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Design, synthesis, and preliminary biological evaluation of 2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine derivatives

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Abstract—We synthesized a series of novel small molecules, 2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine derivatives, by tandem reduction-oxirane opening of 2-nitroaroxymethyloxiranes in moderate or excellent yields. We investigated the effects of all of the compounds on HUVEC apoptosis and A549 cell growth. The results showed that 6,8-dichloro-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine was the most effective small molecule in promoting HUVEC apoptosis and inhibiting A549 cell proliferation, but 6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine could remarkably inhibit HUVEC apoptosis and might induce the formation of microvessel.

The use of small molecules to affect biological phenomena, also known as chemical genetics, has made a significant impact in diverse areas of biology.[1–3] The design of the library is the first and a very crucial step in the forward chemical genetics process, and this step determines the success of the library.[4] In addition to natural products library and known drug-like library, the design and synthesis of novel small molecules library with valuable chemical diversity has been shown to be challenging.[5]

Vascular endothelial cells (VECs) play important roles in angiogenesis that is critical for normal physiological processes such as embryonic development and wound repair. Angiogenesis also promotes tumor growth. The regulation of endothelial cell apoptosis was as a potential therapeutic target to blood vessel disease.[6] Therefore, the current research focuses on the utilization of chemical genetics to discover novel endothelial cell apoptosis promoters or inhibitors. The new candidate promoters and inhibitors will be very useful for us to provide new insights into the mechanisms of angiogenesis, and to advance the development of new antiangiogenic and anti-cancer agents.[7] Cancer is the second leading cause of mortality in developed countries. The discovery and development of new treatments is urgently needed due to problems with currently available treatments, such as toxicities and drug-resistance. It has been reported that the antitumor efficacy of chemotherapeutic agents correlated with their growth-inhibiting, differentiation-inducing or apoptosis-inducing abilities.[8]

In our effort to discover and develop apoptosis inducers as potential new anti-cancer agents, we have identified several classes of molecules as novel apoptosis inducers, including safrole oxide, 1-alkoxy-3-(3′,4′-methylenedioxy)phenyl-2-propanol, γ-lactone, and morpholinone derivatives.[9–20] In an ongoing study in our laboratory on the design and synthesis of the small molecule, we are interested in extending our small molecules library to meet the requirement of our research.

The 2,3-dihydro-1,4-benzodioxine system has been widely used as a substructure of several biologically interesting agents and hence, a variety of reports have been presented for their synthesis and biological

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evaluation of compounds including this ring.[21–25] The 2,3-dihydro-1,4-benzoxazine derivatives, replacement of the one oxygen atom by nitrogen in 2,3-dihydro-1,4-benzodioxine, have been synthesized so far and various pharmacological activities have been reported with this class of molecules.[26–28] However, to the best of our knowledge, only few of 2,3-dihydro-3-substituted-1,4-benzoxazine derivatives, especially 2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine derivatives, have been described in the literature.[29–34]

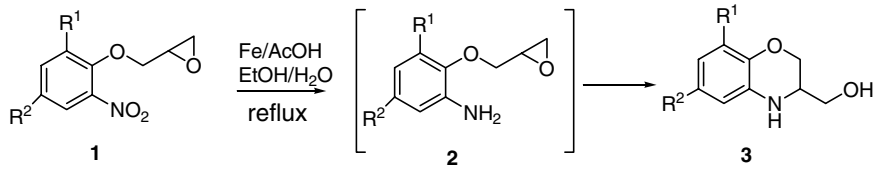
Herein, we report the discovery of 2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine derivatives and the findings of their biological activities in controlling HUVEC apoptosis and A549 cell growth.

