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Novel fatty acid synthase (FAS) inhibitors: Design, synthesis, biological evaluation, and molecular docking studies

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ABSTRACT

Several novel series of **C75** derivatives were synthesized and evaluated for their FAS inhibitory activities. The results showed compound 4-methylene-2-octyl-5-oxo-tetrahydro-thiophene-3-carboxylic acid (**1c**) had more effective FAS inhibitory (IC_{50} was 2.56 μM and T.I. was 9.26) and potent anti-tumor activities on HL60 and Hela cells in vitro (IC_{50} were 5.38 μM and 46.10 μM, respectively).

1. Introduction

Human fatty acid synthase (FAS) is a key enzyme in de novo biosynthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA, and NADPH. It has been an attractive target for anti-obesity[1–5] and cancer.[6–11] Human FAS is a multi-enzyme complex containing seven functional proteins. Among the seven moieties of FAS, the-ketoacyl synthase domain (KS)[12,13] is considered to be the potential target of **C75** (Fig. 1), which has been known as a novel FAS inhibitor. **C75** has been used as a leading compound for discovering the role of FAS in obesity and cancer.

There are few small molecular inhibitors of KS (FAS) have been reported, and **C75** is one of the most extensive study. The SAR of the series of **C75** is pointed at C-2 position and it has indicated that the flexibly saturated chains are favoring substituents.[14]

To obtain the advanced SAR and develop some novel inhibitors of FAS, our research program was started in studying SAR of C-5 and O-3 position of **C75**. We had introduced an isopropylidene- (**2a–f**), dimethyl- (**3a–f**), and benzene ring (**4a–f**) to replace the unstable α-methylene moiety binding to C-5 position of **C75**, and O-3 atom was replaced by the sulfur atom (**1a–f**) (Fig. 1). Some different carbon chains had been introduced also to obtain the SAR of C-2 position. The results of the work on synthesis, the biological activity and the SAR are described herein.

2. Chemistry

The synthesis of compounds **1a–f** had been reported previously[15] (Scheme 1). In a general procedure, the activated ester **6** was created by succinate esters and triethyl phosphate **5**. The Stobbe reaction[16] was used in the condensation of esters **6** and alde-

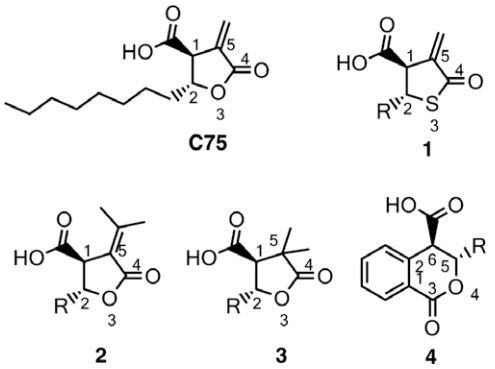

Figure 1. Structures of **C75** and newly designed compounds.

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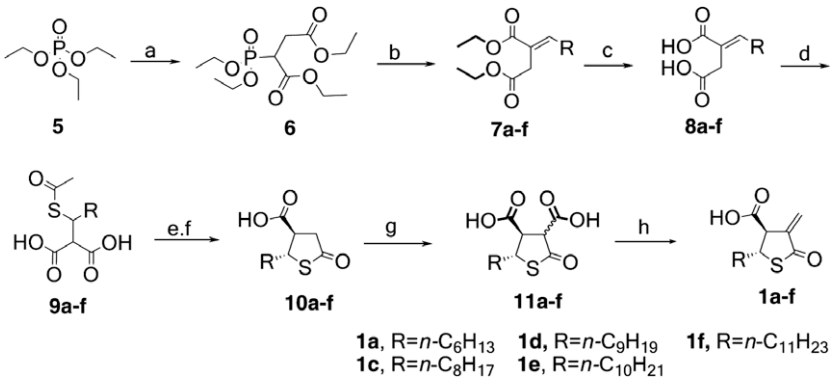


Scheme 1. Reagents and conditions: (a) NaH/THF; $BrCH_2CO_2Et$ 76.5%; (b) LDA/THF; *n*-RCHO; (c) NaOH/H_2O; (b+c) 48–57%; (d) CH_3COSH 80–85 °C/3 days; (e) 6 mol/l HCl reflux; (f) TFA/reflux; (d+e+f) 39–48.5%; (g) $CH_3OCO_2MgOCH_3$ 1.8 mol/l DMF 135–140 °C 3 days; (h) Stock solution; (g+h) 29–50.4%.

hydes of various chain descriptions (**R**) to give the intermediates **7a–e**. Following by the base-catalyzed hydrolysis of diethyl esters 7a–**f** and 1,4-addition with thioacetic acid of **8a–f**, the compounds **9a–f** were obtained. The acetylthio groups of **9a–f** were hydrolyzed to the thiols, and then the intermediates **10a–f** were formed by an acid-catalyzed cyclization.[17,18] The intermediates **11a–f** were obtained by the reaction of **10a–f** and Stiles' reagent (methyl methoxymagnesium), and they were converted to the desired products **1a–f** by diethylamine in formalin (Stock solution).[19,20]

Compounds **2a–f** were prepared from succinic acid diethyl ester **12** (Scheme 2). It was converted to the **13** by reaction with acetone and *t*-BuOK. Followed by the dehydration catalyzed by acetic anhydride, the anhydride **14** was obtained in high yields.[21] Compound **14** reacted with 4-methoxybenzyl alcohol in mild condition to yield the ester **15**, which was condensed with aldehydes by the Aldol reaction and cyclization to gave γ-butyrolactones (**16a–f**).[14] Both the *trans*-isomers (**2a–f)** and *cis*-isomers (**2′a–f)** were obtained by the TFA catalyzed hydrolysis.

Compounds **3c–d** were prepared with the similar synthesis route to **2a–f** from the commercially available 2,2-dimethyl-succinic acid **17** via the intermediate **18** (Scheme 2).

