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Research Article

SYNTHESIS OF 4 –BENZYLAMIDOTHIOSEMICARBAZIDE AND ITS THIOSEMICARBAZONES VIS-À-VIS THEIR ANTIFUNGAL ACTIVITY

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Abstract

The 4 –benzylamidothiosemicarbazide **(BTSC)** has been prepared by hydrazinolysis of 5 –(benzyl)1,3,4-oxadiazol 2-(3H) thione and a number of its Schiff's bases were also prepared and their toxicity examined towards Alternaria solani, Cunninghmella blakesleema and Sclerotium rolfsii. Of these compounds, the **BTSC**, 2-furyl- and 2-thinyl- derivatives of **BTSC** were found to be most effective, toxicity being comparable with that of 8-hydroxyquinoline sulphate. The growth of young mycelium of A. solani and S. rolfsii were checked at 20 ppm and C. blakesleema at 100 ppm. The low toxicity of Schiff's bases and transition metal complexes of **BTSC** is probably due to their low soubility.

Key words: Thiosemicarbazide, thiosemicarbazone, toxicity, Schiff's base, antifungal activity.

Introduction

The thiosemicarbazides and thiosemicarbazones have long been known for their pharmacological activity including anti-carcinogenic[1-2,] antibacterial[3], antituberculostatic[4-7], and fungicidal activities[8-9] . The copper complexes of thiosemicarbazide and thiosemicarbazones have also been reported [10,11] to show antifungal

activity towards various fungi. In order to test their biological activity against various microorganisms **BTSC** and its Schiff's bases and complexes of BTSC with various transition metal ions viz., **Cu(II), Ni(II), Co(III), Fe(II), Fe(III), Mn(II), Mn(III), Cr(III) and VO(IV)** were prepared and characterized[12-17] . The present communication describes the synthesis of these compounds and their antifungal activity towards Alternaria solani, Cunnighamella blakesleema and Sclerotlum rolfsii.

Synthesis of the compounds

The 4- benzylamidothisosemicarbazide **(BTSC)** has been prepared by the hydrazinolysis of 5-(benzyl)1,3,4-oxadiazol 2-(3H) thione in ethanol. For the preparation 5-(benzyl) 1,3,4-

oxadiazol 2-thione(III), phenylacetylthiourea was used as a starting material, and the method outlined by Authur Stempel et al [18] was adopted. When phenylacetylthiourea was chlorinated in the presence of concentrated hydrochloric acid, a product of (IIa) was found (not much stable) which liberated iodine from potassium iodide. The chloro-derivative (IIa) was dissolved in dilute sodium hydroxide which caused rearrangement and cyclization to yield III. The reaction mixture was neutralized and 5 –(benzyl)1,3,4 – oxadiazol-2-Thione isolated in ~55% yield from the phenylacetylthiourea (Scheme 1). A number of Schiff's bases of BTSC were prepared with various aldehydes as a result of condensation of BTSC and aromatic aldehydes in equimolecular proportion in the mixture of acetic acid and ethanol(1:4).

Experimental

(i) 5-(benzyl) 1,3,4-oxadiazol-2(3H) thione

Phenylacetyl thiourea (10 gm) was dissolved in 100 ml of concentrated hydrochloric acid and was cooled in ice-bath and rapid stream of chlorine was passed for 30 minutes through the solution. The solution was diluted up to one litre with cold distilled water, cooled and neutralized with sodiumcarbonate and the poduct(IIa) that separated out was filtered and dried in vacuum dessicator (an aqueous suspension of the compound turned starch paper blue thus suggesting that the product IIa was not much stable). The chloro compound (8 gm) was

reacted with 100 ml of 5% sodium hydroxide solution, After 15 minutes the reaction mixture was neutralized with acetic acid, the solid product (III) was separated and recrystallized with alcohol.

Yield ~50 %, m.p. 271-273 ^{0}C dec., Anal. Calcd. For $C_9H_8N_2O_2$: C,56.25; H,4.17 and N,14.58% found C,56.29; H,4.12 and N,14.54%.

(ii) 4-benzylamidothiosemicarbazide

A mixture of 4 gm of 5-(benzyl)1,3,4-oxadiazol-2-thione (III) in 50 ml ethanol containing 2.5 ml of hydrazine hydrate (99-100%) was refluxed for 1h. On cooling white crystals were separated and recrystallized with ethanol.

Yield -70%, m.p. 172-173 ^{o}C dec. Anal. Calcd. For $C_9H_{12}N_4OS$: C, 48.21; H, 5.36 and N, 25.00%. Found: C, 48.27; H, 5.29 and N,25.08%.

(iii) Thiosemicarbazones of BTSC

The various thiosemcarbazones were prepared by the condensation of 0.005 mole BTSC and corresponding aromatic aldehydes (0.005 mole) in the mixture of alcohol and acetic acid (4:1). Their melting points, analytical data (Table1) and important infrared bands are given in Table 2.

