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SYNTHESIS OF DEHYDRO PEPTIDES AND EVALUATED THEIR ANTIMICROBIAL & ANTHELMENTIC ACTIVITY

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ABSTRACT

Peptides have emerged as sructurally novel anti-microbial and anthelminthics. Therefore various N-2-(Benzoylamino)-3-(substituted ohenyl-1-oxo 2-propenyl) dehydro serines and dehydro threonines. The stuctures of title compounds were synthesized established by IR, [1]HNMR. The newly developed compounds evaluated for their antibacterial and anthelminthic activity.

KEY WORDS: Peptides, Dehydro amino acids, Antimicrobials, Earthworms, Anthelmenthics.

INTRODUCTION

Peptide therapeutics have been touted as a promising new addition to the pharmaceutical armamentarium over the last 10-20 years, but the field has also been viewed as more of an art than a science. New delivery techniques and manufacturing technologies, however, coupled with intesified research and development programs, resulted in the approval of new peptide based drugs such as Fuzeon for HIV.[1] These peptides regulate most physiological processes, acting at some sites as endocrine or paracrine signals and at others as neurotransmitters or growth factors. They are already being used therapeutically in such diverse areas as neurology, endocrinology and heamatology.[2]

Therefore, the challenge of this decade is to produce small molecules which mimic peptides and proteins, n order to overcome the ineffectiveness of peptides as drugs when administered orally. The field is rapidly expanding and further contribution to medical problems definitely contine to increase.[3]

MATERIALS AND METHODS

Chemicals and reagents

Glycine, Benzoyl chloride, Aldehydes, Acetic anhydride, Sodium acetate, 1N Sodium hydroxide, Acetone, Serine, Threonine, 1N hydrochloric acid, Methylene Chloride, Mesyl chloride, Triethyl amine.

ExperimentalMethodology

Step-1: Synthesis of Hippuric acid[4]

Hippuric acid was synthesized by benzoylation of glycine.

Glycine was dissolved in sodium hydroxide solution. Benzoyl chloride was added in five portions to the solution and shaken vigorously after each adddition. Few grams of crushed ice and concentrated HCl was added slowly and with stirring until the mixture become acidic. The crystalline precipitate of benzoylglycine was filtered, washed with cold water. The solid was boiled gently with carbon tetra chloride. The mixture was allowed to cool, filtered. The product was recrystallised from boiling water.

Step-2: General method of synthesis of 2-phenyl-4-(substituted benzylidene) oxzole-5-ones[4]

A mixture of benzoyl glycine , various approriate aldehydes, acetic anhydride, and anhydrous sodium acetate were taken and heated on electric hot plate with constant shaking. As soon as the mixture has liquified completely, the flask was transferred to a water bath and heated for 2hr. the methanol was added slowly, and the mixture allowed to overnight in a refrigerator. The crystalline product separated out was filtered and washed with boiling water.

Step-3: Genersl method of synthesis of N-(2-benzoylamino)-3-(substituted phenyl)-1-oxo-2-propenyl serines\ threonines[5]

To a suspension of serine or threonine in acetone there were added with stirring of NaOH and aldehydrophenylalanine azalactone. After 2-3 hrs, the clear solution was acidified, by addition of HCl. The filterate was extracted with ethyl acetate.The evaporation of ethyl acetate gave dipeptide as colourless crude crstals. It was recrystallized from acetone and water system.

Step-4: Synthesis of N-(2-benzoylamuno) 3- (substituted benzylidene) 1- oxo-2-propenyl dehydroserines\ threonines[5]

Into a solution of dipeptide of serine \threonine and mesyl chloride in methylene chloride in the presence of triethylamine was stirred at 0-2^{0}C for 0.5hrs , the resulting solution was washed with 1M HCl. The evaporation of the CH_2 Cl_2 gave final product. Recrystallised from acetone and water.

ANTIBACTERIAL ACTIVITY

METHOD

cup plate agar diffusion method using Muller Hinton agar.[6]

MARERIALS

Nutrient agar, 18-24hr growth culture in nutrient agar, sterile petridishes, sterile micropipettes, sterile cotton swabs sterile cork borer (8mm) and sterile test tubes.

