Design, synthesis, and biological evaluation of 7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one derivatives as novel acetylcholinesterase inhibitors

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Abstract

The docking study on a novel series of 7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one derivatives with acetylcholinesterase from *Torpedo californica* has demonstrated that the ligands bind to the dual-site of the enzyme. The synthesis and characterization of 7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one derivatives was described. The crystal structure of 6-benzyl-3-{4-[2-(1-piperidinyl)-2-oxoethoxy]phenyl}thiazolo[3,2-*b*]-1,2,4-triazin-7-one has been characterized by X-ray diffraction. All target compounds have been screened for their efficacy as acetylcholinesterase inhibitor. The study of AChE inhibitory activity was carried out using the Ellman colorimetric assay with huperzine-A as a reference against targets. Most of the target compounds exhibit more than 50% inhibition at 10 μM. Some derivatives showed good inhibition against AChE. The preliminary structure-activity relationships were discussed.

Keywords: Acetylcholinesterase, inhibitors, synthesis, characterization heterocycles, crystal structure, docking, thiazole, 1,2,4-triazine

Introduction

Alzheimer's disease (AD), a neurodegenerative disease affecting the elderly population throughout the world, is clinically characterized by an impairment of the cognitive function. The most frequently prescribed anti-Alzheimer's drugs are the acetylcholinesterase (AChE) inhibitors, which promote memory function and delay the cognitive decline without altering the underlying pathology.[1-3] However, the clinical usefulness of marketed AChE inhibitors often cause some adverse effects, for example, donepezil can lead to diarrhea, and rivastigmine can cause vomiting, therefore, it is necessary and urgent to find more effective AChE inhibitors to treat AD.

In order to gain insight into the recognition between the AChE and the ligands, docking simulations were done on the three-dimensional structures of AChE. The three-dimensional structure of AChE from *Torpedo californica* has been determined by x-ray analysis in 1991.[4] AChE is an α/β serine hydrolase consisting of 537 residues with a 12-stranded mixed β sheet surrounded by 14 α helices. The active site is striking in that it is a deep, narrow tunnel approximately 20 Å long. Fourteen highly conserved aromatic residues line a substantial portion of the surface of the gorge. This tunnel penetrates halfway into the enzyme and widens out at the end. At the bottom of the gorge, there is the catalytic active site (CAS), which is assigned to the Ser-His-Glu catalytic triad (Ser200-His440-Glu327). In the middle of the gorge, there is the anionic binding locus, in which the quaternary ammonium functionality of many ligands interacts with the side chains of Trp84, Glu199, and Phe330, and Tyr130. At the entrance to the aromatic gorge, there is the peripheral anionic site (PAS) including Tyr70, Asp72, Tyr121, Glu278, Tyr334 and Trp279 residues.[4-5]

Since three-dimensional structure information of the complex structure of ligands and AChE is available, a docking model built with AutoDock 4.0 software was generated. In previous research in this field,[6] a series of 5*H*-thiazolo[3,2-*a*]pyrimidine derivatives have been designed and synthesized as acetylcholinesterase inhibitors, but these derivatives bind difficultly to the catalytic sites of AChE due to big stereospecific blockade. In order to find some novel AChE inhibitors which could simultaneously bind to catalytic and peripheral sites, active substructures of 5*H*-thiazolo[3,2-*a*]pyrimidine derivatives and some clinical drugs were integrated, using the principles of bioisosterism, hybridization, and structural optimization. A novel series of 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives were thus designed on the basis of docking results.

Results and Discussion

Docking studies

There are a lot of complex structures of *Torpedo californica* AChE (*Tc*AChE) and mouse AChE with inhibitors determined experimentally, such as donepezil (PDB code: 1EVE),[7] tacrine (PDB code: 1ACJ),[8] gallamine (PDB code: 1N5M),[9] huperzine A (PDB code: 1VOT),[10] galanthamine (PDB code: 1DX6),[11] and obidoxime (PDB code: 2GYW).[12] All these structures significantly enhance our understanding of the structure of AChE.

Comparison of the three-dimensional structures of human AChE (1B41) and *Tc*AChE (1EVE) has revealed a high degree of similarity, especially with regard to the active sites. The biggest difference between human AChE and *Tc*AChE in the binding site was that Phe330 of *Tc*AChE was replaced by Tyr337 of human AChE, therefore Phe330 was mutated to Tyr337, which might reflect a real difference between human AChE and *Tc*AChE. Another difference is the sequence number of residues (Figure 1). Structure comparison was prepared by the structure editing tools DockPrep in UCSF Chimera (UCSF Chimera, Version 1, USA).

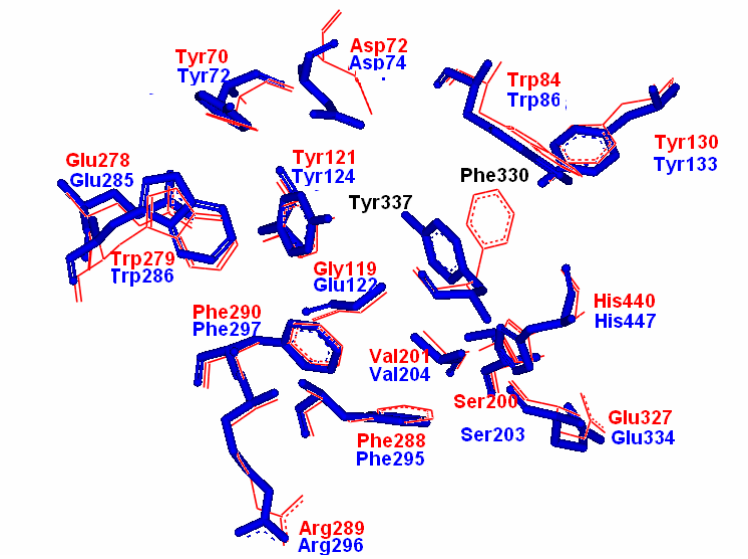


Figure 1. Superimpose result between human AChE (blue) and *Tc*AChE (red).

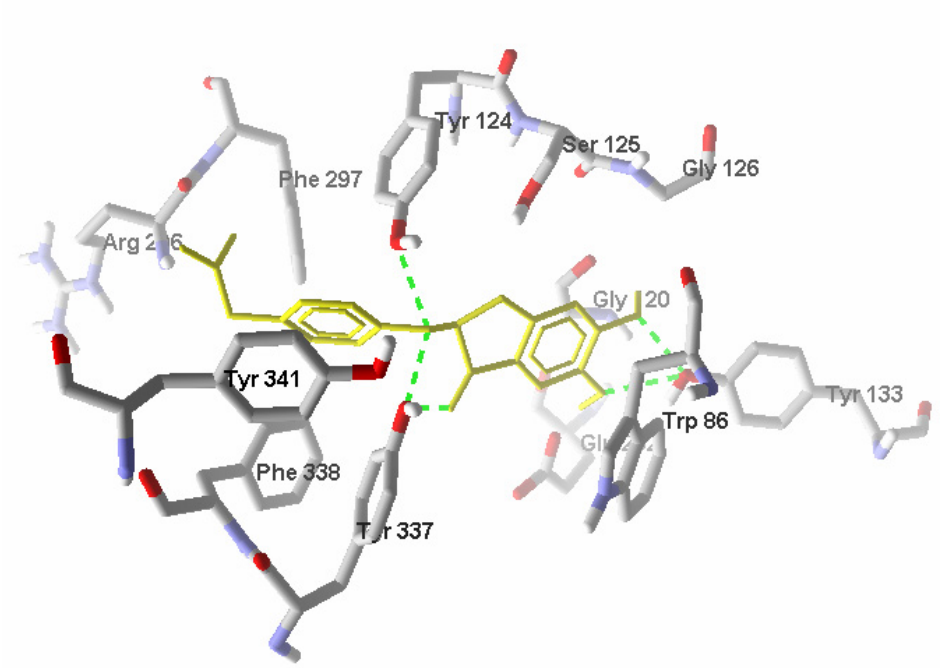


Figure 2. Docking model of donepezil in the active site of human AChE.

