Synthesis, antifungal and antibacterial activity of novel 1,2,4-triazole derivatives

Abstract

A large number of 1,2,4-triazole-containing ring system have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, central nervous system stimulants, antianxiety, and antimicrobial agents. To overcome the rapid development of drug resistance, new agents should preferably have chemical characteristics that clearly differ from those of existing agents. Thus led to the design and synthesize the new antimicrobial agents. A novel series of Schiff bases based on of 4-(benzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol scaffold was prepared by heating thiocarbohydrazide and substituted benzoic acid and subsequently, treating with substituted benzoaldehydes. Seventeen derivatives were synthesized and were biologically screened for antifungal and antibacterial activity. The newly synthesized derivatives of triazole showed antifungal activity against fungal species, Microsporum gypseum; and antibacterial activity against bacterial species, Staphylococcus aureus. It was observed that none of the compounds tested showed positive results for fungi Candida albicans fungi Aspergillus niger, nor against bacterial strain Escherichia coli. Strong antifungal effects were obtained for the synthesized compounds against M. gypseum and were superior or comparable to standard drug ketoconazole. Similarly, all of the synthesized compounds exhibit strong antibacterial activity against S. aureus and were superior or comparable to standard drug streptomycin. It was found that among the triazole derivatives so synthesized, six of them, showed antifungal activity superior to ketoconazole while one of them, showed antibacterial activity superior to streptomycin. Thus, these can be the potential new molecule as an antimicrobial agent.

Key words: Antibacterial activity, antifungal activity, Microsporum gypseum, Schiff bases, Staphylococcus aureus

INTRODUCTION

A large number of 1,2,4-triazole, a heterocyclic derivative exhibits important therapeutic activities such as antifungal,[1] anticonvulsant,[2] anti-tubercular,[3] antioxidant,[4] anti-inflammatory,[5] COX-2 inhibition,[6] anticancer,[7] and antimicrobial activity.[8] Furthermore, 1,2,4-triazole ring system has been incorporated into a wide variety of therapeutically interesting drug candidates like ribavirin (antiviral agent), rizatriptan (antimigraine agent) and fluconazole, itraconazole (an antifungal agent).[9] Thus, there is a need to explore these pharmacophores for the development of novel molecules with different activities.

Fungal and bacterial infections have become an important complication and major cause of mortality.
in immunocompromised individuals suffering from tuberculosis, cancer, AIDS, etc. Amphotericin B is the most frequently used drug in the treatment of systemic mycoses in spite of its toxic effect on humans. Other antifungals like azole derivatives (flucanazole, an orally active triazole agent, and itraconazole), allylamines, thiocarbamates, fluoropyrimidines are some agents actually working in patients with impaired resistance such as those who have AIDS. While these new compounds are often used in treatment of fungal infections, resistance to these drugs is increasing, moreover many of currently available drugs have undesirable side effects, which clearly indicates an urgent need for development of new antimicrobial agents.

Prompted by these observations, triazole derivatives may be the potential candidate to investigate as a safe antimicrobial agent, as these may not affect the host. All newly synthesized triazole derivatives were evaluated for their antifungal activity against fungi *Aspergillus niger*, *Aspergillus albicans*, *Escherichia coli* and *S. aureus*.

**MATERIALS AND METHODS**

Reagents, starting materials and solvents were purchased from common commercial suppliers. The melting points of synthesized compounds were determined by an open capillary method on a Veego digital melting point apparatus. Mass spectral analysis was carried out using Applied Biosystem QTRAP 3200 MS/MS system in ESI mode. The infra-red spectra of the synthesized compounds were recorded on Fourier transformer infra-red spectrophotometer Model Schimadzu 8400S using potassium bromide pellets. 1H NMR spectra were recorded on the Bruker NMR using DMSO-d6, tetramethylsilane as an internal standard.

**Experimental**

**General Procedure for Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (3a-3c)**

A mixture of substituted benzoic acid (0.01 M) and thiocarbohydrazide (0.01 M) was heated until it melted. The mixture was consistently maintained at 145°C for 40 min. The product obtained on cooling was treated with a sodium bicarbonate solution to neutralize the unreacted acid if any. The product was then washed with water and collected by filtration. The solid product was recrystallized from a mixture of ethanol and dimethylformamide.

