

Full Paper

Synthesis and Biological Activity of Peptide Derivatives of Iodoquinazolinones/Nitroimidazoles

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Abstract: Two substituted quinazolinyl/imidazolyl-salicylic acids **5**, **6** were synthesized by the reaction of 6-iodo-2-methylbenzoxazin-4-one/5-nitroimidazole with 5-aminosalicylic acid (5-ASA). Coupling of compounds **5** and **6** with different amino acid ester hydrochlorides, dipeptide and tripeptide methyl esters yielded novel quinazolino/imidazolo-peptide derivatives **5a-f** and **6a-g**. The chemical structures of all newly synthesized compounds were confirmed by means of FT-IR, ¹H- and ¹³C-NMR, MS and elemental analysis. Selected peptide ester derivatives were further hydrolyzed by using lithium hydroxide (LiOH) to afford the corresponding acid derivatives **5b_a-d_a** and **6e_a-g_a**. All peptide derivatives were assayed for antimicrobial and anthelmintic activities against eight pathogenic microbes and three earthworm species. Among the tested compounds, **5e**, **5d**, **6e** and their hydrolyzed analogs **5d_a** and **6e_a** exhibited higher antimicrobial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*, and **5a**, **6g** and **6g_a** displayed better antifungal activity against the dermatophytes *Trichophyton mentagrophytes* and *Microsporum audouinii*. Moreover, **6f** and its hydrolyzed derivative **6f_a** showed good anthelmintic activity against *Megascopex konkanensis*, *Pontoscotex corethruses* and *Eudrilus eugeniae* at dose of 2 mg mL⁻¹.

Keywords: Quinazolinone; imidazole; 5-aminosalicylic acid; peptides; antimicrobial activity; anthelmintic activity.

Introduction

During past decades, compounds bearing heterocyclic nuclei have received much attention due to their chemotherapeutic value in the development of novel antimicrobials and anthelmintics. Quinazolinone and imidazole analogs are associated with a variety of pharmacological activities including antibacterial and antifungal [1-3], anti-inflammatory and analgesic [4-6], antitubercular [7,8], cytotoxic [9-11], antiviral [12, 13], anticonvulsant [14], antimuscarinic [15], insecticidal [16], farnesyltransferase, gastric H^+/K^+ -ATPase and MAP kinase p38 inhibitory properties [17-19]. Furthermore, the literature is enriched with several findings indicating antimicrobial potential of salicylic acid and its analogs [20-22].

Prompted by the chemotherapeutic importance of quinazolinones/imidazoles and salicylic acid derivatives, these two vital moieties were combined together into a single molecule by varying the substitution pattern on heterocyclic moieties to yield 2-hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoic acid (**5**) and 2-hydroxy-5-(5-nitro-1*H*-imidazol-2-yl)benzoic acid (**6**).

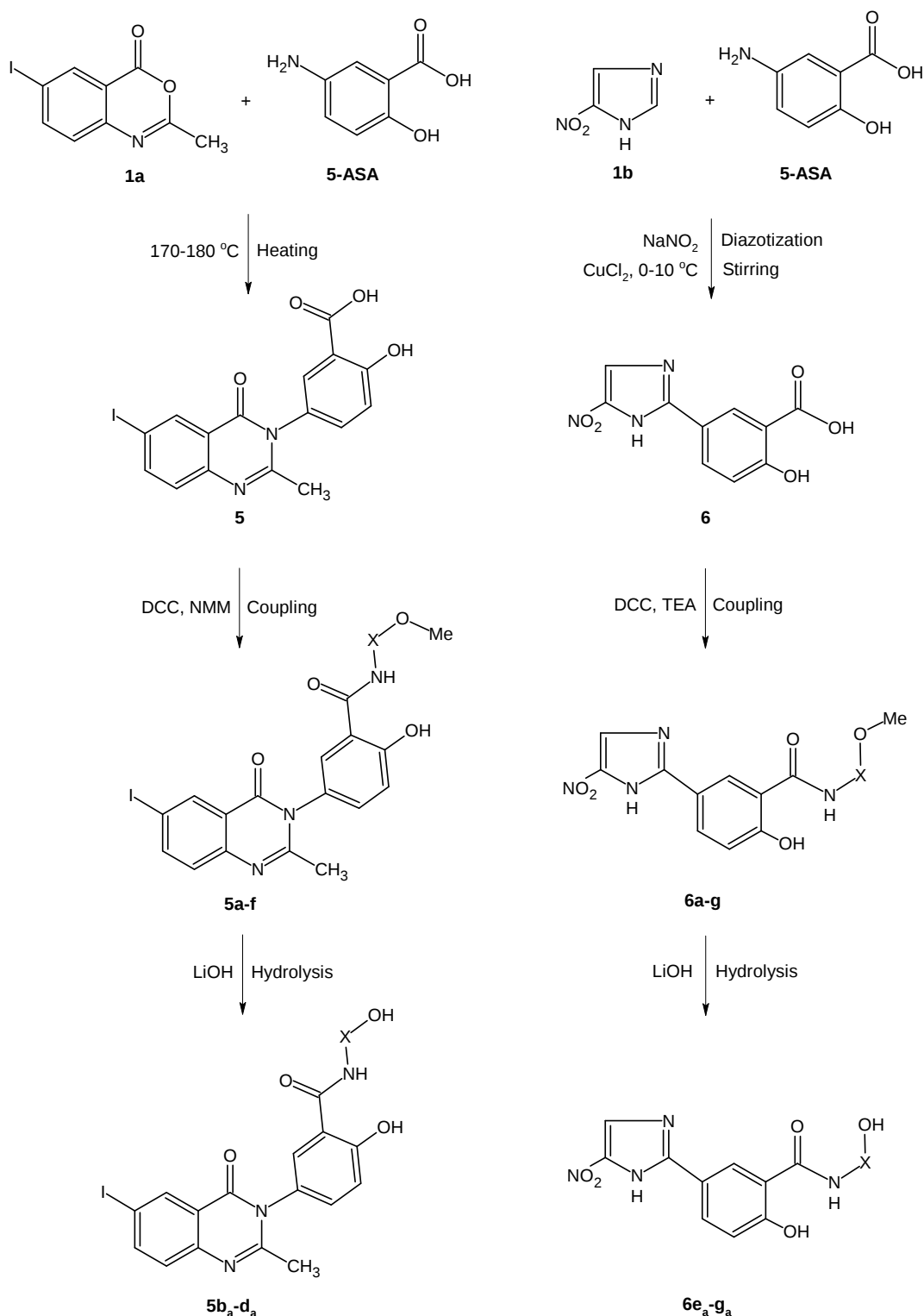
The literature contains several reports on the incorporation of amino acids and peptides into the aromatic and heterocyclic congeners resulting in compounds with potent bioactivities [23-27]. Thus, keeping in mind the pharmacological potential of quinazolinones/imidazoles/salicylic acids as well as taking advantage of biodegradability and biocompatibility of amino acids/peptides and further, in continuation of our earlier work on synthesis of bioactive peptide analogs of aroylbenzoic acids, aryloxyacetic acids, benzimidazoles and furoic acid [28], an attempt was made towards the synthesis of two novel series of peptidyl derivatives of the iodoquinazolinones/nitroimidazoles – 2-hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl amino acids/peptides **5a-f** and 2-hydroxy-5-(5-nitro-1*H*-imidazol-2-yl)benzoyl amino acids/peptides **6a-g**. Selected peptide derivatives were further hydrolyzed to get corresponding acid derivatives **5b_a-d_a** and **6e_a-g_a**. The potential antibacterial, antifungal and anthelmintic activities of the synthesized compounds were also evaluated.

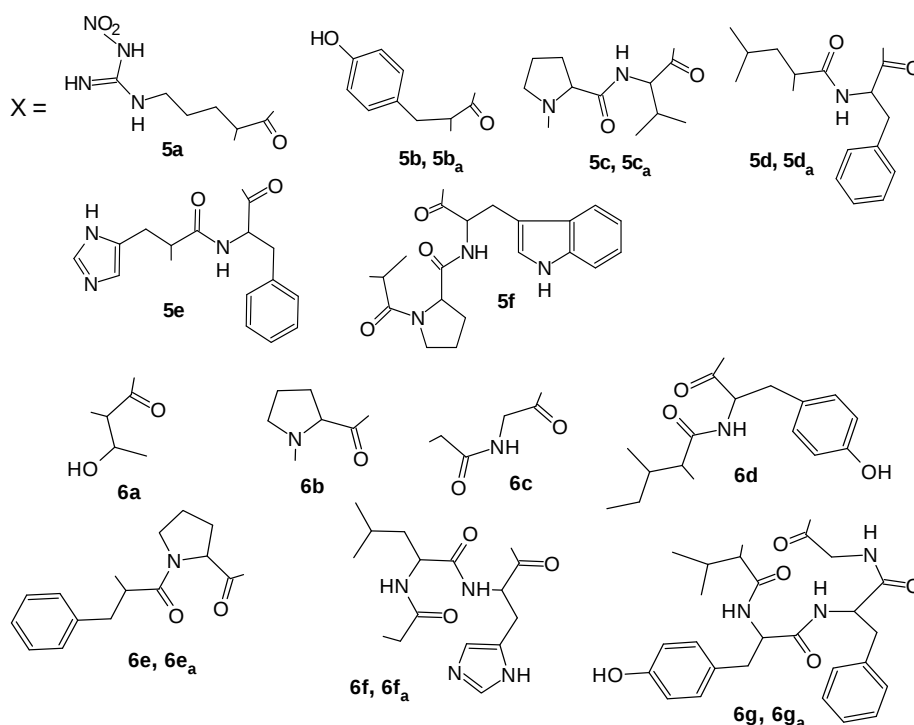
Results and Discussion

6-Iodo-2-methylbenzoxazin-4-one (**1a**) was prepared in good yield according to a literature procedure [16] by refluxing of 5-iodoanthranilic acid and acetic anhydride with stirring. Imidazole was nitrated using nitrating mixture by the standard procedure [29] to afford 5-nitroimidazole (**1b**). Dipeptides Boc-Pro-Val-OMe (**2a**), Boc-Leu-Phe-OMe (**2b**), Boc-His-Phe-OMe (**2c**), Boc-Gly-Gly-OMe (**2d**), Boc-Ile-Tyr-OMe (**2e**), Boc-Phe-Pro-OMe (**2f**) were prepared by coupling Boc-amino acids with the respective amino acid methyl ester hydrochlorides using dicyclohexylcarbodiimide (DCC) as coupling agent and triethylamine (TEA) as base, according to the Bodanzsky and Bodanzsky procedure with suitable modifications [30]. Similarly, tripeptides Boc-Ala-Pro-Tyr-OMe (**3a**), Boc-Gly-Leu-His-OMe (**3b**) and tetrapeptide Boc-Val-Tyr-Phe-Gly-OMe (**4a**) were synthesized by coupling Boc-dipeptides with respective amino acid methyl ester hydrochlorides/dipeptide methyl esters under alkaline conditions. Prior to coupling, all di-/tri- and tetrapeptides were deprotected at the amino end using trifluoroacetic acid (TFA). Compound **5** was prepared by the reaction of **1a** and 5-ASA in ethanol, whereas compound **6** was synthesized by stirring **1b** with a solution of diazotized 5-ASA. Finally, compound **5** was coupled with different amino acid methyl ester hydrochlorides and

peptide methyl esters using DCC and *N*-methylmorpholine (NMM) in THF to afford peptide derivatives **5a-f**. Similarly, coupling of compound **6** with different peptide methyl esters in the presence of DCC and TEA in DMF afforded amino acid/peptide conjugates **6a-g**. Furthermore, amino acid/peptide derivatives **5b-d** and **6e-g** were hydrolyzed by stirring with LiOH to yield corresponding acid derivatives **5b_a-d_a** and **6e_a-g_a** (Scheme 1).

