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Synthesis of some N-phthalimide amino acids derivatives and Evaluation their Biological Activity

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Abstract: In this research six of N-phthalimides of amino acids (**IIIa-f)** have been synthesized, as a first step, through cyclocondensation of o-phthalic acid with amino acids (glycine, L-alanine, L-valine, L-leucine, L-phenylalanine, L-aspartic) in the presence of glacial acetic acid in bath oil (170-180 ^{o}C), the results of this step were compared with the results of traditional method.

In the second step, (**IIIa-b)** were refluxed with o-phenylene diamine (I**Va**) in the present of (HCl,4N) for 2hrs to give N-(1H benzimidazol-2-yl methyl) phthalimide (**Va**) N-[1-(1H benzimidazol-2-yl) ethyl] phthalimide (**Vb**).

All products were characterized by FT-IR, MS and ^{1}H-NMR spectroscopies and elementary analysis.

The synthesized compounds were screened for their antimicrobial activity against four microorganisms: Streptococcus Epidermidis, Escherichia Coli, Mycobacterium Tuberculosis and Candida Albicans.

Keywords: o-phthalic acid, N-phthalimide amino acids, benzimidazole, antimicrobial, anti-mycobacterial.

1. Introduction:

N-phthalimide derivatives are an important class of substrates for biological and chemical applications. They are used for synthesis of lots of biological compounds such as hypoglycemic agents [1,2], antihypertensive agents [3], anticonvulsant [4], anti-inflammatory [5], anti-nociceptive [5], peripheral analgesics[6], anti mycobacterium tuberculosis [7], and antimicrobial activity [8,9]. moreover they also employed as pre vulcanization inhibitor used in sulphurcured rubber polymer systems [10].

Typically, N-phthalimide derivatives are synthesized using the condensation of amine with phthalic anhydride in many methods that used refluxing in organic solvents. High boiling point solvents such as acetic acid [7], water [9], and toluene [11] are commonly used. These solvent were replaced by ionic liquids such as [bmim] [BF4] with acceptable yields to carry out the reaction in the room temperature [12]. There has also been work on using microwave irradiation as a heating method both in the presence [13] or absence [6] of organic solvents. Microwave-mediated and/or solventless syntheses give generally high yields of phthalimides within just a few minutes.

In this paper, we propose an alternative method, to produce N-phthalimide amino acids derivatives from o-phthalic acid and amino acids as the reagents. There are just a few reports of the use of o-phthalic acid as starting material [14], the preparation of anhydrides generally requires long reaction times in an organic solvent under reflux conditions [15], and furthermore, anhydrides are known to undergo hydrolysis in hot water [16]. Therefore, we decided to carry out the reaction with o-phthalic acid as reactant instead.

2. Experimental:

All chemicals were purchased from Sigma Aldrich Chemical Co. and Merck Chemical Co. (Germany). Melting points of the compounds were determined by using an electrothermal digital melting point apparatus and were uncorrected. The infrared (IR) spectra of the compounds were recorded in the region of 4000-400 cm^{-1} using KBr on a FT-IR Perkin-Elmer spectrophotometer. Vibrational transition frequencies were reported in wave number (cm^{-1}).

^{1}H-NMR spectra were recorded on Bruker Specrospin ultra shield magnets 400 MHz instrument using Tetramethyl Silane (TMS) as an internal standard and MeOD as a solvent, Elemental analysis was performed on a Heraeus CHN-O rapid analyzer, The ESI+VE MS spectra were recorded on a Bruker Daltonics LC-MS spectrometer.

2.1. Methods of Preparation:

2.1.1: Synthesis of N-phthalimide amino acids:

These compounds were prepared according to a typical procedure that was shown in scheme 1.

