SYNTHESIS AND CHARACTERIZATION OF CERTAIN THIOUREA DERIVATIVES STARTING FROM 1,2,4-TRIAZOLINE-3-THIONES AS POTENTIAL ANTIBACTERIAL AND ANTIFUNGAL AGENTS

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SUMMARY

Several N-substituted-N'-[4-(4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thioureas were synthesized in order to examine their antibacterial and antifungal activities. The structures and purity of the synthesized compounds were confirmed by UV, IR and mass spectral data and elemental analysis. However, they were found not to possess significant antibacterial or antifungal activity.

KEY WORDS

1,2,4-triazoline-3-thione, substituted thiourea, antifungal activity, agar diffusion method

INTRODUCTION

Remarkable tuberculostatic /1-3/ and antiviral /4/ activities of thiourea and diphenylthiourea compounds have been reported. Certain 1,2,4-triazole derivatives have similar effects /5-7/. The antibacterial activity of thioureas /8,9/ and 1,2,4-triazoles /10,11/ and the antifungal activity of 1,2,4-triazoles /12,13/ have been widely studied.

In a structure-activity relationship study, Galabov and co-workers /4/ showed that the antiviral activity of diphenylthiourea derivatives was due to the -NH-C(=S)-NH- function in the molecule and that

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changes in this activity depended on its substituents. The compounds studied here [**5 a-r**] (Table 1) were considered to have the same functional group.

Moreover the compounds from which substances **5 a-r** were synthesized had some action against certain *Candida* species /13/ making the compounds **5 a-r** worth testing to see whether they had similar properties. Thus, we carried out the syntheses of several N-substituted-N'-[4-(4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl) phenyl]thiourea derivatives with different alkyl/aryl groups either at the fourth position of the triazole ring or at the terminal nitrogen of the thiourea.

The present paper describes the synthesis and characterization of nine compounds having a 1,2,4-triazoline ring combined with a thiourea moiety and their biological evaluation.

MATERIALS AND METHODS

Chemicals and instruments

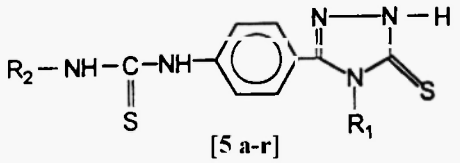
Chemicals used in the experiments were purchased from Merck and Sigma companies. All melting points were determined on a Buchi-530 melting point apparatus and uncorrected. Laboratory solvents were predried using standard procedures. UV spectra were recorded on a Shimadzu UV 2100S spectrophotometer (approx. 1 mg/100 ml ethanol). IR spectra were recorded on a Perkin-Elmer 1420 ratio recording infrared spectrophotometer as KBr pellets. Elemental analysis was performed on a CEST MOD 110 Analyser.

Preparation of 1,2,4-triazoline-3-thiones [4 a-f] starting from benzocaine

Ethyl 4-(benzoylamino)benzoate [**1**] was obtained from benzocaine by the action of benzoylchloride /14/. This product was then reacted with excess hydrazine hydrate to give 4-(benzoylamino)benzoic acid hydrazide [**2**] /14/ (see Fig. 1). In the following step, 1-[4-(benzoylamino)benzoyl]-4-alkyl/aryl thiosemicarbazides [**3 a-f**] were prepared by the reaction of compound **2** with methyl, ethyl, allyl, cyclohexyl, phenyl and phenethyl isothiocyanates /15/. Finally, 5-(4-aminophenyl)-4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones [**4**

TABLE 1

General structure and the suhstitutents of N-alkyl/aryl-N'-[4-(4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea derivatives



[5 a-r]

Compound	R_1	R_2
5 a*	CH_3	CH_3
5 b	CH_3	C_2H_5
5 c*	CH_3	$CH_2CH=CH_2$
5 d	CH_3	C_6H_{11}
5 e*	CH_3	C_6H_5
5 f	C_2H_5	$CH_2CH=CH_2$
5 g	C_2H_5	C_6H_{11}
5 h	$CH_2CH=CH_2$	$CH_2CH=CH_2$
5 i	$CH_2CH=CH_2$	C_6H_5
5 j	C_6H_{11}	$CH_2CH=CH_2$
5 k	C_6H_{11}	C_6H_{11}
5 l*	C_6H_5	CH_3
5 m*	C_6H_5	C_2H_5
5 n*	C_6H_5	$CH_2CH=CH_2$
5 o*	C_6H_5	C_6H_{11}
5 p**	C_6H_5	C_6H_5
5 q*	C_6H_5	C_6H_4Cl (p.)
5 r	$CH_2CH_2C_6H_5$	$CH_2CH=CH_2$

