Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


*Supporting Information*

# Discovery of fluorescent 3-heteroarylcoumarin derivatives as novel inhibitors of anaplastic lymphoma kinase

*Shinmee Mah, Jaebong Jang, Daesun Song, Yoongje Shin, Muhammad Latif, Yongwon Jung* and Sungwoo Hong**

*Department of Chemistry, Korea Advance Institute of Science and Technology (KAIST), Daejeon 34141, Korea*
*Center for Catalytic Hydrocarbon Functionalizations, Institute of Basic Science (IBS), Daejeon 34141, Korea*

**Enzymatic assay.**

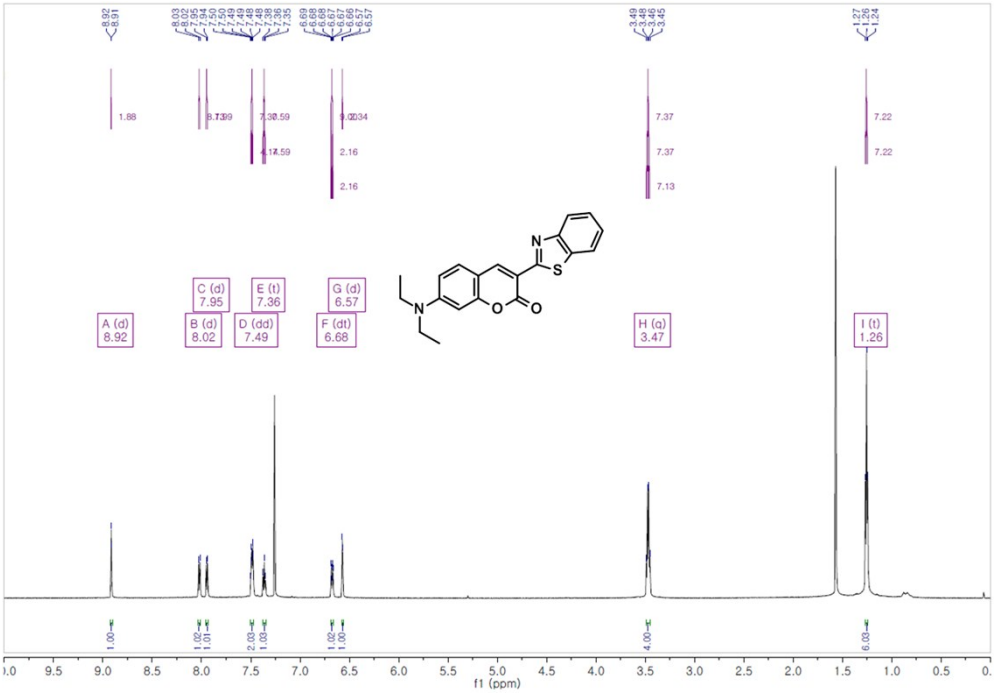
The biochemical potencies of the inhibitors were measured with the radiometric kinase assays ([γ-$^{33}$P]-ATP) at Reaction Biology Corp. (Malvern, PA, USA) using staurosporine as the positive control, which represented the ATP-competitive inhibitors with low-nanomolar activity. Briefly, the enzymatic reaction mixtures contain Human ALK, an artificial peptide substrate, poly[Glu:Tyr] (4:1), in freshly prepared base reaction buffer (20 mM HEPES of pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij-35, 0.02 mg/ml BSA, 0.1 mM $Na_3VO_4$, 2 mM DTT, 1% DMSO). Compounds were delivered into the substrate solution and gently mixed. Each putative inhibitor in 100% DMSO with the indicated concentrations was then delivered to the reaction buffer using the acoustic dispensing system (Echo550; nanoliter range). To initiate the enzymatic reaction, $^{33}$P-ATP with a specific activity of 10 μCi/μL was added to the reaction mixture, which was followed by further incubation of the mixture for 2 h at room temperature. Radioactivity was then monitored by the filter-binding method after reactions were spotted onto P81 ion exchange paper and the filters were washed extensively in 0.75% phosphoric acid. Aurora kinase activity data were expressed as the percent remaining activity in test samples compared to the vehicle (dimethyl sulfoxide) reaction. Finally, IC50 values were calculated based on curve fitting using the PRISM program (GraphPad Software) at 10 different concentrations.

**Cell growth assay.** Ba/F3 EML4-ALK WT (EA WT) were plated at a density of 4,000 cells/well in 96 well plates and incubated for 24 h. The medium was then removed, and cells were treated with different concentrations of compounds for 72 h. Next, 20 μL of MTT solution (2 mg/mL) was added for 4 h at 37 ºC in 5% $CO_2$ incubator. After the media was then removed, 200 μL of DMSO was added to each well, and the plate was incubated for an additional 4 h at 37°C. The formazan crystals that formed in metabolically healthy cells were dissolved in DMSO (200 μL/well) with constant shaking for 30 min. Absorbance of the solution was then measured with a microplate reader at 540 nm.

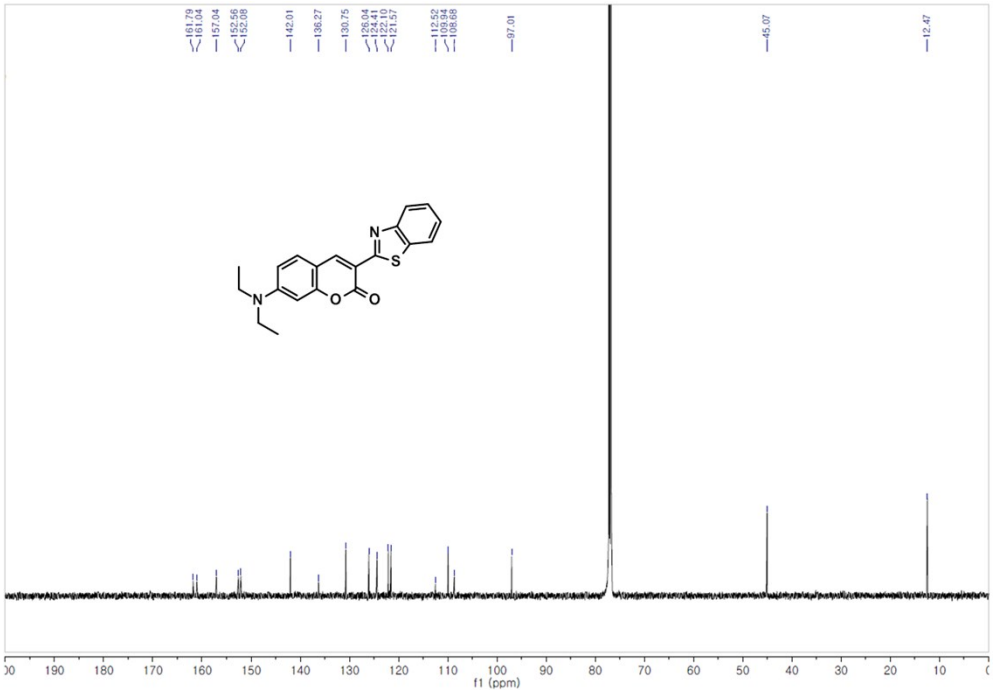
# Spectral Copies of $^{1}H$ and $^{13}C$ NMR Data

