Multistep, Microwave Assisted, Solvent Free Synthesis and Antibacterial Activity of 6-Substituted-2,3,4-trihydropyrimido[1,2-c]9,10,11,12-tetrahydrobenzo[b]thieno[3,2-e]pyrimidines

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A novel, efficient, microwave assisted route for the synthesis of 6-substituted-2,3,4-trihydropyrimido[1,2-c]-9,10,11,12-tetrahydrobenzo[b]thieno[3,2-e]pyrimidines in good yields has been developed. The intermediates, 2-substituted-4-[3-hydroxy(propyl-1-amino)][5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidines were obtained by irradiating 2-substituted-4-chloro-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidines with 1-amino-propanol under basic conditions in a microwave oven. 4-Chlorothieno[2,3-d]pyrimidines were synthesized by microwave irradiation of equimolar mixture of 4-hydroxythieno[2,3-d]pyrimidines and phosphorus oxychloride. The final compounds were screened for antibacterial activity by Kirby Bauer's method using amicacin as the standard against various gram positive and gram negative bacteria. All the compounds showed antibacterial activity comparable with the standard.

Key words pyrimidothienopyrimidine; microwave assisted synthesis; green chemistry; solvent free synthesis; Kirby Bauer’s method

Antibacterial play an important role in the treatment of various bacterial infections. The spectacular success of antibiotics in the treatment of bacterial infection has prompted the expansion of their use from tetracycline’s to fluoroquinolines. However, the emergence of resistant strains, even to fluoroquinolines, has posed a real challenge. Thus researchers are working towards finding new drugs by utilizing the concept of bioisosterism, to defeat resistant strains.

Literature survey revealed that several fused pyrimidines and pyridines like triazole quinazolines and triazoloquinolines have shown good antibacterial activity. Further condensed triazoles have been reported to possess large number of pharmacological activities like fungicidal, pesticidal etc. Thienopyrimidines have been reported to exhibit antimicrobial activities too. Thus it was taught of interest to fuse various heterocyclic moieties like imidazole, triazole ring system to the basic thienopyrimidine ring system and to test their efficacy for their antibacterial activity. Recently, triazolothienopyrimidines have been reported as antibacterial agents from our laboratories. The encouraging antibacterial activity of these compounds gave us an impetus for isosteric replacement of triazole ring in triazolothienopyrimidines by pyrimidine ring. Thus synthesis of some novel pyrimidothienopyrimidines has been worked out.

Since 1986, with the introduction of controlled, precise microwave reactors, microwave-assisted organic synthesis has had a significant impact on synthetic organic chemistry. Thus microwave assisted synthesis has gained popularity due to their enhanced selectivity, improved reaction rates, associated ease of manipulation and ecofriendliness.

Literature survey shows that microwaves are utilized for the synthesis of various heterocyclic compounds like quinolines, pyrazolopyrazoles, xanthines, hydantoin, benzoazines, quinolines, imidazolothienopyrimidines, thiophenes, thieno pyrimidines, etc. but no efforts were made to utilize microwaves for the synthesis of pyrimido-[1,2-c]thieno[3,2-e]pyrimidines. These observations prompted us to attempt the synthesis of these compounds by microwave technique. Herewith we are reporting a novel microwave assisted synthesis and antibacterial activity of 6-substituted-2,3,4-trihydropyrimido[1,2-c]9,10,11,12-tetrahydrobenzo[b]thieno[3,2-e]pyrimidines (Chart 1).

Experimental

Chemistry All reactions were carried out on microwave oven at the power of 960 W [CEM, Discover microwave labstation operating at 2450 MHz under continuous internal temperature control]. Analytical TLC was performed on Silica Gel F254 plates (Merek) with visualization by UV or iodine vapors. Melting points were determined in open capillaries on a Thermonik melting point apparatus, Mumbai, India and are uncorrected. The IR spectra (KBr, vMax, cm⁻¹) were run on Perkin Elmer FTIR Spectrophotometer (577 model). ¹H-NMR (δ ppm, CDCl₃/DMSO-d₆) spectra were recorded using Bruker WM-400 spectrometer (Bruker, Flawil, Switzerland).

Chart 1. Microwave Assisted Synthesis of 6-Substituted-2,3,4-trihydropyrimido[1,2-c]9,10,11,12-tetrahydrobenzo[b]thieno[3,2-e]pyrimidines (5a—f)

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with TMS as internal standard. MS spectra (EI-MS, 70 eV) were recorded on Autospec spectrometer. Elemental analyses were performed on Carlo Erba 1108 elemental analyzer (Hereau, Hanaux, Germany) and were within ±0.4% of theoretical values. All the chemicals used were of analytical grade.

