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Design, synthesis, and antitumor activity of novel paeonol derivatives containing the 1,4-benzoxazinone and 1,2,3-triazole moieties

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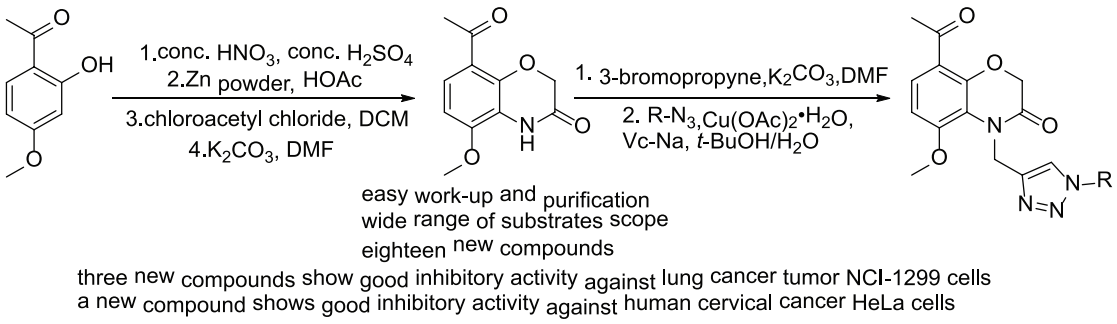
Abstract

A new series of paeonol derivatives containing the 1,4-benzoxazinone and 1,2,3-triazole moieties were synthesized and evaluated for their cytotoxicity in vitro against human non-small cell lung cancer NCI-H1299 cells and human cervical carcinoma HeLa cells. Among them, compared with that of paeonol, compounds 8-acetyl-4-{[(1-(5-chloro-2-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one, 8-acetyl-4-[(1-mesityl-1*H*-1,2,3-triazol-4-yl) methyl]-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one, and 8-acetyl-5-methoxy-4-{[(1-(naphthalen-1-yl)-1*H*-1,2,3-triazol-4-yl]methyl}-2*H*-1,4-benzoxazin-3(4*H*)-one exhibited significant inhibitory activity toward the human non-small cell lung cancer NCI-H1299 cells (IC_{50} = 13.36 ± 0.003, 19.75 ± 0.3, 15.79 ± 0.05 μg mL^{-1}). The last compound also exhibited significant inhibitory activity toward the human cervical carcinoma HeLa cells (IC_{50} = 19.73 ± 1.0 μg mL^{-1}).

Keywords

1,2,3-triazole moieties, 1,4-benzoxazinone, HeLa cells, inhibitory activity, NCI-H1299 cells, paeonol derivatives



Introduction

Paeonol (**1**), the major phenolic component isolated from Moutan Cortex and traditional Chinese medicine widely used in clinical treatment of inflammatory diseases, has various bioactivities such as antineoplastic,[1–4] antiinflammatory,[5,6] antioxidant,[7] and antithrombotic activities.[8] More recent studies have shown that paeonol exhibits promising

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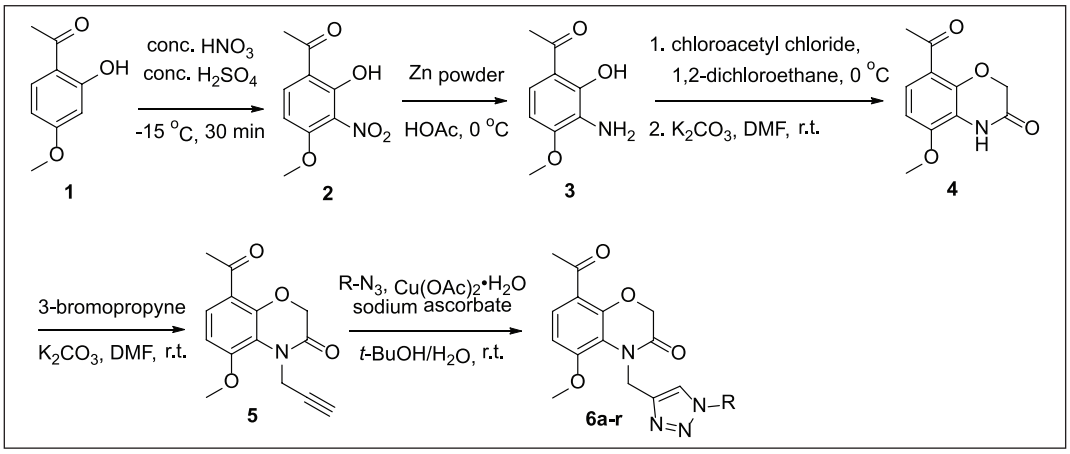


Scheme 1. Synthesis of paeonol derivatives containing the 1,4-benzoxazinone and 1,2,3-triazole moieties.

bioactivities against tumor cells, which indicates that paeonol is a possible therapeutic measure in slowing down the pathogenic processes associated with cancer.[9–12] Efforts to improve the pharmacological and biological activities of paeonol has led to the development of its derivatives by appropriate modification of paeonol as mentioned elsewhere.[13–15]

1,4-Benzoxazinone derivatives have received much attention in medicinal chemistry due to its numerous biological activities,[16–18] especially its anticancer activity.[19,20] Following Khan et al.[21] and Nagavelli et al.,[22] we attempted to enhance the anticancer activity of paeonol through introducing 1,4-benzoxazinone and 1,2,3-triazole moieties into its structure.

