Benzothiazole derivatives substituted in position 2 as biologically active substances with plant growth regulation activity

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ABSTRACT

Thirteen of the new synthetized 2-R substituted benzothiazole derivatives have been tested for plant growth regulatory (PGR) activity. The effect on growth elongation was studied on wheat coleoptile segments *Triticum aestivum* L. cv. Blava, and on the hypocotyl and roots in cucumber *Cucumis sativum* L. cv. Evita. The formation and number of adventitious roots and the length of hypocotyl in *Vigna radiata* (L.) Wilczek and, the effect on the length of stem, fresh and dry mass in buckwheat *Fagopyrum esculentum* Moench. cv. Pyra were evaluated. Cytokinin activity was determined on segments of barley leaves *Hordeum vulgare* L. cv. Jubilant on the basis of senescence inhibition and chlorophyll content. The benzothiazole derivatives were tested in the range of 10^{-3}–10^{-7}M concentrations, and PGR activity was compared with indole-3-acetic acid, indole-3-butyric acid and 6-furfurylaminopurine. All tested derivatives showed different auxine-like effects on elongation growth of plants and the stimulative effects were found to depend on applicable concentrations. At higher concentration rates, derivatives acted as growth retardants and inhibited the length of cucumber hypocotyl and roots. The derivatives increased the formation of adventitious roots of mung bean hypocotyl cuttings, as well as stem elongation and production of fresh and dry mass of buckwheat. Cytokinin activity was confirmed in one derivate only with a significant effect on the inhibition of leaf senescence and higher chlorophyll content. The tested benzothiazole derivatives may be characterized as biologically active substances with dominant auxine-like growth promoting activity.

Keywords: benzothiazole derivatives; elongation growth; root formation, senescence; *Cucumis sativum*; *Fagopyrum esculentum*; *Hordeum vulgare*; *Triticum aestivum*; *Vigna radiata*

Benzothiazole derivatives represent an extensive group of heterocyclic compounds, several of which have already found application in the medical sphere as medicines (Kashiyama et al. 1999) as well as in agriculture (Pulkrábek et al. 1999, Henselová et al. 2001). 3-R-substituted benzothiazole derivatives induced dedifferentiation and morphogenesis of plants *in vitro* (Sekerka et al. 1989), influenced growth in *Triticum aestivum* (Sutoris et al. 1993) and stimulated plant regeneration and peroxidase activity in *Brassica oleraceae* (Havel et al. 1994). Many of 2-R substituted benzothiazoles are known as substances with antibacterial and antifungal activities (Foltínová et al. 1978, Grandolini et al. 1986, Afsah and Nayer 1996) and are reported also to be active as antineoplastics (Krieg and Bilitz 1996) agent. The newly synthesized 2-R substituted benzothiazole compounds were tested under *in vitro* conditions and some of them exhibited antimicrobial activity against *Euglena gracilis* and gram-positive bacteria as *Staphylococcus aureus* and *Bacillus subtilis* (Magdolen et al. 2000a, Buffa et al. 2001). However, none of them have been reported to have plant growth regulation activity. This paper reports the auxine and cytokinin-like activities of thirteen derivatives of benzothiazole substituted in position 2.

MATERIAL AND METHODS

Chemicals. Structures and relative molecular mass of benzothiazole compounds used for bioassays are shown in Figure 1 and Table 1. These compounds were prepared at the Department of Organic Chemistry (Faculty of Natural Sciences, Comenius University, Bratislava). The syntheses of compounds I, II, III, IV, V are described in the papers of Matsumoto et al. (1991), Cuadro et al. (1992), and Nivalkar and Mashraqui (1996). The compounds VI–XIII have been synthesized by the condensation reaction of *p*-X-benzaldehyde with

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Table 1. Relative molecular mass (Mr) of the tested benzothiazole compounds No. I – XIII

Compounds	Relative molecular mass
I	237.3
II	178.2
III	280.4
IV	310.4
V	211.3
VI	457.7
VII	334.5
VIII	267.4
IX	278.4
X	292.4
XI	354.4
XII	304.4
XIII	419.6

the 2-methylbenzothiazole according to Magdolen (1999) and Magdolen et al. (2000b). Because of their low solubility in water, compounds were dissolved in N,N-dimethylsulfoxide (DMSO). The resulting DMSO concentration in the tested samples as well as in the control was adjusted to 0.5% (v/v). The control was a solution containing 0.5% (v/v) DMSO a comparable standards, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 6-furfurylaminopurine (kinetin) (Sigma, St. Louis, MO, USA) were used.

