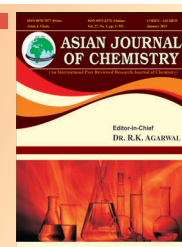




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## Synthesis and Evaluation of Novel Xanthone Derivatives as Potent Dipeptidyl Peptidase-4 Inhibitors

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Two series of xanthone derivatives have been designed, synthesized and evaluated as potent dipeptidyl peptidase-4 inhibitors and among them seven compounds were firstly reported. Most of the inhibitors showed moderate inhibitory activities against dipeptidyl peptidase-4 and compounds **2a**, **2c**, **2d**, **2f** and **2g** exhibited potent activities against dipeptidyl peptidase-4 compared with the positive control diprotin A.

**Keywords:** Xanthone, Synthesis, Dipeptidyl peptidase-4, Structure-activity relationships.

### INTRODUCTION

Diabetes, a chronic disease caused by genetic and environmental factors, threatens the health of millions of people, which has become a global challenge<sup>1</sup>. Therefore, much earlier intervention is required to prevent diabetes, type-2 diabetes particularly. Previous study revealed that glucagon-like peptide-1 is an intestine-derived insulinotropic hormone that stimulated glucose dependent insulin production and secretion from pancreatic  $\beta$ -cells. Glucagon-like peptide-1 also exerted cytoprotective and antiapoptotic effects on  $\beta$ -cells and decreased postprandial blood glucose *via* complicated mechanisms<sup>2,3</sup>. However, glucagon-like peptide-1 was rapidly degraded and inactivated *in vivo* by the serine protease dipeptidyl peptidase-4, which limited their ability to normalize blood glucose levels. Therefore, dipeptidyl peptidase-4 inhibitors could decrease the high levels of blood glucose in type 2 diabetes patients<sup>4,5</sup>.

In recent years, we have focused on synthesizing and evaluating the biological activities of novel xanthone compounds<sup>6-8</sup>. Compound **9**, a small xanthone derivative, was identified to show good dipeptidyl peptidase-4 inhibitory activity from high throughput screening (HTS) through varieties of xanthone derivatives and  $IC_{50}$  value against dipeptidyl peptidase-4 is 7.3  $\mu$ g/mL (Fig. 1). Molecular docking study showed compound **9** could bind with the target enzyme and a pocket existed between the 7-substituted group and the target enzyme, which revealed that an adequate modification to compound **9** could

improve the bind between receptor and ligands and enhanced the inhibition of the xanthone derivatives against dipeptidyl peptidase-4. As shown in Fig. 2, when a hydrophobic group was linked to the xanthone framework at 7-position, the compound could bind with the target enzyme better. Herein, we report our efforts to optimize the inhibitory effects of this compound by analyzing structure-activity relationships.

### EXPERIMENTAL

Melting points were determined with a Yamato MP-21 melting point apparatus and uncorrected. NMR spectra was 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 Erba 1106 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.

**1,3-Dimethoxy-7-nitro-9H-xanthen-9-one (5):** A solution of 5-nitrosalicylic acid (9.1 g, 0.05 mol), 1,3,5-trimethoxybenzene (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 room temperature. After filtration, the solid

