1 **SUPPLEMENTAL MATERIAL**

2

3 **Methods**

4 **Chemical compounds and reagents**

5 Recombinant human Neuregulin 1 (WAKO, #080-09001), fibroblast growth factor 1 (WAKO, #067-
6 05371), BIO (Tocris, #3194), and sulforaphane (LKT, #S8044) were purchased from each of the listed
7 manufacturers. The library of chemicals that were screened was provided by the Chemical Biology
8 Screening Center at Tokyo Medical and Dental University. The purity of each chemical from the library
9 was greater than 90%.

10

11 **Cell cultures**

12 Human embryonic kidney 293 (HEK293) cells, human retinal pigment epithelial ARPE-19 cells, and
13 mouse fibroblast NIH3T3 cells were cultured at 37°C in DMEM media (Sigma) supplemented with
14 10% fetal calf serum (FCS). Human umbilical vein endothelial cells (HUVECs) were cultured at 37°C
15 in Endothelial Cell Growth Medium (EGM) (Lonza) supplemented with EGM™-2 SingleQuots
16 Supplements and Growth Factors (Lonza).

17

18 **Isolation of neonatal CMs and non-CMs**

19 Primary neonatal rat CMs were prepared from 1-day-old Wistar rats (Takasugi Experimental Animal
20 Supply Co.) as previously described (1). The isolated and dispersed cardiac cells were incubated for 80
21 minutes to remove non-CMs. The unattached viable cells, which were rich in CMs, were cultured on
22 gelatin-coated dishes at 37°C in DMEM media supplemented with 10% fetal calf serum (FCS) for 24
23 hours. Non-CMs that attached to the dishes were cultured in DMEM media supplemented with 10%
24 FCS and allowed to grow to confluence.

25

26 **Fluorescence reporter-based assay for YAP-TEADs activators**

27 ARPE-19 cells were transfected with the pLL3.7-K122 FH-YAP1-ires-GFP-TEADs-responsive-
28 promoter-H2B-mCherry reporter using Lipofectamine 2000 (Thermo Fisher Scientific). The YAP1-
29 expressing ARPE-19 cells were treated with 10 µM of each compound for 72 hours and then fixed. The
30 nucleus was visualized with Hoechst 33342, and the H2B-mCherry signal inside the nucleus was
31 measured with ArrayScan VTI (Thermo Fisher Scientific). The assay system is described in more detail
32 elsewhere (2).

33

34 **Sphere formation assay for TAZ activators**

Sphere formation assay was performed as previously described (3). Briefly, human breast epithelial MCF10A cells expressing TAZ (MCF10A-TAZ) were prepared using a pLenti-EF-FH-TAZ-ires-blast lentivirus vector with blasticidin selection. MCF10A-TAZ cells were plated in 96-well Ultra Low Attachment plates (Corning) and cultured with 10 µM of each compound for 10 days in serum-free DMEM/F-12 (Thermo Fisher Scientific) containing 10 ng/ml basic fibroblast growth factor, 20 ng/ml epidermal growth factor, 5 µg/ml insulin, and 0.4% bovine serum albumin. TAZ activators enabled TAZ-expressing MCF10A cells to form spheres, and a cell aggregate with a diameter of more than 150 µm was defined as a sphere.

Luciferase assay

The TEADs reporter assay for 50 putative TAZ activators was performed with HEK293 cells. HEK293 cells were transfected with the 8xGT-IIC-δ51LucII (TEADs reporter) (4), pCMV-alkaline phosphatase (a gift from Sumiko Watanabe), and pFLAG-YAP1 (5) plasmids using Lipofectamine 2000 (Thermo Fisher Scientific). The YAP-expressing HEK293 cells were treated with 10 µM of each compound for 24 hours and harvested. Luciferase activity and alkaline phosphatase activity, as an internal control, were assayed with PicaGene (Toyo Ink) and CDP-star (Roche), respectively, and measured by a microplate-reading luminometer (Wallac ARVO MX 1420; PerkinElmer).

For the luciferase reporter assays in CMs, 8xGTIIC-luciferase reporter (Addgene #34615) (6) and pGL4.49[luc2P/TCF-LEF/Hygro] vectors (Promega) were transfected using Nucleofector™ 2b (Lonza). After 24 hours, cells were starved (0.1% FCS) for 6 hours and subsequently treated with 10 µM of each compound for 40 hours. Dual luciferase activities were measured with the Dual Luciferase Assay System (Promega) on a microplate-reading luminometer (Wallac ARVO MX 1420; PerkinElmer). Reporter activities were normalized to the measured Renilla luciferase activity of pRL-SV40.

Cell proliferation assay

To detect DNA synthesis in proliferating cells, cells were incubated with 5 µM 5-ethynyl-2'-deoxyuridine (EdU) under starved conditions (0.1% FCS). After 40 hours, incorporated EdU in CMs was stained with the Click-iT® EdU detection reagent (Thermo Fisher Scientific) and cardiac troponin T antibody (Thermo Fisher Scientific). To detect nuclear division (karyokinesis) and cell division (cytokinesis), cells were stained with antibodies against phosphohistone H3 (pH3) (Millipore) and aurora B kinase (Cell Signaling), respectively. Cytokinesis was defined by the presence of an aurora B-stained spindle-shaped structure in the middle of the intercellular bridge. The assays were visualized and evaluated using an IN Cell Analyzer 1000 (GE Healthcare) or Olympus FSX 100 fluorescence microscope (Olympus). All images were analyzed using Adobe Photoshop (sizing, brightness, contrast

1 adjustments, etc.). Brightness and contrast were adjusted linearly across the entirety of each image.
2
3 **Cell area analysis**
4 CM size was automatically determined by measuring the surface area of more than 2,500 cells stained
5 with a cardiac troponin T antibody using an IN Cell Analyzer 1000 (GE Healthcare).
6
7 **MTS cell viability assay**
8 CMs under starved conditions (0.1% FCS) were incubated with the indicated compounds or 100 μM
9 hydrogen peroxide (H_2O_2; positive control) for 40 hours, and then cell viability was evaluated using a
10 CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (Promega) and a microplate-reading
11 luminometer (Wallac ARVO MX 1420; PerkinElmer).
12
13 **Analysis of nuclear translocation of YAP and TAZ**
14 Cultured CMs were double-stained with antibodies against cardiac troponin T (Thermo Fisher
15 Scientific) and YAP antibody (Novus Biologicals) or TAZ antibody (Cell Signaling). Nuclei were
16 counterstained with Hoechst 33342. CM nuclei with a strong YAP or TAZ signal were defined as
17 follows: nuclear/cytoplasmic fluorescence intensity ratio of YAP or TAZ was greater than 2, which was
18 analyzed using an automated Operetta High-Content Imaging System (PerkinElmer).
19
20 ***in vitro* reactive oxygen species (ROS) assay**
21 CMs were incubated with 100 μM of an oxidation-sensitive fluorescent probe 2'7'-dichlorofluorescin
22 diacetate (DCFH-DA; Sigma) for 60 minutes, and then excessive amounts methylglyoxal (a precursor
23 of advanced glycation end-products; 100 μM) was added for 90 minutes to induce ROS production.
24 The DCFH-DA probe was converted to highly fluorescent 2,7-dichlorofluorescein (DCF) upon ROS-
25 mediated oxidation, and the cell fluorescence was measured using a microplate-reading luminometer
26 (Wallac ARVO MX 1420; PerkinElmer).
27
28 **Generation of adenoviruses with CM-specific expression**
29 A genomic fragment extending from -497 to +172 bp of Rat cardiac troponin T (*Tnnt2*) promoter was
30 obtained by PCR using rat genomic DNA (7). The promoter fragment was the subcloned into KpnI/SalI-
31 digested pShuttle vector (Agilent, #240005), and human growth hormone (hGH) polyA signal
32 sequences were cloned at the C-terminus (pShuttle-*tnnt2* vector). cDNA fragments coding for Fucci
33 cell cycle indicators were extracted from pFucci-G1 Orange vector (MBL Life science, #AM-V9001M),
34 pFucci-S/G2/M Green vector (MBL Life science, #AM9014M), respectively, and then subcloned into

1 the pShuttle-*tnnt2* vector. Adenoviruses for CM-specific gene expression were generated using
2 AdEasy™ Adenoviral Vector System (Agilent), according to the manufacture's instructions.
3
4 **CM cell cycle analysis**
5 For CM cell cycle analysis, CMs were infected with the CM-specific Fucci expression adenoviruses,
6 *Tnnt2*-Fucci G1 Orange and *Tnnt2*-Fucci S/G2/M Green, at 2 and 10 MOI (multiplicity of infection),
7 respectively, and evaluated 72 hours after incubation using an IN Cell Analyzer 1000 (GE Healthcare).
8 Nuclei with green (Azami-Green1; mAG1) and/or red (Kusabira-Orange2; mKO2) fluorescence were
9 automatically detected. Fucci-expressing CMs were categorized as mono- or binucleated CMs based
10 on the distance between adjacent nuclei. Mono- or binucleated CMs were further classified as cells in
11 the G1-phase, G1/S-transition, or S/G2/M-phase, depending on red, yellow (both red and green), or
12 green fluorescence in the nuclei, respectively.
13
14 **RNA isolation and quantitative real-time PCR**
15 Total RNA from cultured cells was extracted using a RNeasy Mini Kit (Qiagen). Reverse transcription
16 was performed with 1 μg of total RNA, random hexamers, and reverse transcriptase (ReverTraAce,
17 TOYOBO). Real-time PCR was performed using FastStart Essential DNA Green Master (Roche) in a
18 LightCycler 480 System II (Roche). The expression level of each gene was normalized to that of 18s
19 rRNA. The sequences of the PCR primers are available upon request.
20
21 **siRNA-mediated knockdown of neonatal rat CMs**
22 siRNA for *Nfe2l2* (*Nrf2*) (Thermo Fisher Scientific, RSS343557), and control siRNA (Thermo Fisher
23 Scientific, 12935-112) were transfected using the HiPerFect reagent (Qiagen) at a final concentration
24 of 30 nM under starved conditions (0.1% FCS).
25
26 **Western blot analysis**
27 Nuclear and cytoplasmic fractions were separated using a LysoPure Nuclear and Cytoplasmic Extractor
28 Kit (Wako). Extracted proteins were separated by SDS-PAGE and transferred electrophoretically to
29 polyvinylidene fluoride (PVDF) membranes (Millipore). The primary antibodies used included YAP
30 (Cell Signaling), YAP (Abnova), TAZ (Cell Signaling), β-catenin (BD Transduction), active β-catenin
31 (Millipore), GSK3β (Cell Signaling), phospho-GSK3β (Tyr216) (Abcam), phosphor-p44/42 MAPK
32 (ERK1/2) (Thy202/Thy204) (Cell Signaling), p44/42 MAPK (ERK1/2) (Cell Signaling), phospho-Akt
33 (Ser473) (Cell Signaling), Akt (Cell Signaling), Cleaved Caspase 3 (Cell Signaling), NRF2 (Santa Cruz),
34 KEAP1 (Santa Cruz), Actin (Abcam), GAPDH (Cell Signaling), and Lamin A/C (Sigma). Membranes

were exposed to horseradish peroxidase (HRP)-conjugated secondary antibodies, and signals were detected by the Pierce ECL Plus Western Blotting Substrate (Thermo Fisher Scientific). Densitometric analysis of proteins on western blots was performed with ImageJ software (NIH) and normalized to the indicated internal control proteins.

Coimmunoprecipitation assay

HEK293 cells were transfected with the pCDNA3-Myc3-Nrf2 (a gift from Yue Xiong, Addgene plasmid # 21555) (8) and pcDNA3-HA2-Keap1 (a gift from Yue Xiong, Addgene plasmid # 21556) (8) plasmids using Lipofectamine 2000 (Thermo Fisher Scientific). Forty-eight hours later, the NRF2-KEAP1-overexpressing HEK293 cells were treated with 10 µM of TT-10 for 2 hours and harvested. Cells were lysed in immunoprecipitation buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 1 mM EDTA, protease inhibitor cocktail (Roche), and phosphatase inhibitor cocktail (Roche)). Fifty micrograms of cell lysates were immunoprecipitated with mouse IgG, anti-MYC-tag (MBL Life science) or anti-HA-tag (Santa Cruz) antibody. In immunoblots, 5 µg of cell lysates (Input) and immunoprecipitates (IP) were probed for NRF2 and KEAP1 using anti-MYC-tag (MBL Life science) and anti-HA-tag (Santa Cruz) antibodies, respectively.

RNA-Seq

High quality RNA from cultured CMs was sequenced. RNA-seq libraries were constructed according to the Illumina protocol and sequenced on a HiSeq 2500 instrument as previously described (9). The reads per kilobase per million mapped reads (RPKM) method was employed to normalize gene expression.

Heatmap Generation and GO Cluster Analysis

Differentially expressed genes were selected based on the criteria that 1) RPKM value ranges between mean ± SE did not overlap in two groups, 2) RPKM values of all samples were more than 3.25, and 3) the maximum difference between samples was more than 3.25. Genes were clustered by Cluster 3.0 software including $\log_2$-transform. The clustering was visualized in a heatmap using Java TreeView 1.1.6r4 software. Official gene symbols of selected genes were subjected to the Database for Annotation, Visualization and Integrated Discovery (DAVID) Functional Classification Tool (URL: https://david.ncifcrf.gov/summary.jsp) (10) to obtain biological processes related to the genes. We presented GO terms under biological process ranked in the top 9.

MI model

1 MI was performed as previously described (11). MI mice were randomly assigned in a 1:1 ratio to
2 receive vehicle or TT-10 after the MI procedure, according to each experimental protocol as described
3 in detail elsewhere. Briefly, mice were anesthetized by intraperitoneal injection of 2,2,2-
4 tribromoethanol (Sigma) (250 mg/kg body weight) and inhalation of 1% isoflurane. A 23-gauge
5 polyethylene tube was inserted into the trachea, and the animals were ventilated using a volume-cycled
6 rodent ventilator. The chest cavity was opened via incision through the left third intercostal space. The
7 left anterior descending artery (LAD) was doubly ligated by 8-0 nylon suture approximately 1.5 mm
8 below the tip of the left atrial appendage. The occlusion was confirmed by a change in color (paler
9 appearance) of the anterior wall of the left ventricle. Sham-operated mice underwent the identical
10 surgical procedure without LAD ligation.
11
12 **Lineage tracing of existing CMs**
13 Lineage tracing experiments of existing CMs were performed to evaluate clonal proliferation. *αMHC-*
14 *MerCreMer; Rosa26*$^{rbw/+}$ mice were injected intraperitoneally with a single low dose of tamoxifen
15 (Sigma; 5 mg/kg body weight) dissolved in corn oil (Wako). After Cre-mediated recombination, one of
16 the three fluorescent marker proteins (mOrange, mCerulean, and mCherry) was stochastically placed
17 under the control of CAG promoter. mOrange was the brightest and most easily detected using an
18 Olympus FSX 100 fluorescence microscope (Olympus), Olympus BX 51 fluorescence microscope
19 (Olympus), and Zeiss LSM 510 META Confocal Microscope (Carl Zeiss). Thus, we selected mOrange-
20 expressing CMs for subsequent analysis. Mice were randomized to receive vehicle or TT-10 in a 1:1
21 ratio after MI operation. Approximately 3% of existing CMs irreversibly expressed mOrange after
22 tamoxifen treatment, and approximately 2% of mOrange-labeled CMs in MI (-) mice formed a cluster
23 1 week after sham operation, which presumably consisted of both simply adjacent CMs and
24 spontaneously divided CMs. The increase in the proportion of clustered labeled CMs was considered
25 to be attributed to the additional proliferation of existing CMs (clonal expansion) after MI and TT-10
26 treatment, and evaluated. Horizontal sections of the ventricles were freshly embedded in OCT
27 compound, sectioned at a thickness of 10 μm, and collected at 700-μm intervals. Digital images of 5
28 different sections were captured and evaluated. All images were analyzed using Adobe Photoshop
29 (sizing, brightness, contrast adjustments, etc.). Brightness and contrast were adjusted linearly across the
30 entirety of each image.
31
32 **Echocardiography**
33 Two dimensional (2D) guided M-mode echocardiography was performed using an a VisualSonics Vevo
34 2100 echocardiogram. LV diastolic anterior wall (AWth) thickness, LV end-diastolic dimensions

(LVDd), and LV end-systolic dimensions (LVDs) were measured. LV end-diastolic volumes (LVEDV) and LV end-systolic volumes (LVESV) were estimated using the Teichholz method, and ejection fraction (%EF) was calculated as (LVEDV-LVESV)/LVEDV x 100.

Measurement of CM diameter and nuclear density

CM diameter and nuclear density were evaluated on Periodic acid-Shiff (PAS)-stained horizontal sections as previously described (12). The diameter was calculated by measuring the short axis when the visualized CM were circular-shaped with the nucleus. The mean CM size was determined from at least 100 cells.

Histological analysis

Heart sections were prepared as previously described (1) and stained with hematoxylin and eosin for overall morphology. The antibodies used for immunohistochemical (IHC) staining included phosphohistone H3 (pH3) (Millipore), aurora B kinase (Cell Signaling), YAP (Novus Biologicals), phospho-histone H2AX (Ser139) (γH2AX) (Cell Signaling), troponin I (Abcam) and troponin T (Thermo Fisher Scientific). Apoptotic cells were detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay (Roche Diagnostics). All IHC staining was visualized and evaluated using an Olympus FSX 100 fluorescence microscope (Olympus), Olympus BX 51 fluorescence microscope (Olympus), or Zeiss LSM 510 META Confocal Microscope (Carl Zeiss). All images were analyzed using Adobe Photoshop (sizing, brightness, contrast adjustments, etc.). Brightness and contrast were adjusted linearly across the entirety of each image.

Measurement of *in vivo* ROS production

Horizontal sections of the ventricles were freshly embedded in OCT compound, sectioned at a thickness of 10 μm, and collected at 700-μm intervals. Sections were stained with CellROX Green Reagent (Thermo Fisher Scientific) and cardiac troponin T antibody (Thermo Fisher Scientific). All IHC staining was visualized using an Olympus BX 51 fluorescence microscope (Olympus) and digital images of 5 different regions were captured. Nuclei of the infarct border-zone myocardium or MI (-) mouse myocardium were marked by DAPI and mean CellROX fluorescence intensity on those nuclei was calculated using ImageJ software (NIH). Brightness and contrast of inset images were adjusted linearly across the entirety of each image using ImageJ software (NIH).

Data availability

All the data supporting the findings of this study are available within the article and its Online Data

Supplement or from the corresponding author upon reasonable request. RNA-seq data were deposited in the GEO (Gene Expression Omnibus) database at the US National Center for Biotechnology Information (NCBI) under accession number GSE95274.

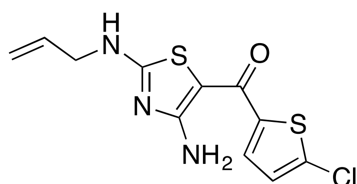
General procedure for the synthesis of analogues of TAZ-12

Synthesis of TAZ-12

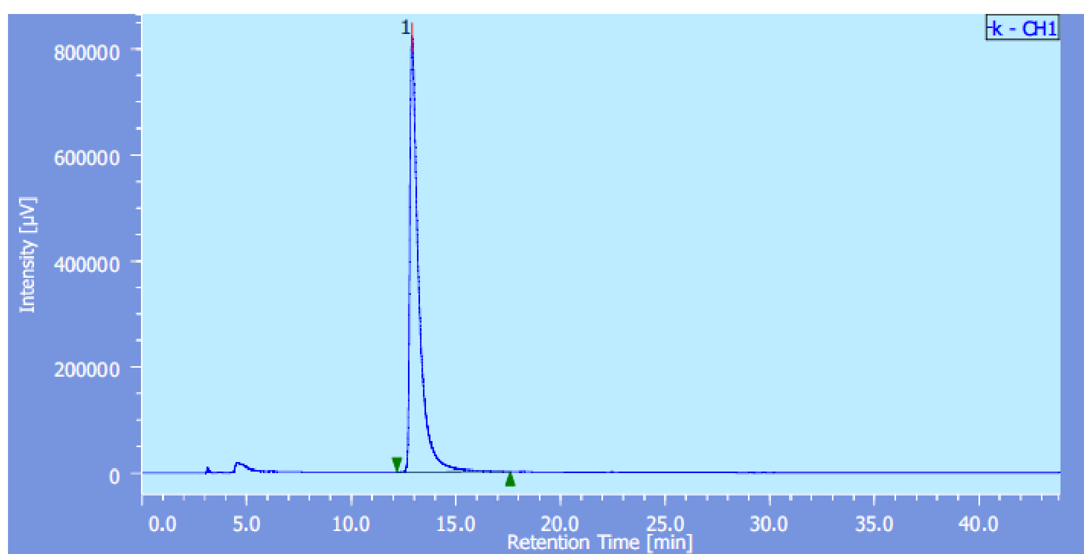
t-BuOK (1.09 g, 9.71 mmol) was added to a solution of allyl isothiocyanate (453.1 μL, 4.43 mmol) and cyanamide (188.1 mg, 4.43 mmol) in THF (17 mL) under an argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(5-chlorothiophen-2-yl)ethan-1-one (1.01 g, 4.22 mmol) was added the solution at 0 °C rinsed with THF, and stirred at rt. After 10 hours, the reaction mixture was diluted with cyclopentyl methyl ether (CPME), and quenched with H_2O. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 1/1) to give TAZ-12 as a yellow solid (581 mg, 46%).

General: All reactions were carried out under an inert atmosphere of dry argon, unless the reaction procedure states otherwise. Column chromatography was performed with silica gel 60 (40-50 μm) purchased from Merck. Tetrahydrofuran (THF) was purchased from Kanto Chemical Co. Inc. The purity of compounds was determined by HPLC analysis and ^{1}H NMR (minimum purity >99%). The HPLC analysis was performed on a JASCO LC-2000 series instrument equipped with a normal-phase column (Senshu, Pegasil Silica SP100, 4.6 φ x 250 mm, 5 μm) at 27°C with UV detection at 254 nm. NMR spectra were recorded on a Bruker AVANCE III HD spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C). Chemical shifts are expressed in δ (ppm) values, and coupling constants are expressed in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad singlet and brd = broad doublet. The value was determined with respect to tetramethylsilane for ^{1}H ($^{1}H(\delta)$ = 0.00 ppm) and solvent for ^{1}H ($CDCl_3$: $^{1}H(\delta)$ = 7.26 ppm, DMSO-d_6: $^{1}H(\delta)$ = 2.50 ppm), ^{13}C ($CDCl_3$: $^{13}C(\delta)$ = 77.16 ppm, DMSO-d6: $^{13}C(\delta)$ = 39.52 ppm). IR spectra were obtained on a JASCO FT/IR-4700 spectrophotometer. ESI-MS was taken on a Bruker micrOTOF-II spectrometer.

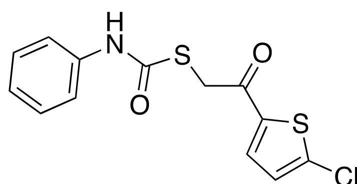
TAZ-12



1 Following the general procedure, the titled compound was obtained as a yellow solid (581 mg, 46%
2 yield).
3 HPLC (Pegasil Silica SP100, hexane/EtOAc= 50/50, flow rate 1.0 ml/min, λ = 254 nm) t_r = 12.90 min,
4 purity >99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.02 (brs, 1H), 8.49–8.09 (br, 1H), 7.31 (d, J = 4.0 Hz,
5 1H), 7.17 (d, J = 4.3 Hz, 1H), 5.89 (ddd, J = 17.1, 10.4, 5.2 Hz, 1H), 5.23 (dd, J = 17.1, 1.2 Hz, 1H),
6 5.16 (dd, J = 10.4, 1.2 Hz, 1H), 3.96 (brs, 2H) (1 proton is missing.); ^{13}C NMR (125 MHz, DMSO-d_6)
7 δ 170.5, 167.8, 146.4, 133.7, 133.1, 128.1 (2C), 127.3, 116.4, 89.8, 46.2; IR (ATR) 2362, 2335, 1584,
8 1543, 1414, 1005 cm^{-1}; mp: 182–186 °C; HRMS calcd for $C_{11}H_{10}ClN_3OS_2Na$ [M+Na]$^+$ 321.9846, found
9 321.9855. Anal. Calcd for $C_{11}H_{10}ClN_3OS_2$: C, 44.07; H, 3.36; N, 14.02%; found C, 43.86; H, 3.50; N,
10 13.76%.