Preparation of nutrient agar

Peptone 0.6g, yeast extract 0.15g, dipotassium dihydrogen phosphate 0.13g were dissolved in 100ml distilled water and p^H was adjusted to 7.2. This solution was srerilized by autoclaving at 15 p.s.i for 20 minutes.

Preparation of subculture

One day prior to these testings, inoculation of the above bacterial cultures were made in the nutrient agar and incubated at 37^{0}C for 18-24hrs.

Preparation of medium (Muller-Hinton Agar)

The 3.8g of agar was dissolved into 100ml of distilled water and the p^H was adjusted to 7.4 ± 0.2 it was sterilized by autoclaving at 15 p.s.i for 20 minutes.

Preparation of test solutions

Each test compound (5g) was dissolved in dimethyl formamide (5ml) to give stock solution of concentration 1000µg/ml. Then 0.1ml 0f this solution was used for testing.

Preparation of standard solution

Standard drug norfloxacin was used. The concentration was 100µg/ml.

Method of testing[7]

Muller – Hinton agar plates were prepared by pouring 10-15ml of the medium into each sterilized petridish and were alllowed to set at room temperature. The cell suspension was standardized to the density of 530nm using spectrophotometer and was inoculated over the surface of agar medium using sterile cotton swab. The eight cups were scooped in each plate using a sterile cork borer of 8mm diameter.

Then the solution of test compounds (0.10ml) were added in cups by using micropipettes and these were incubated at 37^0C for 48hours. The zone of inhibition was measured in mm of each organism.

ANTIFUNGAL ACTIVITY

Method

cup-plate agar difusion method using Sabourand Dextrose agarp.[8]

Materials used

Subourand-Dxtrose agar, 18-24hr growth culture on sabourand dextrose agar, sterile petridishes, sterile micropipettes, sterile cotton swabs sterile cork borer (8mm) and sterile test tubes.

Preparation of Sabouraud-Dextrose Agar

Dextrose (40g), neopeptone (10g), Agar (15g) were dissolved in distilled water (1000ml) and p^H Was adjusted to 5.5-6. This solution was sretilized by autoclaving at 120^0C for 10 minutes.

Preparation of sub-cultures

One day prior to these testings, inoculation of the above fungal cultures were made in the Sabourand Dextrose agar and incubated at 37^0C for 18-24hrs. A suspension of cell from this culture was made in sterile distilled water. Five colonies more than 1mm diameter were mixed with 5ml of normal saline and vortexed for 15sec. The cell density was adjusted using spectrophotometer at 530nm with addition of normal saline.

Preparation of test solutions

Each test compound (5g) was dissolved in dimethyl formamide (5ml) to give stock solution of concentration 1000µg/ml. then 0.1ml 0f this solution was used for testing.

Preparation of standard solution

Standard drug norfloxacin was used. The concentration was 100µg/ml.

Method of testing[8]

Sabouraud-Dextroseagar plates were prepared by pouring 10-15ml of the medium into each sterilized petridish and were alllowed to set at room temperature. The cell suspension was standardized to the density of 530nm using spectrophotometer and was inoculated over the surface of agar medium using sterile cotton swab. The eight cups were scooped in each plate using a sterile cork borer of 8mm diameter, corresponding to control, standard and test solution.

Then the solution of test compounds (0.10ml) were added in cups by using micropipettes and these were incubated at 37^0C for 48hours. The zone of inhibition was measured in mm of each organism.

ANTHELMINTIC ACTIVITY[9,10]

Six indian adult indian earth worms (*Pheretima Postuma*) of nearly equal size 5-8cm in length and 0.2-0.3cm in width were placed in standard drug solution and test compound solutions at room temperature. Normal saline was used as a control. The standard drug and test compounds dissolved in minimum quantity of dimethyl formamide (DMF) and adjusted the volume upto 15ml with normal saline to get the concentraion of (0.1% w/v), (0.2% w/v) and (0.5% w/v).albendazole was used as standard drug.the compounds wre evaluated for the time taken for complete paralysis and death of earthworms. The mean leathal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time.

