Synthesis and anti-tubercular activity of Thieny1 and Furanyl derivatives

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Abstract: The synthesis and anti-tubercular activity of series of acyl hydrazonyl compounds, namely (E)-N’-(arylidene)thiophen-2-carbohydrazide, (E)-N’-(arylidene)furan-2-carbohydrazide, (E)-N-methyl- N’-arylidenethiophen-2-carbohydrazide, and (E)-N-methyl- N’-arylidenethiophen-2-carbohydrazide, [32 compounds in all] are reported. The activities of against Mycobacterium tuberculosis H37Rv (ATCC27294) are compared with those (E)-N’-arylidenethiophen-2-carbohydrazide, previously reported. The most active compounds are (aryl = 5-nitrothien-2-yl), (aryl = 5-nitrofuran-2-yl) and (aryl = 5-nitrothien-2-yl). Moderate activity was displayed by (aryl = 5-nitrofuran-2-yl) and certain derivatives of series where aryl is pyridin-2-yl or an o-hydroxyphenyl derivative. Doubling of certain NMR signals of each compound in solution indicates that a mixture of conformers, ZCONH (E=C=N) and ECONH (E=C=N) about the C(O)-NH=N=C(H, aryl) fragment is present. In contrast, only one form for each compound is present in solution from the single set of NMR signals. It is suggested that this form in solution is the ECONH (E=C=N) form. Only a single set of NMR signals are found for the N-methylated derivatives.

Keywords: Thiényl derivatives; Furanyl derivatives; Acyl hydrazones.

Introduction

Thiophene and its derivatives have been well studied as materials, e.g., in applications in organic electronics and photonics1 and in the medical area. In the medical area, the thiophene nucleus is present in many natural and synthetic products having a wide range of pharmacological activities, such as antiviral2, anticancer3, antibacterial4,5, antifungal5,6, and anti-inflammatory agents7.

Our interests in the biological activities and structural chemistry of heterocyclic compounds have led us to investigate thiophene and its derivatives, which have been found to exhibit tuberculostatic activity8. We have reported the anti-TB activities of acetamido derivatives, 2-(RR’NCOCH2)-thiophene 19-11, and more recently acetohydrazide derivatives of thiophene, 2-(ArCH=N-NHCOCH2)-thiophene 212, see Scheme 1. In the latter study,12, the most active compounds were (2: aryl = 5-nitrothienyl and 5-nitrofuranil), while among the moderately active compounds were (2: aryl = pyridin-2-yl, or 2-hydroxyphenyl). Due to the promising biological results, we have followed up this study with work on further acylhydrazonyl derivatives of thiophene, namely (E)-N’-(arylidene)thiophen-2-carbohydrazide 3, (E)-N-methyl-N’-arylidenethiophen-2-carbohydrazide, 5, and (E)-N-methyl-N’-arylidenethiophen-2(aryl)-carbohydrazide, 6, and in addition, on a series of furanyl compounds, (E)-N’-(arylidene)furan-2-carbohydrazide 4, see Scheme 1.

As well as reporting the synthesis and biological activities of the new thiényl and furanyl derivatives.

Results and Discussion

Chemistry

The synthesis of the compounds, 3 or 4, were achieved by reactions of arylaldehydes with 2-(H2NHHCO-thiophene or furen), 8, generated from methyl thiene-2-carboxylate or methyl furan-2-carboxylate 7, respectively. Methylation of 212 and 3 by methyl iodide produced 5 and 6, respectively, see Scheme 1.

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The choices of the aryl moiety used in this second study were based on the results from our previous study\textsuperscript{12}. Thus derivatives containing 2-hydroxyphenyl, 5-nitrothien-2-yl, 5-nitrofuran-2-yl and 2-pyridinyl as the aryl substituents feature strongly in the current report. Table 1 lists the compounds studied and the biological results obtained. All compounds were characterized by IR and NMR spectroscopy.

