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Imidazopyridyl compounds as aldosterone synthase inhibitors

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ABSTRACT

The inhibition of aldosterone synthase (CYP11B2) may be an effective treatment of hypertension and heart failure, among other ailments. Previously reported benzimidazole CYP11B2 inhibitors led the way for bioisosteric imidazopyridines that are both potent and selective over CYP11B1.

Aldosterone is a principal mineralocorticoid that promotes increased blood pressure, inflammation, fibrosis, and organ damage.[1–4] The final steps of its biosynthesis are catalyzed by a single mitochondrial cytochrome P450 enzyme aldosterone synthase (CYP11B2), found predominantly in zona glomerulosa cells of the adrenal gland.[5,6] Inhibition of CYP11B2 should lower plasma aldosterone levels, potentially providing an effective treatment for a variety of ailments, including hypertension, heart failure, and chronic kidney disease.

A great design challenge to advance a CYP11B2 inhibitor in the clinic is to maintain its high selectivity against other CYPs, in particular cortisol synthase CYP11B1, which share >93% homology with CYP11B2. CYP11B1 catalyzes the biosynthesis of cortisol, and inhibition of CYP11B1 can result in impaired stress response and altered glucose metabolism. Selectivity against other steroidogenic CYPs, such as CYPs 17, 19, and 21, is crucial as well, since they regulate the production of androgens and estrogens.

A host of small molecule CYP11B2 inhibitors have been reported.[7,8,12] Among them, LCI-699 has been shown to lower aldosterone levels and blood pressure in the clinic, providing the proof-of-concept for the therapeutic target. At the same time, undesired dose-limiting impairment of cortisol response was also observed, presumably due to the inhibition of CYP11B1.[9–11]

The results from LCI-699 highlight the need to discover CYP11B2 inhibitors that are both potent and selective against CYP11B1 for clinical development. Our discovery of the benzimidazole series[13] has led us to further explore the isosteric equivalents of the heterocycle. We are pleased to find that the imiadzopyridine analog **2** offers good potency on CYP11B2 while maintaining similar selectivity against CYP11B1 as the initial benzimidazole lead **1** (Fig. 1).[14]

The preparation of the imidazopyridines is illustrated with the synthesis of compound **2** (Scheme 1).[14] 5-Fluoropyridin-2-amine was treated with neat ethyl bromoacetate, and the resultant intermediate **2a** was heated in phosphorus oxychloride to afford the corresponding 2-chloro-6-fluoroimidazo[1,2-*a*]pyridine **2b** after careful quenching and isolation. The intermediate **2b** was halogenated at the 3-position with *N*-iodosuccinimide to yield 2-chloro-6-fluoro-3-iodoimidazo[1,2-*a*]pyridine **2c**. Two iterative Suzuki couplings of **2c**, first with cyclopropylboronic acid and the corresponding pyridineboronic acid pinacol ester, afforded the desired compound **2**.

As of the compounds reported in Table 1, compound **3** was prepared by employing methylboronic acid instead of cyclopropylboronic acid in step d. Compound **4** was prepared directly from **2b** and the corresponding boronate ester. Chlorination of compound **4**

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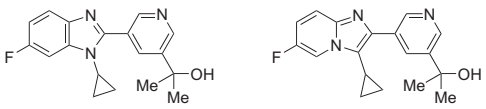
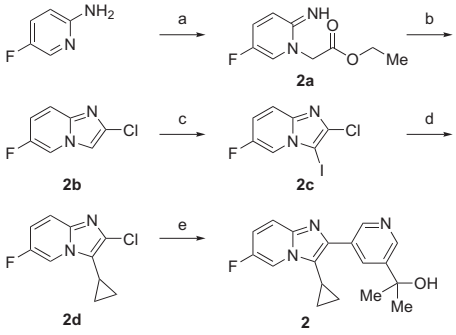


1
CYP11B2 IC_{50} = 1.4 nM
CYP11B1 IC_{50} = 100 nM
CYP11B1/B2 = 75

2
CYP11B2 IC_{50} = 13 nM
CYP11B1 IC_{50} = 830 nM
CYP11B1/B2 = 63

Fig. 1. Comparison of the benzimidazole lead **1** and the imidazopyridine lead **2**.



