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Design, synthesis characterization and *in vitro* biological activity of a series of 3-aryl-6-(bromoarylmethyl)-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one derivatives as the novel acetylcholinesterase inhibitors

He Nan Xu [a,b], Zhe Jin [b], Si Jie Liu [b], Hong Min Liu [a,*], Shuo Li [c], Huang Quan Lin [c], David Chicheong Wan [c], Chun Hu [a,b]

[a] *New Drug Research & Development Center, Zhengzhou University, Zhengzhou 450052, China*
[b] *Key Laboratory of Structure-Based Drug Design Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China*
[c] *Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong SAR, China*

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Abstract

Bromination is used as a strategy to improve biological activity in medicinal chemistry. In order to study on the structure–activity relationships of the novel acetylcholinesterase inhibitors with 7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one scaffold, based on our previous work and molecular modeling, a series of novel 3-aryl-6-(bromoarylmethyl)-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one derivatives were designed by molecular docking, synthesized and characterized by mass spectra, infrared spectra, proton NMR and elemental analyses. The study of AChE inhibitory activity was carried out using the Ellman colorimetric assay with huperzine-A as the positive control. Most of all target compounds exhibited more than 45% inhibition at 10 μmol/L. The preliminary structure–activity relationship was the bromine atoms and the hydroxyl group at the phenyl ring at the C6 position of the parent nucleus played significant roles in the AChE inhibitory activity of the target compounds.

Keywords: Acetylcholinesterase inhibitor; Bromination; Heterocycle; Synthesis; 7*H*-Thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one derivatives

The global population has reached seven billion on Monday, October 31, 2011, accompanied by an aging society [1]. What will life in an aging society be maybe dramatically increased the age-related diseases. Alzheimer's disease (AD) is the most common single cause of dementia in the aging society, and can be diagnosed in people over 65 years of age, although the less-prevalent early-onset Alzheimer's can occur much earlier. In 2006, there were 26.6 million sufferers worldwide. Alzheimer's is predicted to affect 1 in 85 people globally by 2050 [2].

AD is a degenerative brain syndrome characterized by a progressive decline in memory, thinking, comprehension, calculation, language, learning capacity and judgment sufficient to impair personal activities of daily living [3].

* Corresponding author.

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Reduction in the activity of the cholinergic neurons is a well-known feature of Alzheimer's disease. Acetylcholinesterase (AChE) inhibitors are employed to reduce the rate at which acetylcholine (ACh) is broken down, thereby increasing the concentration of ACh in the brain and combating the loss of ACh caused by the death of cholinergic neurons. Now, three acetylcholinesterase inhibitors, donepezil, galantamine and rivastigmine are currently approved by regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to treat the cognitive manifestations of AD [4]. Many of the side effects of the AChE inhibitors are attributable to peripheral cholinergic effects, and nausea, vomiting and diarrhea were the most frequently reported [5]. Lack and side effects of the AChE inhibitors driven us to discover some new acetylcholinesterase inhibitors.

In our previous work, 3-aryl-6-arylmethyl-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one derivatives were designed and synthesized, which exhibited inhibitory activity against AChE, and the molecular docking exhibited 6-arylmethyl-3-phenyl-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one derivatives interacted with the PAS and the CAS of AChE [6]. And 3, 6-diaryl-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-ones were also exhibited inhibitory activity against AChE, the structure activity relationship showed the inhibitory activity could be enhanced with the presence of the halogen atoms [7,8].

Bromination is a frequent modification in medicinal chemistry and can play a significant improvement on the biological activity [9].

In order to rationalize the presence of the bromine atoms influences on the inhibitory activity against AChE, and reveal the interactions between the target molecules and AChE at the molecular level and examine the structure–activity relationship for the 7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one derivatives, a series of 3-aryl-6-(bromoarylmethyl)-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one derivatives were designed and synthesized, and the inhibitory activity against AChE was evaluated.

