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Synthesis of Substituted 2-(Aminoacyl) Amino-6-Halobenzothiazoles as Possible Antimicrobial Agents

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The synthesis of some new 2-(N-Tos-or N-Pht-aminoacyl or Tos-Gly-Gly or aminoacyl hydrochloride or N-Tos or N-Pht-dipeptidyl) amino-6-bromo-(or 6-iodo-) benzothiazole derivatives is described (II–XXVII). All the synthesized compounds were tested against a number of micro-organisms and some compounds were found to possess antimicrobial properties.

INTRODUCTION

Benzothiazoles have been reported to possess antifungal and various antimicrobial activities[1–4]. The presence of halo-substituted phenyl group is shown to increase the activity of the parent compound[5–8]. In our continuing effort directed towards the investigation of the structure-activity relationship of substituted benzothiazole-amino acid derivatives, we encountered a wide variety of benzothiazoles including 6-nitro-, 6-chloro- and 6-methylbenzothiazole amino acid derivatives and studies of their biological properties[9,10].

Herein, we describe the synthesis of some novel 2-(N-protected or free aminoacyl) amino-6-bromo- (or 6-iodo-) benzothiazole derivatives (II-XXVII), and studies on their antimicrobial activities.

RESULTS AND DISCUSSION

For the preparation of 2-(N-Tos- or N-Pht-aminoacyl or Tos-Gly-Gly) amino-6-substituted benzothiazoles (II–XV), N-Tos- or N-Pht-amino acids or Tos-Gly-Gly were reacted with 2-amino-6-bromo- or 2-amino-6-iodobenzothiazole (I-a or I-b) in THF using DCC procedure. The time required for completion of the reaction has been monitored by TLC. All the products (II–XV) were easily isolated, purified, recrystalized and obtained in 52–83% yields (cf. Table I, II–XV).

Hydrazinolysis of 2-(N-Pht-aminoacyl) amino-6-substituted benzothiazoles (IX–XV) in methanol afforded 2-(HCl.N-aminoacyl) amino-6-substituted benzothiazoles (XVI–XXII) as crystalline solids which gave positive ninhydrin and silver nitrate reactions. The products (XVI–XXII) were purified by repeated recrystallizations and obtained in 75–93% yields (cf. Table I, XVI–XXII).

For the preparation of 2-(N-Tos- or N-Pht-dipeptidyl) amino-6-

substituted benzothiazoles (XXIII–XXVII), N-Tos- or N-Pht-amino acids were reacted with 2-(HCl.aminoacyl) amino-6-substituted benzothiazoles (XVI-XXII) in THF-Et_3N medium using DCC procedure. All the synthetic products (XXIII-XXVII) were chromatographically homogeneous when developed with benzidine and iodine solution, and gave negative ninhydrin reaction. The products (XXIII-XXVII) were isolated, purified, and obtained in 66–70% yields (cf. Table 1, XXIII-XXVII).

The antimicrobial activities of the synthesized compounds (II-XXVII) were determined using the hole plate method and filter paper disc method[11-14]. All the products were tested against gram-positive and gram-negative bacteria: *Bacillus cereus* (NRRL-B-569); *Bacillus sphaericus* (159); *Staphellococcus aureus* (ATCC-6538p); *Sarcina species*; *Pseudomonas aeruginosa* (M_2); *Escherichia coli* (NRRL-B-210) and selected fungi: *Candida lypotica*. A qualitative screen was performed in all compounds, while quantitative assays were done on active compounds only (cf. Table II).

2-(N-Tos-L-Val) amino-6-iodobenzothiazole (VII) and 2-(HCl.L-Ala) amino-6-iodobenzothiazole (XXI) were found to possess moderate antimicrobial properties towards *Staph. aureus*, *Sarc. species* and *Bac. sphaericus* with MIC ranging from 50 to 100 μg/ml, but were inactive towards *Esch. coli*, *Pseud. aeruginosa*, *Bac. cerus* and *Cand. lypotica* (MIC > 500 μg/ml).

