Heterocyclization of Thiourea Derivative to Novel Azines and Azoles: Antioxidant and Antimicrobial studies

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Abstract

N,N’-disubstituted thiourea derivative 2 underwent oxidative cyclization with Br2/AcOH mixture to give thiazole 3. Thiadiazole 6 was obtained as the result of chlorination of 2 followed by amination and subsequent intramolecular cyclodehydration. Thermal treatment of N,N’-disubstituted thiourea derivative 2 with TEA in DMF resulted in hydrolysis of 2 to furnish thiourea derivative 8. Treatment of compound 8 with semicarbazide, thiosemicarbazide, diethyl succinate, diethyl malonate / benzaldehyde or urea resulted in the formation of triazoles 11a,b, pyrrole 13, thiazine 16 and oxathiazole 19. The newly synthesized compounds were tested for their antioxidant and antimicrobial activities.

Key words: Thiazole, Triazole, Thiadiazole, Oxathiazole, Anti-oxidant.

1. Introduction

Many studies have shown that thiourea derivatives have a broad spectrum of biological effects profile including antiviral [1], anti-tubercular [2, 3], antibacterial [4], anti-fungal [5], anti-inflammatory [6], antitumor [7] and anti-malarial [9] activities. Nitrogen-containing heterocycles are of great importance to both medicinal and organic chemists, and the design and synthesis of these compounds continue to represent a challenge from both an academic and industrial perspective [10]. Many of these compounds have attracted considerable attention due to the wide spectrum of their pharmacological activities such as anti-inflammatory [11], antimicrobial [12-15], antioxidant [16], anticancer [17], antihypertensive [18], antiviral [19], diuretic [20], anti-HIV [21], anticonvulsant [22], and anti-tubercular agents [23]. Accordingly, we report herein about methods for the synthesis and modification of such ring systems and evaluation of the new synthesized heterocyclic systems as antioxidant and antimicrobial agents.

2. Experimental

Melting points were measured using an Electrothermal IA 9100 apparatus with open capillary tube and are uncorrected. The IR spectrum (KBr disc) was recorded on a Pye Unicomp Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. The (1H NMR) spectrum was measured on a JEOL-JNM-LA 400 MHz spectrometer using DMSO as a solvent. All chemical shifts were expressed on the δ (ppm) scale using TMS as an internal standard reference. Analytical data were obtained from the Microanalysis Center at Cairo University, Giza, Egypt.

4-Chloro-N-(6-chlorobenzo[d]thiazol-2-yl)benzamide (3)

A mixture of compound 2 (0.01 mol), bromine (0.01 mol) in acetic acid (20ml) was heated under reflux for 8 hrs. The solid formed upon pouring onto water was collected by filtration and recrystallized from dilute ethanol to give 3 as brown crystals. Yield 73%, mp178-180 °C. IR spectrum, ν, cm⁻¹: 3334(NH), 1648 (C=O). 1H NMR spectrum, δ, ppm: δ=7.99-7.4
(m, 7H, Ar-H), 10.37(s, 1H, NH). Anal: C_{12}H_{15}ClN_{2}OS (323.20); Calcd: %: C, 52.03; H, 2.49; N, 8.67; Found, %: C, 52.20; H, 2.42; N, 8.69.

5-(4-Chlorobenzylidene)-3-(4-chlorophenyl)-4,5-dihydro-1,2,4-thiadiazole (6)
A mixture of compound 2 (0.01 mol), sodium hydroxide (0.01 mol), sodium hypochlorite (0.01 mol) and ammonium hydroxide (0.01 mol) in DMF (25 ml) was stirred at r.t. for 6 hours. The precipitate formed upon cooling was filtered and recrystallized from DMF to give 6 as black crystals. Yield 62%, mp 159-161°C. IR spectrum, υ, cm⁻¹: 3334(NH), 1648(C=O).

Yield 66%, mp 203-205 °C. IR spectrum, υ, cm⁻¹: 3333(NH), 1648(C=O). Anal: C_{12}H_{17}ClN_{2}OS (342.81); Calcd, %: C, 49.55; H, 2.25; N, 10.50; Found, %: C, 49.55; H, 2.25; N, 10.41.

Hydrolysis method of compound 2 to 1-(4-Chlorophenyl)thiourea (8)
A mixture of compound 2 (0.01 mol), TEA (3 drops) in DMF 20 ml was heated under reflux for 2 hrs. The precipitate formed upon addition of reaction mixture to cold water was collected by filtration and recrystallized from DMF to give 8 as yellow crystals.

Yield 61%, mp180-182°C (as reported). IR spectrum, υ, cm⁻¹: 3449, 3345 (NH, NH). ¹H NMR spectrum, δ, ppm: 6.58 (s, 2H, NH₂), δ=7.43-7.96 (m, 4H, Ar-H), 10.39 (s, 1H, NH). Anal: C_{12}H_{15}ClN_{2}OS (186.66); Calcd, %: C, 45.04; H, 3.78; N, 15.01; Found, %: C, 45.13; H, 3.67; N, 15.09.

