Journal of Medicinal Chemistry

Article

Discovery of N-[5-(6-chloro-3-cyano-1-methyl-1H-indol-2-yl)-pyridin-3-ylmethyl]-ethanesulfonamide, a Cortisol-Sparing CYP11B2 Inhibitor that Lowers Aldosterone in Human Subjects

Julien P.N. Papillon, Changgang Lou, Alok Singh, Christopher M. Adams, Gary M. Ksander, Michael Beil, Wei Chen, Jennifer Leung-Chu, Fumin Fu, Lu Gan, Chii-Whei Hu, Arco Jeng, Daniel LaSala, Guiqing Liang, Dean F Rigel, Kerry S Russell, John A Vest, and Catherine Watson

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b01545 • Publication Date (Web): 05 Nov 2015

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Discovery of *N*-[5-(6-chloro-3-cyano-1-methyl-1*H*-indol-2-yl)-pyridin-3-ylmethyl]-ethanesulfonamide, a Cortisol-Sparing CYP11B2 Inhibitor that Lowers Aldosterone in Human Subjects

Julien P.N. Papillon,[‡] Changgang Lou,[‡,⊥] Alok K. Singh,[‡] Christopher M. Adams,[‡] Gary M. Ksander,[‡,○] Michael E. Beil,[Δ] Wei Chen, [Δ] Jennifer Leung-Chu,[Δ] Fumin Fu,[Δ] Lu Gan, [◊,⊥] Chii-Whei Hu,[Δ,∞] Arco Y. Jeng,[Δ,⊥] Daniel LaSala,[Δ] Guiqing Liang,[‖] Dean F. Rigel,[Δ] Kerry S. Russell, [§] John A. Vest, [§,⊥] Catherine Watson.[§]*

[‡] Global Discovery Chemistry, [‖] Metabolism and Pharmacokinetics, [§] Translational Medicine, [◊] Drug Metabolism and Pharmacokinetics, Novartis Institutes for BioMedical Research, 250 Massachusetts Avenue, Cambridge, MA 02139; [Δ] Cardiovascular and Metabolism, Novartis Institutes for BioMedical Research, One Health Plaza, East Hanover, NJ 07936.

Abstract

Human clinical studies conducted with LCI699 established aldosterone synthase (CYP11B2) inhibition as a promising novel mechanism to lower arterial blood pressure. However, LCI699's low CYP11B1/CYP11B2 selectivity resulted in blunting of adrenocorticotropic hormone-

stimulated cortisol secretion. This property of LCI699 prompted its development in Cushing's disease, but limited more extensive clinical studies in hypertensive populations, and provided an impetus for the search for cortisol-sparing CYP11B2 inhibitors. This paper summarizes the discovery, pharmacokinetics, and pharmacodynamic data in pre-clinical species and human subjects of the selective CYP11B2 inhibitor **8**.

Introduction

Aldosterone is the principal mineralocorticoid in man and is produced in the zona glomerulosa (outer zone) of the adrenal cortex by aldosterone synthase (CYP11B2). Aldosterone is a key component of the renin-angiotensin-aldosterone system (RAAS) and acts primarily at the renal distal convoluted tubules as a critical regulator of fluid and electrolyte homeostasis. Consequently, increases in aldosterone can result in elevated blood pressure and fluid retention. Elevated serum aldosterone has been linked to resistant hypertension,[1] which represents a significant medical need.[2] Patients with an aldosterone excess are more susceptible to premature vascular disease, cardiac fibrosis, vessel wall inflammation, left ventricular hypertrophy, atrial fibrillation, stroke, myocardial infarction, and metabolic syndrome.[3] Aldosterone escape[4] due to inadequate suppression of aldosterone levels during long-term blockade of RAAS with either angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) is also associated with end organ damage. Suppression of aldosterone's untoward effects, therefore, represents an attractive therapeutic approach to reduce blood pressure and cardiovascular disease burden. Mineralocorticoid receptor antagonists (MRAs) spironolactone and eplerenone have demonstrated efficacy in hypertension, primary aldosteronism, obstructive sleep apnea or renal disease. In addition MRAs have shown morbidity and mortality benefits in patients with both mild and severe heart failure on standard therapy,[5] in patients with left ventricular dysfunction

after myocardial infarction,[6] and in heart failure patients with a preserved ejection fraction and elevated brain natriuretic peptide.[7] However, there are significant limitations associated with MRAs. The use of spironolactone is associated with hormonal side effects including breast pain, gynecomastia, impotence in males, and menstrual irregularities in females. Eplerenone is more selective for MR receptors and consequently has a lower incidence of sex hormone-related adverse events. However, eplerenone is less potent than spironolactone, and due to an increased risk of hyperkalemia, is contraindicated in diabetic hypertensive patients with microalbuminuria and various degrees of renal impairment. Importantly, treatment with MRAs is associated with a reactive elevation of circulating aldosterone levels, which is postulated to potentially limit efficacy by activating central mineralocorticoid receptors and increasing sympathetic neural drive in the heart failure setting.[8] Moreover, substantial evidence exists suggesting a pathological role of aldosterone via non-MR mediated pathways.[9] Therefore, therapeutic agents that inhibit the production of aldosterone may confer additional benefits over MRAs.

Early studies with FAD286, an aldosterone synthase inhibitor (ASI), suggested that pharmacological inhibition of CYP11B2 might indeed be clinically useful. Chronic administration of FAD286 in rat models showed positive effects on inflammatory markers, fibrosis, and progression of heart failure.[10] Later studies with LCI699[11] provided additional support for the development of ASIs. Treatment of double-transgenic rats prevented the development of cardiac and renal functional abnormalities and prolonged survival, independent of blood pressure.[12] LCI699 dose-dependently lowered aldosterone in healthy males,[12] and lowered blood pressure in patients with primary hyperaldosteronism,[13] essential hypertension[14] and resistant-hypertension.[15] LCI699 was also found to blunt cortisol secretion in response to adrenocorticotropic hormone (ACTH) stimulation,[16] a finding that is most likely related to potent

inhibition of CYP11B1 by LCI699 (human rCYP11B2 IC_{50} = 0.7 nM, rCYP11B1 IC_{50} = 2.5 nM).[11,12] Although this finding led to cessation of further exploration of the relationship between aldosterone lowering, electrolytes modulation and blood pressure regulation with LCI699, they created an opportunity to address an unmet medical need in Cushing's disease. LCI699 was shown to normalize urinary free cortisol in a 10-week proof-of-concept study in 12 patients.[16] The results from this study may spur further exploration of CYP11B1 selective inhibitors.[17] However, antihypertensive proof-of-concept with a CYP11B2-selective ASI is still needed,[18] and much effort has been devoted to identifying such compounds.[19] Herein, we describe the discovery, pharmacokinetics and pharmacodynamics data in pre-clinical species and human subjects of the cortisol-sparing ASI *N*-[5-(6-chloro-3-cyano-1-methyl-1*H*-indol-2-yl)-pyridin-3-ylmethyl]-ethanesulfonamide (**8**).

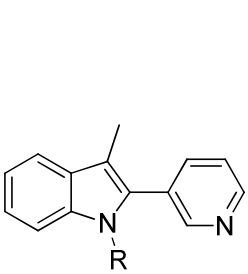
Results and Discussion

The lead compound identified from the Novartis archive was pyridinyl indole **1**, which had been previously described in 1969 as useful in the treatment of hyperaldosteronism by Geigy Chemical Corporation.[20] As shown in Table 1, despite its low molecular weight, **1** showed excellent inhibition of aldosterone secretion in an H295R cellular assay[21] (IC_{50} = 21 nM) and potent inhibition of recombinant CYP11B2[22] (IC_{50} = 3 nM). Most importantly, the selectivity over CYP11B1 was an order of magnitude superior to that of LCI699 (S = 27 *vs.* 3), which led us to optimize **1**. One area of focus was the unacceptable level of aromatase (CYP19) inhibition, namely 94% at 10 μM and 59% at 1 μM. Capping the indole hydrogen with methyl, as in **2**, did not impact aromatase inhibition but had a positive impact on biochemical and cellular potency, as well as selectivity over CYP11B1. We quickly identified that nitrile substitution at the 3-position of the indole, such as in **3**, gave further improvement in selectivity over both CYP11B1

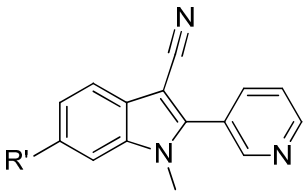
and aromatase, while also improving stability in rat liver microsomes (LM), but at the expense of on-target potency. Further substitution of the indole at the 6-position with chlorine, as in **4**, regained potency and dramatically improved stability in human liver microsomes. While **4** had an attractive combination of potency, CYP selectivity and in vitro metabolic stability, more extensive profiling revealed potent inhibition of monoamine oxidase A (MAO-A), with an IC_{50} = 0.21 μM. MAO-A regulates neurotrasmitter levels by oxidation of biogenic amines, and, like CYP11B2, is expressed in mitochondrial membranes. Intestinal MAO-A inhibition can lead to hypertensive crisis, when foods containing tyramine are consumed (so-called "cheese reaction")[23] or hyperserotonemia, if foods containing tryptophan are consumed.[24] We found that introduction of polar groups at the 5-position of the pyridine ring effectively eliminated MAO-A inhibition. A sulfamate group, as in **5**, not only removed inhibition of MAO-A (IC_{50} >10 μM), but also increased selectivity over CYP11B1 and aromatase. However, **5** showed inadequate in vitro metabolic stability. We surmised that a sulfonamide might afford improved stability. Although sulfonamide **6**, the matched pair of **5**, was a weak ASI, adding the chlorine back to the indole ring afforded **7**, which exhibited improved potency against CY11B2, excellent microsomal stability, and was also devoid of aromatase and MAO-A activity. However, **7** possessed only moderate selectivity over CYP11B1 (S = 43). Further gains in potency and selectivity were achieved with **8**, albeit at the expense of selectivity over aromatase. Nevertheless, aromatase inhibition by **8** (IC_{50} = 3.4 μM) was still nearly 1000-fold weaker than CYP11B2.[25] We also found that the indole core could be replaced with a 7-aza-, but not 5-azabenzimidazole core. Compound **10** showed comparable cellular potency and selectivity over CYP11B1 as **8**, albeit with improved selectivity over aromatase, despite lacking the nitrile at the 3-position of the azaindole ring.

Although much of the early literature around ligand efficiency metrics was being published at the time these studies were conducted and did not impact our decisions, it is interesting to consider a retrospective analysis (Table 1). As optimization proceeded from **1** to **8**, LEI gradually decreased as functionalities were appended to dial out off-target activities (0.53 to 0.32), but LipE remained remarkably constant (5.4 to 5.7).

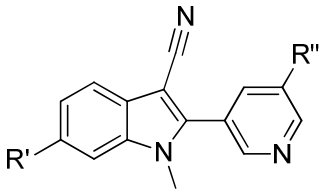
Table 1. Optimization of potency, selectivity and microsomal stability of lead compound **1**



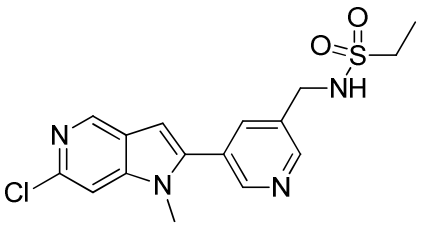
1 R=H
2 R=Me



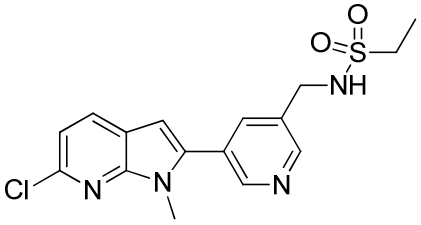
3 R'=H
4 R'=Cl



5 R'=H, R''=OSO_2NMe_2
6 R'=H, R''=$NHSO_2Et$
7 R'=Cl, R''=$NHSO_2Et$
8 R'=Cl, R''=CH_2NHSO_2Et



9



10

	IC_{50} (nM)			CYP11B2 LEI[d]	CYP11B2 LipE[e]	CYP19 %i at 10 μM	LM $t_{1/2}$ (min)	
Compd	Aldo[a]	hB2[b]	S[c]				Rat	Human
1	21	3	27	0.53	5.4	94	-	-
2	9	1.4	38	0.52	5.4	91	5	12
3	90	11	56	0.44	5.1	58	30	10
4	4	2.5	43	0.47	5.4	47	47	259

5	39	2	103	0.35	6.8	27	1	3
6	380	-	-	-	-	-	-	-
7	13	11	43	0.32	4.4	15	176	>400
8	5.8	3.7	100	0.32	5.6	82[f]	28	77
9	169	22	21	0.32	5.7	-	-	-
10	7	1.1	86	0.37	7.1	11	40	21

[a] Aldosterone secretion in H295R cells; [b] Human recombinant CYP11B2; [c] Ratio of CYP11B1 IC_{50} over CYP11B2 IC_{50}; [d] LEI = -log(IC_{50})/HA; [e] LipE = -log(IC_{50}) – clogP; [f] IC_{50} = 3.4 μM.

