Synthesis, characterization, and evaluation of antioxidant activity of new γ- and δ-imino esters

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Abstract: New Schiff bases were synthesized by condensation of methyl-5-(furan-2-yl)-5-oxopentanoate, methyl-4-(furan-2-yl)-4-oxobutanoate, methyl-5-(thiophene-2-yl)-5-oxopentanoate, and methyl-4-(thiophene-2-yl)-4-oxobutanoate with p-anisidine and n-butylamine. The structures of these synthesized compounds were clarified on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, and GC-MS spectroscopic techniques. In addition, antioxidant activity of the synthesized compounds was evaluated on the basis of DPPH radical.

Key words: Imino ester, p-anisidine, n-butylamine, antioxidant activity

1. Introduction

Imine compounds containing the azomethine group (–C=N–) are known as Schiff bases.[1] They are synthesized from the condensation of the primary amines with activated carbonyl compounds. Imines are used for a variety of purposes in many industries, such as paints, pharmaceuticals, and plastics, and also play a role in many biological reactions.[2−6] The imine compounds have an important role in medicinal and pharmaceutical fields due to a broad spectrum of biological activities like antioxidant,[7] antibacterial,[8] antifungal,[9] and antitumor[10] activities.

According to the literature survey, a large number of imine derivatives were synthesized using different catalysts such as montmorillonite, $CuSO_4$, $ZnCl_2$, $MgSO_4$, $TiCl_4$, and molecular sieves as dehydration reagents and a Dean–Stark apparatus.[11,12] Different methods have been developed to synthesize imines containing like infrared irradiation,[13] microwave,[14] ionic liquids,[15] and ultrasound irradiation.[16]

Thiophene and furan derivatives belong to the aromatic heterocyclic group; consequently, they are an important structural fragment in many pharmaceutical and chemical compounds. Amines were also selected from two groups, aliphatic and aromatic, to compare antioxidant activities. Due to the fact that the structures containing γ- and δ-keto esters and amines were known to have a good potential for antioxidant activity from the literature,[17] in this study different heterocyclic γ- and δ-keto esters (**1a–d**) and p-anisidine and n-butylamine were chosen to obtain heterocyclic γ- and δ-imino esters.

Eight novel imino esters (**2a–d, 3a–d**) were synthesized in the presence of $TiCl_4^{18}$ and Et_3N using p-anisidine and n-butylamine in good yields. The synthesized compounds were purified by column chromatography and their structures were determined by spectroscopic methods (elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, and GC-MS) and their isomerization $((E)/(Z)$ ratio) was determined by ^{1}H NMR spectra and their antioxidant activities were investigated by DPPH.

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2. Results and discussion

2.1. Synthesis of γ- and δ-imino esters

Imine compounds have received a great deal of attention due to their chemical and biological properties, especially in the pharmaceutical industry. We aimed to synthesize imino esters, to identify their structure, and to determine their antioxidant activities. In this paper, a total of eight novel imine compounds, four γ-imino esters (**2a,b**; **3a,b**) and four δ-imino esters (**2c,d**; **3c,d**) (Scheme), were obtained in high yields.
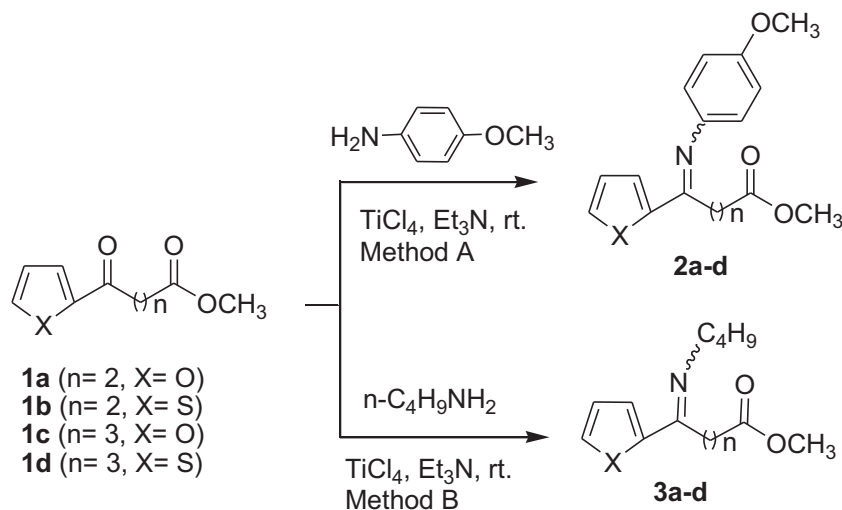
Scheme. Synthesis of γ- and δ-imino esters.

In the literature, some γ- and δ-imino ester derivatives were synthesized using TiCl$_4$,[18] molecular sieves,[19] and Dean–Stark apparatus.[20] We prepared imino esters **2a**–**d** and **3a**–**d** from corresponding keto esters **1a**–**d** with $p-$anisidine and $n-$butylamine in the presence of TiCl$_4$ and Et$_3$N in this study. When molar ratios of keto ester/amine/TiCl$_4$/Et$_3$N are used according to the literature,[18,20] the starting compounds are still present even if the reaction time is long. Therefore, we examined several molar ratios and reaction times to provide optimal conditions. The best performances in the synthesis of the imines with p-anisidine were obtained with keto ester/p-anisidine/TiCl$_4$/Et$_3$N molar ratio of 1:3:1:4, respectively. The γ- and δ-imino esters (**2a**–**d**) were synthesized at room temperature for 24–48 h in 35%–56% yield by these ratios. The results are given in Table 1. Synthesis of imino esters from the reaction of keto ester and n-butylamine (**3a**–**d**) was achieved using 1:3:0.7:4 molar ratios of keto ester/n-butylamine/TiCl$_4$/Et$_3$N in about 40–60 min and yield was around 85%–98%. The results are given in Table 1.

The $(E)/(Z)$ isomer ratios of γ- and δ-imino esters were decided with respect to the literature data.[18−20] The $(E)/(Z)$ isomer ratios of γ- and δ-imino ester derivatives were investigated according to the ^{1}H NMR spectrum and the configuration of the imine was referred to mainly (E) in the literature.[18,20] In our study, the E/Z ratio of obtained γ- and δ-imino esters (**2a**–**d**, **3a**–**d**) were determined by ^{1}H NMR spectrum. The ^{1}H NMR spectra indicated double signals for methoxy group with $(E)/(Z)$ mixtures of γ- and δ- imino esters. As given in the literature, **2a**, **2c**, **3a**, and **3b** were isolated predominantly in (E) isomer $((E/Z)$ 2.5/1, 3.5/1, 2/1, and 2/1, respectively)[18,20] (Table 1). The obtained **2b**, **2d**, and **3c,d** were seen in their ^{1}H NMR spectrum and had only one signal for the methoxy group. As a result, configuration of these imines (**2b**, **2d**, **3c,d**) was determined as E according to the literature[21] (Table 1).

Table 1. Yields and isomer ratios of synthesized γ- and δ-imino esters.

Compound	Yield (%)[a]	Isomer ratio[b] ((E)/(Z))
2a	44	2.5/1
2b	38	E
2c	56	3.5/1
2d	35	E
3a	90	2/1
3b	85	2/1
3c	98	E
3d	91	E

[a] Isolated yield. [b] $(E)/(Z)$ ratio was determined by ^{1}H NMR.

2.2. Antioxidant activity

There are many reports in the literature concerning the antioxidant activity of different Schiff bases.[22] For example, 2-((o-hydroxylphenylimino)-methyl)-phenol and 2-((p-hydroxylphenylimino)-methyl)-phenol using different sources of water-soluble (6-hydroxyl-2,5,7,8- tetramethylchroman-2-carboxylic acid-Trolox, and L-ascorbic acid) or lipophilic (tocopherol and Lascorbyl-6-laurate) antioxidants.

DPPH is used as a free radical to evaluate the antioxidative activity of some natural and synthetic sources. The scavenging of the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The inhibitory effects of different concentrations of synthesized **2a**–**d** and **3a**–**d** on DPPH radical are presented in Table 2.

Table 2. Antioxidant scavenging activity of newly synthesized imines **2a**–**d** and **3a**–**d** at different concentrations.

Compound	DPPH• scavenging activity[a]		
	250 μM/mL	500 μM/mL	1000 μM/mL
2a	90.85 ± 0.27	94.88 ± 0.47	91.32 ± 0.71
2b	56.43 ± 1.63	77.21 ± 1.68	86.20 ± 0.27
2c	50.85 ± 0.27	73.95 ± 0.81	85.89 ± 0.54
2d	65.89 ± 0.71	86.51 ± 1.23	90.54 ± 0.97
3a	51.63 ± 0.93	62.64 ± 0.27	30.08 ± 1.42
3b	43.72 ± 0.93	63.57 ± 0.71	32.87 ± 0.97
3c	20.78 ± 0.71	37.67 ± 0.81	57.99 ± 0.54
3d	24.80 ± 0.54	43.72 ± 1.23	71.63 ± 1.23
BHA	94.57 ± 0.71	96.12 ± 0.71	97.83 ± 0.27
BHT	95.81 ± 0.47	96.12 ± 0.54	97.05 ± 0.27
Ascorbic Acid	96.90 ± 0.27	97.36 ± 0.54	98.29 ± 0.27

[a] Values were the means of three replicates ± S.D.

2.3. DPPH• scavenging activity

DPPH is a stable free radical that can receive an electron or hydrogen radical to turn into a stable diamagnetic molecule. Because of its odd electron, the methanol solution of DPPH indicates a strong absorption band at 517 nm. The DPPH radical reacts with different electron-donating molecules. When electrons become paired off, the DPPH solution is bleached. This results in the formation of the colorless 2,2'-diphenyl1-picryl hydrazine. Reduction of the DPPH radicals can be calculated quantitatively by measuring the decrease in absorbance at 517 nm.

All the tested compounds showed lower free-radical–scavenging activities when BHA, BHT and ascorbic acid were compared. The results of the antioxidant activity of **2a**–**d**, **3a**–**d**, and standards are presented in Table 2. Further preliminary in vitro antioxidant activity of newly synthesized γ- and δ-imino esters exhibited that **2a**–**d** show significant activity in comparison with standard antioxidant BHA, BHT, and ascorbic acid.

2.4. Conclusions

In this article, we modified the imine synthesis method in the literature using different molar ratios of keto ester/amine/TiCl$_4$/Et$_3$N. As a result, we obtained a total of eight original γ- and δ-imino esters by p-anisidine and n-butylamine in the existence of TiCl$_4$ and Et$_3$N in high yields. The synthesized imine compounds were described by IR, ^{1}H NMR, ^{13}C NMR, GC-MS, and elemental analysis. The isomerization $((E)/(Z))$ of obtained imines was clarified by ^{1}H NMR spectra according to the literature data. The results are summarized in Table 1.

The antioxidant activities of synthesized imines were first determined using DPPH scavenging activity and compared with BHA, BHT, and ascorbic acid as the standards. All of the synthesized imino esters (**2a**–**d**, **3a**–**d**) exhibited antioxidant activity at least. According to the results, **2a**–**d** were more effective in terms of antioxidant activity than the other compounds (Table 2). We think that these imine compounds and their antioxidant activity properties can be used in medicinal and pharmaceutical areas.

3. Experimental

3.1. General procedure

The chemicals used in this study were commercially available from Merck (Kenilworth, NJ, USA) and Aldrich (St. Louis, MO, USA) and were used without further purification. Friedel–Crafts acylation was used to synthesize γ- and δ-keto esters.[23,24] The starting compounds and imines were purified by column chromatography on silica gel (particle sizes 0.063–0.200 mm and 0.040–0.063 mm, respectively). ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively) spectra were recorded using Me$_4$Si as the internal standard in CDCl$_3$. Gas chromatography–mass spectrometry (GC-MS) data were recorded on a Shimadzu QP2010 Plus using a GC-MS column Teknokroma TRB-5MS (30 m × 0.25 mm × 0.25 μm I.D.) (Kyoto, Japan). The operating conditions were as follows: injection temperature 250 °C, helium carrier gas flow 20 psi, split ratio 1/100, E.I 70 eV. Temperature programming: 80 °C (5 min) up to 250 °C at 5 °C/min, hold 30 min. FT-IR spectra were recorded on a Bruker Vertex 70 (Billerica, MA, USA). The E/Z ratios of obtained γ- and δ-imino esters (**2a**–**d**, **3a**–**d**) were determined by ^{1}H NMR spectrum according to the literature.[18−20]

3.2. Synthesis of γ- and δ-imino esters using Method A (2a–d)

A solution of the corresponding 10 mmol of keto ester and 30 mmol of p-anisidine in 60 mL of dry ether was added to a dried flask under nitrogen atmosphere. The system was cooled to –15 °C, and 40 mmol Et$_3$N was

added to the solution. Then 10 mmol $TiCl_4$ was added dropwise within 10 min. The reaction mixture was stirred for 24–48 h at room temperature. Then solution quenched with saturated $NaHCO_3$ was stirred for 15 min and filtrated. The filtrate was extracted with ether, washed with brine, and dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel.

3.2.1. Methyl 4-(4-methoxyphenylimino)-4-(furan-2-yl)butanoate (2a)

Chromatographic purification with 10% Et_3N in 150 mL of hexane; yield 44%; yellow oil; Anal. Calcd. for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88. Found: C, 66.85; H, 5.92; N, 4.85. IR (neat, cm^{-1}): 3140, 3116, 2953, 2916, 1737 (C=O), 1627 (C=N), 1498, 1436, 1235, 1194, 1026, 830, 790. ^{1}H NMR (500 MHz, CDCl$_3$, δ/ppm): 2.54 (2H, m, –CH$_2$–), 2.82 (2H, t, J = 7.1 Hz, –CH$_2$–), 3.64 (3H, s, –OCH$_3$), 3.82 (3H, s, arom. –OCH$_3$), 6.53 (1H, dd, J = 3.5 and 1.8 Hz, furan –CH–), 6.64 (1H, d, J= 8.9 Hz, furan –CH–), 6.76 (2H, d, J = 8.9 Hz, arom. –CH–), 6.90 (2H, dd, J = 8.9 and 5.4 Hz, arom. –CH–), 7.57 (1H, dd, J = 1.08 and 0.7 Hz furan –CH–). ^{13}C NMR (125 MHz, CDCl$_3$, δ/ppm): 25.0 (–CH$_2$–), 31.3 (–CH$_2$–), 51.6 (–OCH$_3$–), 55.4 (arom. –OCH$_3$), 114.3 (furan –CH–), 114.8 (furan –CH-), 118.9 (2 × arom. –CH–), 120.4 (2 × arom. –CH–), 143.2 (arom. –CH–), 144.9 (furan –CH–), 156.1 (furan –CH–), 158.9 (arom. –CH–), 172.3 (–C=N–), 173.9 (–C=O). GC-MS (m/z): 64, 77, 92, 107, 115, 137, 200, 213, 228, 256, 272, 287 (M$^+$).

