ORIGINAL ARTICLE

SYNTHESIS OF SOME NEW 4-METHYL-2-(4-PYRIDYL)-THIAZOLE-5-YL-AZOLES AS POTENTIAL ANTIMICROBIAL AGENTS

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Abstract

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

Rezumat

A fost sintetizată o nouă serie de derivați 4-metil-2-(piridin-4-il)-tiazol-5-il-azolici, pornind de la 4-metil-2-(piridin-4-il)-tiazolil-5-carbohidrazida. Compușii nou sintetizați au fost caracterizați prin analize ^{1}H RMN, ^{13}C RMN și spectrometrie de masă. Compușii sintetizați au fost testați, în vederea stabilirii activității antimicrobiene, pe tulpini bacteriene Gram-pozitive și Gram-negative și pe o tulpină fungică (*Candida albicans*).

Keywords: thiazolyl-azoles, antimicrobial activity

Introduction

The incidence of bacterial resistance to antibiotic therapy, including here the emergence of multidrug resistant pathogens, reinforces the need for the development of new antimicrobial drugs. A potential approach to overcome the problem of antibiotic resistance is to design innovative agents with different modes of action so that no cross resistance with present drugs can occur [18].
On the other hand, epidemiological studies confirm the significant impact upon human health by infections caused by pathogenic fungi. In fact, although the genus *Candida* is present as commensally flora in the majority of healthy people, it is also responsible for opportunistic infections and it can become pathogenous because of predisposing conditions related to the host (AIDS, transplants) or excessive prophylaxis with antimicrobial agents [11].
In the recent literature, organic compounds bearing thiazoles, pyrazoles, triazoles and oxadiazoles nuclei were found to possess antibacterial and antifungal activities. They have been investigated for their significant antimicrobial activity against a variety of clinically relevant bacterial and fungal strains. In particular, these studies confirmed that thiazole ring is a good pharmacophore for the design of bioactive molecules [1-6, 8-10, 14-16].
Considering the above-mentioned findings and being involved in a research program aiming to find new compounds with biological activity [12-14], the purpose of the present work was to synthesize and investigate the *in vitro* antimicrobial activity of some novel derivatives comprising mainly the thiazole counterpart substituted with various functionalities and attached to different heterocyclic ring systems (oxadiazolines, oxadiazoles, pyrazoles and triazoles). This combination was suggested in an attempt to investigate the influence of such a structure variation on the anticipated biological activity, aiming to add some synergistic biological significance to the target molecules.

Materials and Methods

Solvents and reagents used for synthesis and purification were purchased from Alfa Aesar (Karlsruhe, Germany). All chemicals were of analysis grade.
The purity of the synthesized compounds was verified by thin layer chromatography (TLC) and was carried out on pre-coated Silica Gel 60F254

sheets using heptan – ethyl-acetate 7:3 as developant and UV absorption for visualization.

The ^{1}H NMR and ^{13}C 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= 2.51ppm, δC 39.98 pm) as solvent and the spectra were recorded using a single excitation pulse of 12 μs. GC-MS analyses were performed with an Agilent gas chromatograph 6890 equipped with an apolar Macherey Nagel Permabond SE 52 capillary column.

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

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.

The synthesis of the desired compounds started with a Hantzsch reaction between 4-pyridil-carbothioamide and the ethyl-2-chloro-acetyl-acetate in order to obtain the ethyl 4-methyl-2-(pyridin-4-yl)-thiazole-5-carboxylate **1** which was transformed into the hydrazide derivative **2** by the hydrazinolysis of the ethyl ester group with hydrazine hydrate (Figure 1).

The structure of compound **2** was confirmed on the basis of its spectral analysis (MS and NMR spectra). The presence in the $^{1}HNMR$ spectrum of two singlet signals at 4.6 and 9.65 ppm due to the NH_2, respectively NH protons, clearly confirms the formation of the compound.

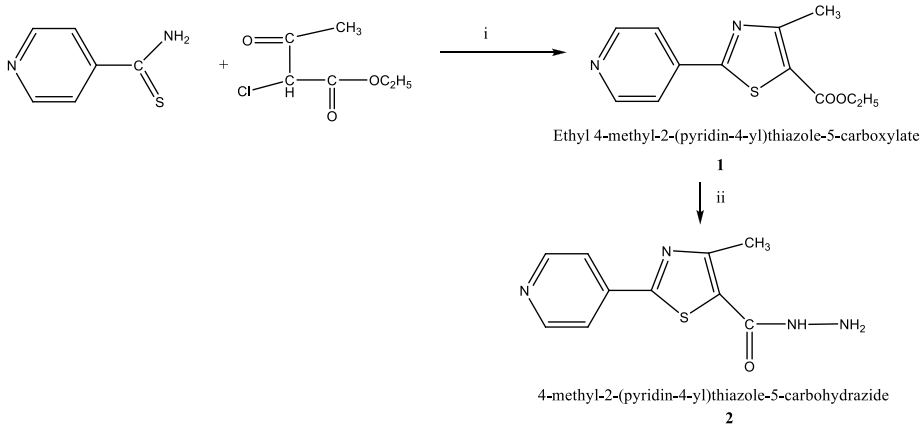

Figure 1.
Reagents and conditions: i. ethanol, reflux; ii. NH_2NH_2 in absolute ethanol, reflux 6h