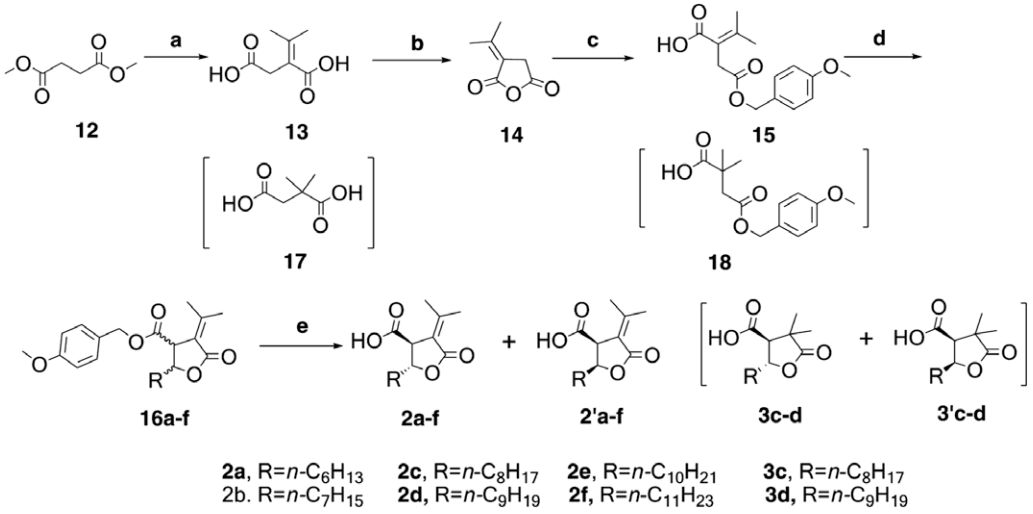
Compounds **4a–f** were synthesized from homophthalic acid dimethyl ester **19** (Scheme 3). The intermediate **20** was formed by **19** and LDA in anhydrous THF at −78 °C, and it was transformed to the intermediate **21** followed by the addition of chlorotitanium triisopropoxide at same temperature.[22] Condensation of **21** and aldehydes, followed by the acid-catalysis cyclization and removal of the methyl group by LiOH[23], both the *trans*-isomers (**4a–f)** and *cis*-isomers (**4′a–f**) were obtained.

The *trans*- and *cis*-isomers were determinated by the coupling constant of hydrogen atoms of C1 and C2 position ($J < 3.5$ Hz in *cis*-isomer and $J > 5.3$ Hz in *trans*-isomer). Each isomer was obtained as racemates.

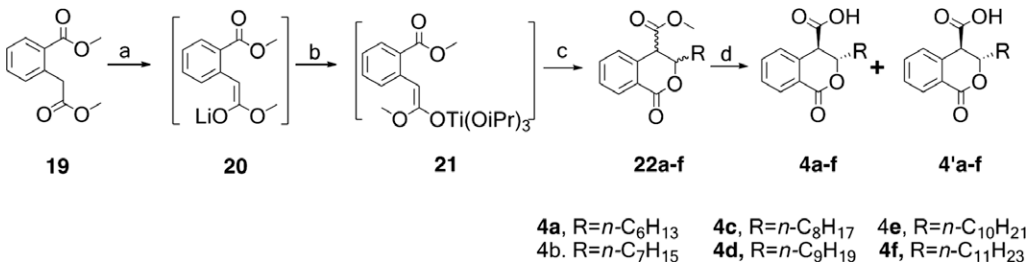
3. Results and discussion

All the synthesized compounds have been evaluated for in vitro FAS inhibitory activities using purified FAS from SD rat liver[24] (Table 1). The lead compound (**C75**) displayed moderate FAS inhibitory activity with an IC_{50} value of 15.53 μM. The FAS inhibitory activity decreased sharply when the α-methylene at C-5 position in **C75** was replaced by dimethyl (**3c** and **3d**) and isopropylidene-(**2a–f**), and the introduction of benzene ring (**4a–f**) remained the inhibitory activity partly. Cheerfully, compounds **1a–f** showed more potent FAS inhibitory activities than **C75**.

We carried out docking of **C75** and representative compounds containing same carbon-chain (**1c, 2c, 3c,** and **4c)** in the active site of KS (Fig. 2, Left), The results (Table 2) indicated that the saturated carbon chain of these compounds could fit well in a linear hydrophobic pocket formed by residues Ala162, Tyr224, Val263,



Scheme 2. Reagents and conditions: (a) *t*-BuOK, CH_3COCH_3; NaOH/H_2O reflux; (b) $(CH_3CO)_2O$ 100 °C/30 min; (c) *p*-methoxybenzyl alcohol, 55–60 °C/40 h; (b+c) 55.3%; (d) LDA/THF, *n*-RCHO, −78 °C; (d) 6 mol/l H_2SO_4; (e) TFA/CH_2Cl_2 12 h, **2′a–f** 9.5–14%; **2a–f** 46.6–52% (**3c–d** and **3′c–d** in 5–7.5% and 36.6–48% yield).



Scheme 3. Reagents and conditions: (a) LDA/THF, −78 °C; (b) (*i*-PrO)$_3$TiCl/THF, −78 °C; (c) *n*-RCHO/THF, −78 °C; 6 mol/l H_2SO_4; (d) LiOH 1 M/H_2O, **4′a–f** 9.21–26%; **4a–f** 35–39%.

Table 1
In vitro inhibitory activities of FAS

Compound	IC_{50}[a] (μM)	TC_{50}[a] (μM)	T.I.[f]
C75	15.53	87.09	5.61
1a	16.73	96.35	5.76
1c	2.56	23.7	9.26
1d	2.28	6.86	
1e	1.19	6.80	
1f	0.79	4.75	
2a	N.E.[d]	N.T.[e]	
2b	N.E.[d]	N.T.[e]	
2c	17.39%[b,c]	N.T.[e]	
2d	38.38%[b,c]	N.T.[e]	
2e	122.40	>200	
2f	27.46	153.22	5.57
3c	16.67%[b,c]	N.T.[e]	
3d	23.81%[b,c]	N.T.[e]	
4a	19.42%[b,c]	N.T.[e]	
4b	35.94%[b,c]	N.T.[e]	
4c	88.64	N.T.[e]	
4d	29.78	>200	
4e	24.15	>200	
4f	19.47	94.21	4.84

[a] The deviations were within <±5%.
[b] Values are means of three experiments.
[c] %Inhibition in 60 μM.
[d] N.E.: no inhibition in 60_ μM.
[e] N.T.: not test.
[f] In vitro therapeutic index (TC50/IC50).

