

## **Synthesis, Characterization and Antimicrobial Studies on Some Newer Imidazole Analogs**

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### **Abstract**

A novel series of 3,5-diiodo-4-(2-methyl-1*H*-imidazol-5-yl)benzoic acid analogs of amino acids, dipeptides and tripeptides was synthesized by using dicyclohexylcarbodiimide and diisopropylcarbodiimide (DCC/DIPC) as coupling agents and triethylamine (TEA) as base. Structures of all the newly synthesized compounds were confirmed by elemental analysis and IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectral data. Imidazolo peptides were investigated for their antimicrobial activity and some of newly synthesized compounds **2c**, **2d**, **2h** and their hydrolyzed analogs **3b**, **3d** exhibited potent bioactivity against pathogenic fungi *Candida albicans* and dermatophytes *Trichophyton mentagrophytes* and *Microsporum audouinii* with MIC values of 12.5-6 µg/ml, as compared to the reference drug – griseofulvin. In addition, moderate activity against gram negative bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was also observed for synthesized imidazolo peptides.

### **Keywords**

Imidazolo peptides • *p*-Amino-3,5-diiodobenzoic acid • Peptide coupling • Antifungal activity • Antibacterial activity

### **Introduction**

In past decades, imidazoles have proved their potential in development of pharmaceutically important organic compounds both of natural and synthetic origin

[1]. Imidazole analogs deal with a variety of bioactivities viz. antitumor [2, 3], anti-HIV [4], antimicrobial [5–8], anticonvulsant [9], antitubercular [10], antiprotozoal [11], anti-inflammatory [12], FTase and MAP kinase p38 inhibitory activities [13, 14]. Literature is enriched with lot of work on synthesis of potent substituted imidazole derivatives with diverse pharmacological activities [15, 16] but only few reports have been received on peptide coupling of imidazoles. Thus keeping in view the biological potency of imidazole derivatives as well as taking advantage of biodegradability and biocompatibility of amino acids/peptides and further, in continuation of our research work on synthesizing peptide derivatives of heterocyclic and other aromatic compounds [17–29], a novel series of 3,5-diiodo-4-(2-methyl-1*H*-5-imidazolyl)benzoyl amino acids and peptides with an anticipation to get potent agents of more therapeutic efficacy with lesser adverse effects.

## Chemistry

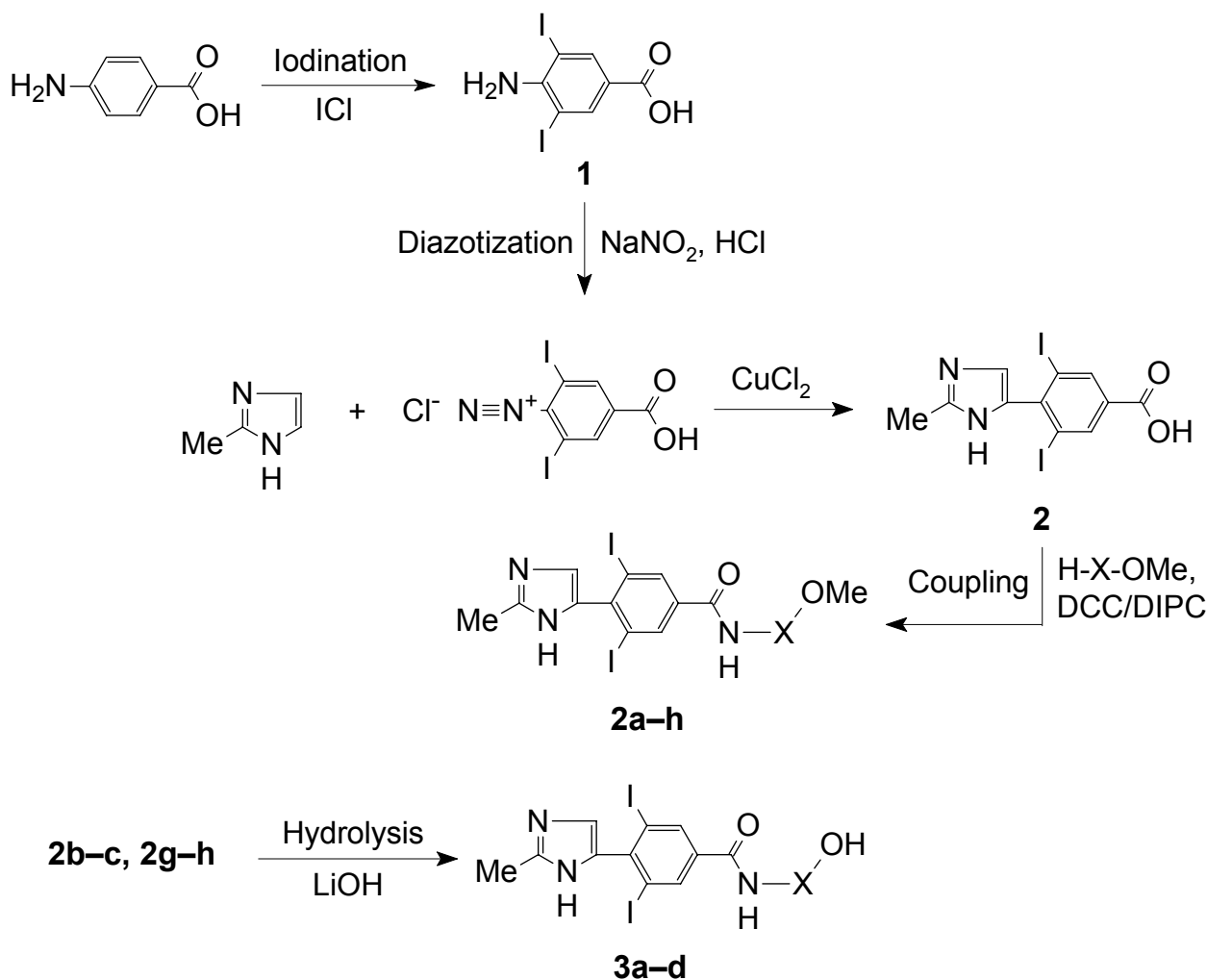
4-Amino-3,5-diiodobenzoic acid (**1**) was prepared according to literature procedure [30] in good yields by iodination of *p*-aminobenzoic acid (PABA) using iodine monochloride. 3,5-Diiodo-4-(2-methyl-1*H*-5-imidazolyl)benzoic acid (**2**) was synthesized by diazotization of compound **1** followed by subsequent interaction with 2-methylimidazole in presence of aqueous cupric chloride. Dipeptides Boc-his-tyr-OMe, Boc-phe-tyr-OMe and Boc-gly-gly-OMe were prepared from the corresponding Boc-amino acids viz. Boc-his-OH, Boc-phe-OH and Boc-gly-OH, and amino acid methyl ester hydrochlorides such as tyr-OMe.HCl, try-OMe.HCl and gly-OMe.HCl using DCC and *N*-methylmorpholine (NMM) in chloroform [31]. Similarly, tripeptides Boc-pro-val-pro-OMe, Boc-ile-phe-leu-OMe and tetrapeptide Boc-gly-his-ala-tyr-OMe were prepared by coupling Boc-dipeptides with corresponding amino acid methyl ester hydrochlorides and dipeptide methyl esters. All Boc-di/tri/tetrapeptide methyl esters were deprotected at amino terminals using trifluoroacetic acid (TFA) prior to coupling.

