Novel N-(2-bromo-phenyl)-2-hydroxy-benzamide Derivatives with Antifugal Activity

IOANA MARIA CARMEN IENASCU[1,2], TIBERIUS BALAES[3], CRISTIANA VIRGINIA PETRE[3], RALUCA OANA POP[4], ADINA CATA[1]*, MARIANA NELA STEFANUT[1], PAUL ALBU[2], MARIOARA POENARU[4]

[1]National Institute of Research and Development for Electrochemistry and Condensed Matter, 144 Dr. A. P. Podeanu Str., 300569, Timisoara, Romania;

[2]Vasile Goldis Western University of Arad, Faculty of Pharmacy, 86 Liviu Rebreanu, 310045, Arad, Romania

[3]Alexandru Ioan Cuza University of Iasi, Anastasie Fatu Botanical Garden, 7-9 Dumbrava Rosie, 700487, Iasi, Romania

[4]University of Medicine and Pharmacy Victor Babes Timisoara, Faculty of Pharmacy, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

In order to increase the biological activity, some novel molecules, esters, hydrazides, hydrazones of N-(2-bromo-phenyl)-2-hydroxy-benzamide, were obtained in good yields (86-93%), working at 150 °C, 500 W, 7-11 min, under microwave irradiation. All synthesized compounds were characterized using modern physico-chemical methods (FTIR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis). Eight dilutions in dimethyl sulfoxide of these derivatives were tested against two phyto-pathogenic fungi, Fusarium oxysporum, Sclerotinia sclerotiorum and one common yeast, Saccharomyces cerevisiae. The antifungal activity was assessed using disc diffusion method, both negative, pure DMSO, and positive control, nystatin, were used. S. cerevisiae was slightly more sensitive than filamentous fungi, the strongest inhibition, MIC=0.3125g/L, was observed for N-(2-bromo-phenyl)-2-hydroxy-benzamide and N-(2-bromo-phenyl)-2-(4-dimethylamino-benzylidene-hydrazinocarbonylmethoxy)-benzamide. The most active compounds against F. oxysporum and S. sclerotiorum were N-(2-bromo-phenyl)-2-hydroxy-benzamide (MIC= 0.625g/L), N-(2-bromo-phenyl)-2-hydrazinocarbonylmethoxy-benzamide (MIC=1.25g/L) and N-(2-bromo-phenyl)-2-(4-dimethylamino-benzylidene-hydrazinocarbonyl-methoxy)-benzamide (MIC=0.625g/L), N-(2-bromo-phenyl)-2-hydrazinocarbonyl-methoxy-benzamide (MIC=1.25g/L), respectively.

Keywords: N-(2-bromo-phenyl)-2-hydroxy-benzamide fungicides, Fusarium oxysporum, Sclerotinia sclerotiorum, Microwave-assisted synthesis, MIC

Fungal pathogens can cause devastating damages of crops and postharvest fruits all over the world [1]. *Fusarium spp.* includes some strains pathogenic to plants and difficult to control [2]. Diseases caused by Fusarium spp., especially Fusarium wilt, Fusarium crown and root rot are among the most studied plant diseases [3]. *Fusarium oxysporum,* a soilborne fungus, is responsible for fusarium wilt disease, one of the most important diseases of tomato, worldwide [4]. *Sclerotinia sclerotiorum* (Lib.) de Bary is a plant pathogen with a global spreading, that can contaminate hundreds of plant species, as well as essential crops like sunflower, rapeseed and soybean [5]. For instance, this fungus causes Sclerotinia stem rot in crops, which is a very damaging disease that can lead to substantial losses [6]. On the other hand, fungi may also be pathogenic to humans. *Fusarium* species cause several infections in immuno-compromised patients, sinusitis in immuno-competent people while dietary, respiratory, dermal, and other exposures to toxic fungal metabolites produce the diseases called mycotoxicoses [7, 8].

Nowadays, fungicides are the principal means for controlling fungal infection on affected crops [9]. However, pollution of surface and underground water with pesticides became a global concern, since most of these compounds are very persistent, bioaccumulative and toxic compounds [10,11]. Because of their enormous populations and high rate of mutation, a large amount of pathogenic fungi acquired resistance to the extensively used fungicides. Some important fungicides belonging to different chemical classes (polyenes, anilinopyrimidine, benzimidazoles, echinocandins, dicarbo-ximide, phenylpyrrole), have lost their efficiency against pathogenic fungi [12]. Thus, the widely use of benzimidazole and dicarboximide fungicides is responsible for fungicide resistance in *S. sclerotiorum* resulted in control failures [13,14]. So, it is required to find more effective fungicides with complementary ways of action and low toxicity to substitute the compounds that are now used to control fungal infections, in order to increase diseases control.

