

## SYNTHESIS AND ANTIMICROBIAL SCREENING OF NOVEL 2, 3 OR 4-[2-ARYL-THIAZOL-YL-METHOXY (OXO-ETHOXY)]-BENZALDEHYDE ISONICOTINOYL HYDRAZONE ANALOGS

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### Abstract

We synthesized new aroyl-hydrazones by condensing some derivatives of 4-[2-(4-methyl-2-phenyl-thiazole-5-yl)-2-oxo-ethoxy]-benzaldehyde and 2, 3 or 4-(2-aryl-thiazol-4-yl-methoxy)-benzaldehyde, respectively with isonicotinoyl hydrazide, in refluxing acetic acid 50%. The purity of the compounds was demonstrated by thin layer chromatography (TLC). The structures of all new compounds were confirmed by elemental analysis and spectroscopic data (infrared - IR, <sup>1</sup>H nuclear magnetic resonance - <sup>1</sup>H NMR, mass spectrometry - MS). A screening of the antimicrobial activity was performed. All the compounds were tested *in vitro* against various Gram-negative and Gram-positive bacteria. They showed poor to moderate antibacterial activity on Gram-negative strains, compound **6** being the only displaying Gram-positive bacterial growth inhibition.

### Rezumat

Au fost sintetizate noi aroil-hidrazone prin condensarea unor derivați de 4-[2-(2-fenil-4-metil-tiazol-5-il)-2-oxo-etoxi]-benzaldehydă, respectiv 2, 3 sau 4-(2-aril-tiazol-4-il-metoxi)-benzaldehydă, cu hidrazidă izonicotinică, prin refluxare în acid acetic 50%. Puritatea compușilor a fost demonstrată prin cromatografie în strat subțire (CSS). Structurile noilor compuși au fost confirmate prin analiză elementală și prin date de spectroscopie (infraroșu - IR, rezonanță magnetică nucleară - <sup>1</sup>H RMN, spectrometrie de masă - MS). A fost realizat un screening al activității antimicrobiene. Toți compușii au fost testați *in vitro* pe diferite tulpini bacteriene Gram-negative și Gram-pozitive. Au prezentat o activitate antibacteriană slabă până la moderată pe tulpinile Gram-negative, iar compusul **6** a fost singurul care a demonstrat o inhibiție a creșterii în cazul tulpinilor Gram-pozitive.

**Keywords:** aroyl-isonicotinoyl hydrazones; antibacterial activity

## Introduction

In the actual context of the continuous growing development of the microorganisms' resistance to chemotherapies, the development of new antibacterial agents represents great interest. Even if the antiinfectious therapeutic arsenal can be considered rich as number of molecules existing in therapy, the appearance of the so-called "superbacteria", which have as a principal characteristic the high level of resistance to antibiotics, creates big problems nowadays for the clinicians, the therapeutical possibilities being often extremely limited. The major bacteria which have a lower and lower sensibility to the antibiotics used today in therapy are: *Streptococcus pneumoniae* penicillin-resistant (PRP), *Staphylococcus aureus* meticiline-resistant (SARM), *Enterococcus* resistant to glycopeptides (VRE), some enterobacteria secreting betalactamases, *Pseudomonas aeruginosa* and *Acinetobacter* multi-drug-resistant [1, 2].

In recent years scientific literature is extremely rich in prospectations of new antibacterial chemotherapeutic molecules, with a structural profile different from that of the chemotherapies available today in the medical practice, molecules able to inhibit new bacterial targets and to have therefore a new mechanism of action. The new molecules belong to some extremely varied structural models, between them being remarked the azolic heterocycles, especially the thiazole, the thiadiazole and the derivatives of these ones (thiazolines, thiazolidines, thiadiazolines, etc.).

Continuing our work focused on obtaining new biological active molecules, we present in this article the synthesis of some new aroyl-hydrazones containing 2 pharmacophore moieties: aryl-thiazole and isonicotinoyl hydrazide. Compounds bearing aryl-thiazole proved an antimicrobial potential [3-7]. Hydrazones derived from isonicotinic acid are known for their antibacterial properties, especially for the tuberculostatic effect. Hydrazones may form active complexes with metals, necessary for the bacterial survival, this representing a possible antimicrobial mechanism [8-10].

Considering these facts, we proposed the synthesis of some new aroyl-hydrazones derivated from isonicotinic acid and the screening of their antibacterial potential.

