Identification of Cyclicsulfonamide Derivatives with an Acetamide Group as 11β-Hydroxysteroid Dehydrogenase 1 Inhibitors

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In the continuation of our 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitor research, cyclic sulfonamide derivatives with an acetamide group at the 2-position were synthesized and evaluated for their abilities to inhibit 11β-HSD1. Among this series, Compound 34 showed good *in vitro* activity toward human 11β-HSD1, selectivity against 11β-HSD2, microsomal stability, good pharmacokinetic and safety profiles human ether-a-go-go related gene (hERG and cytochrome P450 (CYP)). Also, a docking study explained the activity difference between human and mouse 11β-HSD1.

Key words diabetes; anti-diabetic agent; 11β-hydroxysteroid dehydrogenase type 1; cyclic sulfonamide

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an endoplasmic reticulum-associated enzyme that acts predominantly as an reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reductase *in vivo* and converts inactive cortisone to the active glucocorticoid cortisol[1—3] (Fig. 1).

The relation between 11β-HSD1 and type 2 diabetes has been demonstrated in mouse genetic models. Mice overexpressing 11β-HSD1 in adipose showed metabolic syndrome-like phenotypes such as central obesity, glucose intolerance, and insulin resistance.[4,5] In contrast, 11β-HSD1 deficient mice were resistant to the development of high-fat diet-induced obesity and exhibited improved insulin sensitivity and lipid profiles.[6,7] These data suggest that 11β-HSD1 could be a drug target for the treatment of metabolic syndrome as well as type 2 diabetes. During the last few years, small molecule 11β-HSD1 inhibitors have been reported,[8—13] and several candidates including Incyte's compound are in clinical trials.

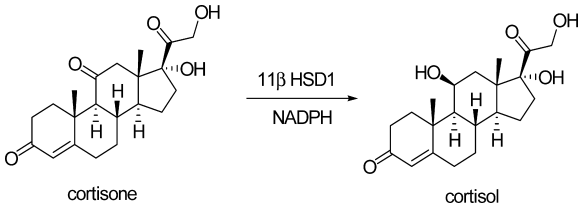
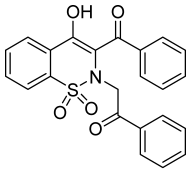


Fig. 1. The Role of 11β-HSD1 between Cortisone and Cortisol

Table 1. *In Vitro* CYP Inhibitions of Compound **A**



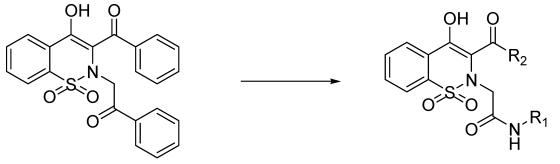
A

CYP subtype	Inhibition at 10 μM (%)
1A2	7.9
2C19	90.5
2D6	79.8
3A4	73.0

In an earlier report, we described the synthesis and biological evaluation of a new series of cyclic sulfonamide derivatives.[14] Among the cyclic sulfonamide derivatives, compound **A** showed good potency, selectivity, reasonable metabolic stability and pharmacokinetic (PK) profiles. Therefore, we have further evaluated toxicity related tests with compound **A**, which was found to inhibit cytochrome P450 (CYP) subtypes (2C19, 2D6, 3A4) as shown in Table 1. CYP enzymes play a major role in metabolizing drug molecules. Many lead candidate molecules in pharmaceutical development fail due to inhibition of one or more isozymic forms of CYP enzymes.

In order to overcome CYP inhibition, a series of new cyclicsulfonamide derivatives with an acetamide group was developed (Fig. 2). We now wish to report here the synthesis and biological evaluation of cyclicsulfonamide derivatives with an acetamide group as 11β-hydroxysteroid dehydrogenase 1 inhibitors.

Synthesis A series of cyclic sulfonamide derivatives was synthesized according to Chart 1. Saccharin sodium salt **1** was reacted with alpha-bromo ketones in *N*,*N*-dimethylformamide (DMF) to provide the *N*-alkylated product **2**. Compound **2** on refluxing in sodium–ethanol resulted in ring expansion and formed cyclic sulfonamide **3**. It was further alkylated with ethyl bromoaceate resulting in the correspon-



A **6-34**

cyclic sulfonamide derivatives with an acetamide group

Fig. 2. Structural Modification

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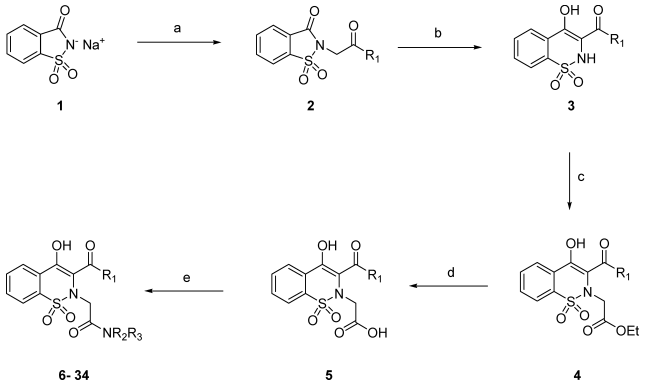


Reagents and conditions: (a) $BrCH_2COR_1$ (R_1=methyl, phenyl), DMF, 100 °C, 4 h; (b) Na, EtOH, reflux, 4 h; (c) 1 M NaOH, ethyl bromoacetate, room temperature, 12 h; (d) LiOH, THF/MeOH/H_2O, room temperature, 5 h; (e) Amines, EDCI, DMAP, CH_2Cl_2 (or DMF), room temperature, 5 h.

