DOI: 10.15386/cjmed-473

SYNTHESIS, LIPOPHILICITY AND ANTIMICROBIAL ACTIVITY EVALUATION OF SOME NEW THIAZOLYL-OXADIAZOLINES

CRISTINA IOANA STOICA[1], IOANA IONUȚ[2], ADRIAN PÎRNĂU[3], CARMEN POP[4], ANCUȚA ROTAR[4], LAURIAN VLASE[5], SMARANDA ONIGA[1], OVIDIU ONIGA[2]

[1]Therapeutical Chemistry Department, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[3]Molecular and Biomolecular Physics Department, National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania
[4]Food Science and Technology Department, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[5]Pharmaceutical Technology and Biopharmaceutics Department, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Abstract

Background and aims. *Synthesis of new potential antimicrobial agents and evaluation of their lipophilicity.*

Methods. *Ten new thiazolyl-oxadiazoline derivatives were synthesized and their structures were validated by 1H-NMR and mass spectrometry. The lipophilicity of the compounds was evaluated using the principal component analysis (PCA) method. The necessary data for applying this method were obtained by reverse-phase thin-layer chromatography (RP-TLC). The antimicrobial activities were tested in vitro against four bacterial strains and one fungal strain.*

Results. *The lipophilicity varied with the structure but could not be correlated with the antimicrobial activity, since this was modest.*

Conclusions. *We have synthesized ten new heterocyclic compounds. After their physical and chemical characterization, we determined their lipophilicity and screened their antimicrobial activity.*

Keywords: thiazolyl-oxadiazoline, lipophilicity, antibacterial, Candida albicans

Background and aims

As an important class of heterocyclic compounds, thiazoles are associated with many types of biological properties, including antitumor, antibacterial, antifungal, antitubercular and anti-inflammatory effects [1-3]. Similarly, 1,3,4-oxadiazoline is a versatile lead molecule for the design of bioactive agents. Compounds containing the 1,3,4-oxadiazoline system have been reported to exhibit various biological activities such as anticancer [4,5], antibacterial, antifungal, anticonvulsant and anti-inflammatory [6-8]. Moreover, the association of the thiazole system and the oxadiazoline backbone in the same molecule may show antimicrobial synergistic effect.

Considering the biological significance of thiazole and 1,3,4-oxadiazoline derivatives we decided to design a series of thiazolyl-oxadiazolines.

The hydrophilic-lipophilic balance of a substance is a fundamental physical property that plays a pivotal role in the absorption, distribution, metabolism and elimination (ADME) of therapeutic drugs. For the determination of the lipophilicity of biologically active

Manuscript received: 17.05.2015
Received in revised form: 02.07.2015
Accepted: 24.07.2015
Address for correspondence: stoica.cristina@umfcluj.ro

compounds, chromatographic techniques are chosen because the behavior and interaction between molecules in chromatographic and biological systems are very similar. Most molecules cross the cell membranes by passive diffusion; they should be lipophilic enough in order to disseminate through the biological membranes, but also hydrophilic enough to penetrate the cytoplasm. The biological activity of the molecules can be associated with the lipophilic character by retention parameters, which can describe or predict the properties of the active substance. These are either calculated by theoretical methods or are experimentally determined (reverse phase-thin layer chromatography) and help us establish the quantitative structure-retention relationship (QSRR). Furthermore, they express the interaction between the cell membranes and the analyzed molecules [9-13].

This prompted us to study the physicochemical properties of this kind of compounds in detail. Herein, we report the synthesis, the study of lipophilicity and the evaluation of the antimicrobial potential of novel 1,3,4-oxadiazolines containing a thiazole ring in their structure, in the hope to provide a deeper insight into the differences in biological activity between them and to suggest synthesis of new active derivatives.

Materials and methods

Chemistry

Solvents and reagents used for synthesis and purification were purchased from Alfa Aesar (Karlsruhe, Germany). All chemicals were of analytical grade. The purity of the synthesized compounds was verified by thin layer chromatography and was carried out on precoated Silica Gel 60F254 sheets using heptan – ethyl-acetate 3:7 as developer and UV absorption for visualization. The melting points were established using an Electrothermal melting point meter and are uncorrected. LC-MS analyses were performed with an Agilent 1100 series and an Agilent Ion Trap SL mass spectrometer. ^{1}H-NMR was performed on a Bruker Avance NMR spectrometer operating at 500 MHz, in DMSO-d6 as solvent. Chemical shift values were reported relative to tetramethylsilane (TMS) as internal standard. The synthesis of the compounds **1, 2** and **3** was previously reported [14]. The chemical structures of compounds ***6a-f, 7a-d*** are presented in Table I, II. Ten compounds with new structures were synthesized (Figure 1) and characterized.

*General procedure for the synthesis of thiazole-5yl-hydrazide-hydrazones (**4a-d, 5a-b**).* [14-16]

To a solution of 2 mmol ***3*** in 5 ml ethanol and a catalytic amount of glacial acetic acid, an equimolar quantity of aldehyde/ketone was added. The reaction mixture was heated under reflux for 3 h. After cooling, the product was filtered off and washed with water.

*General procedure for the synthesis of the thiazolyl-oxadiazolines (**6a-f, 7a-d**).* [14-16]

A mixture of hydrazide-hydrazone ***4 a-d and 5 a-b*** (1 mmol) and propionic/acetic anhydride (6 ml) was heated under reflux for 6 h. After cooling the reaction mixture was poured into ice cold water. The separated product was filtered, washed with water, dried and recrystallized from ethanol.

