New Heterocyclic Compounds from 1,2,4-triazoles and 1,3,4-Oxadiazoles Class Containing 5H-dibenzo[a,d][7]Annulene Moiety

LAURA ILEANA SOCEA¹, STEFANIA FELICIA BARBUCEANU¹*, CONSTANTIN DRAGHICI¹, GEORGE MIHAI NITULESCU¹, GABRIEL SARAMET¹, BOGDAN SOCEA¹, THEODORA VENERA APOSTOL¹

¹University of Medicine and Pharmacy Carol Davila, Faculty of Pharmacy, 6 Traian Vuia Str., 020956, Bucharest, Romania
²Romanian Academy, Organic Chemistry Centre Costin D. Nenitescu, 202B Splaiul Independenței, 060023, Bucharest, Romania
³University of Medicine and Pharmacy Carol Davila, Faculty of General Medicine, St. Pantelimon Emergency Hospital, 340-342 Soseaua Pantelimon Str., 021623, Bucharest, Romania

In this paper we present the synthesis of the new heterocyclic compounds with 5H-dibenzo[a,d][7]annulene moiety obtained by cyclization of 2-acylhydrazinecarbothioamides (2a,b). The acylhydrazinecarbothioamides were obtained by treating 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetoxydiazole (1) with 2,5-difluorophenyl or 3-bromophenyl isothiocyanates. 2-Amino-1,3,4-oxadiazoles (3a,b) were synthesized by cyclization of 2-acylhydrazinecarbothioamides in the presence of mercury oxide. The new 1,2,4-triazole-3-thioles (4a,b) were synthesized by cyclization, in alkaline media, of the corresponding acylhydrazinecarbothioamide. The structures of the new compounds synthesized were investigated by ¹H-NMR, ¹³C-NMR, IR and elemental analysis.

Keywords: acylhydrazinecarbothioamide, 1,2,4-triazol-3-thiole, 2-amino-1,3,4-oxadiazole, dibenzo[a,d] [7]annulene

The synthesis of 1,2,4-triazoles and investigation of their chemical and biological behavior have gained more importance for the drug discovery process. 1,2,4-Triazole and 1,2,4-triazole-3-thioles are known to exhibit a broad spectrum of biological activities such as tubercolostatic, analgesic, antioxidant, antiviral, antitumor, antibacterial, anti-inflammatory, carbonic anhydrase inhibitors [1-11, 34]. Several compounds containing 1,2,4-triazole moiety, for example Flucanazole, Posaconazole and Itraconazole have been used for the treatment of fungal infection disease [12,13].

A few other drugs containing 1,2,4-triazole ring have been used in therapy Vorozole (antineoplastic and immunomodulatory) [14], Lorciparol (anticonvulsant) [15], Ribavirin (antiviral) [16] Rizatriptan (for the treatment of migraine headaches) [17] Alprazolam (anxiolytic) [18].

In the last years research was focused on obtaining biological active products because there is a continuous need for the development of new drugs as the currently available drugs are becoming ineffective due to the drug resistance developed by pathogens [19,20].

The 1,3,4-oxadiazole derivatives have been found to exhibit diverse biological activities such as antimicrobial, antimitotic, antiviral, anti HIV, antitubercular, antimalarial, anti-inflammatory, anticonvulsant, antitumor, antioxidant, muscle relaxant, diuretic, hypnotic, sedative, etc [21-29].

Moreover, life threatening infections caused by pathogenic fungi and bacteria are increasingly becoming very common 1,2,4-triazole and 1,3,4-oxadiazole compounds have shown a great efficacy against antifungal and antibacterial infections. In this situation, discovery of new 1,2,4-triazole and 1,3,4-oxadiazole derivatives with both a pharmaceutical profile and therapeutic safety are of a great interest among researchers.

Bacterial sortases are cysteine transpeptidases that regulate the covalent linkage of several surface protein virulence factors in Gram-positive bacteria. Virulence factors play significant roles in the adhesion, invasion of host tissues, biofilm formation and immune evasion, mediating the bacterial pathogenesis and infectivity. Therefore, sortases are emerging as important targets for the design of new anti-infective agents [30].

For those reasons, we have started a complex study by designing 1,2,4-triazoles or 1,3,4-oxadiazole derivatives that contained the dibenzo[a,d][7]annulene fragment, possible sortase inhibitors. The synthesis of the newly 1,2,4-triazoles or 1,3,4-oxadiazole compounds was realized in order to discover new potent SrtA inhibitors, potential anti-virulence agents targeted against Gram-positive bacteria, including multiresistant strains.

