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Discovery and in Vivo Evaluation of Potent Dual CYP11B2 (Aldosterone Synthase) and CYP11B1 Inhibitors

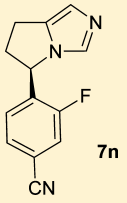
Erik L. Meredith,*[,†] Gary Ksander,[†] Lauren G. Monovich,[†] Julien P. N. Papillon,[†] Qian Liu,[†] Karl Miranda,[†] Patrick Morris,[†] Chang Rao,[†] Robin Burgis,[†] Michael Capparelli,[†] Qi-Ying Hu,[†] Alok Singh,[†] Dean F. Rigel,[‡] Arco Y. Jeng,[‡] Michael Beil,[‡] Fumin Fu,[‡] Chii-Whei Hu,[‡] and Daniel LaSala[‡]

[†]Novartis Institutes for BioMedical Research, 100 Technology Square, Cambridge, Massachusetts 02139, United States

[‡]Novartis Pharmaceuticals Corporation, East Hanover, New Jersey 07936, United States

Supporting Information

ABSTRACT: Aldosterone is a key signaling component of the renin-angiotensin-aldosterone system and as such has been shown to contribute to cardiovascular pathology such as hypertension and heart failure. Aldosterone synthase (CYP11B2) is responsible for the final three steps of aldosterone synthesis and thus is a viable therapeutic target. A series of imidazole derived inhibitors, including clinical candidate **7n**, have been identified through design and structure−activity relationship studies both in vitro and in vivo. Compound **7n** was also found to be a potent inhibitor of 11β-hydroxylase (CYP11B1), which is responsible for cortisol production. Inhibition of CYP11B1 is being evaluated in the clinic for potential treatment of hypercortisol diseases such as Cushing's syndrome.



KEYWORDS: *Inhibitor, CYP11B2, aldosterone synthase, aldosterone, hypertension, enzyme, CYP11B1, Cushing's syndrome, cortisol*

One of the primary functions of aldosterone through the mineralocorticoid receptor (MR) is to effect retention of sodium and excretion of potassium by the kidney.[1] The elevation of aldosterone causes an increase in blood pressure as well as facilitating other cardiac, renal, and vascular damage. Activation of the renin-angiotensin system (RAS) induces aldosterone production. As such, MR antagonists have been used in the treatment of heart failure.[2−4] While both RAS inhibitors and MR antagonists have been shown to reduce some of the pathological effects of aldosterone, there are noted drawbacks. In the case of RAS inhibition, a reduction of aldosterone is realized initially; however, this is not maintained.[5,6] Likewise, MR antagonists do not reduce the level of aldosterone, and in fact, they have been shown to induce aldosterone production.[7] Thus, it was thought that there would be therapeutic benefit in directly inhibiting aldosterone production.

Aldosterone is produced in the *zona glomerulosa* of the adrenal gland by the enzymatic action of aldosterone synthase (CYP11B2) on deoxycorticosterone.[8,9] Clinical observations suggested that the racemic aromatase (CYP19) inhibitor fadrazole affected aldosterone levels and subsequent preclinical studies demonstrated that the *R*-enantiomer (FAD286, Figure 1) was a potent inhibitor of CYP11B2.[10] From this understanding we embarked on a program to investigate the structure−activity relationship of the FAD286 scaffold and to gain a more extensive understanding of the potential of aldosterone synthase inhibition to treat aldosterone-driven pathologies.

Relatively little was known about the impact of substitution at R^1, although we quickly realized that, as with FAD286, chirality at this point of attachment was important. The impact of altering the size of the saturated ring was not understood at the outset. Preliminary structure−activity relationships (SAR) around the phenyl ring indicated that R^2 would be a most promising site for optimization.

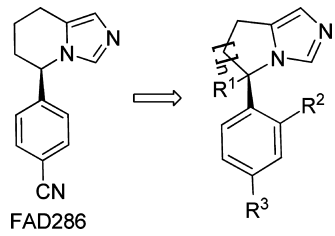


Figure 1. Imidazole derived aldosterone synthase inhibitors.

In general the compounds from series I (n = 1), series II (n = 2), and series III (n = 3) were prepared as outlined in Scheme 1. Intermediates **3** could be prepared by straightforward methods from the corresponding starting materials **1a**, **1b**, or **2**. The imidazole intermediates **3** underwent alkylation with the corresponding substituted benzyl bromide **4** upon heating in acetonitrile. Full removal and scavenging of the trityl group was accomplished by treatment with diethylamine and MeOH. Following alkylation, ring closure for series I and II was readily possible following removal of the TBS protecting group, chlorination, and then treatment with potassium *tert*-butoxide. The racemic product **6** then underwent separation by chiral HPLC to afford enantiomers **7** and **8**.

