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SYNTHESIS, CHARACTERIZATIONS AND ANTIMICROBIAL EVALUATION OF NEW 1-ACYL-4-SUBSTITUTED THIOSEMICARBAZIDE AND 2,5-DISUBSTITUTED 1,3,4-THIADIAZOLE DERIVATIVES

YENİ 1-AÇİL-4-SÜBSTİTÜE TİYOSEMİKARBAZİD VE 2,5-DİSÜBSTİTÜE 1,3,4-TİYADİAZOL TÜREVLERİNİN SENTEZİ, YAPI TAYİNİ VE ANTİMİKROBİAL ETKİLERİNİN ARAŞTIRILMASI

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*5 - (2 - Furyl) - 4 - phenyl - 1,2,4-triazole- 3-mercaptoacetic acid hydrazide (**1**) was reacted with isothiocyanates to afford the corresponding 4 - alkyl/aryl - 1 - [(4 -phenyl- 2,4 - dihydro - 5 - (2 - furyl) - 1,2,4-triazole - 3-yl) thioacetyl] - 3-thiosemicarbazides (**2a-i**) which were cyclized into 2 - alkyl/arylamino- 5 -[4 - phenyl - 2,4 - dihydro - 5- (2 - furyl) - 1,2,4 - triazole - 3-yl] thiomethyl-1,3,4 -thiadiazoles (**3a- h**) in the presence of sulfuric acid. Analytical and spectral data (IR, ^{1}H-NMR and EIMS) confirmed the proposed structures. The title compounds (**2-3**) were evaluated for in vitro antimicrobial activity against Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC 12228, Klebsiella pneumoniae ATCC 4352, Pseudomonas aeruginosa ATCC 1539, Escherichia coli ATCC 8739, Shigella flexneri, Proteus mirabilis and Candida albicans ATCC 10231 using the disc diffusion and macrodilution methods, only **2i** showed antibacterial activity against Staphylococcus aureus with a MIC value of 58.6 µg/ml.*

*5-(2-Furil)-4-fenil-1,2,4-triazol-3-merkaptoasetik asit hidrazidinin (**1**) değişik isotiyosiyanatlarla reaksiyonundan elde edilen 4-alkil/aril-1-[(4-fenil-2,4-dihidro-5-(2-furil) -1,2,4-triazol-3-il)tiyoasetil]-3-tiyosemikar-bazidler (**2a-i**), sülfürik asidli ortamda 2-alkil/arilamino - 5 - [4-fenil - 2,4 - dihidro - 5 - (2 - furil) - 1,2,4 - triazol - 3 - il]tiyometil - 1,3,4 - tiyadiazollere (**3a-h**) siklize edilmiştir. Analitik ve spektral veriler (IR, ^{1}H-NMR ve EIMS) amaçlanan yapıları doğrulamaktadır. Kazanılan maddelerde (**2-3**), Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC 12228, Klebsiella pneumoniae ATCC 4352, Pseudomonas aeruginosa ATCC 1539, Escherichia coli ATCC 8739, Shigella flexneri, Proteus mirabilis ve Candida albicans ATCC 10231' e karşı disk difüzyon ve makrodilüsyon yöntemleri kullanılarak in vitro antimikrobial aktivite araştırması yapılmış, madde **2i**'nin Staphylococcus aureus'a karşı aktif olduğu saptanmıştır (MİK, 58.6 µg/ml).*

Keywords :*1-Acyl-4-substituted thiosemicarbazides; 2,5-Disubstituted 1,3,4-thiadiazoles; Synthesis; Antimicrobial activity*

Anahtar kelimeler :*1-Açil-4-sübstitüe tiyosemikarbazidler; 2,5 - Disübstitüe 1, 3, 4-tiyadiazoller;Sentez; Antimikrobial aktivite*

Introduction

A wide variety of pharmacological properties has been shown to be associated with 1-acyl-4-substituted thiosemicarbazide derivatives. These include antibacterial(1, 2), antifungal(3), antimycobacterial(4,5), antiviral(6), anticonvulsant(7,8), cytostatic(9), cardiovascular(10) and hypoglycemic(11). Heterocycles such as thiadiazoles derived from these thiosemicarbazides are also important chemotherapeutic agents and have been reported to exhibit antibacterial(12-14), antifungal (15, 16), antiviral(17) and antiinflammatory(18) activities. In continuation of our work on the synthesis of heterocycles of pharmaceutical interest (19,20), we report here the synthesis, characterization and biological evaluation of the title compounds.

