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Research Article

SYNTHESIS AND BIOLOGICAL ACTIVITY OF PEPTIDE DERIVATIVES OF 2-HYDROXY-5-(6-IODO-2-METHYL-4-OXOQUINAZOLIN-3(4H)-YL) BENZOIC ACID

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ABSTRACT

Compound named 2-hydroxy-5-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl) benzoic acid (S1) was synthesized by reaction of 6-iodo-2-methylbenzoxazin-4-one with 5-amino salicylic acid (5-ASA). Coupling of compound S1 with different amino acid ester hydrochlorides, dipeptide/tripeptide methyl esters yielded novel quinazolino peptide derivatives S1a-e. The chemical structures of all newly synthesized compounds were confirmed by means of FT-IR, ^{1}H-NMR. The newly synthesized cyclopeptide was screened for its antibacterial, antifungal and anthelmintic activities against pathogenic microbes and earthworm species. The antimicrobial activity was performed against *B.subtilus, S.aureus, E coli* and antifungal activity against *Candida albicans* and *Aspergillus niger*. The anthelmintic activity was performed against *Megascoplex konkanensis*, *Pontoscotex corethruses* and *Eudrilus eugeniea* at dose of 2 mg mL^{-1}.

Keywords: antifungal, heterocyclic ,Quinazolinone, antitubercular.

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 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 [9-10], antiviral [11, 13], anticonvulsant [12], insecticidal [13], farnesyltransferase, gastric H+/K+-ATPase and MAP kinase p38 inhibitory properties [14-15]. Furthermore, the literature is enriched with several findings indicating antimicrobial potential of salicylic acid and its analogs [16]. Prompted by the chemotherapeutic importance of quinazolinones moiety 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-oxoquinazolin-3(4H)-yl) benzoic acid (S1). 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 [17]. Thus, keeping in mind the pharmacological potential of quinazolinones/salicylic acids as well as taking advantage of biodegradability and biocompatibility of amino acids/peptides and further, in continuation of work on synthesis of bioactive peptide analogs of aryloxyacetic acids [18], an attempt was made towards the synthesis of two novel series of peptidyl derivatives of the iodoquinazolinones–2-hydroxy-5-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl) benzoyl amino acids/peptides (5a-e). Further more the synthesized compounds were evaluated for antimicrobial and anthelmintic activity.

MATERIALS AND METHODS

Melting points were determined by the open capillary method and were uncorrected. L-Amino acids, di-tert-butylpyrocarbonate (Boc_2O), dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA), *p*-nitrophenol (pnp) and N-metylmorpholine (NMM) were obtained from Central drug house, New Delhi, India. IR spectra were recorded on a Shimadzu 8700 FTIR spectrophotometer(Shimadzu, Japan) using a thin film supported on KBr pellets and $CHCl_3$ as solvent for intermediate semisolids.^{1}H NMR were recorded on a Bruker AC NMR spectrometer (300MHz), (Brucker, USA) using tetramethylsilane (TMS) as internal standard. Purity of all compounds was checked by TLC on precoated silica gel G plates (Kieselgel 0.25 mm, 60G F254, Merck, Germany). Chloroform/methanol (9:1, *V/V*) was used as the developing solvent system and dark brown spots were detected on exposure to iodine vapours in a tightly closed chamber.

2-hydroxy-5-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl) benzoic acid (S1)

Equimolar amounts (0.025 mol) of 6-iodo-2-methylbenzoxazin-4-one (7.18 g) and 5-amino salicylic acid (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 pure S1.

White solid, mp. 124-125 °C, yield: 71 %, Mol for: $C_{16}H_{11}IN_2O_4$ IR (KBr, $CHCl_3$, v, cm^{-1}): 3365 (O–Hstr, Ar–OH), 3295-2505 (O–Hstr, COOH), 3072-3066, 3052 (Ar–Hstr), 2967, 2875 (C–Hstr, CH3), 1702 (C=Ostr, COOH), 1669 (C=Ostr, ring), 1589, 1575, 1425, 1417 (skeletal bands), 1405 (O–Hdef, COOH), 875, 836, 760, 752, 696 (C–Hdef, oop), 590 (C–Istr) cm–1; ^{1}H NMR (300 MHz, $CDCl_3$, TMS, *δ* ppm): 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, CH_3-*β*, qz).