Equilibrium solubility and pharmacokinetic data for **8** and **10** are summarized in Table 2. The 7-azabenzimidazole afforded much improved solubility over the indole core, however, **10** suffered from high clearance and very short half-life. Despite its low equilibrium solubility, **8** showed good oral exposure and excellent oral bioavailability, which, combined with its cellular potency and favorable selectivity profile, prompted us to select this compound for further evaluation.[26]

Table 2. Equilibrium Solubility and Rat Pharmacokinetics of **8** and **10**.

Compd	Equilibrium solubility (mM) in buffer pH 6.8	In vivo *SD* rat PK[a] - Dose (mg/kg): 3 (po), 1 (iv)			
		CL (mL/min/kg)	$T_{1/2}$	AUC(0-24h) (nM•h) p.o	*F* (%)
8	<0.005	18	1.1	5785	84
10	0.45	68	0.4	750[b]	100

[a] Dosing vehicle for **8**: NMP (10%), PEG400 (30%), cremophor-EL (10%), ETPGS (5%), and **10**: NMP (5%), PEG 300 (10%), Cremophor-EL (5%), WFI. [b] po dose: 1 mg/kg

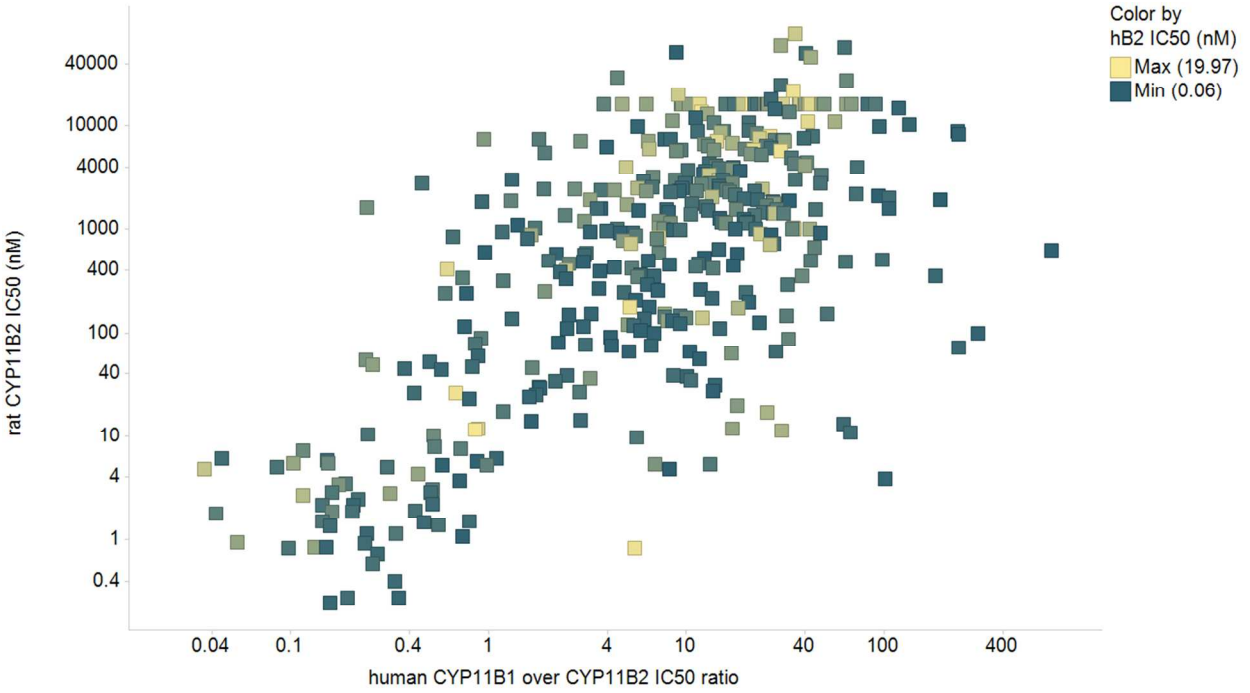
Up to this point, we had relied on a continuous angiotensin II infusion Sprague-Dawley (SD) rat model[27] to evaluate PK/PD relationships, a model successfully used to support the discovery of LCI699,[12] as well as profile follow-up candidates in vivo.[17a] However, profiling of **1** and subsequent analogs in rat recombinant CYP11B2 and CYP11B1[12] revealed a profound disconnect between inhibition of human and rat enzymes (Table 3). Indeed plotting rat CYP11B2 IC_{50} against human CYP11B1/CYP11B2 IC_{50} ratio for 786 internal compounds possessing a human CYP11B2 $IC_{50} < 20$ nM shows a positive correlation between increased human selectivity and loss of potency against rat CYP11B2 (Figure 1).

Table 3. Potency of compounds **1**, **3-5**, **7**, **8** and **10** against human, rat and monkey CYP11B2, and comparison of selectivity ratio for human versus monkey

Compd	CYP11B2 IC_{50} (μM)			S[c]	
	human[a]	Rat[a]	monkey[b]	human	monkey
1	0.003	3.9	-	27	-
3	0.011	10.9	0.009	56	396
4	0.002	8	-	43	-
5	0.002	2	-	103	-
7	0.011	53.1	0.013	43	636
8	0.003	27.4	0.010	100	640
10	0.001	-	0.006	86	99

[a] Recombinant enzyme; [b] Adrenal homogenate; [c] Ratio of CYP11B1 IC_{50} over CYP11B2 IC_{50}

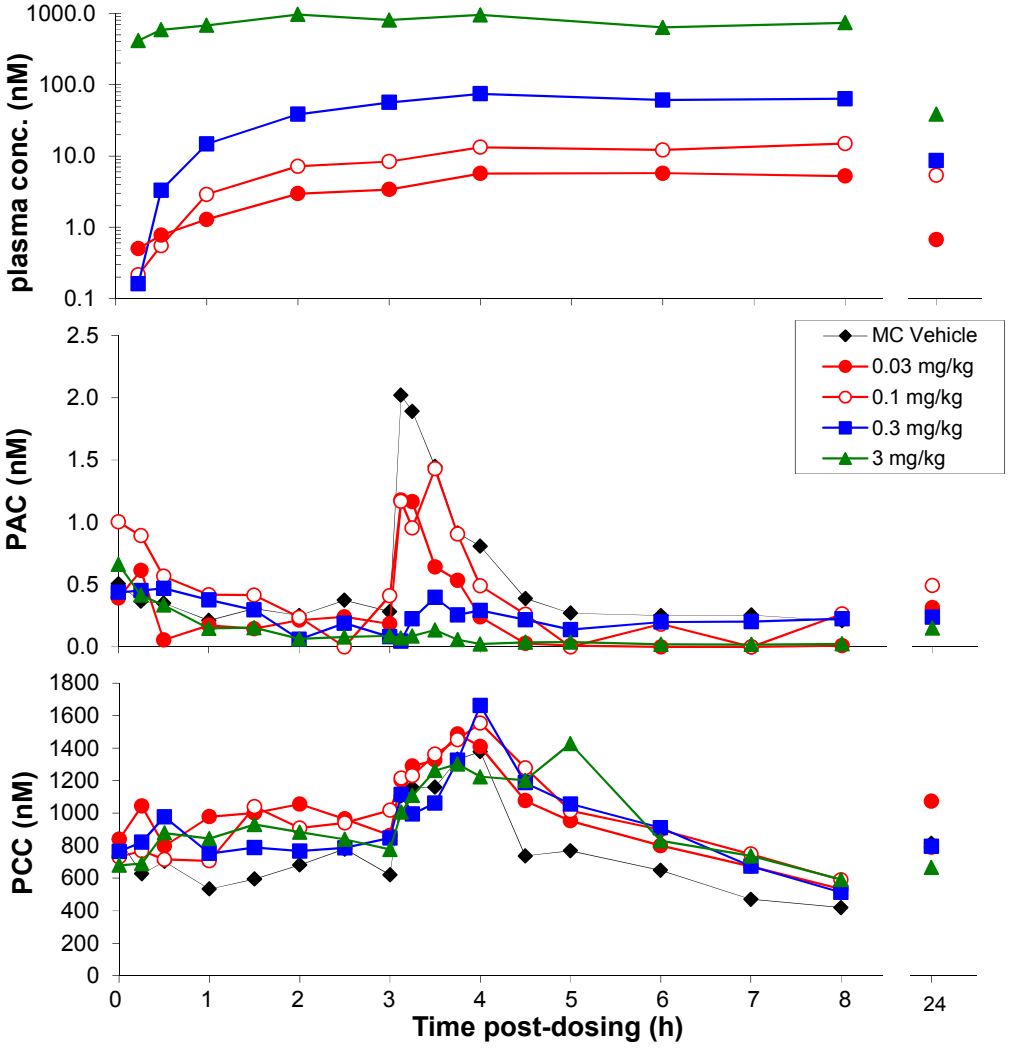
Figure 1. Rat CYP11B2 IC_{50} plotted against human CYP11B2/CYP11B1 IC_{50} ratio for 786 compounds with human CYP11B2 IC_{50} < 20 nM.



Fortunately, a survey of aldosterone secretion from adrenal homogenates of various mammalian species revealed that the activity of human selective ASIs could be assessed in cynomolgus monkeys (*macaca fascicularis*). As shown in Table 3, inhibition of human recombinant CYP11B2 was comparable to inhibition of aldosterone secretion in monkey adrenal homogenates. Selectivity for CYP11B2 over CYP11B1 was also observed in both species. Based on these data, a model was developed using conscious, chair-trained chronically cannulated male cynomolgus monkeys.[12,28] Plasma aldosterone (PAC) and plasma cortisol (PCC) concentrations were measured over time in response to an ACTH challenge (3 μg/kg iv) given 3 h after dosing with the test article. Time-course data for **8** delivered as a methylcellulose suspension at 0.03, 0.1, 0.3, and 3 mg/kg po are shown in Figure 2. Plasma concentrations of **8** dose-dependently

increased following oral dosing with the compound (top panel). At the 3 lowest doses, the maximum concentration (C_{max}) was not achieved until 4-6 h after dosing. In contrast, at the highest dose, the C_{max} was reached rapidly. Plasma concentrations remained high at 8 h and declined by an order of magnitude at 24 h. Group mean baseline PAC ranged from 0.42 to 1.00 nM (Figure 2, middle panel). ACTH injection at 3 h rapidly stimulated an increase in PAC to a peak of 2.02 nM (peak ΔPAC = 1.52 nM) in the vehicle group. Responses were compared to those evoked by vehicle administration in the same animals. Oral dosing with **8** dose-dependently blunted the ACTH-stimulated PAC response. At the highest dose, the peak PAC was 0.14±0.03 nM, which reflects an approximately 95% decrease from the corresponding monkeys' vehicle-control response. The ED_{50} and EC_{50} values of **8** for blunting the 3 h post-dosing ACTH-stimulated PAC response (peak ΔPAC) were 61 μg/kg and 8.5 nM, respectively. Consistent with the low plasma concentrations of **8** at 24 h, ACTH injection 24 h post-dosing showed that the blunting of the PAC response had largely dissipated (data not shown).

Figure 2. Time courses of total compound plasma concentration, PAC and PCC following administration of methylcellulose vehicle (n=6) and **8** at 0.03, 0.1, 0.3 and 3 mg/kg (n=3/dose), and an ACTH challenge 3 h post-dosing (error bars excluded for clarity).



Group mean baseline plasma cortisol concentrations (PCCs) were approximately 1000 times higher than the PACs and ranged from 679 to 839 nM (Figure 2, bottom panel). There were no significant decreases in the baseline or ACTH-stimulated PCC values after administration of **8**, as expected from its high (640) in vitro aldosterone/cortisol selectivity in monkey adrenal homogenate (Table 3). However, LCI699 also had no significant effect on the ACTH-stimulated PCC in this model, despite its poor in vitro selectivity (5).[12] On the basis of the in vivo efficacy, a 30-fold improvement in human in vitro selectivity over LCI699, and a favorable toxicology profile, compound **8** was progressed to human studies.

A double-blind, randomized, placebo-controlled study in healthy male and female volunteers 18-63 years old on a restricted sodium/potassium diet was conducted. Subjects were orally administered bid doses of 30, 60, 100 and 200 mg of **8** or placebo (the ratio of subjects receiving **8** to placebo was 5:2). In these cohorts, median T_{max} was 3-4 h, and plasma exposure, namely AUC_{tau} and C_{max}, both increased under-proportionally between 30 and 200 mg (Table 4). A dose-dependent decrease in plasma aldosterone levels relative to baseline was observed (Figure 3, left panel), with a maximum decrease of 70% at 200 mg bid. Consistent with plasma aldosterone measurements, a dose-dependent decrease in aldosterone excretion measured in urine was observed, also with a maximum of 70% at the highest dose (Figure 3, right panel). Gratifyingly, and in good accord with the 100-fold selectivity for CYP11B2 over CYP11B1 in vitro, no notable decreases in urinary cortisol (Figure 4, left panel) or increases in urinary 11-deoxycortisol (Figure 4, right panel) were observed at any dose. Plasma cortisol, 11-deoxycortisol and ACTH levels were also not affected (data not shown). These data suggest that **8** is a potent and selective inhibitor of aldosterone synthase in humans.

Table 4. Pharmacokinetic parameters in healthy volunteers upon bid dosing of **8**.

