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Investigation of plant growth regulation activity of ([1,2,4]triazolo[1,5-c] quinazolin-2-ylsulfanyl)carboxylic acids and amides on *Cucumis sativus* L. Roots

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Ключові слова: *Cucumis sativus L., рістрегулююча активність, 2-тіо-[1,2,4]тріазоло[1,5-с] хіназоліни.*

Ключевые слова: *Cucumis sativus L., рострегулирующая активность, 2-тио-[1,2,4]триазоло[1,5-с] хиназолины.*

Key words: *Cucumis sativus L., growth regulation activity, 2-thio-[1,2,4] triazolo[1,5-c]quinazolines.*

Роботу присвячено розширенню ряду відомих регуляторів росту рослин серед похідних триазолу, зокрема ([1,2,4]триазоло[1,5-*с*]хіназоліну-2-ілсульфаніл)карбонових кислот та амідів. Біологічну активність останніх досліджено *in vitro* на коренях *Cucumis sativus* L. Кращими стимуляторами росту виявились кислоти **3** (0,02 мг/мл) і **4** (0,5 мг/мл), перевищивши активність гібереніну.

Работа посвящена расширению известных регуляторов роста растений среди производных триазола, а именно ([1,2,4]триазоло[1,5-*с*]хиназолин-2-илсульфанил) карбоновых кислот и амидов. Биологическая активность последних исследована *in vitro* на корнях *Cucumis sativus* L. Лучшими стимуляторами роста оказались кислоты **3** (0,02 мг/мл) и **4** (0,5 мг/мл), превысив активность гиберенина.

The present work is dedicated to the enlargement of the known plant growth regulators among triazole derivatives, namely, ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylsulfanyl) carboxylic acids and amides. The biological activity ща the latter's was investigated *in vitro via* direct organogenesis of *Cucumis sativus* L. roots. The best growth stimulators appeared to be acid **3** (0.02 mg/mL) and acid **4 (**0.5 mg/mL), exceeding the activity of gibberellines.

The chemical contamination of the fields and other components of agrolandscape increases in geometrical progression as a result of successive chemisation programs, mechanization and land-reclamation. Development and introduction of the ecologically oriented systems of management and usage of ecologically clean products are among perspective directions of modern agriculture improvement [1,2]. More than that, it is necessary to stimulate biological, biodynamic and organic low-cost adaptive systems of agriculture and ecologization in agreement to the standards of safety of IFOAM [3]. Most effective and ecologically safe application of nitric, phosphoric and potassium fertilizers is possible only in fulfilling the plants the necessary wide spectrum of other components which provide their growth without decreasing of the soil fertility, such as organic fertilizers, biologics on the basis of the useful ground microorganisms, regulators of growth and oligoelements [4].

Plant growth retardants are synthetic compounds, which are used to reduce the shoot length of plants in a desired way without changing developmental patterns or being phytotoxic [5]. The latter is achieved by reducing the cell elongation and by decreasing of the cell division rate. Considering the effect on the morphological structure of plants, growth retardants are antagonistic to gibberellines (GAs) and auxins – the plant hormones that are primarily responsible for shoot elongation. GAs are tetracyclic diterpenoid growth factors that are essential for normal growth and affect a wide variety of plant developmental processes [6,7]. In recent years, significant progress has been attained in the biochemistry of GAs biosynthesis and in the mechanisms of their regulation. Nowadays four different types of GAs inhibitors are known: onium compounds, substances with an N-containing heterocycle, structural mimics of 2-oxoglutaric acid and 16,17-dihydro-Gas [5].

Therefore, we were interested in the second group, namely, triazole-type compounds. It is known that such GAs inhibitors: paclobutrazol, tetcyclacis, uniconazole-P, triapenthenol, block cytochrome P_{450}-dependent monooxygenases, thereby inhibiting oxidation of entkaurene into entkaurenoic acid [8]. More than that, it is reported, that plant growth retardation can also be a side activity of some triazole-type fungicides such as triadimenol, triadimefon or ipconazole, which inhibit the oxidative demethylation in the fungal ergosterol biosynthesis path and also reduce the formation of 14-demethylatedrols in higher plants blocking the obtusifoliol 14-demethylase.