**Table 1.** Compounds studied and biological results obtained\textsuperscript{a,b}.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µM)</th>
<th>Compound</th>
<th>MIC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a (R=5-O\textsubscript{2}N-thien-2-yl)</td>
<td>Insol.</td>
<td>4e (R=pyridin-2-yl)</td>
<td>Inact.</td>
</tr>
<tr>
<td>3b (R=5-O\textsubscript{2}N-furan-2-yl)</td>
<td>Insol.</td>
<td>4d (R=2-HOC\textsubscript{6}H\textsubscript{5})</td>
<td>Insol.</td>
</tr>
<tr>
<td>3c (R=pyridin-2-yl)</td>
<td>108.2</td>
<td>4e (R=4-HOC\textsubscript{6}H\textsubscript{5})</td>
<td>Inact.</td>
</tr>
<tr>
<td>3d (R=2-HOC\textsubscript{6}H\textsubscript{5})</td>
<td>Insol.</td>
<td>4f (R=2,3(HO)\textsubscript{2}C\textsubscript{6}H\textsubscript{5})</td>
<td>Inact.</td>
</tr>
<tr>
<td>3e (R=4-HOC\textsubscript{6}H\textsubscript{5})</td>
<td>404.8</td>
<td>4g (R=2-HO-4-MeC\textsubscript{6}H\textsubscript{5})</td>
<td>Insol.</td>
</tr>
<tr>
<td>3f (R=2,4-HO\textsubscript{2}C\textsubscript{6}H\textsubscript{5})</td>
<td>380.2</td>
<td>4h (R=2-HO-5-MeC\textsubscript{6}H\textsubscript{5})</td>
<td>Insol.</td>
</tr>
<tr>
<td>3g (R=3,4-(HO)\textsubscript{2}C\textsubscript{6}H\textsubscript{5})</td>
<td>Inact.</td>
<td>4i (R=2-HO-3-MeOC\textsubscript{6}H\textsubscript{5})</td>
<td>401.3</td>
</tr>
<tr>
<td>3h (R=2-HO-4-MeC\textsubscript{6}H\textsubscript{5})</td>
<td>Insol.</td>
<td>4j (R=2-HO-4-MeOC\textsubscript{6}H\textsubscript{5})</td>
<td>Insol.</td>
</tr>
<tr>
<td>3i (R=2-HO-5-MeC\textsubscript{6}H\textsubscript{5})</td>
<td>Inact.</td>
<td>4k (R=4-HO-3-ClC\textsubscript{6}H\textsubscript{5})</td>
<td>Inact.</td>
</tr>
<tr>
<td>3j (R=2-HO-3-MeOC\textsubscript{6}H\textsubscript{5})</td>
<td>180.5</td>
<td>5a (R=5-O\textsubscript{2}N-thien-2-yl)</td>
<td>Insol.</td>
</tr>
<tr>
<td>3k (R=2-HO-4MeOC\textsubscript{6}H\textsubscript{5})</td>
<td>Insol.</td>
<td>5b (R=5-O\textsubscript{2}N-furan-2-yl)</td>
<td>Insol.</td>
</tr>
<tr>
<td>3l (R=2-HO-3-O\textsubscript{2}NC\textsubscript{6}H\textsubscript{5})</td>
<td>171.2</td>
<td>5c (R=pyridine-2-yl)</td>
<td>432.9</td>
</tr>
<tr>
<td>3m (R=2-HO-5-O\textsubscript{2}NC\textsubscript{6}H\textsubscript{5})</td>
<td>Insol.</td>
<td>6a (R=5-O\textsubscript{2}N-thien-2-yl)</td>
<td>10.5</td>
</tr>
<tr>
<td>3n (R=4-HO-3-CIC\textsubscript{6}H\textsubscript{5})</td>
<td>88.8</td>
<td>6b (R=5-O\textsubscript{2}N-furan-2-yl)</td>
<td>179.2</td>
</tr>
<tr>
<td>4a (R=5-O\textsubscript{2}N-thien-2-yl)</td>
<td>Insol.</td>
<td>6c (R=pyridin-2-yl)</td>
<td>Inact.</td>
</tr>
<tr>
<td>4b (R=5-O\textsubscript{2}N-furan-2-yl)</td>
<td>100.3</td>
<td>2b (R=5-O\textsubscript{2}N-furan-2-yl)\textsuperscript{12}</td>
<td>9.0</td>
</tr>
<tr>
<td>2a (R=5-O\textsubscript{2}N-thien-2-yl)\textsuperscript{12}</td>
<td>8.5</td>
<td>Ethambutol</td>
<td>Isoniazide</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Ins = insoluble; \textsuperscript{b}Inact = inactive; >100µg/mL

It has been variously reported that N-acylhydrazones, RCONHN=CHR\textsuperscript{1} can exist in different forms in solution\textsuperscript{13-18}. In our previous study\textsuperscript{11}, the NMR spectra of compounds 2 indicated the presence of two forms in solution, from the doubling of certain signals. A similar finding was observed for compounds 3 in this study. There are, in principle, four possible arrangements about the C(O)NH=N fragment in acylhydrazones, RCONHN=CHR\textsuperscript{1}; these are derived from combinations of two conformers, $E_{C(ONH)/Z_{C(ONH)}}$ (anti-periplanar/syn-periplanar), and two geometric N=C isomers - $E_{C(ON)}/Z_{C(ON)}$, see Figure 1. However the $Z_{C(ON)}$ isomers are generally so much disfavoured for steric reasons, that only $E_{C(ONH)/E_{C(ON)}}$ and $Z_{C(ONH)}/E_{C(ON)}$ are observed by NMR spectroscopy. The two conformers are in equilibrium, with different ratios observed in different solutions and temperatures etc.
Only one set of NMR signals are observed for the N-methylated compounds 5 and 6. Methyl substitution at nitrogen of the C(O)N fragment results in restricted rotation about the (O)C-N(Me) bond and leads to the less hindered rotamer being strongly favoured, which in the cases of 5 and 6, is the \( Z_{(O)NH}{\text{C}(\text{N})} \) conformer. Furthermore, the extra steric hindrance of the methyl substitution would further disadvantage the \( Z_{(N=O)} \) geometric isomer resulting in formation of just the \( E_{(N=C)} \) isomer and elimination of other isomers, at least to the limits of NMR detection.

In contrast to the situation found for the thienyl derivatives 3, the analogous furanyl compounds, 4, in solution exist in a single conformation as only one set of NMR signals are observed in solution. A similar situation was found for 5-(2,5-C\(_3\)H\(_4\))-(2-2-MeOC\(_3\)H\(_4\))-(CH=NH)-furanyl, \( 7 \)^15. To find some collaborative support for this finding, we looked at the single crystal X-ray data for compounds 3 and 4 in the literature.

Various crystal structures for compounds in each of the series 3\(^{19-21} \) and 4\(^{22-24} \) have been reported, albeit briefly. As pointed out above, four possible conformations about the CONH-N=N fragment are potentially possible in solution. In the solid state, rotations about other bonds in the molecule, which are freely allowed in solution, can be frozen out with the result that different conformations are possible. Such additional rotamers, or conformers, arise in the solid state for the furanyl and thienyl derivatives, 3 and 4.

Looking at the molecular conformations of all the reported crystal structures of 3 and 4, four distinct molecular conformations, A-D, are formed, see Figure 2. Forms A and B are one pair of rotamers and C and D are another pair of rotamers, arising from rotation about the (thienyl)C-C(O) and (furanyl)C-C(O) bonds, respectively. A and B are \( E_{(C=O)} \) and \( Z_{(C=O)} \) for both 3 and 4, while C and D are \( E_{(C=O)} \) and \( Z_{(C=O)} \) for compounds having the C(O)-NH-N=CH fragment.

