Synthesis and Characterization of Some New Salicylamide Derivatives with Potential Biological Activity

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In order to obtain biological active compounds, the synthesis of some new O-substituted salicylanilides was achieved. In reaction between N-(3-bromo-phenyl)-2-hydroxy-benzamide and chloro-acetic acid ethyl ester, the appropriate ethyl ester was obtained. The ethyl ester was condensed with hydrazine giving a hydrazide. In the last step of the synthesis, the hydrazide was condensed with chloro-substituted benzaldehyde obtaining a hydrazone. All new synthesized compounds were characterized by modern physico-chemical methods (FTIR, ^{1}H-NMR, ^{13}C-NMR, MS), which confirm their structures.

Keywords: N-(3-bromo-phenyl)-2-hydroxy-benzamide derivatives, ethyl esters, hydrazides, hydrazones, O-substituted-salicylanilide

A variety of reasons, including inappropriate and excessive use of antibiotics, has led to the emergence of pathogenic bacterial strains that are highly resistant to most or all current antibiotics [1,2].

So there is a significant need for discovery of new types of antimicrobials to treat infections caused by resistant organisms. However, there has been relatively limited interest by the pharmaceutical industry in discovery and clinical development of novel types of antimicrobials because of the adequacy of existing antibiotics to treat the majority of infections, the small market at present for newer antimicrobials, the high development costs, and the potential for development of bacterial resistance [3,4].

Salicylanilides have been of interest for synthetic organic chemists for long time due to their biological activity (antimicrobial and tuberculostatic activity). The antibacterial properties of halogenated salicylanilides have been reported previously [5-7]. For instance, 3,5-dibromo-*N*-(3-chloro-phenyl)-benzamide has been reported to have antibacterial and fungicidal activity [5], whereas 4'-bromo analogues of this compound were shown to inhibit *E. coli* growth [6]. Salicylanilides, as well as *O*-substituted salicylanilides, are a class of compounds with a broad spectrum of biological activity [8-10], including the antimicrobial effect against a number of yeast and filamentous fungi. Substitution of phenoxyacetic acid with an electrophile group in *orto* or *para* position increases their activity against human pathogenic fungi [11,12].

The purpose of this research was to synthesize some novel 2-hydroxibenzamide derivatives with potential antimicrobial activity and to obtain their complete characterization , using modern physico-chemical methods.

Experimental part

Materials and methods

The reagents are ethyl chloroacetate, *N*-(3-bromo-phenyl)-2-hydroxy-benzamide (Aldrich, for synthesis); hydrazinium monohydrate ($N_2H_4 \cdot H_2O$) (Merck, for synthesis); 2-chlorobenzaldehyde.

The solvents are absolute ethanol, ethyl methyl ketone, dimethyl-formamide (Merck, analytical purity).

Melting points were measured with a Böetius Carl-Zeiss Jena apparatus. The IR spectra, as KBr pellet, were recorded on Jaskow FT/IR-430 instrument. NMR spectra were recorded in DMSO, on "Bruker Avance 300" instrument. Mass spectra were recorded in methanol on a high capacity ion trap, HCT Ultra PTM instrument (Bruker, Daltonics, Bremen), interfaced to a PC running the Compass™ 1.2. integrated software package, which includes the Hystar™ 3.2.37 module for instrument controlling and spectrum acquisition, Esquire Control™ 6.1.512 and Data Analysis™ 3.4.179 modules for storing the ion chromatograms and processing the MS data, coupled with a NanoMate robot (Advion Biosciences, Inc., USA).

Synthesis of the ethyl ester (**1**) [13]

Ethyl ester (**1**) was obtained by the reaction of *N*-(3-bromo-phenyl)-2-hydroxy-benzamide with ethylchloro-acetate in ethyl methyl ketone. A mixture of *N*-(3-bromo-phenyl)-2-hydroxy-benzamide (0.01 mol) and anhydrous K_2CO_3 (0.01 mol) was refluxed in 50 mL ethyl methyl ketone. Chloro-acetic acid ethyl ester (0.01 mol) was added dropwise. Optimum molar ratio was amide:ester:K_2CO_3 = 1:1:1. The mixture was stirred and heated on a steam bath for 5 h. After cooling at room temperature, the mixture was poured onto water and shaken intensively. The organic phase was separated and dried over $MgSO_4$. After filtration and evaporation of solvent in vacuum, the ethyl ester was crystallized and than re-crystallized from ethanol. Ethyl ester (**1**) (M.p. = 110-112°C) was obtained with 73% yield, after the final purification.

Synthesis of the hydrazide (**2**) [14]

A mixture of ethyl ester (**1**) (0.0075 mol) and hydrazine hydrate (1.65 mL, 0.0075 mol) was refluxed in 25 mL ethanol for 3 h. The reaction mixture was cooled and the separated solid was filtered off, and then re-crystallized from ethanol. Hydrazide (**2**) (M.p. = 163-165°C) was obtained with 92% yield, after the final purification.

