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Synthesis, biological activity evaluation and QSAR studies of novel 3-(aminooxalyl-amino)-and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acid derivatives

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ABSTRACT

A series of novel 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acid derivatives and their corresponding methyl esters were synthesized under Ullmann condensation in the presence of dimethylformamide as a solvent or in a solid phase. Ullmann condensation conditions were optimized for the synthesis thus the products best yields were obtained. The anti-inflammatory and analgesic effects of novel 2-phenylamino-benzoic acid derivatives was evaluated in vivo employing the carrageenan-induced rat paw edema method and acetic acid induced writhing test in mice. The correlation analysis between anti-inflammatory and analgesic activities with different subsets molecular descriptors was carried out for 16 compounds. The regression models derived with QSAR analysis displayed the significant influence of 3D molecular descriptors on the biological activity of the compounds.

Key words: 2-phenylamino-benzoic acid, anti-inflammatory activity, analgesic activity, molecular descriptors, QSAR analysis

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INTRODUCTION

The diverse strategies development for the directed synthesis of potential drug candidates are providing through scaffold hopping towards the hit compounds identifying and further *in silico* techniques application for their rational design.

Molecules designed on anthranilic acid scaffold as drug candidates have attracted great interest in modern medical chemistry during recent years. It had been observed that best known non-steroidal anti-inflammatory drugs (NSAIDs) are acidic in nature. N-phenylantranilic acid derivatives like mefenamic acid and meclofenamates had been used in therapy as potent analgesic and anti-inflammatory agents in the treatment of osteoarthritis, rheumatoid arthritis and other painful musculosketal illnesses [1-4]. Recent literature showed that substitutions at 2-position of anthranilic acid by different aryl or heteroaryl moieties markedly modulated the anti-inflammatory effect [5, 6]. N-phenylanthranilic acid hydrazides were also reported to be effective as analgesic agents and showed more analgesic activity in comparison to mefenamic acid and diclofenac sodium [7].

Anthranilic acid is a good pharmacophore that is frequently used in drug discovery programs [8, 9]. Its surrogates which incorporated the tetrahydro anthranilic acid scaffold led to compounds with improved *in vitro* activity and superior pharmacokinetics profiles. Furthermore, these anthranilic acid analogs also exhibited good selectivity against cytochrome P450 subtypes CYP2C8 and CYP2C9 [10]. Among the wide variety of chemical structures in development of new anticancer drugs a series of novel N-(2-pyridin-4-yl)anthranilic acid derivatives were shown to

possess potent *in vitro* antiproliferative activity against human tumor cells, exhibiting preventive or inhibitory activity on account of their action through different biological mechanisms [11, 12]. The *in vivo* and pharmacokinetic data provided convincing evidence that a series of N-phenyl anthranilic acid analogs could potentially generate clinical candidates for Alzheimer's disease therapy [13].

Quantitative structure-activity relationship (QSAR) analysis is one of the techniques used to investigate the correlation between biological activity and molecular or physicochemical properties of a set of molecules [14]. QSAR models derived provide remarkable information on the structural features of the drug-like molecules and give guidance for the novel drugs design.

The objective of the present work was to synthesize a series of novel 2-phenylamino-benzoic acid derivatives by the structural modification in 3rd position with aminooxalil and carbamoyl moieties substitution and the corresponding methyl esters obtaining for further pharmacological screening *in vivo* as anti-inflammatory and analgesic agents. We are also reporting the above-mentioned biological effects evaluation as the response for further QSAR analysis we thought it being essential to perceive the importance of the molecular properties, which are critical in accentuating the activities.

EXPERIMENTAL SECTION

1.1. Materials

All chemicals were of analytical grade and commercially available. All reagents and solvents were used without further purification and drying.

1.2. Chemistry

All the melting points were determined in an open capillary and are uncorrected. The IR spectra were recorded on «Specord M-80» spectrophotometer, the solid-state samples of novel compounds were prepared by dilution in dry potassium bromide up to the concentration of 1 %, and Fourier transform infrared spectrophotometer «Testcan Shimadsu FTIR 8000 series». ^{1}H NMR spectra of newly synthesized compounds in DMSO-d6 solutions were recorded on a spectrometer Varian Mercury VX-200 (200 MHz) at 298 K. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard, coupling constant *J* are expressed in Hz. The elemental analysis experimental data on contents of carbon, hydrogen and nitrogen were within ±0.4 % of the theoretical values.

1.2.1. General procedure for the synthesis of 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid (1) and its aminooxalil- and phenylamino- moieties substituted derivatives (IV, 2-9)

1.2.1 A. 2-Chloro-3-(aminooxalyl-amino)-benzoic acid or its appropriate aminooxalyl moiety alkyl substituted derivative (**I**) (0.01 mol) was dissolved in dimethylformamide (DMF) (25 mL), phenylamine or its appropriate phenyl moiety substituted derivative (0.04 mol), potassium carbonate (0.01 mol), and copper powder (0.1 g) were then added. The reaction mixture was refluxed for 8-10 h at 145-150 ºC. Water (100 mL) was then added and the mixture was cooled to 60 ºC and acidified with HCl to pH=3. The precipitate that formed was filtered off, washed with hot water and dried. The obtained compounds were recrystallized from ethanol.

1.2.1 B (Solid-phase synthesis). A solid mixture of 2-chloro-3-(aminooxalyl-amino)-benzoic acid or its appropriate aminooxalyl moiety alkyl substituted derivative (**I**) (0.01 mol), phenylamine or its appropriate phenyl moiety substituted derivative (0.01 mol), potassium carbonate (0.01 mol), and copper oxide (0.0005 mol) was heated for 2 h at 180-220 ºC. The reaction mixture was then cooled and ethanol 50 % aqueous solution (10 mL), dioxane-water (2:3) or DMF-water (2:3) was added. The solution was boiled with activated carbon for 15 min. The reaction mixture was filtered off, then HCl aqueous solution (1:1) was added until pH=3. The precipitate was filtered off, water-washed and dried.

1.2.1 C. 3-Amino-2-phenylamino-benzoic acid or its appropriate phenylamino moiety substituted derivative (**II**) (0.01 mol) was treated with pyridine (0.78 g, 0.01 mol) in dry acetone with constant stirring for 30 min. and cooling (0-5^o C). Then ethyl chloro(oxo)acetate (1.4 g, 0.01 mol) was added by drops bringing the solution temperature up to 40^o C. After the reaction mixture cooling it was poured into water to afford respective 3-ethoxyoxalyl-amino derivatives. The precipitate that formed was filtered off, washed with diluted HCl (1:1), dried and re-crystallized from ethanol aqueous solution.

3-(Ethoxyoxalyl-amino)-2-phenylamino-benzoic acid or its appropriate phenylamino moiety substituted derivative (**III**) prepared in the previous stage was dissolved in ethanol (20 mL) and ammonia (for compound (**IV 1**) or appropriate alkylamine 25 % aqueous solution (0.5 g) was then added. The react ion mixture was heated to the boiling point and left standing for 10 h. The precipitate that formed was filtered off, washed with water and dried.

The target compounds (**IV 1**-**5**) are white crystalline powders, well soluble in alcohols, acetone, DMF, DMSO, dioxane, alkalis solutions, almost insoluble in water and hexane.

1.2.1.1. 3-(Aminooxalyl-amino)-2-phenylamino-benzoic acid (IV 1)
Yield 66% (1A protocol), 79% (1B protocol), 87% (1C protocol),, mp 230-231 ºC. IR (KBr): ν 3388 (N-H, *Ph*–NH–COCONH$_2$), 3257 (N-H, *Ph*–NH–COCONH$_2$), 1720, 1655 (C=O, COOH), 1602 (*Ph*), 1250 (C-N, *Ph*–NH–*Ph*), δ 1578 (N-H, *Ph*–NH–*Ph*) cm^{-1}. ^{1}H NMR, δ, ppm: 5.73 (s, 2H, NH$_2$), 6.71-7.74 (m, 8H, C$_6$H$_5$, C$_6$H$_3$), 8.43(s, 1H, *Ph*–NH–COCONH$_2$), 9.73 (s, 1H, *Ph*–NH–*Ph*), 10.25 (s, 1H, COOH). Calcd. for $C_{15}H_{13}N_3O_4$ %: C 60.20; H 4.38; N 14.04. Found %: C 60.45; H 4.51; N 13.95.

1.2.1.2. 3-(Methylaminooxalyl-amino)-2-o-tolylamino-benzoic acid (IV 2)
Yield 67% (1A protocol), 78% (1B protocol), 88% (1C protocol),, mp 219-220 ºC. IR (KBr): ν 3378 (N-H, *Ph*–NH–COCONH$_2$), 3262 (N-H, *Ph*–NH–COCONH$_2$), 1695, 1658 (C=O, COOH), 1605 (*Ph*), 1242 (C-N, *Ph*–NH–*Ph*), δ 1583 (N-H, *Ph*–NH–*Ph*) cm^{-1}. ^{1}H NMR, δ, ppm: 1.35 (s, 3H, -NH–CH$_3$), 2.32 (s, 3H, *Ph*-CH$_3$) 6.96-8.64 (m, 7H, C$_6$H$_4$, C$_6$H$_3$), 8.91 (s, 1H, *Ph*–NH–COCONHCH$_3$), 9.06 (s, 1H, *Ph*–NH–*Ph*), 9.55 (s, 1H, NH-CH$_3$), 12.20 (bs, 1H, COOH). Calcd. for $C_{17}H_{17}N_3O_4$ %: C 62.38; H 5.23; N 12.84. Found %: C 62.10; H 5.35; N 12.93.

1.2.1.3. 3-[(2-Hydroxy-ethylaminooxalyl)-amino]-2-*m*-tolylamino-benzoic acid (IV 3)
Yield 70% (1A protocol), 73% (1B protocol), 82% (1C protocol),, mp 205-207 ºC. IR (KBr): ν 3370 (N-H, *Ph*–NH–COCONH$_2$), 3255 (N-H, *Ph*–NH–COCONH$_2$), 1705, 1652 (C=O, COOH), 1595 (*Ph*), 1238 (C-N, *Ph*–NH–*Ph*), δ 1572 (N-H, *Ph*–NH–*Ph*) cm^{-1}. ^{1}H NMR, δ, ppm: 2.30 (s, 3H, *Ph*-CH$_3$), 3.44 (m, 4H, -CH$_2$–CH$_2$–OH), 4.80 (s, 1H, -CH$_2$–CH$_2$–OH), 6.87-8.65 (m, 7H, C$_6$H$_4$, C$_6$H$_3$), 9.08 (s, 1H, *Ph*–NH–*Ph*), 9.12 (s, 1H, *Ph*–NH–COCONH(CH$_2$)$_2$OH), 10.21 (s, 1H, -NH–(CH$_2$)$_2$–OH), 12.08 (bs, 1H, COOH). Calcd. for $C_{18}H_{19}N_3O_5$ %: C 60.50; H 5.36; N 11.76. Found %: C 60.64; H 5.25; N 11.83.