2.1.1.1 : Method A: Synthesis of N-phthalimide amino acids from phthalic acid (IIIa-f)

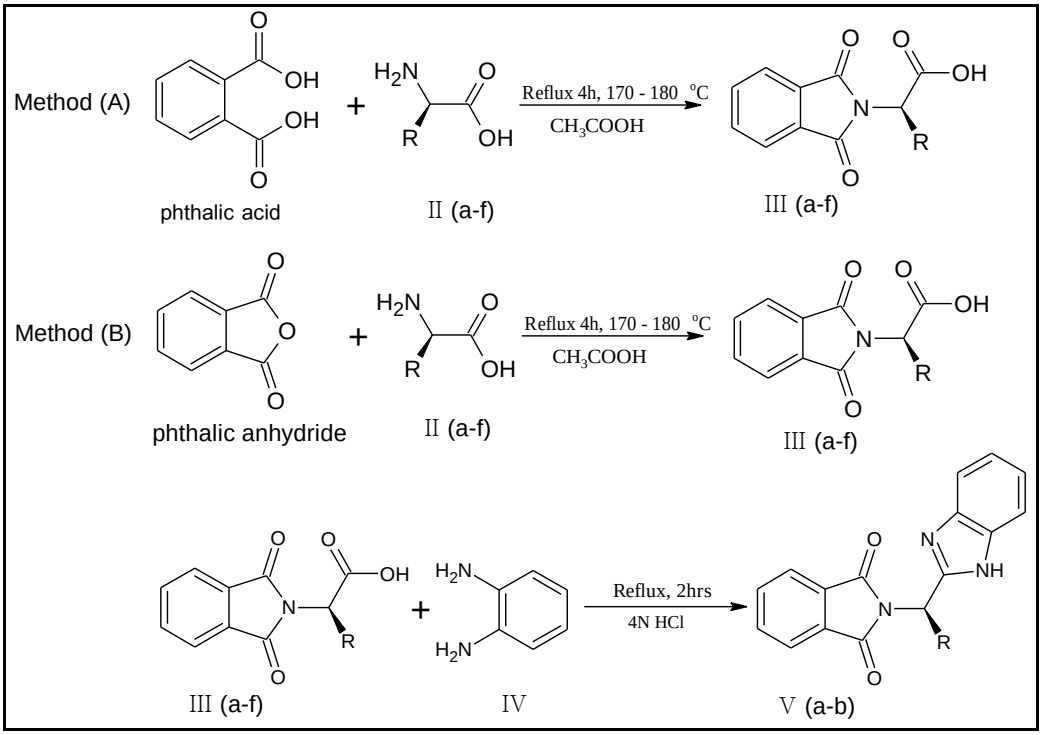
A mixture of 1 g (6 mmole) of phthalic acid and 6.2 mmole of amino acid (0.465g glycine, 0.552g alanine, 1.024g phenylalanine, 0.726g valine, 0.813g leucine, 0.825g aspartic) was heated for 4 h in 8 ml of glacial AcOH (at 170 – 180°C) Then the mixture is cooled to ~20°C and evaporated at a reduced pressure (water-jet pump). The residue is diluted with water and allowed to stand for 12 h at ~20°C. The precipitate is separated by filtration, washed with water, dried in air to obtain compounds (IIIa-f), and recrystallized from ethanol.

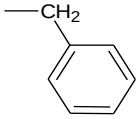
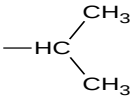
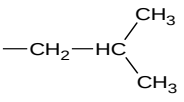
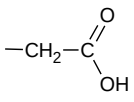
2.1.1.2 : Method B: Synthesis of N-phthalimide amino acids from phthalic anhydride (IIIa-f):

A mixture of 1 g (6.75 mmole) of phthalic anhydride and 6.8 mmole of amino acid (0.510g glycine, 0.605g alanine, 1.123g phenylalanine, 0.796g valine, 0.892g leucine, 0.905g aspartic) were refluxed in (15 ml) glacial AcOH for 2 hours The reaction mixture was filtered off while hot and the solvent was evaporated The solid separated was filtered to obtain compounds (IIIa-f), and recrystallized from ethanol.

