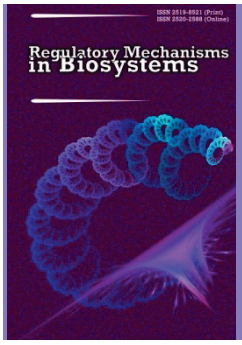

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Influence of derivatives of 2-((6-r-quinolin-4-yl)thio)acetic acid on rhizogenesis of *Paulownia* clones

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In recent years, the demand for effective and low-toxic stimulators of rhizogenesis, which are used in microclonal propagation of plants, has been increasing in Ukraine. One of the promising directions in the search for effective compounds is molecular modeling based on known natural and synthetic compounds. The development of new highly effective and low-toxic biologically active compounds is largely based on derivatives of nitrogen-containing heterocycles, and quinoline occupies a significant place among them. Modern methods of chemometric analysis make it possible to find certain regularities in the "chemical structure – biological activity" and to select the most promising compounds for experimental research. The values of lipophilicity log P for neutral forms and the value of the distribution coefficient log D at pH = 7 were obtained by quantum chemical calculation. The values of log P and log D of the studied compounds are in the most favourable interval for overcoming the biological membranes of the cells of the root system, depending on the pH of the environment. According to Lipinski's "rule of five", all studied compounds can show high biological activity. The toxicity of compounds of 2-((6-R-quinolin-4-yl)thio)acetic acid derivatives was evaluated by computer programs and experimentally. Among the derivatives of 2-((6-R-quinolin-4-yl)thio)acetic acid, the most toxic compounds were those that did not have alkoxy substituents in the 6th position of the quinoline ring. Sodium salts are more toxic than the corresponding acids. This is due to an increase in the bioavailability of ionized compounds. Derivatives of 2-((6-R-quinolin-4-yl)thio)acetic acid (sodium salt of 2-((quinolin-4-yl)thio)acetic acid (QAC-5) showed the greatest toxic effect on the model of the study of progressive sperm motility) and 2-((quinolin-4-yl)thio)acetic acid (QAC-1), which will reduce this indicator by 15–20% compared to intact. The toxicity assessment of the studied compounds made it possible to determine a number of factors of the structure of molecules which affect the level of toxic action of 2-((6-R-quinolin-4-yl)thio)acetic acid derivatives and the directions of creation of non-toxic growth stimulants in this series. 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 and directions of creation of highly active substances in this series was defined. The studied compounds showed a high stimulating effect on rhizogenesis *in vitro* in *Paulownia* explants. It was established that the sodium salt of 2-((quinolin-4-yl)thio)acetic acid was the greatest stimulator of rhizogenesis compared to the corresponding original acid. The presence of alkoxy groups in the 6th position and methyl in the 2nd position of the quinoline ring of 2-((6-R-quinolin-4-yl)thio)acetic acid reduced the activity of the compounds. 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; microclonal propagation 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 molecules and obtain libraries of new promising biologically active compounds (Brazhko et al., 2013).

The study of characteristic pharmacophores and molecular descriptors of the structure of derivatives of 2-((6-R-quinolin-4-yl)thio)acetic acid indicates the possibility of a fairly wide range of biological activity of these 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 (Kalinin, 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, undemanding to the soil (Bergmann, 1998).

Studies have shown that rooting in *Paulownia* is an auxin-dependent process (Matskevich, 2019). In microclonal propagation, *Paulownia* clone *in vitro* 112 needs to optimize and accelerate the rooting process and reduce the stress of regenerating plants when adapting to *in vivo* conditions. The aim of this work was to study the effect of derivatives of 2-((6-R-

quinolin-4-yl)thio)acetic acid on rhizogenesis in vitro in *Paulownia* clone 112 explants and subsequent adaptation of microplants to *in vivo* conditions. Of considerable interest, in terms of creating effective stimulators of rhizogenesis for microclonal plant propagation, is the combination in one molecule of synthons such as heterocycle (6-R-Q and mercaptoalkylcarboxylic acid residue) (Brazhko et al., 2013; Kornet et al., 2021b). Today, such compounds remain poorly studied growth regulators and are a prospect for the creation of new, low-toxic and effective stimulators of rhizogenesis for microclonal plant propagation. Given the above, the preparation of derivatives of 2-((6-R-quinolin-4-yl)thio)acetic acid, and the study of the biological properties of these compounds determine the relevance of the work.

