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SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME NEW BENZOTHIAZOLE DERIVATIVES

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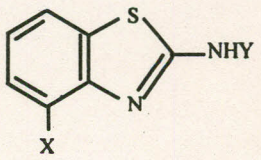
Synthesis of some new 2-(N-Tos-or N-Phtaminoacyl or N-Tos- or N-Pht-dipeptidyl) amino-4-nitro-(or-4-methyl) benzothiazole derivatives has been described (II- XXIX). Some of the synthesized derivatives have been found to possess antimicrobial properties against a number of microorganisms.

Keywords: 2-amino-4-nitrobenzothiazole, 2-amino-4-methylbenzothiazole, Tosyl and phthalyl of some amino acids.

Introduction

In previous communications [1-6] a number of benzothiazole derivatives have been found to exhibit various pharmacological activities. Recently the synthesis of benzothiazole amino acid conjugates with different substituents at 4-position was reported.

The introducing of nitro or methyl group enhanced the biological action of these compounds [7].



I_a (X = NO_2)
Y = H
I_b (X = CH_3)
Y = H

Compounds ($I_{a,b}$)

Experimental

Melting points were determined on an electrothermal melting point apparatus. Thin layer chromatography (TLC) for analytical purposes was carried out through 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 D20 were measured for all compounds in ethanol at λ_{max} 589 nm on Bellingham Stanelly polarimeter using 5 cm tube at 20°.

Infrared spectra υ_{max}; cm^{-1} were taken in KBr discs (pellets) using Schimadzu IR-408 instrument, Ultraviolet spectra λ_{max} nm; logE; in methanol) were measured using Schimadzu UV-240 spectrophotometer and NMR spectra chemical shifts (δ) in ppm; in DMSO - (d_6) and were measured using a Varian EM-360L, 60 MHz Spectrometer and TMS as internal standard. Biological activity experiments were carried out in biology department of Al-Azhar University, A.R. Egypt.

Synthesis of 2-amino-4-nitrobenzothiazole (I_a) and 2-amino-4-methylbenzothiazole (I_b). The title compounds were prepared according to the procedures described earlier [8].

PHYSICAL DATA FOR THE 2-AMINO-4-SUBSTITUTED BENZOTHIAZOLES.

No.	X	Y	Yield	Crst* Solvent	M.P. °C	Refer.
Ia		H	75	a	139-141	7
Ib		H	77	a	163-165	7

* Crystallization. solvent: (a) Methanol - Water

Synthesis of 2-(N-Tos-or N-Pht-aminoacyl) Amino-4-nitro- or 4-methylbenzothiazoles (II-XVII). N-Tosyl- or N-Phthaloylamino acid (0.004 mole) and 2-amino-4-nitro- or 2-amino-4-methylbenzothiazole ($I_{a,b}$ 0.004 mole) were dissolved in tetrahydrofuran (THF). The reaction mixture was cooled at 0°, dicyclo-hexylcarbodiimide (0.004 mole) added and the mixture stirred 1 hr. at 0° and 2 hrs. at 20° and left for 24 hrs at room temperature. The dicyclohexylurea (DCU) was filtered off and the filterate evaporated in vacuo. The residual material was recrystallized from methanol water and acetone water.

The products (II-XVII) were soluble in alcohols and acetone and insoluble in ether. The synthesized compounds (II-XVII) were chromatographically homogeneous when developed with Benzidine or iodine (cf. Table 1, compounds II-XVII).

The IR spectra of the products (II-XVII) showed characteristic bands at: 3360, 3280, 3080 (NH, CONH, SO_2 NH); 1650, 1560, 1360 (amide I, II and III); 1780, 1725 (C=0); 1730, 1550, 1440, 1250 (Ar-NO_2); 2950, 2860, 2760 (Ar-CH_3) in support of the proposed structures.

TABLE . PHYSICAL DATA OF VARIOUS 2-(N-TOS-OR N-PHT-AMINOACYL OR AMINOACYL HYDROCHLORIDE OR PEPTIDE)-AMINO-4-NITRO AND 4-METHYLBENZOTHIAZOLE DERIVATIVES (II-XXIX).