2.1.2: Synthesis of N-substituted benzimidazolyl-phthalimide (Va-b):

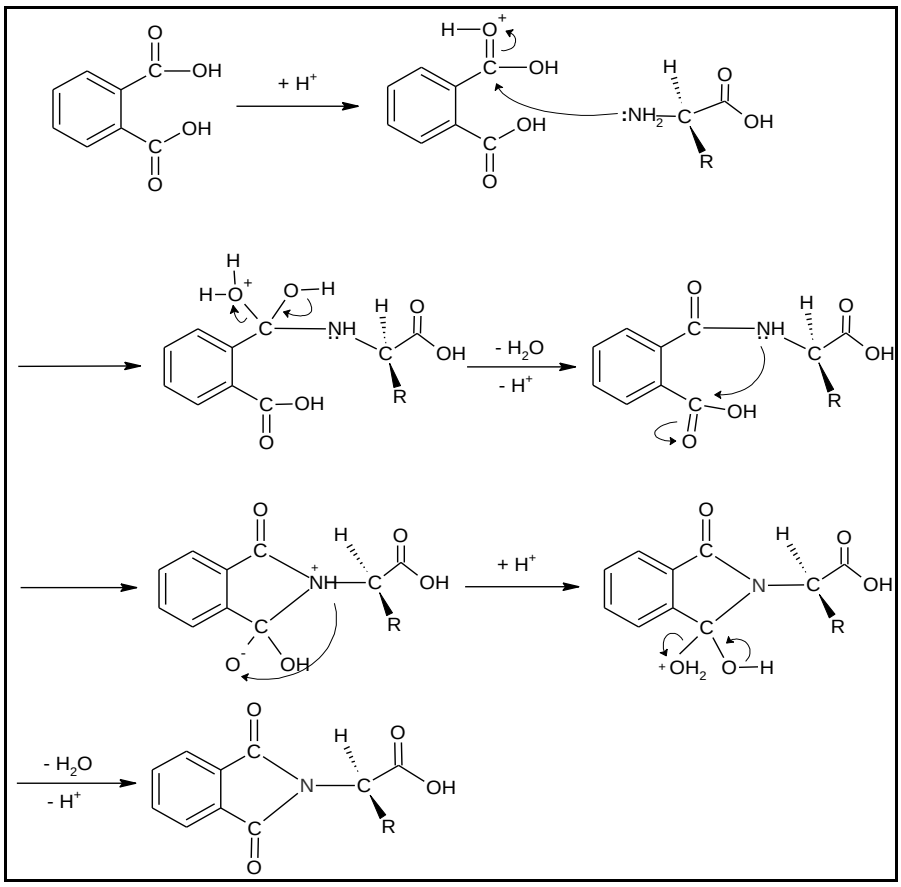
Scheme .1



	a	b	c	d	e	f
R	-H	$-CH_3$				

A mixture of 3.3 mmol of compounds (IIIa-b) of (one by one) and 3.3 mmol of o-phenylene diamine were refluxed in 10 mL. of 4N HCl for 2 hours. The solution on cooling gave a precipitate which was filtered, dried and recrystallized from ethanol. The purity of compounds has been checked by TLC using silica gel and mixture of benzene : ethanol (6:4) was employed as mobile phase.

Scheme .2 The Possible Reaction Mechanism



3. Results and Discussion:

3.1. Chemistry:

Thus, several amino acids (IIa-f) undergo condensation with phthalic acid when exposed to heating and refluxing in present of acetic acid as a condensation agent (Scheme .1). From comparison the results of methods A and B which summarized in (Table 1.) the generality of the reaction is evident, as a variety of amino acid II(a-f) reacts to form N-phthalimide amino acids derivatives (IIIa-f) in satisfactory yields (44.3 -87.1 %).

The elemental analysis values of the resulting polymers are in good agreement with the calculated values for the proposed structures (Table 2.).

The FT-IR spectra of N-phthalimide amino acids showed strong peak between 2500 – 3500 cm^{-1} which was assigned to the (O-H) of acid group in these compounds. Absorption bands between 1700 – 1800 cm^{-1} which were characteristic peaks for two asymmetric and symmetric stretching of carbonyl groups in imide ring and carbonyl groups in acid groups. Absorption bands around 1610 cm^{-1} due to $(C=C)_{arom.}$.