For this study, the model was obtained from 3D structural information, of which the human AChE (*h*AChE) complex with the inhibitor (fasciculin-2), *i.e.* *h*AChE–fasciculin-2 complex (1B41), was available in the RSCB Protein Data Bank [10]. Molecular docking was carried out using Molegro Virtual Docker (MVD) [11]. Based on the Molegro Virtual Docker docking, the schematic diagrams of the interactions between *h*AChE (1B41) after eliminating the inhibitor (fasciculin-2) and 6-(3, 5-dibromo-4-hydroxybenzyl)-3-(2-hydroxy-4-methylphenyl)-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one (**5h**) were shown in Fig. 1, where the hydrogen bonds are indicated with green dotted lines. There were some hydrogen bonds between compound **5h** and His447, Tyr337, and Tyr124 of *h*AChE. The protein residue His447 was located at the CAS, the protein residue Tyr337 was located at the anionic binding site, and Tyr124 was located at the PAS. Therefore, the CAS, the anionic binding site and the PAS of *h*AChE were action sites of **5h**. The similar binding actions between the other target compounds and *h*AChE were identified by molecular docking.

In general, the target compounds (**5a**–**5h**) were obtained in satisfactory yields, and the synthetic pathways are described in Scheme 1. According to our developed procedure [6], the general synthetic procedures for the target

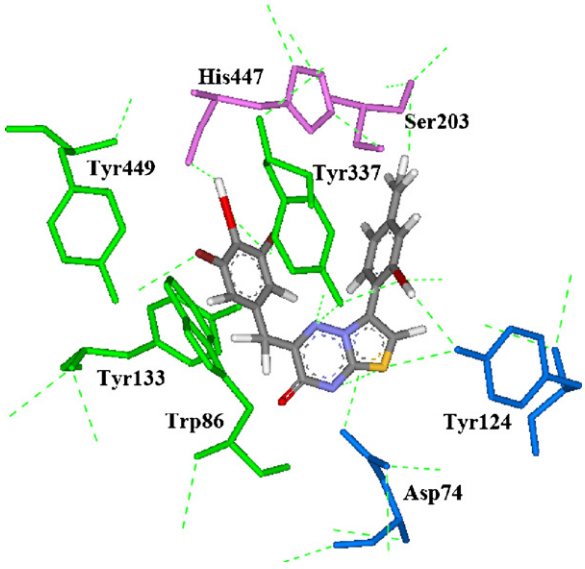
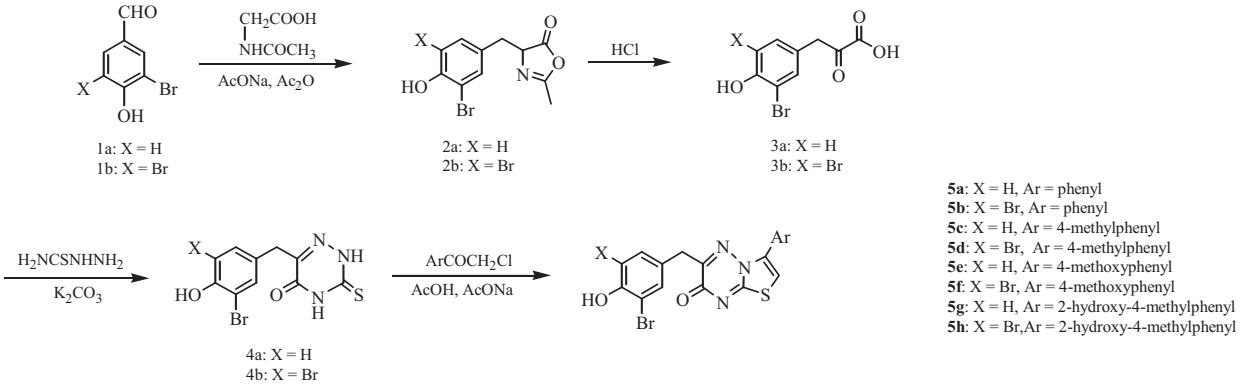


Fig. 1. Docking model of compound **5h** at the active sites of *h*AChE.



Scheme 1. The synthetic route of 3-aryl-6-(bromoarylmethyl)-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives.

Table 1
Inhibition of AChE activities by the targets at 10 μmol/L ($n = 3$).