11
12
13 TT-1

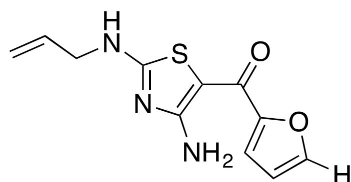


14
15 *t*-BuOK (302 mg, 2.69 mmol) was added to a solution of phenyl isothiocyanate (145.9 mg, 1.22 mmol)
16 and cyanamide (52 mg, 1.22 mmol) in THF (4.3 mL) under an argon atmosphere at 0°C. After stirring
17 at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(5-chlorothiophen-2-yl)ethan-1-one (279.3
18 mg, 1.17 mmol) was added the solution at 0 °C rinsed with THF, and stirred at rt. After 10 hours, the
19 reaction mixture was diluted with cyclopentyl methyl ether (CPME), and quenched with H_2O. The
20 mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*.
21 The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 2/1 to 1/1) to give

TT-16 (Rf = 0.4; n-hexane/EtOAc = 1/1) as a yellow solid (75 mg, 19% yield) and TT-1 (Rf = 0.6; n-hexane/EtOAc = 1/1) as a red solid (87 mg, 24% yield).

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 10.5 (brs, 1H), 8.05 (d, *J* = 4.3 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 2H), 7.36 (d, *J* = 4.3 Hz, 1H), 7.30 (dd, *J* = 8.5, 7.3 Hz, 2H), 7.05 (dd, *J* = 7.3, 7.3 Hz, 1H), 4.43 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d$_6$) δ 186.6, 163.4, 141.3, 138.7, 138.1, 134.3, 129.1(2C), 128.9(2C), 123.6, 118.9, 35.7; HRMS (ESI) calcd for $C_{13}H_{10}NO_2S_2ClNa$ [M+Na]$^+$ 333.9734, found 333.9721; Anal. Calcd for $C_{13}H_{10}NO_2S_2Cl$: C, 50.08; H, 3.23; N, 4.49%, found C, 49.98; H, 3.47; N, 4.43%.

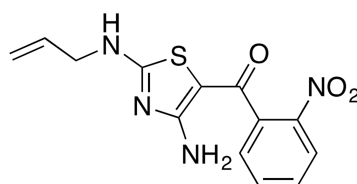
TT-5



t-BuOK (150 mg, 1.34 mmol) was added to a solution of allyl isothiocyanate (63 µL, 683.3 µmol) and cyanamide (26 mg, 683.3 µmol) in DMF (2.1 mL) under an argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(furan-2-yl)ethan-1-one (123 mg, 650.8 µmol) was added the solution at 0 °C rinsed with DMF, and stirred at rt. After 10 hours, the reaction mixture was diluted with cyclopentyl methyl ether (CPME), and quenched with H_2O. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 1/1) to give TT-5 as a yellow solid (51.1 mg, 31% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (dd, *J* = 1.8, 0.6 Hz, 1H), 7.16 (dd, *J* = 3.7, 0.6 Hz, 1H), 6.53 (dd, *J* = 3.7, 1.5 Hz, 1H), 5.93 (brs, 1H), 5.93 (ddd, *J* = 17.1, 10.1, 5.5 Hz, 1H), 5.35 (dd, *J* = 17.1, 2.8, 1.5 Hz, 1H), 5.28 (dd, *J* = 10.4, 2.8, 1.2 Hz, 1H), 3.98 (brs, 2H), 1.61 (brs, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.2, 170.9, 166.8, 153.5, 144.3, 132.5, 118.2, 114.3, 112.3, 92.5, 47.8; IR (ATR) 3127, 2358, 1533, 1410 cm^{-1}; mp 198 °C; HRMS (ESI) calcd for $C_{11}H_{11}N_3O_2SNa$ [M+H]$^+$ 250.0645, found 250.0648.

TT-6

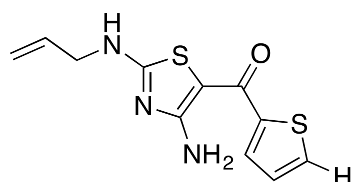


t-BuOK (258.1 mg, 2.30 mmol) was added to a solution of allyl isothiocyanate (107.4 µL, 1.05 mmol)

and cyanamide (44.6 mg, 1.05 mmol) in THF (2.7 mL) under an argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(2-nitrophenyl)ethan-1-one (244 mg, 1.00 mmol) was added the solution at 0 °C rinsed with THF, and stirred at rt. After 10 hours, the reaction mixture was diluted with cyclopentyl methyl ether (CPME), and quenched with H_2O. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 1/1 to 1/2) to give TT-6 as a yellow solid (110 mg, 36% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.87 (brs, 1H), 8.02 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.96 (brs, 1H), 7.77 (ddd, *J* = 7.6, 7.6, 1.2 Hz, 1H), 7.67 (ddd, *J* = 7.9, 7.9, 1.5 Hz, 1H), 7.60 (dd, *J* = 7.3, 1.5 Hz, 1H), 6.20 (brs, 1H), 5.85 (m, 1H), 5.18 (ddd, *J* = 17.1, 3.4, 1.5 Hz, 1H), 5.13 (ddd, *J* = 10.1, 3.1, 1.5 Hz, 1H), 3.89 (brs, 1H) (1 proton is missing.); ^{13}C NMR (125 MHz, DMSO-d_6) δ 178.1, 166.0, 146.7, 137.3, 133.9, 130.4, 128.4(2C), 124.4(2C), 116.4, 93.1, 59.8; IR (ATR) 2359, 1512, 1412, 1343 cm^{-1}; HRMS (ESI) calcd for $C_{13}H_{12}N_4O_3SNa$ $[M+Na]^+$ 327.0522, found 327.0522.

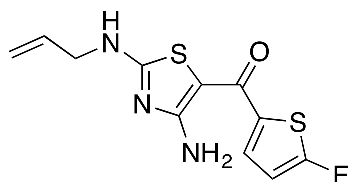
TT-9



t-BuOK (4.00 g, 35.7 mmol) was added to a solution of allyl isothiocyanate (1.67 mL, 16.3 mmol) and cyanamide (684.2 mg, 16.3 mmol) in DMF (55 mL) under an argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(thiophen-2-yl)ethan-1-one (3.18 g, 15.5 mmol) was added the solution at 0 °C rinsed with DMF (2 mL), and stirred at rt. After 10 hours, the reaction mixture was diluted with cyclopentyl methyl ether (CPME), and quenched with H_2O. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 1/1 to 1/2) to give TT-9 as a yellow solid (1.61 g, 39% yield).

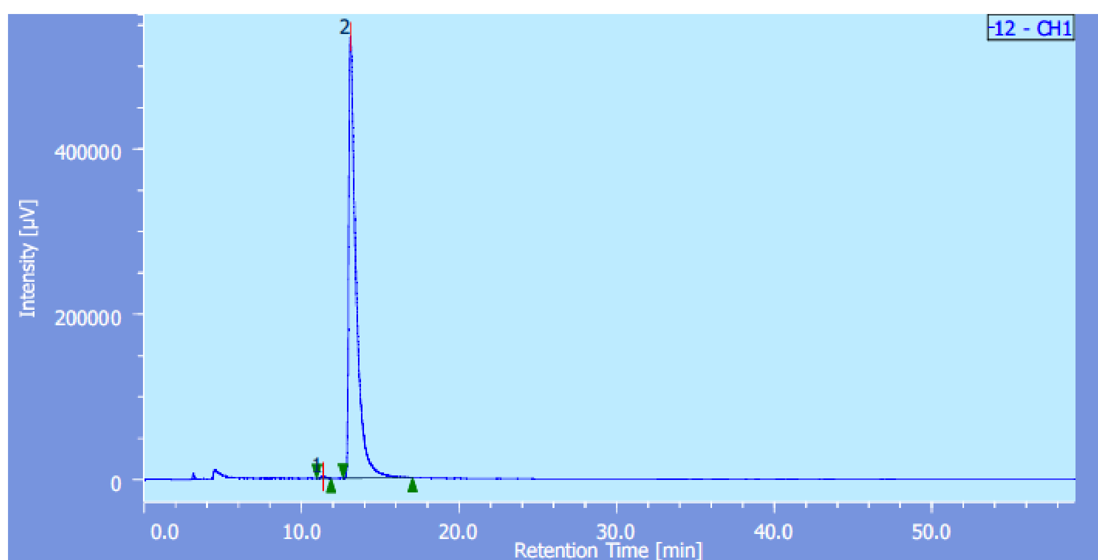
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 3.7, 0.9 Hz, 1H), 7.51 (dd, *J* = 4.9, 1.2 Hz, 1H), 7.10 (dd, *J* = 5.2, 3.7 Hz, 1H), 5.92 (brs, 1H), 5.92 (ddd, *J* = 17.1, 10.4, 5.5 Hz, 1H), 5.35 (dd, *J* = 17.1, 2.8, 1.8 Hz, 1H), 5.28 (dd, *J* = 10.1, 2.4, 1.2 Hz, 1H), 3.97 (dd, *J* = 5.2, 5.2, 2H), 1.59 (brs, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.2, 172.3, 166.2, 146.5, 132.4, 130.6, 128.7, 127.7, 118.4, 92.9, 47.8; IR (ATR) 3146, 2365, 1582, 1521, 1413 cm^{-1}; mp 181-183 °C; HRMS (ESI) calcd for $C_{11}H_{11}N_3OS_2Na$ $[M+Na]^+$ 288.0236, found 288.0239.

1 TT-10



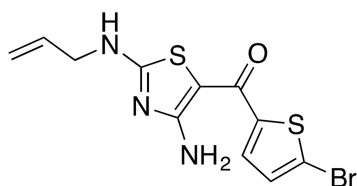
2

3 *t*-BuOK (9.03 g, 80.5 mmol) was added to a solution of allyl isothiocyanate (3.58 mL, 36.8 mmol) and
4 cyanamide (1.55 mg, 36.8 mmol) in DMF (100 mL) under an argon atmosphere at 0°C. After stirring
5 at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(5-fluorothiophen-2-yl)ethan-1-one (7.82 g,
6 35.1 mmol) was added the solution at 0 °C rinsed with DMF (5 mL), and stirred at rt. After 10 hours,
7 the reaction mixture was diluted with cyclopentyl methyl ether (CPME), and quenched with H_2O. The
8 mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*.
9 The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 1/1 to 1/2) to give
10 TT-10 as a yellow solid (2.15 g, 22 % yield).
11 HPLC (Pegasil Silica SP100, hexane/EtOAc= 50/50, flow rate 1.0 ml/min, λ = 254 nm) t_r (minor) =
12 11.36 min, t_r (major) = 13.12 min, purity >99%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (m, 1H), 6.52 (dd,
13 *J* = 4.3, 1.5 Hz, 1H), 5.94 (ddd, *J* = 17.1, 10.1, 5.5 Hz, 1H), 5.75 (brs, 1H), 5.37 (ddd, *J* = 17.1, 2.8, 1.8
14 Hz, 1H), 5.31 (brd, *J* = 10.1, 2.8, 1.2 Hz, 1H), 4.00 (brs, 2H), 1.58 (brs, 2H); ^{13}C NMR (125 MHz,
15 $CDCl_3$) δ 174.4, 172.4, 169.8 (d, $J_{C\text{-}F}$ = 295.2 Hz, 1C), 166.3 (d, $J_{C\text{-}F}$ = 4.5 Hz, 1C), 135.8, 132.3, 126.1,
16 126.1 166.3 (d, $J_{C\text{-}F}$ = 4.5 Hz, 1C), 118.4, 108.9 166.3 (d, $J_{C\text{-}F}$ = 11.8 Hz, 1C), 91.9, 47.9; IR (ATR)
17 2906, 2359, 1541, 1408 cm^{-1}; mp 154-158 °C; HRMS (ESI) calcd for $C_{11}H_{10}FN_3OS_2Na$ [M+Na]$^+$
18 306.0142, found 306.0153. Anal. Calcd for $C_{11}H_{10}FN_3OS_2$: C, 46.63; H, 3.56; N, 14.83%; found C,
19 46.63; H, 3.75; N, 15.02%



20
21

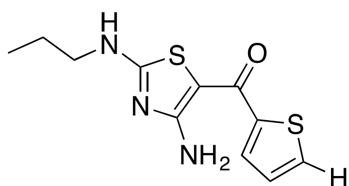
TT-11



t-BuOK (302 mg, 2.69 mmol) was added to a solution of allyl isothiocyanate (125.3 μL, 1.22 mmol) and cyanamide (52 mg, 1.22 mmol) in DMF (4 mL) under an argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(2-nitrophenyl)ethan-1-one (332.2 mg, 1.17 mmol) was added the solution at 0 °C rinsed with DMF (1 mL), and stirred at rt. After 10 hours, the reaction mixture was diluted with cyclopentyl methyl ether (CPME), and quenched with H_2O. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 1/1 to 1/2) to give TT-11 as a yellow solid (147 mg, 36% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.02 (brs, 1H), 8.51 (brs, 1H), 8.10 (brs, 1H), 7.28 (s, 2H), 5.90 (ddd, *J* = 17.1, 10.4, 5.2 Hz, 1H), 5.24 (brd, *J* = 17.1 Hz, 1H), 5.16 (brd, *J* = 10.4 Hz, 1H), 3.97 (brs, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.5, 167.8, 148.9, 133.7(2C), 131.6, 128.1, 117.0, 116.4, 89.8, 46.1; IR (ATR) 3277, 2343, 1587, 1415 cm^{-1}; mp 173-176 °C; HRMS (ESI) calcd for $C_{11}H_{10}N_3OS_2BrNa$ $[M+Na]^+$ 365.9341, found 363.9345.

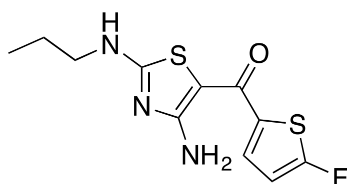
TT-12



Pd/C (10% on carbon, 55% wetted with water, 15.0 mg) was added to a solution of TT-9 (30.0 mg, 113 μmol) in MeOH (2.26 mL). The mixture was stirred for 40 hours at rt under a hydrogen atmosphere. The reaction mixture was filtrated through Celite pad, concentrated *in vacuo*, and the reaction procedure was repeated two times to give TT-12 (28.7 mg, 95% yield) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (dd, *J* = 4.0, 0.9 Hz, 1H), 7.51 (dd, *J* = 4.9, 1.2 Hz, 1H), 7.10 (dd, *J* = 5.2, 4.0 Hz, 1H), 5.67 (brs, 1H), 3.29 (dt, *J* = 6.7, 6.7 Hz, 2H), 1.71 (tq, *J* = 7.3, 7.3 Hz, 2H), 1.02 (t, *J* = 7.3 Hz, 3H) (2 protons are missing.); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 172.7, 166.5, 146.6, 130.5, 128.7, 127.7, 92.8, 47.5, 22.6, 11.5; IR (ATR) 3353, 3153, 2961, 2364, 2338, 1607, 1585, 1417, 1072 cm^{-1}; mp 183-185 °C; HRMS calcd for $C_{11}H_{13}N_3OS_2Na$ $[M+Na]^+$ 290.0392, found 290.0401.

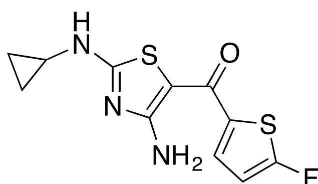
TT-13



Pd/C (10% on carbon, 55% wetted with water, 3.2 mg) was added to a solution of TT-10 (2.9 mg, 10 μmol) in EtOAc (0.3 mL). The mixture was stirred for 48 hours at rt under a hydrogen atmosphere. The reaction mixture was filtrated through Celite pad, concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2; *n*-hexane:EtOAc = 3:1 to 1:1) to give TT-13 (0.8 mg, 28% yield) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (dd, J = 4.3, 3.4 Hz, 1H), 6.49 (dd, J = 4.3, 1.5 Hz, 1H), 5.78 (brs, 1H), 3.29 (dd, J = 7.3, 7.3 Hz, 2H), 1.72 (m, 2H), 1.02 (t, J = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 172.6, 169.8 (d, $J_{C\text{-}F}$ = 295.2 Hz, 1C), 166.5, 135.7, 126.0 (d, $J_{C\text{-}F}$ = 4.5 Hz, 1C), 108.9 (d, $J_{C\text{-}F}$ = 11.8 Hz, 1C), 91.9, 47.5, 22.6, 11.5; IR (ATR) 2363, 1590, 1558, 1456, 1073 cm^{-1}; mp 154-158 °C; HRMS (ESI) calcd for $C_{11}H_{12}FN_3OS_2Na$ $[M+Na]^+$ 308.0298, found 308.0303.

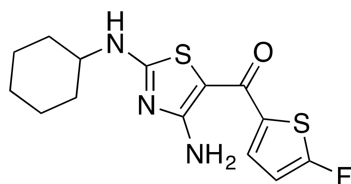
TT-14



NaOMe (39.8 mg, 736.8 μmol), dissolved in MeOH (1.7 mL), was added to a solution of cyclopropyl isothiocyanate (40 μL, 423.6 μmol) and cyanamide (18.0 mg, 428.2 μmol) in MeOH (1.0 mL) under an argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 3 hours, 2-bromo-1-(5-fluorothiophen-2-yl)ethan-1-one (59.9 mg, 268.5 μmol) was added to the solution at rt, and stirred at 50°C. After 1 hour, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 1/1 to 1/2) to give TT-14 as a yellow solid (35.8 mg, 47% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (dd, J = 4.3, 3.4 Hz, 1H), 6.51 (dd, J = 4.3, 1.5 Hz, 1H), 6.07 (brs, 1H), 2.67 (dddd, J = 6.7, 6.7, 3.7, 3.7 Hz, 1H), 0.90 (m, 2H), 0.77 (m, 2H) (2 protons are missing.); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.6, 174.3, 169.9 (d, $J_{C\text{-}F}$ = 295.2 Hz, 1C), 166.2, 135.9, 126.2 (d, $J_{C\text{-}F}$ = 4.5 Hz, 1C), 108.9 (d, $J_{C\text{-}F}$ = 11.8 Hz, 1C), 93.0, 26.3, 7.9 (2C); IR (ATR) 3466, 2875, 1587, 1436, 1198 cm^{-1}; mp 223 °C; HRMS calcd for $C_{11}H_{11}N_3OS_2F$ $[M+H]^+$ 306.0142, found 304.0155. Anal. Calcd for $C_{11}H_{11}N_3OS_2F + 1/3H_2O$: C, 45.66; H, 3.72; N, 14.52%; found C, 45.56; H, 3.64; N, 14.26%

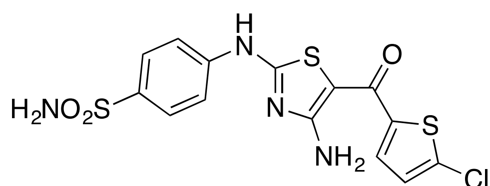
TT-15



NaOMe (120.3 mg, 2.23 mmol), dissolved in MeOH (5.0 mL), was added to a solution of cyclohexyl isothiocyanate (180 μL, 1.27 mmol) and cyanamide (57.3 mg, 1.36 mmol) in MeOH (3.0 mL) under an argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 3 hours, 2-bromo-1-(5-fluorothiophen-2-yl)ethan-1-one (205.5 mg, 921.2 μmol) was added to the solution at rt, and stirred at 50°C. After 1 hour, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 1/1) to give TT-15 as a yellow solid (25.1 mg, 8% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ7.26 (m, 1H), 6.49 (dd, J = 4.3, 1.5 Hz, 1H), 5.54 (brs, 1H), 3.41 (brs, 1H), 2.10-2.07 (m, 2H), 1.81-1.77 (m, 2H), 1.69-1.64 (m, 1H), 1.46-1.37 (m, 2H), 1.34-1.21 (m, 3H) (2 protons are missing.); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 170.9, 169.8 (d, $J_{C\text{-}F}$ = 291.5 Hz, 1C), 166.5, 135.9, 126.0 (d, $J_{C\text{-}F}$ = 4.5 Hz, 1C), 108.8 (d, $J_{C\text{-}F}$ = 11.8 Hz, 1C), 91.6, 55.1, 33.0 (2C), 25.4, 24.7 (2C); IR (ATR) 3188, 2359, 1557, 1430, 1320, 1033 cm^{-1}; mp 192 °C; HRMS calcd for $C_{14}H_{16}N_3OS_2F$ $[M+H]^+$ 326.0792, found 326.0805. Anal. Calcd for $C_{14}H_{16}N_3OS_2F$ + $1/8H_2O$: C, 51.32; H, 5.00; N, 12.82%; found C, 51.52; H, 5.01; N, 12.57%

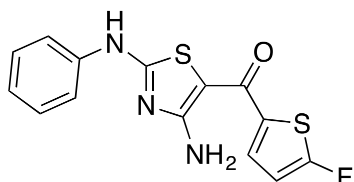
TT-16



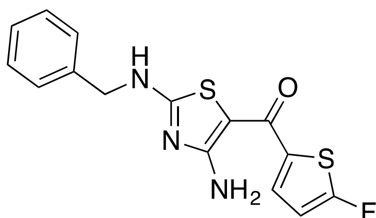
t-BuOK (302 mg, 2.69 mmol) was added to a solution of 4-isothiocyanatobenzenesulfonamide (261.4 mg, 1.22 mmol) and cyanamide (52 mg, 1.22 mmol) in THF (4.3 mL) under an argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(5-chlorothiophen-2-yl)ethan-1-one (279.3 mg, 1.17 mmol) was added the solution at 0 °C rinsed with THF, and stirred at rt. After 10 hours, the mixture was filtrated through a short pad of silica gel and eluted with EtOAc and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc) to give TT-16 as a yellow solid (27 mg, 6% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.3 (brs, 1H), 8.36 (brs, 2H), 7.83 (brd, J = 8.9 Hz, 1H), 7.80 (brd,

1 J = 8.9 Hz, 1H), 7.44 (d, J = 4.3 Hz, 1H), 7.29 (s, 2H), 7.23 (d, J = 4.3 Hz, 1H); ^{13}C NMR (125 MHz,
2 DMSO-d_6) δ 172.2, 166.6, 166.3, 145.7, 142.3, 138.3, 134.1, 128.5(2C), 127.1(2C), 118.5(2C), 90.4;
3 IR (ATR) 3265, 2179, 1594, 1416 cm^{-1}; mp 214 °C (decomposed); HRMS (ESI) calcd for
4 $C_{14}H_{12}N_4O_3S_3Cl$ [M+H]$^+$ 414.9755, found 414.9770; Anal. Calcd for $C_{14}H_{11}N_4O_2S_3Cl$ + 1/4EtOH: C,
5 40.84; H, 2.95; N, 13.14%, found C, 41.04; H, 2.69; N, 12.90%.
6
7 TT-19



8
9 *t*-BuOK (171.7 mg, 1.53 mmol) was added to a solution of phenyl isothiocyanate (107.2 mg, 700 μmol)
10 and cyanamide (29.4 mg, 700 μmol) in DMF (2.0 mL) under an argon atmosphere at 0°C. After stirring
11 at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(5-fluorothiophen-2-yl)ethan-1-one (148.7
12 mg, 667 μmol) was added the solution at 0 °C rinsed with DMF (0.5 mL), and stirred at rt. After 20
13 hours, the mixture was filtrated through a short pad of silica gel and eluted with EtOAc and the filtrate
14 was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-
15 hexane/EtOAc = 3/1) to give TT-19 as a yellow solid (8.1 mg, 4% yield).
16 ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.97 (brs, 1H), 8.31 (brs, 2H), 7.66 (d, J = 7.6 Hz, 2H), 7.39 (dd, J
17 = 7.3, 7.3 Hz, 2H), 7.29 (dd, J = 4.3, 3.7 Hz, 1H), 7.12 (t, J = 7.3 Hz, 1H), 6.84 (dd, J = 4.3, 1.8 Hz,
18 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.3, 169.2, 166.9, 166.8, 139.4, 136.3, 129.2 (2C), 125.9 (d,
19 J = 4.5 Hz), 123.7, 119.2 (2C), 110.1 (d, J = 11.8 Hz), 89.6; ^{19}F-NMR (471 MHz, DMSO-d_6) δ –124.6
20 (s); IR (ATR) 3273, 3084, 2917, 2849, 1702, 1597, 1556, 1422, 1200 cm^{-1}; HRMS calcd for
21 $C_{14}H_{10}FN_3OS_2Na$ [M+Na]$^+$ 342.0142, found 342.0132.
22
23 TT-20

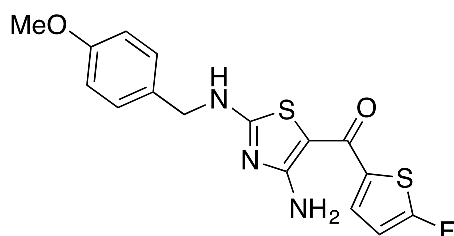


24
25 *t*-BuOK (190.0 mg, 1.69 mmol) was added to a solution of benzyl isothiocyanate (110 μL, 833.1 μmol)
26 and cyanamide (34.3 mg, 815.9 μmol) in THF (2.0 mL) under an argon atmosphere at 0°C. After stirring
27 at 0 °C to room temperature (rt) for 30 minutes, 2-bromo-1-(5-fluorothiophen-2-yl)ethan-1-one (166.5

mg, 746.4 μmol) was added the solution at 0 °C rinsed with THF (1.0 mL), and stirred at rt. After 21 hours, the reaction mixture was diluted with diethyl ether, and quenched with H_2O. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 3/1 to 1/1) to give TT-20 as a yellow solid (1.5 mg, 1% yield).

