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Synthesis and antimicrobial activity of 4-benzyloctahydropyrrolo-[3,4-b][1,4]oxazine derivatives

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ABSTRACT

*The novel bridged bicyclic morpholine derivatives, 4-benzyloctahydropyrrolo-[3,4-b][1,4]oxazine derivatives **(6a-j)** have been synthesized in six steps. The target compounds **(6a-j)** were obtained by reacting active intermediate 4-benzyloctahydropyrrolo-[3,4-b][1,4]oxazine (**5**) with various alkyl or aryl halide substituents in the presence of potassium carbonate. Thesebridged bicyclic morpholineswhich were fused to an additional hetero ring, pyrrolidinewere screened for their antibacterial and antifungal potency. These derivatives,N-substituted with cyclohexomethyl, isopropyl, p-methylbenzyl and phenylethylexhibited promising antimicrobial activity; compounds **6a-c** were effective against the tested bacterial strains; compounds **6a**, **6c**, **6g** were effective against antifungal strains; and compounds **6d** and **6e** were not active for these studies.*

Key words: morpholine; antimicrobial; oxazine; methanone

INTRODUCTION

Bridged bicyclic morpholines are important building blocks in medicinal chemistry research. Morpholines are utilized extensively in drug discovery research. Daniel et al. reported synthesis of a novel morpholine-based building block, 3-Oxa-6-azabicyclo[3.1.1]heptane hydrotosylate [1] and (±)-6-oxa-3-azabicyclo[3.1.1]heptan-2-thione, a potential synthon for the preparation of novel heteroaryl-annulated bicyclic morpholines [2]. Numerous drugs possessing a directly linked morpholine have been approved by the FDA and other regulatory agencies; a snapshot of recently marketed drugs is shown in ***Figure 1***, including linezolid (Zyvox®)[3],gifitinib (Iressa®)[3],reboxetine (Vestra®) [5] and timolol (Betimol®) [6,7].Analogs incorporating a fused morpholine ring have also shown potential for treating various human diseases; some recent preclinical and clinical candidates are shown in ***Figure 2***, including BLI-489[8,9,10],finafloxacin[11], and AGN 193080 [12].In addition, a number of reports detailing analogs incorporating a bridged bicyclic morpholine have been reported in the medicinal chemistry literature [13]. In some instances, the bicyclic analog showed enhanced biological activity compared to the corresponding morpholineanalog[13-18].

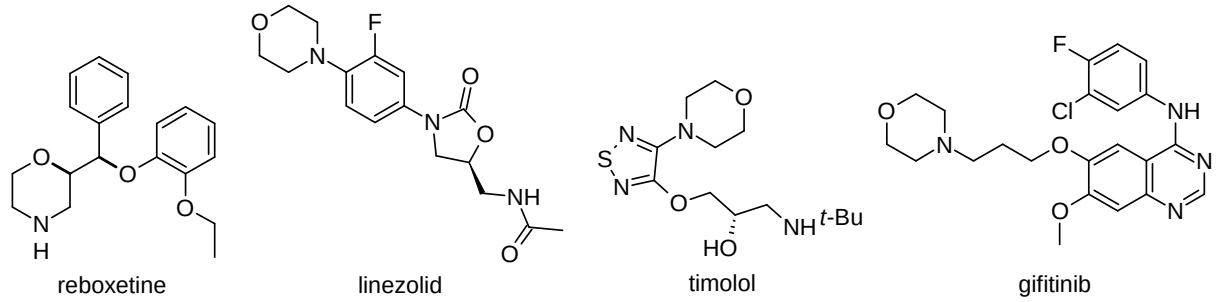


Figure 1 Marketed drugs possessing a directly linked morpholine ring

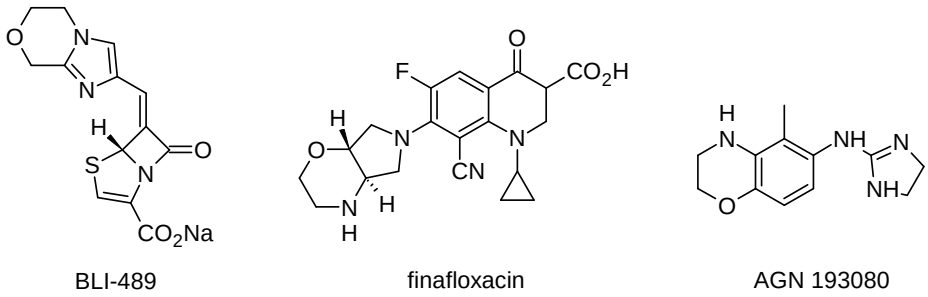


Figure 2 Clinical (BLI-489 and finafloxacin) and preclinical (AGN 193080) candidates possessing a fused morpholine ring