On the other hand, two of N-substituted benzimidazolyl-phthalimides (Va-b) were formed via refluxing two N-phthalimide amino acids with o-phenylene diamine in present of hydrochloric acid (4 N) in good yields.

The FT-IR spectra of N-substituted benzimidazolyl-phthalimide (Va-b) showed peak around 3200 cm^{-1} which was assigned to the (N-H) of benzimidazol ring, Absorption bands between 1766–1749 cm^{-1} which were characteristic peaks for two asymmetric and symmetric stretching of carbonyl groups in imide ring.

The ^{1}H-NMR spectra of (Va-b) multi signals at (7.83-7.88 ppm) due to aromatic protons, ^{1}H-NMR for N-(1H benzimidazol-2-yl methyl) Phthalimide (Va) showed single signal at (4.2 ppm) due to protons of (CH_2) group and single signal at (12.31 ppm) due to proton of (NH) group, and ^{1}H-NMR for (Vb) showed double signal at (1.67 ppm) due to protons of (CH_3) group, quad signal at (4.96 ppm) due to proton of (CH) group and (12.56 ppm) due to proton of (NH) group.

Table 1. The Physical data of synthesized derivatives

Comp. No.	R	Rf*	m.p.	m.p. lit.	$[\alpha]_D^{25}$**	Yields %	
						Method A	Method B
IIIa	H-	0.6	195-197	191-193[a]	-	85.3	88
IIIb	CH_3-	0.65	146-148	144-146[a]	-23.3	80.6	95.8
IIIc	$PhCH_2$-	0.39	180-182	180 -181[a]	-209.9	87.1	89.1
IIId	$(CH_3)_2CH$-	0.43	116- 118	114-115[b]	-68.9	44.3	71.3
IIIe	$(CH_3)_2CHCH_2$-	0.45	115-117	115-118[a]	-27.6	50.2	69.1
IIIf	$HOOCCH_2$-	0.61	221-223	223-225[b]	-57.1	56.4	66.8
Va	H-	0.33	211	-	-	57.2	
Vb	CH_3-	0.25	204	-	-19.3	46.1	

* Mobile phase: Benzen: Ethanole (1:1). a = [17], b = [18].
** Measured at a concentration of 0.5g/dL in EtOH at 25^{o}C.

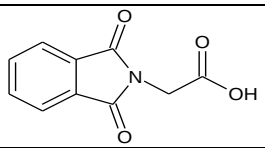
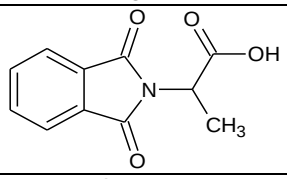
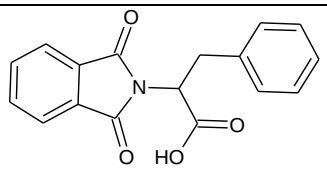
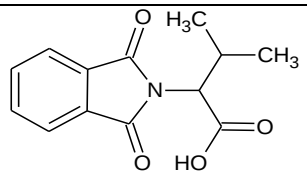
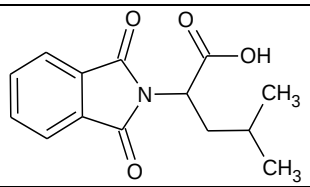
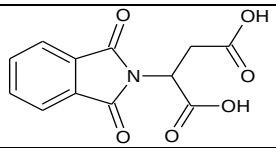
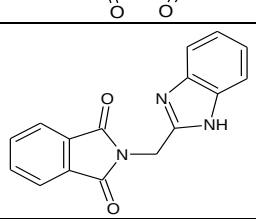
Table 2. Elemental analysis (C.H.N) of synthesized compounds and MS Spectral data.

