ORIGINAL ARTICLE

SYNTHESIS AND ANTIMICROBIAL ACTIVITY EVALUATION OF SOME NEW 2-(3-PYRIDIL)-THIAZOLYL-1,3,4-OXADIAZOLINES

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Abstract

A new series of 4-methyl-2-(pyridin-3-yl)-thiazole-5-yl-oxadiazolines were synthesized starting from 4-methyl-2-(pyridin-3-yl)thiazole-5-carbohydrazide. The newly synthesized compounds were characterized by analytical ^{1}H-NMR, ^{13}C-NMR and mass spectral data. These compounds were screened for their antimicrobial activity against Gram-positive and Gram-negative bacterial strains and one fungal strain (*Candida albicans*).

Rezumat

A fost sintetizată o nouă serie de derivaţi cu structură 4-metil-2-(piridin-3-il)-tiazol-5-il-oxadiazolinică utilizând ca materie primă 4-metil-2-(piridin-3-il) tiazol-5carbohidrazida. Compuşii nou sintetizaţi au fost caracterizaţi prin analize ^{1}H-RMN, ^{13}C-RMN şi spectrometrie de masă. Aceşti compuşi au fost testaţi în vederea stabilirii activităţii antimicrobiene, pe tulpini bacteriene Gram-pozitive şi Gram-negative şi pe o tulpină levurică (*Candida albicans*).

Keywords: thiazole, oxadiazoline, antimicrobial activity

Introduction

The treatment of infectious diseases remains an important issue due to a combinations of factors, including the rapid rise in microbial resistance to the current antibiotics [9, 13]. The 1,3-thiazole heterocycle is an interesting building block for a variety of natural and synthetic compounds found to exhibit a good antimicrobial potential [3, 7, 8]. On the other hand, in recent literature, organic compounds bearing the oxadiazoline nucleus were found to possess antibacterial and antifungal activity [4, 6, 12].

Prompted by the acknowledged biological activity of the above mentioned compounds and as a part of our continuing research on the synthesis of new molecules with biological activity [5, 10, 11], the purpose of the present work was to synthesize and evaluate the antimicrobial activity of some novel 2-(3-pyridine)-thiazole derivatives substituted with an oxadiazoline ring.

Materials and Methods

Chemical protocols

Melting points were determined using open capillary tube method and are uncorrected.

The purity of the synthesized compounds was verified by thin layer chromatography (TLC) and was carried out on pre-coated Silica Gel $60F_{254}$ sheets using heptan – ethyl-acetate 7:3 as mobile phase (v/v) and UV absorption for visualization.

The ^{1}H-NMR spectra were recorded at room temperature on a Bruker Avance NMR spectrometer operating at 500 MHz. Chemical shift values were reported relative to tetramethylsilane (TMS) as internal standard. The samples were prepared by dissolving the synthesized powder of the compounds in DMSO *d6* (δH = 2.51 ppm, δC 39.98 pm) as

solvent and the spectra were recorded using a single excitation pulse of 12 μs. ^{13}C-NMR spectra were recorded on a Bruker Avance NMR spectrometer (Karlsruhe, Germany), operating at 125 MHz, in DMSO-d6, using a waltz 16 decoupling scheme. GC-MS analyses were performed with an Agilent gas chromatograph 6890 equipped with an apolar Macherey Nagel Permabond SE 52 capillary column. Elemental analysis was registered with a Vario El CHNS instrument. All new compounds yielded spectral data consistent with the proposed structure and microanalysis within 0.4% of the theoretical values.

*Synthesis of the arylmethyliden-hydrazides **2 a-g** (general procedure)*

Equimolar quantities of 4-methyl-2-(pyridin-3-yl)-thiazole-5-carbohydrazide and different aromatic or heteroaromatic aldehydes were refluxed in absolute ethanol for 3 h. The solid product, formed after cooling, was filtered and dried. The crude solid was re-crystallised from ethanol.

N'-(2-methoxybenzylidene)-4-methyl-2-(pyridin-3-yl)-thiazole-5-carbohydrazide **2a (**$C_{18}H_{16}N_4O_2S$): 80% yield. M.p. 250 - 253ºC; MS m/z: 353 (M = 1), ^{1}H-NMR (DMSO-d6) δ (ppm): 11.80 (s, 1H, NH), 9.2 (s,1H, pyridyl, C_2), 8.74 (d 1H, pyridyl C_6), 8.37 (d 1H, pyridyl, C_4), 8.1 (s, 1H, -N=CH-Ar), 7.58 (q 1H, pyridyl C_5), 7.4 (t, 1H, phenyl C_4), 7.35 (d 1H, phenyl C_3), 7.15 (d 1H, phenyl C_6), 7 (t 1H, phenyl C_5), 3.8 (s 3H, OCH_3 phenyl C_2), 2.5 (s 3H, CH_3, thiazole C_4); ^{13}C-NMR (DMSO-d6) δ (ppm): 165.66 (amide C=O), 161.87 (thiazole C_2), 160.31 (thiazole C_4), 159.04 (phenyl C_2), 148.87 (pyridyl C_2), 146.86 (pyridyl C_4), 146.49 (pyridyl C_3), 145.06 (CH=N), 134.37 (pyridyl C_6), 129.6 (phenyl C_5), 126.16 (pyridyl C_5), 125.89 (phenyl C_6), 123.64 (phenyl C_1), 121.22 (phenyl C_4), 120.45 (phenyl C_3), 119.2 (thiazole C_5), 56.92 (OCH_3), 19.27 (CH_3 thiazole).