No.	Inhibition (%)	No.	Inhibition (%)	No.	Inhibition (%)	No.	Inhibition (%)
5a	57.66	**5d**	62.13	**5g**	27.56	L1	47.62
5b	66.33	**5e**	48.09	**5h**	55.31	L2	49.04
5c	46.94	**5f**	55.88			Huperzine-A	100.00

compounds were described as follows. The raw material 4-hydroxybenzaldehyde was converted to 3-bromo-4-hydroxybenzaldehyde or 3, 5-dibromo-4-hydroxybenzaldehyde (**1**) by bromination. **1** reacted with *N*-acetylglycine, to obtain 4-(bromoarylmethylene)-2-methyl-5(4*H*)-oxazolones (**2**) in good yield. **2** were then converted into the corresponding arylpyruvic acids (**3**) by treatment with 3 mol/L hydrochloric acid. 6-(Bromoarylmethyl)-3, 4-dihydro-3-thioxo-1, 2, 4-triazin-5(2*H*)-ones (**4**) were prepared by reaction of **3** with thiosemicarbazide in the presence of alcohol and water. And 3-aryl-6-(bromoarylmethyl)-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-ones (**5**) were obtained by reaction of **4** with substituted phenacyl chlorides in the presence of acetic acid. The structures of the target compounds synthesized herein were fully confirmed by mass analysis, infrared spectra, elemental analyses and proton NMR spectroscopic data [12].

The target compounds **5a**–**5h** were initially assessed for the biological activity against *h*AChE, and the positive control drug was huperzine-A by the Ellman assay [13]. The biological activity is summarized in Table 1.

In our previous work, 6-benzyl-3-phenyl-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one (L1) and 6-benzyl-3-(4-methylphenyl)-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one (L2) exhibited inhibitory activity against AChE [6]. Compared with L1 and L2, the bromine atoms and hydroxyl group were introduced into the aromatic nucleus at the C6 position of the target compounds (**5a**–**5d**). The inhibitory activities of **5a**, **5b** and **5d** were stronger than L1 and L2, which may be due to the hydrogen bonds of the halogen atom and hydroxyl group.

Compared with **5e** and **5g**, the bromine atom was introduced into the aromatic nucleus at the C6 position of compounds **5f** and **5h**. The inhibitory activities of **5f** and **5h** were stronger than corresponding **5e** and **5g**, which were due to the hydrogen bonds of the hydroxyl group, showed in Fig. 1.

These phenomena showed that the CAS, the PAS and the anionic binding site were the main active sides of 3-aryl-6-(bromoarylmethyl)-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one derivatives. The hydroxyl group and the halogen atoms at the aromatic ring at the C6 position played significant roles in the AChE inhibitory activities of the target compounds.

In summary, 3-aryl-6-(bromoarylmethyl)-7*H*-thiazolo[3,2-*b*]-1, 2, 4-triazin-7-one derivatives would be a kind of highly active AChE inhibitors. The inhibitory activity could be enhanced with the presence of the hydroxyl groups, the bromine atoms.