3.2.2. Methyl 4-(4-methoxyphenylimino)-4-(thiophen-2-yl)butanoate (2b)

Chromatographic purification with 10% Et_3N in 150 mL of hexane; yield 38%; yellow oil; Anal. Calcd. for $C_{16}H_{17}NO_3S$: C, 63.34; H, 5.65; N, 4.62; S, 10.57. Found: C, 63.29; H, 5.62; N, 4.58; S, 10.52. IR (neat, cm^{-1}): 3104, 3079, 2953, 2916, 1723 (C=O), 1602 (C=N), 1500, 1434, 1230, 1194, 1026, 830, 790. ^{1}H NMR (500 MHz, CDCl$_3$, δ/ppm): 2.54 (2H, m, –CH$_2$–), 2.98 (2H, m, –CH$_2$–), 3.65 (3H, s, –OCH$_3$), 3.83 (3H, s, arom. –OCH$_3$), 6.75 (2H, m, arom. –CH–), 6.91 (2H, m, arom. –CH–), 7.10 (1H, dd, J = 5.1 and 3.7 Hz, thiophen –CH), 7.47 (2H, dd, J = 5.1 and 1.1 Hz, thiophen –CH–). ^{13}C NMR (125 MHz, CDCl$_3$, δ/ppm): 26.1 (–CH$_2$–), 32.3 (–CH$_2$–), 51.9 (–OCH$_3$–), 55.3 (arom. –OCH$_3$), 114.3 (2 × arom. –CH–), 120.2 (2 × arom. –CH–), 127.6 (thiophen –CH–), 128.5 (thiophen –CH–), 129.9 (thiophen –CH–), 143.4 (arom. –CH–), 144.8 (thiophen –CH–), 156.0 (arom. –CH–), 162.7 (–C=N–), 172.1 (–C=O). GC-MS (m/z): 64, 77, 92, 107, 121, 136, 201, 216, 229, 244, 256, 271, 288, 303 (M$^+$).

3.2.3. Methyl 5-(4-methoxyphenylimino)-5-(furan-2-yl)pentanoate (2c)

Chromatographic purification with 10% Et_3N in 150 mL hexane; yield 56%; yellow crystals; mp: 92.6–93.2 °C, Anal. Calcd. for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.71; H, 6.32; N, 4.63. IR (neat, cm^{-1}): 3132, 3116, 2944, 2839, 1724 (C=O), 1622 (C=N), 1500, 1446, 1206, 1104, 1026, 830, 790. ^{1}H NMR (500 MHz, CDCl$_3$, δ/ppm): 1.89 (2H, m, –CH$_2$–), 2.27 (2H, t, J = 7.2 Hz, –CH$_2$–), 2.61 (2H, m, –CH$_2$–), 3.63 (3H, s, –OCH$_3$), 3.81 (3H, s, arom. –OCH$_3$), 6.53 (1H, dd, J = 3.5 and 1.8 Hz, furan –CH–), 6.76 (2H, d, J = 8.9 Hz, arom. –CH–), 6.90 (2H, d, J = 8.9 Hz, arom. –CH–), 7.04 (1H, dd, J = 3.5 and 0.5 Hz, furan –CH–), 7.57 (1H, dd, J = 1.07 and 0.7 Hz furan –CH–). ^{13}C NMR (125 MHz, CDCl$_3$, δ/ppm): 23.7 (–CH$_2$–), 29.2 (–CH$_2$–), 33.5 (–CH$_2$–), 51.6 (–OCH$_3$–), 55.4 (arom. –OCH$_3$), 111.8 (furan–CH–), 113.1 (furan–CH–), 114.6 (2 × arom. –CH–), 120.7 (2 × arom. –CH–), 144.7 (arom. –CH–), 153.0 (furan –CH–), 156.1 (furan –CH–),

160.1 (arom. –C̲H–), 173.2 (–C̲=N–), 173.9 (–C̲=O). GC-MS (m/z): 51, 65, 77, 104, 119, 130, 162, 190, 270, 301 (M$^+$).

3.2.4. Methyl 5-(4-methoxyphenylimino)-5-(thiophen-2-yl)pentanoate (2d)

Chromatographic purification with 10% Et$_3$N in 150 mL of hexane; yield 35%; brown oil; Anal. Calcd. for C$_{17}$H$_{19}$NO$_3$S: C, 64.33; H, 6.03; N, 4.41; S, 10.10. Found: C, 64.29; H, 5.98; N, 4.38; S, 10.05. IR (neat, cm^{-1}): 3107, 3010, 2956, 2839, 1716 (C=O), 1606 (C=N), 1495, 1434, 1230, 1194, 1026, 830, 790. ^{1}H NMR (500 MHz, CDCl$_3$, δ/ppm): 1.92 (2H, m, –C̲H$_2$–), 2.28 (2H, t, J = 7.1 Hz, –C̲H$_2$–), 2.66 (2H, m, –C̲H$_2$–), 3.64 (3H, s, –OC̲H$_3$), 3.82 (3H, s, arom. –OC̲H$_3$), 6.76 (2H, m, arom. –C̲H–), 6.90 (2H, m, arom. –C̲H–), 7.11 (1H, dd, J = 5.1 and 3.7 Hz, thiophen –C̲H), 7.45 (1H, dd, J = 5.1 and 1.1 Hz, thiophen –C̲H–), 7.57 (1H, dd, J = 3.7 and 1.1 Hz thiophen –C̲H–). ^{13}C NMR (125 MHz, CDCl$_3$, δ/ppm): 23.8 (–C̲H$_2$–), 29.9 (–C̲H$_2$–), 33.5 (–C̲H$_2$–), 51.6 (–OC̲H$_3$–), 55.4 (arom. –OC̲H$_3$), 114.6 (2 × arom.–C̲H–), 120.3 (2 × arom.–C̲H–), 120.8 (thiophen –C̲H–), 127.6 (thiophen –C̲H–), 125.6 (thiophen –C̲H–), 129.9 (arom. –C̲H–), 143.5 (thiophen –C̲H–), 156.0 (arom.–C̲H–), 164.1 (–C̲=N–), 173.2 (–C̲=O). GC-MS (m/z): 55, 64, 77, 92, 110, 122, 135, 201, 216, 230, 244, 258, 270, 286, 317 (M$^+$).

3.3. Synthesis of γ- and δ-imino esters using Method B (3a–d)

A solution of the corresponding 10 mmol of keto ester and 30 mmol of n-butylamine in 60 mL of dry ether was added to a dried flask under a nitrogen atmosphere. The system was cooled to –15 °C and 40 mmol Et$_3$N was added to the solution. Then 7 mmol of 1.0 M solution of TiCl$_4$ in CH$_2$Cl$_2$ was added slowly. The reaction mixture was stirred for 40–60 min at room temperature. Then the solution quenched with saturated NaHCO$_3$ was stirred and filtered, and the obtained filtrate was extracted with ether. The organic layers were washed with brine and dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure.

3.3.1. Methyl 4-(butylimino)-4-(furan-2-yl)butanoate (3a)

Yield 90%; brown oil; Anal. Calcd. for C$_{13}$H$_{19}$NO$_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.78; H, 8.01; N, 5.85. IR (neat, cm^{-1}): 3145, 3116, 2956, 2920, 2875, 1710 (C=O), 1622 (C=N), 1573, 1438, 1255, 1156, 1074, 883, 745. ^{1}H NMR (500 MHz, CDCl$_3$, δ, ppm): 0.97 (3H, t, J = 6.0 Hz, –C̲H$_3$) 1.43 (2H, m, –C̲H$_2$–), 1.76 (2H, m, –C̲H$_2$–), 2.55 (2H, m, –C̲H$_2$–), 2.90 (2H, m, –C̲H$_2$), 3.54 (2H, t, J = 6.2 Hz, –C̲H$_2$–), 3.71 (3H, s, –OC̲H$_3$), 6.45 (1H, dd, J = 3.4 and 1.8 Hz, furan –C̲H–), 6.78 (1H, d, J = 3.4 Hz, furan –C̲H–), 7.49 (1H, d, J = 1.7 Hz furan –C̲H–). ^{13}C NMR (125 MHz, CDCl$_3$, δ/ppm): δ 13.5 (–C̲H$_3$–), 20.6 (–C̲H$_2$–), 23.5 (–C̲H$_2$–), 30.6 (–C̲H$_2$–), 32.0 (–C̲H$_2$–), 51.9 (–OC̲H$_3$–), 52.5 (–C̲H$_2$–), 110.8 (furan –C̲H–), 144.0 (furan –C̲H–), 142.7 (furan –C̲H–), 153.1 (furan –C̲H–), 157.6 (–C̲=N–), 172.6 (–C̲=O). GC-MS (m/z): 41, 57, 79, 94, 107, 122, 134, 150, 162, 178, 195, 208, 237 (M$^+$).

3.3.2. Methyl 4-(butylimino)-4-(thiophen-2-yl)butanoate (3b)

Yield 85%; brown oil; Anal. Calcd. for C$_{13}$H$_{19}$NO$_2$S: C, 61.63; H, 7.56; N, 5.53; S, 12.66. Found: C, 61.60; H, 7.50; N, 5.28; S, 12.60. IR (neat, cm^{-1}): 3145, 3116, 2956, 2920, 2854, 1737 (C=O), 1618 (C=N), 1573, 1438, 1255, 1156, 1074, 883, 745. ^{1}H NMR (500 MHz, CDCl$_3$, δ, ppm): 0.97 (3H, t, J = 7.4 Hz, –C̲H$_3$), 1.14 (2H m, –C̲H$_2$–), 1.69 (2H, m, –C̲H$_2$–), 2.56 (2H, m, –C̲H$_2$–), 3.02 (2H, m, –C̲H$_2$–), 3.53 (2H, t, J = 7.1

Hz, =NCH$_2$–), 3.72 (3H, s, –OCH$_3$), 7.03 (1H, dd, $J = 5.1$ and 3.7 Hz, thiophen –CH), 7.31 (1H, dd, $J = 3.7$ and 1.1 Hz, thiophen –CH–), 7.33 (1H, dd, $J = 5.1$ and 1.0 Hz, thiophen –CH–). ^{13}C NMR (125 MHz, CDCl$_3$, δ/ppm): 13.5 (–CH$_3$), 20.9 (–CH$_2$–), 24.1 (–CH$_2$–), 28.4 (–CH$_2$–), 31.7 (–CH$_2$–), 33.2 (–CH$_2$–), 50.7 (–OCH$_3$–), 125.9 (thiophen –CH–), 127.3 (thiophen –CH–), 128.5 (thiophen –CH–), 146.6 (thiophen –CH–), 161.1 (–C=N–), 172.7 (–C=O). GC-MS (m/z): 41, 57, 82, 97, 110, 123, 136, 150, 166, 178, 196, 238, 252 (M$^+$-1).

3.3.3. Methyl 5-(butylimino)-5-(furan-2-yl)pentanoate (3c)

Yield 98%; brown oil; Anal. Calcd. for C$_{14}$H$_{21}$NO$_3$: C, 66.91; H, 8.42; N, 5.57. Found: C, 66.88; H, 8.38; N, 5.52. IR (neat, cm^{-1}): 3145, 3116, 2956, 2920, 2875, 1740 (C=O), 1627 (C=N), 1573, 1438, 1255, 1156, 1074, 883, 740. ^{1}H NMR (500 MHz, CDCl$_3$, δ, ppm): 0.98 (3H, t, $J = 7.4$ Hz, –CH$_3$), 1.42 (2H, m, –CH$_2$–), 1.73 (2H, t, $J = 7.5$ Hz, –CH$_2$–), 1.88 (2H, m, –CH$_2$–), 2.42 (2H, t, $J = 7.1$ Hz –CH$_2$–), 2.64 (2H, m, –CH$_2$–), 3.52 (2H, t, $J = 7.5$ Hz, –CH$_2$–), 3.69 (3H, s, –OCH$_3$), 6.44 (1H, dd, $J = 3.4$ and 1.8 Hz, furan –CH–), 6.78 (1H, d, $J = 3.4$ Hz, furan –CH–), 7.47 (1H, d, $J = 3.6$ Hz furan –CH–). ^{13}C NMR (125 MHz, CDCl$_3$, δ/ppm): 13.5 (–CH$_3$–), 20.4 (–CH$_2$–), 20.6 (–CH$_2$–), 22.6 (–CH$_2$–), 22.7 (–CH$_2$–), 33.2 (–CH$_2$–), 34.0 (–CH$_2$–), 50.7 (–OCH$_3$–), 110.6 (furan –CH–), 111.4 (furan –CH–), 143.7 (furan –CH–), 153.3 (furan –CH–), 158.8 (–C=N–), 173.2 (–C=O). GC-MS (m/z): 41, 55, 81, 94, 109, 123, 136, 150, 164, 178, 192, 208, 251 (M$^+$).

