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## Synthesis of some *N*-phthalimide 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 °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 (**IVa**) in the present of (HCl, 4N) for 2 h to give *N*-(1*H*-benzimidazol-2-yl methyl) phthalimide (**Va**), *N*-[1-(1*H*-benzimidazol-2-yl ) ethyl] phthalimide (**Vb**). All products were characterized by FT-IR, MS and <sup>1</sup>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.

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### 1. Introduction

*N*-phthalimide derivatives are an important class of substrates for biological and chemical applications. These 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 these are 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] [BF<sub>4</sub>] 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 solvent fewer syntheses give generally high yields of phthalimides within just a few minutes.

In this research work, 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 work

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  $\text{cm}^{-1}$  using KBr on a FT-IR Perkin-Elmer spectrophotometer. Vibrational transition frequencies were reported in wave number ( $\text{cm}^{-1}$ ).

$^1\text{H-NMR}$  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<sup>+</sup>VE MS spectra were recorded on a Bruker Daltonics LC-MS spectrometer.

### 2.1. Methods of preparation

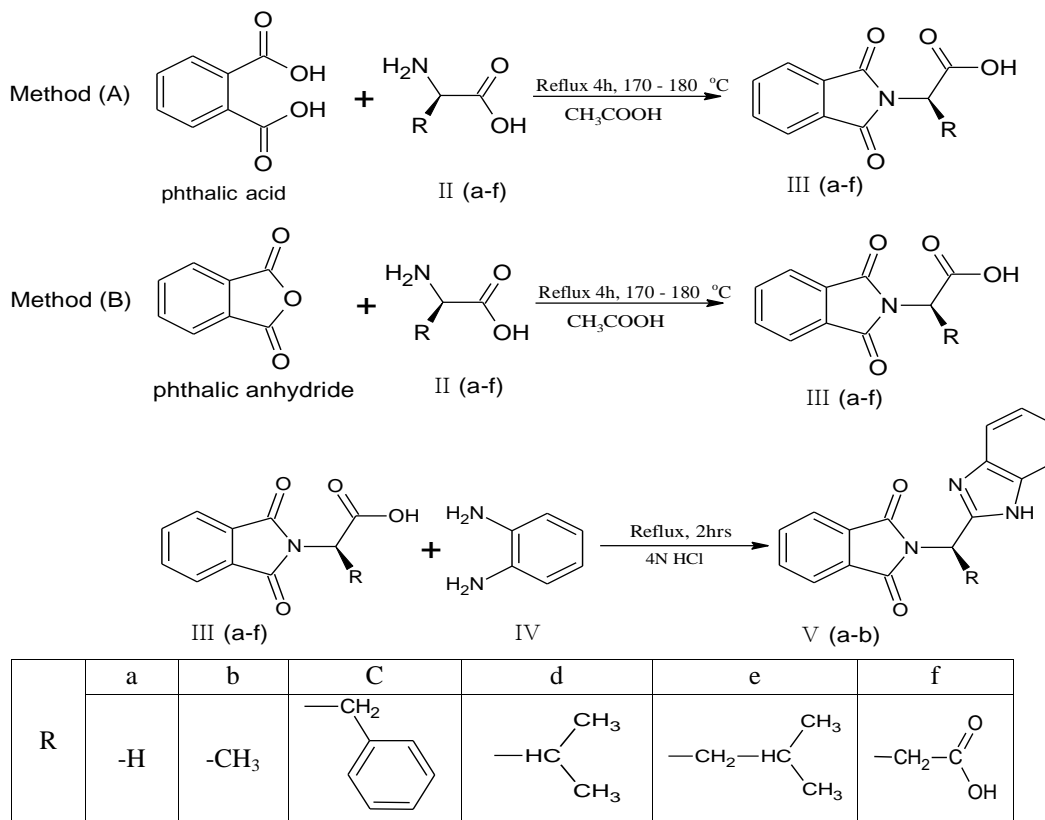
#### 2.1.1: Synthesis of *N*-phthalimide amino acids

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 (0.465 g glycine, 0.552 g alanine, 1.024 g phenylalanine, 0.726 g valine, 0.813 g leucine, 0.825 g aspartic) was heated for 4 h in 8 ml of glacial AcOH (at 170 – 180 °C). Then the mixture was cooled to ~20°C and evaporated at a reduced pressure (water-jet pump). The residue was 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.

