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Synthesis and Evaluation of Novel Xanthone Derivatives as Potent AChE Inhibitors

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A new series of xanthone derivatives have been designed, synthesized and evaluated as potent AChE inhibitors. Some of them showed more potent inhibitory activities to AChE than galanthamine. The most potent inhibitor xanthone derivative **2a** inhibit AChE with a IC_{50} of 0.57 μM and showed good AChE /BuChE inhibition selectivity. Molecular docking studies were also performed to understand the detail information of interaction between AChE and inhibitor.

Keywords: Xanthone, Synthesis, AChE, Structure-activity relationship.

INTRODUCTION

Alzheimer's disease (AD) is a brain disease that causes problems with memory, thinking and behavior[1]. Acetylcholinesterase (AChE) inhibitors has been well known as the key drugs to control alzheimer's disease by improving the levels of acetylcholine (ACh) in brain[2]. AChE is an enzyme that degrades the neurotransmitter ACh, producing choline and an acetate group[3]. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate synaptic transmission.

Meanwhile, a variety of AChE inhibitors have been developed to treat alzheimer's disease (Fig. 1). For example, a series of N-methyl-N-(3-carbamoyloxyphenyl)methylamino xanthone derivatives (**A**) were designed by Rampa and co-workers[4] with good selective inhibition for AChE over butyrylcholinesterase (BuChE). Tang and co-workers[5] had developed some oxoisoaporphine and oxoaporphine derivatives as new dual inhibitors of AChE and BuChE. Furthermore, donepezil, an AChE inhibitor, was approved by the U.S. Food and Drug Administration in 1996 for the treatment of alzheimer's disease.

In recent years, we have focused on synthesizing and evaluating the biological activities of novel xanthone compounds[6-8]. Compound **1a**, a small xanthone molecular, was identified to show good AChE inhibitory activity from high throughput screening (HTS) through varieties of xanthone derivatives. As shown in Fig. 1, the structure of **1a** was simpler than the reported inhibitors and all its pharmacophores could be found in the reported ones. Moreover, it showed a IC_{50} of 0.66 μM in the enzyme based assay, compared to 1.21 μM for the positive control (Galantamine Hydrobromide, an AChE inhibitor). This finding encouraged us to undertake further investigation. Herein, we report our efforts to optimize the inhibitory effects of this compound by analyzing structure-activity relationships(SAR).

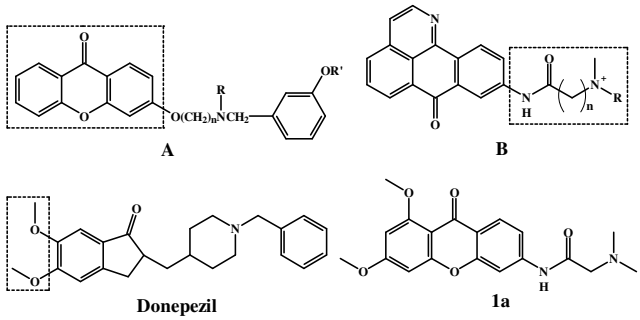


Fig. 1. Chemical structures of some inhibitors of AChE

EXPERIMENTAL

Melting points were determined with a Yamato MP-21 melting point apparatus and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 unless otherwise indicated with a Bruker AC-300P spectrometer, using tetramethylsilane as an internal standard. ESI mass

spectra were performed on an API-3000 LC-MS spectrometer. Elemental analysis was conducted with Carlo Erba1106 auto-apparatus. Column chromatography was carried out on silica gel (200-300 mesh). The solvents and reagents were used as received or dried prior to use as needed. All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. Detection was effected by examination under UV light.

3-Dimethoxy-7-nitro-9*H*-xanthen-9-one (5): A solution of 5-nitro salicylic acid (9.1 g, 0.05 mol), 1,3,5-trimethoxy-benzene (8.5 g, 0.055 mol) and Eaton's solution 100 mL,was stirred at 110 °C for 4 h, the reaction was complete, then cool down to room temperature, some ice water was added and stirred for another 2 h at rt, after filtration, the solid was washed with water, The residue was crystallized from MeOH to afford the compound 5 (12.0 g, 79 %). ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 9.14 (1 H, s, Ar), 8.47 (1 H, d, Ar), 7.50 (1 H, d, Ar), 6.55 (1 H, s, Ar), 6.42 (1 H, s, Ar), 4.01 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3). ESI-MS, *m/z*: $[M + H]^+$, 302.90.