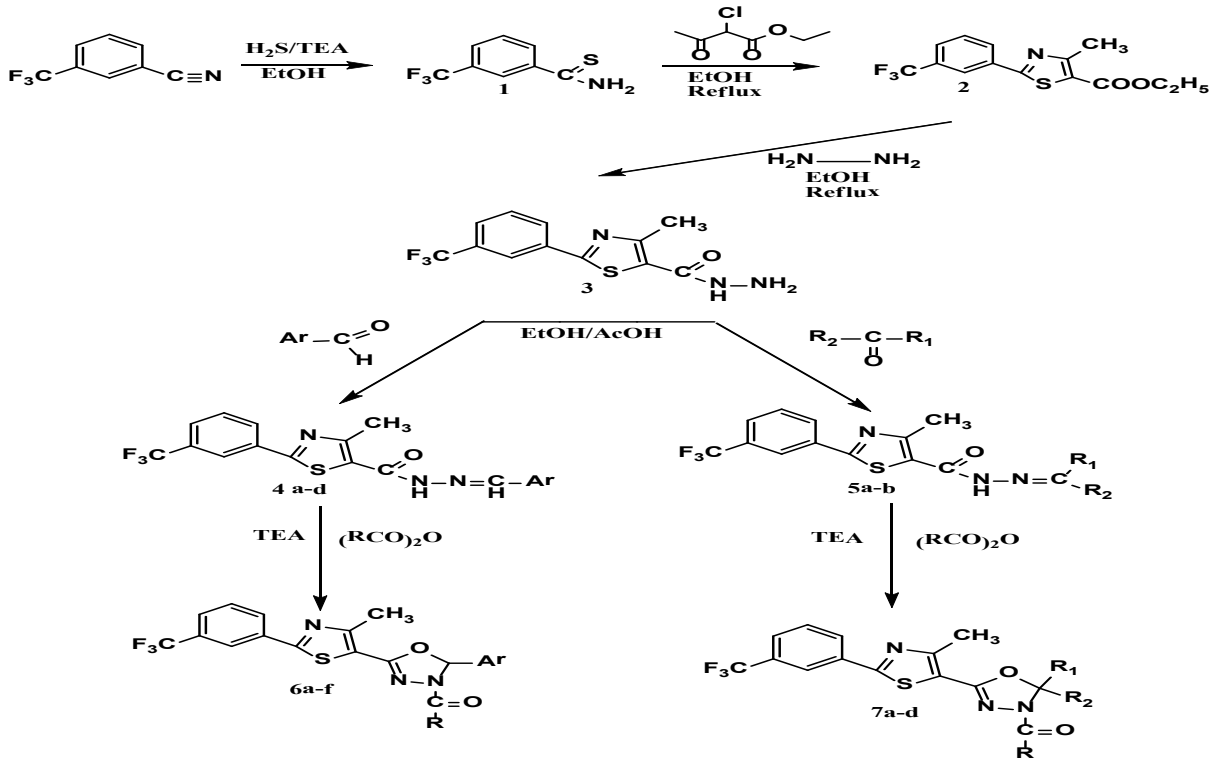
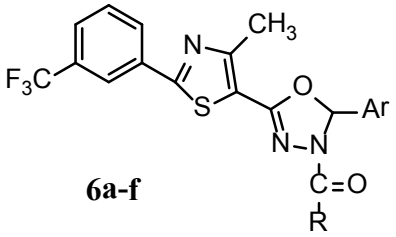
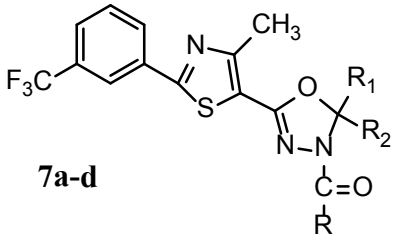


Figure 1. The procedure of the synthesis of the thiazolyl-1,3,4-oxadiazolines.



6a-f



7a-d

Table I. The chemical structures of **6a-f.**

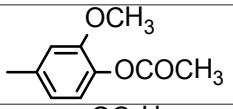
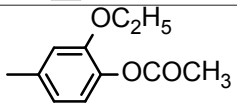
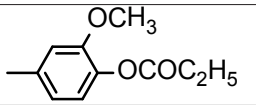
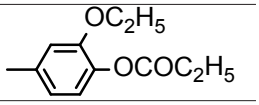
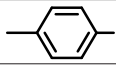
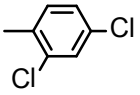
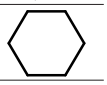
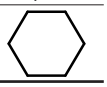
Compound	R	Ar
6a	-CH3	
6b		
6c	-C2H5	
6d		
6e		
6f		

Table II. The chemical structures of **7a-7d.**

Compound	R	R_1+R_2
7a	-CH_3	
7b		
7c	-C_2H_5	
7d		

Lipophilicity evaluation

The synthesized oxadiazolines were subjected to RP-TLC in order to obtain the parameters that are necessary for the estimation of their lipophilic properties.

The chromatographic behavior of the compounds was studied by using standard chromatographic plates Silicagel 60 RP-180F_{254}S (20x20 cm), with a chemically bound C_{18} stationary phase, purchased from Merk (Darmstadt, Germany). Test compounds were dissolved in DMSO to achieve a concentration of 1 mg/ml. These were manually applied as spots at 10 mm from the base and 5 mm from the edges of the plate. The distance between successive spots was 10 mm. The solutions of the investigated compounds were applied using 1 µl standardized chromatographic micropipettes. The mobile phase consisted of a mixture of water and *i*-propanol. Concentrations of *i*-propanol in different proportions, between 60% to 80% with 5% increments, were used. The migration distance of the eluent was 85 mm, in all cases. Before the development, the chamber was saturated with the mobile phase for 15 min. The plates were visually inspected under the UV light (364 nm), each zone was clearly marked, its distance was manually measured and was used to calculate the R_f values.

