# SUPPLEMENTAL MATERIALS

# for

# Design, synthesis and bioevaluation of novel N-heterocyclic hydroxamic acids as histone deacetylase inhibitors and their antitumor activity study

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#### 1. Materials and Methods

#### 1.1. Chemistry

Thin layer chromatography which was performed using Whatman<sup>®</sup> 250  $\mu$ m Silica Gel GF Uniplates and visualized under UV light at 254 and 365 nm, was used to check the progress of reactions and preliminary evaluation of compounds' homogeneity. Melting points were measured using a Gallenkamp Melting Point Apparatus (LabMerchant, London, United Kingdom) and are uncorrected. Purification of compounds was carried out using crystallization methods and/or open silica gel column flash chromatography employing Merck silica gel 60 (240 to 400 mesh) as stationary phase. Nuclear magnetic resonance spectra (<sup>1</sup>H NMR) were recorded on a Bruker 500 MHz spectrometer with DMSO- $d_6$  as solvent unless otherwise indicated. Tetramethylsilane was used as an internal standard. Chemical shifts are reported in parts per million (ppm), downfield from tetramethylsilane. Mass spectra with different ionization modes including electron ionization (EI), Electrospray ionization (ESI), were recorded using PE Biosystems API2000 (Perkin Elmer, Palo Alto, CA, USA) and Mariner® (Azco Biotech, Inc. Oceanside, CA, USA) mass spectrometers, respectively. The elemental (C, H, N) analyses were performed on a Perkin Elmer model 2400 elemental analyzer. All reagents and solvents were purchased from Aldrich or Fluka Chemical Corp. (Milwaukee, WI, USA) or Merck unless noted otherwise. Solvents were used directly as purchased unless otherwise indicated.

#### 1.2. Synthesis of library compounds



Scheme 1. Synthesis of 1*H*-benzo[d]imidazole-based hydroxamic acids *via* reductive amination *General procedures for the synthesis of compounds* 6*a-g*, 9*a-g* 

To a solution of 5-nitro-1*H*-benzo[d]imidazole (1) (4.0 mmol) in dry DMF (10 mL) was added  $K_2CO_3$  (685 mg, 5.0 mmol). The resulting mixture was heated at 60 °C for 60 minutes, then KI (33.2 mg, 0.2 mmol) was added. After stirring for further 15 minutes, methyl 4-bromomethylcinnamate (4.0 mmol) diluted with DMF (2 mL) was dropwise added into the mixture. The reaction mixture was again stirred at 60 °C until the reaction finished (checked by TLC). Subsequently, the mixture was cooled to room temperature, followed by being poured into ice-cold water (30 mL). The obtained light-yellow precipitate was filtered off, washed with water, and dried at 40 °C under a vacuum for 24 hours. The crude product was used directly for the next step without further purification.

Next, the raw material (3 mmol) from the previous step was dissolved in ethyl acetate (30 mL). Then, tin(II) chloride dihydrate (3.38 g, 15 mmol) and 2 drops of glacial acetic acid were added to the mixture and stirring was continued for 4 h. After completion of the reaction, the resulting mixture was cooled, poured into water (50 mL). The aqueous phase was neutralized with sodium carbonate. Celite was used as the filtration medium to remove fine solids such as metal salts and metal hydroxides from reaction mixture. The mixture after filtration was extracted with ethyl acetate (3 x 30 mL). The combined organic layer was washed with brine, dried over sodium sulfate anhydrous, and evaporated under reduced pressure to give the crude products. The residue was purified by column chromatography (silica gel, DCM: MeOH = 98:2) to give two intermediate reductive product isomers 4 and 7.

In the next step, the aromatic amines **4** or **7** (1 mmol) was dissolved in methanol (10 mL), then 2 drops of concentrated acetic acid, followed by aldehydes (1.0 mmol) was added. After stirring for further 15 minutes, sodium cyanoborohydride (NaBH<sub>3</sub>CN) (50 mg) diluted with methanol (1 mL) was dropwise added into the mixture. The mixture was stirred at room temperature until the starting materials were consumed completely (6-8 hours). The resulting mixtures were evaporated under reduced pressure to give the residues, then 10 mL water was added. The aqueous phase was extracted with DCM, and the combined organic layer was evaporated under reduced pressure to give the residues, then 20 mL water was added. The aqueous phase was extracted with DCM, and the combined organic layer was evaporated under reduced pressure to give the residue. The crude material was purified via open chromatography (silica gel, DCM: MeOH = 96:4) to afford the yellowish oil (**5a-g**, **8a-g**).

