Synthesis and Antimicrobial Evaluation of a New Series of Heterocyclic Systems Bearing a Benzosuberone Scaffold

Osama I. Abd El-Salam 1, Ali S. Alsayed 2, Korany A. Ali 1, Ahmed A. Abd Elwahab 1, Abd El-Galil E. Amr 3,∗ and Hassan M. Awad 4,5

Received: 11 September 2015 ; Accepted: 4 November 2015 ; Published: 16 November 2015

Abstract: A series of novel benzosuberone derivatives were synthesized and evaluated as antimicrobial agents by using substituted benzosuberone derivatives 1a, b as starting materials. Treatment of 1a, b with phenyl isothiocyanate in dimethylformamide was followed by treatment with cold HCl solution to afford the thioamides 4a, b, which was reacted with methyl iodide to obtain methylated products 5a, b. Cyclocondensation of 4a, b with chloroacetone 6 and phenacyl chloride 7 gave the corresponding thiophene derivatives 9a–c. Reaction of 4a, b with C-acetyl-N-arylhydrazonoyl chlorides 14a and 14b in boiling EtOH in the presence of triethylamine, afforded the corresponding 1,3,4-thiadiazole derivatives 16a–d. The thioamides 4a, b were reacted with C-ethoxycarbonyl-N-arylhydrazonoyl chlorides 18a, b which afforded 1,3,4-thiadiazole derivatives 19a–d. The benzosuberones 1a, b were treated with 3-mercaptopropanoic acid to give compounds 21a, b, which were cyclized to tricyclic thiopyran-4(5H)-one derivatives 22a, b. The latter compounds 22a, b were reacted with 3-mercaptopropanoic acid to give compounds 23a, b, which were cyclized tetracyclic ring systems 24a, b. Finally, compounds 24a, b were oxidized using hydrogen peroxide under reflux conditions to afford the oxidized form of the novel tetracyclic heterogeneous ring systems 25a, b. The newly synthesized compounds were screened for antimicrobial activities. The structures of new compounds were characterized by 1H-NMR, 13C-NMR, IR, and EI-MS.

Keywords: benzosuberone; nitro-benzosuberone; thioamide; thiophene; thiadiazole; antimicrobial activity

1. Introduction

Thiazolopyrimidine derivatives were studied as potential drug candidates with biological activities [1]. In a previous work, we reported that certain of our newly substituted heterocyclic compounds exhibited androgen receptor antagonists and anticancer activities [2,3]. Also, substituted and condensed cycloalkanones derivatives are of special interest for the preparation of potentially
bioactive compounds as they possess anti-inflammatory, anti-convulsant, anti-pyretic, anti-tumor, and anti-ulcer activities [4]. Benzosuberone moiety is the main scaffold in tricyclic antidepressant drugs such as noxiptiline and amitriptyline (the analogues of imipramine), which mostly affect the central nervous systems [5,6]. On the other hand, heterocyclic sulfur compounds are of special interest in modern medicinal chemistry. For example, thiophene and thiadiazole derivatives are a well-known class of biologically active basic compounds for a large number of new drugs [7–9]. In view of these observations and in continuation of our current interest in the synthesis of poly-substituted heterocycles for biological evaluations [10–14], the present work was planned to prepare some new benzosuberone derivatives bearing thiadiazole and thiophene moiety, in addition to synthesis of new tricyclic and tetracyclic compounds bearing the benzosuberone scaffold. The newly synthesized compounds were investigated as antimicrobial agents.

2. Results and Discussion

2.1. Chemistry

Thioamide and thioanalide intermediates are considered to be a category of versatile intermediates that provide building blocks in the synthesis of poly-substituted thiophene and thiadiazole derivatives [15,16]. Preparation of benzosuberone derivatives substituted with thioamide and thioanalide was achieved by the treatment of the benzosuberone derivatives 1a,b with phenyl isothiocyanate in dimethylformamide, in the presence of potassium hydroxide, followed by treatment with cold HCl solution to afford the thioamide derivatives 4a,b with 81% and 79% yields, respectively (Scheme 1). The 1H-NMR spectrum of the thioamide derivative 4a as an example, revealed the presence of multiple signals at 1.90 ppm and two triplet signals 2.41–3.20 ppm characteristic for 3CH2 protons, in addition to a broad signal at 11.50 ppm due to NH proton. The mass spectrum of compound 4a revealed a peak at m/z 295 corresponding to its molecular ion peak. Treatment of the thioamide derivatives 4a,b with methyl iodide in EtONa solution afforded the methylated products 5a,b (Scheme 1). The 1H-NMR spectrum of compound 5a displayed a new singlet signal at 3.08 ppm characteristic for S-methyl group.

![Scheme 1](image_url)

Scheme 1. Synthetic routes for compounds 4a,b and 5a,b.

Cyclocondensation of the thioamide derivatives 4a and 4b with chloroacetone 6 and phenacyl chloride 7 in refluxing EtOH, in the presence of a catalytic amount of triethylamine furnished, in each case, was only one isolated product. The identities of the isolated products were assigned as the thiophene derivatives 9a-c rather than the isomeric 1,3-thiazoles 10 (Method A, Scheme 2), on the basis of their spectral data. The IR spectrum of compound 9b, as an example of the synthesized compounds, showed the presence of NH and carbonyl bands at 3428 and 1654 cm⁻¹, respectively.
Moreover, the $^1$H-NMR spectrum of compound 9b, revealed multiplets at 1.72 and 2.35–2.59 ppm corresponding to 3CH$_2$ groups, in addition to signals at 3.30 and 9.53 ppm corresponding to CH$_3$ and NH protons, respectively. Compound 9c was also prepared using thioamide derivative 4a and phenacyl chloride 7b in DMF in the presence of potassium carbonate at room temperature (Method B, Scheme 2). The product 9c from the later method was identical in all respects with the previously obtained authentic sample 9c.