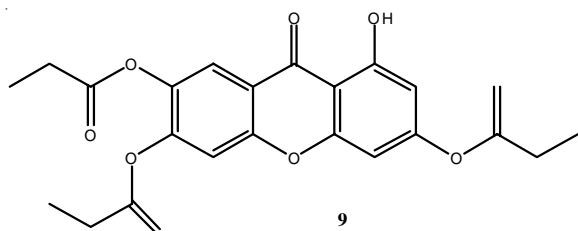
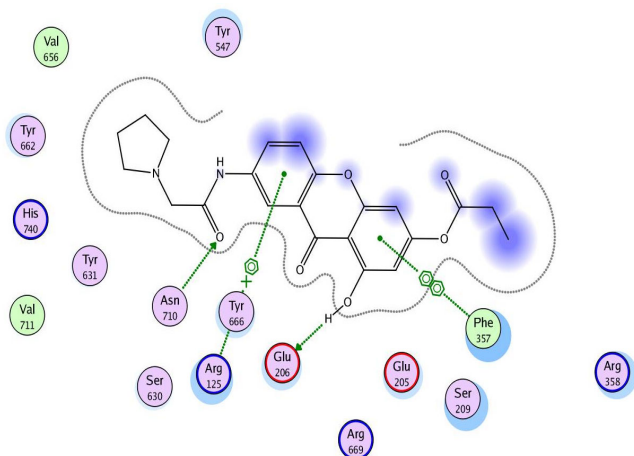
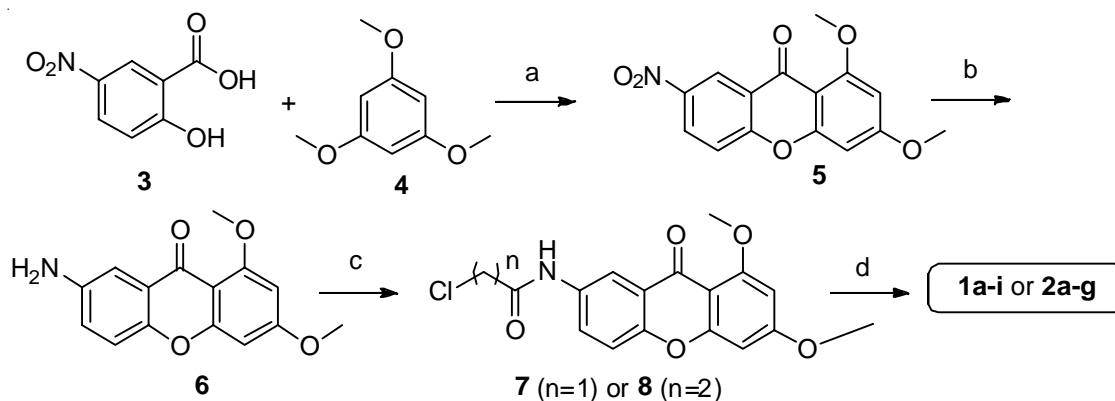


Fig. 1. Structure of compound

Fig. 2. Mode of compound **1i** binding with dipeptidyl peptidase-4

was washed with water, the residue was crystallized from MeOH to afford the compound **5** (12.0 g, 79 %).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ , TMS):  $\delta$  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<sub>3</sub>), 3.95 (3 H, s, OCH<sub>3</sub>). ESI-MS,  $m/z$ : [M + H]<sup>+</sup>, 302.90.

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

Scheme-I: Synthesis of compounds **1a-i** or **2a-g**

**2-Chloro-N-(6,8-dimethoxy-9-oxo-9H-xanthen-2-yl)acetamide (7):** A solution of 7-amino-1,3-dimethoxy-9H-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, removed the excess 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<sub>2</sub>SO<sub>4</sub> 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-9H-xanthen-2-yl)propanamide (8):** The reaction was run similarly to that used to synthesize the compound **8**.

**N-(6,8-Dimethoxy-9-oxo-9H-xanthen-2-yl)-2-(dimethylamino)acetamide (1a):** A solution of 2-chloro-N-(6,8-dimethoxy-9-oxo-9H-xanthen-2-yl)acetamide (694 mg, 2 mmol), K<sub>2</sub>CO<sub>3</sub> (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<sub>2</sub>SO<sub>4</sub> and evaporated. Silica gel column chromatography of the residue afforded the compound **1a** (626 mg, 88 %).