Scheme 1. Synthesis of peptide analogs of iodoquinazolinones/nitroimidazoles **5a-6g_a**.





All peptide derivatives **5a–6g_a** were synthesized in good yields using DCC as coupling agent and TEA/NMM as bases. Presence of bands at 3365–3362, 3305–2505, 1702–1697, 1542, 1349, 590 cm^{-1} in the IR spectra of compounds **5** and **6** clearly indicated presence of functional groups like -COOH , -OH , -NO_2 and -I and the absence of a free -NH_2 group which was present in the starting material 5-ASA. Furthermore, the IR spectra of peptide derivatives **5a–6g** showed amide I and amide II bands at 1660–1637 cm^{-1} and 1538–1525 cm^{-1} , indicating formation of peptide bonds and confirming the success of the coupling reactions. This fact was further supported by the appearance of broad singlets for imino proton of the CO-NH moiety at 8.62–6.50 ppm in the ^1H -NMR spectra and singlets at 177.3–166.2 ppm (for the carbonyl carbon of the CO-NH moiety) in the ^{13}C -NMR spectra of compounds **5a–6g**. Moreover, the presence of a NO_2 group in the peptide analogs of compound **6** was indicated by appearance of medium bands at 1544–1540 cm^{-1} and 1349–1345 cm^{-1} (asymmetric and symmetric NO_2 stretching) in the IR spectra, whereas presence of a iodo group in the peptide derivatives of compound **5** was indicated by the appearance of medium intensity bands at 592–587 cm^{-1} (C-I_{str}) in the IR spectra. The 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 $\text{R-C}\equiv\text{O}^+$ ion peaks at $M^+ - 31$, along with characteristic fragmentation patterns on both sides of 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 the mass spectra of the synthesized peptide derivatives. Structures of hydrolyzed derivatives **5b_a–6g_a** were confirmed by the appearance of strong bands at 1713–1710 cm^{-1} (C=O_{str} , COOH) in the IR spectra, broad singlets at 8.17–7.42 ppm (for hydroxyl proton of COOH) in the ^1H -NMR spectra and singlets at 177.2–175.4 ppm (for carbonyl carbon of COOH) in their ^{13}C -NMR spectra. This fact was further supported by the disappearance of the medium to strong bands at 1752–1742 cm^{-1} (C=O_{str} , ester) and 1272–1268 cm^{-1} (C-O_{str} , ester) in the IR spectra and the singlets at 53.9–52.1 ppm (for the carbonyl carbon of OCH_3) in the ^{13}C -NMR spectra of compounds **5b_a–6g_a**.

All hydrolyzed peptide derivatives showed peaks at $M^+ - 17$ and $M^+ - 45$ in their mass spectra corresponding to the loss of OH and COOH, respectively. Moreover, a $[\text{COOH}^+]$ fragment ion peak appeared at m/z value 45 in the mass spectra of compounds **6f_a** and **6g_a**, along with characteristic fragmentation patterns on both sides of the carbonyl moiety. The newly synthesized compounds were also analyzed for C, H and N content and the results revealed deviations of ± 0.04 from calculated values.

Almost all the synthesized compounds were found to exhibit moderate to good bioactivity against Gram negative bacteria, dermatophytes and *C. albicans*. However, **5b-6g_a** displayed mild to moderate activity against Gram positive bacteria and *A. niger*. Comparison of the antimicrobial activity data suggested that the hydrolyzed peptide derivatives **5b_a-d_a** and **6e_a-g_a** are more potent antimicrobial agents than their corresponding methyl ester derivatives **5b-6g**, but the methyl ester analogs were found to be more potent than the corresponding acid derivatives against dermatophytes.

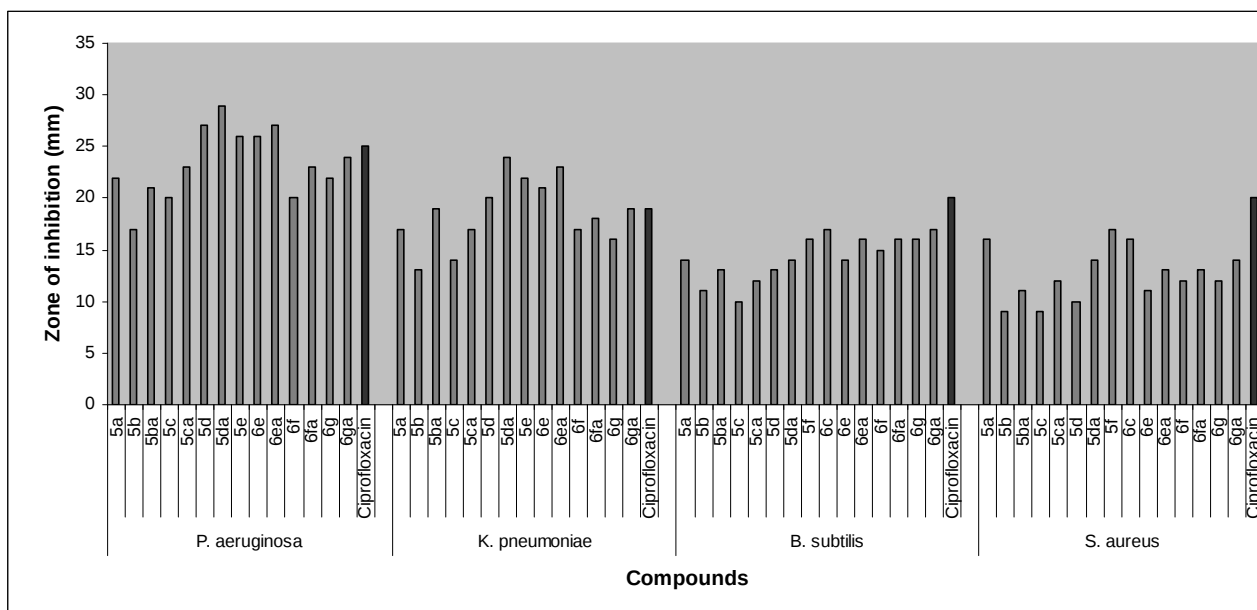
Compounds **5a**, **5d**, **5d_a**, **5e**, **6e**, **6g**, **6g_a** and **6e_a** were found to be the most active compounds, with higher antimicrobial activity against *P. aeruginosa*, *K. pneumoniae*, *C. albicans* and dermatophytes. Against Gram positive bacteria and *A. niger*, only compounds **5a**, **5f** and **6c** exhibited significant activity in comparison to reference drugs - ciprofloxacin and griseofulvin respectively (Table 1, Figure 1).

Table 1. Antimicrobial activity of compounds **5a-6e_a**.

Compound	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>
5a	14(6) [†]	16(12.5)	22(6)	17(12.5)	14(12.5)	19(6)	14(12.5)	22(6)
5b	11(25)	9(25)	17(12.5)	13(25)	15(6)	12(6)	15(25)	15(6)
5c	10(12.5)	9(50)	20(6)	14(12.5)	18(6)	13(6)	16(50)	17(6)
5d	13(25)	10(25)	27(6)	20(12.5)	22(6)	12(6)	16(50)	18(6)
5e	9(12.5)	8(25)	26(6)	22(12.5)	24(6)	9(12.5)	7(12.5)	13(12.5)
5f	16(6)	17(12.5)	20(6)	18(12.5)	11(12.5)	10(6)	15(12.5)	9(25)
5b_a	13(25)	11(25)	21(12.5)	19(25)	17(6)	10(6)	16(25)	14(6)
5c_a	12(12.5)	12(50)	23(6)	17(12.5)	19(6)	11(6)	17(50)	14(6)
5d_a	14(25)	14(25)	29(6)	24(12.5)	25(6)	10(6)	17(50)	16(6)
6a	16(25)	10(50)	24(6)	18(25)	11(12.5)	11(12.5)	14(25)	12(25)
6b	14(50)	12(25)	23(6)	15(12.5)	13(12.5)	9(6)	19(25)	12(6)
6c	17(6)	16(12.5)	23(12.5)	16(12.5)	12(25)	8(25)	14(12.5)	10(6)
6d	17(25)	12(25)	22(6)	18(25)	15(12.5)	10(12.5)	14(25)	12(12.5)
6e	14(25)	11(50)	26(6)	21(12.5)	23(6)	11(6)	13(25)	13(6)
6f	15(50)	12(25)	20(6)	17(12.5)	15(6)	12(6)	15(50)	17(6)
6g	16(25)	12(50)	22(12.5)	16(25)	16(6)	21(6)	14(50)	23(6)
6e_a	16(25)	13(50)	27(6)	23(12.5)	25(6)	10(6)	14(25)	11(6)
6f_a	16(50)	13(25)	23(6)	18(12.5)	16(6)	11(6)	16(50)	15(6)
6g_a	17(25)	14(50)	24(12.5)	19(25)	17(6)	23(6)	15(50)	25(6)
Control*	—	—	—	—	—	—	—	—
Ciprofloxacin	20(6)	20(12.5)	25(6)	19(12.5)	—	—	—	—
Griseofulvin	—	—	—	—	20(6)	17(6)	18(12.5)	20(6)

