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Synthesis and Antimicrobial Activities of Some Substituted Thiosemicarbazides, 1,2,4-Triazole-5-thiones and Their 5-Methyl Derivatives

Erhan PALASKA*°, Gülay ŞAHİN*, Melike EKİZOĞLU**, Meral ÖZALP**

Synthesis and Antimicrobial Activities of Some Substituted Thiosemicarbazides, 1,2,4-Triazole-5-thiones and Their 5-Methyl Derivatives

Summary : *A series of new 1- [(1-naphthyl)oxyacetyl]-4-substituted-thiosemicarbazides, 3-[(1-naphthyl)oxymethyl]-4-substituted-1,2,4-triazole - 5 - thiones and 3-[(1-naphthyl) oxymethyl]-4-substituted-5-methylmercapto-1,2,4-triazoles have been synthesized from 1-naphthol . The chemical structures of the compounds were proved by IR, ^{1}H-NMR and elementary analysis. Antimicrobial activities of the synthesized compounds were investigated against* Staphylococcus aureus, Enterococcus faecalis, Escherichia coli *and* Pseudomonas aeruginosa, Candida albicans, C. krusei *and* C. parapsilosis *by the microdilution method.*

Key Words: *Thiosemicarbazide, 1,2,4-Triazole-5-thione, 5-Methylmercapto-1,2,4-triazole, Antifungal activity, Antibacterial activity.*

Bazı Sübstitüe Tiyosemikarbazitler, 1,2,4-Triazoller ve 5-Metil Türevlerinin Sentezleri ve Antimikrobiyal Aktiviteleri

Özet : *1-Naftolden hareketle yeni 1-[(1-naftil)oksiasetil]-4-sübstitüe-tiyosemikarbazit, 3-[(1-naftil)oksimetil]-4-sübstitüe-1,2,4-triazol-5-tiyon ve 3-[(1-naftil)oksimetil]-4-sübstitüe-5-metilmerkapto-1,2,4-triazol türevleri sentez edilmiştir. Bileşiklerin kimyasal yapıları IR, ^{1}H-NMR ve eleman analizleri ile kanıtlanmıştır. Sentezi yapılan bileşiklerin antifungal ve antibakteriyel aktiviteleri* Staphylococcus aureus, Enterococcus faecalis, Escherichia coli *ve* Pseudomonas aeruginosa, Candida albicans, C. krusei *ve* C. parapsilosis *'e karşı mikrodiliüsyon yöntemi kullanılarak incelenmiştir.*

Anahtar kelimeler : *Tiyosemikarbazit, 1,2,4-Triazol-5-tiyon, 5-Metilmerkapto - 1,2,4 - triazol, Antifungal aktivite, Antibakteriyal c'tivite*

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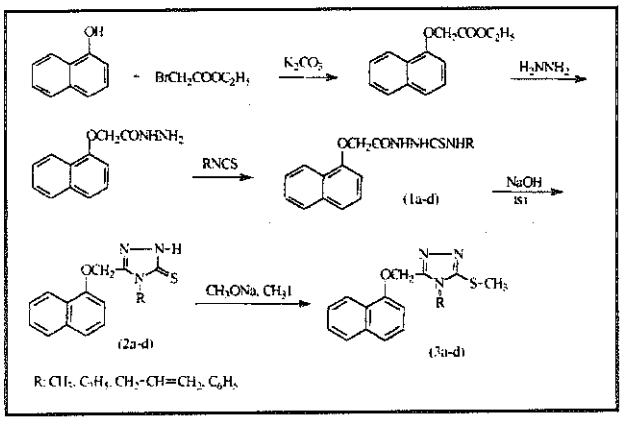
INTRODUCTION

Thiosemicarbazides and corresponding triazole derivatives are reported to possess antimicrobial[1-5], antiinflammatory and anticonvulsant activities[6-7]. In view of these findings, it was thought of interest to synthesize 1-[(1-naphthyl)oxyacetyl]-4-substituted-thiosemicarbazide, 3 - [(1-naphthyl)oxymethyl] - 4 - substituted-1,2,4-triazole-5-thione, 3-[(1-naphthyl) oxymethyl]-4-substituted-5-methylmercapto - 1,2,4 - triazole derivatives and evaluate their antibacterial and antifungal activities.