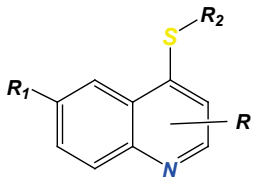
The aim of this work is to select the best among the derivatives of 2-((6-R-quinolin-4-yl)thio)acetic acid by virtual screening and experimental studies of potential stimulators of rhizogenesis for microclonal plant propagation.

Materials and methods

Derivatives of 2-((6-R-quinolin-4-yl)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).

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-ylthio) of acetic acid. The general reaction scheme followed for the synthesis of selected 2-((6-R-quinolin-4-yl)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 acetate-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 1260 Infinity and a proton-ionization diode-matrix probe.

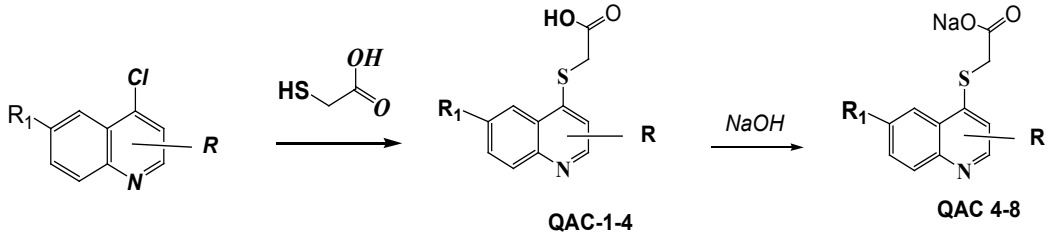


R = H, CH_3, R_1 =H,OCH_3, OC_2H_5, R_2 = CH_2COOH

Fig. 1. General structure of derivatives of 2-((6-R-quinolin-4-yl)thio)acetic acid

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-yl)thio)acetic acid (QAC-1-3, Table 1). The compounds were synthesized and described previously according to the method, sodium salts 2-((quinolin-4-yl)thio)acetic acid (QAC-3-6 Table 1).

On the basis of 4-chloroquinolines (1) synthesis methods for 2-((quinolin-4-yl)thio)acetic acid (QAC1-4, 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-yl)thio)acetic acid (QAC 4–8).



R = H; CH_3; R_1 = H, OCH_3, OC_2H_5

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

The investigated derivatives of 2-((6-R-quinolin-4-yl)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 (mercaptoacetic acid). Derivatives were obtained to increase water solubility – sodium salts 2-((6-R quinolin-4-yl)thio)acetic acid.

Molecular descriptors of structure: gross formula, elemental composition, molecular weight, molecular refractive index, Log P, Log D, investigated compounds were determined using the computer software package ACD-I-Labs. LogP is the partition coefficient of the compound between n-octanol and water, and Log D is the lipophilicity of the compound depending on the pH of the medium (Brazhko et al., 2018; Metelytsia et al., 2020). A key parameter in the study of the relationship between the structure and biological activity of organic compounds is the partition coefficient in the system of n-octyl alcohol – water. Correlations between the value of Pow and toxicity, penetration of artificial and natural membranes, biological activity of non-specific drugs, bioaccumulation, soil adsorption, etc. were found. However, the experimental determination of Pow is very time consuming. Therefore, it is generally accepted to use calculation methods for their evaluation. Adequacy of additive methods of calculation of distribution coefficients, and completeness of a set of experimental values of Pow on which this model is built were calculated.

