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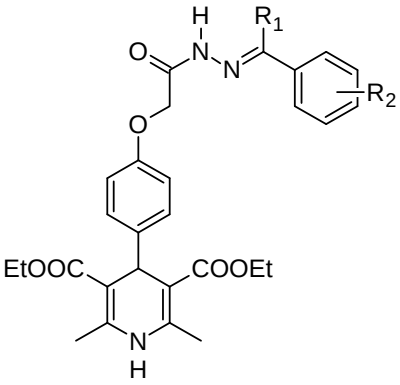

R_1= H, Me

R_2= Alkyl, alkoxy, aryl, OH, NH_2, F, Cl, Br

New DHPs carrying hydrazone pharmacophore have been synthesized as new anticonvulsant and anti-inflammatory agents. Many tested compounds showed good activity and are found to be non-toxic in nature.

Synthesis, anticonvulsant and anti-inflammatory studies of new 1,4-dihydropyridin-4-yl-phenoxyacetohydrazones

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Abstract:

The present work involves design and synthesis of new substituted 1,4-dihydropyridin-4-yl-phenoxyacetohydrazones (**4a-s**, **5a-h**), starting from 4-hydroxybenzaldehyde. The final compounds were screened for their *in vivo* anticonvulsant activity by MES and scPTZ methods, while their anti-inflammatory screening was performed by Carrageenan induced Paw Edema method. The results indicated that compounds carrying electron donating groups are anticonvulsant active, while most of the tested compounds exhibited significant anti-inflammatory activity. Compounds **4k-l, 4p-s**, and **5c** showed rapid anti-inflammatory activity within 30 minutes and appeared as lead compounds. Further, Neurotoxicity study revealed that all the tested compounds are non-toxic up to 300 mg/kg doses. Selected compounds were also subjected to analgesic screening following Tail immersion method and they exhibited good activity.

Key words: Dihydropyridine; Hydrazone; Anticonvulsant; Anti-inflammatory; Analgesic; Neurotoxicity.

1. Introduction

Epilepsy is a major neurological disorder that accounts for 1% of global burden of disease and affects more than 50 million people, all over the world [1]. Even though epilepsy is known to mankind for several thousands of years, rapid advances both in diagnosis and management have been made only in last few decades. Presently, there are many

anticonvulsant drugs are available in the market that deals with control of disease. Nevertheless, about 30% of patients are pharmaco-resistant to available drugs and consequently, these patients experience uncontrolled seizures [2]. Moreover, present antiepileptic treatment demands continuous medication for years together that brings about many side effects [3]. According to literature, some of the available active anti-epileptic drugs do not link with any binding site of the receptor [4], where as many other drugs show their effect via different mechanism of actions [5]. As a result, identification of new therapeutic agents that are devoid of any side effects, with a known mechanism of action has become an active area of research, today in medicinal chemistry.

Dihydropyridines (DHPs) are the largest and most studied calcium channel blockers. In addition to their clinical utility as Ca^{2+} channel blockers, they are also used extensively as tools for study of voltage activated calcium channel structure and function [6]. It is well established that calcium is an important factor responsible for induction of convulsive seizures [7]. As a result, Ca^{2+} channel blockers are significant in controlling the convulsion [8]. In this context, many DHPs are well documented in literature as potential anticonvulsant agents [9, 10]. Further, literature reports support that DHP derivatives are potent anti-inflammatory agents [11-13]. Interestingly, they were found to bind adenosine receptors (A_1, A_2, A_3) effectively in the brain and bring about therapeutic effects [14]. In fact, A_3 adenosine receptor antagonists are being sought as potential anti-inflammatory agents [15]. In our previous study [16] also, good anti-inflammatory activity was observed for new DHPs carrying amide pharmacophore. This clearly shows that DHP is a suitable heterocyclic scaffold for the development of new antiepileptic and anti-inflammatory agents.

Hydrazones containing azomethine (–NHN=CH) protons constitute a vital class of compounds for new drug development [17]. Several heterocyclic hydrazones were reported to possess various biological activities viz. anticonvulsant [18], anti-inflammatory [19, 20], analgesic [21], anti-candida [22], anti-tubercular [23, 24], antimicrobial [25], anticancer [26], anti-proliferative [27] and antiamoebic [28] activities. Particularly, hydrazone moiety attached to heterocyclic systems was shown to offer enhanced activity [29, 30]. Interestingly, aryl hydrazones with terminal electron donating groups possess enhanced hydrogen bonding capabilities which influence their biological activity significantly [31]. Against this background, it has been planned to design and synthesize new dihydropyridine derivatives carrying aromatic hydrazone as an important pharmacophore at C_4 position of DHP through an aryl linker, and to investigate their anticonvulsant as well as anti-inflammatory properties. Further, most of the reported NSAIDs are exhibiting both anti-inflammatory and analgesic

activities. Since their mode of actions are almost same, it has been also contemplated to screen selected target compounds of the present work for *in vivo* analgesic property.

A detailed literature reports on structure-activity relationship study of 1,4-DHP system revealed that unsubstituted free NH group in DHP ring is crucial for its better activity with respect to any medicinal property. Further, presence of methyl groups at 2^{nd} and 6^{th} positions, ester groups at 3^{rd} and 5^{th} positions and an aryl ring at 4^{th} position are essential structural features for prominent biological effect [32, 33]. Based on these observations, new dihydropyridine derivatives carrying azomethine group have been designed as shown in Figure 1. Different aryl rings with various substituents have been incorporated in our new design in order to study the effect of substituents on their pharmacological activity.

Figure 1: Design of new dihydropyridine derivatives

2. Results and discussion:

2.1. Chemistry

All the intermediates and target compounds **4a-s** and **5a-h** were synthesized according to Scheme 1. Required dihydropyridine derivative **1** was constructed following Hantzsch method, from 4-hydroxybenzaldehyde by refluxing it with two equivalents of ethyl acetoacetate and ammonium acetate under ethanol medium. The hydroxyl group was alkylated with ethyl chloroacetate in DMF medium under nitrogen atmosphere to obtain the product **2**. Under similar mild conditions, only phenolic OH group, but not NH group of DHP ring undergoes alkylation. The resulting ester **2** was converted to its hydrazide **3** through nucleophilic substitution reaction by refluxing it with hydrazine hydrate in ethanol for about 4 hrs. Interestingly, stable ester groups on DHP ring remained intact under this condition. The target hydrazones **4a-s** and **5a-h** were obtained by condensing hydrazide **3** with various aldehydes and ketones, in ethanol medium with trace of conc. sulphuric acid as catalyst.

Scheme 1: Synthesis of intermediates and final compounds **4a-s** and **5a-h**

Newly synthesized target compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectral techniques followed by elemental analysis. Formation of DHP ring was confirmed by FTIR spectrum of **1**, where it showed prominent peaks at 3337 cm^{-1} and 1656 cm^{-1}, due to NH/OH and ester groups, respectively. This was further confirmed by its ^{1}H NMR spectrum, where it displayed singlets at δ 9.09 and δ 8.77 ppm, which are attributed to phenolic OH and NH proton of DHP ring, respectively. The presence of two ester groups were confirmed by its ^{1}H NMR spectrum, where in it showed multiplet and triplet at δ 4.04

and δ 1.15 ppm, respectively for ester OCH_2CH_3 group. Appearance of a singlet at δ 4.74 ppm that corresponds to C_4 CH proton further confirms the proposed structure of the product. In ^{1}H NMR spectrum of compound **2**, disappearance of OH peak at δ 9.09 ppm clearly established that phenolic hydroxyl group was alkylated but not NH group of DHP ring. In FTIR spectrum of hydrazide **3**, shifting of carbonyl stretching frequency from 1739 cm^{-1} to lower frequency 1656 cm^{-1} indicated the formation of **3**. Further, its ^{1}H NMR spectrum displayed two singlets at δ 9.24 and δ 4.28 ppm confirming the presence of hydrazidic NH and NH_2 groups. Formation of various hydrazones **4a-s** and **5a-h** from hydrazide **3** was evidenced by their FTIR and ^{1}H NMR spectra. ^{1}H NMR spectrum of **4a** showed a new peak at δ 7.89 ppm which corresponds to –N=CH- proton, in the place of a singlet at δ 4.28 ppm confirming its structure. Further, in ^{1}H NMR spectrum of **5a** appearance of new peak at δ 2.31 ppm which corresponds to allylic methyl group, is the proof for its formation. Similar pattern of peaks were observed for remaining compounds of the series. Furthermore, structures of all the final compounds were confirmed by their ^{13}C NMR, mass spectral and elemental data.

2.2. Biological results

2.2.1 Anticonvulsant and toxicity studies

The preclinical discovery and development of a new bioactive chemical entity for the treatment of epilepsy depends heavily on the use of predictable animal models. The maximal electroshock (MES) [34] and subcutaneous pentylenetetrazole (scPTZ) [35] screening methods are the two important and routinely used *in vivo* animal models for the anticonvulsant studies [36]. They are claimed to detect new bioactive chemical entities affording protection to generalized tonic-clonic seizures and generalized absence seizures, respectively. Further, Rotarod method [37] is an effective route for detecting motor impairment of the new compounds. In this context, we also screened newly synthesized molecules following these methodologies. Amongst twenty seven new derivatives, only eight derivatives exhibited antiepileptic activity in MES method, while all the derivatives were found to be inactive in scPTZ method. The screening results of active target compounds are summarized in Table 1. Additionally, the MES active compounds were further screened by 6 Hz method [38] and these results are summarized in Table 2. These animal studies were performed in accordance with the ethical standards on animal experimentations.

The screening results clearly show that the hydrazone group carrying an electron rich aryl moiety is an essential structural feature for good anticonvulsant activities. The active

compounds **4e**, **4i-k**, **4p**, **5c**, **5f** and **5h** exhibit their activities 4 hour post i.p. injection of test samples, where as they are inactive at 0.5 hour. Amongst arylhydroxy derivatives, compound **4e** containing 4-hydroxy substituent display prominent activity at 100 mg/kg dose. However, its ortho-hydroxyphenyl analogue **4f** is inactive even at high test dose of 300 mg/kg. It may be due to the steric hindrance offered by the ortho hydroxyl group on aryl ring, which can result in poor interaction of pharmacophore with the receptor. Similarly, 2,4-dihydroxyphenyl derivative **4q** does not show any activity. Based on these observations, it can be concluded that a p-hydroxyphenyl group attached to hydrazone linkage is an activity enhancing moiety. Further, it is observed that presence of an electron donating methoxy group adjacent to hydroxy functionality (as in **4j**) does not influence on the activity of **4e**. However, replacement of methoxy by ethoxy as in **4h** causes loss of activity. This could be probably due to steric effect exerted by the bulky ethoxy group. Also, a hydroxynaphthalene derivative **4p** show activity at a dose of 100 mg/kg, confirming the importance of hydroxyl group in enhancing the activity.

It is also observed that the presences of electron rich aryl systems like 4-methyl phenyl (**4i**), indole (**4k, 5c**) display activity at 4 hours at a dose of 300 mg/kg. Interestingly, 4-fluorophenyl derivative **5f** exhibit significant activity at 100 mg/kg dose. However, replacement of fluoro by chloro as in **5h** makes it less potent (300 mg/kg) while the bromo derivative **5d** is found to be inactive. Thus, as the size of halogen increases, their anticonvulsant activity follows in the reverse order. Moreover, the active samples show significant activity only in MES method but not in scPTZ mode, at all tested doses. This clearly shows that new DHP derivatives are capable of preventing seizure spread effectively. Finally, based on the activity profile, selected active compounds, viz. **4e**, **4j**, **4p**, **5c** and **5f** were subjected to 6 Hz screening study at a dose of 100 mg/kg. All these tested compounds except **5c** exhibit activity in 6 Hz method within one hour.

The Rotarod toxicity measurement study is an important route for the establishment of toxicity profile of a newly synthesized compound. In our study, all the final compounds were screened for their toxicity study by taking 30, 100 and 300 mg/kg doses. Interestingly, none of the tested compounds displayed neurotoxicity up to 300 mg/kg. This clearly shows that incorporation of hydrazone pharmacophore into dihydropyridine scaffold will not induce toxicity on the resulting molecules. Thus, the active derivatives of the present series can be concluded as non-toxic antiepileptic agents.

Table 1: Anticonvulsant and toxicity screening results of active compounds

Table 2: Anticonvulsant activity of selected samples in 6 Hz screening method

2.2.2 Anti-inflammatory and analgesic studies

The results of anti-inflammatory activity (Table 3) clearly indicated that almost all newly synthesized final compounds are quite active when compared to standard. It is observed that DHP derivatives with electron donating substituents on aryl ring showed better medicinal efficacy than those with electron withdrawing substituents. Interestingly, compounds **4f**, **4p**, **4q** and **4r** with hydroxyl substituent, **4k** and **5c** carrying indole ring, **4l** and **5b** involving thiophene system, **4s** and **5f** with halogen substituents exhibited good activity. Further, compounds **4k-l, 4p-s**, and **5c** displayed very good anti-inflammatory activity even better than that of standard drug diclofenac sodium. It is exciting to note that these compounds showed superior activity soon after injection of Carrageenan irritant, i.e. within half an hour.

Amongst the hydroxyl substituted derivatives, 2-hydroxy compound **4f** exhibited more activity than 4-hydroxy analogue **4e**. Here the increased activity is probably due to the crucial role played by o-hydroxybenzene moiety, which would involve in chelation with ions along with hydrazone group at the vicinity of receptor [39]. Further, **4q** with two hydroxyl groups displayed the highest activity which may be attributed to the fact that the distance between two hydroxyl groups is comparable with the distance between two functional groups of salicylic acid and acetyl salicylate (Aspirin), so that it can bind to the same receptor site to equal extent [40]. An interesting observation is that compound **4j** carrying electron donating methoxy group adjacent to hydroxyl moiety showed better activity than compound **4e**, while ethoxy substituted compound **4h** displayed less activity, which may be due to steric effect played by the bulky group. This was further confirmed by the result of compound **4d**, where presence of two methoxy groups offered low activity [41].

The influence of halogen attached to aryl group on biological activity is noteworthy [42]. The results clearly indicate that compounds **4r, 4s** and **5f** with bromo and fluoro substituents showed excellent activity, while **5d**, **5f**, and **5h** carrying bromo, fluoro and chloro groups, respectively displayed the activity in the decreasing order, **5f** > **5h** > **5d**. The variation in the activity may be owing to difference in the size of halogens. But, presence of nitro group as in **4c** and **5a** reduced the activity to a considerable extent. Despite the presence of nitro substituent, molecule **4o** showed excellent activity, which may be because of the presence of electron donating furan ring.

Results of analgesic studies (Table 4) of five potent anti-inflammatory agents, viz. **4e**, **4f**, **4k**, **4q**, **5c** revealed that compound **4f** with 2-hydroxy substituent showed better activity than **4e**, containing 4-hydroxy analogue. Compound **4q** with 2,4-dihydroxy substituent and **5c** with indole ring demonstrated excellent analgesic property. In conclusion, compounds **4q** and **5c** have emerged as lead compounds both in the anti-inflammatory and analgesic studies.

Table 3: Anti-inflammatory activity data of compounds **4a-s** and **5a-h**

Table 4: Analgesic activity data of selected hydrazones

3. Conclusion

In the present work, twenty seven new DHP derivatives were designed, synthesized and characterized using spectral and elemental analyses. Their *in vivo* anticonvulsant, anti-inflammatory and analgesic properties were investigated by standard methodologies. DHPs carrying electron rich aryl groups exhibited moderate antiepileptic activity. Interestingly, compounds **4k**, **5c**, **4l**, **4p**, **4q**, **4r**, **4s**, and **5f** containing indole, thiophene, arylhydroxy, haloaryl as active groups displayed potential anti-inflammatory activity. Particularly, compounds **4k-l, 4p-s**, and **5c** showed rapid anti-inflammatory activity within 30 minutes. Active anti-inflammatory agents **4e**, **4f**, **4k**, **4q** and **5c** were further studied for their *in vivo* analgesic property, wherein compounds **4q** and **5c** containing hydroxylphenyl and indole moieties exhibited prominent activity. The neurotoxicity study clearly revealed that all the tested compounds are non-toxic up to 300 mg/kg and so, new compounds can be considered as good templates for further developmental studies.

4. Experimental

4.1 Chemistry

All the chemicals used in the present work procured from Sigma Aldrich and Lanchaster (UK). All the solvents are of analytical grade. They were purchased and used as such without any further purification. The progress of the reaction was monitored by thin layer chromatography, performed on a Silica gel 60 F254 coated aluminium sheet. Melting points were determined on open capillaries using a Stuart SMP3 (BIBBY STERLIN Ltd. UK) apparatus. Infrared spectra of all intermediate compounds and final molecules were recorded on a Nicolet Avatar 5700 FTIR (Thermo Electron Corporation). ^{1}H NMR and ^{13}C NMR spectra were obtained with Bruker-400 MHz and 100 MHz FT-NMR spectrometers, respectively using TMS as internal reference and DMSO-d_6 as solvent. Elemental analyses

were performed on a Flash EA1112 CHNS analyzer (Thermo Electron Corporation). Mass spectra were recorded on LC-MSD-Trap-XCT_Plus Mass Spectrometer.

