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SYNTHESIS AND BIOEVALUATION OF KETOCONAZOLE THIOSEMICARBAZONE ANALOGUES

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ABSTRACT

Ketoconazole (KTZ) is a synthetic antifungal drug used to prevent and treat fungal infections, especially in immunocompromised patients such as those with AIDS. Resistance to ketoconazole has been observed in a number of clinical fungal isolates, including *C. albicans.* Thus new effective agents with less toxicity against fungal infection are urgently required. With this view, ketoconazole thiosemicarbazone analogues (Compounds 1-10) were synthesized wherein condensation of different thiosemicarbazides substituted by different cyclic and aromatic amines with the KTZ was done. Investigation of *in-vitro* antifungal activity of compounds was done by broth microdilution assay method against five pathogenic fungi *Aspergillus niger, Candida albicans, Candida krusei, Candida glabrata* and *Candida tropicalis*. Ketoconazole was used as reference for inhibitory activity against fungi. All the compounds were found potent antifungal agents, while compounds 8, 9 and 10 exhibited excellent *in-vitro* antifungal activity showing importance of halogenated compounds.

INTRODUCTION: Ketoconazole (KTZ) [1-5] is a potent, orally active, broad-spectrum antifungal agent, which was recently developed from imidazole. KTZ derivatives is most often used to treat fungal infections that can spread to different parts of the body through the bloodstream such as yeast infections of the mouth, skin, urinary tract, and blood, and certain fungal infections that begin on the skin or in the lungs and can spread through the body. However, resistances to KTZ in many pathogenic fungi as well as several side effects are also well documented [6].

Thiosemicarbazones are a class of small molecules that have been evaluated for antifungal activity [7-8]. In addition various thiosemicarbazone derivatives have been shown to possess anticancer, anti-proliferative, antioxidant and many other biological properties [9-13].

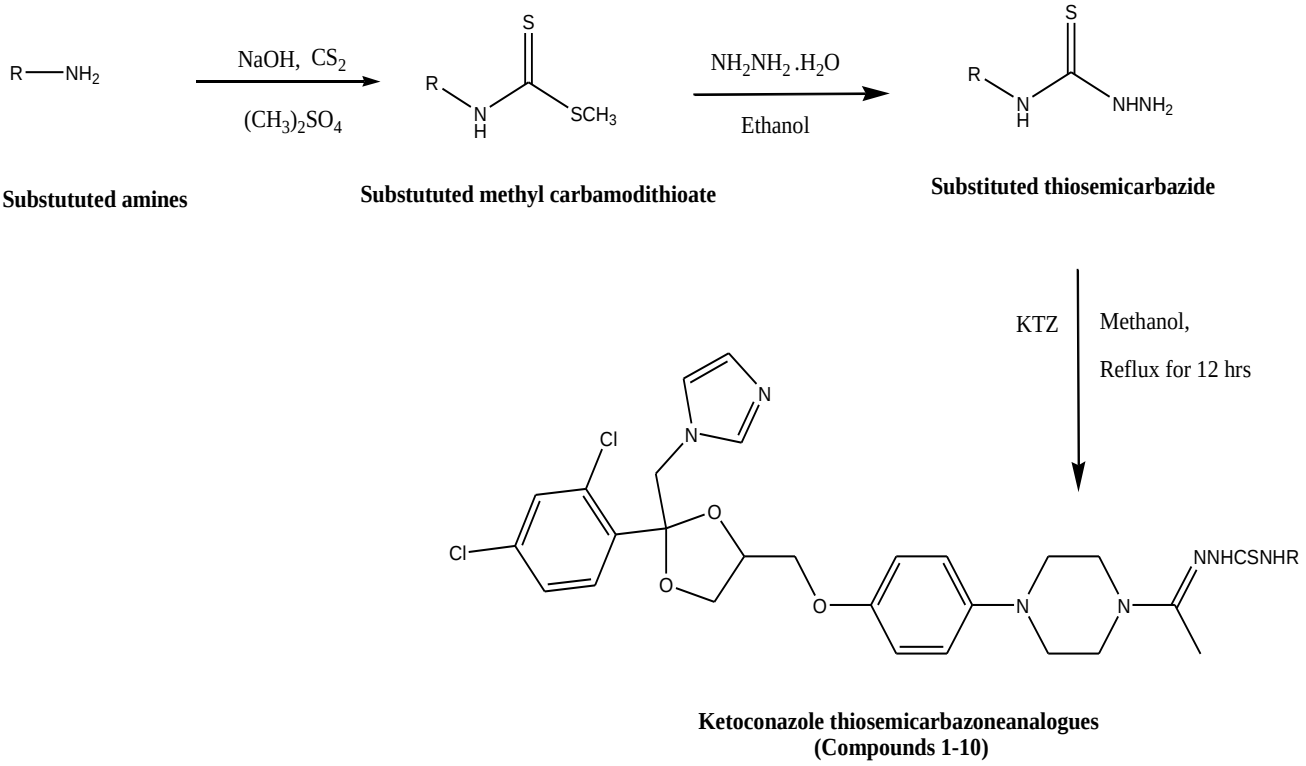
In view of the antifungal activity of the above pharmacophores, it was envisaged that the combined effect of these entities will result in increased antifungal activity. Therefore, it was planned to synthesize ketoconazole thiosemicarbazone analogues (Compounds 1-10) with a hope to obtained potent antifungal agents.

