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New combination of pharmacophoric elements of potent σ_1 ligands: Design, synthesis and σ receptor affinity of aminoethyl substituted tetrahydrobenzothiophenes

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Abstract

The aminoethyl substituted tetrahydrobenzothiophenes **4** resulted from combination of the pharmacophoric elements of the potent σ_1 ligands **2** and **3**. The aminoethyl substituted tetrahydrobenzothiophenes **4** were prepared in an 8-step synthesis starting with thiophene. Whereas the σ_1 affinity of the N-benzyl derivative **4a** is in the medium nanomolar range (K_i = 49 nM), the analogous N-cyclohexylmethyl derivative **4d** exhibits low nanomolar affinity (K_i = 5.0 nM). The reduced σ_1 affinity and σ_2/σ_1 selectivity of tetrahydrobenzothiophenes **4** compared to analogous spirocyclic piperidines **3** is attributed to the increased conformational flexibility of the aminoethyl side chain.

Key words

σ_1 ligands; conformational flexibility; combination of pharmacophoric elements; tetrahydrobenzothiophenes; Horner-Wittig reaction

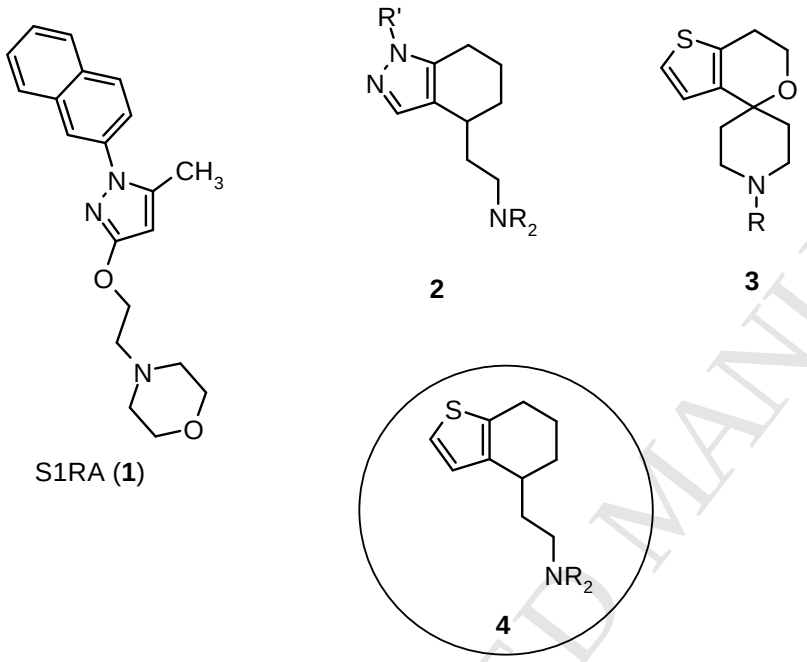
1. Introduction

It is well established that σ receptors represent a unique, non-opioid, non-phencyclidine but haloperidol sensitive receptor family. It consists of two subtypes known as σ_1 and σ_2 receptor. They are widely expressed in the central nervous system, but they are also found in various tissues and organs in the periphery (e.g. heart, liver, lung, and kidney) [1,2]. The gene encoding the σ_1 receptor protein was determined by successful cloning. The protein contains 223 amino acids and has a molecular weight 25.3 kDa. The membrane-bound σ_1 receptor contains two transmembrane domains located at the mitochondrial-associated endoplasmic reticulum. This unique structure of the σ_1 receptor is different from typical G-protein coupled receptors, ion channel receptors and tyrosine kinase receptors. Moreover, a similarity to any mammalian protein on the level of amino acid sequence could not be detected. However, a 30 % homology to the yeast enzyme sterol $\Delta^{8/7}$-isomerase was found [3-7]. The amino acid sequence of the σ_2 receptor subtype is not known and therefore this subtype is less characterized. Very recently, it has been reported that the σ_2 receptor might be identical to the progesterone receptor membrane component 1 (pgrmc1), which has already been cloned [8,9].

It has been shown that σ_1 receptor antagonists have a potential for the treatment of acute and chronic neurological disorders including schizophrenia, neuropathic pain as well as alcohol and cocaine abuse, whereas σ_1 receptor agonists can be used for the treatment of depression, memory disorders, including Alzheimer's disease and stroke [10,11]. The σ_1 receptor antagonist S1RA (**1**) is currently investigated in phase 2 clinical trials for the treatment of neuropathic pain. Therefore σ_1 antagonists possess a high potential as innovative analgesics with reduced side effects [12,13]. Due to the high expression of σ_1 and σ_2 receptors in several tumor cell lines (e.g.

breast, lung, and prostate cancer cell lines), both receptor subtypes represent interesting targets for the development of novel biomarkers for tumor diagnosis and antitumor drugs [14,15].

Figure 1: Development of novel σ_1 receptor ligands **4** by combination of S1RA (**1**), aminoethylindazoles **2** and spirocyclic thienopyrans **3**.



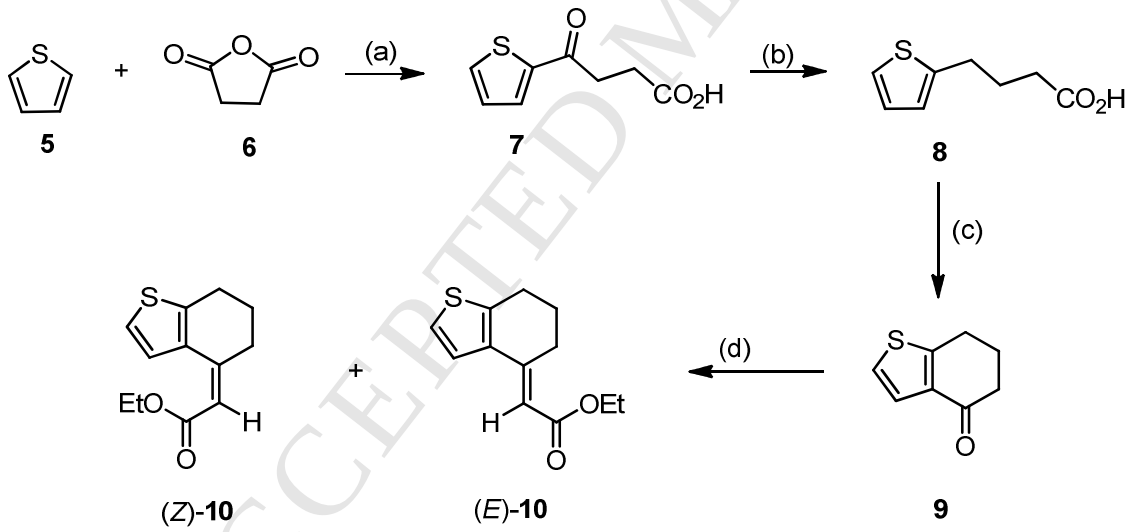
Our interest has been focused on the development of novel ligands with high affinity towards σ_1 receptors and selectivity over related receptors, in particular the σ_2 subtype, but also the phencyclidine binding site of the NMDA receptor. The class of tetrahydroindazoles **2** with an aminoethyl side chain shows high affinity towards the σ_1 receptor. (Figure 1) In particular compound **2a** ($R' = CH_3$, NR_2 = 4-phenylpiperidin-1-yl) represents a very potent and selective σ_1 receptor antagonist (K_i (σ_1) = 7.0 nM, K_i (σ_2) = 39.7 nM) with analgesic activity in the neuropathic pain model [16,17]. The indazoles **2** represent the lead compounds for the development of S1RA (**1**). Bioisosteric replacement of the pyrazole ring of **2** with a thiophene ring and conformational restriction of the flexible aminoethyl side chain led to the spirocyclic thienopyrans **3** with very high σ_1 affinity and selectivity over the σ_2 subtype [18,19]. Therefore

we planned to merge the aminoethylindazole system **2** with the spirocyclic thienopyran system **3** to form aminoethyl-substituted tetrahydrobenzothiophenes of type **4**. In order to fit into the common pharmacophore models [20] arylalkyl residues should be attached to the basic amino moiety.

2. Chemistry

For the synthesis of the aminoethyl substituted tetrahydrobenzothiophenes **4** the ketone **9** was required as key intermediate. According to literature [21,22] ketone **9** was synthesized in three steps starting with thiophene (**5**). (Scheme 1)

Scheme 1: Synthesis of the α,β-unsaturated ester **10** starting with thiophene.



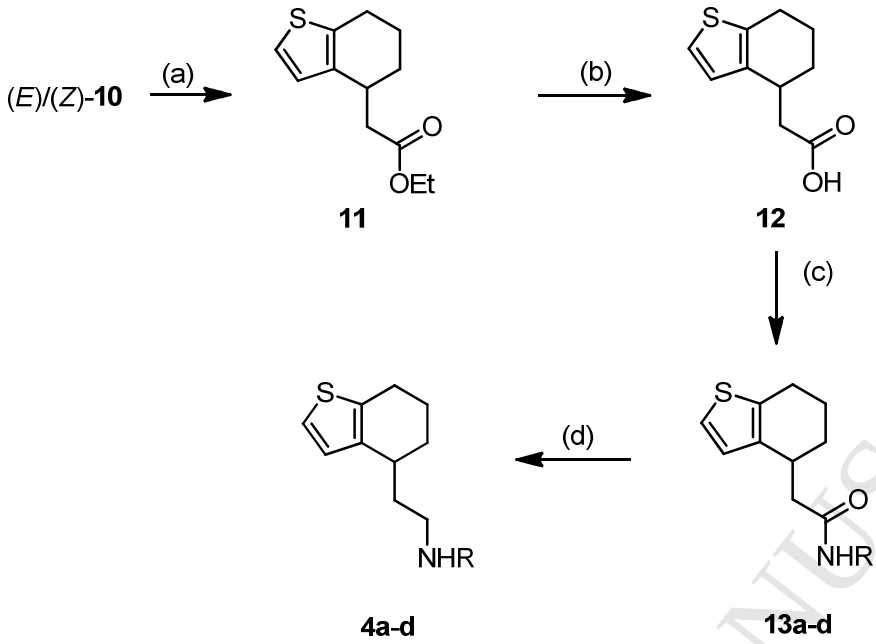
Reagents and reaction conditions: (a) $AlCl_3$, CH_2Cl_2, 0 ^oC, 65 %. (b) NH_2NH_2, KOH, diethylene glycol, reflux, 72 %. (c) (i) $SOCl_2$, CH_2Cl_2, reflux; (ii) $SnCl_2$, EtOAc, rt, 62 %. (d) $EtO_2CCH_2PO(OEt)_2$, NaOEt, THF, rt, 60 %.

Reaction of thiophene (**5**) with succinic anhydride (**6**) and $AlCl_3$ provided the γ-ketoacid **7** in 65 % yield. The Huang-Minlong variation of the Wolff-Kishner reduction transformed the γ-ketoacid **7** into the butyric acid **8**. For this purpose a solution of γ-ketoacid **7**, NH_2NH_2 monohydrate and KOH in diethylene glycol was heated to reflux to obtain the butyric acid **8** in 72 % yield. An intramolecular Friedel-Crafts acylation after conversion of the acid **8** into its acid chloride provided the ketone **9** in 62 % yield.

The synthesis of the α,β-unsaturated ester **10** was achieved by a Horner-Wittig reaction of the ketone **9** with triethyl phosphonoacetate. In the first attempt NaH was used for the deprotonation of $EtO_2CCH_2PO(OEt)_2$ leading to 40 % of a 60:40 mixture of (*E*)-**10** and (*Z*)-**10**. The same ratio of diastereomers was obtained after deprotonation of the phosphonate with NaOEt and conducting the transformation at room temperature. However, performing the condensation of the ketone **9** with the phosphonate at 0 °C led almost exclusively to the (*E*)-configured diastereomer (*E*)-**10**.

The ratio of (*E*):(*Z*) diastereomers and the configuration of isomers of **10** were determined by ^1H NMR spectroscopy. The proton in 3-position of the thiophene ring of (*Z*)-**10** resonates as characteristic doublet at 7.79 ppm. In case of (*E*)-**10** the signal for the 3-CH proton appears at 6.99 ppm. The downfield shift of the 3-CH signal of (*Z*)-**10** is due to the anisotropic effect of the carbonyl moiety of the ester group. Only in case of the (*Z*)-configured isomer (*Z*)-**10** the carbonyl moiety of the planar thiophenylacrylic ester is close to 3-CH of the thiophene ring. This anisotropic effect is not possible for (*E*)-**10**, since the CO_2Et group is oriented opposite to 3-CH.

Scheme 2: Synthesis of aminoethyl tetrahydrobenzothiophenes **4**.



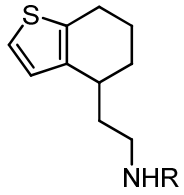
Reagents and reaction conditions: (a) H_2,Pd/C, rt, 24 h, 70 %. (b) LiOH, THF:H_2O, rt, 83 %. (c) RNH_2. CDI, THF, 0 ^oC. (d) $LiAlH_4$, THF, reflux; definition of residues R see Table.

Hydrogenation of the double bond of the α,β-unsaturated ester **10** was performed with H_2 and Pd/C. Since both diastereomers (*E*)-**10** and (*Z*)-**10** were transformed into the same saturated ester **11**, separation of the diastereomeric esters (*E*)-**10** and (*Z*)-**10** was not necessary. The saturated ester **11** was hydrolyzed with LiOH to obtain the acid **12** in 83 % yield. Coupling of acid **12** with various amines using carbonyl-1,1'-diimidazole (CDI) provided the amides **13a-d**. Finally $LiAlH_4$ reduction of the amides **13a-d** afforded the aminoethyl substituted tetrahydrobenzothiophenes **4a-d**. Since at least two hydrophobic substituents are postulated by the pharmacophore models to achieve high σ_1 affinity, amines containing an additional lipophilic aryl or cyclohexyl moiety were selected for the coupling reactions. (see Table 1)

3. σ Receptor affinity

The σ_1 and σ_2 receptor affinities of the aminoethyl substituted tetrahydrobenzothiophenes **4a-d** were determined in competition experiments with the radioligands [^{3}H]-(+)-pentazocine (σ_1 assay) and [^{3}H] ditolylguanidine (σ_2 assay). Membrane preparations of guinea pig brains and rat livers were used as receptor material in the σ_1 and σ_2 assay, respectively. Since ditolylguanidine also interacts with σ_1 receptors, an excess of the selective σ_1 receptor ligand (+)-pentazocine was added in the σ_2 assay to mask the σ_1 receptors [23,24].

Table 1: σ_1 and σ_2 receptor affinities of aminoethyl substituted tetrahydrobenzothiophenes **4** and reference compounds.

	R	K_i ± SEM [nM]		selectivity
		σ_1	σ_2	σ_2/σ_1
3a[18]	$PhCH_2$	0.31 ± 0.06	13 ± 2.5	42
3d[18]	$C_6H_{11}CH_2$	0.66 ± 0.16	3.3 ± 0.3	5
4a	$PhCH_2$	49 ± 2.0	149	3
4b	$PhCH_2CH_2$	126 ± 73	129 ± 20	1
4c	$PhCH_2CH_2CH_2$	132	166	1
4d	$C_6H_{11}CH_2$	5.0 ± 2.0	10 ± 1.0	2
(+)-pentazocine	-	5.7 ± 2.2	-	--
haloperidol	-	6.3 ± 1.6	78 ± 2.3	12
di-*o*-tolylguanidine	-	89 ± 29	58 ± 18	0.7

In Table 1 the σ_1 and σ_2 receptor affinities of the aminoethyl substituted tetrahydrobenzothiophenes **4** are summarized. The benzylamine **4a** reveals a K_i-value of 49 nM (σ_1), which is considerably higher than the K_i-value of the benzyl substituted piperidine **3a** (K_i = 0.31 nM), although the structural elements and distances (e.g. the distance between the basic amino group and the thiophene moiety) are identical in **4a** and **3a**. The reduced σ_1 receptor affinity of the aminoethyl derivative **4a** is explained by entropic factors, i.e. by the increased conformational flexibility of the aminoethyl side chain of **4a** compared to the conformationally restricted piperidine **3a**.

