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Synthesis and biological activity of peptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid

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ABSTRACT

*1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid **(S1)** was prepared by interaction of pyruvic acid and 3,4-diaminobenzoic acid in presence of rectified spirit. Coupling of **(S1)** with different amino acids methyl ester hydrochlorides/dipeptide methyl esters afforded novel quinoxalopeptide derivatives **(S1a-f).** Selected peptide ester derivatives **(S1a-f)** were further hydrolyzed by using lithium hydroxide (LiOH) to afford the corresponding derivatives **S2a-f**. The chemical structure of all newly synthesized compounds were confirmed on the basis of IR, ^{1}HNMR spectral data. All the synthesized peptide derivatives were screened for their antibacterial, antifungal and anthelmintic activity against four pathogenic bacteria, fungus strains and three earthworm species. The newly synthesized peptide derivatives had shown moderate to good anthelmintic activity against three earthworm species Megascoplex konkanensis, Pontoscotex corethruses and Eudrilus eugeniea and exhibited potent bioactivity against gram negative-bacteria Pseudomonas aeruginosa and Klebsiella pneumonia and fungal strains of Candida albicans and Aspergillus niger.*

Keywords: Quinoxaline, antibacterial activity, antifungal activity, anthelmintic activity.

INTRODUCTION

Compounds containing the quinoxaline nucleus exhibit a broad spectrum of biological activity such as antibacterial [11], antifungal [9], anticancer, anticonvulsant [15], anti-tuberculosis[13], anti-amoebic [5] and anti-inflammatory properties[4]. Many researchers have reported the synthesis and biological activity of quinoxaline derivatives[6, 15, 12]. Prompted by the chemotherapeutic importance of quinoxaline derivatives, the vital moieties 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid (S1) was synthesized. 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[14]. Thus, keeping in mind the pharmacological potential of quinoxaline derivatives as well as taking advantage of biodegradability and biocompatibility of amino acids/peptides and further, in continuation of earlier work on the synthesis of bioactive peptide analogs of aroylbenzoic acids, aryloxyacetic acids, benzimidazoles and imidazoles[7], therefore an attempt was made towards the synthesis of novel series of 3-methyl-2-oxo-1,2-dihydro-6-quinoxalinoyl amino acid/peptide derivatives **(S1a-f)**. The synthesized peptide derivatives were further hydrolyzed to get corresponding acid derivatives **(S2a-f)**. The synthesized peptide derivatives were evaluated for their antimicrobial, antifungal and anthelmintic activity.

MATERIALS AND METHODS

Materials and equipments

Melting points were determined by the open capillary method and were uncorrected. IR spectra were recorded on a Shimadzu 8700 FT-IR spectrophotometer (Shimadzu, Japan) using a thin film supported on KBr pellets and $CHCl_3$ as solvent for intermediate semisolids. ^{1}H NMR was recorded on a Bruker AC NMR spectrometer (300MHz), (Bruker, USA) using $CDCl_3$ as solvent. Purity of all compounds was checked by TLC on pre-coated silica gel G plates (Kieselgel 0.25 mm, 60G F254, Merck, Germany) utilizing chloroform/methanol as the developing solvent system in different proportions (9:1, 8:2, 7:3 v/v) and dark brown spots were detected on exposure to iodine vapours in a tightly closed chamber. All the chemicals and solvents were purchased from Merck (Darmstadt, Germany).

*2.1.2. Synthesis of L-amino acid methyl ester hydrochlorides **(1–3)***

Thionyl chloride (3.7 ml, 0.02 mol) was slowly added to methanol (100 ml) at 0 ºC and L-phenylalanine (8.2 gm, 0.02 mol) was added to the above solution. The resulting mixture was refluxed for 8 h at ambient temperature. Methanol was evaporated and the residue was triturated with ether at 0 ºC until excess dimethyl sulphite was removed. The crude solid was crystallized from methanol and ether at 0 ºC to get L-Phenylalanine methyl ester hydrochloride (**1**). Similarly, L-tryptophan methyl ester hydrochloride (**2**) and L-tyrosine methyl ester hydrochloride (**3**) were prepared by refluxing L- tryptophan (10.3 g, 0.02 mol)/L-tyrosine (9.05 g, 0.02 mol) with methanol (100 ml) in the presence of thionyl chloride (3.7 ml, 0.02 mol).

*L-Phenylalanine methyl ester hydrochloride **(1)***

White solid; Yield: 78%; m.p. 160-162 ºC; IR (KBr, $CHCl_3$, ν, cm^{-1}): 3010–2855 (s/br, NH_3^+ str., asym. and sym.), 3076, 3030 (w, CH str., ring), 2926 (m, CH str., asym., aliph., CH_2), 2894 (m, CH str., >CH–), 2828 (m, CH str., OCH_3), 1742 (s, C=O str., ester), 1605, 1503 (s/br, NH_3^+ bend., asym. and sym.), 1205 (s, C–O str., ester), 732, 695 (s, CH bend., out-of-plane, monosub. ring). ^{1}H NMR (300 MHz,$CDCl_3$, *δ*, ppm): 7.66–7.61 (t, 2H, *m*-H's, Phe), 7.53–7.49 (t, 1H, *p*-H, Phe), 7.47–7.45 (d, 2H, *o*-H's, Phe, *J* = 6.5 Hz), 5.17 (br. s, 3H, NH_3^+), 4.15–4.11 (m, 1H, α-H, Phe), 4.09 (s, 3H, OCH_3), 2.31–2.29 (d, 2H, β-H's, Phe, *J* = 4.45 Hz).

*L-tyrosine methyl ester hydrochloride **(2)***

White crystals; yield 83%; m.p.190 °C; IR (KBr, $CHCl_3$, ν, cm^{-1}): 3372 (OH str.), 3011-2863 (s/br, NH_3^+), 2928, 2848 (m, CH_2), 1750 (s, C=O ester), 1588, 1475 (m, skeletal bands, ring), 1227 (s, C–O str., phenolic), 1272 (s, C−O str., ester). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 7.80-7.78 (d, 2H, H-*m*, J=7.4 Hz, J=5.0 Hz), 7.56-7.54 (d, 2H, *o*-H, J=7.5 Hz, J=4.6 Hz), 5.40 (br. s, OH and NH_3^+), 4.13- 4.09 (m, 1H, α-H), 4.12 (s, 3H, OCH_3), 2.20-2.18 (d, 2H, β-H, J=7.15 Hz).

*L-Tryptophan methyl ester hydrochloride **(3)***

White crystals; yield 80%; m.p.185 °C; IR (KBr, $CHCl_3$, ν, cm^{-1}): 3050 (C-H ring str.), 1670 (C=C ring str.), 3300 (NH str.), 1640 (C=O str.), 1240, (C-O str.), 2960 (C-H methyl str.). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 4.09 (s, 3H, OCH_3), 7.42 (d, 1H), 7.39 (d, 1H), 7.16 (d, 1H), 7.14 (d, 1H), 7.04 (t, 1H), 4.24 (t, 1H), 3.66 (s, 3H, NH_3^+), 3.25 (m, 2H, CH_2), 10.1 (s, 1H, NH, Trp).