Dose	N	Median T_{max} (h)	AUC_{tau} (ng*h/mL)	C_{max} (ng/mL)	AUC_{tau}/dose (ng*h/mL)	C_{max}/dose (ng/mL)
30	10	4.0	768	117	25.6	3.9
60	17	3.0	1030	173	17.1	2.8
100	10	4.0	1270	173	12.7	1.7
200	10	4.0	2180	306	10.9	1.5

Figure 3. Plasma aldosterone AUC(0-24h) (left panel) and amount of aldosterone excreted in urine over 24 h (right panel), expressed as mean placebo-adjusted percent of baseline, following 30, 60, 100 and 200 mg bid dosing of **8**.

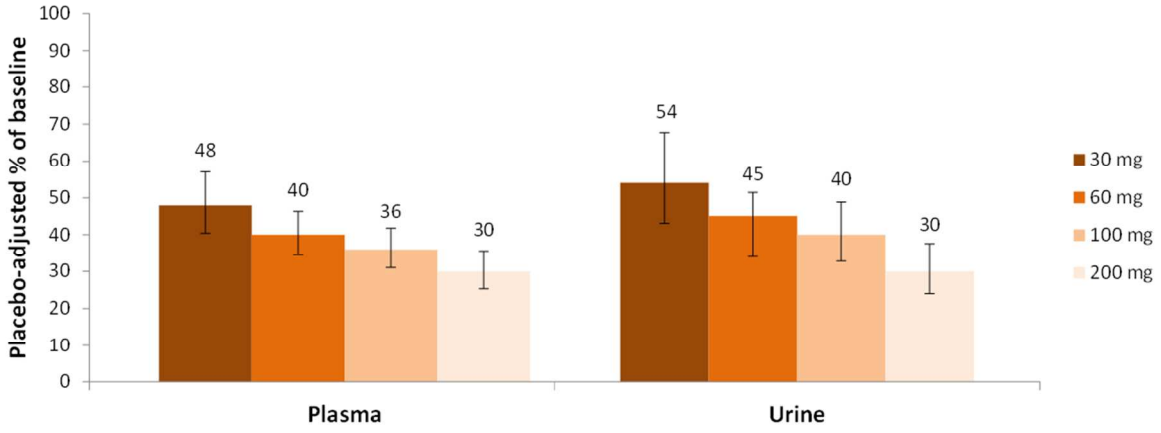
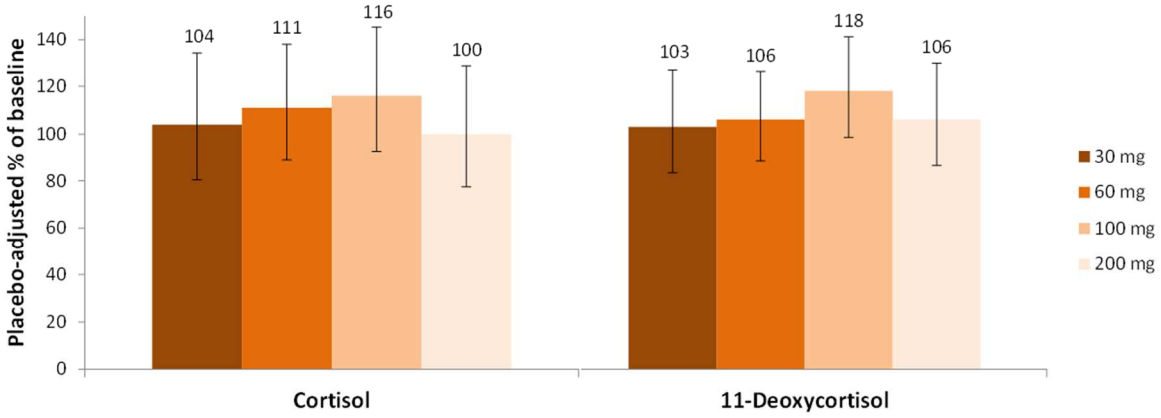


Figure 4. Amount of cortisol (left panel) and 11-deoxycortisol (right panel) excreted in urine over 24 h, expressed as mean placebo-adjusted percent of baseline, following 30, 60, 100 and 200 mg bid dosing of **8**.

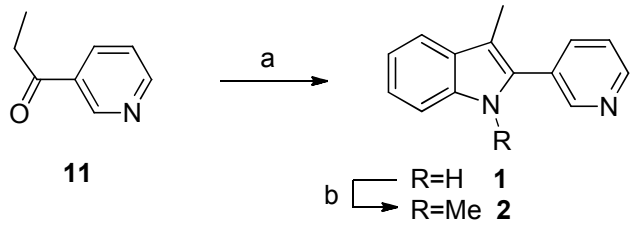


Chemistry

Various methodologies were used to synthesize 3-pyridyl indoles **1-10**. A classic Fischer indole synthesis was employed to prepare **1**, which was converted into **2** using standard conditions

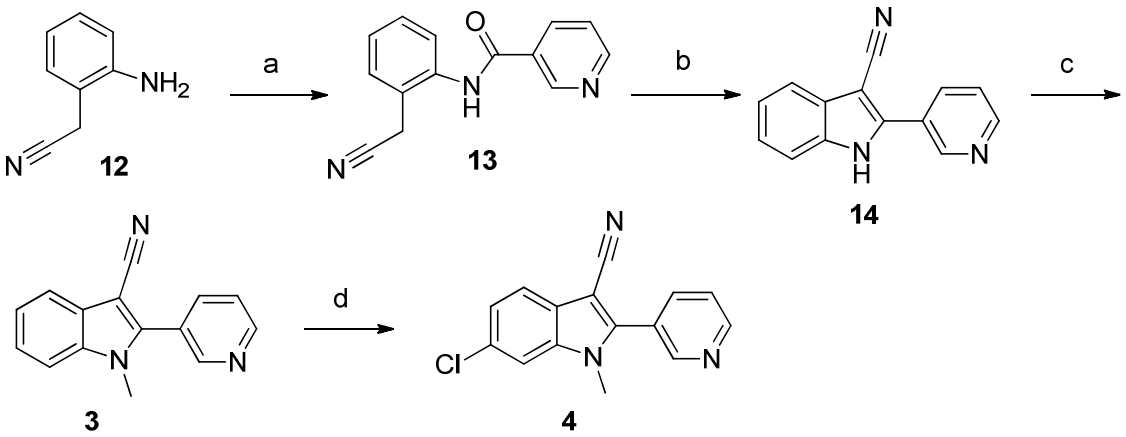
(Scheme 1). 3-Cyanoindoles paired with unsubstituted pyridines were accessed from a Madelung reaction as described in Scheme 2. The product of the Madelung reaction, **14**,[29] was methylated to give **3**, which could be chlorinated with N-chlorosuccinimide to give **4**. For more complex pyridines, we relied on the Suzuki reaction as shown in Scheme 3. Either N-methyl or N-Boc-indol-2-yl boronic acid was used to couple with 5-substituted 3-bromopyridines using anhydrous s-Phos/ Pd_2dba_3 conditions, with the exception of **22**, for which $Pd(PPh_3)_4$ was used. One-pot electrophilic substitution of indole **19**, **21**, **22** and **25** with chlorosulfonyl isocyanate, followed by treatment with DMF was used as a mild method to introduce the nitrile group at the 3-position of the indole ring.[30] Also of note is the use of mild heating and silica gel under vacuum to cleave Boc groups in compounds **23** and **27**. 5-Azaindole **34** was prepared using a Sonogashira/ tBuOK-mediated electrophilic cyclization sequence, and was then converted into indole **9** using standard procedures (Scheme 4). As for 7-azaindole **10**, we reverted back to using Suzuki couplings, given the commercial availability of precursor **37** (Scheme 5).

Scheme 1. Synthesis of ASIs 1 and 2



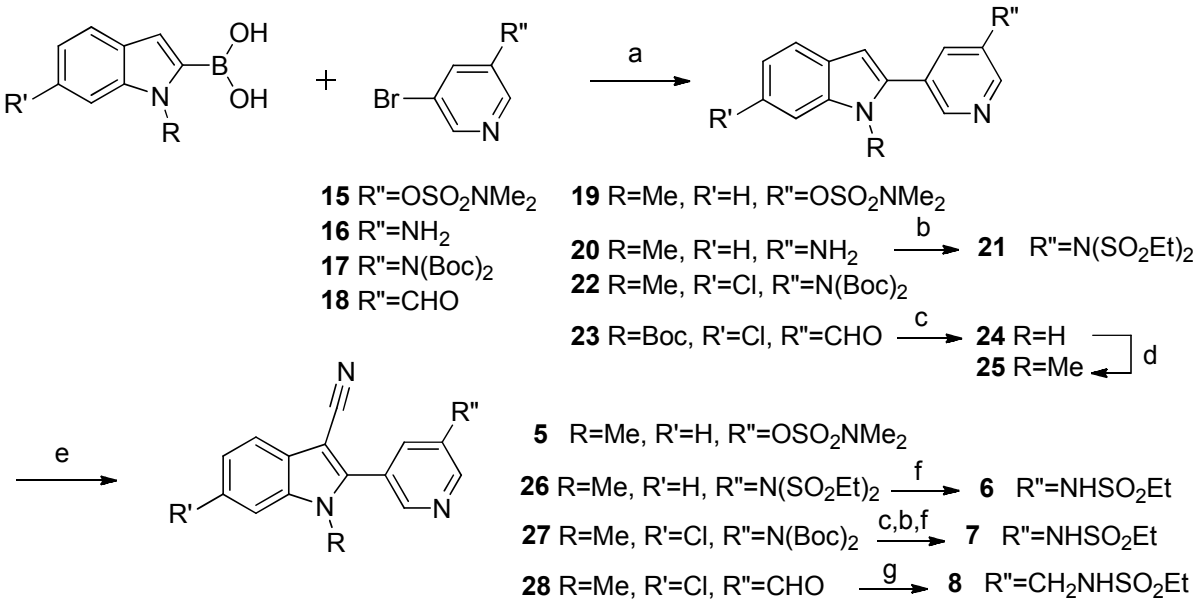
(a) Phenylhydrazine hydrochloride (0.95 equiv), ethanol, reflux, 1 h, then catalytic HCl, reflux, 3 h, 63%; (b) NaH (60%, 2.2 equiv), DMF, 0 °C, 30 min, then MeI (1.1 equiv), 1.5 h, then NaH (60%, 1.4 equiv), 16 h, 16%.

Scheme 2. Synthesis of ASIs 3 and 4



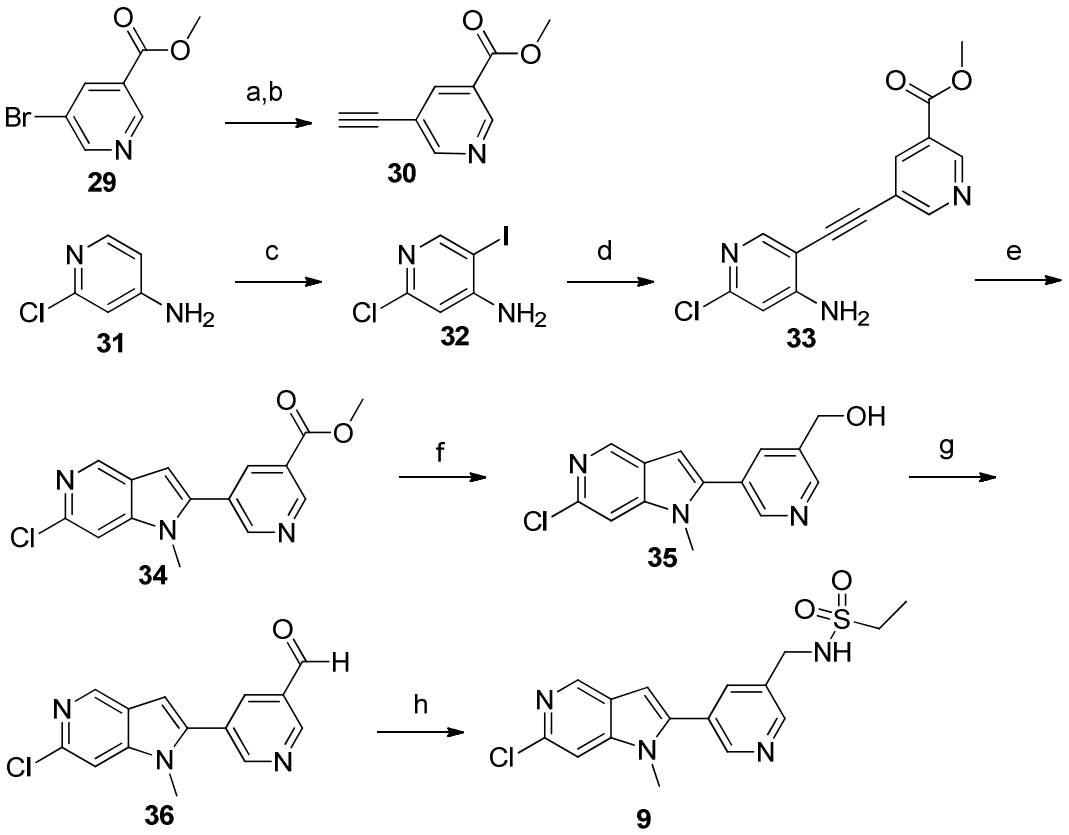
(a) Nicotinoyl chloride (1.1 equiv), diisopropylethylamine (2.5 equiv), DCM, r.t., 16 h, 31%; (b) NaH (60%, 1.0 equiv), DMF, 130 °C, 18 h, 45%; (c) NaH (60%, 3.0 equiv), DMF, 30 min, then MeI (1.5 equiv), r.t., 1 h, 65%. (d) *N*-chlorosuccinimide (2.5 equiv), dichloroethane, reflux, 2 days, 10%.