2-(N-Pht-Gly) amino-6-bromobenzothiazole (IX) was found to be active against *Staph. aureus*, *Cand. lypotica* (MIC 100 μg/ml) and *Esch. coli* (MIC 50 μg/ml), but inactive towards the remaining microorganisms. 2-(HCl.Gly) amino-6-bromobenzothiazole (XVI) possesses high antimicrobial activity towards *Staph. aureus* (MIC 50 μg/ml), *Bac. sphaericus* (MIC 5 μg/ml), *Esch. coli* and *Pseud. aeruginosa* (MIC 100 μg/ml), and antifungal activity towards *Cand. lypotica* (MIC 25 μg/ml), but did not inhibit the growth of the remaining microorganisms.

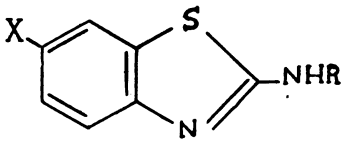
2-(HCL.L-Phe) amino-6-bromobenzothiazole (XX) inhibited the growth of *Staph. aureus* (MIC 100 μg/ml), *Bac. sphaericus* (MIC 5 μg/ml) and *Pseud. aeruginosa* (MIC 50 μg/ml), but was inactive towards all other tested microorganisms. Compounds (II–VI, VIII, X–XV, XVII–XIX and XXII-XXVII) were biologically inactive towards all the tested microorganisms (MIC > 500 μg/ml).

From the above data it is evident that combination of 2-amino-6-iodobenzothiazole residue with 2-(N-Tos-L-Val) or 2-(HCl.L-Ala) moieties gave compounds (VII–XXI) of specific biological properties. However, the corresponding Tos-L-Phe (VIII), Pht-L-Ala, Pht-L-Phe (XIV–XV), HCl.L-Phe (XXII), Tos-L-Val-L-Phe (XXVI) and Pht-Gly-L-Ala (XXVII) were biologically inactive.

On the other hand, the isomeric 6-bromo derivatives, 2-(N-Pht-Gly) amino-6-bromobenzothiazole (IX) and the corresponding HCl.Gly (XVI)

TABLE 1

PHYSICAL DATA OF VARIOUS 2-(N-PROTECTED OR FREE AMINOACYL) AMINO-6-BROMO- or 6-IODOBENZOTHIAZOLE DERIVATIVES (II–XXVII)



Compounds (II–XXVII)

Compd. No.	–R	–X	Cryst. solvent*	M.pt. (°C)	Yield %	R_f	E_{cm}	$(\alpha)_D^{20}$ (c=5 in ethanol)	Molecular formula	Elemental analysis %					
										Required			Found		
										C	H	N	C	H	N
II	Tos-Gly	Br	a	207–209	62	0.78	—	—	$C_{16}H_{14}N_3O_3S_2Br$	43.63	3.18	9.54	43.62	3.20	9.56
III	Tos-L-Ala	Br	a	208–210	79	0.83	—	+76.36	$C_{17}H_{16}N_3O_3S_2Br$	44.93	3.52	9.25	45.00	3.62	9.31
IV	Tos-L-Val	Br	a	218–220	52	0.76	—	+4.52	$C_{19}H_{20}N_3O_3S_2Br$	47.30	4.14	8.71	47.35	4.17	8.72
V	Tos-L-Leu	Br	a	190–192	83	0.81	—	+25.12	$C_{20}H_{22}N_3O_3S_2Br$	48.38	4.43	8.46	48.53	4.40	8.41
VI	Tos-Gly-Gly	Br	a	178–180	75	0.82	—	—	$C_{18}H_{17}N_4O_4S_2Br$	43.46	3.42	11.26	43.49	3.45	11.29
VII	Tos-L-Val	I	b	140–142	57	0.84	—	+40.69	$C_{19}H_{20}N_3O_3S_2I$	43.10	3.78	7.93	43.00	3.69	7.83
VIII	Tos-L-Phe	I	b	110–112	60	0.77	—	+42.70	$C_{23}H_{20}N_3O_3S_2I$	47.83	3.46	7.27	47.82	3.46	7.27
IX	Pht-Gly	Br	a	191–193	66	0.80	—	—	$C_{17}H_{10}N_3O_3SBr$	49.03	2.40	10.09	49.13	2.45	10.05
X	Pht-L-Ala	Br	a	145–147	74	0.84	—	+28.13	$C_{18}H_{12}N_3O_3SBr$	50.23	2.79	9.76	50.24	2.80	9.76
XI	Pht-L-Val	Br	a	187–189	58	0.84	—	+43.20	$C_{20}H_{16}N_3O_3SBr$	52.40	3.49	9.17	52.40	3.42	9.17
XII	Pht-L-Leu	Br	a	205–207	56	0.80	—	+14.06	$C_{21}H_{18}N_3O_3SBr$	53.38	3.81	8.89	53.41	3.85	8.92
XIII	Pht-L-Phe	Br	a	200–202	79	0.85	—	+18.58	$C_{24}H_{16}N_3O_3SBr$	56.91	3.16	8.30	56.97	3.26	8.35
XIV	Pht-L-Ala	I	b	190–192	82	0.78	—	+52.24	$C_{18}H_{12}N_3O_3SI$	45.28	3.35	8.80	45.32	3.36	8.81

