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SYNTHESIS OF SOME NEW 5-CHLORO- AND 3,5- DICHLOROSALICYLAMINO ACID, ESTER AMIDEHYDRAZIDE AND TRIPEPTIDE DERIVATIVES WITH EXPECTED BIOLOGICAL ACTIVITIES

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The synthesis of 5-chloro- and 3,5-dichlorosalicylamino acid methyl esters (VIII-XIII) is described. Acid hydrolysis of the esters gave the corresponding free carboxylic acid derivatives (II-VII). Ammonolysis of the methyl ester derivatives afforded their corresponding amides (VIX-XIX). Action of hydrazine hydrate on the methyl esters gave 5-chloro- and 3,5-dichlorosalicylamino acid hydrazides (XX-XXV). Some tripeptide methyl ester derivatives (XXVI-XXXI) were synthesized through DCC method. All the synthesized compounds (II-XXXI) were found to possess different antimicrobial activities.

Key Words: 5-chloro- and 3,5-dichlorosalicylamino acid; Ester Amide; Hydrazide Tripeptide; Biological Activities

Introduction

Previously salicylic acid, salicylamide, acetylsalicylic acid and some of their derivatives were found to have hypoglycemic, antipyretic, analgesic and other pharmacological activities[1-7]. Moreover, the synthesis of N-salicylsulphonylamino acid was reported and the compounds were found to possess some biological activities[8-12]. However, the effect of variation of the functional groups in both the salicylic and amino acid moieties on the biological and pharmacological activities has not yet been studied.

On the other hand, the synthesis of some new 3,5-dichlorosalicylamino acids, esters, amides, hydrazides and tripeptide ester derivatives were found to possess also some biological activities[13]

The present investigation involved the synthesis of some 5-chloro and 3,5-dichlorosalicylamino acid, ester amides, hydrazide and tripeptide ester derivatives (II-XXXI) and studies of their antimicrobial activities.

Discussion

5-chloro- and 3,5-dichlorosalicylyl chloride (1*a*-1*b*) were prepared by action of

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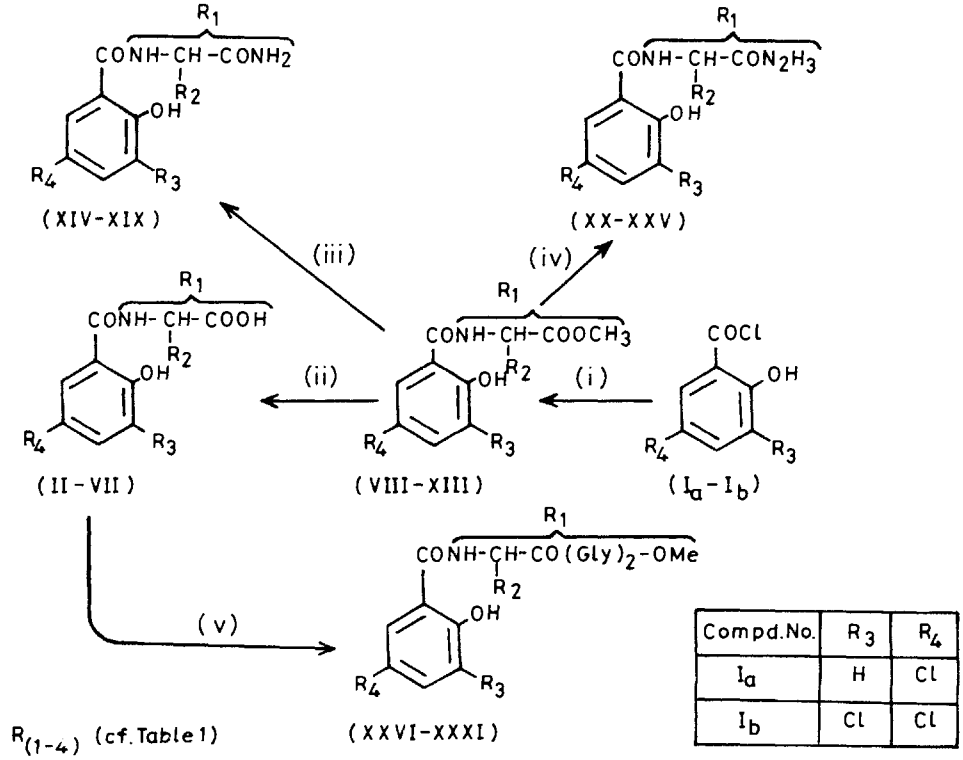
thionyl chloride upon 5-chloro and 3,5-dichlorosalicylic acid in chloroform[14-15]. The reaction of acid chlorides (1*a*-1*b*) with amino acid methyl esters in chloroform/ Et_3N gave the desired 5-chloro and 3,5-dichlorosalicylamino acid methyl esters (VIII-XIII) in crystalline form and chromatographically homogeneous products in (70-90%) yields. the time required for completion of reaction was monitored by TLC.