1-Naphthyloxyacetylhydrazine was prepared by esterification of 1-naphthol with ethyl bromoacetate and anhydrous potassium carbonate in dry acetone, followed by refluxing with hydrazine hydrate in absolute ethanol. The treatment of 1-naphthyl-

* Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100 Ankara-TURKEY
** Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 06100 Ankara-TURKEY
° Correspondence

oxyacetylhydrazine with different substituted isothiocyanates in absolute ethanol gave 1-[(1-naphthyl)oxyacetyl]-4-substituted-thiosemicarbazides (1a-d), which were cyclized to corresponding 3-[(1-naphthyl)oxymethyl]-4-substituted-1,2,4-triazole-5-thiones (2a-d) by heating in 1N sodium hydroxide. 3-[(1-Naphthyl)oxymethyl]-4-substituted-5-methylmercapto-1,2,4-triazoles (3a-d) were obtained by treating appropriate compounds (2a-d) with the methyl iodide in methanol, in the presence of sodium methoxide[8] (Scheme 1).



Scheme 1: Synthesis of the compounds.

The structures of the compounds were confirmed by IR, ^{1}H-NMR and elementary analysis. Formula, melting points, yields % and spectral data of the compounds are given in Table 1.

The antimicrobial activities of twelve compounds were tested against some Gram (+) and Gram (-) bacteria such as *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and yeast like fungi such as *Candida albicans* (ATCC 90028), *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 90018) by using the microdilution method[9].

MATERIALS AND METHODS

Chemistry

The chemicals were supplied by Aldrich and Merck Chemical Co. Melting points were taken in a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded in the Perkin Elmer FT-IR 1720x spectrophotometer (KBr disc). ^{1}H-NMR spectra were run on a Bruker AC 400 MHz FT-NMR spectrometer (DMSO-d_6, $CDCl_3$, TMS). The elementary analysis of the compounds were performed at the Scientific and Technical Research Council of Turkey.

1-Naphthyloxyacetylhydrazine

A mixture of 14.42 g 1-naphthol (0.1 mol), 13.82 g anhydrous potassium carbonate (0.1 mol) and 16.70 g ethyl bromoacetate (0.1 mol) in 50 ml dry acetone were refluxed on an oil bath for 6 h. The reaction mixture was filtered and acetone was removed under reduced pressure. The residue and 1.17 g 65% hydrazine hydrate (0.15 mol) were dissolved in 30 ml ethanol and refluxed for 1h. Precipitated product was filtered off, dried and crystallized from ethanol.

1-[(1-Naphthyl)oxyacetyl]-4-substituted-thiosemicarbazides (1a-d)

4.33 g of 1-naphthyloxyacetylhydrazine (0.02 mol) and 0.02 mol of appropriate substituted isothiocyanate derivatives were refluxed in 50 ml absolute ethanol for 4 h. The crude product which precipitated on cooling was filtered off, washed with diethyl ether, dried and crystallized from suitable solvents.

3-[(1-Naphthyl)oxymethyl]-4-substituted-1,2,4-triazole-5-thiones (2a-d)

1-[(1-Naphthyl)oxyacetyl]-4-substituted-thiosemicarbazides (0.01 mol) were refluxed for 8 h. in 40 ml 1 N aqueous sodium hydroxide solution. The mixture was acidified to pH 2 and the precipitated product was filtered off, washed several times with water and crystallized from suitable solvents.

3-[(1-Naphthyl)oxymethyl]-4-substituted-5-methylmercapto-1,2,4-triazoles (3a-d)

0.22 g of sodium methoxide (0.004 mol) was added to a solution of 3-[(1-naphthyl)oxymethyl]-4-substituted-1,2,4-triazole-5-thiones (2 a-d) (0.004 mol) in ethanol (50 ml) and the mixture was heated under

Table 1 : Formula, melting points, yields %, IR and ^{1}H-NMR spectral data of the compounds.