Experimental:

Instrumentation: Melting points were taken in open capillary tube and were uncorrected. The reaction was monitored by thin layer chromatography on silica gel G plates (Merck Silica-60F_{254}) and the final products were purified by recrystallization from absolute methanol. All the newly synthesized compounds were confirmed by FTIR (Recorded on a FTIR-8400S spectro-photometer, SHIMADZU), ^{1}H NMR (Recorded on

Bruker NMR spectrophotometer in deuterium-substituted DMSO form using TMS as internal standard) and elemental analysis.

Synthetic procedure: Cyclic or aromatic amine on reaction with carbon disulfide in aqueous sodium hydroxide solution and dimethyl sulfate afforded substituted methyl carbamodithioates which on treatment with hydrazine hydrate yielded substituted thiosemicarbazides. Condensation of different substituted thiosemicarbazides with the KTZ was done in methanol (**Scheme 1**).



Substututed amines

Substututed methyl carbamodithioate

Substituted thiosemicarbazide

Ketoconazole thiosemicarbazoneanalogues (Compounds 1-10)

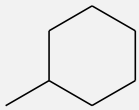
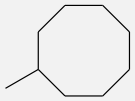
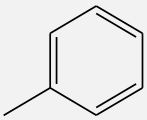
SCHEME 1

Where R = cyclohexyl **(1)**, cyclooctyl **(2)**, phenyl **(3)**, *o*-methyl phenyl **(4)**, *p*-methyl phenyl **(5)**, *o*-nitro phenyl **(6)**, *p*-nitro phenyl **(7)**, *o*-chloro phenyl **(8)**, *p*-chloro phenyl **(9)** and 2,4-dichloro phenyl **(10)**.

The reaction mixture was refluxed for 12 hrs and left overnight at room temperature. After cooling, the solid was filtered and recrystallized from appropriate solvent to give desired ketoconazole thiosemicarbazone analogues 1-10.

The structures of the synthesized compounds have been determined by their elemental analysis, FTIR, ^{1}H NMR spectra. Physicochemical, analytical and spectral data of synthesized compounds are given in **table 1 & 2**.

TABLE 1: PHYSICOCHEMICAL AND ANALYTICAL DATA OF SYNTHESIZED COMPOUNDS (1-10)

R	Mol. Formula	M. wt. / M. pt. (°C)	% Yield	Found (Calc.)		
				C	H	N
	$C_{33}H_{41}Cl_2N_7O_3S$	686.69 (205)	55	57.68 (57.72)	6.08 (6.02)	14.33 (14.28)
	$C_{35}H_{45}Cl_2N_7O_3S$	714.75 (211)	59	58.79 (58.81)	6.21 (6.25)	13.68 (13.72)
	$C_{33}H_{35}Cl_2N_7O_3S$	680.65 (235)	63	58.19 (58.23)	5.23 (5.18)	14.44 (14.40)

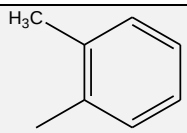
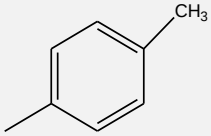
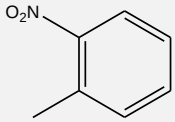
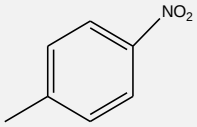
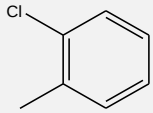
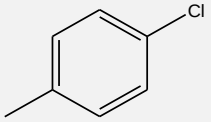
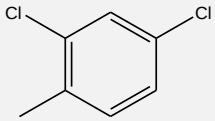
	$C_{33}H_{37}Cl_2N_7O_3S$	694.67 (240)	67	58.75 (58.78)	5.34 (5.37)	14.07 (14.11)
	$C_{33}H_{37}Cl_2N_7O_3S$	694.67 (242)	58	58.73 (58.78)	5.33 (5.37)	14.07 (14.11)
	$C_{33}H_{34}Cl_2N_8O_5S$	725.64 (259)	54	54.65 (54.62)	4.77 (4.72)	15.47 (15.44)
	$C_{33}H_{34}Cl_2N_8O_5S$	725.64 (258)	60	54.67 (54.62)	4.74 (4.72)	15.43 (15.44)
	$C_{33}H_{34}Cl_3N_7O_3S$	715.09 (261)	70	55.47 (55.43)	4.81 (4.79)	13.75 (13.71)
	$C_{33}H_{34}Cl_3N_7O_3S$	715.09 (263)	69	55.47 (55.43)	4.83 (4.79)	13.74 (13.71)
	$C_{33}H_{33}Cl_4N_7O_3S$	749.54 (267)	57	52.85 (52.88)	4.39 (4.44)	13.05 (13.08)