Increasing of the distance between the basic amino moiety and the phenyl ring in the side chain from benzyl (**4a**) over 2-phenylethyl (**4b**) to 3-phenylpropyl (**4c**) led to a reduced σ_1 receptor affinity. This trend correlates nicely with the reduced σ_1 affinities of spirocyclic thienopyrans **3** with the same series of N-substituents. Introduction of the saturated cyclohexylmethyl residue at the N-atom resulted in an increased σ_1 affinity of **4d**. The positive effect of the cyclohexylmethyl moiety on the σ_1 receptor affinity has already been observed for spirocyclic piperidines [18].

In general the selectivity of the σ_1 ligands **4** over the σ_2 receptor subtype is rather low. Even the cyclohexylmethyl derivative **4d** binding in the low nanomolar range to σ_1 receptors shows also high affinity towards the σ_2 receptor (K_i = 10 nM). The reduced subtype selectivity of the aminoethyl derivatives **4** can be attributed to the high conformational flexibility allowing the 2-aminoethyl side chain adapt to different receptor subtypes. However it should be noted that a rather low σ_2/σ_1 selectivity was also observed for spirocyclic piperidines bearing a cyclohexylmethyl moiety at the N-atom.

4. Conclusion

Combining the structural elements of two promising σ_1 ligands (i.e. pyrazoles **2** and spirocyclic piperidines **3**) led to the novel σ_1 ligands **4**. The reduced σ_1 affinity and subtype selectivity of the aminoethyl substituted tetrahydrobenzothiophenes **4** compared to analogous spirocyclic piperidines **3** is explained by increased conformational flexibility of the aminoethyl side chain of **4**. Nevertheless, low nanomolar σ_1 affinity was achieved by introduction of a cyclohexylmethyl moiety at the N-atom (**4d**, K_i (σ_1) = 5.0 nM).

5. Experimental part

5.1. General, chemistry

Unless otherwise mentioned, THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (tlc): Silica gel 60 F_{254} plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 µm (Merck); parentheses include: diameter of the column, length of column, fraction size, eluent, R_f value. Melting point: Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Mercury plus 400 spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. Where necessary, the assignment of the signals in the ^{1}H NMR and ^{13}C NMR spectra was performed using ^{1}H-^{1}H and ^{1}H-^{13}C COSEY NMR spectra. MS: EI = electron impact, ESI = electro spray ionization: MicroTof (Bruker Daltronics, Bremen), Calibration with sodium formate clusters before measurement. HPLC method for determination of the product purity: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; Method: column: LiChrospher® 60 RP-select B (5 µm), 250-4 mm cartridge; flow rate: 1.00

mL/min; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: water with 0.05 % (v/v) trifluoroacetic acid; B: acetonitrile with 0.05 % (v/v) trifluoroacetic acid: gradient elution: (A %): 0-4 min: 90 % , 4-29 min: gradient from 90 % to 0 %, 29-31 min: 0 %, 31-31.5 min: gradient from 0 % to 90 %, 31.5-40 min: 90 %.

5.2. General procedures

5.2.1. General procedure A for the synthesis of benzothiophenacetamides 13

Under N_2 acid **12** (150 mg, 0.77 mmol) was dissolved in dry THF (5 mL). CDI (137 mg, 0.84 mmol) was added slowly at 0 ^oC and the reaction mixture was stirred for 10 min. Then a solution of primary amine (0.77 mmol) was added dropwise and the reaction mixture was stirred at rt for 4 h. Water was added and the solution was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuum and the residue was purified by fc.

5.2.2. General procedure B for the synthesis of amines 4a-d

Under N_2 amide **13** (1.0 equiv.) was dissolved in dry THF (5 mL). Then a solution of 1M $LiAlH_4$ (2.2 equiv.) was added dropwise at 0 ^oC and the reaction mixture was heated to reflux for 12 h. Water was added and the mixture was extracted with EtOAc (3x50 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuum and the residue was purified by fc.

5.3. Synthetic procedures

5.3.1. 4-Oxo-4-(thiophen-2-yl)butanoic acid (7) [21,22]

Under N_2 succinic anhydride (**6**) (11.9 g, 119 mmol) was dissolved in dry CH_2Cl_2 (550 mL). Anhydrous $AlCl_3$ (19.0 g, 142 mmol) was added at 0 ^oC and the reaction mixture was stirred for 30 min. Then thiophene (**5**) (10.0 g, 119 mmol) dissolved in CH_2Cl_2 (100 mL) was added over a

period of 60 min and the reaction mixture was stirred for 4 h at rt. Then water was added and mixture was extracted with CH_2Cl_2 (3 x 300 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuum, (dichloromethane/MeOH = 85/15, R_f = 0.18). Pale yellow solid, mp 72-82 ^oC, yield 14.0 g, (65 %). Crude purity: 92 %, t_R = 10.7 min. FT-IR (neat): ν (cm^{-1}) = 3100 (O-H), 1694 (C=O), 1655 (HOC=O). Exact mass (ESI): *m/z* = calcd. for $(C_8H_8O_3S)H$ 185.0292, found 185.0298. ^1H NMR (DMSO-d_6): δ (ppm) = 2.56 (t, J = 6.7 Hz, 2H, $COCH_2$), 3.18 (t, J = 6.7 Hz, 2H, CH_2CO_2H), 7.23 (t, J = 4.8 Hz, 1H, 4-CH), 7.68 (dd, J = 4.8/0.9 Hz, 1H, 3-CH), 7.72 (dd, J = 4.8/0.9 Hz, 1H, 5-CH), 12.2 (bs, 1H, CO_2H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 27.7 (1C, $COCH_2$), 32.4 (1C, CH_2CO_2H), 128.7 (1C, C-4), 133.1 (1C, C-3), 134.5 (1C, C-5), 143.3 (1C, C-2), 173.6 (1C, CO_2H), 191.6 (1C, C=O).

5.3.2. 4-(Thiophen-2-yl)butanoic acid (8) [21,22]

Compound **7** (12.0 g, 65.2 mmol) was dissolved in diethylene glycol (250 mL). Hydrazine monohydrate (7.18 g, 143 mmol) and KOH (8.05 g, 143 mmol) were added at 0 ^oC and the reaction mixture was heated to reflux for 4 h. The reaction mixture was cooled to rt, acidified to pH 2 by addition of 10 M HCl and extracted with CH_2Cl_2 (3x500 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuum and the residue was purified by fc (dichloromethane/MeOH = 98/2, ø = 5 cm, h = 30 cm, R_f = 0.32). Colorless liquid, yield 8.0 g, (72 %). Purity: 94 %, t_R = 14.3 min. FT-IR (neat): ν (cm^{-1}) = 2936 (O-H), 1701 (HOC=O). Exact mass (ESI): *m/z* = calcd. for $(C_8H_{10}O_2S)H$ 171.0402, found 171.0408. ^1H NMR ($CDCl_3$): δ (ppm) = 1.95 (quint, J = 7.4 Hz, 2H, $CH_2CH_2CH_2CO_2H$), 2.34 (t, J = 7.4 Hz, 2H, $CH_2CH_2CH_2CO_2H$), 2.82 (t, J = 7.4 Hz, 2H, $CH_2CH_2CH_2CO_2H$), 6.72 (dd, J = 3.5/1.1 Hz, 1H, 3-CH), 6.84 (dd, J = 5.1/3.5 Hz, 1H, 4-CH), 7.05 (dd, J = 5.1/1.1 Hz, 1H, 5-CH), 10.7 (bs, 1H, CO_2H). ^{13}C NMR ($CDCl_3$): δ (ppm) = 26.5 (1C, $CH_2CH_2CH_2CO_2H$), 29.0 (1C,

$CH_2CH_2\mathit{C}H_2CO_2H$), 33.1 (1C, $\mathit{C}H_2CH_2CH_2CO_2H$), 123.4 (1C, C-3), 124.6 (1C, C-4), 126.8 (1C, C-5), 143.8 (1C, C-2), 179.9 (1C, CO_2H).

5.3.3. 6,7-Dihydrobenzo[b]thiophen-4(5*H*)-one (9) [21,22]

Under N_2 compound **8** (5.0 g, 29 mmol) was dissolved in dry CH_2Cl_2 (150 mL). Thionyl chloride (3.8 g, 31.9 mmol) was added dropwise at 0 °C and the reaction mixture was heated to reflux for 45 min. The reaction mixture was concentrated in vacuum, the residue was dissolved in EtOAc (100 mL) and anhydrous $SnCl_2$ (6.6 g, 35 mmol) was added over a period of 30 min at 0 °C. The reaction mixture was stirred for 3 h at rt. Water was added and the solution was extracted with EtOAc (3x150 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuum and the product was purified by fc (petroleum ether/EtOAc = 95/5, ø = 3 cm, h = 20 cm, R_f = 0.26). Colorless solid, mp 42 °C, yield 2.8 g, (62 %). Purity: 98 %, t_R = 10.7 min. FT-IR (neat): ν (cm^{-1}) = 2944 (C-H), 1666 (C=O). Exact mass (ESI): *m/z* = calcd. for $(C_8H_8OS)H$ 153.0296, found 153.0298. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.21 (quint, J = 6.5 Hz, 2H, 6-CH_2), 2.56 (t, J = 6.5 Hz, 2H, 5-CH_2), 2.98 (t, J = 6.5 Hz, 2H, 7-CH_2), 7.05 (d, J = 5.3 Hz, 1H, 3-CH), 7.33 (d, J = 5.3 Hz, 1H, 2-CH). ^{13}C NMR ($CDCl_3$): δ (ppm) = 24.5 (1C, C-6), 25.4 (1C, C-5), 37.8 (1C, C-7), 122.9 (1C, C-3), 124.7 (1C, C-3a), 137.1 (1C, C-2), 155.8 (1C, C-7a), 193.1 (1C, C-4).

5.3.4. (*E*)-Ethyl 2-(6,7-dihydrobenzo[b]thiophen-4(5*H*)-ylidene)acetate (10)

Under N_2 $EtO_2CCH_2PO(OEt)_2$ (3.2 g, 14.5 mmol) was dissolved in dry THF (25 mL). At 0 °C NaH (0.37g, 15.8 mmol) was added and the reaction mixture was stirred for 15 min at 0 °C. Then compound **9** (2.0 g, 13.2 mmol) dissolved in THF (10 mL) was added dropwise and the reaction mixture was stirred at rt for 3 h. Water was added to the reaction mixture and the mixture was extracted with EtOAc (3x150 mL). The organic layer was dried (Na_2SO_4), concentrated in

vacuum and the residue was purified by fc (petroleum ether/EtOAc = 90/10, ø = 2.5 cm, h = 15 cm, R_f = 0.29). Colorless liquid, yield 1.9 g, (65 %). Exact mass (ESI): *m/z* = calcd. for $(C_{12}H_{14}O_2S)H$ 223.0715, found 223.0709. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.24 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.88 (quint, J = 6.4 Hz, 2H, 6-CH_2), 2.81 (t, J = 6.4 Hz, 5-CH_2), 3.07 (t, J = 6.5 Hz, 2H, 7-CH_2), 4.12 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 6.04 (s, 1H, C=C*H*(CO_2Et)), 6.99 (d, J = 5.4 Hz, 1H, 3-CH), 7.13 (d, J = 5.4 Hz, 1H, 2-CH). ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.4 (1C, OCH_2CH_3), 23.7 (1C, C-6), 25.5 (1C, C-5), 26.3 (1C, C-7), 59.7 (1C, OCH_2CH_3), 110.4 (1C, C=*C*H$CO_2CH_2CH_3$), 122.7 (1C, C-3), 123.4 (1C, C-2), 134.9 (1C, C-3a), 144.9 (1C, C-7a), 150.6 (1C, C-4), 167.4 (1C, EtO*C*=O).

5.3.5. Ethyl 2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)acetate (11)

Compound **10** (500 mg, 2.25 mmol) was dissolved in EtOH (10 mL), Pd/C (50 %) was added and the mixture was stirred at rt under H_2 atmosphere (balloon) for 24 - 40 h. The mixture was filtered through Celite® bed and the solvent was removed under reduced pressure to obtain a residue which was purified by fc (petroleum ether/EtOAc = 80/20, ø = 2.0 cm, h = 10 cm, R_f = 0.28). Colorless liquid, yield 354 mg, (70 %). Exact mass (ESI): *m/z* = calcd. for $(C_{12}H_{16}O_2S)H$ 225.0875, found 225.0869. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.21 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.43 – 1.49 (m, 1H, 6-CH_2), 1.67 – 1.97 (m, 3H, 6-CH_2/5-CH_2), 2.33 (dd, J = 15.4/8.2Hz, 1H, CH_2CO_2Et), 2.62 (dd, J = 15.4/8.2 Hz, 1H, CH_2CO_2Et), 2.70 (t, J = 6.1 Hz, 2H, 7-CH_2), 3.10 – 3.27 (m, 1H, 4-CH), 4.10 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 6.74 (d, J = 5.2 Hz, 1H, 3-CH), 6.98 (d, J = 5.2 Hz, 1H, 2-CH).

5.3.6. 2-(4,5,6,7-Tetrahydrobenzo[b]thiophen-4-yl)acetic acid (12)

Compound **11** (250 mg, 1.12 mmol) was dissolved in THF (3 mL), a solution of LiOH (80 mg, 1.68 mmol) in water (5 mL) was added at 0 ^{o}C and the reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated in vacuum, the residue was acidified to pH 2 by addition of 1M HCl and the mixture was extracted with EtOAc (3x50 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuum. Colorless solid, mp 89 – 93 ^{o}C, yield 182 mg, (83 %). Exact mass (ESI): *m/z* = calcd. for $(C_{10}H_{12}O_2S)H$ 197.0558, found 197.0552. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.45 – 1.61 (m, 1H, 6-CH_2), 1.67 – 2.02 (m, 3H, 5-CH_2/6-CH_2), 2.41 (dd, J = 15.2/8.1Hz, 1H, C*H*$_2$CO$_2$H), 2.68 (dd, J = 15.2/8.1 Hz, 1H, C*H*$_2$CO$_2$H), 2.72 (t, J = 5.9 Hz, 2H, 7-CH_2), 2.98 - 3.26 (m, 1H, 4-CH), 6.77 (d, J = 5.2 Hz, 1H, 3-CH), 6.99 (d, J = 5.2 Hz, 1H, 2-CH).

5.3.7. N-Benzyl-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)acetamide (13a)

According to general procedure A compound **12** (150 mg, 0.77 mmol) was reacted with benzylamine (83 mg, 0.77 mmol) and CDI (137 mg, 0.84 mmol). The product was purified by fc (dichloromethane/MeOH = 95/5, ø = 2.0 cm, h = 10 cm, R_f = 0.26). Colorless solid, mp 118 – 122 ^{o}C, yield 150 mg, (69 %). Purity: 99.1 %, t_R = 21.1 min. Exact mass (ESI): *m/z* = calcd. for $(C_{17}H_{19}NOS)H$ 286.1298, found 286.1301. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.41 – 1.53 (m, 1H, 6-CH_2), 1.99 – 1.64 (m, 3H, 6-CH_2/5-CH_2), 2.23 (dd, J = 14.2/8.2 Hz, 1H, C*H*$_2$CONHCH$_2$Ph), 2.53 (dd, J = 14.2/8.2 Hz, 1H, C*H*$_2$CONHCH$_2$Ph), 2.67 (t, J = 5.9 Hz, 2H, 7-CH_2), 2.89 – 3.29 (m, 1H, 4-CH), 4.36 (dd, J = 14.7/5.7 Hz, 1H, PhC*H*$_2$NHCO), 4.41 (dd, J = 14.7/5.7 Hz, 1H, PhC*H*$_2$NHCO), 5.63 (d, J = 5.6 Hz, 1H, PhCH$_2$N*H*CO), 6.74 (d, J = 5.2 Hz, 1H, 3-CH), 6.95 (d, J = 5.2 Hz, 1H, 2-CH), 7.30 – 7.14 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ (ppm) = 21.5 (1C, C-6), 25.1 (1C, C-5), 28.7 (1C, *C*H$_2$CONHCH$_2$Ph), 32.9 (1C, C-7), 43.4 (1C, C-4), 43.7 (1C,

Ph*C*H_2NHCO), 122.1 (1C, C-3), 126.4 (1C, C-3a), 127.5 (1C, Ph), 127.9 (2C, o-Ph), 128.7 (2C, m-Ph), 136.6 (1C, C-2), 137.7 (1C, C-7a), 138.2 (1C, Ph), 171.6 (1C, NHC=O).