The thienyl compounds 3 are present in forms 4\(^{20} \), A & B, or C & D, with A the most common. The finding that both \( E_{(C=O)} \) and \( Z_{(C=O)} \) cis- and trans-5-nitrothienyl molecular structural forms occur for 3, is in contrast with the finding for the furanyl compounds, 4, where only \( E_{(C=O)} \) cis-5-nitrothienyl molecular structural forms are found: references for compounds having the C form, C & D and D forms for solid 4 are 22, 23 are 15 & 24, respectively: the most common form for 4 is the D form.

**Biological activity**

Table 1 lists the results of biological activity against *M. tuberculosis* H37Rv (ATTCC27294). Of the 30 or so compounds prepared, 13 were not sufficiently soluble for the screening and 8 were deemed inactive. The most active compounds were the 5-nitrothienyl derivatives, 6\(^a\) and 2\(^a\) and the 5-nitrofuran derivative 2b\(^{12} \) with MIC (\( \mu \text{M} \)) values of 10.8, 8.5 and 9.0, respectively, compared to those of ethambutol of 15.3 and isoniazide of 0.46. The other two 5-nitrothienyl derivatives, 3\(^a\) and 4\(^a\) were insufficiently soluble for the screening, thereby preventing a comparison involving all the 5-nitrothienyl compounds.
Moderate activity was determined for the 5-nitrofuranyl derivatives, 4b and 6b: again a comparison of all the 5-nitrofuranyl derivatives was thwarted by the lack of the adequate solubility of 3b.

Of the remainder of the compounds, moderate activity was also found for 3e and 3n: similar moderate activities were found for the corresponding RCH=NNHCOCH=thiophenederivatives, 2, in the earlier study. The activities determined for the various hydroxyphenyl derivatives, 3d-3n and 4d-4k are so mixed that no general trend can be extracted from the data, apart from the generally lower activity of the furanyl compounds 4, compared to those of the analogous thienyl compounds 3. The activity of the 5-nitro-heterocyclic compounds, 2a, 2b and 6a, good on one hand, and the 2-hydroxyphenyl and pyridine-2-ylderivatives, moderately so on the other, does point to differing modes of action. The latter compounds, for example are potentially good chelators of metal ions, while the former compounds do not possess this property, but could be sources of free radicals via reduction of the nitro groups and references therein.

Conclusion

The most active compounds against M. tuberculosis H37Rv (ATCC27294) are (2a; aryl = 5-nitrothien-2-yl), (2b; aryl = 5-nitrofuran-2-yl) and (6; aryl = 5-nitrothien-2-yl). Moderate activity was displayed by (4b; aryl = 5-nitrofuran-2-yl) and certain derivatives of series 3 where aryl is an o-hydroxyphenyl derivative or pyridin-2-yl. The screening data from this study concurs in the main over substituent effects with our earlier findings. Each compound 3 in solution exists as a mixture of conformers, $Z_{\text{cond}} / E_{\text{C-N}}$ and $E_{\text{cond}} / E_{\text{C-N}}$ about the C(O)-NH-N=C(H, aryl) fragment. In contrast, only one form, $E_{\text{cond}} / E_{\text{C-N}}$, for each compound 4 is present in solution.

Acknowledgements

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Experimental Section

Chemistry

Melting points were determined on a Buchi apparatus and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer in potassium bromide pellets. HRMS were performed on Bruker Compact QTOF mass spectrometer system. NMR spectra were recorded on a Bruker Avance 400 or 500 spectrometers in DMSO-d6 at room temperature. For TLC plates coated with silica gel were run in hexane/acetate mixture and spots were developed in ultraviolet and solution of ninhydrine (0.2% p/v in ethanol). Compounds 7 were prepared as reported.

Synthesis of methyl thiophene-2-carboxylate (7; $E= S$) and methyl furan-2-carboxylate (7; $E= O$)

Thionyl chloride (8.25 mL, 117.2 mmol) was slowly added to methanol (80.0 mL) at 0°C under nitrogen atmosphere. After stirring for 20 minutes, 2-thiophencarboxylic acid or 2-furancarboxylic acid (3.0 g, 23.5 mmol) was added. The mixture was stirred at room temperature for 24 hours, concentrated under reduced pressure, and the residue was neutralized with saturated aqueous solution of sodium bicarbonate. The layers were separated and the aqueous phase was extracted with dichloromethane (3 x 15 mL). The organic phases were combined, dried over magnesium sulfate and concentrated under vacuum to yield 7 as yellow syrup (3.26 g, 98-100%). The crude product was used without further purification. The product (7; $E= O$) was particularly unstable and the NMR spectra were not obtained.

Compound (7; $E= S$)

Yield: 98%; yellow syrup.

$^1$H NMR (400 MHz; DMSO)δ: 7.96 (1H; dd; $J_{HH} = 5.0$ and 1.2 Hz; H-5), 7.82 (1H; dd; $J_{HH} = 3.7$ and 1.2 Hz; H-3), 7.23 (1H; dd; $J_{HH} = 3.8$ and 4.9 Hz; H-4), 3.83 (3H; s; OCH3). $^{13}$C NMR(100 MHz DMSO) δ: 161.9 (C=O), 133.9 (C-2), 133.7 (C-3), 132.7 (C-4), 128.3 (C-5), 52.1 (OCH3). HRMS m/z: 164.9990 [M+Na]+ (calcd for [C6H5O2S+Na]+: 164.9986).

IR $\nu_{\text{max}}$ (cm$^{-1}$; KBr pellets): 1712 (C=O); 1265 (O=CH3).

