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RESEARCH ARTICLE

Synthesis, β-catenin Translocation Capability and ALP Activation Activity of 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one Derivatives

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Abstract: ***Background*:** Osteoporosis (OP) is a common bone disease, most often diagnosed in post-menopausal women. The majority of OP treatments are focused on manipulation of the patient's hormone levels, therefore, they are associated with significant adverse effects.

***Objective*:** The study aimed to design, synthesize and evaluate the β-catenin translocation capability and the alkaline phosphatase (ALP) activation activity of 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives.

***Method*:** The styrene derivatives were synthesized as raw materials, followed by oxidation and condensation reactions, in which 6-aryl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one derivatives (**1**) were obtained. The 3,6-diaryl-7H-thiazolo[3,2-b]-1,2,4-triazin-7-ones (**2**) were obtained by a condensation reaction of compound **1** with substituted phenacyl chlorides in acetic acid. The target compounds 3,6-diaryl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-ones (**3a-3c**) were prepared by compound **2** with substituted alkyl chloride by Williamson reaction. As to 6-benzyl-3-aryl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives as the target compounds, the benzaldehyde and acetylglycine used as raw materials, followed by Erlenmeyer-Plöchl reaction, condensation reaction, hydrolysis reaction, condensation reaction, 6-benzyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one derivatives were obtained, and were converted to the target compounds 6-benzyl-3-(hydroxylaryl)-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives (**5a-5d**) using reaction with substituted α-phenacyl chlorides. Finally, Williamson reaction were used to yield 6-benzyl-3-aryl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-ones as target compounds (**6a-6e**). The β-catenin translocation capability and the ALP activation activity were tested, and the glycogen synthase kinase-3 (GSK-3) inhibition was simulated by molecular docking.

***Results*:** Fourteen 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives were synthesized and characterized by mass spectra, proton NMR and infrared spectra, the β-catenin translocation capability and the ALP activation activities of the target compounds were tested and calculated. The EC_{50} value of the ALP activation activity of 6-(4-chlorobenzyl)-3-{4-[(2-dimethylamino)-2-oxoethoxy]phenyl}-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (**6b**) was 11.283 μM. The molecular docking results have showed that the target compounds would be GSK-3 inhibitors.

***Conclusion*:** Based on the results of the biological activity test, the target compounds have exhibited the β-catenin translocation capability and the ALP activation activity.

Keywords: Acetylglycine, alkaline phosphatase, anti-osteoporotic activity, benzaldehyde, β–Catenin, triazine.

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1. INTRODUCTION

Osteoporosis (OP) is a common bone disease, most often diagnosed in postmenopausal women. One investigation report found that the incidence of OP among people aged 50-60 was 21%, with 60-70 was 58% and with age 70-80 was 100%. Because early clinical manifestations of OP are very subtle, it has been called "the epidemic disease in silence" [1].

At present, the majority of OP treatments are based on estrogen replacement therapy (ERT), selective estrogen receptor modulators (SERMs), bisphosphonates, and calci-

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tonin; among them, ERT, SERMs, and calcitonin were focused at manipulation of the patient's hormone levels, therefore, they were associated with significant adverse effects such as atrial fibrillation, bone pain, atypical fractures, and osteosarcoma [2-4]. Novel OP treatments based on molecular targets, such as the key proteins or nucleic acids with some particular signaling pathways, such as RANKL, Wnt and BMP rather than hormones, are therefore urgently needed.

Wnt protein is a secreted glycoprotein, consisting of a frizzled (Fzd) receptor, low-density lipoprotein receptor-related protein receptor-5/6 (LRP-5/6), disheveled (Dvl) protein, glycogen synthase kinase-3 (GSK-3), *β*–catenin, and alkaline phosphatase (ALP) in C2C12 cells. In the signaling pathway, after Wnt is combined with its receptors, Fzd, LRP-5/6, and Dvl are activated subsequently; then, the GSK-3 is inhibited by Dvl, which increases *β*–catenin translocation and cellular ALP activation [5]. Following the inhibition of a GSK-3, *β*–catenin is translocated from the adhesion junctions between the clusters of C2C12 cells to the nucleus and cytosol, and the ALP activation activity of cell is increased optimally after a 48 h treatment with a GSK-3 inhibitor. The untreated cells display very low cellular activity. The ALP activation activity is a marker for osteoblast differentiation. The *β*–catenin translocation and cellular ALP activation can be measured by laser scanning cytometry [6].

In this work, based on virtual screening, scaffold hopping and structural optimization, a series of 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives was synthesized and evaluated for the *β*-catenin translocation capability and the ALP activation activity, the glycogen synthase kinase-3 (GSK-3) inhibition was simulated by molecular docking.