RP-TLC provides retention data in the form of R_f (1) and corresponding R_M (2) values that can be used to derive chromatographic descriptors for the estimation of lipophilicity: R_{M0}, b and PCA [17,18].

$$\mathbf{R_f = x/f} \quad (1)$$

x represents the migration distance of the solute and **f** represents the migration distance of the solvent.

$$\mathbf{R_M = \log(1/R_f - 1)} \quad (2)$$

$$\mathbf{R_M = R_{M0} + bc} \quad (3)$$

R_{M0}=lipophilicity estimation parameter
R_M = retention of a solute
b = slope
c = concentration of the organic mobile phase modifier (*i*-propanol)

The lipophilic properties of the investigated compounds were evaluated by using the principal component analysis method (PCA, XL-STAT) [19]. For a better interpretation of the results, these were correlated with cLogP values of the compounds, which were generated by the ChemBioDraw 11.0 software.

Antimicrobial Activity

The newly synthesized compounds were screened for their *in vitro* antimicrobial activities against four strains of bacteria (*Staphylococcus aureus* ATCC 49444, *Escherichia coli ATCC 25922, Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853) and one strain of fungi (*Candida albicans* ATCC 10231),

by the disk diffusion method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012) [20]. For the antibacterial assay, Mueller-Hinton agar medium (Oxoid, Basingstoke, UK) was used and for the antifungal assay, *YPD agar (Sigma-Aldrich, Germany)* was used. The solutions of the tested compounds were prepared by dissolving each compound in DMSO in order to obtain a concentration of 10 mg/ml. Sterile filter paper disks (6 mm in diameter), impregnated with the solution in DMSO of the test compounds (25µL solution), were placed on the Petri plates, previously seeded "in layer" with the tested bacterial strain inoculums. Gentamicin (25 µL/disk at concentration of 4 µg/mL) was used as positive control for bacteria. Fluconazole (25 µL/disk disk at concentration of 10 mg/mL) was used as positive control for *Candida albicans* ATCC 10231. A paper disk impregnated with DMSO (25µL/disk) was used as a negative control. The plates that were inoculated with bacteria were incubated for 24 h at 37°C and those inoculated with fungal culture were incubated for 24h at 30°C. The inhibition zone diameters were measured in millimeters. All the tests were performed in duplicate and the average was taken as final reading.

Results

Chemistry

The structures of the newly synthesized compounds were correlated with the data obtained from ^{1}H NMR and mass spectrometry, given below.

N'-(4-hydroxy-3-methoxybenzylidene)-2-(3-(trifluoromethyl)phenyl)-4-methylthiazole-5-carbohydrazide **(4a)**

$C_{20}H_{16}F_3N_3O_3S$ (435,42). Yellow powder. Yield 95%. M.p.: 284°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.29 (d, 1H, Ph), 8.27 (s, 1H, Ph), 7.92 (d, 1H, Ph), 7.8 (s, 1H), 7.78 (t, 1H, Ph), 7.44 (s, 1H, Ph), 7.4 (s, 1H, NH), 7.20 (d, 1H, Ph), 7.07 (d, 1H, Ph), 5.17 (s, 1H, OH), 3.81 (s, 3H, OCH_3), 2.73 (s, 3H, CH_3 thiazole). MS *m/z(%):* 436.8 (M+1, 100).

N'-(3-ethoxy-4-hydroxybenzylidene)-2-(3-(trifluoromethyl)phenyl)-4-methylthiazole-5-carbohydrazide **(4b)**

$C_{21}H_{18}F_3N_3O_3S$ (449,45). Yellow powder. Yield 95%. M.p.: 266°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.32 (d, 1H, Ph), 8.28 (s, 1H, Ph), 7.94 (d, 1H, Ph), 7.85 (s, 1H), 7.77 (t, 1H, Ph), 7.44 (s, 1H, Ph), 7.20 (d, 1H, Ph), 7.07 (d, 1H, Ph), 7.01 (s, 1H, NH), 5.20 (s, 1H, OH), 3.6 (d, 2H, OCH_2CH_3), 2.73 (s, 3H, CH_3 thiazole), 2.5 (t, 3H, OCH_2CH_3). MS *m/z(%):* 450.8 (M+1, 100).

N'-(4-bromobenzylidene) -2-(3-(trifluoromethyl) phenyl)-4-methylthiazole-5-carbohydrazide **(4c)**

$C_{19}H_{13}BrF_3N_3OS$ (468.29). White powder. Yield 90%. M.p.: 273°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.33 (d, 1H, Ph), 8.25 (s, 1H, Ph), 7.93 (d, 1H, Ph), 7.8 (s, 1H), 7.78 (t, 1H, Ph), 7.37 (d, 1H, Ph), 7.35 (d, 1H, Ph), 7.20 (d, 1H, Ph), 7.17 (d, 1H, Ph), 7.05 (s, 1H, NH), 2.7 (s, 3H, CH_3 thiazole). MS *m/z(%):* 469.4 (M+1, 100).

N'-(2,4-dichlorobenzylidene)-2-(3-(trifluoromethyl) phenyl)-4-methylthiazole-5-carbohydrazide **(4d)**

$C_{19}H_{12}Cl_2F_3N_3OS$ (458.28). White powder. Yield 93%. M.p.: 278°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.33 (d, 1H, Ph), 8.25 (s, 1H, Ph), 7.95 (d, 1H, Ph), 7.81 (s, 1H), 7,79 (t, 1H, Ph), 7.75 (d, 1H, Ph), 7.54 (s, 1H, NH), 7.44 (s, 1H, Ph), 7.2 (d, 1H, Ph), 2.73 (s, 3H, CH_3 thiazole). MS *m/z(%):* 459.3 (M+1, 100).