Synthesis of thien-2-yl-2-carboxyhydrazide (8; $E= S$) and furan-2-ylcarboxyhydrazide (8; $E= O$)

To a solution of (7) (0.5 g, 3.4 mmol) in ethanol (5.0 mL) was added hydrazine hydrate (0.46 mL, 5.2 mmol, aqueous solution 55%). After stirring for 18 hours at 80°C, the reaction mixture was concentrated under reduced pressure, and the residue was washed with cold ethanol (2 x 10.0 mL), followed by diethyl ether (2 x 10.0 mL), and dried. Compounds 8 were obtained as solids, in yields of ca 80%.

Compound (8; $E= S$)

Yield: 80%; white solid; m.p. 136-137°C.

$^1$H NMR (400 MHz; DMSO)δ: 9.75 (1H; sl; NH), 7.74 (1H; dd; $J_{HH} = 5.0$ and 0.9 Hz; H-5), 7.71 (1H; dd; $J_{HH} = 3.7$ and 0.9 Hz; H-3), 7.13 (1H; t; $J_{HH} = 3.8$ Hz), 4.57 (2H; sl; NH2). IR $\nu_{\text{max}}$ (cm$^{-1}$; KBr pellets): 1622 (C=O); 3100 (NH2).

Compound (8; $E= O$)

Yield: 80%; light yellow solid;

$^1$H NMR (400 MHz; DMSO)δ: 9.61 (1H; sl; NH), 7.80 (1H; d; $J_{HH} = 1.7$ Hz; H-5), 7.07 (1H; d; $J_{HH} = 3.4$ Hz; H-3), 6.59 (1H; dd; $J_{HH} = 3.4$ and 1.7 Hz; H-4), 4.41 (2H; sl; NH2).
HRMS $m/z$: 149.0330 [M+Na]$^+$ (calcd for [C$_7$H$_6$N$_2$O$_2$+Na]$^+$; 149.0327).
IR $v_{\text{max}}$ (cm$^{-1}$; KBr pellets): 1683 (C=O); 3145 (NH$_2$).

**General procedures for**

(E)-N'-benzylidenethiophene-2-carboxyhydrazide (3)
or (E)-N'-benzylidenefuran-2-carboxyhydrazide (4).

(E)-N'-benzylidenethiophene-2-carboxyhydrazide (3) or (E)-N'-benzylidenefuran-2-carboxyhydrazide (4) derivatives were prepared by reaction between the appropriate carboxyhydrazide 8 (0.2 g, 1.4 mmol) and the appropriate arene-carbaldehyde (1.6 mmol) in ethanol (2.0 mL). The reaction mixture was stirred for 1-72 hours at room temperature. After that, the excess of solvent was concentrated under reduced pressure and the residue was purified by washing with cold diethyl ether (2 x 10.0 mL), leading to the pure derivatives 3a-n and 4a-k as solids in 40-97% yields.

(E)-N'[(5-Nitrothiophen-2-ylmethylene)thiophene-2-carboxyhydrazide](3a)

**Yield:** 80%; orange solid; m.p. 268-269°C.

$^1$H NMR (400 MHz; DMSO) $\delta$: 12.24 (1H; sl; NH), 8.30 (1H; s; H-4'), 8.13 (1H; d; $J_{\text{HN}}$ = 4.0 Hz, H-3), 8.04-7.93 (2H; m; H-8' and H-9'), 7.58 (1H; d; $J_{\text{HN}}$ = 4.2 Hz H-5), 7.25 (1H; d; $J_{\text{HN}}$ = 4.3 Hz; H-4). $^1$C NMR (100 MHz DMSO) $\delta$: 161.3 (C=O), 150.9 (C-4'), 146.3 (C-2), 140.9 (C-5'), 137.0 (C-7'), 135.5 (C-5), 132.5 (C-4), 130.5 (C-3), 129.7 (C-8'), 128.2 (C-9').

HRMS $m/z$: 303.9837 [M+Na]$^+$ (calcd for [C$_{10}$H$_{10}$N$_2$O$_2$+Na]$^+$; 303.9827).

IR $v_{\text{max}}$ (cm$^{-1}$; KBr pellets): 1649 (C=O); 3101 (N-H).

(E)-N'-(5-Nitrofuran-2-ylmethylene)thiophene-2-carboxyhydrazide (3b)

**Yield:** 92%; yellow solid; m.p. 255-256°C.

$^1$H NMR (400 MHz; DMSO) $\delta$: 12.26 (1H; sl; NH), 8.10-7.96 (3H; m; H-4'; H-8' and H-9'), 7.81 (1H; d; $J_{\text{HN}}$ = 3.9 Hz; H-5), 7.28 (1H; d; $J_{\text{HN}}$ = 3.9 Hz; H-4), 7.26-7.24 (1H; m; H-3). $^1$C NMR (100 MHz DMSO) $\delta$: 161.6 (C=O), 157.9 (C-2), 151.6 (C-4'), 137.5 (C-5'), 135.2 (C-3), 132.7 (C-2) 131.4 (C-7'), 129.7 (C-8'), 128.2 (C-9'), 127.1 (C-7').

HRMS $m/z$: 288.0082 [M+Na]$^+$ (calcd for [C$_{10}$H$_{10}$N$_2$O$_2$+Na]$^+$; 288.0055).

IR $v_{\text{max}}$ (cm$^{-1}$; KBr pellets): 1629 (C=O); 3209 (N-H).

