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IN VITRO ANTIBACTERIAL STUDIES OF SOME NEWLY SYNTHESIZED PHENACYL-THIOSEMICARBAZONES

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ABSTRACT

Four different phenacyl-thiosemicarbarone compounds were studied for their antibacterial activity against seventeen different speatrystrains. Of the four compounds under study, *O*-methozy- phenacyt-thiosemiurbuonc was found to be highly active against almast all the cultures under study. The other compounds were also found to posmn antibacterial spectrum, but in very high concentrations for some of the cultures.

Introduction

Thiosemicarbazones, having a general structure $RC=N.NH.CSNH_2$, are monosubstituted urea containing compounds. Their derivatives have long been known to possess antibacterial (Krynkova *et al*, 1977; Verma *et al*, 1980; Goswami *et al*., 1984; Klayman *et al*, 1984; Granchayov et al., 1985; Bobek *et* at, 1986; Marta, 1988; Kudari and Sangapine, 1989), antiviral (Arita, 1979) and antiprotozoal activity (Klayman and Joseph, 1979; Casero *et al.*, 1980; Klayman and Pscovill, 1981). Some of the derivatives have also been used for clinical treatment (Kudari and Sangapine, 1989), while others have proved unsuitable due to their toxicity (Walter *et al.*, 1981; Khan *et al.*, 1986) or effectiveness in high concentration.

In view of the importance of this group of compounds as antimicrobial agents, a number of derivatives are being synthesized to obtain an effective, broad spectrum antimicrobial agent that may be used for therapy. The main object of the present study is to evaluate the comparative antibacterial activity of four structurally related, newly synthesized derivatives of thiosemicarbazones against different species/ strains of bacterial cultures.

Materials and Methods

2,4-Dimethoxy-phenacyl-thiosemicarbazone (DT), O-fluoro-phenacyl-thiosemicarbazone (FT), O-methoxy-phenacyl-thiosemicarbazone (MT) and O-hydroxy-phenacyl-thiosemicarbazone (HT) (Figure 1) were synthesized by Ms. Shoana in Dr. Saify's Laboratory, Faculty of Pharmacy, University of Karachi.

Preparation of stock solution

Compounds insoluble in water were made soluble by preparing their hydrochloride salts by reaming them in HCI saturated ethanol for 1-3 hours on a water bath at 45°C. The compounds were then dissolved in distilled water to obtain a final concentration of 1 mg/ml. The solutions were sterilized by using membrane titer (0.45µm) (Schleicher and Schuell, Germany).

Bacterial cultures

The following cultures were used for the determination of minimum inhibitory concentration (MIC) of the compounds under test.

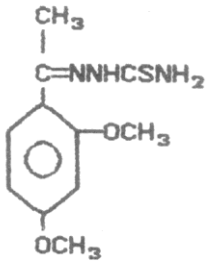
Listeda monocytogenes C-274 serotype 4b, NCTC 5214 m serotype 4a, NCtC 7973 sem type 1/2a, Shigella sonnei ATCC 25931, Klesiella pneumoniae ATCC 13883, local human isolates of *Staphylococcus aureus, Staphylococcus epidennidis, Streptococcus pyogenes, Corynebacterium diphtheriae, Salmonella typhi, Salmonella paralyphi A, Salmonella schottmuelleri, Shigella dysenteriae, Shigella flexeri; Shigella boydii, Pseudomonas aerginosa* and *Vibrio cholerae.*

Grown cultures were stored in tryptose phosphate broth (TPB) (oxoid), pH 73 containing 0.5% agar at 4°C in the screw-capped tubes (Flow labs.). The cultures were activated by transferring a loopful from the stored culture to TPB (pH 73) and incubated overnight at 37°C.

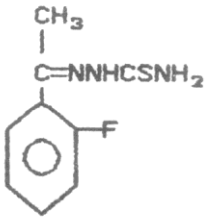
Susceptibility study

MIC values were determined by tube dilution method. The serial dilutions of each compound were prepared in TPB. Overnight broth cultures (activated) were added to each of the tubes, the final bacterial concentration was 10^5-10^6 c.f.u.ml^{-1} (colony forming unit/ml). The tubes were incubated for 24 hours at 37°C. MIC values were determined by visual inspection for turbidity.

