Pakistan Journal of Pharmaceutical Sciences
Vol.9(2), July 1996, pp.37-42

IN VITRO ANTIBACTERIAL STUDIES OF FOUR NEWLY SYNTHESIZED PHENACYL-THIOSEMICARBAZONE

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ABSTRACT

Four different phenacyl-thiosemicarbazone compounds were studied for their antibacterial activity against seventeen different species/strains. Of the four compounds under study O-methoxy-phenacyl-thiosemicarbazone was found to be highly active against almost all the cultures under study. The other compounds also possessed antibacterial spectrum, however in *very* high concentrations for some of the cultures.

Introduction

Thiosemicarbazones, having a general structure RC = N.NH.CS.NH2, are monosubstituted urea containing compounds. The derivatives of which have long been known to possess antibacterial (1-8), antiviral (9) and antiprotozoal activity (10-12). Some of the derivatives have been used clinically (5), while others have proved unsuitable either due to toxicity (13-14) or because of its 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 objective of the present study was to evaluate the antibacterial effectiveness of four structurally related, newly synthesized derivatives of thiosemicarbazones.

Materials and Methods

The Compounds:

2,4-dimcthoxy-phenacyl-thiosemicarbazone (DT), O-fluoro-phenacyl-thiosemicarbazone (FT), O-methoxy-Phenacylthiosemicarbazone (MT) and O-hydroxy-phenacyl-thiosemicarbazone (HT) (Fig. 1).

These compounds were synthesized in Faculty of Pharmacy, University of Karachi by Ms. Shoana in Dr. Saify's laboratory and were used to evaluate their antibacterial spectra.

Preparation of Stock Solution:

Compounds being insoluble in water were made soluble by preparing their hydrochloride salts by refluxing them in HCl saturated ethanol for 1-3 hours in a water bath at 45°C. The compounds were then solubilized in distilled water to obtain a final concentration of 400 pg/ml. The solutions were then membrane filter (0.45 μm) sterilized.

The Organism and Storage

The following cultures were used for minimum inhibitory concentrations (MIC) determination.

Listeria monocytogenes C-274 Serotype 4b, NCTC 5214 m serotype 4a, NCTC 7973 serotype 1/2a, *Shigella sonnei* ATCC 25931, *Klebsiella pnermtoniae* ATCC 13883, local human isolates of *Staphylococcus aureus, Staphylococcus epidennidis, Streptococcus pyogenes, Corynebacterium diphtlienae, Salmonella typhi, Salmonella parapphi A, Salmonella schotumuelleri; Shigella dysenteriae, Shigella flexneri, Shigella boydii, Pseudomonas aeruginosa and Vibrio cholerae.*

The bacterial growth was stored in tryptose phosphatebroth (TPB) (oxoid), pH 7.3 containing 0.5% agar at 4°C in the screw-capped tubes (Flow labs.) in a refrigerator. The culture was activated by transferring a loopful from the stored culture to TPB (oxoid), pH 7.3 and incubated overnight at 37°C.

The 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 deter-mined by visual inspection for turbidity.

MBC defined as 99.9% decrease in c.f.u. relative to inoculum; it was determined by streaking 10 ul culture on test compound free tryptose phosphate agar (TPA) plates, pH 7.3 (15). No significant difference in MBC was observed.

Results

Table 1 illustrates the results for MIC against 17 (seventeen) bacterial cultures used in this study.

DT was found to be relatively effective only against *L. monocytogenes* C-274 having MIC of 25 µg/ml, whereas, exhibited MIC of 200 µg/ml against *L. monocytogenes* 5214 m and 7973, *Salmonella paratyphi* A and *Shigella sonnet* Against other organisms the MIC values was 400pg/ml or above.

FT also possessed some degree of antibacterial activity having relatively higher MIC values i.e. 200 pg/ml for most of the organisms except *L. nonocytogenes* 7973 of 25 pg/ml, *L.* monocytogenes 5214 m of 50 pg/ml and *C. diphtheriae* and *S. paratyphi A* with 100pg/ml.

