Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

< Supplementary Information>

Synthesis and biological evaluation of 2-acetamidothiophene-3-carboxamide derivatives against *Leishmania donovani*

Sangmi Oh,[a] Bosung Kwon,[a] Sunju Kong,[a] Gyongseon Yang,[b] Nakyung Lee,[b] Dawon Han,[b] Junghyun Goo,[b] Jair L. Siqueira-Neto,[b,†] Lucio H. Freitas-Junior,[b,‡] Michel Liuzzi,[c] Jinhwa Lee,[d] Rita Song[a,*]

[a]*Medicinal Chemistry Group, Institut Pasteur Korea, 696 Sampyeong-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463-400, Korea*

[b]*Center for Neglected Diseases Drug Discovery (CND3), Institut Pasteur Korea, 696 Sampyeong-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463-400, Korea*

[c]*Early Discovery Program, Institut Pasteur Korea, 696 Sampyeong-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463-400, Korea*

[d]*Late Discovery Program, Institut Pasteur Korea, 696 Sampyeong-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463-400, Korea*

[†] *Present address: University of California, San Francisco, USA*

[‡] *Present address: Laboratório Nacional de Biociências (LNBio), Brazil*

∗ Corresponding author. Tel.: +82-31-8018-8230; fax: +82-31-8018-8014; e-mail: rsong@ip-korea.org

Table of Contents

I. Experimental Procedure about Biology

1. Parasite and cell cultures

Leishmania donovani (*L. donovani*) parasites MHOM/ET/67/HU3 (ATCC 50127) were cultivated as promastigotes at 28 ℃ in M199 with 40 mM HEPES, 0.1 mM adenine, 0.0001 % biotin and 4.62 mM $NaHCO_3$, supplemented with 10 % heat-inactivated FBS. Parasites were sub-cultured every 2 or 3 days. THP-1 cells (ATCC TIB-202) were cultivated in RPMI supplemented with 10 % FBS at 37 ℃, 5 % CO_2.

2. Intracellular *Leishmania* (amastigote) screening assay

The screening assay for *Leishmania* was performed as previously reported by Siqueira-Neto *et al.*[1] Briefly, *L. donovani* culture was incubated 6 days before infection to generate metacylic promatigote, which was macrophage-infective form. THP-1 cells were differentiated with 50 ng/mL of phorbol 12-myristate 13-acetate (PMA, Sigma P1585) before infection. Cells and parasites were seeded in 384 well plates, using a WellMate™ liquid handler. After 24 hours incubation at 37 ℃, 5 % CO_2, reference drug amphotericin B (Sigma A2942) 4 µM as EC_{100} (positive control), DMSO 1 % (negative control) and the compounds were added to the wells in volume of 10 µL followed by incubation at 37 ℃ and 5 % CO_2 for 3 days. Wells were then washed with PBS, fixed with 4 % paraformaldehyde, and then DNA was stained with Draq5. Automated confocal microscope (Perkin Elmer) was used to take pictures of the plates and then images were analyzed by software developed in house.

3. Extracellular *Leishmania* (promastigote) assay

L. donovani (1×10^6 parasites/mL) were seeded in 384 plate (Evotec™) contained synthesized compound dissolved in DMSO. Compound was exposed to *Leishmania* for 48 hours and 400 µM of resazurin sodium salt (Sigma R7017) was added 20 hours before total incubation time was finished. After 48 hours exposure to compounds, the parasites were fixed with 4 % paraformaldehyde and plates were read in Victor3TM (Perkin Elmer) at 530 nm (excitation) and 590 nm (emission).

4. Data analysis

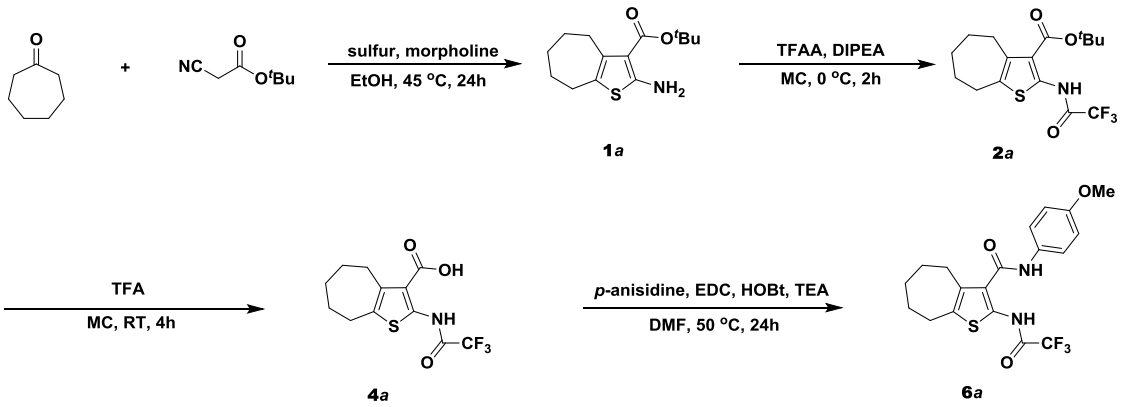
The acquired images were analyzed with in-house software to quantify, cell numbers, parasites numbers, and infection ratio. 4 μM of amphotericin B and 1 % of DMSO were used for drug-positive and negative control, respectively. 2-Fold dilution with 10 points of amphotericin B and miltefosine were tested for assay-to-assay quality control by comparing EC_{50} and DRC graph. Collected data were used to validate activities of synthesized compounds from EC_{50}, CC_{50} and SI value. EC_{50} was calculated by infection ratio normalized by positive and negative controls. CC_{50} value was obtained from counting THP-1 cell numbers, and SI value was determined by EC_{50} / CC_{50} value. All performed assay was quality controlled by Z' value, windows and CV.

II. General Information about Synthesis

^{1}H and ^{13}C NMR spectra were recorded on a Varian High Resolution FT-NMR Spectrometer-400 [Varian Inc., Palo Alto, USA], and chemical shifts were measured in ppm relative to internal tetramethylsilane (TMS) standard or specific solvent signal. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublet); dt (doublet of triplet); td (triplet of doublet); brs (broad singlet), etc. Coupling constants were reported in Hz. Routine mass analyses were performed on Waters LC/MS ZQ2000 [Waters Corp., Milford, USA] system equipped with a reverse phase column (XBridge^{TM} C18 × 3.5 μm, 50 × 2.1 mm) and photodiode array detector using electron spray ionization (ESI). Melting point analyses were performed on BUCHI Melting point M-565 [BUCHI Labortechnik AG, Flawil, Switzerland]. Most reagents in this synthetic procedure were purchase from Sigma-Aldrich [MO, USA], Alfa Aesar [MA, USA], Fluorochem [CA, USA] and TCI [Japan]. The progress of reaction was monitored using thin-layer chromatography (TLC) (silica gel 60 F2540.25 mm), and components were visualized by observation under UV light (254 and 365 nm) or by treating the TLC plates with ninhydrin staining solution followed by heating. Silica gel 60 (0.040–0.063 mm) used in flash column chromatography was purchased from Merck [Germany]. Other solvents and organic reagents were purchased from commercial venders and used without further purification unless otherwise mentioned.

III. Synthetic Procedure

1. Scheme 1



General procedure for the synthesis of 1*a*

To a stirred solution of cycloheptanone (1.00 g, 8.92 mmol), *tert*-butyl cyanoacetate (1.26 g, 8.92 mmol) and sulfur (343 mg, 10.70 mmol) in absolute EtOH (30 mL) was added morpholine (2.4 ml, 26.76 mmol). The reaction mixture was heated to 45 ˚C for 24 hrs. After reaction was completed, the mixture was allowed to cool to room temperature, and filtered, and then filtrate was concentrated *in vacuo*. The resultant oil was dissolved in EA, washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated *in vacuo*. The crude product was purified by flash column chromatography (EA / Hexane) to give **1*a***.

General procedure for the synthesis of 2*a*

To a stirred solution of **1*a*** (500 mg, 1.87 mmol) and TFAA (0.4 ml, 2.81 mmol) in CH_2Cl_2 (15 ml) was added dropwise DIPEA (0.65 ml, 3.74 mmol) under a N_2 atmosphere at 0 ˚C. The reaction mixture was stirred for 2 hrs at the same temperature. After reaction was completed, the mixture poured onto cold water. The organic layer was washed with 1M HCl, water and brine, and then dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography (EA / Hexane) to give **2*a***.