Salicylic acid and its derivatives are used as fungicides and disinfectants. Free salicylic acid is used as preservative for fruits and vegetables, meanwhile, salicilanilide is a widely used fungicide for controlling brown spot of some harvests. Chlorosalicylanilide and dichlorosalicylanilide showed stronger fungicidal activity. Salicylanilides containing bromine in their molecules are also used for the treatment of some fungal diseases [15]. Antifungal activity of salicylanilide derivatives, esters, hydrazides and hydrazones, was tested *in vitro* against *Candida albicans* and *Sacharomyces cerevisiae*. The MIC values obtained for ethyl esters were comparable to those for salicylanilide, but the hydrazides and hydrazones proved to possess superior biological activity in comparison with the control substance [16].

In this study, we investigated the potential of some new compounds, N-(2-bromo-phenyl)-2-hydroxy-benzamide derivatives, against two phyto-pathogenic fungi, *Fusarium oxysporum* and *Sclerotinia sclerotiorum*, and against *Saccharomyces cerevisiae*, one of the most common and studied yeast, so that the results can easily be compared with those reported in similar studies.

*email: adina.cata@yahoo.com

Experimental part

Chemicals and equipment

The reagents used for syntheses are: 2-bromoaniline, phosphorus trichloride (Acros Organics, for synthesis); salicylic acid, ethyl chloroacetate, methyl chloroacetate (Sigma-Aldrich, for synthesis); hydrazine monohydrate, 4-dimethylaminobenzaldehyde, 5-bromosalicylaldehyde (Merck, for synthesis). Solvents, chlorobenzene (Scharlau, analytical purity); absolute ethanol, dimethylformamide, 2-butanone (Merck, analytical purity), and were used with no further purification.

The syntheses were conducted on a Microwave oven Speed Wave MWS–2, Berghof, Germany. Melting points are uncorrected and measured using SMP30 Melting Point apparatus, Stuart, UK. IR spectra (ν_{max} in cm^{-1}) were recorded as KBr pellet, on a Vertex 70 FTIR instrument, Bruker, Germany. The ^{1}H, ^{13}C-NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ on an Avance DRX 400 spectrometer, Bruker, Germany, operating at 400 MHz. Chemical shifts (d values in ppm) are expressed against tetramethylsilane (TMS) as internal standard and coupling constants (*J*) are reported in Hz. Elemental analyses were recorded on an Elemental Combustion System 4010, Costech Instruments, USA. For antifungal activity evaluation, peptone, glucose, agar (Sigma-Aldrich, analytical purity), dimethyl sulfoxide (Merck, analytical purity), laminar flow cabinet (Faster KBN Carbo 600, Combi chemical filter), were used.

Microwave-assisted synthesis

Anilide (**1**). To a mixture of salicylic acid (0.003 mol), 2-bromoaniline (0.003 mol) and phosphorus trichloride (0.001 mol), chlorobenzene (5 mL) was added. The reaction mixture was taken in a sealed Teflon tube, placed in a microwave oven and irradiated at 500 W, 150°C, for 11 min. After cooling at room temperature, the crude product obtained was filtered off, washed three times successively with 5 mL hot water and then three times successively with 5 mL 10% sodium carbonate solution. The solid product was then filtered off, dried and recrystallized from dimethylformamide.

Ethyl/Methyl esters (**2**,**3**). To a mixture of N-(2-bromo-phenyl)-2-hydroxy-benzamide (0.002 mol), chloro-acetic acid ethyl/methyl ester (0.002 mol) and potassium carbonate (0.002 mol), 2-butanone (10 mL) was added. The reaction mixture was taken in a sealed Teflon tube, placed in a microwave oven and irradiated at 500 W, 150 °C, for 11 min. After cooling, the solid product was filtered off, washed with water, dried and recrystallized from ethanol.

Hydrazide (**4**).

To a mixture of ethyl ester (0.002 mol) and hydrazine hydrate (0.002 mol), absolute ethanol (10 mL) was added. The reaction mixture was taken in a sealed Teflon tube, placed in a microwave oven and irradiated at 500 W, 150°C, for 7 min. After cooling, the solid product was filtered off, washed with water, dried and recrystallized from ethanol.

*Hydrazones (**5**,**6**).* To a mixture of hydrazide (0.002 mol) and substituted benzaldehydes (0.002 mol), absolute ethanol (10 mL) was added. The reaction mixture was taken in a sealed Teflon tube, placed in a microwave oven and irradiated at 500 W, 150 °C, for 9 min. After cooling, the solid product was filtered off, washed with water, dried and recrystallized from dimethylformamide.