Comp.	R	m.p. (°C)	Yield %	IR ν (cm^{-1})	^{1}H-NMR (δ ppm)
1a	CH_3	174-6	87.2	3334, 3171 (N-H) 1697 (C=O) 1234 (C=S)	2.84 (3H; d[a]; -C<u>H</u>$_3$), 4.72 (2H; s; Ar-O-C<u>H</u>$_2$-), 6.90-8.39 (7H; m; aromatic prot.), 7.95 (1H; d; CS-N<u>H</u>-CH$_3$), 9.25 (1H; s; CO-NH-N<u>H</u>-CS), 10.05 (1H; s; CO-N<u>H</u>-NH-CS)
1b	C_2H_5	167	61.3	3320, 3296 (N-H) 1663 (C=O) 1237 (C=S)	1.10 (3H; t; CH2-C<u>H</u>$_3$), 3.45-3.52 (2H; q; C<u>H</u>$_2$-CH$_3$), 4.79 (2H; s; Ar-O-C<u>H</u>$_2$-), 6.96-8.45 (7H; m; aromatic prot.), 7.99 (1H; d; CS-<u>NH</u>-CH$_2$-CH$_3$), 9.23 (1H; s; CO-NH-N<u>H</u>-CS), 10.07 (1H; s; CO-N<u>H</u>-NH-CS)
1c	C_3H_5	172	80.0	3327, 3165 (N-H) 1695 (C=O) 1242 (C=S)	4.15 (2H; m; -C<u>H</u>$_2$-CH=CH$_2$), 4.80 (2H; s; Ar-O-C<u>H</u>$_2$-), 5.06 (1H; dd; -CH$_2$-CH=C<u>H</u>$_2$; H_A; J_{AB}: 1.6 Hz, J_{AX}: 10.3 Hz), 5.16 (1H; dd; -CH$_2$-CH=C<u>H</u>$_2$; H_B; J_{AB}: 1.6 Hz, J_{BX}: 17.5 Hz), 5.80-5.88 (1H; m; -CH$_2$-C<u>H</u>=CH$_2$), 6.95-8.45 (7H; m; aromatic prot.), 8.19 (1H; s; CS-N<u>H</u>-CH$_2$-), 9.35 (1H; s; CO-NH-N<u>H</u>-CS), 10.12 (1H; s; CO-N<u>H</u>-NH-CS)
1d	C_6H_5	164-6	77.5	3317, 3131 (N-H) 1694 (C=O) 1237 (C=S)	4.78 (2H; s; Ar-O-C<u>H</u>$_2$-), 6.93-8.40 (7H; m; aromatic prot.), 7.83 (1H; s; CS-<u>NH</u>-C$_6$H$_5$), 9.63 (1H; s; CO-NH-N<u>H</u>-CS), 10.26 (1H; s; CO-N<u>H</u>-NH-C=S)
2a	CH_3	223	88.8	3375 (N-H) 1632 (C=N) 1242 (C=S)	3.51 (3H; d; N-C<u>H</u>$_3$), 5.38 (2H; s; Ar-O-C<u>H</u>$_2$-), 7.11-8.09 (7H; m; aromatic prot.), 13.5-14.2 (1H; bs; N<u>H</u>-C=S)
2b	C_2H_5	211	77.7	3375 (N-H) 1632 (C=N) 1241 (C=S)	1.28 (3H; t; N-CH$_2$-C<u>H</u>$_3$), 4.12 (2H; q; -C<u>H</u>$_2$-CH$_3$), 5.44 (2H; s; Ar-O-C<u>H</u>$_2$-), 7.18-8.13 (7H; m; aromatic prot.), 13.91 (1H; s; N<u>H</u>-C=S)
