SYNTHESIS OF SOME NEW ANTIPYRINE-THIOPHENE HYBRIDS AND THEIR EVALUATIONS AS ANTIOXIDANT AND ANTIBACTERIAL AGENTS

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ABSTRACT. A novel series of antipyrinyl thienyl ketones 4a-c, 7a-c, 10a-c, and 13a-b was chemically synthesized through the cyclocondensation of 4-chloroacetylantipyrine with various 2-substituted-thioacetanilide scaffolds, including 3-aryloxy-4-mercapto-4-phenylamino-buten-2-ones, ethyl 2-aryloxy-3-mercapto-3-phenylamino-acrylate, 2-cyano-3-mercapto-3-phenylamino-N-arylacrylilamide, 4-mercapto-4-phenylamino-buten-3-ene-2-one, and/or ethyl 3-mercapto-3-phenylaminoacrlylate. Indeed, the reaction of 4-chloroacetylantipyrine with 4-hydroxybenzaldehyde followed by refluxing with 2-cyanoacetohydrazide yielded 2-cyano-N’-(2-antipyrinyl-4-ynyl)-2-oxo(thio)benzylidenyl)-acetohydrazide 17 as a building compound, which was used consequentially to synthesize a set of new antipyrinyl thienyl hybrids 19a-d. The chemical structures of newly synthesized compounds were unambiguously confirmed using extensive elemental and spectral data analyses. The newly synthesized compounds were screened for their antioxidant and antimicrobial activities. Compared to the test reference (Ascorbic acid, 88.0%), the antipyrinyl thienyl ketones 13a and 13b substituted with methyl and/or hydroxyl groups at the thiophene ring system displayed excellent antioxidant properties, 87.8% and 87.2%, respectively. Additionally, antipyrinyl thienyl ketones 13a and 13b showed high antibacterial activities, and their relative activity index (which ranges from 68% to 91.7%) was close to that of a reference compound, Ampicillin.

KEY WORDS: 4-Chloroacetylantipyrine, Thioacetanilide, Antipyrinyl thienyl ketones, Antioxidant, Antimicrobial

INTRODUCTION

Thiophene derivatives represent an intriguing class of heterocyclic compounds, which are widely documented in numerous drug discovery studies. They occupy a unique position because advances in their synthesis, availability, stability, and structural simplicity make them useful scaffolds in pharmaceuticals and other therapeutics, like the best-selling drugs Olanzepine and Tinoridine [1–3] (Figure 1). Furthermore, thiophene derived molecules displayed a variety of pharmacological properties, including anti-inflammatory [4], antimicrobial [5], antihypertensive [6], anti-atherosclerotic properties [7], cytotoxicity in several cancer cell lines [8, 9], tubulin polymerization [10, 11], antioxidant [12], inhibitor for acetyl-CoA carboxylase [13], STAT3 inhibitors [14], antidepressant [15], antidiabetic [16], anti-tubercular [17], antifungal [18], enzyme inhibitor [19], anti-malarial drugs [20] and are used in the treatment of asthma [21]. On the other hand, thiophene derivatives are the largest class of industrial chemistry, thermal, optoelectronic properties and material science because of their wide utilized applications, such as materials for electroluminescence devices [22, 23], corrosion inhibitors [24], organic semiconductors [25], organic field-effect transistors (OFETs), and in the fabrication of organic light-emitting diodes (OLEDs) [26], synthesis of fluorescent chemo sensors [27]. Antipyrine is the first pyrazolone derivative that was introduced in 1884 to treat inflammation, pain, and fever [28] (Figure 1). Antipyrine-based derivatives are well-known to be used mainly as analgesics and

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antipyretic drugs. Additionally, they are strong inhibitors of cyclooxygenase isoenzymes, platelet thromboxane synthesis, and prostanoid synthesis [29]. These compounds demonstrate their effect through the inhibition of cyclooxygenases (COXs) because they are involved in the metabolism of arachidonic acid (AA) into prostaglandins (PGs) and thromboxanes (TXs) [30]. These chemical biological activities have also been linked to their ability to scavenge reactive oxygen and nitrogen species, as well as the suppression of neutrophil oxidative burst [31]. Antimicrobial [32, 33], antifungal [34], anti-angiogenic [35], antiviral [36], anticancer [37, 38], anti-ischemic [39], antiproliferative, antioxidant [40], and cytoxic [41] actions of these heterocyclic ring systems lead to other applications. In light of these findings, the current research project aims to synthesise chemically a set of new thiophene scaffolds containing the antipyrine moiety and investigate their antioxidant and antibacterial properties.

Figure 1. Selected biologically active thiophene and antipyrine compounds.

RESULTS AND DISCUSSION

The starting compound, 4-chloroacetylanditipyline (1), was synthesized by heating antipyrine with chloroacetyl chloride for four hours at 95-98 °C [42]. The reaction of 4-chloroacetylanditipyline (1) with 3-arylazo-4-mercapto-4-phenylamino-buten-2-ones 2a-c [43] was proceeded by nucleophilic displacement of the chlorine atom in refluxing ethanol containing sodium ethoxide. The corresponding 4-(4-arylazo-3-methyl-5-phenylamino-thiophene-2-carbonyl)-antipyrine derivatives 4a-c resulted from the intramolecular elimination of water molecule from the generated alkylated intermediates 3a-c (Scheme 1). The IR spectrum of compound 4a revealed two distinct absorptions at 3342 and 1662 cm⁻¹ corresponding to N-H and carbonyl (C=O) groups, respectively. In the 'H NMR spectrum of 4a, singlet signals were observed in the up-field region at δ 2.53, 2.57 and 3.34 ppm, each integrated for three protons, which have been assigned to the protons of three methyl groups (thiophene-CH₃, pyrazole-5-CH₃ and pyrazole-1-CH₃). The ¹³C NMR spectrum of compound 4a exhibited signals for the aliphatic carbon atoms in the expected regions: δ 12.15 (thiophene-CH₃), 13.50 (pyrazole-5-CH₃) and 34.06 ppm (pyrazole-1-CH₃).
addition, the signals of carbonyl groups were resonated at 162.23 (C=O, pyrazole ring) and 182.51 ppm (C=O, ketone). In the mass spectrum of compound 4a, the molecular ion peak was observed at \( m/z = 507.75 \), in agreement with the molecule’s chemical formula \( C_{29}H_{25}N_5O_2S \).

Scheme 1. Synthesis of antipyrinyl thiienyl ketones 4a-c.