N'-(2,4-dichlorobenzylidene)-4-methyl-2-(pyridin-3-yl)thiazole-5-carbohydrazide **2b (**$C_{17}H_{12}Cl_2N_4OS$): 75% yield. M.p. 289 - 291ºC; MS m/z: 393 (m+2), ^{1}H-NMR (DMSO-d6) δ (ppm): 11.78 (s, 1H, NH), 9.22 (s, 1H, pyridyl, C_2), 8.77 (d 1H, pyridyl C_6), 8.42 (d 1H, pyridyl, C_4), 8.15 (s, 1H, -N=CH-Ar), 7.6 (q 1H, pyridyl C_5), 7.79 (d 1H, phenyl C_6), 7.56 (s 1H, phenyl C_3), 7.54 (d 1H, phenyl C_5), 2.7 (s 3H, CH_3, thiazole C_4); ^{13}C-NMR (DMSO-d6) δ (ppm): 166.66 (amide C=O), 161.87 (thiazole C_2), 160.31 (thiazole C_4), 148.37 (pyridyl C_2), 147.64 (pyridyl C_4), 147.13 (pyridyl C_3), 134.37 (pyridyl C_6), 148.06 (CH=N), 134.6 (phenyl C_2), 132.89 (phenyl C_4), 128.79 (pyridyl C_5), 128.64 (phenyl C_1), 127.22 (phenyl C_6), 125.45 (phenyl C_3), 123.12 (phenyl C_5),), 119.2 (thiazole C_5), 19.27 (CH_3 thiazole).

N'-(2,6-dichlorobenzylidene)-4-methyl-2-(pyridin-3-yl)thiazole-5-carbohydrazide **2c (**$C_{17}H_{12}C_{l2}N_4OS$): 75% yield. M.p. 264 - 267ºC; MS m/z: 393 (m+2), ^{1}H-NMR (DMSO-d6) δ (ppm): 11.72 (s, 1H, NH), 9.17 (s 1H, pyridyl, C_2,), 8.74 (d 1H, pyridyl, C_6), 8.4 (d 1H pyridyl, C_4), 8.05 (s, 1H, -N=CH-Ar), 7.58 (q 1H, pyridyl C_5), 7.5 (q 2H, phenyl C_3, C_5), 7.3 (t 1H, phenyl C_4), 2.7 (s 3H, CH_3, thiazole C_4); ^{13}C-NMR (DMSO-d6) δ(ppm): 165.99 (amide C=O), 162.67 (thiazole C_2), 161.31 (thiazole C_4), 148.91 (pyridyl C_2), 147.8 (CH=N), 147.36 (pyridyl C_4), 147.20 (pyridyl C_3), 134.37 (pyridyl C_6), 134.3 (phenyl C_2 and C_6), 132.89 (phenyl C_1), 127.96 (pyridyl C_5), 127.22 (phenyl C_4), 125.45 (phenyl C_3 and C_5), 120.2 (thiazole C_5), 20.27 (CH_3 thiazole).

N'-(4-nitrobenzylidene) 4-methyl-2-(pyridin-3-yl)-thiazole-5-carbohydrazide **2d (**$C_{17}H_{13}N_5O_3S$): 78% yield. M.p. 327 - 330ºC MS m/z: 368 (m+1), ^{1}H-NMR (DMSO-d6) δ (ppm): 11.68 (s, 1H, NH), 9.2 (s 1H, pyridyl, C_2), 8.73 (d 1H, pyridyl, C_6), 8.6 (d 1H pyridyl, C_4), 8.35 (q 2H, phenyl, C_3, C_5), 8.06 (s, 1H, -N=CH-Ar), 7.55 (q 1H, pyridyl, C_5,), 7.5 (q 2H, phenyl C_2, C_6), 2.7 (s 3H, CH_3, thiazole C_4); ^{13}C-NMR (DMSO-d6) δ (ppm): 166.02 (amide C=O), 161.27 (thiazole C_2), 160.39 (thiazole C_4), 148.91 (pyridyl C_2), 147.66 (pyridyl C_4), 147.49 (pyridyl C_3), 147.7 (CH=N), 147.2 (phenyl C_4) 140.88 (phenyl C_1), 134.43 (pyridyl C_6), 128.8 (phenyl C_2 and C_6), 128.46 (pyridyl C_5), 124.85 (phenyl C_3 and C_5), 124.55 (thiazole C_5), 20.27 (CH_3 thiazole).