3-Dimethoxy-7-amino-9*H*-xanthen-9-one (6): A solution of 1,3-dimethoxy-7-nitro-9*H*-xanthen-9-one (3.01 g, 0.01 mol), Raney-nickel 0.5 g and methanol 20 mL was stirred at room temperature, then hydrazine was added dropwised into the reaction slowly and stirred for another 4 h at rt. After filtration, the filtrate was evaporated under reduced pressure. The residue was crystallized from MeOH to afford the compound **6** (1.9 g, 69 %). ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 7.52 (1 H, s, Ar), 7.22 (1 H, d, Ar), 7.00 (1 H, d, Ar), 6.47 (1 H, s, Ar), 6.32 (1 H, s, Ar), 3.98 (3 H, s, OCH_3), 3.91 (3 H, s, OCH_3), 3.77 (2 H, br, NH). ESI-MS, *m/z*: $[2M + Na]^+$, 565.19.

2-Chloro-N-(6,8-dimethoxy-9-oxo-9*H*-xanthen-2-yl)acetamide (7): A solution of 7-amino-1,3-dimethoxy-9*H*-xanthen-9-one (2.71 g, 0.01 mol), chloroacetyl chloride (1.68 g, 0.015 mol) and toluene 50 mL was stirred reflux for about 2 h. The reaction was complete, remove most of toluene under reduced pressure. Water was added to the residue, which was then extracted with ethyl acetate. The extract was washed with saturated NaCl solution, dried over anhydrous Na_2SO_4 and evaporated. Silica gel column chromatography of the residue afforded the compound **7** (3.1 g, 90 %).

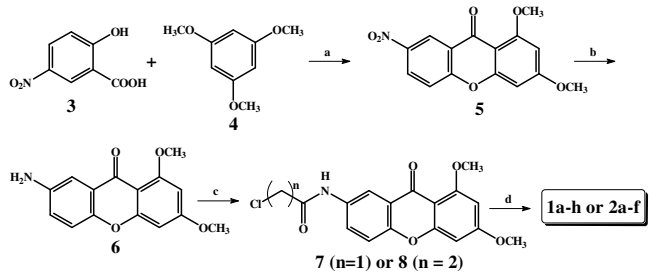
3-Chloro-N-(6,8-dimethoxy-9-oxo-9*H*-xanthen-2-yl)propanamide (8): The reaction was run similarly to that used to synthesize the compound **8**.

N-(6,8-dimethoxy-9-oxo-9*H*-xanthen-2-yl)-2-(dimethyl-amino)acetamide (1a): A solution of 2-chloro-N-(6,8-dimethoxy-9-oxo-9*H*-xanthen-2-yl)acetamide (694 mg, 2 mmol), K_2CO_3 (276 mg, 2 mmol), dimethylamine (180 mL, 4 mmol) and DMF 10 mL was stirred at room temperature for about 10 h. After filtration, the filtrate was extracted with ethyl acetate, washed with water and saturated NaCl solution 3 times , dried over anhydrous Na_2SO_4 and evaporated. Silica gel column chromatography of the residue afforded the compound **1a** (626 mg, 88 %).

The target compounds **1b-h** and **2a-f** were synthesized by the same procedure as the compound **1a**.

RESULTS AND DISCUSSION

Compounds required for the establishment of structure-activity relationship were prepared as shown in **Scheme-I** starting from 2-hydroxy-4-nitrobenzoic acid (**3**). Reaction of **3** with 1,3,5-trimethoxybenzene (**4**) by using Eaton's reagent afforded xanthone (**5**)[9]. Treat **5** with Raney nickel to reduce the nitro group gave **6** in high yield (85 %)[10]. Then, chloroacetyl chloride or 3-chloropropionyl chloride was added dropwisely into the flask containing **6** under reflux to give key intermediate **7** or **8**. Reaction of **7** or **8** with different secondary amines formed the final xanthone sulfonamides **1a-h** and **2a-f** in high yield (Fig 2)[11]. The reactions were carried out in parallel. All new compounds were characterized by NMR and MS.



Reagents and conditions: (a) P_2O_5/HSO_3H, 110 °C, 4 h, 75 %; (b) Raney nickel, hydrazine, MeOH, room temperature, 2 h, 85 %; (c) Chloroacetyl chloride or 3-chloropropionyl chloride, toluene, reflux, 2 h, 90 %; (d) Amine or azole, K_2CO_3, DMF, room temperature, 10 h, 72-88 %