5-((4-chlorophenyl)amino)-3H-1,2,4-triazole-3-one (11a) and 5-((4-chlorophenyl)amino)-3H-1,2,4-triazole-3-thione (11b)
General method:
A mixture of compound (8) (0.01 mol), semicarbazide or thiosemicarbazide (0.01 mol), and sodium carbonate (0.01 mol) in DMF (30 ml) was heated under reflux for 7hrs. The precipitate formed upon dilution with water was filtered and recrystallized from DMF to give 11a,b as yellow crystals.

Compound (11a)
Yield 66%, mp 203-205 °C. IR spectrum, υ, cm⁻¹: 3333 (NH), 1648 (C=O). ¹H NMR spectrum, δ, ppm: δ=7.88-7.36 (m, 4H, Ar-H), 10.38 (s, 1H, exchangeable with D₂O, NH). Anal: C_{12}H_{17}ClN_{2}O (210.62); Calcd, %: C, 46.06; H, 2.42; N, 26.86; Found, %: C, 46.08; H, 2.41; N, 26.84.

Compound (11b)
Yield 58%, mp180-182 °C. ¹H NMR spectrum, δ, ppm: 7.96-7.40 (m, 4H, Ar-H), 10.38 (s, 1H, NH). Anal: C_{12}H_{15}ClN_{2}S (226.69); Calcd, %: C, 42.77; H, 2.24; N, 24.94; Found, %: C, 42.78; H, 2.25; N, 24.92.

2-((4-Chlorophenyl)amino)-4H-1,3-thiazine-4,6(5H)-dione (13)
A ternary mixture of compound (8) (0.01 mol), diethyl succinate (0.01 mol) and 3 drops of TEA in DMF (20 ml) was refluxed for 6 hrs. The precipitate formed upon concentration was collected by filtration and recrystallized from DMF to give compound 13 as yellow crystals. Yield 81%, mp188-190 °C. IR spectrum, υ, cm⁻¹: 3450 (NH), 1519(C=N), 1648 (C=O). ¹H NMR spectrum, δ, ppm: 7.36-7.88 (m, 6H, Ar-H + pyrrole-H), 10.45 (s, 1H, exchangeable with D₂O, NH). Anal: C_{12}H_{15}ClN_{2}O_{2}S (266.71); Calcd, %: C, 49.54; H, 2.65; N, 10.50; Found, %: C, 49.55; H, 2.75; N, 10.41.

5-Benzylidene-2-((4-chlorophenyl)amino)-4H-1,3-thiazine-4,6(5H)-dione (16)
A mixture of compound (8) (0.01 mol), diethyl malonate (0.01 mol), 3 drops of TEA and benzenaldehyde (0.01 mol) in DMF (50 ml) was refluxed for 7 hrs. The solid produced upon cooling was collected by filtration and recrystallized from DMF to give 16 as yellow crystals. Yield 61%, mp 222-224 °C. IR spectrum, υ, cm⁻¹: 1647 (C=O), 1594 (C=N). ¹H NMR spectrum, δ, ppm: 7.41-7.94 (m, 10H, Ar-H + exocyclic olefinic-H), 10.38 (s, 1H, exchangeable with D₂O, NH). Anal: C_{12}H_{17}ClN_{2}O_{2}S (342.81); Calcd: C, 59.56; H, 3.23; N, 8.17; Found, %: C, 59.55; H, 3.22; N, 8.16.

3-((4-Chlorophenyl)amino)-5H-1,2,4-oxathiazol-5-one (19)
A mixture of compound (8) (0.01 mol), urea (0.01 mol) and sodium carbonate (0.01 mol) in DMF (75 ml) was heated under reflux for 5hrs. The precipitate formed upon pouring the reaction mixture with water was collected by filtration and recrystallized from DMF to give 19 as yellow crystals. Yield 74%, mp 265-267 °C. IR spectrum, υ, cm⁻¹: 3347 (NH), 1653 (C=O). ¹H NMR spectrum, δ, ppm: δ=7.96-7.41 (m,
HETEROCYCLIZATION OF THIOUREA DERIVATIVE TO NOVEL...

4H, Ar-H), 10.39 (s, 1H, exchangeable with D₂O, NH). Anal: C₈H₅ClN₂O₂S (228.66); Calcd: C, 42.02; H, 2.20; N, 12.25; Found: C, 42.00; H, 2.21; N, 12.24.

3. Results and Discussion

In the present study, N,N'-disubstituted thiourea derivative 2 was prepared by the addition of p-chloroaniline to p-chlorobenzoyl isothiocyanate [24]. The intramolecular oxidative cyclization of the target 2 to benzothiazole 3 was achieved by treatment of 2 with bromine/ AcOH mixture (Scheme1). The characteristic resonance NH of compound 3 was detected at δ=10.37 ppm, IR Spectrum of 3 displayed the NH and C=O function at 3334 and 1648 cm⁻¹, respectively. The thiadiazole 6 was obtained by keeping 2 in NaOCl in the presence of NaOH/ NH₄OH mixture. The reaction may be started by the formation of sulphenyl chloride that underwent amination forming sulphenamide, followed by intramolecular cyclodehydration. The formation of 6 was potentiated by the absence of C=O frequency and the presence of D₂O exchangeable NH signal at 9.75 ppm (Scheme1).