# Obtained in this study

**3-(1,3-Benzothiazol-2-yl)-7-(diethylamino)chromen-2-one (3h)**

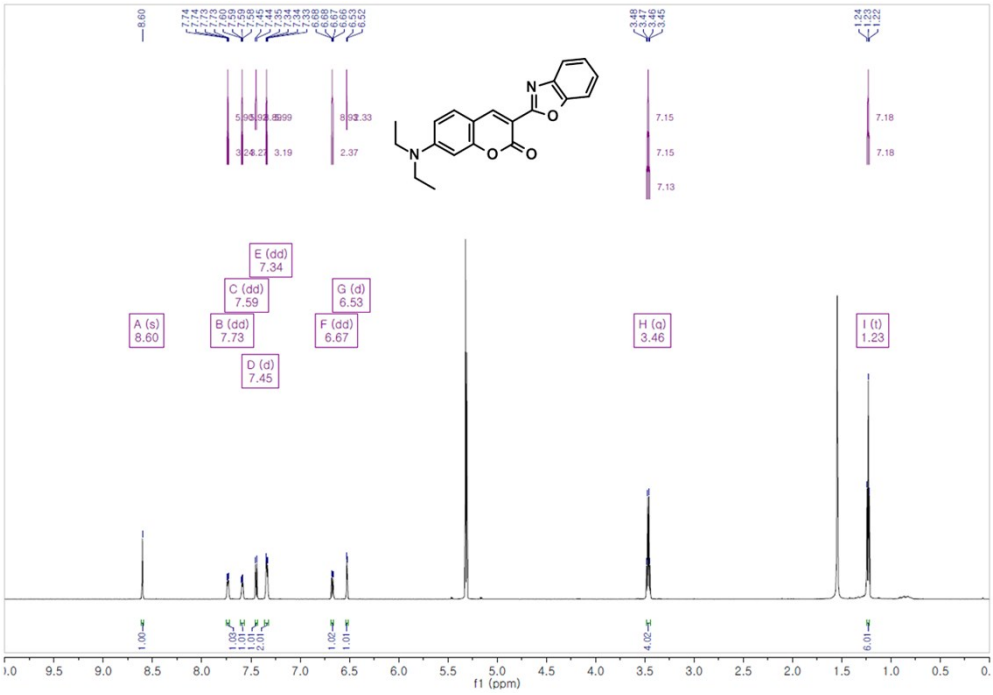



**600 MHz, $^{1}$H NMR in $CDCl_3$.**

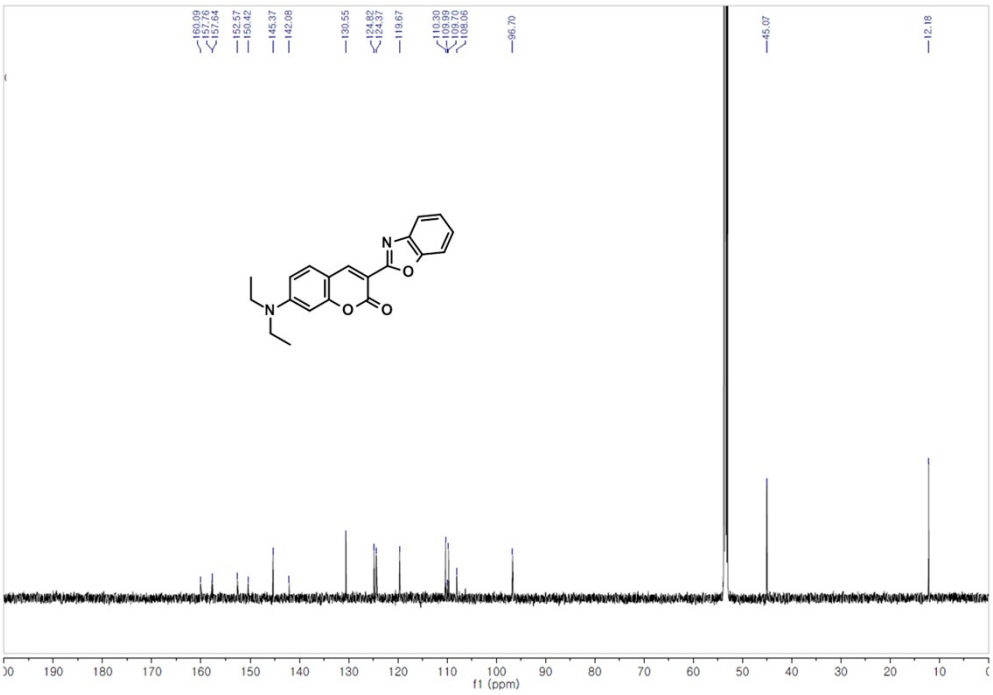



**150 MHz, $^{13}$C NMR in $CDCl_3$.**

**3-(1,3-Benzoxazol-2-yl)-7-(diethylamino)chromen-2-one (4a)**

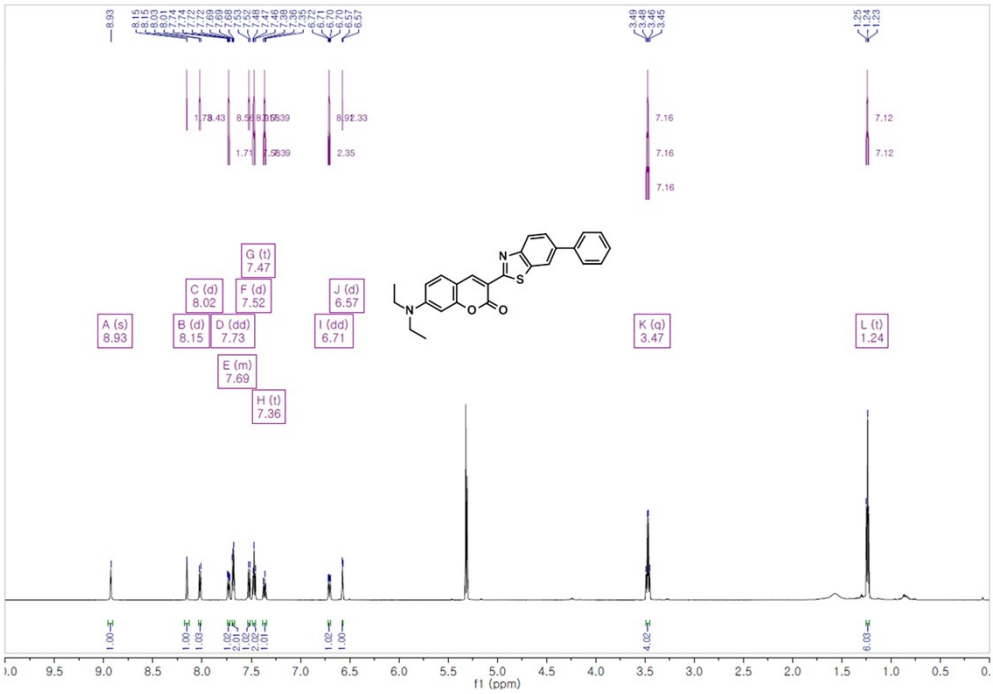