Given the promising biological profiles of analogs possessing a fused morpholine ring and the emerging potential of bridged bicyclic morpholineanalogs, we became interested in preparing bridged bicyclic morpholines that were fused to an additional hetero ring [19], many of these hetero-annulated analogs have shown interesting biological properties [20,21].Considering the scope and requirement of these compounds for the drug development and in continuation to our previous findings on synthesis and pharmacological activities of heterocyclic compounds [22], we have designed and synthesized, 4-benzyloctahydropyrrolo[3,4-*b*][1,4]oxazine derivatives**6a-j**. The structure of these compounds was confirmed by ^{1}H-NMR, ^{13}C-NMR and mass spectral studies. The newly synthesized compounds were screened for their antimicrobial activity.

MATERIALS AND METHODS

1.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX400 spectrometer operating at 400 MHz, with Me_4Si as internal standard. The chemical shifts are expressed as δ values in parts per million (ppm), and the coupling constants (*J*) are given in hertz (Hz). Mass spectra were determined by the EPSRC Mass Spectrometry Centre (Swansea, UK). Flash column chromatography was performed with silica gel 60 (Merck), and TLC carried out on precoated silica plates (kiesel gel 60 F_{254}, BDH). Melting points were determined on an electro thermal instrument and are uncorrected. All reagents involved in the experiments were commercially available and used without further purification. The yields were of purified compounds and were not optimized.

1.1.1.Synthesis of 2,5-dihydro-1H-pyrrol-1-yl(phenyl)methanone (1)

To a stirred solution of benzamide (2.5g, 0.02 mol, 1 eq) in dry toluene (250 ml) was added potassium hydroxide (1.15g, 0.02 mol, 1 eq). Then added TBAB (1.28g, 0.004mol, 0.2 eq) and the contents were heated to 40°C. 1,4-dichloro-2-butene (5.0g, 0.04mol, 2eq) was added drop-wise. The reaction mixture was stirred at 50°C for 5h, then poured into ice-water (20ml) and the organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain 2,5-dihydro-1H-pyrrol-1-yl(phenyl)methanone(**1**) which was taken as such to the next step. Light yellow liquid (79%), b.p: 205-208.5 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm):3.83-3.87(dd,J_1=4.2Hz,J_2=7.6Hz,4H), 5.76(d,*J*=5.6Hz,1H), 5.88(d,*J*=7.2Hz,1H), 7.43(m,2H), 7.80(m,3H). MF=$C_{11}H_{11}NO$, MW=173.21, $[m/z]^{+}$=174.34.

1.1.2.Synthesis [3-bromo-4-(2-hydroxyethoxy)pyrrolidin-1-yl](phenyl)methanone (2)

To 2,5-dihydro-1H-pyrrol-1-yl(phenyl)methanone (**1**) (3.0g, 0.0173mol, 1eq) was added ethyleneglycol (12ml), N-bromsuccinimide (3.39g, 0.019mol, 1.1 eq) and the mixture was stirred under inert atmosphere for 16h. Added water (25ml) to the reaction mass and the product was extracted to dichloromethane (20ml*2). The organic layer was washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound obtained was purified by column chromatography using 60-120 mesh silicagel and chloroform and

methanol as eluent. The product got eluted about 1% of methanol. The pure fractions were concentrated under reduced pressure to obtain 3-bromo-4-(2-hydroxyethoxy)pyrrolidin-1-yl](phenyl)methanone (**2**). Yellow liquid (89%), b.p: 215-218.5 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm):3.65(q,J_1=6.4Hz,J_2=1.6Hz,2H), 3.72(s,2H), 3.8(t,J=5.6Hz,2H), 4.16(t,J=1.6Hz,2H), 4.36(m,1H), 4.48(m,1H), 5.2(bs,1H), 7.36(d,J=7.6Hz,2H), 7.8(m,3H). MF=$C_{13}H_{16}BrNO_3$, MW=314.17, $[m/z]^+$=316.34.

