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Short communication

Synthesis and antimicrobial activity of *N*-[(α-methyl)benzylidene]-(3-substituted-1,2,4-triazol-5-yl-thio) acetohydrazides

Seyhan Ersan [a,*], Sultan Nacak [a], Rukiye Berkem [b]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Hipodrom, 06330 Ankara, Turkey*
[b] *Ankara Hospital, Department of Microbiology and Clinical Microbiology, Cebeci, Ankara, Turkey*

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Abstract

In this study 18 new hydrazones have been synthesized by reacting *ortho*- or *para*-substituted acetophenones with (3-substituted-1,2,4-triazol-5-yl-thio)acetohydrazide in ethanol. The prepared compounds were tested for antimicrobial activity. The prepared compounds exhibited only poor activity against Gram (+) and Gram (−) bacteria with the minimal inhibitory concentration (MIC) ≥ 400 μg/ml. Moderate activity was observed against *Candida* species with MIC in the range 100–400 μg/ml.

Keywords: Antibacterial agents; Antifungal agents; Substituted-triazole derivatives

1. Introduction

Substituted 1,2,4-triazole derivatives have been associated with various types of biological effects such as antiviral, antibacterial, anti-inflammatory and anticonvulsant activity [1–6]. The 1,2,4-triazole derivatives have been reported in the literature to show antifungal activities [7–9]. In addition to these findings it is known that some hydrazones have antimicrobial activity [10].

On the basis of these data we have synthesized some hydrazones of 3-substituted-1,2,4-triazol-5-yl-thioacetohydrazide (Table 1) by reacting (3-substituted-1,2,4-triazol-5-yl-thio)acetohydrazide with substituted acetophenones, and their antimicrobial activities were investigated.

2. Chemistry

For the synthesis of the title compounds the reaction sequences outlined in Scheme 1 were followed, starting from 3-substituted-5-mercapto-1,2,4-triazole [11]. The reaction of 3-substituted-5-mercapto-1,2,4-triazole with ethyl bromoacetate has given ethyl (3-substituted-1,2,4-triazol-5-yl-thio)acetate. The ester has been converted to the corresponding hydrazide which has given the title compounds by the reaction with *ortho*- and *para*-substituted acetophenones.

3. Experimental

3.1. Chemistry

All chemicals were obtained from Aldrich Chemical Co. (Steinheim, Germany). Melting points were determined with an Electrothermal melting point apparatus (Southend, UK) and are uncorrected.

* Corresponding author.

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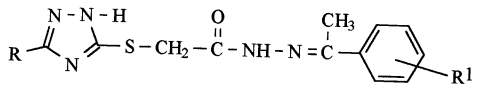
Table 1
Physical and chemical data of hydrazones of (3-substituted-1,2,4-triazol-5-yl-thio)acetohydrazides



Comp.	R	R^1	M.p. (°C)	Yield (%)	Formula	Analysis
1	H	4-Cl	136	63	$C_{12}H_{12}ClN_5OS$	C, H, N, S
2	H	4-OH	143	60	$C_{12}H_{13}N_5O_2S$	C, H, N, S
3	H	4-NO_2	193	50	$C_{12}H_{12}N_6O_3S$	C, H, N, S
4	H	2-Cl	195	47	$C_{12}H_{12}ClN_5OS$	C, H, N, S
5	H	2-OH	190	40	$C_{12}H_{13}N_5O_2S$	C, H, N, S
6	H	2-NO_2	192	35	$C_{12}H_{12}N_6O_3S$	C, H, N, S
7	CH_3	4-Cl	168	50	$C_{13}H_{14}ClN_5OS$	C, H, N, S
8	CH_3	4-OH	198	44	$C_{13}H_{15}N_5O_2S$	C, H, N, S
9	CH_3	4-NO_2	158	50	$C_{13}H_{14}N_6O_3S$	C, H, N, S
10	CH_3	2-Cl	98	65	$C_{13}H_{14}ClN_5OS$	C, H, N, S
11	CH_3	2-OH	177	72	$C_{13}H_{15}N_5O_2S$	C, H, N, S
12	CH_3	2-NO_2	188	66	$C_{13}H_{14}N_6O_3S$	C, H, N, S
13	C_2H_5	4-Cl	116	45	$C_{14}H_{16}ClN_5OS$	C, H, N, S
14	C_2H_5	4-OH	192	32	$C_{14}H_{17}N_5O_2S$	C, H, N, S
15	C_2H_5	4-NO_2	165	68	$C_{14}H_{16}N_5O_3S$	C, H, N, S
16	C_2H_5	2-Cl	148	56	$C_{14}H_{16}ClN_5OS$	C, H, N, S
17	C_2H_5	2-OH	153	57	$C_{14}H_{17}N_5O_2S$	C, H, N, S
18	C_2H_5	2-NO_2	195	67	$C_{14}H_{16}N_5O_3S$	C, H, N, S

