Synthesis and Antimicrobial Evaluation of Novel Thiophene Derivatives

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ABSTRACT
Thiophene nucleus has been established as the potential entity in the largely rising chemical world of heterocyclic compounds possessing promising pharmacological character. A series of tetrahydrobenzothiophene derivatives was synthesized with an purpose to build up novel and potent antimicrobial agents of synthetic origin. The required starting material ethyl-2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (1) was synthesized via a multi component condensation between sulphur, cyclohexanone and ethylcyanoacetate adopting Gewald Reaction. The Compound (1) was changed in to respective Schiff bases (KM1-KM4) by refluxing it with various aromatic aldehydes in dioxanen or 15 hours. The Schiff bases were further processed into the final compounds i.e. thiazolinediones derivatives (KM1-KM4) by treating them with thioglycollic acid in presence of anhydrous ZnCl2 in DMF and refluxing the reaction mixture for 4-5 hours. Synthesized compounds were purified, characterized and evaluated for their antimicrobial activity. Most of the compounds exhibited moderate to significant activities.

Keyword: antibacterial activity, antifungal activity, Schiff base and thiazolidinone

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INTRODUCTION
Heterocyclic compounds are widely distributed in nature and are essential for life. There are vast numbers of pharmacologically active heterocyclic compounds many of which are in regular clinical use[1]. The investigational approaches towards Structure- Activity Relationship focusing the search of optimized candidates have become immensely important. Literature survey reveals that thiophene is parent of a series of compounds that are important in medicinal and industrial chemistry. Thiophene is one of the most important classes of heterocyclic compounds with variety of biological activities. Substituted thiophene and their biheterocycles have received considerable attention during last two decades as they are endowed with wide range of therapeutic properties such as analgesic[2], antibacterial[3], antioxidant & anti-inflammatory[4], antifungal[5], anticancer[6] and local anaesthetic activity[7]. Thiophene can be fused with various heterocyclic nuclei giving rise to newer compounds having enhanced biological activities. Thiopyrimidines occupy special position among these compounds. Many of these derivatives exhibit antiallergic[8], antibacterial[9], antidepressant[10], antidiabetic[11], analgesic and anti-inflammatory[12] activities. In continuation to these efforts and with an objective to develop novel and potent therapeutic agents of synthetic origin, it was decided to synthesize certain thiazolidinone derivatives and evaluate them for their antimicrobial potential.

METHOD AND MATERIAL
The melting points of synthesized compounds were determined in open capillary tubes using Kshiti Innovations melting point apparatus, expressed in °C and are uncorrected. The IR spectra of compounds were recorded on Shimadzu Affinity-1 FTIR in KBr disc and absorption bands are expressed in cm⁻¹. 1H NMR spectra were recorded on Bruker Advance 400.13 MHz NMR Spectrometer (Chemical shift from ppm) using TMS as internal standard. The purity of the compounds was checked by TLC on silica gel G plates using ethyl acetate: nhexane (1:2) solvent system and iodine vapours as a visualizing agent.

Synthesis of ethyl-2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (1)
Sulphur (0.06 mole) was added to a mixture of ethyl cyanooacetate (0.05 mole) and cyclohexanone (0.05 mole) at room temperature with stirring. Diethylamine (0.05 mole) was added to this heterogeneous mixture and the reaction mixture was stirred at 45°C for 2 hours. Completion of reaction was monitored using TLC...
and mixture was kept overnight at room temperature. The precipitate was filtered, washed, dried and recrystallized from ethanol.

**Synthesis of Schiff bases (KM1-KM4)**

Equimolar quantities of Compound 1 (0.1 mole) and suitably chosen substituted aldehydes (0.1 mole) were suspended in 100 ml dioxane and the mixture was refluxed for 14-15 hours. Reaction was monitored by TLC and the mixture was cooled and poured into crushed ice. Solid thus obtained was filtered, washed with water, dried and recrystallized from ethanol.

**Synthesis of thiazolidinones (KM1-KM4)**

Equimolar mixture of Schiff base (0.1 mole) and thioglycolic acid (0.1 mole) were suspended in DMF (60 ml). Catalytic amount of zinc chloride (1 g) was added to it and the mixture was refluxed for 4 hours. Mixture was cooled and poured into crushed ice. Solid thus obtained was filtered, washed, dried and recrystallized from ethanol.

**Chemistry**

IR, 1H-NMR and elemental analysis were consistent with the assigned structure.