5.3.8. N-(2-Phenylethyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)acetamide (13b)

According to general procedure A compound **12** (150 mg, 0.77 mmol) was reacted with 2-phenylethylamine (93 mg, 0.77 mmol) and CDI (137 mg, 0.84 mmol). The product was purified by fc (dichloromethane/MeOH = 95/5, ø = 2.0 cm, h = 10 cm, R_f = 0.27). Yellow solid, mp 98 – 103 ^{o}C, yield 151 mg, (66 %). Purity: 97.2 %, t_R = 19.9 min. Exact mass (ESI): m/z = calcd. for ($C_{18}H_{21}NOS$)H 300.1338, found 300.1326. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.33 – 1.47 (m, 1H, 6-CH_2), 1.61 – 1.92 (m, 3H, 6-CH_2/5-CH_2), 2.11 (dd, J = 14.2/8.2 Hz, 1H, C*H*$_2$CONHC$_2$H$_4$Ph), 2.44 (dd, J = 14.2/8.3 Hz, 1H, C*H*$_2$CONHC$_2$H$_4$Ph), 2.66 (t, J = 5.9 Hz, 2H, 7-CH_2), 2.73 (t, J = 6.7 Hz, 2H, PhC*H*$_2$CH$_2$NHCO), 3.11 – 3.27 (m, 1H, 4-CH), 3.45 (dt, J = 7.8/6.7 Hz, 1H, PhCH$_2$C*H*$_2$NHCO), 3.49 (dt, J = 7.8/6.7 Hz, 1H, PhCH$_2$C*H*$_2$NHCO), 5.41 (t, J = 6.7 Hz, 1H, NHCO), 6.71 (d, J = 5.2 Hz, 1H, 3-CH), 6.94 (d, J = 5.2 Hz, 1H, 2-CH), 7.06 – 7.27 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ (ppm) = 21.4 (1C, C-6), 25.1 (1C, C-5), 28.6 (1C, HNCO*C*H$_2$), 32.8 (1C, C-7), 35.7 (1C, NHCH$_2$*C*H$_2$Ph), 40.5 (1C, NH*C*H$_2$CH$_2$Ph), 43.5 (1C, C-4), 121.9 (1C, C-3), 126.4 (1C, C-3a), 126.5 (1C, Ph), 128.6 (2C, o-Ph), 128.7 (2C, m-Ph), 136.5 (1C, C-2), 137.8 (1C, C-7a), 138.8 (1C, Ph), 171.7 (1C, NHC=O).

5.3.9. N-[(3-Phenylpropyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)]acetamide (13c)

According to general procedure A compound **12** (150 mg, 0.77 mmol) was reacted with 3-phenylpropylamine (104 mg, 0.77 mmol) and CDI (137 mg, 0.84 mmol). The product was purified by fc (dichloromethane/MeOH = 97/3, ø = 2.0 cm, h = 10 cm, R_f = 0.29). Colorless solid, mp 127 – 131 ^{o}C, yield 164 mg, (68 %). Purity: 95.3 %, t_R = 18.7 min. Exact mass (ESI):

m/z = calcd. for $(C_{19}H_{23}NOS)H$ 315.1498, found 315.1502. ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.38 – 1.50 (m, 1H, 6-CH_2), 1.62 – 1.77 (m, 3H, 6-CH_2/5-CH_2), 1.76 – 1.89 (m, 2H, $PhCH_2CH_2CH_2NH$), 2.12 (dd, J = 14.2, 7.8 Hz, 1H, CH_2CONH), 2.50 (dd, J = 14.2, 7.8 Hz, 1H, CH_2CONH), 2.58 (t, J = 7.6 Hz, 2H, $PhCH_2CH_2CH_2NH$), 2.68 (t, J = 6.2 Hz, 2H, 7-CH_2), 3.16 – 2.99 (m, 3H, $PhCH_2CH_2CH_2NH$/4-CH), 6.86 (d, J = 5.2 Hz, 1H, 3-CH), 7.22 – 7.06 (m, 5H, Ph), 7.28 (d, J = 5.2 Hz, 1H, 2-CH), 7.91 (t, J = 6.7 Hz, 1H, HNCO).^{13}C NMR (DMSO-d_6): δ (ppm) = 20.9 (1C, C-6), 24.5 (1C, C-5), 28.0 (1C, $NHCH_2CH_2CH_2Ph$), 30.9 (1C, $NHCOCH_2$), 32.4 (1C, $NHCH_2CH_2CH_2Ph$), 32.5 (1C, C-7), 38.0 (1C, $NHCH_2CH_2CH_2Ph$), 40.5 (1C, C-4), 121.9 (1C, C-3), 125.7 (1C, C-3a), 126.6 (1C, Ph), 128.1 (2C, o-Ph), 128.2 (2C, m-Ph), 135.1 (1C, C-2), 138.4 (1C, C-7a), 141.7 (1C, Ph), 170.9 (1C, NHC=O).

5.3.10. N-(Cyclohexylmethyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)acetamide (13d)

According to general procedure A compound **12** (150 mg, 0.77 mmol) was reacted with cyclohexylmethylamine (87 mg, 0.77 mmol) and CDI (137 mg, 0.84 mmol). The product was purified by fc (dichloromethane/MeOH = 95/5, ø = 2.0 cm, h = 10 cm, R_f = 0.28). Colorless solid, mp 87 – 91 °C, yield 145 mg, (65 %). Purity: 97.8 %, t_R = 19.6 min. Exact mass (ESI): m/z = calcd. for $(C_{17}H_{25}NOS)H$ 292.1658, found 292.1662. ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.79 – 0.94 (m, 2H, C_6H_{11}), 1.07 – 1.26 (m, 3H, C_6H_{11}), 1.32 – 1.50 (m, 2H, C_6H_{11}), 1.54 – 1.75 (m, 6H, C_6H_{11}, 6-CH_2), 1.91 – 1.72 (m, 2H, 5-CH_2), 2.12 (dd, J = 14.2/8.2 Hz, 1H, $CH_2CONHCH_2C_6H_{11}$), 2.46 (dd, J = 14.2/8.2 Hz, 1H, $CH_2CONHCH_2C_6H_{11}$), 2.68 (t, J = 6.2 Hz, 2H, 7-CH_2), 2.90 (dt, J = 12.6/7.9 Hz, 1H, $COHNCH_2C_6H_{11}$), 2.93 (dt, J = 12.9/7.9 Hz, 2H, $COHNCH_2C_6H_{11}$), 3.15 – 3.07 (m, 1H, 4-CH), 6.85 (d, J = 5.2 Hz, 1H, 3-CH), 7.19 (d, J = 5.2 Hz, 1H, 2-CH), 7.81 (t, J = 7.9 Hz, 1H, NHCO). ^{13}C NMR (DMSO-d_6): δ (ppm) = 20.9 (1C, $CH_2C_6H_{11}$), 24.5 (1C, C-6), 25.3 (1C, $CH_2C_6H_{11}$), 26.01, 26.02 (2C, $CH_2C_6H_{11}$), 28.0 (1C, C-5),

30.3 (1C, $CH_2C_6H_{11}$), 30.4 (1C, CHC_6H_{11}), 32.4 (1C, C-7), 37.3, (1C, COHN*C*$H_2C_6H_{11}$), 42.1 (1C, NHCO*C*H_2), 44.7 (1C, C-4), 121.8 (1C, C-3), 126.7 (1C, C-3a), 135.1 (1C, C-2), 138.4 (1C, C-7a), 170.9 (1C, NHC=O).

5.3.11. N-Benzyl-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)ethanamine (4a)

According to general procedure B compound **13a** (150 mg, 0.77 mmol) was reacted with $LiAlH_4$ (73 mg, 1.93 mmol) and the product was purified by fc (dichloromethane/MeOH = 95/5, ø = 1.5 cm, h = 10 cm, R_f = 0.27). Colorless oil, yield 90 mg, (63 %). Purity: 99.1 %, t_R = 21.1 min. Exact mass (ESI): m/z = calcd. for $(C_{17}H_{21}NS)H$ 272.1598, found 272.1504. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.35 – 1.47 (m, 1H, 5-CH_2), 1.53 – 1.72 (m, 2H, CHC*H*$_2$$CH_2$NH), 1.72 – 1.97 (m, 3H, 6-CH_2/5-CH_2), 2.60 – 2.69 (m, 4H, 7-CH_2/CHCH_2C*H*$_2$NH), 2.70 – 2.77 (m, 1H, 4-CH), 3.74 (d, J = 13.2 Hz, 1H, HNC*H*$_2$Ph), 3.76 (d, J = 13.2 Hz, 1H, HNC*H*$_2$Ph), 6.76 (d, J = 5.2 Hz, 1H, 3-CH), 6.95 (d, J = 5.2 Hz, 1H, 2-CH), 7.28 – 7.14 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ (ppm) = 21.7 (1C, C-6), 25.2 (1C, C-5), 28.3 (1C, C-7), 33.8 (1C, CH*C*$H_2$$CH_2$NH), 36.2 (1C, C-4), 47.2 (1C, CHCH_2*C*H_2NH), 54.1 (1C, Ph*C*H_2NH), 121.5 (1C, C-3), 126.7 (1C, C-3a), 126.9 (1C, Ph), 128.2 (2C, o-Ph), 128.4 (2C, m-Ph), 135.8 (1C, C-2), 139.1 (1C, C-7a), 140.3 (1C, Ph).

5.3.12. N-(2-Phenylethyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)ethanamine (4b)

According to general procedure B compound **13b** (150 mg, 0.50 mmol) was reacted with $LiAlH_4$ (47.0 mg, 1.25 mmol) and the product was purified by fc (dichloromethane/MeOH = 95/5, ø = 1.5 cm, h = 10 cm, R_f = 0.26). Colorless oil, yield 45 mg, (31 %). Purity: 96.2 %, t_R = 20.5 min. Exact mass (ESI): m/z = calcd. for $(C_{18}H_{23}NS)H$ 286.1682, found 286.1680. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.35 – 1.48 (m, 1H, 5-CH_2), 1.54 – 1.70 (m, 2H, CHC*H*$_2$$CH_2$NH), 1.74 – 1.94 (m, 3H, 6-CH_2/5-CH_2), 2.48 – 2.56 (m, 1H, 4-CH), 2.59 – 2.76 (m, 4H, 7-CH_2/ PhC*H*$_2$$CH_2$NH), 2.73 –

2.93 (m, 4H, $PhCH_2CH_2NH/NHCH_2$), 6.75 (d, J = 5.2 Hz, 1H, 3-CH), 6.95 (d, J = 5.2 Hz, 1H, 2-CH), 7.16 – 7.10 (m, 3H, Ph), 7.26 – 7.19 (m, 2H, Ph). ^{13}C NMR ($CDCl_3$): δ (ppm) = 21.7 (1C, C-6), 25.1 (1C, C-5), 33.9 (1C, $CHCH_2CH_2NH$), 34.1 (1C, C-7), 35.9 (1C, $PhCH_2CH_2NH$), 36.1 (1C, C-4), 47.6 (1C, $CHCH_2CH_2NH$), 51.2 (1C, $PhCH_2CH_2NH$), 121.6 (1C, C-3), 126.2 (1C, Ph), 126.7 (1C, C-3a), 128.5 (2C, o-Ph), 128.7 (2C, m-Ph), 135.9 (1C, C-2), 138.9 (1C, C-7a), 139.8 (1C, Ph).

5.3.13. 3-Phenyl-N-[2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)ethyl]propan-1-amine (4c)

According to general procedure B compound **13c** (120 mg, 0.38 mmol) was reacted with $LiAlH_4$ (35 mg, 0.95 mmol) and the product was purified by fc (dichloromethane/MeOH = 95/5, ø = 1.5 cm, h = 10 cm, R_f = 0.28). Colorless liquid, yield 48 mg, (42 %). Purity: 97.2 %, t_R = 19.4 min. Exact mass (ESI): m/z = calcd. for $(C_{19}H_{25}NS)H$ 300.1841, found 300.1839. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.33 – 1.48 (m, 1H, 6-CH_2), 1.54 – 1.72 (m, 2H, 5-CH_2), 1.74 – 1.97 (m, 5H, 6-CH_2/$CHCH_2CH_2NH$/$PhCH_2CH_2CH_2NH$), 2.21 (bs, 1H, NH), 2.55 – 2.63 (m, 5H, 4-CH/7-CH2/$PhCH_2CH_2CH_2NH$), 2.64 – 2.73 (m, 4H, CH_2CH_2NH/CH_2NH), 6.77 (d, J = 5.2 Hz, 1H, 3-CH), 6.95 (d, J = 5.2 Hz, 1H, 2-CH), 7.01 – 7.14 (m, 3H, Ph), 7.15 – 7.25 (m, 2H, Ph). ^{13}C NMR ($CDCl_3$): δ (ppm) = 21.8 (1C, C-6), 25.2 (1C, C-5), 28.3 (1C, $PhCH_2CH_2CH_2NH$), 31.4 (1C, C-7), 33.7 (1C, $CHCH_2CH_2NH$), 33.9 (1C, $PhCH_2CH_2CH_2NH$), 35.9 (1C, 4-CH), 47.7 (1C, $CHCH_2CH_2NH$), 49.5 (1C, $PhCH_2CH_2CH_2NH$), 121.6 (1C, C-3), 125.8 (1C, Ph), 126.7 (1C, 3a), 128.3 (2C, o-Ph), 128.4 (2C, m-Ph), 135.9 (1C, C-2), 138.9 (1C, 7a), 141.9 (1C, Ph).

5.3.14. N-(Cyclohexylmethyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)ethanamine (4d)

According to general procedure B compound **13d** (140 mg, 0.48 mmol) was reacted with $LiAlH_4$ (43 mg, 1.20 mmol) and the product was purified by fc (dichloromethane/MeOH = 97/3, ø = 1.5

cm, h = 10 cm, R_f = 0.27). Colorless liquid, yield 56 mg, (42 %). Purity: 95.6 %, t_R = 19.6 min. Exact mass (ESI): m/z = calcd. for $(C_{17}H_{27}NS)H$ 278.1953, found 278.1967. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.77 – 0.91 (m, 2H, C_6H_{11}), 1.02 – 1.25 (m, 4H, C_6H_{11}), 1.33 – 1.50 (m, 2H, 6-CH_2), 1.53 – 1.73 (m, 7H, 5-CH_2/C_6H_{11}), 1.78 – 1.94 (m, 3H, CHC*H*$_2$CH$_2$NH/C_6H_{11}), 2.28 – 2.35 (m, 1H, HNC*H*$_2C_6H_{11}$), 2.38 – 2.45 (m, 1H, HNC*H*$_2C_6H_{11}$), 2.63 (dt, 2H, J = 12.1/6.1 Hz, 1H, CHCH$_2$C*H*$_2$NH), 2.66 – 2.70 (m, 2H, 7-CH_2), 2.72 – 2.78 (m, 1H, 4-CH), 2.65 (t, J = 6.5 Hz, 2H, CH-5), 6.78 (d, J = 5.2 Hz, 1H, CH-2), 6.96 (d, J = 5.2 Hz, 1H, CH-2). ^{13}C NMR ($CDCl_3$): δ (ppm) = 21.7 (1C, C-6), 25.2 (1C, C-5), 25.8, 26.1, 26.7, 28.3, 29.7, 31.5, (6C, C_6H_{11}), 33.9 (1C, CH*C*H$_2$CH$_2$NH), 36.4 (1C, C-7), 38.0 (1C, CHCH$_2$*C*H$_2$NH), 48.1 (1C, C-4), 57.0 (1C, HN*C*H$_2C_6H_{11}$), 121.5 (1C, C-3), 126.8 (1C, C-3a), 135.8 (1C, C-2), 139.2 (1C, C-7a).

5.4. Receptor binding studies

5.4.1. Materials

The guinea pig brains and rat liver for the σ_1 and σ_2 receptor binding assays were commercially available (Harlan-Winkelmann, Borchen, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany). Cooling centrifuge model Rotina 35R (Hettich, Tuttlingen, Germany) and High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Multiplates: standard 96-well multiplates (Diagonal, Muenster, Germany). Shaker: self-made device with adjustable temperature and tumbling speed (scientific workshop of the institute). Vortexer: Vortex Genie 2 (Thermo Fisher Scientific, Langenselbold, Germany). Harvester: MicroBeta FilterMate-96 Harvester. Filter: Printed Filtermat Typ A and B. Scintillator: Meltilex (Typ A or B) solid state scintillator. Scintillation analyzer: MicroBeta Trilux (all Perkin Elmer LAS, Rodgau-Jügesheim, Germany). Chemicals and reagents were purchased from different commercial sources and of analytical grade.

