Synthesis and Antimicrobial Assessment of Some New 2-(Thiazol-5-yl)-1,3,4-oxadiazoles

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Considering the promising antimicrobial activity of compounds bearing the thiazole or the oxadiazole rings in their structures, we set out to obtain new antimicrobial molecules bearing the 2-(thiazol-5-yl)-1,3,4-oxadiazole scaffold. The structures of the 8 new compounds obtained was confirmed by physicochemical characterization including: 1H-NMR, MS and elemental analysis. Antimicrobial activity was investigated against 5 Gram-positive bacterial strains, 2 Gram-negative bacterial strains and 2 fungal strains. The newly synthesized compounds showed modest antimicrobial activity.

Keywords: thiazole, oxadiazole, antibacterial, antifungal

Developing new antimicrobial drugs is a key concern as the emergence of multi-drug, extended-drug and pan-drug resistance among bacterial and fungal pathogens is a worldwide health threat [1]. Thiazole containing molecules have a highly versatile biological activity. The thiazole nucleus is present in some already approved medicines like: xanthine oxidase inhibitors (Febuxostat), NSAIDs (meloxicam), H, receptor antagonists (nizatidine), leukotriene receptor antagonists (cinalukast), dopamine agonists (pramipexol) as well as a series of antimicrobial agents (abafungin, acinotrazole, astreonam, ceftriaxone) or antineoplastic drugs (bleomycin, tiazofurin) [2,3]. However, a great deal of interest is still bestowed upon this molecular moiety by current research [4] that aims at identifying new lead compounds to act as: antineoplastic agents [5–9], prostaglandin receptor antagonists [10], antiprotozoal drugs [11,12] or antimicrobial agents [13]. Alongside thiazole, oxadiazole is also a versatile moiety present in a series of antimicrobial agents [14,15]. Given our research group’s previous expertise concerning thiazole based antimicrobial agents [16–24], we decided to synthesize a new series of antimicrobial agents bearing the 2-(thiazol-5-yl)-1,3,4-oxadiazole scaffold. The structures of the 8 new compounds obtained was confirmed by physicochemical characterization including: 1H-NMR, MS and elemental analysis. Antimicrobial activity was investigated against 5 Gram-positive bacterial strains, 2 Gram-negative bacterial strains and 2 fungal strains. The newly synthesized compounds showed modest antimicrobial activity.

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Experimental part

Chemistry
All chemical reagents and solvents used in the synthesis, isolation and purification process were of analytical grade purity and were purchased from Alfa Aesar (Karlsruhe, Germany). Initial reaction progress monitoring and initial purity assessment were performed using Silica Gel thin layer chromatography sheets and UV visualization. The melting points were uncorrected and were obtained by using an Electrothermal 9100 melting point apparatus. The 1H-NMR were recorded on a Bruker Avance NMR spectrometer, operating at 500MHz, in DMSO-d6 as solvent. Chemical shift values are reported in δ units, relative to TMS as internal standard. Elemental analyses were performed by an Elemental Analyser Systeme GmbH VarioEL. MS spectra were recorded on a LC-MSD-Trop-VL mass spectrophotometer.

Synthesis of ethyl 4-methyl-2-(pyridin-3-yl)-thiazole-5-carboxylate (A)
To a solution of thiobenzamide (13.7 g, 0.1 mol) in 40 mL ethanol an equimolar quantity of ethyl-2-chloroacetacetate (16.45 g, 0.1 mol) was added and it was refluxed for 5 h. The reaction mixture was cooled to room temperature and neutralized with sodium hydrogen carbonate. The solid obtained was filtered, washed with water and then with acetone and recrystallised from ethanol [25,26].

Synthesis of the 4-methyl-2-phenylthiazole-5-carboxyhydrazide (B)
The solution obtained by dissolving 10 mM of the corresponding ester in 4 mL ethanol was treated with 5 mL hydrazine hydrate and refluxed for 5 h. The resulting mixture was allowed to cool overnight and then poured over ice water. The resulting solid was filtered and washed with water in order to yield the pure compound [25,26].

