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Preparation, Characterization and Evaluation of Some New Amides as Antimicrobial Agents

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ABSTRACT

The some new amide derivatives 1(a-c) and, 2d were synthesized by the two-step N-acylation of 4-nitroaniline or heterocyclic amine derivatives with acyl chlorides. All of the products were determined using ^{13}C NMR, 1H NMR, FT-IR spectroscopies and elemental analysis. Antimicrobial activities of the molecules were evaluated against various bacterial and fungal species. The results show that the some new compounds exhibit good antibacterial and antifungal activities.

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Keywords:
Amides; Secondary amides; N-acylation, Antimicrobial activity; Characterization.

INTRODUCTION

Amides are an important class of organic compounds in which a carbonyl group is connected to a nitrogen atom. These compounds and those similar possess various excellent biological activities including antibacterial, antifungal [1-6], antioxidant [7-11], insecticide [12], anticonvulsant, analgesic, and antitumor agents [13-17].

As is known, amide formation does not occur spontaneously at room temperature and for this reason, it is necessary to pre-activated the carboxylic acids such as acid chlorides. For this purpose, the corresponding amides were synthesized the pre-activation the carboxyl group using thionyl chloride and then, in the presence of triethylamine (TEA) the interaction of those activation products with amine derivatives. The obtained compounds were characterized using ^{13}C NMR, ^{1}H NMR, FT-IR spectroscopies and elemental analysis. Antimicrobial activities of the synthesized compounds were evaluated against various bacterial and fungal species. These target molecules were tested for their antibacterial and antifungal activities using serial dilution technique. As a result, compounds 1b and 2d showed good antibacterial and antifungal activities.

MATERIAL AND METHODS

Measurement and Reagent

All chemicals were purchased from Sigma-Aldrich, Merck or ABCR and directly used without further purification other than commercial thionyl chloride. It was twice distilled; colorless product of high purity was obtained (b.p. 77 °C/760 mmHg). Melting points were determined using Stuart SMP 30 apparatus. The FT-IR spectra were obtained on Bruker Vertex 80V spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded a Bruker/Biospin 400 MHz spectrometer instrument using $CDCl_3$ as solvent and TMS as internal standard. The elemental analyses were carried out on a Costech, ECS 4010 elemental analyser.

Preparation of new amide compounds 1(a-c) and 2d

The newly amide compounds 1(a-c) and 2d were prepared as a result of the two-step reaction shown in Fig. 1. In the firstly step, activation step, the acid chloride intermediate was formed by the interaction of thionyl chloride and carboxylic acid by the procedure as previously described in the literature [18]. In the second step, the acylation step: Heterocyclic amine or 4-nitroaniline derivatives (12 mmol) was dissolved in THF (6 mL) and triethylamine (8 mmol) was added dropwise. Then, to mixture was added dropwise (14 mmol) of 3-acetoxy-2-methylbenzoyl chloride or 2-thiophene carbonyl chloride in 8 mL of THF at room temperature [19]. After this mixture was allowed to stir for 14 hours at room temperature, the resulting white salt precipitate was filtered and washed several times with water. The filtrate was then precipitated with water and the obtained the white crude product was recrystallized from acetonitrile.

