Synthesis and biological activity of 6-alkyl/chloro-3-{4-(6-alkyl/chloro-2H-benzo[e][1,3]oxazin-3(4H)-yl)phenyl}-3,4-dihydro-2H-benzo[e][1,3]oxazines

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The efficient synthesis of symmetrical bis-benzoxazines using microwave irradiation is described and the possibility of a multicomponent approach to the target molecule has also been explored. The antimicrobial studies on the synthesized benzoxazines have been investigated.

Keywords: Bis-benzoxazine, sodium borohydride, microwave irradiation, multicomponent reaction, formalin, p-phenylenediamine

Bis-benzoxazines exhibit various biological activities including antibacterial1,2, antitumor3, fungicidal, tuberculostatic4 and plant growth regulative properties. Polybenzoxazines have been used for the preparation of resins by ring-opening reactions. The popular method of synthesizing benzoxazine is the Mannich reaction involving the condensation of phenol, formaldehyde and primary amine5-7. Bis-benzoxazine8 monomers containing phenylphosphine oxide have been synthesized from phosphorus containing bisphenol compounds, primary amine and formaldehyde. 2-Naphthol when treated with poly(propyleneoxide) amines and p-formaldehyde gave naphthoxazine functionalized poly(propylene-oxide)9. After successfully synthesizing benzoxazines with alkyl, aryl and halo substituents in the 3rd position of 2,3-dihydro-1,3-benzoxazines10, it has been planned to synthesize bis-benzoxazines – two benzoxazines connected by a phenyl ring. There is only one report11 in literature, which provides the single crystal X-ray structural data of a bis benzoxazine, related to that proposed in the present work.

After successfully synthesizing benzoxazines with alkyl, aryl and halo substituents in the 3rd position of 2,3-dihydro-1,3-benzoxazines11, it has been planned to synthesize bis-benzoxazines – two benzoxazines connected by a phenyl ring. There is only one report12 in literature, which provides the single crystal X-ray structural data of a bis benzoxazine, related to that proposed in the present work.

The starting material for the synthesis of the target benzoxazines are the bis secondary amines, 2-(4-{(2-hydroxy-5-substitutedbenzyl)amino}anilino)methyl)-4-substituted benzenols 2, which can be obtained by the sodium borohydride reduction of the Schiff bases, 2-[4-{1-(2-hydroxy-5-substituted phenyl)methylidene]aminophenyl]imino] methyl-4-substituted phenols. These Schiff bases 1, in turn, can be prepared by the reaction of p-phenylenediamine with 2-hydroxy-4-substituted benzaldehydes. Thus the target bis-benzoxazines 3 were successfully synthesized following the sequence described in Scheme I. All the reduction products 2, except 2f, 2g and 2h, have been reported in literature13,14. Unfortunately, compounds 2 were not soluble enough in the usual NMR solvents to record the NMR spectra. The reaction of 2 with formalin to yield 3 is clean, both by conventional (reflux in ethanol) and under microwave irradiation, giving only one product. The reaction has also been carried out in a multicomponent fashion involving p-phenylenediamine, para-substituted phenols and formaldehyde in 1:2:4 to get the target bis-benzoxazines in quantitative yield (Scheme II, Table I).

Complete assignment of all carbons and hydrogens for 3c (Figure 1) is possible by one and two dimensional NMR spectra. As the hydrogen at δ 1.22 has a HMBC contour with the carbon at δ 141.2, the latter carbon is assigned as the C-6 carbon, carbon ipso to the isopropyl group. The hydrogen at δ 4.55 has HMBC contours with the carbons at δ 125.8 and obviously this carbon is C-7 carbon. The singlet at δ 7.06 gives a contour with the methine carbon at δ 120.0 and this is the ring carbon (C-10) of the linking phenyl group. The left out
Scheme I