In the case of series I, the formation of the saturated ring in a similar manner to series II and III was problematic as the corresponding alkyl chloride derivative underwent elimination

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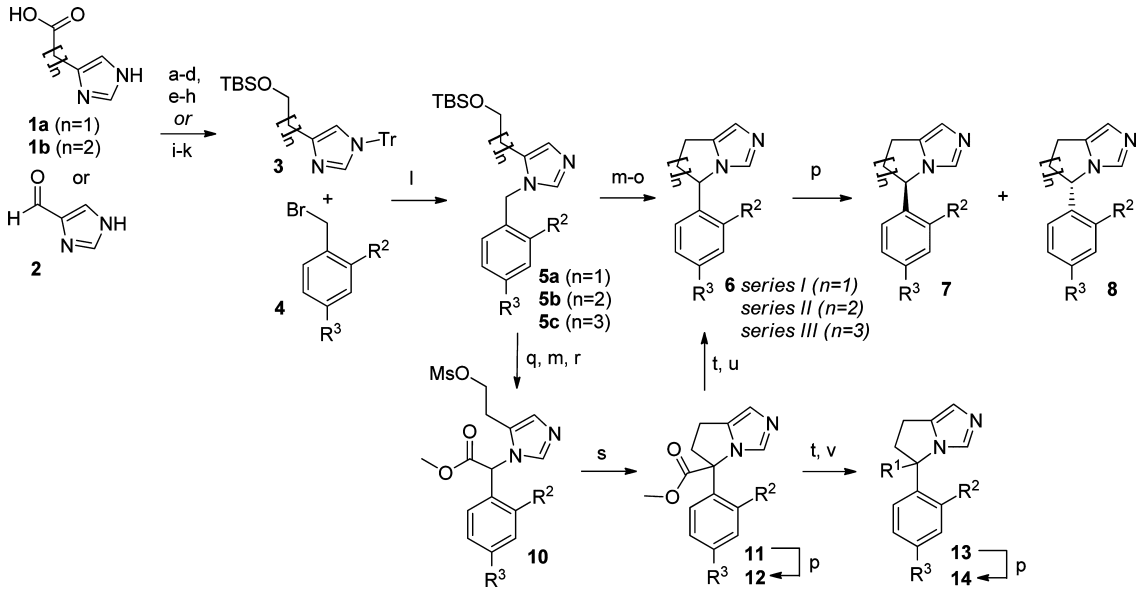
Scheme 1. General Synthetic Scheme[a]



[a]Reagents and conditions: (a) **1a** or **1b**, MeOH, HCl. (b) TrCl, Et_3N, CH_3CN. (c) $LiAlH_4$, THF, 0 °C. (d) TBSCl, imidazole, CH_2Cl_2. (e) **1a** or **1b**, TrCl, pyridine. (f) BH_3·THF, THF, 0 °C. (g) Ethanolamine, 90 °C. (h) TBSCl, imidazole, CH_2Cl_2. (i) **2**, TrCl, Et_3N, DMF (j) Ph_3P^+–$CH_2CH_2CH_2OTBS$, *n*BuLi, THF. (k) 5% Pd/C, H_2, EtOH. (l) CH_3CN, 80 °C; then MeOH/Et_2NH, 80 °C. (m) **5b** or **5c**, 4 M HCl in dioxane, 0 °C. (n) $SOCl_2$. (o) *t*BuOK, THF. (p) Chiral semiprep HPLC. (q) **5a**, NCC(O)OMe, LiHMDS, THF, −78 °C. (r) MsCl, Et_3N, CH_2Cl_2, 0 °C. (s) NaI, K_2CO_3, Et_3N, DMF, 80 °C. (t) LiOH, THF/H_2O (3:2); HCl neutralization. (u) **12**, Et_3N, DMSO, 100 °C. (v) Oxalyl chloride, cat. DMF, CH_2Cl_2; then Et_3N, amine, or alcohol.

rather than cyclization. To overcome this problem, the benzylic position of **5a** was activated by the installation of an ester moiety (**10**) prior to cyclization. This gave facile conversion to the desired five-membered ring. Compound **11** could then be separated by chiral HPLC to give **12** and provide the first data regarding the tolerance of substitution at R^1. Alternatively, the ester functionality could be removed by hydrolysis followed by decarboxylation to give **6** (n = 1), which then underwent chiral HPLC separation to provide the corresponding enantiomers **7** and **8**.