4.1.1 *Procedure for synthesis of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**1**): A mixture of 4-hydroxybenzaldehyde (2 g, 16.3 mmol) ethyl acetoacetate (2.1 mL, 32.7 mmol) and ammonium acetate (1.9 g, 24.5 mmol) in 20 mL of ethanol was refluxed for 12 hrs. The reaction mixture was cooled on ice bath and solid separated was filtered, washed with ethanol and dried under vacuum. The product was recrystallized using ethanol/DMF mixture. Yield 60%, m.p. 239-241 ^{o}C. FTIR (ATR, cm^{-1}): 3337, 2980, 1656, 1221. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.09 (s, 1H), 8.77 (s, 1H), 6.93 (d, 2H, *J*=12 Hz), 6.58 (d, 2H, *J*=12 Hz), 4.74 (s, 1H), 3.96 (m, 4H), 2.23 (s, 6H), 1.13 (t, 6H, 8.0 Hz). MS (m/z): 346.4. Anal. Calcd. for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 65.94; H, 6.69; N, 4.05.

4.1.2 *Procedure for synthesis of diethyl 4-(4-(2-ethoxy-2-oxoethoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**2**): To a clear solution of **1** (3 g, 8.7 mmol) in 30 mL of DMF, ethyl chloroacetate (1.1 mL, 9.01 mmol) and K_2CO_3 (2.4 g, 17.3 mmol) were added with stirring. The reaction mixture was stirred at 80 °C for 20 hrs under nitrogen and cooled to room temperature. It was then quenched to ice cold water with stirring. Resulting solid was filtered, washed with water and dried. This was recrystallized with hot ethanol. Yield 80%, m.p. 105-107 ^{o}C. FTIR (ATR, cm^{-1}): 3351, 2979, 2905, 1739, 1686, 1221. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.73 (s, 1H), 7.03 (d, 2H, *J*=6.8 Hz), 6.73 (d, 2H, *J*=6.8 Hz), 4.78 (s, 1H), 4.66 (s, 2H), 4.13 (m, 2H), 3.96 (m, 4H), 2.23 (s, 6H), 1.18 (t, 3H, *J*=7.2 Hz), 1.12 (t, 3H, *J*=6.8 Hz). MS (m/z): 432.2. Anal. Calcd. for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.77; N, 3.25. Found: C, 63.88; H, 6.74; N, 3.23.

4.1.3 *Procedure for synthesis of 2-(4-(3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy) acetic acid hydrazide* (**3**): To a clear solution of **2** (2.5 g, 5.8 mmol) in ethanol (25 mL), hydrazine hydrate (0.5 mL, 10 mmol) was added and the mixture was refluxed for 4 hrs. It was then cooled to room temperature to get crude hydrazide **3**. The resulting solid was filtered, washed with ethanol and recrystallized from ethanol/DMF mixture. Yield 90%, m.p. 191-193 ^{o}C. FTIR (ATR, cm^{-1}): 3348, 3228, 3224, 3099, 2978, 1672, 1656, 1203. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.24 (s, 1H), 8.73 (s, 1H), 7.03 (d, 2H, *J*=8.8 Hz), 6.76 (d, 2H, *J*=8.8 Hz), 4.78 (s, 1H), 4.39 (s, 2H), 4.28 (s, 2H), 3.97 (m, 4H),

2.23 (s, 6H), 1.12 (t, 6H, *J*=7.0 Hz). MS (m/z): 418.1. Anal. Calcd. for $C_{21}H_{27}N_3O_6$. C, 60.42; H, 6.52; N, 10.07. Found: C, 60.32; H, 6.50; N, 10.05.

4.1.4 *General procedure for synthesis of hydrazones* (**4a-s, 5a-h**): To a clear solution of **3** (0.5 g, 1.2 mmol) in ethanol (10 mL), 1.2 mmol of aldehyde / ketone was added and refluxed for 8 hrs. Catalytic amount (0.2 mL) of glacial acetic acid was used for the synthesis of **5a-h**. Resulting solid was filtered, washed with ethanol and finally recrystallized with methanol/chloroform mixture. The characterization and spectral data of final compounds are given below.

4.1.4.1 *Diethyl 4-(N'-(3,4,5-trimethoxybenzylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4a**): Yield 88%, m.p. 212-214 ^oC. FTIR (ATR, cm^{-1}): 3352, 3000, 2941, 1663, 1620, 1492, 1211. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.47 (s, 1H, CONH), 8.72 (s, 1H, NH), 7.89 (s, 1H, CH), 7.07-6.72 (m, 6H, ArH), 5.06 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 3.79 (s, 6H, OCH_3), 3.68 (s, 3H, OCH_3), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=8.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.2, 168.3, 157.3, 148.8, 145.1, 136.4, 131.4, 126.8, 125.4, 119.7, 116.5, 112.2, 102.4, 68.7, 62.5, 56.7, 43.3, 18.4, 16.3. MS (m/z) 596.1. Anal. Calcd. for $C_{31}H_{37}N_3O_9$: C, 62.51; H, 6.26; N, 7.05. Found: C, 62.40; H, 6.23; N, 7.02.

4.1.4.2 *Diethyl 4-(N'-(4-chloro benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4b**): Yield 90%, m.p. 246-248 ^oC. FTIR (ATR, cm^{-1}): 3285, 3231, 3058, 2957, 1660, 1610, 1217. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.2 (s, 1H, CONH), 8.73 (s, 1H, NH), 7.97 (s, 1H, CH), 7.71 (d, 2H, ArH, *J*=7.2 Hz), 7.50 (d, 2H, ArH, *J*=7.2 Hz), 7.06 (d, 2H, ArH, *J*=8.8 Hz), 6.81 (d, 2H, ArH, *J*=8.8 Hz), 5.09 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.33 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=8.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.4, 163.4, 158.7, 145.3, 136.7, 134.6, 132.0, 127.1, 123.2, 119.1, 116.5, 112.4, 101.9, 68.7, 62.9, 43.2, 18.1, 16.5. MS (m/z) 540.1. Anal. Calcd. for $C_{28}H_{30}ClN_3O_6$: C, 62.28; H, 5.60; N, 7.78. Found: C, 62.21; H, 5.58; N, 7.75.

4.1.4.3 *Diethyl 4-(N'-(4-nitro benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4c**): Yield 91%, m.p. 244-246 ^oC. FTIR (ATR, cm^{-1}): 3309, 3237, 1667, 1608, 1224. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.80 (s, 1H, CONH), 8.73 (s, 1H, NH), 8.09 (s, 1H, CH), 8.28-6.75 (m, 8H, ArH), 5.09 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=7.8 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.2, 163.7, 161.4, 146.7, 135.7, 134.2, 132.4, 127.3, 123.4,

118.5, 116.5, 112.9, 101.9, 68.7, 63.1, 43.2, 18.1, 16.5. MS (m/z) 551.2. Anal. Calcd. for $C_{28}H_{30}N_4O_8$. C, 61.08; H, 5.49; N, 10.18. Found: C, 60.97; H, 5.49; N, 10.14.

4.1.4.4 *Diethyl 4-(N'-(3,4-dimethoxy benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4d**): Yield 92%, m.p. 213-215 ^oC. FTIR (ATR, cm^{-1}): 3230, 3094, 2969, 1667, 1612, 1496, 1221, 1122. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.35 (s, 1H, CONH), 8.72 (s, 1H, NH), 7.95 (s, 1H, CH), 7.29-6.72 (m, 7H, ArH), 5.04 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 3.78 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=7.6 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 172.4, 165.2, 161.4, 158.4, 146.5, 134.7, 131.8, 123.4, 120.4, 118.5, 117.2, 102.8, 69.1, 62.3, 58.4, 43.2, 18.2, 16.5. MS (m/z) 566.6. Anal. Calcd. for $C_{30}H_{35}N_3O_8$. C, 63.70; H, 6.24; N, 7.43. Found: C, 63.57; H, 6.24; N, 7.41.

4.1.4.5 *Diethyl 4-(N'-(4-hydroxy benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4e**): Yield 87%, m.p. 237-239 ^oC. FTIR (ATR, cm^{-1}): 3384, 3282, 3224, 1662, 1609, 1222. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.28 (s, 1H, CONH), 9.44 (s, 1H, NH), 8.72 (s, 1H, CH), 7.86 (s, 1H, OH), 7.25-6.71 (m, 8H, ArH), 5.02 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=8.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 170.4, 164.2, 161.4, 158.4, 146.8, 134.7, 132.4, 127.3, 123.4, 118.5, 117.2, 102.4, 69.1, 62.8, 43.2, 18.2, 16.5. MS (m/z) 522.3. Anal. Calcd. for $C_{28}H_{31}N_3O_7$. C, 64.48; H, 5.99; N, 8.06. Found: C, 64.39; H, 5.95; N, 8.02.

4.1.4.6 *Diethyl 4-(N'-(2-hydroxy phenyl)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4f**): Yield 92%, m.p. 270-272 ^oC. FTIR (ATR, cm^{-1}): 3354, 3280, 3225, 1657, 1614, 1218. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.04 (s, 1H, CONH), 8.73 (s, 1H, NH), 8.53 (s, 1H, OH), 8.28 (s, 1H, CH), 7.68-6.73 (m, 8H, ArH), 5.01 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=8.8 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 168.7, 166.9, 157.3, 148.3, 145.0, 136.3, 131.1, 126.4, 119.3, 116.2, 113.7, 101.9, 66.5, 58.9, 43.2, 18.1, 14.1. MS (m/z) 522.2. Anal. Calcd. for $C_{28}H_{31}N_3O_7$. C, 64.48; H, 5.99; N, 8.06. Found: C, 64.40; H, 5.92; N, 8.03.

4.1.4.7 *Diethyl 4-(N'-(4-dimethylamino benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4g**): Yield 81%, m.p. 217-219 ^oC. FTIR (ATR, cm^{-1}): 3286, 3228, 1663, 1605, 1222. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.15 (s, 1H, CONH), 8.72 (s, 1H, NH), 7.85 (s, 1H, CH), 7.49-6.70 (m, 8H, ArH), 4.99 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.95 (s, 6H, CH_3), 2.33 (s, 6H, CH_3), 1.13 (t, 6H, CH_3,

J=8.8 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 171.4, 164.5, 158.9, 151.3, 146.7, 137.6, 133.2, 132.7, 127.3, 123.5, 118.9, 116.4, 112.9, 101.9, 68.7, 63.3, 43.8, 42.7, 37.8, 18.1, 16.6. MS (m/z) 549.2. Anal. Calcd. for $C_{30}H_{36}N_4O_6$. C, 65.68; H, 6.61; N, 10.21. Found: C, 65.54; H, 6.62; N, 10.17.

4.1.4.8 *Diethyl 4-(N'-(4-hydroxy-3-ethoxy benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4h**): Yield 86%, m.p. 207-209 ^{o}C. FTIR (ATR, cm^{-1}): 3308, 3226, 1675, 1602, 1212. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.27 (s, 1H, CONH), 9.37 (s, 1H, OH), 8.72 (s, 1H, NH), 7.85 (s, 1H, CH), 7.23-6.79 (m, 7H, ArH), 5.02 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 4.00 (m, 6H, OCH_2), 2.32 (s, 6H, CH_3), 1.33 (t, 3H, CH_3, *J*=7.2 Hz), 1.12 (t, 6H, CH_3, *J*=9.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.1, 163.8, 158.7, 151.2, 149.2, 138.6, 134.7, 127.3, 124.6, 123.4, 118.5, 117.2, 113.4, 102.4, 69.1, 64.3, 62.8, 43.2, 18.2, 16.5, 14.7. MS (m/z) 566.2. Anal. Calcd. for $C_{30}H_{35}N_3O_8$. C, 63.70; H, 6.24; N, 7.43. Found: C, 63.56; H, 6.21; N, 7.41.

4.1.4.9 *Diethyl 4-(N'-(4-methyl benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4i**): Yield 90%, m.p. 251-253 ^{o}C. FTIR (ATR, cm^{-1}): 3291, 3232, 3099, 2997, 1662, 1612, 1219. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.41 (s, 1H, CONH), 8.73 (s, 1H, NH), 7.95 (s, 1H, CH), 7.57-6.72 (m, 8H, ArH), 5.03 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.32 (s, 3H, CH_3), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=9.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.5, 163.2, 158.9, 151.8, 142.9, 139.6, 137.6, 132.4, 130.7, 128.9, 122.3, 116.4, 101.9, 69.3, 64.2, 43.7, 18.1, 16.2. MS (m/z) 520.2. Anal. Calcd. for $C_{29}H_{33}N_3O_6$. C, 67.04; H, 6.40; N, 8.09. Found: C, 66.92; H, 6.39; N, 8.02.

4.1.4.10 *Diethyl 4-(N'-(4-hydroxy-3-methoxy benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4j**): Yield 85%, m.p. 213-215 ^{o}C. FTIR (ATR, cm^{-1}): 3459, 3318, 3247, 1676, 1612, 1217. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.28 (s, 1H, CONH), 9.44 (s, 1H, OH), 8.72 (s, 1H, NH), 7.86 (s, 1H, CH), 7.25-6.71 (m, 7H, ArH), 5.02 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 3.77 (s, 3H, OCH_3), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=9.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 171.2, 163.4, 158.5, 152.5, 147.3, 142.7, 136.7, 132.9, 128.7, 123.2, 121.3, 118.5, 116.6, 113.1, 101.9, 68.7, 62.9, 56.7, 43.3, 18.1, 16.5. MS (m/z) 552.3. Anal. Calcd. for $C_{29}H_{33}N_3O_8$. C, 63.15; H, 6.03; N, 7.62. Found: C, 63.03; H, 5.96; N, 7.57.

4.1.4.11 *Diethyl 4-(N'-(1H-indol-3-yl)methylene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4k**): Yield 88%, m.p. 231-233 ^{o}C. FTIR (ATR, cm^{-1}): 3259, 3201, 3089, 2977, 1675, 1610, 1216. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.52 (s, 1H, CONH), 11.12 (s, 1H, NH), 8.72 (s, 1H, NH), 8.04 (s, 1H, CH), 7.78-6.82 (m, 9H, ArH), 5.07 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=9.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.3, 167.5, 159.8, 152.5, 142.3, 138.4, 132.2, 130.5, 128.7, 125.7, 122.1, 120.9, 118.6, 116.5, 114.7, 112.1, 106.4, 102.1, 67.1, 61.8, 42.0, 18.1, 14.3. MS (m/z) 545.6. Anal. Calcd. for $C_{30}H_{32}N_4O_6$. C, 66.16; H, 5.92; N, 10.29. Found: C, 66.02; H, 5.90; N, 10.25.

4.1.4.12 *Diethyl 4-(N'-(thiophen-2-yl methylene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4l**): Yield 83%, m.p. 261-263 ^{o}C. FTIR (ATR, cm^{-1}): 3303, 3218, 3067, 2977, 1671, 1641, 1216. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.14 (s, 1H, CONH), 8.73 (s, 1H, NH), 8.16 (s, 1H, CH), 7.65-6.70 (m, 7H, ArH), 4.95 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.33 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*= 9.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 168.4, 164.5, 158.2, 142.3, 140.3, 138.4, 132.2, 130.7, 128.1, 126.3, 124.5, 123.7, 122.1, 120.9, 117.7, 114.3, 102.3, 67.8, 62.1, 42.3, 18.1, 14.5. MS (m/z) 512.6. Anal. Calcd. for $C_{26}H_{29}N_3O_6S$. C, 61.04; H, 5.71; N, 8.21. Found: C, 60.92; H, 5.68; N, 8.22.

4.1.4.13 *Diethyl 4-(N'-(pyridin-4-ylmethylene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4m**): Yield 94%, m.p. 216-218 ^{o}C. FTIR (ATR, cm^{-1}): 3301, 3259, 2975, 1665, 1607, 1222. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.76 (s, 1H, CONH), 8.73 (s, 1H, NH), 7.96 (s, 1H, CH), 7.65-6.74 (m, 8H, ArH), 5.07 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=9.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.6, 162.3, 157.8, 147.6, 146.7, 141.3, 140.3, 132.2, 131.3, 125.7, 121.5, 117.7, 114.3, 102.1, 67.8, 62.1, 42.5, 18.1, 14.3. MS (m/z) 507.2. Anal. Calcd. for $C_{27}H_{30}N_4O_6$. C, 64.02; H, 5.97; N, 11.06. Found: C, 63.89; H, 5.98; N, 11.02.