N'-(2-hydroxybenzylidene)-4-methyl-2-(pyridin-3-yl)-thiazole-5-carbohydrazide **2e (**$C_{17}H_{14}N_4O_2S$): 85% yield. M.p. 248 - 250ºC MS m/z: 313.4 (m+1), ^{1}H-NMR (DMSO-d6) δ (ppm): 11.8 (s, 1H, NH), 10.3 (s 1H OH, phenyl C_2), 9.22 (s 1H, pyridyl, C_2), 8.73 (d 1H, pyridyl, C_6), 8.5 (d 1H pyridyl, C_4), 8.1 (s, 1H, -N=CH-Ar),7.6 (q 1H, pyridyl, C_5,), 7.55 (t 1H, phenyl, C_4), 7.3 (t 1H, phenyl C_5), 7.25 (d 1H, phenyl C_3), 2.7 (s 3H, CH_3, thiazole C_4); ^{13}C-NMR (DMSO-d6) δ (ppm): 169.06 (amide C=O), 166.31 (thiazole C_2), 165.04 (thiazole C_4), 157.87 (phenyl C_2), 149.51 (pyridyl C_2), 148.66 (pyridyl C_4), 147.49 (pyridyl C_3), 146.86 (CH=N), 133.43 (pyridyl C_6), 131.8 (phenyl C4), 130.8 (phenyl C_6), 127.26 (pyridyl C_5), 128.04 (phenyl C_1), 126.55 (phenyl C_5), 120.4 (phenyl C_3), 120.18 (thiazole C_5), 17.87 (CH_3 thiazole).

N'-(4-hydroxybenzylidene)-4-methyl-2-(pyridin-3-yl)-thiazole-5-carbohydrazide **2f** (**$C_{17}H_{14}N_4O_2S$):** 80% yield. M.p. 300 - 305ºC MS m/z: 313.4 (m+1), ^{1}H-NMR (DMSO-d6) δ (ppm): 11.74 (s, 1H, NH), 10 (s 1H OH, phenyl C_4), 9.17 (s 1H, pyridyl, C_2), 8.75 (d 1H, pyridyl, C_6), 8.64 (d 1H pyridyl, C_4), 8 (s, 1H, -N=CH-Ar), 7,63 (q 2H, phenyl, C_2, C_6), 7.57 (q 1H, pyridyl, C_5,), 6,91 (q 2H, phenyl, C_3, C_5), 2,79 (s 3H, CH_3, thiazole C_4); ^{13}C-NMR (DMSO-d6) δ (ppm): 167,66 (amide C=O), 161,87 (thiazole C_2), 161,84 (thiazole C_4), 159,96 (phenyl C_4), 149.91 (pyridyl C_2), 148.86 (pyridyl C_4), 147.49 (pyridyl C_3), 145,4 (CH=N), 134.43 (pyridyl C_6), 129,69 (phenyl C_2 and C_6), 126.86 (pyridyl C_5), 125,31 (phenyl C_1), 120.64 (phenyl C_3 and C_5), 120,46 (thiazole C_5), 19,27 (CH_3 thiazole).

4-methyl-N'-((2-phenylthiazol-4-yl)methylene)-2-(pyridin-3-yl)thiazole-5-carbohydrazide **2g** (**$C_{20}H_{15}N_5OS_2$**): 65% yield. M.p. 220 - 224ºC; MS m/z: 406 (m+1), ^{1}H-NMR (DMSO-d6) δ (ppm): 11.4 (s, 1H, NH), 9.22 (s 1H, pyridyl, C_2), 8.72 (d 1H, pyridyl, C_6), 8.68 (d 1H pyridyl, C_4), 8.1 (s, 1H, -N=CH-Ar), 7.92 (q, phenyl C_2, C_6), 7.58 (q 1H, pyridyl, C_5,), 7.54 (s 1H, thiazole C_5), 7.45 (q 2H, phenyl C_3, C_5), 7.3 (t 1H, phenyl C_4), 2.76 (s 3H, CH_3, thiazole C_4); ^{13}C-NMR (DMSO-d6) δ (ppm): 167.74 (amide C=O), 166.32 (pyridyl-thiazole C_2), 158.5, (phenyl-thiazole C_2), 152.96 (pyridyl-thiazole C_4), 152.1 (phenyl-thiazole C_4), 148.91 (pyridyl C_2), 148.66 (pyridyl C_4), 147.49 (pyridyl C_3), 146.2 (CH=N), 134.34 (phenyl C_2 and C_6), 133.43 (pyridyl C_6) 132.12 (phenyl C_1), 131.8 (phenyl-thiazole C_5), 128.62 (phenyl C_4), 126.86 (pyridyl C_5), 126.45 (phenyl C_3 and C_5), 120.81 (thiazole C_5), 17.45 (CH_3 thiazole).