The cucumber bioassay of Halevy and Cathey (1960) was used. Cucumber seeds cv. Evita were sown in Petri dishes on 2 layers of Whatman No. 2 filter paper, which were saturated with water (control), or with the solution of the above mentioned benzothiazole compounds in concentrations from 1.10^{-3} to 1.10^{-7}M. The seeds were incubated in the dark in a thermostatically controlled chamber at a constant temperature of 26°C and relative air humidity (RH) at 80% for 7 days. The length of the roots and hypocotyl was measured to the nearest millimeter.

The coleoptile growth bioassay according to Shimabukuro et al. (1978) was used in the following modification: wheat (*Triticum aestivum* cv. Blava) seeds were soaked for 4 h in distilled water in darkness. The seeds were left to germinate in the thermostatically controlled chamber for three days at 25°C and 80% RH. The seedlings with a coleoptile length of 10 to 11 mm were selected. Coleoptile segments (2 mm) were excised from 2 mm below

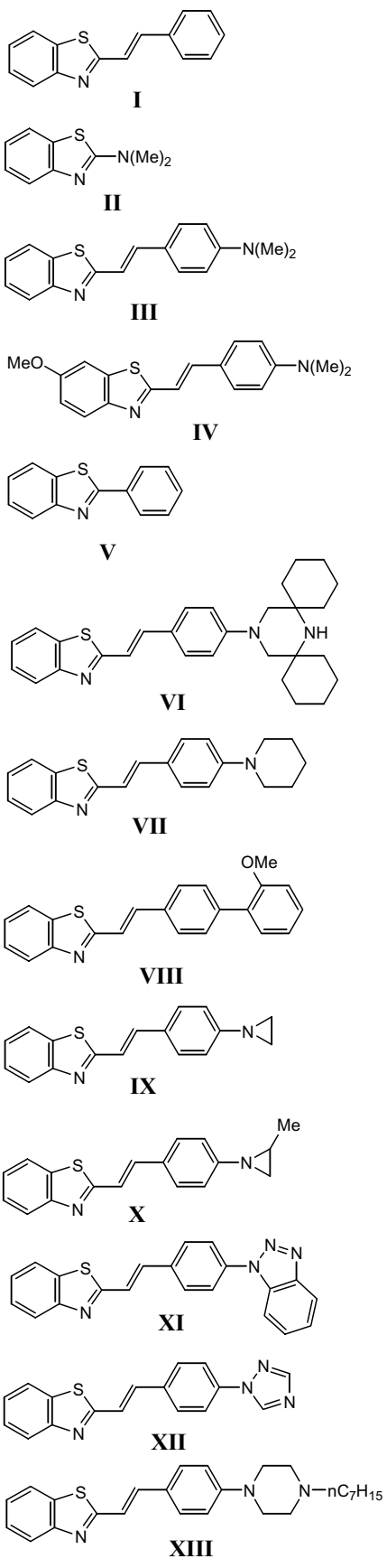

Figure 1. Structures of the tested benzothiazole compounds No. I–XIII

the apical tip and floated for 2 h in distilled water in the dark. Twenty coleoptile segments per variant in three repetitions were incubated in 10 cm^3 of 3% (w/v) sucrose solution containing different concentrations of the tested compounds. The length of the segments was measured after an incubation period of 24 h in the dark at 25°C. Elongation of the coleoptile sections was measured in the dim green safe light. Segments were projected, enlarged ten times and measured with a map marker.

The mung bean bioassay of Pan and Zhao's method (1994) was used. The seedlings of mung bean *Vigna radiata* (L.) Wilczek were grown from seeds at 26 ± 2°C in the dark for 72 h. Material was prepared by cutting the seedlings 5 cm below the cotyledons. Twenty hypocotyl cuttings per treatment with three repetitions were dipped in solutions of tested benzothiazole compounds at various concentrations or in water (control) for 24 h. After treatment, the cuttings were maintained in a growth chamber (Klimabox 1300, Cita, Slaný, Czech Republic) at 26 ± 1°C, 65 ± 5% RH, 12 h irradiance at 80 μmol/m^2/s PAR in substrate (sand + peat in the ratio 3:1) and watered every day. The number of adventitious roots and length of hypocotyl were evaluated 5 days later.