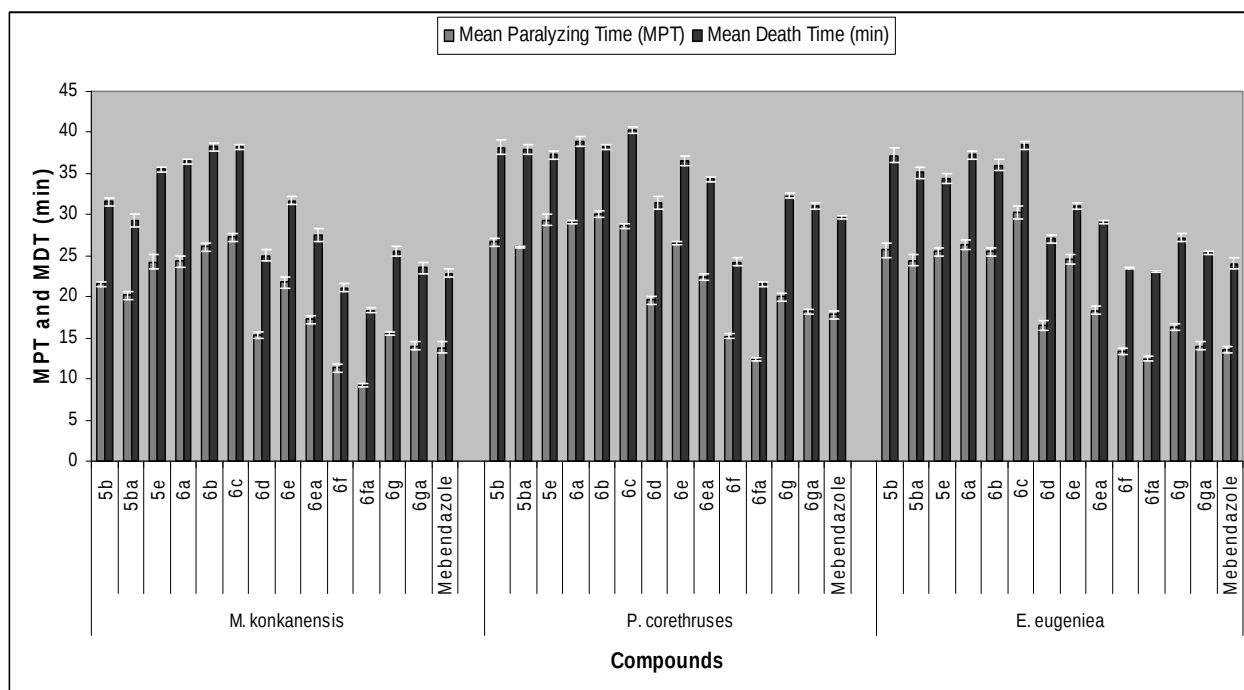
[†] Values in bracket are MIC values ($\mu\text{g mL}^{-1}$).

* Dimethylformamide (DMF) / Dimethylsulfoxide (DMSO).

Figure 1. Comparison of antimicrobial activity of quinazolino/imidazolo peptide derivatives.**Table 2.** Anthelmintic activity of compounds 5a–6e_a

Compound	Earthworm species					
	<i>M. konkanensis</i>		<i>P. corethruses</i>		<i>E. eugeniea</i>	
	Mean paralyzing time (min) [‡]	Mean death time (min) [‡]	Mean paralyzing time (min)	Mean death time (min)	Mean paralyzing time (min)	Mean death time (min)
5a	40.38 ± 0.52	52.58 ± 0.59	42.57 ± 0.26	54.26 ± 0.42	39.25 ± 0.23	49.34 ± 1.62
5b	21.56 ± 0.28	31.56 ± 0.45	26.65 ± 0.44	38.22 ± 0.87	25.67 ± 0.82	37.21 ± 0.82
5c	42.34 ± 0.59	55.40 ± 0.84	41.17 ± 0.88	55.40 ± 0.43	40.73 ± 0.49	51.54 ± 0.93
5d	44.68 ± 0.12	52.59 ± 0.72	44.55 ± 0.23	54.18 ± 0.17	41.49 ± 0.32	51.28 ± 0.44
5e	24.22 ± 0.21	35.48 ± 0.16	29.35 ± 0.65	37.24 ± 0.54	25.45 ± 0.58	34.34 ± 0.62
5f	43.25 ± 0.44	50.52 ± 0.43	42.22 ± 0.24	57.50 ± 0.42	43.59 ± 0.41	55.10 ± 0.60
5b _a	20.15 ± 0.52	29.29 ± 0.76	26.04 ± 0.12	37.96 ± 0.54	24.38 ± 0.69	35.12 ± 0.71
5c _a	40.29 ± 0.89	53.27 ± 0.92	39.61 ± 0.21	50.39 ± 0.20	39.80 ± 0.85	49.24 ± 0.55
5d _a	40.54 ± 0.83	52.18 ± 0.28	40.47 ± 0.47	52.02 ± 0.18	37.44 ± 0.89	47.08 ± 0.38
6a	24.28 ± 0.67	36.46 ± 0.28	29.14 ± 0.23	38.82 ± 0.58	26.34 ± 0.56	37.30 ± 0.49
6b	26.04 ± 0.53	38.28 ± 0.71	30.10 ± 0.38	38.29 ± 0.30	27.50 ± 0.51	36.02 ± 0.66
6c	27.23 ± 0.56	38.23 ± 0.38	28.60 ± 0.33	40.32 ± 0.37	30.22 ± 0.75	38.45 ± 0.48
6d	15.33 ± 0.40	25.04 ± 0.77	19.57 ± 0.56	31.42 ± 0.86	16.43 ± 0.60	27.02 ± 0.53
6e	21.73 ± 0.64	31.73 ± 0.49	26.50 ± 0.23	36.58 ± 0.63	24.48 ± 0.59	31.08 ± 0.37
6f	11.31 ± 0.43	21.10 ± 0.44	15.19 ± 0.31	24.24 ± 0.45	13.39 ± 0.33	23.45 ± 0.12
6g	15.55 ± 0.21	25.56 ± 0.62	20.02 ± 0.50	32.32 ± 0.34	16.27 ± 0.38	27.15 ± 0.46
6e _a	17.25 ± 0.52	27.48 ± 0.83	22.38 ± 0.33	34.33 ± 0.28	18.37 ± 0.44	29.06 ± 0.21
6f _a	09.21 ± 0.22	18.34 ± 0.30	12.41 ± 0.15	21.55 ± 0.26	12.45 ± 0.33	23.09 ± 0.10
6g _a	14.05 ± 0.46	23.52 ± 0.66	18.19 ± 0.36	31.03 ± 0.44	14.02 ± 0.43	25.34 ± 0.22
Control [#]	—	—	—	—	—	—
Mebendazole	13.85 ± 0.64	22.85 ± 0.53	17.82 ± 0.43	29.60 ± 0.22	13.54 ± 0.45	24.05 ± 0.62

[‡] Data are given as mean ± S.D. (n = 3).[#] Tween 80 (0.5 %) in distilled water.

Figure 2. Comparison of anthelmintic activity of quinazolino/imidazolo peptide derivatives.

All imidazolo peptide derivatives **6a-g_a** showed moderate to good anthelmintic activity at 2 mg mL⁻¹ concentration in Tween 80 (0.5 %) and distilled water, whereas quinazolino peptide analogs **5a-d_a** displayed mild to moderate activity. Comparison of anthelmintic activity data revealed that hydrolyzed peptide derivatives **5b_a-6g_a** are slightly more active than their corresponding ester derivatives **5b-6g**. Compound **6f** and its hydrolyzed derivative **6f_a** were found to exhibit higher bioactivity against all three earthworm species, in comparison to the standard drug - mebendazole. Compounds **6d**, **6g** and **6g_a** showed good anthelmintic activity whereas compounds **6a-c**, **6e** and **6e_a** displayed only moderate level of activity. Among quinazolino peptides, no compound exhibited any significant anthelmintic activity except moderate activity for compounds **5e**, **5b** and its hydrolyzed derivative **5b_a** (Table 2, Figure 2).

Conclusions

The present study reports the successful synthesis of the title compounds in good yields via coupling reactions. For peptide coupling, the method employing DCC/TEA in DMF solvent proved to be better than the DCC/NMM method utilizing THF as solvent, providing 10-15 % additional yields. Gram negative bacteria proved to be more sensitive in comparison to Gram positive bacteria towards newly synthesized peptide derivatives. Greater anthelmintic activity was found in derivatives with histidine and tyrosine constituents in their amino acid chain. Hydrolyzed derivatives exhibited more antimicrobial and anthelmintic activity in comparison to their corresponding methyl ester derivatives except against dermatophytes. Among the tested compounds, **5a**, **5d**, **5d_a**, **5e**, **5f**, **6c**, **6g**, **6g_a**, **6e** and **6e_a** showed good antimicrobial activity and, **6f** and **6f_a** exhibited better anthelmintic activity. On passing toxicity tests, these compounds may prove good candidates for clinical studies and may be potential antimicrobial and anthelmintic agents of future.

Experimental

General

Melting points were determined by the open capillary method and are uncorrected. L-Amino acids, di-*tert*-butylpyrocarbonate (Boc₂O), dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA), triethylamine (TEA) and *N*-methylmorpholine (NMM) were procured from Spectrochem Limited (Mumbai, India). IR spectra were recorded on a Shimadzu 8700 FTIR spectrophotometer using a thin film supported on KBr pellets or chloroform solutions. ¹H-NMR and ¹³C-NMR spectra were recorded on Bruker AC NMR spectrometer (300 MHz) using CDCl₃ as solvent and TMS as internal standard. The mass spectra were recorded on a JMS-DX 303 mass spectrometer (Jeol, Tokyo, Japan) operating at 70 eV using the electron spray ionization technique (ESI MS). Optical rotation of synthesized peptide derivatives was measured on automatic polarimeter in a 2 dm tube at 25 °C using sodium lamp and methanol as solvent. Elemental analyses of all compounds were performed on Vario EL III elemental analyzer. Purity of all synthesized compounds was checked by TLC on precoated silica gel G plates utilizing chloroform/methanol in different ratios (9:1 / 7:3 v/v) as developing solvent system and spots were detected on exposure to iodine vapours in a tightly closed chamber.

