Synthesis and In vitro Antibacterial Activity of Some 1,2,4-triazoles and 1,3,4-oxadiazoles Derivatives

LAURA-ILEANA SOCEA*, GABRIEL SARAMET†, CRISTINA ELENA DINU-PIRVU‡, CONSTANTIN DRAGHICI‡, BOGDAN SOCEA‡

1 University of Medicine and Pharmacy “Carol Davila”, Faculty of Pharmacy, Organic Chemistry Department, 6 Traian Vaia Str., 020956, Bucharest, Romania
2 University of Medicine and Pharmacy “Carol Davila”, Faculty of Pharmacy, Pharmaceutical Technology Department, 6 Traian Vaia Str., 020956, Bucharest, Romania
3 University of Medicine and Pharmacy “Carol Davila”, Faculty of Pharmacy, Physical and Colloidal Chemistry Department, 6 Traian Vaia Str., 020956, Bucharest, Romania
4 Romanian Academy, Organic Chemistry Centre “Costin D. Nenitescu”, 202B Splaiul Independenței, 060023, Bucharest, Romania
5 Carol Davila University of Medicine and Pharmacy, Faculty of General Medicine, St. Pantelimon Emergency Hospital, 340-342, Soseaua Pantelimon, 021623, Bucharest, Romania

A new series of the 2-amino-1,3,4-oxadiazoles 6a-c and 1,2,4-triazoles 8a-c substituted with 5H-dibenzo[a,d][7]annulene moiety were synthesised following the reaction sequences depicted in Scheme 1 and evaluated for antibacterial activity against Gram-positive and Gram-negative bacteria. All the newly synthesized compounds were characterized by their spectral data IR-, UV-, 1H-NMR and 13C-NMR spectroscopy and elemental analysis.

Keywords: 5H-dibenzo[a,d][7]annulene, 2-amino-1,3,4-oxadiazole, S-alkyl-1,2,4-triazole, antibacterial activity

Bacteria that develop resistance to antibiotics are one of society greatest future threats and are having a major impact on our ability to use various medical treatments. The spread of resistance is no longer a local problem in hospitals, antibiotic-resistant bacteria are also spreading to and throughout the environment. The rising incidence of bacterial infections, along with the emergence of resistance to conventionally-utilized antibiotics has added considerable urgency to the pursuit of safe and effective therapies in the last decade. So, the increasing clinical importance of drug resistant microbial pathogens has additional urgency in microbiological and antifungal research. A wide variety of heterocyclic systems have been exploited for developing new antimicrobial agents. The 1,3,4-oxadiazole derivatives have been found to exhibit diverse biological activities such as antimicrobial, anti-tubercular, anti-malarial, anti-inflammatory, anticonvulsant, and antitumor, anti HIV, muscle relaxant, antimitotic, diuretic, hypnotic, sedative etc [1-14]. Furthermore, it is well documented that 1,2,4-triazol derivatives possess antimicrobial, antifungal, analgesic, tuberculostatic, carbonic anhydrase inhibitors activities [15-20].

The 5H-dibenzo[a,d][7]annulene ring is incorporated in many biologically active compounds used in therapeutics as antimicrobial, anticonvulsive, anticholinergic, miorelaxant, antihistaminic, antifungal, analgesic agents, carbonic anhydrase inhibitors, but mostly they are used as antidepressant drugs [21-29].

For that reason we report in the present investigation the synthesis, characterization and antibacterial testing of the new 1,3,4-oxadiazole and 1,2,4-triazole derivatives.

Experimental part

All reagents were purchased from the Merck, Sigma-Aldrich and Fluka Companies. Melting points were determined on a Böetius apparatus and were uncorrected.

The UV spectra were determined on a SPECCORD 40 Analytik Jena spectrophotometer, using methanolic solutions (2.5.10^-5 M). The IR spectra were recorded in KBr discs on a Vertex 70 Bruker spectrometer. The 1H- and 13C-NMR spectra were recorded on a Varian Gemini 300BB spectrometer (300 MHz for H and 75 MHz for C), using CDCl3 as solvent and tetramethylsilane (TMS) as internal standard. The content of C, H, and N was assayed using a ECS-40-10-Costech microdosimeter.

Synthesis of compounds

2-(5H-Dibenzo[a,d][7]annulen-5-yl)acetohydrazide 3 is the starting material for the synthesis of all derivatives (Scheme 1) and was prepared according to the reported method [28]. By treatment of hydrazide (3) with various alkyl isothiocyanates gave hydrazinecarbothioamides 4a-c [28, 30].

For the synthesis of 1,3,4-oxadiazoles 6a-c, two methods were used: first method consists in the cyclization of hydrazinecarbothioamides 4a-c in the presence of mercury oxide in methanol and the second method consists in the treatment of the compounds 4a-c with methyl iodide in the presence of potassium hydroxide. The reaction probably takes place with the intermediate formation of 5a-c compounds which we could not isolate in a pure form.