**600 MHz, $^{1}$H NMR in $CD_2Cl_2$.**

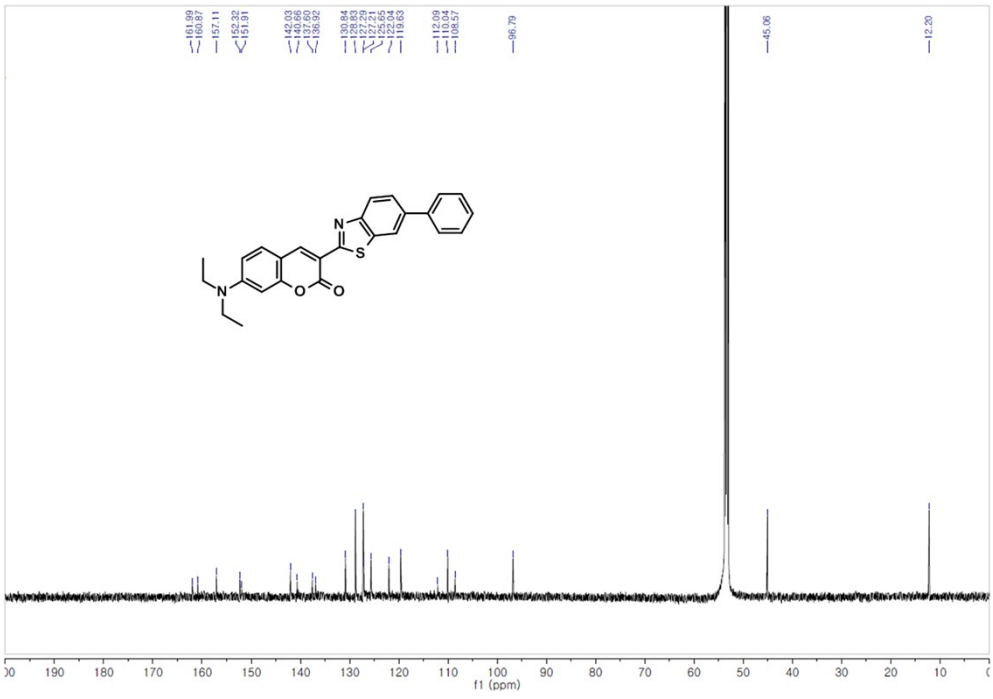



**150 MHz, $^{13}$C NMR in $CD_2Cl_2$.**

**7-(Diethylamino)-3-(6-phenyl-1,3-benzothiazol-2-yl)chromen-2-one (4b)**

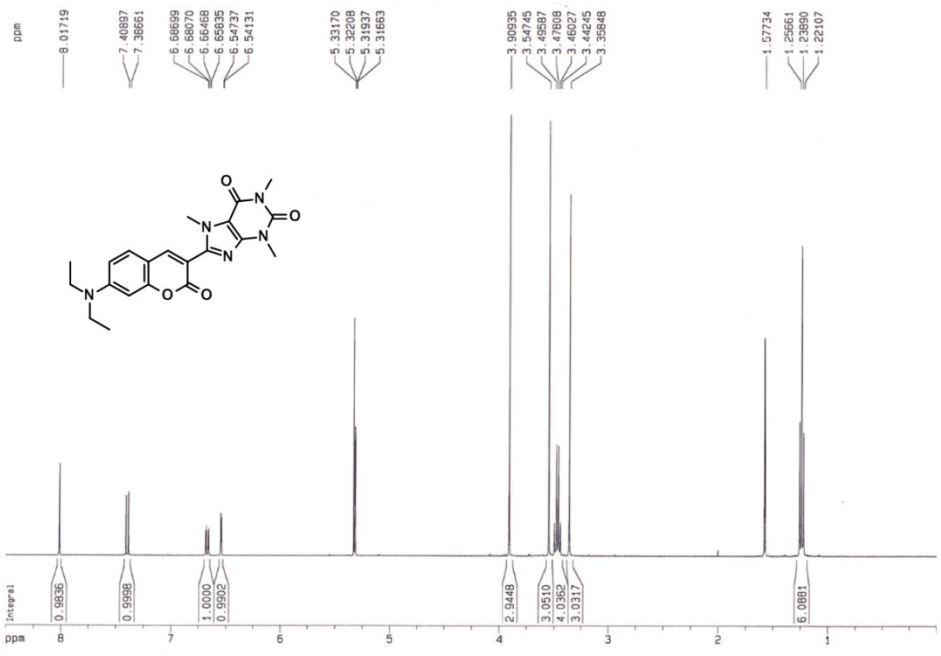



**600 MHz, $^{1}$H NMR in $CD_2Cl_2$.**

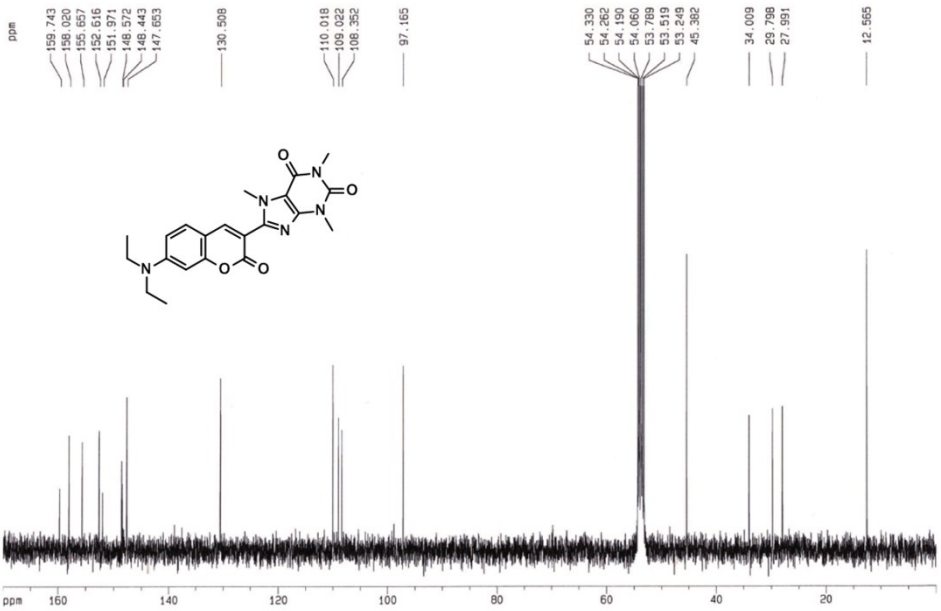