Based on all the above considerations and as an extension of our research on the development of novel paeonol derivatives, a new series of paeonol derivatives containing the 1,4-benzoxazinone and 1,2,3-triazole moieties, which could connect more pharmacophores for constructing bioactive and functional molecules,[23–28] were synthesized and exhibited preferable inhibitory activity toward human non-small cell lung cancer NCI-H1299 cells and human cervical carcinoma HeLa cells.

Results and discussion

Chemistry

A new series of paeonol derivatives containing the 1,4-benzoxazinone and 1,2,3-triazole moieties were synthesized in five steps and the synthetic routine is shown in Scheme 1. Paeonol (**1**) was first selectively introduced nitro group by reacting with HNO_3/H_2SO_4 at the C-3 position to form compound **2**,[29] which could be further converted to intermediate **3** by reduction with zinc powder in acetic acid.[30] After successfully synthesizing compound **3** via amidation and cyclization reaction, intermediate **4** containing the 1,4-benzoxazinone moiety was obtained.[30] Then, compound **4** was used for the synthesis of compound **5** via *N*-alkylation reaction.[31] With compound **5** in hand, a series of paeonol derivatives linked with the 1,2,3-triazole moiety (**6a–r**) were synthesized by reacting with organic azides via 1,3-dipolar Huisgen cycloaddition reaction.[32,33]

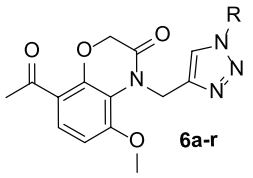
In vitro anticancer activity

All the synthesized paeonol derivatives containing the 1,4-benzoxazinone and 1,2,3-triazole moieties (**6a–r**) were evaluated for anticancer activity in in vitro mode using the MTT method on the human non-small cell lung cancer NCI-H1299 cells and the human cervical carcinoma HeLa cells. Cells were obtained from the cell bank of typical culture preservation committee of Chinese Academy of Sciences. Paeonol was used as a positive control (Table 1). The results of cytotoxic activity in vitro were expressed as IC_{50} values ($\mu g\,mL^{-1}$; Table 1). The preliminary screening results showed that most compounds exhibited moderate or high activity against human non-small cell lung cancer NCI-H1299 cells and the human cervical carcinoma HeLa cells. Almost all the tested compounds showed inhibitory activity against human non-small cell lung cancer NCI-H1299 cells, while only five of them showed inhibitory activity against the human cervical carcinoma HeLa cells. Among the tested compounds, compounds **6k**, **6l**, and **6o** exhibited good inhibitory activity toward human non-small cell lung cancer NCI-H1299 cells ($IC_{50} = 13.36 \pm 0.003$, 19.75 ± 0.3, $15.79 \pm 0.05\ \mu g\,mL^{-1}$; Table 1) compared with that of paeonol ($IC_{50} = 122.5 \pm 0.03\ \mu g\,mL^{-1}$). And compound **6o** exhibited good inhibitory activity toward the human cervical carcinoma HeLa cells ($IC_{50} = 19.73 \pm 1.0\ \mu g\,mL^{-1}$).

Conclusion

In summary, a new series of paeonol derivatives containing the 1,4-benzoxazinone and 1,2,3-triazole moieties were synthesized and their anticancer activities were evaluated. The anticancer activities were greatly enhanced by introducing the 1,4-benzoxazinone and 1,2,3-triazole moieties into the structure of paeonol. Three compounds exhibited good activity against the human non-small cell lung cancer NCI-H1299 cells and one compound showed good activity against the human cervical carcinoma HeLa cells, for which the IC_{50} value was less than 20 $\mu g\,mL^{-1}$. The research established here will accelerate structure–activity relationship studies on paeonol derivatives containing the 1,4-benzoxazinone and 1,2,3-triazole scaffolds for potential use in bioassays in vitro.

Table 1. Antitumor activity of compounds **6a–r** against human non-small cell lung cancer NCI-H1299 cells and the human cervical carcinoma HeLa cells[a] (IC_{50} μg mL^{-1}).