![Scheme 2](image)

**Scheme 2.** Synthetic routes for compounds 9a–c.

Further evidence for the proposed structure of compound 9a was obtained by an independent synthesis via treatment of thioamide derivative 4a with 3-chloroacetylacetone in refluxing EtOH, in the presence of a catalytic amount of triethylamine. The obtained product 9a identical in all respects (mp, TLC, and spectra) with that obtained from the reaction of the thioamide derivative 4a with chloroacetone. A reasonable mechanism of the latter reaction is outlined in Scheme 3, the addition of the haloketone to 4a with the elimination of HCl gave 11 followed by intramolecular cyclization to give 12, which under the effect of hydronium ion gave 13 and elimination of acetic acid gave 9a.

![Scheme 3](image)

**Scheme 3.** Mechanism of formation compound 9a.
We have investigated the behavior of the thioamide derivatives 4a,b toward hydrazonoyl halide derivatives to prompt our synthetic strategy toward new heterocyclic systems attached to the benzo-suberone scaffold. Thus, treatment of the thioamide derivatives 4a and 4b with C-acetyl-N-arylhydrazonoyl chlorides 14a and 14b in boiling EtOH in the presence of triethylamine, afforded in each case only one isolated product. The identities of the isolated products were assigned as the 1,3,4-thiadiazoline derivatives 16a–d rather than the arylhydrazono-thiazole derivative 17 on the basis of their spectroscopic data (Scheme 4). For example, compound 16c showed characteristic IR bands at 1671, 1653 cm$^{-1}$ due to two carbonyl groups. The $^1$H-NMR spectrum of 16c revealed multiplets at 1.53, 2.38–2.46 ppm due to the protons of 3CH$_2$ groups and signal at 3.43 ppm due to the acetyl protons. In addition, its mass spectrum revealed a peak at m/z 380 corresponding to its molecular ion peak.

In a similar manner, the thioamides 4a and 4b were reacted with C-ethoxycarbonyl-N-arylhydrazonoyl chlorides 18a and 18b under the same reaction condition and afforded 1,3,4-thiadiazoline derivatives 19a–d rather than the thiazole-4-one derivative 20 on the basis of their spectroscopic data (Scheme 5). For example, compound 19a showed characteristic IR bands at 1718, 1675 cm$^{-1}$ due to two carbonyl groups. The $^1$H-NMR spectrum of 19a was revealed triplet and quartet signals in the region 2.36–2.8 ppm due to the protons of 3CH$_2$ groups. The mass spectrum of compound 19a revealed a peak at m/z 392 corresponding to its molecular ion.

The reaction of 1a,b with 3-mercaptopropanoic acid in refluxing benzene, in the presence of 4-toluenesulfonic acid (PTSA), results in compounds 21a,b which undergo intermolecular cyclization under reflux temperature using phosphorus pentoxide to afford the novel tricyclic thiopyran-4(5H)-one derivatives 22a,b (Scheme 6). The structures of the latter products were established on the basis of their elemental analysis and spectral data.
Treatment of the thiopyran-4(5H)-one derivatives 22a,b in step 1 with 3-mercaptopropanoic acid in refluxing benzene, in the presence of 4-toluenesulfonic acid (PTSA), afford the non-isolated intermediates 23a,b that undergo intramolecular cyclization under reflux temperature using phosphorus pentoxide to afford the novel tetracyclic ring systems 24a,b (Scheme 7). The structures of compounds 24a,b were established on the basis of their elemental analysis and spectral data. The IR spectrum of compound 24a showed the presence of the carbonyl band at 1692 cm\(^{-1}\). The \(^1\)H-NMR spectrum of 24 revealed multimples at 2.25–2.50 and 3.25–3.38 ppm corresponding to 5 CH\(_2\) groups, in addition to the signal at 3.73 ppm corresponding to CH\(_2\) of the dioxothiopyran protons. The later products were oxidized using hydrogen peroxide under reflux condition to afford the oxidized form of the novel tetracyclic derivatives 25a,b (Scheme 7).

Due to the oxidation of compounds 24a,b to compounds 25a,b, the carbonyl band in the IR spectrum of compound 25a for example shifted to 1695 cm\(^{-1}\). The \(^1\)H-NMR spectrum of compound 25a revealed multimples near 1.76, 2.15, 2.63 and 3.27 ppm corresponding to 5 CH\(_2\) groups, in addition to signal at 2.63 ppm corresponding to CH\(_2\) of the dioxi-thiopyran protons.
2.2. Antimicrobial Screening

The in vitro antimicrobial activity of the synthesized compounds was screened against gram-positive (Bacillus subtilis) and gram-negative (Escherichia coli) bacteria, yeast (Candida albicans) and filamentous fungi (Aspergillus niger). The tested products have shown a strong to moderate effect against most of the tested pathogens.

Most of the compounds showed a moderate inhibitory effect against Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus and Enterococcus faecalis) except compounds 25b and 22a, which showed strong inhibition effect in comparison with the standard antibiotics used (Table 1). On the other hand, five compounds 25b, 9c, 19b, 21b and 5a showed a strong inhibitory effect (15–25 mm) against Escherichia coli, as an example of Gram-negative bacteria. While the others showed a weak to a moderate inhibition effect against Pseudomonas aeruginosa, Proteus sp. The antifungal activities are presented in Table 1. In case of unicellular fungi most of the compounds showed a strong antifungal effect against Candida albicans. These compounds are 22a, 25b, 21a, 9c and 19b while the other compounds were characterized by moderate antifungal effect. In the case of filamentous fungi, compounds 22a, 25b, 4b, 5a, 5b, 9c, 19d, 22b and 24a have a higher antifungal effect against Aspergillus niger and a moderate activity against A. flavus in comparison with the antifungal standard antibiotic (Neomycin) used in this study. Finally, the demonstration of the activity of compound 25b against gram-positive bacteria, gram-negative bacteria, and fungi is an indication that this compound can be used in the treatment of the tested pathogens due to its broad spectrum effect.