Finally, each of the intermediate esters (**5a-g**, **8a-g**) was dissolved in methanol (10 mL). Then, hydroxylamine hydrochloride (685 mg, 10 mmol) was added, followed by dropwise addition of a solution of NaOH (400 mg in 1 mL of water). The mixture was stirred at 0 °C until the reaction completed (1-2 h). At the end of this reaction, the resulting reaction mixture was poured into ice-cold water, neutralised to pH~7, then acidified by dropwise addition of a solution of HCl 5% to induce the maximum precipitation. The precipitates were filtered, dried and re-crystalised in methanol to give the desired compounds **6a-g** and **9a-g**.

#### General procedures for the synthesis of compounds 14a-g, 17a-g

Compounds **14a-g** and **17a-g** were synthesized by a similar synthetic pathway described for **6a-g**, **9a-g**, except that methyl 7-bromoheptanoate was used instead of methyl (*E*)-4-bromomethylcinnamate (Scheme 1).

#### General procedures for the synthesis of compounds 22a-g, 25a-g

Compounds 22a-g and 25a-g were prepared following a synthetic route analogous to that outlined for 6a-g and 9a-g, with the only difference being the substitution of methyl 4-bromomethylbenzoate for methyl (*E*)-4-bromomethylcinnamate, as illustrated in Scheme 1.



Scheme 2. Synthesis of 1H-benzo[d]imidazole-based hydroxamic acids via amide coupling reaction

General procedures for the synthesis of compounds 27a-c, 29a-c

Compounds **27a-c** and **29a-c** were synthesized via a four-step pathway as illustrated in Scheme 2. The first two reactions in the synthetic pathway of these compounds were similar to those in the synthesis of **6a-g**, **9a-g**.

In the next step, the aromatic amines 4 or 7 (1 mmol) were dissolved in 30 mL DCM, and DMAP (122 mg, 1 mmol) was added. After stirring for 10 minutes, a respective benzoyl chloride (1.2 mmol) was added. The reaction mixture was stirred at 40 °C for 12 hours. After the reaction was finished (monitored by TLC), the solvent was removed in vacuo. A solution of NaHCO<sub>3</sub> 5% was gradually added to adjust pH to 7, which led to the formation of white solids. The solids were filtered, washed with cold water and dried at 60 °C. The crude product was further purified by column chromatography (DCM/methanol = 95:5) to give the corresponding derivatives **26a-c** and **28a-c**, yields 72-81%.

In the final step, each of the intermediate esters **26** or **28** (0.5 mmol) was dissolved in methanol (10 mL). Then, hydroxylamine hydrochloride (343 mg, 5 mmol) was added, followed by dropwise addition of a solution of NaOH (200 mg in 1 mL of water). The mixture was stirred at 0 °C until the reaction completed (1-2 h, checked by TLC). At the end of this reaction, the resulting reaction mixture was poured into ice-cold water, neutralised to pH~7 and acidified by dropwise addition of a solution of HCl 5% to induce the maximum precipitation. The precipitates were filtered, dried and re-crystalised in methanol to give the designed hydroxamic acids **27a-c**, **29a-c**.

#### General procedures for the synthesis of compounds 31a-c, 33a-c

Compounds **31a-c** and **33a-c** were produced following a synthetic route akin to the one delineated for **27a-c** and **29a-c**, with the modification of employing methyl 4-bromoheptanoate instead of methyl (E)-4-bromomethylcinnamate, as depicted in Scheme 2.

#### 1.3. Biology

#### Cytotoxicity assay

The cytotoxicity of the synthesized compounds was evaluated against three cell lines, including SW620 (colon cancer), MDA-MB-231 (breast cancer), MRC-5 (human fetal lung fibroblast cells). The cell lines were purchased from a Cancer Cell Bank at the Korea Research Institute of Bioscience and Biotechnology (KRIBB). The media, sera and other reagents that were used for cell culture in this assay were obtained from GIBCO Co. Ltd. (Grand Island, New York, USA). The cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) until confluence. The cells were then trypsinized and suspended at  $3 \times 10^4$  cells/mL of cell culture medium. On day 0, each well of the 96-well plates was seeded with 180 µL of cell suspension. The plates were then incubated in a 5% CO<sub>2</sub> incubator at 37 °C for 24 h. Compounds were initially dissolved in dimethyl sulfoxide (DMSO) and diluted to appropriate concentrations by culture medium. Then 20 µL of each compounds' samples, which were prepared as described above, were added to each well of the 96-well plates, which had been seeded with cell suspension and incubated for 24-h, at various concentrations. The plates were further incubated for 48 h. Cytotoxicity of the compounds was measured by the colorimetric method, as described previously [26] with slight modifications [27-29]. The IC<sub>50</sub> values were calculated using a Probits method [30] and were averages of three independent determinations (SD  $\leq$  10%).

