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Synthesis of Biologically Active 3-Benzalphthalide Derivatives

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Abstract □ Synthesis of series of 3-benzalphthalidyl-amino acids and their corresponding methyl esters, dipeptides and tripeptide methyl esters **2a-7c** is decribed. All 3-benzalphthalidynamino acids **2a-g** were found to possess a remarkable antimicrobial properties against a number of microorganisms and fungi.

Keywords □ 3-benzalphthalide, amino acid derivatives, antimicrobial activity.

Recently the synthesis of many substituted nitro, amino and halophthaloylamino acid and peptide derivatives was reported[1-4]. All these compounds were found to possess specific antimicrobial activity[1-4]. This promoted me to synthesize some novel 3-benzalphthalidylamino acids, esters, dipeptides and tripeptide derivatives and evaluate their antimicrobial activity.

EXPERIMENTAL

Melting points were determined on an electrothermal apparatus and are uncorrected. Thin layer chromatography (R_f values) for analytical purposes was taken on silica gel-G (BDH) and developed with benzene-ethyl acetate (3 : 2) as solvent system and iodine -KI (20%) as detection reagent. Paper chromatography (spot reactions) was carried out using Whatman No. 1 paper and *n*-butanol-pyridine-acetic acid- water (30 : 20 : 4 : 18) as the solvent system. Benzdine, ninhydrin, silver nitrate and hydroxamate reactions were used for detection of amino acid derivatives. The IR spectra were recorded on Unicam SP 1200 using KBr discs. ^{1}H-NMR spectra were recorded on a Varian EM-360 L spectrophotometer using DMSO-D_6 as solvent and TMS as internal standard. Optical activities $[\alpha]_D^{20}$ were taken in Zeiss polarimeter with 1 dm tube, C=5 in ethanol. The microanalyses were performed by microanalytical center at Cairo University.

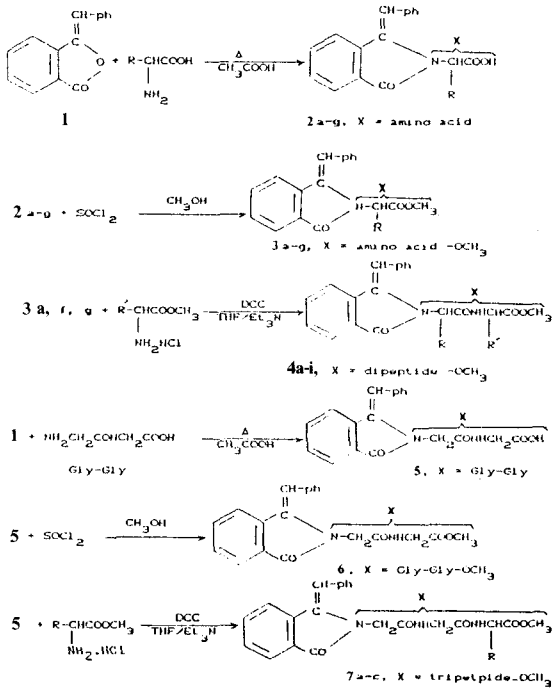
General procedure for the synthesis of 3-benzalphthalidylamino acids 2a-g and 3-benzalphalidylglyceryl-glycine 5

A mixture of the amino acid or glycyl glycine (0.1 mole), 3-benzalphthalide (I; 0.1 mole) and glacial acetic acid (50 m*l*) was shaken for 30 min at 20℃ and refluxed for 6-8 hr at 120℃. The reaction mixture was cooled to 10℃. The solid product was filtered and recrystallized from acetic acid-water. All the products were chromatographically homogeneous when developed with iodine solution, benzidine and gave negative ninhydrin reaction.

The IR spectra of compounds **2a-g**, **5** showed characteristic bands at cm^{-1}: 1780, 1450. 1250, (COOH); 1760, 1720 (C=O); for compound **5**: 3320, 3120 (NH, CONH); 1660, 1540, 1330 (amide I, II and III) and other bands characteristic of amino acid and 3-benzalphthalide moreties. The NMR spectra of compounds **2a-g**, **5** exhibited (δ) at: 10.8 (s, 1H, COOH), 8.1-8.4 (s, 4H, aromatic protons); for compound **5** 4.25 (s, 1H, NH) and other protons assignable to each individiual amino acid residues.