Chart 1

ding coupled product **4**. Alkaline hydrolysis then provided acid **5**, which was further derivatized with diverse amines to obtain the final amides **6**—**34**.

Results and Discussion

In vitro inhibition activity of 11β-HSD1 was assessed by a Homogeneous Time Resolved Fluorescence (HTRF) cortisol assay. Human microsomes were incubated with cortisone, NADPH and compound. The IC_{50} values of the compounds were determined from concentration-dependent inhibition curves. Carbenoxolone was used as a standard 11β-HSD1 inhibitor.[15)]

First, acid **5** was inactive toward 11β-HSD1, but adamantyl amide **6** was modestly potent with an IC_{50} of 3.5 μM as shown in Table 2. Also, cyclohexyl amide **7** showed submicromolar activity (0.9 μM). Therefore, we further derivatized with aliphatic amines, however, their activities (**8**—**10**) were detrimental.

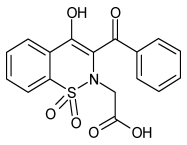
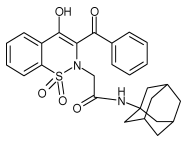
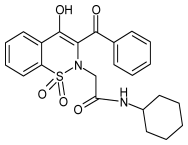
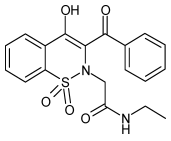
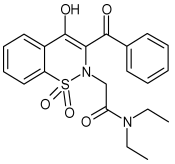
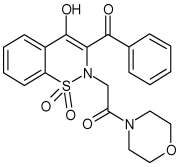
We then focused our attention on aromatic amides as shown in Table 3. Benzyl amide (**11**) showed a good *in vitro* activity with an IC_{50} value of 174 nM. Whereas, heteroaryl amides (**12**, **13**) were found to be less potent. Other substituted benzyl amide derivatives (**14**—**18**) exhibited weak to moderate inhibitory activities (0.264—8.06 μM).

Among the aromatic amides, anilides were synthesized and evaluated for their *in vitro* activity. As shown in Table 4, compound **19**, which is a simple aromatic anilide, showed a good inhibitory activity with an IC_{50} value of 102 nM. Therefore, we chose **19** as a prototype compound for further derivatization. The substituent effect of **19** at the 3-postion was investigated. The acetyl (**20**) and oxime (**21**) derivatives resulted in loss of *in vitro* activity. *N*-Methylanilide (**22**) and heteroaryl amide (**23**) also showed poor activities.

Compound **19** was evaluated for its CYP inhibition. Fortunately, **19** was found to show no CYP inhibitions as shown in Table 5. Based on these results, we further synthesized anilide derivatives having phenyl ring substituents.

The substituent effect of anilide derivatives was evaluated as shown in Table 6. Methyl substituents at *ortho*, *meta* and *para* positions (**24**—**26**) were similar or less potent than unsubstituted anilide **19** with IC_{50} values in the range of 129—795 nM. Also, although methoxy and fluoro substituted anilide derivatives (**27**—**30**) showed good *in vitro* potencies

Table 2. *In Vitro* 11β-HSD1 Inhibitory Activity of Cyclic Sulfonamide Derivatives

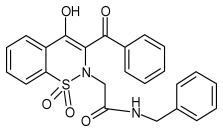
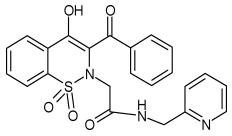
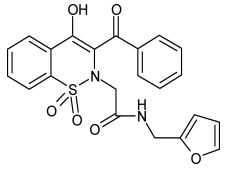
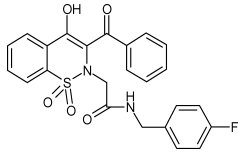
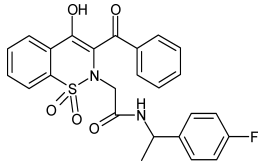
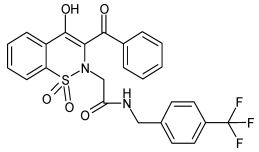
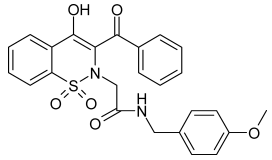
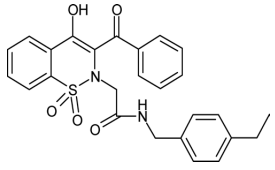
Compound	Structure	IC_{50}, μM[a)]
5		Not active 34% at 10 μM
6		3.5
7		0.9
8		Not active 45% at 10 μM
9		Not active 4% at 10 μM
10		Not active 44% at 10 μM
Carbenoxolone		0.5

a) IC_{50} values were determined by GraphPad Prism software.

(122—272 nM), unsubstituted anilide **19** was still more potent. Compound **31** is the first compound to improve on **19**'s activity with an IC_{50} value of 83 nM. Furthermore, compound **34**, which is the most potent in this series, showed better *in vitro* activity with an IC_{50} value of 41 nM.