When 4-chloroacetylantipyrine (1) was refluxed in sodium ethoxide with ethyl 2-arylazo-3-mercapto-3-phenylamino-acrylate derivatives 5a-c, the corresponding 4-(4-arylazo-3-hydroxy-5-phenylamino-thiophene-2-carbonyl)-antipyrine derivatives 7a-c were formed (Scheme 2). The antipyrinyl thienyl ketones 7a-c were obtained by intramolecular elimination of the ethanol molecule from the alkylated sulphide intermediates 6a-c. The structures of compounds 7a, 7b, and 7c were secured based on their interpreted spectroscopic analyses. The IR spectrum of compound 7a revealed two key absorption bands at broad 3447, 3382, and 1652 cm\(^{-1}\) corresponding to the O-H, N-H, and carbonyl (C=O) groups, respectively. The \( ^1\)H NMR spectrum of antipyrinyl thienyl ketone 7a exhibited three singlet signals at δ 2.38, 2.63 and 3.34 ppm to characterize the protons of three methyl groups (aromatic-CH\(_3\), antipyrine-5-CH\(_3\) and antihpirene-1-CH\(_3\)), respectively. The aromatic protons resonated as multiplet signals in the region from δ 7.17 to 7.68 ppm. The two singlet signals at δ 13.80 and 15.18 ppm clearly indicated the protons of the -NH and -OH groups, respectively. The \( ^{13}\)C NMR spectrum of 7a displayed signals for the aliphatic carbon atoms in the expected regions: 12.46 (pyrazole-5-CH\(_3\)), 21.26 (phenylene-CH\(_3\)), and 33.47 ppm (pyrazole-1-CH\(_3\)). Also, the signals for aromatic carbon atoms were indicated by their chemical shifts, 117.83, 120.11, 121.43, 122.43, 122.61, 125.24, 125.78, 126.16, 126.79, 127.02, 128.86, 129.10, 129.28, 129.59, 129.68, 129.76, and 129.93 ppm. In addition, the signals of carbonyl groups were resonated at δ 163.42 (C=O, pyrazole ring) and 187.78 ppm (C=O, ketone). The molecular ion peak at \( m/z = 523.33 \) (M\(^+\)), which matches the compound's molecular formula \( C_{29}H_{25}N_5O_2S\), was observed in the mass spectrum of compound 7a with a relative intensity of 43.1%.
The 2-cyano-3-mercapto-3-(phenylamino)-acrylamide derivatives 8a-c have been prepared by stirring their corresponding 2-cyanoacetamides with phenyl isothiocyanate in dry dimethyl formamide and KOH [44]. Refluxing of compounds 8a-c with 4-chloroacetylantipyrine (1) proceeded in ethanol and triethylamine to produce the 4-amino-5-(antipyrine-4-carbonyl)-N-aryl-2-(phenylamino)thiophene-3-carboxamide derivatives 10a-c (Scheme 3). According to the following mechanistic consideration, the production of 10a-c can be explained by the nucleophilic attack of active methylene on the cyano group. The alkylated sulphide intermediate 9 had little effect on molecule elimination, but it did undergo intramolecular methylene group addition on the nitrile function to create the antipyryl thienyl ketones 10a–c. The IR spectrum of compound 10a exhibited absorptions at 3371, 3273 and 1654 cm⁻¹ corresponding to the (NH₂ and N-H) and carbonyl (C=O) groups, respectively. The ¹H NMR spectrum of compound 10a exhibited three singlet signals at δ 2.29, 2.66 and 3.37 ppm characteristic for the protons of three methyl groups (aromatic-CH₃, pyrazole-5-CH₃; and pyrazole-1-CH₃). The aromatic protons resonated as multiplet signals in the region from δ 7.03 to 7.55 ppm. One singlet signal was observed at δ 9.50 ppm to characterize two protons of the –NH₂ group. The protons of two N-H groups resonated as two singlet signals at δ 10.05 and 11.71 ppm. The ¹³C NMR spectrum of 10a displayed signals for the aliphatic carbon atoms in the expected regions, 12.51 (pyrazole-5-CH₃), 20.80 (aromatic-CH₃), and 33.23 ppm (pyrazole-1-CH₃). The signals for aromatic carbon atoms were indicated by their chemical shifts, 120.21, 121.23, 124.30, 127.17, 127.53, 127.85, 128.93, 129.18, 129.91, 130.22, 132.76, and 135.99 ppm. In addition, the signals of carbonyl groups were resonated at δ 162.68 (C=O, pyrazole ring), 164.50 (C=O, amide), and 186.30 ppm (C=O, ketone).
Scheme 3. Synthesis of antipyrinyl thienyl ketones 10a-c.

Treatment of the 4-chloroacetylantipyrine (1) with various thioacetanilide derivatives 11a and 11b in boiling ethanol and a catalytic amount of triethylamine furnished the corresponding 4-(3-substituted-5-phenylamino-thiophene-2-carbonyl)-antipyrine derivatives 13a and 13b, respectively (Scheme 4). Spectroscopic techniques and adequate elemental analysis were used to determine the structures of antipyrinyl thienyl ketones 13a and 13b. The IR spectrum of 13a displayed absorptions at 3239 and 1626 cm\(^{-1}\) corresponding to imino (N-H) and carbonyl (C=O) groups, respectively. The \(^1\)H NMR signals were identified as singlet signals at 2.33, 2.36 and 3.25 ppm attributed to the protons of three methyl groups (thiophene-CH\(_3\), pyrazole-5-CH\(_3\), and pyrazole-1-CH\(_3\)). In addition, the thiophene proton resonated as a singlet at 6.38 ppm, while the proton of the N-H group resonated as a singlet signal at 9.70 ppm. While the \(^13\)C NMR spectrum of 13a displayed several signals for the aliphatic carbon atoms in the expected regions, 11.51 (pyrazole-5-CH\(_3\)), 16.31 (thiophene-CH\(_3\)), and 34.26 (pyrazole-1-CH\(_3\)). The signals for aromatic carbon atoms were indicated by their chemical shifts, 116.69, 121.33, 123.35, 125.68, 127.54, 129.26, 129.48, and 134.56 ppm. The signals at 108.51 and 155.07 ppm indicated the carbon atoms of pyrazole-C4 and pyrazole-C5, respectively. The carbon signals of the thiophene ring were observed at \(\delta\) 113.04 (C-4), 141.88 (C-2), 145.49 (C-3), and 154.05 (C-5) ppm. In addition, the signals of carbonyl groups were resonated at 161.94 (C=O, pyrazole ring) and 179.35 ppm (C=O, ketone). The molecular ion peak at \(m/z = 403.13\) (M\(^+\)) with a relative intensity of 33.7%, which matches the molecular formula of the compound C\(_{23}\)H\(_{15}\)N\(_3\)O\(_2\)S, was observed in the mass analysis of compound 13a.
Scheme 4. Synthesis of antipyrinyl thienyl ketones 13a and 13b.