General procedure for the synthesis of 3-benzalphthalidylamino acid methyl esters 3a-g and 3-benzalphalidylglyceyl-glycine methyl ester 6

A suspension of 3-benzalphthalidylamino acid (0.01 mole) or 3-benzalphalidylglycyl-glycine (**5**; 0.01 mole) in abs. methanol (30 m*l*) was cooled to −5℃ and pure thionyl chloride (0.01 mole) was added dropwise during 1 hr. The temperature of the



Scheme 1. General scheme for the synthesis of various 3-benzalphthalidylamino acids 2a-g, corresponding methyl esters 3a-g, dipeptides 4a-6 and tripeptide methyl esters 7a-c.

mixture was kept below 5℃ during the addition of thionyl chloride and the reaction mixture was then stirred for 3-4 hr at 5-10℃, kept overnight at room temperature and the solvent was removed *in vacuo*. Methanol was added and reevaporated several times. The solid residue was recrystallized from ethanol-water (1 : 1) mixture. Compounds **3a-g**, **6** were chromatographically homogeneous when developed with iodine solution, benzidine and gave positive hydroxamate reaction.

The IR spectra of compounds **3a-g**, **6** showed characteristic bands at cm^{-1}: 1780, 1740, (C=O); 1760, 1450, 1370, 1080 (-$COOCH_3$); for compound **5**: 3320, 3110 (NH, CONH) and other bands characteristic to amino acid residues. The NMR spectra of compounds **3a-g**, **6** exhibited (δ) at: 3.7 (s, 3H, $COOCH_3$), and other signals in suport of their assigned structures.

General procedure for the synthesis of 3-benzalphthidyldipeptide methyl esters 4a-i

3-Benzalphthalidylamino acid (0.01 mole) and amino acid methyl ester hydrochloride (0.01 mole) were dissolved in THF (30 m*l*) containing triethylamine (0.013 mole). The mixture was shaken for 5 minutes at 20℃, cooled to 0℃ and dicyclohexyl carbodiimide (DCC, 0.01 mole) was added. The mixture was stirred for 3 hr at 0℃ and kept 24 hr at 0℃ and another 24 hr at room temperature. The dicyclohexylura was filtered off and glacial acetic acid (1 m*l*) was added. After 24 hr, the reaction mixture was filtered and the solvent evaporated *in vacuo*. The solid residue was purified by several recrystallizations from ethanol-water (1 : 1) mixture. The compounds **4a-i** gave negative ninhydrin reaction but respornded positively to iodine solution, benzidine and hydroxamate reaction.

General procedure for the synthesis of 3-benzalphthidyltripeptide methyl esters 7a-c

3-Benzalphalidylglyceyl-glycine (**5**; 0.01 mole) and amino acid methyl ester hydrochloride (0.011 mole) were dissolved in THF (20 m*l*) containing triethylamine (0.013 mole). The remaining procedure was as described for the preparation of compounds **4a-i**. Crystallization solvent for compounds **7a-c** was ethanol-water (1 : 1) mixture. All the tripeptide derivatives were chromatograophically homogeneous when developed with iodine solution, benzidine and gave ninhydrin negative reaction.

The IR spectra of compounds **4a-i**, **7a-c** showed characteristic bands at cm^{-1}; 3340, 3080 (NH, CONH); 1760, 1720 (C=O); 1780, 1460, 1080 ($COOCH_3$); 1650, 1560, 1340 (amide I, II and III) and other characteristic bands due to peptide and 3-benzalphthalide residues. The NMR of compounds **4a-i**, **7a-c** exhibited (δ) at: 4.3 (s, 1H, NH); 3.7 (s, 3H, $COOCH_3$) and other signals in support of their proposed structures.

RESULTS AND DISCUSSION

Chemistry

3-Benzalphthalidylamino acids **2a-g** were readily prepared by condensation of 3-benzalphthalide **1** with the appropriate amino acid in glacial acetic acid for 6-8 hr at 120℃ (cf. Scheme 1 and Table I). The time required for the completion of the reaction was checked up by TLC. Compounds **2a-g** were purified by repeated recrystallizations and chromatographically homogeneous material were obtained in 45-80% yields.