We performed individual stages of calculation of molecular descriptors of the structure using a number of software tools, such as: framework JSDraw, OpenBabel, PaDEL-Descriptor, McQSAR, Pandoc, ACD-I-Labs. The following molecular descriptors of the structure were calculated: 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 stoxy group in the structure of 2-((quinolin-4-yl) 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.

Table 1
Chemical structures of synthesized compounds

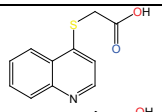
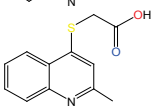
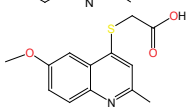
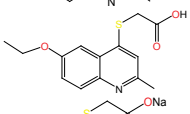
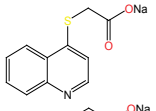
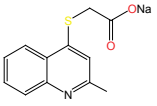
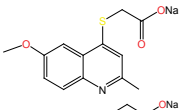
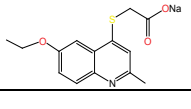
Compound	Chemical structure	Molecular formula, molecular mass (MW)	Chemical name
QAC-1		$C_{11}H_9NO_2S$ (219.04)	2-((quinolin-4-yl)thio)*acetic* acid
QAC-2		$C_{12}H_{11}NO_2S$ (233.29)	2-((2-methylquinolin-4-yl)thio) *acetic* acid
QAC-3		$C_{14}H_{15}NO_3S$ (263.31)	2-((6-methoxy-2-methylquinolin-4-yl)thio) *acetic* acid
QAC-4		$C_{14}H_{15}NO_3S$ (277.08)	2-((6-ethoxy-2-methylquinolin-4-yl)thio) *acetic* acid
QAC-5		$C_{11}H_8NNaO_2S$ (241.24)	Sodium 2-((quinolin-4-yl)thio) *acetic* acid
QAC-6		$C_{12}H_{10}NNaO_2S$ (255.27)	Sodium 2-((2-methylquinolin-4-yl)thio) *acetic* acid
QAC-7		$C_{13}H_{12}NNaO_3S$ (285.04)	Sodium 2-((6-methoxy-2-methylquinolin-4-yl)thio) *acetic* acid
QAC-8		$C_{14}H_{14}NNaO_3S$ (299.32)	Sodium 2-((6-ethoxy-2-methylquinolin-4-yl)thio) *acetic* acid

Table 2
Molecular descriptors of synthesized compounds

Compounds	M_r, g/mol	log P (neutral form)	log D, (pH = 7)	ClogP	MR, cm^3/mol
QAC-1	219.26	1.82 ± 1.04	–1.70	1.95	60.76
QAC-2	233.29	2.53 ± 1.01	–1.10	2.45	65.54
QAC-3	263.31	2.40 ± 1.02	–1.02	2.74	71.68
QAC-4	277.34	2.74 ± 1.02	–0.48	3.27	76.32
QAC-5	241.24	–	–	1.70	82.91
QAC-6	255.27	–	–	2.20	–
QAC-7	285.29	–	–	2.50	–
QAC-8	299.32		–	3.03	–
NAA	186.21	2.53 ± 1.01	0.068	2.51	54.03

The lipophilicity of such a system will be determined by the partition coefficient log D – the ratio of the sums of activities of all components of the organic and aqueous phases. For comparison, we obtained quantum-chemical values of lipophilicity log P for neutral forms 2-((6-R-quinolin-4-yl)thio)acetic acid (compounds I-4) and the value of the partition coefficient log D at pH = 7. This characteristic is most often used to assess the ability of the substance to overcome biological membranes of cells of the root system depending on the pH of the medium. The pH of most plant cloning media is maintained in the range of 6.5 to 7.5.