3.3.4. Methyl 5-(butylimino)-5-(thiophen-2-yl)pentanoate (3d)

Yield 91%; brown oil; Anal. Calcd. for C$_{14}$H$_{21}$NO$_2$S: C, 62.89; H, 7.92; N, 5.24; S, 11.99. Found: C, 62.84; H, 7.86; N, 5.20; S, 11.95. IR (neat, cm^{-1}): 3145, 3116, 2956, 2920, 2854, 1732 (C=O), 1618 (C=N), 1573, 1438, 1255, 1156, 1074, 883, 793. ^{1}H NMR (500 MHz, CDCl$_3$, δ/ppm): 1.04 (3H, t, $J = 6.6$ Hz, Aliphatic –CH$_3$), 1.42 (2H, m, –CH$_2$–), 1.78 (2H, m, –CH$_2$–), 2.03 (2H, m, –CH$_2$–), 2.42 (2H, t, $J = 7.7$ Hz, –CH$_2$–), 2.46 (2H, t, $J = 5.7$ Hz, –CH$_2$–), 3.74 (3H, s, –OCH$_3$), 3.82 (2H, t, 5.3 Hz, =NCH$_2$–), 7.13 (1H, t, $J = 7.5$ Hz, thiophen –CH), 7.26 (1H, dd, $J = 7.5$ and 1.6 Hz, thiophen –CH–), 7.51 (1H, dd, $J = 7.4$ and 1.5 Hz, thiophen –CH–). ^{13}C NMR (125 MHz, CDCl$_3$, δ/ppm): 13.8 (–CH$_3$), 20.9 (–CH$_2$–), 22.7 (–CH$_2$–), 28.4 (–CH$_2$–), 33.2 (–CH$_2$–), 33.7 (–CH$_2$–), 51.0 (–OCH$_3$–), 51.6 (–CH$_2$), 126.2 (thiophen –CH–), 127.0 (thiophen –CH–), 128.5 (thiophen –CH–), 147.4 (thiophen –CH–), 162.2 (–C=N–), 173.5 (–C=O). GC-MS (m/z): 41, 57, 82, 97, 110, 123, 139, 153, 166, 180, 194, 238, 266 (M$^+$-1).

3.4. Determination of antioxidant activity by the scavenging of the stable radical DPPH

Equal volumes of 0.02% DPPH in methanol were added to different concentrations of test compounds (250–1000 μM/mL) in methanol, mixed well, and kept in dark for 30 min. The absorbance at 517 nm was measured.[25] Plotting the percentage DPPH • scavenging against concentration gave the standard curve and the percentage scavenging was calculated from the following equation:

$$\text{DPPH radical scavenging activity } (\%) = (A_0 - A_1/A_0) \times 100$$

A_0 and A_1 are the absorbance of blank (without sample) and test sample, respectively. Ascorbic acid, BHA, and BHT were used as standards for comparison.

References

1. Schiff, H. *Justus Liebings Ann. Chem.* **1864**, *131*, 118-119.

2. Eftekhari-Sis, B.; Zirak, M. *Chem. Rev.* **2017**, *117*, 8326-8419.

3. Balsells, J.; Mejorado, L.; Phillips, M.; Ortega, F.; Aguirre, G.; Somanathan, R. et al. *Tetrahedron-Asymmetry* **1998**, *9*, 4135-4142.

4. S. Krishnaraj, M.; Viswanathamurthi, P.; Sivakumar, S. *Transition Metal Chem.* **2008**, *33*, 643-648.

5. Eswaran, S.; Adhikari, A. V.; Shetty, N. S. *Eur. J. Med. Chem.* **2009**, *44*, 4637-4647.

6. Przybylski, P.; Huczynski, A.; Pyta, K.; Brzezinski, B.; Bartl, F. *Curr. Org. Chem.* **2009**, *13*, 124-148.

7. Varma, M.; Pandeya, S., N.; Singh, K. N.; Stables, J. P. *Acta Pharm.* **2004**, *54*, 49-56.

8. Wang, X.; Yin, J.; Shi, L.; Zhang, G. P.; Song, B. *Eur. J. Med. Chem.* **2014**, *77*, 65-74.

9. Ashraf M. A.; Wajid, A.; Mahmood, K.; Maah, M. J.; Yusoff, I. *Oriental Journal of Chemistry* **2011**, *27*, 363-372.

10. Proetto, M.; Liu, W. K.; Hagenbach, A.; Abram, U.; Gust, R. *Eur. J. Med. Chem.* **2012**, *53*, 168-175.

11. Chakraborti, A. K.; Bhagat, S.; Rudrawar, S. *Tetrahedron Lett.* **2004**, *45*, 7641-7644.

12. Qin, W. L.; Long, S.; Panunzio, M.; Biondi, S. *Molecules* **2013**, *18*, 12264-12289.

13. Vazquez, M. A.; Landa, M.; Reyes, L.; Miranda, R.; Tamariz, J.; Delgado, F. *Synthetic Commun.* **2004**, *34*, 2705-2718.

14. Gopalakrishnan, M.; Sureshkumar, P.; Kanagarajan, V.; Thanusu, J. *Res. Chem. Intermediat.* **2007**, *33*, 541-548.

15. Law, M. C.; Cheung, T. W.; Wong, K. Y.; Chan, T. H. *J. Org. Chem.* **2007**, *72*, 923-929.

16. Guzen, K. P.; Guarezemini, A. S.; Orfao, A. T. G.; Cella, R.; Pereira, C. M. P; Stefani, H. A. *Tetrahedron Lett.* **2007**, *48*, 1845-1848.

17. Meotti, F. C.; Silva, D. O.; dos Santos, A. R. S.; Zeni, G.; Rocha, J. B. T.; Nogueira, C. W. *Environmental Toxic. Pharm.* **2003**, *37*, 37-44.

18. Xue, Z. Y.; Liu, L. X.; Jiang, Y.; Yuan, W. C.; Zhang, X. M. *Eur J. Org. Chem.* **2012**, 251-255.

19. Cheemala, M. N.; Knochel, P. *Org. Lett.* **2007**, *9*, 3089-3092.

20. Malkov, A. V.; Vrankova, K.; Stoncius, S.; Kocovsky, P. *J. Org. Chem.* **2009**, *74*, 5839-5849.

21. Wakchaure, V. N.; Kaib, P. S. J.; Leutzsch, M.; List, B. *Angew. Chem. Int. Edit.* **2015**, *54*, 11852-11856.

22. Tang, Y. Z.; Liu, Z. Q. *Cell Biochem. Function.* **2008**, *26*, 185-191.

23. Maekawa, T.; Sakai, M.; Hiroyuki, T.; Murase, K.; Hazama, M.; Sugiyama, Y. Momose, Y. *Chem. Pharm. Bull.* **2003**, *51*, 565-573.

24. Breusch F. L.; Oğuzer, M. *Chem. Berichte* **1954**, *87*, 1225-1228.

25. Bondet, V.; Brand-Williams, W.; Berset, C. *Lebensmittel-Wissenschaft und Technologie* **1997**, *30*, 609-615.

SUPPORTING INFORMATION

Synthesis, characterization, and evaluation of antioxidant activity of new γ- and δ-imino

esters

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1) Content

2. General procedure

The chemicals used in this study were commercially available from Merck (Kenilworth, NJ, USA) and Aldrich (St. Louis, MO, USA) and were used without further purification. Friedel–Crafts acylation was used to synthesize γ- and δ-keto esters. The starting compounds and imines were purified by column chromatography on silica gel (particle sizes 0.063–0.200 mm and 0.040–0.063 mm, respectively). ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively) spectra were recorded using Me$_4$Si as the internal standard in CDCl$_3$. Gas chromatography–mass spectrometry (GC-MS) data were recorded on a Shimadzu QP2010 Plus using a GC-MS column Teknokroma TRB-5MS (30 m × 0.25 mm × 0.25 μm I.D.) (Kyoto, Japan). The operating conditions were as follows: injection temperature 250 °C, helium carrier gas flow 20 psi, split ratio 1/100, E.I 70 eV. Temperature programming: 80 °C (5 min) up to 250 °C at 5 °C/min, hold 30 min. FT-IR spectra were recorded on a Bruker Vertex 70 (Billerica, MA, USA). The *E/Z* ratios of obtained γ- and δ-imino esters (**2a–d**, **3a–d**) were determined by ^{1}H NMR spectrum according to the literature

2.1. Synthesis of γ- and δ-imino esters using Method A (2a-d)

A solution of the corresponding 10 mmol of keto ester and 30 mmol of *p*-anisidine in 60 mL of dry ether was added to a dried flask under nitrogen atmosphere. The system was cooled to −15 °C, and 40 mmol Et$_3$N was added to the solution. Then 10 mmol TiCl$_4$ was added dropwise within 10 min. The reaction mixture was stirred for 24–48 h at room temperature. Then solution quenched with saturated NaHCO$_3$ was stirred for 15 min and filtrated. The filtrate was extracted with ether, washed with brine, and dried over anhydrous Na$_2$SO$_4$, concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel.

2.2. Synthesis of γ- and δ-imino esters using Method B (3a-d)

A solution of the corresponding 10 mmol of keto ester and 30 mmol of n-butylamine in 60 mL of dry ether was added to a dried flask under a nitrogen atmosphere. The system was cooled to −15 °C and 40 mmol Et_3N was added to the solution. Then 7 mmol of 1.0 M solution of $TiCl_4$ in CH_2Cl_2 was added slowly. The reaction mixture was stirred for 40–60 min at room temperature. Then the solution quenched with saturated $NaHCO_3$ was stirred and filtered, and the obtained filtrate was extracted with ether. The organic layers were washed with brine and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure.

Spectral Data
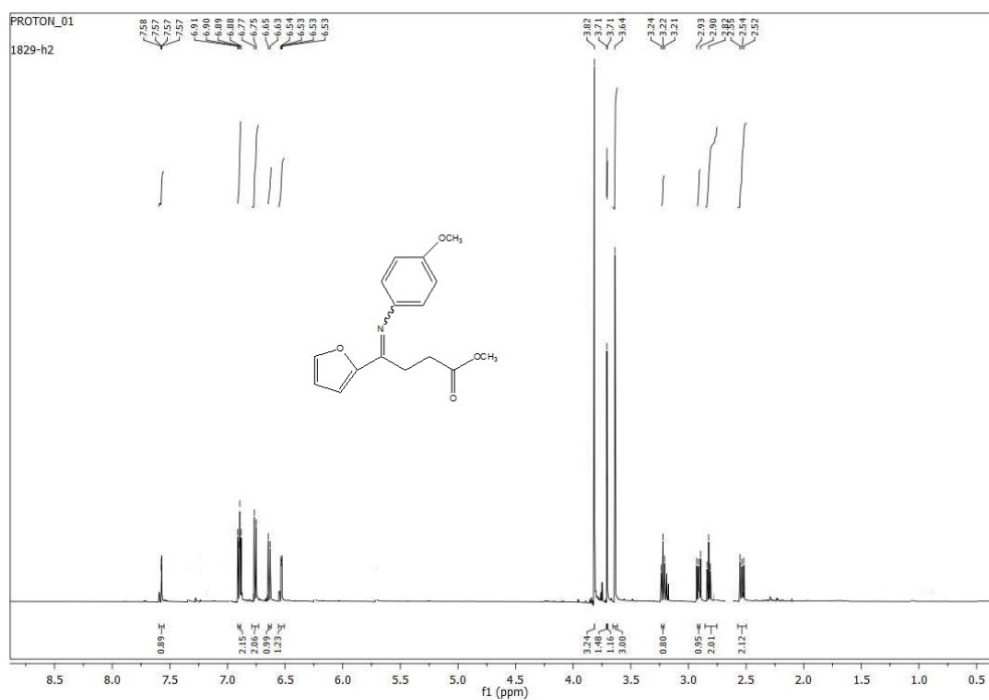
Figure S1. ^{1}H NMR spectra of compound **2a** (500 MHz, CDCl$_3$).
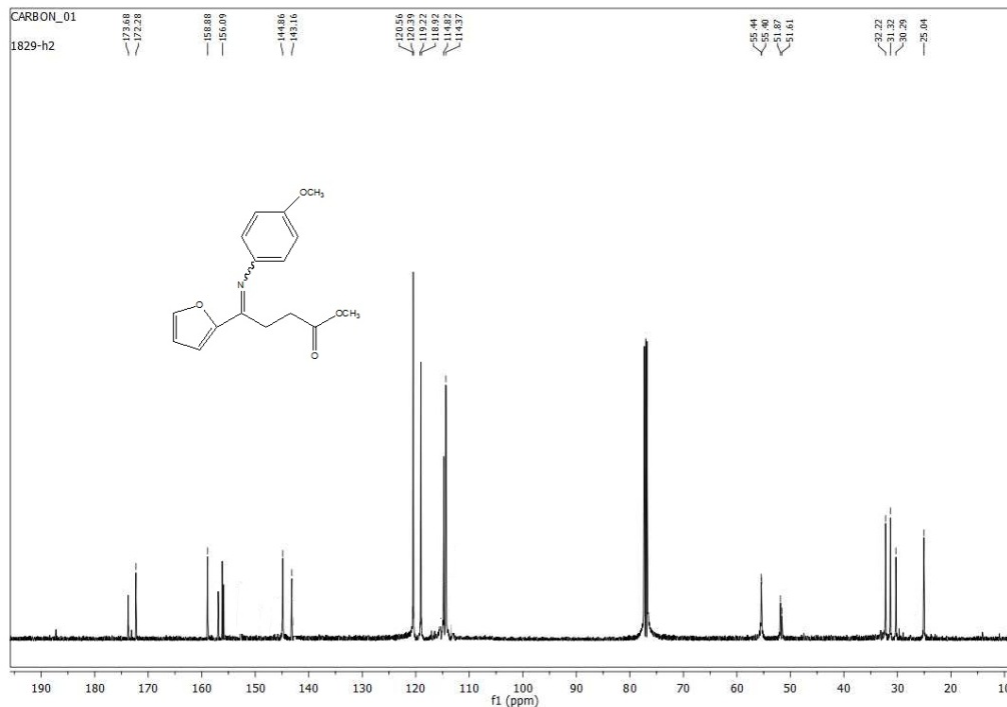
Figure S2. ^{13}C NMR spectra of compound **2a** (125 MHz, CDCl$_3$).