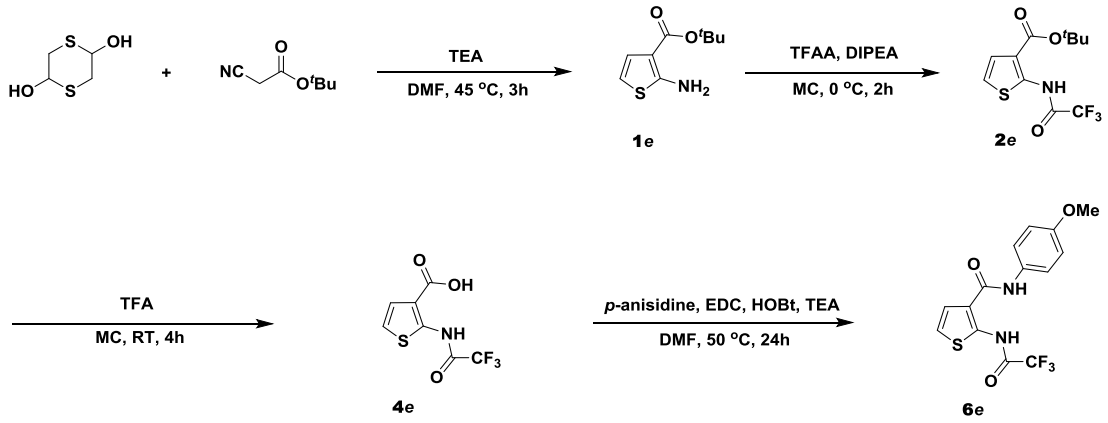
General procedure for the synthesis of 4*a*

To a stirred solution of **2*a*** (220 mg, 0.60 mmol) in CH_2Cl_2 (5 ml) was added dropwise TFA (0.25 ml, 3.00 mmol) under N_2 atmosphere. The reaction mixture was stirred 4 hrs at room temperature. After reaction was completed, the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash column chromatography (EA / Hexane) to give **4*a***.

General procedure for the synthesis of 6*a*

To a stirred solution of **4*a*** (20 mg, 0.07 mmol), *p*-anisidine (7 mg, 0.08 mmol), EDC (25 mg, 0.13 mmol), HOBt (18 mg, 0.13 mmol) in DMF (0.5 ml) was added TEA (0.03 ml, 0.20 mmol) under N_2 atmosphere. The reaction mixture was heated to 50 ˚C for overnight. After reaction was completed, the reaction mixture was diluted with CH_2Cl_2 and washed with water and aq. NH_4Cl solution. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography (EA / Hexane) to give **6*a***.

2. Scheme 2



General procedure for the synthesis of 1*e*

To a stirred solution of *tert*-butyl cyanoacetate (1 g, 7.08 mmol) and 1,4-dithiane-2,5-diol (0.54 g, 3.54 mmol) in DMF (5 mL) was added TEA (1.43 g, 14.2 mmol). The reaction mixture was heated to 45 ˚C for 3 hrs. After reaction was completed, the reaction mixture was diluted with EA. The organic layer was washed with water and brine and then dried over anhydrous Na_2SO_4 and concentrated *in vacuo*.

The crude product was purified by flash column chromatography (EA / Hexane) to give **1*e***.

General procedure for the synthesis of 2*e*

The procedure for **2*e*** was followed by procedure of **2*a***.

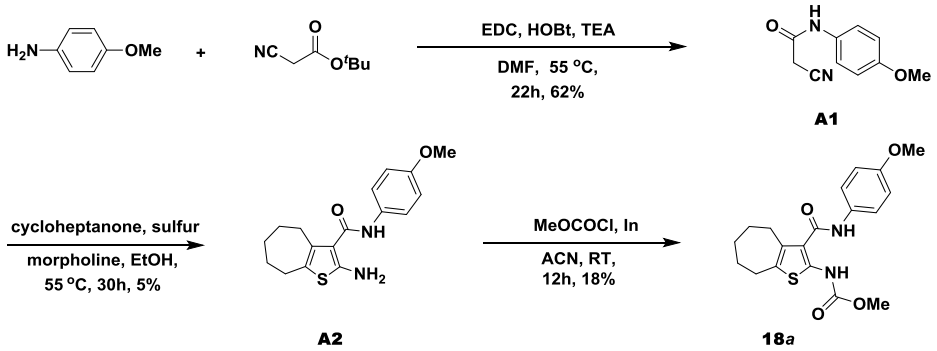
General procedure for the synthesis of 4*e*

The procedure for **4*e*** was followed by procedure of **4*a***.

General procedure for the synthesis of 6*e*

The procedure for **6*e*** was followed by procedure of **6*a***.

3. Scheme 3



General procedure for the synthesis of A1

The procedure for **A1** was followed by procedure of **6*a***.

General procedure for the synthesis of A2

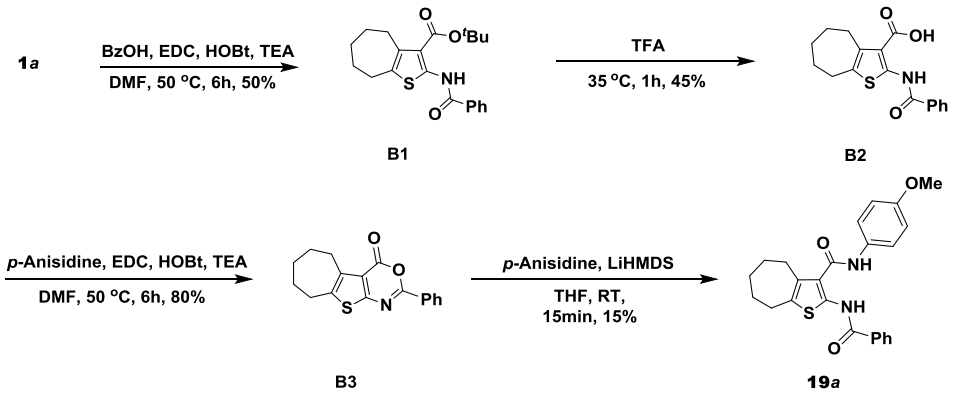
The procedure for **A2** was followed by procedure of **1*a***.

General procedure for the synthesis of 18*a*

To a stirred solution of **A2** (100 mg, 0.32 mmol) in ACN (5 ml) was added methyl chloroformate (33 mg, 0.35 mmol) and indium (25 mg, 0.22 mmol) under N_2 atmosphere. The reaction mixture was stirred

for 12 hrs at room temperature. After reaction was completed, the reaction mixture was diluted with CH_2Cl_2 and washed with sat. $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (EA / Hexane) to give 1**8*a***.

4. Scheme 4



General procedure for the synthesis of B1

The procedure for **B1** was followed by procedure of **6*a***.

General procedure for the synthesis of B2

The procedure for **B2** was followed by procedure of **4a**.

General procedure for the synthesis of B3

The procedure for **B3** was followed by procedure of **6*a***.

General procedure for the synthesis of 19*a*

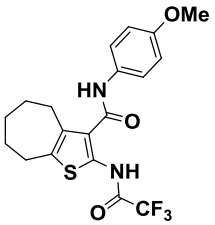
To a stirred solution of 2-phenyl-6,7,8,9-tetrahydro-4*H*,5*H*-cyclohepta[4,5]thieno[2,3-*d*][1,3]oxazin-4-one (75 mg, 0.25 mmol) in THF (0.35 mL) was added *p*-anisidine (31 mg, 0.25 mmol) and 1.0 M lithium hexamethyl disilazide in THF (0.25 mmol). The reaction mixture was stirred for 15 min. After reaction was completed, the reaction mixture was quenched with aq. NH_4Cl solution and then extracted with EA.

The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (EA / Hexane) to give **9*a***.

IV. Compound Characterizations

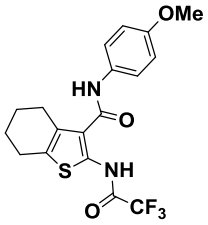
Compound **1*a***, **6*a*–6*c***, **8*a*–12*a***, **17*a***, **19*a*** are already known molecules.

<u>*N*-(4-Methoxyphenyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (6*a*)</u>



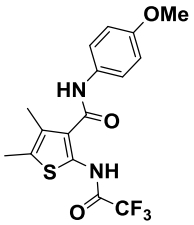
White solid; mp = 191.2 ˚C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 11.61 (brs, 1H), 9.13 (brs, 1H), 7.65 (d, J = 9.2 Hz, 2H), 6.94 (d, J = 8.8 Hz, 2H), 3.80 (s, 3H), 2.95–2.91 (m, 2H), 2.83–2.80 (m, 2H), 1.91–1.85 (m, 2H), 1.72–1.63 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.30, 157.42, 153.81, 153.42, 138.56, 135.00, 133.38, 129.72, 122.85, 120.62, 114.61, 55.70, 31.75, 29.19, 28.94, 27.62, 27.30; LRMS (electrospray) m/z $(M+H)^+$ 413.

<u>*N*-(4-Methoxyphenyl)-2-(2,2,2-trifluoroacetamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (6*b*)</u>



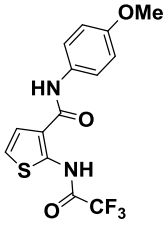
White solid; mp = 172.1 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.73 (m, 4H), 1.82 (brs, 2H), 2.67 (brs, 4H), 3.72 (s, 3H), 6.89 (m, 2H), 7.59 (m, 2H), 9.77 (s, 1H), 12.00 (s, 1H); LRMS (electrospray) m/z $(M+H)^+$ 399.

