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Synthesis, structure elucidation and antimicrobial activity of some 3-hydroxy-2-naphthoic acid hydrazide derivatives

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Abstract

In this study, some 1,4-disubstituted thiosemicarbazide, 1,2,4-triazole and 1,3,4-thiadiazole type novel compounds derived from 3-hydroxy-2-naphthoic acid hydrazide were synthesized to screen for their antimicrobial activity. The structures of these substances were elucidated using elemental analysis and UV, ^{1}H NMR, and mass spectral methods. All of these compounds were tested in vitro for their antibacterial and antifungal activity.

Keywords: Antimicrobial activity; 3-Hydroxy-2-naphthoic acid hydrazide derivatives

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1. Introduction

Some aroylthiosemicarbazide derivatives have recently been reviewed. The considerable biological activities of aroylthiosemicarbazides are responsible for this interest. Furthermore, the chemistry of these structures is very interesting and 1,2,4-triazoline-5-thione and 1,3,4-thiadiazole rings can be obtained from these compounds [1,2]. The investigations showed that 1-aroyl-4-substituted thiosemicarbazides [3–8], 3,4-disubstituted-1,2,4-triazoline-5-thiones [6–9] and 2,5-disubstituted-1,3,4-thiadiazoles [3,10] have antimicrobial activity. Therefore 16 original compounds derived from 3-hydroxy-2-naphthoic acid hydrazide, which have these structures, have been synthesized to establish their possible antibacterial and antifungal activity.

2. Chemistry

For the synthesis of 1-aroyl-4-substituted thiosemicarbazides **IIa–d**, 3-hydroxy-2-naphthoic acid hydrazide **I** was added to ethyl-, cyclohexyl-, benzyl- and *p*-fluorophenylsothiocyanates. 1,3,4-Thiadiazole **IVa–d** and 1,2,4-triazoline-5-thione **IIIa–d** derivatives were obtained by the cyclization of the thiosemicarbazides in acidic medium and alkaline medium, respectively. Furthermore, acetylated derivatives of the 1,2,4-triazoline-5-thiones **IIIe–h** have also been synthesized. All of the triazoles underwent acetylation from the hydroxyl group, but two compounds without a methylene group attached to the N4 nitrogen of the triazole ring also underwent diacetylation from the N2 nitrogen of the triazole (**IIIf,h**). According to these results, electronic properties and/or spatial arrangements of groups linked to the N4 nitrogen of the triazole and also the heating time can be important in acetylation reactions. The synthetic route to these compounds is presented in Scheme 1 and the physicochemical properties of the synthesized compounds are listed in Table 1.

The structures of these compounds were confirmed by UV, ^{1}H NMR and mass (**IIIa,g** and **IVd**) spectral methods together with elemental analysis.

According to UV spectroscopic data, the K band of the triazole ring was observed in the range 226–236 nm. All of the compounds, which have benzyl- and *p*-fluorophenyl moieties, exhibited the E band of the benzene ring at 204–207 nm. The monoacetylation products (**IIIe,g**) showed maximum absorbance at about 255–256 nm which was different from the diacetylated ones.

The ^{1}H NMR spectra of the compounds showed the signals of aromatic protons in the range 6.92–8.75 ppm. For the thiosemicarbazides, the signals of the protons linked to N1–N2 and N3 nitrogens were shown at 11.16–11.44 and 7.76–9.85 ppm, respectively. The signals of the hydroxyl protons

