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# Synthesis and biological evaluation of some novel triazol-3-ones as antimicrobial agents

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ABSTRACT

A new series of triazol-3-one derivatives bearing 4-methyl-4*H*-thieno[3′,2′: 5,6]thiopyrano [4,3-d][1,3]thiazolyl or 4-(thiophene-3-yl) thiazolyl moiety at 4-position and alkyl substitution at 2-position are synthesized. All the synthesized compounds are characterized by elemental analysis, IR, $^{1}$H NMR, $^{13}$C NMR, and mass spectral data. The newly synthesized compounds are screened for antifungal and antibacterial activities.



In the past decades sulfur containing heterocycles have emerged as an important class of drugs used in various applications. Thiazole and their derivatives have attracted continuing interest over the years because of their varied biological activities.[1–8] Thiophene, in particular, has been investigated in great detail. Thiophene nucleus play active role in several biological active compounds.[9–14] Moreover, thienothiopyran derivatives also represent important building blocks in pharmaceutical chemistry and have been tested as potential antihypertensive agent, CNS receptor modulators, and anti glaucoma drugs.[15–17] Thienothiopyran-2-sulfonamides have shown to be carbonic anhydrase inhibitor.[16]

1,2,4-triazole and 1,2,4-triazol-3-one are important heterocycles because they have been incorporated into a wide variety of drugs like Fluconazole, Itraconazole, Voriconazole, Posaconazole, Letrozole, and Anastrazole used in medical therapy.[18–31]

The increase in resistance to existing antimicrobial treatment has resulted in urgent demand for a new class of antimicrobial agent with a different mode of action and has led medicinal chemist to explore a wide variety of chemical structures. The current trend in antimicrobial drug design[32] is towards clubbing two or three heterocyclic rings having different sites or mode of action. Infact, compounds with two or three heterocyclic rings, for example, 2-substituted 4-(2,5-dichloro thienyl)-1,3-thiazole derivatives have shown to exhibit good antimicrobial activity.[33] Considering the biological significance of thiazole, thiophene, and thienothiopyran and in continuation of our work on synthesis of pharmacologically significant heterocycles,[34] two novel series of triazol-3-one bearing thiophene-3-yl thiazolyl or thieno thiopyrano[4,3-d][1,3]thiazolyl moiety at $C_4$ position and alkyl substituent at $C_2$ position (Schemes 1 and 2) has been synthesized. The thiazolyl thiophene and thiazolyl thienothiopyran moeities were incorporated on triazol-3-ones to evaluate their role and compare their effect, if any, on the antimicrobial activity of the triazolo-3-ones. It was also worthwhile to investigate the effect of N-alkylation of triazol-3-one on biological activity. The synthesized compounds are novel and evaluated for in vitro antimicrobial activity.

The synthetic strategies adopted for the synthesis of title compounds **5**, **6**, **11**, and **12** are depicted in Schemes 1 and 2. 3-(Bromoacetyl) thiophene **1** and 5-bromo-6-methyl-5,6-dihydrothieno[2,3-b]thiopyran-4-one **7**, the key starting materials were prepared by bromination of corresponding ketone with NBS in acetonitrile using *p*-toluensulfonic acid.[35] Amino thiazoles **2** and **8** were prepared by reacting corresponding α-bromoketones **1** and **7** with thiourea in ethanol in good yield.[36] The reaction of aminothiazoles **2** and **8** with phenychloroformate was accomplished in acetonitrile using potassium carbonate to obtain carbamates **3** and **9** in excellent yield (80% and 75%, respectively). However, when reaction was carried out in chloroform using pyridine as base, yield of carbamates **3** and **9** were 60% and 58%, respectively.[30] The semicarbazides **4** and **10** were synthesized by reacting hydrazine hydrate with corresponding carbamates **3** and **9** which in turn cyclized with formamidine acetate in DMF gave 4-substituted triazole-3-ones **5** and **11**, respectively.[30] N-alkylation of triazol-3-ones **5** and **11** was achieved by reaction with alkyl halide in DMSO using