The precursor, 4-(2-(antipyrid-4-yl)-2-oxoethoxy)-benzaldehyde (15) has been prepared by stirring of 4-chloroacetylantipyrine (1) with 4-hydroxybenzaldehyde (14) in dimethyl sulfoxide (DMSO) and potassium carbonate. Spectroscopic techniques and adequate elemental analysis were used to determine the structure of compound 15. The IR spectra exhibited absorption bands corresponding to carbonyl (3 C=O) groups at 1677 and 1655 cm\(^{-1}\). The \(^1\)H NMR signals were presented at their expected regions; two singlet signals were observed at δ 2.58 and 3.38 ppm, indicating the protons of two methyl groups (pyrazole-5-CH\(_3\)) and (pyrazole-1-CH\(_3\)) and a singlet for two protons of methylene group (-OCH\(_2\)-) at δ 5.33 ppm. The aromatic protons appeared as doublet and triplet signals at δ 7.01-7.83 ppm. The proton of the formyl group (-CHO) was observed as a singlet at δ 9.83 ppm. Refluxing of compound 15 with 2-cyanoacetohydrazide (16) in ethanol yields 2-cyano-N'-(4-(2-(antipyrid-4-yl)-2-oxoethoxy)benzylidene)acetohydrazide (17), which undergoes stirring with phenyl isothiocyanate in dimethyl formamide and potassium hydroxide to furnish 2-cyano-N'-(4-(2-(antipyrid-4-yl)-2-oxoethoxy)benzylidene)-3-mercapto-3-(phenylamino)-acrylohydrazide (18). The structures of 17 and 18 were elucidated by spectroscopic techniques and satisfactory elemental analysis. The IR spectrum of 18 revealed the characteristic absorptions of N-H functions at 3206 cm\(^{-1}\). The absorption frequencies of nitrile (C≡N) and carbonyl (C=O) groups were observed at 2178 and broad near 1655 and 1630 cm\(^{-1}\), respectively. The \(^1\)H NMR spectrum of compound 18 exhibited two singlet signals at δ 2.58 and 3.17 ppm, indicating the protons of two methyl groups (pyrazole-5-CH\(_3\)) and (pyrazole-1-CH\(_3\)) and two protons of a methylene group (OCH\(_2\)) at δ 5.24 ppm. The aromatic protons are observed as multiplet signals in the region from δ 7.14 to 7.57 ppm. The proton of the imine function (CH=N) is resonated as a singlet at δ 7.91 ppm. The protons of the N-H groups are resonated as singlet signals at δ 10.57 and 11.67 ppm and sulfanyl proton (-SH) is exhibited as singlet signal at δ 12.71 ppm.

The corresponding 3-aminothiophene derivatives 19a-d were obtained by treating thiocarbamoyl derivative 18 with several alpha-chlorocarbonyl reagents (namely, chlorooacetone, ethyl chloroacetate, ethyl 4-chloroacetoacetate, and/or phenacyl chloride) in boiling ethyl alcohol and triethylamine (Scheme 5). The chemical structures of 3-aminothiophenes 19a-d have been

confirmed using spectroscopic data. The IR spectrum of all thiophene derivatives displayed absorption bands at a range of 3425-3419 cm\(^{-1}\) indicating the amino (-NH\(_2\)) groups of the newly furnished 3-aminothiophene moiety. Also, the presence of broad absorption bands at 1636, 1636, 1638, and 1633 cm\(^{-1}\) is referred to carbonyl groups of \(19a, 19b, 19c,\) and \(19d,\) respectively. The mass spectrum of compound \(19a\) displayed the molecular ion peaks at \(m/z = 622.05 (M^+)\), which agrees with the molecular formula of the compound \(C_{33}H_{30}N_6O_5S\). The mass analysis of thiophene \(19d\) has the molecular ion peak at \(m/z\) group 684.17 (\(M^+\)), which matches the formula \(C_{38}H_{32}N_6O_5S\) followed by a subsequent and synchronous fragmentation to give the basic fragment peak at \(m/z = 224.06 (100\%)\).

Scheme 5. Synthesis of antipyrine thiophene hybrids \(19a-d\).

**Antioxidant activity**

The antioxidant activity of the produced antipyrine-thiophene analogues was assessed using the ABTS method, which uses a stable free radical generated from 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) as a radical cation [45, 46]. The results (Table 1) clearly demonstrated that the antipyrinyl thiienyl ketones \(13a\) and \(13b,\) which were substituted with a methyl or hydroxyl group at the third position of the thiophene ring, had excellent antioxidant activity, with percentage inhibition of 87.2% and 87.7%, respectively, when compared to the test reference (ascorbic acid, 88.0%). The next potent antioxidant compound is antipyrinyl thiienyl ketone \(4c,\) substituted by a methyl group at position-3 of the thiophene ring and methoxy at the phenylene moiety with percentage inhibition of 83.5%. The antioxidant activity of antipyrinyl
thienyl ketone $^{10b}$, which was substituted by an amino group at position-3 of the thiophene ring and methoxy at the phenylene moiety, was moderate, with a percentage inhibition of 64.9%. Unfortunately, the introduction of the thiophene ring system into 2-cyano-$N^1$-(4-2-(antipyrin-4-yl)-2-oxoethoxy)benzylideneacetohydrazide (17), percentage inhibition 67.8%, did not promote the antioxidant activity and resulted in weak percentage inhibitions (49.4-28.8%) for the antipyrrine thiophene analogues $^{19a-d}$.

Table 1. *In vitro* Antioxidant activity of the synthesized antipyrine thiophene analogues $^4$, $^7$, $^{10}$, $^{13}$, and $^{19}$.

<table>
<thead>
<tr>
<th>Method</th>
<th>Compound</th>
<th>Absorbance of samples</th>
<th>ABTS</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control of ABTS</td>
<td>0.510</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>0.206</td>
<td>59.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>0.193</td>
<td>62.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4c</td>
<td>0.084</td>
<td>83.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td>0.271</td>
<td>46.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7b</td>
<td>0.285</td>
<td>44.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7c</td>
<td>0.396</td>
<td>22.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10a</td>
<td>0.326</td>
<td>36.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10b</td>
<td>0.179</td>
<td>64.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10c</td>
<td>0.423</td>
<td>17.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13a</td>
<td>0.065</td>
<td>87.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13b</td>
<td>0.062</td>
<td>87.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.406</td>
<td>20.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0.164</td>
<td>67.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0.237</td>
<td>53.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19a</td>
<td>0.363</td>
<td>28.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19b</td>
<td>0.298</td>
<td>41.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19c</td>
<td>0.258</td>
<td>49.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19d</td>
<td>0.340</td>
<td>33.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.061</td>
<td>88.0%</td>
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</tbody>
</table>

**Antibacterial activities**

The new antipyrine thiophene analogues were tested in vitro against two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). Table (2) shows the results for antibacterial activity and indicates that there are substantial changes in the diameter of inhibition zones. The new antipyrine thiophene hybrids $^4$, 17 and 18 showed good antibacterial action, inhibiting the development of bacteria with relative activity indexes ranging from 52 to 82.6%. On the other hand, the antipyrinyl thienyl ketones $^{13a}$ and $^{13b}$, which have a methyl or hydroxyl group at the third position of the thiophene ring, have extremely high antibacterial activity, inhibiting the growth of bacteria (P. *aeruginosa*, S. *aureus*, and B. *subtilis*) with relative activity indexes ranging from 75.0% to 91.7%. They had an action similar to that of Ampicillin (a typical inhibitor), which inhibits bacteria with inhibition zones of 23 mm to 25 mm.
### Table 2. Diameter of inhibited zones (I.Z.D) in mm as a criterion of antibacterial activity of the synthesized antipyrine thiophene analogues 4, 7, 10, 13, and 19 at a concentration level of 20 mg/mL.