## Materials and methods

### *General procedure for the synthesis of aroyl-hydrazones 1-6*

4-[2-(4-Methyl-2-phenyl-thiazol-5-yl)-2-oxo-ethoxy]-benzaldehyde or 2, 3 or 4-(2-Aryl-thiazol-4-ylmethoxy)-benzaldehyde (1 mmol) was

dissolved in 10 mL of acetic acid 50%. Separately, a suspension of isonicotinic-hydrazide (HIN) (1 mmol) in 10 mL acetic acid 50% was prepared. This suspension of HIN was added under stirring to the solution of benzaldehyde. The reaction mixture was stirred for 1.5 hours. For improving the solubility of products in acetic acid, a small quantity of ethanol was added. After 1.5 hours of refluxing, the reaction mixture was cooled off. The crude product was filtered under reduced pressure, washed with water on the filter and purified by crystallization from ethanol.

*Isonicotinic acid {4-[2-(4-methyl-2-phenyl-thiazol-5-yl)-2-oxo-ethoxy]-benzylidene}-hydrazide (1).*

Yield 70%, white solid, mp: 210-214 °C. IR (KBr,  $\text{cm}^{-1}$ ): 3397 (NH), 3028 ( $\text{CH}_{\text{arom}}$ ), 1669 ( $\text{C=O}$ ), 1655 ( $\text{C=O}$  Hydrazide), 1610 ( $\text{C=C}$ ), 1552 ( $\text{C=N}$ ).  $^1\text{H}$  NMR (DMSO-*d*6, 500 MHz, ppm):  $\delta$  11.995 (s, 1H, NH); 8.787 (dd, 2H, Ar-H); 8.43 (s, 1H, -CH=); 8.044 (dd, 2H, Ar-H); 7.823 (dd, 2H, Ar-H); 7.715 (d, 2H, Ar-H); 7.592-7.546 (m, 3H, Ar-H); 7.122 (d, 2H, Ar-H); 5.435 (s, 2H, -CH<sub>2</sub>-); 2.785 (s, 3H, -CH<sub>3</sub>). Anal. Calcd. (%) for  $\text{C}_{25}\text{H}_{20}\text{N}_4\text{O}_3\text{S}$  (456.51): C, 65.77; H, 4.42; N, 12.27; S, 7.02. Found: C, 65.59; H, 4.41; N, 12.24; S, 7.01. MS (EI, 70 eV):  $m/z$  457.5 (M<sup>+</sup>), 217 [ $\text{C}_{12}\text{H}_{10}\text{NSO}$ ], 106 [ $\text{C}_7\text{H}_5\text{O}$ ], 104 [ $\text{C}_7\text{H}_5\text{N}$ ], 71 [ $\text{C}_2\text{HNS}$ ].

*Isonicotinic acid {2-[2-(4-bromo-phenyl)-thiazol-4-ylmethoxy]-benzylidene}-hydrazide (2).*

Yield 78.3%, white solid, mp: 244 °C. IR (KBr,  $\text{cm}^{-1}$ ): 3202 (NH), 3094 ( $\text{CH}_{\text{arom}}$ ), 2920 and 2849 ( $\text{CH}_{\text{aliph}}$ ), 1658 ( $\text{C=O}$ ), 1610 ( $\text{C=C}$ ), 1555 ( $\text{C=N}$ ), 1070 (C-Br).  $^1\text{H}$  NMR (500 MHz, DMSO-*d*6, ppm):  $\delta$  11.95 (s, 1H, NH); 8.79 (d, 2H, Ar-H); 8.432 (s, 1H, -CH=); 8.01 (dd, 2H, Ar-H); 7.92-7.86 (m, 3H, Ar-H); 7.68-7.56 (m, 2H, Ar-H); 7.45 (s, 1H, H-Thiazole); 7.32 (m, 1H, Ar-H); 7.21 (d, 2H, Ar-H); 5.3 (s, 2H, -CH<sub>2</sub>-). Anal. Calcd. (%) for  $\text{C}_{22}\text{H}_{16}\text{BrN}_4\text{O}_2\text{S}$  (493.37): C, 55.99; H, 3.47; N, 11.36; S, 6.50. Found: C, 55.79; H, 3.47; N, 11.332; S, 6.512. MS (EI, 70 eV):  $m/z$  492-494 (isotopic contribution of  $^{81}\text{Br}/^{79}\text{Br}$ ) [M+1], 373 [M-  $\text{C}_6\text{H}_5\text{N}_2\text{O}$ ], 356-358 [M-  $\text{C}_6\text{H}_5\text{N}_3\text{O}$ ], 252-254 [ $\text{C}_{10}\text{H}_7\text{NSBr}$ ], 240 [M-  $\text{C}_{10}\text{H}_7\text{NSBr}$ ], 181-183 [ $\text{C}_7\text{H}_4\text{NBr}$ ], 106 [ $\text{C}_7\text{H}_5\text{O}$ ], 71 [ $\text{C}_2\text{HNS}$ ].