Materials and Methods

Mp.s were determined in a Büchi 530 apparatus in open glass capillary tubes and were uncorrected. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. IR (KBr) and ^{1}H-NMR spectra were recorded on Perkin-Elmer 577 (Grating) and Bruker AC 200 (200 MHz) instruments, respectively. EIMS were recorded at the Pennsylvania State University, USA.

*1. General procedure for the preparation of 4-alkyl/aryl-1-[(4-phenyl-2,4-dihydro-5-(2-furyl)-1,2,4-triazole -3-yl)thioacetyl]-3-thiosemicarbazides (**2a-i**)*

A mixture of hydrazide **1** (0.01 mol) and EtOH (100

ml) was warmed to effect solution. The appropriate isothiocyanate (0.01 mol) was added and the mixture was refluxed for 3h. The precipitate which formed after cooling was collected by filtration. Crystallization from EtOH afforded the pure compounds.

2b: IR (KBr) $\bar{\nu}$ (cm^{-1}) : 3320, 3160 (N-H), 1700 (C=O), 1520, 1480, 1470, 1440 (C=N, C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 10.22 (s, 1H, CONH), 9.33 (s, 1H, NHCS), 8.24 (s, 1H, NH-CH_2), 7.79 (s, 1H, C5-H), 7.66-7.63 (m, 4H, Ar-H), 7.54-7.50 (m, 1H, Ar-H), 6.52 (dd, J=3.19 Hz, 1.77 Hz, 1H, C4-H), 6.13 (d, J=3.43 Hz, 1H, C3-H), 3.90 (s, 2H, SCH_2), 3.53 (q, J=6.66 Hz, 2H, N-CH_2), 1.11 (t, J=7.02 Hz, 3H, N-CH_2-CH_3).

2g: IR (KBr) $\bar{\nu}$ (cm^{-1}): 3280, 3180 (N-H), 1700 (C=O), 1620, 1590, 1520, 1450 (C=N, C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 10.31 (s, 1H, CONH), 9.60 (s, 1H, NHCS), 9.58 (s, 1H, NH-Ar), 7.75 (s, 1H, C5-H), 7.65-7.62 (m, 4H, Ar-H), 7.53-7.48 (m, 1H, Ar-H), 7.39 (d, J=8.23 Hz, 2H, Ar-H), 7.13 (d, J=8.23 Hz, 2H, Ar-H), 6.51 (dd, J=3.27 Hz, 1.77 Hz, 1H, C4-H), 6.17 (d, J=3.30 Hz, 1H, C3-H), 3.98 (s, 2H, SCH_2), 2.29 (s, 3H, Ar-CH_3).

*2. General procedure for the preparation of 2-alkyl/arylamino-5-[4-phenyl-2,4-dihydro-5-(2-furyl)-1,2,4-triazole-3-yl]thiomethyl-1,3,4-thiadiazoles (**3a-h**)*

The appropriate thiosemicarbazide **2** (0.005 mol) was dissolved in 5.3 ml of H_2SO_4 (96%) and allowed to stand for 30 min. The solid then was poured in crushed ice and neutralized with Na_2CO_3. The precipitate thus obtained was filtered and recrystallized from aqueous EtOH.

3b: IR (KBr) $\bar{\nu}$ (cm^{-1}): 3184 (N-H), 1577, 1518, 1497, 1446 (C=N, C=C). ^{1}H-NMR (DMSO-d_6) δ ppm:7.73 (d, J=1.03 Hz, 1H, C5-H), 7.66-7.49 (m, 4H, Ar-H), 7.44-7.37 (m, 2H, Ar-H and NH), 6.50 (dd, J=3.44 Hz, 1.65 Hz, 1H, C4-H), 6.17 (d, J=3.38 Hz, 1H, C3-H), 4.55 (s, 2H, SCH_2), 3.31-3.18 (m, N-CH_2 and H_2O), 1.14 (t, J=7.17 Hz, 3H, N-CH_2-CH_3).