4.1.4.14 *Diethyl 4-(N'-((5-methylthiophen-2-yl)methylene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4n**): Yield 88%, m.p. 261-263 ^{o}C. FTIR (ATR, cm^{-1}): 3291, 3231, 3027, 2975, 1665, 1605, 1219. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.34 (s, 1H, CONH), 8.72 (s, 1H, NH), 8.06 (s, 1H, CH), 7.22-6.69 (m, 6H, ArH), 4.91 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.44 (s, 3H, CH_3), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=9.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 166.8, 163.1,

158.7, 141.3, 140.3, 138.6, 131.3, 128.6, 125.7, 116.4, 114.2, 102.4, 67.8, 63.1, 41.8, 18.1, 15.2, 14.3. MS (m/z) 526.2. Anal. Calcd. for $C_{27}H_{31}N_3O_6S$. C, 61.70; H, 5.94; N, 7.99. Found: C, 61.55; H, 5.89; N, 7.97.

4.1.4.15 *Diethyl (N'- 4-((5-nitrofuran-2-yl)methylene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4o**): Yield 84%, m.p. 231-233 ^{o}C. FTIR (ATR, cm^{-1}): 3301, 3234, 3065, 2974, 1674, 1611, 1213. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.89 (s, 1H, CONH), 8.73 (s, 1H, NH), 7.94 (s, 1H, CH), 7.78-6.72 (m, 6H, ArH), 5.02 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=9.8 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 173.1, 164.5, 158.5, 154.3, 152.7, 136.7, 135.4, 132.9, 123.2, 121.3, 118.8, 116.4, 101.8, 68.7, 63.1, 43.1, 18.2, 16.5. MS (m/z) 541.2. Anal. Calcd. for $C_{26}H_{28}N_4O_9$. C, 57.77; H, 5.22; N, 10.37. Found: C, 57.62; H, 5.19; N, 10.39.

4.1.4.16 *Diethyl (N'-4-((1-hydroxynaphthalen-2-yl)methylene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4p**): Yield 88%, m.p. 260-262 ^{o}C. FTIR (ATR, cm^{-1}): 3306, 3263, 3058, 2978, 1664, 1617, 1219. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.78 (s, 1H, CONH), 9.41 (s, 1H, OH), 8.74 (s, 1H, NH), 8.19 (s, 1H, CH), 7.93-6.88 (m, 10H, ArH), 5.05 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=9.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 173.8, 164.8, 161.3, 158.5, 151.8, 143.2, 135.4, 132.7, 131.7, 126.4, 123.6, 121.3, 116.9, 114.7, 102.3, 68.7, 62.9, 42.8, 18.2, 16.6. MS (m/z) 572.1. Anal. Calcd. for $C_{32}H_{33}N_3O_7$. C, 67.24; H, 5.82; N, 7.35. Found: C, 67.10; H, 5.79; N, 7.29.

4.1.4.17 *Diethyl 4-(N'-(2,4-dihydroxy benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4q**): Yield 81%, m.p. 271-273 ^{o}C. FTIR (ATR, cm^{-1}): 3380, 3229, 3094, 2973, 1661, 1621, 1223. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.21 (s, 1H, CONH), 9.92 (s, 1H, OH), 8.71 (s, 1H, NH), 8.38 (s, 1H, OH), 8.15 (s, 1H, CH), 7.46-6.30 (m, 7H, ArH), 4.95 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=9.6 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.1, 165.4, 161.4, 158.3, 149.2, 133.6, 131.2, 128.2, 124.7, 122.4, 115.7, 113.7, 109.7, 102.0, 66.4, 59.8, 41.2, 18.2, 14.1. MS (m/z) 538.3. Anal. Calcd. for $C_{28}H_{31}N_3O_8$. C, 62.56; H, 5.81; N, 7.82. Found: C, 62.42; H, 5.76; N, 7.79.

4.1.4.18 *Diethyl 4-(N'-(4-bromo-2-hydroxybenzylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4r**): Yield 89%, m.p. 248-250 ^{o}C. FTIR

(ATR, cm^{-1}): 3325, 3272, 3013, 2976, 1671, 1612, 1212. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.06 (s, 1H, CONH), 10.32 (s, 1H, OH), 8.73 (s, 1H, NH), 8.21 (s, 1H, CH), 7.81-6.72 (m, 7H, ArH), 5.05 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=9.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 168.3, 164.6, 159.2, 149.5, 142.3, 133.6, 131.2, 125.7, 124.7, 123.2, 119.7, 115.7, 113.7, 109.7, 102.0, 66.4, 59.8, 41.2, 18.2, 14.1. MS (m/z) 599.8. Anal. Calcd. for $C_{28}H_{30}BrN_3O_7$. C, 56.01; H, 5.04; N, 7.00. Found: C, 55.86; H, 5.05; N, 6.96.

4.1.4.19 *Diethyl 4-(N'-(4-bromo benzylidine)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**4s**): Yield 90%, m.p. 235-237 ^{o}C. FTIR (ATR, cm^{-1}): 3307, 3272, 3058, 2957, 1667, 1610, 1228. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.55 (s, 1H, CONH), 8.73 (s, 1H, NH), 7.96 (s, 1H, CH), 7.65-6.73 (m, 8H, ArH), 5.04 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=9.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 173.8, 165.2, 158.5, 151.8, 142.9, 135.4, 132.7, 131,2, 126.4, 114.7, 102.3, 68.7, 62.9, 42.8, 18.2, 16.6. MS (m/z) 584.1. Anal. Calcd. for $C_{28}H_{30}BrN_3O_6$. C, 57.54; H, 5.17; N, 7.19. Found: C, 57.46; H, 5.15 N, 7.15.

4.1.4.20 *Diethyl 4-(-N'-(1-(4-nitrophenyl)ethylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**5a**): Yield 91%, m.p. 225-227 ^{o}C. FTIR (ATR, cm^{-1}): 3309, 3237, 1682, 1667, 1497, 1224. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.1 (s, 1H, CONH), 8.77 (s, 1H, NH), 8.24 (d, 2H, ArH, *J*=12.0 Hz), 8.06 (d, 2H, ArH, *J*=12.0 Hz), 7.06-6.78 (m, 4H, ArH), 5.14 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.31 (s, 3H, CH_3), 2.24 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=10.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.9, 166.9, 163.8, 161.4, 156.3, 147.2, 144.9, 140.6, 134.4, 128.3, 115.2, 113.7, 102.0, 65.0, 58.9, 37.8, 18.2, 14.1. 13.4. MS (m/z) 566.2. Anal. Calcd. for $C_{29}H_{32}N_4O_8$: C, 61.69; H, 5.71; N, 9.92. Found: C, 61.55; H, 5.67; N, 9.88.

4.1.4.21 *Diethyl 4-(N'-(1-(thiophen-2-yl)ethylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**5b**): Yield 83%, m.p. 218-220 ^{o}C. FTIR (ATR, cm^{-1}): 3294, 3094, 2969, 1689, 1652, 1206. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.81 (s, 1H, CONH), 8.72 (s, 1H, NH), 7.58-6.70 (m, 7H, ArH), 4.97 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.97 (m, 4H, CH_2), 2.26 (s, 3H, CH_3), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=7.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 168.2, 164.5, 160.2, 155.8, 136.7, 134.3, 133.2, 128.7, 123.5, 121.7, 117.4, 115.3, 102.1, 64.6, 59.8, 40.2, 18.1, 14.4. 13.3. MS (m/z) 526.3. Anal. Calcd. for $C_{27}H_{31}N_3O_6S$. C, 61.70; H, 5.94; N, 7.99. Found: C, 61.57; H, 5.92; N, 7.93.

4.1.4.22 *Diethyl 4-(N'-(1-(1H-indol-3-yl)ethylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**5c**): Yield 89%, m.p. 226-228 ^{o}C. FTIR (ATR, cm^{-1}): 3277, 3210, 3080, 2966, 1684, 1641, 1209. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.42 (s, 1H, CONH), 10.55 (s, 1H, NH), 8.72 (s, 1H, NH), 8.56-6.76 (m, 9H, ArH), 5.11 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.27 (s, 3H, CH_3), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=7.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 170.4, 167.3, 164.5, 156.5, 138.4, 136.8, 134.2, 128.7, 126.9, 124.1, 120.7, 114.2, 113.5, 102.1, 67.1, 59.3, 42.0, 18.1, 14.3. 13.7. MS (m/z) 559.1. Anal. Calcd. for $C_{31}H_{34}N_4O_6$. C, 66.65; H, 6.13; N, 10.03. Found: C, 66.51; H, 6.12; N, 10.04.

4.1.4.23 *Diethyl 4-(N'-(1-(4-bromophenyl)ethylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**5d**): Yield 90%, m.p. 144-146 ^{o}C. FTIR (ATR, cm^{-1}): 3295, 3237, 2976, 1687, 1648, 1202. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.84 (s, 1H, CONH), 8.72 (s, 1H, NH), 7.86-6.72 (m, 8H, ArH), 5.07 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.25 (s, 3H, CH_3), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=7.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.2, 166.7, 162.3, 158.9, 138.5, 136.1, 134.7, 131.4, 128.7, 126.9, 116.3, 114.2, 102.1, 67.1, 59.7, 42.1, 18.1, 14.4. 13.3. MS (m/z) 598.2. Anal. Calcd. for $C_{29}H_{32}BrN_3O_6$. C, 58.20; H, 5.39; N, 7.02. Found: C, 58.03; H, 5.39; N, 6.98.

4.1.4.24 *Diethyl 4-(N'-(1-phenylethylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-pihydro pyridine-3,5-dicarboxylate* (**5e**): Yield 87%, m.p. 215-217 ^{o}C. FTIR (ATR, cm^{-1}): 3296, 3218, 2976, 1684, 1618, 1208. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.78 (s, 1H, CONH), 8.72 (s, 1H, NH), 7.79-6.72 (m, 9H, ArH), 5.07 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.25 (s, 3H, CH_3), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=7.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.2, 166.4, 159.7, 135.4, 134.7, 132.9, 130.4, 123.5, 121.7, 118.9, 117.6, 116.4, 102.1, 67.4, 59.7, 41.1, 18.1, 14.4. 13.3. MS (m/z) 520.3. Anal. Calcd. for $C_{29}H_{33}N_3O_6$. C, 67.04; H, 6.40; N, 8.09. Found: C, 66.89; H, 6.36; N, 8.05.

4.1.4.25 *Diethyl 4-(N'-(1-(4-fluorophenyl)ethylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**5f**): Yield 91%, m.p. 191-193 ^{o}C. FTIR (ATR, cm^{-1}): 3342, 2976, 1693, 1642, 1207. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.79 (s, 1H, CONH), 8.72 (s, 1H, NH), 7.85-6.72 (m, 8H, ArH), 5.07 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.24 (s, 3H, CH_3), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=7.8 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.9, 166.8, 156.4, 147.2, 144.9, 140.9,

134.4, 128.3, 115.2, 113.7, 102.0, 65.3, 62.3, 38.2, 18.2, 14.1. 13.4. MS (m/z) 538.2. Anal. Calcd. for $C_{29}H_{32}FN_3O_6$. C, 64.79; H, 6.00; N, 7.82. Found: C, 64.63; H, 5.95; N, 7.79.

4.1.4.26 *Diethyl 4-(N'-(1-(furan-2-yl)ethylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydro pyridine-3,5-dicarboxylate* (**5g**): Yield 92%, m.p. 161-163 ^{o}C. FTIR (ATR, cm^{-1}): 3282, 2956, 1683, 1632, 1217. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.74 (s, 1H, CONH), 8.72 (s, 1H, NH), 7.67-6.57 (m, 7H, ArH), 4.99 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.27 (s, 3H, CH_3), 2.23 (s, 6H, CH_3), 1.13 (t, 6H, CH_3, *J*=7.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 168.2, 164.2, 156.4, 154.3, 147.2, 142.7, 141.8, 126.4, 124.8, 123.1, 116.2, 113.7, 102.2, 66.7, 60.4, 37.9, 17.9, 14.2. 13.4. MS (m/z) 510.4. Anal. Calcd. for $C_{27}H_{31}N_3O_7$. C, 63.64; H, 6.13; N, 8.25. Found: C, 63.52; H, 6.15; N, 8.23.

4.1.4.27 *Diethyl 4-(N'-(1-(4-chlorophenyl)ethylidene)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate* (**5h**): Yield 91%, m.p. 205-207 ^{o}C. FTIR (ATR, cm^{-1}): 3295, 3237, 2976, 1687, 1648, 1202. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.84 (s, 1H, CONH), 8.72 (s, 1H, NH), 7.86-6.72 (m, 8H, ArH), 5.07 (s, 1H, CH), 4.78 (s, 2H, OCH_2), 3.98 (m, 4H, CH_2), 2.25 (s, 3H, CH_3), 2.23 (s, 6H, CH_3), 1.12 (t, 6H, CH_3, *J*=7.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.2, 166.4, 162.3, 160.2, 158.7, 135.1, 134.2, 132.6, 123.4, 121.5, 118.6, 117.3, 116.3, 102.1, 67.4, 59.7, 42.1, 18.1, 14.4. 13.3. MS (m/z) 554.2. Anal. Calcd. for $C_{29}H_{32}ClN_3O_6$. C, 62.87; H, 5.82; N, 7.58. Found: C, 62.71; H, 5.80; N, 7.56.

4.2. Pharmacology

4.2.1 Anticonvulsant study

4.2.1.1 Maximal Electroshock Seizure test

Anticonvulsant evaluations were performed by the Anticonvulsant Screening Program at the Epilepsy Branch of the National Institutes of Neurological Disorders, and Stroke (NINDS) in Rockville, USA. Groups of six to ten mice (20–40 g each) were used to test the samples by MES method using phenytoin as a standard drug. An electrical stimulus of 0.2 s in duration (50 mA at 60 Hz) was delivered via corneal electrodes, which was primed with an electrolytic solution containing anaesthetic agent. Animals were tested at 0.5 and 4 hour after i.p. injection of 30, 100 and 300 mg/kg doses. Disappearance of the hindlimb tonic extensor was used as positive criterion.

4.2.1.2 Subcutoneous pentylene tetrazole (scPTZ) test

Similar to MES method, a group of six to ten mice were taken for scPTZ screening by using sodium valproate as a standard drug. Animals were pre-treated with various doses of the test compound. A standard dose of 85 mg/kg of Metrazol (Tetrazole) was injected subcutoneously. These animals were placed in isolation cages to minimize stress and observed for the next 30 min to see the absence of a seizure. Failure to observe any seizure in this duration was defined as protection.

4.2.1.3 Minimal clonic seizure (6 Hz) test

In this method, test compounds (100 mg/kg) were pre-administered to mice via i.p. injection. At varying times, individual mice (four mice per time point) were challenged with sufficient current delivered through corneal electrodes to elicit a psychomotor seizure in 97% of animals (32 mA for 3 sec). Untreated mice would display seizures characterized by a minimal clonic phase followed by stereotyped, automatistic behaviours described originally as being similar to the aura of human patients with partial seizures. Animals not displaying this behaviour were considered as protected.

4.2.1.4 Neurotoxicity study

Rotarod test was performed to detect the motor deficit in mice. Animals were divided in groups of 4 animals and trained to stay on an accelerating rotarod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Trained animals (able to stay on the rotarod for at least two consecutive periods of 90 s) were given an i.p injection of the test compounds at doses of 30, 100 and 300 mg/kg. Neurological deficit was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. The dose at which animal fell off the rod, was determined.

4.2.2. Anti-inflammatory study

Newly synthesized title compounds were screened for their *in vivo* anti-inflammatory properties following Carrageenan induced Paw Edema method [43] by using Diclophenac sodium as standard drug. Male or female Wistar strain rats with a body weight between 100 and 150 g were used. The animals were weighed and divided into different groups (control, standard and the test groups) of five rats each. The animals were starved overnight. To insure uniform hydration, the rats received 5 mL of water by stomach tube (controls) or the test drug (20 mg/kg) dissolved or suspended in the same volume. Thirty minutes later, the rats were given a subcutaneous injection of 0.05 mL of 1% solution of Carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured

plethysmographically immediately after injection, after 30, 60, 120 and 180 min. Results were expressed as mean, by one way ANOVA analysis followed by Dunnet's-t-test.