* /16/; ** /16,18,21/

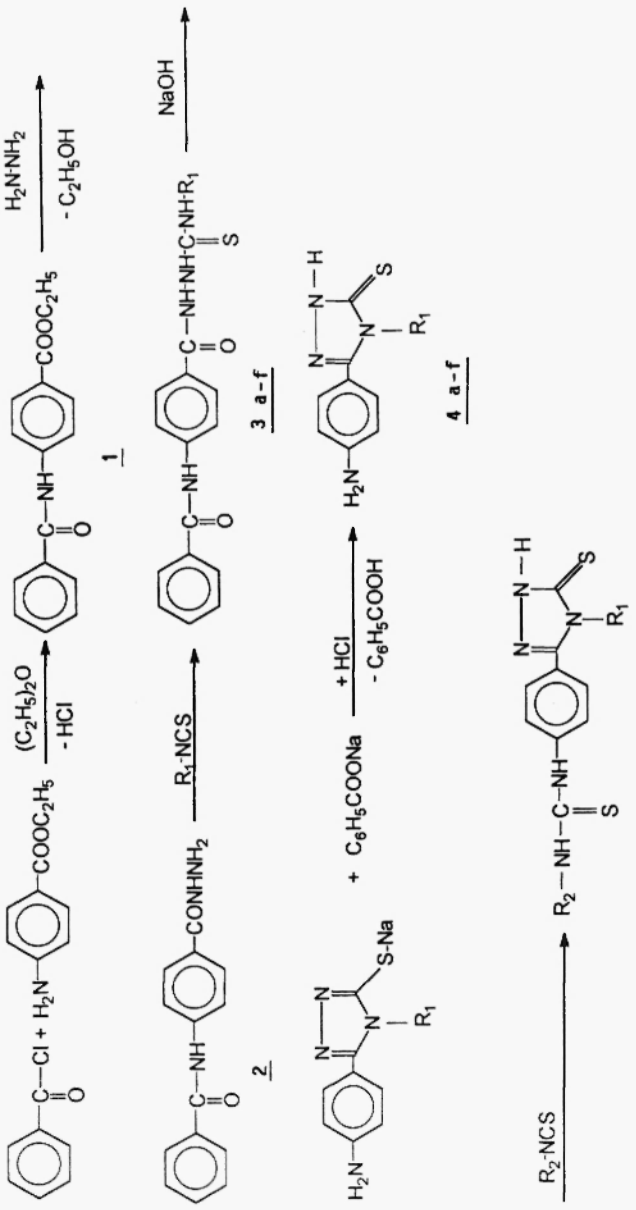


Fig. 1: Synthetic route for N-alkyl/aryl-N'-[4-(4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl] thiourea derivatives.

a-f] were synthesized by refluxing these thiosemicarbazides in the presence of 2N aqueous NaOH /13/.

Syntheses of N-substituted-N'-[4-(4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thioureas [5 a-r]

Compounds **4 a-f** (0.003 mol) and an equimolar quantity of the appropriate isothiocyanate were heated under reflux in 15 ml of dioxane-methanol (1:2, v/v) for 4 h. The solvent mixture was then evaporated and the crude product was recrystallized from (or washed with) boiling ethanol several times /16/.

N-Ethyl-N'-[4-(4-methyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea [**5b**] (96%): M.Wt. 293.415; M.p. 193°C; UV 214, 255.2, 294.8 nm; IR 3305, 1550, 1515, 1345, 1325, 1290, 1255, 1215, 1190 cm^{-1}; Analysis for $C_{12}H_{15}N_5S_2$ (%, calculated/found): 49.12/48.84 (C), 5.15/5.02 (H), 23.87/23.57 (N).

N-Cyclohexyl-N'-[4-(4-methyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea [**5d**] (91%): M.Wt. 347.507; M.p. 209°C; UV 214, 256.4, 295.4 nm; IR 3300, 1555-1525, 1349, 1325, 1285, 1260, 1205 cm^{-1}; Analysis for $C_{16}H_{21}N_5S_2$ (%, calculated/found): 55.30/54.71 (C), 6.09/6.37 (H), 20.15/19.93 (N).

