Anti-MRSA activity of aldehyde Schiff base N-aryl thiosemicarbazones

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ABSTRACT

Eight different newly synthesised aldehyde Schiff base N-aryl thiosemicarbazones, differing in R, R' groups, are tested on 25 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and a standard strain. Antibacterial activity was carried out by a well-diffusion method in concentrations of 15–500 µg/well. Compounds 1, 2, 3, 4 and 6 showed good inhibition of MRSA. Increasing concentration of the test compounds enlarged the inhibition zone. Determination of minimum inhibitory concentration (MIC) was carried out using a dilution susceptibility test in concentrations of 4–512 µg/mL of the medium. The lowest MIC value (16 µg/mL) was produced by compound 4.

KEY WORDS: Antibacterial agents.
Schiff bases.
Staphylococcus aureus.
Thiosemicarbazones.
Methicillin resistance.

Introduction

Staphylococcus aureus is an important cause of serious infection in hospitals and in the community. *S. aureus* is the most frequently isolated pathogen causing bloodstream infections, skin and tissue infections, and pneumonia.[1–3] Unfortunately, this pathogen is particularly efficient at developing resistance to antimicrobial agents. Since the first isolation of methicillin-resistant *S. aureus* (MRSA) in the United Kingdom in 1961, increasing rates of methicillin resistance among *S. aureus* strains have been a cause for concern, especially in developed countries.[4]

Currently, MRSA is a predominant and dangerous nosocomial pathogen. Unfortunately, infections caused by this organism are becoming more difficult to treat as further evolution of drug resistance occurs. Vancomycin has become the drug of choice for treating MRSA infection; however, treatment failures, adverse side effects and the emergence of vancomycin resistance are leading to urgent requirements for alternative anti-MRSA therapies.[5]

Linezolid is a new agent developed recently for Gram-positive infections, including MRSA. However, it is alarming to know that resistance to this drug is already developing.[6] Therefore, much attention is focused on the search for new antimicrobial agents.

In this study, eight different aldehyde Schiff base thiosemicarbazones, differing in R, R' groups, are synthesised and screened for their biological activity against MRSA.

Materials and methods

Isolate collection

Twenty-five clinical isolates of MRSA and a standard strain (in-house PCR confirmed) were obtained from the clinical microbiology diagnostic laboratory, PSG Institute of Medical Science & Research and PSG Hospitals, Coimbatore, Tamil Nadu, India. They were isolated from different clinical samples (e.g., pus, tracheal aspirate, sputum, blood, throat secretions and wound swabs). Sensitivity testing for these isolates was performed using Kirby-Bauer's disc-diffusion method using oxacillin (1 µg) and cephoxitin (30 µg).

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Stock culture and preparation of inoculum

Two to three colonies from a bacterial culture were stab-inoculated in semi-solid Mueller Hinton agar (MHA) in test tubes and incubated for 16–24 h at 37°C and stored at 4°C. An inoculum was prepared by subculturing the isolate on MHA from the stock cultures and incubated at 37°C for 16–24 h. One colony of the bacterial subculture was inoculated into 4 mL peptone water and incubated at 37°C for 2–6 h. The inoculum was standardised using a 0.5% McFarland standard and inoculated on sterile medium to make a lawn culture.[7]

Test compounds

Eight test compounds (1–8) were synthesised in the Department of Chemistry, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, India.

Synthesis of N-aryl thiosemicarbazones

N-aryl thiosemicarbazones were synthesised in two stages. Stage I was the preparation of N-aryl thiosemicarbazides. Stage II was the preparation of N-aryl thiosemicarbazones. Molecular weights of the prepared compounds were determined by a Rast micro method, the melting points were determined, and the infrared (IR) spectra were recorded.

Preparation of N-aryl thiosemicarbazide

Aromatic primary amine (0.1 mol/L) was dissolved in 50 mL 95% ethanol, and 20 mL ammonium hydroxide was added.

After cooling the reaction mixture below 30°C, 8 mL carbon disulphide (CS_2) was added slowly for 15 min with shaking. After the complete addition of CS_2, the solution was allowed to stand for an hour, and then 0.1 mol/L sodium chloroacetate solution was added. During this addition, the reaction was found to be exothermic and colour change was observed from red to yellow/green. Then, 20 mL 50% solution of hydrazine hydrate was added. The mixture was warmed gently and kept overnight. The thiosemicarbazide product was filtered and recrystallised from ethanol (Fig. 1).

