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Facile synthesis and antibacterial evaluation of 2-phenyl-3, 4-dihydro-2h-1, 3-benzoxazine derivatives

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Abstract
A method for the synthesis of 3,4-dihydro-1,3-benzoxazines that involves the neat reaction of salicylaldehyde and benzyl amine to give an imine followed by reduction of the imine to a 2-(aminomethyl) phenol derivative and subsequently cyclisation through a reaction with various benzaldehydes is reported. The highlights of this procedure include the short reaction times, easy work-up methods and the reliance on recrystallization for the purification of the products. The 1, 3-benzoxazines were prepared in yields of 51-75% over the three steps. The prepared 1, 3-benzoxazines showed promising activity against gram negative bacteria *Salmonella enterica*, and *Klebsiella pneumonia* as compared to the reference antibiotic gentamycin.

Keywords: 1, 3-benzoxazine, benzaldehydes, 2-(aminomethyl) phenol, imine, benzyl amine, antibacterial

Introduction
3,4-Dihydro-1,3-benzoxazines are interesting heterocyclic compounds that polymerizes upon heating to give thermosetting resins whose applications include casting of airplane parts to adhesives [1]. In addition, these compounds have been shown to exhibit a number of biological activities such as fungicidal activity [2], Histone Deacetylases inhibitors [3], anti-reserpine activity [4], antibacterial activity [5, 6] and anti-tuberculosis activity [7]. Our interest on 2-phenyl-3,4-dihydro-1,3-benzoxazines is due to the close similarity between their basic structure and that of natural occurring flavans, a class of compounds that has recently attracted our attention [8] The only difference is that the carbon-3 of flavans is replaced by nitrogen in the 1,3-benzoxanes. The literature general procedure for the synthesis of 1,3-benzoxazines involve a three component reaction of one mole of phenol, one mole of a primary amine and 2 moles of an aldehyde [5, 9, 10]. The limitation of the literature procedure is that two different aldehydes cannot be involved in the process. In this paper we report a stepwise efficient synthesis of benzoxazines that involves different aldehydes and assessment of their antimicrobial activity.

Results and Discussion
Synthesis of 1, 3-benzoxazines
In our earlier work we reported the grinding induced reaction of 2-aminobenzyl alcohol and benzyladehydes as a route for the preparation of 3, 1-benzoxazine derivatives [11]. It was therefore envisaged that the preparation of 1, 3-benzoxazines will be achieved by the grinding induced reaction of 2-(aminomethyl) phenol with benzaldehydes. However, the high price of 2-(aminomethyl) phenol (EUR164 per 250 mg) [12] made this route unavailable to us. The recognition that it should be possible to prepare 2-(aminomethyl) phenol derivatives from readily available salicylaldehyde 1 and benzyl amine 2 is an elegant and central feature of our strategy. In the case at hand, 1 and 2 were mixed in a mortar without any catalyst and the resulting yellow solid was ground with a pestle to give pure imine 3 in quantitative yield. It is important to note that in the literature aniline instead of benzyl amine was used in the synthesis of related imines and the reaction is performed under reflux for a much longer time [2, 13-16]. Subsequent reduction of imine 3 using $NaBH_4$ gave 2-(aminomethyl) phenol derivative 4 in 90% yield, scheme 1. Through the two reactions discussed above, 2-(aminomethyl) phenol derivative 4 was prepared in gram quantities in a short period of time without any need for lengthy purification procedures. The ^1H NMR spectrum of imine 3 is shown in figure 1 and that of amine 4 is shown in figure 2.

An observation worth noting is that the spectrum of imine 3 exhibit a chelated hydroxy proton resonating at δ13.47 while that for amine 4 does not show such a chelation.

