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Phenylcyclobutyl triazoles as selective inhibitors of 11β-hydroxysteroid dehydrogenase type I

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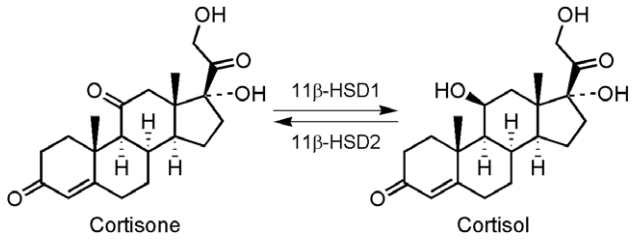
Abstract—3-(Phenylcyclobutyl)-1,2,4-triazoles were identified as selective inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). These were active both in vitro and in an in vivo mouse pharmacodynamic (PD) model. Fluorine substitution of the cyclobutane ring improved the pharmacokinetic profile significantly. The synthesis and structure–activity relationships are presented.

11β-Hydroxysteroid dehydrogenase enzymes (11β-HSD) interconvert cortisol and cortisone in man, and corticosterone and 11-dehydrocorticosterone in rodents. The type I isoform (11β-HSD1), which is located in the membranes of the endoplasmic reticulum, converts 11-keto steroid into active glucocorticoids in the presence of NADPH.[1] The type II isoform (11β-HSD2) utilizes NAD^{2} to convert glucocorticoids to 11-keto compounds which have low affinity for glucocorticoid and mineralocorticoid receptors[3] (Scheme 1).



Scheme 1. Enzymatic interconversion of cortisone and cortisol.

Glucocorticoids are important regulators of glucose and lipid homeostasis, acting largely via intracellular glucocorticoid receptors in the liver, adipose tissue, and muscle. Glucocorticoid excess, epitomized by Cushing's syndrome in humans, leads to insulin resistance/type 2 diabetes, dyslipidemia, and a redistribution of fat to visceral depots associated with increased cardiovascular risk.[4,5] Therefore, selective inhibition of the 11β-HSD1 enzyme may lead to an effective treatment for metabolic syndrome. The selectivity over 11β-HSD2 is very important since the inhibition of 11β-HSD2 is known to result in sodium retention, hypokalemia and hypertension.[6,7] The discovery and development of selective 11β-HSD1 inhibitors is an important area of academic and pharmaceutical research.[8]

Previously, we identified adamantyl triazole **1a** as a potent and selective inhibitor of human and mouse 11β-HSD1 by high-throughput screening. It was determined to have an IC_{50} versus human 11β-HSD1 of 7.8 nM (98 nM against the mouse enzyme) using a SPA-based assay.[9] In a human 11β-HSD2 counterscreen, the compound had an IC_{50} of >3000 nM (>10,000 nM against the mouse). A pharmacodynamic (PD) mouse model of 11β-HSD1 inhibition was developed to measure the in vivo activity of the inhibitors. In this model, a test

Keywords: 11β-HSD1; Phenylcyclobutyl triazole; Metabolic syndrome.
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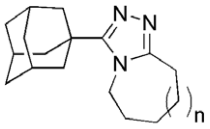
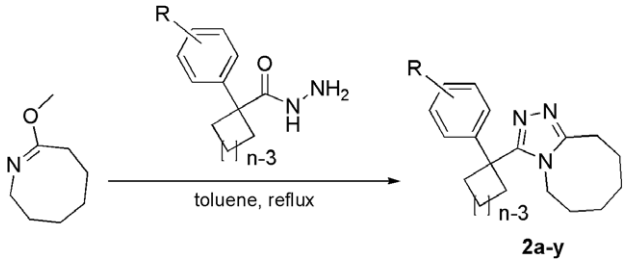
compound or vehicle is administered orally (typically at 10 mg/kg) and after a predetermined time interval, [^{3}H]-cortisone is injected intravenously via the tail vein. After 2 min, blood is collected by cardiac puncture. Steroids are then extracted from the serum and analyzed by HPLC. The relative levels of [^{3}H]-cortisone and [^{3}H]-cortisol are measured, and the percent inhibition of conversion of 11-ketosteroid to 11-hydroxysteroid is calculated.[9] In the PD assay, **1a** showed 59% inhibition at 1 h and 17% inhibition at 4 h.