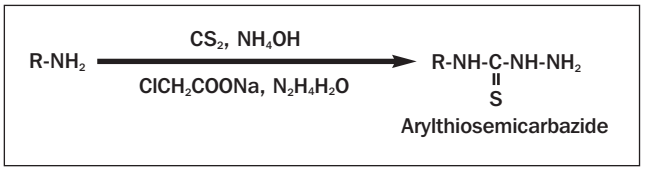

Fig. 1. Preparation of N-aryl thiosemicarbazide.

Preparation of N-aryl thiosemicarbazones
Aromatic aldehyde (0.01 mol/L) and 0.01 mol/L thiosemicarbazide were dissolved in 50 mL ethanol in a round-bottomed flask. The mixture was refluxed for 3–4 h on a water bath. The product was cooled, filtered and recrystallised from ethanol (Fig. 2).

Preparation of the stock solution of the test compound
Stock solution (1%) of each test compound was prepared using the solvent dimethyl formamide (DMF). This stock was diluted serially to obtain concentrations of 15.6 µg, 31.2 µg, 62.5 µg, 125 µg, 250 µg and 500 µg per 50 µL solvent.

Antibacterial assay by well-diffusion method
Antibacterial assay of the test compounds was performed by the well-diffusion method[8] using concentrations of 15.6–500 µg. The negative (DMF) and positive (vancomycin, 30 µg) controls were maintained. The standard MRSA strain was used for comparison. Triplicates were maintained.

Determination of minimum inhibitory concentration
Dilution susceptibility testing[9] was used to determine the MIC of the test compounds. The lowest concentration of test compound required to inhibit microorganisms was determined qualitatively and quantitatively. Concentrations of test compounds made were 4 µg, 8 µg, 16 µg, 32 µg, 64 µg, 128 µg, 256 µg and 512 µg per mL medium. A solvent control was kept. Triplicates were maintained.

Results

Effect of N-aryl thiosemicarbazones against MRSA strains
In antibacterial assay, test compounds 1, 2, 3, 4 and 6 showed significant inhibition of MRSA. Increasing the concentration

Table 1. Determination of MIC of compound 1 for MRSA strains.

No.	Laboratory No. of MRSA strain	Minimum inhibitory concentration* (µg/mL medium)								
		0†	4	8	16	32	64	128	256	512
1	89	+	+	+	+	+	+	+	–	–
2	124	+	+	+	+	+	+	+	–	–
3	168	+	+	+	+	+	+	+	–	–
4	224	+	+	+	+	+	+	+	–	–
5	370	+	+	+	+	+	+	+	–	–
6	378	+	+	+	+	+	+	+	–	–
7	359	+	+	+	+	+	+	+	–	–
8	492	+	+	+	+	+	+	+	–	–
9	354	+	+	+	+	+	+	+	–	–
10	306	+	+	+	+	+	+	+	–	–
11	249	+	+	+	+	+	+	+	–	–
12	324	+	+	+	+	+	+	+	–	–
13	258	+	+	+	+	+	+	+	–	–
14	344	+	+	+	+	+	+	+	–	–
15	369	+	+	+	+	+	+	+	–	–
16	48	+	+	+	+	+	+	+	–	–
17	74	+	+	+	+	+	+	+	–	–
18	25	+	+	+	+	+	+	+	–	–
19	101	+	+	+	+	+	+	+	–	–
20	402	+	+	+	+	+	+	+	–	–
21	62	+	+	+	+	+	+	+	–	–
22	191	+	+	+	+	+	+	+	–	–
23	332	+	+	+	+	+	+	+	–	–
24	408	+	+	+	+	+	+	+	–	–
25	161	+	+	+	+	+	+	+	–	–
26	Standard strain (in-house PCR confirmed)	+	+	+	+	+	+	+	–	–

*Triplicate observation, †Solvent control

of the test compounds increased the size of the inhibition zone. The diameters of the inhibition zones of the test compounds at 30 µg were comparatively greater than the inhibition zone diameter produced by vancomycin at the same concentration. Compounds 5, 7 and 8 did not show good inhibition of MRSA isolates (Fig. 3). The solvent control did not inhibit the MRSA isolates.