2. MATERIAL AND METHODS

2.1. Chemistry

All reagents and solvents were purchased from common commercial suppliers and were used without further purification. Melting points were obtained using a Koffler hot-plate apparatus. Mass spectra (MS) were determined on an electrospray (ESI) ion source in a Waters spectrometer at 3.5 kV spray voltage, and acetonitrile was used as the solvent. ^{1}H-NMR spectra were determined in DMSO-d_6 or $CDCl_3$ on a Bruker spectrometer operating at 300 MHz or 600 MHz. The IR spectra were obtained using a Bruker AFS55 spectrometer.

The target compounds 6-aryl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (**1**) and 6-arylmethyl-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2*H*)-one (**4**) have been reported previously [7-10]. The synthesis of compounds **2**, **3**, **5**, **6** was as follows:

2.1.1. General Procedure for Synthesis of 3,6-diaryl-7H-thiazolo[3,2-b]-1,2,4-triazin-7-ones (2)

A mixture of 6-aryl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (10 mmol) and substituted phenacyl chloride (10 mmol) was dissolved in glacial acetic acid (20 mL) and refluxed for 12-24 h. When the solution was cooled down to room temperature, the solid was precipitated and filtered and then collected and crystallized from ethanol to obtain the target compounds **2a** and **2b**.

2.1.2. 6-(4-Bromophenyl)-3-(4-hydroxyphenyl)-7H-thiazolo [3,2-b]-1,2,4-triazin-7-one (2a)

This compound was obtained as a yellow powder, 23% yield; mp: 292-294°C; ESI-MS (m/z): 397.9 ([M-H]$^-$), 399.8 ([M+2-H]$^-$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.02 (2H, d, J = 8.52 Hz), 7.69 (2H, d, J = 8.52 Hz), 7.58 (2H, d, J = 8.55 Hz), 7.39 (1H, s), 6.90 (2H, d, J = 8.55 Hz); IR (KBr): υ 3424, 3114, 2445, 1704, 1610, 1589, 1505, 1464, 1384, 1275, 1174, 1076, 1011, 833, 647 cm^{-1}.

2.1.3. 6-(4-Bromophenyl)-3-(4-hydroxy-3-methoxyphenyl)-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (2b)

This compound was obtained as a yellow powder, 17% yield; mp: 232-234°C; ESI-MS (m/z): 427.9 ([M-H]$^-$), 429.8 ([M+2-H]$^-$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.06 (2H, d, J = 8.4 Hz), 7.72 (2H, d), 7.46 (1H, s), 7.39 (1H, s), 7.21 (1H, d), 6.94 (1H, d), 3.82 (3H, s); IR (KBr): υ 3423, 3103, 2933, 1664, 1587, 1513, 1469, 1384, 1267, 1180, 1034, 1010, 838, 816, 657, 525 cm^{-1}.

2.1.4. General Procedure for Synthesis of 3,6-diaryl-7H-thiazolo[3,2-b]-1,2,4-triazin-7-ones (3)

A mixture of 6-(4-Bromophenyl)-3-(4-hydroxy-3-aryl)-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (**2a** or **2b**, 10 mmol), substituted alkyl chlorides (10 mmol), potassium carbonate (6.9 g, 50 mmol), potassium iodide (0.2 g, 1 mmol), and acetone (25 mL) was added in a round bottom flask, heated and refluxed for 12-24 h. Then the mixture was poured into a flask holding cold water (100 mL) and stirred for 12 h. The solid was collected and recrystallized from absolute ethanol, to obtain the target compounds **3a**-**3c**.

2.1.5. 6-(4-Bromophenyl)-3-{4-[(2-benzylamino)-2-oxo-ethoxy]phenyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (3a)

This compound was obtained as a yellow powder, 44% yield; mp: 220-222°C; ESI-MS (m/z): 547.1 ([M+H]$^+$), 569.1 ([M+Na]$^+$), 571.1 ([M+2+Na]$^+$), 585.1 ([M+K]$^+$), 587.1 ([M+2+K]$^+$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.72 (1H, t), 8.02 (2H, d), 7.73 (2H, d), 7.69 (2H, d), 7.49 (1H, s), 7.22~7.31 (5H, m), 7.14 (2H, d), 4.66 (2H, s), 4.36 (2H, s); IR (KBr): υ 3424, 3268, 3074, 2922, 1660, 1631, 1534, 1505, 1464, 1384, 1235, 1178, 1078, 1009, 834, 738, 552, 527 cm^{-1}.