Compounds **3a**, **3b** and **4a** were synthesized by coupling Boc-dipeptides with respective amino acid methyl ester hydrochlorides/dipeptide methyl esters under alkaline conditions.

tert-Butyloxycarbonyl-alanyl-prolyl-tryptophan methyl ester (**3a**). Semisolid mass; yield 82 %; [α]_D −55.6°; R_f - 0.81; IR (CHCl₃): ν 3491 (N–H_{str}, ring), 3122, 3117 (N–H_{str}, amide), 3077, 3053 (C–H_{str}, ring), 2994–2989 (C–H_{str}, CH₂, pro), 2962, 2925 (C–H_{str}, asym, CH₃ and CH₂), 2872, 2849, (C–H_{str}, sym, CH₃ and CH₂), 1750 (C=O_{str}, ester), 1680, 1670, 1645 (C=O_{str}, 3° and 2° amide), 1585, 1482 (skeletal bands), 1538, 1534 (N–H_{bend}, 2° amide), 1390, 1372 (C–H_{bend}, butyl-*t*), 1267 (C–O_{str}, ester), 732, 675 (C–H_{bend}, out-of-plane (oop), ring), 478 (C–C_{bend}, aliphatic) cm^{−1}; ¹H-NMR: δ 8.95 (1H, s, NH, ring), 8.65 (1H, br. s, NH), 7.50 (1H, s, H-β, ring), 7.20–6.98 (4H, m, H-δ-η, ring), 6.90 (1H, br. s, NH), 5.38–5.31 (1H, m, H-α, try), 4.52–4.43 (2H, m, H-α, ala and pro), 3.64 (2H, t, H-δ, pro), 3.55 (3H, s, OCH₃), 3.21 (2H, d, *J* = 6.8 Hz, H-β, try), 2.72–2.66 (2H, q, H-β, pro), 1.98–1.89 (2H, m, H-γ, pro), 1.52 (9H, s, butyl-*t*), 1.35 (3H, d, *J* = 5.2 Hz, H-β, ala) ppm; Anal. Calcd. for C₂₅H₃₄N₄O₆: C, 61.7; H, 7.1; N, 11.5. Found: C, 61.5; H, 7.3; N, 11.44 %.

tert-Butyloxycarbonyl-glycyl-leucyl-histidine methyl ester (**3b**). Semisolid mass; yield 71 %; [α]_D −19.2°; R_f - 0.72; IR (CHCl₃): ν 3495 (N–H_{str}, ring), 3129 (N–H_{str}, amide), 3076 (C–H_{str}, ring), 2928, 2852 (C–H_{str}, asym and sym, CH₂), 1748 (C=O_{str}, ester), 1641 (C=O_{str}, amide), 1583, 1488 (skeletal bands), 1533, 1526 (N–H_{def}, amide), 1390, 1368 (C–H_{def}, butyl-*t*), 932, 925 (CH₃rocking, butyl-*t* and propyl-*i*), 910 (C–H_{def}, oop, ring) cm^{−1}; ¹H-NMR (CDCl₃): δ 9.10 (1H, br. s, NH, ring), 7.73 (1H, d, *J* = 7.25 Hz, H at C₃, ring), 7.59 (1H, s, H at C₂, ring), 7.54 (1H, br. s, NH), 7.02 (1H, br. s, NH), 6.50 (1H, br. s, NH), 4.56–4.53 (1H, m, H-α, leu), 4.14–4.09 (1H, m, H-α, his), 3.55 (3H, s, OCH₃), 3.52 (2H, d, *J* = 4.8 Hz, CH₂, gly), 3.06 (2H, d, *J* = 6.65 Hz, H-β, his), 1.84 (2H, t, H-β, leu), 1.55 (9H, s, butyl-*t*), 1.51–1.43 (1H, m, H-γ, leu), 0.98 (6H, d, *J* = 6.15 Hz, H-δ, leu) ppm; Anal. Calcd. for C₂₀H₃₃N₅O₆: C, 54.66; H, 7.57; N, 15.93. Found: C, 54.65; H, 7.58; N, 15.95 %.

tert-Butyloxycarbonyl-valyl-tyrosinyl-phenylalanyl-glycine methyl ester (**4a**). Semisolid mass; Yield 79 %; $[\alpha]_D +37.7^\circ$; R_f - 0.66; IR (CHCl₃): ν 3375 (O–H_{str}, tyr), 3127–3122 (N–H_{str}, amide), 3075–3069, 3035 (C–H_{str}, rings), 2929–2925, 2856–2849 (C–H_{str}, asym and sym, CH₂), 1748 (C=O_{str}, ester), 1644–1640, 1639–1637 (C=O_{str}, 2° amide), 1585–1578, 1425–1420 (skeletal bands, rings), 1532–1528 (N–H_{bend}, 2° amide), 1465 (C–H_{bend} (scissoring), CH₂), 1392, 1370 (C–H_{bend}, butyl-*t*), 1383, 1359 (C–H_{bend}, propyl-*i*), 1268 (C–O_{str}, ester), 930, 922 (CH₃rocking, butyl-*t* and propyl-*i*), 829, 730, 695 (C–H_{def}, oop) cm^{−1}; ¹H-NMR: δ 8.19 (1H, br. s, NH, phe), 7.60 (1H, br. s, NH, tyr), 7.20–7.16 (2H, tt, H-*m*, phe), 7.02–6.96 (3H, m, H-*m*, tyr and H-*p*, phe), 6.90–6.82 (4H, m, H-*o*, tyr and phe), 6.52 (1H, br. s, NH, gly), 6.45 (1H, br. s, NH, val), 5.95 (1H, br. s, OH, tyr), 4.52–4.48 (1H, q, H- α , tyr), 4.20 (1H, t, H- α , val), 4.15–4.11 (1H, q, H- α , phe), 4.04 (2H, d, J = 5.45 Hz, H- α , gly), 3.66 (3H, s, OCH₃), 2.95–2.83 (4H, m, H- β , tyr and phe), 1.86–1.74 (1H, m, H- β , val), 1.54 (9H, s, butyl-*t*), 1.06 (6H, d, J = 4.6 Hz, H- γ , val) ppm; Anal. Calcd. for C₃₁H₄₂N₄O₈: C, 62.19; H, 7.07; N, 9.36. Found: C, 62.22; H, 7.05; N, 9.35 %.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoic acid (**5**)

Equimolar amounts (0.025 mol) of 6-iodo-2-methylbenzoxazin-4-one (**1a**, 7.18 g) and 5-ASA (3.83 g) were heated at 170–180 °C for 2 h in an oil bath. The separated jelly-like mass solidified upon cooling. The crude product was finally crystallized from ethanol to give a 71 % yield of pure **5** as a white solid; m.p. 124–125 °C; R_f - 0.52; IR (KBr): ν 3365 (O–H_{str}, Ar–OH), 3295–2505 (O–H_{str}, COOH), 3072–3066, 3052 (Ar–H_{str}), 2967, 2875 (C–H_{str}, CH₃), 1702 (C=O_{str}, COOH), 1669 (C=O_{str}, ring), 1589, 1575, 1425, 1417 (skeletal bands), 1405 (O–H_{def}, COOH), 875, 836, 760, 752, 696 (C–H_{def}, oop), 590 (C–I_{str}) cm^{−1}; ¹H-NMR: δ 11.43 (2H, br. s, OH and COOH), 8.42 (1H, s, H- ϵ , quinazolinone moiety (qz)), 8.14 (1H, d, J = 7.15 Hz, H- η , qz), 7.89 (1H, s, H- ζ , o-hydroxybenzoic acid moiety (hba)), 7.65 (1H, d, J = 6.9 Hz, H- θ , qz), 7.50 (1H, d, J = 6.65 Hz, H- δ , hba), 6.98 (1H, d, J = 6.9 Hz, H- γ , hba), 8.42 (3H, s, CH₃- β , qz) ppm; ¹³C-NMR: δ 172.3 (C=O, COOH), 163.9 (C-2, hba), 158.7 (C=O, qz), 153.4, 148.5 (2C, C-2 and C-2', qz), 141.7, 137.8 (2C, C-7 and C-5, qz), 135.0, 131.5 (2C, C-4 and C-5, hba), 128.4, 124.1 (2C, C-8 and C-3', qz), 123.3, 117.5 (2C, C-6 and C-3, hba), 114.0 (C-1, hba), 95.1 (C-6, qz), 19.8 (CH₃, qz) ppm; Anal. Calcd. for C₁₆H₁₁IN₂O₄ (422): C, 45.52; H, 2.63; N, 6.64. Found: C, 45.49; H, 2.60; N, 6.65 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoic acid (**6**)

A mixture of 5-ASA (7.65 g), dilute hydrochloric acid (15%, 30 mL) and water (45 mL) was heated to give a clear solution. After cooling to 0 °C, the solution was diazotized by addition of sodium nitrite solution (30%, 12 mL). To the filtrate of diazotized salt solution, 5-nitroimidazole (**1b**, 5.65 g) and aqueous cupric chloride solution (1.3 g in 5 mL water) were added with stirring, followed by slow addition of water (25 mL), maintaining the temperature between 5–10 °C. Stirring was continued for 6 h and finally the reaction mixture was 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 give a 77 % yield of pure **6** as a pale-yellow solid; m.p. 108–110 °C; R_f - 0.67; IR (KBr): ν 3488

(N-H_{str}, ring), 3362 (O-H_{str}, Ar-OH), 3305-2509 (O-H_{str}, COOH), 3077, 3054 (Ar-H_{str}), 1697 (C=O_{str}, COOH), 1588, 1576, 1428, 1419 (skeletal bands), 1542, 1349 (NO_{2str}), 1408 (O-H_{def}, COOH), 872, 838, 822, 698 (C-H_{def}, oop) cm⁻¹; ¹H-NMR: δ 10.89 (2H, br. s, OH and COOH), 10.85 (1H, br. s, NH, imidazole moiety (imz)), 9.10 (1H, s, H- ζ , hba), 8.80 (1H, d, J = 6.5 Hz, H- δ , hba), 8.52 (1H, s, H- δ , imz), 6.83 (1H, d, J = 6.75 Hz, H- γ , hba) ppm; ¹³C-NMR: δ 173.8 (C=O, COOH), 164.2 (C-2, hba), 155.4 (C-2, imz), 148.7, 139.4 (2C, C-5 and C-4, imz), 135.1, 128.6 (2C, C-6 and C-4, hba), 125.0, 122.3 (2C, C-5 and C-3, hba), 117.6 (C-1, hba) ppm; Anal. Calcd. for C₁₀H₇N₃O₅ (249): C, 48.20; H, 2.83; N, 16.86. Found: C, 48.22; H, 2.84; N, 16.83 %.