Compound	R=	Yield (%)[b]	NCI-H1299 (μg mL^{-1})	HeLa (μg mL^{-1})
6a	C_6H_5	52	24.19 ± 1.7	131.81 ± 15.3
6b	3-NO_2-C_6H_4	94	47.12 ± 18.7	112.81 ± 0.3
6c	4-NO_2-C_6H_4	87	33.45 ± 1.0	106.29 ± 7.0
6d	2-OH-C_6H_4	61	21.08 ± 0.6	104.42 ± 14.5
6e	5-$COCH_3$-4-OH-2-OCH_3-C_6H_2	78	83.89 ± 2.4	152.01 ± 10.0
6f	2-OH-4-OCH_3-C_6H_3	61	99.94 ± 0.5	186.06 ± 8.3
6g	4-Br-C_6H_4	60	114.62 ± 6.9	118.02 ± 2.2
6h	2-CH_3-5-NO_2-C_6H_3	83	34.02 ± 0.1	140.78 ± 20.5
6i	2-F-C_6H_4	75	20.35 ± 0.01	54.39 ± 8.0
6j	4-Cl-2-NO_2-C_6H_3	42	39.24 ± 0.07	143.81 ± 8.9
6k	5-Cl-2-NO_2-C_6H_3	74	13.36 ± 0.003	32.05 ± 3.5
6l	2,4,6-CH_3-C_6H_2	55	19.75 ± 0.3	106.17 ± 5.2
6m	2-CH_3-C_6H_4	89	31.93 ± 0.04	55.05 ± 4.6
6n	2,6-$CH(CH_3)_2$-C_6H_3	72	28.91 ± 1.3	47.43 ± 0.5
6o	$C_{10}H_7$	86	15.79 ± 0.05	19.73 ± 1.0
6p	CH_2-C_6H_5	85	32.75 ± 2.4	114.14 ± 11.0
6q	4-F-C_6H_4	86	29.08 ± 0.7	147.18 ± 7.9
6r	4-CH_3-C_6H_4	86	27.83 ± 0.3	130.26 ± 2.3
Paeonol	–	–	122.5 ± 0.03	122.2 ± 0.2

SEM: standard error of mean.
[a]Values are expressed as mean ± SEM.
[b]Percentage yield.

Experimental

All reagents were purchased from commercial sources and used without further treatment unless otherwise indicated. The unknown products were characterized using ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, melting points, infrared (IR) spectra, and high-resolution mass spectrometry (HRMS). Melting points were determined on a YUHUA X-3 melting point apparatus and were uncorrected. IR spectra were recorded on a Bio-Rad FTS-40 spectrometer. ^{1}H and ^{13}C NMR spectra were determined on a Bruker Avance (400 MHz) or Bruker Avance HD (600 MHz) using $CDCl_3$ or DMSO-d_6 as the solvent. Data are represented as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, dd = double of doublets, t = triplet, q = quartet, m = multiplet, br = broad), and coupling constants (*J*) in Hertz. HRMS was performed on a micrOTOF-Q II mass spectrometer with an electrospray ionization (ESI) source (Waters, Manchester, UK).

*1-(2-hydroxy-4-methoxy-3-nitrophenyl) ethanone (**2**)*

Paeonol (**1**) (120 mmol, 19.94 g) was dissolved in conc. H_2SO_4 (100 mL) and cooled to −15 °C. Then, conc. HNO_3 (120 mmol, 5.4 mL) was added dropwise to the above solution. After 30 min, the mixture was poured into ice water (500 mL) with stirring and precipitated a large amount of granular solids. The mixture was filtered and washed with water until neutral. The resulting solid was purified by silica gel column chromatography (petroleum/ethyl acetate = 4/3) to give compound **2** (5.91 g, 23%) as yellow granular crystal.[34] m.p. 211.0–212.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H, OH), 8.17 (d, *J* = 9.1 Hz, 1H, ArH), 6.93 (d, *J* = 9.1 Hz, 1H, ArH), 4.00 (s, 3H, CH_3), 2.65 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 204.7, 156.4, 154.4, 135.7, 130.4, 114.8, 104.25, 57.9, 27.2; IR (KBr) (ν_{max} cm^{-1}): 3107, 2852, 1643, 1505, 1373, 1280, 1090, 791, 607; HRMS: *m/z* calcd for $C_9H_9NO_5$ $(M + H)^+$ 212.0559, found 212.0558.

*1-(3-amino-2-hydroxy-4-methoxyphenyl) ethanone (**3**)*

Compound **2** (25 mmol, 5.66 g) was dissolved in HOAc (25 mL), to which zinc powder (0.25 mol, 16.35 g) was slowly added. The reaction mixture was stirred at room temperature and monitored by thin-layer chromatography (TLC). After the reaction finished, the mixture was neutralized with a saturated sodium carbonate solution, extracted with ethyl acetate, and dried (Na_2SO_4). The residue was chromatographed over silica gel (petroleum/ethyl acetate = 4/3) to

yield **3** (2.62 g, 58%) as a yellow solid.[35] m.p. 115.0–115.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.46 (s, 1H, OH), 7.19 (d, J=8.9 Hz, 1H, ArH), 6.45 (d, J=8.9 Hz, 1H, ArH), 3.92 (s, 4H, CH_3), 3.84 (s, 2H, NH_2), 2.56 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.5, 151.9, 150.5, 124.3, 120.6, 114.4, 102.1, 55.8, 26.3; IR (KBr) (ν_{max} cm^{-1}): 3334, 2842, 1633, 1440, 1281, 1143, 1056, 764, 568; HRMS: *m*/*z* calcd for $C_9H_{11}NO_3$ $(M+H)^+$ 182.0817, found 182.0823.