2.1.6. 6-(4-Bromophenyl)-3-{4-[2-(4-chlorobenzylamino)-2-oxoethoxy]phenyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (3b)

This compound was obtained as a yellow powder, 66% yield; mp: 269-271°C; ESI-MS (m/z): 567.1 ([M+H]$^+$), 589.0 ([M+Na]$^+$), 591.0 ([M+2+Na]$^+$), 605.0 ([M+K]$^+$), 607.0 ([M+2+K]$^+$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ 11.08 (1H, s), 8.02 (2H, d, J = 7.8 Hz), 7.68~7.73 (6H, m), 7.50 (1H, s), 7.38 (2H, d, J = 7.8 Hz), 7.16 (2H, d, J = 7.8 Hz), 4.87 (2H, s); IR (KBr): υ 3406, 3075, 1694, 1584, 1538, 1492, 1466, 1401, 1385, 1248, 1179, 1079, 1011, 829, 649, 554, 523 cm^{-1}.

2.1.7. 6-(4-Bromophenyl)-3-{3-methoxy-4-[2-(4-chlorobenzylamino)-2-oxoethoxy]phenyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (3c)

This compound was obtained as a yellow powder, 77% yield; mp: 226-229°C; ESI-MS (m/z): 594.8 ([M-H]$^-$), 596.8 ([M+2-H]$^-$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.48 (1H, s), 8.05 (2H, d, J = 8.4 Hz), 7.70 (2H, d, J = 8.4 Hz), 7.68 (2H, d, J = 9.0 Hz), 7.52 (1H, d), 7.49 (1H, s), 7.38 (2H, d, J = 8.4 Hz), 7.31 (1H, s), 7.09 (1H, d), 4.82 (2H, s), 3.88 (3H, s); IR (KBr): υ 3432, 2928, 1642, 1541, 1492, 1468, 1401, 1258, 1145, 1081, 1033, 1012, 833, 527, 506 cm^{-1}.

2.1.8. General Procedure for Synthesis of 6-arylmethyl-3-aryl-7H-thiazolo[3,2-b]-1,2,4-triazin-7- ones (5)

A mixture of 6-arylmethyl-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2*H*)-one (**4**, 10 mmol), substituted phenacyl chloride (10 mmol) and AcONa 2.0 g (24.4 mmol), was dissolved in glacial acetic acid 20 mL and refluxed for 10-24 h. The progress of the reaction was monitored by TLC, the reaction liquid was cooled down to room temperature, which was filtered and crystallized from ethanol to obtain the target compounds (**5**).

2.1.9. 6-Benzyl-3-(4-hydroxyphenyl)-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (5a)

This compound was obtained as a pale yellow crystal, 85% yield; mp: 245-247°C; ESI-MS (m/z): 335.9 ([M+H]$^+$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.92 (1H, s), 7.46 (2H, d, J = 8.4 Hz), 7.33 (1H, s), 7.23-7.30 (5H, m), 6.78 (2H, d, J = 8.4 Hz), 3.98 (2H, s); IR (KBr): υ 3229, 3035, 2941, 1604, 1557, 1511, 1472, 1440, 1386, 1345, 1299, 1275, 1229, 1206, 1177, 1127, 1071, 842, 776, 757, 749, 722, 702 cm^{-1}.

2.1.10. 6-(4-Chlorobenzyl)-3-(4-hydroxyphenyl)-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (5b)

This compound was obtained as a white powder, 65% yield; mp: 219-221°C; ESI-MS (m/z): 369.9 ([M+H]$^+$), 762.8 ([2M+Na]$^+$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.93 (1H, s), 7.43 (2H, d, J = 8.6 Hz), 7.30-7.37 (5H, m), 6.78 (2H, d, J = 8.6 Hz), 3.98 (2H, s); IR (KBr): υ 3425, 1647, 1611, 1509, 1480, 1384, 1277, 1236, 1179, 1092, 1050, 1016, 837, 808, 751 cm^{-1}.

2.1.11. 6-(4-Methoxybenzyl)-3-(4-hydroxyphenyl)-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (5c)

This compound was obtained as a pale yellow powder, 64% yield; mp: 188-189°C; ESI-MS (m/z): 366.0 ([M+H]$^+$), 752.8 ([2M+Na]$^+$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.0 (1H, s), 7.48 (2H, d, J = 8.4 Hz), 7.33 (1H, s), 7.21 (2H, d, J = 8.4 Hz), 6.87 (2H, d, J = 8.4 Hz), 6.80 (2H, d, J = 8.4 Hz), 3.91 (2H, s), 3.76 (3H, s); IR (KBr): υ 3119, 1610, 1511, 1476, 1385, 1279, 1247, 1176, 1123, 1029, 838, 752 cm^{-1}.