3,5-Diiodo-4-(2-methyl-1*H*-5-imidazolyl)benzoic acid (**2**) was coupled with various L-amino acid methyl ester hydrochlorides/peptide methyl esters using

DCC/DIPC and TEA to get newly synthesized 3,5-diiodo-4-(2-methyl-1*H*-5-imidazolyl)benzoyl amino acid methyl esters (**2a–b**), dipeptide methyl esters (**2c–e**) and tri/tetrapeptide methyl esters (**2f–h**). Finally, compounds **2b–c** and **2g–h** were hydrolyzed with lithium hydroxide (LiOH) to get the corresponding acid derivatives **3a–d**. Structures of all the newly synthesized compounds were confirmed by IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and mass spectral data. Elemental analysis of the novel compounds was performed for carbon, hydrogen and nitrogen content. The synthesis of compounds **2a–3d** is mentioned in Scheme 1 and their physico-chemical data is described in Table 1.

## Results and Discussion

All imidazolo peptide derivatives **2a–h** were synthesized in good yields using DCC/DIPC as coupling agents and TEA as base. Presence of bands at 3307–2499, 1696 and 593  $\text{cm}^{-1}$  in IR spectrum of compound **2** clearly indicated presence of functional groups like  $-\text{COOH}$  and  $-\text{I}$  but no free  $-\text{NH}_2$  group which was present in starting material *p*-amino-3,5-diiodobenzoic acid (**1**). Furthermore, IR spectra of peptide derivatives **2a–h** showed Amide I and Amide II bands at 1662–1639  $\text{cm}^{-1}$  and 1538–1531  $\text{cm}^{-1}$  indicating formation of peptide bonds and successfulness of coupling reaction. This fact was further confirmed by appearance of broad singlets at 9.32–6.96 ppm (for imino proton of  $\text{CO}-\text{NH}$  moiety) in  $^1\text{H}$  NMR spectra and singlets at 179.5–167.2 ppm (for carbonyl carbon of  $\text{CO}-\text{NH}$  moiety) in  $^{13}\text{C}$  NMR spectra of compounds **2a–h**. Mass spectra of peptide ester derivatives showed molecular ion peaks along with isotopic peaks at  $m/z$  values, consistent with their respective molecular formulas. All peptide ester derivatives showed easily distinguishable  $\text{R}-\text{C}\equiv\text{O}^+$  ion peaks at  $M - 31$  along with characteristic fragmentation pattern after and before the carbonyl moiety in their respective structures. Furthermore,  $[\text{CH}_3\text{O}^+]$  and  $[\text{CH}_3\text{OCO}^+]$  fragment ion peaks appeared at  $m/z$  values 31 and 59 in mass spectra of peptide ester derivatives.



**Sch. 1.** X = pro (**2a**), leu (**2b**), his-tyr (**2c**), phe-try (**2d**), gly-gly (**2e**), pro-val-pro (**2f**), ile-phe-leu (**2g**), gly-his-ala-try (**2h**), leu (**3a**), his-tyr (**3b**), ile-phe-leu (**3c**), gly-his-ala-try (**3d**).

Structures of hydrolyzed analogs **3a-d** were confirmed by appearance of strong bands at 1713–1708  $\text{cm}^{-1}$  (C=O str,  $\underline{\text{COOH}}$ ) in IR spectra, broad singlets at 8.69 ppm (for hydroxyl proton of  $\underline{\text{COOH}}$ ) in  $^1\text{H}$  NMR spectra and singlets at 175.0 ppm (for carbonyl carbon of  $\underline{\text{COOH}}$ ) in  $^{13}\text{C}$  NMR spectra. This fact was further supported by disappearance of medium to strong bands at 1754–1748  $\text{cm}^{-1}$  (C=O str, ester) and 1274–1269  $\text{cm}^{-1}$  (C–O str, ester) in IR spectra and singlets at 52.5 ppm (for carbonyl carbon of  $\underline{\text{OCH}_3}$ ) in  $^{13}\text{C}$  NMR spectra of compounds **3a-d**.

All hydrolyzed peptide derivatives showed peaks at  $M - 17$  and  $M - 45$  in their mass spectra by loss of OH and COOH respectively. Moreover,  $[\text{COOH}^+]$  fragment ion peak appeared at  $m/z$  value 45 in mass spectra of compound **3c** and **3d** along with characteristic fragmentation pattern after and before the carbonyl moiety. Moreover, the results of elemental analysis revealed the variation by a factor of  $\pm 0.03$  from calculated values.

### Biological activity

All the newly synthesized compounds **2a–3d** were evaluated for its antimicrobial activity against four bacterial strains *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), *Pseudomonas aeruginosa* (NCIM 2034) and *Klebsiella pneumoniae* (NCIM 2011) and four fungal strains *Microsporum audouinii* (MUCC 545), *Trichophyton mentagrophytes* (MUCC 665), *Candida albicans* (MUCC 29) and *Aspergillus niger* (MUCC 177) at  $50\text{--}6\ \mu\text{g ml}^{-1}$  concentration, according to modified Kirby-Bauer's disk diffusion method [32]. MIC values of test compounds were determined by tube dilution technique. The solvents DMF/DMSO were used as negative controls and ciprofloxacin/griseofulvin were used as standards. Calculated average diameters (for triplicate sets) of the zones of inhibition (in mm) for test samples were compared with that produced by the standard drugs.

Almost, all the synthesized compounds were found to exhibit moderate to good antifungal activity and mild to moderate antibacterial activity. Analysis of antimicrobial data suggested that compounds **2c**, **2d**, **2h**, **3b** and **3d** possessed higher antifungal activity against dermatophytes and *Candida* sp. in comparison to standard drug.

Compd.	X	Mol. formula (Mol. wt.)	M.p. (°C)	Yield (%)	R <sub>f</sub> Value*	Elemental analysis [Calcd. (found)]		
						%C	%H	%N
<b>2</b>	–	C <sub>11</sub> H <sub>8</sub> I <sub>2</sub> N <sub>2</sub> O <sub>2</sub> (454)	118	72	0.79 <sup>1</sup>	29.10 (29.09)	1.78 (1.80)	6.17 (6.19)
<b>2a</b>	pro	C <sub>17</sub> H <sub>17</sub> I <sub>2</sub> N <sub>3</sub> O <sub>3</sub> (565)	–	77	0.58	36.13 (36.11)	3.03 (3.06)	7.44 (7.46)
<b>2b</b>	leu	C <sub>18</sub> H <sub>21</sub> I <sub>2</sub> N <sub>3</sub> O <sub>3</sub> (581)	105	82	0.67	37.20 (37.23)	3.64 (3.62)	7.23 (7.25)
<b>2c</b>	his-tyr	C <sub>27</sub> H <sub>26</sub> I <sub>2</sub> N <sub>6</sub> O <sub>5</sub> (768)	120	75	0.81	42.21 (42.19)	3.41 (3.44)	10.94 (10.95)
<b>2d</b>	phe-try	C <sub>32</sub> H <sub>29</sub> I <sub>2</sub> N <sub>5</sub> O <sub>4</sub> (801)	84	79	0.74	47.96 (47.98)	3.65 (3.65)	8.74 (8.77)
<b>2e</b>	gly-gly	C <sub>16</sub> H <sub>16</sub> I <sub>2</sub> N <sub>4</sub> O <sub>4</sub> (582)	142	71	0.51	33.01 (33.04)	2.77 (2.80)	9.62 (9.59)
<b>2f</b>	pro-val-pro	C <sub>27</sub> H <sub>33</sub> I <sub>2</sub> N <sub>5</sub> O <sub>5</sub> (761)	–	80	0.59	42.59 (42.58)	4.37 (4.35)	9.20 (9.23)
<b>2g</b>	ile-phe-leu	C <sub>33</sub> H <sub>41</sub> I <sub>2</sub> N <sub>5</sub> O <sub>5</sub> (841)	–	84	0.72	47.10 (47.10)	4.91 (4.92)	8.32 (8.29)
<b>2h</b>	gly-his-ala-try	C <sub>34</sub> H <sub>35</sub> I <sub>2</sub> N <sub>9</sub> O <sub>6</sub> (919)	–	69	0.61 <sup>2</sup>	44.41 (44.42)	3.84 (3.85)	13.71 (13.69)
<b>3a</b>	leu	C <sub>17</sub> H <sub>19</sub> I <sub>2</sub> N <sub>3</sub> O <sub>3</sub> (567)	63	86	0.49	36.00 (35.98)	3.38 (3.41)	7.41 (7.40)
<b>3b</b>	his-tyr	C <sub>26</sub> H <sub>24</sub> I <sub>2</sub> N <sub>6</sub> O <sub>5</sub> (754)	159	89	0.66	41.40 (41.43)	3.21 (3.19)	11.14 (11.15)
<b>3c</b>	ile-phe-leu	C <sub>33</sub> H <sub>41</sub> I <sub>2</sub> N <sub>5</sub> O <sub>5</sub> (841)	–	92	0.63	46.45 (46.46)	4.75 (4.77)	8.46 (8.44)
<b>3d</b>	gly-his-ala-try	C <sub>33</sub> H <sub>33</sub> I <sub>2</sub> N <sub>9</sub> O <sub>6</sub> (905)	–	90	0.85 <sup>2</sup>	43.77 (43.75)	3.67 (3.68)	13.92 (13.90)