Hydrazinecarbothioamides 4a-c were cyclized in alkaline conditions to 1,2,4-triazol-3-thioles 7a-c [28-32]. The thioethers 8a-c were prepared by alkylation of 7a-c with ethyl bromide [23, 28, 31].

Synthesis of 2-(aminoalkyl)-5-[5H-dibenzo[a,d][7] annulen-5-ylmethyl]-1,3,4-oxadiazoles 6a-c

a1) For 0.001 mol hydrazinecarbothioamide in methanol, 0.002 mol of HgO are added, and was refluxed for 3h. The resulted product is filtered in order to remove the HgS, and after cooling the solution, the corresponding 1,3,4-oxadiazoles precipitate.
2) 0.002 mol KOH and 0.001 mol methyl iodide are added at 0°C to 0.001 mol of each hydrazine-carbothioamides 4a-c solved in ethanol. The solution is magnetically stirred at room temperature for 12 h. The solid compound, that was obtained, is filtered, washed with water and boiled in ethanol for 6 h, to result the corresponding 1,3,4-oxadiazole.

2-(Aminethyl)-5-[5H-dibenzo[a,d][7]annulen-5-ylmethyl]-1,3,4-oxadiazole 6a
m.p. 157-159°C; yield: 29% (using CH3I). 65.2% (using HgO); elemental analysis: anal. calcd. for (317.39 g/mol): C, 75.69; H, 6.03; N, 13.24; found: C, 75.66; H, 6.05; N, 13.62; UV (methanol, \( \lambda_{max} \), nm): 3438 (N–H stretching), 1635, 1586, 1490 (C=C stretching, C=N stretching); 1H-NMR (300 MHz, CDCl3, \( \delta \)/ppm): 163.21 (C2); 158.26 (C5); 138.84 (C4a', C6a'); 133.68 (C10a', C11a'); 130.94 (C10', C11'); 129.90 (C1', C9'); 129.58 (C4', C6'); 129.07 (C4', C6'); 126.88 (C3', C7'); 52.75 (C5'); 38.44 (C15'); 26.33 (C12'); 51.31 (C16').

2-(Aminomethyl)-5-[5H-dibenzo[a,d][7]annulen-5-ylmethyl]-1,3,4-oxadiazole 6b
m.p. 101-103°C; yield: 40% (using CH3I). 65% (using HgO); elemental analysis: anal. calcd. for (317.39 g/mol): C, 75.69; H, 5.81; N, 12.76; found: C, 75.63; H, 5.80; N, 12.75; UV (methanol, \( \lambda_{max} \), nm): 220.3; 292.5; IR (KBr, cm-1): 3044 (N–H stretching), 2928, 2850 (CH3 stretching), 1657, 1588, 1493 (C=C stretching, C=N stretching); 1H-NMR (300 MHz, CDCl3, \( \delta \)/ppm): 163.39 (C2); 159.07 (C5); 138.95 (C4a', C6a'); 134.08 (C10a', C11a'); 131.11 (C10', C11'); 130.78 (C1', C9'); 129.65 (C4', C6'); 129.07 (C4', C6'); 128.75 (C3', C7'); 52.75 (C5'); 38.44 (C15'); 26.33 (C12'); 13.21 (C16').

b) Synthesis of 5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-3-ethylthio-4-alkyl-4H-1,2,4-triazoles 8a-c
To a 10 ml absolute ethanol add 0.004 mol Na and stir magnetically at room temperature. After obtaining a clear solution add 0.004 mol of the 1,2,4-triazole 7a-c and continue stirring for 30 min. Then add stoechiometrically ethyl bromide and stir at room temperature for 12 hours. The precipitate is filtered off and washed with water.

5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-3-ethylthio-4-ethyl-4H-1,2,4-triazole 8a
m.p. 96-98°C; yield: 81.35%; elemental analysis: anal. calcd. for (361.51 g/mol): C, 73.09; H, 6.41; N, 11.62; S, 8.87; found: C, 73.08; H, 6.43; N, 11.62; S, 8.86; UV (methanol, \( \lambda_{max} \), nm): 211.0; 226.5; IR (KBr, cm-1): 3044, 3022 (C–H stretching of aromatic ring), 2978, 2869 (CH3 stretching), 2930, 2823 (CH2 stretching), 1512, 1493 (C=C stretching, C=N stretching); 1H-NMR (300 MHz, CDCl3, \( \delta \)/ppm): 7.20-7.27.
Results and discussions

Chemistry

For the synthesis of new 1,3,4-oxadiazoles 6a-c, two methods were used: first method consists in the cyclization of hydrazinecarbothioamides 4a-c in the presence of mercury oxide in methanol. The best yields in 1,3,4-oxadiazoles were obtained by the first method. The losses recorded for the second method is due to the secondary reaction product which have not been isolated in pure form.