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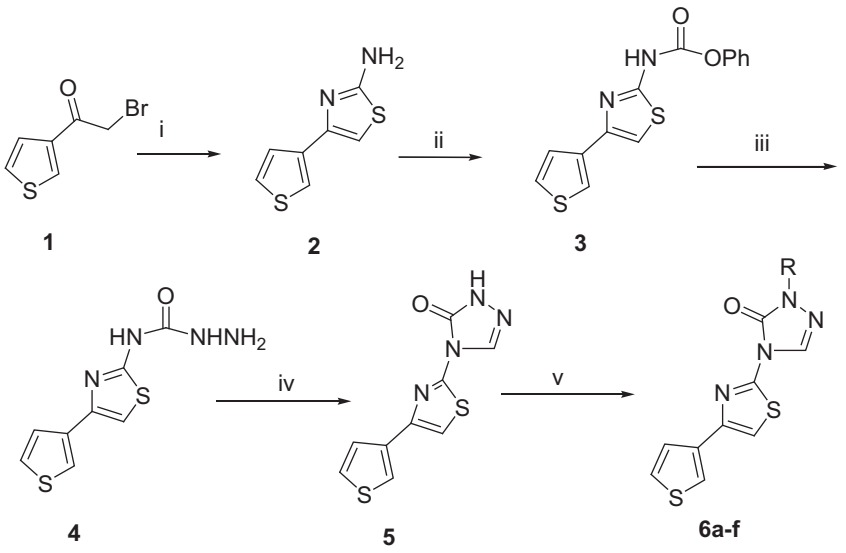
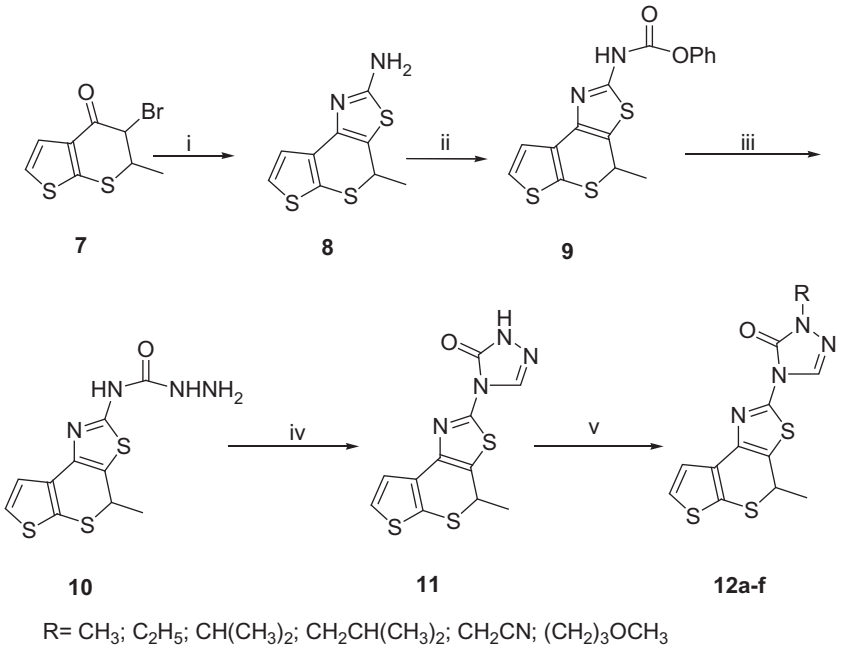



**Scheme 1.** *Reagents:* (i) thiourea, ethanol; (ii) acetonitrile, potassium carbonate, phenyl chloroformate; (iii) dioxane, hydrazine hydrate; (iv) DMF, formamidine acetate; and (v) DMSO, potassium hydroxide, alkylhalide.




**Scheme 2.** *Reagents:* (i) thiourea, ethanol; (ii) acetonitrile, potassium carbonate, phenyl chloroformate; (iii) dioxane, hydrazine hydrate; (iv) DMF, formamidine acetate; and (v) DMSO, potassium hydroxide, alkylhalide.

potassium hydroxide. The yields of target compounds **6a–f** and **12a–f** were obtained in the range of 60–85%.

All the synthesized compounds were screened for in vitro antibacterial and antifungal activity,[37,38] against a panel of gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*) and pathogenic fungus (*Fusarium solani*, *Aspergillus niger*, and *Candida albicans*). The MIC's of these compounds were carried out using microdilution susceptibility method. The antibiotic chloramphenicol and ketoconazole were used as reference for antibacterial and antifungal activity, respectively.