\* (CHCl<sub>3</sub>:MeOH / 9:1), <sup>1</sup>(CHCl<sub>3</sub>:AcOH:H<sub>2</sub>O / 3:2:5), <sup>2</sup>(CHCl<sub>3</sub>:MeOH / 7:3)

**Tab. 2.** Antimicrobial activity of synthesized compounds

Compd.	Diameter of zone of inhibition (mm)							
	Bacterial strains				Fungal strains			
	<i>B. sub.</i>	<i>S. aur.</i>	<i>P. aeru.</i>	<i>K. pneu.</i>	<i>C. alb.</i>	<i>M. audo.</i>	<i>A. niger</i>	<i>T. menta.</i>
<b>2a</b>	9(25) <sup>†</sup>	11(50)	11(6)	10(12.5)	14(6)	14(6)	12(12.5)	14(6)
<b>2b</b>	11(25)	13(25)	17(6)	13(12.5)	15(6)	13(6)	14(12.5)	13(6)
<b>2c</b>	10(12.5)	9(50)	14(6)	11(12.5)	21(6)	19(6)	12(12.5)	21(6)
<b>2d</b>	15(6)	10(12.5)	20(6)	13(12.5)	23(6)	22(6)	15(12.5)	24(6)
<b>2e</b>	16(6)	9(12.5)	24(6)	19(12.5)	17(6)	12(6)	9(12.5)	16(12.5)
<b>2f</b>	15(12.5)	13(12.5)	25(6)	18(12.5)	11(12.5)	10(6)	12(12.5)	9(25)
<b>2g</b>	13(25)	11(25)	17(12.5)	14(12.5)	17(12.5)	10(6)	14(12.5)	11(25)
<b>2h</b>	11(12.5)	10(25)	13(6)	12(12.5)	22(6)	18(6)	15(12.5)	21(6)
<b>3a</b>	15(25)	16(25)	19(6)	16(12.5)	17(6)	15(6)	15(12.5)	17(6)
<b>3b</b>	16(12.5)	10(50)	17(6)	15(12.5)	25(6)	20(6)	14(12.5)	23(6)
<b>3c</b>	16(25)	13(25)	19(12.5)	16(12.5)	19(12.5)	14(6)	16(12.5)	14(25)
<b>3d</b>	13(12.5)	12(25)	18(6)	15(12.5)	25(6)	21(6)	17(12.5)	23(6)
Control <sup>‡</sup>	–	–	–	–	–	–	–	–
Ciprofloxacin	20(6)	20(12.5)	25(6)	19(12.5)	–	–	–	–
Griseofulvin	–	–	–	–	20(6)	17(6)	18(12.5)	20(6)

<sup>†</sup> Values in bracket are MIC values (µg/ml).

<sup>‡</sup> Dimethylformamide (DMF) / Dimethylsulfoxide (DMSO)

Moreover, all the synthesized compounds exhibited moderate activity against gram negative bacteria. Compounds **2e** and **2f** exhibited bioactivity equivalent or closer to reference compound, against *P. aeruginosa* and *K. pneumoniae*. Moreover, comparison of antimicrobial data further suggested that amino acid and peptide derivatives **3a–d** possessed more antimicrobial activity as compared to corresponding methyl ester derivatives **2b–c** and **2g–h** (Table 2).

Newly synthesized compounds **2a–3d** were found to be more potent in comparison to previously synthesized diiododimethylbenzimidazole analogs against *K. pneumoniae* and *M. audouinii*. Even some of the synthesized analogs **2c**, **2d**,

**2h**, **3b** and **3d** displayed better activity against *P. aeruginosa* and compounds **2d–f** exhibited better activity against *C. albicans* when compared to their corresponding dimethylbenzimidazolepeptides [18]. However, activity of compounds **2a–3d** against gram positive bacteria was found to be less in comparison to earlier synthesized analogs. Moreover, presence of iodine atoms in nucleus was found to confer more antimicrobial activity especially against *P. aeruginosa*, *K. pneumoniae*, *C. albicans* and *dermatophytes*, as evident from previously synthesized non-iodinated benzimidazolepeptide analogs [26].

## Experimental

Melting points were determined by open capillary method and are uncorrected. L-amino acids, DIPC, DCC, TFA, NMM and TEA were procured from SpectroChem Ltd, Mumbai, India. IR spectra were recorded on Shimadzu 8700 fourier transform infrared spectrophotometer using a thin film supported on KBr pellets for solids and  $\text{CHCl}_3$  as solvent for semisolids.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on Bruker AC NMR spectrometer (300 and 50 MHz) using  $\text{DMSO}-d_6$  and  $\text{CDCl}_3$  as solvents and TMS as internal standard. Mass spectra were recorded on Jeol JMS DX 303 Mass spectrometer operating at 70 eV. Elemental analysis of all compounds was performed on Elementar vario EL III. Purity of all the compounds was checked by TLC on precoated silica gel G plates.

## Preparation of peptides

Amino acid methyl ester hydrochloride/dipeptide methyl ester (0.01 mol) was dissolved in  $\text{CHCl}_3$  (15 ml). To this, NMM (0.021 mol) was added at 0 °C and the reaction mixture was stirred for 10 min. Boc-amino acid/dipeptide (0.01 mol) in  $\text{CHCl}_3$  (15 ml) and DCC (0.01 mol) were added with stirring. After 36 h, the reaction mixture was filtered and the residue was washed with  $\text{CHCl}_3$  (30 ml) and added to the filtrate. The filtrate was washed with 25 ml each of 5%  $\text{NaHCO}_3$  and saturated NaCl solutions. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and evaporated in vacuum. The crude product was crystallized from a mixture of



chloroform and *n*-hexane followed by cooling at 0 °C. Resulting Boc-di/tri/tetrapeptide methyl ester (0.01 mol) was dissolved in CHCl<sub>3</sub> (15 ml) and treated with trifluoroacetic acid (TFA, 0.02 mol). The mixture was stirred at RT for 1 h and washed with 25 ml of saturated NaHCO<sub>3</sub> solution. The resulting organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Finally, crude product was purified by recrystallization with CHCl<sub>3</sub> and petroleum ether (b.p. 40–60 °C) to get the deprotected di/tri/tetrapeptide methyl esters.