4.2.3 Analgesic study

The analgesic study of active target compounds was done by Tail immersion method [44]. The animals were weighed and divided into different groups (control, standard and the test groups) of five rats each. Young female Wistar rats (170–210 g body weight) were used for the study. They were placed into individual restraining cages leaving the tail hanging out freely. The animals were allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water of exactly 55 ℃. Within a few seconds the rat reacts by withdrawing the tail. The reaction time was recorded in 0.5 sec units by a stopwatch. After each determination the tail was carefully dried. The reaction time was determined before and periodically after either oral or subcutaneous administration of the test substance, e.g. after 0, 30, 60 and 90 mins. The cut off time of the immersion was 15 sec. The withdrawal time of untreated animals was between 1 and 5.5 sec. A withdrawal time of more than 6 sec therefore was regarded as a positive response.

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Figure captions

Table 1: Anticonvulsant screening results of active compounds.

Sample	R	MES[a]	
		0.5 h	4.0 h
4e	4-Hydroxyphenyl	-	100
4i	p-Tolyl	-	300
4j	Vanilinyl	-	100
4k	3-Indolyl	-	300
4p	1-Hydroxy-naphthalein-2-yl	-	100
5c	Indole-3-yl	-	300
5f	4-Fluorophenyl	-	100
5h	4-Chlorophenyl	-	300
Phenytoin	-	30	30
Sodium valproate	-	x	x

[a]Doses of 30, 100, 300 mg/kg of the compounds were administered and the protection was measured after 0.5 and 4.0 hours. The figures indicate the minimal concentration of sample required to cause protection in at least 50% of animals. The dash (-) indicates the absence of activity, while (x) denotes not tested.

Table 2: Anticonvulsant activity of selected samples in 6 Hz screening method.[a]

Compound name	Time (hours)				
	0.25	0.5	1.0	2.0	4.0
4e	0/4	1/4	0/4	0/4	0/4
4j	0/4	1/4	1/4	0/4	0/4
4p	1/4	0/4	2/4	0/4	0/4
5c	0/4	0/4	0/4	0/4	0/4
5f	0/4	0/4	2/4	0/4	0/4

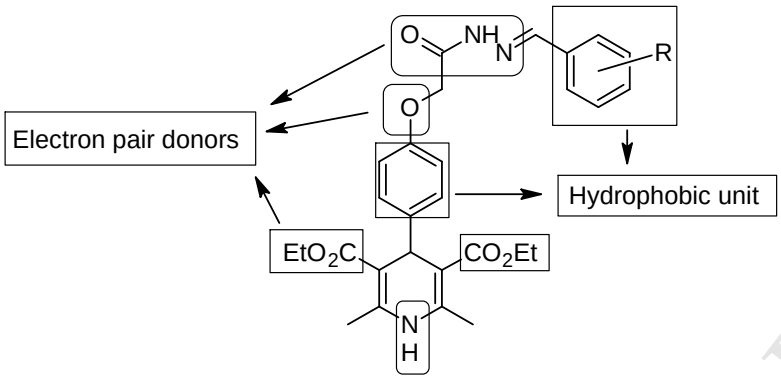
[a]A dose of 100 mg/kg was used. Anticonvulsant screening was done after 0.25, 0.5, 1.0, 2.0 and 4.0 hour against 4 mice each. The data (a/b) indicates the number of animals displayed activity against number of tested animals.

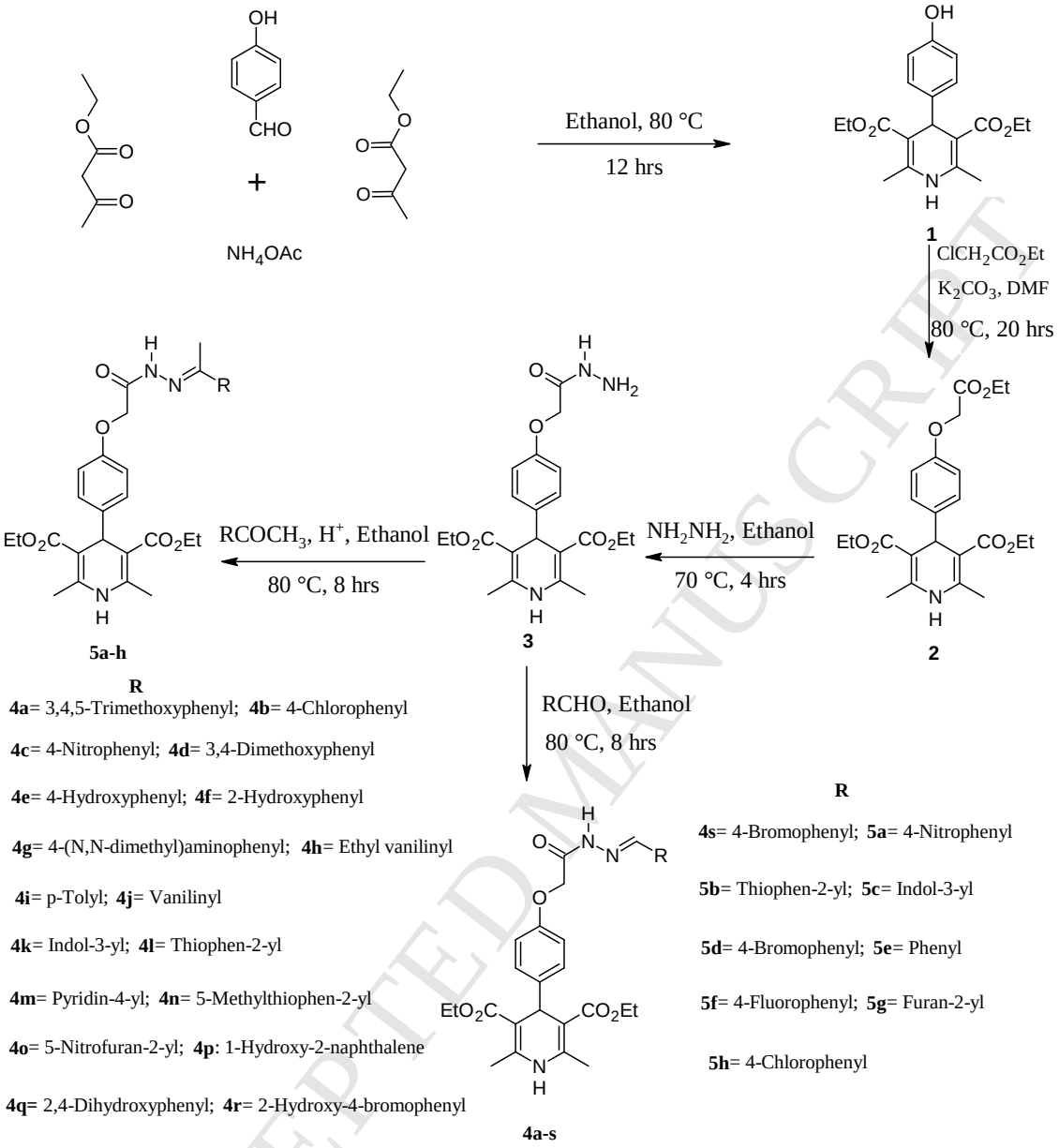
Table 3: Anti-inflammatory activity data of compounds **4a-s** and **5a-h**

Compounds	Percentage Inhibition			
	1/2h	1 h	2 h	3 h
Diclophenac	16.67	41.03	61.11	65.26
4a	12.88	42.31	52.78	53.29
4b	10.61	39.74	57.22	56.28
4c	11.36	15.38	29.44	49.10
4d	8.333	16.03	8.889	31.13
4e	18.18	39.1	53.89	55.09
4f	31.82	46.79	57.22	58.08
4g	15.91	26.28	47.78	53.89
4h	13.64	32.69	46.11	48.50
4i	9.091	29.49	44.44	44.91
4j	34.09	42.31	57.22	59.28
4k	43.18	49.36	58.89	60.47
4l	43.18	51.28	61.67	61.07
4m	16.67	35.9	46.67	47.30
4n	37.12	48.08	57.22	56.28
4o	43.94	55.13	62.22	61.07
4p	43.94	53.21	62.22	61.07
4q	51.52	60.9	68.89	66.46
4r	50	58.97	65.56	64.67
4s	50	58.33	67.78	66.46
5a	13.64	33.33	47.78	52.69
5b	42.42	49.36	59.44	57.48
5c	47.73	54.49	64.44	65.86
5d	37.12	45.51	58.89	56.29
5e	34.85	42.95	57.78	56.88
5f	42.42	54.49	61.67	61.078
5g	39.39	48.08	62.78	67.06
5h	38.64	46.79	58.89	56.88

Table 4: Analgesic activity data of selected hydrazones

Compound	Tail flick latency in secs			
	0 min	30 min	60 min	90 min
Control	3.35±0.028	3.23±0.005	3.34±0.005	3.25±0.005
Standard	3.23±0.017	6.25±0.02	6.95±0.02	7.45±0.02
4e	3.17±0.017	4.56±0.008	5.73±0.020	6.8±0.017
4f	3.25±0.028	4.92±0.005	6.1±0.057	6.9±0.028
4k	3.25±0.005	4.99±0.101	6.11±0.063	6.92±0.014
4q	3.23±0.033	5.41±0.005	6.30±0.018	7.23±0.020
5c	3.24±0.003	5.15±0.028	5.91±0.023	7.12±0.012





R

4a= 3,4,5-Trimethoxyphenyl; **4b**= 4-Chlorophenyl

4c= 4-Nitrophenyl; **4d**= 3,4-Dimethoxyphenyl

4e= 4-Hydroxyphenyl; **4f**= 2-Hydroxyphenyl

4g= 4-(N,N-dimethyl)aminophenyl; **4h**= Ethyl vanilinyl

4i= p-Tolyl; **4j**= Vanilinyl

4k= Indol-3-yl; **4l**= Thiophen-2-yl

4m= Pyridin-4-yl; **4n**= 5-Methylthiophen-2-yl

4o= 5-Nitrofuran-2-yl; **4p**: 1-Hydroxy-2-naphthalene

4q= 2,4-Dihydroxyphenyl; **4r**= 2-Hydroxy-4-bromophenyl

R

4s= 4-Bromophenyl; **5a**= 4-Nitrophenyl

5b= Thiophen-2-yl; **5c**= Indol-3-yl

5d= 4-Bromophenyl; **5e**= Phenyl

5f= 4-Fluorophenyl; **5g**= Furan-2-yl

5h= 4-Chlorophenyl

Highlights:

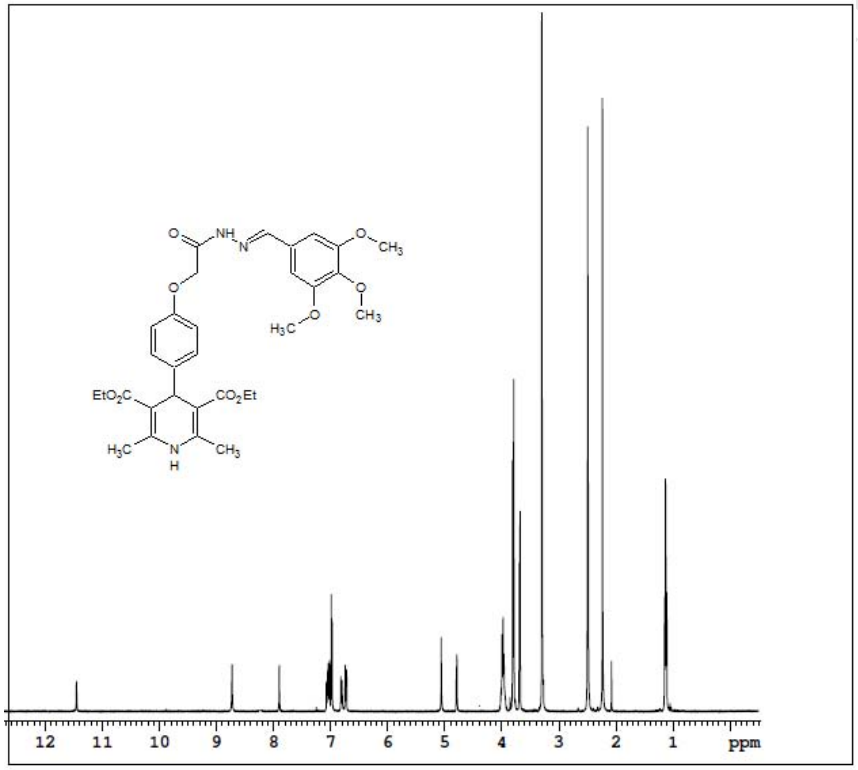
- New dihydropyridines were synthesized and characterized by spectral techniques.
- Target compounds were screened for their biological studies.
- DHPs carrying electron donating groups exhibited good antiepileptic activity.
- Few derivatives displayed rapid anti-inflammatory activity.
- New compounds were found to be non-toxic in nature.

Synthesis, anticonvulsant and anti-inflammatory studies of new 1,4-dihydropyridin-4-yl-phenoxyacetohydrazones

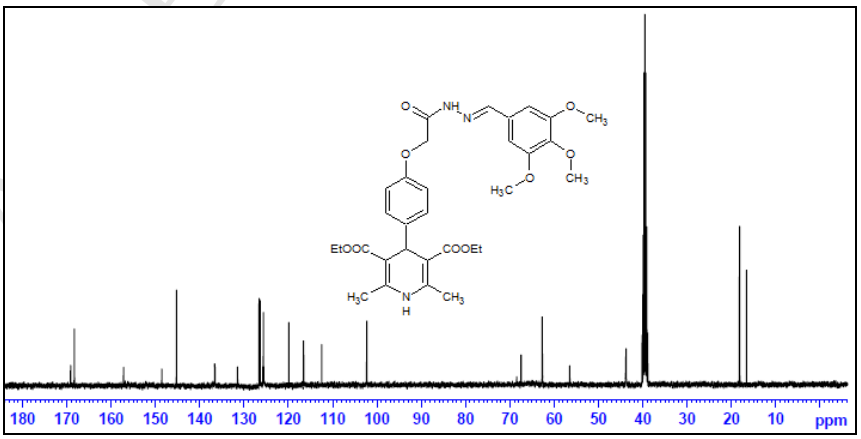
Shrikanth Ulloora, Ramakrishna Shabaraya, Rajesh Ranganathan, Airody Vasudeva Adhikari

Supplementary material

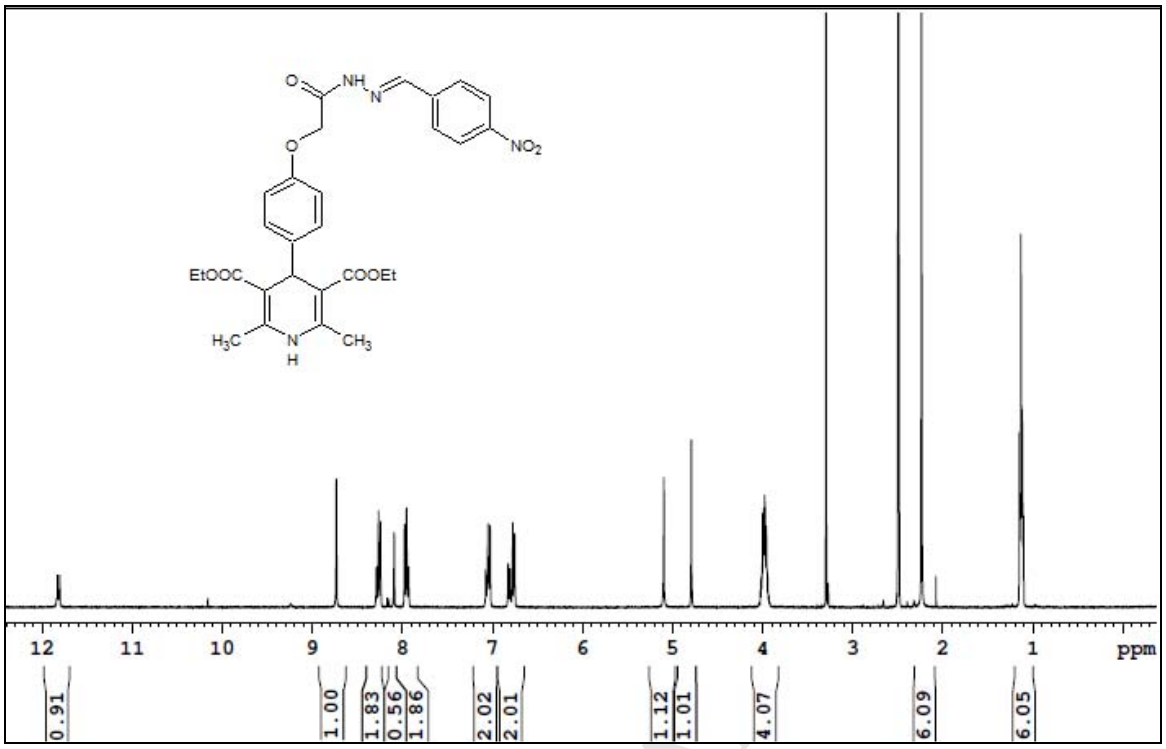
The ^{1}H NMR and ^{13}C NMR spectrograms of few representative target compounds are given below.