HT was has invariably exhibited MIC of 200 pg/ml against the organisms.

MT was found to be extremely effective against S. *epidennidis* and *C. diphlheriae* with a MIC of 1.56 pg/ml. The MIC against *L. mono*cy*togenes* 7973, *S. paratyphi* A, *S. flexneri* and *S. boydif* was 12.5 pg/ml and 25 pg/ml against rest of the organisms.

Discussion

The data showed considerable differences in the antibacterial activity of DT, FT, MT and HT. The introduction of O-methoxy group into the phenacyl-thiosemicarbazone increased the antibacterial activity of the compound compared to the other three. The other three compounds have shown good selective antibacterial activity against some species and their strains, although without significant antibacterial activity.

The reason for increased activity of O-methoxy-phenacyl-thiosemicarbazone compared to other three have not yet been established.

Table 1
Minimum inhibitory concentration values and minimum bacterial concentrations in $\mu g/ml^{-1}$ for a series of 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
S. 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
S. dysentriae	> 400	> 400	200	200	25.0	25.0	200	200
Sh. sonneri	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

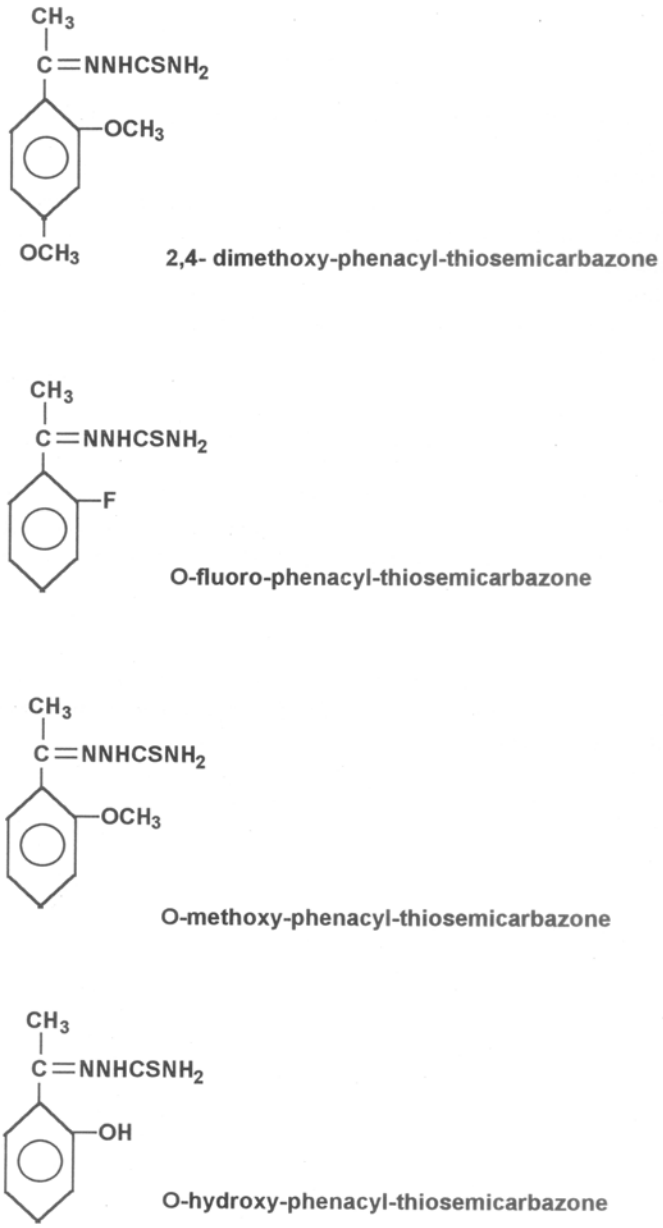

Fig. 1: Structural formula of Phenacyl-thiosemicarbazones

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