Until now, the structure of the complex of human AChE and inhibitors is not available, so donepezil was docked into human AChE. The proposed binding model of donepezil with the key residues in the gorge site is shown in Figure 2. The binding model suggests that donepezil is bound near the bottom of the gorge, and it shows parallel π-π stacking against the six-membered ring of Trp86 indole. The piperidinic nitrogen of the ligand, which contains a positive charge,

complexes by cation-π interaction with the phenyl group of Tyr341 of the enzymatic cavity. The donepezil was located as a linker and forms three hydrogen bonds with Tyr124, Tyr133 and Tyr337.

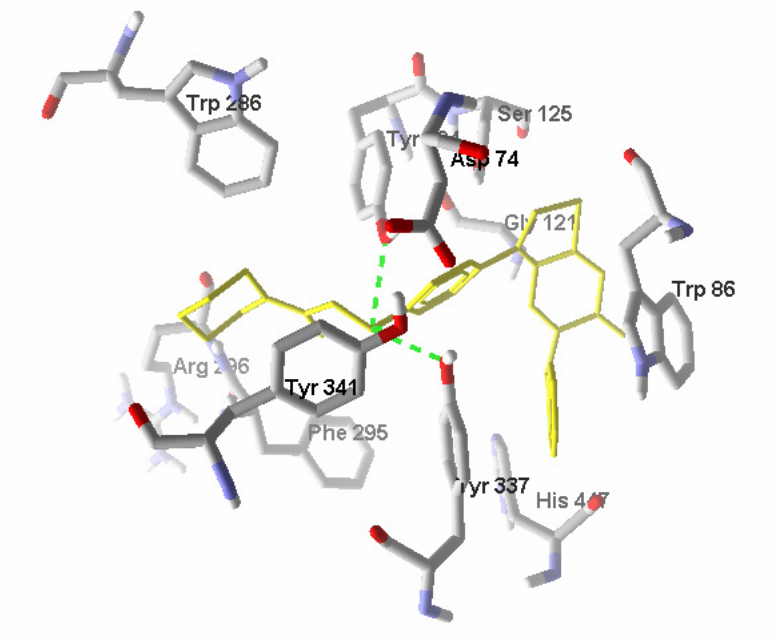


Figure 3. Docking model of **6d** in the active site of human AChE.

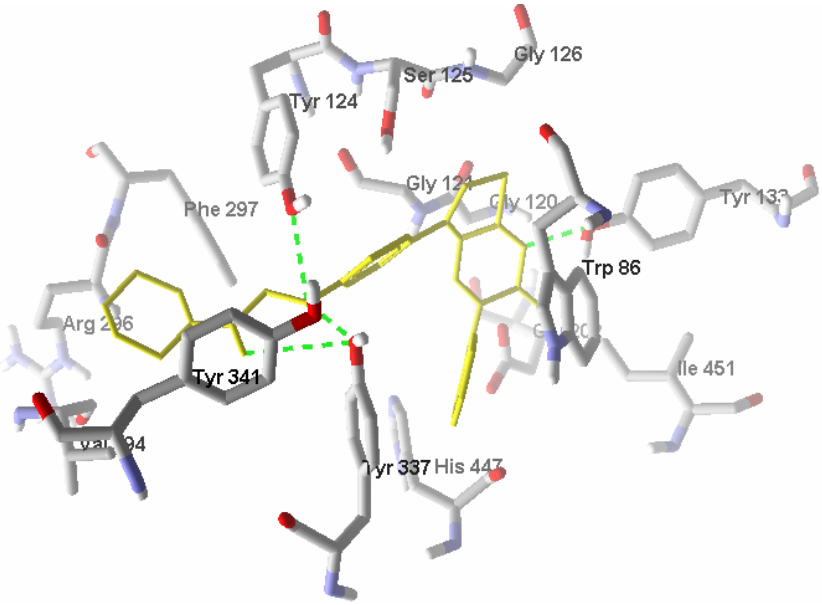


Figure 4. Docking model of **6e** in the active site of human AChE.

