Synthesis and Characterization of some Potential Biologically Active Niclosamide Derivatives

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Abstract
Compounds with 2-hydroxy-benzanilide core are recognized for their biological effects. Niclosamide, in particular, is authorized for its anti-helmintic properties and, recently, demonstrated additional effects like antitumoral and antiviral ones. Thus, we considered worthwhile to synthesize some niclosamide derivatives, which could possess enhanced biological activity. Starting from 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide and methyl/ethyl α-halogenated acid esters were obtained methyl/ethyl esters. In order to prove the structural identity of the newly synthesized compounds, modern physico-chemical methods (FTIR, ^{1}H-NMR, ^{13}C-NMR) were used. The data obtained for the analyzed compounds proved their identity and confirm their structure.

Introduction
Worldwide, the drug and pharmaceutical products industry is trying to obtain some products, with high biological activity, a broad spectrum of action, minimal toxicity and side effects. Thus, finding new biologically active compounds has been a challenge for researchers, since the incidence of disease and the action spectrum of pathogens was constantly increasing.
Salicylanilides and their derivatives proved antifungal, antibacterial, antimycobacterial, analgesic and antiinflammatory effects beeing used in various pharmaceutical and biochemical fields [1-4].
Niclosamide (5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide) belongs to the salicylanilides family and appeared on the market in 1960 under the trade name *Bayluscide (Bayer 73) for* treating infections caused by gastrointestinal tapeworm, both in humans and animals [5]. Niclosamide is now an anti-helmintic compound used in human therapy which is approved by FDA [6]. It has an acute oral toxicity in rats (LD_{50}) larger than 5 g/kg body weight and a marginal decrease in haemoglobin concentration and erythrocyte count occurred when rats were given niclosamide at 5 g/kg/day for four weeks [7]. Recently, Niclosamide received renewed attention due to its antiviral effects against severe acute respiratory syndrome virus [8] and human rhinovirus [9], anti-neoplastic activity [10] and anti-anthrax toxin effects [11].
Thus, attempting to combine these properties for a better biological activity and fewer side effects, some new Niclosamide derivatives were obtained and characterized.

Experimental
Reagents and solvents: ethyl chloroacetate, methyl chloroacetate, methyl 2-chloro-propionate, 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide (Sigma-Aldrich, for synthesis);

absolute ethanol, 2-butanone (Merck, analytical purity); sodium carbonate, magnesium sulfate (Sigma-Aldrich).

Apparatus: Melting points are uncorrected and measured Stuart Melting point Apparatus SMP 30. IR spectra (ν_{max} in cm^{-1}) were recorded as KBr pellet, on a Jaskow FTIR-430 instrument. The ^{1}H, ^{13}C-NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ on a Bruker Avance DRX 400 spectrometer, operating at 400 MHz. Chemical shifts (δ values) are expressed in ppm against tetramethylsilane (TMS) as internal standard and coupling constants (*J*) are reported in Hz.

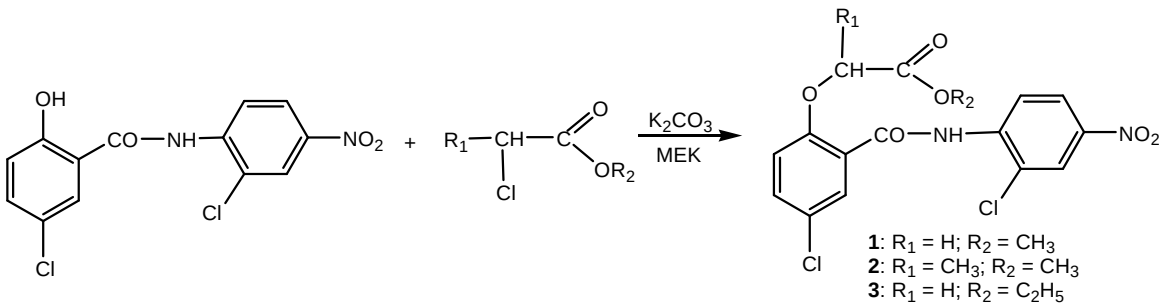
Synthesis of methyl/ethyl esters of 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide [12]. A mixture of 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide and anhydrous K_2CO_3 was refluxed in 2-butanone. Ethyl/methylchloro-acetate/propionate was added dropwise. Optimum molar ratio was amide:ester:K_2CO_3 = 1:1:1. The mixture was stirred and heated on a steam bath for 5 h. After cooling at room temperature, the mixture was poured into water and shook intensively. The organic phase was dried over $MgSO_4$. After filtration and evaporation of solvent in vacuum, the esters crystallized and were recrystallized from ethanol.