*Synthesis of 4-methyl-2-(pyridin-3-yl)thiazol-5-yl)-1,3,4-oxadiazolines **3a-g** (general procedure)*

0.5 mmol of arylmethyliden-hydrazides **2a-g** were refluxed with acetic anhydride (5 mL) for 3 h. After cooling, the reaction mixture was poured into ice cold water. The obtained precipitate was filtered off, washed with water and re-crystallised in ethanol to give the products.

1-(2-(2-methoxyphenyl)-5-(4-methyl-2-(pyridin-3-yl)thiazol-5-yl)-1,3,4oxadiazol-3(2H)-yl)ethanone **3a** ($C_{20}H_{18}N_4O_3S$): 75% yield. M.p. 175 - 178ºC; MS m/z: 395 (m+1), ^{1}H-NMR (DMSO-d6) δ (ppm): 9.2 (s 1H, pyridyl, C_2), 8.7 (d 1H, pyridyl, C_6), 8.55 (d 1H, pyridyl, C_4), 7.5 (q 1H, pyridyl, C_5), 7.46 (t, 1H, phenyl C_4), 7.35 (d 1H, phenyl C_3), 7.27 (s 1H, oxadiazoline C_2) 7.15 (d 1H, phenyl C_6), 7.02 (t 1H, phenyl C_5), 3.82 (s 3H, OCH_3 phenyl C_2), 2.7 (s 3H, CH_3, thiazole C_4), 2.2 (s 3H, N-$COCH_3$); ^{13}C-NMR (DMSO-d6) δ (ppm): 166.99 (acetyl C=O), 165.04 (thiazole C_2), 158.15 (oxadiazoline C_5), 157.51 (thiazole C_4), 148.91 (pyridyl C_2), 147.66 (pyridyl C_4), 147.49 (pyridyl C_3), 134.43 (pyridyl C_6), 132.16 (phenyl C_5), 128.89 (phenyl C_6), 128.46 (pyridyl C_5), 124.84 (thiazole C_5), 123.64 (phenyl C_1), 120.92 (phenyl C_4), 117.5 (phenyl C_3), 90.25 (oxadiazoline C_2), 56.32 (OCH_3), 21.6 (CH_3-acetyl), 17.69 (CH_3 thiazole).

1-(2-(2,4-dichlorophenyl)-5-(4-methyl-2-(pyridin-3-yl)thiazol-5-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone **3b** ($C_{19}H_{14}Cl_2N_4O_2S$)**:** 70% yield. M.p. 160 - 165ºC; MS m/z: 434 (m+2), ^{1}H-NMR (DMSO-d6) δ (ppm): 9.19 (s 1H, pyridyl, C_2), 8.72 (d 1H, pyridyl, C_6), 8.6 (d 1H, pyridyl, C_4), 7.79 (d 1H, phenyl C_6), 7.6 ppm (s 1H, phenyl C_3), 7.58 ppm (d 1H, phenyl C_5), 7.56 (q 1H, pyridyl, C_5), 7.34 (s 1H, oxadi-azoline C_2) 2.67 (s 3H, CH_3, thiazole C_4), 2.26 ppm (s 3H, N-$COCH_3$); ^{13}C-NMR (DMSO-d6) δ (ppm): 167.56 (acetyl C=O), 165.42 (thiazole C_2), 157.55 (oxadiazoline C_5), 150.16 (thiazole C_4), 148.91 (pyridyl C_2), 147.66 (pyridyl C_4), 147.49 (pyridyl C_3), 134.43 (pyridyl C_6), 136.24 (phenyl C_4), 133.74 (phenyl C_2), 131.98 (phenyl C_3), 131.15 (phenyl C_6), 130.35 (phenyl C_5), 128.63 (phenyl C_1), 128.46 (pyridyl C_5), 117.03 (thiazole C_5), 90.55 (oxadiazoline C_2), 21.59 (CH_3-acetyl), 17.75 (CH_3 thiazole).