<table>
<thead>
<tr>
<th>Cpd. No.</th>
<th>Gram-negative bacteria</th>
<th>Gram-positive bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli (mm) Activity index (%) I.Z.D</td>
<td>P. aeruginosa (mm) Activity index (%) I.Z.D</td>
</tr>
<tr>
<td>4a</td>
<td>7 28</td>
<td>13 56.5</td>
</tr>
<tr>
<td>4b</td>
<td>9 36</td>
<td>15 65.2</td>
</tr>
<tr>
<td>4c</td>
<td>4 56</td>
<td>19 82.6</td>
</tr>
<tr>
<td>7a</td>
<td>4 16</td>
<td>9 39.1</td>
</tr>
<tr>
<td>7b</td>
<td>5 20</td>
<td>18 35.8</td>
</tr>
<tr>
<td>7c</td>
<td>NA ----</td>
<td>NA ----</td>
</tr>
<tr>
<td>10a</td>
<td>NA ----</td>
<td>5 21.7</td>
</tr>
<tr>
<td>10b</td>
<td>10 40</td>
<td>16 69.6</td>
</tr>
<tr>
<td>10c</td>
<td>NA ----</td>
<td>NA ----</td>
</tr>
<tr>
<td>13b</td>
<td>19 76</td>
<td>21 81.3</td>
</tr>
<tr>
<td>15</td>
<td>NA ----</td>
<td>NA ----</td>
</tr>
<tr>
<td>17</td>
<td>10 22</td>
<td>17 73.9</td>
</tr>
<tr>
<td>18</td>
<td>10 40</td>
<td>12 52.2</td>
</tr>
<tr>
<td>19a</td>
<td>NA ----</td>
<td>2 8.7</td>
</tr>
<tr>
<td>19b</td>
<td>2 8</td>
<td>6 26.1</td>
</tr>
<tr>
<td>19c</td>
<td>8 32</td>
<td>10 43.5</td>
</tr>
<tr>
<td>19d</td>
<td>NA ----</td>
<td>3 13</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>25</td>
<td>23</td>
</tr>
</tbody>
</table>

* NA → No Activity.

### EXPERIMENTAL

A Gallenkamp electric instrument was used to figure the melting points. Thermo Scientific Nicolet iS10 FTIR spectrometer was used to measure IR spectra (KBr discs). $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectra were acquired using a Joel spectrometer. Mass spectra were recorded on a GC-MS QP-1000 EX Shimadzu instrument by EI mode at 70 eV. Elemental analyses were determined on PerkinElmer 2400 analyzer.

**Synthesis of 4-(3-methyl-5-(phenylamino)-4-(4-substitutedphenylazo)thiophene-2-carbonyl)antipyrine derivatives 4a-c.** A suspension of 4-chloroacetylantipyrine (1) (0.27 g, 0.001 mol) and thiocarbamoyl compounds, for example, 4-mercapto-4-(phenylamino)-3-phenylazo-but-3-en-2-one (2a) or 4-mercapto-4-phenylamino-3-(p-tolylazo)but-3-en-2-one (2b) or 3-(p-anisylazo)-4-mercapto-4-(phenylamino)but-3-en-2-one (2c) (0.001 mol) was stirred for 15 minutes in sodium ethoxide (prepared by dissolving 0.05 g sodium metal in 20 mL ethanol), and then refluxed for additional 2 h. The mixture was cooled, the solid that obtained was collected, dried, and recrystallized from ethanol to give the target antipyrinyl thienyl ketones 4a-c.

**4-(3-Methyl-5-phenylamino-4-(phenylazo)-thiophene-2-carbonyl)antipyrine (4a).** Red crystals, yield 69%, mp 228-230 °C. IR (KBr): 3342 (N-H), 1662 cm$^{-1}$ (2C=O). $^1$H NMR (CDCl$_3$) $\delta$/ppm: 2.53 (s, 3H, CH$_3$), 2.57 (s, 3H, CH$_3$), 3.34 (s, 3H, NCH$_3$), 7.16-7.20 (m, 2H), 7.31-7.33 (m, 4H), 7.37-7.41 (m, 5H), 7.48 (t, $J$ = 7.5 Hz, 2H), 7.55 (d, $J$ = 7.00 Hz, 2H). $^{13}$C NMR (DMSO-d$_6$) $\delta$/ppm: 12.15, 13.50, 34.06, 109.08, 117.42 (2C), 120.64 (2C), 125.17, 125.84, 126.15 (2C), 128.23, 129.28 (2C), 129.41 (2C), 128.45 (3C), 134.13, 137.44, 140.12, 145.76, 164.05, 155.64, 156.97, 162.23, 182.51. MS (m/z, %): 507 (M$^+$, 21.4), 449 (23.2), 396 (28.7), 371 (22.5), 362

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(28.4), 345 (49.1), 316 (25.5), 292 (34.5), 266 (38.2), 224 (31.2), 214 (40.4), 199 (43.5), 174 (44.0), 170 (23.2), 143 (27.8), 141 (43.5), 120 (66.4), 112 (22.8), 91 (100.0), 75 (34.3), 54 (53.8).

Anal. calcd. for C$_2$H$_2$N$_2$O$_5$S (507.17): C, 68.62; H, 4.96; N, 13.80%. Found: C, 68.79; H, 4.90; N, 13.88%.

4-(3-Methyl-5-phenylamino-4-(p-tolylazo)-thiophene-2-carbonyl)antipyrine (4b). Red crystals, yield 82%, mp 220-222°C. IR (KBr): 3349 (N–H), 1672 cm$^{-1}$ (2C=O). $^1$H NMR (CDCl$_3$) δ/ppm: 2.37 (s, 3H, CH$_3$), 2.55 (s, 3H, CH$_3$), 2.58 (s, 3H, CH$_3$), 3.33 (s, 3H, NCH$_3$), 7.17 (t, J = 7.50 Hz, 1H), 7.20 (d, J = 8.00 Hz, 2H), 7.33 (d, J = 8.00 Hz, 4H), 7.39-7.43 (m, 3H), 7.48 (d, J = 8.00 Hz, 2H), 7.50 (d, J = 8.00 Hz, 2H). $^{13}$C NMR (CDCl$_3$) δ/ppm: 12.07, 13.60, 21.10, 34.07, 109.30, 118.12 (2C), 120.32 (2C), 124.83, 126.01 (2C), 126.31, 128.06, 129.38 (2C), 129.69 (2C), 129.82 (2C), 134.05, 136.10, 136.34, 141.93, 144.38, 145.26, 155.25, 155.56, 162.25, 182.15. Anal. calcd. for C$_{16}$H$_{14}$N$_2$O$_5$S (521.19): C, 69.08; H, 5.22; N, 13.43%. Found: C, 69.00; H, 5.18; N, 13.36%.