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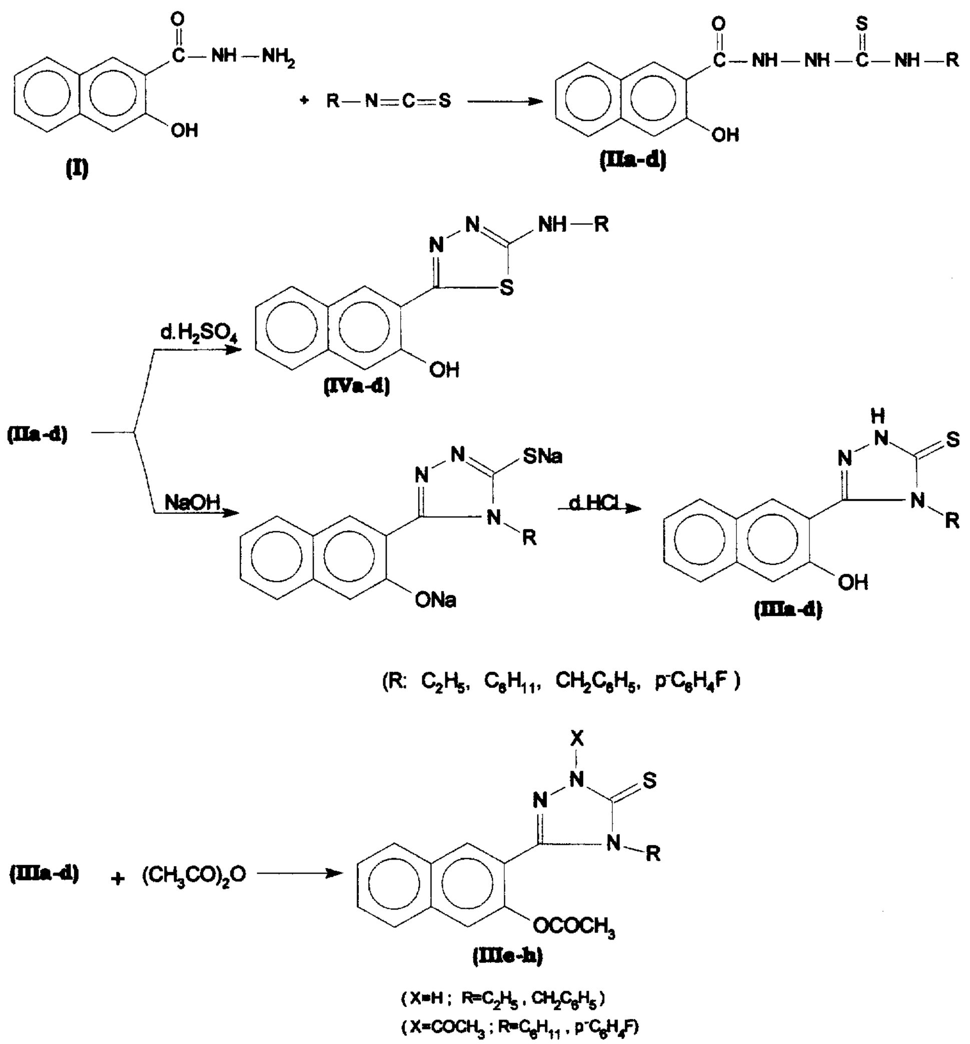


Scheme 1. Synthetic route to compounds **IIa–d**, **IIIa–h** and **IVa–d**.

of the naphthoyl ring were observed at 9.49–9.85 and 10.22–10.73 ppm in thiosemicarbazides and nonacetylated triazoles, respectively; in thiosemicarbazides, the signals of the hydroxyl protons appeared lower field than nonacetylated triazoles. While the NH proton of the secondary amine of thiadiazole was observed at higher field, the NH proton of the nonacetylated triazole ring shifted lower field and it was observed at 13.72–14.05 ppm as a singlet. In the diacetylated triazoles, the signal of the NH proton was not observed and the methyl protons of the acetyl groups (*N*-acetyl and *O*-acetyl) showed two singlets at 2.23–2.73 ppm. In the monoacetylated triazoles, only one singlet was observed at 2.18–2.16 ppm. These results supported the accuracy of our compounds. The ^1H NMR and mass spectral data were consistent with the proposed structures.

The mass spectra of **IIIa,g** and **IVd** revealed the molecular ion (M^+) peaks (Table 2). Each of them showed fragments characteristic of the substituted naphthol moiety at *m/z* 169, 141, 140 and 114. The main mass fragmentation patterns of the compounds are shown in Scheme 2.