2.1.12. 6-Benzyl-3-(3-methyl-4-hydroxyphenyl)-7H-thiazolo [3,2-b]-1,2,4-triazin-7-one (5d)

This compound was obtained as a pale yellow powder, 73% yield; mp: 224-225°C; ESI-MS (m/z): 350.0 ([M+H]$^+$), 720.9 ([2M+Na]$^+$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.83 (1H, s), 7.34 (1H, s), 7.22-7.30 (7H, m), 6.79 (1H, d), 3.99 (2H, s), 2.11 (3H, s); IR (KBr): υ 3226, 1624, 1604, 1559, 1475, 1405, 1351, 1268, 1199, 1126, 1052, 897, 867, 819, 738, 702 cm^{-1}.

2.1.13. General Procedure for Synthesis of 6-arylmethyl-3-aryl-7H-thiazolo[3,2-b]-1,2,4-triazin-7- ones (6)

6-Arylmethyl-3-(4-hydroxyaryl)-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (**5a**-**5d**, 10 mmol) was dissolved in acetone (20 mL). Potassium carbonate (2.0 g, 14.5 mmol) and substituted alkyl chloride (10 mmol) were added, and the mixture was refluxed for 8-24 h, until the TLC indicated the completion of the reaction. The mixture was filtered to obtain crude product which was evaporated to remove solvents and was collected and recrystallized from ethanol, to give the target compound **6**.

2.1.14. 6-Benzyl-3-{4-[2-(1-piperidinyl)ethoxy]phenyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (6a)

This compound was obtained as a pale yellow powder, 52% yield; mp: 185-187°C; ESI-MS (m/z): 447.0 ([M+H]$^+$), 893.0 ([2M+H]$^+$), 915.9 ([2M+Na]$^+$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.46 (2H, d, J = 8.8 Hz), 7.25-7.36 (5H, m), 6.92 (2H, d, J = 8.8 Hz), 6.72 (1H, s), 4.18 (2H, t), 4.12 (2H, s), 2.83 (2H, t), 2.56 (4H, t), 1.65 (4H, m), 1.48 (2H, m); IR (KBr): υ 3109, 2931, 2851, 1634, 1579, 1507, 1480, 1416, 1386, 1356, 1293, 1253, 1173, 1036, 933, 824, 767 cm^{-1}.

2.1.15. 6-(4-Chlorobenzyl)-3-{4-[(2-dimethylamino)-2-oxoethoxy]phenyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (6b)

This compound was obtained as a white powder, 42% yield; mp: 192-194°C; ESI-MS (m/z): 454.9 ([M+H]$^+$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ 7.51 (2H, d, J = 8.4 Hz), 7.41 (1H, s), 7.37 (2H, d, J = 8.4 Hz), 7.32 (2H, d, J = 8.4 Hz), 6.92 (2H, d, J = 8.4 Hz), 4.89 (2H, s), 3.96 (2H, s), 3.02 (3H, s), 2.86 (3H, t); IR (KBr): υ 3124, 2921, 1655, 1608, 1579, 1491, 1417, 1384, 1357, 1309, 1297, 1271, 1249, 1183, 1151, 1106, 1062, 1014, 950, 883, 815, 788, 767, 743, 729 cm^{-1}.

2.1.16. 6-(4-Methoxybenzyl)-3-{4-[2-(1-piperidinyl)ethoxy]phenyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (6c)

This compound was obtained as a pale yellow powder, 37% yield; mp: 171-173°C; ESI-MS (m/z): 477.7 ([M+H]$^+$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.47 (2H, d, J = 8.7 Hz), 7.27 (2H, d, J = 8.4Hz), 6.94 (2H, d, J = 8.7 Hz), 6.83 (2H, d, J = 8.4 Hz), 6.72 (1H, s), 4.20 (2H, t), 4.05 (2H, s), 3.79 (3H, s), 2.86 (2H, t), 2.59 (4H, s), 1.64 (4H, s), 1.49 (2H, s); IR (KBr): υ 3020, 2933, 1632, 1580, 1512, 1479, 1385, 1355, 1300, 1252, 1180, 1118, 1035, 820, 760 cm^{-1}.