General procedure for synthesis of amino acid / peptide derivatives of 2-hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoic acids 5a-f

Amino acid methyl ester hydrochloride/di/tri/tetrapeptide methyl ester (0.01 mol) was dissolved in THF (75 mL). To this, NMM (2.3 mL) was added at 0 °C and the reaction mixture was stirred for 15 min. Compound **5** (4.22 g, 0.01 mol) in THF (75 mL) and DCC (2.1 g) were added to the above mixture with stirring. After 36 h, the reaction mixture was filtered and the residue was washed with THF (25 mL). Then, filtrate was washed with 5% NaHCO₃ and saturated NaCl solutions (15 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and *n*-hexane followed by cooling at 0 °C.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl nitro(arginine) methyl ester (5a). Semisolid mass; yield 62 %; [α]_D +34.8° (*c*, 0.2 in MeOH); R_f - 0.63; IR (CHCl₃): ν 3362 (O-H_{str}, Ar-OH), 3340, 3328 (N-H_{str}, amine), 3122 (N-H_{str}, amide), 3075-3069, 3056 (Ar-H_{str}), 2965, 2872 (C-H_{str}, CH₃), 2926, 2919, 2856, 2850 (C-H_{str}, CH₂), 1738 (C=O_{str}, ester), 1672 (C=O_{str}, ring), 1643 (C=O_{str}, amide), 1587, 1576, 1422, 1415 (skeletal bands), 1551 (NO_{2str}, asym), 1532 (N-H_{bend}, amide), 1468, 1459 (C-H_{bend} (scissoring), CH₂), 1370 (NO_{2str}, sym), 1269 (C-O_{str}, ester), 1156 (C-N_{str}, amine), 870, 833, 763, 751, 694 (C-H_{def}, oop), 588 (C-I_{str}) cm⁻¹; ¹³C-NMR: δ 173.5 (C=O, ArCO), 171.8 (C=O, arg), 160.6 (C=NH), 158.3 (C=O, qz), 156.1 (C-2, hba), 152.8, 148.9 (2C, C-2 and C-2', qz), 141.9, 139.0 (2C, C-7 and C-5, qz), 137.4, 132.2 (2C, C-4 and C-5, hba), 127.7 (C-8, qz), 123.1 (C-1, hba), 122.4 (C-3', qz), 120.7, 118.0 (2C, C-6 and C-3, hba), 96.6 (C-6, qz), 54.2 (C- α , arg), 50.9 (OCH₃), 40.1 (C- δ , arg), 28.0 (C- β , arg), 24.3 (C- γ , arg), 20.6 (CH₃, qz) ppm; Anal. Calcd. for C₂₃H₂₄IN₇O₇ (637): C, 43.34; H, 3.80; N, 15.38. Found: C, 43.36; H, 3.79; N, 15.42 %.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl tyrosine methyl ester (5b). Pale-yellow solid; m.p. 163-164 °C; yield 65 %; [α]_D +17.7° (*c*, 0.2 in MeOH); R_f - 0.81; IR (KBr): ν 3364, 3359 (O-H_{str}, Ar-OH), 3126 (N-H_{str}, amide), 3072-3068, 3058-3053 (Ar-H_{str}), 2967, 2874 (C-H_{str}, CH₃), 2922, 2852 (C-H_{str}, CH₂), 1742 (C=O_{str}, ester), 1670 (C=O_{str}, ring), 1640 (C=O_{str}, amide), 1587-1578, 1425-1417 (skeletal bands), 1535 (N-H_{bend}, amide), 1272 (C-O_{str}, ester), 876, 835-824, 764-753, 698 (C-H_{def}, oop), 588 (C-I_{str}) cm⁻¹; ¹H-NMR: δ 8.43 (1H, s, H- ϵ , qz), 8.12 (1H, d, J = 7.2 Hz, H- η , qz), 7.67 (1H, d, J = 6.85 Hz, H- θ , qz), 7.39 (1H, d, J = 6.7 Hz, H- δ , hba), 7.35 (1H, s, H- ζ , hba), 7.18 (1H, d, J = 6.95 Hz, H- γ , hba), 6.90 (2H, dd, J = 8.55, 5.25 Hz, H-*o*, tyr), 6.77 (2H, dd, J = 8.6, 4.9 Hz, H-*m*, tyr), 6.50 (1H, br. s, NH), 5.12 (2H, br. s, OH, tyr and hba), 4.68-4.62 (1H,

m, H- α , tyr), 3.56 (3H, s, OCH₃), 2.82 (2H, d, J = 4.9 Hz, H- β , tyr), 2.58 (3H, s, CH₃- β , qz) ppm; Anal. Calcd. for C₂₆H₂₂IN₃O₆ (599): C, 52.10; H, 3.70; N, 7.01. Found: C, 52.09; H, 3.72; N, 7.05 %.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl prolyl-valine methyl ester (5c). Off-white solid; m.p. 102–103 °C; yield 69 %; $[\alpha]_D$ −53.2° (c , 0.2 in MeOH); R_f - 0.72; IR (KBr): ν 3367 (O–H_{str}, Ar–OH), 3124 (N–H_{str}, amide), 3069, 3055 (Ar–H_{str}), 2995–2989 (C–H_{str}, CH₂, pro), 2965, 2876 (C–H_{str}, CH₃), 1746 (C=O_{str}, ester), 1668 (C=O_{str}, ring), 1659, 1642 (C=O_{str}, 3° and 2° amide), 1582, 1423 (skeletal bands), 1531 (N–H_{bend}, amide), 1385, 1362 (C–H_{bend}, propyl- i), 1270 (C–O_{str}, ester), 1224 (C–O_{str}, phenolic), 875, 832, 767, 750, 692 (C–H_{def}, oop), 589 (C–I_{str}) cm^{−1}; ¹³C-NMR: δ 174.9 (C=O, val), 172.7 (C=O, pro), 171.9 (C=O, ArCO), 158.1 (C=O, qz), 155.7 (C-2, hba), 152.5, 149.3 (2C, C-2 and C-2', qz), 141.6, 137.7 (2C, C-7 and C-5, qz), 137.1, 131.9 (2C, C-4 and C-5, hba), 128.8 (C-8, qz), 122.5 (C-1, hba), 121.7 (C-3', qz), 120.5, 117.2 (2C, C-6 and C-3, hba), 95.3 (C-6, qz), 70.7 (C- α , pro), 58.3 (C- α , val), 52.1 (OCH₃), 45.5 (C- δ , pro), 31.4, 29.8 (2C, C- β , val and pro), 28.7 (C- γ , pro), 21.2 (2C, C- γ , val), 19.9 (CH₃, qz) ppm; Anal. Calcd. for C₂₇H₂₉IN₄O₆ (632): C, 51.28; H, 4.62; N, 8.86. Found: C, 51.30; H, 4.61; N, 8.85 %.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl leucyl-phenylalanine methyl ester (5d). White crystals; m.p. 90–92 °C; yield 71 %; $[\alpha]_D$ +4.8° (c , 0.2 in MeOH); R_f - 0.60; IR (KBr): ν 3365 (O–H_{str}, Ar–OH), 3129–3125 (N–H_{str}, amide), 3067, 3059–3955 (Ar–H_{str}), 2966, 2874 (C–H_{str}, CH₃), 2928–2924, 2853 (C–H_{str}, CH₂), 1749 (C=O_{str}, ester), 1669 (C=O_{str}, ring), 1645, 1641 (C=O_{str}, 2° amide), 1587–1583, 1426–1422 (skeletal bands), 1534, 1531 (N–H_{bend}, amide), 1387, 1363 (C–H_{bend}, propyl- i), 1268 (C–O_{str}, ester), 1226 (C–O_{str}, phenolic), 878, 830, 765–754, 712–695 (C–H_{def}, oop), 587 (C–I_{str}) cm^{−1}; ¹H-NMR: δ 8.41 (1H, s, H- ϵ , qz), 8.10 (1H, d, J = 7.15 Hz, H- η , qz), 7.65 (1H, d, J = 6.9 Hz, H- θ , qz), 7.61 (1H, br. s, NH), 7.58 (1H, s, H- ζ , hba), 7.38 (1H, d, J = 6.65 Hz, H- δ , hba), 7.14 (1H, t, J = 6.15 Hz, H- p , phe), 7.05 (1H, br. s, NH), 6.98 (2H, m, H- m , phe), 6.95 (1H, d, J = 6.9 Hz, H- γ , hba), 6.84 (2H, dd, J = 8.75, 4.1 Hz, H- o , phe), 4.48–4.42 (1H, m, H- α , leu), 4.27 (1H, br. s, OH), 3.94–3.89 (1H, m, H- α , phe), 3.55 (3H, s, OCH₃), 2.98 (2H, d, J = 5.6 Hz, H- β , phe), 2.60 (3H, s, CH₃- β , qz), 1.72 (2H, t, J = 4.9 Hz, H- β , leu), 1.50–1.42 (1H, m, H- γ , leu), 0.98 (6H, d, J = 6.2 Hz, H- δ , leu) ppm; Anal. Calcd. for C₃₂H₃₃IN₄O₆ (696): C, 55.18; H, 4.78; N, 8.04. Found: C, 55.19; H, 4.80; N, 8.02 %.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl-histidinyl-phenylalanine methyl ester (5e). Semisolid mass; yield 63 %; $[\alpha]_D$ −14.9° (c , 0.2 in MeOH); R_f - 0.55; IR (CHCl₃): ν 3489 (N–H_{str}, ring), 3368 (O–H_{str}, Ar–OH), 3127–3123 (N–H_{str}, amide), 3068–3063, 3058–3954 (Ar–H_{str}), 2964, 2876 (C–H_{str}, CH₃), 2929–2923, 2852 (C–H_{str}, CH₂), 1751 (C=O_{str}, ester), 1667 (C=O_{str}, ring), 1644, 1639 (C=O_{str}, 2° amide), 1589–1582, 1428–1423 (skeletal bands), 1536, 1533 (N–H_{bend}, amide), 1269 (C–O_{str}, ester), 1224 (C–O_{str}, phenolic), 878, 848–839, 765, 758, 714–698 (C–H_{def}, oop), 589 (C–I_{str}) cm^{−1}; ¹³C-NMR: δ 173.2 (C=O, his), 169.8 (C=O, phe), 170.5 (C=O, ArCO), 157.6 (C=O, qz), 156.0 (C-2, hba), 153.3, 149.1 (2C, C-2 and C-2', qz), 146.7 (C-2, imz), 141.5 (C-7, qz), 137.1 (C- γ , phe), 136.4 (C-5, qz), 135.1, 133.9 (2C, C-4 and C-5, hba), 129.1 (2C, C- o , phe), 128.4 (2C, C- m , phe), 127.2 (C- p , phe), 126.4 (C-5, imz), 124.9 (C-8, qz), 122.6 (C-3', qz), 120.2 (C-1, hba), 119.4, 117.7 (2C, C-6 and C-3, hba), 115.3 (C- γ , his), 94.9 (C-6, qz), 60.6 (C- α , his),