Scheme 1

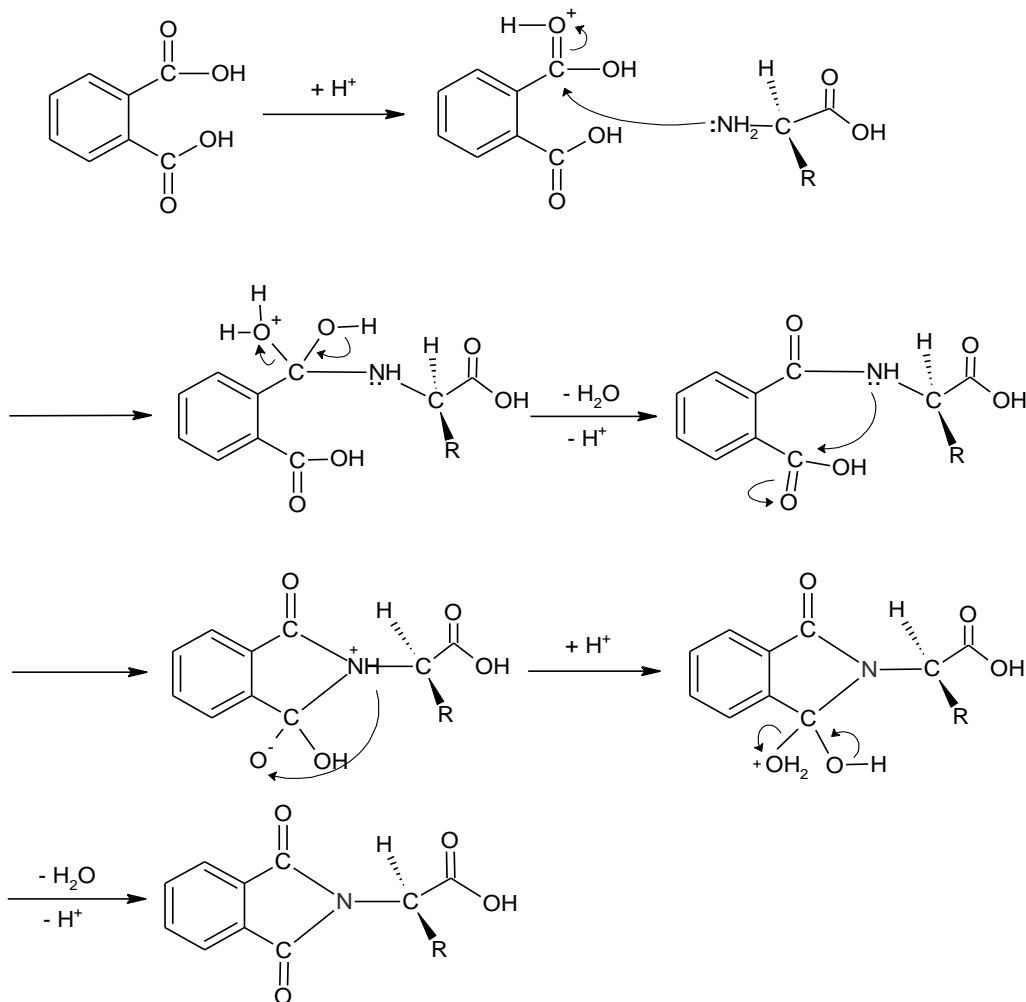


**2.1.1.2: Method B: Synthesis of *N*-phthalimide amino acids from phthalic anhydride (IIIa-f):**

A mixture of 1 g (6.75 mmole) of phthalic anhydride and 6.8 mmole of amino acid (0.510 g glycine, 0.605 g alanine, 1.123 g phenylalanine, 0.796 g valine, 0.892 g leucine, 0.905 g aspartic) were refluxed in (15 ml) glacial AcOH for 2 h. 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):**