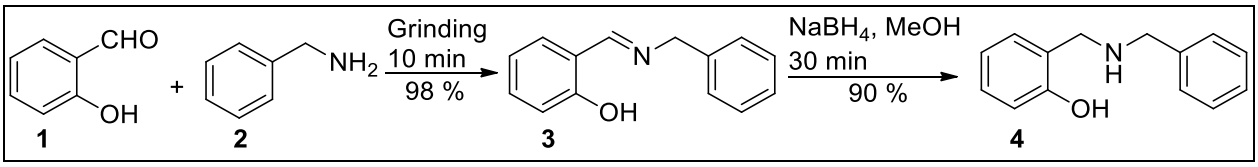


Fig 1: Synthesis of 2-(aminomethyl) phenol derivative 4

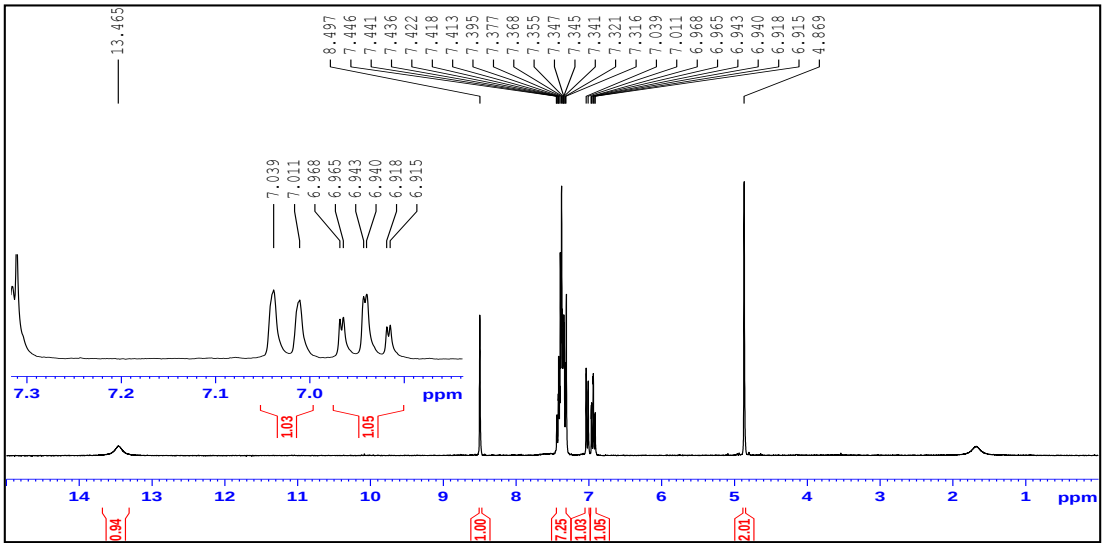


Fig 2: ^{1}H NMR spectrum of imine 3

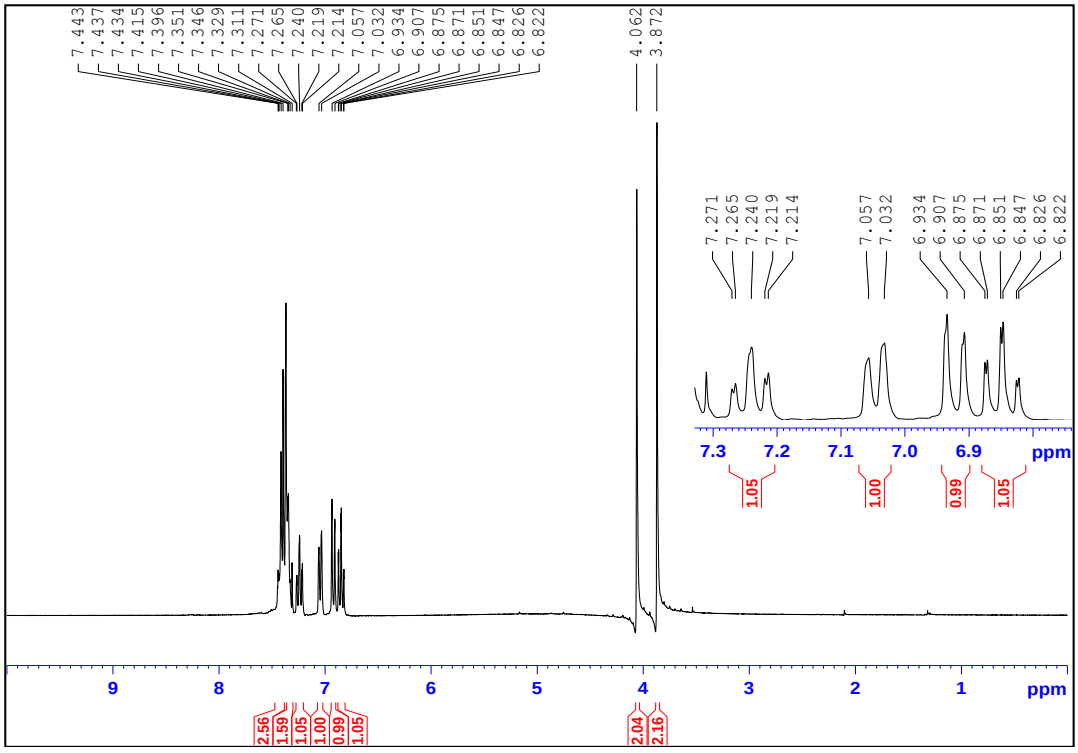


Fig 3: ^{1}H NMR spectrum of 4

The preparation of 2-(aminomethyl) phenol 4 set the stage for the crucial cyclisation reaction. In the event, refluxing of a solution of 4 and benzaldehyde 5 in methanol followed by cooling gave benzoxazine 15 as a white solid in 68% yield, scheme 2. The product was characterized on the basis of NMR experiments, mass spectrometry measurements, melting point determination and IR spectroscopy. The ^{1}H spectrum of 15 for example exhibited a characteristic singlet peak at δ 6.05 that was assigned to H-2 and a multiplet peak intergrating for four protons at δ3.91 due to H-4 and the adjacent two benzylic protons.

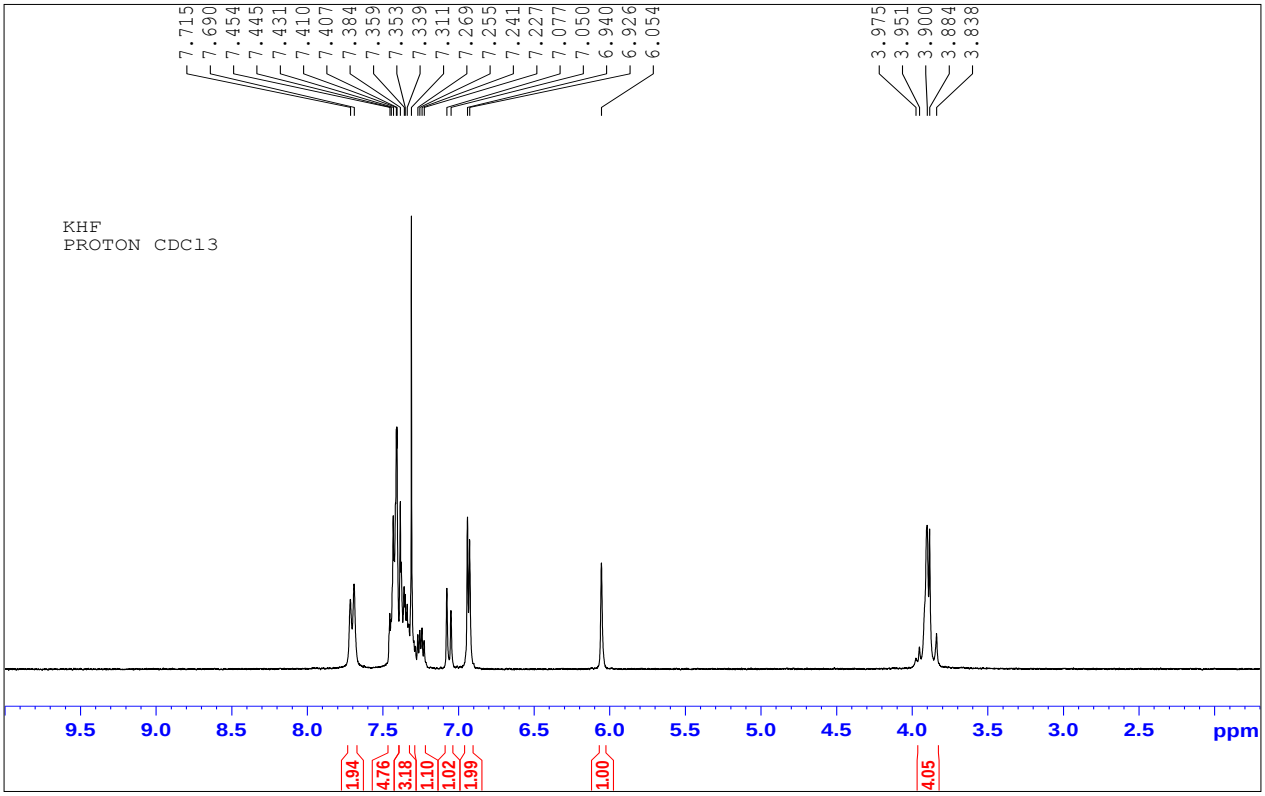
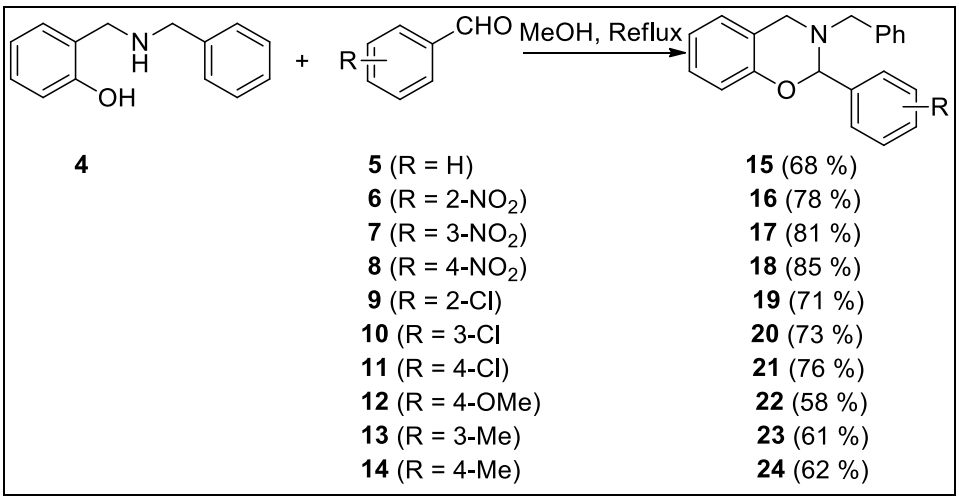


