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ORIGINAL ARTICLE

CONVENTIONAL AND GREEN SYNTHESIS UNDER SOLVENT-FREE MICROWAVE IRRADIATION OF 2-(4-(PHENYLDIAZENYL)PHENOXY) ACETIC ACID DERIVATIVES AND THEIR BIOLOGICAL ACTIVITY

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Abstract

Several 2-(4-(phenyldiazenyl)phenoxy)acetic acid derivatives were prepared by refluxing method in an aqueous medium, and solvent-free and catalyst-free microwave-assisted method. Retrosynthetic approaches and structural analysis of the target compounds were performed. Spectral characterization (IR, ^{1}H NMR, ^{13}C NMR) is reported. The antibacterial activity of the products was tested, the strongest effect being against *S. aureus*.

Rezumat

În acest articol este prezentată obținerea unor derivați ai acidului 2-(4-(fenildiazenil)fenoxi) acetic prin două metode: la reflux în mediu apos, respectiv cu ajutorul microundelor fără solvent și fără catalizator. Compușilor țintă li s-a efectuat analiza retrosintetică și analiza structurală. Produșii au fost caracterizați spectral (IR, RMN-^{1}H, RMN-^{13}C). Activitatea antibacteriană a compușilor a fost testată, efectul cel mai puternic fiind față de *S. aureus*.

Keywords: 2-(4-(phenyldiazenyl)phenoxy) acetic acid, microwave, spectroscopic analysis, antibacterial activity

Introduction

Phenylacetic acid derivatives are valuable compounds having practical uses in varied fields of human activity. Many pharmacological agents such as analgesic [1, 2], hypolipidemic [3], anti-inflammatory [2, 4], antifungal [5, 6], antibacterial [7, 8] and new anticancer agents [9] contain the phenoxy acetic acid scaffold.

Herbicides based on phenoxyacetic acid derivatives are widely used due to the low price and water solubility. In addition, they are easily degradable, biological or photolytic. They are employed in agriculture and on recreational surfaces [10, 11].

The use of phenoxyacetic acid derivatives as precursors of side chains plays an important role in industrial biosynthesis of penicillins [12]. The specific production rate of penicillin V as a function of the phenoxyacetic acid concentration followed Michaelis-Menten-type kinetics. Thus, the higher is the concentration of phenoxyacetic acid, the higher becomes the productivity of penicillin V [13].

Based on these data, we synthesized some phenoxyacetic acid derivatives with azobenzene skeleton (Figure 1).

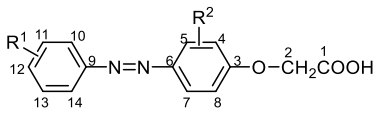

Figure 1.
Phenoxyacetic acid derivatives with azobenzene scaffold

Materials and Methods

The substances used are all commercial products from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and were used without any prior purification. Melting points were measured with the help of a Gallenkamp digital melting point apparatus manufactured by Sanyo Electric Co (Osaka, Japan). IR/FT spectra were recorded with an ALPHA FTIR/ATR spectrometer produced by Bruker Optics GmbH (Ettlingen, Germany). Ultraviolet-visible spectra were recorded in 2.5 x 10^{-4} mol/L 1,4-dioxane solution using a Cary 50 UV-Vis spectrophotometer manufactured by Varian Inc. (Darmstadt, Germany). A Rohnson P-2012 microwave, manufactured by Rohnson International Limited (Prague, Czech Republic), was employed. A digital infrared IR laser thermometer version 900-En-00 produced by Shenzhen Jumaoyuan Science and Technology Co. Ltd. (Shenzhen, China), was utilized. ^{1}H NMR spectra were recorded in $CDCl_3$ solution with TMS as internal reference, using a BRUKER ARX 400 spectrometer produced by Bruker Optics GmbH (Ettlingen, Germany), in a magnetic field of induction B = 9.33T (frequency of 400.13 MHz). ^{13}C NMR spectra were obtained in solution of $CDCl_3$ with TMS as internal reference on a BRUKER ARX 400 spectrometer using a magnetic field with induction B = 2.34T (frequency of 100.62 MHz). The chemical shifts (δ) were recorded in parts *per* million (ppm), compared to the internal standard (TMS) and coupling constants (J) in Hz. The following