1-(2-(2,6-dichlorophenyl)-5-(4-methyl-2-(pyridin-3-yl)thiazol-5-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone **3c** ($C_{19}H_{14}Cl_2N_4O_2S$): 70% yield. M.p. 172 - 175ºC; MS m/z: 434 (m+2), ^{1}H-NMR (DMSO-d6) δ (ppm): 9.22 (s 1H, pyridyl, C_2), 8.75 (d 1H, pyridyl, C_6), 8.35 (d 1H, pyridyl, C_4), 7.6 (q 1H, pyridyl, C_5), 7.58 (s 1H, oxadiazoline C_2), 7.45 (q 2H, phenyl C_3, C_5), 7.3 (t 1H, phenyl C_4), 2.73 (s 3H, CH_3, thiazole C_4). 2.21 (s 3H, N-$COCH_3$; ^{13}C-NMR (DMSO-d6) δ (ppm): 169.16 (acetyl C=O), 166.32 (thiazole C_2), 157.85 (oxadiazoline C_5), 151.96 (thiazole C_4), 148.91 (pyridyl C_2), 147.66 (pyridyl C_4), 147.49 (pyridyl C_3), 134.43 (pyridyl C_6), 134.34 (phenyl C_2 and C_6), 132.12 (phenyl C_1), 128.92 (phenyl C_4), 128.46 (pyridyl C_5), 126.45 (phenyl C_3 and C_5), 121.81 (thiazole C_5), 90.85 (oxadiazoline C_2), 22.29 (CH_3-acetyl), 17.85 (CH_3 thiazole).

1-(5-(4-methyl-2-(pyridin-3-yl)thiazol-5-yl)-2-(4-nitrophenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone **3d** ($C_{19}H_{15}N_5O_4S$**)** 70% yield. M.p. 142 - 148ºC; MS m/z: 410.6 (m+1), ^{1}H-NMR (DMSO-d6) δ (ppm): 9.17 (s 1H, pyridyl, C_2), 8.73 (d 1H, pyridyl, C_6), 8.37 (d 1H, pyridyl, C_4), 8.32 (q 2H, phenyl C_2, C_6), 7.84 (q 2H, phenyl C_3, C_5), 7.58 (q 1H, pyridyl, C_5), 7.37 (s 1H, oxadiazoline C_2) 2.72 (s 3H, CH_3, thiazole C_4), 2.26 (s 3H, N-$COCH_3$); ^{13}C-NMR (DMSO-d6) δ (ppm): 167.6 (acetyl C=O), 165.02 (thiazole C_2), 157.75 (oxadiazoline C_5), 157.51 (thiazole C_4), 152.3 (phenyl C_1), 152.13 (phenyl C_4), 148.91 (pyridyl C_2), 147.66 (pyridyl C_4), 147.49 (pyridyl C_3), 134.43 (pyridyl C_6), 128,82 (phenyl C_3 and C_5), 124.58 (phenyl C_2 and C_6), 128.46 (pyridyl C_5), 124.84 (thiazole C_5), 91.24 (oxadiazoline C_2), 21.62 (CH_3-acetyl), 17.81 (CH_3 thiazole).

2-(3-acetyl-5-(4-methyl-2-(pyridin-3-yl)thiazol-5-yl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)phenyl acetate **3e** ($C_{21}H_{18}N_4O_4S$*)*: 65% yield. M.p. 189 - 190ºC, MS m/z: 423.6 (m+1), ^{1}H-NMR (DMSO-d6) δ (ppm): 9.2 (s 1H, pyridyl, C_2), 8.75 (d 1H, pyridyl, C_6), 8.4 (d 1H, pyridyl, C_4), 7.58 (d 1H phenyl C_6) 7.55 (t 1H, phenyl, C_4), 7.5 (q 1H, pyridyl, C_5), 7.36 (t 1H, phenyl C_5), 7.25 (d 1H, phenyl C_3), 7.22 (s 1H, oxadiazoline C_5), 2.7 (s 3H, CH_3, thiazole C_4), 2.2 (s 3H, N-$COCH_3$), 1.9 (s 3H, $OCOCH_3$, phenyl C_2); ^{13}C-NMR (DMSO-d6) δ (ppm): 172.45 (O-C=O), 169.06 (acetyl C=O), 166.9 (thiazole C_2), 161.64 (oxadiazoline C_5), 157.37 (thiazole C_4), 149.4 (phenyl C_2), 148.41 (pyridyl C_2), 146.46 (pyridyl C_4), 145.49 (pyridyl C_3), 134.43 (pyridyl C_6), 131.8 (phenyl C_4), 130.31 (phenyl C_5), 128.04 (phenyl C_6), 126.96 (pyridyl C_5), 126.55 (phenyl C_1), 124.54 (phenyl C_3), 122.18

(thiazole C_5), 90.93 (oxadiazoline C_2), 21.51 (CH_3-N-acetyl), 21.11 (CH_3-O-acetyl) 17.7 (CH_3 thiazole).