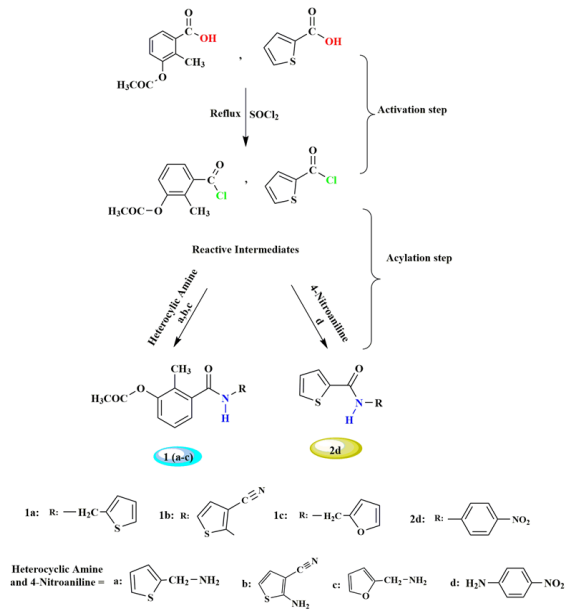


Figure 1. General synthesis of compounds 1(a-c) and 2d

Antimicrobial activity

Four new synthesized molecules were exhibited antimicrobial activities against the following eight microorganisms including Gram-staining-positive (Bacillus subtilis ATCC 6633; Staphylococcus aureus ATCC 25923; Enterococcus faecalis ATCC 29212), Gram-staining-negative (Escherichia coli ATCC 25922; Klebsiella pneumoniae ATCC 70060; Pseudomonas aeruginosa ATCC 27853) bacteria and fungi (Aspergillus niger ATCC 16404; Candida albicans ATCC 1023). Antimicrobial activities were performed using the microdilution method (MIC) [20] by the broth microdilution method carried out in 96-well microplates. Synthesized compounds were dissolved in DMSO at the appropriate concentration. The cultures were obtained from nutrient broth for all the bacterial strains after 24 h of incubation at 28 °C. Fungi were maintained in nutrient broth after incubation for 24 h at 37 °C. Bacterial and fungi cells were homogenized in nutrient broth. The turbidity of bacterial and fungi suspensions was set at a concentration of approximately 106 cells/ml. Only inoculated broth was used as controls. 100 µL suspension of each microorganism and 100 µL suspension of compound tested were added into the wells. The microplate with no growth of microorganism was recorded to represent the MIC enounced in µg/mL. Amoxicillin and Tetracycline were used as the reference standard for antibacterial activity while Ketoconazole was used as the reference standard for antifungal activity, the MIC value were showed in Table 5.

RESULTS AND DISCUSSION

Physical characteristics

The some physical, chemical properties, and elemental analysis results of the newly synthesized molecules are given in Tables 1 and 2.

Table 1. The physical, chemical properties of prepared molecules (1a-1c) and 2d

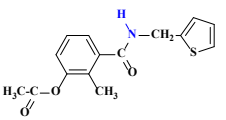
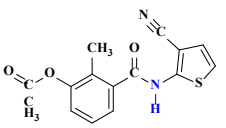
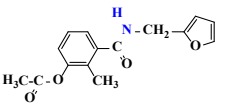
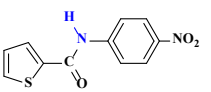
Code	Structure)	Melting point (°C)	Yield (%)
1a		123-126	62
1b		109-111	51
1c		96-98	58
2d		207-210	63

Table 2. The results for elemental analysis of prepared compounds (1a-1c) and 2d

Compound	Calculated				Experimental			
	N %	C %	H %	S %	N %	C %	H %	S %
1a	4.84	62.22	5.18	11.06	4.74	62.71	5.06	10.75
1b	9.32	59.94	3.99	10.65	8.74	60.45	3.88	10.13
1c	5.12	65.87	5.49	-	5.01	66.07	4.87	-
2d	11.28	53.18	3.22	12.89	10.84	53.33	2.89	12.11

IR Spectra

The infrared spectrum of compound 1a displayed a significant vibrational band at 3271 cm^{-1} for the presence of a seconder amide. The absorption for an amide carbonyl (-NHC=O) was observed at 1637 cm^{-1} while an absorption for the carbonyl of ester was observed at 1751 cm^{-1}.