Phe202, Phe258, and Glu335, and the lactone moiety interacted with a hydrophilic pocket formed by residues Ser114, Cys163, Pro336, and Phe397.

The structure of sp^3 hybridization methyl of C-5 position in **2c** and **3c** were unfavorable for the activity because they decreased the hydrophilic of the lactones moiety and weakened the ability of π–π effect formation. Most importantly, the steric bulky effect had lead to the configuration change of C-1 carboxyl and they were devoid of H-bond interactions.

Compared **4c**, C75 and 3-Octyl-1-oxo-isothiochroman-4-carboxylic acid that we reported previously[25], the formation of π–π effect and H-bond at C-6 carboxyl of **4c** were not been influenced because of its planar feature. But the FAS inhibitory activity was lower because of benzene ring decreased the hydrophilic of the lactones moiety perhaps.

Compared **1c** and **C75**, there were most similar manner of interactions to the protein (Fig. 2, Right), and the introduction of sulfur atom to replace O-3 of **C75** could increase the inhibitory activity remarkably because it had stronger polarity.

In each series compounds, the enhancement of activities was found following by the carbon-chain elongation from *n*-C_6H_{13}– to *n*-$C_{11}H_{23}$–. It showed that with the extension of carbon chain, and its affinity with the protein also further increased. Unfortunately, the cytotoxicity was increased significantly.

Compound **1c**, which had more potent activity and higher therapeutic index (T.I., 9.26) than **C75**, was chosen to perform the advanced assay. It had performed potent anti-tumor activity on HL60 cells and Hela cells in vitro (IC_{50} were 5.38 ± 1.95 μM and 46.10 ± 2.30 μM, respectively) with a dose-dependent manner and the results were plotted in Figure 3.

4. Conclusion

In an attempt to discover new potent FAS inhibitors, several series of **C75** analogs were synthesized and evaluated. The results of the SAR studies indicated that there a little tolerance for changes at C-5 position of **C75**, and it was unfavorable of the hydrophobic

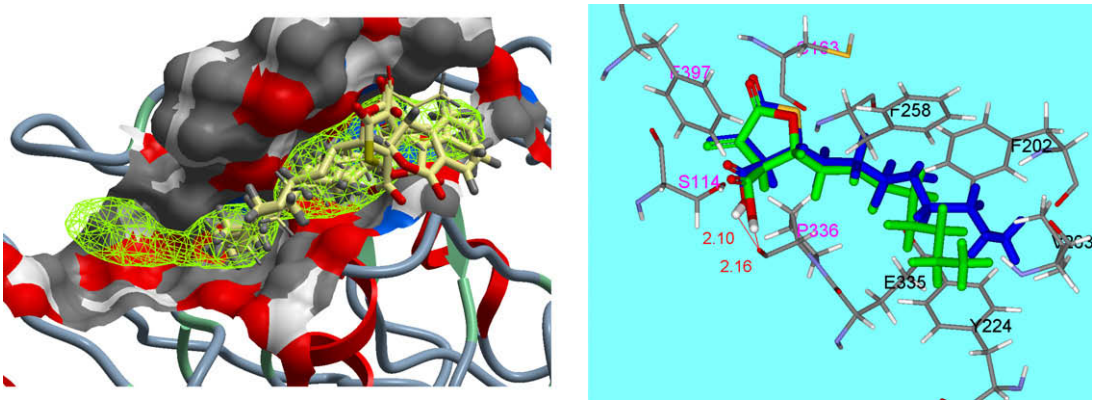


Figure 2. Left: The Docking model of compounds **C75**, **1c**, **2c**, **3c**, and **4c** with active site of KS domain. Right: **1c** (blue) and **C75** (green) in the active site. The red lines and numbers show the potential hydrogen bonds and bond length (2.10 and 2.16 Å, respectively). The α-methylene approached the aromatic moiety of Phe397 and π–π effect would be present. Figure was generated with Ds ViewPro 5.0.

Table 2
The results of docking compounds **C75**, **1c**, **2c**, **3c**, and **4c** into the active site

Compound	Energy (kcal/mol)					RMSD	H bond formation
	Van Der waals	Repulsion	Dispersion	Coulomb	Total		
C75	−30.4957	16.0382	−46.5244	−4.2395	−34.7291	1.09	Yes
1c	−30.8321	14.6643	−47.4937	−6.7366	−39.5630	0.96	Yes
2c	−25.7895	18.0467	−43.8339	−2.7217	−28.5089	1.14	No
3c	−24.7157	16.3109	−40.0195	−2.7792	−26.4815	1.82	No
4c	−29.2920	19.5318	−49.8140	−4.0198	−34.3020	1.14	Yes

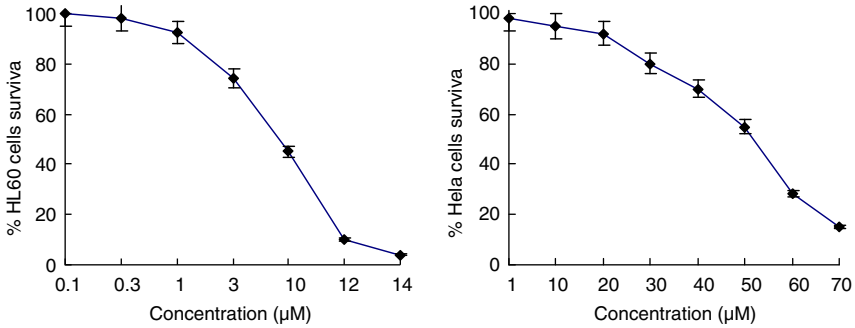


Figure 3. Effect of **1c** on the HL60 cells (left) and Hela cells (right). The EC_{50} were 5.38 ± 1.95 μM and 46.10 ± 2.30 μM, respectively.

and bulky groups that could prevent the formation of H-bond of carboxyl (C-1 position in **4a–f**, and C-5 in others) with protein, which was essential for activity. It was helpful to the activity to increase the polarity of γ-butyrolactone moiety such as compounds **1a–f**. Consequently, 4-methylene-2-octyl-5-oxo-tetrahydro-thiophene-3-carboxylic acid (**1c**) performed higher FAS inhibitory and therapeutic index, and it showed potent anti-tumor activity on HL60 cells and Hela cells in vitro.

5. Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz on JNM-ECA-400 instrument in the solvent indicated below, respectively. Proton and carbon chemical shifts are expressed in ppm relative to internal tetramethylsilane and coupling constants (*J*) are expressed in Hertz. Melting points were determined using a RY-1 apparatus. Thin-layer chromatography (TLC) was carried out on silica gel GF/UV 254 and the chromatograms were performed on silica gel (200–300 mesh) visualized under UV light at 254 and 365 nm.

5.1. General procedures for 6a–e

Triethylphosphonoacetate (**5**) (100 mmol) in dry THF was added dropwise to NaH (125 mmol) in dry THF at 0 °C under N_2. The reaction mixture was stirred overnight at room temperature and ethyl bromoacetate (100 mmol) was added dropwise at 0 °C. The mixture was stirred for 24 h at room temperature. The solution was concentrated and diluted with ethyl acetate. The organic layer was washed with brine and dried with $MgSO_4$. The intermediate **6** was obtained as a yellow liquid in 76% yield.

Phosphonate **6** (15 mmol) was dissolved in dry THF at 0 °C under N_2 followed by the addition of LDA (15 mmol). The reaction mixture was stirred at 0 °C for 30 min and then the aldehyde (15 mmol) was added dropwise. The reaction solution was warmed to room temperature and stirred overnight. Water was added and the solvent was removed under reduced pressure. The residue was diluted with water and CH_2Cl_2, and the organic phase was dried, filtered, and evaporated to dryness. The resulting oil was dissolved in trifluoroacetic/water (9:1) and stirred at room temperature for 3 h. The solvent was removed and the residue was treated with 10% aqueous NaOH (25 ml) and ethanol (20 ml). The solution was stirred by refluxing overnight and acidified with concd HCl. The reaction mixture was extracted with ethyl acetate and the organic phase was dried and evaporated. Compounds **8a–f** was obtained by silica gel chromatography (ethyl acetate/hexanes/acetic acid 30:70:1) as a white solid in 48–57% yield.

5.1.1. 2-Heptylidene-succinic acid (8a)
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (t, 1H, *J* = 5.8 Hz), 3.33 (s, 2H), 1.1–1.6 (m, 10H), 0.88 (t, 3H, *J* = 6.64 Hz); FAB MS(*m*/*e*) $[M]^+$ 214.1.

5.1.2. 2-Nonylidene-succinic acid (8c)
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.58 (t, 1H, *J* = 5.7 Hz), 3.36 (s, 2H), 1.1–1.6 (m, 14H), 0.88 (t, 3H, *J* = 6.66 Hz); FAB MS(*m*/*e*) $[M]^+$ 242.3.

5.1.3. 2-Decylidene-succinic acid (8d)
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.58 (t, 1H, *J* = 6.2 Hz), 3.39 (s, 2H), 1.1–1.6 (m, 16H), 0.89 (t, 3H, *J* = 6.58 Hz); FAB MS(*m*/*e*) $[M]^+$ 256.3.

5.1.4. 2-Undecylidene-succinic acid (8e)
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48 (t, 1H, *J* = 6.2 Hz), 3.38 (s, 2H), 1.1–1.6 (m, 18H), 0.88 (t, 3H, *J* = 6.64 Hz); FAB MS(*m*/*e*) $[M]^+$ 270.2.

5.1.5. 2-Dodecylidene-succinic acid (8f)
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.46 (t, 1H, *J* = 6.2 Hz), 3.39 (s, 2H), 1.1–1.6 (m, 20H), 0.89 (t, 3H, *J* = 6.56 Hz); FAB MS(*m*/*e*) $[M]^+$ 284.1.

5.2. General procedures for 10a–f

To a stirred solution of **8a–f** (20 mmol) in dry THF was added AcSH (6 ml) and it was heated to reflux and stirred for 3 days. The solution was concentrated under reduced pressure to afford 9a–**f** without purification. The resulting solution **9a–f** was dissolved in 6 M HCl (20 ml) and heated to reflux under N_2 for 6 h. The reaction was concentrated and dissolved in TFA (10 ml) and refluxed for another 1 h. The TFA was removed and compounds **10a–f** was obtained by silica gel chromatography (ethyl acetate/hexanes/acetic acid 2:10:0.05) as a white solid in 39–48.5% yield.

5.2.1. 2-Hexyl-5-oxo-tetrahydro-thiophene-3-carboxylic acid (10a)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (m, 1H), 3.09 (m, 1H), 2.84–3.04 (m, 2H), 1.1–2.1 (m, 10H), 0.86 (t, 3H, J = 6.86 Hz); FAB MS(m/e) $[M]^+$ 230.3.

5.2.2. 2-Octyl-5-oxo-tetrahydro-thiophene-3-carboxylic acid (10c)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (m, 1H), 3.10 (m, 1H), 2.83–3.04 (m, 2H), 1.1–2.1 (m, 14H), 0.86 (t, 3H, J = 6.88 Hz); FAB MS(m/e) $[M]^+$ 258.1.

5.2.3. 2-Nonyl-5-oxo-tetrahydro-thiophene-3-carboxylic acid (10d)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (m, 1H), 3.10 (m, 1H), 2.84–3.05 (m, 2H), 1.1–2.1 (m, 16H), 0.86 (t, 3H, J = 6.88 Hz); FAB MS(m/e) $[M]^+$ 272.2.

5.2.4. 2-Decyl-5-oxo-tetrahydro-thiophene-3-carboxylic acid (10e)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14 (m, 1H), 3.12 (m, 1H), 2.84–3.05 (m, 2H), 1.1–2.1 (m, 18H), 0.86 (t, 3H, J = 6.89 Hz); FAB MS(m/e) $[M]^+$ 286.1.

