Bioorganic & Medicinal Chemistry Letters 20 (2010) 4324–4327

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The discovery of potent inhibitors of aldosterone synthase that exhibit selectivity over 11-β-hydroxylase

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ARTICLE INFO

Article history:
Received 11 April 2010
Revised 12 June 2010
Accepted 15 June 2010
Available online 19 June 2010

Keywords:
CYP11B2
Aldosterone
CYP11B1
Aldosterone synthase inhibitor
Hypertension.

ABSTRACT

Aldosterone, the final component of the renin–angiotensin–aldosterone system, plays an important role in the pathophysiology of hypertension and congestive heart failure. Aldosterone synthase (CYP11B2) catalyzes the last three steps of aldosterone biosynthesis, and as such appears to be a target for the treatment of these disorders. A sulfonamide–imidazole scaffold has proven to be a potent inhibitor of CYP11B2. Furthermore, this scaffold can achieve high levels of selectivity for CYP11B2 over CYP11B1, a key enzyme in the biosynthesis of cortisol.

The renin–angiotensin–aldosterone system (RAAS) is a key regulator of blood pressure and extracellular volume. Aldosterone, the final component of the RAAS, is a potent mineralocorticoid. Elevated aldosterone levels play an important role in the pathophysiology of hypertension and congestive heart failure.[1] As such, aldosterone has attracted much attention from both the pharmaceutical and clinical communities. Until recently, the majority of this effort has focused on mineralocorticoid receptor (MR) antagonists, such as spironolactone and eplerenone.[2,3] However, current evidence indicates that the deleterious effects of elevated aldosterone levels are not solely mediated by the MR and that non-genomic effects may play a critical role.[4,5] Therefore, directly inhibiting the synthesis of aldosterone may prove advantageous.[6]

The final three steps of aldosterone biosynthesis are catalyzed by a single cytochrome P450 enzyme, CYP11B2, which is expressed in *zona glomerulosa* cells of the adrenal gland.[7] Importantly, fadrozole, an inhibitor of CYP11B2, has been shown to reduce aldosterone levels in humans.[8] Fadrozole was initially developed as an aromatase (CYP19) inhibitor, but it was later discovered that the *R*-enantiomer of fadrozole (FAD286, Fig. 1) is a potent aldosterone synthase inhibitor.[9]

Subsequent studies have shown that FAD286 is also an inhibitor of 11-β-hydroxylase (CYP11B1). CYP11B1 is also expressed in the adrenal gland and catalyzes the last step in the biosynthesis of cortisol, the primary glucocorticoid.[7] Cortisol mediates the stress response affecting energy mobilization and the immune system. Therefore, a selective CYP11B2 inhibitor may be desirable. FAD286 exhibits IC_{50} values of 1.6 and 9.9 nM in recombinant human CYP11B2 and CYP11B1 enzyme assays, respectively.[10] This small degree of selectivity is not surprising, considering that the amino acid sequences of these two enzymes are 95% identical in the coding regions.[11] Homology models of the two enzymes

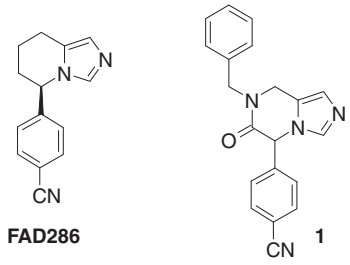

Figure 1. The *R*-enantiomer of fadrozole (FAD286) and a representative analog from the lactam series (**1**).

