SYNTHESIS, PHARMACOLOGICAL EVALUATION AND COMPUTATIONAL STUDIES OF SOME NOVEL HYDRAZONE DERIVATIVES OF THIOPHENE CHALCONE AS ANTIMICROBIAL AND ANTIOXIDANT AGENTS

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ABSTRACT

The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistant pathogens, functioning an ever-increasing used for new drugs. In an effort to develop antimicrobial agents a series of hydrazones derivative of thiophene were synthesized from benzylidene hydrazine and various thiophene chalcones. Benzylidene hydrazine was reacted with various thiophene chalcones with varying substitution at benzaldehyde portion of chalcones in the presence of HCl and hydrazone derivatives were synthesized. The synthesized hydrazones were characterized on the basis of basis of physical, chemical tests and spectroscopic data and were evaluated for their antimicrobial activity against various bacterial and fungal strains using cup plate method using nutrient agar media and also for antioxidant activity using DPPH assay and Nitric oxide scavenging methods. Furthermore the assessment of structural similarity of the target compounds with various standard drugs was done. Evaluation of the compounds revealed remarkable antibacterial, antifungal and antioxidant activity.

Keywords: Hydrazone, Thiophene chalcones, Antimicrobial activity, Antioxidant activity, Computational studies.

1. INTRODUCTION

A dramatic increase in microbial infection and microorganisms resistant to multiple antimicrobial agents is a serious problem worldwide; especially gram positive bacteria which triggered a clear need for the discovery of new antibacterial rather than analogs of existing
ones (Foroumadi et al., 2003; Papyne et al., 2004; Richet et al., 2001). Traditionally small molecules have been a reliable source for discovering novel biologically active compounds. Although a lot of work has been done on heterocycles, they remained an active area of research. Nitrogen and sulphur heterocyclic system families are very interesting due to their versatile pharmacological activities, such as antitumor, diuretics, fungicides, bactericides, antihelmintic, antiallergic, anti-ulcer and local analgesic (Kucukbay et al., 2004, 2003; Singh et al., 2000), especially in the sense of design of new drugs. Studies on thiophene and hydrazone like compounds have served as a feasible field of research in perusal of biologically active compounds. Hydrazones and molecules having hydrazide residues in their structures are capable of showing antifungal, antibacterial activities and in the treatment of tuberculosis infections (Abdel-Aziz, Abdel-Rahman, 2010; Banerjee et al., 2009).

On the other hand, an increasing interest in antioxidants, particularly in those intended to prevent the mischievous effects caused by free radicals in the human body is attracting one. The free radicals are also believed to be associated with carcinogenesis, mutagenesis, arthritis, diabetes, inflammation, cancer and genotoxicity (Buyukokuroglu et al., 2001; Kourounakis et al., 1999) due to oxidative stress, which arises as a result of imbalance between free radical generations. Thiophenes and hydrazones are also known to have considerable antioxidant activity and can be expressed by means of various in vitro models (Kumar et al., 2006; Padmashali, 2005).

Thus hydrazones of thiophene chalcone analogs might have antibacterial, antifungal and antioxidant activity. To test this idea we have synthesized 2-Benzylidene-1-(3-(4-substituted-phenyl)-1-(thiophen-2-yl) allylidene) hydrazine analogues [TS1-TS6] by reactions of benzylidene hydrazine [6] to thiophene chalcones [3a-f] in presence of acid and investigated in-vitro antibacterial, antifungal and antioxidant activities of synthesized compounds.

2. EXPERIMENTAL

2.1 Materials and Methods

All the reagents and chemicals were purchased from commercial sources (Thomas baker, Spectro chem, Lobe chemie etc.) Melting point of newly synthesized compounds was determined on Digital melting point apparatus and were found uncorrected. The solubility of synthesized compounds was tested in various solvents. IR spectra were recorded on a BRUKER ATR spectrometer. $^1$H NMR spectra were measured in CDCl$_3$ and recorded on Bruker ADVANCE II 400 MHz NMR spectrometer. The $\lambda_{\text{max}}$ was calculated by using UV-
Visible 1800 Shimadzu spectrophotometer. The progress of the reaction was monitored by thin layer chromatography (TLC) and spots were visualized in UV chamber.