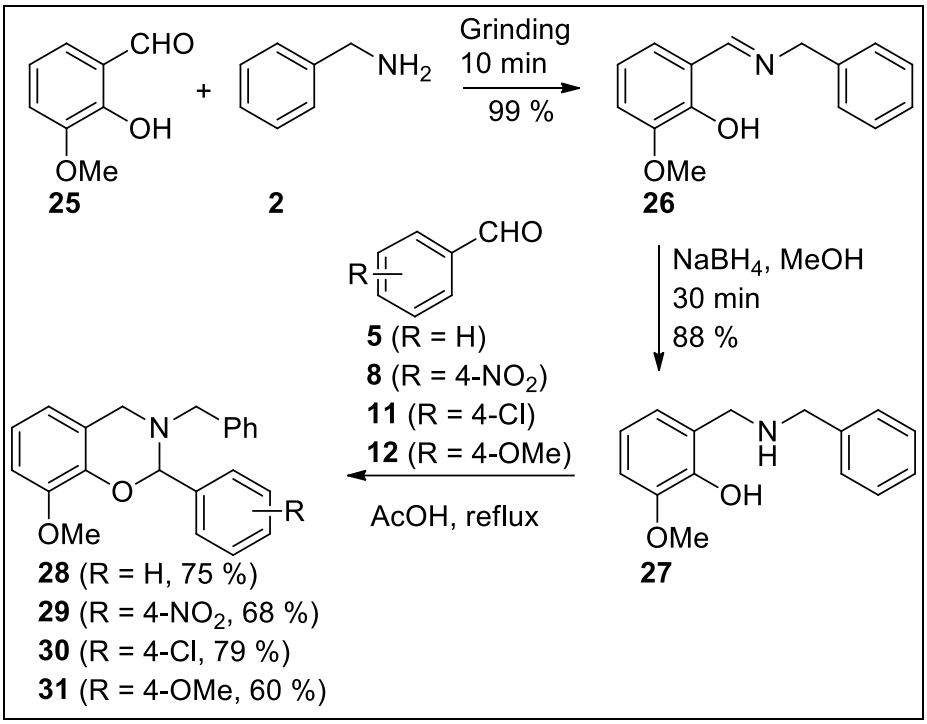
Fig 4: ^{1}H NMR spectrum of 1,3-benzoxazine 15

To extend the scope and generality of this method, an array of substituted benzaldehydes were employed in the cyclisation reaction. Thus, benzaldehydes with electron withdrawing groups 6, 7, 8, 9, 10, and 11 participated in the cyclisation reaction with 4 to give the corresponding benzoxazines in 71-85% yields. Likewise, benzaldehydes with electron donating groups 12, 13 and 14 also took part in the cyclisation reaction to afford the benzoxazine derivatives 22, 23 and 24 respectively in 58-62% yields scheme 2. It is instructive to note that the more electrophilic benzaldehydes with electron withdrawing groups consistently gave better yields that the ones with electron donating groups.



Scheme 1: Synthesis of 1, 3-benzoxazines 16-26

In a similar fashion, 3-methoxysalicylaldehyde 25 reacted with benzylamine 2 to give imine 26 and reduction of the imine gave 2-(aminomethyl) phenol derivative 27 in 87%. Subsequent reaction of 27 with benzaldehydes 5, 8, 11 and 12 gave the corresponding 1, 3-benzoxazines 28, 29, 30 and 31 in 75, 68, 79 and 60% yields respectively, scheme 3. It is noteworthy that the methoxy substitution on the salicylaldehyde did not show any significant effect on the percentage yields of the three reactions.



Scheme 2: Synthesis of 2-(aminomethyl) phenol derivative 27 and 1,3-benzoxazines 29-31

Antibacterial activity

The prepared benzoxazines were screened for their antibacterial activities against *Staphylococcus aureus* (NCTC 65710), *Listeria monocytogenes* (ATCC 7644), *Bacillus cereus* (ATCC 33019), *Salmonella enterica* (ATCC 13076), *Klebsiella pneumonia* (NCTC 9633). Gentamycin was used as a positive control while DMSO was used as a negative control. The zones of inhibition were determined using the agar overlay disc diffusion method [17-20] while the minimum inhibitory concentrations were determined using the resazurin microtitre plate assay [21, 22]. All the prepared 1, 3-benzoxazines showed no growth inhibition for gram positive bacteria *S. aureus*, *L. monocytogenes* and *B. cereus*. Further, the 8-methoxybenzoxazines 28-31 showed no activity against both gram positive and gram negative bacteria. Benzoxazines 15-24 showed interesting growth inhibition activity against the gram negative bacteria *S. enterica* and *K. pneumonia*. The results are summarized in table 1.

Table 1: Antimicrobial activities of the benzoxazines

Compound (100pg disk)	S. enterica		K. pneumonia	
	ZI (mm)	MIC (ughtL)	ZI (mm)	MIC (pea)
15	11	03000	12	02500
16	13	0.2500	12	0.1250
17	8	1.000	9	0.5000
18	10	02500	11	02500
19	10	02500	11	02500
20	9	02500	12	02500
21	11	02500	11	02500
22	12	0.5000	12	05000
23	14	05000	12	02500
24	13	0.1250	12	02500
DMSO	0		0	
Gentamycin	29	02500	9	0.0625

Note: ZI = Zone of inhibition, MIC = Minimum inhibitory concentration

The growth inhibition results against *S. enterica* plotted in figure 4 showed that the reference antibiotic gentamycin exhibited a significantly higher zone of inhibition (29 mm) than any of the prepared benzoxazines. However, in terms of the lowest concentration that inhibited the growth of *S. enterica*, the prepared benzoxazines compared favourable with the reference antibiotic. For example, benzoxazines 16, 18, 20 and 21 have an MIC of 0.2500 µg/µL and this was identical to that of the reference antibiotic. It is important to note that benzoxazines 16, 18, 20 and 21 have electron withdrawing groups NO_2 or Cl attached to their ring B. The best MIC of 0.1250 µg/µL that was better than that of the reference antibiotic was achieved when benzoxazine 24 was used. Benzoxazine 24 has an electron donating 4-methyl group attached to its ring B.

Another observation worth noting was that benzoxazines 21-24 with electron donating groups attached to ring B gave bigger zones of inhibition (12-14 mm) than that of the unsubstituted benzoxazine 15 (11 mm). In the contrary, benzoxazines 17-21 with electron withdrawing groups exhibited zones of inhibition that were smaller or equal (7-11 mm) to that of benzoxazine 15. The exception to this observation was benzoxazine 16 with a 2-NO_2 group on ring B that showed a zone of inhibition of 13 mm and bigger than that of the unsubstituted benzoxazine 15.