Preparation of L-amino acid methyl ester hydrochlorides (2c-e)

Thionyl chloride (1.4 ml, 20 mmol) was added to methanol (100 ml) slowly at 0°C and L-amino acid (20 mmol) was added to above solution. The resulting mixture was refluxed for 8-10 h at ambient temperature. Solvent was evaporated and the residue was triturated with ether at 0°C until excess dimethyl sulphite was removed. The crude product was crystallized from methanol and ether at 0°C to obtain the pure amino acid methyl ester hydrochloride viz. tyrosine methyl ester hydrochloride, phenyl alanine methyl ester hydrochloride, glycine methyl ester hydrochloride respectively (2c-e).

Tyrosine methyl ester hydrochloride

White crystals, mp 190 °C, yield: 84%, Mol for:$C_{10}H_{14}ClNO_3$ IR (KBr, $CHCl_3$, v, cm^{-1}): 3372 (m/br, 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), 825 (s, CH def, oop, ring). ^{1}H NMR (300 MHz, $CDCl_3$, TMS, *δ* ppm): 7.80-7.78 (dd, 2H, H-*m*, J=7.4 Hz, J=5.0 Hz), 7.56-7.54 (dd, 2H, H-*o*, J=7.5 Hz, J=4.6 Hz), 5.40 (br. s, OH and NH_3^+), 4.13-4.09 (m, 1H, H-α), 4.12 (s, 3H, OCH_3) 2.20-2.18 (d, 2H, H-β, J=7.15 Hz).

Phenylalanine methyl ester hydrochloride:

White solid, m.p: 160-162 ^{0}C, Yield: 78%, Mol. For: $C_{10}H_{14}ClNO_2$. IR (KBr, $CHCl_3$, v, 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,$CDCl_3$, TMS, *δ* ppm): 7.66–7.61 (tt, 2H, *m*-H's, Phe), 7.53–7.49 (t, 1H, *p*-H, Phe), 7.47–7.45 (dd, 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).

Glycine methyl ester hydrochloride:

White solid, mp 174-175 °C, yield: 90%, Mol for: $C_3H_8ClNO_2$ IR (KBr, $CHCl_3$, v, cm^{-1}): 3015-2853 (s/br, NH_3^+ str, asym and sym); 2925, 2847 (m, CH str, asym and sym, CH_2); 1744 (s, C=O str, ester); 1599, 1502 (s/br, NH_3^+ bend, asym and sym), 1268 (s, C–O str, ester). H NMR (300 MHz, $CDCl_3$, TMS, *δ* ppm): 4.75 (br. s, NH_3^+); 4.19 (s, 3H, OCH_3); 3.87-3.85 (d, 2H, H-α, J=5.5 Hz, gly).

Preparation of linear peptide fragments (2-3):

L-Amino acid methyl ester hydrochloride (2c-d) (0.01 mol) was dissolved in $CHCl_3$ (20 ml). To this, NMM (2.8 ml, 0.02 mol) was added at 0 °C and the reaction mixture was stirred for 15 min. Boc-L-amino acid (Phenyl alanine, Proline,) (0.01 mol) in $CHCl_3$ (20 ml) and dicyclohexylcarbodiimide (DCC) (2.1 g, 0.01 mol) were added with stirring. After 24 h, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30 ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ and saturated NaCl solutions. 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 petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to obtain Boc-Phe-Tyr-OMe (**2**), Boc-Pro-Phe-OMe (**3**).

Deprotection of dipeptide methyl esters (2a-b)

Compound 2 (4.42 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 r.t. for 1 h and washed with saturated $NaHCO_3$ solution (25 mL). The organic layer was dried over anhydrous 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 ^{0}C) to give pure Phe-Tyr-OMe (2a). The same procedure was adopted for synthesis of compound Pro-Phe-OMe (2b) from compound 3.