2-Substituted-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-ones (2a–f). General Procedure

2-Amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-one (2e) Reaction time: 150 s. Yield 75%; colorless needle; mp 286—288 °C; IR (KBr) cm⁻¹: 1654 (C=O), 3417 (–NH), 2921 (–CH₃), 3008 (Ar-H), 1046 (–C–N). ¹H-NMR (CDCl₃) δ: 1.75—1.95 (4H, m), 2.65—2.95 (4H, m), 4.12 (2H, s), 8.34—9.15 (4H, m), 11.4 (1H, brs). MS m/z: 331.5 (M⁻+1). Anal. Calc. for C₁₅H₁₉N₃SO: C, 61.65; H, 5.43; N, 18.72. Found: C, 61.35; H, 4.45; N, 18.10.

2-(Pyridyl)-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-one (2f) Reaction time: 150 s. Yield 72%; colorless needle; mp 302—304 °C; IR (KBr) cm⁻¹: 3434 (–OH), 3355 (–NH), 2924 (–CH₃), 1048 (–C–N), 1650 (–C=O). ¹H-NMR (CDCl₃) δ: 1.85—2.15 (4H, m), 2.25—2.30 (2H, pentet, J = 6.3 Hz), 2.85—2.88 (2H, t, J = 5.9 Hz), 2.95—2.98 (2H, t, J = 5.9 Hz), 3.65—3.70 (2H, t, J = 6.2 Hz), 3.90—3.93 (2H, t, J = 6.2 Hz), 6.30—6.32 (2H, t, J = 6.2 Hz), 4.14 (2H, s), 5.62 (1H, s), 8.30—8.32 (2H, d, J = 3.5 Hz). MS m/z: 385 (M⁺+1). Anal. Calc. for C₁₅H₁₉N₃SO: C, 61.65; H, 5.35; N, 10.57. Found: C, 61.93; H, 5.67; N, 12.83.

2-(Pyridyl)-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-one (2d) Reaction time: 15 s. Yield 88%; colorless needle; mp 230—232 °C; IR (KBr) cm⁻¹: 3436 (–OH), 3355 (–NH), 2924 (–CH₃), 1048 (–C–N), 1650 (–C=O). ¹H-NMR (CDCl₃) δ: 1.85—2.15 (4H, m), 2.25—2.30 (2H, pentet, J = 6.3 Hz), 2.85—2.88 (2H, t, J = 5.9 Hz), 2.95—2.98 (2H, t, J = 5.9 Hz), 3.65—3.70 (2H, t, J = 6.2 Hz), 3.90—3.93 (2H, t, J = 6.2 Hz), 6.30—6.32 (2H, t, J = 6.2 Hz), 4.14 (2H, s), 5.62 (1H, s), 8.30—8.32 (2H, d, J = 3.5 Hz). MS m/z: 341 (M⁺+1). Anal. Calc. for C₁₅H₁₉N₃SO: C, 66.95; H, 5.85; N, 12.05. Found: C, 67.25; H, 6.19; N, 12.38.

2-(Pyridyl)-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-one (2c) Reaction time: 15 s. Yield 88%; colorless needle; mp 230—232 °C; IR (KBr) cm⁻¹: 3436 (–OH), 3355 (–NH), 2924 (–CH₃), 1048 (–C–N), 1650 (–C=O). ¹H-NMR (CDCl₃) δ: 1.85—2.15 (4H, m), 2.25—2.30 (2H, pentet, J = 6.3 Hz), 2.85—2.88 (2H, t, J = 5.9 Hz), 2.95—2.98 (2H, t, J = 5.9 Hz), 3.65—3.70 (2H, t, J = 6.2 Hz), 3.90—3.93 (2H, t, J = 6.2 Hz), 6.30—6.32 (2H, t, J = 6.2 Hz), 4.14 (2H, s), 5.62 (1H, s), 8.30—8.32 (2H, d, J = 3.5 Hz). MS m/z: 341 (M⁺+1). Anal. Calc. for C₁₅H₁₉N₃SO: C, 66.95; H, 5.85; N, 12.05. Found: C, 67.25; H, 6.19; N, 12.38.

2-(Pyridyl)-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-one (2b) Reaction time: 15 s. Yield 88%; colorless needle; mp 230—232 °C; IR (KBr) cm⁻¹: 3436 (–OH), 3355 (–NH), 2924 (–CH₃), 1048 (–C–N), 1650 (–C=O). ¹H-NMR (CDCl₃) δ: 1.85—2.15 (4H, m), 2.25—2.30 (2H, pentet, J = 6.3 Hz), 2.85—2.88 (2H, t, J = 5.9 Hz), 2.95—2.98 (2H, t, J = 5.9 Hz), 3.65—3.70 (2H, t, J = 6.2 Hz), 3.90—3.93 (2H, t, J = 6.2 Hz), 6.30—6.32 (2H, t, J = 6.2 Hz), 4.14 (2H, s), 5.62 (1H, s), 8.30—8.32 (2H, d, J = 3.5 Hz). MS m/z: 341 (M⁺+1). Anal. Calc. for C₁₅H₁₉N₃SO: C, 66.95; H, 5.85; N, 12.05. Found: C, 67.25; H, 6.19; N, 12.38.