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

## RESULTS AND DISCUSSION

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

**Evaluation of biological activities:** To determine dipeptidyl peptidase-4 inhibitory activities, all the compounds were measured *in vitro* according to the modified Ellman method with diprotin A as the positive control. Table-1 showed that

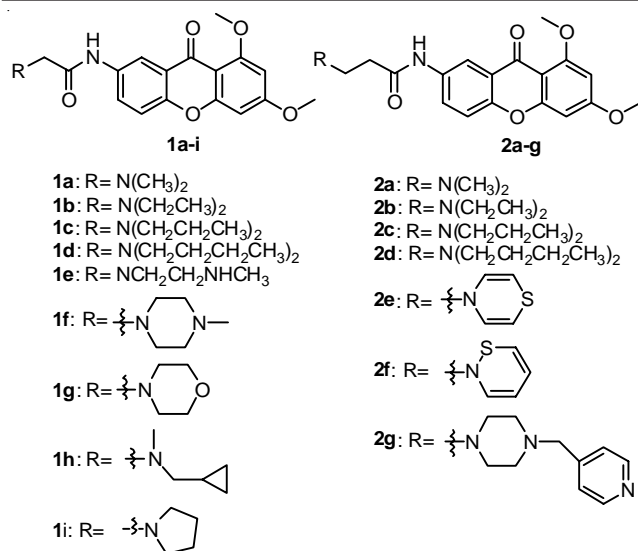


Fig. 3. Target xanthone derivatives

TABLE-1  
DIPEPTIDYL PEPTIDASE-4 INHIBITION RATE OF  
COMPOUNDS **1a-1h** AND **2a-2g** AT 50 µg/mL CONCENTRATION

Sample	Inhibition (%)	Sample	Inhibition (%)
<b>1a</b>	88	<b>2a</b>	94
<b>1b</b>	75	<b>2b</b>	88
<b>1c</b>	85	<b>2c</b>	96
<b>1d</b>	71	<b>2d</b>	96
<b>1e</b>	33	<b>2e</b>	81
<b>1f</b>	89	<b>2f</b>	93
<b>1g</b>	75	<b>2g</b>	91
<b>1h</b>	85	<b>Diprotin A<sup>a</sup></b>	91 <sup>b</sup>
<b>1i</b>	65	–	–

<sup>a</sup>Positive control; <sup>b</sup>the inhibition rate at 17 µg/mL concentration

most of the tested compounds demonstrated good inhibitory activities against dipeptidyl peptidase-4. Compounds **2a**, **2c**, **2d**, **2f** and **2g** exhibited potent activities against dipeptidyl peptidase-4.

**Structure-activity relationship:** According to the results above, a preliminary structure-activity relationships could be concluded: Series compound **2a-g** exhibited better inhibitory activities against dipeptidyl peptidase-4 than series compound **1a-I**, which demonstrated that length of 7'-substituted ethylamine group could fit with the pocket of the target enzyme. In

addition, alkyl group substituted compounds exhibited better inhibitory activities against dipeptidyl peptidase-4.

## Conclusion

In summary, two series of new xanthone derivatives (**1a-i**) and (**2a-g**) as potential dipeptidyl peptidase-4 inhibitors were synthesized in high yields. The biological screening of these compounds resulted in the identification of several potent dipeptidyl peptidase-4 inhibitors and compounds **2a**, **2c**, **2d**, **2f** and **2g** exhibited potent activities against dipeptidyl peptidase-4 compared with the positive control diprotin A. This observation was fitted to a molecular model resulting from the computational docking simulation, which showed that compound **1i** could fit into the hydrophobic pocket of dipeptidyl peptidase-4.

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- Representative Analytical Data for Compound **2a**, Yield 72%, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>, TMS): δ 10.41 (1 H, br, NH), 8.25 (1 H, s, Ar), 7.96 (1 H, br, Ar), 7.48 (1 H, d, Ar), 6.66 (1 H, s, Ar), 6.49 (1 H, s, Ar), 3.88 (3 H, s, OCH<sub>3</sub>), 3.83 (3 H, m, OCH<sub>3</sub>), 3.83 (2 H, br, CH<sub>2</sub>), 2.88-2.32 (6 H, m, CH<sub>2</sub>), 1.51-1.37 (6 H, m, CH<sub>2</sub>). ESI-MS, *m/z*: Calcd. 410.2; found, 411.1 [M + H]<sup>+</sup>.