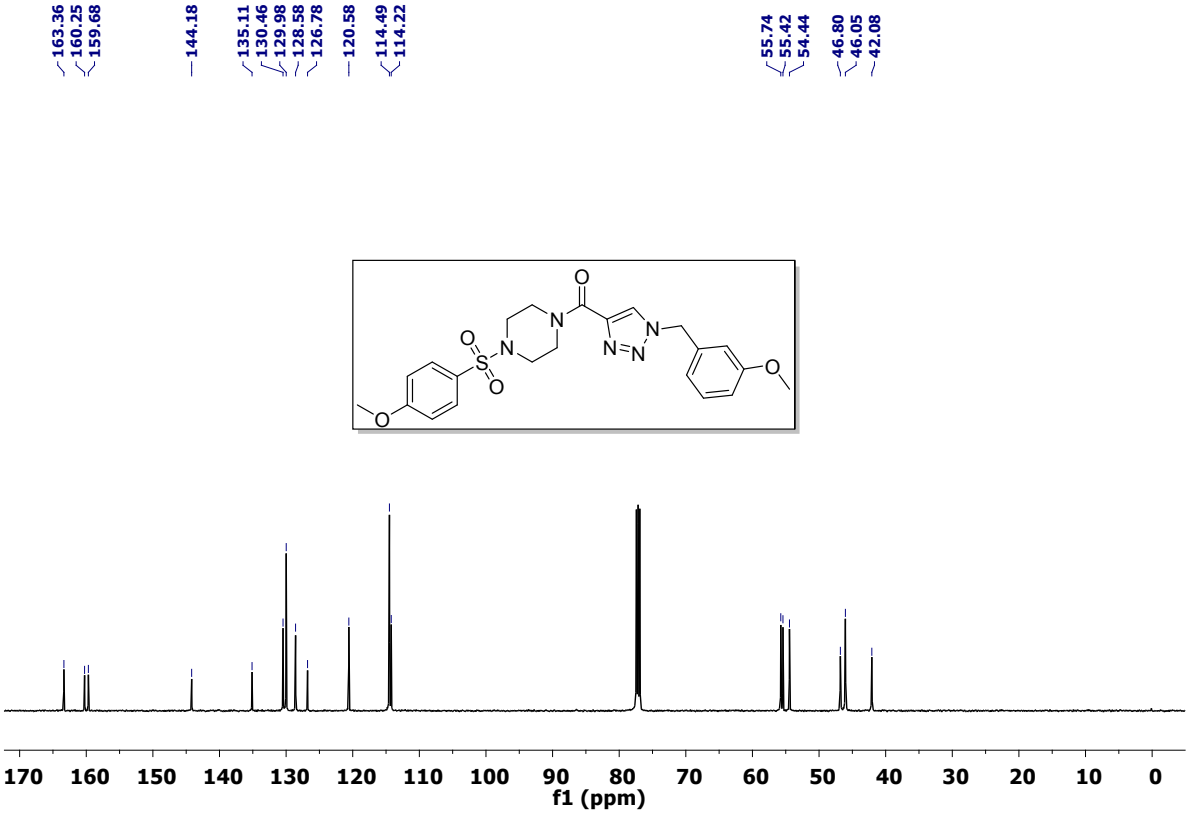
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ae**



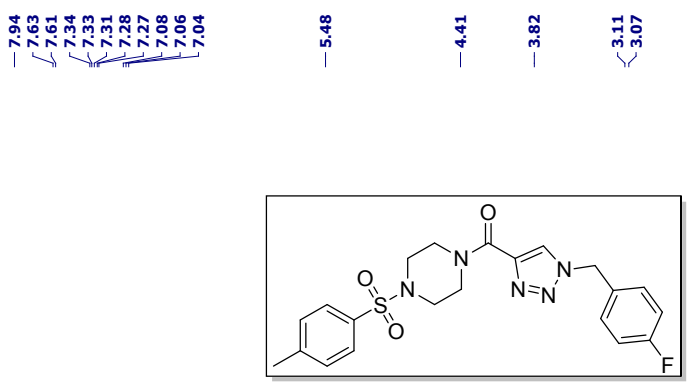


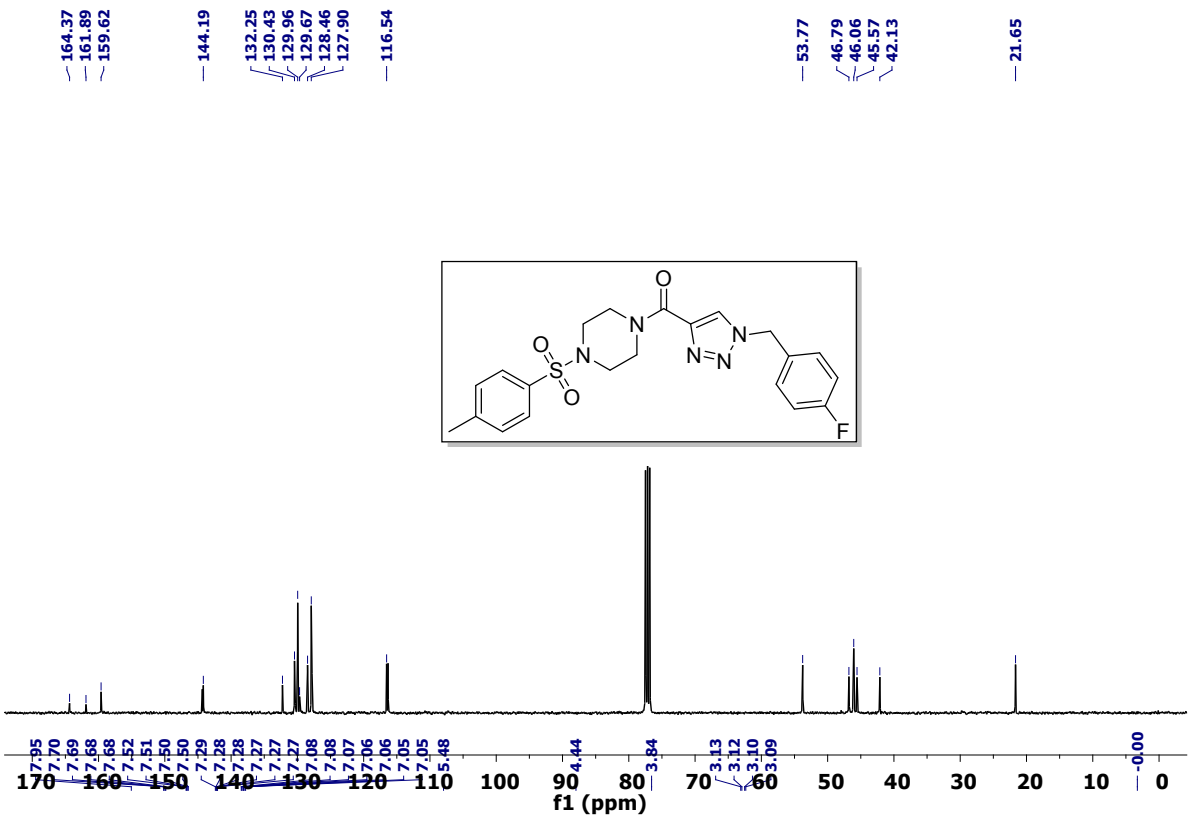
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10af**



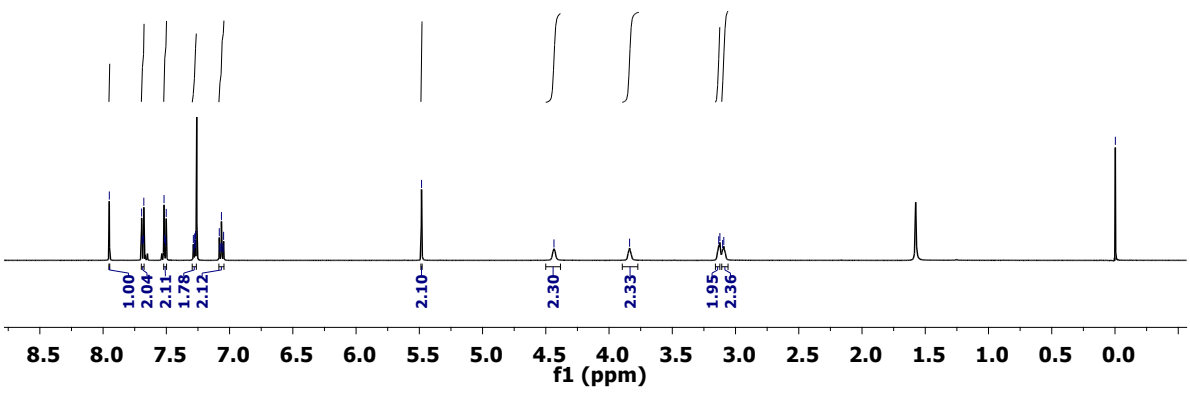


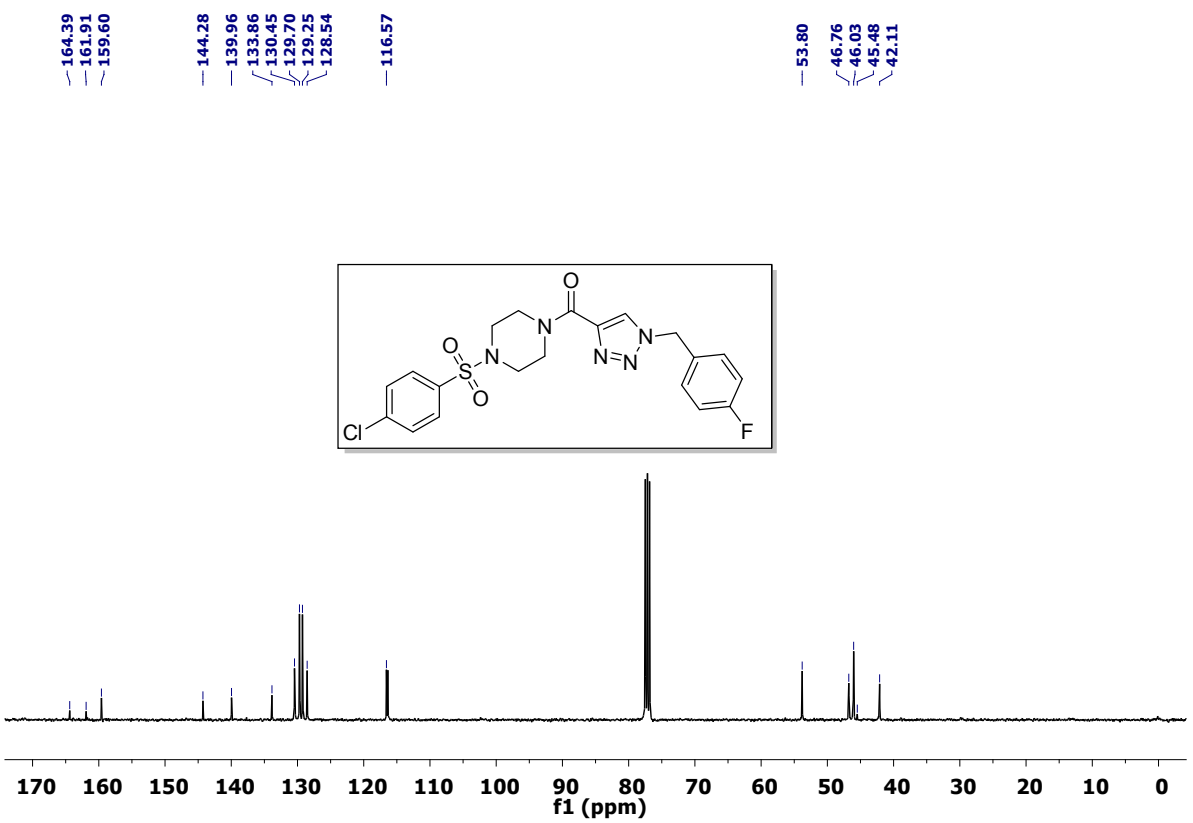
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ba**



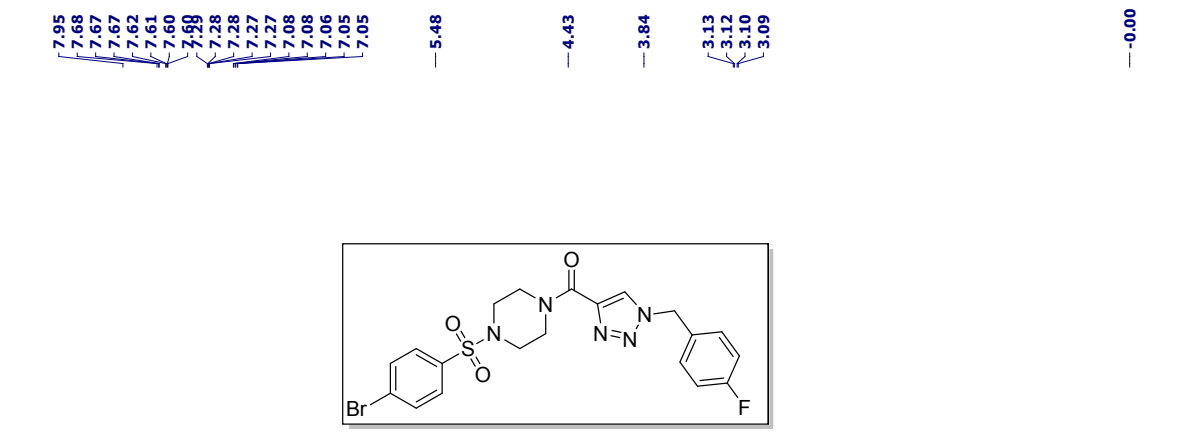


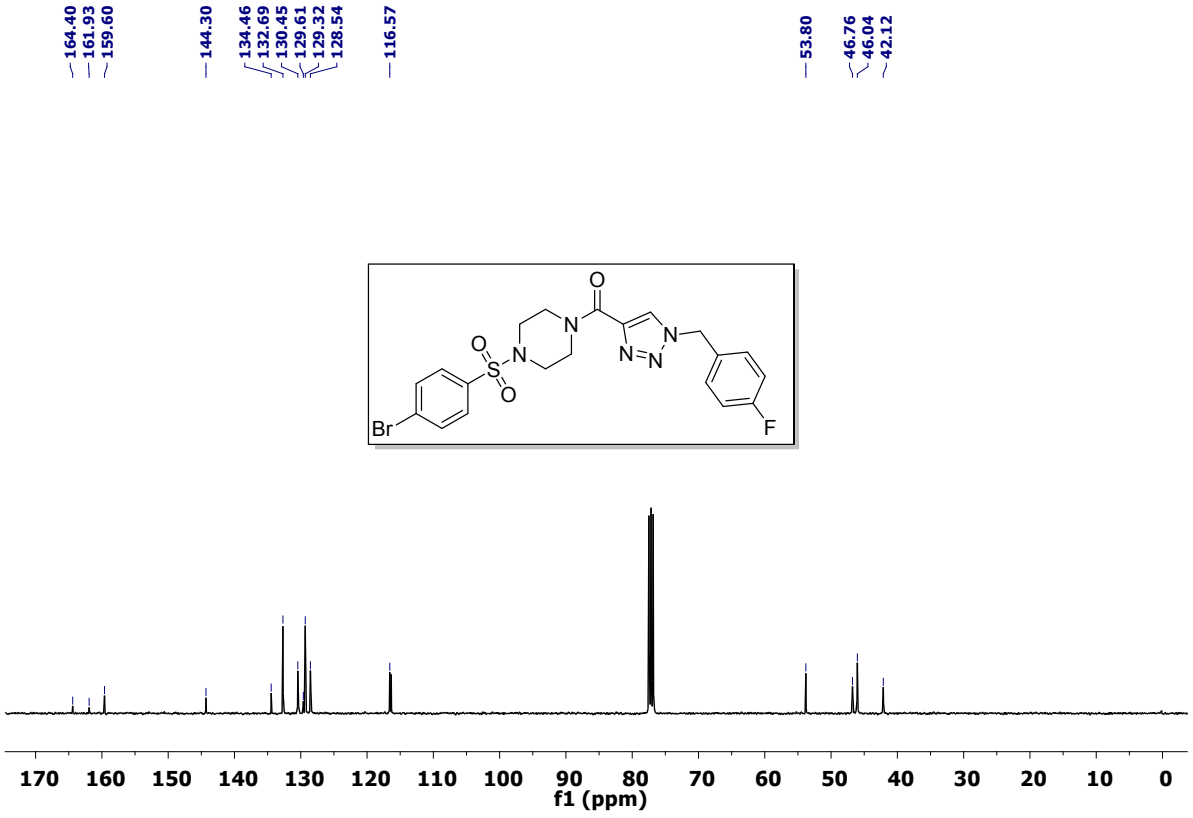
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10bb**