The structure-activity relationships (SAR) of these synthesized compounds (Tetracyclic derivatives) may be due to their ability for inhibiting the cell growth by inhibiting the protein synthesis [17,18].

The minimum inhibitory concentration (MIC) of the synthesized compounds is presented in the Table 2. The MIC varied from 50 to 500 µg/mL based on the tested compounds. The MIC of the compound 25b was 50, 50, 75 and 100 µg/mL against tested pathogens E. coli, C. albicans, B. subtilis and A. niger. On the other hand, the MIC of the compound 22a was 100 µg/mL against Bacillus subtilis, while the MIC of the compound 9c was 200 µg/mL against Bacillus subtilis. Finally, we can interpret that some of samples have strongest antifungal and antibacterial activities. The demonstration of the activity against gram-positive bacteria, gram-negative bacteria, and fungi is an indication that the compounds have a broad spectrum effect. A few of the test compounds possessed a broad spectrum of activity having MIC values ranging from 50 to 200 µg/mL.
Table 1. Antimicrobial activity of compounds at 10 mg/mL.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>E. faecalis</th>
<th>P. aeroginosa</th>
<th>Proteus sp.</th>
<th>C. albicans</th>
<th>A. niger</th>
<th>A. flavus</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>17</td>
<td>17</td>
<td>14</td>
<td>17</td>
<td>14</td>
<td>15</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>4b</td>
<td>15</td>
<td>15</td>
<td>12</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td>00</td>
<td>20</td>
</tr>
<tr>
<td>5a</td>
<td>00</td>
<td>18</td>
<td>15</td>
<td>18</td>
<td>15</td>
<td>14</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>5b</td>
<td>00</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>9a</td>
<td>12</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>9b</td>
<td>12</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>9c</td>
<td>22</td>
<td>20</td>
<td>17</td>
<td>20</td>
<td>18</td>
<td>17</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>16a</td>
<td>20</td>
<td>16</td>
<td>14</td>
<td>16</td>
<td>15</td>
<td>15</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>16b</td>
<td>12</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>16c</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>16d</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>11</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>19a</td>
<td>12</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>19b</td>
<td>22</td>
<td>20</td>
<td>17</td>
<td>20</td>
<td>17</td>
<td>18</td>
<td>17</td>
<td>00</td>
</tr>
<tr>
<td>19c</td>
<td>12</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>19d</td>
<td>20</td>
<td>17</td>
<td>14</td>
<td>17</td>
<td>14</td>
<td>15</td>
<td>00</td>
<td>20</td>
</tr>
<tr>
<td>21a</td>
<td>17</td>
<td>15</td>
<td>13</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td>20</td>
<td>00</td>
</tr>
<tr>
<td>21b</td>
<td>14</td>
<td>20</td>
<td>18</td>
<td>20</td>
<td>15</td>
<td>17</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>22a</td>
<td>25</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>22b</td>
<td>15</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>24a</td>
<td>15</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>24b</td>
<td>12</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>15</td>
<td>00</td>
</tr>
<tr>
<td>25a</td>
<td>17</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>25b</td>
<td>27</td>
<td>25</td>
<td>19</td>
<td>25</td>
<td>19</td>
<td>20</td>
<td>25</td>
<td>29</td>
</tr>
</tbody>
</table>

* S = Streptomycin; TE = Tetracycline; N = Neomycin and T = Oxytetracycline.

Table 2. Minimum inhibitory concentration (MIC) of different compounds against tested pathogens.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Inhibition Zone Diameters (mm)</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
<td>E. coli</td>
</tr>
<tr>
<td>25b</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>22a</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>9c</td>
<td>11</td>
<td>-</td>
</tr>
</tbody>
</table>

3. Experimental Section

3.1. Chemistry

All melting points were measured on a Gallenkamp melting point apparatus (Weiss Gallenkamp, London, UK). The infrared spectra were recorded in potassium bromide disks on a PyeUnicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers (PyeUnicam Ltd., Cambridge, UK and Shimadzu, Tokyo, Japan, respectively). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, Palo Alto, CA, USA). \(^1\)H spectra were run at 300 MHz and \(^{13}\)C spectra were run at 75.46 MHz in deuterated chloroform (CDCl\(_3\)) or dimethyl sulfoxide (DMSO-\(d_6\)). (Sigma-Aldrich, St. Louis, MO, USA). Chemical shifts are given in parts per million and were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer (Shimadzu, Tokyo, Japan) at 70 eV. Elemental analyses were carried out at the Micro-analytical Centre of Cairo University, Giza, Egypt and recorded on Elementar-Vario EL (ELTRA GmbH, Haan, Germany) automatic analyzer. Compounds 1b, 14a–c and 18a–c were prepared by following the reported procedures in the literature [19–22]. The in vitro antimicrobial screening was performed by Chemistry of Natural and Microbial Products Dept., National Research Centre, Cairo-12622, Cairo, Egypt.
3.1.1. Preparation of the Thioamide Derivatives 4a,b

A solution of finely ground KOH (0.12 g, 2 mmol) and benzosuberone derivatives 1a,b (2 mmol) in DMF (10 mL), was stirred for 2 h. Phenyl isothiocyanate (0.27 g, 10.0 mmol) was then added drop-wise and the mixture was stirred for 10–12 h. The mixture was poured onto cold water acidified with 1N HCl. The solid product obtained was filtered off, washed with water, dried, and finally recrystallized from the prober solvent to afford the thioamide derivatives 4a,b.

