Synthesis and Antibacterial Evaluation of Some N-Amino Acid -3- Phthalimidine Amino Acid Esters

M.S. Al-Ajely
College of Education

H.A.Basheer
College of Teachers
Mousl University

S.M. Sulaman
College of Dentistry

Abstract:

Some new amino acid derivatives were prepared from the reaction of amino acids esters (glycine, tyrosine and cystine) with 2-thioxo phthaloyl of the following amino acids (leucine, valine, glycine and alanine), The reaction products were tested against six types of bacteria. The results indicated that N-valyl-3- phthalimidine ethyl tyrosinate and N-anyl-3- phthalimidine ethyl cystinate showed remarkable activity against *Pseudomonas araginosa*. The synthesized compounds were characterized by using elemental analysis. IR spectral methods and their melting points.

تحضير بعض N- حامض أميني -3- فثاليميدين استر حامض أميني ودراسة فعالياتها البايولوجية

محمد سلمان العجيلي
كلية التربية

هدى أحمد بشير
كلية المعلمين
جامعة الموصل

صبا مؤيد سليمان
كلية طب الاسنان

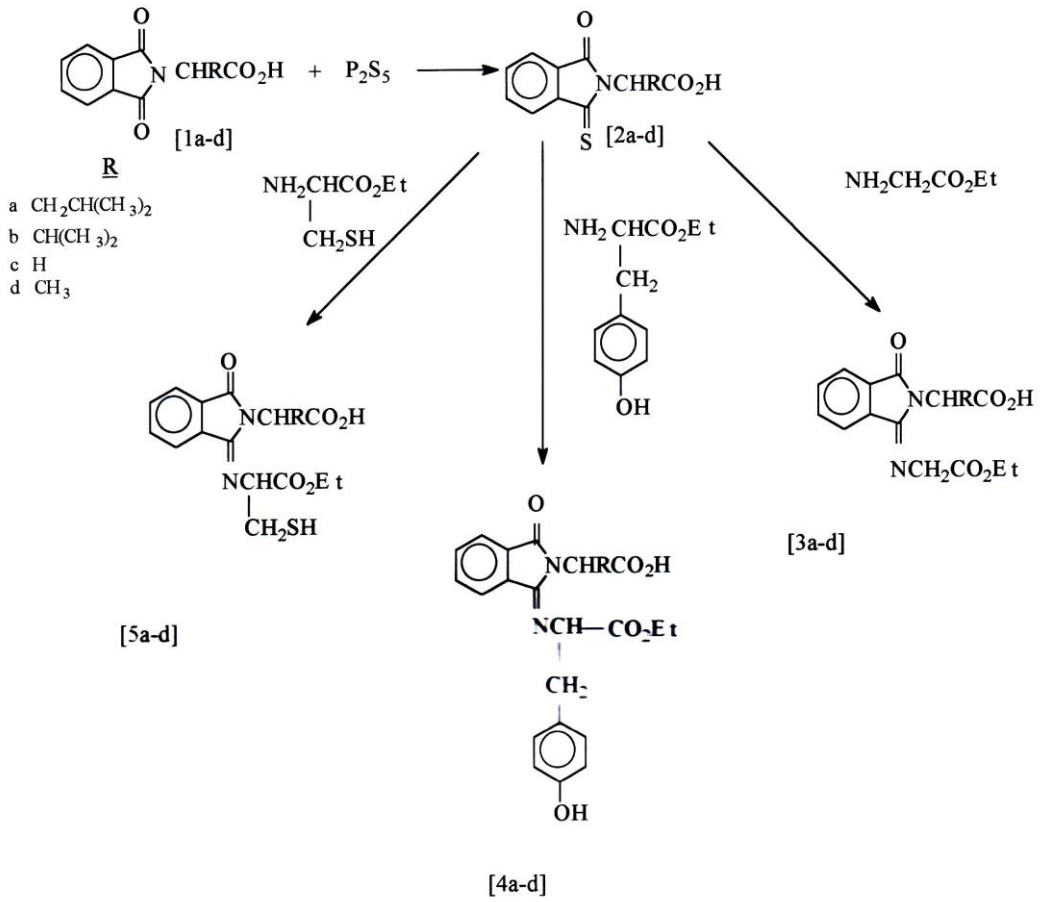
ملخص البحث :

تم تحضير مشتقات جديدة للاحماض الأمينية من تفاعل استرات الأحماض الأمينية (كلايسين – تايروسين – سايستين) مع 2– ثايواوكوفثالويل للأحماض الأمينية (ليوسين وفالين وكلايسين والانين) .