### Preparation of 3,5-diiodo-4-(2-methyl-1*H*-imidazol-5-yl)benzoic acid (2)

A mixture of 4-amino-3,5-diiodobenzoic acid (**1**) (38.9 g, 0.1 mol), dil. hydrochloric acid (15%, 60 ml) and water (90 ml) was heated to get a clear solution. After cooling, the solution was diazotized by addition of sodium nitrite solution (30%, 24 ml). To the filtrate of diazotized salt solution, 2-methylimidazole (8.2 g, 0.1 mol) and aqueous cupric chloride solution (2.5 g in 10 ml of water) were added with stirring followed by slow addition of water (50 ml). Stirring was continued for 4 h and kept overnight in the refrigerator. The separated solid was collected by filtration and washed with cold water. The crude product was recrystallized from acetone to get the title compound. White crystals; m.p. 118 °C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3488 (N–H str, ring), 3307–2499 (O–H str, COOH), 3074, 3054 (Ar–H str), 2969, 2873 (C–H str, CH<sub>3</sub>), 1696 (C=O str, COOH), 1587, 1425, 1422 (skeletal bands), 869, 833, 748 (C–H def, out-of-plane (oop)), 593 (C–I str); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.22 (2H, br. s, NH, imz and OH, COOH), 8.15 (2H, s, H-2 and H-6, iodobenzoic acid moiety (iba)), 7.45 (1H, s, H- $\delta$ , imidazole moiety (imz)), 2.64 (3H, s,  $\beta$ -CH<sub>3</sub>, imz); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 170.2 (C=O, COOH), 159.0 (C-2, imz), 150.1 (C-4, imz), 149.3 (C-4, iba), 146.7 (C-5, imz), 141.2 (2C, C-2 and C-6, iba), 124.4 (C-1, iba), 105.8 (2C, C-3 and C-5, iba), 18.9 ( $\beta$ -CH<sub>3</sub>, imz).

### General procedure for synthesis of 3,5-diiodo-4-(2-methyl-1*H*-imidazol-5-yl)benzoyl amino acid and peptide methyl esters (2a–h)

To a mixture of amino acid methyl ester hydrochloride/di/tri/tetrapeptide methyl ester (0.01 mol) in 50 ml of DMF, 2.8 ml of TEA was added at 0 °C with stirring. Compound **2** (4.54 g, 0.01 mol) in DMF (50 ml) and DCC/DIPC (0.01 mol) were added to the above mixture and stirring was done for 24 h. After this, reaction mixture was filtered and filtrate was diluted with equal proportion of water and aqueous layer was washed with ether (3 × 50 ml). The organic layer was separated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The product obtained was dissolved in chloroform, washed with 30 ml each of 10% HCl, saturated NaHCO<sub>3</sub> solution and water followed by evaporation in vacuum. Crude product was crystallized from a mixture of ethyl acetate and petroleum ether (b.p. 40–60 °C).

***Methyl 1-[3,5-diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl]-L-prolinate (2a)***

IR (CHCl<sub>3</sub>)  $\nu$  (cm<sup>-1</sup>): 3486 (N–H str, ring), 3077, 3052 (Ar–H str), 2997–2992 (C–H str, CH<sub>2</sub>, pro), 2968, 2875 (C–H str, CH<sub>3</sub>), 1744 (C=O str, ester), 1658 (C=O str, 3° amide), 1589, 1421 (skeletal bands), 1272 (C–O str, ester), 865, 838, 752 (C–H def, out-of-plane (oop)), 590 (C–I str); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.32 (1H, s, H- $\delta$ , imz), 8.20 (2H, s, H-2 and H-6, iba), 7.59 (1H, br. s, NH, imz), 4.17 (1H, t, *J* = 6.9 Hz, H- $\alpha$ , pro), 3.72 (2H, t, *J* = 7.15 Hz, H- $\delta$ , pro), 3.62 (3H, s, OCH<sub>3</sub>), 2.65 (3H, s,  $\beta$ -CH<sub>3</sub>, imz), 2.20–2.14 (2H, m, H- $\gamma$ , pro), 2.05–2.01 (2H, q, H- $\beta$ , pro); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 174.4 (C=O, pro), 173.9 (C=O, ArCO), 159.0 (C-2, imz), 151.7 (C-4, iba), 146.6 (C-5, imz), 141.2 (2C, C-2 and C-6, iba), 137.9 (C-1, iba), 137.4 (C-4, imz), 102.6 (2C, C-3 and C-5, iba), 60.2 (C- $\alpha$ , pro), 54.4 (OCH<sub>3</sub>), 50.1 (C- $\delta$ , pro), 28.9 (C- $\beta$ , pro), 27.0 (C- $\gamma$ , pro), 19.1 ( $\beta$ -CH<sub>3</sub>, imz).

***Methyl 1-[3,5-diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl]-L-leucinate (2b)***

IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3489 (N–H str, ring), 3134 (NH str, amide), 3075, 3048 (Ar–H str), 2966 (C–H str, CH<sub>3</sub>), 2929, 2853 (C–H str, CH<sub>2</sub>), 1742 (C=O str, ester), 1641 (C=O str, 2° amide), 1586, 1424 (skeletal bands), 1533 (N–H def, amide), 1385, 1362 (C–H bend, propyl-*i*), 1269 (C–O str, ester), 862, 839, 756 (C–H def, oop), 588 (C–I str); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.35 (2H, s, H-2 and H-6, iba),

8.32 (1H, s, H- $\delta$ , imz), 7.63 (1H, br. s, NH, imz), 6.05 (1H, br. s, NH, leu), 4.23–4.16 (1H, m, H- $\alpha$ , leu), 3.60 (3H, s, OCH<sub>3</sub>), 2.68 (3H, s,  $\beta$ -CH<sub>3</sub>, imz), 1.50–1.44 (3H, m, H- $\beta$  and H- $\gamma$ , leu), 0.95 (6H, d,  $J$  = 6.2 Hz, H- $\delta$ , leu); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 179.5 (C=O, leu), 168.9 (C=O, ArCO), 159.2 (C-2, imz), 152.1 (C-4, iba), 145.9 (C-5, imz), 140.4 (2C, C-2 and C-6, iba), 138.2 (C-1, iba), 136.7 (C-4, imz), 104.8 (2C, C-3 and C-5, iba), 52.5 (OCH<sub>3</sub>), 51.3 (C- $\alpha$ , leu), 45.5 (C- $\beta$ , leu), 27.2 (C- $\gamma$ , leu), 26.4 (2C, C- $\delta$ , leu), 19.4 ( $\beta$ -CH<sub>3</sub>, imz).

***Methyl 1-[3,5-diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl]-L-histidyl-L-tyrosinate (2c)***

IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3488, 3483 (N–H str, rings), 3359 (O–H str, Ar–OH), 3079–3075, 3055–3051 (Ar–H str), 2967, 2872 (C–H str, CH<sub>3</sub>), 2932, 2928, 2855, 2851 (C–H str, CH<sub>2</sub>), 1749 (C=O str, ester), 1645–1639 (C=O str, amide), 1592–1588, 1426–1419 (skeletal bands), 1270 (C–O str, ester), 1224 (C–O str, phenolic), 867–863, 835, 788, 750, 654 (C–H def, oop), 589 (C–I str); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 9.32 (1H, br. s, NH, his), 8.40 (2H, s, H-2 and H-6, iba), 8.31 (1H, s, H- $\delta$ , imz), 7.67 (1H, s, H-4, his), 7.55 (3H, br. s, NH, imz, his and OH, tyr), 7.38 (1H, d,  $J$  = 7.75 Hz, H-2, his), 6.96 (1H, br. s, NH, tyr), 6.89 (2H, dd,  $J$  = 8.6, 5.25 Hz, H-o, tyr), 6.77 (2H, dd,  $J$  = 8.65, 4.9 Hz, H-m, tyr), 5.05–5.01 (1H, m, H- $\alpha$ , his), 3.94–3.89 (1H, m, H- $\alpha$ , tyr), 3.56 (3H, s, OCH<sub>3</sub>), 3.02 (2H, d,  $J$  = 5.45 Hz, H- $\beta$ , tyr), 2.94 (2H, d,  $J$  = 5.8 Hz, H- $\beta$ , tyr), 2.66 (3H, s,  $\beta$ -CH<sub>3</sub>, imz); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 172.3 (C=O, his), 171.6 (C=O, ArCO), 170.1 (C=O, tyr), 159.0 (C-2, imz), 153.9 (C-p, tyr), 150.4 (C-4, iba), 148.8 (C-2, his), 145.0 (C-5, imz), 143.6 (2C, C-m, tyr), 139.2 (2C, C-2 and C-6, iba), 137.1 (C-1, iba), 136.5 (C-4, imz), 135.4 (C-4, his), 129.7 (2C, C-o, tyr), 126.5 (C- $\gamma$ , tyr), 117.2 (C-5, his), 105.5 (2C, C-3 and C-5, iba), 60.3 (C- $\alpha$ , his), 56.1 (C- $\alpha$ , tyr), 52.8 (OCH<sub>3</sub>), 38.5 (C- $\beta$ , tyr), 20.2 (C- $\beta$ , his), 19.1 ( $\beta$ -CH<sub>3</sub>, imz).