Based on the above data, we chose three representative compounds (**31**, **32**, **34**) for further biological evaluations which include mouse 11β-HSD1 inhibition potency, selectivity, metabolic stability, human ether a-go-go related gene (hERG), CYP assay and PK study in rat (Table 7). All compounds showed good *in vitro* activities toward human 11β-HSD1 and no significant CYP inhibition as we wished. However, *in vitro* activities toward mouse 11β-HSD1 were at micromolar levels with IC_{50} values in the range of 3—7 μM. In order to better understand the activity difference between human and mouse 11β-HSD1, we investigated a binding mode of compound **34** in both human and mouse 11β-HSD1

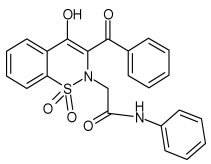
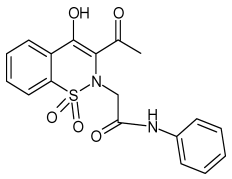
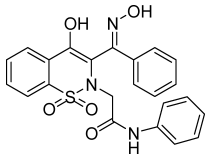
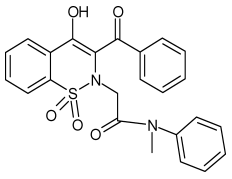
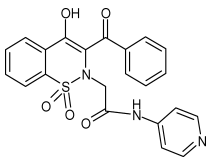
Table 3. *In Vitro* 11β-HSD1 Inhibitory Activity of Cyclic Sulfonamide Derivatives

Compound	Structure	IC_{50}, μM[a)]
11		0.174
12		0.978
13		2.58
14		1.61
15		8.06
16		1.61
17		2.20
18		0.263
Carbenoxolone		0.5

a) IC_{50} values were determined by GraphPad Prism software.

(Fig. 3). Our model suggests that compound **34** interacts through H-bonding with S170 and Y183 residues in the active sites in 11β-HSD1, and also shows the hydrophobic interaction with Y177 in human form. However, in mouse sterically unfavorable ineraction with Y284 residues in the B-chain resulted in lowering activity.

Table 4. *In Vitro* 11β-HSD1 Inhibitory Activity of Cyclic Sulfonamide Derivatives

Compound	Structure	IC_{50}, μM[a)]
19		0.102
20		Not active 25% at 10 μM
21		Not active 5% at 10 μM
22		10.6
23		0.342
Carbenoxolone		0.5

a) IC_{50} values were determined by GraphPad Prism software.

Table 5. *In Vitro* CYP Inhibition of Compound **19**

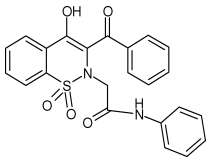
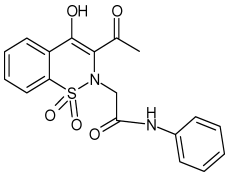
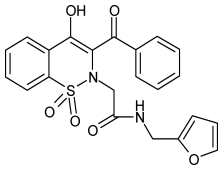
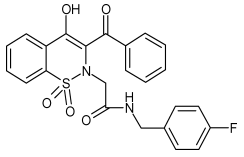
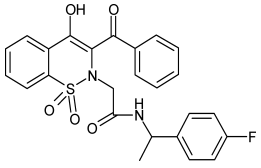
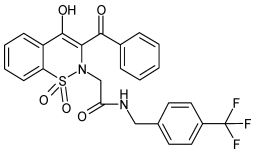
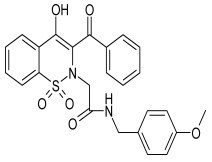
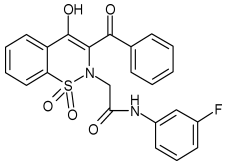
CYP subtype	Inhibition at 10 μM (%)
1A2	5
2C19	0
2D6	8
3A4	10

Compound **34** was the most potent *in vitro* toward both human and mouse 11β-HSD1. Also **34** showed a good selectivity, metabolic stability and no binding with hERG. The rat PK profiles of compound **34** showed good systemic exposure and oral bioavailability with an acceptable clearance and half-life.

Conclusion

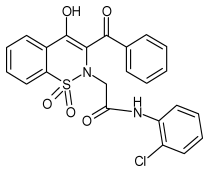
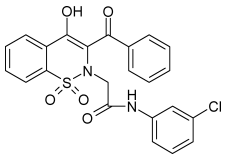
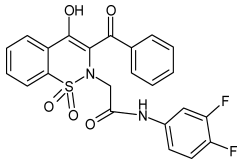
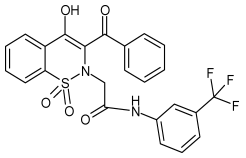
We have developed a series of cyclic sulfonamide derivative with an acetamide group as 11β-HSD1 inhibitors. Compound **34** showed good *in vitro* activity toward human 11β-HSD1, selectivity toward 11β-HSD2, metabolic stability,

Table 6. *In Vitro* 11β-HSD1 Inhibitory Activity of Cyclic Sulfonamide Derivatives

Compound	Structure	hHSD1 IC_{50}, $\mu M^{a)}$
19		0.102
24		0.278
25		0.129
26		0.795
27		0.124
28		0.256
29		0.122
30		0.272

good PK and safety profile such as hERG and CYP. Also, a docking study explained the activities difference between human and mouse 11β-HSD1.

Table 6. (Continue)

Compound	Structure	hHSD1 IC_{50}, $\mu M^{a)}$
31		0.083
32		0.092
33		0.079
34		0.041
Carbenoxolone		0.5

a) IC_{50} values were determined by GraphPad Prism software.