Determination of minimum inhibitory concentration
Minimum inhibitory concentrations (MIC90) for test compounds 1, 2, 3, 4 and 6 (Tables 1–5) were 256 µg, 64 µg, 32 µg, 16 µg and 32 µg, respectively (Table 6). The MIC90 of compound 4 was lowest (16 µg) compared to other compounds for all MRSA isolates tested (Fig. 4).

Discussion

Heterocyclic compounds have been the centre of study in drug design for the past four decades because of their potent antimicrobial activity, showing a broad spectrum of biological activity (e.g., antituberculous, antibacterial, antifungal, antiviral, anti-inflammatory and antineoplastic).[10–13] In the synthesis of heterocycles, several intermediates are separated and a few have been reported to have antimicrobial activity.[14] One such intermediate is the Schiff base thiosemicarbazones.

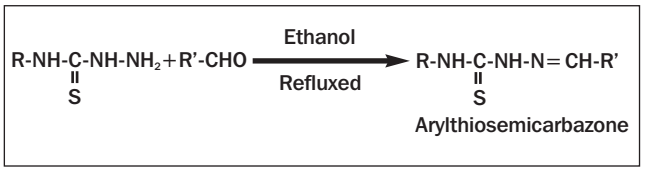

Fig. 2. Preparation of N-aryl thiosemicarbazones.

Schiff base is an important class of compounds in the medicinal and pharmaceutical field, showing a wide variety of biological activity (e.g., antibacterial, antifungal, anticancer and herbicidal).[15–21] Certain polymeric Schiff bases have been reported to possess antitumour activity.[22]

In the present study, eight different newly synthesised aldehyde Schiff base N-aryl thiosemicarbazones derived from primary amine were screened for their biological activity against MRSA. Antibacterial activity of N-aryl thiosemicarbazones on 25 clinical isolates and a standard strain of MRSA showed that five (compounds 1, 2, 3, 4 and 6) of the eight compounds were more effective than vancomycin (30 µg), the standard antibiotic control. The other three (compounds 5, 7 and 8) were less effective on MRSA isolates (Fig. 3).

From the MIC results, it was apparent that compound 4 had stronger anti-MRSA potency, being effective at half the concentration of the vancomycin (Table 1, Fig. 4).

Study on the structures of the eight N-aryl

Table 2. Determination of MIC of compound 2 for MRSA strains.

No.	Laboratory No. of MRSA strain	Minimum inhibitory concentration* (µg/mL medium)								
		0†	4	8	16	32	64	128	256	512
1	89	+	+	+	+	+	–	–	–	–
2	124	+	+	+	+	+	–	–	–	–
3	168	+	+	+	+	+	–	–	–	–
4	224	+	+	+	+	+	–	–	–	–
5	370	+	+	+	+	+	–	–	–	–
6	378	+	+	+	+	+	–	–	–	–
7	359	+	+	+	+	+	–	–	–	–
8	492	+	+	+	+	+	–	–	–	–
9	354	+	+	+	+	+	–	–	–	–
10	306	+	+	+	+	+	–	–	–	–
11	249	+	+	+	+	+	–	–	–	–
12	324	+	+	+	+	+	–	–	–	–
13	258	+	+	+	+	+	–	–	–	–
14	344	+	+	+	+	+	–	–	–	–
15	369	+	+	+	+	+	–	–	–	–
16	48	+	+	+	+	+	–	–	–	–
17	74	+	+	+	+	+	–	–	–	–
18	25	+	+	+	+	+	–	–	–	–
19	101	+	+	+	+	+	–	–	–	–
20	402	+	+	+	+	+	–	–	–	–
21	62	+	+	+	+	+	–	–	–	–
22	191	+	+	+	+	+	–	–	–	–
23	332	+	+	+	+	+	–	–	–	–
24	408	+	+	+	+	+	–	–	–	–
25	161	+	+	+	+	+	–	–	–	–
26	Standard strain (in-house PCR confirmed)	+	+	+	+	+	–	–	–	–