Phe-Tyr-OMe (2a)

White solid, m.p 115 ^{0}C, yield: 78%, Mol for: $C_{19}H_{22}N_2O_4$, IR (KBr, $CHCl_3$, v, cm^{-1}): 3078, 3022 (w, CH str, rings); 2929, 2925, 2847 (m, CH str, asym and sym, CH2); 1752 (s, C=O str, ester); 1633, 1629 (s, C=O str, amide); 1586, 1482, 1472 (m, skeletal bands, ring); 1538 (m, NH bend, amide); 1385, 929 (w, CH_3 rock, butyl-*t*); 1269 (s, C–O str, ester); 1229 (s, C–O str, phenolic), ^{1}H NMR (300 MHz, $CDCl_3$, TMS, *δ* ppm): 7.50-7.46 (tt, 2H, H-*m*, phe); 6.95-6.92 (t, 1H, H-*p*, phe); 6.91-6.89 (dd, 2H, H-*o*, J=7.55 Hz, J=4.6 Hz); 6.84-6.82 (dd, 2H, H-*o*, J=7.85 Hz, J=4.5 Hz, phe), 6.78-6.76 (dd, 2H, H-*m*, J=7.5 Hz, J=4.95 Hz); 6.69 (br. s, 1H, NH); 6.56 (br. s, 1H, NH); 5.98 (br. s, 1H, OH); 4.75-4.69 (m, 1H, H-α, phe).

Pro-Phe-OMe (2b)

Brown semi solid, m.p 118 ^{0}C, yields: 61 %, Mol. For: $C_{15}H_{20}N_2O_3$. IR (KBr, $CHCl_3$, v, cm^{-1}): 3325 (s, NH str, amide), 3087, 3033 (w, CH str, ring), 2992, 2986 (m, CH str, cyclic CH_2 and CH), 2927 (m, CH str, asym, aliph. CH_2), 2895 (m, CH str, >CH–), 2823 (m, CH str, OCH_3), 1752 (s, C=O str, ester), 1669, 1206 (s, C–O str, ester), 732, 693 (s, CH bend, oop, ring). ^{1}H NMR (300 MHz, $CDCl_3$, TMS, *δ* ppm): 7.53–7.46 (m, 2H**,** *m*-H's, Phe), 6.95–6.90 (t, 1H, *p*-H, Phe), 6.86–6.83 (dd, 2H, *o*-H's, Phe, *J* = 6.5 Hz), 6.44 (br. s, 1H, NH), 4.34 – 4.29 (t, 1H, δ-H of Pro), 3.15–3.13 (d, 2H, β-H's, Phe, *J* = 4.5 Hz),

General procedure for synthesis of amino acid / peptide derivatives of 2-hydroxy-5-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl) benzoic acid (S1a-e)

Amino acid methyl ester hydrochloride (2a-e) (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% NaHCO3 and saturated NaCl solutions (15 mL). The organic layer was dried over anhydrous Na_2SO4, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and *n*-hexane followed by cooling at 0 °C to get (S1a-e).

2-hydroxy-5-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl) benzoyl phenyl alaninyl tyrosine methyl ester hydrochloride (*S*1a)

Off white solid, mp. 102-103 °C, yield: 68 %, Mol. For:$C_{35}H_{32}ClIN_4O_7$, IR (KBr, $CHCl_3$, v, cm^{-1}): 3365 (O–Hstr, Ar–OH), 3295-2505 (O–Hstr, COOH), 3072-3066, 3052 (Ar–Hstr), 2967, 2875 (C–Hstr, CH3), 1702 (C=Ostr, COOH), 1669 (C=Ostr, ring), 1589, 1575, 1425, 1417 (skeletal bands), 1405 (O–Hdef, COOH), 875, 836, 760, 752, 696 (C–Hdef, oop), 590 (C–Istr) cm–1; ^{1}H NMR (300 MHz, $CDCl_3$, TMS, *δ* ppm): 11.43 (2H, br. s, OH and COOH), 4.69 (m, 1H, H-α, phe), 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)), 7.50-7.46 (tt, 2H, H-*m*, phe),7.65 (1H, d, *J* = 6.9 Hz, H-*θ*, qz), 6.69 (br. s, 1H, NH); 6.56 (br. s, 1H, NH); 5.98 (br. s, 1H, OH),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, CH_3-*β*, qz), 6.95-6.92 (t, 1H, H-*p*, phe); 6.91-6.89 (dd, 2H, H-*o*, J=7.55 Hz, J=4.6 Hz); 6.84-6.82 (dd, 2H, H-*o*, J=7.85 Hz, J=4.5 Hz, phe), 6.78-6.76 (dd, 2H, H-*m*, J=7.5 Hz, J=4.95 Hz).