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doi:10.1016/j.bmcl.2010.06.086

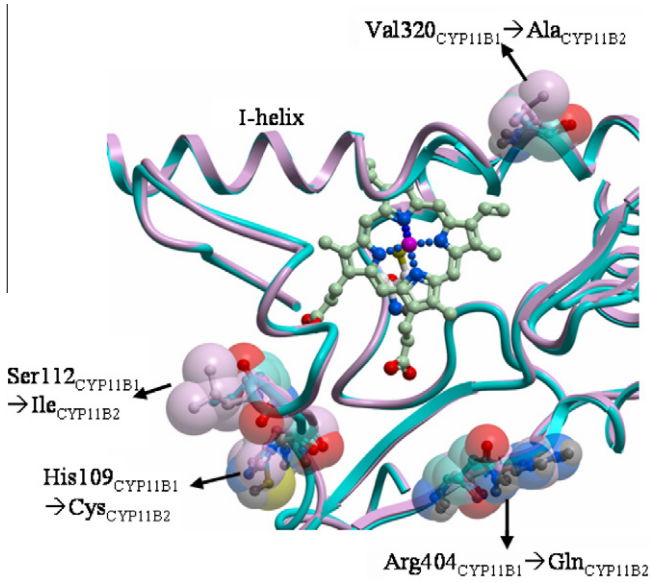


Figure 2. Homology models of CYP11B1 (pink) and CYP11B2 (cyan). Active sites depicted; residues that lie within 10 Å of heme, and are different between CYP11B1 and CYP11B2, are labeled.

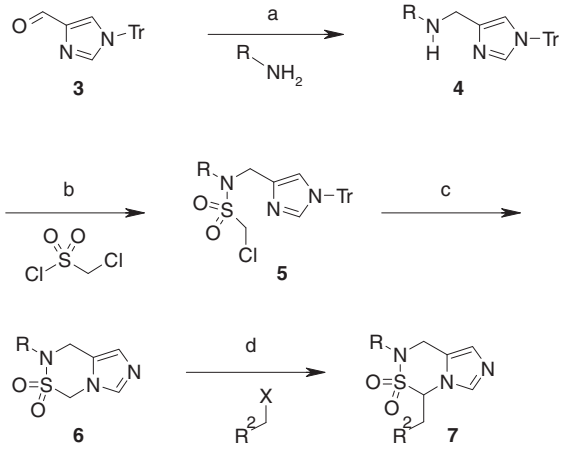
provided further evidence that achieving high levels of selectivity would be challenging.[12] Our modeling indicated that only four residues differed between the two enzymes within 10 Å of the heme, the presumed substrate binding site (Fig. 2).

Having recognized the ability of FAD286 to inhibit CYP11B2, we began a program to discover novel analogs of this compound with improved properties. Early efforts focused on modifying the saturated portion of the fused ring system of fadrozole. One promising series, which contains a lactam moiety exemplified by **1**[13,14] (Fig. 1), was found to have excellent potency against CYP11B2 (IC_{50}: 40 nM).[15] However, compounds from this series were also potent CYP11B1 inhibitors (e.g., IC_{50} = 2 nM for **1**).

The potency of the lactam series led us to explore a bioisosteric sulfonamide series. This Letter describes the development of cyclic sulfonamide analogs with the aim of greatly improving selectivity for CYP11B2 over CYP11B1.

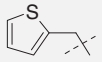
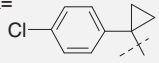
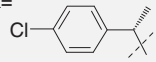
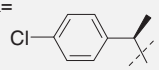
Sulfonamide **2** (Table 1), one of the earliest molecules synthesized in this series, displays modest CYP11B1/CYP11B2 selectivity. However, it is also plagued by poor solubility (<0.5 μM), low metabolic stability in human liver microsomes (HLM, $t_{1/2}$ <5 min),[16] and activity against CYP19 (39% inhibition at 1 μM).[17] In an attempt to improve upon this profile we initially explored the space around the nitrogen of the sulfonamide. From a synthetic perspective this required a four-step sequence starting from commercially available aldehyde **3** (Scheme 1).

A series of reductive aminations with commercially available primary amines permitted access to a diverse array of amino-imidazoles. Sulfonylation of the resulting secondary amines with chloromethanesulfonyl chloride, followed by microwave-mediated cyclization afforded sulfonamides of type **6** in modest yields. Alkylation with the appropriate alkyl halide or epoxide completes the



Scheme 1. Reagents and conditions: (a) $NaBH(OAc)_3$, DCM, rt; (b) DIPEA, DCM, −78 °C; (c) DMF, EtOH, DIPEA (3:1:1), microwave irradiation at 200 °C for 1 h, 10–30% yield for the three steps; (d) KHMDS, THF, −78 °C to rt 10–70%.