*Isonicotinic acid {3-[2-(4-bromo-phenyl)-thiazol-4-ylmethoxy]-benzylidene}-hydrazide (3).*

Yield 78%, white solid, mp: 192-7 °C. IR (KBr,  $\text{cm}^{-1}$ ): 3346 (NH), 3047 ( $\text{CH}_{\text{arom}}$ ), 2917 and 2840 ( $\text{CH}_{\text{aliph}}$ ), 1656 ( $\text{C=O}$ ), 1620 ( $\text{C=C}$ ), 1553 ( $\text{C=N}$ ), 1068 (C-Br).  $^1\text{H}$  NMR (500 MHz, DMSO-*d*6, ppm): 11.99 (s, 1H, NH); 8.81 (d, 2H, Ar-H); 8.36 (s, 1H, -CH=); 7.972 (dd, 2H, Ar-H); 7.81 (d, 2H, Ar-H); 7.794-7.778 (m, 3H, Ar-H); 7.535 (s, 1H, H-Thiazole); 7.39-7.19 (m, 3H, Ar-H); 5.32 (s, 2H, -CH<sub>2</sub>-). Anal. Calcd. (%)  $\text{C}_{22}\text{H}_{16}\text{BrN}_4\text{O}_2\text{S}$

(493.37): C, 55.99; H, 3.47; N, 11.36; S, 6.50. Found: C, 55.81; H, 3.47; N, 11.35; S, 6.507. MS (EI, 70 eV):  $m/z$  492-494 (isotopic contribution of  $^{81}\text{Br}/^{79}\text{Br}$ )  $[M+1]$ , 373  $[M-\text{C}_6\text{H}_5\text{N}_2\text{O}]$ , 356-358  $[M-\text{C}_6\text{H}_5\text{N}_3\text{O}]$ , 252-254  $[\text{C}_{10}\text{H}_7\text{NSBr}]$ , 240  $[M-\text{C}_{10}\text{H}_7\text{NSBr}]$ , 181-183  $[\text{C}_7\text{H}_4\text{NBr}]$ , 106  $[\text{C}_7\text{H}_5\text{O}]$ , 71  $[\text{C}_2\text{HNS}]$ .

*Isonicotinic acid {4-[2-(4-bromo-phenyl)-thiazol-4-ylmethoxy]-benzylidene}-hydrazide (4).*

Yield 90%, yellow solid, mp: 236-7 °C. IR (KBr,  $\text{cm}^{-1}$ ): 3340 (NH), 3072 ( $\text{CH}_{\text{arom}}$ ), 2922 and 2843 ( $\text{CH}_{\text{aliph}}$ ), 1655 (C=O), 1618 (C=C), 1554 (C=N), 1068 (C-Br).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ , ppm):  $\delta$  11.97 (s, 1H, NH); 8.791 (d, 2H, Ar-H); 8.432 (s, 1H, -CH=); 7.914 (dd, 2H, Ar-H); 7.869 (s, 1H, H-Thiazole); 7.827 (d, 2H, Ar-H); 7.734 (d, 2H, Ar-H); 7.717 (d, 2H, Ar-H); 7.188 (d, 2H, Ar-H); 5.31 (s, 2H, -CH<sub>2</sub>-). Anal.Calcld.(%) for  $\text{C}_{23}\text{H}_{17}\text{BrN}_4\text{O}_2\text{S}$  (493.37): C, 55.99; H, 3.47; N, 11.36; S, 6.50. Found: C, 55.78; H, 3.46; N, 11.32; S, 6.49. MS (EI, 70 eV): 492-494 (isotopic contribution of  $^{81}\text{Br}/^{79}\text{Br}$ )  $[M+1]$ , 252-254  $[\text{BrC}_{10}\text{H}_7\text{NS}]$ , 181-183  $[\text{C}_7\text{H}_4\text{NBr}]$ , 106  $[\text{C}_7\text{H}_5\text{O}]$ , 71  $[\text{C}_2\text{HNS}]$ .