Newly compounds were prepared starting from of 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide (1) according with scheme 1.

Experimental part
Materials and methods

All reactants and solvents were obtained commercially with the highest purity and were used without further purification. Melting points were determined on a Boetius apparatus and are uncorrected. The UV-Vis spectra were recorded on a SPECORD 40 Analytik Jenova spectrometer, in methanol (2.5x10⁻³ M) in the wavelength range 200-600 nm. IR spectra were recorded on a FT-IR-100 Perkin Elmer spectrometer, in solid phase by ATR and frequencies are expressed in cm⁻¹. ¹H-NMR and ¹³C-NMR spectra are recorded on Varian Gemini 300 BB spectrometer (300 MHz for ¹H and 75 MHz for ¹³C) using DMSO-d₆ as solvent for hydrazinecarbothioamides and CDCI₃, for 1,2,4-triazole and 1,3,4-oxadiazole compounds, chemical shifts are given in ppm relative to TMS as internal standard. Complementary spectra: 2D-NMR and decoupling. Coupling constants, J, are expressed in Hertz (Hz). The content of C, H, and N was assayed using an ECS-40-10-Costeh microdosimeter.

* email:sbarbuceanu@gmail.com; stefaniagelicabarbuceanu@yahoo.com; Phone: 0722763428
**Synthesis and characterization of compounds**

The first step was the preparation of 2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-arylhydrazinecarbothioamides (2a,b) from 2,5-difluorophenyl or 3-bromophenyl isothiocyanate and 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide (1), in ethanol at room temperature, by the methods described in the our previously papers [31-33]. On treatment with HgO, acylhydrazinecarbothioamides (2a,b) yielded 2-aminodifluorophenyl or 3-bromophenyl isothiocyanate in ethanol (20 mL) was heated to reflux for 8 h. The reaction mixture was cooled and the separated product was filtered off, dried and recrystallized from ethanol.

2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(2,5-difluorophenyl)hydrazinecarbothioamide (2a): Yield: 89%; m.p.: 194-196°C; elemental analysis: anal. calcd. for C_{24}H_{19}F_{2}N_{3}O (435.50 g/mol): C, 66.19%; H, 4.40%; N, 8.78%; found: C, 71.80%; H, 4.27%; N, 10.49% IR (KBr, cm-1): 3415, 3067, 3022, 2915, 2868, 1673, 1595, 1569, 611; 1H-NMR (CDCl3, δ ppm, J, Hz): 8.90 (s, NH); 6.95-7.40 (12H, m, aromatic); 7.03 (2H, s, H^{10'}, H^{11'}); 3.24 (2H, d, 8.1, H 12'); 13C-NMR (CDCl3, δ ppm): 160.25 (C 2), 158.90 (C 5), 158.99 (d, C 6), 134.16 (C 1', C 3'), 133.75, 131.28 (CH), 130.80 (2CH), 129.63 (2CH), 129.52 (2CH), 128.75 (2CH), 128.71 (CH), 128.07 (C 12'), 127.52 (CH), 126.49 (2CH), 122.91 (CH), 116.66 (CH), 114.27 (CH), 44.99 (C'), 34.49 (C") - axial isomer, 33.15 (C") - equatorial isomer.

**Synthesis of 2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-arylhydrazinecarbothioamides (2a,b)**

An equimolar mixture of hydrazide 1 (2 mmol) and 2,5-difluorophenyl or 3-bromophenyl isothiocyanate in ethanol (20 mL) was heated to reflux for 8 h. The reaction mixture was cooled and the separated product was filtered off, dried and recrystallized from ethanol.

2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(2,5-difluorophenyl)-1,3,4-oxadiazol-2-amine (3a): Yield: 44.2%; m.p.: 186-187°C; elemental analysis: anal. calcd. for C_{24}H_{17}F_{2}N_{3}O (401.42 g/mol): C, 71.81%; H, 4.27%; N, 10.47%; found: C, 71.80%; H, 4.27%; N, 10.49% IR (KBr, cm-1): 3145, 3087, 3020, 2948, 2883, 1812, 1599, 1510, 1476, 1422, 1383, 1244, 1224, 1087, 1012, 775; 1H-NMR (CDCl3, δ ppm, J, Hz): 9.45 (s, NH); 6.95-7.40 (12H, m, aromatic); 7.03 (2H, s, H^{10'}, H^{11'}); 3.24 (2H, d, 8.1, H 12'); 13C-NMR (CDCl3, δ ppm): 160.77 (C 2), 159.75 (C 5), 159.80 (d, C 6), 134.10 (C 1', C 3'), 133.51, 131.28 (CH), 130.80 (2CH), 129.63 (2CH), 129.52 (2CH), 128.75 (2CH), 128.71 (CH), 128.07 (C 12'), 127.52 (CH), 126.49 (2CH), 122.91 (CH), 116.66 (CH), 114.27 (CH), 44.99 (C'), 34.49 (C") - axial isomer, 33.15 (C") - equatorial isomer.

