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Synthesis and Biological Activity of Some New β-(2-Benzothiazolyl)-Acryloylamino Acid Derivatives

T. M. IBRAHIM*, A. M. GOMMAA, M. A. EL-GAZZAR
A. M. ABD EL-SALAM AND A. H. M. RADI

Chemistry Department, Faculty of Science,
Al-Azhar University, Nasr City, Cairo, Egypt

The synthesis of β-(2-benzothiazolyl) acryloylamino acid methyl esters (II-VII), hydrazides (VIII-XIII) and dipeptide methyl esters (XIV-XIX) are described. The derivatives containing the residues of Ser, Phe, Tyr and Try were found to be active against several microorganisms.

INTROUCTION

Previously, several benzothiazole derivatives were found to possess antibacterial and antifungal properties[1-3]. Later on, we reported that the acryloyl moiety is incorporated in a wide variety of compounds possessing biological properties[4-6].

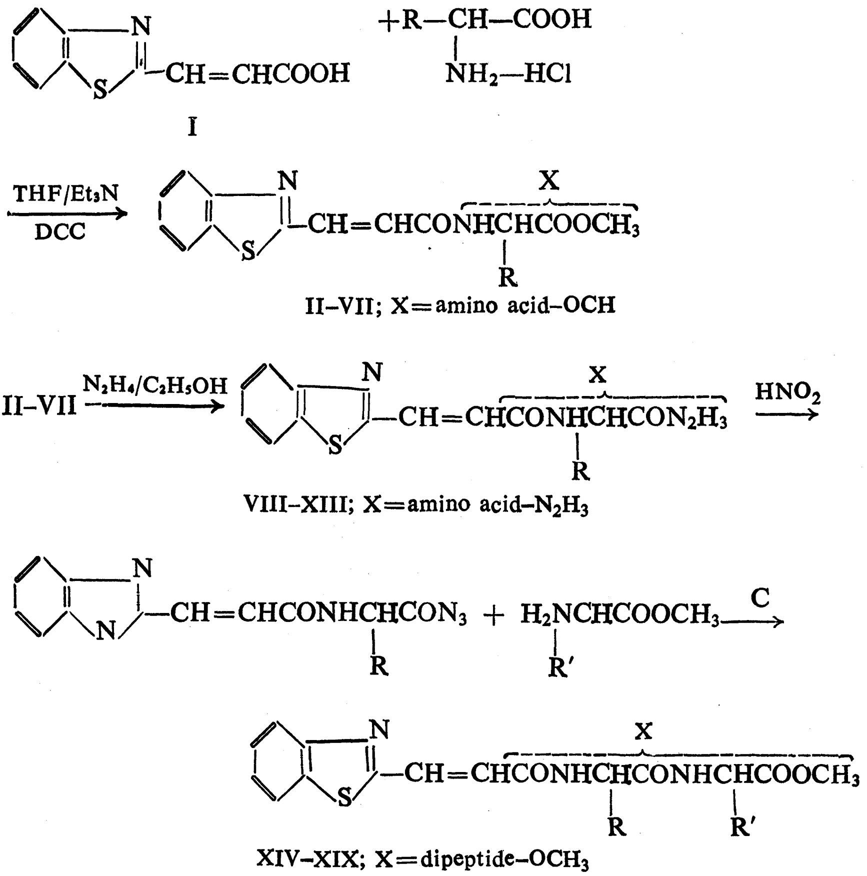
In continuation of our studies in this field, we now report the synthesis of a series of β-(2-benzothiazolyl acryloylamino acid methyl esters (II-VII), corresponding hydrazides (VIII-XIII), and dipeptide methyl esters (XIV-XIX) (cf. Scheme I). These compounds were also tested for biological activity.

RESULTS AND DISCUSSION

Coupling of β-(2-benzothiazolyl) acrylic acid[7] (I) with amino acid methyl ester hydrochlorides in THF containing triethylamine and using the dicyclohexylcarbodiimide (DCC) technique[8] afforded the desired β-(2-benzothiazolyl) acryloylamino acid methyl esters (II-VIII) (cf. Scheme I and Table 1). Synthesis of the Ser, Tyr and Try derivatives did not require the prior protection of the side chain groups and side reactions were observed. The products (II-VII) were easily isolated, purified, recrystallized and obtained in 41-65% yields. Hydrazinolysis of the methyl esters (II-VII) in ethanol gave the corresponding hydrazides (VIII-XIII) (cf. Scheme I and Table 1) as crystalline solids which gave the positive benzidine, iodine and silver nitrate reactions. The products (VIII-XIII) were purified by repeated recrystallizations and obtained in 46-63% yields.

Complete acid hydrolysis (6M-HCl, 24 hrs, 100°C) of (IV) or (X) followed by subsequent paper chromatography afforded a ninhydrin positive spot of valine.