Table 1
Exploration of the space around the sulfonamide nitrogen

	hCYP11B2 IC_{50}, (nM) (Ref. 10)	hCYP11B1 IC_{50}, (nM) (Ref. 10)	hCYP19 % Inhib @ 1 μM (Ref. 16)	HLM $t_{1/2}$, (min) (Ref. 15)
2, R = 4-F-Ph-CH_2–	2.5	26.1	39	5
8, R = 4-Cl-Ph-CH_2–	5.2	50.2	51	8
9, R = Ph-CH_2–	18.6	103	14	3.
10, R = 4-CF_3O-Ph-CH_2–	104	544	13	7
11,[19] R = 4-CF_3-Ph-CH_2–	121	566	0	13
12, R = c-C_3H_5–	>1000	930	9	>405
13, R = c-C_3H_5-CH_2–	758	122	19	55
14, R= 	117	55.7	13	2
15, R= 	628	119	71	19
16, R= 	9.4	76.0	56	6
17, R= 	61.7	204	24	5

synthesis. The resulting stereoisomers could be readily separated via HPLC employing a chiral stationary phase.

With this synthesis in hand, we generally found that one enantiomer proved to be more potent against CYP11B2 and/or more selective.[14] We also determined that benzylic moieties are preferred and that halogen substitution at the para position affords maximal CYP11B2 potency and selectivity over CYP11B1 (e.g., **2** and **8**, Table 1). Unfortunately, changes in this region were not able to significantly improve metabolic stability without dramatic decreases in potency. Of particular note are compounds **12** and **13**, which exhibit vastly improved metabolic stability, but only weakly inhibit CYP11B2. Based on the superior metabolic stability of compounds **12** and **13**, we theorized that the benzylic position of compounds such as **8** may be metabolically labile. This was confirmed by metabolite identification studies.[18] We next synthesized compounds **15**–**17**[13] with the intention of decreasing the rate of benzylic oxidation (Table 1). Only **15** showed any improvement in metabolic stability, and only **16** maintained single digit nanomolar potency against CYP11B2.

Surprised by the lack of improvement in metabolic stability, we initiated further metabolite identification studies with **16**. These studies indicated that the installation of the benzylic methyl group did in fact block metabolism at this site, but that the isobutyl group was now the major site of metabolism. We therefore began exploring replacements for the isobutyl group. We anticipated that incorporating polar functional groups in this region may not only serve to slow metabolism, but would also improve solubility and attenuate CYP19 activity. To our delight, not only did this strategy lead to improvements in all of these areas, but they also greatly improved selectivity over CYP11B1 (Table 2).

The hydroxyl of **18** specifically blocked the most likely site of metabolism, resulting in an improvement in the HLM $t_{1/2}$ to greater than 300 min. In addition, the added polarity improved solubility to 500 μM (pH 6.8), abrogated CYP19 activity, and afforded CYP11B1/CYP11B2 selectivity of 42-fold while maintaining adequate potency. Ethers, such as **20** and **21**, also improved CYP11B1/CYP11B2 selectivity but failed to provide significant benefits with respect to metabolic stability. Incorporating more metabolically stable alkyl groups (e.g., **22**) reintroduced CYP19 activity and did not address the solubility concerns. The greatest levels of selectivity were achieved by alkylating intermediate **6** with styrene epoxide. Reaction with (R)-styrene epoxide furnished **24**, which was greater than 600-fold selective for CYP11B2 over CYP11B1, and exhibited negligible CYP19 inhibition.

Despite the selectivity achieved over CYP11B1 and CYP19, this particular scaffold exhibited strong inhibition of CYP3A4 in vitro. Inhibition of CYP3A4-mediated oxidation of both testosterone and midazolam is observed (Table 3).[20] The SAR relating to midazolam competition is particularly flat. Modifications to the northern hemisphere, including replacement of the benzyl moiety with a thiophene (e.g., **14**) failed to improve selectivity over CYP3A4. Adding polarity to the southern hemisphere also proved ineffective (e.g., **18**, **19**).

