SYNTHESIS AND PLANT GROWTH ACTIVITY OF 2-(4-OXOCHROMEN-3-YL)BENZOTHIAZOLIUM AND - BENZOXAZOLIUM BROMIDES

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Abstract: A facile synthesis of novel 2-(4-oxochromen-3-yl) benzothiazolium bromides **4** and benzoxazolium bromide **5** using the one-pot condensation of substituted 4-oxochromene-3-carboxaldehydes **1** with 2-methylbenzothiazole (**2a**) or 2-methylbenzoxazole (**2b**) and benzyl bromide is described. The effects of benzothiazolium bromides **4** on the growth stimulation of the cucumber and retardant activity on corn seedlings were investigated. They inhibited growth of cucumber (root and hypocotyl) and shoots of corn at the range of 10 – 100 ppm and stimulated at 0.1 – 1 ppm concentrations.

Keywords: plant growth activity, benzothiazolium, benzoxazolium salts, 4-oxochromene-3-carboxaldehydes

1. Introduction

Benzothiazole derivatives represent a large group of heterocyclic substances, which were tested for a different biological activity (SUTORIS *et al*., 1988; KRÁĽOVÁ *et al*., 1994; EL-SHAAER *et al.,* 1998). 2-R substituted benzotiazole derivatives manifested antibacterial and antifungal activities (AFSAH and NAYER 1996; MAGDOLEN *et al*., 2000; BUFFA *et al*., 2001) and are reported also to be active as antineoplastics agent (KRIEG and BILLITZ 1996). According ŠIMONOVÁ *et al*., (2005) ones were characterized as biologically active substances with dominant auxin-like growth promoting activity. 3-R substituted benzothiazole derivatives influenced the prolongation of growth, stimulated the plant regeneration, activity of peroxidase, and may induce the dedifferentiation and morphogenesis of plants in *in vitro* conditions (SUTORIS *et al*., 1993; HAVEL *et al*., 1994). Benzothiazole derivatives were also observed as protective agents against UV-induced mutagenesis in *Euglena gracilis* FOLTÍNOVÁ *et al*., (2003) and also against of cadmium stress HENSELOVÁ *et al*., (2007); KLECOVÁ - ŠIMONOVÁ *et al*. (2008) in *Phytollaca dioica*, *Vigna radiata* and *Brassica juncea* plants.

A large number of 4-oxochromene derivatives occur in nature and exhibit variety of biological activities, e.g. antialgal (KRÁĽOVÁ *et al*., 1998), antifungal, antimycobacterial and antiparasitic (CAUJOLLE *et al*., 1993; LÁCOVÁ *et al*., 1995; GAŠPAROVÁ *et al*., 1997; NAWROT-MODRANKA *et al*., 2006), however, none of

them have been reported to have plant growth regulation activity. This paper described the auxin and retardant-like growth activity of newly synthesized oxochromene benzothiazole compounds.

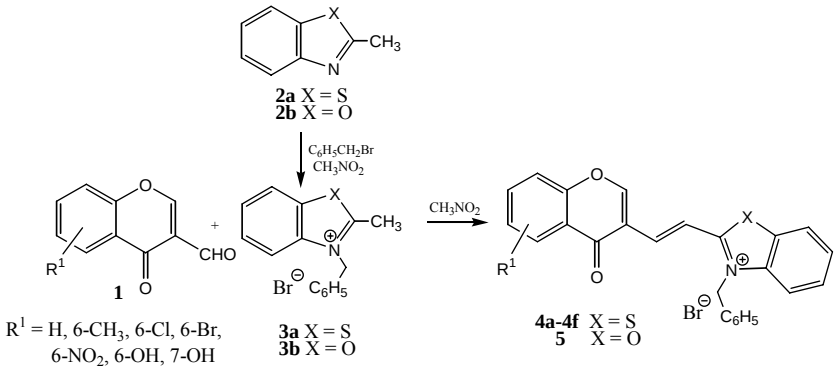
2. Materials and methods

2.1 Chemistry

Scheme 1 shows the synthetic pathway to benzothiazolium and benzoxazolium salts. More synthetic details as well as the characteristic data of synthesized compounds are described (KLEŠTINEC *et al.*, 2008).

General procedure for synthesis 4a-5

A stirred mixture of 4-oxochromene-3-carboxaldehyde **1a** (1 mmol), 2-methylbenzothiazole **2a** (1 mmol) (or 2-methyloxazole **2b**) and benzylbromide (1 mmol) in anhydrous nitromethane (2 dm^3) was irradiated in microwave oven at 270 W for 10 min. After cooling, the solid product was filtered off, washed with warm acetone and crystallized from acetonitrile to give 45 – 81% yields of products.



