https://doi.org/10.31925/farmacia.2021.4.13

ORIGINAL ARTICLE

DEVELOPMENT OF NEW 2-METHYL-4-SALICYLAMIDE THIAZOLE DERIVATIVES: SYNTHESIS, ANTIMICROBIAL ACTIVITY EVALUATION, LIPOPHILICITY AND MOLECULAR DOCKING STUDY

BIANCA POP [1], IOANA IONUȚ [1], GABRIEL MARC [1]*, DAN CRISTIAN VODNAR [2], ADRIAN PÎRNĂU [3], LAURIAN VLASE [1], OVIDIU ONIGA [1]

[1] *"Iuliu Haţieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, 12 Ion Creangă Street, 400010, Cluj-Napoca, Romania*
[2] *University of Agricultural Sciences and Veterinary Medicine, Department of Food Science and Technology, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania*
[3] *National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donath Street, 400293, Cluj-Napoca, Romania*

**corresponding author: marc.gabriel@umfcluj.ro*

Manuscript received: March 2021

Abstract

A series of 15 new thiazolyl-salicylamide ethers were obtained through an alkylation reaction in alkaline medium. The compounds were physico-chemically and spectrally characterized. The evaluation of their antimicrobial activity highlighted the antifungal effect of compound **5n**, which was equivalent to that of fluconazole. A molecular docking study revealed the structurally important elements for a better interaction with target lanosterol 14α-fungal demethylase. Given the importance of the lipophilicity for the penetration of biological membranes of bioactive molecules, this was evaluated *in silico*.

Rezumat

O serie de 15 noi eteri tiazolil-salicilamidici au fost obținuți prin alchilare în mediu bazic. Compușii au fost caracterizați fizico-chimic și spectral. Evaluarea activității antimicrobiene a evidențiat efectul antifungic al compusului **5n**, echivalent cu al fluconazolului. Un studiu de andocare moleculară a evidențiat elementele structurale importante pentru o bună interacțiune cu lanosterol 14α-demetilaza fungică. Datorită importanței pe care o are lipofilia asupra penetrabilității prin membrane a moleculelor bioactive, aceasta a fost evaluată *in silico*.

Keywords: antifungal, thiazole, salicylamide, molecular docking, fluconazole

Introduction

Microbial infections represent a global crisis due to the extension of microbial resistance that leads to a progressive reduction of therapy efficacy. The improper use of antibiotics has led to the selection of microbial strains with high degrees of resistance [2, 9]. The incidence of infections with multidrug resistant micro-organisms is constantly increasing, being the cause of many deaths within the hospital environment. Consequently, the need for discovering new active molecules against resistant bacteria or fungi is a priority of many researchers in the field of medicinal chemistry [5, 12, 17]. Research into new molecules with potential antimicrobial activity is intense, addressing various research pathways to combat resistance [3, 4]. The present paper is a continuation of our group's research in developing new thiazole derivatives as antimicrobial agents, with various structural features. In our previous report, a series of 5-(2-(phenylamino)-thiazol-4-yl)benzamide ethers were synthesized and evaluated against some microbial strains [1]. In this paper, we report the development of a new series of thiazolyl-salicylamide derivatives, using different halide-based alkylating agents, in order to obtain the desired ethers. The phenyl-amino moiety from the position 2 of the thiazole from our previous report, was replaced with a methyl residue [1]. The present research was encouraged by the favourable results obtained by other researchers in the field of obtaining thiazole derivatives with antimicrobial activity [7, 8, 11, 14].

Materials and Methods

Chemistry

Reagents and solvents used, produced by Merck (Darmstadt, Germany) and Alfa Aesar (Karlsruhe, Germany), were of analytical grade purity and were purchased from local suppliers and used without any further purification. The chemical synthesis was monitored by thin layer chromatography (TLC). The purity of the compounds was evaluated using TLC and RP-HPLC. The uncorrected melting points were determined using an MPM-H1 melting point meter (Schorpp Gerätetechnik, Überlingen, Germany) in open

glass capillary. MS analyses were performed on an Agilent 1100 series, in positive ionization with an Agilent Ion Trap SL mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded using a Jasco FT/IR 6100 spectrometer (Jasco, Cremella, Italy), after compression in anhydrous KBr under *vacuum*. ^{1}H NMR analysis were performed in DMSO-d_6 (δ_H = 2.51 ppm) as solvent, on an Avance NMR spectrometer (Bruker, Karlsruhe, Germany), using tetramethylsilane (TMS) as internal standard.