5.2.5. 2-Undecyl-5-oxo-tetrahydro-thiophene-3-carboxylic acid (10f)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14 (m, 1H), 3.13 (m, 1H), 2.84–3.05 (m, 2H), 1.1–2.1 (m, 20H), 0.86 (t, 3H, J = 6.90 Hz); FAB MS(m/e) $[M]^+$ 300.1.

5.3. General procedures for 1a–f

A solution of **10a–f** (10 mmol) in methyl methoxymagnesium carbonate in DMF (20 mmol) was stirred under argon at 135–140 °C for 3 days. The reaction mixture was then added to 10% aqueous HCl in the presence of CH_2Cl_2, and the CH_2Cl_2 layer was separated, dried, and evaporated below 30 °C. The crude diacid **11a–f** was obtained and it was treated with 2.0 ml of stock solution (prepared from 20 ml of acetic acid, 15 ml of 37% formaldehyde in water, 5.20 ml of *N*-methylaniline and 600 mg of sodium acetate) and stirred under argon at 20 °C for 2 h. The crude product was extracted with ether and purified by column silica gel chromatography (ethyl acetate/hexanes/acetic acid 2:10:0.05) to give the products **1a–f** as white solid in 29–50.4% yield.

5.3.1. 2-Hexyl-4-methylene-5-oxo-tetrahydro-thiophene-3-carboxylic acid (1a)

^{1}H NMR (400 MHz, CD_3Cl) δ 6.18 (d, 1H, J = 2.24 Hz), 5.61 (d, 1H, J = 2.0 Hz), 4.08 (dt, 1H, J = 9.04, 5.28 Hz), 3.71 (dt, 1H, J = 2.0, 5.32 Hz), 1.1–2.1 (m, 10H), 0.88 (t, 3H, J = 6.56 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.4, 27.6, 28.7, 31.5, 37.0, 47.0, 53.01, 120.1, 142.0, 175.8, 195.3; FAB MS(m/z) $[M+1]^+$ 243.1. Anal. Calcd for $C_{12}H_{18}O_3S$: C, 59.48; H, 7.49; O, 19.81; S, 13.23. Found: C, 59.45; H, 7.51; S 13.25.

5.3.2. 2-Octyl-4-methylene-5-oxo-tetrahydro-thiophene-3-carboxylic acid (1c)

^{1}H NMR (400 MHz, CD_3Cl) δ 6.18 (d, 1H, J = 2.36 Hz), 5.60 (d, 1H, J = 2.12 Hz), 4.08 (dt, 1H, J = 5.08, 9.28 Hz), 3.71 (dt, 1H, J = 2.28, 5.32 Hz), 1.1–2.1 (m, 14H), 0.86 (t, 3H, J = 6.68 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.6, 27.6, 28.3, 29.1, 29.2, 31.7, 36.9, 47.0, 53.0, 120.1, 142.1, 175.3, 195.4; FAB MS(m/z) $[M+1]^+$ 271.1. Anal. Calcd for $C_{14}H_{22}O_3S$: C, 62.19; H, 8.20; O, 17.75; S, 11.86. Found: C, 62.21; H, 8.17; S 11.81.

5.3.3. 2-Nonyl-4-methylene-5-oxo-tetrahydro-thiophene-3-carboxylic acid (1d)

^{1}H NMR (400 MHz, CD_3Cl) δ 6.17 (d, 1H, J = 2.48 Hz), 5.59 (d, 1H, J = 2.28 Hz), 4.08 (dt, 1H, J = 9.28, 5.32 Hz), 3.72 (dt, 1H, J = 5.52, 2.04 Hz), 1.1–2.1 (m, 16H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.6, 27.6, 29.2, 29.3, 29.4, 29.6, 31.8, 37.0, 47.1, 53.0, 120.0, 142.1, 175.6, 195.4; FAB MS(m/z) $[M+1]^+$ 285.1. Anal. Calcd for $C_{15}H_{24}O_3S$: C, 63.35; H, 8.51; O, 16.88; S, 11.27. Found: C, 63.37; H, 8.51; S, 11.23.

5.3.4. 2-Decyl-4-methylene-5-oxo-tetrahydro-thiophene-3-carboxylic acid (1e)

^{1}H NMR (400 MHz, CD_3Cl) δ 6.18 (d, 1H, J = 2.48 Hz), 5.61 (d, 1H, J = 2.28 Hz), 4.08 (dt, 1H, J = 9.28, 5.32 Hz), 3.71 (dt, 1H, J = 5.42, 2.14 Hz), 1.1–2.1 (m, 18H), 0.89 (t, 3H, J = 6.68 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 27.6, 29.2, 29.3, 29.4, 29.5, 29.6, 31.8, 37.0, 47.1, 53.1, 120.0, 142.2, 175.5, 195.5; FAB MS(m/z) $[M+1]^+$ 299.1. Anal. Calcd for $C_{16}H_{26}O_3S$: C, 64.39; H, 8.78; O, 16.08; S, 10.74. Found: C, 64.34; H, 8.80; S, 10.77.

5.3.5. 2-Undecyl-4-methylene-5-oxo-tetrahydro-thiophene-3-carboxylic acid (1f)

^{1}H NMR (400 MHz, CD_3Cl) δ 6.18 (d, 1H, J = 2.4 Hz), 5.59 (d, 1H, J = 2.12 Hz), 4.08 (dt, 1H, J = 9.28, 5.32 Hz), 3.71 (dt, 1H, J = 2.12, 5.32 Hz), 1.1–2.1 (m, 20H), 0.86 (t, 3H, J = 6.64 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 27.6, 29.1, 29.3(×2), 29.4, 29.5(×2), 31.8, 37.0, 47.0, 52.9, 120.1, 142.0, 175.4, 195.2; FAB MS(m/z) $[M+1]^+$ 313.1. Anal. Calcd for $C_{17}H_{28}O_3S$: C, 65.35; H, 9.03; O, 15.35; S, 10.26. Found: C, 65.39; H, 8.99; S, 10.22.