standard abbreviations for signal multiplicity were used for the ^{1}H NMR spectra: s (singlet), sbr (broad singlet), d (doublet), t (triplet), q (quartet), spt (septet), m (multiplet), dd (double doublet), td (triple doublet). The glassware was purchased from BTC Glass Design SRL (Bucharest, Romania). A Vilber Lourmat UV lamp (λ = 254 nm) and CN6 darkroom for chromatographic plates fabricated by VILBER LOURMAT Germany GmbH (Eberhardzell, Germany) was employed. Elemental analyses were accomplished with a Carlo Erba model 1106 elemental analyser fabricated by Carlo Erba SpA (Milano, Italy). A BOECO rotary evaporator RVO 400 SD made by Boeckel Germany GmbH (Hamburg, Germany) was utilized. An Extech EA10 dual input digital thermometer manufactured by Extech Instruments Corporation (Nashua, NH, USA) was employed.

Synthesis of intermediates para-hydroxyazobenzene derivatives

Diazotization reaction. To a 100 mL beaker is added 0.06 mol of primary aromatic amine in 15 mL of H_2O. Under magnetic stirring and ice cooling, 16.5 mL of 32% HCl solution (d = 1.16; 0.167 mol HCl) is added gradually to the initial suspension or emulsion. The resulting mixture is cooled to a temperature below 5°C. Sodium nitrite (6,279 g, 0.091 mol) is dissolved in water (16.5 mL), and then this solution is cooled to a temperature below 5°C and added dropwise under intense magnetic stirring. During diazotization, the temperature of the medium should not exceed 5°C. After completion of the addition of aqueous sodium nitrite, the reaction is perfected at the same temperature (T < 5°C) during 45 min. At the end of the reaction, a solution containing the diazonium salt of the amine arose. Urea (1.3 g, 0.021 mol) is added in the solution of diazonium salts and continues stirring for 10 minutes. With starch-iodide paper (it turns blue, if nitrous acid is in excess), the absence of HNO_2 is checked (otherwise, more urea is added to destroy nitrous acid).

Coupling reaction. To a 500 mL beaker with magnetic stirring, we added 210 mL of H_2O, 0.06 mole of phenol derivative and 14.28 g (0.105 mole) of CH_3COONa x $3H_2O$. With a 20% NaOH solution, the pH is brought to 11 (at this value the phenols used are completely dissolved). The resulting solution is cooled with ice to a temperature below 5°C, afterwards the solution cooled with diazonium salt is gradually added. During coupling reaction, the temperature of the reaction medium is strictly maintained below 5°C. After adding all of the diazonium salt solution, the reaction is kept for 45 minutes at the same temperature. The precipitate formed is filtered off, dried and purified by recrystallization from acetic acid. Using this protocol (**a** and **b**) the yield is quite good.

4-(phenyldiazenyl)phenol (I). m.p. = 150 - 151°C; Lit. m.p. = 149 - 151°C [14] yield 85%; Anal. Calc. for $C_{12}H_{10}N_2O$: C 72.72, H 5.05, N 14.14. Found: C 72.68, H 5.00, N 14.08.

^{1}H NMR δ/ppm: 6.92 - 7.89 (m, *J* = 8 Hz, 9H, H-4,5,7,8,10,11,12,13,14), 9.21 (sbr, 1H, OH); ^{13}C NMR δ/ppm: 116.2 (C-4, C-8), 129.9 (C-10, C-14), 125.4 (C-5, C-7), 129.2 (C-11, C-13), 130.8 (C-12), 146.7 (C-6), 153.2 (C-9), 161.1 (C-3); IR (cm^{-1}): 3400-3135m, 1505vi, 1488m, 1417i, 1145i, 1108m, 806 m; UV-Vis (λ nm): 344 nm (π-π*), 436 nm (n-π*).