Comp. No.	Y	X	Yield %	M.P. (°C)	Cryst.* solvent	Rf (TLC)	(α) 20 D (C=5 ethanol)	Molecular Formula	Elemental analysis Calcd. C	H	N	Found C	H	N
II	Tos-L-Ala	NO_2	65	186-8	a	0.68	+76.36	$C_{17}H_{16}N_4O_5S_2$	48.57	3.80	13.33	48.28	3.71	12.99
III	Tos-L-Val	NO_2	84	155-7	a	0.83	+4.55	$C_{19}H_{20}N_4O_5S_2$	50.89	4.46	12.50	51.10	4.51	12.71
IV	Tos-L-Leu	NO_2	57	210-12	a	0.72	+25.42	$C_{20}H_{22}N_4O_5S_2$	51.94	4.76	12.12	52.01	4.82	12.32
V	Tos-L-Phe	NO_2	87	216-18	a	0.78	+36.12	$C_{23}H_{20}N_4O_5S_2$	55.64	4.03	11.29	55.73	4.21	11.40
VI	Tos-L-Ala	CH_3	80	177-9	a	0.81	+8.96	$C_{18}H_{19}N_4O_5S_2$	49.65	4.36	12.87	49.80	4.43	12.63
VII	Tos-L-Val	CH_3	62	152-4	a	0.77	+41.72	$C_{20}H_{23}N_3O_3S_2$	57.55	5.51	10.07	57.51	5.49	10.18
VIII	Tos-L-Leu	CH_3	66	195-7	a	0.80	+42.78	$C_{21}H_{25}N_3O_3S_2$	58.46	5.80	9.74	58.44	5.78	9.79
IX	Tos-L-Phe	CH_3	72	218-20	a	0.84	+33.15	$C_{24}H_{23}N_3O_3S_2$	61.93	4.94	9.03	62.05	5.01	9.12
X	Pht-L-Ala	NO_2	79	86-8	a	0.86	+27.34	$C_{18}H_{12}N_4O_5S$	54.54	3.03	14.14	54.57	3.08	14.21
XI	Pht-L-Vel	NO_2	64	108-10	a	0.75	+29.15	$C_{20}H_{16}N_4O_5S$	56.60	3.77	13.20	56.63	3.79	13.22
XII	Pht-L-Leu	NO_2	60	140-42	a	0.76	+18.56	$C_{21}H_{18}N_4O_5S$	57.53	4.10	12.78	57.43	4.11	12.82
XIII	Pht-L-Phe	NO_2	83	184-6	a	0.79	+60.50	$C_{24}H_{16}N_4O_5S$	61.01	3.38	11.86	61.09	3.43	11.91
XIV	Pht-L-Ala	CH_3	88	206-8	a	0.89	+13.75	$C_{19}H_{15}N_3O_3S$	62.46	4.10	11.50	62.38	4.06	11.45
XV	Pht-L-Val	CH_3	69	162-4	a	0.85	+6.75	$C_{21}H_{19}N_3O_3S$	64.12	4.83	10.68	64.13	4.84	10.71
XVI	Pht-L-Leu	CH_3	92	167-9	a	0.78	+82.15	$C_{22}H_{21}N_3O_3S$	64.36	5.15	10.31	64.35	5.15	10.31
XVII	Pht-L-Phe	CH_3	86	181-3	a	0.73	+49.45	$C_{25}H_{19}N_3O_3S$	68.02	4.30	9.52	67.98	4.25	9.47
XVIII	L-Ala HCL	NO_2	85	205-7	b	0.85	+61.72	$C_{10}H_{11}N_4O_3SCl$	39.66	3.60	18.51	39.75	3.68	18.52
XIX	L-Val HCL	NO_2	80	211-13	b	0.81	+45.25	$C_{12}H_{15}N_4O_3SCl$	43.57	4.53	16.94	43.58	4.53	16.95
XX	L-Leu HCl	NO_2	86	222-4	b	0.88	+38.18	$C_{13}H_{17}N_4O_3SCl$	45.28	4.93	15.25	45.26	4.94	15.23
XXI	L-PheHCl	NO_2	83	214-16	b	0.72	+44.16	$C_{16}H_{15}N_4O_3SCl$	50.72	3.96	14.79	50.78	4.02	14.83
XXII	L-AleHCl	CH_3	87	221-3	b	0.80	+19.60	$C_{11}H_{14}N_3OSCl$	48.61	5.15	15.46	48.67	5.21	15.53
XXIII	L-ValHCl	CH_3	73	231-3	b	0.71	+14.06	$C_{13}H_{18}N_3OSCl$	52.08	6.01	14.02	52.11	6.05	14.06
XXIV	L-Leu Hcl	CH_3	66	209-11	b	0.78	+16.58	$C_{14}H_{20}N_3OSCl$	53.58	6.37	13.39	53.57	6.36	13.40
XXV	L-Phe HCL	CH_3	71	262-4	b	0.69	+61.46	$C_{17}H_{18}N_3OSCl$	58.70	5.17	12.08	58.72	5.17	12.06
XXVI	Tos-L-Ala-L-Val	NO_2	70	203-5	a	0.77	+45.72	$C_{22}H_{25}N_5O_6S_2$	50.81	4.81	13.48	50.87	4.84	13.52
XXVII	Tos-L-Val-L-Leu	NO_2	71	177-9	a	0.83	+18.95	$C_{25}H_{31}N_5O_6S_2$	53.47	5.52	12.49	53.50	5.53	12.51
XXVIII	Tos-L-Leu-L-Phe	CH_3	78	250-2	a	0.84	+31.35	$C_{30}H_{34}N_4O_4S_2$	62.27	5.88	9.68	62.31	5.90	9.70
XXIX	Pht-L-Phe-L-Phe	CH_3	72	226-8	a	0.79	+27.49	$C_{35}H_{30}N_4O_4S$	69.76	4.98	9.30	69.77	4.97	9.32

