Influence of derivatives of 2-((6-R-quinolin-4-yl)thio)acetic acid on rhizogenesis of Paulownia clones

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Abstract

The impact on rhizogenesis during microclonal reproduction in vitro in explants Paulownia clone 112 and further adaptation of microplants in vivo hybrid molecules of quinoline and acetic acid, which are analogues of known growth stimulants, was studied. A number of factors influencing the level of influence on rhizogenesis of the action of derivatives of 2-((6-R-quinolin-4-yl)thio)acetic acid (QAC-5) and 2-quinolinyl-4-thio)acetic acid (QAC-1) were determined. The selection of new effective, low-toxic, less expensive substances was carried out for further testing as potential stimulators of rhizogenesis for microclonal propagation of plants.

Keywords: hybrid molecules; quinoline derivatives; stimulation of rhizogenesis; bioavailability factors; lipophilicity; toxicity; progressive motility; micropropagation of plants.

Introduction

One of the modern approaches in creating bioregulators for clonal micropropagation of plants is the molecular design of natural and synthetic compounds that combine derivatives of nitrogen-containing heterocycles and carboxylic acid residues. Heterocyclic systems of nitrogen-containing heterocycles have highly reactive positions, which allows one to modify and carboxylic acid residues. Heterocyclic systems of nitrogen-containing compounds. It was found that the studied derivatives (quinolin-4-ylthio) of acetic acid are promising as potential growth regulators. With the help of molecular design, potential bioactive molecules were created for experimental research (Kalnin, 1984; Kornet et al., 2021; Yakovleva-Nosar et al., 2022). It is known that Paulownia is a genus of evergreen and semi-evergreen deciduous trees, which is well adapted to the soil and climatic conditions of Ukraine. Popular in garden and park design, Paulownia clone in vitro 112 of Spanish selection can withstand low temperatures – 25...–27 °C and has high decorative properties. This is an artificially bred and cloned tree, able to survive and develop in extreme conditions, under-
The 4-chloroquinolines (1) ("IBS") were used as starting materials, as well as reagents and solvents ("UkrOrgSynthesis", Ukraine) for the synthesis of derivatives (quinolin-4-yI)thio) of acetic acid. The general reaction scheme followed for the synthesis of selected 2-(6-R-quinolin-4-yI)thio) acetic acid derivatives is presented in Figure 2.

The reactions and the purity of the synthesized compounds were controlled by the TLC on Sorbton-2 plates (Russia). As an eluent, mixtures of chloroform-methanol (1:1) and acetone-water (1:1) were used. Manifestations of chromatograms were performed using UV rays. The 1H NMR spectra were recorded on the "Bruker AC-300" (manufacturer Bruker, 2010, 300 MHz) device in DMSO-d6 and D2O. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling constants (J) are reported in Hertz (Hz). LC-MS spectra were recorded on a high-performance liquid chromatography module of the HPLC system for Agilent 1200 Infinity and a proton-ionization diode-matrix probe.

![Fig. 1. General structure of derivatives of 2-(6-R-quinolin-4-yI)thio)acetic acid](image)

### Materials and methods

Derivatives of 2-(6-R-quinolin-4-yI)thio)acetic acid were synthesized to the Department of Chemistry of the Zaporizhia National University and the Department of Horticulture of the Khortytsk National Academy (Fig. 1).

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All compounds were synthesized according to the well-known method (Brazhko et al., 2013; Metelytsia et al., 2020) with the corresponding physico-chemical and spectral data, which correspond to the literature. The compounds were prepared according to a previously described procedure (Brazhko et al., 2012; Brazhko et al., 2013e, 2018). 2-(quinolin-4-yI)thio)acetic acid (QAC 1-3, Table 1). The compounds were synthesized and described previously according to the method, sodium salts 2-(quinolin-4-yI)thio)acetic acid (QAC 3-6 Table 1).

On the basis of 4-chloroquinolines (1) synthesis methods for 2-(quinolin-4-yI)thio)acetic acid (QAC 1-4, Fig. 2) were developed and it was shown to be a convenient precursor for obtaining a variety of functional derivatives. Neutralization of sodium hydroxide acid synthesized corresponding to water-soluble compounds – sodium salts 2-(quinolin-4-yI)thio)acetic acid (QAC 4-8).