*Triplicate observation, †Solvent control

thiosemicarbazones revealed that compounds 1, 2, 3, 4 and 6 have either a –Cl or an $–NO_2$ group in one or both of their aromatic rings, while they are not present in the other three ineffective compounds. Presence of –Cl and/or $–NO_2$ groups in the ring is reported as one of the reasons behind the effective antimicrobial activity of Schiff base compounds.[23]

Compound 4 showed maximum inhibitory activity because it has an $–NO_2$ group on both the aromatic rings. The $–NO_2$ groups must be in planar with the aromatic rings so that they contribute effectively towards the inhibitory activity. This is proved by the fact that compound 3, with only one $–NO_2$ group, showed lower anti-MRSA activity than did compound 4. □

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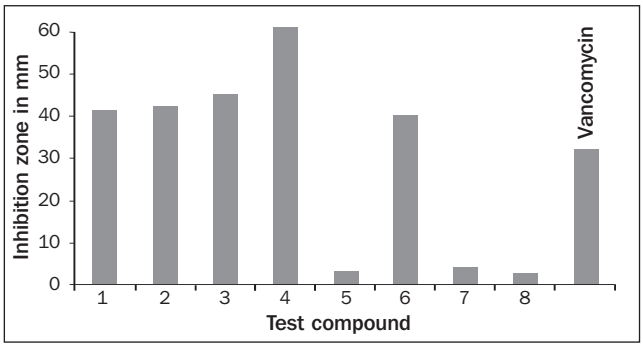

Fig. 3. Antibacterial activity of eight different N-aryl thiosemicarbazones (30 µg) on MRSA isolates and comparison to vancomycin (30 µg). Values are average of triplicates of 25 MRSA clinical isolates and one standard strain.

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Table 3. Determination of MIC of compound 3 for MRSA strains.

No.	Laboratory No. of MRSA strain	Minimum inhibitory concentration* (µg/mL medium)								
		0†	4	8	16	32	64	128	256	512
1	89	+	+	+	+	+	–	–	–	–
2	124	+	+	+	+	+	–	–	–	–
3	168	+	+	+	+	+	–	–	–	–
4	224	+	+	+	+	+	–	–	–	–
5	370	+	+	+	+	+	–	–	–	–
6	378	+	+	+	+	+	–	–	–	–
7	359	+	+	+	+	+	–	–	–	–
8	492	+	+	+	+	+	–	–	–	–
9	354	+	+	+	+	+	–	–	–	–
10	306	+	+	+	+	+	–	–	–	–
11	249	+	+	+	+	+	–	–	–	–
12	324	+	+	+	+	+	–	–	–	–
13	258	+	+	+	+	+	–	–	–	–
14	344	+	+	+	+	+	–	–	–	–
15	369	+	+	+	+	+	–	–	–	–
16	48	+	+	+	+	+	–	–	–	–
17	74	+	+	+	+	+	–	–	–	–
18	25	+	+	+	+	+	–	–	–	–
19	101	+	+	+	+	+	–	–	–	–
20	402	+	+	+	+	+	–	–	–	–
21	62	+	+	+	+	+	–	–	–	–
22	191	+	+	+	+	+	–	–	–	–
23	332	+	+	+	+	+	–	–	–	–
24	408	+	+	+	+	+	–	–	–	–
25	161	+	+	+	+	+	–	–	–	–
26	Standard strain (in-house PCR confirmed)	+	+	+	+	+	–	–	–	–

*Triplicate observation, †Solvent control

Table 4. Determination of MIC of compound 4 for MRSA strains.