*8-acetyl-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**4**)*

To a solution of compound **3** (15 mmol, 2.72 g) in dichloroethane (40 mL) was added chloroacetyl chloride (16.5 mmol, 1.23 mL) at 0 °C and the reaction was monitored by TLC. After the reaction finished, the solvent was evaporated. To the residue, dimethylformamide (DMF) (30 mL) and anhydrous K_2CO_3 (18 mmol, 2.49 g) were successively added. The mixture was reacted at room temperature and monitored by TLC. The mixture was washed three times with water, extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (petroleum/ethyl acetate=5/1) to give **4** (2.84 g, 86%) as a white solid. m.p. 196.3–197.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H, NH), 7.56 (d, J=8.9 Hz, 1H, ArH), 6.63 (d, J=8.9 Hz, 1H, ArH), 4.69 (s, 2H, CH_2), 3.94 (s, 3H, CH_3), 2.58 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 163.5, 149.8, 144.3, 125.4, 120.9, 115.8, 104.7, 67.1, 56.3, 31.6; IR (KBr) (ν_{max} cm^{-1}): 3178, 2996, 1681, 1448, 1361, 1284, 1106, 779, 543; HRMS: *m*/*z* calcd for $C_{11}H_{11}NO_4$ $(M+H)^+$ 222.0766, found 222.0766.

*8-acetyl-5-methoxy-4-(prop-2-yn-1-yl)-2*H*-1,4-benzooxazin-3(4*H*)-one (**5**)*

Compound **4** (10 mmol, 2.21 g), 3-bromopropyne (12 mmol, 1.43 g), and anhydrous K_2CO_3 (12 mmol, 1.66 g) were suspended in DMF (20 mL) and the reaction was monitored by TLC. After the reaction finished, the mixture was washed three times with water, extracted with ethyl acetate, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography to afford **5** (2.51 g, 97%) as a white solid. m.p. 104.6–105.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J=9.0 Hz, 1H, ArH), 6.74 (d, J=9.0 Hz, 1H, ArH), 4.87 (d, J=2.4 Hz, 2H, CH_2), 4.58 (s, 2H, CH_2), 3.96 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 2.12 (t, J=2.4 Hz, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4, 165.0, 153.6, 149.9, 127.3, 121.3, 117.9, 106.6, 78.4, 71.5, 68.5, 56.3, 34.1, 31.7; IR (KBr) (ν_{max} cm^{-1}): 3264, 3008, 2122, 1690, 1390, 1277, 1107, 810, 547; HRMS: *m*/*z* calcd for $C_{14}H_{13}NO_4$ $(M+H)^+$ 260.0923, found 260.0912.

General procedure for the synthesis of the paeonol derivatives containing 1,2,3-triazole (6a–r)

Compound **5** (0.5 mmol, 130 mg), *t*-BuOH/H_2O (2.0 mL, *v*/*v*=1/1), phenyl azide (0.75 mmol), sodium ascorbate (0.1 mmol, 20 mg), and $Cu(OAc)_2{\cdot}H_2O$ (0.05 mmol, 10 mg) were sequentially added into a 25-mL round flask. The reaction was stirred at room temperature and monitored by TLC. After the reaction finished, the mixture was evaporated, extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography to afford the compounds **6a–r**.

Antitumor activity

The logarithmic phase of human non-small cell lung cancer NCI-H1299 cells in RPMI 1640 medium and human cervical carcinoma HeLa cells in Dulbecco's Modified Eagle Medium (DMEM) was diluted into 5×10^4 cells/mL and then vaccinated in 96-well plates at 37 °C in 5% CO_2 environment to cultivate for 24 h. And then after adding different concentrations (NCI-H1299: 0.8 μM, 4 μM, 20 μM, 100 μM, 250μM, 500μM; HeLa: 0.16 μM, 0.8 μM, 4 μM, 20 μM, 100 μM, 500 μM) of the sample, each group 3 holes hatched 72 h. Before the end of the experiment of 4 h, to each hole was added a concentration of 0.5 mg mL^{-1} determined by 20 μL MTT solution and continued to develop for 4 h. The supernatant fraction was discarded and 150 μL DMSO was added into each hole. The absorbance of each hole (A=562 nm) was determined by the enzyme calibration instrument. After obtaining the inhibition rate, the IC_{50} value was calculated by the logit method.