NH₂

R

CHO

OH

R

EtOH

Δ

NaBH₄/Silica gel

NH₂

R

HO

R

2CH₂O

MW or thermal

R

R

N

N

R

OH

R

OH

R

MW or thermal

Scheme II

H₂N

R

NH₂

OH

R

4CH₂O

MW

Table I — Reaction time and yield under different conditions for the formation of 3

<table>
<thead>
<tr>
<th>Compd</th>
<th>Melting point (°C)</th>
<th>Under microwave irradiation</th>
<th>Under reflux in ethanol</th>
<th>Multicomponent under microwave</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Yield (%)</td>
<td>Reaction time (min)</td>
<td>Yield (%)</td>
</tr>
<tr>
<td>3a</td>
<td>170-71</td>
<td>99</td>
<td>4</td>
<td>97</td>
</tr>
<tr>
<td>3b</td>
<td>172-73</td>
<td>74</td>
<td>4</td>
<td>92</td>
</tr>
<tr>
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</tr>
<tr>
<td>3d</td>
<td>153-55</td>
<td>97</td>
<td>6</td>
<td>96</td>
</tr>
<tr>
<td>3e</td>
<td>85-86</td>
<td>95</td>
<td>6</td>
<td>95</td>
</tr>
<tr>
<td>3f</td>
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<tr>
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<td>178-79</td>
<td>72</td>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td>3h</td>
<td>255-56</td>
<td>90</td>
<td>6</td>
<td>87</td>
</tr>
</tbody>
</table>
methine carbon is assigned to C-8. The interesting aspect is the H,H-COSY contour between C-2 and C-4 hydrogens. Though they appear as two different singlets with no apparent coupling, there seems to be a long range four bond coupling between them. Single crystal X-ray studies confirm the structure of compound 3a (Ref. 15). (Figure 2).

The synthesized compounds were tested for their in vitro antibacterial activity against some Gram-Positive (Staphylococcus aureus and Bacillus subtilis) and Gram Negative (Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa) bacteria. Pencillin G and Streptomycin were used as standard drugs. Only compounds 3d, 3e, 3f and 3g show significant activity in vitro against E. coli. Unfortunately, all the other compounds do not show any significant activity in vitro against the other tested organisms.

**Experimental Section**

All chemicals were of reagent grade and used without further purification. All melting points were recorded in open capillaries and are uncorrected. The $^1$H and $^{13}$C NMR spectra were recorded on a Bruker 300 MHz spectrometer at 300 MHz and 75 MHz respectively in CDCl$_3$/DMSO-d$_6$ using TMS as internal standard. Microanalyses were carried out on a Perkin-Elmer instrument. IR spectra were recorded on a Jasco 32 instrument and the mass spectra were recorded on a Thermo Fischer LC-MS instrument. Microwave assisted reactions were carried out in a Biotage Microwave Synthesizer. All chromatographic

![Figure 1 — Complete assignment of $^1$H and $^{13}$C NMR signals for 3c](image1)

![Figure 2 — ORTEP diagram of 3a](image2)
General procedure for the preparation of 2-{(4-
[2-hydroxy-5-substituted benzyl)amino]anilino}-
 methyl)-4-substituted benzenols, 2. A mixture of 2-
[4-[2-hydroxy-5-substitutedphenyl)methylidene]-
anilinomethyl]methyl-4-substituted phenol
(0.001 mole), sodium borohydride (0.004 mole) and
2 g of silica gel (60-120 mesh) was ground effectively
with a few drops of chloroform followed by few
drops of water. After vigorous stirring for 10 to 20
min, the reduction product 2 was extracted with hot
ethyl acetate and purified by recrystallization from
ethyl acetate.

4-Ethyl-2-{(4-[ethyl-2-hydroxybenzyl)amino]-
anilinomethyl}benzenol 2f. This compound was
obtained as brown crystals (ethyl acetate), yield 98%,
m.p. 137-38°C, time 15 min. Anal. Calcd for
C₃₂H₃₈N₂O₂: C, 75.83; H, 6.94; N, 8.04. Found: C,
75.94; H, 7.02; N, 8.13.