Scheme 1. Reagents and conditions for preparation of compound **2**: (a) ethyl bromoacetate, neat, room temperature; (b) phosphorous oxychloride, neat, 110 °C; (c) *N*-iodosuccinimide, MeCN, room temperature; (d) *c*-PrB(OH)$_2$, Pd(PPh$_3$)$_2$(OAc)$_2$ (0.1 equiv), Cs_2CO_3, THF, 100 °C; (e) 5-(1-hydroxy-1-methylethyl) pyridine-3-boronic acid pinacol ester, Pd(OAc)$_2$ (0.1 equiv), S-Phos (0.2 equiv), K_3PO_4, THF, 100 °C.

by *N*-chlorosuccinimide afforded compound **5** The precursor for compound **6** (**6d**) was prepared through lithiation of intermediate **2c** (Scheme 2).

Our investigation focused on improving selectivity over CYP11B1 and to bring forward a suitable candidate for *in vivo* studies. In the benzimidazole series, N-substitution was found to exert a substantial effect on CYP11B2 inhibition, with optimal activity limited to a small cyclopropyl-containing group.[13] Here in the imidazopyridine series, we observed a similar SAR pattern (Table 1).

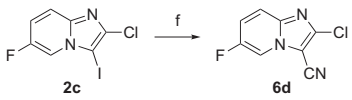
Poor activity was observed when the corresponding C-3 position of the imidazopyridine is unsubstituted (compound **4**). Both the methyl group (compound **3**) and the chloro group (compound **5**) give compounds that are more potent than the corresponding cyclopropyl compound at CYP11B2 but less selective against CYP11B1. The pseudohalide cyano group leads to a less potent and less selective compound (compound **6**).

Prior work in the benzimidazole series has established that substitution at the 2- or the 6-position of the pyridine is not tolerated by CYP11B2.[13] With the cyclopropyl group confirmed as the optimal one balancing potency at CYP11B2 and selectivity against CYP11B1, we proceeded to examine the effect of different substituents R^2 on the pyridine (Table 2). Compounds **7**–**17** were synthesized similarly as compound **2**, with the appropriate boronate ester used in the second Suzuki coupling.

Compounds **7** and **8**, bearing a tertiary alcohol with one of the methyl groups replaced by a trifluoromethyl group, show similar activity on CYP11B2 with similar levels of selectivity against CYP11B1 as compound **2**. The carboxylate ester group improves the CYP11B2 activity while decreasing the CYP11B1 activity (compound **9**), resulting in a highly selective compound, but it does not offer enough metabolic stability to go forward. Electron-withdrawing groups such as fluoro or trifluoromethyl (compounds **10**, **12**) have little effect on either the CYP11B2 activity or CYP11B1 selectivity, while the cyano group (compound **11**) leads to some erosion of CYP11B2 activity. Groups such as methoxy, methyl or phenyl improve the CYP11B2 activity and CYP11B1 selectivity slightly (compounds **13**–**15**). Finally, replacing the entire pyridine with isoquinoline gives a potent CYP11B2 inhibitor with similar selectivity as compound **2** against CYP11B1 (compound **17**).

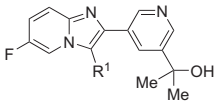
We then sought to investigate the effect of different R^4 and R^5 on the imidazopyridine. We investigated two series, one with the pyridine bearing a tertiary alcohol at the 5-position of the pyridine (Table 3) and another with the pyridine bearing a 5-fluoro group (Table 4). In the 5-tertiary alcohol series, when the fluorine is moved from the 6-position to the 7-position (compound **18**), reduction in potency at CYP11B2 and selectivity against CYP11B1 was observed. Replacing the fluoro with a cyano group at the 6-position results in little change in potency and selectivity (compound **19**).