6,7,8,9-Tetrahydro-6-(mercaptophenylamino)methylenebenzo[7]annulen-5-one 4a. Yield: (0.24 g, 81%); mp: 185–187 °C; as a pale yellow crystals (MeOH). IR (KBr, cm⁻¹): ν 3430 (NH), 1637 (C=O). 1H-NMR (DMSO-d6): δ 1.90 (m, 2H, CH2), 2.41–3.20 (m, 4H, 2CH2), 6.99–7.90 (m, 9H, Ar-H), 8.50 (br s, 1H, NH, D2O exchangeable). MS m/z (%): 296 [M + 1]+ (5), 295 [M]+ (25), 219 (10), 205 (40), 160 (30), 92 (100), 77 (50). Anal. Calcd. for C_{18}H_{17}NOS (295.40): C, 73.19; H, 5.80; N, 4.74; S, 10.85. Found: C, 73.44; H, 5.89; N, 4.62; S, 10.79.

6,7,8,9-Tetrahydro-6-(mercapto(phenylamino)methylene)-3-nitrobenzo[7]annulen-5-one 4b. Yield: (0.27 g, 79%); mp: 215–217 °C; yellow crystals (EtOH). IR (KBr, cm⁻¹): ν 3433 (NH), 1663 (C=O). 1H-NMR (DMSO-d6): δ 1.94–3.30 (m, 6H, 3CH2), 7.01–8.27 (m, 8H, Ar-H), 9.20 (br s, 1H, NH, D2O exchangeable). 13H-NMR (DMSO-d6): δ 25.41, 25.60, 39.90, 115.68, 119.98, 120.39, 124.39, 129.51, 130.88, 138.88, 139.05, 142.92, 147.15, 155.41, 189.93. MS m/z (%): 341 [M + 1]+ (3), 340[M]+ (15), 338 (100), 262 (35), 205 (60). Anal. Calcd. for C_{18}H_{16}N_{2}O_{3}S (340.40): C, 63.51; H, 4.74; N, 8.23; S, 9.42. Found: C, 63.44; H, 4.69; N, 7.97; S, 9.48.

3.1.2. General Procedure for Preparation of the S-Methylated Thioamide Derivatives 5a,b

To a stirred solution of the thioamide derivatives 4a,b (1 mmol) and potassium carbonate (0.14 g, 2 mmol) was added and stirring was continued for another 12 h. The mixture was poured onto crushed ice and the solid product obtained was filtered off, washed with water, dried, and finally recrystallized from the prober solvent to afford colorless crystals of compounds 5a,b.

6,7,8,9-Tetrahydro-6-(methylthio(phenylamino)methylene)-3-nitrobenzo[7]annulen-5-one 5a. Yield: (0.26 g, 84%); mp: 195–197 °C; buff powder (MeOH/dioxan). IR (KBr, cm⁻¹): ν 3400(NH), 1674 (C=O). 1H-NMR (DMSO-d6): δ 1.77–2.51 (m, 6H, 3CH2), 3.08 (s, 3H, CH3), 6.86–7.79 (m, 9H, Ar-H), 8.31 (br s, 1H, NH, D2O exchangeable). MS m/z (%): 310 [M + 1]+ (8), 309 [M]+ (45), 261 (30), 217 (20), 115 (70), 91 (50), 77 (100). Anal. Calcd. for C_{19}H_{19}NOS (309.43): C, 73.75; H, 6.19; N, 4.53; S, 10.36. Found: C, 73.52; H, 6.10; N, 4.62; S, 10.44.

6,7,8,9-Tetrahydro-6-(methylthio(phenylamino)methylene)-3-nitrobenzo[7]annulen-5-one 5b. Yield: (0.31 g, 84%); mp: 225–227 °C; pale yellow crystals (EtOH/DMF). IR (KBr, cm⁻¹): ν 3430 (NH), 1664 (C=O). 1H-NMR (DMSO-d6): δ 1.77 (m, 2H, CH2), 2.30–2.51 (m, 4H, 2CH2), 3.30 (s, 3H, CH3), 7.18–8.57 (m, 8H, Ar-H), 9.01 (br s, 1H, NH, D2O exchangeable). MS m/z (%): 355 [M + 1]+ (5), 354 [M]+ (25), 205 (35), 159 (40), 93 (100), 77(70). Anal. Calcd. for C_{19}H_{18}N_{2}O_{3}S (354.42): C, 64.39; H, 5.12; N, 7.90; S, 9.05. Found: C, 64.26; H, 5.03; N, 7.78; S, 9.14.

3.1.3. Reaction of Thioamide Derivatives 4 with α-Halo Carbonyl Compounds: General Procedure for the Preparation of 9a–c

Method A: To a solution of the thioamide derivatives 4a and 4b (1 mmol) and 1 mmol of chloroacetone 6 or phenacyl chloride 7 in EtOH (10 mL), 0.2 mL of triethylamine were added. The reaction mixture was refluxed for 10–15 h and then allowed to cool. The solid product obtained was filtered off, washed with EtOH, dried, and finally recrystallized from the prober solvent to afford the corresponding thiophenes 9a–c, respectively.
By the same method, 4a (1 mmol) was reacted with 3-chloroacetyl acetone (1 mmol) to afford 9a, which is identical in all respects (mp, TLC and spectra) in comparison with an authentic sample that obtained from the reaction of 4a and chloroacetone.

Method B: To a mixture of the thioamide derivatives 4a,b (1 mmol) and chloroacetone 6 or phenacyl chloride 7 (1 mmol) in DMF (5 mL), 0.19 g potassium carbonate was added. The reaction mixture was stirred at ambient temperature for 10 h, and then poured onto ice cold water acidified with 1N HCl. The solid product obtained was filtered off, washed with water, dried and finally recrystallized from the prober solvent to afford products identical in all respect with compounds 9a–d.