2-hydroxy-5-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl) benzoyl prolinyl phenyl alanine methyl ester (S1b)

Brown solid, m.p 114-115 ^{0}C, yields: 64 %, Mol. For:$C_{31}H_{29}IN_4O_6$ IR (KBr, $CHCl_3$, v, cm^{-1}): 3365 (O–Hstr), 3325 (s, NH str, amide), 590 (C–Istr), 3087, 3033 (w, CH str, ring), 2992, 2823 (m, CH str, OCH_3), 1752 (s, C=O str, ester), 1669, 1206 (s, C–O str, ester), 732, 693 (s, CH bend, oop, ring), 2986 (m, CH str, cyclic CH_2 and CH), 2927 (m, CH str, asym, aliph. CH_2), 2895 (m, CH str, >CH–). ^{1}H NMR (300 MHz, $CDCl_3$, TMS, *δ* ppm): 7.53–7.46 (m, 2H**,** *m*-H's, Phe), 4.69 (m, 1H, H-α, phe), 8.42 (1H, s, H-*ε*, quinazolinone moiety (qz)), 8.14 (1H, d, *J* = 7.15 Hz, H-η, qz), 6.95–6.90 (t, 1H, *p*-H, Phe), 6.86–6.83 (dd, 2H, *o*-H's, Phe, *J* = 6.5 Hz), 6.44 (br. s, 1H, NH), 3.15–3.13 (d, 2H, β-H's, Phe, *J* = 4.5 Hz).

2-hydroxy-5-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl) benzoyl tyrosine methyl ester hydrochloride (S1c)

Pale-yellow solid, m.p. 163-164 °C; yield: 65 %, Mol. for: $C_{26}H_{22}IN_3O_6$, (KBr, $CHCl_3$, v, cm^{-1}): 3364, 3359 (O–Hstr, Ar–OH), 3126 (N–Hstr, amide), 3072-3068, 3058-3053 (Ar–Hstr), 2967, 2874 (C–Hstr, CH_3), 2922, 2852 (C–Hstr, CH_2), 1742 (C=Ostr, ester), 1670 (C=Ostr, ring), 1640 (C=Ostr, amide), 1587-1578, 1425-1417 (skeletal bands), 1535 (N–Hbend, amide), 1272 (C–Ostr, ester), 876, 835-824, 764-753, 698 (C–Hdef, oop), 588 (C–Istr) cm–1, ^{1}H NMR (300 MHz, $CDCl_3$, TMS, *δ* ppm): 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, OCH_3), 2.82 (2H, d, *J* = 4.9 Hz, H-*β*, tyr), 2.58 (3H, s, CH3-*β*, qz).

2-hydroxy-5-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl) benzoyl phenyl alanine methyl ester hydrochloride (S1d)

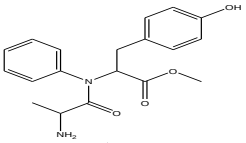
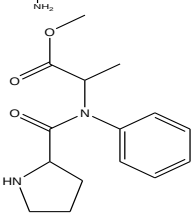
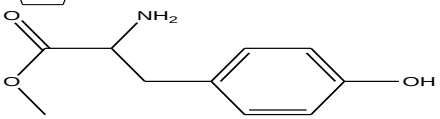
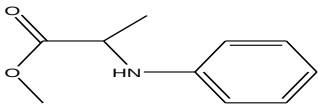
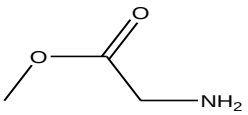
White crystals, m.p. 159-160 °C; yield: 62 %, Mol. for: $C_{26}H_{23}ClIN_3O_6$,3126 (N–Hstr, amide), 3072-3068, 3058-3053 (Ar–Hstr), 2967, 2874 (C–Hstr, CH_3), 2922, 2852 (C–Hstr, CH_2), 1742 (C=Ostr, ester), 1670 (C=Ostr, ring), 1640 (C=Ostr, amide), 1587-1578, 1425-1417 (skeletal bands), 1535 (N–Hbend, amide), 1272 (C–Ostr, ester), 876, 835-824, 764-753, 698 (C–Hdef, oop), 588 (C–Istr) cm–1, ^{1}H NMR (300 MHz, $CDCl_3$, TMS, δ ppm): 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) 4.09 (s, 3H, OCH_3), 2.31–2.29 (d, 2H, β-H's, Phe, J = 4.45 Hz).