It was found that the values of log D for the tested compounds are much smaller than the values of log P, this is due to the consideration in the second case of acid-base equilibrium, which is in solution of the test substances. The change in lipophilicity of the substance from the ability to dissociate into ions in aqueous solution is explained as follows. Since water is a polar solvent (μ = 1.86 D) and the dipole moment of octanol is much smaller (it can be taken as a non-polar solvent), the ions that will be formed in the aqueous medium will hardly diffuse into the organic layer and the concentration of ions in it will be caused mainly by the transition of uncharged molecules of matter, resulting in a significant reduction in the concentration of matter in the organic phase.

At introduction in 6 positions of a quinoline cycle of methoxy group insignificant increase in lipophilicity of a molecule is observed (Δ log D = 0.07–0.08). 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.

Toxicity studies of derivatives 2-((6-R-quinolin-4-yl)thio)acetic acid were performed virtually and experimentally. To evaluate the toxic effect of in silico compounds, software solutions were used to build structure-toxicity models and predict LD_{50} using models GUSAR (Germany), TEST (USA) (Martin 2016b; Brazhko et al., 2018b).

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; Tiuzikov, 2013). Measurements were performed on the sperm fertility analyzer "AFS-500-2" (NPF "Biola"). 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 10–6M – 10 μL;

– to the third aliquot – ATC at a concentration of 10^{-6} M – 10 μL;

– to the fourth aliquot – the test substance (quinoline derivative) at a concentration of 10^{-6} M – 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} M – 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} M – 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-progressive 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 x 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-Skuga 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 growth regulators (MC 0). 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.

The tables and figures show the arithmetic mean values and their standard error (x ± SE). The certainty of differences between the samples was assessed using ANOVA ($P < 0.05$).

Results

Toxicity study of 4-thioquinolines using the GUSAR 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.

According to chemometric calculations, the greatest toxic effects on these calculation models were shown by derivatives 2-((quinolin-4-yl)thio)acetic acid (sodium salt 2-((quinolin-4-yl)thio) acetic acid (QAC-5) and 2-((quinolin-4-yl)thio)acetic acid (QAC-1)). Derivatives 2-((quinolin-4-yl)thio)acetic acid, which contain in the second position a metal radical (QAC-2, QAC-6) will have moderate toxicity. Number (QAC-3, QAC-4, QAC-7, QAC-8) compounds containing in the 6th position alkoxy substituents (-OCH_3, -OC_2H_5) on the contrary will be low-toxic.

Table 3
Toxicity indicators of the studied compounds

Compounds	TEST computer program	GUSAR computer program			Progressive
	oral rat LD_{50}, mg/kg	intravenous administration, mg/kg	oral administration, mg/kg	subcutaneous injection, mg/kg	sperm motility, %
QAC-1	352.90	259.01	715.00	394.20	33.2
QAC-2	452.69	355.42	976.41	590.00	36.5
QAC-3	610.12	364.41	758.55	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, (sodium salt 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) show 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 (-OCH_3, -OC_2H_5), 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. For rhizogenesis we prepared nutrient medium Murashige-Skuga, which contained 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 growth regulators (MS0). 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. Murashige T. Scoog medium was prepared for rhizogenesis, which contained 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 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. The data obtained show that the compounds of all tested compounds when added to the nutrient medium for rhizogenesis containing ½ MS and 1 mg/L of the compound caused the maximum increase in rhizogenesis of *Pavlovna* clone 112.

Table 4
Indicators of root formation of *Paulownia* clone 112 on the 28th day of cultivation

Options for environments	Number of roots	Length of roots, mm	Frequency of rhizogenesis,%
MC 0 (control, environment without growth stimulants)	0.81 ± 0.61	2.14 ± 1.38	71.3
QAC-1	4.41 ± 0.70**	6.40 ± 1.11***	85.4
QAC-2	4.02 ± 0.51**	6.74 ± 1.41***	81.2
QAC-3	3.43 ± 0.81*	5.23 ± 1.42**	75.3
QAC-4	4.92 ± 0.54*	6.73 ± 1.31***	78.1
QAC-5	4.32 ± 0.43**	6.12 ± 0.80***	86.0
QAC-6	3.50 ± 0.82**	4.81 ± 2.23**	77.2
QAC-7	3.32 ± 0.42*	5.15 ± 0.82**	78.3
QAC-8	3.11 ± 0.41**	4.74 ± 0.71**	74.1
NAA (comparison drug – 2 - (naphthalene-5-yl) acetic acid	3.73 ± 0.42**	5.14 ± 1.42***	78.0

Note: differences compared to controls (MS0 medium without growth stimulants): * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$.

