ISSN 0975-6299 **Vol.1/Issue-4/Oct-Dec.2010**

International Journal of Pharma and Bio Sciences

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF A N-PHTHILIMIDO AND ACETIMIDO DERIVATIVES FROM AMINO ACIDS AND ANHYDRIDES

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ABSTRACT

The phthaloyl and acetyl group are common protecting groups from are common protecting group for amines in organic synthesis[1] and are also important pharmacophores[2]. Common method are imide synthesis include dehydrative condensation of an anhydride and amino acid at high temperature, the acid-catalyzed cyclization of N-substituted amic acid. Abdol Reza Hajipour[3], rapidly synthesised Phthalimide derivatives by microwave irradiation. A simple extremely fast, and high yielding method for the reaction of phthalic anhydride with a number of amino acids using microwave irradiation under solventless dry condition has been developed. The aim of the study was to design, synthesize and investigate the antimicrobial and antifungal activities, anticancer activities of some α N-Phthilimido and acetylated derivatives of amino acids. The chemical structures of the titled compound were confirmed by IR, ^{13}CNMR and elemental analysis. All the compounds were screened for antimicrobial activity against gram positive, gram negative bacteria (*Escherichia coli, Klebsiella, Staphylococcus epidermitis, Bacillus cereus, Micrococcus leteus, Staphylococcus aureus)* and fungal strains *(Candida albicans, Aspergillus niger)*

KEY WORDS

α N-Phthilimido and acetylated amino acids, Antibacterial and Antifungal Activity.

INTRODUCTION

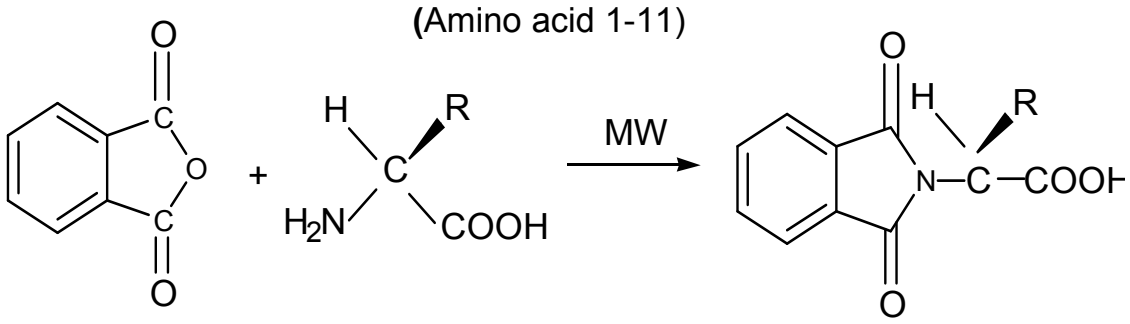
Non-essential amino acids play important key role in tumour growth by supplying its amide nitrogen atoms in the biosyntheses of other amino acid, purine, pyrimidine bases, aminosugar and coenzymes via a family comprised of 16 amido transferases with diversified mechanisms. It also plays the central role in multiple metabolic pathway and considered to be the most essential component of tissue culture media for not only the nitrogen sourse but also as carbon source. In view of the importance of amino acids metabolic processes and translation of information, they have been an important target in the design of antimetabolites. However the number of amino acid analogs, that have been shown to have significant chemotherapeutic activity is rather small. This may be due, in part, to the high serum concentration that is needed, which is difficult to maintain over long periods, and the possibility of inter conversions among amino acids, which help to overcome metabolic blocks easily of their resemblance with natural

amino acids (Glutamine, glycine, alanine, aspartic acid, glutamic acid, arginine, serine, cysteine, thyrosine, methionine and histidine), these amino acid inhibitors are thought to interfere with amino acid metabolism.