Chemistry. The method used to synthesize the 2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine derivatives (**3**) is shown in Scheme 1. Substituted 2-nitrophenoxymethyloxiranes (**1**) were prepared according to the known method.[35] The tandem reduction-oxirane opening of substituted 2-nitrophenoxymethyloxiranes (**1**) in the presence of iron powder and acid gave substituted 2,3-dihydro-3-hydroxymethyl-1,4-benzoxazines (**3**) in moderate or excellent yields. All the seven synthesized compounds gave satisfactory spectral data. Representatively, the structure of **3a** was confirmed by ^{1}H NMR and ^{13}C NMR data,[36] which showed the presence of two CH_2–O signals at 75.2 and 68.4 ppm, and a CH–N signal at 52.2 ppm (assigned to C-3), confirming the formation of only one regioisomer.[37] The one-pot synthesis provides a novel and facile method of preparing the 2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine derivatives.

Effects of the compounds **3a**–**g** *on HUVEC apoptosis.* In order to evaluate the effects of the compounds on HUVEC[38] apoptosis, we used the given model of HUVEC apoptosis induced by deprivation of growth factors.[39,40] The results showed that compounds **3a**–**f** promoted HUVEC apoptosis at the concentrations of 25–200 μM to a certain extent. Among the six compounds, the most effective one was compound **3f** (Figs. 1A–F). Contrarily, compound **3g** inhibited the apoptosis of HUVECs from 50 to 200 μM (Fig. 1G).

Effects of the compounds **3a**–**g** *on the morphology*[41] *of HUVEC.* Morphological changes are associated with the physiological and pathological processes in HUVECs. When the cells detach from the culture dish bottom and become round, they will enter into apoptosis processes.[39] If HUVECs elongate and bend into rings, they will form microvessels.[42] In our experiments, we found that compounds **3a**–**f** first promoted the detachment of HUVECs from the dish bottom and then triggered them becoming round and forming apoptotic bodies (Figs. 2D and E). Compound **3g** induced HUVEC to elongate and form into loop-like structures (Fig. 2F). The data suggested that compound **3g** might be capable of promoting the formation of microvessel.

Effects of compounds **3a**–**g** *on the viability of A549 cells.* The data obtained by MTT assay showed that exposure of A549 cells to compound **3c** 400 μM for 24 h resulted in cell viability decrease from 100% to 77.8% ($p < 0.05$) (Fig. 3A). When the exposure continued on to 48 h, compared with the vehicle group, cell viability reduced more significantly from 100% to 59.5% ($p < 0.01$) (Fig. 3A). If the cells were treated with compound **3c** 200 μM for 48 h, cell viability could be also decreased to 72.6% ($p < 0.01$) (Fig. 3A). The data suggested that compound **3c** inhibited cell growth in a dose dependent manner from 200 to 400 μM. Compound **3d** also obviously inhibited the cell growth at 200 μM after 48 h of the treatment and its growth inhibitory effect was more obvious ($p < 0.05$) at 400 μM (Fig. 3B). When A549 cells were exposed to compound **3f** at 50 μM for 48 h or 200 μM for 24 and 48 h, the viability of the cells was decreased obviously ($p < 0.05$) (Fig. 3C). When the cells were treated with compound **3f** 400 μM for 24 or 48 h, the cell growth was remarkably suppressed ($p < 0.01$). Surprisingly, the apoptosis-promoting activity of compound **3f** at a concentration of 50 μM was higher than that at 100 μM (Fig. 3C). The results showed that the apoptosis-promoting activity of a compound was not always linear to concentration. Compounds **3a**, **3b**, **3e** and **3g** had no effect on A549 cell growth ($p > 0.05$). Taken together, the results showed that compounds **3c**, **3d**, and **3f** had obviously inhibitory effects on A549 cell growth at 200 and 400 μM, but the compounds **3a**, **3b**, **3e**, and **3g** had no effects on



1a: $R^1 = R^2 = H$
1b: $R^1 = H$, $R^2 = CH_3$
1c: $R^1 = H$, $R^2 = C(CH_3)_3$
1d: $R^1 = H$, $R^2 = Cl$
1e: $R^1 = Cl$, $R^2 = H$
1f : $R^1 = R^2 = Cl$
1g: $R^1 = H$, $R^2 = NO_2$

3a: $R^1 = R^2 = H$
3b: $R^1 = H$, $R^2 = CH_3$
3c: $R^1 = H$, $R^2 = C(CH_3)_3$
3d: $R^1 = H$, $R^2 = Cl$
3e: $R^1 = Cl$, $R^2 = H$
3f : $R^1 = R^2 = Cl$
3g: $R^1 = H$, $R^2 = NH_2$

Scheme 1. Synthesis of 2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine derivatives.