3. Experimental

3.1. Chemistry

Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. UV spectra were obtained on a Shimadzu UV 2100S spectrophotometer (1 mg/100 ml in ethanol). The elemental analyses were performed on Carlo-Erba 1106 and Leco CHNS-932 instruments and the results were within ±0.4% of the theoretical values. ^1H NMR spectra were taken on a Bruker AVANC-DPX 400 spectrometer in DMSO with TMS as internal reference; chemical shifts δ are expressed in ppm. Mass spectra of **IIIa,g** and **IVd** were obtained on a Fisons Instruments VG Platform II LS–MS spectrometer.

3-Hydroxy-2-naphthoic acid hydrazide, ethylisothiocyanate and cyclohexylisothiocyanate were purchased from Sigma. Benzylisothiocyanate and *p*-fluorophenylisothiocyanate were supplied from Fluka and Aldrich, respectively. Sodium hydroxide, sulfuric acid, hydrochloric acid, acetic

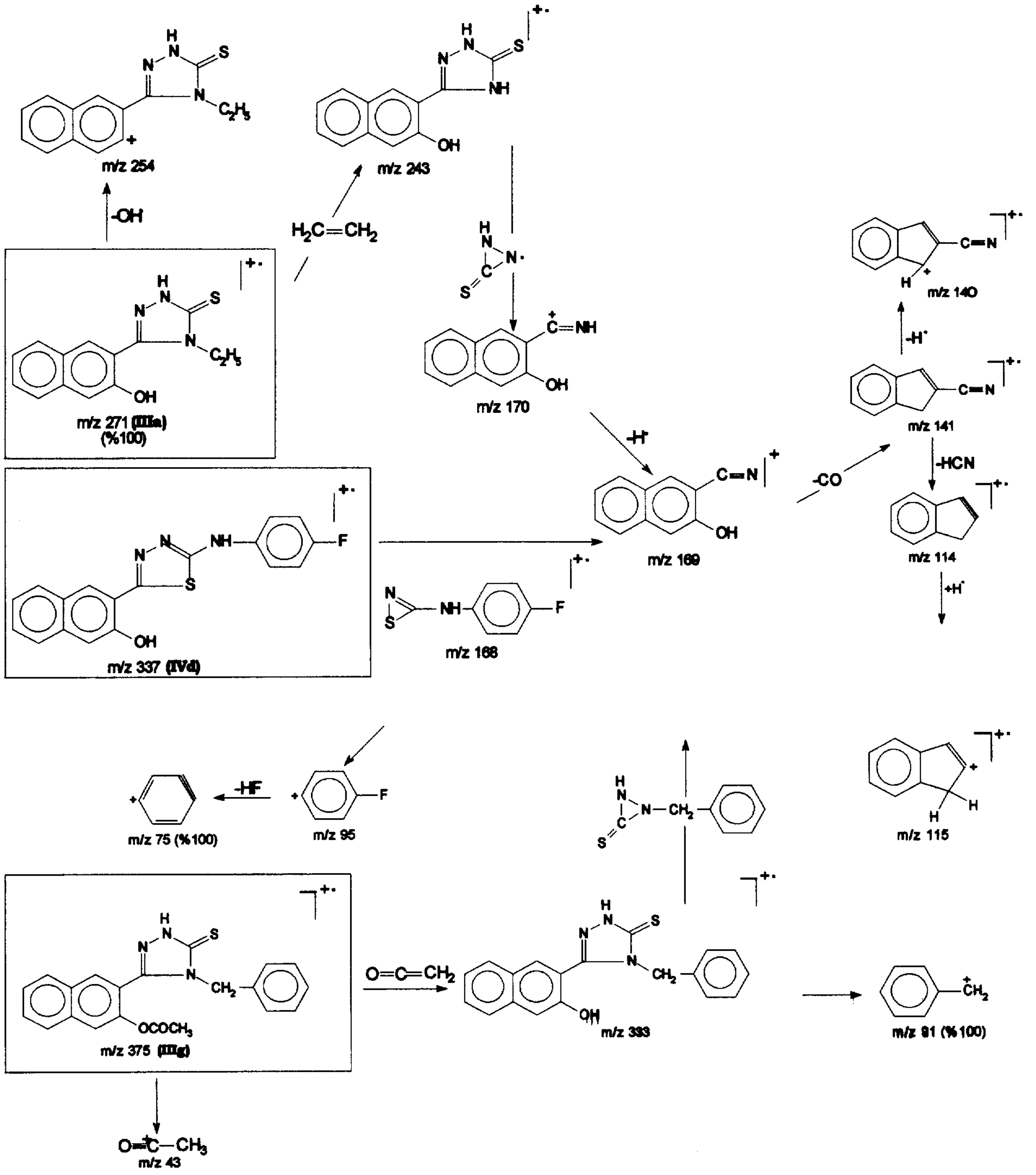
Table 1
The melting points, yields and spectral data of the compounds