In the 5-fluoro series, moving the halogen from the 6-position to the 7-position has a similar effect in loss of potency at CYP11B2 (compound **20**). Replacing the fluoro with a chloro group generates a compound with a similar profile (compound **21**). By introducing an extra halogen at the 7-position (R^5), we obtained a compound with better potency at CYP11B2 while maintaining similar selectivity at CYP11B1 (compound **22**). Incorporating additional alkyl



Scheme 2. Reagents and conditions for preparation of intermediate **6d**: (f) *n*-butyllithium, THF, −78 °C, 5 min; *p*-toluenesulfonyl cyanide.

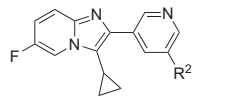
Table 1
Effect of R^1 substitution on CYP11B2/CYP11B1 inhibition.[a]



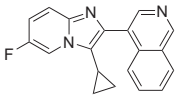
Compound	R^1	hCYP11B2 (IC_{50}, nM)	hCYP11B1 (IC_{50}, nM)	B1/B2[b]
2	Cyclopropyl	13	830	63
3	Me	1.2	37	31
4	H	52	880	17
5	Cl	1.2	33	27
6	CN	23	600	27

[a] All IC_{50}'s reported in this table correspond to n ⩾ 2, reported as their geometric mean.
[b] Ratio of hCYP11B1 IC_{50}/hCYP11B2 IC_{50}.

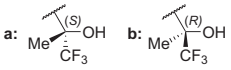
Table 2
Effect of R^2 substitution on CYP11B2/CYP11B1 inhibition.



7-16



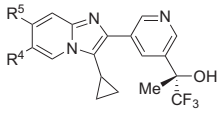
17



Compound	R^2	hCYP11B2 (IC_{50}, nM)	hCYP11B1 (IC_{50}, nM)	B1/B2[a]
2	$CMe_2(OH)$	13[b]	830[b]	63
7	a	6.6[c]	460[c]	69
8	b	4.7[c]	250[c]	53
9	CO_2Me	2.5[b]	640[b]	>260
10	F	25[b]	1800[b]	71
11	CN	160[b]	>8700[b]	>55
12	CF_3	10[b]	590[b]	60
13	OMe	4.0[b]	530[b]	130
14	Me	3.3[b]	280[b]	85
15	Ph	6.6[b]	300[b]	46
16	CH_2NHSO_2Et	33[b]	490[c]	15
17		0.6[b]	100[b]	190

[a] Ratio of hCYP11B1 IC_{50}/hCYP11B2 IC_{50}.
[b] IC_{50}'s determined as $n \geqslant 2$, reported as their geometric mean.
[c] IC_{50}'s determined as n = 1.

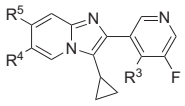
Table 3
Effect of R^4 and R^5 substitution on CYP11B2/ CYP11B1 inhibition in the 5-tertiary alcohol series.[a]



Compound	R^4	R^5	hCYP11B2 (IC_{50}, nM)	hCYP11B1 (IC_{50}, nM)	B1/B2[b]
7	F	H	6.6	460	69
18	H	F	26	630	25
19	CN	H	5.8	220	37

[a] All IC_{50}'s reported in this table correspond to $n \geqslant 2$, reported as their geometric mean.
[b] Ratio of hCYP11B1 IC_{50}/hCYP11B2 IC_{50}.

Table 4
Effect of R^3, R^4 and R^5 substitution on CYP11B2 /CYP11B1 inhibition in the 5-fluoro pyridine series.[a]



Compound	R^4	R^5	R^3	hCYP11B2 (IC_{50}, nM)	hCYP11B1 (IC_{50}, nM)	B1/B2[b]
10	F	H	H	25	1800	71
20	H	F	H	98	4600	47
21	Cl	H	H	23	1100	47
22	Cl	F	H	7.3	760	100
23	F	H	Me	7.9	930	120
24	F	H	Et	6.6	800	120

[a] All IC_{50}'s reported in this table correspond to $n \geqslant 2$, reported as their geometric mean.
[b] Ratio of hCYP11B1 IC_{50}/hCYP11B2 IC_{50}.

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Table 5
Activity of selected compounds at related CYP targets (IC_{50} in nM).