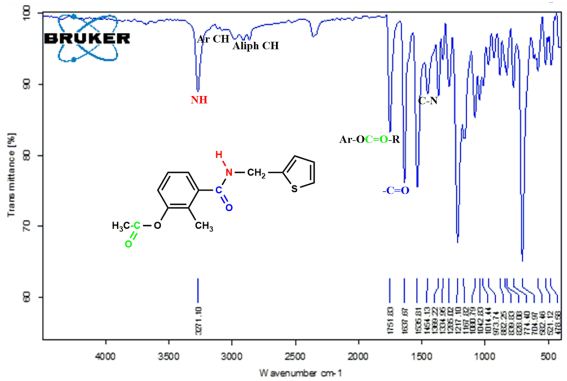


Figure 2. FT-IR spectrum of compound 1a

Due to resonance the aromatic ring with oxygen atom, the strong C=O stretching vibration of ester carbonyl is (~1740 cm^{-1}) higher than normal stretching vibration of ester carbonyl. The other remarkable band at around 1454 cm^{-1} belongs to C-N stretching vibration as shown in Fig. 2. In addition, important IR absorptions of the synthesized molecules are given in the Table 3. These spectral data are consistent with similar structures given in the literature [21, 22].

NMR Spectra

In the ^{1}H NMR spectra of molecule 1a there are two singlets at 2.26 ppm (s, Ar-CH_3) and 2.34 ppm (s, -OCOCH_3) belong to the methyl protons on the benzene ring and methyl protons bound to ester carbonyl respectively. The characteristic NH peak for amides was observed as a singlet at 6.27 ppm (s, -NHC=O). The methylene protons in the structure of compound 1a interacted with the amide proton and were observed as a doublet at 4.79 ppm. The signals of the phenyl ring protons (H1-H3) appeared at between 7.25-6.97 ppm. Of phenyl ring protons, the H2 proton coupled to the H3 proton show a doublet and gives a triplet by coupling the H1 and the H3 as being 7.28 ppm. The signals of the thiophene protons resonated in slightly lower up-field compared to the phenyl protons. These thiophene protons, labeled as H4, H5, and H6, showed two doublets and a triple signals observed in the range of 7.11-6.95 ppm (Fig. 3). These values obtained are in

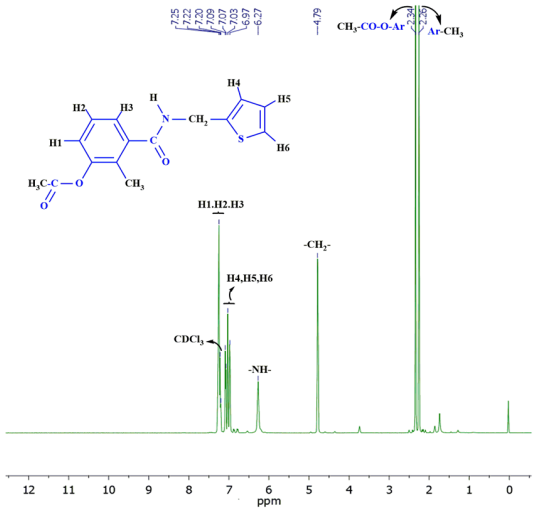


Figure 3. ^{1}H NMR spectrum of compound 1a in $CDCl_3$

consistent with similar compounds in the literature [21]. In the Table 4 are illustrated the chemical shift values of the other compounds.

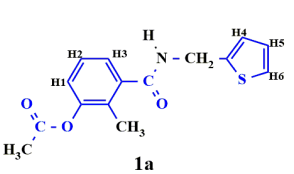
^{13}C NMR Spectra

The ^{13}CNMR spectrum of compound 1a recorded in $CDCl_3$ showed 15 different carbon signals. Two of these signals belong to ester carbonyl carbon and amide carbonyl carbon, was observed at 169.3 ppm and 168.9 ppm, respectively. The phenyl ring carbons (C1-C6) were detected at 149.7, 124.4, 126.1, 123.8, 138.1 and 128.6 ppm respectively. The carbons (C7-C10) belonging to the thiophene ring were resonated at 140.5, 127.0, 126.6 and 125.4 ppm, respectively. While the methyl carbon atom attached to the ester carbonyl group was observed at 20.7 ppm, the other methyl carbon atom attached to the phenyl ring resonated at 12.9 ppm (Fig. 4) The methylene carbon atom (-CH_2-) was observed at 38.7 ppm. These chemical shift values are compatible with the literature and confirm the formation of the target molecule [21]. The carbon chemical shifts values of other synthesized molecules are illustrated in the Table 5.