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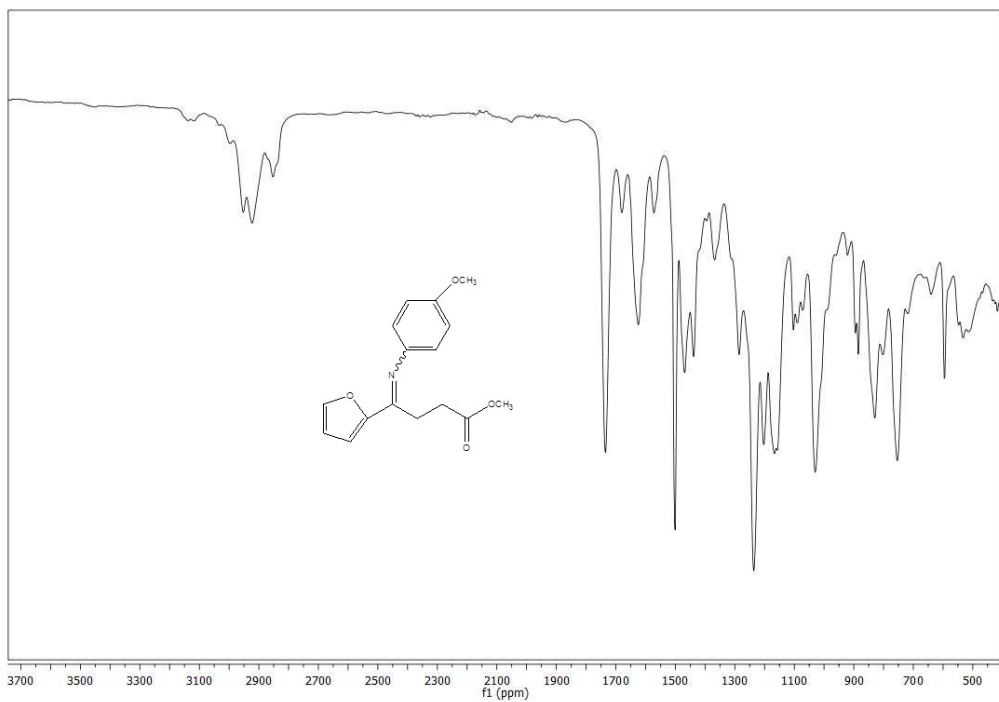
Figure S3. IR spectra of compound **2a**.
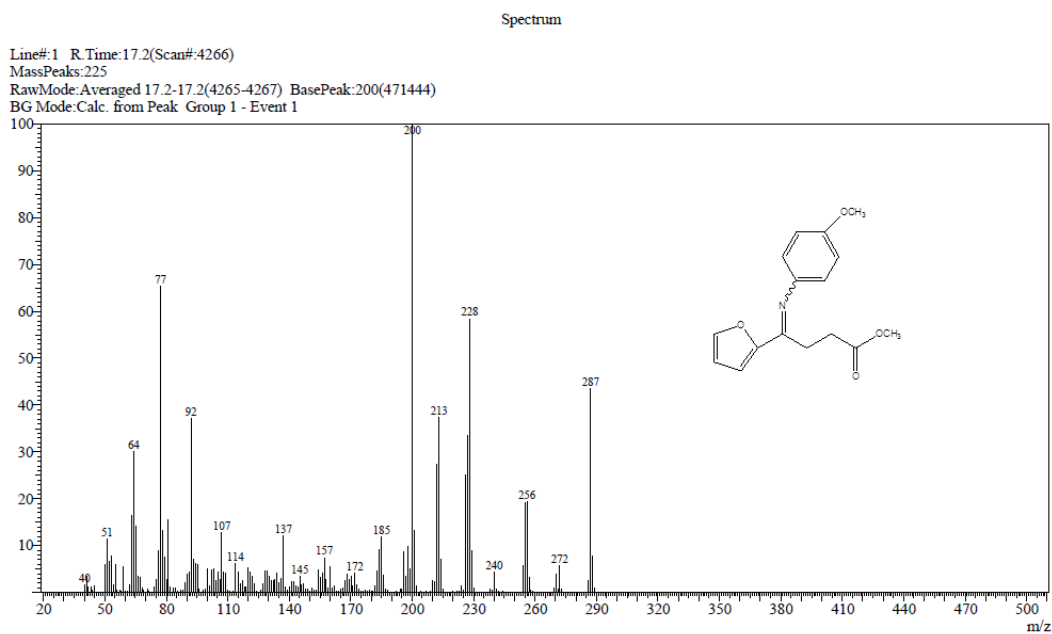
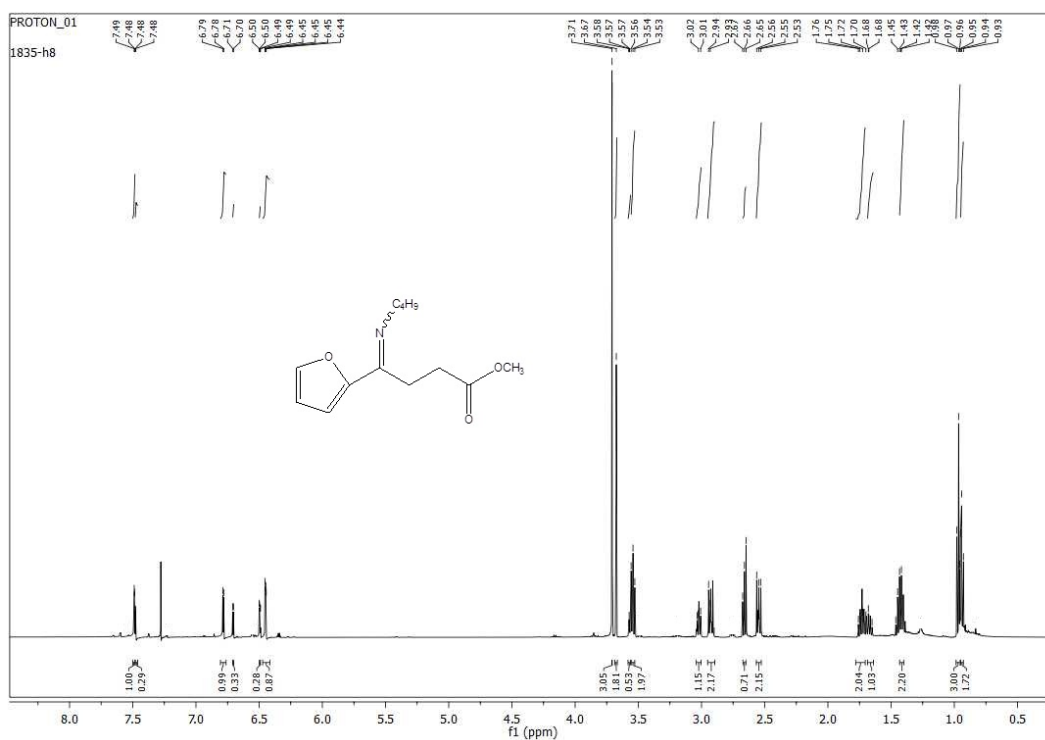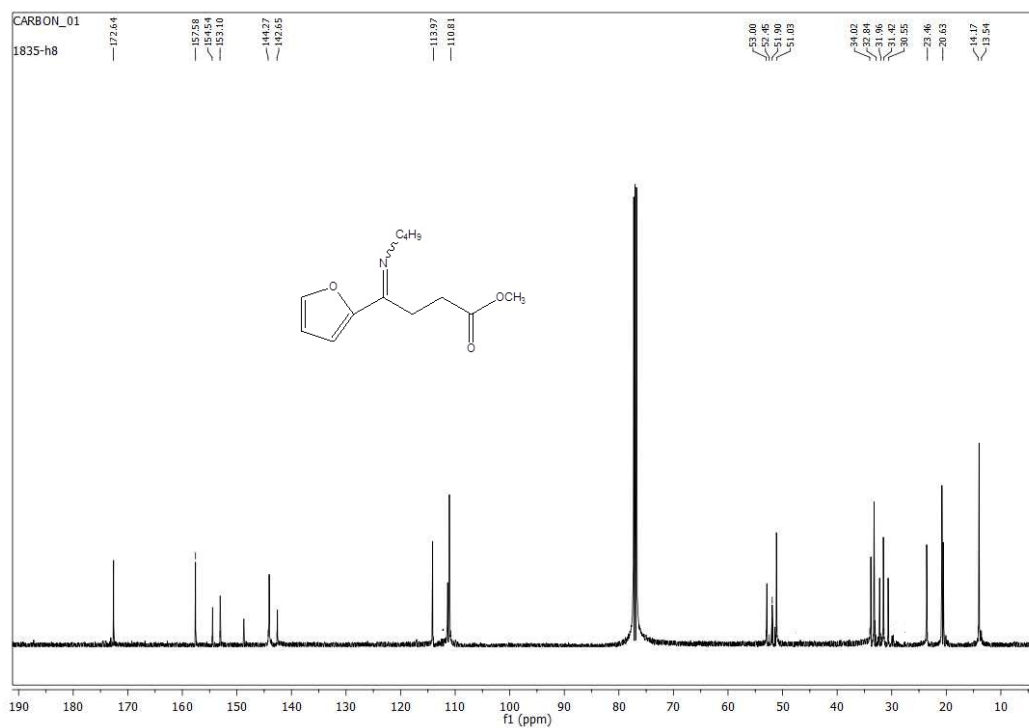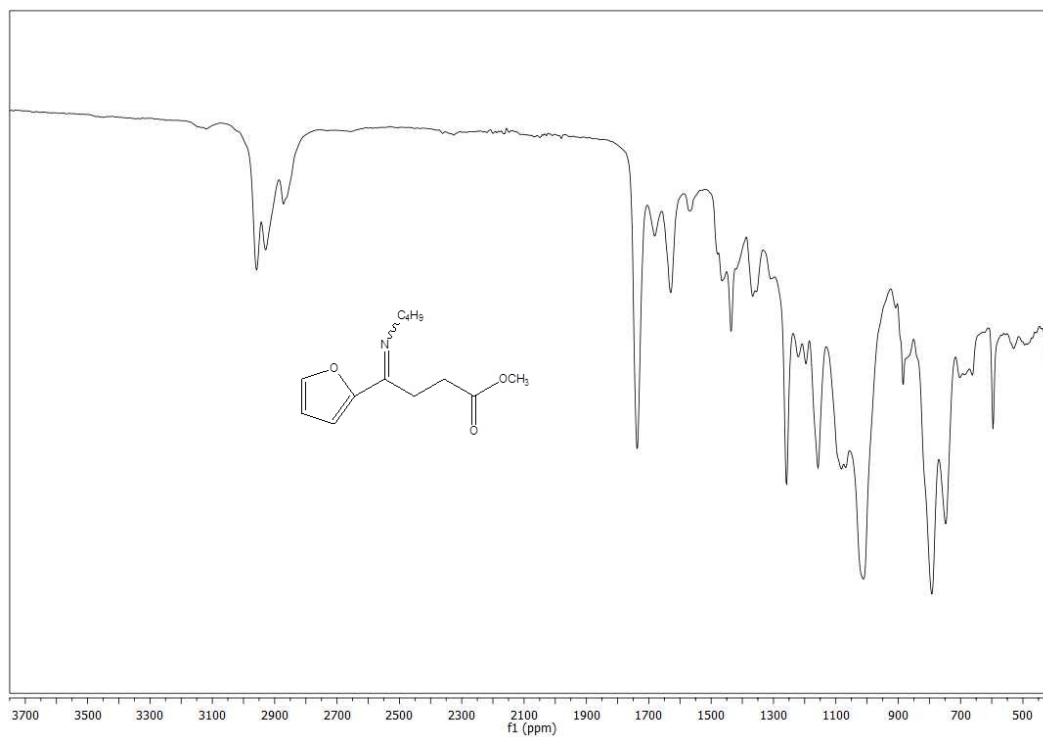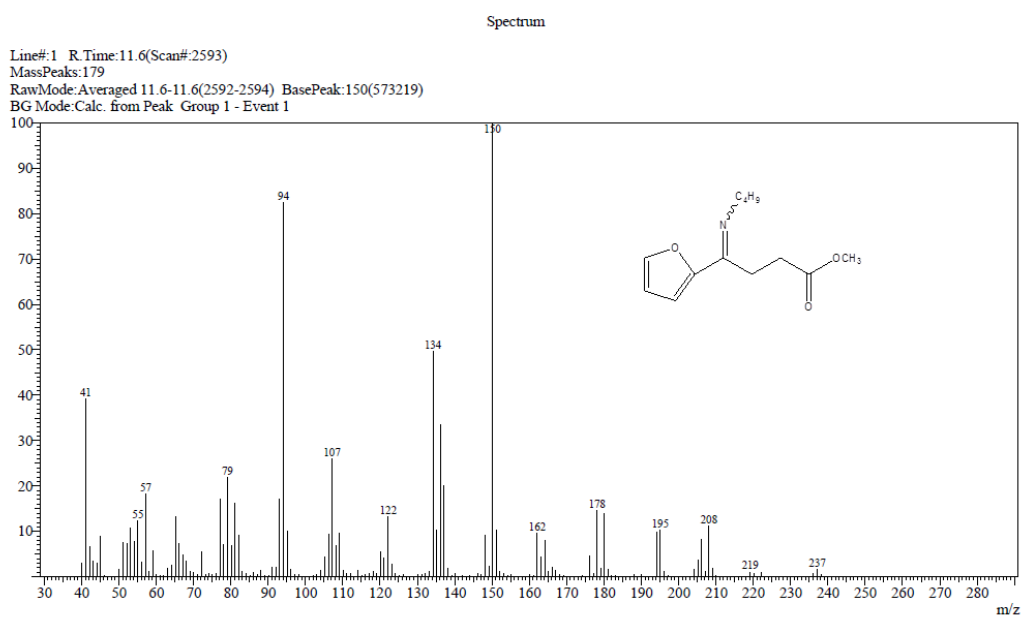**Figure S4.** GC-MS spectra of compound **2a**.
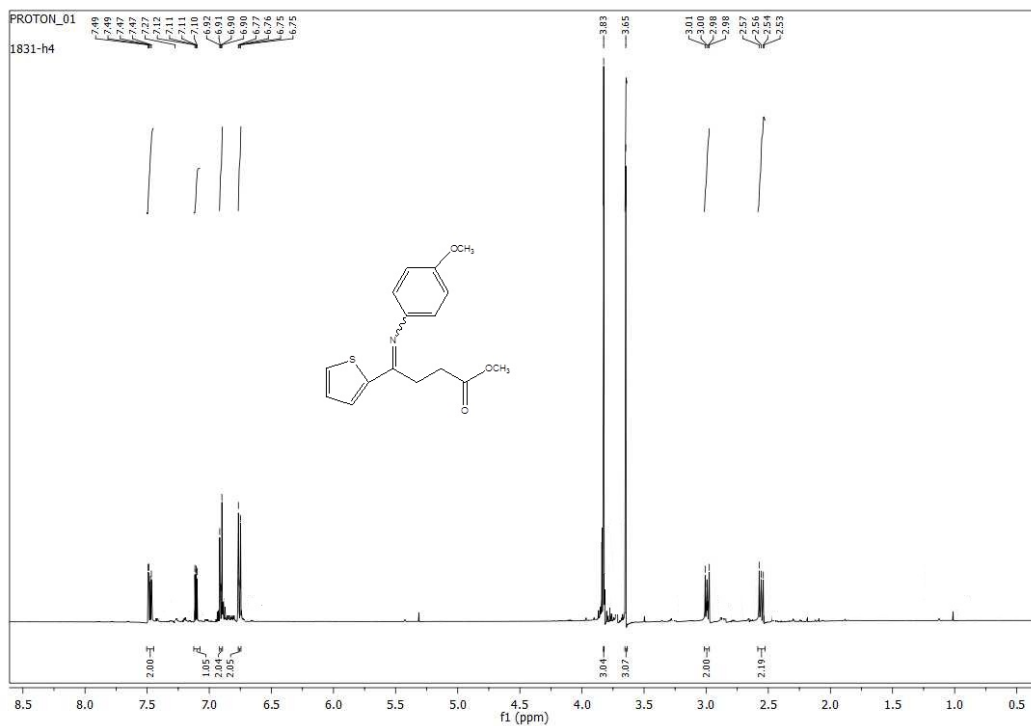