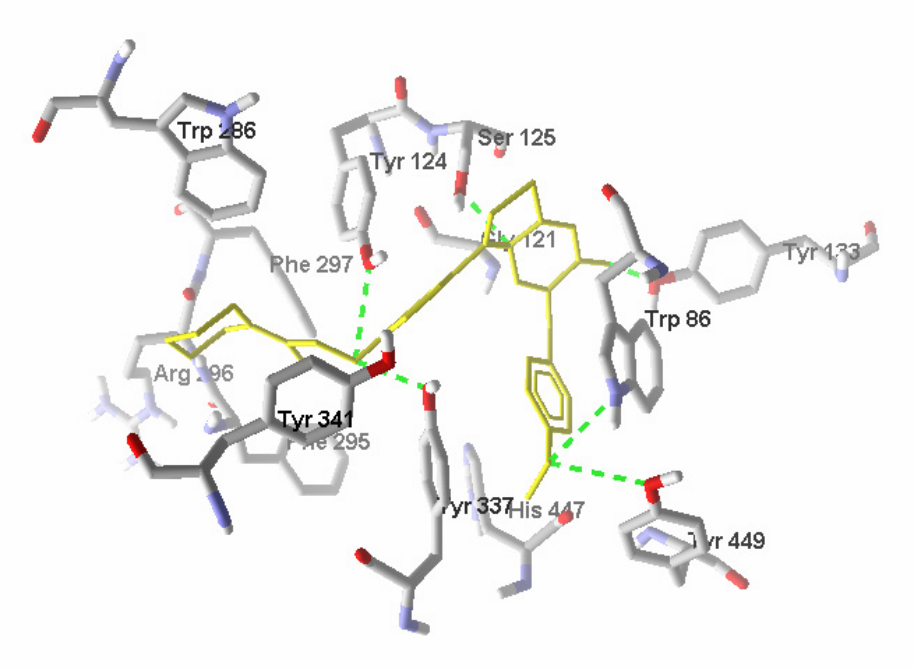
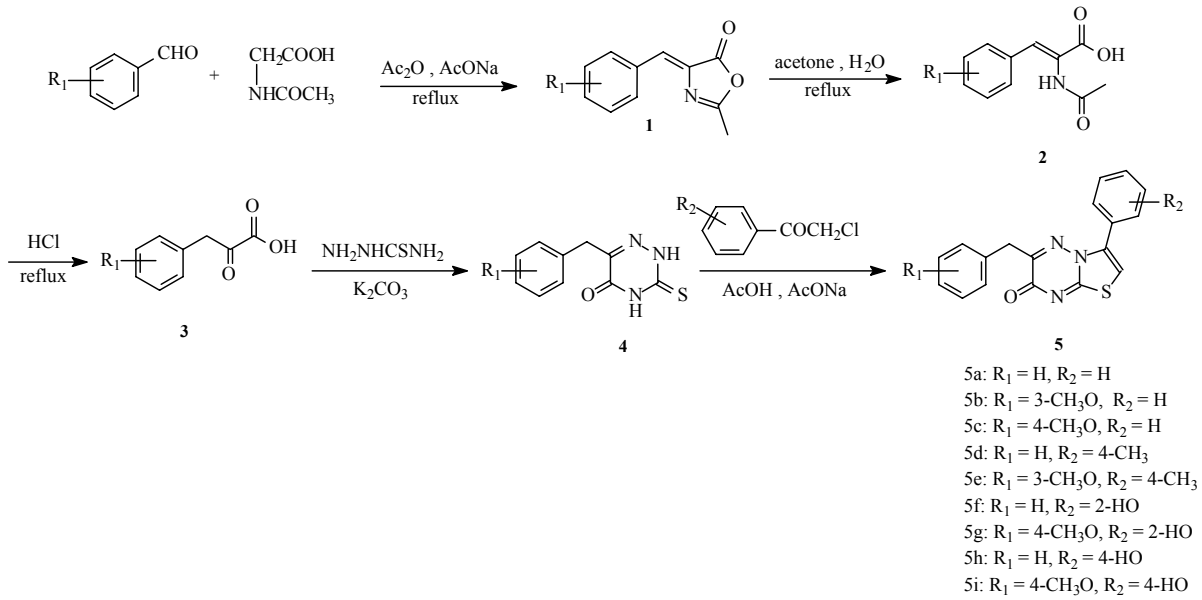


Figure 5. Docking model of **6f** in the active site of human AChE.

Based on the docking, all the complexes between the enzymes and the target molecules suggest this kind of interactions. The inhibitors are depicted as sticks in Figure 3, 4 and 5. They are located in the active site of AChE so as to maximize the favorable contacts. The hydrogen bonds and the van der Waals forces are the main features of the interactions of 6-benzyl-3-{4-[2-(1-piperidinyl)-2-oxoethoxy]phenyl}thiazolo[3,2-b]-1,2,4-triazin-7-one **6d**, 6-benzyl-3-{4-[2-(4-morpholinyl)-2-oxoethoxy]phenyl}thiazolo[3,2-b]-1,2,4-triazin-7-one **6e** and 6-(4-methoxybenzyl)-3-{4-[2-(1-piperidinyl)-2-oxoethoxy]phenyl}thiazolo [3,2-b]- 1,2,4-triazin-7-one **6f** with the Tyr124, Tyr133 and Tyr337 of AChE. However, the π-π interaction plays an important role, giving the ligand-AChE complexes high stability and at the same time improving the recognition process between this enzyme and the target molecules. The π-π interaction is formed between the aromatic ring of the target molecules and the aromatic ring from the Trp86 of AChE.

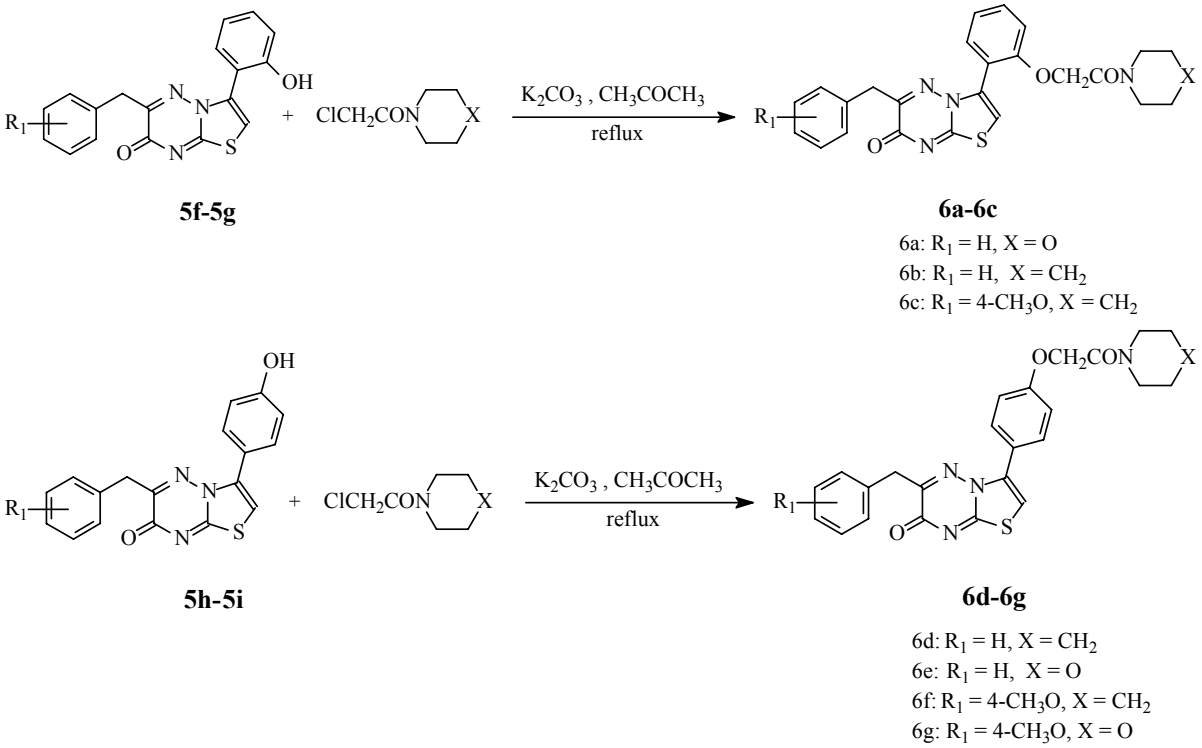
Chemistry

The target compounds **5a-5e** were obtained in satisfactory yields, and the synthetic pathways are described in Schemes 1.



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

The target compound 3-substituted aryl 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives **6a-6g** could be obtained with ordinary Williamson reaction, illustrated in Scheme 2.



Scheme 2. The synthesis of 3-substituted 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives.