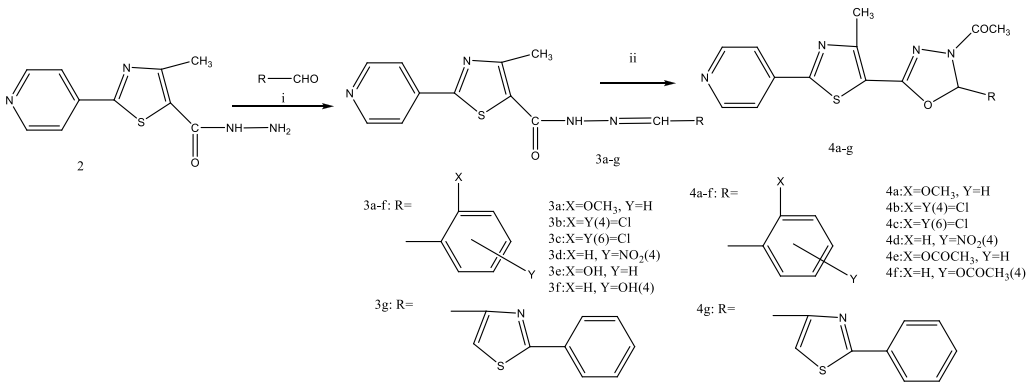

Figure 2.
Reagents and conditions: i. absolute ethanol, reflux, 3h; ii. Ac_2O, reflux, 2h

Synthesis of ethyl 4-methyl-2-(pyridin-4-yl)-thiazole-5-carboxylate 1

Compound 1 was synthesized by refluxing a mixture of pyridine-4-carbothioamide (30 mmol) with ethyl 2-chloro-3-oxobutanoate (30 mmol) in absolute ethanol (30 mL) for 5 hours. After cooling, the mixture was poured in cold water, neutralized with sodium bicarbonate solution (10%) and the obtained solid was filtered out, washed with water and crystallized from water.

Synthesis of 4-methyl-2-(pyridin-4-yl)thiazole-5-carbohydrazide 2

A mixture of 1 (0.001 mol) and hydrazine hydrate (1 ml) was refluxed for 6 h in absolute ethanol (10 mL). The reaction mixture was cooled and the crystalline mass obtained was recrystallised from ethanol and obtained as yellow crystals in 74% yield.

The hydrazide derivative **2** can be used as a precursor for the synthesis of various five member heterocyclic compounds. The type of the heterocycle depends on the reagent used. Thus, to obtain various oxadiazolines derivatives, the hydrazide **2** was first condensed with a series of aromatic or heteroaromatic aldehydes, leading to the aryl-methyliden-hydrazides **3a-g.** The latter compounds were transformed into oxadiazolines derivatives **4a-g** by cyclization
with acetic anhydride (Figure 2).

Synthesis of the arylmethyliden-hydrazides 3a-g (general procedure)

Equimolar quantities of 4-methyl-2-(pyridin-4-yl)-thiazole-5-carbohydrazide and different aldehydes were refluxed in absolute ethanol for 4 h. The solid product formed after cooling was filtered and dried. The crude solid was recrystallised in ethanol to give the products.

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

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

Heating the hydrazide **2** with acetic anhydride or propionic anhydride gave the corresponding methyl-oxadiazole **5a** and ethyl-oxadiazole **5b** (Figure 3).

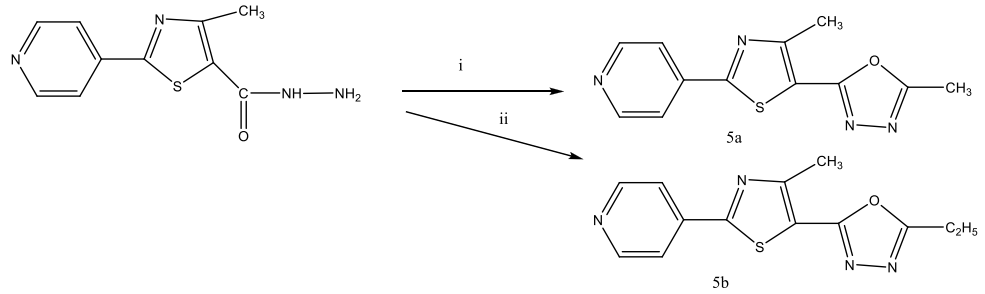

Figure 3.
Reagents and conditions: i. acetic anhydride, reflux 6h; ii. propionic anhydride, reflux 6h

Synthesis of 2-methyl-5-(4-methyl-2-(pyridin-4-yl)-thiazol-5-yl)-1,3,4-oxadiazole 5a

A mixture of **2** (0.001 mol) and acetic anhydride (5 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 recrystallised in ethanol.

Synthesis of 2-ethyl-5-(4-methyl-2-(pyridin-4-yl)-thiazol-5-yl)-1,3,4-oxadiazole 5b

A mixture of **2** (0.001 mol) and propionic anhydride (5 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 in ethanol.