1.1.3.Synthesis of 2-[(1-benzoyl-4-bromopyrrolidin-3-yl)oxy]ethyl-4-methylbenzenesulfonate (3)

To a stirred solution of [3-bromo-4-(2-hydroxyethoxy)pyrrolidin-1-yl](phenyl)methanone (**2**) (3.7g, 0.0117mol, 1eq) in tolunene (40ml), was added triethylamine (1.54g, 0.0153mol, 1.3eq) and 4-N,N'-dimethylaminopyridine (37g). p-toluenesulfonylchloride (2.9g, 0.015mol, 1.3eq) was added drop-wise into the reaction mixture. The reaction mass was stirred under inert atmosphere ambient temperature for 12h. Added water (25ml), separated the organic layer, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product obtained was purified by column chromatography using 60-120 mesh silicagel and ethylacetate and petroleum ether as eluent. The product got eluted at about 30% of ethylacetate. The pure fractions were concentrated under reduced pressure to obtain 2-[(1-benzoyl-4-bromopyrrolidin-3-yl)oxy]ethyl-4-methylbenzenesulfonate (**3**). Yellow solid (65%), m.p: 195-198.5 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm):2.65(s,3H), 3.63(q,J_1=6.4Hz,J_2=5.6Hz,2H), 3.67(s,2H), 3.8(t,J=6.0Hz,2H), 4.36(t,J=1.6Hz,2H), 4.39(m,1H), 4.4(m,1H), 7.38(d,J=7.6Hz,2H), 7.87(m,3H), 7.92(d,J=6.8Hz,2H), 8.02(dd,J_1=8.0Hz,J_2=1.6Hz,2H).MF=$C_{20}H_{22}BrNO_5S$, MW=468.36; $[m/z]^+$=470.87.

1.1.4.Synthesis of (4-benzylhexahydropyrrolo[3,4-*b*][1,4]oxazin-6(2*H*)-yl)(phenyl)methanone (4)

To a stirred solution of 2-[(1-benzoyl-4-bromopyrrolidin-3-yl)oxy]ethyl-4-methylbenzenesulfonate (**3**) (4.0g, 0.0085mol, 1 eq) in xylene (40ml) was added benzylamine (2.75g, 0.0256mol, 3eq). The contents were heated to reflux under inert atmosphere for 12h. The product was filtered and purified by column chromatography using 60-120 mesh silicagel and ethylacetate and petroleum ether as eluent. The product got eluted at about 30% of ethylacetate. The pure fractions were concentrated under reduced pressure to obtain (4-benzylhexahydropyrrolo[3,4-b][1,4]oxazin-6(2H)-yl)(phenyl)methanone (**4**). Pale yellow solid (75%), m.p: 175-178.3 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm):2.42(t,J=5.6Hz,2H), 3.35(t,7.2Hz,2H), 3.58(d,J=0.8Hz,2H), 3.86(d,J=12Hz,2H), 4.1(m,1H), 4.23(dd,J_1=9.6Hz,J_2=1.6Hz,1H), 4.43(s,2H), 7.38(d,J=7.6Hz,2H), 7.87-7.90(m,4H), 7.92(d,J=7.6Hz,2H), 8.05(dd,J_1=8.0Hz, J_2=2.0Hz,2H).MF=$C_{20}H_{22}N_2O_2$, MW=322.40; $[m/z]^+$=323.47.

1.1.5.Synthesis of 4-benzyloctahydropyrrolo[3,4-*b*][1,4]oxazine (5)

To a stirred solution of (4-benzylhexahydropyrrolo[3,4-b][1,4]oxazin-6(2H)-yl)(phenyl)methanone (**4**) (3.0g, 0.0093mol, 1eq) in con.HCl (20ml), added water (5ml) and the contents were refluxed for 10h. The by-product benzoic acid formed was filtered and the filtrate was washed with ethyl acetate. The aqueous layer was treated with charcoal, filtered and basified with sodium hydroxide to pH about 11 using 5% sodium hydroxide solution. The product was extracted to dichloromethane, dried over Na_2SO_4 and concentrated under reduced pressure to residue to obtain 4-benzyloctahydropyrrolo[3,4-b][1,4]oxazine (**5**). Pale yellow solid (75%), m.p: 175-178.3 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm):2.42(t,J=5.6Hz,2H), 3.35(t,7.2Hz,2H), 3.58(d,J=0.8Hz,2H), 3.86(d,J=12Hz,2H), 4.1(m,1H), 4.23(dd,J_1=9.6Hz,J_2=1.6Hz,1H), 4.43(s,2H), 4.72(bs,1H), 7.38(d,J=7.6Hz,2H), 7.87-7.90(m,3H). MF=$C_{13}H_{18}N_2O$,MW=218.29; $[m/z]^+$=219.45.

1.1.6.Synthesis of 4-benzyloctahydropyrrolo[3,4-b][1,4]oxazine derivatives (6a-j)

To a stirred solution of 4-benzyloctahydropyrrolo[3,4-b][1,4]oxazine (**5**) (500mg, 0.0022mol, 1eq) in ACN (5ml), was added alkyl or aryl halide (1eq) and potassium carbonate (0.94g, 0.0068mol, 3eq). The contents were stirred at 50°C for 8h. Added water (15ml) and the product was extracted to ethyl acetate, washed with brine solution, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product obtained was purified by column chromatography using 60-120 mesh silicagel and ethylacetate and petroleum ether as eluent. The pure fractions were concentrated under reduced pressure to obtain the title compounds.