![Scheme 1: Synthetic pathway for benzothiazole 3 and thiadiazole 6](image1)

In attempt to synthesize quinazoline 7; thiourea derivative 2 was heated in DMF in presence of TEA. However, the product was identified to be N-aryl thiourea 8 not the desired quinazoline 7. Base-induced cyclization of 8 with semicarbazide and/or thiosemicarbazide yielded triazoles 11a,b (Scheme2). The formation of the latter compounds was confirmed by their spectral data; for example, IR spectrum of triazolone 11a clarified a C=O absorption band at 1648 cm⁻¹, whereas its 1H NMR revealed a D₂O-exchangeable signal at 10.38 ppm for NH. Treatment of compound 8 with diethyl succinate leads to condensation, followed by dehydrogenation providing pyrrole 13. Compound 13 showed ¹HNMR signal at 10.45 ppm for NH function.

![Scheme 2: Synthetic pathway for triazoles 11a,b](image2)

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Thermal reaction of ternary mixture of 8, diethylmalonate and benzaldehyde in basic medium resulted in cyclocondensation followed by condensation with benzaldehyde to produce thiazine 16. Compound 16 showed stretching frequency at 1647 cm\(^{-1}\) for C=O. The \(^1\)H NMR clarified a singlet at 10.38 ppm for NH. Finally, heterocyclization of 2 with urea in refluxing DMF yielded oxathiazole 19 whose IR spectrum revealed a stretching band at 1653 cm\(^{-1}\) for C=O, and its \(^1\)H NMR clarified a D\(_2\)O-exchangeable singlet at 10.39 ppm for NH (Scheme 3).

Scheme 3: Synthetic pathway for pyrrole 13 and thiazine 16 and oxathiazole 19

**Antioxidant Evaluation**

The antioxidant activities of the synthesized compounds were determined and listed in Table 1.

**Table 1: Antioxidant assay for the tested new compounds**

<table>
<thead>
<tr>
<th>compounds</th>
<th>Absorbance of compounds</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.225</td>
<td>54.6</td>
</tr>
<tr>
<td>11a</td>
<td>0.136</td>
<td>72.8</td>
</tr>
<tr>
<td>13</td>
<td>0.081</td>
<td>83.8</td>
</tr>
<tr>
<td>16</td>
<td>0.062</td>
<td>87.6</td>
</tr>
<tr>
<td>Ascorbic acid (standard)</td>
<td>0.058</td>
<td>88.4</td>
</tr>
</tbody>
</table>

The results given in table (1) revealed that all compounds were found to be potent. Moreover, the results showed that compound 16 and 13 had the most potent levels of activity. Additionally, compound 11a was found to have moderate activity. While compound 6 was found to have the lowest potent levels

**Antimicrobial Evaluation**

The antimicrobial activities of the newly synthesized compounds were determined in vitro against a group of microorganisms using disc diffusion method. Tetracycline and Econazole were used as antibacterial and antifungal agent standards, respectively. DMSO was used as solvent. The zone of inhibition of bacterial growth was observed.
Table 2: Antibacterial Activity for the tested new compounds

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>DMSO (-ve control)</th>
<th>Tetracycline (standard)</th>
<th>5</th>
<th>6</th>
<th>11a</th>
<th>13</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>9.5</td>
<td>10.5</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>0</td>
<td>27</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>9</td>
<td>10.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0</td>
<td>25</td>
<td>10</td>
<td>11.5</td>
<td>0</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>0</td>
<td>25.5</td>
<td>0</td>
<td>12</td>
<td>12.5</td>
<td>9</td>
<td>13.5</td>
</tr>
</tbody>
</table>

The results given in tables (2 and 3) showed that most of the tested compounds have weak to moderate activity against the tested microorganisms.

Table 3: Antifungal Activity for the tested new compounds

<table>
<thead>
<tr>
<th>Fungi</th>
<th>DMSO (-ve control)</th>
<th>Econazole (standard)</th>
<th>5</th>
<th>6</th>
<th>11a</th>
<th>13</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pencillium sp.</td>
<td>0</td>
<td>23.5</td>
<td>9.5</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillussochraceus</td>
<td>0</td>
<td>23</td>
<td>9.5</td>
<td>0</td>
<td>12</td>
<td>8</td>
<td>9.5</td>
</tr>
<tr>
<td>Aspergillusfalvus</td>
<td>0</td>
<td>21</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10.5</td>
<td>9</td>
</tr>
<tr>
<td>Aspergillusparasiticus</td>
<td>0</td>
<td>26</td>
<td>12.5</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

4. References