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 h. 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 (IIa-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  $\text{cm}^{-1}$  which was assigned to the (O-H) of acid group in these compounds. Absorption bands between 1700 – 1800  $\text{cm}^{-1}$  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  $\text{cm}^{-1}$  due to  $(\text{C}=\text{C})_{\text{arom.}}$ .

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  $\text{cm}^{-1}$  which was assigned to the (N-H) of benzimidazol ring, Absorption bands between 1766–1749  $\text{cm}^{-1}$  which were characteristic peaks for two asymmetric and symmetric stretching of carbonyl groups in imide ring.

The  $^1\text{H-NMR}$  spectra of (Va-b) multi signals at (7.83-7.88 ppm) due to aromatic protons,  $^1\text{H-NMR}$  for N-(1H benzimidazol-2-yl methyl). Phthalimide (Va) showed single signal at (4.2 ppm) due to protons of  $(\text{CH}_2)$  group and single signal at (12.31 ppm) due to proton of (NH) group, and  $^1\text{H-NMR}$  for (Vb) showed double signal at (1.67 ppm) due to protons of  $(\text{CH}_3)$  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 <sup>a</sup>  | -                    | 85.3     | 88       |
| IIIb      | CH <sub>3</sub> -                                   | 0.65 | 146-148  | 144-146 <sup>a</sup>  | -23.3                | 80.6     | 95.8     |
| IIIc      | PhCH <sub>2</sub> -                                 | 0.39 | 180-182  | 180 -181 <sup>a</sup> | -209.9               | 87.1     | 89.1     |
| IIId      | (CH <sub>3</sub> ) <sub>2</sub> CH-                 | 0.43 | 116- 118 | 114-115 <sup>b</sup>  | -68.9                | 44.3     | 71.3     |
| IIIe      | (CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> - | 0.45 | 115-117  | 115-118 <sup>a</sup>  | -27.6                | 50.2     | 69.1     |
| IIIf      | HOOCCH <sub>2</sub> -                               | 0.61 | 221-223  | 223-225 <sup>b</sup>  | -57.1                | 56.4     | 66.8     |
| Va        | H-  | 0.33 | 211      | -                     | -                    | 57.2     |          |
| Vb        | CH <sub>3</sub> -                                   | 0.25 | 204      | -                     | -19.3                | 46.1     |          |

\* Mobile phase: Benzen: Ethanol (1:1). a = [17], b = [18].

\*\* Measured at a concentration of 0.5g/dL in EtOH at 25°C.