A typical synthesis was developed for our target compounds described in Scheme 1, the aromatic aldehydes were easily converted to 4-arylmethylideneoxazol-5(4*H*)-ones **1** by cyclization in good yield. Subsequently, the hydrolysis of **1** in the acetone aqueous solution resulted in α-(acetylamino)cinnamic acids **2**, in practice, it was found that addition of a small amount of sodium acetate exhibited a remarkable enhancement in hydrolyzing **1** to **2**, and the yields of hydrolysis ranged from 85 to 92%. **2** were then converted into the corresponding aryl pyruvic acids (**3**) by treatment with 1 $mol.L^{-1}$ hydrochloric acid aqueous solution. The reaction of **3** with thiosemicarbazone gave the cyclized products 3-thioxo-1,2,4-triazin-5(2H)-ones **4**. 6-Arylmethyl-3-aryl-7*H*-thiazolo[3,2-b]-1,2,4-triazin-7 -one derivatives **5** were prepared by reaction of **4** with substituted phenacyl chlorides in the presence of acetic acid. The target compounds **6** could be obtained with ordinary Williamson reaction in Scheme 2.[13-15]

The chemical structures of all novel compounds synthesized herein were fully characterized by mass analysis, infrared spectra, and proton NMR spectroscopic data reported in the experimental section.

Crystallography

The final X-ray measurement has been carried out by using an BRUKER SMART 1000CCD diffractometer with graphite monochromated MoKa (λ=0.71073Å) radiation. Intensity data were collected in the range of 1.77-25.01° using a moving crystal, moving detector (ω/2θ) scan technique. A total of 3858 independent 'observed' reflections were used in structural analysis. The atomic scattering factors were taken from the International Tables for X-ray Crystallography. All non-hydrogen atoms were refined with anisotropy thermal parameters and the hydrogen atoms were subjected to isotropic refinement. The structure was solved by direct methods using SHELXL-97 refined anisotropically. A summary of the crystal data and structure refinement parameters are collected in Table 1. The structure of **6d** is shown in Figure 6.

In the stable crystal structures, the intermolecular interaction is very important. An X-ray diffraction study demonstrated that crystalline **6d** was packed as dimers, where two molecules are linked to each other by O-H and N-H hydrogen bonds through a crystallographic center of symmetry (Figure 7).

Complete crystallographic data for the structure of target compound **6d** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as a CIF file (CCDC 732102). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336-033, e-mail: deposit@ccdc.cam.ac.uk).

Table 1. Crystal data and details of the structure determination of **6d**

Identification code	**6d**
Empirical formula	$C_{25}H_{24}N_4O_3S$
Formula weight	460.54
Temperature	113(2) K
Wavelength	0.71073 Å
Crystal system, space group	Triclinic, P-1
Unit cell dimensions	a = 9.6575(19) Å alpha = 74.94 (3) deg b = 10.099(2) Å beta = 82.67 (3) deg c = 11.970(2) Å gamma = 81.20 (3) deg.
Volume	1109.4(4) A^3
Z, Calculated density	2, 1.379 Mg/m^3
Absorption coefficient	0.182 mm^{-1}
F(000)	484
Crystal size	0.22 x 0.18 x 0.10 mm
Theta range for data collection	1.77 to 25.01 deg.
Limiting indices	$-11\leq h\leq 10$, $-12\leq k\leq 11$, $-14\leq l\leq 12$
Reflections collected / unique	6407 / 3858 [R(int) = 0.0406]
Completeness to theta = 25.02	98.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9820 and 0.9610
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3858 / 0 / 298
Goodness-of-fit on F^2	1.043
Final R indices [I>2sigma(I)]	R1 = 0.0487, wR2 = 0.1307
R indices (all data)	R1 = 0.0648, wR2 = 0.1388
Largest diff. peak and hole	0.527 and -0.338 $e.A^{-3}$

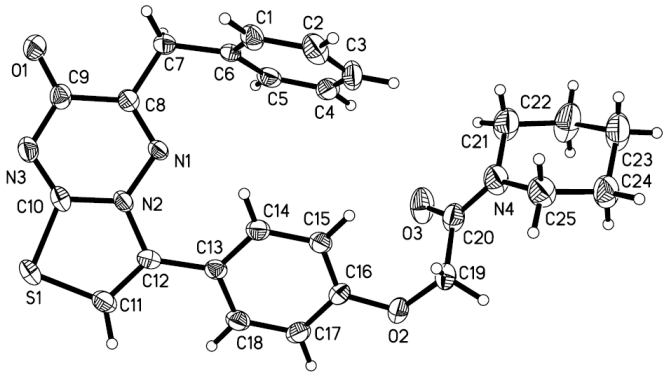


Figure 6. The structure of the **6d**, showing 50% probability displacement ellipsoids and the atom-numbering scheme.

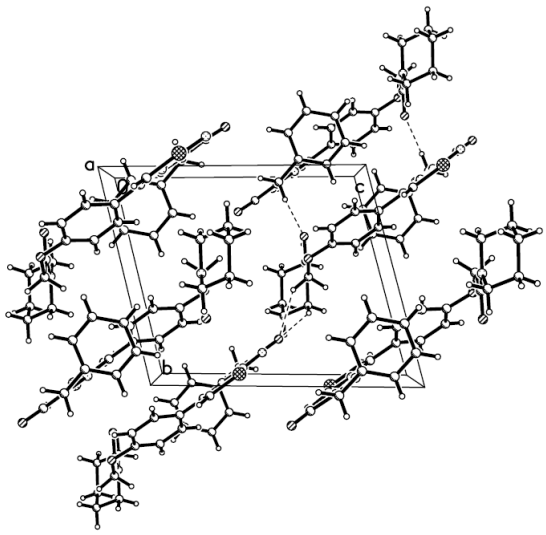


Figure 7. The molecular packing of **6d**, the molecular packing viewed along the a axis. Intermolecular C-H...O and C-H...N interactions are shown as dashed lines.

Inhibition of AChE

Table 2 illustrates the biological activity of the target compounds against human AChE, in comparison to the huperzine-A.

Table 2. Inhibition of AChE activities by the targets at 10 μM (n=2)

No.	Inhibition (%)	No.	Inhibition (%)	No.	Inhibition (%)
5a	47.62	5b	55.34	5c	49.27
5d	49.04	5e	53.83	5f	63.63
5g	64.36	5h	62.20	5i	62.43
6a	29.11	6b	48.29	6c	42.41
6d	67.81	6e	31.46	6f	71.09
6g	45.13				
huperzine-A (10 μM)	100				

Human AChE (Sigma C-1682) 0.5unit was used. Each performed in double. The incubation time was 20 min, with gentle shake.

The compounds **5f-5i** showed moderate activity, this was probably due to the presence of a hydroxy group, which would be able to form a hydrogen bond with the AChE receptor. While the compounds **6a**, **6e** and **6g** with 2-(4-morpholinyl)-2-oxoethoxy group led to a huge decrease in activity than that bearing other groups on the phenyl ring at the C3 position of the parent nucleus (the compounds **6d** and **6f**), indicating that the binding of some compounds to the active site in the enzyme could be limited by hydrophobic interactions.

The compounds **6d** and **6f** are more potent than **6b** and **6c**, the reason is that probably compounds **6b** and **6c** both have big substituents at the *ortho* position on the phenyl ring at C3 position of the parent nucleus, resulting in a larger steric hindrance which limits the access of the target molecules to the enzyme-binding site.

