

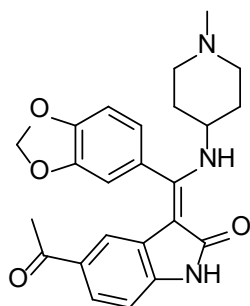


GSK-3 Inhibitor | BI-5521

Synthesis of BI-5521 (Patent No. US 2005/0203104)

The compound numbers mentioned herein are a reference to the numbering system employed in: Gollner A., Heine C., Hofbauer K. S. Kinase Degradors, Activators, and Inhibitors: Highlights and Synthesis Routes to the Chemical Probes on [opnMe.com](https://opnme.com), Part 1. *ChemMedChem* **2023**, 18, e202300031. DOI: [10.1002/cmdc.202300031](https://doi.org/10.1002/cmdc.202300031), [PubMed](https://pubmed.ncbi.nlm.nih.gov/).

BI-5521 (Compound 43)

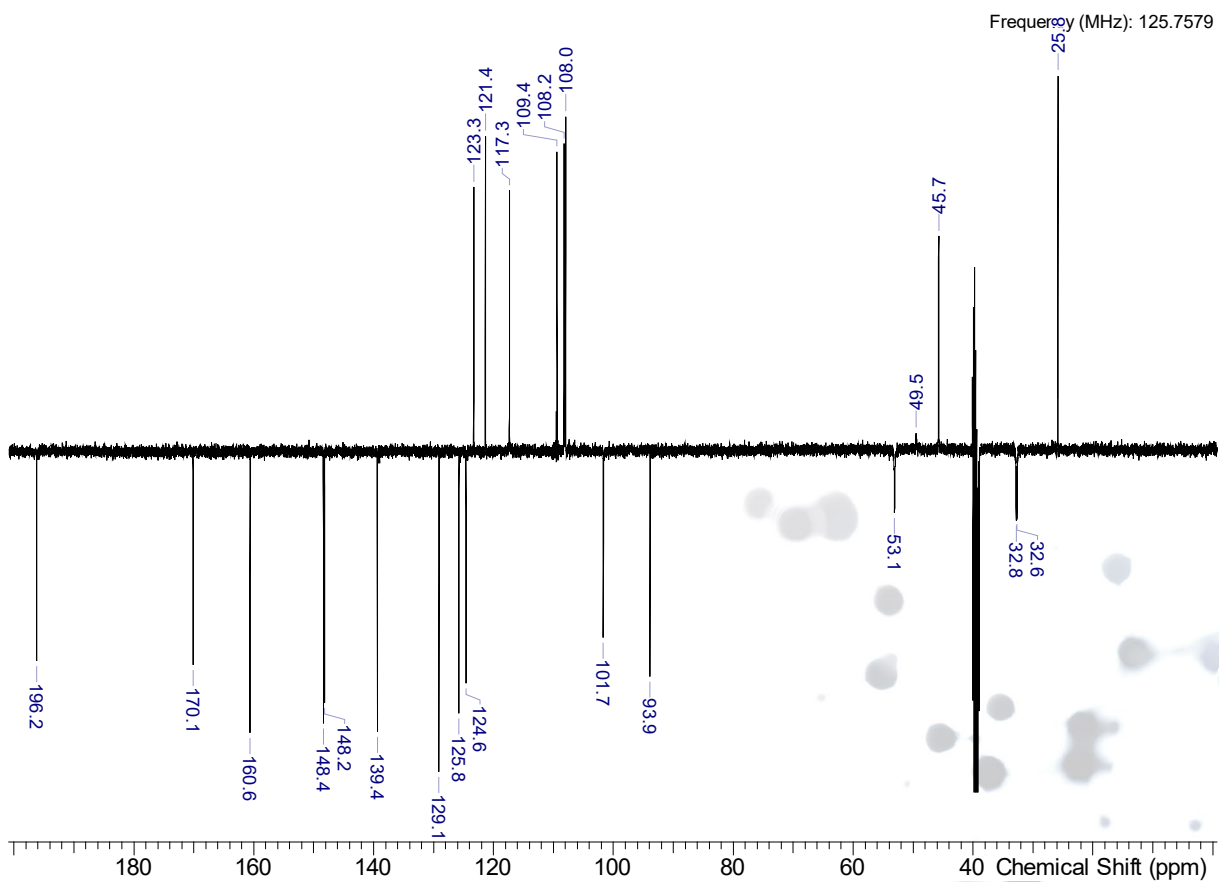
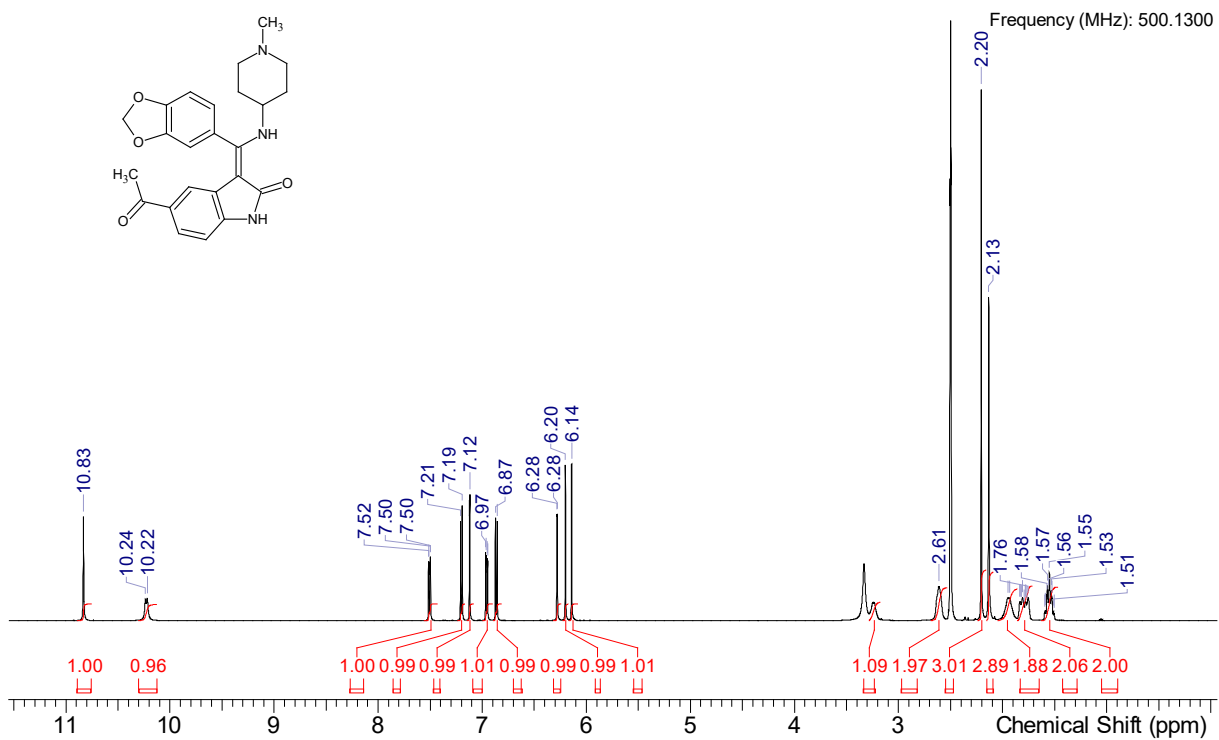