Results and discussion

The synthesized compounds, 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide derivatives, are presented in Table 1. Molecular formula / weight, melting points and yields are also presented in Table 1. The synthesized compounds (**1**-**3**) are white-yellow or brick-red, crystalline substances. The synthetic route for preparation of the synthesized compounds is outlined in Scheme 1. The final purification was achieved by recrystallization from absolute ethanol. The target compounds were obtained in yields between 63-75%.

Table 1. Titled compounds characteristics

No.	Compound name	Molecular formula / weight	M.p. (ºC)	Yield (%)
1	[4-Chloro-2-(2-chloro-4-nitro-phenylcarbamoyl)-phenoxy]-acetic acid methyl ester	$C_{16}H_{12}Cl_2N_2O_6$ 399.18	187-188	65
2	2-[4-Chloro-2-(2-chloro-4-nitro-phenylcarbamoyl)-phenoxy]-propionic acid methyl ester	$C_{17}H_{14}Cl_2N_2O_6$ 413.21	284-288	63
3	[4-Chloro-2-(2-chloro-4-nitro-phenylcarbamoyl)-phenoxy]-acetic acid ethyl ester	$C_{17}H_{14}Cl_2N_2O_6$ 413.21	293-296	75



Scheme 1. The synthesis of 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide derivatives

The structures of the synthesized compounds were elucidated by IR, ^{1}H-NMR and ^{13}C-NMR analysis. In order to facilitate the NMR spectra interpretation, the numbering of the aromatic rings is presented in Figure 1.

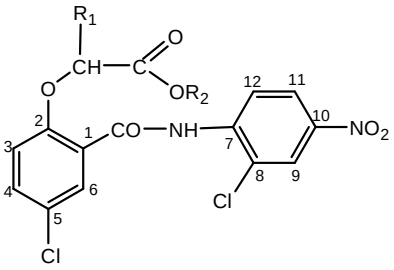


Figure 1. Numbering of aromatic rings

The spectral data of the synthesized compounds are listed below.

([4-chloro-2-(2-chloro-4-nitro-phenylcarbamoyl)-phenoxy]-acetic acid methyl ester (1)

IR ν(cm^{-1}) KBr pellet: 3317; 3087; 2958; 1735; 1685; 1583; 1548; 1502; 1481; 1400; 1340; 1274; 1205; 1116; 1047; 989; 804; 742;

^{1}H-NMR [δ(ppm)]: 3.73 (s, 3H, COOC$\underline{H}_3$); 5.22 (s, 2H, OC$\underline{H}_2$CO); 7.34 (d, 1H, H_3, J=8.0); 7.65 (dsc, 1H, H_4, J=8.0); 7.98 (ssc, 1H_6); 8.28 (dsc, 1H, H_{12}, J=8.0); 8.39 (ssc, 1H, H_9); 8.64 (d, 1H, H_{11}, J=8.0); 10.60 (s, 1H, CON$\underline{H}$);

^{13}C-NMR [δ(ppm)]: 51.78 (COO$\underline{C}H_3$); 66.06 (O$\underline{C}H_2$CO); 115.96 (C_3); 121.78 (C_1); 122.86 (C_{11}); 123.15 (C_{12}); 123.43 (C_9); 124.36 (C_5); 126.01 (C_8); 130.34 (C_6); 133.21 (C_4); 140.41 (C_7); 143.02 (C_{10}); 154.27 (C_2); 161.70 ($\underline{C}$ONH); 168.94 ($\underline{C}$OOCH$_3$);

2-[4-chloro-2-(2-chloro-4-nitro-phenylcarbamoyl)-phenoxy]-propionic acid methyl ester (2)

IR ν(cm^{-1}) KBr pellet: 3292; 3103; 1745; 1679; 1581; 1544; 1475; 1436; 1402; 1336; 1257; 1193; 1157; 1056; 891; 703;