MATERIAL AND METHOD

Melting point of all synthesized compound were determined by using open capillary method and are uncorrected. The precoated alumina plates with silica gel GF254 (E.Merck) were used for purity determination and pet: ethyl acetate (1:2) was employed as irrigate. IR spectra were recorded in (cm^{-1})ABB BOMEM FT-IR Spectrometer using KBr pellet technique. ^{1}H-NMR and ^{13}C-NMR were recorded (in ppm) on BRUKER AV 400 using TMS as internal standard.

Synthesis of α N-Phthilimido amino acids(Compound 1-11): Equimolar quantities of phthalic anhydride, amino acids(Glutamine, Glycine, Alanine, aspartic acid, glutamic acid, Arginine, serine, cysteine, thyrosine, methionine and histidine) and distilled water. The above was mixed well and heated on a heating mantle and refluxed for two & half-hours. After reflux, the hot mixture was transferred and kept in a beaker at room temperature, over night. The crystalline product, which was found to be insoluble in benzene and chloroform but soluble in hot water, was collected by filtered & dried to remove moisture. The unreacted raw materials were removed by repeatedly shaking with 50ml portions of benzene A.R. and then with 50ml portions of chloroform A. R. filtered, dried and recrystallised from hot water. The pure product was collected by filtration and dried to remove moisture.



Synthesis of α N-acetyl amino acids(12-22): Equimolar quantities acetic anhydride, amino acid(Glycine, aspartic acid, glutamine, glutamic acid, Arginine, serine, cysteine, alanine, thyrosine, methionine and histidine) and distilled water. The above was mixed well and heated on a heating mantle and refluxed for two & half-hours. After reflux, the hot mixture was transferred and kept in a beaker at room temperature, over night. The crystalline product, which was found to be insoluble in benzene and chloroform but soluble in hot water, was collected by filtered & dried to remove moisture. The unreacted raw materials were removed by repeatedly shaking with 50ml portions of benzene A.R. and then with 50ml portions of chloroform A. R. filtered, dried and recrystallised from hot water. The pure product was collected by filtration and dried to remove moisture.

