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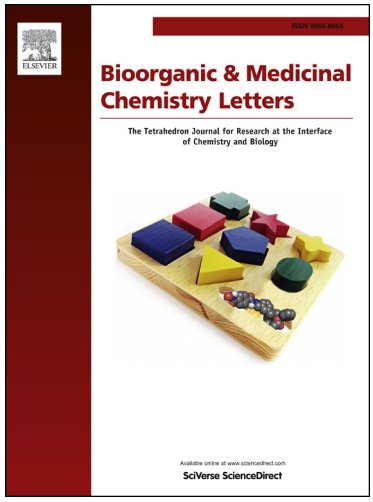

Mono-carbonyl curcumin analogues as 11β-hydroxysteroid dehydrogenase 1 inhibitors

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Mono-carbonyl curcumin analogues as 11β-hydroxysteroid dehydrogenase 1 inhibitors

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Abbreviations

11β-HSD1 11β-hydroxysteroid dehydrogenase isoform
11β-HSDs 11β-hydroxysteroid dehydrogenases
11DHC 11-dehydrocorticosterone
CORT corticosterone
CUR curcumin

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Abstract

A series of structurally novel mono-carbonyl curcumin analogues have been synthesized and biologically evaluated to test their inhibitory potencies and the structure–activity relationship (SAR) on human and rat 11β-hydroxysteroid dehydrogenase isoform (11β-HSD1) activities. 11β-HSD1 selective inhibitors have been discovered and compound A10 is discovered as a very potent with an IC_{50} value of 97 nM without inhibiting 11β-HSD2.

Keywords: 11β-HSD1; 11β-HSD2; mono-carbonyl curcumin analogues; inhibitors

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Excessive glucocorticoids under conditions such as Cushing's disease cause a spectrum of clinical features similar to the metabolic syndrome.[1] The treatment of the metabolic syndrome in Cushing's disease can be achieved by reducing circulating glucocorticoids.[2] Glucocorticoids have been demonstrated to increase the risks of metabolic syndromes. Intracellular levels of glucocorticoids (cortisol in the human or corticosterone, CORT, in the rat) are regulated by two 11β-hydroxysteroid dehydrogenases (11β-HSDs). 11β-HSD1 is an NADPH-dependent oxidoreductase that activates glucocorticoid by converting biologically inactive 11keto glucocorticoids, cortisone (in the human) or 11-dehydrocorticosterone (11DHC, in the rat) into the active 11β-hydroxyl glucocorticoids (cortisol or CORT). In the contrast, 11β-HSD2 is an NAD+-dependent unidirectional enzyme that does the opposite reaction. 11β-HSD2 is a critical enzyme that acts to prevent cortisol from stimulating the mineralocorticoid receptor, thus preventing from apparent mineralocorticoid excess (Figure 1).[3, 4]

Recently, 11β-HSD1 reductase inhibition has gained attention as a potentially effective method for treating the metabolic syndrome, including type 2 diabetes.[5] 11β-HSD1 knockout mice are resistant to diet-induced obesity and glucose intolerance, and the 11β-HSD1 over-expression in fat and liver tissues causes the metabolic syndrome.[6, 7] Selectivity against the 11β-HSD2 is expected to be critical as deficiency of the enzyme leads to apparent mineralocorticoid excess caused by the occupancy of mineralocorticoid receptor by cortisol.[4] Therefore, many classes of 11β-HSD1 inhibitors without any effects on 11β-HSD2 have been reported [8-10]. In the present study, we report such a new class of inhibitors.

Curcumin (CUR) was identified as an initial hit by high-throughput screening. CUR had significant level of potency on the human 11β-HSD1 (IC50= 4501 nM, Figure 2), when the enzyme activity was measured in intact CHOP cells transfected with human 11β-HSD1 cDNA (*HSD11B1*). CUR showed an equivalent selectivity of inhibiting human liver microsomal 11β-HSD1 (IC_{50} = 15,983 nM) and human kidney microsomal 11β-HSD2 (IC_{50} = 15,696 nM).