2-chloro-4-(phenyldiazenyl) phenol (II). m.p. = 85 - 85.5°C; Lit. m.p. = 84.5 - 85.5°C [15]; yield 66%; Anal. Calc. for $C_{12}H_9N_2OCl$: C 61.93, H 3.87, N 12.04; found: C 61.75, H 3.82, N 11.78; ^{1}H NMR δ/ppm: 6.94 (d, *J* = 8.2 Hz, 1H, H-8), 7.41 - 7.51 (m, 3H, H-11,12,13), 7.60 (dd, *J* = 8.2 Hz, *J* = 2.0 Hz, 1H, H-7), 7.62 - 7.70 (m, 3H, H-5,10,14), 7.77 (sbr, 1H, OH); ^{13}C NMR δ/ppm: 117.0 (C-8), 122.1 (C-4), 122.5 (C-10, C-14), 122.9 (C-7), 125.1 (C-5), 129.2 (C-11, C-13), 130.3 (C-12), 143.1 (C-6), 152.7 (C-9), 153.6 (C-3); IR (cm^{-1}): 3350-3001m, 1510vi, 1490m, 1418w, 1139m, 1105w, 798m; UV-Vis (λ nm): 350 nm (π-π*), 440 nm (n-π*).

*2-allyl-4-(phenyldiazenyl) phenol (III)***.** m.p. = 90 - 91°C; Lit. m.p. = 89 - 91 [16]; yield 74%). Anal. Calc. for $C_{14}H_{12}N_2O$: C 75, H 5.35, N 12.5; found: C 75.21, H 5.73, N 12.62; ^{1}H NMR δ/ppm: 5.08 (dd, *J* = 10 Hz, *J* = 2.1 Hz, 2H, =CH_2), 5.14 (dd, *J* = 16.6 Hz, *J* = 2.1 Hz, 2H, H-7,8), 6.08 (ddt, *J* = 16.6 Hz, *J* = 10.1 Hz, *J* = 7.7 Hz, 1H, =CH), 7.03 - 8.03 (m, 6H, H-5, 10,11,12,13,14), 9.25 (sbr, 1H, OH); ^{13}C NMR δ/ppm: 34.2 (CH_2), 116.1 (=CH_2), 117.1 (C-8), 122.5 (C-10, 14), 122.8 (C-7), 124.1 (C-5), 127.2 (C-4), 129.2 (C-11, 13), 130.3 (C-12), 136.7 (=CH), 142.4 (C-6), 152.6 (C-9), 156.1 (C-3); IR (cm^{-1}): 3450-3250m, 1638w, 1511i, 1495m, 1416w, 1140w, 1100m, 800i; UV-Vis (λ nm): 350 (π-π*), 445 (n-π*).

4-(para-tolyldiazenyl) phenol (IV). m.p. = 143 - 143.5°C; Lit. m.p. = 143 - 144°C [14]; yield 88%; Anal. Calc. for $C_{13}H_{12}N_2O$: C 73.58, H 5.66, N 13.20; found: C 73.38, H 5.28, N 13.11; ^{1}H NMR δ/ppm: 2.39 (s, 3H, CH_3), 6.93 (d, *J* = 10 Hz, 2H, H-4,8), 7.35 (d, *J* = 10 Hz, 2H, H-11,13), 7.72 (d, *J* = 10 Hz, 2H, H-5,7), 7.77 (d, *J* = 10 Hz, 2H, H-10,14), 10.26 (s, 1H, OH); ^{13}C NMR δ/ppm: 21.1 (CH_3), 116.5 (C-4, 8), 120.2 (C-10,14), 125 (C-5,7), 130.5 (C-11, 13), 141 (C-12), 147.1 (C-6), 148.2 (C-9), 161.4 (C-3); IR (cm^{-1}): 3421-3997i, 1562i, 1467m, 1412w, 1130m, 987i; UV-Vis (λ nm): 350 (π-π*), 447 (n-π*).