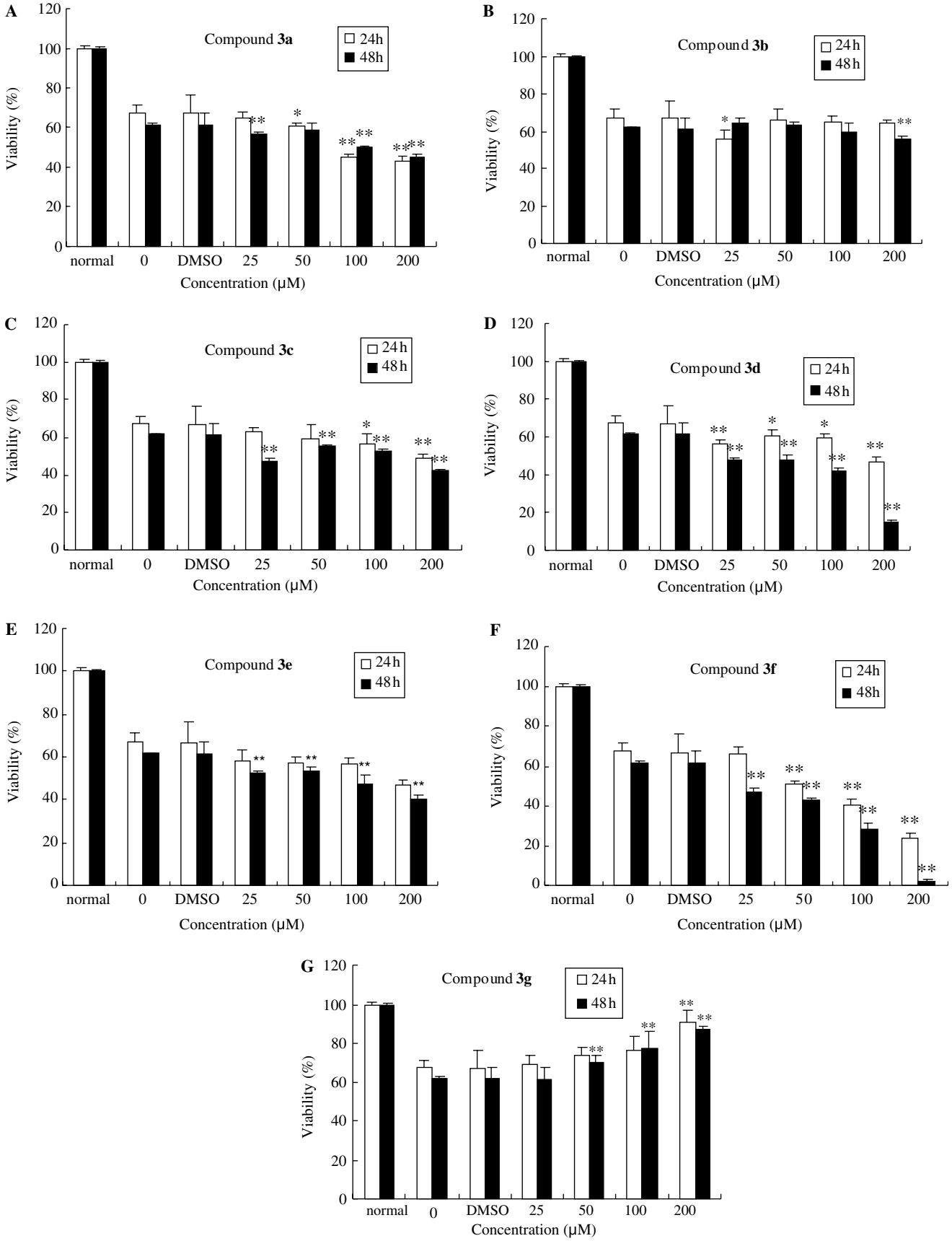


Figure 1. Effects of compounds **3a**–**g** on apoptosis of HUVECs. Data are means ± SE from three independent experiments. (∗$P < 0.05$ vs the control group; ∗∗$P < 0.01$ vs the control group).

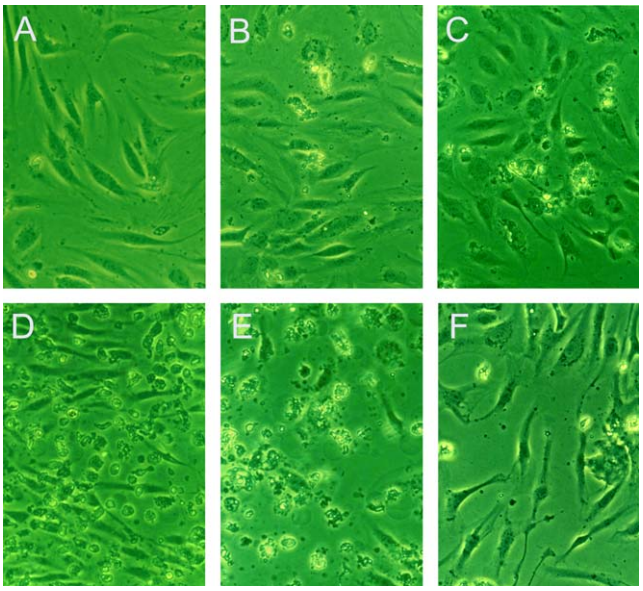


Figure 2. Morphology image of HUVEC treated with compounds **3d**, **3f**, and **3g** (200 μM) for 24 