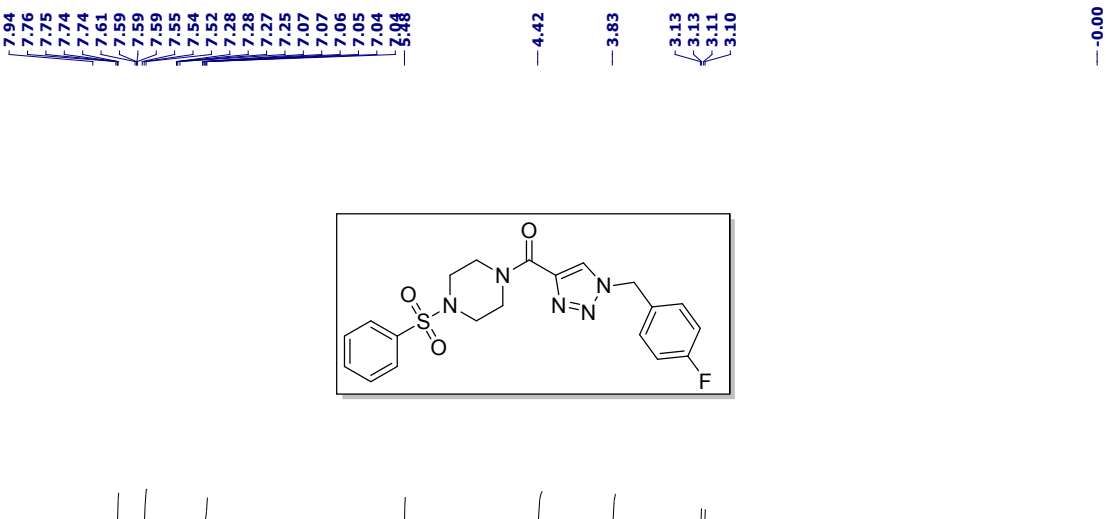


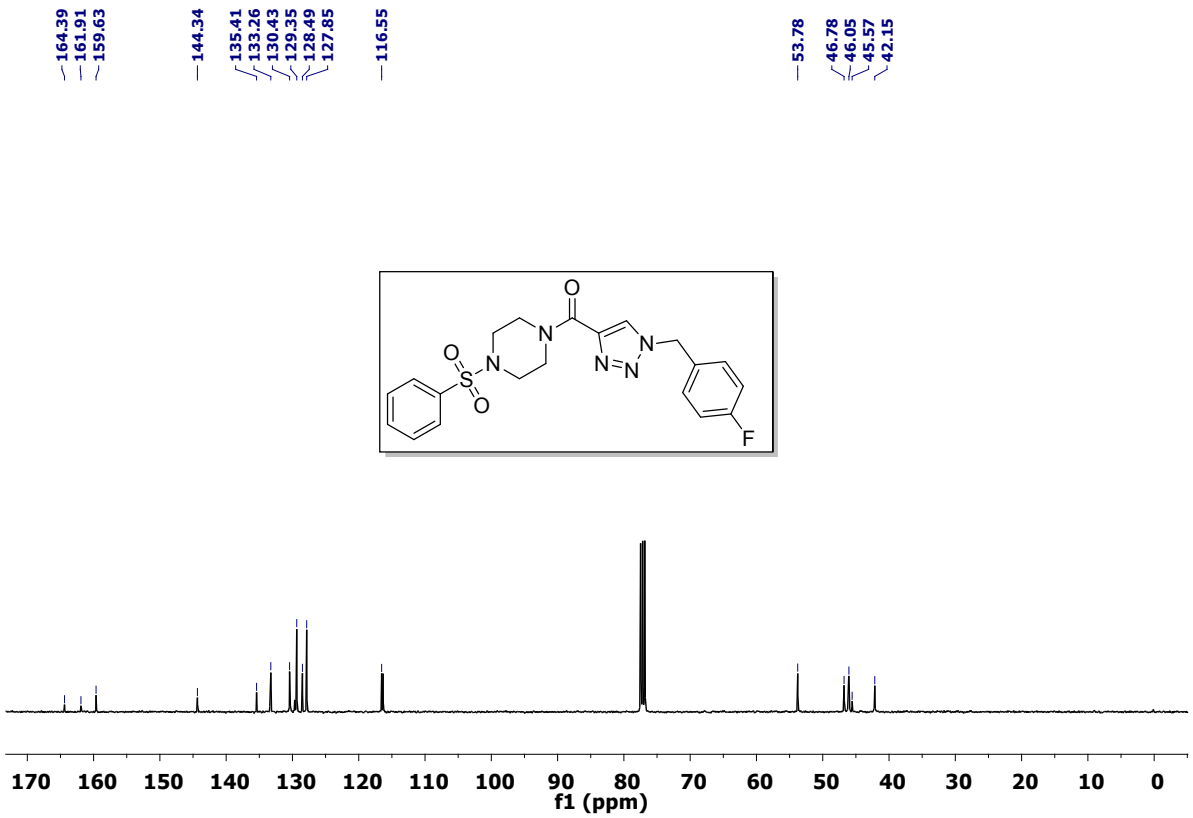
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10bc**



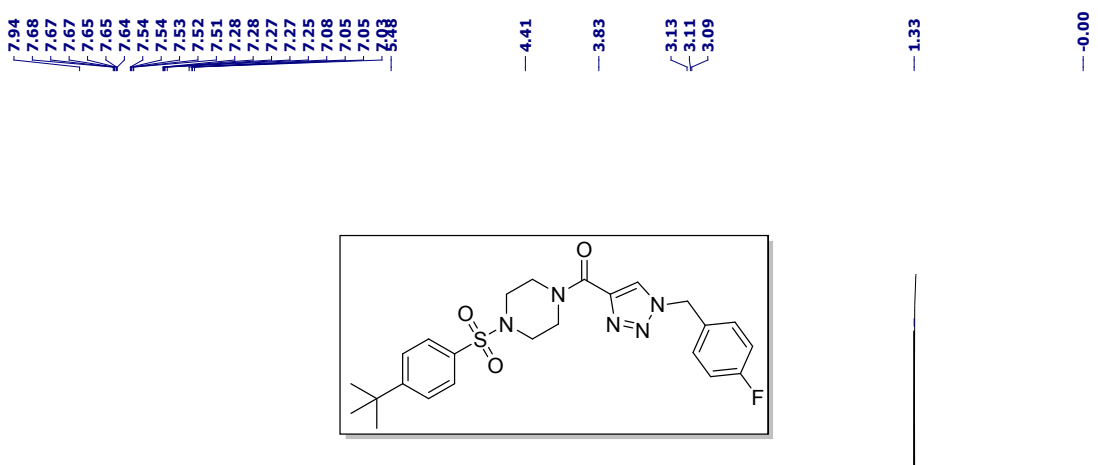


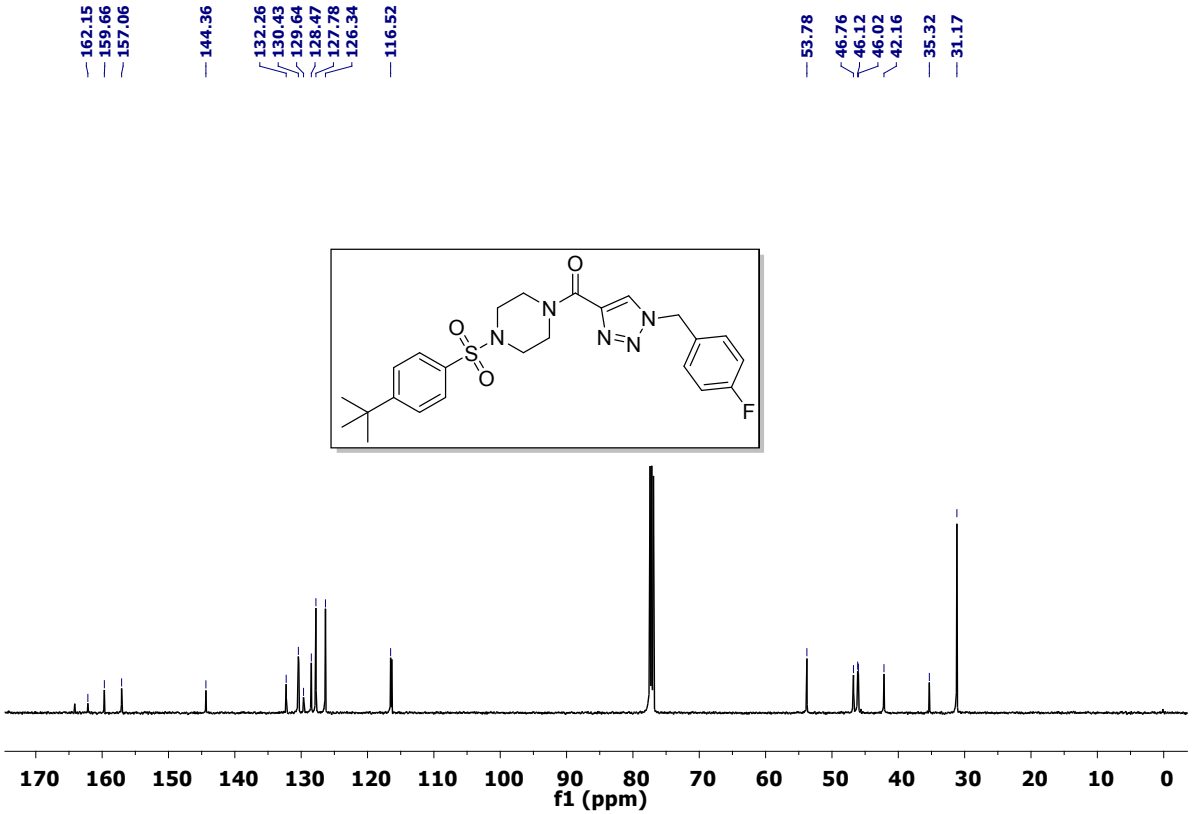
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10bd**



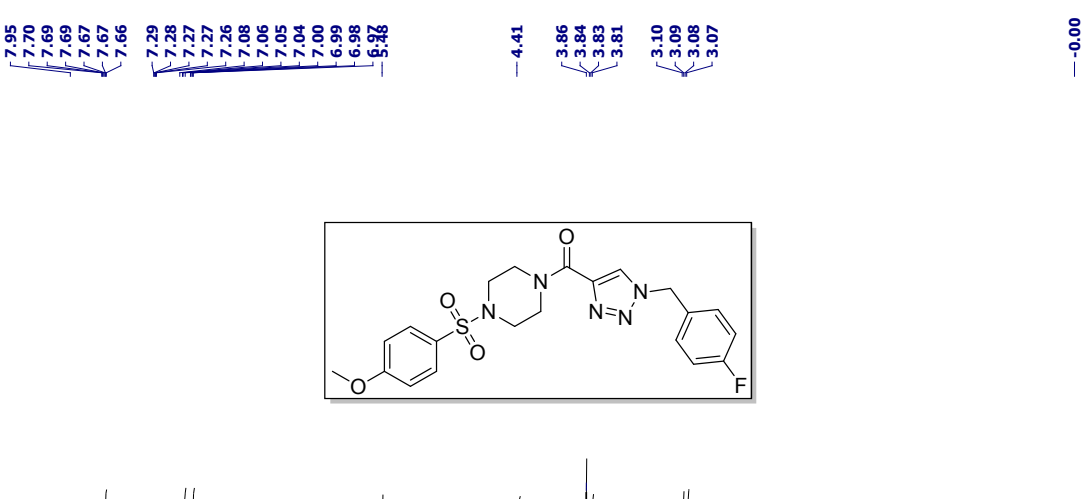


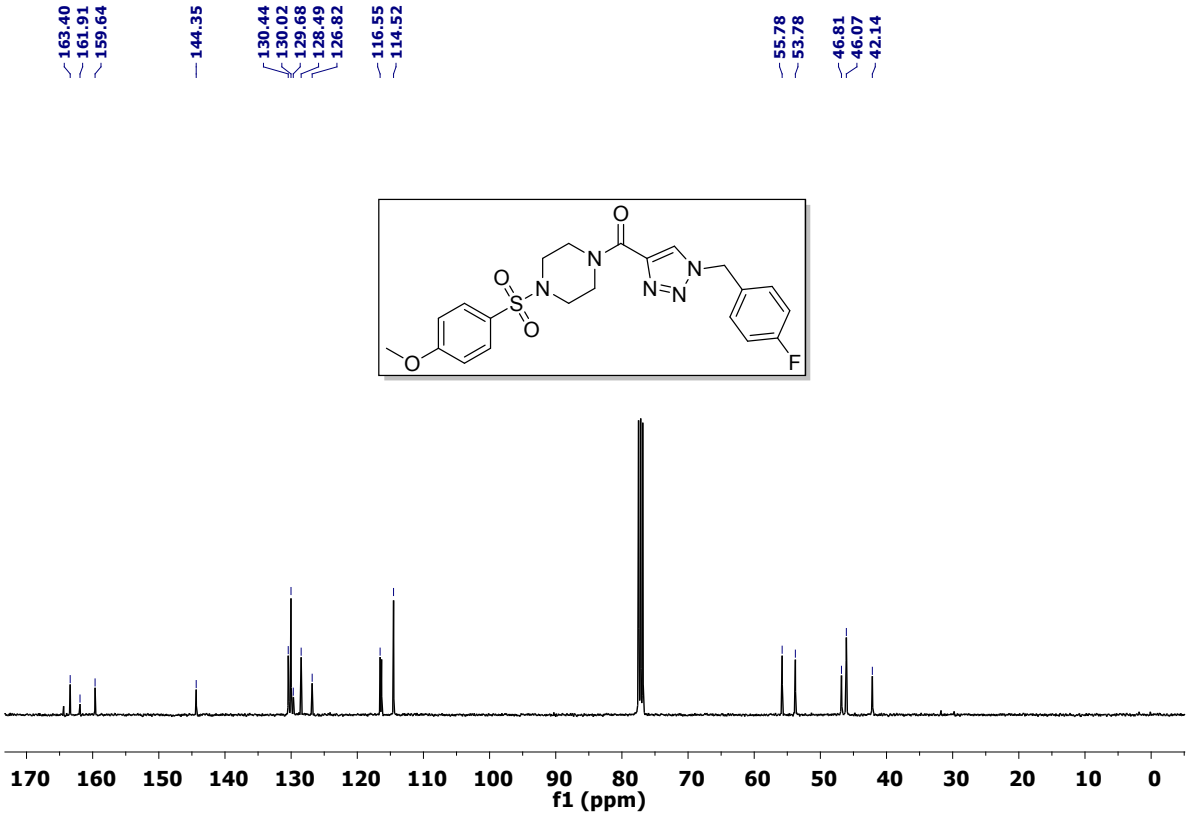
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10be**