2c	C_3H_5	159-60	57.9	3379 (N-H) 1631 (C=N) 1243 (C=S)	4.70 (2H; d; N-C<u>H</u>$_2$-CH=CH$_2$), 4.98 (1H; dd; -CH$_2$-CH=C<u>H</u>$_2$; H_B; J_{AB}: 1.2 Hz, J_{BX}: 17.2 Hz), 5.06 (1H; dd; -CH$_2$-CH=C<u>H</u>$_2$; H_A; J_{AB}: 1.2 Hz, J_{AX}: 10.3 Hz), 5.31 (2H; s; Ar-O-C<u>H</u>$_2$-), 5.81-5.90 (1H; m; -CH$_2$-C<u>H</u>=CH$_2$), 7.08-8.07 (7H; m; aromatic prot.) 13.6-14.4 (1H; bs; N<u>H</u>-C=S)
2d	C_6H_5	238-9	39.9	1632 (C=N) 1243 (C=S)	5.21 (2H; s; Ar-O-C<u>H</u>$_2$-), 6.97-7.84 (12H; m; aromatic prot.) 13.80-14.35 (1H; bs; N<u>H</u>-C=S)
3a	CH_3	168	81.5	1632 (C=N) 737 (C-S)	2.89 (3H; s; S-C<u>H</u>$_3$), 3.78 (3H; s; N-C<u>H</u>$_3$) 5.7 (2H; s; Ar-O-C<u>H</u>$_2$-), 7.04-8.08 (7H; m; arom. prot.) Anal: Calc. C:63.13, H:5.30, N:14.73, Found: C:63.27, H:5.16, N:14.55
3b	C_2H_5	85-6	73.6	1631 (C=N) 690 (C-S)	1.3 (3H; t; -CH$_2$-C<u>H</u>$_3$), 2.58 (3H; s; S-C<u>H</u>$_3$) 4.14 (2H; q; -C<u>H</u>$_2$-CH$_3$), 5.23 (2H; s; Ar-O-C<u>H</u>$_2$-), 7.02-8.11 (7H; m; aromatic prot.). Anal: Calc. C:64.19, H:5.72, N:14.04, Found: C:63.98, H:5.49, N:14.21
3c	C_3H_5	143	45.5	1631 (C=N) 684 (C-S)	2.93 (3H; s; S-C<u>H</u>$_3$), 4.81 (2H; d; -C<u>H</u>$_2$-CH=CH$_2$), 5.16 (1H; dd; -CH$_2$-CH=C<u>H</u>$_2$; H_B; J_{BX}: 17.04 Hz), 5.25 (1H; dd; -CH$_2$-CH=C<u>H</u>$_2$; H_A; J_{AX}: 10.3 Hz), 5.71 (2H; s; Ar-O-C<u>H</u>$_2$-), 5.76-5.80 (1H; m; -CH$_2$-C<u>H</u>=CH$_2$), 7.01-8.06 (7H; m; aromatic prot.). Anal: Calc. C:65.57, H:5.50, N:13.49, Found: C:65.67, H:5.48, N:13.55
3d	C_6H_5	116-7	88.4	1632 (C=N) 776 (C-S)	2.75 (3H; s; S-C<u>H</u>$_3$), 5.21 (2H; s; Ar-O-C<u>H</u>$_2$-), 6.78-7.61 (12H; m; aromatic prot.) Anal: Calc. C:69.14, H:4.93, N:12.09, Found: C:69.22, H:4.88, N:12.35