2-(Pyridyl)-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-one (2a) Reaction time: 15 s. Yield 88%; colorless needle; mp 230—232 °C; IR (KBr) cm⁻¹: 3436 (–OH), 3355 (–NH), 2924 (–CH₃), 1048 (–C–N), 1650 (–C=O). ¹H-NMR (CDCl₃) δ: 1.85—2.15 (4H, m), 2.25—2.30 (2H, pentet, J = 6.3 Hz), 2.85—2.88 (2H, t, J = 5.9 Hz), 2.95—2.98 (2H, t, J = 5.9 Hz), 3.65—3.70 (2H, t, J = 6.2 Hz), 3.90—3.93 (2H, t, J = 6.2 Hz), 6.30—6.32 (2H, t, J = 6.2 Hz), 4.14 (2H, s), 5.62 (1H, s), 8.30—8.32 (2H, d, J = 3.5 Hz). MS m/z: 341 (M⁺+1). Anal. Calc. for C₁₅H₁₉N₃SO: C, 66.95; H, 5.85; N, 12.05. Found: C, 67.25; H, 6.19; N, 12.38.

### Results and Discussion

2-Amino-3-carboxy-4,5,6,7-tetrahydrobenzo[b]thiophene (1) was synthesized by following the reported procedures using microwave oven. The substituted 4-hydroxy-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidines (2a—f) were characterized as 6-substituted-2,3,4-trihydropyrimido[1,2-c]9,10,11,12-tetrahydrobenzo[b]thieno[3,2-e]pyrimidines. The elemental analyses of the newly synthesized compounds showed that the purity of the compounds were within ±0.4% limits. Thus the usage of present controlled, precise microwave reactor [CEM, Discover] has not only resulted in simple reaction conditions and easy work-up procedures but also improved yields over conventional methods. The synthesized compounds were evaluated for antimicrobial activity against various gram-positive and gram-negative bacteria like K. pneumoniae, P. aeruginosa, B. subtilis and S. citrus using Kirby Bauer’s Method. The negative control did not show any zone of inhibition in all the bacterial strains used for the study. B. subtilis was found to be the most susceptible and K. pneumoniae was the most resistant organism.

<table>
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<th>Compd.</th>
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<tr>
<td>5a</td>
<td>50 μg</td>
<td>10.0±0.42</td>
</tr>
<tr>
<td>5b</td>
<td>50 μg</td>
<td>11.0±0.36</td>
</tr>
<tr>
<td>5c</td>
<td>50 μg</td>
<td>12.0±0.36</td>
</tr>
<tr>
<td>5d</td>
<td>50 μg</td>
<td>11.0±0.28</td>
</tr>
<tr>
<td>5e</td>
<td>50 μg</td>
<td>11.0±0.14</td>
</tr>
<tr>
<td>5f</td>
<td>50 μg</td>
<td>11.0±0.18</td>
</tr>
<tr>
<td>Amikacin</td>
<td>50 μg</td>
<td>20.0±0.35</td>
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### Table 1. Antibacterial Activity of 6-Substituted-2,3,4-trihydropyrimido[1,2-c]9,10,11,12-tetrahydrobenzo[b]thieno[3,2-e]pyrimidines (5a—f)

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*P. aeruginosa* and *S. citrus*, showed intermediate activity.

All the compounds of the series (5a—f) were found to be equipotent against with that of the standard against *B. subtilis* which indicates the susceptibility of the organism. Compounds 5b (9.0±0.45) and 5d (9.0±0.28) showed efficacy similar to that of amikacin (10.0±0.20) against *S. citrus*. Compound 5a showed least activity in the series.

The activity of the compounds (5a—f) [zone of inhibition 10—12 mm] against *K. pneumoniae* were not comparable with that of amikacin (20±0.35) indicating the resistance of the organism. Compounds of the series were moderately active against *P. aeruginosa*. Compound 5f (9.0±0.44) was the most active of the series.

As all the compounds showed antibacterial activity against the bacteria tested, it indicates that this basic moiety can be a potential scaffold for antibacterial drugs. However *B. subtilis* was the only susceptible organism and other organisms were found to be bit resistant. Thus further lead optimization is required to get wide spectrum of activity.

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References and Notes