**150 MHz, $^{13}$C NMR in $CD_2Cl_2$.**

**8-[7-(Diethylamino)-2-oxo-chromen-3-yl]-1,3,7-trimethyl-purine-2,6-dione (4c)**

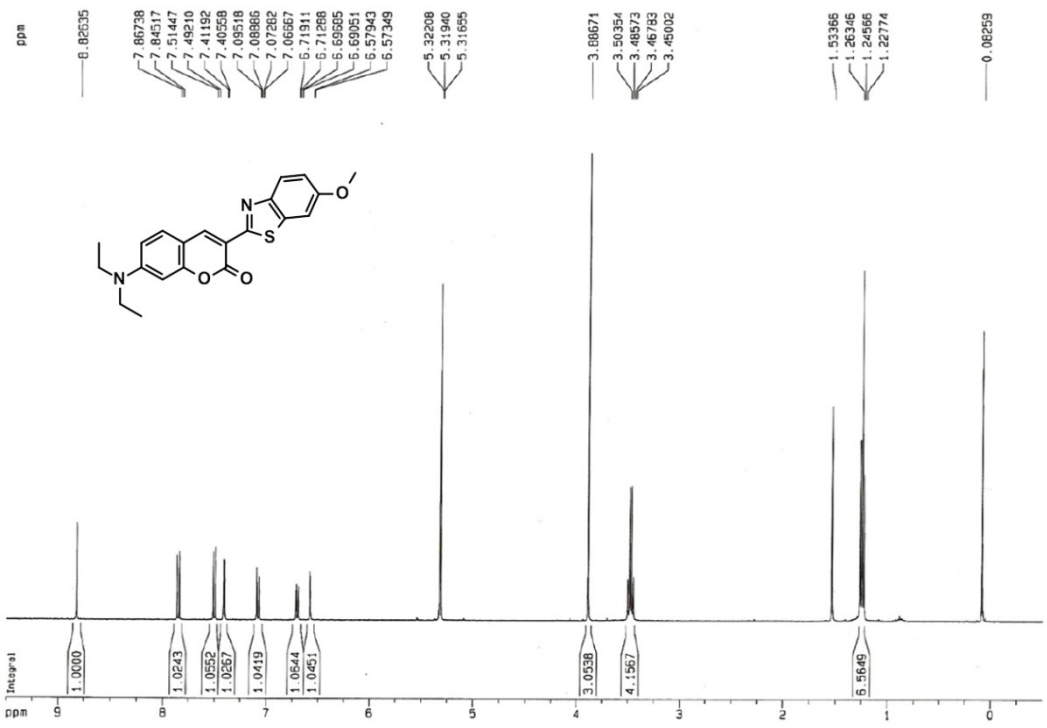



**400 MHz, $^{1}$H NMR in $CD_2Cl_2$.**

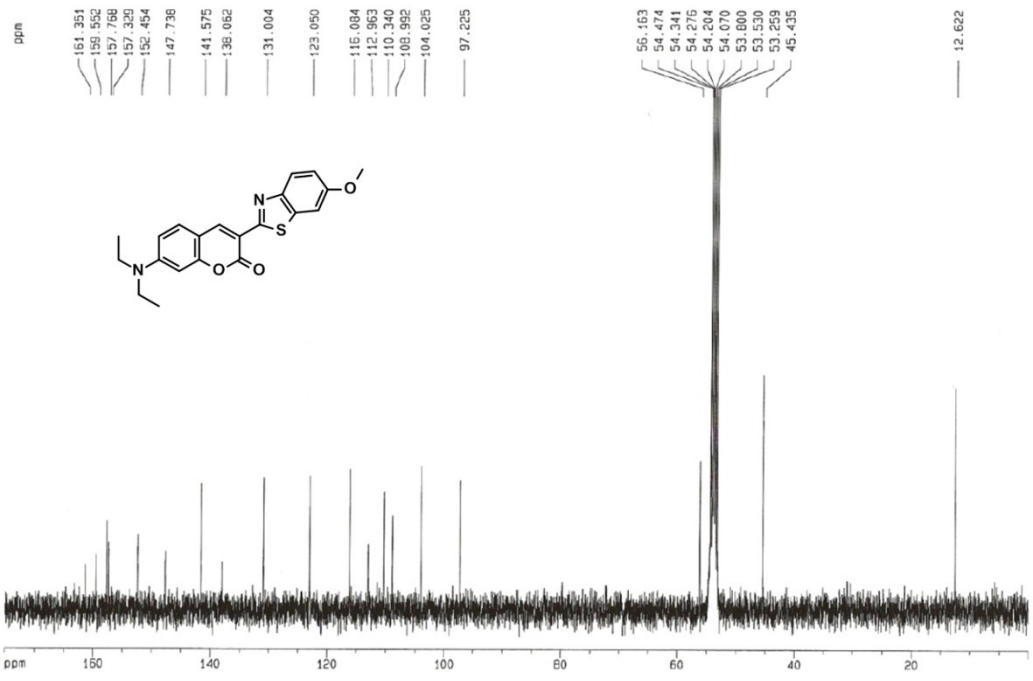