This compound is attractive, because it is a natural product with low toxicity.[11] However, CUR has several disadvantages in pharmacokinetics such as structural instability, poor bioavailability and fast metabolism, which limit its applications.[11] Due to the simplicity of the scaffold, a large collection of mono-carbonyl curcumin analogues with better pharmacokinetics profiles and improved structural stability have been synthesized (scheme 1).[12-14] These mono-carbonyl curcumin analogues include cyclopentanones (A), 1,5-diaryl-1,4-pentadiene-3-ones (B) and cyclohexanones (C). These compounds were synthesized to examine the role of different substituents on the benzene ring and their influence on inhibitory potencies of 11β-HSD1 without affecting 11β-HSD2.

The synthesis and spectral properties of compounds A01-A17, B01-B17 and C01-C17 were reported in our previous papers.[12, 15] General procedure for synthesizing compounds A01-C17 is described in the following section.

An amount of 7.5 mmol acetone (B-class), cyclopentanone (A-class), or cyclohexanone (C-class) was added to a solution of 15 mmol arylaldehyde in methanol (10 ml). The solution was stirred at room temperature for 20 min, followed by adding NaOCH3/CH3OH (1.5 ml, 7.5 mmol). The mixture was stirred at room temperature and monitored with thin layer chromatography. When the reaction was finished, the residue was poured into saturated NH4Cl solution and filtered. The precipitate was washed with water and cold ethanol, and dried in vacuum. The solid was purified by chromatography over silica gel using CH2Cl2/CH3OH as the

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eluent to yield compounds. Details of the reaction routes, yields, melting points, NMR and electrospray ionization mass spectrometry (ESI-MS) analysis were reported as described.[12, 15]

The synthesized analogues were evaluated for the inhibition of human and rat 11β-HSD1 reductase activities in either microsome (human liver or rat testis) or intact cell (CHOP cells transfected with human *HSD11B1* or intact isolated rat Leydig cell).

11β-HSD1 reductase assay in human liver and rat testis microsomes was performed as described.[16] Briefly, 11β-HSD1 activity assay tubes contained 25 nM substrate 11DHC (for rat) or cortisone (for human), spiked with 60,000 dpm of their respective 3H-steriods. 11DHC or cortisone was used as the substrate to measure 11β-HSD1 activity. 25 nM of steroid substrate was used because this concentration was within the physiological concentration range. Human liver (4 µg) or rat testis (10 µg) microsomes were incubated with 11keto steroids, 0.2 mM NADPH and various concentrations (10^{-10}-10^{-5} M) of curcumin analogues at 37C for 60-90 mins. The inhibitory potency of curcumin analogues was measured relative to control (DMSO solvent). Curcumin analogues were dissolved in DMSO with final concentration of 0.4%, at this concentration DMSO did not inhibit enzyme activity. At the end of the reaction, the reaction was stopped by adding 10 µl of 1 mM glycyrrhentic acid (that terminates the reaction [17]) and 1 ml ice-cold ether. The steroids were extracted by ether, and the organic layer was dried under nitrogen. The steroids were separated chromatographically on the thin layer plate in chloroform and methanol (90:10, v/v), and the radioactivity was measured using a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC) as described previously.[16] The percentage conversion of cortisone to cortisol or 11DHC to CORT was calculated by dividing the radioactive counts identified as 11-OH-steroids by the total counts. The liver or testis were selected because of the abundance of expression of 11β-HSD1 in these tissues.[18]

The 11β-HSD1 activity was also measured in intact cells, in which no exogenous NADPH was added as described.[16] Adult rat Leydig cells were isolated from 90-day-old Sprague Dawley rats as described in our previous study.[19] The human 11β-HSD1 activity was achieved by transfecting human *HSD11B1* into the CHOP cell line as described.[20] 0.01×10^6 CHOP cells or 0.025×10^6 rat Leydig cells were used for the cell-based assays.