***Methyl 1-[3,5-diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl]-L-phenylalanyl-L-tryptophanate (2d)***

IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3487, 3483 (N–H str, rings), 3138-3135 (NH str, amide), 3079-3075, 3049-3046 (Ar–H str), 2965 (C–H str,  $\text{CH}_3$ ), 2927, 2923, 2855, 2851 (C–H str,  $\text{CH}_2$ ), 1746 (C=O str, ester), 1644-1639 (C=O str, amide), 1589-1585, 1428-1422 (skeletal bands), 1538, 1532 (N–H def, amide), 1267 (C–O str, ester), 868-861, 839, 759-756, 685 (C–H def, oop), 585 (C–I str);  $^1\text{H}$ -NMR ( $\text{DMSO}-d_6$ )  $\delta$  (ppm): 9.35 (1H, br. s,  $\text{NH}$ , phe), 8.42 (2H, s, H-2 and H-6, iba), 8.30 (1H, s, H- $\delta$ , imz), 8.27 (2H, br. s,  $\text{NH}$ , imz and try), 7.50 (1H, d,  $J = 7.8$  Hz, H-2, try), 7.22 (2H, tt,  $J = 7.8$ , 4.65 Hz, H-m, phe), 7.16-7.04 (4H, m, H-4–7, try), 6.99 (1H, t,  $J = 6.15$  Hz, H-p, phe), 6.92 (1H, br. s,  $\text{NH}$ , try), 6.82 (2H, dd,  $J = 8.75$ , 4.1 Hz, H-o, phe), 4.60-4.56 (1H, m, H- $\alpha$ , phe), 4.22-4.17 (1H, m, H- $\alpha$ , try), 3.56 (3H, s,  $\text{OCH}_3$ ), 3.20 (2H, d,  $J = 5.75$  Hz, H- $\beta$ , try), 2.89 (2H, d,  $J = 5.6$  Hz, H- $\beta$ , phe), 2.66 (3H, s,  $\beta\text{-CH}_3$ , imz);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 175.2 (C=O, try), 172.6 (C=O, phe), 165.4 (C=O,  $\text{ArCO}$ ), 158.7 (C-2, imz), 152.0 (C-4, iba), 140.8 (C- $\gamma$ , phe), 144.4 (C-5, imz), 140.1 (2C, C-2 and C-6, iba), 138.3 (C-1, iba), 137.8 (C-4, imz), 136.2 (C-2', try), 130.4 (2C, C-o, phe), 129.2 (C-2', try), 128.6 (2C, C-m, phe), 127.1 (C-p, phe), 122.9 (C-2, try), 121.6, 119.5, 118.2 (3C, C-6, C-5, C-4, try), 112.4, 109.5 (2C, C-7 and C-3, try), 105.0 (2C, C-3 and C-5, iba), 56.2 (C- $\alpha$ , try), 53.6 (C- $\alpha$ , phe), 51.4 ( $\text{OCH}_3$ ), 40.8 (C- $\beta$ , phe), 28.3 (C- $\beta$ , try), 19.1 ( $\beta\text{-CH}_3$ , imz).

***Methyl N-[3,5-diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl]glycylglycinate (2e)***

IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3488 (N–H str, ring), 3075, 3050 (Ar–H str), 2969, 2870 (C–H str,  $\text{CH}_3$ ), 2928, 2923, 2856, 2852 (C–H str,  $\text{CH}_2$ ), 1747 (C=O str, ester), 1644-1640 (C=O str, amide), 1587, 1425 (skeletal bands), 1536, 1532 (N–H def, amide), 1268 (C–O str, ester), 868, 839, 751 (C–H def, oop), 592 (C–I str);  $^1\text{H}$ -NMR ( $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.65 (1H, br. s,  $\text{NH}$ , gly-2), 8.46 (1H, br. s,  $\text{NH}$ , gly-1), 8.42 (2H, s, H-2 and H-6, iba), 8.30 (1H, s, H- $\delta$ , imz), 7.65 (1H, br. s,  $\text{NH}$ , imz), 4.05 (2H, d,  $J = 5.15$  Hz, H- $\alpha$ , gly-2), 3.90 (2H, d,  $J = 5.2$  Hz, H- $\alpha$ , gly-1), 3.53 (3H, s,  $\text{OCH}_3$ ), 2.69 (3H, s,  $\beta\text{-CH}_3$ , imz);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 176.6 (C=O, gly-1), 170.2 (C=O, gly-2), 168.4 (C=O,  $\text{ArCO}$ ), 158.7 (C-2, imz), 151.6 (C-4, iba), 146.3 (C-5,

imz), 141.2 (2C, C-2 and C-6, iba), 138.8 (C-1, iba), 137.4 (C-4, imz), 105.0 (2C, C-3 and C-5, iba), 54.5 (OCH<sub>3</sub>), 46.3 (C- $\alpha$ , gly-1), 41.4 (C- $\alpha$ , gly-2), 17.8 ( $\beta$ -CH<sub>3</sub>, imz).

***Methyl 1-[3,5-diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl]-L-prolyl-L-valyl-L-prolinate (2f)***

IR (CHCl<sub>3</sub>)  $\nu$  (cm<sup>-1</sup>): 3486 (N-H str, ring), 3136 (N-H str, amide), 3078, 3047 (Ar-H str), 2998-2993 (C-H str, CH<sub>2</sub>, pro), 2969, 2872 (C-H str, CH<sub>3</sub>), 1746 (C=O str, ester), 1662-1657, 1643 (C=O str, 3° and 2° amide), 1588, 1426 (skeletal bands), 1535 (N-H def, amide), 1386, 1360 (C-H bend, propyl-*i*), 1269 (C-O str, ester), 863, 839, 755 (C-H def, oop), 587 (C-I str); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.25 (1H, br. s, NH, val), 8.36 (2H, s, H-2 and H-6, iba), 8.30 (1H, s, H- $\delta$ , imz), 7.63 (1H, br. s, NH, imz), 4.70 (1H, dd,  $J$  = 5.9, 4.35 Hz, H- $\alpha$ , val), 4.08 (1H, t,  $J$  = 6.9 Hz, H- $\alpha$ , pro-1), 3.66 (1H, t,  $J$  = 6.85 Hz, H- $\alpha$ , pro-2), 3.61 (3H, s, OCH<sub>3</sub>), 3.45 (2H, t,  $J$  = 7.15 Hz, H- $\delta$ , pro-1), 3.45 (2H, t,  $J$  = 7.2 Hz, H- $\delta$ , pro-2), 2.65 (3H, s,  $\beta$ -CH<sub>3</sub>, imz), 2.62 (2H, q, H- $\beta$ , pro-1), 2.10-2.05 (1H, m, H- $\beta$ , val), 2.02-1.96 (4H, m, H- $\gamma$ , pro-1 and pro-2), 1.93 (2H, q, H- $\beta$ , pro-2), 1.05 (6H, d,  $J$  = 4.65 Hz, H- $\gamma$ , val); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 176.4 (C=O, pro-1), 173.1 (C=O, pro-2), 170.8 (C=O, ArCO), 167.2 (C=O, val), 159.0 (C-2, imz), 150.9 (C-4, iba), 146.6 (C-5, imz), 141.5 (2C, C-2 and C-6, iba), 138.0 (C-1, iba), 136.6 (C-4, imz), 104.2 (2C, C-3 and C-5, iba), 66.2 (C- $\alpha$ , pro-1), 60.7 (C- $\alpha$ , pro-2), 54.2 (OCH<sub>3</sub>), 53.0 (C- $\alpha$ , phe), 45.3 (C- $\delta$ , pro-2), 43.1 (C- $\delta$ , pro-1), 30.2 (C- $\beta$ , val), 29.3 (C- $\beta$ , pro-2), 27.7 (C- $\beta$ , pro-1), 27.0 (C- $\gamma$ , pro-1), 23.5 (C- $\gamma$ , pro-2), 19.8 (2C, C- $\gamma$ , val), 18.8 ( $\beta$ -CH<sub>3</sub>, imz).