Conventional synthesis of 2-(4-(phenyldiazenyl)-phenoxy)acetic acid derivatives

In a 50 mL Erlenmeyer flask, it was dissolved chloro-acetic acid (0.472 g, 5 mmol) in 2.5 mL of H_2O. Separately, a basic solution is prepared in a 25 mL Erlenmeyer flask by dissolving NaOH (0.4 g, 10 mmol) in 10 mL of H_2O. The aqueous chloroacetic acid solution was gradually neutralized with half of the NaOH solution with stirring and cooling into ice-water bath, ensuring

that the temperature did not exceed 5°C. Into 50 mL round bottom flask, 1-neck, outer joint, size NS 14/23, fitted with an Allihn reflux condenser, socket inner joint, size NS 14/23 and jacket length 40 cm and magnetic stirrer 5 mmol of aromatic *para*-hydroxyazo compound was mixed with the other half of the NaOH solution. After cooling to room temperature, the chloro-acetic acid salt solution was poured into the aromatic solution of the *para*-hydroxyazo compound. The reaction medium was heated to moderate refluxing in a silicone oil bath on a magnetic stirrer hot plate for 3.5 h. The mixture obtained was acidified with a 10% HCl solution until pH = 3. The resulting precipitate was filtered, dried and purified by recrystallization from acetic acid. The progress of the reaction was tracked by TLC using Merck silica gel 60 F_{254} plates (eluent, ethanol:benzene = 2:1)

Synthesis of 2-(4-(phenyldiazenyl)phenoxy)acetic acid derivatives using microwave irradiation

In a 25 mL round bottom flask, NaOH (0.08 g, 2 mmol) was mixed with 0.8 mL H_2O and stirred until a solution was obtained. In this alkaline solution, 2 mmol of *para*-hydroxyazo derivative was added and stirred (magnetically) until complete dissolution of the *para*-hydroxyazo derivative. The water is evaporated under vacuum in a rotary evaporator and sodium azophenate salt brought out. A 50% concentration solution was prepared by dissolving sodium azophenate salt in acetone and this mixture was poured into a 25 mL round bottom flask containing sodium chloro-acetate (0.233 g, 2 mmol). The acetone was removed under vacuum in a rotary evaporator and the resulting mixture was introduced in a 5 mL microwave-vial. A few drops of EtOH were added. The resulting paste was irradiated in a microwave reactor (λ = 12.2 cm, 400 W) for required time. The mixture was poured into a 50 mL Berzelius beaker containing 30 mL of H_2O and 10% HCl solution was added until pH = 3. The resulting precipitate is filtered, dried and purified by recrystallization from CH_3COOH.

2-(4-(phenyldiazenyl)phenoxy)acetic acid (V). m.p. = 225°C; yield 48% (conventional synthesis) and 90% (microwave synthesis, 5 min.); Anal. Calc. for $C_{14}H_{12}N_2O_3$: C 65.62, H 4.68, N 10.93; found: C 65.59, H 4.57, N 10.33; ^{1}H NMR δ/ppm: 4.73 (s, 2H, CH_2), 7.40 - 7.48 (m, *J* = 8 Hz, 4H, H-4,8,11,13), 7.83 - 7.86 (m, 5H, H-5,7,10,12,14), 11.4 (sbr, 1H, COOH); ^{13}C NMR δ/ppm: 65.2 (C-2), 115.4 (C-4,8), 122.5 (C-10,14), 123 (C 5,7), 129.2 (C-11,13), 130.4 (C-12), 146.9 (C-6), 152.2 (C-9), 158.7 (C-3), 170.1 (C-1); IR (cm^{-1}): 3455-3360m, 1734i, 1617i, 1591i, 1499i, 1428i, 1377w, 1250vi, 1153i, 1087i, 848i; UV-Vis (λ nm): 238 (π-π*), 350 (n-π*), 440 (n-π*).

2-(2-chloro-4-(phenyldiazenyl)phenoxy)acetic acid (VI). m.p. = 134 - 135°C; yield 47% (conventional synthesis) and 81% (microwave synthesis, 6 min.); Anal. Calc. for $C_{14}H_{11}ClN_2O_3$: C 57.83, H 3.78, N 9.63; found: C 57.67, H 3.71, N 9.52; ^{1}H NMR δ/ppm: 4.75 (s, 2H, CH_2), 7.06 (d, *J* = 7.6 Hz, 1H, H-8), 7.51 - 7.39 (m, 2H, H-11,13), 7.60 (dd, *J* = 7.6, *J* = 2.2 Hz, 1H, H-12), 7.66 (d, *J* = 2.3 Hz, 1H, H-7), 7.72 - 7.68 (m, 3H, H-5,10,14); 11 (sbr, 1H, COOH); ^{13}C NMR δ/ppm: 65.2 (C-2), 114.7 (C-8), 121.2 (C-7), 122.4 (C-10,14), 124 (C-4), 124.3 (C-5), 129.1 (C-11,13), 130.2 (C-12), 145.6 (C-6), 152.6 (C-9), 155.3 (C-3), 169.5 (C-1); IR (cm^{-1}): 3470-3350, 1733, 1588, 1414, 1262,1039, 875; UV-Vis (λ nm): 241 (π-π*), 349 (n-π*), 439 (n-π*).