*L-tyrosine methyl ester hydrochloride **(3)***

White crystals; yield 83%; m.p.190 °C; IR (KBr, $CHCl_3$, ν, cm^{-1}): 3372 (OH str.), 3011-2863 (s/br, NH_3^+), 2928, 2848 (m, CH_2), 1750 (s, C=O ester), 1588, 1475 (m, skeletal bands, ring), 1227 (s, C–O str., phenolic), 1272 (s, C−O str., ester). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 7.80-7.78 (d, 2H, H-*m*, J=7.4 Hz, J=5.0 Hz), 7.56-7.54 (d, 2H, *o*-H, J=7.5 Hz, J=4.6 Hz), 5.40 (br. s, OH and NH_3^+), 4.13- 4.09 (m, 1H, α-H), 4.12 (s, 3H, OCH_3), 2.20-2.18 (d, 2H, β-H, J=7.15 Hz).

*Synthesis of Boc-amino acids **(4–6)***

L-Alanine (4.45gm, 0.02 mol) was dissolved in 1mol L^{-1} NaOH (20 ml) and iso-propanol (20 ml). Boc_2O (6 ml, 0.026 mol) in iso-propanol (10 ml) was added followed by 1mol L^{-1} NaOH (20 ml) to the resulting solution. The solution was stirred at room temperature for 2h and washed with light petroleum ether (b.p. 40–60 °C) (20 ml), acidified to pH 3.0 with 1 mol L^{-1} H_2SO_4 and finally extracted with chloroform (3 x 20 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure to give the crude product, which was crystallized from chloroform and petroleum ether (b.p.40 - 60 °C) to get pure Boc-Alanine (**4**). Similarly Boc-

Phenyl alanine (**5**) and Boc-Proline (**6**) and were prepared by stirring Boc_2O (6 ml, 0.026 mol) with L-Proline (5.75 g, 0.02 mol) and L-Phenylalanine (8.2 g, 0.02 mol), respectively.

*Boc-Alanine **(4)***
White crystals; yield 79%; m.p.79-81 °C; IR (KBr, $CHCl_3$, ν, cm^{-1}):) 3350 (NH str.), 1750 (C=O str.), 3300 (OH str.), 1250 (C-O str., COOH), 925 (w, CH_3 rocking, *t*-butyl), 2950 (CH str., CH_3). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 12.02 (br. s, 1H, OH), 1.49 (s, 9H, *t*-butyl), 11.80 (s, NH), 1.43 (s, CH_3).

*Boc-Phenylalanine **(5)***
White crystals; yield 78%; mp: 85-86 °C; IR (KBr, $CHCl_3$, ν, cm^{-1}): 3298–2485 (m/br, OH str., COOH), 3089, 3034 (w, CH str., ring), 2928 (m, CH str, asym, aliph. CH_2), 2897 (m, CH str, >CH–), 1715 (s, C=O str., COOH), 1582, 1482 (m, skeletal bands, ring), 1542 (m, NH bend., amide), 1387, 1365 (m, CH bend., *t*-butyl), 933 (w, CH_3 rocking, *t*-butyl), 730, 693 (s, CH bend, out-of-plane, monosub., ring). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 10.48 (s, 1H, OH, COOH), 7.30–7.26 (t, 2H, m-H's, Phe), 7.09–7.07 (dd, 2H, o-H's, Phe, J = 6.45 Hz), 7.00–6.96 (t, 1H, p-H, Phe), 5.89 (br. s, 1H, NH), 1.54 (s, 9H, *t*-butyl).

*Boc-Proline **(6)***
White crystals; yield 72%; mp: 130-131 °C; IR (KBr, $CHCl_3$, ν, cm^{-1}): 2960 (CH ring str.), 1650 (C=C ring str.), 870 (C=C ring bend), 1160 (C-C str.), 2950 (CH str., CH_3), 3350 (NH str.), 1750 (C=O str.), 3300 (OH str.), 1250 (C-O str., COOH). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 4.15 (s, CH-COOH), 3.01-2.98 (m, CH_2), 10.80 (s, NH), 11.05 (OH), 1.32 (s, 9H, *t*-butyl).

*Synthesis of Boc-dipeptide methyl esters **(7–9)***
A mixture of compound **1** (L-Phenylalanine methyl ester hydrochloride, 4.1gm, 0.01mol) in $CHCl_3$ (20 ml), NMM (2.3 ml, 0.021mol) was added at 0 °C. The reaction mixture was stirred for 15 min. Compound **4** (Boc-Alanine, 2.22 gm, 0.01mol) in $CHCl_3$ (20 ml) and DCC (2.1gm, 0.01mol) was added under stirring to the above mixture. After 36 h the reaction mixture was filtered and the residue was washed with $CHCI_3$ (30 ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ and saturated NaCl solution (25 ml each). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was crystallized from a mixture of chloroform and petroleum ether (b.p. 40–60 °C), followed by cooling at 0 °C to get Boc-Ala-Phe-OMe (**7**). Similarly, Boc-Phe-Tyr-OMe (**8**) and Boc-Pro-Trp-OMe (**9**) were prepared by stirring compounds **5** and **6** with amino acid methyl ester hydrochlorides **2** and **3** respectively, in the presence of DCC and NMM.

*Boc-Ala-Phe-OMe **(7)***
Semi solid mass; yield 61 %; IR (KBr, $CHCl_3$, ν, cm^{-1}): 3123 (N-H str., amide), 3052 (C-H str., arom. ring), 2956, 2926 (C-H str., asym., CH_3 and CH_2), 1750 (C=O str., ester), 1643 (C=O str., amide), 1584, 1475 (skeletal bands, arom. ring), 1535 (N-H bend., amide), 1390, 1367 (C-H bend., *t-b*utyl), 1269 (C-O str., ester), 710, 690 (C-H bend., Phenyl ring). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 7.13-7.09 (t, 1H, *p*-H, Phe), 7.00-6.97 (t, 2H, *m*- H, Phe), 6.89 (s, 1H, N-H), 6.86-6.84 (d, 2H, *o*-H, Phe), 6.55 (s, 1H, NH), 4.60- 4.48 (m, 2H, α-H, Phe and Ala), 3.54 (s, 3H, OCH_3), 3.00-2.98 (d, 2H, β-H, Phe), 1.59-1.57 (d, 3H, β-H, Ala); 1.54 (s, 9H, *t*-butyl group).