These phenomena show that the steric effects and hydrophobic effect might play a significant role in influencing the biological activity of 6-arylmethyl-3-aryl- 7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one derivatives.

Conclusions

In conclusion, 6-arylmethyl-3-aryl-7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one derivatives represent a class with a novel scaffold for highly active and selective AChE inhibitors. Further efforts aiming at developing potent AChE inhibitors based on modification of the compounds **5** and **6** will be continued in our laboratory.

Experimental Section

Docking studies

Preparation of the enzyme

The coordinates for the enzyme were those deposited in the Protein Data Bank for the human acetylcholinesterase (1B41) after eliminating the inhibitor (Fasciculin-2) and water molecules. The missing residues were built and the polar hydrogen atoms to amino acid residues were added. This work was completed by SPDB Viewer. (GSK SPDB Viewer, Version3.7, Swiss). Kollman charges were assigned to all atoms of the enzyme in AutoDock.

Preparation of ligands

The structures of 7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one derivatives were sketched by ISIS/draw 2.1 and saved in MOL2 format. Gasteiger-Hückel charges were add to the target molecules.

Molecular docking

For docking studies, the latest version of AutoDock (4.0) was chosen because its algorithm allows full flexibility of small ligands. It has been shown that it successfully reproduces many crystal structure complexes and includes an empirical binding free energy evaluation. Docking to AChE was carried out using the hybrid Lamarckian Genetic Algorithm, with an initial population of 100 randomly placed individuals and a maximum number of 1.0×10^7 energy evaluations. The dimension of the active site box was set at 60 Å ×60 Å ×60 Å, which ensured an appropriate size of the accessible space. Active site box centre was set at the centre of active gorge of AChE. Resulting docked orientations within a root-mean square deviation of 1.0 Å were clustered

together. The lowest energy cluster returned by AutoDock for each compound was used for further analysis. All other parameters were maintained at their default settings.[16]

Chemistry

Melting points were determined on Koffler hot-plate apparatus and are uncorrected. ^{1}H-NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 on a Bruker spectrometers instrument operating at 300 MHz or 600 MHz. The mass spectra (MS) were obtained by electronic impact (EI) at 70 eV in an Agilent spectrometer (with direct insertion probe) or by electrospray (ESI) in a Waters spectrometer. The IR spectra were obtained using a Bruker AFS55 spectrometer. The C, H and N analyses were performed on a Perkin Elmer 240C elemental analyzer.

Synthesis of 4-(arylmethylene)-2-methyl-5(4H)-oxazolones (1). Aromatic aldehydes (100 mmol), N-acylglycine (10.7 g, 120 mmol), sodium acetate (51 g, 500 mmol) were heated in acetic anhydride for 7h. The solution was cooled and filtered. The resulting yellow solid were collected and dried to give 4-(arylmethylene)-2-methyl-5(*4H*)-oxazolone as a solid.

4-(Phenylmethylene)-2-methyl-5(*4H*)-oxazolone (1a). Yellow solid, 100% yield; mp: 149-151°C (lit.[17] mp: 151-152 °C).

4-[(3-Methoxyphenyl)methylene]-2-methyl-5(*4H*)-oxazolone (1b). Yellow solid, 100% yield; mp: 123-124°C.

4-[(4-Methoxyphenyl)methylene]-2-methyl-5(*4H*)-oxazolone (1c). Yellow solid, 100% yield; mp: 156-158°C (lit.[18] mp: 158°C).

Synthesis of 2-acetamido-3-aryl-2-propenoic acids (2)

To a mixture of 4-(arylmethylene)-2-methyl-5(*4H*)-oxazolone **1** (10.85 g, 50 mmol) and sodium acetate, acetone (85 mL) and H_2O (70 mL) were added. The mixture was then refluxed for 10 h with stirring. After cooling, then the solvent was evaporated to give 2-acetamido-3-aryl-2-propenoic acid as a solid.

2-Acetamido-3-phenyl-2-propenoic acid (2a). Yellow solid, 89% yield; mp: 193-194°C (lit[17] mp: 192-193°C).

2-Acetamido-3-(3-methoxyphenyl)-2-propenoic acid (2b). Yellow solid, 90% yield; mp: 187-188°C.

2-Acetamido-3-(4-methoxyphenyl)-2-propenoic acid (2c). Yellow solid, 93% yield; mp: 219-221°C (lit.[19] mp: 220-221°C).

Synthesis of aryl pyruvic acids (3)

The compound **2** (10 mmol) was added to 60 mL 1mol/L HCl aqueous solution. The solution was refluxed for 7h. The solution was cooled and extract with EtOAc (3×20 mL). The organic layer was dried and evaporated to give aryl pyruvic acid as a solid.

Phenylpyruvic acid (3a). Yellow solid, 89% yield; mp: 153°C(dec) (lit.[20] mp: 153-155°C (dec).
3-Methoxyphenylpyruvic acid (3b). Yellow solid, 87% yield; mp: 138-141°C (lit.[21] mp: 143-145°C).
4-Methoxyphenylpyruvic acid (3c). Yellow solid, 85% yield; mp: 184-185°C (lit.[22] mp: 184-186°C).

Synthesis of 6-(arylmethyl)-3,4-dihydro-3-thioxo-1,2,4-triazin-5(*2H*)-ones (4)
A mixture of aryl pyruvic acid (10 mmol), thiosemicarbazone (10 mmol), potassium carbonate and water (25 mL) was refluxed for 2 h, cooled to room temperature and treated with HCl until effervescence to go to completion, the solid was collected and crystallized from ethanol.
6-(Phenylmethyl)-3,4-dihydro-3-thioxo-1,2,4-triazin-5(*2H*)-one (4a). White solid, 93% yield; mp: 185-187°C (lit.[14] mp: 188°C).
6-(3-Methoxybenzyl)-3-thioxo-1,2,4-triazin-5(*2H*)-ones (4b). This compound was obtained as a white solid, 91% yield; mp:137-138 °C (lit.[23] mp: 140-141°C).
6-[(4-Methoxyphenyl)methyl]-3,4-dihydro-3-thioxo-1,2,4-triazin-5(*2H*)-one (4c). White solid, 92% yield; mp: 166-167°C (lit.[14] mp: 167-168°C).