To ascertain the death of motionless worms, they were frequently applied with external stimuli, which stimulate and induce movements in the worms, if alive.

RESULTS AND DISCUSSION

Spectral data

N- (2- benzoylamino) 3-(4-chloro benzylidene) 1-oxo-2-propenyl dehydro serine. (A_2)

TLC: R_f 0.72 (Ethyl acetate: Ethanol) (3:1)

UV (methanol): λ_{max} 245.2 nm

IR (KBr): 3250 (N-H), 3060 (O-H), 1794 & 1651 (C=O), 1512 cm^{-1} (c=c)

PMR (DMSO-d_6): δ 2.7 (s, 2H, CH_2), 5.7-8.1 (m, 12H, -NH, Ar-H & Olefinic), 9.18 (s, 1H, -COOH)

N-(2-benzoylamino) 3-(4- methoxy benzylidene) 1-oxo-2-propenyl dehydro serine. (A_4)
UV (methanol): λ_{max} 305.4 nm IR (KBr): 3258 (N-H), 1647 & 1602 (C=0), 1511 Cm^{-1} (c==c)
PMR (DMSO- d_6): δ 2.7 (s, 2H, CH_2), 3.8 (s, Ar-OCH_3), 5.9-8.1 (m, 12H, -NH, Ar-H & Olefinic), 9.18 (s, 1H, - COOH)

N- (2- benzoylamino) 3- (4- isopropyl benzylidene) 1-oxo-2-propenyl dehydro serine. (A_6)
TLC: R_f 0.58 (Ethylacetate: Ethanol) (3:1) UV (methanol): λ_{max} 289.6 nm PMR (DMSO-d_6): 1.4 (m, 1H), 2.7 (s, 2H, CH_2), 3.7-3.9 (d, 6H, (CH_3)), 6.7-8.5 (m, 12H, -NH,Ar-H & Olefinic), 9.75 (1H,-COOH)

N- (2- benzoylamino) 3- (4-hydroxy,3,5-dimethoxy benzylidene) 1-oxo-2-propenyl dehydro serine. (A_8)

TLC: R_f 0.76 (Ethyl acetate: Ethanol (3:1))
UV (methanol): λ_{max} 288,8nm,

IR (KBr): 3376 (N-H), 2938 (O-H), 1731&1651 (C=0), 1513 cm^{-1}
PMR (DMSO-d_6): δ 2.6 (s, 2H, CH_2), 3.8 (Br, 6H, $(OCH_3)_2$, 6.5-8.2(m, 12H, -NH, Ar-H & Olefinic.

N-(2-benzoylamino) 3-(4-methoxy benzylidene) 1- oxo-2-propenyl dehydro threonine.(B_7)
TLC: R_f 0.42 (Ethyl acetate: Ethanol) (3:1)

IR (KBr): 3249 (N-H), 2934 (O-H), 1647 & 1601 (C=O), 1511 cm^{-1}
PMR (DMSO-d_6): δ 1.1-1.4 (q, 1H, CH), 1.8 (d, 3H, CH_3), 3.8 (S, Ar-OCH_3), 6.7-8.5 (m, 12H, -NH, Ar-H & Olefinic), 9.8 (s, 1H,-COOH).