وتم اختبار نواتج التفاعل ضد ستة أنواع من البكتريا وقد أظهر المركبات N– فاليل – 3– فثاليميدين اثيل تايروسينينو و N– الأثيل –3– فثاليميدين اثيل سايستينين فعالية ضد بكتريا بسيدوموناس اراجينوسا ، وقد شخصت المركبات الناتجة بالتحليل الدقيق للعناصر وطيف الأشعة تحت الحمراء ودرجات الانصهار .

Introduction:

Phthaloyl group was used as amino acid protecting agent[1-7] (phthaloyl amino acid synthesis). Amino acids especially peptides were widely investigated by many researchers and were found to have different biological effects[7-13]. Different studies showed that phthalimides have some biological activities, Determination of antimicrobial effectiveness against specific pathogens is essential to proper therapy[14-15], Also it was found to be effective against insecticide by modifing the phoshatidylinositol on the prion protein[16]. According to the above finding. We made a combination of amino acid and the phthaloyl nucleus in one organic compound namely N-amino acid-3- phthalimidine amino acid esters compounds (3a-d),(4a-d) and (5a-d) respectively (Scheme 1). It was found that the above synthesized compounds have remarkable activities toward different types of gram + ve and gram-ve bacteria.



Scheme (1)

Experimental:

Uncorrected melting points were determined using electrothermal 9300 melting point apparatus. IR. Spectra were recorded by pye-unicam sp 1100 spectrometer as KBr disc. Phthaloylamino acids and phthalic anhydride and following the same literature reported procedure[17].

Preparation of 2. thioxo phthaloylamino acids [2a-d]:

Phthaloyl amino acid (0.01 mole) and phosphrous penta sulphide (0.01 mole) were heated under reflux in dry dioxin (100 ml) for tow hours. The mixture was filtered hot, allowed to cool, and evaporated to dryness giving thick oil.

Preparation of N- amino acid phthalimidine amino acid esters [3a-d], [4a-d] and [5a-d]:
General procedure:

2. thioxo phthaloylamino acid (0.001 mole) was stirred in dry dioxin (50 ml) with ethyl glycinate, ethyl tyrosinate and ethyl cysteinate (0.001 mole each) for three hours at room temperature, (expulsion of H_2S).

The solvent was evaporated to leave thick oil which was recrystallized from ethanol/water to afford a brown solid.

The biological methods:
1. Bacteria :

the bacteria species used are listed in Table (3), All strins were human type and were obtained from Biology department, college of Science, Mosul university. They grown up to the stationary phase in a nutrient bath at 37^o C and a sample of 0.5 ml of each bacteria was spread over a surface of a nutrient agar plate[18].

2. Antibacterial assay:
Disc method:

Discs of filter paper (6 mm diameter) were sterilized at 140 ^{o} C for 1 hr. And impregnated with (1 ml) of stock solution (10 mg/ml, 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml) of each compound and then dried. Ethanol was used as solvent for compounds (4a, 4b, 4d, 5b and 5d). The same solvent (absolute ethanol) was used for antibiotics, Table (3). Blank paper dices of absolute ethanol was used as control, the inculated plates were incubated at 37^{o}C for 24 hrs., and the inhibition zones (mm) were measured[19]. In all experiments, the mean of each triplicate was then measured.

Table (1) : IR spectral and physical data for compounds (2a-d)

Comp. No.	m.p.	%yield	IR ν cm^{-1}			
			-C=S	N.C=O imide	-C=O acid	-OH
2a	Oil	65	1110	1780	1725	3300
2b	=	62	1080	1770	1730	3280
2c	=	68	1140	1775	1725	3310
2d	=	65	1090	1785	1720	3300

Table (2) : IR spectral data, melting point and C.H.N. analysis for compounds (3a-d), (4a-d) and (5a-d)

