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Synthesis and SAR evaluation of the phytochemical activity of new N-pyrrolylcarboxylic acids

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Two new N-pyrrolylcarboxylic acids were synthesized via Paal-Knorr cyclization by condensation of γ-aminobutyric acid and 1,4-dicarbonyl compounds. The obtained structures were elucidated by IR and ^{1}H-NMR spectral data and their purity was proven by TLC characteristics and melting points. The corresponding phytochemical activity of the obtained compounds on wheat and cucumber cultivars in three concentrations was studied. Both molecules were tested at 4 concentrations for herbicidal activity whereat no concentration dependence of the herbicidal effects was established. The inhibition of growth of the aerial parts and roots at the lowest (0.001 mM) and the highest (1 mM) concentration of the compounds was comparable.

In addition a number of structural parameters were calculated for the target compounds and some analogues thereof. A second degree polynomial structure-activity dependency on the herbicidal effects from the corresponding miLogP was observed with R^2 in the range of 0.796 to 0.894.

Keywords: pyrroles, phytochemical activity, structure-activity relationship.

INTRODUCTION

Herbicide use is increasingly being adopted around the world. In many parts of the world, herbicides are being increasingly used to replace tillage in order to improve environmental conditions. In comparison with tillage, herbicide use reduces erosion, fuel use, greenhouse gas emissions and nutrient run-off and conserves water [1,2].

Thousands herbicides are known of the chemists, but widely used in agriculture are approximately 100. After the imposition of the widespread use of herbicide glyphosate, there is no introducing of new herbicides in the last 20 years [3,4]. The search for new herbicides is necessary mainly due to the great "plasticity" of the weeds, which results in adjustment to the herbicides used. In this regard, the synthesis and study of the activity of novel compounds with a potential herbicidal activity is especially important.

As a continuation of previous research in our laboratory [5], the current study offers 2 new derivatives of pyrrole for evaluation of phytochemical activity as potential herbicides.

RESULTS AND DISCUSSIONS

Synthesis of the targeted structures

Paal-Knorr pyrrole synthesis, well known as a *powerful reaction* in a retrosynthetic context, was chosen as a reliable access to the targeted structures. The reaction was performed according to Scheme 1 as a cyclization between preliminary prepared 1,4-dicarbonyl compounds and γ-aminobutyric acid. The selection of γ-aminobutyric acid, acting as amino-partner, was based on its involvement of a number of reactions plants such as: control the balance of carbon and nitrogen for the plants, regulate pH of the cells involved in the defense of plants against oxidative stress, and attacks from insects, such as acting osmoregulator, and also as a signal molecule in plants.

The intermediate 1,4-dicarbonyl compounds were synthesized by condensation of R-substituted ω-bromoacetophenones with relevantly R1-substituted commercially available β-dicarbonyl compounds [6]. Conditions for C-alkylation were afforded to suppress the concurrent O-targeted reaction intrinsic to this class of ambident compounds [7,8]. The ω-bromoacetophenones, well known as strong lachrymators, were prepared in our laboratory [9].

The structural diversity **R1**= $N(C_2H_5)_2$ and **R1**= CH_3 aimed changes in hydrophobicity and molar volume of the molecules:

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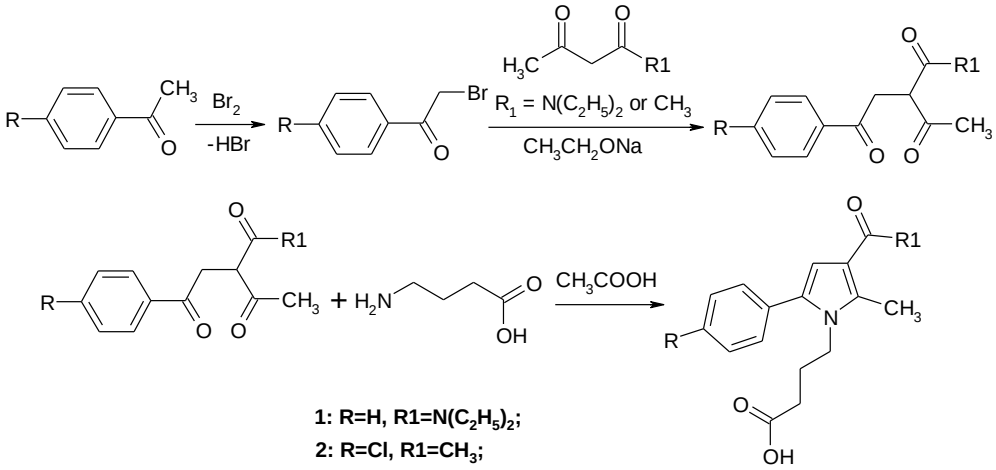
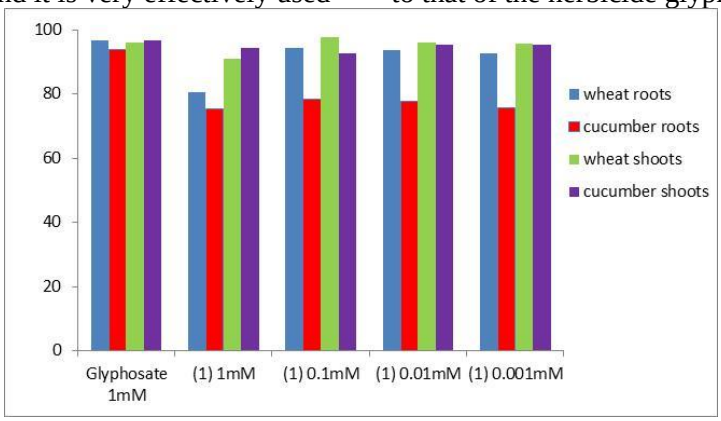


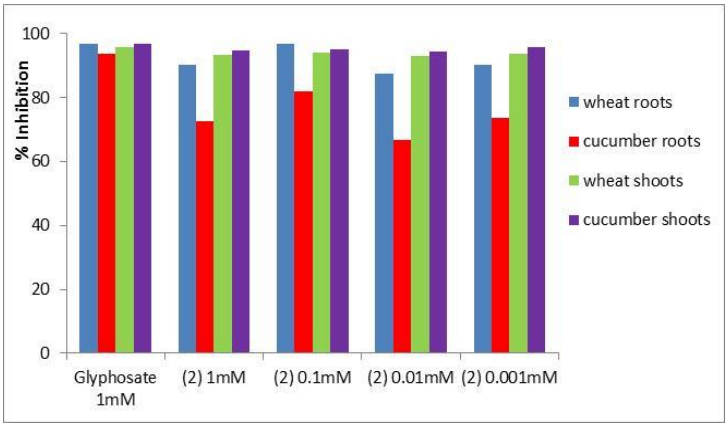
Scheme 1. A synthetic access to the targeted products.

Phytochemical activity of newly synthesized N-pyrrolylcarboxylic acids

The growth-regulating activity of the newly synthesized N-pyrrolylcarboxylic acids was tested by bioassays with wheat (monocotyledonous) and cucumber (dicotyledonous) cultivars. The phytochemical activity was compared to the activity of herbicide glyphosate [(N-phosphonomethyl)glycine]. Glyphosate was chosen since it is one of the most widely distributed herbicide in the world and it is very effectively used in both agricultural and nonagricultural lands. It is very toxic to practically all plant species, but monocotyledonous are more sensitive to its action [10,11]. The prime activity of glyphosate is that it inhibits the biosynthesis of proteins that is essential for plant growth. The results of the bioassays showed that both newly synthesized compounds inhibit the growth of the coleoptile (hypocotyle respectively) and roots of seedlings, and in the experiments with wheat, the effect of all compounds was comparable to that of the herbicide glyphosate (Graphics 1,2):



Graphic 1. Influence of compound 1 on the wheat growth (*Triticumaestivum* L.), cv. Sadovo-1 and cucumber growth (*Cucumissativus* L.), cv. Gerganaseedlings, grown in the dark (4 days, 25±1 °C)



Graphic 2. Influence of compound 2 on the wheat growth (*Triticumaestivum* L.), cv. Sadovo-1 and cucumber growth (*Cucumissativus* L.), cv. Gerganaseedlings, grown in the dark (4 days, 25±1 °C)

Both compounds inhibited the growth of the roots and shoots of cucumber to a lesser extent to the one measured for the reference glyphosate, where the influence on the cucumber shoots was the weakest. It is visible, that the inhibition of roots and shoots in the evaluated wheat cultures is comparable with the one for glyphosate.