Figure S5. ^{1}H NMR spectrums of compound **2b** (500 MHz, CDCl$_3$).
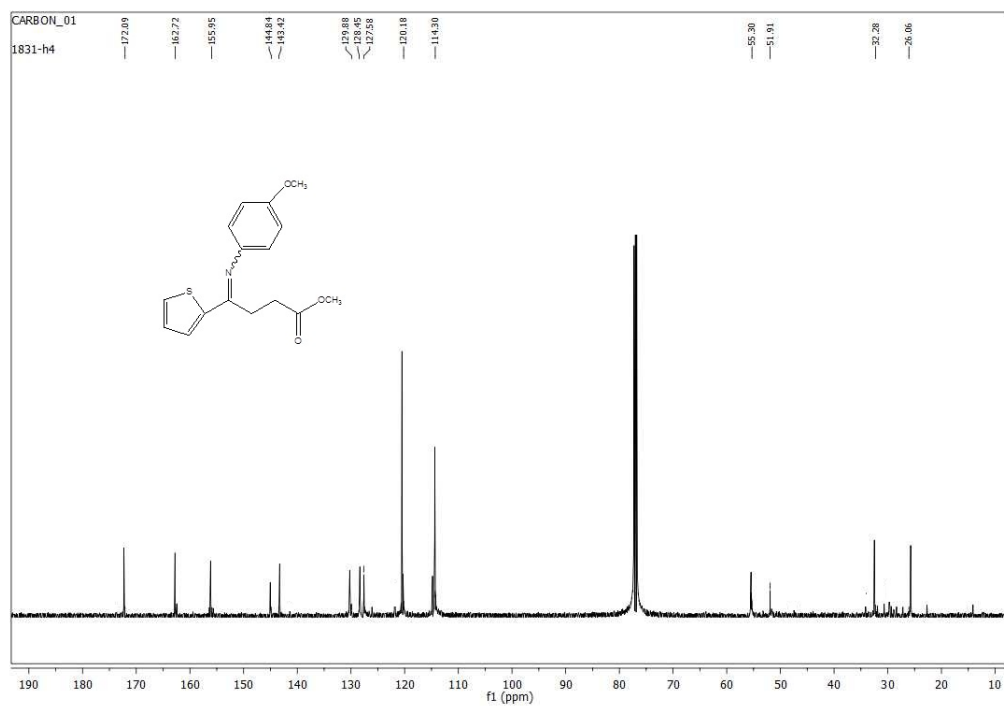
Figure S6. ^{13}C NMR spectra of compound **2b** (125 MHz, CDCl$_3$).
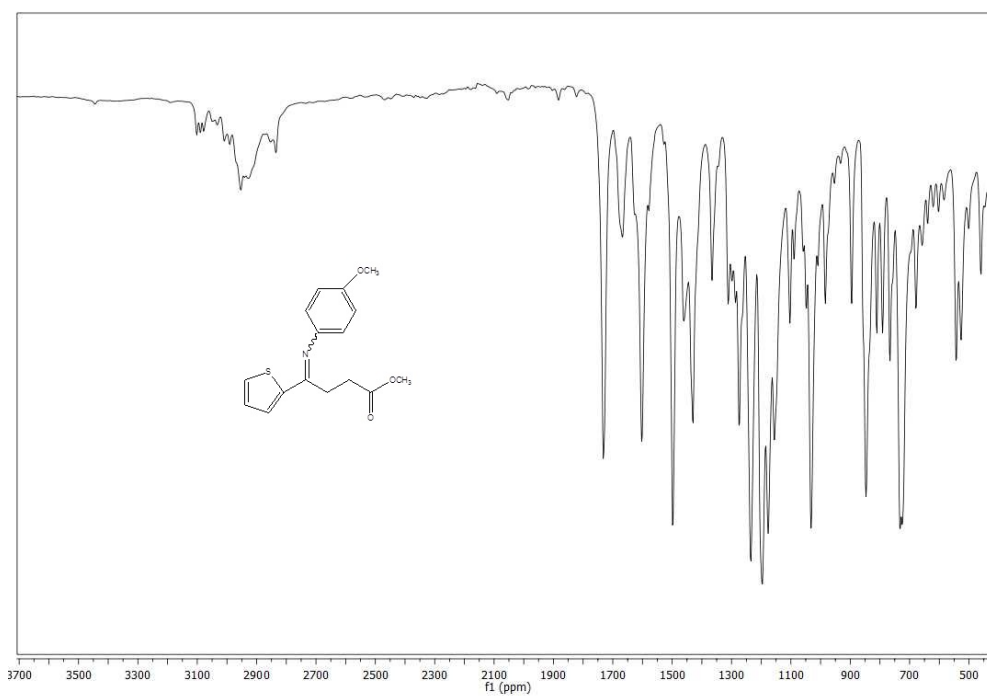
Figure S7. IR spectra of compound **2b**.
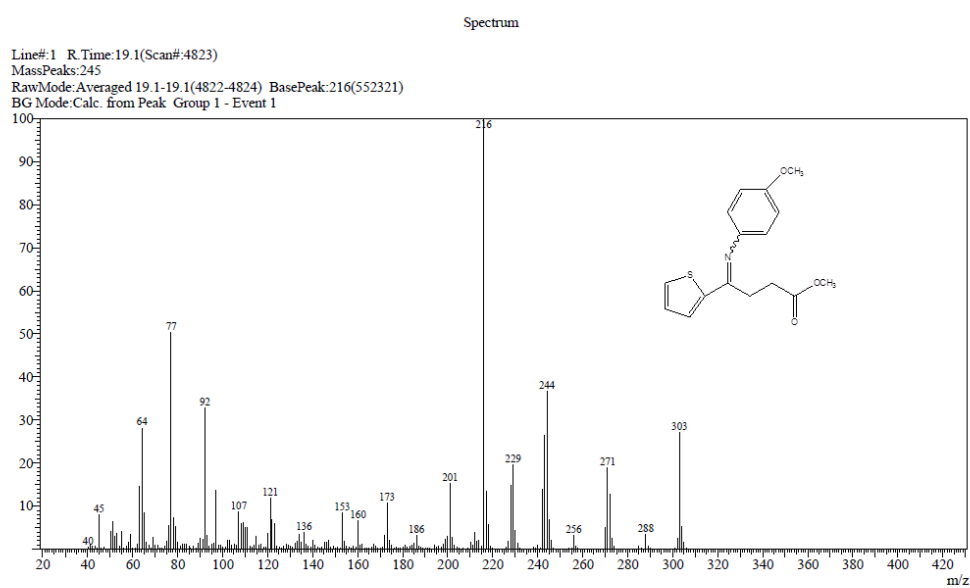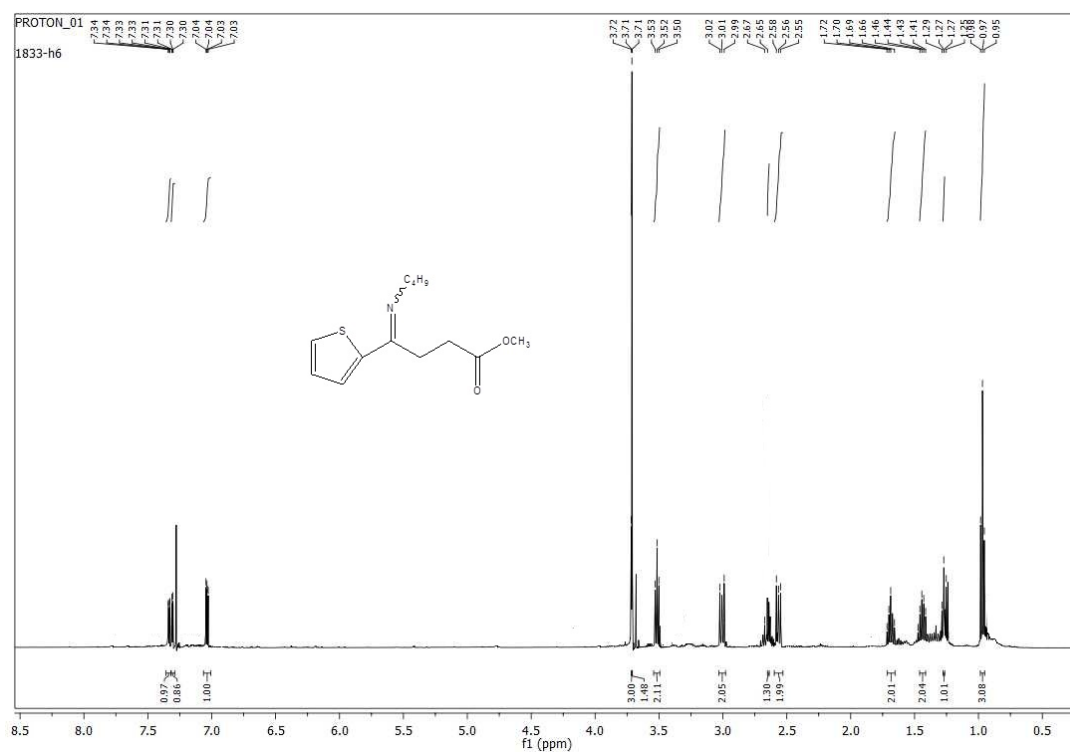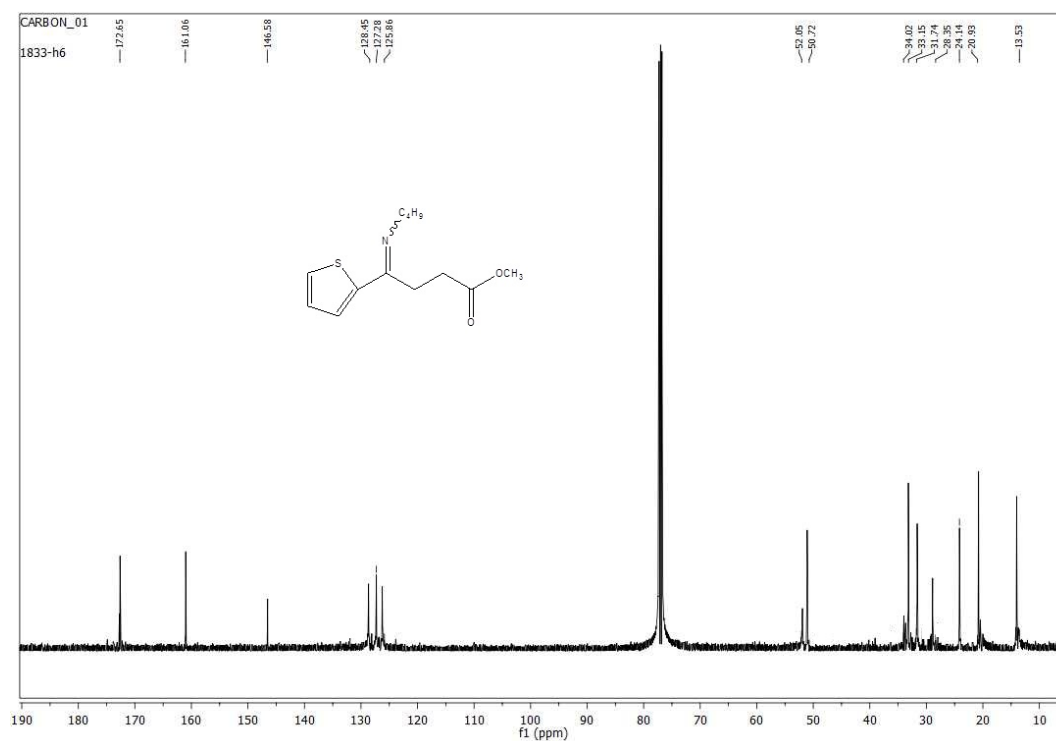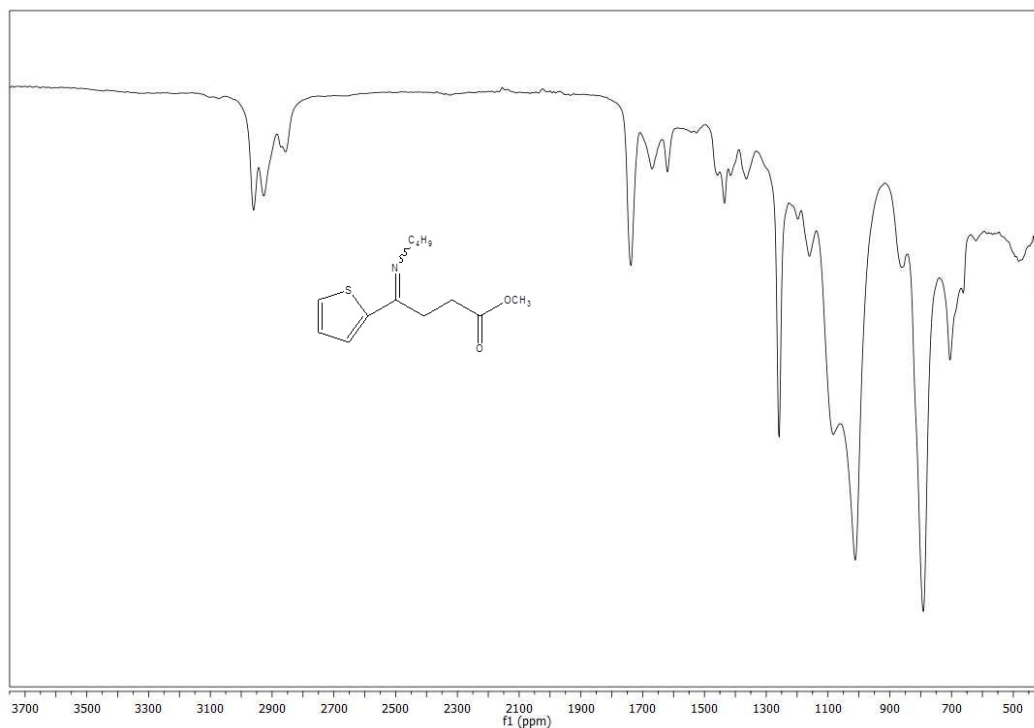

