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SYNTHESIS OF PEPTIDE ANALOGS OF 4-[2-(3-BROMOPHENYL)-7-NITRO-4-OXO-3,4-DIHYDRO-3-QUINAZOLINYL] BENZOIC ACIDS AS POTENT ANTIFUNGAL AGENTS

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A novel series of 4-[2-(3-bromophenyl)-7-nitro-4-oxo-3,4-dihydro-3-quinazolinyl] benzoic acid derivatives of amino acids and di/tripeptides was synthesized by using diisopropylcarbodiimide as coupling agent and N-methylmorpholine as base. Structures of all the newly synthesized compounds were elucidated using IR, $^{1}H/^{13}C$ NMR and MS spectral data and evaluated for antimicrobial potential. Most of the compounds exhibited potent antifungal activity against pathogenic *Candida albicans* and dermatophytes, in comparison to reference compound. Moderate bioactivity was also observed against Gram-negative bacteria for newly synthesized peptide derivatives.

Literature is enriched with several reports on synthesis of potent quinazolinones and benzoic acid derivatives with diverse bioactivities[1-3] but no report has been yet received on peptide coupling of quinazolinone derivatives. Moreover, incorporation of amino acids and peptides into aromatic nuclei have resulted in compounds with potent bioactivities[4-6]. So, keeping in view the pharmacological potential of quinazolinone and benzoic acid derivatives, both moieties were coupled in single nucleus and taking advantage of biodegradability and biocompatibility of amino acids and peptides, a series of 4-[2-(3-bromophenyl)-7-nitro-4-oxo-3,4-dihydro-3-quinazolinyl] benzoyl amino acids and peptides was synthesized to get novel bioactive agents with less side effects.

