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Synthesis of New Triazolyl-*N,N*-Dialkyldithiocarbamates as Antifungal Agents

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N,N-Dialkylditihiocarbamate derivatives have been well known as broad-range fungicides. In this study, the triazole derivatives of ten new *N,N*-disubstituted dithiocarbamates (**3a-j**) were synthesized and their structures were identified by spectral and elemental analysis. Results of the antifungal activity studies showed that some of the compounds tested were active against *M. canis*, *M. gypseum*, and *T. rubrum* at the concentration of 12.5 μg/mL when clotrimazol was used as a standard.

Key words: Triazole derivatives, Dithiocarbamates, Antifungal agents

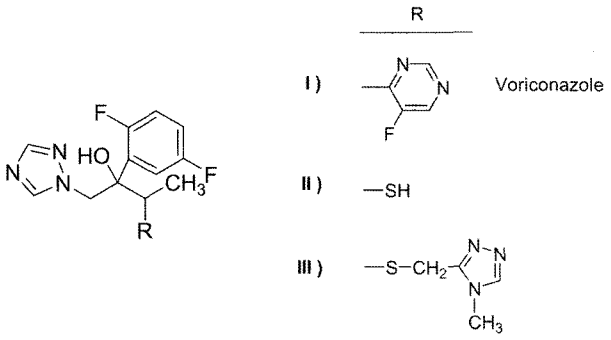
INTRODUCTION

The clinical relevance of fungal diseases has increased over the past 30 years due to an increasing population of immunocompromised patients who have cancer, AIDS or have received transplants. Antifungal azoles, fluconazole and itraconazole are inhibitors of lanosterol –14α-demethylase and have been widely used in antifungal chemotherapy. Widespread use of antifungal agents has led to the development of drug resistance (Georgopapadakou, 2002).

There have been several reports on the synthesis and evaluation of new azoles with antifungal activity against fluconazole resistant fungi.(Meerpoel *et al.*, 2004; Takahata *et al.*, 2005). It has been shown that introduction of sulfur containing groups in the antifungal compounds improved the activity. For example sulfhydryl and thioalkyl analogs of voriconazole (**I-III**) (Scheme 1) were reported to have efficacies in animal models comparable or superior to fluconazole (Tasaka *et al.*, 1994). The antifungal activities of 5,8-quinolinediones were significantly improved by the introduction of *S*-(aryl)thio moieties in the compounds (Ryu *et al.*, 2002).

Dithiocarbamates were first developed as herbicides and fungicides (Kuhr and Dorough, 1976). However, the synthesis of new derivatives has increased the application of these substances dramatically. Considerable interest has been focused on the dithiocarbamates that have been shown to possess a broad spectrum of biological activities such as antifungal (Mishra *et al.*, 1992; Farghaly and Moharram, 1999; Xu *et al.*, 2002) anticholinergic (Safak *et al.*, 1992), and antibacterial (Chourasia and Tyagi, 1999) effects. Development of new antifungal agents will have a significant impact on the management of drug resistance in fungal infections (Lamb *et al.*, 1999).

In order to take the advantage of the antifungal properties of both triazoles and dithiocarbamates, we synthesized new triazole derivatives with dithiocarbamate side chain and evaluated antifungal activities of these compounds. Structural elucidation of these compounds was performed



Scheme 1. Structure of voriconazole and its sulfhydryl and thioalky derivatives

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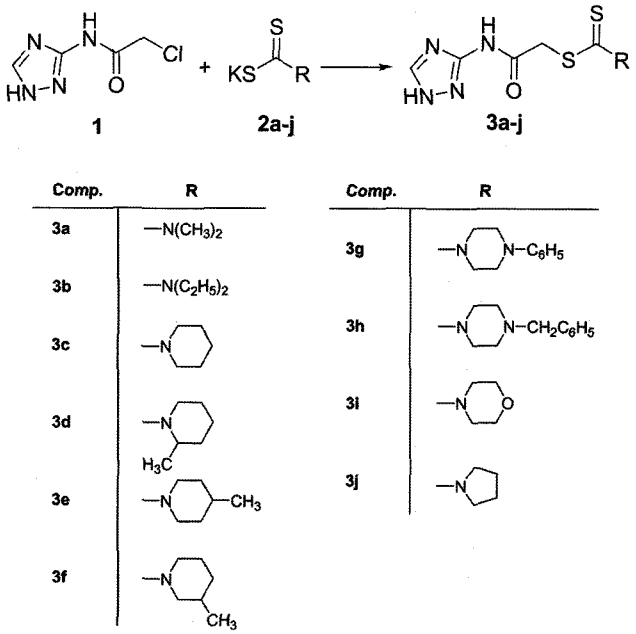


Scheme 2. General synthetic scheme of compounds **3a-j**

by UV, IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy, and elemental analysis (Scheme 2).