**100 MHz, $^{13}$C NMR in $CD_2Cl_2$.**

**7-(Diethylamino)-3-(6-methoxybenzo[d]thiazol-2-yl)-2H-chromen-2-one (4d)**



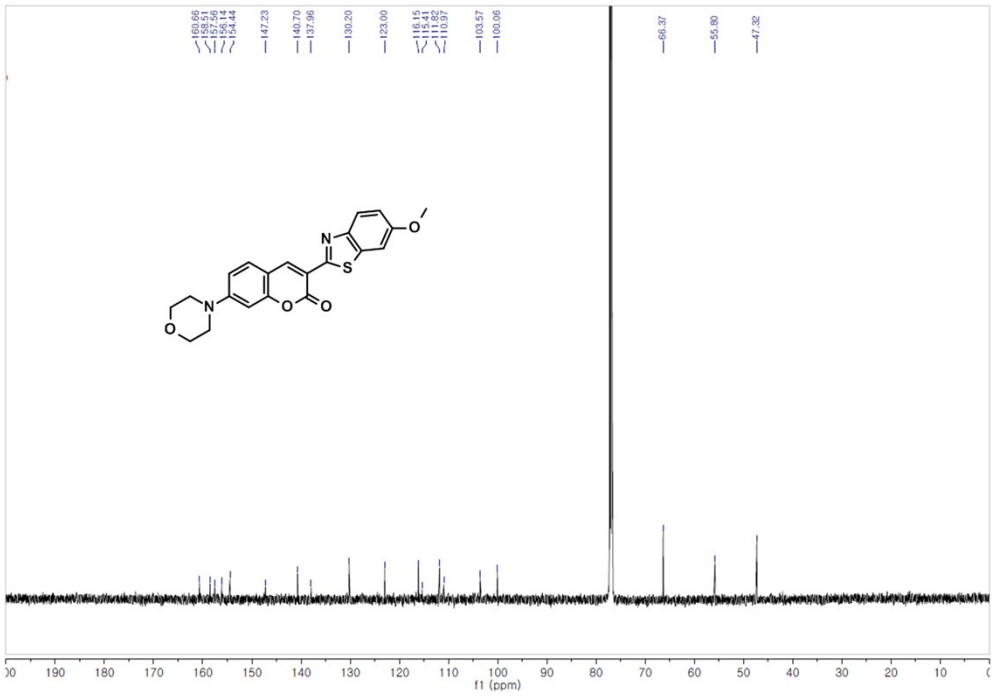

**400 MHz, $^{1}H$ NMR in $CD_2Cl_2$.**




**100 MHz, $^{13}C$ NMR in $CD_2Cl_2$.**

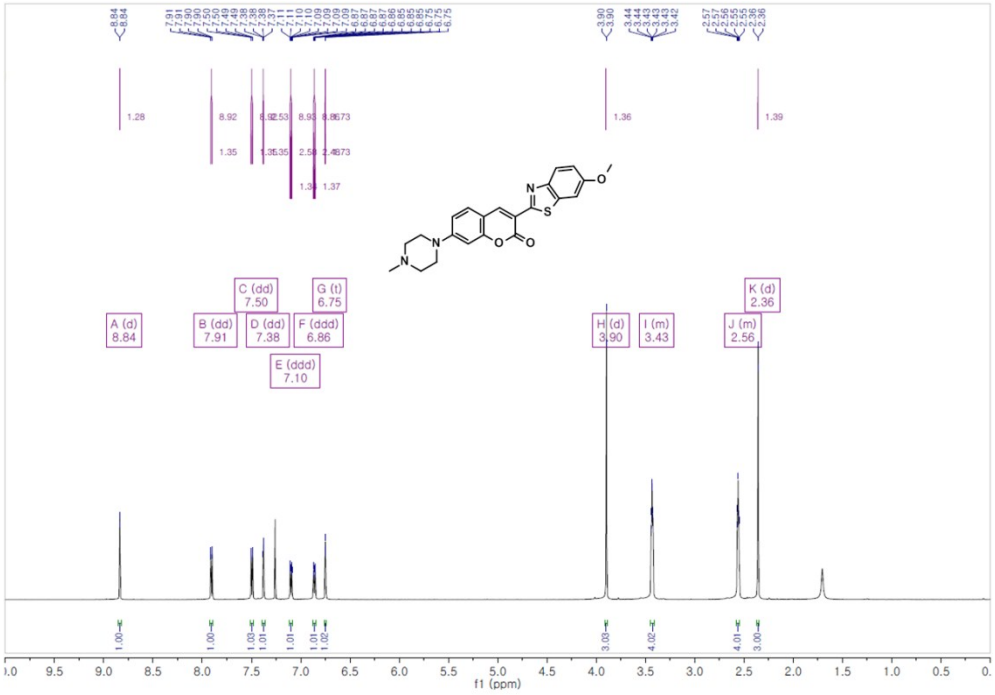
**3-(6-Methoxy-1,3-benzothiazol-2-yl)-7-morpholino-chromen-2-one (5a)**