N'-cyclopentylidene-2-(3-(trifluoromethyl)phenyl)-4-methylthiazole-5-carbohydrazide **(5a)**

$C_{17}H_{16}F_3N_3OS$ (367,39). White powder. Yield 80%. M.p.: 223°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.27 (s, 1H, Ph), 8.26 (d, 1H, Ph), 7.92 (d, 1H, Ph), 7.77 (t, 1H, Ph), 7.1 (s, 1H, NH), 2.7 (s, 3H, CH_3 thiazole), 2.35-1.3 (m, 8H, cyclopentane). MS *m/z(%):* 368.8 (M+1, 100).

(N'-cyclohexylidene-2-(3-(trifluoromethyl)phenyl)-4-methylthiazole-5-carbohydrazide) **(5b)**

$C_{18}H_{18}F_3N_3OS$ (381.42). White powder. Yield 90%. M.p.: 244°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.27 (d, 1H, Ph), 8.24 (s, 1H, Ph), 7.91 (d, 1H, Ph), 7.77 (t, 1H, Ph), 6.9 (s, 1H, NH), 2.7 (s, 3H, CH_3 thiazole), 2.4-1.4 (m, 10H, cyclohexane). MS *m/z(%):* 382.5 (M+1, 100).

4-(3-acetyl-5-(-2-(3-(trifluoromethyl)phenyl)-4-methylthiazol-5-yl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl acetate (**6a**)

$C_{24}H_{20}F_3N_3O_5S$ (519,49). Yellow powder. Yield 60%. M.p.: 135°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.29 (d, 1H, Ph), 8.27 (s, 1H, Ph), 7.92 (d, 1H, Ph), 7.78 (t, 1H, Ph), 7.28 (s, 1H, Ph), 7.21 (s, 1H), 7.17 (d, 1H, Ph), 7.08 (d, 1H, Ph), 3.81 (s, 3H, OCH_3), 2.73 (s, 3H, CH_3 thiazole), 2.289 (s, 3H, $COCH_3$), 2.27 (s, 3H, $OCOCH_3$). MS *m/z(%)*: 520.5 (M+1, 100), 478.7 (M+1-$COCH_3$, 33.4).

4-(3-acetyl-5-(-2-(3-(trifluoromethyl)phenyl)-4-methylthiazol-5-yl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)-2-ethoxyphenyl acetate (**6b**)

$C_{25}H_{22}F_3N_3O_5S$ (533,12). Yellow powder. Yield 65%. M.p.: 140°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm): δ, 8.28 (d, 1H, Ph), 8.27 (s, 1H, Ph), 7.92 (d, 1H, Ph), 7.78 (t, 1H, Ph), 7.20 (s, 1H, Ph), 7.20 (s, 1H), 7.17 (d, 1H, Ph), 7.07 (d, 1H, Ph), 4.07 (d, 2H, $-CH_2CH_3$), 2.728 (s, 3H, CH_3 thiazole), 2.28 (s, 3H, $COCH_3$), 2.27 (s, 3H, $OCOCH_3$), 1.29 (t, 3H, $-CH_2CH_3$). MS *m/z(%):* 534.3 (M+1, 100), 492.3 (M+1-$COCH_3$, 47.5).

2-methoxy-4-(5-(-2-(3-(trifluoromethyl)phenyl)-4-methylthiazol-5-yl)-3-propionyl-2,3-dihydro-1,3,4-oxadiazol-2-yl)phenyl propionate (**6c**)

$C_{26}H_{24}F_3N_3O_5S$ (547.55). Yellow powder. Yield 60%. M.p.: 136°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.28 (d, 1H, Ph), 8.27 (s, 1H, Ph), 7.92 (d, 1H, Ph), 7.78 (t, 1H, Ph), 7.27 (s, 1H, Ph), 7.20 (s, 1H), 7.17 (d, 1H, Ph), 7.07 (d, 1H, Ph), 3.80 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3 thiazole), 2.7 (d, 2H, $OCOCH_2CH3$), 2.6 (d, 2H, $COCH_2CH_3$), 1.13 (t, 3H, $OCOCH_2CH3$), 1.1 (t, 3H,

$COCH_2CH_3$). MS: *m/z(%):* 548.7 (M+1, 100), 492.7 (M+1-COC_2H_5, 17.39).

2-ethoxy-4-(5-(-2-(3-(trifluoromethyl)phenyl)-4-methylthiazol-5-yl)-3-propionyl-2,3-dihydro-1,3,4-oxadiazol-2-yl)phenyl propionate (**6d**)

$C_{27}H_{26}F_3N_3O_5S$ (561,57). Yellow powder. Yield 50%. M.p.: 142°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.28 (d, 1H, Ph), 8.27 (s, 1H, Ph), 7.91 (d, 1H, Ph), 7.77 (t, 1H, Ph), 7.25 (s, 1H), 7.19 (s, 1H, Ph), 7.17 (d, 1H, Ph), 7.06 (d, 1H, Ph), 4.065 (d, 2H, -OCH_2-CH_3), 2.72 (s, 3H, CH_3 thiazole), 2.67 (d, 2H, $COCH_2CH_3$), 2.59 (d, 2H, $OCOCH_2CH_3$), 1.28 (t, 3H, -OCH_2-CH_3), 1.15 (t, 3H, $COCH_2CH_3$), 1.087 (t, 3H, $OCOCH_2CH_3$). MS *m/z(%):* 563.2 (M+1, 100), 506.4 (M+1-COC_2H_5, 45.32).