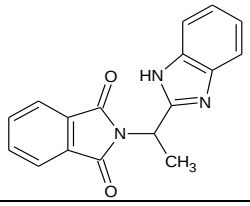
Comp. No.	Formula M_w	MS (*m/z*) $(M\text{-}H^+)^-$	elemental analysis (C.H.N)			
			%	H%	C%	N%
IIIa	$C_{10}H_7NO_4$ 205.166	204	Calc.	3.44	58.54	6.83
			Found	3.344	58.58	6.71
IIIb	$C_{11}H_9NO_4$ 219.193	218	Calc.	4.14	60.27	6.36
			Found	3.942	59.95	6.25
IIIc	$C_{17}H_{13}NO_4$ 295.289	294	Calc.	4.44	69.15	4.74
			Found	4.210	69.33	4.69
IIId	$C_{13}H_{13}NO_4$ 247.246	246	Calc.	5.30	63.15	5.67
			Found	5.277	62.90	5.45
IIIe	$C_{14}H_{15}NO_4$ 261.273	260	Calc.	5.79	64.36	5.36
			Found	5.610	64.09	5.34
IIIf	$C_{12}H_9NO_6$ 263.202	262	Calc.	3.45	54.76	5.32
			Found	3.304	54.77	5.21
Va	$C_{16}H_{11}N_3O_2$ 277.277	276	Calc.	4.00	69.31	11.54
			Found	3.99	69.46	11.25
Vb	$C_{17}H_{13}N_3O_2$ 291.304	290	Calc.	4.50	70.09	14.42
			Found	4.36	69.99	14.28

Table 3. The FT-IR spectral data of synthesized derivatives

Comp. No.	$(O-H, \text{str.})$	$(C-H, \text{str.})$	$(C=O)$	$(C=C)_{\text{arom}}$	Others
IIIa	2500-3500	3062	1774–1750	1602	1464 (CH_2, ben.)
IIIb	2500-3500	2926	1771–1702	1610	1466 (CH_3, ben.)
IIIc	2500-3500	3027	1747–1798	1610	1451 (CH_2, ben.)
IIId	2500-3500	2998	1758–1692	1609	1451 (CH_2, ben.)
IIIe	2500-3500	2964	1712	1609	1466 (CH_2 and CH_3, ben.)
IIIf	2500-3500	3039	1725	1611	1466 (CH_2, ben.)
Va	-	3055	1766	1603	3201 (N-H, Str.)
Vb	-	3026	1749	1612	3198 (N-H, Str.)

Table 4. The ^{1}H-NMR spectral data of synthesized derivatives (Va-b)

Comp. No.	Comp. Structure	^{1}H-NMR chemical shifts *ppm*
IIIa		4.25 (s, 2H, CH_2), 6.96-7.01 (br, 1H, OH), 7.96-8.21 (m, 4H, $H_{arom.}$)
IIIb		1.48-1.66 (d, 3H, CH_3), 4.24-4.51 (q, 1H,CH), 6.96-7.01 (br, 1H, OH), 8.02-8.53 (m, 4H, $H_{arom.}$)
IIIc		2.83-3.01 (dd, 2H, CH_2), 4.43-4.72 (t, 1H, CH), 6.72-6.93(s, br, 1H, OH), 7.02-8.21 (m, 9H, $H_{arom.}$)
IIId		1.20-1.25 (d, 6H, $2CH_3$), 2.60-2.80 (m, 1H, C<u>H</u>-CH_3), 4.90-4.97 (m,1H, CH), 6.40-6.45 (s, br, 1H, OH), 8.00-8.20 (m, 4H, $H_{arom.}$)
IIIe		0.90-95 (d, 6H, $2CH_3$), 2.40-2.60 (m, 1H, C<u>H</u>-CH_3), 4.20-4.30 (t, 2H, CH), 6.50-6.60 (s, br, 1H, OH), 8.10-8.20 (m, 4H, $H_{arom.}$)
IIIf		2.93-3.21 (m, 2H, CH_2), 4.69-4.81 (t, 1H,CH), 8.01-8.23 (m, 4H, $H_{arom.}$) 8.29-8.48 (s, br, 2H)
Va		4.22 (s, 2H, CH_2), 7.84-7.90 (m, 8H, $H_{arom.}$), 12.31 (s, H, NH)