(2-Acetyl-5-phenylimino)thiophen-2-ylbenzo[7]lanulene 9a. Yield: (0.29 g, 87%); mp: 187–190 °C; buff powder (MeOH). IR (KBr, cm
-1): \(\nu = 3430\) (NH), 1691 (C=O). \(^1\)H-NMR (DMSO-d_6): \(\delta = 1.72\) (m, 2H, CH_2), 2.15–2.64 (m, 4H, 2CH_2), 2.80 (s, 3H, CH_3), 6.97–7.59 (m, 9H, Ar-H), 8.60 (s, 1H, NH, D_2O exchangeable). MS m/z (%): 334 [M + 1]^+ (7), 333 [M]^+ (35), 308 (50), 293 (15), 194 (20), 118 (100), 92 (15), 77 (40). Anal. Calcd. for C_{21}H_{19}NOS (333.45): C, 75.64; H, 5.74; N, 4.20; S, 9.62. Found: C, 75.32; H, 5.61; N, 4.05; S, 9.74.

(2-Benzoyl-5-phenylimino)thiophen-2-ylbenzo[7]lanulene 9b. Yield: (0.30 g, 80%); mp: 200–202 °C; yellow crystals (EtOH). IR (KBr, cm
-1): \(\nu = 3428\) (NH), 1654 (C=O). \(^1\)H-NMR (DMSO-d_6): \(\delta = 1.72\) (m, 2H, CH_2), 2.35–2.59 (m, 4H, 2CH_2), 3.30 (s, 3H, CH_3), 6.68–7.82 (m, 8H, Ar-H), 9.53 (s, 1H, NH, D_2O exchangeable). \(^1\)H-NMR (DMSO-d_6): \(\delta = 21.35, 28.23, 34.12, 39.45, 116.30, 119.35, 122.21, 123.10, 125.50, 129.60, 136.10, 138.50, 141.33, 143.31, 145.21, 150.13, 187.21. MS m/z (%): 379 [M + 1]^+ (4), 378 [M]^+ (25), 343 (10), 258 (40), 212 (100), 200 (35), 142 (70), 91 (65), 77 (60). Anal. Calcd. for C_{21}H_{18}N_2O_3S (378.44): C, 66.65; H, 4.79; N, 7.40; S, 8.47. Found: C, 66.51; H, 4.73; N, 7.47; S, 8.55.

3.1.4. Reactions of Thioamide Derivatives 4a,b with C-Acetyl-N-arylhydrazonoyl Chlorides 14a,b and C-Ethoxycarbonyl-N-arylhydrazonoyl chlorides 18a,b

The reactions of the thioamide derivatives 4a and 4b with hydrazonoyl chlorides 14a,b and/or 18a,b, were carried out as described above for the synthesis of thiophene derivatives (method A), to afford the corresponding 1,3,4-thiadiazol derivatives 16a–d and 19a–d, respectively.

6-(5-Acetyl-3-p-toly1,3,4-thiadiazol-2(3H)-ylidene)-6,7,8,9-tetrahydrobenzo[7]annelulen-5-one 16a. Yield: (0.3 g, 79%); mp: 225–227 °C; yellow powder (MeOH/dioxan). IR (KBr, cm
-1): \(\nu = 3428\) (NH), 1668, 1653 (C=O). \(^1\)H-NMR (DMSO-d_6): \(\delta = 1.57\) (m, 2H, CH_2), 2.40–2.46 (m, 4H, 2CH_2), 3.10 (s, 3H, CH_3), 3.43 (s, 3H, CH_3), 7.10–7.50 (m, 8H, Ar-H). MS m/z (%): 377 [M + 1]^+ (9), 376 [M]^+ (75), 187 (45), 160 (20), 91 (80), 86 (100). Anal. Calcd. for C_{22}H_{20}N_2O_5S (376.47): C, 70.19; H, 5.35; N, 7.44; S, 8.52. Found: C, 69.98; H, 5.19; N, 7.66; S, 8.47.

6-(5-Acetyl-3-p-toly1,3,4-thiadiazol-2(3H)-ylidene)-6,7,8,9-tetrahydro-3-nitrobenzo[7]annelulen-5-one 16b. Yield: (0.38 g, 90%); mp: 260–262 °C; brown crystals (EtOH/dioxan). IR (KBr, cm
-1): \(\nu = 3428\) (NH), 1690, 1645 (C=O). \(^1\)H-NMR (DMSO-d_6): \(\delta = 1.70\) (m, 2H, CH_2), 2.40–2.46 (m, 4H, 2CH_2), 3.10 (s, 3H, CH_3), 3.43 (s, 3H, CH_3), 7.30–7.90 (m, 7H, Ar-H). MS m/z (%): 423 [M + 2]^+ (11), 421 [M]^+ (55), 287 (15), 203 (30), 159 (20), 91 (35). Anal. Calcd. For C_{22}H_{19}N_3O_4S (421.47): C, 62.69; H, 4.54; N, 9.97; S, 7.61. Found: C, 62.35; H, 4.39; N, 10.14; S, 7.41.

6-(5-Acetyl-3-(4-fluorophenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-6,7,8,9-tetrahydrobenzo[7]annelulen-5-one 16c. Yield: (0.32 g, 85%); mp: 240–242 °C; gray powder (dioxan). IR (KBr, cm
-1): \(\nu = 3428\) (NH), 1671, 1653 (C=O). \(^1\)H-NMR (DMSO-d_6): \(\delta = 1.53\) (m, 2H, CH_2), 2.38–2.46 (m, 4H, 2CH_2), 3.43 (s, 3H, CH_3), 6.76–7.40 (m, 8H, Ar-H). \(^1\)H-NMR (DMSO-d_6): \(\delta = 23.13, 23.80, 25.41, 38.90, 116.44, 117.60, 119.90, 126.23, 128.75,
Ethyl 4,5-Dihydro-5-(5,6-dihydro-9-oxo-5H-benzo[7]annulen-8(7H)-ylidene)-4-phenyl-1,3,4-thiadiazole-2-carboxylate 19a. Yield: (0.31 g, 79%); mp: 218–220 °C; brown crystals (EtOH/DMF). IR (KBr, cm⁻¹): ν 1718, 1675 (C=O). 1H-NMR (DMSO-d₆): δ 1.57 (m, 2H, CH₂), 2.19–2.56 (m, 4H, 2CH₂), 2.26–2.80 (m, 6H, 3CH₂), 3.43 (s, 3H, CH₃), 4.27 (m, 2H, CH₂), 6.83–7.49 (m, 8H, Ar-H), 13H-NMR (DMSO-d₆): δ 13.80, 21.35, 23.35, 35.55, 46.39, 64.90, 125.44, 127.60, 128.13, 133.67, 135.13, 136.28, 138.75, 139.13, 141.35, 150.25, 151.20, 154.31, 156.15. MS m/z (%): 438 [M + 1]⁺ (4), 437 [M]⁺ (19), 333 (25), 315 (100), 242 (45), 158 (90), 91 (50). Anal. calcd. for C₂₂H₂₀N₂O₃ (437.47): C, 67.96; H, 5.46; N, 6.89; S, 7.89. Found: C, 67.52; H, 5.28; N, 7.06; S, 8.13.