Compound	CYP17[a]	CYP19[a]	CYP21[a]	CYP3A4[b]
7	>10,000	7700	>10,000	>50,000
10	ND	2200	>10,000	>50,000
17	ND	790	>10,000	3500
22	>10,000	2300	ND	>25,000
23	>10,000	420	>10,000	11,000
24	5700	120	>10,000	2800

ND not determined.
[a] IC_{50}'s determined as $n \geqslant 2$, reported as their geometric mean.
[b] IC_{50}'s determined as n = 1.

Table 6
Metabolic stability of selected compounds in liver microsomes.[a]

Compound	Human	Rat	Rhesus
7	88%	38%	31%
8	96%	33%	31%
9	ND	1%	0%
10	93%	70%	29%
16	92%	31%	45%
21	90%	77%	37%
22	93%	80%	87%
23	88%	56%	46%
24	37%	2%	0%

[a] Reported as % parent remaining 30 min after incubating a 1 μM solution with 250 μg/mL of liver microsomes and NADPH.

groups at the pyridine (compounds **23**, **24**) has little impact on the potency at CYP11B1.

It is important to confirm that the CYP11B2 inhibitors progressing into development do not interfere against CYPs that play a role in androgen and estrogen biosynthesis (CYPs 17, 19, and 21) as well as hepatic CYPs such as CYP3A4. As shown in Table 5, most of the compounds in the chemical series are selective against the related CYP targets. They do not inhibit CYP17 or CYP 21, and for exemplified compounds such as **7** and **22**, there is a good window (>1000-fold) for the inhibition of CYP19. The erosion of CYP19 selectivity is evident when alkyl groups are introduced into the 4-position of the pyridine (compounds **23**, **24**), or when the pyridine is replaced by isoquinoline (compound **17**). With regard to the hepatic CYPs, compounds **7** and **22** are selective against CYP 3A4 ($IC_{50} > 25$ μM), CYP 2D6 ($IC_{50} > 25$ μM), and CYP 2C9 ($IC_{50} = 6$, >25 μM respectively).

To identify development candidates that display reasonable oral bioavailability, we have screened some of the compounds for their metabolic stability against liver microsomes (Table 6). Most of the compounds have good metabolic stability against human microsomes. Stereochemistry at the tertiary alcohol has little impact on the metabolic stability of compound **7** vs **8**. Compound **9** has an excellent in-vitro profile but unfortunately displayed poor metabolic stability across human, rat and rhesus. Metabolic stability against rhesus microsomes is moderate for the selected compounds, which appeared to be improved by extra halogens on the imidazopyridine ring (compound **10** vs compound **22**). The introduction of alkyl groups in the 4-position of the pyridine (compounds **23**, **24**) is detrimental to metabolic stability across multiple species.

Table 7
Rat pharmacokinetic profile for compound **7**[a] (1 mg kg^{-1} IV[b]/2 mg kg^{-1} PO[c]).

Parameters	
Cl_p (mL min^{-1} kg^{-1})	23
Vd_{ss} (L kg^{-1})	0.82
MRT (h)	0.58
$t^{1/2}$ (h)	0.61
PO AUCN (μM h kg mg^{-1})	0.92 ± 0.54
C_{max} (μM)	0.7 ± 0.5
F%	47%

[a] Male Wistar Han rats.
[b] n = 2, fasted rats, compound dosed in ethanol/PEG300/ water (10/60/30).
[c] n = 3, fasted rats, compound dosed in 0.5% methylcellulose.

To confirm that the liver microsome stability screen can identify compounds with good pharmacokinetic profiles, we have submitted compound **7** for rat PK determination (Table 7). As expected, compound **7** displays moderate plasma clearance and good oral exposure, which would allow the compound to be further profiled *in vivo*.

In summary, we have demonstrated that imidazopyridines are a suitable bioisostere of benzimidazoles for inhibition of aldosterone synthase (CYP11B2). Further optimization of the series improved activity on CYP11B2 with the necessary selectivity on remaining CYPs. By studying the SAR on the series, we brought forth a lead candidate for *in vivo* studies.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.12.003.

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