General procedure for the synthesis of the 2-(4-methyl-2-phenylthiazol-5-yl)-5-aryl-1,3,4-oxadiazoles (C1–8)
A mixture of the 4-methyl-2-phenylthiazole-5-carboxyhydrazide (0.001 M) and the appropriate aromatic acid (0.001 M) was refluxed in 3 mL phosphorus oxychloride for 8 h. The reaction mixture was allowed to cool down and then slowly poured over crushed ice. The resulting mixture was neutralized with solid sodium hydrogen carbonate and kept overnight. The solid that separated was filtered, washed with cold water and recrystallised from methanol.

Biological Assays: The in vitro qualitative screening of the antimicrobial activity
The in vitro qualitative screening of the antimicrobial activity was carried out by an adapted agar disk diffusion technique using a bacterial suspension of 0.5 McFarland obtained from 24 h cultures. The antimicrobial activities of the newly synthesized compounds were determined

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against 5 reference Gram-positive microbial strains (Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 6538, Staphylococcus aureus BAA 1026, Staphylococcus saprophyticus, Bacillus subtilis ATCC 6633), 2 reference Gram-negative microbial strains (Escherichia coli ATCC 8739 and Pseudomonas aeruginosa ATCC 27853) as well as 2 fungal strains (Candida albicans ATCC 10231 and Candida parapsilosis ATCC 22019).

The antimicrobial activity was tested on Mueller-Hinton Agar (MHA) medium, while yeast peptone glucose (YPG) medium was used in the case of Candida spp. The compounds or standards (ciprofloxacin, fluconazole) were solubilized in dimethylsulfoxide to a final concentration of 10 µg/mL. A volume of 5 µL of each tested compound solution was distributed directly on the solid medium previously seeded with the microbial inocula. The inoculated plates were incubated for 24 h at 37 °C. Antimicrobial activity was assessed by measuring the growth inhibition zones diameters expressed in mm

Results and discussions

Chemistry

The 3 step reaction path used proved very efficient as it was characterized by high yields. The first step of the synthesis was a Hantzsch condensation between thiobenzamide and the alpha-keto carbonyl component (ethyl-2-chloroacetoacetate). This led to the formation of a new thiazole ring with an ethyl carboxylate substituent in the 5 position. The ester derivative (A) was then transformed in the corresponding 5-carbohydrazide by reflux with hydrazine hydrate. The structural variety represented by ethyl-2-chloroacetoacetate. This led to the formation of a new thiazole ring with an ethyl carboxylate substituent in the 5 position. The ester derivative (A) was then transformed in the corresponding 5-carbohydrazide (B) by reflux with hydrazine hydrate. The structural variety of the 2-(thiazol-5-yl)-1,3,4-oxadiazoles final products was due to the various aromatic carboxylic acids used in the last condensation reaction, as shown in figure 1.

All new compounds presented spectral data consistent with the proposed structure and elemental microanalysis within 0.4% of the theoretical values, as described in the following paragraphs.

2-(4-Methyl-2-phenylthiazol-5-yl)-5-phenyl-1,3,4-oxadiazole (C1)

Yellow green powder. Yield 65%. m.p. 221 °C. 1H NMR (DMSO-d6, 500 MHz, ppm): δ 8.67 (s, 1H, C-Ph-NO2), 8.47 (m, 2H, C and C dib Ph-NNO2), 8.02 (dd, 2H, Ph), 7.57 (m, 3H, Ph), 7.35 (dd, 2H, Ph-Cl) 2.84 (s, 3H, Tz-CH3). Anal. calcd. (%) for C24H25N3OS (365,46): C, 71.43; H, 6.24; N, 10.41; S, 7.95. Found: C, 71.30; H, 6.16; N, 10.48; S, 7.76. MS (El, 70eV): m/z 354,4 (M+1).

2-(4-Chlorophenyl)-5-(4-methyl-2-phenylthiazol-5-yl)-1,3,4-oxadiazole (C2)

Yellow powder. Yield 75%. m.p. 285 °C. 1H NMR (DMSO-d6, 500 MHz, ppm): δ 8.02 (dd, 2H, 5-Ph), 7.52 (m, 3H, 5-Ph), 7.28 (dd, 2H, 2-Ph), 7.17 (dd, 2H, 2-Ph), 4.57 (q, 1H, CH-CH3), 3.33 (dd, 3H, CH-CH3), 2.85 (s, 3H, Tz-CH3), 2.45 (d, 2H, -CH-CH-CH3), 1.7 (m, 1H, CH-CH-CH3), 0.85 (dd, 6, -CH-CH-CH3). Anal. calcd. (%) for C18H17N3OS (403,17): C, 71.43; H, 6.24; N, 10.41; S, 7.95. Found: C, 71.30; H, 6.16; N, 10.48; S, 7.76. MS (El, 70eV): m/z 404,4 (M+1).