Scheme 3. Synthesis of ASIs 5-8.



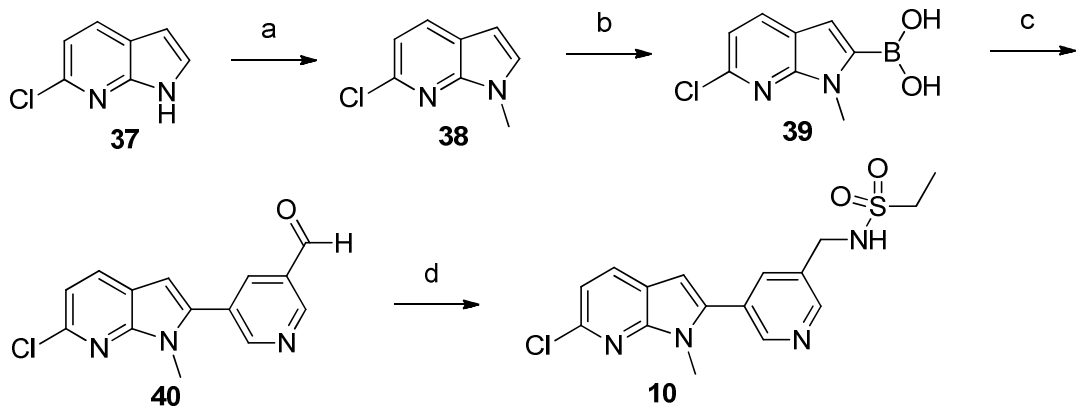
(a) K_3PO_4 (2.0 equiv), s-Phos (0.05 equiv), Pd_2dba_3 (0.02 equiv), toluene, 85 °C, 45-90 min, 87% (**19**), 72% (**20**) [$Pd(PPh_3)_4$ (0.05 equiv) in DMF was used for **22**, 89%]; (b) ethanesulfonyl chloride (3-5 equiv), di-isopropylethylamine (3-4 equiv), DCM, r.t., 1-16 h, 98% (**21**); (c) silica gel, 60-65 °C under high vacuum, 2-48 h, 73% (2 steps, **24**); (d) MeI (1.4 equiv), NaH (60%, 1.6 equiv), DMF, 0 °C to r.t., 1 h, quant. (**25**); (e) chlorosulfonyl isocyanate (3-5 equiv), DCM or acetonitrile, r.t., 10-45 min, then DMF, 10-120 min, 19% (**5**); (f) aqueous NaOH, methanol, r.t., 1 h, 51% (2 steps, **6**), 53% (4 steps, **7**); (g) ethanesulfonamide (2 equiv), $Ti(OiPr)_4$ (1.5 equiv), toluene, reflux, 16 h then $NaBH_4$ (2 equiv), DCM, MeOH, 0 °C, 30 min, 37% (2 steps, **8**).

Scheme 4. Synthesis of ASI 9



(a) trimethylsilylacetylene (3.0 equiv), CuI (0.05 equiv), $Pd(PPh_3)_2Cl_2$ (0.05 equiv), TEA, 50 °C, 1 h; (b) K_2CO_3 (0.25 equiv), MeOH, r.t., 30 min, 67% (2 steps); (c) ICl (1.05 equiv), NaOAc (6 equiv), acetic acid, 60 °C, 2 h, 45%; (d) **30** (1.0 equiv), CuI (0.05 equiv), $Pd(PPh_3)_2Cl_2$ (0.05 equiv), TEA, 100 °C, 3 h; (e) KOtBu (3.0 equiv), NMP, r.t., 16 h, then MeI (3.0 equiv), 0 °C, 30 min; (f) LAH (2.0 equiv), THF, 0 °C to r.t., 45 min, 11% (3 steps); (g) MnO_2 (10 equiv), dioxane, reflux, 16 h; (h) ethanesulfonamide (2 equiv), $Ti(OiPr)_4$, (1.5 equiv), toluene, reflux, 16 h then $NaBH_4$ (4 equiv), DCM, MeOH, 0 °C, 30 min, 17% (2 steps).

Scheme 5. Synthesis of ASI 10



(a) NaH (2.0 equiv), DMF, 0 °C, 20 min then MeI (1.2 equiv), 0 °C to r.t., 2 h; (b) tBuLi (2.5 equiv), THF, −78 °C, 2 h, then $B(OMe)_3$ (1.5 equiv), −78 °C to r.t., 16 h; (c) 5-bromo-3-pyridine carboxaldehyde (0.66 equiv), 2M aqueous K_2CO_3 (2 equiv), PS-$Pd(PPh_3)_4$ (0.03 equiv), dioxane, 85 °C, 2 h, 31% (3 steps); (d) ethanesulfonamide (2 equiv), $Ti(OiPr)_4$, (1.5 equiv), toluene, reflux, 16 h then $NaBH_4$ (4 equiv), DCM, MeOH, 0 °C, 30 min, 20%.

Conclusion

N-[5-(6-chloro-3-cyano-1-methyl-1*H*-indol-2-yl)-pyridin-3-ylmethyl]-ethanesulfonamide (**8**) is an orally available CYP11B2 inhibitor with 100-fold selectivity over CYP11B1 that reduces plasma and urinary aldosterone in healthy volunteers. The lack of concomitant effect on cortisol, 11-deoxycortisol or ACTH in humans effectively validates the selectivity for CYP11B2 over CYP11B1 observed in biochemical assays using recombinant proteins.

Experimentals

Pharmacology Studies. Measurement of cellular aldosterone synthase inhibition,[17a] aromatase inhibition,[11] recombinant human CYP11B1 and human and rat CY11B2 inhibition[22,12,17a] were conducted as previously described. The monkey in vitro assays were conducted similarly, using adrenal homogenates and 11-DOC as a substrate.[12] The microsomal stability assay was previously described.[17a]

The study protocols for the monkey model have been described.[12] Briefly, thirty minutes before the start of the experiment, a Huber needle was inserted transdermally into the vascular access port for the collection of blood samples and injection of ACTH. Blood samples (0.3 mL in 15 U/mL heparin) for baseline pharmacokinetic and pharmacodynamic assessments were collected at 0.5 h, 0.25 h and immediately before dosing. Compound **8** or vehicle was administered followed 3 h later by ACTH(1–24) (Cortrosyn; Amphastar Pharmaceuticals, Inc., Rancho Cucamonga, CA, USA) 3000 ng/kg i.v. in 0.1 mL/kg (over ~2 min). Blood samples were collected at 0.125, 0.25, 0.5, 0.75 and 1 h after ACTH injection to assess the time course of plasma aldosterone and cortisol stimulation. Further blood samples were collected up to 8 h and at 23.5 and 24 h after **8**/vehicle administration. Plasma aldosterone and cortisol were determined by radioimmunoassay.[22] Plasma concentration of **8** was measured by a liquid chromatography separation coupled with tandem mass spectrometric detection (LC/MS/MS) method (LLOQ 0.26 nmol/L). Samples with compound **8** concentrations below the LLOQ were treated as zero.

Rat Pharmacokinetic Studies. Male Sprague Dawley rats (200−300 g) (Harlan Laboratories Inc., Indianapolis, IN, USA) were used in the experiments. All animal experiments were performed in accordance with IACUC protocol. Two rats received 0.3 mg/1 mL/kg (free base equivalents) by slow intravenous injection via the jugular vein, and three rats received 1 mg/5 mL/kg or 3 mg/5 mL/kg in solution via oral gavage. Approximately 0.2 mL of venous whole

blood was collected from the jugular vein catheter of each animal at 5 min (iv dose only) and 0.25, 0.5, 1, 2, 4, 6, and 8 h postdose and transferred to a EDTA tube. The blood was centrifuged at 3000 rpm, and the plasma was transferred to a polypropylene tube, capped, and stored frozen (−20 °C) for parent compound analysis. Protein precipitation was employed for sample preparation. A 25 μL aliquot of sample was subjected to protein precipitation using 150 μL of acetonitrile containing 100 ng/mL of internal standard (Glyburide). After vortex and centrifugation for 5 min at 4000 rpm, the supernatant (125 μL) was transferred to a 1 mL 96-well plate, followed by the addition of 50 μL of water. The analysis was conducted by using HPLC separation coupled with mass spectrometric detection. All pharmacokinetic (PK) parameters were derived from concentration−time data by noncompartmental analyses. All pharmacokinetic parameters were calculated with the computer program WinNonlin (Enterprise, version 5.2) purchased from Pharsight Corporation (St. Louis, MO).

General Chemical Methods. Starting materials, reagents, and solvents were obtained from commercial sources and used as received. THF and diethyl ether were anhydrous grade. Progress of the reactions was monitored by thin-layer chromatography (TLC) analysis (Merck, 0.2 mm silica gel 60 F254 on glass plates) or by analytical LC−MS using an Agilent 1100 series with UV detection at 214 and 254 nm and an electrospray mode (ESI) coupled with a Waters ZQ single quad mass detector. Purification of intermediates and final products was carried out on normal phase using an ISCO CombiFlash system or an Analogix Intelliflash 280 or a Biotage SP1 system and prepacked SiO2 cartridges eluted with optimized gradients of either heptane−ethyl acetate mixture or dichloromethane−methanol as described. Preparative high pressure liquid chromatography (HPLC) was performed on Waters or Gilson instruments. Systems were run with either a 5−95% or 10−90% acetonitrile/water gradient with either a 0.1% TFA or 0.1%

NH_4OH modifier. All target compounds had purity of >95% as established by analytical HPLC. NMR spectra were recorded on a Bruker AV400 (Avance 400 MHz) instrument. Chemical shifts (δ) are reported in parts per million (ppm) relative to deuterated solvent as the internal standard ($CDCl_3$ 7.26 ppm, DMSO-d6 2.50 ppm, CD_3OD 3.31), and coupling constants (J) are in hertz (Hz). Peak multiplicities are expressed as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m), and broad singlet (br s).

3-Methyl-2-pyridin-3-yl-1H-indole (1). A flask was charged with 3-propionylpyridine (1.004 g, 7.279 mmol), phenylhydrazine hydrochloride (1.010 g, 6.915 mmol) and ethanol (15 mL). The mixture was heated to reflux for 1 h and cooled to room temperature. To a portion of the reaction mixture (2.5 mL) was added HCl (4M in dioxane, 1 mL, 4 mmol) and the mixture was heated to reflux. After 3 h, the mixture was concentrated *in vacuo*. The solid was dissolved in the minimum amount of boiling methanol (17 mL) and allowed to cool slowly to room temperature. A solid precipitated and after cooling to 0 °C for 30 min, the solid was filtered off, washed with portions of cold methanol and dried under high vacuum to give **1** as the hydrochloride salt (0.177 g, 0.723 mmol, 63%) as yellow needles. ^{1}H NMR (400 MHz, MeOD) δ ppm (HCl salt) 2.56 (s, 3 H), 7.09 - 7.13 (m, 1 H), 7.23 - 7.26 (m, 1 H), 7.43 (d, *J*=8.1 Hz, 1 H), 7.63 (d, *J*=8.1 Hz, 1 H), 8.14 - 8.18 (m, 1 H), 8.74 (d, *J*=5.8 Hz, 1 H), 8.83-8.85 (m, 1 H), 9.08 (s, 1 H).

1,3-Dimethyl-2-(pyridin-3-yl)-1H-indole (2). To a suspension of **1** hydrochloride (205 mg, 0.838 mmol) in DMF (10 mL) at 0 °C was added NaH (60% in mineral oil, 73 mg, 1.84 mmol) and the mixture was stirred at 0 °C for 30 min. MeI (131 mg, 0.92 mmol) was added and after 1.5 h, another portion of NaH (60% in mineral oil, 50 mg, 1.22 mmol) was added. The mixture was stirred overnight, whereupon it was quenched with methanol (2 mL). The mixture was purified by reverse phase HPLC to give **2** (30 mg, 0.134 mmol, 16%) as a beige solid. ^{1}H NMR

(400 MHz, DMSO-d_6) δ ppm 2.23 (s, 3 H), 3.62 (s, 3 H), 7.06 - 7.12 (m, 1 H), 7.22 (td, *J*=7.6, 1.1 Hz, 1 H), 7.48 (d, *J*=8.3 Hz, 1 H), 7.55 - 7.61 (m, 2 H), 7.93 (dt, *J*=7.9, 2.0 Hz, 1 H), 8.66 (dd, *J*=4.8, 1.5 Hz, 1 H), 8.69 (dd, *J*=2.3, 0.8 Hz, 1 H). HRMS: (ESI) m/z 223.1236 [(M+H)+ Calcd for $C_{15}H_{15}N_2$: 223.1230].