(E)-N'-(Pyridin-2-ylmethylene)thiophene-2-carboxyhydrazide (3c)

**Yield:** 74%; pale yellow syrup; m.p. 149-150°C.

$^1$H NMR (400 MHz; DMSO) $\delta$: 12.05 (1H; sl; NH), 8.64 (1H; d; $J_{\text{HN}}$ = 4.7 Hz; H-7'), 8.47 (1H; s; H-4'), 8.17-7.93 (4H; m; H-5; H-8'; H-9' and H-10'), 7.44 (1H; dd$J_{\text{HH}}$ = 6.5 and 5.2 Hz; H-3), 7.25 (1H; t; $J_{\text{HN}}$ = 4.4 Hz H-4). $^1$C NMR (100 MHz DMSO) $\delta$: 161.5 (C=O), 152.9 (C-4'), 149.4 (C-7'), 147.4 (C-2), 137.1 (C-9'), 135.0 (C-5), 132.3 (C-4), 129.3 (C-3), 128.2 (C-8'), 126.8 (C-10') 124.4 (C-8').

HRMS $m/z$: 254.0382 [M+Na]$^+$ (calcd for [C$_{10}$H$_{10}$N$_2$O$_2$+Na]$^+$; 254.0364).

IR $v_{\text{max}}$ (cm$^{-1}$; KBr pellets): 1649 (C=O); 3101 (N-H).
(E)-N’-(3,4-Dihydroxybenzylidene)thiophene-2-carboxyrazide (3g)
Yield: 97%; yellow solid; m.p. 226-227°C.

^1^H NMR (400 MHz; DMSO): δ: 11.64 (1H; s; NH), 11.59 (1H; s; NH), 8.24 (1H; s; H-4’), 8.03 (1H; s; H-4’), 7.95-7.93 (2H; m; H-4 and H-5), 7.86 (2H; dd; J_HH = 10.5 and 4.3 Hz; H-4 and H-5), 7.29 (1H; s; H-6’), 7.24-7.21 (3H; m; H-6’, H-9’ and H-10’), 6.96 (2H; dd; J_HH = 12.5 and 7.9 Hz; H-9’ and H-10’), 6.81 (2H; d; J_HH = 5.2; H-3). ^13^C NMR (100 MHz DMSO) δ: 161.0 & 160.5 (C=O), 148.9 & 148.2 (C-4’ and C-7’), 134.7 & 134.4 (C-8’), 131.5 (C-5), 128.6 (C-3), 128.1 (C-4), 126.6 & 125.1 (C-5’), 121.9 & 120.6 (C-10’), 115.7 & 113.9 (C-6’), 113.2 & 112.7 (C-9’).

HRMS m/z: 285.0334 [M+Na]^+ (calcd for C_{12}H_{11}NO_S+Na]^+; 285.0310).

IR vmax (cm^-1; KBr pellets): 1641 (C=O); 3265 (N-H).

(3H; s; OCH_3). ^13^C NMR(100 MHz DMSO) δ: 162.8 & 161.1 (C=O), 157.6 & 148.5 (C-4’), 148.5 & 147.6 (C-2), 147.1 & 146.1 (C-7’), 140.8 & 137.7 (C-6’), 134.6 & 133.2 (C-3), 132.2 & 129.2 (C-5), 128.3 & 126.7 (C-4’), 122.1 & 120.9 (C-5’), 119.1 & 118.4 (C-10’), 117.6 & 115.3 (C-9’), 113.8 & 112.9 (C-8’), 55.7 (OCH_3).

HRMS m/z: 299.0482 [M+Na]^+ (calcd for C_{15}H_{16}N_{2}O_3S+Na]^+; 299.0466).

IR vmax (cm^-1; KBr pellets): 1641 (C=O); 3554 (N-H).

(3H; s; OCH_3). ^13^C NMR(100 MHz DMSO) δ: 162.8 & 161.1 (C=O), 157.6 & 148.5 (C-4’), 148.5 & 147.6 (C-2), 147.1 & 146.1 (C-7’), 140.8 & 137.7 (C-6’), 134.6 & 133.2 (C-3), 132.2 & 129.2 (C-5), 128.3 & 126.7 (C-4’), 122.1 & 120.9 (C-5’), 119.1 & 118.4 (C-10’), 117.6 & 115.3 (C-9’), 113.8 & 112.9 (C-8’), 55.7 (OCH_3).

HRMS m/z: 299.0482 [M+Na]^+ (calcd for C_{15}H_{16}N_{2}O_3S+Na]^+; 299.0466).

IR vmax (cm^-1; KBr pellets): 1641 (C=O); 3554 (N-H).

(3H; s; OCH_3). ^13^C NMR(100 MHz DMSO) δ: 162.8 & 161.1 (C=O), 157.6 & 148.5 (C-4’), 148.5 & 147.6 (C-2), 147.1 & 146.1 (C-7’), 140.8 & 137.7 (C-6’), 134.6 & 133.2 (C-3), 132.2 & 129.2 (C-5), 128.3 & 126.7 (C-4’), 122.1 & 120.9 (C-5’), 119.1 & 118.4 (C-10’), 117.6 & 115.3 (C-9’), 113.8 & 112.9 (C-8’), 55.7 (OCH_3).

HRMS m/z: 299.0482 [M+Na]^+ (calcd for C_{15}H_{16}N_{2}O_3S+Na]^+; 299.0466).

IR vmax (cm^-1; KBr pellets): 1641 (C=O); 3554 (N-H).

(3H; s; OCH_3). ^13^C NMR(100 MHz DMSO) δ: 162.8 & 161.1 (C=O), 157.6 & 148.5 (C-4’), 148.5 & 147.6 (C-2), 147.1 & 146.1 (C-7’), 140.8 & 137.7 (C-6’), 134.6 & 133.2 (C-3), 132.2 & 129.2 (C-5), 128.3 & 126.7 (C-4’), 122.1 & 120.9 (C-5’), 119.1 & 118.4 (C-10’), 117.6 & 115.3 (C-9’), 113.8 & 112.9 (C-8’), 55.7 (OCH_3).