Human and rat kidney microsomes were used to measure 11β-HSD2 as described.[21] In brief, 11β-HSD2 activity assay tubes contained 25 nM substrate (cortisol for human and CORT for rat). Kidney microsome was incubated with substrate and 0.2 mM NAD+ for 30 min. The percentage conversion of cortisol to cortisone (human) or CORT to 11DHC (rat) was calculated by dividing the radioactive counts identified as 11keto steroid product by the total counts associated with both substrate and product.

The inhibitory potencies of chemicals on 11β-HSD1 reductase in intact cell preparations were included in Table 1, and those values of inhibiting 11β-HSD1 and 11β-HSD2 activities of microsomes were listed in Table 2.

The results indicate that curcumin analogues inhibited 11β-HSD1 activity to various degrees as judged by the results from intact CHOP cells transfected with human *HSD11B1*. Among these compounds, A02, A06, A10, B02, B06, B13, B14, C02, C06 and C13 are potent inhibitors (Table 1). Halogenation of benzene ring such as 4-fluorobenzyl (A02, B02 and C02), 2-bromobenzyl (A06, B06 and C06) and 3-bromobenzyl (A10) analogues improved 9-46 fold in potency of inhibiting human 11β-HSD1 in intact cells compared to CUR (Table 1). Hydroxylation of benzene ring at 4'-position for B and C groups such as 4-hydroxybenzyl (B13 and C13) and 3-methoxy-4-hydroxybenzyl (B14) analogues also showed 5-23 fold increases of potency compared to CUR. Apparently, this hydroxylation at 4'-position for A-group

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compounds even lowered the inhibitory potency (Table 1). For example, 4-hydroxybenzyl (A13) and 3-methoxy-4-hydroxybenzyl (A14) at maximum concentration (100 μM) only inhibited human 11β-HSD1 of intact cells by 49% and 12%, respectively (Table 1). Interestingly, the halogenated curcumin analogues showed greater selectivity for 11β-HSD1 because they almost did not inhibit human and rat 11β-HSD2 activities at 100 μM (Table 2). When B06 was selected for the mode of inhibition of 11β-HSD1, it was found that B06 was a competitive inhibitor against substrate cortisone (Figure 3).

Although the synthesis of several analogues has been reported previously,[12, 15, 22, 23] the inhibition on 11β-HSD1 and 11β-HSD2 have not been explored. Interestingly, some of these analogues have been examined for the inhibition on another hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase isoform 3 and data showed that there were completely different structure activity responses with potent inhibition of this enzyme by C03 (IC_{50} = 100 nM), which did not inhibit human 11β-HSD1 and 11β-HSD2 activities at all.[23] Furthermore, these compounds have been showed to have different anti-inflammatory properties with SAR difference from the inhibition of 11β-HSD1 (Table 3).[12, 14, 15, 22, 23] Liang et al. has reported that some these compounds showed cytotoxic effects on several cell lines including HeLa cell (Table 3).[14] According to the described method by Liang et al[14], we examined the cytotoxic effects of compound B06 and C06 on CHOP cells and rat Leydig cells, and we did not find any cytotoxic effects of these compounds (data not shown). This indicates that these curcumin analogues showed selectivity of inhibition on 11β-HSD1 activity.

In summary, three series of mono-carbonyl analogues of curcumin were synthesized. Their structures were identified by NMR analysis and the inhibitory potencies of curcumin analogues on human and rat 11β-HSD1 in intact cells and microsomes were examined. The selectivity against 11β-HSD2 was compared. SAR studies of these chemicals have resulted in the identification of novel potent 11β-HSD1 inhibitors A02, A06, A10, B02, B06, B13, B14, C02, C06 and C13 with IC_{50} values between 97-830 nM. These compounds displayed selectivity for 11β-HSD1 compared to human and rat 11β-HSD2.

Acknowledgment

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Legends

Figure 1. The catalysis of 11β-HSD1 and 11β-HSD2 in the human.

Figure 2. Initial hit curcumin discovered by high-throughput screening (HTS).