4-(3-acetyl-5-(4-methyl-2-(pyridin-3-yl)thiazol-5-yl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)phenyl acetate **3f** ($C_{21}H_{18}N_4O_4S$): 72% yield. M.p. 230 - 232ºC; MS m/z: 423.4 (m+1), ^{1}H-NMR (DMSO-d6) δ (ppm): 9.22 (s 1H, pyridyl, C_2), 8.75 (d 1H, pyridyl, C_6), 8.55 (d 1H, pyridyl, C_4), 7.6 (q 1H, pyridyl, C_5), 7.57 (q 2H, phenyl, C_2, C_6), 7.24 (q 2H, phenyl, C_3, C_5), 7.22 (s 1H, oxadiazoline C_5) 2.73 (s 3H, CH_3, thiazole C_4), 2.27 (s 3H, N-$COCH_3$), 2.1 (s 3H, $OCOCH_3$, phenyl C_4); ^{13}C-NMR (DMSO-d6) δ (ppm): 171.95 (O-C=O), 169.46 (acetyl C=O), 165.92 (thiazole C_2), 161.68 (oxadiazoline C_5), 159.37 (thiazole C_4), 147.91 (pyridyl C_2), 147.66 (pyridyl C_4), 146.89 (pyridyl C_3), 133.43 (pyridyl C_6), 145.08 (phenyl C_4), 129.69 (phenyl C_2 and C_6), 125.31 (phenyl C_1), 127.46 (pyridyl C_5), 121.61 (phenyl C_3 and C_5), 118.46 (thiazole C_5), 90.43 (oxadiazoline C_2), 22.51 (CH_3-N-acetyl), 21.61 (CH_3-O-acetyl) 17.72 (CH_3 thiazole).

1-(5-(4-methyl-2-(pyridin-3-yl)thiazol-5-yl)-2-(2-phenylthiazol-4-yl)-1,3,4-oxadiazol-3(2H)-yl)-ethanone **3g** ($C_{22}H_{17}N_5O_2S_2$): 75% yield. M.p. 140 - 143°C; MS m/z: 448 (m+1), ^{1}H-NMR (DMSO-d6) δ (ppm): 9.10 (s 1H, pyridyl, C_2), 8.72 (d 1H, pyridyl, C_6), 8.65 (d 1H, pyridyl, C_4), 7.92 (q, phenyl C_2, C_6), 7.6 (q 1H, pyridyl, C_5), 7.58 (s 1H, thiazole C_5), 7.45 (q 2H, phenyl C_3, C_5), 7.3 (t 1H, phenyl C_4), 7.2 (s 1H, oxadiazoline C_5), 2.73 (s 3H, CH_3, thiazole C_4). 2.21 (s 3H, N-$COCH_3$); ^{13}C-NMR (DMSO-d6) δ (ppm): 169.82 (acetyl C=O), 167.74 (phenyl-thiazole C_2), 166.32 (pyridyl-thiazole C_2), 157.85 (oxadiazoline C_5), 152.96 (pyridyl-thiazole C_4), 152.1 (phenyl-thiazole C_4), 148.11 (pyridyl C_2), 147.86 (pyridyl C_4), 147.28 (pyridyl C_3), 134.83 (pyridyl C_6), 134.34 (phenyl C_2 and C_6), 132.12 (phenyl C_1), 131.8 (phenyl-thiazole C_5), 128.62 (phenyl C_4), 126.86 (pyridyl C_5), 126.45 (phenyl C_3 and C_5), 119.81 (thiazole C_5), 90.65 (oxadiazoline C_2), 22.49 (CH_3-acetyl), 17.45 (CH_3 thiazole).

The in vitro qualitative screening of the anti-microbial activity

The *in vitro* qualitative screening of the anti-microbial activity was carried out by an adapted agar disk diffusion technique [1], using a microbial suspension of 0.5 McFarland density obtained from 24 h cultures. The antimicrobial activities of the newly synthesized compounds were determined against ATCC reference microbial strains: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6683; "Cantacuzino" Institute reference microbial strain: *Klebsiella pneumoniae* IC 13420; as well as clinical strains, recently isolated from different clinical specimens: *Candida albicans* 393, *Enterococcus faecium* E5.

The compounds were solubilized in dimethylsulfoxide to a concentration of 10 mg/mL. A volume of 5 µL of each tested compounds solution was distributed directly on the solid medium previously seeded with the microbial inoculum. The inoculated plates were incubated for 24 h at 37°C for bacterial strains and 48 h at 28°C for the fungal strain. Antimicrobial activity was assessed by measuring the growth inhibition zones diameters expressed in mm. Ciprofloxacin as an antibacterial agent and fluconazole as an antifungal agent were used as references to evaluate the potency of the tested compounds under the same conditions.

The in vitro quantitative screening of the anti-microbial activity

The quantitative assay of the minimal inhibitory concentration (MIC) was based on liquid medium two-fold micro dilutions method [2] and performed in 96 multi-well plates. For this purpose, serial binary dilutions of the tested compounds were performed in a 200 µL volume of nutrient broth/ YPG and each well was seeded with 20 µL microbial inoculum of 0.5 McFarland density. The plates were incubated for 24 h at 37°C for bacterial strains, respectively for 48 h at 28°C for fungal strains. The MICs were read as the lowest concentration of the tested compound, expressed in µg/mL, which inhibited the visible microbial growth.