The modified buckwheat bioassay of Mitchel and Livingston (1968) was employed. The *Fagopyrum esculentum* Moench. cv. Pyra plants were grown from seeds in a substrate prepared by mixing sandy-clay soil with humus in the ratio 3:1 (w/w) in a greenhouse [day/night temperature 28 ± 2°C/16 ± 2°C, RH 60 ± 5%, maximum irradiance (PAR) 1200 μmol/m^2/s, photoperiod 14 h]. In the cotyledon stage, the plants were transferred to the growth chamber and the tested compounds were applied twice. The first application was in the stage of cotyledons and the second 7 days later. Ten cm^3 of solution of the tested compounds (10^{-3}–10^{-5}M) in Tween 80 (1%, v/v) as a surfactant were applied per pot with 30 plants. The control set was sprayed with an equal amount of water with Tween 80. Two weeks after the second application to the treated and untreated plants stem length was measured and the fresh and dry mass of the plants determined. Dry mass was determined after drying in an oven at 80–105°C to a constant dry mass with precision to ± 0.1 mg.

The senescence barley bioassay. Senescence was studied on segments of barley leaves using a modified method of Osborne and McCalla (1961). The plants of barley cv. Jubilant were grown in pots with a soil mixture. The plants were cultivated in a greenhouse (day/night temperature 20 ± 2°C/12 ± 2°C, RH 50 ± 5%, maximum irradiance (PAR) 600 μmol/m^2/s, photoperiod 10) h up to the developmental stage of three leaves. Sub-apical segments 2 cm long from the first leaves cut 1.5 cm below the apex were used. The segments were weighed and placed abaxial side down into Petri dishes with 6 cm^3 of the tested solutions or in water (control). The segments were cultivated in the dark at 25°C for five days. The segments from each Petri dish were homogenized and repeatedly extracted with 80% acetone until complete extraction of chlorophyll. The chlorophyll content was determined spectrophotometrically (Jenway 6400 Great Britain) as described by Vernon (1960).

Statistical analysis. Means ± SE at $P \leq 0.05$ and $P \leq 0.01$ were calculated using the Student's *t*-test.

RESULTS

A 0.5% dimethylsulfoxide solution had no measurable effect on auxin-stimulated elongation of the hypocotyls and roots of cucumber. All thirteen benzothiazole derivatives showed concentration-dependent stimulation activity in growth elongation of cucumber hypocotyl and roots (Table 2). The highest significant growth stimulation was achieved following application tested substances in a concentration range of 1×10^{-5}M to 1×10^{-6}M for roots, and at 1×10^{-5}M for hypocotyls. Growth elongation of hypocotyls was increased from 22% (XIII) to 69.5% (XI). In the case of root growth, maximum stimulation was achieved following the application of 1×10^{-6}M of compound No. IV (20.4%) and No. XII (31.9%). Eleven substances exhibited significant stimulative effect on root growth at a concentration of 1×10^{-5}M, ranging from 21.9%. (IV), up to 76.1% (XI) (Table 2). However, all the compounds were weaker, than IAA, which exhibited maximum effect at a concentration fifty times lower.

The majority of tested compounds (I, II, III, V, VIII, XI, XII and XIII) showed retardation activity in elongation growth of cucumber plants at the highest concentration (1×10^{-3}M). This effect, in dependence on the compound structure, amounted up to 29% in hypocotyl and to 27% in root elongation as compared to the control. At this concentration, benzothiazole compounds exhibited a higher retardation effect and also exhibited a formative effect evident in the thickening of the root and hypocotyl and also as in the dwarfing of cotyledons. The effect of the tested benzothiazole derivatives on cucumber growth declined in the order XI, I, X, VII, III, XII, IV, XIII, II, VIII and V; the effect of the four most active derivatives was verified in other types of bioassays. Within the application range of 1×10^{-4} to 1×10^{-7}M, stimulation activity was recorded for substances I, VII, X and XI also for wheat coleoptile growth which increased by 9.5% (compound No. I) at a concentration of 1×10^{-6}M, by up to 33.2% for compound No. XI

Table 2. The effect of benzothiazole derivatives and indole-3-acetic acid (IAA) on root and hypocotyl length in cucumber cv. Evita seven days after treatment of seeds; data are the means ± *SE* of three independent experiments; values followed by the letter (a) are significantly different at $P \leq 0.01$ and the ones followed by the letter (b) are significantly different at $P \leq 0.05$ level against to the control determined by Student's *t*-test