Synthesis of 6-arylmethyl-3-aryl-7*H*-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(2H)-ones (10 mmol), substituted phenacyl chloride (10 mmol) and AcONa/AcOH (2 g/20 mL) was heated under refluxed for 8 h. The solution was cooled to room temperature and filtered. The solid was collected and crystallized from ethanol.
6-Benzyl-3-phenyl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (5a). White crystals, 78% yield; mp: 179-181°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.43~7.55 (5H, m), 7.26~7.33 (5H, m), 6.82 (1H, s), 4.13 (2H, s); MS (m/z): 44, 63, 77, 90, 102, 117, 134, 136, 159, 174, 187, 202, 206, 228, 242, 265, 291, 319, 335(M)$^+$; IR (KBr): υ 3066, 1638, 1571, 1482, 1384, 1292, 1206, 1188, 1152, 1113, 1052, 1029, 805, 786, 754, 709 cm^{-1}; Anal. calcd for $C_{18}H_{13}N_3OS$: C, 67.69; H, 4.10; N 13.16. Found: C, 67.56; H, 4.02; N 13.03.
6-(3-Methoxybenzyl)-3-phenyl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (5b). White crystals, 68% yield; mp: 175-177°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.43~7.56 (5H, m), 7.22 (1H, t), 6.92 (2H, t), 6.80 (2H, d), 4.10 (2H, s), 3.76 (3H, s); MS (m/z): 51, 63, 77, 89, 104, 116, 134, 147, 159, 174, 202, 248, 321, 334, 349(M)$^+$; IR (KBr): υ 3111, 3058, 2959, 1632, 1602, 1576, 1479, 1408, 1385, 1357, 1319, 1294, 1260, 1185, 1166, 1114, 1033, 883, 786, 758, 708 cm^{-1}; ^{13}C-NMR (600 MHz, DMSO-d_6): δ 164.4, 159.1, 158.2, 153.0, 137.4, 137.2, 129.6, 129.1, 128.6, 128.2, 127.6, 121.6, 115.0, 111.9, 106.1, 54.8, 36.4; Anal. calcd for $C_{19}H_{15}N_3O_2S$: C, 65.31; H, 4.33; N , 12.03. Found: C, 65.22; H, 4.24; N , 11.89.
6-(4-Methoxybenzyl)-3-phenyl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (5c). White crystals, 84% yield; mp: 179-181°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42~7.56 (5H, m), 7.27 (2H, d), 6.83 (3H, t), 4.01 (2H, s), 3.79 (3H, s); MS (m/z): 51, 63, 77, 89, 104, 121, 134, 146, 159, 174, 186, 202, 218, 246, 263, 288, 306, 321, 334, 349(M)$^+$; IR (KBr): υ 3073, 2835, 1633, 1567, 1546,

1513, 1483, 1418, 1385, 1358, 1301, 1250, 1220, 1180, 1119, 1033, 920, 878, 813, 763 cm^{-1}; Anal. calcd for $C_{19}H_{15}N_3O_2S$: C, 65.31; H, 4.33; N, 12.03. Found: C, 65.28; H, 4.26; N, 11.91.

6-Benzyl-3-(4-methylphenyl)-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (5d). White crystals, 65% yield; mp: 229-232°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42~7.45 (2H, d, *J* = 8.2Hz), 7.22~7.38 (7H, m), 6.77 (1H, s), 4.12 (2H, s), 2.33 (3H, s); MS (m/z): 51, 65, 77, 91, 104, 115, 130, 147, 159, 190, 202, 216, 242, 262, 279, 304, 329, 333$(M)^+$; IR (KBr): υ 3111, 1639, 1577, 1486, 1384, 1358, 1189, 1113, 819, 770, 750, 707 cm^{-1}; Anal. calcd for $C_{19}H_{15}N_3OS$: C, 68.45; H, 4.53; N, 12.60. Found: C, 68.31; H, 4.46; N, 12.50.

6-(3-Methoxybenzyl)-3-(4-methylphenyl)-7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one (5e). White crystals, 77% yield; mp: 217-220°C; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.45 (2H, d), 7.20~7.25 (3H, m), 6.94 (1H, d), 6.91 (1H, s), 6.80 (1H, d), 6.77 (1H, s), 4.10 (2H, s), 3.76 (3H, s), 2.43 (3H, s) ; ESI-MS (m/z): 364.0 $(M+H)^+$, 748.9 $(2M+Na)^+$; IR (KBr): υ 3110, 2927, 1636, 1613, 1579, 1484, 1454, 1385, 1356, 1321, 1296, 1260, 1187, 1154, 1114, 1049, 890, 818, 782, 750, 709 cm^{-1}; Anal. calcd for $C_{20}H_{17}N_3O_2S$: C, 66.10; H, 4.71; N, 11.56. Found: C, 66.00; H, 4.66; N, 11.42.

6-Benzyl-3-(2-hydroxyphenyl)-7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one (5f). White crystals, 73% yield; mp: 227-230°C; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.00 (1H, s), 7.38 (1H, s), 7.18~7.35 (7H, m), 6.99 (1H, d), 6.88 (1H, t), 3.88 (2H, s); MS (m/z): 51, 65, 77, 90, 103, 121, 132, 149, 150, 168, 185, 218, 220, 335$(M)^+$; IR (KBr): υ 3219, 1697, 1646, 1618, 1482, 1384, 1350, 1296, 1258, 1240, 1181, 1157, 1108, 1073, 1028, 929, 861, 838, 752, 703 cm^{-1}; Anal. calcd for $C_{18}H_{13}N_3O_2S$: C, 64.46; H, 3.91; N, 12.53. Found: C, 64.32; H, 3.84; N, 12.48.

6-(4-Methoxybenzyl)-3-(2-hydroxyphenyl)-7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one (5g). White crystals, 73% yield; mp: 218-219°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.99 (1H, s), 7.37 (1H, s), 7.32~7.58 (2H, m), 7.18 (2H, d, *J* = 8.7Hz), 6.99 (1H, d), 6.89 (1H, t), 6.81 (2H, d, *J* = 8.7Hz), 3.80 (2H, s), 3.72 (3H, s); MS (m/z): 51, 63, 77, 89, 104, 121, 132, 147, 159, 174, 192, 193, 218, 231, 282, 350, 365$(M)^+$; IR (KBr): υ 3102, 2965, 1663, 1621, 1571, 1513, 1486, 1384, 1294, 1267, 1243, 1180, 1127, 1107, 1047, 1025, 943, 921, 879, 842, 803, 773 cm^{-1}; Anal. calcd for $C_{19}H_{15}N_3O_3S$: C, 62.45; H, 4.14; N, 11.50. Found: C, 62.32; H, 4.10; N, 11.40.

6-Benzyl-3-(4-hydroxyphenyl)-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (5h). White crystals, 85% yield; mp: 245-247°C; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.92 (1H, s), 7.46 (2H, d, *J* = 8.4Hz), 7.33 (1H, s), 7.23~7.30 (5H, m), 6.78 (2H, d, *J* = 8.4Hz), 3.98 (2H, s); ESI-MS (m/z): 335.9 $(M+H)^+$; IR (KBr): υ 3229, 3035, 2941, 1604, 1557, 1511, 1472, 1440, 1386, 1345, 1299, 1275, 1229, 1206, 1177, 1127, 1050, 928, 842, 805, 776, 757, 742, 722, 701 cm^{-1}; ^{13}C-NMR (600 MHz, DMSO-d_6): δ 165.1, 159.4, 158.9, 153.9, 138.2, 136.8, 130.9, 130.2, 128.9, 127.1, 119.1, 115.8, 104.5, 37.3; Anal. calcd for $C_{18}H_{13}N_3O_2S$: C, 64.46; H, 3.91; N, 12.53. Found: C, 64.33; H, 3.86; N, 12.46.