4-(4-(p-Anisylazo)-3-methyl-5-phenylaminothiophene-2-carbonyl)antipyrine (4e). Reddish brown crystals, yield 87%, mp 240-242°C. IR (KBr): 3347 (N–H), 1657 cm$^{-1}$ (2C=O). $^1$H NMR (DMSO-d$_6$) δ/ppm: 2.46 (s, 3H, CH$_3$), 2.51 (s, 3H, CH$_3$), 3.32 (s, 3H, NCH$_3$), 3.80 (s, 3H, OCH$_3$), 7.04 (d, J = 9.00 Hz, 2H), 7.22 (t, J = 7.50 Hz, 1H), 7.34 (d, J = 7.50 Hz, 2H), 7.41 (t, J = 7.50 Hz, 3H), 7.46-7.54 (m, 4H), 7.75 (d, J = 9.00 Hz, 2H), 13.26 (s, 1H, NH). $^{13}$C NMR (DMSO-d$_6$) δ/ppm: 11.69, 13.55, 34.05, 55.60, 114.77 (2C), 120.52 (4C), 121.27, 126.54 (2C), 128.18, 129.42 (2C), 129.85 (4C), 131.04, 132.22, 134.31, 142.13, 144.54, 145.26, 151.57, 154.93, 161.89, 181.17. Anal. calcd. for C$_{16}$H$_{14}$N$_2$O$_5$S (537.18): C, 67.02; H, 5.06; N, 13.03%. Found: C, 66.86; H, 5.12; N, 13.14%.

Synthesis of 4-(3-hydroxy-5-(phenylamino)-4-(4-substitutedphenylazo)-thiophene-2-carbonyl)antipyrines 5a-c. Each thiocarbamoyl compound, 2-(p-tolylidrazono)-2-ethoxy carbonylthioacetanilide (5a) (0.34 g, 0.001 mol), 2-(p-anisylidrazono)-2-ethoxy carbonylthioacetanilide (5b) (0.36 g, 0.001 mol) or 2-(p-chlorophenylidrazono)-2-ethoxy carbonylthioacetanilide (5c) (0.36 g, 0.001 mol), was dissolved in sodium ethoxide solution (0.05 g sodium metal in 20 mL ethanol). To this solution, 4-chloroacetylanthipyrine (1) (0.27 g, 0.001 mol) was added and refluxing was continued for 2-3 h. The mixture was cooled, then poured into ice cold water and neutralized with 0.1N HCl. To obtain the desired antipyryl thiényl ketones 7a-c, the solid that formed was filtered, dried and then recrystallized from ethanol.

4-(3-Hydroxy-5-phenylamino-4-(p-tolylazo)thiophene-2-carbonyl)antipyrine (7a). Red crystals; yield 46%, mp 238-240°C. IR (KBr): broad at 3447, 3382 (O-H and N-H), broad at 1652 cm$^{-1}$ (2C=O). $^1$H NMR (CDCl$_3$) δ/ppm: 2.38 (s, 3H, CH$_3$), 2.63 (s, 3H, CH$_3$), 3.34 (s, 3H, NCH$_3$), 7.17-7.68 (m, 14H), 13.80 (s, 1H, NH), 15.18 (s, 1H, OH). $^{13}$C NMR (CDCl$_3$) δ/ppm: 12.46, 21.26, 33.47, 117.83 (2C), 120.11, 121.43 (2C), 122.61, 125.24, 125.78, 126.16, 126.79, 127.02 (2C), 128.86, 129.10, 129.28, 129.59 (2C), 129.68 (2C), 129.76, 129.93 (2C), 146.25, 155.09, 163.42, 187.78. MS (m/z, %): 523 (M$^+$, 43.1), 490 (18.6), 448 (26.8), 430 (50.8), 429 (42.6), 427 (32.6), 418 (27.1), 408 (32.3), 355 (28.0), 350 (24.1), 345 (31.6), 342 (52.0), 282 (24.6), 225 (30.7), 222 (30.5), 189 (36.8), 178 (32.3), 149 (25.6), 135 (43.5), 106 (88.2), 90 (24.0), 81 (30.7), 74 (59.3), 67 (100.0), 58 (27.3), 54 (27.1), 52 (32.4). Anal. calcd. for C$_2$H$_2$N$_2$O$_5$S (523.61): C, 66.52; H, 4.81; N, 13.38%. Found: C, 66.42; H, 4.77; N, 13.30%.

4-(4-(p-Anisylazo)-3-hydroxy-5-phenylaminothiophene-2-carbonyl)antipyrine (7b). Deep red crystals; yield 35%, mp 160-162°C. IR (KBr): broad at 3445, 3390 (O-H and N-H), broad at 1643 cm$^{-1}$ (2C=O). $^1$H NMR (CDCl$_3$) δ/ppm: 2.64 (s, 3H, CH$_3$), 3.31 (s, 3H, NCH$_3$), 3.85 (s, 3H, OCH$_3$), 6.95 (d, J = 9.00 Hz, 2H), 7.34 (d, J = 8.00 Hz, 3H), 7.41-7.36 (m, 6H), 7.54-7.48 (m.

4-(4-(p-Chlorophenylazo)-3-hydroxy-5-phenylaminothiophene-2-carbonyl)antipyrine (7c).

Brown crystals, yield 90%, mp 180-182 °C. IR (KBr): broad at 3445, 3394 (O-H and N-H), broad at 1642 cm⁻¹ (C=O). ¹H NMR (CDCl₃) δ ppm: 2.65 (s, 3H, CH₃), 3.35 (s, 3H, NCH₃), 7.20 (t, J = 8.00 Hz, 1H, Ar-H), 7.45-7.35 (m, 7H, Ar-H), 7.5-6.49 (m, 2H, Ar-H), 7.70 (s, 1H, Ar-H), 13.61 (s, 1H, NH), 14.98 (s, 1H, OH). ¹³C NMR (CDCl₃) δ ppm: 12.45, 33.94, 118.85, 120.91, 121.57 (2C), 125.56, 126.18, 126.74, 127.09 (2C), 129.30, 129.42, 129.48, 129.69 (4C), 129.74 (4C), 131.94, 132.86, 133.75, 155.09, 163.14, 186.19. Anal. calcd. for C₂₇H₂₉N₅O₂S (544.03): C, 61.82; H, 4.08; N, 12.87%. Found: C, 61.76; H, 4.04; N, 12.77%.

4-Amino-(antipyrine-4-carbonyl)-2-(phenylamino)-N-substituted thiophenes 10a-

c. A mixture of 4-chloroacetanilide (1) (0.27 g, 0.001 mol) and 2-cyano-3-mercapto-3-(phenylamino)-N-(p-tolyl)acrylamide (8a), 2-cyano-3-mercaptop-N-(4-methoxyphenyl)-3-(phenylamino)acrylamide (8b) or N-(4-chlorophenyl)-2-cyano-3-mercaptop-3-(phenylamino)acrylamide (8c) (0.001 mol) in ethanol (25 mL) and triethylamine (0.5 mL) was refluxed for 5-6 h. Then, the mixture was cooled at room temperature; the formed precipitate was filtered off, dried well and recrystallized from ethanol to afford 4-amino-(antipyrine-4-carbonyl)-2-(phenylamino)-N-substituted thiophenes 10a, 10b, and 10c, respectively.