Scheme 1

2.2 Study of the Plant Growth Activity

The solutions of salts **4 - 5** were prepared in dimethylsulfoxide (DMSO). The final concentration of DMSO in experiments, including the control, was held constant at 1% by volume. This concentration of DMSO had no detectable effect on the growth of plants under experimental conditions. For determination of stimulated growth activity-like indole-3-acetic acid (IAA) we used the bioassay as described by MERRILL (1989) in this modification: Twenty seeds of cucumber (*Cucumis sativus* L. cv. Regina) were placed in 12 cm Petri-dishes on two layers of filter paper Whatman № 2, wetted with 15 ml of distilled water (control), or of the indicated benzothiazole

solution at 0.1, 1 and 10 ppm concentrations. The Petri-dishes were placed to a dark box and incubated at 28 °C. The length of the root and hypocotyl seedlings was measured to the nearest milimeter after 7 days. The stimulated effect of tested compounds was evaluated and compared with the control and standard IAA in the range from 0.001 to 10 ppm concentrations. For corn bioassay in nutrient culture the seeds of corn (*Zea mays* L. cv. Greta) were pregerminated by soaking them in water for 24 hours. Twenty-five seeds were placed on wet filter paper in the Petri-dishes of 20 cm diameter for 48 hours at 25 °C to the dark box. Seeds selected for treatment had a coleoptile emergence of 10 to15 mm. For determination of retardant activity-like abscisic acid (ABA) we used the seedlings bioassay as described by DATHE *et al.*, (1978): Twenty seedlings were chosen per every variant and placed after two seedlings in glass flasks containing 4 ml of half nutrient solution prepared according BROWN and DALTON (1970) with application of testing compounds in 1, 10 and 100 ppm concentrations. The treated and untreated corn plants were cultivated under glass cover in climatic box (temperature 25/15 °C during 16 hours photoperiod and light intensity of 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a relative humidity of 80%). The level of the solutions in the flasks was maintained daily by adding nutrient solution. The length of the corn shoots was measured after 7 days and compared with the control and standard ABA. The standards IAA and ABA come from Sigma, St. Louis, MO, USA. All dates are presented as arithmetic averages of the individual experiments. Bioassay results were statistically evaluated using the Student's *t*-test.

3. Results and discussion

3.1 Study of inhibition of germination and early growth of cucumber and corn seedlings

It is obvious from our experimental data that the biological growth effects depend on concentrations of tested compounds. The cucumber growth test and corn bioassay have been used as a specific and sensitive tests for verification of auxin and retardant activities of tested benzothiazole compounds. The differences in auxin (IAA) and retardant (ABA) like growth activities between tested derivatives are evident from Tables 1 and 2. In general, the inhibition activity was observed at higher (10-100 ppm), and the stimulation activity at lower (0.1-1 ppm) concentrations. All tested compounds showed concentration-dependent stimulation activity in growth elongation of cucumber hypocotyl and root (Table 1). Growth elongation of hypocotyls was increased from 6% (compound No.5) to 42% (No. 4a). In the case of root growth, maximum stimulation was achieved following the application of 0.1ppm concentration from 14.3% (No. 4e) to 45% (No. 4c). Their effects were similar not only to those of synthetic auxin IAA, but also 2,4-dichlorophenoxyacetic acid (YORK *et al*., 1984; SUTORIS *et al*., 1993) which stimulated elongation of pea stem and wheat coleoptile segments. The highest significant growth stimulation was achieved following application-tested substances in a concentration range of 0.1 to 1 ppm for both hypocotyl and root. Compounds No. 4b and 5 have no stimulation effect on root growth (Table 1). However, all the compounds were weaker, than IAA, which

exhibited maximum effect (61- 65%) at a concentration one hundred times lower (0.001 ppm). Similar effects achieved SUTORIS *et al*., (1993) at testing of 3–phenoxycarbonyl-methyl- and 3-aryloxycarbonyl-methyl-2-benzothiazolinones and (ŠIMONOVÁ *et al*., (2005) at 2-R substituted benzothiazole derivatives. In the range of 10^{-3} - 10^{-7} M concentrations the compounds showed different auxin-like effects on elongation growth of wheat coleoptile segments and the formation and number of adventitious roots and the length of hypocotyl in mung bean.

Tab. 1. Effect of 4a-5 benzothiazole compounds and indole-3-acetic acid (IAA) on root and hypocotyl length in cucumber cv. Regina.