*8-acetyl-5-methoxy-4-[(1-phenyl-1*H*-1,2,3-triazol-4-yl) methyl]-2H-1,4-benzoxazin-3(4*H*)-one* (**6a**): Yellow solid; m.p. 121.0–121.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H, CH), 7.67 (d, J=8.0 Hz, 2H, ArH), 7.61 (d, J=9.2 Hz, 1H, ArH), 7.47 (t, J=7.8 Hz, 2H, ArH), 7.39 (t, J=7.4 Hz, 1H, ArH), 6.69 (d, J=8.8 Hz, 1H, ArH), 5.37 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 3.94 (s, 3H, CH_3), 2.56 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 165.8, 153.5, 149.7, 145.3, 137.0, 129.7, 128.7, 127.3, 121.1, 121.0, 120.4, 118.9, 106.6, 68.7, 56.1, 40.8, 31.6; IR (KBr) (ν_{max} cm^{-1}): 3020, 1693, 1652, 1594, 1500, 1465, 1278, 1115, 807; HRMS: *m*/*z* calcd for $C_{20}H_{18}N_4O_4$ $(M+H)^+$ 379.1406, found 379.1452.

*8-acetyl-5-methoxy-4-[(1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methyl]-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6b**): Yellow solid; m.p. 162.9–163.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H, ArH), 8.28 (d, J=8.3 Hz, 1H, ArH), 8.13 (d, J=8.8 Hz, 1H, ArH), 8.11 (s, 1H, CH), 7.72 (t, J=8.2 Hz, 1H, ArH), 7.64 (d, J=8.9 Hz, 1H, ArH), 6.73 (d, J=9.0 Hz, 1H, ArH), 5.36 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 3.96 (s, 3H, CH_3), 2.59 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 165.9, 153.4, 149.6, 148.9, 146.2, 137.7, 130.9, 127.4, 125.7, 123.1, 121.2, 121.1, 118.9, 115.2, 106.6, 68.7, 56.1, 41.0, 31.5; IR (KBr) (ν_{max} cm^{-1}): 3082, 1679, 1596, 1538, 1455, 1357, 1265, 1109, 821; HRMS: *m*/*z* calcd for $C_{20}H_{17}N_5O_6$ $(M+H)^+$ 424.1257, found 424.1288.

*8-acetyl-5-methoxy-4-{[(1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6c**): Yellow granular solid; m.p. 179.2–180.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J=8.9 Hz, 2H, ArH), 8.11 (s, 1H, CH), 7.95 (d, J=8.9 Hz, 2H, ArH), 7.64 (d, J=8.9 Hz, 1H, ArH), 6.73 (d, J=9.0 Hz, 1H, ArH), 5.36 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 3.96 (s, 3H, CH_3), 2.58 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 166.0, 153.4, 149.6,

147.2, 146.4, 141.1, 127.4, 125.5, 121.2, 121.2, 120.4, 118.9, 106.7, 68.7, 56.1, 41.0, 31.5; IR (KBr) (ν_{max} cm^{-1}): 3094, 1687, 1601, 1525, 1456, 1347, 1295, 1109, 821; HRMS: *m/z* calcd for $C_{20}H_{17}N_5O_6$ $(M + Na)^+$ 446.1077, found 446.1098.

*8-acetyl-4-[(1-(2-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl]methyl)-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6d**): Orange solid; m.p. 220.0–220.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (s, 1H, OH), 8.09 (s, 1H, CH), 7.64 (d, *J* = 8.9 Hz, 1H, ArH), 7.36 (d, *J* = 8.1 Hz, 1H, ArH), 7.29 (d, *J* = 7.9 Hz, 1H, ArH), 7.17 (d, *J* = 8.2 Hz, 1H, ArH), 6.97 (t, *J* = 7.7 Hz, 1H, ArH), 6.72 (d, *J* = 9.0 Hz, 1H, ArH), 5.38 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 3.94 (s, 3H, CH_3), 2.59 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.5, 166.1, 153.7, 149.8, 145.6, 144.6, 133.9, 130.8, 130.4, 127.9, 127.5, 125.7, 124.1, 121.2, 118.9, 106.7, 68.8, 56.2, 41.3, 31.7; IR (KBr) (ν_{max} cm^{-1}): 3364, 3058, 1693, 1665, 1507, 1234, 1115, 809; HRMS: *m/z* calcd for $C_{20}H_{18}N_4O_5$ $(M + H)^+$ 395.1355, found 395.1355.

