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Antimicrobial activity of 1-Substituted-2-thio-(1H)-4-(3-phenylthio-carbamido-1-yl)-6-(1-substitutedguanidino-3-yl)-1,2-dihydro- S-triazine.

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Novel series of 1-substituted-2-thio-(1H)-4-(3-phenylthiocarbamido-1-yl)-6-(1-substituted-guanidino-3-yl)-1,2-dihydro-s-triazine [4a(i) to 4f(ii)] **has been obtained by the isomerisation of 2-(1- substitutedguanidino-3-yl)-4-(3-phenylthiocarbamido-1-yl)-6- substitutedimino-1,3,5-thiadiazine** [3a(i) to 3f(ii)] **in presence of ethanolic sodium bicarbonate solution, which have been obtained by basification of their hydrochlorides** [2a(i) to 2f(ii)] **which are synthesized by the interaction of 1-Formamidino- (N-_substitutedthioamido)-5-phenyl-2-thio-4-iminobiuret** (1a-f) **and N-aryl/alkylisocyanodichlorides. The latter were prepared initially by the condensation of N-aryl/alkylisothiocyanate with N-phenylformamidinoformamidino-thiocarbamide. The structure of all these compounds was established on the basis of elemental analysis, IR and PMR spectral data. All the synthesized compounds have been screened for their antimicrobial activity against both gram-positive and gram-negative human pathogens.**

Keywords: N-phenylformamidinoformamidinothiocarbamide, 1,3,5-thiadiazines, S-triazines, antimicrobial activity.

The literature survey reveals that the heterocyclic compounds containing nitrogen and nitrogen and sulphur have gain immense importance in human life due to their variety of applications in agricultural, medicinal, pharmacological and industrial value. It has also been found that the heterocyclic compounds containing S-triazine in the nucleus have been successfully tested against several pathogens and found that they possess insecticidal[1], medicinal, industrial, pharmaceutical, agricultural and bactericidal [2-4] properties. Some triazino compounds show remarkable biological activity[5-7] and help to find better alternative against drug.

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MATERIALS AND METHODS

Any chemical moiety which inhibit the growth of microorganism or kill it is called as antimicrobial activity.

All S-triazine compounds were screened for their antibacterial activity using cup plate diffusion method.[8-9] The bacterial organisms used include both gram positive and gram negative strains like *S. aureus*, *S. typhi, A. aerogenes, E. coli* and *B. subtilis*.

The medium was prepared by dissolving 28 gm of ingredients in one liter of distilled water and was sterilized at 121°C temperature and 15 lbs/inch pressure in an autoclave for 15 minutes. After sterilization it was cooled down to 50°C and poured into sterile petriplates and allowed to solidify. The media plates were then seeded with 24 hrs old active nutrient growth culture of the test organism in order to obtain lawn culture. The compounds were dissolved in 50%

dimethylformamide (DMF) solvent at fix concentration 100 µg/ml . To these added 2 drops of test solutions of synthesised compounds. Plane DMF solvent was used as control. The plates were then incubated at 37°C for 24 hrs. After incubation the zones of inhibition were recorded around the wells and result are cited in Table 1.

EXPERIMENTAL

All chemicals used were of analar grade. Aryl/alkylisothiocyanate, Aryl/alkylisocyanodichlorides were prepared according to literature method.[10] Melting points of all synthesized compounds were determine in open capillary. IR spectra were recorded on Perkin-Elmer spectrometer in the range 4000-400 cm^{-1} in KBr pellets. PMR spectra were recorded with TMS as internal standard using $CDCl_3$ and DMSO-d_6. TLC checked the purity of the compounds on silica gel-G plates with layer thickness of 0.3 mm.

Synthesis of 2-(1-phenylguanidino-3-yl)-4-(3-phenylthiocarbamido-1-yl)-6-phenylimino-1,3,5-thiadiazine [3a(i)].

1-Formamidino-(N-phenylthioamido)-5-phenyl-2-thio-4-iminobiuret (0.01m) (1a) was suspended in carbon tetrachloride medium (25ml). To this a solution of phenylisocyanodichlorides (0.01m) was added in 1:1 molar proportions. The reaction mixture was refluxed on water bath for 4 hr. During heating evolution of hydrogen chloride gas was observed and tested with moist blue litmus paper. Cooling the reaction mixture and distilled off excess solvent needle shape crystals were separated out. And crystallized from aqueous ethanol. Yield 82 %; m.p.272^{0}C and identified as of 2-(1-phenylguanidino-3-yl)-4-(3-phenylthiocarbamido-1-yl)-6-phenylimino-1,3,5-thiadiazine hydrochloride [2a(i)] , which on basification with aqueous ammonium hydroxide solution afforded free base [3a(i)]. It was recrystallised from aqueous ethanol. Yield 72% m.p.265^{0}C.IR spectrum of compound showed ν(N-H) 3396.6 cm^{-1}, ν(CH-Ar) 2924.7 cm^{-1}, ν(C=N) 1661.1 cm^{-1}, ν(C=S) grouping 1101.1 cm^{-1}, ν(C-S) 778.2 cm^{1}. The PMR spectrum of compound showed signals due to Ar-NH protons at δ 6.16 ppm, N-H protons at δ 4.00 ppm and Ar-H protons at δ 7.22 ppm. Similarly others compounds [2a(ii) to 2f (ii)] [3a(ii) to 3f(ii)] were synthesized by above mention method.