*Isonicotinic acid [4-(2-phenyl-thiazol-4-ylmethoxy)-benzylidene]-hydrazide (5).*

Yield 80%, white solid, mp: 193-5 °C. IR (KBr,  $\text{cm}^{-1}$ ): 3201 (NH), 3047 ( $\text{CH}_{\text{arom}}$ ), 2918 and 2851 ( $\text{CH}_{\text{aliph}}$ ), 1655 (C=O), 1609 (C=C), 1555 (C=N).  $^1\text{H}$  NMR (DMSO- $d_6$ , 500 MHz, ppm):  $\delta$  11.99 (s, 1H, NH); 8.79 (d, 2H, Ar-H); 8.44 (s, 1H, -CH=); 7.971-7.957 (dd, 2H, Ar-H); 7.836-7.828 (m, 3H, Ar-H); 7.734 (d, 2H, Ar-H); 7.526 (s, 1H, H-Thiazol); 7.511 (d, 2H, Ar-H); 7.195 (d, 2H, Ar-H); 5.31 (d, 2H, -CH<sub>2</sub>-). Anal.Calcld.(%) for  $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_2\text{S}$  (414.48): C, 66.65; H, 4.38; N, 13.52; S, 7.73. Found: C, 66.43; H, 4.37; N, 13.49; S, 7.75. MS (EI, 70 eV):  $m/z$  414  $[M]$ , 174  $[\text{C}_{10}\text{H}_8\text{NS}]$ , 106  $[\text{C}_7\text{H}_5\text{O}]$ , 104  $[\text{C}_7\text{H}_5\text{N}]$ , 71  $[\text{C}_2\text{HNS}]$ .

*Isonicotinic acid [3-(2-phenyl-thiazol-4-ylmethoxy)-benzylidene]-hydrazide (6).*

Yield 75%, yellow solid, mp: 87-90 °C. IR (KBr,  $\text{cm}^{-1}$ ): 3218 (NH), 3058 ( $\text{CH}_{\text{arom}}$ ), 2922 and 2858 ( $\text{CH}_{\text{aliph}}$ ), 1652 (C=O), 1607 (C=C), 1553 (C=N).  $^1\text{H}$  NMR (DMSO- $d_6$ , 500 MHz, ppm):  $\delta$  12 (s, 1H, NH); 8.84 (d, 2H, Ar-H); 8.4 (s, 1H, -CH=); 7.984-7.962 (dd, 2H, Ar-H); 7.857-7.833 (m, 3H, Ar-H); 7.78-7.65 (m, 3H, Ar-H); 7.53 (s, 1H, H-Thiazole); 7.172 (m, 1H, Ar-H); 5.34 (d, 2H, -CH<sub>2</sub>-). Anal.Calcld.(%) for  $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_2\text{S}$  (414.48): C, 66.65; H, 4.38; N, 13.52; S, 7.73. Found: C, 66.42; H, 4.37; N, 13.48; S, 7.75. MS (EI, 70 eV):  $m/z$  414  $[M]$ , 308  $[M-\text{C}_6\text{H}_4\text{NO}]$ , 238  $[\text{C}_{15}\text{H}_9\text{O}_2]$ , 174  $[\text{C}_{10}\text{H}_8\text{NS}]$ , 106  $[\text{C}_7\text{H}_5\text{O}]$ , 104  $[\text{C}_7\text{H}_5\text{N}]$ , 71  $[\text{C}_2\text{HNS}]$ .

### *Chemistry*

Analytical thin layer chromatography was carried out on precoated Silica Gel 60F<sub>254</sub> sheets using UV absorption for visualization. The melting points were established using two melting point meters, Electrothermal and MPM-H1 Schorpp and were uncorrected. FT-IR spectra were recorded on a Nicolet 210 FT-IR spectrometer with a MCT detector and an Omnic 4.1b soft system, using potassium bromide. The <sup>1</sup>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 dimethyl sulfoxide - DMSO-*d*<sub>6</sub> ( $\delta_{\text{H}} = 2.51\text{ppm}$ ) as solvent and the spectra were recorded using a single excitation pulse of 12  $\mu\text{s}$ . Gas chromatography – mass spectrometry (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 performed with a Vario El CHNS instrument.