2.2 Synthesis

2.2.1 General procedure for the synthesis of 3-(4-substituted phenyl)-1-(thiophen-2-yl) prop-2-en-1-one [3a-3f] (Kalirajan et al., 2009)

To a solution of 2-acetyltiophene (0.015 mol, 1.71 ml) [1] in ethanol, 5ml 5% KOH was added dropwise with continuous stirring on an ice bath. To this equimolar amount of benzaldehyde [2a] or 4-nitrobenzaldehyde [2b] or 4-methoxybenzaldehyde [2c] or 4-methylbenzaldehyde [2d] or 4-cyanobenzaldehyde [2e] or 4-fluorobenzaldehyde [2f] was added dropwise and the mixture was stirred until it became cloudy. Then the mixture was poured slowly into 400 ml of ice water, the crude solid so obtained was filtered, washed with water, dried and purified by recrystallizing them from suitable solvents.

2.2.2 General procedure for the synthesis of 1-benzylidenehydrazine [6]

A mixture of benzaldehyde (0.04 mol, 4.28 ml) [4] and hydrazine hydrate (0.03 mol, 1.265 ml) [5] in ethanol in 250 ml round bottom flask was stirred on an ice bath, until it became cloudy. Then the mixture was poured slowly into 400 ml of ice water, the crude solid so obtained was filtered, washed with water, dried and purified by recrystallizing them from ethanol.

2.2.3 General procedure for the synthesis of 2-benzylidene-1-(3-(4-substituted-phenyl)-1-(thiophen-2-yl) allylidene) hydrazine analogues [TS1-TS6]

To a solution of 1-benzylidenehydrazine [6] in rectified spirit, compound [3a or 3b or 3c or 3d or 3e or 3f], few drops of HCl was added and it was heated under reflux for 8 hours. The progress of the reaction was monitored by TLC. The solid formed after cooling was filtered off, dried and subjected to column chromatography with ethyl acetate/hexane and finally recrystallized by using suitable solvents.

a) 2-benzylidene-1-[E]-3-phenyl-1-(thiophen-2-yl) allylidene] hydrazine [TS1]

White powder (from ethanol), Yield: 82.14 %, M.pt. 236 ±2 °C; IR (ν cm⁻¹): 1584.21 (C=N-N=C) ¹HNMR (400 MHz, CDCl₃, δ ppm): 8.27 (s, 1H CH=N), 7.88 and 7.24 (d, 1H CH), 7.43 and 7.30 (d, 2H, Ar-H), 7.19 (d, 1H, C₆H₅S), 7.11 and 7.02 (t, 1H, C₆H₅), 7.10 and 7.10 (t, 2H, C₆H₅), 6.87 (t, 1H, C₆H₅S).
b) Synthesis of 2-benzylidene-1-[(E)-3-(4-nitrophenyl)-1-(thiophen-2-yl) allylidene] hydrazine [TS2]
Pale yellow powder (from ethanol), Yield: 85.00 %, M.pt. 317±2 °C; IR (v cm⁻¹): 1528.96 (C=N-N=C), 1578.19 (Ar-NO₂); ¹HNMR (400 MHz, CDCl₃, δ ppm): 8.27 (s, 1H CH=N), 7.88 and 7.24 (d, 1H CH), 7.43 and 7.30 (d, 2H, Ar-H), 7.19 (d, 1H, C₆H₅S), 7.11 and 7.02 (t, 1H, C₆H₅), 7.10 and 7.10 (t, 2H, C₆H₅), 6.87 (t, 1H, C₆H₅).

c) Synthesis of 2-benzylidene-1-[(E)-3-(4-methoxyphenyl)-1-(thiophen-2-yl) allylidene] hydrazine [TS3]
Yellow crystals (from methanol), Yield: 80.52 %, M.pt. 325±2 °C; IR (v cm⁻¹): 1515.11 (C=N-N=C), 1237.43 (OCH₃); ¹HNMR (400 MHz, CDCl₃, δ ppm): 8.13 (s, 1H CH=N), 7.82 and 7.12 (d, 1H CH), 7.46 and 7.30 (d, 2H, Ar-H), 7.28 and 7.21 (d, 1H, C₆H₅S), 7.12 (t, 1H, C₆H₅), 7.07 (t, 2H, C₆H₅), 6.60 (t, 1H, C₆H₅S), 6.60 (d, 2H, C₆H₅), 3.39 (s, 3H, OCH₃).