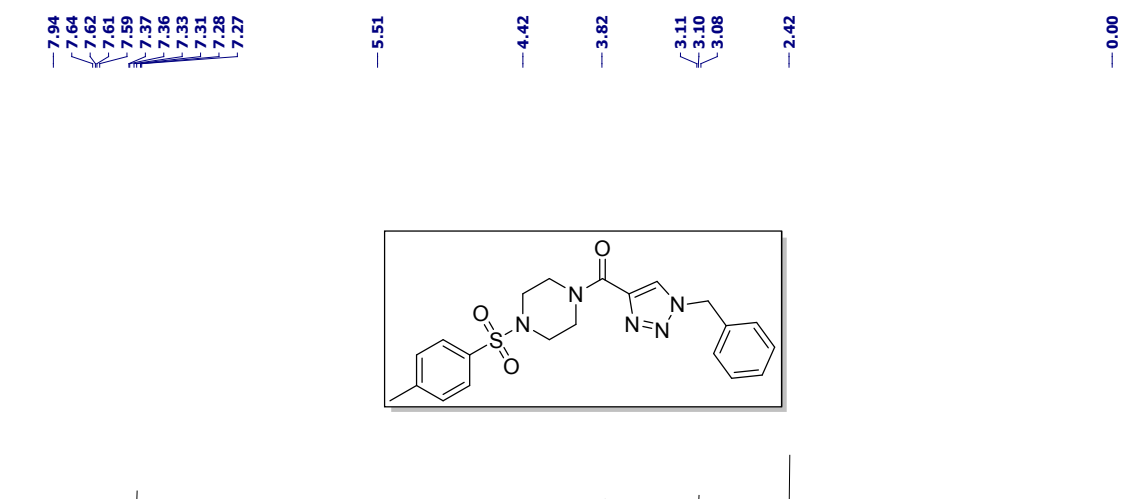


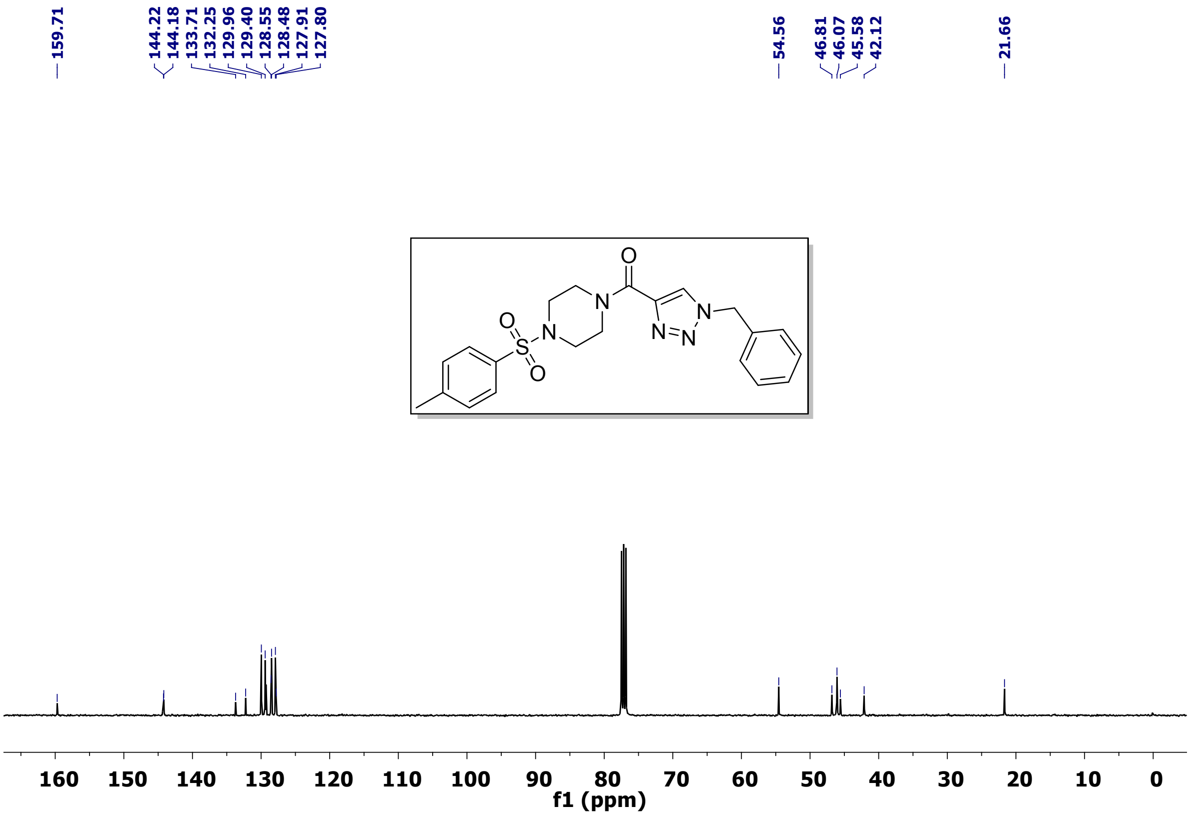
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10bf**



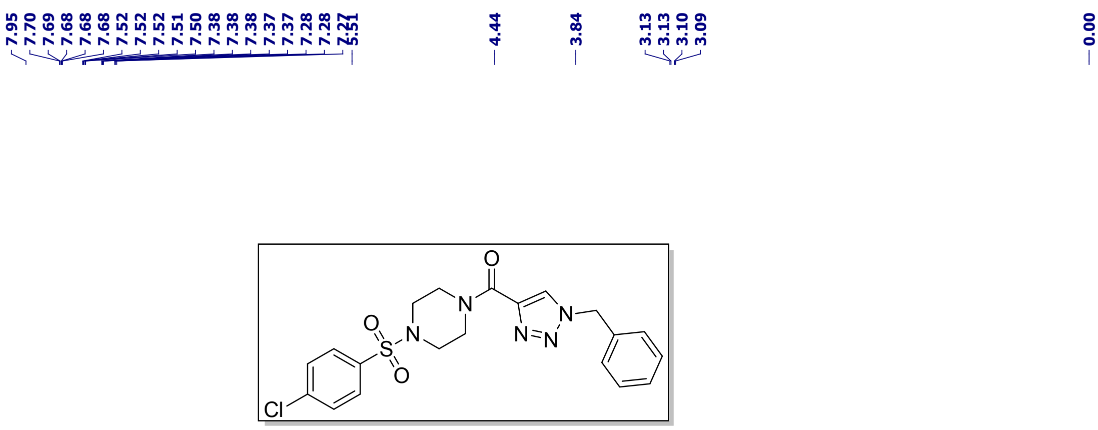


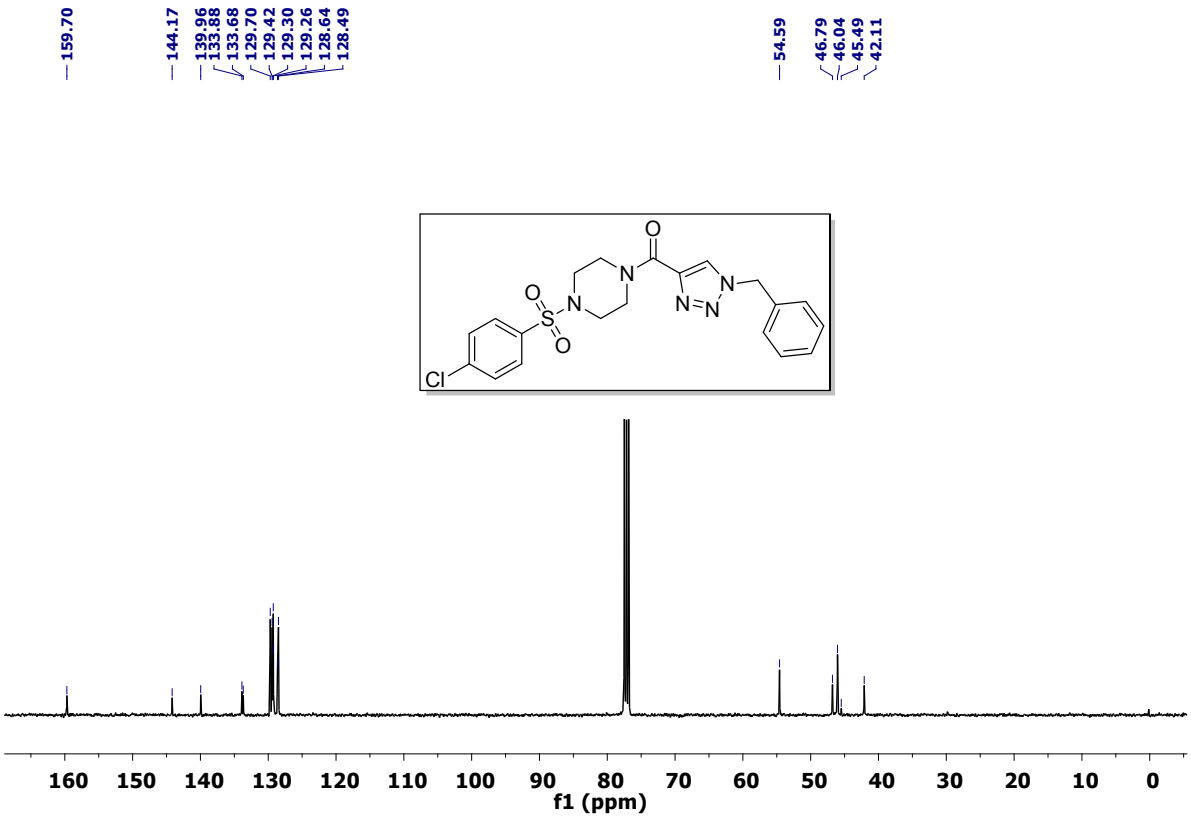
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ca**



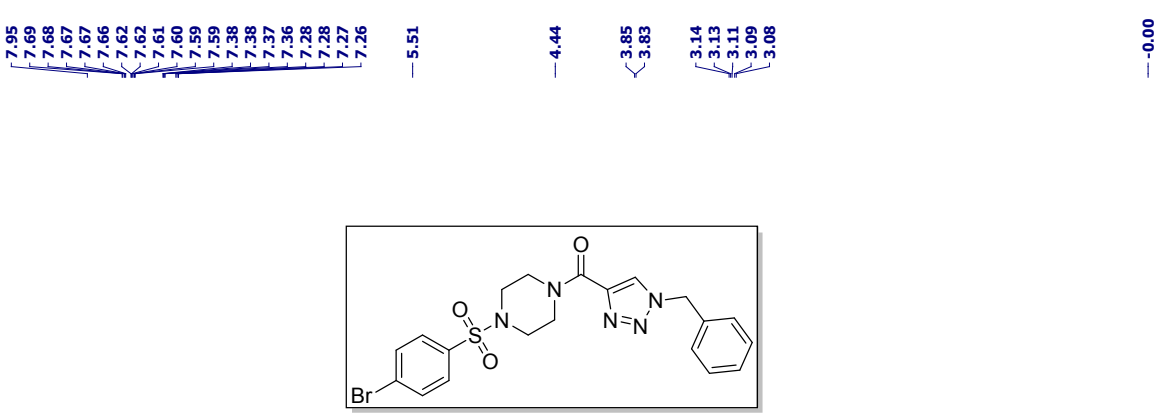


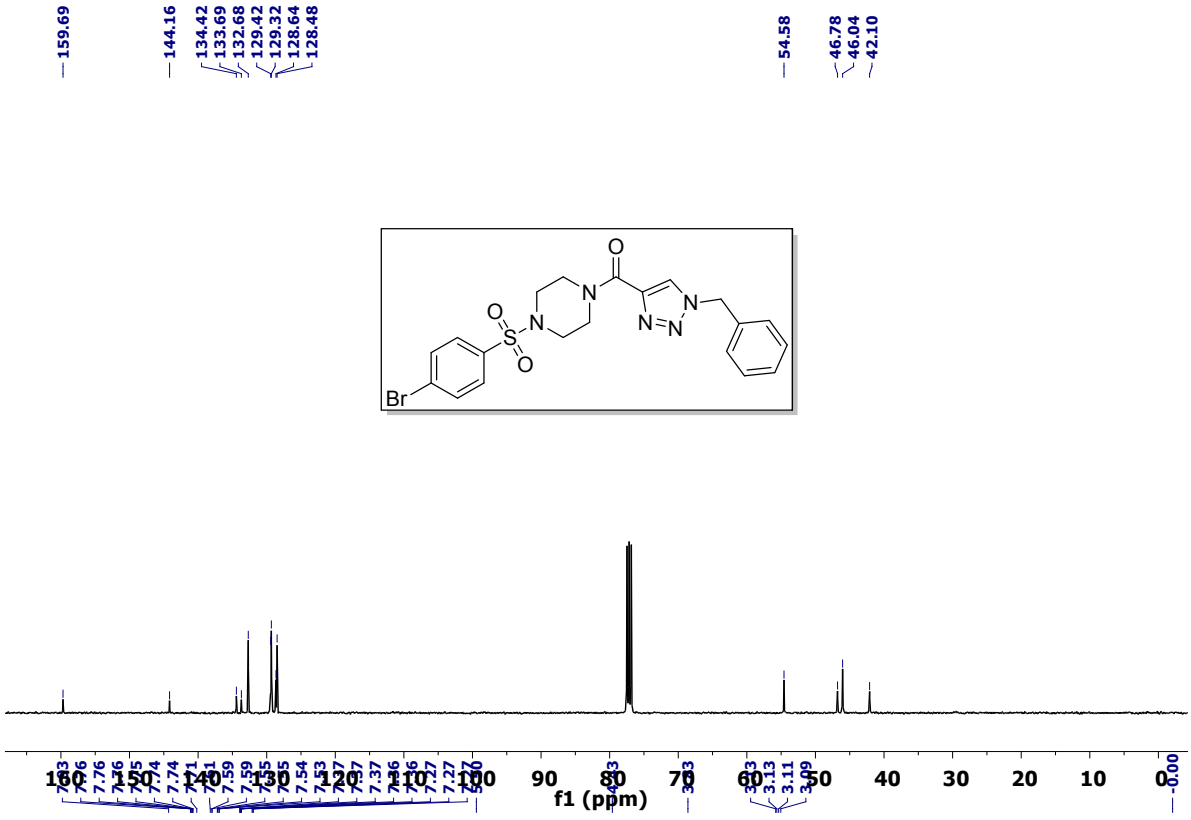
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10cb**