Comp.	M.p. (°C)	Yield (%)	Mol. formula (Mol. weight)	UV_{max}, ϵ(EtOH) (1 mg/100 ml)	^{1}H NMR (DMSO)
IIa	198–199	92.05	$C_{14}H_{15}N_3O_2S$ (289.35)	330.4 (15046), 288.6 (14901), 236.2 (51432)	1.10 (t, 3H, CH_3), 3.49 (q, 2H, CH_2), 7.30–8.48 (m, 6H, Ar), 8.06 (s, 1H, N_3), 9.49 (s, 1H, OH), 11.26 (s, 2H, N_1 and N_2)
IIb	206	81.88	$C_{18}H_{21}N_3O_2S$ (343.45)	330.2 (17301), 289.0 (16099), 236.6 (48298)	1.04–1.88 (m, 10H, CH_2), 4.09 (s, 1H, CH), 7.30–8.40 (m, 6H, Ar), 7.76 (d, 1H, N_3), 9.55 (s, 1H, OH), 11.16 (s, 2H, N_1, N_2)
IIc	203	90.91	$C_{19}H_{17}N_3O_2S.H_2O$ (369.45)	316.8 (9348), 287.4 (12265), 237.4 (50219), 205.6 (16095)	4.79 (s, 2H, CH_2), 7.23–8.68 (m, 11H, Ar), 8.53 (s, 1H, N_3), 9.66 (s, 1H, OH), 11.44 (s, 2H, N_1 and N_2)
IId	199	91.19	$C_{18}H_{14}FN_3O_2S$ (355.39)	321.8 (13327), 236.8 (51531), 204.0 (17059)	7.16–8.54 (m, 10H, Ar), 9.85 (s, 2H, N_3 and OH), 11.44 (s, 2H, N_1 and N_2)
IIIa	228–230	94.74	$C_{14}H_{13}N_3OS$ (271.33)	229.8 (57217)	1.05 (t, 3H, CH_3), 3.90 (q, 2H, CH_2), 7.34–8.00 (m, 6H, Ar), 10.49 (s, 1H, OH), 13.82 (s, 1H, NH)
IIIb	273	94.44	$C_{18}H_{19}N_3OS$ (325.43)	256.0 (28697), 229.4 (62349)	0.88–2.23 (m, 10H, CH_2), 4.09 (s, 1H, CH), 7.30–7.96 (m, 6H, Ar), 10.46 (s, 1H, OH), 13.72 (s, 1H, NH)
IIIc	234	94.37	$C_{19}H_{15}N_3OS$ (333.41)	234.2 (46811), 205.1 (13570)	5.26 (s, 2H, CH_2), 6.92–7.77 (m, 11H, Ar), 10.73 (s, 1H, OH), 13.90 (s, 1H, NH)
IIId	259–261	90.88	$C_{18}H_{12}FN_3OS$ (337.38)	236.4 (53677), 205.0 (15013)	7.07–8.03 (m, 10H, Ar), 10.22 (s, 1H, OH), 14.05 (s, 1H, NH)
IIIe	160–162	37.6	$C_{16}H_{15}N_3O_2S$ (313.38)	254.7 (31366), 226.6 (69618)	1.12 (t, 3H, CH_3), 2.18 (s, 3H, $OCOCH_3$), 3.94 (q, 2H, CH_2), 7.63–8.35 (m, 6H, Ar), 13.98 (s, 1H, NH)
IIIf	135	84	$C_{22}H_{23}N_3O_3S$ (409.50)	226.4 (76269)	0.82–2.01 (m, 10H, CH_2), 2.23 (s, 3H, $OCOCH_3$), 2.61 (s, 3H, $NCOCH_3$), 4.34 (s, 1H, CH), 7.65–8.34 (m, 6H, Ar)
IIIg	215–217	98.05	$C_{21}H_{17}N_3O_2S$ (375.45)	256.1 (26582), 232.4 (48621), 205.1 (14980)	2.16 (s, 3H, $OCOCH_3$), 5.23 (s, 2H, CH_2), 6.97–7.99 (m, 11H, Ar), 14.16 (s, 1H, NH)
IIIh	194	89.75	$C_{22}H_{16}FN_3O_3S$ (421.45)	234.8 (49520), 207.0 (15846)	2.29 (s, 3H, $OCOCH_3$), 2.73 (s, 3H, $NCOCH_3$), 7.22–8.08 (m, 10H, Ar)
IVa	210	78.37	$C_{14}H_{13}N_3OS \cdot \frac{1}{2}H_2SO_4 \cdot \frac{1}{2}C_2H_5OH$ (343.40)	339.0 (15231), 290.1 (15324), 241.8 (24235), 216.2 (17236)	1.25 (t, 3H, CH_3), 3.50 (2H, CH_2) [a], 7.32–8.49 (m, 6H, Ar), 8.41(s, 1H, NH), 11.21 (s, 1H, OH)
IVb	225–230	87.37	$C_{18}H_{19}N_3OS \cdot H_2SO_4$ (423.51)	342.1 (14766), 289.7 (11756), 243.0 (26238), 212.8 (16800)	1.20–2.03 (m, 1OH, CH_2), 3.50 (1H, CH) [a], 7.36–8.64 (m, 6H, Ar), 13.43 (s, 2H, OH and NH)
IVc	225	95.92	$C_{19}H_{15}N_3OS$ (333.41)	326.8 (23339), 285.2 (16304), 238.9 (30540), 205.1 (16437)	4.58 (d, 2H, CH_2), 7.27–8.46 (m, 11H, Ar), 8.41 (t, 1H, NH), 10.93 (s, 1H, OH)
IVd	268	97.54	$C_{18}H_{12}FN_3OS$ (337.38)	344.8 (28441), 247.2 (32490), 206.1 (19804)	7.20–8.64 (m, 10H, Ar), 10.44 (s, 1H, OH), 11.12 (s, 1H, NH)