Following hydrolysis of ester **11**, both amide and ester derivatives **14** could be prepared by treatment of the carboxylic acid with oxalyl chloride and then the corresponding amine or alcohol.

As had been noted with FAD286, chiral separation proved to be critical as it was shown early on that for the majority of compounds in all three series (I, II, and III), only one enantiomer inhibited CYP11B2 activity, while the opposite enantiomer inhibited CYP19 activity. Some exceptions to this trend are described below (Table 1).

A number of modifications were made to the R^2 position of series II, and the potency was assessed in an assay measuring inhibition of aldosterone secretion in NCI-H295R cells.[12] The inclusion of a phenyl group at this position such as **7a** (56 nM) and **7b** (37nM) provided good potency. These compounds also demonstrate that the inclusion of a pheny group at R^2 allows for removal of the nitrile at R^3 without loss of activity. However, much to our surprise, other very simple modifications at R^2 such as fluoro (**7c**; 87 nM), chloro (**6a**; 792 nM), methyl (**6b**; 464 nM), and methoxy (**6c**; 390 nM) led to loss in potency.

In parallel with SAR studies on series II, the exploration of the effect of the saturated ring size on activity was pursued. Because of the synthetic challenges with forming the five-membered ring analogues (vide supra), the more readily prepared seven-membered analogues were explored first. In this case, as was seen with series II, substitution of the phenyl ring at R^2 with a substituted (**7d** and **7e**) or unsubstituted phenyl ring (**7f**) provided potent inhibition of CYP11B2. In fact, these analogues were among the most potent compounds both in vivo and in vitro, yet they tended to be less selective for other P450 enzymes. As with series II, a phenyl substituent at R^2 allowed for removal of the nitrile without a loss in potency (**7d** vs **7e**). Encouraged by the tolerance for expansion of ring size, we pursued additional modifications at the R^2 position in series III. In contrast to series II, nonphenyl substitution with small groups such as fluorine (**7g**) or chlorine (**7h**) were well tolerated with IC_{50} values of 42 and 16 nM, respectively. The methoxy derivative (**7i**), however, provided much lower inhibition. More troubling for series III was significant CYP19 inhibition with both **7g** and **7h** being >6-fold more potent than FAD286.

Given that the general SAR for CYP11B2 inhibition thus far strongly suggested that analogues with a saturated seven-membered ring were more tolerant of substitution than those with a six-membered ring, it would be expected that the five-membered analogues, if they fit the trend, would be less active than the six. With a reasonable synthesis for the five-membered analogues now in hand, it was decided to determine if this indeed was the case.

As mentioned above, an ester moiety needed to be incorporated into **5a** to facilitate the eventual ring closure. Following resolution of the enantiomers, the simple chloro-substituted compound **12a** provided good inhibition of CYP11B2 with IC_{50} of 17 nM. Unfortunately, **12a** also demonstrated very potent inhibition of CYP19. However, the surprising tolerance for substitution at R^1 in series I encouraged us to pursue the exploration of the phenyl ring. In contrast to

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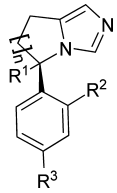
Table 1. Inhibition of Cellular Aldosterone Production and Aromatase Enzymatic Function (CYP19)[a]



compd	*n*	R^1	R^2	R^3	AS[c] cellular IC_{50} (nM)	CYP19[d] %inh @ 1 μM* or IC_{50} (μM)[§]
FAD286	2	H	H	CN	30	6[§]
7a	2	H	Ph	CN	56	
7b	2	H	Ph	H	37	
7c	2	H	F	CN	87	20*
6a[b]	2	H	Cl	CN	792	99*
6b[b]	2	H	Me	CN	464	100*
6c[b]	2	H	−OMe	CN	390	100*
7d	3	H	4-F−Ph−	CN	1	
7e	3	H	4-F−Ph−	H	2	
7f	3	H	Ph	H	2	
7g	3	H	F	CN	42	1.1[§]
7h	3	H	Cl	CN	16	100*
7i[a]	3	H	−OMe	CN	520	
12a	1	−C(O)OMe	Cl	CN	17	0.050[§]
12b	1	−C(O)OMe	−OMe	CN	3	
12c	1	−C(O)OMe	F	CN	351	
14a	1	4-F-Ph−CH_2−O(O)C-	Cl	CN	1.5	95*
14b	1	4-F−Ph−CH_2(Me)N(O)C−	−OMe	CN	5	45*
7j	1	H	Ph	H	441	
7k	1	H	4-F−Ph−	H	140	
7l	1	H	Cl	CN	13	
7m	1	H	OMe	CN	5	80*
7n	1	H	F	CN	9	6[§]
7o	1	H	Me	CN	8	87*
7p	1	H	Br	CN	10	47*