*2-(2-allyl-4-(phenyldiazenyl)phenoxy)acetic acid (VII)***.** m.p. = 82 - 83°C; yield 43% (conventional synthesis) and 78% (microwave synthesis, 5 min.); Anal. Calc. for $C_{17}H_{16}N_2O_3$: C 68.91, H 5.40, N 9.45 found: C 68.88, H 5.35, N 9.39; ^{1}H NMR δ/ppm: 4.69 (s, 2H, CH_2), 5.07 (dd, *J* = 10 Hz, *J* = 2.1 Hz, 2H, $=CH_2$), 5.14 (dd, *J* = 16.6 Hz, *J* = 2.1 Hz, 2H, H-7,8), 6.09 (ddt, *J* = 16.6 Hz, *J* = 10.1Hz, *J* = 7.7 Hz, 1H, =CH), 8.02 - 7.04 (m, 6H, H-5, 10,11,12,13,14), 11.8 (sbr, 1H, COOH); ^{13}C NMR δ/ppm: 66.3 (C-2), 114.5 ($=CH_2$), 116.2 (C-8), 121.2 (C-5), 122.7 (C-10,14), 123.2 (C-7), 127.2 (C-4), 129.1(C-11, 13), 130.3 (C-12), 136.6 (=CH), 144.7 (C-6), 152.8 (C-9), 157.2 (C-3), 171.6 (C-1); IR (cm^{-1}): 3460-3330m, 1730vi, 1569m, 1590i, 1416i, 1270vi, 1060i, 863m; UV-Vis (λ nm): 231 (π-π*), 351 (n-π*), 451 (n-π*).

*2-(4-(para-tolyldiazenyl)phenoxy)acetic acid (VIII)***.** m.p. = 217°C; yield 42% (conventional synthesis) and 85% (microwave synthesis, 6 min.); Anal. Calc. for $C_{15}H_{14}N_2O_3$: C 66.66, H 5.18, N 10.37 found: C 66.61, H 5.01, N 10.12; ^{1}H NMR δ/ppm: 4.72 (s, 2H, CH_2), 2.40 (s, 3H, CH_3), 7.76 (m, *J* = 7.8 Hz, *J* = 1.2 Hz, 2H, H-4,8)), 7.27 (m, 2H, H-11,13), 7.01 (m, *J* = 6.6 Hz, *J* = 3.6 Hz, 2H, H-10,14), 7.27 (m, 2H, H-5,7), 11.5 (sbr, 1H, COOH); ^{13}C NMR δ/ppm: 21.2 (CH_3), 65.1 (C-2), 115.4 (C-4,8), 120.1 (C-10,14), 129.8 (C-5,7), 130.5 (C-11,13), 141.2 (C-12), 146.9 (C-6), 148.4 (C-9), 158.7 (C-3), 170.1 (C-1); IR (cm^{-1}): 3430-3250m, 3020w, 2914m, 1731i, 1600vi, 1583vi, 1497vi, 1427vi, 1370m, 1240vi, 1143i, 1085i, 830vi; UV-Vis (λ nm): 233 (π-π*), 352 (n-π*), 453 (n-π*).

Results and Discussion

Before proceeding to the preparation of the compounds we had to perform a retrosynthetic analysis. In this way, we have identified the synthons and the corresponding commercially available synthetic equivalent (accessible reagents).

Retrosynthetic analysis of the target molecule 2-(4-(phenyldiazenyl) phenoxy) acetic acid derivatives showed us two possible pathways (A and B) for the compound synthesis (Figure 2).