Although the compounds were tested at 4 concentrations no concentration dependence of the herbicidal effects was established, and the inhibition of growth of the aerial parts and roots at the lowest (0.001 mM) and the highest (1 mM) concentration of the compounds was comparable.

From the results presented here, it is difficult to explain the exact mode of the herbicide action of the newly synthesized compounds tested. Further investigation will give more information on this problem and will trace the possibilities of their usage in agriculture.

SAR EVALUATION

In an attempt to identify any structure activity relationships a number of structural parameters were calculated for the synthesized compounds. Some analogues thereof, previously synthesized and analyzed by us [5], were added for statistical evaluation. It was of interest to establish the dependency of the herbicidal activity from the structural parameters as: molecular mass, miLogP, volume. From the obtained results was found, that only in the means of miLogP a structure-activity relationship may be drawn. The corresponding dependencies of the evaluated herbicidal effects from miLogP are presented on Figures 1 to 4 below as follows:

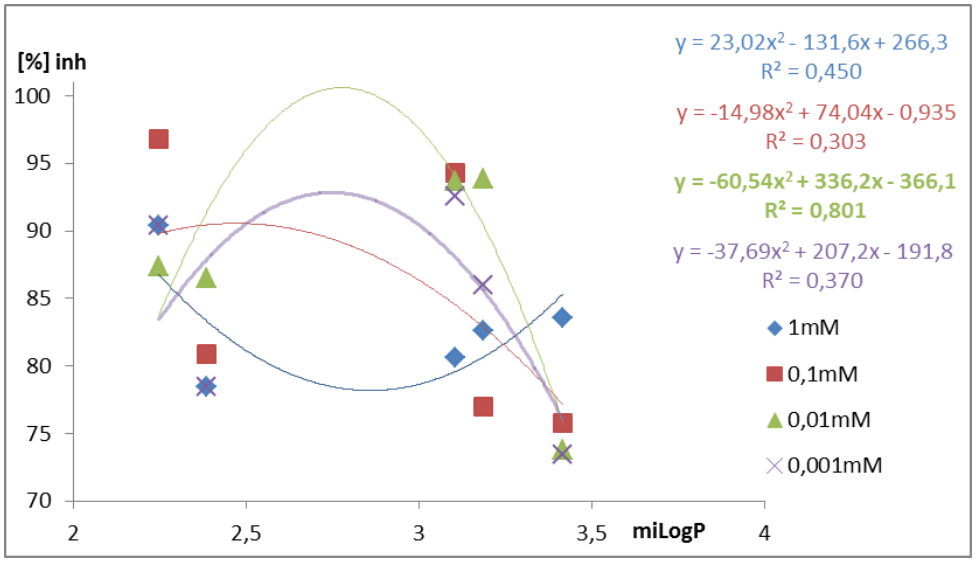


Fig. 1. SAR dependency of inhibition of wheat roots from miLogP.