1-(2-(4-bromophenyl)-5-(-2-(3-(trifluoromethyl) phenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-3(2H)-yl) propan-1-one (**6e**)

$C_{22}H_{17}BrF_3N_3O_2S$ (524,35). Yellow powder. Yield 70%. M.p.: 155 °C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.27 (d, 1H, Ph), 8.26 (s, 1H, Ph), 7.91 (d, 1H, Ph), 7.77 (t, 1H, Ph), 7.68 (d, 1H, Ph), 7.66 (s, 1H), 7.47 (d, 1H, Ph), 7.47 (d, 1H, Ph), 7.20 (d, 1H, Ph), 2.67 (s, 3H, CH_3 thiazole), 2.50 (d, 2H, $COCH_2$-CH_3), 1.06 (t, 3H, $COCH_2CH_3$). MS *m/z(%):* 525.8 (M+1, 100).

1-(2-(2,4-dichlorophenyl)-5-(-2-(3-(trifluoromethyl)phenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-3(2H)-yl)propan-1-one (**6f**)

$C_{22}H_{16}Cl_2F_3N_3O_2S$ (514,35). Yellow powder. Yield 55% .M.p.: 182°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.29 (d, 1H, Ph), 8.27 (s, 1H, Ph), 7.92 (d, 1H, Ph), 7.79 (d, 1H, Ph), 7.78 (t, 1H, Ph), 7.55 (s, 1H, Ph), 7.55 (d, 1H, Ph), 7.35 (s, 1H), 2.7 (s, 3H, CH_3 thiazole), 2.68 (d, 2H, $COCH_2$-CH_3), 1.08 (t, 3H, $COCH_2CH_3$). MS *m/z(%):* 515.3 (M+1, 100).

1-(3-(-2-(3-(trifluoromethyl)phenyl)-4-methylthiazol-5-yl)-4-oxa-1,2-diazaspiro[4.4]non-2-en-1-yl)ethanone (**7a**)

$C_{19}H_{18}F_3N_3O_2S$ (409,43). Yellow powder. Yield 60%. M.p.: 144°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, Yellow powder. Yield 65%. M.p: 143°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.29 (s, 1H, Ph), 8.27 (d, 1H, Ph), 7.91 (d, 1H, Ph), 7.77 (t, 1H, Ph), 2.69 (s, 3H, CH_3 thiazole), 2.22 (s, 3H, $COCH_3$), 2.51-1.77 (m, 8H, cyclopentane). MS *m/z(%):* 410.6 (M+1, 100).

1-(3-(-2-(3-(trifluoromethyl)phenyl)-4-methylthiazol-5-yl)-4-oxa-1,2-diazaspiro[4.5]dec-2-en-1-yl)ethanone (**7b**)

$C_{20}H_{20}F_3N_3O_2S$ (423,45). Yellow powder. Yield 65%. M.p.: 143°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.28 (d, 1H, Ph), 8.26 (s, 1H, Ph), 7.91 (d, 1H, Ph), 7.77 (t, 1H, Ph), 2.7 (s, 3H, CH_3 thiazole), 2.18 (s, 3H, $COCH_3$), 1.87-1.03 (m, 10H, cyclohexane). MS *m/z(%):* 424.2 (M+1, 100).

1-(3-(-2-(3-(trifluoromethyl)phenyl)-4-methylthiazol-5-yl)-4-oxa-1,2-diazaspiro[4.4]non-2-en-1-yl)propan-1-one (**7c**)

$C_{20}H_{20}F_3N_3O_2S$ (423,45). Yellow powder. Yield 60%. M.p.: 113°C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.3 (s, 1H, Ph), 8.28 (d, 1H, Ph), 7.92 (d, 1H, Ph), 7.78 (t, 1H, Ph), 2.69 (s, 3H, CH_3 thiazole), 2.69 (t, 3H, $COCH_2CH_3$), 2.61 (d, 2H, $COCH_2CH_3$), 1.98-1.06 (m, 8H, cyclopentane). MS *m/z(%)*: 424.6 (M+1, 100).

1-(3-(-2-(3-(trifluoromethyl)phenyl)-4-methylthiazol-5-yl)-4-oxa-1,2-diazaspiro[4.5]dec-2-en-1-yl)propan-1-one (**7d**)

$C_{21}H_{22}F_3N_3O_2S$ (437,48). Yellow powder. Yield 50%. M.p.: 144 °C. ^{1}H NMR (DMSO-d6, 500 MHz, ppm) δ, 8.28 (d, 1H, Ph), 8.23 (s, 1H, Ph), 7.90 (d, 1H, Ph), 7.77 (t, 1H, Ph), 2.7 (s, 3H, CH_3 thiazole), 2.6 (d, 2H, $COCH_2CH_3$), 2.5 (t, 3H, $COCH_2CH_3$), 1.91-1.03 (m, 10H, cyclohexane). MS *m/z(%):* 438 (M+1, 100).

Lipophilicity evaluation

The R_f and R_M values, as well as the results of the regression analysis of compounds ***6a-f*** and ***7a-d***, are shown in Table III and V.