Synthesis of 4-Benzyl-6-(cyclohexylmethyl)octahydropyrrolo[3,4-b][1,4]oxazine(6a)

Pale brown solid (69%), m.p: 185-188.5 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.15 (q, J = 5.2 Hz, 2H), 1.19-1.27 (m, 4H), 1.58-1.76 (m, 5H), 2.39 (t, J = 11.2 Hz, 1H), 2.63 (t, J = 7.5 Hz, 1H), 2.88 (s, 2H), 3.16-3.39 (m, 4H), 3.49-3.62 (M, 4H), 3.78 (dd, J_1 = 0.8 Hz, J_2 = 11.4 Hz, 1H), 4.04 (s, 1H), 7.24-7.33 (m, 5H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm):135.5, 128.8, 128.5, 127.3, 82.9, 64.5, 60.7, 59.6, 58.4, 58.2, 54.9, 51.0, 36.2, 31.3, 28.3, 25.5; Anal. calcd. for $C_{20}H_{30}N_2O$: C, 76.39; H, 9.62; N, 8.91%; found: C, 76.37; H, 9.61; N, 8.91%. MF = $C_{20}H_{30}N_2O$; MW = 314.46; $[m/z]^+$= 314.2.

*Synthesis of 4-Benzyl-6-isopropyloctahydropyrrolo[3,4-b][1,4]oxazine(**6b**)*

Brown solid (71%), m.p: 125-129 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 0.94 (d, J = 6.4 Hz, 6H), 2.29-2.37 (m, 2H), 2.47-2.52 (m, 1H), 2.62 (t, J = 7.2 Hz, 1H), 2.75 (t, J = 8.4 Hz, 1H), 2.95-3.0 (m, 2H), 3.51-3.55 (m, 3H),

3.63-3.68 (m, 1H), 3.84 (t, *J* = 4.0 Hz, 1H), 7.22-7.25 (m, 1H), 7.30-7.35 (m, 5H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 135.5, 128.8, 128.5, 127.3, 83.2, 64.5, 59.9, 58.2, 57.6, 51.8, 51.6, 51.0, 21.5; Anal. calcd. for $C_{16}H_{24}N_2O$: C, 73.81; H, 9.29; N, 10.76%; found: C, 73.80; H, 9.29; N, 10.76%. MF = $C_{16}H_{24}N_2O$; MW = 260.37; $[m/z]^+$= 260.2.

*Synthesis of 4-Benzyl-6-(4-methylbenzyl) octahydropyrrolo[3,4-b][1,4]oxazine(**6c**)*
Pale brown solid (63%), m.p: 229-235 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.26 (s, 3H), 2.31 (d, *J* = 11.6 Hz, 1H), 2.39-2.42 (m, 1H), 2.51-2.57 (m, 1H), 2.67 (t, *J* = 5.2 Hz, 1H), 2.86 (d, *J* = 2.8 Hz, 1H), 3.01 (dd, J_1 = 4.4 Hz, J_2 = 11 Hz, 1H), 3.20 (d, *J* =5.6 Hz, 1H), 3.45-3.70 (m, 6H), 3.89 (t, *J* = 4 Hz, 1H), 7.09-7.15 (m, 4H), 7.17-7.23 (m, 1H), 7.29-7.32 (m, 4H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 136.9, 135.5, 132.5, 128.8, 128.5, 127.3, 82.9, 64.5, 60.4, 60.0, 59.6, 58.2, 54.2, 51.0, 24.3; Anal. calcd. for $C_{21}H_{26}N_2O$: C, 78.22; H, 8.13; N, 8.69%; found: C, 78.20; H, 8.13; N, 8.68%. MF = $C_{21}H_{26}N_2O$; MW = 322.44; $[m/z]^+$= 322.2.

*Synthesis of 4-Benzyl-6-(1,3-dioxolan-2-ylmethyl) octahydropyrrolo[3,4-b][1,4]oxazine(**6d**)*
Yellow solid (74%), m.p: 214-218.5 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.31-2.34 (m, 1H), 2.41-2.43 (m, 1H), 2.54-2.57 (m, 1H), 2.62 (dd, J_1 = 4.4 Hz, J_2 = 10.8 Hz, 2H), 2.71 (t, *J* = 7.2 Hz, 1H), 2.85 (m, 2H), 3.32-3.42 (m, 2H), 3.45-3.47 (m, 2H), 3.59-3.66 (m, 4H), 3.92 (t, *J* = 2.4 Hz, 1H), 4.78 (t, *J* = 4.8 Hz, 1H), 5.08 (t, *J* = 4 Hz, 1H), 7.22-7.25 (m, 1H), 7.30-7.33 (m, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 135.5, 128.8, 128.5, 127.3, 100.6, 82.9, 66.2, 64.5, 61.1, 60.7, 59.6, 58.2, 54.2, 51.0; Anal. calcd. for $C_{17}H_{24}N_2O_3$: C, 67.08; H, 7.95; N, 9.20%; found: C, 67.07; H, 7.95; N, 9.20%. MF = $C_{17}H_{24}N_2O_3$; MW = 304.38; $[m/z]^+$= 304.2.