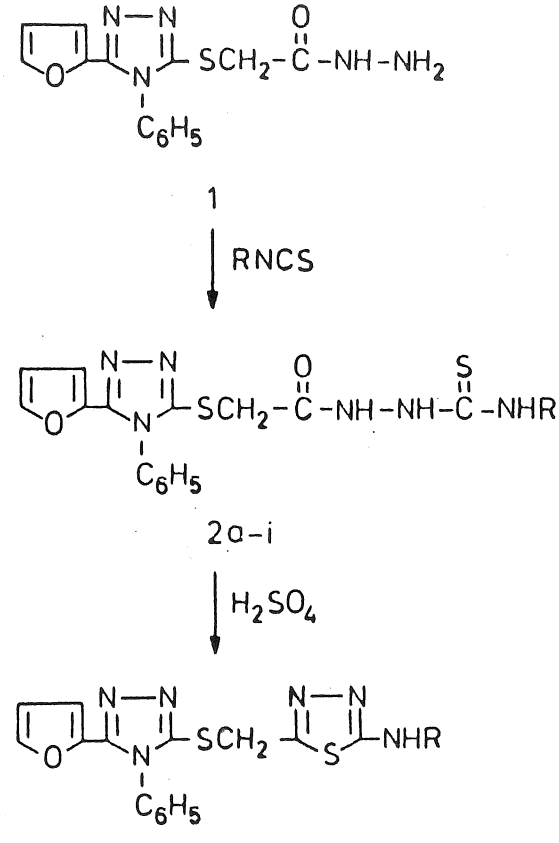
3f: IR (KBr) $\bar{\nu}$ (cm^{-1}): 3500 (O-H), 3250 (N-H), 1610, 1565, 1510, 1435 (C=N, C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 10.11 (s, 1H, NH), 7.74 (d, J=0.94 Hz, 1H, C5-H), 7.66-7.53 (m, 4H, Ar-H), 7.47-7.40 (m, 3H, Ar-H), 7.13 (d, J=8.34 Hz, 2H, Ar-H), 6.51 (dd, J=3.33 Hz, 1.70 Hz, 1H, C4-H), 6.19 (d, J=3.38 Hz, 1H, C3-H), 4.64 (s, 2H, SCH_2), 2.26 (s, 3H, Ar-CH_3).

Determination of antimicrobial activity

The title compounds were evaluated for antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Escherichia coli* ATCC 8739, *Shigella flexneri, Proteus mirabilis* and *Candida albicans* ATCC 10231 using the disc diffusion and macrodilution methods (21). Only **2i** showed antibacterial activity against *Staphylococcus aureus* with a MIC value of 58.6 µg/ml.

Results and Discussion

1-Acyl-4-substituted thiosemicarbazides (**2a-i**) were synthesized by the addition of