**General procedure for the synthesis of 4-(benzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (5a-5q)**

To a suspension of substituted benzaldehyde (4) (0.2 M) in ethanol (1 ml), an equimolar amount of corresponding 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (3a-3c) was added. The suspension was heated until a clear solution was obtained. Then few drops of concentrated sulfuric acid were added, and the solution was refluxed for 6 h on a water-bath. The precipitated solid was filtered off and recrystallized from a mixture of dimethylformamide and ethanol.

**4-(4-bromobenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (5a)**

77% yield – M.P. =142–144°C, IR (KBr) v/cm: 3109.04, 2935.46, 1504.37, 1446.51. 1H NMR (DMSO-d6, 400 MHz): δ 7.26–7.92 (m, 1H), 10.07 (s, 1H), 13.72 (s, 1H). MS-API: [M + H]+360.02 (calculated 361) Anal. calculated for C13H12BrN3S: C, 49.87; H, 3.43; Br, 22.12; N, 15.53; S, 8.78.

**4-(2,4-dichlorobenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (5b)**

79% yield – M.P. =125–127°C, IR (KBr) v/cm: 3107.04, 2932.26, 1505.17, 1443.53. 1H NMR (DMSO-d6, 400 MHz): δ 7.33–7.88 (m, 1H), 10.42 (s, 1H), 13.86 (s, 1H). MS-API: [M + H]+350.02 (calculated 351) Anal. calculated for C13H12Cl2N3S: C, 49.53; H, 3.23; Cl, 20.59; N, 15.01; S, 8.28.

**4-(4-fluorobenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (5c)**

81% yield – M.P. =167–169°C, IR (KBr) v/cm: 3108.02, 2934.44, 1501.20, 1445.45. 1H NMR (DMSO-d6, 400 MHz): δ 7.18–7.93 (m, 1H), 10.02 (s, 1H), 13.12 (s, 1H). MS-API: [M + H]+340.08 (calculated 341) Anal. calculated for C13H13FN3S: C, 59.98; H, 4.36; F, 6.33; N, 18.65; S, 10.68.

**4-(3-chlorobenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (5d)**

78% yield – M.P. =155–158°C, IR (KBr) v/cm: 3106.00, 2931.46, 1502.12, 1436.13. 1H NMR (DMSO-d6, 400 MHz): δ 7.44–7.92 (m, 1H), 10.22 (s, 1H), 13.89 (s, 1H). MS-API: [M + H]+313.05 (calculated 314) Anal. calculated for C13H12Cl2N3S: C, 56.87; H, 4.14; Cl, 11.19; N, 17.68; S, 10.12.

**4-(4-chlorobenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (5e)**

82% yield – M.P. =192–195°C, IR (KBr) v/cm: 3112.08, 2938.26, 1514.17, 1426.25. 1H NMR (DMSO-d6, 400 MHz): δ 7.42–7.97 (m, 1H), 10.17 (s, 1H), 13.92 (s, 1H). MS-API: [M + H]+309.05 (calculated 310) Anal. calculated for C13H12Cl2N3S: C, 56.87; H, 4.14; Cl, 11.19; N, 17.68; S, 10.12.

**4-(4-fluorobenzylideneamino)-5-(2,4-dichlorophenyl)-4H-1,2,4-triazole-3-thiol (5f)**

75% yield – M.P. =143–145°C, IR (KBr) v/cm: 3223.12, 2921.96, 1508.23, 1226.64. 1H NMR (DMSO-d6, 400 MHz): δ 7.41–7.98 (m, 1H), 10.12 (s, 1H), 13.93 (s, 1H). MS-API: [M + H]+309.05 (calculated 310) Anal. calculated for C13H12Cl2N3S: C, 56.87; H, 4.14; Cl, 11.19; N, 17.68; S, 10.12.
4-4-bromobenzylideneamino)-5-(2,4-dichlorophenyl)-4H-1,2,4-triazole-3-thiol (5g)
78% yield – M. P. = 187–189°C, IR (KBr) v/cm: 3323.22, 2911.95, 1505.28, 1229.12. 1H NMR (DMSO-d6, 400 MHz): δ 7.26–7.37 (m, 9H), 10.02 (s, 1H), 13.12 (s, 1H). MS-API: [M + H]+383.98 (calculated 385) Anal. calculated for C15H12BrFN4S: C, 53.81; H, 3.67; Br, 21.05; F, 5.67; N, 16.70; S, 9.58 Found: C, 53.80; H, 3.55; Br, 16.73; S, 9.58.

