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1-Oxo-3-substitute-isothiochroman-4-carboxylic acid compounds: Synthesis and biological activities of FAS inhibition

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ABSTRACT

A new series 1-oxo-3-substitute-isothiochroman-4-carboxylic acid compounds have been designed and synthesized. Screening of these molecules for FAS inhibition in vitro has indicated that compounds **2c** and **2d** showed more effective FAS inhibition activities and higher therapeutic index than C75.

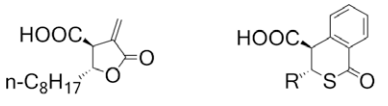
Obesity has emerged as a worldwide health, economy and social psychology problem. It has been one of the most common metabolic pathologies in contemporary society, that is, associated with increased risk of type II diabetes, cardiovascular and cerebrovascular diseases.[1]

Fatty acid synthase (FAS) is the key enzyme in de novo lipogenesis from acetyl-CoA, malonyl-CoA and NADPH, which has been proved to be a new appetite and body weight regulation target in mice. FAS is a multi-enzyme complex containing seven proteins as functional domains.[2] Among the seven moieties of FAS, the ketoacyl synthase domain (KS) is the potential target of C75 (**1** in Fig. 1), which has been known as a novel inhibitor of FAS and it can lead a distinct decrease of food intake and body weight of mice without significant toxity.[3]

Our interest was initially kindled by the novel mechanism and the structure of C75. We have planned to introduce a benzene ring to replace the unstable α-methylene moiety, which has been known as a key pharmacophore of C75. Some different carbon-chains and sulfur atom have also been introduced based on bio-isosteric rule to investigate structure–function relationship of FAS inhibitors. The rationally designed molecules (**2a–f** in Fig. 1) has been synthesized and evaluated for their FAS inhibitory activities.

The synthesis of the compounds **2a** and **3a** has been described as a sample. Commercially available 2-Carboxymethyl-benzoic acid **4** was transferred to the dimethylester **5** by reaction with conc. sulfuric acid and methanol. Compound **5** reacted with NaH in THF at 0 °C for 1 h, followed by the addition of heptaldehyde keeping on stirring for another 4–6 h at same temperature, after usual acidification and purification, provided the expected compound **6a** as a white solid in 90% yields (Scheme 1). The reaction has been used generally in organic synthesis named aldol reactions.[4]

1,4-Addition of thioacetic acid to the compound **6a** gave the corresponding product **7a** according to Holmberg (Scheme 1).[5] A solution of **6a** and thioacetic acid (1:2) was stirred at 80–85 °C in dry pyridine for 3 days, intermediates **7a** was obtained as a thick liquid. The liquid was hydrolyzed from acetylthio group to the thiol in HCl solution (6 mol/L) without purification, and then it was cyclized to the mixture of two diastereomers by refluxing with TFA for 24 h. The mixture of **8a** and **9a**, the *trans*- and *cis*-isomers, were obtained as a white solid in total 48% yield (Scheme 2).



1 **2a-f**

2a. R=*n*-C_6H_{13}
2b. R=*n*-C_7H_{15}
2c. R=*n*-C_8H_{17}
2d. R=*n*-C_9H_{19}
2e. R=*n*-$C_{10}H_{21}$
2f. R=*n*-$C_{11}H_{23}$

Figure 1.

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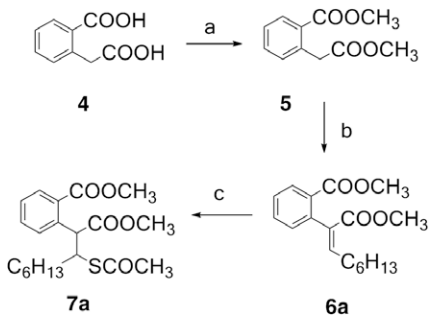
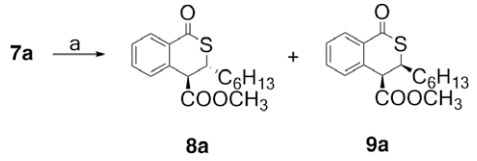


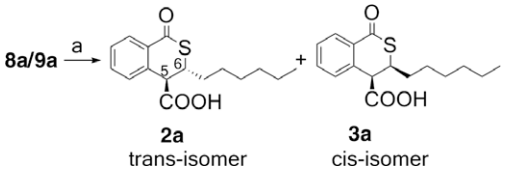
Scheme 1. Reagents and conditions: (a) $CH_3OH{\cdot}H_2SO_4$, 90%; (b) NaH/THF, n-$C_6H_{13}CHO$, 90%; (c) CH_3COSH/pyridine, 80–85 °C/3days, 57%.



