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In vitro anti-microbial activity of some 2-(substituted)amino-5-aryl-1,3,4-thiadiazoles

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ABSTRACT

In vitro anti-microbial activity of the 28 synthesized compounds [2-(Substituted)amino-5-aryl-1,3,4-thiadiazoles)] was carried out by Liquid dilution method. For antibacterial study three Gram negative strains i.e., Proteus mirabilis (MTCC 425), Shigella flexneri (MTCC 1457), Pseudomonas aeruginosa (MTCC 424) and two Gram positive strains i.e., Staphylococcus aureus (MTCC 96) and Bacillus subtilis (MTCC 619) were taken. For antifungal study Aspergillus niger (MTCC 1344) and Candida albicans (MTCC 227) were used. Results indicate that compounds studied for anti-bacterial and anti-fungal activity, appreciable activity was shown by compounds 24, 10, 11 and 2 against P. aeruginosa i.e., 24, 36, 54 and 58 µg/ml concentrations (MIC) respectively. In addition compound 2, 3, 17, 9, 10 and 11 showed activity against S. flexneri. Compound 20 showed activity against P. mirabilis. Compound 24 and 26 showed activity against B. subtilis. It was observed that none of the compounds inhibit the growth of the bacterial strain S. aureus. None of the compounds showed any anti-fungal activity.

Keywords: 1,3,4-thiadiazoles, Anti-microbial activity.

INTRODUCTION

1,3,4-Thiadiazole, the heterocyclic nucleus of the present work is a versatile pharmacophore, which exhibits a wide variety of biological activities. A few of them, which are worthy of mention are CNS depressants [1,2], hypoglycaemic [3,4], anti-bacterial [5-11], anti-fungal [12,13] and anti-tumor activities [14].

In continuation of earlier work on 1,3,4-thiadiazoles [15-17], synthesized 2-(Substituted) amino-5-aryl-1,3,4-thtiadiazoles were evaluated for assessing their potential as antimicrobial agents. Liquid dilution method [18,19] was used for determining the minimum inhibitory concentration (MIC) of the synthesized compounds, for a particular organism, which is the lowest concentration that prevents growth of that organism. In liquid dilution method, graded concentrations of test compounds are prepared in broth and accurate volume of a suspension of the organism is added to each. After shaking to mix, the dilutions are incubated for the specified period at the optimum temperature, and examined for growth. The MIC is then supposed to lie between the highest concentration showing growth and the lowest concentration inhibiting growth. The exact value of MIC is then determined by repeating the procedure using a range of dilution between these two values.

MATERIALS AND METHODS

Experimental

1,3,4-Thiadiazoles: The synthesized compounds, 2-(Substituted)amino-5-aryl-1,3,4-thtiadiazoles [15-17] were evaluated for antimicrobial activity [20-24] in order to find out their potential as antimicrobial agents.

Microbial Strains: For antibacterial study *Proteus mirabilis*, *Shigella flexneri*, *Pseudomonas aeruginosa* (Gram negative strains), *Staphylococcus aureus* and *Bacillus subtilis* (Gram positive strains) were taken. *Aspergillus niger* and *Candida albicans* were used for antifungal studies. Microorganisms used for examining antimicrobial activity were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India (Table 1).

Table 1: List of microbial strains* used in the present study

S No.	MTCC No.	Microbial Strain	Incubation temp. (^{o}C)	Incubation period
1	425	*Proteus mirabilis*	37	24 hrs.
2	1457	*Shigella flexnari*	37	24 hrs.
3	424	*Pseudomonas aeruginosa*	37	24hrs.
4	96	*Staphylococcus aureus*	30	48 hrs.
5	619	*Bacillus subtilis*	26	24 hrs.
6	1344	*Aspergillus niger*	25	5 days
7	227	*Candida albicans*	25	48 hrs.