4-Amino-(antipyrine-4-carbonyl)-2-(phenylamino)-N-(p-tolyl)thiophene-3-carboxamide (10a).

Red crystals, yield 46%, mp 180-182 °C. IR (KBr): 3371, 3273 (2N=H and NH₂), broad at 1654 cm⁻¹ (C=O). ¹H NMR (CDCl₃) δ ppm: 2.29 (s, 3H, CH₃), 2.66 (s, 3H, CH₃), 3.37 (s, 3H, NCH₃), 7.03-7.15 (m, 5H, 7.29-7.35 (m, 4H), 7.44-7.55 (m, 5H), 9.50 (s, 2H, NH₂), 10.05 (s, 1H, N-H), 11.71 (s, 1H, N-H). ¹³C NMR (CDCl₃) δ ppm: 12.51, 20.80, 53.23, 99.74, 103.03, 120.21 (2C), 121.23 (2C), 124.30, 127.17, 127.53, 127.85 (2C), 128.93 (2C), 129.18 (2C), 129.91 (2C), 130.22, 132.76, 135.99, 150.12, 154.89, 162.68, 163.84, 164.50, 170.36, 186.30. Anal. calcd. for C₁₈H₁₇N₅O₂S (377.64): C, 67.02; H, 5.06; N, 13.03%. Found: C, 67.20; H, 4.98; N, 13.14%.

4-Amino-(antipyrine-4-carbonyl)-2-(phenylamino)-N-(p-anisyl)thiophene-3-carboxamide (10b).

Red crystals, yield 38%, mp 174-176 °C. IR (KBr): 3424, 3343, 3264 (2N=H and NH₂), 1656 cm⁻¹ (C=O). ¹H NMR (CDCl₃) δ ppm: 2.70 (s, 3H, CH₃), 3.38 (s, 3H, NCH₃), 3.78 (s, 3H, OCH₃), 6.82-6.88 (m, 3H), 7.17-7.20 (m, 2H), 7.27-7.43 (m, 3H), 7.46-7.56 (m, 6H), 11.08 (s, 2H, NH₂), 11.50 (s, 1H, NH), 12.87 (s, 1H, NH). ¹³C NMR (CDCl₃) δ ppm: 12.69, 33.41, 55.45, 92.69, 100.14, 103.37, 113.95 (2C), 121.31 (2C), 122.00 (2C), 124.34, 127.79 (2C), 129.00 (2C), 130.07 (2C), 130.31, 131.60, 131.81, 150.26, 155.26, 155.75, 162.89, 163.81, 164.43, 170.11, 186.31. Anal. calcd. for C₁₉H₁₇N₅O₂S (553.64): C, 65.08; H, 4.92; N, 12.65%. Found: C, 65.21; H, 4.96; N, 12.57%.

4-Amino-(antipyrine-4-carbonyl)-2-(phenylamino)-N-(4-chlorophenyl)thiophene-3-carboxamide (10c).

Green crystals, yield 61%, mp 170-172 °C. IR (KBr): 3326, 3212 (N-H, NH₂), broad at 1664 cm⁻¹ (C=O). ¹H NMR (CDCl₃) δ ppm: 2.59 (s, 3H, CH₃), 3.37 (s, 3H, NCH₃), 7.01 (t, J = 8.50 Hz, 2H, Ar-H), 7.31-7.63 (m, 10H, Ar-H), 7.66 (d, J = 9.00 Hz, 2H, Ar-H), 9.22 (s, 1H, NH), 11.04 (s, 1H, NH), 11.93 (s, 2H, NH₂). ¹³C NMR (CDCl₃) δ ppm: 12.12, 33.49, 96.85, 100.99, 120.89 (2C), 121.02, 121.24, 124.46, 127.14, 128.55, 128.83 (4C), 128.92 (2C), 129.16 (2C), 129.39 (2C), 129.73, 132.97, 132.92, 149.97, 152.95, 162.28, 164.06, 172.24, 186.03. MS (m/z, %): 558 (M⁺, 22.3), 543 (36.1), 521 (47.2), 500 (46.4), 481 (37.4), 460 (34.4), 458 (22.9), 409 (36.9), 389 (49.0), 372 (70.4), 347 (36.0), 346 (47.2), 339 (37.5), 336 (44.2), 331 (84.5), 326

Hz, 2H recrystallized from ethanol to furnish the benzaldehyde compound DMSO (15 mL) and anhydrous K. 7.00 Hz, 1H 46%, mp 180 °C, 186 (37.9), 179 (68.0), 163 (35.9), 139 (68.7), 121 (46.6), 107 (29.66), 93 (42.7), 91 (100.0). 84 403 129.26 (2C), 129.48 (2C), 134.56, 141.88, 145.49, 154.05, 155.07, 161.94, 179.35. MS (m/z, %): (δ/ppm 35%, 56 (57.5). 6 1.9), 159 (44.4), 149 (100.0), 132 (44.0), 126 (42.7), 118 (41.8), 102 (44.9), 63 (76.3), 61 (45.3), 56 (57.5). Anal. calcd. for C$_2$H$_5$ClO$_2$S (558.05): C, 62.42; H, 6.35; N, 12.55%. Found: C, 62.58; H, 6.38; N, 12.46%.

Synthesis of 4-(3-substituted-5-(phenylamino)thiophene-2-carbonyl)-antipyrines 13a and 13b: 2-Acetyl-3-oxo-N-phenylbutanethioamide (11a) (0.47 g, 0.002 mol) or ethyl 3-(phenylamino)-3-thioxopropionate (11b) (0.45 g, 0.002 mol) was added to a suspension of 4-chloroacetylantipyrine (1) (0.52 g, 0.002 mol) in 20 mL ethanol and triethylamine (0.5 mL). The mixture was refluxed for 2-4 h and then allowed to cool. The solid being filtered was recrystallized from ethanol to get the 4-(3-methyl/3-hydroxy-5-(phenylamino)thiophene-2-carbonyl)-antipyrine derivatives 13a and 13b.

4-(3-Methyl-5-(phenylamino)thiophene-2-carbonyl)-antipyrine (13a). Yellow crystals; yield 35%, mp 240-242 °C. IR (KBr): 3239 (N-H), broad at 1626 cm$^{-1}$ (2C=O). $^1$H-NMR (DMSO-d$_6$): δ/ppm: 2.33 (s, 3H, CH$_3$), 2.36 (s, 3H, CH$_3$), 3.25 (s, 3H, NCH$_3$), 6.38 (s, 1H, thiophene-H), 6.93 (t, J = 7.50 Hz, 1H, Ar-H), 7.19 (d, J = 7.50 Hz, 2H, Ar-H), 7.31 (t, J = 8.00 Hz, 4H, Ar-H), 7.39 (t, J = 7.50 Hz, 1H, Ar-H), 7.52 (t, J = 7.50 Hz, 2H, Ar-H), 9.70 (s, 1H, NH). $^{13}$C NMR (DMSO-d$_6$) δ/ppm: 115.1, 163.1, 34.26, 108.51, 113.04, 116.69 (2C), 121.33, 123.35, 125.68 (2C), 127.54, 129.26 (2C), 129.48 (2C), 134.56, 141.88, 145.49, 154.05, 155.07, 161.94, 179.35. MS (m/z, %): 403 (M$^+$, 33.7), 367 (30.7), 313 (68.7), 296 (34.3), 270 (48.8), 265 (68.7), 253 (39.0), 210 (57.6), 186 (37.9), 179 (68.0), 163 (35.9), 139 (68.7), 121 (46.6), 107 (29.69), 93 (42.7), 91 (100.0). 84 (48.8), 77 (69.1). Anal. calcd. for C$_2$H$_5$N$_2$O$_2$S (403.50): C, 68.46; H, 5.25; N, 10.41%. Found: C, 68.38; H, 5.22; N, 10.34%.