*Boc-Phe-Tyr-OMe **(8)***
Semi solid mass; yield 65 %; IR (KBr, $CHCl_3$, ν, cm^{-1}): 3373 (OH str., Tyr), 3125 (NH str., amide), 3065, 3052 (CH str., rings), 2925, 2846 (CH str., asym. and sym., CH_2), 1749 (C=O str., ester), 1645, 1638 (C=O str., amide), 1587, 1479, 1476 (skeletal bands, rings), 1536, 1526 (NH bend, amide), 1390, 1365 (CH bend, *t*-butyl), 1272 (C−O str, ester), 825, 710, 695 (CH def., rings). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 7.51-7.47 (t, 2H, H-*m*, Phe), 6.92-6.88 (m, 3H, H-*p*, Phe and H-*o*, Tyr), 6.86-6.84 (d, 2H, H-*o*, Phe, J=6.45 Hz, J=4.15 Hz), 6.80-6.78 (d, 2H, H-*m*, Tyr, J=7.45 Hz, J=5.1 Hz), 6.66 (s, 1H, NH), 5.95 (s, 1H, OH, Tyr), 4.72-4.69 (q, 1H, α-H, Phe), 4.60- 4.56 (m, 1H, α-H, Tyr), 3.54 (s, 3H, OCH_3), 3.02-2.76 (m, 4H, β-H, Phe and Tyr); 1.55 (s, 9H, *t*-butyl).

*Boc-Pro-Trp-OMe **(9)***
Semi solid mass, Yield 79%; IR (KBr, ν, cm^{-1}): 3065, 3052 (CH str., rings), 2925, 2846 (CH str., asym. and sym., CH_2), 1749 (C=O str., ester), 1645, 1638 (C=O str., amide), 1587, 1479, 1476 (skeletal bands, rings), 1536, 1526 (NH bend, amide), 1390, 1365 (CH bend, *t*-butyl), 1272 (C−O str., ester), 825, 710, 695 (CH def., rings), 3125 (NH str., amide). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 4.73–4.70 (t, 1H, δ-H of Pro), 4.02–3.97 (t, 2H, α-H's, Pro), 2.48–

2.41 (m, 2H, γ-H's, Pro), 2.40–2.31 (m, 2H, β-H's, Pro), 1.54 (s, 9H, *t*-butyl), 3.66 (s, 3H, OCH_3), 7.39 (s, 1H, NH, Trp), 7.42-7-41(d, 1H, Trp), 3.25 (m, 2H, CH_2), 7.16-7.15(d,1H, Trp).

yield: 60 %, Mol. For: $C_{21}H_{28}N_3O_5$. ^{1}H NMR (300 MHz, $CDCl_3$, TMS, δ ppm): 1.49 (s, 9H, butyl-*t*), 4.31- 4.26 (t, 1H, H-α), 1.90-1.85 (m, 2H, H-β), δ 7.42 (d, 1H), 7.39 (d, 1H), 7.16 (d, 1H), 7.14 (d, 1H), 7.04 (t, 1H), 4.24 (t, 1H), 3.66 (s, 3H), 8.00 (s, 1H, NH), IR (KBr, $CHCl_3$, ν, cm^{-1}): 2998, 2986 (m, CH str, CH_2, pro); 1715 (s, C=O str), 3300(NH str).

*Deprotection of dipeptide methyl esters at amino end **(7a-9a)***
Compound **7** (3.42 g, 0.01 mol) was dissolved in $CHCl_3$ (15 ml) and treated with trifluoroacetic acid (2.28 g, 0.02 mol). The resulting solution was stirred at room temperature for 1 h and washed with saturated $NaHCO_3$ solution (25 ml). The organic layer was dried over an-hydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by crystallization from $CHCl_3$ and petroleum ether (b.p. 40–60 °C) to give pure Ala-Phe-OMe (**7a**). The same procedure was adopted for synthesis of compound **8a** (Phe-Tyr-OMe) and **9a** (Pro-Trp-OMe) from compound **8** and **9** respectively.

*Ala-Phe-OMe **(7a)***
Semi solid mass; Yield 72%; IR (KBr, ν, cm^{-1}): 3123 (NH str., amide), 3052 (CH str., aromatic ring), 2956, 2926 (CH str., asym., CH_3 and CH_2), 1750 (C=O str, ester), 1643 (C=O str., amide), 1584, 1475 (skeletal bands, aromatic ring), 1535 (NH bend., amide), 1269 (C-O str., ester), 710, 690 (CH bend., phenyl ring). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 7.13-7.09 (t, 1H, *p*-H, Phe), 7.00-6.97 (t, 2H, *m*- H, Phe), 6.89 (s, 1H, NH), 6.86-6.84 (d, 2H, *J* = 6.45 Hz, *o*-H, Phe), 6.55 (s, 1H, NH), 4.60- 4.48 (m, 2H, α-H, Phe and Ala), 3.54 (s, 3H, OCH_3), 3.00-2.98 (d, 2H, *J* = 5.9 Hz, β-H, Phe), 1.59-1.57 (d, 3H, *J* = 4.2 Hz, β-H, Ala).

*Phe-Tyr-OMe **(8a)***
Semi solid mass; yield 62 %; IR (KBr, $CHCl_3$, ν, cm^{-1}): 3373 (OH str., Tyr), 3125 (NH str., amide), 3065, 3052 (CH str., rings), 2925, 2846 (CH str., asym. and sym., CH_2), 1749 (C=O str., ester), 1645, 1638 (C=O str., amide), 1587, 1479, 1476 (skeletal bands, rings), 1536, 1526 (NH bend, amide), 1390, 1272 (C−O str, ester), 825, 710, 695 (CH def., rings). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 7.51-7.47 (t, 2H, H-*m*, Phe), 6.92-6.88 (m, 3H, H-*p*, Phe and H-*o*, Tyr), 6.86-6.84 (d, 2H, H-*o*, Phe, J=6.45 Hz, J=4.15 Hz), 6.80-6.78 (d, 2H, H-*m*, Tyr, J=7.45 Hz, J=5.1 Hz), 6.66 (s, 1H, NH), 5.95 (s, 1H, OH, Tyr), 4.72-4.69 (q, 1H, α-H, Phe), 4.60- 4.56 (m, 1H, α-H, Tyr), 3.54 (s, 3H, OCH_3), 3.02-2.76 (m, 4H, β-H, Phe and Tyr).

*Pro-Trp-OMe **(9a)***
Semi solid mass; Yield: 71%; IR (KBr, ν, cm^{-1}): 3065, 3052 (CH str., rings), 2925, 2846 (CH str., asym. and sym., CH_2), 1749 (C=O str., ester), 1645, 1638 (C=O str., amide), 1587, 1479, 1476 (skeletal bands, rings), 1536, 1526 (NH bend., amide), 1272 (C−O str., ester), 825, 710, 695 (s, CH def., rings), 3125 (m, NH str., amide). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 4.73–4.70 (t, 1H, δ-H, Pro), 4.02–3.97 (t, 2H, α-H, Pro), 2.48–2.41 (m, 2H, γ-H, Pro), 2.40–2.31 (m, 2H, β-H, Pro), 3.66 (s, 3H, OCH_3), 7.39 (s, 1H, NH, Trp. ring), 7.42-7.41(d, 1H, Trp), 3.25 (m, 2H, CH_2).