**400 MHz, $^{1}H$ NMR in $CDCl_3$.**

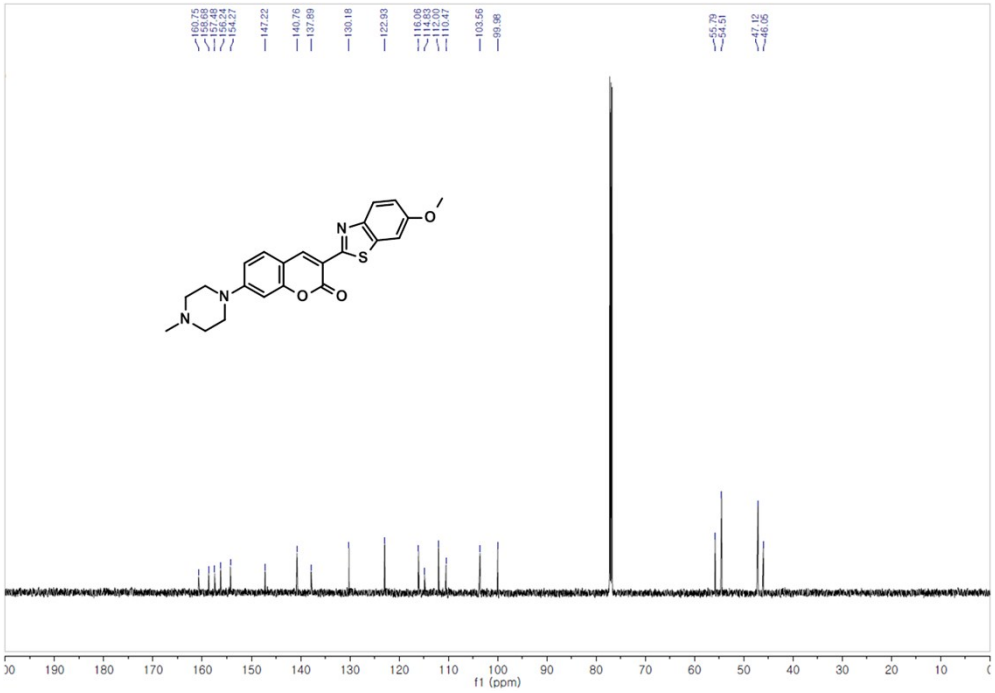



**150 MHz, $^{13}C$ NMR in $CDCl_3$.**

**3-(6-Methoxy-1,3-benzothiazol-2-yl)-7-(4-methylpiperazin-1-yl)chromen-2-one (5b)**

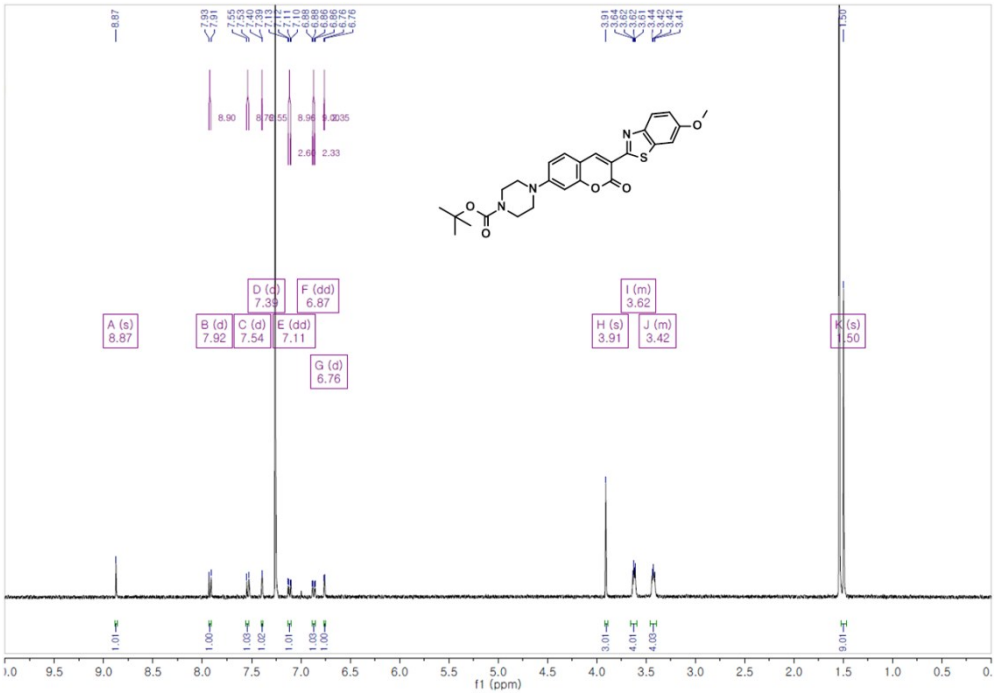



**600 MHz, $^{1}$H NMR in $CDCl_3$.**

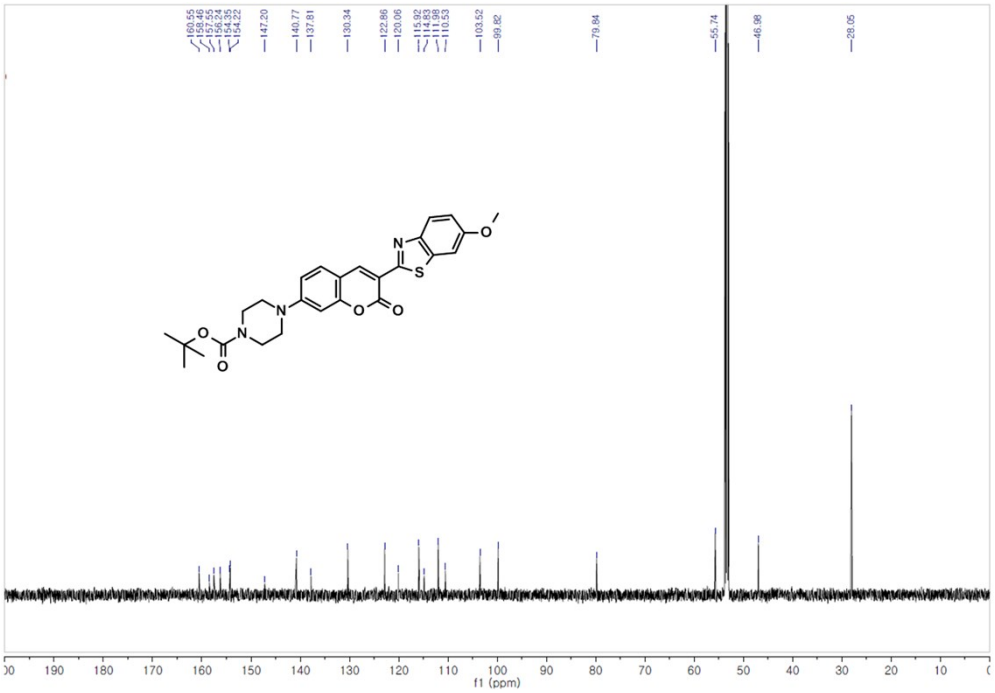



**150 MHz, $^{13}$C NMR in $CDCl_3$.