Antifungal activity

Culture media and inoculum. For both obtaining inoculum and for testing plates, Sabouraud medium has been used (10 $g \cdot L^{-1}$ peptone, 20 $g \cdot L^{-1}$ glucose, 5 $g \cdot L^{-1}$ agar), on Petri dishes of 9 cm diameter.

Fungal species

Three fungal species: *Saccharomyces cerevisiae*, *Sclerotinia sclerotiorum* (isolated from celery root), *Fusarium oxysporum* f. sp. *lilii* (isolated from daffodil bulbs) have been tested. All the strains are deposited in the Culture collection of *Laboratory for Research of Fungi with Applications in Soil Reconstruction-RECOSOL,* Faculty of Biology, Alexandru Ioan Cuza University of Iasi.

Inocula. For the test plates with *S. cerevisiae*, a suspension of 10^6 CFU/mL from 72 h old culture has been prepared as inoculum, used to flood Petri dishes of 9 cm diameter with 1 mL of suspension and the plate has been exposed under laminar flow cabinet for 20 min in order to let the excess of water evaporate. In case of *S. sclerotiorum* and *F. oxysporum*, agar discs of 9 mm diameter, covered with mycelium, from 7 days old cultures, were cut from the edges of colonies and placed in the center of each test plate.

Screening. The antifungal activity was tested through disc diffusion method [17], placing sterile filter paper discs of 9 mm diameter at an equal distance to the center of the test plate (the point of inoculation), followed by covering each disc with 100 µL solution. An additional method, well diffusion method [18], was used, each well of 9 mm diameter being fulfilled with 100 µL solution. The plates were incubated for 72 h at 28 °C in case of *Saccharomyces cerevisiae* and for 10 days at 25 °C in case of *Fusarium oxysporum* and *Sclerotinia sclerotiorum*. Both negative control consisting in pure DMSO and a positive control consisting in nystatin were used. Experiments were carried out in triplicate.

Results and discussions

The title compounds were obtained using microwave-assisted synthesis. Starting from salicylic acid and 2-bromoaniline, anilide (**1**), ethyl/methyl esters (**2**,**3**), hydrazide (**4**) and hydrazones (**5**,**6**), were obtained. Except N-(2-bromo-phenyl)-2-hydroxy-benzamide (**1**), derivatives **2-6** are novel compounds. The synthesized compounds (**1-6**) are white/yellow, crystalline substances, needles or prisms. The synthetic route for preparation of the title compounds is presented in figure 1. The anilide and hydrazones were purified by re-crystallization from dimethylformamide, meanwhile the esters and hydrazide were purified by re-crystallization from absolute ethanol. After the final purification, the compounds were obtained in good yields (86-93%). *O*-subtituted-hydroxybenzamide derivatives were prepared previously, by our research group, using conventional heating synthesis [19,20]. Higher yields and especially less reaction times, indicate the importance of microwave method over the conventional method. Microwave-enhanced chemical reaction rates were faster than those of conventional heating method used to obtain related compounds by at least 25 fold.

IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis were used to confirm the structures of the synthesized derivatives. Molecular formula, molecular weight, melting points and yields, along spectral data characteristics, for each compound, are shown below. The numbering of the aromatic rings used for the NMR spectra interpretation is presented in figure 2.

*N-(2-Bromo-phenyl)-2-hydroxy-benzamide (**1**).* Yield: 86%; MP: 158-160 °C; Anal. Calcd. for $C_{13}H_{10}BrNO_2$ (292.13 g/mol): C, 53.45, H, 3.45, N, 4.79 %. Found C, 53.34, H, 3.19,