4-(3-Hydroxy-5-(phenylamino)thiophene-2-carbonyl)-antipyrine (13b). Yellow crystals, yield 46%, mp 180-182 °C. IR (KBr): 3449 (O-H), 3223 (N–H), 1655, 1616 cm$^{-1}$ (2C=O). $^1$H NMR (CDCl$_3$) δ/ppm: 2.19 (s, 3H, CH$_3$), 3.08 (s, 3H, NCH$_3$), 6.21 (s, 1H, thiophene-H), 7.06 (t, J = 7.00 Hz, 1H), 7.24-7.27 (m, 1H), 7.32 (d, J = 8.00 Hz, 2H), 7.35 (t, J = 8.00 Hz, 2H), 7.47-7.43 (m, 4H), 10.16 (s, 1H, NH). $^{13}$C NMR (CDCl$_3$) δ/ppm: 11.83, 36.37, 106.90, 107.79, 110.32, 118.78 (2C), 123.01, 123.20 (2C), 125.90, 128.77, 129.00 (2C), 129.41 (2C), 135.55, 140.79, 153.32, 159.72, 164.62, 166.21. Anal. calcd. for C$_2$H$_5$N$_2$O$_2$S (405.11): C, 65.17; H, 4.72; N, 10.36%. Found: C, 65.07; H, 4.77; N, 10.46%.

Synthesis of 4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzaldehyde (15). A mixture of 4-chloroacetylantipyrine (1) (0.53 g, 0.002 mol) and 4-hydroxybenzaldehyde (0.25 g, 0.002 mol) in DMSO (15 mL) and anhydrous K$_2$CO$_3$ (0.28 g, 0.002 mol) was stirring for 4 hrs. Then, the mixture poured into ice cold water and neutralized with 0.1N HCl. The separated solid was filtered and recrystallized from ethanol to furnish the benzaldehyde compound 15. Orange crystals, yield 44%, mp 130-132 °C. IR (KBr): 1677, 1655 broad cm$^{-1}$ (3C=O). $^1$H NMR (DMSO-d$_6$) δ/ppm: 2.58 (s, 3H, CH$_3$), 3.38 (s, 3H, CH$_3$), 5.73 (s, 2H, -OCH$_2$-), 7.01 (d, J = 9.00 Hz, 2H), 7.38 (d, J = 7.00 Hz, 2H), 7.47 (t, J = 7.00 Hz, 1H), 7.56 (t, J = 7.75, 2H), 7.83 (d, J = 10.00 Hz, 2H), 9.83 (s, 1H, H-C=O). $^{13}$C NMR (DMSO-d$_6$) δ/ppm: 11.19, 33.48, 71.35, 102.10, 114.93 (2C), 127.33 (2C), 128.80, 129.47 (2C), 129.67 (2C), 131.78, 132.41, 154.17, 162.91, 163.40, 188.06, 191.32. MS (m/z, %): 350 (M$^+$, 29.0), 319 (31.7), 295 (33.0), 284 (64.1), 283 (69.1), 281 (35.8), 255 (72.1), 216 (33.7), 192 (38.1), 164 (100.0), 163 (63.3), 153 (39.9), 132 (35.8), 101 (46.0), 74 (36.7), 60 (33.3). Anal. calcd. for C$_{20}$H$_{18}$N$_4$O$_2$ (350.37): C, 68.56; H, 5.18; N, 8.00%. Found: C, 68.42; H, 5.14; N, 8.09%.

Synthesis of 2-cyano-N'-4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene-acetohydrazide (17). A solution of 4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzaldehyde (15) (0.35 g, 0.001 mol) and 2-

cyanoacetoxyhydrazide (16) (0.10 g, 0.001 mol) in 20 mL ethanol was refluxed for 3-4 h. The mixture was then cooled to room temperature, and the resulting precipitate was filtered out, thoroughly dried, and crystallized from ethanol to yield compound 17. Brown crystals, yield 59%, mp 160-162 °C. IR (KB): 3205 (N=O), 2260 (C=N), 1692, 1609 cm⁻¹ (C=`O), 1 H NMR (DMSO-d₆) δ/ppm: 2.58 (s, 3H, CH₃), 3.17 (s, 3H, NCH₃), 5.24 (s, 2H, OCH₂), 7.14-7.57 (m, 1H), 7.91 (s, 1H, NH), 10.57 (s, 1H, NH), 11.67 (s, 1H, NH), 12.71 (s, 1H, SH). MS (m/z, %): 568 (M⁺ + 2, 10.2), 566 (M⁺, 38.1), 545 (66.1), 538 (57.0), 476 (42.0), 472 (20.0), 455 (40.2), 436 (54.6), 404 (31.7), 362 (54.5), 341 (35.8), 338 (28.7), 311 (98.4), 283 (78.2), 263 (28.7), 257 (24.5), 134 (100.0), 132 (37.6), 120 (46.60), 117 (84.7), 107 (58.9), 94 (79.2), 81 (30.0), 75 (45.6), 63 (60.9), 51 (50.8). Anal. calcd. for C₁₁H₁₃N₄O₃S: C, 63.59; H, 4.73; N, 14.83%. Found: C, 63.45; H, 4.69; N, 14.88%.

Synthesis of 2-cyano-N¹-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-3-mercapto-3-(phenylamino)acrylohydrazide (18)

To a solution of compound 2-cyano-N¹-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-3-mercapto-3-(phenylamino)acrylohydrazide (18) (0.57 g, 0.001 mol) in 20 mL ethanol and triethylamine (0.5 mL), chloroacetonitrile (0.10 mL, 0.001 mol), ethyl bromoacetate (0.17 mL, 0.001 mol), ethyl-4-chloroacetacetate (0.14 mL, 0.001 mol), or phenacyl chloride (0.16 mL, 0.001 mol) was added. The mixture was refluxed for 2-3 h and then cooled to room temperature. The formed precipitate was filtered off, dried and crystallized from ethanol to afford the corresponding antipyrine thiophene hybrids 19a-d.

