Synthesis and Biological Evaluation of Novel Thiazole Hydrazines as Antimicrobial and Antimalarial Agents

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Abstract: Synthesis of some novel thiazole hydrazine derivatives from thiosemicarbazones of salicylaldehyde and 5-chlorosalicylaldehyde with phenacyl bromide in ethanol under reflux condition is reported. The synthesized compounds were characterized by spectral analysis and further screened against - S. aureus, E. coli, P. aeruginosa, S. pyogenus bacteria, and against C. albicans, A. clavatus, and A. niger fungal strains. Most of the compounds were active against E. coli and C. albicans. Antimalarial screening against Plasmodium falciparum showed moderate to the good activity of the synthesized compounds but less than the standard quinine. One of the synthesized compounds (4c) exhibited promising antimalarial activity against Plasmodium falciparum with IC50 close to the standard quinine.

Keywords: thiazole hydrazines; antimicrobial; antimalarial.

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1. Introduction

Thiazole heterocycles are well known for biological activities [1]. Thiazole and thiazolidinone rings are the core structures in various synthetic pharmaceuticals that are associated with diverse biological activities such as antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, analgesic and calcium antagonistic activities [2-3]. Many compounds with a thiosemicarbazone moiety exhibit significant biological properties because the thiourea unit (NHCSNH), which can easily form chelation with metal ions like iron, zinc, magnesium, etc. [4]. In particular, thiazolyl-hydrazine derivatives are an important class of heterocyclic compounds obtained by incorporating the hydrazino-thiazolyl group and the phenothiazine structures in a single molecule. Thus, thiazoles containing hydrazone functionality are of increasing interest due to their significant role in medicinal chemistry.

Infectious diseases have always remained severe major health hazards and significant problems of the world population [5-6]. The rapid development of microbial resistance to the existing antimicrobial drugs is the prime cause of the global issue. It is an important challenge to the medicinal chemists for the development of better antimicrobial drugs having a different mechanism of action to combat the problem of multidrug resistance [7]. The currently used powerful antibiotics may not be suitable in the future when used to treat some severe infections. In many cases, resistance to synthetic antimicrobial agents is due to variations displayed by
individual microorganisms on account of genetic changes and mutations. As a consequence, the search for new antimicrobial agents becomes inevitable [8].

Malaria has remained a common and life-threatening challenge all over the world, particularly in Sub-Sahara and Africa, which can be transmitted through international travelers also [9]. In 2017, the World Health Organization (WHO) declared around 219 million cases of malaria worldwide, with an increase of 2 million cases per year [10]. Among the different species of malaria, Plasmodium falciparum is mainly responsible for the disease, which may result in death within hours or few days of infection, particularly in people with low immunity, including children and pregnant women [11]. In pregnant women, it may lead to miscarriage, developmental disabilities, and other severe problems in newly born babies. Although the number of malarial cases has been reduced to around 35%, recent literature reveals a slow plateauing in the disease, emphasizing the development of antimalarial agents as a prevention strategy. Thus, in continuation of our previous work for the development of bioactive entities [12-13], in the present work, we report the synthesis, characterization, the antimicrobial, and antimalarial activity of thiazoles containing salicylaldehyde and 5-chlorosalicylaldehyde.

![Scheme 1. General scheme for the synthesis of thiazoles.](https://nanobioletters.com/)