2-(4-[2-Hydroxy-5-methylbenzyl)amino]anilino-
methyl)-4-methylbenzenol 2g. This compound was
obtained as brown crystals (ethyl acetate), yield 98%.
m.p. 220-21°C, time 20 min. Anal. Calcd for
C₂₉H₃₀N₂O₂: C, 76.72; H, 6.01; N, 8.13. Found: C,
76.82; H, 6.07; N, 8.11.

4-Chloro-2-{(4-[chloro-2-hydroxybenzyl)amino]-
anilinomethyl}benzenol 2h. This compound was
obtained as brown crystals (ethyl acetate), yield 98%.
m.p. 220-21°C, time 20 min. Anal. Calcd for
C₂₉H₂₈ClN₂O₂: C, 68.21; H, 5.49; N, 7.86. Found: C,
68.22; H, 5.50; N, 7.89.

General procedure for the synthesis of 6-sub-
tituted-3-{(4-[6-substituted-2H-benzo[e][1,3]oxa-
in-3(4H)-yl)phenyl]-3,4-dihydro-2H-benzo[e][1,3]oxa-
zines 3. A mixture of 2-{(4-[2-hydroxy-5-substituted
benzyl)amino]anilino}methyl)-4-substituted benzenols
(0.005 mole) and formalin (35%, w/v, 0.010
mole) was irradiated under microwaves for 4 to 6
min. The progress of the reaction was monitored on
TLC. After completion, ice-cold water (50 mL) was
added to the reaction mixture and the compound
was extracted with chloroform, the combined organic
layers were dried over anhydrous sodium sulfate and
solvent evaporated. The crude mixture was purified
by column chromatography using petroleum ether-
ethyl acetate (95:5) mixture as eluent to afford the
pure benzoxazines 3 (ethyl acetate) in good yields.

Under conventional method: A mixture of 2-[{(4-
[2-hydroxy-5-substituted benzyl)amino]anilino}me-
thyl)-4-substituted benzenol 2 (0.005 mole) and
formalin (35%, w/v, 0.010 mole) in 30 mL ethanol
was refluxed for 4 to 5 hr. The reaction mixture
was worked up as described earlier to give 3.

Multi-component approach: A mixture of 4-substituted phenol (0.003 mole), p-phenylen-
diamine (0.003 mole) and formalin (35%, w/v, 0.006
mole) was subjected to microwave irradiation for 3 to
5 min. The progress of the reaction was monitored on
TLC. After completion, ice-cold water (50 mL) was
added to the reaction mixture. The crude product was
filtered off and purified by recrystallization from ethyl
acetate to yield 3.

3-{(2H-Benzo[e][1,3]oxazin-3(4H)-yl)phenyl}-
3,4-dihydro-2H-benzo[e][1,3]oxazine 3a. IR (CH₂Cl₂):
2896, 1519, 939 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ
4.45 (s, 4H), 5.29 (s, 4H), 6.79 (d, J = 7.8 Hz, 2H),
6.88 (t, J = 7.8 Hz, 2H), 6.99 (d, J = 7.8 Hz, 2H),
7.04 (s, 4H), 7.11 (t, J = 7.8, 2H); ¹³C NMR (75 MHz,
CDCl₃): δ 50.8, 80.2, 116.8, 120.0, 120.7, 120.8,
126.7, 127.8, 143.3, 154.3. Anal. Calcd for
C₂₃H₂₂N₂O₂: C, 78.51; H, 7.54; N, 6.56%. LC-MS:
m/z 457.34 [(M+1)⁺].