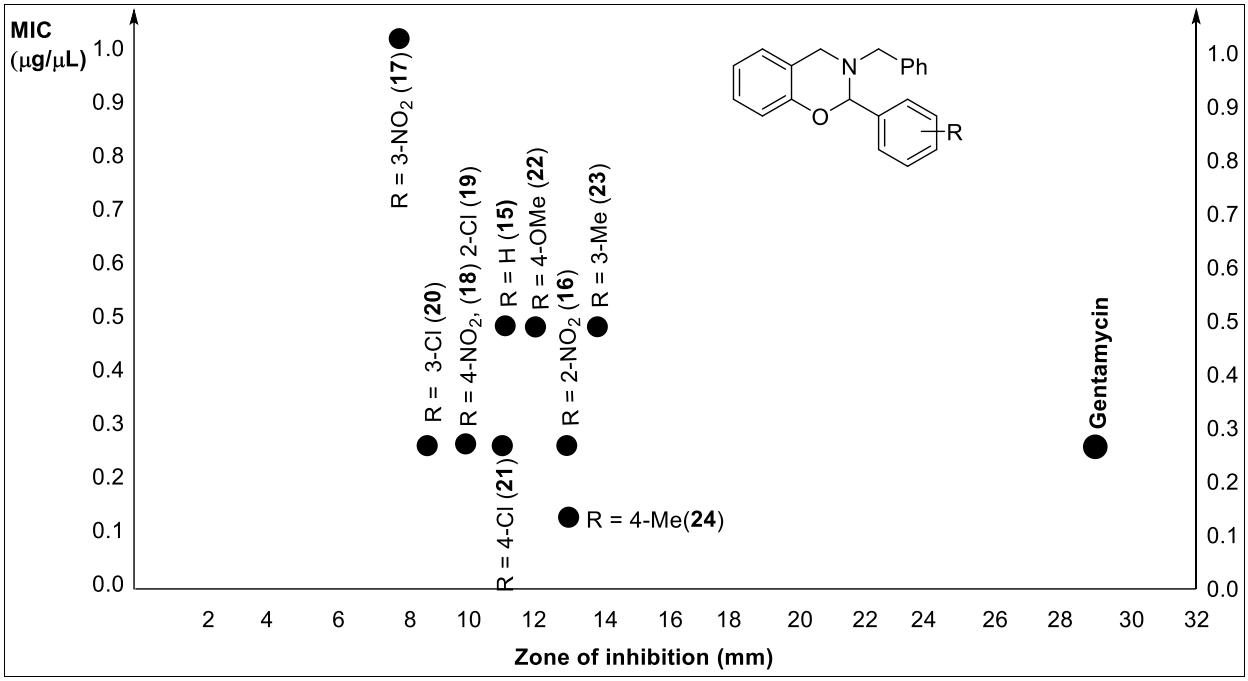


Fig 5: Growth inhibition activity of benzoxazines against *Salmonella enterica*

The results for the growth inhibition of *K. pneumonia* by the prepared benzoxazines are summarized as a plot of zone of inhibition against minimum inhibitory concentration in figure 5. The zone of inhibition for benzoxazine 17 was 9 mm and the same as that of the reference antibiotic gentamycin while the zones on inhibition for all the other active benzoxazines were bigger than that of gentamycin. However, the minimum concentrations of the benzoxazines (0.1250-0.5000 µg/µL) that inhibited the growth of *K. pneumonia* were significantly higher than that of the reference antibiotic (0.0625 µg/µL). The benzoxazine that exhibited the lowest MIC against *K. pneumonia* was 16 with an electron withdrawing 2-NO_2 group on ring B. However, the benzoxazines with electron donating groups showed bigger zones of inhibition than most of those with electron withdrawing groups. The exceptions were benzoxazine 16 with a 2-NO_2 group on ring B and 20 with a 3-Cl group on ring B. These two exhibited zones of inhibition (12 mm) that were the same as those for benzoxazines with electron donating methoxy and methyl groups.

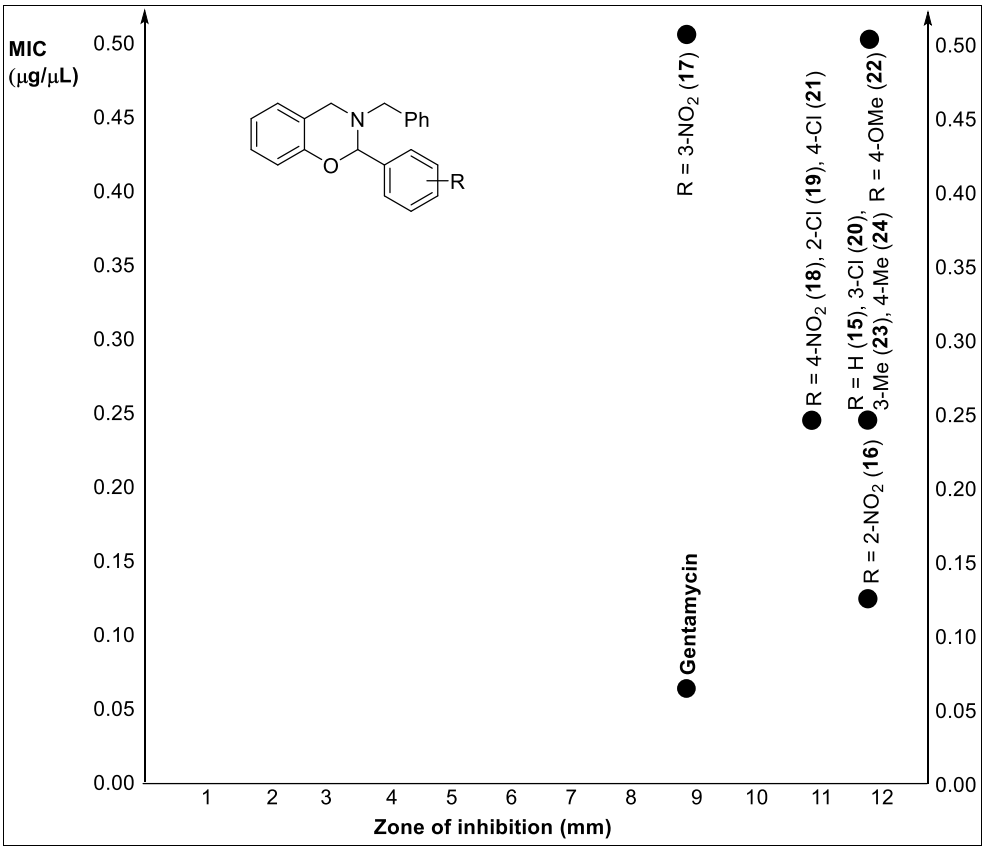


Fig 6: Growth inhibition activity of benzoxazines against *Klebsiella pneumonia*

Conclusion

In conclusion, a procedure for the synthesis of 1,3-benzoxazines that involves the instant reaction between benzaldehyde and benzylamine as a critical step has been described. The procedure distinguishes itself by using minimal organic solvents, convenience and high yields of the 1,3-benzaxozine products. Further, the prepared 1,3-benzoxazines were found to have a bigger zone of inhibition against the gram negative bacteria *Klebsiella pneumonia* that the standard antibiotic gentamycin. Benzoxazine 24 was found to inhibit the growth of *S. enterica* at a lower concentration than that of the reference antibiotic. All the prepared compounds were inactive against gram positive bacteria and the benzoxazines with an 8-methoxy group on ring A were inactive against all test bacteria.

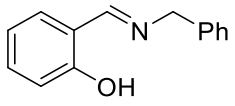
Experimental

Chemistry

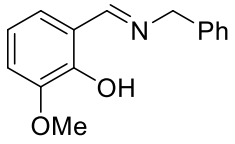
General experimental conditions: Laboratory grade chemicals and solvents were procured from Sigma-Aldrich and used without any further purification. Reactions were monitored by TLC using Merck's TLC Silica gel 60 F254 aluminium sheets. Melting point measurements were determined on a Stuart melting point apparatus and are uncorrected. Infrared spectra were recorded neat on a Perkins Elmer FT-IR spectrophotometer 1000. High resolution mass spectra were recorded on a Thermo LTQ Orbitrap mass spectrometer. NMR spectra were recorded on a Bruker Avance DPX 300 MHz NMR spectrometer with TMS as an internal standard.

Typical procedure for the synthesis of imine intermediate: Salicylaldehyde (0.26 ml, 2.46 mmol) and benzylamine (0.27 ml, 2.46 mmols) were transferred to a mortar and an instant yellow paste formed. The paste was mixed thoroughly with a pestle for about 10 minutes to give imine intermediate 3.

(E)-2-((Benzylimino) methyl) phenol 3: yellow solid, 98% yellow solid; m.p. 31.4-32.8 ^{o}C; IR (neat) ν: 3055, 3026, 1578, 1451 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 4.87 (2H, s, NC<u>H</u>$_2$), 6.94 (1H, dd, J = 8.4 and 0.9 Hz, H-6), 7.02 (1H, d, J = 8.4 Hz, H-4), 6.915-7.45 (7H, m, Ar-H), 8.50 (1H, s, N=CH), 13.47 (1H, s, OH). ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 63.2 (N<u>C</u>H$_2$), 117.1 (C-6), 118.6 (C-4), 118.9 (C-2), 127.4 (C-4'), 127.8 (C-2' and 6'), 128.7 (C-3' and 5'), 131.4 (C-3), 132.4 (C-5), 138.2 (C-1'), 161.2 (C=N), 165.6 (C-1). HR-LTQ ESI-MS (m/z) calcd for [$C_{14}H_{13}NO$ + H]: 212.1102; found, 212.1025 [M+H].