1.2.1.4. 3-(Butylaminooxalyl-amino)-2-(3,4-dimethyl-phenylamino)-benzoic acid (IV 4)
Yield 62% (1A protocol), 68% (1B protocol), 80% (1C protocol),, mp 220-222 ºC. IR (KBr): ν 3370 (N-H, *Ph*–NH–COCONH$_2$), 3245 (N-H, *Ph*–NH–COCONH$_2$), 1725, 1644 (C=O, COOH), 1600 (*Ph*), 1235 (C-N, *Ph*–NH–*Ph*), δ 1568 (N-H, *Ph*–NH–*Ph*) cm^{-1}. ^{1}H NMR, δ, ppm: 1.23 (t, *J* = 8.0 Hz, 3H, -NH–CH$_2$–CH$_2$–CH$_2$–CH$_3$), 1.82 (m, 4H, -NH–CH$_2$–CH$_2$–CH$_2$–CH$_3$), 2.18 (s, 3H, *Ph*-3-CH$_3$), 2.25 (s, 3H, *Ph*-4-CH$_3$), 3.50 (d, *J* = 5.6 Hz, 2H, -NH–CH$_2$–CH$_2$–CH$_2$–CH$_3$), 6.61-7.70 (m, 6H, C$_6$H$_3$, C$_6$H$_3$), 8.56 (s, 1H, *Ph*–NH–COCONHCH$_3$), 9.60 (s, 1H, *Ph*–NH–*Ph*), 10.15 (s, 1H, -NH–(CH$_2$)$_3$–CH$_3$), 10.22 (bs, 1H, COOH). Calcd. for $C_{21}H_{25}N_3O_4$ %: C 65.78; H 6.57; N 10.96. Found %: C 66.65; H 6.65; N 11.01.

1.2.1.5. 2-(4-Chloro-phenylamino)-3-(methylaminooxalyl-amino)-benzoic acid (IV 5)
Yield 66% (1A protocol), 77% (1B protocol), 87% (1C protocol),, mp 172-174 ºC. IR (KBr): ν 810 (C–Cl), 2956 (C–H, CH$_3$ asym), 2842 (C–H, CH$_3$ sym), 3350 (N-H, *Ph*–NH–COCONH$_2$), 3238 (N-H, *Ph*–NH–COCONH$_2$), 1700, 1642 (C=O, COOH), 1598 (*Ph*), 1232 (C-N, *Ph*–NH–*Ph*), δ 1572 (N-H, *Ph*–NH–*Ph*) cm^{-1}. ^{1}H NMR, δ, ppm: 2.79 (s, 3H, -NH–CH$_3$), 6.76-7.74 (m, 7H, C$_6$H$_3$, C$_6$H$_4$), 8.57 (s, 1H, *Ph*–NH–COCONHCH$_3$), 9.02 (s, 1H, *Ph*–NH–*Ph*), 9.73 (bs, 1H, COOH), 10.23 (s, 1H, -NH–CH$_3$). Calcd. for $C_{16}H_{14}ClN_3O_4$ %: C 55.26; H 4.06; N 12.08. Found %: C 55.19; H 4.14; N 12.14.

1.2.2. General procedure for the synthesis of 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acid carbamoyl- and phenylamino- moieties substituted derivatives (IX 6-8)

2.2.2 A. An appropriate 2-chloro-3-(3-carbamoyl-propionylamino)-benzoic acid carbamoyl- moiety alkyl or hydroxylalkyl substituted derivative (**V**) (0.01 mol) was dissolved in dimethylformamide (DMF) (25 mL), phenylamine appropriate phenyl moiety substituted derivative (0.04 mol), potassium carbonate (0.01 mol), and copper powder (0.1 g) were then added. The reaction mixture was refluxed for 8-10 h at 145-150 ºC. Water (100 mL) was then added and the mixture was cooled to 60 ºC and acidified with HCl to pH=3. The precipitate that formed was filtered off, washed with hot water and dried. The obtained compounds were recrystallized from ethanol.

1.2.2 B (Solid-phase synthesis). A solid mixture of the appropriate 2-chloro-3-(3-carbamoyl-propionylamino)-benzoic acid carbamoyl- moiety alkyl or hydroxylalkyl substituted derivative (**V**) (0.01 mol), phenylamine appropriate phenyl moiety substituted derivative (0.01 mol), potassium carbonate (0.01 mol), and copper oxide (0.0005 mol) was heated for 2 h at 180-220 ºC. The reaction mixture was then cooled and ethanol 50 % aqueous solution (10 mL), dioxane-water (2:3) or DMF-water (2:3) was added. The solution was boiled with activated carbon for 15 min. The reaction mixture was filtered off, then HCl aqueous solution (1:1) was added until pH=3. The precipitate was filtered off, water-washed and dried.

1.2.2 C. The appropriate 3-Amino-2-phenylamino-benzoic acid appropriate phenylamino moiety substituted derivative (**VI**) (0.01 mol) was dissolved in glacial acetic acid (10 mL) and the obtained solution was then treated with dihydro-furan-2,5-dione (1.0 g, 0.01 mol), anhydrous sodium acetate (0.5 g) and acetic anhydride (1.02 g, 0.01 mol). The reaction mixture was refluxed for 1 h to afford respective 3-(2,5-dioxo-pyrrolidin-1-yl)-2-phenylamino-benzoic acid derivatives (**VII**). The reaction mixture was then cooled and diluted with water. The precipitate that formed was filtered off, washed with water and dried.

3-(2,5-Dioxo-pyrrolidin-1-yl)-2-phenylamino-benzoic acid derivatives (**VII**) (0.01 mol) prepared in the previous stage were dissolved in methanol (20 mL) and appropriate methylamine or hydroxoethylamine 25 % aqueous solution (0.5 g, 0.01 mol) was then added. The reaction mixture was heated up to the complete reactants dissolving and left standing for 1.5 h at rt. The reaction mixture was then diluted with water and acidified with HCl 1:1 solution. The precipitate that formed was filtered off, washed with water and dried. The precipitate was recrystallized from aqueous dioxane solution.

1.2.2 D. 3-Amino-2-phenylamino-benzoic acid appropriate phenylamino moiety substituted derivative (**VI**) (0.01 mol) was dissolved in dry acetone (10 mL), triethylamine (1.4 mL, 0.01 mol) was then added. The obtained solution was then treated with 3-chlorocarbonyl-propionic acid methyl ester (1.4 mL, 0.01 mol) in the presence of pyridine at cooling and constant stirring. The reaction mixture was heated to homogenization and left standing for 2 h. Acetone was then partially distilled off, the residue was diluted with water to afford respective 3-(4-oxo-pentanoylamino)-2-phenylamino-benzoic acid derivatives (**VIII**). The precipitate that formed was filtered off, washed with water and dried. The precipitate was recrystallized from methanol.

3-(4-Oxo-pentanoylamino)-2-phenylamino-benzoic acid derivatives (**VIII**) (0.01 mol) prepared in the previous stage were dissolved in methanol (20 mL) and appropriate methylamine or hydroxoethylamine 25 % aqueous solution (0.5 g, 0.01 mol) was then added. The reaction mixture was heated up to the complete reactants dissolving and left standing for 12 h at rt. The reaction mixture was then diluted with water and acidified with HCl 1:1 solution for pH=3-4. The precipitate that formed was filtered off, washed with water and dried.

The target compounds (**IX 6-8**) are white crystalline powders, well soluble in dioxane, DMF, DMSO, acetone, alkalis solutions, alcohols at heating, almost insoluble in water and hexane.

1.2.2.1. 3-[3-(2-Hydroxy-ethylcarbamoyl)-propionylamino]-2-o-tolylamino-benzoic acid (IX 1)
Yield 67% (2A protocol), 82% (2B protocol), 78% (2C protocol), 75% (2D protocol), mp 195-196 ºC. IR (KBr): ν 3374 (N-H, *Ph*–NH–COCONH$_2$), 3248 (N-H, *Ph*–NH–COCONH-), 1715, 1648 (C=O, COOH), 1595 (*Ph*), 1232 (C-N, *Ph*–NH–*Ph*), 2955 (C–H, CH_3 asym), 2842 (C–H, CH_3 sym); δ 1568 (N-H, *Ph*–NH–*Ph*), 1405 (C–H, CH_2) cm^{-1}. ^{1}H NMR, δ, ppm: 2.25 (s , 3H, *Ph*–CH_3), 2.51 (d, *J* = 4.0 Hz, 2H, CO–CH_2–CH_2-), 2.77 (d, *J* = 4.0 Hz, 2H, CO–CH_2–CH_2-), 3.41 (m , 2H, -NH–CH_2–CH_2–OH), 3.59 (m , 2H, *Nh*–CH_2–CH_2–OH), 4.80 (s, 1H, OH), 6.52 (s, 1H, -NH–$(CH_2)_2$–OH), 6.68-8.44 (m, 7H, C_6H_3, C_6H_4), 8.51 (s, 1H, *Ph*–NH–CO), 8.94 (s, 1H, *Ph*–NH–*Ph*), 12.20 (bs, 1H, COOH). Calcd. for $C_{20}H_{23}N_3O_5$ %: C 62.33; H 6.02; N 10.90. Found %: C 62.26; H 6.10; N 10.98.

1.2.2.2. 3-(3-Methylcarbamoyl-propionylamino)-2-p-tolylamino-benzoic acid (IX 2)
Yield 65% (2A protocol), 80% (2B protocol), 76% (2C protocol), 71% (2D protocol), mp 162-164 ºC. IR (KBr): ν 3370 (N-H, *Ph*–NH–COCONH$_2$), 3240 (N-H, *Ph*–NH–COCONH-), 1720, 1648 (C=O, COOH), 1595 (*Ph*), 1245 (C-N, *Ph*–NH–*Ph*), 2952 (C–H, CH_3 asym), 2840 (C–H, CH_3 sym); δ 1574 (N-H, *Ph*–NH–*Ph*), 1403 (C–H, CH_2) cm^{-1}. ^{1}H NMR, δ, ppm: 2.30 (s , 3H, *Ph*–CH_3), 2.52 (d, *J* = 4.0 Hz, 2H, CO–CH_2–CH_2-), 2.74 (t, *J* = 7.0 Hz, 3H, -NH–CH_3), 2.79 (d, *J* = 4.0 Hz, 2H, CO–CH_2–CH_2-), 6.89 (s, 1H, -NH–CH_3), 6.85-8.48 (m, 7H, C_6H_3, C_6H_4), 8.50 (s, 1H, *Ph*–NH–CO), 8.94 (s, 1H, *Ph*–NH–*Ph*), 12.29 (bs, 1H, COOH). Calcd. for $C_{19}H_{21}N_3O_4$ %: C 64.21; H 5.96; N 11.82. Found %: C 64.28; H 5.89; N 11.78.