HRMS m/z: 299.0482 [M+Na]^+ (calcd for C_{15}H_{16}N_{2}O_3S+Na]^+; 299.0466).

IR vmax (cm^-1; KBr pellets): 1641 (C=O); 3554 (N-H).

(3H; s; OCH_3). ^13^C NMR(100 MHz DMSO) δ: 162.8 & 161.1 (C=O), 157.6 & 148.5 (C-4’), 148.5 & 147.6 (C-2), 147.1 & 146.1 (C-7’), 140.8 & 137.7 (C-6’), 134.6 & 133.2 (C-3), 132.2 & 129.2 (C-5), 128.3 & 126.7 (C-4’), 122.1 & 120.9 (C-5’), 119.1 & 118.4 (C-10’), 117.6 & 115.3 (C-9’), 113.8 & 112.9 (C-8’), 55.7 (OCH_3).

HRMS m/z: 299.0482 [M+Na]^+ (calcd for C_{15}H_{16}N_{2}O_3S+Na]^+; 299.0466).

IR vmax (cm^-1; KBr pellets): 1641 (C=O); 3554 (N-H).

(3H; s; OCH_3). ^13^C NMR(100 MHz DMSO) δ: 162.8 & 161.1 (C=O), 157.6 & 148.5 (C-4’), 148.5 & 147.6 (C-2), 147.1 & 146.1 (C-7’), 140.8 & 137.7 (C-6’), 134.6 & 133.2 (C-3), 132.2 & 129.2 (C-5), 128.3 & 126.7 (C-4’), 122.1 & 120.9 (C-5’), 119.1 & 118.4 (C-10’), 117.6 & 115.3 (C-9’), 113.8 & 112.9 (C-8’), 55.7 (OCH_3).
(E)-N’-(3-Chloro-4-hydroxybenzylimidene)thiophene-2-carboxyhydrazide (3n)

Yield: 69%; light yellow solid; m.p. 212-213°C.

\(^1^H\) NMR (400 MHz; DMSO): 11.80 (1H; s; NH), 8.30 (1H; s; H-4’), 8.03-7.97 (1H; m; H-9’), 7.90-7.87 (1H; m; H-10’), 7.75-7.71 (1H; m; H-6’), 7.61-7.53 (1H; m; H-3), 7.22 (1H; dd; \(J = 4.9\) and 3.9 Hz; H-5), 7.10-7.05 (1H; m; H-4’).

\(^1^C\) NMR (100 MHz DMSO) \(\delta\): 161.1 (C=O), 154.8 & 146.6 (C-8’), 154.7 (C-4’), 143.0 & 138.3 (C-2), 134.9 (C-3), 133.0 (C-5), 131.7 (C-4), 128.8 & 128.7 (C-5’), 128.4 & 128.1 (C-6’), 127.3 & 126.6 (C-10’), 126.5 & 120.3 (C-7’), 117.0 & 116.9 (C-9’).

HRMS m/z: 314.0230 [M+Na]\(^+\) (calcd for [C\(_4\)H\(_4\)N\(_2\)O\(_5\)+Na\(^+\)]: 314.0212).

IR v max (cm\(^{-1}\); KBr pellets): 1624 (C=O); 3292 (N-H).

(\(E\))-N’-(3-Chloro-4-hydroxybenzylimidene)thiophene-2-carboxyhydrazide (4n)

Yield: 40%; yellow solid; m.p. 209-210°C.

\(^1^H\) NMR (400 MHz; DMSO): 12.24 (1H; s; NH), 8.66 (1H; s; H-4’), 8.13 (1H; d; \(J_{HH} = 4.3\) Hz; H-8’), 7.99 (1H; s; H-3), 7.57 (1H; d; \(J_{HH} = 4.3\) Hz; H-9’), 7.36 (1H; s; H-5), 6.74 (1H; s; H-4). \(^1^C\) NMR (100 MHz DMSO) \(\delta\): 154.2 (C=O), 150.8 (C-7’), 146.6 (C-4’), 146.4 (C-2), 146.2 (C-5), 141.1 (C-1’), 130.5 (C-9’), 129.6 (C-5’), 115.8 (C-11’), 112.3 (C-3).

HRMS m/z: 288.0073 [M+Na]\(^+\) (calcd for [C\(_4\)H\(_4\)N\(_2\)O\(_5\)+Na\(^+\)]: 288.0055).

IR v max (cm\(^{-1}\); KBr pellets): 1659 (C=O); 3155 (N-H).

(\(E\))-N’-(2,3-Dihydroxybenzimidene)furan-2-carboxyhydrazide (4f)

Yield: 56%; light yellow solid; m.p. 212-122°C.

\(^1^H\) NMR (400 MHz; DMSO): 12.14 (1H; s; NH), 8.66 (1H; s; H-4’), 7.97 (1H; d; \(J_{HH} = 4.6\) Hz; H-8’), 7.49 (1H; s; H-5), 7.37 (1H; s; H-3), 7.28 (1H; d; \(J_{HH} = 3.9\) Hz; H-9’), 6.74 (1H; dd; \(J_{HH} = 3.3\) and 1.5 Hz; H-4’). \(^1^C\) NMR (100 MHz DMSO) \(\delta\): 128.4 (C=O), 151.8 (C-5), 151.6 (C-4’), 146.3 (C-2), 146.0 (C-5’), 135.4 (C-8’), 115.2 (C-9’), 114.6 (C-4), 112.2 (C-3).

HRMS m/z: 272.0292 [M+Na]\(^+\) (calcd for [C\(_6\)H\(_8\)N\(_2\)O\(_4\)+Na\(^+\)]: 272.0283).

IR v max (cm\(^{-1}\); KBr pellets): 1666 (C=O); 3124 (N-H).