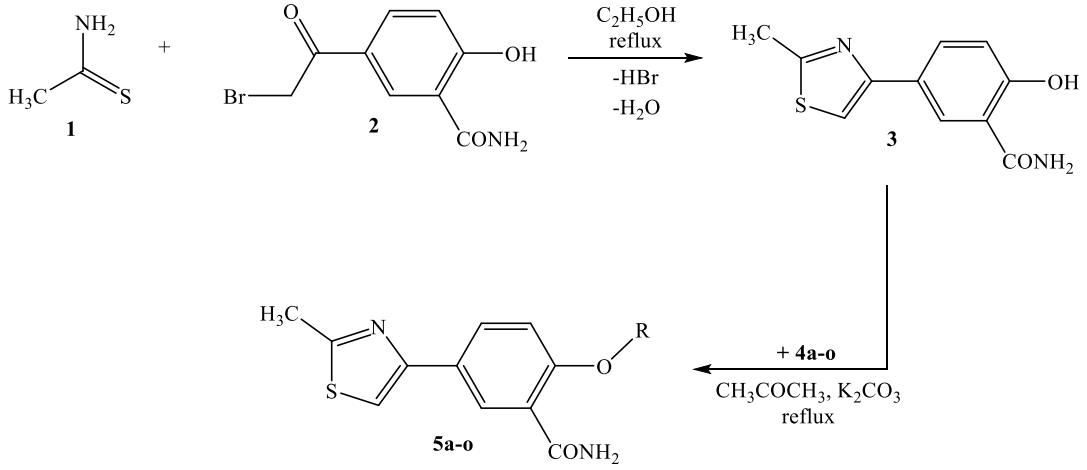
*Synthesis of 2-hydroxy-5-(2-methylthiazol-4-yl)-benzamide (**3**)*

In a round bottom flask, 25 mmol (1.875 g) of thioacetamide (compound **1**) were dissolved in 15 mL of ethanol. Later, 25 mmol (6.425 g) of α-bromoketone (compound **2**) were added, in order to obtain a thiazole ring through a Hantzsch cyclisation. The mixture was refluxed for 3 hours. The resulted precipitate was filtered under *vacuum* after cooling. The resulted solid was placed in a beaker and neutralized using a sodium bicarbonate solution and filtered again. The resulted precipitate was filtered and dried using *vacuum*. The final pure product **3** was obtained after recrystallization from ethanol. Compound **3** was previously reported in the literature [18].

2-hydroxy-5-(2-methylthiazol-4-yl)benzamide (**3**): white solid; mp = 199°C; yield = 61%; FT IR (KBr) ν_{max} cm^{-1}: 1670 (C=O amide), 1632 (C=N), 1373 (O-H bending); MS: *m/z* = 236.0 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.70 (s, 3H, -CH_3), 7.03 (d, 1H, Ar), 7.81 (s, 1H, Th), 8.06 (d, 1H, Ar), 8.19 (br, 1H, -NH_2), 8.61 (s, 1H, Ar), 8.78 (br, 1H, -NH_2), 13.62 (br, 1H, -OH).

*General procedure for synthesis of ethers **5a-o** (Figure 1)*

1 mmol (0.234 g) of intermediate compound **3**, 5 mmol (0.69 g) of anhydrous K_2CO_3 and 1 mmol of the corresponding **4a-o** halide compound were suspended in 15 mL of anhydrous acetone. With the exception of compound **5d**, because compound **4d** was iodoacetamide, for the synthesis of the other fourteen compounds, 1 mmol (0.166 g) of anhydrous KI was added in order to perform a Finkelstein *in situ* transhalogenation reaction. The compounds **4b**, **4e** and **4g** were aliphatic chlorine derivatives, while others were aliphatic bromine derivatives (compounds **4a**, **4c**, **4f** and **4h-o**). Supplementary, 1 mmol (0.138 g) of anhydrous K_2CO_3 was added for the synthesis of the compound **5g**, to neutralize the acidity given by the carboxyl group. The mixture was refluxed for three hours. After the disappearance of the compound **3** from the reaction flask, confirmed by TLC, the acetone was removed using a rotational evaporator under *vacuum*. The remaining solid was mixed with water and a 10% sulphuric acid was added until the complete precipitation of the final product. The resulted precipitate was filtered under *vacuum* and dried. The impure solid was crystallized from methanol, in order to give the pure desired products **5a-o**. The purity of the final compounds was confirmed using TLC and RP-HPLC.



Compound	R	Compound	R
5a	-CH_2-$COOCH_2$-CH_3	**5h**	-CH_2-CO-C_6H_4-*p*NO_2
5b	-CH_2-CO-CH_3	**5i**	-CH_2-CO-C_6H_5
5c	-CH_2-CH_2-CH_3	**5j**	-CH_2-CO-C_6H_4-*p*Cl
5d	-CH_2-CO-NH_2	**5k**	-CH_2-CO-*β*-$C_{10}H_7$
5e	-CH_2-CH_2-N(CH_2-CH_3)$_2$	**5l**	-CH_2-CO-C_6H_4-*p*OCH_3
5f	-CH_2-CH_2-CH_2-CH_3	**5m**	-CH(CH_3)-CO-C_6H_5
5g	-CH_2-COOH	**5n**	-CH_2-CO-C_6H_4-*p*Br
		5o	-CH_2-CO-C_6H_4-*p*CN

Figure 1.
The synthetic route followed in order to obtain the thiazolyl-salicylamide ethers **5a-o**

Ethyl 2-(2-carbamoyl-4-(2-methylthiazol-4-yl)phenoxy)-acetate (**5a)**: white solid; mp = 152°C; yield = 88%; FT IR (KBr) ν_{max} cm^{-1}: 1744 (C=O ester), 1672 (C=O amide), 1639 (C=N), 1257 (C-O-C ether asymm str), 1026 (C-O-C ether symm str); MS: *m/z* = 322.1 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 1.29 (t, 3H, -CH_3), 2.71 (s, 3H, -CH_3), 4.24 (q, 2H, -CH_2-), 5.11 (s, 2H, -CH_2-), 7.28 (d, 1H, Ar), 7.86 (s, 1H, Th), 8.09 (d, 1H, Ar), 8.58 (m, 2H, Ar, -NH_2), 9.09 (br, 1H, -NH_2).