1.2.2.3. 3-[3-(2-Hydroxy-ethylcarbamoyl)-propionylamino]-2-p-tolylamino-benzoic acid (IX 3)
Yield 63% (2A protocol), 78% (2B protocol), 70% (2C protocol), 73% (2D protocol), mp 187-189 ºC. IR (KBr): ν 3355 (N-H, *Ph*–NH–COCONH$_2$), 1710, 1658 (C=O, COOH), 1604 (*Ph*), 1230 (C-N, *Ph*–NH–*Ph*), 2944 (C–H, CH_3 asym), 2836 (C–H, CH_3 sym); δ 1578 (N-H, *Ph*–NH–*Ph*), 1402 (C–H, CH_2) cm^{-1}. ^{1}H NMR, δ, ppm: 2.30 (s , 3H, *Ph*–CH_3), 2.50 (d, *J* = 4.0 Hz, 2H, CO–CH_2–CH_2-), 2.77 (d, *J* = 4.0 Hz, 2H, CO–CH_2–CH_2-), 3.39 (m, 2H, -NH–CH_2–CH_2–OH), 3.59 (m , 2H, *Ph*–CH_2–CH_2–OH), 4.80 (s, 1H, OH), 6.51 (s, 1H, -NH–$(CH_2)_2$–OH), 6.86-8.46 (m, 7H, C_6H_3, C_6H_4), 8.51 (s, 1H, *Ph*–NH–CO), 9.06 (s, 1H, *Ph*–NH–*Ph*), 12.21 (bs, 1H, COOH). Calcd. for $C_{20}H_{23}N_3O_5$ %: C 62.33; H 6.02; N 10.90. Found %: C 62.38; H 5.96; N 10.86.

1.2.3. General procedure for the synthesis of 3-(aminooxalyl-amino)- and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids methyl esters and their aminooxalil, carbamoyl and phenylamino moieties substituted derivatives (X 1-8)

A mixture of 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid or its appropriate aminooxalil and phenylamino moieties substituted derivatives (0.01 mol) with concentrated sulfuric acid (0.75 mL) in anhydrous methanol (30 mL) was heated for 5 h at hot water bath. The reaction mixture was then cooled and poured into water. The precipitate that formed was filtered off, washed with water and dried. The obtained compounds were recrystallized from aqueous methanol solution or dioxane.

In the same way 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids carbamoyl and phenylamino moieties substituted derivatives methyl esters were obtained.

1.2.3.1. 3-(Aminooxalyl-amino)-2-phenylamino-benzoic acid methyl ester (X 1)

Yield 84 %, mp 145-148 ºC. IR (KBr): ν 3350 (N-H, *Ph*–NH–COCONH$_2$), 3242 (N-H, *Ph*–NH–COCONH$_2$), 1680 (C=O, *Ph*–NH–CO–CO–), 1700 (C=O, *Ph*–CO–OCH$_3$), 1292 (C=O, –NH–CO–CO–), 1155 (C-O, *Ph*-CO–OCH$_3$), 1600 (C–*Ph*), δ 1572 (N-H, *Ph*–NH–*Ph*), δ 1415 (C–H, CH$_2$) cm^{-1}. ^{1}H NMR, δ, ppm: 3.60 (s, 3H, COOCH$_3$), 5.72 (s, 2H, NH$_2$), 6.54-7.75 (m, 8H, C$_6$H$_5$, C$_6$H$_3$), 7.95 (s, 1H, *Ph*–NH–COCONH$_2$), 9.47 (bs, 1H, *Ph*–NH–*Ph*). Calcd. for $C_{16}H_{15}N_3O_4$ %: C 61.34; H 4.83; N 13.41. Found %: C 61.45; H 4.97; N 13.26.

1.2.3.2. 3-(Butylaminooxalyl-amino)-2-(3,4-dimethyl-phenylamino)-benzoic acid methyl ester (X 2)

Yield 75, mp 152-154 ºC. IR (KBr): ν 3334 (N-H, *Ph*–NH–COCONH$_2$), 3218 (N-H, *Ph*–NH–COCONH$_2$), 1670 (C=O, *Ph*–NH–CO–CO–), 1705 (C=O, *Ph*-CO-OCH$_3$), 1280 (C=O, –NH–CO–CO–), 1158 (C-O, *Ph*-CO-OCH$_3$), 2970 (C–H, CH$_3$ asym), 2862 (C–H, CH$_3$ sym), 1599 (C–*Ph*), δ 1578 (N-H, *Ph*–NH–*Ph*), δ 1416 (CH$_2$) cm^{-1}. ^{1}H NMR, δ, ppm: 1.25 (t, *J* = 8.0 Hz, 3H, –NH–CH$_2$–CH$_2$–CH$_2$–CH$_3$), 1.80 (m, 4H, -NH–CH$_2$–CH$_2$–CH$_2$–CH$_3$), 2.12 (s, 3H, *Ph*-3-CH$_3$), 2.27 (s, 3H, *Ph*-4-CH$_3$), 3.49 (d, *J* = 5.6 Hz, 2H, –NH–CH$_2$–CH$_2$–CH$_2$–CH$_3$), 3.61 (s, 3H, COOCH$_3$), 6.40 (t, *J* =7,5, 1H, CO-NH-), 6.55-7.74 (m, 6H, C$_6$H$_3$, C$_6$H$_3$), 8.05 (s, 1H, *Ph*–NH–COCONH$_2$), 9.45 (bs, 1H, *Ph*–NH–*Ph*). Calcd. for $C_{22}H_{27}N_3O_4$ %: C 66.48; H 6.85; N 10.57. Found %: C 66.34; H 6.93; N 10.68.

1.2.3.3. 2-(4-Ethoxy-phenylamino)-3-(methylaminooxalyl-amino)-benzoic acid methyl ester (X 3)

Yield 84 %, mp 122-125 ºC. IR (KBr): ν 3330 (N-H, *Ph*–NH–COCONH$_2$), 3215 (N-H, *Ph*–NH–COCONH$_2$), 1665 (C=O, *Ph*–NH–CO–CO–), 1692 (C=O, *Ph*–CO–OCH$_3$), 1275 (C=O, –NH–CO–CO–), 1154 (C-O, *Ph*-CO–OCH$_3$), 2984 (C–H, CH$_3$ asym), 2870 (C–H, CH$_3$ sym), 1594 (C–*Ph*), δ 1568 (N-H, *Ph*–NH–*Ph*), δ 1404 (C–H, CH$_2$) cm^{-1}. ^{1}H NMR, δ, ppm: 1.33 (t, *J* = 8.0 Hz, 3H, –*Ph*–OCH$_2$–CH$_3$), 2.79 (s, 3H, –NH–CH$_3$),), 2.80 (s, 2H, *Ph*–OCH$_2$–CH$_3$), 3.49 (d, *J* = 5.6 Hz, 2H, –NH–CH$_2$–CH$_2$–CH$_2$–CH$_3$), 3.67 (s, 3H, COOCH$_3$), 6.42 (t, *J* =7,5, 1H, –CO–NH–), 6.60-7.72 (m, 7H, C$_6$H$_3$, C$_6$H$_4$), 8.07 (s, 1H, *Ph*–NH–COCONH$_2$), 9.44 (bs, 1H, *Ph*–NH–*Ph*). Calcd. for $C_{19}H_{21}N_3O_5$ %: C 61.45; H 5.70; N 11.31. Found %: C 61.61; H 5.59; N 11.47.

1.2.3.4. 2-(2-Chloro-phenylamino)-3-(methylaminooxalyl-amino)-benzoic acid methyl ester (X 4)

Yield 84 %, mp 158-161 ºC. IR (KBr): ν 3350 (N-H, *Ph*–NH–COCONH$_2$), 3228 (N-H, *Ph*–NH–COCONH$_2$), 1672 (C=O, *Ph*–NH–CO–CO–), 1695 (C=O, *Ph*–CO–OCH$_3$), 1280 (C=O, –NH–CO–CO–), 1158 (C-O, *Ph*-CO–OCH$_3$), 2982 (C–H, CH$_3$ asym), 2876 (C–H, CH$_3$ sym), 1598 (C–*Ph*), 790 (C–Cl), δ 1578 (N-H, *Ph*–NH–*Ph*), δ 1414 (C–H, CH$_2$) cm^{-1}. ^{1}H NMR, δ, ppm: 2.80 (s, 3H, –NH–CH$_3$), 3.61 (s, 3H, COOCH$_3$), 6.54 (t, *J* =7.5 Hz, 1H, CO-NH-), 6.57-7.70 (m, 7H, C$_6$H$_3$, C$_6$H$_4$), 8.06 (s, 1H, *Ph*–NH–COCONH$_2$), 10.40 (bs, 1H, *Ph*–NH–*Ph*). Calcd. for $C_{17}H_{16}ClN_3O_4$ %: C 56.44; H 4.46; N 11.61. Found %: C 56.37; H 4.56; N 11.55.

1.2.3.5. 3-(3-Methylcarbamoyl-propionylamino)-2-o-tolylamino-benzoic acid methyl ester (X 5)

Yield 84 %, mp 175-177 ºC. IR (KBr): ν 3340 (N-H, *Ph*–NH–COCONH$_2$), 3212 (N-H, *Ph*–NH–COCONH$_2$), 1692 (C=O, *Ph*–NH–CO–CO–), 1708 (C=O, *Ph*–CO–OCH$_3$), 1288 (C=O, –NH–CO–CO–), 1150 (C-O, *Ph*-CO–OCH$_3$), 2976 (C–H, CH$_3$ asym), 2880 (C–H, CH$_3$ sym), 1605 (C–*Ph*), δ 1580 (N-H, *Ph*–NH–*Ph*), δ 1408 (C–H, CH$_2$) cm^{-1}. ^{1}H NMR, δ, ppm: 2.07 (s, 3H, *Ph*-2-CH$_3$), 2.62 (s, 3H, –NH–CH$_3$), 2.72 (t, *J* = 4.0 Hz, 2H, CO–CH$_2$–CH$_2$-), 2.84 (t, *J* = 4.0 Hz, 2H, CO–CH$_2$–CH$_2$-), 3.62 (s, 3H, COOCH$_3$), 3.99 (t, *J*=6.0 Hz, 1H, *Ph*–NH–CO(CH$_2$)$_2$– CONH$_2$), 6.56-7.72 (m, 7H, C$_6$H$_3$, C$_6$H$_4$), 7.15 (t, *J*=7.0 Hz, 1H, CO-NH-), 9.33 (bs, 1H, *Ph*–NH–*Ph*). Calcd. for $C_{20}H_{23}N_3O_4$ %: C 65.03; H 6.28; N 11.37. Found %: C 65.22; H 6.13; N 11.48.