Vb		1.67(d, 3H, CH3), 4.96 (q, 1H, CH), 7.83-7.88 (m, 8H, $H_{arom.}$), 12.56 (s, H, NH)

3.2. Biological activity:

3.2.1. Antimicrobial activity in vitro determination:

To determine the antibacterial activity of the synthesized compounds the cup plate method [19] was used with Gentamicin, Ampicillin and Nystatin as the reference antibiotics. The prepared compounds were examined at a concentration of (100, 250, 500, 1000 ppm) in (DMF), against two strains of bacteria, one is gram positive bacteria (S. epidermises), and another is gram negative (E.coli), for one kind of fungi (Candida albicans). The zone of inhibition was compared after 24 h of incubation at 37° against suitable antibiotics.

The results indicated that all compounds exhibited good inhibitory activity against tested pathogenic microorganism (Figures 1, 2 and 3).

3.2.2. Anti-mycobacterial activity in vitro determination:

To determine the anti-mycobacterial activity of the synthesized compounds the tube method [20] was used with Isoniazid (INH) as the reference antibiotics. The synthesized compounds were evaluated at a concentration of (100, 1000, 2000 ppm) in (DMF), against M. tuberculosis (isolated from positive sputum patient), the suspended bacterial dilutions were (1/10 and 1/1000) and the lowest drug concentration complete inhibition of bacterial growth (MIC) was determined after 72 days of incubation at 37°.

The results indicated that (Va-b) exhibited complete inhibition of bacterial growth at 2000 ppm against M. tuberculosis (Table .5).