Scheme-I: Synthesis of compounds **1a-h** or **2a-f**

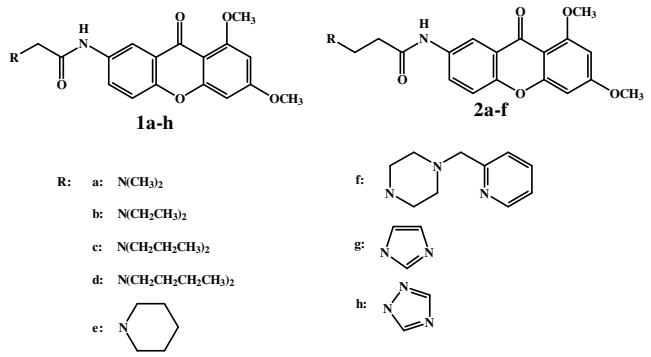


Fig. 2. Target xanthone derivatives

Evaluation of biological activities: To determine AChE and BChE inhibitory activities, all the compounds were measured *in vitro* according to the modified Ellman method with galanthamine as the positive control[12]. The selectivity of the compounds was also tested by determining their inhibitory activity against BuChE. The results were summarized in Table-1. From the results, most of the tested compounds demonstrated good inhibitory activities against AChE. Compound **2a** and **2e** showed higher inhibitory activity than the positive control. Furhtermore, all the compounds showed weak activity against BuChE.

Structure-activity relationship (SAR): To explain the results, we proposed a likely binding mode for **1e** to the active site of AChE based on computational docking results (Fig. 3)[13]. The designed compound could fit into the hydrophobic pocket formed by Tyr70, Asp72, Tyr121, Tyr334, Trp279. Moreover, the amide group could generate two hydrogen bond interaction with Asp72 and Tyr121. These results may provide some guidance for the development of novel AChE inhibitory lead structures.

TABLE-1
in vitro ChE (AChE AND BuChE) INHIBITION
ASSAY DATA FOR COMPOUNDS **1a-h** and **2a-f**

Compound	AChE IC_{50} (μM)	BuChE Inhibitory ratio at 25 μM
1a	39.2[a]	7.75
1b	14.0	0.62
1c	33.0[a]	-
1d	18.1[a]	-
1e	0.66	11.2
1f	15.0	-
1g	-[b]	-
1h	-	-
2a	0.57	26.0
2b	2.93	-
2c	50.6[a]	-
2d	52.6[a]	-
2e	0.59	-
2f	23.0[a]	7.44
Galanthamine[c]	1.21	58.2

[a]inhibitory ratio at 12.5 μM, [b]no inibition observed, [c]positive control

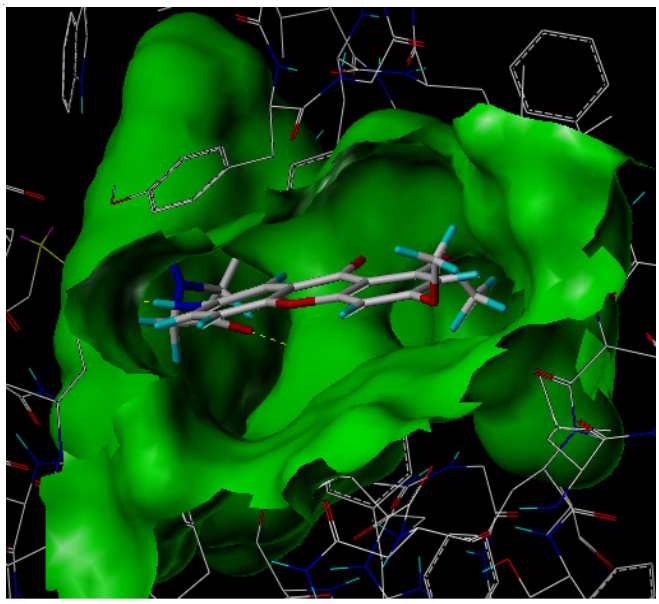


Fig. 3. Computed binding geometry of **1e** in the active site of AChE

Conclusion

In summary, a series of new xanhtone derivatives **1a-h**, **2a-f** as potential AChE inhibitors were synthesized in high yields. The biological screening of these compounds resulted in the identification of several potent AChE inhibitors with higher BChE/AChE selectivity, particularly compound **2n** which is more potent than the established AChE inhibitor galanthamine. This observation was fitting to a molecular model resulting from the computational docking simulation, which showed that **1e** could fit into the hydrophobic pocket of AChE. More effort was aimed at further SAR optimization and next round biological investigations, meanwhile our job identifying lead compounds in the half way will be well done soon and results will be reported in near future.

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11. Representative analytical data for compound **2a**, yield 72 %, ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 10.41 (1H, br, NH), 8.25 (1H, s, Ar), 7, 96 (1H, br, Ar), 7.48 (1H, d, Ar), 6.66 (1H, s, Ar), 6.49 (1H, s, Ar), 3.88 (3H, s, OCH_3), 3.83 (3H, m, OCH_3), 3.83 (2H, br, CH_2), 2.88-2.32 (6H, m, CH_2), 1.51-1.37 (6H, m, CH_2). ESI-MS, *m/z*: Calcd. 410.2, Found, 411.1 $[M + H]^+$.
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