1.2.3.6. 3-[3-(2-Hydroxy-ethylcarbamoyl)-propionylamino]-2-o-tolylamino-benzoic acid methyl ester (X 6)

Yield 82 %, mp 154-155 ºC. IR (KBr): ν 3338 (N-H, *Ph*–NH–COCONH$_2$), 3210 (N-H, *Ph*–NH–COCONH$_2$), 1685 (C=O, *Ph*–NH–CO–CO–), 1712 (C=O, *Ph*–CO–OCH$_3$), 1290 (C=O, –NH–CO–CO–), 1152 (C-O, *Ph*-CO–OCH$_3$), 2974 (C–H, CH$_3$ asym), 2875 (C–H, CH$_3$ sym), 1603 (C–*Ph*), δ 1582 (N-H, *Ph*–NH–*Ph*), δ 1405 (C–H, CH$_2$) cm^{-1}. ^{1}H NMR, δ, ppm: 2.07 (s, 3H, *Ph*-2-CH$_3$), 2.72 (t, *J* = 4.0 Hz, 2H, CO–CH$_2$–CH$_2$-), 2.82 (t, *J* = 4.0 Hz, 2H, CO–

CH_2–$\underline{CH_2}$ –), 3.61 (s, 3H, COO$\underline{CH_3}$), 3.98 (t, *J*=6 1H, *Ph*–$\underline{NH}$–CO$(CH_2)_2$CONH_2), 6.65 (s, 1H, CH_2–$\underline{OH}$), 6.55-7.51 (m, 7H, C_6H_3, C_6H_4), 7.10 (t, *J*=7, 1H, CO-$\underline{NH}$-), 9.30 (bs, 1H, *Ph*–$\underline{NH}$–*Ph*). Calcd. for $C_{21}H_{25}N_3O_5$ %: C 63.15; H 6.31; N 10.52. Found %: C 63.25; H 6.12; N 10.65.

1.2.3.7. 2-(3,4-Dimethyl-phenylamino)-3-(3-methylcarbamoyl-propionylamino)-benoic acid methyl ester (X 7)
Yield 88 %, mp 125-128 ºC. IR (KBr): ν 3340 (N-H, *Ph*–$\underline{NH}$–COCONH_2), 3218 (N-H, *Ph*–NH–COCO$\underline{NH_2}$), 1670 (C=O, *Ph*–NH–$\underline{CO}$–CO–), 1715 (C=O, *Ph*–$\underline{CO}$–OCH_3), 1280 (C=O, –NH–CO–$\underline{CO}$–), 1154 (C-O, *Ph*-CO–$\underline{OC}H_3$), 2982 (C–H, CH_3 asym), 2884 (C–H, CH_3 sym), 1608 (C–*Ph*), δ 1580 (N-H, *Ph*–NH–*Ph*), δ 1412 (C–H, CH_2) cm^{-1}. ^{1}H NMR, δ, ppm: 2.19 (s, 3H, *Ph*-3-$\underline{CH_3}$), 2.24 (s, 3H, *Ph*-4-$\underline{CH_3}$), 2.63 (s, 3H, –NH–$\underline{CH_3}$), 2.72 (t, *J* = 4.0 Hz, 2H, CO–$\underline{CH_2}$–CH_2-), 2.84 (t, *J* = 4.0 Hz, 2H, CO–CH_2–$\underline{CH_2}$-), 3.61 (s, 3H, COO$\underline{CH_3}$), 3.95 (t, *J*=6.0 Hz, 1H, *Ph*–$\underline{NH}$–CO$(CH_2)_2$ –CONH_2), 6.88-7.74 (m, 6H, C_6H_3, C_6H_3), 7.17 (t, *J*=7.2 Hz, 1H, CO-$\underline{NH}$-), 9.39 (bs, 1H, *Ph*–$\underline{NH}$–*Ph*). Calcd. for $C_{21}H_{25}N_3O_4$ %: C 65.78; H 6.57; N 10.96. Found %: C 65.89; H 6.43; N 10.85.

1.2.3.8. 2-(4-Chloro-phenylamino)-3-(3-methylcarbamoyl-propionylamino)-benzoic acid methyl ester (X 8)
Yield 88 %, mp 167-169 ºC. IR (KBr): ν 3352 (N-H, *Ph*–$\underline{NH}$–COCONH_2), 3225 (N-H, *Ph*–NH–COCO$\underline{NH_2}$), 1655 (C=O, *Ph*–NH–$\underline{CO}$–CO–), 1690 (C=O, *Ph*–$\underline{CO}$–OCH_3), 1270 (C=O, –NH–CO–$\underline{CO}$–), 1140 (C-O, *Ph*-CO–$\underline{OC}H_3$), 2970 (C–H, CH_3 asym), 2884 (C–H, CH_3 sym), 1602 (C–*Ph*), δ 1572 (N-H, *Ph*–NH–*Ph*), δ 1414 (C–H, CH_2) cm^{-1}. ^{1}H NMR, δ, ppm: 2.61 (s, 3H, –NH–$\underline{CH_3}$), 2.70 (t, *J* = 4.0 Hz, 2H, CO–$\underline{CH_2}$–CH_2-), 2.86 (t, *J* = 4.0 Hz, 2H, CO–CH_2–$\underline{CH_2}$-), 3.60 (s, 3H, COO$\underline{CH_3}$), 3.98 (t, *J*=6.0 Hz, 1H, *Ph*–$\underline{NH}$–CO$(CH_2)_2$–CONH_2), 6.61-7.75 (m, 7H, C_6H_3, C_6H_4), 7.20 (t, *J*=7.2 Hz, 1H, CO-$\underline{NH}$-), 9.46 (bs, 1H, *Ph*–$\underline{NH}$–*Ph*). Calcd. for $C_{19}H_{20}ClN_3O_4$ %: C 58.54; H 5.17; N 10.78. Found %: C 58.43; H 5.28; N 10.87.

1.3. Pharmacology assays

1.3.1. Anti-inflammatory activity

Anti-inflammatory activity was evaluated using carrageenan induced rat paw edema method in rats [15]. Outbred (male/female) white rats weighing 180–220 g were used for the edema test. Animals were divided into 17 groups comprising five rats per group. One group was kept as the control and remaining 16 groups (test groups) were used to determine the anti-inflammatory activity elicited by the 16 drug candidates, respectively. Rats were kept in the animal house under standard conditions of light and temperature on the general diet prior to the experiment. The standard drug, Diclofenac (8 mg/kg body weight) and the test drugs (20 mg/kg body weight) were suspended in CMC and administrated through intraperitoneal route. CMC was injected to the control group. 30 minutes later, 0.1 ml of 2 % carrageenan solution in saline was injected in the sub-plantar region of the right hind paw of each rat. After 4h of the carrageenan injection, the volume of paw edema (in mL) was measured using water plethysmometer and paw edema decreasing was compared between control group and drug-tested groups. National Pharmaceutical University, Kharkiv, ethics committee, constituted by the Ministry of Health of Ukraine, approved the experimental protocols for anti-excudative and analgesic activities evaluation. The inflammatory reaction inhibition was expressed as percent of paw volume reduction and it was calculated using the following formula:

$$\%\ \text{Inhibition} = \frac{V_{\text{control}} - V}{V_{\text{control}}} \cdot 100\ \%,$$

where V_{control} is the increase in paw volume in control group animals;
V is the increase in paw volume in animals injected with the test substances.

1.3.2. Analgesic activity

Analgesic activity was tested by the acetic acid induced writhing method [16]. The mice were divided into 17 groups of ten animals each. A 0.6 % aqueous acetic acid solution (intraperitoneal injection, 0.1 mL) was used as a writhing induced agent. Mice were kept individually in the test cage before acetic acid injection and habituated for 30 min. Screening of analgesic activity was performed after i.p. administration of test drugs at a dose of 20 mg/kg body weight and the reference drug, Analgin, at a dose of 55 mg/kg. All the compounds were injected as CMC suspensions (1 %) with Tween-80 emulsifier. One group was kept as control and received 1 % CMC. After 20 minutes of drugs administration, 0.1 mL of 0.6 % acetic acid solution was given to mice inrtaperitoneally. Severity of the writhing response was recorded for 20 min after administration of acetic acid solution. The analgesic activity was expressed in terms of % protection.

1.4. QSAR methods

The QSAR studies workflow included a few stages. Firstly 2*D* structures of all molecules were drawn with ISISDraw tools and converted to 3*D* structures. Molecular modeling studies were performed using the HyperChem 7.5 software [17]. We pre-optimized the molecular structures with the Molecular Mechanics Force Field (MM+)

procedure, and refined the resulting geometries by means of the semi-empirical Method AM1 from the Molecular Orbitals Theory using the Polak-Ribiere algorithm and a gradient norm limit of 0.01 kcal· $Å^{-1}$. The minimized 3*D* geometries of all compounds were then converted into SD File format with Open Babel version 2.3.2 software [18] and were used as the input for molecular 3*D* descriptors calculation. On the next stage >1600 molecular descriptors were generated with DRAGON E-version software [19, 20] for the whole structures set.

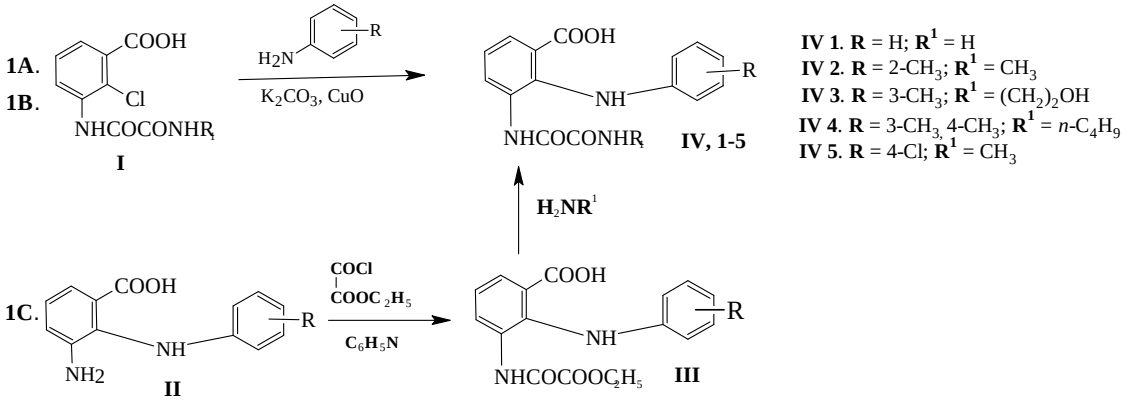
Stepwise variables selection procedure for the statistically significant QSAR models development as multiple linear regression equations was performed with BuildQSAR software [21] application. Multiply regression analysis was carried out and statistical quality of the equations was justified by correlation coefficient *R*, standard deviation *s*, and *F*-ratio test.

The predictive ability of the deriver models was also estimated with leave-one-out (LOO) cross-validation technique. The predicted residual sums of squares standard deviation (S_{PRESS}), standard deviation error in prediction (S_{DEP}) and cross-validation coefficient Q^2 were used for the predictive ability validation of the QSAR-models.