Treatment of compounds **2a-g** with thionyl chlo-

Table I. Physical data of various 3-benzalphthalidylamino acid derivatives 2a-7c

Comp. No.	X	Yield* (%)	M.P. ℃	R_f	$[\alpha]_D^{20}$**	Molecular formula	Elemental analysis% Calculated/Found					
							C	H	N	C	H	N
2a	Gly	45	90-2	0.75	−	$C_{17}H_{13}NO_3$	73.11	4.65	5.01	73.3	4.8	5.2
b	β-Ala	80	104-6	0.83	−	$C_{18}H_{15}NO_3$	73.72	5.11	4.77	73.8	5.2	4.9
c	L-Ala	62	117-19	0.75	+23.1	$C_{18}H_{15}NO_3$	73.72	5.11	4.77	73.9	5.3	4.8
d	L-Val	77	133-35	0.61	+25.5	$C_{20}H_{19}NO_3$	74.76	5.91	4.36	74.8	5.9	4.4
e	DL-Leu	61	195-97	0.71	−	$C_{21}H_{21}NO_3$	75.22	6.26	4.17	75.4	6.3	4.2
f	L-Met	79	109-10	0.69	+30.4	$C_{20}H_{19}NO_3S$	67.98	5.38	3.96	68.1	5.5	4.1
g	L-Phe	73	130-32	0.82	+36.5	$C_{24}H_{19}NO_3$	78.04	5.14	3.79	78.2	5.2	3.9
3a	Gly-OMe	75	69-71	0.84	−	$C_{18}H_{15}NO_3$	73.72	5.11	4.77	73.9	5.3	4.9
b	β-Ala-OMe	61	81-3	0.90	−	$C_{19}H_{17}NO_3$	74.26	5.53	4.56	74.3	5.6	4.8
c	L-Ala-OMe	62	99-101	0.87	+27.5	$C_{19}H_{17}NO_3$	74.26	5.53	4.56	74.4	5.5	4.6
d	L-Val-OMe	70	101-3	0.77	+39.3	$C_{21}H_{21}NO_3$	75.22	6.26	4.17	75.4	6.3	4.3
e	DL-Leu-OMe	65	160-62	0.73	−	$C_{22}H_{23}NO_3$	75.64	6.59	4.01	75.6	6.7	4.2
f	L-Met-OMe	69	89-91	0.75	+43.5	$C_{21}H_{21}NO_3S$	68.66	5.72	3.81	68.9	5.9	3.7
g	L-Phe-OMe	72	111-13	0.78	+39.7	$C_{25}H_{21}NO_3$	78.32	5.48	3.65	48.4	5.6	3.7
4a	Gly-β-Ala-OMe	80	77-9	0.82	−	$C_{21}H_{20}N_2O_4$	69.23	5.49	7.69	69.4	5.7	7.9
b	Gly-DL-Val-OMe	75	70-2	0.72	−	$C_{23}H_{24}N_2O_4$	70.40	6.12	7.14	70.8	6.4	7.3
c	Gly-L-Tyr-OMe	67	81-3	0.59	+50.2	$C_{27}H_{24}N_2O_5$	71.05	5.26	6.14	71.3	5.5	6.2
d	L-Met-β-Ala-OMe	70	101-2	0.61	−51.7	$C_{24}H_{26}N_2O_4S$	65.75	5.93	6.39	65.9	6.1	6.6
e	L-Met-DL-Val-OMe	59	117-19	0.70	+33.7	$C_{26}H_{30}N_2O_4S$	66.95	6.43	6.01	67.1	6.5	6.3
f	L-Met-L-Tyr-OMe	81	123-25	0.43	−17.8	$C_{30}H_{30}N_2O_5S$	67.92	5.66	5.28	68.2	5.8	5.4
g	L-Phe-β-Ala-OMe	59	140-42	0.81	−60.1	$C_{28}H_{26}N_2O_4$	74.01	5.72	6.16	74.2	5.9	6.4
h	L-Phe-DL-Val-OMe	57	119-21	0.89	−70.5	$C_{30}H_{30}N_2O_4$	74.68	6.22	5.81	74.9	6.4	5.9
i	L-Phe-Tyr-OMe	55	114-16	0.67	+66.8	$C_{34}H_{30}N_2O_5$	74.72	5.49	5.12	74.9	5.7	5.4
5	Gly-Gly	84	90-2	0.63	−	$C_{19}H_{16}N_2O_4$	67.85	4.76	8.33	68.1	4.9	8.5
6	Gly-Gly-OMe	89	66-8	0.71	−	$C_{20}H_{18}N_2O_4$	68.57	5.14	8.00	68.7	5.3	8.2
7a	Gly-Gly-β-Ala-OMe	50	177-79	0.75	−	$C_{23}H_{23}N_3O_5$	65.55	5.46	9.97	65.7	5.6	10.1
b	Gly-Gly-DL-Val-OMe	41	166-68	0.83	−	$C_{25}H_{27}N_3O_5$	66.81	6.01	9.35	66.9	6.4	9.5
c	Gly-Gly-L-Tyr-OMe	54	190-92	0.53	+47.2	$C_{29}H_{27}N_3O_6$	67.83	5.26	8.18	68.1	5.5	8.4

*Crystallization solvent for compounds **2a-g** and **5**: acetic acid-water and for compounds **3a-g**, **4a-i** and **6-7c**: ethanol-water.