Scheme 1. Reagents and conditions: cyclopentanone (A), acetone (B), or cyclohexanone (C), NaOH/EtOH, rt.

Figure3. The mode of B06 on 11β-HSD1 in human liver microsome. Lineweaver–Burk plotting in presence of substrate cortisone shows the competitive inhibition.

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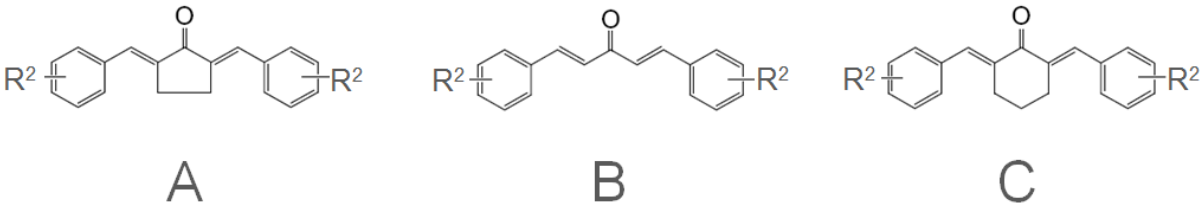
Table 1. Structure and the inhibition of compounds (Com.) on 11β-HSD1 in intact cells.



Com.	R^2 group	Rat		Human	
		% inhibition [a]	IC_{50} (nM) [b]	% inhibition [a]	IC_{50} (nM) [b]
CUR	-	-	3,921	-	4501
A01	4-ethanoxy	27%	-	14%	-
A02	4-fluoro	-	3,469	-	310
A03	3,4,5-trimethoxy	18%	-	31%	-
A04	4-dimethylamino	13%	-	16%	-
A06	2-bromo	-	1,448	-	144
A07	4-tetrahydro-2H-pyran-2-yloxy	22%	-	9%	-
A08	2-fluoro-3-trifluoromethyl	22%	-	24%	-
A10	3-bromo	-	1,147	-	97
A11	3-methoxy-4-tetrahydro-2H-pyran-2-yloxy	18%	-	26%	-
A12	4-3-dimethyl aminopropoxy	-	4,171	49%	-
A13	4-hydroxy	21%	-	12%	-
A14	4-hydroxy-3-methoxy	19%	-	17%	-
A16	4-allyloxy	10%	-	23%	-
A17	4-allyloxy-3-methoxy	17%	-	31%	-
B01	4-ethanoxy	-	9945	9%	-
B02	4-fluoro	-	229	-	507
B03	3,4,5-trimethoxy	5%	-	40%	-
B04	4-dimethylamino	10%	-	29%	-
B06	2-bromo	-	129	-	115
B07	4-tetrahydro-2H-pyran-2-yloxy	-	17,239	54%	-
B11	3-methoxy-4-tetrahydro-2H-pyran-2-yloxy	-	52,476	28%	-
B13	4-hydroxy	-	986	-	832
B14	4-hydroxy-3-methoxy	-	292	-	257
B16	4-allyloxy	22%	-	4%	-
B17	4-allyloxy-3-methoxy	18%	-	1%	-
C01	4-ethanoxy	26%	-	16%	-
C02	4-fluoro	-	1,111	-	263
C03	3,4,5-trimethoxy	42%	-	14%	-
C04	4-dimethylamino	37%	-	26%	-
C06	2-bromo	-	148	-	346
C07	4-tetrahydro-2H-pyran-2-yloxy	28%	-	28%	-
C11	3-methoxy-4-tetrahydro-2H-pyran-2-yloxy	8%	-	40%	-
C13	4-hydroxy	-	633	-	195
C16	4-allyloxy	8%	-	18%	-
C17	4-allyloxy-3-methoxy	6%	-	16%	-

[a]% inhibition at the maximum concentration of each compound used; [b]IC_{50} value for inhibitory potency; All potency data are reported as the mean of 2-3 determinations.