Synthesis of 1, 2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic *carboxylic acid **(S1)***
A warm solution of pyruvic acid (0.88 gm, 10 mmol) was dissolved in 8 ml of rectified spirit and added to the solution of 3, 4-diaminobenzoic acid (1.52 gm, 10 mmol) in 8 ml of rectified spirit. The resulting mixture was refluxed for 1.5 h at 80ºC temperature, add water until a slight cloudiness persists. After cooling, mixture was filtered and finally crude product was recrystallized from aqueous ethanol.

White crystals; Yield: 72%; m.p. 295-296 ºC; IR (KBr, ν, cm^{-1}): 3467.38 (O–H str., COOH), 3350.71 (NH str.), 3078.80 (C–H str., aromatic ring), 1506 (C-C Str., Aromatic ring), 1036 (C-H bend.), 2864.49 (C–H str., CH_3), 1700.52 (C=O str., COOH), 1681.69 (C=O str.). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 2.411 (s, 3H, CH_3), 7.293-8.165 (m, 3H, aromatic H), 9.281 (s, 1H, aromatic NH), 12.5 (s, 1H, COOH).

*General procedure for synthesis of amino acid / peptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid **(S1a-f)***
Amino acid methyl ester hydrochloride/dipeptide methyl ester (**1, 2, 3, 7a, 8a, 9a**) (0.01 mol) was dissolved in THF (75 ml) separately. To the solution of these compounds, NMM (2.3 ml) was added at 0 °C and the reaction mixture was stirred for 15 min. Compound **S1** (4.22 g, 0.01 mol) in THF (75 ml) and DCC (2.1 g) were added to the above

mixtures 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% $NaHCO_3$ and saturated NaCl solutions (15 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and *n*-hexane followed by cooling at 0 °C.

Compound ***S1a***

White crystals, yield 78%; m.p. 250-251°C; IR (KBr, ν, cm^{-1}): 3350.71 (NH str.); 3078.80 (C–H str., aromatic ring); 1506 (C-C Str., Aromatic ring); 1036 (C-H bend.); 2864.49 (C–H str., CH_3); 1681.69 (C=O str.), 3076, 3030 (w, CH str., ring), 2926 (m, CH str., asym., aliph., CH_2), 2828 (m, CH str., OCH_3), 1742 1205 (s, C–O str., ester), 732, 695 (s, CH bend., out-of-plane, monosub. ring). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 2.411 (s, 3H, CH_3); 7.293-8.165 (m, 3H, aromatic H); 9.281 (s, 1H, NH, Quinoxaline ring); 1.03(s, CH_3, Quinoxaline ring), 7.66–7.61 (t, 2H, *m*-H's, Phe), 7.53–7.49 (t, 1H, *p*-H, Phe), 4.09 (s, 3H, OCH_3).

Compound ***S1b***

White solid, yield 82%; m.p. 260-261°C; IR (KBr, ν, cm^{-1}): 3350.71 (NH str.), 3078.80 (C–H str., aromatic ring), 1506 (C-C Str., Aromatic ring), 1036 (C-H bend.), 2864.49 (C–H str., CH_3), 3050 (C-H ring str.), 1670 (C=C ring str.), 3300 (NH str.), 1640 (C=O str.), 1240, (2960 (C-H methyl str.). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 4.09 (s, 3H, OCH_3), 7.42 (d, 1H), 7.39 (d, 1H), 7.16 (d, 1H), 7.14 (d, 1H), 7.04 (t, 1H), 4.24 (t, 1H), 3.25 (m, 2H, CH_2), 2.411 (s, 3H, CH_3); 7.293-8.165 (m, 3H, aromatic H); 9.28 (s, 1H, NH, Quinoxaline ring).

Compound ***S1c***

White crystals, yield 78%; m.p. 247-248 °C; IR (KBr, ν, cm^{-1}): 3350.71 (NH str.), 3078.80 (C–H str., aromatic ring), 1506 (C-C Str., Aromatic ring), 1036 (C-H bend.), 2864.49 (C–H str., CH_3), 3050 (C-H ring str.), 1670 (C=C ring str.), 3300 (NH str.), 3372 (OH str.), 2928, 2848 (m, CH_2), 1750 (s, C=O ester), 1588, 1475 (m, skeletal bands, ring), 1227 (s, C–O str., phenolic), 1272 (s, C−O str., ester). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 5.40 (br. s, OH, Tyr), 4.09 (s, 3H, OCH_3), 4.13- 4.09 (m, 1H, α-H, Tyr), 4.12 (s, 3H, OCH_3), 2.20-2.18 (d, 2H, β-H, Tyr, J=7.15 Hz), 9.28 (s, 1H, NH, Quinoxaline ring), 1.24 (s, CH_3, Quinoxaline ring), 7.8-7.6 (d, CH, Quinoxaline ring).

Compound ***S1d***

Brown solid, yield 74%; m.p. 310-311 °C; IR (KBr, ν, cm^{-1}): 3123 (NH str., amide), 3052 (CH str., aromatic ring), 1750 (C=O str, ester), 1643 (C=O str., amide), 1535 (NH bend. amide), 1269 (C-O str., ester), 710, 690 (CH bend., phenyl ring). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 7.13-7.09 (t, 1H, *p*-H, Phe), 7.00-6.97 (t, 2H, *m*- H, Phe), 6.89 (s, 1H, NH), 6.86-6.84 (d, 2H, *J* = 6.45 Hz, *o*-H, Phe), 6.55 (s, 1H, NH), 4.60- 4.48 (m, 2H, α-H, Phe and Ala), 3.54 (s, 3H, OCH_3), 3.00-2.98 (d, 2H, *J* = 5.9 Hz, β-H, Phe), 1.59-1.57 (d, 3H, *J* = 4.2 Hz, β-H, Ala), 8.10 (s, CH, Quinoxaline ring), 9.20 (s, NH, Quinoxaline ring).

Compound ***S1e***

Yellow brown, yield 74%; m.p. 319-321 °C; IR (KBr, ν, cm^{-1}): 3373 (OH str., Tyr), 3125 (NH str., amide), 3065, 3052 (CH str., rings),1645, 1638 (C=O str., amide), 1526 (NH bend, amide), 1390, 1272 (C−O str, ester), 825, 710, 695 (CH def., rings). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 8.10 (s, CH, Quinoxaline ring), 9.20 (s, NH, Quinoxaline ring), 1.24 (s, 3H, CH_3, quinoxaline ring), 7.51-7.47 (t, 2H, H-*m*, Phe), 6.92-6.88 (m, 3H, H-*p*, Phe and H-*o*, Tyr), 6.86-6.84 (d, 2H, H-*o*, Phe, J=6.45 Hz, J=4.15 Hz), 6.80-6.78 (d, 2H, H-*m*, Tyr, J=7.45 Hz, J=5.1 Hz), 6.66 (s, 1H, NH), 5.95 (s, 1H, OH, Tyr), 4.72-4.69 (q, 1H, α-H, Phe), 4.60- 4.56 (m, 1H, α-H, Tyr), 3.54 (s, 3H, OCH_3), 3.02-2.76 (m, 4H, β-H, Phe and Tyr).