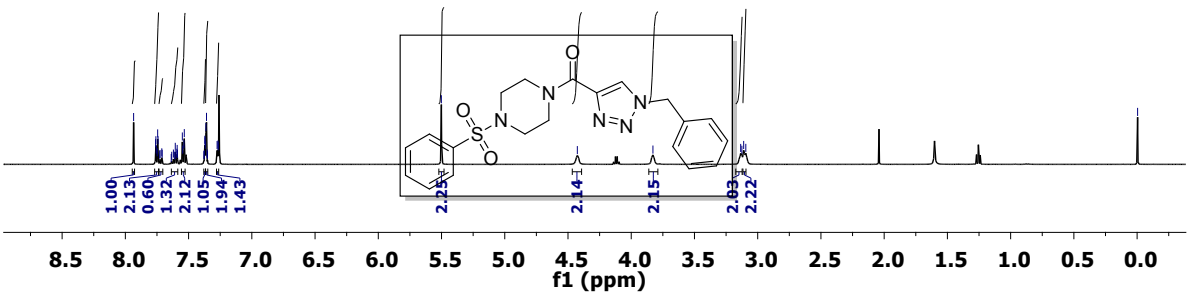


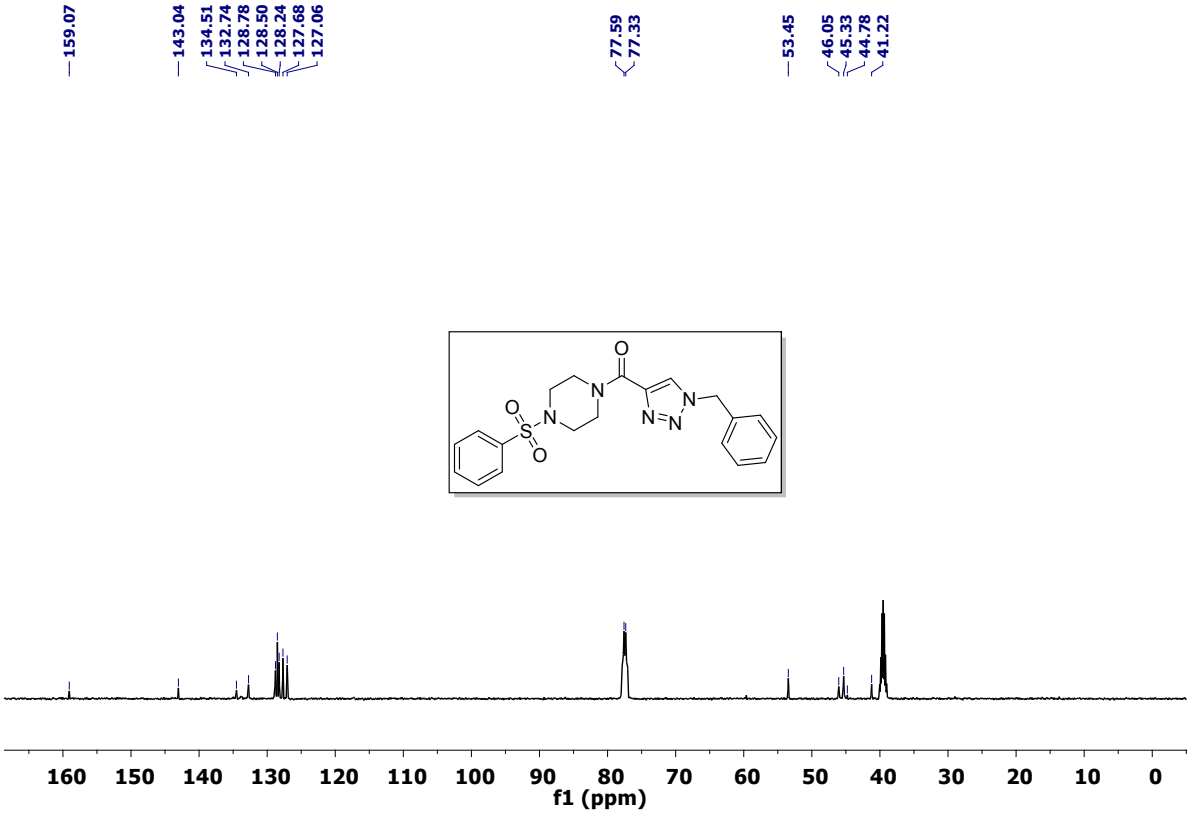
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10cc**



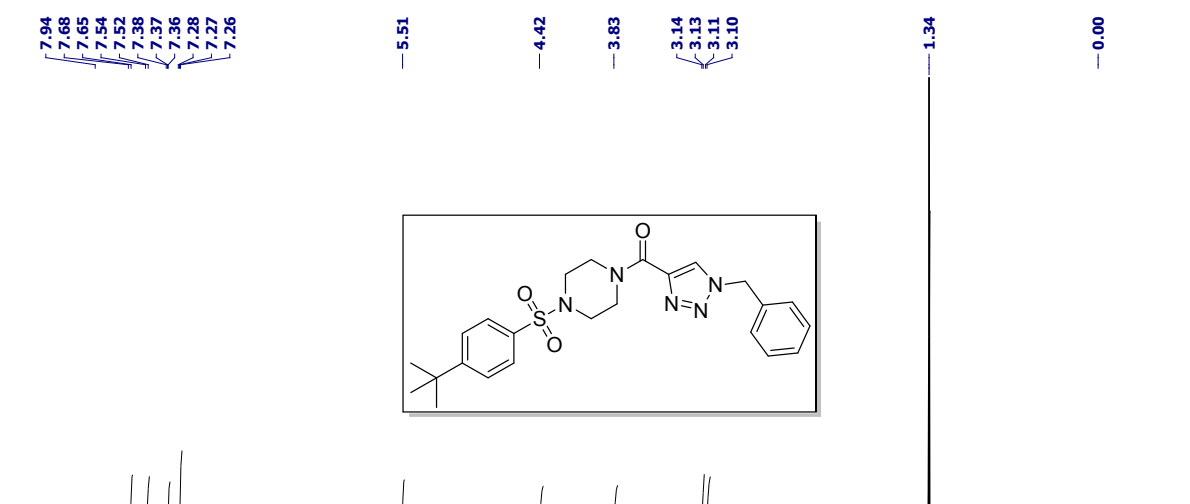


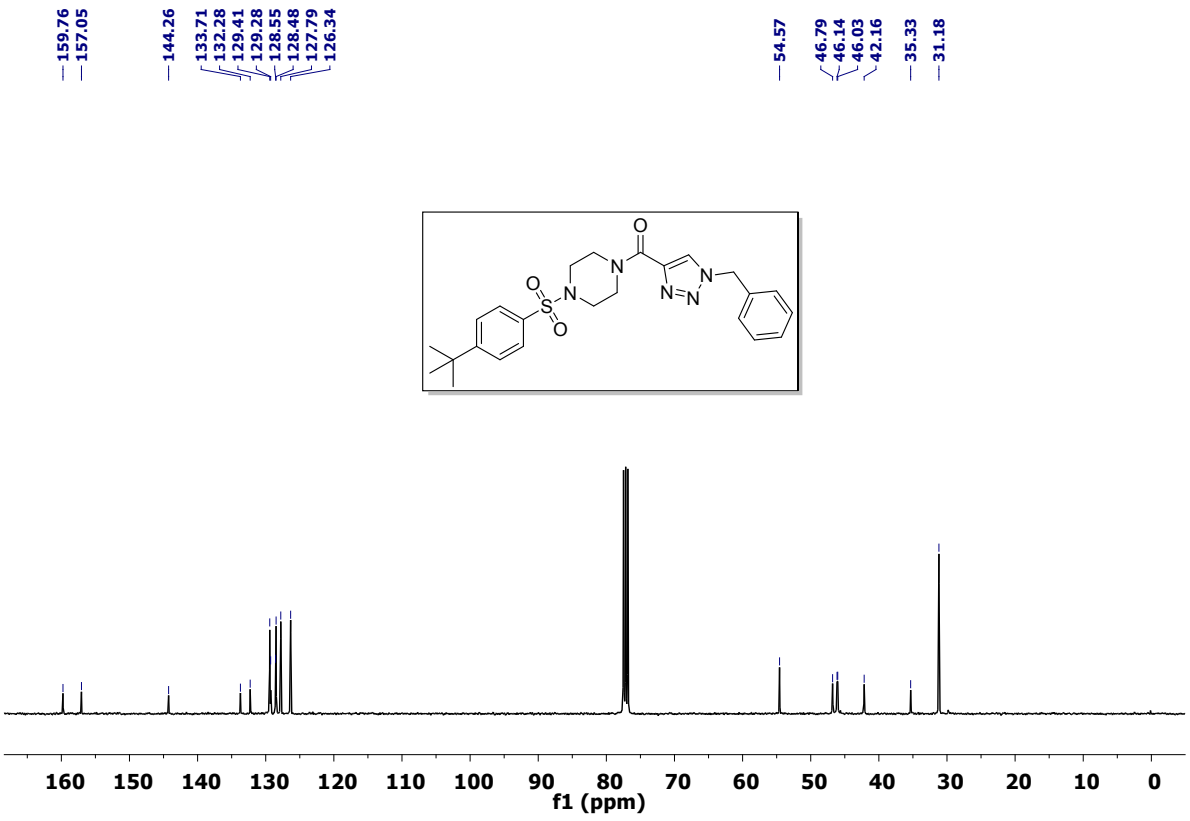
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10cd**



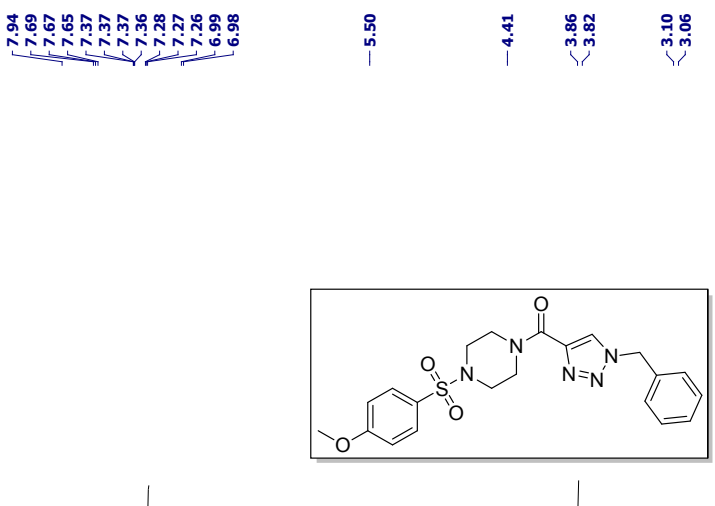


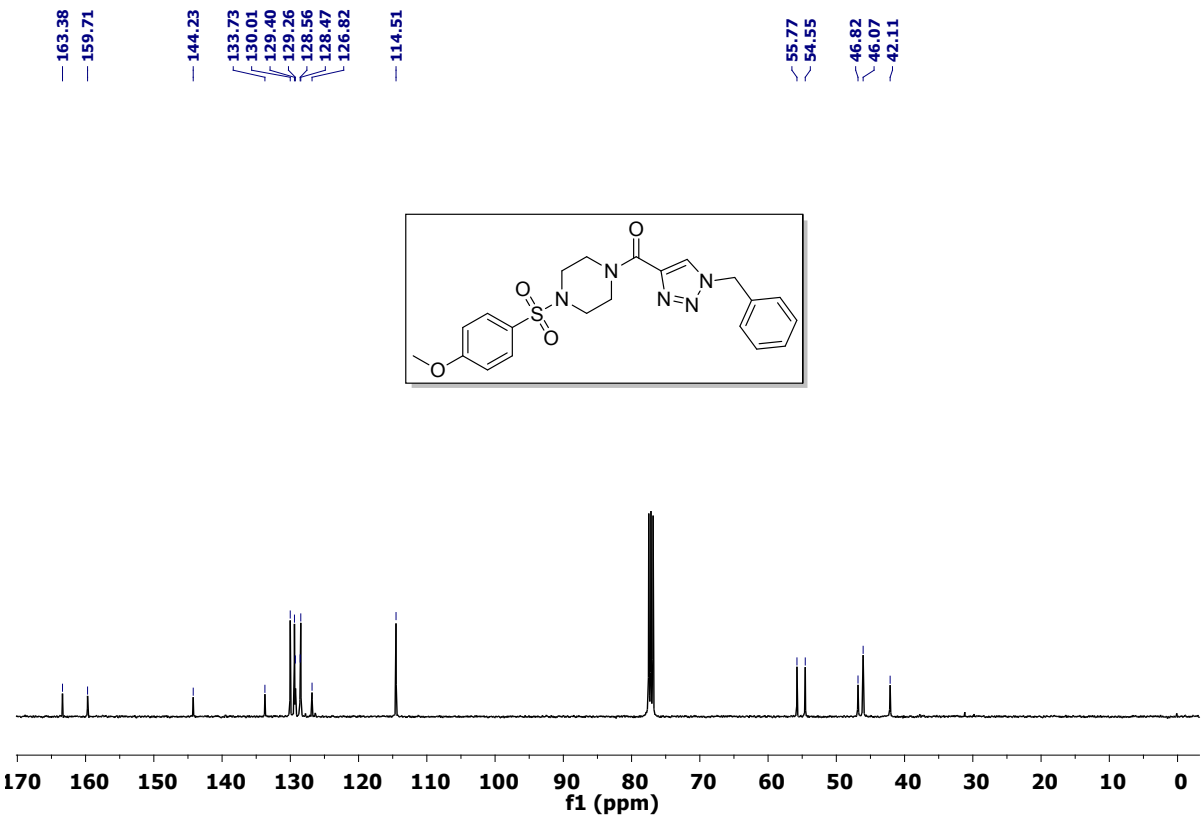
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ce**