**

***tert*-Butyl 4-[3-(6-methoxy-1,3-benzothiazol-2-yl)-2-oxo-chromen-7-yl]piperazine-1-carboxylate (5c)**

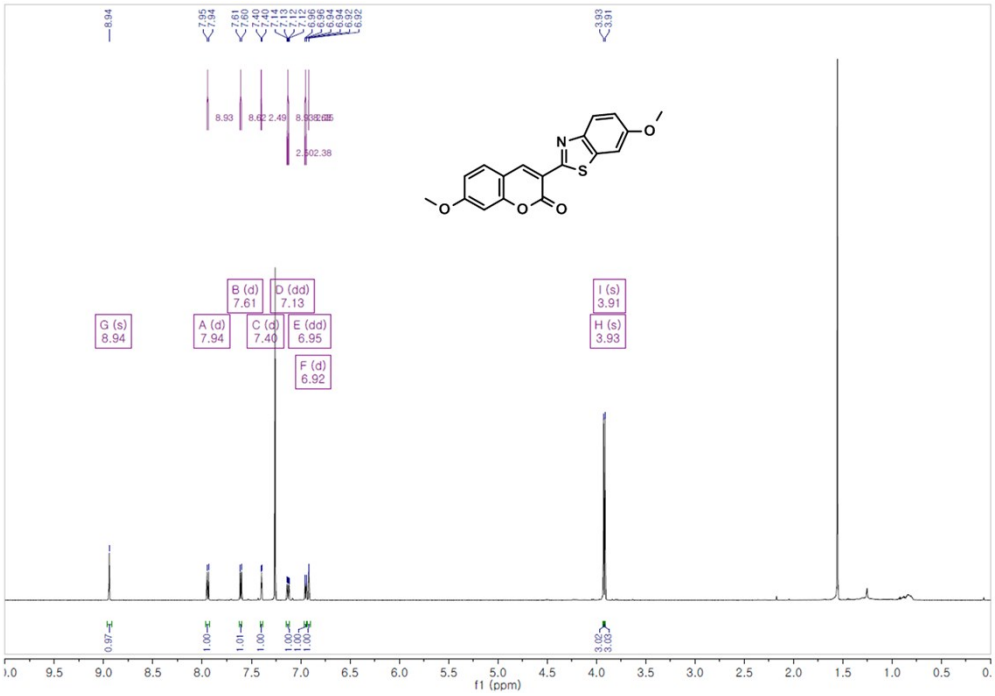



**400 MHz, $^{1}H$ NMR in $CDCl_3$.**

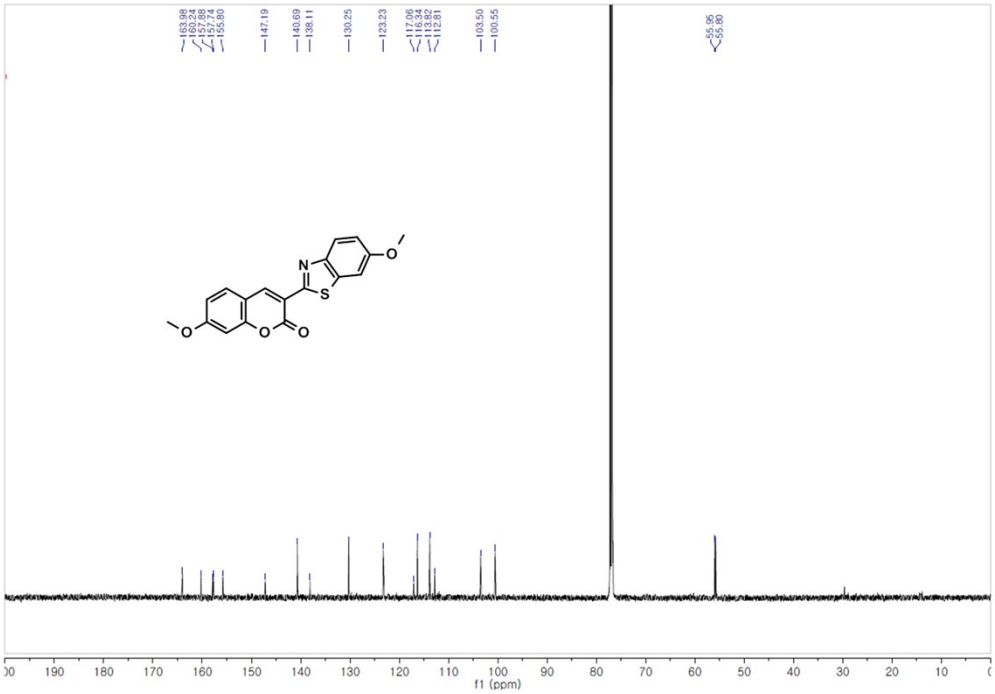



**150 MHz, $^{13}C$ NMR in $CD_2Cl_2$.**

**7-Methoxy-3-(6-methoxy-1,3-benzothiazol-2-yl)chromen-2-one (6a)**

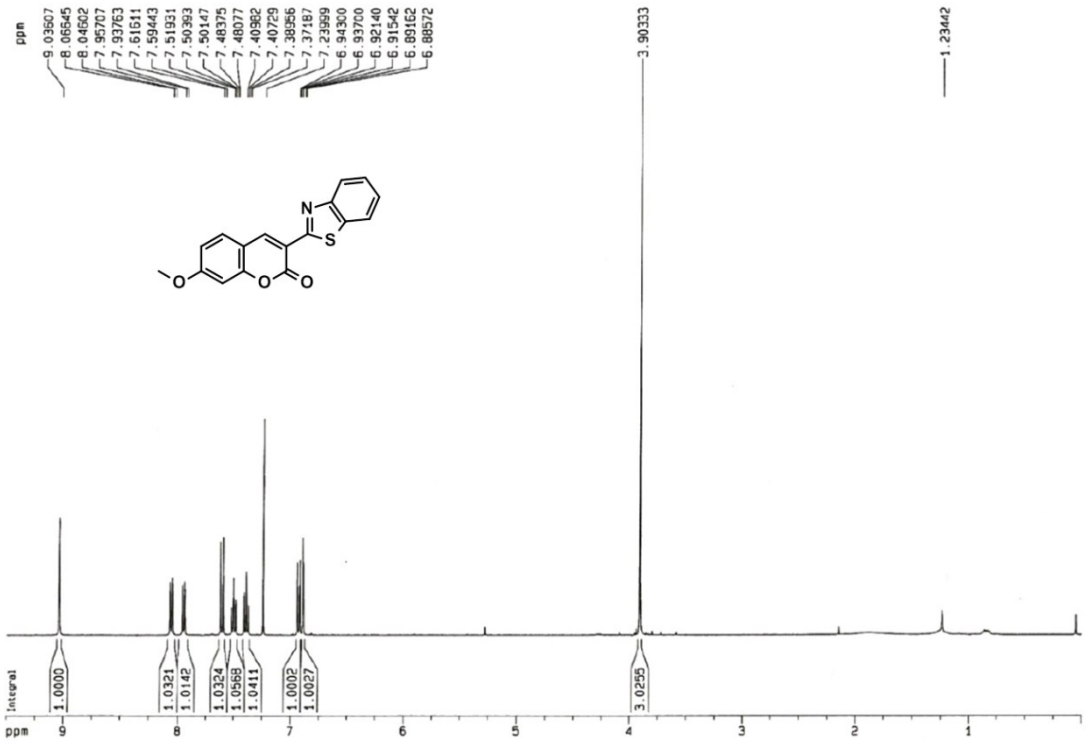



**600 Mhz, $^{1}$H NMR in $CDCl_3$.**

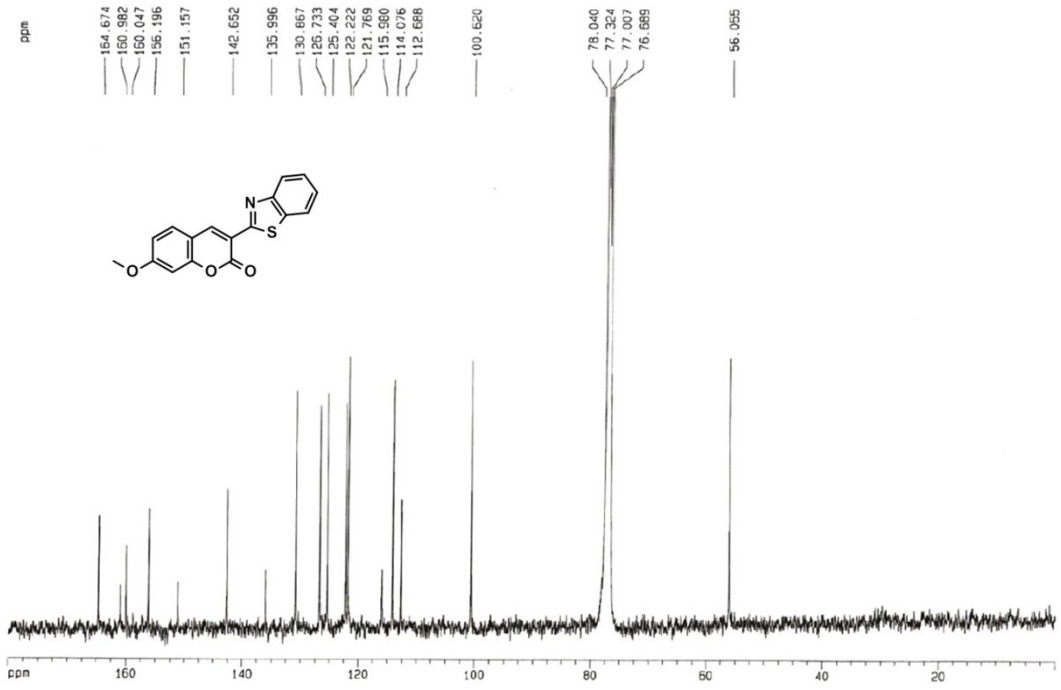