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38 (m, 6H), 7.25 (dd, J = 4.3, 3.4 Hz, 1H), 6.48 (dd, J = 4.3, 1.5 Hz, 1H), 5.88 (brs, 1H), 4.54 (brs, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 174.5, 172.2, 169.9 (d, J_{C-F} = 295.2 Hz, 1C), 166.2, 136.2, 135.8, 129.2 (2C), 128.5, 127.8 (2C), 126.1 (d, J_{C-F} = 4.5 Hz, 1C), 108.9 (d, J_{C-F} = 11.8 Hz, 1C), 91.8, 49.6; IR (ATR) 3489, 2912, 1584, 1406, 1199 cm^{-1}; mp 193 °C; HRMS calcd for $C_{15}H_{13}N_3OS_2F$ [M+H]$^+$ 334.0479, found 334.0495.

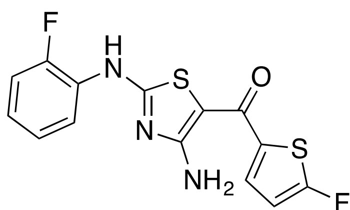
TT-21



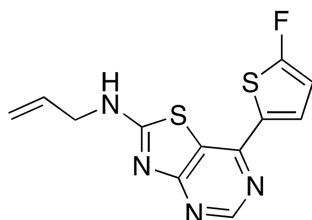
NaOMe (39.2 mg, 725.7 μmol), dissolved in MeOH (1.7 mL), was added to a solution of 4-methoxybenzyl isothiocyanate (65 μL, 427.9 μmol) and cyanamide (17.9 mg, 425.6 μmol) in MeOH (1.0 mL) under an argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 3 hours, 2-bromo-1-(5-fluorothiophen-2-yl)ethan-1-one (67.4 mg, 302.2 μmol) was added to the solution at rt, and stirred at rt. After 15 hours, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 1/1) to give TT-21 as a yellow solid (27.0 mg, 25% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29-7.23 (m, 3H), 6.31 (d, J = 8.5 Hz, 1H), 6.48 (dd, J = 4.3, 1.5 Hz, 1H), 5.87 (brs, 1H), 4.45 (brd, J = 4.6 Hz, 1H), 3.82 (s, 3H), 3.53 (brs, 1H) (1 proton is missing.); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 172.0, 169.8 (d, J_{C-F} = 295.2 Hz, 1C), 166.3, 159.8, 135.8, 129.3 (2C), 128.0, 126.1 (d, J_{C-F} = 4.5 Hz, 1C), 114.6 (2C), 108.9 (d, J_{C-F} = 11.8 Hz, 1C), 91.8, 55.5, 49.2; IR (ATR) 2361, 1585, 1408, 1246, 1177 cm^{-1}; mp 174 °C; HRMS calcd for $C_{16}H_{15}N_3O_2S_2F$ [M+H]$^+$ 364.0584, found 364.0596. Anal. Calcd for $C_{16}H_{15}N_3O_2S_2F$ + 1/2H_2O: C, 51.60; H, 4.06; N, 11.28%; found C, 51.77; H, 4.18; N, 11.36%

TT-22

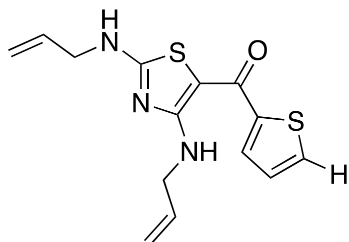
1 

2 NaOMe (38.0 mg, 703.4 μmol), dissolved in MeOH (1.7 mL), was added to a solution of *o*-fluorophenyl
3 isothiocyanate (50 μL, 408.0 μmol) and cyanamide (17.2 mg, 409.1 μmol) in MeOH (1.0 mL) under an
4 argon atmosphere at 0°C. After stirring at 0 °C to room temperature (rt) for 3 hours, 2-bromo-1-(5-
5 fluorothiophen-2-yl)ethan-1-one (68.2 mg, 305.7 μmol) was added to the solution at rt, and stirred at
6 50°C. After 2 hours, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica
7 gel column chromatography (n-hexane/EtOAc = 2/1) to give TT-22 as a yellow solid (34.7 mg, 34%
8 yield).
9 ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (dd, J = 7.9, 7.9 Hz, 1H), 7.56 (brs, 1H), 7.29 (dd, J = 4.3, 3.7 Hz,
10 1H), 7.25-7.12 (m, 3H), 6.50 (dd, J = 4.3, 1.2 Hz, 1H) (3 protons are missing); ^{13}C NMR (125 MHz,
11 $CDCl_3$) δ 175.3, 170.2 (d, J_{C-F} = 297.9 Hz, 1C), 167.6, 165.1, 153.1 (d, J_{C-F} = 245.2 Hz, 1C), 135.4,
12 126.9 (d, J_{C-F} = 10.9 Hz, 1C), 126.7 (d, J_{C-F} = 4.5 Hz, 1C), 125.5 (d, J_{C-F} = 7.3 Hz, 1C), 125.1 (d, J_{C-F} =
13 3.6 Hz, 1C), 121.1, 115.9 (d, J_{C-F} = 19.1 Hz, 1C), 109.1 (d, J_{C-F} = 11.8 Hz, 1C), 92.3; IR (ATR) 3425,
14 3304, 2956, 1625, 1414, 1258 cm^{-1}; mp 178 °C; HRMS calcd for $C_{14}H_{10}N_3OS_2F_2$ $[M+H]^+$ 338.0228,
15 found 338.0237; Anal. Calcd for $C_{14}H_9N_3O_2S_2F_2$: C, 49.84; H, 2.69; N, 12.46%; found C, 49.80; H,
16 2.90; N, 12.49%.
17
18 TT-23

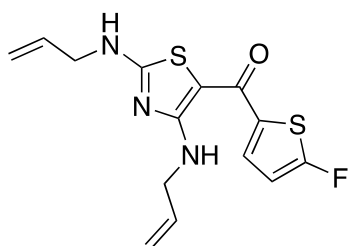
19 

20 Ammonium acetate (6.4 mg, 70.0 μmol) and ethyl orthoformate (13.4 mg, 70.0 μmol) were added to a
21 solution of TT-10 (4.0 mg, 14.0 μmol) in toluene (1 mL). After stirring under reflux for 17 hours before
22 concentration under reduced pressure. The residue was purified by flash chromatography (SiO_2; *n*-
23 hexane:EtOAc = 1:1) to give TT-23 (1.7 mg, 41% yield) as a yellow solid.
24 ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (s, 1H), 7.30 (dd, J = 4.0, 3.7 Hz, 1H), 6.62 (dd, J = 4.3, 1.8 Hz,
25 1H), 6.12 (brs, 1H), 5.99 (ddd, J = 17.1, 10.1, 5.8 Hz, 1H), 5.41 (brd, J = 17.1 Hz, 1H), 5.32 (brd, J =
26 10.1 Hz, 1H), 4.22 (brd, J = 5.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 167.6 (d, J_{C-F} =
27 291.5 Hz, 1C), 155.3, 148.6, 133.7, 130.77, 130.75, 126.3 (d, J_{C-F} = 4.5 Hz, 1C), 116.77, 116.75, 110.9

1 (d, $J_{C\text{-}F}$ = 11.8 Hz, 1C), 46.4; IR (ATR) 2871, 1618, 1479, 1383, 767 cm^{-1}; mp 236 °C; HRMS calcd for
2 $C_{12}H_{10}N_4S_2F$ $[M+H]^+$ 293.0325, found 293.0334.
3
4 TT-24

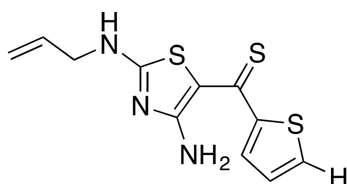


5
6 *t*-BuOK (11.2 mg, 100 µmol) was added to a solution of TT-9 (26.5 mg, 100 µmol) in THF/DMF (3/1,
7 400 µL) at rt under an argon atmosphere. After stirring at rt for 1 hour, allyl bromide (6.8 µL, 80.0 µmol)
8 was added to the mixture, and stirred at rt for 2 hours. The reaction mixture was quenched with H_2O,
9 extracted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue
10 was purified by flash column chromatography (SiO_2; *n*-hexane:EtOAc = 5:1 to 4:1) to give TT-24 (14.3
11 mg, 47% yield) as a yellow solid.
12 ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (dd, *J* = 3.7, 0.9 Hz, 1H), 7.49 (dd, *J* = 4.9, 0.9 Hz, 1H), 7.09 (dd,
13 *J* = 4.9, 3.7 Hz, 1H), 5.82 (ddt, *J* = 17.1, 10.1, 5.8 Hz, 2H), 5.27 (d, *J* = 11.3 Hz, 2H), 5.24 (d, *J* = 17.1
14 Hz, 2H), 4.09 (brs, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.8, 172.0, 166.7, 146.9, 131.3 (2C), 130.4,
15 128.6, 127.7, 118.9 (2C), 93.4, 52.7 (2C); IR (ATR) 3330, 3262, 3157, 2360, 2341, 1605, 1538, 1415
16 cm^{-1}; mp 99-100 °C; HRMS calcd for $C_{14}H_{15}N_3OS_2Na$ $[M+Na]^+$ 328.0549, found 328.0552; Anal.
17 Calcd for $C_{14}H_{15}N_3OS_2$: C, 55.06; H, 4.95; N, 13.76%, found C, 55.06; H, 5.06; N, 13.54%.
18
19 TT-25

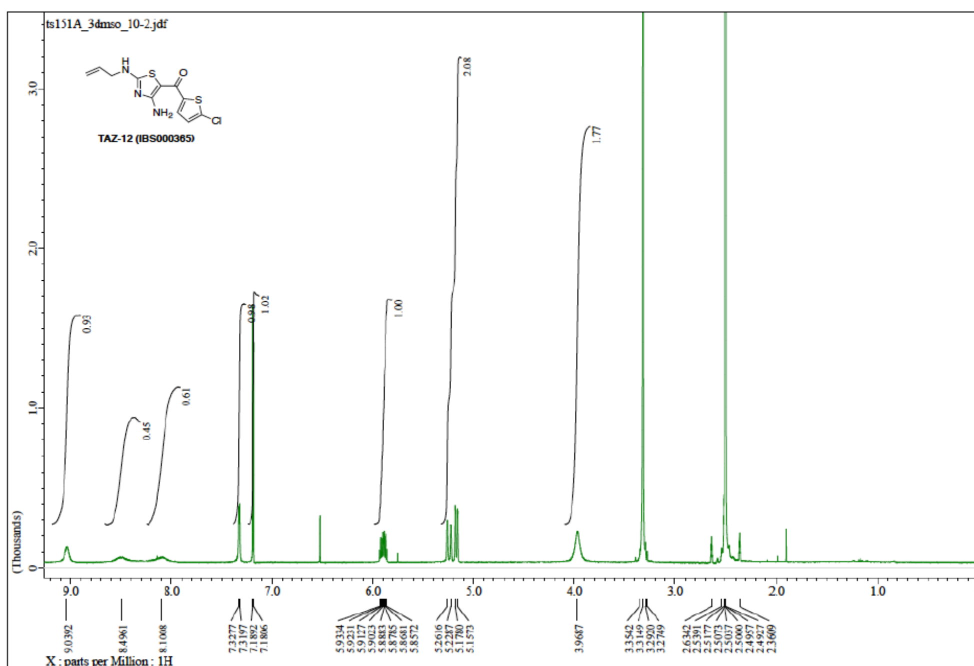


20
21 *t*-BuOK (11.7 mg, 104.3 µmol) was added to a solution of TT-10 (30.3 mg, 106.9 µmol) in THF/DMF
22 (3/1, 440 µL) at rt under an argon atmosphere. After stirring at rt for 5 minutes, allyl bromide (10.7 mg,
23 88.4 µmol) was added to the mixture, and stirred at rt for 3 hours. The reaction mixture was quenched
24 with sat. NH_4Cl aq., extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated *in*
25 *vacuo*. The residue was purified by flash column chromatography (SiO_2; *n*-hexane:EtOAc = 4:1 to 2:1)
26 to give TT-25 (13.2 mg, 46% yield) as a yellow solid.

1 ^1H NMR (500 MHz, $CDCl_3$) δ 7.26 (dd, J = 4.3, 3.4 Hz, 1H), 6.48 (dd, J = 4.3, 1.5 Hz, 1H), 5.82 (ddd,
2 J = 17.1, 10.4, 5.5 Hz, 2H), 5.29-5.23 (m, 4H), 4.10 (brs, 4H) (1 proton is missing.); ^{13}C NMR (125
3 MHz, $CDCl_3$) δ 174.0, 172.0, 169.7 (d, J_{C-F} = 295.2 Hz, 1C), 166.8, 136.1, 131.2 (2C), 125.9 (d, J_{C-F} =
4 4.5 Hz, 1C), 119.0 (2C), 108.8 (d, J_{C-F} = 11.8 Hz, 1C), 92.5, 52.6 (2C); IR (ATR) 3357, 3253, 3193,
5 2360, 1609, 1519, 1411, 1201, 924 cm^{-1}; mp 105 °C; HRMS calcd for $C_{14}H_{15}N_4OS_2F$ $[M+H]^+$ 324.0635,
6 found 324.0637. Anal. Calcd for $C_{14}H_{15}N_4OS_2F$ + 1/4H_2O: C, 51.28; H, 4.46; N, 12.81%; found C,
7 51.42; H, 4.34; N, 12.54%
8
9 TT-26



10
11 Lawesson's Reagent (22.0 mg, 54.5 μmol) was added to a solution of TT-9 (21.9 mg, 82.5 μmol) in
12 toluene (330 μL) at rt under an argon atmosphere. The mixture was stirred at 85 °C for 16 hours, and
13 then concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2; *n*-
14 hexane:EtOAc = 3:1 to 0:1) two times to give TT-26 (4.1 mg, 18% yield) as a yellow solid.
15 ^1H NMR (500 MHz, DMSO) δ 11.4 (brs, 1H), 9.34 (brs, 1H), 7.65 (dd, *J* = 5.2, 0.9 Hz, 1H), 7.32(d, *J*
16 = 3.4 Hz, 1H), 7.07 (dd, *J* = 5.2, 3.7 Hz, 1H), 6.30 (brs, 1H), 5.91 (m, 1H), 5.23 (brd, *J* = 17.1 Hz, 1H),
17 5.18 (d, *J* = 10.1 Hz, 1H), 3.82 (brs, 2H); ^{13}C NMR (125 MHz, DMSO) δ 175.4, 170.3, 169.5, 154.2,
18 133.6, 130.4, 127.1, 124.5, 116.5, 110.3, 45.6; IR (ATR) 3200, 3051, 2920, 2359, 2334, 1611, 1572,
19 1421 cm^{-1}; mp 156-158 °C; HRMS calcd for $C_{11}H_{11}N_3S_3Na$ $[M+Na]^+$ 304.0007, found 304.0006; Anal.
20 Calcd for $C_{11}H_{11}N_3S_3$ + 1/3EtOH: C, 47.22; H, 4.42; N, 14.16%; found C, 47.03; H, 4.20; N, 14.15%.
21
22
23 Data of TAZ-12 (13), TT-2 (13), TT-3 (13), TT-4 (13), TT-7 (13), TT-8 (13), TT-17 (14), and TT-18
24 (15) have been already reported.
25



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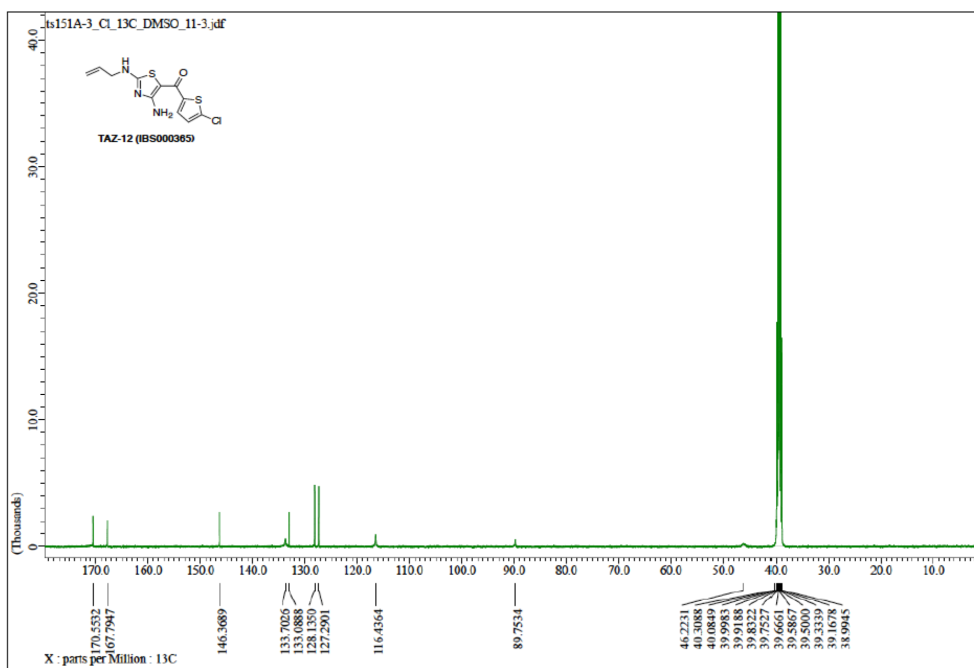

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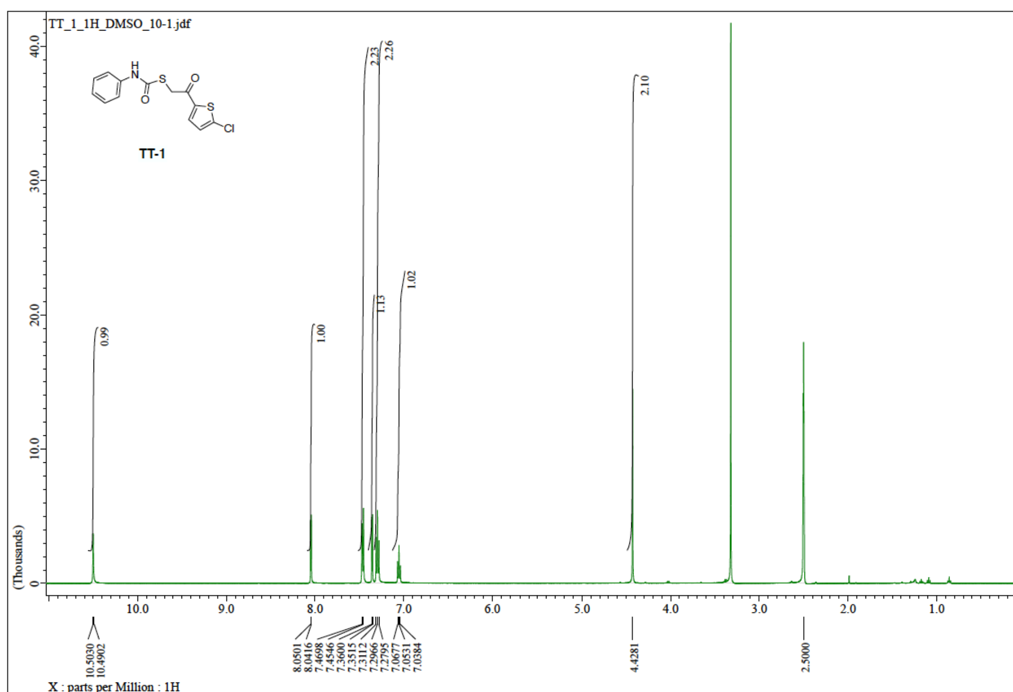

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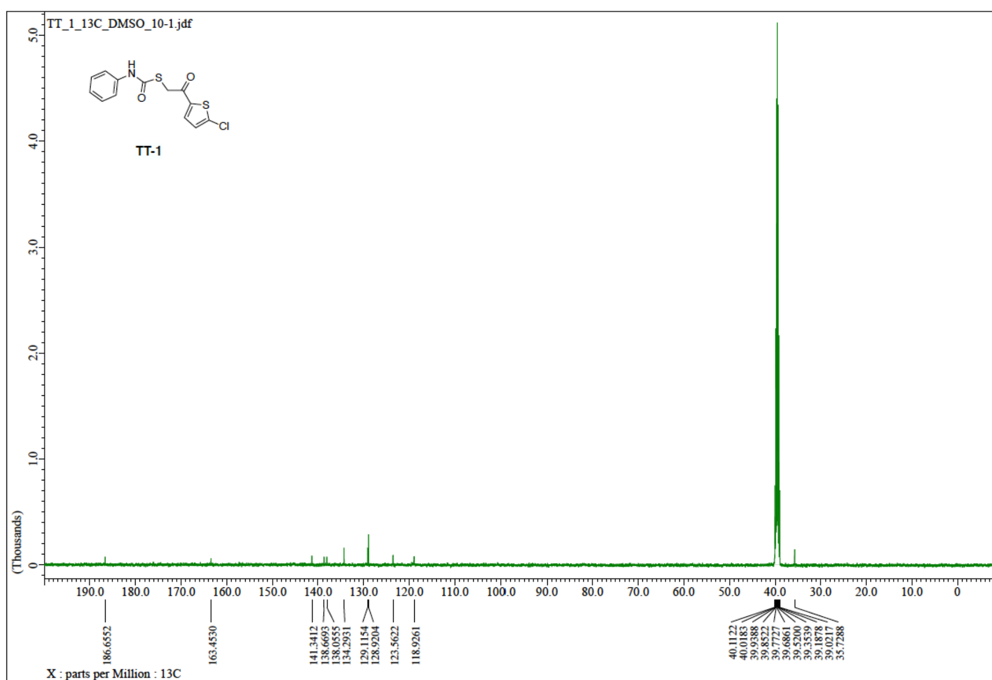

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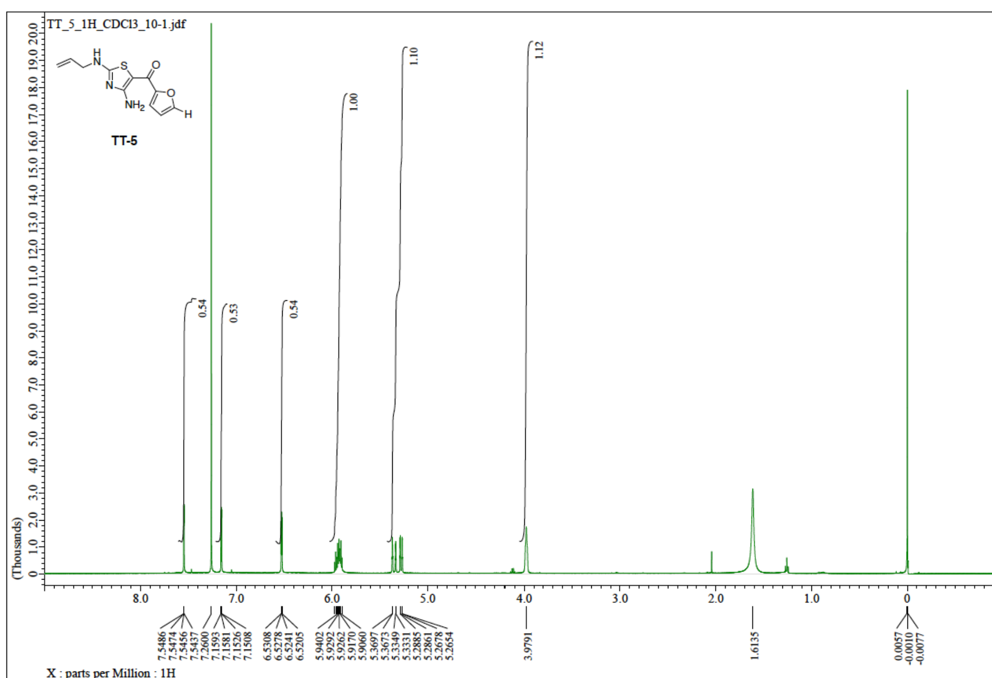