5.4. 2-Isopropylidene-succinic acid (13)

Potassium (4.73 g, 0.12 mol) was added into dry *t*-butyl alcohol (120 ml) stirring, and it was warmed up to 60–65 °C slowly. A clear liquid was obtained and diethyl succinate (30.8 g 0.177 mol), acetone (7 g 0.12 mol) and *t*-butyl alcohol (10 ml) was added. The reaction solution was refluxed for 20 min diluted with water (100 ml) and the aqueous layer was separated and acidified with concd HCl. The solution was extracted with ether and evaporated yielding red oil, and then it was refluxed with NaOH solution (2 M, 100 ml) overnight. The mixture was acidified with HCl and extracted with acetic ether. The organic phase was evaporated under reduced pressure and **13** was obtained as a white solid in 82% yield. Mp 159–162 °C, ^{1}H NMR (400 Hz, $CDCl_3$) δ 1.99 (s, 3H), 2.27 (s, 3H), 3.58 (s, 2H); FAB MS(m/z) $[M+1]^+$ 159.1.

5.5. 2-Isopropylidene-succinic acid 4-(4-methoxy-benzyl) ester (15)

Compound **13** (0.063 mol) was dissolved in 25 ml acetic anhydride and refluxed for 5–6 h **14** was obtained at 125–130 °C by distilled under 5 mmHg as a white solid (yield of 84.5%, mp 161–163 °C). A mixture of **14** and *p*-methoxybenzyl alcohol (15 ml) was stirred at 55–60 °C for 40 h. The reaction mixture was diluted with ether and the solution was poured into saturated aqueous $NaHCO_3$. The aqueous layer was separated and acidified with concd HCl. The product **15** was obtained in 55.3% yield by filtration and recrystallization from ethyl acetate–hexane as a white solid.

Mp 80–81 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 7.25 (d, 2H, J = 8.96 Hz), 6.87 (dd, 2H, J = 1.96, 8.68 Hz), 5.03 (s, 2H), 3.78 (s, 3H), 2.62 (s, 2H), 1.28 (s, 6H); FAB MS(m/z) $[M+1]^+$ 279.1.

5.6. General procedures for 2a–f

Lithiumdiisopropyl amide (40 mmol) was added to a solution of 20 mmol of **15** in anhydrous THF cooled at −78 °C. After stirring for 1 h, the aldehyde (20 mmol) in anhydrous THF was added and the solution was stirred at −78 °C for another 4 h. The solution was quenched with 6 N H_2SO_4 and extracted with ether. The ether layer was dried over anhydrous $MgSO_4$ and evaporated under reduced

pressure yielding a gummy solid **16a–f**, and then it was taken up in CH_2Cl_2 and treated with 1.5 ml of trifluoroacetic acid. The mixture was stirred at room temperature for 12 h. Aqueous $NaHCO_3$ was added, then the aqueous layer was separated and acidified with concd HCl and extracted with ether. The organic layers were dried with anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The mixture of *cis*-(**2′a**–**f**) and *trans*-diastereoisomers (**2a–f**) was separated by silica gel chromatography (ethyl acetate/hexanes/acetic acid 30:70:1) in 9.5–14% and 46.6–52% yield, respectively.

5.6.1. *trans*-4-Isopropylidene-2-hexyl-5-oxo-tetrahydro-furan-3-carboxylic acid (2a)

Mp 76.5–77.5 °C; ^{1}H NMR (400 MHz, CD_3Cl) δ *4.45 (m,* 1H), 3.90 (d, 1H, J = 7.2 Hz), 2.30 (s, 3H), 1.93 (s, 3H), 1.60–1.84 (m, 3H), 1.24–1.59 (m, 7H), 0.88 (t, 3H, J = 6.6 Hz); FAB MS(m/e) $[M+H]^+$ 255.1. Anal. Calcd for $C_{14}H_{22}O_4$ (254.33): C, 66.12; H, 8.72. Found: C, 66.40; H, 8.62.

5.6.2. *cis*-4-Isopropylidene-2-hexyl-5-oxo-tetrahydro-furan-3-carboxylic acid (2′a)

Mp 78–80 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 4.60 (br, 1H), 3.58 (br, 1H), 2.31 (s, 3H), 1.96 (s, 3H), 1.50–1.70 (m, 2H), 1.15–1.50 (m, 8H), 0.88 (t, 3H, J = 7.2 Hz); FAB MS(m/e) $[M+H]^+$ 255.1. Anal. Calcd for $C_{14}H_{22}O_4$ (254.33): C, 66.12; H, 8.72. Found: C, 66.38; H, 8.54.

5.6.3. *trans*-4-Isopropylidene-2-heptyl-5-oxo-tetrahydro-furan-3-carboxylic acid (2b)

Mp 75–77 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 4.45 (m, 1H), 3.90 (d, 1H, J = 7.8 Hz), 2.31 (s, 3H), 1.93 (s, 3H), 1.60–1.84 (m, 3H), 1.26–1.60 (m, 9H), 0.88 (t, 3H, J = 6.6 Hz); FAB MS(m/e) $[M+H]^+$ 269.1.

5.6.4. *trans*-4-Isopropylidene-2-octyl-5-oxo-tetrahydro-furan-3-carboxylic acid (2c)

Mp 75.5–77 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 4.45 (m, 1H), 3.91 (d, 1H, J = 7.2 Hz), 2.31 (s, 3H), 1.93 (s, 3H), 1.67–1.84 (m, 3H), 1.26–1.61 (m, 11H), 0.88 (t, 3H, J = 7.2 Hz); FAB MS(m/e) $[M+H]^+$ 283.1. Anal. Calcd for $C_{16}H_{26}O_4$ (282.38): C, 66.06; H, 9.28. Found: C, 65.60; H, 9.69.

5.6.5. *trans*-4-Isopropylidene-2-nonyl-5-oxo-tetrahydro-furan-3-carboxylic acid (2d)

Mp 79.5–81 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 4.45 (m, 1H), 3.91 (d, 1H, J = 7.2 Hz), 2.31 (s, 3H), 1.93 (s, 3H), 1.67–1.82 (m, 3H), 1.26–1.60 (m, 13H), 0.88 (t, 3H, J = 7.2 Hz); FAB MS(m/e) $[M+H]^+$ 297.1.