### *Antimicrobial screening*

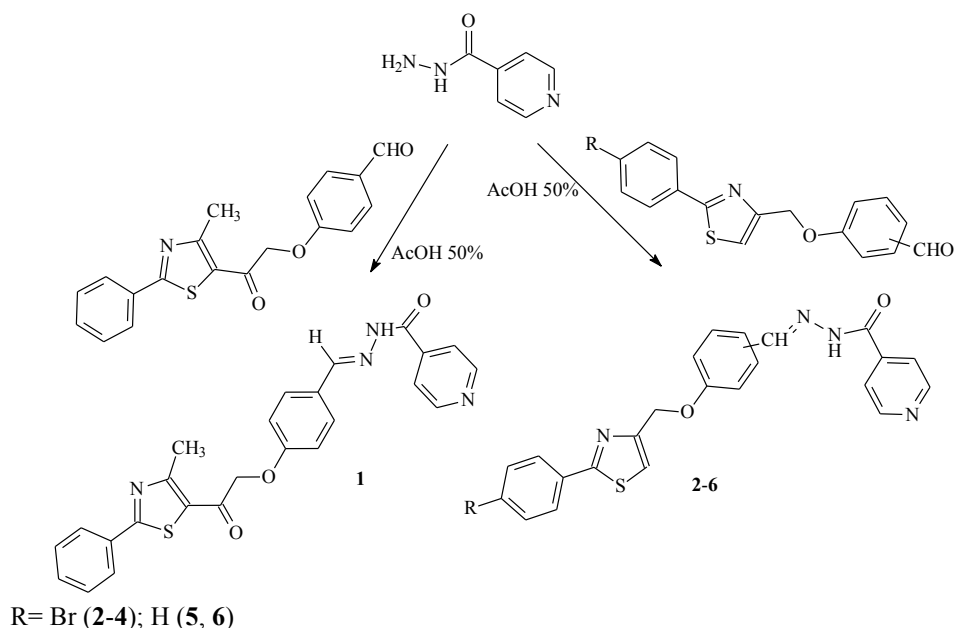
For the antimicrobial screening, the agar disk diffusion method was used. The solutions of the testing compounds were prepared by dissolving the corresponding amount of compounds in DMSO, in order to have a final concentration of 10mg/mL. Each paper disk of 6mm diameter was impregnated with 10 $\mu\text{L}$  of solution, in order to have 100 $\mu\text{g}$  compound/disk.

The filter paper disks were placed on Petri dishes previously seeded “in layer” with the tested bacterial strain inoculums. The Petri dishes were left at room temperature to ensure the equal diffusion of the compound in the medium and afterwards the dishes were incubated at 37°C for 24 hours. Inhibition zones were measured after 24 hours of incubation.

## **Results and Discussion**

### *Chemistry*

The new hydrazones were obtained by condensing isonicotinic hydrazide (HIN) with 4-[2-(4-Methyl-2-phenyl-thiazol-5-yl)-2-oxo-ethoxy]-benzaldehyde or 2, 3 or 4-(2-Aryl-thiazol-4-ylmethoxy)-benzaldehyde, in refluxing acetic acid 50% (Figure 1). The synthesis of the 2-aryl-thiazol-yl benzaldehyde derivatives used as reactants was previously presented [11]. The purity of the compounds was demonstrated by thin layer chromatography (TLC). The structures of all new compounds were confirmed by elemental analysis and spectroscopic data (IR, <sup>1</sup>H NMR, MS).

**Figure 1**

Synthesis of new isonicotinic hydrazide (HIN) derivatives.

### ***Spectral data***

#### ***MS spectra***

The characteristic peaks were observed in the mass spectra of the compounds. Molecular ion peaks ( $M^+$ ) provided the molecular formula of all synthesized compounds. Characteristic  $M^{+2}$  isotope peaks were observed in the mass spectra of the compounds **2-4**, because of the isotopic contribution of halogen in their molecules. The major fragments that were encountered in almost all the spectra were formed by the fragmentation of the thiazole ring, the cleavage of  $-H_2C-O-$  and  $CH=N$  bonds and by different fragmentations of the hydrazide moiety.