6-tert-Butyl-3-{(4-tet-buty-2H-benzo[e][1,3]oxa-
in-3(4H)-yl)phenyl}-3,4-dihydro-2H-benzo[e]-
[1,3]oxazine 3b. IR (CH₂Cl₂): 2865, 1502, 944 cm⁻¹;
¹H NMR (300 MHz, CDCl₃): δ 1.27 (s, 18 H), 4.56 (s,
4H), 5.27 (s, 4H), 6.72 (d, J = 8.7 Hz, 2H), 6.98 (d,
J = 2.4 Hz, 2H), 7.06 (s, 4H), 7.15 (dd, J = 8.7, 2.4 Hz,
2H); ¹³C NMR (75 MHz, CDCl₃): δ 31.5, 34.1, 51.2,
80.0, 116.3, 120.0, 123.2, 124.8, 141.6, 143.4, 143.5,
151.9. Anal. Calcd for C₂₃H₂₂N₂O₂: C, 79.81; H, 7.95;
N, 6.13. Found: C, 78.99; H, 7.98; N, 6.16%. LC-MS:
m/z 457.34 [(M+1)⁺].

3,4-Dihydro-6-isopropyl-3-{(4-isopropyl-2H-
benzo[e][1,3]oxazin-3(4H)-yl)phenyl}-2H-benzo[e]-
[1,3]oxazine 3c. IR (CH₂Cl₂): 2865, 1508, 944 cm⁻¹;
¹H NMR (300 MHz, CDCl₃): δ 1.22 (d, J = 6.9 Hz,
12H), 2.82 (sep, J = 6.9 Hz, 2H), 4.55 (s, 4H), 5.27 (s,
4H), 6.74 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 1.8 Hz,
2H), 6.99 (dd, J = 8.4 Hz, 1.8 Hz 2H), 7.06 (s, 4H);
¹³C NMR (75 MHz, CDCl₃): δ 24.1, 33.3, 51.0, 80.0,
116.6, 120.0, 124.3, 125.8, 141.2, 143.3, 152.2. Anal.
Calcd for C₂₃H₂₃N₂O₂: C, 78.47; H, 7.53; N,
6.54. Found: C, 78.51; H, 7.54; N, 6.56%. LC-MS:
m/z 429.15 [(M+1)⁺].

6-tet-Pentyl-3-{(4-tet-pentyl-2H-benzo[e][1,3]oxa-
in-3(4H)-yl)phenyl}-3,4-dihydro-2H-benzo[e]-
[1,3]oxazine 3d. IR (CH₂Cl₂): 2871, 1508, 956 cm⁻¹;
¹H NMR (300 MHz, CDCl₃): δ 0.68 (t, J = 7.5 Hz,
6H), 1.23 (s, 12H), 1.58 (q, J = 7.5 Hz, 4H), 4.55 (s,
4H), 5.26 (s, 4H), 6.72 (d, J = 8.7 Hz, 2H), 6.91 (d, J
= 2.1 Hz, 2H), 7.05 (s, 4H), 7.15 (dd, J = 8.7, 2.4 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 9.2, 28.5, 36.9, 37.3, 51.1, 80.0, 116.2, 119.9, 123.9, 125.4, 141.8, 143.2, 143.4, 151.8. Anal. Calcd for C$_{23}$H$_{24}$N$_2$O$_2$: C, 79.30; H, 8.32; N, 5.78. Found: C, 79.36; H, 8.35; N, 5.88%. LC-MS: m/z 485.23 [(M+H)$^+$].

3.4-Dihydro-6-(2-phenylpropan-2-yl)-3-(4-(6-phenylpropan-2-yl)-2H-benzo[e][1,3]oxazin-3(4H)-yl)phenyl)-2H-benzo[e][1,3]oxazine 3e. IR (CH$_3$Cl$_2$): 2869, 1509, 943 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 1.64 (s, 12H), 4.51 (s, 4H), 5.27 (s, 4H), 6.77 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 2.1 Hz, 2H), 6.95 (dd, J = 8.7, 2.1 Hz, 2H), 7.04 (s, 4H), 7.16-7.31 (m, 10H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 30.9, 42.3, 51.0, 79.9, 116.3, 119.8, 120.0, 124.7, 125.5, 126.4, 126.7, 127.9, 143.1, 143.3, 150.7, 152.1. Anal. Calcd for C$_{49}$H$_{40}$N$_2$O$_2$: C, 82.72; H, 6.94; N, 4.82. Found: C, 82.77; H, 6.97; N, 4.89%.