5.4.2. Preparation of membrane homogenates from guinea pig brain

5 guinea pig brains were homogenized with the potter (500-800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200 x *g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 23,500 x *g* for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 7.4) and centrifuged again at 23,500 x g (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5-6 volumes of buffer and frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

5.4.3. Preparation of membrane homogenates from rat liver

Two rat livers were cut into small pieces and homogenized with the potter (500-800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x *g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000 x *g* for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 8.0) and incubated at room temperature for 30 min. After the incubation, the suspension was centrifuged again at 31,000 x *g* for 20 min at 4 °C. The final pellet was resuspended in 5-6 volumes of buffer and stored at -80,°C in 1.5 mL portions containing about 2 mg protein/mL.

5.4.4. Protein determination

The protein concentration was determined by the method of Bradford [25], modified by Stoscheck [26]. The Bradford solution was prepared by dissolving 5 mg of Coomassie Brilliant Blue G 250 in 2.5 mL of EtOH (95 %, v/v). 10 mL deionized H_2O and 5 mL phosphoric acid (85%, m/v) were added to this solution, the mixture was stirred and filled to a total volume of

50.0 mL with deionized water. The calibration was carried out using bovine serum albumin as a standard in 9 concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 and 4.0 mg /mL). In a 96-well standard multiplate, 10 µL of the calibration solution or 10 µL of the membrane receptor preparation were mixed with 190 µL of the Bradford solution, respectively. After 5 min, the UV absorption of the protein-dye complex at λ = 595 nm was measured with a platereader (Tecan Genios, Tecan, Crailsheim, Germany).

5.4.5. General protocol for the binding assays

The test compound solutions were prepared by dissolving approximately 10 µmol (usually 2-4 mg) of test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution was diluted with the respective assay buffer. The filtermats were presoaked in 0.5% aqueous polyethylenimine solution for 2 h at room temperature before use. All binding experiments were carried out in duplicates in 96-well multiplates. The concentrations given are the final concentrations in the assay. Generally, the assays were performed by addition of 50 µL of the respective assay buffer, 50 µL test compound solution in various concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} mol/L), 50 µL of corresponding radioligand solution and 50 µL of the respective receptor preparation into each well of the multiplate (total volume 200 µL). The receptor preparation was always added last. During the incubation, the multiplates were shaken at a speed of 500-600 rpm at the specified temperature. Unless otherwise noted, the assays were terminated after 120 min by rapid filtration using the harvester. During the filtration each well was washed five times with 300 µL of water. Subsequently, the filtermats were dried at 95 °C. The solid scintillator was melted on the dried filtermats at a temperature of 95 °C for 5 min. After solidifying of the scintillator at room temperature, the trapped radioactivity in the filtermats was measured with the scintillation

analyzer. Each position on the filtermat corresponding to one well of the multiplate was measured for 5 min with the [^{3}H]-counting protocol. The overall counting efficiency was 20%. The IC_{50}-values were calculated with the program GraphPad Prism® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. Subsequently, the IC_{50} values were transformed into K_i-values using the equation of Cheng and Prusoff [26]. The K_i-values are given as mean value ± SEM from three independent experiments.

5.4.6. Protocol of the σ_1 receptor binding assay

The assay was performed with the radioligand [^{3}H]-(+)-pentazocine (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation of guinea pig brain cortex (about 100 µg of protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and TRIS buffer (50 mM, pH 7.4) at 37 °C. The non-specific binding was determined with 10 µM unlabeled (+)-Pentazocine. The K_d-value of (+)-pentazocine is 2.9 nM [28].

5.4.7. Protocol of the σ_2 receptor binding assay

The assays were performed with the radioligand [^{3}H]DTG (specific activity 50 Ci/mmol; ARC, St. Louis, MO, USA). The thawed membrane preparation of rat liver (about 100 µg of protein) was incubated with various concentrations of the test compound, 3 nM [^{3}H]DTG and buffer containing (+)-pentazocine (500 nM (+)-pentazocine in 50 mM TRIS, pH 8.0) at room temperature. The non-specific binding was determined with 10 µM non-labeled DTG. The K_d value of [^{3}H]DTG is 17.9 nM [29].

Acknowledgement

This work was done within the framework of the NRW International Graduate School of Chemistry, Münster, which is funded by the Government of the state Nordrhein-Westfalen and Westfälische Wilhelms-University Münster. The financial support is deeply acknowledged.

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List of captions of Figures, Schemes and Tables

Figure 1

Development of novel σ_1 receptor ligands **4** by combination of S1RA (**1**), aminoethylindazoles **2** and spirocyclic thienopyrans **3**.

Scheme 1

Synthesis of the α,β-unsaturated ester **10** starting with thiophene.

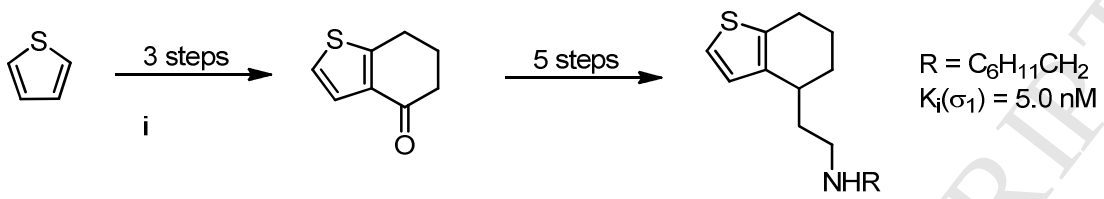
Scheme 2

Synthesis of aminoethyl tetrahydrobenzothiophenes **4**.

Table 1

σ_1 and σ_2 receptor affinities of aminoethyl substituted tetrahydrobenzothiophenes **4** and reference compounds.

Graphical Abstract



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Datum

06.06.2013

Research Highlights

> Pharmacophoric elements of different compounds were combined.

> Benzothiophenes with a flexible aminoethyl side chain were prepared.

> The most potent compound showed low nanomolar σ_1 affinity.

> Relationships between the structure and the σ_1 affinity were elaborated.

Supporting Information

New combination of pharmacophoric elements of potent σ_1 ligands: Design, synthesis and σ receptor affinity of aminoethyl substituted benzothiophenes

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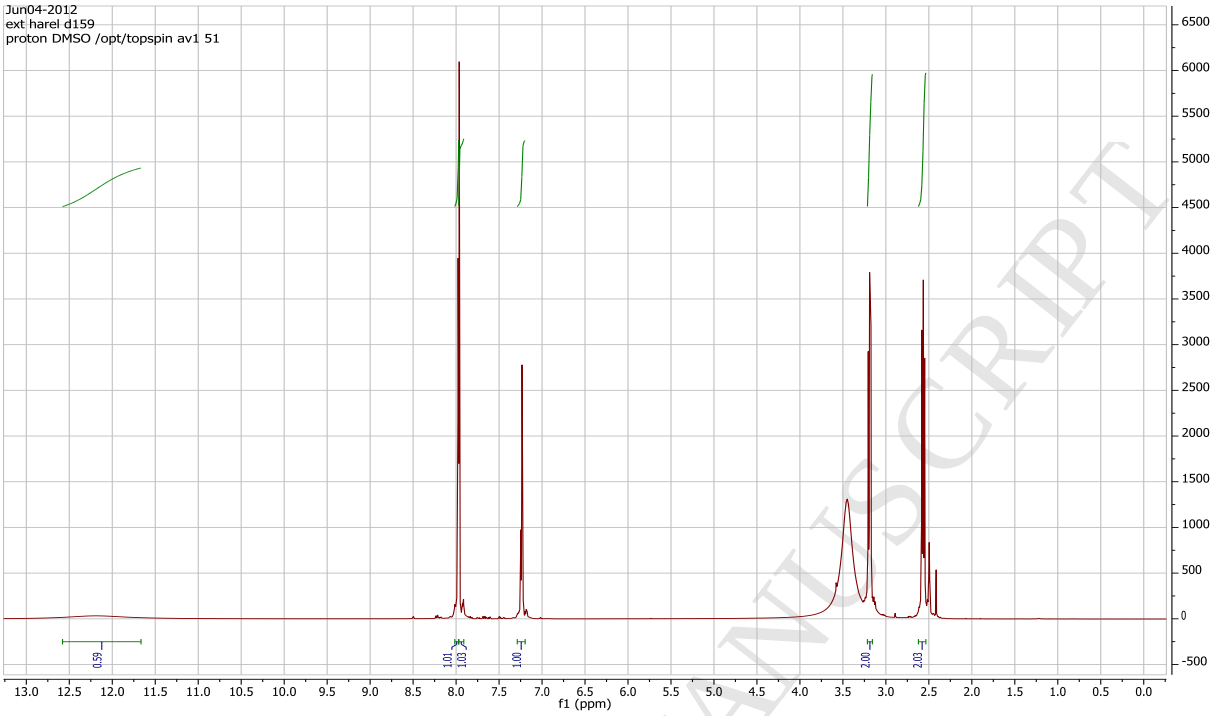
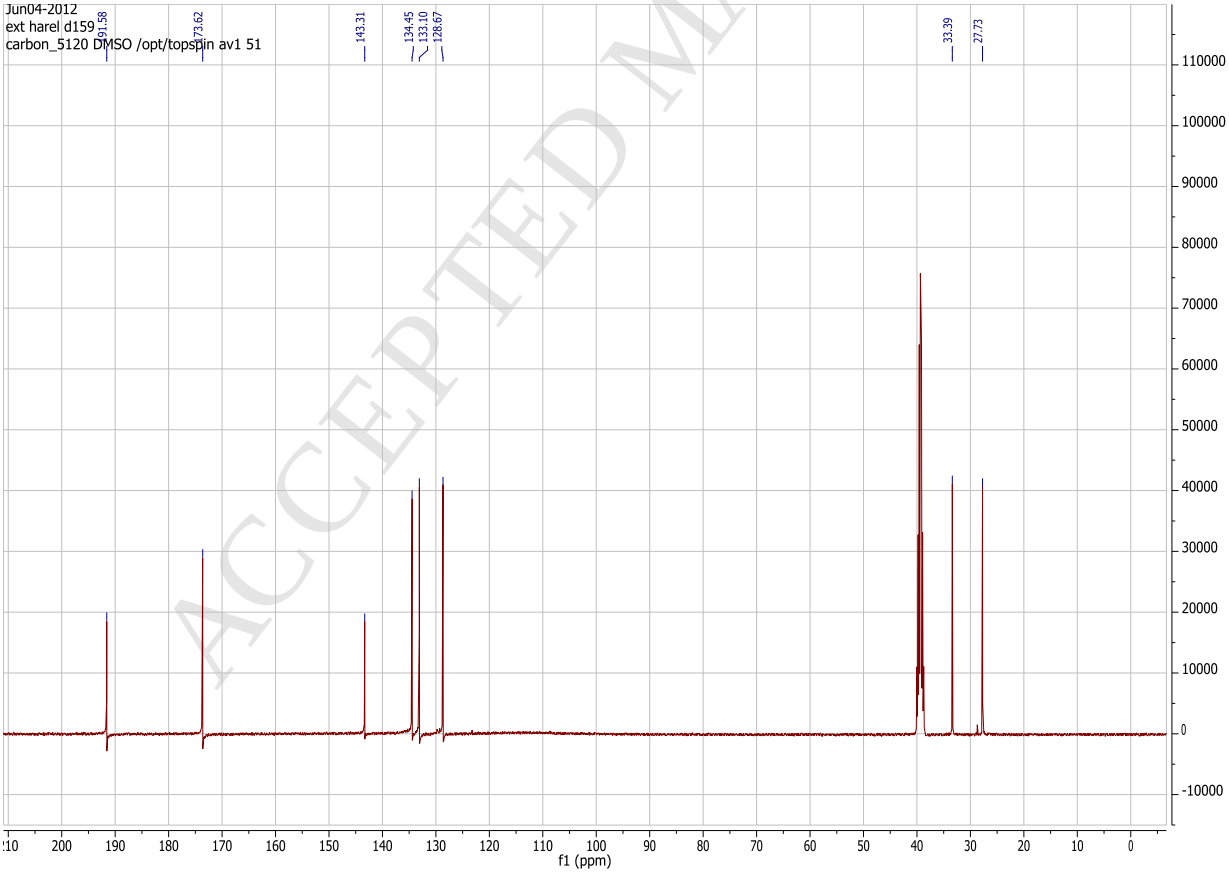
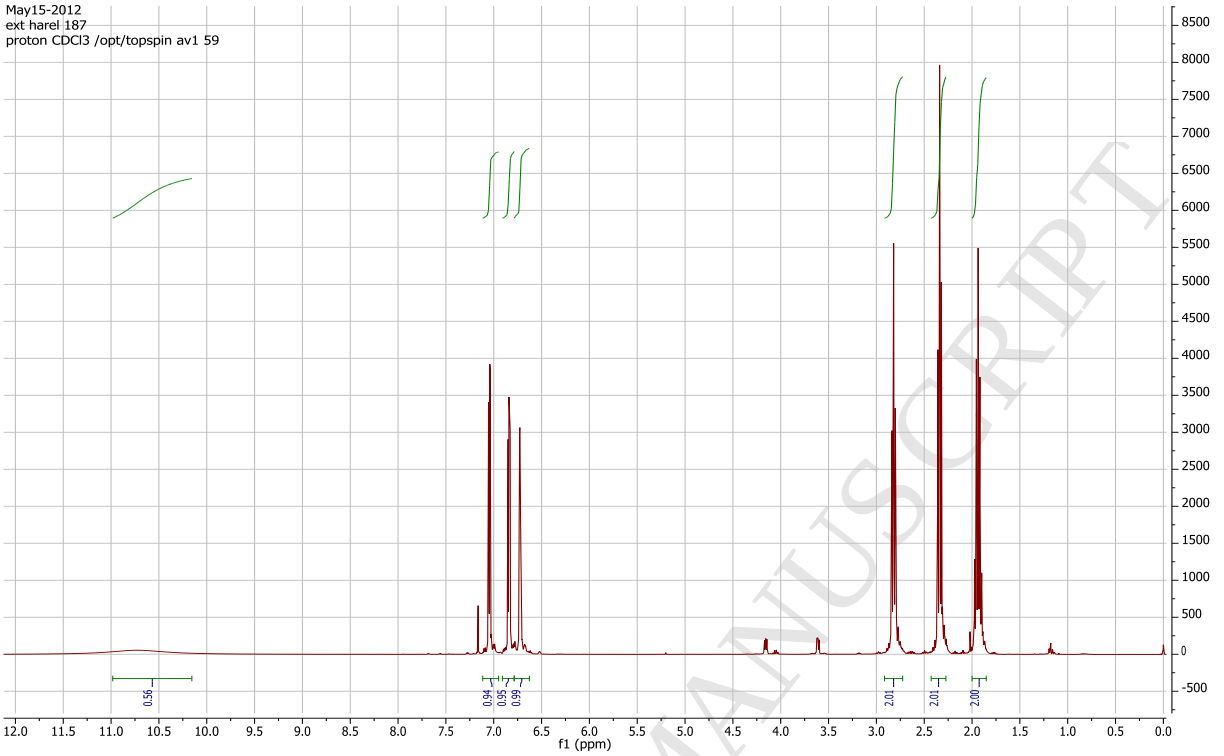
^{1}H NMR and ^{13}C NMR data