d) Synthesis of 2-benzylidene-1-[(E)-3-(methylphenyl)-1-(thiophen-2-yl) allylidene] hydrazine [TS4]
Off white powder (from ethanol), Yield: 75.36 %, M.pt. 283±2 °C; IR (v cm⁻¹): 1590.33 (C=N-N=C), 773.11 (CH₃); ¹HNMR (400 MHz, CDCl₃, δ ppm): 8.36 (s, 1H CH=N), 7.80 and 7.21 (d, 1H CH), 7.52 and 7.21 (d, 2H, Ar-H), 7.37 and 7.28 (d, 1H, C₆H₅S), 7.20 (t, 1H, C₆H₅), 7.19 (t, 2H, C₆H₅), 6.97 (t, 1H, C₆H₅S), 6.81 (d, 2H, C₆H₅), 2.13 (s, 3H, CH₃).

e) Synthesis of 2-benzylidene-1-[(E)-3-(cyanophenyl)-1-(thiophen-2-yl) allylidene] hydrazine [TS5]
Pale yellow powder (from ethanol), Yield: 73.65 %, M.pt. 279±2 °C; IR (v cm⁻¹): 1519.50 (C=N-N=C), 1647.36 (C≡N); ¹HNMR (400 MHz, CDCl₃, δ ppm): 8.35 (s, 1H CH=N), 7.83 and 7.18 (d, 1H CH), 7.53, 7.45 and 7.29 (d, 2H, Ar-H), 7.33 and 7.24 (d, 1H, C₆H₅S), 7.17 (t, 1H, C₆H₅), 7.15 (t, 2H, C₆H₅), 6.96 (t, 1H, C₆H₅).

f) Synthesis of 2-benzylidene-1-[(E)-3-(4-fluorophenyl)-1-(thiophen-2-yl) allylidene] hydrazine [TS6]
White powder (from ethanol), Yield: 85.62 %, M.pt. 252±2 °C; IR (v cm⁻¹): 1532.65 (C≡N-N=C), 1258.09 (C-F); ¹HNMR (400 MHz, CDCl₃, δ ppm): 8.24 (s, 1H CH=N), 7.71 and 7.09 (d, 1H CH), 7.42, 7.30 and 6.75 (d, 2H, Ar-H), 7.28 and 7.17 (d, 1H, C₆H₅S), 7.00 (t, 1H, C₆H₅), 6.96 (t, 2H, C₆H₅), 6.83 (t, 1H, C₆H₅S), 6.75 (d, 2H Ar-H).
Scheme-1  Synthesis of 2-benzylidene-1-(3-(4-substituted-phenyl)-1-(thiophen-2-yl)allylidene)hydrazine analogues [TS₁-TS₆]

1.2.4 Physical Properties of Synthesized Compounds

Table 1 and 2 shows all the physical data like color, percentage yield, molecular formula, molecular weight, solubility, melting point, $\lambda_{\text{max}}$, $R_f$ value of synthesized compounds.

**Table-1 Physical data of various synthesized compounds**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical structure</th>
<th>Color</th>
<th>%age yield</th>
<th>Mol. Formula</th>
<th>Mol. weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS₁</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>White powder</td>
<td>82.14</td>
<td>C$<em>{20}$H$</em>{16}$N$_2$S</td>
<td>316.429</td>
</tr>
<tr>
<td>TS₂</td>
<td><img src="image2" alt="Chemical structure" /></td>
<td>Pale yellow powder</td>
<td>85.00</td>
<td>C$<em>{20}$H$</em>{15}$N$_3$O$_2$S</td>
<td>361.427</td>
</tr>
<tr>
<td>TS₃</td>
<td><img src="image3" alt="Chemical structure" /></td>
<td>Yellow crystals</td>
<td>80.52</td>
<td>C$<em>{21}$H$</em>{18}$N$_2$OS</td>
<td>346.456</td>
</tr>
</tbody>
</table>
Table-2 Physical properties and UV-Visible analysis of various test compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solubility</th>
<th>Melting point (°C)</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt;</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS&lt;sub&gt;4&lt;/sub&gt;</td>
<td>EtOH, MeOH, CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>75.36</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>330.456</td>
</tr>
<tr>
<td>TS&lt;sub&gt;5&lt;/sub&gt;</td>
<td>MeOH, CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>73.65</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;15&lt;/sub&gt;N&lt;sub&gt;3&lt;/sub&gt;S</td>
<td>341.439</td>
</tr>
<tr>
<td>TS&lt;sub&gt;6&lt;/sub&gt;</td>
<td>EtOH, MeOH, CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>85.62</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;FN&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>334.420</td>
</tr>
</tbody>
</table>

TLC mobile phase- Ethyl acetate: Hexane (9:1)