Compound	Concentration (M)	Hypocotyle length (cm)	% of control	Root length (cm)	% of control
Control	0	7.68 ± 0.66	100.0	6.77 ± 0.34	100.0
	1×10^{-6}	8.83 ± 0.44	115.0[b]	8.80 ± 0.74	130.0[b]
I	1×10^{-5}	12.13 ± 1.47	157.9[b]	9.59 ± 0.56	141.7[b]
	1×10^{-4}	9.69 ± 0.83	126.2[b]	7.71 ± 0.56	113.9[b]
	1×10^{-6}	8.96 ± 0.62	116.7[b]	8.12 ± 1.09	119.9[b]
II	1×10^{-5}	9.52 ± 1.00	124.0[b]	8.73 ± 1.00	129.0[b]
	1×10^{-4}	9.35 ± 0.94	121.7[b]	5.46 ± 0.34	80.6[b]
	1×10^{-6}	8.88 ± 0.65	115.6[b]	7.36 ± 0.31	108.7
III	1×10^{-5}	10.26 ± 0.68	133.6[b]	8.39 ± 0.46	123.9[b]
	1×10^{-4}	8.52 ± 0.37	110.9[b]	7.62 ± 0.74	112.6[b]
	1×10^{-6}	9.71 ± 0.47	126.4[b]	6.82 ± 0.66	100.7
IV	1×10^{-5}	9.86 ± 0.76	128.4[b]	8.25 ± 0.56	121.9[b]
	1×10^{-4}	8.48 ± 0.54	110.4	7.76 ± 0.56	114.6[b]
	1×10^{-6}	8.09 ± 0.57	105.3	8.42 ± 0.58	124.4[b]
V	1×10^{-5}	9.54 ± 0.76	124.2[b]	9.18 ± 0.74	135.6[b]
	1×10^{-4}	8.79 ± 0.89	114.5[b]	8.20 ± 0.87	121.1[b]
	1×10^{-6}	8.25 ± 0.83	107.4	8.15 ± 0.62	120.4[b]
VI	1×10^{-5}	9.74 ± 0.89	126.8[b]	7.95 ± 0.78	117.4[b]
	1×10^{-4}	9.42 ± 0.86	122.7[b]	7.14 ± 0.74	105.5
	1×10^{-6}	10.40 ± 0.90	135.4[b]	8.03 ± 0.79	118.6[b]
VII	1×10^{-5}	11.46 ± 0.88	149.2[b]	8.94 ± 0.40	132.1[b]
	1×10^{-4}	10.15 ± 0.74	132.2[b]	7.50 ± 0.68	110.8
	1×10^{-6}	9.48 ± 0.85	123.4[b]	8.76 ± 0.27	129.4[b]
VIII	1×10^{-5}	9.95 ± 0.94	129.5[b]	9.07 ± 0.77	134.0[b]
	1×10^{-4}	9.88 ± 0.59	128.6[b]	8.61 ± 0.57	127.2[b]
	1×10^{-6}	9.29 ± 0.77	121.0[b]	8.64 ± 0.72	127.6[b]
IX	1×10^{-5}	9.57 ± 0.83	124.6[b]	8.97 ± 0.88	132.5[b]
	1×10^{-4}	8.86 ± 0.61	115.3[b]	8.49 ± 0.94	125.4[b]
	1×10^{-6}	9.11 ± 0.72	118.6[b]	8.07 ± 0.49	119.2[b]
X	1×10^{-5}	10.88 ± 1.36	141.7[a]	9.27 ± 0.79	136.9[b]
	1×10^{-4}	9.91 ± 0.88	129.0[b]	7.64 ± 0.55	112.9[b]
	1×10^{-6}	9.88 ± 1.05	128.6[b]	8.58 ± 0.45	126.7[b]
XI	1×10^{-5}	13.02 ± 1.20	169.5[a]	11.92 ± 1.15	176.1[a]
	1×10^{-4}	10.91 ± 0.91	142.1[a]	8.94 ± 0.85	132.1[a]
	1×10^{-6}	8.26 ± 0.59	107.5	8.93 ± 0.76	131.9[b]
XII	1×10^{-5}	9.75 ± 0.67	126.9[b]	8.51 ± 0.62	125.7[b]
	1×10^{-4}	9.29 ± 0.73	121.0[b]	7.76 ± 0.57	114.6[b]
	1×10^{-6}	7.84 ± 0.55	102.1	7.39 ± 0.66	109.2
XIII	1×10^{-5}	9.37 ± 0.81	122.0[b]	9.13 ± 0.92	134.9[b]
	1×10^{-4}	8.79 ± 0.50	114.5[b]	7.59 ± 0.72	112.1[b]
	1×10^{-7}	11.66 ± 1.06	151.8[a]	8.88 ± 0.86	131.2[b]
IAA	2×10^{-7}	15.17 ± 1.39	197.5[a]	10.27 ± 1.41	151.7[a]
	3×10^{-7}	13.02 ± 0.79	169.5[a]	9.20 ± 1.31	135.9[b]

Table 3. The effect of benzothiazole derivatives and indole-3-acetic acid (IAA) on the coleoptile elongation of wheat cv. Blava incubated in 3% sucrose solution with content of tested compounds for 24 h; data are the means ± *SE* of 20 coleoptile segments of three independent experiments; values followed by the letter (a) are significantly different at $P \leq 0.01$ and the ones followed by the letter (b) are significantly different at $P \leq 0.05$ level against to the control determined by Student's *t*-test