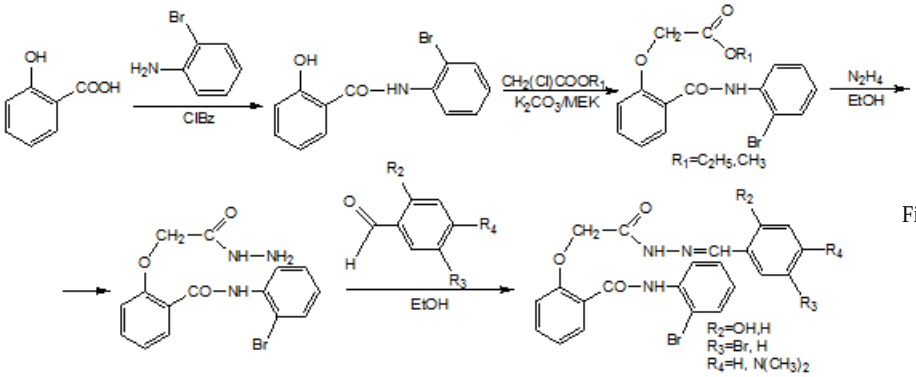

Fig. 1. Synthesis of the N-(2-bromo-phenyl)-2-hydroxy-benzamide derivatives

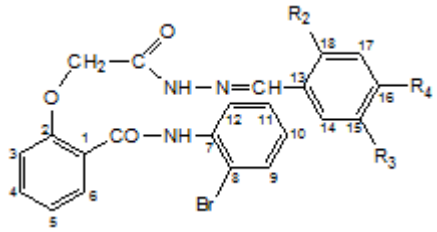

Fig. 2. Numbering of aromatic rings

N, 44.81 %; IR (KBr, cm^{-1}): 3105m,l, 1635i, 1609i, 1588i, 1544i, 1498m, 1454i, 1435i, 1365m, 1275s, 1234m, 1157s, 1136s, 1119s, 1095s, 1047s, 1028m, 955s, 904s, 823s, 759m, 741i, 693m, 677m, 588s, 527s, 439s; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 6.96 (tsc, 1H, H_{10}, J=8.0); 7.06 (dsc, 1H, H_3, J=8.0); 7.12 (tsc, 1H, H_5, J=8.0); 7.44 (m, 2H, H_4, H_{11}, J=8.0); 7.10 (dsc, 1H, H_9, J=8.0); 8.05 (d_{sc}, 1H, H_{12}, J=8.0); 8.35 (d_{sc}, 1H, H_6, J=8.0); 10.99 (s, 1H, CONH); 12.05 (s, 1H, OH); ^{13}C-NMR (400 MHz, $CDCl_3$, δ / ppm): 114.56 (C_8); 117.18 (C_3); 117. 81 (C_1); 119.23(C_5); 123.69 (C_{12}); 125.75 (C_{10}); 128.24 (C_{11}); 130.49 (C_6); 132.57 (C_9); 133.78 (C_4); 136.63 (C_7); 157.45 (C_2); 164.41 (CONH).

*[2-(2-Bromo-phenylcarbamoyl)-phenoxy]-acetic acid ethyl ester (**2**).* Yield: 88 %; MP: 86-88 °C; Anal. Calcd. for $C_{17}H_{16}BrNO_4$ (378.22 g/mol): C, 53.99, H, 4.26, N, 3.70 %. Found C, 54.18, H, 4.32, N, 3.71 %; IR (KBr, cm^{-1}): 3325m, 3074s, 1755i, 1654i, 1601m, 1584i, 1535i, 1483i, 1457i, 1438i, 1380m, 1361s, 1313i, 1277s, 1195i, 1064m, 752i; ^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): 1.17 (t, 3H, $COOCH_2CH_3$, J=7.2); 4.17 (q, 2H, $COOCH_2CH_3$, J=7.2); 5.22 (s, 2H, OCH_2COO); 7.13 (tsc, 1H, H_{10}, J=8.0); 7.24 (t, 1H, H_5, J=7.6); 7.24 (d, 1H, H_3, J=7.6); 7.44 (tsc, 1H, H_{11}, J=8.0);7.59 (tsc, 1H, H_4, J=7.6); 7.71 (dsc, 1H, H_9, J=8.0); 8.09 (d_{sc}, 1H, H_{12}, J=8.0); 8.30 (d, 1H, H_6, J=7.6); 10.38 (s, 1H, CONH); ^{13}C-NMR (400 MHz, $CDCl_3$, δ / ppm): 13.86 ($COOCH_2CH_3$); 61.02 ($COOCH_2CH_3$); 65.63 (OCH_2CO); 113.55 (C_3); 114.88 (C_8); 121.52 (C_1); 121.93 (C_5); 123.69 (C_{12}); 125.93 (C_{10}); 128.20 (C_{11}); 131.58 (C_6); 132.53 (C_9); 133.63 (C_4); 136.40 (C_7); 155.53 (C_2); 162.67 (CONH); 168.30 ($COOCH_2CH_3$).