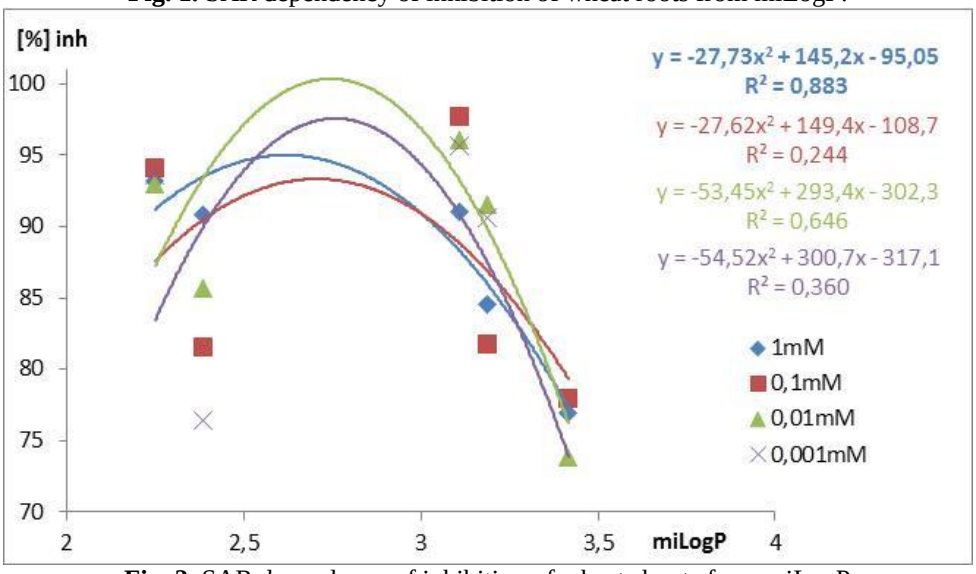


Fig. 2. SAR dependency of inhibition of wheat shoots from miLogP.

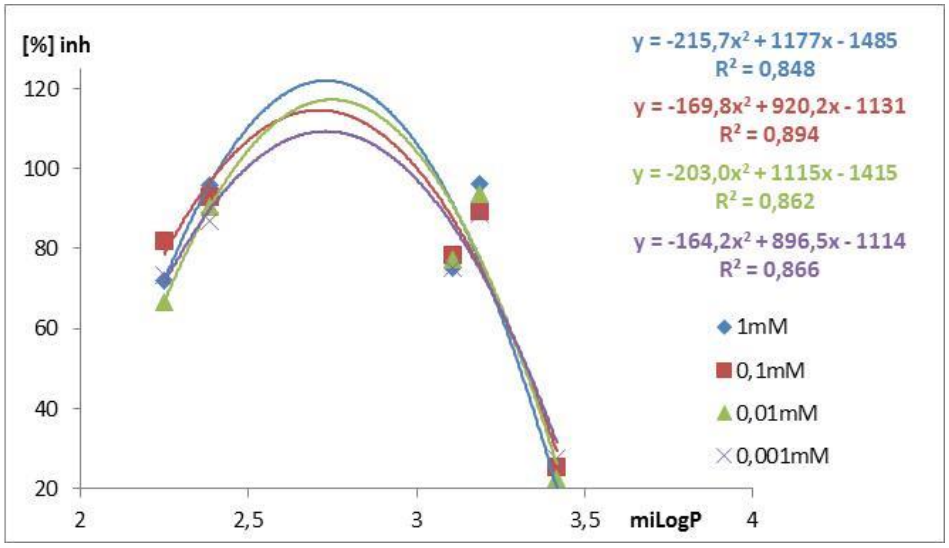


Fig. 3. SAR dependency of inhibition of cucumber roots from miLogP.

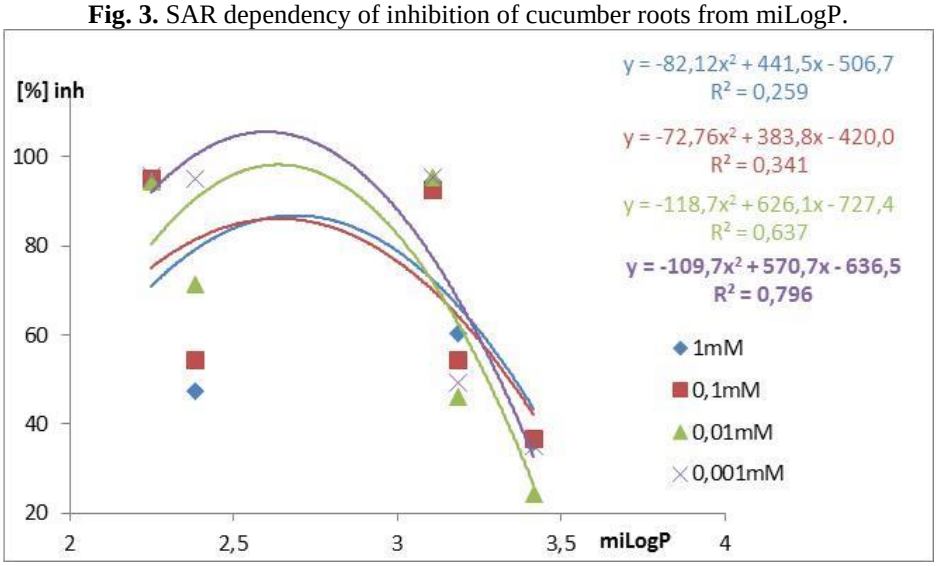


Fig. 4. SAR dependency of inhibition of cucumber shoots from miLogP.