* Crystallization solvent : (a) methanol-water. (b) acetone-water.

The UV spectra of (II-XVII) in ethanol showed λ_{max} (log. ε) at: 208 nm (5.27), 227 nm (5.41) and 268 nm (4.79) characteristic for the benzothiazole chromophore.

The NMR spectra of (II-XVII) exhibited chemical shifts (δ in ppm) at: aromatic protons in the range 7.3-7.8, the NH amide at 5.68, methyl protons at 1.9-1.0 and other protons assignable to aromatic and amino acid or peptide residues.

Synthesis of 2-(N-aminoacyl) Amino-4-nitro or-4-methylbenzothiazoles hydrochloride (XVIII-XXV). 2-(N-phthaloylaminoacyl) amino-4-nitro-or 4-methyl-benzo-thiazoles (X-XVII), 0.01 mole) wee dissolved in 100 ml methanol containing 10 ml alcoholic hydrazine hydrate (85%). The reaction mixture was refluxedfor 1 hr. and to the residue obtained after evaporation of the solvent, 50 ml 2 N-HCl has been added and the mixture was heated for 10 min. at 50°. The contents were allowed to cool and the insoluble phthalyl hydrizide filtered off, the filterate evaporated and the residue was recrystallized from methanol. Products (XVIII-XXV) were chromatographically homogeneous when developed with benzidene or iodine solution and gave positive ninhydrin test (cf. Table 1, compounds XVII-XXV).

The IR spectra of all the hydrochloride (XVIII-XXV) displayed characteristic bands at: 3360, 3280 (NH, CONH); 1650, 1560, 1360 (amide I, II and III); 1780, 1725 ($\rangle$C=0) and other bands characteristic of the amino acids and the UV spectra of (XVIII-XXV) showed bands (log e) at: 208 nm (5.27), 227 nm(5.41) and 268 nm (4.79)Characteristic for the benzothiazole chromophore.

The NMR spectra of compounds (XVIII-XXV) 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.

Synthesis of 2-(N-Tos- or N-Pht-dipeptidyl) Amino-4-nitro-or-4-methyl benzothiazoles (XXVI-XXIX). 2-(N-aminoacyl amino-4-substituted benzothiazoles hydrochloride (XVIII-XXV), 0.001 mole). were dissolved in 40 ml tetrahydrofuran containing 1 ml triethylamine and stirred for 30 min, then N-tosyl-or N-phthaloylamino acid (0.001 mole) was added and the reaction mixture was cooled to 0°. Dicyclohexylcarbediimide (DCC) (0.001 mole) was added and the reaction mixture was stirred at 0-5° for 3 h, then left to stand at room temperature overnight. The precipitated

dicyclohexylurea (DCU) was removed by filteration and a few drops of acetic acid were added to the filterate which was left for 3 h, then filtered again. The solvent was evaporated in a vacuum and the residual product was recrystallized from methanol-water.