No.	Laboratory No. of MRSA strain	Minimum inhibitory concentration* (μg/mL medium)								
		0†	4	8	16	32	64	128	256	512
1	89	+	+	+	–	–	–	–	–	–
2	124	+	+	+	–	–	–	–	–	–
3	168	+	+	+	–	–	–	–	–	–
4	224	+	+	+	–	–	–	–	–	–
5	370	+	+	+	–	–	–	–	–	–
6	378	+	+	+	–	–	–	–	–	–
7	359	+	+	+	–	–	–	–	–	–
8	492	+	+	+	–	–	–	–	–	–
9	354	+	+	+	–	–	–	–	–	–
10	306	+	+	+	–	–	–	–	–	–
11	249	+	+	+	–	–	–	–	–	–
12	324	+	+	+	–	–	–	–	–	–
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16	48	+	+	+	–	–	–	–	–	–
17	74	+	+	+	–	–	–	–	–	–
18	25	+	+	+	–	–	–	–	–	–
19	101	+	+	+	–	–	–	–	–	–
20	402	+	+	+	–	–	–	–	–	–
21	62	+	+	+	–	–	–	–	–	–
22	191	+	+	+	–	–	–	–	–	–
23	332	+	+	+	–	–	–	–	–	–
24	408	+	+	+	–	–	–	–	–	–
25	161	+	+	+	–	–	–	–	–	–
26	Standard strain (in-house PCR confirmed)	+	+	+	–	–	–	–	–	–

*Triplicate observation, †Solvent control

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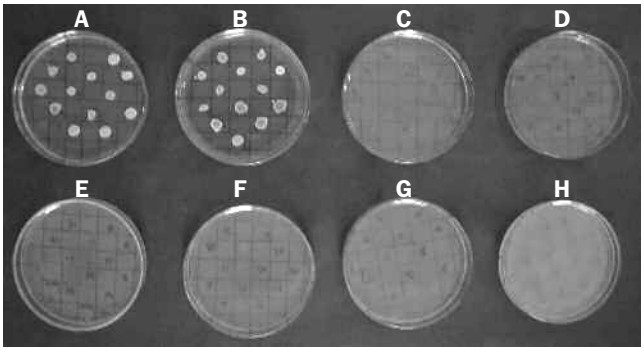

Fig. 4. Determination of minimum inhibitory concentration (MIC) of compound 4 on MRSA isolates (n=13). Concentration of the medium: A: 4 μg/mL, B: 8 μg/mL, C: 16 μg/mL, D: 32 μg/mL, E: 64 μg/mL, F: 128 μg/mL, G: 256 μg/mL and H: 512 μg/mL.

Table 5. Determination of MIC of compound 6 for MRSA strains.

No.	Laboratory No. of MRSA strain	Minimum inhibitory concentration* (μg/mL medium)								
		0†	4	8	16	32	64	128	256	512
1	89	+	+	+	+	–	–	–	–	–
2	124	+	+	+	+	–	–	–	–	–
3	168	+	+	+	+	–	–	–	–	–
4	224	+	+	+	+	–	–	–	–	–
5	370	+	+	+	+	–	–	–	–	–
6	378	+	+	+	+	–	–	–	–	–
7	359	+	+	+	+	–	–	–	–	–
8	492	+	+	+	+	–	–	–	–	–
9	354	+	+	+	+	–	–	–	–	–
10	306	+	+	+	+	–	–	–	–	–
11	249	+	+	+	+	–	–	–	–	–
12	324	+	+	+	+	–	–	–	–	–
13	258	+	+	+	+	–	–	–	–	–
14	344	+	+	+	+	–	–	–	–	–
15	369	+	+	+	+	–	–	–	–	–
16	48	+	+	+	+	–	–	–	–	–
17	74	+	+	+	+	–	–	–	–	–
18	25	+	+	+	+	–	–	–	–	–
19	101	+	+	+	+	–	–	–	–	–
20	402	+	+	+	+	–	–	–	–	–
21	62	+	+	+	+	–	–	–	–	–
22	191	+	+	+	+	–	–	–	–	–
23	332	+	+	+	+	–	–	–	–	–
24	408	+	+	+	+	–	–	–	–	–
25	161	+	+	+	+	–	–	–	–	–
26	Standard strain (in-house PCR confirmed)	+	+	+	+	–	–	–	–	–

*Triplicate observation, †Solvent control

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Table 6. Determination of MIC for five N-aryl thiosemicarbazones against MRSA strains.

Test compound	Concentration of compound in medium at MIC90 (μg/mL)								
	0*	4	8	16	32	64	128	256	512
1	+	+	+	+	+	+	+	–	–
2	+	+	+	+	+	–	–	–	–
3	+	+	+	+	–	–	–	–	–
4	+	+	+	–	–	–	–	–	–
6	+	+	+	+	–	–	–	–	–

*Solvent control, MRSA clinical isolates (n=25), standard strain (n=1)