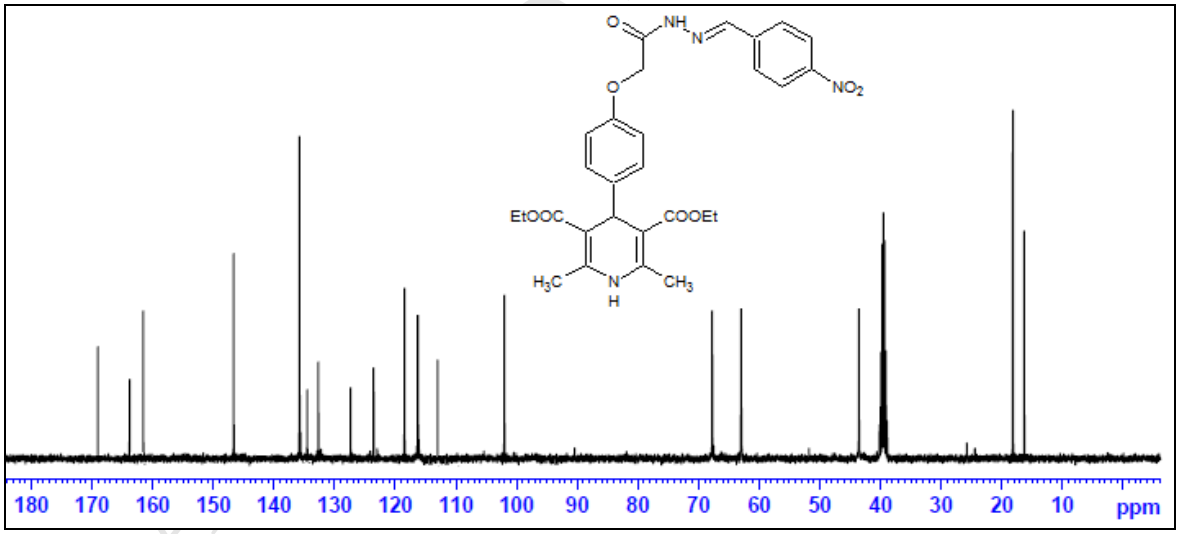
^{1}H NMR spectrum of **4a**



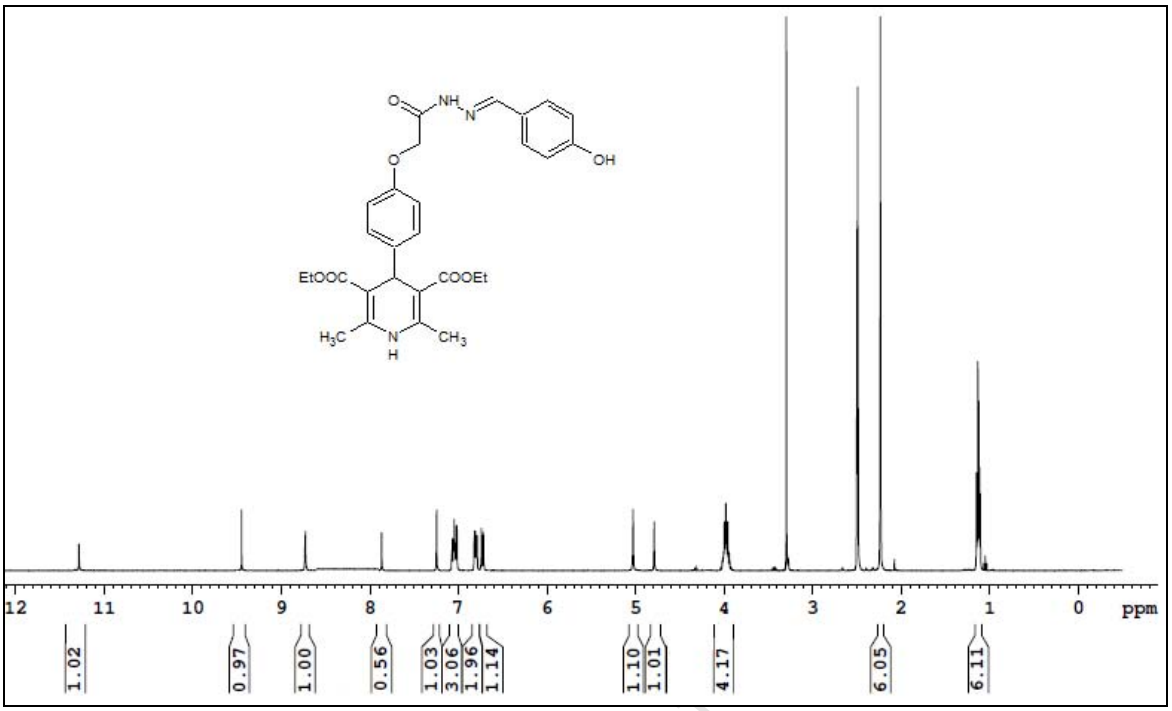
^{13}C NMR spectrum of **4a**



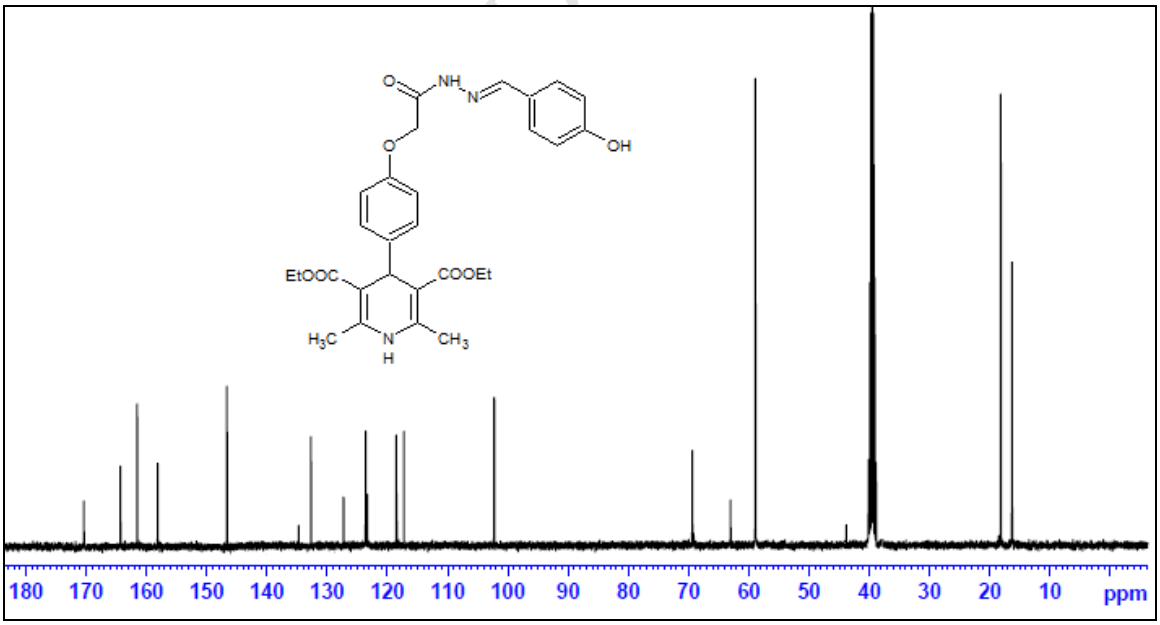
^{1}H NMR spectrum of **4c**



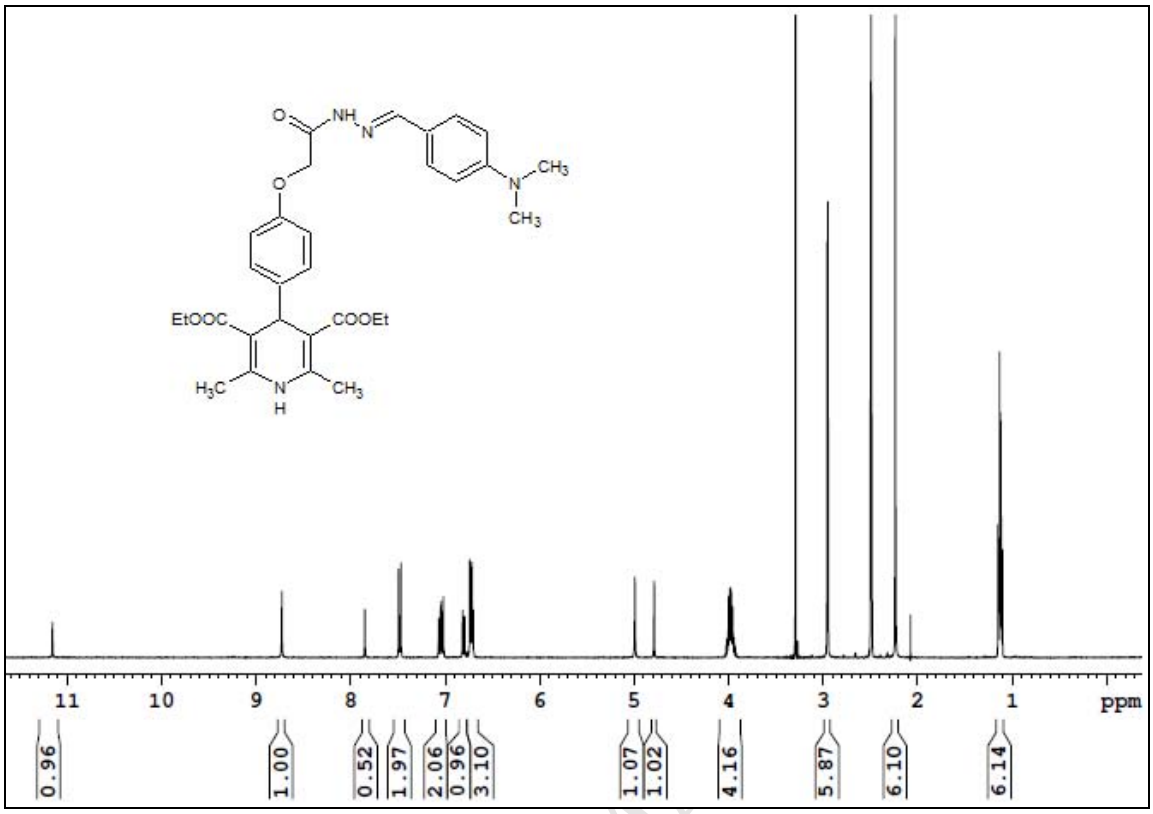
^{13}C NMR spectrum of **4c**



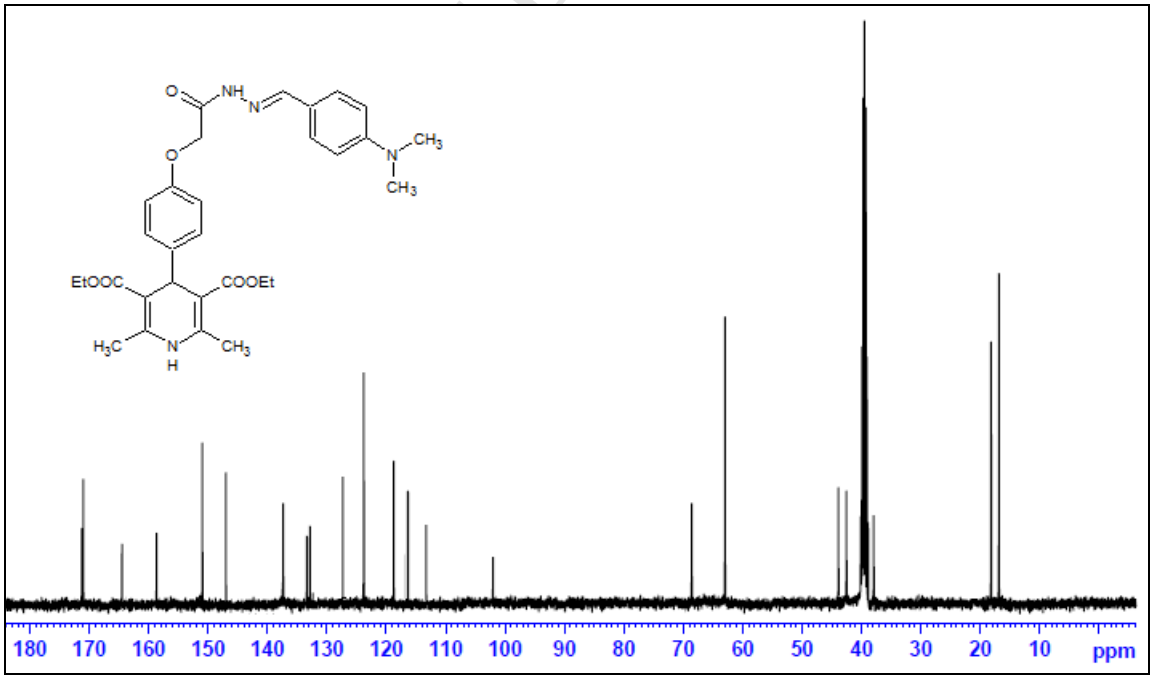
^{1}H NMR spectrum of **4e**



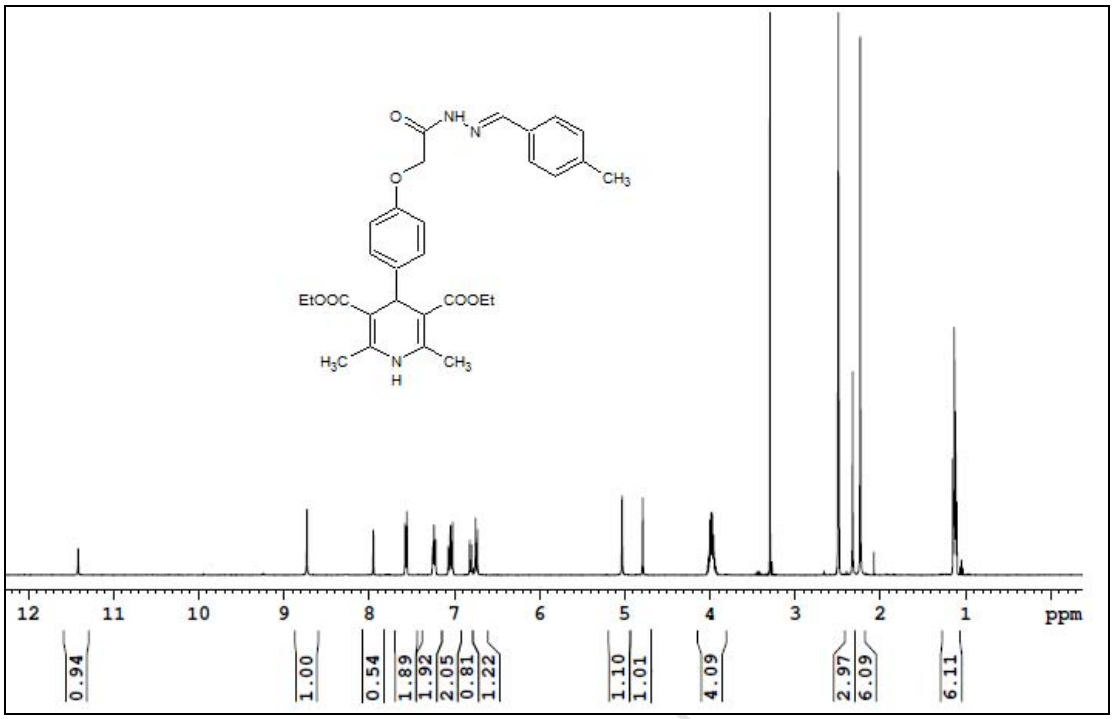
^{13}C NMR spectrum of **4e**



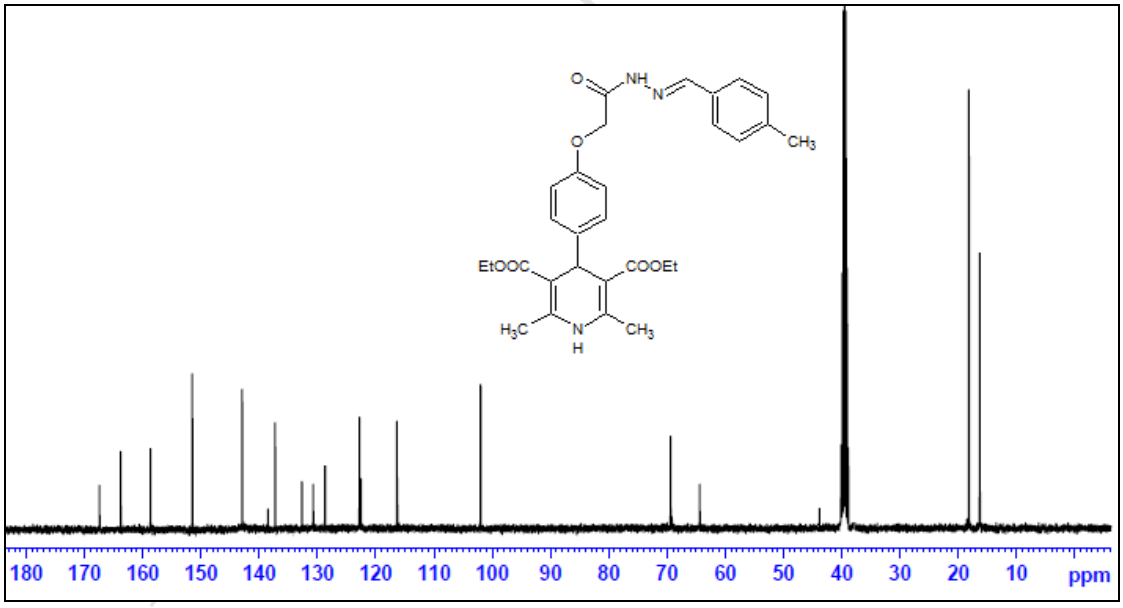