The investigated derivatives of 2-(6-R-quinolin-4-yI)thio)acetic acid are shown in Table 1. The investigated compounds are acids – hybrid molecules of the quinoline heterocycle and the remainder of thiocarboxylic acid (mercaptocarboxylic acid). Derivatives were obtained to increase water solubility – sodium salts 2-(6-R-quinolin-4-yI)thio)acetic acid.

Molecular descriptors of structure: gross formula, elemental composition, molecular weight, molecular refractive index, Log P, Log D, ClogP (Table 2).

Based on this, we can say that the introduction in the 6th position of the methoxy group and styryl group in the structure of 2-(quinolin-4-yI) thio)acetic acid and its structural analogues leads to increased molar refraction. This trend is easily explained by the fact that such a change in the structure of the molecule increases the effective radii of the molecules, the molar mass, and thus increases the molar refraction. A particularly important characteristic of any biologically active substance is lipophilicity (hydrophobicity) – a model of the distribution of the substance studied between two phases that do not mix (most often used octanol – water). This characteristic is easily modulated by the use of an appropriate descriptor and is most often used to assess the ability of a substance to overcome the biological membranes of cells.

When the test substance is in the aqueous phase in the form of molecules (uncharged particles) to characterize lipophilicity we use the indicator log P (P – partition coefficient at the boundary of octane – water).

If the test substance in the aqueous solution is partially dissociated in the form of charged particles (ions), there will be a certain dynamic equilibrium between the different forms of the compound, which will vary depending on the pH of the medium.

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**Fig. 2. Synthesis of 2-(6-R-quinolin-4-yI)thio)acetic acid of investigated derivatives**

R = H, CH3; R1 = H, OCH3, OC2H5; R2 = CH3COOH
Thus, lipophilicity (log D) is an important characteristic for assessing the ability to penetrate cell biological membranes and stimulate rooting derivatives 2-(6-R-quinolin-4-yl)thio)acetic acid and its derivatives, which may exist as ions in aqueous solution.

All of the compounds tested (compounds 1-8) according to Lipinski’s "rule five" can show high biological activity.

To conduct a study of the toxic effects of compounds using native material we used ejaculate of fertile men (normozoospermia). To do this, we pre-evaluated the standard spermogram according to generally accepted methods in accordance with WHO criteria (Stefanov, 2001; Tiuziko, 2013). Measurements were performed on the sperm fertility analyzer "AFS-500-2" ("NPF "Biolit"). The selected ejaculate was aliquoted by 100 μL, aliquots were numbered, and the following was added:

- to the first aliquot – saline solution – 10 μL (intact);
- to the second aliquot – ascorbic acid (AA) at a concentration of 10–6 – 10 μL;
- to the third aliquot – ATC at a concentration of 10–6 – 10 μL;
- to the fourth aliquot – the test substance (quinoline derivative) at a concentration of 10–6 – 10 μL;
- to the fifth – saline solution – 10 μL, then hydrogen peroxide at a concentration of 200 μM – 0.5 μL (reference);
- to the sixth – hydrogen peroxide at a concentration of 200 μM – 0.5 μL, then AA at a concentration of 10–6 – 10 μL;
- to the seventh – hydrogen peroxide at a concentration of 200 μM – 0.5 μL, then ATC at a concentration of 10–6 M;
- to the eighth – hydrogen peroxide at a concentration of 200 μM – 0.5 μL, then the test substance at a concentration of 10–6 – 10 μL;
- the obtained samples were incubated at 37 °C for 2 hours. Immediately after incubation, the quality criteria of sperm were studied: concentration, movement, vital activity.

Measured indicators: total sperm concentration; total number of sperm in the ejaculate; rapid progressive motility (A); slow progressive motility (B); progressive motility (A + B); relative number of sperm with normal morphology; concentration of functional sperm; concentration of sperm with progressive motility; concentration of immotile sperm; the total number of sperm with progressive motility; total number of functional sperm; total number of immotile and non-functional sperm; average speed (A + B) of motile sperm; index of normal motile sperm.

To address the issues of differentiation of living and dead sperm, Bloom’s supravital staining is performed. The researchers evaluated the presence or absence of cell membrane permeability for eosin dye (EO; 1% aqueous solution) according to WHO guidelines, followed by counting living and dead cells. Live sperm were not stained (transparent), dead were stained in pink. To prepare a smear, 1 drop of ejaculate and 1 drop of eosin dye are applied to a medical glass, the drops are mixed with each other with another glass just like the blood sample, and a smear is complete. After the smear were dried in air, the number of live and dead sperm was counted by microscopy under an immersion lens (x 100) with 10 binoculars. 100 stained and unstained sperm were counted and the percentage of living and dead sperm was determined.