<u>*N*-(4-Methoxyphenyl)-4,5-dimethyl-2-(2,2,2-trifluoroacetamido)thiophene-3-carboxamide (6*d*)</u>



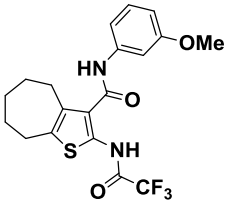
White solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.1 (s, 3H), 2.3 (s, 3H), 3.73 (s, 3H), 6.89 (m, 2H), 7.59 (m, 2H), 9.92 (s, 1H), 11.90 (s, 1H); LRMS (electrospray) m/z $(M+H)^+$ 373.

<u>*N*-(4-Methoxyphenyl)-2-(2,2,2-trifluoroacetamido)thiophene-3-carboxamide (6*e*)</u>



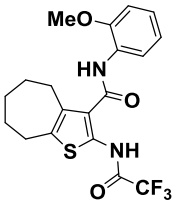
Brown solid; mp = 170.9 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.78 (s, 3H), 6.95 (d, 2H), 7.36 (brs, 1H), 7.57 (d, 2H), 7.79 (brs, 1H), 10.17 (brs, 1H), 13.42 (brs, 1H); LRMS (electrospray) m/z $(M+H)^+$ 345.

<u>*N*-(3-Methoxyphenyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (7*a*)</u>



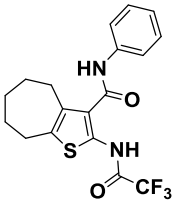
White solid; mp = 159.1 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54 (m, 4H), 1.60 (brs, 2H), 2.74 (m, 4H), 3.74 (s, 3H), 6.64 (m, 1H), 7.21 (m, 2H), 7.43 (s, 1H), 10.15 (brs, 1H), 11.84 (brs, 1H); LRMS (electrospray) m/z $(M+H)^+$ 413.

<u>*N*-(2-Methoxyphenyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (8*a*)</u>



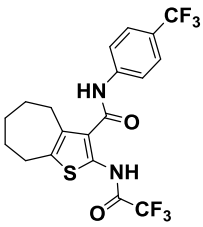
White solid; mp = 167.1 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.62 (m, 4H), 1.84 (brs, 2H), 2.78 (m, 4H), 3.79 (s, 3H), 6.95 (m, 1H), 7.08 (m, 2H), 7.99 (s, 1H); LRMS (electrospray) m/z $(M+H)^+$ 413.

<u>*N*-Phenyl-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (9*a*)</u>



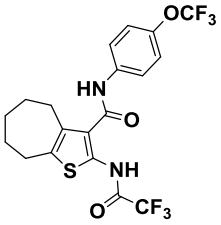
White solid; mp = 181.2 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54 (m, 4H), 1.82 (brs, 2H), 2.76 (m, 4H), 7.05 (m, 1H), 7.31 (d, 2H), 7.70 (d, 2H), 10.18 (brs, 1H), 11.86 (brs, 1H); LRMS (electrospray) m/z $(M+H)^+$ 383.

2-(2,2,2-Trifluoroacetamido)-*N*-(4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (12*a*)



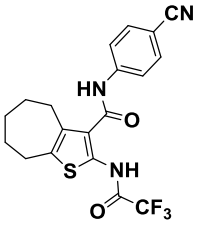
Brown solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54 (d, 4H), 1.82 (s, 2H), 2.72 (d, 4H), 7.68 (d, 2H), 7.94 (d, 2H), 10.58 (brs, 1H), 11.98 (brs, 1H); LRMS (electrospray) m/z $(M+H)^+$ 451.

2-(2,2,2-Trifluoroacetamido)-*N*-(4-(trifluoromethoxy)phenyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (13*a*)



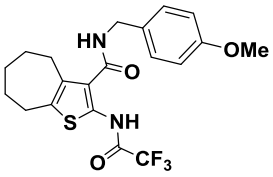
Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54 (m, 4H), 1.82 (brs, 2H), 2.5 (m, 4H), 7.32 (d, 2H), 7.80 (d, 2H), 10.40 (brs, 1H), 11.89 (brs, 1H); LRMS (electrospray) m/z $(M+H)^+$ 467.

***N*-(4-Cyanophenyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (14*a*)**



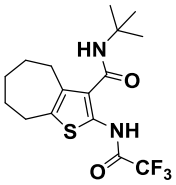
Brown solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.53 (m, 4H), 1.60 (brs, 2H), 2.4 (m, 4H), 7.78 (d, 2H), 7.87 (d, 2H), 10.64 (brs, 1H), 11.96 (brs, 1H); LRMS (electrospray) m/z $(M+H)^+$ 408.

***N*-(4-Methoxybenzyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (15*a*)**



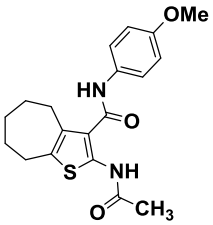
White solid; mp = 151.9 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.58 (m, 4H), 1.80 (brs, 2H), 2.78 (m, 4H), 3.72 (s, 3H), 4.34 (m, 2H), 6.8 (d, 2H), 7.21 (d, 2H), 8.57 (brs, 1H), 11.75 (brs, 1H); LRMS (electrospray) m/z $(M+H)^+$ 427.

_N_-(_tert_-Butyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4_H_-cyclohepta[_b_]thiophene-3-carboxamide (16_a_)



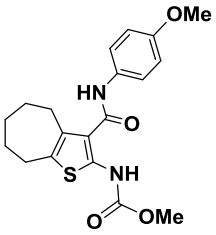
White solid; mp = 199.4 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (brs, 9H), 1.54 (m, 4H), 1.8 (brs, 2H), 2.73 (d, 4H), 7.46 (brs, 1H), 11.33 (brs, 1H); LRMS (electrospray) m/z $(M+H)^+$ 363.

2-Acetamido-_N_-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4_H_-cyclohepta[_b_]thiophene-3-carboxamide (17_a_)



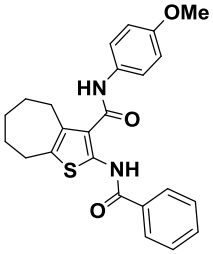
Brown solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.53 (m, 4H), 1.79 (brs, 2H), 2.06 (brs, 3H), 2.62 (m, 4H), 3.72 (s, 3H), 6.88 (d, 2 H), 7.6 (d, 2H), 9.96 (s, 1H), 10.37 (s, 1H); LRMS (electrospray) m/z $(M+H)^+$ 359.

Methyl (3-((4-methoxyphenyl)carbamoyl)-5,6,7,8-tetrahydro-4_H_-cyclohepta[_b_]thiophen-2-yl)carbamate (18_a_)



Pale brown solid; mp = 189.3 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.53 (m, 4H), 1.59 (brs, 2H), 1.79 (brs, 2H), 1.98 (brs, 2H), 3.64 (s, 3H), 3.74 (s, 3H), 6.89 (d, 2H), 7.59 (d, 2H), 9.8 (brs, 2H); LRMS (electrospray) m/z $(M+H)^+$ 375.

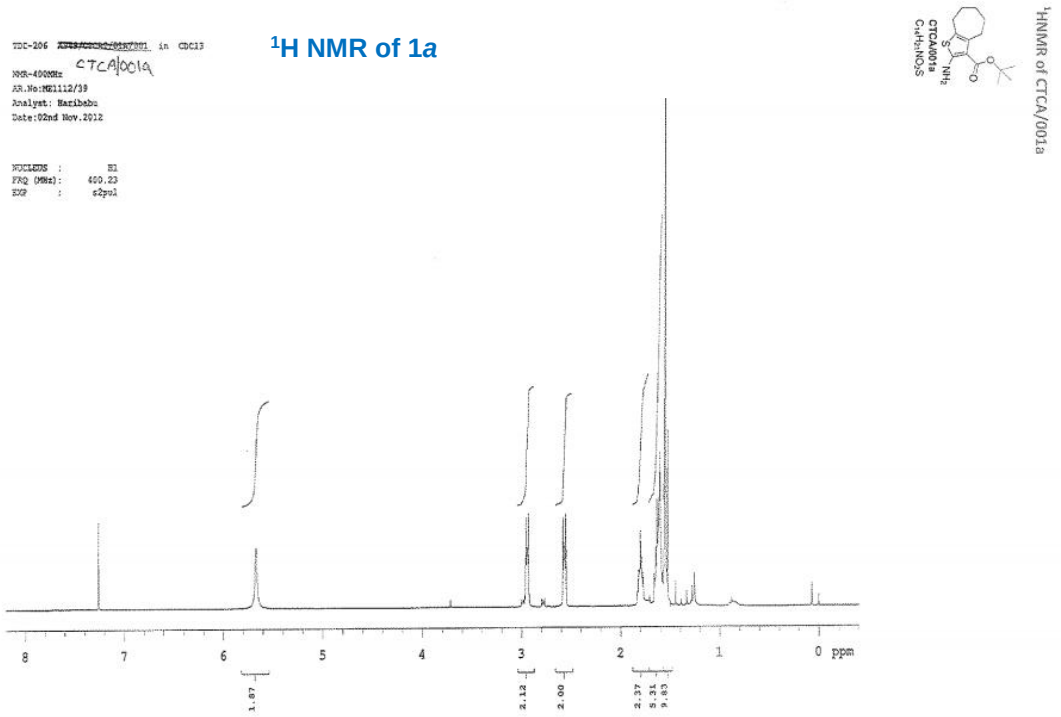
2-Benzamido-_N_-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4_H_-cyclohepta[_b_]thiophene-3-carboxamide (19_a_)