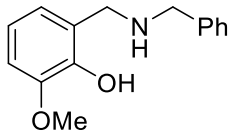
(E)-2-((Benzylimino) methyl)-6-methoxyphenol 26: orange solid, 99%; m.p. 63.5-63.8 ^{o}C; IR (neat) ν: 2991, 2835, 1631, 1435 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.95 (3H, s, OC<u>H</u>$_3$), 4.89 (2H, s, NC<u>H</u>$_2$), 6.84-7.05 (2H, m, H-4 and 5), 7.33-7.43 (6H, m, H-3, 1', 2', 3', 4', 5' and 6'), 8.48 (1H, s, C<u>H</u>=N), 9.97 (1H, s, O<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 56.2 (OCH_3), 62.8 (NCH_2), 114.2 (C-5), 118.0 (C-4), 118.7 (C-3), 123.1 (C-2, 127.4 (C-4'), 127.7 (C-2' and 6'), 128.7 (C-3' and 5')), 138.1 (C-1'), 148.6 (C-6), 151.9 (C-1), 165.7 (<u>C</u>=N). HR-LTQ ESI-MS (m/z) calcd for [$C_{15}H_{15}NO_2$ + H]: 242.1121; found 242.1112 [M+H].



Typical procedure for reduction of imine: Imine intermediate 3 (0.30 g, 1.42 mmol) was dissolved in methanol (10 ml) in a round bottom flask. $NaBH_4$ (0.11 g, 2.84 mmol) was then added to the solution and the mixture was stirred for 30 minutes. The solvent was removed under pressure and the resulting crude product was treated water (20 ml), transferred to a separating funnel and extracted three times with ethyl acetate (15 ml). The organic layers were combined and the solvent was removed under pressure to give amine 4.

2-((Benzylamino)methyl)phenol 4: yellow oil, 90%; IR (neat) ν: 3304, 3021, 2842, 1587 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$): δ_H 3.87 (2H, s, NC<u>H</u>$_2$), 4.06 (2H, s, NC<u>H</u>$_2$), 6.85 (1H, dd, J = 8.4 and 1.2 Hz, H-6), 6.92 (1H, d, J = 8.4 Hz, H-4), 7.04 (1H, dd, 1 H, J = 9.3 and 1.8 Hz, H-3), 7.33-7.44 (5H, m, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 51.9 (N<u>C</u>H$_2$), 52.6 (N<u>C</u>H$_2$), 116.4 (C-6), 119.2 (C-4), 122.3 (C-2), 127.6 (C-4'), 128.4 (C-2' and 6'), 128.5 (C-3' and 5'), 128.8 (C-5), 128.9 (C-3), 138.4 (C-1'), 158.2 (C-1). HR-LTQ ESI-MS (m/z) calcd for [$C_{14}H_{15}NO$ + H]: 214.1250; found, 214.1241 [M+H].

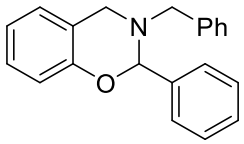
2-((benzylamino)methyl)-6-methoxyphenol 27: white solid, 88%; m.p. 95.1-95.5 ^{o}C; IR (neat) ν: 3298, 1500, 1232 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δH 3.90 (3H, s, OC<u>H</u>$_3$), 3.93 (2H, s, NC<u>H</u>$_2$), 4.07 (2H, s, NC<u>H</u>$_2$), 6.72-6.89 (3H, m, H-3, 4 and 5,), 7.34-7.40 (5H, m, H-2', 3', 4', 5' and 6'); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 56.1 (OC<u>H</u>$_3$), 62.3 9 (N<u>C</u>H$_2$), 114.1 (C-5), 118.0 (C-3), 118.7 (C-4), 123.1 (C-2), 127.4 (C-4'), 127.7 (C-2' and 6'), 128.6 (C-3' and 5'), 138.1 (C-1'), 151.8 (C-1), 165.7 (C-6); HR-LTQ ESI-MS (m/z) calcd for [$C_{15}H_{17}NO_2$ + H]: 243.1322; found, 244.1321 [M+H].



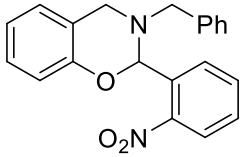
Typical procedure for the reaction of amine 4 with benzaldehyde: A solution of amine 4 (0.25 g, 1.17 mmol) in ethanol (10 ml) was treated with benzaldehyde (0.12 ml, 1.17 mmol) in a round bottom flask. The mixture was refluxed for 5 hours and monitored by TLC. The reaction mixture was allowed to cool at room temperature and then in an ice bath to give a precipitate. The solid precipitate was recrystallized from ethanol to give benzoxazine 16

3-Benzyl-2-phenyl-3, 4-dihydro-2H-1, 3-benzoxazine 16: white solid, 68%; m.p. 108.9-109.3 ^{o}C; IR (Neat) ν: 3030, 2553, 1583, 1483 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.84-3.98 (4H, m, H-4 and NC<u>H</u>$_2$), 6.05 (1H, s, H-2), 6.93 (2H, m, H-6 & H-8) 7.06 (1H, d, J = 8.1 Hz, H-5), 7.25 (1H, dd, J = 8.4 and 4.2 Hz, H-7), 7.34-7.45 (8H, m, Ar-H), 7.70 (2H, d, J = 7.5, H-2' and 6'); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 46.9 (C-4), 53.4 (-N<u>C</u>H$_2$-), 90.5 (C-2), 116.6 (C-8), 119.8 (C-6), 120.6 (C-4a), 126.7 (C-4'), 127.2 (C-4''), 127.7 (C-3' and 5'), 127.8 (C-3'' and 5''), 127.9 (C-7), 128.4 (C-2' and 6'), 128.7 (C-2'' and 6''),138.9 (C-5), 139.1 (C-1'), 153.7 (C-8a). HR-LTQ

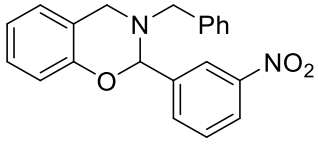
ESI-MS (m/z) calcd for [$C_{21}H_{19}NO$ + H]: 301.1493; found, 301.1493.



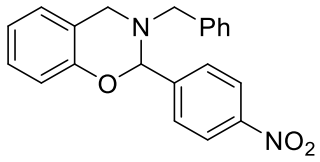
3-Benzyl-2-(2-nitrophenyl)-3,4-dihydro-2H-1,3-benzoxazine 17: white solid, 78%; m.p. 165.2-165.6 °C; IR (Neat) ν: 3066, 2918, 1527, 1487, 1361 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$): δ_H 3.69-3.91 (4H, m, H-4 and NC<u>H</u>$_2$), 6.71 (1H, s, H-2), 6.87 (1H, d, J = 6.0 Hz, H-6), 6.95 (1H, ddd, J = 7.2, 7.5 and 1.2 Hz, H-7) 7.08 (1H, d, J=8.1 Hz, H-8), 7.23-7.37 (5H, m, Ar-H), 7.47-7.60 (2H, m, H-4' and 6'), 7.82 (2H, d, J=7.8, H-3' and 5'). ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 44.9 (C-4), 54.2 (N<u>C</u>H$_2$), 87.7 (C-2), 116.3 (C-8), 119.4 (C-7), 121.1 (C-3'), 124.5 (C-5'), 127.3 (C-4a), 127.9 (C-4''), 128.1 (C-4'), 128.3 (C-7), 128.4 (C-3'' and 5''), 128.8 (C-2'' and 6''), 129.0 (C-5), 131.9 (C-1' and 6'), 133.1 (C-1''), 138.0 (C-2'), 153.0 (C-8a). HR-LTQ ESI-MS (m/z) calcd for [$C_{21}H_{19}N_2$ + H]: 347.1389; found, 347.1391 [M+H].