XV	Pht-L-Phe	I	b	209–211	64	0.79	—	+62.80	$C_{24}H_{16}N_3O_3SI$	52.07	2.89	7.59	52.00	2.82	7.53
XVI	Gly-HCl	Br	c	140–142	93	0.85	2.9	—	$C_9H_9N_3OSClBr$	33.48	2.79	13.02	33.50	2.79	13.00
XVII	L-Ala-HCl	Br	c	188–190	87	0.82	4.9	+37.17	$C_{10}H_{11}N_3OSClBr$	35.66	3.26	12.48	35.68	3.29	12.42
XVIII	L-Val-HCl	Br	c	205–207	80	0.87	4.5	+37.68	$C_{12}H_{15}N_3OSClBr$	39.50	4.11	11.52	39.48	4.10	11.51
XIX	L-Leu-HCl	Br	c	236–238	86	0.78	3.9	+42.70	$C_{13}H_{17}N_3OSClBr$	41.21	4.49	11.09	41.23	4.53	11.13
XX	L-Phe-HCl	Br	c	200–202	75	0.76	3.2	+17.58	$C_{16}H_{15}N_3OSClBr$	46.54	3.63	10.18	46.55	3.63	10.17
XXI	L-Ala-HCl	I	c	206–208	83	0.80	4.5	+61.79	$C_{10}H_{11}N_3OSClI$	31.29	2.86	10.95	31.32	2.89	10.96
XXII	L-Phe-HCl	I	c	197–199	87	0.78	2.0	+67.82	$C_{16}H_{15}N_3OSClI$	41.78	3.26	9.14	41.75	3.22	9.10
XXIII	Tos-Gly-L-Val	Br	a	188–190	66	0.77	—	+13.06	$C_{21}H_{23}N_4O_4S_2Br$	46.75	4.26	10.38	46.70	4.25	10.39
XXIV	Tos-L-Leu-L-Phe	Br	a	186–188	73	0.83	—	+46.72	$C_{29}H_{31}N_4O_4S_2Br$	56.95	5.07	9.16	57.00	5.11	9.17
XXV	Pht-L-Ala-L-Val	Br	a	170–172	78	0.84	—	+16.57	$C_{23}H_{21}N_4O_4SBr$	52.17	3.96	10.58	52.00	3.81	10.43
XXVI	Tos-L-Val-L-Phe	I	a	190–192	70	0.78	—	+77.36	$C_{28}H_{29}N_4O_4S_2I$	49.70	4.28	8.28	49.69	4.25	8.27
XXVII	Pht-Gly-L-Ala	I	a	207–209	71	0.79	—	+56.26	$C_{20}H_{15}N_4O_4SI$	44.94	2.80	10.48	44.95	2.82	10.51

*Crystallization solvents: (a) methanol-water, (b) acetone-water and (c) methanol.

and HCl.L-Phe (XX) showed improved and verified biological action. But the remaining 6-bromo derivatives containing the N-tosyl derivatives of Gly, L-Ala, L-Val, L-Leu, Gly-Gly (II–VI) and the N-phthalyl derivatives of L-Ala, L-Val, L-Leu and L-Phe (XX–III) residues were biologically inactive.