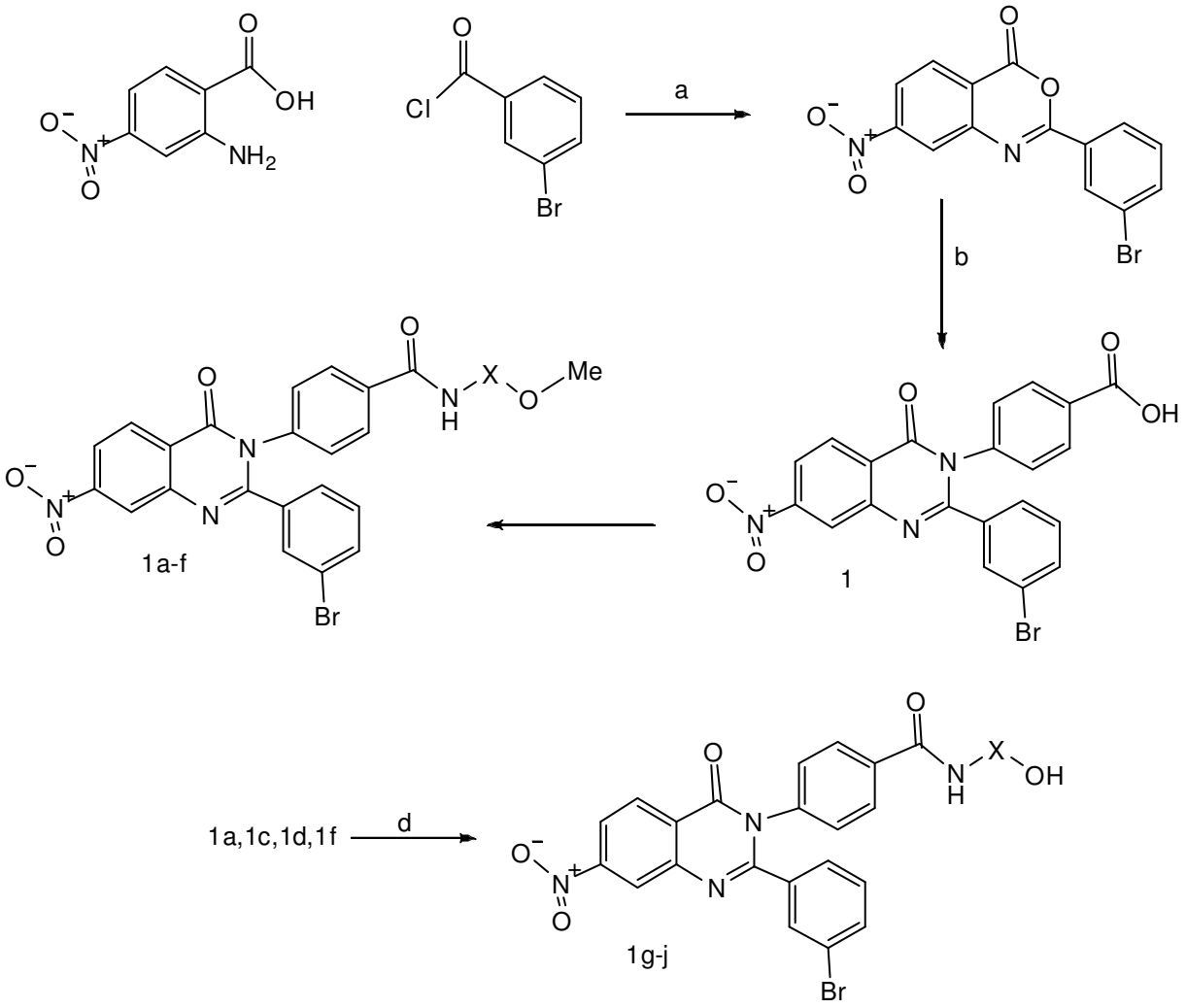
4-[2-(3-Bromophenyl)-7-nitro-4-oxo-3,4-dihydro-3-quinazolinyl] benzoic acid (**1**) was prepared by interaction of *p*-aminobenzoic acid (PABA) and 2-(3-bromophenyl)-7-nitro-4*H*-3,1-benzoxazin-4-one, which was in turn prepared from 4-nitroanthranilic acid and 3-bromobenzoyl chloride at $0\text{-}5^{0}$ in presence of pyridine[7]. Dipeptides Boc-Pro-Pro-OMe, Boc-Try-His-OMe, Boc-His-Phe-OMe, were prepared from the corresponding Boc-amino acids and amino acid methyl ester hydrochlorides using dicyclohexylcarbodiimide (DCC) and triethylamine (TEA) in dichloromethane (DCM). Similarly, Boc-Phe-Ile-Pro-OMe and Boc-His-Tyr-His-OMe were prepared by coupling Boc-Phe/Boc-His with Ile-Pro-OMe/Tyr-His-OMe in alkaline conditions.

4-[2-(3-Bromophenyl)-7-nitro-4-3,4-oxo-dihydro-3-quinazolinyl] benzoic acid (**1**) was coupled with various L-amino acid methyl ester hydrochlorides using DIPC as the coupling agent to get newly synthesized 4-[2-(3-bromophenyl)-7-nitro-4-oxo-3,4-dihydro-3-quinazolinyl] benzoyl amino acid methyl ester (**1**a). Similarly, dipeptide and tripeptide coupling was done to get dipeptide (**1**a-d) and tripeptide (**1**e, **1**f) methyl ester derivatives. Finally, selected compounds were hydrolyzed with lithium hydroxide to get the corresponding acid derivatives (**1**g-j).

Structures of all the novel compounds were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectra. In addition, elemental analysis of the newly synthesized compounds was performed for carbon, hydrogen and nitrogen content (Table-1).