h. (A) normal; (B) control; (C) DMSO; (D) **3d**; (E) **3f**; and (F) **3g**.

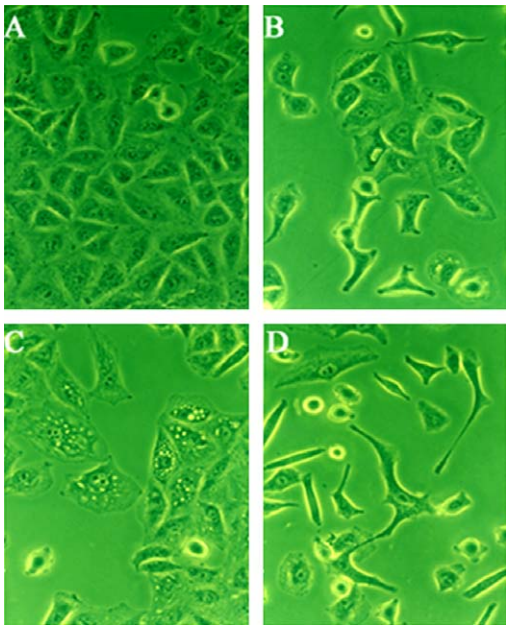


Figure 4. Morphology image of A549 treated with compounds **3c**, **3d**, and **3f** (200 μM) for 48 h. (A) DMSO; (B) **3c**; (C) **3d**; and (D) **3f**.

the cell growth. Among compounds **3c**, **3d** and **3f**, compound **3f** was the most effective one.

