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1,3-DITHIAN-2-YLIDENE AND THIAZOLIDIN-2-YLIDENE DERIVATIVES AS A NOVEL CLASS OF ANTIMYCOTIC AGENTS†

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Abstract: Several 1,3-dithian-2-ylidenes (**2a-e,3**), (4-methyl-1,3-dithiol-2-ylidene)(imidazol-1-yl) acetonitrile (**4**) and thiazolidin-2-ylidene derivatives (**5a-d**) were synthesized and evaluated for *in vitro* antifungal activity .

Introduction: Because of the wide spread prevalence of mycoses due to soilborne or airborne transmission of the systemic fungal pathogens, and lack of clinically available vaccines or useful antisera for mycotic infections, search for new safe and highly effective chemotherapeutic agents become inevitable.

Introduction of ketoconazole[1,2] in clinical use initiated an imidazole class of antifungal therapy[3]. An attempt for further improvement in efficacy and reduction in hormonal effects led to the synthesis of several imidazole[3] and triazole[4-6] derivatives which replaced ketoconazole for specific use. Both the classes of compounds impair the biosynthesis of ergosterol for cytoplasmic membrane and increase the accumulation of 14-α-methyl sterols. A critical structure survey of azole class of fungicides revealed that at least all of them possess azol-1-yl moiety linked with either 1,3-dioxolane or 1,3-dithiolane at position-2 in their molecular make up. The activity of these compounds is potentiated by the presence of certain functionality or group attached at specific position. Thorough literature survey revealed that 1,3-dithian-2-ylidene (**2a-e**, **3**), 4-methyl-1,3-dithiol-2-ylidene (**4**) and thiazolidin-2-ylidene (**5a-d**) derivatives are not so far evaluated for their antimycotic property.

All the synthesized compounds were evaluated for antifungal activity against *Aspergillus fumigatus* (Af), *Candida albicans* (Ca), *Cryptococcus neoformans* (Cn), *Trichophyton mentagrophytes* (Tm) and *Sporothrix schenckii* (Ss) by two fold serial dilution technique. The activity of these compounds was expressed in terms of minimum inhibitory concentration (MIC), considering ketoconazole as a standard drug. The most potent compound amongst all the screened compounds was **2b** having MIC of 0.19, 50 and 3.12 µg/ml against Af, Ca and Tm respectively. The other two compounds **2e** and **4** were equipotent and highly effective at 12.5 µg/ml concentration against Af and Tm. Even compounds **5a** and **5b** were also found to be active but inferior to ketoconazole, a standard drug. The data are presented in Table 1.

Synthesis : 1,3-Dithian-2-ylidene derivatives (**2,3**) were prepared[7,8] by stirring a reaction mixture of **1**, CS_2 and

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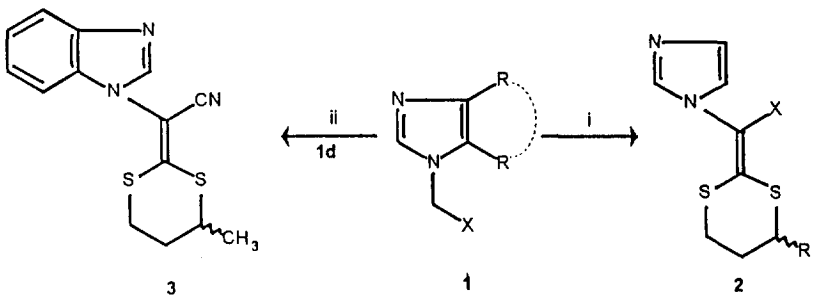
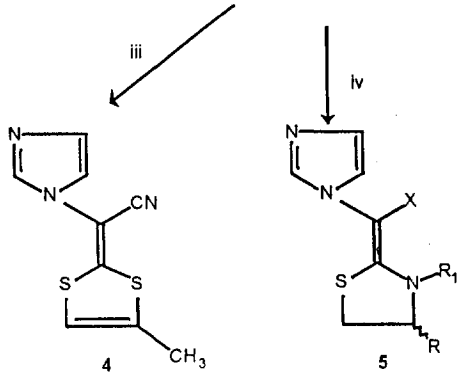
powdered KOH in DMSO. The cold solution on reaction with 1,3-dihaloalkane led to the formation of **2** and **3** as major product and 1,3-dithian-2-thione as minor but under phase transfer condition using benzyltriethylammonium chloride as a catalyst, **2** and **3** were isolated as minor constituents and 1,3-dithian-2-thione as major. (4-Methyl-1,3-dithiol-2-ylidene)(imidazol-1-yl) acetonitrile (**4**) was prepared using propargyl chloride as alkylating agent in place of dihaloalkane. This reaction was initiated by substitution followed by addition to multiple bond. 1,3-thiazolidin-2-ylidene derivatives (**5**) were prepared from the reaction of **1** and alkyl/arylisothiocyanate in alkaline medium followed by reaction with dihaloalkane (Scheme 1).