Ethyl 4,5-Dihydro-5-(6,7-dihydro-9-oxo-5H-benzo[7]annulen-8(9H)-ylidene)-4-phenyl-1,3,4-thiadiazole-2-carboxylate 19b. Yield: (0.33 g, 75%); mp: 235–2375 °C; pale yellow crystals (EtOH/DMF). IR (KBr, cm⁻¹): ν 1735, 1670 (C=O). 1H-NMR (DMSO-d₆): δ 1.35 (t, J = 6.9 Hz, 3H, CH₃), 1.92–2.61 (m, 6H, 3CH₂), 3.28 (s, 3H, CH₃), 3.87 (q, J = 7.1 Hz, 2H, CH₂), 4.37 (q, J = 7.2 Hz, 2H, CH₂), 7.22–8.28 (m, 7H, Ar-H), 13H-NMR (DMSO-d₆): δ 13.35, 23.25, 25.90, 26.11, 38.23, 61.25, 116.32, 121.23, 125.75, 126.61, 127.67, 129.25, 131.93, 134.31, 143.45, 148.20, 150.60, 155.62, 162.20, 185.32. MS m/z (%): 452 [M + 1]⁺ (12), 451 [M]⁺ (50), 360 (15), 234 (61), 199 (36), 125 (29), 77 (100). Anal. calcd. for C₂₃H₂₁N₂O₅ (451.49): C, 61.01; H, 4.43; N, 9.54; S, 7.10. Found: C, 61.18; H, 4.69; N, 9.31; S, 7.31.

3-(E)-6,7-Dihydro-5H-benzol[7]annulen-9-ythio)propanoic acid 21a. Yield: (0.21 g, 84%); mp: 193–195 °C; White crystals (MeOH). IR (KBr, cm⁻¹): ν 1722 (C=O). 1H-NMR (DMSO-d₆): δ 1.65 (m, 2H, CH₂), 1.96

3.1.5. General Procedure for the Preparation of Compounds 21a,b

A mixture of the 1a,b (5 mmol), mercaptoproic acid (5 mmol) and p-toluenesulfonic acid (PTSA) in dry benzene (50 mL) was refluxed for 72 h and allowed to cool to room temperature then diluted with water (30 mL). The mixture was washed with saturated NaHCO₃ followed by 0.1N HCl solution. The organic layer was separated and dried over anhydrous sodium sulfate, then evaporated under reduced pressure. The solid products were collected by filtration, washed with EtOH, dried and recrystallized from the prober solvent to afford compounds 21a,b.
(t, J = 6.7 Hz, 2H, CH$_2$), 2.65–3.14 (m, 6H, CH$_2$), 6.70–7.50 (m, 5H, Ar-H + CH=CH=C), 11.5 (s, 1H, OH). MS m/z (%): 249 [M + 1]$^+$ (25), 248 [M]$^+$ (100), 175 (36), 143 (75). Anal. Calcd. for C$_{14}$H$_{16}$O$_2$S (248.34): C, 67.71; H, 6.49; S, 12.91. Found: C, 67.55; H, 6.33; S, 13.17.

3-((E)-6,7-Dihydro-5H-benzo[7]annulen-9-ylthio)propanoic acid 21b. Yield: (0.35 g, 86%); mp: 208–210 °C; pale yellow crystals (EtOH). IR (KBr, cm$^{-1}$): $\nu$ 1735 (C=O). $^1$H-NMR (DMSO-d$_6$): $\delta$ 1.65 (m, 2H, CH$_2$), 2.70–2.80 (m, 4H, 2CH$_2$), 2.80 (m, 2H, CH$_2$), 3.41 (m, 2H, CH$_2$), 7.01–7.50 (m, 4H, Ar-H). MS m/z (%): 294 [M + 1]$^+$ (25), 293 [M]$^+$ (100), 220 (70), 188 (40), 143 (36). Anal. Calcd. for C$_{14}$H$_{15}$NO$_2$S (293.34): C, 57.32; H, 5.15; N, 477; S, 10.93. Found: C, 57.44; H, 5.04; N, 4.87; S, 11.16.

3.1.6. General Procedure for the Preparation of Compounds 22a,b

To a solution of the appropriate mercapto propanoic acid derivatives 21a,b in benzene (thiophene free) (100 mL), phosphorous pentoxide (1 mmol) was added. The resulting mixture was refluxed for 72 h and allowed to cool to room temperature then diluted with water (30 mL), and washed with a saturated NaHCO$_3$ solution, then separated the water and HCl was added. The solid products that formed were collected by filtration, washed with water, dried, and recrystallized from the ethanol to afford the newly tricyclic compounds 22a,b.