In vitro antibacterial and antifungal activity, assay (Table 1) indicated that all synthesized compounds (**5**, **11**, **6a**–**f**, and **12a–f**) show moderate to excellent activity against all tested gram positive as well as gram negative bacterial and fungal pathogens determined at concentrations (100 and 200 μg/mL). From the antibacterial activity data, it is observed that compounds **6a**, **6e**, and **12b** are the most active among the tested compounds against gram positive bacteria while all other compounds show less antibacterial activity comparable to chloramphenicol. The data indicated that N-alkylation of triazole-3-ones **5** and **11** played a crucial role in determining the antimicrobial activity. For the series thiophene thiazolyl **5** and **6a–f**, N-$CH_3$ at 2-position (**6a**) increases the antibacterial activity while N-$CH(CH_3)_2$ (**6d**) reduces the activity compared to parent derivative **5** against gram positive bacteria. In the case of the other series thieno thiopyarano-thiazolyl **11** and

**Table 1**
Antimicrobial activity of synthesized compounds **5**, **6a–f**, **11** and **12a–f**

| Compd | R | Conc. (μg/ml) | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|---|---|
| **5** | H | 100 | 25 | 27 | 24 | 22 | 32 | 34 | 35 |
| | | 200 | 28 | 29 | 26 | 26 | 37 | 39 | 38 |
| **6a** | Methyl | 100 | 32 | 34 | 23 | 26 | 40 | 39 | 36 |
| | | 200 | 35 | 36 | 25 | 28 | 43 | 42 | 40 |
| **6b** | Ethyl | 100 | 24 | 21 | 23 | 26 | 29 | 21 | 23 |
| | | 200 | 32 | 27 | 29 | 28 | 32 | 24 | 29 |
| **6c** | Isopropyl | 100 | 25 | 20 | 22 | 26 | 27 | 30 | 22 |
| | | 200 | 27 | 23 | 24 | 29 | 29 | 33 | 24 |
| **6d** | Isobutyl | 100 | 16 | 16 | 17 | 15 | 26 | 26 | 17 |
| | | 200 | 20 | 18 | 21 | 18 | 30 | 27 | 21 |
| **6e** | Cyanomethyl | 100 | 28 | 23 | 26 | 16 | 28 | 27 | 28 |
| | | 200 | 31 | 26 | 29 | 21 | 34 | 29 | 32 |
| **6f** | 3-Methoxy propane | 100 | 24 | 23 | 24 | 25 | 34 | 26 | 24 |
| | | 200 | 26 | 26 | 28 | 28 | 36 | 28 | 28 |
| **11** | H | 100 | 18 | 20 | 15 | 17 | 22 | 26 | 25 |
| | | 200 | 21 | 23 | 20 | 20 | 24 | 29 | 28 |
| **12a** | Methyl | 100 | 14 | 16 | 18 | 16 | 24 | 20 | 22 |
| | | 200 | 16 | 19 | 21 | 19 | 26 | 23 | 24 |
| **12b** | Ethyl | 100 | 25 | 28 | 22 | 24 | 28 | 23 | 26 |
| | | 200 | 29 | 31 | 27 | 26 | 31 | 26 | 29 |
| **12c** | Isopropyl | 100 | 14 | 13 | 15 | 14 | 17 | 17 | 16 |
| | | 200 | 16 | 15 | 18 | 16 | 19 | 22 | 21 |
| **12d** | Isobutyl | 100 | 12 | 13 | 12 | 10 | 16 | 16 | 17 |
| | | 200 | 15 | 16 | 14 | 12 | 20 | 18 | 21 |
| **12e** | Cyanomethyl | 100 | 13 | 15 | 11 | 11 | 15 | 16 | 14 |
| | | 200 | 17 | 18 | 13 | 14 | 18 | 19 | 18 |
| **12f** | 3-Methoxy propane | 100 | 23 | 31 | 22 | 21 | 39 | 37 | 36 |
| | | 200 | 28 | 33 | 25 | 24 | 44 | 41 | 39 |
| Chloromphenicol | | 100 | 35 | 38 | 40 | 42 | — | — | — |
| | | 200 | 39 | 41 | 44 | 45 | — | — | |
| Ketoconazole | | 100 | — | — | — | — | 38 | 41 | 36 |
| | | 200 | — | — | — | — | 42 | 44 | 39 |

(A) *Staphylococcus aureus*; (B) *Bacillus subtilis*; (C) *Escherichia coli*; (D) *Klebsiella pneumoniae*; (E) *Fusarium solanil*; (F) *Aspergillus niger*; and (G) *Candida albicans*.