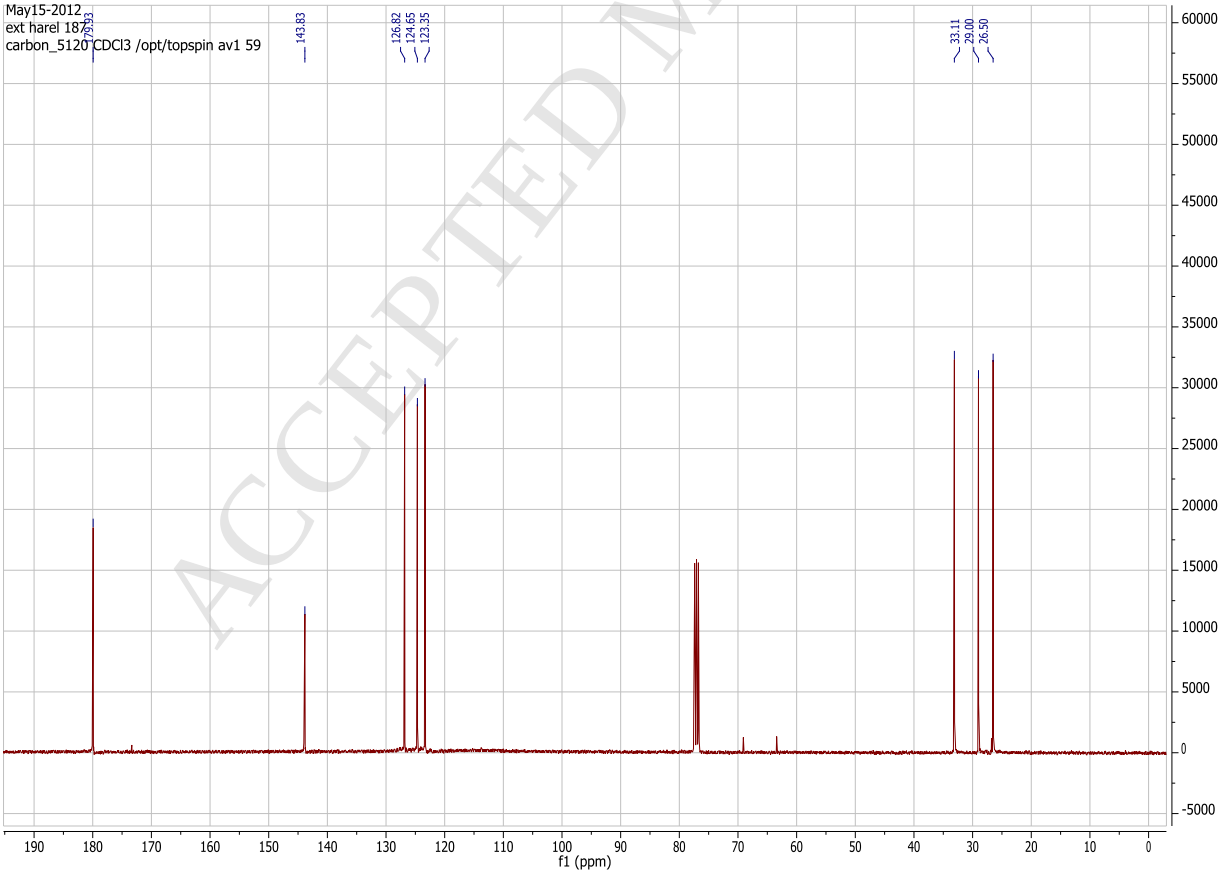
5.3.1. 4-Oxo-4-(thiophen-2-yl)butanoic acid (7)



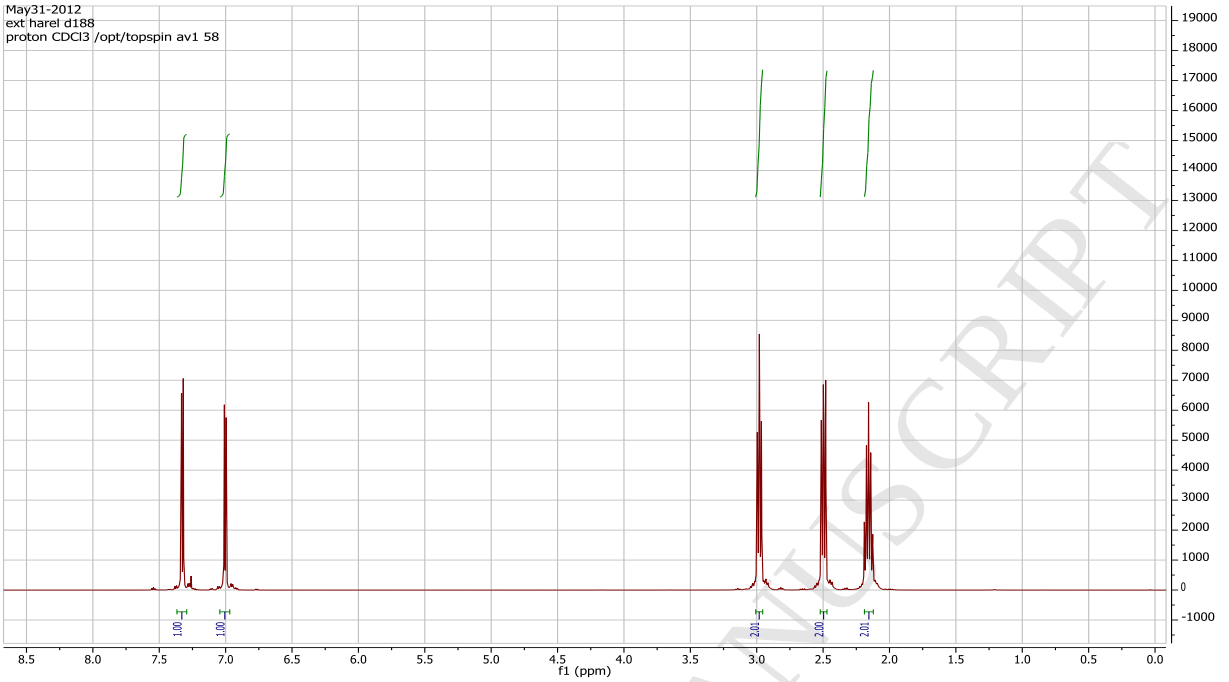


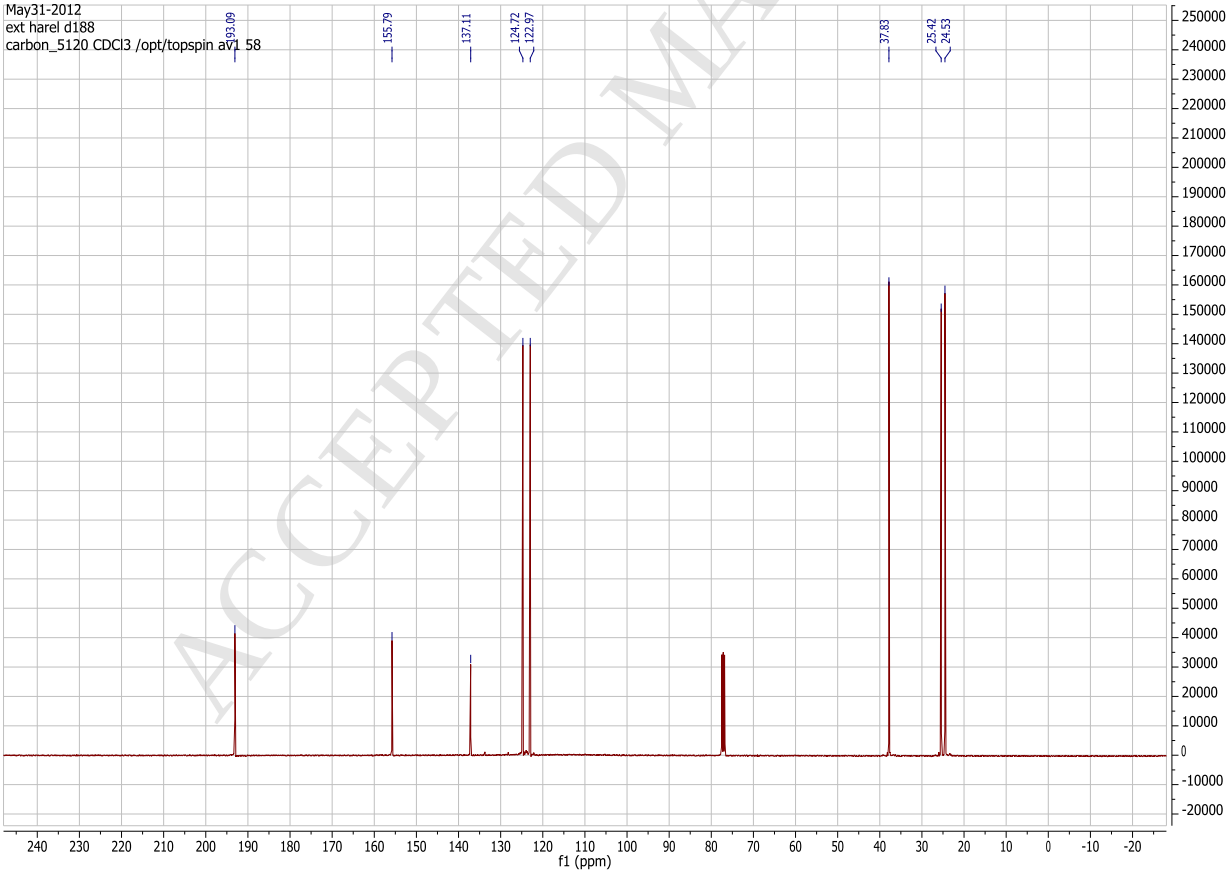
5.3.2. 4-(Thiophen-2-yl)butanoic acid (8)



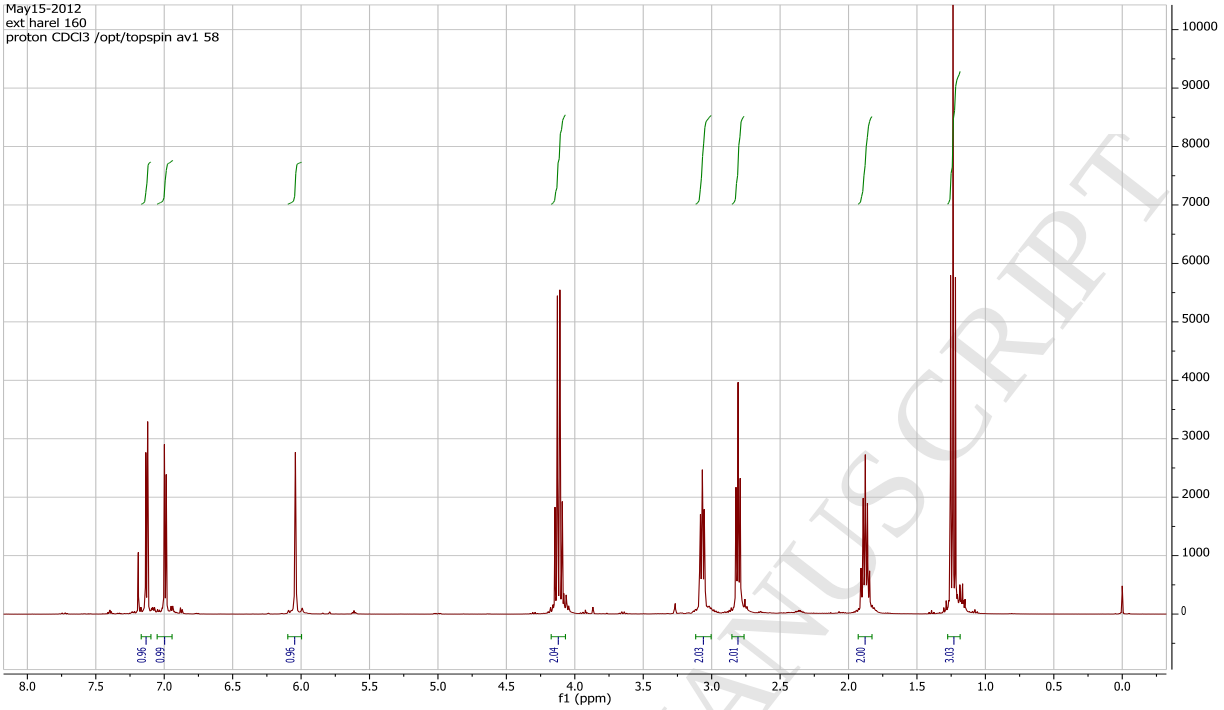


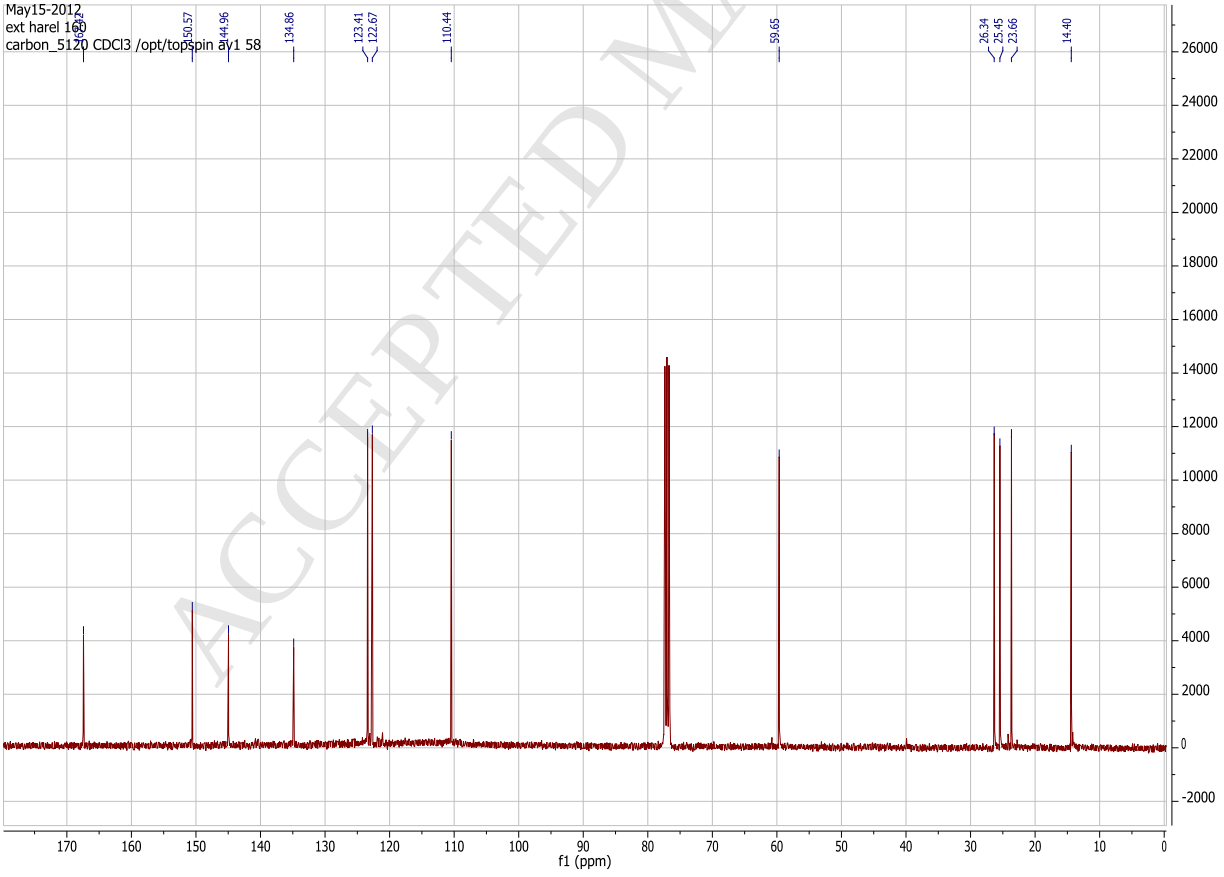
5.3.3. 6,7-Dihydrobenzo[b]thiophen-4(5*H*)-one (9)