4-(2-chlorobenzylideneamino)-5-(4-fluorophenyl)-4H-1,2,4-triazole-3-thiol (5m)
83% yield – M. P. = 168–171°C, IR (KBr) v/cm: 3107.08, 2927.31, 1506.23, 1415.30. 1H NMR (DMSO-d6, 400 MHz): δ 7.16–7.32 (m, 10H), 10.37 (s, 1H), 13.85 (s, 1H). MS-API: [M + H]+334.05 (calculated 334) Anal. calculated for C15H12ClFN4S: C, 53.81; H, 3.67; Cl, 10.59; F, 5.67; N, 16.73; S, 9.58 Found: C, 53.80; H, 3.55; Cl, 10.50; F, 5.65; N, 16.70; S, 9.50.

Antifungal activity
The antifungal activity of triazoles was evaluated by cup-plate method[13] against three fungal species: C. albicans
Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol

Scheme 1: Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol

Scheme 2: Synthesis of 4-(benzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol

ATCC 10231, A. niger ATCC 1015 and M. gypseum C 115 2000, dermatophyte fungal species. Stock solutions of synthesized compounds were prepared in DMSO. Aliquots of the stock solution were used to prepare series of subsequent concentration. Control experiments were performed under similar conditions without the synthesized compounds. Ketoconazole was used as a standard for antifungal activity.

Antibacterial activity
The antibacterial activity of triazoles was evaluated by the cup-plate method against two bacterial strain E. coli ATCC 25922 and S. aureus ATCC 25923. By this method, minimal inhibitory concentration (MIC) was found out using Streptomycin as a standard drug. All stock solutions were prepared in DMSO. Aliquots of the stock solution were used to prepare series of subsequent concentration. Control experiments were performed under similar conditions without the synthesized compounds.

RESULTS AND DISCUSSION

Chemistry
The synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (3a-3c) was carried out from benzoic acid derivatives (1) and thiocarbohydrazide (2) as per the literature. Intermediate compound (3) was treated with substituted aromatic aldehydes (4) in the presence of concentrated H2SO4 and yielded Schiff bases (5) The structures of the synthesized compounds were confirmed by NMR, IR, Mass and elemental analysis [Schemes 1 and 2].

The purity of the compounds was checked by TLC-using Silicagel-G (Merck). Their structures were established with IR, NMR and mass spectrometry analysis.

Biological evaluation
All the synthesized compounds underwent antifungal evaluation against fungal species: C. albicans, A. niger and M. gypseum and antibacterial evaluation against bacterial strain E. coli and S. aureus. The results obtained from the evaluation study are provided in Table 1 and Figures 1 and 2. The results so obtained indicated that monochloro, 2,4 dichloro and 4 fluoro derivative have antifungal activity superior to ketoconazole against M. gypseum while other synthesized derivatives showed comparable antifungal activity as ketoconazole against M. gypseum. None of the synthesized derivatives was found to be effective against fungal species: C. albicans, and A. niger.

4-chloro derivative showed antibacterial activity superior to streptomycin against bacterial species, S. aureus and other synthesized derivatives showed antibacterial activity comparable to streptomycin against bacterial species, S. aureus. None of the synthesized derivative was found to be effective against bacterial strain E. coli.

Structure-activity relationship
On studying the effect of the substituents on the antifungal activity, an interesting structure activity relationship can be seen. An electron withdrawing group, that is, Cl group when placed in the para position as in the synthesized compound, showed minimum inhibitory concentration better than ketoconazole indicating better potency than ketoconazole. Presence of Cl group at ortho and para position as in the synthesized compound has potency superior to ketoconazole, similar results were found when fluorine group was placed at para position or 3-chloro derivative as their MIC were found to be comparable. The presence of the OCH3 group as in the synthesized compound showed decrease in potency. On considering the relationship of the antifungal activity of substituted triazole derivatives with the planarity of their molecules, it was observed that as substituent increased, that is, it turned into a bulky group, activity of the compound was observed to be lower as compared to the less bulky triazole derivative.

This shows that the stearic hindrance may reduce the activity.