Figure S8. GC-MS spectra of compound **2b**.
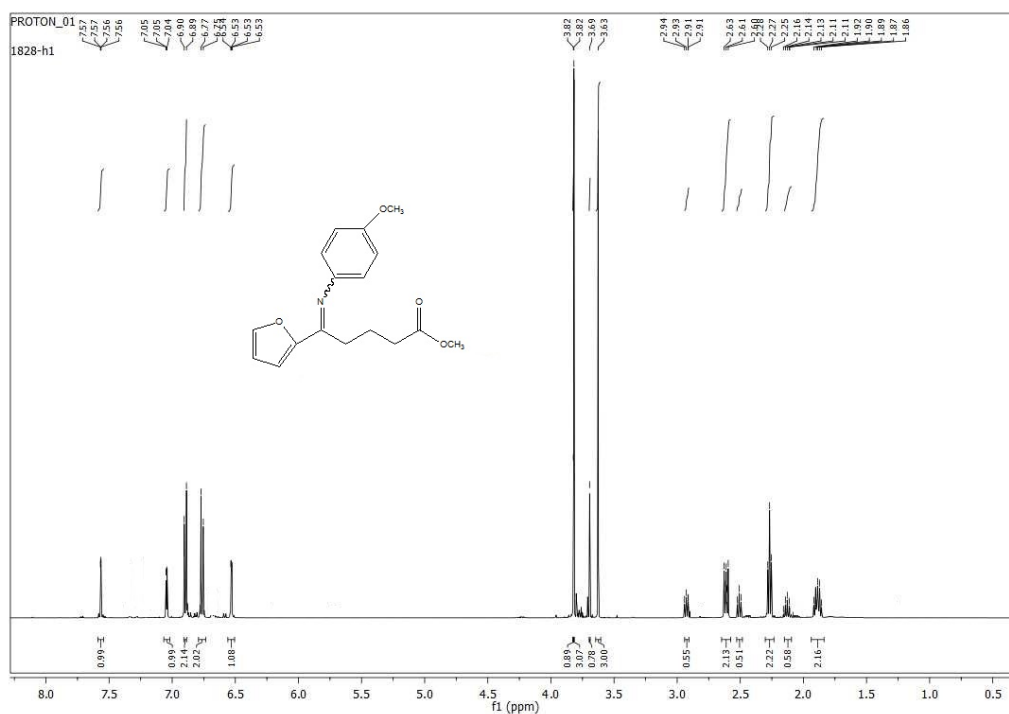
Figure S9. ^{1}H NMR spectra of compound **2c** (500 MHz, CDCl$_3$).
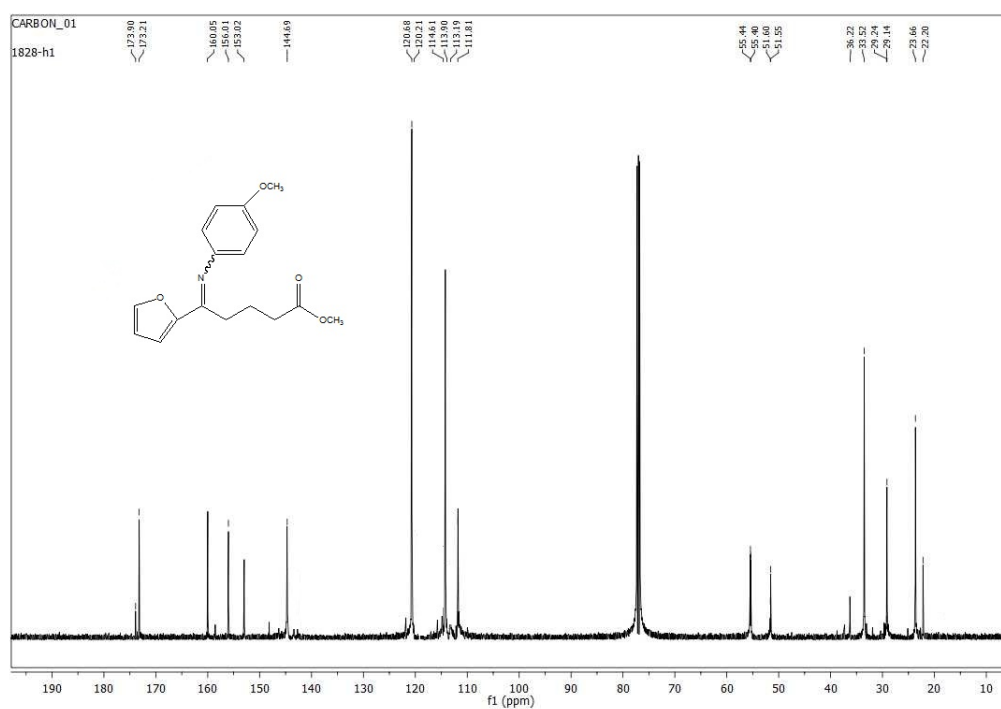
Figure S10. ^{13}C NMR spectra of compound **2c** (125 MHz, CDCl$_3$).
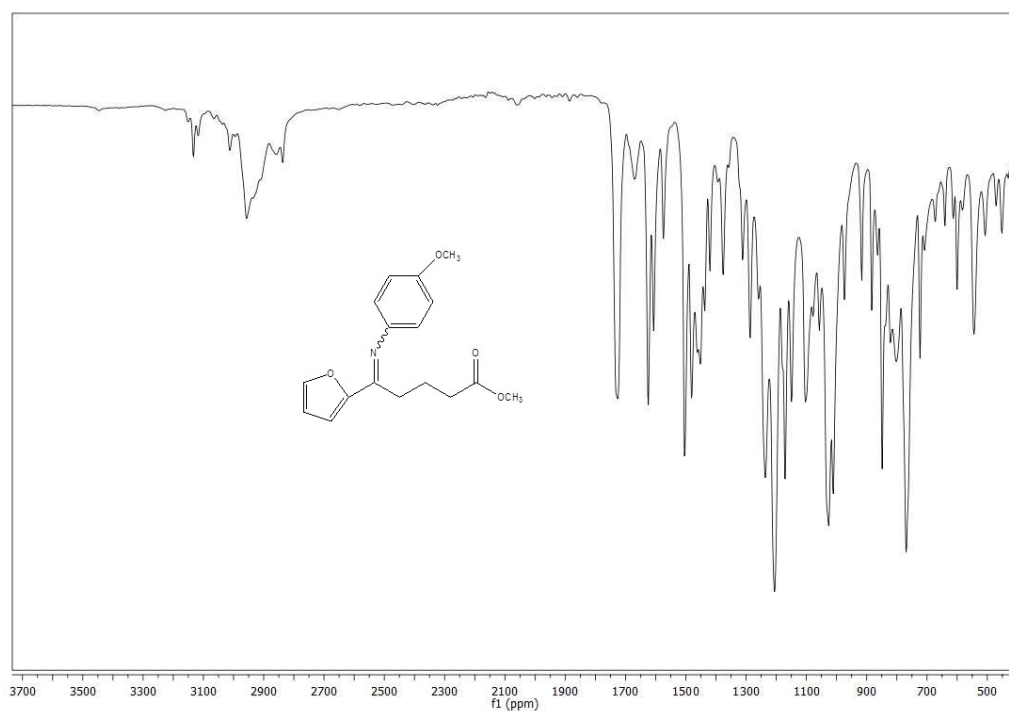
Figure S11. IR spectra of compound **2c**.