^{1}H-NMR [δ(ppm)]: 1.68 (d, 3H, OCH(C$\underline{H}_3$)COO); 3.48 (q, 1H, OC$\underline{H}$(CH$_3$)COO); 3.69 (s, 3H, COOC$\underline{H}_3$); 6.93 (d, 1H, H_3, J=8.0); 7.26 (dsc, 1H, H_4, J=8.0); 7.86 (ssc, 1H_6); 8.17 (dsc, 1H, H_{12}, J=8.0); 8.27 (ssc, 1H, H_9); 8.88 (d, 1H, H_{11}, J=8.0); 10.26 (s, 1H, CON$\underline{H}$);

^{13}C-NMR [δ(ppm)]: 18.12 (OCH($\underline{C}$H$_3$)COO); 55.78 (COO$\underline{C}$H$_3$); 82.20 (O$\underline{C}$H(CH$_3$)COO); 114.22 (C_3); 119.09 (C_1); 120.35 (C_{11}); 120.48 (C_{12}); 122.13 (C_9); 123.01 (C_5); 124.23 (C_8); 128.97 (C_6); 132.71 (C_4); 141.26 (C_7); 143.21 (C_{10}); 155.16 (C_2); 161.66 ($\underline{C}$ONH); 164.60 ($\underline{C}$OOCH$_3$);

[4-chloro-2-(2-chloro-4-nitro-phenylcarbamoyl)-phenoxy]-acetic acid ethyl ester (3)

IR ν(cm^{-1}) KBr pellet: 3105; 2904; 1749; 1641; 1600; 1541; 1500; 1473; 1407; 1319; 1242; 1178; 1118; 1045; 889; 825;

^{1}H-NMR [δ(ppm)]: 1.19 (t, 3H, COOCH$_2$C$\underline{H}_3$, J=8.0); 4.19 (q, 2H, COOC$\underline{H}_2$CH$_3$, J=8.0); 5.18 (s, 2H, OC$\underline{H}_2$COO); 6.91 (d, 1H, H_3, J=8.0); 7.27 (dsc, 1H, H_4, J=8.0); 7.87 (ssc, 1H_6); 8.18 (dsc, 1H, H_{12}, J=8.0); 8.29 (ssc, 1H, H_9); 8.88 (d, 1H, H_{11}, J=8.0); 10.60 (s, 1H, CON$\underline{H}$);

^{13}C-NMR [δ(ppm)]: 13.87 (COOCH$_2$$\underline{C}H_3$); 60.83 (COO$\underline{C}H_2CH_3$); 65.27 (O$\underline{C}H_2$CO); 114.23 ($C_3$); 119.06 ($C_1$); 119.11 ($C_{11}$); 120.40 ($C_{12}$); 122.18 ($C_9$); 123.00 ($C_5$); 124.22 ($C_8$); 128.91 ($C_6$); 132.67 ($C_4$); 141.21 ($C_7$); 143.36 ($C_{10}$); 155.14 ($C_2$); 161.51 ($\underline{C}$ONH); 164.65 ($\underline{C}$OOCH$_2CH_3$);

The IR spectral data of the esters show the existence of an ether bond between the phenolic hydroxyl group and the alkyl α-C atom of the ester by signals in the range 1200–1260 and 1040–1060 cm^{-1}. The carbonyl groups from the esters appear in the range 1730–1750 cm^{-1}. The vibrations of the amide and hydrazide group appear as signals between 3100–3400 and 1640–1690 cm^{-1}, respectively.

The 1H-NMR shifts of the methyl protons from the methyl ester were observed in the spectra as singlet between 3.6 and 3.8, whereas the ethyl group from the ethyl ester appears in the range 1.1-4.2 ppm. The proton of the amide group, in all analyzed compounds, was observed as singlet between 10.2 and 10.6 ppm.
The 13C-NMR signals corresponding to the carbons from the amide group appear in the range 161–162 ppm and those of the aromatic carbons between 114 and 156 ppm.

Conclusion

Three new compounds, 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide derivatives, were synthesized in order to expand the collection of potential biologically active compounds.
The target compounds belonging to esters group were obtained with good yields (>65%) and characterized using modern analytical methods.
All spectral data proved the identity and provided the elemental composition of the analyzed compounds.

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