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Research Article

Evaluation of Antibacterial Activity of Thiazolo-Thiourea Sydnones

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Abstract: Sydnones are the mesoionic compounds having wide range of biological activities. Structural modifications at various positions have provided different class of sydnones, thiazolo-thiourea sydnones are one among them. Nowadays Greenfield chemistry revolves around the design and development of chemical compounds in feasible economical and nonhazardous way. Ten different thiazolo thiourea sydnones compounds were tested for their antibacterial activity. They were tested on *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas pyocyneous* using agar well method. Out of the ten different Thiazolo thiourea sydnones derivatives compounds 2, 3 and 10 showed promising results others also had some antibacterial activity. It can be concluded from the above study that Thiazolo thiourea compounds have antibacterial property.
Keywords: Antibacterial property, Thiazolo-thiourea sydnones, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas pyocyneous*

INTRODUCTION

Sydnones are a novel class of meso-ionic compounds with unique chemical and physical properties. A vast array of sydnone derivatives have been found to show varied biological properties [1], antioxidant activity [2], and liquid crystalline properties [3]. Numbers of meso-ionic compounds have been reported to have biological activities. Sydnones are earliest known members of the meso-ionic heterocycles [4]. Following are some of the biological activities claimed for sydnones: antibacterial [5], antifungal [6], antimalarial [7], anti-inflammatory [4, 8], analgesic [9], anticonvulsant [10], antihyertensive [11], Antithrombotic [12], Antitumor [13] etc.

Cephalosporin derivatives of sydnones have anti-streptococcal and anti-staphylococcal activities in vivo [14]. Sydnofen and sydnocarb have shown antidepressant activity [15] and molsidomine has antianginal and antiischemic activity [16].

The available methods for antibacterial sensitivity testing are (A) Paper diffusion disk method [17] (B) Ditch or Well procedure [18, 19]; (C) Cylinder plate method [20]. The agar well method was chosen in this study; because of problem of solubility of test compounds, the ease of diffusiblity in agar well and to overcome the problem of preparing the drug concentrations. This agar well method is a modification of the ditch or well procedure, and it is widely used for antibiotic assays [21, 22].

MATERIALS AND METHODS

General procedure for the synthesis of 4-substituted-3-phenylsydnones (Friedel-Crafts acylations) [23]: in a pressure tube 4-substituted phenyl sydnone ($6.167x10^{-4}$ mol), bismuth triflate ($1.542x10^{-4}$ mol, 25 mol %), and acetic anhydride ($2.467x10^{-4}$mol) were mixed and the reaction mixture was kept inside a microwave oven (BPL make-model, BMO: 700T) operating at 160 W for about fifteen minutes. After completion of the reaction the product was poured in water and then allowed to cool to room temperature. The reaction mixture was extracted with methylene chloride (3 x 10 mL), and the combined extracts were removed *in vacuo*. The resulting solid was recrystallized from hot ethanol. The progress of the reaction was monitored by TLC after every 3 minutes. In this study the antibacterial activity of the test compounds was carried out against four pathogenic organisms viz, *Staphylococcus aureus* (gram positive), *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas pyocyneous* (all gram negative).

Preparation of subcultures

4 to 5 hour prior to testing, the above mentioned four cultures were inoculated in 20ml of nutrient broth in screw capped test tube, and were incubated for 5-6 hours at 37^0C. nutrient broth was prepared by dissolving Peptone-(0.5%), Yeast extract-(0.15%), Beef extract-(0.15%), Sodium chloride-(0.35%), Potassium Dihydrogen phosphate-(0.13%) and Potassium Monohydrogen phosphate-(0.13%) in distilled water. The PH of this solution was adjusted to

7.2 by using 1N sodium hydroxide solution and was autoclaved for 20 minutes at 15 lbs pressure. So this was added, 10% scitz filtered sterilized glucose solution, 1ml to each 100 ml media.

Preparation of nutrient agar

Nutrient agar which served as the basal medium was prepared by dissolving; Bacteriological peptone (0.6%), Yeast extract (0.3%), Beef extract (0.13%) and Agar (2.1%) in distilled water. pH of solution was adjusted to 7.2 solutions and was autoclaved for 20 minutes at 15 lbs pressure. To this was added sterilized 10% glucose solution, 1 ml to 100ml of media.