*Synthesis of 4-Benzyl-6-propyl) octahydropyrrolo[3,4-b][1,4]oxazine (**6e**)*
Pale yellow solid (67%), m.p: 139-142 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): δ 0.84-0.92 (m, 3H), 1.69-1.73 (m, 4H), 2.45 (d, J = 12.4 Hz, 1H), 2.71-2.73 (m, 1H), 3.15-3.27 (m, 4H)3.48-3.66 (m, 2H), 3.75-3.80 (m, 2H), 3.82-3.91 (m,1H), 4.14 (s, 1H), 7.27-7.36 (m, 5H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 135.5, 128.8, 128.5, 127.3, 82.9, 64.5, 60.4, 59.6, 58.2, 56.6, 54.6, 51.0, 21.4, 11.8; Anal. calcd. for $C_{16}H_{24}N_2O$: C, 73.81; H, 9.29; N, 10.76%; found: C, 73.80; H, 9.29; N, 10.75%. MF = $C_{16}H_{24}N_2O$; MW = 260.37; $[m/z]^+$= 260.2.

*Synthesis of 4,6-dibenzyl octahydropyrrolo[3,4-b][1,4]oxazine (**6f**)*
Pale brown solid (64%), m.p: 209-215 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): δ 1.83 (s, 2H), 2.04 (d, *J* = 2.4 Hz, 1H), 2.45-2.52 (m, 2H), 3.52 (d, *J* = 9.6 Hz, 2H), 3.83-3.89 (m, 2H), 4.05 (t, *J* = 7.2 Hz, 1H), 4.33 (d, *J* = 10.8 Hz, 1H), 5.04 (d, *J* = 10.8 Hz, 1H), 5.59 (d, *J* = 12.4 Hz, 1H), 5.72 (d, *J* = 12.4 Hz, 1H), 7.13-7.29 (m, 6H), 7.42-7.47 (m, 3H), 7.67 (d, *J* = 5.2 Hz, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 135.5, 128.8, 128.5, 127.3, 82.9, 64.5, 60.4, 60.0, 59.6, 58.2, 54.2, 51.0; Anal. calcd. for $C_{20}H_{24}N_2O$: C, 77.89; H, 7.84; N, 9.08%; found: C, 77.88; H, 7.84; N, 9.08%. MF = $C_{20}H_{24}N_2O$; MW = 308.42; $[m/z]^+$= 308.2.

*Synthesis of 4-Benzyl-6-(2-phenylethyl) octahydropyrrolo[3,4-b][1,4]oxazine(**6g**)*
Brown solid (81%), m.p: 215-220.5 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): δ 2.39 (dd, J_1 = 1.6 Hz, J_2 = 12 Hz, 1H), 2.55 (t, *J* = 10 Hz, 1H), 2.77 (d, *J* = 10 Hz, 1H), 3.01-3.13 (m, 5H), 3.42-3.55 (m, 4H), 3.69-3.77 (m, 2H), 4.02 (t, *J* = 3.2 Hz, 1H), 7.15-7.28 (m, 11H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 139.4, 135.5, 128.8, 128.7, 128.5, 127.7, 127.3, 126.0, 82.9, 64.5, 60.4, 59.6, 55.7, 54.6, 51.0, 33.9; Anal. calcd. for $C_{21}H_{26}N_2O$: C, 78.22; H, 8.13; N, 8.69%; found: C, 78.21; H, 8.13; N, 8.69%. MF = $C_{21}H_{26}N_2O$; MW = 322.44; $[m/z]^+$= 322.2.

*Synthesis of 1-(4-benzylhexahydropyrrolo[3,4-b][1,4]oxazin-6(2H)-yl)acetone (**6h**)*
Yellow solid (73%), m.p: 185-188 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): δ 0.83 (d, *J* = 6.8 Hz, 1H), 1.21-1.28 (m, 6H), 2.54 (t, *J* = 7.6 Hz, 1H), 2.78 (d, *J* = 9.6 Hz, 1H), 3.09-3.14 (m, 4H), 3.41-3.59 (m, 4H), 7.23-7.33 (m, 5H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 200.8, 135.5, 128.8, 128.5, 127.3, 82.6, 64.5, 59.9,59.3, 58.2, 54.1, 51.0, 28.6; Anal. calcd. for $C_{16}H_{22}N_2O_2$: C, 70.04; H, 8.08; N, 10.21%; found: C, 70.03; H, 8.08; N, 10.20%. MF = $C_{16}H_{22}N_2O_2$; MW = 274.36; $[m/z]^+$= 274.2.