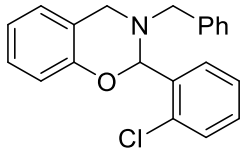
3-Benzyl-2-(3-nitrophenyl)-3,4-dihydro-2H-1,3-benzoxazine 18: white solid, 81%; m.p. 134.5-135.8 °C; IR (neat) ν: 3029, 2902, 1577, 1525, 1346 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$): δ_H 3.83-4.03 (4H, m, H-4 and NC<u>H</u>$_2$), 6.07 (1H, s, H-2), 6.96 (2H, d, J = 6.3 Hz, H-6 & H-8), 7.10, (1H, d, J = 8.1 Hz, H-7), 7.35-7.63 (6H, m, Ar-H), 7.60 (1H, dd, J = 7.8 Hz, H-5'), 8.07 (1H, d, J = 7.8, H-6'), 8.22 (1H, d, J = 7.8 Hz, H-4'), 8.58 (1H, s, H-2'). ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 46.8 (C-4), 53.6 (N<u>C</u>H$_2$), 89.1 (C-2), 116.7 (C-8), 119.4 (C-6), 121.2 (C-2'), 122.2 (C-4'), 123.1 (C-4a), 127.5 (C-4''), 127.8 (C-7), 128.2 (C-5), 128.6 (C-3'' and 5''), 128.7 (C-2'' and 6''), 129.5 (C-5'), 132.9 (C-6'), 138.2 (C-1'), 141.5 (C-1''),148.6 (C-3'), 152.9 (C-8a). HR-LTQ ESI-MS (m/z) calcd for [$C_{21}H_{18}N_2O_3$ + H]: 347.1389; found, 347.1388.



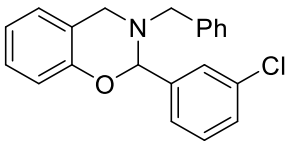
3-Benzyl-2-(4-nitrophenyl)-3, 4-dihydro-2H-1, 3-benzoxazine 19: white solid, 85%; m.p.: 90.2-91.5 °C; IR (neat) ν: 3030, 2848, 1606, 1581 cm^{-1}. ^{1}HNMR (300 MHz, $CDCl_3$): δ_H 3.81-3.98 (4H, m, H-4 and NC<u>H</u>$_2$), 6.06 (1H, s, H-2), 7.01 (2H, m, H-7 and 8), 7.08 (1H, d, J=8.1 Hz, H-5), 7.25-7.42 (H, m, Ar-H), 7.88 (2H, d, J = 8.1 Hz, H-2' and 6'), 7.27(2H, d, J = 8.1 Hz, H-3' and 5'); ^{13}C NMR (75 MHz, $CDCl_3$): δ_c 46.7 (C-4), 53.9 (N<u>C</u>H$_2$), 89.2 (C-2), 116.6 (C-8), 119.4 (C-6), 121.2 (C-4a), 123.7 (C-2' and 6'), 127.5 (C-4''), 127.8(C-3' and 5'), 128.2 (C-3'' and 5''), 128.6 (C-7), 128.7 (C-2'', 6'' and 5), 138.2 (C-1''), 146.4 (C-1''), 147.83 (C-4'), 152.9 (C-8a). HR-LTQ ESI-MS (m/z) calcd for [$C_{21}H_{18}N_2O_3$ + H]: 347.1389; found, 347.1389 [M+H].



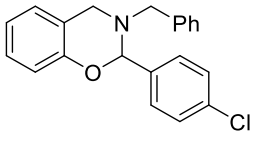
3-Benzyl-2-(2-chlorophenyl)-3, 4-dihydro-2H-1, 3-benzoxazine 20: white solid, 71%. m.p. 102.8-103.1 °C; IR (neat) ν: 3066, 2887, 1586, 1487-925 cm^{-1}. ^{1}HNMR (300 MHz, $CDCl_3$): δ_H 3.72-3.88 (4H, m, 4 H, H-4 and NC<u>H</u>$_2$), 6.28 (1H, s, H-2), 6.97 (2H, m, H-6 & H-7), 7.24-7.34 (8H, m, Ar-H), 7.48 (1H, m, H-4'), 7.72 (1H, m, H-3'); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c: 46.8 (C-4), 52.1 (N<u>C</u>H$_2$), 89.5 (C-2), 119.8 (C-8), 120.1 (C-6), 126.3 (C-4a), 127.1 (C-5'), 127.8 (C-4''), 127.9 (C-7), 128.2 (C-3'), 128.7 (C-4'), 129.5 (C-5 and 6'), 130.1 (C-3'' and 5''), 133.1 (C-2'' and 6''), 135.9 (C-1' and C-2'), 138.2 (C-1''), 154.3 (C-8a). HR-LTQ ESI-MS (m/z) calcd for [$C_{21}H_{18}NOCl$ + H]: 336.11453; found, 336.11496 [M+H].



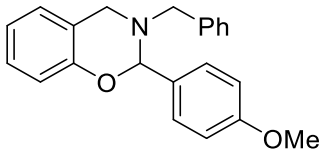
3-Benzyl-2-(3-chlorophenyl)-3, 4-dihydro-2H-1, 3-benzoxazine 21: white solid, 73%. m.p. 80.1-81.2 °C; IR (neat): 3030, 2892, 1581, 1485 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$): δ_H 3.83-3.93 (4H, m, H-4 and NC<u>H</u>$_2$), 5.99 (1H, s, H-2), 6.94 (2H, m, H-7 & H-8), 7.06 (1H, d, J = 7.8 Hz, H-5), 6.94 (1H dd, J = 9.0 and 5.1 Hz, H-6), 7.34-7.41 (7H, m, Ar-H), 7.58 (1H, d, J = 6.0 Hz, H-4'), 7.70 (1H, s, H-2'); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c: 46.7 (C-4), 53.6 (NCH_2), 89.6 (C-2), 116.6 (C-8), 119.6 (C-6), 120.9, 124.9 (C-4a), 127.0 (C-6'), 127.3 (C-4''), 127.8 (C-4'), 127.9 (C-7), 128.2 (C-2'), 128.5 (C-5'), 128.7 (C-3''and 5''), 129.8 (C-2'', 5 and 6''), 134.5 (C-3'), 138.5 (C-1'), 141.3 (C-1''), 153.3 (C-8a). HR-LTQ ESI-MS (m/z) calcd for [$C_{21}H_{18}NOCl$ + H]: 336.11453; found, 336.11485 [M+H].



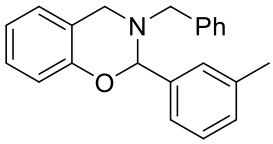
3-Benzyl-2-(4-chlorophenyl)-3, 4-dihydro-2H-1, 3-benzoxazine 22: White solid, 79%; m.p.: 116.0-116.5 °C; IR (NEAT): ν $_{cm^{-1}}$ 2988, 2884, 1485, 1455-951. ^{1}HNMR (300 MHz, $CDCl_3$): d_H 3.82-3.91 (4H, m, H-4 and NCH_2), 6.00 (1H, s, H-2), 6.93 (2H, m, H-6 & H-7), 7.05 (1H, d, J=7.8 Hz, H-8), 7.23-7.41 (7H, m, Ar-H), 7.64 (2Hd, J=8.4 Hz, H-3 and 5'); ^{13}C NMR (75 MHz, $CDCl_3$) d_c 46.7 (C-4), 53.5 (NCH_2), 89.8 (C-2), 116.6 (C-8), 119.6 (C-6), 120.8 (C-4a), 127.3 (C-4''), 127.7 (C-7), 127.9 (C-3' and 5'), 128.2 (C-3'' and 5''), 128.5 (C-2'' and 6''), 128.6 (C-7), 133.9 (C-1' 2', 4' and 6'), 137.7 (C-5), 138.6 (C-1''), 153.3 (C-9). HR-LTQ ESI-MS (m/z) calcd for [$C_{21}H_{18}NOCl$ + H]: 336.1153; found, 336.11485 [M+H].