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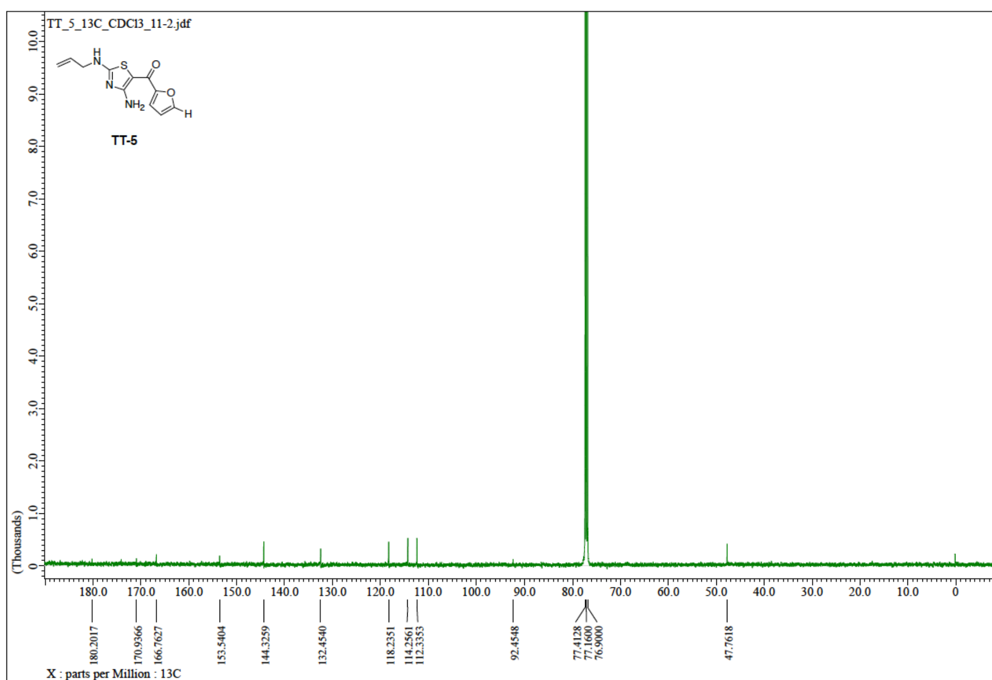

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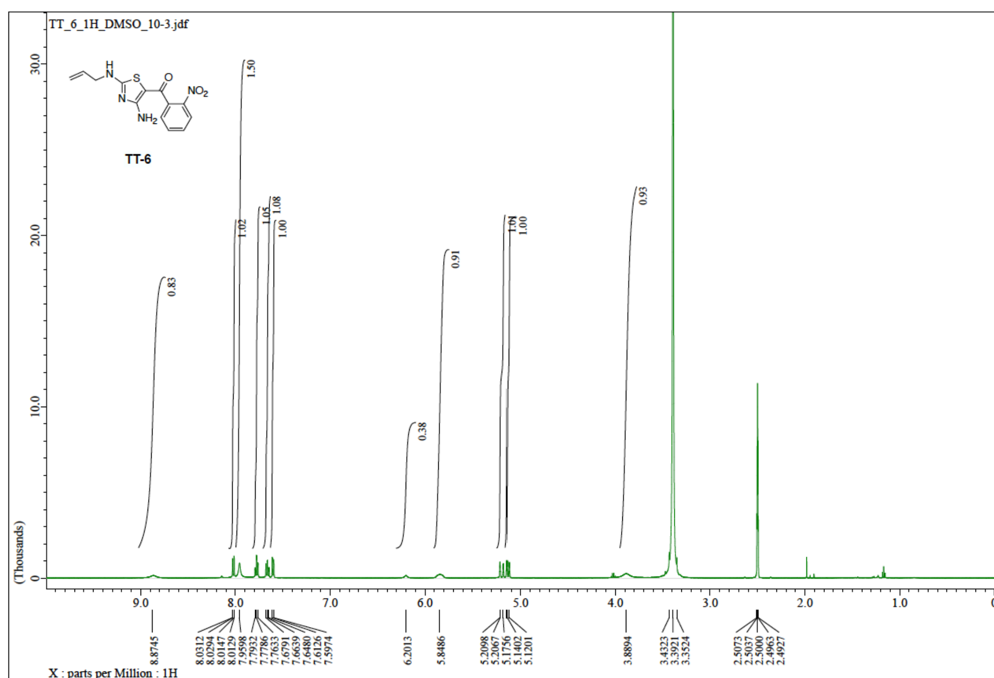

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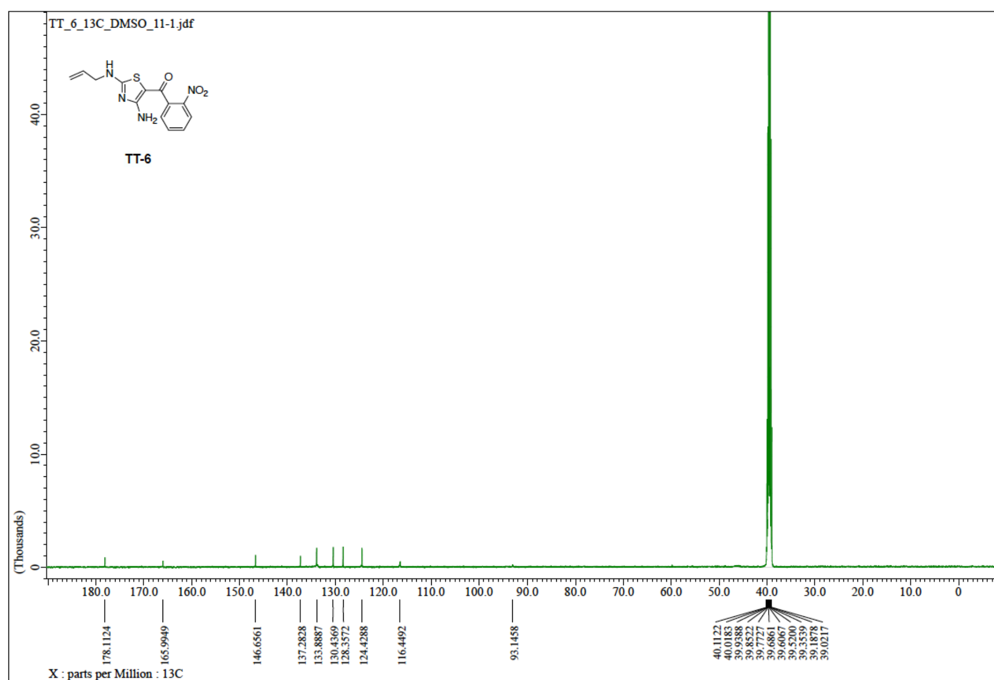

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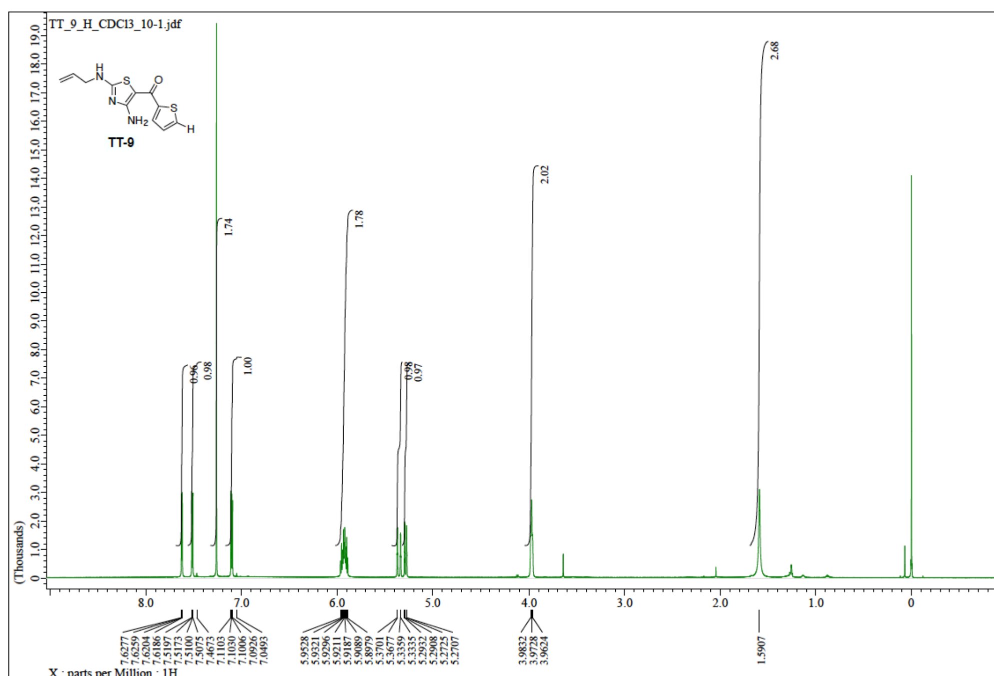

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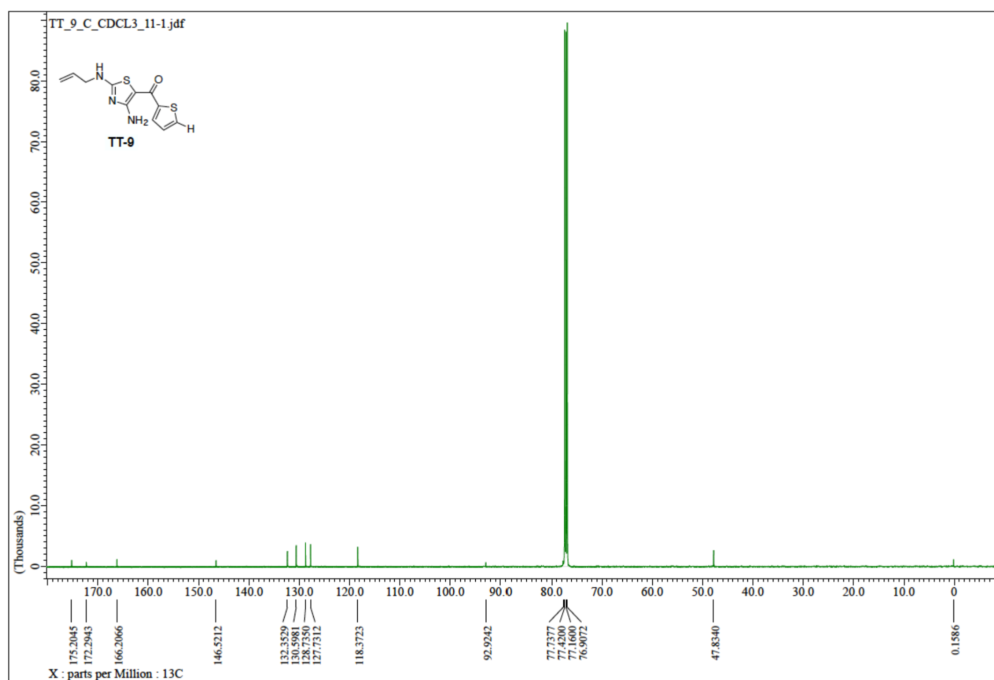

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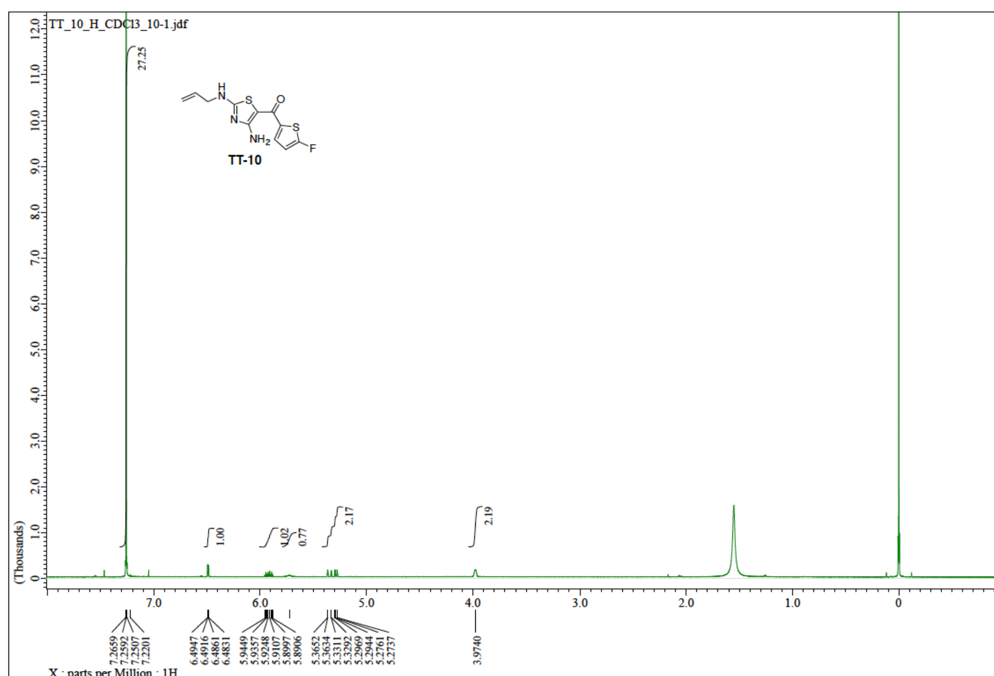

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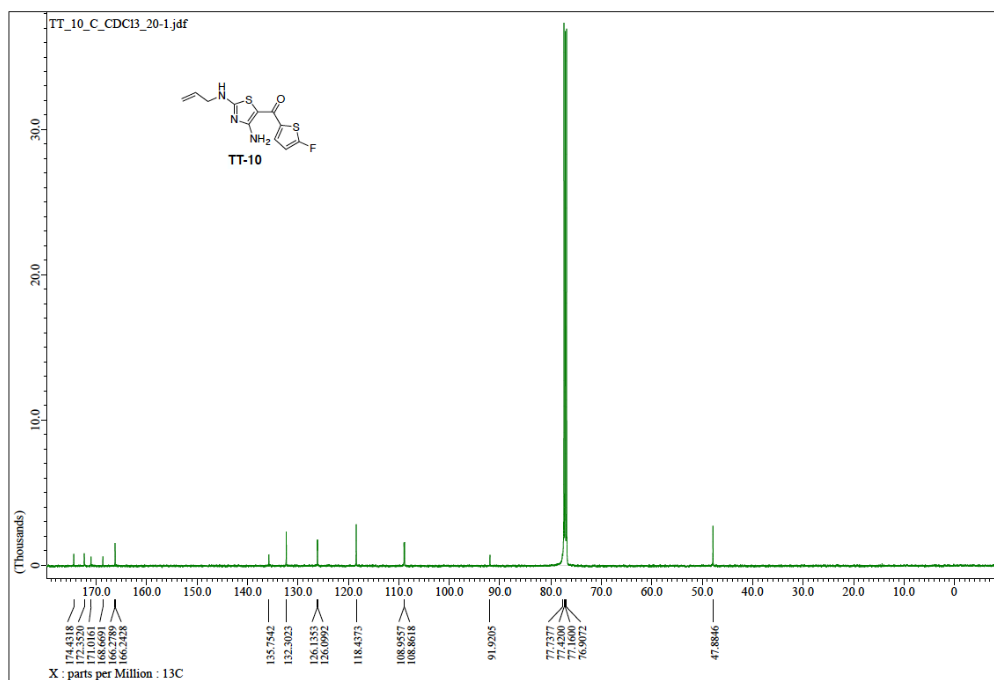

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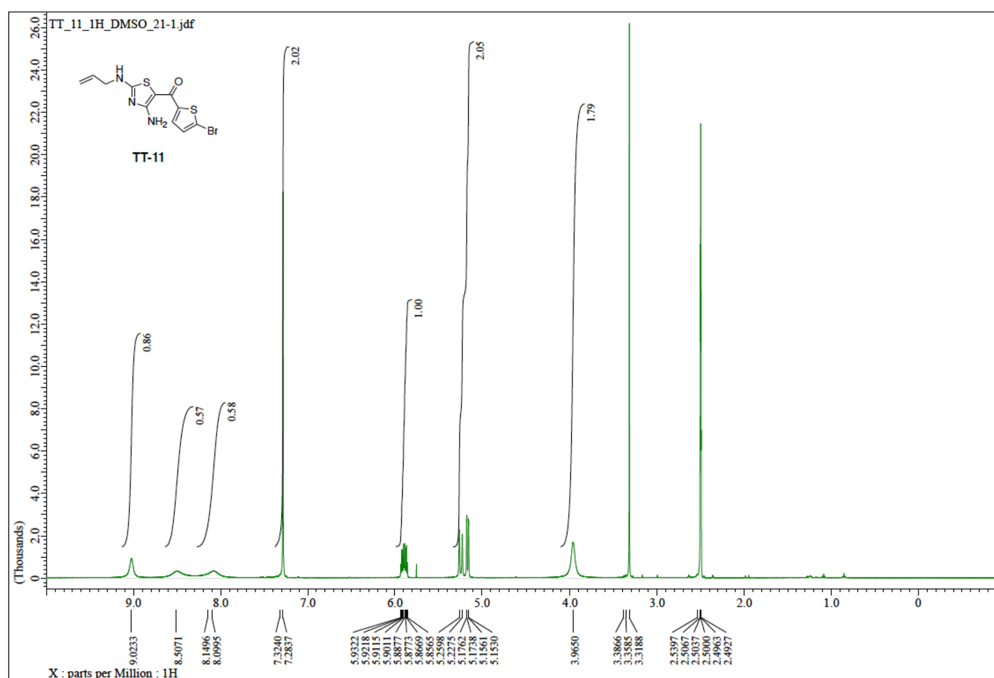

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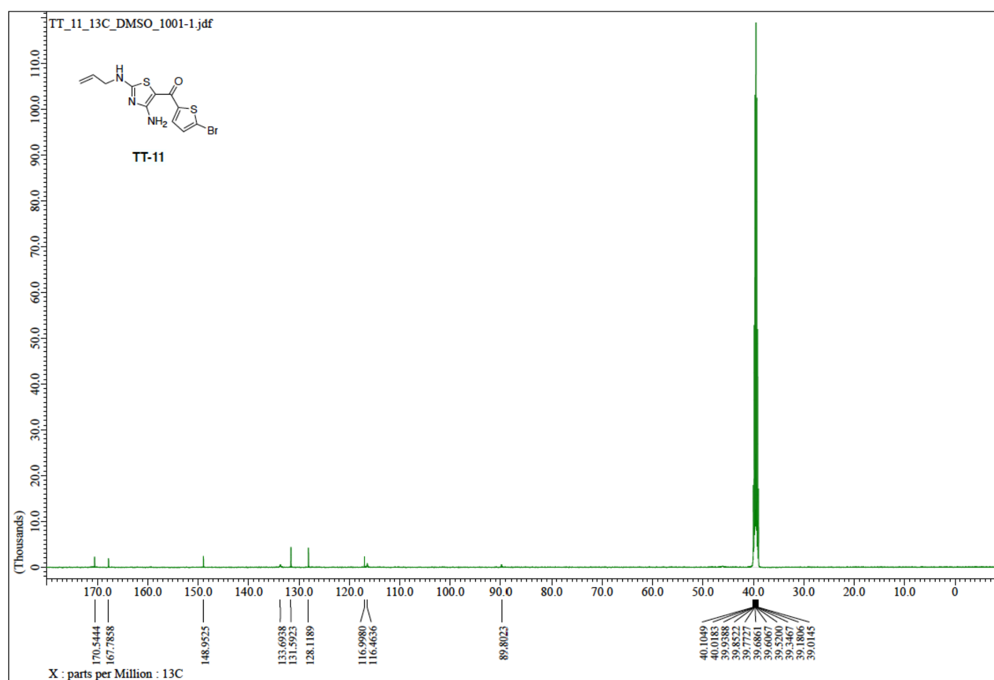

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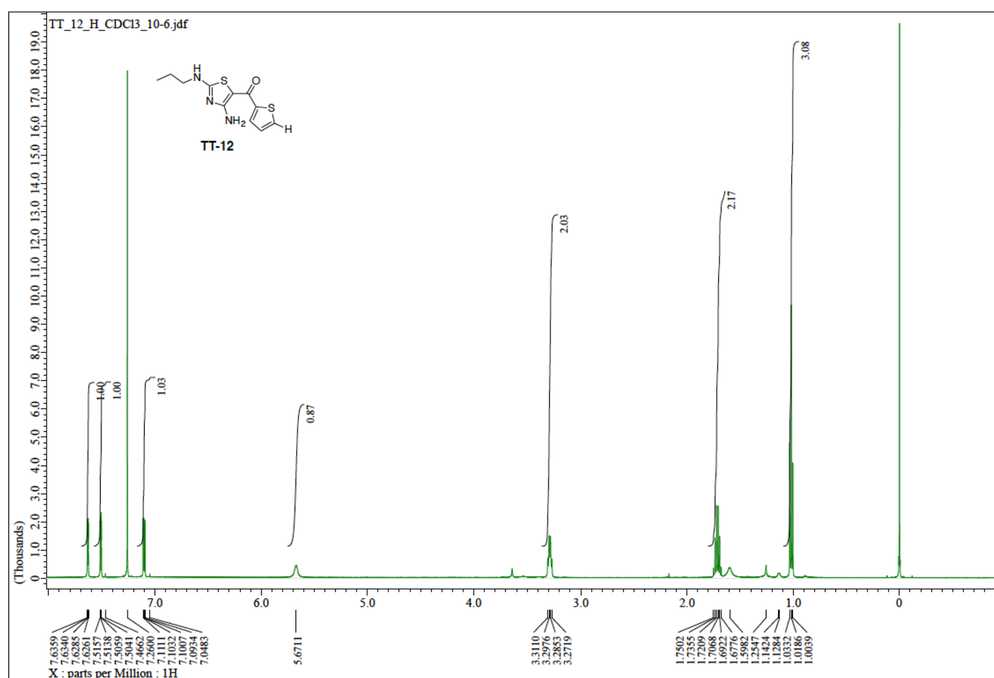

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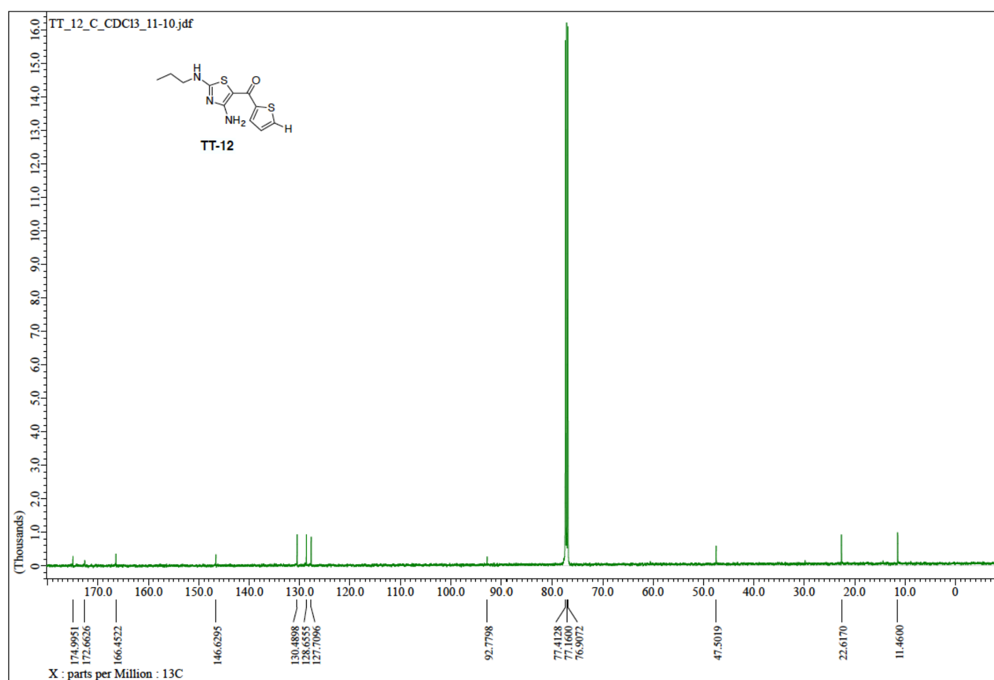

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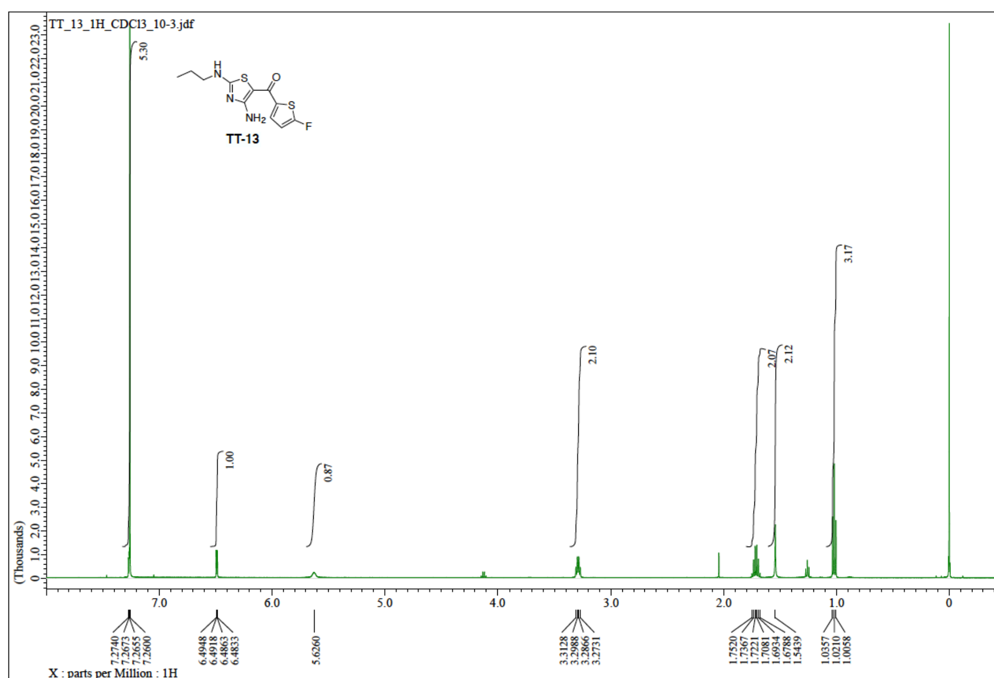