Comp. No.	Melting point c° %yield	IR cm^{-1}					C.H.N. analysis Cal./found		
		C=N	C=O acid	C=O Ester	C=O lmide	OH	C	H	N
3a	218-220 70	1600	1705	1725	1775	3300	65.042 65.240	6.672 6.660	8.430 8.530
3b	232-234 75	1610	1725	1745	1800	3400	64.135 64.425	6.332 6.330	8.801 8.706
3c	240-242 66	1600	1705	1750	1775	3250	60.862 60.971	5.108 5.006	10.142 10.138
3d	220dec. 65	1610	1705	1735	1780	3350	62.059 62.259	5.555 5.535	9.652 9.750
4a	Oil 65	1590	1705	1740	1800	3400	68.474 68.674	6.436 6.426	6.390 6.390
4b	182-184 70	1620	1710	1725	1800	3400	67.907 68.002	6.649 6.638	6.601 6.610
4c	180-182 72	1600	1715	1730	1775	3350	52.284 52.480	4.179 4.169	5.808 5.800
4d	174-176 70	1605	1710	1725	1780	3400	66.653 66.741	5.594 5.583	7.068 7.006
5a	274-276 65	1600	1700	1725	1800	3400	60.289 60.480	6.347 6.256	7.403 7.404
5b	262-264 65	1600	1700	1725	1775	3400	59.363 60.041	6.084 6.000	7.687 7.666
5c	236-238 72	1610	1725	1750	1800	3200	55.883 56.033	5.002 5.000	8.691 8.610
5d	230dec. 74	1610	1705	1730	1800	3300	57.120 57.032	5.393 5.382	8.329 8.419

Note: the upper values of the elemental analysis are the calculated one

Results and discussion

Compounds (2a-d) were characterized by sodium fussion test which gave + ve results for nitrogen and sulfur. They gave + ve iodide iodate test. The IR. Spectral data (Table 1) also support the above finding through the main stretching vibration absorption bands of – C=S at (1080-1110 cm), -N-C=O of imides carbonyl at (1770-1785cm) , -C=O of acid moieties at (1720-1725cm) and the hydroxyl groups absorbed at (3280-3310cm) as indicated in Table (1) Compound (2a) showed the

following absorption bands; 1110, 1780, 1725 and 3300 cm^{-1} related to the –C=S stretching, -C=O stretching of amide, -C=O stretching of acid and –OH stretching vibration. Compounds (3a-d), (4a-d) and (5a-d) were synthesized from the reaction of the corresponding 2-thioxo phthaloylamino acids (2a-d) with glycine, tyrosine and cytsteine ethyl esters respectively (Scheme 1). Their structure illucidation comes from elemental C.H.N. analysis Table (2) and sodium fussion test (-ve sulfur contents) and the IR Spectral results Table (2). The IR data as indicated in the above table gave evidence for the presence of the following groups; C=N of phthalimidine absorbed at (1590-1610 cm-1) including the aromatic ring C=C stretching vibration, C=O of acid and ester absorbed at (1700-1710 cm) and (1725-1750 cm) , -C=O of imide at (1775-1800cm^{-1}) and finally the hydroxyl group of the acid moiety absorbed at (3250-3400 cm^{-1}). Compound (3a) showed the following main absorption vibration bands; 1600, 1705, 1725, 1775 and 3300 cm^{-1} for the –C=N and C=C aromatic, -C=O of acid, ester, amide and finally the hydroxyl groups while compound 4a gave the following absorption for same above groups, while compound 4a gave the following absorption for same above groups; 1590, 1705, 1740, 1800 and 3400 $cm^{-1}$and compound 5a also gave the following characteristic stretching vibrational absorption bands for the above mentioned groups at 1600, 1700, 1725, 1800 and 3400 cm^{-1}.

The antibacterial studies revealed that the most of the compounds are effective against different microorganisms. These results showed that compounds (4b, 4d and 5d) were active in inhibiting the growth of nearly all the organisms used. The diameter of inhibition zone of the tested chemicals showed that compounds (4b and 5d) have the highest activity against *staph aureus*, *bacillus subtilis and pseudomonas aeruginosa* which is known as a resistant bacteria toward the commercial antibiotics,

while compounds (5a and 5b) had less effect. As a conclusion to the above study. Compounds (4b and 5d) gave promissing results and need further studies in VIVO.

