SYNTHESIS OF NOVEL 2-ARYLBENZOTHIAZOLE DERIVATIVES AND BIOLOGICAL EVALUATION OF ANTIOXIDANT, ANTIBACTERIAL AND ANTITUMOR ACTIVITY

SINTEZA NOVIH 2-ARILBENZOTIAZOLSKIH DERIVATA I BIOLOŠKA ISPITIVANJA ANTIOKSIDATIVNE, ANTIBAKTERIJSKE I ANTITUMORSKE AKTIVNOSTI

Livio Racané[1], Vesna Tralić-Kulenović[1], Glorija Fajdetić[1], Marko Klobučar[2], Sandra Kraljević-Pavelić[2], Mihaela Perić[3], Hana Čipčić Paljetak[3], Donatella Verbanac[3], Kristina Starčević[4]

[1]Department of Applied Chemistry, Faculty of Textile Technology, University of Zagreb, Prilaz baruna Filipovića 28a, 10000 Zagreb, Croatia; [2]Department of Biotechnology, Centre for high-throughput technologies, University of Rijeka, Radmile Matejčić 2, 51000 Rijeka, Croatia; [3]Center for Translational and Clinical Research, School of Medicine, University of Zagreb, Šalata 2, 10000 Zagreb, Croatia: [4]Department of Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

INTRODUCTION

Studies of benzothiazole derivatives are a rapidly developing field of research due to their interesting pharmacological properties. Benzothiazole analogues offer a high degree of structural diversity and were studied extensively for their antimicrobial, antitumor, and antiviral activities [1]. Besides, the benzothiazole core and its numerous potential biological implications, the central rings and attached substituents play a major role in the binding affinity and selectivity. In connection with our previous studies focused on synthesis and antitumor activity of 2-arylbenzothiazole derivatives [2-4] we now put our attention on the synthesis and biological evaluation of a series of novel 2-hydroxyphenyl- and 2-methoxyphenylbenzothiazole compounds.

RESULTS AND DISCUSSION

SYNTHESIS Efficient syntheses of 2-hydroxyphenyl- and 2-methoxyphenylbenzothiazole derivatives were carried out by reactions outlined in Scheme 1. Different substituents at 6-position of the benzothiazole skeleton were introduced through condensation reactions involving 5-substituted-2-aminothiphenols (**2-4** and **5a,b**) and the corresponding benzaldehydes in glycerol for preparation of compounds **6a,b-8a,b** or acetic acid for preparation of compounds **9a,b-10a,b**. The 6-amino- derivatives **11a** and **11b** were prepared by reduction of the corresponding 6-nitro- derivatives **7a** and **7b**. To improve the solubility of 6-amidino- (**9a,b-10a,b**), and 6-amino- derivatives (**11a** and **11b**) the compounds were isolated as monocationic mesylate salts.

ANTIBACTERIAL EVALUATION The *in vitro* antibacterial activity of benzothiazole derivatives **6a,b–11a,b** against gram-negative bacteria *Escherichia coli* (TolC-) and *Moraxella catarrhalis* (ATCC 23246) and gram-positive bacteria *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC29212) was evaluated and presented as MICs in Table 1. The evaluated compounds showed very poor antibacterial activity with exception of amidino substituted derivatives **9a,b** and **10b** which exhibited moderated to low activity towards all bacterial strains examined.

ANTITUMOR EVALUATION Antiproliferative activities of the benzothiazole derivatives **6a,b – 11a,b** were evaluated against four human tumor cell lines: cervical carcinoma (HeLa), colorectal metastatic adenocarcinoma (SW620), breast metastatic epithelial adenocarcinoma (MCF-7), lung carcinoma (A549) cell lines and against human skin fibroblasts (HFF) and the results are summarized in Table 2. Overall, 6-substituted benzothiazole compounds except **7b** showed higher antiproliferative activity on against HeLa cell line (IC_{50} value ranging from 0.2-20 μM) in comparison with unsubstituted 2-(2-hydroxyphenyl)- (**6a**), and 2-(2-methoxyphenyl)-bezothiazole (**6b**) derivatives. Compounds bearing an amidino group (**9a,b-10a,b**) showed the strongest antitumor activity but without any selectivity for cancer cell lines except **10a**, the least active among this group. The most interesting selective compounds were several novel 6-substituted benzothiazole compounds showing the highest antiproliferative activity on HeLa cells (**8a**, **11a** and **11b**). Compounds from the same group, **7a** and **10a**, were selective on HeLa and MCF-7 cells with a concomitant lack of cytotoxicity on normal human skin fibroblasts (HFF).

ANTIOXIDANT EVALUATION Antioxidant properties of benzothiazole derivatives **6a,b – 11a,b** were evaluated by; DPPH and ABTS stable radicals as well as ferric reducing ability/antioxidant power (FRAP) *in vitro* assays (Table 3). The results obtained by DPPH method were presented as IC_{50} values with exception of compounds **7b**, **8a,b**, **9a** and **10a,b**. They did not react under assay condition while compound **6a,b**, **7a** and **9b** showed poor antioxidant activity with very high IC_{50} (from 500 μM to 1mM). The best activity by DPPH method was measured for two 6-amino- substituted compounds **11a** and **11b** which showed lower IC_{50} values (4,8 and 51 μM) and for compound **11a** antioxidative activity was more pronounced than BHT used as control. The results obtained by ABTS method showed that all 2-hydroxyphenyl-substituted derivatives **6a**, **7a**, **8a**, **9a**, **10**a and **11a** exhibited good antioxidant activity (from 38 to 117 μM) while 2-methoxyphenyl derivatives **6b**, **7b**, **8b**, **9b**, **10b** showed very low ability towards stabilization of $ABTS^{\bullet+}$ radical (>200 μM). The reducing ability of compounds tested by the (FRAP) assay showed, the best activity among tested compounds for 2-hydroxyphenyl-6-amino derivative **11a** (553±4 $mmolFe^{2+}/mmol_C$). It is clear that presence of phenolic group and amino protonated group attached to benzotiazole moiety are essential for antioxidant activity.