Products (XXVI-XXIX) were chromatographically homogeneous when developed with benzidine or iodine and gave negative ninhydrin test (cf. Table I, compounds XXVI-XXIX).

Results and Discussion

Antimicrobial activities of the synthesized compounds (II-XXIX) were determined using the hole plate and filter paper disc method [9-12]. All the products were tested against gram-positive and gram negative bacteria: *B. spaericus* (159); *S. aureus* (ATCC-6538 P); *S. species; P. aeruginosa* (M_2); *E. coli* (NRRL-B210). A qualitative screen was performed in all compounds, while quantitative assays were done on active compounds only (cf. Table 2).

TABLE 2. MINIMAL INHIBITORY CONCENTRATION (MIC μg/ml) OF THE BIOLOGICALLY ACTIVE COMPOUNDS.

Comp. No.	*S.aureus*	*S.species*	*B.sphaericus*	*E.coli*	*P.aeruginosa*
IV	50	75	600	550	--
X	25	--	700	50	500
XVIII	--	50	100	550	650
XXV	100	700	5	600	50

2-(N-Tos-L-Leu) amino-4-nitro benzothiazole (IV) was found to possess moderate antimicrobial properties towards *S. aureus* and *S. species* with MIC ranging 50-75 μg/ml but were inactive against *E.coli.*

2-(N-Pht-Ala) amino-4-methyl benzothiazole (XIV) was found to be active against *S. aureus* and *E. coli* with MIC ranging 25-50 μg/ml, but inactive against the remaining microorganisms.

2 (HCl-L-Ala) amino-4-nitro benzothiazole (XVIII) was found to possess moderate antimicrobial properties towards *S. species* and *B. sphaericus* with MIC ranging 50-100 μg/ml.

2 (HCl-L-Phe) amino-4-methyl benzothiazole (XXV) inhibited the growth of *S. aureus* (MIC 100ug/ml), *B. sphaericus* (MIC 5 μg/ml) and *P.aeruginosa* (MIC 50 μg/ml), but was inactive against all other tested organisms. All the remaining compounds were biologically inactive towards all the tested microorganisms. (MIC > 500 μg/ml).

From the above data, it is evident that combination of 2-amino-4-nitro benzothiazole residue with 2-(N-Tos-L-Leu) or 2-(HCl-L-Ala) moieties gave compounds (IV, XVIII) of specific biological properties. On the other hand, the isomeric 4-methyl derivatives with 2-(N-Pht-L-Ala) amino-4-methyl benzothiazole (XIV) showed improved and varified biological action. But the remaining 4-methyl derivatives were biologically inactive.

Removal of the N-Phthalyl protecting group enhanced the biological properties of the L-Ala derivatives (XVIII) and L-Phe derivatives (XXV) but did not improve the biological properties of the L-Val;, L-Leu derivatives, Elongation of the peptide chain did not affect the biological properties of the substituted benzothiazole derivatives. Other biological properties are in progress.

References

1. F. Kurzer, Organic Compounds of Sulphur, **1**,378(1970).
2. J.V. Metzger, Chem. Heterocycl. Compound, **34**, 1 (1979).
3. P.A. Lowe, Heterocyclic Chemistry, Chemical Society, London, **1**, 119 (1980).
4. Elderfield, Journal Organic Chemistry, **18**, (1953).
5. A.M. El-Naggar, M.R. Zahr, A.H. Bedair and N.S. Khalaf, Acta Pharm. Jugosl, **34**, 75 (1984).
6. A.M. El-Naggar, M.R. Zahr and N.S. Khalaf, IL Farmaco, **39**, 154 (1984).
7. Kaufmann, Arch. Pharm., **48**, 273 (1935).
8. K. Papke and R. Pohloudek, Pharmazie, **22**,229 (1967).
9. J.G. Carlson, H.G. Douglas and H.d. Bissel, J. Bacteriol, **55**, 607 (1948).
10. G.W. Irvin, T.D. Fontaine and S.P. Doolittle, J. Bacteriol., **52**, 10 (1946).
11. J.G. Vincent and H.W. Vincent, Proc. Soc. Exptl. Biol. Med. **55**, 162 (1944).
12. J.A. Epstein, E.J. Foley, I. Perrine and S.W. Lee, J. Lab. Clin. Med., **29**, 319 (1944).