Spectrum

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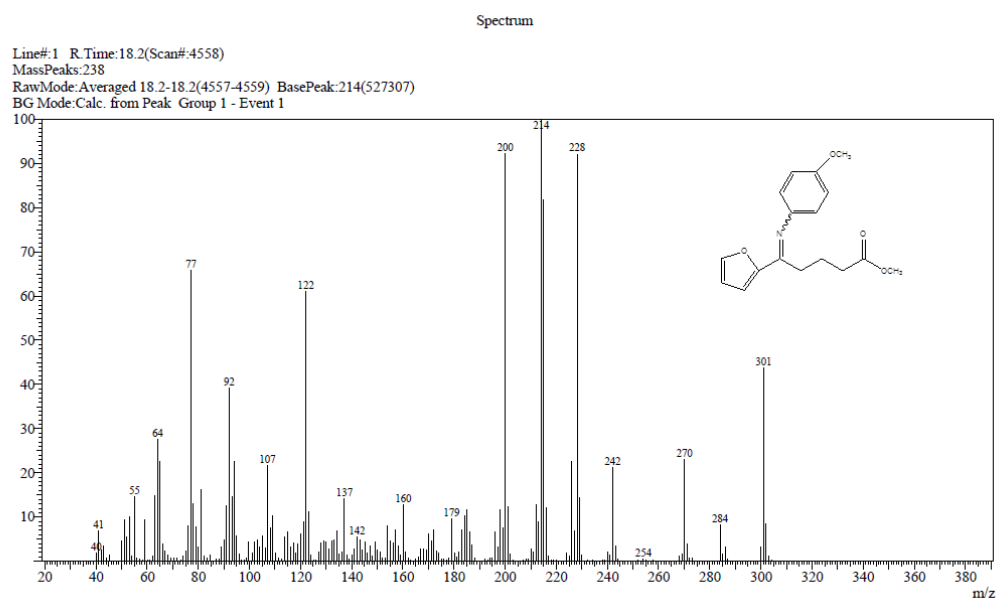
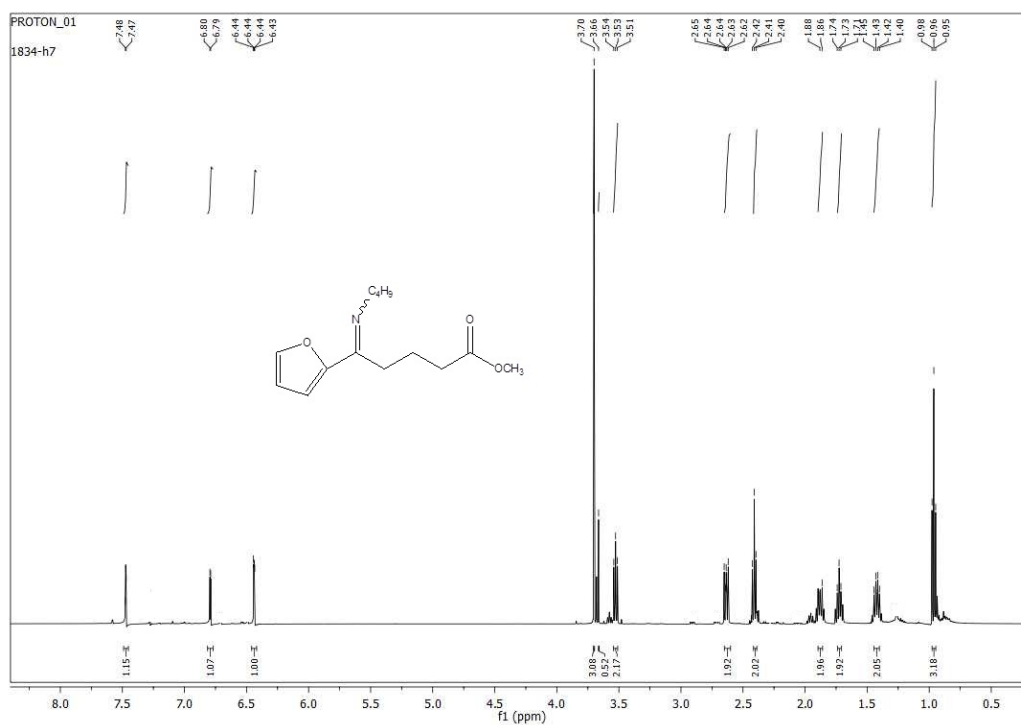**Figure S12.** GC-MS spectra of compound **2c**.
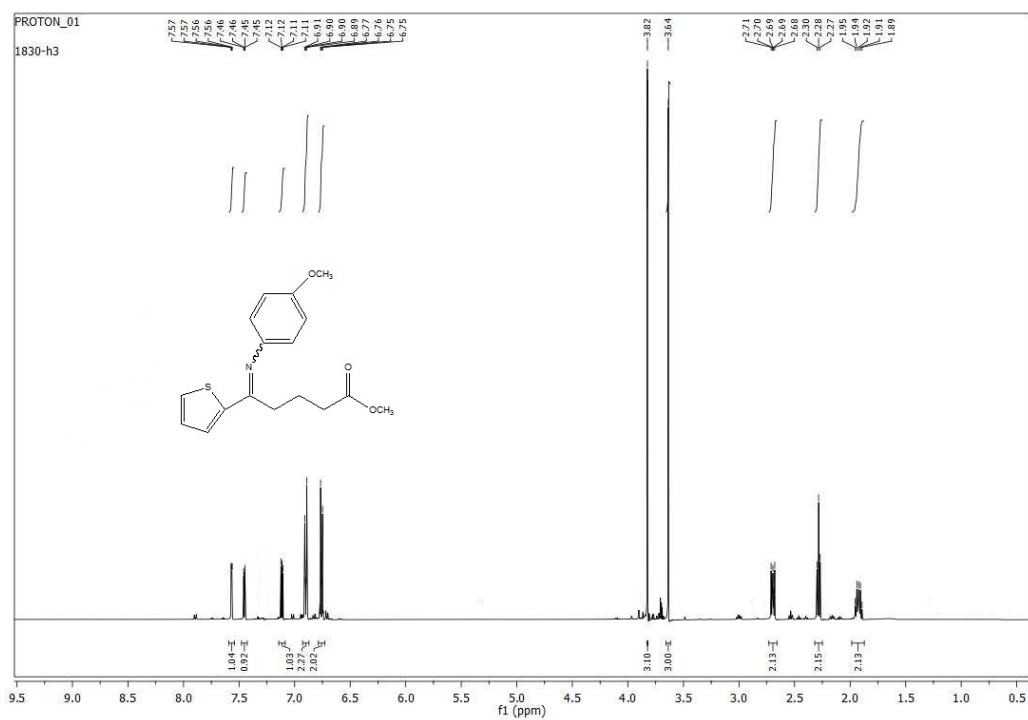
Figure S13. ^{1}H NMR spectra of compound **2d** (500 MHz, CDCl$_3$).
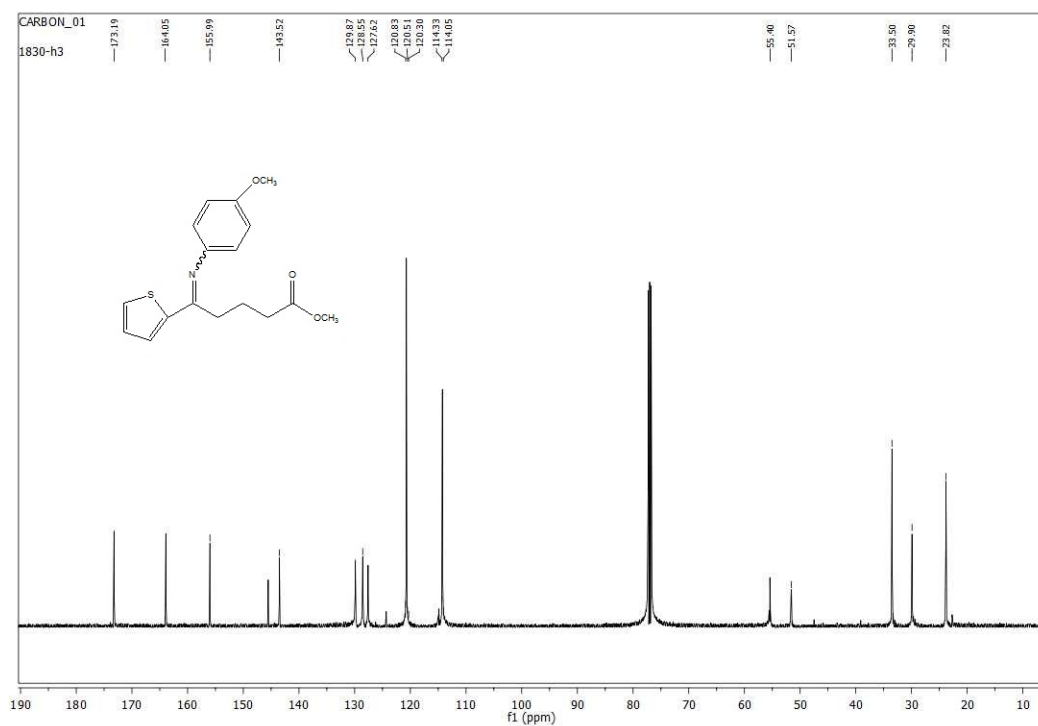
Figure S14. ^{13}C NMR spectra of compound **2d** (125 MHz, CDCl$_3$).
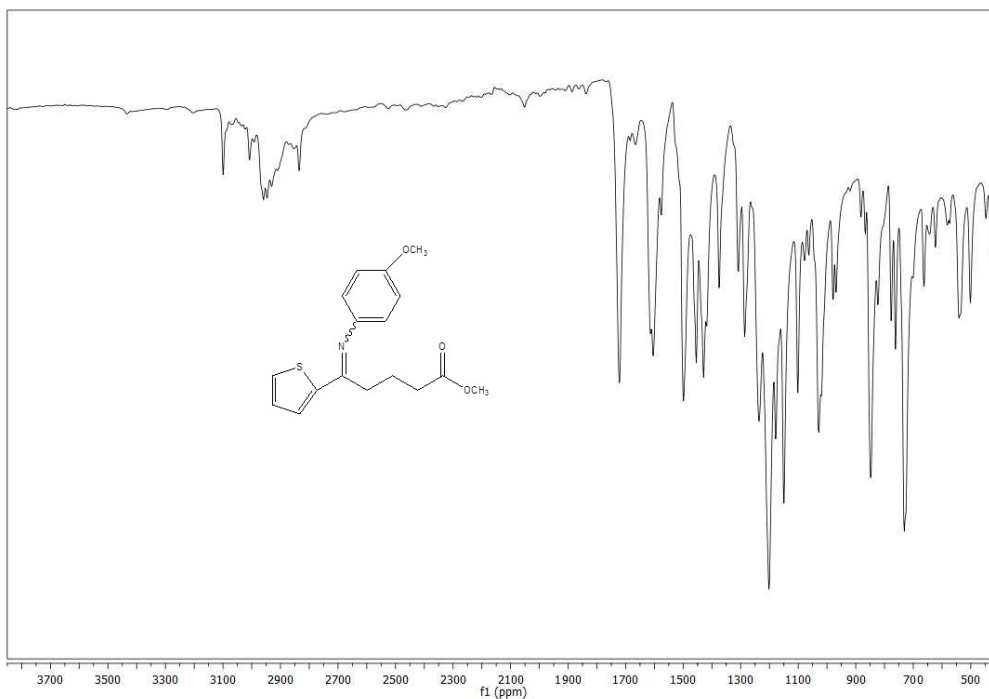
Figure S15. IR spectra of compound **2d**.

Spectrum

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MassPeaks:245
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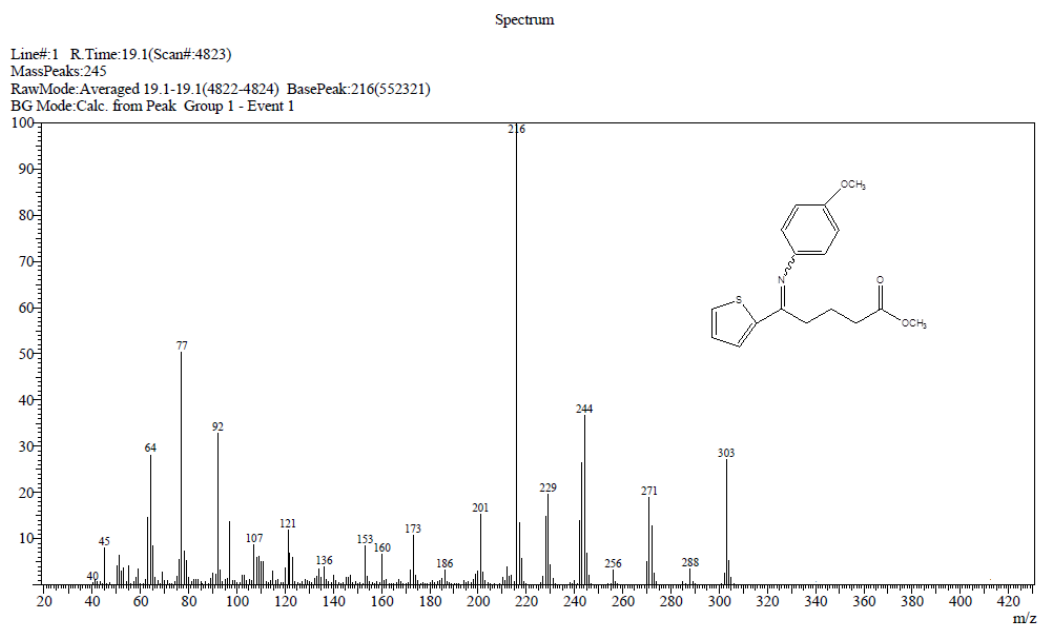
Figure S16. GC-MS spectra of compound **2d**.

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Figure S17. ^{1}H NMR spectra of compound **3a** (500 MHz, CDCl$_3$).
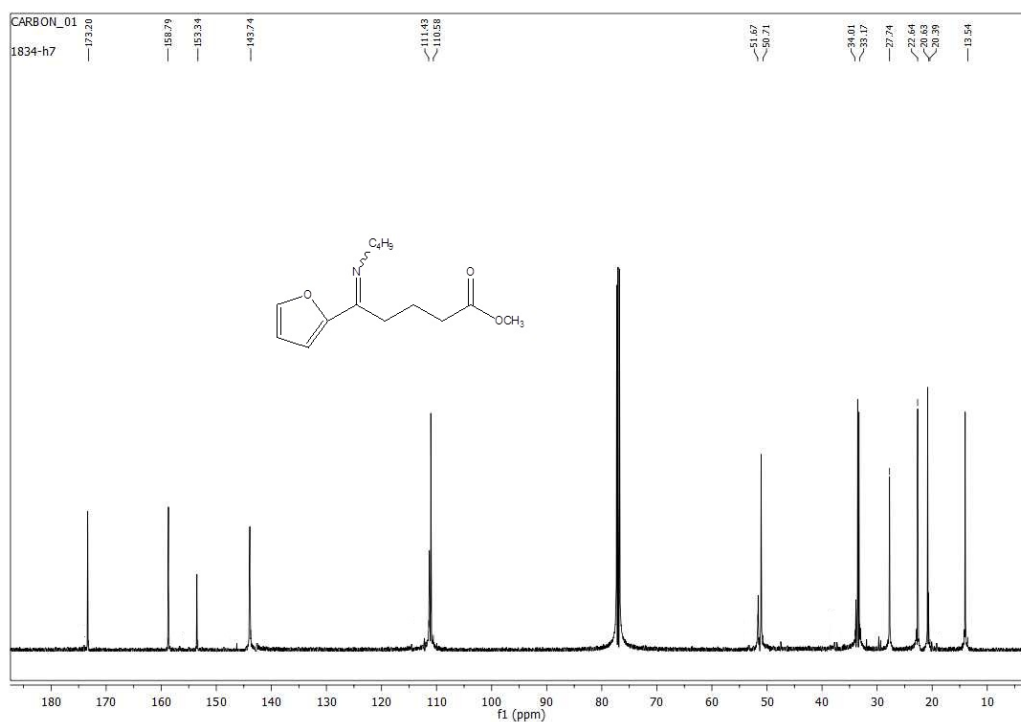

Figure S18. ^{13}C NMR spectra of compound **3a** (125 MHz, CDCl$_3$).
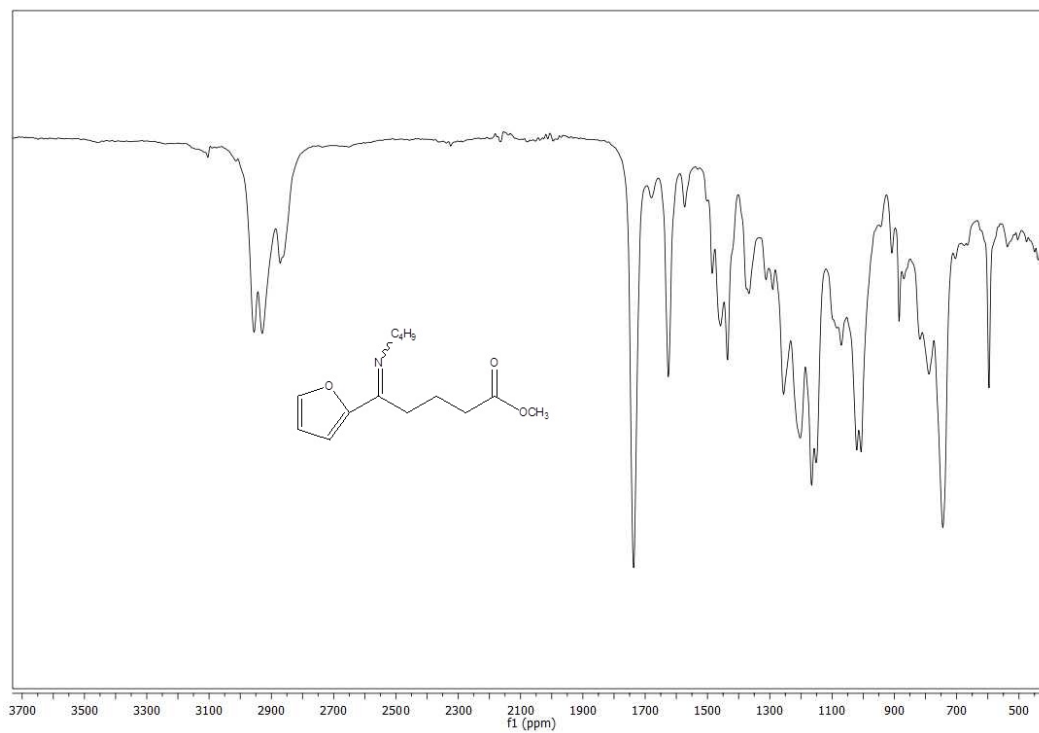
Figure S19. IR spectra of compound **3a**.

Figure S20. GC-MS spectra of compound **3a**.

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Figure S21. ^{1}H NMR spectra of compound **3b** (500 MHz, CDCl$_3$).

Figure S22. ^{13}C NMR spectra of compound **3b** (125 MHz, CDCl$_3$).