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

| Comp. No. | Formula<br>M <sub>w</sub>                                  | MS (m/z)<br>(M-H <sup>+</sup> ) <sup>-</sup> | elemental analysis (C.H.N) |       |       |      |
|-----------|--|--|----------------------------|-------|-------|------|
|           |  |  | %                          | H%    | C%    | N%   |
| IIIa      | C <sub>10</sub> H <sub>7</sub> NO <sub>4</sub><br>205.166  | 204  | Calc.                      | 3.44  | 58.54 | 6.83 |
|           |  |  | Found                      | 3.344 | 58.58 | 6.71 |
| IIIb      | C <sub>11</sub> H <sub>9</sub> NO <sub>4</sub><br>219.193  | 218  | Calc.                      | 4.14  | 60.27 | 6.36 |
|           |  |  | Found                      | 3.942 | 59.95 | 6.25 |
| IIIc      | C <sub>17</sub> H <sub>13</sub> NO <sub>4</sub><br>295.289 | 294  | Calc.                      | 4.44  | 69.15 | 4.74 |
|           |  |  | Found                      | 4.210 | 69.33 | 4.69 |
| IIId      | C <sub>13</sub> H <sub>13</sub> NO <sub>4</sub><br>247.246 | 246  | Calc.                      | 5.30  | 63.15 | 5.67 |
|           |  |  | Found                      | 5.277 | 62.90 | 5.45 |
| IIIe      | C <sub>14</sub> H <sub>15</sub> NO <sub>4</sub><br>261.273 | 260  | Calc.                      | 5.79  | 64.36 | 5.36 |
|           |  |  | Found                      | 5.610 | 64.09 | 5.34 |
| IIIf      | C <sub>12</sub> H <sub>9</sub> NO <sub>6</sub><br>263.202  | 262  | Calc.                      | 3.45  | 54.76 | 5.32 |
|           |  |  | Found                      | 3.304 | 54.77 | 5.21 |

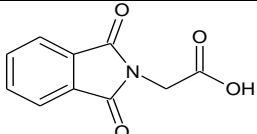
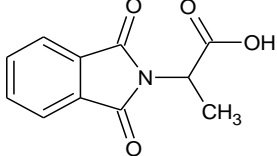
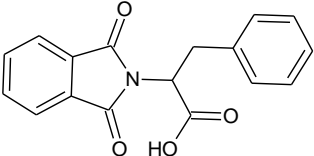
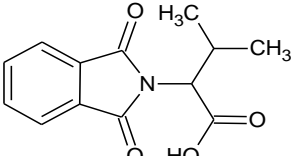
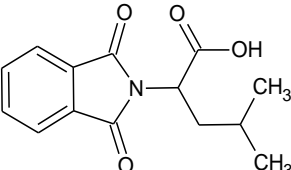


|    |  |     |       |      |       |       |
|----|--|-----|-------|------|-------|-------|
| Va | C <sub>16</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub><br>277.277 | 276 | Calc. | 4.00 | 69.31 | 11.54 |
|    |  |     | Found | 3.99 | 69.46 | 11.25 |
| Vb | C <sub>17</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub><br>291.304 | 290 | Calc. | 4.50 | 70.09 | 14.42 |
|    |  |     | Found | 4.36 | 69.99 | 14.28 |

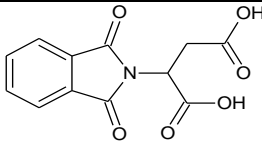
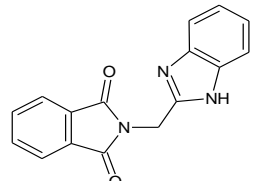
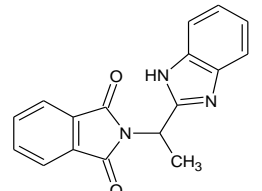
Table 3: The FT-IR spectral data of synthesized derivatives

| Comp. No. | (O – H, str.) | (C – H, str.) | (C = O)   | (C = C) <sub>arom</sub> | Others  |
|-----------|---------------|---------------|-----------|-------------------------|---|
| IIIa      | 2500-3500     | 3062          | 1774–1750 | 1602                    | 1464 (CH <sub>2</sub> , ben.)                     |
| IIIb      | 2500-3500     | 2926          | 1771–1702 | 1610                    | 1466 (CH <sub>3</sub> , ben.)                     |
| IIIc      | 2500-3500     | 3027          | 1747–1798 | 1610                    | 1451 (CH <sub>2</sub> , ben.)                     |
| III d     | 2500-3500     | 2998          | 1758–1692 | 1609                    | 1451 (CH <sub>2</sub> , ben.)                     |
| IIIe      | 2500-3500     | 2964          | 1712      | 1609                    | 1466 (CH <sub>2</sub> and CH <sub>3</sub> , ben.) |
| III f     | 2500-3500     | 3039          | 1725      | 1611                    | 1466 (CH <sub>2</sub> , ben.)                     |
| Va        | -             | 3055          | 1766      | 1603                    | 3201 (N-H, Str.)                                  |
| Vb        | -             | 3026          | 1749      | 1612                    | 3198 (N-H, Str.)                                  |