In may 2011 Germany was suffering from the 330 cases of serious infection, most of which have occurred in the north of the country. The cause has been identified as Shiga toxin-producing *Echerichia coli* O104:H4, that was firstly said to be found on Spanish *Cucumis sativus*, which is a member of the *Cucurbitaceae* plant kingdom family, and one of the widely eaten foods, especially in the hot summer months and finally – on the German bean sprouts Shiga toxin-producing *E. coli* causes: diarrhea (frequently bloody), vomiting and fever [9].

Hence, taking into account the afore-mentioned facts, we were aimed to find growth regulators with GAs-like activity among new derivatives of S-substituted 2-thio-([1,2,4]triazolo[1,5-*c*]quinazoline, namely carboxylic acids and amides *via* direct organogenesis of *Cucumis sativus* L.,

using the stem elongation assay that has been reported to be used in order to identify GA-binding activity in the soluble protein fractions of hypocotyl as well as to prevent people from other food diseases and farmers from sales loss [10-15]. It is worth mentioning, that Sulphur in the molecule of our synthesized substances plays important role for showing the antimicrobial activity, because it is known, that among active antibacterial constituents of *Allium cepa* are alkylcysteine sulfoxides and Sulphur containing γ-glutamic acids [16]. More than that, in our previous research investigated ([1,2,4] triazolo[1,5-*c*]quinazolin-2-ylsulfanyl)carboxylic acids and their amides have already demonstrated antifungal activity [17, 18].

Materials and methods

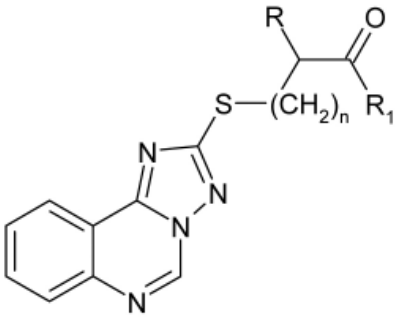
Explants were cultured on Petri dishes, containing 10 mL of water with 0.01 mL of Tween-80. Each Petri dish contained 15 seeds of Cucumis sativus L. (kind "Konkurent"). Investigated compounds were added to dishes to acquire solutions of different concentrations (0.001, 0.005, 0.02, 0.1, 0.5 mg/mL). For every concentration 2 dishes were used. Control solution contained water with 0.01 mL of Tween-80. GA5 was used as reference. Cultures were incubated in a growth room at 30±2 ºC and at relative air humidity 80% for 72 h in darkness. The length of hypocotyl, main root, growth area and number of adventitious roots were measured under a dissecting microscope. The experiment was repeated for three times. Means are at $P \leq 0.05$ and were calculated using the Student's t-test.

Results and discussion

Cucumber sativus L. was selected from many species (bean, pea) as an indicator of growth activity causes it gives the most uniform and rapid response for the necessary measurements of root and hypocotyl. Structures of ([1,2,4] triazolo[1,5-*c*]quinazolin-2-ylsulfanyl)carboxylic acids and amides used for bioassays are shown in Figure 1. The detailed synthesis of compounds **1**-**8** and "structure-activity" relationship of their antibacterial and antimicrobial activity were reported earlier [17, 18]. It worth mentioning, that substances **1**-**4** possess antifungal activity against *Candida albicans* in concentration 1-40 mg/mL and **5**-**7** – against *Aspergillus niger* in concentration 5 mg/mL.

It's interesting, that the effect of hormesis, a dose-response relationship phenomenon characterized by low-dose stimulation and high-dose inhibition, was observed during the investigation [19]. The findings that low doses of herbicides can stimulate plant growth already had implications concerning herbicidal drift and their effects on adjacent crops [20, 21]. The increasing of the concentration to 0.5 mg/mL resulted in total inhibition of all Cucumber sativus L. growth parameters *(Tables 1-4)*.

The highest significant hypocotyl growth stimulation was achieved by 3-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylsulfanyl) propionic (**3**) and 5-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylsulfanyl)pentanoic (**4**) acids in the concentrations 0.001 mg/mL (39.32% and 33.90%), 0.005 mg/mL (36.75% and 26.21%) and 0.02 mg/mL (47.29% and 17.38%). Thus,



Cmpd.	n	R	R_1
1	0	H	OH
2	0	CH_3	OH
3	1	H	OH
4	3	H	OH
5	0	H	$N(C_2H_5)_2$
6	0	H	$-N(CH_2)_5-$
7	0	CH_3	NHC_4H_9
8	0	CH_3	NH(m-OCH_3-Ph)

Fig. 1. Structure of the ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylsulfanyl)carboxylic acids and amides.