***Methyl 1-[3,5-diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl]-L-isoleucyl-L-phenylalanyl-L-leucinate (2g)***

IR (CHCl<sub>3</sub>)  $\nu$  (cm<sup>-1</sup>): 3484 (N-H str, ring), 3079, 3052-3047 (Ar-H str), 2973, 2968, 2870 (C-H str, CH<sub>3</sub>), 2929-2925, 2855-2852 (C-H str, CH<sub>2</sub>), 1748 (C=O str, ester), 1647-1642 (C=O str, amide), 1587, 1422 (skeletal bands), 1538-1531 (N-H def, amide), 1386, 1360 (C-H bend, propyl-*i*), 1274 (C-O str, ester), 869, 832, 786-781, 748 (C-H def, oop), 590 (C-I str); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.39 (2H, s, H-

2 and H-6, iba), 8.30 (1H, s, H- $\delta$ , imz), 7.98 (1H, br. s,  $\text{NH}$ , phe), 7.65 (1H, br. s,  $\text{NH}$ , imz), 7.20 (2H, tt,  $J = 7.75, 4.8$  Hz, H-m, phe), 7.12 (1H, br. s,  $\text{NH}$ , phe), 6.98 (1H, t,  $J = 6.2$  Hz, H-p, phe), 6.84 (2H, dd,  $J = 8.7, 4.15$  Hz, H-o, phe), 6.74 (1H, br. s,  $\text{NH}$ , leu), 4.59 (1H, dd,  $J = 5.35, 3.7$  Hz, H- $\alpha$ , ile), 4.32 (1H, q, H- $\alpha$ , phe), 3.60 (3H, s,  $\text{OCH}_3$ ), 3.48 (1H, q, H- $\alpha$ , leu), 2.80 (2H, d,  $J = 5.65$  Hz, H- $\beta$ , phe), 2.62 (3H, s,  $\beta\text{-CH}_3$ , imz), 2.02-1.96 (1H, m, H- $\beta$ , ile), 1.65-1.59 (2H, m, H- $\gamma$ , ile), 1.50-1.43 (3H, m, H- $\beta$  and H- $\gamma$ , leu), 1.02 (3H, d,  $J = 5.9$  Hz, H- $\gamma'$ , ile), 0.98 (3H, d,  $J = 5.9$  Hz, H- $\delta$ , ile), 0.95 (6H, d,  $J = 6.25$  Hz, H- $\delta$ , leu);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 178.2 (C=O, leu), 171.5 (C=O, phe), 168.7 (C=O, ile), 168.0 (C=O,  $\text{ArCO}$ ), 159.4 (C-2, imz), 152.2 (C-4, iba), 139.9 (2C, C-2 and C-6, iba), 146.5 (C-5, imz), 137.8 (C-1, iba), 137.2 (C-4, imz), 134.6 (C- $\gamma$ , phe), 130.9 (2C, C-o, phe), 130.2 (2C, C-m, phe), 128.5 (C-p, phe), 105.6 (2C, C-3 and C-5, iba), 58.4 (C- $\alpha$ , ile), 52.3 ( $\text{OCH}_3$ ), 50.7 (C- $\alpha$ , leu), 49.5 (C- $\alpha$ , phe), 45.2 (C- $\beta$ , leu), 38.8 (C- $\beta$ , phe), 36.5 (C- $\beta$ , ile), 31.2 (C- $\gamma$ , ile), 26.4 (C- $\gamma$ , leu), 25.6 (2C, C- $\delta$ , leu), 17.9 ( $\beta\text{-CH}_3$ , imz), 14.4 (C- $\gamma'$ , ile), 10.5 (C- $\delta$ , ile); MASS  $m/z$  (% rel. int.): 843(2.3), 842(3.8), 841( $\text{M}^+$ , 4.5), 826(12.5), 810(33.6), 782(10.9), 697(59.2), 669(18.7), 550(100), 522(25.8), 437(65.4), 408(24.5), 328(10.9), 203(11.7), 126(6.5), 120(11.5), 91(12.4), 86(19.5), 82(13.1), 81(10.4), 65(17.2), 59(16.5), 57(14.2), 56(17.2), 55(11.6), 43(16.2), 42(11.7), 31(14.4), 29(10.2), 15(3.7).

***Methyl N-[3,5-diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl]glycyl-L-histidyl-L-alanyl-L-tryptophanate (2h)***

IR ( $\text{CHCl}_3$ )  $\nu$  ( $\text{cm}^{-1}$ ): 3489-3483 (N-H str, rings), 3078-3073, 3053 (Ar-H str), 2964, 2872, 2869 (C-H str,  $\text{CH}_3$ ), 2929-2922, 2855, 2850 (C-H str,  $\text{CH}_2$ ), 1754 (C=O str, ester), 1645-1641 (C=O str, amide), 1589-1584, 1428-1423 (skeletal bands), 1538-1533 (N-H def, amide), 1272 (C-O str, ester), 869-864, 838-835, 749 (C-H def, oop), 590 (C-I str);  $^1\text{H}$ -NMR ( $\text{DMSO}-d_6$ )  $\delta$  (ppm): 9.10 (1H, br. s,  $\text{NH}$ , gly), 8.58 (3H, br. s,  $\text{NH}$ , imz, his and try), 8.42 (2H, s, H-2 and H-6, iba), 8.30 (1H, s, H- $\delta$ , imz), 8.15 (1H, br. s,  $\text{NH}$ , ala), 7.78 (1H, br. s,  $\text{NH}$ , his), 7.65 (1H, s, H- $\delta$ ,