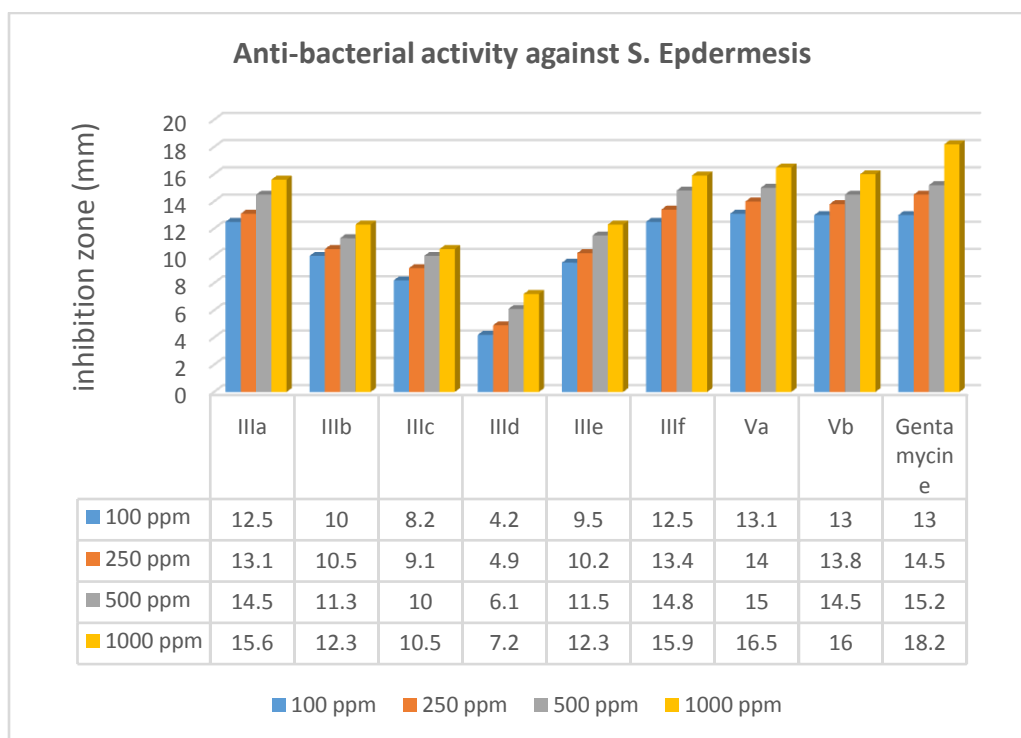
Table 4: The <sup>1</sup>H-NMR spectral data of synthesized derivatives (Va-b)

| Comp. No. | Comp. Structure   | <sup>1</sup> H-NMR chemical shifts ppm   |
|-----------|---|--|
| IIIa      |   | 4.25 (s, 2H, CH <sub>2</sub> ),<br>6.96-7.01 (br, 1H, OH),<br>7.96-8.21 (m, 4H, H <sub>arom</sub> .)   |
| IIIb      |  | 1.48-1.66 (d, 3H, CH <sub>3</sub> ),<br>4.24-4.51 (q, 1H, CH),<br>6.96-7.01 (br, 1H, OH),<br>8.02-8.53 (m, 4H, H <sub>arom</sub> .)  |
| IIIc      |  | 2.83-3.01 (dd, 2H, CH <sub>2</sub> ),<br>4.43-4.72 (t, 1H, CH),<br>6.72-6.93(s, br, 1H, OH),<br>7.02-8.21 (m, 9H, H <sub>arom</sub> .)   |
| III d     |  | 1.20-1.25 (d, 6H, 2CH <sub>3</sub> ),<br>2.60-2.80 (m, 1H, CH-CH <sub>3</sub> ),<br>4.90-4.97 (m, 1H, CH),<br>6.40-6.45 (s, br, 1H, OH),<br>8.00-8.20 (m, 4H, H <sub>arom</sub> .) |
| IIIe      |  | 0.90-0.95 (d, 6H, 2CH <sub>3</sub> ),<br>2.40-2.60 (m, 1H, CH-CH <sub>3</sub> ),<br>4.20-4.30 (t, 2H, CH),<br>6.50-6.60 (s, br, 1H, OH),<br>8.10-8.20 (m, 4H, H <sub>arom</sub> .) |