*[2-(2-Bromo-phenylcarbamoyl)-phenoxy]-acetic acid methyl ester (**3**).* Yield: 87 %; MP: 119-120 °C; Anal. Calcd. for $C_{16}H_{14}BrNO_4$ (364.19 g/mol): C, 52.77, H, 3.87, N, 3.85 %. Found C, 52.99, H, 3.66, N, 3.76 %; IR (KBr, cm^{-1}): 3315m, 3075s, 1740i, 1660i, 1600m, 1585i, 1537i, 1486m, 1459m, 1435i, 1351m, 1314i, 1263m, 1204i,1060m, 755i; ^{1}H-NMR (400 MHz, DMSO-d_6, δ/ ppm): 3.72 (s, 3H, $COOCH_3$); 5.24 (s, 2H, OCH_2COO); 7.13 (tsc, 1H, H_{10}, J=8.0); 7.20 (t, 1H, H_5, J=8.4); 7.23 (d, 1H, H_3, J=8.4); 7.44 (tsc, 1H, H_{11}, J=8.0); 7.58 (tsc, 1H, H_4, J=8.4); 7.71 (dsc, 1H, H_9, J=8.0); 8.08 (d_{sc}, 1H, H_{12}, J=8.0); 8.29 (d, 1H, H_6, J=8.4); 10.37 (s, 1H, CONH); ^{13}C-NMR (400 MHz, $CDCl_3$, δ / ppm): 52.09 ($COOCH_3$); 65.47 (OCH_2CO); 113.46 (C_3); 114.86 (C_8); 121.50 (C_1); 121.92 (C_5); 123.67 (C_{12}); 125.93 (C_{10}); 128.19 (C_{11}); 131.58 (C_6); 132.53 (C_9); 133.62 (C_4); 136.40 (C_7); 155.48 (C_2); 162.66 (CONH); 168.82 ($COOCH_3$).

*N-(2-Bromo-phenyl)-2-hydrazinocarbonylmethoxy-benzamide (**4**).* Yield: 93 %; MP: 156 °C; Anal. Calcd. for $C_{15}H_{14}BrN_3O_3$ (364.19 g/mol): C, 49.47, H, 3.87, N, 11.54 %. Found C, 50.46, H, 3.73, N, 11.70 %; IR (KBr, cm^{-1}): 3307m, 2975s, 2933s, 1667i, 1625m, 1600m, 1588i, 1537i, 1483m, 1454m, 1261s, 1211m, 1056s, 977s, 939s, 750m; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 4.35 (s, 2H, $NH-NH_2$); 4.90 (s, 2H, OCH_2COO); 7.16 (m, 3H, H_{10}, H_3, H_5); 7.44 (tsc, 1H, H_{11}, J=8.0); 7.56 (tsc, 1H, H_4, J=8.4); 7.71 (dsc, 1H, H_9, J=8.0); 8.01 (d_{sc}, 1H, H_{12}, J=8.0); 8.20 (d, 1H, H_6, J=8.4); 9.51 (s, 1H, $CONH-NH_2$); 10.48 (s, 1H, CONH-Ar); ^{13}C-NMR (400 MHz, $CDCl_3$, δ / ppm): 66.84 (OCH_2CO); 113.46 (C_3); 115.64 (C_8); 121.65 (C_1); 122.37 (C_5); 124.40 (C_{12}); 126.10 (C_{10}); 128.12 (C_{11}); 131.27 (C_6); 132.58 (C_9); 133.31 (C_4); 136.56 (C_7); 155.80 (C_2); 163.23 (CONH-Ar); 166.43 ($CONHNH_2$).

*2-(5-Bromo-2-hydroxy-benzylidene-hydrazino-carbonylmethoxy)-N-(2-bromo-phenyl)-benzamide (**5**).* Yield: 89 %; MP: 272-273 °C; Anal. Calcd. for $C_{22}H_{17}Br_2N_3O_4$ (547.20 g/mol): C, 48.29, H, 3.13, N, 7.68%. Found C, 49.53, H, 2,93, N, 7,30%; IR (KBr, cm^{-1}): 3437m,l, 3329m, 1686i, 1607i, 1588m, 1533i, 1484m, 1452m, 1279m, 1211m, 1066m, 811s, 745i; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 5.61 (s, 2H, OCH_2COO); 6.88 (d, 1H, H_{17}, J=8.8); 7.17 (m, 2H, H_{10}, H_5); 7.31 (d, 1H, H_3, J=8.4); 7.43 (m, 1H, H_{11}, H_6); 7.57 (tsc, 1H, H_4, J=8.4); 7.70 (dsc, 1H, H_9, J=8.0); 7.95 (s_{sc}, 1H, H_{14}, J=8.0); 8.07 (d_{sc}, 1H, H_{12}, J=8.0); 8.27 (d, 1H, H_6, J=8.4); 8.28 (s, 1H, -N=CH-); 10.39(s, 1H, OH); 10.67 (CO-NH-Ar); 11.73 (s, 1H, CONH-N=CH-); ^{13}C-NMR (400 MHz, $CDCl_3$, δ / ppm): 66.13 (OCH_2CO); 110.84 (C_3); 113.86 (C_8); 115.11 (C_{15}); 118.32 (C_{17}); 121.51 (C_1); 121.60 (C_{13}); 122.40 (C_5); 123.98 (C_{12}); 125.89 (C_{10}); 127.98 (C_{11}); 128.12 (C_6); 131.50 (C_9); 132.57 (C_4); 133.52 (C_{14}); 133.56 (C_{16}); 136.63 (C_7); 139.75 (-N=CH-); 155.58 (C_{18}); 156.22 (C_2); 163.00 (CONH-Ar); 168.76 (CONH-N=CH-).