*Synthesis of 2-(4-benzylhexahydropyrrolo[3,4-b][1,4]oxazin-6(2H)-yl)-1-(4-bromophenyl)ethanone(**6i**)*
Brown solid (82%), m.p: 255-258.5 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): δ 2.40 (dd, J_1 = 1.9 Hz, J_2 = 12.1 Hz, 1H), 2.53 (t, *J* = 9.6 Hz, 1H), 2.76 (d, *J* = 9.8 Hz, 1H), 3.01-3.09 (m, 3H), 3.41-3.59 (m, 4H), 3.69-3.76 (m, 2H), 4.09 (t, *J* = 3.19 Hz, 1H), 7.14-7.27 (m, 10H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 195.3, 135.7, 135.5, 131.5, 131.0, 128.8, 128.5, 127.3, 82.6, 67.8, 64.5, 59.9, 59.3, 58.2, 54.1, 51.0; Anal. calcd. for $C_{21}H_{23}BrN_2O_2$: C, 60.73; H, 5.58; N, 6.74%; found: C, 60.72; H, 5.58; N, 6.74%. MF = $C_{21}H_{23}BrN_2O_2$; MW = 415.32; $[m/z]^+$= 414.09.

*Synthesis of 4-benzyl-6-(4-chlorobenzyl)octahydropyrrolo[3,4-b][1,4]oxazine(**6j**)*
Pale brown solid (79%), m.p: 245-250 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): δ 2.30-2.35 (m, 2H), 2.51-2.64 (m, 2H), 2.82 (t, *J* = 4 Hz, 1H), 2.99 (dd, J_1 = 4.8 Hz, J_2 = 10.8 Hz, 1H), 3.10 (d, *J* = 3.2 Hz, 1H), 3.41-3.51 (m, 2H),

3.58-3.62 (m, 2H), 3.68-3.71 (m, 2H), 3.89 (t, *J* = 4 Hz, 1H), 7.23-7.35 (m, 9H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 135.5, 133.6, 132.8, 130.2, 128.8, 128.5, 127.3, 82.9, 64.5, 60.4, 60.0, 59.6, 58.2, 54.2, 51.0; Anal. calcd. for $C_{20}H_{23}ClN_2O$: C, 70.06; H, 6.76; N, 8.17%; found: C, 70.05; H, 6.76; N, 8.17%. MF = $C_{20}H_{23}ClN_2O$; MW = 342.86; $[m/z]^+$= 342.1.

1.2. Pharmacology

The newly synthesized compounds were screened for their antimicrobial and antioxidant properties to study the effect of substitution at pyrrolidine ring on these activities.

1.2.1.Antimicrobial activity

The following bacteria and fungi were used for the experiment. Bacteria: S*taphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. All bacterial strains were maintained on nutrient agar medium at ±37°C. Fungi: *Aspergillusflavus*, *Chrysosporiumkeratinophilum* and *Candida albicans* MTCC 227 is used in this study. All fungi strains were maintained on potato dextrose agar (PDA) at ±25 °C. These cultures are obtained from the Department of Microbiology, Kuvempu University.

1.2.1.1. Antibacterial activity

The compounds **3a-i** were tested against a panel of pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus* and P*seudomonas aeruginosa*. Microorganism strains were maintained on nutrient agar medium at 37°C. The cultures were inoculated in fresh 10 ml Nutirent Broth to yield an initial suspension of approximately 10–100 cfu/ml. All broths were then incubated statically at the aforementioned temperatures for microorganisms, for 18–24 h so that all cells were in the stationary phase. Susceptibility of the test organism to the extract was determined by employing in the well plate technique. The bacterial suspensions were diluted tenfold in distilled water, and 0.1 ml from the appropriate dilution was spread plated on nutrient agar in order to give a population of approximately 106 cfu/plate. The wells were dug in each Petri plate by sterilized cork borer. The compounds were dissolved in DMSO, and appropriate dilutions were made (1mg/ml and 0.5mg/ml). The same procedure was repeated for different micro-organisms. Each experiment was carried out in triplicate. After the inoculation and addition of organism and compound, the Petri plates were incubated in inverted position for 18 h at 37°C. After the incubation, the zone of inhibition was measured and the values for Dimethylsulphoxide (DMSO) were subtracted to get the actual values. Streptomycin was used as a positive control at concentrations of 0.5mg/ml and 1mg/ml.