#### HDAC enzymes assay

The HDAC enzymes (Hela cell nuclear extract) were purchased from Enzo Life Sciences Inc. (Farmingdale, New York, USA). The HDAC enzymatic assay was performed using a Fluorogenic HDAC Assay Kit (Enzo Life Sciences Inc.) according to the manufacturer's instructions. Briefly, HDAC enzymes were incubated with vehicle or various concentrations of the assayed samples or SAHA for 30 min at  $37^{\circ}$ C in the presence of an HDAC fluorometric substrate. The HDAC assay developer (which produces a fluorophore in reaction mixture) was added, and the fluorescence was measured using VICTOR (PerkinElmer, Waltham, MA, USA) with excitation at 360 nm and emission at 460 nm. The measured activities were subtracted by the vehicle-treated control enzyme activities and IC<sub>50</sub> values were calculated using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

#### Cell cycle analysis

SW620 human colon cancer cells ( $2 \times 10^{5}$ /ml per well) were seeded in 6-well culture plates and allowed to adhere for either 2 hours or 24 hours. Subsequently, the cells were treated with various concentrations of compounds for either 24 hours or 48 hours, respectively, followed by harvesting. The harvested cells underwent two washes with ice-cold PBS, were fixed in 75% ice-cold ethanol, and then stained with propidium iodide (PI) along with RNase at room temperature for 30 minutes. The stained cells were then subjected to DNA content analysis using a FACScalibur flow cytometer (BD Biosciences, San Jose, CA, USA), and the resulting data were processed utilizing Cell Quest Pro software (BD Biosciences).

#### Apoptosis assay

The Annexin V-FITC/PI dual staining assay was employed to assess the proportion of apoptotic cells. SW620 human colon cancer cells ( $2 \times 10^5$ /ml per well) were seeded in 6-well culture plates and allowed to adhere for either 2 hours or 24 hours. Subsequently, the cells were treated with varying concentrations of compounds for either 24 hours or 48 hours, respectively, followed by harvesting. The harvested cells underwent two washes with ice-cold PBS and were then incubated in the dark at room temperature in 100 µl of 1× binding buffer containing 1 µl Annexin V-FITC and 12.5 µl PI. After a 15-minute incubation period, the cells were analyzed for the percentage undergoing apoptosis using a FACScalibur flow cytometer (BD Biosciences). The resulting data were processed utilizing Cell Quest Pro software (BD Biosciences).

#### 1.4. Molecular docking studies

Compound structures were generated by ChemDraw version 9.0 and subsequently subjected to minimize energy within a rms gradient of 0.1 kcal<sup>-1</sup>.mol<sup>-1</sup>.Å<sup>-1</sup> by MOE 2015 package. The force field was set to the 94s variant of the Merck Molecular force field (MMFF94s). The hydroxamic acid groups were deprotonated according to the

previous reports [31-33]. The X-ray crystallographic structure of SAHA in complex with HDAC2 and HDAC6 were taken from Protein Data Bank with ID of 4LXZ and 5EEI, respectively [34-35]. The structure was prepared using the QuickPrep tool in MOE for adding hydrogen, protonating, deleting water, and editing atom types, assigning AMBER FF99 charges according to the same procedures mentioned before [32-33]. For the docking setting, simulations of flexible-ligand rigid-protein were performed using the method of MOE Triangle matcher placement with retaining 30 poses for analysis. The results that showed appropriate binding geometry with Zn<sup>2+</sup> ion was considered. The finally selected conformations were scored using London dG (Score1) and GBVI/WSA dG functions (Score2) to estimate the free binding energy between the ligands and the enzyme. All the selected poses were visualized using BIOVIA Discovery Studio v3.5 program.