2.1.17. 6-Benzyl-3-{3-methyl-4-[2-(1-piperidinyl)ethoxy]phenyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (6d)

This compound was obtained as a white powder, 43% yield; mp: 168-169°C; ESI-MS (m/z): 461.0 ([M+H]$^+$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ 7.43 (1H, d), 7.38 (2H, s), 7.23~7.30 (5H, m), 6.98 (1H, d), 4.13 (2H, t), 3.88 (2H, s), 2.70 (2H, t), 2.46 (4H, t), 2.13 (3H, s), 1.50 (4H, t), 1.39 (2H, s); IR (KBr): υ 3109, 2931, 2785, 1634, 1575, 1478, 1385, 1359, 1305, 1257, 1134, 1037, 957, 883, 859, 815, 799, 767, 750, 708 cm^{-1}.

2.1.18. 6-Benzyl-3-{3-methyl-4-[2-(4-morpholinyl)ethoxy] phenyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (6e)

This compound was obtained as a white powder, 38% yield; mp: 174-176°C; ESI-MS (m/z): 463.2 ($[M+H]^+$), 925.3 ($[2M+H]^+$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.24~7.39 (7H, m), 6.83 (1H, d), 6.70 (1H, s), 4.20 (2H, t), 4.13 (2H, s), 3.77 (4H, t), 2.90 (2H, t), 2.65 (4H, t), 2.24 (3H, s); IR (KBr): υ 3088, 2949, 2923, 2856, 2799, 1626, 1573, 1560, 1472, 1386, 1357, 1303, 1261, 1213, 1170, 1137, 1116, 1069, 1041, 960, 939, 900, 859, 802, 703 cm^{-1}.

2.2. Biological Activity

The target compounds were evaluated for the β-catenin translocation capability and the ALP activation activity using the Eli Lilly Open Innovation Drug Discovery Platform. The Wnt3A conditioned media were prepared as described [11]. C2C12 cells seeded in 384-well plates were treated with Wnt3A, bone morphogenetic protein 4, and the target compounds at the indicated concentration for either 24 h or 48 h respectively, before measuring β-catenin located in the cell nuclei or cell-associated ALP activation activity [6]. The β-catenin translocation capability (% stimulation) of the target compounds at 10 μM concentration was tested and calculated. Then, for the target compounds whose β-catenin translocation capability (% stimulation) values were higher than 60%, the EC_{50} values of the ALP activation activities were tested and calculated.

2.3. Molecular Docking

GSK-3 is a multifunctional serine-threonine kinase, which plays an important role in glycogen metabolism, Wnt and hedgehog signal transduction pathways. Some crystal structure complexes which consist of GSK-3 and its small molecule inhibitor have been reported in Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank. The PDB ID of the complexes was 4IQ6 [12], 1Q3W [13], 4ACD [14], 4B7T [15], and so on.

Complex 4IQ6 consists of GSK-3 and the small molecule GSK-3 inhibitor 6-chloro-*N*-cyclohexyl-4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyridin-2-amine (**7**) [12]. Compound **8** was confirmed as a GSK-3 inhibitor [16]. The complex 4IQ6 was used for taking molecular docking, the interactions of GSK-3 and **6b**, GSK-3 and **7**, and GSK-3 and **8** were analyzed by using Molegro Virtual Docker (MVD) software, and the docking results were compared. The parameters of MVD were all default values.