2,3-Dihydrothiopyran-4-one[b,f]benzo[7]9,10,11-trihydroanulen 22a. Yield: (0.20 g, 86%); mp: 210–212 °C; buff powder (EtOH). IR (KBr, cm$^{-1}$): $\nu$ 1695 (C=O). $^1$H-NMR (DMSO-d$_6$): $\delta$ 2.05 (m, 2H, CH$_2$), 2.26–2.50 (m, 4H, 2CH$_2$), 2.80 (m, 2H, CH$_2$), 3.41 (m, 2H, CH$_2$), 7.01–7.50 (m, 4H, Ar-H). MS m/z (%): 231 [M + 1]$^+$ (25), 230 [M]$^+$ (100), 214 (36), 142 (75). Anal. Calcd. for C$_{14}$H$_{14}$OS (230.33): C, 73.01; H, 6.13; S, 13.92. Found: C, 72.79; H, 5.95; S, 14.26.

2,3-Dihydrothiopyran-4-one[b,f]3-nitrobenzo[7]9,10,11-trihydroanulen 22b. Yield: (0.23 g, 83%); mp: 227–230 °C; gray crystals (Dioxane). IR (KBr, cm$^{-1}$): $\nu$ 1683 (C=O). $^1$H-NMR (DMSO-d$_6$): $\delta$ 2.15–2.70 (m, 6H, 3CH$_2$), 3.25 (m, 2H, CH$_2$), 4.07 (t, J = 7.0 Hz, 2H, CH$_2$), 7.71–8.47 (m, 3H, Ar-H). $^{13}$H-NMR (DMSO-d$_6$): $\delta$ 23.50, 25.9, 28.80, 39.20, 38.50, 126.00, 126.7, 128.01, 128.7, 132.0, 135.17, 138.99, 196.03. MS m/z (%): 276 [M + 1]$^+$ (23), 275 [M]$^+$ (100), 219 (70), 186 (40), 115 (36), 63 (29). Anal. calcd. for C$_{14}$H$_{13}$NO$_2$S (275.32): C, 71.07; H, 4.76; N, 5.09; S, 11.65. Found: C, 69.93; H, 4.63; N, 5.21; S, 11.89.

3.1.7. General Procedure for the Preparation of Compounds 24a,b

A mixture of the compounds 22a and/or 22b (2 mmol), mercaptoacetic acid (2 mmol) and PTSA (2 mmol) in dry benzene (100 mL) was refluxed for 24 h and allowed to cool to room temperature. Phosphorous pentoxide (1 mmol) was then added and the resulting mixture was refluxed for additional 5 h and allowed to cool. The mixture was then diluted with water (30 mL), washed by saturated NaHCO$_3$ and 0.1N HCl. The organic layer was separated and dried over anhydrous sodium sulfate, then evaporated under reduced pressure. The solid products that formed were collected by filtration, washed with water, dried, and recrystallized from the prober solvent to afford compounds 24a,b.

2,3-Dihydrothiopyran[3,2-b]thiopyran-4(8H)-one[d,f]benzo[7]lanulen 24a. Yield: (0.23 g, 86%); mp: 280–282 °C; white crystal (EtOH/Dioxan). IR (KBr, cm$^{-1}$): $\nu$ 1692 (C=O). $^1$H-NMR (DMSO-d$_6$): $\delta$ 2.25–2.50 (m, 6H, 3CH$_2$), 3.25–3.38 (m, 4H, 2CH$_2$), 3.73 (s, 2H, CH$_2$), 7.30–7.57 (m, 4H, Ar-H). $^{13}$H-NMR (DMSO-d$_6$): $\delta$ 24.1, 24.9, 35.50, 38.40, 38.90, 126.00, 126.7, 128.01, 128.7, 132.0, 135.17, 138.99, 196.03. MS m/z (%): 301 [M + 1]$^+$ (23), 300 [M]$^+$ (100), 211 (50), 142 (35). Anal. calcd. for C$_{17}$H$_{16}$OS$_2$ (300.44): C, 67.96; H, 5.37; S, 21.35. Found: C, 67.84; H, 5.26; S, 21.66.

2,3-Dihydrothiopyran[3,2-b]thiopyran-4(8H)-one[d,f]3-nitrobenzo[7]lanulen 24b. Yield: (0.31 g, 90%); mp: >300 °C; brown crystals (EtOH/DMF). IR (KBr, cm$^{-1}$): $\nu$ 1698 (C=O). $^1$H-NMR (DMSO-d$_6$): $\delta$ 2.30–2.78 (m, 6H, 3CH$_2$), 3.27 (t, J = 6.4 Hz, 2H, CH$_2$), 3.33 (t, J = 6.8 Hz, 2H, CH$_2$), 3.89 (s, 2H, CH$_2$), 7.64–8.37 (m, 3H, Ar-H). MS m/z (%): 346 [M + 1]$^+$ (19), 345 [M]$^+$ (100), 299 (15), 257 (40), 209 (20).
60 (25). Anal. Calcd. for C\textsubscript{17}H\textsubscript{15}NO\textsubscript{3}S\textsubscript{2} (345.44): C, 59.11; H, 4.38; N, 4.05; S, 18.56. Found: C, 58.97; H, 4.26; N, 4.15; S, 18.86.

3.1.8. General Procedure for the Preparation of Compounds 25a,b

A mixture of the compound 24a and/or 24b (1 mmol) and hydrogen peroxide (2 mmol) was refluxed for 24 h and allowed to cool to room temperature then diluted with water (30 mL). The solid products that formed were collected by filtration, washed with water, dried and recrystallized from the ethanol to afford compounds 25a,b.

\textbf{2,3-Dihydro-dioxothiopyrano[3,2-b]dioxothiopyran-4(8H)-one[d,f]benzo[7]anulen 25a.} Yield (0.23 g, 65%); mp: > 300 °C; gray crystals (Dioxan). IR (KBr, cm\textsuperscript{-1}): v 1689 (C=O). \textsuperscript{1}H-NMR (DMSO-\textit{d}_6): \(\delta\) 1.76 (m, 2H, CH\textsubscript{2}), 2.15–2.30 (m, 4H, 2CH\textsubscript{2}), 2.63 (s, 2H, CH\textsubscript{2}), 3.27–3.52 (m, 4H, 2CH\textsubscript{2}), 7.01–7.47 (m, 4H, Ar-H). MS m/z (%): 366 [M + 2]\textsuperscript{+} (3), 364 [M]\textsuperscript{+} (25), 246 (30), 142 (70), 115 (100). Anal. Calcd. for C\textsubscript{17}H\textsubscript{16}O\textsubscript{5}S\textsubscript{2} (364.44): C, 56.03; H, 4.43; S, 17.60. Found: C, 55.76; H, 4.28; S, 18.04.