#### ***IR spectra***

The IR spectra of all newly synthesized HIN derivatives showed the appearance of the absorption bands at about  $1658-1652\text{ cm}^{-1}$  characteristic for the carbonyl group in hydrazide moiety and  $\nu(N-H)$  bands in CONH were observed in the  $3397-3201\text{ cm}^{-1}$  region. Each of the new aryl-hydrazones displayed a strong and sharp band in the region  $1555-1552\text{ cm}^{-1}$  ascribed to  $\nu(C=N)$  of azomethine group, providing evidence for the condensation reaction between the aldehyde group and the hydrazide. The absorption band for  $\nu(C=O)$  (aldehyde) did not occur for the synthesized compounds. The band at  $1669\text{ cm}^{-1}$  was assigned to  $\nu(C=O)$  group in the 5

position of the thiazole ring for compound **1**. The  $\nu(\text{Ar-Br})$  absorption bands in the  $1070\text{--}1068\text{ cm}^{-1}$  region were observed for the compounds **2-4**.

#### *<sup>1</sup>H NMR spectra*

The exhibited chemical shifts obtained from <sup>1</sup>H NMR spectra confirmed the proposed structures of the compounds. The chemical shift of the NH proton appeared in the form of singlet peak at  $\delta$  12-11.95 ppm. The N=CH proton signal appeared at  $\delta$  8.44-8.36 ppm in the form of singlet peak. The aromatic protons signals were registered in the  $\delta$  8.84-7.122 ppm region in the form of multiple peaks (doublet, double doublets or multiplet). The proton in the 5 position of thiazole ring signal appeared in the range of  $\delta$  7.869-7.45 ppm as singlet peak for the compounds **2-6**. One singlet signal appeared in the  $\delta$  5.435-5.3 ppm region is consistent with the two protons of the methylene group. The chemical shift of the three protons from methyl group belonging to compound **1** appeared at  $\delta$  2.785 ppm in the form of a singlet peak. The chemical shifts for all the new synthesized compounds were in good agreement with those calculated. Calculations of the <sup>1</sup>H NMR chemical shifts were performed using ACD/HNMR Predictor software [12].

#### *The structure of the compounds*

The resulting double bond  $\text{--HC=N--}$  following the condensation reaction, contributes to the formation of geometrical isomers *Z* and *E*. Geometrical isomerism may have an important role in the bioactivity of the aroyl-hydrazones and in their capability of bonding with the target. One distinctive feature to be remarked is the amide proton resonance which is well downfield of all other peaks. Thus, the <sup>1</sup>H NMR spectra indicated the chemical shift of the NH proton at  $\delta$  12-11.95 ppm in the form of a singlet peak of an intense signal, assigned to the NH proton of *E* isomer. The presence of single downfield resonating (8.44-8.36 ppm) CH=N signal accounts for formation of *E*-isomers exclusively. The dominating isomer possesses an *E*-configuration, according to the literature [13-15].

Hydrazones display prototropic amido-imido tautomerism. The possibility of this type of tautomerism involving a hydroxilic group was excluded for all the compounds, by the evident absence of typical signals of an OH group in the IR and <sup>1</sup>H NMR spectra. The IR absorption of the hydrazone  $\nu(\text{NH})$  group at  $3397\text{--}3201\text{ cm}^{-1}$ , together with a strong band at about  $1658\text{--}1652\text{ cm}^{-1}$  for the  $\nu(\text{C=O})$  group, confirms the amido tautomeric form in solid state.

#### *Antimicrobial testing*

All the newly synthesized HIN derivatives **1-6** were tested for their *in vitro* antimicrobial activity against three Gram-positive bacteria namely, *Bacillus cereus* (ATCC 13061), *Staphylococcus aureus* (ATCC 9144) and

*Enterococcus faecalis* (CIP 103907), and eight Gram-negative bacteria strains namely, *Escherichia coli* (clinical), *Salmonella typhimurium* (clinical), *Salmonella typhimurium* (ATCC 13311), *Salmonella enterica* (CIP 105150), *Shigella dysenteriae* (CIP 5154), *Shigella flexneri* (clinical), *Proteus mirabilis* (ATCC 35659), *Enterobacter aerogenes* (CIP 104725).

The screening was performed using the disk diffusion method. Ampicillin, Ciprofloxacin, Gentamicin and Co-trimoxazole were used as reference antibacterial agents. In all the determinations, the results were registered as a mean of at least three determinations and are presented in Table I.

The compounds indicated no inhibitory activity against Gram-positive bacteria, with the exception of compound **6** which showed an inhibitory effect against *Bacillus cereus* (ATCC 13061) and *Staphylococcus aureus* (ATCC 9144). This compound was also the only inhibiting the growth of *Proteus mirabilis* (ATCC 35659).