Experimental

Chemistry All reported yields are isolated yields after column chromatography or crystallization. ^{1}H-NMR spectra were obtained on FT-NMR Bruker AVANCE-300 with tetramethylsilane (TMS) as an internal reference. High-resolution mass spectra were obtained on the Autospec magnetic sector mass spectrometer (Micromass, Manchester, U.K.). Low-resolution mass spectra were obtained on the liquid chromtography-mass spectrometer (LC-MS, Waters, U.S.A.). General Procedure for the Synthesis of compound **34**.

2-(2-Oxo-2-phenylethyl)benzo[*d*]isothiazol-3(2*H*)-one-1,1-dioxide (2) Saccharin sodium salt (10 g, 0.049 mol) was dissolved in DMF (100 ml) and was treated with 2-bromoacetophenone (0.059 mol) at room temperature. The mixture was stirred for 4 h at 100 °C. The solution was poured into ice/water, and the resulting solid was collected and dried *in vacuo* to give compound **2** (12.98 g, 88%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.12 (d, J=6.6 Hz, 1H), 8.01 (m, 5H), 7.65 (t, J=7.8 Hz, 1H), 7.53 (t, J=7.8 Hz, 2H), 5.15 (s, 2H).

(4-Hydroxy-1,1-dioxido-2H-benzo[*e*][1,2]thiazin-3-yl)(phenyl)methanone (3) A solution of NaOEt, prepared from 2.3 g (33.18 mmol) of sodium in 100 ml of ethanol was heated to 40 °C and 5.0 g (16.59 mmol) of compound **2** was added all at once as the powder. The mixture was quickly heated to 50—55 °C and maintained at this temperature for 3 h. It was then quickly cooled to 25 °C and 10 ml of 9% HCl was added as rapidly as possible while maintaining the temperature at 30—35 °C. The crystals which separated from solution were collected by filtration, washed with 50% aq. EtOH, and dried *in vacuo* at 60 °C for 1 h to yield compound **3** (3.9 g, 80%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.25 (d, J=7.8 Hz, 1H), 8.05 (d, J=6.9 Hz, 2H), 7.94 (m, 1H), 7.82 (m, 2H), 7.58 (m, 3H), 5.82 (brs, 1H).

Ethyl 2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)acetate (4) Compound **3** (1 g, 3.32 mmol) was dissolved in ethanol (25 ml) and was treated with 1 M aqueous NaOH (5 ml) followed by ethyl bromoacetate (665 mg, 3.98 mmol) and was stirred at room temperature for 12 h. The resulting suspension was filtered, and the solid was washed with water and dried *in vacuo* to give compound **4** (587 mg, 45%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.13—8.08 (m, 1H), 7.88—7.81 (m, 5H), 7.60—

Table 7. *In Vitro* Inhibition, Metabolic Stability, hERG and PK Study of Cyclic Sulfonamide Derivatives

Entry	hHSD1 IC_{50}, μM[a)]	mHSD1 IC_{50}, μM[a)]	hHSD2 IC_{50}, μM[a)]	Microsomal stability 30 min after	hERG	CYP inhibition % at 10 μM		PK in rat
34	0.041	3.0	20% at 10 μM	85% (h) 69% (r)	>100 μM	1A2 2C19 2D6 3A4	0% 17% 20% 7%	PO C_{max}=1.1 μg/ml $t_{1/2}$=5.5 h *CL* (l/h/kg)=0.4 *F*=48.1%
33	0.079	6.3	NT[b)]	63% (h) 66% (r)	26 μM	1A2 2C19 2D6 3A4	0% 0% 0% 0%	*F*=84.6%
31	0.083	7.0	NT	85% (h) 68% (r)	57 μM	1A2 2C19 2D6 3A4	0% 24% 35% 32%	*F*=84.6%
Carben-oxolone	0.5							

a) IC_{50} values were determined by GraphPad Prism software. *b*) Not tested.

7.48 (m, 3H), 3.92 (s, 2H), 3.69 (q, *J*=7.1 Hz, 2H), 0.78 (t, *J*=7.1 Hz, 3H); LC-MS (*m*/*z*): 388 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)acetic Acid (5) To a solution of **4** (340 mg, 0.878 mmol) in tetrahydrofuran (THF)/MeOH (5 ml/5 ml), was added a solution of LiOH (185 mg, 4.39 mmol) in H_2O (5 ml). The reaction mixture was stirred for 5 h, and then the solvent was evaporated. Ice was poured into this residue, and acidified by 2 N HCl to adjust it to pH 3. The resulting solution was extracted with EtOAc and the organic layer was separated, dried and evaporated under reduced pressure to give **5** (300 mg, 95%). mp 201—203 °C; IR (KBr): 1692, 1727 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.67 (s, 1H), 8.18—8.15 (m, 1H), 8.00—7.98 (m, 2H), 7.93—7.90 (m, 3H), 7.72—7.58 (m, 3H), 3.80 (s, 2H); LC-MS (*m*/*z*): 360 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-[3-(trifluoromethyl)phenyl]acetamide (34) To a solution of **5** (50 mg, 0.139 mmol) and *m*-trifluoromethylaniline (26.9 mg, 0.167 mmol) in CH_2Cl_2, were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (40 mg, 0.209 mmol) and 4-dimethylaminopyridine (DMAP) (cat). The reaction mixture was stirred for 5 h at room temperature and then brine and CH_2Cl_2 were added. The organic layer was separated, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give **34** (21 mg, 30%). mp 292—294 °C; IR (KBr): 3359 (NH) cm^{-1}, 1692, 1712 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 15.07 (s, 1H), 10.17 (s, 1H), 8.19—7.18 (m, 13H), 4.00 (s, 2H); high resolution-mass spectra (HR-MS) ($C_{24}H_{17}F_3N_2O_5S$): Calcd 502.0810, Found 502.0807.