imz, his), 7.52 (1H, d,  $J = 7.75$  Hz, H-2, try), 7.36 (1H, d,  $J = 7.8$  Hz, H-2, his), 7.15-7.04 (4H, m, H-4-7, try), 6.77 (1H, br. s,  $\text{NH}$ , try), 4.30-4.25 (1H, m, H- $\alpha$ , ala), 4.23-4.17 (1H, m, H- $\alpha$ , try), 4.15-4.11 (1H, m, H- $\alpha$ , his), 4.08 (2H, d,  $J = 5.1$  Hz, H- $\alpha$ , gly), 3.57 (3H, s,  $\text{OCH}_3$ ), 3.05-2.96 (4H, m, H- $\beta$ , his and try), 2.66 (3H, s,  $\beta\text{-CH}_3$ , imz), 1.49 (3H, d,  $J = 5.85$  Hz, H- $\beta$ , ala);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 177.8 (C=O, gly), 175.2 (C=O, try), 174.4 (C=O, ala), 170.7 (C=O,  $\text{ArCO}$ ), 169.9 (C=O, his), 159.1 (C-2, imz), 152.0 (C-4, iba), 148.6 (C-2, his), 146.2 (C-5, imz), 141.4 (2C, C-2 and C-6, iba), 137.7 (C-2', try), 136.8 (C-4, imz), 136.0 (C-1, iba), 135.6 (C-4, his), 129.3 (C-3', try), 122.8 (C-2, try), 121.4, 119.7, 118.2 (3C, C-4-6, try), 117.1 (C-5, his), 112.4, 109.5 (2C, C-7 and C-3, try), 105.2 (2C, C-3 and C-5, iba), 63.2 (C- $\alpha$ , his), 60.5 (C- $\alpha$ , ala), 55.6 (C- $\alpha$ , try), 51.7 ( $\text{OCH}_3$ ), 48.2 (C- $\alpha$ , gly), 28.5 (C- $\beta$ , try), 20.1 (C- $\beta$ , ala), 19.6 (C- $\beta$ , his), 18.7 ( $\beta\text{-CH}_3$ , imz); MASS  $m/z$  (% rel. int.): 920(2.5), 919( $\text{M}^+$ , 3.6), 904(14.9), 888(30.9), 860(10.7), 702(59.8), 674(16.6), 631(100), 603(20.4), 494(72.5), 466(23.3), 437(38.6), 408(12.7), 328(10.6), 203(12.2), 159(17.4), 130(9.9), 126(8.8), 116(8.5), 110(14.8), 82(13.3), 81(19.7), 67(5.9), 59(15.8), 56(17.4), 55(9.6), 44(12.2), 31(10.9), 30(5.7), 15(3.4).

### ***Preparation of 3,5-diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl amino acids and peptides (3a-d)***

To a solution of the amino acid/peptide methyl esters (0.01 mol) in THF :  $\text{H}_2\text{O}$  (1 : 1, 36 ml), LiOH (0.36 g, 0.015 mol) was added at 0 °C. The mixture was stirred at RT for 1 h and then acidified to pH 3.5 with 1N  $\text{H}_2\text{SO}_4$ . The aqueous layer was extracted with diethyl ether ( $3 \times 25$  ml). The combined organic extracts were dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The crude products were crystallized from methanol and ether to get hydrolyzed peptide derivatives.

### ***3,5-Diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl-L-leucine (3a)***

IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3486 (N-H str, ring), 3298-2508 (O-H str, COOH), 3137 (NH str, amide), 3078, 3046 (Ar-H str), 2965 (C-H str,  $\text{CH}_3$ ), 2927, 2852 (C-H str,  $\text{CH}_2$ ),

1713 (C=O str, COOH), 1644 (C=O str, amide), 1588, 1423 (skeletal bands), 1536 (N–H def, amide), 1406 (O–H def, COOH), 1387, 1360 (C–H bend, propyl-*l*), 860, 836, 759 (C–H def, oop), 585 (C–I str);  $^1\text{H-NMR}$  (DMSO- $d_6$ )  $\delta$  (ppm): 10.05 (2H, br. s,  $\text{NH}$ , imz and  $\text{OH}$ , COOH), 8.32 (2H, s, H-2 and H-6, iba), 8.30 (1H, s, H- $\delta$ , imz), 8.07 (1H, br. s,  $\text{NH}$ , leu), 4.38-4.33 (1H, m, H- $\alpha$ , leu), 2.69 (3H, s,  $\beta\text{-CH}_3$ , imz), 1.98-1.85 (1H, m, H- $\gamma$ , leu), 1.55 (2H, t, H- $\beta$ , leu), 0.97 (6H, d,  $J = 6.15$  Hz, H- $\delta$ , leu);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 175.0 (C=O, leu), 168.2 (C=O,  $\text{ArCO}$ ), 159.0 (C-2, imz), 154.5 (C-4, iba), 146.4 (C-5, imz), 140.2 (2C, C-2 and C-6, iba), 137.6 (C-4, imz), 136.6 (C-1, iba), 106.7 (2C, C-3 and C-5, iba), 49.2 (C- $\alpha$ , leu), 39.9 (C- $\beta$ , leu), 24.4 (C- $\gamma$ , leu), 19.9 (2C, C- $\delta$ , leu), 18.3 ( $\beta\text{-CH}_3$ , imz).

### **3,5-Diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl-L-histidyl-L-tyrosine (3b)**

IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3489, 3482 (N–H str, rings), 3356 (O–H str, Ar–OH), 3294-2503 (O–H str, COOH), 3077-3072, 3054 (Ar–H str), 2965, 2870 (C–H str,  $\text{CH}_3$ ), 2930, 2929, 2852, 2848 (C–H str,  $\text{CH}_2$ ), 1708 (C=O str, COOH), 1644-1640 (C=O str, amide), 1590-1587, 1425-1419 (skeletal bands), 1409 (O–H def, COOH), 866-862, 839, 789, 754, 651 (C–H def, oop), 585 (C–I str);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 9.38 (1H, br. s,  $\text{NH}$ , his), 8.69 (4H, br. s,  $\text{NH}$ , imz, his and  $\text{OH}$ , tyr, COOH), 8.42 (2H, s, H-2 and H-6, iba), 8.32 (1H, s, H- $\delta$ , imz), 7.65 (1H, s, H-4, his), 7.39 (1H, d,  $J = 7.8$  Hz, H-2, tyr), 7.25 (1H, br. s,  $\text{NH}$ , tyr), 7.17 (2H, dd,  $J = 8.65, 5.3$  Hz, H-o, tyr), 6.79 (2H, dd,  $J = 8.7, 4.95$  Hz, H-m, tyr), 5.80–5.75 (1H, m, H- $\alpha$ , his), 4.20–4.15 (1H, m, H- $\alpha$ , tyr), 3.10 (2H, d,  $J = 5.5$  Hz, H- $\beta$ , tyr), 2.90 (2H, d,  $J = 5.85$  Hz, H- $\beta$ , tyr), 2.65 (3H, s,  $\beta\text{-CH}_3$ , imz);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 173.4 (C=O, tyr), 171.6 (C=O, his), 171.2 (C=O,  $\text{ArCO}$ ), 159.2 (C-2, imz), 154.4 (C-p, tyr), 151.6 (C-4, iba), 149.3 (C-2, his), 146.2 (C-5, imz), 142.1 (2C, C-m, tyr), 139.6 (2C, C-2 and C-6, iba), 137.1 (C-4, imz), 136.4 (C-1, iba), 135.0 (C-4, his), 128.8 (2C, C-o, tyr), 127.2 (C- $\gamma$ , tyr), 117.0 (C-5, his), 105.2 (2C, C-3 and C-5, iba), 65.5 (C- $\alpha$ , his), 54.3 (C- $\alpha$ , tyr), 37.7 (C- $\beta$ , tyr), 19.8 (C- $\beta$ , his), 18.6 ( $\beta\text{-CH}_3$ , imz).