5-(2-Methylthiazol-4-yl)-2-(2-oxopropoxy)benzamide (**5b)**: yellow solid; mp = 188°C; yield = 62%; FT IR (KBr) ν_{max} cm^{-1}: 1731 (C=O ketone), 1671 (C=O amide), 1638 (C=N), 1278 (C-O-C ether asymm str), 1064 (C-O-C ether symm str); MS: *m/z* = 291.1 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.29 (s, 3H, -CH_3), 2.71 (s, 3H, -CH_3), 4.99 (s, 2H, -CH_2-), 7.21 (d, 1H, Ar), 7.84 (s, 1H, Th), 8.19 (d, 1H, Ar), 8.28 (m, 2H, Ar, -NH_2), 8.55 (s, 1H, Ar), 9.06 (br, 1H, -NH_2).

5-(2-Methylthiazol-4-yl)-2-propoxybenzamide (**5c)**: white solid; mp = 206°C; yield = 75%; FT IR (KBr) ν_{max} cm^{-1}: 1670 (C=O amide), 1637 (C=N), 1256 (C-O-C ether asymm str), 1067 (C-O-C ether symm str); MS: *m/z* = 277.3 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 1.01 (t, 3H, -CH_3), 1.69 (m, 2H, -CH_2-), 2.75 (s, 3H, -CH_3), 3.96 (s, 2H, -CH_2-), 7.16 (d, 1H, Ar), 7.90 (s, 1H, Th), 8.10 (d, 1H, Ar), 8.26 (m, 2H, Ar, -NH_2), 8.50 (s, 1H, Ar), 8.99 (br, 1H, -NH_2).

2-(2-Amino-2-oxoethoxy)-5-(2-methylthiazol-4-yl)-benzamide (**5d)**: white solid; mp = 233 - 224°C; yield = 51%; FT IR (KBr) ν_{max} cm^{-1}: 1670 (C=O amide), 1636 (C=N), 1268 (C-O-C ether asymm str), 1048 (C-O-C ether symm str); MS: *m/z* = 292.1 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.73 (s, 3H, -CH_3), 4.58 (s, 2H, -CH_2-), 7.01 (br, 2H, -NH_2) 7.15 (d, 1H, Ar), 7.80 (s, 1H, Th), 8.16 (d, 1H, Ar), 8.25 (m, 2H, Ar, -NH_2), 8.50 (s, 1H, Ar), 9.51 (br, 1H, -NH_2).

2-(2-(Diethylamino)ethoxy)-5-(2-methylthiazol-4-yl)benzamide (**5e)**: pale yellow solid; mp = 128°C; yield = 29%; FT IR (KBr) ν_{max} cm^{-1}: 1674 (C=O amide), 1637 (C=N), 1273 (C-O-C ether asymm str), 1053 (C-O-C ether symm str); MS: *m/z* = 334.5 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 1.05 (t, 6H, -CH_3), 2.59 (t, 2H, -CH_2-), 2.65 (m, 4H, -CH_2-), 2.71 (s, 3H, -CH_3), 3.99 (d, 2H, -CH_2-), 7.22 (d, 1H, Ar), 7.88 (s, 1H, Th), 8.18 (m, 2H, Ar, -NH_2), 8.50 (s, 1H, Ar), 8.99 (br, 1H, -NH_2).

2-Butoxy-5-(2-methylthiazol-4-yl)benzamide (**5f)**: white solid; mp = 139°C; yield = 63%; FT IR (KBr) ν_{max} cm^{-1}: 1672 (C=O amide), 1637 (C=N), 1245 (C-O-C ether asymm str), 1035 (C-O-C ether symm str); MS: *m/z* = 291.2 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 1.01 (t, 3H, -CH_3), 1.40 (m, 2H, -CH_2-), 1.69 (m, 2H, -CH_2-), 2.70 (s, 3H, -CH_3), 3.91 (d, 2H, -CH_2-), 7.19 (d, 1H, Ar), 7.81 (s, 1H, Th), 8.13 (d, 1H, Ar), 8.54 (s, 1H, Ar), 8.65 (br, 1H, -NH_2), 9.28 (br, 1H, -NH_2).

2-(2-Carbamoyl-4-(2-methylthiazol-4-yl)phenoxy)-acetic acid (**5g)**: white solid; mp = 162°C; yield = 41%; FT IR (KBr) ν_{max} cm^{-1}: 1711 (C=O acid), 1671 (C=O amide), 1637 (C=N), 1257 (C-O-C ether asymm str), 1026 (C-O-C ether symm str); MS: *m/z* = 293.1 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.74 (s, 3H, -CH_3), 4.81 (d, 2H, -CH_2-), 7.29 (d, 1H, Ar), 7.88 (s, 1H, Th), 8.18 (d, 1H, Ar), 8.52 (s, 1H, Ar), 8.17 (br, 1H, -NH_2), 8.85 (br, 1H, -NH_2).