**12a–f**, N-$C_2H_5$ (**12b**) and N-$(CH_2)_3$-$OCH_3$ (**12f**) at 2 position of triazol-3-one increases the antibacterial activity while N-$CH(CH_3)_2$ (**12d**) reduces the antibacterial activity compared to parent derivative **11** against gram positive bacteria *S. aureus* and *B. subtills*. It was also observed that for both series, there are subtle difference in activity of N-substituted derivatives (**6a–f**) and (**12a–f**) compared to their parent compound **5** and **11** against gram negative bacteria *E. coli*, *K. pneumoniae*. However, it is worth mentioning that the synthesized compounds are more active against fungal pathogens as compared to bacterial strains. Interestingly, it was found that thiophene thiazolyl derivatives **5** and (**6a–f**) were more active than thieno thiopyarano-thiazolyl derivatives **11** and (**12a–f**) against both bacterial and fungal pathogens. From the antifungal activity data it was concluded that compounds **5**, **6a**, and **12f** are the most active among all the synthesized compounds against most of the tested organisms comparable with standard drug ketoconazole. For the thiophene thiazolyl series, N-$CH_3$ (**6a**) increases the antifungal activity while other substitution marginally reduces the activity compared with parent compound **5**. Introduction of the N-$(CH_2)_3$-$OCH_3$ (**12f**) at $C_2$ of triazol-3-one in thieno thiopyarano-thiazolyl series substantially increases the antifungal activity compared with unsubstituted parent compound **11**. Introduction of isopropyl group (**12c**), isobutyl group (**12d**), and cyanomethyl group (**12e**) reduce the antifungal activity whereas there is no effect of ethyl (**12b**) and methyl (**12a**) substitution compared with parent compound **11** against all the tested pathogens.

Based on the activity data for Triazol-3-one derivatives **5** and **11**, it is concluded that triazol-3-one bearing (thiophene-3-yl) thiazolyl moiety exhibits better antibacterial and antifungal activity than 4-methyl-4H-thieno[3′,2′: 5,6]thiopyrano[4,3-d][1,3]thiazolyl moiety. Moreover, N-alkylation of triazol-3-one ring seems to have marginal effect on biological activity. Infact, upon N-alkylation the biological activity marginally decreases except in case of N-methylation. The results also reveal that all the synthesized compounds exhibit better antifungal activity than antibacterial activity, in line with the fact that sulfur containing compounds are good antifungal agents.

The antimicrobial activity of all the synthesized compounds could be attributed to the presence of triazol-3-one ring. This ring is incorporated into a wide variety of drugs used in medical therapy. Infact, contribution towards biological activity by thiazole and thiophene or thienothiopyran ring could not be ruled out. However, based on the observation, it is immature to arrive at any conclusion on structure activity aspect of these molecules and further evaluation is needed.

In summary, we have synthesized two series of novel triazol-3-one bearing (thiophene-3-yl) thiazolyl and 4-methyl-4*H*-thieno[3′,2′: 5,6]thiopyrano[4,3-d][1,3]thiazolyl moiety at $C_4$ and alkyl substitution at $C_2$ for their in vitro antimicrobial evaluation.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.049.

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37. The minimum inhibitory concentrations (MICs) were determined by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guidelines. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 5th ed.; Approved standard: NCCLS Document M7-A5, 2000.
38. Preliminary antimicrobial activities were determined by disc diffusion method against panel of gram positive bacteria (*S. aureus*, *B. subtilis*), gram negative bacteria (*E. coli*, *K. pneumoniae*) and pathogenic fungus (*F. solani*, *A. niger*, and *C. albicans*). The antibiotic chloramphenicol (10 μg/disc) and ketoconazole (100 μg/disc) were used as control for antibacterial and antifungal activity, respectively. The samples were dissolved in DMSO and used for the antimicrobial activities. The bacterial cultures of known inoculums size ($1 \times 108$ bacteria/mL) of test microorganisms were spread on Muller Hinton agar plates, while the fungi cultures of known inoculums size ($1 \times 106$ bacteria/mL) of test organisms were spread on sabourad dextrose agar plates. The Whatman filter paper discs of 6 mm were placed and sample of appropriate concentration was added to the inoculated plate. The plates were incubated for 24 h at 37 °C for antibacterial activity and for 48 h at 37 °C for antifungal activity.
The MIC's of the compound were carried out using microdilution susceptibility method. The antibiotic chlorampenicol and ketoconazole was used as reference standard for antibacterial and antifungal activity, respectively.
The test compound, chlorampenicol, and ketoconazole were dissolved in DMSO at concentration of 800 μg/ml. The two fold dilution of the solution prepared (400, 200, 100, ... 6.25 μg/ml). The microorganism suspension was inoculated to the corresponding wells. The plates were incubated at 37 °C for 24 and 48 h for bacterial and fungi, respectively. The minimal inhibitory concentration was taken from the concentration of the lowest dosed test tube showing visually no growth of inoculated bacteria and fungi.