Effects of the compounds **3a**–**g** *on the morphology of A549 cells.* Concomitant with cell growth inhibition, compounds **3c**, **3d**, and **3f** induced the changes of A549 cell morphology. Compounds **3a**, **3b**, **3e**, and **3g** had no effects on the cell morphology. As shown in Figures 4B and D, when exposed to compound **3c** and **3f** 200 μM for 48 h, A549 cells became slender and longer, the effect of compound **3f** was much stronger than that of **3c**. The data suggested that compounds **3c** and **3f** not only could inhibit A549 cell growth, but also might induce the cell differentiation to type I lung epithelial cells in morphology. When A549 cells were treated with compound **3d**, the cells vacuolated gradually as the

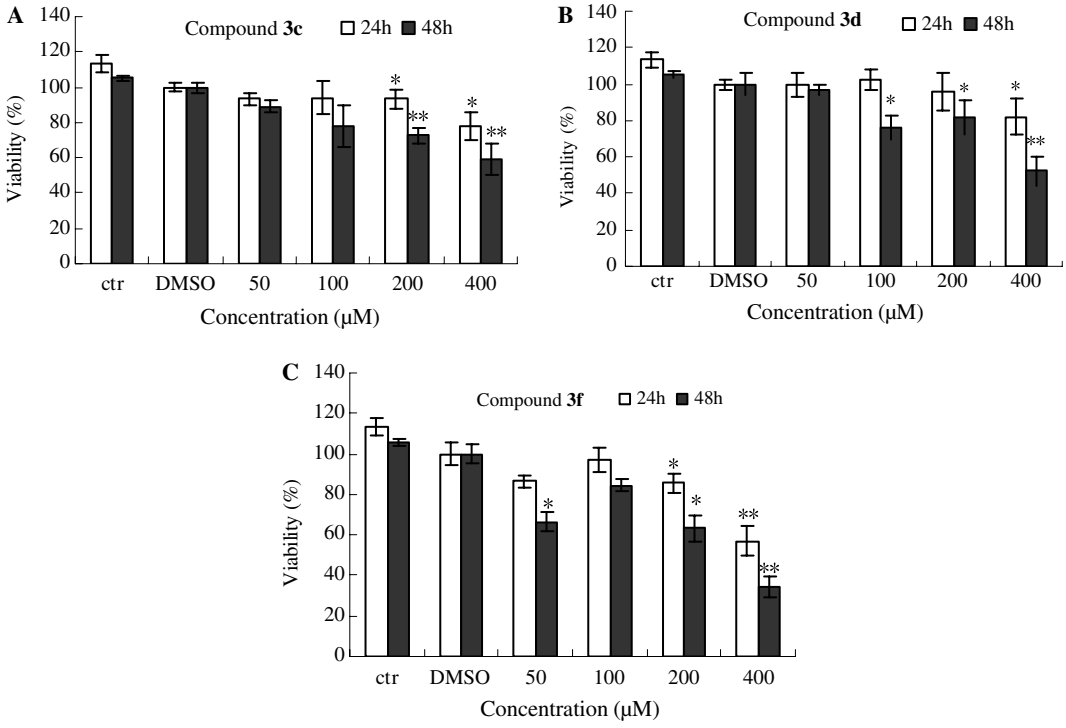


Figure 3. Inhibition of compounds **3c**, **3d**, and **3f** on A549 cell growth. Data are means ± SE from three independent experiments. (∗$P < 0.05$ vs the control group; ∗∗$P < 0.01$ vs the control group).

concentration increased and the time elongated (Fig. 4C). The results told us that compound **3d** might induce A549 cell death, because vacuolation is a common event in many cell death processes including both apoptosis and necrosis.[43]

In summary, we have described a facile approach to prepare 2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine derivatives by tandem reduction-oxirane opening of 2-nitroaroxymethyloxiranes, and we found two very interesting compounds. 6,8-Dichloro-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine **3f** was the most effective small molecule in promoting HUVEC apoptosis and inhibiting A549 cell proliferation. 6-Amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine **3g** could remarkably inhibit HUVEC apoptosis and might induce the formation of microvessel. The findings lead us to find their targets and to investigate the mechanisms of the small molecules acting in controlling cell proliferation, differentiation and apoptosis in our next researches.

