Synthesis of thiazolyltriazole substituted azetidinones as antimicrobial agents

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The reaction of ethyl 2-amino-4-methylthiazole-5-carboxylate 1 with acetic anhydride followed by reaction with hydrazine hydrate yields the ethyl 2-acetamido-4-methylthiazole-5-carboxylate 2 and N-[5-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methylthiazol-2-yl]acetamide 3, respectively. The compound 3 on further reaction with alcoholic potassium hydroxide-carbon disulphide followed by cyclization with hydrazine hydrate gives N-[5-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methylthiazol-2-yl]acetamide 5. The compound 5 is then condensed with different aromatic aldehydes to offer Schiff bases 6a-h. The Schiff bases on cyclization with chloroacetyl chloride in presence of triethylamine as catalyst furnish the azetidin-2-one 7a-h. The compounds are synthesized in good yield and the chemical structures of the compounds are elucidated from their IR, 1H NMR, and elemental analysis. All the synthesized compounds have been screened for their antimicrobial activity.

Keywords: Thiazolyl-triazole, Schiff base, azetidin-2-one, antimicrobial activity

The chemistry of heterocyclic compounds is an interesting area in medicinal chemistry as it offers challenging tasks in the development of novel synthetic strategies\(^1\). Literature survey shows that thiazole derivatives play a very important role in biological fields such as antimicrobial\(^2\),\(^5\), antidiabetic\(^6\), antiviral\(^1\), antiinflammatory\(^8\), antituberculosis\(^3\) and anticancer\(^10\) activities. 1,2,4-triazoles, are among the various heterocycles that have received the most attention during the last two decades as potential antimicrobial agents\(^11\)-\(^14\). Schiff base has good antimicrobial\(^15\), antifungal\(^16\) activity and it can be prepared by the acid catalysed reaction of aldehyde or ketone with amines\(^17\). Further azetidin-2-one derivatives have been exhibited to possess biological properties like antimicrobial\(^18\),\(^19\), antifungal\(^20\) and antiinflammatory\(^21\) activities. The development of resistance to existing antimicrobial treatment has resulted in urgent demand for a new class of antimicrobial agent with a different mode of action and it has led medicinal chemists to explore a wide variety of chemical structures. Today, the trend in antimicrobial drug design\(^22\) is towards clubbing of two or three heterocyclic molecules having different sites or mechanisms of action. This inspired the construction of compounds containing thiazole, triazole and azetidinones ring systems in the same matrix to serve as a new scaffold towards the development of novel antimicrobial agents. In view of the foregoing discussion, the synthesis of thiazolyltriazole substituted azetidinones was aimed towards development of novel antimicrobial agents.

Results and Discussion

In the present work, a series of new compounds were synthesized. Thus, starting from the ethyl 2-amino-4-methylthiazole-5-carboxylate 1 was synthesized as per reported procedure\(^23\). The reaction of 1 with acetic anhydride followed by reaction with hydrazine hydrate yielded the ethyl 2-acetamido-4-methylthiazole-5-carboxylate 2 and N-[5-(4-amino-4-hydrazinecarbonyl)-4-methylthiazol-2-yl]acetamide 3 respectively. The compound 3 on further reaction with alcoholic potassium hydroxide-carbon disulphide followed by cyclization with hydrazine hydrate gave N-[5-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methylthiazol-2-yl]acetamide 5. The N-[5-(4-benzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl]-4-methyl thiazol-2-yl]acetamide 6a-h were prepared by reacting with different aromatic aldehyde. The Schiff bases on cyclization with chloroacetyl chloride in presence of triethylamine as catalyst furnished N-[5-(4-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methylthiazol-2-yl]acetamide 7a-h as shown in Scheme I.

