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Heterocyclic Amides Derived from 2-Thiopheneacetic Acid: Synthesis, Characterization and Antimicrobial Activity Evaluation

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Abstract: Synthesized the heterocyclic amide derivatives (I-IV) were from 2-thiophene acetic acid in two steps. In the first step, the intermediate acylation agent formed and isolated, then subjected to aminolysis and obtained the corresponding amide derivatives. The structures of compounds obtained were characterized by FT-IR, ^{13}C NMR, ^{1}H NMR, spectroscopies and elemental analysis techniques. The antimicrobial activities of these four compounds against Gram-negative bacteria, Gram-positive bacteria and fungi were investigated using the minimum inhibition concentration method. As a result, compounds (I and III) exhibited better good antibacterial activities against *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* compared to the commercially standard antibacterial agent of amoxicillin.

Keywords : Acyl chloride, heterocyclic amides, antimicrobial activity, aminolysis, spectroscopic evaluation

2-Tiyofenasetik Asitten Türetilen Heterosiklik Amitler: Sentez, Karakterizasyon ve Antimikrobiyal Aktivitesinin Değerlendirilmesi

Öz: Sentezlenen heterosiklik amit türevleri (I-IV), 2-tiyofenasetik asitten iki aşamada elde edildi. İlk aşamada, ara açilasyon ajanı oluştu ve izole edildi, ardından aminolize tabi tutuldu ve ilgili amit türevleri elde edildi. Elde edilen bileşiklerin yapıları FT-IR, ^{13}C NMR, ^{1}H NMR, spektroskopi ve element analiz teknikleri ile karakterize edildi. Bu dört bileşiğin Gram negatif bakterilere, Gram pozitif bakterilere ve mantarlara karşı antimikrobiyal aktivitesi minimum inhibisyon konsantrasyon yöntemi kullanılarak araştırıldı. Sonuç olarak, bileşikler (I ve III), ticari olarak temin edilebilen amoksisilinin antibakteriyel standardına göre *Staphylococcus aureus*, *Enterococcus faecalis* ve *Pseudomonas aeruginosa*'ya karşı daha iyi antibakteriyel aktivite sergilediği gözlemlenmiştir.

Anahtar Kelimeler: Asit klorür, heterosiklik amit, antimikrobiyal aktivite, aminoliz, spektroskopik değerlendirme

1. Introduction

Heterocyclicles are an interesting family of organic compounds because of their reaction behaviour, excellent application as useful material and pharmaceutical activity. The cyclic systems, especially heterocyclic compounds, possess enhanced biological properties [1-9].

Amides are generally as considered to be carboxylic acids derivatives in which an amine or ammonia replaces the hydroxy group. The presence of heteroatoms in their structure causes amide compounds to exhibit different biological properties like antioxidant, antifungal, antibacterial,

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anti-HSV, analgesic, anti-inflammatory and anti-cancer, antitumor properties [10-16]. Since a wide variety of amides are known to have high biological activity and are useful as pharmaceuticals, one can also conclude that the amide functionality may be important.

Based on this observation, we designed some amide compounds containing a heteroatom. For this, were prepared as a result of the interaction of different heterocyclic amines with 2-thiopheneacetyl chloride within a slightly basic medium. Determined the structures of the synthesized compounds were by using IR, ^{1}H NMR and ^{13}C NMR spectroscopies, and elemental analysis techniques. The in vitro antimicrobial effect of all the compounds were done according to the MIC method.

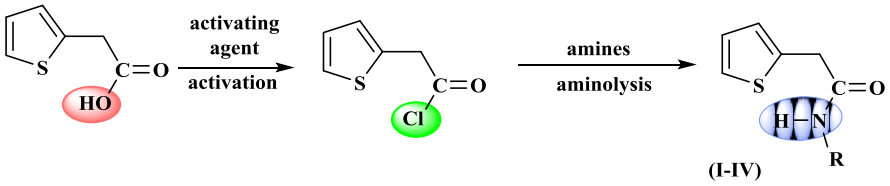
2. Experimental Methods

2.1. Chemicals and Apparatus

All chemicals and solvents were obtained from Sigma-Aldrich, Merck or ABCR Chemical Company and used without purification except thionyl chloride. Obtained the elemental analyses were on a Costech, ECS 4010 elemental analyser. Melting points are taken using Stuart SMP 30 apparatus. Recorded the Infrared spectra were on Bruker Vertex 80V spectrometer. Used a Bruker/Biospin 400 MHz spectrometer was to take ^{1}H NMR and ^{13}C NMR spectra.