IR spectra were recorded on a Perkin–Elmer 1330 spectrometer (Uberlingen, Germany) [KBr, ν (cm^{-1})]. ^1H NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer (Rheinstetten, Karlsruhe, Germany) using TMS as internal standard and DMSO-d_6. All chemical shifts were reported as δ (ppm) values. Elemental analyses were performed with Leco-932 (C, H, N, S elemental analyzer, St. Joseph, USA) at the Scientific and Technical Research Council of Turkey, Instrumental Analysis Center (Ankara, Turkey), and the results were within $\pm 0.4\%$ of the calculated values.

3.1.1. (3-Substituted-1,2,4-triazol-5-yl-thio)-acetohydrazides

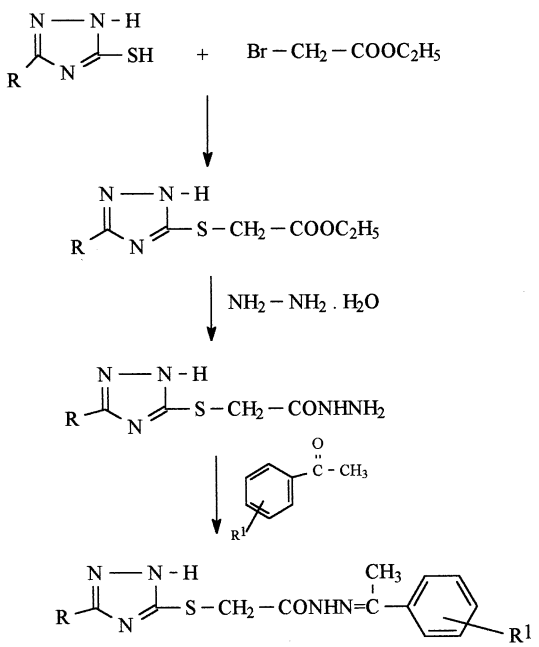
To the solution of ethyl (3-substituted-1,2,4-triazol-5-yl-thio)acetate [10] in ethanol, an equimolar amount of hydrazine hydrate was added and the solution was then stirred. The mixture was heated under reflux for 8 h. After evaporating ethanol, a solid compound was recrystallized from absolute ethanol.

3.1.2. Hydrazones of (3-substituted-1,2,4-triazol-5-yl-thio)acetohydrazides

(3-Substituted-1,2,4-triazol-5-yl-thio)acetohydrazide (0.003 mol) was dissolved in 10 ml ethanol and 0.2 ml acetic acid and 0.004 mol of *o*- or *p*-substituted acetophenone was added. The mixture was refluxed for 6 h, then left overnight at room temperature. The solid material which precipitated was filtered, dried and recrystallized from ethanol. Data concerning these compounds are presented in Table 1.