**Ethyl 2-[2-(2-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (KM1):**

Yield: 68.75%; Melting Point: 115°C; IR (KBr, cm⁻¹): 2854 (C-H str.), 1501 (C=O str.), 1282 (C-O str.), 3072 (Ar-H str.), 1358 (C-N str.), 791 (C str.), 1686 (C=O str.), 3297 (O-H str.); 1H NMR (CDCl3, δ ppm): 1.408-1.444 (t, 3H, OCH2CH3), 1.754-1.803 (m, 4H, C5 and C6), 2.503 - 2.518 (d, 2H, C4), 2.765 - 2.734 (d, 2H, C7), 3.470 (s, 2H, CH2 of thiazolidine), 4.249 - 4.302 (q, 2H, OCH2CH3), 5.421 (s, 1H, OH), 5.900 (s, 1H, NH), 6.920 - 7.021 (m, 4H, Ar H); Anal. Calcd. for C20H21NO4S2: C (59.23), H (5.78), N (3.40), O (15.81), S (15.82); found: C (58.62), H (6.36), N (4.00), O (15.72), S (15.12); Mol. Wt.: 409.

**Ethyl 2-[2-(4-methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (KM2):**

Yield: 70.5%; Melting Point: 120°C; IR (KBr, cm⁻¹): 2846 (C-H str.), 1282 (C-O str.), 3024 (Ar-H str.), 1391 (C-N str.), 780 (C str.), 1614 (C=O str.), 1125 (C=O str. in C-O-C); 1H NMR (CDCl3, δ ppm): 1.316 - 1.392 (t, 3H, OCH2CH3), 1.790 - 1.798 (m, 4H, C5 and C6), 2.481 - 2.499 (d, 2H, C4), 2.682 - 2.700 (d, 2H, C7), 3.416 (s, 2H, CH2 of thiazolidine), 3.739 (s, 3H, OCH3), 4.222 - 4.275 (q, 2H, OCH2CH3), 5.939 (s, 1H, N=CH), 6.442 - 7.248 (m, 4H, Ar-H); Anal. Calcd. for C21H23NO4S2: C (60.56), H (5.42), N (3.09), O (15.66), S (15.83); found: C (59.95), H (5.22), N (4.09), O (15.31), S (15.35); Mol. Wt.: 444.

**Ethyl 2-[3-(3,4-dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-4,5,6,7-tetrahydro1benzothiophene-3-carboxylate (KM3):**

Yield: 68.4%; Melting Point: 130°C; IR (KBr, cm⁻¹): 2856 (C-H str.), 1262 (C-O str.), 3043 (Ar-H str.), 1278 (C-N str.), 759 (C str.), 1627 (C=O str.), 1136 (C=O str. in C-O-C); 1H NMR (CDCl3, δ ppm): 1.324 - 1.362 (t, 3H, OCH2CH3), 1.749 - 1.756 (m, 4H, C5 and C6), 2.667 - 2.731 (m, 4H, C and C7), 3.442 (s, 2H, CH2 of thiazolidine), 3.945 (s, 6H, OCH3), 4.228 - 4.281 (q, 2H, OCH2CH3), 5.964 (s, 1H, N=CH), 6.452 - 7.042 (m, 3H, Ar-H); Anal. Calcd. for C22H25NO5S2: C (59.14), H (5.12), N (3.17), O (17.78), S (14.34); found: C (58.63), H (6.14), N (3.60), O (17.58), S (14.17); Mol. Wt.: 444.

**Ethyl 2-[2-(4-dimethylaminophenyl)-4-oxo-1,3-thiazolidin-3-yl]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (KM4):**

Yield: 56.2%; Melting Point: 135°C; IR (KBr, cm⁻¹): 2842 (C-H str.), 1532 (C=O str.), 1281 (C-O str.), 3076 (Ar-H str.), 1364 (C-N str.), 759 (C str.), 1662 (C=O str.); 1H NMR (CDCl3, δ ppm): 1.324 - 1.359 (t, 3H, OCH2CH3), 1.750 - 2.689 (m, 8H, C4, C5, C6 and C7), 3.050 (s, 6H, CH3), 3.875 (s, 2H, CH2 of thiazolidine), 4.241 - 4.387 (q, 2H, OCH2CH3), 5.940 (s, 1H, N=CH), 6.679 - 7.723 (m, 4H, Ar-H); Anal. Calcd. for C22H26N2O3S2: C (61.30), H (6.01), N (6.50), O (11.17), S (14.80); found: C (60.93), H (6.51), N (6.75), O (10.86), S (14.94); Mol. Wt.: 431.

**Biological Screening**

All the synthesized compounds were subjected to antimicrobial screening at a concentration of 100 µg/ml involving three Gram-ve bacteria (Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae) and three Gram+ve bacteria (Seratarietricula Bacillus subtilis and Streptococcus pneumoniae) and two fungal strains (P. aeruginosa and C. albicans) using Ampicillin as standard at the same concentration. The work, in reference, was carried out by Agar disc diffusion method [13]. The response of organisms to the synthesized compounds were measured in terms of zone of inhibition and compared with that obtained with standard.

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Preparation of Mueller Hinton agar media
Mueller Hinton Agar Media was used for antimicrobial screening and its composition is as: Casein Acid Hydrolysate 17.50gm, Beef Heart Infusion 2.00gm, Starch soluble 1.50gm and Agar 17.00gm. For preparing Mueller Hinton Agar (MHA) Media, 36gm of Mueller Hinton Agar No.2 was dissolved in 1000ml distilled water. It was mixed properly and heated to boil to dissolve the medium completely. It was autoclaved at 15lbs pressure (121°C) for 15 minutes. It was then cooled and poured into sterilized plates. All the plates were kept for 4-5 hours in laminar airflow until the media got solidified. The plates were then kept in an incubator at 37°C.