^{1}H NMR spectrum of **4g**



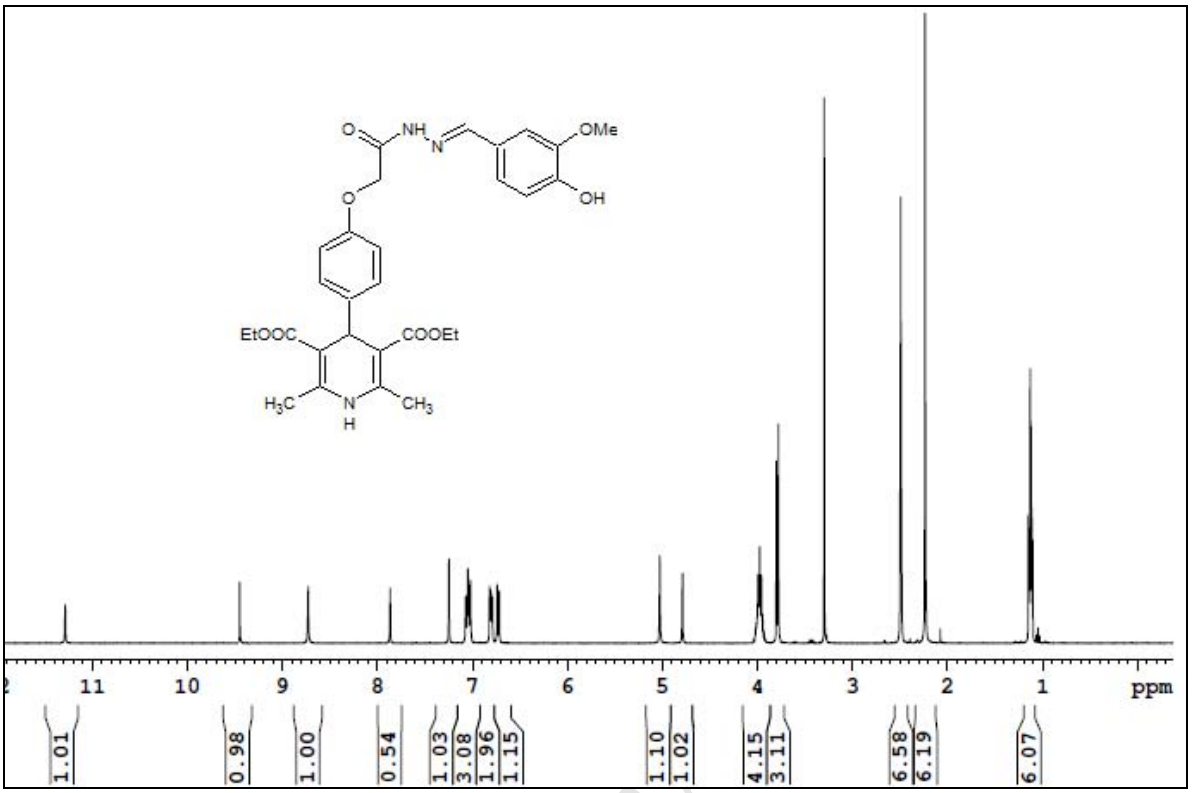
^{13}C NMR spectrum of **4g**



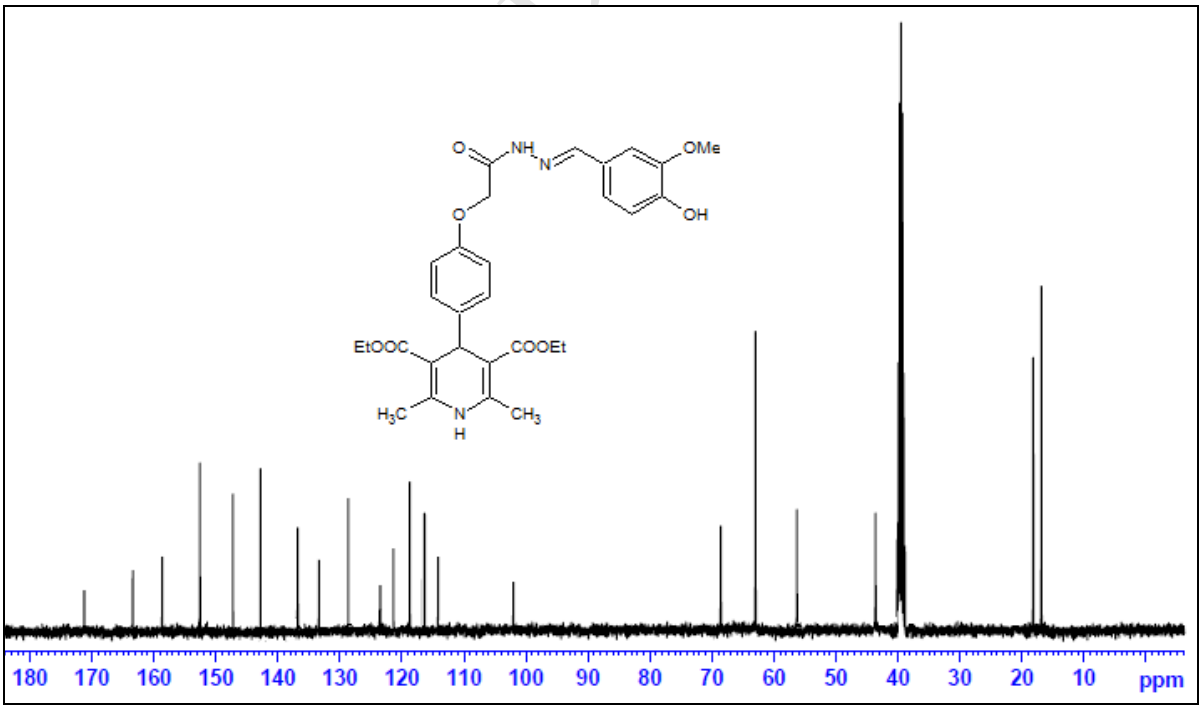
^{1}H NMR spectrum of **4i**



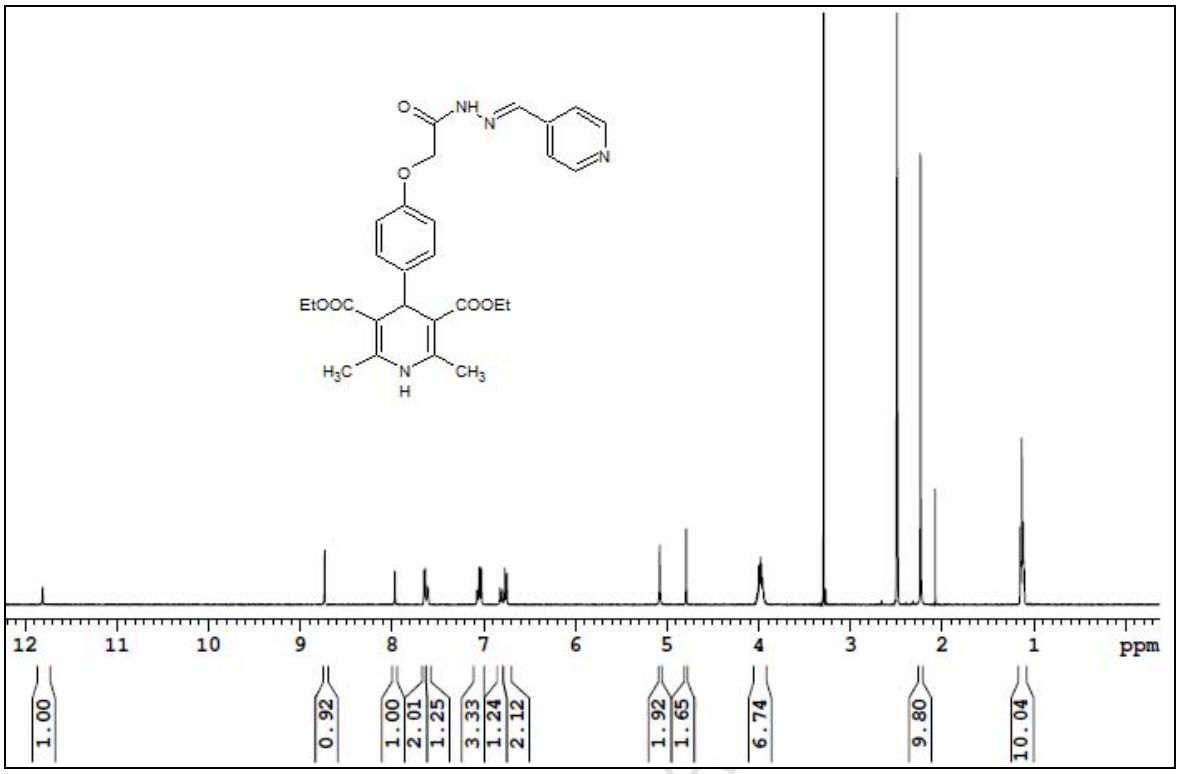
^{13}C NMR spectrum of **4i**



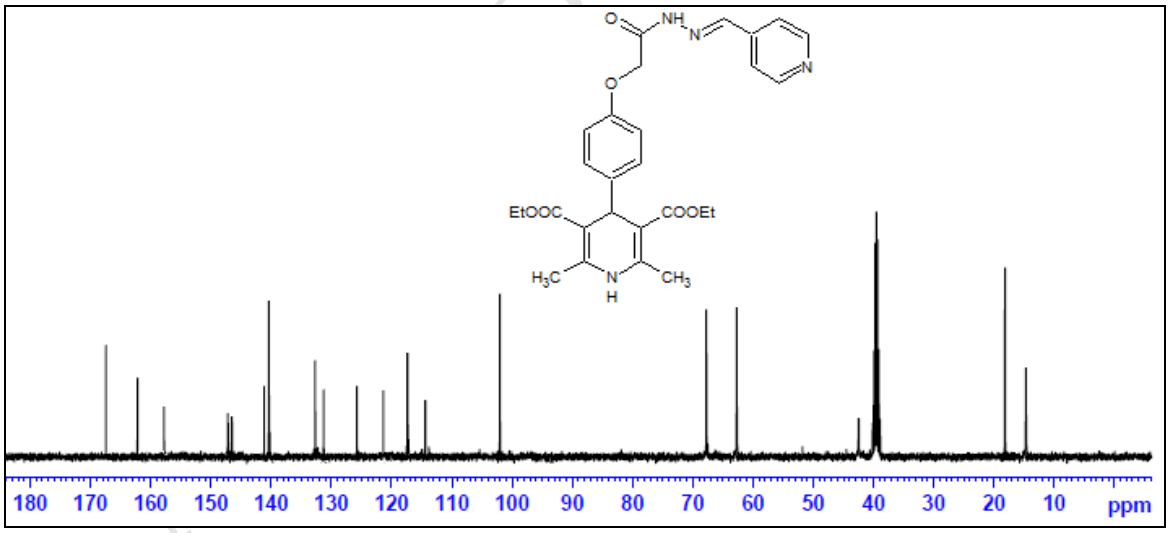
^{1}H NMR spectrum of **4j**



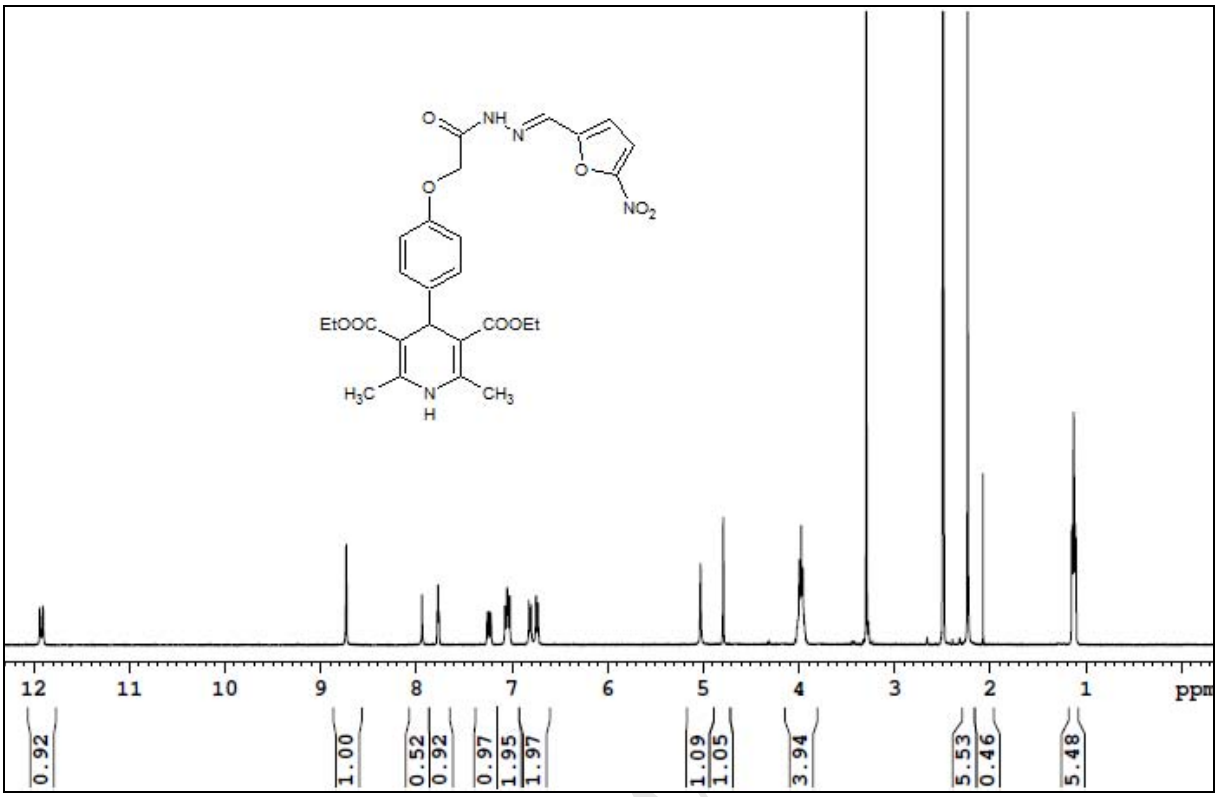
^{13}C NMR spectrum of **4j**



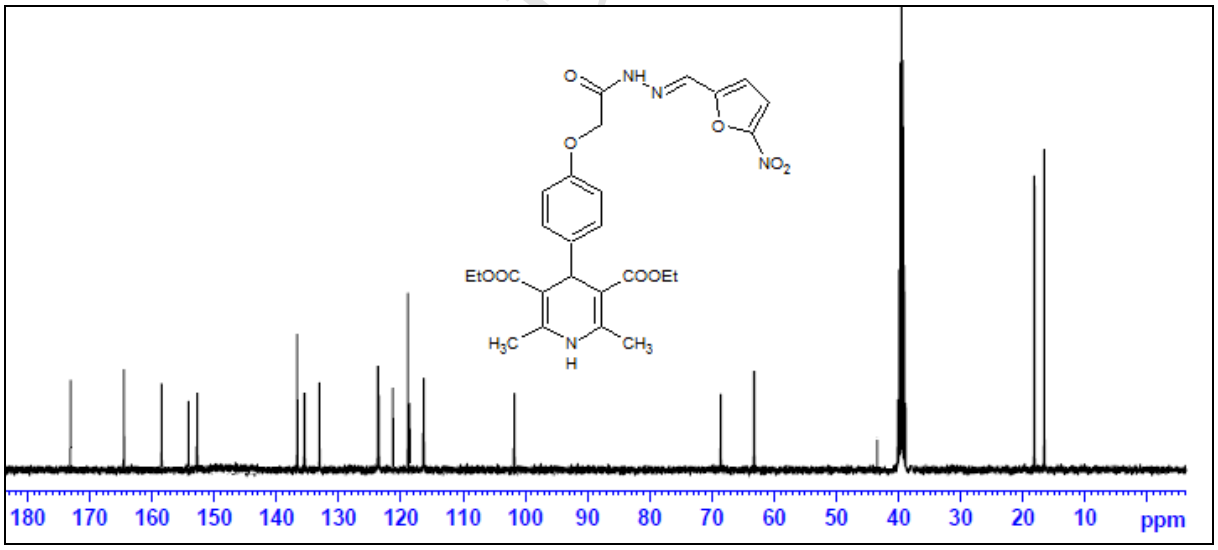
^{1}H NMR spectrum of **4m**



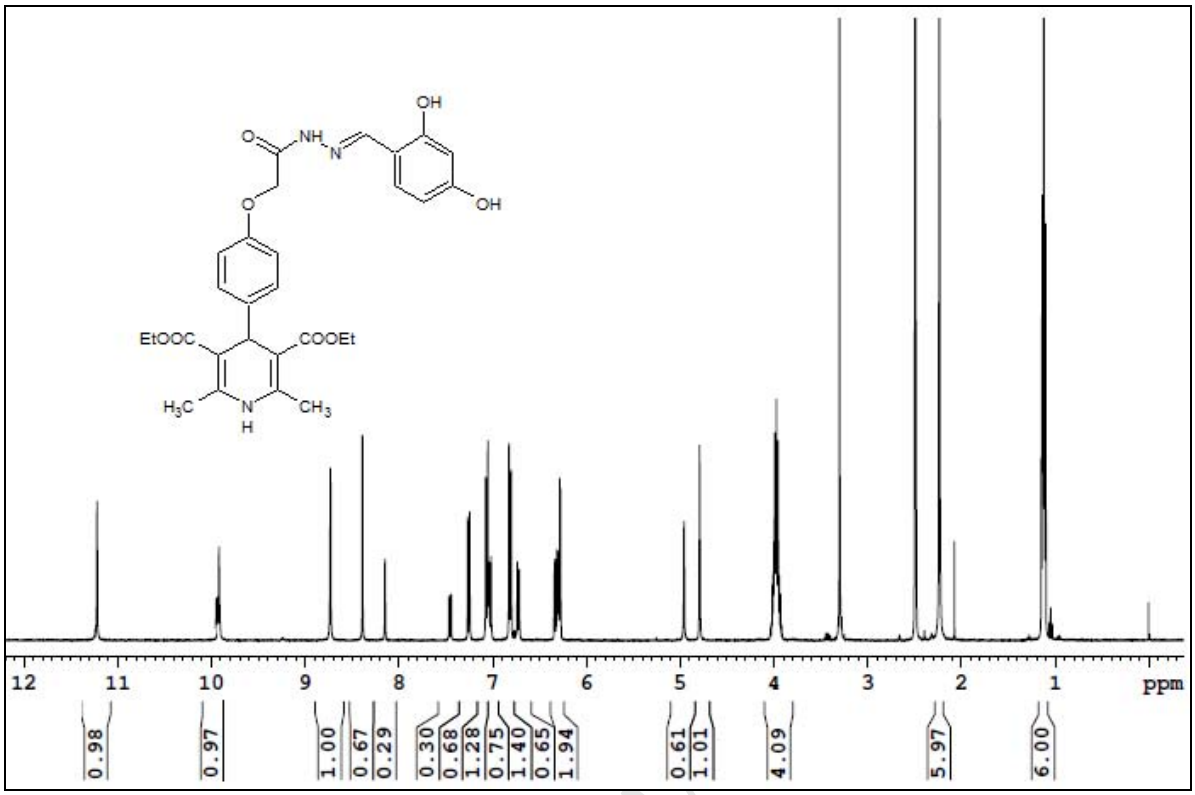