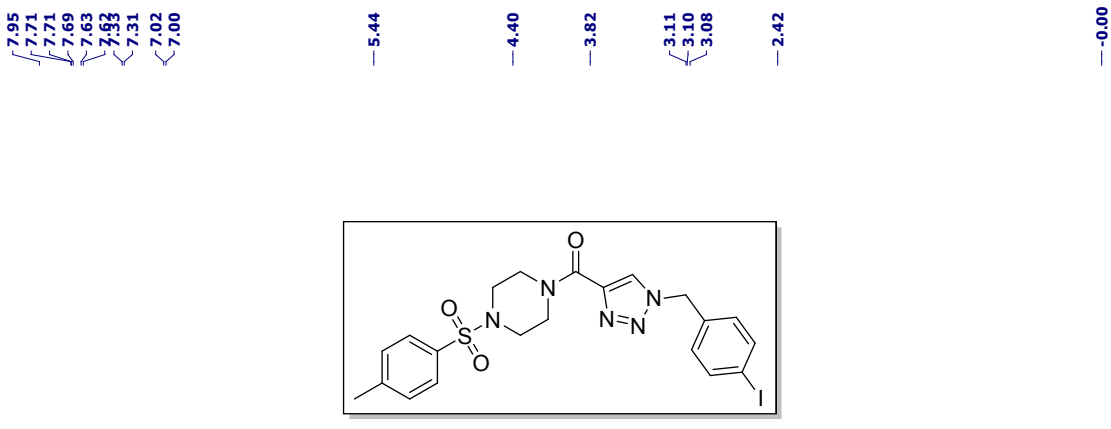


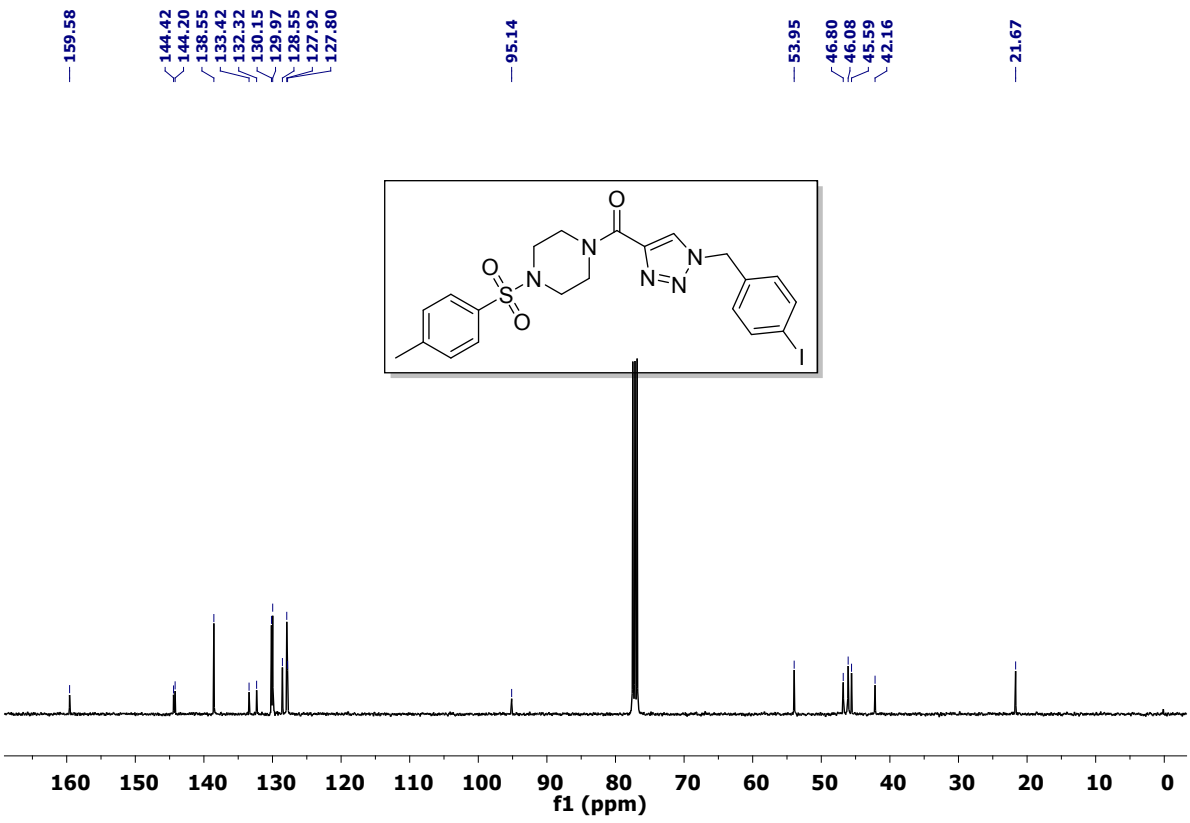
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10cf**



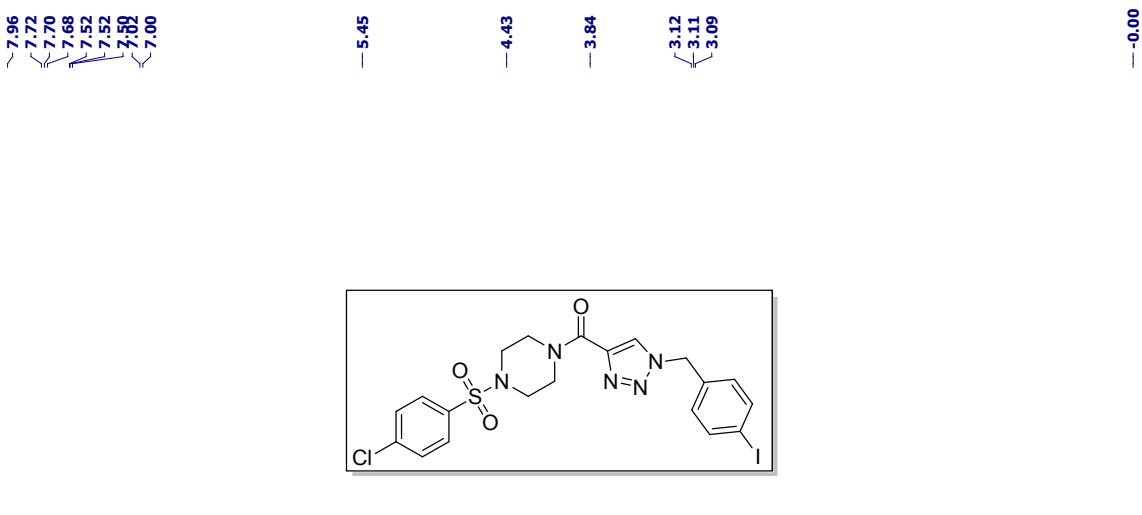


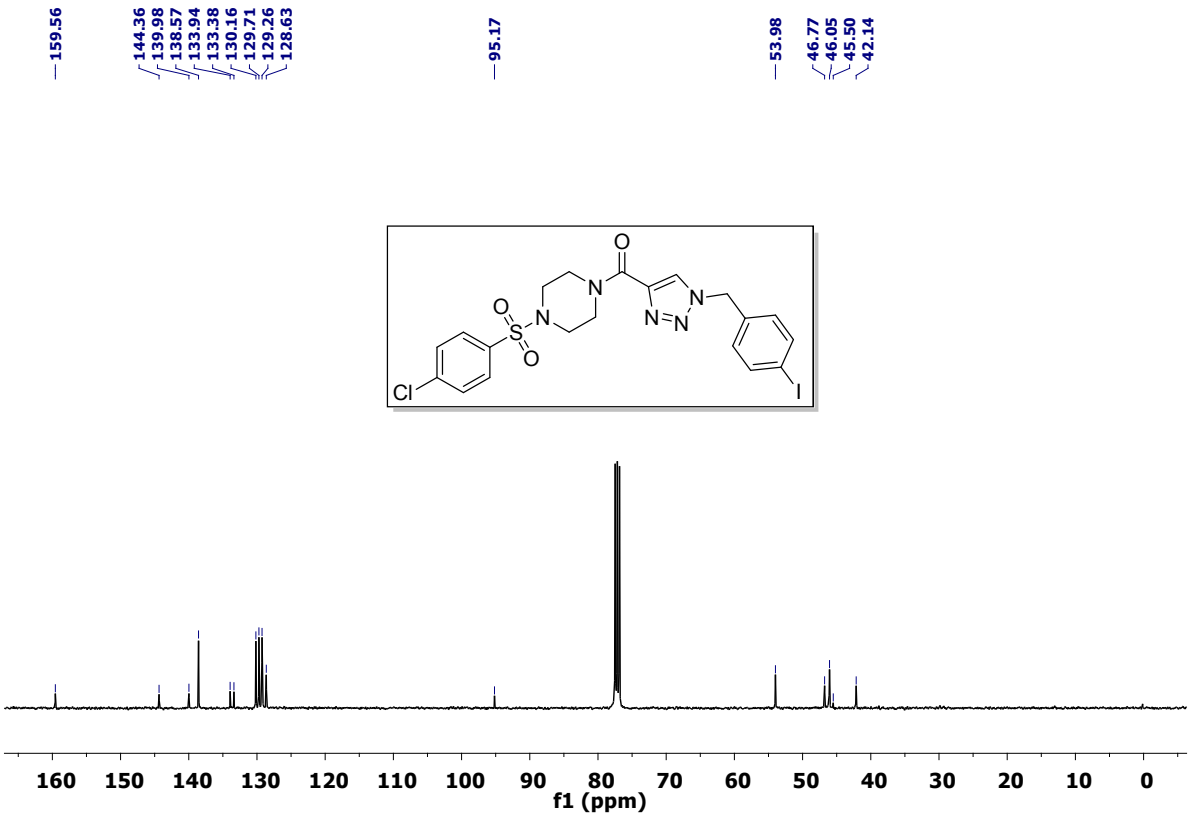
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10da**



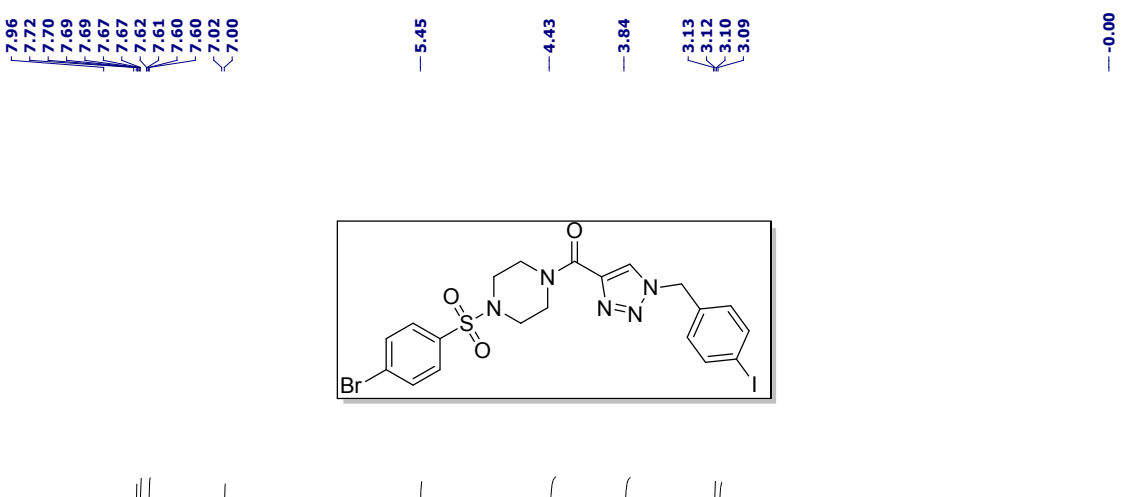


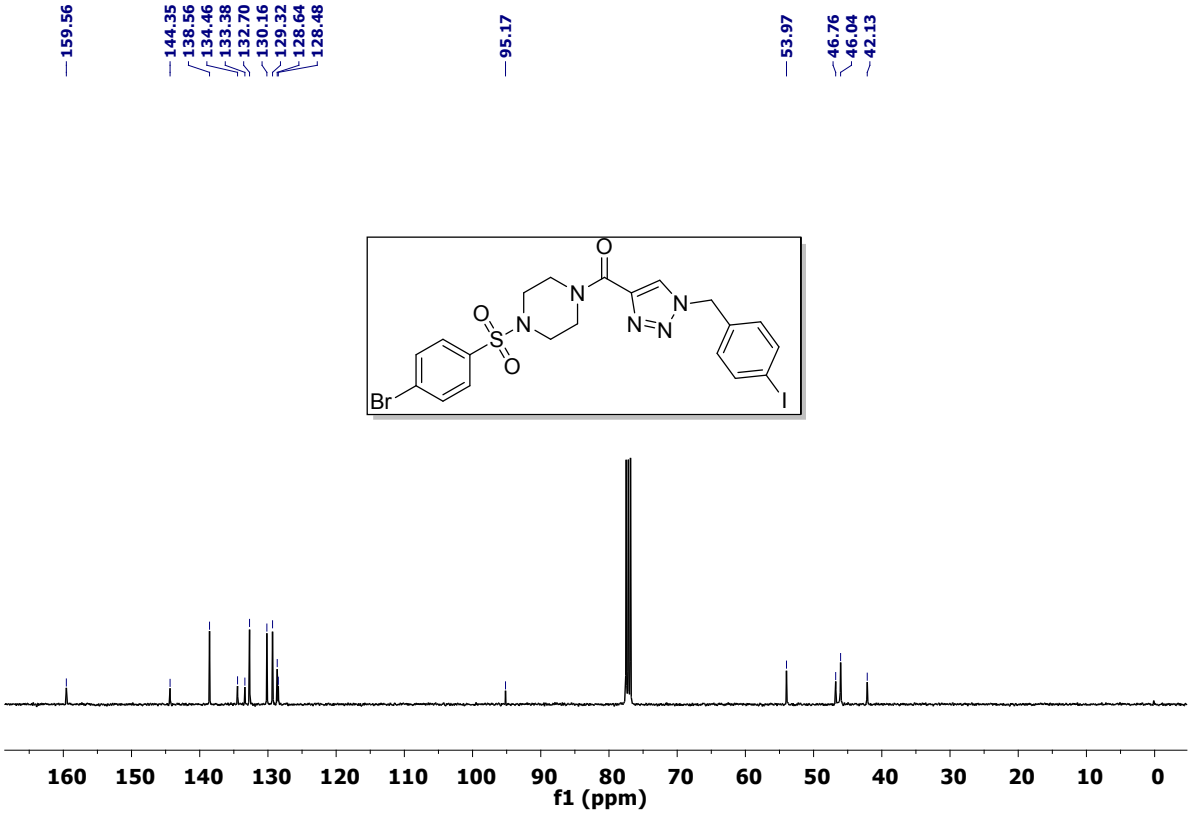
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10db**



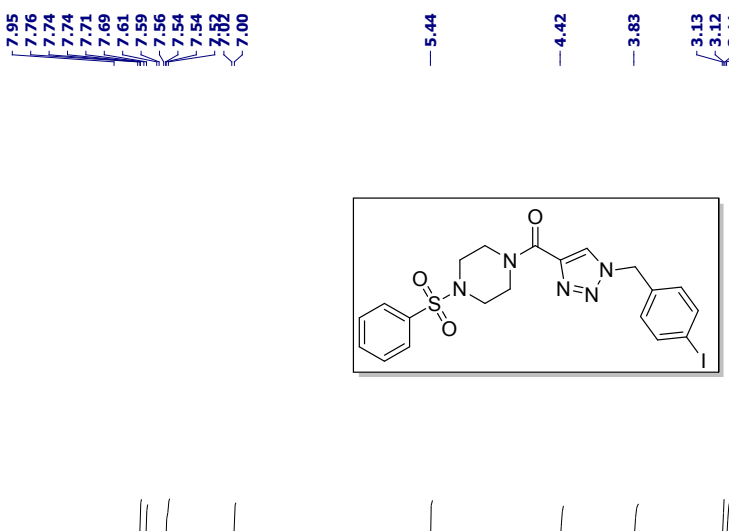


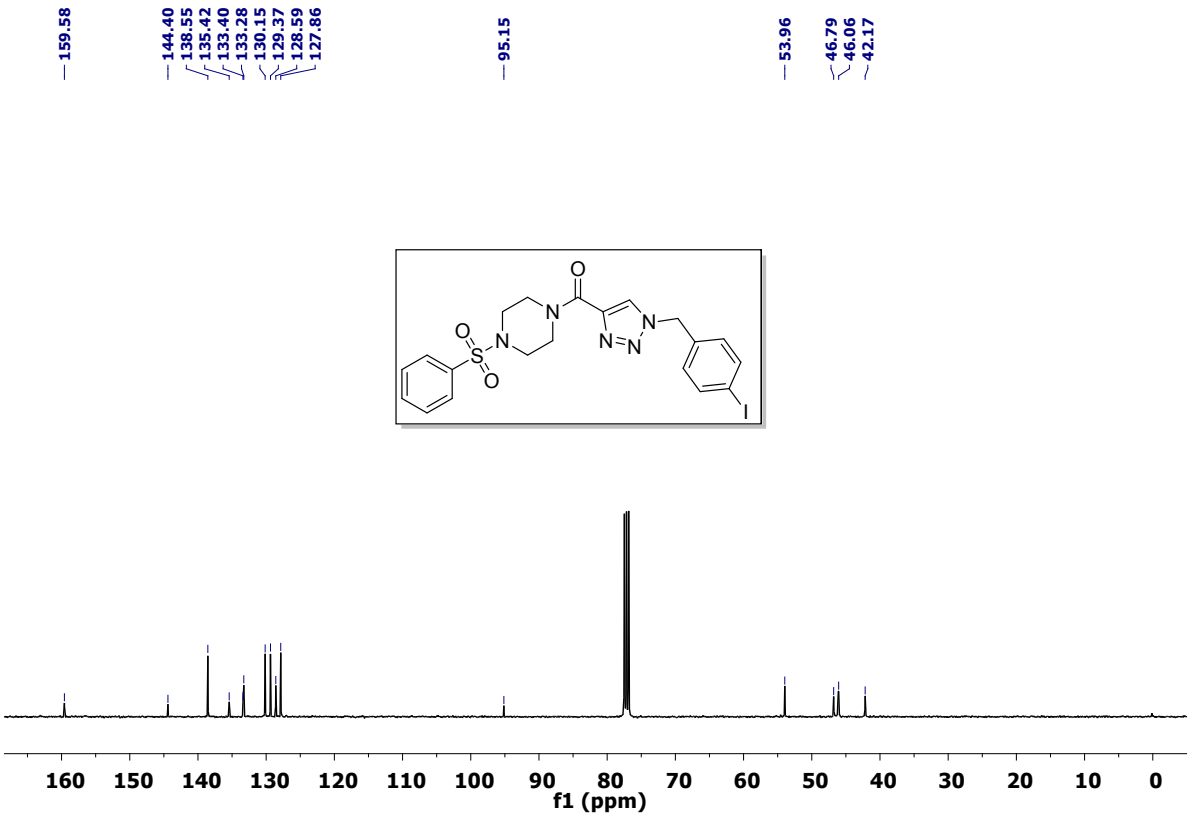
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10dc**