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

ride in excess methanol gave the corresponding methyl esters **3a-g** in 61-75% yields (cf. Scheme 1 and Table I). All the methyl ester derivatives were chromatographically homogeneous and gave positive hydroxamate reaction.

Complete acid hydrolysis (6M-HCl, 24 hr, 100℃) of **2c** or **3** followed by subsequent paper chromatography afforded a ninhydrin positive spot of alanine.

Coupling of 3-benzalphthalidylamino acid **3a**, **f** and **g** with amino acid methyl ester in THF-triethylamino medium and using (DCC) technoque[5] afforded the dipeptide derivatives **4a-i**. 3-Benzalphalidylglycel-glycine **5** was prepared by coupling 3-benzalphalide **1** with Gly-Gly using the same procedure described for the preparation of compounds **2a-g**. 3-Benzalphalidyl-gly-gly-OMe **6** was also prepared by esterification of the corresponding acid **5** using the same procedure described for preparation the methyl esters **3a-g**. (cf. Scheme 1 and Table I).

Complete acid hydrolysis (6M-HCl, 24 hr, 100℃) of **6i** followed by subsequent paper chromatography yielded two positive spots of phenylanine and tyrosine. The dipeptide derivatives **4a-6** gave a deep blue coloured 1 : 1 copper II complexes exhibiting λ_{max} 650-670 nm, characteristic for normal dipeptide[6].

3-Benzalphthalidyltripeptide methyl esters **7a-c**

Table II. Minimal inhibitory concentration (MIC in μg /ml) of the biologically active compounds*

Comp. No.	*B. sutilis*	*B. mycoides*	*E. coli*	*S. typhose*	Cand. utilis
1	500	−**	500	−	−**
2a	250	250	250	500	500
2b	100	100	100	100	250
2c	50	50	50	50	100
2d	25	25	25	50	100
2e	250	250	250	500	500
2f	25	25	25	25	25
2g	25	25	25	50	100

*Compounds **3a-7c** were found to possess very low antimicrobial activities (MIC 500 μg/ml) against some of tested microorganisms or were biologically inactive.
**Biologically inactive compound (MIC>500 μg/ml).

were easily prapared by coupling 3-benzalphthalidyl-Gly-Gly **5** with amino acid methyl ester hydrochloride in THF-triethylamine medium using (DCC) method (cf. Scheme 1 and Table I). Compounds **7a-c** were easily isolated, purified by recrystallization and obtained in 41-54% yields.

Biology

The antimicrobial activities of the synthesized compounds were tested using the hole plate and filter paper disc methods[7,8]. All compounds were tested against gram-positive and gram-negative bacteria: *Bacillus subtilis* (ICC-strain); Bacillus mycoides (USSR); Escherichia coli (NRRL-B-210); Salmonella typhosa (NRRL-B-573) and selected fungi: Candida utilis. The results were compared with the activity of the parent compound **1**, which found to possess a very low antimicrobial action against *B. subtilis* (500 μg/ml) and *E. coli* (500 μg/ml). The data for the minimal inhibitory concentrations (MIC in μg/ml) of the active compounds are summarized in Table II.

3-Benzalphthalidyl-Gly **2a** and the corresponding β-Ala **2b**, L-Ala **2c**, L-Val **2d**, DL-Leu **2e**, L-Met **2f** and L-Phe **2g** were found to possess various antimicrobial activity at a minimal inhibitory concentration (MIC) of 25-500 μg/ml against all tested microorganisms (cf. Table II).

All the synthesized 3-benzalphthalidylamino acid methyl esters **3a-g**, corresponding dipeptides **4a-6** and tripeptide derivatives **7a-c** were found to be inactive against all tested microorganisms.

The present investigations indicated that the introduction of amino acid moieties to 3-benzalphthalide in combination with Gly, β-Ala, L-Ala, L-Val, DL-Leu, L-Met, and L-Phe residues induce specific biological properties of 3-benzalphthalidylamino acids. Esterification of the C-terminal of carboxyl group, abolished completely the antimicrobial activities of 3-benzalphthalidylamino acid methyl esters **3a-g**. Also the elongation of the peptide chain gave biologically inactive compounds **6a-7c**. Other pharmacological studies are now in progress.

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