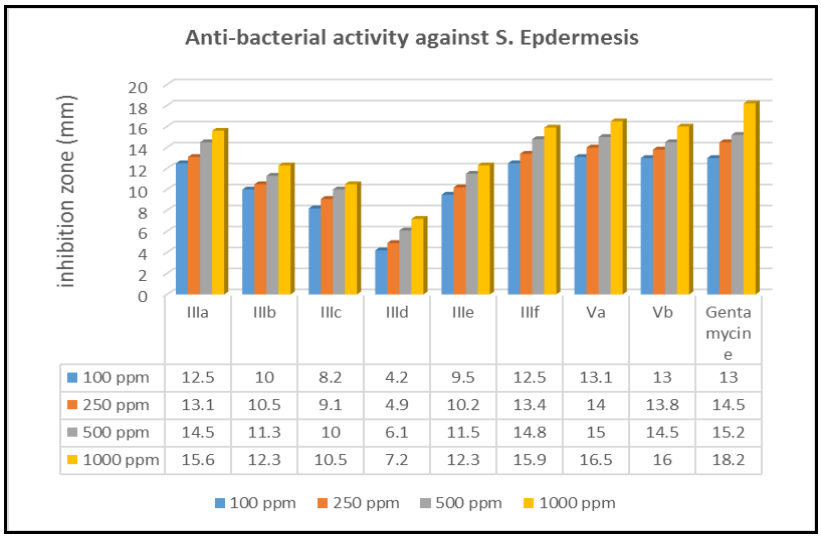


Figure 2. Inhibitory activity against S. Epidermises

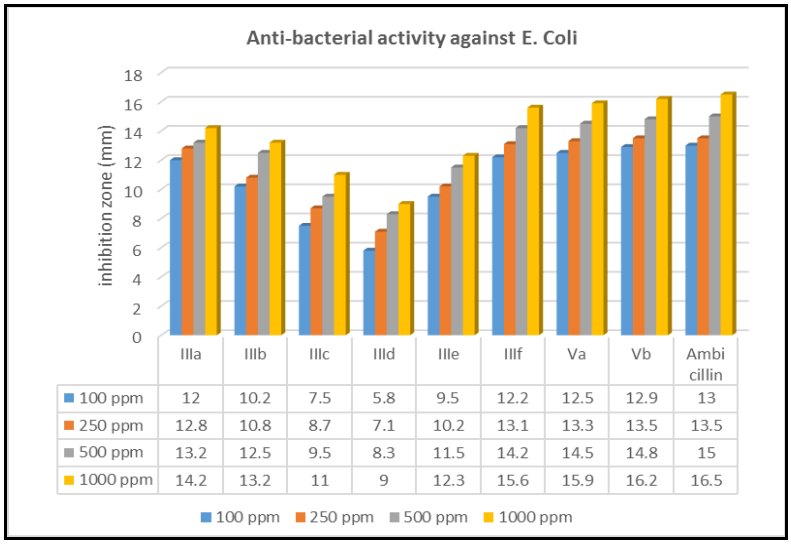


Figure 2. Inhibitory activity against E. Coli

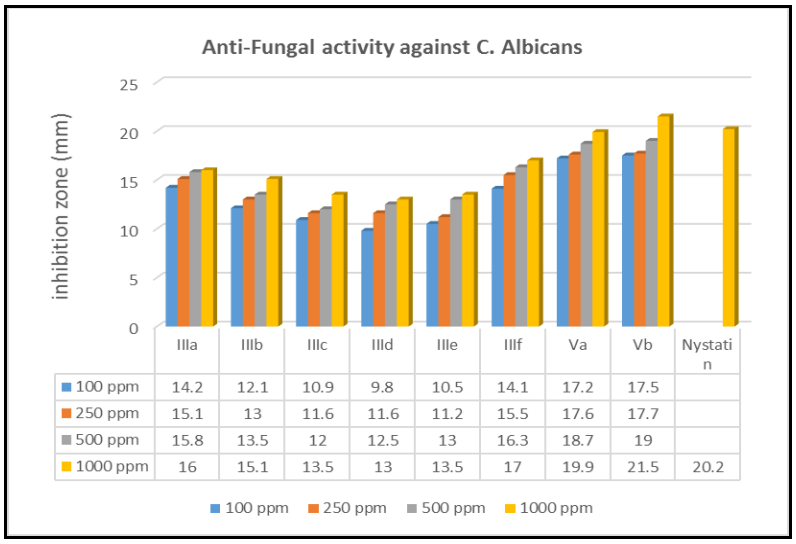


Figure 2. Inhibitory activity against C. Albicans

Table 5. Anti-Mycobacterial activity of the synthesized compounds

Bacterial suspended dilutions	**1/10**			**1/1000**		
Concentrations	**20 *ppm***	**200 *ppm***	**2000 *ppm***	**20 *ppm***	**200 *ppm***	**2000 *ppm***
IIIa	R	R	R	R	R	R
IIIb	R	R	R	R	R	R
IIIc	R	R	R	R	R	R
IIId	R	R	R	R	R	R
IIIe	R	R	R	R	R	R
IIIf	R	R	R	R	R	R
Va	R	R	S	R	R	S
Vb	R	R	S	R	R	S
Positive standard (INH)	R	S	S	R	S	S
Negative standard (DMF)	R	R	R	R	R	R

R: Resistant, S: Sensitive.

4. Conclusion:

In this study a series of N-phthalimide amino acids derivatives are synthesized. Via refluxing of phthalic acid and amino acids in the presence of glacial acetic acid at oil bath this method gave satisfactory yields comparing the traditional method phthalic acid, on the other hand two derivatives of benzimidazole moiety were synthesized.

The biological activity showed that all compound – except IIId - have high activity against S. Epdermesis, E. Coli and C. Albicans at all studied concentrations, and the anti-mycobacterial activity showed that the compounds Va-Vb have an activity against studied Mycobacteria at (2000 ppm) concentration.