**Table 1.** Yields and melting points of the synthesized thiazoles.

| Entry | R  | R'   | Product | Comp. | Yield (%) | M. P. (°C) |
|-------|----|------|---------|-------|-----------|------------|
| 1.    | H  | H    | ![Product Image](https://nanobioletters.com/) | 4a    | 82        | 204-206    |
| 2.    | H  | 4-OMe| ![Product Image](https://nanobioletters.com/) | 4b    | 85        | 190-192    |
| Entry | R | R^1 | Product | Comp. | Yield\(^\circ\) (%) | M. P. (°C) |
|-------|---|-----|---------|-------|-------------------|------------|
| 3.    | H | 4-F | ![image](https://doi.org/10.33263/LIANBS101.18461855) | 4c    | 82                | 195-197    |
| 4.    | H | 4-Cl | ![image](https://doi.org/10.33263/LIANBS101.18461855) | 4d    | 80                | 192-194    |
| 5.    | H | 4-NO₂ | ![image](https://doi.org/10.33263/LIANBS101.18461855) | 4e    | 80                | 194-196    |
| 6.    | H | Naphthyl | ![image](https://doi.org/10.33263/LIANBS101.18461855) | 4f    | 85                | 205-207    |
| 7.    | 5-Cl | H | ![image](https://doi.org/10.33263/LIANBS101.18461855) | 4g    | 83                | 193-195    |
| 8.    | 5-Cl | 4-OMe | ![image](https://doi.org/10.33263/LIANBS101.18461855) | 4h    | 88                | 188-190    |
| 9.    | 5-Cl | 4-F | ![image](https://doi.org/10.33263/LIANBS101.18461855) | 4i    | 82                | 208-210    |
| 10.   | 5-Cl | 4-Cl | ![image](https://doi.org/10.33263/LIANBS101.18461855) | 4j    | 82                | 188-190    |
Entry | R      | R¹      | Product | Comp. | Yield (%) | M. P. (°C) |
-------|--------|---------|---------|-------|-----------|------------|
11.    | 5-Cl   | 4-NO₂  | ![Product](image) | 4k    | 87        | 202-204    |
12.    | 5-Cl   | Naphthyl | ![Product](image) | 4l    | 90        | 210-212    |

*Yields isolated for the reaction of salicylaldehyde (3 mmol), thiosemicarbazide (3 mmol), and phenacyl bromide (3 mmol) in EtOH (5 mL) under reflux.

2. Materials and Methods

The chemicals were procured from Sigma Aldrich, or SD fine Ltd. Progress of the reaction was monitored on silica gel precoated F254 Merck made TLC plates. The developed TLC plates were examined under ultra-violet light. Melting points were recorded on a digital melting point apparatus in capillaries open at one end and were uncorrected.$^1$H NMR spectra were recorded on a 400-MHz Bruker Advance NMR spectrometer. Chemical shifts were reported in terms of δ ppm units using tetramethylsilane as the internal standard. The following abbreviations were used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

2.1. General procedure for the synthesis of thiazole hydrazines (4a-4l):

A mixture of salicylaldehyde (3 mmol) or 5-chlorosalicylaldehyde (3 mmol) and thiosemicarbazide (3 mmol) was refluxed in ethanol (3 mL) in the presence of 2-3 drops of HCl for 3 h. The resulting solid was filtered off, washed with cold ethanol (2 mL), and dried to get thiosemicarbazone. Further, the thiosemicarbazone was refluxed with an equimolar amount of different phenacyl bromide in ethanol (3 mL). The reaction progress was monitored by TLC (30% Ethyl acetate: n-Hexane) indicated completion of the reaction after 4 h. Further, the reaction mass was poured onto crushed ice, basified with an aqueous saturated cold solution of sodium bicarbonate, and then filtered off. The resulting solid was purified by recrystallization from ethanol and characterized by $^1$H NMR and mass spectral data.

2.1.1. Spectral data.

Spectral data of the synthesized compounds is mentioned below:
Thiazole hydrazine(4a): Yellow solid, Yield = 82%; Melting point: 204-206 °C; $^1$H NMR (400 MHz, DMSO-d₆): δ ppm 6.9 (q, 2H), 7.2 (t, 1H), 7.3 (t, 2H), 7.43 (t, 2H), 7.65 (d, 1H), 7.8 (d, 2H), 8.34 (s, 1H), 10.1 (br, s, 1H); Mass: ESIMS = 296.1013 (M+1)$^+$. Thiazole hydrazine(4b): Yellow solid, Yield = 85%; Melting point: 190-192 °C; $^1$H NMR (400 MHz, DMSO-d₆): δ ppm 3.59 (s, 3H), 6.9 (q, 2H), 6.96 (d, 2H), 7.1 (s, 1H), 7.2 (t, 1H), 7.62 (d, 1H), 7.79 (d, 2H), 8.33 (s, 1H), 10.2 (br, s, 1H); Mass: ESIMS = 326.1433 (M+1)$^+$. 