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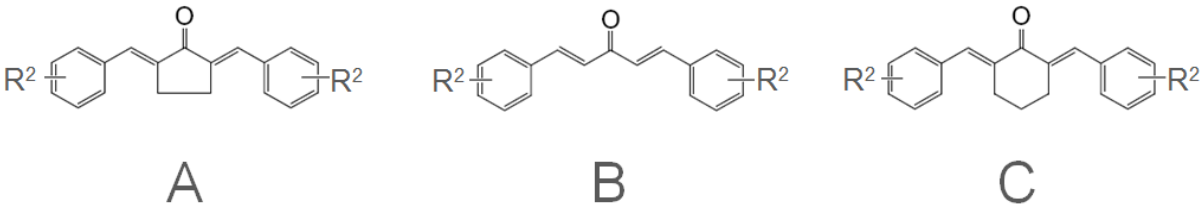
Table 2. Structure and the inhibition of compounds (Com.) on 11β-HSD1 and 11β-HSD2 in microsomes.



Com	R^2 group	11β-HSD1 at 100μM				11β-HSD2 at 100μM	
		Rat %inhibition[a]	IC_{50} (nM) [b]	Human %inhibition[a]	IC_{50} (nM) [b]	Rat %inhibition	Human %inhibition
CUR	-	-	4,125	-	15983	(14,939) [c]	(15,696) [c]
A01	4-ethanoxy	0	-	14%	-	10%	6%
A02	4-fluoro	-	21,987	-	17,393	9%	26%
A03	3,4,5-trimethoxy	12%	-	16%	-	9%	5%
A04	4-dimethylamino	0	-	15%	-	1%	0
A06	2-bromo	-	2,882	-	6,109	22%	36%
A07	4-tetrahydro-2H-pyran-2-yloxy	11%	-	30%	-	13%	0
A08	2-fluoro-3-trifluoromethyl	2%	-	23%	-	5%	0
A10	3-bromo	-	599	-	3,512	3%	23%
A11	3-methoxy-4-tetrahydro-2H-pyran-2-yloxy	13%	-	26%	-	11%	0
A12	4-3-dimethyl aminopropoxy	2%	-	15%	-	12%	24%
A13	4-hydroxy	32%	-	39%	-	17%	0
A14	4-hydroxy-3-methoxy	52%	-	49%	-	34%	13%
A16	4-allyloxy	9%	-	28%	-	15%	0
A17	4-allyloxy-3-methoxy	14%	-	13%	-	0	10%
B01	4-ethanoxy	7%	-	36%	-	27%	8%
B02	4-fluoro	2%	-	37%	-	22%	26%
B03	3,4,5-trimethoxy	0	-	16%	-	11%	1%
B04	4-dimethylamino	0	-	24%	-	13%	0
B06	2-bromo	-	288	-	529	39%	53%
B07	4-tetrahydro-2H-pyran-2-yloxy	13%	-	36%	-	24%	13%
B11	3-methoxy-4-tetrahydro-2H-pyran-2-yloxy	19%	-	28%	-	16%	0
B13	4-hydroxy	41%	-	-	4,423	7%	44%
B14	4-hydroxy-3-methoxy	-	16,077	-	70,901	35%	39%
B16	4-allyloxy	0	-	35%		16%	2%
B17	4-allyloxy-3-methoxy	9%	-	14%		19%	13%
C01	4-ethanoxy	17%	-	17%	-	14%	11%
C02	4-fluoro	-	13,502	-	2,973	16%	24%
C03	3,4,5-trimethoxy	35%	-	21%	-	23%	13%
C04	4-dimethylamino	16%	-	14%	-	20%	21%
C06	2-bromo	-	218		1,111	39%	33%
C07	4-tetrahydro-2H-pyran-2-yloxy	45%	-	14%	-	48%	17%
C11	3-methoxy-4-tetrahydro-2H-pyran-2-yloxy	-	65,430	37%	-	14%	6%
C13	4-hydroxy	63%	-	-	1,944	24%	0
C16	4-allyloxy	19%	-	18%	-	23%	6%
C17	4-allyloxy-3-methoxy	15%	-	7%	-	8%	6%

[a]% inhibition at the maximum concentration of each compound used; [b]IC_{50} value for inhibitory potency; [c]() shows the IC_{50} values (nM) for inhibitory potency of curcumin on 11β-HSD2; All potency data are reported as the mean of at least two determinations.