(E)-N’-(2-Hydroxybenzimidene)furan-2-carboxyhydrazide (4d)

Yield: 66%; light yellow solid; m.p. 146-147°C.

\(^1^H\) NMR (400 MHz; DMSO): 12.11 (1H; s; NH), 11.14 (1H; s; NH), 8.64 (1H; s; H-4’), 7.96 (1H; d; \(J_{HH} = 1.0\) Hz; H-5), 7.53 (1H; d; \(J_{HH} = 7.4\) Hz; H-3), 7.30 (2H; d; \(J_{HH} = 7.8\) Hz; H-8’ and H-10’), 6.93 (2H; d; \(J_{HH} = 7.9\) Hz; H-7’ and H-9’), 6.72 (1H; dd; \(J_{HH} = 3.4\) and H-4’). \(^1^C\) NMR (100 MHz DMSO) \(\delta\): 157.4 (C=O), 154.0 (C-6’), 148.3 (C-4’), 146.3 (C-3), 146.1 (C-5), 131.4 (C-10’), 129.4 (C-9’), 119.4 (C-8’), 118.8 (C-5’), 116.4 (C-7’), 115.3 (C-4), 112.2 (C-3).

HRMS m/z: 253.0567 [M+Na] (calcd for [C\(_3\)H\(_4\)ClN\(_2\)O\(_5\)+Na\(^+\)]: 253.0589).

IR v max (cm\(^{-1}\); KBr pellets): 1621 (C=O); 3130 (N-H).
Hz; H-7'), 7.30 (1H; d; J_HH = 3.1 Hz; H-5), 6.81-6.79 (1H; m; H-3), 6.76-6.74 (2H; m; H-9' and H-10'), 6.72-6.71 (1H; m; H-4), 2.28 (3H; s; CH_3).

^1C NMR (100 MHz DMSO): δ: 153.8 (C=O), 148.3 (C-4'), 146.2 (C-2'), 145.9 (C-5), 143.7 (C-6'), 130.8 (C-8'), 129.3 (C-10'), 120.3 (C-5'), 116.6 (C-9'), 115.5 (C-7'), 115.0 (C-4'), 112.0 (C-3), 21.1 (CH_3).

HRMS m/z: 267.0746 [M+Na]^+ (caled for [C_7H_9NO_4^+Na]^+; 267.0746).

IR ν_max (cm^-1; KBr pellets): 1607 (C=O); 3191 (N-H).

(3-Cloro-4-hyroxybenzylidene)thiophene-2-carboxyhydrazide (4k)

Yield: 42%; yellow solid; m.p. 202-203°C.

^1H NMR (400 MHz; DMSO): δ: 11.76 (1H; s; NH), 10.70 (1H; s; NH), 8.30 (1H; s; H-4'), 7.79 (1H; d; J_HH = 1.0 Hz; H-5), 7.51 (1H; dd; J_HH = 8.4 and 1.8 Hz; H-10'), 7.28 (1H; s; H-6'), 7.07 (1H; dd; J_HH = 3.2 Hz; H-3), 7.04 (1H; dd; J_HH = 8.4 Hz; H-9'), 6.59 (1H; dd; J_HH = 3.5 and 1.7 Hz; H-4').

^13C NMR (100 MHz DMSO): δ: 157.9 (C=O), 154.1 (C-8'), 147.0 (C-4'), 146.7 (C-2'), 145.7 (C-5), 128.4 (C-5'), 127.2 (C-6'), 126.6 (C-10'), 120.3 (C-7'), 114.8 (C-9'), 112.0 (C-4), 111.5 (C-3).


IR ν_max (cm^-1; KBr pellets): 1640 (C=O); 3127 (N-H).

General Synthesis of 5 and 6.

The appropriate derivative 2^1 or 3 (0.2 g, 1.0 equiv.) was suspended in acetone (5.0 mL) and potassium carbonate (4.0 equiv.) was added. The reaction mixture was stirred at room temperature for 30 minutes and methyl iodide (4.0 equiv.) was added. The temperature of the reaction mixture was increased to 40°C over 2-2.5 hours. The reaction mixture was rotary evaporated to leave a residue which was dissolved in water (20.0 mL) and extracted with ethyl acetate (3 x 10.0 mL). The organic phases were washed, dried with anhydrous magnesium sulfate, filtered and then evaporated at reduced pressure. The derivatives 5 and 6 were obtained as solids in 65-89% yields.

(2-Hydroxy-3-methoxybenzylidene)furan-2-carboxyhydrazide (4i)

Yield: 57%; yellow solid; m.p. 122-123°C.

^1H NMR (400 MHz; DMSO): δ: 12.10 (1H; s; NH), 10.80 (1H; s; NH), 8.67 (1H; s; H-4'), 7.96 (1H; dd; J_HH = 1.7 and 0.7 Hz; H-5), 7.32 (1H; d; J_HH = 2.9 Hz; H-3'), 7.15 (1H; d; J_HH = 7.6 Hz; H-8'), 7.04 (1H; dd; J_HH = 7.9 and 0.9 Hz; H-10'), 6.86 (1H; t; J_HH = 7.9 Hz; H-9'), 6.71 (1H; dd; J_HH = 3.5 and 1.7 Hz; H-4'), 3.82 (3H; s; CH_3).

^13C NMR (100 MHz DMSO): δ: 154.0 (C=O), 148.0 (C-6'), 147.9 (C-6'), 147.1 (C-7'), 146.3 (C-2'), 145.6 (C-4'), 144.9 (C-5), 120.6 (C-10'), 119.1 (C-5'), 115.2 (C-9'), 113.8 (C-3 or C-8'), 112.2 (C-3 or C-8'), 111.5 (C-4), 55.8 (CH_3).