2.2. General Synthesis of Heterocyclic Amide Molecules (I-IV)

The starting material, 2-thiophenacetyl chloride, was prepared by the reaction of 2-thiophene acetic acid with thionyl chloride according to the method given in the literature [17]. To a solution of the appropriate heterocyclic amine (20-60 mmol) and added triethylamine (20 mmol) in THF (35 mL) was dropwise a THF solution of 2-thiophenacetyl chloride (20 mmol) at room temperature. Stirred the reaction mixture was for 15 h, and then added 150 mL water. The mixture was stirred for 30 min, then THF was removed under reduced pressure. The precipitate was filtered off and washed several times with water to remove an excess of the heterocyclic amine derivative and triethylamine hydrochloride salt. The crude product was crystallized from THF/acetonitrile [18, 19, 20]. The stepwise synthesis illustrated in Figure 1.



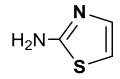
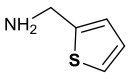
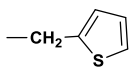
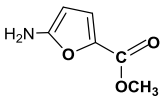
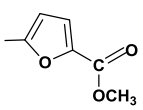
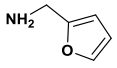
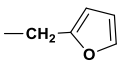
Amines	R	Compound
		I
		II
		III
		IV

Figure 1. Stepwise synthesis of the heterocyclic amides (**I-IV**)

2.3. Antimicrobial Activity

The antimicrobial activities of the obtained compounds evaluated using serial dilution technique. The compounds (I-IV) were tested for their antibacterial activities against *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70060, *Pseudomonas aeruginosa* ATCC 27853, and antifungal activities against *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 1023. The antibacterial activities of the compounds (I-IV) were estimated by the minimum inhibition concentration (MIC)[21]. The obtained compounds dissolved in DMSO at the appropriate concentration. While the cultures were grown from the nutrient broth for all the bacterial strains after 24 h of incubation at 37 °C, fungi were grown in the nutrient broth after incubation for 24 h at 28 °C. Bacterial and fungi cells were homogenized in nutrient broth. The density of bacterial and fungi suspensions was set at a concentration of approximately 10^6 cells/mL. As a control, used inoculated borth. 100 μL suspension of each microorganism and 100 μL suspension of the 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, tetracycline and ketoconazole were used as the reference standard for antimicrobial activities.

3. Results and Discussion

3.1. Physical and Elemental Data

The physical constants and elemental analysis data of the synthesized molecules recorded in Table 1.

Table 1. The physical and elemental data of synthesized compounds **(I-IV**)

Comp.	Chem. formula	Colour	Yield (%)	MP (◦C)	% of C, H, N, S calculated (found)			
					C	H	N	S
I	$C_9H_8N_2OS_2$	Light Brownish	54	179-181	48.19(48.48)	3.60(3.66)	12.49(12.41)	28.59(28.62)
II	$C_{11}H_{11}NOS_2$	Light Yellow	81	119-121	55.67(55.85)	4.67(4.08)	5.90(5.95)	27.02(26.53)
III	$C_{12}H_{11}NO_4S$	Light Yellow	45	112-114	54.33(54.43)	4.18(4.04)	5.28(5.32)	12.09(11.86)
IV	$C_{11}H_{11}NO_2S$	White	74	115-117	59.71(60.26)	5.01(4.82)	6.33(6.39)	14.49(14.29)

3.2. Vibrational Frequencies

The IR spectrum of compound (I) exhibit the important characteristic absorption bands shown in Figure 2. In the FT-IR spectrum of compound (I) the stretching band of the amide group (N-H) has appeared at 3285 cm^{-1}. The characteristic vibrational band associated with the C=O stretching vibration (amide I) was observed at 1688 cm^{-1}. The other characteristic absorption bands are amide II, and amide III appeared at 1567 cm^{-1} and 1332 cm^{-1}, respectively. The amide II mode is due to the N-H bending vibration while amide III absorption mode consists of the C-N stretching and N-H in-plane bending vibrations.