Compound ***S1f***

Dark brown solid, yield 70%; m.p.325-326 °C; IR (KBr, ν, cm^{-1}): 3065, 3052 (CH str., rings), 2925, 2846 (CH str., asym. and sym., CH_2), 1749 (C=O str., ester), 1645, 1638 (C=O str., amide), 1587, 1479, 1476 (skeletal bands, rings), 1536, 1526 (NH bend., amide), 1272 (C−O str., ester), 825, 710, 695 (s, CH def., rings), 3125 (m, NH str., amide). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 4.73–4.70 (t, 1H, δ-H, Pro), 4.02–3.97 (t, 2H, α-H, Pro), 2.48–2.41 (m, 2H, γ-H, Pro), 2.40–2.31 (m, 2H, β-H, Pro), 3.66 (s, 3H, OCH_3), 7.39 (s, 1H, NH, Trp. ring), 7.42-7.41(d, 1H, Trp), 3.25 (m, 2H, CH_2), 9.20 (s, NH, Quinoxaline ring), 1.24 (s, 3H, CH_3, quinoxaline ring), 7.81-7.83 (d, CH, Quinoxaline ring).

*General method for hydrolysis of amino acid /peptide derivatives of 1,2-dihydro-3-methyl-2 oxoquinoxaline-6-carboxylic acid **(S2a-f)***

To a solution of the compound **(S1a-f)** (0.01 mol) in THF-H_2O (1:1, 36 ml), LiOH (0.36 g) was added at 0 °C. The mixture was stirred at room temperature for 1 h and then acidified to pH 3.5 with 1N H_2SO_4. The aqueous layer was extracted with Et_2O (3 x 25 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude products were recrystallized from methanol and ether to afford hydrolyzed peptide derivatives **(S2a-f)**.

*Compound **S2a***

White solid, yield 74%; m.p. 242-243°C; IR (KBr, ν, cm^{-1}): 3350.71 (NH str.), 3078.80 (C–H str., aromatic ring), 1506 (C-C Str., Aromatic ring), 1036 (C-H bend.), 2864.49 (C–H str., CH_3),3480 (OH str.), 732, 695 (s, CH bend., out-of-plane, monosub. ring). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 2.411 (s, 3H, CH_3); 7.293-8.165 (m, 3H, aromatic H); 9.281 (s, 1H, NH, Quinoxaline ring); 1.03(s, CH_3, Quinoxaline ring), 7.66–7.61 (t, 2H, *m*-H's, Phe), 7.53–7.49 (t, 1H, *p*-H, Phe), 11.0 (s, 1H, OH).

*Compound **S2b***

White crystals, yield 78%; m.p.255-256°C; IR (KBr, ν, cm^{-1}): 3350.71 (NH str.), 3078.80 (C–H str., aromatic ring), 1506 (C-C Str., Aromatic ring), 1036 (C-H bend.), 2864.49 (C–H str., CH_3), 3050 (C-H ring str.), 1670 (C=C ring str.), 3300 (NH str.), 3450 (OH str.). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 4.09 (s, 3H, OCH_3), 7.42 (d, 1H), 7.39 (d, 1H), 7.16 (d, 1H), 7.14 (d, 1H), 7.04 (t, 1H), 4.24 (t, 1H), 3.25 (m, 2H, CH_2), 2.411 (s, 3H, CH_3); 7.293-8.165 (m, 3H, aromatic H); 9.28 (s, 1H, NH, Quinoxaline ring), 11.0 (s, 1H, OH).

*Compound **S2c***

White crystals, yield: 73 %; m.p. 240-241 °C; IR (KBr, ν, cm^{-1}): 3350.71 (NH str.), 3078.80 (C–H str., aromatic ring), 1506 (C-C Str., Aromatic ring), 1036 (C-H bend.), 2864.49 (C–H str., CH_3), 3050 (C-H ring str.), 1670 (C=C ring str.), 3300 (NH str.), 3372 (OH str.), 2928, 2848 (m, CH_2), ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 5.40 (br. s, OH, Tyr), 4.13- 4.09 (m, 1H, α-H, Tyr), 2.20-2.18 (d, 2H, β-H, Tyr, J=7.15 Hz), 9.28 (s, 1H, NH, Quinoxaline ring), 1.24 (s, CH_3, Quinoxaline ring), 7.8-7.6 (d, CH, Quinoxaline ring), 11.0 (s, 1H, OH).

*Compound **S2d***

Brown solid, yield 70%; m.p. 290-291 °C; IR (KBr, ν, cm^{-1}): 3123 (NH str., amide), 3052 (CH str., aromatic ring), 1643 (C=O str., amide), 1535 (NH bend., amide), 3372 (OH str.), 710, 690 (CH bend., phenyl ring). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 7.13-7.09 (t, 1H, *p*-H, Phe), 7.00-6.97 (t, 2H, *m*- H, Phe), 6.89 (s, 1H, NH), 6.86-6.84 (d, 2H, *J* = 6.45 Hz, *o*-H, Phe), 6.55 (s, 1H, NH), 4.60- 4.48 (m, 2H, α-H, Phe and Ala), 3.00-2.98 (d, 2H, *J* = 5.9 Hz, β-H, Phe), 1.59-1.57 (d, 3H, *J* = 4.2 Hz, β-H, Ala), 8.10 (s, CH, Quinoxaline ring), 9.20 (s, NH, Quinoxaline ring), 11.0 (s, 1H, OH).

*Compound **S2e***

Yellow brown, yield 70%; m.p. 195-296 °C; IR (KBr, ν, cm^{-1}): 3373 (OH str., Tyr), 3125 (NH str., amide), 3065, 3052 (CH str., rings),1645, 1638 (C=O str., amide), 1526 (NH bend, amide). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 8.10 (s, CH, Quinoxaline ring), 9.20 (s, NH, Quinoxaline ring), 1.24 (s, 3H, CH_3, quinoxaline ring), 7.51-7.47 (t, 2H, H-*m*, Phe), 6.92-6.88 (m, 3H, H-*p*, Phe and H-*o*, Tyr), 6.86-6.84 (d, 2H, H-*o*, Phe, J=6.45 Hz, J=4.15 Hz), 6.80-6.78 (d, 2H, H-*m*, Tyr, J=7.45 Hz, J=5.1 Hz), 6.66 (s, 1H, NH), 5.95 (s, 1H, OH, Tyr), 4.72-4.69 (q, 1H, α-H, Phe), 4.60- 4.56 (m, 1H, α-H, Tyr), 3.54 (s, 3H, OCH_3), 3.02-2.76 (m, 4H, β-H, Phe and Tyr), 11.02 (S, 1H, OH).