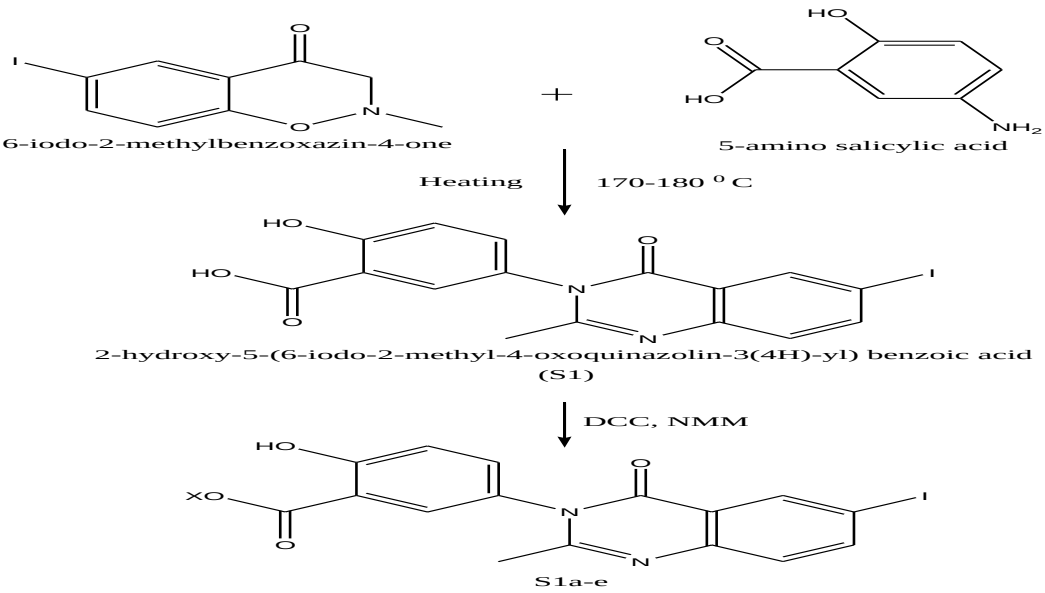
2-hydroxy-5-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl) benzoyl glycine methyl ester hydrochloride (S1e)

White solid, mp 165-166 °C, yield: 64 %, Mol for: $C_{19}H_{17}ClIN_3O_5$, IR (KBr, $CHCl_3$, v, cm^{-1}): 2925, 2847 (m, CH str, asym and sym, CH_2); 1744 (s, C=O str, ester), 588 (C–Istr), 1268 (s, C–O str, ester). ^{1}H

NMR (300 MHz, $CDCl_3$, TMS, δ ppm): 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), 4.19 (s, 3H, OCH_3); 3.87-3.85 (d, 2H, H-α, J=5.5 Hz, gly).

Table 1: List of synthesized compounds

S.NO	X
2a	
2b	
2c	
2d	
2e	



Scheme

BIOLOGICAL ACTIVITY

Anti microbial activity

All the newly synthesized compounds S1a-e were evaluated for their antimicrobial activity against three bacterial strains *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), *E.coli* (ATCC-11775) and two fungal strains *Candida albicans* (MUCC 29) and *Aspergillus niger* (MUCC 177) at 50-6 µg mL^{-1} concentration, according to the modified Kirby-Bauer disk diffusion method [19]. MIC values of test compound were determined by the tube dilution technique. Solvents 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 2 and figure 1. Antifungal data shown in table 2 and figure 2.