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 4.92 ± 0.54 and 4.32 ± 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.

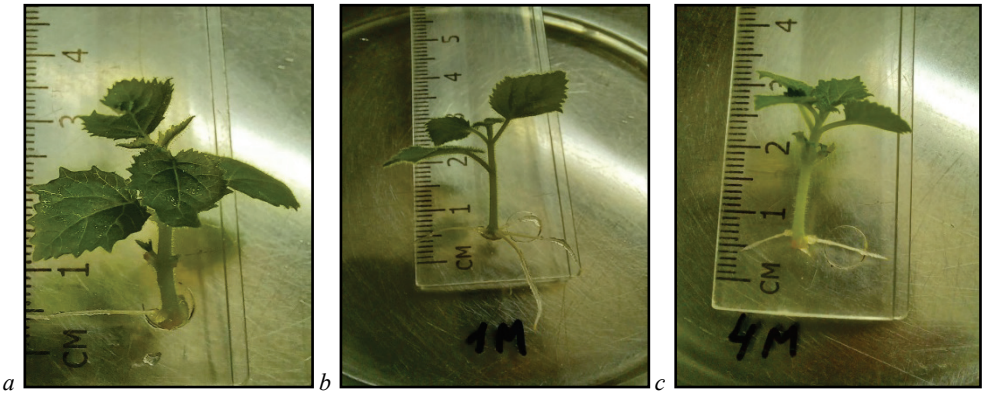


Fig. 3. Plants of *Paulownia* (species) clone 112 for 28 days: *a* – on a nutrient medium with the addition of 2-(naphthalene-5-yl)acetic acid; *b* – with the addition of QAC-1; *c* – with the addition of QAC-5

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 (Arteta 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 synthetic 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 significant number of works by domestic and foreign authors are devoted to the study of virtual and experimental methods for obtaining low-toxic substances and methods of reducing the toxicity of quinoline derivatives (Brazhko et al., 2020; Yepes et al., 2021; Lenin et al., 2022).

Various quinoline derivatives are used both as synthons in organic synthesis and molecular design, and as known effective biologically active compounds. During the synthesis of a new compound, there is a need to calculate the molecular descriptors of the structure, physical and chemical properties, the main constants that affect the manifestation of toxic effects. Such problems are solved using modern chemometric research methods.

The studied derivatives of 2-((6-R-quinolin-4-yl)thio)acetic acid are synthetic analogues of known growth stimulants, such as 2-(naphthalene-5-yl)acetic acid. From the change in rhizogenesis indicators, it is clear that the studied compounds exhibit auxin properties. Therefore, the conducted research has a high practical potential for obtaining new effective, low-toxic, less expensive substances for plant propagation, in the conditions of microlonal production.

Conclusion

The results of the study spread the idea of the influence of 2-((6-R-quinolin-4-yl)thio)acetic acid on rhizogenesis. It was determined that the molecular descriptors correspond to Lipinski's rule, the compounds are low-toxic and have optimal bioavailability, so they can be used as a basis for further research as growth stimulators of rhizogenesis in the microclonal propagation of plants of other species, especially ornamental plants, the propagation of which is very costly under standard conditions.

The research results can be implemented in the reproduction of both agricultural and ornamental plants. The most effective compounds were selected, which can be recommended as new effective competitive stimulators of rhizogenesis in microclonal propagation of plants.

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