5 g (13.2 mmol) 1,5-diacetyl-3-[2H-1,3-benzodioxol-5-yl(methoxy)methylidene]indol-2-one were suspended in 50 ml of dimethylformamide and stirred overnight at ambient temperature with 1.5 g (13.2 mmol) 4-amino-N-methylpiperidine. The acetyl-protected intermediate product was combined with 2 ml of conc. ammonia without purification and stirred at ambient temperature for 30 min. Then the mixture was evaporated and the residue was chromatographed through a silica gel column with the eluant methylene chloride/methanol 4:1. A total of 4.8 g (86%) was obtained.

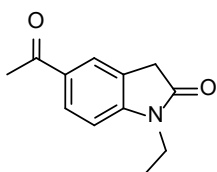
^1H NMR (DMSO- d_6 , 500 MHz) δ 10.83 (s, 1H), 10.23 (br d, 1H, $J=9.1$ Hz), 7.51 (dd, 1H, $J=1.9$, 8.2 Hz), 7.20 (d, 1H, $J=7.9$ Hz), 7.12 (d, 1H, $J=1.6$ Hz), 6.96 (dd, 1H, $J=1.6$, 7.9 Hz), 6.86 (d, 1H, $J=8.2$ Hz), 6.28 (d, 1H, $J=1.3$ Hz), 6.20 (s, 1H), 6.14 (s, 1H), 3.2-3.3 (m, 1H), 2.5-2.7 (m, 2H), 2.20 (s, 3H), 2.13 (s, 3H), 1.94 (br d, 2H, $J=7.9$ Hz), 1.7-1.9 (m, 2H), 1.5-1.6 (m, 2H);

^{13}C NMR (DMSO- d_6 , 125 MHz) δ 196.2, 170.1, 160.6, 148.4, 148.2, 139.4, 129.1, 125.8, 124.6, 123.3, 121.4, 117.3, 109.4, 108.2, 108.0, 101.7, 93.9, 53.1, 49.5, 45.7, 32.8, 32.6, 25.8;

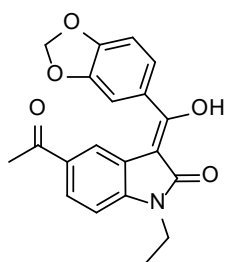
HRMS (m/z): $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{24}\text{H}_{25}\text{N}_3\text{O}_4$, 420.19178; found, 420.19256;



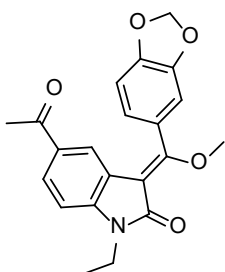
GSK-3 Inhibitor Negative Control, BI-4481 (Compound 44)



10.71 g (0.14 mol) Acetyl chloride was added drop wise to a stirred suspension of 33 g (0.25 mol) anhydrous AlCl₃ in 520 ml carbon disulfide. Stirring was allowed to continue at room temperature for 15 min. 20 g (0.12 mol) 1-ethyl-3H-indol-2-one was then added, and the mixture was brought to reflux for 2 h, then cooled on ice. After the solvent was decanted, the remaining thick brown residue was treated with ice cold water. The resulting precipitate was recovered by filtration, washed with water, and dried under vacuum to provide pure xox1 as a white solid. Yield: 15 g (59% of theory).



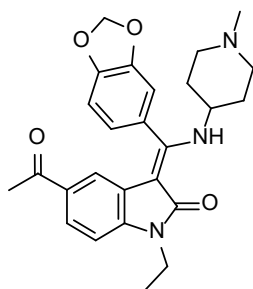
To a solution of 5.0 g (25 mmol) 5-acetyl-1-ethyl-3H-indol-2-one, 4.09 g (25 mmol) piperonylic acid, 8.7 g (27 mmol) TBTU (O-benzotriazol-1-yl-N,N',N'- tetramethyluronium tetrafluoroborate) and 7.6 ml (54 mmol) triethylamine in 50 ml DMF (dimethylformamide), 2.13 g (89 mmol) sodium hydride were added at 0°C. The reaction mixture was stirred overnight at ambient temperature, then acidified (pH~5-6) with acetic acid. The precipitate was filtered off and dried. The compound was purified by column chromatography (DCM/MeOH) and required compound eluted at 2% MeOH/DCM. Yield: 3.0g (34% of theory).



3.5 g (10 mmol) 5-acetyl-3-[2H-1,3-benzodioxol-5-yl(hydroxy)methylidene]-1-ethylindol-2-one were suspended in 35 ml methylene chloride and combined with 2.93 g (20 mmol) trimethyloxonium

tetrafluoroborate as well as 5.2 ml (30 mmol) DIPEA (ethyl-diisopropylamine) at ambient temperature. The solution was stirred overnight at ambient temperature. The reaction mixture was washed three times with water, the organic phase was dried over sodium sulphate, filtered and concentrated by rotary evaporation. Yield: 3.5 g (96% of theory).

BI-4481

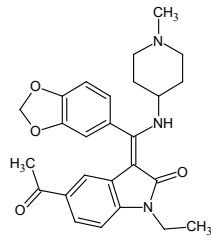


To a solution of 3 g (8.2 mmol) 5-acetyl-3-[2H-1,3-benzodioxol-5-yl(methoxy)methylidene]-1-ethylindol-2-one in 30 ml of dimethylformamide, 1.03g 4-amino-N-methylpiperidine was added the reaction mixture was stirred overnight at ambient temperature. Then the mixture was concentrated and the residue was chromatographed through a silica gel column with the eluant methylene chloride/methanol 90:10. Yield: 1.33 g (36% of theory) of **BI-4481**.