1a n = 1
1b n = 2

We have also described the SAR on the right-hand side of **1a** and the superior 11β-HSD1 inhibitory activity of the corresponding eight-membered ring analog **1b**. The adamantyl group was identified as a metabolic liability and therefore, we sought to replace it with a more inert left-hand fragment. Phenyl cyclohexane is a bulky hydrophobic group similar to adamantane and not surprisingly, compound **2a** was a potent and selective inhibitor of 11β-HSD1 in our in vitro assays. A series of 1-phenyl-1-triazolylcycloalkane analogs based on **2a** were prepared from the corresponding acylhydrazides and the eight-membered imino ether in a one pot reaction that has previously been described (Scheme 2).[10] In the phenylcyclohexyl series, substitution on phenyl did not improve in vivo activity as desired. However, we found that reducing the ring size resulted in the improvement of both in vitro and in vivo activity (Table 1). Going from cyclohexane to cyclopentane, the in vitro activity improved about tenfold (**2k** vs **2d** or **2g** vs **2b** in Table 1). From cyclopentane to cyclobutane, a further improvement in in vitro and in vivo activity was achieved (**2l**–**2u**). The four-membered ring size was optimal for mouse since the cyclopropyl analogs **2v**–**2y** resulted in a loss of in vitro activity against the mouse enzyme. A number of analogs with phenyl substituents were prepared and evaluated. The *para*-chloro analog in the cyclobutyl series (**2p**) possessed the best overall activity as measured in the in vitro and pharmacodynamic assays. This compound was further optimized as described below.



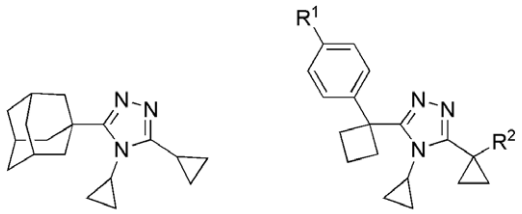
Scheme 2. Synthesis of phenylcyclobutyl triazoles.

Table 1. SAR of substituted phenylcycloalkyl triazoles

Compound	Ring size		11β-HSD1 IC_{50} (nM)		11β-HSD2 IC_{50} (nM)		Mouse pharmacodynamic assay[a]	
	n =	R =	Human	Mouse	Human	Mouse	1 h	4 h
2a	6	H	13	100	1037	>4000	25	0
2b	6	*p*-F	27	494	1174	>4000	—[b]	—
2c	6	*p*-Cl	32	454	613	>4000	—	—
2d	6	*p*-Me	15	374	216	>4000	—	—
2e	5	H	2	12	1035	>4000	73	37
2f	5	*o*-F	1	27	1270	>4000	57	18
2g	5	*p*-F	8	18	>4000	>4000	58	30
2h	5	*p*-Cl	2	41	NT	NT	—	—
2i	5	*m*-OH	43	61	>4000	>4000	3	21
2j	5	*m*-Me	10	57	1961	>4000	20	22
2k	5	*p*-Me	2	25	503	>4000	35	3
2l	4	H	1	8	4000	>4000	88	72
2m	4	*o*-F	1	16	<4000[c]	>4000	90	53
2n	4	*m*-F	2	19	4000	>4000	87	68
2o	4	*p*-F	3	37	>4000	>4000	68	55
2p	4	*p*-Cl	1	14	>4000	>4000	91	77
2q	4	*o*-Me	1	78	3318	>4000	23	21
2r	4	*m*-Me	2	52	>4000	>4000	17	22
2s	4	*p*-Me	2	19	2006	>4000	48	28
2t	4	*o*-CF_3	5	355	>4000	>4000	—	—
2u	4	*p*-CF_3	2	115	>4000	>4000	—	—
2v	3	H	8	172	>4000	>4000	63	45
2w	3	*p*-Cl	2	230	>4000	>4000	—	—
2x	3	*p*-Me	1	332	>4000	>4000		
2y	3	*o*,*p*-di-Cl	2	199	1964	>4000	—	—