Furthermore, the characteristic absorption frequencies of all synthesized compounds given in Table 2. These results confirm that heterocyclic amide compounds have synthesized successfully. There are the chemical shift values that were identical with those in the spectrum of similar compounds [19, 22].

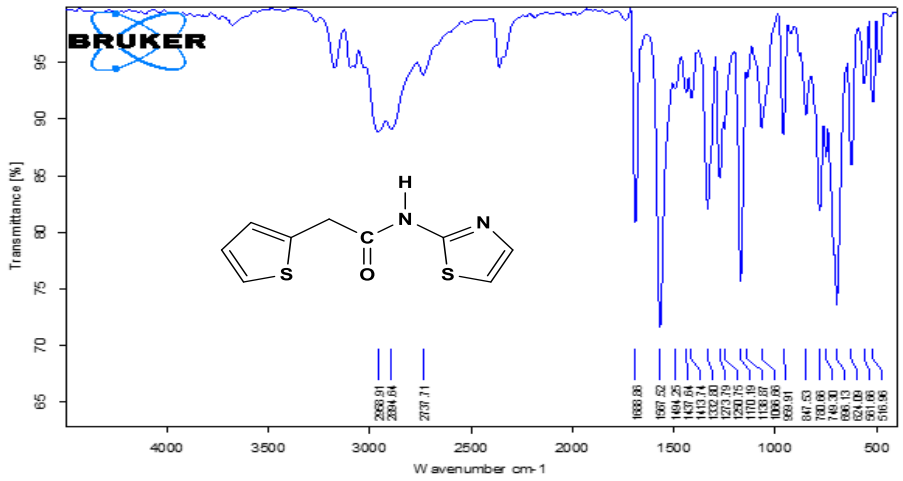


Figure 2. FT-IR spectrum of compound **(I)**

Table 2. The characteristic IR absorption frequencies of the compounds (cm^{-1})

Comp.	Amide υ_{N-H}	Arom υ_{CH}	Aliph υ_{CH}	Amide I $\upsilon_{C=O}$	Amide II υ_{N-H}	Amide III υ_{C-N}	Ester $\upsilon_{C=O}$
I	3285	3087-3045	2958-2894	1688	1567	1332	-
II	3279	3008	2977-2804	1636	1547	1318	-
III	3271	3100-3015	2993-2845	1688	1530	1320	1707
IV	3270	3078	2931-2858	1633	1555	1318	-

3.3. ^{1}H NMR Spectra

Figure 3 give ^{1}H NMR spectrum of compound (I). In this figure, the signals at 4.10 ppm assigned to the methylene protons, and the broad signal appeared at 11.34 ppm for secondary amide proton. The protons of the thiazole ring (H4 and H5) resonated in a lower field compared to the protons of the thiophene ring (H1, H2 and H3). The H4 proton was coupled to the H5 proton shown as doublet peak at 7.49 ppm. The other ring protons (H1, H2 and H3) showed a triplet and a doublet peak at 7.30-7.04 ppm. These results are in accordance with previously similar molecules in the literature [19, 22]. ^{1}H NMR of all compounds the chemical shift values given in Tablo 3.

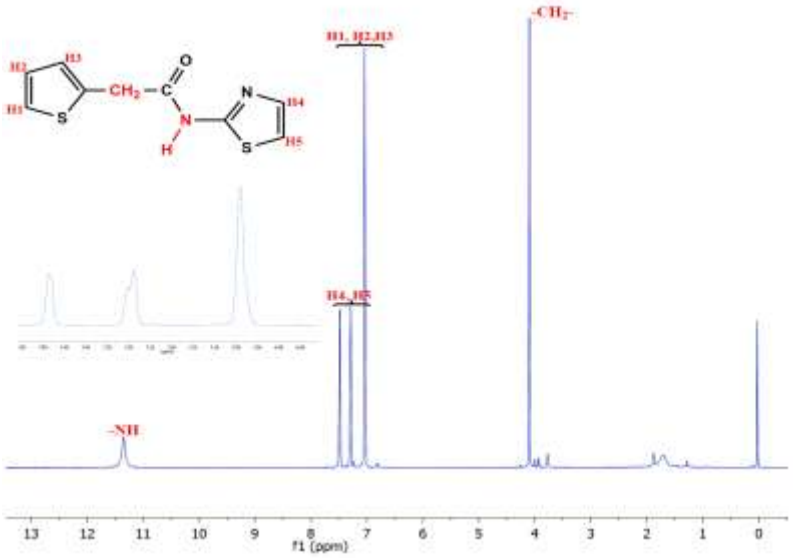
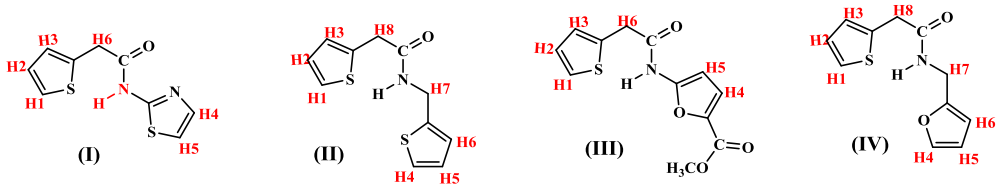