2-(4-Chlorophenyl)-5-(4-(4-methyl-2-phenylthiazol-5-yl)-3-nitrophenyl)-1,3,4-oxadiazole (C3)

Yellow powder. Yield 75%. m.p. 190 °C. 1H NMR (DMSO-d6, 500 MHz, ppm): δ 8.80 (dd, 2H, C and C, dib Ph-Cl), 8.02 (dd, 2H, Ph), 7.50 (m, 3H, Ph), 7.35 (dd, 2H, Ph-PF-Cl), 2.84 (s, 3H, Tz-CH3). Anal. calcd. (%) for C18H13ClN3OS (353,04): C, 61.10; H, 3.42; Cl, 10.02; N, 11.88; S, 9.06. Found: C, 61.25; H, 3.2; Cl, 10.15; N, 11.98; S, 9.16. MS (El, 70eV): m/z 354,4 (M+1).

2-(4-Chlorophenyl)-5-(4-(4-methyl-2-phenylthiazol-5-yl)-1,3,4-oxadiazole (C4)

White powder. Yield 65%. m.p. 221 °C. 1H NMR (DMSO-d6, 500 MHz, ppm): δ 8.67 (s, 1H, C-Ph-NO2), 8.47 (m, 2H, C and C dib Ph-NNO2), 8.02 (dd, 2H, Ph), 7.91-7.94 (t, 1H, C-Ph-NO2), 7.54 (m, 3H, Ph), 2.84 (s, 3H, Tz-CH3). Anal. calcd. (%) for C18H13N3OS (365,06): C, 59.33; H, 3.32; N, 15.38; S, 8.80. Found: C, 59.53; H, 3.35; N, 15.18; S, 8.89. MS (El, 70eV): m/z 365,4 (M+1).

2-(2-Chlorophenyl)-5-(4-(4-methyl-2-phenylthiazol-5-yl)-1,3,4-oxadiazole (C5)

Yellow powder. Yield 70%. m.p. 219 °C. 1H NMR (DMSO-d6, 500 MHz, ppm): δ 8.15 (d, 1H, C-Ph-PF), 8.0 (dd, 2H, Ph), 7.52 (m, 3H, Ph), 7.30-7.40 (m, 3H, Ph-Cl), 2.84 (s, 3H, Tz-CH3). Anal. calcd. (%) for C18H13N3OS (353,04): C, 61.10; H, 3.42; Cl, 10.02; N, 11.88; S, 9.06. Found: C, 61.15; H, 3.28; Cl, 10.19; N, 11.94; S, 9.26. MS (El, 70eV): m/z 354,7 (M+1).

2-(4-Methyl-2-phenylthiazol-5-yl)-5-(2-nitrophenyl)-1,3,4-oxadiazole (C6)

White powder. Yield 75%. m.p. 179 °C. 1H NMR (DMSO-d6, 500 MHz, ppm): δ 8.25 (m, 3H, C, Ph-PF), 8.04 (dd, 2H, Ph), 7.55 (m, 3H, Ph), 7.30 (t, 1H, Ph-PF-NO2), 2.84 (s, 3H, Tz-CH3). Anal. calcd. (%) for C18H13N3OS (365,06): C, 59.33; H, 3.32; N, 15.38; S, 8.80. Found: C, 59.45; H, 3.59; N, 15.28; S, 8.71. MS (El, 70eV): m/z 365,7 (M+1).
2-(4-methoxyphenyl)-5-(4-methyl-2-phenylthiazol-5-yl)-1,3,4-oxadiazole (C7)
Yellow powder. Yield 75%. p.t. 285°C. 1H NMR (DMSO-d6, 500 MHz, ppm): δ 8.04 (dd, 2H, Ph), 7.75 (dd, 2H, Ph-OCH3), 7.55 (m, 3H, Ph), 7.0 (dd, 2H, Ph-OCH3), 3.75 (s, 3H, -OCH3), 2.80 (s, 3H, Tz-CH3). Anal. calcld. (%) for C19H15N3O2S (349.09): C, 65.31; H, 4.33; N, 12.03; S, 9.18 . Found: C, 65.39; H, 4.35; N, 12.23; S, 9.38. MS (EI, 70eV): m/z 350.7 (M+1).