As seen from the presented figures a second degree polynomial structure-activity dependency on the herbicidal effects from the corresponding miLogP is observed with R^2 in the range of 0.796 to 0.894.

Conclusion. Two new N-pyrrolylcarboxylic acids were synthesized *via* Paal-Knorr cyclization. The obtained structures were elucidated by IR and ^{1}H-NMR spectral data and their purity was proven by TLC characteristics and melting points. The corresponding phytochemical activity of the obtained compounds on wheat and cucumber cultivars in three concentrations was studied. No concentration dependence of the herbicidal effects was established for the evaluated compounds.

In addition a second degree polynomial structure-activity dependency of the herbicidal effects from the corresponding miLogP was determined with R^2 in the range of 0.796 to 0.894.

EXPERIMENTAL PART

All commercial chemicals used in this study as starting materials and reagents were purchased from "Merck" (Darmstadt, Germany). The melting points were determined with a capillary digital melting point apparatus IA 9200 Electrothermal AZ9003MK4, Southend-on-Sea, UK. The IR spectra were registered on Specord IR-71, Carl Zeiss, Jena, Germany (KBr). The ^{1}H NMR spectra (250 MHz, 20 ^{o}C) were registered on a BrukerSpectrospin WM250 spectrometer (Faenlanden, Switzerland), using TMS as internal standard. All OH protons were D_2O exchangeable.

TLC characteristics of the products were measured on aluminum sheets of silica gel 60 F_{254}, Merck 1.05554 at ambient temperature using a mobile phase chloroform-ethanol (*Rf* value for the new compounds at the relevant $CHCl_3$-C_2H_5OH ratio is given below).

General procedure for the synthesis of 1,4-dicarbonyl compounds. Sodium (0.10 moles) was

dissolved in anhydrous ethanol (50 ml) and to the resulting solution, cooled to 20-25 °C, relevant 1,3-dicarbonyl compounds (0.10 moles) were added, ensuring that the temperature didn't exceed 30 °C. The mixture was stirred for 15-20 minutes. After cooling, the corresponding α-brominated acetophenone (0.10 moles) was added in portions at a temperature not exceeding 30 °C. The mixture was stirred for 30-40 minutes, benzene (100 ml) were added and the resulting solution was washed successively with 5% HCl and water. The organic layer was dried with anhydrous sodium sulfate. The solvent was removed by rotary vacuum evaporator at a temperature below 45 °C. The residue consisted in the relevant 1,4-dicarbonyl compound as an oil, which was used directly in the next stage of condensation.

General procedure for the synthesis of targeted N-pyrrolylcarboxylic acids: 1,4-dicarbonyl compound (0.10 moles) and the γ-aminobutyric acid (0.12 moles) were dissolved in glacial acetic acid (50 ml). For the preparation of compound **1**, the reaction was performed at the boiling point of the mixture and for the preparation of compound **2** - at 60 ºC. The reaction development was monitored by TLC. After reaction completion, the mixture was poured into water. The separated precipitate was filtered off, washed with water, dried and recrystallized from warm ethanol. The reaction time was varied from 2.30 hours (compound **1**) to 4.30 hours (compound **2**) (TLC control).

All compounds were soluble in warm ethanol, chloroform and dimethylsulfoxide, but insoluble in water and hexane.