Table III. R_f and R_M values for compounds ***6a-f, 7a-d.***

Compound	*i*-PROPANOL : WATER (v:v %)									
	60:40		65:35		70:30		75:25		80:20	
	R_f	R_M	R_f	R_M	R_f	R_M	R_f	R_M	R_f	R_M
6a	0.323	0.321	0.420	0.140	0.508	-0.013	0.594	-0.165	0.680	-0.327
6b	0.270	0.431	0.366	0.238	0.461	0.067	0.552	-0.090	0.639	-0.247
6c	0.211	0.572	0.295	0.378	0.384	0.205	0.488	0.020	0.616	-0.205
6d	0.164	0.707	0.236	0.510	0.343	0.282	0.458	0.073	0.575	-0.131
6e	0.100	0.954	0.165	0.704	0.235	0.512	0.335	0.297	0.447	0.092
6f	0.082	1.049	0.142	0.781	0.200	0.602	0.300	0.367	0.411	0.156
7a	0.156	0.733	0.241	0.498	0.307	0.353	0.411	0.156	0.511	-0.019
7b	0.145	0.770	0.229	0.527	0.282	0.405	0.394	0.186	0.482	0.031
7c	0.117	0.877	0.200	0.602	0.260	0.454	0.364	0.242	0.465	0.060
7d	0.104	0.935	0.176	0.670	0.236	0.510	0.335	0.297	0.441	0.102

Table IV. The eigenvalues and the ratios of the variance explained by the five components using a covariance matrix: results of PCA.

Component	Eigenvalue	Difference	Proportion%	Cumulative%
1	4.925	4.86	**98.504**	98.504
2	0.065	0.015	**1.308**	**99.812**
3	0.005	0.002	0.110	99.921
4	0.003	0.002	0.051	99.972
5	0.001		0.028	100

Table V. Regression data and scores on the first two principal components for compounds ***6a-f, 7a-d.***

Compound	R_{M0}	b	Linear correlation coefficient (R^2)	PC_1	PC_2
6a	0.471	-0.160	0.999	4.450	-0.262
6b	0.586	-0.168	0.998	3.167	-0.054
6c	0.768	-0.191	0.997	1.590	0.378
6d	0.922	-0.211	0.999	0.408	0.610
6e	1.151	-0.213	0.998	-2.197	0.046
6f	1.250	-0.219	0.997	-2.971	-0.096
7a	0.898	-0.184	0.995	-0.361	-0.028
7b	0.930	-0.181	0.991	-0.855	-0.175
7c	1.045	-0.199	0.991	-1.537	-0.043
7d	1.114	-0.203	0.995	-2.138	-0.095

Table IV contains the eigenvalues of the covariance matrix, disposed from largest to smallest; the third column of this table displays the difference between each eigenvalue and the next smaller eigenvalue and the fourth column shows the proportion.

For a correct assessment of the initial data set, the first two principal components were retained; this explains 99.812% of the total information of the initial data. The first component (PC_1) explains 98.504% of the total variance and the second 1.308%.

The 2-D scatterplot of the scores corresponding to the first two principal components was generated by the graphic representation of $PC_2=f(PC_1)$ (Figure 2).

For a better interpretation of the lipophilicity parameters PC_1 and R_{M0}, cLogP values were generated (Table VI).

The variations of cLogP, R_{M0} and PC_1 values in the five mobile phases are represented in Figure 3.

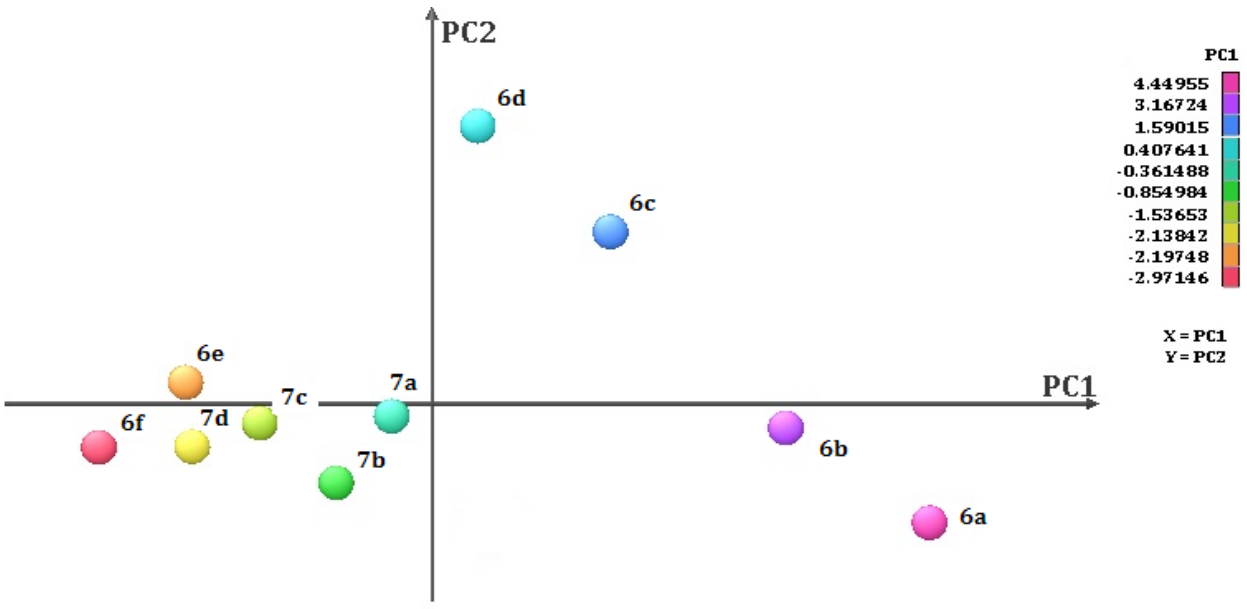


Figure 2. 2-D scatterplot of scores corresponding to the first two principal components.

Table VI. cLogP values for compunds ***6a-f, 7a-d.***

Compound	cLogP
6a	4.163
6b	4.692
6c	4.54
6d	5.75
6e	6.467
6f	7.03
7a	4.75
7b	5.308
7c	5.278
7d	5.837

While compounds **6f, 6e, 7d, 6d, 7b** and **7c** exhibited high values of the lipophilicity parameters, compounds **7a, 6b, 6c** and **6a** had lower values.