Results and Discussion

Chemistry

The synthesis of the target compounds is outlined in schemes 1-2. The synthesis of the intermediate 4-methyl-2-(pyridin-3-yl)-thiazole-5-carbohydrazide **1** was reported in our previous work [11].

The presence of the carbohydrazide group in the thiazole compound **1** allows it to be used as a key intermediate for the synthesis of 1,3,4-oxadiazolines. Thus, condensation of carbohydrazide **1** with aromatic or heteroaromatic aldehydes, in absolute ethanol, afforded the corresponding Schiff bases **2a-g** (Figure 1).

The obtained Schiff bases **2a-g** were cyclised into 1,3,4-oxadiazolines, **3a-g,** by refluxing them with acetic anhydride (Figure 2).

The structures of the newly synthesized compounds were elucidated by the combined use of ^{1}H-NMR, ^{13}C-NMR, mass spectral data and elemental analysis.

The ^{1}H-NMR spectra of compounds **2a-g** showed a singlet signal at δ 8 - 8.2 ppm range corresponding to CH of the benzylidene group and another singlet signal at δ 11.6 - 11.8 ppm range due to the NH proton, hence confirming the formation of Schiff bases hydrazones.

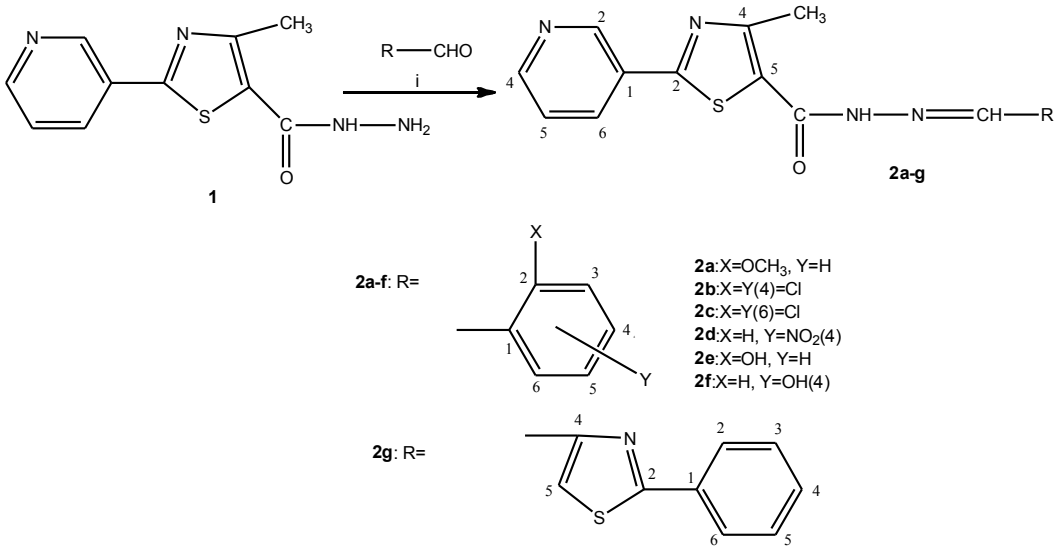

Figure 1.
Reagents and conditions: i: Absolute ethanol reflux 3 h

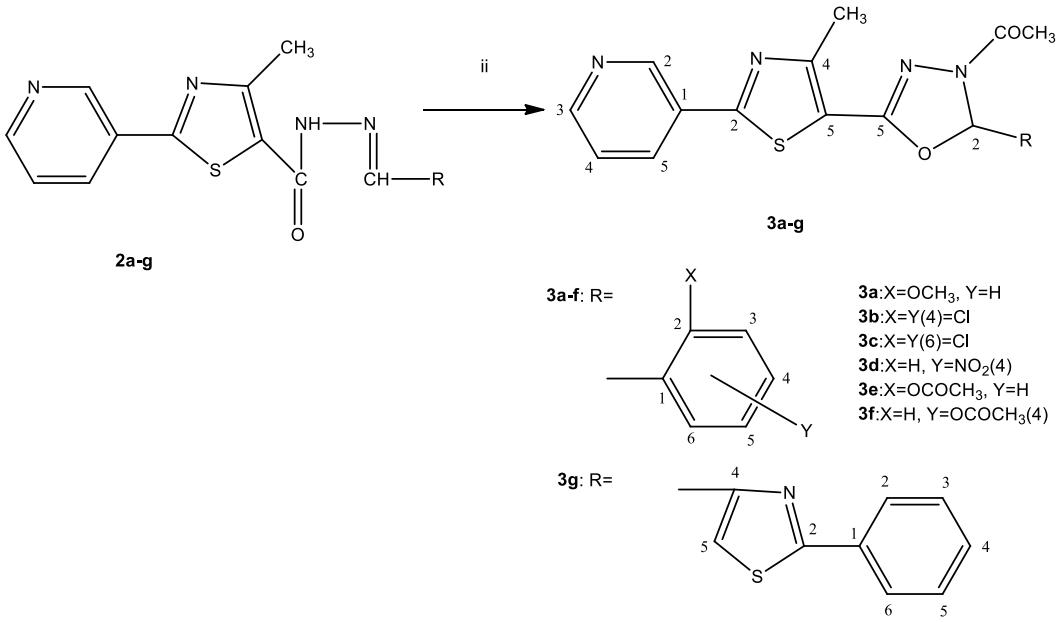

Figure 2.
Reagents and conditions: ii: Ac_2O, reflux, 2 h

The ^1H-NMR spectra of compounds **3a-g** revealed the disappearance of azomethine and hydrazide protons and the occurrence of some new singlet signals at 7.2 - 7.6 ppm (1H) indicating CH resonance of the oxadiazoline ring and 2.2 - 2.3 ppm (3H), which were attributed to the acetyl group protons (N-$COCH_3$- in the 4 position of the oxadiazoline ring). Thus, the ^1H-NMR spectra confirmed the cyclisation of the arylmethyliden-hydrazides **2a-g** into the corresponding 1,3,4-oxadiazolines **3a-g**.

Biological evaluation

The antimicrobial activity of the synthesized compounds was assessed on seven microbial strains: three Gram-positive bacterial strains (*E. faecium*, *S. aureus*, *B. subtillis*), three Gram-negative bacterial strains (*P. aeruginosa*, *K. pneumoniae*, *E. coli*) and one fungal strain (*C. albicans*), using the qualitative agar-diffusion method and the quantitative broth dilution technique [1, 2].

Qualitative screening assay

The qualitative screening of the susceptibility spectra of the tested compounds revealed a low antimicrobial activity expressed especially against Gram-positive bacteria and fungi. Antimicrobial results (inhibition zone diameters in mm) of the compounds are given in Table I. The DMSO did not induce the occurrence of any growth inhibition diameter.