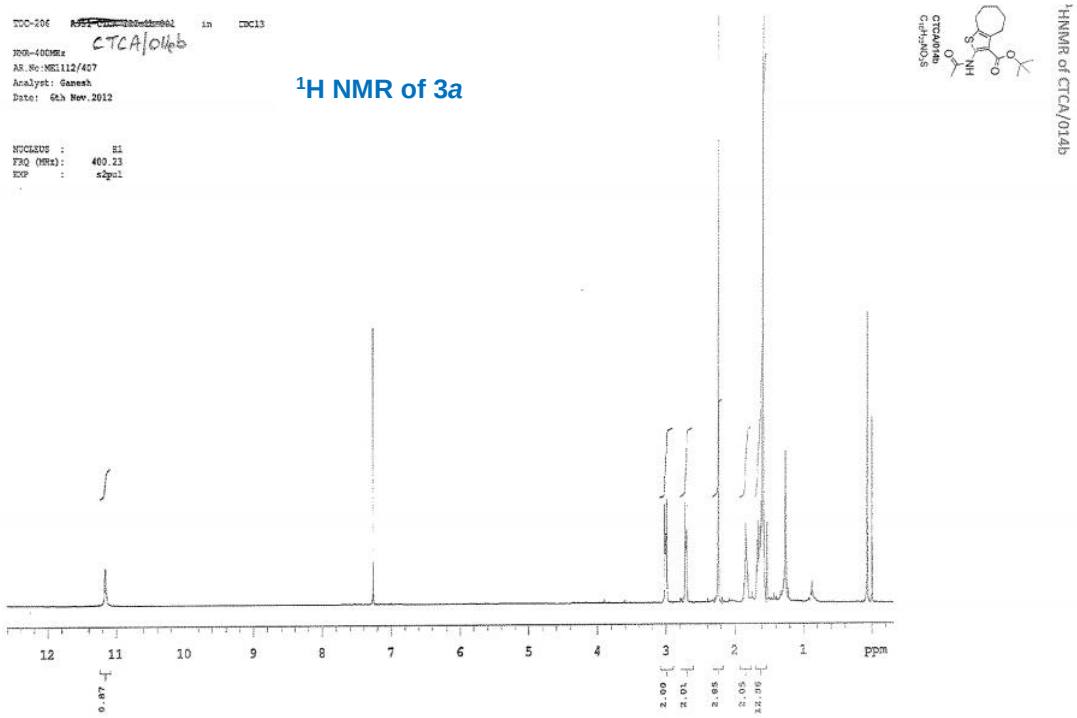
White solid; mp = 218.0 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.6 (m, 4H), 1.82 (brs, 2H), 2.76 (brs, 4H), 3.73 (s, 3H), 6.92 (d, 2 H), 7.53 (m, 5H), 7.85 (d, 2H), 9.951 (s, 1H), 11.06 (s, 1H); LRMS (electrospray) m/z $(M+H)^+$ 421.