3 PHARMACOLOGICAL EVALUATION

3.1 Antimicrobial activity

All the synthesized compounds were screened for their in-vitro antimicrobial activity against various bacterial strains; Staphylococcus aureus, Streptococcus thermophilus (Gram +ve bacteria) Escherichia coli, Pseudomonas aeruginosa (Gram –ve bacteria), fungal strain Candida albicans and yeast Kluyveromyces marxianus by Cup plate method using different concentrations (1600, 800, 400, 200, 100, 50, 25, 12.5, 6.5 µg/ml) of the test compounds. Ciprofloxacin (antibacterial) and Fluconazole (antifungal) was used as standards and DMF was used as control for all the strains. MIC (Minimum inhibitory concentration) was recorded for synthesized compounds as well as for standard and shown in Table-3 (IP, 1996; Panda, chowdary, 2008; Swamy, Agasimudin, 2008).
Table-3: Minimum inhibitory concentration (MIC) in µg/ml of synthesized compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC</th>
<th>PA</th>
<th>SA</th>
<th>ST</th>
<th>CA</th>
<th>KM</th>
</tr>
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<tbody>
<tr>
<td>TS1</td>
<td>50</td>
<td>21</td>
<td>31</td>
<td>42</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>TS2</td>
<td>200</td>
<td>50</td>
<td>20</td>
<td>28</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>TS3</td>
<td>12.5</td>
<td>28</td>
<td>12.5</td>
<td>15</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>TS4</td>
<td>25</td>
<td>30</td>
<td>25</td>
<td>12.5</td>
<td>20</td>
<td>12.5</td>
</tr>
<tr>
<td>TS5</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>19</td>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td>TS6</td>
<td>100</td>
<td>30</td>
<td>38</td>
<td>37</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>6.25</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

EC- Escherichia coli; PA- Pseudomonas aeruginosa; SA- Staphylococcus aureus; ST- Streptococcus thermophilus; CA- Candida albicans; KM- Kluyveromyces marxianus

3.2 Antioxidant Activity

All the synthesized hydrazones are evaluated for their *in vitro* antioxidant activity by following methods

3.2.1 DPPH Assay

The anti-oxidant potential of any compound can be determined on the basis of its scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical as described by Sadhu *et al* (Sadhu *et al.*, 2003). DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquot of the different concentrations (5-500 µg/mL) of the test sample is added to 3 mL of a 0.004% ethanolic solution of DPPH. Absorbance at 517 nm is determined after incubation in dark for 30 min, and IC$_{50}$ (Inhibitory concentration to scavenge 50% free radicals) is also determined. IC$_{50}$ value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.

The equation used to measure free radical scavenging is:

\[
\text{% DPPH Scavenging Activity} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100
\]

The experiment is performed in triplicate and average absorption is noted for each concentration. Ascorbic acid is used as a positive control. Results are expressed as mean inhibitory concentration (IC$_{50}$). A lower value of IC$_{50}$ indicates a higher free radical scavenging activity (Molyneux, 2004). % scavenging activity of the test compounds and standard are shown in Figure-1. Results are expressed as mean inhibitory concentration (IC$_{50}$) and shown in Table-4.
Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction according to the method of Marcocci. The chemical source of NO was sodium nitroprusside (5 mM) in 0.5 M phosphate buffer, pH 7.4, spontaneously generates nitric oxide in aqueous solution. Nitric oxide interacts with oxygen to produce stable products, leading to the production of nitrites (Marcocc et al., 1994).

About 1 ml sodium nitroprusside (5 mM) in 0.5 M phosphate buffer was mixed with 3.0 ml of different concentrations (20-100µg/ml) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 min. Ascorbic acid was used as standard. The capability to scavenge the NO radical was calculated using the following equation:

\[
\% \text{ NO Scavenging Activity} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100
\]

% scavenging activity of the test compounds is shown in Figure-2. Results are expressed as mean inhibitory concentration (IC\textsubscript{50}) and shown in Table-4.
Table-4 IC\textsubscript{50} (µg/ml) values of antioxidant activity of different synthesized compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Nitric oxide scavenging activity (IC\textsubscript{50} µg/ml)</th>
<th>DPPH Scavenging Activity (IC\textsubscript{50} µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS\textsubscript{1}</td>
<td>129</td>
<td>150</td>
</tr>
<tr>
<td>TS\textsubscript{2}</td>
<td>88</td>
<td>71</td>
</tr>
<tr>
<td>TS\textsubscript{3}</td>
<td>70</td>
<td>52</td>
</tr>
<tr>
<td>TS\textsubscript{4}</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>TS\textsubscript{5}</td>
<td>110</td>
<td>88</td>
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<td>TS\textsubscript{6}</td>
<td>130</td>
<td>100</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>70</td>
<td>40</td>
</tr>
</tbody>
</table>

3.3 Assessment of Structural Similarity of Target Compounds to Standard Drugs

Assessment of structural similarity of target compounds to standard drugs (Nikolova, 2003) involves the study of physiochemical and steric similarity between the standard drugs and new analogues designed. Assessment of structural similarity is a prerequisite for good spatial compatibility and effective binding to the site of action as lock and key model.