Antimicrobial activity

All the synthesized compounds were evaluated for *in vitro* antimicrobial activity against Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and cutaneous fungi *Trichophyton mentagrophytes, Microsporum audouinii* and diamorphic fungi *Candida albicans* (Table-2) using modified Kirby-Bauer disk diffusion method[8] at 10 mcg/ml concentration. Almost, all the synthesized compounds were found to exhibit potent antifungal activity and moderate antibacterial activity against Gram-negative bacteria. Compounds (**1**c, **1**f) and their



SCHEME-1

X= try (**1**a,**1**g), Pro-Pro (**1**b), Try-His (**1**c, **1**h), His-Phe (**1**d, **1**i), Phe-Ile-Pro (**1**e), His-Try-His (**1**f-**1**j)
a = pyridine, 0^0
b = PABA, 170^0, 2hr
c= DIPC, NMM, DCM, RT, 24 hr
d = LiOH, THF:H_2O (1:1), RT, 1hr

Table-1
Physical data of the synthesized compounds

Compd	X	M.P. (^{0}C)	Yield (%)
1a	L-Try	122	78
1b	L-Pro-L-Pro	-	89
1c	L-Try-L-His	188	79
1d	L-His-L-Phe	207	80
1e	L-Phe-L-Ile-L-Pro	166	95
1f	L-His-L-Try-L-His	223	92
1g	L-Try	159	70
1h	L-Try-L-His	145	67
1i	L-His-L-Phe	192	71
1j	L-His-L-Try-L-His	179	89

hydrolyzed derivatives (**1**h, **1**j) were found to be more active than standard drug Griseofulvin against pathogenic fungus *C. albicans* and dermatophytes whereas compounds (**1**a, **1**g) displayed good activity against bacteria *E. coli* and *P. aeruginosa*. Comparison of antimicrobial data has suggested that amino acid and peptide derivatives (**1**g-j) were more potent antifungal and antibacterial agents than corresponding methyl ester derivatives (**1**a, **1**c, **1**d and **1**f).

Experimental

Melting points were determined by open capillary method and are uncorrected. L-Amino acids, di-tert-butylpyrocarbonate (Boc_2O), diisopropylcarbodiimide (DIPC), trifluoroacetic acid (TFA), N-methylmorpholine (NMM) and triethylamine (TEA) were obtained from Spectrochem Limited, Mumbai. IR spectra were recorded on FT infrared spectrophotometer (Jasco, Japan) using KBr pellets/$CHCl_3$ for all the synthesized compounds. ^{1}H NMR (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on Bruker spectrospin spectrometer using $CDCl_3$ as solvent and TMS as an internal standard. Mass spectra were recorded on Micromass Quattro II triple quadrupole mass spectrometer at 70eV. Elemental analysis of all compounds were performed on CHN analyzer (Elementar, Germany). Purity of all the compounds was checked by TLC on precoated silica gel G. plates.

Preparation of peptides

Peptide units were prepared by stirring a mixture of amino acid methyl ester hydrochloride and Boc-amino acid/dipeptide (0.01 mol each) with DCC (0.01 mol), TEA (0.021 mol) and DCM (50 ml) for 24 hr. Then, reaction mixture was filtered and the residue was washed with DCM (25 ml). After washing the filtrate with 5% $NaHCO_3$ and saturated NaCl solutions, organic layer was dried over anhyd. Na_2SO_4 and evaporated in vacuum after filtration. The crude product was recrystallized from a mixture of chloroform and petroleum ether followed by cooling at 0^0.

Resulting Boc-di/tripeptide methyl ester (0.01 mol) was dissolved in $CHCl_3$ (25 ml) and treated with trifluoroacetic acid (0.02 mol). The mixture was stirred at RT for 1 hr and washed with saturated $NaHCO_3$ solution. The resulting organic layer was dried over anhyd Na_2SO_4 and concentrated under reduced pressure. Finally, crude product was purified by recrystallization from $CHCl_3$ and petroleum ether to get the deprotected di/tripeptide methyl ester.

For protecting L-amino acids at amino end, Boc_2O was used whereas the carboxyl group of L-amino acids was protected by esterification with methanol using thionyl chloride. Peptides were prepared by Bodanszky method with certain modifications[9]. Furthermore, trifluoroacetic acid was used for the removal of Boc group and ester group was removed by alkaline hydrolysis with lithium hydroxide.