*N-(2-Bromo-phenyl)-2-(4-dimethylamino-benzylidene-hydrazinocarbonylmethoxy)-benzamide (**6**).* Yield: 92%; MP: 220-221°C; Anal. Calcd. for $C_{24}H_{23}BrN_4O_3$ (495.37 g/mol): C, 58.19, H, 4.68, N, 11.31%. Found C, 57,34, H, 4,49, N, 11,16%; IR (KBr, cm^{-1}): 3424m,l, 3308m, 1711i, 1696i, 1643i, 1601m,1587i, 1537i, 1479i, 1436m, 1394m, 1344s, 1319m, 1298m, 1281m, 1271i, 1209i, 1078m, 828m, 751i, 731m; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.98 (s, 6H, $-N(CH_3)_2$); 5.53 (s, 2H, OCH_2CO); 6.74 (d, 2H, H_{15}, H_{17}, J=8.8); 7.17 (m, 2H, H_5, H_{10}); 7.27 (d, 1H, H_3, J=8.4); 7.46 (m, 2H, H_4, H_{11}); 7.56 (d, 2H, H_{14}, H_{18}, J=8.8); 7.70 (dsc, 1H, H_9, J=8.0); 7.91 (s, 1H, -N=CH-); 8.07 (dsc, 1H, H_{12}); 8.25 (d, 1H, H_6, J=7.2); 10.70 (s, 1H, CONH-Ar); 11.46 (s, 1H, CONH-N=CH-); ^{13}C-NMR (400 MHz, $CDCl_3$, δ / ppm): 38.85-40.10 ($N(CH_3)_2$); 66.07 (OCH_2CO); 111.65 (C_{15}, C_{17}); 113.70 (C_3); 115.26 (C_8); 121.11 (C_1); 121.47 (C_{13}); 121.60 (C_5); 124.17 (C_{12}); 125.94 (C_{10}); 128.11 (C_{11}); 128.33 (C_{14}, C_{18}); 128.46 (C_6); 132.57 (C_9); 133.53 (C_4); 136.64 (C_7); 145.28 (C_{16}); 151.44 (-N=CH-); 156.23 (C_2); 163.00 (CONH-Ar); 168.11 (CONH-N=CH-).

Saccharomyces cerevisiae, 72 hours after inoculation									
Conc.	Tested compounds						Negative control (DMSO)	Positive control (Nystatin)	
	1	2	3	4	5	6		Conc.	Inhibition halo (mm)
20	+++	++	0	+	++	+	0	3.5	5
10	++	+	0	+	++	+		2.0	5
5.0	++	+	0	+	+	+		1.0	4.9
2.5	++	+	0	+	+	0		0.1	4.5
1.25	+	0	0	+	+	0		0.01	3.8
0.625	+	0	0	0	+	0		0.05	2.9
0.3125	+	0	0	0	+	0		0.0025	1.3
0.156	0	0	0	0	0	0		0.00125	0.8
								0.000625	0
Fusarium oxysporum, 10 days after inoculation									
Conc.	Tested compounds						Negative control (DMSO)	Positive control (Nystatin)	
	1	2	3	4	5	6		Conc.	Inhibition zone (mm)
20	+++	0	0	++	++	++	0	3.5	3.9
10	++	0	0	+	+	++		2	3.6
5	++	0	0	+	+	++		1	3.4
2.5	++	0	0	+	0	+		0.1	3.1
1.25	++	0	0	+	0	0		0.01	3
0.625	+	0	0	0	0	0		0.05	2.8
0.3125	0	0	0	0	0	0		0.0025	2.4
0.156	0	0	0	0	0	0		0.00125	2
								0.000625	1.2
Sclerotinia sclerotiorum, 10 days after inoculation									
Conc.	Tested compounds						Negative control (DMSO)	Positive control (Nystatin)	
	1	2	3	4	5	6		Conc.	Inhibition zone (mm)
20	+	+	0	+++	+++	0	0	3.5	4.3
10	+	+	0	++	+++	0		2	3.6
5	+	+	0	++	++	0		1	3
2.5	0	0	0	+	++	0		0.1	0.7
1.25	0	0	0	+	+	0		0.01	0.4
0.625	0	0	0	0	+	0		0.05	0.2
0.3125	0	0	0	0	0	0		0.0025	0
0.156	0	0	0	0	0	0		0.00125	0
								0.000625	0