[a]All compounds in the table are single enantiomers except where noted. With the exception of **7n**, the absolute configuration was not determined. [b]Racemic compound. [c]Aldosterone production assay in human adrenocortical carcinoma (NCI-H295R) cells. [d]Enzymatic aromatase (CYP19) assay. See Supporting Information for more detail.

series II, the substitution of R^2 with a methoxy (**12b**) provided an increase in potency, whereas fluoro substitution (**12c**) caused a loss in potency. Additional ester and amide derivatives such as **14a** and **14b** were prepared and found to provide good inhibition of CYP11B2, yet most had an undesirable level of CYP19 inhibition.

The apparent increase in tolerance for substitution within series I vs series II and III warranted further exploration of analogues of type **7**. As phenyl substitution at the R^2 in both series II and III provided good potency in vitro and in vivo, we prepared such analogues in series I. Once again, we observed a disconnect in the SAR as the corresponding analogues **7j** and **7k** showed a substantial loss in potency against CYP11B2. Gratifyingly, **7l**, obtained by decarboxylation of **12a** followed by chiral separation, was very potent against CYP11B2 (IC_{50} 13 nM). Again, no translation of SAR between the series was observed, as the direct analogues of **7l** in the six-membered series (**6a**) had low activity (792 nM), while the seven-membered analogue (**7h**) provided similar inhibition (16 nM).

Building on this result, additional R^2 analogues were prepared including methoxy (**7m**, 5 nM), fluoro (**7n**, 9 nM), methyl (**7o**, 8 nM), and bromo (**7p**, 10 nM), among others. All maintained potency against CYP11B2. Equally important was the fact that several of these analogues had attenuated potency against CYP19, with **7n** inhibiting CYP19 comparable to FAD286.

Compound **7n** was found to have good selectivity against other P450 isoforms (IC_{50}s: CYP3A 4.8 μM; CYP2D6 >10 μM; CYP2C9 >10 μM). CYP11B1, which is highly homologous to CYP11B2 and is responsible for cortisol production, was a notable exception.[11] As shown in Table 2,

Table 2. Inhibition of Recombinant Human and Rat CYP11B2 and CYP11B1 Enzyme Activity

compd	hCYP11B2[a] IC_{50} (nM)	rCYP11B2[b] IC_{50} (nM)	hCYP11B1[a] IC_{50} (nM)	rCYP11B1[b] IC_{50} (nM)
FAD286	1.8	118	9.5	686
7d	0.4	1	0.3	0.3
7g	3.2		7.2	
7n	0.7	111	2.5	495
7o	0.9	24	1.5	386
14a	3.8		<0.1	

[a]See ref 11 for details on protein preparation and enzymatic assay conditions. [b]Recombinant rat CYP11b2 and CYP11b1 prepared similarly to the corresponding human enzymes as in ref 11.

some compounds in this series, such as **14a**, demonstrated greater inhibition of CYP11B1 enzymatic activity than that seen for CYP11B2. However, compound **7n** along with most other compounds in the series provided modest selectivity over CYP11B1. On this basis, it was desirable to determine how well the compounds reduced aldosterone in vivo and to evaluate the impact of CYP11B1 inhibition.

An efficient pharmacokinetic−pharmacodynamic (PK−PD) model in Sprague−Dawley (SD) rat was established to enable rapid compound screening and the development of in vivo SAR for the CYP11B2 inhibitors.[13] Analogous to the cellular assay, an angiotensin-II (Ang-II) infusion was used to stimulate an increase in aldosterone production and establish an experimental baseline plasma aldosterone concentration (PAC). Following oral administration of various CYP11B2 inhibitors the PAC was measured at given time points. As shown in Table 3, compounds in series I (e.g., **7l** and **7n**) provided better oral exposure in general than either series II or III. Compound **7n** provided both good exposure after oral dosing (%F = 42) and strong reduction of PAC (65%) over the duration of the study. Compounds **7d**, **7g**, and **12a** provided good reduction in PAC (66 and 81%, respectively) despite having very low oral exposure. Given that the cellular and enzymatic potency for these compounds are in line with the others, the in vivo efficacy for **7d**, **7g**, and **12a** may be in part due to the generation of active metabolites.