TABLE 2: SPECTRAL DATA OF SYNTHESIZED COMPOUNDS (1-10)

IR (KBr) cm^{-1}	^1H NMR ($CDCl_3$)
3221 (NH), 1642 (C=N), 1583 (C=N imidazole), 1206 (C-N), 1185 (C=S)	8.38 (s, 1H, NH), 7.85 (s, 1H, NH), 7.59 (d, 1H), 7.49 (s, 1H), 7.39 (d, 1H), 7.21-6.48 (m, 7H, Ar), 4.37 (dd, 2H), 4.25 (m, 1H, CH), 4.12 (m,1H), 3.86 (m, 2H), 3.74 (m, 4H), 3.5(d, 2H), 3.01 (m, 4H), 2.1 (s, 3H, CH_3), 1.12-2.57 (m, 10H, CH_2)
3314 (NH), 1630 (C=N), 1590 (C=N imidazole), 1182 (C-N), 1092 (C=S)	8.87 (s, 1H, NH), 8.34 (s, 1H, NH), 7.45 (d, 1H), 7.38 (s, 1H), 7.15 (d, 1H), 7.01-6.08 (m, 7H, Ar), 4.38 (dd, 2H), 4.14 (m, 1H, CH), 4.01 (m,1H), 3.36 (m, 2H), 3.12 (m, 4H), 3.09 (d, 2H), 3.02 (m, 4H), 2.71 (s, 3H, CH_3), 1.24-2.84 (m, 14H, CH_2)
3232 (NH), 1629 (C=N), 1588 (C=N imidazole), 1181 (C-N), 1095 (C=S)	8.71 (s, 1H, NH), 8.10 (s, 1H, NH), 7.59 (d, 1H), 7.49 (s, 1H), 7.39 (d, 1H), 7.23-6.41 (m, 12H, Ar), 4.37 (dd, 2H), 4.12 (m,1H), 3.86 (m, 2H), 3.74 (m, 4H), 3.5(d, 2H), 3.01 (m, 4H), 2.1 (s, 3H, CH_3)
3275 (NH), 1663 (C=N), 1584 (C=N imidazole), 1262 (C-N), 1187 (C=S)	8.54 (s, 1H, NH), 8.17 (s, 1H, NH), 7.73 (d, 1H), 7.53 (s, 1H), 7.35 (d, 1H), 7.41-6.72 (m, 11H, Ar), 4.58 (dd, 2H),4.32 (m,1H), 3.72 (m, 2H), 3.69 (m, 4H), 3.53 (d, 2H), 3.47 (m, 4H), 2.74 (s, 3H, CH_3, Ar), 2.46 (s, 3H, CH_3)
3224 (NH), 1630 (C=N), 1586 (C=N imidazole), 1181 (C-N), 1092 (C=S)	8.52 (s, 1H, NH), 7.98 (s, 1H, NH), 7.83 (d, 1H), 7.60 (s, 1H), 7.28 (d, 1H), 7.34-6.48 (m, 11H, Ar), 4.47 (dd, 2H),4.24 (m,1H), 3.57 (m, 2H), 3.24 (m, 4H), 3.15 (d, 2H), 3.11 (m, 4H), 2.62 (s, 3H, CH_3, Ar), 2.21 (s, 3H, CH_3)
3217 (NH), 1663 (C=N), 1565 (C=N imidazole), 1270 (C-N), 1183 (C=S)	8.98 (s, 1H, NH), 8.38 (s, 1H, NH), 7.72 (d, 1H), 7.44 (s, 1H), 7.41 (d, 1H), 7.83-6.81 (m, 11H, Ar), 4.73 (dd, 2H),4.42 (m,1H), 3.46 (m, 2H), 3.32 (m, 4H), 3.31 (d, 2H), 3.21 (m, 4H), 2.23 (s, 3H, CH_3)
3265 (NH), 1630 (C=N), 1583 (C=N imidazole), 1183 (C-N), 1061 (C=S)	8.87 (s, 1H, NH), 8.24 (s, 1H, NH), 7.87 (d, 1H), 7.62 (s, 1H), 7.52 (d, 1H), 7.69-6.78 (m, 11H, Ar), 4.45 (dd, 2H),4.36 (m,1H), 3.56 (m, 2H), 3.34 (m, 4H), 3.29 (d, 2H), 3.19 (m, 4H), 2.13 (s, 3H, CH_3)
3312 (NH), 1659 (C=N), 1529 (C=N imidazole), 1183 (C-N), 1082 (C=S)	8.19 (s, 1H, NH), 7.89 (s, 1H, NH), 7.76 (d, 1H), 7.54 (s, 1H), 7.34 (d, 1H), 7.12-6.19 (m, 11H, Ar), 4.34 (dd, 2H),4.21 (m,1H), 3.72 (m, 2H), 3.56 (m, 4H), 3.39 (d, 2H), 3.23 (m, 4H), 2.15 (s, 3H, CH_3)
3265 (NH), 1641 (C=N), 1584 (C=N imidazole), 1267 (C-N), 1184 (C=S)	8.72 (s, 1H, NH), 7.94 (s, 1H, NH), 7.58 (d, 1H), 7.51 (s, 1H), 7.24 (d, 1H), 7.53-6.17 (m, 11H, Ar), 4.63 (dd, 2H),4.32 (m,1H), 3.92 (m, 2H), 3.62 (m, 4H), 3.42 (d, 2H), 3.13 (m, 4H), 2.32 (s, 3H, CH_3)
3221 (NH), 1642 (C=N), 1583 (C=N imidazole), 1206 (C-N), 1185 (C=S)	8.80 (s, 1H, NH), 8.02 (s, 1H, NH), 7.63 (d, 1H), 7.49 (s, 1H), 7.23 (d, 1H), 7.31-6.12 (m, 10H, Ar), 4.53 (dd, 2H),4.18 (m,1H), 3.83 (m, 2H), 3.45 (m, 4H), 3.20 (d, 2H), 3.09 (m, 4H), 2.38 (s, 3H, CH_3)