Compd.No.	R_3	R_4
I_a	H	Cl
I_b	Cl	Cl

Reagents : (i) amino acid methyl ester (ii) 2N-HCl
(iii) NH_3 / abs. methanol (iv) 85% alc. NH_2NH_2
(v) Gly-Gly.OMe/Dcc.

Synthesis of 5-chloro and 3,5-dichlorosalicylamino acids (II-VII) was achieved through acid hydrolysis (2N HCl and 4-5 hours). Alkaline hydrolysis of the methyl ester derivatives (1N NaoH, 5hrs.) gave impure products with very poor yield (20-25%). The free carboxylic acid derivatives (II-VII) were easily isolated as chromatographically homogeneous solid materials in 60-90% yields.

Treatment of the methyl ester derivatives (VIII-XIII) with methanol, previously saturated with ammonia gas at 0 °C, afforded 5-chloro and 3,5-dichlorosalicylamino acid amides (XIV-XIX). The amide derivatives were obtained as crystalline materials in 59-73% yield.

Hydrazinolysis of the methyl esters (VIII-XIII) with 85% hydrazine hydrate in methanol gave the corresponding hydrazides (XX-XXV) as crystalline solids which gave positive benzidine and silver nitrate reactions.

Reaction of 5-chloro- and 3,5-dichlorosalicylamino acid with Gly-Gly. OMe-HCl in tetrahydrofuran-triethylamine medium using dicyclohexyl carbodiimide (DCC) method afforded the tripeptide methyl ester derivatives (XXVI-XXXI) (cf. Table I).

Biological Screening Results

The antimicrobial activities of compounds (II-XXXI) were tested using the hole plate and filter paper disc methods[16-19]. The microorganisms included gram positive: *Staphylococcus aureus* (NCTC-7447), *Staphylococcus saprophyticus* (NCTC-7292); gram negative: *Escherichia coli* (BPP-01), *proteus morganii* (ATCC-25830) and Fungi, *Aspergillus Fumigatus*, (International mycological London).

Qualitative screening were done for all compounds while quantitative determination were performed for the biologically active compounds only. The results were compared with that of (1*a* and 1*b*).

The synthesized 5-chloro- and 3,5-dichlorosalicylamino acids (II-VII) were found to possess high to moderate antimicrobial activities towards all the tested microorganisms (MIC 25-125μg/ml) (cf. Table II).

Esterification of the carboxyl group of 5-chloro- and 3, 5-dichlorosalicylamino acids afforded the methyl ester derivatives (VIII-XIII) and led to decrease of the anti-microbial activity.

Ammonolysis of the methyl esters (VIII-XIII) gave 5-chloro- and 3,5-dichlorosalicylamino acid amides (XIV-XIX) with modified antimicrobial activities (cf. Table II).

Hydrazinolysis of the methyl ester groups or elongation of the peptide chain with Gly-Gly moiety led to remarkable decrease of the antimicrobial activities of the hydrazides or tripeptide derivatives compounds (XX-XXXI) (cf. Table II).

The present investigation revealed that introduction of 5-chloro- and 3,5-dichlorosalicyl moiety in combination with amino acids gave compound with moderate to high biologically active products. However, esterification of the carboxyl group of 5-chloro- and 3, 5-dichlorosalicylamino acid decreased the biological activity of compounds under investigations.