MATERIALS AND METHODS

Chemistry

3-Amino-1,2,4-triazole, chloroacetyl chloride, dimethylamine, diethylamine, piperidine, 2-methylpiperidine, 3-methylpiperidine, 4-methylpiperidine,1-phenylpiperazine, 1-benzylpiperazine, morpholine, and pyrrolidine were purchased from Fluka AG. M.p.'s were determined with a Buchi 530 apparatus in open glass capillary tubes and were uncorrected. UV spectra in ethanol were taken on a Shimadzu Model UV 2100S UV spectrophotometer. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. IR spectra were recorded on KBr discs using a Perkin Elmer 1600 FTIR spectrometer. ^{1}H-NMR and ^{13}C-NMR proton decoupled-DEPT 135 spectra were taken on Bruker AC 200 and a Bruker ARX 300 (^{1}H: 200 MHz, ^{13}C: 75.5 MHz, spectrometer). [d_6] DMSO was used for the NMR experiments. EIMS was recorded on a VG Zab Spec instrument at 70 eV.

General Procedure for the synthesis of 1

Equimolar amounts of 3-amino-1,2,4-triazole and chloroacetyl chloride were refluxed in anhydrous benzene for 6 h. The solution was evaporated to dryness under reduced pressure and neutralized with sodium bicarbonate.

General procedure for the synthesis of 2a-j

The secondary amine (10 mmol) was added to an ethanolic solution of KOH (10 mmol/100 mL). The mixture was cooled in an ice bath and CS_2 (100 mmol) was added drop by drop. The reaction mixture was agitated for 1 h at room temperature. The solvent was evaporated under the reduced pressure. Dry ether was added to obtain **2a-j** as the precipitate (Cesur *et al.*, 1994).

General procedure for the synthesis of 3a-j

The ethanolic solution of 3-chloroacetamido-1,2,4-triazole (**1**) (10 mmol) and **2a-j** (10 mmol) were refluxed for 1 h. After evaporation of the solvent in vacuo, products were washed with water and purified by recrystallization from ethanol.

RESULTS

Chemistry

^{1}H-NMR, ^{13}C-NMR, and Mass spectra of compounds are listed below.

3-[(*N,N*-Dimethylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3a)

^{1}H-NMR [DMSO-d_6] δ ppm, 3.33 (s, 3H, C*H*$_3$), 3.37 (s, 3H, C*H*$_3$), 4.21 (s, 2H, -C*H*$_2$S-), 7.76 (s, 1H, triazole 5-H); ^{13}C-NMR proton decoupled DEPT 135: 40.81 (-CH$_2$S-), 41.76 (N-CH$_3$), 45.55 (N-CH$_3$), 147.05 (triazole *C*-3/*C*-5), 166.30 (*C*=O), 194.73 (*C*=S).

3-[(*N,N*-Diethylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3b)

^{1}H-NMR [DMSO-d_6] δ ppm, 1.17-1.26 (m, 6H, C*H*$_3$), 3.79 (q, J= 6.70 Hz, 2H, N-C*H*$_2$-), 3.94 (q, J= 6.55 Hz, 2H, N-C*H*$_2$-), 4.29 (s, 2H, -C*H*$_2$S-), 7.69 (s, 1H, triazole 5-H), 11.73 (s, 1H, -N*H*CO), 13.31 (s, 1H, triazole -N*H*-); EI/MS [m/z (rel. int. %)]: 273.1 (20) M$^+$, 190 (23), 157 (1), 148 (33), 126 (100), 116 (88), 111 (10), 88 (72), 84 (37), 72 (61), 60 (47).

3-[(1-Piperidinylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3c)

^{1}H-NMR [DMSO-d_6] δ ppm, 1.62 (m, 6H, piperidine -*H*), 3.92 (s, 2H, piperidine -*H*), 4.21 (s, 2H, piperidine -*H*), 4.30 (s, 2H, -C*H*$_2$S-), 7.90 (s, 1H, triazole 5-H); ^{13}C-NMR proton decoupled DEPT 135: 40.31 (-CH$_2$S-), 149.01 (triazole *C*-3/*C*-5), 168.10 (*C*=O), 193.21 (*C*=S).