Acknowledgments

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References

[1] H.E. Nasser, http://www.usatoday.com/news/world/story/2011-10-30/world-population-hits-seven-billion/51007670/1 (30.10.11).
[2] R. Brookmeyer, E. Johnson, K. Ziegler-Graham, et al. Alzheimer's Dementia 3 (2007) 186.
[3] A. Kumar, S. Dogra, Drugs Future 33 (2008) 433.
[4] C. Geula, M.M. Mesulam, Alzheimer Dis. Assoc. Disord. 2 (Suppl. 9) (2012) 23.
[5] S.J. Liu, L. Yang, X.G. Liu, et al. Lett. Drug Des. Dis. 7 (2010) 5.
[6] S.J. Liu, L. Yang, Z. Jin, et al. ARKIVOC (2009) 333.
[7] Z. Jin, L. Yang, S.J. Liu, et al. Arch. Pharm. Res. 33 (2010) 1641.
[8] Z. Jin, L. Yang, H.N. Xu, et al. Sci. China Chem. 53 (2010) 2297.
[9] C.S. Neumann, D.G. Fujimori, C.T. Walsh, Chem. Biol. 15 (2008) 99.
[10] G. Kryger, M. Harel, K. Giles, et al. Acta Crystallogr. Sect. D 56 (2000) 1385.
[11] R. Thomsen, M.H. Christensen, J. Med. Chem. 49 (2006) 3315.
[12] The characteristic data for the target compounds. **5a**: white solid, 56.4% yield, mp: 244–245 °C. ESI–MS (*m*/*z*): 411.8 $(M-H)^-$, 413.8 $(M+2-H)^-$. 1H NMR (300 MHz, DMSO-d_6): δ 3.87 (s, 2H), 6.86 (d, 1H, J = 8.1 Hz), 7.07 (dd, 1H, J1 = 2.1 Hz, J2 = 8.4 Hz), 7.40–7.48 (m, 4H), 7.52 (s, 1H), 7.62–7.65 (m, 2H), 10.07 (s, 1H). Anal. Calcd. for $C_{18}H_{12}O_2N_3SBr$ (%): C, 52.19; H, 2.92; N, 10.14. Found: C, 52.10; H, 2.87; N, 10.22. IR (KBr): υ 3150, 1602, 1508, 1473, 1417, 1355, 1287, 1265, 1236, 1214, 1132, 1047, 899, 824, 793, 765, 733, 687 cm^{-1}. **5b**: white solid, 62.0% yield, mp: 236–237 °C. ESI–MS (*m*/*z*): 489.8 $(M-H)^-$, 491.8 $(M+2-H)^-$, 493.7 $(M+4-H)^-$. 1H NMR (300 MHz, DMSO-d6): δ 3.92 (s, 2H), 7.39–7.48 (m, 5H), 7.52 (s, 1H), 7.60–7.63 (m, 2H), 9.78 (s, 1H). Anal. Calcd. for $C_{18}H_{11}O_2N_3SBr_2$ (%): C, 43.84; H, 2.25; N, 8.52. Found: C, 43.75; H, 2.21; N, 8.45. IR (KBr): δ 3122, 1635, 1577, 1556, 1480, 1409, 1385, 1278, 1241, 1168, 1148, 1120, 750, 735, 691 cm^{-1}. **5c**: white solid, 67.2% yield, mp: 246–247 °C. ESI–MS (*m*/*z*): 425.8 $(M-H)^-$, 427.8 $(M+2-H)^-$. 1H NMR (300 MHz, DMSO-d6): δ 2.35 (s, 3H), 3.86 (s, 2H), 6.86 (d, 1H, J = 8.4 Hz), 7.06 (dd, 1H, J1 = 2.1 Hz, J2 = 8.4 Hz), 7.25 (d, 2H, J = 8.4 Hz), 7.42 (d, 1H, J = 2.1 Hz), 7.46 (s, 1H), 7.51–7.54 (m, 2H), 10.08 (s, 1H). Anal. Calcd. for $C_{19}H_{14}O_2N_3SBr$ (%): C, 53.28; H, 3.29; N, 9.81. Found: C, 53.21; H, 3.32; N, 9.75. IR (KBr): δ 3044, 2769, 2712, 2640, 2575, 1613, 1505, 1468, 1423, 1299, 1254, 1222, 1186, 1118, 1043, 876, 809, 784, 746, 715, 667 cm^{-1}. **5d**: yellowish solid, 48.5% yield, mp: 255–256 °C. ESI–MS (*m*/*z*): 503.8 $(M-H)^-$, 505.8 $(M+2-H)^-$, 507.8 $(M+4-H)^-$. 