Table I

Physical data of-various 5-chloro and 3-5 dichlorosalicylamino acid, ester, amide, hydrazide and tripeptide derivatives (II-XXXI)

Compound No.	R_1	R_2	R_3	R_4	M.P°C	Cryst Solvent	Yield %	R_f	$[2]^{20}$ C=5 acetone	Molecular Formula	Elemental analysis Calc/Found C	H	N
II	Gly	H	H	Cl	180-182	a	90	0.92	–	$C_9H_8NO_4Cl$	47.05	3.48	6.10
											47.00	3.50	6.00
III	DL-Ala	CH_3	H	Cl	196-198	a	87	0.82	–	$C_{10}H_{10}NO_4Cl$	49.28	4.10	5.75
											49.30	4.00	5.70
IV	L-Phe	CH_2-C_6H_5	H	Cl	169-171	a	60	0.79	+83	$C_{16}H_{14}NO_4Cl$	60.10	4.38	4.38
											60.00	4.00	4.40
V	Gly	H	Cl	Cl	208-210	b	80	0.77	–	$C_9H_7NO_4Cl_2$	40.90	2.65	5.30
											41.00	2.60	5.20
VI	DL-Ala	CH_3	Cl	Cl	145-147	b	82	0.91	–	$C_{10}H_9NO_4Cl_2$	43.16	3.23	5.03
											43.10	3.20	5.00
VII	L-Phe	CH_2-C_6H_5	Cl	Cl	70-72	c	79	0.90	+110	$C_{16}H_{13}NO_4Cl_2$	54.23	3.67	3.95
											54.20	3.70	3.90
VIII	Gly-OMe	H	H	Cl	135-137	a	75	0.65	–	$C_{10}H_{10}NO_4Cl$	49.28	4.10	5.75
											49.30	4.00	5.80
IX	DL-Ala-OMe	CH_3	H	Cl	95-97	a	79	0.66	–	$C_{11}H_{12}NO_4Cl$	51.26	4.66	5.43
											51.30	4.70	5.40
X	L-Phe-OMe	CH_2-C_6H_5	H	Cl	106-108	b	82	0.68	+120	$C_{17}H_{16}NO_4Cl$	61.17	4.79	4.19
											61.10	4.80	4.20
XI	Gly-OMe	H	Cl	Cl	98-100	c	70	0.92	–	$C_{10}H_9NO_4Cl_2$	43.16	3.23	5.03
											43.00	3.10	5.10
XII	DL-Ala-OMe	CH_3	Cl	Cl	110-112	a	90	0.85	–	$C_{11}H_{11}NO_4Cl_2$	45.20	3.76	4.76
											45.00	3.80	4.80
XIII	L-Phe-OMe	CH_2-C_6H_5	Cl	Cl	118-120	a	81	0.80	+30	$C_{17}H_{15}NO_4Cl_2$	55.43	4.07	3.80
											55.40	4.00	3.70
XIV	Gly-NH_2	H	H	Cl	179-181	a	73	0.75	–	$C_9H_9N_2O_3Cl$	47.26	3.93	12.25
											47.30	3.90	12.30
XV	DL-Ala-NH_2	CH_3	H	Cl	213-215	b	59	0.79	–	$C_{10}H_{11}N_2O_3Cl$	49.48	4.53	11.54
											49.50	4.50	11.50
XVI	L-Phe-NH_2	CH_2-C_6H_5	H	Cl	205-207	c	70	0.80	+15	$C_{16}H_{15}N_2O_3Cl$	60.28	4.71	8.80
											60.00	4.60	8.70
XVII	Gly-NH_2	H	Cl	Cl	173-175	c	71	0.92	–	$C_9H_8N_2O_3Cl_2$	41.06	3.04	10.64
											41.00	3.00	10.50
XVIII	DL-Ala-NH_2	CH_3	Cl	Cl	176-178	c	66	0.95	–	$C_{10}H_{10}N_2O_3Cl_2$	43.32	3.61	10.10
											43.20	3.50	10.00

Table Contd.

