New Phenoxyacetic Acid Analogues with Antimicrobial Activity

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This paper presents experimental data regarding the synthesis and the antimicrobial activity of new 4-phenylazo-phenoxyacetic acids. All compounds were characterized by IR, UV-Vis, mass spectral data and by elemental analysis. These compounds were tested for their antibacterial activity against Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and for their antifungal activity against Candida albicans by disk diffusion method.

Key words: 4-phenylazo-phenoxyacetic acids, IR spectra, UV-Vis spectra, mass spectra, antimicrobial activity

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The derivatives of phenoxyacetic acid have been studied intensively due to their pharmacological and phytobiological activity. 2,4-D (2,4-dichlorophenoxyacetic acid) was introduced in 1944 as the first of the “phenoxy herbicides”. It is highly selective for broadleaf weeds and was translocated throughout the plant. Several compounds belong to this class are 2,4,5-T, 2,4-DB, MCPA and silvex [1]. Ethacrynic acid (2-[2,3-dichloro-4-(2-ethylprop-2-enoyl)phenoxy]ethanoic acid), a monosulfonyl diuretic, differs from other thiazide diuretics in that a double ring system is incorporated into its structure. Ethacrynic acid inhibits sodium ion transport across the renal tubular epithelium. By increasing the delivery of sodium to the renal tub, ethacrynic acid indirectly increases potassium excretion via the sodium-potassium exchange mechanism [2].

Centrophenoxine (acetic acid, (4-chlorophenoxy)-2-(dimethylamino)ethyl ester), also known as Lucidril® and meclofenoxate, is one of the older nootropic drugs- it was developed in 1959 at the French National Scientific Research Center [3]. Beneficial therapeutic effects of centrophenoxine have been observed in various human disorders such as cerebral atrophy, brain injury, post apoplectic status, chronic alcoholism and barbiturate intoxication [4].

We have recently reported the synthesis of 4-phenylazo-phenoxyacetic acids by the condensation of some 4-phenylazo-phenols with chloroacetic acid [5, 6].

In this respect, as the most recent results are continuing our concern in the chemistry of azo dyes, and in the present paper we report the synthesis, structure and antimicrobial activity of several new 4-phenylazo-phenoxyacetic acids.

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Experimental part

Materials

The novel 4-phenylazo-phenoxyacetic acids have been synthesised starting from 4-phenylazo-phenols and monochloroacetic acid using a method described in previous papers [5, 6]. As intermediates: 2-chloro-4-phenylazo-phenol, 2-allyl-4-(4-chloro-phenylazo)-phenol, 2-bromo-4-phenylazo-phenol, 4-(4-cyano-phenylazo)-phenol, have been used. They have been obtained by the coupling of adequate diazonium salts with different phenols. The aromatic amines, the phenols and the monochloroacetic acid were Merck products.

Techniques

The melting points have been established in capillaries and verified with a Boetius apparatus. Elemental analyses of carbon, hydrogen and nitrogen have been performed using a Carlo-Erba O/EA, 1108, analyser. The electronic spectra have been carried in dioxane, with a UV-Vis Jasco spectrophotometer, within 200-700nm. FTIR spectra were recorded on a Avatar Nicolet spectrophotometer in KBr pellets, within the range 3500-400cm\(^{-1}\). Mass spectra have been obtained using a HPGC-MS 5890 MD 5971 spectrometer at 70eV, with carrier gas He at 2mL/min.

General procedure

All compounds were tested for antimicrobial activity against 6 microorganisms: Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli and Candida albicans. Test compounds were dissolved in ethanol. Concentrations of 0.2% of the test compounds were obtained.

Tests of different isolates of microorganisms used were carried out by pouring 15mL sterile Mueller Hinton agar in each Petri discs of 9 cm diameter. After solidification, the plates were placed in an incubator at 37°C for 30 min to remove excessive moisture.

Overnight broth culture was streaked evenly onto medium in three directions using a wooden stick cotton swab. Excess suspension was removed from the swab by rotating it firmly against the side of the tube before seeding the plate surface using sterile forceps. The plates were inoculated aerobically at 37°C within 15 min. After 24 h incubation, the diameters of the inhibition zones were measured (including the 6 mm diameter of the disc) with a rule [7].

Synthesis of 2-chloro-4-phenylazo-phenoxyacetic acid

In a 100mL three necked flask, equipped with a condenser, stirrer and thermometer are added 1.16g (5mmol) of 2-chloro-4-phenylazo-phenol and 5mL sodium hydroxide solution 10N. The reaction mixture is stirred for 90 min, while the azophenol reacts with sodium hydroxide.