Scheme 1. Synthesis of compounds **6a,b-11a,b**

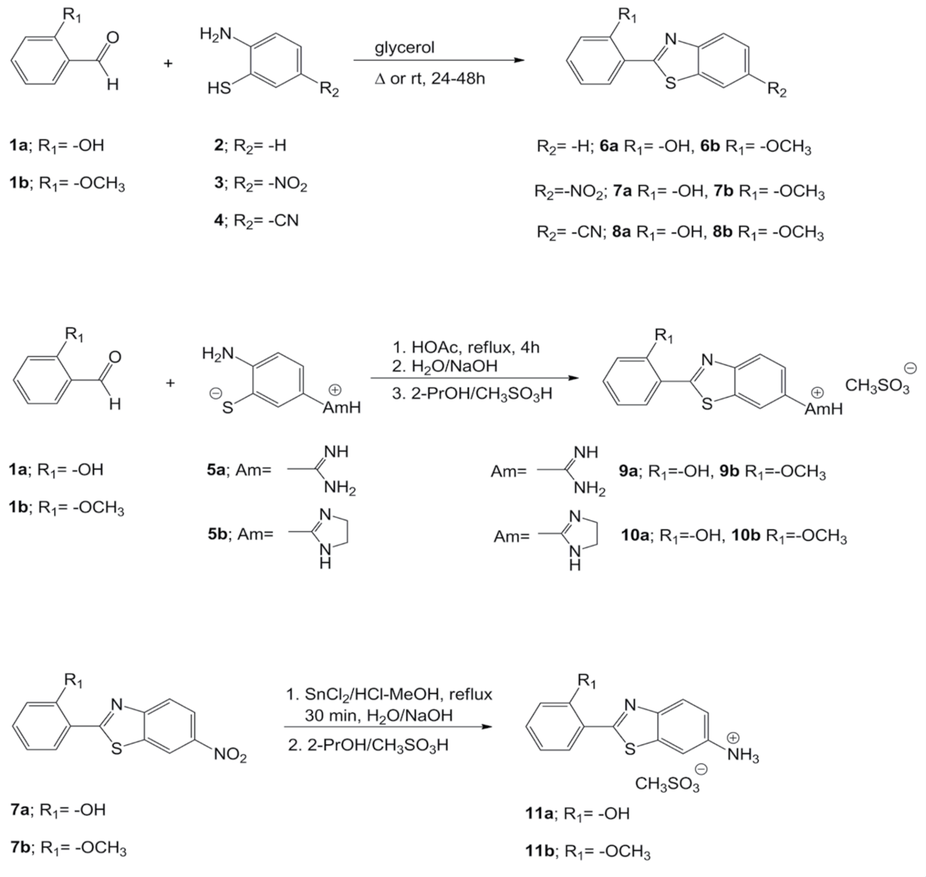

Table 1. Antibacterial activity

Comp.	MICs* (μg/mL)			
	S. aureus	*M. catarrhalis*	*E. faecalis*	*E. coli*
6a	>256	4	>256	>256
6b	>256	<16	>256	>256
7a	>256	>256	>256	>256
7b	>256	>256	>256	>256
8a	>256	>256	>256	>256
8b	>256	>256	>256	>256
9a	32	4	64	32
9b	128	4	128	64
10a	256	256	256	256
10b	128	8	64	64
11a	>256	>256	>256	>256
11b	>256	>256	>256	>256
AZT**	1	0,06	2	0,5

* minimal inhibitory concentrations
** Azithromycin

Table 2. Antiproliferative activity

Comp.	IC_{50}* (μM)				
	A549	HeLa	MCF-7	SW 620	HFF
6a	84±2,1	42±2,2	86±13	>100	70±13
6b	49±4,8	21±4,3	55±22	70±16	36±4,9
7a	>100	5,9±0,7	5,0±5,4	>100	>100
7b	>100	34±4,9	>100	>100	>100
8a	90±9,0	0,23±0,00	66±15	>100	>100
8b	5,7±0,15	2,3±2,1	14±7,3	>100	6,7±3,7
9a	6,8±0,27	3,0±0,77	3,8±2,1	4,7±0,50	24±1,6
9b	4,7±0,27	0,80±0,12	4,4±2,1	5,7±0,23	4,4±1,4
10a	6,9±1,7	22±6,3	4,2±3,3	>100	>100
10b	5,2±0,62	1,9±0,31	3,8±0,74	5,9±0,36	3,7±0,50
11a	>100	9,5±0,54	78±7,4	>100	>100
11b	>100	14,6±7,1	>100	>100	>100

* IC_{50} values are the concentrations that cause 50% inhibition of cancer cell growth (μM).

Table 3. Antioxidant activity

Comp.	DPPH	FRAP	ABTS
	IC_{50} (μM)	$mmolFe^{2+}/mmol_C$	IC_{50} (μM)
6a	>100	317±7	38±3,6
6b	>100	78,8±4	>200
7a	>100	299±3	117±16
7b	no	342±5	>200
8a	no	174±3	61±5,0
8b	no	326±3	>200
9a	no	34,7±0,4	55±3,1
9b	>100	6±1	>200
10a	no	16,9±1,0	48±0,02
10b	no	2,8±0,6	>200
11a	4,8±0,97	553±4	42±1,3
11b	51±0,9	244±8	45±0,00
BHT	25±4,2	2089±56	-

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