5-(2-Methylthiazol-4-yl)-2-(2-(4-nitrophenyl)-2-oxoethoxy)benzamide (**5h)**: brown solid; mp = 199°C; yield = 59%; FT IR (KBr) ν_{max} cm^{-1}: 1670 (C=O amide), 1637 (C=N), 1524 (N=O nitro asymm str), 1349 (N=O nitro symm str), 1258 (C-O-C ether asymm str), 1062 (C-O-C ether symm str); MS: *m/z* = 398.3 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.73 (s, 3H, -CH_3), 5.20 (s, 2H, -CH_2-), 7.18 (d, 1H, Ar), 7.84 (s, 1H, Th), 8.17 (d, 1H, Ar), 8.29 (m, 3H, Ar, -NH_2), 8.36 (d, 2H, Ar), 8.55 (s, 1H, Ar), 9.06 (br, 1H, -NH_2).

5-(2-Methylthiazol-4-yl)-2-(2-oxo-2-phenylethoxy)-benzamide (**5i)**: white solid; mp = 204°C; yield = 83%; FT IR (KBr) ν_{max} cm^{-1}: 1673 (C=O amide), 1637 (C=N), 1238 (C-O-C ether asymm str), 1074 (C-O-C ether symm str); MS: *m/z* = 353.2 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.73 (s, 3H, -CH_3), 5.24 (s, 2H, -CH_2-), 7.37 (d, 1H, Ar), 7.62 (d, 2H, Ar), 7.74 (t, 1H, Ar), 7.79 (br, 1H, -NH_2), 7.89 (s, 1H, Th), 8.05 (d, 1H, Ar), 8.11 (d, 2H, Ar), 8.46 (br, 1H, -NH_2), 8.58 (s, 1H, Ar).

2-(2-(4-Chlorophenyl)-2-oxoethoxy)-5-(2-methyl-thiazol-4-yl)benzamide (**5j)**: white solid; mp = 232°C; yield = 42%; FT IR (KBr) ν_{max} cm^{-1}: 1691 (C=O ketone), 1670 (C=O amide), 1638 (C=N), 1230 (C-O-C ether asymm str), 1052 (C-O-C ether symm str); MS: *m/z* = 387.6 (M+1, with specific aspect due to chlorine isotopes ^{35}Cl and ^{37}Cl in approximately 3:1 ratio); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.70 (s, 3H, -CH_3), 5.17 (s, 2H, -CH_2-), 7.25 (d, 1H, Ar), 7.54 (d, 2H, Ar), 7.85 (s, 1H, Th), 8.06 (m, 3H, Ar), 8.59 (s, 1H, Ar), 8.90 (br, 1H, -NH_2), 9.21 (br, 1H, -NH_2).

5-(2-Methylthiazol-4-yl)-2-(2-(naphthalen-2-yl)-2-oxoethoxy)benzamide (**5k)**: yellow solid; mp = 196°C; yield = 40%; FT IR (KBr) ν_{max} cm^{-1}: 1697 (C=O ketone), 1672 (C=O amide), 1638 (C=N), 1273 (C-O-C ether asymm str), 1069 (C-O-C ether symm str); MS: *m/z* = 403.2 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.70 (s, 3H, -CH_3), 5.18 (s, 2H, -CH_2-), 7.29 (d, 1H, Ar), 7.65 (m, 2H, Ar), 7.82 (m, 2H, Ar, Th), 7.92 (d, 1H, Ar), 8.06 (m, 3H, Ar), 8.30 (m, 2H, Ar, -NH_2), 8.59 (s, 1H, Ar), 8.89 (br, 1H, -NH_2).

2-(2-(4-Methoxyphenyl)-2-oxoethoxy)-5-(2-methyl-thiazol-4-yl)benzamide (**5l)**: yellow solid; mp = 199°C; yield = 52%; FT IR (KBr) ν_{max} cm^{-1}: 1695 (C=O

ketone), 1671 (C=O amide), 1638 (C=N), 1260 (C-O-C ether asymm str), 1058 (C-O-C ether symm str); MS: *m/z* = 383.3 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.74 (s, 3H, -CH_3), 3.88 (s, 3H, -OCH_3), 5.15 (s, 2H, -CH_2-), 7.12 (d, 1H, Ar), 7.28 (d, 2H, Ar), 7.83 (s, 1H, Th), 8.01 (d, 2H, Ar), 8.15 (d, 1H, Ar), 8.24 (br, 1H, -NH_2), 8.50 (s, 1H, Ar), 9.51 (br, 1H, -NH_2).

5-(2-Methylthiazol-4-yl)-2-((1-oxo-1-phenylpropan-2-yl)oxy)benzamide (**5m)**: pale yellow solid; mp = 193°C; yield = 44%; FT IR (KBr) ν_{max} cm^{-1}: 1696 (C=O ketone), 1672 (C=O amide), 1637 (C=N), 1256 (C-O-C ether asymm str), 1056 (C-O-C ether symm str); MS: *m/z* = 367.3 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 1.49 (s, 3H, -CH_3), 2.71 (s, 3H, -CH_3), 5.28 (m, 1H, CH), 7.21 (d, 1H, Ar), 7.63 (t, 2H, Ar), 7.73 (s, 1H, Ar), 7.82 (s, 1H, Th), 8.11 (m, 3H, Ar), 8.50 (s, 1H, Ar), 8.64 (br, 1H, -NH_2), 9.03 (br, 1H, -NH_2).