**Synthesis of 5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-N-aryl-1,3,4-oxadiazol-2-amines (3a,b)**

To a solution of 2-acylhydrazinecarbothioamide (2a,b) (1 mmol) in ethanol yellow mercuric oxide (2 mmol) was added. The mixture was refluxed for 9 h. The resulted product was filtered of in order to remove the HgS, and after cooling, the corresponding 2-arylaminol-1,3,4-oxadiazole precipitate was obtained.

5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-N-(2,5-difluorophenyl)-1,3,4-oxadiazol-2-amine (3b): Yield: 49.4%; m.p.: 168-170°C; elemental analysis: anal. calcd. for C_{24}H_{18}BrN_{3}O (444.43 g/mol): C, 71.81%; H, 4.27%; N, 10.47%; found: C, 71.80%; H, 4.27%; N, 10.49% IR (KBr, cm-1): 3415, 3077, 3022, 2915, 2868, 1656, 1585, 1510, 1494, 1196, 1H-NMR (CDCl3, δ ppm, J, Hz): 8.90 (s, NH); 7.58 (m, 1H, Harom); 7.05-7.38 (m, 3H, Harom); 7.02 (s, 2H, H^{10'}); 6.65 (m, 1H, Harom); 3.23 (d, 8.0, H^{12'}); 6.91 (d, 8.5, H^{10'}); 4.54 (t, 8.1, H 5'); 3.24 (2H, d, 8.1, H 12'); 13C-NMR (CDCl3, δ ppm): 160.25 (C'), 159.80 (C'), 159.99 (d, C'), 241.61 (C', F), 147.86 (d, 128.38 (2C'), 134.10 (2C'), 131.13 (CH), 130.21 (CH), 129.58 (CH), 129.23 (CH), 127.29 (CH), 115.48 (dd, 24.3, 7.3), 108.74 (dd, 24.3, 7.3), 106.27 (31.3), 52.80 (C'), 26.32 (C').

5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-N-(3-bromophenyl)-1,3,4-oxadiazol-2-amine (3b): Yield: 44.2%; m.p.: 184-185°C; elemental analysis: anal. calcd. for C_{24}H_{19}BrN_{3}O (444.33 g/mol): C, 68.48%; H, 4.08; N, 9.46%; found: C, 64.88%; H, 4.09; N, 9.46% IR (KBr, cm-1): 3411, 3063, 2916, 2866, 1673, 1595, 1569, 1161, 1H-NMR (CDCl3, δ ppm, J, Hz): 9.45 (s, NH); 6.95-7.40 (12H, m, Haromatic); 7.02 (s, H^{10'}); 4.51 (t, 8.0, H 5'); 3.24 (d, 8.0, H^{12'}); 13C-NMR (CDCl3, δ ppm): 160.77 (C'), 159.75 (C'), 159.36 (2C'), 138.63 (2C'), 131.09 (C'), 130.59 (13C), 130.17 (2CH), 129.79 (2CH), 129.51 (2CH), 129.19 (2CH), 127.21 (2CH), 122.98 (CH), 120.07 (CH), 116.21 (CH), 53.02 (C'), 26.40 (C').
Synthesis of 5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-aryl-4H-1,2,4-triazole-3-thioles (4a,b)

Acylhydrazinecarbothioamides (2a,b) (1 mmole) was added to 10 mL of NaOH 8% solution and the reaction mixture was heated under reflux for 9 h. After cooling, the solution was acidified with acetic acid. The obtained white precipitate was filtered, recrystallized from CHCl₃-petroleum ether (1:2/v:v).