Condensation of the hydrazide **2** with dicarbonyl compounds such as acetyl-acetone, afforded the corresponding dimethyle-pyrazole derivative **6** (Figure 4). The ^{1}HNMR spectrum presents characteristic singlet signals at 2.23, 2.59 ppm (CH_3 groups' position 3 and 5 pyrazole nucleus) and at 6.35 ppm (CH position 4 pyrazole nucleus).

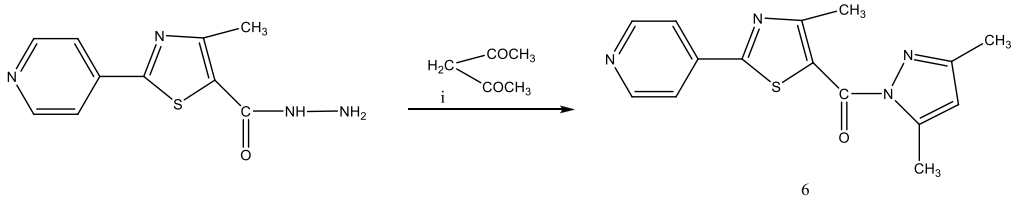

Figure 4.
Reagents and conditions: i: absolute ethanol, reflux, 6h

Synthesis of (3,5-dimethyl-1H-pyrazol-1-yl)(4-methyl-2-(pyridin-4-yl)-thiazol-5-yl)-methanone 6

0.001 mol acid hydrazide **2** was refluxed with 0.001 mol acetyl-acetone in absolute ethanol for 3 h. The product formed is filtered, washed with water and recrystallised from ethanol.

Treatment of hydrazide **2** with allyl-isothiocyanate in refluxing ethanol yielded a product identified as

N-allyl-2-(4-methyl-2-(pyridin-4-yl)-thiazole-5-carbonyl)-hydrazinecarbothioamide **7**. The latter product undergoes intramolecular cyclization when treated with potassium hydroxide 5% in ethanol and gave the corresponding allyl-1,2,4-triazole-5-thione derivative **8** (Figure 5). Assignment of the structure **8** is based on MS and the ^{1}HNMR spectrum which showed a singlet signal at 14.31ppm (NH 4, triazole ring) and the corresponding signals for the allyl group.

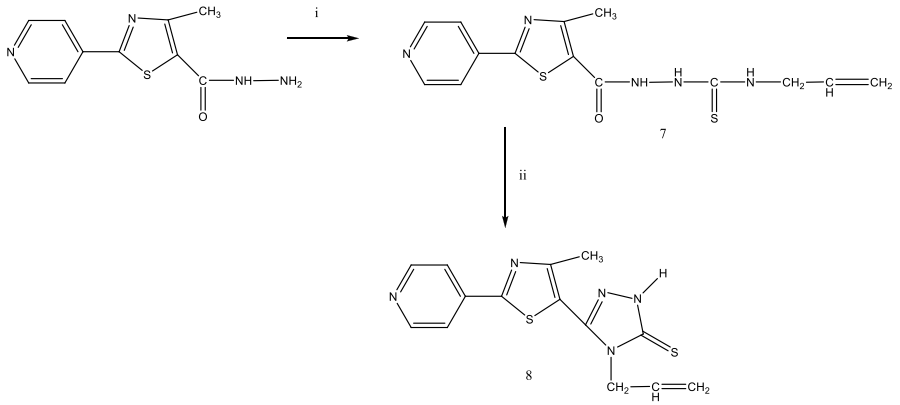

Figure 5.
Reagents and conditions: i. Allylisothiocyanate in absolute ethanol, reflux 6h; ii KOH 5% in absolute ethanol, reflux 6h

Synthesis of N-allyl-2-(4-methyl-2-(pyridin-4-yl)-thiazole-5-carbonyl)-hydrazinecarbothioamide 7
A mixture of acid hydrazide **2** (0.001 mol) and allylisothiocyanate (0.001 mol) in absolute ethanol was refluxed for 3h. After cooling, the formed solid was filtered and recrystallised from ethanol.
Synthesis of 4-allyl-3-(4-methyl-2-(pyridin-4-yl)-thiazol-5-yl)-1H-1,2,4-triazole-5(4H)-thione 8
(0.001 mol) N-allyl-2-(4-methyl-2-(pyridin-4-yl)-thiazole-5-carbonyl)-hydrazine-carbothioamide **7** was refluxed 3h in a solution of potassium hydroxide (5%) in absolute ethanol (5 mL). After cooling, the solution was neutralized with a solution of hydrochloric acid 55 and the resulted solid was filtered and recrystallised from ethanol.
Antibacterial/antifungal activity
The antimicrobial activities of the newly synthesized compounds were determined against ATCC reference microbial strains, *i.e.*, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311, *Listeria monocytogenes,* ATCC 35152, *B. cereus* ATCC 13061, *Candida albicans* ATCC 90028.
The *in vitro* antimicrobial activity was determined using the cup-plate agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. [7]. For antibacterial testing, Mueller-Hinton agar medium was used whereas for antifungal testing, Mueller-Hinton medium supplemented with 2% glucose (providing adequate growth of yeasts) and 0.5 mg/mL methylene blue (providing a better definition of the inhibition zone diameter) was used. A 24-48 hours old culture of selected bacteria was mixed with sterile distilled water to adjust the cell density to a 0.5 McFarland standard scale (corresponding to a population of 1-5x10^6 CFU/mL) by measuring the absorbance in a spectrophotometer at a wavelength of 530 nm. The inoculum was spread on the surface of the solidified media and after drying for 10-15 minutes, six-millimeter diameter wells were cut from the agar using a sterile cork-borer, and a volume of 10 µL of each compound solution (5 mg/mL in dimethyl sulfoxide - DMSO) were delivered into the wells (50µg/well). Ciprofloxacin (50µg/well) and fluconazole (50µg/well) were used as standard drugs. The controls were performed with only sterile broth, overnight culture and 10µL of DMSO. The plates inoculated with bacteria were incubated 24 h at 37°C and the fungal culture was incubated 48h at 25°C. Antimicrobial activity was assessed by measuring the growth inhibition zones diameters expressed in mm.
The solvent used for the preparation of each compound stock solution, DMSO (Merck, Germany) exhibited no inhibitory activity against the tested bacterial and fungal strains.