4-(3-diehtylcarbamoyl-2-methyl-5-phenyl-pyrrol-1-yl)-butyric acid (1): White solid, Yield 76%, mp 96-98 ºC, R_f 0.48 (10:0.4). IR spectrum (KBr), ν, cm^{-1}: 3600-2300 (COOH), 3350 (O-H), 1700, 1580 (C=O), 780, 730 (C_6H_5); ^{1}H NMR (250 MHz, $CDCl_3$) δ, ppm: 1.20 (t, 6H, *J* 7.1 Hz, 2□NCH$_2$C<u>H$_3$</u>), 1.70-1.81 (m, 2H, CH$_2$<u>CH$_2$</u>CH$_2$COOH), 2.15 (s, 3H, CH$_3$-2), 2.30 (t, *J* 2.0 Hz, 2H, CH$_2$CH$_2$<u>CH$_2$</u>COOH), 3.50 (q, 4H, *J*=7.1, 2□N<u>CH$_2$</u>CH$_3$), 3.95 (t, *J* 3.0 Hz, 2H, <u>CH$_2$</u>CH$_2$CH$_2$COOH), 6.10 (s, 1H, H-4), 7.40–7.50 (m, 5H, C_6H_5), 8.10 (s, 1H, COOH).

4-[3-acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-butyric acid (2): White solid, Yield 84%, mp 162-164 ºC, R_f 0.46 (10:0.3). IR spectrum (KBr), ν, cm^{-1}: 3600-2400 (COOH), 3300 (O-H), 1720, 1695 (C=O), 830 (*p*-C_6H_4); ^{1}H NMR (250 MHz, $CDCl_3$) δ, ppm: 1.79-1.84 (m, 2H, CH$_2$<u>CH$_2$</u>CH$_2$COOH), 2.17 (t, *J* 2.0 Hz, 2H, CH$_2$CH$_2$<u>CH$_2$</u>COOH), 2.30 (s, 3H, COCH$_3$), 2.50 (s, 3H, CH$_3$-2), 3.98 (t, *J* 3.0 Hz, 2H, <u>CH$_2$</u>CH$_2$CH$_2$COOH), 6.30 (s, 1H, H-4), 7.15-7.25 (m, 4H, C_6H_4), 7.44 (s, 1H, COOH).

Phytochemical analyses

Both newly synthesized compounds were tested for potential herbicidal activity in a series of bioassays with the representatives of the monocotyledonous (wheat - *Triticumaestivum* L., cv. Sadovo-1) and dicotyledonous (cucumber - *Cucumissativus* L., cv. Gergana) cultivars. The growth-regulating activity of compounds tested was determined in accordance with their influence on the growth of intact seedlings, grown in dark for 96 h (25±1 °C) by measuring the coleoptile (hypocotyl respectively) and root length. The compounds were tested at 1 mM, 0.1 mM, 0.01 mM, and 0.001 mM concentrations. As a standard in the experiments was used total herbicide glyphosate (active ingredient in Roundup formulation, production of international company Monsanto) at 1 mM concentration.

All experiments were repeated three times with three replications for each concentration tested. The results reported in the tables are means of the values with a standard error (SE).

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СИНТЕЗ И ОЦЕНКА НА ВРЪЗКАТА СТРУКТУРА-ФИТОХИМИЧНА АКТИВНОСТ НА НОВИ N-ПИРОЛИЛ КАРБОКСИЛНИ КИСЕЛИНИ

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(Резюме)

Две нови N-пиролилкарбоксилни киселини са синтезирани чрез Паал-Кнор кондензация на γ-аминомаслена киселина и 1,4-дикарбонилни съединения. Структурите на получените съединения са изяснени чрез ИЧ и ^{1}Н-ЯМР спектрални анализи, а чистотата им бе доказана посредством тънкослойна хроматография и температури на топене. Проучена е фитохимичната активност на съединенията спрямо сортове пшеница и краставица. И двете молекули са тествани при 4 концентрации за хербицидна активност, при което не е установена концентрационна зависимост. Инхибирането на растежа на надземните части и корените на най-ниската (0.001 mM) и най-високата (1 mM) концентрация на съединенията е съизмерима.

В допълнение бяха изчислени редица структурни параметри за целевите съединения и някои техни аналози. Зависимостта на хербицидните ефекти от съответния miLogP се описва с полином от втора степен, като R^2 е в диапазона от 0.796 до 0.894.