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-adamantylacetamide (6) mp 146—148 °C; IR (KBr): 3323 (NH) cm^{-1}, 1667, 1680 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.67 (s, 1H), 8.18—8.15 (m, 1H), 8.00—7.98 (m, 2H), 7.93—7.90 (m, 3H), 7.72—7.58 (m, 3H), 3.80 (s, 2H); LC-MS (*m*/*z*): 360 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-cyclohexylacetamide (7) mp 145—147 °C; ^{1}H-NMR (300 MHz, CD_3OD) δ: 8.23—8.18 (m, 1H), 8.08—8.03 (m, 2H), 7.84—7.73 (m, 2H), 7.66—7.54 (m, 4H), 3.68 (s, 2H), 3.18—3.12 (m, 1H), 1.62—0.85 (m, 10H); LC-MS (*m*/*z*): 441 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-ethylacetamide (8) mp 155—157 °C; IR (KBr): 3273 (NH) cm^{-1}, 1685, 1716 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.13 (m, 1H), 8.00—7.98 (m, 2H), 7.90—7.82 (m, 4H), 7.68—7.58 (m, 3H), 3.69 (s, 2H), 2.74 (m, 2H), 0.76 (t, *J*=7.2 Hz, 3H); LC-MS (*m*/*z*): 387 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*,*N*-diethylacetamide (9) mp 163—165 °C; IR (KBr): 1654, 1697 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.23 (d, *J*=6.6 Hz, 1H), 8.09 (d, *J*=7.2 Hz, 2H), 7.85—7.82 (m, 1H), 7.80—7.71 (m, 3H), 7.62—7.50 (m, 3H), 4.22 (s, 1H), 3.53 (s, 1H), 2.95—2.88 (m, 4H), 0.90 (t, *J*=7.1 Hz, 3H), 0.79 (t, *J*=7.1 Hz, 3H); LC-MS (*m*/*z*): 415 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-1-morpholinoethanone (10) mp 134—136 °C; IR (KBr): 1662, 1718 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.14 (m, 1H), 8.00 (d, *J*=7.2 Hz, 2H), 7.90—7.85 (m, 3H), 7.68—7.58 (m, 3H), 4.02 (s, 2H), 3.34 (s, 4H), 3.03 (s, 4H); LC-MS (*m*/*z*): 429 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-benzylacetamide (11) mp 233—235 °C; IR (KBr): 3358 (NH) cm^{-1}, 1675, 1702 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.85—7.83 (m, 1H), 7.67—7.59 (m, 3H), 7.31—7.21 (m, 10H); LC-MS (*m*/*z*): 449 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(pyridin-2-ylmethyl)acetamide (12) mp 176—178 °C; IR (KBr): 3377 (NH) cm^{-1}, 1687, 1695 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.50—8.40 (m, 2H), 8.13—8.09 (m, 1H), 8.02—7.99 (m, 2H), 7.87—7.86 (m, 3H), 7.72—7.59 (m, 4H), 7.23—7.19 (m, 1H), 7.00 (br d, 1H), 4.05 (d, *J*=7.2 Hz, 2H), 3.86 (m, 2H); LC-MS (*m*/*z*): 450 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(furan-2-ylmethyl)acetamide (13) IR (KBr): 3335 (NH) cm^{-1}, 1678, 1701 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.24 (s, 1H). 8.13—8.09 (m, 1H), 8.00—7.97 (m, 2H), 7.89—7.81 (m, 3H), 7.69—7.57 (m, 3H), 7.48—6.47 (m, 1H), 6.30—6.29 (m, 1H), 6.03—6.02 (m, 1H), 3.91 (d, *J*=5.2 Hz, 2H), 3.76 (s, 2H); LC-MS (*m*/*z*): 439 (MH^+).

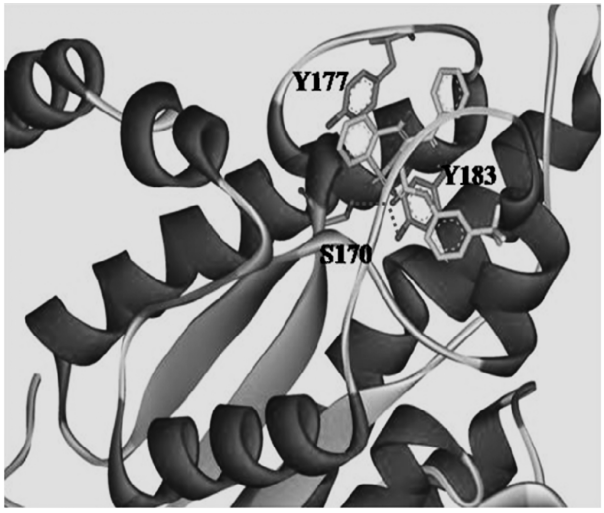
2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(4-fluorobenzyl)acetamide (14) IR (KBr): 3352 (NH) cm^{-1}, 1670, 1697 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.31 (s, 1H). 8.12—8.08 (m, 1H), 8.01—7.99 (m, 2H), 7.90—7.82 (m, 3H), 7.70—7.57 (m, 3H), 7.09—6.98 (m, 4H), 3.92 (d, *J*=5.5 Hz, 2H), 3.79 (s, 2H); LC-MS (*m*/*z*): 467 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(1-(4-fluorophenyl)ethyl)acetamide (15) mp 162—164 °C; IR (KBr): 3302 (NH) cm^{-1}, 1660, 1692 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.27—8.24 (m, 1H), 8.07—7.99 (m, 3H), 7.85—7.80 (m, 3H), 7.70—7.58 (m, 3H), 7.10—7.01 (m, 4H), 4.52—4.47 (m, 1H), 3.76 (s, 2H), 1.08 (d, *J*=6.9 Hz, 3H); LC-MS (*m*/*z*): 481 (MH^+).