Determination of *in vitro* Antifungal Activity: All the compounds were evaluated for their *in-vitro* antifungal activity against *Aspergillus niger, Candida albicans, Candida krusei, Candida glabrata* and *Candida tropicalis* using broth microdilution assay method [14]. Ketoconazole was used as reference for inhibitory activity against fungi and was also screened under similar conditions for comparison. Serial dilutions of the test compounds and KTZ were prepared in Mueller-Hinton agar. Suspensions of each microorganism were prepared to contain 10^5 cfu/mL (colony forming unit/mL) and the plates were incubated at 35°C for 24 h and 48 h for all fungal strains tested and recorded as the MIC expressed in (µg/mL).

These experiments were duplicated to define the MIC values. The MIC was defined as at least 80 % inhibition of the growth of control. The data of activity is summarized in **table 3**.

TABLE 3: MICS VALUES (µg/ml) FOR FUNGI OF THE SYNTHESIZED COMPOUNDS (1-10)

Fungal strain	Incubation time (Hrs)	Compound No.										
		1	2	3	4	5	6	7	8	9	10	KTZ
Aspergillus niger	24	31.05	31.45	30.01	30.12	30.13	30.21	30.33	28.66	28.54	27.94	29.74
	48	31.11	31.57	30.05	30.19	30.57	30.24	30.39	28.68	28.59	28.03	30.05
Candida albicans	24	0.43	0.42	0.38	0.29	0.23	0.19	0.18	0.09	0.10	0.08	0.14
	48	0.44	0.42	0.40	0.29	0.24	0.20	0.21	0.10	0.10	0.09	0.15
Candida krusei	24	0.37	0.36	0.34	0.30	0.31	0.29	0.28	0.24	0.22	0.20	0.25
	48	0.38	0.36	0.35	0.31	0.31	0.32	0.29	0.24	0.23	0.21	0.27
Candida glabrata	24	0.41	0.42	0.39	0.37	0.37	0.40	0.42	0.33	0.31	0.30	0.35
	48	0.43	0.44	0.41	0.38	0.39	0.41	0.44	0.34	0.33	0.32	0.39
Candida tropicalis	24	0.29	0.25	0.26	0.23	0.24	0.27	0.28	0.18	0.17	0.15	0.19
	48	0.31	0.27	0.29	0.27	0.25	0.26	0.25	0.19	0.19	0.17	0.23

RESULT AND DISCUSSION: In the present study, ketoconazole thiosemicarbazone derivatives have been synthesized by using different substituted thiosemicarbazides with KTZ. TLC confirmed the purity of the synthesized compounds. The structural elucidation was done by using spectral and elemental analysis data. The data given in table 3 reveal good *in-vitro* antifungal activity of synthesized compounds (1-10) against the tested fungal strains. When compared to KTZ, compounds 8, 9 and 10 are more active with less MIC value showing importance of halogenated compounds.

CONCLUSION: The study on ketoconazole thiosemicarbazones as candidate antifungals suggests the beneficial potential of these leads that need to be further explored in order to discover and develop better and yet safer therapeutic agents for fungal infections.

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