Compound No.	R_1	R_2	R_3	R_4	M.P°C	Cryst Solvent	Yield %	R_f	$[2]^{20}$ C=5 acetone	Molecular Formula	Elemental analysis Calc/Found C	H	N
XIX	L-Phe-NH_2	CH_2-C_6H_5	Cl	Cl	238-240	a	65	0.90	+150	$C_{16}H_{14}N_2O_4Cl_2$	54.40	3.96	7.93
											54.50	3.90	8.00
XX	Gly-N_2H_3	H	H	Cl	>300	a	62	0.95	–	$C_9H_{10}N_3O_3Cl$	44.35	4.10	17.24
											44.30	4.00	17.20
XXI	DL-Ala-N_2H_3	CH_3	H	Cl	230-232	b	60	0.90	–	$C_{10}H_{12}N_3O_3Cl$	46.60	4.66	16.31
											46.50	4.60	16.20
XXII	L-Phe-N_2H_3	CH_2-C_6H_5	H	Cl	260-262	c	55	0.87	+17	$C_{16}H_{16}N_3O_3Cl$	57.57	4.79	12.60
											57.50	4.80	12.50
XXIII	Gly-N_2H_3	H	Cl	Cl	190-192	a	53	0.81	–	$C_9H_9N_3O_3Cl_2$	38.84	3.24	15.10
											38.80	3.20	15.00
XXIV	DL-Ala-N_2H_3	CH_3	Cl	Cl	181-183	a	59	0.82	–	$C_{10}H_{11}N_3O_3Cl_2$	41.10	3.76	14.38
											41.20	3.70	14.30
XXV	L-Phe-N_2H_3	CH_2-C_6H_5	Cl	Cl	168-170	b	54	0.90	+90	$C_{16}H_{15}N_3O_3Cl_2$	52.17	4.07	11.41
											52.10	4.10	11.40
XXVI	Gly-Gly-Gly-OMe	H	H	Cl	115-117	c	50	0.69	–	$C_{14}H_{16}N_3O_6Cl$	46.99	4.47	11.75
											47.00	4.50	11.70
XXVII	Dl-Ala-Gly-Gly-OMe	CH_3	H	Cl	125-127	d	46	0.79	–	$C_{15}H_{18}N_3O_6Cl$	48.45	4.84	11.30
											48.40	4.80	11.20
XXVIII	L-Phe-Gly-Gly-OMe	CH_2-C_6H_5	H	Cl	111-113	d	44	0.81	+77	$C_{21}H_{22}N_3O_6Cl$	56.31	4.91	9.38
											56.30	4.90	9.30
XXIX	Gly-Gly-Gly-OMe	H	Cl	Cl	135-137	d	48	0.92	–	$C_{14}H_{15}N_3O_6Cl_2$	42.85	3.82	10.71
											42.80	3.80	10.70
XXX	DL-Ala-Gly-Gly-OMe	CH_3	Cl	Cl	150-152	d	52	0.90	–	$C_{15}H_{17}N_3O_6Cl_2$	44.33	4.18	10.34
											44.30	4.20	10.30
XXXI	L-Phe-Gly-Gly-OMe	CH_2-C_6H_5	Cl	Cl	171-173	d	39	0.85	+45	$C_{21}H_{21}N_3O_6Cl_2$	52.28	4.35	8.71
											52.30	4.30	8.70

* Crystallization solvent a = Ethanol, b = Ethanol-water, c = Methanol, d = Dioxane

Table 2

Biological activities of the synthesized compounds (MIC μg/ml).