Preparation of drug solution

The test compounds (10mg each) were dissolved in 10 ml of dimethyl sulfoxide (DMSO) to give a drug solution of 1000 microgram/ml and 0.1 ml of the solution was taken for testing. From this solution drug concentrations of 50 microgram/0.1 ml and 25 micrograms/0.1 ml were prepared.

Method of testing

Nutrient agar, while hot was poured into sterilized petridishes and allowed to cool and solidify at room temperature. The agar plates were inoculated with 4 to 5 drops of prepared subcultures and this was spread uniformly all over the agar surface, by using sterile glass rod. Plates were allowed to dry in the inverted position in the incubator for 30 minutes.10 mm wells -3 in numbers were punched by using a sterile cork borer and the agar was scooped out by using a template, taking care to see that, the surrounding agar from the plates was not lifted when removing the agar cores. The plates were marked for the drug concentrations corresponding to the wells and the solutions of the test compounds corresponding to the concentrations were delivered to the wells using sterile 0.1 ml pipette. The plates were incubated I the upright position, overnight at 37 ^{0}C. After incubating the antibacterial activity was found out by measuring zones of inhibition in mms, by using calipers and scale. These values were compared with values obtained from two standards taken viz phenol and sulfanilamide. The antibacterial activity of the solution (DMSO) was tested for above organisms by pipetting out 0.1 ml of solvent into corresponding wells of the agar plate.

RESULTS

In this study Thiazolo-thiourea substituted 10 sydnone compounds were tested for anti-inflammatory and analgesic activities in the earlier study. These ten compounds were tested for antibacterial activities using 4 organisms' viz., *Staphylococcus aureus*; *Pseudomonas pyocyneous*; *Proteus vulgaris*; *Escherechia coli* and compared with the effectiveness of sulphanilamide and phenol over the above mentioned organisms. To know the effects of DMSO (solvent) if any, it was also tested in these organisms, which has not shown any effects on any of the organisms chosen for testing.

The compound no's 2 3 and 10 have shown comparatively satisfactory results against *S. aureus*, *P. pyocynaceous*, *P. vulgaris* and *E. coli*. They have shown even better effectiveness than sulfanilamide over these organisms. Zone of inhibition with these compounds, reveals that they are effective against both; gram positive and gram negative organisms, in the concentration of 25,50 and 100 micrograms. The maximum zone of inhibition was achieved at 100 microgram concentration. The remaining seven compounds have also shown zone of inhibition on the same organisms but not as effective as compounds 2, 3 and 10. However the inhibition effect of these seven compounds cannot be neglected or ignored as they have shown little more zone of inhibition than sulphanilamide and phenol.

Compound no 8 has shown less effect on *S.aureus* and *E. coli* as the zone of inhibition is lesser. Compound 3 has shown larger zone of inhibition than sulfanilamide on *P.vulgaris*, *E.coli* and *S. aureus*. Compound 5 has shown better inhibition on *P. vulgaris* and *E. coli* but not as much as compound 3 against these organisms. All these compounds have shown larger zone of inhibition than sulfanilamide on S.aureus except compound no 8. Sulphanilamide has not shown much effectiveness on *E. coli*, phenol has shown nil effect on *P. pyocynosis* and very little effect on *E. coli*. The solvent DMSO has not shown any effect on any of the organisms tested.

DISCUSSION

In the present study the Thiazolo-thiourea sydnone compounds were tested for antibacterial activity against *S.aureus*, *P pyocynaeceous, P. vulgaris* and *E. coli.* Out of the four well known methods for antibacterial sensitivity testing agar well method is adopted for this study, because of ease of diffusiblity of test compounds in agar, problem of solubility of compounds and to overcome the problem of preparing various drug concentrations. The zone of inhibition (in mm) shown by compounds 2, 3 and 10 are significant and worth appreciating as compared to sulfanilamide, on above mentioned organisms. The organisms were selected to represent both gram positive and gram negative.

