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Synthesis and antifungal activity of 1, 3-bis morpholinomethyl) - 2 - imidazolidone and 1, 3-bis (morpholinomethyl) - imidazolidine - 2-thione compounds

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ABSTRACT

Substituted cyclic ureas and thioureas having aliphatic homocyclic and heterocyclic rings were synthesized. They were screened for their antifugal activity against *Cladosporium herbarum* and *Fusarium oxysporum*.1,3 – Bis (morpholinomethyl) – imidazolidine 2- thione (MMIT) and $COCl_2$.MMIT showed total inhibition to *C.herbarum* at 250, 500 and 1000 ppm concentrations, whereas 1,3-bis (morpholinomethyl)-2-imidazolidone (MMIZ) and $ZnCl_2$.MMIZ showed total inhibition at 1000 ppm concentration towards *C.herbarum.* Against *Fusarium oxysporum* only $ZnCl_2$.MMIT showed total inhibition at 1000 ppm concentration. Biosorption of metals on the mycelium of *F. oxysporum* were decreased in the other cases tried.

Key words: 1,3-bis (morpholinomethyl) -2-imidazolidone (MMIZ), 1,3-bis (morpholinomethyl) imidazolidine – 2 thione (MMIT), antifungal activity, biosorption.

INTRODUCTION

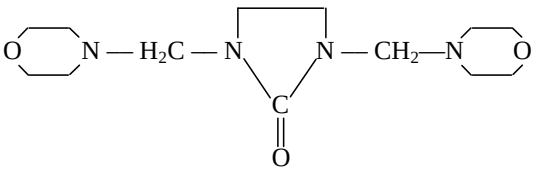
Cyclic ureas and cyclic thioureas have the ability to destroy or inhibit microorganisms. The presence of aliphatic cyclic substituents on cyclic urea and cyclic thiourea moiety can effectively alter the microbial metabolism. The synthesis and fungicidal activities of many chemical compounds were reported by many workers.[1-5]

The present work deals with testing the substituted cyclic ureas and cyclic thioureas namely 1,3 -bis (morpholinomethyl)-2-imidazolidone (MMIZ) and 1,3-bis-(morpholino-methyl) – imidozolidine-2-thione (MMIT) for their antifungal activity against *Cladosporium herbarum* NCBT 146 and *Fusarium oxysporum*, NCBT 156.

MATERIAL AND METHODS

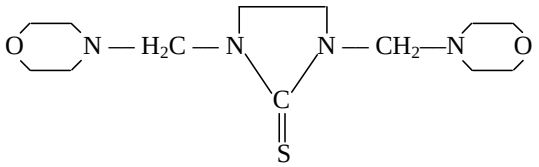
Synthesis and Characterization of 1,3–Bis (morpholinomethyl) – 2 – imidazolidone (MMIZ)

MMIZ was synthesized by replacing the two hydrogen atoms of reasonable activity at the equivalent N(1) and N(3) positions of 2-imidazolidone (ethyleneurea) with morpholinomethyl moiety by employing the Mannich reaction. The crude product was recrystallised form 2-propanol (yield 78.65%). The melting point was found to be 92 °C with molecular formula as C_{13} H_{24} N_4 O_3 (shown below).



Synthesis and Characterization of 1, 3-Bis (morpholinomethyl)-imidazolidine-2-thione (MMIT)

MMIT was synthesized by introducing morpholinomethyl moiety at the 1,3 positions of ethylenethiourea by taking recourse to the Mannich reaction[6,7]. The crude product was recrystallised from water (yield 92%). The melting point was found to be 152° C with molecular formula as C_{13} H_{24} N_4 O_2 S. The substrate ethylenethiourea (bifunctional) contains two active hydrogen atoms attached to the N(1) and N(3) atoms as shown below.



Fungal Cultures

Cladosporium herbarum NCBT 146 and *Fusarium* oxysporum NCBT 156 cultures maintained in immobilized condition in the laboratory were used in this work.