2-(2-(4-Bromophenyl)-2-oxoethoxy)-5-(2-methyl-thiazol-4-yl)benzamide (**5n)**: pale yellow solid; mp = 237°C; yield = 51%; FT IR (KBr) ν_{max} cm^{-1}: 1693 (C=O ketone), 1671 (C=O amide), 1638 (C=N), 1275 (C-O-C ether asymm str), 1068 (C-O-C ether symm str); MS: *m/z* = 431.1 (M+1, with specific aspect due to bromine isotopes ^{79}Br and ^{81}Br in approximately 1:1 ratio); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.70 (s, 3H, -CH_3), 5.16 (s, 1H, CH), 7.17 (d, 1H, Ar), 7.60 (d, 2H, Ar), 7.82 (s, 1H, Th), 7.98 (d, 2H, Ar), 8.09 (d, 1H, Ar), 8.22 (br, 1H, -NH_2), 8.52 (s, 1H, Ar), 9.02 (br, 1H, -NH_2).

2-(2-(4-Cyanophenyl)-2-oxoethoxy)-5-(2-methylthiazol-4-yl)benzamide (**5o)**: yellow solid; mp = 193°C; yield = 25%; FT IR (KBr) ν_{max} cm^{-1}: 2229 (C≡N), 1706 (C=O ketone), 1671 (C=O amide), 1638 (C=N), 1278 (C-O-C ether asymm str), 1072 (C-O-C ether symm str); MS: *m/z* = 378.2 (M+1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.74 (s, 3H, -CH_3), 5.12 (s, 1H, CH), 7.19 (d, 1H, Ar), 7.79 (d, 2H, Ar), 7.81 (s, 1H, Th), 8.15 (d, 2H, Ar), 8.19 (d, 1H, Ar), 8.23 (br, 1H, -NH_2), 8.52 (s, 1H, Ar), 8.98 (br, 1H, -NH_2).

Antimicrobial activity

The antimicrobial evaluation was performed as previously reported, based on the serial double microdilution technique on 96 well microplates [10]. By dissolving the test compounds**,** the reference antifungal agent (fluconazole) and the reference antibacterial agent (streptomycin) in sterile DMSO, stock solutions (1 mg/mL) were prepared. The resulted solutions were stored in a refrigerator at 4°C until using. The solvent used (DMSO) showed no inhibition of the fungal growth and was used as negative control. The micro-organisms used for this assay were obtained from the Food Biotechnology Laboratory, Life Sciences Institute, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania and cultured on Muller-Hinton broth. The MIC and MMC values were determined against cultures of *S. aureus* ATCC 49444, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. typhimurium* ATCC 14028, *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 and *C. zeylanoides* ATCC 201082. The cell suspensions were adjusted with sterile saline to a concentration of approximately 3 x 10^5 CFU/mL in a final volume of 100 µL *per* well. The samples were carried out over the wells containing 100 µL MHB and afterward, 10 µL of inoculum was added to all the wells. The microplates were incubated for 18 h at 37°C. The microbicidal activity of the samples was detected following the addition of 20 µL of resazurin solution (0.2 mg/mL) to each well, and the plates were incubated 2 h at 37°C. A change from blue to pink indicates a reduction of resazurin and therefore microbial growth. The results of the antimicrobial activity were expressed as the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC), depending on the changing of the colour of the solution from wells.

Molecular docking study

A molecular docking study was performed using AutoDock 4.2 [13], using as target the fungal lanosterol 14α-demethylase built by homology modelling, as previously reported [10]. The preparation protocol of the ligands and of the target macromolecule performed as previously reported, using AutoDockTools 1.5.6 [13]. In order to have a better understanding of the interactions of the ligands with the target macro-molecule, the number of searched conformations was increased to 200, compared to the previously reported experiments. The other parameters were left unchanged, as previously reported [10]. Depiction of the interactions between the target and the ligand was made using UCSF Chimera 1.10.2 [15].

Lipophilicity evaluation

Given the importance of lipophilicity for the penetration of microbial membranes by bioactive compounds [16], the theoretical logP of the newly synthesized thiazolyl-salicylamide derivatives **5a-o** was computationally estimated using Swiss-ADME [6].

Results and Discussion

Chemistry

As we hoped, spectral data confirmed the structures of the obtained compounds **5a-o**. Compound **3** spectral signals confirmed its obtaining. In the IR spectrum, the appearance of a strong signal at 1670 cm^{-1} given by the stretching of the amide C=O bond and the presence of another signal given by the bending of phenolic O-H confirmed the presence of the salicylamide fragment in the newly obtained compound. In addition, the stretching of the C=N bond, a new type of bond, compared to the starting compound **1**, confirmed the successful formation of the thiazole nucleus through the Hantzsch reaction. The molecular peak of the

compound was found in its mass spectra, confirming one more time the obtaining of the desired intermediate compound **3**. ^{1}H NMR spectra was consistent with the expected data, in terms of number of protons, their deshielding and multiplicity. For example, at 7.81 ppm a strong sharp singlet signal of the C_5-H proton confirms the formation of the thiazole ring, while the appearance of three broad signals, corresponding of one exchangeable proton each, confirms again the successful obtaining of the intermediate compound **3**. Two of the signals are given by the amide protons and one from the phenol proton.