*8-acetyl-4{[(1-(5-acetyl-4-hydroxy-2-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6e**): Brick red solid; m.p. 191.9–192.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.83 (s, 1H, OH), 8.01 (s, 1H, ArH), 7.86 (s, 1H, CH), 7.61 (d, *J* = 9.0 Hz, 1H, ArH), 6.70 (d, *J* = 9.0 Hz, 1H, ArH), 6.55 (s, 1H, ArH), 5.44 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 3.96 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 2.57 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.8, 196.4, 165.8, 165.1, 157.8, 153.6, 149.9, 143.9, 128.1, 127.2, 124.9, 121.1, 118.7, 118.7, 113.2, 106.6, 100.8, 68.7, 56.5, 56.1, 40.3 31.5, 26.5; IR (KBr) (ν_{max} cm^{-1}): 3438, 3184, 1686, 1640, 1513, 1454, 1276, 1109, 819; HRMS: *m/z* calcd for $C_{23}H_{22}N_4O_7$ $(M + Na)^+$ 489.1386, found 489.1411.

*8-acetyl-4-({1-[2-(2-hydroxy-4-methoxyphenyl)-2-oxoethyl]-1*H*-1,2,3-triazol-4-yl}methyl)-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6f**): White solid; m.p. 175.4–176.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.87 (s, 1H, OH), 7.61 (s, 1H, CH), 7.58 (d, *J* = 4.6 Hz, 2H, ArH), 6.67 (d, *J* = 9.0 Hz, 1H, ArH), 6.49 (dd, *J* = 9.2, 2.4 Hz, 1H, ArH), 6.44 (d, *J* = 1.8 Hz, 1H, ArH), 5.69 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 4.60 (s, 2H, CH_2), 3.87 (d, *J* = 16.1 Hz, 6H, CH_3), 2.57 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4, 193.1, 167.2, 165.8, 165.8, 153.6, 149.8, 144.9, 130.3, 127.2, 124.5 121.0, 118.9, 111.2, 108.9, 106.6, 101.3, 68.7, 56.1, 55.8, 54.2, 40.9, 31.6; IR (KBr) (ν_{max} cm^{-1}): 3438, 3088, 1690, 1598, 1506, 1456, 1240, 1110, 812; HRMS: *m/z* calcd for $C_{23}H_{22}N_4O_7$ $(M + H)^+$ 467.1567, found 467.1568.

*8-acetyl-4-{[1-(4-bromophenyl)-1*H*-1,2,3-triazol-4-yl]methy})-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6g**): Yellow solid; m.p. 202.7–203.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H, CH), 7.61 (q, *J* = 8.9 Hz, 5H, ArH), 6.71 (d, *J* = 9.0 Hz, 1H, ArH), 5.35 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 3.94 (s, 3H, CH_3), 2.57 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 165.9, 153.5, 149.7, 145.6, 136.0, 132.9, 127.3, 122.3, 121.8, 121.1, 121.0, 118.9, 106.6, 68.7, 56.1, 40.9, 31.5; IR (KBr) (ν_{max} cm^{-1}): 3101, 1690, 1662, 1498, 1458, 1266, 1118, 808; HRMS: *m/z* calcd for $C_{20}H_{17}BrN_4O_4$ $(M + H)^+$ 457.0511, found 457.0475.

*8-acetyl-5-methoxy-4-{[1-(2-methyl-5-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6h**): Light yellow solid; m.p. 71.6–72.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 8.6 Hz, 1H, ArH), 8.20 (s, 1H, ArH), 7.77 (s, 1H, CH), 7.63 (d, *J* = 9.0 Hz, 1H, ArH), 7.54 (d, *J* = 8.4 Hz, 1H, ArH), 6.71 (d, *J* = 9.0 Hz, 1H, ArH), 5.40 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 3.95 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.27 (s, 3H, CH_3).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 165.9, 153.5, 149.8, 146.5, 145.2, 141.4, 136.8, 132.5, 127.4, 124.3, 124.3, 121.2, 121.2, 118.9, 106.6, 68.7 56.1, 40.9, 31.5, 18.5; IR (KBr) (ν_{max} cm^{-1}): 3087, 1691, 1598, 1527, 1502, 1456, 1353, 1283, 1283, 1109, 802; HRMS: *m/z* calcd for $C_{21}H_{19}N_5O_6$ $(M + H)^+$ 438.1414, found 438.1436.

*8-acetyl-4-{[1-(2-fluorophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6i**): Yellow viscous liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H, CH), 7.90 (t, *J* = 7.7 Hz, 1H, ArH), 7.62 (d, *J* = 9.0 Hz, 1H, ArH), 7.40 (q, *J* = 7.3, 6.8 Hz, 1H, ArH), 7.31–7.23 (m, 2H, ArH), 6.70 (d, *J* = 9.0 Hz, 1H, ArH), 5.42 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 3.94 (s, 3H, CH_3), 2.58 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4, 165.8, 154.5, 153.5, 152.0, 149.9, 144.9, 130.1 (d, *J* = 7.7 Hz), 127.3, 125.2 (d, *J* = 3.7 Hz), 124.8, 124.0 (d, *J* = 8.2 Hz), 121.1, 118.8, 117.0 (d, *J* = 19.8 Hz), 106.6, 68.7, 56.1, 40.6, 31.6; IR (KBr) (ν_{max} cm^{-1}): 3082, 1686, 1668, 1596, 1508, 1274, 1106, 815; HRMS: *m/z* calcd for $C_{20}H_{17}FN_4O_4$ $(M + H)^+$ 397.1312, found 397.1316.