Acknowledgments

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36. *2,3-Dihydro-3-hydroxymethyl-[1,4]benzoxazine* (**3a**): light yellow solid, mp 97–99 °C; IR (KBr) *v*: 3360, 3282, 2960, 2925, 1501, 1289, 1059 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.19 (dd, J = 2.4, 12.9 Hz, 1H), 3.23 (br s, 2H), 3.36 (dd, J = 4.8, 12.9 Hz, 1H), 3.88 (dd, J = 2.0, 12.8 Hz, 1H), 3.91–3.97 (m, 1H), 4.26 (dd, J = 3.8, 12.8 Hz, 1H), 6.76 (dd, J = 1.7, 7.6 Hz, 1H), 6.83 (td, J = 1.7, 7.6 Hz, 1H), 6.90 (td, J = 1.5, 7.6 Hz, 1H), 7.0 (dd, J = 1.5, 7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 52.2 (CH, C-3), 68.4 (CH_2, CH_2–O), 75.2 (CH_2, CH_2–O), 119.7 (CH, C-5), 121.8 (CH, C-8), 122.0 (CH, C-7), 123.9 (CH, C-6), 141.8 (C, C-10), 150.5 (C, C-9); EIMS (*m*/*z*, %): 165 (M^+, 54), 121 (56), 120 (100), 93 (15), 65 (15); Anal. Calcd for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.16; H, 6.76; N, 8.23.
2,3-Dihydro-3-hydroxymethyl-6-methyl-[1,4]benzoxazine (**3b**): white solid, mp 122–123 °C; IR (KBr) *v*: 3361, 3272, 2952, 2920, 1520, 1305, 1289, 1066 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.24 (s, 3H), 3.14 (dd, J = 1.9, 12.8 Hz, 1H), 3.28 (br s, 2H), 3.37 (dd, J = 3.9, 12.8 Hz, 1H), 3.80 (dd, J = 1.5, 12.3 Hz, 1H), 3.89–3.95 (m, 1H), 4.25 (dd, J = 2.7, 12.3 Hz, 1H), 6.60 (s, 1H), 6.64 (d, J = 8.0 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H); EIMS (*m*/*z*, %): 179 (M^+, 48), 135 (54), 134 (100), 107 (13), 77 (13); Anal. Calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.80; H, 7.36; N, 7.49.
2,3-Dihydro-3-hydroxymethyl-6-tert-butyl-[1,4]benzoxazine (**3c**): white solid, mp 152–154 °C; IR (KBr) *v*: 3441, 3342, 2961, 2924, 1520, 1491, 1306, 1258, 1052 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.27 (s, 9H), 3.18 (dd, J = 2.2, 12.8 Hz, 1H), 3.36 (br s, 2H), 3.39 (dd, J = 4.7, 12.8 Hz, 1H), 3.84 (dd, J = 1.9, 12.3 Hz, 1H), 3.89–3.97 (m, 1H),

4.25 (dd, J = 3.7, 12.3 Hz, 1H), 6.80 (d, J = 2.2 Hz, 1H), 6.86 (dd, J = 2.2, 8.3 Hz, 1H), 6.94 (d, J = 8.3 Hz, 1H); EIMS (*m*/*z*, %): 221 (M^+, 67), 206 (24), 177 (64), 162 (100), 133 (30), 105 (16), 77 (14); Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.56; H, 8.65; N, 6.33. Found: C, 69.83; H, 8.63; N, 6.18.