N-Allyl-N'-[4-(4-ethyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl) phenyl]thiourea [**5f**] (85%): M.Wt. 319.453; M.p. 194°C; UV 214, 256, 290.4 nm; IR 3295, 1555, 1530, 1330, 1280, 1250, 1200 cm^{-1}; Analysis for $C_{14}H_{17}N_5S_2$ (%, calculated/found): 52.64/51.99 (C), 5.36/5.19 (H), 21.92/21.53 (N).

N-Cyclohexyl-N'-[4-(4-ethyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea [**5g**] (80%): M.Wt. 361.534; M.p. 239°C; UV 214, 257.2, 291.6 nm; IR 3300, 1555, 1545, 1530, 1320, 1280, 1260, 1205-1195 cm^{-1}; Analysis for $C_{17}H_{23}N_5S_2$ (%, calculated/found): 56.48/56.45 (C), 6.41/6.37 (H), 19.37/19.59 (N).

N-Allyl-N'-[4-(4-allyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl) phenyl]thiourea [**5h**] (91%): M.Wt. 331.464; M.p. 175°C; UV 213.8, 257, 289.8 nm; IR 3305, 1565, 1530, 1355, 1325, 1280, 1250, 1200 cm^{-1}; Analysis for $C_{15}H_{17}N_5S_2$ (%, calculated/found): 54.35/54.68 (C), 5.17/5.17 (H), 21.13/21.44 (N).

N-Phenyl-N'-[4-(4-allyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea [**5i**] (90%): M.Wt. 385.513; M.p. 176°C; UV 215.6, 257.6, 301.4 nm; IR 3180-3100, 1560, 1525, 1360, 1325, 1270, 1200 cm^{-1}; Analysis for $C_{18}H_{17}N_5S_2.H_2O$ (%, calculated/found): 56.08/56.76 (C), 4.96/4.43 (H), 18.16/19.35 (N).

N-Allyl-N'-[4-(4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione -5-yl)phenyl]thiourea [**5j**] (88%): M.Wt. 373.545; M.p. 215°C; UV 214.8, 256.4, 285.5 (shoulder) nm; IR 3300-3160, 1560-1540, 1520, 1355-1340, 1270-1255, 1190 cm^{-1}; Analysis for $C_{18}H_{23}N_5S_2$ (%, calculated/found): 57.87/57.48 (C), 6.21/6.10 (H), 18.75/18.68 (N).

N-Cyclohexyl-N'-[4-(4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea [**5k**] (94%): M.Wt. 415.627; M.p. 218°C; UV 214, 258.8, 288 (shoulder) nm; IR 3280, 1540, 1520, 1350-1340, 1270-1255, 1195-1185 cm^{-1}; Analysis for $C_{21}H_{29}N_5S_2$ (%, calculated/ found): 60.69/59.90 (C), 7.03/7.00 (H), 16.85/16.82 (N).

N-Allyl-N'-[4-(4-phenethyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea [**5r**] (63%): M.Wt. 395.552; M.p. 197°C; UV 213.8, 256.2, 288 nm; IR 3300, 1550-1520, 1355, 1320, 1290, 1260, 1190 cm^{-1}; Analysis for $C_{20}H_{21}N_5S_2$ (%, calculated/found): 60.73/60.55 (C), 5.35/5.22 (H), 17.70/17.23 (N).

Testing for antimicrobiological activity

Antibacterial and antifungal activities of the compounds **5 a-r** were examined using the agar diffusion method /17/ against *Pseudomonas mirabilis* ATCC 14153, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC 4352, *Escherichia coli* ATCC 11229, *Candida krusei* KUEN 1001, *Candida tropicalis* KUEN 1021, *Candida pseudotropicalis* KUEN 1012, *Candida albicans* ATCC 10231, and *Cryptococcus neoformans* KUEN 1048.

Saboraud dextrose agar and Saboraud dextrose broth for yeast-like fungi and Mueller-Hinton agar and Mueller-Hinton broth for bacteria were used as growth media (DIFCO).

Overnight fresh cultures of 10^7 CFU/ml of strain dilutions were inoculated into broth media. Compounds to be examined were dissolved in DMSO (5 mg/ml). Solutions of the compounds were then placed onto agar media (100 μl).