Similar findings were seen in the case of antibacterial activity. On studying the effect of the substituents on the antibacterial activity, an interesting structure-activity relationship can be seen. Cl group which is electron withdrawing group when placed in the para position as in the synthesized compound showed minimum inhibitory concentration better than streptomycin indicating better potency than streptomycin. It was observed that as
Table 1: Antifungal and antibacterial activity of triazoles

| Compound code | R¹ | R¹¹ | R₂ | R₃ | R₄ | MIC (µg/ml)€ | MIC (µg/ml)§ | MIC (µg/ml)# | MIC (µg/ml)## |
|---------------|----|-----|----|----|----|--------------|--------------|--------------|--------------|
| 5a            | H  | H   | H  | H  | Br | 12.5         | -            | 25           |              |
| 5b            | H  | H   | Cl | H  | Cl | 6.25         | -            | 12.5         |              |
| 5c            | H  | H   | H  | H  | F  | 6.25         | -            | 12.5         |              |
| 5d            | H  | H   | Cl | H  | H  | 3            | -            | 12.5         |              |
| 5e            | H  | H   | H  | Cl | H  | 6.25         | -            | 6.25         |              |
| 5f            | Cl | Cl  | H  | H  | F  | 12.5         | -            | 12.5         |              |
| 5g            | Cl | Cl  | H  | H  | Br | 25           | -            | 50           |              |
| 5h            | Cl | Cl  | Cl | H  | H  | 50           | -            | >50          |              |
| 5i            | Cl | Cl  | Cl | Cl | H  | 50           | -            | >50          |              |
| 5j            | Cl | Cl  | Cl | H  | Cl | 25           | -            | 50           |              |
| 5k            | Cl | Cl  | H  | H  | Cl | 50           | -            | 25           |              |
| 5l            | F  | H   | Cl | H  | Cl | 12.5         | -            | 50           |              |
| 5m            | F  | H   | Cl | H  | H  | 6.25         | -            | 25           |              |
| 5n            | F  | H   | Cl | Cl | H  | 6.25         | -            | 50           |              |
| 5o            | F  | H   | H  | F  | H  | 12.5         | -            | 25           |              |
| 5p            | F  | H   | H  | Br | H  | 12.5         | -            | 12.5         |              |
| 5q            | F  | H   | H  | Cl | H  | 12.5         | -            | 25           |              |
| Ket*          |    |     |    |    |    | 6.25         | 12.5         | 12.5         |              |
| Strept**      | 12.5 | 12.5 |     |    |    |              |              |              |              |

*Ketoconazole, **Streptomycin, ¹Candida albicans ATCC 10231, ²Aspergillus niger ATCC 1015, ³Microsporum gypseum C 115 2000, ⁴Staphylococcus aureus ATCC 25923, ⁵Escherichia coli ATCC 25922. MIC: Minimal inhibitory concentration

Figure 1: Antifungal activity of triazoles, Ket*: Ketoconazole used as standard, MIC: Minimum inhibitory concentration in (µg/ml)

Figure 2: Antibacterial activity of triazoles. Strept**: Streptomycin used as standard. MIC: Minimum inhibitory concentration in (µg/ml)

substituent increased that is the presence of bulky group reduced the antibacterial effect.

CONCLUSION

A novel series of Schiff base was successfully synthesized and tested for antifungal activity against three fungal strains and antibacterial activity against two bacterial strains. The results of the biological studies revealed that among the three fungal strains, *M. gypseum* was found to be more sensitive to the studied 4-(benzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol. In fact, six (5b, 5c, 5d, 5e, 5m, 5n) among the 17 compounds tested were more effective than the clinical candidate ketoconazole. *M. gypseum* is a type of fungi which causes dermatomycoses, a type of infection difficult to treat, hence,
the studied compounds, specifically, (5b, 5c, 5d, 5e, 5m, 5n) could be promising lead molecules for development of more potent and safer antifungal drugs for the treatment of dermatomycoses.

From the study, it was concluded that the 4-(benzyldieneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol derivatives showed antibacterial activity against bacterial species, *S. aureus*. Amongst 17 compounds synthesized, 5e showed antibacterial effect superior to clinical candidate streptomycin, others also had a significant antibacterial effect. Hence, it may be the better pharmacophore to explore the development of new bioactive moieties.

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**Conflicts of interest**
There are no conflicts of interest.

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