[a] s: singlet, bs: broad singlet, d: doublet, dd: doublet of doublets, t: triplet, q: quarted, m: multiplet

reflux for 10 min. 0.57 g of methyl iodide (0.004 mol) was added to the mixture and stirred for 3 h. The mixture was poured into cold water (50 ml) and the solid separated out was filtered, dried and crystallized.

Microbiology

Antibacterial activities of the compounds were tested against Gram (+) and Gram (-) bacteria such as *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and the antifungal activities of the compounds against some yeast like fungi such as *Candida albicans* (ATCC 90028), *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 90018) were evaluated in vitro by using the microdilution method[9]. Ceftazidine and fluconazole were used as reference compounds. The stock solutions of the compounds tested were prepared in dimethylsulfoxide. The solutions in the test medium furnished the required concentration ranging from 512 - 0.5 µg/ml. The microtiter plates were incubated at 35 °C and read visually after 24 h. but for Candida species at 48 h. The minimum inhibitory concentration (MIC) values were recorded as the lowest concentrations of the substances that had no visible turbidity.

RESULTS AND DISCUSSION

1-[(1-Naphthyl)oxyacetyl]-4-substituted-thiosemicarbazides (1a-d) were prepared by the condensation of 1-naphthyloxyacetylhydrazine with alkyl or aryl isothiocyanates in yields varying from 61.3 to 87.2 %. Ring closure of 1-[(1-naphthyl)oxyacetyl]-4-substituted-thiosemicarbazides in 1N aquoeus sodium hydroxide gave 3-[(1-naphthyl)oxymethyl]-4-substituted-1,2,4-triazole-5-thiones (2a-d) in yields of 88.8-39.9 %. 3-[(1-Naphthyl)oxymethyl]-4-substituted-5 - methylmercapto -1,2,4-triazoles (3a-d) were obtained by treating compounds 2a-d with methyl iodide in methanol, in the presence of sodium methoxide[8] in yields of 45.5-88.4 %.

The IR spectral characteristics of the thiosemicarbazides were assigned at 3334-3131 cm^{-1}, 1697-1663 cm^{-1} and 1234-1237 cm^{-1} for N-H, C=O and C=S, respectively. The ^{1}H-NMR spectra of the compounds displayed three singlet at around 7.83-8.19, 9.23-9.63, and 10.05-10.12 ppm. These signals were attributed to N-H protons.

1,2,4-Triazole-5-thione derivatives (2a-d) may exist in thiole and thione forms. We observed that the thione form was prefered in the solution and solid states. The IR spectra of compounds 2a-d showed N-H bands in the region of 3375-3379 cm^{-1} instead of S-H bands occurred at around 2600-2550 cm^{-1}. In addition, the C=S absorption bands were exhibited in the region of 1241-1243 cm^{-1}. Otherwise in the ^{1}H-NMR spectra of 2a-d, the N-H protons were observed as a singlet at 13.5-14.35 ppm. The conversions were monitored by the disappearance of C=O stretching bands of thiosemicarbazides at 1697-1663 cm^{-1} and the appearance of a strong band at 1645-1609 cm^{-1} for C=N stretching in the IR spectra of the compounds 2a-d and 3a-d.

The ^{1}H-NMR spectra, methylmercapto-1,2,4-triazoles (3a-d) showed a singlet of three-proton intensity at δ 2.58-2.93 ppm for the methyl group, whereas in the corresponding 1,2,4-triazole-3-thione, the signal for the NH proton was at low field (13.5-14.4ppm).

The elementary analyses results of new compounds (3a-d) were within ± 0.4 % theoretical values.

In order to test antibacterial activity of the compounds against bacteria, such as *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) and yeast like fungi, such as *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 90018), the microdilution method was used. As can be seen in Tables 2 and 3; compounds 3a and 3b were moderately active against *S. aureus* at 64 µg/ml, compounds 2a, 2c and 2d were active against *E. faecalis* at 64, 32, 64 µg/ml, respectively. Except for 2a, 2c and 2d, all the compounds showed antibacterial activity between at 128-512 µg/ml. Compounds 3a and 3c showed activity against *C. krusei* at 64 µg/ml and all the compounds were active against *C. albicans* and *C. parapsilosis* at 64-256 µg/ml concentration.

Table 2: Antibacterial activities of the compounds.

Comp. (10mg/kg)	*Staphylococcus aureus* (ATCC 25923)	*Escherichia coli* (ATCC 25922)	*Enterococcus faecalis* (ATCC 29212)	*Pseudomonas aeruginosa* (ATCC 27853)
1a	128	256	128	256
1b	256	512	128	256
1c	128	256	128	256
1d	256	256	128	256
2a	256	256	64	256
2b	256	256	128	256
2c	128	256	32	256
2d	128	256	64	256
3a	64	128	256	256
3b	64	256	128	256
3c	>512	256	512	512
3d	256	256	256	256
Ceftazidine	2	0.25	n.a.	8

n.a.: No activitiy.

Table 3: Antifungal activities of the compounds.

Comp. (10mg/kg)	*Candida albicans* (ATCC 90028)	*Candida krusei* (ATCC 6258)	*Candida Parapsilosis* (ATCC 90018)
1a	128	128	128
1b	128	128	64
1c	>512	>512	64
1d	128	128	64
2a	128	128	128
2b	256	128	128
2c	128	256	128
2d	256	128	128
3a	128	64	128
3b	128	128	128
3c	64	64	64
3d	128	128	128
Fluconazole	0.25	32	0.5

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