Compound R[1]	**Concentration** [ppm]	**Mean length** [mm]		**% of control**	
		Root	**Hypocotyl**	**Root**	**Hypocotyl**
control	—	63.5	36.6	100.0	100.0
4a H	0.1	83.0	52.0	130.7[a]	142.1[a]
	1.0	77.0	48.5	121.2[a]	132.5[a]
	10.0	63.0	44.7	99.2	122.1
4b 6-CH_3	0.1	59.0	44.7	92.9	122.1[a]
	1.0	52.2	38.2	82.2[a]	104.4
	10.0	40.0	26.2	63.0[a]	71.6[a]
4c 6-Cl	0.1	92.0	46.2	144.9[a]	126.2[a]
	1.0	71.8	39.8	113.1[b]	108.7
	10.0	45.8	26.8	72.1[a]	73.2[a]
4d 6-Br	0.1	80.0	50.5	126.0[a]	137.9[a]
	1.0	63.0	47.0	99.2	128.4[a]
	10.0	61.0	10.7	96.1	111.2[b]
4e 6-NO_2	0.1	72.6	47.0	114.3[b]	128.4[a]
	1.0	51.8	44.2	81.6[a]	120.8[a]
	10.0	48.6	35.2	76.5[a]	96.2
4f 6-OH	0.1	89.7	49.2	141.2[a]	134.4[a]
	1.0	79.7	47.5	125.5[a]	129.8[a]
	10.0	64.0	43.2	100.8	118.0[a]
4g 7-OH	0.1	74.6	41.2	117.5[b]	112.6[a]
	1.0	70.4	35.8	110.9[b]	97.8
	10.0	48.8	33.7	76.8[b]	92.1
5 6-CH_3	0.1	61.2	38.8	96.4	106.0
	1.0	39.0	25.4	61.4[a]	69.4[a]
	10.0	31.2	19.2	49.1[a]	52.4[a]
IAA standard	0.001	104.8	58.9	165.0[a]	160.9[a]
	0.01	94.8	51.2	149.3[a]	139.9[a]
	0.1	86.4	44.6	136.1[a]	121.8[a]
	1.0	79.8	43.1	125.7[a]	117.7[a]
	10.0	32.2	27.9	50.7[a]	76.2[a]

[a] Values are significantly different at the 1%; [b]-values are significantly different at the 5% level against the control as determined by Student's *t*-test.

Present studies thus show that the compounds retarded shoot growth of corn, although not necessarily at the same rate (Table 2). The majority of tested compounds showed retardation activity in elongation shoot growth of corn seedlings in the range

of 10 -100 ppm concentrations. This effect, in dependence on the compound structure, amounted up to 82% at No.5 (Table 2). At the highest concentration (100 ppm) the compounds exhibited a morfogenic effect evident in the dwarfing of corn coleoptile and cucumber hypocotyle. It is known that the synthetic auxins at higher concentrations causes inhibition of growth, stem twisting, and other formative effects in susceptible plants (CHANG and FOY 1983; FARGAŠOVÁ 1994). On the other hand however, stimulates both root and shoot growth of many plants at rates lower, what confirmed also our results. The effect of tested substances was comparable with that of ABA (Table 2) with exception of compounds No. 4b and 5 that exhibited higher retardant activity (63-82%) in compare to the standard (42.8%) at the same concentration of 100 ppm. Three compounds (4a, 4e and 4f) had no retardant effect on corn shoot growth in the range of tested concentrations (Table. 2).

Tab. 2. Effect of 4a – 5 benzothiazole compounds and abscisic acid (ABA) on shoot length of corn cv. Greta.

Compound	**Concentration** (ppm)	**Mean length of the shoot** (mm)	**% of control**
control	—	121.4	100.0
4a	1	155.5	128.1[a]
	10	143.0	117.8[a]
	100	142.0	116.9[a]
4b	1	113.0	93.1
	10	65.0	53.5[a]
	100	45.0	37.1[a]
4c	1	185.0	152.4[a]
	10	141.0	116.1[a]
	100	98.5	81.1[a]
4d	1	125.0	102.9
	10	142.0	116.9[a]
	100	93.0	76.6[a]
4e	1	135.0	111.2[b]
	10	145.0	119.4[a]
	100	121.5	100.1
4f	1	132.0	108.7
	10	146.0	120.3[a]
	100	143.5	118.2[a]
5	1	126.0	103.8
	10	101.0	83.2[a]
	100	22.0	18.1[a]
ABA standard	1	95.4	78.6[a]
	10	77.4	63.7[a]
	100	45.2	57.2[a]

[a] Values are significantly different at the 1%; [b]-values are significantly different at the 0.5% level against the control as determined by Student's *t*-test.

The enhancement of growth of cucumber and corn seedlings at the lower concentrations and, on the other hand, their inhibition effect at higher concentrations

observed in tested of benzothiazole derivatives are according to MERRILL'S characteristic growth pattern for auxins. Auxins generally showed the best induction of adventitious roots and stimulated the elongation growth on various plants (MÜLLER 2000; WANG *et al*., 2003; KOLLÁROVÁ *et al*., 2005). Mode(s) of action of growth stimulators, especially auxin-like substances as were tested compounds, have often been hypothesized as being involved in the regulation of synthesis or degradation of endogenous auxin indole-3-acetic acid (IAA) in plants (DAVIES 1995).

4. Conclusions

The effects of 3-Benzyl-2-[(6-R-4-oxochromen-3-yl)ethenyl]benzothiazolium bromides **4a – 4g** and 3-Benzyl-2-[(6-R-4-oxochromen-3-yl)ethenyl]benzoxazolium bromide and **5** were tested on the germination and early growth of cucumber and corn seedlings. The tested oxochromene benzothiazole derivatives may be characterized as biologically active substances with auxin and retardant growth promoting activity. They inhibited growth of cucumber (root and hypocotyl) and shoots of corn at the range of 10 – 100 ppm and stimulated at 0.1 – 1 ppm concentrations. The most effective compounds with stimulating activity on cucumber seedlings was **4a** (R = H), **4f** (R = OH) and the best retardant activity on corn seedlings showed **5** (R = CH_3) and **4b** (R = CH_3).

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