2-(4-methyl-2-phenylthiazol-5-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazole (C8)
Pale yellow powder. Yield 75%. m.p. >360 °C. 1H NMR (DMSO-d6, 500 MHz, ppm): δ 8.47 (dd, 2 H, C3 and C5 from Py), 8.43 (dd, 2H, C2 'i C6 Py), 8.02 (dd, 2H, Ph), 7.54 (m, 3H, Ph), 2.84 (s, 3H, Tz-CH3). Anal. calcld. (%) for C17H12N4OS (320.07): C, 63.73; H, 3.78; N, 17.49; S, 10.01. Found: C, 63.53; H, 3.88; N, 17.59; S, 10.21. MS (EI, 70eV): m/z 321.6 (M+1).

Biological evaluation
The in vitro qualitative screening of the antimicrobial activity showed an overall modest activity for the tested compounds, as can be seen from table 1. Antibacterial activity was varied with the most promising being that of C1 against B. subtilis. Generally the tested molecules performed better against Gram-positive strains than against Gram-negative strains.

The weakest activity was observed against the fungal strains, with practically no activity against C. parapsilosis.

Conclusions
A series of 8 new thiazolyl-oxadiazoles were synthesized and characterized via 1H-NMR, mass spectrometry and elemental analyses. The structures of all new compounds were verified and confirmed. Also, a preliminary biological evaluation was performed under the form of an in vitro qualitative antimicrobial activity screening. Despite their structural similarities with other active compound the new molecule seem to have limited antimicrobial effect. Further molecular modeling must be undertaken in order to generate new molecules with improved antimicrobial effect.

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References
1. KARP, BE., TATE, H., PLUMBLEE, JR., DESSAI, J., WHICHARD, JM., THACKER, EL. Foodborne. Pathog. Dis., 2017, epub ahead of print.
2. POLA, S., Significance of Thiazole-based Heterocycles for Bioactive Systems. In: Scope of Selective Heterocycles from Organic and Pharmaceutical Perspective. InTech; 2016.
3. ROUF, A., TANYELI, C., Eur. J. Med. Chem., 97, nr. 1, 2015, p. 911.