[a] % Inhibition of the conversion of [^{3}H]-cortisone to [^{3}H]-cortisol after a single oral dose of compound at 10 mg/kg.
[b] Not tested.
[c] 60% inhibition at 4 μM.

Table 2. SAR of small group substitutions of 3-phenylcyclobutyl-4,5-dicyclopropyl-1,2,4-triazoles



3 **4a-e**

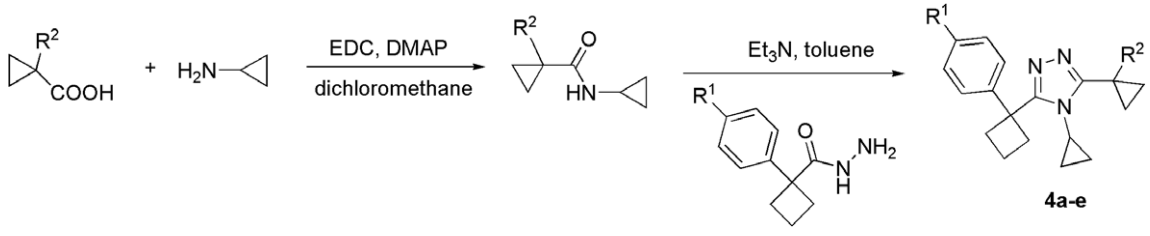
Compound	R^3 =	11β-HSD1 IC_{50} (nM)[a]		Mouse pharmacodynamic assay[b]	
		Human	Mouse	1 h	4 h
5a	*anti*-F	5	16	92	91
5b	*syn*-F	22	38	— [c]	—
5c	F,F	35	107	—	—

[a] The human and mouse 11β-HSD2 IC_{50}s were >4000 nM for all the compounds.
[b] % Inhibition of the conversion of [^{3}H]-cortisone to [^{3}H]-cortisol after oral dosing with compound at 10 mg/kg.
[c] Not tested.

Previous SAR studies on the right-hand side of triazole **1a** demonstrated that replacing the ring with acyclic forms such as 4-methyl-5-phenyl- or 4,5-dicyclopropyl-1,2,4-triazole improved in vivo activity significantly.[10,11] In the mouse pharmacodynamic assay, compound **3** showed 74% inhibition 4 h after an oral dose of 10 mpk while **1b** showed only 24% inhibition. Therefore, a series of phenylcyclobutyl triazole analogs possessing two cyclopropyl substituents were prepared. Improved in vitro and in vivo activity was observed by replacing the eight-membered ring with two cyclopropyl substituents in the phenylcyclobutyl series (Table 2). The synthesis of phenyl cyclobutyl–dicyclopropyl triazoles was similar to the adamantyl triazoles (Scheme 3).