4-[2-(3-Bromophenyl)-7-nitro-4-oxo-3,4-dihydro-3-quinazolinyl] benzoic acid (1)

To a cold solution of 4-nitroanthranilic acid (0.01 mol) in pyridine (30 ml) maintained at 0^0, a solution of 3-bromobenzoyl chloride (0.02 mol) in pyridine (30 ml) was added slowly with constant stirring. The reaction mixture was further stirred for 30 min at RT and set aside for 1 hr. The pasty mass obtained was diluted with water (50 ml) and treated with aq sodium bicarbonate solution. The precipitate obtained was filtered off, washed with water and finally dried, Equimolar amounts (0.025 mol) of precipitate and *p*-aminobenzoic acid (PABA) were heated at 170-180^0 for 2 hr in an oil bath. The separated jelly like mass

Table-2
Antimicrobial activity data

Compd	Zone of inhibition (in mm)						
	B. subtilis	*E. coli*	*S. aureus*	*P. aeruginosa*	*M. audouinii*	*T. mentagrophytes*	*C. albicans*
1a	11	19	11	17	13	11	12
1b	14	-	13	15	-	12	14
1c	10	16	10	14	16	14	17
1d	10	16	10	14	13	13	14
1e	-	14	-	16	11	13	-
1f	9	16	11	14	15	15	17
1g	12	21	14	19	14	13	14
1h	11	17	11	16	18	16	19
1i	12	18	12	17	14	14	15
1j	11	17	14	16	16	17	20
Ciprofloxacin	18	22	19	20	-	-	-
Griseofulvin	-	-	-	-	15	14	16

solidified upon cooling. The crude product was finally recrystallized from ethanol to give a good yield of title compound.

Pale yellow solid : yield 78%, m.p. 193^0; IR (KBr): 3298-2509 (O-H str, COOH), 3078-3069, 3054-3049 (C-H str, rings), 1706 (C=O str, COOH), 1667 (C=O str, ring), 1596 (C=N str, ring), 1584-1575, 1422-1414 (C=C str, rings), 1515, 1350 (NO_2 str, asym and sym), 1402 (O-H def, COOH), 863 (C-N str, Ar-NO_2), 779, 735, 721, 702, 698, 663 (C-H def, oop, rings), 685, 622 (C-Br str) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.76 (1H, s, OH, COOH), 10.76 (1H, s, H-8, quinazolione ring [qze]), 8.34 (1H, d, J=7.4 Hz, H-6, qze), 8.28 (1H, d, J=7.6 Hz, H-5, qze), 8.15 (1H, s, H-2, bromobenzene moiety [bbz]), 7.91-7.89 (1H, d, J=6.9 Hz, H-2, bbz), 7.85-7.83 (2H, dd, J=6.95 Hz, 7.2 Hz, m-H's, benzoic acid moiety [bza]), 7.42-7.38 (3H, m, *o*-H's, bza and H-4, bbz), 7.42-7.38 (1H, t, J=6.6 Hz, H-5, bbz) ppm; ^{13}C NMR ($CDCl_3$, 70 MHz): δ 171.5 (C=O, COOH), 168.2 (C-2, qze), 157.9 (C-4, qze), 154.6 (C-7, qze), 149.2 (C-2′, qze), 138.6 (C-1, bza), 133.7 (C-1, bbz), 133.2 (2C, m-C's, bza), 132.6 (2C, *o*-C's bza), 130.4 (C-4, bbz), 129.9 (*p*-C, bza), 129.2 (C-2, bbz), 128.8 (C-5, bbz), 128.2 (C-5, bbz), 127.7 (C-5, qze), 125.1 (C-3′, qze), 123.4 (C-3, bbz), 118.8 (C-6, qze), 116.5 (C-8,qze) ppm.