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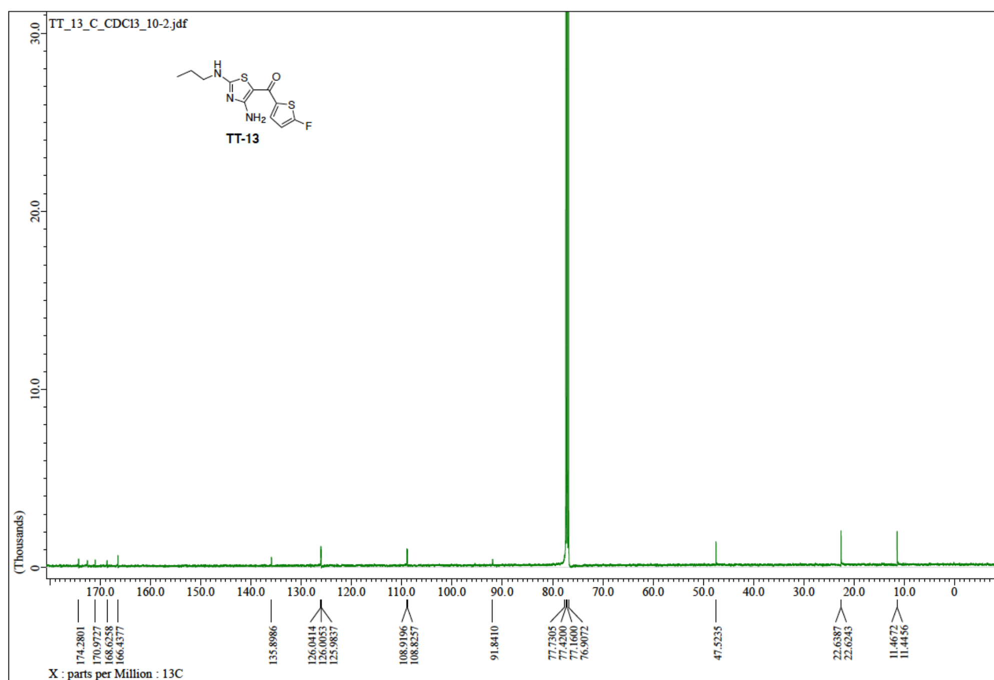

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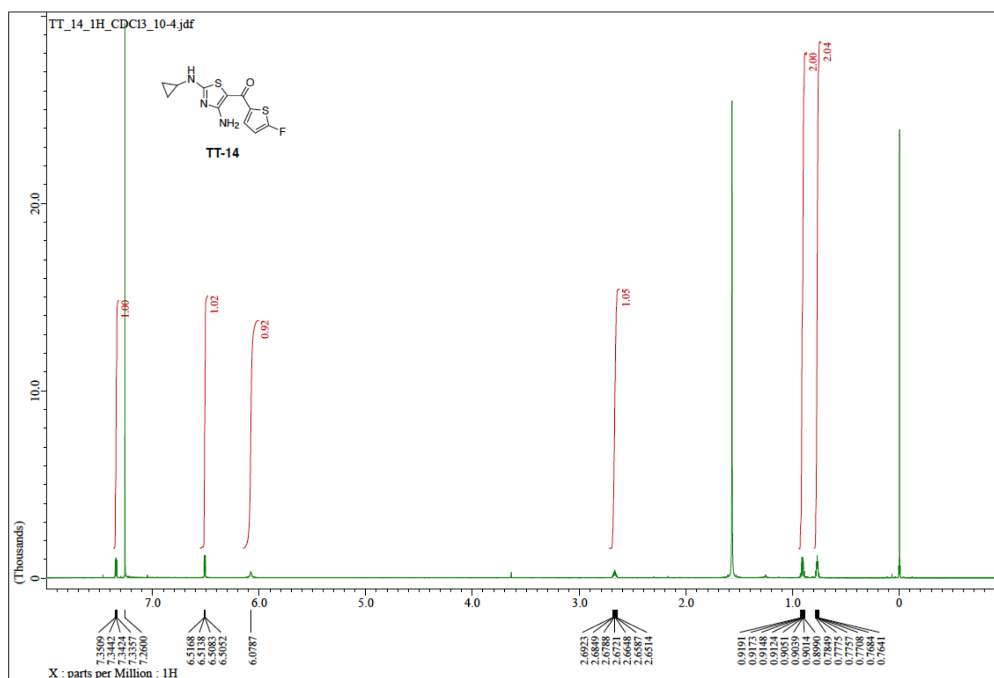

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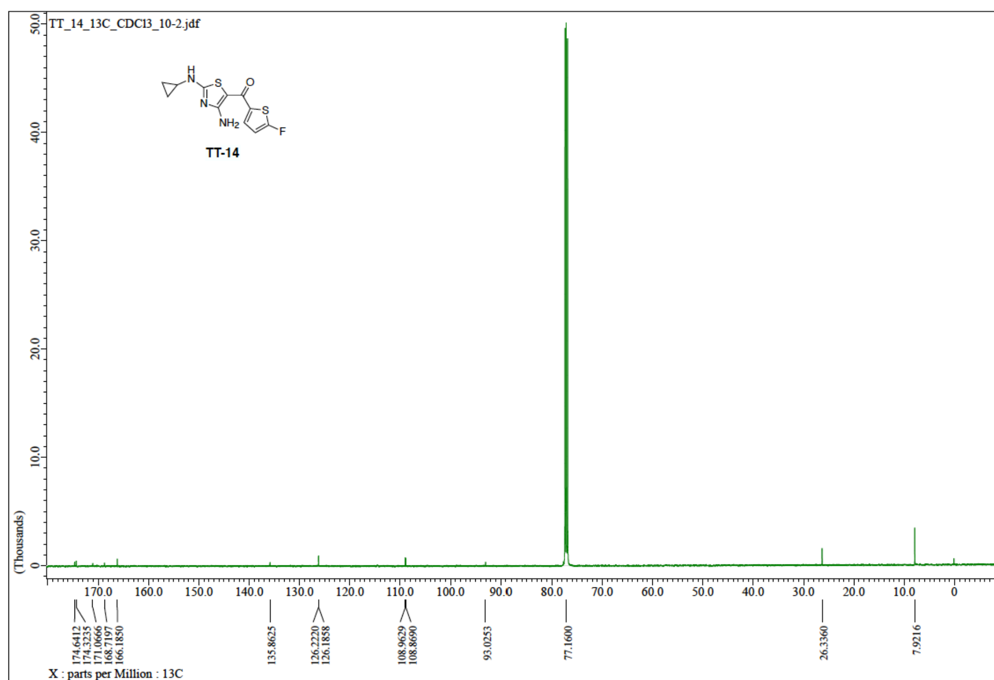

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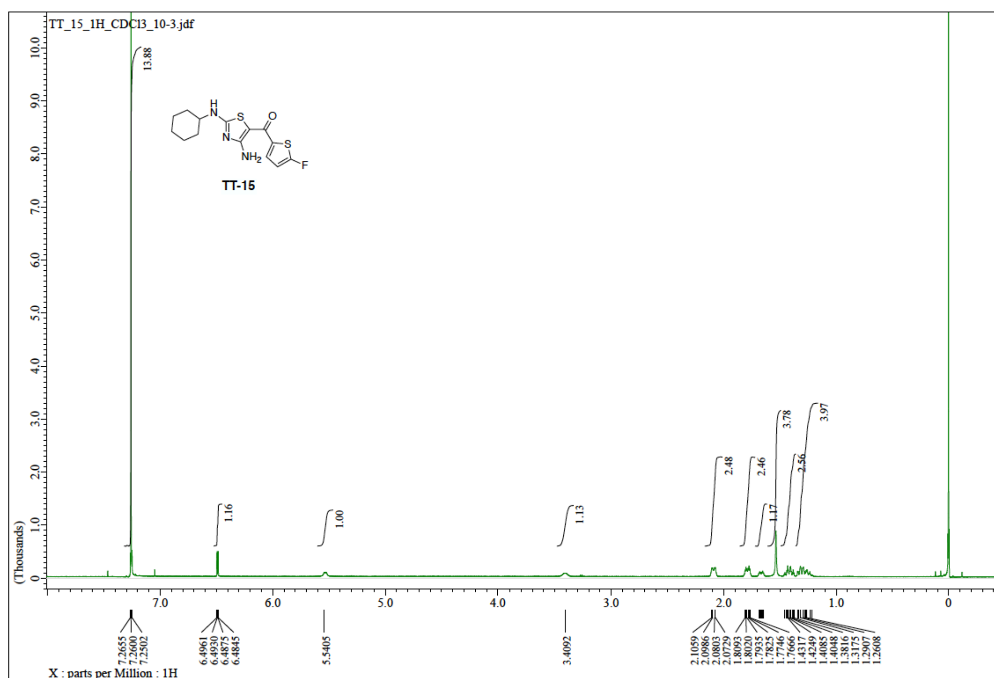

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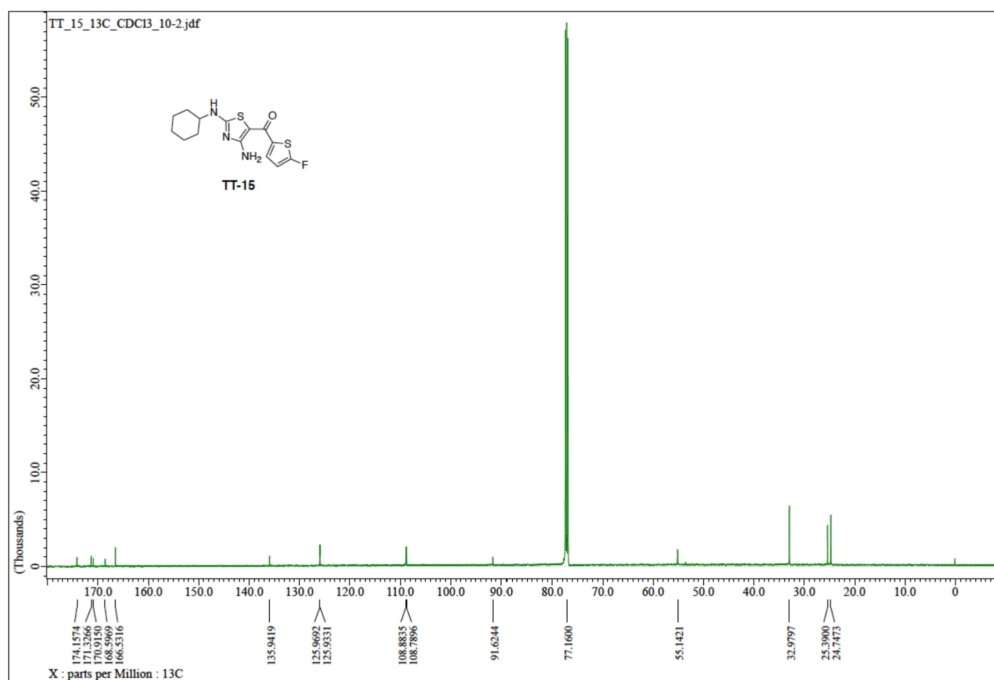

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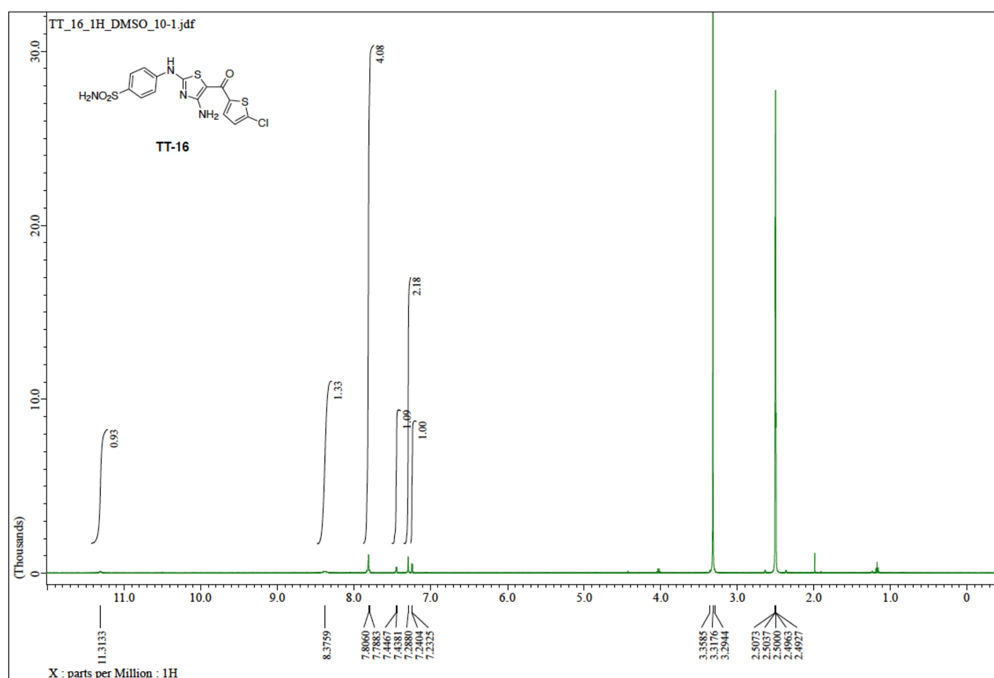

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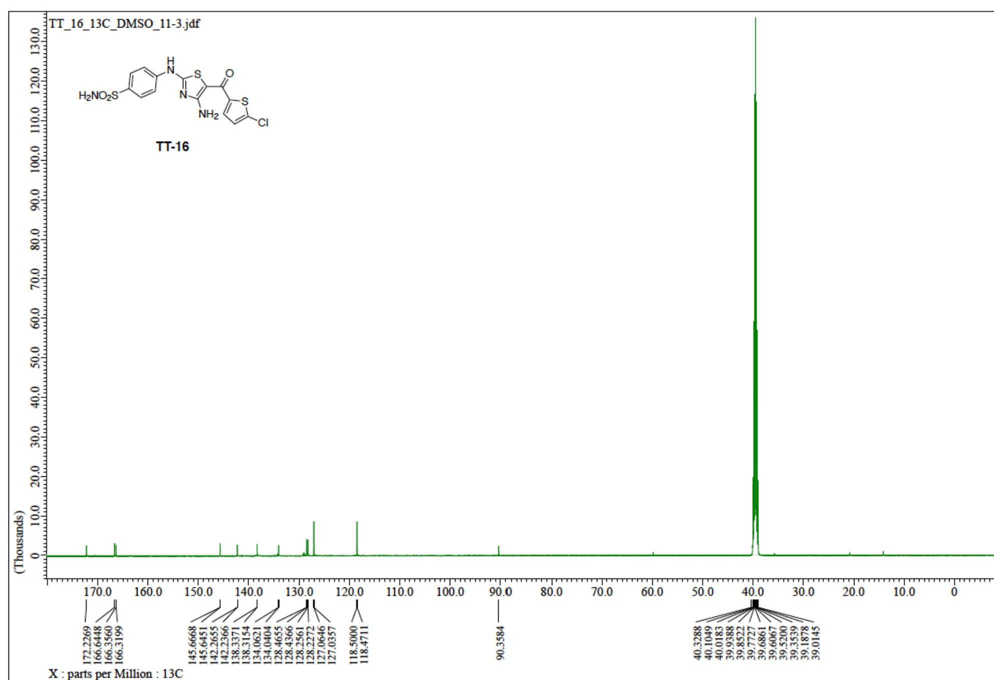

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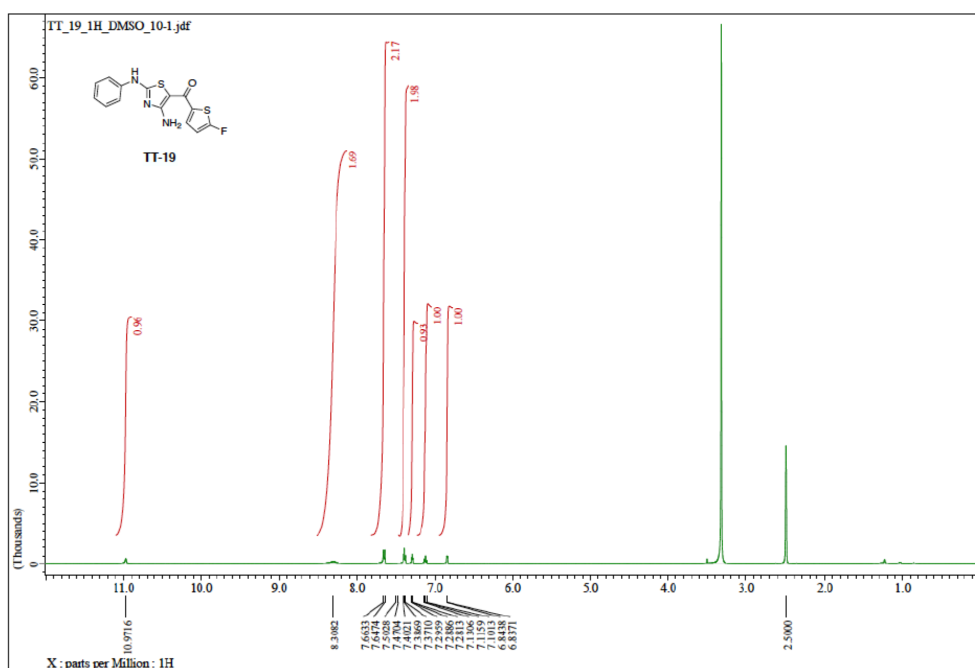

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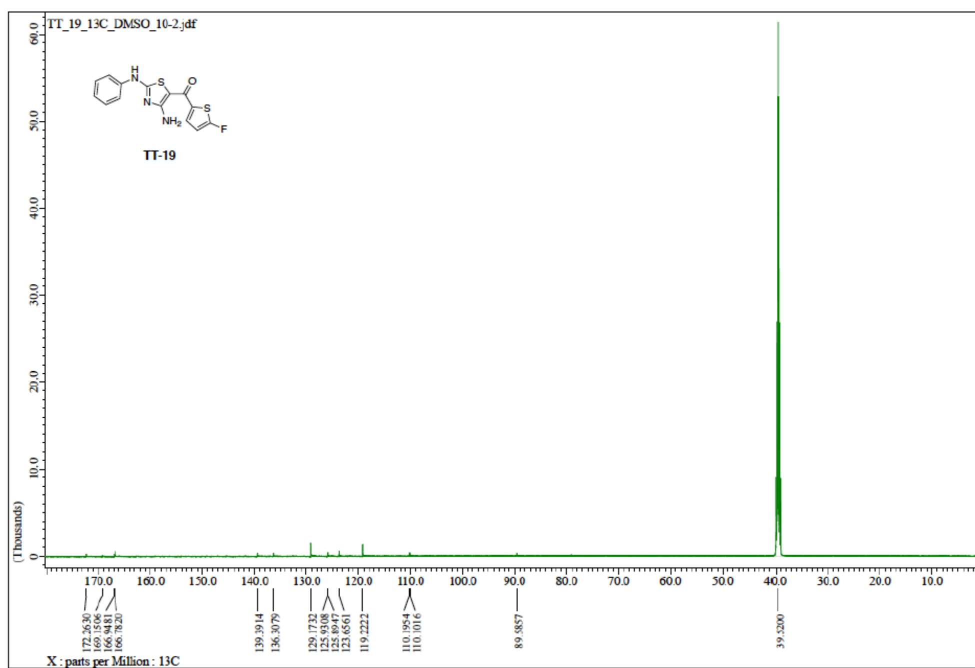

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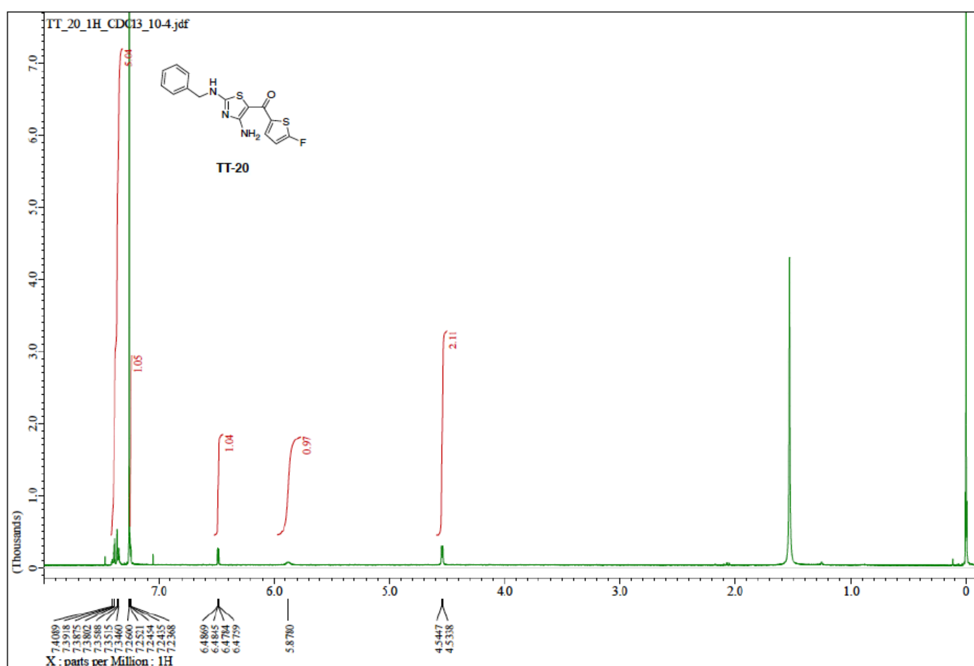

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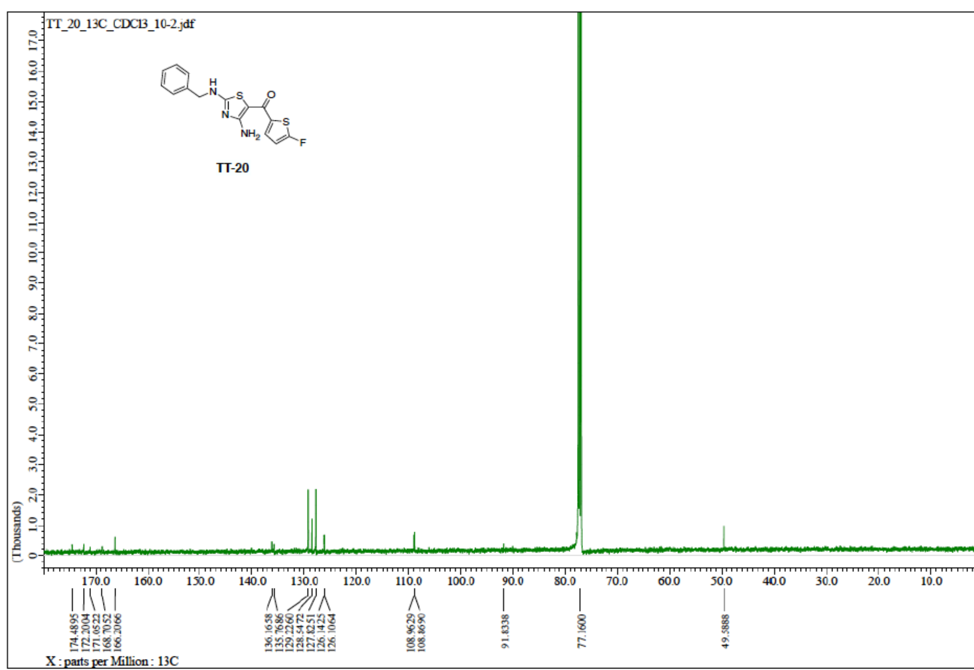