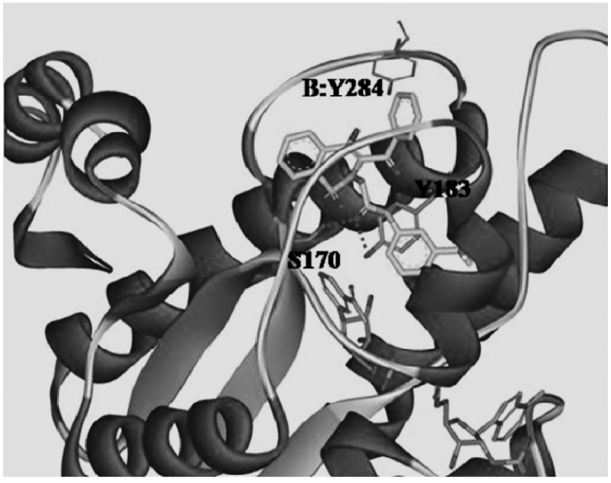
2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(4-(trifluoromethyl)benzyl)acetamide (16) IR (KBr): 3288 (NH) cm^{-1}, 1672, 1700 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.42 (s, 1H). 8.10—7.99 (m, 3H), 7.85—7.82 (m, 3H), 7.70—7.58 (m, 5H), 7.21—7.19 (m, 2H), 4.03 (d, *J*=5.3 Hz, 2H), 3.82 (s, 2H); LC-MS (*m*/*z*): 517 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(4-(methoxy)benzyl)acetamide (17) IR (KBr): 3312 (NH) cm^{-1}, 1683, 1711 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.74 (s, 1H), 8.18—8.17 (m, 1H), 8.05—8.04 (m, 2H), 7.93—7.86 (m, 3H), 7.70—7.62 (m, 3H), 7.12—7.10 (m, 2H), 6.76—6.74 (m, 2H), 3.95 (s, 2H), 3.65 (s, 3H); LC-MS (*m*/*z*): 465 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(4-ethylbenzyl)acetamide (18) IR (KBr): 3306 (NH) cm^{-1}, 1667, 1708 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.76 (s, 1H), 8.17—8.14 (m, 1H), 8.04—8.01 (m, 2H), 7.90—7.84 (m, 4H), 7.68—7.58 (m, 3H),



(a)



(b)

Fig. 3. The Binding Mode of **34** Was Shown for Human (a)[16] and Mouse (b) 11β-HSD1

The used reference structure for 11β-HSD1 complex was obtained from protein data bank (pdb entry; 3FRJ for human, 1Y5M for mouse). The calculation for docking was carried out using LigandFit[17] interfaced with Accelrys DiscoveryStudio2.5.

7.09—6.96 (m, 4H), 3.95 (s, 2H), 2.44 (q, J=7.5 Hz, 2H), 1.05 (t, J=7.5 Hz, 3H); LC-MS (m/z): 463 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-phenylacetamide (19) mp 128—130 °C; IR (KBr): 3280 (NH) cm^{-1}, 1660, 1680 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.85 (br s, 1H), 9.13—7.85 (m, 6H), 7.85 (m, 3H), 7.18—7.16 (m, 4H), 6.96—6.92 (m, 1H), 3.95 (s, 2H); HR-MS ($C_{23}H_{18}N_2O_5S$): Calcd 434.0936, Found 434.0936.

2-(3-Acetyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-phenylacetamide (20) mp 188—190 °C; IR (KBr): 3345 (NH) cm^{-1}, 1620, 1671 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.04—7.99 (m, 1H), 7.81—7.66 (m, 3H), 7.48—7.40 (m, 2H), 7.29—7.21 (m, 2H), 7.03—6.97 (m, 1H), 4.13—3.89 (m, 2H), 2.48—2.47 (m, 3H); LC-MS (m/z): 373 (MH^+).