2,3-Dihydro-3-hydroxymethyl-6-chloro-[1,4]benzoxazine (**3d**): white solid, mp 108–110 °C; IR (KBr) *v*: 3353, 3275, 2962, 2923, 1493, 1288, 1060 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.19 (br s, 2H), 3.21 (dd, J = 2.5, 13.0 Hz, 1H), 3.39 (dd, J = 4.8, 13.0 Hz, 1H), 3.87 (dd, J = 2.1, 12.3 Hz, 1H), 3.93–4.00 (m, 1H), 4.25 (dd, J = 3.8, 12.3 Hz, 1H), 6.76 (d, J = 2.5 Hz 1H), 6.77 (dd, J = 2.5, 8.2 Hz, 1H), 6.91 (d, J = 8.2 Hz, 1H); EIMS (*m*/*z*, %): 199 (M^+, 45), 157 (22), 155 (58), 154 (100), 127 (10), 92 (14); Anal. Calcd for $C_9H_{10}ClNO_2$: C, 54.15; H, 5.05; N, 7.02. Found: C, 53.86; H, 5.02; N, 6.90.

2,3-Dihydro-3-hydroxymethyl-8-chloro-[1,4]benzoxazine (**3e**): light yellow oil; IR (film) *v*: 3371, 2924, 1593, 1476, 1221, 1083 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.22 (dd, J = 2.5, 13.1 Hz, 1H), 3.33 (br s, 2H), 3.41 (dd, J = 4.8, 13.1 Hz, 1H), 3.93 (dd, J = 2.2, 12.3 Hz, 1H), 3.93–4.05 (m, 1H), 4.37 (dd, J = 3.7, 12.3 Hz, 1H), 6.68 (dd, J = 1.5, 8.0 Hz, 1H), 6.81 (t, J = 8.0 Hz, 1H), 6.93 (dd, J = 1.5, 8.0 Hz, 1H); EIMS (*m*/*z*, %): 199 (M^+, 60), 157 (37), 156 (52), 155 (67), 154 (100), 127 (18), 99 (19), 92 (17); Anal. Calcd for $C_9H_{10}ClNO_2$: C, 54.15; H, 5.05; N, 7.02. Found: C, 54.22; H, 5.06; N, 6.78.

2,3-Dihydro-3-hydroxymethyl-6,8-dichloro-[1,4]benzoxazine (**3f**): light yellow oil; IR (film) *v*: 3380, 2964, 2925, 1589, 1472, 1303, 1055 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.90 (br s, 1H), 3.25 (dd, J = 2.8, 13.2 Hz, 1H), 3.40 (dd, J = 4.9, 13.2 Hz, 1H), 3.65 (br s, 1H), 3.95 (dd, J = 2.4, 12.3 Hz, 1H), 3.98-4.20 (m, 1H), 4.34 (dd, J = 3.7, 12.3 Hz, 1H), 6.65 (d, J = 2.5 Hz, 1H), 6.92 (d, J = 2.5 Hz, 1H); EIMS (*m*/*z*, %): 233 (M^+, 55), 193 (12), 192 (22), 189 (72), 188 (100), 161 (12), 126 (15), 99 (11); Anal. Calcd for $C_9H_9Cl_2NO_2$: C, 46.18; H, 3.88; N, 5.98. Found: C, 46.76; H, 4.16; N, 5.56.

2,3-Dihydro-3-hydroxymethyl-6-amino-[1,4]benzoxazine (**3g**): light yellow solid, mp 154–156 °C; IR (KBr) *v*: 3399, 3337, 2955, 2923, 1561, 1414, 1213, 1053 cm^{-1}; ^1H NMR (DMSO, 400 MHz) δ: 2.88 (dd, J = 7.7, 12.6 Hz, 1H), 3.20 (d, J = 12.6 Hz, 1H), 3.58 (dd,J = 5.6, 11.8 Hz, 1H), 3.72–3.81 (m, 1H), 4.17 (dd, J = 4.0, 11.8 Hz, 1H), 4.50 (br s, 3H), 5.00 (br s, 1H), 5.82 (dd, J = 2.6, 8.3 Hz, 1H), 5.94 (d, J = 2.6 Hz, 1H), 6.44 (d, J = 8.3 Hz, 1H); EIMS (*m*/*z*, %): 180 (M^+, 80), 137 (10), 136 (86), 135 (100), 108 (15), 95 (21), 80 (28).

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