The IR spectra of the newly acylhydrazinecarbothioamides (2a,b) do not show the absorption band characteristic of C=O group, confirming that the cyclization reaction of these new intermediates (2a,b) took place. The IR spectra of these compounds showed three NH proton singlet signals in region 8.79-9.86 ppm and the ¹³C-NMR spectra of these compounds showed two characteristic signals of carbon atoms from C=O and C=S groups at ~170 ppm and ~181 ppm respectively. In the ¹³C-NMR spectra of compounds (2a), the carbon atoms signals of C¹⁰⁺ and C¹⁰⁻ appear as a doublet because of the strong germinal coupling (J₁ = 239 Hz and 241 Hz) due to the bridge of the strong germinal coupling (J₁ = 239 Hz and 241 Hz) because of the strong germinal coupling (J₁ = 239 Hz and 241 Hz). The IR spectra of compounds (3a,b) and (4a,b) obtained from acylhydrazinecarbothioamides (2a,b) do not show the absorption band characteristic of C=O group, confirming that the cyclization reaction of these new intermediates (2a,b) took place. The IR spectra of these compounds showed three NH proton singlet signals in region 8.79-9.86 ppm and the ¹³C-NMR spectra of these compounds showed two characteristic signals of carbon atoms from C=O and C=S groups at ~170 ppm and ~181 ppm respectively. In the ¹³C-NMR spectra of compounds (2a), the carbon atoms signals of C¹⁰⁺ and C¹⁰⁻ appear as a doublet because of the strong germinal coupling (J₁ = 239 Hz and 241 Hz) due to the bridge of the strong germinal coupling (J₁ = 239 Hz and 241 Hz). The IR spectra of compounds (3a,b) and (4a,b) obtained from acylhydrazinecarbothioamides (2a,b) do not show the absorption band characteristic of C=O group, confirming that the cyclization reaction of these new intermediates (2a,b) took place. The IR spectra of these compounds showed three NH proton singlet signals in region 8.79-9.86 ppm and the ¹³C-NMR spectra of these compounds showed two characteristic signals of carbon atoms from C=O and C=S groups at ~170 ppm and ~181 ppm respectively. In the ¹³C-NMR spectra of compounds (2a), the carbon atoms signals of C¹⁰⁺ and C¹⁰⁻ appear as a doublet because of the strong germinal coupling (J₁ = 239 Hz and 241 Hz) due to the bridge of the strong germinal coupling (J₁ = 239 Hz and 241 Hz).
3-thioles appears a new quaternary carbon signal (for C\(^3\)) at \(\delta = 167.87-167.83\) ppm (scheme 3) and a signal for C\(^2\) at \(\delta = 151.57-151.84\) ppm.

In 1,2,4-triazole (4a) substituted with the 2,5-difluorophenyl substituent, the double bond protons H\(^1\) and H\(^1\)\(^{'1}\) appears as an AB system with a vicinal coupling constant \(J = 11\) Hz, because of to the axial configuration. The double bond protons H\(^1\) and H\(^1\)\(^{'1}\) are shielded with \(= 0.4\) ppm comparative with the hydrazinecarbothioamide moieties. The chemical structure was determined by spectral characterization of six new compounds, respectively two 1,2,4-triazoles with 5H-dibenzo[a,d][7]annulene acylhydrazinecarbothioamides, two 1,3,4-oxadiazoles and some new potent SrtA inhibitors.

Conclusions

In this work we described the synthesis and characterization of six new compounds, respectively two acylhydrazinecarbothioamides, two 1,3,4-oxadiazoles and two 1,2,4-triazoles with 5H-dibenzo[a,d][7]annulene moiety. The chemical structure was determined by spectral analysis. The inhibitory activities of the newly compounds against SrtA will be evaluated.

Acknowledgements: The authors acknowledge the financial support offered by Romanian National Authority for Scientific Research, UEFISCDI, through grant PN-II-PU-TE-2014-4-1670, no. 342/2015.

References

4. ZAHARIA V., IMRE S., PALIBRODA N., Rev. Chim.(Bucharest), 58, no.4., 2009, p. 391
5. TOMA A., LEONTE D., ZAHARIA V., FARMACIA, 65, no. 1, 2017, p.23
7. ZAHARIA V., IMRE S., PALIBRODA N., Rev. Chim.(Bucharest), 58, no.4., 2009, p. 391
17. MILLISON D., TEPPER S., Expert Opin. Pharmacother. 1, no. 3, 2000, p. 391
32. SOCEA, L. I., SARATEM, G., DRAGHICI C., BARBUCANU S.F., ILIES D.C., SOCEA, B., Rev. Chim. (Bucharest), 67, no 1, 2016, p. 17
33. SOCEA, L., SARATEM, I., SOCEA, B., DRAGHICI, C., Rev.Chim. (Bucharest), 58, no.3, 2007, p. 328

Manuscript received: 5.07.2017