The study of rhizogenesis was performed in vitro, with the addition of synthesized compounds at a concentration of 1 mg/L in the nutrient medium. Murashige-Skaguter nutrient medium was prepared for rhizogenesis (Murashige, 1962), containing half the concentration of macrosalts and trace elements and 2% sucrose. The compounds were added before sterilization of the nutrient medium. The control was nutrient media without trace elements and 2% sucrose. The compounds were added before sterilization of the nutrient medium. The study of rhizogenesis was performed in vitro, with the addition of synthesized compounds at a concentration of 1 mg/L in the nutrient medium. Murashige-Skaguter nutrient medium was prepared for rhizogenesis (Murashige, 1962), containing half the concentration of macrosalts and trace elements and 2% sucrose. The compounds were added before sterilization of the nutrient medium. The control was nutrient media without trace elements and 2% sucrose. The compounds were added before sterilization of the nutrient medium. The study of rhizogenesis was performed in vitro, with the addition of synthesized compounds at a concentration of 1 mg/L in the nutrient medium. Murashige-Skaguter nutrient medium was prepared for rhizogenesis (Murashige, 1962), containing half the concentration of macrosalts and trace elements and 2% sucrose. The compounds were added before sterilization of the nutrient medium. The control was nutrient media without trace elements and 2% sucrose. The compounds were added before sterilization of the nutrient medium.
Results

Toxicity study of 4-thioquinolines using the GUSPAP program (Germany) and TEST (USA) showed that they are low-toxic (Table 3). Among the derivatives of 2-(6-R-quinolin-4-yl)thio)acetic acid the most toxic compounds were those that did not have in the 6-th position of the quinoline cycle alkoxy substituents (QAC-1 and QAC-5). Sodium salts are more toxic than the corresponding acids. This is due to the increased bioavailability of ionized compounds.

Table 3
Toxicity indicators of the studied compounds

| Compounds | TEST computer program oral rat LD₅₀, mg/kg | intravenous administration, mg/kg | GUSPAP computer program oral administration, mg/kg | subcutaneous injection, mg/kg | Progressive sperm motility, % |
|-----------|------------------------------------------|----------------------------------|---------------------------------------------|-------------------------------|-----------------------------|
| QAC-1     | 5529.00                                  | 259.01                           | 715.00                                      | 394.20                        | 33.2                         |
| QAC-2     | 452.90                                   | 355.42                           | 976.41                                      | 590.00                        | 36.5                         |
| QAC-3     | 610.12                                   | 364.41                           | 788.55                                      | 1045.01                       | 41.4                         |
| QAC-4     | 879.47                                   | 415.92                           | 866.04                                      | 1079.01                       | 46.0                         |
| QAC-5     | 386.76                                   | 271.10                           | 308.41                                      | 638.81                        | 29.1                         |
| QAC-6     | 402.42                                   | 246.90                           | 608.21                                      | 725.03                        | 31.4                         |
| QAC-7     | 518.02                                   | 377.11                           | 901.33                                      | 799.00                        | 39.3                         |
| QAC-8     | 545.01                                   | 443.22                           | 936.82                                      | 813.01                        | 44.1                         |
| Intact    |                                         |                                  |                                             |                               | 37.0                         |

The total number of sperm with progressive motility is an important indicator of the toxic effect of compounds, the value of which is directly proportional to the value of the toxic effect of the substance. The study uses native material – ejaculate of fertile men (normozoospermia). The following derivatives showed the greatest toxic effects in this model: 2-(quinolin-4-yl)thio)acetic acid (QAC-5) and 2-(quinolin-4-yl)thio)acetic acid (QAC-1), which will reduce this figure by 15-20% compared to intact (Table 3). Derivatives 2-(quinolin-4-yl)thio)acetic acid, which contain in the second position a metal radical (QAC-2, QAC-6) showed moderate toxicity, and reduce the rate of progressive mobility by 12-20%. Number (QAC-3, QAC-4, QAC-7, QAC-8) compounds containing in the 6th position alkoxy substituents (-OCH₃, -OCH₂H₃) on the contrary increase the rate of progressive mobility, which means that they are non-toxic.