** The strains were purchased from Institute of Microbial Technology (IMTECH), Chandigarh.*

Culture Medium: Nutrient broth and agar were used as culture media [25] for bacterial strains. Czapek Yeast Extract/agar and Malt Yeast/agar were used for *A. niger* and *C. albicans* respectively. Stock culture of corresponding microbial strains was prepared from the original lyophilized strains using method of IP [26]. For the preparation of agar media, 2 % agar was added to the formulae.

Sterilization: Sterilization of media, boiling tubes and other materials was done by autoclaving them at 15 lb/sq. inch pressure for 20 minutes.

Incubation: Incubation was done in an electrically heated incubator at the temperature and time period specified in Table 1.

Preparation of Test Organism Suspension: Test organisms[11] were transferred on slants of nutrient agar medium, under asceptic condition. Then incubation was done as mentioned in Table 1. The surface of the slants were washed with 3 mL of saline solution and transferred onto a large agar surface of medium, in a one litre conical flask containing 250 mL of nutrient agar and incubated. After incubation, the surface of the medium was washed 50.0 mL of saline solution and transferred the washings into other conical flask. Then it was stored in a refrigerator.

Preparation of Solutions of Standard Drug: A stock solution of Norfloxacin (1 mg/mL) was prepared in dimethylformamide (DMF). The required concentration of the standard drug was prepared by appropriately diluting the stock solution with DMF.

Preparation of Solutions of Synthesized Compounds: A stock solution of each synthesized compound (1 mg/mL) was prepared in dimethylformamide (DMF). The required concentrations of the synthesized compounds were prepared by appropriately diluting the stock solution with DMF.

Determination of Minimum Inhibitory Concentration (MIC) of the Standard Drug and Synthesized Compounds: Different concentrations of Norfloxacin (0.5 mL) was added to a set of ten boiling tubes containing 9.5 mL of sterilized nutrient broth aseptically under Laminar flow bench, so that the final concentration of drug solution ranges from 2 to 20 µg/mL. Test organism suspension (0.1 mL) was added to each tube under similar condition. Three control tubes were also taken. One is containing only nutrient broth to check the sterility of the media. Second containing nutrient broth and 0.5 mL of solvent, for confirming, if there is any interaction between the solvent molecules and the ingredients of the medium. Third containing nutrient broth, solvent and 0.1 ml of test organism suspension to check the suppression of growth of the strain if any due to solvent i.e., DMF.

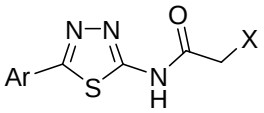
Tubes were then incubated in an incubator. After incubation, 0.5 mL of formaldehyde solution was added to arrest the growth of test organism further and then examined for any inhibition. The concentration inhibiting the growth of the test organism and that allowing the growth of the test organism were noted. The exact MIC was determined by repeating the experiment using a range of dilution between these two values.

Same procedure as for Norfloxacin was followed with the concentration of the synthesized compounds in the range of 20-200 µg/mL. The final results of MIC determination of standard drug and synthesized compounds are given in Table 2.

RESULTS AND DISCUSSION

Microorganisms used in the present study for antimicrobial study is shown in Table 1. Results of the antimicrobial activity (MIC) of the synthesized compounds i.e., 1,3,4-thiadiazoles and standard drugs is shown in Table 2.