The obtaining of the final compounds **5a-o** was confirmed by the appearance in their IR spectra of two characteristic signals, given by the stretching of the newly resulted etheric bond: asymmetric, between 1230 - 1278 cm^{-1}, and a symmetric stretching between 1026 - 1074 cm^{-1}, respectively. The presence of the amidic C=O bond stretching at almost identical wave-numbers as for the starting compound, proved that the amide fragment was not subjected to chemical reaction and the chemical modulation took place only at the phenol moiety. Other signals given by ester (compound **5a**), ketone (compound **5b**), acid (compound **5g**), nitro (compound **5h**) or nitrile (compound **5o**) could be easily identified in the IR spectrum, proving that the insertion of those fragments was successful. The mass spectra were concordance with the expected data for the final compounds, presenting even the characteristic pattern given by halide atoms (compounds **5j** and **5n**) for the specified compounds. The ^{1}H NMR data of compounds 5**a-o** were consistent with the expected data, in terms of number of protons, their deshielding and multiplicity. From the three broad signals given by the salicylamide fragment, the phenol signal disappeared, remaining only the two broad signals given by the exchangeable protons of the amide moiety.

Antimicrobial activity

The results of the antimicrobial screening of compounds **5a-o** are presented in Table I as minimum inhibitory concentration (MIC) and in Table II as minimum microbicidal concentration (MMC), against the microbial strains taken into the antimicrobial assay. With the exception of compound **5n**, most compounds exhibited low antibacterial and antifungal activities, with MIC and MMC values higher than those of the reference drugs, fluconazole and streptomycin, respectively. Compound **5n** exhibited a much stronger antifungal activity compared to the other newly synthesized compounds from the present series. Against *C. albicans* ATCC 10231 and *C. zeylanoides* ATCC 201082 strains, derivative **5n** was equally active as the reference compound fluconazole, with a 15.62 µg/mL MIC value, and a 31.24 µg/mL MMC value, respectively. Against *C. parapsilosis* ATCC 22019 strain, compound **5n** was slightly less active than fluconazole, with a MIC value equal to 15.62 µg/mL *versus* 7.81 µg/mL for fluconazole. The fungicide activity expressed as MMC values against all the three fungal strains were of 31.24 µg/mL, identical with those obtained for the reference drug against *C. albicans* ATCC 10231 and *C. zeylanoides* ATCC 201082 strains and double against *C. parapsilosis* ATCC 22019 strain.

Table I

Minimum inhibitory concentration (µg/mL) values for compounds **5a-o**

Compound	*S. aureus* ATCC 49444	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. typhimurium* ATCC 14028	*C. albicans* ATCC 10231	*C. parapsilosis* ATCC 22019	*C. zeylanoides* ATCC 201082
5a	125	125	125	125	62.5	62.5	125
5b	125	125	125	125	62.5	62.5	125
5c	125	125	125	125	62.5	62.5	125
5d	125	125	125	125	62.5	62.5	125
5e	62.5	62.5	125	125	62.5	62.5	125
5f	125	125	125	125	62.5	62.5	125
5g	62.5	62.5	125	125	62.5	62.5	125
5h	125	125	125	125	62.5	62.5	125
5i	125	125	125	125	62.5	62.5	62.5
5j	125	125	125	125	62.5	62.5	62.5
5k	125	62.5	125	125	62.5	62.5	125
5l	62.5	62.5	125	125	62.5	62.5	125
5m	62.5	62.5	125	125	62.5	62.5	62.5
5n	125	125	125	125	**15.62**	**15.62**	**15.62**
5o	62.5	62.5	125	125	62.5	62.5	125
Fluconazole	NT	NT	NT	NT	15.62	7.81	15.62
Streptomycin	0.03	0.12	0.06	0.06	NT	NT	NT

NT = not tested

Table II

Minimum microbicidal concentration (µg/mL) of compounds **5a-o**

Compound	*S. aureus* ATCC 49444	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. typhimurium* ATCC 14028	*C. albicans* ATCC 10231	*C. parapsilosis* ATCC 22019	*C. zeylanoides* ATCC 201082
5a	250	250	250	250	125	125	250
5b	250	250	250	250	125	125	250
5c	250	250	250	250	125	125	250
5d	250	250	250	250	125	125	125
5e	125	125	250	250	125	125	250
5f	250	250	250	250	125	125	250
5g	125	125	250	250	125	125	250
5h	250	250	250	250	125	125	250
5i	250	250	250	250	125	125	250
5j	250	250	250	250	125	125	250
5k	250	125	250	250	125	125	250
5l	125	125	250	250	125	125	250
5m	125	125	250	250	125	125	125
5n	250	250	250	250	**31.24**	**31.24**	**31.24**
5o	125	125	250	250	125	125	250
Fluconazole	NT	NT	NT	NT	31.24	15.62	31.24
Streptomycin	0.06	0.24	0.12	0.12	NT	NT	NT

NT = not tested

Molecular docking study

The results of the molecular docking study expressed as variation of Gibbs free energy (ΔG) and the consequent predicted inhibition constants are presented in Table III. Also, a clustering analysis was performed, taking into account the top binding conformation of each compound into the catalytic site of lanosterol 14α-demethylase.