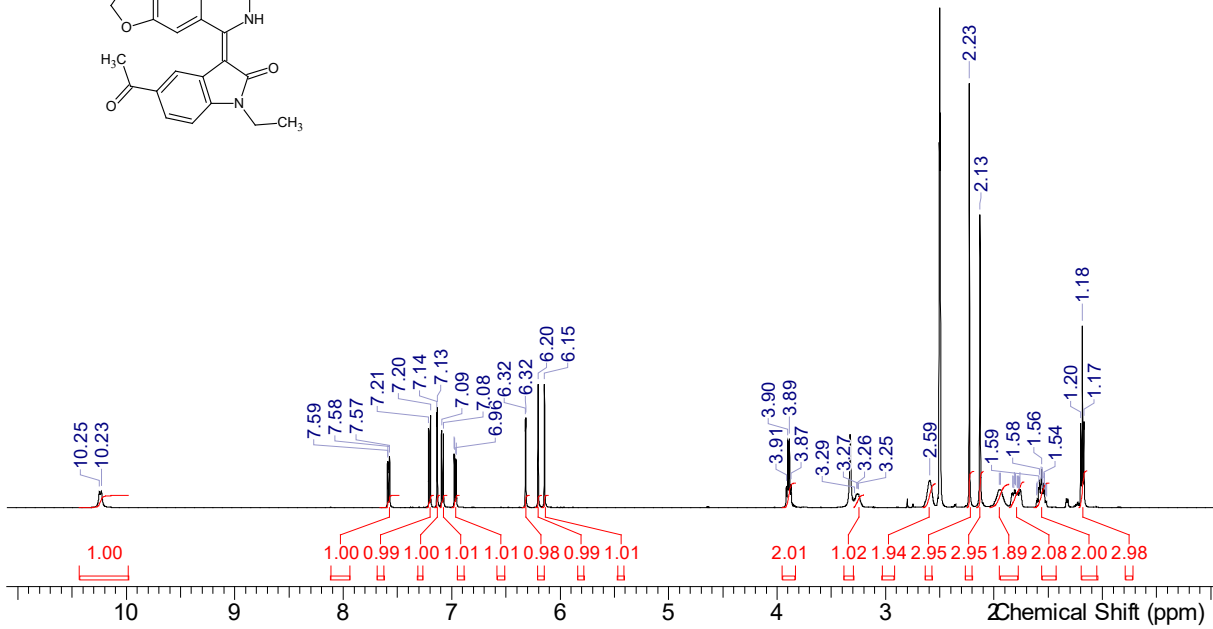
^1H NMR (DMSO- d_6 , 500 MHz) δ 10.24 (br d, 1H, $J=9.1$ Hz), 7.58 (dd, 1H, $J=1.6, 8.2$ Hz), 7.20 (d, 1H, $J=7.9$ Hz), 7.13 (d, 1H, $J=1.6$ Hz), 7.09 (d, 1H, $J=8.2$ Hz), 6.97 (dd, 1H, $J=1.6, 7.9$ Hz), 6.32 (d, 1H, $J=1.3$ Hz), 6.20 (s, 1H), 6.15 (s, 1H), 3.89 (q, 2H, $J=7.1$ Hz), 3.2-3.3 (m, 1H), 2.5-2.7 (m, 2H), 2.23 (s, 3H), 2.13 (s, 3H), 1.9-2.0 (m, 2H, $J=7.3$ Hz), 1.7-1.9 (m, 2H), 1.5-1.6 (m, 2H), 1.18 (t, 3H, $J=7.1$ Hz);

^{13}C NMR (DMSO- d_6 , 125 MHz) δ 196.3, 167.9, 160.7, 148.5, 148.2, 139.3, 129.4, 125.6, 123.8, 123.3, 121.4, 117.2, 109.4, 108.0, 107.0, 101.7, 93.0, 53.0, 49.5, 45.8, 33.6, 32.8, 32.6, 25.9, 13.4;

HRMS (m/z): $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{26}\text{H}_{29}\text{N}_3\text{O}_4$, 448.22308; found, 448.22381;



Frequency (MHz): 500.1300



Frequency (MHz): 125.7579