***N*-(2-Cyanomethyl-phenyl)-nicotinamide (13).** 2-aminophenylacetonitrile (5.0 g, 37.1 mmol) and nicotinoyl chloride (7.5 g, 40.8 mmol) were taken up in dry dichloromethane (200 mL) and diisopropylethylamine (12.1 g, 92.7 mmol) was added while cooling the mixture with a cold water bath. The mixture was stirred overnight at room temperature, whereupon it was washed twice with saturated aqueous sodium bicarbonate. The combined aqueous layer was back-extracted with ethyl acetate. The combined organic phase was dried over $MgSO_4$ and concentrated *in vacuo* to give a residue, which was purified by silica gel flash chromatography (dichloromethane-methanol, 1:0 to 19:1) to give **13** (2.70 g, 11.37 mmol, 31%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.02 (s, 2 H), 7.32 - 7.37 (m, 1 H), 7.39 - 7.44 (m, 2 H), 7.50 (d, *J*=7.1 Hz, 1 H), 7.59 (dd, *J*=8.1, 4.8 Hz, 1 H), 8.30 - 8.33 (m, 1 H), 8.78 (dd, *J*=4.8, 1.5 Hz, 1 H), 9.14 (d, *J*=1.8 Hz, 1 H), 10.36 (s, 1 H).

2-Pyridin-3-yl-1H-indole-3-carbonitrile (14). 13 (0.095 g, 0.384 mmol) was dissolved in DMF (3 mL). NaH (60%, 0.015 g, 0.384 mmol) was added and the mixture was heated to 130 °C. After 18 h, the mixture was cooled down, diluted with ethyl acetate, and washed with 1M aqueous sodium hydroxide. The combined washings were back-extracted with ethyl acetate. The combined organic phase was dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (dichloromethane-methanol, 99:1 to 19:1) to give **14** (0.038 mg, 0.17 mmol, 45%) as a brown solid. ^{1}H NMR (400 MHz, MeOD) δ ppm 7.29 - 7.32 (m, 1 H), 7.34 - 7.39 (m, 1 H), 7.57 (d, *J*=8.0 Hz, 1 H), 7.66 (dd, *J*=8.0, 4.9 Hz,

1 H), 7.70 (d, *J*=7.8 Hz, 1 H), 8.39 - 8.44 (m, 1 H), 8.68 (dd, *J*=4.8, 1.5 Hz, 1 H), 9.15 (d, *J*=1.5 Hz, 1 H).

1-Methyl-2-pyridin-3-yl-1H-indole-3-carbonitrile (3). To **14** (1.0 g, 4.56 mmol) in DMF (17 mL) was added 60% sodium hydride in mineral oil (547 mg, 13.68 mmol) and the suspension was stirred for 30 min. Iodomethane (971 mg, 6.84 mmol) was then added to the reaction mixture, which was stirred at ambient temperature for 1 h. Aqueous $NaHCO_3$ (3 mL) was added and the mixture was concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (dichloromethane-methanol gradient) to give **3** (0.70 g, 3.00 mmol, 65%) as a white solid. ^{1}H NMR (400 MHz, MeOD) δ ppm 3.83 (s, 3 H), 7.35 (t, *J*=7.5 Hz, 1 H), 7.40 - 7.46 (m, 1 H), 7.64 (d, *J*=8.3 Hz, 1 H), 7.67 - 7.74 (m, 2 H), 8.16 (dt, *J*=8.0, 2.0, 1.9 Hz, 1 H), 8.76 (dd, *J*=5.1, 1.5 Hz, 1 H), 8.86 (d, *J*=1.5 Hz, 1 H). HRMS (ESI) m/z 234.1029 [$(M+H)^+$ Calcd for $C_{15}H_{12}N_3$: 234.1031].

6-Chloro-2-pyridin-3-yl-1-methyl-1H-indole-3-carbonitrile (4). *N*-chlorosuccinimide (0.086 g, 0.644 mmol) was added to **3** (150 mg, 0.644 mmol) in dichloroethane (10 mL) and the mixture was refluxed for 2 days. Three additional portions of *N*-chlorosuccinimide (0.043 g, 0.322 mmol) were added at 1 hour intervals, whereupon the volatiles were removed *in vacuo*. Purification by reverse phase HPLC afforded **4** (18 mg, 0.067 mmol, 10%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.79 (s, 3 H), 7.37 (dd, *J*=8.5, 1.9 Hz, 1 H), 7.69 (dd, *J*=7.5, 4.4 Hz, 1 H), 7.73 (d, *J*=8.6 Hz, 1 H), 7.98 (d, *J*=1.5 Hz, 1 H), 8.17 (dt, *J*=8.1, 2.0, 1.8 Hz, 1 H), 8.82 (dd, *J*=4.9, 1.6 Hz, 1 H), 8.90 (d, *J*=1.5 Hz, 1 H). HRMS (ESI) m/z 268.0653 [$(M+H)^+$ Calcd for $C_{15}H_{11}ClN_3$: 268.0642].

Dimethyl-sulfamic acid 5-bromo-pyridin-3-yl ester (15). A flask was charged with 5-bromo-pyridin-3-ol (0.200 g, 1.126 mmol), potassium phosphate (0.631 g, 2.884 mmol) and acetone (5 mL) and cooled to 0 °C. Dimethylsulfamoyl chloride (0.261 g, 1.802 mmol) was then

added dropwise and the cooling bath was removed. After 2 h, the mixture was diluted with acetone, filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in THF (30 mL) and polymer-supported trisamine (3.85 mmol/g, 0.7 g, 2.7 mmol) was added. After 1 h, the mixture was filtered. Concentration *in vacuo* gave a residue which was purified by silica gel flash chromatography (heptane-ethyl acetate, 9:1 to 4:1) to give **15** (0.228 g, 0.811 mmol, 72%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.05 (s, 3 H), 7.86 (m, 1 H), 8.50 (d, *J*=2.3 Hz, 1 H), 8.62 (d, *J*=1.9 Hz, 1 H).

Dimethyl-sulfamic acid 5-(1-methyl-1H-indol-2-yl)-pyridin-3-yl ester (19). A flask was charged with **15** (0.225 g, 0.76 mmol), N-methyl-indole boronic acid (0.200 g, 1.14 mmol), s-Phos (0.016 g, 0.038 mmol), finely crushed potassium phosphate (0.323 g, 1.52 mmol) and toluene (5 mL). After sparging with nitrogen for 15 min, Pd_2dba_3 (0.014 g, 0.015 mmol) was added, the flask was flushed with nitrogen and the mixture was heated to 85 °C. After 45 min, the mixture was allowed to cool to r.t., diluted with ethyl acetate and filtered through a plug of silica gel (elution with ethyl acetate). The volatiles were removed *in vacuo* and the residue was purified by silica gel flash chromatography (heptane-ethyl acetate, 3:7 to 0:1) to give to give **19** (220 mg, 0.664 mmol, 87%); MS (ESI) *m/z* 332.1 $(M+H)^+$.

Dimethyl-sulfamic acid 5-(3-cyano-1-methyl-1H-indol-2-yl)-pyridin-3-yl ester (5). A flask was charged with **19** (220 mg, 0.664 mmol) and dichloromethane (10 mL). Chlorosulfonyl isocyanate (0.282 g, 1.99 mmol) was added and the reaction was stirred for 45 min, whereupon DMF (3 mL) was added. After another 2 h, the reaction was concentrated in vacuo and the residue was purified by reverse phase HPLC to give **5** (46 mg, 0.129 mmol, 19%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.99 (s, 6 H), 3.82 (s, 3 H), 7.35 - 7.40 (m, 1 H), 7.43 - 7.48 (m, 1 H), 7.74 (d, *J*=7.8 Hz, 1 H), 7.79 (d, *J*=8.3 Hz, 1 H), 8.24 (dd, *J*=2.5, 1.8 Hz, 1 H), 8.85 (d, *J*=2.5 Hz,

1 H), 8.91 (d, *J*=1.8 Hz, 1 H). HRMS (ESI) m/z 357.1018 [$(M+H)^+$ Calcd for $C_{17}H_{17}N_4O_3S$: 357.1021].

2-(5-Amino-pyridin-3-yl)-1-methyl-1H-indole (20). A flask was charged with 3-amino-5-bromopyridine (4.05 g, 22.71 mmol), *N*-methyl-indole-2-boronic acid (5.27 g, 29.52 mmol), s-Phos (0.625 g, 1.476 mmol), finely crushed potassium phosphate (9.74 g, 45.41 mmol) and toluene (75 mL). After sparging with nitrogen for 60 min, Pd_2dba_3 (0.420 g, 0.454 mmol) was added, the flask was flushed with nitrogen and the flask was lowered into a preheated oil bath at 85 °C. After 1.5 h, the mixture was allowed to cool to r.t., diluted with ethyl acetate and filtered through a plug of silica gel (elution with ethyl acetate). The residue was purified by silica gel flash chromatography (heptane-ethyl acetate, 3:7 to 0:1) to give **20** (3.64 g, 16.30 mmol, 72%) as an off-white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.74 (s, 3 H), 5.49 (s, 2H), 6.56 (s, 1H), 7.05-7.09 (m, 1H), 7.09-7.10 (m, 1H), 7.17-7.21 (m, 1H), 7.49 (d, *J*=7.6 Hz, 1 H), 7.57 (d, *J*=7.6 Hz, 1 H), 7.94 (d, *J*=2.0 Hz, 1 H), 7.99 (d, *J*=2.5 Hz, 1 H). MS (ESI) *m/z* 224 $(M+H)^+$.

Ethanesulfonic acid [5-(1-methyl-1H-indol-2-yl)-pyridin-3-yl]-*N*-ethanesulfonyl-amide (21). To a solution of **20** (223 mg, 1.0 mmol) in dichloromethane (20 mL) was added ethanesulfonyl chloride (386 mg, 3.0 mmol) and diisopropylethylamine (517 mg, 4.0 mmol). The mixture was stirred at room temperature for 1 h. Saturated $NaHCO_3$ in water (0.5 mL) and silica gel (10 g) were added and the mixture was concentrated *in vacuo*. The residue was purified by silica gel chromatography (heptane-ethyl acetate, 1:0 to 1:9) to give **21** (400 mg, 0.98 mmol, 98%) as a white solid. MS (ESI) *m/z* 408.1 $(M+H)^+$.

Ethanesulfonic acid [5-(3-cyano-1-methyl-1H-indol-2-yl)-pyridin-3-yl]-amide (6) Chlorosulfonyl isocyanate (0.162 g, 1.15 mmol) was added to **21** (96 mg, 0.23 mmol) in acetonitrile (10 mL) and the mixture was stirred for 20 min before adding DMF (1 mL). After 10

min, the solvent was removed and the crude mixture was used with no further purification; MS (ESI) *m/z* 432.96 $(M+H)^+$. The crude mixture in DMF (2 mL), methanol (10 mL) and 5 M NaOH in water (1 mL) was stirred at room temperature for 1 h. The mixture was purified by reverse phase HPLC on Xbridge C18 eluting with a 1:9 to 9:1 acetonitrile-water gradient to give **6** (40 mg, 0.117 mmol, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.25 (t, *J*=7.3 Hz, 3 H), 3.24 - 3.32 (m, 2 H), 3.80 (s, 3 H), 7.32 - 7.38 (m, 1 H), 7.43 (td, *J*=7.8, 1.1 Hz, 1 H), 7.70 (d, *J*=7.8 Hz, 1 H), 7.75 (d, *J*=8.3 Hz, 1 H), 7.90 (t, *J*=2.3 Hz, 1 H), 8.58 - 8.62 (m, 2 H), 10.42 (s, 1 H). HRMS (ESI) *m/z* 341.1078 [$(M+H)^+$ Calcd for $C_{17}H_{17}N_4O_2S$: 341.1072].

3-[Bis[[(1,1-dimethylethyl)oxy]carbonyl]amino]-5-bromo-pyridine (17). To 5-amino-3-bromo-pyridine (1.73 g, 10 mmol) and Boc_2O (4.8 g, 22 mmol) in acetonitrile (100 mL) at ambient temperature was added DMAP (212 mg, 1 mmol) and the reaction mixture was heated to 60 °C and stirred overnight. The reaction mixture was then cooled to room temperature. The solvent was removed *in vacuo* and the residue was purified by silica gel flash chromatography (heptane-ethyl acetate, 1:0 to 7:3) to afford **17** (3.0 g, 8.04 mmol, 80%) as a white solid. MS (ESI) *m/z* 374.9 $(M+H)^+$.

3-[Bis[[(1,1-dimethylethyl)oxy]carbonyl]amino]-5-(6-chloro-1-methyl-1H-indol-2-yl)-pyridine (22). A flask was charged with **17** (3.0 g, 8.0 mmol), *N*-methyl-6-chloroindole-2-boronic acid (2.0 g, 9.65 mmol), finely crushed potassium phosphate (3.4 g, 16.0 mmol) and DMF (40 mL). After sparging with nitrogen for 15 min, $Pd(PPh_3)_4$ (465 mg, 0.402 mmol) was added. The flask was flushed with nitrogen and the mixture was heated to 90 °C and stirred overnight. The mixture was then cooled to room temperature and poured into water (300 mL). The mixture was extracted with EtOAc three times and the combined organic phase was then dried over Na_2SO_4 and concentrated. The residue was purified by silica gel flash chromatography

(ethyl acetate-heptane, 0:1 to 1:9) to give **22** (3.3 g, 7.2 mmol, 89%). MS (ESI) *m/z* 458.1 $(M+H)^+$.