MBC, defined as 99.9% decrease in c.f.u. relative to inoculum, was determined by streaking 10 µl culture showing no turbidity on test compound free iryptose phosphate agar (TPA) plates, pH 73 (Thrupp, 1981). No significant difference in MBC was observed.



2,4-dimethoxy-phenacyl-thiosemicarbazone



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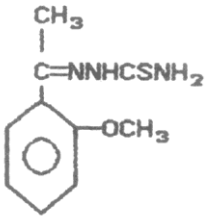
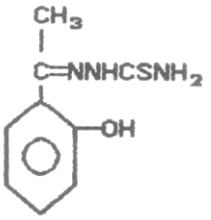


O-methoxy-phenacyl-thiosemicarbazone



O-hydroxy-phenacyl-thiosemicarbazone

Figure 1. Structural formula of phenacyl-thiosemicarbazones

Results and Discussion

Table 1 illustrates the results for MIC against seventeen bacterial cultures used in this study.

DT was found to be relatively effective only against L. monocytogenes C-274 with MIC of 2 pg/ml. It's MIC against *L.* monocytogenes 5214 m and *L. monocytogenes* 7973, *Salmonella paratyphi* A and *Shigella sonnei* was 200 µg/ml and against other organisms was 400 pg/ml or above.

Antibacterial activity of FT was also determined against these organisms. It's MIC against *L.* monocytogenes 7973 and L. monocytogenes 5214 m was 25 pg/ml and 50 pg/ml respectively, while against C. diph heriae and *S. paratyphi* A was 100 pglml. The MIC against the remaining 13 cultures was found to be 200 pg/ml. The MIC of HT against all these organisms was 200 pg/ml.

MT was found to be extremely effective against *S. epidennidis* and *C. diphetheriae* with MIC of 136 pg/ml. The MIC against *L. monocytogenes* 7973, *S. paratyphi* A, *Sh. flexneri* and *Sh. bodydii* was 12.5 pg/ml, and against rest of the organisms 25 pg/ml.

The data show considerable differences in the antibacterial activity of DT, Fr, MT and HT. The introduction of O-methoxy group into the phenacyl-thiosemicarbazone (DT) increases the antibacterial activity of the compound as compared to the other analogues. These compounds (Fr, MT and HT) have shown significant antibacterial activity against some species/strains, and insignificant activity against others.

The reason for increased activity of O-methoxy- phenacylthiosemicarbazone compared to other three compounds is yet to be established.

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Table 1

Minimum inhibitory concentration and minimum bactericidal concentrations in $\mu g\ ml^{-1}$ for phenacyl-thiosemicarbazone compounds.

Organisms	2,4-dimethoxy-phenacyl-thiosemi-carbazone		*O*-fluoro-phenacyl-thiosemi carbazone		*O*-methoxy-phenacyl-thiosemi-carbazone		*O*-hydroxy-phenacyl-thiosemi-carbazone	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
L. monocytogenes (NCTC 5214)	200	200	50	100	25.0	25.0	200	200
L. monocytogenes (C-274)	25	25	200	200	25.0	25.0	200	200
L. monocytogenes (NCTC-7973)	200	25	25	25	12.5	25.0	200	200
S. aureus	400	400	200	200	25.0	25.0	200	400
S. epidermidis	400	400	200	200	1.56	3.12	200	200
S. pyogenes	400	400	200	200	25.0	25.0	200	200
C. diphtheriae	400	400	100	200	1.56	1.56	200	400
S. typhi	>400	>400	200	400	25.0	25.0	200	200
S. paratyphi A	200	200	100	100	12.5	25.0	200	200
S. schottmuelleri	>400	>400	200	200	25.0	25.0	200	200
Sh. dysentriae	>400	>400	200	200	25.0	25.0	200	200
Sh. sonnei	200	400	200	400	25.0	25.0	200	200
Sh. flexneri	400	400	200	200	12.5	12.5	200	200
Sh. boydii	400	>400	200	200	12.5	12.5	200	200
V. cholerae	>400	>400	200	200	25.0	50.0	200	200
Ps. aeruginosa	>400	>400	200	400	25.0	50.0	200	400
K. pneumoniae	400	>400	200	200	25.0	25.0	200	200

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