Figure 3. ^{1}H NMR spectrum of compound **(I)** in $CDCl_3$

Table 3. ^{1}H NMR spectral values of the synthesized molecules (δ, ppm, in $CDCl_3$)



Comp.	H1	H2	H3	N-H	H4	H5	H6	H7	H8	-COOCH$_3$
I	7.30-7.29(d)	7.30-7.26 (t)	7.29-7.26 (d)	11.34 (s)	7.49 (d)	7.49 (d)	4.10 (s)	-	-	-
II	7.01-6.96 (d)	7.01-6.93 (t)	6.96-6.93 (d)	6.05 (s)	7.26-7.22 (d)	7.26-7.22 (d)	7.26-7.22 (d)	4.62-4.61 (d)	3.83(s)	-
III	7.34-7.19 (d)	7.19-7.04 (t)	7.07-7.04 (d)	8.27 (s)	6.59 (d)	6.59 (d)	3.87 (s)	-	-	4.01 (s)
IV	7.34-7.26 (d)	7.34-7.26 (t)	7.01-6.96 (d)	6.02 (s)	6.32 (d)	6.19 (d)	6.19 (d)	4.44-4.43 (d)	3.82(s)	-

3.4. ^{13}C NMR Spectra

In the ^{13}C-NMR spectrum of compound (I), there are a total of 9 different carbons with chemical shift values between 37.09-167.64 ppm. The signal at 37.09 ppm is the only aliphatic carbon atom belonging to the methylene group (-CH_2) in the structure of the molecule. The signal at the lowest field at 167.64 ppm that can be assigned to the amide carbonyl carbon (-CONH-). The carbons of the thiazole ring (C5, C6 and C7) were seen at 159.21, 134.10 and 114.07 ppm, respectively. The carbons (C1, C2, C3 and C4) of the thiophene ring, another ring in the structure of compound (I), resonated at 125.98, 127.46, 127.68 and 136.73 ppm, respectively (Figure 4). The spectral data of compound (I) are fully compatible with similar structures in the literature [19, 22]. ^{13}C NMR values for all the other synthesized substances illustrated in Table 4.

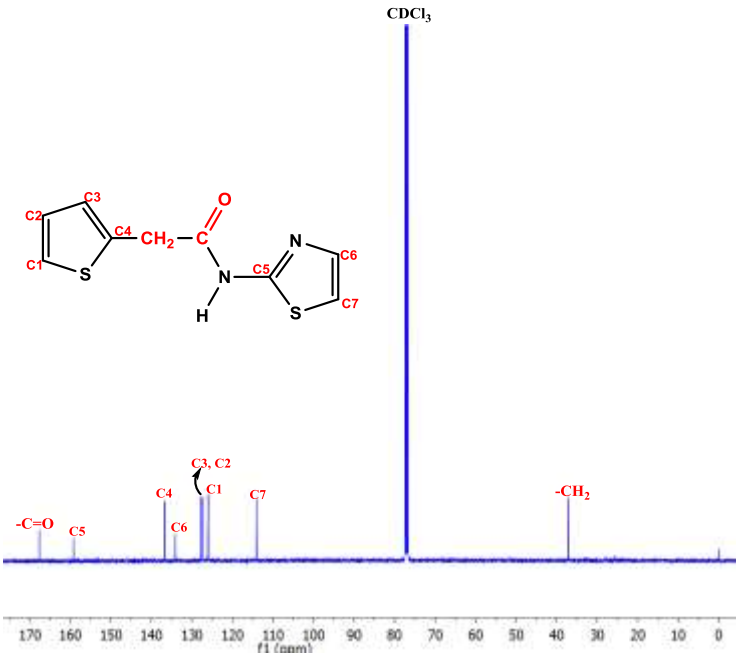
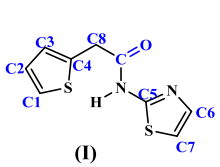
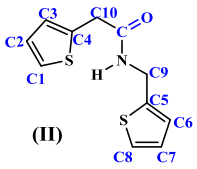