Preparation of 4-[2-(3-bromophenyl)-7-nitro-4-oxo-3,4-dihydro-3-quinazolinyl] benzoyl amino acid and peptide derivatives (1a-j)

Title compounds **1**a-j were prepared by coupling 4-[2-(3-bromophenyl)-7-nitro-4-oxo-3,4-dihydro-3-quinazolinyl] benzoic acid (**1**) with amino acid methyl ester hydrochlorides/peptide methyl esters by using DIPC and NMM in DCM by following the same procedure as that adopted for peptide synthesis to get the amino acid and peptide derivatives (**1**a-j). Amino acid (**1**a), dipeptide (**1**c, **1**d) and tripeptide (**1**f)

derivatives were further hydrolyzed with LiOH to get the corresponding free acids (**1**a-j).

For removal of the ester group, peptide ester derivative (0.01 mol) was dissolved in THF:H_2O (1:1) and LiOH (0.015 mol) was added to the solution at 0^0. The resulting mixture was stirred at RT for 1 hr and then acidified to pH 3.5 with 1N H_2SO_4. The aqueous layer was extracted with Et_2O (2x20 ml) and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to get the desired hydrolyzed compound.

1a: ^{13}C NMR ($CDCl_3$, 70 MHz): δ 172.3 (C=O ester), 169.0 (C-2, qze), 168.6 (C=O, bza), 158.3 (C-4, qze), 154.1 (C-7, qze), 148.9 (C-2′, qze), 139.7 (C-1, bze), 137.8 (C-2′, indole ring [idr]), 134.2 (C-1, bbz), 131.9 (C-4, bbz), 130.8 (C-2, bbz), 129.2 (C-5, bbz), 128.7 (C-6, bbz), 128.0 (C-5, qze), 127.5 (2C, *m*-C's bza), 127.0 (2C, *o*-C's, bza), 126.8 (C-3′, idr), 125.6 (*p*-C, bza), 124.8 (C-3′, qze), 123.1 (C-3, bbz), 122.6 (C-2, idr), 121.7 (C-6, idr), 119.5 (C-5, idr), 118.7 (C-4, idr), 117.9 (C-6, qze), 116.6 (C-8, qze), 114.4 (C-3, idr), 112.9 (C-7, idr), 55.6 (C-α, Trp), 51.7 (OCH_3), 19.9 (C-β, Trp). [Found : C, 59.49, H, 3.59, N, 10.52 $C_{33}H_{25}BrN_5O_6$ requires C, 59.47, H, 3.63, N, 10.51%].

1b: ^1H NMR ($CDCl_3$, 300 MHz): 8.64 (1H, s, H-8, qze), 8.44-8.39 (2H, m, *m*-H's, bza), 8.31-8.26 (2H, m, H-5 and H-6, qze), 8.11 (1H, s, H-2, bbz), 7.92-7.89 (1H, t, J=6.5 Hz, H-6, bbz), 7.62-7.58 (2H, m, *o*-H's bza), 7.40-7.38 (1H, d, J=6.85 Hz, H-4, bbz), 7.29-7.26 (1H, t, J=6.7 Hz, H-5, bbz), 4.41-4.38 (1H, t, J=6.9 Hz, H-α, Pro-1), 4.26-4.23 (1H, t, J=6.85 Hz, H-α, Pro-2), 3.72-3.69 (2H, t, J=7.15 Hz, H-δ, Pro-2), 3.62 (3H, s, OCH_3), 3.45-3.42 (2H, t, J=7.2 Hz, H-δ, Pro-1), 2.69-2.64 (2H, m, H-β, Pro-1), 2.05-2.01 (2H, m, H-β, Pro-2), 1.97-1.92 (4H, m, H-γ, Pro-1 and Pro-2) ppm. [Found : C, 56.99, H, 4.15, N, 10.40 $C_{32}H_{28}BrN_5O_7$ requires C, 56.98, H, 4.18, N, 10.38%].