Ethanesulfonic acid [5-(6-chloro-3-cyano-1-methyl-1H-indol-2-yl)-pyridin-3-yl]-amide (7). Chlorosulfonyl isocyanate (4.08 g, 28.8 mmol) was added to a solution of **22** (3.3 g, 7.2 mmol) in acetonitrile (200 mL) and the reaction mixture was stirred for 10 min. DMF (3 mL) was added and the reaction mixture was stirred for 2 h. 20 g silica gel was added to the mixture and the solvent was removed *in vacuo*. The resulting solid was heated to 65 °C under high vacuum for 2 h. The mixture was cooled to room temperature and then purified by silica gel flash chromatography (dichloromethane-methanol-triethylamine, 83:8:9) to afford 2-(5-amino-pyridin-3-yl)-6-chloro-1-methyl-1H-indole-3-carbonitrile mixed with triethylammonium chloride. The combined fractions were concentrated and taken up in DCM. After washing with water and drying over Na_2SO_4, a residue was obtained (1.8 g), which was used with no further purification; MS (ESI) *m/z* 283.0 $(M+H)^+$. A portion (512 mg) was dissolved in DCM (10 mL). Di-isopropylethylamine (0.898 g, 6.88 mmol) and ethanesulfonyl chloride (0.68 g, 5.16 mmol) were added and the reaction mixture was stirred overnight. Another portion of ethanesulfonyl chloride (0.68 g, 5.16 mmol) was added, and after 2 h, the mixture was concentrated and purified by silica gel flash chromatography (heptane-ethyl acetate 1:1) to give a residue (260 mg) which was used with no further purification. A portion (200 mg) was redissolved in methanol (5 mL). Aqueous 1M NaOH (4 mL) was added and the reaction mixture was stirred for 1 h. The pH was adjusted to 5 with 1M aqueous HCl and the mixture was extracted with DCM twice. The organic phase was dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by silica gel flash chromatography (methanol-dichloromethane, 1:19) to give **7** (115 mg, 0.306 mmol, 53%, 4 steps) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.25 (t, *J*=7.3 Hz, 3 H),

3.23 - 3.33 (m, 2 H), 3.79 (s, 3 H), 7.36 (dd, *J*=8.6, 1.8 Hz, 1 H), 7.72 (d, *J*=8.6 Hz, 1 H), 7.90 (t, *J*=2.3 Hz, 1 H), 7.95 (d, *J*=1.5 Hz, 1 H), 8.61 (d, *J*=2.3 Hz, 2 H), 10.43 (s, 1 H). HRMS (ESI) *m/z* 375.0692 [$(M+H)^+$ Calcd for $C_{17}H_{16}ClN_4O_2S$: 375.0682].

5-(6-Chloro-1H-indol-2-yl)-pyridine-3-carbaldehyde (24). A flask was charged with *N*-(*tert*-butoxycarbonyl)-6-chloro-1H-indol-2-ylboronic acid (10 g, 33.8 mmol), 5-bromonicotinaldehyde (5.25 g, 28.2 mmol), K_3PO_4 (11.97 g, 56.4 mmol), s-Phos (0.579 g, 1.410 mmol) and $Pd_2(dba)_3$ (0.516 g, 0.564 mmol), and the flask was flushed with N_2. Toluene (200 mL) was added, and the mixture was heated to 90 ºC for 1 h. The mixture was cooled to room temperature. Ethyl acetate (250 mL) was added and the mixture was filtered through a pad of silica gel, which was washed with EtOAc. Silica gel was added to the combined filtrate, which was concentrated *in vacuo*. The residue was placed under high vacuum at 60 ºC for 2 days, and after elution with ethyl acetate, **24** (5.6 g, 20.7 mmol, 73%) was obtained. MS (ESI) *m/z* 257.0 and 258.9 $(M+H)^+$.

6-Chloro-2-(5-formyl-pyridin-3-yl)-1-methyl-1H-indole (25). A flask was charged with **24** (5.6 g, 17.3 mmol), MeI (3.41 g, 24.0 mmol) and DMF (200 mL), and 60% NaH in mineral oil (1.134 g, 28.4 mmol) was added at 0 ºC. The mixture was stirred for 1 h. Water (200 mL) was added. The mixture was extracted with EtOAc (2 × 300 mL) and the combined organic phase was washed with water (3 × 100 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (heptane-ethyl acetate, 3:2 to 1:1) to give **25** (5.2 g, 17.3 mmol, quant.). MS (ESI) *m/z* 271.0, 272.9 $(M+H)^+$.

***N*-[5-(6-chloro-3-cyano-1-methyl-1H-indol-2-yl)-pyridin-3-ylmethyl]-ethanesulfonamide (8).** A flask was charged with **25** (3.00 g, 11.1 mmol) and acetonitrile (300 mL), and chlorosulfonyl isocyanate (4.73 g, 33.4 mmol) was added at 0 ºC. The mixture was stirred for 10

min. DMF (4.01 g, 54.9 mmol) was added at 0 ºC, and the mixture was stirred for another 10 min. Saturated $NaHCO_3$ (30 mL) was added and the volatiles were removed *in vacuo*. The mixture was taken up in ethyl acetate and washed with water. The aqueous phase was extracted with ethyl acetate, the combined organic phase was washed with water, dried over sodium sulfate. After filtration and concentration, **28** (2.0 g) was obtained, which was used with no further purification; MS (ESI) *m/z* 296.0, 297.8 $(M+H)^+$. To a solution of **28** (2.0 g, 6.09 mmol) and ethanesulfonamide (1.33 g, 12.17 mmol) in toluene (250 mL) was added titanium(IV) isopropoxide (2.59 g, 9.13 mmol). The mixture was refluxed overnight, then concentrated *in vacuo*. The residue was taken up in DCM (150 mL) and MeOH (150 mL), and $NaBH_4$ (0.461 g, 12.17 mmol) was added at 0 ºC. The mixture was stirred at 0 ºC for 30 min. Water (50 mL) was added and the mixture was stirred for 5 min. The suspension was filtered through a pad of celite. The celite layer was washed with DCM (3 × 50 mL). The combined organic phase was dried over Na_2SO_4 and concentrated to give a residue was purified by silica gel flash chromatography (ethyl acetate). The resulting fractions containing the product were concentrated and repurified by silica gel flash chromatography (dichloromethane-methanol, 1:0 to 97:3). The concentrated product was redissolved in MeOH (500 mL) at 60 ºC and concentrated *in vacuo* to give **8** (1.60 g, 4.11 mmol, 37%, 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.20 (t, *J*=7.3 Hz, 3 H), 3.06 (q, *J*=7.3 Hz, 2 H), 3.78 (s, 3 H), 4.34 (d, J=6.3 Hz, 2 H), 7.37 (dd, *J*=8.6, 1.8 Hz, 1 H), 7.73 (d, *J*=8.3 Hz, 1 H), 7.77 (t, *J*=6.2 Hz, 1 H), 7.97 (d, *J*=1.8 Hz, 1 H), 8.09 (t, *J*=2.1 Hz, 1 H), 8.78 (d, *J*=1.8 Hz, 1 H), 8.80 (d, *J*=1.8 Hz, 1 H). HRMS (ESI) *m/z* 389.0853 $[(M+H)^+$ Calcd for $C_{18}H_{17}ClN_4O_2S$: 389.0839].

Methyl 5-ethynylnicotinate (30). To a solution of methyl 5-bromonicotinate (3.0 g, 13.89 mmol) in TEA (50 mL) under N_2 was added ethynyltrimethylsilane (4.09 g, 41.7 mmol), copper

iodide (0.132 g, 0.694 mmol) and bis(triphenylphosphine)palladium(II)chloride (0.487 g, 0.694 mmol). The reaction was stirred at 50°C for 1 h, cooled to room temperature and filtered to remove solids, which were washed with ethyl acetate. The filtrate was taken and washed with water thrice and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated in vacuo to afford a black solid. The crude was dissolved in MeOH (50 mL) and potassium carbonate (0.480 g, 3.47 mmol) was added. The reaction was stirred for 30 min, filtered and purified by silica gel flash chromatography (DCM-MeOH, 9:1) to afford **30** (1.5 g, 9.31 mmol, 67%) as a light yellow solid.

2-Chloro-5-iodopyridin-4-amine (32). To 2-chloropyridin-4-amine (3.0 g, 23.34 mmol) in acetic acid (60 mL) was added sodium acetate (11.49 g, 140 mmol) and iodine monochloride (3.98 g, 24.50 mmol). The reaction was stirred at 60°C for 2 h, whereupon it was cooled to room temperature and quenched with 1M aqueous sodium bisulfite and extracted with dichloromethane. The organic layer was separated, dried over sodium sulfate and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (DCM-MeOH, 19:1) to afford **32** (2.7 g, 10.61 mmol, 45%) and 2-chloro-3,5-diiodopyridin-4-amine (3.0 g, 11.79 mmol, 50%) as white solids; MS (ESI) *m/z* 255.0 $(M+H)^+$.

(5-(6-chloro-1-methyl-1H-pyrrolo[3,2-c]pyridin-2-yl)pyridin-3-yl)methanol (35). To **32** (1.0 g, 3.93 mmol) in TEA (50 mL) under N_2 was added **30** (0.633 g, 3.93 mmol), copper iodide (0.037 g, 0.196 mmol) and bis(triphenylphosphine)palladium(II) chloride (0.138 g, 0.196 mmol). The reaction was stirred at 100°C for 3 h, cooled to room temperature and filtered to remove solids, which were washed with ethyl acetate. The filtrate was taken and washed with water thrice and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated in vacuo to afford **28** (0.95 g) as a brown solid which was dissolved in NMP (15

mL). Potassium t-butoxide (1.11 g, 9.91 mmol) was added. The reaction was stirred for 16 h at room temperature, cooled to 0°C and methyl iodide (0.619 mg, 9.91 mmol) was added. The reaction was stirred for 30 min, then quenched with water and extracted with ethyl acetate twice. The organic layer was dried over sodium sulfate and concentrated in vacuo to afford **29** (0.78 g) as a brown oil which was dissolved in THF (10 mL). The solution was cooled to 0°C and LAH (1M in THF, 5.17 mL, 5.17 mmol) was added. The reaction was stirred at room temperature for 45 min, then quenched with water (1 mL), then washed with 2M NaOH solution (2 mL) and extracted with DCM twice. The organic layer was separated, dried over sodium sulfate and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (DCM-MeOH, 9:1) to afford **35** (125 mg, 0.457 mmol, 11%, 3 steps) as a light yellow solid. MS (ESI) *m/z* 274.1 $(M+H)^+$.

N-((5-(6-chloro-1H-pyrrolo[3,2-c]pyridin-2-yl)pyridin-3-yl)methyl)ethanesulfonamide (9). To **35** (128 mg, 0.468 mmol) in dioxane (5 mL) was added manganese dioxide (0.407 g, 4.68 mmol). The mixture was refluxed for 16 h. The reaction was cooled to room temperature and filtered through celite. The celite layer was washed with methanol. The filtrate was concentrated in vacuo to afford **36** (120 mg) as a yellow solid. A portion (58 mg, 0.213 mmol) was dissolved in toluene (5 mL) and ethanesulfonamide (46 mg, 0.427 mmol) was added. Titanium(IV) isopropoxide (91 mg, 0.320 mmol) was added drop wise and the reaction mixture was stirred at 120°C for 16 h. The mixture was concentrated in vacuo and the residue was then dissolved in DCM-MeOH 1:1 (10 mL), cooled to 0°C and sodium borohydride (32.2 mg, 0.854 mmol) was added. The mixture was stirred at 0°C for 30 min and then quenched with water (1 mL). After 5 min, DMF (3.5 mL) was added, the solids were filtered off and the filtrate was purified using Xbridge C18 eluting with a 10 to 100% ACN-water to afford **9** as a yellow solid (14 mg, 0.038

mmol, 17%, 2 steps). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 1.38 (t, *J*=7.5 Hz, 3 H), 3.15 (q, *J*=7.3 Hz, 2 H), 3.83 (s, 3 H), 4.44 (s, 2 H), 6.91 (s, 1 H), 7.66 (s, 1 H), 8.14 (t, *J*=1.9 Hz, 1 H), 8.67 (s, 1 H), 8.69 (d, *J*=2.0 Hz, 1 H), 8.75 (d, *J*=2.0 Hz, 1 H). MS (ESI) *m/z* 365.1 $(M+H)^+$

5-(6-Chloro-1-methyl-1H-pyrrolo[2,3-b]pyridin-2-yl)nicotinaldehyde (40). To a solution of 6-chloro-1H-pyrrolo[2,3-b]pyridine (1.00 g, 6.55 mmol) in DMF (30 mL) at 0°C was added sodium hydride (60%, 0.524 g, 13.11 mmol) and the mixture was stirred for 20 min at 0°C. Methyl iodide (0.512 mL, 8.18 mmol) was added at 0°C and the mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched with water and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated in vacuo to afford **38** (1.01 g) as a brown oil, which was dissolved in THF (60 mL) and cooled to −78°C. 1.7 M t-BuLi in pentane (9 mL, 15.31 mmol) was added. After 2 h, trimethyl borate (0.954 g, 9.18 mmol) was added. The mixture was allowed to warm to room temperature and stirred for 16 h. The reaction was quenched with water (2 mL) and concentrated in vacuo to afford **39** (2.1 g) as a brown solid which was taken in the next step with no further purification. To a portion (1.00 g) in dioxane (40 mL) under N_2 was added 5-bromonicotinaldehyde (0.589 g, 3.17 mmol), aqueous 2M potassium carbonate (4.75 ml, 9.50 mmol) and PS-Pd$(PPh_3)_4$ (1.760 g, 0.158 mmol). The reaction was stirred for 2 h at 85°C, then cooled to room temperature. The solids were filtered off and the precipitate washed thoroughly with methanol. The filtrate was concentrated in vacuo to give a residue which was purified by silica gel flash chromatography (DCM-MeOH, 9:1) to afford **40** (280 mg, 1.03 mmol, 31%, 3 steps) as a solid. MS (ESI) *m/z* 272.1 $(M+H)^+$.