*8-acetyl-4-{[1-(4-chloro-2-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6j**): Yellow solid; m.p. 134.7–135.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (s, 1H, CH), 7.73 (d, *J* = 11.2 Hz, 2H, ArH), 7.63 (d, *J* = 8.8 Hz, 1H, ArH), 7.54 (d, *J* = 8.3 Hz, 1H, ArH), 6.70 (d, *J* = 8.8 Hz, 1H, ArH), 5.35 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 3.89 (s, 3H, CH_3), 2.58 (s, 3H, CH_3); ^{13}C NMR (150MHz, $CDCl_3$) δ 196.4, 166.0, 153.5, 149.6, 145.7, 144.6, 136.7, 133.8, 128.8, 128.7, 127.4, 125.8, 124.0, 121.1, 118.8, 106.6, 68.7, 56.1, 41.2, 31.5; IR (KBr) (ν_{max} cm^{-1}): 3097, 1694, 1670, 1598, 1506, 1358, 1264, 1112, 834; HRMS: *m/z* calcd for $C_{20}H_{16}ClN_5O_6$ $(M + H)^+$ 458.0867, found 458.0880.

*8-acetyl-4-{[1-(5-chloro-2-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6k**): Yellow solid; m.p. 146.0–147.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.7 Hz, 1H, ArH), 7.75 (s, 1H, CH), 7.65–7.61 (m, 3H, ArH), 6.71 (d, *J* = 9.0 Hz, 1H, ArH), 5.35 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 3.89 (s, 3H, CH_3), 2.59 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4, 166.0, 153.5, 149.6, 145.8, 142.5, 140.1, 131.2, 130.7, 128.0, 127.4, 126.8, 124.0, 121.1, 118.8, 106.6, 77.2, 77.0, 68.7, 56.1, 41.2, 31.6; IR (KBr) (ν_{max} cm^{-1}): 3076, 1702, 1669, 1560, 1501, 1362, 1286, 1111, 857; HRMS: *m/z* calcd for $C_{20}H_{16}ClN_5O_6$ $(M + H)^+$ 458.0867, found 458.0852.

*8-acetyl-4-[(1-mesityl-1*H*-1,2,3-triazol-4-yl)methyl]-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6l**): Yellow solid; m.p. 134.9–135.3 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 (d, *J* = 8.9 Hz, 1H, ArH), 7.36 (s, 1H, CH), 6.92 (s, 2H, ArH), 6.64 (d, *J* = 8.9 Hz, 1H, ArH), 5.48 (s, 2H, CH_2), 4.61

(s, 2H, CH_2), 3.90 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 1.78 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.2, 165.8, 153.7, 150.2, 144.1, 140.0, 134.9, 133.3, 129.0, 127.3, 124.1, 120.9, 118.5, 106.4, 68.8, 56.1, 40.6, 31.6, 21.1, 17.0; IR (KBr) (ν_{max} cm^{-1}): 3089; 1692, 1598, 1499, 1456, 1283, 1108, 806; HRMS: *m/z* calcd for $C_{23}H_{24}N_4O_4$ $(M + H)^+$ 421.1876, found 421.1864.

*8-acetyl-5-methoxy-4-[(1-o-tolyl-1*H*-1,2,3-triazol-4-yl) methyl]-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6m**): Yellow viscous liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64–7.62 (m, 2H, CH, ArH), 7.38 (dd, *J* = 6.3, 1.8 Hz, 1H, ArH), 7.33–7.26 (m, 3H, ArH), 6.69 (d, *J* = 9.0 Hz, 1H, ArH), 5.44 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 3.94 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.08 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 165.8, 153.6, 149.9, 144.3, 136.4, 133.6, 131.4, 129.8, 127.3, 126.8, 125.9, 124.2, 121.1, 118.8, 106.6, 68.7, 56.1, 40.8, 31.6, 17.7; IR (KBr) (ν_{max} cm^{-1}): 3124, 1682, 1666, 1595, 1504, 1284, 1106, 818; HRMS: *m/z* calcd for $C_{21}H_{20}N_4O_4$ $(M + H)^+$ 393.1563, found 393.1551.

*8-acetyl-4-{[1-(2,6-diisopropylphenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6n**): Light pink solid; m.p. 140.0–141.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.8 Hz, 1H, ArH), 7.43 (t, *J* = 7.5 Hz, 1H, ArH), 7.37(s, 1H, CH), 7.21 (d, *J* = 7.6 Hz, 2H, ArH), 6.64 (d, *J* = 8.8 Hz, 1H, ArH), 5.55 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 3.92 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 1.00 (dd, *J* = 34.6, 6.5 Hz, 12H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.0, 165.8, 153.7, 150.3, 145.9, 143.8, 133.1, 130.8, 127.4, 125.0, 123.7, 120.83, 118.2, 106.4, 68.8, 56.1, 40.1, 31.6, 28.3, 24.1, 23.8; IR (KBr) (ν_{max} cm^{-1}): 2967, 1681, 1662, 1597, 1456, 1283, 1107, 814; HRMS: *m/z* calcd for $C_{26}H_{30}N_4O_4$ $(M + H)^+$ 463.2345, found 463.2330.