Overall, analysing the results obtained for compounds **5a-o**, an obvious difference between then can be observed. Thiazolyl-salicylamide derivatives **5h-o,** possessing an aromatic substituent on the etheric moiety, had a greater affinity for the enzyme, compared to **5a-g** having a non-aromatic substituent. Between the molecules bearing an aromatic substituent, the best binding to target lanosterol 14α-demethylase was predicted for **5k** and **5n** with ΔG values of -10.99 and -10.95 kcal/mol, respectively.

Thus, these two compounds are predicted to have the lowest inhibition constants from the present series: -8.79 kcal/mol and -9.41 kcal/mol, respectively. From the aromatic derivatives, the most homogenous binding was found for compound **5n**, with the highest number of conformations in the top binding cluster of the top binding conformation. This idea can be confirmed by the low number (9 conformations) of un-clustered residual conformations. The predicted binding conformation of compound **5n** into the catalytic site of fungal lanosterol 14α-demethylase is depicted in Figure 2.

Two important amino acid residues from the polar area of access channel to the active site are predicted to interact with the **5n** ligand. Tyr132 is predicted to create a hydrogen bond with the ligand through the oxygen atom from the ketone, while Tyr118 is predicted to interact with the salicylamide fragment of the ligand. Those polar contacts are considered to be important for a good interaction with the macromolecule. The lipophilic side of the thiazole-salicylamide moiety is oriented into a lipophilic pocket from the access channel, created by the Leu376, Val509, Met508, Ile304, Pro230 and Phe233 sidechains.

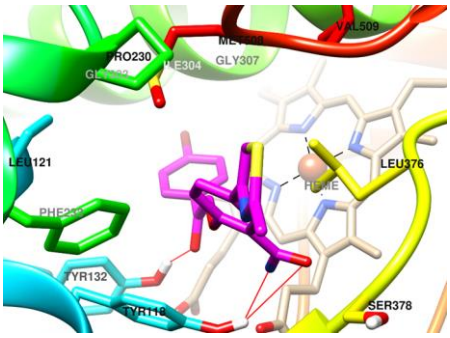


Figure 2.

Compound **5n** docked into the catalytic site of fungal lanosterol 14α-demethylase. Carbon atoms of the ligand in depicted in magenta, while the hydrogen bonds with red lines. Unnecessary amino acid residues were removed to ensure a better view.

The *p*-bromoacetophenone fragment of the **5n** ligand is oriented into the depth of the catalytic site. The bromine atom fits well into a large lipophilic sub-pocket found between the heme side and two alpha helices (Asn136-Ala149 sequence, respectively Asp294-Gly324 sequence). The oxymethylene bridge acts like a hinge, giving the molecule flexibility to ensure a better interaction with the biological target.

Lipophilicity evaluation

Lipophilicity results evaluations of compounds 5a-o, expressed as logP, are presented in Table III. The most lipophilic compounds from our series were **5e**, **5n**, **5f** and **5k** with logP values ranging between 3.05 - 2.79.

Table III

Results of the lipophilicity and the molecular docking studies for compounds **5a-o**

Compound	LogP	The cluster with the top binding conformation			Multi membered clusters	Residual conformations
		Best binding conformation		NoC		
		ΔG (kcal/mol)	Ki (nM)			
5a	2.71	-8.19	992.31	67	18	16
5b	2.04	-8.15	1061.61	22	21	10
5c	2.76	-7.73	2156.90	7	14	**6**
5d	1.08	-7.98	1414.42	22	13	11
5e	**3.05**	-9.05	232.41	86	20	**8**
5f	**2.88**	-8.21	959.37	73	15	13
5g	1.38	-8.75	385.62	17	19	10
5h	1.84	-10.26	30.15	18	30	22
5i	2.52	-10.67	15.09	25	23	11
5j	2.47	-10.69	14.59	13	28	15
5k	**2.79**	**-10.99**	**8.79**	15	23	12
5l	2.58	-10.42	23.02	15	30	15
5m	2.67	-10.55	18.48	27	25	**9**
5n	**2.95**	**-10.95**	**9.41**	**39**	26	**9**
5o	2.00	-10.58	17.57	23	28	17

NoC represents "number of conformations"

Conclusions

In order to develop new antimicrobial agents, in the present study we report the synthesis of 15 novel 4-thiazolyl-salicylamide derivatives. The compounds were physico-chemically and spectrally characterized. With the exception of one molecule, most derivatives exhibited low antibacterial and antifungal activities. The *para*-bromine substituted aromatic compound **5n** exhibited good antifungal activity against *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 and *C. zeylanoides* ATCC 201082, comparable or equal to that of fluconazole. A molecular docking study revealed the structural features which influence the interaction with lanosterol 14α-demethylase. The most active compound, **5n**, was highly lipophilic and had good interactions with the target enzyme, knowing that these parameters are one of the most important for the antifungal effect.

Acknowledgement

The work reported in this paper was financially supported by 1526/39/18.01.2019 PCD grant of the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania and PN 19 35 02 01 grant, through the Core Program (Program Nucleu).

Conflict of interest

The authors declare no conflict of interest.