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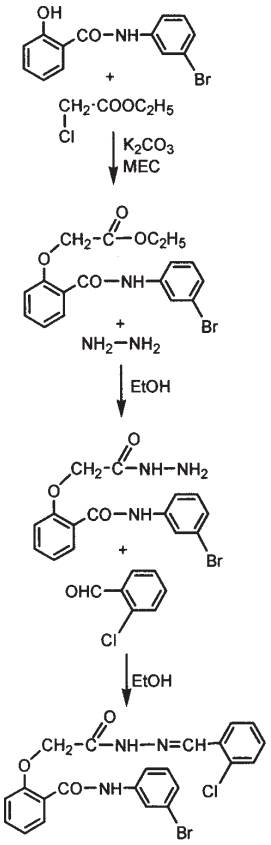

[2-(3-Bromo-phenylcarbamoyl)-phenoxy]-acetic acid ethyl ester (**1**)
$C_{17}H_{16}BrNO_4$ (Mol. Wt. = 377.03)

N-(3-Bromo-phenyl)-2-hydrazinocarbonyl-methoxy-benzamide (**2**)
$C_{15}H_{14}BrN_3O_3$ (Mol. Wt. = 363.02)

N-(3-Bromo-phenyl)-2-(2-chloro-benzylidene-hydrazinocarbonyl-methoxy)-benzamide (**3**)
$C_{22}H_{17}BrClN_3O_3$ (Mol. Wt. = 485.01)

Fig. 1. The obtaining pathways of the synthesized compounds.

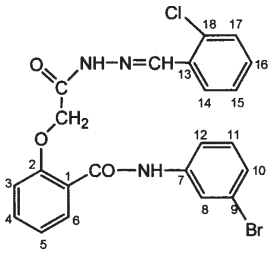

Fig. 2. Numbering of the aromatic rings

Synthesis of the hydrazone (**3**) [14]

To a solution of hydrazide (**2**) (0.003 mol) in 25 mL ethanol, the appropriate benzaldehyde (0.003 mol) was added. The reaction mixture was refluxed for 5 h. The solid obtained after cooling was filtered off, washed with water and re-crystallized from dimethylformamide. Hydrazone (**3**) (M.p. = 175-177°C) was obtained with 78% yield, after the final purification.

Results and discussions

The synthesized compounds are white-grey crystalline substances (needless or prisms) and were obtained with yields ranged between 70-92%.

The obtaining pathways of the synthesized compounds are presented in figure 1. The experimental results suggested that the *N*-(3-bromo-phenyl)-2-hydroxy-benzamide derivatives were readily separated and gave pure compounds.