5.6.6. *trans*-4-Isopropylidene-2-decyl-5-oxo-tetrahydro-furan-3-carboxylic acid (2e)

Mp 82.5–83.5 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 4.45 (m, 1H), 3.91 (d, 1H, J = 7.2 Hz), 2.31 (s, 3H), 1.93 (s, 3H), 1.67–1.84 (m, 3H), 1.26–1.61 (m, 15H), 0.88 (t, 3H, J = 7.2 Hz); FAB MS(m/e) $[M+H]^+$ 311.1.

5.6.7. *trans*-4-Isopropylidene-2-undecyl-5-oxo-tetrahydro-furan-3-carboxylic acid (2f)

Mp 85–86 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 4.45 (m, 1H), 3.91 (d, 1H, J = 7.2 Hz), 2.31 (s, 3H), 1.93 (s, 3H), 1.68–1.82 (m, 3H), 1.26–1.60 (m, 17H), 0.88 (t, 3H, J = 7.2 Hz); FAB MS(m/e) $[M+H]^+$ 325.1.

5.7. General procedures for 3c–d

Compounds **3c–d** were prepared with the similar synthesis route to **2a–f** from the commercially available 2,2-dimethyl-succinic acid **17** via the intermediate **18**.

5.7.1. 2,2-Dimethyl-succinic acid 4-(4-methoxy-benzyl) ester (18)

Mp 74–75.5 °C, ^{1}H NMR (400 Hz, $CDCl_3$) δ 1.29 (s, 6H), 2.64 (s, 2H), 3.80 (s, 3H), 5.05 (s, 2H), 6.88 (d, 2H, J = 8.4 Hz), 7.27 (d, 2H, J = 8.2 Hz); FAB MS(m/z) $[M+1]^+$ 266.3.

5.7.2. *trans*-4,4-Dimethyl-2-octyl-5-oxo-tetrahydrofuran-3-carboxylic acid (3c)

^{1}H NMR (400 Hz, $CDCl_3$) δ 4.61 (m, 1H), 2.94 (d, 1H, J = 7.8 Hz), 1.29 (s, 6H), 1.28–1.54 (m, 14H), 0.88 (t, 3H, J = 6.6 Hz); FAB MS(m/e) $[M+H]^+$ 271.1. Anal. Calcd for $C_{15}H_{26}O_4$: C, 66.64; H, 9.69; O, 23.67. Found: C, 66.60; H, 9.72; mp 105.5–107 °C.

5.7.3. *cis*-4,4-Dimethyl-2-octyl-5-oxo-tetrahydrofuran-3-carboxylic acid (3′c)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.58 (m, 1H), 3.29 (m, 1H), 1.29 (s, 6H), 1.28–1.54 (m, 14H), 0.88 (t, 3H, J = 6.8 Hz); FAB MS(m/z) $[M+1]^+$ 271.3. Anal. Calcd for $C_{15}H_{26}O_4$: C, 66.64; H, 9.69; O, 23.67. Found: C, 66.61; H, 9.74; mp 64–66 °C.

5.7.4. *trans*-4,4-Dimethyl-2-nonyl-5-oxo-tetrahydrofuran-3-carboxylic acid (3d)

^{1}H NMR (400 Hz, $CDCl_3$) δ 4.63 (m, 1H), 2.98 (d, 1H, J = 7.6 Hz), 1.29 (s, 6H), 1.28–1.54 (m, 16H), 0.88 (t, 3H, J = 6.6 Hz); FAB MS(m/e) $[M+H]^+$ 285.1. Anal. Calcd for $C_{16}H_{28}O_4$: C, 67.57; H, 9.92; O, 22.50. Found: C, 67.60; H, 9.88; mp 116.5–118.5 °C.

5.8. General procedures for 4a–f

LDA (20 mmol) was added to a solution of 20 mmol of **19** in anhydrous THF cooled at −78 °C. After stirring for 1 h, $(i\text{-PrO})_3TiCl$ (20 mmol) in 20 ml anhydrous THF was added dropwise at same temperature and stirred for 2 h. Then the aldehyde (20 mmol) in anhydrous THF was added and the solution was stirred for another 4 h. The solution was quenched with 6 N H_2SO_4 and extracted with ether. The ether layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure yielding a gummy solid **22a–f**, and then it was treated with LiOH (1 M in water and methanol) at room temperature for 4 h. The solution was acidified with concd HCl and extracted with ether. The ether was concentrated and **4a–f** (*trans*-isomers) and **4′a–f** (*cis*-isomers) was separated by silica gel chromatography (ethyl acetate/hexanes/acetic acid 1:10:5%) in 35–39% and 21–26% yield, respectively.

5.8.1. *trans*-3-Hexyl-1-oxo-isochroman-4-carboxylic acid (4a)

Mp 76.5–77.5 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 8.16 (d, 1H, J = 6.72 Hz), 7.59 (m, 1H), 7.50 (m, 1H), 7.33 (d, 1H, J = 7.28 Hz), 4.61 (m, 1H), 3.85 (d, 1H, J = 3.08 Hz), 1.98 (m, 1H), 1.82 (m, 1H), 1.62 (m, 1H), 1.49 (m, 1H), 1.2–1.4 (m, 6H), 0.88 (t, 3H, J = 6.74 Hz); FAB MS(m/e) $[M+1]^+$ 277.1. Anal. Calcd for $C_{16}H_{20}O_4$ (276.14): C, 69.54; H, 7.30. Found: C, 69.56; H, 7.29.

5.8.2. *cis*-3-Hexyl-1-oxo-isochroman-4-carboxylic acid (4′a)

Mp 78–80 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 8.15 (d, 1H, J = 7.56 Hz), 7.62 (m, 1H), 7.50 (m, 1H), 7.32 (d, 1H, J = 7.56), 4.92 (m, 1H), 3.90 (d, 1H, J = 5.32 Hz), 1.78 (m, 1H), 1.61 (m, 1H), 1.1–1.5 (m, 8H), 0.86 (t, 3H, J = 6.72 Hz); FAB MS(m/e) $[M+1]^+$ 277.1. Anal. Calcd for $C_{16}H_{20}O_4$ (276.14): C, 69.54; H, 7.30. Found: C, 69.59; H, 7.28.