Scheme 1



1	RR	X
a	H	CN
b	H	$COOC_2H_5$
c	H	$4\text{-}ClC_6H_4CO$
d	$(\text{-}CH{=}CH\text{-})_2$	CN

2	X	R
a	CN	H
b	CN	CH_3
c	$COOC_2H_5$	H
d	$COOC_2H_5$	CH_3
e	$4\text{-}ClC_6H_4CO$	H



5	X	R	R_1
a	CN	H	C_2H_5
b	CN	CH_3	C_2H_5
c	$4\text{-}ClC_6H_4CO$	H	C_6H_5
d	$4\text{-}ClC_6H_4CO$	H	$4\text{-}ClC_6H_4$

Reagents/Conditions: i) CS_2/1,3-Dihaloalkane/KOH/DMSO/0°C; ii) CS_2/1,3-Dichlorobutane/KOH/DMSO/0°C; iii) $CH{\equiv}C\text{–}CH_2Cl/CS_2$/KOH/DMSO/0°C; iv) R_1NCS/1,2-Dihaloalkane/KOH/DMSO/0°C

Biological Activity: The *in vitro* antifungal activity was evaluated by two-fold serial dilution technique[9] against five human pathogenic fungi viz. *A. fumigatus, C. albicans, C. neoformans, S. schenckii* and *T. mentagrophytes*. Sabouraud's dextrose agar (SDA) slant growth of the pathogens for 24-48 h (yeasts) or 7 days (mycelial fungi) were suspended in Sabouraud's dextrose broth (SDB). The colony forming units (cfu) of the seeded broth were determined by dilution and plating technique and adjusted in the range of 10^4-10^5 cfu/ml.

A solution of 0.2 ml (1mg/ml in DMSO) of test compound was added to 1.8 ml seeded broth to form the first dilution in assay tubes (size 13x100mm). One ml of this was further diluted with 1 ml of seeded broth to make second dilution and so on till 10 such dilutions were obtained. A set of assay tubes with seeded broth and solvent were kept as control. The tubes were incubated in a BOD incubator at 28±1°C. The minimum inhibitory concentration (MIC) was recorded by visual observation after 24-48 h (yeasts) and 72-96 h (mycelial fungi) incubation. Ketoconazole was used as standard drug. The antifungal activity profile of 1,3-dithian-2-ylidene derivatives depends upon the heterocyclic ring attached to it. As it is evident from the activity profile that a mere change of imidazole in **2b** by benzimidazole in **3** displayed low order of activity only against *A. fumigatus*.

Table 1: *In vitro* antifungal activity of 1,3-dithian-2-ylidene (**2 a-e, 3**), (4-methyl-1,3-dithiol-2-ylidene)(imidazol-1- yl)acetonitrile (**4**) and thiazolidin-2-ylidene (**5a-d**) derivatives against human pathogenic fungi.

Compound No.	Minimum inhibitory concentration (MIC) in µg/ml				
	Af	Ca	Cn	Ss	Tm
2a	3.1	100	>100	>100	25
2b	0.19	50	>100	>100	3.12
2c	>100	>100	>100	>100	>100
2d	50	>100	>100	>100	25
2e	12.5	>100	>100	>100	12.5
3	50	>100	>100	>100	>100
4	12.5	>100	>100	>100	12.5
5a	25	>100	>100	>100	25
5b	25	>100	>100	>100	50
5c	>100	>100	>100	>100	>100
5d	>100	>100	>100	>100	>100
Ketoconazole (standard drug)	0.7	0.1	0.3	0.1	25

The substituent X in structure **2** is also responsible for high order of antifungal activity. An exchange of CN in **2a** by COOEt (**2c**) led to almost complete loss of activity against all the fungi. A similar change from **2b** to **2d** attenuated the antifungal activity several folds against *A. fumigatus* (50µg/ml) and *T. mentagrophytes* (25µg/ml).

A further change of cyano function by 4-chlorobenzoyl group activity against *A. fumigatus* (12.5μg/ml) and *T. mentagrophytes* (12.5μg/ml) was retained to a certain extent. (4-Methyl-1,3-dithiol-2-ylidene)(imidazol-1-yl) acetonitrile (**4**) also displayed same level of activity against *A. fumigatus* (12.5μg/ml) and *T. mentagrophytes* (12.5μg/ml). Presence of methyl substituent at position 4 in **2,3** and **4** ameliorated the antifungal activity.

In the case of thiazolidin-2-ylidene derivatives, only N-ethyl substituted derivatives (**5a , b**), demonstrated antimycotic activity against *A. fumigatus* (25μg/ml) and *T. mentagrophytes* (25-50μg/ml). These compounds were found to be inactive against rest of the fungi.

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