3.1.3. Spectral data of the compounds

The IR spectra of hydrazones of (3-substituted-1,2,4-triazol-5-yl-thio)acetohydrazides showed absorption bands at 3260 and 3000 cm^{-1} due to the 1,2,4-triazole moiety. Moreover C=O, amide II and C=N– stretching absorption bands were observed between 1685–1655, 1565–1510 and 1440–1380 cm^{-1}, respectively.



Scheme 1. Synthetic route of *N*-[(α-methyl)benzylidene]-(3-substituted-1,2,4-triazol-5-yl-thio)acetohydrazides.

Table 2
Antifungal activity of hydrazones of (3-substituted-1,2,4-triazol-5-yl-thio)acetohydrazides (MIC in μg/ml)[a]

Compound	Organisms					
	C. albicans		*C. parapsilosis*		*C. krusei*	
	24 h	48 h	24 h	48 h	24 h	48 h
1	200	200	200	400	400	400
2	200	200	200	400	200	400
3	100	400	100	400	100	200
4	200	200	200	400	200	400
5	800↑	800↑	800↑	800↑	400	800↑
6	200	400	100	400	200	200
7	200	200	200	400	200	400
8	200	200	200	400	200	400
9	100	200	100	200	200	400
10	200	200	100	400	200	400
11	800↑	800↑	800↑	800↑	800↑	800↑
12	200	400	200	400	200	400
13	100	400	100	400	200	200
14	200	400	200	400	200	200
15	100	400	100	400	200	400
16	200	400	100	400	200	200
17	200	200	200	400	200	400
18	200	400	100	200	400	400
Clotrimazole	0.5	1.0	0.5	1.0	0.5	1.0

[a] MIC, minimal inhibitory concentration.

The absorption bands associated with other functional groups appeared in the expected regions.

In the ^{1}H NMR spectra of title compounds, there are two singlets at about 3.95 and 4.30 ppm (2H) of the CH_2 group of the thioacetyl moiety. This phenomenon may be attributed to the folding of the side chain on to the sulfur bridge in solution. In other words these two singlets might represent two rotamers. In accordance with our suggestions, –CON*H*–N signals also appear as two singlets at 11.44–11.88 ppm. The same feature of similar compounds has been reported by Gürsoy et al. [12].

3.2. Pharmacology

3.2.1. Materials

The microdilution susceptibility test in Mueller-Hinton Broth (Oxoid Ltd., Basingstoke, Hampshire, UK) and Sabouraud Liquid Medium (Difco, Detroit, Michigan, USA) were used for the determination of antimicrobial activity [13–15]. Test organisms were: *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 as Gram (+) bacteria, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as Gram (−) bacteria, and *Candida albicans* ATCC 90028, *C. parapsilosis* ATCC 90018 and *C. krusei* ATCC 6258 as yeast. Antibacterial and antifungal screening were carried out using the same method.

3.2.2. Methods

The reference compounds penicillin G, ampicillin, gentamicin, vancomycin and clotrimazole were dissolved in DMSO at 800 μg/ml. Two-fold diluted solutions of the compounds and references were prepared (800, 400, 200, 100,..., 3.15 μg/ml). Microorganism suspensions at 10^6 CFU (colony forming units)/ml were inoculated to the wells. The plates were incubated at 36°C for 24–48 h. The incubation chamber was kept sufficiently humid. The minimal inhibitory concentrations (MIC) values were determined at the end of the incubation period.

4. Results and discussion

The antibacterial activity was present in almost all the prepared compounds, but it was rather poor with MIC around 400 μg/ml for both Gram (+) and Gram (−) strains, thus quite far from MIC of reference drugs (penicillin G, ampicillin, gentamicin, vancomycin) that were in the range 0.5–8 μg/ml. Compounds **5**, **10** and **11** were completely inactive.

Somewhat better results were obtained against yeasts (Table 2). Apart from compounds **5** and **11** (once more completely inactive), the tested compounds exhibited MIC in the range 100–400 μg/ml. The highest activities were shown by compounds **3**, **9**, **13** and **15**, all carrying a Cl or NO_2 group on the benzylidene moiety.

Acknowledgements

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