5.8.3. *trans*-3-Heptyl-1-oxo-isochroman-4-carboxylic acid (4b)

Mp 75–77 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 8.16 (d, 1H, J = 6.56 Hz), 7.58 (m, 1H), 7.50 (m, 1H), 7.33 (d, 1H, J = 7.28 Hz), 4.61 (m, 1H), 3.84 (d, 1H, J = 3.08 Hz), 1.97 (m, 1H), 1.82 (m, 1H), 1.62 (m, 1H), 1.48 (m, 1H), 1.2–1.4 (m, 8H), 0.88 (t, 3H, J = 6.88 Hz); FAB MS(m/e) $[M+1]^+$ 291.1.

5.8.4. *trans*-3-Octyl-1-oxo-isochroman-4-carboxylic acid (4c)

Mp 75–77 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 8.16 (d, 1H, J = 6.72 Hz), 7.59 (m, 1H), 7.50 (m, 1H), 7.34 (d, 1H, J = 7.28 Hz), 4.61 (m, 1H), 3.84 (d, 1H, J = 3.08 Hz), 1.98 (m, 1H), 1.83 (m, 1H), 1.63 (m, 1H), 1.49 (m, 1H), 1.2–1.4 (m, 10H), 0.88 (t, 3H, J = 6.74 Hz); FAB MS(m/e) $[M+1]^+$ 305.1. Anal. Calcd for $C_{18}H_{24}O_4$ (304.39): C, 71.03; H, 7.95. Found: C, 71.39; H, 8.21.

5.8.5. *trans*-3-Nonyl-1-oxo-isochroman-4-carboxylic acid (4d)

Mp 75–77 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 8.15 (d, 1H, J = 7.56 Hz), 7.58 (m, 1H), 7.50 (m, 1H), 7.32 (d, 1H, J = 7.56 Hz), 4.61 (m, 1H), 3.84 (d, 1H, J = 3.08 Hz), 1.97 (m, 1H), 1.83 (m, 1H), 1.60 (m, 1H), 1.45 (m, 1H), 1.0–1.4 (m, 12H), 0.87 (t, 3H, J = 6.74 Hz); FAB MS(m/e) $[M+1]^+$ 319.1.

5.8.6. *trans*-3-Decyl-1-oxo-isochroman-4-carboxylic acid (4e)

Mp 75–77 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 8.15 (d, 1H, J = 7.00 Hz), 7.58 (m, 1H), 7.50 (m, 1H), 7.32 (d, 1H, J = 7.56 Hz), 4.61 (m, 1H), 3.84 (d, 1H, J = 3.08 Hz), 1.98 (m, 1H), 1.83 (m, 1H), 1.60 (m, 1H), 1.49 (m, 1H), 1.0–1.4 (m, 14H), 0.87 (t, 3H, J = 6.86 Hz); FAB MS(m/e) $[M+1]^+$ 333.1.

5.8.7. *trans*-3-Undecyl-1-oxo-isochroman-4-carboxylic acid (4f)

Mp 75–77 °C, ^{1}H NMR (400 MHz, CD_3Cl) δ 8.16 (d, 1H, J = 6.72 Hz), 7.59 (m, 1H), 7.50 (m, 1H), 7.34 (d, 1H, J = 7.56 Hz), 4.61 (m, 1H), 3.85 (d, 1H, J = 5.60 Hz), 1.99 (m, 1H), 1.83 (m, 1H), 1.62 (m, 1H), 1.49 (m, 1H), 1.0–1.4 (m, 16H), 0.88 (t, 3H, J = 6.76 Hz); FAB MS(m/e) $[M+1]^+$ 347.1.

5.9. Biology

FAS was purified from rat liver[24] and it was 90% pure as estimated from SDS/PAGE with Coomassie blue staining. The purified FAS, compounds, NADPH and acetyl-CoA were incubated in K_2HPO_4 buffer (pH 7.0) at 37 °C for 30 min then malonyl-CoA was added. The reaction was assayed for an additional 3 min to determine FAS-dependent oxidation of NADPH and the absorbance was monitored at 340 nm in a heated chamber S500P spectrophotometer at 37 °C.[26] Linear-regression-analysis was used to calculate the IC_{50} values and values were reported as means of triplicate experiments. The cytotoxicity was evaluated by human embryonic lung fibroblast (HLF) cells using the MTT method.

The exponential growth HL60 cells were suspended in 1640 medium. Approximately 2×10^4 cells were plated in each well of a 24-well plate and the gradient diluted **1c** dissolved in DMSO was added individual and they were incubated in 5% CO_2 at 37 °C for 2 h, and the number of viable cells was determined using the MTT method.

Hela cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Hyclone) supplement with 10% fetal calf serum (FBS) at 37 °C in an atmosphere of 5% CO_2 for 24 h. Approximately 2.5×10^4 cells were plated in each well and different concentrations of **1c** dissolved in DMSO were added to into the wells for 20 h. The cells were trypsinized, and measured by trypan-blue exclusion. A single experiment was repeated three times to calculate the standard deviation.

5.10. Docking study

KS domain (402 amino acid residues) of human FAS was built on InsightII/Homology module using *Escherichia coli*. KASII protein (PDB code 1FJ8) and Synechocystis SP KASII protein (PDB code 1E5M) as template. They had about 23% homology. The result was optimized under CVFF on Discover module. The geometry optimization was operated using 800 steps of steepest decent and 3000 steps of conjugated gradient and followed by 20 ps molecular dynamics optimizations. Then the energy optimization was carried out using 500 steps of steepest decent and 3000 steps of conjugated gradient and the minimal gradient of energy was 89.54 kJ/nm mol. The scores of Profile 3D is 149.4, and the RMSD is 0.25.

The active site was defined based on the interaction site of 1FJ8 and Cerulenin. Docking was carried out on Discovery/Affinity, and only residues within 5.0 Å of substrates were allowed to move during the geometry optimizations under CVFF using 500 steps of steepest decent and 1000 steps of conjugated gradient.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.01.050.

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