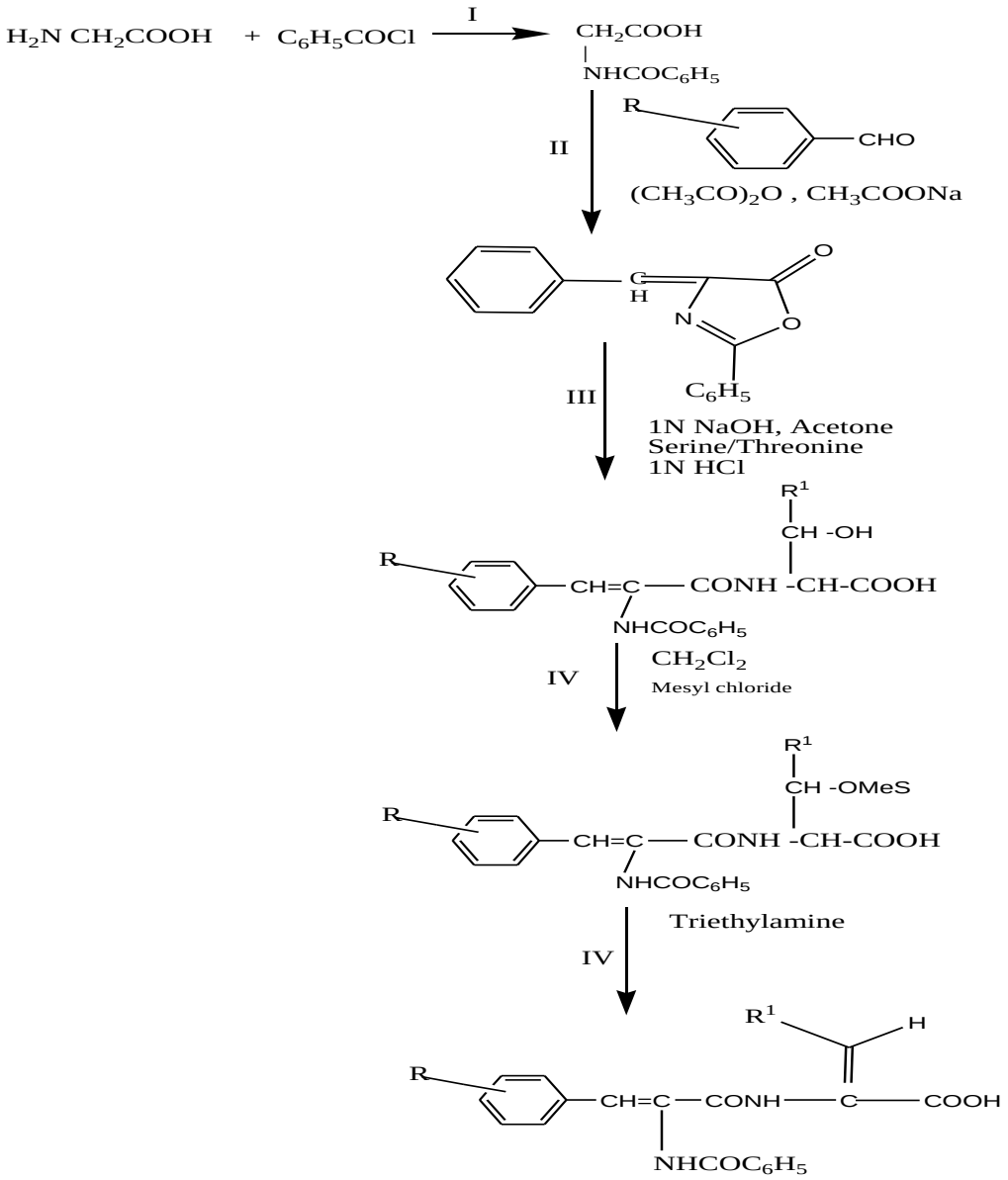

Table 1: Physical Data and Yields of title compounds.

Compound	R	R^1	m.p0C	Yield %	Formula
1	H	H	177-178	68	$C_{19}H_{16}O_4N_2$
2	4-Cl	H	158-161	72	$C_{19}H_{16}O_4N_2Cl$
3	4-OH	H	172-174	56	$C_{19}H_{16}O_5N_2$
4	4-OCH_3	H	135-137	60	$C_{20}H_{18}O_4N_2$
5	4-CH_3	H	162-163	65	$C_{20}H_{18}O_4N_2$
6	4-CH $(CH_3)_2$	H	151-152	80	$C_{22}H_{21}O_4N_2$
7	5-Br, 4-0H, 3-OCH_3	H	144-146	32	$C_{22}H_{19}O_7N_2Br$
8	4-OH, 3,5-$(OCH)_3$	H	178-180	75	$C_{21}H_{21}O_7N_2$
9	4-NO_2	H	124-127	71	$C_{19}H_{16}O_6N_2$
10	4-OH,3-OCH_3	H	137-139	68	$C_{22}H_{19}O_7N_2$
11	H	CH_3	1156-158	63	$C_{20}H_{18}O_4N_2$
12	4-Cl	CH_3	138-140	66	$C_{20}H_{18}O_4N_2Cl$
13	4-OH	CH_3	135-137	65	$C_{20}H_{18}O_5N_2$