55.9 (C- α , phe), 53.7 (OCH₃), 38.2, 26.5 (2C, C- β , phe and his), 18.7 (CH₃, qz) ppm; Anal. Calcd. for C₃₂H₂₉IN₆O₆ (720): C, 53.34; H, 4.06; N, 11.66. Found: C, 53.35; H, 4.06; N, 11.68 %.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl alanyl-prolyl-tryptophan methyl ester (5f). Semisolid mass; yield 66 %; [α]_D −102.6° (c, 0.5 in MeOH); R_f - 0.76; IR (CHCl₃): ν 3483 (N-H_{str}, ring), 3358 (O-H_{str}, Ar-OH), 3133-3126 (N-H_{str}, amide), 3069-3065, 3058 (Ar-H_{str}), 2996-2989 (C-H_{str}, CH₂, pro), 2968, 2963, 2876 (C-H_{str}, CH₃), 2926, 2854 (C-H_{str}, CH₂), 1747 (C=O_{str}, ester), 1667 (C=O_{str}, ring), 1658, 1643, 1639 (C=O_{str}, 3° and 2° amide), 1589-1582, 1428-1424 (skeletal bands), 1536, 1533 (N-H_{bend}, amide), 1274 (C-O_{str}, ester), 1223 (C-O_{str}, phenolic), 878, 835-829, 768, 715, 698-693 (C-H_{def}, oop), 592 (C-I_{str}) cm^{−1}; MS (m/z, %): 792 (2.8), 791 (3.4), 790 (M⁺, 5.9), 775 (9.2), 759 (26.5), 731 (8.1), 573 (100), 545 (11.4), 476 (72.7), 448 (33.1), 405 (54.9), 377 (20.6), 285 (19.3), 228 (26.6), 159 (14.7), 130 (10.3), 126 (11.5), 116 (7.7), 93 (9.7), 70 (8.2), 59 (21.8), 44 (17.4), 42 (10.5), 31 (11.9), 15 (9.2); Anal. Calcd. for C₃₆H₃₅IN₆O₇ (790): C, 54.69; H, 4.46; N, 10.63. Found: C, 54.72; H, 4.45; N, 10.65 %.

General procedure for synthesis of amino acid/peptide derivatives of 2-hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoic acids 6a-g

To a mixture of amino acid methyl ester hydrochloride/di/tri/tetrapeptide methyl ester (0.01 mol) in DMF (50 mL), TEA (2.8 mL) was added at 0 °C with stirring. Compound **6** (2.5 g, 0.01 mol) in DMF (50 mL) and DCC (2.1 g) were added to the above mixture and stirring was done for 24 h. After 24 h, the reaction mixture was filtered. To the filtrate, water was added in equal proportions and the aqueous layer was washed with ether (3 × 50 mL). The organic layer was separated and dried over anhydrous Na₂SO₄, filtered and evaporated under vacuum. The product obtained was dissolved in chloroform, washed with 10 % HCl, saturated NaHCO₃ solution and water (25 mL each) followed by evaporation under vacuum. The crude product was recrystallized from a mixture of ethyl acetate and petroleum ether.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl threonine methyl ester (6a). Semisolid mass; yield 79 %; [α]_D −22.4° (c, 0.35 in MeOH); R_f - 0.53; IR (CHCl₃): ν 3485 (N-H_{str}, ring), 3366 (O-H_{str}, Ar-OH), 3132, 3127 (N-H_{str}, amide), 3075, 3052 (Ar-H_{str}), 2969, 2874 (C-H_{str}, CH₃), 1744 (C=O_{str}, ester), 1642 (C=O_{str}, 2° amide), 1586, 1424 (skeletal bands), 1540, 1348 (NO_{2str}), 1532 (N-H_{bend}, amide), 1422, 1329 (O-H_{def}), 1271 (C-O_{str}, ester), 1225 (C-O_{str}, phenolic), 870, 839, 820, 696 (C-H_{def}, oop) cm^{−1}; ¹H-NMR: δ 9.38 (1H, s, H- δ , imz), 8.90 (1H, s, H- ζ , hba), 8.85 (1H, d, *J* = 6.5 Hz, H- δ , hba), 7.05 (1H, d, *J* = 6.85 Hz, H- γ , hba), 6.50 (1H, br. s, NH), 5.75 (3H, br. s, OH, hba and thr, NH, imz), 4.65-4.57 (1H, m, H- β , thr), 4.52-4.48 (1H, m, H- α , thr), 3.60 (3H, s, OCH₃), 1.32 (3H, d, *J* = 4.95 Hz, H- γ , thr) ppm; Anal. Calcd. for C₁₅H₁₆N₄O₇ (364): C, 49.45; H, 4.43; N, 15.38. Found: C, 49.49; H, 4.40; N, 15.39 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl proline methyl ester (6b). Semisolid mass; yield 85 %; [α]_D +71.5° (c, 0.35 in MeOH); R_f - 0.69; IR (CHCl₃): ν 3482 (N-H_{str}, ring), 3368 (O-H_{str}, Ar-OH), 3133 (N-H_{str}, amide), 3077, 3055 (Ar-H_{str}), 2998-2992 (C-H_{str}, CH₂, pro), 1742 (C=O_{str}, ester), 1660,

1644 (C=O_{str}, 3° and 2° amide), 1584, 1421 (skeletal bands), 1544, 1347 (NO_{2str}), 1535 (N–H_{bend}, amide), 1269 (C–O_{str}, ester), 1223 (C–O_{str}, phenolic), 869, 835, 824, 695 (C–H_{def}, oop) cm^{−1}; ¹³C-NMR: δ 178.5 (C=O, ArC=O), 170.3 (C=O, pro), 159.6 (C-2, hba), 153.3 (C-2, imz), 154.2, 138.0 (2C, C-5 and C-4, imz), 133.7 (C-4, hba), 130.6 (C-1, hba), 126.9, 123.7 (2C, C-6 and C-3, hba), 120.8 (C-5, hba), 65.8 (C-α, pro), 54.0 (OCH₃), 46.5 (C-δ, pro), 29.2 (C-β, pro), 26.0 (C-γ, pro) ppm; Anal. Calcd. for C₁₆H₁₆N₄O₆ (360): C, 53.33; H, 4.48; N, 15.55. Found: C, 53.32; H, 4.50; N, 15.58 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl glycyl-glycine methyl ester (6c). White solid; m.p. 155 °C; yield 80 %; [α]_D −57.4° (c, 0.35 in MeOH); R_f - 0.66; ¹H-NMR: δ 9.35 (1H, s, H-δ, imz), 9.15 (1H, s, H-ζ, hba), 8.88 (1H, d, *J* = 6.45 Hz, H-δ, hba), 8.62 (1H, br. s, NH), 8.15 (1H, br. s, NH), 6.98 (2H, br. s, OH, hba and NH, imz), 6.87 (1H, d, *J* = 6.8 Hz, H-γ, hba), 4.05 (2H, d, *J* = 5.15 Hz, H-α, gly-2), 3.90 (2H, d, *J* = 5.2 Hz, H-α, gly-1), 3.48 (3H, s, OCH₃) ppm; ¹³C-NMR: δ 177.3, 171.5 (2C, C=O, gly-1 and gly-2), 165.7 (C=O, ArC=O), 161.9 (C-2, hba), 156.0 (C-2, imz), 149.5, 139.9 (2C, C-5 and C-4, imz), 134.3 (C-4, hba), 129.2 (C-1, hba), 125.6, 122.4 (2C, C-6 and C-5, hba), 119.5 (C-3, hba), 54.1 (OCH₃), 47.2 (C-α, gly-1), 41.6 (C-α, gly-2) ppm; Anal. Calcd. for C₁₅H₁₅N₅O₇ (377): C, 47.75; H, 4.01; N, 18.56. Found: C, 47.78; H, 3.98; N, 18.55 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl isoleucyl-tyrosine methyl ester (6d). Semisolid mass; yield 88 %; [α]_D +29.4° (c, 0.35 in MeOH); R_f - 0.58; ¹³C-NMR: δ 172.0, 170.6 (2C, C=O, ile and tyr), 166.8 (C=O, ArC=O), 157.6 (C-2, hba), 154.0 (C-*p*, tyr), 153.4 (C-2, imz), 146.2, 139.2 (2C, C-5 and C-4, imz), 134.0 (C-4, hba), 133.3 (2C, C-*m*, tyr), 130.6 (2C, C-*o*, tyr), 127.9 (C-1, hba), 125.0, 123.7 (2C, C-6 and C-5, hba), 118.9 (C-3, hba), 117.5 (C-γ, tyr), 58.2 (C-α, ile), 55.7 (C-α, tyr), 53.1 (OCH₃), 39.7 (C-β, tyr), 34.2 (C-β, ile), 30.1 (C-γ, ile), 14.5 (C-γ', ile), 10.2 (C-δ, ile) ppm; ¹H-NMR: δ 9.37 (1H, s, H-δ, imz), 9.10 (1H, s, H-ζ, hba), 8.85 (1H, d, *J* = 6.5 Hz, H-δ, hba), 7.50 (1H, br. s, NH), 7.16 (1H, br. s, NH), 6.92 (1H, d, *J* = 6.75 Hz, H-γ, hba), 6.88 (2H, dd, *J* = 8.6, 5.3 Hz, H-*o*, tyr), 6.80 (2H, dd, *J* = 8.55, 4.85 Hz, H-*m*, tyr), 6.65 (3H, br. s, OH, hba and tyr, NH, imz), 4.79-4.75 (1H, m, H-α, ile), 3.96-3.92 (1H, m, H-α, tyr), 3.55 (3H, s, OCH₃), 2.78 (2H, d, *J* = 4.85 Hz, H-β, tyr), 1.59-1.47 (3H, m, H-β and H-γ, ile), 0.98 (3H, t, *J* = 7.8 Hz, H-δ, ile), 0.95 (3H, d, *J* = 5.9 Hz, H-γ', ile) ppm; Anal. Calcd. for C₂₆H₂₉N₅O₈ (539): C, 57.88; H, 5.42; N, 12.98. Found: C, 57.90; H, 5.40; N, 13.02 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl phenylalanyl-proline methyl ester (6e). Semisolid mass; yield 86 %; [α]_D −109.2° (c, 0.35 in MeOH); R_f - 0.71; ¹³C-NMR: δ 174.5, 168.1 (2C, C=O, pro and phe), 165.4 (C=O, ArC=O), 158.3 (C-2, hba), 156.6 (C-2, imz), 154.7, 139.0 (2C, C-5 and C-4, imz), 135.4 (C-γ, phe), 132.5 (C-4, hba), 131.5 (2C, C-*o*, phe), 129.9 (2C, C-*m*, phe), 127.7 (C-1, hba), 127.0 (C-*p*, phe), 134.2, 124.8, 122.0 (3C, C-6, C-5, C-3, hba), 58.6 (C-α, pro), 53.9 (OCH₃), 52.1 (C-α, phe), 45.6 (C-δ, pro), 38.5 (C-β, phe), 29.5, 23.8 (2C, C-β and C-γ, pro) ppm; ¹H-NMR: δ 9.38 (1H, s, H-δ, imz), 9.13 (1H, s, H-ζ, hba), 8.88 (1H, d, *J* = 6.55 Hz, H-δ, hba), 7.86 (1H, br. s, NH), 7.22 (2H, m, H-*m*, phe), 7.05 (1H, t, *J* = 6.2 Hz, H-*p*, phe), 6.95 (2H, br. s, OH, hba and NH, imz), 6.90 (1H, d, *J* = 6.8 Hz, H-γ, hba), 6.82 (2H, dd, *J* = 8.8, 4.1 Hz, H-*o*, phe), 4.69-4.65 (1H, m, H-α, phe), 3.90 (1H, t, *J* = 6.9 Hz, H-α, pro), 3.62 (3H, s, OCH₃), 3.40 (2H, t, *J* = 7.2 Hz, H-δ, pro), 2.78