Table 1

Influence of the investigated compounds at the *Cucumis sativus* L. hypocotyl length

Compd.	Control [mm]	Concentration [mg/mL]									
		0.001		0.005		0.02		0.1		0.5	
		Hypocotyl length									
		mm	%	mm	%	mm	%	mm	%	mm	%
1	7.20	7.15	-0.69	6.60	-8.33	7.51	4.31	6.73	-6.53	2.22	-68.75
2	7.20	6.70	-6.94	6.40	-11.11	7.20	0	6.10	-15.28	3.90	-45.83
3	3.51	4.89	39.32	4.80	36.75	5.17	47.29	3.70	5.40	2.44	-30.48
4	3.51	4.70	33.90	4.43	26.21	4.12	17.38	3.61	2.85	2.55	-27.35
5	3.51	3.56	1.42	4.66	32.76	3.51	0	1.11	-68.38	0	-100.00
6	4.25	4.24	-0.24	3.96	-6.82	3.36	-20.94	2.10	-50.59	1.36	-68.00
7	4.25	4.02	-5.41	4.29	0.94	3.25	-23.53	2.33	-45.18	1.57	-63.06
8	3.51	3.58	1.99	4.42	25.93	4.06	15.67	4.04	15.10	3.32	-5.41
GA_5	4.60	4.03	-12.50	6.34	37.82	5.90	28.26	5.83	26.74	6.05	31.52

Table 2

Influence of the investigated compounds at the *Cucumis sativus* L. root length

Compd.	Control [mm]	Concentration [mg/mL]									
		0.001		0.005		0.02		0.1		0.5	
		Root length									
		mm	%	mm	%	mm	%	mm	%	mm	%
1	5.00	4.17	-16.60	3.44	-31.20	4.68	-6.40	4.44	-11.20	2.84	-43.20
2	5.00	5.00	0	4.40	-12.00	5.70	14.00	4.80	-4.00	4.30	-14.00
3	4.01	3.98	-2.92	4.20	2.44	5.46	33.17	4.64	13.17	2.58	-37.07
4	4.01	2.47	-38.40	4.97	23.90	4.20	4.74	4.10	2.24	2.01	-49.87
5	4.01	2.98	-25.69	2.32	-42.14	2.78	-30.67	1.47	-63.34	0	-100.00
6	4.27	4.37	2.34	4.48	4.92	3.49	-18.27	2.84	-33.49	2.20	-48.48
7	4.27	4.14	-3.04	4.20	-1.64	2.20	-48.48	1.44	-66.28	1.20	-71.90
8	4.01	3.90	-2.74	4.40	9.70	4.32	7.73	4.29	6.98	2.41	-39.90
GA_5	2.24	2.96	32.14	3.97	77.23	3.60	60.71	3.30	47.32	2.99	33.48

compound **3** even exceeded the GA5 influence on hypocotyl except in the concentration of 0.5 mg/mL. Close values to that results showed diethylamide of acetic (**5**) (32.76%) and N-(m-methoxyphenyl)amide of propionic acid (**8**) (25.93%) at the latter concentration. Acids **1** and **2** almost hadn't affected the processes of hypocotyls' growth (*Table 1*). Amide **6** showed a cytotoxic activity in all concentrations, detaining elongation.

The length of roots during the experiment changed insignificantly. Not a single compound surpassed a GA_5 by activity. The best results of roots elongation were achieved by the substance **3** in concentration of 0.02 mg/mL (33.17%) and compound **4 -** at 0.5 mg/mL (23.90%). A weak positive action was shown by the acetic (**6**) and α-propionic acid (**8**) amides (*Table 2*). Substances **5** and **7** caused the delay of root growth.