**150 MHz, $^{13}$C NMR in $CDCl_3$.**

**3-(Benzo[d]thiazol-2-yl)-7-methoxy-2H-chromen-2-one (6b)**

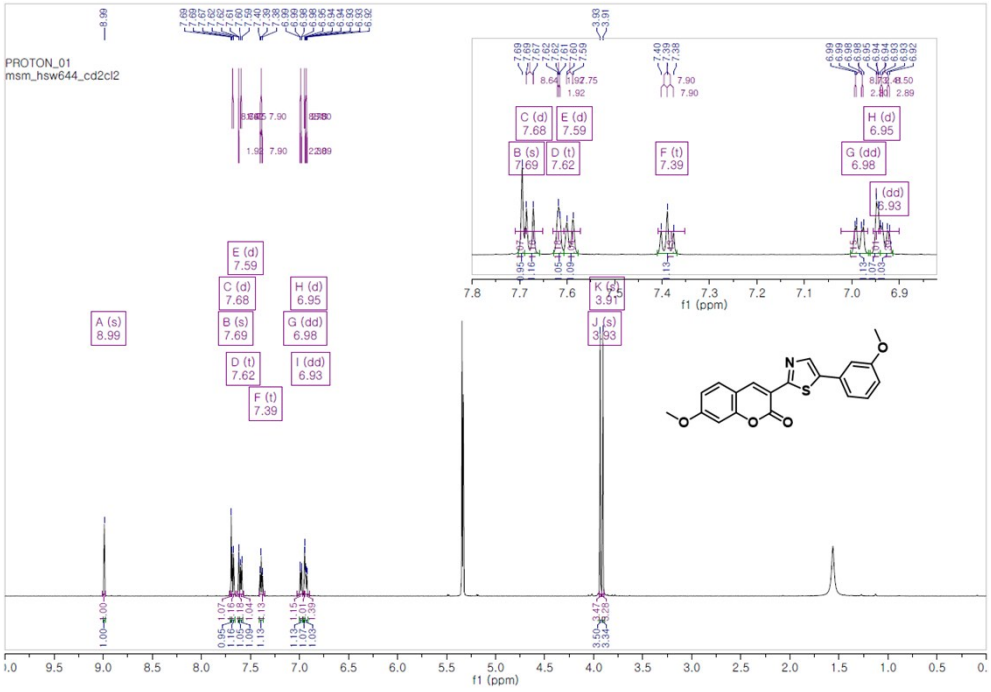



**400 Mhz, $^{1}$H NMR in $CDCl_3$.**

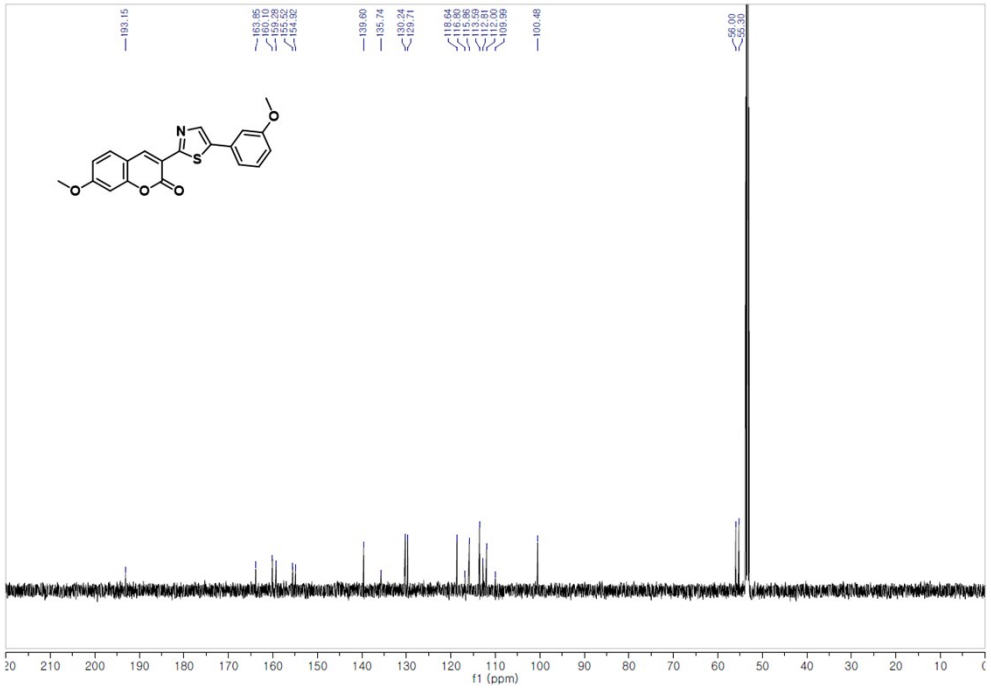



**100 MHz, $^{13}$C NMR in $CDCl_3$.**

**7-Methoxy-3-[5-(2-methoxyphenyl)thiazol-2-yl]chromen-2-one (6c)**




**600 Mhz, $^{1}$H NMR in $CDCl_3$.**




**150 MHz, $^{13}$C NMR in $CDCl_3$.**