TABLE 2

MINIMAL INHIBITORY CONCENTRATION (MIC μg/ml) OF THE BIOLOGICALLY ACTIVE COMPOUNDS

Compd. No.	*Staph. aureus*	*Sarc. species*	*Bac. sphaericus*	*Esch. coli*	*Pseud. aeruginosa*	*Cand. lypotica*
VII	100	50	100	—	—	—
IX	100	—	—	50	—	100
XVI	50	—	5	100	100	25
XX	100	—	5	—	50	—
XXI	100	100	100	—	—	—

Removal of the N-phthalyl protecting group enhanced and verified the biological properties of the Gly and L-Phe derivatives (XVI and XX), but did not improve the biological properties of the L-Ala, L-Val and L-Leu derivatives (XVII-XIX and XXII). Elongation of the peptide chain did not affect the biological properties of the substituted benzothiazole derivatives, but hydrazinolysis of the N-phthalyl protecting group verified the biological properties of some of the synthesized amino acid derivatives. Other biological properties are in progress.

EXPERIMENTAL

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Thin layer chromatography (R_f values) for analytical purposes was taken on silica gel G-1 plastic sheets and developed with (n-butanol : acetic acid : water) (4 : 1 : 1) using iodine, ninhydrin and benzidine as spraying agents. Optical rotations $(\alpha)_D^{20}$ were measured for all compounds in ethanol at λ_{max} 589 nm on Bellingham Stanelly polarimeter using 5 cm tube at 20°C.

The infrared spectra (ν_{max}; cm^{-1}) were taken in KBr discs (pellets) using Schimadzu IR-408 instrument, the ultraviolet spectra (λ_{max} nm; log ϵ; in methanol) were measured using Schimadzu UV-240 spectrophotometer and NMR spectra (chemical shifts δ in ppm; in DMSO-d_6) were measured using Varian EM-360L, 60-MHz spectrometer and TMS as internal standard.

Paper electrophoresis were carried out on Whatman No. 1 paper by

the method of (Horizontal paper electrophoresis, Type Helena Lab Beaumant, Texas) with E. pyridine-acetate buffer (4 ml pyridine + 1 ml acetic acid in one litre of water; pH = 5.6) at 450 volts for 2 hrs, silver nitrate reaction was used for development; (E refers to electrophoretic mobility).

Synthesis of 2-amino-6-bromobenzothiazole (I-a) and 2-amino-6-iodo benzothiazole (I-b)

The title compounds were prepared according to the procedures described earlier[15].

General Procedure for Synthesis of 2-(N-Tos- or N-Pht-aminoacyl or Tos-Gly-Gly) Amino-6-bromo-or 6-iodobenzothiazoles (II-XV)

N-Tosyl- or N-phthaloylamino acid or Tos-Gly-Gly (0.004 mole) and 2-amino-6-bromo- or 2-amino-6-iodobenzothiazole (I-a or I-b, 0.004 mole) were dissolved in tetrahydrofuaran (THF). The reaction mixture was cooled at 0°C, dicyclohexylcarbodiimide (0.004 mole) added and the mixture stirred 1 hr at 0°C and 2 hrs at 20°C and left at room temperature. The dicyclohexylurea (DCU) was filtered off and the filtrate evaporated in vacuo. The residual material was recrystallized from methanol-water and acetone-water. The products (II–XV) were soluble in alcohols, acetone, DMF and dioxane, and insoluble in ether and petroleum ether. The synthesized compounds (II–XV) were chromatographically homogeneous when developed with benzidine or iodine (cf. Table I, compounds II–XV).