[a] Interfered with H_2O peak of the solvent.

Table 2
The mass spectral data of **IIIa,g** and **IVd**

Comp.	M^+ (relative abundance in %)	Characteristic ions (relative abundance in %)
IIIa	271 (100)	273 ($M+2$) (15), 272 ($M+1$) (66), 270 (21), 254 (54), 243 (32), 170 (19), 169 (28), 152 (15), 141 (40), 140 (23), 126 (15), 115 (14), 114 (33), 113 (23), 69 (19), 60 (19), 59 (19), 45 (15), 42 (23)
IIIg	375 (23)	376 ($M+1$) (14), 334 (15), 333 (54), 169 (12), 141 (14), 140 (19), 114 (14), 92 (12), 91 (100), 65 (16), 43 (85)
IVd	337 (46)	338 ($M+1$) (12), 187 (14), 169 (39), 168 (44), 155 (22), 141 (70), 140 (59), 128 (90), 115 (86), 114 (97), 109 (36), 102 (16), 95 (86), 88 (29), 83 (70), 75 (100), 63 (43), 57 (29), 51 (21)



Scheme 2. The main mass fragmentation patterns of **IIIa,g** and **IVd**.

anhydride and methanol used in the experiments were all purchased from Merck.

*3.1.1. Synthesis of 1-(3-hydroxy-2-naphthoyl)-4-substituted thiosemicarbazides **IIa–d***

0.0025 mol of **I** in 20 ml of ethanol was heated until it dissolved. Ethyl-, cyclohexyl-, benzyl- or *p*-fluorophenyl-isothiocyanate (0.003 mol) was added and the mixtures were refluxed for 3 h. The crystalline product was recrystallized from ethanol.