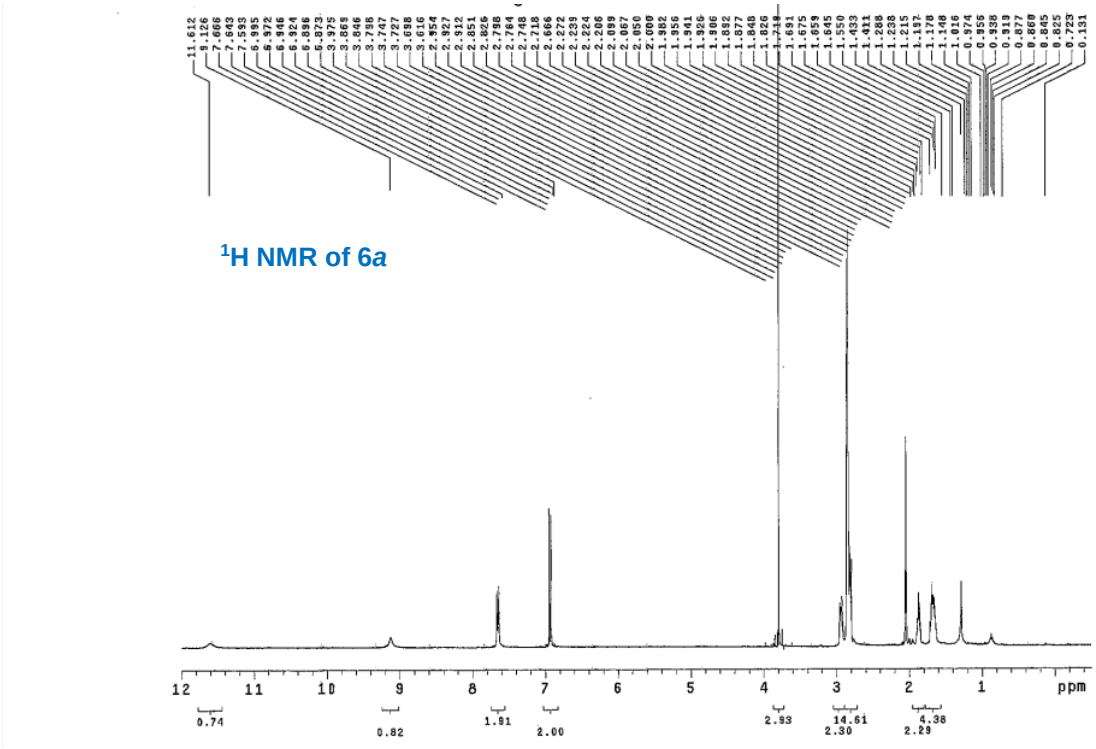
V. NMR Data

^{1}H NMR of 1*a*

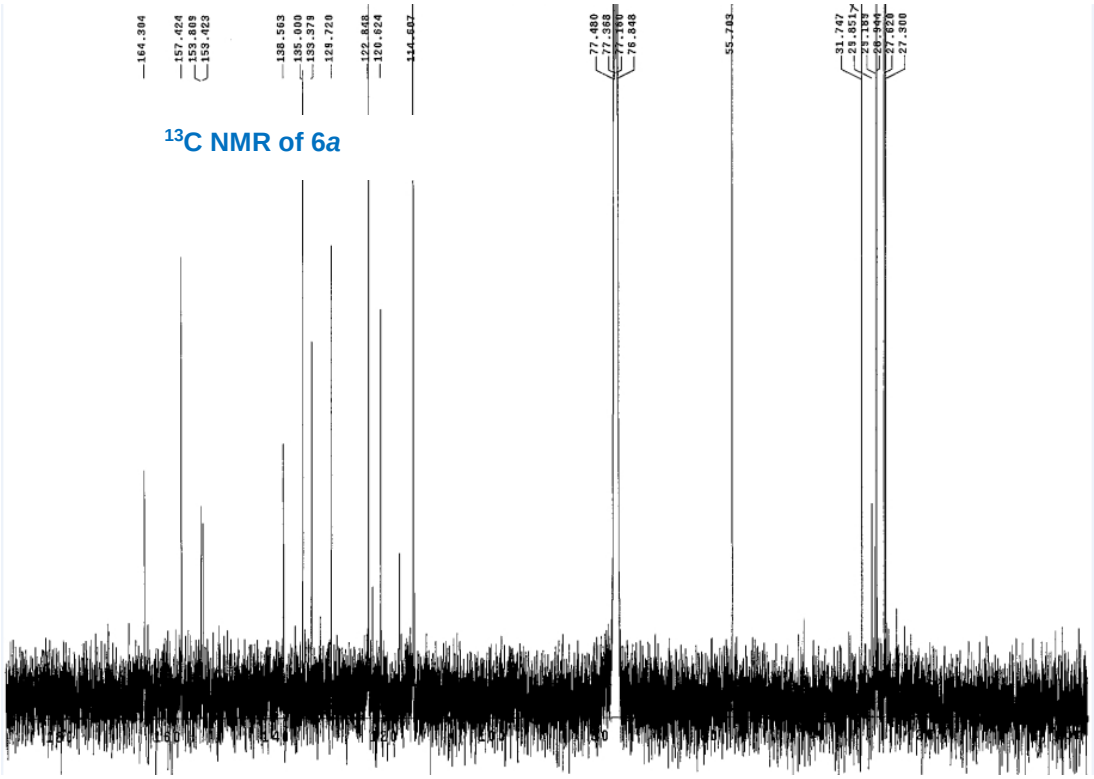


^{1}H NMR of 3*a*



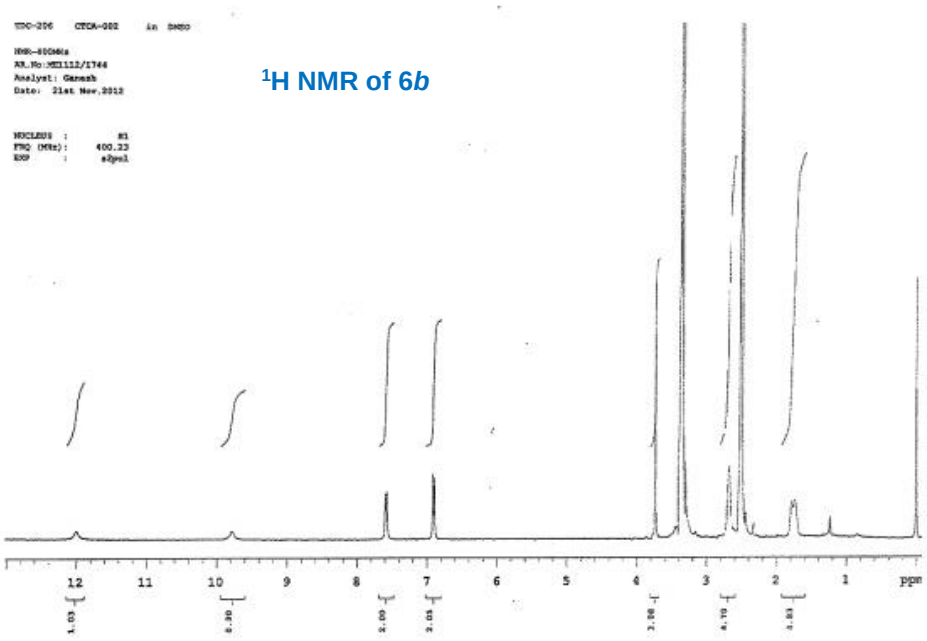


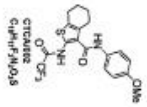
^{1}H NMR of 6*a*



^{13}C NMR of 6*a*

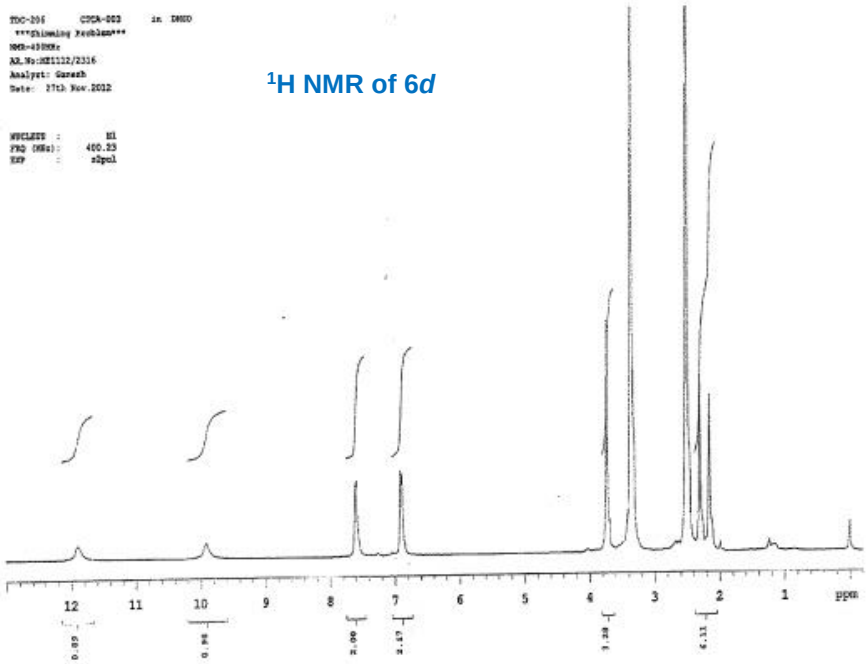
^{1}H NMR of 6*b*

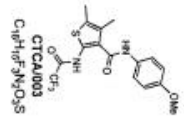




^{1}H NMR of N-(4-methoxyphenyl)-2-(2,2,2-trifluoroacetamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (CTCA/002):

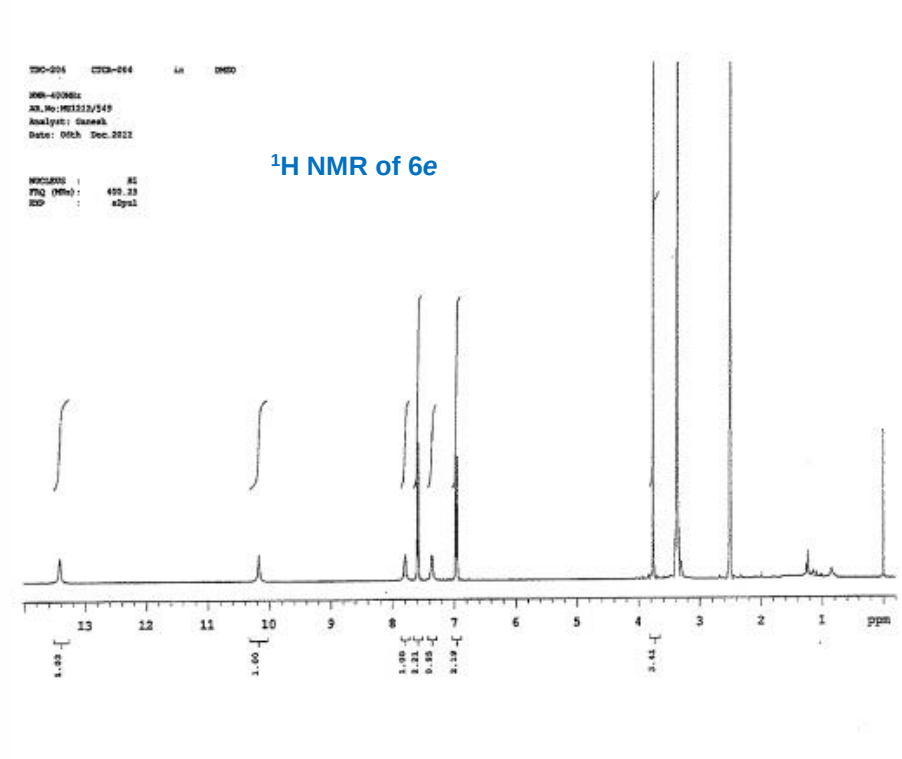
^{1}H NMR of 6*d*



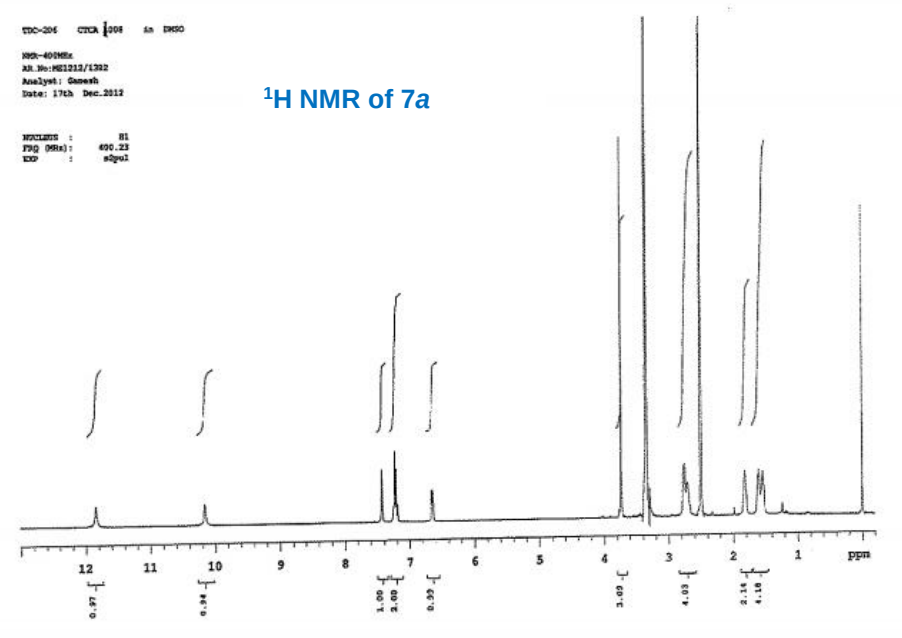


^{1}H NMR of N-(4-methoxyphenyl)-4,5-dimethyl-2-(2,2,2-trifluoroacetamido)thiophene-3-carboxamide (CTCA/003):