^{13}C NMR spectrum of **4m**



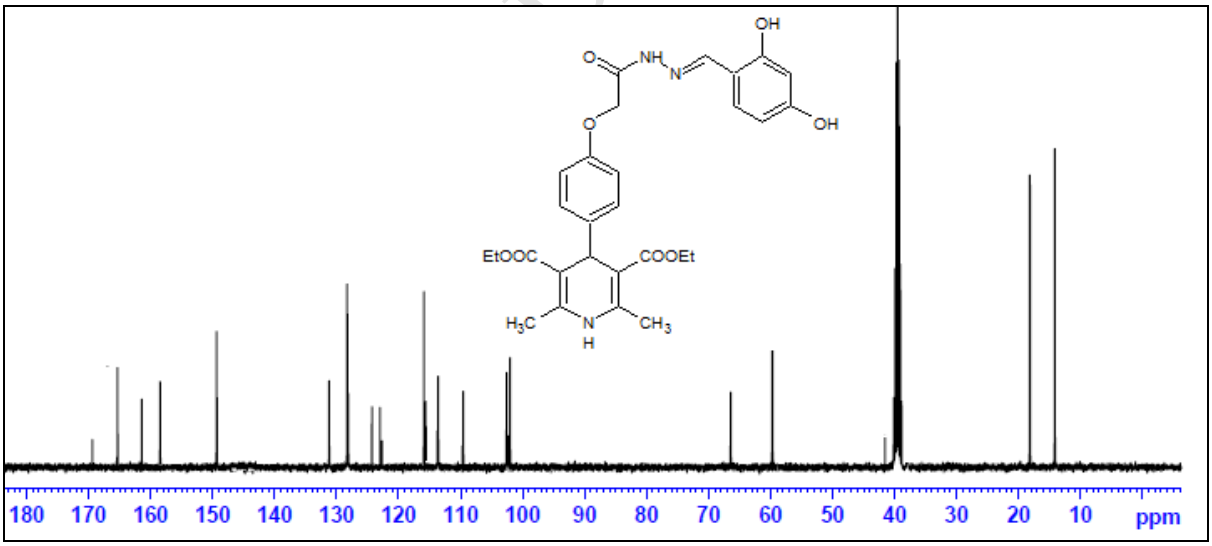
^{1}H NMR spectrum of **4o**



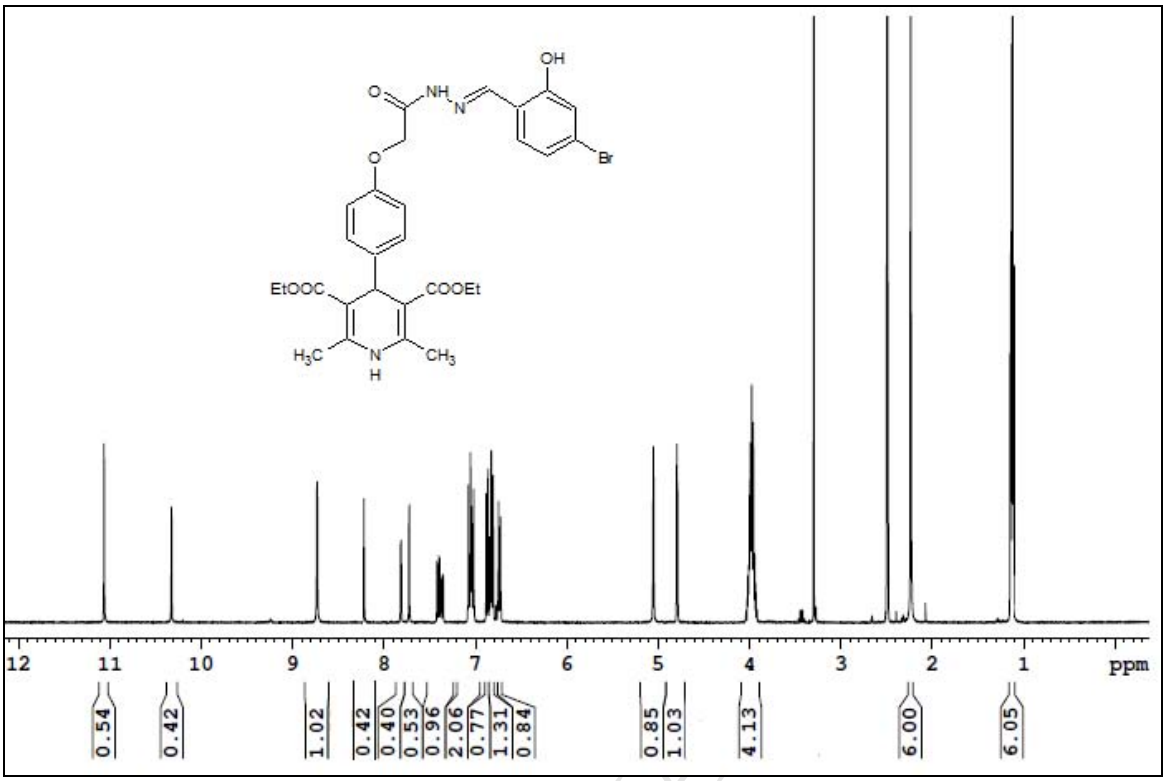
^{13}C NMR spectrum of **4o**



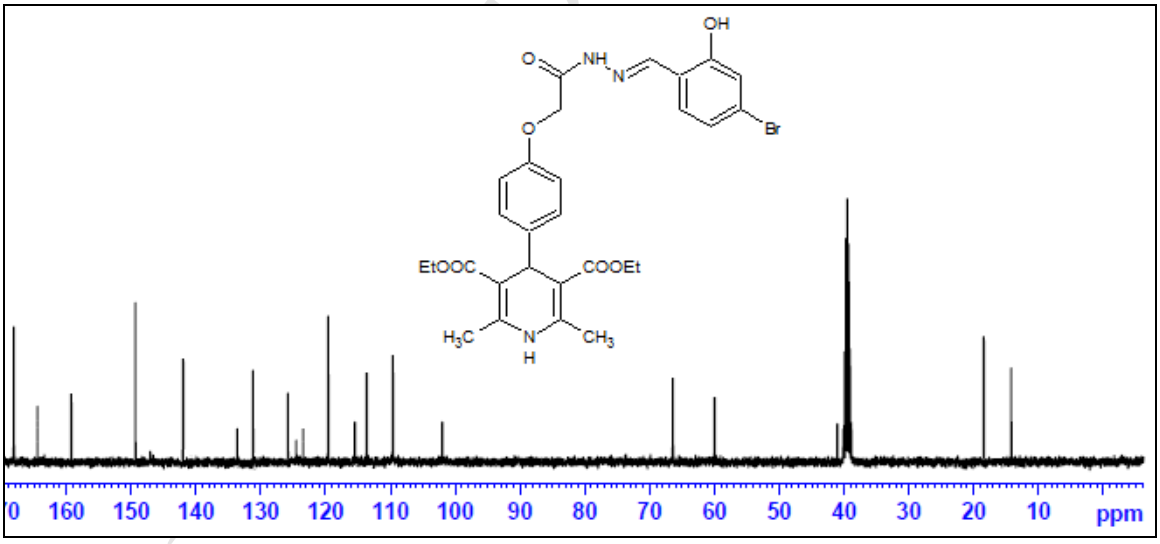
^{1}H NMR spectrum of **4q**



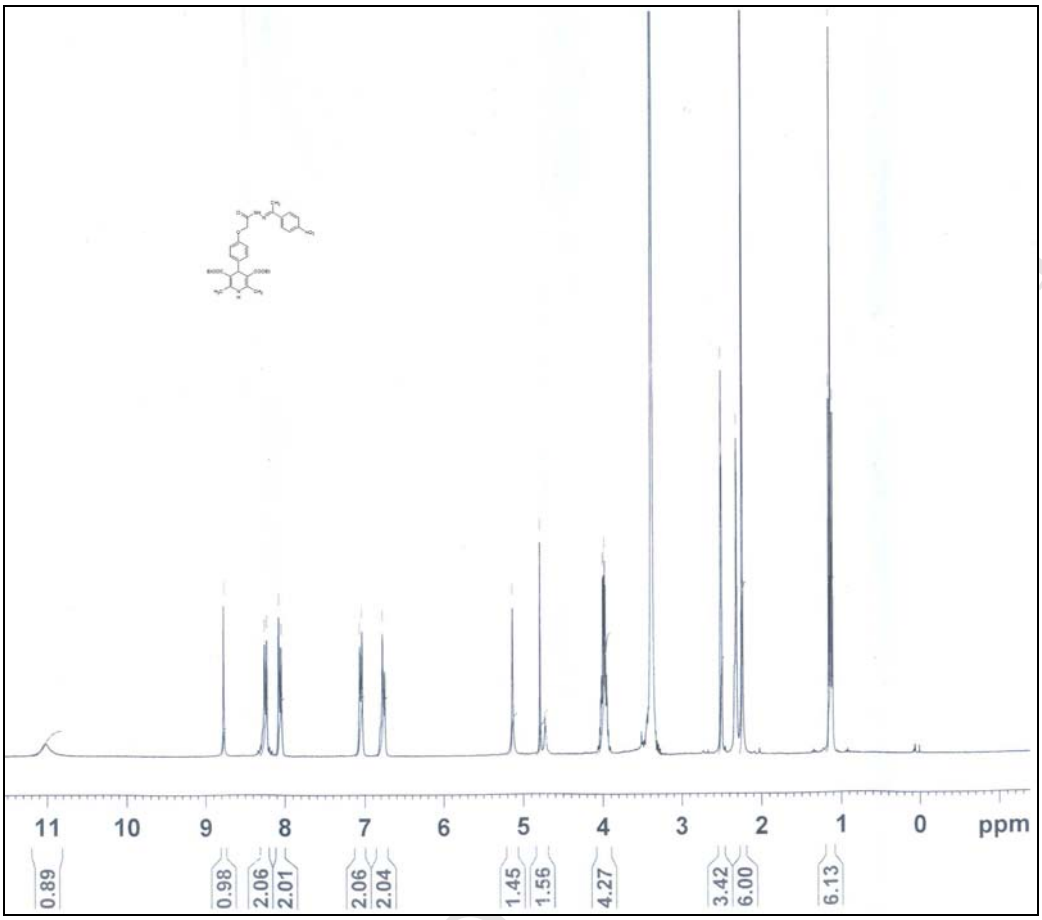
^{13}C NMR spectrum of **4q**



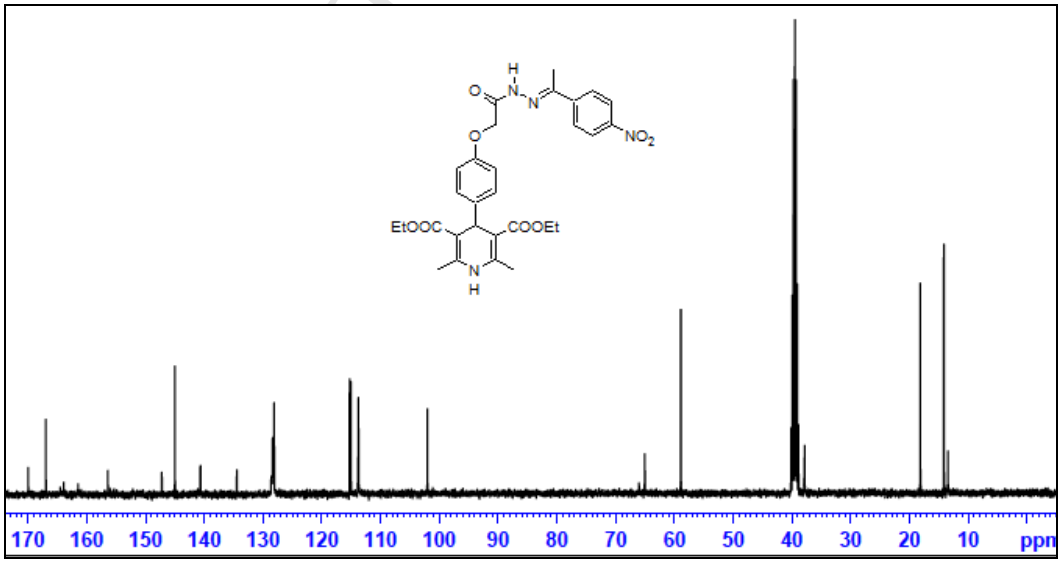
^{1}H NMR spectrum of **4r**



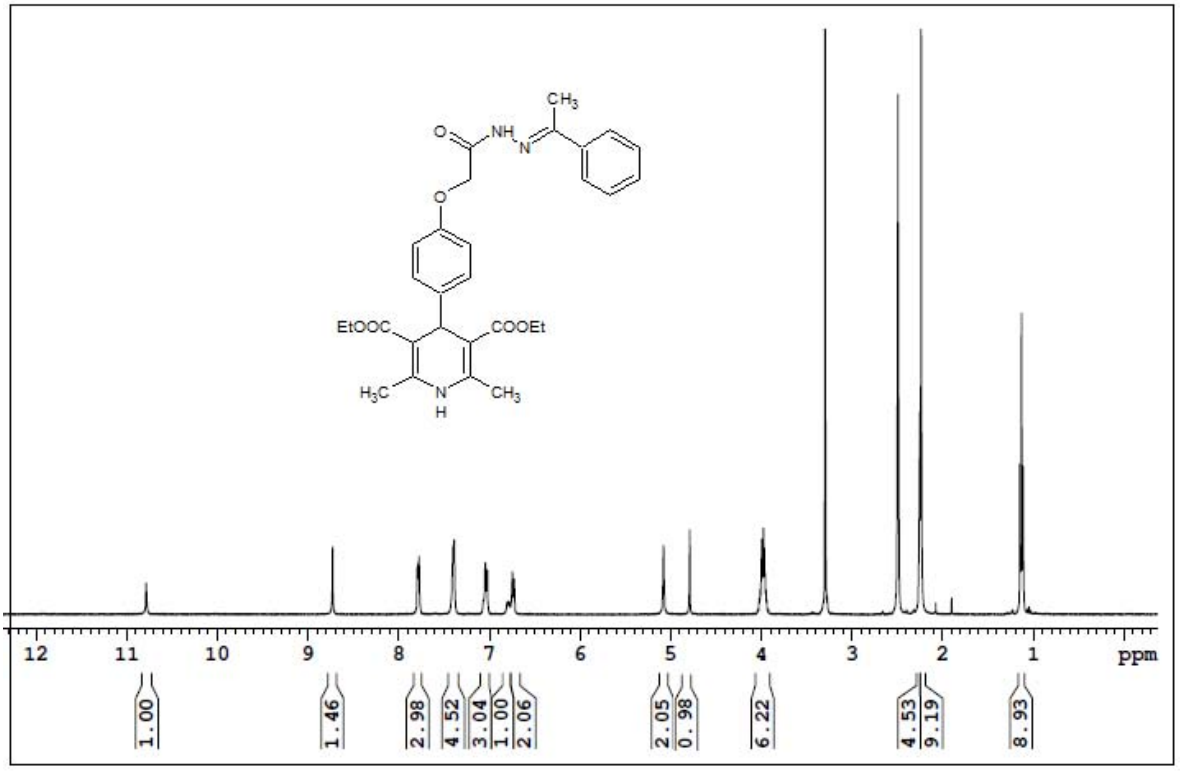
^{13}C NMR spectrum of **4r**