Figure 4. ^{13}C NMR spectrum of compound **(I)** in $CDCl_3$

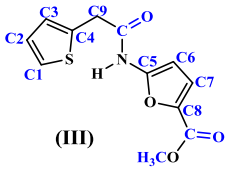
Table 4. ^{13}C NMR spectral values for the synthesized substances (δ, ppm, in $CDCl_3$)



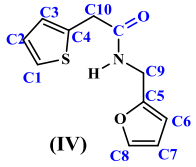
(I)



(II)



(III)



(IV)

Comp	C1	C2	C3	C4	C5	C6	C=O Ester	C=O Amide	C7	C8	C9	C10	-$COOCH_3$
I	125.9	127.4	127.6	136.7	159.2	134.1	-	167.6	114.0	37.0	-	-	-
II	125.7	127.4	127.5	135.7	140.8	125.8	-	169.5	126.8	125.2	38.5	37.4	-
III	126.6	127.9	128.4	134.0	148.9	96.7	159.0	166.2	121.1	136.5	37.6	-	51.9
IV	125.7	127.4	127.5	135.9	151.0	107.4	-	169.6	110.4	142.2	36.7	37.4	-

3.5. Antimicrobial Activities

The four synthesized substances were screened *in vitro* for antimicrobial activities against three Gram-staining-positive, three Gram-staining-negative bacterial strains and two fungi strains. The commercial antimicrobial agents (amoxicillin, tetracycline and ketoconazole) were used as controls, and the results illustrated in Table 5. While compounds (II and IV) have not displayed antimicrobial activity, compounds (I and III) showed antimicrobial activities (Table 5). Compounds (I and III) showed better antimicrobial activities against *S. aureus*, *E. faecalis* and *P. aeruginosa* than the amoxicillin standard. Compounds (I and III) were equipotent against *K. pneumoniae* compared to the reference amoxicillin. Compounds (I and III) showed low activity against *B. subtilis*, *E. coli*, *A. niger* and *C. albicans* compared to the references amoxicillin, Tetracycline, Ketoconazole.

In recent years, varied types of substance with amide bonds have shown excellent antibacterial activities [22, 23].

Table 5. Antimicrobial activities of the compounds **(I-IV)** (μg/mL)

Compd. No.	A	B	C	D	E	F	G	H
I	1000	500	500	1000	1000	500	1000	2000
II	-	-	-	-	-	-	-	-
III	500	500	500	500	1000	500	500	1000
IV	-	-	-	-	-	-	-	-
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

A: *Bacillus subtilis*, B: *Staphylococcus aureus*, C: *Enterococcus faecalis*, D: *Escherichia coli*, E: *Klebsiella pneumoniae*, F: *Pseudomonas aeruginosa*, G: *Aspergillus niger*, H: *Candida albicans;* NT: Not tested.

4. Conclusions

In summary, we have reported that heterocyclic amide derivatives (I-IV) synthesized from 2-thiophene acetic acid in two steps. The first step involved activation with thionyl chloride to form 2-thiophenacetyl chloride. This step was followed by the aminolysis step to give four heterocyclic amide derivatives, and obtained products yields are appreciable (45-81%). All of the compounds structural analysis were evaluated by FT-IR, ^1H NMR, ^{13}C NMR, spectroscopies and elemental

analyses techniques. Moreover, in vitro antibacterial and antifungal activities of the compounds were evaluated using the serial dilution technique. The relationship between the structure-activity has shown that compounds (I and III) are more active than other compounds. The compound III exhibited a more significant activity than compound I due to the acetoxy group (OAc) on the furan ring.

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