After cooling, to azophenoxide are added 0.47 g (5mmol) chloroacetic acid, previously neutralized with 5% solution

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of sodium hydroxide. The mixture is refluxed for five hours, then cooled and neutralized with 10% HCl solution, when precipitates 2-chloro-4-phenylazo-phenoxyacetic acid. After repeated recrystallizations from the acetic acid a pure compound was afforded, with m.p. 136°C, yield 73%. All 4-phenylazo-phenoxyacetic acids 1-4 were obtained using the same experimental procedures [5].

2-chloro-4-phenylazo-phenoxyacetic acid 1
m.p. = 136°C (acetic acid); yield 73%; calcd. for C_{14}H_{11}N_{2}O_{3}Cl: C 57.83, H 4.57, N 8.47; found: C 57.90, H 4.45, N 8.32.
IR (KBr; cm^{-1}): 3472, 1740, 1590, 1414, 1263,1037.
UV (dioxane, λ_{max} (nm), ε_{max} (1000cm^{-1}/mol)): 240 (14358); 347 (26795); 438 (1739).
MS spectra (base peak m/z = 100%): 77.

2-bromo-4-phenylazo-phenoxyacetic acid 2
m.p. = 83°C (acetic acid); yield 58%; calcd. for C_{14}H_{11}N_{2}O_{3}Br: C 50.17, H 3.31, N 8.36; found: C 50.05, H 3.24, N 8.29.
IR (KBr; cm^{-1}): 3462, 1729, 1568, 1417, 1269, 1059.
UV (dioxane, λ_{max} (nm), ε_{max} (1000cm^{-1}/mol)): 230 (89928); 351 (10490); 452 (5490).
MS spectra (base peak m/z = 100%): 111.

2-allyl-4-(4-chloro-phenylazo)-phenoxyacetic acid 3
m.p. = 74°C (acetic acid); yield 65%; calcd. for C_{15}H_{11}N_{3}O_{3}: C 64.05, H 3.94, N 14.94; found: C 63.94, H 3.89, N 14.72.
IR (KBr; cm^{-1}): 3453, 1732, 1562, 1414, 1257, 1014.
UV (dioxane, λ_{max} (nm), ε_{max} (1000cm^{-1}/mol)): 230 (22300); 346 (20715); 437 (1255).
MS spectra (base peak m/z = 100%): 77.

4-(4-cyano-phenylazo)-phenoxyacetic acid 4
m.p. = 180°C (acetic acid); yield 79%; calcd. for C_{14}H_{11}N_{2}O_{3}: C 57.93, H 4.39, N 8.39; found: C 58.46, H 4.38, N 8.39.
IR (KBr; cm^{-1}): 3473, 2234, 1707, 1572, 1406, 1276, 1078.
UV (dioxane, λ_{max} (nm), ε_{max} (1000cm^{-1}/mol)): 220 (22300); 344 (13160); 432 (10820).
MS spectra (base peak m/z = 100%): 102.

Results and discussions
In this paper it is presented the synthesis of four 4-phenylazo-phenoxyacetic acids by the condensation of sodium salts of corresponding substituted 4-phenylazo-phenoxyacids with chloroacetic acid in alkaline medium, using Williamson method.

scheme 1

The melting temperatures of 4-phenylazo-phenoxyacetic acids depend on the nature and position of the existing on the aromatic nuclei. The presence of cyano group in para position explains the increasing of melting points of 4-(4-cyano-phenylazo)-phenoxyacetic acid.

The yields are generally high, depending on the raw materials purity and on solubility of novel acids in the solvent used for recrystallisation.

The structure of these compounds has been investigated on the basis of UV-Vis, IR and mass spectra.

The IR spectra show absorption bands specific to –COOH, –N=N-, Ar-O-CH_{2}, Ar-CN groups, and also for the aromatic rings.

Carboxylic acids show two characteristic IR absorption that make the COOH group easily identifiable. In our case, the OH bond of the carboxy group gives rise to a very broad absorption over the range 3473-3453cm^{-1} and the C=O bond shows an absorption between 1740-1707cm^{-1}.

Aromatic rings show a characteristic series of peaks in the 1520-1470cm^{-1} of the infrared spectra.