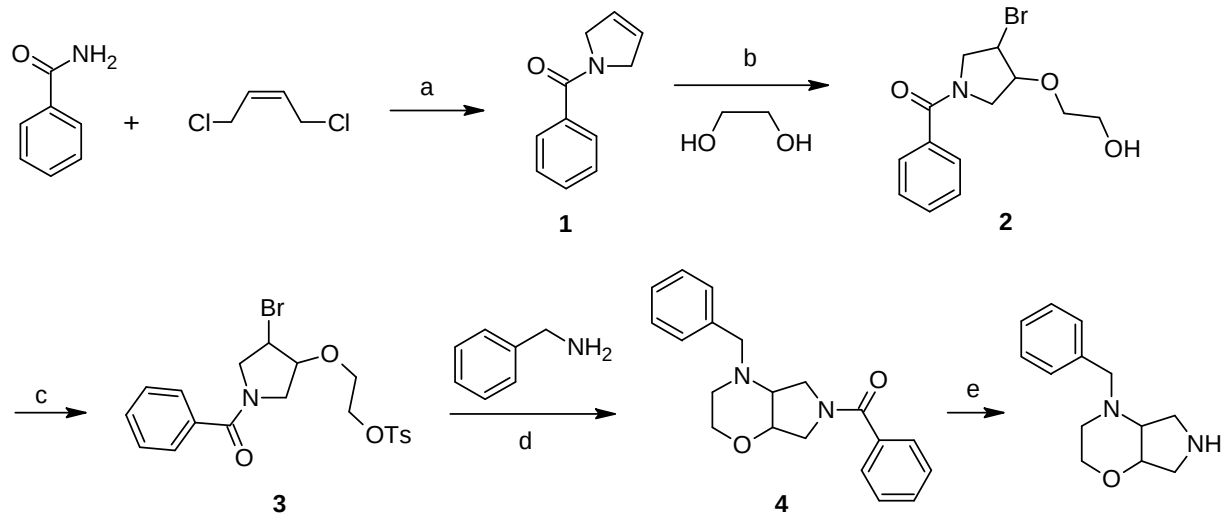
1.2.1.2. Antifungal activity

The fungal strains used in this study were *Candida albicans*, *AspergillusFlavus* and *ChrysosporiumKeratinophilum*. The required amounts of each fungal strain were removed from the stock and suspended in 5ml of distilled water with two drops of Tween 80. This suspension was uniformly spread on Petri plates containing potato dextrose agar media using sterile swabs. After applying the samples into the wells formed by using the same technique for tests on bacteria, the plates were incubated at 25 °C for three days. The plates were then examined for the presence of zones of inhibition, and the results were recorded. Fluconazole was used as a positive control at concentrations of 0.5 and 1 mg/ml.

RESULTS AND DISCUSSION

2,5-dihydro-1H-pyrrol-1-yl(phenyl)methanone (**1**) was obtained by cyclisation reaction of benzamide with 1,4-dichlorobut-2-ene using KOH as base in the presence of catalytic amount of tetra *n*-butylammonium bromide (TBAB) in toluene. The bromo ether derivative (**2**) formed by treating compound **1** with N-bromosuccinimide (NBS) and ethylene glycol. The protection of bromo ether derivative (**2**) as its tosylate (**3**) followed by cyclisation with 1-phenylmethanamine produced (4-benzylhexahydropyrrolo[3,4-b][1,4]oxazin-6(2H)-yl)(phenyl)methanone (**4**). The active intermediate 4-benzyloctahydropyrrolo[3,4-*b*][1,4]oxazine (**5**) was obtained by deprotection of phenyl methanone under acidic conditions. The target compounds **6a-j** were obtained by the reaction of this intermediate with various alkyl or aryl halide substituents in the presence of potassium carbonate as base. The synthesis methodology is described in (**Scheme 1**).

Scheme 1Synthesis of 4-benzyloctahydropyrrolo[3,4-b][1,4]oxazine derivatives (6a-j)



	R
6a	cyclohexylmethyl
6b	propan - 2 - yl
6c	4 - methylbenzyl
6d	(1,3-dioxolan-2-yl)methyl
6e	propyl
6f	benzyl
6g	phenethyl
6h	1-propan-2-one
6i	4-bromophenacyl
6j	4-chlorobenzyl

Reagents and conditions: (a) TBAB, toluene, KOH, 50°C, 5h; (b) NBS, rt, 16h; (c) TsCl, DMAP, TEA, toluene, rt, 12h; (d) xylene, reflux, 12h; (e) Con.HCl, reflux, 10h; (f) K_2CO_3, ACN, 50°C, 8h

4-benzyloctahydropyrrolo[3,4-b][1,4]oxazine derivatives were synthesized and screened for their antimicrobial activity to study the effect of substitution on additional pyrrolidine ring on these activities. Compounds **6a**, **6b** and **6c** showed pronounced antibacterial activity (**Table 1**); compounds **6a**, **6c**, and **6g** showed considerable antifungal activity (**Table 2**); and compounds **6d** and **6e** were not active for these studies.