The IR spectra of the products (II–XV) showed characteristic bands at: 3360, 3280 3080 (NH, CONH, SO_2NH); 1650, 1560, 1360 (amide I, II and III); 1780, 1725 ($\rangle C=O$); 1060, 1020, 980, 920, 830, 780, 680 (Ar—Br, Ar—I) and other bands in support of the proposed structures. The UV spectra of (II-XV) showed bands λ_{max} (log ϵ) at: 208 nm (5.27), 227 nm (5.41) and 268 nm (4.79) characteristic for the benzothiazole chromophore. NMR spectra of compounds (II–XV) exhibited chemical shifts (δ in ppm) at: aromatic protons in the range 7.3–7.8, the NH amide at: 5.68, methyl of tolyl at 1–1.9 and other bands assignable to the remaining amino acids and benzothiazole residues.

General Procedure for Synthesis of 2-(N-aminoacyl hydrochloride) Amino-6-bromo- or 6-iodobenzothiazoles (XVI-XXII)

2-(N-phthaloylaminoacyl) amino-6-bromo- or 6-iodobenzothiazoles (IX–XV, 0.01 mole) were dissolved in 100 ml methanol containing 10 ml alcoholic hydrazine hydrate (85%). The reaction mixture was refluxed for 1 hr and to the residue obtained after evaporation of the solvent, 50 ml 2N–HCl has been added and the mixture was heated

for 10 min. at 50°C. The reaction mixture was allowed to cool and the insoluble phthalyl hydrazide filtered off, the filtrate evaporated and the residue was recrystallized from methanol. The products (XVI–XXII) were chromatographically homogeneous when developed with benzidine or iodine solution and gave positive reaction with ninhydrin (cf. Table I, compounds XVI–XXII).

The IR spectra of all the hydrochlorides (XVI-XXII) displayed characteristic bands at: 3360, 3280 (NH, CONH); 1650, 1560, 1360 (amide I, II and III); 1780, 1725 (>C=O) and other bands characteristic of the amino acids and aromatic moeities. The UV spectra of (XVI–XXII) showed bands (log ϵ) at: 208 nm (5.27), 227 nm (5,41) and 268 nm (4.79) characteristic for the benzothiazole chromophore. NMR spectra of compounds (XVI–XXII) exhibited at 7.3–7.8 (aromatic protons); 5.68 (NH amide); 6–8.3 (NH_3^+) and other signals in support of their assigned structures.

General Procedure for Synthesis of 2-(N-Tos- or N-Phtdipeptidyl) Amino-6-bromo- or 6-iodobenzathiozoles (XXIII-XXVII)

2-(N-Aminoacyl hydrochloride) amino-6-substituted benzothiazoles (XVI–XXII, 0.001 mole) were dissolved in 40 ml THF containing 1 ml triethylamine and stirred for 30 minutes, then N-tosyl- or N-phthaloyl-amino acid (0.001 mole) was added and the reaction mixture was cooled to 0°C. Dicyclohexylcarbodiimide (DCC) (0.001 mole) was added and the reaction mixture was stirred at 0–5°C for 3 hrs, then left to stand at room temperature overnight. The precipitated dicyclohexylurea (DCU) was removed by filtration and few drops of acetic acid were added to the filtrate and leave it for 3 hrs, then filter again. The solvent was evaporated under vacuum and the residual product was recrystallized from methonol-water. The products (XXIII–XVII) were found to be chromatographically homogeneous when developed with benzidine or iodine and gave ninhydrin negative reaction (cf. Table I, compounds XXIII–XXVII).

The IR spectra of the synthesized compounds (XXIII–XXVII) showed characteristic bands at: 3360, 3280, 3080 (NH, CONH, SO_2NH); 1650, 1560, 1360 (amide I, II and III); 1780, 1725 (>C=O) and other bands in support of their structures. The UV spectra of the products (XXIII–XXVII) showed bands (log ϵ) at: 208 nm (5.27), 227 nm (5.41) and 268 nm (4.79) characteristic for the benzothiazole chromophore. NMR spectra of (XXIII–XXVII) exhibited chemical shifts (δ in ppm) at : 7.3–7.8 aromatic protons, 5.68 the NH amide and other signals assignable to the proposed structures.

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