N-((5-(6-chloro-1H-pyrrolo[3,2-c]pyridin-2-yl)pyridin-3-yl)methyl)ethanesulfonamide (10). To **40** (91 mg, 0.335 mmol) in toluene (5 mL) was added ethanesulfonamide (73.1 mg, 0.670 mmol). Titanium(IV) isopropoxide (143 mg, 0.502 mmol) was added and the reaction

mixture was refluxed for 16 h. The mixture was concentrated in vacuo to give a residue which was then dissolved in DCM-MeOH (1:1) (10 mL), cooled to 0°C and sodium borohydride (50.7mg, 1.34 mmol) was added. The mixture was stirred at 0°C for 30 min and then quenched with water (1 mL). The resulting residue was diluted with DMF (3.5 mL), and the mixture was filtered. The filtrate was purified using Xbridge C18 eluting with a 10 to 100% ACN-water to afford **10** (25 mg, 0.068 mmol, 20%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 1.38 (t, *J*=7.3 Hz, 3 H), 3.15 (q, *J*=7.3 Hz, 2 H), 3.89 (s, 3 H), 4.44 (s, 2 H), 6.77 (s, 1 H), 7.19 (d, *J*=8.3 Hz, 1 H), 8.03 (d, *J*=8.3 Hz, 1 H), 8.15 (d, *J*=2.0 Hz 1 H), 8.67 (d, *J*=1.8 Hz, 1 H), 8.76 (d, *J*=2.0 Hz, 1 H). MS (ESI) *m/z* 365.2 $(M+H)^+$.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 617 871 7653. E-mail: julien.papillon@novartis.com

Present Addresses

⊥ C.L.: Takeda Pharmaceuticals, Cambridge, MA 02139; L.G.: Boston Biomedical, Cambridge, MA 02139; J.V.: Alnylam Pharmaceuticals, Cambridge, MA 02142; A.Y.J.: Golda Och Academy, West Orange, NJ 07052.

Notes

The authors declare no competing financial interest.

∞ C.-W.H.: retired.

○ G.M.K.: deceased.

ACKNOWLEDGMENT

We thank Prof. Joël Ménard for his work on the ASI program. We thank Dr. Lac Lee and Dr. Toshiyuki Honda for their contributions to the IC_{50} shifts and reversibility studies with **8**.

ABBREVIATIONS

ACTH: adrenocorticotropic hormone; ACN: acetonitrile; ASI: aldosterone synthase inhibitor; CYP: cytochrome P450; DCM: dichloromethane; DMF: N,N-dimethylformamide; DOC: deoxycorticosterone; ESI: electronspray ionization; LAH: lithium aluminum hydride; LEI: ligand efficiency index; LipE: lipophilic efficiency; LM: liver microsomes; MAO: monoamine oxidase; MC: methylcellulose; MR: mineralocorticoid receptor; PAC: plasma aldosterone concentration; PCC: plasma cortisol concentration; RAAS: renin-angiotensin-aldosterone system; SD: Sprague Dawley; TEA: triethylamine; THF: tetrahydrofuran; WFI: water-for-injection.

REFERENCES

(1) (a) Vasan, R. S.; Evans, J. C.; Larson, M. G.; Wilson, P. W.; Meigs, J. B.; Rifai, N.; Benjamin, E. J.; Levy, D. Serum aldosterone and the incidence of hypertension in non-hypertensive persons. *N. Engl. J. Med.* **2004**, *351*, 33−41. (b) Xanthakis, V.; Vasan, R. S. Aldosterone and the risk of hypertension. *Curr. Hypertens. Rep.* **2013**, *15*, 102−107. (c) Clark, D., III; Ahmed, M. I.; Calhoun, D. A. Resistant hypertension and aldosterone: an update. *Can. J. Cardiol.* **2012**, *28*, 318−325. (d) Calhoun, D. A.; Jones, D.; Textor, S.; Goff, D. C.; Murphy, T. P.; Toto, R. D.; White, A.; Cushman, W. C.; White, W.; Sica, D.; Ferdinand, K.; Giles, T. D.; Falkner, B.; Carey, R. M. Resistant hypertension: Diagnosis, evaluation, and treatment. A scientific statement from the American Heart

Association Professional Education Committee of the Council for High Blood Pressure Research. *Hypertension* **2008**, *51*, 1403−1419.

(2) Daugherty, S. L.; Powers, J. D.; Magid, D. J.; Tavel, H. M.; Masoudi, F. A.; Margolis, K. L.; O'Connor, P. J.; Selby, J. V.; Ho, P. M. Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* **2012**, *125*, 1635−1642.

(3) (a) He, B. J.; Anderson, M. E. Aldosterone and cardiovascular disease: the heart of the matter. *Cell* **2013**, *24*, 21−30. (b) Briet, M.; Schiffrin, E. L. Vascular actions of aldosterone. *J. Vasc. Res.* **2013**, *50*, 88−89. (c) Young, M. J. Mechanisms of mineralocorticoid receptor mediated cardiac fibrosis and vascular inflammation. *Curr. Opin. Nephrol. Hypertens.* **2008**, *17*, 174−180. (d) Brown, N. J. Aldosterone and end-organ damage. *Curr. Opin. Nephrol. Hypertens.* **2005**, *14*, 235−241. (e) Stowasser, M.; Sharman, J.; Leano, R.; Gordon, R. D.; Ward, G.; Cowley, D.; Marwick, T. H. Evidence for abnormal left ventricular structure and function in normotensive individuals with familial hyperaldosteronism type 1. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 5070−5076. (f) Milliez, P.; Girerd, X.; Plouin, P. F.; Blacher, J.; Safar, M. E.; Mourad, J. J. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J. Am. Coll. Cardiol.* **2005**, *45*, 1243−1248.

(4) (a) Staessen, J.; Lijnen, P.; Fagard, R.; Verschueren, L. J.; Amery, A. Rise in plasma concentration of aldosterone during long-term angiotensin II suppression. *J. Endocrinol.* **1981**, *91*, 457−465. (b) Struthers, A. D. Aldosterone escape during ACE inhibitor therapy in chronic heart failure. *Eur. Heart J.* **1995**, *16* (Suppl. N), 103−106.

(5) (a) Pitt, B.; Zannad, F.; Remme, W. J.; Cody, R.; Castaigne, A.; Perez, A.; Palensky, J.; Wittes, J.; Randomized Aldactone Evaluation Study Investigators.. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. *N. Engl. J. Med.* **1999**, *341*, 709−717. (b) Zannad, F.; McMurray, J.J.; Krum, H.; van Veldhuisen, D. J.; Swedberg, K.; Shi, H.; Vincent, J.; Pocock, S. J.; Pitt, B. Eplerenone in patients with systolic heart failure and mild symptoms. *N. Engl. J. Med.* **2011**, *364*, 11-21.

(6) Pitt, B.; Remme, W.; Zannad, F.; Neaton, J.; Martinez, F.; Roniker, B.; et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N. Engl. J. Med.* **2003**, *348*, 1309−1321.

(7) Pitt, B.; Pfeffer, M. A.; Assmann, S. F. et al. Spironolactone for heart failure with preserved ejection fraction. *N. Engl. J. Med.* **2014**, *370*, 1383-1392.

(8) Yu, Y.; Wei, S.-G.; Zhang, Z.-H.; Gomez-Sanchez, E.; Weiss, R.M.; Felder, R.B. Does aldosterone upregulate the brain renin- angiotensin system in rats with heart failure? *Hypertension* **2008**, *51*, 727-733.

(9) Williams, J. S. Evolving research in nongenomic actions of aldosterone. *Curr. Opin. Endocrinol. Diabetes Obes.* **2013**, *20*, 198−203.

(10) (a) Mellin, V.; Mulder, P.; DiMeglio, B.; Henry, J. P.; Jeng, A. Y.; Webb, R. L.; Thuillez, C. Long-term aldosterone synthase inhibition improves cardiac function and myocardial structure in chronic heart failure. *Circulation* **2004**, *110* (17, Suppl. 3), III-102. (b) Fiebeler, A.; Nussberger, J.; Shagdarsuren, E.; Rong, S.; Hilfenhaus, G.; Al-Saadi, N.; Dechend, R.; Wellner, M.; Meiners, S.; Maser-Gluth, C.; Jeng, A. Y.; Webb, R. L.; Luft,

F. C.; Muller, D. N. Aldosterone synthase inhibitor ameliorates angiotensin II-induced organ damage. *Circulation* **2005**, *111*, 3087−3094. (c) Rigel, D. F.; Fu, F.; Beil, M.; Hu, C.-W.; Liang, G.; Jeng, A. Y. Pharmacodynamic and pharmacokinetic characterization of the aldosterone synthase inhibitor FAD286 in two rodent models of hyperaldosteronism: Comparison with the 11β-hydroxylase inhibitor metyrapone. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 232−243. (d) Lea1, W. B.; Kwak, E. S.; Luther, J. M.; Fowler, S. M.; Wang, Z.; Ma, J.; Fogo, A. B.; Brown, N. J. Aldosterone antagonism or synthase inhibition reduces end-organ damage induced by treatment with angiotensin and high salt. *Kidney Int.* **2009**, *75*, 936−944. (e) Mulder, P.; Mellin, V.; Favre, J.; Vercauteren, M.; Remy-Jouet, I.; Monteil, C.; Richard, V.; Renet, S.; Henry, J. P.; Jeng, A. Y.; et al. Aldosterone synthase inhibition improves cardiovascular function and structure in rats with heart failure: a comparison with spironolactone. *Eur. Heart J.* **2008**, *29*, 2171−2179. (f) Huang, B. S.; White, R. A.; Ahmad, M.; Jeng, A. Y.; Leenen, F. H. H. Central infusion of aldosterone synthase inhibitor prevents sympathetic hyperactivity and hypertension by central Na+ in Wistar rats. *Am. J. Phys.* **2008**, *295*, R166−R172. (g) Minnaard-Huiban, M.; Emmen, J. M. A.; Roumen, L.; Beugels, I. P. E.; Cohuet, G. M. S.; van Essen, H.; Ruijters, E.; Pieterse, K.; Hilbers, P. A. J.; Ottenheijm, H. C. J.; Plate, R.; de Gooyer, M. E.; Smits, J. F. M.; Hermans, J. J. R. Fadrozole reverses cardiac fibrosis in spontaneously hypertensive heart failure rats: discordant enantioselectivity versus reduction of plasma aldosterone. *Endocrinology* **2008**, *149*, 28−31. (h) Ménard, J.; Pascoe, L. Can the dextroenantiomer of the aromatase inhibitor fadrozole be useful for clinical investigation of aldosterone-synthase inhibition? *J. Hypertens.* **2006**, *24*, 993−997.

(11) Meredith, E. L.; Ksander, G.; Monovich, L. G.; Papillon, J. P. N.; Liu, Q.; Miranda, K.; Morris, P.; Rao, C.; Burgis, R.; Capparelli, M.; Hu, Q.-Y.; Singh, A.; Rigel, D. F.; Jeng, A. Y.; Beil, M.; Fu, F.; Hu, C.-W.; LaSala, D. Discovery and in vivo evaluation of potent dual CYP11B2 (aldosterone synthase) and CYP11B1 inhibitors. *ACS Med. Chem. Lett.* **2013**, *4*, 1203−1207

(12) Ménard, J.; Rigel, D. F.; Watson, C.; Jeng, A. Y.; Fu, F.; Beil, M.; Liu, J.; Chen, W.; Hu, C.-W.; Leung-Chu, J.; LaSala, D.; Liang, G.; Rebello, S.; Zhang, Y.; Dole, W. P. Aldosterone synthase inhibition: cardiorenal protection in animal disease models and translation of hormonal effects to human subjects. *J. Trans. Med.* **2014**, *12*, 340-361.

(13) (a) Amar, L.; Azizi, M.; Ménard, J.; Peyrard, S.; Watson, C.; Plouin, P. F. Aldosterone synthase inhibition with LCI699: a proof-ofconcept study in patients with primary aldosteronism. *Hypertension* **2010**, 831−838. (b) Amar, L.; Azizi, M.; Ménard, J.; Peyrard, S.; Plouin, P.-F. Sequential comparison of aldosterone synthase inhibition and mineralocorticoid blockade in patients with primary aldosteronism. *J. Hypertens.* **2013**, *31*, 624−629.

(14) (a) Calhoun, D. B.; White, W. B.; Krum, H.; Guo, G.; Bermann, G.; Trapani, A.; Leftkoowitz, M. P.; Ménard, J. Effects of a novel aldosterone synthase inhibitor for treatment of primary hypertension: Results of a randomized, double-blind, placebo. *Circuation* **2011**, *124*, 1945−1955. (b) Andersen, K.; Hartman, D.; Peppard, T.; Hermann, D.; Van Ess, P.; Lefkowitz, M.; Trapani, A. The effects of aldosterone synthase inhibition on aldosterone and cortisol in patients with hypertension: a phase II,

randomized, double-blind, placebo-controlled, multicenter study. *J. Clin. Hypertens.* **2012**, *14*, 580−587.