Table 1Antibacterial activity: Zone of inhibition and Minimum inhibitory concentration of 4-benzyloctahydropyrrolo[3,4-b][1,4]oxazine derivatives (6a-j)

Compound	*Escherichia coli*		*Staphylococcus Aureus*		*Pseudomonas Aeruginosa*	
Con in mg/ml	1	0.5	1	0.5	1	0.5
Control	ND		ND		ND	
Streptomycin*	18±0.2	14±0.1	16±0.2	12±0.2	16±0.2	13±0.2
6a	15±0.2	13±0.1	10±0.2	08±0.1	11±0.1	09±0.2
6b	13±0.3	10±0.1	09±0.2	07±0.1	10±0.2	09±0.1
6c	12±0.1	08±0.2	12±0.3	10±0.1	08±0.1	06±0.1
6d	ND	ND	ND	ND	ND	ND
6e	ND	ND	ND	ND	ND	ND
6f	04±0.2	02±0.1	05±0.1	03±0.2	03±0.2	02±0.1
6g	07±0.2	05±0.1	08±0.2	06±0.1	08±0.2	05±0.1
6h	05±0.2	02±0.1	04±0.1	02±0.1	02±0.1	01±0.1
6i	04±0.1	03±0.2	05±0.1	03±0.2	06±0.1	03±0.2
6j	05±0.1	03±0.2	04±0.2	02±0.1	06±0.1	03±0.2

ND: Not developed, Standard*

Table 2Antifungal activity: Zone of inhibition and Minimum inhibitory concentration of 4-benzyloctahydropyrrolo[3,4-b][1,4]oxazine derivatives (6a-j)

Compound	*AspergillusFlavus*		*Chrysosporium Keratinophilum*		*Candida Albicans*	
Con in mg/ml	1	0.5	1	0.5	1	0.5
Control	ND		ND		ND	
Flucanazole*	14±0.2	10±0.1	16±0.2	14±0.2	23±0.2	20±0.2
6a	10±0.1	08±0.2	10±0.1	07±0.3	11±0.1	08±0.2
6b	05±0.2	03±0.1	04±0.1	02±0.2	05±0.1	02±0.2
6c	08±0.2	06±0.1	07±0.2	05±0.1	09±0.1	07±0.2
6d	ND	ND	ND	ND	ND	ND
6e	ND	ND	ND	ND	ND	ND
6f	ND	ND	05±0.2	03±0.1	04±0.1	02±0.2
6g	07±0.2	05±0.2	05±0.1	02±0.2	06±0.2	04±0.1
6h	04±0.2	01±0.1	05±0.1	03±0.2	03±0.1	02±0.1
6i	06±0.1	04±0.1	07±0.2	05±0.3	06±0.2	04±0.1
6j	ND	ND	ND	ND	ND	ND

ND: Not developed, Standard*

The N-substitution of pyrrolidine ring by clohexomethyl (**6a**), isopropyl (**6b**), p-methylbenzyl (**6c**) and phenylethyl (**6g**) enhanced the antibacterial activity. The compound **6a,**substituted with cyclohexomethyl showed better activity compared to the benzyl (**6f)**, p-methylbenzyl (**6c**) and phenylethyl (**6g**) derivative. The compounds substituted with 1,3-dioxolan-2-ylmethyl **(6d)** and propyl (**6e)** were inactive against with the tested strains. The results thus obtained, reveal that the nature of substituent hetero ring has a considerable impact to enhance antimicrobial property.

CONCLUSION

In this investigation, we have reported a convenient, economically viable and useful method of synthesis of 4-benzyloctahydropyrrolo[3,4-b][1,4]oxazine derivatives, which are biologically active molecules possessing antimicrobial property. The preliminary biological screening revealed that compounds **6a-c**, **6g** exhibited a potential antimicrobial activity. The structure and biological activity relationship of the title compounds showed that the N-substitution of pyrrolidine ring with clohexomethyl (**6a**), isopropyl (**6b**), p-methylbenzyl (**6c**) and phenylethyl (**6g**) are responsible for increased antimicrobial activity. Hence, it can be concluded that, this new class of compounds certainly holds a greater promise in discovering a potent antimicrobial agent.

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