1H NMR (300 MHz, DMSO-d6): δ 2.35 (s, 3H), 3.90 (s, 2H), 7.23 (d, 2H, J = 7.8 Hz), 7.46–7.50 (m, 5H), 9.78 (s, 1H). Anal. Calcd. for C19H13O2N3SBr2 (%): C, 44.99; H, 2.58; N, 8.28. Found: C, 44.91; H, 2.62; N, 8.33. IR (KBr): δ 3106, 1651, 1573, 1549, 1480, 1403, 1381, 1353, 1306, 1282, 1235, 1147, 1112, 819, 769, 734, 695 cm^{-1}. **5e**: white solid, 56.6% yield, mp: 248–250 °C. ESI–MS (*m*/*z*): 441.7 $(M-H)^-$, 443.7 $(M+2-H)^-$. 1H NMR (300 MHz, DMSO-d6): δ 10.09 (s, 1H), 7.53–7.58 (m, 2H), 7.40 (s, 1H), 7.07 (d, 2H), 6.98 (d, 1H), 6.95 (d, 1H), 6.87 (d, 1H), 3.87 (s, 2H), 3.81 (3H, s). Anal. Calcd. for $C_{19}H_{14}O_3N_3SBr$ (%): C, 51.36; H, 3.18; N, 9.46. Found: C, 51.29; H, 3.21; N, 9.41. IR (KBr): δ 3115, 2768, 2640, 2570, 1615, 1561, 1505, 1475, 1421, 1391, 1302, 1264, 1250, 1224, 1177, 1117, 1028, 875, 834, 808, 744, 716, 683 cm^{-1}. **5f**: greenish solid, 51.6% yield, mp: 247–248 °C. ESI–MS (*m*/*z*): 519.8 $(M-H)^-$, 521.8 $(M+2-H)^-$, 523.7 $(M+4-H)^-$. 1H NMR (300 MHz, DMSO-d6): δ 3.81 (s, 3H), 3.92 (s, 2H), 6.92–6.97 (m, 2H), 7.40 (s, 1H), 7.48 (s, 2H), 7.50–7.55 (m, 2H), 9.81 (1H, s). Anal. Calcd. for $C_{19}H_{13}O_3N_3SBr_2$ (%): C, 43.62; H, 2.50; N, 8.03. Found: C, 43.70; H, 2.45; N, 8.07. IR (KBr): δ 3118, 1644, 1605, 1574, 1551, 1507, 1478, 1419, 1354, 1262, 1237, 1181, 1146, 1027, 836, 769, 735, 694 cm^{-1}. **5g**: white solid, 56.9% yield, mp: 246–247 °C. ESI–MS (*m*/*z*): 444.5 $(M+H)^+$, 446.5 $(M+2+H)^+$, 468.5 $(M+Na)^+$. 1H NMR (600 MHz, DMSO-d6): δ 2.28 (s, 3H), 3.78 (s, 2H), 6.70 (d, 1H, J = 7.8 Hz), 6.79 (s, 1H), 6.83 (d, 1H, J = 8.4 Hz), 7.04 (dd, 1H, J1 = 2.4 Hz, J2 = 8.4 Hz), 7.22 (d, 1H, J = 7.8 Hz), 7.32 (s, 1H), 7.36 (d, 1H, J = 2.4 Hz), 9.82 (s, 1H), 10.07 (s, 1H). Anal. Calcd. for $C_{19}H_{14}O_3$ N3SBr (%): C, 51.36; H, 3.18; N, 9.46. Found: C, 51.43; H, 3.21; N, 9.40. IR (KBr): δ 3361, 1609, 1498, 1463, 1417, 1296, 1255, 1121, 1044, 964, 810, 767, 720, 675 cm^{-1}. **5h**: white solid, 28.6% yield, mp: 261–262 °C. ESI–MS (*m*/*z*): 520.0 $(M-H)^-$, 521.9 $(M+2-H)^-$, 523.8 $(M+4-H)^-$. 1H NMR (300 MHz, DMSO-d6): δ 2.28 (s, 3H), 3.82 (s, 2H), 6.66–6.78 (m, 2H), 7.19 (d, 1H, J = 7.8 Hz), 7.33 (s, 1H), 7.40 (s, 2H), 9.74 (s, 1H), 9.75 (s, 1H). Anal. Calcd. for $C_{19}H_{13}O_3N_3SBr_2$ (%): C, 43.62; H, 2.50; N, 8.03. Found: C, 43.53; H, 2.45; N, 8.07. IR (KBr): δ 3497, 3318, 1611, 1580, 1416, 1247, 1158, 1128, 1047, 872, 808, 770, 733, 713, 682 cm^{-1}.
[13] P. Elsinghorst, C. Tanarro, M. Gutschow, J. Med. Chem. 49 (2006) 7540.