Scheme 2. Reagents and conditions: (a) HCl (6 mol/L), reflux, TFA in CH_2Cl_2/reflux, 48%.

The mixture of **8a** and **9a** were easily hydrolyzed by LiOH (1 mol/L) in CH_3OH–H_2O (1:2) on stirring at room temperature overnight (Scheme 3),[6] and the products **2a** and **3a** were obtained in the ratio of 3:1 by the usual workup and chromatography separation. The *cis*- and *trans*-isomer was determinated by the coupling constant of hydrogen atoms of C5 and C6 position (J = 3.08 Hz in *cis*-isomer and J = 3.36 Hz in *trans*-isomer).[7] Each isomer was obtained as racemates. Compounds **2b–f** and **3b–f** were synthesized by the same procedure using different aldehydes.

Compound **2a–f and 3a–f** have been evaluated for in vitro FAS inhibitory activities using purified FAS from SD rat liver.[8] All these compounds have showed comparatively activities of FAS inhibitory in vitro (Table 1). Just like C75, the *trans*-isomers perform higher inhibitory activities than *cis*-isomers, but the distinction become unremarkable in **2e/3e** and **2f/3f**. Otherwise, the enhancement of activities has been found following by the elongation of carbon-chain at the C5 position from n-C_6H_{13}– (**2a**) to n-$C_{11}H_{23}$– (**2f**). The inhibitory activities rised immensely when the carbon-chain of substituent arrived at n-$C_{10}H_{21}$–, and it was probably correlated



Scheme 3. Reagents and conditions: (a) LiOH (1 mol/L), 85%.

Table 1
In vitro FAS inhibitory activities of compounds **2a–f**, **3a–f** and C75 in 60 μmol

Compound	% Inhibition ± SD	Compound	% Inhibition ± SD
C75	52.75 ± 2.51		
2a	40.44 ± 4.26	**3a**	11.03 ± 3.76
2b	43.07 ± 2.84	**3b**	11.59 ± 4.53
2c	50.93 ± 6.51	**3c**	40.16 ± 1.42
2d	77.54 ± 2.46	**3d**	43.48 ± 2.08
2e	90.98 ± 3.76	**3e**	90.18 ± 3.42
2f	96.72 ± 1.42	**3f**	95.65 ± 3.40

Table 2
Classical complement inhibition, cytotoxicity and apoptosis assay results for compounds **2a–f**

Compound	IC_{50}[a] (μM)	TC_{50}[a] (μM)	T.I.[b]
C75	15.53(±2.26)	87.09(±14.25)	5.61
2a	36.73(±4.03)	>200	
2b	26.86(±3.76)	>200	
2c	12.32(±1.80)	84.18(±15.37)	6.83
2d	8.29(±1.24)	88.58(±15.77)	10.69
2e	4.11(±1.03)	11.99(±2.26)	2.92
2f	3.62(±0.46)	9.67(±2.23)	2.67

[a] Values are means of three experiments, standard deviation is given in parentheses.
[b] In vitro therapeutic index (TC_{50}/IC_{50}).

with the cytotoxic estimated by the MTT method of HLF cell (Table 2). Finally, it will be a favorite substituent when the carbon-chain at C5 position is n-C_8H_{17}– or n-C_9H_{19}–, and compounds **2c** and **2d** showed more effective FAS inhibition activities and higher therapeutic index than C75.

The crystal structure of KS of human FAS has not been reported. In order to investigate the interactions of these compounds in the active site of KS, the three-dimensional structure of KS domain (402 amino acid residues) of human FAS was modeled preliminary on InsightII/Homology module using the X-ray structure of *Escherichia coli.* KASII protein (PDB code 1FJ8) and *Synechocystis SP* KASII protein (PDB code 1E5M) as template. The scores of Profile 3D is 149.4, and the RMS deviation of protein backbone is 0.25.

We carried out docking of compounds C75 and **2c** in the target by means of the Affinity modular of InsightII. The result indicated that these molecules can fit in the KS active site in the same fashion, and the RMS deviation is 0.35 and 0.40 (Fig. 2). The active site includes a hydrophobic pocket (13–15 Å) formed by residues Ala162, Tyr224, Val263, Phe202, Phe258, Glu335, etc., and a hydrophilic pocket formed by residues Ser114, Cys163, Pro336, Phe397, etc.