(*E*)-2-{1,1-Dioxo-4-hydroxy-3-[(hydroxyimino)(phenyl)methyl]-2*H*-benzo[*e*][1,2]thiazin-2-yl}-*N*-phenylacetamide (21) mp 214—216 °C; IR (KBr): 3375 (NH) cm^{-1}, 1651 (C=O) cm^{-1}, 1604 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) δ: 8.08—8.06 (m, 1H), 7.91—7.89 (m, 3H), 7.84—7.79 (m, 1H), 7.67—7.62 (m, 1H), 7.43—7.37 (m, 3H), 7.28—7.20 (m, 4H), 7.09—7.05 (m, 1H), 3.85 (s, 2H); LC-MS (m/z): 450 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-methyl-*N*-phenylacetamide (22) mp 186—188 °C; IR (KBr): 1668, 1744 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.28—8.25 (m, 1H), 7.90—7.85 (m, 3H), 7.80—7.75 (m, 2H), 7.47—7.34 (m, 3H), 7.26—7.22 (m, 3H), 6.85—6.82 (m, 3H), 2.94 (s, 3H); LC-MS (m/z): 448.96 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(pyridin-4-yl)acetamide (23) mp 150—152 °C; IR (KBr): 3262 (NH) cm^{-1}, 1660, 1726 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.36—7.18 (m, 13H), 4.02 (s, 2H); LC-MS (m/z): 436 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-*o*-tolylacetamide (24) IR (KBr): 3293 (NH) cm^{-1}, 1670, 1692 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.27 (s, 1H). 8.13—8.03 (m, 3H), 7.88—7.84 (m, 3H), 7.72—7.60 (m, 3H), 7.08—6.98 (m, 4H), 4.00 (s, 2H), 1.92 (s, 3H); LC-MS (m/z): 449 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-*m*-tolylacetamide (25) mp 134—136 °C; IR (KBr): 3257 (NH) cm^{-1}, 1693, 1708 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.76 (s, 1H). 8.18—8.15 (m, 1H), 8.03—8.01 (m, 2H), 7.92—7.85 (m, 3H), 7.70—7.58 (m, 3H), 7.06—6.97 (m, 3H), 6.77—6.75 (m, 1H), 3.95 (s, 2H), 2.14 (s, 3H); LC-MS (m/z): 449 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-*p*-tolylacetamide (26) mp 133—135 °C; IR (KBr): 3342 (NH) cm^{-1}, 1688, 1707 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.22 (dd, J=7.1, 2.0 Hz, 1H), 8.05 (dd, J=7.7, 1.7 Hz, 2H), 7.88 (dd, J=6.9, 1.8 Hz, 1H), 7.74 (p, J=7.4 Hz, 2H), 7.53—7.46 (m, 3H), 7.18 (s, 1H), 6.98 (d, J=8.4 Hz, 2H), 6.91 (d, J=8.7 Hz, 2H), 3.76 (s, 2H), 2.26 (s, 3H); LC-MS (m/z): 449 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(2-methoxyphenyl)acetamide (27) mp 212—214 °C; IR (KBr): 3335 (NH) cm^{-1}, 1673, 1702 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.21 (dd, J=7.2, 1.5 Hz, 1H), 8.07 (dd, J=8.1, 1.5 Hz, 2H), 7.84 (d, J=7.2 Hz, 1H), 7.77—7.64 (m, 4H), 7.50—7.42 (m, 3H), 6.98 (t, J=8.7 Hz, 1H), 6.77 (t, J=8.4 Hz, 2H), 3.86 (s, 5H); LC-MS (m/z): 465 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(3-methoxyphenyl)acetamide (28) mp 153—155 °C; IR (KBr): 3298 (NH) cm^{-1}, 1678, 1712 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.22 (d, J=7.2 Hz, 1H), 8.05 (d, J=7.5 Hz, 2H), 7.89 (d, J=7.2 Hz, 1H), 7.75 (p, J=7.5 Hz, 2H), 7.54—7.48 (m, 3H), 7.22 (s, 1H), 7.08 (t, J=8.4 Hz, 1H), 6.78 (s, 1H), 6.59 (t, J=8.4 Hz, 2H), 3.77 (s, 2H), 3.73 (s, 3H); LC-MS (m/z): 465 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(2-fluorophenyl)acetamide (29) IR (KBr): 3362 (NH) cm^{-1}, 1666, 1706 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 9.72 (s, 1H), 8.17 (m. 1H), 8.04 (d, J=7.5 Hz, 2H), 7.97—7.85 (m, 3H), 7.73—7.61 (m, 3H), 7.52—7.47 (m, 1H), 7.23—6.98 (m, 3H), 3.92 (s, 2H); LC-MS (m/z): 453 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(3-fluorophenyl)acetamide (30) mp 180—182 °C; IR (KBr): 3287 (NH) cm^{-1}, 1669, 1701 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.23 (dd, J=7.1, 2.0 Hz, 1H), 8.05 (dd, J=6.0, 1.8 Hz, 2H), 7.89 (dd, J=7.4, 1.7 Hz, 1H), 7.78 (d, J=7.2 Hz, 2H), 7.54—7.46 (m, 3H), 7.32 (s, 1H), 7.19—7.11 (m, 1H), 6.99 (dt, J=10.7, 2.1, 1H), 6.79—6.72 (m, 2H), 3.78 (s, 2H); LC-MS (m/z): 453 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(2-chlorophenyl)acetamide (31) mp 169—171 °C; IR (KBr): 3253 (NH) cm^{-1}, 1676, 1706 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.54 (s, 1H), 8.15—8.14 (m, 1H), 8.04 (m, 2H), 7.90—7.88 (m, 3H), 7.71—7.64 (m, 3H), 7.40—7.38 (m, 1H), 7.31 (m, 1H), 7.20—7.13 (m, 1H), 7.12—7.09 (m, 1H), 4.08 (s, 2H); HR-MS ($C_{23}H_{17}ClN_2O_5S$): Calcd 468.0547, Found 468.0512.