The synthesis of β-(2-benzothiazolyl) acryloldipeptide methyl esters (XIV–XIX) was achieved starting from the hydrazides (VIII–XIII) which were converted into the corresponding azides. The azides on coupling with the free amino acid methyl esters furnished the dipeptides (XIV–XIX) (cf. Scheme I and Table 1), which were isolated, purified and obtained in 30–56% yields. All the products (XIV–XIX) were chromatographically homogeneous. Complete acid hydrolysis of (XVII) (6M–HCl, 24 hrs, 100°C) followed by subsequent paper chromatography yielded two positive spots of phenylalanine and valine. The dipeptide methyl esters (XIV–XIX) gave deep blue complexes with Cu(II) λ_{max} 650–670 nm, characteristic for normal dipeptide copper complexes.[9]



Scheme 1 General scheme for the synthesis of various β-(2-benzothiazolyl) acrylamino acid methyl esters (II–VII), corresponding hydrazides (VIII–XIII), and dipeptide methyl esters (XIV–XIX).

TABLE 1

PHYSICAL DATA OF VARIOUS β-(2-BENZOTHIAZOLYL)-ACRYLOYLAMINO ACID DERIVATIVES (II–XIX)

Compd. No.	X	Yield* %	M.pt. °C	R_f	$[\alpha]_D^{20}$ (C=5, ethanol	Molecular formula	Elemental analysis (%) Calculated/Found C	H	N
II	L–Ala–OMe	65	135–36	0.73	−67.1	$C_{14}H_{14}N_2O_3S$	57.93 58.03	4.82 4.90	9.65 9.71
III	L–Ser–OMe	63	128–29	0.77	+75	$C_{14}H_{14}N_2O_4S$	54.90 54.98	4.57 4.62	9.15 9.20
IV	L–Val–OMe	56	120–22	0.78	+73	$C_{16}H_{18}N_2O_3S$	60.37 60.43	5.66 5.71	8.99 8.91
V	L–Phe–OMe	41	171–73	0.72	+79.5	$C_{20}H_{18}N_2O_3S$	65.57 65.59	4.91 4.95	7.65 7.67
VI	L–Tyr–OMe	51	124–25	0.74	+83.4	$C_{20}H_{18}N_2O_4S$	62.82 63.03	4.71 4.81	7.32 7.41
VII	DL–Try–OMe	52	143–44	0.80	—	$C_{22}H_{19}N_3O_3S$	65.18 65.31	4.69 4.74	10.37 10.43
VIII	L–Ala–N_2H_3	63	110–12	0.70	−72.5	$C_{13}H_{14}N_4O_2S$	53.79 53.82	4.82 4.90	19.31 19.33
IX	L–Ser–N_2H_3	53	99–100	0.73	+69.9	$C_{13}H_{14}N_4O_3S$	50.98 51.03	4.57 4.66	18.30 18.37
X	L–Val–N_2H_3	50	103–104	0.74	+77.1	$C_{15}H_{18}N_4O_2S$	56.60 56.66	5.66 5.68	17.61 17.63
XI	L–Phe–N_2H_3	57	116–17	0.68	+82.3	$C_{19}H_{18}N_4O_2S$	62.29 62.35	4.91 5.01	15.30 15.41

TABLE 1 (Contd.)

Compd. No.	X	Yield* %	M.pt. °C	R_f	$[\alpha]_D^{20}$ (C=5, ethanol)	Molecular formula	Elemental analysis (%) Calculated/Found C	H	N
XII	L-Try-N_2H_3	55	310-3	0.71	+87.5	$C_{19}H_{18}N_4O_2S$	59.68 59.75	4.71 4.78	14.65 14.72
XIII	DL-Try-N_2H_3	46	125-27	0.62	—	$C_{21}H_{19}N_5O_2S$	62.72 62.28	4.69 4.73	17.28 17.31
XIV	L-Ala-Val-OMe	30	114-15	0.79	+77	$C_{19}H_{23}N_3O_4S$	58.61 58.67	5.91 5.95	10.79 10.82
XV	L-Ser-L-Val-OMe	40	220-22	0.75	+89.5	$C_{19}H_{23}N_3O_5S$	56.29 56.41	5.67 5.70	10.37 10.41
XVI	L-Val-L-Val-OMe	47	203-204	0.88	+81	$C_{21}H_{27}N_3O_4S$	60.43 60.47	6.47 6.50	10.07 10.12
XVII	L-Phe-L-Val-OMe	45	97-98	0.64	+86	$C_{25}H_{27}N_3O_4S$	64.51 64.91	5.80 5.93	9.03 9.11
XVIII	L-Tyr-L-Tyr-OMe	56	185-87	0.62	+94.6	$C_{29}H_{27}N_3O_6S$	63.85 63.90	4.95 5.01	7.70 7.83
XIX	DL-Try-L-Phe-OMe	43	197-99	0.80	+80.1	$C_{31}H_{28}N_4O_4S$	67.39 67.41	5.07 5.11	10.14 10.20

*Crystallization solvent for compounds (II-VII and XIV-XIX)=ethanol-water and for compounds (VIII-XIII)=abs. ethanol.