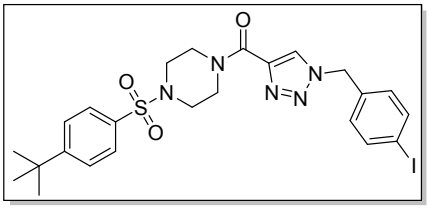


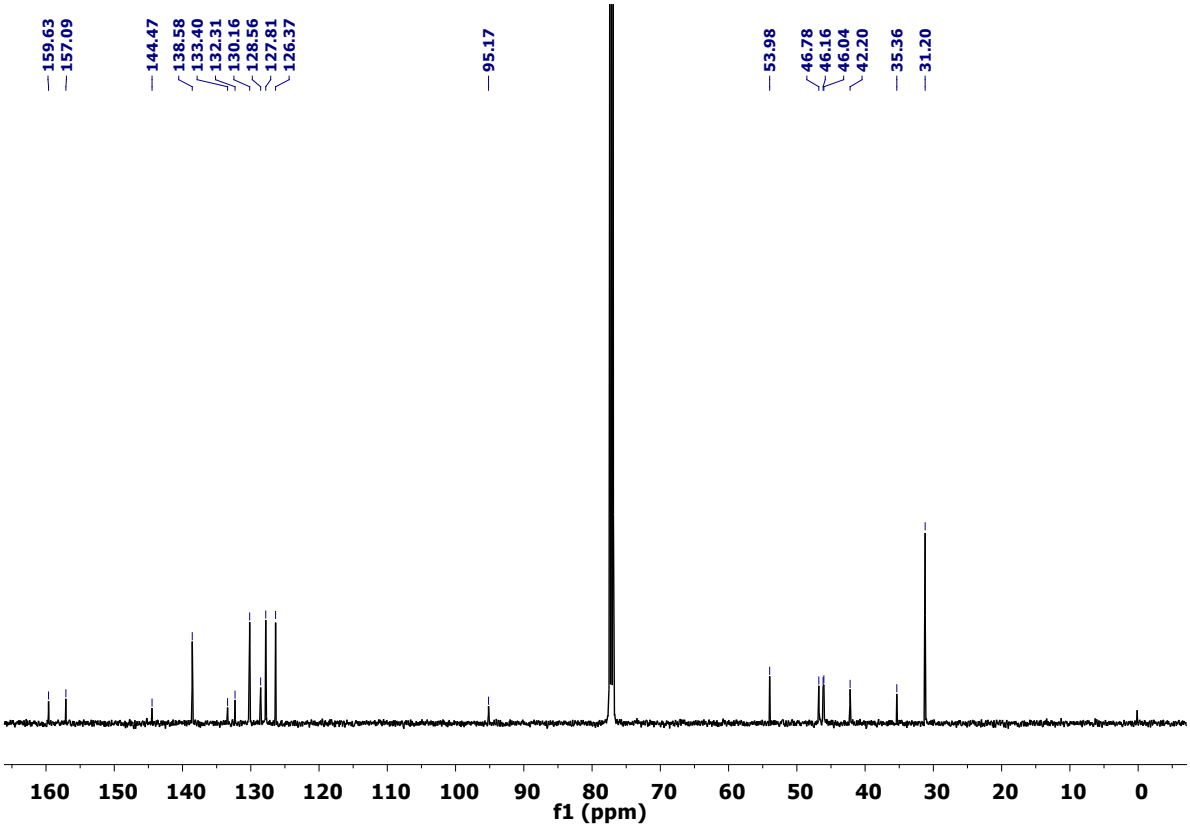
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10dd**



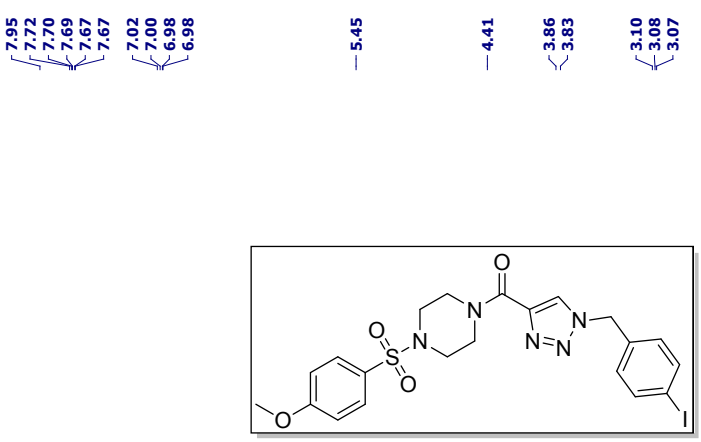


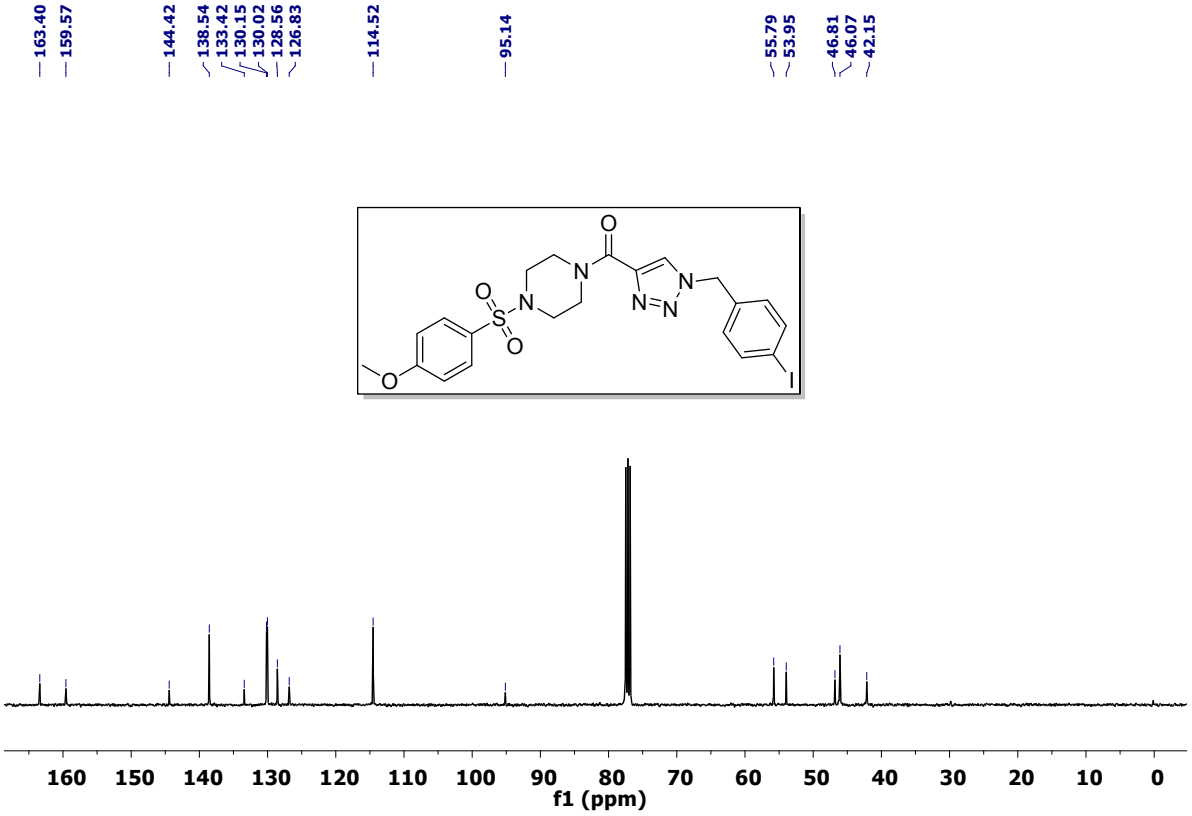
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10de**



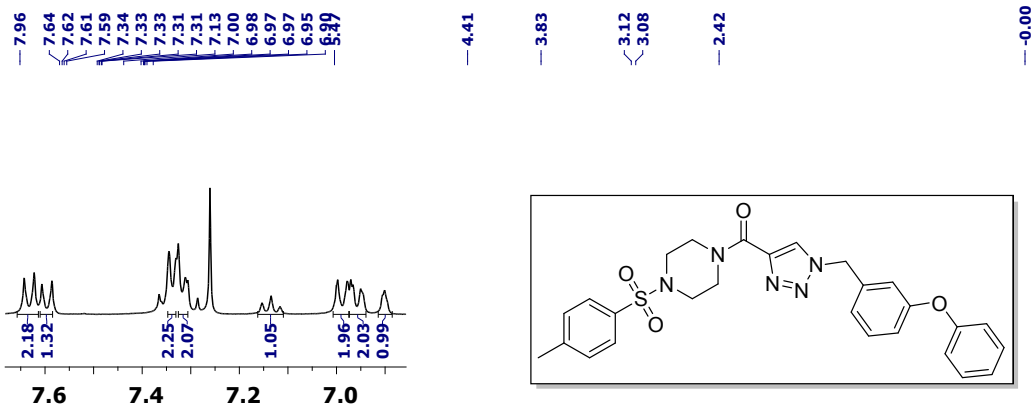


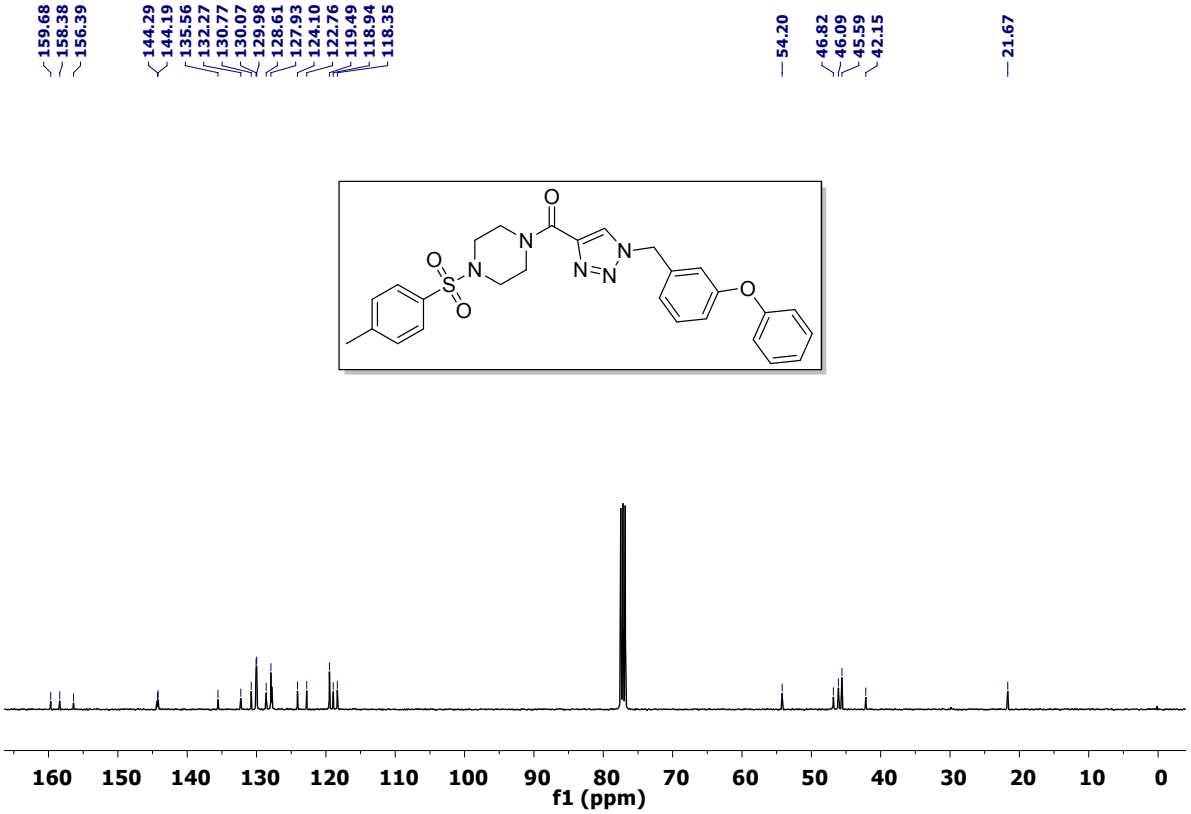
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10df**