Anthelmintic activity

Anthelmintic activity studies were carried out against three different species of earthworms *Megascoplex konkanensis* (ICARBC 211),

Pontoscotex corethruses (ICARBC 117) and *Eudrilus eugeniea* (ICARBC 042) at 2 mg mL^{-1} concentration following Garg's method [10]. 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 ^{0}C) which stimulated the movement, if the worm was alive. The anthelmintic study results are tabulated in Table 3 and figure 3.

Table 2: Antimicrobial activity data of synthesized compounds (S1a-e)

Compound	Diameter of zone of inhibition (mm)				
	Bacterial strains			Fungal strains	
	B. subtilus	*S. aureus*	*E. Coli*	*C. albicans.*	*A. niger*
S1a	14(6)	16(12.5)	22(6)	14(12.5)	14(12.5)
S1b	11(25)	9(25)	17(12.5)	15(6)	15(25)
S1c	10(12.5)	9(50)	20(6)	18(6)	16(50)
S1d	13(25)	10(25)	27(6)	22(6)	16(50)
S1e	9(12.5)	8(25)	26(6)	24(6)	7(12.5)
Control	-	-	-	-	-
Ciprofloxacin	20(6)	20(12.5)	25(6)	-	-
Griseofulvin	-	-	-	20(6)	18(12.5)

Values in brackets are MIC values (µg/mL), Control: DMSO

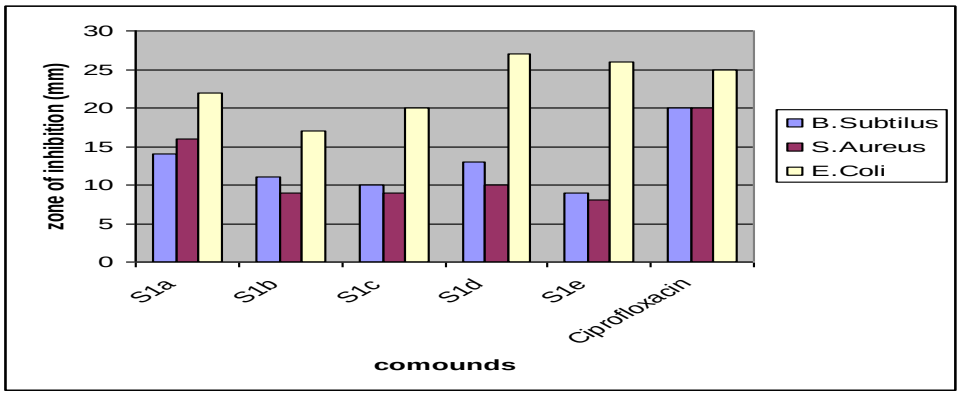


Figure 1: Antimicrobial activity data of synthesized compounds (S1a-e).

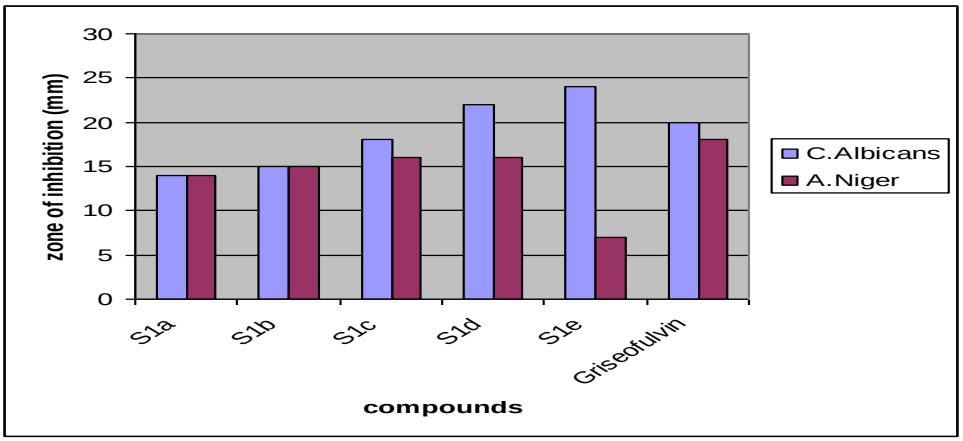


Figure 2:Antifungal activity data of synthesized compounds (S1a-e)