Experimental Procedure

Potato-dextrose-agar (PDA) medium was prepared[8] and streptomycin (50 mg) was added to 250 ml medium to inhibit the bacterial growth. Different concentrations of MMIT, $CoCl_2$.MMIT, $Cu(NO_3)_2$.MMIT2H_2O and $ZnCl_2$.MMIT (250, 500 and 1000 ppm) were individually prepared in distilled water and sterilized in an autoclave along with the medium. Similarly, different concentrations (250, 500 and 1000 ppm) of MMIZ, Co_2Cl_4.MMIZ, Cu_2Br_4.MMIZ 2H_2O and $ZnCl_2$.MMIZ were prepared in distilled water and sterilized.

The sterile medium (20 ml) and the sterile solution (5 ml of 1250 ppm) of MMIT were mixed under sterile condition to obtain the effective concentration of MMIT in the medium as 250 ppm. Similarly 5 ml of 2500 ppm solution of MMIT and 20 ml of the medium were mixed in another Petri dish so that the effective concentration was 500 ppm. In the same way 5 ml of 5000 ppm solution of MMIT and 20 ml of the medium were mixed in a Petri dish to get an effective concentration of 1000 ppm. The same procedure was adopted in the case of $CoCl_2$.MMIT, $Cu(NO_3)_2$.MMIT 2H_2O and $ZnCl_2$.MMIT as well for the MMIZ, Co_2Cl_4.MMIZ, Cu_2Br_4.MMIZ 2H_2O and $ZnCl_2$.MMIZ compounds. Control was prepared by pouring 20 ml of the medium and 5 ml of sterile distilled water. After the medium solidified, each Petri dish was inoculated with spores from a pure culture of *C.herbarum* (NCBT 146) and *F. oxysporum* (NCBT 156) fungal strains and incubated for 4 days at a temperature of 28 ± 2°C in the dark.

RESULTS AND DISCUSSION

MMIT and its metal complexes tested for antifungal activity on *C.herbarum* (NCBT 146), revealed total inhibition in the case of $CoCl_2$.MMIT at 1000 ppm concentration. $Cu(NO_3)_2$.MMIT 2H_2O and $ZnCl_2$.MMIT are ineffective Table 1.

In the case of *F. oxysporum* (NCBT 156), $ZnCl_2$.MMIT at 1000 ppm concentration showed total inhibition, whereas in other cases the activity was not effective (Table-2). But biosorption of metals especially in $Cu(NO_3)_2$.MMIT 2H_2O is very high.MMIZ and its metal complexes tested for antifungal activity on *C. herbarum* (NCBT 146), revealed total inhibition in the case of $ZnCl_2$.MMIZ at 1000 ppm concentration, whereas MMIZ and CO_2Cl_4.MMIZ showed the same quantum of inhibition at 1000 ppm, but Cu_2Br_4.MMIZ2H_2O was almost insensitive at all concentrations (Table-1). In the case of *F. oxysporum* (NCBT 156), the MMIZ and its complexes are not effective (Table-2). But biosorption of metals especially in the case of $Cu(NO_3)_2$.MMIZ 2H_2O is very high.

Imidazolidine -2- thione and its derivatives are interesting ligands because of their potential binding sites.[9] The fungal cell wall polymers provide a multitude of chemical groups such as hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate and phosphodiester.[10] These chemical groups of the biopolymers in turn harbour the binding sites, which provide the ligand atoms to form complexes with metal ions which lead to inhibition of fungal growth.[11,12] Such factors are perhaps involved in the inhibition of growth against *C. herbarum* and *F. oxysporum* in the case of MMIT and MMIZ complexes. The biosorption of metals from

Table 1: Antifungal effect on *Cladosporium herbarum* NCBT 146

Compound	Concentration (ppm)	Time 96h	Compound	Concentration (ppm)	Time 96h
MMIT	Control	++++	MMIZ	Control	++++
	250	++++		250	+++
	500	++		500	+++
	1000	+		1000	+
$CoCl_2$MMIT	Control	++++	Co_2Cl_4MMIT	Control	++++
	250	-		250	+++
	500	-		500	++
	1000	-		1000	+
$Cu(No_3)_2$	Control	++++	Cu_2Br_4	Control	++++
MMIT $2H_2O$	250	++++	MMIZ	250	++++
	500	++++		500	+++
	1000	++		1000	+++
$ZnCl_2$ MMIT	Control	++++	$ZnCl_2$ MMIZ	Control	++++
	250	+++		250	+++
	500	+++		500	+
	1000	+++		1000	-