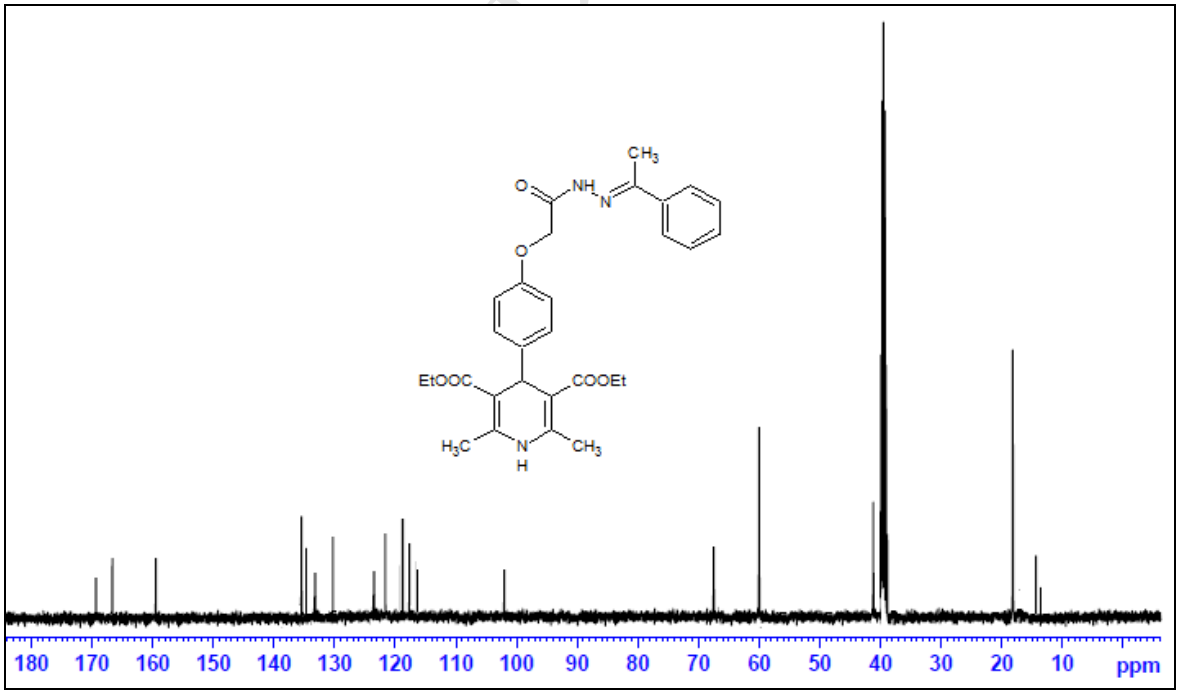
^{1}H NMR spectrum of **5a**



^{13}C NMR spectrum of **5a**



^{1}H NMR spectrum of **5e**



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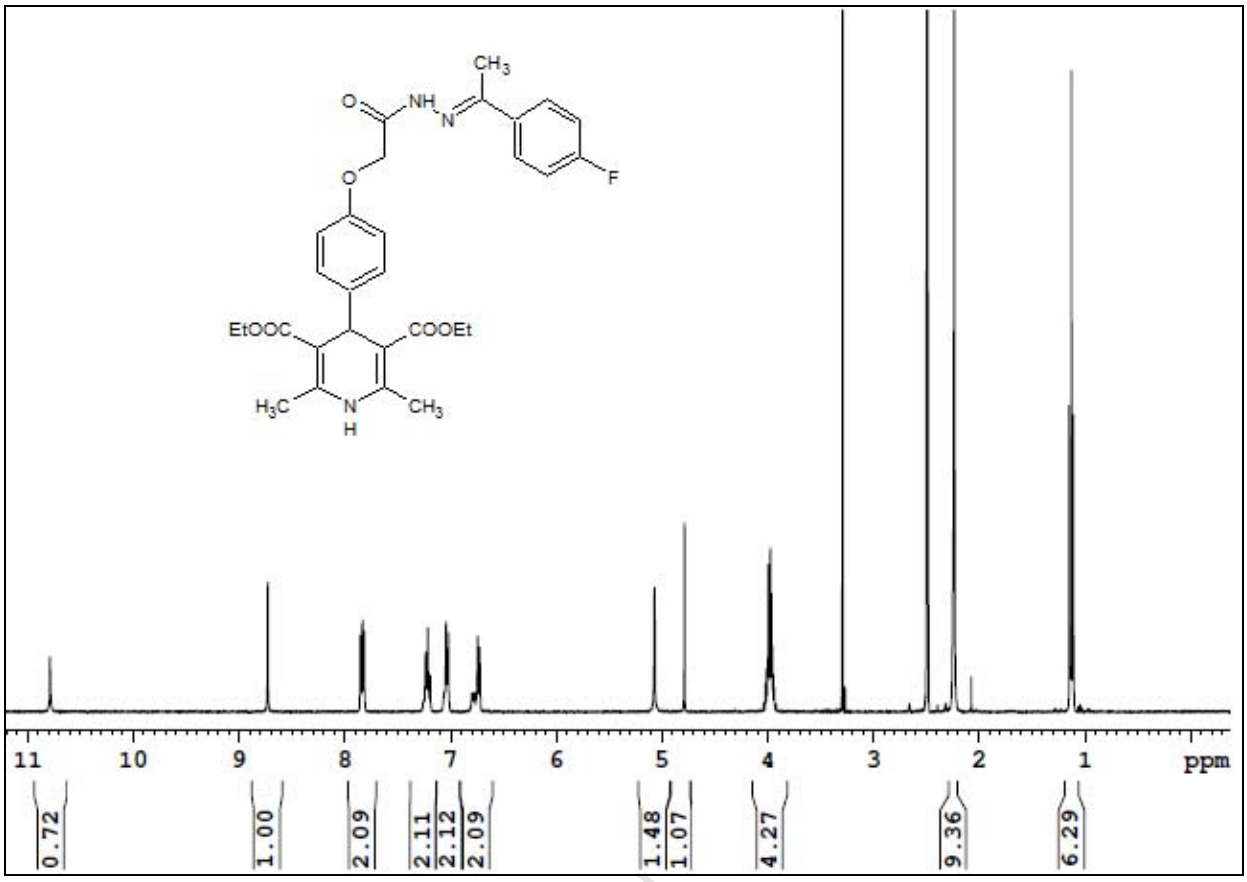
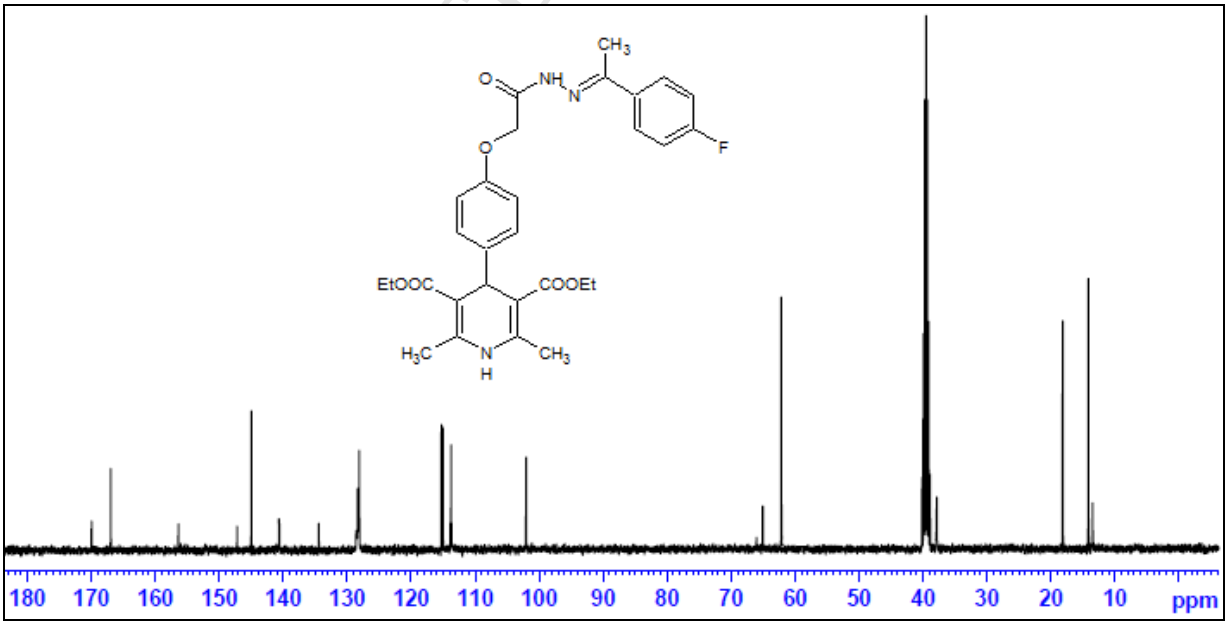
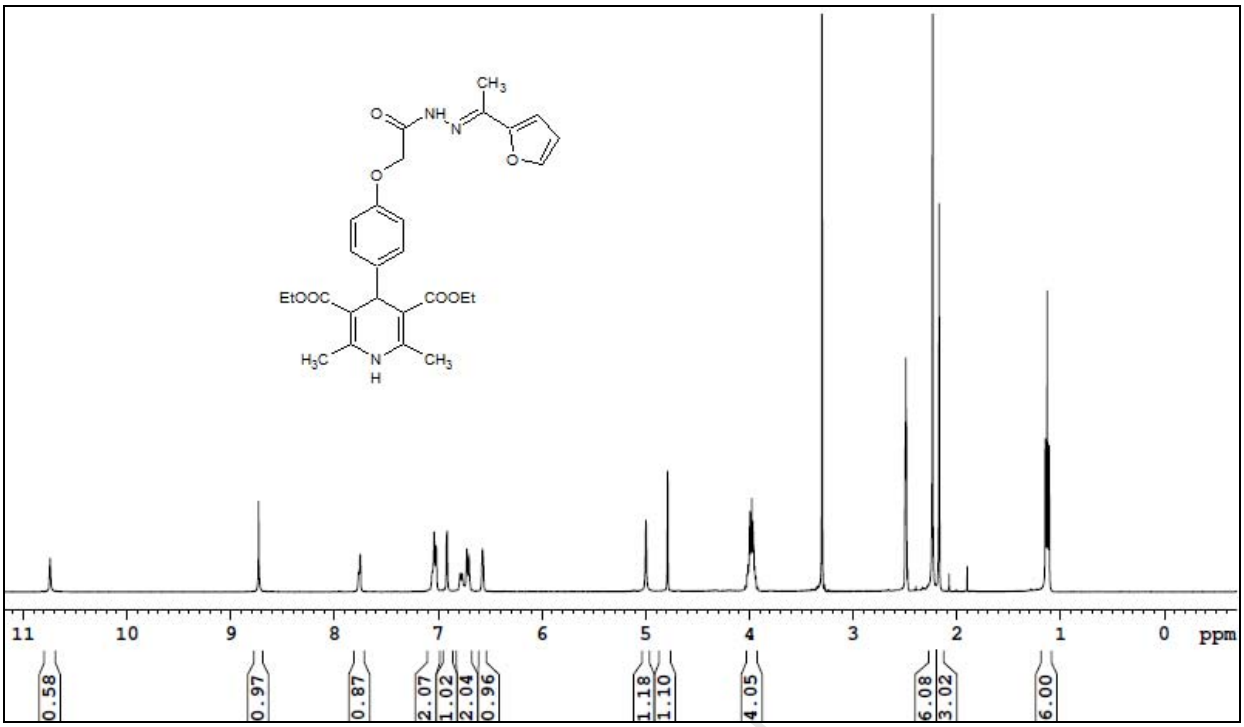


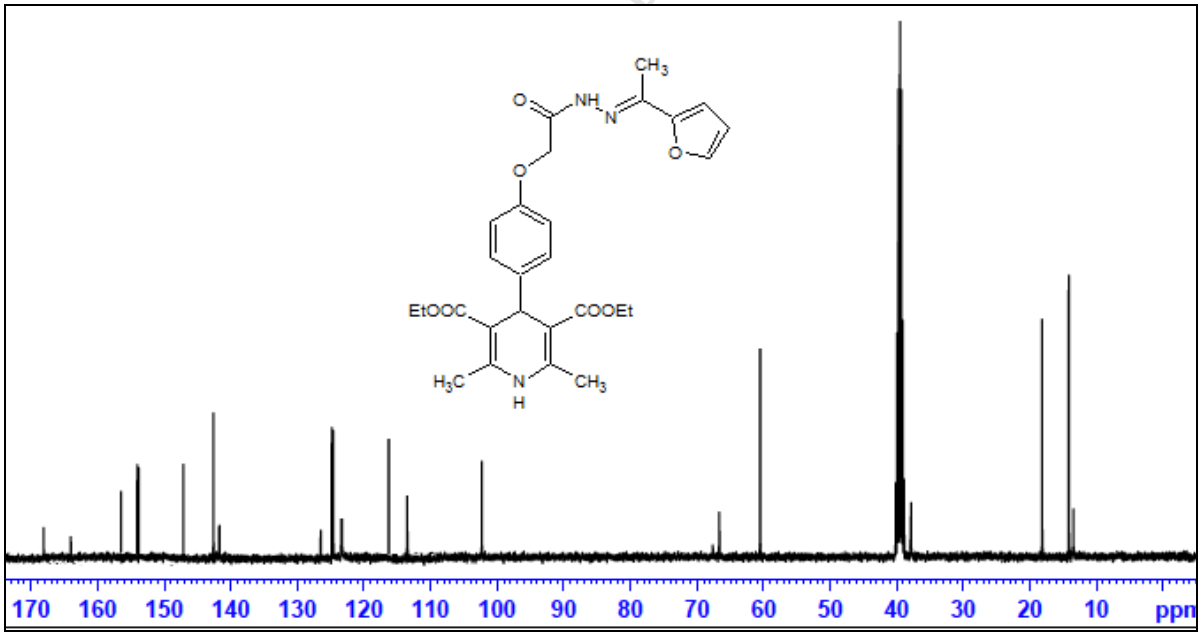
^{1}H NMR spectrum of **5f**



^{13}C NMR spectrum of **5f**



^{1}H NMR spectrum of **5g**



^{13}C NMR spectrum of **5g**