Compound	Concentration (M)	Coleoptile length (mm)	% of control
Control	0	2.11 ± 0.039	100.0
I	1×10^{-7}	2.26 ± 0.043	107.1[b]
	1×10^{-6}	2.31 ± 0.083	109.5[b]
	1×10^{-5}	2.18 ± 0.045	103.3
VII	1×10^{-7}	2.68 ± 0.080	127.0[a]
	1×10^{-6}	2.45 ± 0.137	116.1[b]
	1×10^{-5}	2.40 ± 0.108	113.7[b]
X	1×10^{-7}	2.35 ± 0.055	111.4[a]
	1×10^{-6}	2.23 ± 0.040	105.7
	1×10^{-5}	2.19 ± 0.041	103.8
XI	1×10^{-7}	2.26 ± 0.057	107.1[b]
	1×10^{-6}	2.81 ± 0.157	133.2[a]
	1×10^{-5}	2.36 ± 0.159	111.8[b]
IAA	2.0×10^{-5}	3.66 ± 0.070	173.5[a]
	2.5×10^{-5}	4.03 ± 0.132	191.0[a]
	3.0×10^{-5}	3.57 ± 0.134	169.2[a]

at a concentration of 1×10^{-5}M. However, none of the compounds attained the stimulative level of IAA where, at a concentration of 2.5×10^{-5}M, growth elongation in the coleoptile increased by 91.0% (Table 3). Compounds Nos. I, VII, X and XI at concentrations of 1×10^{-4}M and 1×10^{-5}M, also significantly stimulated elongation growth of the hypocotyls and also that of adventitious root formation in mung beans. The number of roots per hypocotyl cutting ranged from 16.9 ± l.53 to 32.0 ± 2.77, which is an increment of 1.8% to 92.8% against the control with a mean number of 16.6 ± 1.26 roots (Figure 2A). The greatest effect on root formation was obtained from the action of derivative No. I at a concentration of 1×10^{-4}M, however, it proved essentially weaker than that of IBA at a concentration producing 62.2 ± 3.54 roots per cutting. The differences in root effectiveness between derivative No. I and IBA are also evident from Figure 3. The auxinoid character of the effect of benzothiazole compounds was also manifested in the fact that while in treated cuttings roots were produced along the whole length of the stimulated hypocotyl, in the controls they appeared solely at the base of the cutting (Figure 3). Besides the evaluated roots with a mean length from 0.2 to 4.5 cm, hypocotyl cuttings treated with derivatives No. XI and X, also had in addition to developed roots, a certain number of visible root bases smaller than 0.2 cm. Likewise, the length of the treated hypocotyl cuttings significantly increased under the action of benzothiazole compounds – from 0.5 cm in compound No. X, up to 1.7 cm in compound No. I, representing an increase of 7.7 up to 26.5% (mean hypocotyl length in the control was 6.48 ± 1.67 cm). In the case of stimulation of hypocotyl elongation, the effect of the tested substances was comparable with that of IBA; under the action of