3. RESULTS AND DISCUSSION

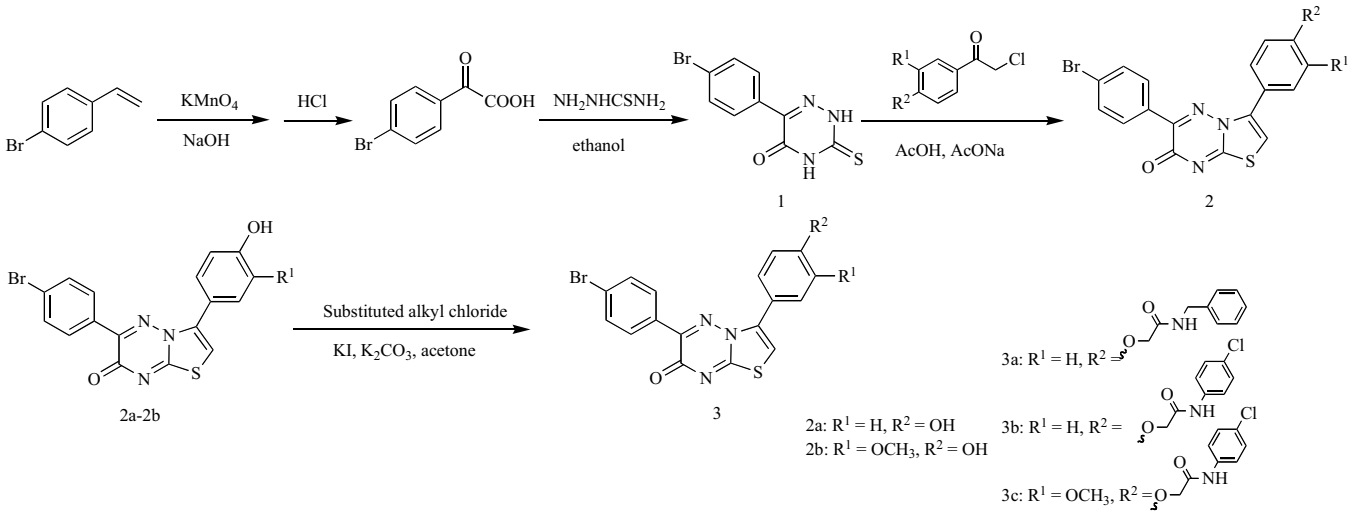
3.1. Chemistry

The synthesis of the target compounds 6-aryl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (**1**) and 6- arylmethyl-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2*H*)-one (**4**) has been reported [7-10].

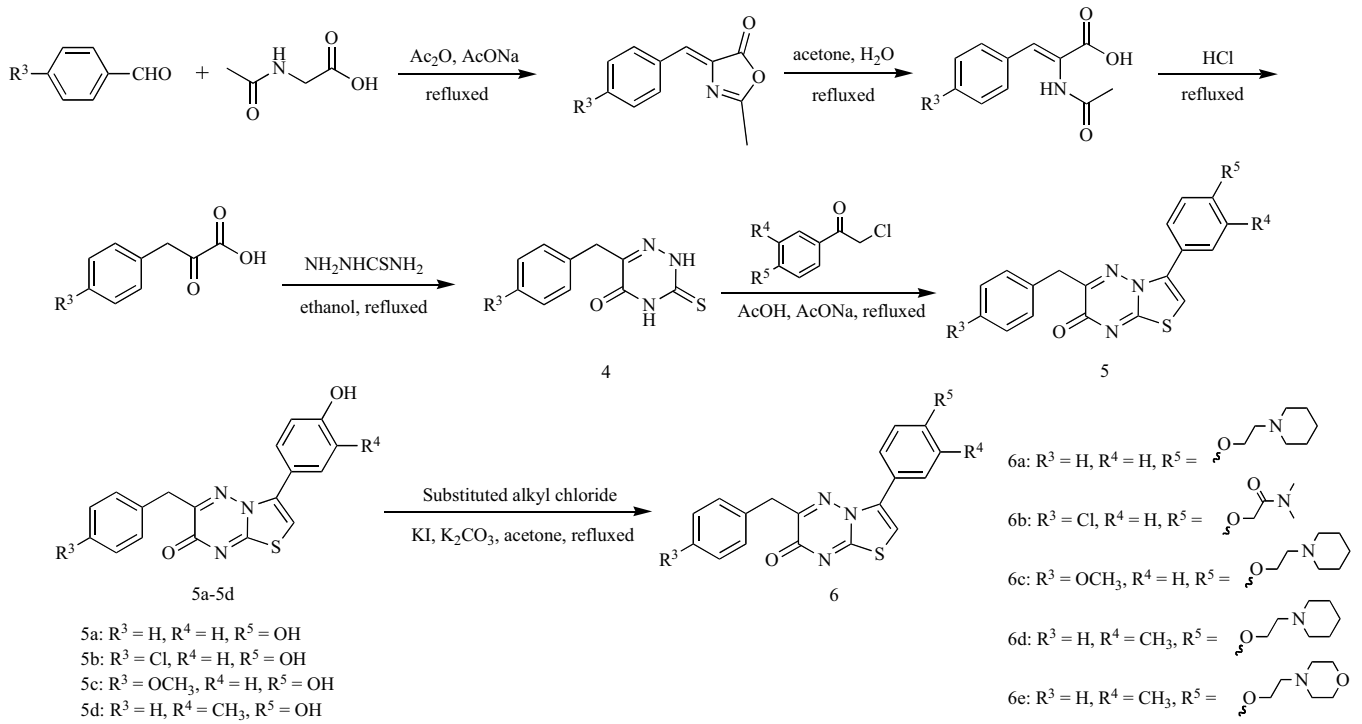
The general synthesis procedure is described as follows: The target compounds 3,6-diaryl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-ones (**2**) were prepared by **1** reacted with substituted phenacyl chlorides (2-chloro-1-(4-hydroxyphenyl) ethan-1-one or 2-chloro-1-(4-hydroxy-3- methoxyphenyl) ethan-1-one) in acetic acid to give 17% and 23% yield, respectively. The target compounds 3,6-diaryl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-ones (**3**) were obtained by the classical Williamson reaction of **2** reacted with substituted alkyl chlorides, potassium carbonate, and potassium iodide in acetone with 44%-77% yield. The synthetic route is shown in Scheme **1**.