Table 1&2: Analytical Data of 4-benzylamidothiosemicarbazide and its Thiosemicarbazones

$C_6H_5CH_2CO.NH.NH.C.NH.NH_2$ + O==CH-R-------> $C_6H_5CH_2CO.NH.NH.C.NH.N$ =CHR

S S

Ch.No.	Chemical R	m.p°.c (dec.)	Yield (%)	Molecular Formula	Anal. Data (%)					
					Found			Calc.		
					C	H	N	C	H	N
1	1-Phenyl-	159-160	75	$C_{16}H_{16}N_4OS$	61.63	5.04	18.03	61.54	5.13	17.95
2	2-chlorophenyl-	171-173	65	$C_{16}H_{15}N_4OSCl$	55.4	4.3	16.21	55.41	4.32	16.16
3	3-Chlorophenyl-	169-170	55	$C_{16}H_{15}N_4OSCl$	55.61	4.27	16.09	55.41	4.32	16.16
4	4-chlorophenyl-	176-179	65	$C_{16}H_{15}N_4OSCl$	55.49	4.37	16.19	55.41	4.32	16.16
5	2-Nitrophenyl-	165-167	70	$C_{16}H_{15}N_5O_3S$	53.83	4.23	19.72	53.78	4.2	19.61
6	3-Nitrophenyl-	168-170	65	$C_{16}H_{15}N_5O_3S$	53.69	4.18	19.62	53.78	4.2	19.61
7	4-Nitrophenyl-	159-160	70	$C_{16}H_{15}N_5O_3S$	53.71	4.16	19.69	53.78	4.2	19.61
8	2-Methozyphenyl-	167-168	60	$C_{17}H_{18}N_4O_2S$	60.01	5.29	16.32	59.65	5.26	16.37
9	4-Methoxyphenyl-	197-199	70	$C_{17}H_{18}N_4O_2S$	59.59	5.22	16.29	59.65	5.26	16.37
10	3:4-Dimethoxyphenyl-	207-208	55	$C_{18}H_{20}N_4O_3S$	58.07	5.39	15.13	58.06	5.38	15.05
11	2:4-Dimethoxyphenyl-	199-201	65	$C_{18}H_{20}N_4O_3S$	58.1	5.41	15.09	58.06	5.38	15.05
12	2-Hydroxyphenyl-	186-187	70	$C_{16}H_{15}N_4O_2S$	58.69	4.63	17.27	58.72	4.59	17.13
13	3-Hydroxyphenyl-	192-193	55	$C_{16}H_{15}N_4O_2S$	58.79	4.61	17.17	58.72	4.59	17.13
14	4-Hydroxyphenyl-	205-207	65	$C_{16}H_{15}N_4O_2S$	58.76	4052	17.21	58.72	4.59	17.13
15	2:4:6-Trihydroxyphenyl-	225-227	65	$C_{16}H_{15}N_4O_2S$	53.6	4.48	15.59	53.33	4.44	15.36
16	2-Hydroxyl-3-nitrophenyl-	233-234	70	$C_{16}H_{15}N_5OS$	51.49	4.07	15.69	51.47	4.02	18.77
17	2-Hydroxy-5-Nitrophenyl-	299-301	65	$C_{16}H_{15}N_5OS$	51.59	4.01	18.74	51.47	4.02	18.77
18	Dimethylaminophenyl-	165-167	60	$C_{18}H_{21}N_5OS$	60.97	5.87	19.67	60.85	5.92	19.72
19	4-Methylpenyl-	195-197	55	$C_{17}H_{18}N_4OS$	62.61	5.56	17.09	62.58	5.52	17.18
20	2-Furyl-	164-165	65	$C_{14}H_{14}N_4O_2S$	55.71	4.59	18.58	55.63	4.64	18.54
21	2-thinyl-	174-176	70	$C_{14}H_{14}N_4OS$	52.91	4.47	17.7	52.83	4.4	17.61
22	2-Cinnamyl-	161-163	50	$C_{17}H_{17}N_4OS$	62.92	4.99	17.21	62.77	5.23	17.23
23	BTSC	172-173	45	$C_9H_{12}N_4OS$	48.27	5.29	25.08	48.21	5.36	25.00

(B) Antifungal Activity of Compounds

Material and Methods

The test organisms used were Cunninghamella blakesleema Lendner, obtained from the culture collection of the Indian Agricultural Reseach Institute, New Delhi, and Sclerotium rolfsii Sacc. And Alternaria solani (Ell. And G.Mart.) Sor. Isolated from peanut and potato crops. Potatodeztro agar (PDA) of standard composition was used through out these studies and the pH of the medium was adjusted to 6.0. This was found to be satisfactory for normal growth of the three test fungi.