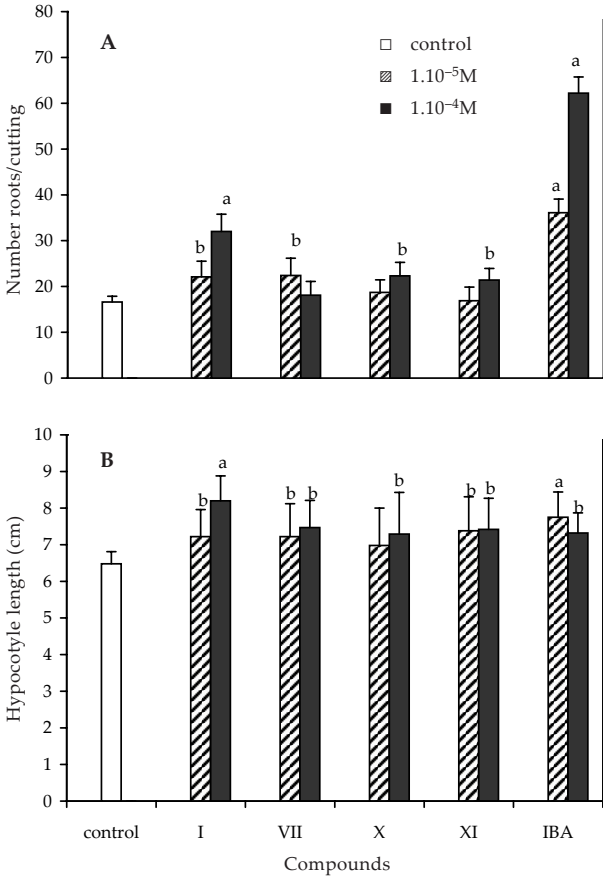

Figure 2. The effect of benzotiazole derivatives No. I, VII, X, XI, and indole-3-butyric acid (IBA) on number of roots per cutting (A) and hypocotyl length (B) of mung bean five days after treatment with of 1×10^{-4} and 1×10^{-5}M concentrations; data are the means ± *SE* of 20 cuttings of three independent experiments; values with the same letter in a vertical column are significantly different at $P \leq 0.01$ (a) and $P \leq 0.05$ (b) level against to the control determined by Student's *t*-test

Figure 3. The effect of benzotiazole derivative No. I and indole-3-butyric acid (IBA) on formation of adventitious roots of mung bean cuttings five days after treatment (bar = 1 cm)

A – control; B – cutting treated with derivate No. I at 1×10^{-4}M concentration; C – cutting treated with IBA at 1×10^{-4}M concentration

the latter, the hypocotyl became prolonged by l.3 cm i.e. by 19.6% (Figure 2B).

The most efficient derivative No. XI was also verified in the *in vivo* system on buckwheat. Its application on leaves confirmed its effect on growth parameters. The effect of the compound at concentrations of 1×10^{-4}M was noted by a significant elongation of the stem by 1.44 cm against the control, i.e. by 20.3%, and in an increase of fresh mass by 2.7 g, i.e. by 30.7%, and also an increase in dry mass by 0.21 g, i.e. by 22.1%.

The significant inhibition effect on senescence in leaf segments of barley under the effect of benzothiazole derivatives at concentrations from 1×10^{-3}M to 1×10^{-7}M failed to be confirmed in the twelve compounds. Chlorophyll content in the treated segments ranged from 36.1 ± 4.10 mg/g FW to 40.2 ± 2.21 mg/g FW, which, in comparison with the control (38 ± 1.71 mg/g FW), was an insignificant change in chlorophyll content. An exception was compound No. II in which a highly significant inhibition effect on senescence was determined in leaf segments. In this compound, at a concentration of 1×10^{-4}M, a significant increase in chlorophyll content against the control was established, amounting to 76.5 mg/g FW, i.e. 201.3%, and under the effect of kinetin at a concentration lower by a degree, this increase was higher by 140.8 mg/g FW, i.e. by 370.4% against the control (Figure 4).

DISCUSSION

Synthetic auxinoids are known to profoundly affect plant metabolism, as it is evident from their effects on growth, development and morphology of plants (Procházka and Šebánek 1997). The results of our experiments showed that the tested 2-R substituted benzothiazole derivatives also influenced growth in cucumber, wheat coleoptile, mung bean and buckwheat and may be characterized as biologically active substances with dominant auxine-like growth activity. Present studies thus show that the compounds stimulated both radicle and hypocotyl growth of cucumber, although not necessarily at the same rate. The effects of the compounds were similar to those of some growth herbicides, such as diclofop and MCPA (Olson et al. 1981), 2,4-D (Sekerka et al. 1989) and picloram (Chang and Foy 1983), therefore, at higher concentrations, the compounds cause inhibition of hypocotyl and root elongation growth with different formative effects, similar to those of MCPA and 2,3,6-TBA-sodium (Kingham and Fletcher 1963). On the other hand they stimulated both roots and hypocotyls at rates lower than those for herbicidal uses. Cucumber cv. Evita was selected from many species (bean, pea) as an indicator of growth activity as it gives the most uniform and rapid response for the necessary measurements of radicle and hypocotyl length to the tested compounds. Mode of the action of growth stimulators, especially auxine-like substances, have often been hypothesized as being involved in the regulation of synthesis or degradation of endogenous auxin indole-3-acetic acid (IAA) in plants Lee (1972). It is known that root and shoot growth is an auxin-stimulated response, which may be reinforced by natural or synthetic auxine compounds (Sutoris et al. 1993).