Table 1
SYNTHESIZED COMPOUNDS CHARACTERISTICS

Comp. No.	Spectral data
1.	**IR** ν(cm^{-1}): 3322i, 1758i, 1651i, 1536i, 1217i, 1216i, 1069m, 1026s, 1106m, 753m, 679m, 600s, 532s, 435s; **^{1}H-NMR** [δ(ppm)]: 1.15 (t, 3H, COOCH$_2$C<u>H$_3$</u>); 4.15 (q, 2H, COO<u>C</u>H$_2$CH$_3$); 4.91 (s, 2H, O<u>C</u>H$_2$CO); 7.04 (d, 1H, H$_3$); 7.09 (t, 1H, H$_5$); 7.22 (m, 2H, H$_{10}$, H$_{11}$); 7.46 (t, 1H, H$_4$); 7.67 (d, 1H, H$_{12}$); 7.79 (d, 1H, H$_6$); 8.10 (s, 1H, H$_8$); 10.44 (s, 1H, CO<u>N</u>H); **^{13}C-NMR** [δ(ppm)]: 14.45 (COOCH$_2$<u>C</u>H$_3$); 61.63 (COO<u>C</u>H$_2$CH$_3$); 66.23 (O<u>C</u>H$_2$CO); 114.03 (C$_3$); 119.10 (C$_1$); 122.10 (C$_{12}$); 122.59 (C$_5$); 123.34 (C$_9$); 126.74 (C$_8$); 129.98 (C$_{10}$); 131.22 (C$_6$); 133.56 (C$_{11}$); 137.01 (C$_4$); 140.85 (C$_7$); 155.52 (C$_2$); 164.01 (<u>C</u>ONH); 169.30 (<u>C</u>OOC$_2$H$_5$); **MS^1** (m/z): 378.1 ([M+H]$^+$), 400.1([M+Na]$^+$), 416.1([M+K]$^+$); **MS^n** (m/z): 378.1, 225.0, 207.0, 179.1, 151.1, 121.2, 95.4;
2.	**IR** ν(cm^{-1}): 3342m,l, 3288m, 3051s, 1663i, 1540i, 1286s, 1227s, 1046m, 751i, 712s, 668s, 617s, 517s, 445s; **^{1}H-NMR** [δ(ppm)]: 4.73 (s, 2H, NH-<u>NH$_2$</u>); 5.12 (s, 2H, O<u>C</u>H$_2$CO); 7.05 (d, 1H, H$_3$); 7.21 (m, 3H, H$_5$, H$_{10}$, H$_{12}$); 7.46 (t, 1H, H$_{11}$); 7.69 (t, 1H, H$_4$); 8.02 (s, 1H, H$_8$); 8.12 (d, 1H, H$_6$); 9.39 (s, 1H, CO<u>N</u>H-NH$_2$); 10.81 (s, 1H, CO<u>N</u>H-Ar); **^{13}C-NMR** [δ(ppm)]: 67.72 (O<u>C</u>H$_2$CO); 114.30 (C$_3$); 119.04 (C$_8$); 122.09 (C$_1$); 122.37 (C$_{10}$); 124.58 (C$_5$); 126.69 (C$_{12}$); 131.15 (C$_6$); 131.23 (C$_{11}$); 133.35 (C$_9$); 140.99 (C$_4$); 141.11 (C$_7$); 155.66 (C$_2$); 164.67 (<u>C</u>ONH-Ar); 167.72 (<u>C</u>ONHNH$_2$); **MS^1** (m/z): 364.1 ([M+H]$^+$), 386.1([M+Na]$^+$), 402.1([M+K]$^+$); **MS^n** (m/z): 364.1, 292.0, 211.1, 193.1, 165.1, 121.2;
3.	**IR** ν(cm^{-1}): 3356m, 3297m, 1702i, 1644m, 1586i, 1247m, 1231m, 1065m, 785s, 768s, 752i, 680i, 516m, 444s; **^{1}H-NMR** [δ(ppm)]: 5.36 (s, 2H, O<u>C</u>H$_2$CO); 7.08 (t, 1H, H$_5$); 7.25 (m, 2H, H$_3$, H$_{10}$); 7.33 (m, 2H, H$_{15}$, H$_{16}$); 7.48 (t, 2H, H$_{11}$, H$_4$); 7.93 (d, 2H, H$_{14}$, H$_{17}$); 8.15 (d, 2H, H$_6$, H$_{12}$); 8.36 (s, 1H, H$_8$); 8.88 (s, 1H, -N=<u>C</u>H-); 11.01 (s, 1H, CO<u>N</u>H-Ar); 12.10 (s, 1H, CO<u>N</u>H-N=CH-); **^{13}C-NMR** [δ(ppm)]: 66.97 (O<u>C</u>H$_2$CO); 114.51(C$_3$); 119.14 (C$_1$); 122.07 (C$_{12}$); 122.15 (C$_5$); 122.49(C$_9$); 126.55(C$_8$); 127.73(C$_{15}$); 128.04(C$_{10}$); 130.34 (C$_6$); 131.13 (C$_{17}$); 131.46 (C$_{14}$); 132.08 (C$_{11}$); 132.23 (C$_{13}$); 133.59 (C$_{16}$); 134.02 (C$_4$); 141.07 (C$_{18}$); 141.18 (C$_7$); 141.40 (-N=<u>C</u>H-); 156.17 (C$_2$); 163.70 (<u>C</u>ONH-Ar); 170.32 (<u>C</u>ONHNH=CH-); **MS^1** (m/z): 486.1 ([M+H]$^+$); **MS^n** (m/z): 486.1, 313.1, 285.1, 165.0, 138.1, 121.2, 93.4;