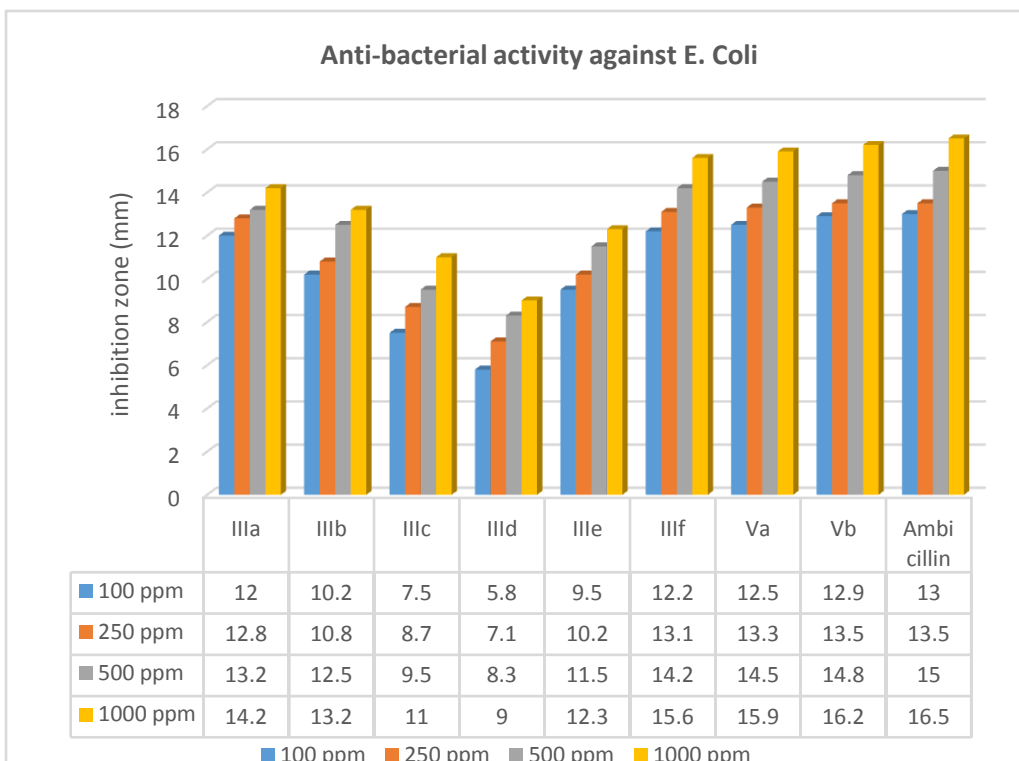
|              |   |   |
|--------------|---|---|
| <b>III f</b> |  | 2.93-3.21 (m, 2H, CH <sub>2</sub> ),<br>4.69-4.81 (t, 1H, CH),<br>8.01-8.23 (m, 4H, H <sub>arom.</sub> )<br>8.29-8.48 (s, br, 2H) |
| <b>V a</b>   |  | 4.22 (s, 2H, CH <sub>2</sub> ),<br>7.84-7.90 (m, 8H, H <sub>arom.</sub> ),<br>12.31 (s, H, NH)                                    |
| <b>V b</b>   |  | 1.67(d, 3H, CH <sub>3</sub> ),<br>4.96 (q, 1H, CH),<br>7.83-7.88 (m, 8H, H <sub>arom.</sub> ),<br>12.56 (s, H, NH)                |