Results and Discussion

The target compounds were synthesized with good yields, following the aforementioned procedure. The results for elemental analysis data for the new compounds were within ± 0.4% of theoretical values and are in agreement with the proposed chemical structure. The structures of the newly synthesized compounds were elucidated by spectral data.
1: M.p. 52-55 ^{0}C, MS m/z: 248; ^{1}H NMR (DMSO-d6) δ: 8.74 (q 2H, pyridyl, C_2, C_6), 7.94 (q 2H, pyridyl, C_3, C_5), 4.87 (q, 2H, CH_2, OC_2H_5), 2.58 (s

3H, CH_3, thiazole C_4), 1.9 (t, 3H, CH_3, OC_2H_5); ^{13}C NMR (DMSO-d6) δ (ppm): 163.43 (C=O), 160.2 (thiazole C_2), 155.35 (thiazole C_4), 151.86 (pyridyl C_3 and C_5), 134.2 (pyridyl C_1), 128.1 (pyridyl C_2 and C_6), 124.2 (thiazole C_5), 62.4 (CH_2, OC_2H_5), 17.4 (CH_3 thiazole). 15.4 (CH_3, OC_2H_5).

2: M.p. 214-215 ^{0}C, MS m/z: 234; ^{1}H NMR (DMSO-d6) δ: 9.65 (s, 1H, NH), 8.70 (q 2H, pyridyl, C_2, C_6), 7.92 (q 2H, pyridyl, C_3, C_5), 4.59 (s, 2H, NH_2), 2.62 (s 3H, CH_3, thiazole C_4); ^{13}C NMR (DMSO-d6) δ(ppm): 163.43 (C=O), 161.3 (thiazole C_2), 155.35 (thiazole C_4), 151.86 (pyridyl C_3 and C_5), 134.2 (pyridyl C_1), 128.1 (pyridyl C_2 and C_6), 124.2 (thiazole C_5), 17.4 (CH_3 thiazole).

All the arylmethyliden-hydrazides **3a-g** showed in ^{1}HNMR spectra characteristic singlet signals in 8-8.2 ppm range (CH) and 11.6-11.8 ppm range (NH). The ^{13}C NMR spectra showed characteristic signals in 145-148 ppm range (CH=N). The cyclization of the arylmethyliden-hydrazides **3a-g** into the oxadiazolines derivatives **4a-g** was established by the absence of the singlet signal at 11.6-11.8 ppm (NH) and the apparition of two new signals at 2.2-2.35 ppm (N-$COCH_3$) and at 7.2-7.6 ppm (CH, position 5, oxadiazoline ring).

3a: 80% yield. M.p. 292-295 ^{0}C; MS m/z: 352, ^{1}H NMR (DMSO-d6) δ (ppm): 11.80 (s, 1H, NH), 8.75 (q 2H, pyridyl, C_2, C_6), 8.1 (s, 1H, -N=CH-Ar), 7.92 (q 2H, pyridyl, C_3, 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), 151.34 (pyridyl C_3 and C_5), 145.06 (CH=N), 139.7 (pyridyl C_1), 129.6 (phenyl C_5), 125.89 (phenyl C_6), 123.64 (phenyl C_1), 121.22 (phenyl C_4), 120.64 (pyridyl C_2 and C_6), 116.45 (phenyl C_3), 115.2 (thiazole C_5), 56.92 (OCH_3), 19.27 (CH_3 thiazole).

3b: 84% yield. M.p. 305 ^{0}C; MS m/z:393 (m+2), ^{1}H NMR (DMSO-d6) δ (ppm): 11.78 (s, 1H, NH), 8,72 (q 2H, pyridil, C_2,C_6), 8.15 (s, 1H, -N=CH-Ar), 7.92 (q 2H, pyridyl, C_3, 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), 151.04 (pyridyl C_3 and C_5), 148.06 (CH=N), 140.7 (pyridyl C_1), 134.6 (phenyl C_2), 132.89 (phenyl C_4), 128.64 (phenyl C_1), 127.22 (phenyl C_6), 125.45 (phenyl C_3), 123.12 (phenyl C_5), 121.64 (pyridyl C_2 and C_6), 114.2 (thiazole C_5), 19.27 (CH_3 thiazole).