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(3-chlorophenyl)acetamide (32) IR (KBr): 3266 (NH) cm^{-1}, 1674, 1714 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.23 (dd, J=7.7, 1.4 Hz, 1H), 8.04 (dd, J=7.8, 1.7 Hz, 2H), 7.90 (dd, J=7.4, 1.4 Hz, 1H), 7.78 (p, J=7.5 Hz, 2H), 7.56—7.47 (m, 3H), 7.29 (s, 1H), 7.20—7.17 (m, 2H), 7.09—7.04 (m, 2H), 3.77 (s, 2H); LC-MS (m/z): 469 (MH^+).

2-(3-Benzoyl-4-hydroxy-1,1-dioxido-2*H*-benzo[*e*][1,2]thiazin-2-yl)-*N*-(3,4-difluorophenyl)acetamide (33) mp 194—196 °C; IR (KBr): 3475 (NH) cm^{-1}, 1631, 1710 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) δ: 8.17—8.13 (m, 1H), 7.99—7.96 (m, 2H), 7.77—7.71 (m, 3H), 7.54—7.42 (m, 3H), 7.33—7.27 (m, 1H), 6.97—6.89 (m, 1H), 6.68—6.61 (m, 1H), 3.89 (s, 2H); HR-MS ($C_{23}H_{16}F_2N_2O_5S$): Calcd 470.0748, Found 470.0749.

Biological Evaluation. *in Vitro* Study Human 11β-HSD1: To assay microsomal 11β-HSD1 activity, 10 μg of human microsome was added in an assay buffer (100 μl) containing 250 μM NADPH, 160 nM cortisone, 20 mM Tris–HCl, and 5 mM ethylenediaminetetraacetic acid (EDTA, pH 6.0) with or without compounds (in dimethyl sulfoxide (DMSO), final 1%) and allowed

to incubate for 3 h at 37 °C. Small aliquots (2 μl) of the reaction mixtures were removed and subjected to HTRF cortisol assay according to the manufacturer's instructions (Nihon Schering, Tokyo, Japan). The specific signal is expressed as percentage of Delta F, which is a value calculated from the ratio of 665 nm/615 nm [$(R_{sample}-R_{negative})/R_{negative}\times 100$], and is inversely proportional to the concentration of cortisol in the sample or the calibrator. The cortisol concentration was calculated from the calibration curve obtained from Delta F *versus* the standard solution. IC_{50} values of the compounds were determined from the concentration-dependent inhibition curves by GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, U.S.A.).

Mouse 11β-HSD1: C2Cl2 cells (ATCC CRL-1772, mouse skeletal muscle cell line) were grown and maintained in Dulbecco's modified Eagle's medium high glucose containing 10% fetal bovine serum and 1% antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) in a 5% CO_2 environment, and then differentiated by the addition of the same medium containing 2% fetal bovine serum for 4 d. Fully differentiated C2C12 cells (1×10^5 cells/500 μl/well in 24 well plate) were treated with 160 nM cortisone plus compounds for 24 h, and then samples of the medium (2 μl) were taken to measure enzyme activity using HTRF cortisol assay kit (Nihon Schering, Tokyo, Japan).

Selectivity Human 11β-HSD2 overexpressed HEK-293 cells (2.5×10^4 cells/100 μl/well in 96 well plate) were treated with 100 nM cortisol plus compounds for 24 h, and then samples of the medium (10 μl) were taken to measure enzyme activity using HTRF cortisol assay kit (Nihon Schering, Tokyo, Japan).

CYP Assay The CYP450 enzyme (1A2, 2C19, 2D6, 3A4) assays were carried out using fluorometric enzyme assays with Vivid CYP enzymes assay kit (PanVera, CA, U.S.A.) in a 96-well microtiter plate following the manufacturer's instruction with some modification. Test compounds including the ketoconazole, α-naphthoflavone, sulfaphenazole and quinidine as known as CYP3A4, 1A2, and 2D6 inhibitors, respectively, were prepared in acetonitrile to give final concentrations of 10 μM. Briefly, to each well of the microtiter plate was added NADP generating solution (1.0 mM $NADP^+$, 3.3 mM glucose 6-phosphate, 3.3 mM $MgCl_2\cdot6H_2O$, and 0.4 U/ml glucose 6-phosphate dehydrogenase in 10 mM KPO_4, pH 8.0) followed by the vehicle acetonitrile (control) and the test samples. Typically, for each P450 study, each plate containing one standard inhibitor was constructed. Plates were covered and then incubated at 37 °C for 20 min. The enzyme reaction was initiated by the addition of an enzyme/substrate (E/S) mixture (each CYP450 enzymes for 0.5 pmol and fluorogenic substrates for 5 μM substrate CYP3A4 Green, CYP1A2 Blue, CYP2C19 Blue and CYP2D6 Blue). The plate was further incubated for 20 min, followed by the addition of the stop solution to terminate the enzyme activity. Background reading was measured in a similar manner except for the E/S mixture which was added after the enzyme reaction was terminated. The fluorescence of substrate metabolite fluorescein was measured on a fluorescence plate reader with an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The effect of test compounds on CYP450 enzymes were calculated as a percentage of the enzyme activity.

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