Physical constants of the title compounds are shown in Table I. The structures of the various synthesized compounds were assigned on the basis of elemental analysis, IR and \(^1\)H NMR spectral data. The compounds are evaluated for their antimicrobial activity and the results are summarised in Table II. From the antimicrobial screening it was observed that
Scheme I

R = -H -2-CH -4-Cl -3-NC -2-NC -4-CH -4-(CH₂)₂N -3 4 5-(CCH₃)₂
all the compounds exhibited activity against all the organisms employed. Looking from the structure activity relationship angle, marked inhibition in bacterial growth was observed by the compounds bearing R = H-, 4-Cl-, 2-OH-, 4-(CH$_3$)$_2$N-, 3,4,5-(OCH$_3$)$_3$- (7a, 7b, 7c, 7g, 7h) substituents whereas compounds 7d, 7e, 7f showed moderate to good activity. Fungicidal screening data also revealed that compounds bearing R = H-, 4-Cl-, 2-OH-, 4-(CH$_3$)$_2$N-, 3,4,5-(OCH$_3$)$_3$- (7a, 7b, 7c, 7g, 7h) imparted maximum activity to the compounds, whereas compounds 7d, 7e, 7f showed moderate to good activity.

**Experimental Section**

The melting points were recorded on electrothermal apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer model-983. $^1$H NMR spectra were recorded on Varian Mercury 300 MHz instrument using DMSO-d$_6$ as solvent (chemical shift in $\delta$, ppm), using TMS as internal standard. Elemental analysis was performed on a Heraeus CHN analyzer. Analyses indicated by the symbols of the elements of the elements were within ±0.4% of the theoretical values.

**Synthesis of ethyl 2-acetamido-4-methylthiazole-5-carboxylate, 2**

Compound 1 (0.01 mole) was mixed with acetic anhydride (0.02 mole) in a 250 mL round bottom flask. The reaction mixture was refluxed for 1 hr. Then the solution was poured into ice-cold water with vigorous stirring. A solid precipitated out. The mixture was heated to boiling to decompose excess of acetic anhydride and cooled. The solid obtained was filtered, washed with water, dried and purified by recrystallization from ethanol. Pure white crystals of 2 were obtained. Yield 82%, m.p. 178°C.

**Synthesis of N-[5-(hydrazinecarbonyl)-4-methylthiazol-2-yl]acetamide, 3**

Compound 2 (0.01 mole) and hydrazine hydrate (0.02 mole) in a 250 mL round bottom flask. The reaction mixture was refluxed for 10 min. To it ethanol (20 mL) was added till both the layers were miscible. Then refluxing was continued for 6 hr. The excess of ethanol and unreacted hydrazine hydrate were distilled out and the contents poured into a beaker. The solid obtained was filtered, washed with water, dried and purified by recrystallization from ethanol. Pure white crystals of 2 were obtained. Yield 76%, m.p. 178°C.

**Synthesis of potassium dithiocarbazinate, 4**

Potassium hydroxide (0.03 mole) was dissolved in absolute ethanol (50 mL). The solution was cooled in an ice bath and hydrazide 3 (0.02 mole) was added with stirring. To this, carbon disulfide (0.025 mole) was added in small portions with constant stirring.

<table>
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<tr>
<th>Compd</th>
<th>R</th>
<th>Yield (%)</th>
<th>m.p. (°C)</th>
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<tr>
<td>6a</td>
<td>H-</td>
<td>85</td>
<td>245</td>
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<tr>
<td>6b</td>
<td>2-OH-</td>
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<td>248</td>
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<tr>
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<td>4-Cl-</td>
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<td>238</td>
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<tr>
<td>6d</td>
<td>3-NO$_2$-</td>
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<td>246</td>
</tr>
<tr>
<td>6e</td>
<td>2-NO$_2$-</td>
<td>74</td>
<td>242</td>
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<td>6f</td>
<td>4-OH-</td>
<td>76</td>
<td>250</td>
</tr>
<tr>
<td>6g</td>
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<td>83</td>
<td>249</td>
</tr>
<tr>
<td>6h</td>
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<td>76</td>
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<table>
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<td></td>
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<td>S.a</td>
<td>P.a</td>
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<td>H-</td>
<td>13</td>
<td>14</td>
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<td>2-OH-</td>
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<tr>
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<td>4-(CH$_3$)$_2$N-</td>
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<td>7h</td>
<td>3,4,5-(OCH$_3$)$_3$.</td>
<td>10</td>
<td>12</td>
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</table>

Penicillin  —   22       20       21       19       —       —
Griesofulvin  —   —        —        —        20       22       —

$^a$Zone of inhibition is measured in mm.