**Optical rotations $[\alpha]_D^{20}$ were measured (C=5) in ethanol.

The IR spectra for compounds (II–XIX) in KBr showed characteristic bands at cm^{-1} : 3340, 3210 (NH, CONH); 1770, 1705 (>C=O); 1650, 1530, 1350 (amide I, II and III) and other bands characteristic of amino acid and benzothiazole residues.

The NMR spectra of compounds (II–XIX) in DMSO–d_6 exhibited the chemical shifts at δ 5.9 (s, 1H, amide, –NH); 7.9–8.2 (s, 4H, for aromatic protons); for compounds (II–VII) and (XIV–XIX) 2.7 (s, 3H, $-COOCH_3$ methyl ester protons); for compounds (VIII–XIII), 4.7–4.9 (s, 3H, NH–NH_2 hydrazide protons) and other protons assignable to each individual amino acid moiety, thus confirming the proposed structure.

Compounds (II–XIX) were prepared and characterized for the first time and their structures were assigned on the basis of elemental analysis. chromatographic studies, spot tests, IR and NMR spectra.

The antimicrobial activities of the synthesized compounds were tested using the hole plate and filter paper disc methods[10–14]. All the synthesized compounds were tested against the following bacteria: *Bacillus subtilis* (ICC strain); *Staphylococcus aureus* (USSR); *Sarcine luteoe* (NRRL–B–569) and selected fungi *Penicillum chrysogenum.* The data for the minimal inhibitory concentrations (MIC in μg/ml) of the active compounds are summarized in Table 2.

β-(2-Benzothiazolyl) acryloyl–L–Ser–OMe (III) and the corresponding L–Phe–OMe (V), L–Tyr–OMe (VI), DL–Try (VII), L–Ser–N_2H_3 (IX), L–Phe–N_2H_3 (XI), L–Tyr–N_2H_3 (XIII) DL–Try–N_2H_3 (XIII), L–Ser–L–Val–OMe (XV), L–Phe–L–Val–OMe (XVII), L–Tyr–L–Tyr–OMe (XVIII), and DL–Try–L–Phe–OMe (XIX) were found to possess a various antimicrobial activities towards *Bac. subtilis*, *Staph. aureus*, and *Sar. luteoe*, MICs ranging from 25 to 500 μg/ml (cf. Table 2).

β-(2-Benzothiazolyl)acryloyl–L–Tyr–OMe (VI) and the corresponding DL–Try–OMe (VII), L–Tyr–N_2H_3 (XII), DL–Tyr–N_2H_3 (XIII), L–Tyr–L–Tyr–OMe (XVIII), and DL–Tyr–L–Phe–OMe (XIX) product antifungal effect against *Penicillum chrysogenum* only (MIC 100–500 μg/ml) (cf. Table 2).

The remaining β-(2-benzothiazolyl)acryloylamino methyl esters, hydrazides and dipeptide methyl esters were found to possess very low antimicrobial (MIC 500 μg/ml) activity towards the tested microorganisms or were biologically inactive.

It is interesting to point out that β-(2-benzothiazolyl) acryloyl–L–Ser–OMe and the corresponding L–Phe–OMe, L–Tyr–OMe, DL–Tyr–OMe, their hydrazides, and dipeptide methyl esters show a noteworthy antimicrobial activity when compared with the activity of the L–Ala and L–Val derivatives.

In other words, the presence of β-(2-benzothiazolyl)acryloyl residue in combination with an aromatic or hydroxy or heterocyclic amino acid such as L–Phe, L–Tyr–, DL–Try and L–Ser gives rise to specific biological properties in β-(2-benzothiazolyl)acryloylamino acid methyl esters, hydrazides, and dipeptide methyl esters, which need further pharmacological investigation to be completely defined.

TABLE 2

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

Compd. No.	Bac. subtilis	Staph. aureus	Sar. luteoe	Pen. chrysogenum
III	100	100	150	—**
V	100	100	150	—
VI	100	100	100	250
VII	100	150	150	500
IX	50	25	50	—
XI	50	50	100	—**
XII	25	25	25	100
XIII	50	100	125	250
XV	250	250	250	—
XVII	250	500	250	—
XVIII	100	100	250	250
XIX	100	250	250	500

*Compounds (II, IV, VIII, X, XIV and XVI) were found to possess very low antimicrobial activities (MIC 500 μg/ml) against some of the tested microorganisms or were biologically inactive.