5-Acetyl-4-amino-N¹-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-3-(phenylamino)thiophene-3-carboxamide (19a)

Brown dark crystals, yield 40%, mp 288-290 °C. IR (KB): 3419 (2N=H, NH), 1636 broad cm⁻¹ (C=O), 1 H NMR (DMSO-d₆) δ/ppm: 2.34 (s, 3H, COCH₃), 2.56 (s, 3H, CH₃), 3.31 (s, 3H, NCH₃), 5.42 (s, 2H, -OCH₂), 6.16 (s, 2H, NH₂), 7.02 (d, J = 9.00 Hz, 2H), 7.13-7.54 (m, 10H), 7.87 (d, J = 9.00 Hz, 2H), 8.17 (s, 1H, N=CH), 10.28 (s, 1H, NH), 11.46 (s, 1H, NH), 14.46 (s, 1H, NH). MS (m/z, %): 922 (M⁺, 28.5), 605 (65.9), 570 (31.3), 555 (56.4), 469 (63.3), 418 (82.2), 362 (70.6), 240 (36.4), 179 (100.0), 177 (75.4), 148 (83.6), 119 (43.3), 109 (54.7), 75 (43.9), 48 (21.3). Anal. calcd. for C₂₁H₂₆N₄O₃S (622.20): C, 63.65; H, 4.86; N, 13.50%. Found: C, 63.50; H, 4.81; N, 13.59%.

Ethyl 3-amino-4-(2-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)hydrazine-1-carbonyl)-5-(phenylamino)thiophene-2-carboxylate (19b). Brown crystals, yield 43%, mp 300-302°C. IR (KBr): 3425 broad (2N–H, NH$_2$), broad near 1636 cm$^{-1}$ (C=O). $^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 1.23 (t, $J$ = 7.50 Hz, 3H, CH$_3$), 2.56 (s, 3H, CH$_3$), 3.41 (s, 3H, NCH$_3$), 4.18 (q, $J$ = 7.50 Hz, 2H, CH$_2$), 5.44 (s, 2H, -OCH$_2$), 6.71 (s, 2H, NH$_2$), 6.96 (d, $J$ = 9.00 Hz, 2H, Ar-H), 7.11-7.62 (m, 10H), 7.85 (d, $J$ = 9.00 Hz, 2H), 8.23 (s, 1H, N=CH), 10.41 (s, 1H, NH), 12.05 (s, 1H, NH). MS (m/z, %): 694 (M$^+$, 26.5), 659 (36.2), 650 (27.4), 623 (37.9), 614 (31.0), 611 (59.3), 598 (50.5), 597 (43.3), 594 (39.5), 586 (57.1), 579 (30.6), 565 (36.0), 505 (44.1), 503 (63.5), 502 (31.2), 495 (32.1), 395 (41.2), 298 (34.9), 293 (42.4), 226 (41.8), 213 (30.5), 195 (33.4), 173 (31.8), 168 (100.00), 166 (31.4), 157 (30.6), 117 (36.2), 85 (46.3), 77 (46.0), 68 (53.0), 62 (60.4), 57 (37.3), 52 (31.1). Anal. calc. for C$_{38}$H$_{34}$N$_2$O$_8$S (694.76): C, 62.42; H, 4.93; N, 12.10%. Found: C, 62.07; H, 4.88; N, 12.23%.

4-Amino-5-benzoyl-N’-(4-(2-(antipyrine-4-yl)-2-oxoethoxy)benzylidene)-2-(phenylamino)thiophene-3-carbohydrazone (19d). Red crystals, yield 58%, mp 280-282°C. IR (KBr): broad near 3423 (2N–H, NH$_2$), broad near 1633 cm$^{-1}$ (C=O). $^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 3.38 (s, 3H, NCH$_3$), 3.42 (s, 2H, -OCH$_2$), 6.11 (s, 2H, NH$_2$), 6.94 (d, $J$ = 9.00 Hz, 2H), 7.08-7.64 (m, 15H), 7.88 (d, $J$ = 9.00 Hz, 2H), 8.22 (s, 1H, N=CH), 10.28 (s, 1H, NH), 11.52 (s, 1H, NH). MS (m/z, %): 684 (M$^+$, 42.5), 650 (36.9), 639 (47.5), 638 (97.4), 621 (40.6), 540 (53.1), 522 (39.81), 495 (44.7), 451 (52.9), 438 (43.9), 432 (36.4), 396 (41.6), 392 (52.0), 390 (66.7), 388 (43.7), 386 (39.0), 378 (50.6), 377 (41.3), 373 (48.2), 363 (52.5), 349 (66.1), 344 (66.5), 337 (58.1), 319 (96.8), 307 (52.8), 304 (47.8), 287 (44.5), 269 (89.0), 262 (47.2), 249 (58.7), 248 (51.7), 241 (56.8), 225 (96.4), 224 (100.00), 182 (35.4), 126 (78.3), 111 (53.9), 85 (84.2), 44 (44.4), 40 (48.9). Anal. calc. for C$_{38}$H$_{34}$N$_2$O$_8$S (684.77): C, 66.65; H, 4.71; N, 12.27%. Found: C, 66.52; H, 4.77; N, 12.18%.

Anti-oxidant activity screening assay - ABTS method

The radical cation was created by combining 2 mL of ABTS solution (60 µM) with 3 mL of MnO$_2$ (25 mg/mL) in 5 mL of aqueous phosphate buffer solution (pH = 7). The resulting suspension was agitated for a few minutes before being centrifuged and filtered. The absorbance (A control) of the resultant green-blue solution (ABTS radical solution) was measured at $\lambda_{max}$ 734 nm. Then, 50 mL of (2 mM) solution of the tested antipyrine thiophene analogue in spectroscopic grade MeOH/phosphate buffer (1:1) was added to the ABTS solution. The absorbance (A test) was measured and the reduction in color intensity was expressed as % inhibition. The % inhibition for each compound is calculated from the following equation: % Inhibition = [A (control) – A (test)]/A (control) x 100. Ascorbic acid was used as standard antioxidant (positive control) [45, 46].

In vitro Antibacterial activities

Each of the antipyrine thiophene analogue was dissolved in DMSO and solution of the concentration 1 mg/mL was prepared separately paper discs of Whatman filter paper were prepared with standard size (5 cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were places aseptically in the petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with S. aureus, Bacillus subtilis, E. coli and P. aeruginosa. The petri dishes were incubated at 36 °C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic ampicillin was also recorded using the same procedure as mentioned above [47]. The % activity index for the complex was calculated by the formula as under:

\[
\%\text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100
\]

CONCLUSION

This study focused on the synthesis of novel antipyrine thiophene analogues with antioxidant and antibacterial properties. Based on screening results, the antioxidant activity ABTS technique was shown to be balanced with the antibacterial activity. The methyl and hydroxyl functionality-bearing on thiophene derivative 13a and 13b to the parent antipyrinyl nucleus showed the best results. They displayed the highest inhibitory effects against bacteria (P. aeruginosa, S. aureus, and B. subtilis) with relative activity index, which ranges from 68% to 91.7%). Additionally, they exhibited excellent antioxidant activity, with percentage inhibition 87.2% and 87.7%, respectively when compared to the test reference (Ascorbic acid, 88.0%). The presence of a methyl substituent on the third position of the thiophene ring and methoxy at the phenylene moiety of compound 4c reduced the percentage inhibition (83.5%).

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