The study of rhizogenesis was performed in vitro, with the addition of synthesized compounds QAC-1–8 at a concentration of 1 mg/L in the nutrient medium. Murashige T. Scoog medium was prepared for rhizogenesis, which contained half the concentration of macro- salts and trace elements and 2% sucrose. The compounds were added before sterilization of the nutrient medium. The control was nutrient media without growth regulators (MS0). Comparison drug – 2-(naphthalene-5-yl)acetic acid (NAA). The nutrient medium was sterilized by autoclaving under a pressure of 0.11 MPa for 30 minutes. The explants were cultivated at an air temperature of 22–24 °C with a photoperiod of 16 hours, a relative humidity of 65–70% and an illumination of 2.5 thousand lux. The results were recorded for 28 days and took into account the number, length of roots, frequency of rhizogenesis.

In vitro rhizogenesis was studied with the obtained compounds, with the addition of synthesized compounds QAC-1–8 at a concentration of 1 mg/L in the nutrient medium. The results were recorded for 28 days and took into account the number, length of roots, frequency of rhizogenesis. The data obtained show that the compounds of all tested compounds caused a significantly higher number of roots (P < 0.05), and media with compounds QAC-1, QAC-5 had the maximum number (P < 0.001) of roots compared to control. The length of the roots was dominated by nutrient media containing compounds QAC-1 and QAC-5 (P < 0.001) in comparison with the comparison drug (Table 1, Fig. 3).

Thus, on the nutrient medium QAC-1 and QAC-5 was there a maximum stimulation of rhizogenesis, which exceeded the effect of the comparison drug – 2-(naphthalene-5-yl)acetic acid. Paulownia clones formed 492 ± 0.54 and 432 ± 0.43 roots (P < 0.05), respectively. This environment contributed the most to the formation of 7-8 roots and the frequency of rhizogenesis was more than 80%. Significantly the longest roots were observed on the medium QAS-1 (P < 0.05), QAC-5 (P < 0.001) in comparison with the control and the comparison drug.

The engraftment of plants on the substrate peat universal:sand:vermiculite in a ratio of 2:1:1 was 73%. Paulownia clone 112 on hormone-free nutrient medium initiates the minimum number and length of roots from all studied variants of media. In contrast, with media containing compounds, all tested compounds caused a significantly higher number of roots (P < 0.05), and media with compounds QAC-1, QAC-5 had the maximum number (P < 0.001) of roots compared to control. The length of the roots was dominated by nutrient media containing compounds QAC-1 and QAC-5 (P < 0.001) in comparison with the comparison drug (Table 1, Fig. 3).

Thus, the addition of sodium salts to the nutrient medium for rhizogenesis of QAC-1–5 compounds significantly increased the number and length of roots (P < 0.001) with the maximum percentage of rhizogenesis frequency (Fig. 3) compared to the corresponding starting acids.
Discussion

The selection of substances for the substrate during microclonal propagation of plants is an urgent problem. Stimulation of rhizogenesis is the most problematic task for the efficiency of plant reproduction (Amer et al., 2019; Anjos et al., 2021). It is known that each plant has its own individual characteristics that affect the composition of the nutrient medium for explants. The nutrient medium was sterilized by autoclaving under a pressure of 0.11 MPa. Therefore, the compounds that are added to the nutrient medium must have the necessary chemical resistance to decomposition (Artefa et al., 2018; Awada et al., 2020).

The most important moment in the clonal micropropagation of any culture is the planting of plants in the substrate, it is at this stage that there is a danger of the death of plants—regenerants, therefore it is important to obtain an optimal root system that will provide nutrition and growth of regenerants (Ivashchuk et al., 2018; Grishchenko et al., 2020). It is known that when explants of plants without roots or with poorly developed root systems were planted in the substrate, 34% of plants took root, which made production unprofitable. Stimulation of root formation is the result of the interaction of a substance with plant cells. It depends on the characteristics of the substance (molecular structure, physical and chemical properties), biological object and mode of action. It is known that the toxicological problem is associated with the use of synthetically biologically active substances. It is associated with the presence of many of them as side effects, that is, undesirable effects (Vostrikova, 2020; Koprulu, 2021). A new cytokinin-like compounds as a tool to improve rooting and establishment of micropropagated plantlets. Ac- tu Horticulturae, 6th International Symposium on Production and Establishment of Micropropagated Plants, Sardinia, Italy, 497–503.

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Fig. 3. Plants of Paulownia (species) clone 112 for 28 days: a – on a nutrient medium with the addition of 2-(naphthalene-5-yl)acetic acid; b – with the addition of QAC-1; c – with the addition of QAC-5

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