3c: 78% yield. M.p. 305-307 ^{0}C; MS m/z:393 (m+2), ^{1}H NMR (DMSO-d6) δ (ppm): 11.72 (s, 1H, NH), 8.7 (q 2H, pyridyl, C_2, C_6), 8.05 (s, 1H, -N=CH-Ar), 7.96 (q 2H, pyridyl, C_3, 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), 152.11 (pyridyl C_3 and C_5), 147.8 (CH=N), 140.7 (pyridyl C_1), 134.3 (phenyl C_2 and C_6), 132.89 (phenyl C_1), 127.22 (phenyl C_4), 125.45 (phenyl C_3 and C_5), 120.94 (pyridyl C_2 and C_6), 116.2 (thiazole C_5), 20.27 (CH_3 thiazole).

3d: 78% yield. M.p. 317-320 ^{0}C MS m/z: 368 (m+1) ^{1}H NMR (DMSO-d6) δ (ppm): 11.72 (s, 1H, NH), 8.71 (q 2H, pyridyl, C_2, C_6), 8.35 (q 2H, phenyl, C_3, C_5), 8.06 (s, 1H, -N=CH-Ar), 7.85 (q 2H, pyridyl, C_3, C_5), 7.5 (q 2H, phenyl C_2, C_6), 2.7 (s 3H, CH_3, thiazole C_4); ^{13}CNMR (DMSO-d6) δ (ppm): 166.02 (amide C=O), 161.27 (thiazole C_2), 160.39 (thiazole C_4), 152.11 (pyridyl C_3 and C_5), 147.2 (CH=N), 147.7 (phenyl C_4) 142.22 (pyridyl C_1), 140.88 (phenyl C_1), 128.8 (phenyl C_2 and C_6), 124.85 (phenyl C_3 and C_5), 120.75 (pyridyl C_2 and C_6), 115.85 (thiazole C_5), 20.27 (CH_3 thiazole).

3e: 85% yield. M.p. 230 ^{0}C 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), 8.74 (q 2H, pyridyl, C_2, C_6), 8.1 (s, 1H, -N=CH-Ar), 7.92 (q 2H, pyridyl, C_3, C_5), 7.6 (d 1H phenyl C_6) 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), 151.37 (pyridyl C_3 and C_5), 146.86 (CH=N), 138.87 (pyridyl C_1), 131.8 (phenyl C4), 130.8 (phenyl C_6), 128.04 (phenyl C_1), 126.55 (phenyl C_5), 124.50 (pyridyl C_2 and C_6), 120.4 (phenyl C_3), 117.18 (thiazole C_5), 17.87 (CH_3 thiazole).

3f: 80% yield. M.p. 296-300 ^{0}C 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), 8.74 (q 2H, pyridyl, C_2, C_6), 8 (s, 1H, -N=CH-Ar), 7,96 (q 2H, pyridyl, C_3, C_5), 7,63 (q 2H, phenyl, C_2, C_6), 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), 151,34 (pyridyl C_3 and C_5), 145,4 (CH=N), 139,7 (pyridyl C_1), 129,69 (phenyl C_2 and C_6), 125,31(phenyl C_1), 121,21 (pyridyl C_2 and C_6), 120.64 (phenyl C_3 and C_5), 116,46 (thiazole C_5), 19,27 (CH_3 thiazole).

3g: 75% yield. M.p. 248-250 ^{0}C; MS m/z: 406 (m+1), ^{1}H NMR (DMSO-d6) δ (ppm): 11.4 (s, 1H, NH), 8.72 (q 2H, pyridyl, C_2, C_6), 8.1 (s, 1H, -N=CH-Ar), 7.98 (q 2H, pyridyl, C_3, C_5), 7.92 (q, phenyle C_2, C_6), 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, (phenyle-thiazole C_2), 152.96 (pyridyl-thiazole C_4), 152.1 (phenyle-thiazole C_4), 151.37 (pyridyl C_3 and C_5), 146.2 (CH=N), 138.12 (pyridyl C_1), 134.34 (phenyl C_2 and C_6), 132.12 (phenyl C_1), 131.8 (phenyl-thiazole C_5), 128.62 (phenyl

C_4), 126.45 (phenyl C_3 and C_5), 121.94 (pyridyl C_2 and C_6), 119.81 (thiazole C_5), 17.45 (CH_3 thiazole).