# 2. All <sup>1</sup>H & <sup>13</sup>C NMR spectra of the compounds



<sup>&</sup>lt;sup>13</sup>C NMR of compound 6a



#### <sup>1</sup>H NMR of compound 6b



# <sup>1</sup>H NMR of compound 6c



# <sup>1</sup>H NMR of compound 6d



# <sup>13</sup>C NMR of compound 6d







<sup>&</sup>lt;sup>13</sup>C NMR of compound 6f



# <sup>1</sup>H NMR of compound 6g



#### <sup>13</sup>C NMR of compound 6g





<sup>&</sup>lt;sup>13</sup>C NMR of compound 9a



#### <sup>1</sup>H NMR of compound 9b

TAnh-122B



#### <sup>13</sup>C NMR of compound 9b





# <sup>1</sup>H NMR of compound 9d



#### <sup>13</sup>C NMR of compound 9d







<sup>13</sup>C NMR of compound 9f



#### <sup>1</sup>H NMR of compound 9g



## <sup>13</sup>C NMR of compound 9g



## <sup>1</sup>H NMR of compound 14a

TAnh-126A



#### <sup>13</sup>C NMR of compound 14a

TAnh-126A



## <sup>1</sup>H NMR of compound 14b

TAnh-126B B JK ÉR 8.661 9.661 1.481 1.481 1.452 1.452 1.452 1.218 6.595 6. 1.920 1.955 1.890 1.615 1.625 1.625 1.625 1.625 1.6399 1.639 1.639 1.639 1.639 1.639 1.639 1.639 1.639 1.639 1.639 Current Data Parameters NAME TAnh-126B EXPNO 31 PROCNO 1 
 PROCNO
 1

 F2 - Acquisition Parameters Date\_\_\_\_20220819

 Time
 9.42

 INSTRUM
 spect

 PROBHD
 5 mm PABBO BB/

 PULPROG
 65536

 SOLVENT
 DMSO

 NS
 16

 DS
 2,230

 SULVENT
 DMSO

 NS
 16

 DS
 2,230

 SWH
 10000.000 Hz

 FIDRES
 0.152588 Hz

 AQ
 3.2767999 sec

 RG
 191.38

 DW
 50.000 usec

 DE
 6,50 usec

 TE
 297.0 K

 TO
 1.00000000 sec

 TO
 1.00001000
 SFO1 NUC1 P1 PLW1 --- CHANNEL f1 ------500.1330885 MHz 1H 9.80 usec 24.0000000 W F2 - Processing parameters SI 65536 SF 500.1300043 MHz WDW EM SSB 0 EM GB 0 0.30 Hz GB 0 1.00 ++/ 2.62 2.02 2.60 2.78 5.53 1.31 3.99 0.98 1000 8 9 6 4 3 2 10 8 7 5 ppm 1

#### <sup>13</sup>C NMR of compound 14b

TAnh-126B



TAnh-126C B IK ÉR 1.933 1.919 1.919 1.919 1.919 1.646 1.646 1.618 1.437 1.437 1.422 1.422 1.422 1.422 1.212 1.212 1.12577 1.1257 1.1257 1.1257 1.125 Current Data Parameters NAME TAnh-126C EXPNO 20 PROCNO 1 1 
 PROCNO
 1

 F2 - Acquisition Parameters Date\_\_ 20220922

 Time
 9.24

 INSTRUM
 Spect

 PROBHD
 5 mm PABBO BB/ PULPROG
 230

 TD
 65536

 SOLVENT
 DMSO

 NS
 16

 DS
 20000 Hz

 FIDRES
 0.152588 Hz

 AQ
 3.2767999 sec

 RG
 191.38

 DW
 50.000 usec

 DE
 6.50 usec

 TE
 296.7 K

 TO
 1.00000000

 RG
 191.38

 DW
 50.000 usec

 DE
 6.50 usec

 TE
 1.00000000 sec

 TO
 1.00000000 sec
 SFO1 NUC1 P1 PLW1 ---- CHANNEL f1 ------1H 9.80 usec 24.0000000 W F2 - Processing parameters SI 65536 SF 500.1300000 MHz WDW EM SSB 0 EM GB 0 0.30 Hz GB 0 1.00 h 2.02 1.20 2.07 2.27 2.13 5.14 9 8 5 4 3 2 ppm 7 6 1