Several computational modules are available to predict molecular properties such as log P, polar surface area, molecular refractivity, ovality etc. this information can be related to prediction of biological activity for important drug targets. Therefore, we calculated a number of parameters for test compounds using Chem 3D ultra (version 8.0.3, Cambridge software) and compared them to the values obtained for standard compounds. The standard drugs used for assessment of similarity with target compounds are ticonazole, nifuroxazide (intestinal antiseptic), sertaconazole, pyridoxal salicyloyl hydrazone (antimycobacterial) and cefixime (Rollas, 2007; Singh et al., 1992). All the calculated parameters are given in Table-5.

The distance \( d_i \) of a particular target compound \( i \) could be presented according to the formula

\[
d_i^2 = \frac{\sum_{j=1}^{n} (X_{i,j} - X_{i,\text{standard}})^2}{n}
\]

Where, \( X_{i,j} \) is the value of molecular parameter \( i \) for compound \( j \).
\( X_{i,\text{standard}} \) is the value of same molecular parameter \( i \) for standard drug.
\( n \) is the total number of the considered molecular parameters.

Then the similarity of the compounds was calculated according to the formula

\[
\text{Similarity} (\%) = (1-R) \times 100
\]

Where \( R= \) quadratic mean (also known as the root mean square) and can be calculated as

\[
R = \sqrt{d_i^2}
\]

The %age similarity between test compound and standard drugs was given in Table-6.
Table 5: Calculation of Various Molecular Parameters of the Target Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>SAS^a</th>
<th>MS^b</th>
<th>SEV^c</th>
<th>Ovality</th>
<th>MR^d</th>
<th>MTL^e</th>
<th>WI^f</th>
<th>BI^g</th>
<th>MW^h</th>
<th>Log P</th>
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<tr>
<td>TS1</td>
<td>566.478</td>
<td>303.62</td>
<td>250.305</td>
<td>1.58063</td>
<td>98.830</td>
<td>9182</td>
<td>1170</td>
<td>305884</td>
<td>304.418</td>
<td>4.8605</td>
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<tr>
<td>TS2</td>
<td>601.753</td>
<td>325.329</td>
<td>269.737</td>
<td>1.61143</td>
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<td>572204</td>
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<td>275.436</td>
<td>1.60654</td>
<td>105.293</td>
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<td>1523</td>
<td>470214</td>
<td>334.444</td>
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<td>379995</td>
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<td>307.661</td>
<td>253.953</td>
<td>1.58642</td>
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<td>9977</td>
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<td>298.138</td>
<td>277.813</td>
<td>1.4479</td>
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<td>80.3531</td>
<td>8198</td>
<td>1156</td>
<td>387032</td>
<td>303.319</td>
<td>---</td>
</tr>
<tr>
<td>Std.5</td>
<td>650.956</td>
<td>361.159</td>
<td>337.033</td>
<td>1.54205</td>
<td>105.886</td>
<td>13778</td>
<td>2067</td>
<td>856808</td>
<td>427.505</td>
<td>---</td>
</tr>
</tbody>
</table>

Where,

- ^a^ Connolly Solvent Accessible Surface Area
- ^b^ Connolly Molecular Surface Area
- ^c^ Connolly Solvent Excluded Volume
- ^d^ Molar Refractivity
- ^e^ Molecular Topological Index
- ^f^ Wiener Index
- ^g^ Balaben Index
- ^h^ Molecular Weight