\textbf{2,3-Dihydro-dioxothiopyrano[3,2-b]dioxothiopyran-4(8H)-one[d,f]-3-nitrobenzo[7]anulene 25b.} Yield (0.30 g, 75%); mp: > 300 °C; buff crystals (Dioxan). IR (KBr, cm\textsuperscript{-1}): v 1695 (C=O). \textsuperscript{1}H-NMR (DMSO-\textit{d}_6): \(\delta\) 1.90 (m, 2H, CH\textsubscript{2}), 2.15–2.30 (m, 4H, 2CH\textsubscript{2}), 2.53 (s, 2H, CH\textsubscript{2}), 3.27–4.09 (m, 4H, 2CH\textsubscript{2}), 7.71–8.47 (m, 3H, Ar-H). MS m/z (%): 410 [M + 1]\textsuperscript{+} (10), 409 [M]\textsuperscript{+} (100), 289 (15), 187 (40). Anal. Calcd. for C\textsubscript{17}H\textsubscript{15}NO\textsubscript{7}S\textsubscript{2} (409.43): C, 49.87; H, 3.69; N, 3.42; S, 15.66. Found: C, 49.63; H, 3.57; N, 3.52; S, 15.89.

3.2. Biological Evaluation

3.2.1. Antimicrobial Activity

The ability to inhibit the growth of Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi was observed using an overlay method [17].

Sample Preparation

All samples were dissolved in dimethyl sulfoxide (RFCL Limited, New Delhi, India) DMSO at 10 mg/mL concentration as shown in the Table 1 in comparison with different standard antibiotics. Antibiotic discs of Streptomycin (S) (10 µg), Oxytetracycline (T) (30 µg) and Tetracycline (TE) (30 µg) were used as positive controls for bacteria. Neomycin (N) (30 µg), was used for fungi. The experiment was performed in triplicate.

Strains and Media Used

The common pathogenic and food spoilage microorganisms were selected for their relevance in bakery products and other food: the gram-positive bacteria; \textit{Bacillus subtilis} NRRL-B-4219, \textit{Staphylococcus aureus} ATCC 6538, \textit{Enterococcus faecalis} ATCC 19433 and the gram negative bacteria; \textit{Escherichia coli} ATCC 25922, \textit{Pseudomonas aeruginosa} ATCC 9027, \textit{Proteus} sp., yeasts such as \textit{Candida albicans} ATCC 10231, fungi \textit{Aspergillus niger} NRRL 2766 (equivalent to ATCC16888), and \textit{Aspergillus flavus} ATCC 16883.

The bacteria were slanted on nutrient agar (Merck, Darmstadt, Germany), Yeast was slanted and mentioned on Sabaroud’s agar medium (Lab M., Bury, Lancashire, UK) and the fungi was slanted and mentioned on the potato Dextrose Agar medium (Lab M Limited, Bury, Lancashire, UK). Mueller-Hinton agar (Lab M, Bury, Lancashire, UK) following the manufacturer’s instructions was used for the assay.

Bioassay

The antibacterial screening was essentially by the well diffusion agar method [23,24]. The organisms were streaked in radial patterns on the agar plates. Plates were incubated under aerobic conditions at 37 °C and 28 °C for 24 h and 48 h for bacteria and fungi, respectively. In order to
obtain comparable results, all prepared solutions were treated under the same conditions under the same incubated plates. All tests were performed in triplicate. Plates were examined for evidence of antimicrobial activities, represented by a zone of inhibition of the microorganism’s growth around the holes, and diameters of clear zones were expressed in millimeters [25].

3.2.2. Determination of Minimum Inhibitory Concentration (MIC)

The in vitro minimum inhibitory concentration (MIC) of the synthesized compounds was determined by the agar well diffusion method. DMSO was used to prepare different concentrations ranging from 50 to 500 µg/mL by serial dilutions. The media were inoculated with 100 µL of each of the 10^6 cfu/mL bacterial and fungal strains, and the assay was applied by an agar well diffusion method. Blank DMSO was used as negative control. The plates were incubated aerobically in an incubator at 37 °C for 24 h for bacterial strains and 25 °C for 48 h for fungal strains. The MIC was taken as the lowest concentration in the series dilution that prevented bacterial growth.

4. Conclusions

An efficient one step method for the synthesis of substituted thiophene derivatives 9 from thioamides 4 and α-haloketones has been performed. Reaction of 4 with C-acetyl-N-arylhydrazonoyl chlorides 14 and C-ethoxycarbonyl-N-arylhydrazonoyl chlorides 18 under basic conditions, afforded the corresponding 1,3,4-thiadiazoline derivatives 16 and 19, respectively. Benzosuberones 1 underwent condensation followed by cyclization to obtain tricyclic thiopyran-4(5H)-one derivatives and tetracyclic ring systems 24 and 25. The newly synthesized compounds showed to be active when tested as antimicrobial agents.

Acknowledgments: The project was financially supported by King Saud University, Vice Deanship of Research Chairs.

Author Contributions: The listed authors contributed to this work as described in the following. Osama I. Abd El-Salam, Aly S. Alsayed, Korany A. Ali, Abd El-Galil E. Amr gave the concepts of the work, interpreted the results and prepared the manuscript, Ahmed A. Abd Elwahab carried out the experimental part (this work is a part of his PhD thesis) and Hassan M. Awad tested the antimicrobial activities. All authors have read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References


**Sample Availability:** Samples of the compounds are not available from the authors.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).