The *Avena* coleoptile growth test, a bioassay based on auxin-induced cell elongation (Sirois 1966), has been used as a specific and sensitive test for the next verification of auxin-like activity of benzothiazole compounds. Their effects were similar to those of synthetic auxin IAA and 2,4-dichlorophenoxyacetic acid (Sutoris et al. 1993). The changes in endogenous growth rates according Evans and Schmitt

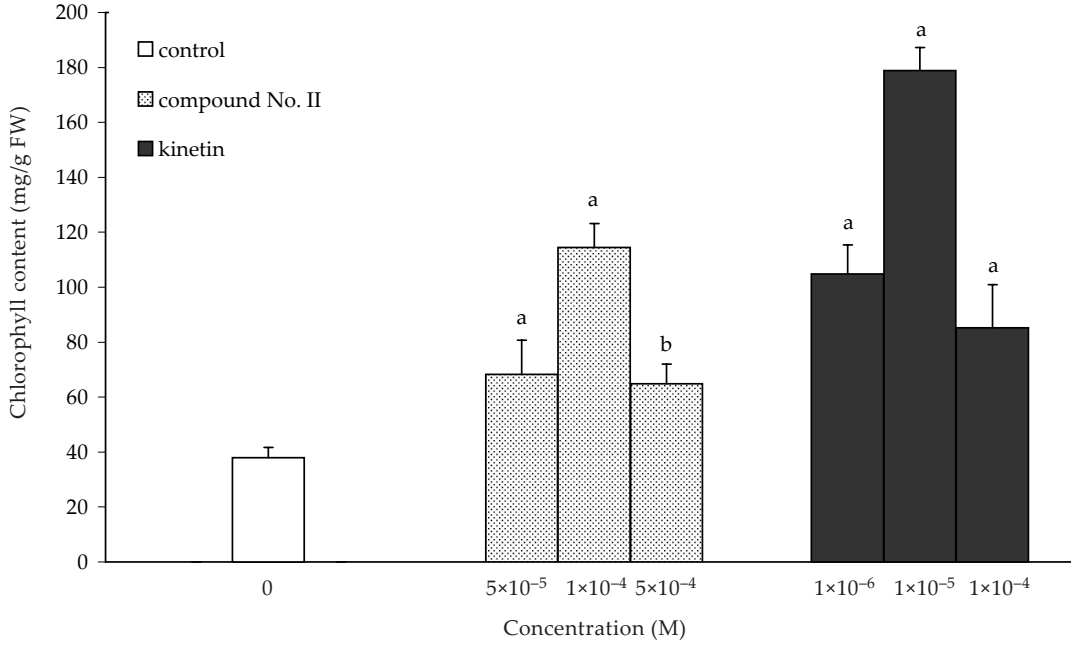

Figure 4. The effect of benzotiazole derivative No. II and 6-furfurylaminopurin (kinetin) on the chlorophyll content in segments of barley leaves cv. Jubilant incubated in different concentrations for five days; data are the means ± *SE* of three independent experiments; values with the same letter in a vertical column are significantly different at $P \leq 0.01$ (a) and $P \leq 0.05$ (b) level against to the control determined by Student's *t*-test

(1975) correlate with the relative activity of IAA synthetase. The activity of IAA synthetase began to increase 2 to 3 hours after removal of the coleoptile tip and then growth rate began to increase. These findings confirmed that excised wheat coleoptiles responded to applied benzothiazole compounds. The fact that changes in endogenous growth rate can be correlated with the activity of IAA synthetase makes the coleoptile an excellent system to study the mode of action of auxine-like substances. We suppose that benzothiazole compounds such as synthetic auxinoids may substitute for natural auxin, e.g. IAA in stimulating root and hypocotyl growth of cucumber and cell elongation of wheat coleoptile, or reinforcing its effect.

Auxins are known to increase adventitious root formation and play a direct role in promoting rooting (Gaspar and Hofinger 1988). We found that treatment of mung bean hypocotyl cuttings with benzothiazole compounds induced rhizogenesis very rapidly within five days and better formation of roots as against controls. The effects of compounds at the same concentrations were less effective than IBA in adventitious roots development and thus it confirms that IBA is one of the most widely used root promoters (Davis 1986).

The effect obtained with IBA at a concentration of 1×10^{-4}M, was comparable to that reported by Kollárová et al. (2004) on a similar species *Vigna radiata* and higher by 12% than that obtained by Pan and Zhao (1994) in the species *Phaseolus aureus*. Root formation along the entire length of the stimulated hypocotyls under the action of both the benzothiazole compounds and also of IBA confirmed the auxinoide character of the tested substances.

Many regulating chemicals cause an increase or decrease in the rate of growth of the main stems of plants Mitchel and Livingston (1968). The effect on the length of the stems is therefore one means of evaluating the resulting properties of the tested substances. The application of the most effective derivate No. XI had a stimulating effect on elongation of the main stem and on increased fresh and dry mass in buckwheat. The application of this derivative at the stage of cotyledons could act in different ways upon the meristematic tissues and mature cells of treated *Fagopyrum* plants. This fact corroborates the opinion that biologically active substances in many cases affect the quantitative growth and yield-related parameters in the plants and also alter their natural hormonal status (Caldiz et al. 1991, Beckett and Van Staden 1992, Chernyad'ev 1994). Buckwheat plants proved a suitable model for indicating growth activity of tested compounds, because it was consistently a more sensitive seedling than bean seedlings.