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Table 3. Structure and various activities of compounds (Com.)

Com	Inhibition of TNF-α Formation at 10 μM	Cytotoxic to HeLa	Inhibition on human enzyme at 100μM		
			11β-HSD1	11β-HSD2	17β-HSD3
	Ref.12	Ref.14	This paper		Ref.23
CUR	NI[a]	(17.5μM)[c]	(16.0μM)[c]	(15.7μM)[c]	(67.3μM)[c]
A01	<50%[b]	(24.0 μM)	<50%[b]	<50%[b]	NI[a]
A02	NI	(1.2 μM)	(17.4μM)	<50%	<50%
A03	NI	-	<50%	<50%	NI
A04	NI	-	<50%	NI	NI
A06	-	-	(6.1μM)	<50%	<50%
A07	NI	(39.1μM)	<50%	NI	NI
A08	-	-	<50%	NI	NI
A10	-	-	(3.5μM)	<50%	NI
A11	NI	-	<50%	NI	NI
A12	-	-	<50%	<50%	<50%
A13	-	-	<50%	NI	<50%
A14	-	-	<50%	<50%	-
A16	-	-	<50%	NI	-
A17	-	-	<50%	NI	-
B01	-	-	<50%	<50%	<50%
B02	NI	NI	<50%	<50%	<50%
B03	NI	(95.0μM)	<50%	<50%	NI
B04	NI	-	<50%	NI	(2.4μM)
B06	>50%[b]	(34.2μM)	(0.5μM)	<50%	(2.7μM)
B07	NI	-	<50%	<50%	NI
B11	-	-	<50%	NI	<50%
B13	-	(217.0μM)	(4.4μM)	<50%	<50%
B14	-	NI	(70.9μM)	<50%	-
B16	-	-	<50%	<50%	-
B17	-	-	<50%	<50%	-
C01	-	NI	<50%	<50%	NI
C02	NI	-	(3.0μM)	<50%	<50%
C03	NI	(27.5μM)	<50%	<50%	(0.1μM)
C04	NI	-	<50%	<50%	<50%
C06	>50%[b]	NI	(1.1μM)	<50%	<50%
C07	NI	-	<50%	<50%	NI
C11	-	-	<50%	<50%	<50%
C13	-	-	(1.9μM)	NI	<50%
C16	-	(27.5μM)	<50%	<50%	-
C17	-	-	<50%	<50%	-

NI=no inhibition; [b]% inhibition at the maximum concentration of each compound used;
[c]() shows the IC_{50} values for inhibitory potency.

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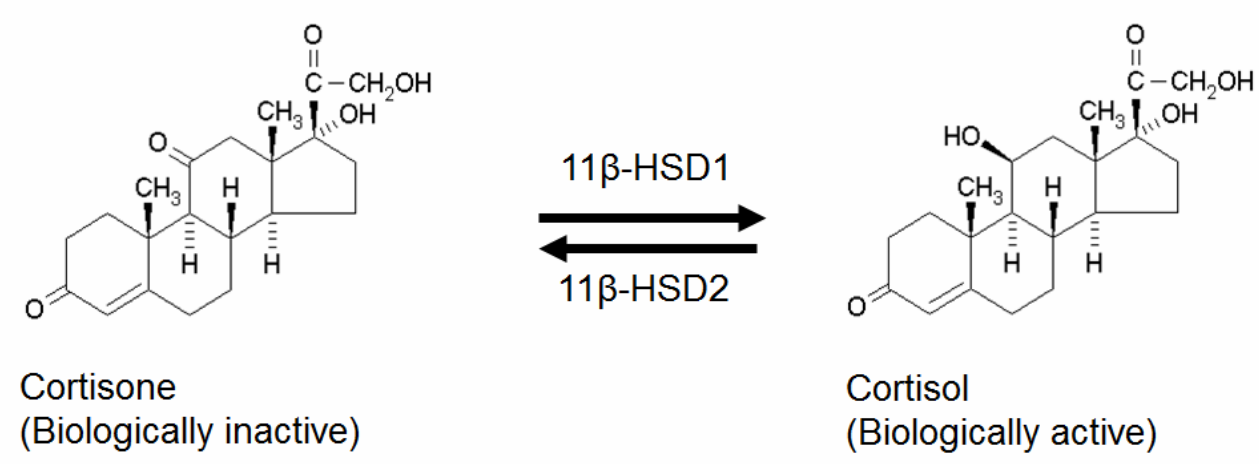
Figure 1