*Compound **S2f***

Dark brown solid, yield 67%; m.p.312-313 °C; IR (KBr, ν, cm^{-1}): 3065, 3052 (CH str., rings), 2925, 2846 (CH str., asym. and sym., CH_2), 1645, 1638 (C=O str., amide), 1587, 1479, 1476 (skeletal bands, rings), 1536, 1526 (NH bend., amide), 825, 710, 695 (s, CH def., rings), 3125 (m, NH str., amide). ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 4.73- 4.70 (t, 1H, δ-H, Pro), 4.02–3.97 (t, 2H, α-H, Pro), 2.48–2.41 (m, 2H, γ-H, Pro), 2.40–2.31 (m, 2H, β-H, Pro), 3.66 (s, 3H, OCH_3), 7.39 (s, 1H, NH, Trp. ring), 7.42-7.41(d, 1H, Trp), 3.25 (m, 2H, CH_2), 11.02 (S, 1H, OH), 9.20 (s, NH, Quinoxaline ring), 1.24 (s, 3H, CH_3, quinoxaline ring), 7.81-7.83 (d, CH, Quinoxaline ring).

Biological Screening

Anthelmintic studies

Anthelmintic activity studies were carried out against three different species of earthworms *M. konkanensis*, (ICARBC 211), *P. Corethruses* (ICARBC 117) and *E. Eugeniea* (ICARBC 042) following Garg's method[10] at 2mg/ml concentration. Suspensions of samples were prepared by triturating synthesized compounds (100 mg) with tween 80 (0.5%) and distilled water and the resulting mixtures were stirred using mechanical stirrer for 30 min. The suspensions were diluted to contain 0.2% w/v of test samples. Suspension of reference drug, tiabendazole, was prepared with same concentration in a similar manner. Three sets of five earthworms of almost similar sizes (2 inch in length) were placed in petri plates for 4 inch diameter containing 50 ml of suspension of test samples and reference drug at room temperature. Another set of five earthworms was kept as control in 50 ml suspension of distilled water and Tween 80 (0.5%). The paralyzing and death time were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50ºC) which stimulated the movement, if the worm was alive. The results of anthelmintic studies were presented in (Table 1).

Antibacterial Studies

The synthesized compounds **(S1a-f** and **S2a-f)** were screened for their antibacterial activity against two gram positive bacterial strains *B. Subtilis* (NCIM 2063), *S. Aureus* (NCIM 2079) and two gram negative bacterial strains *P. Aeruginosa* (NCIM 2034), *K. Pneumonia* (NCIM 2011) by using modified Kirby-Bauer disc diffusion method [2]. MIC values of test compounds were determined by tube dilution technique [1]. All the synthesized compounds were dissolved separately to prepare a stock solution of 1 mg/ml using DMF. Stock solution was aseptically transferred and suitably diluted with sterile broth medium to have seven different concentrations of each test compound ranging from 200 to 3.1 µg/ml in different test tubes. All the tubes were inoculated with one loopful of one of the test bacteria. The process was repeated with different test bacteria and different samples. Tubes inoculated with bacterial cultures were incubated at 37 ºC for 18 h and the presence/absence of growth of bacteria was observed. From these results, MIC of each test compound was determined against each test bacterium. A spore spore suspension in sterile distilled water was prepared from five-days-old culture of the test bacteria growing on nutrient broth media. About 20 ml of the growth medium was transferred into sterile petri plates and inoculated with 1.5 ml of spore suspension (spore concentration- 6 x 10^4 spores ml^{-1}). Filter paper disc of 6 mm diameter and 2 mm thickness was sterilized by autoclaving at 121ºC (15 psig) for 15 min. Each petri plate was divided into 5 equal portions along the diameter to place one disc. Three disc for test sample were placed on three portions together with one discs with reference drug gatifloxacin and a disc impregnated with solvent (DMF) as negative control. Test and reference drugs were tested at concentration of 10 µg/ml. The petri plates inoculated with bacterial cultures were incubated at 37 ºC for 18 h. Diameters of zone of inhibition (mm) were measured and the average diameters of test samples were calculated in triplicate sets. The diameters obtained for the test sample were compared with that produced by standard drug gatifloxacin. The results of antibacterial studies were presented in (Table 2).

Antifungal studies

Serial plate dilution method [8] was used in the present study for the assessment of antifungal activity against diamorphic fungal strains *C. Albicans* (MUCC 29), *A.niger* (MUCC 29) and two cutaneous fungal strains *M. audouinii* (MUCC 545) and *T. Mentagrophytes* (MUCC 665). MIC values of test compounds were determined by employing the same technique as used for antibacterial studies using DMSO in place of DMF and tubes inoculated with fungal cultures were incubated at 37 ºC for 48 h. After incubation, the presence/absence of growth of the fungi was observed and MIC of test compounds was determined against each fungal strains. A spore suspension in normal saline was prepared from culture if test fungi on sabouraud's broth media. After transferring growth medium, petri plates were inoculated with spore suspension. After drying, wells were made using an agar punch and test samples, reference drug and negative control were placed in labelled wells in each petri plate. Test samples **(S1a-f** and **S2a-f)** along with reference drug (griseofulvin) were tested at 10 µg/ml. The petri plates inoculated with fungal cultures were incubated at 37 ºC for 48 h. Antifungal activity was determined by measuring diameters of zone of inhibition (mm) in triplicate sets. The diameters obtained for the test sample were compared with that produced by standard drug griseofulvin. The results of antibacterial studies were presented in (Table 3).

RESULTS AND DISCUSSION

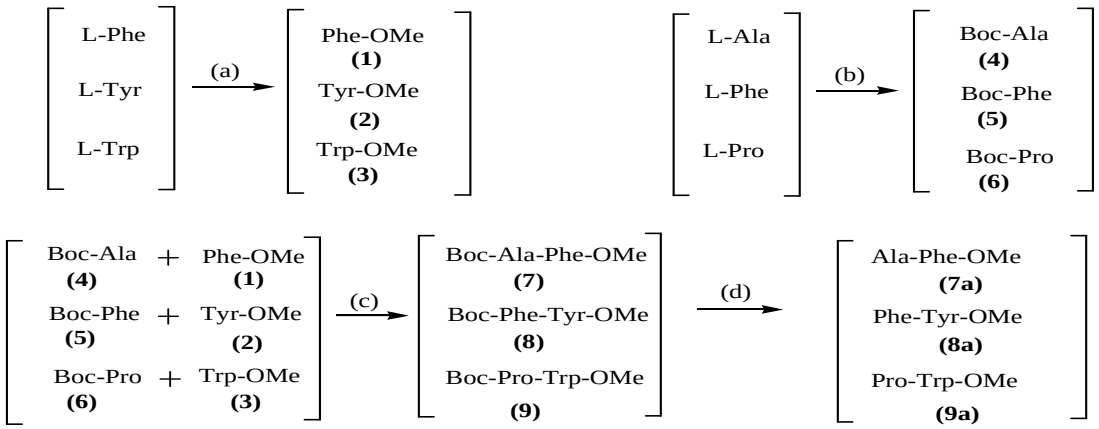
The field of synthesizing customizable peptide chains is considered as an attractive field in modern drug discovery and proteomic research, it allowed the development of useful molecules and derivative of biological interest. The synthetic sequence is relatively convenient, consisting of steps of protection, coupling and deprotection, which upon

repetition would produce the desired peptide. Based on this, the process of producing customizable dipeptide chain was initiated first, followed by the addition of Quinoxaline derivative (S1) in later stage.