*3.1.2. Synthesis of 1,4-dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-5H-1,2,4-triazoline-5-thiones **IIIa–d***

A solution of 0.0015 mol **IIa–d** in 2N sodium hydroxide solution (4 ml) was heated under reflux for 2.5 h. After cooling at room temperature, concentrated hydrochloric acid was added. The precipitate was filtered and washed several times with distilled water. The pure compounds were obtained following crystallization from ethanol.

*3.1.3. Synthesis of 1,4-dihydro-3-(3-acetyloxy-2-naphthyl)-4-substituted-5H-1,2,4-triazoline-5-thiones **IIIe,g** and 1,4-dihydro-1-acetyl-3-(3-acetyloxy-2-naphthyl)-4-substituted-5H-1,2,4-triazoline-5-thiones **IIIf,h***

A mixture of 0.0015 mol **IIIa–d** and acetic anhydride (2.5 ml) was heated under reflux for 30 min. After cooling, distilled water was added. The resulting precipitate was filtered and washed with distilled water. The residue was purified by recrystallization with methanol.

*3.1.4. Synthesis of 5-(3-hydroxy-2-naphthyl)-2-substituted amino-1,3,4-thiadiazoles **IVa–d***

0.0015 mol **IIa–d** was dissolved in concentrated sulfuric acid. The mixture was stirred at room temperature for 30 min. It was poured into cooling water and washed with distilled water. The precipitate was recrystallized from ethanol.

3.2. Microbiology

The antibacterial and antifungal activity of the 16 original substances (**IIa–d**, **IIIa–h**, **IVa–d**) and the starting compound **I** were tested by the microdilution method [11–13]. In the determinations of antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans*, Mueller-Hinton broth (pH 7.3) and yeast nitrogen base, respectively, were used.

All of the compounds were dissolved in DMSO and concentrations of 0.25–500 μg/ml were examined. The results from these experiments were compared with those of ceftriaxone (Roche) and miconazole (Sigma) as references for antibacterial and antifungal agents, respectively. The concentration ranges of ceftriaxone for *Staph. aureus*, *E. coli* and *Ps. aeruginosa* were 0.015–32, 0.0019–3.84 and 0.12–256 μg/ml, respectively. For *C. albicans*, the concentration limits were 0.05–100 μg/ml.

4. Results and discussion

The minimum inhibitory concentrations (MICs) of ceftriaxone for *Staph. aureus*, *E. coli* and *Ps. aeruginosa*, are 2, 0.06 and 8 μg/ml, respectively, and the MIC of miconazole for *C. albicans* is 0.05 μg/ml. These values are within the limits given by the National Committee for Clinical Laboratory Standards (NCCLS) [13].

The MIC values of the compounds are listed in Table 3. For nine compounds, the antibacterial activity against *Staph. aureus* is better than that of the starting compounds **I**. The

Table 3
MIC values (μg/ml) of the compounds

Comp.	*Staph. aureus* ATCC 29213	*E. coli* ATCC 25922	*Ps. aeruginosa* ATTC 27853	*C. albicans* ATCC 2091
I	250	500	500	250
IIa	64	500	500	250
IIb	64	500	500	500
IIc	500	500	>500	250
IId	64	125	500	250
IIIa	250	500	500	250
IIIb	250	250	500	250
IIIc	250	500	500	125
IIId	250	500	500	250
IIIe	125	500	500	250
IIIf	125	250	250	250
IIIg	500	500	>500	250
IIIh	64	500	500	500
IVa	125	125	125	125
IVb	250	500	500	250
IVc	125	125	125	125
IVd	125	250	250	125

thiadiazole derivatives, except for **IVb**, have better activity than **I** against all the examined bacteria and the fungus. When the acetylated triazoles are compared with the nonacetylated triazoles, it is observed that the acetylated ones (especially **IIIh**), except for **IIIg**, are more potent against *Staph. aureus*. **IIc** and **IIIg**, which have the benzyl moiety, were found to exhibit less antibacterial activity against *Staph. aureus* and *Ps. aeruginosa* than **I**. The antibacterial activity against *Staph. aureus* of the thiosemicarbazide derivatives, except for **IIc**, is about four times higher than that of **I**. As a result, **IIa,b,d** and **IIIh** were the most active compounds against *Staph. aureus*, and may be considered promising for the development of new antibacterial agents.

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