6-(4-Methoxybenzyl)-3-(4-hydroxyphenyl)-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (5i). White crystals, 63% yield; mp: 188-189°C; ^{1}H-NMR (600 MHz, DMSO): δ 10.0 (1H, s), 7.48 (2H, d, *J* = 8.4Hz), 7.33 (1H, s), 7.21 (2H, d, *J* = 8.4Hz), 6.87 (2H, d, *J* = 8.4Hz), 6.80 (2H, d, *J* = 8.4Hz), 3.91 (2H, s), 3.76 (3H, s); ESI-MS (m/z): 366.0 $(M+H)^+$, 752.8 $(2M+Na)^+$; IR (KBr): υ

3119, 3035, 2941, 1719, 1610, 1511, 1476, 1385, 1279, 1247, 1176, 1123, 1029, 838, 752 cm^{-1}; Anal. calcd for $C_{19}H_{15}N_3O_3S$: C, 62.45; H, 4.14; N, 11.50. Found: C, 62.30; H, 4.08; N, 11.39.

Synthesis of 6-arylmethyl-3-aryl-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-ones 6

A mixture of 6-arylmethyl-3-aryl-7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one (10 mmol), *N*-(chloroacetyl)morpholine or *N*-(chloroacetyl)piperidine (10 mmol), potassium carbonate (50 mmol), KI (1 mmol) and acetone (25 ml) was heated under refluxed for 24h. The solvent was evaporated, a residual solid was given and recrystallized form EtOH.

6-Benzyl-3-{2-[2-(4-morpholinyl)-2-oxoethoxy]phenyl}-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (6a). White crystals, 56% yield; mp: 212-214°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.47 (1H, t), 7.37 (1H, d), 7.21~7.26 (5H, m), 7.08 (1H, t), 6.93 (2H, t), 4.47 (2H, s), 4.05 (2H, s), 4.58 (2H, br), 3.50 (2H, br), 3.27 (2H, br); ESI-MS (m/z): 463.1440 $(M+H)^+$, 485.1260 $(M+Na)^+$, 947.2622 $(2M+Na)^+$; IR (KBr): υ 3084, 2981, 2919, 2866, 1671, 1644, 1566, 1482, 1442, 1383, 1366, 1337, 1298, 1272, 1241, 1167, 1124, 1064, 1033, 957, 863, 845, 780, 743 cm^{-1}; Anal. calcd for $C_{24}H_{22}N_4O_4S$: C, 62.32; H, 4.79; N, 12.11. Found: C, 62.20; H, 4.71; N, 12.01.

6-Benzyl-3-{2-[2-(1-piperidinyl)-2-oxoethoxy]phenyl}-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (6b). White crystals, 52% yield; mp: 178-180°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.45 (1H, t), 7.37 (1H, d), 7.21~7.35 (5H, m), 7.05 (1H, t), 7.03 (1H, s), 6.94 (1H, t), 4.48 (2H, s), 4.05 (2H, s), 3.49 (2H, t), 3.20 (2H, t), 1.59 (2H, br), 1.49 (2H, br), 1.39 (2H, br); ESI-MS (m/z): 461.0 $(M+H)^+$; IR (KBr): υ 3071, 2933, 2858, 1649, 1580, 1478, 1448, 1429, 1384, 1344, 1301, 1248, 1226, 1170, 1131, 1066, 1006, 857, 822, 752, 704 cm^{-1}; Anal. calcd for $C_{25}H_{24}N_4O_3S$: C, 65.20; H, 5.25; N, 12.17. Found: C, 61.05; H, 5.20; N, 12.06.

6-(4-Methoxybenzyl)-3-{2-[2-(1-piperidinyl)-2-oxoethoxy]phenyl}-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (6c). White crystals, 81% yield; mp: 151-153°C; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 7.43~7.49 (3H, m), 7.12 (2H, d, J = 8.4Hz), 7.06 (2H, t), 6.79 (2H, d, J = 8.4Hz), 4.76 (2H, s), 3.80 (2H, s), 3.71 (3H, s), 3.35 (2H, br), 3.25 (2H, br), 1.53 (2H, br), 1.37 (2H, br); ESI-MS (m/z): 513.1573 $(M+Na)^+$, 1003.3198 $(2M+Na)^+$, 1493.4687 $(3M+Na^+)$; IR (KBr): υ 2932, 2854, 1642, 1488, 1384, 1247, 1124, 1025, 746 cm^{-1}; ^{13}C-NMR (600 MHz, $CDCl_3$): δ 165.2, 164.1, 159.1, 158.5, 156.5, 154.0, 135.7, 131.9, 131.6, 130.7, 127.9, 121.2, 116.9, 113.7, 112.3, 106.7, 67.3, 55.3, 45.9, 43.1, 35.9, 26.4, 25.4, 24.3; Anal. calcd for $C_{26}H_{26}N_4O_4S$: C, 63.66; H, 5.34; N, 11.42. Found: C, 63.51; H, 5.30; N, 11.29.

6-Benzyl-3-{4-[2-(1-piperidinyl)-2-oxoethoxy]phenyl}-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (6d). White crystals, 72% yield; mp: 207-208°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.47 (2H, d, J = 8.0Hz), 7.25~7.36 (5H, m), 6.98 (2H, d, J = 8.0Hz), 6.72 (1H, s), 4.76 (2H, s), 4.12 (2H, s), 3.55 (4H, d), 2.56 (4H, t), 1.63 (6H, br); ESI-MS (m/z): 461.0 $(M+H)^+$; IR (KBr): υ 3109, 2947, 2853, 1668, 1650, 1488, 1419, 1384, 1356, 1313, 1301, 1245, 1224, 1183, 1141, 1108, 1073, 1013, 952, 825, 784, 752, 705 cm^{-1}; ^{13}C-NMR (600 MHz, $CDCl_3$): δ 166.0, 164.6, 159.1, 158.8, 158.6, 154.6, 138.2, 130.7, 130.5, 127.6, 120.9, 114.7, 113.9, 103.5, 67.4, 66.8, 66.7, 55.3, 45.8, 42.5, 36.3; Anal. calcd for $C_{25}H_{24}N_4O_3S$: C, 65.20; H, 5.25; N, 12.17. Found: C, 65.08; H, 5.20; N, 12.08.

6-Benzyl-3-{4-[2-(4-morpholinyl)-2-oxoethoxy]phenyl}-7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one (6e). White crystals, 71% yield; mp: 202-205°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.48 (2H, d, *J* = 8.5Hz), 7.28~7.35 (5H, m), 6.98 (2H, d, *J* = 8.5Hz), 6.73 (1H, s), 4.78 (2H, s), 4.12 (2H, s), 3.66 (8H, br); ESI-MS (m/z): 463.0 $(M+H)^+$, 496.8 $(2M+Na)^+$; IR (KBr): υ 3101, 2921, 1652, 1488, 1384, 1300, 1227, 1184, 1109, 751, 704 cm^{-1}; Anal. calcd for C24H22N4O4S: C, 62.32; H, 4.79; N, 12.11. Found: C, 62.20; H, 4.70; N, 12.08.