3-[(2-Methyl-1-piperidinylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3d)

^{1}H-NMR [DMSO-d_6] δ ppm, 1.37 (s, 3H, C*H*$_3$), 1.53-1.90 (m, 6H, piperidine -*H*) 4.44 (s, 2H, -C*H*$_2$S-), 5.05 (s, 1H, piperidine -*H*), 5.75 (s, 2H, piperidine -*H*), 7.86 (s, 1H, triazole 5-*H*), 10.70 (s, 1H, -N*H*CO); ^{13}C-NMR proton decoupled DEPT 135: 40.02 (-CH$_2$S-), 148.9 (triazole *C*-3/*C*-5), 166.49 (*C*=O), 193.84 (C=S).

3-[(4-Methyl-1-piperidinylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3e)

^{1}H-NMR [DMSO-d_6] δ ppm, 0.70 (d, 3H, C*H*$_3$), 0.80-1.02 (m, 5H, piperidine -*H*), 4.09 (s, 2H, piperidine -*H*), 4.30 (s, 2H, -C*H*$_2$S-), 5.05 (s, 2H, piperidine -*H*), 7.65(s, 1H, triazole 5-*H*).

3-[(3-Methyl-1-piperidinylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3f)

^{1}H-NMR [DMSO-d_6] δ ppm, 0.70 (d, 3H, C*H*$_3$), 1.05-1.80 (m, 5H, piperidine -*H*), 4.20 (s, 2H, piperidine -*H*), 4.30 (s, 2H, -C*H*$_2$S-), 5.02 (m, 2H, piperidine -*H*), 7.07 (s, 1H, triazole 5-*H*).

3-[(4-Phenyl-1-piperazinylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3g)

^{1}H-NMR [DMSO-d_6] δ ppm, 2.66-2.68 (m, 4H, piperazine -*H*), 4.30 (s, 2H, -C*H*$_2$S-), 4.52 (m, 4H, piperazine -*H*), 7.01 (t, 1H, phenyl -*H*), 7.13 (m, 2H, phenyl -*H*), 7.41 (m, 2H, phenyl -*H*), 8.00 (s, 1H, triazole 5-*H*); ^{13}C-NMR proton decoupled DEPT 135: 40.28 (-S-CH$_2$), 150.37 (triazole *C*-3/*C*-5), 166.20 (*C*=O), 194.76 (*C*=S).

3-[(4-Benzyl-1-piperazinylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3h)

^{1}H-NMR [DMSO-d_6] δ ppm, 2.48 (m, 4H, piperazine -*H*), 3.54 (s, 2H, -N-C*H*$_2$-), 4.08 (t, 4H, piperazine -*H*), 4.32 (s, 2H, -S-C*H*$_2$-), 7.19-7.38 (m, 5H, phenyl), 7.73 (s, 1H, triazole 5-*H*), 11.72 (s, 1H, -N*H*CO), 13.32 (s, 1H, triazole -N*H*-); EI/MS [m/z (rel. int. %)]: 376.2 (M$^+$), 293 (20), 251 (100), 219 (74), 200 (60), 175 (70), 159 (42), 157 (8), 146 (96), 134 (92), 126 (65), 111 (34), 91 (79), 84 (46), 65 (56).

3-[(4-Morpholinylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3i)

^{1}H-NMR [DMSO-d_6] δ ppm, 3.68 (t, J=4.81 Hz, 4H, morpholine -*H*), 4.08 (s, 4H, morpholine -*H*), 4.33 (s, 2H, -S-C*H*$_2$-), 7.70 (s, 1H, triazole 5-*H*), 11.72 (s, 1H, -N*H*CO), 13.32 (s, 1H, triazole -N*H*-); EI/MS [m/z (rel. int. %)]: 287.2 (M$^+$), 204 (41), 200 (20), 162 (24), 157 (53), 130 (100), 126 (81), 111 (16), 99 (26), 86 (97), 84 (43), 60 (24).