Preparation of Standard antibiotic solution
A solution [100μg/ml] of standard drug (Ampicillin) was prepared in sterile water.

Preparation of Test solution
10mg of the synthesized compound(s) was dissolved in 10ml of DMF. 1ml of this solution was taken and diluted to 10ml (with DMF) so that the concentration of the test solution became 100μg/ml.

Preparation of inoculums
For the preparation of inoculums, 5g of nutrient agar was dissolved in 100ml of distilled water and the pH was adjusted at 7.2±0.2. It was poured in test-tubes as per requirement and then sterilized by autoclaving at 121°C. A 24 hour old culture was used for the preparation of bacterial suspension. Likewise suspension of all the organisms were prepared as per standard procedure.

Preparation of Discs
Discs of 6-7mm in diameter were punched from No.1 Whatmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140°C one hour. Standard and test solutions were added separately to these discs which were air dried lateron.

Method of testing
Inoculums were added to the prepared media plates and allowed to solidify. The previously prepared discs were carefully kept on the solidified media by using sterilized forceps. These petridishes were kept for one-hour diffusion at room temperature and then for incubation at 37°C for 24 hours in an incubator. The zones of inhibition after 24 hours were measured in millimeters. The results obtained are shown in Table1 and Table2.

RESULT AND DISCUSSION
According to Gewald [14], heating under stirring of a mixture of ethylcyanoacetate, sulphur and cyclohexanone in diethylamine for 2-3 hours afforded ethyl-2-amino-4.5.6.7-tetrahydro-1-benzothiophene-3-carboxylate (1). Substantial proof for the formation of Schiff base (KM1-KM4) has been provided by differences in melting points and yield values from that of parent compound. Compound 1 on reaction with various substituted aldehydes yielded various Schiff bases which on cyclization withthioglycollic acid in catalytic amount of Cl2 yielded novel thiophene derivatives (KM1-KM4). The primary structural difference within this series involves the nature of various substituted aldehydes. Synthesized compounds were found to be crystalline in nature and easily soluble in chloroform, ethylacetate, benzene, DMSO and DMF but in soluble in hexane and toluene. With the help of analytical techniques such as meltingpoint, IRan1, HNMR, synthesized derivatives were characterized. These compounds showed abandat1646cm-1 for cyclic> C=O group [15]. All the compounds showed NMR signals for different kind of protons at their respective positions.

| Table 1: Antibacterial activity of synthesized thiazolidinones (KM1-KM4) | Zone of Inhibition in mm |
|---|---|---|---|---|---|---|
| Sample | E. coli | S. reticulata | S. aureus | B. subtilis | S. pneumoniae | K. pneumoniae |
| KM1  | -  | 14  | 15  | 16  | 10  | 11  |
| KM2  | 13 | 20  | 14  | 14  | 15  | 15  |
| KM3  | 14 | 18  | -   | -   | 13  | 13  |
| KM4  | 10 | 17  | 16  | 16  | 13  | 9   |
| Ampicillin | 22 | 30  | 24  | 25  | 22  | 20  |
| DMF  | -  | -   | -   | -   | -   | -   |

All compounds have been screened for their antimicrobial activity. From the screening results it was observed that the presence of electron withdrawing group and ester linkage made the compounds to exhibit moderate to significant activity in comparison to standard drug Ampicillin. Compound KM2 and
KM4 exhibited promising antibacterial activity while compound KM3 exhibited promising antifungal activity.

Table 2: Antifungal activity of synthesized thiazolidiones

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone of Inhibition in mm</th>
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<tbody>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td>KM1</td>
<td>10</td>
</tr>
<tr>
<td>KM2</td>
<td>9</td>
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<td>KM3</td>
<td>13</td>
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<tr>
<td>KM4</td>
<td>12</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>21</td>
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<tr>
<td>DMF</td>
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However other compounds of the series also exhibited moderate to significant activity against the microorganisms as mentioned above. Therefore compound KM2, KM3 and KM4 can be recommended for further studies. The above results established the fact that thiophene substituted with various aldehydes (substituted) can be studied further to explore out newer antimicrobial compounds.

CONCLUSION

The analytical and other informational data, available in literature so far, have rendered thiophene significantly important class of heterocyclic compounds and their applications in ever challenging chemotherapy of various ailments/ infections etc. since last two decades immensely hiked interests of medicinal chemist and biochemist. This particular research study, in reference, would extend great deal of help to researchers in reckoning and determining the best and most productive, economical, suggestive and conclusive access to various thiophenes of clinical importance superseding other compounds of their class. Further combinatorial libraries of these compounds can be generated which can be screened for optimal pharmacological activities by optimization techniques using 2D and 3D QSAR investigation.

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