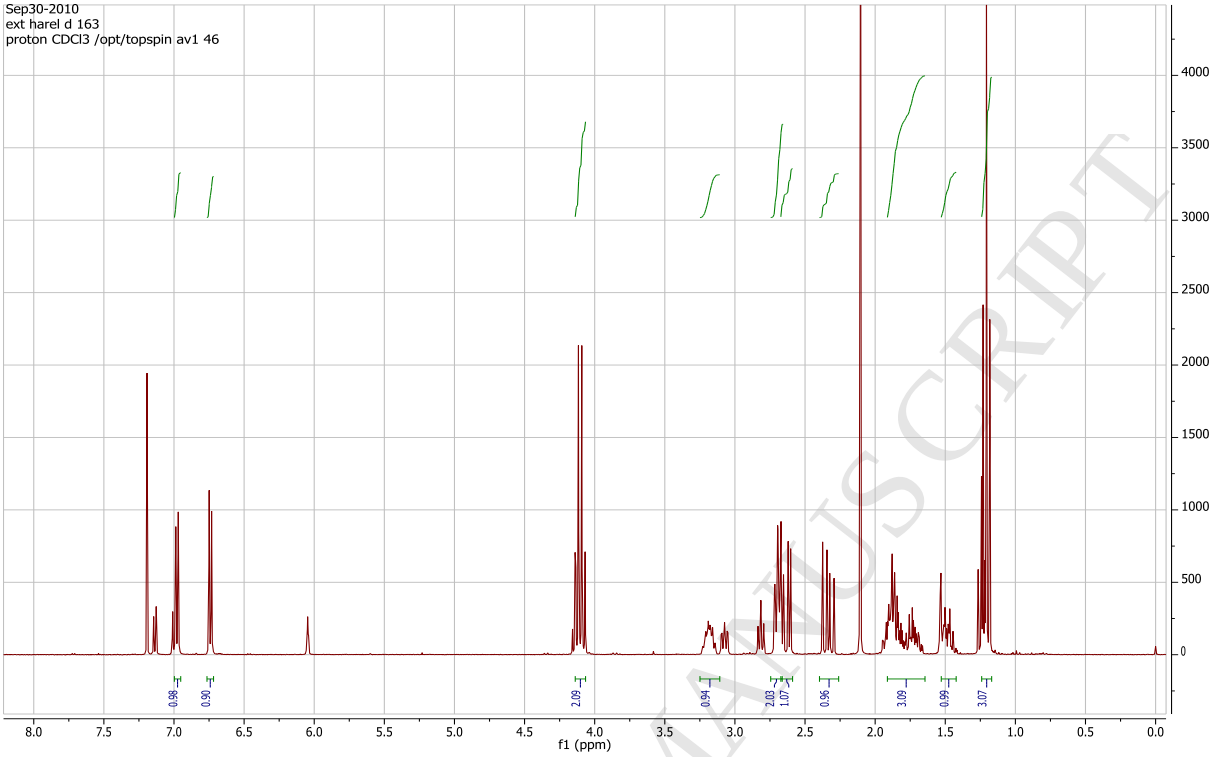


5.3.4. (*E*)-Ethyl 2-(6,7-dihydrobenzo[b]thiophen-4(5*H*)-ylidene)acetate (10)

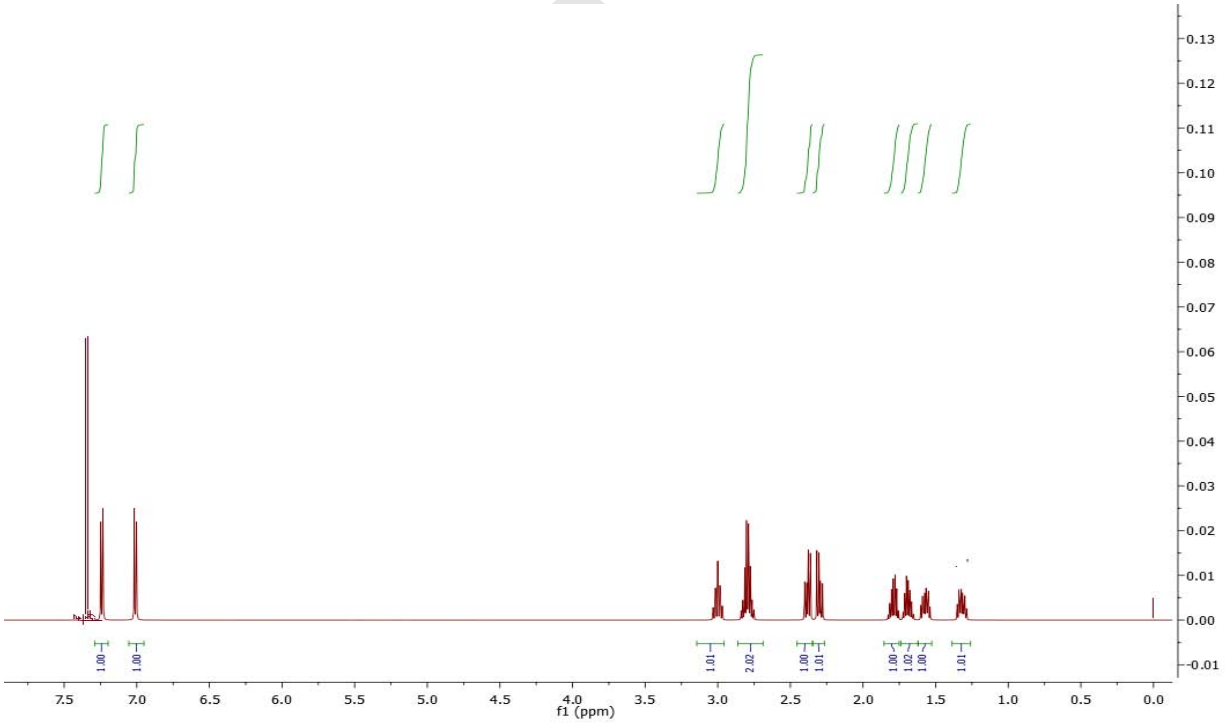




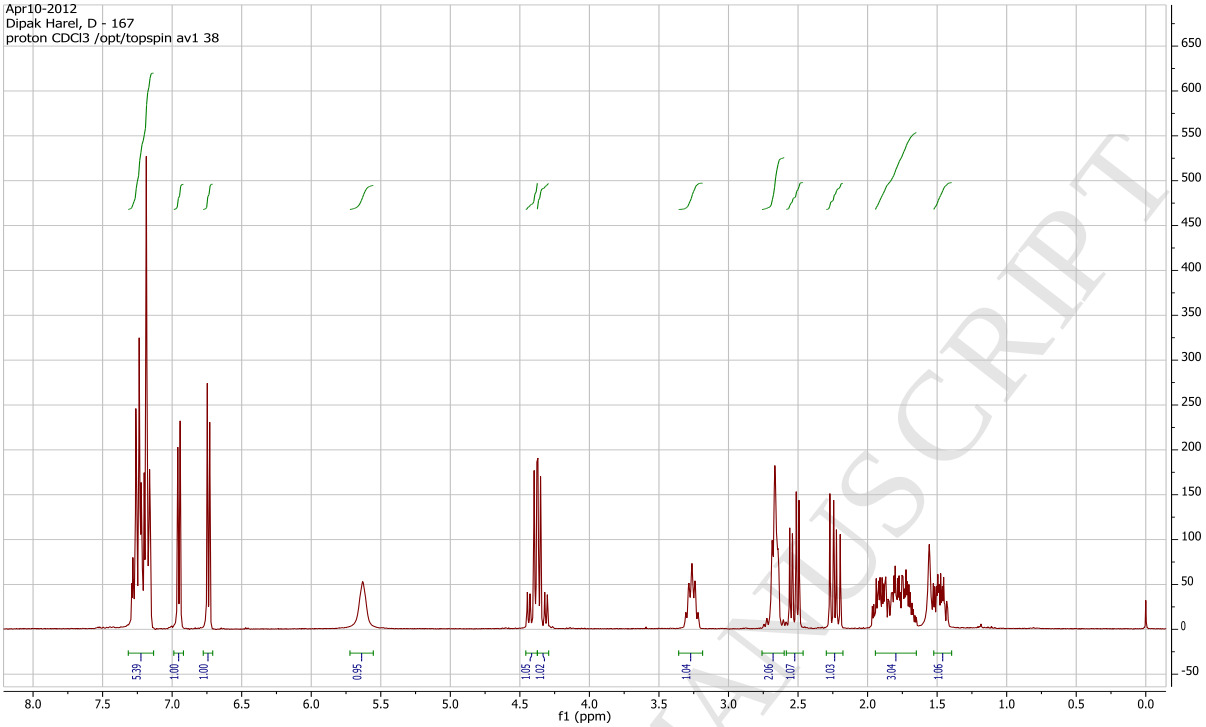
5.3.5. Ethyl 2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)acetate (11)

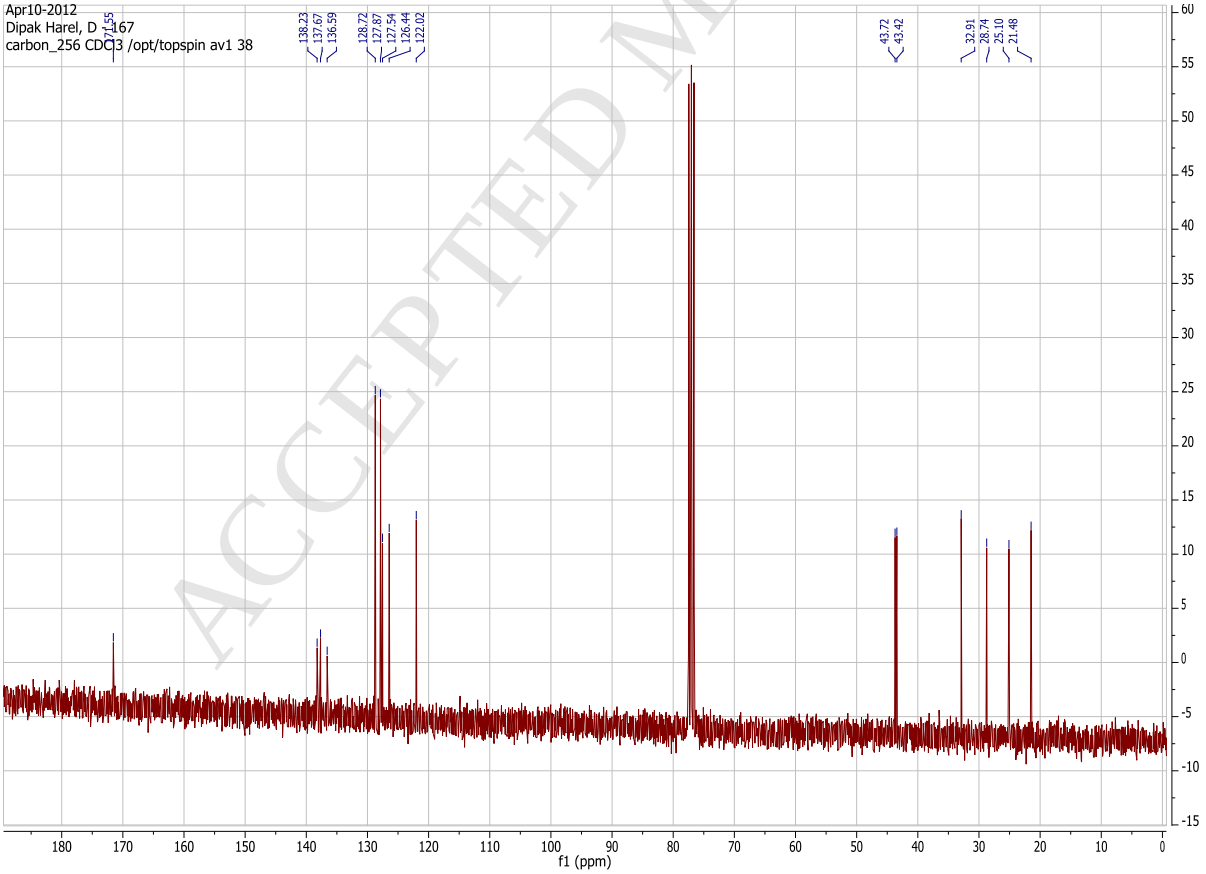


5.3.6. 2-(4,5,6,7-Tetrahydrobenzo[b]thiophen-4-yl)acetic acid (12)

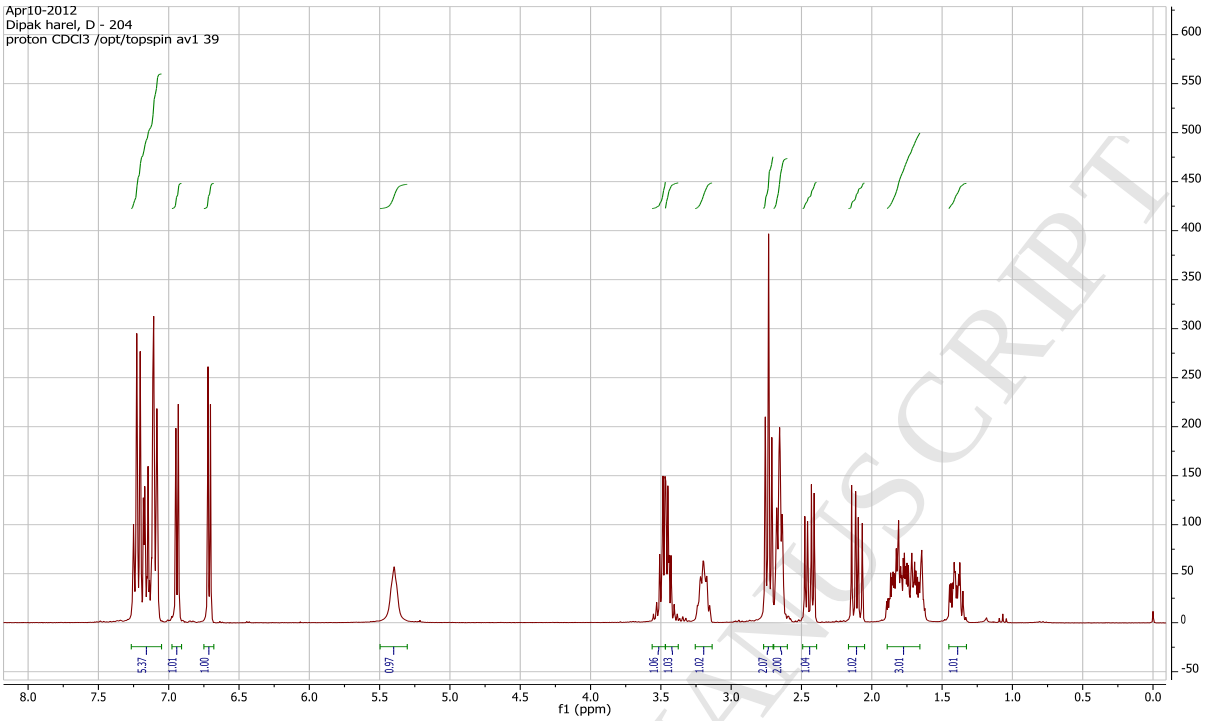


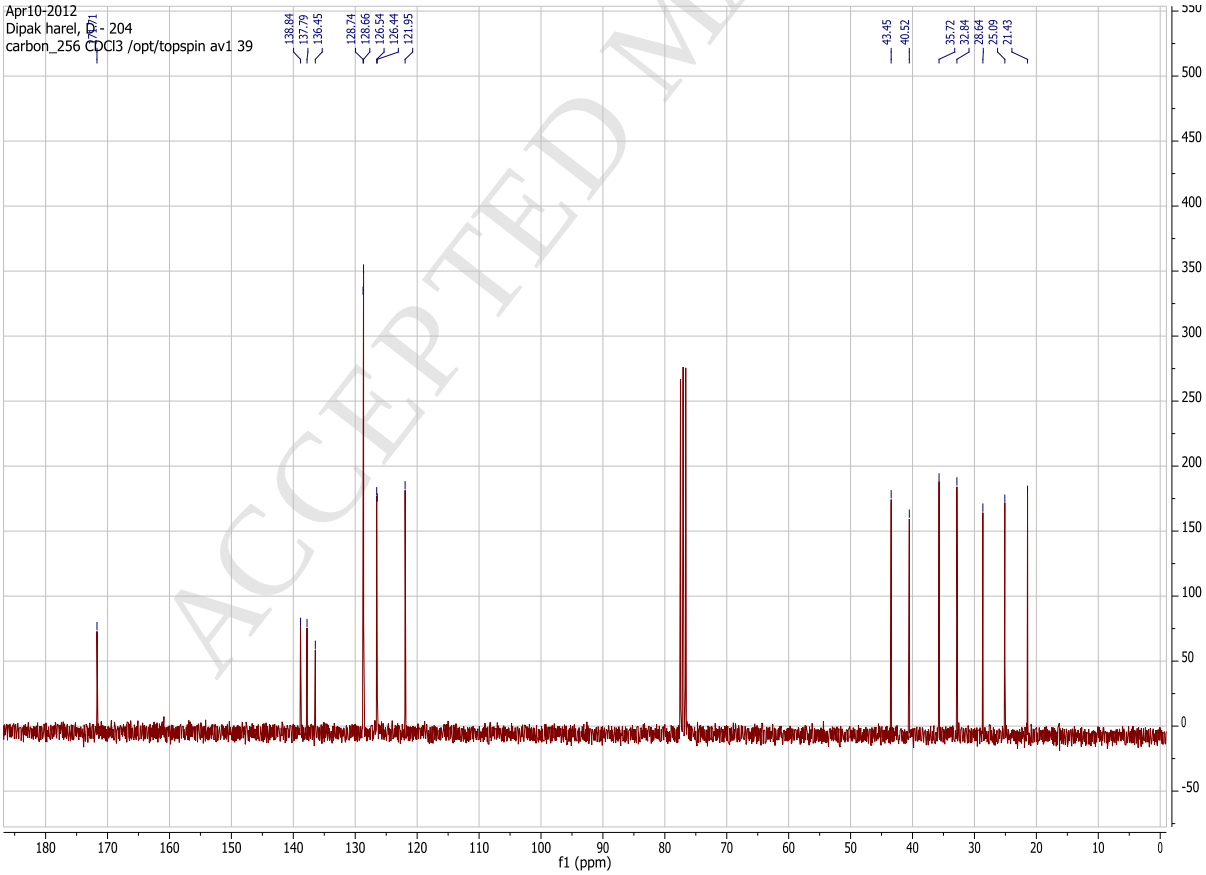
5.3.7. N-Benzyl-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)acetamide (13a)