It is known, that excised leaves usually attain senescence rapidly. Visibly, they turn yellow or lose their color. This is a consequence of the deg-

radation of chlorophyll and proteins and can be retarded, to some extent, if the excised leaves are treated with cytokinins (Richmond and Lang 1957), or substances with anti senescence effects (Satler and Thimann 1980, Thomas and Katterman 1986, Cavender et al. 1988). Significantly cytokinin-like activity manifested solely by derivate No. II with an anti senescence effect and higher chlorophyll content in leaf segments of barley. Compound No. II differs in its structure – in comparison with the other compounds – by its simplicity, its typical feature being the N-C-N bond fragment, where N-C belongs to the heterocyclic ring. This structural feature does not appear in the other studied derivatives, but it is known in adenine derivatives with cytokinin activity. This may have given rise to the determined activity similar to that of kinetin. The relationship between auxin and senescence has long been known, therefore auxins, according to Osborne and Hallaway (1960), retard protein degradation in separated leaves. However, no such relation was found in the twelve 2-R substituted benzothiazole derivatives, for they showed no inhibiting effect on chlorophyll degradation in leaf segments of barley. We found it present only in derivative No. II. Similarly as Klíčová et al. (1994) did in oats with the preparation Rastim 30 DKV (the active substance 3-benzyloxycarbonyl-methyl 2-benzothiazolinone) and IAA. Rastim and IAA retarded chlorophyll degradation on an average by 64 and 109%, respectively, while derivative No. II inhibited it by as much as 201%.

The results obtained will be utilized not only to study the relationship between the chemical structure and the biological activity of the compounds investigated here, but also for regulating the ongoing targeted synthesis of substances with biological growth activity.

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ABSTRAKT

Benzothiazolové deriváty substituované v pozici 2 jako biologicky aktivní sloučeniny s růstově-regulační aktivitou

Třináct novosyntetizovaných 2-R substituovaných derivátů benzothiazolu bylo testováno na růstovou biologickou aktivitu typu auxinu a cytokininu. Auxinoidní aktivita sloučenin byla sledována na prodlužovacím růstu u koleoptilových segmentů pšenice *Triticum aestivum* L. cv. Blava a na hypokotylu a kořenech okurky *Cucumis sativum* L. cv. Evita. Produkce adventivních kořenů a délka hypokotylu byly hodnoceny u fazole mungo *Vigna radiata* (L.) Wilczek, vliv na délku stonku a produkci čerstvé a suché hmotnosti u rostlin pohanky *Fagopyrum esculentum Moench*. cv. Pyra. Cytokininová aktivita byla stanovena na listových segmentech ječmene *Hordeum vulgare* L. cv. Jubilant na

základě inhibice senescence a obsahu chlorofylu. Sloučeniny benzothiazolu byly testovány v koncentračním rozpětí 1×10^{-3}–1×10^{-7} M a jejich aktivita byla srovnávána s kyselinou indolyloctovou (IAA), kyselinou indolyl máslovou (IBA) a 6 furfuryl aminopurinem (kinetinem). Všechny testované deriváty projevily aktivitu podobnou IAA na prodlužovacím růstu rostlin, přičemž stimulační účinek byl závislý na aplikační koncentraci. Při vyšších koncentracích působily sloučeniny jako růstové retardanty, inhibovaly prodlužovací růst hypokotylu a kořene okurky, naopak při nižších koncentracích růst stimulovaly, podobně stimulovaly i prodlužovací růst koleoptilu pšenice. Sloučeniny č. I, VII, X, a XI zvyšovaly tvorbu adventivních kořenů u fazole mungo a sloučenina č. XI též pozitivně ovlivnila délku stonku a produkci čerstvé a suché hmotnosti u pohanky. Aktivitu podobnou kinetinu projevil pouze derivát č. II, který vysoce průkazně inhiboval senescenci listových segmentů ječmene oproti kontrole. Benzothiazolové deriváty substituované v pozici 2 lze charakterizovat jako biologicky aktivní sloučeniny s dominantní růstovou aktivitou podobnou auxinu.

Klíčová slova: deriváty benzothiazolu; prodlužovací růst; tvorba kořenů; senescence; *Cucumis sativum*; *Fagopyrum esculentum*; *Hordeum vulgare*; *Triticum aestivum*; *Vigna radiata*

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