RESULTS AND DISCUSSION

2.1. Chemistry

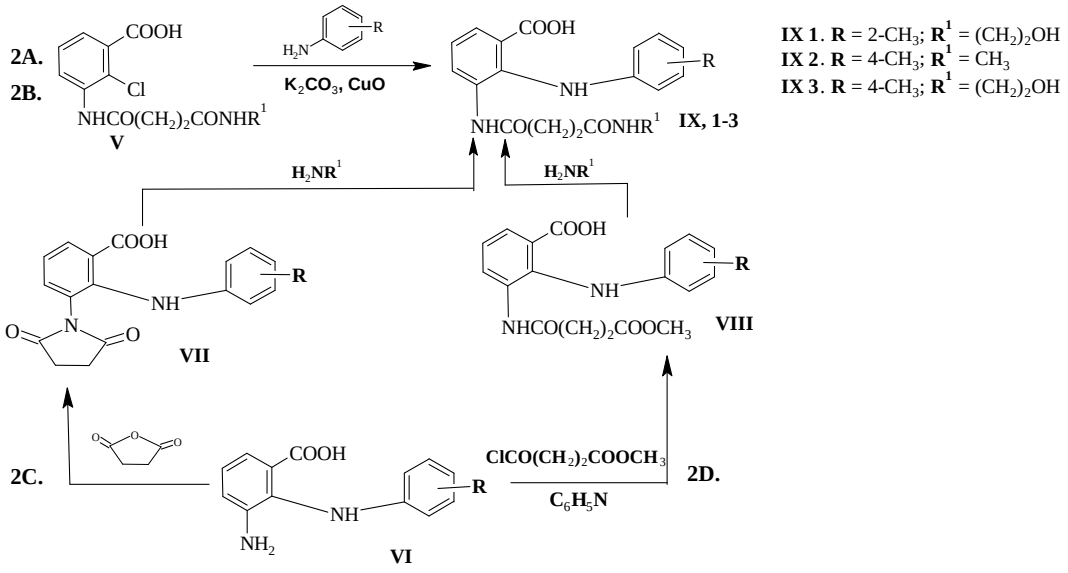
The synthetic reactions leading to 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid (**IV 1**) and its aminooxalil- and phenylamino- moieties substituted derivatives (**IV**, **2-5**) as they are outlined in Scheme 1 belong to Ullmann condensation in the presence of dimethylformamide (DMF) as a solvent (1A protocol) or in a solid phase (1B protocol). Ullmann condensation conditions were optimized for the synthesis thus the products best yields were obtained with 0.01 mol of 2-chloro-3-(aminooxalyl-amino)-benzoic acid or its appropriate aminooxalyl moiety alkyl substituted derivative (**I**), 0.04 mol of phenylamine or its appropriate phenyl moiety substituted derivative, 0.01 mol of potassium carbonate, 0.1 g of copper, 145-150 ºC reaction mixture refluxing and a reaction time of 8-10 h. For the solid-phase condensation 2-chloro-3-(aminooxalyl-amino)-benzoic acid or its appropriate derivative was treated with phenylamine or its appropriate phenyl moiety substituted derivative as 0.01: 0.01 by moles and 0.0005 mol of copper oxide instead of copper was used. In this case the reaction time was reduced up to 2 h nevertheless the mixture heating at 180-220 ºC was ensured.



Scheme 1. Synthesis of 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid (IV 1) and its aminooxalil- and phenylamino- moieties substituted derivatives (IV 2-5)

For 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid and its aminooxalil- and phenylamino- moieties substituted derivatives preparation we developed also the alternative cross synthesis two-stages procedure (Scheme 1, 1C protocol) which began with 3-amino-2-phenylamino-benzoic acid or its appropriate phenylamino moiety substituted derivative (**II**) acylation by treating it with ethyl chloro(oxo)acetate in concentrated acetic acid medium in the presence of pyridine to afford respective 3-ethoxyoxalyl-amino esters (**III**). On the second stage the esters were involved into the reaction with appropriate alkylamine 25 % aqueous solution in ethanol medium.

The synthetic protocol employed for 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acid carbamoyl- and phenylamino- moieties substituted derivatives (**IX 1-3**) preparation was based on the appropriate 2-chloro-3-(3-carbamoyl-propionylamino)-benzoic acid carbamoyl- moiety alkyl or hydroxylalkyl substituted derivatives (**V**) treatment with the respective methylphenylamine in DMF medium (2A protocol) or as a solid-phase synthetic protocol 2B in the presence of potash and CuO catalyst as it is depicted in Scheme 2.

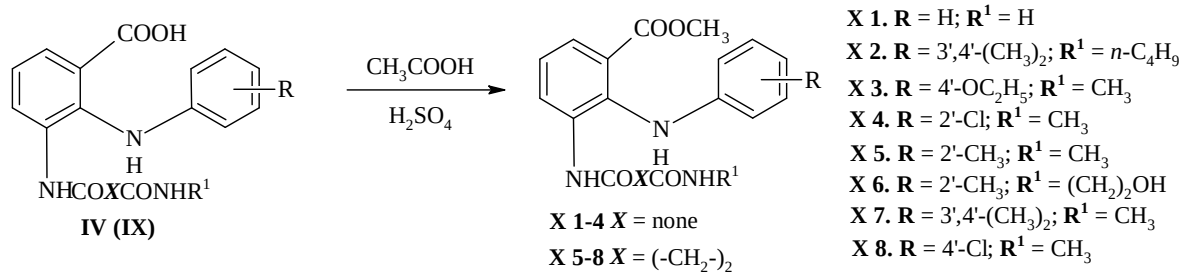


Scheme 2. Synthesis of 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acid carbamoyl - and phenylamino- moieties substituted derivatives (IX 1-3)

The direct amidation reaction also enabled a simple and efficient preparation of 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acid derivatives (**IX 1-3**) from the corresponding 3-(2,5-dioxo-pyrrolidin-1-yl)-2-phenylamino-benzoic acid derivatives (**VII**) (2C protocol) or 3-(4-oxo-pentanoylamino)-2-phenylamino-benzoic acid derivatives (**VIII**) (2D protocol) by their treatment with the appropriate aliphatic amines (Scheme 2).

Therefore, we proved that the solid phase synthesis (2B protocol) at the temperature of 180-220 ^{o}C was the most convenient way to access 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acid derivatives (**IX 1-3**) as the temperature increasing led to the target compounds **IX 1-3** and the starting 2-chloro-3-(3-carbamoyl-propionylamino)-benzoic acid derivatives **V** decarboxylation.

In order to afford 3-(aminooxalyl-amino)- and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids methyl esters the corresponding acids or their aminooxalil-, carbamoyl- and phenylamino- moieties substituted derivatives were treated with anhydrous methanol in the presence of concentrated sulfuric acid (Scheme 3).



Scheme 3. Synthesis of 3-(aminooxalyl-amino)- and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids methyl esters and their aminooxalil-, carbamoyl- and phenylamino- moieties substituted derivatives (X 1-8)

The structures of the obtained compounds were confirmed by IR and ^{1}H NMR spectroscopy, elemental analysis and approved *via* cross synthesis.

The IR spectra of 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid (**1**) and its aminooxalil- and phenylamino-moieties substituted derivatives (**IV**, **2-5**) showed the expected bands for the characteristic groups which were present in the compounds. The broad band for OH-group stretching vibration centered at ~ 3100-3150 cm^{-1} was shifted to the low-frequency spectrum region that proved the intermolecular associates formation. The broad low-frequency spectrum region shifted band for N-H in the secondary amine group at 3200-3400 cm^{-1} proved its participation in intermolecular hydrogen binding formation. All compounds had carboxyl group C=O stretching bands in the range of 1725-1674. The strong absorption bands at the 1250 to 1234 cm^{-1} region came from secondary amine group bonds with two phenyl rings stretching. Deformative vibrations for N-H were shown as a weak absorption band in the range of 1583-1568 cm^{-1}.

The IR spectra of 2-phenylamino-benzoic acids methyl esters showed the bands for N-H in the secondary amine group at 3360-3210 cm^{-1} while all esters provided carbonyl C=O stretching bands in the range of 1680-1655 cm^{-1} for carboxyl group and in the range of 1715-1690 cm^{-1} for the ester group. Deformative vibrations for methylene and secondary amine groups were shown as absorption bands in the ranges of 1415-1405 cm^{-1} and 1580-1570 cm^{-1}, respectively. The bands for CH_3-group stretching asymmetric and symmetric vibrations were present at 2988-2862 cm^{-1}.

In the ^{1}H-NMR spectral data for 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid and its aminooxalil- and phenylamino- moieties substituted derivatives, all protons were seen according to the expected chemical shift and integral values. The aromatic protons of both phenyl rings appeared as multiplet peaks within the range of 6.61-8.65 ppm. The singlet signals derived from secondary amine group in diphenylamine moiety appeared around 9.02-9.06 and 9.60-9.73 ppm. The ^{1}H-NMR spectra of all compounds showed two secondary amine groups protons in aminooxalyl-amino or alkylaminooxalyl-amino moiety signals as singlets in the δ 8.43–9.12 ppm and 5.73 (compound **1**) or around 10.15-10.23 ppm, respectively. The carboxylic group proton resonated as broad singlet at 9.73-12.25 ppm. Methyl group protons in tolyl fragment of compounds **IV 2** and **IV 3** were observed around 2.30-2.32 ppm while protons of two methyl groups in dimethyl phenyl fragment of compound **IV 3** appeared at 2.18 and 2.25 ppm. The protons signals derived with ethylene and hydroxyl fragments structures in hydroxy-ethylaminooxalyl moiety of compound **IV 3** appeared as a multiplet at 3.44 ppm and as a singlet at 4.80 ppm, respectively. The ^{1}H-NMR spectrum of compound **IV 4** displayed signals due to butyl substituent in aminooxalyl-amino moiety as a triplet at 1.23 ppm, multiplet at 1.82 ppm, and dublet at 3.50 ppm.

The ^{1}H NMR spectra of 3-(aminooxalyl-amino)-2-phenylamino-benzoic acids methyl esters showed the protons signals of secondary amine groups attached to phenyl ring of benzoic acid as singlets at 7.95–8.07 ppm. All esters spectra contained characteristic aromatic multiplet signals used to identify two phenyl rings in the δ broad area of 6.54-7.75 ppm. The ester functional group $COOCH_3$ signal was recorded as a singlet at the δ area of about 3.60 ppm. The protons signals as triplets at about 6.40 ppm were used to locate secondary amine group of aminooxalyl moiety. The amine group attached to both aromatic rings was responsible for a broad singlet at 9.47 ppm.