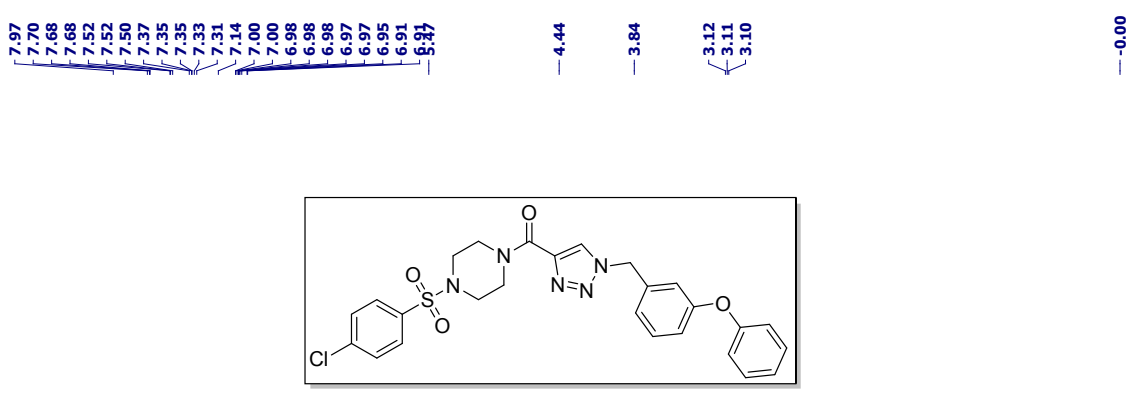


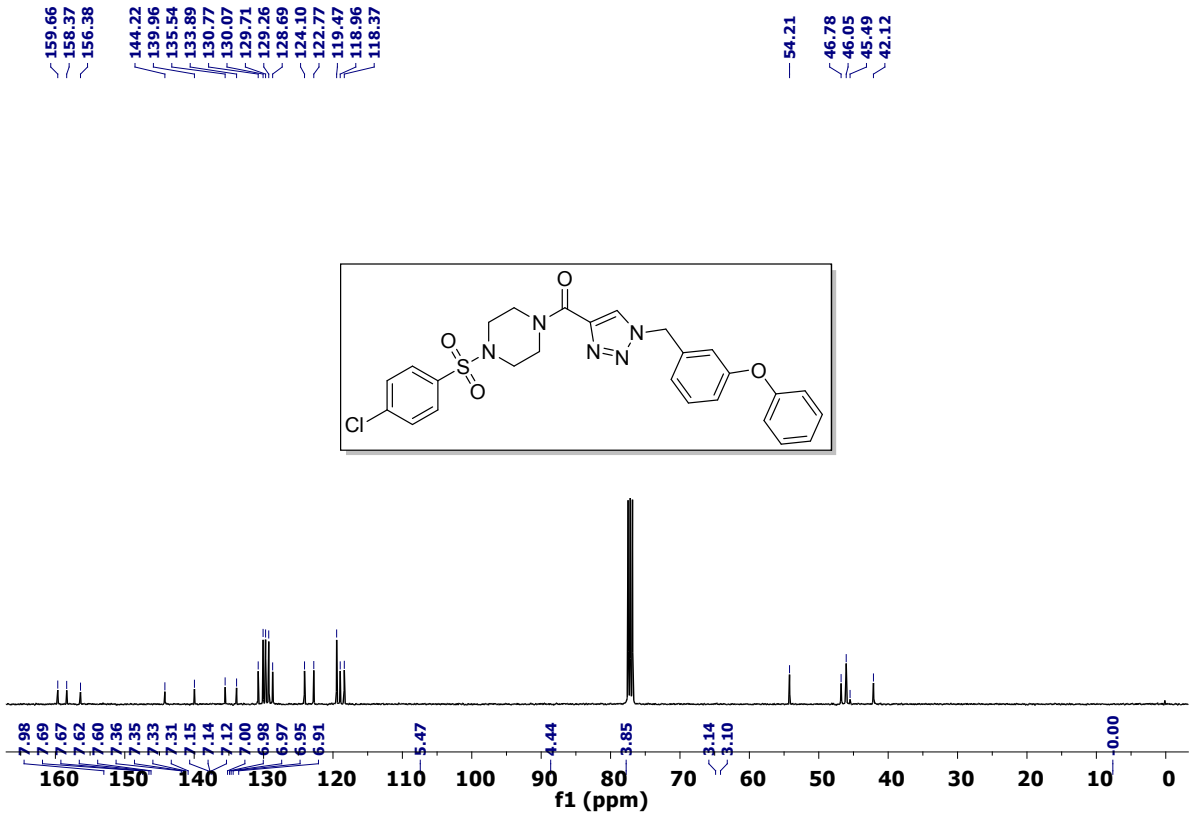
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ea**



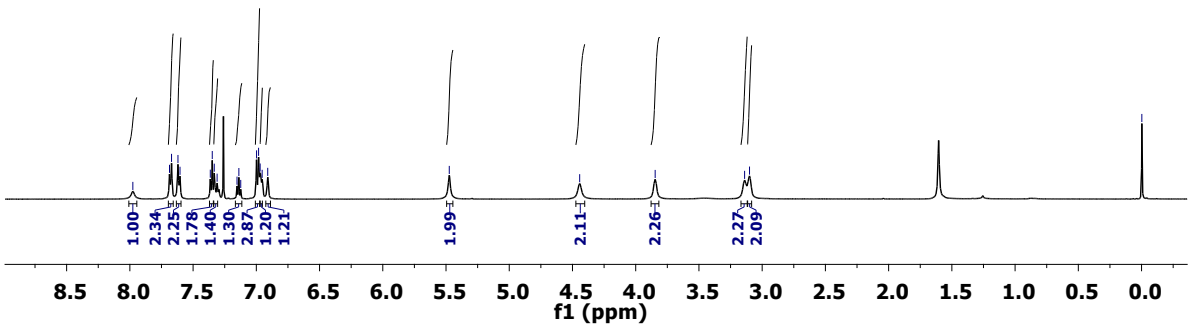


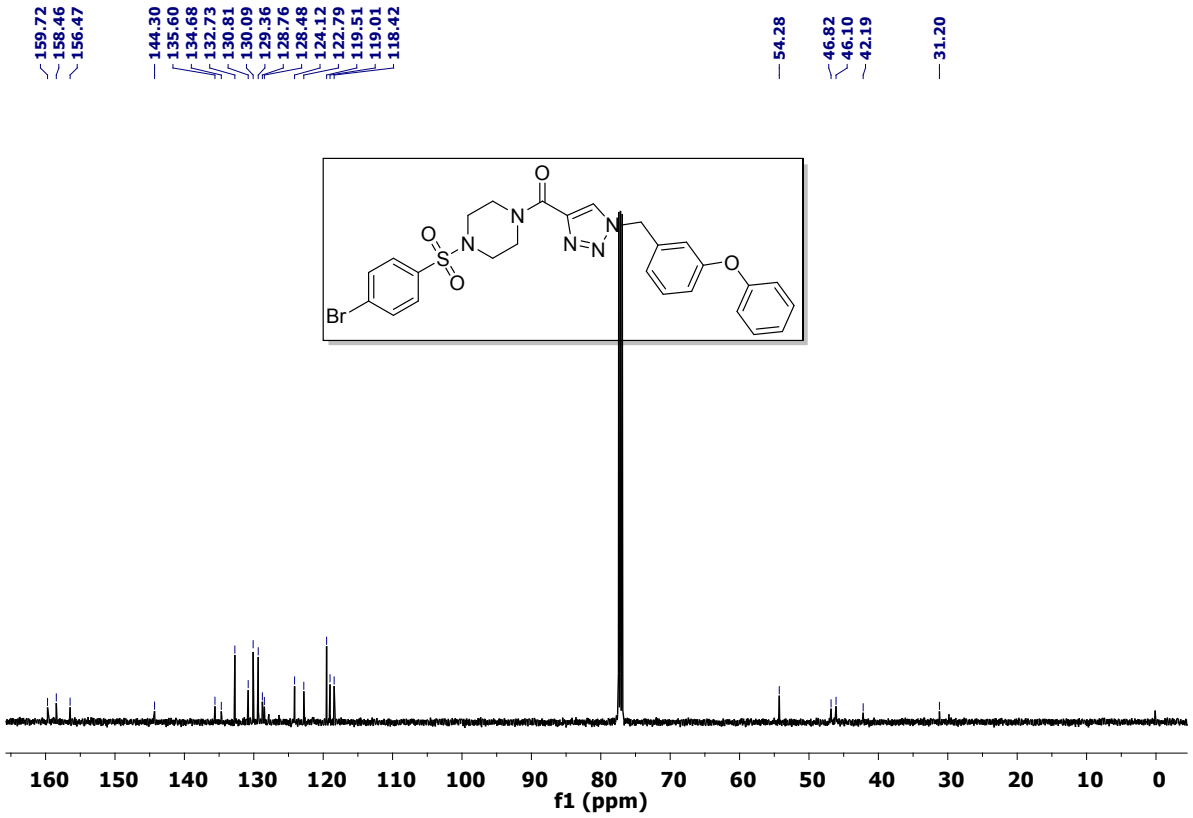
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10eb**



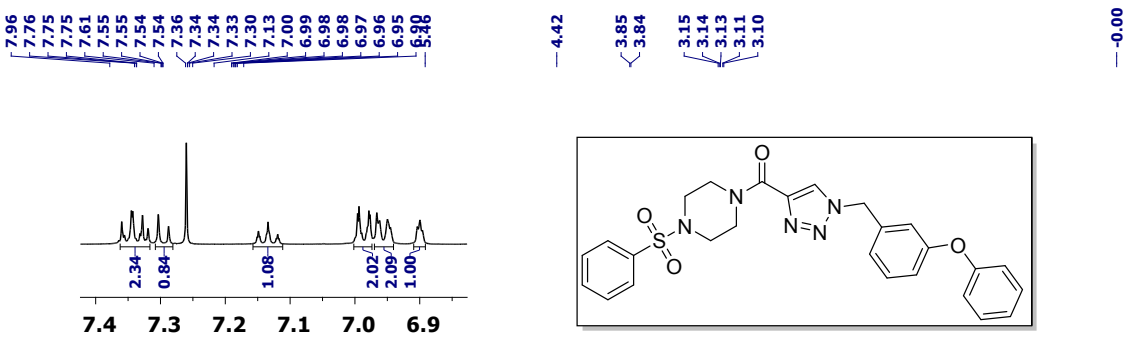


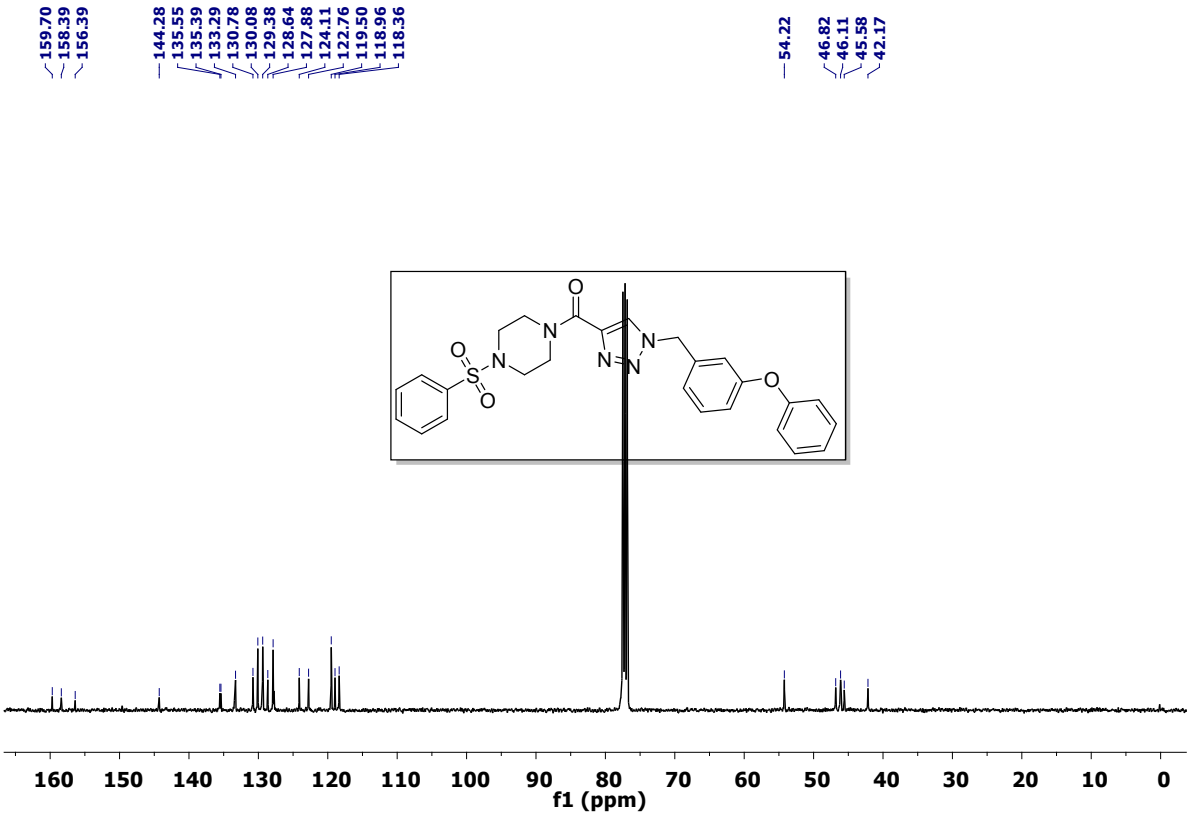
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ec**



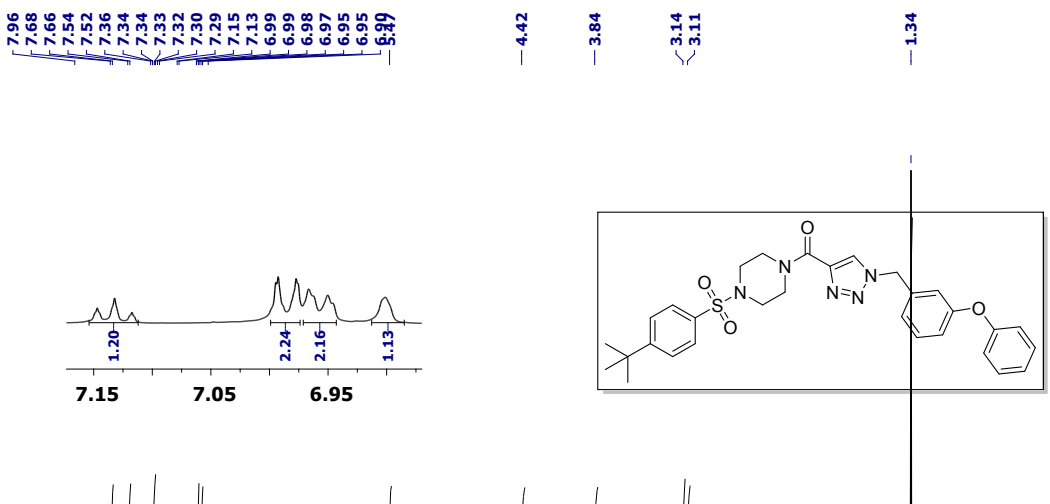


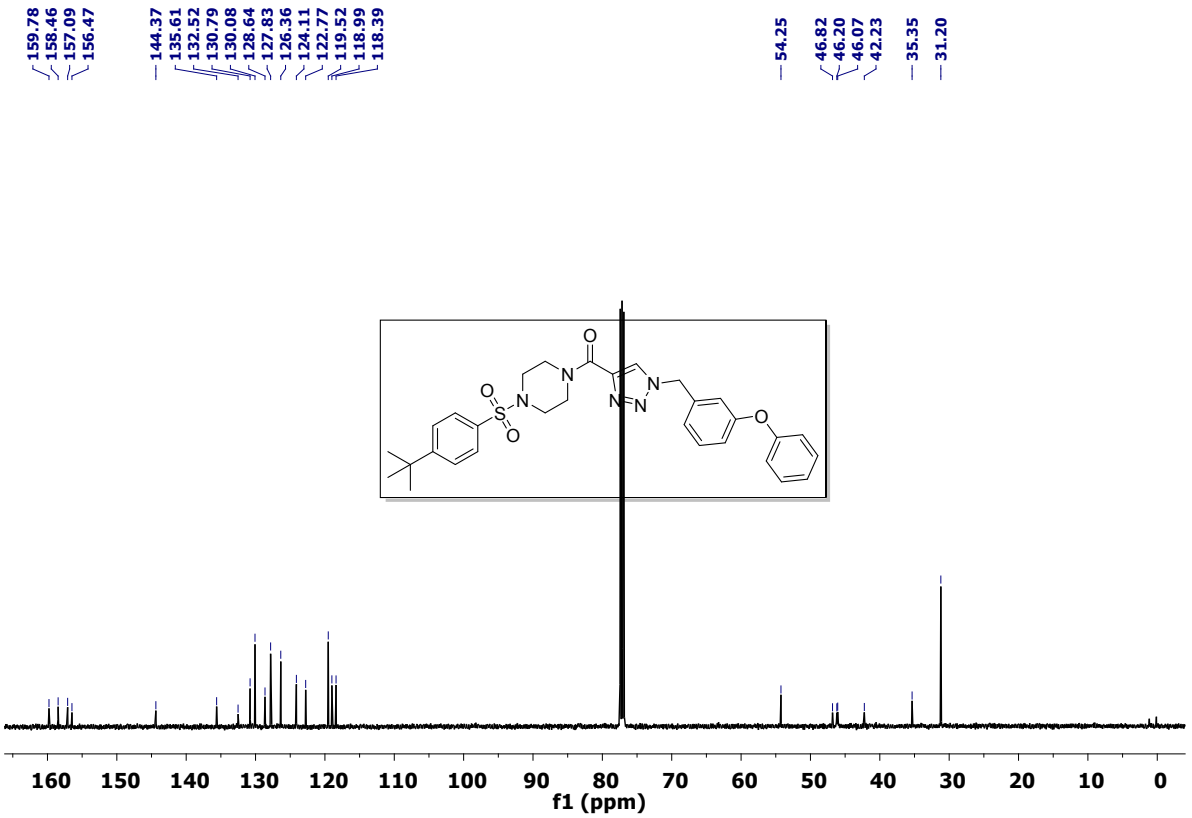
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ed**