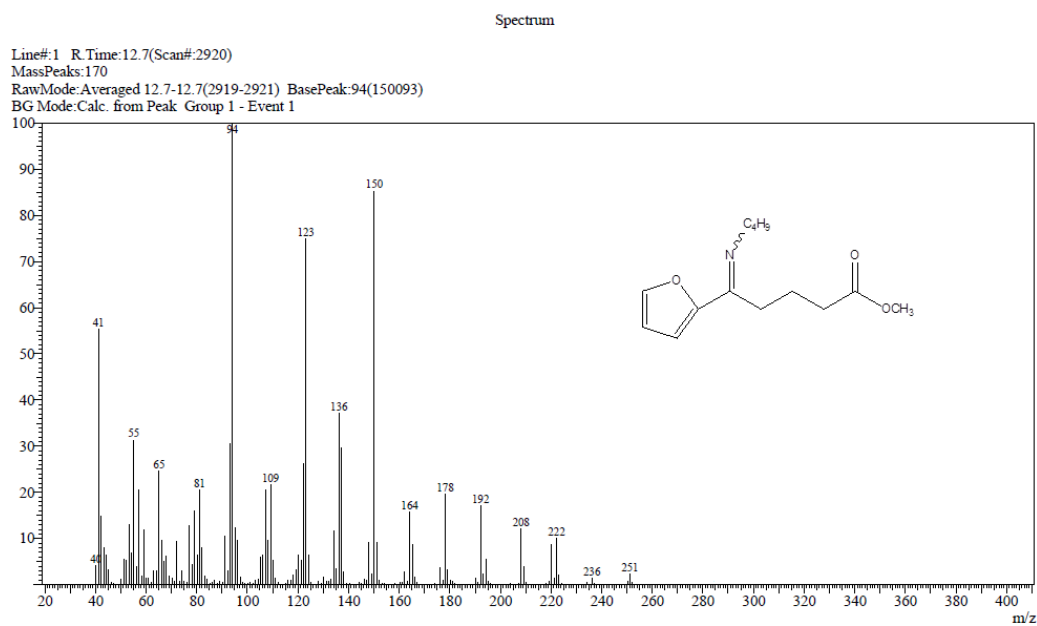**Figure S23.** IR spectra of compound **3b**.
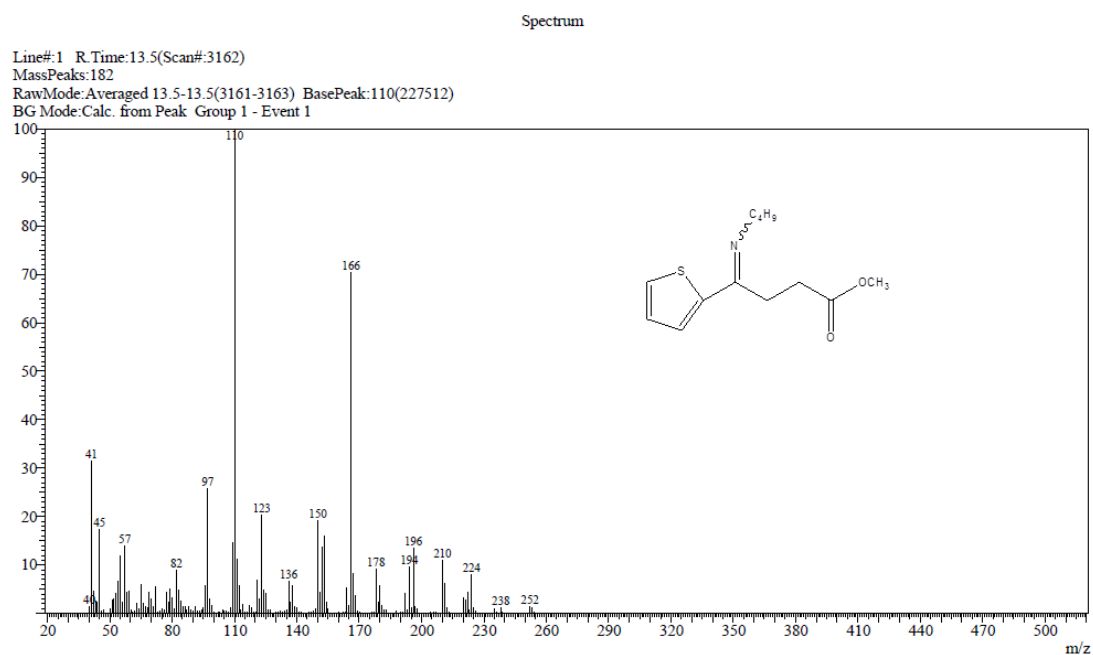
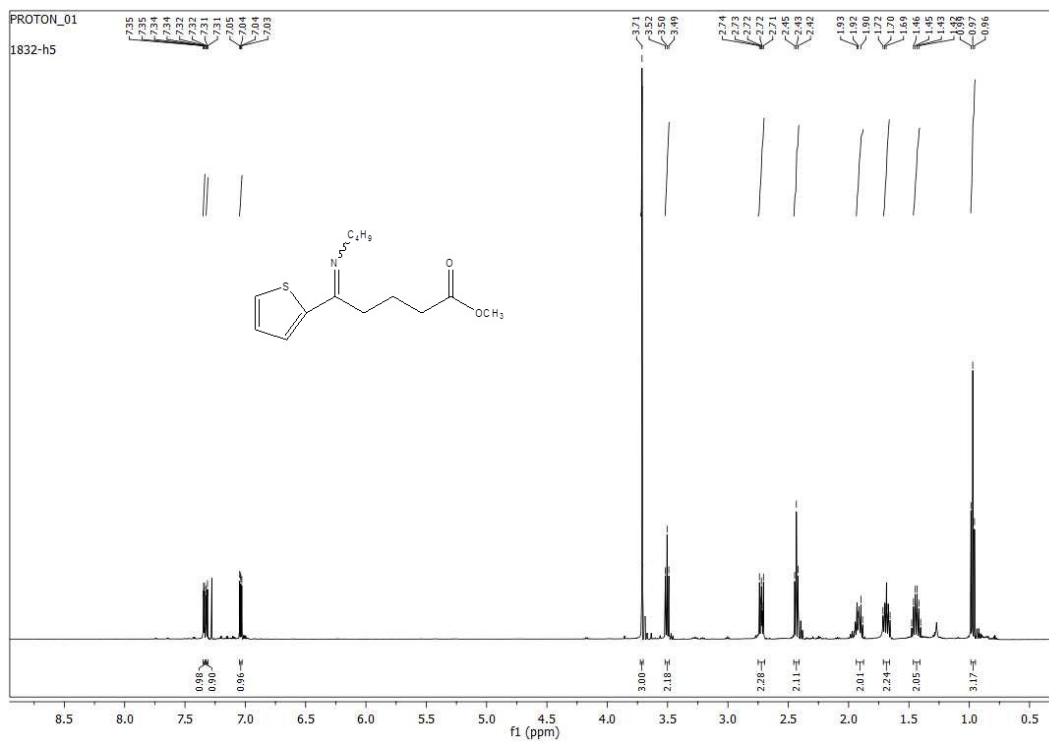**Figure S24.** GC-MS spectra of compound **3b**.

Figure S25. ^{1}H NMR spectra of compound **3c** (500 MHz, CDCl$_3$).

Figure S26. ^{13}C NMR spectra of compound **3c** (125 MHz, CDCl$_3$).

Figure S27. IR spectra of compound **3c.**

Figure S28. GC-MS spectra of compound **3c**.

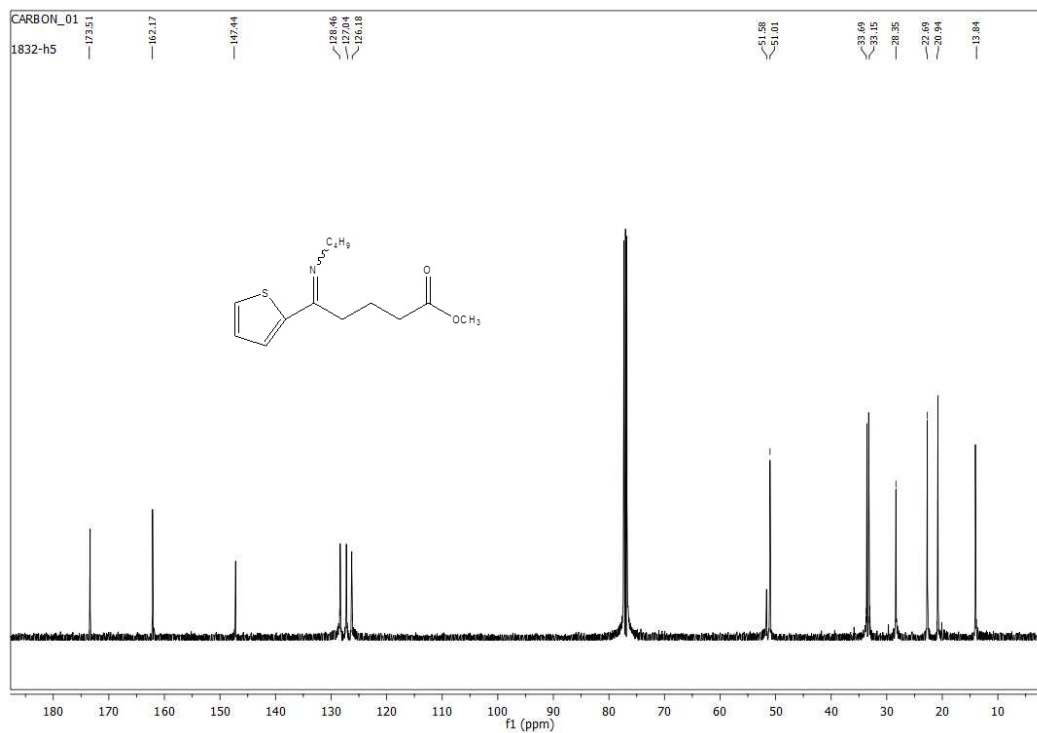**Figure S29.** ^{1}H NMR spectra of compound **3d** (500 MHz, CDCl$_3$).

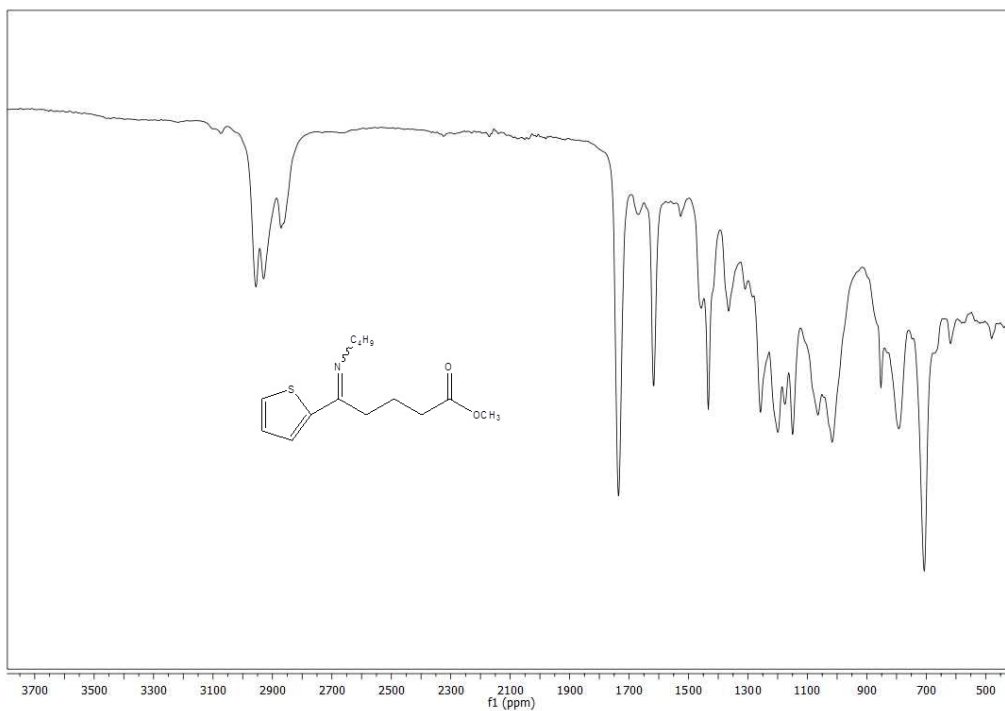**Figure S30.** ^{13}C NMR spectra of compound **3d** (125 MHz, CDCl$_3$).

Figure S31. IR spectra of compound **3d**.

Spectrum

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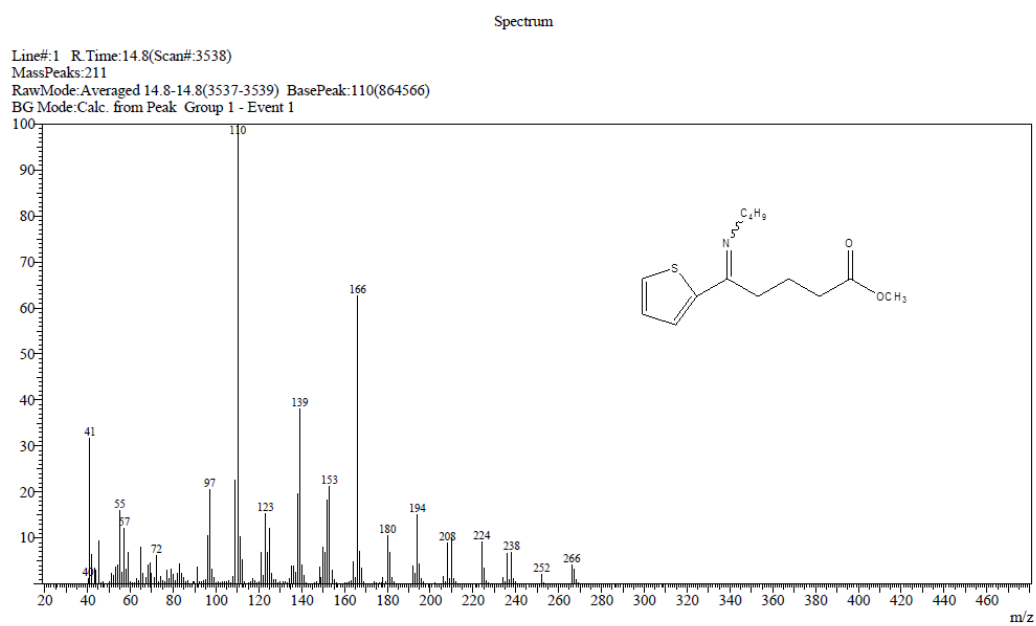BG Mode:Calc. from Peak Group 1 - Event 1

Figure S32. GC-MS spectra of compound **3d**.