In the region 1600-1400 cm^{-1} available the bands due to the vibrations of azo group and those due to the stretching vibrations íC=C for the C-C linkages in aromatic nuclei. The absorptions bands due to the azo group appear in two regions, namely at 1600-1550 cm^{-1}and 1430-1400 cm^{-1}. The intensive bands of the first region are a result of conjugation of azo group with aromatic nuclei. The weak bands of region 1430-1400 cm^{-1}are due to the vibration of azo group and agree with the data presented in literature. [8-10]

In conformity with our previous papers, the examination of the infrared spectra of compounds 1-4 reveals that the azo group shows a stronger absorption in region 1590-1562cm^{-1} than in 1417-1406cm^{-1}[5, 6].

Very important and relevant are the bands due to the etheric vibrations; normally, these compounds should have two bands because of antisymmetrical and symmetrical vibrations. Very intense bands were attributed from 1276-1257cm^{-1} to the antisymmetrical valence vibrations and much weaker from 1078-1014cm^{-1} to the etheric symmetrical valency vibrations.

In the case of 4-(4-cyano-phenylazo)-phenoxyacetic acid, a characteristic band for CN group at 2234cm^{-1} is shown.

The strong absorption band at 1190-1110 cm^{-1} is characteristic to the vibration of the Ph-N band [9-10].

On the other hand, the moderate absorption band at 870-856cm^{-1} can be assigned to the vibration of the C-Cl bond.

In the UV-Vis spectra we remark the presence of some absorption bands: from middle intensity at 220-248nm, benzenoid-type E- or B-bands, consequently to the π electrons conjugation from the aromatic rings, intense absorption K-bands 344-362nm, as the result of the conjugated system Ar-N=N-Ar, and low intensity R-bands at 430-452nm, due to the -N=N- chromophore [11-12].

Further evidence for the 4-phenylazo-phenoxy acetic acids structure was obtained from mass spectrum.

For 2-chloro-4-phenylazo-phenoxyacetic acid, scheme 1 show the fragmentation process specific to the phenoxyacetic acids by the cleavage of the bond between the C of the methylene group and C atoms of the carboxyl group followed by CO_{2} elimination.
The mass spectrum of 2-chloro-4-phenylazo-phenoxyacetic acid, for example, showed the peak at m/z 232, consistent with molecular formula C_{12}H_{9}N_{2}O. The peak at m/z 226 can be assigned due to the loss of C_{2}H_{2}O_{2} from the parent ion (scheme 2).

The cleavage of the C-N bonds in this fragmentation produced fragments at m/z 155, m/z 77 (base peak), m/z 105 and m/z 127, respectively (scheme 3).

The tropilium ion, m/z 91, obtained by skeletal transposition, characteristic of the monoalkyl aromatic compounds, could be eliminate acetylene, and formed the fragment m/z 65 (ciclopentadienilium), which by itself losing an other acetylene molecule generate the ion ciclopropenilium (m/z 39) (scheme 6).

All fragmentation process can support the structure formula assigned to the compounds and are in agreement with the literature and with fragmentation of phenoxyacetic acid [13 - 19].

The fragmentation described in schemes 1-6 for 2-chloro-4-phenylazo-phenoxyacetic acid, are characteristic for all compounds 1-4.

**Antimicrobial activity**

The 4-phenylazo-phenoxyacetic acids thus described were subjected to antimicrobial activity screening against two gram-positive bacteria (Staphylococcus aureus and Streptococcus pyogenes), three gram-negative bacteria (Pseudomonas aeruginosa, Proteus vulgaris and Escherichia coli) and one fungi species (Candida albicans) employing the disk diffusion technique.

2-chloro-4-phenylazo-phenoxyacetic acid exhibit antibacterial activity against Staphylococcus aureus (diameter of zone of inhibition=12mm), Escherichia coli. (diameter of zone of inhibition=7mm) and Pseudomonas aeruginosa, (diameter of zone of inhibition=15mm). The best efficiency was exhibited by 2-bromo-4-phenylazo-phenoxyacetic and 2-allyl-4-(4-chloro-4-phenylazo)-phenoxyacetic acid against Staphylococcus aureus (diameter of zone of inhibition=16mm, respectively 18mm). All compounds tested are inactive against Streptococcus pyogenes, Proteus vulgaris and species Candida albicans.

**Conclusions**

Four novel 4-phenylazo-phenoxyacetic acids have been obtained by SN2 reaction, and their structure have been confirmed by elemental analysis, IR, UV-Vis and mass spectra. All compounds are tested from antimicrobial activity. The investigation on the structure-activity relationships confirmed the importance of the nature of substituent in the antimicrobial activity. The best efficiency was exhibited by all compounds having chloro substituent.

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