**3,5-Diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl-L-isoleucyl-L-phenylalanyl-L-leucine (3c)**

IR (CHCl<sub>3</sub>)  $\nu$  (cm<sup>-1</sup>): 3487 (N–H str, ring), 3308-2513 (O–H str, COOH), 3077, 3050-3046 (Ar–H str), 2971, 2966, 2872 (C–H str, CH<sub>3</sub>), 2928-2922, 2856-2853 (C–H str, CH<sub>2</sub>), 1711 (C=O str, COOH), 1646-1640 (C=O str, amide), 1588, 1420 (skeletal bands), 1536-1530 (N–H def, amide), 1408 (O–H def, COOH), 1388, 1362 (C–H bend, propyl-*l*), 867, 830, 785-780, 749 (C–H def, oop), 588 (C–I str); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 10.05 (2H, br. s, NH, imz and OH, COOH), 8.41 (2H, s, H-2 and H-6, iba), 8.30 (1H, s, H- $\delta$ , imz), 7.99 (1H, br. s, NH, phe), 7.22 (2H, tt, *J* = 7.8, 4.75 Hz, H-*m*, phe), 7.15 (1H, br. s, NH, leu), 7.10 (1H, br. s, NH, phe), 7.02 (1H, t, *J* = 6.15 Hz, H-*p*, phe), 6.82 (2H, dd, *J* = 8.75, 4.15 Hz, H-*o*, phe), 4.84 (1H, q, H- $\alpha$ , phe), 4.58 (1H, dd, *J* = 5.4, 3.75 Hz, H- $\alpha$ , ile), 3.65 (1H, q, H- $\alpha$ , leu), 2.82 (2H, d, *J* = 5.65 Hz, H- $\beta$ , phe), 2.66 (3H, s,  $\beta$ -CH<sub>3</sub>, imz), 2.05-1.98 (1H, m, H- $\beta$ , ile), 1.95-1.88 (1H, m, H- $\gamma$ , leu), 1.67-1.55 (4H, m, H- $\gamma$ , ile and H- $\beta$ , leu), 1.05 (3H, d, *J* = 5.85 Hz, H- $\gamma'$ , ile), 0.99 (3H, d, *J* = 5.95 Hz, H- $\delta$ , ile), 0.94 (6H, d, *J* = 6.3 Hz, H- $\delta$ , leu); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 174.3 (C=O, leu), 171.0 (C=O, phe), 168.9 (C=O, ile), 168.4 (C=O, ArCO), 159.7 (C-2, imz), 152.0 (C-4, iba), 140.4 (2C, C-2 and C-6, iba), 146.0 (C-5, imz), 137.5 (C-1, iba), 136.8 (C-4, imz), 135.2 (C- $\gamma$ , phe), 130.7 (2C, C-*o*, phe), 130.0 (2C, C-*m*, phe), 128.2 (C-*p*, phe), 105.4 (2C, C-3 and C-5, iba), 58.6 (C- $\alpha$ , ile), 51.1 (C- $\alpha$ , leu), 50.7 (C- $\alpha$ , phe), 39.9 (C- $\beta$ , leu), 38.6 (C- $\beta$ , ile), 38.0 (C- $\beta$ , phe), 32.3 (C- $\gamma$ , ile), 24.8 (C- $\gamma$ , leu), 22.1 (2C, C- $\delta$ , leu), 18.3 ( $\beta$ -CH<sub>3</sub>, imz), 14.5 (C- $\gamma'$ , ile), 10.2 (C- $\delta$ , ile); MASS *m/z* (% rel. int.): 828(2.9), 827(M<sup>+</sup>, 3.7), 810(30.9), 782(12.5), 697(55.7), 669(19.2), 550(100), 522(23.7), 437(68.5), 408(22.9), 328(11.2), 203(10.9), 126(8.7), 120(10.6), 91(10.9), 86(18.2), 82(13.7), 81(11.2), 65(15.7), 57(13.6), 56(16.4), 55(10.9), 45(13.5), 43(15.5), 42(12.2), 29(9.8), 17(3.8), 15(4.2).

**N-[3,5-Diiodo-4-(2-methyl-1H-imidazol-5-yl)benzoyl]glycyl-L-histidyl-L-alanyl-L-tryptophan (3d)**

IR (CHCl<sub>3</sub>)  $\nu$  (cm<sup>-1</sup>): 3487-3482 (N-H str, rings), 3295-2502 (O-H str, COOH), 3077-3072, 3050 (Ar-H str), 2965, 2870, 2869 (C-H str, CH<sub>3</sub>), 2927-2920, 2855, 2852 (C-H str, CH<sub>2</sub>), 1709 (C=O str, COOH), 1644-1640 (C=O str, amide), 1589-1585, 1427-1422 (skeletal bands), 1536-1532 (N-H def, amide), 1405 (O-H def, COOH), 867-863, 839-834, 747 (C-H def, oop), 586 (C-I str); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.45 (4H, br. s, NH, imz, his, try and OH, COOH), 9.11 (1H, br. s, NH, gly), 8.43 (2H, s, H-2 and H-6, iba), 8.29 (1H, s, H- $\delta$ , imz), 8.16 (1H, br. s, NH, ala), 7.76 (1H, br. s, NH, his), 7.67 (1H, s, H- $\delta$ , imz, his), 7.56 (1H, d, *J* = 7.7 Hz, H-2, try), 7.53 (1H, br. s, NH, try), 7.38 (1H, d, *J* = 7.75 Hz, H-2, his), 7.13-6.98 (4H, m, H-4-7, try), 4.77-4.72 (1H, m, H- $\alpha$ , ala), 4.48-4.42 (1H, m, H- $\alpha$ , try), 4.16-4.12 (1H, m, H- $\alpha$ , his), 4.10 (2H, d, *J* = 5.15 Hz, H- $\alpha$ , gly), 3.08-2.97 (4H, m, H- $\beta$ , his and try), 2.67 (3H, s,  $\beta$ -CH<sub>3</sub>, imz), 1.51 (3H, d, *J* = 5.9 Hz, H- $\beta$ , ala); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 178.3 (C=O, gly), 172.8 (C=O, ala), 172.3 (C=O, try), 170.8 (C=O, ArC=O), 170.2 (C=O, his), 159.3 (C-2, imz), 151.7 (C-4, iba), 149.2 (C-2, his), 145.9 (C-5, imz), 141.8 (2C, C-2 and C-6, iba), 138.5 (C-2', try), 137.4 (C-4, imz), 136.7 (C-1, iba), 135.3 (C-4, his), 128.9 (C-3', try), 124.4 (C-2, try), 123.1, 119.4, 118.0 (3C, C-4-6, try), 116.8 (C-5, his), 113.6, 110.5 (2C, C-7 and C-3, try), 105.7 (2C, C-3 and C-5, iba), 60.0 (C- $\alpha$ , ala), 59.2 (C- $\alpha$ , his), 54.3 (C- $\alpha$ , try), 49.1 (C- $\alpha$ , gly), 28.8 (C- $\beta$ , try), 20.6 (C- $\beta$ , ala), 19.8 (C- $\beta$ , his), 18.9 ( $\beta$ -CH<sub>3</sub>, imz); MASS *m/z* (% rel. int.): 906(3.3), 905(M<sup>+</sup>, 4.2), 888(28.9), 860(11.9), 702(55.9), 674(15.2), 631(100), 603(23.2), 494(70.2), 466(23.9), 437(36.8), 408(11.9), 328(11.7), 203(10.5), 159(16.7), 130(8.7), 126(7.9), 116(9.3), 110(13.6), 82(12.8), 81(17.9), 67(6.2), 56(16.8), 55(7.9), 45(11.9), 44(11.6), 30(4.9), 17(3.6), 15(3.9).

## Conclusion

Present investigation describes successful synthesis of title compounds via coupling reaction in good yields. DCC proved to be effective coupling agent both economically and yieldwise, in comparison to DIPC. Greater antifungal activity was found in derivatives with tryptophan and histidine constituents in their amino acid

chain. Gram negative bacteria were found to be more sensitive in comparison to gram positive bacteria towards the newly synthesized peptide derivatives. Hydrolyzed peptide derivatives exhibited more antimicrobial activity when compared to corresponding methyl ester analogs. Among tested compounds, **2c**, **2d**, **2e**, **2f**, **2h**, **3b** and **3d** possessed better antimicrobial activity as compared to standard drugs. On passing toxicity tests, these compounds may prove good candidates for clinical studies and may be potential antifungal and antibacterial agents of future.

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