(15) Kams, A. D.; Bral, J. M.; Hartman, D.; Peppard, T.; Schumacher, C. Study of aldosterone synthase inhibition as an add-on therapy in resistant hypertension. *J. Clin. Hypertens.* **2013**, *15*, 186−192.

(16) Bertagna, X.; Pivonello, R.; Fleseriu, M.; Zhang, Y.; Robinson, P.; Taylor, A.; Watson, C. E.; Maldonado, M.; Hamrahian, A. H.; Boscaro, M.; Biller, B. M. K. LCI699, a potent 11-hydroxylase inhibitor, normalizes urinary cortisol in patients with Cushing's disease: results from a multicenter, proof-of-concept study. *J. Clin. Endocrinol. Metab.* **2014**, *99*, 1375−1383.

(17) (a) Papillon, J. P. N.; Adams, C. M.; Hu, Q.-Y.; Lou, C.; Singh, A. K.; Zhang, C.; Carvalho, J.; Rajan, S.; Amaral, A.; Beil, M. E.; Fu, F.; Gangl, E.; Hu, C.-W.; Jeng, A. Y.; LaSala, D.; Liang, G.; Logman, M.; Maniara, W. M.; Rigel, D. F.; Smith, S. A.; Ksander, G. M. Structure−Activity Relationships, Pharmacokinetics, and in Vivo Activity of CYP11B2 and CYP11B1 Inhibitors *J. Med. Chem.* **2015**, *58*, 4749-4770. (b) Emmerich, J.; Hu, Q.; Hanke, N.; Hartmann, R. W. Cushing's syndrome: development of highly potent and selective CYP11B1 inhibitors of the (pyridylmethyl)pyridine type. *J. Med. Chem.* **2013**, *56*, 6022−6032. (c) Hille, U. E.; Zimmer, C.; Haupenthal, J.; Hartmann, R. W. Optimization of the first selective steroid-11β-hydroxylase (CYP11B1) inhibitors for the treatment of cortisol dependent diseases. *ACS Med. Chem. Lett.* **2011**, *2*, 559−564. (d) Yin, L.; Lucas, S.; Maurer, F.; Kazmaier, U.; Hu, Q.; Hartmann, R. W. Novel imidazol-1-ylmethyl substituted 1,2,5,6-tetrahydro-pyrrolo-[3,2,1-ij]quinolin-4-

ones as potent and selective CYP11B1 inhibitors for the treatment of Cushing's syndrome. *J. Med. Chem.* **2012**, *55*, 6629−6633.

(18) (a) Azizi, M.; Amar, L.; Menard, J. Aldosterone synthase inhibition in humans. *Nephrol., Dial., Transplant.* **2013**, *28*, 36−43. (b) Hu, Q.; Yin, L.; Hartmann, R. W. Aldosterone synthase inhibitors as promising treatments for mineralocorticoid dependent cardiovascular and renal diseases. *J. Med. Chem.* **2014**, *57*, 5011−5022. (c) Cerny, M. A. Progress towards clinically useful aldosterone synthase inhibitors. *Curr. Top. Med. Chem.* **2013**, *13*, 1385−1401.

(19) (a) Hoyt, S. B.; Park, M. K.; London, C.; Xiong, Y.; Tata, J.; Bennett, D. J.; Cooke, A.; Cai, J.; Carswell, E.; Robinson, J.; MacLean, J.; Brown, L.; Belshaw, S.; Clarkson, T. R.; Liu, K.; Liang, G.-B.; Struthers, M.; Cully, D.; Wisniewski, T.; Ren, N.; Bopp, C.; Sok, A.; Cai, T.-Q.; Stribling, S.; Pai, L.-Y.; Ma, X.; Metzger, J.; Verras, A.; McMasters, D.; Chen, Q.; Tung, E.; Tang, W.; Salituro, G.; Buist, N.; Kuethe, J.; Rivera, N.; Clemas, J.; Zhou, G.; Gibson, J.; Maxwell, C. A.; Lassman, M.; McLaughlin, T.; Catro-Perez, J.; Szeto, D.; Forrest, G.; Hajdu, R.; Rosenbach, M.; Ali, A. Discovery of Benzimidazole CYP11B2 Inhibitors with in Vivo Activity in Rhesus Monkeys. *ACS Med. Chem. Lett.* **2015**, *6*, 573-578. (b) Hu, Q.; Yin, L.; Ali, A.; Cooke, A. J.; Bennett, J.; Ratcliffe, P.; Lo, M. M.; Metzger, E.; Hoyt, S.; Hartmann, R. W. Novel Pyridyl Substituted 4,5-Dihydro-[1,2,4]triazolo[4,3‑a]quinolines as Potent and Selective Aldosterone Synthase Inhibitors with Improved in Vitro Metabolic Stability *J. Med. Chem.* **2015**, *58*, 2530-2537. (c) Grombein, C. M.; Hu, Q.; Heim, R.; Rau, S.; Zimmer, C.; Hartmann, R. W. 1-Phenylsulfinyl-3-(pyridin-3-yl)naphthalen-2-ols: a new class of potent and selective

aldosterone synthase inhibitors. *Eur. J. Med. Chem.* **2015**, 89, 597−605. (d) Grombein, C. M.; Hu, Q.; Rau, S.; Zimmer, C.; Hartmann, R. W. Heteroatom insertion into 3,4-dihydro-1H-quinolin-2-ones leads to potent and selective inhibitors of human and rat aldosterone synthase. *Eur. J. Med. Chem.* **2015**, *90*, 788−796. (e) Yin, L.; Hu, Q.; Emmerich, J.; Lo, M. M.; Metzger, E.; Ali, A.; Hartmann, R. W. Novel pyridyl- or isoquinolinyl-substituted indolines and indoles as potent and selective aldosterone synthase inhibitors. *J. Med. Chem.* **2014**, *57*, 5179−5189. (f) Lucas, S.; Negri, M.; Heim, R.; Zimmer, C.; Hartmann, R. W. Fine-tuning the selectivity of aldosterone synthase inhibitors: insights from studies from studies of heteroaryl substituted 1,2,5,6-tetrahydropyrrolo[3,2,1-ij]quinoline-4-one derivatives. *J. Med. Chem.* **2011**, *54*, 2307−2319. (g) Roumen, L.; Peeters, J. W.; Emmen, J. M. A.; Beugels, I. P. E.; Custers, E. M. G.; de Gooyer, M.; Plate, R.; Pieterse, K.; Hilbers, P. A. J.; Smits, J. F. M.; Vekemans, J. A. J.; Leysen, D.; Ottenheijm, H. C. J.; Janssen, H. M.; Hermans, J. J. R. Synthesis, biological evaluation, and molecular modeling of 1-benzyl-1H-imidazoles as selective inhibitors of aldosterone synthase (CYP11B2). *J. Med. Chem.* **2010**, *53*, 1712−1725. (h) Adams, C. M.; Hu, C.-W; Jeng, A. Y.; Karki, R.; Ksander, G. M.; LaSala, D.; Leung-Chu, J.; Liang, G.; Liu, Q.; Meredith, E. L.; Rao, C.; Rigel, D. F.; Shi, J.; Smith, S.; Springer, C.; Zhang, C. The discovery of potent inhibitors of aldosterone synthase that exhibit selectivity over 11-β-hydroxylase. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4324−4327. (i) Lucas, S.; Heim, R.; Ries, C.; Schewe, K. E.; Birk, B.; Hartmann, R. W. In vivo active aldosterone synthase inhibitors with improved selectivity: lead optimization providing a series of pyridine substituted 3,4-dihydro-1H-quinolin-2-one derivatives. *J. Med. Chem.* **2008**, *51*, 8077−8087. (j) Voets, M.; Antes, I.; Scherer, C.;

Müller-Vieira, U.; Biemel, K.; Barassin, C.; Marchais-Oberwinkler , S.; Hartmann, R. W. Heteroaryl-Substituted Naphthalenes and Structurally Modified Derivatives: Selective Inhibitors of CYP11B2 for the Treatment of Congestive Heart Failure and Myocardial Fibrosis. *J. Med. Chem.* **2005**, *48*, 6632-6642.

(20) Pfenninger, H. A. Certain 2-pyridyl-substituted indole derivatives. US 3468894, Sept. 23, 1969.

(21) Bird, L.; Hanley, N.; Word, R.; Mathis, J.; Mason, J.; Rainey, W. Human NCI-H295 adrenocortical carcinoma cells: a model for angiotensin-II responsive aldosterone secretion. *Endocrinology* **1993**, *133*, 1555−1561.

(22) LaSala, D.; Shibanaka, Y.; Jeng, A. Y. Coexpression of CYP11B2 or CYP11B1 with adrenodoxin and adrenodoxin reductase for assessing the potency and selectivity of aldosterone synthase inhibitors. *Anal. Biochem.* **2009**, *394*, 56−61.

(23) Anderson, M. C.; Hasan, F.;, McCrodden, J. M.; Tipton, K. F. Monoamine oxidase inhibitors and the cheese effect. *Neurochem. Res.* **1993**, *18*, 1145-1149.

(24) Sun-Edelstein, C.; Tepper, S. J.; Shapiro, R. E. Drug-induced serotonin syndrome: a review. *Expert Opin. Drug Saf.* **2008**, *7*, 587–596.

(25) Greater than 1000-fold selectivities over CYP3A4/5, CYP2D6, CYP2C9 and CYP1A2 enzymes were also observed.

(26) **8** is a reversible inhibitor of CYP11B2 with a $k_{off} = 0.013 \pm 0.003$ min^{-1} (n=4) and residence half-life $t_{1/2} = 52 \pm 9$ min (n=4); **8** is a substrate-competitive inhibitor, as

determined from IC_{50} shifts experiments with increasing 11-deoxycorticosterone concentrations.

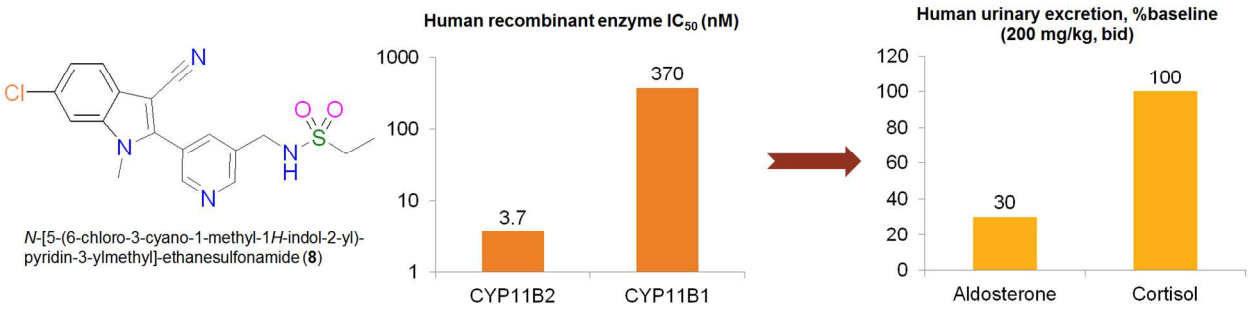
(27) Rigel, D. F.; Fu, F.; Beil, M.; Hu, C.-W.; Liang, G.; Jeng, A. Y. Pharmacodynamic and pharmacokinetic characterization of the aldosterone synthase inhibitor FAD286 in two rodent models of hyperaldosteronism: Comparison with the 11β-hydroxylase inhibitor metyrapone. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 232−243.

(28) After these studies were completed, a similar model was published: Cai, T. -Q.; Stribling, S.; Tong, X.; Xu, L.; Wisniewski, T.; Fontenot, J. A.; Struthers, M; Akinsanya, K. O. *J. Pharmacol. Toxicol. Meth.* **2015**, *71*, 137-146.

(29) Wacker, D. A.; Kasireddy, P. Efficient solid-phase synthesis of 2,3-substituted indoles. *Tetrahedron Lett.* **2002**, *43*, 5189-5191.

(30) Mehta, G. Reaction of indoles with chlorosulphonyl isocyanate; a versatile route to 3-substituted indoles. *Synthesis* **1978**, 374-376.

Table of contents graphic



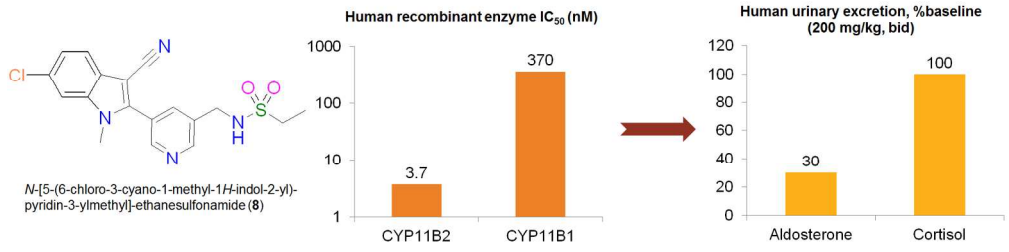


Table of content graphic
435x109mm (120 x 120 DPI)