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Thiazole hydrazine (4c): Yellow solid, Yield = 82%; Melting point: 195-197°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 6.9 (q, 2H), 7.22 (q, 3H), 7.30 (s, 1H), 7.64 (d, 1H), 7.9 (t, 2H), 8.34 (s, 1H), 10.1 (br, s, 1H), 12.1 (br, s, 1H); Mass: ESIMS = 314.1319 (M+1)+.

Thiazole hydrazine (4d): Yellow solid, Yield = 80%; Melting point: 192-194°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 6.9 (q, 2H), 7.2 (t, 1H), 7.3 (s, 1H), 7.47 (d, 2H), 7.64 (d, 1H), 7.88 (d, 2H), 8.34 (s, 1H), 10.1 (s, 1H), 12.1 (br, s, 1H); Mass: ESIMS = 330.1234 (M+1)+.

Thiazole hydrazine (4e): Yellow solid, Yield = 80%; Melting point: 194-196°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 6.9 (q, 2H), 7.23 (t, 1H), 7.66 (d, 2H), 7.73 (t, 1H), 8.17 (d, 1H), 8.34 (t, 2H), 8.68 (s, 1H), 10.1 (s, 1H), 12.2 (s, 1H); Mass: ESIMS = 341.1639 (M+1)+.

Thiazole hydrazine (4f): Yellow solid, Yield = 85%; Melting point: 205-207°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 6.9 (q, 2H), 7.22 (t, 1H), 7.5 (m, 3H), 7.67 (d, 1H), 7.9 (m, 3), 8.0 (d, 1H), 8.39 (d, 2H), 8.68 (s, 1H), 10.1 (br, s, 1H), 12.2 (br, s, 1H); Mass: ESIMS = 346.1946 (M+1)+.

Thiazole hydrazine (4g): Yellow solid, Yield = 83%; Melting point: 193-195°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 6.92 (d, 1H), 7.22 (d, 1H), 7.3 (t, 2H), 7.42 (t, 2H), 7.64 (d, 1H), 7.86 (d, 1H), 7.89 (t, 2H), 8.29 (s, 1H), 10.4 (br, s, 1H); Mass: ESIMS = 330.1323 (M+1)+.

Thiazole hydrazine (4h): Yellow solid, Yield = 88%; Melting point: 188-190°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 3.78 (s, 3H), 6.9 (d, 1H), 6.98 (d, 2H), 7.14 (s, 1H), 7.25 (d, 1H), 7.62 (d, 1H), 7.79 (d, 2H), 8.2 (s, 1H), 10.4 (br, s, 1H), 12.2 (br, s, 1H); Mass: ESIMS = 360.1657 (M+1)+.

Thiazole hydrazine (4i): Yellow solid, Yield = 82%; Melting point: 208-210°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 6.9 (s, 1H), 7.22 (m, 3H), 7.30 (s, 1H), 7.62 (d, 2H), 7.88 (m, 2H), 8.27 (s, 1H), 10.3 (br, s, 1H), 12.2 (br, s, 1H); Mass: ESIMS = 346.1431 (M+1)+.

Thiazole hydrazine (4j): Yellow solid, Yield = 82%; Melting point: 188-190°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 6.9-8.29 (m, 9H), 10.4 (br, s, 1H), 12.3 (br, s, 1H); Mass: ESIMS = 364.1431 (M+1)+.

Thiazole hydrazine (4k): Yellow solid, Yield = 87%; Melting point: 202-204°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 6.9 (d, 2H), 7.3 (q, 3H), 7.35 (s, 3H), 7.65 (d, 1H), 7.75 (q, 2H), 8.4 (s, 1H); Mass: ESIMS = 375.1772 (M+1)+.

Thiazole hydrazine (4l): Yellow solid, Yield = 90%; Melting point: 210-212°C; 1H NMR (400 MHz, DMSO-d6): δ ppm 6.93 (d, 1H), 7.26 (d, 1H), 7.5 (m, 3H), 7.6 (d, 1H), 7.9 (m, 3H), 8.0 (d, 1H), 8.31 (s, 1H), 8.39 (s, 1H), 10.4 (br, s, 1H), 12.3 (br, s, 1H); Mass: ESIMS = 380.1763 (M+1)+.