3-[(1-Pyrrolidinylthiocarbamoylthio)acetylamino]-1,2,4-triazole (3j)

^{1}H-NMR [DMSO-d_6] δ ppm, 2.13-2.71 (m, 4H, pyrrolidine-H), 3.90 (t, 2H, pyrrolidine-H) 3.97 (t, 2H, pyrrolidine-H), 4.52 (s, 2H, -S-C*H*$_2$-), 8.05 (s, 1H, (s, 1H, triazole 5-*H*); ^{13}C-NMR proton decoupled DEPT 135: 39.90 (-S-*C*H$_2$),150.60 (triazole *C*-3/*C*-5), 168.98(*C*=O), 190.32 (*C*=S).

Table I. Some characteristics of compounds **3a-j**

Comp.	Formula (Mol. wt.)	M.p. (°C)	Yield (%)	UV (EtOH) λ_{max}	IR (KBr) cm^{-1}		Analysis (calc./found)		
					C=O	C=S	C	H	N
3a	$C_7H_{11}N_5OS_2$ (245.32)	265-266	75	274, 238, 215	1686	1252	34.27 33.96	4.52 4.40	28.55 28.23
3b	$C_9H_{15}N_5OS_2$ (273.37)	224-226	87	277, 243, 217	1698	1209	39.54 39.30	5.53 5.59	25.62 25.45
3c	$C_{10}H_{15}N_5OS_2$ (285.38)	265-267	54	279, 245, 217	1694	1239	42.09 42.57	5.30 5.15	24.54 24.31
3d	$C_{11}H_{17}N_5OS_2$ (299.41)	175-176	33	278, 244, 218	1686	1231	44.12 43.85	5.72 5.50	23.39 23.12
3e	$C_{11}H_{17}N_5OS_2$ (299.41)	250-251	76	278, 216	1691	1218	44.12 43.96	5.72 5.53	23.39 23.50
3f	$C_{11}H_{17}N_5OS_2$ (299.41)	247-251	76	279, 245, 217	1695	1241	44.12 43.16	5.72 5.26	23.39 23.10
3g	$C_{15}H_{18}N_6OS_2$ (362.46)	260-262	63	278, 249, 202	1691	1222	49.70 49.60	5.01 5.04	23.19 23.60
3h	$C_{16}H_{20}N_6OS_2$ (376.49)	228-231	73	279, 245, 209	1687	1218	51.04 50.98	5.35 5.51	22.32 22.32
3i	$C_9H_{13}N_5O_2S_2$ (287.35)	264-266	81	280, 248, 215	1691	1227	37.62 37.18	4.56 4.67	24.37 24.52
3j	$C_9H_{13}N_5OS_2$ (271.35)	265-267	98	274, 240, 216	1693	1252	38.33 38.81	4.83 4.87	25.81 25.94

DISCUSSION

Reaction of 2-amino-1,2,4-triazole (**1**) with the corresponding *N,N*-disubstituted potassium dithiocarbamates (**2a-j**) in ethanolic medium produced 3-[(*N,N*-disubstituted thiocarbamoylthio)acetylamino]-1,2,4-triazoles (**3a-j**). The structures of **3a-j** were confirmed by physical and spectral (IR, ^{1}H-NMR, ^{13}C-NMR, EIMS) data (Table I).

IR spectra of **3a-j** showed two bands resulting from amide C=O and thiocarbamoyl group C=S stretchings in the 1686-1698 and 1209-1252 cm^{-1} regions, respectively (Ates *et al.*, 2003).