Normal Growth (++++) 25% growth inhibition (+++) 50% growth inhibition (++)
75% growth inhibition (+) 100% inhibition (-)

Table 2: Antifungal effect on *Fusarium oxysoprum* NCBT 156

Compound	Concentration (ppm)	Time 96h	Compound	Concentration (ppm)	Time 96h
MMIT	Control	++++	MMIZ	Control	++++
	250	++++		250	++++
	500	++++		500	++++
	1000	+++		1000	++++
$CoCl_2$MMIT	Control	++++	Co_2Cl_4MMIZ	Control	++++
	250	++++		250	++++
	500	++++		500	++++
	1000	++++		1000	+++
$Cu(No_3)_2$	Control	++++	Cu_2Br_{42}	Control	++++
MMIT $2H_2O$	250	++++	MMIZ	250	++++
	500	+++		500	++++
	1000	+++		1000	+++
$ZnCl_2$ MMIT	Control	++++	$ZnCl_2$ MMIZ	Control	++++
	250	++++		250	++++
	500	+++		500	++++
	1000	-		1000	++++

Normal Growth (++++) 25% growth inhibition (+++) 50% growth inhibition (++)
75% growth inhibition (+) 100% inhibition (-)

$Cu(NO_3)_2$.MMIT$2H_2O$ and $Cu(NO_3)_2$.MMIZ $2H_2O$ are different in the case of *F.oxysporum* and *C. herbarum*. The mechanism of activity in the case of *F. oxysporum* is ionic binding of the fungal cell membrane[13], but in the case of *C. herbarum* it is adsorption and microprecipitation of the fungal cell wall[14].

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REFERENCES

1. Rajvir Singh Suman Sharma and Om Prakash Malik Synthesis of some Alkyl / Chloro/Methyoxy-substituted-4- (substituted-phenoxy methyl) -2H-1-benzopyran - 2- ones as Potential Antifungal Agents. *J.Indian Chem. Soc.* **69**: 338-339 (1992).
2. Roy N K and Taneja H K Synthesis and Antifungal Activity of O-Methyl -O-Aryl-2-Chloraethyl Phosphonates and Bisaryl -2-Chloroethyl Phosphonates, *J. Indian Chem. Soc.* **69**: 42-43 (1992).
3. Sridharan K Abubacker M N and Muthuswamy S Antifungal activity of-α-amino acid dithricarbomates. *Indian Journal of Microbiology.* **35**: 243-247 (1995).
4. Bhat A R Varadaraj Bhat G and Gautham Shenoy G Synthesis and *in vitro* antimicrobial activity of new 1,2,4-triazoles. *Journal of Pharmacy and Pharmacology.* **53**: 267-272 (2001).
5. Vingkar S K Bobade AS and Khadse B G Synthesis and Antimicrobial activity of 3-2 (Alkyl / Aryl, 4-substituted Thiazolo)-6-Fluorocinnoline-4 ones. *Indian Drugs*. **38**: 573-575 (2001).
6. Sawlewicz, J., Wisterowicz, K. and Vogel S *Acta. Pol. Pharm.* **32**: 435 (1975).
7. Rhone-Poulene, S.A. *Chem. Abst.* **63**: 612 (1965).
8. Harrigan W F and Mc Cance M E Laboratory Methods in Microbiology. Academic Press, London, 297 (1969).
9. Isab A A Hussain M S Synthesis C^{13} NMR and IR spectroscopic studies of Gold (I) complexes of imidazolidine -2- thione and its derivatives. *Polyhedron*. **4**: 1683-1688 (1985).
10. Schiewer S and Volesky B In : Environmental Microbe- metal interaction. Ed. D.R. Lovely, ASM Press, Washington DC, 329 (2000).
11. Hunt S In:Immobilization of ions by biosorption. Ed H Eccles and S. Hunt, Ellis Horwood, Chichester, UK, 15 (1986).
12. Gadd G M Metals and Microorganisms: A problem of definition, *FEMS Microbiol. Lett.* **100** : 197 (1992).
13. Remacle J In: Biosorption of heavy metals Ed. B Volesky CRC Press, *Boca Ration*, 84 (1990).
14. Gadd G M In: Microbial mineral recovery. Ed. HL Ehrlich and C.L. Brierley, Mc Graw publishing Co. New York, 249 (1990).