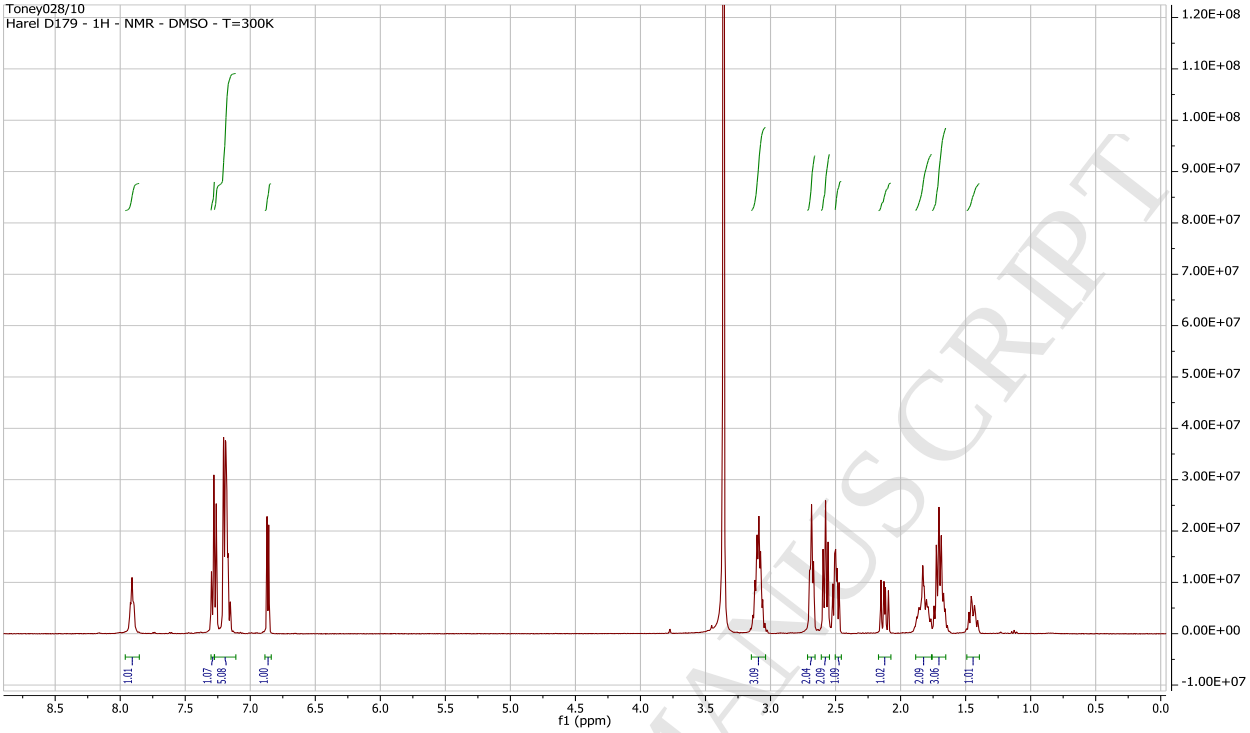


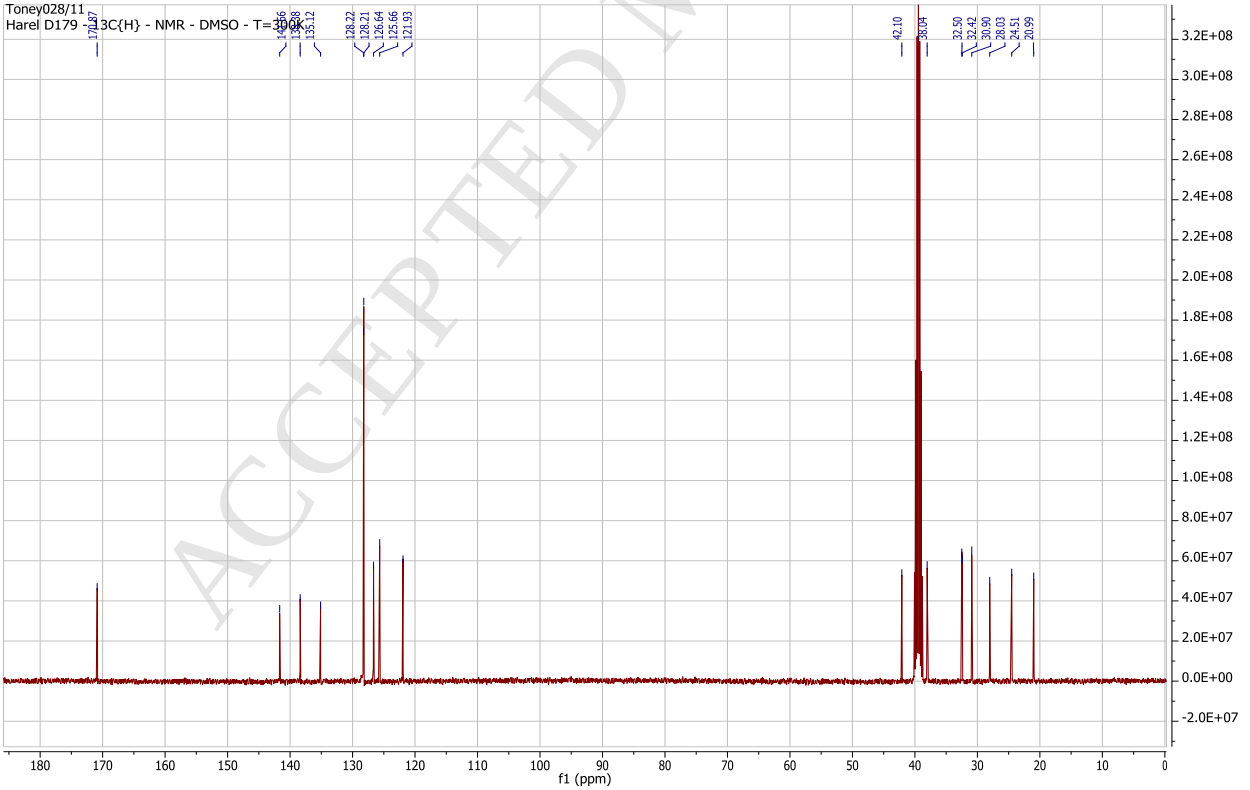
5.3.8. N-(2-Phenyl ethyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)acetamide (13b)



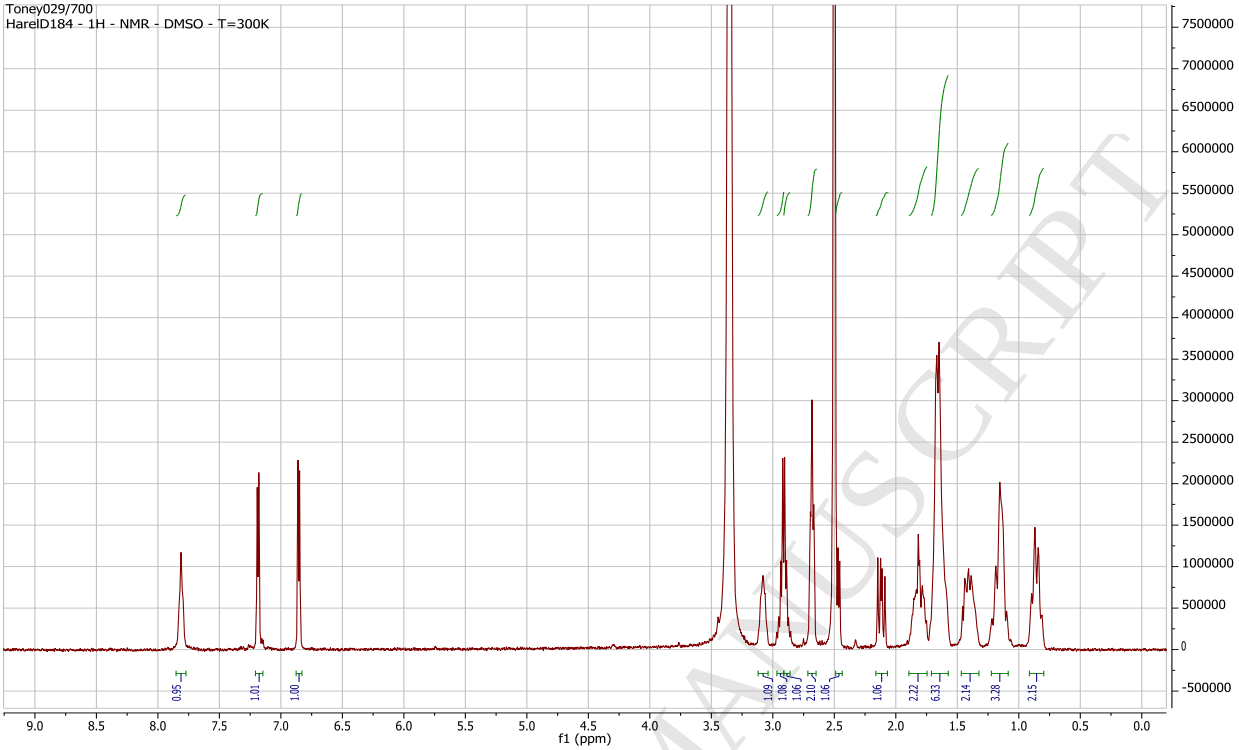


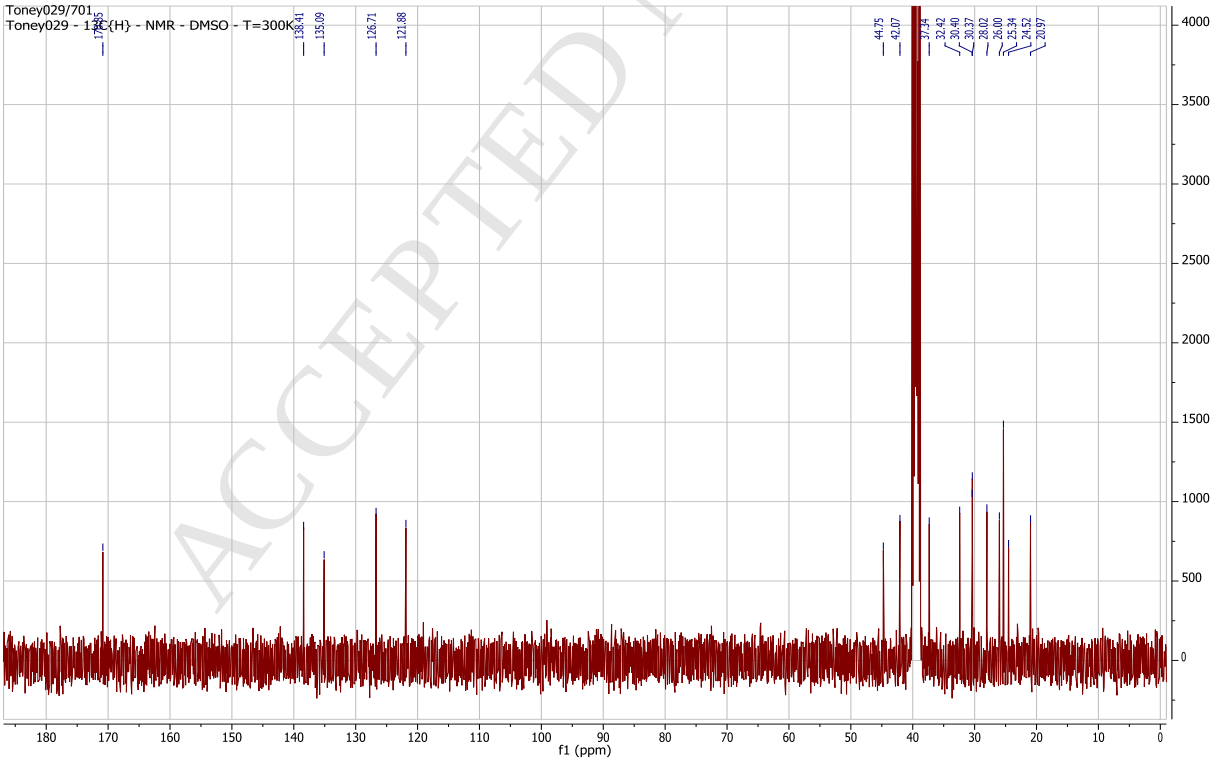
5.3.9. N-[(3-Phenylpropyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)]acetamide (13c)