## <sup>13</sup>C NMR of compound 14c

TAnh-126C



TAnh-126D JKÉR BÌ 8.657
8.657
8.657
8.30
8.7333
8.7333
8.7333
8.7333
8.7333
8.7333
8.7333
8.7233
8.7233 1.929 11.929 11.638 1.638 1.624 1.449 1.449 1.449 1.449 1.1420 1.178  $\begin{array}{c}
4.314 \\
4.302 \\
4.021 \\
4.007 \\
3.993
\end{array}$ Current Data Parameters NAME TAnh-126D EXPNO 20 PROCNO 1 
 PROCNO
 1

 F2 - Acquisition Parameters Date\_\_\_\_20220819

 Time
 9.38

 INSTRUM
 spect

 PROBHD
 5 mm PABBO BB/

 PULPROG
 2303

 TD
 65536

 SOLVENT
 DMSO

 NS
 16

 DS
 202

 SWH
 10000.000

 FIDRES
 0.152588

 AQ
 3.2767999

 RG
 191.38

 DW
 50.000

 TE
 2.97.1

 R
 1.00000000

 TE
 2.97.1

 TO
 1.0000000
 SF01 NUC1 P1 PLW1 ---- CHANNEL f1 ------500.1330885 MHz 1H 9.80 usec 24.0000000 W 
 F2 - Processing parameters

 SI
 65536

 SF
 500.1300042 MHz

 WDW
 EM

 SSB
 0

 GB
 0

 PC
 1.00
 2.19 2.33 2.33 1.13 2.24 8 5.69 1.18 8 10 9 7 5 4 3 2 ppm 8 6 1

#### <sup>13</sup>C NMR of compound 14d

TAnh-126D ВŘ ÚKÉR 145.406 141.683 139.963 131.502 129.660 128.603 120.055 46.913 44.159 32.624 29.386 28.548 26.289 25.429 111.061 91.661 . Current Data Parameters NAME TAnh-126D EXPNO 21 PROCNO 1 
 FROCING
 F2
 Acquisition Parameters

 F2
 20220819

 Time
 13.30

 INSTRUM
 spect

 PROBHD
 5 mm

 PABEO
 BB/

 PULPROG
 5536

 SOLVENT
 DMSO

 NS
 2048

 DS
 2048
 PULPROG TD SOLVENT NS SWH FIDRES AQ RG DW DE TE D1 D11 TD0 2048 4 31250.000 Hz 0.476837 Hz 1.0485760 sec 191.38 16.000 usec 6.50 usec 298.2 K 2.00000000 sec 0.03000000 sec SFO1 NUC1 P1 PLW1 = CHANNEL f1 ------125.7703637 MHz 13C 10.20 usec 90.00000000 W SF02 NUC2 CPDPRG[2 PCPD2 PLW2 PLW12 PLW13 
 F2 Processing parameters

 SI
 65536

 SF
 125.7577885 MHz

 WDW
 EM

 SSB
 0

 LB
 1.00 Hz

 GB
 0

 PC
 1.40
 180 160 140 120 100 80 60 40 20 ppm

TAnh-126E B IKÉR 8.677 7.4834 7.433 7.433 7.1329 7.1329 7.1150 7.1150 6.1150 6.526 6.526 6.526 4.298 4.293 4.028 4.014 4.000 1.931 1.917 1.902 1.653 1.653 1.454 1.454 1.1426 1.1230 1.1215 Current Data Parameters NAME TAnh-126E EXPNO 10 PROCNO 1 
 PROCNO
 1

 F2 - Acquisition Parameters Date\_\_\_\_20221201

 Time
 10.40

 INSTRUM
 spect

 PROBHD
 5 mm PABBO BB/ PULPROG
 zg30

 TD
 65536

 SOLVENT
 DMSO

 NS
 16

 DS
 2,230

 SWH
 10000.000

 FIDRES
 0.152588

 AQ
 3.2767999

 RG
 191.38

 DW
 50.000

 TE
 2.97.1 K

 DI
 1.0000000

 TO
 1
 SFO1 NUC1 P1 PLW1 ---- CHANNEL f1 -------500.1330885 MHz 1H 9.80 usec 24.0000000 W F2 - Processing parameters SI 65536 SF 500.1300045 MHz WDW EM SSB 0 EM GB 0 0.30 Hz GB 0 1.00 2.15 1.97 2.27 4.66 0.97 5.2 5.20 8 6 8 7 5 4 3 2 ppm 10 9 6 1