Boc-Dipeptide methyl esters (compounds **7-9**, **scheme 1**) were prepared by coupling Boc-amino acids with the respective amino acid methyl ester hydrochlorides using dicyclohexylcabodiimide (DCC) as coupling agent and N-methylmorpholne (NMM) as base according to bodanzsky and bodanzsky procedure with some suitable modifications [3]. Deprotection of the dipeptides (compounds **7-9**) at the amino end was done by using trifluoroacetic acid (TFA) to get Ala-Phe-OMe **(7a)**, Phe-Tyr-OMe **(8a)** and Pro-Trp-OMe **(9a)**. Later, 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid (**S1**) was prepared by refluxing equal milimoles of pyruvic acid and 3,4-diaminobenzoic acid in rectified spirit. Subsequently, the compound (**S1**) was coupled with different amino acids/dipeptide methyl esters by using DCC/Triethylamine (TEA) in THF to get the peptide derivatives **(S1a-f)** respectively (**scheme 2**).

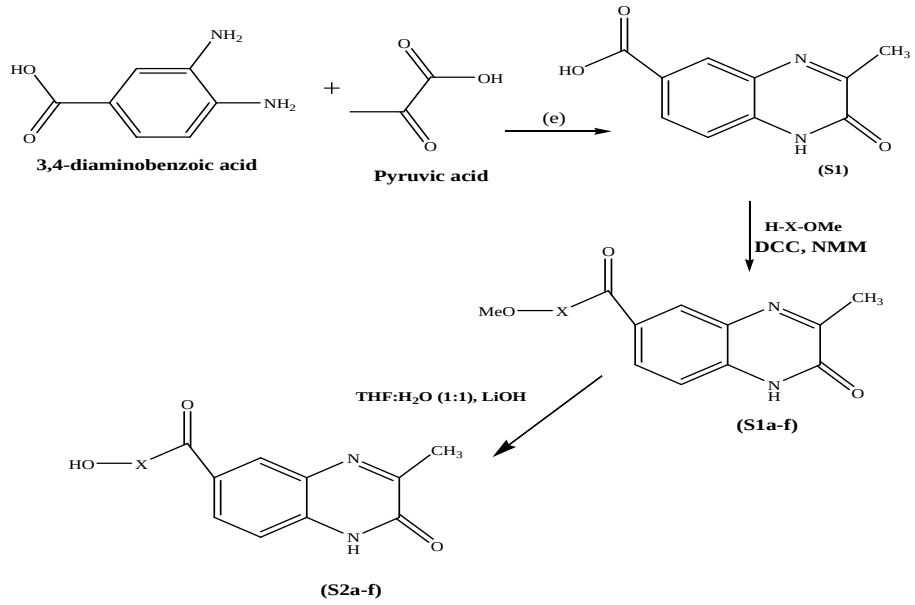
Furthermore, synthesized compounds **(S1a-f)** were hydrolyzed by stirring with LiOH to yield corresponding acid derivatives **(S2a-f)** respectively. All the structures of newly synthesized peptide derivatives were confirmed by IR, ^{1}HNMR spectral analysis.

Coupling reaction was accomplished and confirmed by appearance of bands of medium and strong intensity at 1660-1630 cm^{-1} (C=O str., amide I band), 1536-1520 cm^{-1} (N-H def., amide II band), 1200-1210 cm^{-1} (C-O str., ester) and 1536-1520 cm^{-1} in IR spectra of all dipeptide methyl esters. This is further confirmed by appearance of broad singlets at 9.98-6.25 ppm (for imino proton of CO-NH moiety) in ^{1}HNMR. The deprotection of dipeptides at amino terminal was indicated by disappearance of singlets at 1.55-1.50 ppm (for nine protons of *t*-butyl group). Structure of Compounds **(S1a-f)** were confirmed by appearance of bands at 1500-1450 cm^{-1} (-C=N str., Quinoxaline ring), 1200-1210 cm^{-1} (C-O str., ester), 1536-1520 cm^{-1} (N-H def., amide II band) and presence of aromatic rings in structures of compounds was confirmed by strong out-of-plane deformation bands (C-H bending) at 886-820 cm^{-1}. Moreover, the structures of compounds were also confirmed by appearance of broad singlet at 9.98-6.25 ppm (for imino proton of CO-NH moiety) in ^{1}HNMR. The structure of compounds **(S2a-f)** was confirmed by the appearance of bands of medium and strong intensity at 330-2700 cm^{-1} (O-Hstr., COOH) and 1760-1650 cm^{-1} (C=Ostr., COOH) and disappearance of bands at 1273-1262 cm^{-1} (C-O str., ester) in IR spectra of compounds, this is further confirmed by appearance of broad singlets at 9.72-9.80 ppm (for one proton of Hydroxyl group).



Scheme 1. Steps for the synthesis of different amino acid/dipeptide methyl esters

Reagents and conditions: (a)Thionyl chloride, Methanol, Reflux, 8h; (b) Iso-propanol, NaOH, Boc_2O; (c) DCC, NMM, $CHCl_3$, r.t., 36 h; (d) TFA, $CHCl_3$, r.t., 1h.



X = amino acid/dipeptides
S1a: X= Phe, S1b: X= Trp, S1c: X= Tyr,
S1d: X= Ala-Phe, S1e: X= Phe-Tyr, S1f: X= Pro-Trp

Scheme 2. Chemical synthesis for the different amino acid/di-peptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid (S1a-f) and (S2a-f). Reagents and conditions: (e) rectified spirit, reflux 1.5 h.

All the peptide derivatives **(S1a-i** and **S2a-f)** showed moderate to good anthelmintic activity (Table 1) at 2 mg/ml in Tween 80 (0.5%) and distilled water. Comparison of anthelmintic activity data revealed that the compounds **(S2a-f)** showed more potent anthelmintic activity compared to compounds **(S1a-f)**. **S2f** and **S1f** are most active and possessed higher activity against *M. konkanensis*, *P. corethruses* and *E. Eugeniea* species in comparison to standard drug and compounds **S1e** and **S2e** and **S2d** showed anthelmintic activity comparable to that of standard drug tiabendazole.