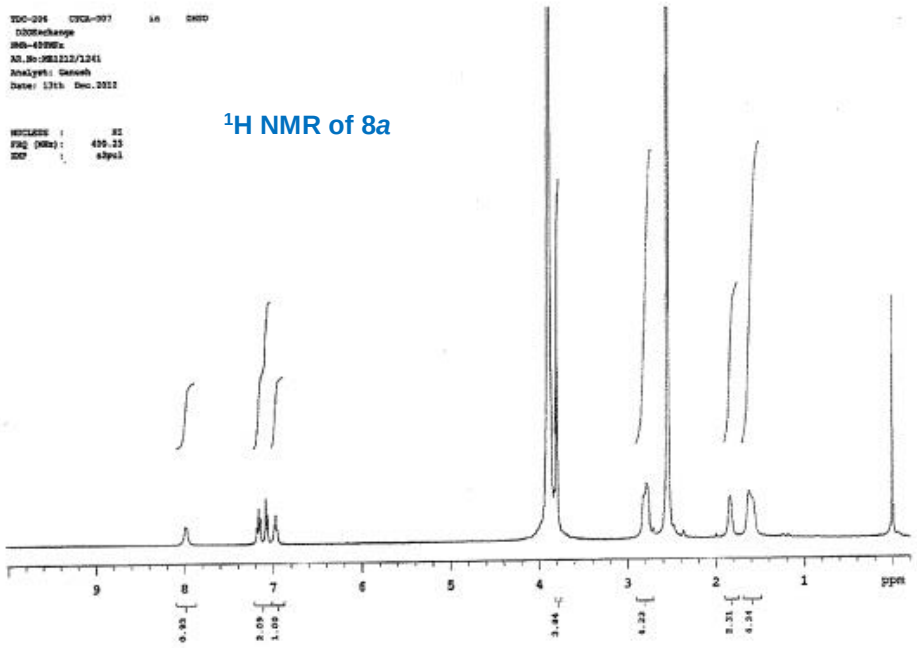
^{1}HNMR of N-(4-methoxyphenyl)-2-(2,2,2-trifluoroacetamido)thiophene-3-carboxamide (CTCA/004):



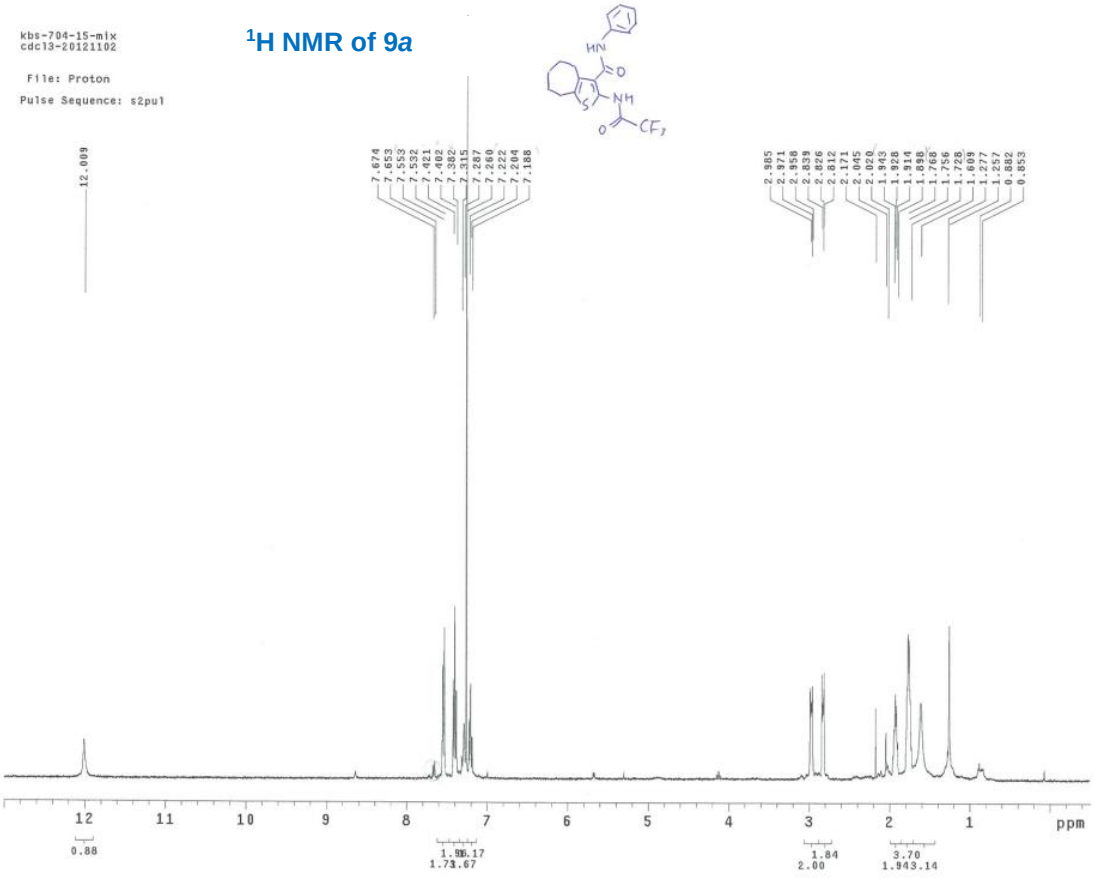
^{1}HNMR of N-(3-methoxyphenyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4H-cycloheptа[b]thiophene-3-carboxamide (CTCA/008):



^{1}H NMR of 8*a*

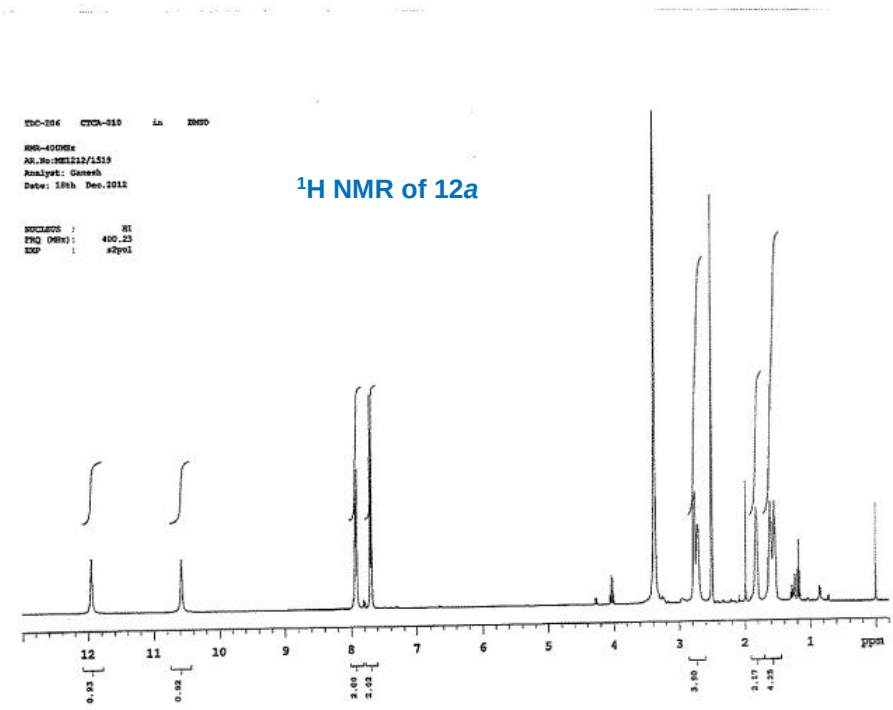


^{1}H NMR of 9*a*

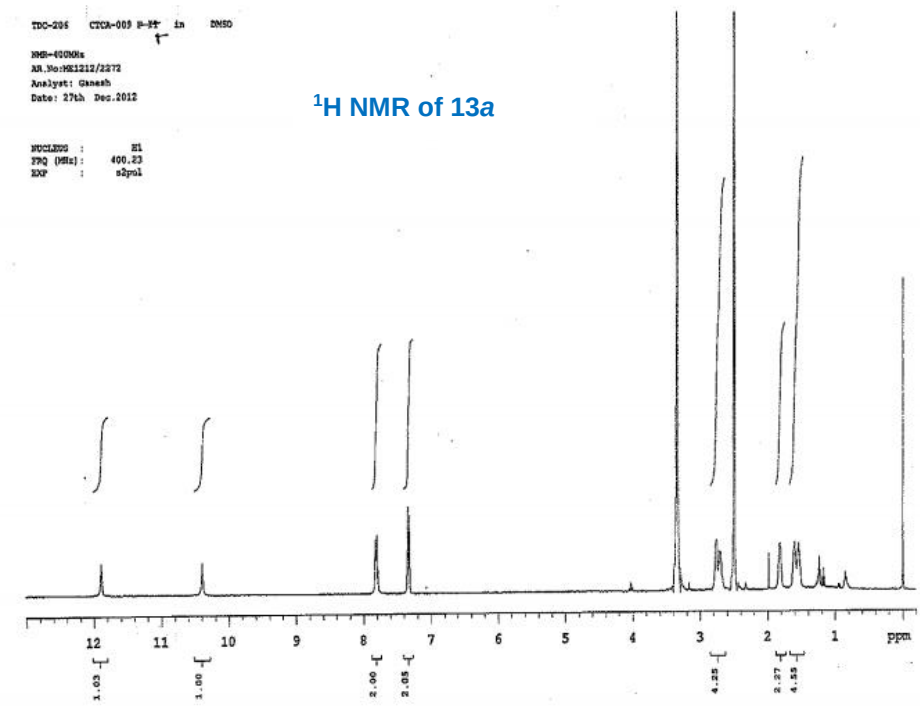


^{1}HNMR of N-(2-methoxyphenyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxamide (**CTCA/007**):