4a: 75% yield. M.p. 175-178 ^{0}C; MS m/z: 395 (m+1), ^{1}H NMR (DMSO-d6) δ (ppm): 8.75 (q 2H, pyridyl, C_2, C_6), 7.92 (q 2H, pyridyl, C_3, C_5), 7.46 (t, 1H, phenyl C_4), 7.35 (d 1H, phenyl C_3), 7.27 (s 1H, oxadiazoline C_5) 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 (oxadiazoline C_5), 157.11 (phenyl C_2), 151.35 (pyridyl C_3 and C_5), 150.31 (thiazole C_4), 138.96 (pyridyl C_1), 132.16 (phenyl C_5), 128.89 (phenyl C_6), 123.64 (phenyl C_1), 120.92 (phenyl C_4), 120.57 (pyridyl C_2 and C_6), 117.5 (phenyl C_3), 112.45 (thiazole C_5), 90.25 (oxadiazoline C_2), 56.32 (OCH_3), 21.6 (CH_3-acetyl), 17.69 (CH_3 thiazole).

4b: 78% yield. M.p. 193-195 ^{0}C; MS m/z:434 (m+2), ^{1}H NMR (DMSO-d6) δ (ppm): 8.72 ppm (q 2H, pyridyl, C_2, C_6), 7.92 ppm (q 2H, pyridyl, C_3, C_5), 7.79 (d 1H, phenyl C_6), 7.56 ppm (s 1H, phenyl C_3), 7.54 ppm (d 1H, phenyl C_5), 7.34 (s 1H, oxadiazoline C_5) 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), 151.37 (pyridyl C_3 and C_5), 150.16 (thiazole C_4), 138.87 (pyridyl C_1), 136.24 (phenyl C_4), 133.74 (phenyl C_2), 131.98 (phenyl C_3), 131.15 (phenyl C6), 130.35 (phenyl C_5), 128.63 (phenyl C_1) 121.64 (pyridyl C_2 and C_6), 117.03 (thiazole C_5), 90.55 (oxadiazoline C_2), 21.59 (CH_3-acetyl), 17.75 (CH_3 thiazole).

4c: 74% yield. M.p. 180-182 ^{0}C; MS m/z:434 (m+2), ^{1}H NMR (DMSO-d6) δ (ppm): 8.75 (q 2H, pyridyl, C_2, C_6), 7.93 (q 2H, pyridyl, C_3, C_5), 7.58 (s 1H, oxadiazoline C_5), 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), 151.5 (pyridyl C_3 and C_5), 138 (pyridyl C_1), 134.34 (phenyl C_2 and C_6), 132.12 (phenyl C_1), 128.92 (phenyl C_4), 126.45 (phenyl C_3 and C_5), 121.94 (pyridyl C_2 and C_6), 119.81 (thiazole C_5), 90.85 (oxadiazoline C_2), 22.29 (CH_3-acetyl), 17.85 (CH_3 thiazole).

4d: 78% yield. M.p. 194-196 ^{0}C MS m/z: 410.6 (m+1) ^{1}H NMR (DMSO-d6) δ (ppm): 8.71 (q 2H, pyridyl, C_2, C_6), 8.32 (q 2H, phenyl, C_3, C_5), 7.83 (q 2H, pyridyl, C_3, C_5), 7.57 (q 2H, phenyl C_2, C_6), 7.36 (s 1H, oxadiazoline C_5) 2.7 (s 3H, CH_3, thiazole C_4), 2.27 (s 3H, N-$COCH_3$); ^{13}C NMR (DMSO-d6) δ (ppm): 167.59 (acetyl C=O), 165.29 (thiazole C_2), 161.64 (oxadiazoline C_5), 157.75 (thiazole C_4), 152.1 (pyridyl C_3 and C_5), 150.63 (phenyl C_4), 147.47 (phenyl C_1), 142.9 (pyridyl C_1), 134.38 (phenyl C_2 and C_6), 124.42 (phenyl C_3 and C_5), 120.75 (pyridyl C_2 and C_6), 115.81 (thiazole C_5), 90.7 (oxadiazoline C_2), 21.29 (CH_3-acetyl), 17.85 (CH_3 thiazole.

4e: 70% yield. M.p. 172-173 ^{0}C MS m/z: 423.6 (m+1) ^{1}H NMR (DMSO-d6) δ (ppm): 8.8 (q 2H, pyridyl, C_2, C_6), 7.92 (q 2H, pyridyl, C_3, C_5), 7.58 (d 1H phenyl C_6) 7.55 (t 1H, phenyl, C_4), 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), 151.34 (pyridyl C_3 and C_5), 149.4 (phenyl C_2), 138.87 (pyridyl C_1), 131.8 (phenyl C_4), 130.31 (phenyl C_5), 128.04 (phenyl C6), 126.55 (phenyl C_1), 124.54 (phenyl C_3), 120.6 (pyridyl C_2 and C_6), 117.18 (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).

4f: 72% yield. M.p. 180-182 ^{0}C MS m/z:423.4 (m+1) ^{1}H NMR (DMSO-d6) δ (ppm): 8,75 (q 2H, pyridyl, C_2, C_6), 7.93 (q 2H, pyridyl, C_3, 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), 151.34 (pyridyl C_3 and C_5), 145.08 (phenyl C_4), 138.7 (pyridyl C_1), 129.69 (phenyl C_2 and C_6), 125.31 (phenyl C_1), 121.61 (phenyl C_3 and C_5), 120.64 (pyridyl C_2 and C_6), 116.46 (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).