Table (3) : the antibacterial activity of compounds (4a, 4b,4d,5b and 5d) diameter of inhibition zone (mm)

compound		4d	5d	4d	5d	4a	Erythromycin	Chloramphaniclol	Control (Ethanol)
Staph. aureus	10	4.5	3.5	2.5	-	-	2.5	1.5	-
	1	3.6	2.2	1.8	-	-	1.8	1	-
	0.1	2.2	1	1	-	-	1	0.8	-
	0.01	1.3	0.8	0.8	-	-	-	-	-
Bacillus subtilis	10	4.1	2.5	1.5	-	-	2.2	1.3	-
	1	2.8	1.6	1	-	-	1.3	0.8	-
	0.1	1.2	0.9	0.8	-	-	-	-	-
	0.01	0.8	-	-	-	-	-	-	-
Pesudomonas Aeruginosa	10	1.6	1	-	-	-	-	-	-
	1	0.8	-	-	-	-	-	-	-
	0.1	-	-	-	-	-	-	-	-
	0.01	-	-	-	-	-	-	-	-
Proteus mirabilis	10	3.5	3.4	2.2	2.5	1.2	0.8	1.2	-
	1	2	2.7	1.6	1.6	0.8	-	0.8	-
	0.1	1.2	1.3	0.8	1	-	-	-	-
	0.01	-	0.8	-	-	-	-	-	-
E.coli	10	3.6	1.6	2.4	1	2.3	1.8	2.8	-
	1	2	0.9	1.7	0.8	1.5	1.2	2	-
	0.1	1.3	-	1	-	0.8	0.8	1	-
	0.01	0.8	-	-	-	-	-	-	-
K.pneumoniae	10	1.4	2	2.5	-	2.5	-	1	-
	1	0.8	1.1	2	-	1.4	-	1	-
	0.1	-	0.8	1.5	-	0.8	-	-	-
	0.01	-	-	0.8	-	-	-	-	-

References :

1. J.C. Sheehan and V.S. Frank, J. Am. Chem. Soc., 71,1856 (1952).
2. F.E. King and D.A. Kidd, J. Chem. Soc., 71, 3315 (1949).
3. O.H. Emerson, U.S.P. 2, 498, 665 (1950), Chem. Abstr., 44, 4926 (1950).
4. J.C. Sheehan, D.W. Chapman and R.W. Roth, J.Am. Chem. Soc., 74, 3822 (1952).
5. S. Shankman and Y. Schvo, J.Am. Chem. Soc., 80, 1164 (1958).
6. B. Acott, R.b. bates and C.D. Green, Tetrahedron Lett. 3465 (1969).
7. S. Shankman, S. Higa and V. Gold, J. Am. Chem. Soc., 82, 990 (1960).
8. S.Shankman, S. Higa, H.A. Florsheim, Y. Schvo and V. Gold, Arch. Bio. Chem. Biophys. 86, 204 (1960) Chem. Abstr., 54, 17541 (1960).
9. D.E. Nitecki and B. Halpern, Aust. J. Chem., 22, 871 (1968).
10. M. Cushman, J. Jurayi and J.D. Moyer, J. Org. Chem., 55, 3186 (1990).
11. L. Guy, J. Vidal and A. Collet, J. Med. Chem., 41 (24), 2843 (1998).
12. J. Alsina, K.J. Jensen, F. Allbericio and G. Barany, Chem. European. J. 5 (10) 2787 (1999).
13. A.A. Khayrollah, K.Y. Saour and J.A. Faraj 1st Conference of pharmaceutical Science Univ. of Mousl, College of Pharmacy 12-13 June, Mosul , Iraq, (2000).
14. L.M. Perescott, J.P. Harley and D.A. Klein, "Microbiology", 3rded., W.M.C. Brown Communication, Inc. Australia, Oxford, P. 313-391 (1996).
15. L.M. Prescott, J.P. Harley and D.A. Klein, "Microbiology", 2rded. W.M.C. Brown Communication, Inc. Australia, Oxford, P. 412-414 (1996).
16. C. Harcourt brace Co. Ltd. Medical hypotheses 50, 91 (1998).

17. H.D. Jakubkw and H. Jeschkit “Amino acid peptides and proteins” Vol. 2 (1977) The Macmillan press LTD.
18. H.W. Seely and P.J. Vandemerk, Microbes in action, 3rd ed, Freeman and company, San Francisco, 187 (1981).
19. L.P. Garrod, H.P. Lamberat, D. Grady and P. Waterworth, “Antibiotic and chemotherapy”, 5th ed., Churchill Livingstons (1981).