1c: IR (KBr): 3486-3479 (N-H str, heterocyclic rings), 3129, 3122 (N-H str, amide), 3085-3069, 3055, 3047-3042 (C-H str, rings), 2925 (C-H str, asym, CH_2), 2844 (C-H str, sym, CH_2), 1745 (C=O str, ester), 1664 (C=O str, ring), 1639-1634 (C=O str, 2^0 amide), 1599-1595 (C=N str, rings), 1594-1573, 1438-1423 (C=C str, rings), 1537, 1532 (N-H bend, 2^0 amide), 1515, 1357 (NO_2 str, asym and sym), 1274 (C-O str, ester), 864 (C-N str, Ar-NO2), 854-843, 775, 729-714, 692, 657-653 (C-H def, oop, rings), 681, 622 (C-Br str) cm^{-1}. [Found : C, 58.27, H, 3.92, N, 13.95 $C_{39}H_{31}BrN_8O_7$ requires C, 58.29, H, 3.89, N, 13.94%].

1d: ^{13}C NMR ($CDCl_3$, 7- MHz): 171.6 (C=O, His), 170.3 (C=O ester), 169.4 (C-2, qze), 167.7 (C=O, bza), 158.0 (C-4, qze), 152.3 (C-7, qze), 149.1 (C-2', qze), 142.8 (C-2, imidazole ring [imz]), 140.2 (C-1, bza), 137.3 (C-γ, Phe), 134.7 (C-1, bbz), 133.5 (C-4, bbz), 132.4 (C-2, bbz), 130.2 (C-5, bbz), 129.4 (2C, *o*-C's, Phe), 128.9 (C-6, bbz), 128.3 (2C, m-C's, Phe), 128.1 (2C, o-C's, bza), 127.9 (C-5, qze), 127.5 (2C, m-C's, bza), 126.7 (*p*-C, Phe), 125.6 (C-4, imz), 125.0 (*p*-C, bza), 124.5 (C-3′, qze), 123.6 (C-3, bbz), 120.5 (C-6, qze), 118.8 (C-8, qze), 116.6 (C-5, imz), 59.2 (C-α, His), 55.8 (C-α, Phe), 52.7 (OCH_3), 38.6 (C-β, Phe), 18.8 (C-b, His), ppm. [Found : C, 58.09, H, 3.96, N, 12.85 $C_{37}H_{34}BrN_5O_7$ requires C, 58.12, H, 3.95, N, 12.82%].

1e: MS: m/z (rel. int.) 14 (4.7), 15 (14.2), 29 (9.8), 31 (9.6), 59 (12.4), 70 (11.5), 77 (16.4), 86 (17.1), 91 (21.5), 120 (13.9), 145 (11.8), 156 (11.4), 190 (16.9), 345 (24.5), 421 (32.6), 449 (68.2), 568 (10.7), 596 (100), 681 (24.7), 709 (74.7), 778 (16.6), 806 (24.2), 822 (19.4), 837 (M^+, 2.9), 838 (M+, 0.6). [Found : C, 60.19, H, 4.95, N, 10.05 $C_{42}H_{41}BrN_6O_8$ requires C, 60.22, H, 4.93, N, 10.03%].

1f: MS m/z (rel. int.) 14 (3.9), 15 (3.6), 17 (4.7), 31 (9.9), 59 (13.3), 67 (15.7), 81 (22.9), 93 (9.4), 107 (11.8), 110 (19.5), 136 (14.8), 145 (12.6), 156 (10.9), 190 (16.9), 345 (21.2), 421 (29.4), 449 (65.6), 558 (14.3), 586 (100), 721 (14.6), 749 (70.2), 858 (15.9), 886 (33.7), 902 (17.2), 917 (M^+, 2.6), 918 (M^+, 0.8). [Found : C, 56.25, H, 4.08, N, 15.25 $C_{43}H_{37}BrN_{10}O_9$ requires C, 56.28, H, 4.06, N, 15.26%].