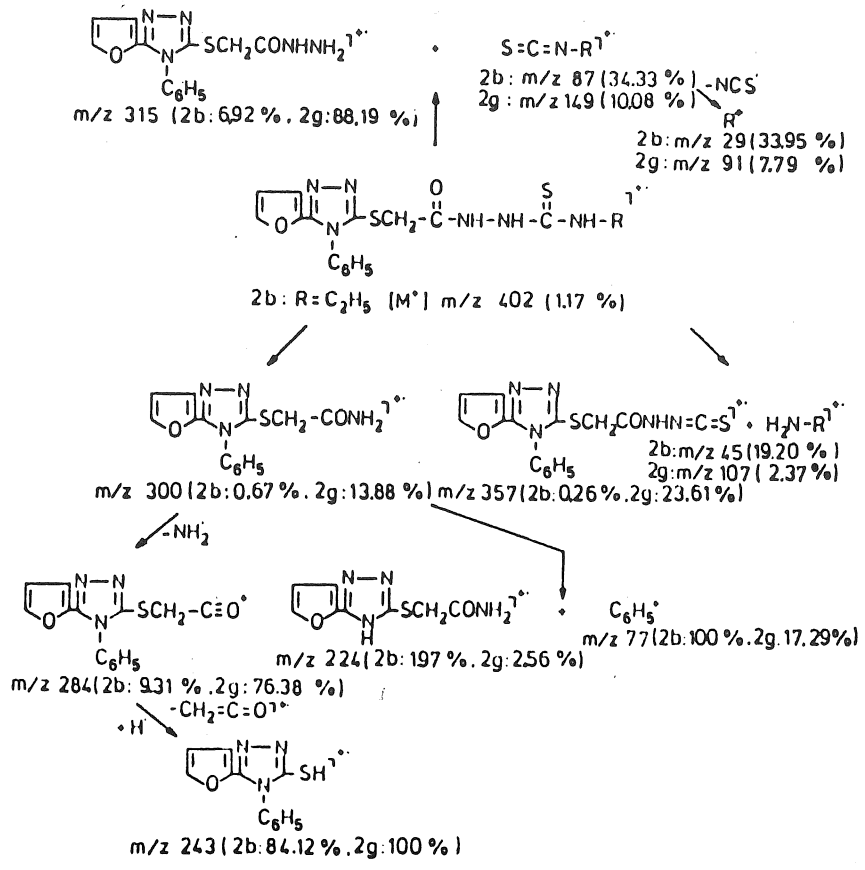


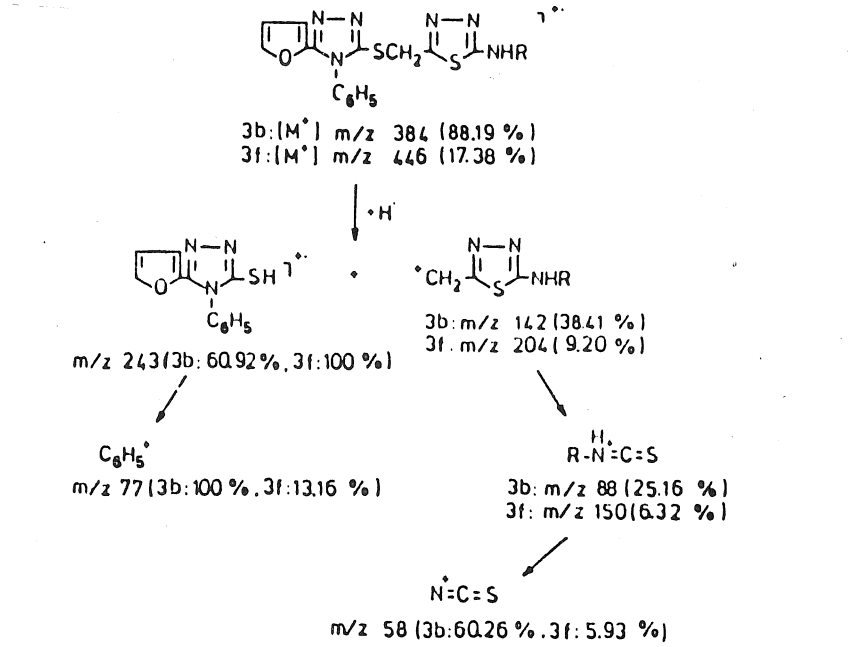
Scheme 1

5-(2-furyl)-4-phenyl-1,2,4- triazole- 3-mercaptoacetic acid hydrazide (**1**) (20) to alkyl/arylisothiocyanates. Cyclization of **2a-i** in conc. sulfuric acid afforded 2,5-disubstituted 1,3,4-thiadiazoles (**3a-h**) (22) (Scheme 1).

Table. Some characteristics of compounds **2-3**

Comp.	R	Formula (mol. wt.)	M.p. [°C]	Yield (%)	Analysis calcd./found C	H	N
2a	CH_3	$C_{16}H_{16}N_6O_2S_2$ (388.47)	204-5	87.11	49.47 48.88	4.15 4.28	21.63 21.59
2b	C_2H_5	$C_{17}H_{18}N_6O_2S_2$ (402.49)	216-17	92.59	50.73 50.86	4.50 4.45	20.88 21.03
2c	$CH_2CH_2C_6H_5$	$C_{23}H_{22}N_6O_2S_2$ (478.59)	235-36	82.63	57.72 57.45	4.63 4.13	17.55 17.02
2d	CH_2=CH-CH_2	$C_{18}H_{18}N_6O_2S_2$ (414.50)	201-2	90.71	52.15 52.18	4.37 4.36	20.27 20.62
2e	C_4H_9	$C_{19}H_{22}N_6O_2S_2$ (430.55)	199-200	72.09	53.00 53.02	5.15 4.95	19.51 19.37
2f	C_6H_5	$C_{21}H_{18}N_6O_2S_2$ (450.53)	194-95	84.78	55.98 55.81	4.02 3.71	18.65 18.33
2g	$C_6H_4CH_3$(4-)	$C_{22}H_{20}N_6O_2S_2$ (464.56)	206-7	87.15	56.87 56.33	4.33 4.00	18.09 17.73
2h	C_6H_4Cl(4-)	$C_{21}H_{17}ClN_6O_2S_2$ (484.98)	219-20	86.41	52.00 51.89	3.53 3.50	17.32 17.31
2i	C_6H_4Br(4-)	$C_{21}H_{17}BrN_6O_2S_2$ (529.44)	208	85.56	47.64 47.11	3.23 3.08	15.87 15.72
3a	CH_3	$C_{16}H_{14}N_6OS_2$ (370.45)	184-85	92.85	51.87 51.76	3.80 3.67	22.68 22.00
3b	C_2H_5	$C_{17}H_{16}N_6OS_2$ (384.48)	195-96	88.06	53.10 52.64	4.19 4.26	21.85 21.72
3c	CH_2=CH-CH_2	$C_{18}H_{16}N_6OS_2 \cdot ½\ H_2O$ (405.49)	141-42	81.16	53.31 53.29	4.22 4.13	20.72 20.65
3d	C_4H_9	$C_{19}H_{20}N_6OS_2$ (412.53)	120-21	94.28	55.31 55.10	4.88 4.80	20.37 20.17
3e	C_6H_5	$C_{21}H_{16}N_6OS_2 \cdot ½\ H_2O$ (441.52)	188-89	96.40	57.12 57.50	3.88 3.74	19.03 19.08
3f	$C_6H_4CH_3$(4-)	$C_{22}H_{18}N_6OS_2 \cdot ½\ H_2O$ (455.54)	176-77	95.05	58.00 58.07	4.20 3.94	18.44 18.37
3g	C_6H_4Cl(4-)	$C_{21}H_{15}ClN_6OS_2$ (466.96)	206-7	90.63	54.01 53.54	3.23 3.09	17.99 17.55
3h	C_6H_4Br(4-)	$C_{21}H_{15}BrN_6OS_2$ (511.42)	209-10	97.90	49.31 49.32	2.95 2.88	16.43 16.35