4g: 75% yield. M.p. 204-208 ^{0}C; MS m/z: 448 (m+1), ^{1}H NMR (DMSO-d6) δ (ppm): 8.75 (q 2H, pyridyl, C_2, C_6), 8.5 (q 2H, pyridyl, C_3, C_5), 7.92 (q, phenyle C_2, C_6), 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 (phenyle-thiazole C_2), 166.32 (pyridyl-thiazole C_2), 157.85 (oxadiazoline C_5), 152.96 (pyridyl-thiazole C_4), 152.1 (phenyle-thiazole C_4), 151.37 (pyridyl C_3 and C_5), 138.12 (pyridyl C_1), 134.34 (phenyl C_2 and C_6), 132.12 (phenyl C_1), 131.8 (phenyl-thiazole C_5), 128.62 (phenyl C_4), 126.45 (phenyl C_3 and C_5), 121.94 (pyridyl C_2 and C_6), 119.81 (thiazole C_5), 90.65 (oxadiazoline C_2), 22.49 (CH_3-acetyl), 17.45 (CH_3 thiazole).

Assignments of structures 5a-b are based on the correct elemental analyses, MS spectra and the ^{1}HNMR spectra which revealed characteristic signals for CH_3 and C_2H_5 groups.

5a: 70% yield. M.p. 246-250 ^{0}C MS m/z: 259 (m+1) ^{1}H NMR (DMSO-d6) δ (ppm): 8.75 (q 2H, pyridyl, C_2, C_6), 7.95 (q 2H, pyridyl, C_3, C_5), 2.78 (s 3H, CH_3, thiazole C_4), 2.61 (s 3H, CH_3, oxadiazole C_2); ^{13}C NMR (DMSO-d6) δ (ppm): 165.55 (thiazole C_2), 164.37 (oxadiazole C_2), 159.28 (oxadiazole C_5), 156.56 (thiazole C_4),

151.41 (pyridyl C_3 and C_5), 138.9 (pyridyl C_1), 120.63 (pyridyl C_2 and C_6), 116.68 (thiazole C_5), 17.5 (CH_3 thiazole), 11.03 (CH_3 oxadiazole).

5b: 74% yield. M.p. 168-170 ^{0}C MS m/z: 273 (m+1) ^{1}H NMR (DMSO-d6) δ: 8.74 ppm (q 2H, pyridil, C_2, C_6), 7.93 ppm (q 2H, pyridil, C_3, C_5), 2.98 (q 2H, CH_2 from C_2H_5 oxadiazole C_2) 2.76 (s 3H, CH_3, thiazole C_4), 1.34 ppm (t 3H, CH_3, from C_2H_5 oxadiazole C_2); ^{13}C NMR (DMSO-d6), δ (ppm): 166.13 (thiazole C_2), 165.5 (oxadiazole C_2), 159.16 (oxadiazole C_5), 156.57 (thiazole C_4), 151.37 (pyridil C_3 and C_5), 138.88 (pyridil C_1), 120.6 (pyridil C_2 and C_6), 116.68 (thiazole C_5), 18.79 (CH_2, C_2H_5-oxadiazole), 17.5 (CH_3 thiazole), 10.8 (CH_3 C_2H_5-oxadiazole).

6: 70% yield. M.p. 195-197 ^{0}C MS m/z:299 (m+1) ^{1}H NMR (DMSO-d6) δ: 8.76 ppm (q 2H, pyridil, C_2, C_6), 7.98 ppm (q 2H, pyridil, C_3, C_5), 6.35 (s 1H, pyrazole C_4) 2.83 (s 3H, CH_3, thiazole C_4), 2.59 ppm (s 3H, CH_3, pyrazole C_5). 2.23 ppm (s 3H, CH_3, pyrazole C_3). ^{13}C NMR (DMSO-d6) δ (ppm): 165.7 (thiazole C_2), 165.12 (C=O), 156.57 (thiazole C_4), 151.39 (pyridil C_3 and C_5), 138.88 (pyridil C_1), 134.8 (pyrazole C_3), 128.4 (pyrazole C_5), 120.7 (pyridil C_2 and C_6), 116.8 (thiazole C_5), 112.6 (pyrazole C_4), 19.85 (CH_3 thiazole), 14.73 (CH_3 pyrazole C_3). 13.98 (CH_3 pyrazole C_5).

7: 78% yield. M.p. 222-224 ^{0}C MS m/z: 334 (m+1) ^{1}H NMR (DMSO-d6) δ: 10.2 ppm (s 1H, NH, -CO-NH), 9.5 ppm (s 1H, NH, -NH-C=S), 8.75 ppm (q 2H, pyridil, C_2, C_6), 8.38 ppm (s, 1H, NH –NH-CH_2-), 7.90 ppm (q 2H, pyridil, C_3, C_5), 5.85 ppm (m 1H, CH, -CH=), 5.16 (d 1H, CH, =CH_2), 5.07 ppm (d 1H CH, =CH_2), 4.13 ppm (d 2H, CH_2, –NH-CH_2-), 2.63 (s 3H, CH_3, thiazole C_4); ^{13}C NMR (DMSO-d6) δ (ppm): 181.2 (C=S), 164.44 (thiazole C_2), 161.3 (C=O), 158.07 (thiazole C_4), 151.4 (pyridil C_3 and C_5), 138.32 (pyridil C_1), 135.38 (-CH=), 125.8 (=CH_2), 120.53 (pyridil C_2 and C_6), 115.74 (thiazole C_5), 46.43 (-CH_2 -), 17.84 (CH_3 thiazole).