Incubation time was 24 h at 37°C for bacteria and 48 h at 37°C for yeasts. Fluconazole for yeast-like fungi and penicillin G-sodium for bacteria were included in each experiment as active reference standards.

Antimicrobial activities of compounds **5 a-r** were determined by subtracting the diameters of inhibition zones of DMSO from those of the test compounds.

RESULTS AND DISCUSSIONS

The present method yielded the desired N-alkyl/aryl-N'-[4-(4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea derivatives in a pure state according to the route shown in Figure 1.

Syntheses of 1,2,4-triazoline-3-thiones have been carried out by cyclization of 1-acyl-4-alkyl/arylthiosemicarbazides in alkaline medium as reported by Rollas *et al.* /13,18-20/. In addition, by the benzoylation of benzocaine as the first step, protection of the amino group was achieved during the following two reactions. The free amino function was then regained by heating and the alkali used in the cyclizations of **3 a-f** to give **4 a-f**. Thus, compounds carrying different substituents at the fourth position of the triazole ring and at the terminal nitrogen of thiourea were synthesized.

Of the **5 a-r** series, all the compounds except compound **5p,** which had been previously synthesized, were original. However, compound **5p** has been reported to be synthesized by a different procedure /18,21/. In the above-mentioned studies, a number of thiosemicarbazides were obtained by reacting *p*-aminobenzoic acid hydrazide with various isothiocyanates. Basic cyclizations of these thiosemicarbazides were then carried out (Fig. 2). It has to be understood that only compounds bearing the same substituents at the N-4 of triazole and at the terminal nitrogen of thiourea could be obtained according to this route.

In order to synthesize N,N'-disubstituted thiourea derivatives, the amine compounds have been reported to be reacted with a variety of isothiocyanates in certain solvents, such as ethanol, benzene, DMF, acetone, o-dichlorobenzene, pyridine-water (1:1) and dioxane /1-3,8,9,18,22-24/. In the present study, dioxane-methanol (1:2, v/v) was tried and proved useful /16/.

UV data of the **5** series exhibit similarities with the main structure arising from the starting compounds **4 a-f**. Absorption bands between

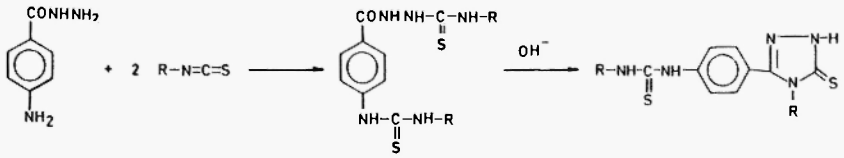


Fig. 2: Cyclization of thiosemicarbazides

255 and 259 nm have been reported as characteristic for 1,2,4-triazoline-3-thiones /13,16,25,26/. Third absorption bands have also been attributed to the same structure and proposed to exist by the chromophoric effect of the additional C=S function. Thiourea and its derivatives have been reported to give absorption bands between 255 and 320 nm /27/. Third absorption maxima of the **5** series were observed to undergo a bathochromic shift when the aromaticity increased /16/.

IR spectra of the synthesized compounds display the characteristic absorption bands of thiolactam C=S, aromatic C=C and triazoline C=N stretchings within the expected region /9,13,19,28,29/. Thiourea C=S stretching bands were observed at 1310-1360 cm^{-1}. N-H stretchings due to thiourea and 1,2,4-triazole-3-thiones were detected within the 3100-3380 cm^{-1} region. These findings are consistent with those reported in the hterature /9,30/.

In addition, the presence of absorption of the triazoline C=S at 1185-1205 cm^{-1} and the lack of absorption bands at around 2600 cm^{-1} supported the thione form of the 1,2,4-triazoline structures /19/.

The fact that the starting compounds **4 a-f** of the thioureas have been shown to possess positive responses against *Candida albicans* and *Candida tropicalis* /13/ led us to investigate further possible antimicrobial activity.

Compounds **5 a-r** were screened for antibacterial and antifungal activity *in vitro*. An agar diffusion method was utilised in the present study as a preliminary test. Biological evaluation showed that the resulting thioureas obtained from compounds **4 a-f** showed a tendency to exhibit antifungal rather than antibacterial activity. However, none of the tested compounds showed a significant activity against the strains used in the present study. Only compounds **5b**, **5d** and **5f** showed a slight activity against *Candida pseudotropicalis* KUEN 1012 when compared to that of Fluconazole.

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