Compound **4** proved to have a more potent inhibitory activity than the reference drugs Ampicillin and Ciprofloxacin against Gram-negative bacteria *Enterobacter aerogenes* (CIP 104725). Against *Salmonella typhimurium* (ATCC 13311), compound **3** indicated a more potent inhibitory activity than Ampicillin. The other HIN-derivatives showed a poor to moderate inhibitory effect on Gram-negative bacterial strains. The compounds presented no inhibitory effect on *Shigella dysenteriae* (CIP 5154), nor on *Enterococcus faecalis* (CIP 103907).

**Table I**  
*In vitro* antibacterial activity of newly synthesized compounds **1-6** by the disk diffusion assay technique

| Compound              | Diameter of growth inhibition zone <sup>a</sup> (mm) |      |        |      |      |     |     |      |        |      |      |
|-----------------------|------------------------------------------------------|------|--------|------|------|-----|-----|------|--------|------|------|
|                       | E.c                                                  | B. c | S. t 1 | S. d | S. a | P.m | E.a | S. f | S. t 2 | E. f | S. e |
| <b>1</b>              | 12                                                   | -    | -      | -    | -    | -   | 11  | -    | -      | -    | -    |
| <b>2</b>              | 12                                                   | -    | -      | -    | -    | -   | -   | 9    | 10     | -    | 10   |
| <b>3</b>              | -                                                    | -    | 14     | -    | -    | -   | 11  | -    | 17     | -    | -    |
| <b>4</b>              | -                                                    | -    | -      | -    | -    | -   | 17  | -    | 12     | -    | -    |
| <b>5</b>              | 11                                                   | -    | -      | -    | -    | -   | -   | -    | -      | -    | -    |
| <b>6</b>              | 12                                                   | 17   | 15     | -    | 15   | 13  | -   | -    | -      | -    | 15   |
| <b>Ampicillin</b>     | 21                                                   | -    | 21     | 12   | 16   | 24  | 10  | 18   | 12     | 15   | -    |
| <b>Ciprofloxacin</b>  | 34                                                   | 32   | 38     | 39   | 31   | 36  | 10  | 31   | 41     | 24   | 42   |
| <b>Gentamicin</b>     | 25                                                   | 32   | 31     | 31   | 27   | 20  | 21  | 34   | 36     | 34   | 35   |
| <b>Co-trimoxazole</b> | 28                                                   | 24   | -      | 33   | 35   | 29  | -   | 30   | 43     | 34   | 35   |

-: No activity.

<sup>a</sup> Mean of three replicates. E.c, *Escherichia coli* (clinical); B.c, *Bacillus cereus* (ATCC 13061); S.t.1, *Salmonella typhimurium* (clinical); S.t.2, *Salmonella typhimurium* (ATCC 13311); S.d, *Shigella dysenteriae* (CIP 5154); S.a, *Staphylococcus aureus* (ATCC 9144); P.m, *Proteus mirabilis* (ATCC 35659); E.a, *Enterobacter aerogenes* (CIP 104725); S.f, *Shigella flexneri* (clinical); E.f, *Enterococcus faecalis* (CIP 103907); S.e, *Salmonella enterica* (CIP 105150).

### Conclusions

The synthesis and the screening of the antimicrobial activity of a new series of HIN derivatives are reported in this paper. The compounds were obtained by the reaction between a series of benzaldehyde-derivatives and HIN, in acetic acid 50%. The new structures were confirmed by TLC, elemental analysis and spectroscopic data (MS, IR,  $^1\text{H}$  NMR).

Regarding the antimicrobial activity, the compounds showed no inhibitory effect on *Shigella dysenteriae* (CIP 5154), nor on *Enterococcus faecalis* (CIP 103907). Only compound **6** developed an inhibitory effect on Gram-positive bacteria and also on *Proteus mirabilis* (ATCC 35659). The newly synthesized molecules were found to be poorly to moderately potent against the other microorganisms. Compound **4** proved to have a more potent inhibitory activity than the reference drugs Ampicillin and Ciprofloxacin against Gram-negative bacteria *Enterobacter aerogenes* (CIP 104725). Against *Salmonella typhimurium* (ATCC 13311), compound **3** showed a more potent inhibitory activity than Ampicillin.

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