#### <sup>13</sup>C NMR of compound 14e

TAnh-126E



TAnh-126F BÌ IKÉR 8.650 8.650 9.655959 6.5595959 6.5595959 6.559599 6.55959 6.55959 6.55959 6 Current Data Parameters NAME TAnh-126F EXPNO 20 PROCNO 1 20 20 1 
 PROCNO
 1

 F2 - Acquisition Parameters Date\_\_\_\_20220812

 Time
 14.35

 INSTRUM
 spect

 PROBHD
 5 mm PABBO BB/

 PULPROG
 2303

 TD
 65536

 SOLVENT
 DMSO

 NS
 16

 DS
 202

 SWH
 10000.000

 FIDRES
 0.152588

 AQ
 3.2767999

 RG
 191.38

 DW
 50.000

 TE
 2.97.2

 R
 1.00000000

 TD
 1.00000000
 SFO1 NUC1 P1 PLW1 --- CHANNEL f1 ------500.1330885 MHz 1H 9.80 usec 24.0000000 W F2 - Processing parameters SI 65536 SF 500.1300043 MHz WDW EM SSB 0 EM GB 0 0.30 Hz GB 0 1.00 2.07 2.52 5.84 1.22 2.35 3.27 ē. 1.06 2.29 8 10 8 5 4 3 2 ppm 9 7 6 1

#### <sup>13</sup>C NMR of compound 14f

TAnh-126F



TAnh-126G



#### <sup>13</sup>C NMR of compound 14g

TAnh-126G



TAnh-127A



#### <sup>13</sup>C NMR of compound 17a

TAnh-127A BRÚKÉR 144.974 143.323 143.323 129.066 128.966 128.901 126.976 126.976 126.9775 169.520 -47.832 -44.409 -32.602 -29.713 -28.506 -26.289 -25.444 . Current Data Parameters NAME TAnh-127A EXPNO 11 PROCNO 1  $\left| \right|$  $\mathbb{N}$  
 F2 - Acquisition Parameters

 Date\_
 20221012

 Time
 16.28

 INSTRUM
 spect

 PROBHD
 5 mm PABBO BB/

 PULPROG
 65536

 COLUEND
 DMSO
 PULPROG TD SOLVENT NS DS SWH FIDRES AQ RG DW DE TE D1 D11 TD0 DMSO 1433 1433 4 31250.000 Hz 0.47683760 sec 191.38 16.000 usec 6.50 usec 2.0000000 sec 0.3000000 sec 1 SFO1 NUC1 P1 PLW1 CHANNEL f1 ------125.7703637 MHz 13C 10.20 usec 90.00000000 W \_\_\_\_ SF02 NUC2 CPDPRG[2 PCPD2 PLW2 PLW12 PLW13 F2 - Processing parameters SI 65536 SF 125.7577885 MHz WDW EM SSB 0 LB 1.00 Hz GB 0 PC 1.40 170 160 150 140 130 120 110 100 90 80 70 60 **50** 40 30 20 ppm



#### <sup>13</sup>C NMR of compound 17b

TAnh-127B



TAnh-127C



#### <sup>13</sup>C NMR of compound 17c

TAnh-127C





#### <sup>13</sup>C NMR of compound 17d

TAnh-127D



TAnh-127E B JK ÉR 4.238 4.257 4.2439 4.2439 4.2439 4.2439 4.1208 4.1208 1.934 1.919 1.934 1.777 1.9195 1.234 10.373 8:542 7:838 7:838 7:4462 7:4462 7:4462 7:417 7:422 7:417 7:417 7:4163 7:1145 7:1145 7:1145 7:1145 6:839 6:844 6:641 Current Data Parameters NAME TAnh-127E EXPNO 100 PROCNO 1 
 PROCING
 Image: Transform of the second 65536 DMS0 16 2 10000.000 Hz 0.152588 Hz 3.276799 sec 191.38 50.000 usec 296.9 K 1.0000000 sec 1 AQ RG DW DE TE D1 TD0 SFO1 NUC1 P1 PLW1 --- CHANNEL f1 ------500.1330885 MHz 1H 9.80 usec 24.0000000 W F2 - Processing parameters SI 65536 SF 500.1300031 MHz WDW EM SSB 0 EM GB 0 0.30 Hz GB 0 1.00 1.18 3.27 2.36 0.96 2.05 2.73 6.66 0.76 8 3.76 10 7 6 5 3 2 ppm 9 8 4 1