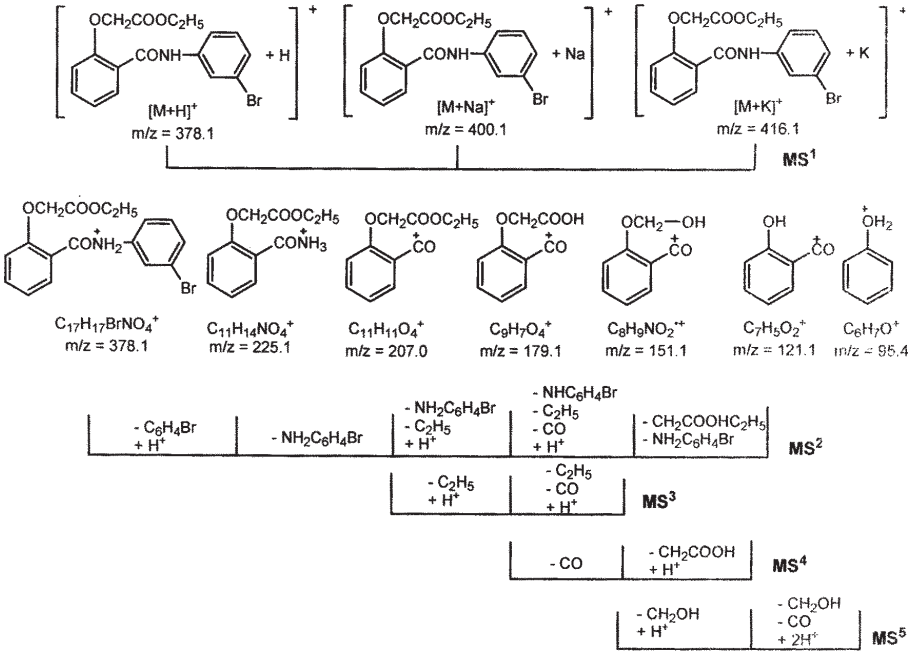

Fig. 3. The assignments of the detected positive ions and their fragments corresponding to the [2-(3-bromo-phenylcarbamoyl)-phenoxy]-acetic acid ethyl ester (**1**)

IR spectral data of ethyl ester (table 1) indicate the presence of ether bond between phenolic hydroxyl and alkyl α-C atom of ester by signals at 1216 cm^{-1} (ν^{as}COC aromatic) and 1106 cm^{-1} (ν^{as}COC alifatic). Carbonyl group from esters (νC=O) appears 1758 cm^{-1}, but, in IR spectra of the hydrazide, this band is missing, proving the conversion of the ester into hydrazide. The signals corresponding to the vibrations of the amidic and hydrazidic group appear between 3290-3350 cm^{-1} (νNH) and 1640-1670 cm^{-1} (νC=O).

The obtained compounds were also analyzed by ^{1}H- and ^{13}C-NMR in DMSO. In order to facilitate the NMR data interpretation, in figure 2, the numbering of the aromatic rings is presented. The ^{1}H-NMR shifts of ethyl group from ester appear between 1.15-4.15 ppm, that of amidic group between 10.44-11.01 ppm, that of hydrazidic group, from both, hydrazides and hydrazones, between 9.39-12.10 ppm, and that of iminic group at 8.88 ppm. The ^{13}C-NMR signals corresponding to both carbons from hydrazidic and amidic groups appear between 163-171 ppm and those for aromatic carbons between 114-157 ppm.

All synthesized compounds were characterized by chip-electrospray ionization (chip-ESI) mass spectrometry, which was employed by using the coupling of a high capacity ion trap (HCT) mass spectrometer instrument and a NanoMate robotic system.

To analyze samples, the NanoMate uses a conductive pipette tip to draw sample from a 96 well-plate. The sample-filled tip aligns with a nozzle inlet on the back of the disposable ESI chip, creating a tight seal. Each pipette tip and nozzle is used only once, providing a unique path into the mass spectrometer and eliminating sample carryover. The coupling generates a system that combines automatic chip-based ESI infusion with ultra-fast screening and multistage fragmentation at superior sensitivity. For MS analysis, the samples were dissolved in methanol (spectrophotometric grade, ≥ 99.9%) to a concentration of approximately 20-30 pmol/μL and infused into MS by chip-ESI.

Both (+) ESI MS^{1} and tandem mass spectra (+) ESI MS^{n} (n=2-5) were acquired. MS data for the synthesized compounds are presented in table 1.MS^{1} and mass calculation revealed only the presence of the molecular ions corresponding to monoprotonated molecules, $[M+H]^{+}$, and/or species exhibiting sodiated and potassiated adducts: $[M+Na]^{+}$ and $[M+K]^{+}$. Unlike classical tandem MS in a single dissociation phase, the consecutive fragmentation episodes of up to MS^{5} applied to the precursor ion and its derived fragments, provided not only a strict control upon the dissociation process but also the unique possibility to resequence small fragment ions of the same precursor until unequivocal structure elucidation.

Interpretation of MS^{2}-MS^{5} spectra showed that obtained multistage sequencing data unambiguously corroborate the structure of the synthesized compounds. In figure 3, the assignments of the detected positive ions and their fragments corresponding to the ethyl ester product (**1**) are presented.

Conclusions

In the present work, novel compounds belonging to *O*-substituted salicylanilides class were obtained and will be analyzed for their biological activity in our future experiments.

The methods used for all synthesis are relatively simple and require short reaction time. The 1:1 molar ratio for reagents gave good yields (>70%) after final purification.

To provide the identity of all new synthesized compounds, these were characterized by IR, NMR and chip-electrospray ionization mass spectrometry (chip-ESI-MS).

As a novelty in the field, NanoMate robot was coupled to a high capacity ion trap mass spectrometer (HCT MS) to create a system merging automatic chip-based ESI infusion, ultra-fast ion detection and multistage sequencing at superior sensitivity.

The employed analytical methods prove the identity and provide the elemental composition of all investigated compounds.

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