Table 2: Anti-microbial activity of synthesized compounds



S No.	Ar	X	MIC* (µg/mL)				
			S. flexneri **MTCC 1457**	***P. mirabilis*** **MTCC 425**	***P.aeruginosa*** **MTCC 424**	***B. subtilis*** **MTCC 619**	***S. aureus*** **MTCC 96**
1	C_6H_5-	Dicyclohexylamino	>200	>200	>200	>200	>200
2	.,,..	Morpholino	**56**	>200	**58**	>200	>200
3	.,,..	4-methyl piperidino	96	>200	80	>200	>200
4	.,,..	Piperidino	>200	>200	>200	>200	>200
5	.,,..	4-methyl piperazino	>200	>200	94	>200	>200
6	.,,..	Pyrrolidino	>200	>200	192	>200	>200
7	.,,..	Pyrrolidine-2-one-1-yl	>200	>200	>200	>200	>200
8	*p*-$CH_3OC_6H_4$-	Dicyclohexylamino	>200	>200	>200	>200	>200
9	.,,..	Morpholino	124	>200	84	>200	>200
10	.,,..	4-methyl piperidino	130	>200	**36**	>200	>200
11	.,,..	Piperidino	138	>200	**54**	>200	>200
12	.,,..	4-methyl piperazino	>200	>200	86	>200	>200
13	.,,..	Pyrrolidino	>200	>200	>200	>200	>200
14	.,,..	Pyrrolidine-2-one-1-yl	>200	>200	>200	>200	>200
15	*p*-$CH_3C_6H_4$-	Dicyclohexylamino	>200	>200	>200	>200	>200
16	.,,..	Morpholino	196	>200	70	>200	>200
17	.,,..	4-methyl piperidino	108	>200	72	>200	>200
18	.,,..	Piperidino	>200	>200	>200	>200	>200
19	.,,..	4-methyl piperazino	>200	>200	>200	>200	>200
20	.,,..	Pyrrolidino	>200	78	>200	>200	>200
21	.,,..	Pyrrolidine-2-one-1-yl	>200	>200	>200	>200	>200
22	*p*-ClC_6H_4-	Dicyclohexylamino	>200	>200	>200	>200	>200
23	.,,..	Morpholino	>200	>200	>200	>200	>200
24	.,,..	4-methyl piperidino	>200	>200	**24**	118	>200
25	.,,..	Piperidino	>200	>200	>200	>200	>200
26	.,,..	4-methyl piperazino	>200	>200	68	126	>200
27	.,,..	Pyrrolidino	>200	>200	>200	>200	>200
28	.,,..	Pyrrolidine-2-one-1-yl	>200	>200	>200	>200	>200
Norfloxacin (Standard drug)			4	6	10	16	4

For A. niger & C. albicans MIC 4 and 14 µg/mL respectively (Clotrimazole used as the standard drug for fungal strains).

A perusal of Table 2 shows that out of all the compounds studied for antibacterial and anti-fungal activity, appreciable activity was shown by compounds 24, 10, 11 and 2 against *P. aeruginosa* i.e., 24, 36, 54 and 58 µg/ml concentration (MIC) respectively. In addition compound 2, 3, 17, 9, 10 and 11 showed activity against *S. flexneri*. Compound 20 showed activity against *P. mirabilis*. Compound 24 and 26 showed activity against *B. subtilis*. It was observed that all the compounds failed to inhibit the growth of the bacterial strain *S. aureus.* None of the compounds showed any anti-fungal activity.

CONCLUSION

In vitro anti-microbial activity of the synthesized compounds [2-(Substituted)amino-5-aryl-1,3,4-thiadiazoles)] was carried out by Liquid dilution method. For antibacterial study three Gram negative strains i.e., *Proteus mirabilis* (MTCC 424), *Shigella flexneri* (MTCC 1457),

Pseudomonas aeruginosa (MTCC 424) and two Gram positive strains i.e., *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 619) were taken. For antifungal study *Aspergillus niger* (MTCC 1344) and *Candida albicans* (MTCC 227) were used. Results indicates that compounds studied for antibacterial and anti-fungal activity, appreciable activity was shown by compounds 24, 10, 11 and 2 against *P. aeruginosa* i.e., 24, 36, 54 and 58 µg/ml concentration (MIC) respectively. In addition compound 2, 3, 17, 9, 10 and 11 showed activity against *S. flexneri*. Compound 20 showed activity against *P. mirabilis*. Compound 24 and 26 showed activity against *B. subtilis*. It was observed that all the compounds failed to inhibit the growth of the bacterial strain *S. aureus.* None of the compounds showed any anti-fungal activity.

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