2.1.2. Biological activity.

The effectiveness of the synthesized thiazole hydrazines to act as antimicrobial agents was studied against four bacteria - *S. aureus* (MTCC 96), *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 1688), *S. pyogenes* (MTCC 442), and three fungi - *C. albicans* (MTCC 227), *A. clavatus* (MTCC 1323) and *A. niger* (MTCC 282) using broth dilution method [14]. Ampicillin and chloramphenicol were used as the standards for antibacterial activity, whereas griseofulvin, and nystatin were used as the standards for antifungal activity. The results were expressed in terms of Minimum Inhibitory Concentration (MIC) values. The minimum inhibitory concentration of a drug is the lowest sample concentration that inhibits the visible growth of the microbe.

The efficiency of synthesized thiazole hydrazines to act as antimalarial hits was studied in-vitro against *Plasmodium falciparum* in 96-well microtitre plate according to the reported method of Rieckmann and co-workers with minor modifications [15]. Quinine was used as the...
standard, and the results were reported in terms of IC<sub>50</sub> values. All the screening was performed at Microcare Laboratory, Surat, Gujarat, India.

3. Results and Discussion

For the synthesis of thiazole hydrazines, initially, a mixture of salicyaldehyde (3 mmol) or 5-chlorosalicylaldehyde (3 mmol) was refluxed with thiosemicarbazide (3 mmol) in the presence of concentrated HCl (2-3 drops) in ethanol (3 mL) under reflux condition. After 3 h, the resulting solid was filtered off, dried, and refluxed with phenacyl bromide (3 mmol) in ethanol (3 mL) for further 4 h. The progress of the reaction was monitored by Thin Layer Chromatography (TLC) using 30% ethyl acetate: n-hexane as the mobile phase. After completion of the reaction, the reaction mixture was cooled, basified with aqueous saturated sodium bicarbonate, filtered off to get thiazole hydrazine, which was further purified by recrystallization from ethanol and well-characterized using <sup>1</sup>H NMR and mass spectral data.

Alternatively, the thiazole hydrazines can be synthesized in a one-pot three-component manner as in our previous work [12]. However, with the present strategy, we found a more clean reaction profile and reduced reaction time by around 1 hour for the cyclization reaction. Hence with these optimized reaction conditions in hand, we employed salicyaldehyde or 5-chlorosalicylaldehyde, thiosemicarbazide, and various substituted phenacyl bromides for the synthesis of target compounds as shown in Scheme-1 (Table 1).

The synthesized compounds were screened for antimicrobial and antimalarial activity. Antibacterial activity was carried using the broth dilution method against <i>E. coli</i> (MTCC 443), <i>P. aeruginosa</i> (MTCC 1688), <i>S. aureus</i> (MTCC 96), <i>S. pyogenus</i> (MTCC 442) bacterial strains using Ampicillin and Chloramphenicol as the standard drugs; antifungal activity was studied against <i>C. albicans</i> (MTCC 227), <i>A. niger</i> (MTCC) 282, <i>A. clavatus</i> (MTCC 1323) fungal strains. Compounds (4b) and (4h) (MIC = 62.5 µg/mL) with methoxy group at the para position of the phenacyl bromide exhibited a better antibacterial activity against <i>E. coli</i> than the standard ampicillin (100 µg/mL). All the compounds showed less antibacterial activity against <i>P. aeruginosa</i> and <i>S. pyogenus</i> as compared to the standard chloramphenicol (Table 2). The compounds were less active against <i>S. aureus</i> than the standard chloramphenicol; but the thiazole hydrazines (4a), (4b), (4c), (4e), (4g), and (4k) were more potent than the standard ampicillin as they have lower MIC values than the standard (Figure 1).