Cortisone
(Biologically inactive)

Cortisol
(Biologically active)

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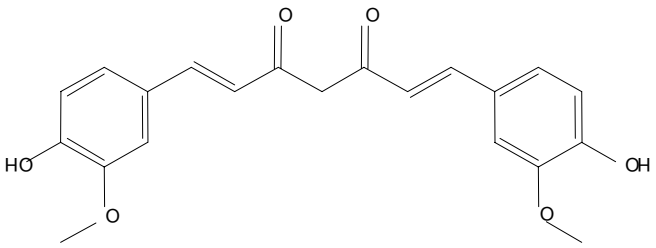
Figure 2



IC_{50} = 4501 nM

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Scheme 1

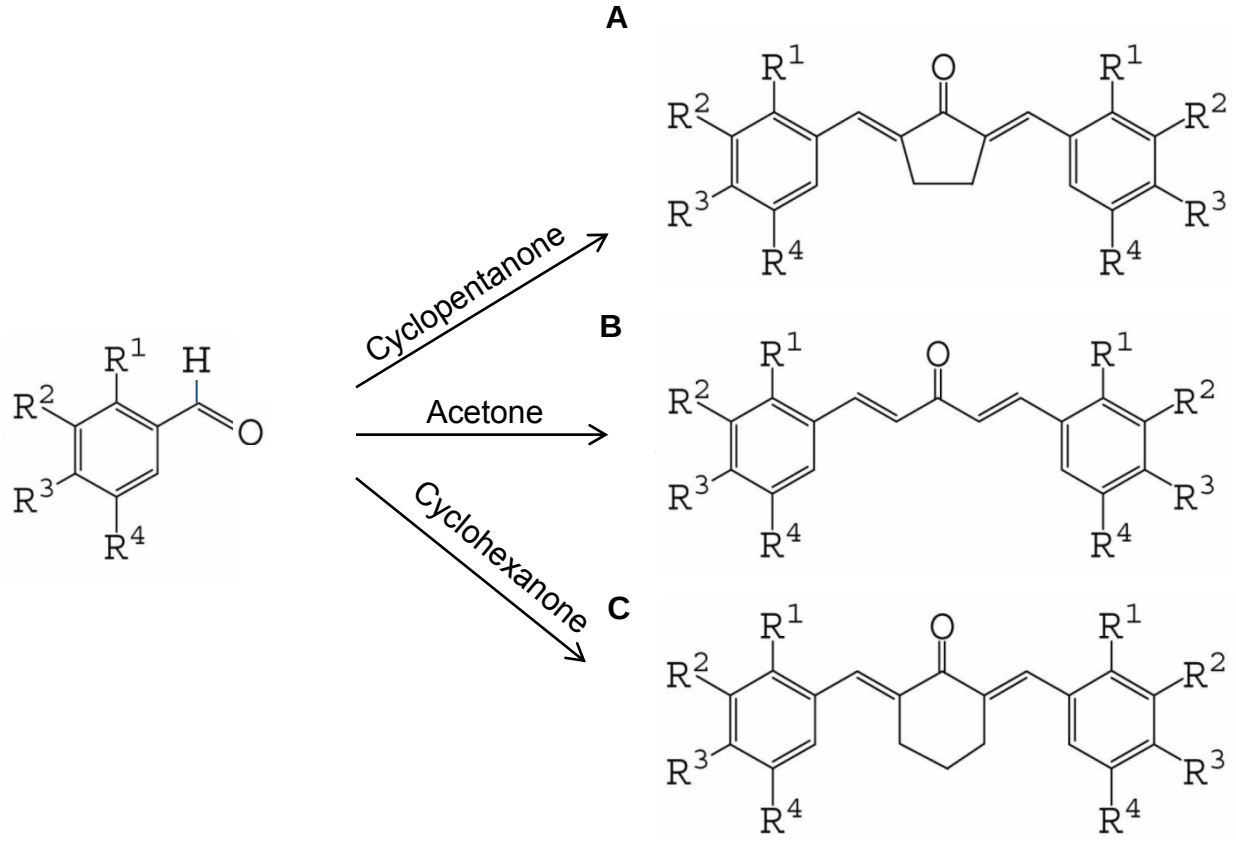

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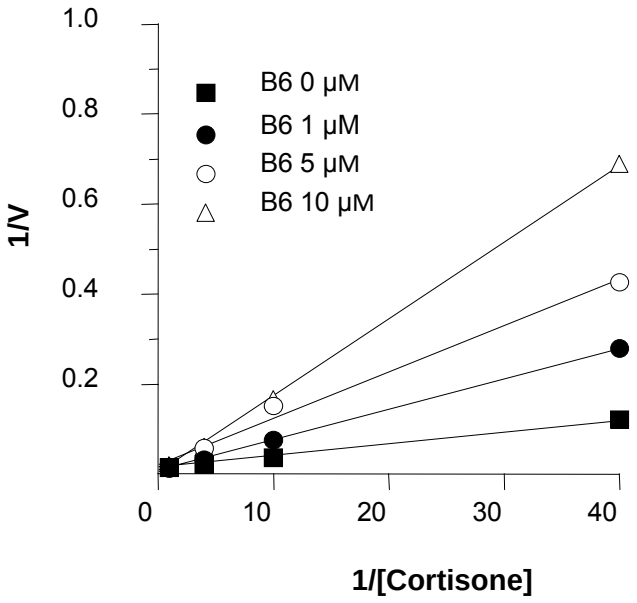
Figure 3



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Legends

Figure 1. The catalysis of 11 -HSD1 and 11 -HSD2 in the human.