5 . Acknowledgments:

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References:

1. James M. Chapman, Jr., et al., Hypolipidemic Activity of Phthalimidee 1,3-Dione Derivatives. 1. N-Substituted Phthalimidee 1,3-Dione Derivatives, *Journal of Medicinal Chemistry*, Vol. 22, No. 11 1399, 1979, North Carolina, USA.
2. James M. Chapman, et al., Hypolipidemic Activity of Pht halimide Derivatives. 2. N-Phenylpht halimide and Derivatives, *Journal of Medicinal Chemistry*, Vol. 26, No. 2, 1983, North Carolina, USA.
3. R Raghubir, et al., January 2004, Thiadiazolyl Quinazolones as Potential Antihypertensive Agents, *Indian Journal of Chemistry*, Vol.43B, pp. 180 – 183.
4. Priya Ahuja, et al., 2014, Essential Aminoacid Incorporated GABA–Phthalimide Derivatives: Synthesis and Anticonvulsant Evaluation, *Medicinal Chemistry Research*, New York, USA.
5. Lucky O. Okunrobo, et al., April 2006, Reaction of Phthalimido Alkyl Acids with Isopropylamine: Synthesis, Anti-Inflammatory and Antinociceptive Properties, *African Journal of Biotechnology*, Vol. 5 (8), pp. 643-647.
6. Roberto Antunes, et al., 2003, Synthesis, Ch.aracterization and Interaction Mechanism of New Oxadiazolo-Phthalimides as Peripheral Analgesics. IV, *Journal of molecular structure*, Vol.660, pp. 1-13.
7. Jean L. Santos, et al, 2009, Synthesis And In Vitro Anti Mycobacterium Tuberculosis Activity of a Series of Phthalimide Derivatives, Bioorganic & Medicinal Chemistry 17 (2009) 3795–3799, Araraquara, SP, Brazil.
8. Vijay K. Salvi, et al., 2006, Synthesis and antimicrobial activity of some 2-[1-(4-oxo-3,4-dihydrophthalazine-1-yl)alkyl]-1H-isoindole-1,3(2H)-dione and their imidoxy derivatives, *General Papers ARKIVOC*, (xiv) 133-140.
9. R. Srinivasan, et al, 2010, Synthesis and Antimicrobial Activity of some New α N-Phthilimido Amino Acids Analogues, International Journal of ChemTech Research, Vol.2, No.2, pp 895-898, April-June 2010, USA.
10. J. Geier, H. Lessmann, P.J. Frosch and A. Schnuch, Contact Dermattis, 2003, 48,1.
11. Dong Chu Chen, et al, 2007, Ionic liquid [bmim][BF4] acts as solvent and promoter for synthesis of halo-containing N-arylphthalimides, Chinese Chemical Letters 18 (2007) 27–29, Changsha, China.
12. Dong Chu Chen, et al, 2007, Ionic liquid [bmim][BF4] acts as solvent and promoter for synthesis of halo-containing N-arylphthalimides, Chinese Chemical Letters 18 (2007) 27–29, Changsha, China.
13. Abdol Reza Hajipour, et al., 2001, Microwave-assisted rapid synthesis of phthalimide devatives, Indian journal of chemistry, Vol. 40B, March 2001, pp. 250-251, Isfahan, Iran.
14. Joan Fraga, et al, 2007, Rapid and clean synthesis of phthalimide derivatives in high-temperature, high-pressure H2O/EtOH mixtures, Green Chem., 2007, 9, 1067–1072, Nottingham, UK.
15. D. V. Jacob, R. J. Kunnath and M. Lopez,Res. J. Chem. Environ.,2006,10, 45.
16. W. C. Bell, K. S. Booksh and M. L. Myrick,Anal. Chem., 1998,70,332.
17. Abdol Raza Hajipour et al., 2001, Microwave-assisted rapid synthesis of phthalimide derivatives, Indian Journal of Chemistry, Vol. 40B, March, 2001, pp. 250-251.
18. Bhushan M Khadilkar et al., 2002, Protection of amino group as N-phthlyl derivative using microwave irradiation, , Indian Journal of Chemistry, Vol. 41B, May, 2002, pp.1083-1085.

19. Anyonyya Mallam et al., 2012, antimicrobial activty of sarcostemma acidum voigt. (apocynaceae) stem, International Journal of Biological & Pharmaceutical Research. 2012; 3(6): 752-757.
20. Hamoud et al., 2009, Guidelines of Internal and External Quality Control of Tuberculosis Laboratory, world health organization and ministry of health, Damascus, Syria.

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