References

1. Bikobo DSN, Vodnar DC, Stana A, Tiperciuc B, Nastasă C, Douchet M, Oniga O, Synthesis of 2-phenylamino-thiazole derivatives as antimicrobial agents. *J Saudi Chem Soc.*, 2017; 21(7): 861-868.
2. Blejan IE, Diaconu CC, Arsene AL, Udeanu DI, Ghica M, Drăgănescu D, Burcea Dragomiroiu GTA, Rădulescu M, Maltezou HC, Tsatsakis AM, Papasavva M, Drakoulis N, Popa DE, Antibiotic resistance in community-acquired pneumonia. A Romanian perspective. *Farmacia*, 2020; 68(3): 512-520.
3. Breijyeh Z, Jubeh B, Karaman R, Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. *Molecules*, 2020; 25(6): 1340: 1-23.
4. Cebrián R, Rodríguez-Cabezas ME, Martín-Escolano R, Rubiño S, Garrido-Barros M, Montalbán-López M, Rosales MJ, Sánchez-Moreno M, Valdivia E, Martínez-Bueno M, Marín C, Gálvez J, Maqueda M, Preclinical studies of toxicity and safety of the AS-48 bacteriocin. *J Adv Res.*, 2019; 20: 129-139.
5. Chatterjee A, Modarai M, Naylor NR, Boyd SE, Atun R, Barlow J, Holmes AH, Johnson A, Robotham JV, Quantifying drivers of antibiotic resistance in humans: a systematic review. *Lancet Infect Dis.*, 2018; 18(12): e368-e378.
6. Daina A, Michielin O, Zoete V, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.*, 2017; 7: 42717: 1-13.
7. Grossman S, Soukarieh F, Richardson W, Liu R, Mashabi A, Emsley J, Williams P, Cámara M, Stocks MJ, Novel quinazolinone inhibitors of the *Pseudomonas aeruginosa* quorum sensing transcriptional regulator PqsR. *Eur J Med Chem.*, 2020; 208: 112778: 1-15.
8. Hannoun MH, Hagras M, Kotb A, El-Attar A-AMM, Abulkhair HS, Synthesis and antibacterial evaluation of a novel library of 2-(thiazol-5-yl)-1,3,4-oxadiazole derivatives against methicillin-resistant *Staphylococcus aureus* (MRSA). *Bioorg Chem.*, 2020; 94: 103364: 1-10.
9. Manciuc C, Mihai IF, Filip-Ciubotaru F, Lacatusu GA, Resistance profile of multidrug-resistant urinary tract infections and their susceptibility to carbapenems. *Farmacia*, 2020; 68(4): 715-721.

10. Marc G, Stana A, Pîrnău A, Vlase L, Vodnar DC, Duma M, Tiperciuc B, Oniga O, 3,5-Disubstituted Thiazolidine-2,4-Diones: Design, Microwave-Assisted Synthesis, Antifungal Activity, and ADMET Screening. *SLAS Discov.*, 2018; 23(8): 807-814.
11. Mir F, Shafi S, Zaman MS, Kalia NP, Rajput VS, Mulakayala C, Mulakayala N, Khan IA, Alam MS, Sulfur rich 2-mercaptobenzothiazole and 1,2,3-triazole conjugates as novel antitubercular agents. *Eur J Med Chem.*, 2014; 76: 274-283.
12. Molnar A, Antimicrobial Resistance Awareness and Games. *Trends Microbiol.*, 2019; 27(1): 1-3.
13. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ, AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J Comput Chem.*, 2009; 30(16): 2785-2791.
14. Osman H, Yusufzai SK, Khan MS, Abd Razik BM, Sulaiman O, Mohamad S, Gansau JA, Ezzat MO, Parumasivam T, Hassan MZ, New thiazolyl-coumarin hybrids: Design, synthesis, characterization, X-ray crystal structure, antibacterial and antiviral evaluation. *J Mol Struct.*, 2018; 1166: 147-154.
15. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE, UCSF Chimera—A visualization system for exploratory research and analysis. *J Comput Chem.*, 2004; 25(13): 1605-1612.
16. Stoica CI, Ionuț I, Vlase L, Tiperciuc B, Marc G, Oniga S, Araniciu C, Oniga O, Lipophilicity evaluation of some thiazolyl-1,3,4-oxadiazole derivatives with antifungal activity. *Biomed Chromatogr.*, 2018; 32(7): e4221: 1-7.
17. Tacconelli E, Sifakis F, Harbarth S, Schrijver R, van Mourik M, Voss A, Sharland M, Rajendran NB, Rodríguez-Baño J, Bielicki J, de Kraker M, Gandra S, Gastmeier P, Gilchrist K, Gikas A, Gladstone BP, Goossens H, Jafri H, Kahlmeter G, Leus F, Luxemburger C, Malhotra-Kumar S, Marasca G, McCarthy M, Navarro MD, Nuñez-Nuñez M, Oualim A, Price J, Robert J, Sommer H, von Cube M, Vuong C, Wiegand I, Witschi AT, Wolkewitz M, Surveillance for control of antimicrobial resistance. *Lancet Infect Dis.*, 2018; 18(3): e99-e106.
18. Wang WL, Chai SC, Huang M, He HZ, Hurley TD, Ye QZ, Discovery of Inhibitors of Escherichia coli Methionine Aminopeptidase with the Fe(II)-Form Selectivity and Antibacterial Activity. *J Med Chem.*, 2008; 51(19): 6110-6120.