The target compounds 6-arylmethyl-3-aryl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives (**5**) were obtained by **4** reacted with different substituted phenacyl chlorides in acetic acid and sodium acetate to give the corresponding target compounds **5** in 64%-85% yield. The target compounds 6-arylmethyl-3-aryl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-ones (**6**) were prepared by the Williamson reaction of **5** reacted with substituted alkyl chloride in acetone and potassium carbonate with 37%-55% yield. The synthetic route is shown in Scheme **2**.

The chemical structures of all target compounds were fully characterized by mass spectra (MS), ^{1}H-NMR, and infrared spectra data.



Scheme 1. The synthetic route of **2a-2b** and **3a-3c**.



Scheme 2. The synthetic route of **5a-5d** and **6a-6e**.

Table 1. The *β*-catenin translocation capability of the target compounds (3a-3c, 5b, 6a-6e).

Compound No.	Stimulation, %	SD	Compound No.	Stimulation, %	SD
3a	12.94	0.135	**6b**	74.15	0.296
3b	7.43	0.0831	**6c**	3.39	0.0598
3c	2.72	0.0357	**6d**	9.29	0.0865
5b	64.16	0.372	**6e**	6.31	0.0527
6a	63.86	0.323	**-**	-	-

Table 2. EC_{50} values of the ALP activation activity of the target compounds (5b, 6a, 6b).

Compound No.	EC_{50}, μM	Compound No.	EC_{50}, μM	Compound No.	EC_{50}, μM
5b	>20	**6a**	14.751	**6b**	11.283

3.2. Biological Activity

The *β*-catenin translocation capability (% stimulation) of the tested compounds at the indicated concentration (10 μM) was tested and calculated.

The experimental results are listed in Table **1**.

The target compounds **5b**, **6a**, **6b** were chosen for further screening. The compounds at the indicated concentration showed higher *β*-catenin translocation capability. The EC_{50} values of the ALP activation activities in C2C12 cells were tested and calculated. The experimental results were listed in Table **2** and Fig. (**1**).

From the biological data of Table **2** and (Fig. **1**), the EC_{50} values of the three target compounds were calculated. For the target compound **6b,** which had a stronger ALP activation activity than others, the EC_{50} value was 11.283 μM.

3.3. Molecular Docking

The interactions of GSK-3 and the target compound **6b**, GSK-3 and the active ligand **7**, GSK-3 and its inhibitor **8**, were analyzed by MVD software and compared as shown in Fig. (**2**) and Table **3**.

The results of the molecular docking showed that the interaction at the active sites and the interaction modes of GSK-3 and **6b**, GSK-3 and **7**, and GSK-3 and **8** were similar. Hydrogen bond interactions were found between **6b** and GSK-3, **7** and GSK-3, **8** and GSK-3 at amino acid residues Tyr140 and Ser147.

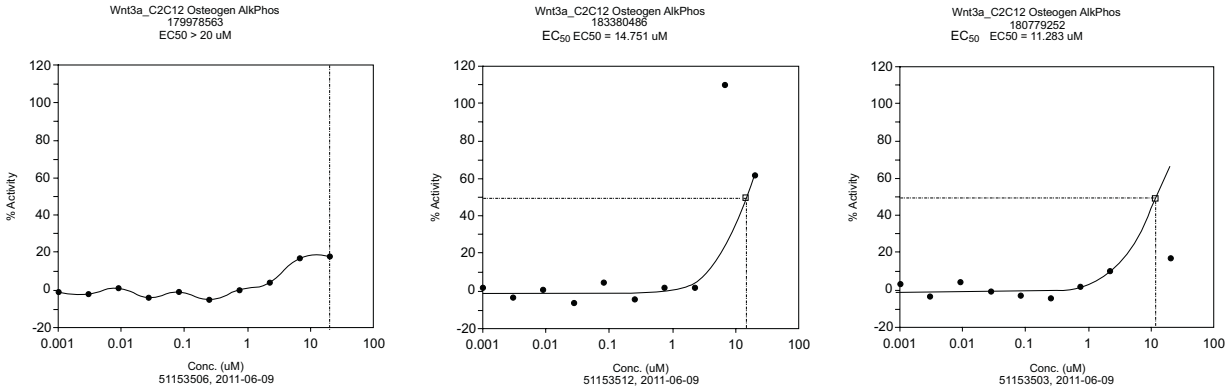


Fig. (1). EC_{50} values of the ALP activation activity of the target compounds (**5b**, **6a**, **6b**).