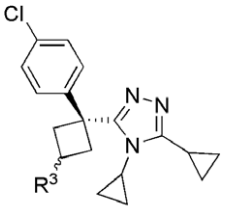
Despite the excellent in vitro and in vivo activity that was observed in this series, pharmacokinetic measurements in mice showed high plasma clearance rates. In liver microsome studies, it was determined that the hydroxylation of the cyclobutane was a significant process. In order to improve the pharmacokinetic profile, we sought to block this metabolic site. Fluoro-cyclobutane derivatives of **4b**, **4c**, and **4d** were prepared. The fluoro derivative of **4b,** where the fluorine atom was *anti*- to the triazole (**5a**), was superior to the other three analogs (data not shown). The *syn*- and difluoro analogs **5b** and **5c** were less potent inhibitors of 11β-HSD1 than **5a** (see Table 3). The preparation of the *anti*-fluoro-derivatives of **4b**, **4c** and **4d** is similar to that shown for **5a** in Scheme 4. Briefly, the hydroxycyclobutane fragment was prepared by reaction of the lithium anion of commercially available (4-chlorophenyl)acetonitrile with epichlorohydrin followed by the regeneration of the anion with methyl magnesium iodide. The resultant syn- and anti-hydroxy isomers (syn:anti ≈ 1:3.3) were separated through their benzoyl derivatives. DAST was used to convert the hydroxyl-cyclobutane to the corresponding fluoro-cyclobutane with an inversion of configuration. The fluoro-cyclobutane nitrile was then reduced to the aldehyde which was oxidized to carboxylic acid,[12] which was further converted to the corresponding methyl ester and hydrazide. The hydrazide was reacted with the in situ generated imino ether of cyclopropyl cyclopropamide to give the desired triazoles as described previously.[10] The di-fluoro analog **5c** was prepared from the ketone derivative of hydroxyl triazoles **5a** or **5b** using DAST.

Regarding the pharmacokinetic properties of the fluoro- versus non-fluoro-analogs, a comparison of compounds **4b** and **5a** shows comparable in vitro activity but better PD activity for **5a**. This compound had much higher plasma concentrations than the non-fluoro analog when dosed at 10 mg/kg (Fig. 1). More importantly, this compound had an excellent pharmacokinetic profile including low clearance with improved half life and oral bioavailability. Compound **5a** was selected for more extensive biological evaluation and will be the subject of a future communication (Table 4).



Scheme 3. Synthesis of phenylcyclobutyl-dicyclopropyl triazoles.

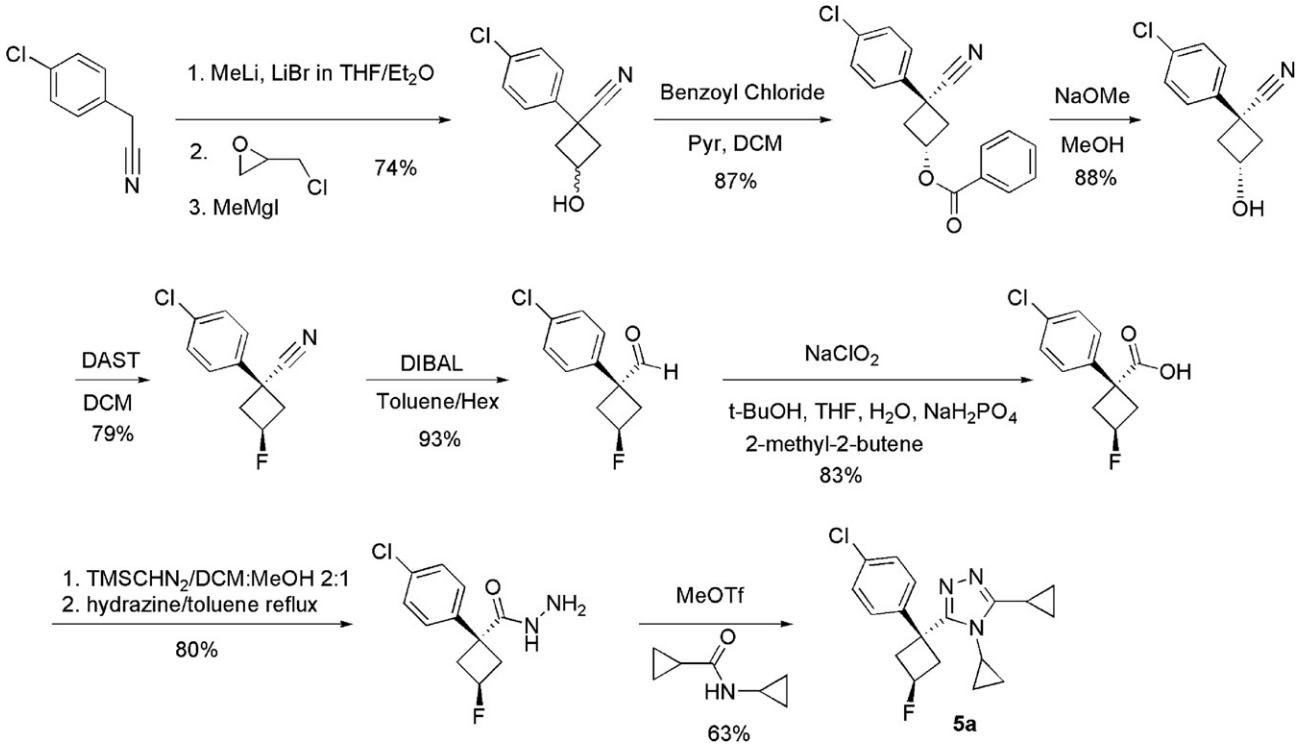
Table 3. SAR of substitutions on cyclobutane of 3-phenylcyclobutyl-4,5-dicyclopropyl-1,2,4-triazoles