Compound No.	*Staphylococcus aureus*	*Staphylococcus saprophyticus*	*Escherichia coli*	*Proteus morganii*	*Aspergillus fumigatus*
	MIC	MIC	MIC	MIC	MIC
II	75	75	50	125	125
III	75	75	50	25	—
IV	25	25	125	25	—
V	75	50	50	75	125
VI	50	75	125	75	—
VII	125	25	125	125	125
VIII	250	125	250	250	—
IX	250	125	125	125	—
X	125	75	125	125	—
XI	125	75	125	250	250
XII	75	75	250	125	—
XIII	125	125	125	125	125
XIV	75	75	125	125	—
XV	125	125	125	125	—
XVI	125	125	50	125	250
XVII	50	50	75	125	—
XVIII	75	125	125	75	250
XIX	75	75	75	75	—
XX	125	125	75	250	250
XXI	250	75	250	125	—
XXII	250	125	125	—	—
XXIII	75	75	125	125	250
XXIV	75	75	250	—	—
XXV	75	75	—	125	250
XXVI	250	—	125	250	—
XXVII	250	250	125	—	—
XXVIII	—	250	—	—	—
XXIX	250	—	250	—	—
XXX	250	250	—	250	—
XXXI	250	250	—	—	—

Moreover, the corresponding amide derivatives showed modified biologically active compounds compared with that of free acid or methyl ester derivatives of compounds under investigation. Finally, hydrazinolysis or elongation of peptide chain led to remarkable decrease in biological properties.

Experimental

Melting points were determined on an electrothermal melting point apparatus which are uncorrected. Thin layer chromatography TLC 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 sparing agents. Optical rotations $[a]_D^{20}$ were measured for all compounds in DMF at λ 589nm on Bellingham Stanely polarimeter using 5cm tube at 20°C.

The infrared spectra (υ_{max} cm^{-1}) were taken in KBr, discs (Pellets) using, Shimadzu 1R 408 instrument, NMR spectra (Chemical shifts (δ) in ppm, in DMSO-d_6 or acetone-d_6) were measured using variant EM-360 spectrometer with TMS as internal standard.

5-Chloro- and 3,5-Dichlorosalicylyl Chloride (Ia and 1b)

The title compound was prepared by the reaction of 5-chloro and 3,5-dichlorosalicylic acid (0.1 mol) and thionyl chloride (0.1 mol) at 90-100 °C for 4 hrs in chloroform[13,14].

General Procedure for the Synthesis of 5-Chloro- and 3,5-Dichlorosalicyl-Amino Acid Methyl Ester Derivatives (VIII-XIII)

To a suspension or a solution of amino acid methyl ester hydrochloride (0.0043 mole) in chloroform (30ml) was added triethylamine (2ml). The solution was stirred at 20°C for 15min and cooled to 0°C. To the mixture at 0°C was added 5-chloro- or 3,5-dichlorosalicylyl chloride (I_a or I_b) (0.0042 mole) and the reaction mixture was stirred for 1 hr at 0°C and 3-4hrs at room temperature and then kept overnight at room temperature. The precipitated triethylamine hydrochloride and unreacted materials were filtered off and the filtrate was evaporated *in vacuo*. The residual solid was recrystallized from the proper solvent. Compounds (VIII-XIII) were chromatographically homogeneous when detected with benzidine and all gave a negative test with ninhydrin.

IR spectra of compounds (VIII-XIII) showed characteristic bands at: 3340, 3140cm^{-1} (-CONH), 1660, 1560, 1360 cm^{-1} (amide I, II and III), 1660 cm^{-1} (C=O)

and 1660, 1360cm^{-1} (-COOCH$_3$) while their NMR spectrum of compound (XI) showed peaks at δ: 3.6-3.8(s, 3H, OCH$_3$), 5.6-5.8(*s*, 1H, NH), 6.1-6.3(*s*, 1H, OH), 7.4-7.6 (*s*, 2H, Ar-H).

General Procedure for Synthesis of 5-Chloro-or 3,5-Dichlorosalicylamino Acid Derivatives (II-VII)

To an ethanolic solution of 5-chloro- or 3,5-dichlorosalicylamino acid methyl ester (VIII-XIII) (0.003 mole) was added 10ml 1N HCl and the reaction mixture was stirred for 4-5 hours at room temperature. The precipitated solid material was filtered and recrystallized from ethanol and ethanol-water. The isolated products (II-VII) were chromatographically homogeneous when developed with benzidine or iodine solution.