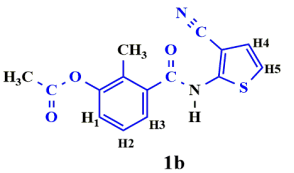
Table 3. Important IR bands of synthesis compounds (cm^{-1})

Comp.	-NH	Ar CH	Aliph CH	Amide C=O	Ester C=O	C-N	Ar-C≡N	Ar-NO2 N=O (Asym. Stretch) N=O (Sym. Stretch)	Ar-NO2 C-N
1a	3271	3070-3040	2970-2845	1637	1751	1454	-	-	-
1b	3244	3113-3081	2981-2934	1755	1787	1457	2229	-	-
1c	3264	3075-3012	2978-2907	1644	1744	1441	-	-	-
2d	3358	3138-3106	-	1641	-	1412	-	1538 (Asym.) 1322 (Sym.)	852

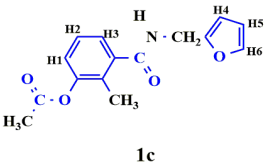
Table 4. ^{1}H NMR spectral values of the synthesized molecules (δ, ppm, in $CDCl_3$)



1a



1b

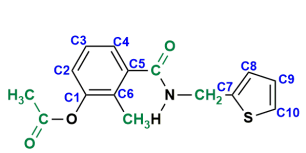


1c

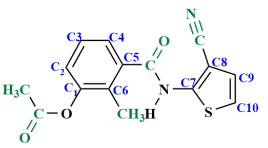
2d

Comp	H1	H2	H3	N-H	H4	H5	H6	H7	-CH2-	-COCH3	Ar-CH3
1a	7.24-7.20 (d)	7.28-7.20 (t)	7.28-7.24 (d)	6.27 (s)	7.11-7.07 (d)	7.11-6.95 (t)	7.02-6.95 (d)	-	4.78-4.77 (d)	2.34 (s)	2.26 (s)
1b	7.30-7.28 (d)	7.36-7.31 (t)	7.38-7.36 (d)	8.92 (s)	7.95-7.93 (d)	7.96-7.94 (d)	-	-	-	2.52 (s)	2.35 (s)
1c	7.24-7.22 (d)	7.28-7.24 (t)	7.39-7.35 (d)	6.12 (s)	6.36-6.31 (d)	7.10-7.08 (d)	7.24-7.22 (d)	-	4.64-4.62 (d)	2.35 (s)	2.25 (s)
2d	7.45-7.43 (d)	7.28-7.20 (m)	8.29-8.27 (d)	7.90 (s)	7.67-7.65 (d)	7.86-7.84 (d)	7.77-7.76 (d)	8.01-7.77 (d)	-	-	-

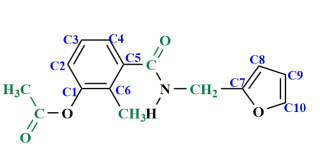
Table 5. ^{13}C NMR spectral data for the synthesized compounds (δ, ppm, in $CDCl_3$)



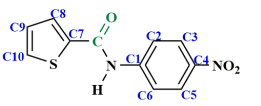
1a



1b



1c



2d

Comp	C1	C2	C3	C4	C5	C6	C=O-Ester	C=O-Amide	C7	C8	C9	C10	-CH2-	CO-CH3	Ar-CH3	R
1a	149.7	124.4	126.1	123.8	138.1	128.6	169.3	168.2	140.5	126.6	127.0	125.4	38.7	20.7	12.9	-
1b	150.3	126.4	127.7	125.7	134.6	129.6	169.0	162.1	134.6	118.6	129.0	125.4	-	20.9	13.3	124.0
1c	150.8	124.5	126.7	123.7	138.1	128.7	169.2	168.8	149.8	110.5	107.6	142.3	36.8	20.7	12.9	-
2d	136.0	119.3	125.1	135.2	125.1	119.3	-	156.6	132.0	128.1	128.4	129.3	-	-	-	-