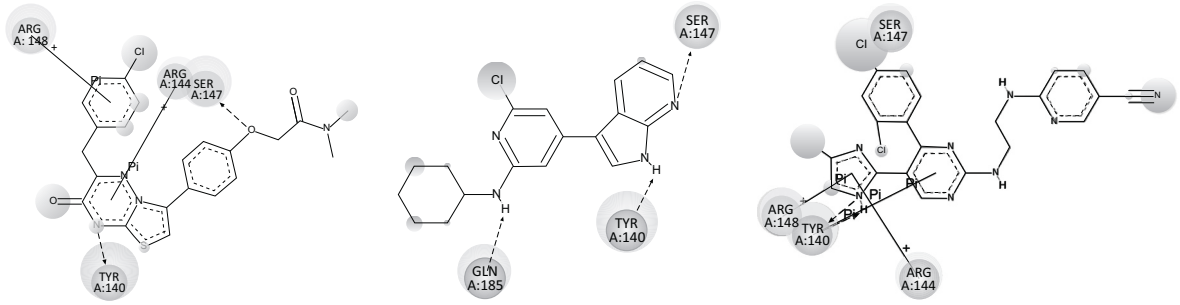


Fig. (2). The docking model of **6b** (left), **7** (middle), and **8** (right) at the active sites of GSK-3.

Table 3. The interaction active sites of GSK-3 and 6b, GSK-3 and 7, GSK-3 and 8.

No.	Amino Acid Residues (Hydrogen Bonds Between Molecule and GSK-3)	Amino Acid Residues (Other Interactions Between Molecule and GSK-3)
6b	**Tyr140**, **Ser147**	**Arg144 (charge), Arg148(charge)**
7	**Tyr140**, **Ser147**, **Gln185**	——
8	**Tyr140**	**Arg144 (charge), Arg148(charge)**

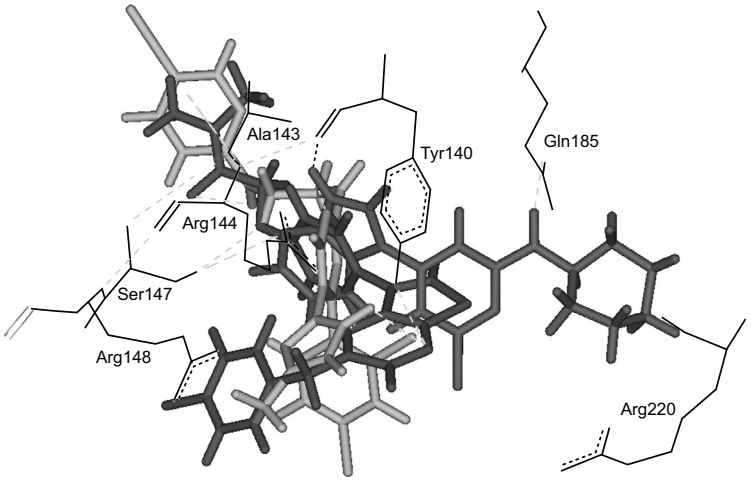


Fig. (3). The 3D active conformations of compounds **6b** (red), 7 (violet) and **8** (green) at the active sites of GSK-3.

The similarity of interaction active sites was further proved and the three-dimensional active conformations of compound **6b**, **7** and **8** at the active site are shown in Fig. (**3**).

Comparison of the three-dimensional active conformations of the active molecule **7**, the GSK-3 inhibitor **8** and the target compound **6b** at the active site of GSK-3 showed that

although the structures of parent nucleus and side chains of the three molecules were very different, all molecules were located in the same active site cavity of GSK-3. The interactional active sites of compounds **6b** and **8** were unbelievably coincident. Similarly, we speculated that compound **6b** seemed to have the feature of GSK-3 inhibitory activity.

Therefore, our further work will focus on testing GSK-3 inhibitory activity and the anti-osteoporotic activity in the near future.

CONCLUSION

In conclusion, 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives are considered as a novel class of compounds with the *β*-catenin translocation capability and the ALP activation activity with the EC_{50} value of the ALP activation activity of the target compound **6b** being 11.283 μM. The molecular docking result showed that **6b** was supposed to be a GSK-3 inhibitor. Further efforts aimed at developing potent anti-osteoporotic drugs based on the modification of the target compounds would be continued in our laboratory.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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