As noted above, one of the key questions was how the modest in vitro CYP11B2/CYP11B1 selectivity would translate to an effect on corticosterone levels in vivo. To address this question, a second PK−PD model in SD rat was developed to

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Table 3. Pharmacokinetic–Pharmacodynamic Parameters for Selected Compounds[a]

compd	dose (mg/kg)[b]	TWA_{0-8} [C] (nM)[d]	TWA_{0-8} % reduction of PAC[e]	%F[f]
7d	1[b]	6 ± 1	66 ± 4	1
7f	1[b]	4 ± 2	27 ± 17	<1
7e	1[b]	152 ± 72	73 ± 8	8
7g	1[b]	BQL[g]	69 ± 3	n.d.[h]
12a	1[c]	6 ± 2	81 ± 1	1
7l	1[c]	152 ± 21	51 ± 7	59
7n	1[c]	377 ± 27	65 ± 2	42
7m	1[b]	33 ± 8	50 ± 15	18
14b	1[c]	15 ± 8	35 ± 10	8

[a]Sprague–Dawley rat (n = 3). [b]Compound dosed in corn starch/water. [c]Compound dosed in HCl (1.5 equiv of 1 N/cornstarch/water). [d]Time-weighted average (TWA) compound concentration from 0 to 8 h. [e]TWA % reduction in plasma aldosterone concentration (PAC) from baseline. [f]Calculated from 0.3 mg/kg i.a. dose. [g]Below quantitation limit. [h]Not calculated since oral exposure was BQL.

evaluate the effect of **7n** on plasma corticosterone concentrations (PCC; unlike in humans, corticosterone is the primary corticosteroid in rats).[13] In this model, an increase in baseline corticosterone level was stimulated with ACTH, followed by treatment with compound. Although compound **7n** showed a dose-dependent reduction in PCC following ACTH stimulation, the effects on PAC levels were consistently greater on both a dose and exposure basis.[14] On the basis of the ability of **7n** to effectively reduce aldosterone levels in vivo and its generally favorable profile, the compound was selected for initial human proof-of-concept studies and to understand any limitations of the potential concurrent cortisol reduction.

In human studies, treatment with **7n** was well tolerated and effective in reducing aldosterone levels to provide sustained lowering of blood pressure in patients with primary aldosteronism,[15] primary hypertension,[16] and resistant hypertension.[17] It was found that **7n** provided selective reduction of plasma aldosterone levels without an effect on baseline morning cortisol levels.[15,16] However, suppression of stimulated cortisol levels was seen at doses above 0.5 mg, which can be attributed to the modest selectivity for CYP11B2 over CYP11B1.

While the inhibition of cortisol synthesis by **7n** has limited its development to indications where this effect is either desired or neutral, it provided a valuable initial proof-of-concept for the ability of a CYP11B2 inhibitor to lower blood pressure in patients. In addition, the extensive profiling of **7n** in hypertensive patients afforded an opportunistic approach to safely and effectively lower cortisol levels, which has led to investigation of the compound as a potential therapy for Cushing's syndrome,[18] a disease characterized by elevated levels of cortisol.

■ ASSOCIATED CONTENT

Supporting Information

Procedures for the preparation of **7n** and associated analytical data for **7n** and other representative compounds described herein. Protocols for the aldosterone synthase cellular and CYP19 enzymatic assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(E.L.M.) Tel: 617-871-7586. Fax: 617-871-7045. E-mail: erik.meredith@novartis.com.

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CYP11B2 or AS, aldosterone synthase; CYP11B1, 11β-hydroxylase; CYP19, aromatase; MR, mineralocorticoid receptor; RAS, renin-angiotensin system; PAC, plasma aldosterone concentration; PCC, plasma corticosterone concentration; SAR, structure–activity relationship; TWA, time-weighted average; ACTH, adrenocorticotropic hormone; PK–PD, pharacokinetic–pharmacodynamic; SD, Sprague–Dawley; [C], compound concentration

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