Figure 2. Initial hit curcumin discovered by HTS.

Scheme 1. Reagents and conditions: cyclopentanone (A), acetone (B), or cyclohexanone (C), NaOH/EtOH, rt.

Figure3. The mode of B06 on 11 -HSD1 in human liver microsome. Lineweaver–Burk plotting in presence of substrate cortisone shows the competitive inhibition.

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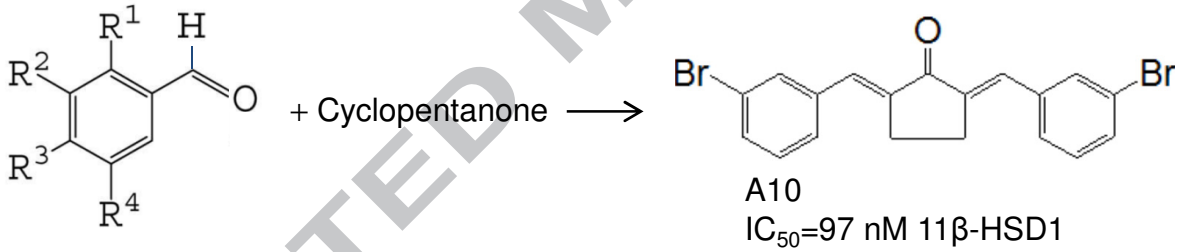

A10
IC_{50}=97 nM 11β-HSD1
IC_{50}>100 μM 11β-HSD2

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