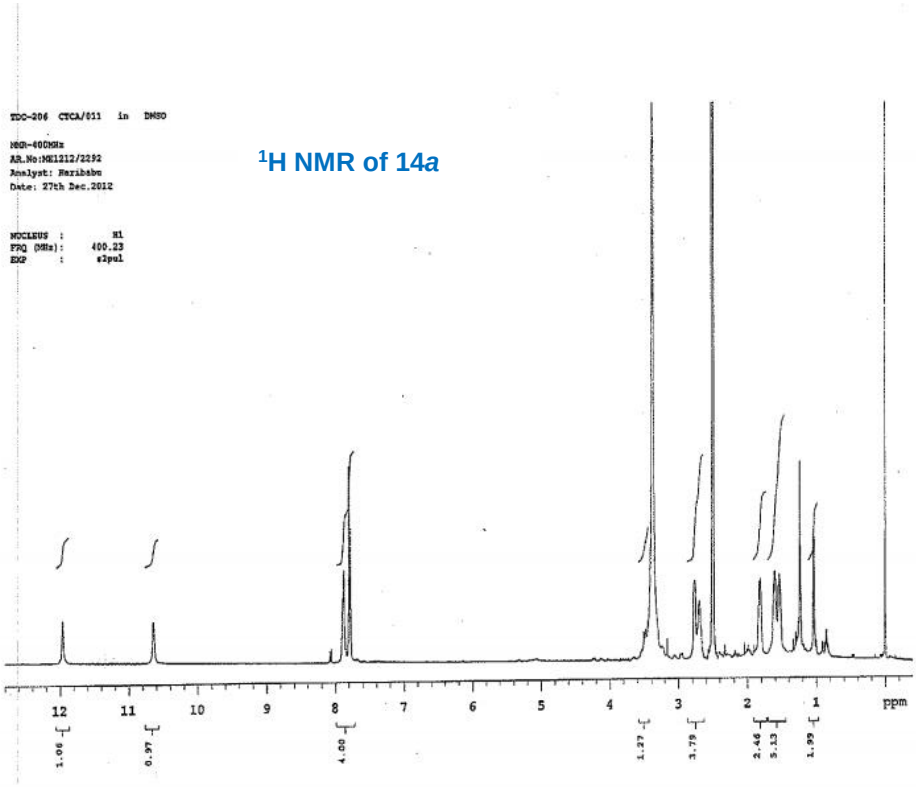
^{1}HNMR of 2-(2,2,2-trifluoroacetamido)-N-(4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxamide (CTCA/010):



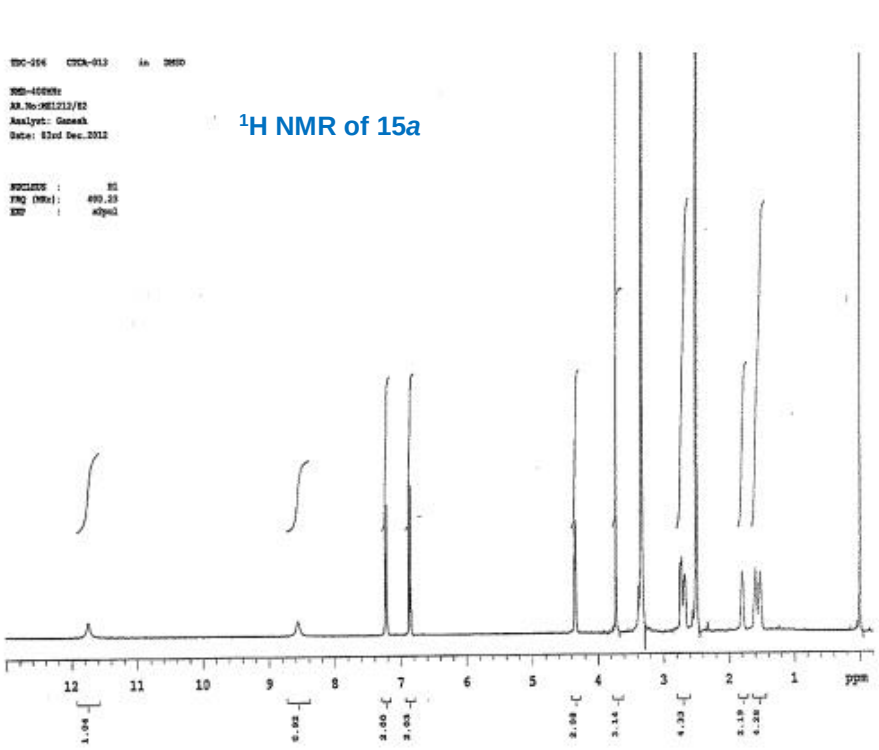
^{1}HNMR of 2-(2,2,2-trifluoroacetamido)-N-(4-(trifluoromethoxy)phenyl)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxamide (CTCA/009):



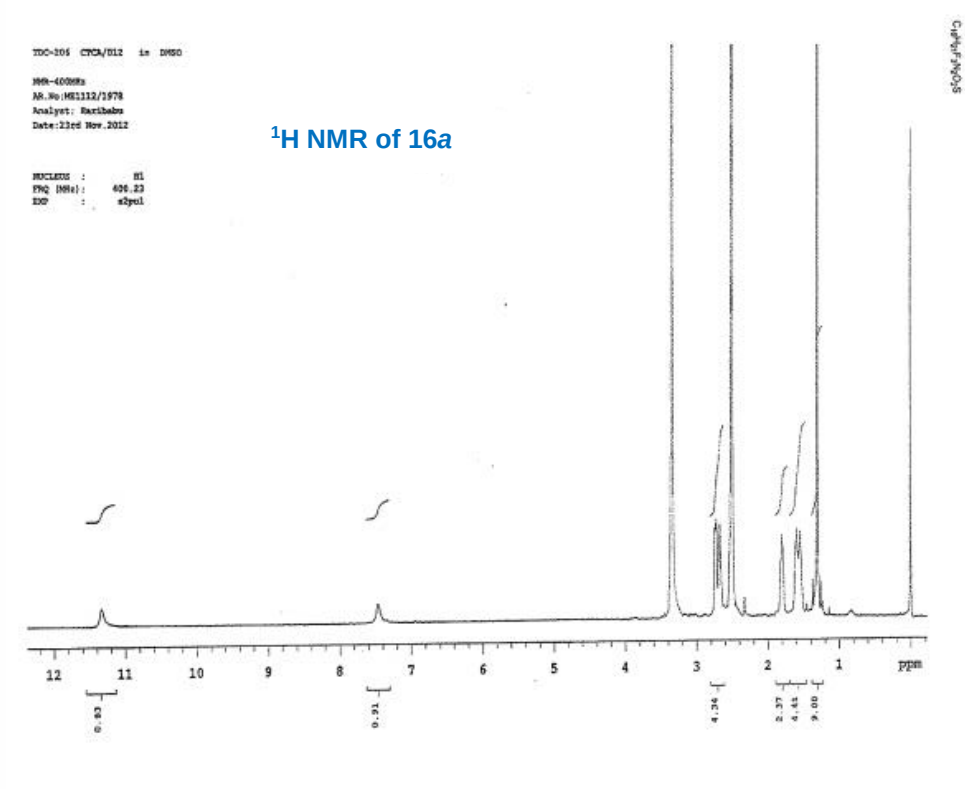
^{1}HNMR of N-(4-cyanophenyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxamide (CTCA/011):



^{1}HNMR of N-(4-methoxybenzyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxamide (CTCA/013):