2.2. Pharmacological screening evaluations

2.2.1. Anti-inflammatory activity in vivo evaluation

Carrageenan-induced paw edema is the most widely used animal model of acute inflammation. *In vivo* studies of novel 3-(aminooxalyl-amino)- and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids, their aminooxalil-, carbamoyl- and phenylamino- moieties substituted derivatives and respective methyl esters were carried out for anti-inflammatory activity employing the carrageenan-induced rat paw edema method. Marked paw edema was produced in rats with sub-planter injection of 0.1 mL 2 % carrageenan. The test compounds were dissolved in CMC and injected intraperitoneally in the dose of 20 mg/kg body weight 30 minutes prior to carrageenan injection. The NSAID drug Diclofenac in its effective therapeutic dose (8 mg/kg) was tested in parallel as an activity references. Anti-inflammatory activity was defined by measuring the paw edema volume 4 h after the carrageenan injection. Results of paw edema decreasing were expressed as the mean ± standard deviation and compared statistically with the control group using Student's t-test. A level of $p<0.05$ was adopted as the test of significance (Table 1). The percentage protection against inflammation was calculated as % inhibition by comparison between CMC injected control group and drugs-tested groups.

Evaluation indicated that 3 compounds (**IV 3, IX 3, X 7**) showed rather significant decrease in edema, the inhibition rate for them was observed at the level of 31.4-39.5 % as compared to control group. A series of the newly synthesized compounds possessed low anti-inflammatory activity in the range of 9.8-29.5 % protection to inflammation. The anti-inflammatory evaluation tests for compounds **IV 4** and **X 2** gave the results as 0 % inhibition indicating the compounds showed no any anti-excudative effect.

The results of the pharmacological tests were analyzed with respect of the compounds structure. Among 3-(aminooxalyl-amino)- and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids and their aminooxalil-, carbamoyl- and phenylamino- moieties substituted derivatives the nature and length of the alkyl substituent R^1 was found to be essential for their anti-inflammatory effects. Thus 2-hydroxy-ethyl substituent presence in aminooxalyl and carbamoyl moieties of compounds **IV 3** and **IX 3** was resulted into their anti-inflammatory activity enhancement. While the same substituent presence in carbamoyl moiety of compound **X 6** (3-[3-(2-Hydroxy-ethylcarbamoyl)-propionylamino]-2-o-tolylamino-benzoic acid methyl ester) caused its activity significant decreasing. The long chain substituents at the same positions of 3-(aminooxalyl-amino)- and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids were resulted into their anti-inflammatory activity decreasing up to 0 % like it was observed for compounds **IV 4** and **X 2** containing *n*-butyl substituents in their aminooxalyl- and carbamoyl- moieties.

Table 1. Anti-inflammatory and analgesic effects of 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acid, their aminooxalil-, carbamoyl- and phenylamino- moieties substituted derivatives and respective methyl esters

Compound ID	*Anti-inflammatory activity*		*Analgesic activity*	
	Paw edema volume (mL) ± SEM*	% Inhibition of paw edema	No of writhes in 20 min after treatment (Mean ± SEM*)	% Inhibition
	after 4 h	after 4 h	after 20 min	after 20 min
Control	2.200 ± 0.050	–	36 ± 3.0	–
IV 1	1.810 ± 0.035	17.7	29 ± 2.5	19.4
IV 2	1.550 ± 0.030	29.5	21 ± 1.5	41.7
IV 3	1.330 ± 0.025	39.5	18 ± 1.0	50.0
IV 4	2.200 ± 0.050	0.0	36 ± 2.5	0.0
IV 5	1.588 ± 0.030	27.8	25 ± 2.0	30.6
IX 1	1.868 ± 0.035	15.1	27 ± 2.0	25.4
IX 2	1.819 ± 0.035	17.3	36 ± 2.5	0.0
IX 3	1.463 ± 0.025	33.5	22 ± 1.5	38.9
X 1	1.973 ± 0.035	10.3	27 ± 2.5	25.0
X 2	2.20 ± 0.050	0.0	33 ± 3.0	8.3
X 3	1.800 ± 0.035	18.2	26 ± 2.5	27.8
X 4	1.859 ± 0.040	15.5	23 ± 2.0	36.1
X 5	1.863 ± 0.035	15.3	28 ± 2.5	22.2
X 6	1.984 ± 0.040	9.8	26 ± 2.0	27.8
X 7	1.509 ± 0.025	31.4	22 ± 1.5	38.9
X 8	1.927 ± 0.040	12.4	29 ± 2.5	19.4
Diclofenac	1.375± 0.020	37.5	–	–
Analgin	–	–	17 ± 2.5	52.8

**SEM denotes standard error of mean*

2.2.2. Analgesic activity in vivo evaluation

The analgesic activity of the synthesized compounds was studied by using acetic acid induced writhing test in mice. The analgesic effect was evaluated after intraperitoneal administration of test drugs or reference drug (Analgin in the case of standard group) at a dose of 20 mg/kg body weight (55 mg/kg for Analgin) and expressed as % protection of writhing (Table 1).

Analgesic activity evaluation indicated that two compounds (**IV 2** and **IV 3**) showed significant analgesic effect, the severity of writhing response for them was recorded as 41.7 % and 50.0 %, respectively. Four compounds namely **IV 5**, **IX 3**, **X 4** and **X 7** possessed the analgesic effect in the range of 30.6-38.9 %. For the series of the newly synthesized compounds their writhing inducing effect was insignificant (8.3-27.8 %). The writhing response evaluation tests for compounds **IV 4** and **IX 2** gave the results as 0 % indicating the compounds showed no any analgesic effect.

2.3. QSAR studies

The objectives of the present work were the QSAR studies of 2-phenylaminobenzoic acids derivatives and their methyl esters as novel anti-inflammatory and analgesic agents. We are reporting the percentage protection against inflammation and the writhing severity of synthesized compounds as the response for further QSAR analysis we thought it being essential to perceive the importance of the molecular properties, which are critical in accentuating the biological activity.

HyperChem 7.5 [17] software was used for the molecular modeling and energy optimization. The molecular mechanics MM+ and semi-empirical algorithm with Hamiltonian Austin Model 1 (AM1) force field at 0.01 RMS gradients were used to optimize the molecules. A large number of theoretical molecular descriptors available in E-DRAGON software package [20] were calculated to define the structural property of the molecules needed to perform QSAR analysis.

3*D* molecular descriptors had been shown to be very useful in QSAR problems in order to perform a rational analysis of different pharmacological activities [22]. DRAGON E-version software allowed 6 subsets of 3*D* molecular descriptors calculating including: Randic molecular profiles, Geometrical descriptors, RDF descriptors, 3D-MoRSE, WHIM and GETAWAY descriptors.

Multiple linear regression (MLR) analysis implemented into BuildQSAR software application [21] was used to perform the QSAR studies. The statistical processing of the QSAR models obtaining was carried out by using systematic search algorithm within each descriptors subset firstly [23]. The most significant descriptors from each

set were then included into single 3*D* descriptors set followed by the systematic search procedure in order to confirm that the selected descriptors were the most optimal for describing the biological properties. The statistical significance of the models was determined by examining the correlation coefficients, the standard deviations, the number of variables, F-test ratio and the residuals analysis. The rule of thumb was applied to select number of descriptors in the models: the number of compounds used for the model generation (n) and the number of parameters under consideration (M) should undergo the ratio $n/M \geq 5$.

The significant models selected were further undergone validation study by internal leave-one-out (LOO) cross-validation method [24, 25]. In the LOO approach, each predicted compound is deleted from the n compounds and its activity is computed. The square of LOO cross-validation coefficient Q^2 can be considered as a criterion of both predictive ability and robustness of the model as well as its stability. For a reliable model, the square of cross-validation coefficient Q^2 should be ≥ 0.5 [26].

The best QSAR models obtained with 3*D* descriptors for anti-inflammatory activity are given below with the statistical parameters of the regressions:

Anti-inflam. % = - 43.1077 (± 12.8104) RDF145m + 8.7476
(± 4.2622) RDF030p - 5.4994 (Eq. 1.1)
(± 2.1802) RDF055p + 38.8314
(n = 16; R = 0.910; s = 5.255; F = 19.385; p = 0.0001; Q^2 = 0.745; S_{PRESS} = 6.416; S_{DEP} = 5.739)

Anti-inflam. % = + 1.5879 (± 0.7923) RDF070u - 31.8222
(± 9.3093) RDF145m - 4.7014 (Eq. 1.2)
(± 1.9356) RDF055v + 29.8435 (± 14.2924)
(n = 16; R = 0.910; s = 5.269; F = 19.253; p = 0.0001; Q^2 = 0.757; S_{PRESS} = 6.261; S_{DEP} = 5.600)

Anti-inflam. % = - 31.8534 (± 9.4600) RDF145m - 4.6275
(± 1.9454) RDF055v + 1.6787 (Eq. 1.3)
(± 0.8564) RDF070e + 27.4866 (± 15.0025)
(n = 16; R = 0.907; s = 5.341; F = 18.636; p = 0.0001; Q^2 = 0.753; S_{PRESS} = 6.315; S_{DEP} = 5.649)

where n is the number of compounds included in the model, R is the correlation coefficient, s is the standard deviation of the regression, F is the Fisher ratio, p is significance of the variables in the model, Q^2 is the correlation coefficient of cross-validation, S_{PRESS} is the predicted residual sums of squares standard deviation, and S_{DEP} is the standard deviation error in prediction.

The best models for anti-inflammatory activity were obtained using RDF descriptors explaining more than 90 % of activity variance. The variables in these models are related to the polarizabilities, van der Waals volumes, electronegativities and atomic masses (Table 2).

Table 2. Symbols of the descriptors used in the models 1.1-1.3 and their definitions

Symbol	*Definition*
RDF145m	Radial Distribution Function 14.5 / weighted by atomic masses
RDF030p	Radial Distribution Function 3.0 / weighted by atomic polarizabilities
RDF055p	Radial Distribution Function 5.5 / weighted by atomic polarizabilities
RDF055v	Radial Distribution Function 5.5 / weighted by atomic van der Waals volumes
RDF070u	Radial Distribution Function 7.0 / unweighted
RDF070e	Radial Distribution Function 7.0 / weighted by atomic Sanderson electronegativity

The values of selected RDF descriptors used for the models 1.1-1.3 obtaining are given in Table 3.

Analysis of the residuals and standard deviations of residuals for equations 1.1-1.3 (Table 4) allowed to identify compound **IX 3** as a significant outlier for models 1.2 and 1.3.