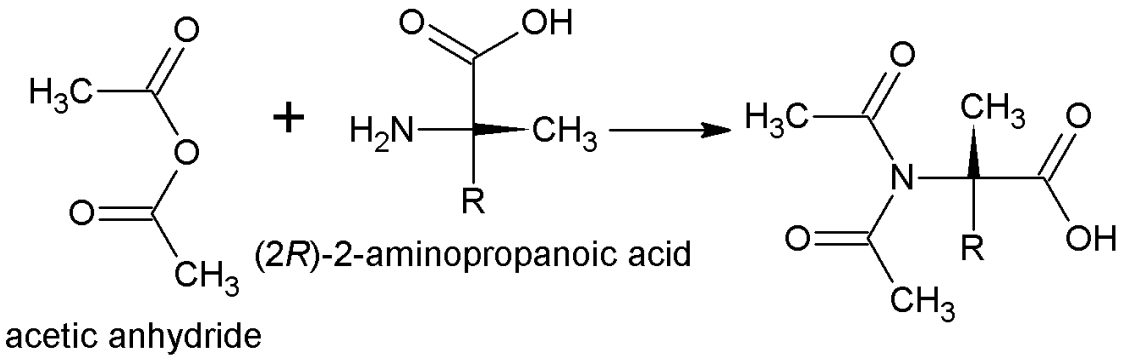


Table 1
Physicochemical data of synthesized compound

S.No.	Compound	% yield	Mol. Wt	Molecular Formula	Elementary Analysis				
					Carbon	Hydrogen	Nitrogen	Oxygen	Sulphur
1	Compound 1	90%	279.31	$C_{13}H_{13}NO_4S$	55.90	4.69	5.01	22.91	11.48
2	Compound 2	92%	285.25	$C_{14}H_{11}N_3O_4$	58.95	3.89	14.73	22.44	
3	Compound 3	85%	311.28	$C_{17}H_{13}NO_5$	65.59	4.21	4.50	25.70	
4	Compound 4	88%	249.21	$C_{12}H_{11}NO_5$	57.83	4.45	5.62	32.10	
5	Compound 5	91%	205.16	$C_{10}H_7NO_4$	58.54	3.44	6.83	31.19	
6	Compound 6	80%	263.20	$C_{12}H_9NO_6$	54.76	3.45	5.32	36.47	
7	Compound 7	85%	277.22	$C_{13}H_{11}NO_6$	56.32	4.00	5.05	34.63	
8	Compound 8	85%	304.30	$C_{14}H_{16}N_4O_4$	55.62	5.30	18.41	21.03	
9	Compound 9	90%	276.24	$C_{13}H_{12}N_2O_5$	56.52	4.38	10.14	28.96	
10	Compound 10	92%	251.25	$C_{11}H_9NO_4S$	52.58	3.61	5.57	25.47	12.76
11	Compound 11	85%	219.19	$C_{11}H_9NO_4$	60.27	4.14	6.39	29.20	
12	Compound 12	88%	233.28	$C_9H_{13}NO_4S$	46.34	6.48	6.00	27.43	30.75
13	Compound13	91%	239.22	$C_{10}H_{13}N_3O_4$	50.21	5.48	7.50	26.75	
14	Compound 14	80%	265.26	$C_{13}H_{15}NO_5$	58.86	5.70	5.28	30.16	
15	Compound 15	85%	305.19	$C_8H_{13}NO_5$	47.29	6.45	6.89	39.75	
16	Compound 16	85%	159.13	$C_6H_9NO_4$	45.28	5.70	8.80	40.21	
17	Compound 17	90%	217.17	$C_8H_{13}NO_6$	44.24	5.11	6.45	44.20	
18	Compound18	92%	231.20	$C_9H_{13}NO_6$	46.75	5.67	6.06	41.52	
19	Compound 19	85%	258.27	$C_{10}H_{18}N_4O_4$	46.50	7.02	21.69	24.78	
20	Compound 20	88%	232.19	$C_8H_{12}NO_6$	41.38	5.21	12.60	41.34	
21	Compound 21	91%	205.23	$C_7H_{11}NO_4S$	40.27	5.40	6.82	31.18	15.62
22	Compound 22	80%	173.16	$C_7H_{11}NO_4$	48.55	6.40	8.09	56.96	

Antimicrobial Activity: All synthesized compound were screened for antimicrobial activities by disc diffusion method using Mueller-Hinton agar medium to study the preliminary antibacterial activity against *Escherichia coli, Klebsiella, Staphylococcus epidermitis, Bacillus cereus, Micrococcus leteus and Staphylococcus aureus*. The agar medium was purchased in HI media laboratories Ltd., Mumbai, India. Nutrient broth, subculture, base layer medium, agar medium and peptone water were prepared as per the standard procedure. Each test compound was dissolved in 5ml of dimethyl sulfoxide (1000ug/ml) Volume of 0.05ml and 0.1ml of each compound were used for testing.

The cup plate method using PDA medium was employed to study the preliminary antifungal activity of *Candida albicans, Aspergillus niger*. The PDA medium was purchased from HI media laboratories Ltd., Mumbai, India. Nutrient broth, subculture, base

layer medium, agar medium and peptone water were prepared as per the standard procedure. Each test compound was dissolved in 5ml of dimethyl sulfoxide (1000ug/ml) volume of and 1mg/ml of each compound were used for testing. Ciprofloxacin and ketocanazole were used as standard drug (50 & 100ug/ml) and dimethyl sulfoxide as a control. The observed zone of inhibition was measured in mm and results are present in table.