The results of antifungal activity reveal that; almost all the synthesized compounds showed lower antifungal activity as compared to the standard Nystatin. However, compounds (4b), (4g), and (4j) had better antifungal activity (MIC values 250 µg/mL each) against <i>C. albicans</i> than the standard Griseofulvin (MIC = 500 µg/mL). The compounds were less active against the <i>A. niger</i> and <i>A. clavatus</i> fungal strains in comparison with both the standards (Table 3).

| Sr. No. | Entry | Minimal Inhibition Concentration [µg/mL] |
|--------|-------|----------------------------------------|
|        |       | <i>S. aureus</i> | <i>E. coli</i> | <i>P. aeruginosa</i> | <i>S. pyogenus</i> |
| 1      | 4a    | MTCC 96        | 125           | MTCC 443     | 100          | 200          | 200          |
| 2      | 4b    | MTCC 1688     | 125           | MTCC 442     | 62.5         | 100          | 125          |
| 3      | 4c    | MTCC 442      | 100           | MTCC 1688     | 125          | 200          | 125          |
| 4      | 4d    | MTCC 442      | 200           | MTCC 442     | 100          | 200          | 125          |
| 5      | 4e    | MTCC 442      | 100           | MTCC 442     | 100          | 62.5         | 125          |
Table 3. Antifungal activity of the synthesized compounds (4a-l).

| Sr. No. | Entry | C. albicans [µg/mL] | A. clavatus [µg/mL] | A. niger [µg/mL] |
|---------|-------|---------------------|---------------------|-----------------|
|         |       | MTCC 227            | MTCC 1323           | MTCC 282        |
| 1       | 4a    | 500                 | 1000                | 1000            |
| 2       | 4b    | 250                 | 500                 | 500             |
| 3       | 4c    | 500                 | >1000               | >1000           |
| 4       | 4d    | 500                 | 1000                | 500             |
| 5       | 4e    | 1000                | 500                 | 500             |
| 6       | 4f    | >1000               | 500                 | 500             |
| 7       | 4g    | 250                 | 1000                | 1000            |
| 8       | 4h    | 1000                | >1000               | >1000           |
| 9       | 4i    | 1000                | 1000                | 1000            |
| 10      | 4j    | 250                 | 1000                | 1000            |
| 11      | 4k    | 500                 | 1000                | 1000            |
| 12      | 4l    | >1000               | 250                 | 250             |
| 13      | Nystatin | 100                 | 100                 | 100             |
| 14      | Griseofulvin | 500                | 100                 | 100             |

Figure 1. Comparison of MIC values of thiazole hydrazines against *S. aureus* with ampicillin standard.
Figure 2. Antimalarial activity of thiazole hydrazines and the standard quinine.

Table 4. Antimalarial activity of the synthesized compounds (4a-l).

| Sr. No. | Compound | Plasmodium falciparum MeanIC50a |
|---------|----------|---------------------------------|
| 1       | 4a       | 1.02                            |
| 2       | 4b       | 0.96                            |
| 3       | 4c       | 0.60                            |
| 4       | 4d       | 0.84                            |
| 5       | 4e       | 1.10                            |
| 6       | 4f       | 1.16                            |
| 7       | 4g       | 0.95                            |
| 8       | 4h       | 1.06                            |
| 9       | 4i       | 0.75                            |
| 10      | 4j       | 0.79                            |
| 11      | 4k       | 0.95                            |
| 12      | 4l       | 1.03                            |
| 13      | Quinine  | 0.268                           |

* Mean values of experiments performed in duplicate.

The antimalarial activity was carried against *Plasmodium falciparum*; one of the species mainly responsible for malaria. The compound (4c) showed good antimalarial activity. The results of antimalarial activity revealed that all the compounds were moderately active but less potent than the standard quinine, which as graphically represented in (Figure 2, Table 4).

4. Conclusions

In summary, we report the synthesis, characterization, antimicrobial and antimalarial activity of some thiazole hydrazines derived from salicylaldehyde and 5-chlorosalicylaldehyde. Compounds (4b) and (4h) showed promising antibacterial activity against *E. coli* as they had lower MIC values in comparison with the standard ampicillin. Thiazole hydrazines (4a), (4b), (4c), (4e), (4g), and (4k) exhibited better antibacterial activity than the standard drug ampicillin. Compounds (4b), (4g), and (4j) were better in terms of antifungal activity against *C. albicans* having lower MIC values than the standard Griseofulvin. All the compounds were less active against *A. niger* and *A. clavatus*. The compounds exhibited moderate antimalarial activity but less than the standard quinine. Thus, the present studies reveal salicylaldehyde incorporated thiazole hydrazines as promising antibacterial and antimalarial entities highlighting their future significance in medicinal chemistry.
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Conflicts of Interest

The authors declare no conflict of interest.

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