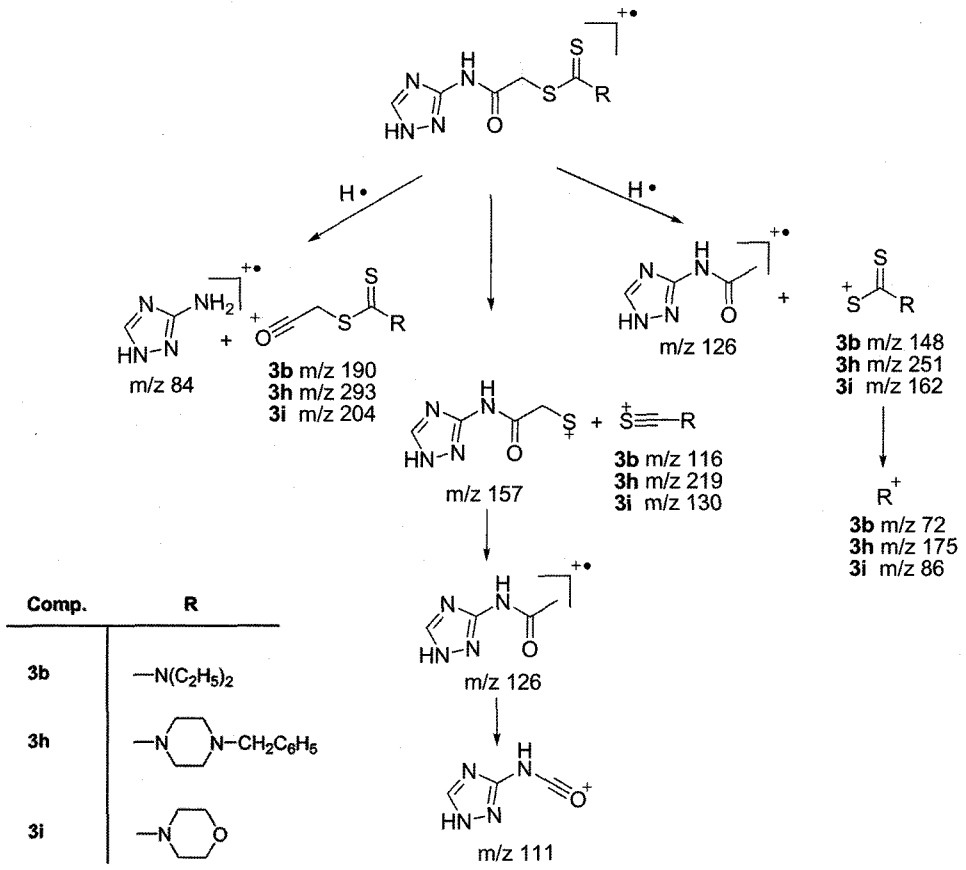
In the ^{1}H-NMR spectra of **3a-j**, -C*H*$_2$S-protons were observed as a singlet in the 4.21-4.52 ppm region (Karali *et al.*, 1999) and triazole 5-*H* was observed as singlet in the 7.07-8.00 ppm region (Dash and Elmquist, 2001) Amide N-*H*, and triazole N-*H* protons were observed in 10.70-11.76 ppm region and at 13.31 ppm respectively (Ates *et al.*, 1998; Ergenc *et al.*, 1999).

In the ^{13}C-NMR spectra of compounds, -CH_2S-, C=O, and C=S peaks which verified the proposed dithiocarbamic acid ester structure appeared as singlets at 39.89-40.81, 166-168 and 190.32-194.76 ppm regions respectively (Crews *et al.*, 1998).

EIMS of **3b**, **3h**, and **3i** were chosen as prototypes showed (M^+) molecular ions. The major fragmentation pathway involved the cleavage of C-S or C-N bonds of the dithiocarbamate moiety (Capan *et al.*, 1993). The proposed fragmentation patterns of these compounds are depicted in Scheme 3.

Antifungal activity

Antifungal activity against *Micosporum canis*, *Micosporum gypseum* (NCPF-580) and *Tricophyton rubrum* were determined using a microdilution method (Shadomy *et al.*, 1991; Granade and Artis, 1980). A yeast nitrogen base (YNB, Difco) and a nutrient broth media were used during the experiments. Clotrimazole was used as the standard drug. Compounds were dissolved in DMSO and solutions of 25±0.2 μg/mL were prepared. The minimum concentration at which no growth was observed was taken as the MIC value. Compounds **3b-j** were active against *Micosporum canis,* compounds **3b** and **3f-h** were active against *Micosporum gypseum*, compounds **3b-e** and **3j**, were active against *Tricophyton rubum* at 12.5 mg/mL MIC value. The results are given in Table II.



Scheme 3. Proposed mass fragmentation pattern of compounds **3b**, **3h**, and **3i**

Table II. Antifungal activity of compounds **3a-j** tested on *M. canis*, *M. gypseum*, and *T. rubrum*, clotrimazole as the standard

Compound	Micosporum canis	Micosporum gypseum	Tricophyton rubrum
3a	12.5	12.5	12.5
3b	<12.5	<12.5	<12.5
3c	<12.5	12.5	<12.5
3d	<12.5	12.5	<12.5
3e	<12.5	12.5	<12.5
3f	<12.5	<12.5	12.5
3g	<12.5	<12.5	12.5
3h	<12.5	<12.5	12.5
3i	<12.5	12.5	12.5
3j	<12.5	12.5	<12.5
Clotrimazole	<0.4	<0.4	<0.4

MIC (μg/mL)

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