(2H, t, $J = 5.6$ Hz, H- β , phe), 2.05–1.97 (4H, m, H- γ and H- δ , pro) ppm; Anal. Calcd. for $C_{25}H_{25}N_5O_7$ (507): C, 59.17; H, 4.97; N, 13.80. Found: C, 59.20; H, 4.94; N, 13.82 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl glycyl-leucyl-histidine methyl ester (6f). Semisolid mass; yield 83 %; $[\alpha]_D +7.2^\circ$ (c , 0.5 in MeOH); R_f - 0.76; IR ($CHCl_3$): ν 3493, 3489 (N-H_{str}, ring), 3365 (O-H_{str}, Ar-OH), 3132–3128 (N-H_{str}, amide), 3076–3072, 3056 (Ar-H_{str}), 2929–2923, 2854–2851 (C-H_{str}, CH₂), 1752 (C=O_{str}, ester), 1645–1641 (C=O_{str}, amide), 1589–1575, 1429–1422 (skeletal bands), 1535–1531, 1527 (N-H_{def}, amide), 1544, 1346 (NO_{2str}), 1385, 1362 (C-H_{bend}, propyl-*i*), 1272 (C-O_{str}, ester), 1227 (C-O_{str}, phenolic), 912, 869, 839–828, 696 (C-H_{def}, oop) cm^{-1} ; MS (m/z , %): 572 (1.9), 571 (2.7), 570 (M^+ , 4.1), 555 (6.9), 539 (17.7), 511 (15.1), 402 (59.3), 374 (11.3), 289 (100), 261 (38.6), 232 (31.4), 204 (15.9), 110 (13.8), 86 (17.4), 81 (10.2), 55 (6.7), 54 (4.4), 112 (16.3), 93 (14.4), 86 (12.8), 85 (7.5), 59 (17.9), 57 (10.5), 46 (7.7), 43 (11.3), 42 (3.9), 30 (6.6), 31 (12.7), 15 (3.6); Anal. Calcd. for $C_{25}H_{30}N_8O_8$ (570): C, 52.63; H, 5.30; N, 19.64. Found: C, 52.65; H, 5.29; N, 19.66 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl valyl-tyrosinyl-phenylalanyl-glycine methyl ester (6g). Semisolid mass; yield 90 %; $[\alpha]_D -49.1^\circ$ (c , 0.5 in MeOH); R_f - 0.85; IR ($CHCl_3$): ν 3492 (N-H_{str}, ring), 3365, 3362 (O-H_{str}, Ar-OH), 3132–3125 (N-H_{str}, amide), 3079–3072, 3057 (Ar-H_{str}), 2966, 2874 (C-H_{str}, CH₃), 2925, 2922, 2857, 2852 (C-H_{str}, CH₂), 1748 (C=O_{str}, ester), 1645–1638 (C=O_{str}, 2° amide), 1589–1575, 1429–1422 (skeletal bands), 1544, 1347 (NO_{2str}), 1539–1532 (N-H_{bend}, amide), 1469, 1457 (CH_{bend} (scissoring), CH₂), 1388, 1362 (C-H_{bend}, propyl-*i*), 1268 (C-O_{str}, ester), 1226 (C-O_{str}, phenolic), 870, 839–831, 758–752, 695 (C-H_{def}, oop) cm^{-1} ; MS (m/z , %): 730 (3.2), 729 (M^+ , 4.7), 714 (8.6), 698 (19.9), 670 (12.7), 641 (69.6), 613 (10.2), 494 (100), 466 (13.5), 331 (57.3), 303 (20.9), 232 (32.6), 204 (16.7), 136 (13.9), 120 (11.4), 112 (14.3), 107 (17.2), 93 (19.8), 91 (15.1), 86 (11.3), 85 (7.9), 72 (9.7), 66 (11.5), 59 (19.5), 46 (8.9), 43 (12.8), 42 (5.7), 30 (7.1), 31 (13.2), 15 (4.5); Anal. Calcd. for $C_{36}H_{39}N_7O_{10}$ (729): C, 59.25; H, 5.39; N, 13.44. Found: C, 59.28; H, 5.40; N, 13.45 %.

General method for hydrolysis of amino acid /peptide derivatives of 2-hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoic acid (5) / 2-hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoic acid (6)

To a solution of the amino acid / peptide methyl ester (0.01 mol) in THF-H₂O (1:1, 36 mL), LiOH (0.36 g) was added at 0 °C. The mixture was stirred at RT for 1 h and then acidified to pH 3.5 with 1N H₂SO₄. The aqueous layer was extracted with Et₂O (3 × 25 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude products were recrystallized from methanol and ether to afford hydrolyzed peptide derivatives **5b_a–d_a** and **6e_a–g_a**.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl tyrosine (5b_a). Yellowish solid, m.p. 195 °C; yield 70 %; $[\alpha]_D +30.3^\circ$ (c , 0.25 in MeOH); R_f - 0.64; IR (KBr): ν 3366, 3357 (O-H_{str}, Ar-OH), 3298–2509 (O-H_{str}, COOH), 3122 (N-H_{str}, amide), 3075, 3069, 3055 (Ar-H_{str}), 2965, 2872 (C-H_{str}, CH₃), 2920, 2855 (C-H_{str}, CH₂), 1710 (C=O_{str}, COOH), 1672 (C=O_{str}, ring), 1644 (C=O_{str}, amide), 1589–1582, 1424–1418 (skeletal bands), 1533 (N-H_{bend}, amide), 1408 (O-H_{def},

COOH), 879, 832-826, 765-752, 695 (C-H_{def}, oop), 589 (C-I_{str}) cm⁻¹; ¹H-NMR: δ 8.42 (1H, s, H-ε, qz), 8.32 (1H, br. s, NH), 8.10 (1H, d, *J* = 7.15 Hz, H-η, qz), 7.61 (1H, d, *J* = 6.9 Hz, H-θ, qz), 7.42 (3H, br. s, OH, tyr, hba and COOH), 7.38 (1H, d, *J* = 6.65 Hz, H-δ, hba), 7.33 (1H, s, H-ζ, hba), 7.19 (2H, dd, *J* = 8.6, 5.25 Hz, H-o, tyr), 7.08 (1H, d, *J* = 7.0 Hz, H-γ, hba), 6.75 (2H, dd, *J* = 8.6, 4.85 Hz, H-m, tyr), 4.92-4.86 (1H, m, H-α, tyr), 2.91 (2H, d, *J* = 4.85 Hz, H-β, tyr), 2.59 (3H, s, CH₃-β, qz) ppm; Anal. Calcd. for C₂₅H₂₀IN₃O₆ (585): C, 51.30; H, 3.44; N, 7.18. Found: C, 51.30; H, 3.47; N, 7.15 %.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl prolyl-valine (5c_a). Semi-solid mass; yield 73 %; [α]_D -81.9° (*c*, 0.25 in MeOH); R_f - 0.54; IR (CHCl₃): ν 3366 (O-H_{str}, Ar-OH), 3306-2512 (O-H_{str}, COOH), 3126 (N-H_{str}, amide), 3066, 3052 (Ar-H_{str}), 2994-2988 (C-H_{str}, CH₂, pro), 2962, 2874 (C-H_{str}, CH₃), 1712 (C=O_{str}, COOH), 1669 (C=O_{str}, ring), 1658, 1644 (C=O_{str}, 3° and 2° amide), 1587, 1422 (skeletal bands), 1536 (N-H_{bend}, amide), 1405 (O-H_{def}, COOH), 1386, 1360 (C-H_{bend}, propyl-*i*), 1228 (C-O_{str}, phenolic), 876, 835, 765-753, 690 (C-H_{def}, oop), 586 (C-I_{str}) cm⁻¹; ¹³C-NMR: δ 175.4 (C=O, COOH), 171.9 (C=O, pro), 170.2 (C=O, ArCO), 157.8 (C=O, qz), 156.1 (C-2, hba), 152.7, 150.1 (2C, C-2 and C-2', qz), 141.2, 136.8 (2C, C-7 and C-5, qz), 135.3, 131.5 (2C, C-4 and C-5, hba), 127.3 (C-8, qz), 124.0 (C-1, hba), 123.5 (C-3', qz), 120.8, 116.4 (2C, C-6 and C-3, hba), 95.1 (C-6, qz), 71.5 (C-α, pro), 57.7 (C-α, val), 47.1 (C-δ, pro), 30.8, 28.9 (2C, C-β, val and pro), 28.3 (C-γ, pro), 18.6 (2C, C-γ, val), 16.9 (CH₃, qz) ppm; Anal. Calcd. for C₂₆H₂₇IN₄O₆ (618): C, 50.50; H, 4.40; N, 9.06. Found: C, 50.48; H, 4.43; N, 9.05 %.