14	4-OCH_3	CH_3	117-120	76	$C_{21}H_{20}O_5N_2$
15	4-CH_3	CH_3	130-132	70	$C_{21}H_{18}O_4N_2$
16	4-CH $(CH_3)_2$	CH_3	159-161	78	$C_{24}H_{24}O_4N_2$
17	5-Br, 4-0H, 3-OCH_3	CH_3	148-151	53	$C_{23}H_{20}O_7N_2Br$
18	4-OH, 3,5-$(OCH)_3$	CH_3	175-177	52	$C_{24}H_{23}O_8N_2$
19	4-NO_2	CH_3	125-127	65	$C_{20}H_{18}O_7N_3$
20	4-OH,3-OCH_3	CH_3	201-203	30	$C_{23}H_{20}O_7N_2$

Table 2: Antibacterial activity of title compound

SI.NO	Compounds	Zone of Inhibition (in mm)			
		B. subtillis	***S. aureus***	***E.coli***	***p. vulgaris***
1	A_1	15	14	14	13
2	A_2	20	21	20	19
3	A_3	18	22	19	18
4	A_4	19	20	20	17
5	A_5	14	15	12	16
6	A_6	22	21	17	19
7	A_7	22	22	18	20
8	A_8	22	20	19	18
9	A_9	19	18	17	19
10	A_{10}	16	15	11	10
11	B_1	17	14	13	15
12	B_2	19	18	20	19
13	B_3	16	18	19	17
14	B_4	18	19	19	18
15	B_5	20	23	19	19
16	B_6	22	21	20	18
17	B_7	21	20	18	17
18	B_8	20	21	18	18
19	B_9	21	20	20	19
20	B_{10}	17	16	12	11
Standard	Norfloxacin	23	25	21	20

Table 3: Antifungal activity of title compounds

SI.NO	Compounds	Zone of Inhibition (in mm)			
		C.albicans	***A.niger***	***C.verticulate***	***A. flavus***
1	A_1	19	22	20	17
2	A_2	18	20	20	16
3	A_3	20	23	20	18
4	A_4	20	22	19	16
5	A_5	22	23	21	19
6	A_6	23	24	20	18
7	A_7	24	25	22	19
8	A_8	16	15	13	10
9	A_9	13	14	12	11
10	A_{10}	19	15	15	14
11	B_1	14	17	15	17

12	B_2	19	18	20	16
13	B_3	14	16	12	13
14	B_4	18	20	19	17
15	B_5	24	23	21	18
16	B_6	22	22	20	17
17	B_7	23	24	20	19
8	B_8	12	15	11	9
19	B_9	18	17	19	15
20	B_{10}	17	15	16	13
Standard	Griseofulvin	25	26	24	20

Table 4: Anthelminthic activity of3-(substituted benzylidene) 1-oxo,2-propenyl dehydro serine peptides