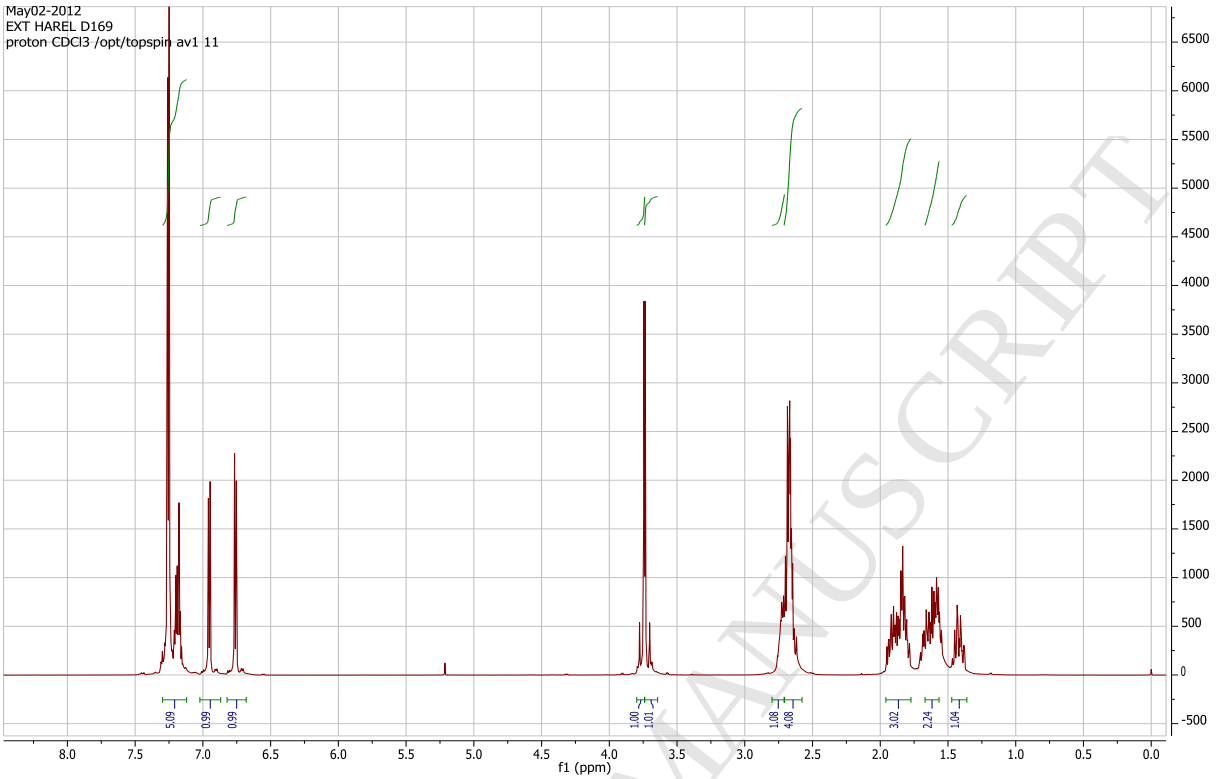


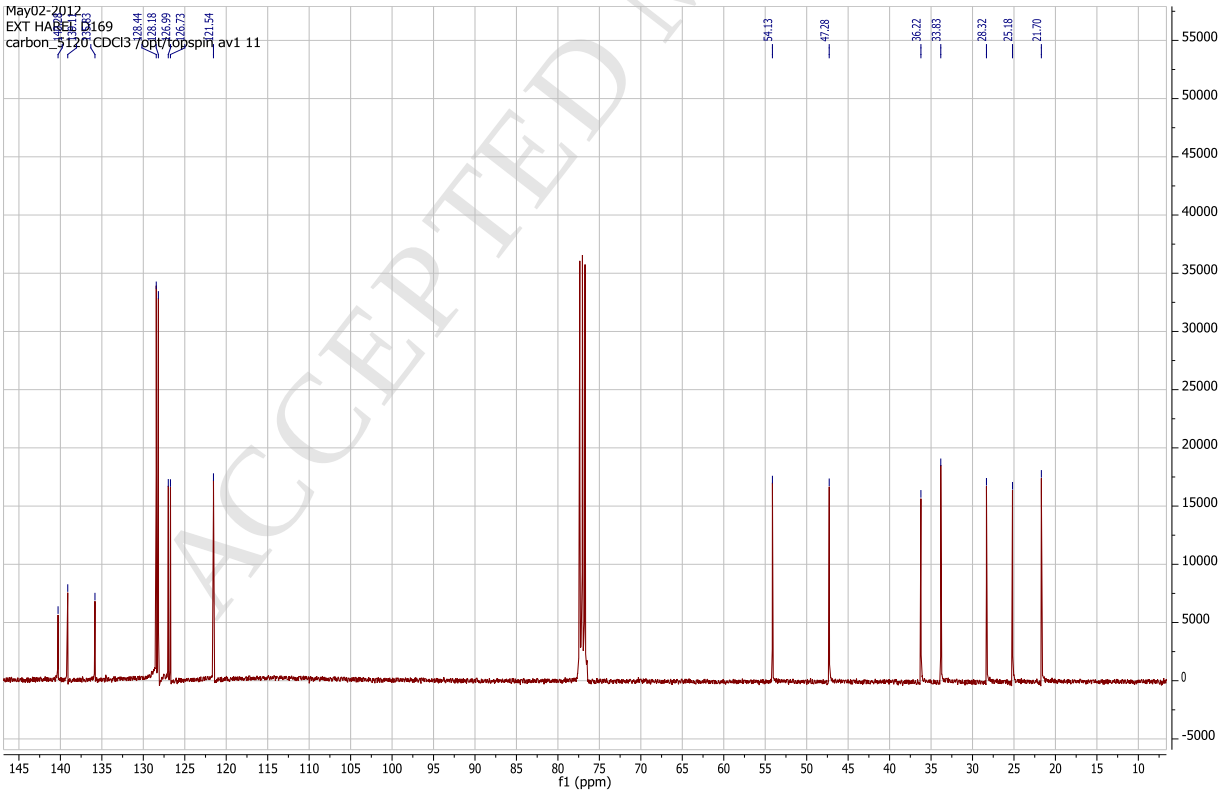
5.3.10. N-(Cyclohexylmethyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)acetamide (13d)



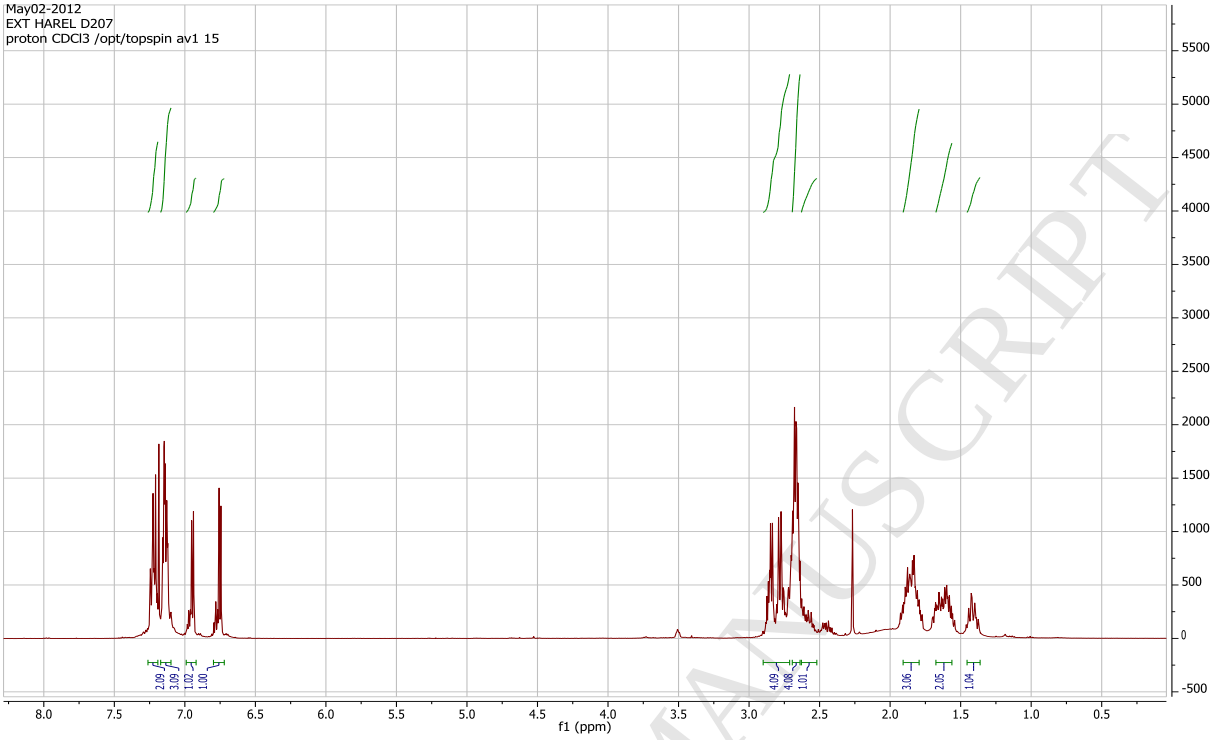


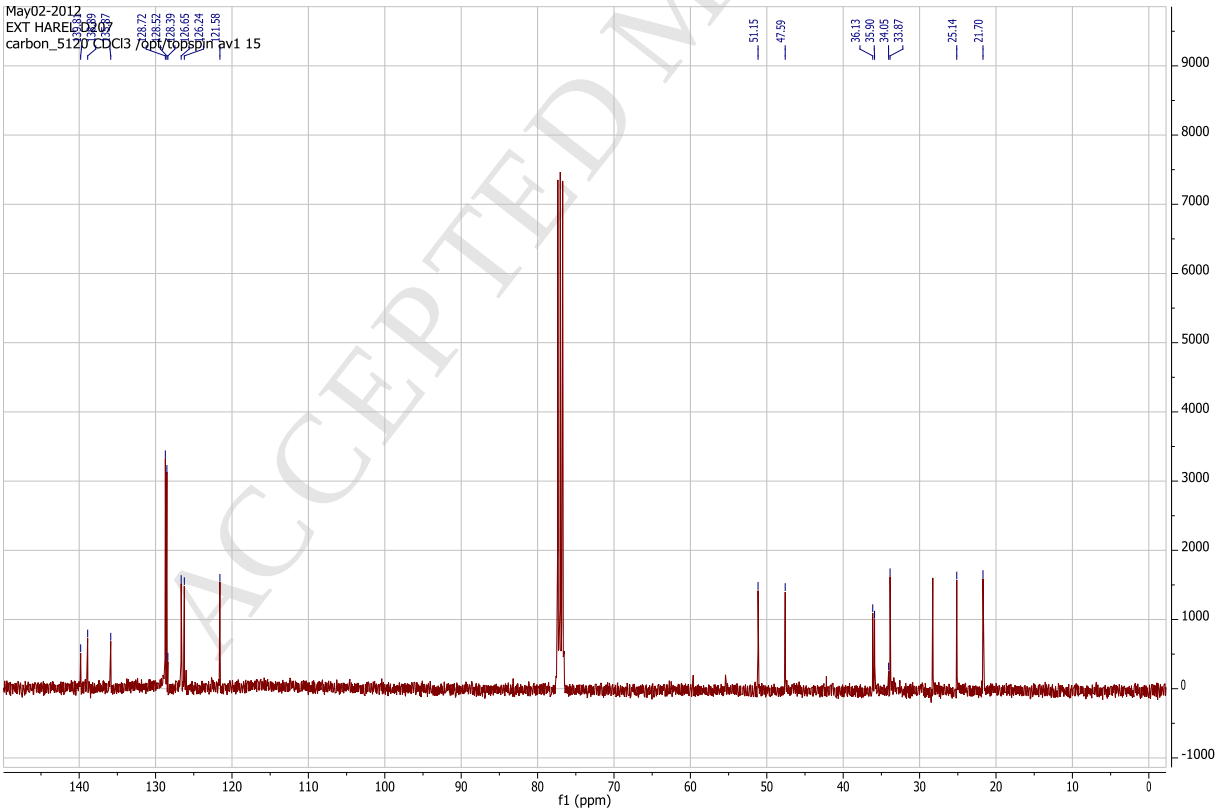
5.3.11. N-Benzyl-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)ethanamine (4a)



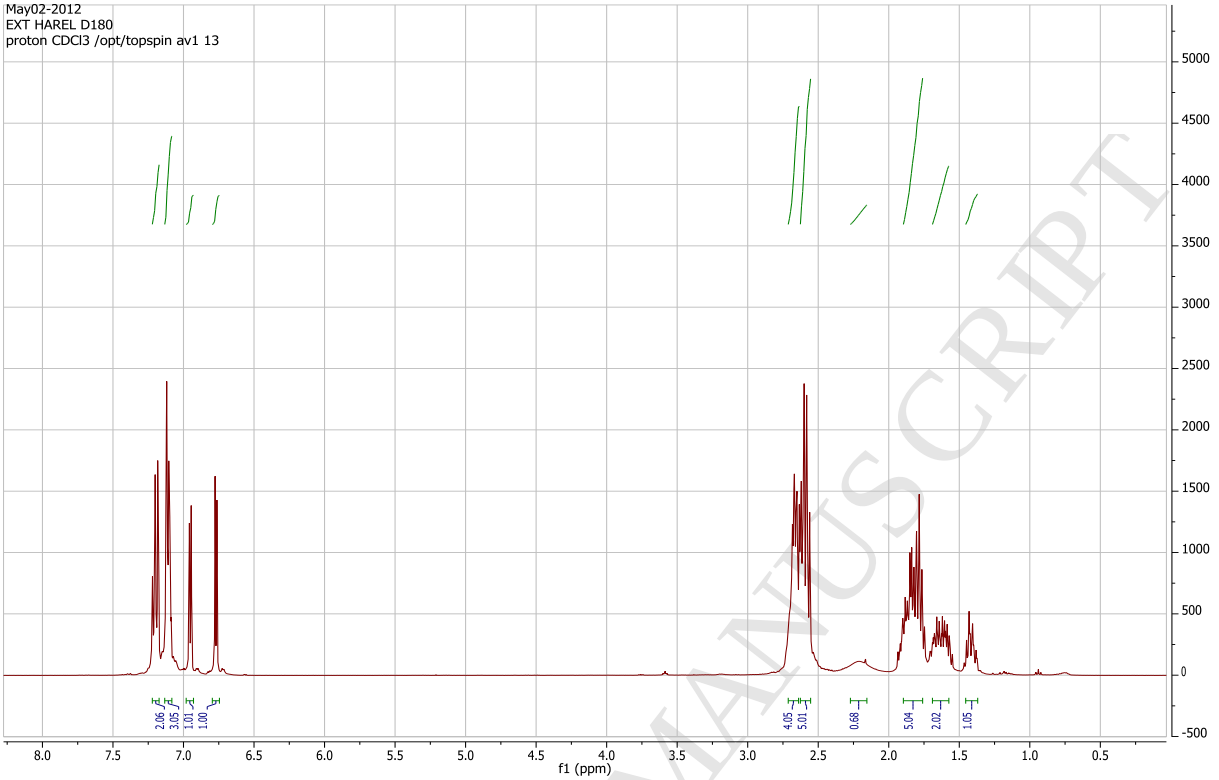


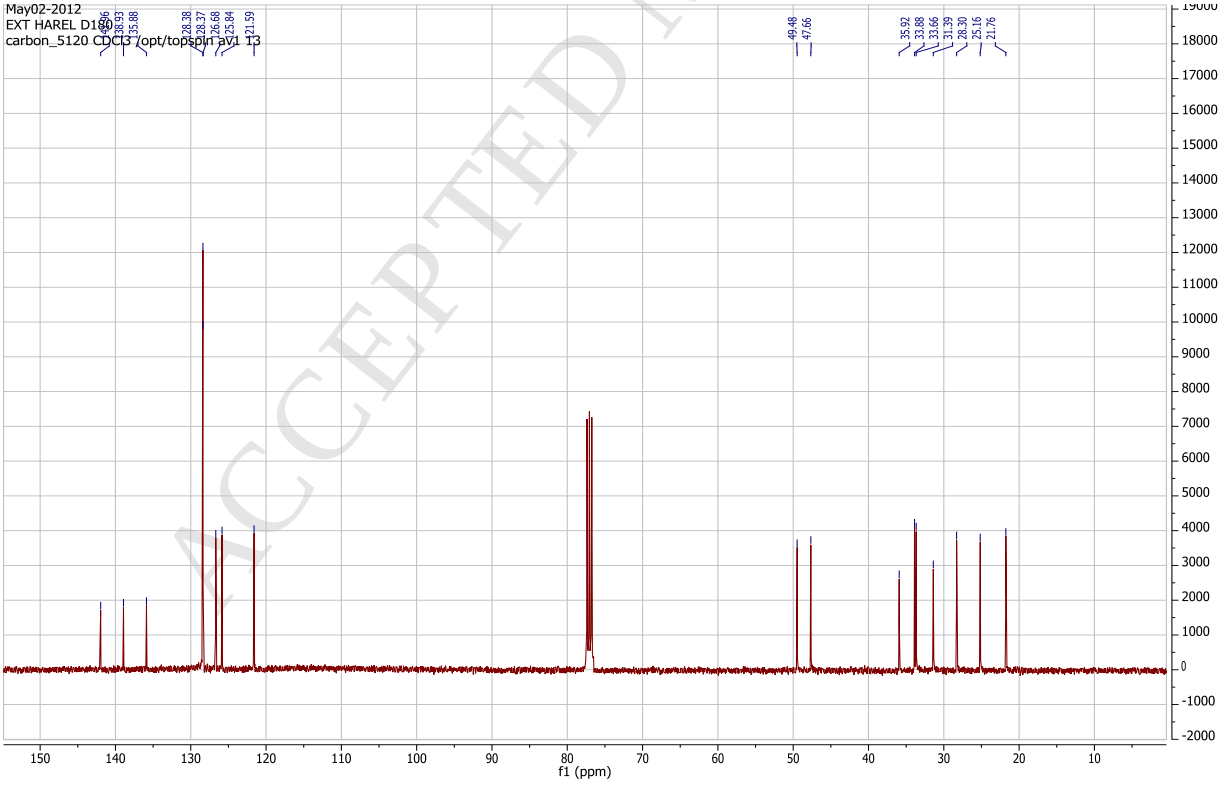
5.3.12. N-(2-Phenylethyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)ethanamine (4b)





5.3.13. 3-Phenyl-N-[2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)ethyl]propan-1-amine (4c)





5.3.14. N-(Cyclohexylmethyl)-2-(4,5,6,7-tetrahydrobenzo[b]thiophen-4-yl)ethanamine (4d)