6-Ethyl-3-(4-(6-ethyl-2H-benzo[e][1,3]oxazin-3(4H)-yl)phenyl)-3,4-dihydro-2H-benzo[e][1,3]oxazine 3f. IR (CH$_3$Cl$_2$): 2865, 1496, 950 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 1.19 (t, J = 7.5 Hz, 6H), 2.54 (q, J = 7.5 Hz, 4H), 4.53 (s, 4H), 5.26 (s, 4H), 6.71 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 1.5 Hz, 2H), 6.93 (d, J = 8.4 Hz, 1.5 Hz, 2H), 7.04 (s, 4H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 15.8, 28.0, 50.9, 80.2, 116.6, 120.0, 120.5, 125.7, 127.2, 136.5, 143.3, 152.1. Anal. Calcd for C$_{25}$H$_{26}$N$_2$O$_2$: C, 77.97; H, 7.05; N, 6.99. Found: C, 77.95; H, 7.07; N, 7.08%. LC-MS: m/z 400.63 [(M+H)$^+$].

3.4-Dihydro-6-methyl-3-(4-(6-methyl-2H-benzo[e][1,3]oxazin-3(4H)-yl)phenyl)-2H-benzo[e][1,3]oxazine 3g. IR (CH$_3$Cl$_2$): 2900, 1498, 948 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 2.24 (s, 6H), 4.50 (s, 4H), 5.25 (s, 4H), 6.68 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 2.4 Hz, 2H), 6.90 (dd, J = 8.4, 2.4 Hz, 2H), 7.02 (s, 4H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 20.6, 50.8, 80.2, 116.5, 120.0, 120.4, 127.0, 128.4, 129.9, 143.3, 152.0. Anal. Calcd for C$_{22}$H$_{22}$N$_2$O$_2$: C, 77.39; H, 6.49; N, 7.52. Found: C, 77.42; H, 6.55; N, 7.55%. LC-MS: m/z 373.25 [(M+H)$^+$].

6-Chloro-3-(4-(6-chloro-2H-benzo[e][1,3]oxazin-3(4H)-yl)phenyl)-3,4-dihydro-2H-benzo[e][1,3]oxazine 3h. IR (CH$_3$Cl$_2$): 2987, 1498, 829 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-d$_6$): δ 4.49 (s, 4H), 5.26 (s, 4H), 6.65 (d, J = 8.4 Hz, 2H), 6.94-6.97 (m, 6H), 7.02 (d, J = 7.5 Hz, 2H); $^{13}$C NMR (75 MHz, DMSO-d$_6$): δ 47.7, 78.2, 116.1, 117.5*, 122.5, 124.8, 125.7, 140.5, 151.0 (*One carbon merged with other). Anal. Calcd for C$_{22}$H$_{18}$Cl$_3$N$_2$O$_2$: C, 63.93; H, 4.39; N, 6.78. Found: C, 63.95; H, 4.37; N, 6.76%.

**Antibacterial studies on 3**

The *in vitro* activity of the compounds were tested in Muller Hinton agar for bacteria by the two-fold serial dilution method. The test compounds were dissolved in dimethyl sulfoxide to obtain 5 mg mL$^{-1}$ stock solutions. Seeded broth (broth containing microbial spores) was prepared in Nutrient broth from 24 old bacterial cultures on Nutrient agar at 37°C. The final inoculum’s size was 10$^5$ (colony forming units) cfu mL$^{-1}$ for antibacterial assay. Testing was performed at pH 7.4. The minimum inhibitory concentrations were recorded by visual observations after 24 hr (for bacteria). Penicillin G and Streptomycin were used as standards.

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**References**