Table 3

Influence of the investigated compounds at the *Cucumis sativus* L. root growth area length

Compd.	Control [mm]	Concentration [mg/mL]									
		0.001		0.005		0.02		0.1		0.5	
		Growth area length									
		mm	%	mm	%	mm	%	mm	%	mm	%
1	2.30	2.40	4.35	1.36	-40.87	2.17	-5.65	1.75	-23.91	0	-100.00
2	2.30	2.10	-8.70	2.30	0	3.20	39.13	0.70	-69.57	0	-100.00
3	1.98	1.92	-3.03	2.02	2.02	2.34	18.18	0.90	-54.55	0	-100.00
4	1.98	1.52	-23.23	2.46	24.24	1.61	-18.69	0.92	-53.54	0.24	-87.88
5	1.98	1.76	-11.11	1.58	-20.20	1.69	-14.65	0.73	-63.13	0	-100.00
6	2.03	1.92	-5.42	2.24	10.34	2.01	-0.99	1.55	-23.65	1.37	-32.51
7	2.03	2.64	30.05	-1.52	-25.12	0.99	-51.23	0.15	-92.61	0	-100.00
8	1.98	1.80	-9.09	2.08	5.05	1.94	-2.02	1.81	-8.59	1.38	-30.30
GA_5	1.76	1.99	13.07	1.78	1.14	1.65	-6.25	1.47	-16.48	1.51	-14.20

Table 4

Influence of the investigated compounds at the number of *Cucumis sativus* L. adventitious roots

Compd.	Control [num]	Concentration [mg/mL]									
		0.001		0.005		0.02		0.1		0.5	
		Number of adventitious roots									
		num.	%	num.	%	num.	%	num.	%	num	%
1	14.30	15.30	6.99	15.20	6.29	16.50	15.38	14.80	3.50	0	-100.00
2	14.30	14.00	-2.10	14.40	0.70	15.60	9.09	3.10	-78.32	0	-100.00
3	12.50	12.20	-2.40	11.40	-8.80	11.55	-7.60	5.00	-60.00	0	-100.00
4	12.50	12.18	-2.56	12.45	-0.40	9.64	-22.88	5.64	-54.88	2.27	-81.84
5	12.50	11.70	-6.40	10.50	-16.00	10.40	-16.80	4.50	-64.00	0	-100.00
6	14.00	13.50	-3.57	14.50	3.57	11.20	-20.00	8.30	-40.71	8.30	-40.71
7	14.00	9.40	-32.86	11.70	-16.43	8.70	-37.86	1.70	-87.86	0	-100.00
8	12.50	12.40	-0.80	11.50	-8.00	11.20	-10.40	9.80	-21.60	7.40	-40.80
GA_5	9.70	10.25	5.67	10.40	7.22	10.51	8.35	10.70	10.31	8.50	-12.37

In concentration 0.001 mg/mL amide **7** and in 0.02 mg/mL acid **2** considerably exceeded the influence of GA_5 and promoted the growth root at 39.13% and 30.05% accordingly. The moderate lengthening of adventitious roots area was influenced by acids **3**, **4** and amides **6**, **8** in concentration 0.5 mg/mL (*Table 3*).

Almost all obtained compounds negatively affected the number of adventitious roots. Only acetic (**1**) and α-propionic (**2**) acids showed insignificant growth stimulating effect from 0.7 to 15.38%. Substance **1** even exceeded effect of GA5 in concentration 0.02 mg/mL *(Table 4)*.

Conclusion

To conclude, this is the first report about the growth regulation activity of ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylsulfanyl)carboxylic acids and amides on the *Cucumis sativus* L. cultivation. Almost all investigated substances inhibited values of above-mentioned parameters of growth in the concentration 0.5 mg/mL. Exceptions were the amides of acetic (**5**) and α-propionic acids (**7**), which showed the strongest cytotoxic activity inhibiting the cell growth of main and adventitious roots, demonstrating growth retardant properties. The best growth stimulators appeared to be acid **3** in the concentration of 0.02 mg/mL and **4** in the concentration of 0.5 mg/mL, which promoted *Cucumus sativus* L. hypocotyl growth at 47.29% and 26.21%, main root at 33.17% and 23.90%, adventitious roots at 18.18% and 24.24% accordingly, exceeding the activity of GA_5.

Thus, the investigated compounds can be used as novel plant growth regulators or as inhibitors in high concentrations (>0.5 mg/mL) or as promoters in low concentrations (0.001-0.02 mg/mL), which additionally protect seeds from growth of *Aspergillus niger* and *Candida albicans*.

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