### 3.2. Biological activity:

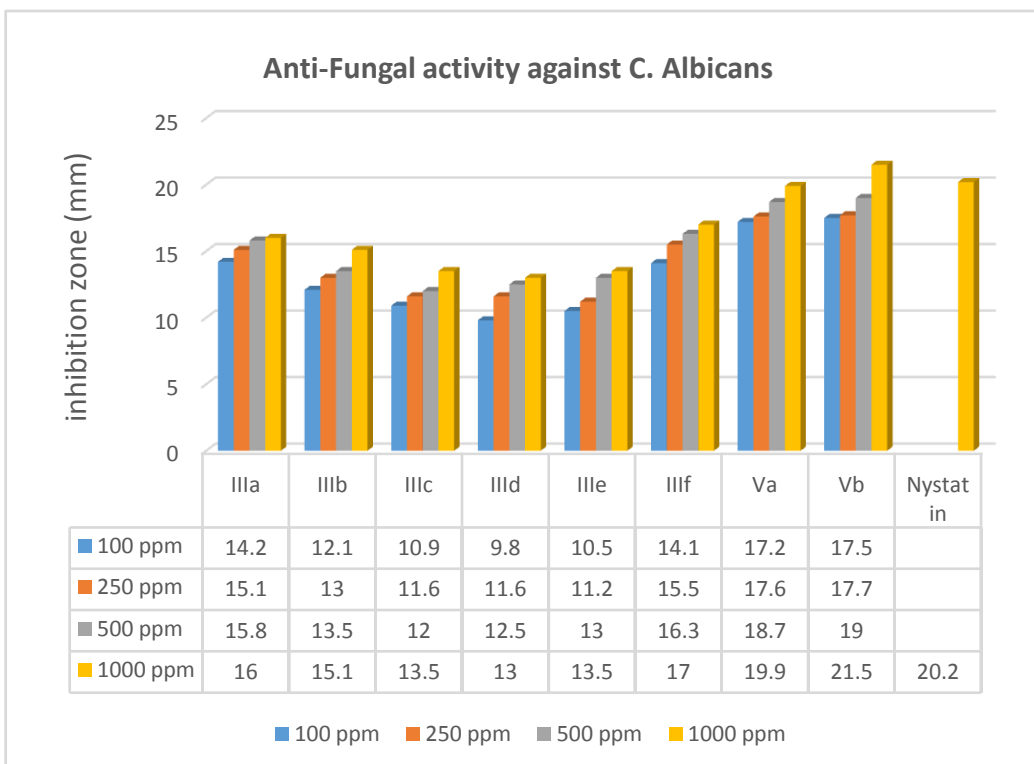


**Figure 1:** Inhibitory activity against *S. Epidermises*





**Figure 2:** Inhibitory activity against E. Coli



**Figure 3:** Inhibitory activity against C. Albicans

### 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 °C 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 °C.

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

**Table 5:** Anti-Mycobacterial activity of the synthesized compounds

| Bacterial suspended dilutions | 1/10   |         |          | 1/1000 |         |          |
|-------------------------------|--------|---------|----------|--------|---------|----------|
|                               | 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        |
| III d                         | R      | R       | R        | R      | R       | R        |
| IIIe                          | R      | R       | R        | R      | R       | R        |
| III f                         | 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 III d - 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:

We are grateful to Department of Chemistry, Faculty of Sciences, Damascus University, and to Laboratories of Researches and Control Drugs Directorate, Ministry of Health, Syria for their supports of this research.

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