5a-c

Compound	R^3 =	11β-HSD1 IC_{50} (nM)[a]		Mouse pharmacodynamic assay[b]	
		Human	Mouse	1 h	4 h
5a	*anti*-F	5	16	92	91
5b	*syn*-F	22	38	—[c]	—
5c	F,F	35	107	—	—

[a] The human and mouse 11β-HSD2 IC_{50}s were >4000 nM for all the compounds.
[b] % Inhibition of the conversion of [^{3}H]-cortisone to [^{3}H]-cortisol after oral dosing with compound at 10 mg/kg.
[c] Not tested.



Scheme 4. Stereoselective Synthesis of **5a**.

Table 4. Pharmacokinetic parameters for **4b** and **5a** in C57B6 mice

Compound	IV $nAUC_{0\rightarrow 24\ h}$ (μM h/mg/kg)	Clp (mL/min/kg)	$t_{1/2}$ (h)	Oral $nAUC_{0\rightarrow 24\ h}$ (μM h/mg/kg)	F_{oral} (%)
4b	0.70	81	0.3	0.19	27
5a	4.68	10.7	1.5	5.44	~100

In conclusion, a novel class of potent and selective inhibitors of mouse and human 11β-HSD1 has been identified. These analogs showed potent in vivo 11β-HSD1 activity in a mouse pharmacodynamic model. It was found that fluoro-substitution on the cyclobutyl group improved the pharmacokinetic profile dramatically while maintaining excellent in vitro activity. Compound **5a** was selected for more extensive biological evaluation.

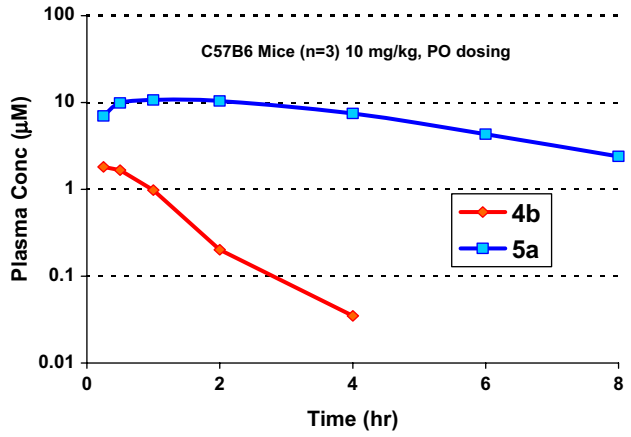


Figure 1. Plasma exposure in mice of non-fluoro analog **4b** and fluoro analog **5a**.

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