Table I

Results of the qualitative screening for the newly synthesized compounds (diameter of the growth inhibition zones expressed in mm)

Compound	*B. subtillis*	*E. faecium*	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*E. coli*	*C. albicans*
2a	5	0	5	4	5	0	5
2b	4	0	4	4	4	4	4
2c	4	0	0	4	0	4	4
2d	5	0	0	5	0	0	5
2e	4	0	4	0	4	4	0
2f	6	6	5	5	5	5	6
2g	4	0	4	4	0	0	4
3a	10	6	11	0	0	5	5
3b	12	6	10	6	0	0	10
3c	11	6	10	0	0	0	6
3d	6	8	6	0	0	0	6
3e	6	0	8	0	0	5	6
3f	5	0	11	5	0	5	6
3g	5	5	0	5	5	0	5
DMSO	0	0	0	0	0	0	0
Ciprofloxacin	22	20	20	21	22	24	-
Fluconazole	-	-	-	-	-	-	17

Minimal inhibitory concentration assay (MIC)

For a more accurate biological characterisation of the synthesized compounds, the antimicrobial potential has been further evaluated by determining the minimum inhibition concentration values by the broth dilution method.

The broth dilution technique was performed for the compounds that proved to be the most active on different microbial strains (inducing growth inhibition zone diameters of at least 10 mm) in the qualitative screening assay. The results of the quantitative assay revealed an overall insignificant inhibitory activity on the microbial growth in liquid culture medium, with MIC values > 62 µg/mL. Compound **3b** proved to be the most active against the clinically isolated *C. albicans* fungal strain, exhibiting the lowest MIC value, i.e. 31 µg/mL (Table II).

Table II

Results of the quantitative assay (MIC values expressed in µg/mL)

Compound	Microbial strain			
	B. subtillis	*E. faecium*	*S. aureus*	*C. albicans*
3a	> 250	NT	250	NT
3b	> 250	NT	62	31
3c	> 250	NT	250	NT
3f	NT	NT	250	NT
Ciprofloxacin	1	2	0.5	-
Fluconazole	-	-	-	8

NT – not tested

Conclusions

In the present work, a novel series of 4-methyl-2-(pyridin-3-yl)-thiazole-5-yl-oxadiazolines were synthesized. According to the physicochemical and spectral data obtained by ^{1}H-NMR, ^{13}C-NMR, mass spectrometry and elemental analysis all the compounds presented the proposed structures. All the newly synthesized compounds were screened for their antibacterial activity against *Enterococcus faecium*, *Staphylococcus aureus, Bacillus subtillis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and for their antifungal activity against *Candida albicans*. The antimicrobial activity is low and spectrum comprises particularly the Gram positive bacterial strains.

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