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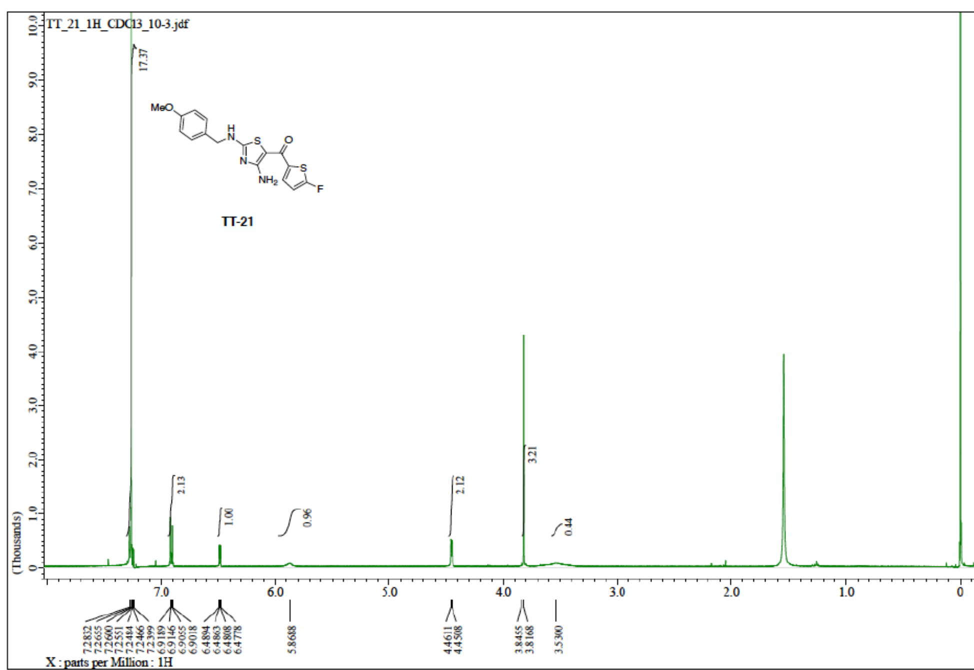

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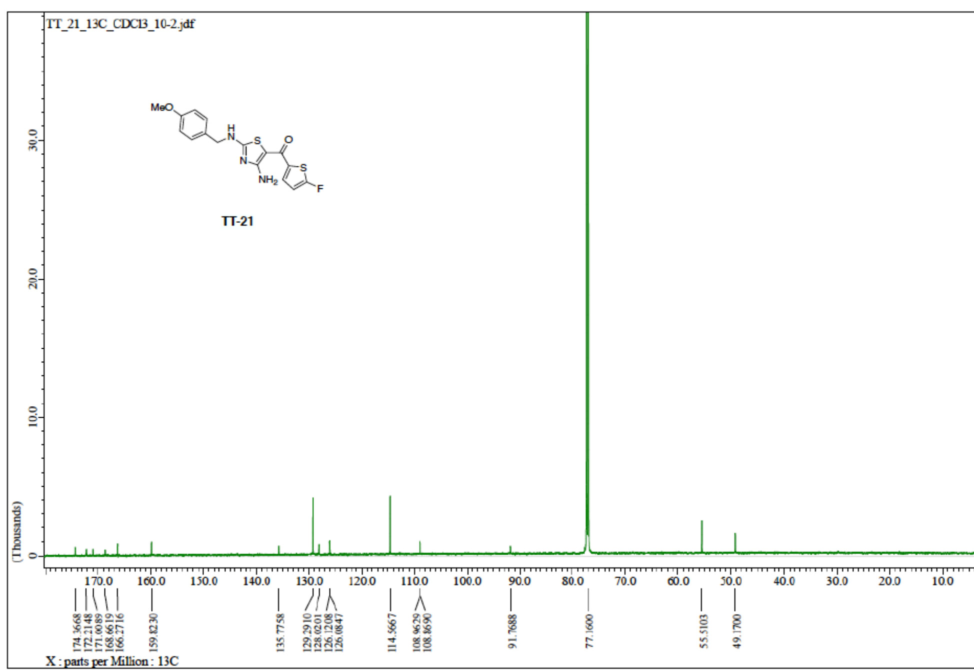

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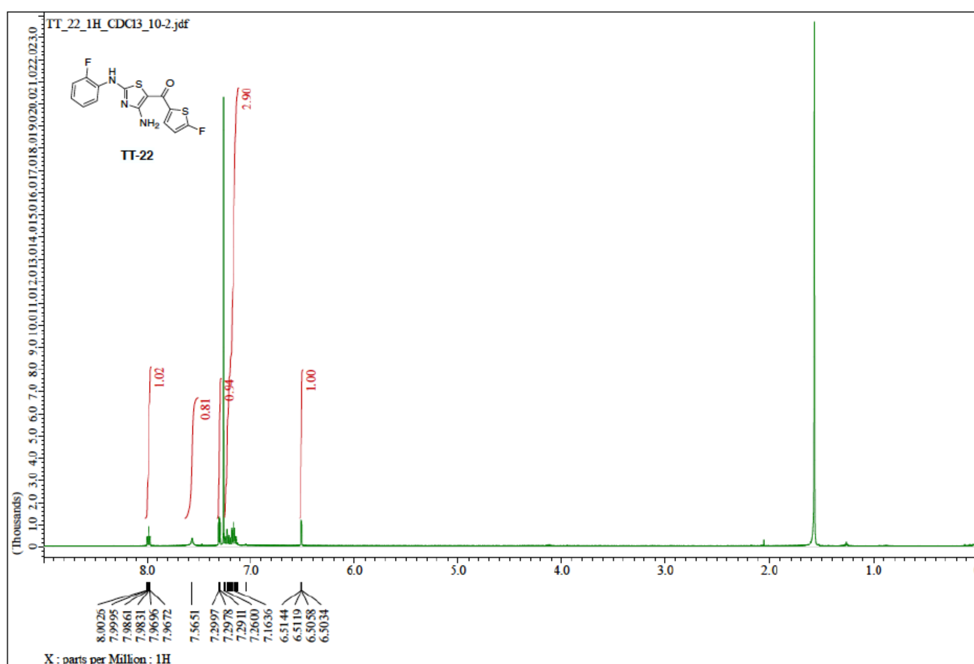

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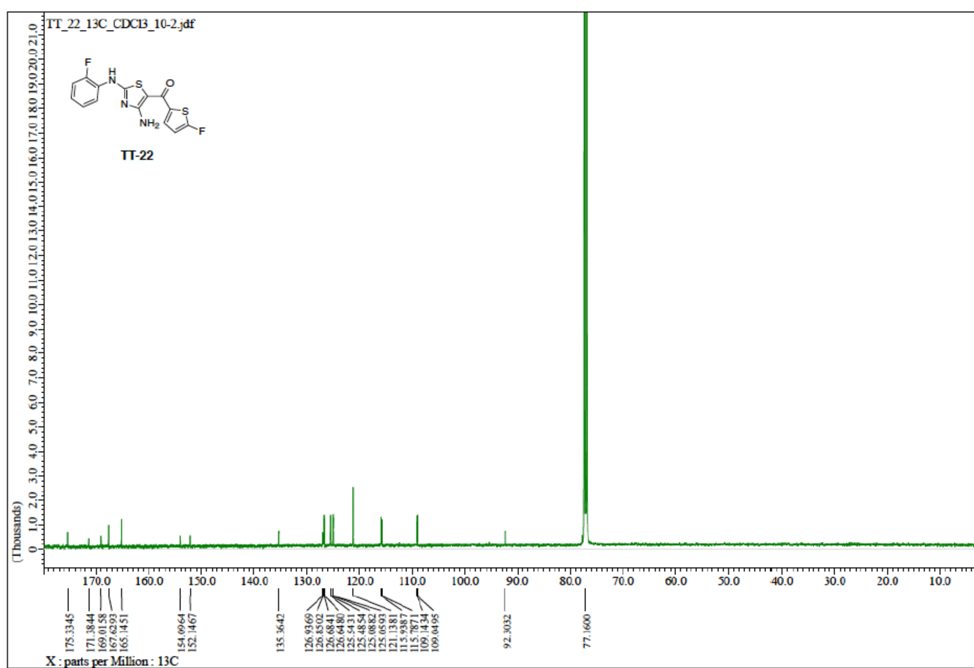

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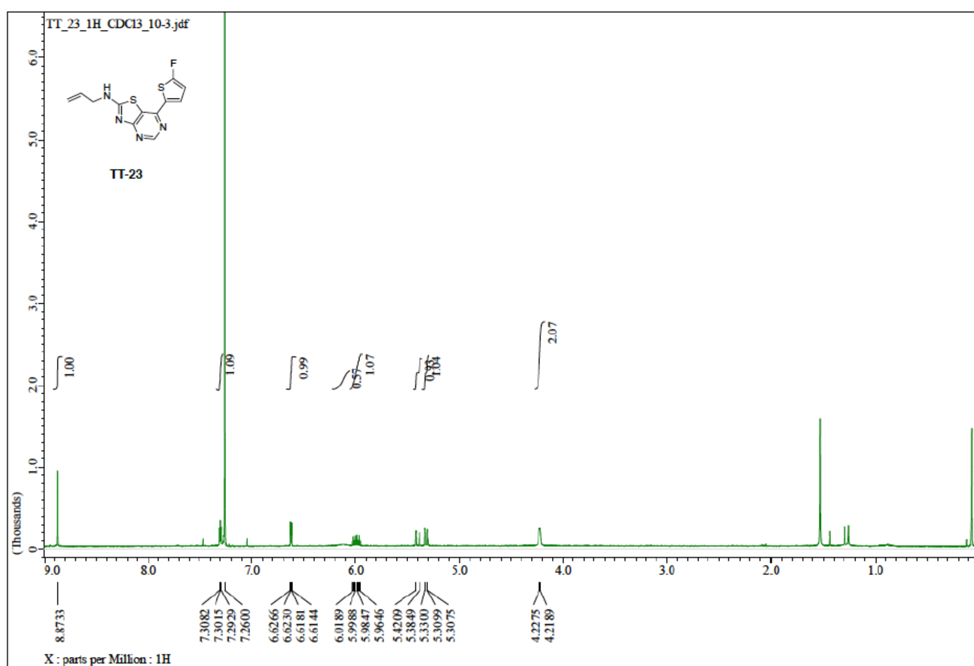

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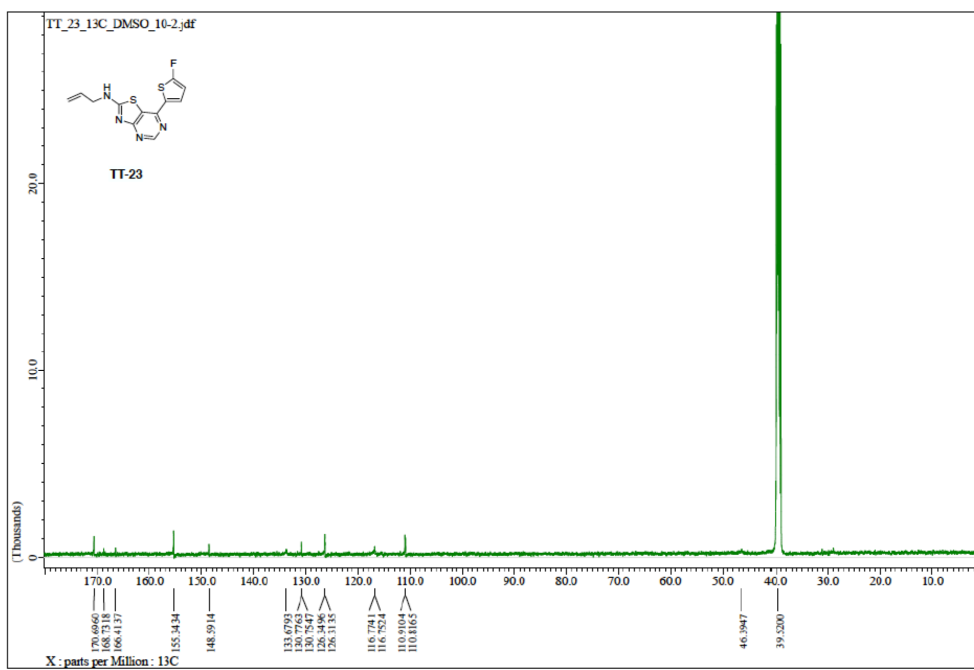

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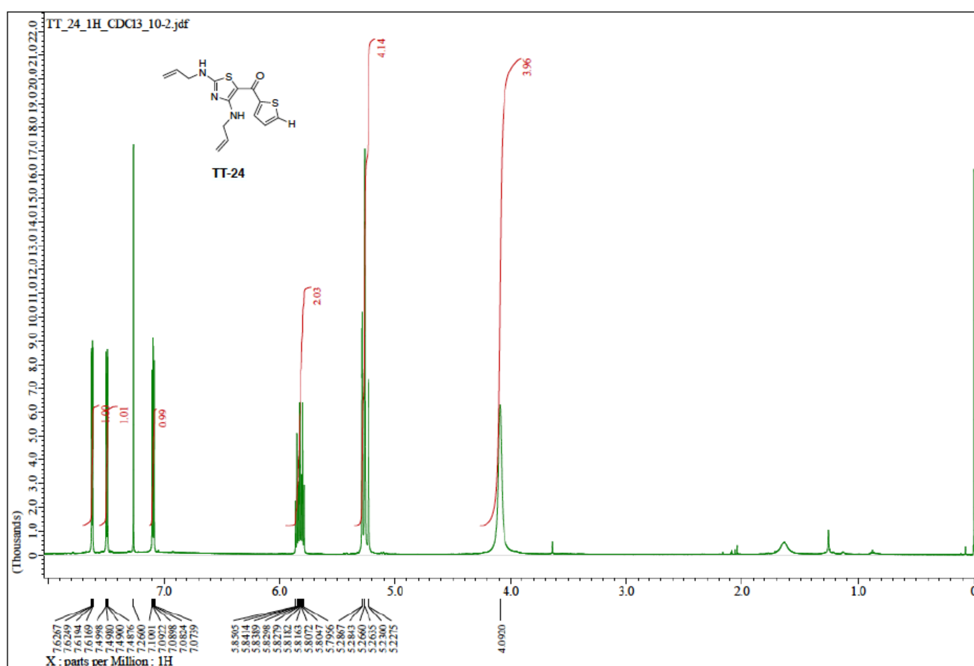

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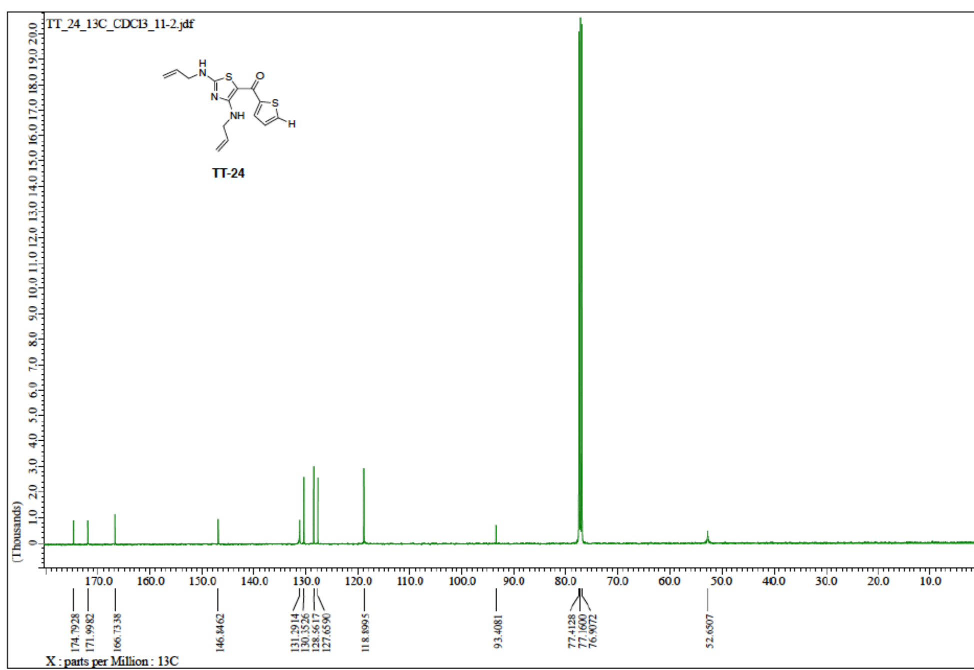

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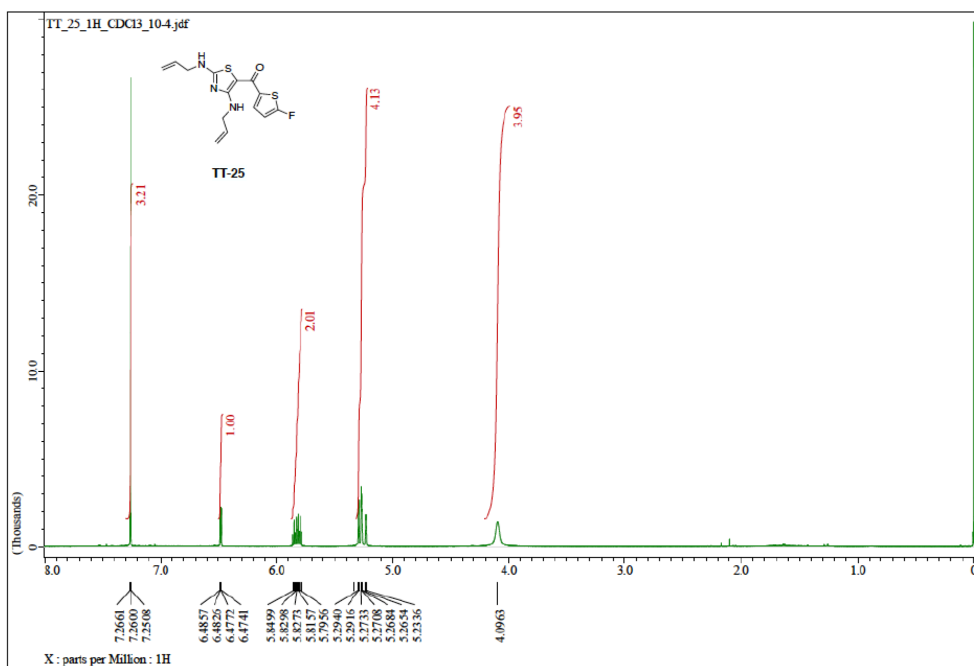

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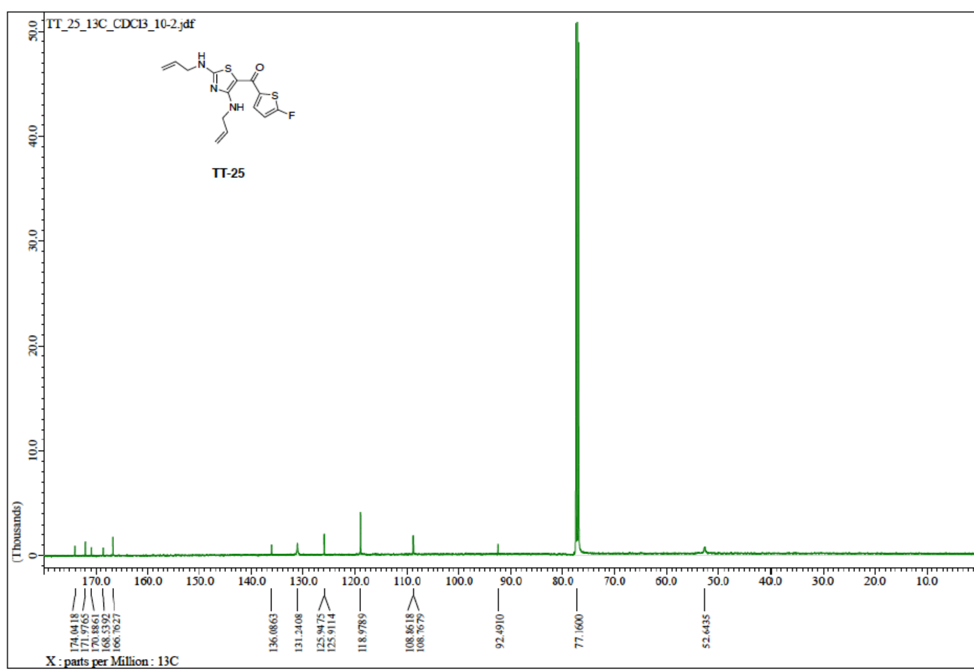

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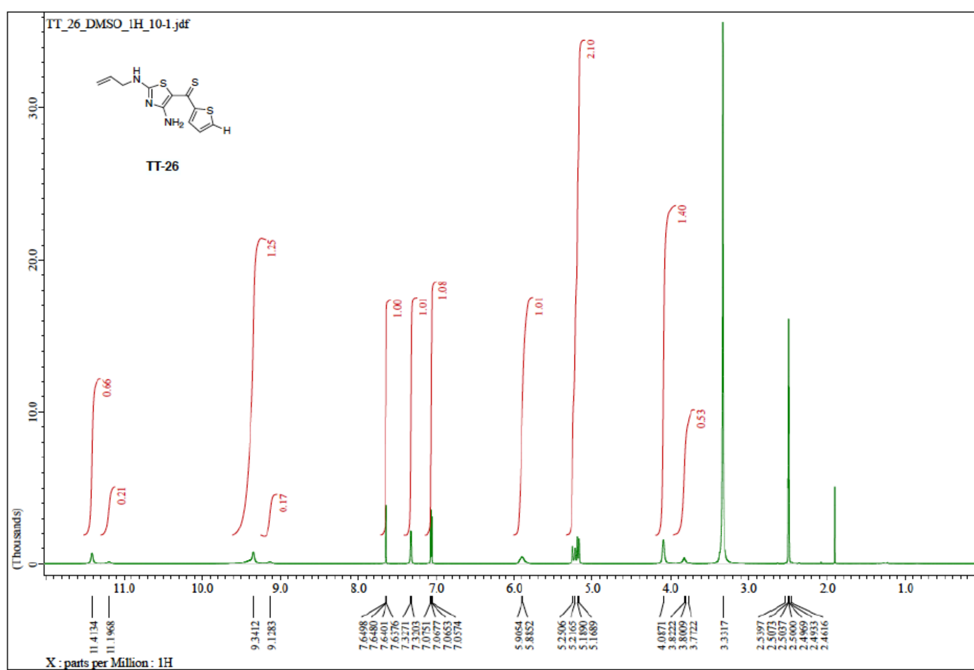

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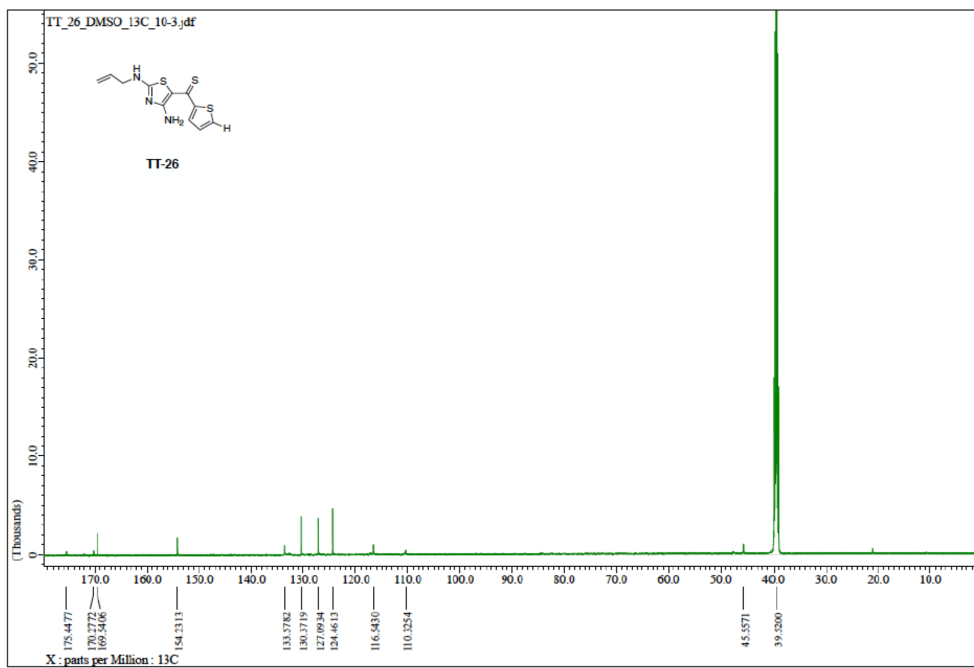

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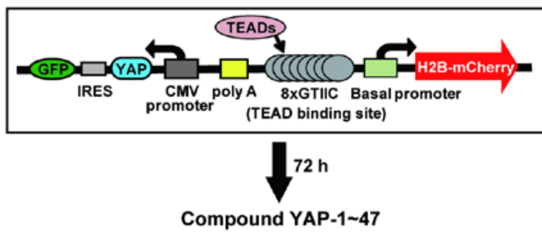
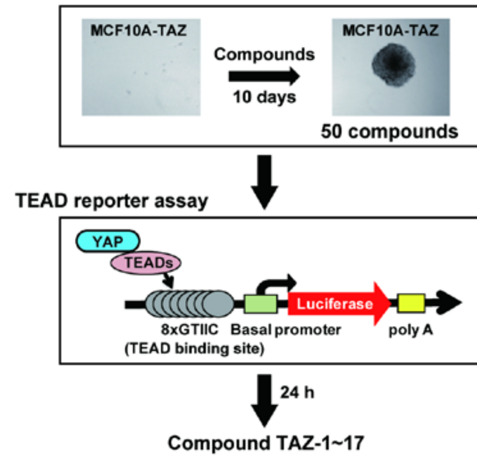
Supplemental Figures

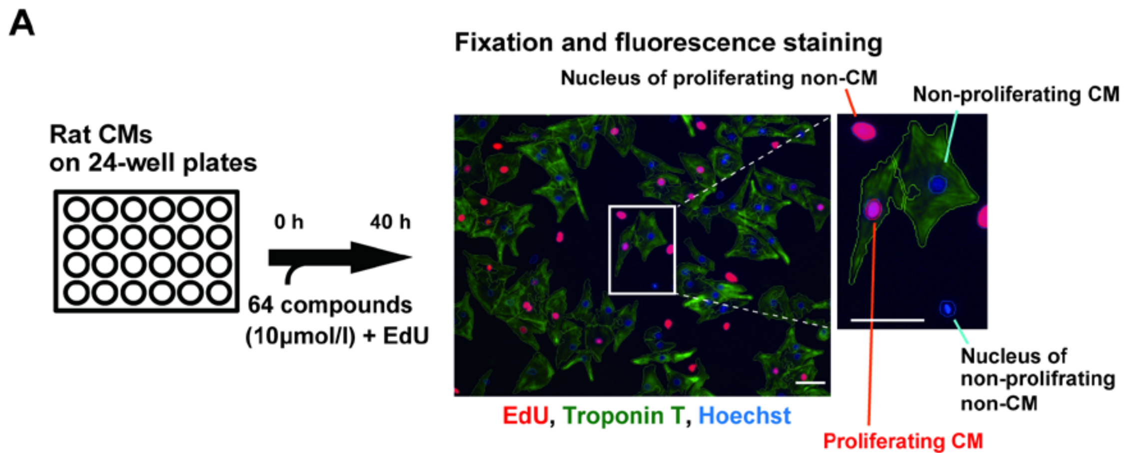




Supplemental Figure 1. High-throughput screening workflows to identify activators of YAP-TEADs complex

(A, **B)** Screening workflow for the fluorescence reporter-based assay for screening of YAP-TEADs activators in YAP-expressing ARPE-19 cells **(A)**, and sphere formation assay for TAZ-activators in MCF10A-TAZ cells and subsequent luciferase assay for YAP-TEADs activators in HEK293 cells **(B)**.