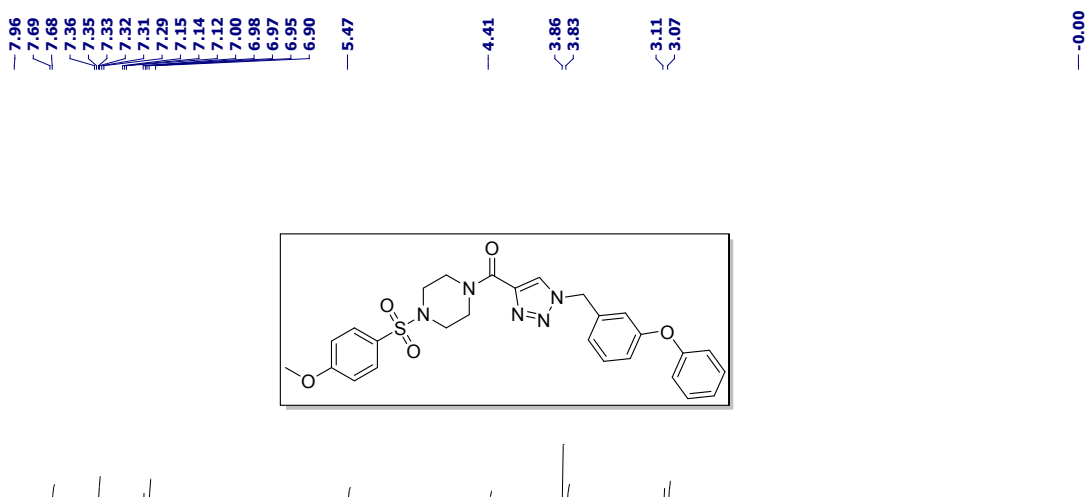


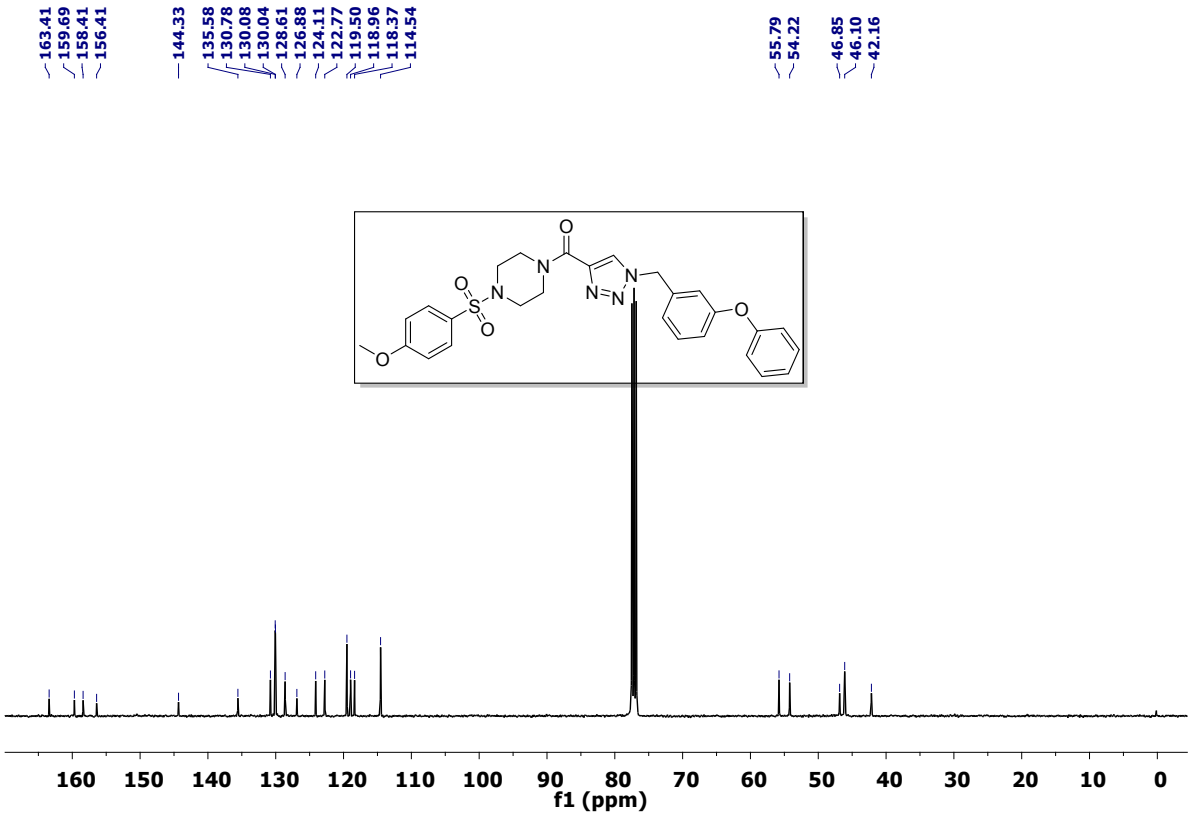
Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ee**





Copy of ^{1}H NMR and ^{13}C NMR spectra of **10ef**





Pharmacology

MTT Assay

The anticancer activity of all newly synthesized compounds was determined by using MTT ((3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl tetrazolium bromide) assay which measures the formation of formazon. The cells were seeded in 96 well plate at a density ranging from 3000-4500 per well. Then after 24 h, the tested compounds were initially dissolved in DMSO and made 20 mM stock. From the stock

solution, further dilutions were made in the respective medium and ensured that the final DMSO concentration is <0.1%. Then, the compounds were treated in a twofold serial dilution ranging from (0.625 to 20 μM. After 48 h of incubation, drugs containing media was removed and 100 μL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (0.5 mg/mL) was added to each well and the plates were further incubated for 4 h. Then, the supernatant from each well was carefully removed, formazon crystals were dissolved in 200 μL of DMSO and absorbance was recorded by using a spectrophotometric microtiter plate reader (Spectra Max, M4 Molecular devices, USA) at 570 nm wavelength.

Tubulin Polymerization Assay

Tubulin polymerization kit was purchased from Cytoskeleton, Inc. (BK011). To evaluate the effect of compound **10ec** on tubulin assembly, fluorescence based *in vitro* tubulin polymerization assay was conducted by following the manufacturer's protocol. The reaction mixture having 2 mg/mL tubulin in 80 mM PIPES at pH 6.9, 2.0 mM $MgCl_2$, 0.5 mM EGTA, 1.0 mM GTP and 10% glycerol in the presence and absence of **10ec** compound (1μM and 5 μM) was prepared. The sample without compound **10ec** is considered as control. Experiment was performed three times and its inhibition data has been included in the manuscript. Tubulin polymerization was followed by a time dependent increase in fluorescence due to the incorporation of a fluorescence reporter into microtubules as the polymerization proceeds. Fluorescence emission at 420 nm (excitation wavelength is 360 nm) was measured by using F-7000 fluorescence spectrofluorimeter from Hitachi. Fluorescence intensity measurement was recorded up to 80 min (at 5 min interval for each measurement) at 37 ^{o}C. Besides untreated sample, tubulin treated with 5 μM Paclitaxol and Nocodazole was also used as controls in the assay.

Immunohistochemistry Assay

Breast cancer cell line, BT-474 cells were seeded on glass cover slips and incubated for 48 h in the presence or absence of 2 μM concentration of test compound **10ec** and Combretastatin A-4 (**CA-4**), a known tubulin inhibitor. CA-4 is considered as a positive control in the present assay. After treatment, the cover slips were fixed with a paraformaldehyde solution (4% in 1×PBS) for 20 min at room temperature. Cell permeabilization was achieved by administration of a Triton X-100 solution (0.2% in 1×PBS) for 5 min. The cover slips were left in 100% MeOH overnight at 4 °C. Subsequently, the cover slips were blocked with a 1% bovine serum albumin (BSA) solution for 60 min and then incubated with α-tubulin antibody (1:1000) at room temperature for 2 h. The slides were washed three times for 5 min each with PBST. Next, the cover slips were incubated with FITC conjugated anti-mouse secondary antibody (Cell signaling technology) for 1 h and then washed three times with PBST solution. Finally, the cells were observed under

a confocal microscope with 20 µm magnification, and the pictures were analyzed for the integrity of the microtubule network.

Morphological observations using phase contrast microscope

BT-474 cancer cells were seeded in 12 well plates at a density 5×10^4 cells/well, after 24 h, cells were treated with **10ec** with increasing concentration (0.5, 1 and 2.5 µM). After 48 h treatment, cells were observed for the morphological changes and photographs were captured under a phase contrast microscope (Nikon, Inc. Japan).

Acridine orange - ethidium bromide (AO/EB) staining

BT-474 cells were seeded in 12 well plates at a density 5×10^4 cells/well after 24 h, cells were treated with **10ec** and the plates were incubated for 48 h in an atmosphere of 5% CO_2 at 37 ^oC. 10 µL of Acridine Orange (AO) and Ethidium Bromide (EB) dye were added into each well in equal volumes (10 µg/mL) respectively. Then the cells were visualized under fluorescence microscope (Nikon, Inc. Japan) at 200x magnification.

DAPI staining

BT-474 cells were seeded in 12 well plates at a density 5×10^4 cells/well after 24 h, cells were treated with **10ec** further cells are washed with PBS and permeabilized with 0.1% Tween 20 for 10 min and fixed with 4% paraformaldehyde followed by staining with 1 µM DAPI. Control and treated cells were observed with fluorescence microscope (Model: Nikon, Japan) with excitation at 359 nm and emission at 461 nm using DAPI filter at 200x magnification.

Clonogenic growth inhibition assay

BT-474 cancer cells were seeded in 6-well plates at a density 100 cells/well. After overnight incubation, cells were treated with or without **10ec** at determined concentrations for 12 h. After 12 h, medium was replaced with fresh media and the cells were kept at 37 °C in 5% CO_2 for 14 days, and the culture medium was changed every 2 days. After 7 days of incubation, the colonies were fixed and stained with 1% crystal violet in methanol for 3 h. The number of stained colonies was counted under chemdoc imaging system (Vilber Fusion Fx, France) and the software used was cSeries Capture Software, Azure Biosystems, Inc, USA. Colony formation was calculated as a percentage to untreated control cultures.

Cell cycle Analysis

1 x 10^5 BT-474 cells were seeded in 6 well plates after 24 h, cells were starved for 6 h for synchronization, then plain medium (without FBS) was replaced with complete medium (with FBS) and the cells are treated with increasing concentration (0.5, 1 and 2.5 µM) Untreated and treated cells were harvested, washed with phosphate buffered saline (PBS), fixed in ice-cold 70% ethanol, and stained with propidium iodide (50 µg/mL) with RNase A for 20 min at 37 ^oC in dark according to the manufacturer instructions and about 10,000 events are analyzed by using flow cytometry (BD accuri c6, USA).

Measurement of mitochondrial membrane potential

BT-474 cells (1x10^{6}cells/mL) were seeded in 6 well plates and allowed to adhere for overnight. The cells were incubated with increasing concentrations (0.5, 1 and 2.5 μM) of compound **10ec** for 48 h. Cells were collected and washed with PBS and resuspended in solution of JC-1 dye (1 μM) and incubated for 30 min in incubator at 37 ^{o}C. The cells were washed twice with PBS and cells were trypsinized, centrifuged and analysed by flow cytometer.

Annexin V binding assay

BT-474 cells were seeded in a 12 well plate with a density of 1×10^5 and treated with 0.5, 1 and 2.5 μM concentration of compound **10ec** for 48 h. Treated and untreated cells were stained with Annexin V-alexa flour 488 and Propidium Iodide (PI) using Dead cell apoptosis detection kit (Thermofisher Scientific, USA) and the samples were analyzed by flow cytometry.

Binding energy calculations for compound 10ec

MM/GBSA (Molecular mechanics/generalized born surface area) technology available in Prime module of Schrodinger software was employed to calculate ligand-binding energies based on docking complex. For this study, we have considered four major binding sites available in tubulin protein and their docked complexes of compound **10ec** i.e 1) Colchicine binding site/**10ec** complex 2) Laulimalide binding site/**10ec** complex 3) Taxane binding site/**10ec** complex and 4) Vinca binding site/**10ec** complex. Further, we have performed binding energy calculations of these complexes and the results are as depicted in **Table S1**. It was clearly observed that the compound **10ec** showed very good binding energy (-44.140 Kcal/mol) with colchicine binding site as compared to that of other binding sites of tubulin. From this study we are anticipating that compound **10ec** has the highest proximity of binding at the colchicine binding site.

Table S1. Binding energies (ΔG_{bind}) of compound **10ec** at various binding sites of tubulin protein.

Ligand ID	**Name of the Binding Site**	**PDB ID**	**Binding Energy (Kcal/mol)**
10ec	Colchicine	3E22	-44.140
	Laulimalide	4O4H	-41.054
	Taxane	1JFF	-36.641
	Vinca	5J2T	-22.205

Table 2 *In silico* physico chemical properties of selected compounds.

Physicochemical parameters

Compd.	clogp	nON	nOHNH	nrotb	MW	TPSA	No. of violations	clogS	Drug likeness
10db	3.69	8	0	5	571.83	88.41	1	-7.05	1.45
10dc	3.82	8	0	5	616.28	88.41	1	-7.24	1.14
10de	4.71	8	0	6	593.49	88.41	1	-7.99	1.32
10eb	4.33	9	0	7	538.03	97.64	1	-7.23	1.53
10ec	4.46	9	0	7	582.48	97.64	1	-7.42	1.25
10ee	5.36	9	0	8	559.69	97.64	2	-8.17	1.14
10ef	3.71	10	0	8	533.61	106.88	1	-6.53	1.29
Nocodazole	2.79	6	2	4	301.33	84.09	0	-4.41	0.94

Table 3 GLIDE docking results for compound **10ec** at the active site of α/β-tubulin (PDB ID: 3E22).

S.No	Ligand id	Docking score	Interactions	
			H-bonds	Hydrophobic
1	**10ec**	-7.844	Ser178, Ala250, Lys254	Val177, Ala180, Tyr224, Cys241, Leu248, Ala250, Leu252, Leu255, Ala316, Ala317, Val318, Met325, Ala354, Val355, Ile378

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