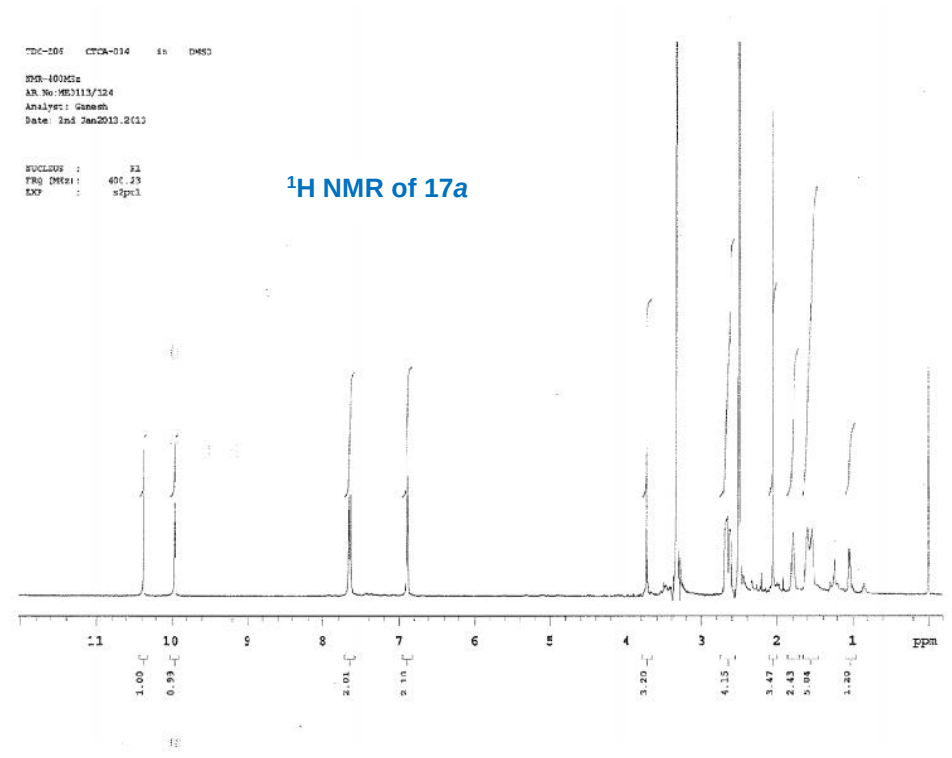


^{1}H NMR of 16*a*



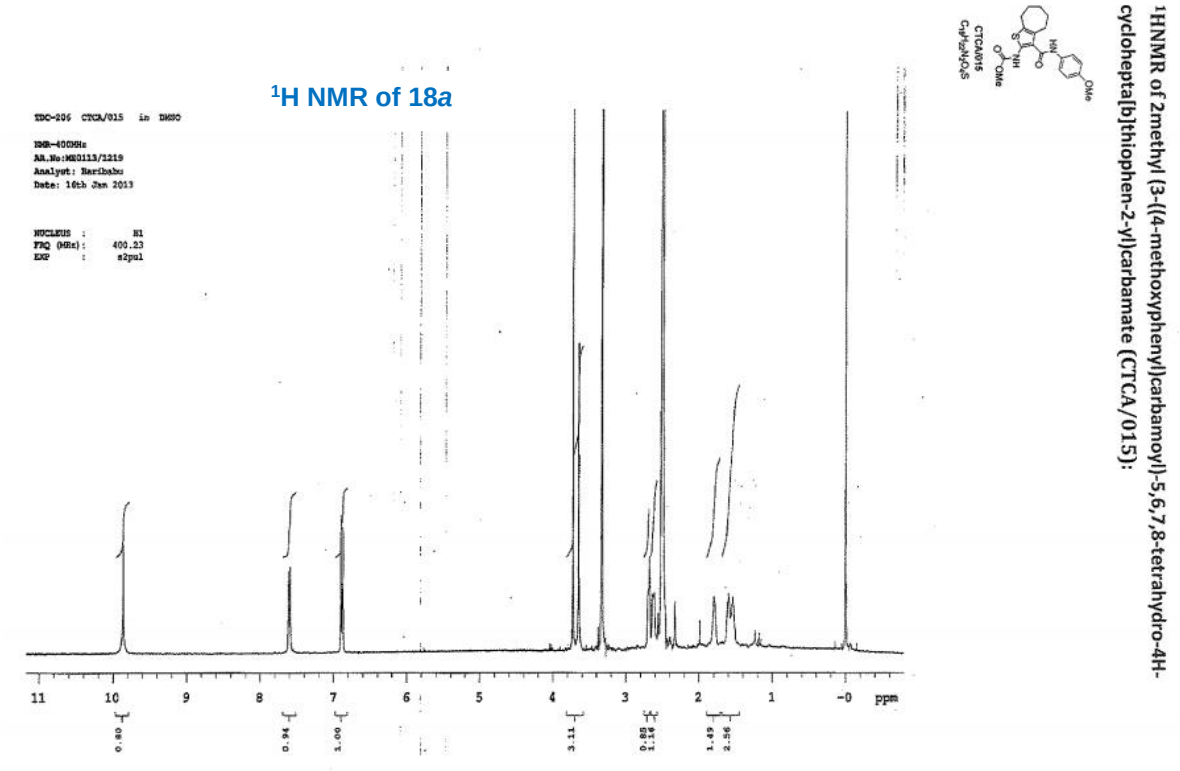
^{1}HNMR of N-(tert-butyl)-2-(2,2,2-trifluoroacetamido)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxamide (CTCA/012)

^{1}H NMR of 17*a*



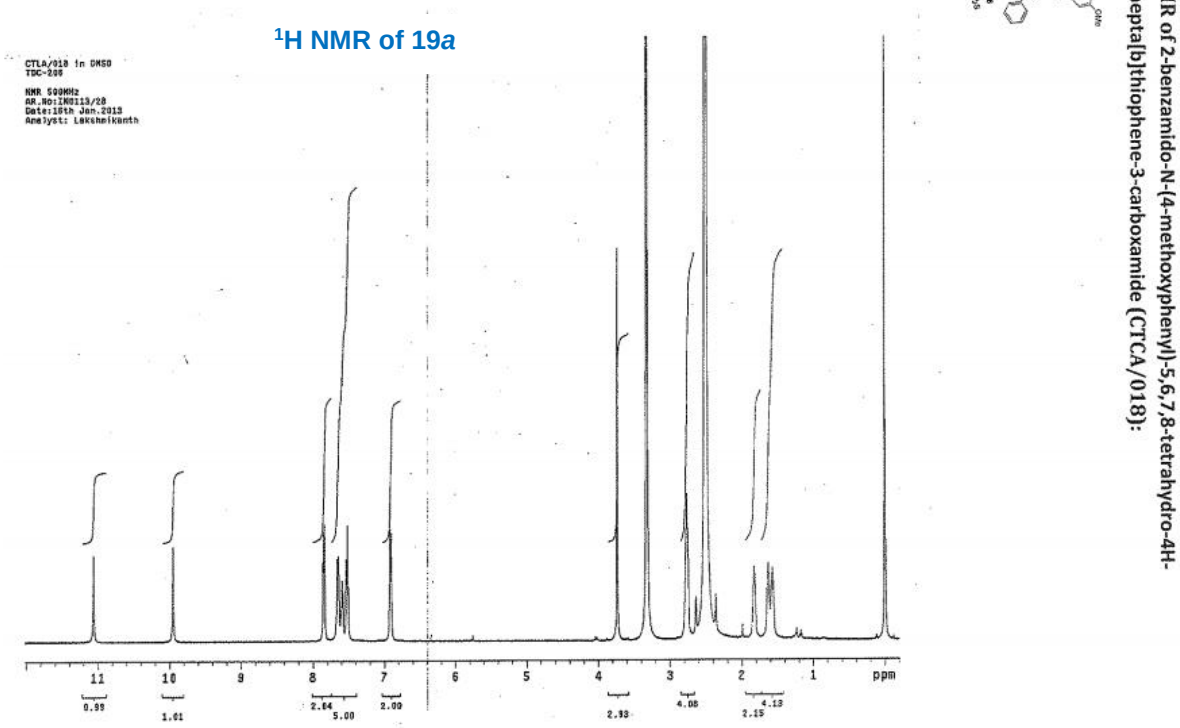
^{1}HNMR of 2-acetamido-N-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxamide (CTCA/014):

^{1}H NMR of 18*a*



^{1}HNMR of 2methyl (3-{(4-methoxyphenyl)carbamoyl}-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophen-2-yl)carbamate (CTCA/015):

^{1}H NMR of 19*a*



^{1}HNMR of 2-benzamido-N-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxamide (CTCA/018):

VI. Reference

1. Siqueira-Neto, J. L.; Moon, S.; Jang, J.; Yang, G.; Lee, C.; Moon, H. K.; Chatelain, E.; Genovesio, A.; Cechetto, J.; Freitas-Junior, L. H. *PLoS Negl. Trop. Dis*. **2012**, *6*, e1671.