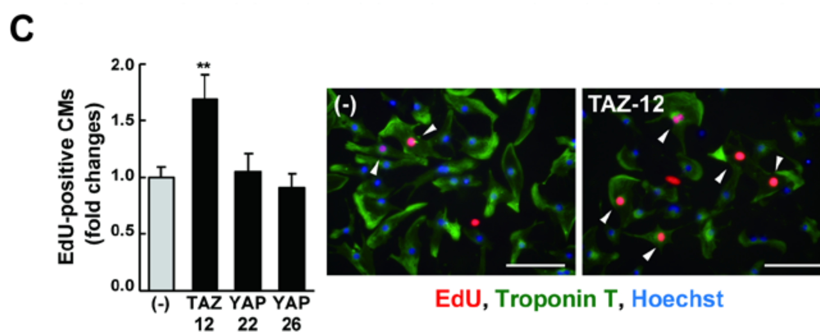
A



B

Compounds	1st evaluation	2nd evaluation	Compounds	1st evaluation	2nd evaluation	Compounds	1st evaluation	2nd evaluation
TAZ-1	0.85 ± 0.18		YAP-5	0.39 ± 0.06		**YAP-26**	**1.22 ± 0.40**	**1.30 ± 0.05**
TAZ-2	0.78 ± 0.11		YAP-6	0.36 ± 0.07		YAP-27	0.24 ± 0.08	
TAZ-3	1.13 ± 0.32	0.81 ± 0.18	YAP-7	0.29 ± 0.29		YAP-28	0.95 ± 0.15	
TAZ-4	0.64 ± 0.09		YAP-8	0.16 ± 0.02		YAP-29	0.44 ± 0.10	
TAZ-5	1.24 ± 0.22	0.98 ± 0.19	YAP-9	0.14 ± 0.05		YAP-30	0.01 ± 0.01	
TAZ-6	1.12 ± 0.09	0.71 ± 0.20	YAP-10	0.43 ± 0.07		YAP-31	0.45 ± 0.19	
TAZ-7	0.93 ± 0.16		YAP-11	0.58 ± 0.17		YAP-32	0.12 ± 0.05	
TAZ-8	1.25 ± 0.24	0.65 ± 0.15	YAP-12	0.22 ± 0.10		YAP-33	0.51 ± 0.13	
TAZ-9	0.95 ± 0.25		YAP-13	0.59 ± 0.13		YAP-34	0.64 ± 0.09	
TAZ-10	1.21 ± 0.12	0.96 ± 0.11	YAP-14	0.34 ± 0.18		YAP-35	0.72 ± 0.12	
TAZ-11	1.21 ± 0.20	0.78 ± 0.11	YAP-15	0.39 ± 0.02		YAP-36	1.36 ± 0.41	0.86 ± 0.08
TAZ-12	**2.80 ± 0.41**	**1.70 ± 0.34**	YAP-16	0.08 ± 0.04		YAP-37	0.67 ± 0.17	
TAZ-13	1.07 ± 0.12	0.76 ± 0.18	YAP-17	0.07 ± 0.03		YAP-38	0.53 ± 0.12	
TAZ-14	0.36 ± 0.09		YAP-18	1.59 ± 0.34		YAP-39	0.15 ± 0.23	
TAZ-15	1.12 ± 0.17	0.60 ± 0.17	YAP-19	0.09 ± 0.04		YAP-40	0.35 ± 0.28	
TAZ-16	1.14 ± 0.07	0.76 ± 0.09	YAP-20	0.13 ± 0.02		YAP-41	0.41 ± 0.03	
TAZ-17	1.07 ± 0.17	0.70 ± 0.12	YAP-21	1.62 ± 0.28	0.81 ± 0.24	YAP-42	0.59 ± 0.19	
YAP-1	0.01 ± 0.02		**YAP-22**	**1.58 ± 0.29**	**1.12 ± 0.16**	YAP-43	0.79 ± 0.24	
YAP-2	0.31 ± 0.09		YAP-23	0.80 ± 0.06		YAP-44	0.05 ± 0.03	
YAP-3	0.24 ± 0.15		YAP-24	0.14 ± 0.01		YAP-45	0.06 ± 0.01	
YAP-4	1.41 ± 0.28	0.84 ± 0.11	YAP-25	1.32 ± 0.14	0.80 ± 0.21	YAP-46	0.01 ± 0.01	
						YAP-47	0.94 ± 0.20	

C



Supplemental Figure 2. Screening workflow for evaluating CM proliferation

(A) EdU incorporation in DNA synthesizing CMs stained with cardiac troponin T antibody were evaluated. Scale bar: 50 µm. **(B)** Effects of 64 compounds (10 µM) on DNA synthesis. Data are shown as the ratio of EdU-incorporated CMs as compared to untreated CMs under starved conditions (0.1% FCS). The second evaluation was conducted for 17 compounds that showed equivalent or more effective DNA forming activities in the first evaluation. YAP-18 was excluded because of low CM viability. n = 3 per group. **(C)** Ratios of EdU-positive CMs after treatment with 3 compounds (TAZ-12, YAP-22, and

1 YAP-26). A representative image of TAZ-12-treated CMs is shown. Arrow heads, positive CMs. n = 3

2 per group. Scale bar: 100 µm. $^{**}p < 0.01$ versus the untreated control.

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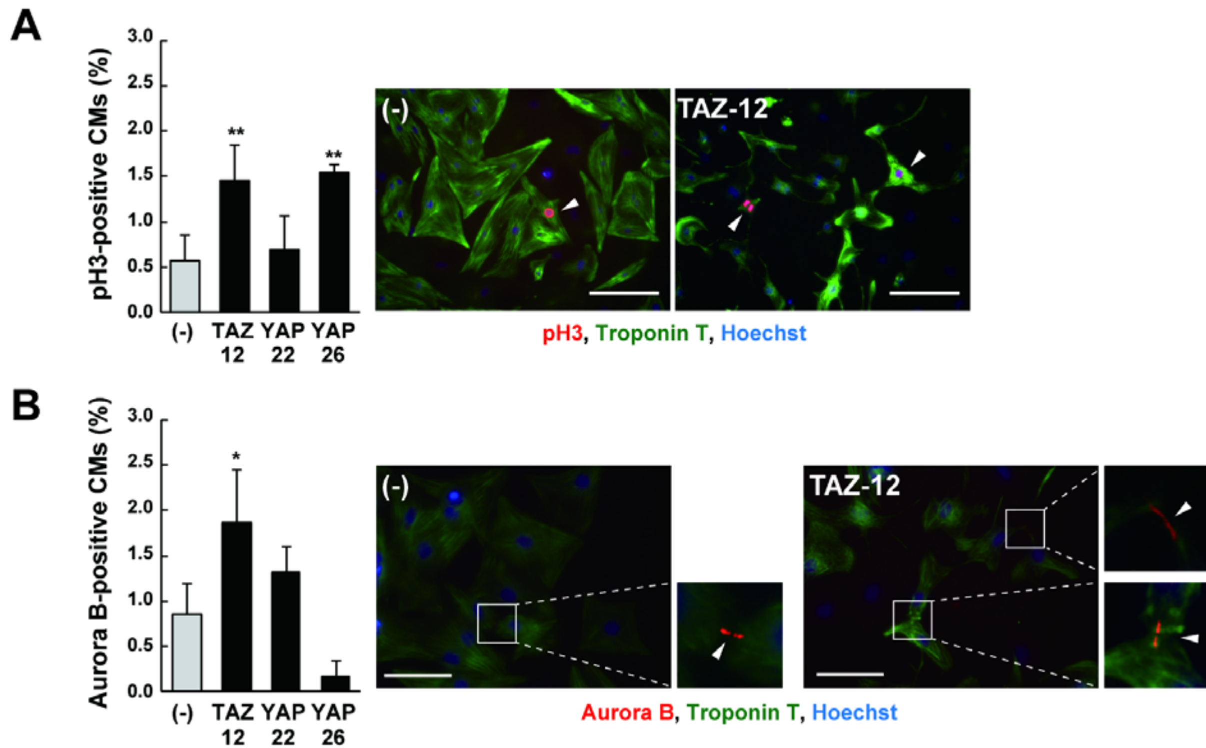

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2 **Supplemental Figure 3. Effects of compounds on CM karyokinesis, cytokinesis, and viability**

3 **(A, B)** Percentages of pH3-positive (A) and Aurora B-positive (B) CMs after treatment with 3

4 compounds (TAZ-12, YAP-22, and YAP-26). A representative image of TAZ-12-treated CMs is shown.

5 Arrow heads, positive CMs. n = 3 or 6 (A), and 3 (B) per group. Scale bars: 100 µm in A; 50 µm in B.

6 $^{*}p < 0.05$, $^{**}p < 0.01$ versus the untreated control.

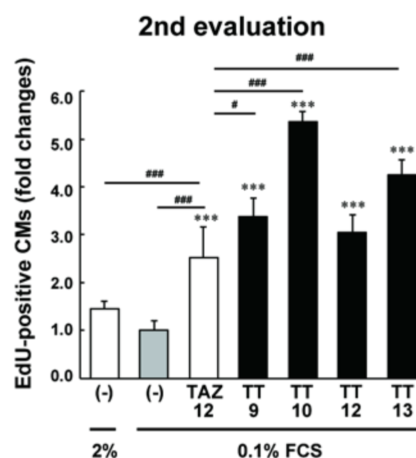
7

A

1st evaluation

Compounds	ratios to control	ratios to TAZ-12
TT-1	0.59 ± 0.07	0.24 ± 0.03
TT-2	1.19 ± 0.36	0.75 ± 0.23
TT-3	1.33 ± 0.40	0.93 ± 0.28
TT-4	1.34 ± 0.29	0.67 ± 0.14
TT-5	1.47 ± 0.08	0.82 ± 0.04
TT-6	0.25 ± 0.06	0.12 ± 0.03
TT-7	1.02 ± 0.14	0.51 ± 0.07
TT-8	1.09 ± 0.03	0.60 ± 0.02
TT-9	**2.78 ± 0.55**	**1.50 ± 0.29**
TT-10	**3.53 ± 0.30**	**1.65 ± 0.14**
TT-11	1.64 ± 0.33	0.88 ± 0.18
TT-12	**2.32 ± 0.11**	**1.45 ± 0.07**
TT-13	**3.47 ± 0.00**	**1.76 ± 0.00**
TT-14	0.67 ± 0.41	0.26 ± 0.16
TT-15	0.20 ± 0.13	0.10 ± 0.06
TT-16	1.68 ± 0.24	0.68 ± 0.10
TT-17	0.36 ± 0.19	0.15 ± 0.08
TT-18	0.21 ± 0.04	0.12 ± 0.02
TT-19	0.07 ± 0.04	0.04 ± 0.02
TT-20	0.97 ± 0.10	0.49 ± 0.05
TT-21	0.85 ± 0.31	0.28 ± 0.10
TT-22	1.73 ± 0.14	0.57 ± 0.04
TT-23	0.80 ± 0.10	0.41 ± 0.05
TT-24	0.12 ± 0.05	0.08 ± 0.04
TT-25	0.18 ± 0.02	0.06 ± 0.01
TT-26	0.06 ± 0.02	0.03 ± 0.01

B



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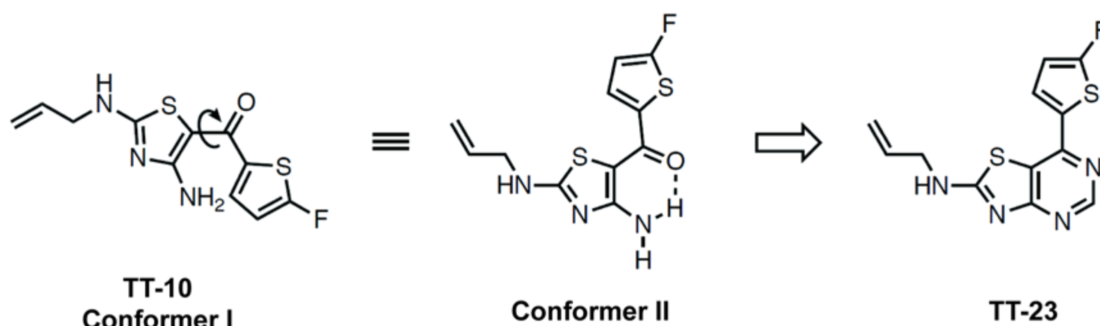
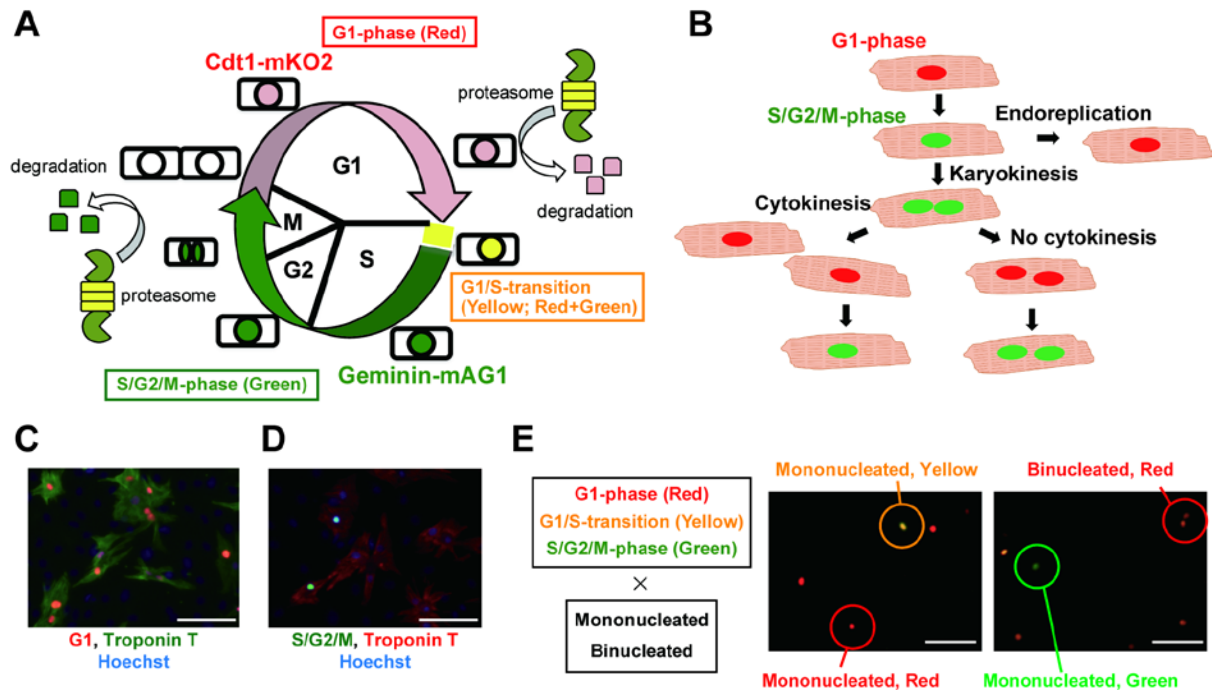


Supplemental Figure 4. Effects of 26 analogues on CM proliferation

(A) Effects of 26 analogues (10 μM) on DNA synthesis in CMs. Data are shown as the ratio of EdU-incorporated CMs as compared to untreated CMs. n = 3–4 per group. **(B)** Ratios of EdU-positive CMs after treatment with the indicated compounds. n = 5 per group. **(C)** The motivation of synthesis of tethered structure, TT-23. TT-10 can form either conformer I or conformer II, and TT-23 was synthesized to understand the influence of these conformers on CM proliferation. ****p* < 0.001 versus the untreated control, #*p* < 0.05, ###*p* < 0.001 versus TAZ-12.



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2 **Supplemental Figure 5. CM-specific expression of Fucci indicators**

3 **(A)** Fluorescent ubiquitination-based cell cycle indicator (Fucci) system. The Fucci probe was
4 generated by fusing Kusabira-Orange2 (mKO2, red fluorescent protein) and Azami-Green1 (mAG1,
5 green fluorescent protein) to the ubiquitylation domains of human Cdt1 [hCdt1(30/120)] and Geminin
6 [hGem(1/110)], respectively. The two chimeric proteins, mKO2-hCdt1(30/120) and mAGhGem(1/110),
7 accumulate reciprocally in the nuclei during the cell cycle, labeling individual G1-phase nuclei in red
8 and S/G2/M-phase nuclei in green, which is mediated by the ubiquitin proteasome system. **(B)** Scheme
9 illustrating proliferation of CMs transfected with CM-specific Fucci expression adenoviruses. **(C, D,**
10 **E)** Establishment of adenoviruses for CM-specific Fucci expression. CMs were infected with the CM-
11 specific Fucci expression adenovirus, *Tnnt2*-Fucci G1 Orange (C) or *Tnnt2*-Fucci S/G2/M Green (D)
12 for 72 hours and then stained with troponin T antibody. Note the CM-specific Fucci expression. (E)
13 CMs infected with two Fucci adenoviruses were classified based on two criteria: cell cycle and nuclear
14 number. Scale bar: 100 µm.

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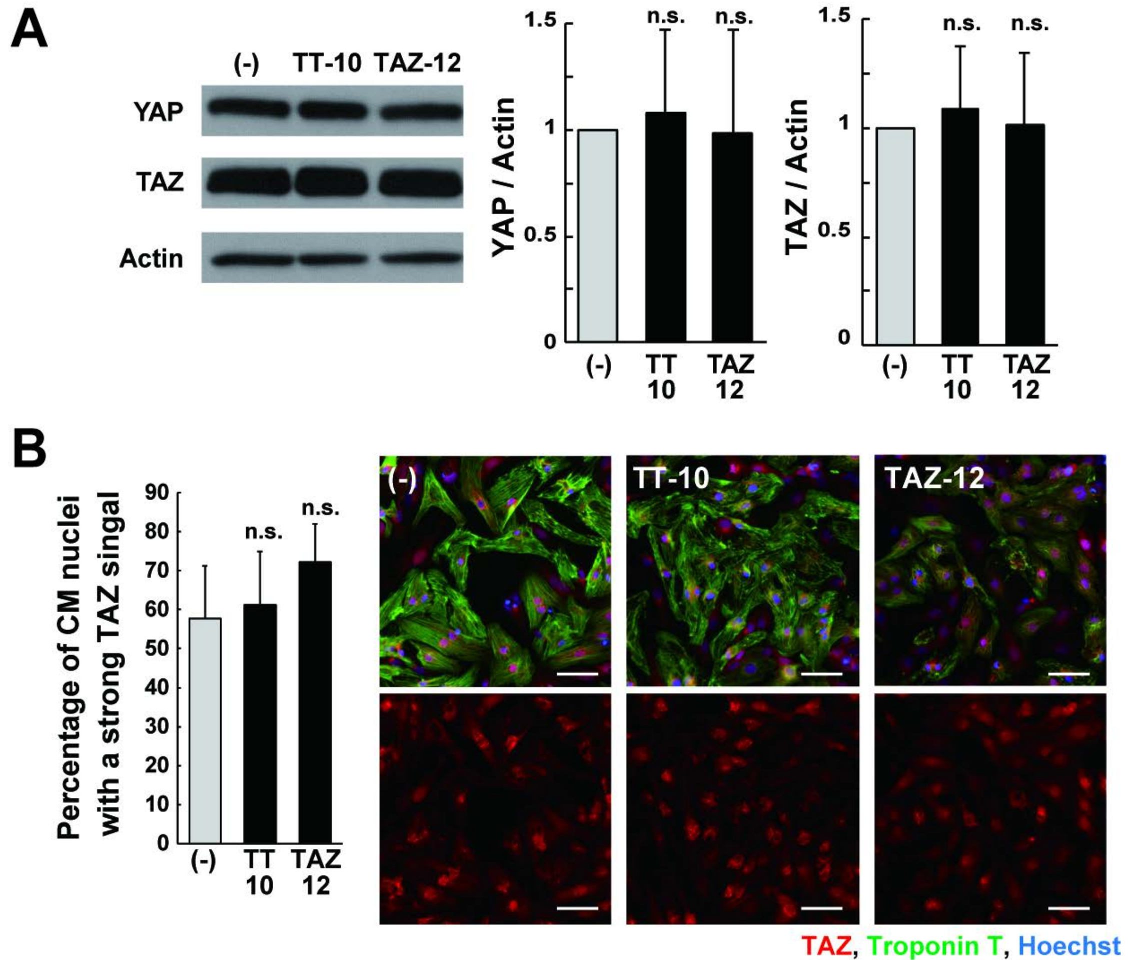

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2 **Supplemental Figure 6. Effects of TT-10 on protein expression of YAP and TAZ**

3 **(A)** Western blot analysis of YAP and TAZ. CMs were treated with 10 μM of each compound for 6

4 hours under starved conditions. n = 4 per group. **(B)** Quantitative imaging assay for TAZ nuclear

5 translocation. CMs were treated with the indicated reagents at the following concentrations for 24 hours:

6 TT-10, 10 μM; TAZ-12, 10 μM. n = 6 per group. Scale bar: 50 μm. n.s. $p > 0.05$ versus the untreated

7 control.