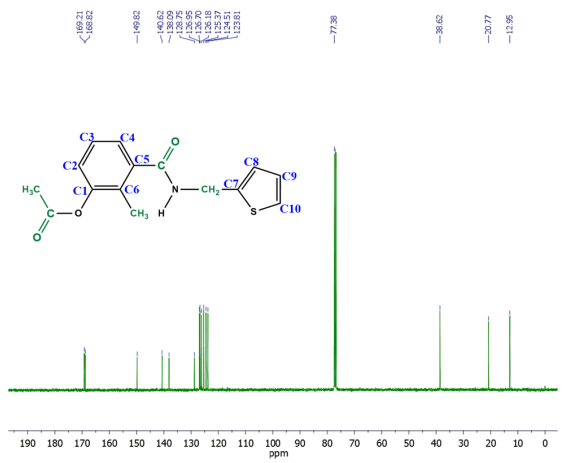


Figure 4. ^{13}C NMR spectrum of compound 1a in $CDCl_3$

Antimicrobial activities

The four newly synthesized molecules were tested in vitro for antimicrobial activity against three Gram-staining-positive, three Gram-staining-negative bacterial strains and two fungi strains. While 1a and 1c compounds did not show antimicrobial activity, 2d and 1b compounds showed antimicrobial activity (Table 6). The MIC values of 2d and 1b were determined between the dose of 500–1000 μg/mL and 125–500 μg/mL, respectively, against Gram-positive, Gram-negative bacteria and fungus species. The 2d and 1b compounds showed better antimicrobial activity against S. aureus, E. faecalis, K. pneumoniae and P. aeruginosa than the Amoxicillin standard.

CONCLUSION

In this article, four new amide molecules (1a-1c) and 1d were successfully prepared by two-step synthesis reactions consisting of activation and acylation steps. The structural analysis of the obtained molecules was made using FT-IR, ^{1}H NMR, ^{13}C NMR, spectroscopy and elemental analyses techniques. All of the target molecules were screened for their antibacterial and antifungal activities using serial dilution technique. As a result, among tested compounds 1b and 2d were exhibited good anti-

Table 6. The minimum inhibition concentrations (MIC's) of the tested molecules

Sample	Minimum inhibition concentration (µg/mL)							
	Gram-staining-positive			Gram-staining-negative			Fungi	
	B. subtilis	S. aureus	E. faecalis	E. coli	K. pneumoniae	P. aeruginosa	A.niger	C. albicans
1a	-	-	-	-	-	-	-	-
1b	125	125	125	125	125	125	125	500
1c	-	-	-	-	-	-	-	-
2d	500	500	500	500	500	500	1000	1000
Amoxicillin	<2	>1000	>1000	32	>1000	>1000	NT	NT
Tetracycline	<2	8	8	<2	8	4	NT	NT
Ketoconazole	NT	NT	NT	NT	NT	NT	1	2

NT: not tested

microbial activity. This antimicrobial activity can be the directly related to the nature of the substituents on the ring of compounds 1b and 2d.