In pathway A for the synthesis of the target molecule, starting reagents are substitute derivatives of phenol. These are nitrosated in the *para* position, and the

nitrosophenols etherify with chloracetic acid to *para*-nitrosophenoxyacetic acids. The latter, through a Miller reaction [17] with primary aromatic amines, form desired products (Figure 3).

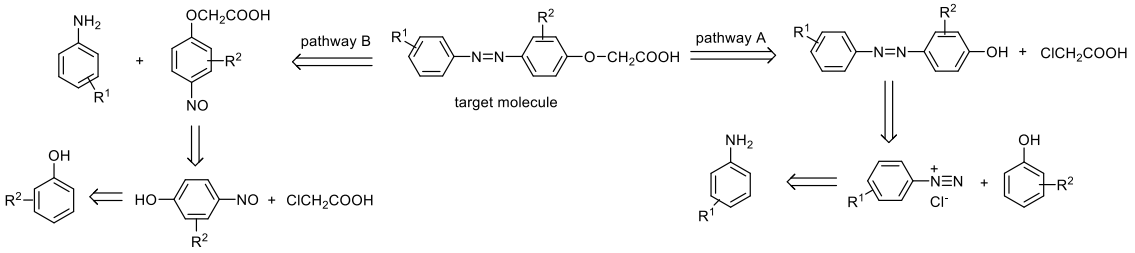

Figure 2.
Retrosynthetic analysis of 2-(4-(phenyldiazenyl)phenoxy) acetic acid derivatives

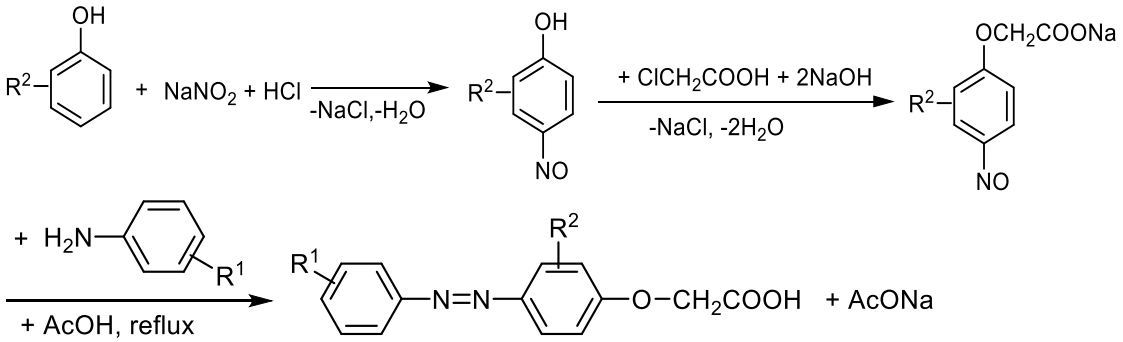

Figure 3.
Synthesis of 2-(4-(phenyldiazenyl)phenoxy) acetic acid derivatives from phenols

In path B, starting reagents are the primary aromatic amines that are converted into diazonium salts. These are coupled by an electrophilic substitution with phenols generating hydroxyazo dyes. Etherification of the latter with chloroacetic acid will lead to the wished products (Figure 4).