2-Hydroxy-5-(6-iodo-2-methyl-4-oxo-3,4-dihydro-3-quinazolinyl)benzoyl leucyl-phenylalanine (5d_a). White solid; m.p. 151-152 °C; yield 69 %; [α]_D +15.8° (*c*, 0.25 in MeOH); R_f - 0.68; IR (CHCl₃): ν 3368 (O-H_{str}, Ar-OH), 3296-2502 (O-H_{str}, COOH), 3129-3126 (NH_{str}, amide), 3065, 3058-3953 (Ar-H_{str}), 2968, 2876 (C-H_{str}, CH₃), 2929-2925, 2852 (C-H_{str}, CH₂), 1711 (C=O_{str}, COOH), 1668 (C=O_{str}, ring), 1646, 1643 (C=O_{str}, 2° amide), 1589-1583, 1425-1420 (skeletal bands), 1537, 1532 (N-H_{bend}, amide), 1402 (O-H_{def}, COOH), 1387, 1362 (C-H_{bend}, propyl-*i*), 1225 (C-O_{str}, phenolic), 879, 833, 768-756, 711-692 (C-H_{def}, oop), 588 (C-I_{str}) cm⁻¹; ¹H-NMR: δ 8.43 (1H, s, H-ε, qz), 8.17 (2H, br. s, OH, hba and COOH), 8.12 (1H, d, *J* = 7.2 Hz, H-η, qz), 7.65 (1H, d, *J* = 6.85 Hz, H-θ, qz), 7.60 (1H, br. s, NH), 7.57 (1H, s, H-ζ, hba), 7.39 (1H, d, *J* = 6.7 Hz, H-δ, hba), 7.29 (1H, br. s, NH), 7.10 (1H, t, *J* = 6.2 Hz, H-*p*, phe), 7.05 (2H, dd, *J* = 8.8, 4.15 Hz, H-*o*, phe), 6.98 (1H, d, *J* = 6.85 Hz, H-γ, hba), 6.95 (2H, m, H-*m*, phe), 5.41-5.37 (1H, m, H-α, leu), 4.19-4.14 (1H, m, H-α, phe), 2.94 (2H, d, *J* = 5.55 Hz, H-β, phe), 2.58 (3H, s, CH₃-β, qz), 1.70 (2H, t, *J* = 4.85 Hz, H-β, leu), 1.51-1.44 (1H, m, H-γ, leu), 0.99 (6H, d, *J* = 6.15 Hz, H-δ, leu) ppm; Anal. Calcd. for C₃₁H₃₁IN₄O₆ (682): C, 54.55; H, 4.58; N, 8.21. Found: C, 54.58; H, 4.58; N, 8.24 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl phenylalanyl-proline (6e_a). White crystals; m.p. 123-125 °C; yield 68 %; [α]_D -87.4° (*c*, 0.35 in MeOH); R_f - 0.51; ¹³C-NMR: δ 177.2 (C=O, COOH), 168.8 (C=O, ArCO), 166.2 (C=O, phe), 158.0 (C-2, hba), 156.2 (C-2, imz), 152.9, 140.9 (2C, C-5 and C-4, imz), 140.4 (C-γ, phe), 134.1 (C-4, hba), 131.1 (2C, C-*o*, phe), 129.4 (2C, C-*m*, phe), 127.5 (C-1, hba), 127.2 (C-*p*, phe), 126.2, 124.1, 121.8 (3C, C-6, C-5, C-3, hba), 61.2 (C-α, pro), 51.5 (C-α, phe), 45.2 (C-δ, pro), 36.6 (C-β, phe), 30.2, 24.5 (2C, C-β and C-γ, pro) ppm; ¹H-NMR: δ 9.35 (1H, s, H-δ,

imz), 9.11 (1H, s, H- ζ , hba), 8.86 (1H, d, J = 6.6 Hz, H- δ , hba), 8.15 (3H, br. s, OH, hba and COOH, NH, imz), 7.85 (1H, br. s, NH), 7.20 (2H, m, H- m , phe), 7.02 (1H, t, J = 6.15 Hz, H- p , phe), 6.92 (1H, d, J = 6.75 Hz, H- γ , hba), 6.84 (2H, dd, J = 8.75, 4.15 Hz, H- o , phe), 5.89–5.85 (1H, m, H- α , phe), 4.07 (1H, t, J = 6.85 Hz, H- α , pro), 3.38 (2H, t, J = 7.15 Hz, H- δ , pro), 2.80 (2H, t, J = 5.55 Hz, H- β , phe), 2.07–1.98 (4H, m, H- γ and H- δ , pro) ppm; Anal. Calcd. for C₂₄H₂₃N₅O₇ (493): C, 58.42; H, 4.70; N, 14.19. Found: C, 58.39; H, 4.70; N, 14.22 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl glycyl-leucyl-histidine (6f_a). Semisolid mass; yield 71 %; $[\alpha]_D +19.5^\circ$ (c , 0.35 in MeOH); R_f - 0.84; IR (CHCl₃): ν 3492, 3489 (N–H_{str}, ring), 3368 (O–H_{str}, Ar–OH), 3298–2510 (O–H_{str}, COOH), 3130–3125 (N–H_{str}, amide), 3077–3073, 3055 (Ar–H_{str}), 2929–2922, 2855–2851 (C–H_{str}, CH₂), 1712 (C=O_{str}, COOH), 1644–1640 (C=O_{str}, amide), 1588–1576, 1427–1423 (skeletal bands), 1538–1533, 1525 (N–H_{def}, amide), 1543, 1345 (NO_{2str}), 1409 (O–H_{def}, COOH), 1385, 1360 (C–H_{bend}, propyl- i), 1222 (C–O_{str}, phenolic), 910, 867, 838, 829, 698 (C–H_{def}, oop) cm^{−1}; MS (m/z , %): 557 (1.9), 556 (M⁺, 3.2), 539 (22.9), 511 (17.3), 402 (55.3), 374 (10.6), 289 (100), 261 (39.2), 232 (27.7), 204 (12.8), 110 (11.5), 86 (15.9), 81 (11.8), 55 (8.8), 54 (3.7), 112 (12.9), 93 (19.3), 86 (11.5), 85 (7.9), 57 (12.7), 46 (8.9), 45 (14.4), 43 (10.2), 42 (4.5), 30 (5.8), 17 (3.6), 15 (2.9); Anal. Calcd. for C₂₄H₂₈N₈O₈ (556): C, 51.80; H, 5.07; N, 20.13. Found: C, 51.77; H, 5.05; N, 20.15 %.

2-Hydroxy-5-(5-nitro-1H-imidazol-2-yl)benzoyl valyl-tyrosinyl-phenylalanyl-glycine (6g_a). Semisolid mass; yield 70 %; $[\alpha]_D -81.9^\circ$ (c , 0.35 in MeOH); R_f - 0.61; IR (CHCl₃): ν 3489 (N–H_{str}, ring), 3362, 3359 (O–H_{str}, Ar–OH), 3298–2510 (O–H_{str}, COOH), 3133–3127 (N–H_{str}, amide), 3077–3072, 3053 (Ar–H_{str}), 2965, 2876 (C–H_{str}, CH₃), 2925–2921, 2856, 2850 (C–H_{str}, CH₂), 1713 (C=O_{str}, COOH), 1643–1637 (C=O_{str}, 2° amide), 1587–1574, 1427–1422 (skeletal bands), 1542, 1345 (NO_{2str}), 1537–1532 (N–H_{bend}, amide), 1467, 1459 (C–H_{bend} (scissoring), CH₂), 1407 (O–H_{def}, COOH), 1389, 1360 (C–H_{bend}, propyl- i), 1224 (C–O_{str}, phenolic), 872, 838, 830, 756–753, 698 (C–H_{def}, oop) cm^{−1}; MS (m/z , %): 715 (M⁺, 3.9), 698 (25.2), 670 (18.9), 641 (63.8), 613 (8.9), 494 (100), 466 (11.2), 331 (59.4), 303 (25.4), 232 (38.2), 204 (14.6), 136 (13.2), 120 (16.1), 112 (11.8), 107 (16.7), 93 (18.9), 91 (13.6), 86 (16.7), 85 (11.6), 72 (7.9), 66 (10.7), 46 (6.7), 45 (10.9), 43 (11.2), 42 (5.9), 30 (6.8), 17 (2.8), 15 (3.6); Anal. Calcd. for C₃₅H₃₇N₇O₁₀ (715): C, 58.74; H, 5.21; N, 13.70. Found: C, 58.75; H, 5.20; N, 13.74 %.

Antimicrobial activity

Experimental details of the antimicrobial and anthelmintic activity test procedures are given in our previously published reports [28]. All the newly synthesized compounds **5a–6g_a** were evaluated for their 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: *Microsporium audouinii* (MUCC 545), *Trichophyton mentagrophytes* (MUCC 665), *Candida albicans* (MUCC 29) and *Aspergillus niger* (MUCC 177) at 50–6 $\mu\text{g mL}^{-1}$ concentration, according to the modified Kirby-Bauer disk diffusion method [31]. MIC values of test compound were determined by the tube dilution technique. The

solvents DMF/DMSO were used as negative controls and ciprofloxacin/griseofulvin were used as standards. Diameters of the zones of inhibition (in mm) were measured and the average diameters for test sample were calculated for triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug - ciprofloxacin. The antibacterial study results are presented in Table 1.

Anthelmintic activity

Anthelmintic activity studies were carried out against three different species of earthworms: *Megascolex konkanensis* (ICARBC 211), *Pontoscotex corethruses* (ICARBC 117) and *Eudrilus eugeniae* (ICARBC 042) at 2 mg mL⁻¹ concentration following Garg's method [32]. Tween 80 (0.5%) in distilled water was used as control and mebendazole was used as a reference compound. The paralysis and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 °C) which stimulated the movement, if the worm was alive. The anthelmintic study results are tabulated in Table 2.

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