m/z 315 (2b: 6.92 %, 2g: 88.19 %)
2b: m/z 87 (34.33 %)
2g: m/z 149 (10.08 %)
-NCS
2b: m/z 29 (33.95 %)
2g: m/z 91 (7.79 %)
2b: R = C2H5 [M+] m/z 402 (1.17 %)
m/z 300 (2b: 0.67 %, 2g: 13.88 %)
2b: m/z 45 (19.20 %)
2g: m/z 107 (2.37 %)
m/z 357 (2b: 0.26 %, 2g: 23.61 %)
-NH2
m/z 284 (2b: 9.31 %, 2g: 76.38 %)
m/z 224 (2b: 19.7 %, 2g: 2.56 %)
m/z 77 (2b: 100 %, 2g: 17.29 %)
-CH2=C=O
+H
m/z 243 (2b: 84.12 %, 2g: 100 %)


3b: [M+] m/z 384 (88.19 %)
3f: [M+] m/z 446 (17.38 %)
+H
m/z 243 (3b: 60.92 %, 3f: 100 %)
3b: m/z 142 (38.41 %)
3f: m/z 204 (9.20 %)
m/z 77 (3b: 100 %, 3f: 13.16 %)
3b: m/z 88 (25.16 %)
3f: m/z 150 (6.32 %)
m/z 58 (3b: 60.26 %, 3f: 5.93 %)

Scheme 2

The structures of the compounds were confirmed by IR, ^{1}H-NMR, EIMS and elemental analyses. The physical and analytical data of the compounds are shown in the Table.

The IR spectra of **2a-i** showed characteristic broad N-H stretching bands in the 3320-3040 cm^{-1} region. The C=O stretchings were observed at 1710-1670 cm^{-1}. ^{1}H-NMR spectra of **2b** and **2g** displayed the N^1-H resonance in the 10.22, 10.31; N^2-H in the 9.33, 9.60 and N^4-H in the 8.24, 9.58 ppm regions, respectively. The C5-H, C4-H and C3-H resonances of the furyl residue appear in the 7.79, 7.75; 6.52, 6.51; 6.13, 6.17 ppm regions with the expected splitting patterns, respectively (19). All the other protons were observed in the expected regions.

Although the EIMS spectra of representative examples **2b** and **2g** either showed a very weak molecular ion (**2b**) or did not show any molecular ion at all (**2g**), the molecules fragmented via the routes proposed for similar structures (23,24) (Scheme 2). Fragments at m/z 77 and m/z 243 were the base peaks. Formation of the base peak in **2g** involved the cleavage of the thioether bond and subsequent protonation yielded the fragment at m/z 243.

IR spectra provided evidence for the cyclization to the thiadiazole structure as the NH stretching bands of the starting materials absorbing as two separate bands absorbed as a single band (3340-3175 cm^{-1}). In the ^{1}H-NMR spectra of **3b** and **3f**, the NH proton at 5-position of 1,3,4-thiadiazole ring appeared at 7.44-7.37 ppm together with Ar-H as a multiplet and 10.11 ppm as a singlet, respectively. After the cyclization, absence of resonances assigned to the N^1-H and N^2-H protons in thiosemicarbazides provided confirmatory evidence to thiadiazole formation. The protons of the furan nucleus and the other protons resonated at the expected regions.

The EIMS of selected compounds **3b** and **3f** showed the molecular ions at m/z 384 and m/z 446 with different intensities and a fragmentation pattern cited for similar structures (22, 25). The fragmentation pattern is shown in Scheme 2.

2a-i and **3a-h** were evaluated for in vitro antimicrobial action against some bacteria and one fungus using the disc diffusion and macrodilution methods. Only **2i** showed antibacterial activity against *Staphylococcus aureus* with a MIC value of 58.6 μg/ml.

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