# <sup>13</sup>C NMR of compound 17e





#### <sup>13</sup>C NMR of compound 17f

TAnh-127F





#### <sup>13</sup>C NMR of compound 17g

TAnh-127G ВŘ ÚKÉR -145.020 -137.937 -135.931 -129.266 -129.266\_\_\_\_\_112.199 \_\_\_\_110.745 47.601 44.428 32.614 29.706 28.508 26.286 25.437 21.132 . Current Data Parameters NAME TAnh-127G EXPNO 22 PROCNO 1 
 FRCUR
 F2 - Acquisition Parameters

 Date\_\_\_\_\_\_20220819

 Time
 7.15

 INSTRUM
 spect

 PROBHD
 5 mm

 PABBO BE
 PULPROG

 SOLVENT
 0450

 NS
 4096

 DST
 2040
 PULPROG TD SOLVENT NS SWH FIDRES AQ RG DW DE TE D1 D11 TD0 4096 4 31250.000 Hz 0.476837 Hz 1.0485760 sec 191.38 16.000 usec 6.50 usec 298.5 K 2.00000000 sec 0.03000000 sec SFO1 NUC1 P1 PLW1 = CHANNEL f1 ------125.7703637 MHz 13C 10.20 usec 90.00000000 W SF02 NUC2 CPDPRG[2 PCPD2 PLW2 PLW12 PLW13 
 F2 Processing parameters

 SI
 65536

 SF
 125.7577885 MHz

 WDW
 EM

 SSB
 0

 LB
 1.00 Hz

 GB
 0

 PC
 1.40
 180 160 140 120 100 80 60 40 20 ppm
# <sup>1</sup>H NMR of compound 22a

TAnh-121A



# <sup>13</sup>C NMR of compound 22a





<sup>&</sup>lt;sup>13</sup>C NMR of compound 22b



# <sup>1</sup>H NMR of compound 22c

TAnh-121C JKÉR BF 11.167 9.046 9.046 9.049 9.049 9.049 9.049 9.049 9.049 9.049 9.049 9.049 9.049 9.049 9.049 9.049 9.049 9.049 9.0566 7.7.289 9.7.280 9.7.289 9.7.299 9. ~ Current Data Parameters NAME TAnh-121C EXPNO 40 PROCNO 1 
 PROCNO
 1

 F2 - Acquisition Parameters Date\_\_\_\_20220105

 Time
 8.11

 INSTRUM
 spect

 PROBHD
 5 mm PABBO BB/ PULPROG
 230

 TD
 65536

 SOLVENT
 DMSO

 NS
 16

 DS
 202

 SWH
 10000.000 Hz

 FIDRES
 0.152588 Hz

 AQ
 3.2767999 sec

 RG
 191.38

 DW
 50.000 usec

 DE
 6.50 usec

 TE
 2.97.0 K

 TO
 1.00000000 sec

 TO
 1.00000000 sec

 CHANNEL fl

 SF01
 500.1330885

 NUC1
 1H

 P1
 9.80
 usec

 PLW1
 24.0000000 W
 W
1H 9.80 usec 24.0000000 W F2 - Processing parameters SI 65536 SF 500.1300052 MHz MDW EM SSB 0 EM GB 0 0.30 Hz GB 0 1.00 10 9 7 6 3 2 ppm 11 5 4 8 1 1.04 6.46 6.46 1.03 1.01 1.03 2.09 8 2.07 8

# <sup>13</sup>C NMR of compound 22c



# <sup>1</sup>H NMR of compound 22d



# <sup>1</sup>H NMR of compound 22e

TAnh-121E JKÉR BF 9.027 9. 11.167 ~ Current Data Parameters NAME TAnh-121E EXPNO 10 PROCNO 1 
 PROCNO
 1

 F2 - Acquisition Parameters Date\_\_\_\_20220106

 Time
 8.57

 INSTRUM
 spect

 PROBHD
 5 mm PABBO BB/ PULPROG
 g30

 TD
 65536

 SOLVENT
 DMSO

 NS
 16

 DS
 2

 SWH
 10000.000 Hz

 FIDRES
 0.152588 Hz

 AQ
 3.2767999 sec

 RG
 191.38

 DW
 50.000 usec

 DE
 6.50 usec

 TE
 226.6 K

 TO
 1.00000000 sec

 TO
 1.00000000 sec

 CHANNEL fl

 SF01
 500.1330885

 NUC1
 1H

 P1
 9.80
 usec

 PLW1
 24.0000000 W
 W
1H 9.80 usec 24.0000000 W F2 - Processing parameters SI 65536 SF 500.1300050 MHz WDW EM SSB 0 EM GB 0 0.30 Hz GB 0 1.00 11 10 9 4 3 2 7 6 5 1 ppm 8 2.06 1.02 1.81 1.00