S No	Compound	Concentration	Time in minutes Mean SD	
			For Paralysis	For death
	Control	0.7		
	Standard	0.1	55 ± 0.51	75 ± 0.37
		0.2	48 ± 0.28	69 ± 0.22
		0.5	37 ± 0.62	52 ± 0.16
1	A_1	0.1	50 ± 0.17	159 ± 0.35
		0.2	46 ± 0.12	148 ± 0.28
		0.5	40 ± 0.23	141 ± 0.21
2	A_2	0.1	58 ± 0.23	147 ± 0.52
		0.2	47 ± 0.18	149 ± 0.54
		0.5	38 ± 0.85	139 ± 0.24
3	A_3	0.1	77 ± 0.18	171 ± 0.29
		0.2	70 ± 0.28	191 ± 0.23
		0.5	63 ± 0.20	173 ± 0.13
4	A_4	0.1	63 ± 0.42	173 ± 0.31
		0.2	66 ± 0.19	170 ± 0.37
		0.5	58 ± 0.36	164 ± 0.37
5	A_5	0.1	66 ± 0.52	162 ± 0.35
		0.2	60 ± 0.43	160 ± 0.28
		0.5	53 ± 0.26	152 ± 0.36
6	A_6	0.1	50 ± 0.59	149 ± 0.33
		0.2	47 ± 0.43	142 ± 0.41
		0.5	36 ± 0.32	133 ± 0.41
7	A_7	0.1	45 ±0.52	138 ± 0.31
		0.2	40 ± 0.53	133± 0.43
		0.5	36 ±0.25	129 ± 0.37
8	A_8	0.1	68 ±0.59	164 ± 0.54
		0.2	60 ±0.30	159 ± 0.63
		0.5	52 ± 0.27	151 ± 0.37
9	A_9	0.1	55± 0.43	145 ± 0.39
		0.2	46 ± 0.38	138 ±0.33
		0.5	42 ± 0.51	127 ±0.63
10	A_{10}	0.1	73 ± 0.42	179 ±0.21
		0.2	68 ± 0.17	180 ± 0.18
		0.5	61 ± 0.20	159 ± 0.10

Table 5: Anthelminthic activity of 3-(substituted benzylidene)1-oxo,2-propenyl dehydro threonines peptides

1	B_1	0.1	53 ± 0.27	167 ± 0.24
		0.2	50 ± 0.33	165 ± 0.17
		0.5	39 ± 0.37	152 ± 0.21
2	B_2	0.1	73 ± 0.52	185 ± 0.28
		0.2	70 ± 0.19	177 ± 0.49
		0.5	59 ± 0.97	170 ± 0.38
3	B_3	0.1	55 ± 0.33	163 ± 0.23
		0.2	49 ± 0.32	153 ± 0.37
		0.5	41 ± 0.61	146 ± 0.22
4	B_4	0.1	59 ± 0.12	174 ± 0.39
		0.2	64 ± 0.27	157 ± 0.17
		0.5	52 ± 0.33	147 ± 0.35
5	B_5	0.1	53 ± 0.53	124 ± 0.22
		0.2	45 ± 0.49	143 ± 0.30
		0.5	36 ± 0.25	159 ± 0.37
6	B_6	0.1	49 ± 0.60	153 ± 0.31
		0.2	43 ± 0.25	138 ± 0.61
		0.5	33 ± 0.51	127 ± 0.20
7	B_7	0.1	51 ± 0.38	147 ± 0.44
		0.2	42 ± 0.51	138 ± 0.22
		0.5	38 ± 0.28	126 ± 0.27
8	B_8	0.1	68 ± 0.82	183 ± 0.37
		0.2	57 ± 0.23	172 ± 0.25
		0.5	59 ± 0.31	169 ± 0.33
9	B_9	0.1	58 ± 0.32	173 ± 0.38
		0.2	63 ± 0.62	158 ± 0.63
		0.5	53 ± 0.23	146 ± 0.47
10	B_{10}	0.1	63 ± 0.53	171 ± 0.63
		0.2	59 ±0.29	176 ± 0.18
		0.5	52 ± 0.61	146 ± 0.51

ANTIMICROBIAL ACTIVITY

The newly synthesized compounds by scheme, were subjected for their antibacterial & antifungal activity. Among all the compounds $A_{5\text{-}8,}$ $B_{5\text{-}8}$ showed significant antibacterial activity. Compounds $A_{5\text{-}7}$, $B_{5\text{-}7}$ have shown excellent antifungal activity.

ANTHELMINTIC ACTIVITY

The anthelmintic activity of all compounds by schemes was carried out using adult earthworms. In serine amino acid series the compounds A_2, $A_{5\text{-}7}$& in threonine sries $B_{5\text{-}7}$ have showed good paralytic time, on earthworms compared to standard drug at 0.1%, 0.2% and 0.5% concentration of the test compounds.

CONCLUSION

A new series of N-2-(Benzoylamino)-3-(substituted phenyl-1-oxo, 2-propenyl) dehydro serines and dehydro threonine derivtives were synthesized. The synthesized comounds were active as Antimicrobial and anthelmintic agents.

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