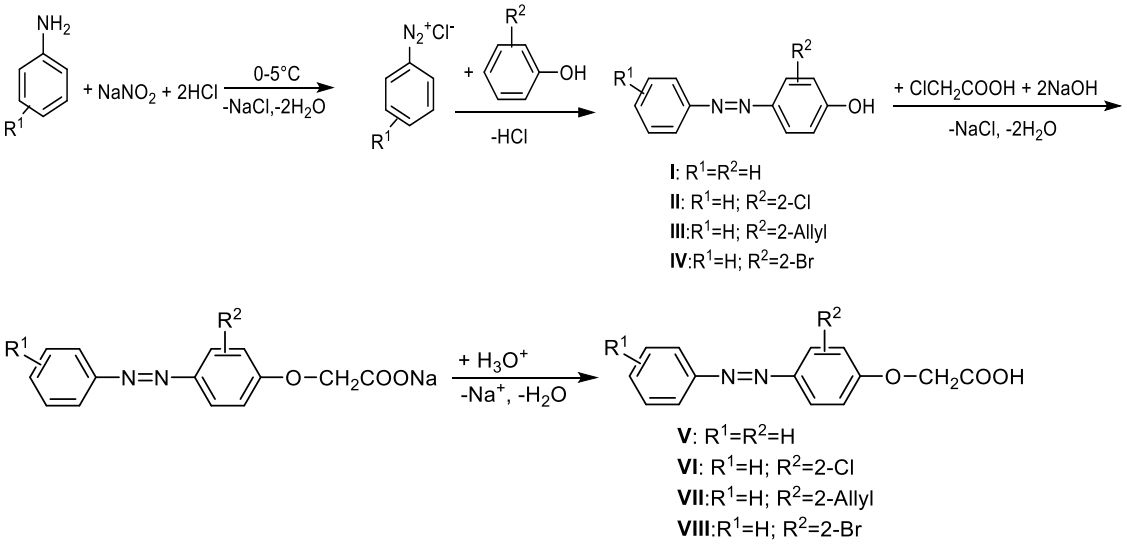

Figure 4.
Synthesis of 2-(4-(phenyldiazenyl)phenoxy) acetic acid derivatives from primary aromatic amines

From the two variants of preparation of the compounds we chose path B (Figure 4), accessibility to reagents being one of the reasons. Azophenols (compounds **I**, **II**, **III** and **IV**) were prepared in two stages: diazotization and azo coupling reaction. Both steps are carried out in an aqueous medium. In order to avoid the decomposition of the diazonium cation, the temperature must be strictly controlled with a dual digital thermocouple thermometer Extech EA10. The yield of azophenol synthesis varies from 66% to 88% (4-(*para*-tolyldiazenyl) phenol, **IV**). Obtaining the 2-(4-(phenyldiazenyl)phenoxy) acetic acid derivatives

was performed by the classical method of refluxing the reaction medium and by microwave assisted synthesis. Four products were obtained using the two techniques (**V**, **VI**, **VII** and **VIII**). Compounds prepared by reflux method are obtained with yields between 42% and 48% for 3.5 h reaction time. The reaction is carried out in basic aqueous solution. Under these conditions, the hydroxyl anion is capable of causing the hydrolysis of chlorine from chloro-acetic acid and thus reduces significantly the yield of the synthesis.

Synthesis of compounds carried out in heterogeneous environment, without solvent, using microwaves is advantageous [18, 19]. Previously, we have shown that in these conditions, inside the reaction medium, there are catalytic centres around which the chemical reaction develops [20, 21]. We applied these findings to the synthesis of our compounds. The reaction medium consists of sodium azophenate and sodium chloro-acetate. The reaction medium is highly polar and easily absorbs electromagnetic microwaves. Furthermore, the absence of hydroxyl ions prevents the hydrolysis reaction of chlorine from sodium chloroacetate. The reaction time becomes of the order of the minutes, and the yield of the 2-(4-(phenyldiazenyl)phenoxy) acetic acid derivative synthesis increases significantly. 2-(4- (phenyldiazenyl)phenoxy) acetic acid (**V**) was obtained with a yield of 90% during 5 min. of irradiation. The synthesis method implemented is superior to the other methods, both in terms of duration and yield [3].

Both the chemical structures of the intermediate azo-phenols (**I** to **IV**) and the 2-(4-(phenyldiazenyl)phenoxy) acetic acid derivatives (**V** to **VIII**) were confirmed by spectroscopic measurements (see the synthesis of compounds).

Antibacterial assay

The antibacterial activity of the compounds was tested against two gram-positive bacterium *Staphylococcus aureus* and *Streptococcus pyogenes* and two gram-negative bacteria *Escherichia coli* and *Salmonella enteritidis*. The antibacterial test was performed using disk diffusion assay - Kirby Bauer method [22]. The reference antibiotic was chloramphenicol.

Compounds were dissolved in aqueous solutions of 0.15% concentration. As microbiological growth medium, the Mueller-Hinton agar (20 mL) was used in the Petri dishes (9 cm diameter). Mueller Hinton agar has a solidifying agent, agar powder that causes the medium solidify at room temperature. Therefore, after solidification at room temperature, the Petri dishes are incubated at 37°C for 30 minutes to take out excess moisture. Sterile filter paper discs (measuring 6 mm in diameter) containing the concentrations of the compounds are placed at equal distances on the agar plate. Aerobic incubation of Petri dishes lasts for 24 hours at 37°C, and then the diameter of the inhibition zones is measured.