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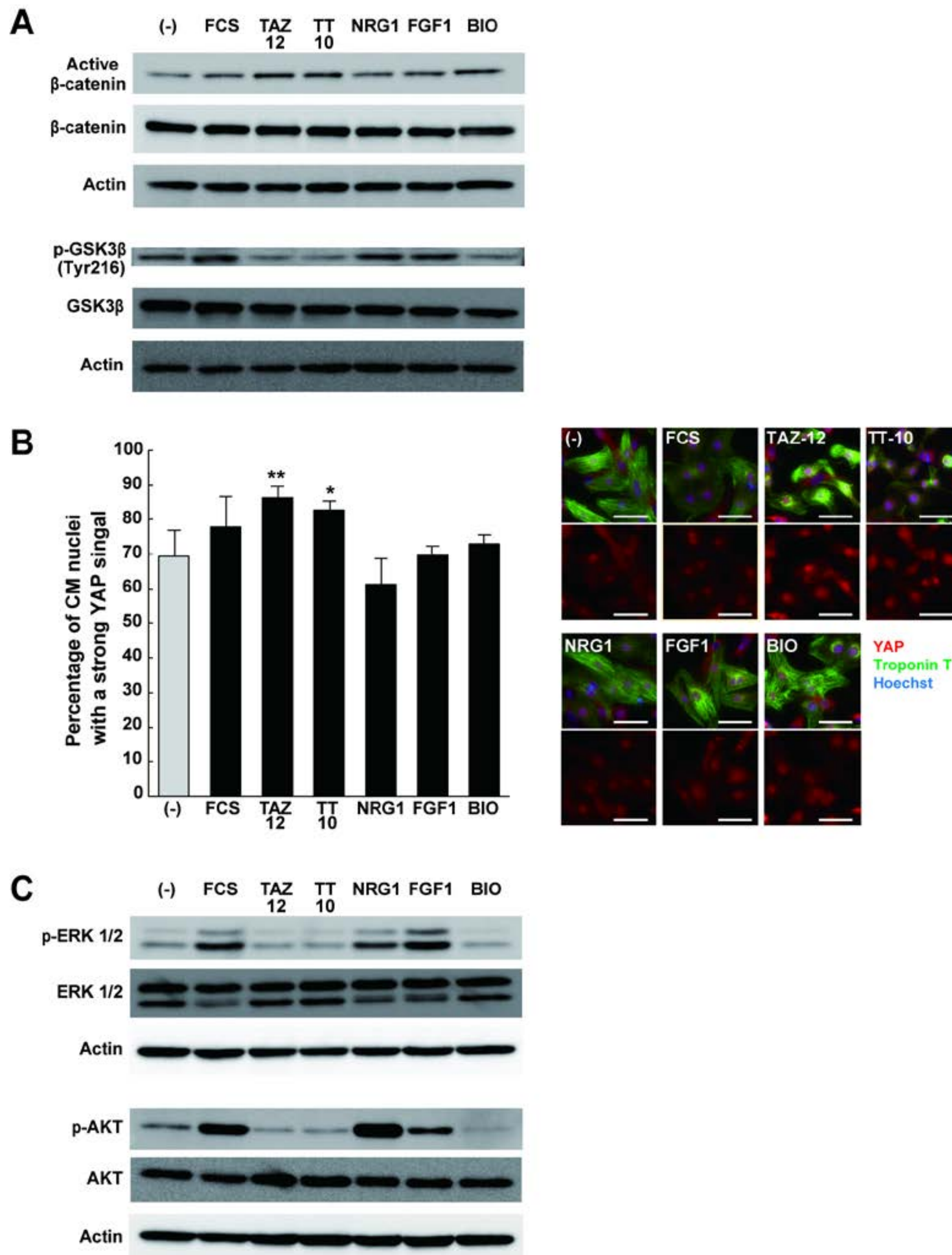

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2 **Supplemental Figure 7. Effects of TT-10 on CM proliferative signals**

3 **(A)** Western blot analysis for Wnt/β-catenin signaling pathway. CMs were treated with the indicated

4 reagents for 6 hours under starved conditions. FCS, 2%; TAZ-12, 10 μM; TT-10, 10 μM; NRG1, 100

5 ng/ml; FGF1, 100 ng/ml; BIO, 1μM. **(B)** Quantitative imaging assay for YAP nuclear translocation.

6 CMs were treated with the indicated reagents at the following concentrations for 24 hours. n = 5 per

7 group. Representative images are shown in the right panel. Scale bar: 50 μm. **(C)** Western blot analysis

8 for ERK and AKT signaling pathway. CMs were treated with the indicated reagents for 15 minutes

9 under starved conditions. *$p < 0.05$, **$p < 0.01$ versus the untreated control.

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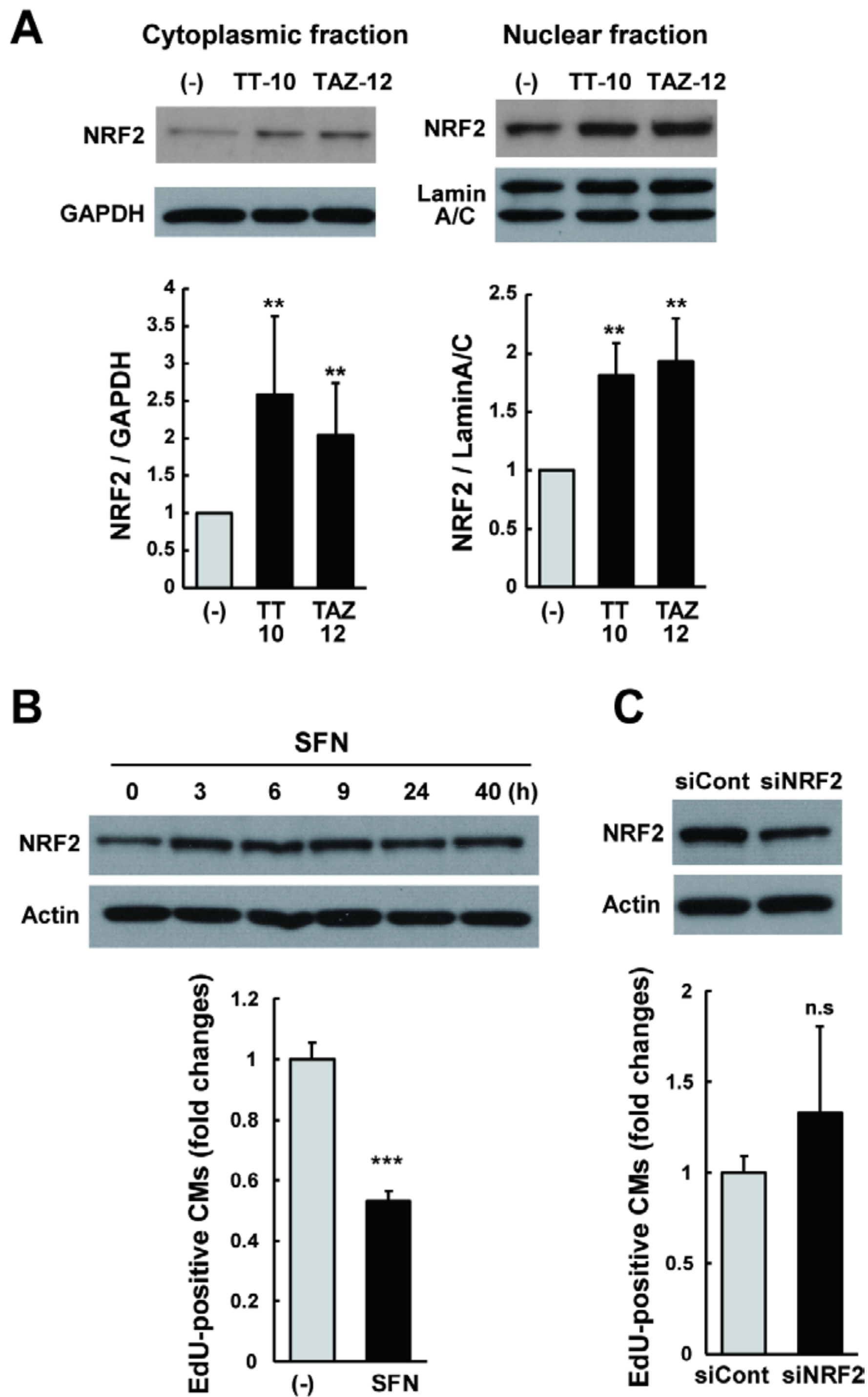

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2 **Supplemental Figure 8. Effects of NRF2 on CM proliferation**

3 **(A)** Western blot analysis of NRF2 in cytoplasmic and nuclear fractions. CMs were treated with 10 μM
4 of each compound for 6 hours under starved conditions. n = 6 per group. **(B)** Effects of sulforaphane
5 (SFN 5 μM; a NFR2 activator) on NRF2 expression and DNA synthesis in CMs. NRF2 expression was
6 analyzed at the indicated hours after treatment (upper panel). An EdU-incorporation assay (lower panel)
7 was conducted over 40 hours. n = 3 per group. **(C)** Effects of NRF2 knockdown (upper panel) on DNA

1 synthesis in CMs (lower panel). CMs transfected with NRF2 siRNA were treated with EdU for 40 hours.

2 n = 4 per group. n.s. $p > 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus the untreated control.

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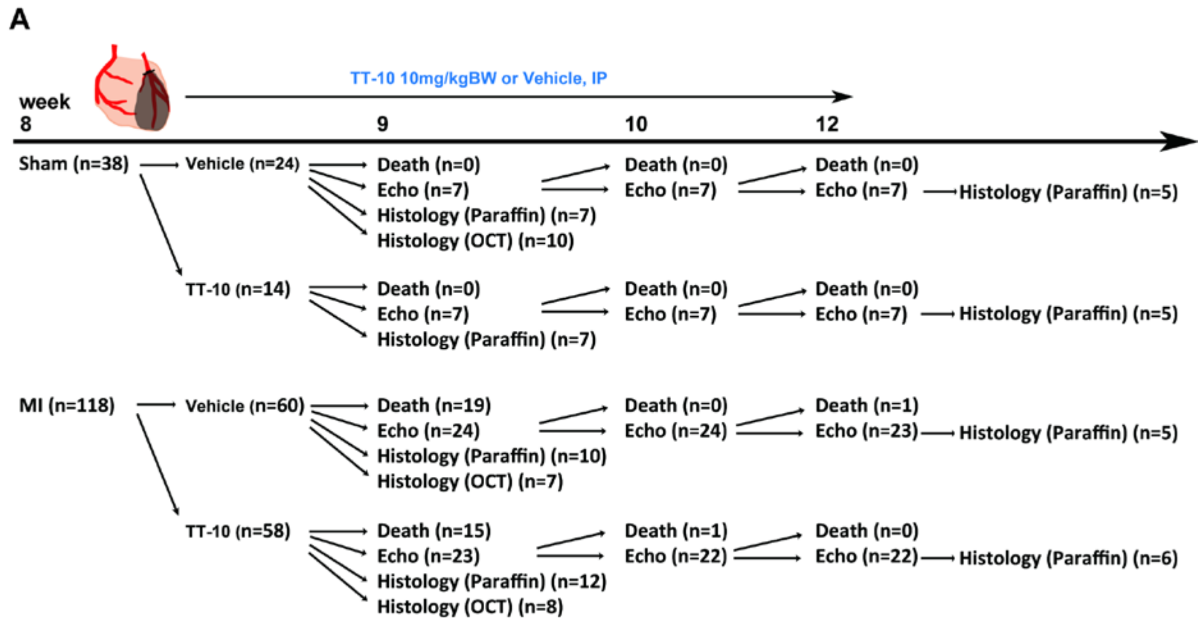
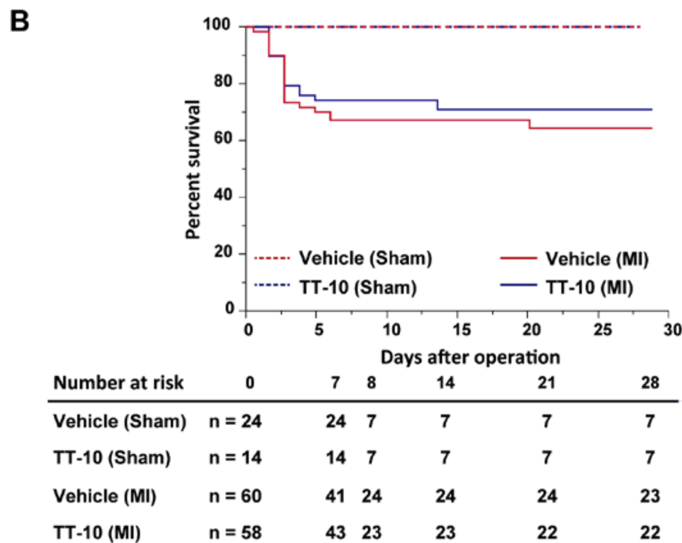
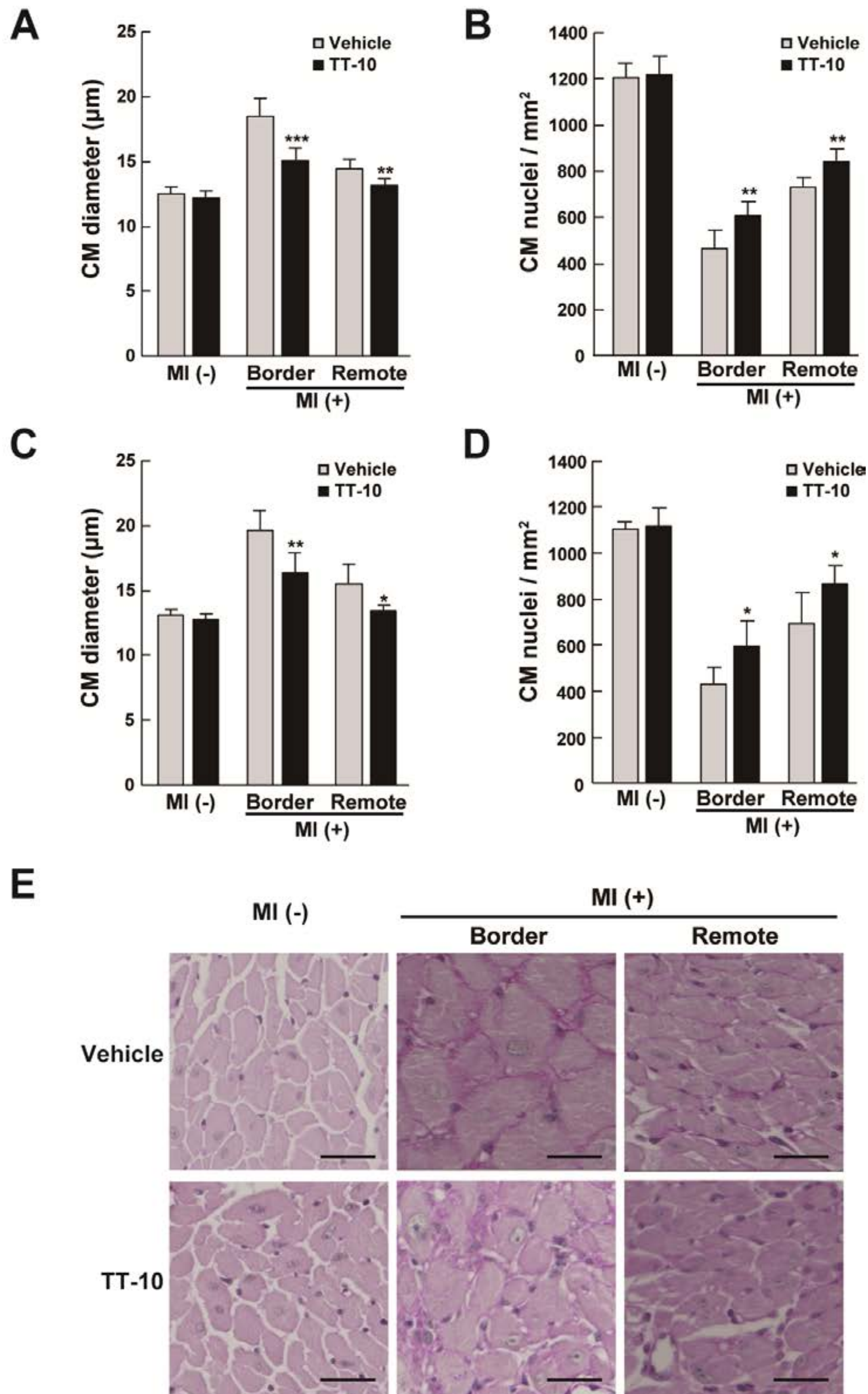




Number at risk	0	7	8	14	21	28
Vehicle (Sham)	n = 24	24	7	7	7	7
TT-10 (Sham)	n = 14	14	7	7	7	7
Vehicle (MI)	n = 60	41	24	24	24	23
TT-10 (MI)	n = 58	43	23	23	22	22

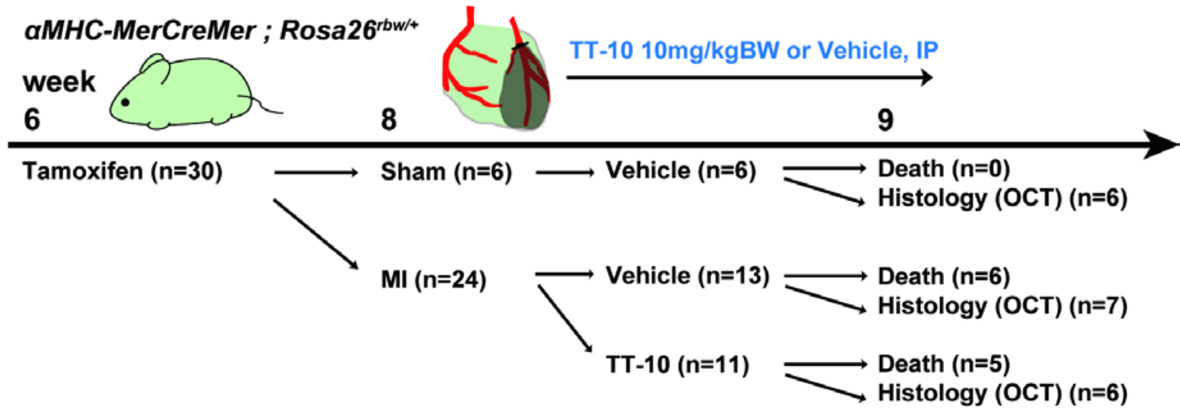
Supplemental Figure 9. Experimental design and effect of TT-10 on 30-day survival rate after MI **(A)** Experimental design. Mice were randomly to assigned in a 1:1 ratio to receive vehicle or TT-10 intraperitoneally after the MI procedure. **(B)** Kaplan-Meier survival curve for mice treated with vehicle or TT-10. There was no significant difference in 30-day survival after the MI procedure between the treatment and control groups (p = 0.5322, log-rank test).



Supplemental Figure 10. TT-10 ameliorates MI-induced CM hypertrophy and decreased CM nuclear density

(A-D) CM diameter (A, C) and nuclear density (B, D) were evaluated on PAS-stained tissues of the infarct border and the remote zone one (A, B) and four (C, D) weeks after the MI procedure. (A, B) n

1 = 5 (MI(-)), 6 (MI(+) + vehicle), and 7 (MI(+) + TT-10). (C, D) n = 5 (MI(-)), 5 ((MI(+) + vehicle), and
2 6 (MI(+) + TT-10). **(E)** Representative images of the myocardium at 1 week following the MI procedure.
3 Scale bars: 25 µm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle control.
4



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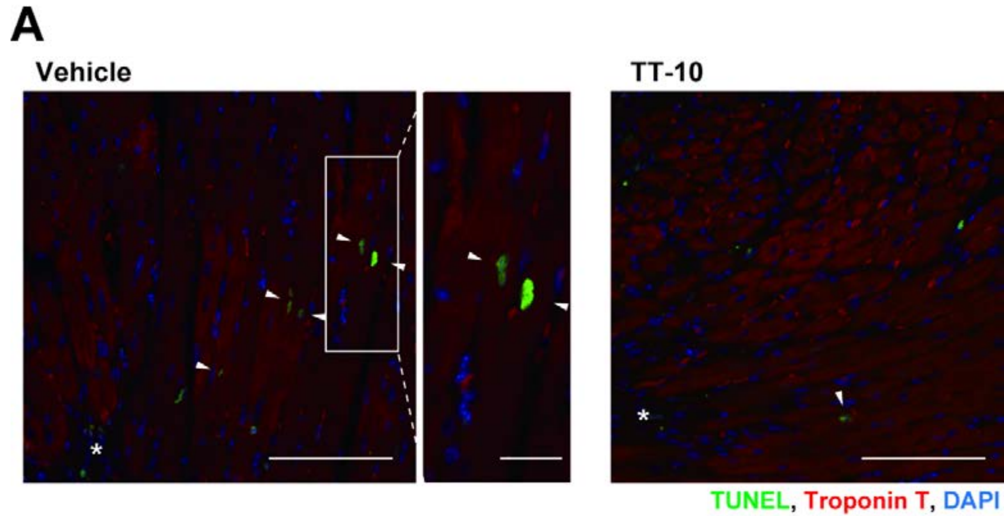
2 **Supplemental Figure 11. Experimental design of the lineage tracing experiment of existing CMs**

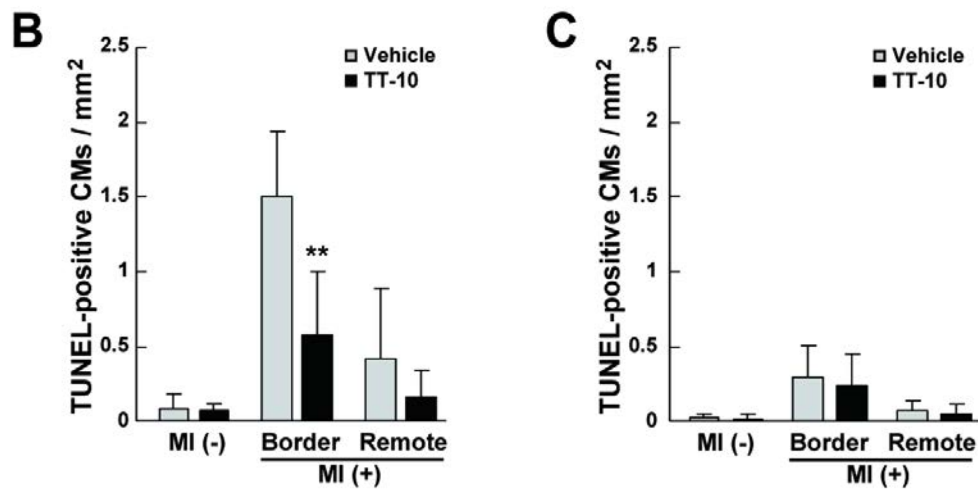
3 *αMHC-MerCreMer*; *Rosa26*$^{rbw/+}$ mice were used in the lineage tracing experiment of existing CMs.

4 Mice were randomly assigned in a 1:1 ratio to receive vehicle or TT-10 after the MI procedure.

5

6





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2 **Supplemental Figure 12. TT-10 reduces MI-induced TUNEL-positive apoptosis**

3 **(A)** Representative images of the myocardium at 1 week following the MI procedure. Arrow heads,
4 TUNEL-positive CMs; asterisk, infarct area. Scale bars: 100 µm; 25 µm in inset. **(B, C)** TUNEL-
5 positive CMs were evaluated in the infarct border and the remote zone one (B) and four (C) weeks after
6 the MI procedure. (B) n = 7 (MI(-)), 6 (MI(+) + vehicle), and 7 (MI(+) + TT-10). (C) n = 5 (MI(-)), and
7 7 ((MI(+)). $^{**}p < 0.01$ versus vehicle control.

8

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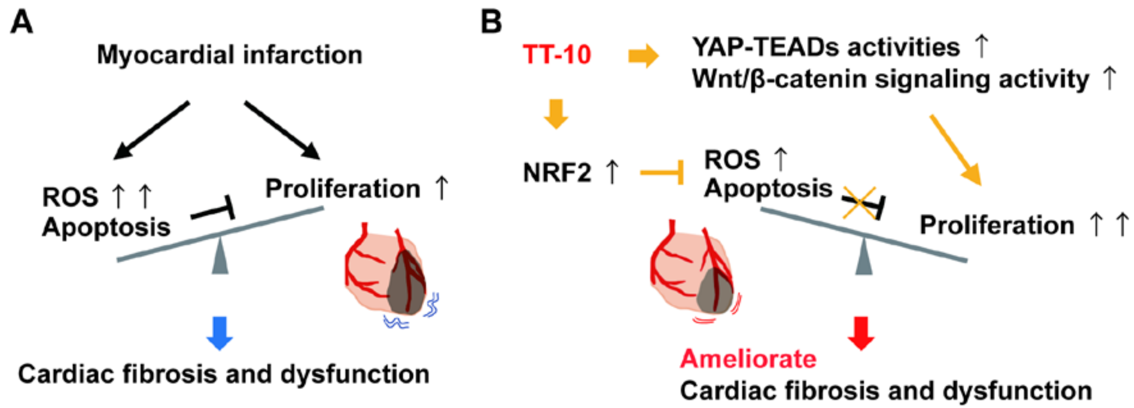

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2 **Supplemental Figure 13 Schematic diagram of the effects of TT-10**

3 **(A)** MI stimulates both ROS generation and apoptosis in CMs and CM proliferation, however, the

4 excessive ROS production rather inhibits CM regenerative capacity and promotes cardiac dysfunction.

5 **(B)** A novel fluorine compound TT-10 protects CMs from ROS and resultant apoptosis by activating

6 NRF2 transcription factor, and increases CM proliferation activity by enhancing YAP-TEADs activities

7 and Wnt/β-catenin signaling. TT-10 can ameliorate cardiac remodeling after MI.

8

9

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