Table 1. Anthelmintic activity data of synthesized compounds (S1a-f and **S2a-f)**

Compound	Earthworm species					
	M. konkanensis		***P. corethruses***		***E. eugeniea***	
	Mean paralyzing time (min)[a]	Mean death time (min)[a]	Mean paralyzing time (min)[a]	Mean death time (min)[a]	Mean paralyzing time (min)[a]	Mean death time (min)[a]
S1a	40.38 ± 0.52	52.58 ± 0.59	42.57 ± 0.26	54.26 ± 0.42	39.25 ± 0.23	49.34 ± 1.62
S1b	36.56 ± 0.28	42.56 ± 0.45	34.65± 0.44	42.22 ± 0.87	35.67 ± 0.82	42.21 ± 0.82
S1c	42.34 ± 0.59	55.40 ± 0.84	41.17 ± 0.88	55.40 ± 0.43	40.73 ± 0.49	51.54 ± 0.93
S1d	44.68 ± 0.12	52.59 ± 0.72	44.55 ± 0.23	54.18 ± 0.17	41.49 ± 0.32	51.28 ± 0.44
S1e	24.22 ± 0.21	35.48 ± 0.16	29.35 ± 0.65	37.24 ± 0.54	25.45 ± 0.58	34.34 ± 0.62
S1f	33.25 ± 0.44	50.52 ± 0.43	33.22 ± 0.24	57.50 ± 0.42	34.59 ± 0.41	37.10 ± 0.60
S2a	20.15 ± 0.52	29.29 ± 0.76	26.04 ± 0.12	37.96 ± 0.54	24.38 ± 0.69	35.12 ± 0.71
S2b	40.29 ± 0.89	53.27 ± 0.92	39.61 ± 0.21	50.39 ± 0.20	39.80 ± 0.85	49.24 ± 0.55
S2c	40.54 ± 0.83	52.18 ± 0.28	40.47 ± 0.47	52.02 ± 0.18	37.44 ± 0.89	47.08 ± 0.38
S2d	24.28 ± 0.67	36.46 ± 0.28	29.14 ± 0.23	38.82 ± 0.58	26.34 ± 0.56	37.30 ± 0.49
S2e	26.04 ± 0.53	38.28 ± 0.71	30.10 ± 0.38	38.29 ± 0.30	27.50 ± 0.51	36.02 ± 0.66
S2f	27.23 ± 0.56	38.23 ± 0.38	28.60 ± 0.33	40.32 ± 0.37	30.22 ± 0.75	38.45 ± 0.48
Control	–	-	-	-	-	-
tiabendazole	13.85 ± 0.64	22.85 ± 0.53	17.82 ± 0.43	29.60 ± 0.22	13.54 ± 0.45	24.05 ± 0.62

[a]*Data are given as mean ±S.D. (n=3)*

Comparison of antibacterial (Table 2) and antifungal (Table 3) screening data suggested that all the compounds **(S1a-f)** and **(S2a-f)** exhibited maximum activity against gram-negative bacteria *P. aeruginosa*, *K. pneumoniae* and pathogenic fungi *C. albicans*, *A. niger* whereas *S. aureus* and *M. audouinii* are found to be least sensitive bacterial and fungal strains. Compounds **S2f**, **S2d**, **S1f** and **S1b** are most active against *P. aeruginosa* , *B.subtilis* and *C.*

albicans in comparison to standard drugs at 10 µg/ml in DMF and DMSO, respectively. However, compounds **S2d**, **S2e**, **S1f** possessed good antifungal activity against *A. niger*. Compound **S2f** possessed high antibacterial and antifungal activity as compared to other derivatives.

Table 2. Antibacterial activity of compounds (S1a-f)and (S2a-f) against different pathogenic bacterial strains

Compound	Diameter of zone of inhibition (mm)			
	Bacterial strains			
	B. subtilis	***S. aureus***	***P. aeruginosa***	***K. pneumoniae***
S1a	13 (6)	11 (12.5)	22 (6)	17 (12.5)
S1b	14 (12.5)	9 (25)	23 (6)	17 (12.5)
S1c	10 (6)	9 (12.5)	20 (6)	14 (12.5)
S1d	14 (6)	10 (12.5)	19 (12.5)	15 (25)
S1e	13 (12.5)	8 (25)	20 (6)	16 (25)
S1f	14 (6)	10 (12.5)	20 (6)	18 (12.5)
S2a	16 (6)	13 (12.5)	27 (6)	25 (6)
S2b	16 (6)	13 (12.5)	23 (6)	16 (6)
S2c	17 (6)	13 (25)	24 (12.5)	17 (6)
S2d	16 (12.5)	11 (12.5)	22 (6)	18 (6)
S2e	14 (6)	11 (25)	21 (6)	19 (6)
S2f	18 (6)	10 (12.5)	24 (6)	20 (6)
Control	-	-	-	-
Gatifloxacin	20 (6)	20 (12.5)	25 (6)	19 (12.5)

Values in bracket are minimal inhibitory concentration (MIC) values (µg/ml)

Table 3. Antifungal activity of compounds (S1a-f and S2a-f)

Compound	Diameter of zone of inhibition (mm)			
	Fungal strains			
	C. albicans	***M. audouinii***	***A. niger***	***T. Mentagrophytes***
S1a	16 (6)	9 (25)	17 (12.5)	13 (6)
S1b	15 (12.5)	18 (12.5)	15 (12.5)	12 (12.5)
S1c	18(6)	10 (25)	16 (25)	12 (6)
S1d	16 (12.5)	8 (25)	16 (12.5)	13 (6)
S1e	16 (6)	9 (12.5)	14 (12.5)	13 (6)
S1f	19 (25)	10 (6)	19 (25)	9 (25)
S2a	18 (6)	10 (25)	14 (12.5)	13 (6)
S2b	16 (6)	11 (12.5)	15 (25)	15 (6)
S2c	17 (6)	11 (12.5)	15 (12.5)	13 (6)
S2d	18 (6)	12 (12.5)	17 (12.5)	15 (6)
S2e	19 (6)	11 (6)	17 (12.5)	15 (6)
S2f	20(6)	11 (6)	16 (12.5)	17 (6)
Control	-	-	-	-
Griseofulvin	20(6)	17 (6)	18 (12.5)	20 (6)

Values in bracket are minimal inhibitory concentration MIC values (µg/ml).

CONCLUSION

This study reported the successful synthesis of title compounds *via* coupling reaction in good yield. For peptide coupling, employing DCC/NMM reagent in THF solvent proved to be good coupling method both economically and yield wise. The synthesized compounds showed moderate to good antimicrobial activity against all tested microbial strains except *S. aureus* and *M. audouinii*. The presence of quinoxaline nucleus in the peptide derivatives confers greater antibacterial activity. Gram-negative bacteria proved to be more sensitive in comparison to gram-positive bacteria towards peptide derivatives. The results of anthelmintic studies indicated good level of activity for the synthesized peptide derivatives when compared to the standard drug, tiabendazole. Greater anthelmintic activity was measured in derivatives having Tryptophan constituent in their amino-acid chain.

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