The relative percentage of inhibition relative to chloramphenicol was calculated with the help of the following relation [23]:

$$P = \frac{(S_1 - S_2)}{S_3 - S_2} x100\% ,$$

where: P, relative percentage inhibition of the test sample; S_1 [mm^2], surface of inhibition of the tested compound; S_2 [mm^2], surface of inhibition of the solvent, S_3 [mm^2], surface of inhibition of the standard drug. The surfaces were calculated using the circle area formula, πr^2, where r is the radius of inhibition zone.

Table I

Antimicrobial activity of 2-(4-(*para*-tolyldiazenyl)phenoxy)acetic acids

Compound	Relative percentage inhibition, P[%]			
	Staphylococcus aureus	*Streptococcus pyogenes*	*Escherichia coli*	*Salmonella enteritidis*
V	50	0	35	1
VI	59	0	40	0
VII	65	0	44	7
VIII	70	0	52	10

The test results show that 2-(4-(*para*-tolyldiazenyl)-phenoxy) acetic acid derivatives have antibacterial activity. The highly active antibacterial compounds contain a carboxyl group, as well as aliphatic chains in their structure. The most potent antibacterial agent is 2-(4-(*para*-tolyldiazenyl)phenoxy) acetic acid (**VIII**), the percentage of effective inhibition being 70% against *Staphylococcus aureus* (Table I). This suggests that the possibility of making intramolecular hydrogen bonding is probably essential to the interaction of these molecules with the microorganism cell. None of the compounds has any action against *Streptococcus pyogenes*.

Regarding the mechanism of action of the 2-(4-(*para*-tolyldiazenyl)phenoxy) acetic acid derivatives we can assume that it is similar to the phenoxyacetic acid derivatives. The following biochemical processes are possible [24]: a) the compound damage the cell membrane of the bacterium; b) the compound interfere with the cellular metabolism in the biochemical processes in which acetylcoenzyme A (acetyl-CoA) participates; c) decoupling oxidative phosphorylation reactions as a consequence of intracellular membrane disruption by the compound and the interference of compounds in cellular metabolism.

Another mechanism to consider is the induction of cell death [25]. The compound produces a mitochondrial

transmembrane disturbance, inhibiting the oxidative dephosphorylation reaction. These cause a decrease in the concentration of ATP in the mitochondria. Moreover, by opening permeability transition pores, cytochrome C is released which activates the caspases by forming complexes with the activating factor of the apoptotic protease and triggering cell apoptosis (Figure 5).

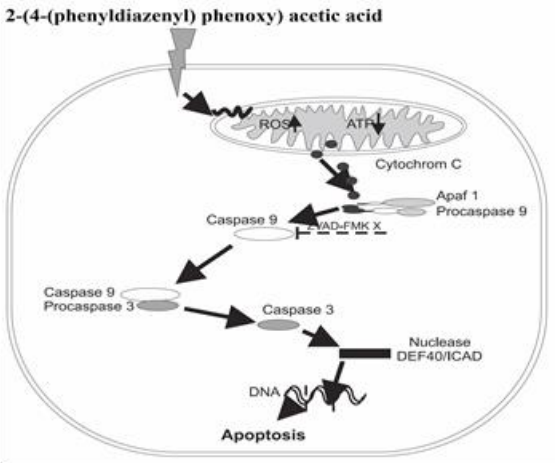

Figure 5.
Possible mechanism of action of 2-(4-(*para*-tolyldiazenyl)phenoxy) acetic acid derivatives [26]

Conclusions

The derivatives of 2-(4-(*para*-tolyldiazenyl)phenoxy) acetic acid were synthesized by the conventional method and with the help of microwaves. The syntheses performed under microwaves are green, eco-friendly, easy to achieve, and take place in a short time with high efficiency.

Compounds were biologically evaluated as antibacterial agents against some Gram-positive and Gram-negative bacteria. Biological activity is much more intense against Gram-positive bacteria.

Conflict of interest

The authors declare no conflict of interest.

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