**=Biologically inactive compound (MIC>500 μg/ml).

EXPERIMENTAL

Melting points were determined on an electothermal melting point apparatus and are uncorrected. Thin layer chromatography (TLC, R_f values) was performed on Silica gel-G using benzene-ethyl acetate (2 : 1) as the solvent system and iodine-KI (20%) as a detection reagent. Paper chromatography (spot reactions) was carried out using Whatman No. 1 paper and *n*-butanol-pyridine-acetic acid-water (30 : 20 : 6 : 24) as solvent system. Benzidine, ninhydrin, silver nitrate and hydroxyamate reactions were used for visualizing the spots. IR spectra (λ_{max} in cm^{-1}) were measured with a Unicam SP 1200 in KBr pellets and NMR data were obtained on a Varian EM–360L spectrophotometer in DMSO–d_6 and shifts are reported in (δ) ppm relative to internal T.M.S. Optical rotations $[\alpha]_D^{20}$ were taken in a Zeiss polarimeter with 1 dm tube (C=5), in ethanol and

λ 589 nm. The microanalyses were performed by the microanalytical center at Cairo University.

β-(2-Benzothiazolyl)Acrylic Acid (I)

The tilte compound was prepared by literature method[7].

General Procedure for the Synthesis of β-(2-Benzothiazolyl)Acryloylamino Acid Methyl Esters (II-VII)

To a solution of amino acid methyl ester hydrochloride (0.01 mole) in THF (20 ml) was added triethylamine (3 ml). The solution was stirred at room temperature for 30 min. and cooled to 0°C. The precipitated triethylamine hydrochloride was filtered off. To the filterate at 0°C was added β-(2-benzothiazolyl)acrylic acid (I; 0.01 mole) in THF (20 ml) and N,N′-dicyclohexylcarbodiimide (DCC) (0.01 mole) in the order. The reaction mixture was stirred for 3 hrs. at 0°C and left 24 hrs. at the same temperature. A few drops of acetic acid (2 ml) were added to the reaction mixture, N,N′-dicyclohexylurea was filtered off and the filterate evaporated in vacuo. The residual oil was recrystallized from ethanol-water (1 : 1) mixture. The compounds (II–VII) were chromatographically homogeneous when developed with benzidine, iodine solution and gave positive hydroxamate reactions and ninhydrin negative test (cf. Table 1; compounds II–VII).

General Procedure for the Synthesis of β-(2-Benzothiazolyl)Acryloylamino Acid Hydrazides (VIII–XIII)

A solution of β-(2-benzothiazolyl)acryloylamino acid methyl ester (II–VII; 0.01 mole) in ethanol (35 ml) and hydrazine hydrate (85%; 0.01 mole) in ethanol (20 ml) was first kept 24 hrs. at 0°C and then for another 24 hrs. at room temperature. The crystalline hydrazides (VIII–XIII) were filtered, washed with cold methanol and recrystallized from absolute ethanol. The hydrazides (VIII–XIII) were chromatographically homogeneous when detected with iodine solution, benzidine and silver nitrate reactions (cf. Table 1; compounds VIII–XIII).

General Procedure for the Synthesis of β-(2-Benzothiazolyl)Acryloyl Dipeptide Methyl Esters (XIV–XIX)

The β-(2-benzothiazolyl)acryloylamino acid hydrazide (VIII–XIII; 0.001 mole) was dissolved in a mixture of acetic acid (3 ml), 5M–HCl (2 ml) and water (15 ml) add cooled to –5°C. Sodium nitrite (0.32 g) in water (4 ml) was added and the mixture stirred for 10 min. at 0°C. The azide was extracted with ethyl acetate (30 ml) and the extract washed successively with water, sodium bicarbonate (3%) and water and dried (Na_2SO_4). Compounds (XIV–XIX) were prepared by the addition of the corresponding azide to a cooled (–5°C) solution of the free amino acid methyl ester (prepared from 0.01 mole of the amino acid methyl ester hydrochloride and 1.5 ml triethylamine). The reaction mixture was stirred 6 hrs, at 0°C and kept for 24 hrs, at 0°C and for another 24 hrs. at room temperature, then washed with HCl (0.5 M), water, sodium bicarbonate (3%) and water in the order and dried (Na_2SO_4). The solvent was removed and the residual material was recrystallized from ethanol-water (1 : 1) mixture. All the dipeptide methyl ester derivatives (XIV–XIX) were found to be chromatographically homogeneous (TLC gave a single spot with benzidine or iodine solution), and showed negative ninhydrin and silver nitrate reactions (cf. Table 1; compounds XIV–XIX).

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