In C75, the hydroxyl H atom of carboxy is present at a distance of 2.16 Å from the oxygen of carbonyl moiety of Pro336, and the distance is 1.95 Å in compound **2c**, which interacts through H-bond formation. Further, the α-methylene group approaches the aromatic moiety of Phe397 at a distance of 2.45 Å, which indicates that a π–π effect would be present. In compound **2c**, the benzene ring performs a similar π–π effect with Phe397 residue to C75 at a distance of 4 Å (Fig. 3).

While similar types of interactions are observed during the docking of compounds **2a–f** in the active site of KS. The thiobuty-

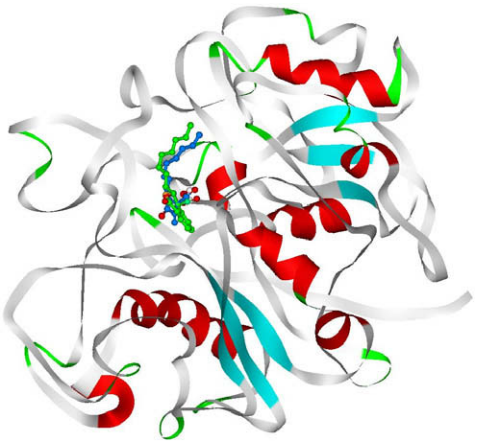


Figure 2. C75 (blue) and compound **2c** (green) docked in the active site with similar fashion in the modulated structure of KS. Figure was generated with Ds ViewPro 5.0 (http://www.accelrys.co).

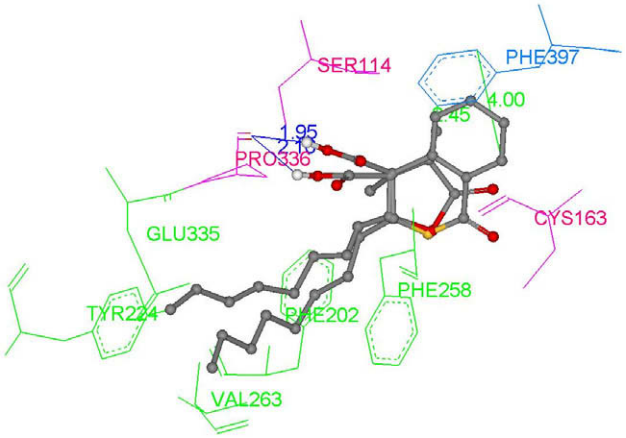


Figure 3. C75 and compound **2c** docked in the active site: some important amino acid residues in hydrophobic pocket (green), hydrophilic pocket (red), H-bond interactions (Pro336) and π–π effect (Phe397, blue) are present.

rolactones structure can fit in the hydrophilic pocket just like C75, and the hydrophobic pocket have enough space to hold a linear carbon-chain containing 6–11 carbon atoms. Compounds **3a–f** are devoid of H-bond interactions because of the *cis*-configuration.

In conclusion, a novel series of 1-oxo-3-substitute-isothiochroman-4-carboxylic acid compounds were designed and synthesized, and these compounds performed similar interactions to C75 in the active site of KS, which was modeled preliminary. Compounds **2c** and **2d** showed more remarkable FAS inhibition activities and higher therapeutic index than C75. The substituent such as *n*-C_8H_{17}– and *n*-C_9H_{19}– at C5 position and *trans*-configuration will be favorite for high activity and low toxicity, and this research would lead to the discovery of new compounds for further investigations and also support the design of these molecules.

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7. *Selected data for compound* ***2a:*** ^{1}H NMR (400 MHz, CD_3Cl) δ = 7.98 (d, 1H, *J* = 6.72 Hz); 7.57 (m, 1H); 7.49 (m, 1H); 7.25 (d, 1H, *J* = 6.16 Hz); 4.06 (d, 1H, *J* = 3.36 Hz); 3.83 (m, 1H); 1.63 (m, 2H); 1.1–1.6 (m, 8H); 0.88 (t, 3H, *J* = 6.72 Hz). MS (FAB) $[M+1]^+$: *m*/*z* = 293.1.*Compound* ***3a:*** ^{1}H NMR (400 MHz, CD_3Cl) δ = 7.98 (dd, 1H, *J* = 7.84, 1.40 Hz); 7.53 (m, 1H); 7.47 (m, 1H); 7.27 (d, 1H, *J* = 9.52 Hz); 4.10 (d, 1H, *J* = 3.08 Hz); 3.93 (m, 1H); 1.99 (m, 1H); 1.84 (m, 1H); 1.1–1.6 (m, 8H); 0.88 (t, 3H, *J* = 6.72 Hz). MS (FAB) $[M+1]^+$: *m*/*z* = 293.1.
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