The reaction mixture was stirred continuously for 16 hr at RT. The precipitated potassium dithiocarbinate was collected by filtration, washed with anhydrous ether and dried in vacuum. The potassium salt thus obtained was used in the next step without further purification.

**Synthesis of N-[5-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methylthiazol-2-yl]acetamide, 5**

A suspension of potassium dithiocarbinate 4 (0.02 mole) and hydrazine hydrate (99%, 0.04 mole) in ethanol (50 mL) was refluxed for 10-15 hr with occasional shaking. The colour of the reaction mixture changed to light green with evolution of hydrogen sulfide gas. A homogenous mixture was obtained was used in the next step without further purification.

Other Schiff bases 6b-h were obtained in a similar manner.

**Synthesis of N-[5-(4-(2-Hydroxybenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methyl thiazol-2-yl]acetamide, 6b**

To a stirred solution of compound 6a (0.01 mole) in 25 mL 1,4-dioxane, triethylamine (0.01 mole) and chloroacetyl chloride (0.01 mole) were added slowly drop-wise with stirring at 0-20°C. The reaction mixture was kept at RT for 30 min, then refluxed for 5 hr. The excess of solvent was distilled off and the residue was poured into ice-cold water. The solid separated was filtered and purified by recrystallization from DMSO to give colourless crystals of 7a. IR (KBr): 3196 (NH), 3010 (Ph-CH), 2608 (SH), 1725 (C=O), 1342 (C-N), 755 (C-Cl); $\delta$ 2.0 (s, 3H, CH$_3$), 2.4 (s, 3H, CH$_3$), 4.7 (d, 1H, CH, azetidin-2-one), 5.2 (d, 1H, CH-Cl, azetidin-2-one), 7.0-7.28 (m, 5H, ArH). Anal. Calcd for C$_{17}$H$_{15}$ClN$_2$O$_2$: C, 46.75; H, 3.41; N, 19.28.

Other azetidin-2-ones 7b-h were synthesized in a similar manner.

$\begin{align*} N\text{-}[5-(4-(2-Hydroxybenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methyl thiazol-2-yl]acetamide, 6b: IR (KBr): & 3181 (N-H), 2833 (SH), 2724 (C=O), 1733 (C=N), 1348 (C-N), 728 (C-Cl) \delta 2.0 (s, 3H, CH$_3$), 2.4 (s, 3H, CH$_3$), 4.7 (d, 1H, CH, azetidin-2-one), 5.2 (d, 1H, CH-Cl, azetidin-2-one), 7.0-7.28 (m, 5H, ArH). Anal. Calcd for C$_{17}$H$_{15}$ClN$_2$O$_2$: C, 46.75; H, 3.41; N, 19.28. \end{align*}$
Antimicrobial activity

The synthesized compounds 7a-h were screened for their in-vitro antimicrobial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi, and antifungal activity against Aspergillus niger and Candida albicans by measuring the zone of inhibition in mm. The antimicrobial activity was performed by cup plate method at a concentration of 100 µg/mL in DMSO and reported in Table II for antimicrobial and antifungal activities. Nutrient agar was employed as culture medium and DMSO was used as solvent control for antimicrobial activity. Penicillin and griseofulvin were used as standard drugs for antibacterial and antifungal activities respectively.

Conclusion

In the present study, a highly efficient and simple procedure for the synthesis of \( N-[5-(4-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methyl thiazol-2-yl]acetamide \) 7a-h, where 1,2,4-triazole and thiazole rings are attached, was developed. The adopted method is simple, inexpensive and gave good yields. When all the results obtained from antibacterial and antifungal tests together are considered, it can be said with confidence that the entire series of compounds tested are active towards bacteria and fungi.

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References