Synthesis of 1-phenyl-2-thio- (1H)-4-(3-phenylthiocarbamido-1-yl)-6-(1-phenylguanidino-3-yl)-1,2-dihydro-s-triazine [4a(i)].

2-(1-phenylguanidino-3-yl)-4-(3-phenylthiocarbamido-1-yl)-6-phenylimino-1,3,5-thiadiazine [3a(i)] was suspended in 5% aqueous ethanolic sodium bicarbonate solution and refluxed for 2 hr. during heating reactant went in to solvent. Then excess solvent was distilled off, a needle shape pale yellow crystals were separated out and crystallized from glacial acetic acid. Yield 61% m.p.258^{0}C and identified 1-phenyl-2-thio-(1H)-4-(3-phenylthiocarbamido-1-yl)-6-(1-phenylguanidino-3-yl)-1,2-dihydro-S-triazine [4a(i)]. IR spectrum of compound showed ν(N-H) 3387.3 cm^{-1}, ν(CH-Ar) 3147.7 cm^{-1}, ν(C=N) 1666.3 cm^{-1}, ν(C-N) 1305.3 cm^{-1}, ν(C=S) grouping 1178.0 cm^{-1}, ν(C-S) 746.9 cm^{-1}. The PMR spectrum of compound showed signals due to NH protons at δ 3.21-3.42 ppm, Ar-NH protons at δ 7.87-8.60 ppm, Ar-H protons at δ 6.8-7.2 and the signal at δ 2.51-2.53 ppm is due to moisture in DMSO-d_6. and δ 2.24 ppm is due to DMSO. Similarly others compounds [4a(ii) to 4f(ii)] were synthesized by above mention method and enlisted in Table 1.

RESULTS AND DISCUSSION

All the bacterial organisms studied are human pathogens. The activity is compared with standard drug ciprofloxacine at the same concentration. From the experimental data it has been observed that the compounds 4a(i), 4a(ii), 4d(i), 4e(i) and 4f(i) shows high activity against *S. typhi* and compounds 4b(i), 4c(i), 4d(ii) and 4f(ii) shows moderate activity while remaining compounds are inactive against same pathogen. Similarly compound 4a(ii), 4b(ii) and 4d(ii) shows high activity while compound 4c(i), 4e(ii) and 4f(ii) shows moderate activity and remaining compounds 4b(i), 4c(ii), 4d(ii) and 4e(i) shows weakly activity against *S. aureus.*

In case of Gram-negative bacteria like *E. coli* the compound 4a(i), 4b(ii) and 4f(ii) shows moderately activity while compound 4e(ii) shows

Table 1. Physical data and antimicrobial activity of the compounds [3a(i) to 3f(ii)]*

Compd.	R	R_1	Yield	m.p. (^{0}C)	Gram Positive		Gram Negative		
					S. aureus	*B. subtilis*	*A. aerogenes*	*E. coli*	*S. typhi*
[4a(i)]	Phenyl	Phenyl	61	258	-	+	++	++	+++
[4a(ii)]	Phenyl	Ethyl	59	246	+++	++	+	-	+++
[4b(i)]	*p*-Chlorophenyl	Phenyl	65	249	+	+++	-	+	++
[4b(ii)]	*p*-Chlorophenyl	Ethyl	69	244	+++	+	+	++	-
[4c(i)]	*p*-Tolyl	Phenyl	72	262	++	-	++	+	++
[4c(ii)]	*p*-Tolyl	Ethyl	69	267	+	-	-	-	+
[4d(i)]	Ethyl	Phenyl	73	256	+++	-	+++	+	+++
[4d(ii)]	Ethyl	Ethyl	66	231	+++	++	-	-	++
[4e(i)]	Methyl	Phenyl	65	221	+	-	+	+	+++
[4e(ii)]	Methyl	Ethyl	77	209	++	-	-	+++	+
[4f(i)]	*t*-Butyl	Phenyl	70	219	++	++	-	-	+++
[4f(ii)]	*t*-Butyl	Ethyl	71	252	+	-	+	++	++

* All Compounds gave satisfactory C, H, N, and S analysis.
(-) = Inactive (Less than 10 mm) (+) = Weakly Active (10-14 mm)
(++) = Moderately Active (15-18 mm) (+++) = Highly Active (19-35 mm)

high activity against the same bacteria. The compound 4b(i) and 4d(i) were effective against the *B. subtilis* and *A. aerogenes* organisms respectively. As newly s-triazines shows remarkable antimicrobial activity, these compounds can be easily used as alternative drugs for the treatment of diseases like typhoid.

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