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APPENDIX

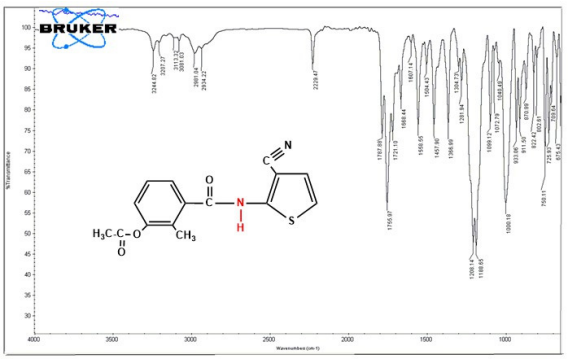


Figure S1. FT-IR spectrum of compound 1b

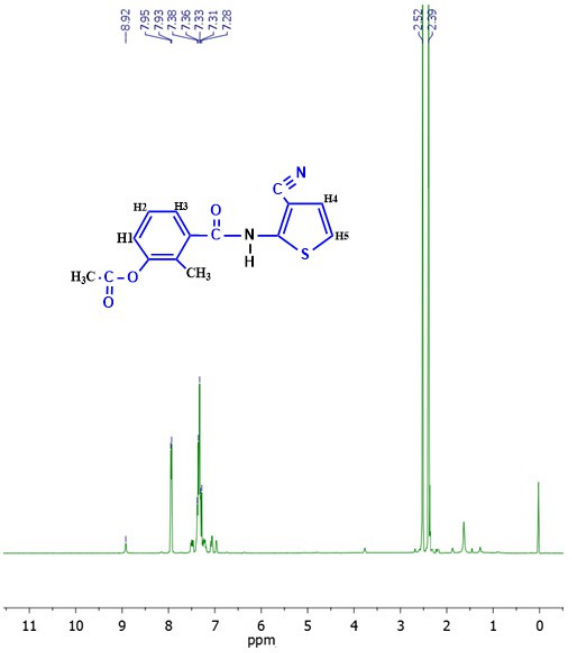


Figure S2. ^{1}H NMR spectrum of compound 1b in $CDCl_3$

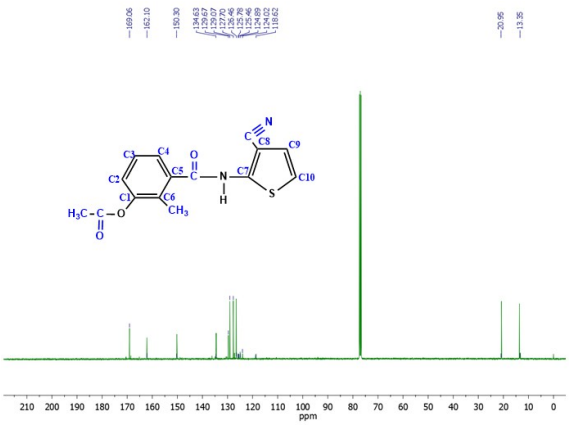


Figure S3. ^{13}C NMR spectrum of compound 1b in $CDCl_3$

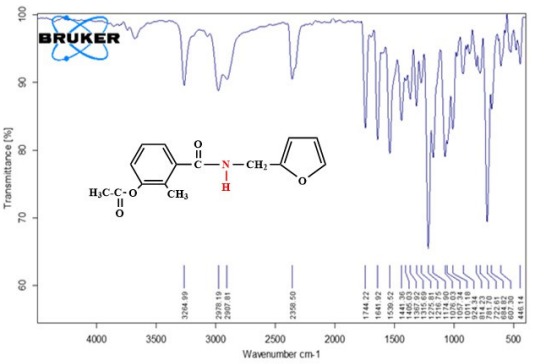


Figure S4. FT-IR spectrum of compound 1c

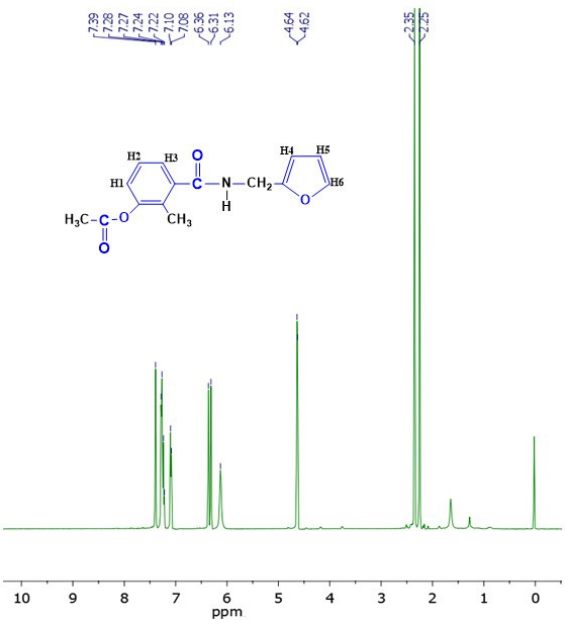