# <sup>13</sup>C NMR of compound 22e



# <sup>1</sup>H NMR of compound 22f



# <sup>13</sup>C NMR of compound 22f



# <sup>1</sup>H NMR of compound 22g



# <sup>1</sup>H NMR of compound 25a



# <sup>13</sup>C NMR of compound 25a







<sup>&</sup>lt;sup>13</sup>C NMR of compound 25c



# <sup>1</sup>H NMR of compound 25d

TAnh-123D JKÉR BF 11.152 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.038 9.1556 9.1556 9.1239 Current Data Parameters NAME TAnh-123D EXPNO 30 PROCNO 1 
 PROCNO
 1

 F2 - Acquisition Parameters Date\_\_\_\_20220106

 Time
 9.05

 INSTRUM
 Spect

 PROBHD
 5 mm PABBO BB/ PULPROG

 FULPROG
 65536

 SOLVENT
 DMSO

 NS
 16

 DS
 2

 SWH
 10000.000 Hz

 FIDRES
 0.152588 Hz

 AQ
 3.2767999 sec

 RG
 191.38

 DW
 50.000 usec

 DE
 6.50 usec

 TE
 226.6 K

 TO
 1.00000000 sec

 TO
 1.00000000 sec
SFO1 NUC1 P1 PLW1 ---- CHANNEL f1 ------500.1330885 MHz 1H 9.80 usec 24.0000000 W F2 - Processing parameters SI 65536 SF 500.1300046 MHz WDW EM SSB 0 EM GB 0 0.30 Hz GB 0 1.00 10 9 8 7 6 5 3 2 ppm 11 4 1 1.03 0.68 0.68 1.36 1.67 0.78 1.09 2.44 **8** 1.59

# <sup>13</sup>C NMR of compound 25d



#### <sup>1</sup>H NMR of compound 25e



# <sup>1</sup>H NMR of compound 25f



#### <sup>13</sup>C NMR of compound 25f



#### <sup>1</sup>H NMR of compound 25g



# <sup>1</sup>H NMR of compound 27a



# <sup>13</sup>C NMR of compound 27a



#### <sup>1</sup>H NMR of compound 27b



<sup>&</sup>lt;sup>13</sup>C NMR of compound 27b



### <sup>1</sup>H NMR of compound 27c



# <sup>13</sup>C NMR of compound 27c



# <sup>1</sup>H NMR of compound 29a



# <sup>13</sup>C NMR of compound 29a



#### <sup>1</sup>H NMR of compound 29b



# <sup>13</sup>C NMR of compound 29b



#### <sup>1</sup>H NMR of compound 29c



# <sup>13</sup>C NMR of compound 29c



### <sup>1</sup>H NMR of compound 31a



# <sup>13</sup>C NMR of compound 31a

TAnh-128A



# <sup>1</sup>H NMR of compound 31b



# <sup>13</sup>C NMR of compound 31b

TAnh-128B



# <sup>1</sup>H NMR of compound 31c



# <sup>13</sup>C NMR of compound 31c

TAnh-128C





<sup>&</sup>lt;sup>13</sup>C NMR of compound 33a



# <sup>1</sup>H NMR of compound 33b



# <sup>13</sup>C NMR of compound 33b





<sup>&</sup>lt;sup>13</sup>C NMR of compound 33c



#### HRMS of compound 6a



#### HRMS of compound 6b







### HRMS of compound 6d











### HRMS of compound 6g



HRMS of compound 9a







#### HRMS of compound 9c











#### HRMS of compound 9f



# HRMS of compound 9g







### HRMS of compound 14b







#### HRMS of compound 14d



# HRMS of compound 14e











# HRMS of compound 17a











# HRMS of compound 17d











# HRMS of compound 17g



#### HRMS of compound 22a






# HRMS of compound 22c











### HRMS of compound 22f



HRMS of compound 22g



## HRMS of compound 25a



# HRMS of compound 25b







## HRMS of compound 25d



## HRMS of compound 25e











# HRMS of compound 27a











## HRMS of compound 29a



## HRMS of compound 29b



## HRMS of compound 29c



## HRMS of compound 31a











# HRMS of compound 33a



# HRMS of compound 33b