Antimicrobial Activity

The thiazolyl-1,3,4-oxadiazolines were screened for their antimicrobial activities against one Gram-positive (*Staphylococcus aureus* ATCC 49444) and three Gram-negative (*Escherichia coli ATCC 25922, Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 27853) bacterial strains. The antifungal activity was tested against a strain of *Candida albicans* ATCC 10231. The results of the antimicrobial evaluation are summarized in Table VII.

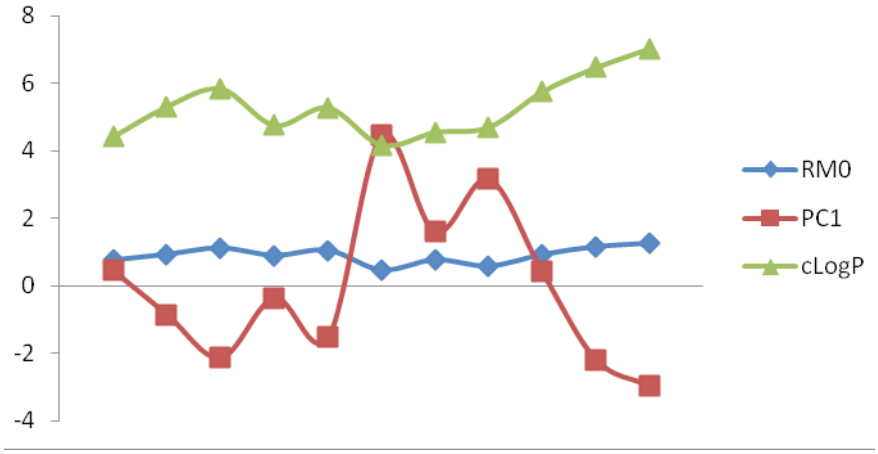


Figure 3. Values profile of cLogP, R_{M0} and PC_1.

Table VII. Antimicrobial activity of thiazolyl-1,3,4 oxadiazolines against bacterial and fungal species tested by disc diffusion assay.

Samples	Inhibition Zone (mm)				
	Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC 49444	*Salmonella typhimurium* ATCC 14028	*Pseudomonas aeruginosa* ATCC 27853	*Candida albicans* ATCC 10231
6a	7.8±0.4	7.8±0.4	7.3±0.4	6.0±0.0	7.3±0.4
6b	7.3±0.4	7.3±0.4	7.5±0.7	6.3±0.4	7.5±0.7
6c	8.1±0.1	8.0±0.0	8.0±0.0	7.3±0.4	8.0±0.0
6d	8.3±0.4	7.0±0.0	9.3±0.4	7.8±0.4	7.3±0.4
6e	8.0±0.0	8.5±0.0	9.5±0.7	6.8±0.4	8.0±0.0
6f	-	-	-	7.3±0.4	6.0±0.0
7a	9.0±0.0	9.3±0.4	8.0±0.0	6.8±0.4	7.0±0.0
7b	8.1±0.1	8.3±0.4	7.3±0.4	6.0±0.0	6.8±0.4
7c	7.8±0.4	8.3±0.4	6.3±0.4	6.0±0.0	6.3±0.4
7d	8.8±0.4	8.8±0.4	8.8±0.4	6.8±0.4	7.8±0.4
Gentamicin	18.8±0.4	25.3±0.4	20.0±1.4	13.8±0.4	NT
DMSO	-		-	-	-
Fluconazole	NT	NT	NT	NT	21.5±0.7

Each value is the mean ± SD of two independent measurements
NT- Not Tested

Discussion

After the structural validation by ^{1}H-NMR and mass spectrometry of the newly synthesized thiazolyl-oxadiazolines, we determined their lipophilicity. The linear dependency between the values of R_{M0} (lipophilic parameter) and the values of b (specific hydrophobic surface) show that the thiazolyl-1,3,4-oxadiazolines form a particular series of compounds. The values of the R^2 parameter showed the linear dependency between the R_M parameter and the concentrations of the organic component of the mobile phase (Table V). This interconnection showed us that the chromatographic experiments were appropriately conducted and, moreover, it helped us to assert that we could estimate the lipophilic character of the studied compounds by analyzing the R_{M0} parameter. High values of the R_{M0} parameter indicate a high lipophilicity.

As it can be seen from the data provided in Table V, the first principal component (PC_1) can replace the R_{M0} parameter for the estimation of the lipophilicity. By using the values of the two principal components, a relative lipophilic scale was created (Figure 2). A compound with a high lipophilicity is characterized by a low value of the PC_1 parameter.

By the graphic representation of $PC_2=f(PC_1)$ (Figure 2), this made possible the repartition of the studied compounds into two congeneric series.

- Class 1: 6a, 6b, 6c, 6d
- Class 2: 6e, 6f, 7a, 7b, 7c, 7d

A good linear correlation was obtained between the lipophilicity evaluated by RP-TLC and cLogP values (partition coefficient) that were theoretically determined (Table VI). A high lipophilicity is characterized by high cLogP and R_{M0} values and by low PC1 values. The R_{M0}, PC_1 and cLogP profiles could be well correlated (Figure 3).

The results obtained revealed that the presence of halogen atoms, with a high atomic volume, on the phenyl ring situated in position 5 of the oxadiazoline system and also of non-polar substituents (cyclopentyl, cyclohexyl), raised lipophilicity. Meanwhile, the substitution of the same phenyl ring with polar groups (-OCH_3, -OC_2H_5) led to lower values of the lipophilicity parameters.