*8-acetyl-5-methoxy-4-{[1-(naphthalen-1-yl)-1*H*-1,2,3-triazol-4-yl]methyl}-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6o**): Brown viscous liquid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (d, *J* = 7.1 Hz, 1H, ArH), 7.93 (d, *J* = 8.1 Hz, 1H, ArH), 7.81 (s, 1H, CH), 7.67 (d, *J* = 8.9 Hz, 1H, ArH), 7.55 (dd, *J* = 11.5, 6.8 Hz, 3H, ArH), 7.46 (t, *J* = 7.5 Hz, 1H, ArH), 7.31 (d, *J* = 8.4 Hz, 1H, ArH), 6.73 (d, *J* = 8.9 Hz, 1H, ArH), 5.51 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 3.97 (s, 3H, CH_3), 2.58 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.3, 165.9, 153.6, 150.0, 144.4, 134.1, 133.6, 130.4, 128.4, 128.3, 127.9, 127.3, 127.1, 125.4, 125.0, 123.5, 122.0, 121.1, 118.7, 106.6, 68.8, 56.2, 40.7, 31.6; IR (KBr) (ν_{max} cm^{-1}): 3058, 1690, 1598, 1505, 1454, 1282, 1108, 803; HRMS: *m/z* calcd for $C_{24}H_{20}N_4O_4$ $(M + H)^+$ 429.1563, found 429.1544.

*8-acetyl-4-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6p**): Yellow viscous liquid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.9 Hz, 1H, ArH), 7.32 (d, *J* = 11.7 Hz, 4H, ArH), 7.13 (s, 2H, CH, ArH), 6.62 (d, *J* = 8.9 Hz, 1H, ArH), 5.42 (s, 2H, CH_2), 5.30 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 3.85 (s, 3H, CH_3), 2.54 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.3, 165.7, 153.5, 144.8, 134.7, 129.1, 128.7, 127.8, 127.2, 122.5, 120.9, 118.7, 106.5, 77.0, 68.7, 56.0, 54.1, 40.6, 31.6; IR (KBr) (ν_{max} cm^{-1}): 3137, 1688, 1598, 1501, 1456, 1284, 1108, 804; HRMS: *m/z* calcd for $C_{21}H_{20}N_4O_4$ $(M + H)^+$ 393.1563, found 393.1556.

*8-acetyl-4-{[(1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl] methyl}-5-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6q**): Yellow powdery solid; m.p. 164.2–165.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H, CH), 7.69–7.59 (m, 3H, ArH), 7.18 (t, *J* = 8.4 Hz, 2H, ArH), 6.70 (d, *J* = 9.0 Hz, 1H, ArH), 5.35 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 3.94 (s, 3H, CH_3), 2.57 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4, 165.9, 163.6, 161.1, 153.5, 149.7, 145.5, 133.3, 127.4, 122.4(d, *J* = 8.6 Hz), 121.3, 121.1, 118.9, 116.8, 116.6, 106.7, 68.7, 56.1, 40.9, 31.6; IR (KBr) (ν_{max} cm^{-1}): 3021, 1705, 1653, 1593, 1460, 1234, 1114, 844; HRMS: *m/z* calcd for $C_{20}H_{17}FN_4O_4$ $(M + H)^+$397.1312, found 397.1307.

*8-acetyl-5-methoxy-4-[(1-p-tolyl-1*H*-1,2,3-triazol-4-yl) methyl]-2*H*-1,4-benzoxazin-3(4*H*)-one* (**6r**): Yellow solid; m.p. 137.3–138.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H, CH), 7.61 (d, *J* = 9.0 Hz, 1H, ArH), 7.55 (d, *J* = 8.3 Hz, 2H, ArH), 7.27 (d, *J* = 8.2 Hz, 2H, ArH), 6.70 (d, *J* = 9.0 Hz, 1H, ArH), 5.37 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 3.94 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 165.8, 153.6, 149.8, 145.1, 138.8, 134.7, 130.2, 127.3, 121.1, 121.0, 120.3, 118.9, 106.6, 68.7, 56.1, 40.9, 31.5, 21.1; IR (KBr) (ν_{max} cm^{-1}): 3021, 1698, 1653, 1594, 1501, 1279, 1113, 823; HRMS: *m/z* calcd for $C_{21}H_{20}N_4O_4$ $(M + H)^+$ 393.1563, found 393.1559.

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