Table 1
ANTIFUNGAL ACTIVITY OF THE TITLE COMPOUNDS TESTED WITH THE DISC DIFFUSION METHOD (100 µL SOLUTION PER DISC; CONCENTRATION EXPRESSED AS $g \cdot L^{-1}$)

The IR spectral data of the anilide indicate the presence of a large band corresponding to the vibration of phenolic hydroxyl group at 3105 cm^{-1}, band that is absent in the IR spectra of the esters. The presence of the ether bond between the phenolic hydroxyl group and the alkyl α-C atom of the ester is proved by signals in the range 1060-1070 and 1310-1320 cm^{-1}. The signals between 1740–1760 cm^{-1} correspond to the ester carbonyl group. This band is missing in the IR spectra of the hydrazide, which indicates the conversion of the ethyl ester into hydrazide. The signals corresponding to the vibrations of the amide and hydrazide group appear between 3100-3440 and 1620-1720 cm^{-1}.

The ^{1}H-NMR shifts of the phenolic hydroxyl from the anilide appears at 12.05 ppm, that of the methyl group from the methyl ester at 3.72 ppm, that of ethyl group from the ethyl ester appear between 1.1 and 4.2 ppm, that of the amide group between 10.3 and 11.0 ppm, that of the hydrazide group, from both hydrazides and hydrazones, between 9.5 and 11.8 ppm and that of the imine group between 7.9 and 8.3 ppm.

The ^{13}C-NMR signals corresponding to the carbons from the hydrazide and amide groups appear in the range 162–169 ppm and those of the aromatic carbons between 110 and 158 ppm.

The elemental analysis data are in agreement with the theoretical values.

Eight dilutions in dimethyl sulfoxide of the 6 synthesized compounds were used in the antifungal screening. Nystatin, as positive control, has been used in different concentrations compared with the tested compounds, based on available literature concerning its minimum inhibitory concentration over yeast and phyto-pathogenic fungi [21-23]. Dimethyl sulfoxide was used as negative control.

The results, obtained using *disc diffusion method*, are presented in table 1. The antifungal activity of the tested compounds, expressed as minimum inhibitory concentration (MIC), varied from 0.3 up to 5.0 $g \cdot L^{-1}$. Due to low solubility of the analyzed compounds, their diffusion in medium was reduced, and therefore the inhibition halo has not exceeded 3-4 mm around the disc. Even when the concentration of the compound strongly increased compared to the smallest concentration at which the inhibition was observed, the halo did not became larger. The halo was more distinct in the case of *S. cerevisiae*, where the colonies were very dens outside the halo. In the case of filamentous fungi, the control discs (pure DMSO) were covered with mycelium, while the discs containing tested compounds produced a halo around them, smaller or larger depending on the used concentration.

In case of *Saccharomyces cerevisiae*, the strongest activity (MIC = 0.3125 $g \cdot L^{-1}$) was observed for both N-(2-bromo-phenyl)-2-hydroxy-benzamide (**1**) and 2-(5-bromo-

2-hydroxy-benzylidene-hydrazinocarbonylmethoxy)-N-(2-bromo-phenyl)-benzamide (**5**). *Fusarium oxysporum* was most strongly inhibited by N-(2-bromo-phenyl)-2-hydroxy-benzamide (**1**) (MIC = 0.625 g·L^{-1}) followed by N-(2-bromo-phenyl)-2-hydrazinocarbonylmethoxy-benzamide (**4**) (MIC = 1.25 g·L^{-1}). 2-(5-Bromo-2-hydroxy-benzylidene-hydrazinocarbonylmethoxy)-N-(2-bromo-phenyl)-benzamide (**5**) (MIC = 0.625 g·L^{-1}) and N-(2-bromo-phenyl)-2-hydrazinocarbonylmethoxy-benzamide (**4**) (MIC = 1.25 g·L^{-1}) showed the lowest MIC's when tested against *Sclerotinia sclerotiorum*. In comparison, the positive control (nystatin) produced larger inhibition halos. The antifungal activity of the tested compounds was not as strong as for nystatin, but the inhibition was positive.

Among the synthesized compounds, only the *[2-(2-bromo-phenylcarbamoyl)-phenoxy]-acetic acid methyl ester (**3**)* proved no antifungal activity against the tested microorganisms.

Although proved to be efficient [18,24], the well diffusion method was not effective in our study, no inhibition being observed in any of the experiments. We believe that the low solubility of the compounds is a barrier against diffusion in the substrate (culture medium).

Conclusions

Five new N-(2-bromo-phenyl)-2-hydroxy-benzamide derivatives, esters, hydrazides and hydrazones, were obtained in higher yield and purity using microwave irradiation synthesis.