Unfortunately, a correlation between the values of the lipophilic parameters and the antimicrobial activity could not be established, given that the inhibition zone was modest. This correlation between lipophilicity and antibacterial activity does not rule out the importance of the existence of other structural factors involved in the activity.

Conclusions

In this paper, we have presented the synthesis of ten new thiazolyl-oxadiazolines, whose structures were confirmed by ^{1}H-NMR and mass spectrometry. We evaluated the lipophilicity of the newly synthesized compounds by RP-TLC using the PCA method. The therapeutic potential was investigated by screening their antimicrobial activity, but the activity was low and could not be correlated with the lipophilicity of the compounds.

Acknowledgements

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/136893.

References

1. Siddiqui N, Arshad MF, Ahsan W, Alam MS. Thiazoles: A Valuable Insight into the Recent Advances and Biological Activities. Int J Pharm Sci Drug Res. 2009;1(3):136-143.
2. Beale JM, Block JH. Wilson's and Gisvold's textbook of organic medicinal and pharmaceutical Chemistry. 12th ed. Philadelphia: Lippincott Williams and Wilkins; 2011.
3. Bondock S, Fadaly W, Metwally MA. Synthesis and antimicrobial activity of some new thiazole, thiophene and pyrazole derivatives containing benzothiazole moiety. Eur J Med Chem. 2010;45(9):3692-3701.
4. Chaaban I, El Khawass el SM, Abd El Razik HA, El Salamouni NS, Redondo-Horcajo M, Barasoain I, et al. Synthesis and biological evaluation of new oxadiazoline-substituted naphthalenyl acetates as anticancer agents. Eur J Med Chem. 2014;87:805-813.
5. Hu Y, Lu X, Chen K, Yan R, Li QS, Zhu HL. Design, synthesis, biological evaluation and molecular modeling of 1,3,4-oxadiazoline analogs of combretastatin-A4 as novel antitubulin agents. Bioorg Med Chem. 2012;20(2):903-909.
6. Shyma PC, Kalluraya B, Peethambar SK, Telkar S, Arulmoli T. Synthesis, characterization and molecular docking studies of some new 1,3,4-oxadiazolines bearing 6-methylpyridine moiety for antimicrobial property. Eur J Med Chem. 2013;68:394-404.
7. Rajak H, Kharya MD, Mishra P. Synthesis of some novel oxadiazole and oxadiazoline analogues for their antiinflammatory activity. Yakugaku Zasshi. 2007;127(10):1757-1764.
8. Somani Rakesh R, Agrawal Anuj G, Kalantri Pushkar P, Savarkar Pratibha S, Clerq Erik De. Investigation of 1,3,4-Oxadiazole Scaffold as Potentially Active Compounds. IJDDD. 2011;2(1):353-360.
9. Janicka M, Sztanke M, Sztanke K. Reversed-phase liquid chromatography with octadecylsilyl, immobilized artificial membrane and cholesterol columns in correlation studies with *in silico* biological descriptors of newly synthesized antiproliferative and analgesic active compounds. J Chromatogr A. 2013;1318:92-101.
10. Starek M, Komsta L, Krzek J. Reversed-phase thin-layer chromatography technique for the comparison of the lipophilicity of selected non-steroidal anti-inflammatory drugs. J Pharm Biomed Anal. 2013;85:132-137.
11. Janicka M, Stepnik K, Pachuta-Stec A. Quantification of Lipophilicity of 1,2,4-Triazoles Using Micellar Chromatography. Chromatographia. 2012;75(9-10):449-456.
12. Ionuţ IA, Tiperciuc B, Oniga O, Szefler B, Matieş R. Correlating study on physico-chemical and biological properties of some thiosemicarbazone and thiadiazoline derivatives. Studia Universitatis Babeş-Bolyai Chemia 2012;57(3):109-119.
13. Ilijaš M, Malnar I, Gabelica Marković V, Stepanić V. Study of

lipophilicity and membrane partition of 4-hydroxycoumarins by HPLC and PCA. J Pharm Biomed Anal. 2013;76:104-111.
14. Tiperciuc B, Pârvu A, Palage M, Oniga O, Ghiran D. The synthesis and the study of the anti-inflammatory activity of some new 3-N-acetyl-2-R-5-(2`-aryl-4`-methyl-thiazole-5`-yl)-Δ_2-1,3,4-oxadiazoline. Farmacia 1999;47(5):77-84.
15. Oniga S, Oniga O, Tiperciuc B, Palage M, Mureșan A, Ghiran D. Synthesis and antimicrobial activity of some new 5-dithiazolyl-2-R-1,3,4-Δ_4-oxadiazoline derivatives. Farmacia. 2000;48(3):65-69.
16. Oniga O, Tiperciuc B, Oniga S, Ioanoviciu A, Săndulescu I, Mogoșanu C. The synthesis and anticonvulsant activity of new 2-(1,3,4- Δ_4-oxadiazoline)-4`- thiazole derivatives. Farmacia 2000;48(4):77-83.
17. Rutkowska E, Pajak K, Jozwiak K. Lipophilicity-methods of determination and its role in medicinal chemistry. Acta Pol Pharm. 2013;70(1):3-18.
18. Kovačević SZ, Podunavac-Kuzmanović SO, Jevrić LR. Multivariate regression modelling of antifungal activity of some benzoxazole and oxazolo[4,5-*b*]pyridine derivatives. Acta Chim Slov. 2013;60:756-762.
19. Mackiewicz A, Ratajczak W. Principal components analysis (PCA). Computer & Geoscience. 1993;19:303-342.
20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty Second Informational Supplement. CLSI: Wayne, PA, USA, 2012.