The compounds in test were incorporated in ethylene glycol solution and added to the medium at 60^{o}C, the concentration of ethylene glycol in the medium being 0.2%.All the chemicals were tested at 20 ppm, 100 ppm and 500 ppm with three replications for each concentration. Aliquits of 20 ml of the medium were poured in to each petridish by means of a cornwall syringe pipette.

Proper checks were maintained with no test chemical present in the medium, but with the concentration of ethylene glycol being maintained 0.2%. The dishes were inoculated with 5 mm discs cut from the periphery of 48 h old cultures of the three test fungi and incubated at room temperature. Mycelial growth was measured on each palte from the centre of the inoculum disc

to the outer extreme of the longest radial strand of mycelium at the end of 48 and 72 h. The average measurements for a particular concentration of a given chemical and three figures were deducted from the control averages. The percentage of control averages. Where no growth occurred at the end of 72 h, the agar discs used for inoculating the plates were removed and washed with the sterile distilled water on a strainer. The mycelial mats form the experiments free from agar were placed in PDA containing no test chemicals and incubated for one week at room temperature. If no growth appeared during this period, the compound was considered fungicidal and not static fungi. The results were expressed in terms of the (percentage growth inhibition by comparing with growth on control plate. Thus).

Percentage inhibition=(C-T)×100. Where, C = Diameter (in mm.) of the fungal colony in control plate, T = diameter (in mm.) of the fungal colony in treated plate.

Results and Discussion

Twenty two thisosemicarbazones (derived from BTSC), BTSC and various transition metal complexes of BTSC were tested towards Alternaria solani, Cunninghamella blakesleema and Sclerotium rolfsii fungi at 20 ppm, 100 ppm and 500 ppm. The growth of young mycelium of S.rolfsii was found to be reduced to 75-85% with test chemicals number 3,4,8,9 and 18 at 500 ppm and rest of the chemicals completely inhibited the growth of the same fungus at the same concentration. Similarly the growth of A.solani was found to be completely quenched with all chemicals except test chemicals number 2,8,9 and 19, which inhibited the growth up to 70-80% at 500 ppm. There were only seven chemicals, which could completely inhibit the growth of C.blakesleema (Table 3). Out of these chemicals, only three viz. BTSC, 2-furyl- and 2-thinyl-derivatives were found to be most effective and completely inhibited the growth of A.solani, S.rolfsii at 20 ppm and C.blakesleema at 100 ppm in PDA medium. Only copper BTSC complex was found to be effective in checking the growth of A.soloni, S.rolfsii and C.blakesleema at 20 ppm, 100 ppm and 500 ppm, respectively. The solubilities of BTSC, 2-furyl-and 2-thinyl-] derivatives are relatively higher than other thiosemicarbazones and similarly the solubility of copper –BTSC complex was higher than other complexes. The compounds having relatively greater solubility are more effective as compared to the less soluble. The relative effect of the introduction of the various groups in aromatic ring and also at various positions i.e. ortho-, para- and meta- has been seen at 100 ppm. The order of increasing inhibition with substitution in benzene ring was as follows: dimethyl metonym ~ amino <methyl ~ chloro < nitro < phenolic. Thus, the phenolic group is most effective. The effect could not be observed at 500 ppm where most of these compounds completely inhibit the growth of the fungi.

Table 3.Antifungal Activity of Chemicals(Average % inhibition after 72 h at 30°C).

Ch.No/	Alternaria soloni			selerotium rolfsii			cunninghamella blakeshleema		
	20ppm	100ppm	500ppm	20ppm	100ppm	500ppm	20ppm	100ppm	500ppm
1	90	92	99	96	99	100	98	99	100
2	92	94	98	95	98	99	98	99	100
3	95	97	99	96	98	76	98	99	100
4	95	98	99	98	99	78	-	-	-
5	96	98	66	98	99	100	98	99	100
6	96	98	99	97	99	100	98	95	100
7	95	97	99	98	99	100	-	-	-
8	94	98	78	98	99	80	88	95	99
9	96	99	74	98	100	82	88	96	98
10	95	98	99	96	99	100	89	97	99
11	97	98	99	98	99	100	89	98	99
12	98	99	100	99	99	99	88	98	99
13	98	99	100	98	99	100	85	99	100
14	96	98	99	97	98	99	89	98	99
15	96	98	99	97	98	99	89	98	99
16	97	98	99	98	99	100	92	96	99
17	98	99	100	98	99	100	93	97	99
18	97	98	100	98	99	81	99	100	100
19	100	100	78	98	99	100	98	99	100
20	98	99	100	97	98	99	98	99	100
21	97	99	100	98	99	100	98	99	100
22	98	99	100	98	99	100	99	100	100
23	97	98	99	97	98	99	98	99	100

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