HRMS m/z: 283.0700 [M+Na]^+ (caled for [C_7H_11NO_3^+Na]^+; 283.0695).

IR ν_max (cm^-1; KBr pellets): 1651 (C=O); 3131 (N-H).

(2-Hydroxy-4-methoxybenzylidene)furan-2-carboxyhydrazide (4j)

Yield: 62%; yellow solid; m.p. 152-153°C.

^1H NMR (400 MHz; DMSO): δ: 12.00 (1H; s; NH), 11.47 (1H; s; NH), 8.55 (1H; s; H-4'), 7.95 (1H; d; J_HH = 1.0 Hz; H-5), 7.41 (1H; d; J_HH = 8.5 Hz; H-10'), 7.29 (1H; d; J_HH = 3.1 Hz; H-3'), 6.71 (1H; dd; J_HH = 3.5 and 1.7 Hz; H-4'), 6.54-6.49 (2H; m; H-7' and H-9'), 3.78 (3H; s; CH_3).

^13C NMR (100 MHz DMSO): δ: 162.0 (C=O), 159.2 (C-8'), 153.7 (C-6'), 148.6 (C-4'), 146.2 (C-2), 145.5 (C-5), 130. (C-10'), 114.9 (C-5'), 112.0 (C-4), 111.7 (C-3), 106.6 (C-9'), 101.0 (C-7'), 55.2 (CH_3).

HRMS m/z: 317.9990 [M+Na]^+ (caled for [C_7H_11NO_3Na]^+; 317.9983).

IR ν_max (cm^-1; KBr pellets): 1637 (C=O); 1168 (C-N).

(E)-N-Methyl-4-(5-nitrofuran-2-yl)methylenethiophene-2-carboxyhydrazide (5b)

Yield: 76%; dark yellow solid; m.p. 165-166°C.

^1H NMR (400 MHz; DMSO): δ: 8.09 (2H; m; H-4' and H-5), 7.99 (1H; d; J_HH = 4.4 Hz; H-3), 7.82 (1H; d; J_HH = 3.8 Hz; H-8'), 7.24 (1H; d; J_HH = 3.9 Hz; H-9'), 7.20 (1H; t; J_HH = 4.5 Hz; H-4'),
Yield: 88%; brown solid; m.p. 90-91°C.

1H NMR (400 MHz; DMSO) δ: 8.86 (1H; d; J_HH = 4.5 Hz; H-7'), 8.18 (1H; d; J_HH = 7.9 Hz; H-9'), 8.09 (1H; dd; J_HH = 3.8 and 1.2 Hz; H-5), 8.05 (1H; s; H-4'), 8.00-7.94 (2H; m; H-3 and H-10'), 7.45 (1H; dd; J_HH = 7.2 and 5.6 Hz; H-8'), 7.22 (1H; t; J_HH = 4.8; H-4) 3.55 (3H; s; CH3).

13C NMR (100 MHz DMSO) δ: 161.4 (C-0), 153.2 (C-4'), 149.6 (C-7'), 142.1 (C-5'), 137.0 (C-9'), 136.0 (C-2), 135.6 (C-3), 132.4 (C-4), 126.6 (C-5), 124.1 (C-10'), 120.8 (C-8'), 29.4 (CH3).


IR ν_max (cm⁻¹); KBr pellets: 1641 (C=O); 1166 (C-N).

(E)-N-Methyl-N’-(pyridin-2-ylmethylene)-2-thiophen-2-ylacetohydrazide (6a):

Yield: 86%; brown solid; m.p. 127-128°C.

1H NMR (400 MHz; DMSO) δ: 8.24 (1H; s; H-5'), 8.14 (1H; d; J_HH = 4.3 Hz; H-9'), 7.51 (1H; d; J_HH = 4.4 Hz; H-10'), 7.38 (1H; dd; J_HH = 5.0 and 1.3 Hz; H-5), 6.98-6.96 (2H; m; H-3 and H-4), 4.31 (2H; s; H-1'), 3.32 (3H; s; CH3).

13C NMR (100 MHz DMSO) δ: 171.6 (C-0), 150.4 (C-5'), 147.1 (C-2), 136.4 (C-6'), 134.3 (C-8'), 130.6 (C-5), 128.9 (C-3), 126.7 (C-4), 126.5 (C-9'), 125.3 (C-10'), 33.9 (C-1'), 28.5 (CH3).


IR ν_max (cm⁻¹); KBr pellets: 1671 (C=O); 1138 (C-N).

(E)-N-Methyl-N’-(5-nitrofuranyl-2-ylmethylene)-2-thiophen-2-ylacetohydrazide (6b):

Yield: 65%; brown solid; m.p. 103-104°C.

1H NMR (400 MHz; DMSO) δ: 8.31 (1H; s; H-5'), 7.82-7.81 (3H; m; H-3 and H-4), 7.38-7.36 (4H; m; H-9' and H-10'), 7.25-7.24 (3H; m; H-3 and H-4), 4.34 (4H; s; H-1').

13C NMR (100 MHz DMSO) δ: 171.0 (C-0), 153.6 & 152.3 (C-5'), 151.6 & 143.4 (C-6'), 136.5 & 129.1 (C-8'), 129.0 (C-2), 127.0 (C-3), 126.6 (C-5'), 125.3 (C-4), 114.8 & 114.7 (C-10'), 113.8 & 113.7 (C-9'), 34.0 (C-1'), 28.7 & 28.4 (CH3).


IR ν_max (cm⁻¹); KBr pellets: 1683 (C=O); 1255 (C-N).

References:


Biological activity

The activity against M. tuberculosis H37Rv (ATCC27294) was assessed using the micro plate alamar blue assay (MABA)15,16. This nontoxic methodology uses a thermally stable reagent, and shows a good and proportional correlation with BACTEC radiometric methods17,18.