Table 1
| Compound                          | E. coli ATCC 25922 | S. aureus ATCC 6538 | S. typhimurium ATCC 11110 | B. subtilis ATCC 6633 | E. coli ATCC 9532 | P. aeruginosa ATCC 27853 | C. albicans ATCC 10231 | C. parapsilosis ATCC 2019 |
|----------------------------------|--------------------|---------------------|---------------------------|-----------------------|-------------------|-------------------------|-------------------------|--------------------------|
| C1                               | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| C2                               | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| C3                               | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| C4                               | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| C5                               | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| C6                               | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| C7                               | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| C8                               | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| Fluconazole                      | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| Ciprofloxacin                    | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
| DMSO                             | 0                  | 0                   | 0                         | 0                     | 0                 | 0                       | 0                       | 0                        |
4. AYATI, A., EMAMI, S., ASADIPOUR, A., SHAHFEE, A., FOROUMADI, A., Eur. J. Med. Chem., 97, 2015, p. 699.
5. PANTSAR, T., SINGHA, P., NEVALAINEN, T.J., KOSHEVOY, I., LEPPÄNEN, J., POSO, A., Eur. J. Pharm. Sci., 107, no.17, 2017, p. 97.
6. DOS SANTOS SILVA, T.D., BOMFIM, L.M., DA CRUZ RODRIGUES, A.C.B., DIAS, R.B., SALES, C.B.S., ROCHA, C.A.G., Toxicol. Appl. Pharmacol., 329, no.8, 2017, p. 212.
7. DOS SANTOS, T.A.R., DA SILVA, A.C., SILVA, E.B., GOMES PAT DE, M., ESPINDOLA, J.W.P., CARDOZO M.V. DE, O., Biomed, Pharmacother., 82, no. 8, 2016, p. 555-60.
8. ZHAO, H., CUI, G., JIN, J., CHEN, X., XU, B., Bioorg. Med. Chem., 24, no. 22, 2016 p. 5911.
9. CHEN, C., SONG, J., WANG, J., XU, C., CHEN, C., GU, W., Bioorg. Med. Chem. Lett., 27, no. 4, 2017, p. 845.
10. UMEI, K., NISHIGAYA, Y., TATANI, K., KOHNO, Y., TANAKA, N., SETO, S., Bioorg. Med. Chem., 25, no. 13, 2017, p. 3406.
11. ALIANÇA A.S. DOS, S., OLIVEIRA, A.R., FEITOSA, A.P.S., RIBEIRO, K.R.C., DE CASTRO, M.C.A.B., LEITE, A.C.L., Eur. J. Pharm. Sci., 105, no.7, 2017, p. 1.
12. DA SILVA, E.B., OLIVEIRA E SILVA, D.A., OLIVEIRA, A.R., DA SILVA MENDES, C.H., DOS SANTOS, T.A.R, DA SILVA, A.C., Eur. J. Med. Chem., 130, no.4, 2017, p. 39.
13. DAS, D., SIKDAR, P., BAIRAGI, M., Eur. J. Med. Chem., 109, no. 2, 2016, p. 89.
14. JANARDHANAN, J., CHANG, M., MOBASHERY, S., Curr. Opin. Microbiol., 33, no. 10, 2016, p. 13.
15. RAZUS, A.C., BIRZAN, L., CRISTEA, M., TECUCEANU, V., DRAGHICI, C., Rev. Chim.(Bucharest), 66, no. 7, 2015, p.1074.
16. ARANICIU, C., PALAGE, M., ONIGA, S., PIRNAU, A., VERITE, P., ONIGA, O., Rev. Chim.(Bucharest), 66, no. 10, 2016, p.1067.
17. ARANICIU, C., ONIGA, S., ONIGA, O., PALAGE, M., CHIFIRIUC, M.C., MARUTESCU, L., Farmacia, 63, no. 1, 2015, p. 40.
18. ONIGA, S., DUMA, M., ONIGA, O., TIPERCIUC, B., PIRNAU, A., ARANICIU, C., Farmacia, 63, no. 2, 2015, p. 171.
19. ARANICIU, C., MARUTESCU, L., ONIGA, S., ONIGA, O., CHIFIRIUC, M.C., PALAGE, M., Dig. J. Nanomater. Biostructures., 9, no.1, 2014, p. 123.
20. STOICA, C.I., MARC, G., PIRNAU, A., VLASE, L., ARANICIU, C., ONIGA, S., ET AL. Farmacia, 64, no.3, 2016, p. 390.
21. ONIGA, S., ARANICIU, C., PALAGE, M., STOICA, C., CHIFIRIUC, M.C., MARUTESCU, L., Rev. Chim.(Bucharest), 67, no. 3, 2016, p. 426.
22. ONIGA, S.D., ARANICIU, C., STOICA, C.I., PALAGE, M.D., VLASE, L., PIRNAU, A., ET AL. Farmacia., 65, no. 4, 2017, p. 501.
23. STANA, A., VODNAR, D., TAMAÍAN, R., PIRNAU, A., VLASE, L., IONU, I., ET AL. Int. J. Mol. Sci., 18, no. 1, p. 177.
24. TIPERCIUC, B., ZAHARIA, V., COLOSI, I., MOLDOVAN, C., CRĂIAN, O., PIRNAU, A., ET AL. J. Heterocycl. Chem., 49, no. 12, 2012, p. 1413.
25. TAZOO, D., ONIGA, O., BOHLE, D.S., CHUA, Z., DONGO, E., J. Heterocycl. Chem., 49, no. 4, 2012, p. 768.
26. TIPERCIUC, B., ZAHARIA, V., COLOSI, I., MOLDOVAN, C., CRĂIAN, O., PIRNAU, A., ET AL. J. Heterocycl. Chem., 49, no. 6, 2012, p. 1407.
27. LIMBAN, C., CHIFIRIUC, M.C., Int. J. Mol. Sci., 28, no.12, 2011, p. 6432.

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