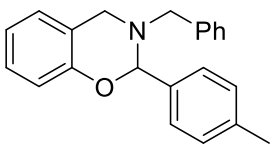
3-Benzyl-2-(4-methoxyphenyl)-3, 4-dihydro-2H-1, 3-benzoxazine 23: yield 58%. White solid, m.p.: 113.8-114.3^{0}C; 1HNMR (300 MHz, $CDCl_3$): d_H 3.85 (s, 4 H, H-4 & -NCH_2-), 3.89 (s, 3 H, OCH_3), 6.01 (s, 1 H, H-2), 6.92-6.97 (m, 4 H, Ar-H), 7.03 (d, 1 H, J=8.1, Ar-H), 7.22-7.42 (m, 6 H, Ar-H), 7.61 (d, 2 H, J=8.4, Ar-H); ^{13}C NMR (300 MHz, $CDCl_3$) d_c 48.2 (C-4), 55.1 (OCH_3), 55.6 (-NCH_2-), 91.3 (C-2), 112.3 (C-8), 115.1 (C-3' and 5'), 118.7 (C-6), 120.3 (C-4a), 121.2 (C-1'), (HR-LTQ Orbitrap ESI-MS m/z: 332.16448 [M+H] (calculated for $C_{22}H_{21}NO_2$, 331.42135), 214.12243 C_{14} H_{16}ON. IR (NEAT): $\nu_{cm^{-1}}$ 3030, 2958, 1582, 1484, 1246-754.



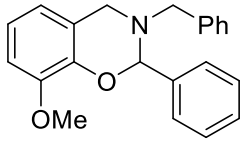
3-Benzyl-2-(*m*-tolyl)-3,4-dihydro-2H-1,3-benzoxazine 24: yield 61%. White solid, m.p.: 94.9-96.2^{0}C; ^{1}HNMR (300 MHz, $CDCl_3$): d_H 2.42 (s, 3 H, CH_3), 3.81-3.93 (m, 4 H, -NCH_2-), 6.02 (s, 1 H, H-2), 6.92-6.94 (m, 2 H, H-7 & H-8), 7.05 (d, 1 H, J= 8.1, H-5), 7.16 (d, 1 H, J= 7.5, Ar-H), 6.92-7.43 (m, 7 H, Ar-H), 7.50 (d, 2 H, J= 8.1, Ar-H); ^{13}C NMR (300 MHz, $CDCl_3$) d_c 21.6 (CH_3), 47.0 (C-4), 53.2 (-NCH_2-), 90.6 (C-2), 116.5 (C-8), 119.9 (C-6), 120.6, 123.7 (C-10), 127.2 (C-7), 127.3, 127.7, 127.8, 128.3 (C-5), 128.4, 128.7, 128.8, 138.1, 138.9, 139.0, 153.7 (C-9). HR-LTQ Orbitrap ESI-MS m/z: 316.14098 [M+H] (calculated for $C_{22}H_{21}NO$, 315.16043), 214.12245 C_{14} H_{16}ON. IR (NEAT): $\nu_{cm^{-1}}$ 3033, 2854, 1585, 1486, 1458-739.



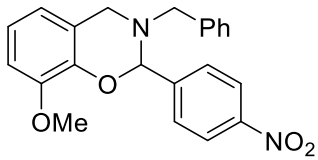
3-Benzyl-2-(*p*-tolyl)-3,4-dihydro-2H-1,3-benzoxazine 25: yield 62%. White solid, m.p.: 91.4-92.6^{0}C; ^{1}HNMR (300 MHz, $CDCl_3$): d_H 2.93 (s, 3 H,-CH_3), 3.82-3.94 (m, 4 H, -NCH_2-),6.03 (s, 1 H, H-2), 6.92 (d, 2 H, J= 4.2,H-7 & H-8),7.05 (d, 1 H, J= 8.1, H-5), 7.22-7.43 (m, 8 H, Ar-H), 7.59 (d, 2 H, J= 8.1, Ar-H); ^{13}C NMR (300 MHz, $CDCl_3$) d_c 21.2 (CH3), 46.9 (C-4), 53.3 (-NCH_2-), 90.6 (C-2), 116.5 (C-8), 119.9 (C-6), 120.6 (C-10), 126.6, 127.1 (C-7), 127.7 (2C), 127.8, 128.4 (C-5), 128.7 (2C), 129.1 (2C0, 136.2, 137.7, 139.0, 153.7, 159.5 (C-9). HR-LTQ Orbitrap ESI-MS m/z: 316.14099 [M+H] (calculated for $C_{22}H_{21}NO$, 315.16053), 214.12259 C_{14} H_{16}ON. IR (NEAT): $\nu_{cm^{-1}}$ 3674, 2988, 2901, 1581, 1483, 1456-737.



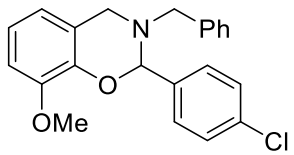
3-Benzyl-8-methoxy-2-phenyl-3,4-dihydro-2H-1,3-benzoxazine 28: white solid, 75%; m.p.: 123.8-124.1^{o}C; IR (neat) υ: 3036, 2938, 1581, 1483 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.92 (4H, m, H-4 and NCH_2), 4.02 (3H, s, OCH_3), 6.14 (1H, s, H-2) 6.52 (1H, dd, J= 5.4 and 5.4 Hz, H-6), 6.86(2H, m, H-5 and 7), 7.33-7.47 (8H, m, Ar-H), 7.65 (2H, d, J=7.8 and 0.9 Hz, H-2' and 6'). ^{13}C NMR (75 MHz, $CDCl_3$): δ_c 45.7($N\underline{C}H_2$), 54.8 (C-4), 56.0 ($O\underline{C}H_3$), 90.9 (C-2), 109.6 (C-7), 119.9 (C-6), 119.9 (C-5), 120.4 (C-4a), 126.6 (C-3'' and C-5''), 127.2 (C-4'), 127.9 (C-4''), 128.4 (C-3' and 5''), 128.5 (C-2' and 6'), 128.7 (C-2'' and 6''), 138.9 (C-1'), 139.2 (C-1''), 142.6 (C-8), 147.9 (C-8a). HR-LTQ ESI-MS (m/z) calcd for [$C_{22}H_{21}NO_2$ + H]: 331.1700; found, 331.12564 [M+H].



3-Benzyl-8-methoxy-2-(4-nitrophenyl)-3,4-dihydro-2H-1,3-benzoxazine 29: yellow solid, 68%; m.p.: 176.5-177.7 ^{o}C; IR (neat) υ: 2987, 2909, 1603, 1584, 1516, 1484 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.82-3.97 (4H, m, H-4 and $NC\underline{H}_2$), 4.04 (3H, s, $OC\underline{H}_3$), 6.14 (1H, s, H-2), 6.52 (1H, dd, J = 5.1 and 5.1 Hz, H-6), 6.90 (2H, m, H-5 and 7), 7.35-7.47 (5H, m, Ar-H), 7.85 (2H, d, J= 8.4, H-2' and 6'), 8.25 (2H, d, J= 8.4, H-3' and 5'). ^{13}C NMR (75 MHz, $CDCl_3$): δ_c 45.6 (NCH_2), 55.2 (C-4), 56.0 ($O\underline{C}H_3$), 89.7 (C-2), 109.9 (C-7), 119.5 (C-6), 120.0(C-5), 120.6 (C-4a), 123.8 (C-2' and 6'), 127.5 (C-4''), 127.8 (C-3' and 5'), 128.6 (C-3'' and 5''), 128.8 (C-2'' and 6''), 138.1 (C-1''), 141.8 (C-1'), 146.4 (C-8, 147.9 (C-4'), 148.0 (C-8a). HR-LTQ ESI-MS (m/z) calcd for [$C_{22}H_{20}N_2O_4$ + H]: 377.15011; found, 377.14963 [M+H].