For the synthesis of new 1,3,4-oxadiazoles 6a-c, two methods were used: first method consists in the cyclization of hydrazinecarbothioamides 4a-c in the presence of mercury oxide in methanol. The best yields in 1,3,4-oxadiazoles were obtained by the first method. The losses recorded for the second method is due to the secondary reaction product which have not been isolated in pure form.

The 1,2,4-triazoles 7a-c were converted to their corresponding thioclides 8a-c by the reaction of their salts with the ethyl bromide (scheme 1).

The UV spectrum of 1,3,4-oxadiazoles 6a-c is characterized by two or three absorption peaks at 211 and 292 nm.

The absence of carbonyl absorption in the IR spectra of the 1,3,4-oxadiazole as compared to corresponding hydrazinecarbothioamide and the appearing of a new band at 1657 cm⁻¹, indicated that cyclization reaction had occurred.

In the 1H-NMR spectrum of the 1,3,4-oxadiazole 6a-c the appearance of a new band at 163 ppm, simultaneously with the disappearing of a signal at 181 ppm for to a thionic hydrazinecarbothioamide group.

Antibacterial activity

The in vitro activities of new compounds 6a-c and 8a-c were determined for a collection of the most frequently isolated bacterial pathogens from St. Pantelimon Emergency Hospital from patients with acute peritonitis, different abscesses, and phlegmons.

The tested compounds were solved in DMSO. To determine the antibacterial activity, we tested the inhibitory activity of the new compounds on the pathogenic bacterial strains of Bacillus Proteus, Staphylococcus aureus, Pseudomonas Aeruginosa, Escherichia coli and Klebsiella pneumoniae in vitro, using the Mueller-Hinton plate. To determine the MIC (minimum inhibitory concentration), the dilution method was used in liquid environment [32]. The observed MIC for the respective microorganisms is listed in the table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>B. Proteus</th>
<th>S. aureus</th>
<th>B. Aeruginosa</th>
<th>E. Coli</th>
<th>Klebsiella pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>&gt;1024</td>
<td>128</td>
<td>64</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>6b</td>
<td>64</td>
<td>128</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>6c</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>8a</td>
<td>64</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>8b</td>
<td>&gt;1024</td>
<td>32</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>8c</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
</tbody>
</table>

**ABTIBACTERIAL SCREENING RESULTS OF COMPOUNDS (MIC-μg/mL)**
Instead of the signal of the amidic >C=O group at 170 ppm, we noticed the presence of a signal at 158-159 ppm, which belongs to a C=C carbon from the oxadiazole nucleus. The experimental results showed that the antibacterial activity of the new compounds is negligible, the compound 6a, 6b and 8b present weak inhibitory activity over the Staphylococcus aureus, the compounds 6b and 8a over Proteus bacilli and the compound 6a over the Pseudomonas aeruginosa bacilli.

To note that the presence of 1,3,4-oxadiazole 6b and also of 1,2,4-triazole 8b determine a moderate antibacterial action upon Bacillus Proteus, and Staphylococcus aureus. In the case of Staphylococcus aureus strain the value of MIC of 32 µg/mL presented by the 8b compound is to be considered for further research. Presentation of the ethyl radical in the case of 1,2,4-triazole 8a and of 1,3,4-oxadiazole 6a determines an increase of the antibacterial activity upon Staphylococcus aureus and Pseudomonas Aeruginosa, for 8a and Bacillus Proteus for 6a. None of the tested compounds has antibacterial activity upon Escherichia coli and Klebsiella pneumoniae.

Conclusions

This study reports the synthesis, characterization and antibacterial activity of six new compounds: three 1,3,4-oxadiazoles and three 1,2,4-triazoles with 5H-dibenz[a,d][7]annulene nucleus. The chemical structure was determined by elemental analysis and spectral methods. The antimicrobial activity of these compounds on gram-positive and gram-negative bacterial strains was tested in vitro, and demonstrated that some of them possess a weak antibacterial activity.

References

7.HABIB N. S., FAHMY S., EL-KHAWASS S. M., , ABDEL AZIEM T, Pharmazie, 55, no. 12, 2000, p. 900
10.GHIRAN D., SCHWARZ I., SIMITI I., Farmacia, 22, 1974, p. 141;
23.SOCCEA L., SARAFET I., SOCCEA B., DRAGHICI C., Rev. Chim. (Bucharest), 57, no.11, 2006, p. 1123
27.MIHALCEA F., BĂRBUCEANU S. F., SOCCEA L. I., DRAGHICI C., CRISTEA C., DRAGHICI C., ENACHE-PREOTEASA C., SARAFET I., Rev. Chim. (Bucharest), 64, no. 2, 2013, p. 127
31. SOCCEA L. I., SARAFET G., SOCCEA B., DRAGHICI C., Rev. Chim. (Bucharest), 57, no. 12, 2006, p. 1242
32. SOCCEA L., SARAFET I., SOCCEA B., DRAGHICI C., Rev. Chim. (Bucharest), 58, no. 3, 2007, p. 328

Manuscript received: 22.11.2013