Figure S5. ^{1}H NMR spectrum of compound 1c in $CDCl_3$

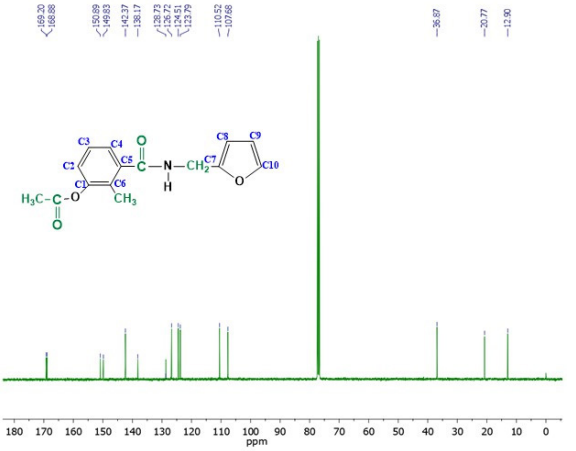


Figure S6. ^{13}C NMR spectrum of compound 1c in $CDCl_3$

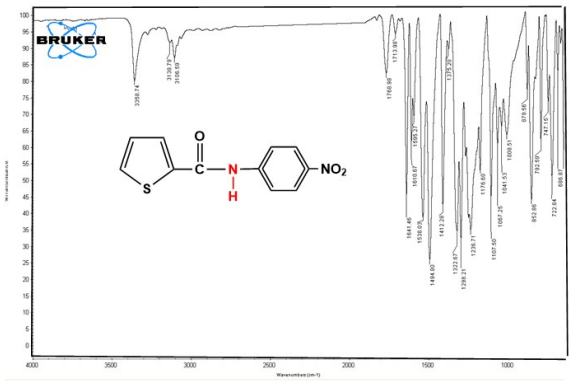
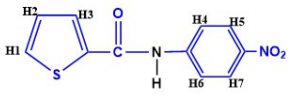


Figure S7. FT-IR spectrum of compound 2d



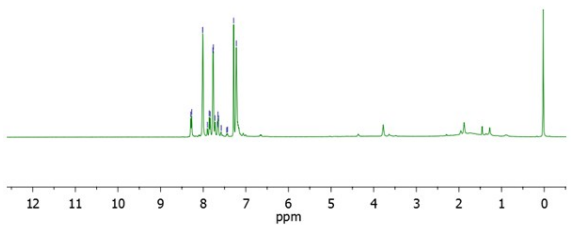


Figure S8. ^{1}H NMR spectrum of compound 2d in $CDCl_3$

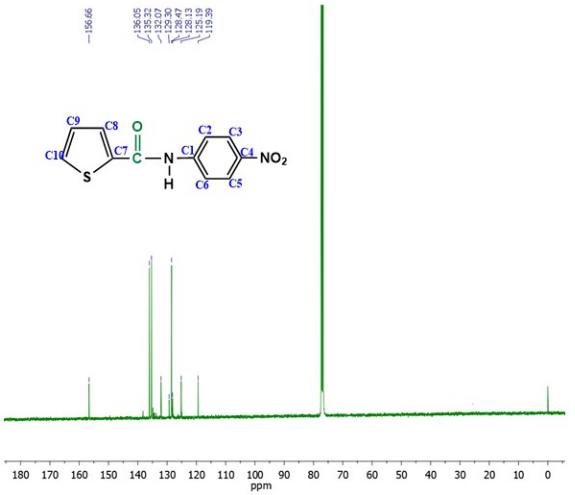


Figure S9. ^{13}C NMR spectrum of compound 2d in $CDCl_3$