IR spectra of compounds (II-VII) showed the presence of bands at 2850 cm^{-1} (OH of COOH) and the disappearance of the methyl ester group bands. Similarly NMR spectrum of compound (V) showed peaks at δ: 10.7-10.3 (COOH) and no peaks for (COOCH$_3$) which confirm the structure of (V).

General Procedure for Synthesis of 5-Chloro- or 3,5-Dichlorosalicylamino Acid Amide Derivatives (XIV-XIX)

A solution of (0.005 mol) 5-chloro- or 3,5-dichlorosalicylamino acid methyl ester (VIII-XIII) in 75ml of methanol, previously saturated with ammonia gas at 0° C, was stored in a pressure bottle at room temperature for 48 hours. After this time, the reaction mixture was concentrated under reduced pressure and the residual material was recrystallized from the proper solvent. The amide derivatives (XIV-XIX) were chromatographically homogeneous when developed with iodine or benzidine solutions.

IR Spectrum of Compounds (XVII) showed characteristic bands at 3340, 3140 cm^{-1} (NH, CONH); 1660, 1550, 1350 cm^{-1} (amide I, II and III); 1680 cm^{-1} ($\rangle$C=O) in support of its structure. The other amides (XIV-XIX) showed similar bands in support of their assigned structures.

NMR spectra of compounds (XVIII) showed peaks at δ; 8.15 (*s*, 1H, -CONH); 7.26 δ (*a*, 2H, CONH$_2$) and 3.68 δ (d, 2H, CH$_3$) confirming its structure. The remaining amide derivatives (XIV-XIX) showed similar NMR peaks in support of their structures.

General Procedure for Synthesis of 5-Chloro-or 3,5-Dichlorosalicylamino Acid Hydrazide Derivatives (XX-XXV)

Each methyl ester derivative (VIII-XIII) (0.002 mole) was dissolved in absolute methanol (50ml) and treated with hydrazine hydrate (85%; 1.5 ml). The mixture was stirred for 3 hours at room temperature, then left for 24 hours at 20° C. The solvent was removed in vacuo and the residue was treated with ethanol and reevaporated several times, the crude hydrazide was recrystallized from the proper solvent. The hydrazides (XX-XXV) were chromatographically homogeneous (detection with benzidine) and gave a positive silver nitrate reaction.

NMR spectra of the hydrazide derivatives (XX-XXV) showed peaks at δ: 8.8-9.1 (*m*) characteristic for hydrazide group, thereby confirming their structures.

General procedure for the synthesis of 5-chloro-or 3,5-dichlorosalicyl-tripeptide methyl esters (XXVI-XXXI)

To a solution of amino acid methyl esters hydrochloride (0.0082 mole) in THF (50 ml was added triethylamine (2 ml). The solution was stirred at 20°C for 30 minutes and cooled to 0°C. 5-chloro or 3,5-dichlorosalicylamino acid (0.008 mole) in THF (25 ml) and dicyclohexylcarbodiimide (DCC; 1.62 gm) were added to the above mixture. The reaction mixture was stirred for 2 hrs. at 0°C and for another 2 hrs at room temperature. Dicyclohexylurea was filtered off, acetic acid (1 ml) added, the solution was filtered and the filtrate was evaporated in vacuo. The residue was recrystallized from methanol-water. The products (XXVI-XXXI) were easily soluble in alcohols, DMF, dioxane and insoluble in water and ether. Compounds (XXVI-XXXI) were chromatograpically homogeneous when detected with iodine or benzidine and gave a negative ninhydrin reaction. The IR spectra of tripeptides (XXVI-XXXI) showed bands at 3260, 3180 cm^{-1} (NH, CONH), 1760, 1720 cm^{-1} (>C=O), 1430, 1320 cm^{-1} ($COOCH_3$) and other bands characteristic of tripeptide residues. The NMR spectrum of tripeptide (XXVI) showed signals at δ 3.5(3H, $COOCH_3$); 5.9 (1H, NH); 6.1-6.3 (s, 1H, OH), 7.4-7.6(5, 2H, Ar-H) and other protons in support of the assigned structures.

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