Table 3. Values of selected RDF descriptors calculated for 3-(aminooxalyl-amino)- and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids derivatives and their methyl esters

Compounds	RDF145m	RDF030p	RDF055p	RDF055v	RDF070u	RDF070e
IV 1	0	2.15	6.111	5.938	12.475	12.916
IV 2	0	2.261	6.855	6.656	20.773	20.712
IV 3	0	3.113	4.884	4.659	18.514	18.418
IV 4	1.286	4.972	5.087	4.797	21.579	21.355
IV 5	0	2.252	6.068	5.908	11.826	12.413
IX 1	0.018	4.497	10.496	9.782	18.874	18.693
IX 2	0	4.365	9.304	8.805	21.247	21.014
IX 3	0.01	4.569	9.361	8.855	21.626	21.31
X 1	0	2.149	7.501	7.318	14.248	14.637
X 2	0.968	5.313	7.671	7.422	22.071	22.354
X 3	0	2.379	8.091	7.988	18.888	19.37
X 4	0	2.935	8.91	8.639	14.251	14.863
X 5	0	4.283	11.878	11.094	22.548	21.973
X 6	0.044	4.488	11.935	11.144	22.915	22.256
X 7	0	5.634	10.651	10.127	31.261	30.745
X 8	0	3.609	10.407	9.949	19.706	19.671

Table 4. Observed and predicted anti-inflammatory activities, residuals and standard deviations of residuals for the compounds according to the equations 1.1 – 1.3

Com-po-unds	Obser-ved activity, %	Model 1.1			Model 1.2			Model 1.3		
		Predic-ted activity, %	Resi-dual	St. dev. of resid.	Predic-ted activity, %	Resi-dual	St. dev. of resid.	Predic-ted activity, %	Resi-dual	St. dev. of resid.
IV 1	17.7	24.032	-6.332	-1.205	21.735	-4.035	-0.766	21.691	-3.991	-0.747
IV 2	29.5	20.911	8.589	1.634	31.536	-2.036	-0.386	31.455	-1.955	-0.366
IV 3	39.5	39.204	0.296	0.056	37.337	2.163	0.41	36.845	2.655	0.497
IV 4	0	-1.087	1.087	0.207	0.632	-0.632	-0.12	0.174	-0.174	-0.032
IV 5	27.8	25.161	2.639	0.502	20.846	6.954	1.32	20.985	6.815	1.276
IX 1	15.1	19.672	-4.572	-0.87	13.251	1.849	0.351	13.027	2.073	0.388
IX 2	17.3	25.848	-8.548	-1.627	22.185	-4.885	-0.927	22.018	-4.718	-0.883
IX 3	33.5	26.888	6.612	1.258	22.233	11.267	2.138	21.965	11.535	2.16
X 1	10.3	16.379	-6.079	-1.157	18.062	-7.762	-1.473	18.194	-7.894	-1.478
X 2	0	1.393	-1.393	-0.265	-0.809	0.809	0.153	-0.167	0.167	0.031
X 3	18.2	15.146	3.054	0.581	22.28	-4.08	-0.774	23.038	-4.838	-0.906
X 4	15.5	15.506	-0.006	-0.001	11.857	3.643	0.691	12.46	3.04	0.569
X 5	15.3	10.976	4.324	0.823	13.489	1.811	0.344	13.035	2.265	0.424
X 6	9.8	10.559	-0.759	-0.144	12.437	-2.637	-0.5	11.877	-2.077	-0.389
X 7	31.4	29.541	1.859	0.354	31.87	-0.47	-0.089	32.235	-0.835	-0.156
X 8	12.4	13.169	-0.769	-0.146	14.359	-1.959	-0.372	14.469	-2.069	-0.387

Among the large amount of 3*D* molecular descriptors Radial Distribution Function descriptors (RDF) have demonstrated their potential as useful tools for modeling different physicochemical and biological properties [27-29]. These descriptors are based on the distances distribution in the geometrical representation of a molecule and constitute a radial distribution function code [22]. Formally, the radial distribution function of an assembly of *N* atoms can be interpreted as the probability distribution of finding an atom in a spherical volume of radius *R*. The general code of the radial distribution function is represented by:

$$\mathrm{RDF}(R,\ A) = f \sum_{i=1}^{N-1} \sum_{j>i}^{N} A_i A_j e^{-B(R-R_{ij})^2} ,$$

where *f* is the scaling factor and *N* is the number of atoms. $A \in \{u, m, v, e, p\}$, where *A* is the characteristic atomic property of the atoms *i* and *j* (unweighted, atomic masses, van der Waals volumes, electronegativities and polarizabilities). The exponential term contains the distance *Rij* between the atoms *i* and *j* and the smoothing parameter *B*, which defines the probability distribution of the individual distances. RDF is calculated for a number of discrete points as $10 \le R \le 155$ with the defined interval of 5. RDF descriptors are independent of the number of atoms, they are unique regarding the three-dimensional arrangement of the atoms, and they are invariant against translation and rotation of the entire molecule. Besides information about interatomic distances in the entire molecule, RDF descriptors provide further valuable information about bond distances, ring types, planar and non-planar systems and atom types.

The negative contributions to the anti-inflammatory activity were made with RDF descriptors weighted by atomic masses, polarizabilities and van der Waals volumes, but the most significant behavior was that these descriptors corresponded to the radii of 14.5 Å and 5.5 Å. In this sense, according to our models, spherical molecular volumes with these dimensions could have certain restrictions to the addition of bulky substituents. This interpretation suggested that 3-(aminooxalyl-amino)- and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids derivatives were unable to accommodate large substituents or long chains as well as atoms with the large van der Waals volumes or polarizabilities like Chlorine at 3-(aminooxalyl-amino)- or 3-(carbamoyl-propionylamino)- moieties and 2-phenylamino ring at the same time, for example.

On the other hand, the variables RDF030p, RDF070u and RDF070e had positive influence in the anti-inflammatory activity of the synthesized compounds. These descriptors were unweighted, weighted with atomic polarizabilities and electronegativities and suggested that 2-phenylamino-benzoic acids derivatives might accommodate atoms or atom groups in different positions such as Oxygen and phenyl ring that possessed certain dimensional restrictions, because very bulky substituents were not be tolerate.

The best QSAR models obtained with 3*D* descriptors for analgesic activity are given below with the statistical parameters of the regressions:

Analgesic, % = - 4.6441 (± 1.5945) RDF110p
- 1743.5312 (± 671.9031) G1e (Eq. 2.1)
+ 312.1270 (± 107.2398)
(n = 16; R = 0.890; s = 6.981; F = 24.815; p < 0.0001; Q^2 = 0.727; S_{PRESS} = 8.000; S_{DEP} = 7.447)

Analgesic, % = - 5.1595 (± 1.8488) RDF110v
- 1753.1821 (± 696.6210) G1e (Eq. 2.2)
+ 313.6951 (± 111.2488)
(n = 16; R = 0.882; s = 7.206; F = 22.885; p = 0.0001; Q^2 = 0.712; S_{PRESS} = 8.21; S_{DEP} = 7.649)

Analgesic, % = - 35.1316 (± 10.5409) RDF145m - 34.1078
(± 18.0870) Mor08v - 1285.0314 (Eq. 2.3)
(± 559.8458) G1e + 212.5715 (± 87.2599)
(n = 16; R = 0.920; s = 6.266; F = 21.908; p < 0.0001; Q^2 = 0.762; S_{PRESS} = 7.781; S_{DEP} = 6.959)

Analgesic, % = - 18.7341 (± 10.6403) RDF145m + 148.8529
(± 88.1409) Mor28v - 1457.3133 (Eq. 2.4)
(± 615.9861) G1e + 240.7505 (± 94.3489)
(n = 16; R = 0.909; s = 6.664; F = 18.910; p = 0.0001; Q^2 = 0.733; S_{PRESS} = 8.237; S_{DEP} = 7.367)

Analgesic, % = - 19.4439 (± 10.7888) RDF145m + 117.9013
(± 73.3287) Mor28p - 1521.6644 (Eq. 2.5)
(± 645.5612) G1e + 256.5712 (± 99.1499)
(n = 16; R = 0.903; s = 6.835; F = 17.773; p = 0.0001; Q^2 = 0.723; S_{PRESS} = 8.394; S_{DEP} = 7.508).

The derived two- and three-variables models for analgesic activity were obtained using 3*D* descriptors of RDF, 3D-MoRSE and WHIM subsets explaining more than 88 % of activity variance. The variables in these models were related to the atomic masses, polarizabilities, van der Waals volumes and electronegativities (Table 5).

Table 5. Symbols of the descriptors used in the models 2.1-2.5 and their definitions

Symbol	***Definition***
RDF145m	Radial Distribution Function 14.5 / weighted by atomic masses
RDF110p	Radial Distribution Function 11.0 / weighted by atomic polarizabilities
RDF110v	Radial Distribution Function 11.0 / weighted by atomic van der Waals volumes
G1e	1st component directional WHIM index / weighted by atomic Sanderson electronegativity
Mor08v	3D-MoRSE – signal 08 / weighted by atomic van der Waals volumes
Mor28v	3D-MoRSE – signal 28 / weighted by atomic van der Waals volumes
Mor28p	3D-MoRSE – signal 28 / weighted by atomic polarizabilities

The values of selected RDF, 3D-MoRSE and WHIM descriptors used for the models 2.1-2.5 obtaining are given in Table 6.

Table 6. Values of selected 3*D* descriptors calculated for 3-(aminooxalyl-amino)- and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acids derivatives and their methyl esters

Compounds	RDF110p	RDF110v	G1e	Mor08v	Mor28v	Mor28p
IV 1	0	0	0.163	-0.701	0.143	0.152
IV 2	0.081	0.085	0.157	-0.781	0.178	0.164
IV 3	1.556	1.577	0.154	-1.002	0.208	0.206
IV 4	7.749	7.015	0.158	-0.997	0.101	0.084
IV 5	0.488	0.536	0.16	-0.528	0.109	0.101
IX 1	2.503	2.31	0.15	-0.562	0.084	0.062
IX 2	2.276	1.943	0.173	-0.352	0.098	0.084
IX 3	3.115	2.875	0.15	-0.455	0.087	0.072
X 1	1.03	0.935	0.16	-0.59	0.136	0.128
X 2	10.319	9.269	0.147	-0.606	-0.013	-0.06
X 3	3.416	2.955	0.152	-0.429	0.094	0.062
X 4	1.549	1.511	0.157	-0.513	0.073	0.045
X 5	2.909	2.541	0.16	-0.363	0.096	0.062
X 6	3.701	3.423	0.157	-0.505	0.084	0.048
X 7	3.582	3.161	0.149	-0.308	0.099	0.067
X 8	3.896	3.408	0.153	-0.24	0.078	0.044

Analysis of the residuals and standard deviations of residuals for two-variable equations 2.2 (Table 4) allowed to identify compound **IV 3** as the significant outlier while compound **IX 1** was identified as an outlier for three-variable model 2.3 (Table 8).