6-(4-Methoxybenzyl)-3-{4-[2-(1-piperidinyl)-2-oxoethoxy]phenyl}-7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one (6f). White crystals, 68% yield; mp: 204-205°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.54 (2H, d, *J* = 9.0Hz), 7.27 (2H, d, *J* = 8.4Hz), 7.00 (2H, d, *J* = 9.0Hz), 6.84 (2H, d, *J* = 8.7Hz), 6.71 (1H, s), 4.77 (2H, s), 3.80 (3H, s), 3.60 (2H, br), 3.52 (4H, br), 1.64 (6H, br); ESI-MS (m/z): 491.1 $(M+H)^+$; IR (KBr): υ 2935, 1636, 1479, 1384, 1248, 1034, 812 cm^{-1}; Anal. calcd for $C_{26}H_{26}N_4O_4S$□C, 63.66; H, 5.34; N, 11.42. Found: C, 63.50; H, 5.30; N, 11.32.

6-(4-Methoxybenzyl)-3-{4-[2-(4-morpholinyl)-2-oxoethoxy]phenyl}-7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one (6g). White crystals, 76% yield; mp: 176-178°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.50 (2H, d, *J* = 8.7Hz), 7.26 (2H, d, *J* = 8.7Hz), 7.00 (2H, d, *J* = 8.7Hz), 6.84 (2H, d, *J* = 8.7Hz), 6.72 (1H, s), 4.77 (2H, s), 4.05 (2H, s), 3.80 (3H, s), 3.67 (8H, br); ESI-MS (m/z): 492.9 $(M+H)^+$; IR (KBr): υ 3113, 2954, 2858, 1643, 1576, 1513, 1486, 1384, 1364, 1327, 1299, 1246, 1212, 1181, 1115, 1065, 1026, 996, 960, 921, 876, 815, 792, 766, 747, 716 cm^{-1}; Anal. calcd for $C_{25}H_{24}N_4O_5S$: C, 60.96; H, 4.91; N, 11.37. Found: C, 60.80; H, 4.84; N, 11.26.

Crystal growth

The solubility of the compound is determined by adding the solvent to a known amount of compound till it is completely dissolved. It is found that the target compound **6d** is sparingly in water, and moderately soluble in ethanol and methanol. It is easily soluble in dichloromethane, chloroform, *N,N*-dimethylformamide. Crystals were grown by the slow evaporation technique at room temperature by using ethanol as a solvent. An aqueous solution of MNC was prepared in a vessel covered with perforated sheet, and kept in a dust free atmosphere. At the period of super saturation, tiny crystals were nucleated[24]. They were allowed to grow to a maximum dimension and then harvested. Thus, grown light yellowish transparent crystals of dimension 13×2×5 mm.

Biology. Inhibition of AChE

The inhibitory potency against AChE was evaluated by means of an Ellman's test[25]. AChE stock solution was prepared by dissolving human AChE 0.5 unit in 100 mM PBS buffer (pH 7.4). The tested target compounds (10 μM) were prepared in DMSO. The assay solution consisted of 100mM PBS buffer (pH 7.4), with the addition of 10 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent), AChE (5 μl), drug (10 μl), and 12.5 mM acetylthiocholine iodide water solution. The final assay volume was 900 μl. Incubate the reaction at 37°C for 15 min with continuous gentle shaking. Add 50 ml acetylthiocholine iodide and 50 ml DTNB. Incubate at 37°C for about 20 min with continuous gentle shake, wait until the yellow color developed. Measure at 412 nm. Calculate the specific inhibition rates.

References and Notes

1. Johannsen, P. *CNS Drugs* **2004**, *18*, 757.
2. Carreiras, M. C.; Marco, J. L. *Curr. Pharm. Des.* **2004**, *10*, 3167.
3. Michael, F. *Drug Discovery Today* **2005**, *10*, 1333.
4. Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872.
5. Asaad, K.; Zaheer-ul-Haq, Shazia, A.; Riaz, M. K. *Bioorg. Med. Chem*. **2004**, *12*, 1995.
6. Zhi, H.; Chen, L-M; Zhang, L-L; Liu, S-J; Wan, D. C. C.; Lin, H-Q; Hu, C. *ARKIVOC* **2008**, *(xiii)*, 266.
7. Kryger, G.; Silman, I.; Sussman, J. L. *Structure Fold. Des.* **1999**, *7*, 297.
8. Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P. H.; Silman, I.; Sussman, J. L. *Proc.Natl.Acad.Sci.USA.* **1993**, *90*, 9031-9035.
9. Bourne, Y.; Taylor, P.; Radic, Z.; Marchot , P. *EMBO J.* **2003**, *22***,** 1.
10. Raves, M. L.; Harel, M.; Pang, Y. P.; Silman, I.; Kozikowski, A. P.; Sussman, J. L. *Nat. Struct. Biol.* **1997**, *4*, 57.
11. Greenblatt, H. M.; Kryger, G.; Lewis, T.; Silman, I.; Sussman, J. L. *FEBS Lett.* **1999**, *463*, 321.
12. Ekstrom, F.; Pang, Y. P.; Boman, M.; Artursson, E. *Biochem. Pharm.* **2006**, *72*, 597.
13. Ashryv, E. S. H.; Ramadan, E. S.; Hamid, H. A.; Hagar, M. *Lett Org Chem.* **2005**, *2*, 415.
14. Roshan, A.; Aurangzeb, H.; Saleh, A. *Indin J. Chem.* **1987**, *26B*, 393.
15. Kchien, L.; Shih, B. J.; Lee, C. H. *J. Heterocycl. Chem*. **1992**, *29*, 97.
16. Morris, G. M.; Goodsell D. S.; Halliday, R. S. *J. Comput. Chem.* **1998**, *19*, 1639.
17. Wong, H. N. C.; Xu, Z. L.; Chang, H. M.; Lee, C. M. *Synthesis* **1992**, *8*, 793.
18. Muhammad, A. M.; Shagufta, R.; Khalid, M. K.; Zia-Ullah; Muhammad, I. C.; Shahnaz, M. Zakiah, I. *Bioorganic & Medicinal Chemistry* **2004**, *12*, 2049.
19. Chen, Y. L.; Chen, S. J.; Lee, K. H.; Huang, B. R.; Tzeng, C. C. *Nucleosides & Nucleotides* **1993**, *9*, 925.
20. Fones, W. S. *J. Org. Chem*. **1952**, *17*, 1534.
21. Asano, M.; Huziwara, S. *Yakugaku Zasshi* **1939**, *59*, 141.
22. Sugasawa, S.; Tsuda, T. *Yakugaku Zasshi* **1935**, *55*, 1050.
23. Brown, T. H.; Ife, R. J. DE 2 811 477 (Sept 21, 1978); Smith Kline and French Laboratories Ltd., UK.; *Chem. Abstr.* **1978**, *89*, 215441.
24. Vijayan, N.; Ramesh, R.; Gopalakrishnan, R.; Ramasamy, P.; Harrison, W. T. A. *J. Cryst. Growth.* **2004**, 262, 490.
25. Paul, W. E.; Camino, M. G. T.; Michael, G. *J. Med. Chem.* **2006**, *49*, 7540.