Compound 2 differs from compound 3 in possessing p.chlorophenyl group whereas compound 3 is having p.bromophenyl group, and rest of the structure being the same, the antibacterial efficacy of both these have a small range of difference. The encouraging results obtained by compound 10, having 4(4''-(3-P-Chlorophenylsydnoyl) thiazole-2-amino allyl thioureas, has made us to show deeper interest regarding its antimicrobial activity in comparison with the standard used, i.e., sulphanilamide and phenol.

The compounds 2,3 and 10 deserves the testing over other possible organisms, as they have shown better effectiveness on 4 organisms tested in the study. But the scanty availability of these compounds and restricted facilities for testing their antibacterial activities by other methods has restricted our work, to only four organisms in the present study. It would be worthy to test these compounds against other colonies of bacteria provided there is sufficient amount of compounds and facilities to go ahead with other testing.

Table-1: Results of antibacterial study of Thiazolo-thiourea substituted 10 sydnone compounds

Sl. No.	Name of compound	Zone of inhibition in mms											
		Staphylococcus aureus			***Pseudomonas pyocyneous***			***Proteus vulgaris***			***Escherichia coli***		
		100	**50**	**25**	**100**	**50**	**25**	**100**	**50**	**25**	**100**	**50**	**25**
1	4(4'(3-P-Phenyl Sydnonyl) Thiozolo-2-Aminophenyl Thiourea	20	16	13	24	24	21	21	19	17	20	18	17
2	4(4'(3-P-Phenyl Sydnonyl) Thiozolo-2-Amino P. Chlorophenyl Thiourea	43	31	23	27	19	17	46	33	22	33	26	22
3	4(4'(3-P-Phenyl Sydnonyl) Thiozolo-2- Amino P. Bromophenyl Thiourea	48	43	40	28	27	23	50	47	43	34	33	28
4	4(4'(3-P-Phenyl Sydnonyl) Thiozolo-2-Amino Benzyl Thiourea	23	21	18	24	23	22	21	19	18	29	22	18
5	4(4'(3-P-Chlorophenyl Sydnonyl) Thiozolo-2-Amino Phenyl Thiourea	24	22	17	25	22	23	32	29	28	35	30	27
6	4(4'(3-P-Toly Sydnonyl) Thiozolo-2-Amino-p-Chlorophenyl Thiourea	28	23	14	24	22	17	31	28	18	22	18	14
7	4(4'(3-P-Toly Sydnonyl) Thiozolo-2-Amino -Benzoyl Thiourea	21	21	16	28	26	24	20	18	16	29	28	26
8	4(4'(3-P-Chlorophenyl Sydnonyl) Thiozolo-2-Amino –p-Chlorophenyl Thiourea	16	17	16	25	24	23	21	20	18	18	17	16
9	4(4'(3-P-Chlorophenyl Sydnonyl) Thiozolo-2-Amino p-Bromophenyl Thiourea	20	21	18	19	17	16	22	19	18	29	20	18
10	4(4'-(3-P-Chlorophenyl Sydnonyl) Thiozolo-2-Aminoallyl Thiourea	40	36	31	29	26	23	36	31	27	34	32	29
11	Sulphanilamide	20	18	16	18	17	16	20	19	18	4	--	--
12	Phenol	17	16	16	--	--	--	19	19	18	14	13	--
13	DMSO-0.1ml	--	--	--	--	--	--	--	--	--	--	--	--

CONCLUSION

The meso-ionic compounds, mainly the sydnones have got wide range of biological activities. To mention are anti-inflammatory, analgesic, antifungal, antimalarial antibacterial, hypotensive, diuretic antidepressant and sedative. This large spectrum of activities of the sydnones has made investigators to show deeper interest in these compounds. In the present study Thiazolo thiourea substituted sydnones were tested for antibacterial activities on gram positive and gram negative organisms in comparison with sulphanilamide and phenol. All these compounds have shown maximum zone of inhibition on these organisms at 100 micrograms concentration.

Compounds 2, 3 and 10 have shown promising results compared to sulphanilamide. Structural modifications at various positions, detail analysis of their antibacterial activities against wide range of

organisms by using different methods will give us further idea about their antibacterial properties. The data obtained by above mentioned techniques and details will definitely give a path to study the accurate and specific activities of these compounds, for their various biological properties.

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