Std.1 - Ticonazole
Std.2 - Sertaconazole
Std.3 - Nifuroxazide
Std.4 - Pyridoxal salicyloyl hydrazide
Std.5 - Cefoxitin
Table-6 %age Similarity of target compounds with standard drugs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ticonazole (1-R)100</th>
<th>Sertaconazole (1-R)100</th>
<th>Nifuroxazide (1-R)100</th>
<th>Pyridoxal salicyloyl hydrazide (1-R)100</th>
<th>Cefoxitin (1-R)100</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS1</td>
<td>89.18</td>
<td>74.29</td>
<td>65.63</td>
<td>88.88</td>
<td>61.77</td>
</tr>
<tr>
<td>TS2</td>
<td>69.54</td>
<td>89.62</td>
<td>45.16</td>
<td>70.49</td>
<td>84.57</td>
</tr>
<tr>
<td>TS3</td>
<td>78.96</td>
<td>74.45</td>
<td>56.77</td>
<td>73.51</td>
<td>80.28</td>
</tr>
<tr>
<td>TS4</td>
<td>85.60</td>
<td>80.61</td>
<td>64.87</td>
<td>84.38</td>
<td>75.16</td>
</tr>
<tr>
<td>TS5</td>
<td>74.79</td>
<td>83.36</td>
<td>57.50</td>
<td>78.06</td>
<td>79.71</td>
</tr>
<tr>
<td>TS6</td>
<td>79.72</td>
<td>71.75</td>
<td>69.16</td>
<td>87.84</td>
<td>74.14</td>
</tr>
</tbody>
</table>

4. RESULTS AND DISCUSSION

Synthesis of hydrazone derivatives of thiophene chalcones (TS1-TS6) were carried out through base catalyzed claisen-schmidt condensation of 2-acetylthiophene with substituted benzoaldehyde in ethanol using at room temperature. The chalcones were obtained in high yields (>80%). Followed by subsequent treatment of thiophene chalcones derivatives with benzylidene hydrazine in the presence of HCl yielded 2-Benzylidene-1-(3-(4-substituted-phenyl)-1-(thiophen-2-yl) allylidene) hydrazine derivatives (TS1-TS6). Which were purified by crystallization technique. The final yield of the derivatives was in the range of 75-86% (Table 1). The compounds have been characterized by UV, IR and $^1$H-NMR.

Comparison of the compounds activity with that of standard antibiotic ciprofloxacin (for antibacterial activity) and fluconazole (for antibacterial activity) is effectively represented in the Table 3. Almost all compounds demonstrate the significant activity against all microorganisms, but only TS1 was more active than fluconazole. While studying MIC against fungal strains, compound TS3 was as active as fluconazole. TS1 was most active against C. albicans as compared to any other bacterial and fungal strain. TS1, TS3, and TS5 were moderately active against K. marxianus. TS3 was least active against C. albicans. TS1, TS2 and TS6 were more active against P. aeruginosa than E. coli. TS2, TS3, TS4 and TS5 were more active against S. aureus as compared to P. aeruginosa. TS2, TS6 were least active against E. coli. TS3, TS4 were most active against S. thermophilus. TS4, TS5, TS6 were more active against S. thermophilus than S. aureus. It was found that compounds containing methyl and methoxy group were more active against all bacterial and fungal strains. Structure and biological activity relationship of these compounds showed that presence of electron donating
group such as –OCH₃ and –CH₃ on aromatic ring enhanced the activity compared to unsubstituted aromatic ring.

While studying antioxidant activity by using DPPH method TS₂, TS₃ were the most active compounds (Table 4). TS₃ was as active as Ascorbic acid by NO method. TS₆ and TS₁ were least active compounds. TS₄ and TS₅ showed moderate antioxidant activity by both methods. While studying antioxidant activity by Nitric oxide scavenging method TS₂ and TS₃ were most active compounds. It was found that nitro and methoxy group enhanced the antioxidant activity of the synthesized compounds.

Assessment of structural similarities of target compounds with standard drugs showed that all compounds have good percentage similarity. All the synthesized compounds showed good structural similarity with ticonazole, sertaconazole and pyridoxal salicyloyl hydrazide as compared to other standard drugs. It was found that synthesized compounds have least similarity to nifuroxazide. Least structural similarity of target compounds to nifuroxazide was due to the absence of thiophene moiety in the standard compound.

5. CONCLUSION
We have synthesized new hydrazone derivatives from thiophene chalcone and evaluated for their antimicrobial (antibacterial and antifungal) activity and antioxidant activity. It has been found that the presence of methyl and methoxy group on phenyl group enhanced antimicrobial activity of the test compounds and the presence of nitro and methoxy group enhanced the antioxidant activity of the synthesized compounds. All the synthesized compounds showed good structural similarity with ticonazole, sertaconazole and pyridoxal salicyloyl hydrazide as compared to other standard drugs. It was found that synthesized compounds have least similarity to nifuroxazide.

REFERENCES