Table 3:Anthelmintic activity data of synthesized compounds (S1a-e)

Compound	Earthworm species					
	M. konkanensis		*P. corethruses*		*E. eugeniea*	
	Mean paralyzing time (min)	Mean death time (min)	Mean paralyzing time (min)	Mean death time (min)	Mean paralyzing time (min)	Mean death time (min)
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	21.56 ± 0.28	31.56 ± 0.45	26.65± 0.44	38.22 ± 0.87	25.67 ± 0.82	37.21 ± 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
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 ± SD. (n=3)

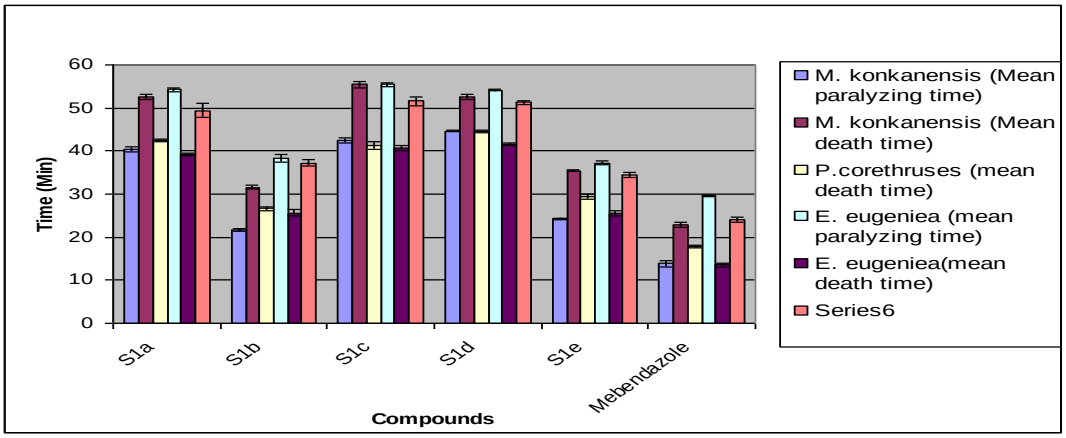


Figure 3:Anthelmintic activity data of synthesized compounds (S1a-e)

RESULT AND DISCUSSION

6-Iodo-2-methylbenzoxazin-4-one (1) was prepared in good yield according to a literature procedure [16] by refluxing of 5-iodoanthranilic acid and acetic anhydride with stirring. Dipeptides, Boc-Phe-Tyr-OMe (2a), Boc-Pro-Phe-OMe (2b), Boc-Pro-Phe-Gly-OMe (2c), were prepared by coupling Boc-amino acids with the respective amino acid methyl ester hydrochlorides using dicyclohexylcarbodiimide (DCC) as coupling agent and N-methyl morpholine (NMM) as base, according to the Bodanzsky and Bodanzsky procedure with suitable modifications. Amino acid methyl esters, Tyr-OMe (2d), Phe-OMe(2e), and Gly-OMe(2f) were synthesized according to the procedure given in literature[10]. Prior to coupling, all di-/tri-peptides were deprotected at the amino end using trifluoroacetic acid (TFA). Finally, compound S1 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 S1a-e (scheme-1). All peptide derivatives S1a-f were synthesized in good yields using DCC as coupling agent and NMM as bases.

All the synthesized compounds were found to exhibit good to moderate antimicrobial and anthelmintic activity against different bacterial, fungal strains and earth worm species. The compound S1a-e show good activity against *E.Coli*, among them S1d show high potency against *B.Subtilus*, Compound S1a was found to be highly active against *B.Subtilus* and *S.Aureus*. The compounds S1b and S1d show good anthelmintic activity against the earth worm species.

CONCLUSION

The present study reports the successful synthesis of the title compounds in good yields via coupling reactions. DCC/NMM method utilizing THF as solvent, providing good yields.

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