The title compounds were characterized using IR, NMR and elemental analysis techniques. The obtained data prove the identity and create a complete characterization of newly synthesized compounds.

In order to evaluate the antifungal activity, all synthesized compounds were tested *in vitro* against two phyto-pathogenic fungi and one yeast. The MIC values were lower than 5.0 g·L^{-1}, which demonstrates that the compounds are moderately active against the tested fungal strains. Beside the anilide (**1**), the most active compounds proved to be the hydrazide (**4**) and hydrazone (**5**). *S. cerevisiae* was slightly more sensitive than filamentous fungi.

These results are promising, so further functionalization of this key intermediate is needed for obtaining new derivatives with higher antimicrobial activities.

References

1. WANG, H. , YAN, Y., WANG, J., ZHANG, H., QI, W., PLoS ONE 7 nr. 1, 2012, p. e29452.
2. YUAN, J., RAZA, W., SHEN, Q., HUANG, Q., Appl. Environ. Microbiol., 78 nr. 16, 2012, p. 5942.
3. MCGOVERN, R. J., Crop Protection, 73, 2015, p.78.
4. AMINI, J., SIDOVICH, D., J. Plant Prot. Res., 50 nr. 2, 2010, p. 172
5. BOLAND, G., HALL, R., Can. J. Plant Pathol., 16, 1994, p. 93.
6. LI, G., HUANG, H., MIAO, H., ERICKSON, R., JIANG, D., XIAO, Y., Eur. J. Plant Pathol., 114, 2006, p. 345.
7. NUCCI, M., ANAISSIE, E., Clin. Microbiol. Rev., 20 nr. 4, 2007, p. 695.
8. BENNETT, J. W., KLICH, M., Clin. Microbiol. Rev., 16 nr. 3, 2003, p. 497.
9. BARDIN, S., HUANG, H., Can. J. Plant Pathol., 23, 2001, p. 88.
10. NEZIRI, A., SHABANI, Z., J. Environ. Prot. Ecol., 14, nr. 2, 2013, p. 447.
11. NEZIRI, A., MALOLLARI, I., LAHO, E., GOLEMI, S., J. Environ. Prot. Ecol., 14, nr. 3, 2013, p. 851.
12. YANG, L., XIE J., JIANG, D., FU, Y., LI, G., LIN, F., World J. Microb. Biot., 24, 2008, p. 909.
13. MA, H. X., FENG, X. J., CHEN, Y., CHEN, C. J., ZHOU, M. G., Plant Dis., 3, 2009, p. 36.
14. ZHANG, X., SUN, X., ZHANG, G., Chin. J. Pest. Sci. Administration, 24, 2003, p.18.
15. MELNIKOV, N. N., Chemistry of Pesticides, Aromatic carboxylic acids and their derivatives, Spriger-Verlag, Gunther, F. A., Gunther, J. D. (Eds.), New York Inc., 1971, p. 148-149.
16. IENASCU, I., LUPEA, A. X., HADARUGA, D., HADARUGA, N., POPESCU, I., Rev. Chim.(Bucharest), **59**, no. 2, 2008, p. 247.
17. YAMAC, M., BILGILI, F., Pharmaceutical Biology, 44, nr. 9, 2006, p. 660.
18. SEN, A., BATRA, A., Int. J. Curr. Pharm. Res., 4, nr. 2, 2012, p. 67.
19. IENASCU, I. M. C., POPESCU, I. M., STEFANUT, M. N., CATA, A., TANASIE, C., BALCU, I., Rev. Roum. Chim.(Bucharest), **60**, nr. 5-6, 2015, p. 615.
20. IENASCU, I. M. C., LUPEA, A. X., POPESCU, I. M., PĂDURE, M. A., ZAMFIR, A.D., J. Serb. Chem. Soc., 74, nr. 8–9, 2009, p. 847.
21. MAHMOUDABADI, A. Z., DRUCKER, D. B., J. Nat. Pharm. Prod., 1, 2006, p. 18.
22. KAPLANCIKLI, Z. A., ALTINTOP, M. D., DEMIREL, R., Turk J. Pharm. Sci., 10, nr. 1, 2013, p. 145.
23. TZATZARAKIS, M. N., TSATSAKIS, A. M., CHARVALOS, E., VAKALOUNAKIS D., J. Environ. Sci. Health. B., 36, nr. 3, 2001, p. 331.
24. VALGAS, C., DE SOUZA, S.M., SMÂNIA, E. F. A, SMÂNIA, A., Braz. J. Microbiol., 38, 2007, p. 369.

Manuscript received: 16.01.2018