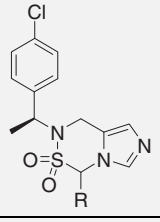
Although attenuating potency against CYP3A4 has been a challenge for this particular scaffold, this work further[6] demonstrates that outstanding selectivities over CYP11B1 can be achieved, in spite of its very high homology with CYP11B2.

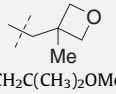
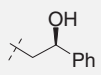
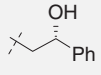
Table 3
CYP3A4 assay results against both midazolam and testosterone

Compounds	hCYP11B2 IC_{50}, (nM)	CYP3A4 (m)[a] IC_{50}, (nM)	CYP3A4 (t)[b] IC_{50}, (nM)
8	5.2	<68	87
9	18.6	100	1400
10	104	<68	490
11	121	<68	280
14	117	<68	1160
16	9.4	<68	<68
17	61.7	<68	NA
18	11.4	<68	<68
19	7.8	<68	<68
20	0.7	<68	530
21	0.8	<68	120
22	1.3	<68	180
23	2.4	100	900
24	0.3	<68	1920

[a] m = Midazolam.
[b] t = Testosterone; see Ref. 19 for assay details.

Table 2
Incorporating polarity into the southern hemisphere



	hCYP11B2 IC_{50}, (nM) (Ref. 10)	hCYP11B1 IC_{50}, (nM) (Ref. 10)	hCYP19 % Inhib @ 1 μM (Ref. 16)	HLM $t½$, (min) (Ref. 15)
18, R = $-CH_2C(CH_3)_2OH$	11.4	471	4	>300
19, R= 	7.8	340	35	76
20, R = $-CH_2C(CH_3)_2OMe$	0.9	20.6	17	12
21, R = $-CH_2$-O-$CH(CH_3)_2$	0.8	222	23	5
22, R = $-CH_2$-c-C_3H_5	1.3	245	39	25
23, R= 	2.4	234	6	24
24, R= 	0.3	182	6	15

Acknowledgements

We thank Dr. Bing Wang, and Karl Gunderson for assistance in securing and interpreting high-field NMR spectra, and Patrick Cronan for assistance in obtaining high resolution mass spectra.

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13. All compounds depicted are single isomers (i.e., >98% de and/or >98% ee) unless otherwise noted. The separation of stereoisomers was accomplished via HPLC, employing columns with chiral stationary phases.
14. Typically, the enantiomeric configuration was not determined. The data reported in this manuscript relates to the enantiomer with the preferred in vitro profile.
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16. Human Liver Microsomal Stability Assay: The half life ($t_{1/2}$) in human liver microsomes (HLM) (BD Gentest, Woburn, MA) was determined based on published method (*vide infra*). In brief, 1 μM test compound was incubated in pooled HLM (containing 0.5 mg microsomal protein per mL) in the presence of 25 μg of alamethacin per mg protein, 1.0 mM NADPH, 1.0 mM UDPGA, and 2 mM $MgCl_2$. The half-life ($t_{1/2}$) was derived by monitoring the disappearance of test compound over a short period of time (e.g., 30 min). See: (a) Obach, R. S. *Drug Metab. Dispos.* **1999**, *27*, 1350; (b) Kalvass, J. C.; Tess, D. A.; Giragossian, C.; Linhares, M. C.; Maurer, T. S. *Drug Metab. Dispos.* **2001**, *29*, 1332; (c) Chauret, N.; Gauthier, A.; Nicoll-Griffith, D. A. *Drug Metab. Dispos.* **1997**, *26*, 1; (d) Fisher, M. B.; Campanale, K.; Ackermann, B. L.; VandenBranden, M.; Wrighton, S. A. *Drug Metab. Dispos.* **2000**, *28*, 560.
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