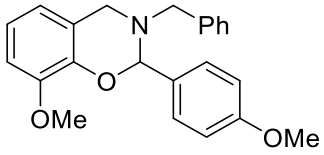


3-Benzyl-2-(4-chlorophenyl)-8-methoxy-3,4-dihydro-2H-1,3-benzoxazine 30: White solid, 79%, m.p.: 137.5-138.5 ^{o}C; IR (neat) υ: 2954, 2834, 1584, 1483, 1257 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.73-3.99 (4H, m, H-4 and $NC\underline{H}_2$), 4.02 (3H, s, $OC\underline{H}_3$), 6.08 (1H, s, H-2), 6.52 (1H, dd, J= 4.9 and 4.8 Hz, H-6), 6.87 (2H, m, H-5 and 7), 7.34-7.45 (7H, m, Ar-H), 7.60 (2H, d, J= 8.4, H-3' and 5'). ^{13}C NMR (75 MHz, $CDCl_3$) δ_c: 45.6 ($N\underline{C}H_2$), 54.8 (C-4), 56.0 ($O\underline{C}H_3$), 90.3 (C-2), 109.8 (C-7), 119.5 (C-6), 120.1 (C-5), 120.2 (C-4a), 127.3 (C-4''), 128.1 (C-3'' and 5''), 128.5 (C-3' and 5'), 128.7 (C-2'' and 6''), 128.8 (C-2' and 6'), 133.9 (C-1'), 137.7 (C-4'), 138.6 (C-1''), 142.2 (C-8), 147.9 (C-8a). HR-LTQ ESI-MS (m/z) calcd for [$C_{22}H_{20}NO_2Cl$ + H]: 366.12544; found, 366.12564 [M+H].



3-Benzyl-8-methoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1,3-benzoxazine 31: White solid, 60%; m.p.: 123.2-124.7 ^{o}C; IR (neat) υ: 3029, 2935, 1581, 1515, 1485 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.83 (3H, s, 4'-$OC\underline{H}_3$), 3.87-3.98 (4H, m, H-4 and $NC\underline{H}_2$), 4.01 (3H, s, 8-$OC\underline{H}_3$), 6.10 (1H, s, H-2), 6.51 (1H, dd, 1 H, J= 5.4 and 5.4 Hz, H-6), 6.84 (2H, m, H-5 and 7), 6.93 (2H, dd, J= 8.7 and 2.1 Hz, H-3' and 5'), 7.34-7.46 (5H, m, Ar-H), 7.58 (2H, dd, J= 8.7 and 2.1 Hz, H-2' and

6'); ^{13}C NMR (75 MHz, $CDCl_3$): δ_c 45.7 ($O\underline{C}H_3$), 54.6 ($N\underline{C}H_2$), 55.2 (C-4), 56.0 ($O\underline{C}H_3$), 90.9 (C-2), 109.8 (C-7), 113.8 (C-3' and 5'), 119.5 (C-7), 119.8 (C-6), 120.4 (C-4a), 127.2 (C-1' and 4''), 127.8 (C-3'' and 5''), 128.4 (C-2'' and 6''), 128.7 (C-2' and 6'), 131.3 (C-1''), 131.9 (C-8), 138.9 (C-8a), 142.9 (C-4'). HR-LTQ ESI-MS (m/z) calcd for [$C_{23}H_{23}NO_3$ + H]: 362.44765; found, 362.92639 [M+H].



Biology

Preparation of disks: for the 100 µg of each chemical compound was dissolved in 50 µl of DMSO (Highveld Biological) and each compound was adsorbed into a blank sterilised disk (Oxoid, UK) so that the final concentration for each test compound was 100 µg/disk. The disks were dried in an incubator at 37 ^{0}C for 18 hours before being used for sensitivity test.

Preparation of bacteria cultures: The bacterial strains used in study were the gram positive *Staphylococcus aureus* (NCTC 65710), *Bacillus cereus* (ATCC 33019) and *Listeria monocytogenes* (ATCC7644) plus the gram negative *Klebsiella pneumonia* (NCTC 9633) and *Salmonella enterica (ATCC 13076).* Prior to sensitivity testing, each of the bacteria test strains were cultured onto a nutrient agar plate and incubated for 18 hours at 37 ^{0}C. A single colony was then cultured in 5 ml of Muller Hinton broth (Oxoid, UK) and incubated for 4 hours at 37 ^{0}C. The density of each of the bacteria test strains was adjusted to an absorbance of 0.5 (approx 1.0 x 10^8 cfu/ml) using saline, at a spectrometer setting of 600 nm.

Agar overlay disc diffusion method: Each of the adjusted bacteria culture was swab onto Muller Hinton agar (Oxoid, UK) to obtain an even lawn. The plates were then dried for 15 minutes. The compound containing disks were then placed on the agar plates with each plate containing Gentamicyn (Minema, RSA) and DMSO disk as the negative control. The plates were then incubated at 37 ^{0}C for 18 hours after which the inhibition zones were examined and measured using callipers.

Preparation of standardized bacterial suspension of cells: Using aseptic techniques, a single colony was transferred into a 100mL bottle of isosensitest broth, capped and placed in incubator overnight at 35 °C. After 12–18 h of incubation, using aseptic preparation and the aid of a centrifuge, a clean sample of bacteria was prepared. The broth was spun down using a centrifuge set at 4000 rpm for 5min with appropriate aseptic precautions. The supernatant was discarded into an appropriately labelled contaminated waste beaker. The pellet was re-suspended using 20mL of sterile normal saline and centrifuged again at 4000 rpm for 5min. This step was repeated until the supernatant was clear. The pellet was then suspended in 20mL of sterile normal saline, and was labelled as Bs. The optical density of the Bs was recorded at 500 nm, and serial dilutions were carried out with appropriate aseptic techniques until the optical density was in the range of 0.5–1.0. The actual number of colony-forming units per mL (cfu/mL) was determined using the Standard Plate Count technique. The dilution factor needed was calculated and the dilution was carried out to obtain a concentration of 5×10^6 cfu/mL.

Preparation of microtitre plates: To all wells of the micro titre plate 50 µl of the nutrient broth was added. To the first row, 100 µl (1 µg/µL) of antibiotic or test compounds were dispensed and mixed with the agar. 50 µl of the mixture from the first row was withdrawn and dispensed into the second row and this was considered to be a concentration of 0.5000 ug/uL. Further, from the second row mixture, 50 µl was withdrawn and dispensed into the third row to give a concentration of 0.2500 ug/uL. This procedure was repeated down to the last row to give subsequent concentrations of 0.1250 ug/uL, 0.0625 ug/uL, 0.03125 ug/uL, 0.015625 ug/uL, etc. The 50 µl withdrawn from the last row was discarded. 10 µl of adjusted bacterial suspension, 10 µl of resazurin indicator solution were added to each well. Control 1 was the blank test without any antibiotic or test compound. Control 2 contained the reference antibiotic. After 18 hours of incubation at 37 ^{0}C the plates were assessed.

Acknowledgement

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References

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