Table 7. Observed and predicted analgesic activities, residuals and standard deviations of residuals for the compounds according to the models 2.1 and 2.2

Compounds	Observed activity, %	*Model 2.1*			*Model 2.2*		
		Predicted activity, %	Residual	St. dev. of resid.	Predicted activity, %	Residual	St. dev. of resid.
IV 1	19.4	27.931	-8.531	-1.222	27.926	-8.526	-1.183
IV 2	41.7	38.016	3.684	0.528	38.007	3.693	0.512
IV 3	50	36.397	13.603	1.949	35.568	14.432	2.003
IV 4	0	0.662	-0.662	-0.095	0.498	-0.498	-0.069
IV 5	30.6	30.896	-0.296	-0.042	30.42	0.18	0.025
IX 1	25.4	38.973	-13.573	-1.944	38.799	-13.399	-1.859
IX 2	0	-0.074	0.074	0.011	0.37	-0.37	-0.051
IX 3	38.9	36.131	2.769	0.397	35.884	3.016	0.419
X 1	25	28.379	-3.379	-0.484	28.362	-3.362	-0.467
X 2	8.3	7.906	0.394	0.056	8.154	0.146	0.02
X 3	27.8	31.246	-3.446	-0.494	31.965	-4.165	-0.578
X 4	36.1	31.199	4.901	0.702	30.649	5.451	0.756
X 5	22.2	19.652	2.548	0.365	20.076	2.124	0.295
X 6	27.8	21.205	6.595	0.945	20.784	7.016	0.974
X 7	38.9	35.706	3.194	0.458	36.162	2.738	0.38
X 8	19.4	27.273	-7.873	-1.128	27.875	-8.475	-1.176

Table 8. Observed and predicted analgesic activity, residuals and standard deviations of residuals for the compounds according to the models 2.3 - 2.5

Compounds	Observed activity, %	*Model 2.3*			*Model 2.4*			*Model 2.5*		
		Predicted activity, %	Residual	St. dev. of resid.	Predicted activity, %	Residual	St. dev. of resid.	Predicted activity, %	Residual	St. dev. of resid.
IV 1	19.4	27.021	-7.621	-1.216	24.494	-5.094	-0.764	26.461	-7.061	-1.033
IV 2	41.7	37.46	4.24	0.677	38.448	3.252	0.488	37.006	4.694	0.687
IV 3	50	48.853	1.147	0.183	47.286	2.714	0.407	46.523	3.477	0.509
IV 4	0	-1.637	1.637	0.261	1.437	-1.437	-0.216	1.047	-1.047	-0.153
IV 5	30.6	24.975	5.625	0.898	23.805	6.795	1.02	25.013	5.587	0.817
IX 1	25.4	38.353	-12.953	-2.067	34.32	-8.92	-1.339	35.281	-9.881	-1.446
IX 2	0	2.267	-2.267	-0.362	3.223	-3.223	-0.484	3.227	-3.227	-0.472
IX 3	38.9	34.985	3.915	0.625	34.916	3.984	0.598	36.616	2.284	0.334
X 1	25	27.09	-2.09	-0.334	27.824	-2.824	-0.424	28.196	-3.196	-0.468
X 2	8.3	10.334	-2.034	-0.325	6.456	1.844	0.277	6.991	1.309	0.192
X 3	27.8	31.879	-4.079	-0.651	33.231	-5.431	-0.815	32.588	-4.788	-0.7
X 4	36.1	28.319	7.781	1.242	22.819	13.281	1.993	22.975	13.125	1.92
X 5	22.2	19.348	2.852	0.455	21.87	0.33	0.049	20.415	1.785	0.261
X 6	27.8	26.5	1.3	0.207	23.632	4.168	0.626	22.474	5.326	0.779
X 7	38.9	31.607	7.293	1.164	38.347	0.553	0.083	37.743	1.157	0.169
X 8	19.4	24.148	-4.748	-0.758	29.392	-9.992	-1.499	28.944	-9.544	-1.396

The negative contributions to the analgesic activity were made with RDF descriptors corresponding to the radius of 10.0 Å, weighted by atomic polarizabilities and van der Waals volumes (models 2.1 and 2.2) and RDF descriptor, corresponding to the radius of 14.5 Å, weighted by atomic masses (model 2.3-2.5). According to the derived models, spherical molecular volumes with these dimensions could have certain restrictions to the addition of bulky substituents, for example long chains as well as atoms with the large van der Waals volumes or polarizabilities at 3-(aminooxalyl-amino)- or 3-(carbamoyl-propionylamino)- moieties in the same manner as it was concluded for anti-inflammatory activity.

WHIM descriptors (Weighted Holistic Invariant Molecular descriptors) are geometrical descriptors based on statistical indices calculated on the projections of the atoms along principal axes [22]. WHIM descriptors are built in such a way to capture relevant molecular 3*D* information regarding molecular size, shape, symmetry and atom distribution with respect to invariant reference frames. They are divided into two main classes: directional WHIM descriptors and global WHIM descriptors.

Within the WHIM approach, a molecule is seen as a configuration of points (the atoms) in the three-dimensional space defined by the Cartesian axes (x, y, z). To obtain a unique reference frame, principal axes of the molecule are calculated. Projections of the atoms along each of the principal axes are then performed and their dispersion and distribution around the geometric center are evaluated. Thus the WHIM approach can be viewed as a generalization of searching for the principal axes with respect to the defined atomic property (the weighting scheme).

The axial symmetry of the atoms in the molecule by the new internal coordinate axes (axes of the three major components) can be calculated by the equation:

$$\gamma_m = \left\{1 - \left[\frac{n_s}{A} \cdot \log_2 \frac{n_s}{A} + n_a \cdot \left(\frac{1}{A} \cdot \log_2 \frac{1}{A}\right)\right]\right\}^{-1}, \; m = 1, 2, 3,$$

where n_s is the number of symmetrical atoms along the principal components axis, n_a is the number of unsymmetrical atoms, A – the number of atoms in the molecule.

Then the index of overall symmetry of the molecule is calculated as follows:

$$G = (\gamma_1 \cdot \gamma_2 \cdot \gamma_3)^{1/3}.$$

Overall symmetry index values are directing to 1 if the atoms in the molecule are symmetrically situated along each axis and 0 if the atoms have low symmetry with respect to at least one of the principle component axes.

All derived models for analgesic activity comprised 1st component directional WHIM index weighted by atomic Sanderson electronegativity (G1e) which made a negative contribution into the activity. This fact could be related to the importance in the lack of symmetry keeping with the atoms possessing high electronegativity, like Oxygen or Chlorine, along the 1st principle component axe.

3D-MoRSE descriptors were also incorporated into three-variable models 2.3-2.5 for analgesic activity of the synthesized compounds.

3D-MoRSE (3*D* Molecule Representation of Structures based on Electron diffraction) descriptors are based on the idea of obtaining information from the 3*D* atomic coordinates by use of the transform used in electronic diffraction studies for preparing theoretical scattering curves [22]. A generalized scattering function, called the molecular transform, can be used as the functional basis for deriving, from a known molecular structure, the specific analytical relationship of both X-ray and electron diffraction. The general molecular transform is:

$$G(s) = \sum_{i=1}^{A} f_i \cdot \exp(2\pi_i \cdot r_i \cdot s),$$

where s represents the s scattering in various directions by a collection of A atoms located at points r_i; f_i is a form factor taking into account the direction dependence of scattering from a spherical body of finite size. The scattering value, s, measures the scattering angle as: $s = 4\pi \cdot \sin(\theta/2)/\lambda$, where θ is the scattering angle and λ is the wavelength of the electron beam.

The equation for $G(s)$ is usually used in the modified form. On substituting the form factor by the atomic property w, considering the molecule to be rigid and setting the instrumental constant equal to unity, the following expression is obtained:

$$Mor(s,w) = I(s,w) = \sum_{i=2}^{n} \sum_{j=1}^{i-1} w_i w_j \sin(sr_{ij}) / (sr_{ij}),$$

where $I(s,w)$ is the scattered electron intensity, w is an atomic property, chosen as the atomic number, r_{ij} are the interatomic distances between the ith and jth atoms, and A is the number of atoms. For atomic weightings w, various physicochemical properties such as atomic mass, partial atomic charge, or atomic polarizability are considered.

To obtain uniform length descriptors, the intensity distribution $I(s)$ is made discrete, its value being calculated as a sequence of evenly distributed values, e.g. 32 or 64 values in the range of 1-31 $Å^{-1}$. Clearly, the more values are chosen, the finer becomes the resolution in the representation of the molecule.

The MoRSE descriptors have been shown to have good modeling power for different biological and physicochemical properties and can be used even for the simulation of infrared spectra.

The derived model 2.3 contained Mor08v descriptor weighted by atomic van der Waals volume, this variable had a negative influence on the biological activity of the compounds. In relation to this, the highest values of the descriptor corresponded to analgesic activity decreasing. Finally, two more 3D-MoRSE descriptors were also incorporated into 2.4 and 2.5 QSAR models, namely Mor28v and Mor28p (signal 28 descriptors), weighted by atomic van der Waals volumes and polarizabilities. Both these descriptors had a positive influence on analgesic activity (i.e. an increase of their values increased activity). Thus, the increase in activity occurred when the electron beam scattering with the group of atoms would be mainly on account of atoms with high van der Waals volumes and polarizabilities.

CONCLUSION

Novel 3-(aminooxalyl-amino)-2-phenylamino-benzoic acid and 3-(carbamoyl-propionylamino)-2-phenylamino-benzoic acid derivatives and their corresponding methyl esters possessing anti-inflammatory and analgesic activities were synthesized under Ullmann condensation. Anti-inflammatory and analgesic effects *in vivo* evaluations indicated that 3 compounds (**IV 3, IX 3, X 7**) showed rather significant decrease in edema, the inhibition rate for them was observed at the level of 31.4-39.5 % as compared to control group, while two compounds (**IV 2** and **IV 3**) showed significant analgesic effect, the severity of writhing response for them was recorded as 41.7 % and 50.0 %, respectively. The pharmacology screening allowed identifying **IV 3** as a lead compound for further structural optimization to improve both biological activities.

QSAR studies for the synthesized compounds were performed incorporating 3*D* descriptors into respective models as their computation involved integration of the relevant molecular 3*D* information regarding molecular size, shape, symmetry, atoms and distances distribution in the geometrical representation of the molecules. The interpretation of the QSAR models derived with multiply linear regression technique revealed that the spherical molecular volumes with the dimensions of 10.0 Å and 14.5 Å could have certain restrictions in the sense of bulky substituents addition into the molecules for anti-inflammatory and analgesic actions enhancing. Moreover, the increase in analgesic activity was observed in the case of insufficient symmetry keeping with the atoms possessing high electronegativity, like Oxygen or Chlorine, along the 1st principle component axe, and also when the electron beam scattering with the group of atoms would be mainly on account of atoms with high van der Waals volumes and polarizabilities. It had been demonstrated statistically that achieved models could be used for identifying novel anti-inflammatory and analgesic agents based on the same congeneric series. Taking into consideration the QSAR studies results, the importance given to 3*D* molecular descriptors in modulating the biological activity profiles, was well reflected.

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