8: 70% yield. M.p. 261-264 ^{0}C MS m/z:316 (m+1) ^{1}H NMR (DMSO-d6) δ: 14.31 ppm (s 1H, NH, N_1-triazole), 8.75 ppm (q 2H, pyridil, C_2, C_6), 7.92 ppm (q 2H, pyridil, C_3, C_5), 5.86 ppm (m 1H, CH, -CH=), 4.91 (d 1H, CH, =CH_2), 4.88 ppm (d 1H CH, =CH_2), 4.66 ppm (d 2H, CH_2, N_4-triazole), 2.48 (s 3H, CH_3, thiazole C_4); ^{13}C NMR (DMSO-d6) δ (ppm): 168.11 (C=S), 165,93 (thiazole C_2), 157.36 (thiazole C_4), 151.36 (pyridil C_3 and C_5), 144.11 (triazole C_2), 139.13 (pyridil C_1), 131.85 (-CH=), 120.59 (pyridil C_2 and C_6), 117.87 (=CH_2) 116.69 (thiazole C_5), 46.32 (-CH_2), 16.96 (CH_3 thiazole).

Biological evaluation

All compounds were tested for their antibacterial activity against two Gram-negative (*Salmonella typhimurium* ATCC 13311, *Escherichia coli* ATCC 25922) and three Gram-positive (*Listeria monocytogenes* ATCC 35152, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 13061) bacterial strains. Antifungal activity of the above compounds was evaluated against a strain of *Candida albicans* ATCC 90028.

The results of the antimicrobial evaluation are summarized in Table I.

Table I

Antimicrobial data of the synthesized compounds (mm inhibition zone)

Compounds / Microbial strains	Inhibition zones (mm)					
	I	II	III	IV	V	VI
3a	12	14	16	8	12	12
3b	12	14	14	12	18	12
3c	14	12	14	10	12	12
3d	12	12	12	6	10	14
3e	10	12	12	6	10	14
3f	12	12	12	6	10	14
3g	12	10	14	8	12	12
4a	18	12	10	14	15	14
4b	16	14	8	12	14	16
4c	12	12	10	12	14	14
4d	12	14	14	12	12	16
4e	22	16	12	20	12	22
4f	14	14	12	10	12	18
4g	14	12	10	12	14	12
5a	22	12	10	14	12	14
5h	18	15	10	6	10	18
6	12	13	10	12	10	14
7	18	14	14	14	20	24
8	15	8	14	14	20	24
Ciprofloxacin	-	22	26	24	22	22
Fluconazole	28	-	-	-	-	-

I = *Candida albicans* ATCC 90028, II = *Salmonella typhimurium* ATCC 13311, III = *E. coli* ATCC 25922, IV = *S. aureus* ATCC 25923, V = *Listeria monocytogenes* ATCC 35152, , VI = *B. cereus* ATCC 13061.

Concerning the antimicrobial activity of the arylmethyliden-hydrazides **3a-g** and oxadiazolines derivatives **4a-g (**Figure 2**)**, the compound **4e** (2-(3-acetyl-5-(4-methyl-2-(pyridin-4-yl)thiazol-5-yl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)phenyl acetate) showed good antimicrobial activity (inhibition zone diameters 20-22 mm) against *Candida albicans, S.aureus and B.cereus.*

For the 2(4-pyridil)-5-thiazolyl-carbonyl-3,5-dimethyle-pyrazole and 2(4-pyridil)-5-thiazolyl-1,3,4-oxadiazoles series (Figures 3 and 4), the results revealed that 2-methyl-5-(4-methyl-2-(pyridin-4-yl)thiazol-5-yl)-1,3,4-oxadiazole **5a** exhibited good antifungal activity.

The 2(4-pyridil)-5-thiazolyl-N-allyl-hidrazine-carbo-thioamide **7** and 2(4-pyridil)-5-thiazolyl-1,3,4-triazol-5-thione **8** (Figure 5) showed

promising antibacterial activity against *Listeria monocytogenes and B.cereus*.

Conclusions

In this present work, novel series of 4-methyl-2-(pyridin-4-yl)-thiazole-5-yl-azoles were synthesized. Presented synthetic figures illustrate the way used for the synthesis of target compounds. The 1H NMR, 13C NMR, mass spectral data and elemental analysis results are in agreement with the proposed structures. All the physicochemical and spectral data of the compounds are presented in detail.

All the synthesized compounds were screened for their anti-bacterial activity against *Salmonella typhimurium, S. aureus*, *Listeria monocytogenes*, *E. coli*, *B. cereus,* and antifungal activity against *Candida albicans.* The tested compounds presented a modest to good antibacterial and antifungal activity compared with the standard drugs.

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