1g: ^{13}C NMR ($CDCl_3$, 70 MHz): 175.8 (C=O, -COOH), 169.1 (C=O, bza), 168.6 (C-2, qze), 157.8 (C-4, qze), 152.9 (C-7, qze), 148.0 (C-2′, qze), 140.5 (C-1, bza), 136.9 (C-2′, idr), 133.7 (C-1, bbz), 132.3 (C-4, bbz), 130.4 (C-2, bbz), 130.1 (C-6, bbz), 129.6 (C-5, bbz), 128.4 (C-5, qze), 128.0 (2C, *m*-C's, bza), 127.6 (2C, *o*-C's, bza), 127.1 (C-3′, idr), 125.4 (C-3′, qze), 124.5 (*p*-C, bza), 123.5 (C-2, idr), 123.0 (C-3, bbz), 122.2 (C-6, idr), 119.2 (C-5, idr), 118.2 (C-6, qze), 117.7 (C-4, idr), 117.2 (C-8, qze), 115.4 (C-3, idr), 111.5 (C-7, idr), 54.9 (C-α, Trp), 19.4 (C-β, Trp), ppm. [Found : C, 58.89, H, 3.43, N, 10.75 $C_{32}H_{22}BrN_5O_6$ requires C, 58.91, H, 3.40, N, 10.73%].

1h: IR (KBr): 3488-3481 (N-H str, heterocyclic rings), 3298-2487 (O-H str-COOH), 3127, 3120 (N-H str, amide), 3087-3072, 3058, 3044-3036 (C-H str, rings), 2928 (C-H str, asym, CH_2), 2845 (C-H str, sym,

CH_2), 1716 (C=O str, COOH), 1668 (C=O str, ring), 1639-1632 (C=O str, 2^0 amide), 1596-1592 (C=N str, rings), 1597-1577, 1439-1425 (C=C str, rings), 1535, 1530 (N-H bend, 2^0 amide) , 1516, 1359 (NO_2 str, asym and sym), 868 (C-N str, Ar-NO_2), 856-842, 777, 726-717, 698, 655-649 (C-H def, oop, rings), 685, 626 (C-Br str). [Found : C, 57.78, H, 3.73, N, 14.20 $C_{38}H_{29}BrN_8O_7$ requires C, 57.80, H, 3.70, N, 14.19%].

1i: ^{13}C NMR ($CDCl_3$, 70 MHz): 173.5 (C=O, COOH), 171.4 (C=O, His), 169.2 (C-2, qze), 168.3 (C=O, bza), 158.6 (C-4, qze), 152.0 (C-7, qze), 148.8 (C-2′, qze), 141.9 (C-2, imz), 138.1 (C-γ, Phe), 137.7 (C-1, bza), 134.2 (C-1, bbz), 133.9 (C-4, bbz), 132.0 (C-2, bbz), 130.6 (C-5, bbz), 129.7 (2C, o-C's, Phe), 128.8 (C-5, qze), 128.5 (2C, *o*-C's, bza), 128.3 (C-6, bbz), 127.9 (2C, m-C's, bza), 127.5 (2C, m-C's, Phe), 126.3 (C-4, imz), 125.2 (*p*-C, bza), 124.8 (C-3', qze), 124.5 (*p*-C, Phe), 124.0 (C-3, bbz), 120.9 (C-6, qze), 119.0 (C-8, qze), 115.8 (C-5, imz), 58.9 (C-α, His), 54.5 (C-α, Phe), 37.8 (C-β, Phe), 18.9 (C-β, His) ppm. [Found : C, 57.59, H, 3.75, N, 13.09 $C_{36}H_{28}BrN_7O_7$ requires C, 57.61, H, 3.76, N, 13.06%].

1j: MS m/z (rel. int) 14 (3.5), 17 (8.2), 45 (10.8), 67 (15.4), 81 (22.2), 93 (9.8), 107 (12.5), 110 (20.2), 136 (14.5), 145 (12.9), 156 (11.4), 190 (16.2), 345 (20.6), 421 (27.9), 449 (65.2), 558 (14.2), 586 (100), 721 (14.9), 749 (69.7), 858 (15.5), 886 (32.9), 903 (M^+, 3.8), 904 (M^+, 0.5). [Found : C, 55.79, H, 3.92, N, 8.85 $C_{42}H_{35}BrN_{10}O_9$ requires C, 55.82, H, 3.90, N, 8.84%].

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