Table 2

Antibacterial Activity of Amino acid analogues:

	Compound	Conc.	E.coli	Kieb.	S.Epid	B.cereu	M.leteu	S.aureu	Candida albicans	Aspergillus Nigar
1	**Ciproflaxacin (Standard)**	1mg/ml	-	20	22	20	*24*	22	8.25	7.25
2	**Compound 1**	1 mg/ml	15	13	15	13	12	12	-	5.25
3	**Compound 2**	1 mg/ml	13	13	13	15	13	12	-	5.21
4	**Compound 3**	1 mg/ml	12	12	12	13	12	15	-	5.40
5	**Compound 4**	1mg/ml	-	20	22	20	*24*	22	8.25	7.25
6	**Compound 5**	1mg/ml	15	13	15	13	12	12	18	16
7	**Compound 6**	1 mg/ml	13	13	13	15	13	12	22	21
8	**Compound 7**	1 mg/ml	12	12	12	13	12	15	18	20
9	**Compound 8**	1mg/ml	-	20	22	20	*24*	22	8.25	7.25
10	**Compound 9**	1 mg/ml	18	16	14	15	16	15	17	17
12	**Compound 10**	1 mg/ml	17	15	19	20	21	18	18	19
14	**Compound 11**	1 mg/ml	12	12	12	13	12	15	10	15
15	**Compound 12**	1 mg/ml	-	12	12	13	*14*	15	10	12
16	**Compound 13**	1 mg/ml	15	13	15	16	14	13	10	5.25
17	**Compound 14**	1mg/ml	13	13	13	15	13	12	-	5.21
18	**Compound 15**	1 mg/ml	12	10	15	17	17	15	-	5.40
19	**Compound 16**	1 mg/ml	-	20	22	20	*24*	22	15	18
20	**Compound 17**	1 mg/ml	15	13	15	22	12	21	24	22
21	**Compound 18**	1mg/ml	13	18	17	15	15	22	18	19
22	**Compound 19**	1 mg/ml	12	15	12	13	12	15	20	19
23	**Compound 20**	1 mg/ml	15	17	15	22	12	21	24	22
24	**Compound 21**	1mg/ml	13	18	17	15	20	22	18	19
25	**Compound 22**	1 mg/ml	12	15	21	13	18	16	20	19
23	**DMSO**	1 mg/ml	-	-	-	-	-	-	-	-

RESULT AND DISCUSSION

In present study a series of amino acid analogs are synthesized. Phthalic anhydride, amino acids and distilled water are used to produce α N-Phthilimido of amino acids(scheme 1,2,3). The structure of the compound was characterized by IR, ^{13}C NMR. All the synthesized compound are active against all micro organisms when compared to standards. From the result it is evident that the compounds Phthalimido & acetimido (Glutamine, Glycine, Alanine, aspartic acid, glutamic acid, Arginine, serine, cysteine, thyrosine, methionine and histidine) exhibit significant antibacterial activity at concentration of 1mg/ml with reference to ciprofloxacin at a 1mg/ml concentration. The compound show significant effect with Aspergillus nigar and on effective against Candid albicans with reference to Ketaconazole at a 1mg/ml concentration.

CONCLUSION

A very ambitious attempt was made to synthesis a simple but useful antimetabolite molecule, by mostly utilizing the facilities

available in our laboratories and also to do the chemical and biological characterization. The urge was to avoid a very common and conventional method of preparation and to try out a different method with the aim of preparing some analogues or antimetabolites.

In this project analogues of amino acids molecules were prepared as antimetabolites to inhibit the cancer cell growth. Enormous effort was made to overcome many limitations and handicaps faced and finally the preparations of 3 antimetabolites were achieved successfully.

The structures of synthesised compounds were confirmed by Infra red and Nuclear magnetic resonance Spectra. These spectral values show the expected peak in the spectra.

All the synthesised compounds has to undergo antibacterial activity against Escherichia coli, Klebsiella, Staphylococcus epidermitis, Bacillus cereus, Micrococcus leteus, Staphylococcus aureus at1mg/ml and antifungal activity against Aspergillus niger at 1mg/ml.

It is suggested to in future to evaluate their anticancer activities.

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