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Article

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Discovery of 3-Pyridyl Isoindolin-1-one Derivatives as Potent, Selective and Orally Active Aldosterone Synthase (CYP11B2) Inhibitors

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Abstract: Aldosterone synthase (CYP11B2) inhibitors were explored in recent years as an alternative therapeutic option to MR antagonists to reduce elevated aldosterone levels, which are associated with deleterious effects on various organ systems including heart, vasculature, kidney, and CNS. A benzamide pyridine hit derived from a focused screen was successfully developed into a series of potent and selective 3-pyridyl isoindolin-1-ones CYP11B2 inhibitors. Our systematic structure-activity relationship study enabled us to identify unique structural features that result in high selectivity against the closely homologous cortisol synthase (CYP11B1). We evaluated advanced lead molecules,

exemplified by compound **52**, in an *in vivo* cynomolgus monkey acute ACTH challenge model and demonstrated a superior 100-fold *in vivo* selectivity against CYP11B1.

Keywords: Aldosterone synthase inhibitor, CYP11B2, CYP11B1, selectivity, 3-pyridyl isoindolin-1-one

INTRODUCTION

The renin angiotensin aldosterone system (RAAS) is an endocrine system that regulates body's blood pressure and fluid balance. Its dysregulation contributes to the pathogenesis of a number of cardiovascular, renal, and metabolic diseases. Therefore, it is a pathway that has been successfully targeted by many RAAS inhibitors, including angiotensin AT1 receptor blockers (ARBs), angiotensin-converting-enzyme inhibitors (ACEIs), renin inhibitors, and mineralocorticoid receptor antagonists (MRAs).[1] Aldosterone, as one component of RAAS, besides its well-known function in regulating fluid and electrolyte homeostasis, has recently been recognized as one important contributor to end-organ damage in various cardiovascular and renal diseases.[2-5] An elevated aldosterone level, especially in conjunction with high sodium/oxidative stress is associated with deleterious effects on various organ systems including heart, vasculature, kidney, and central nervous system (CNS).[6] In addition, in hypertensive patients that are treated with either ACEIs or ARBs for a longer duration, an "aldosterone breakthrough" phenomenon leading to rebound aldosterone levels after an initial decline is associated with end-organ damage.[7]

Antagonizing the aldosterone effect by an MR antagonist has been shown to reduce the risk of death in congestive heart failure (CHF) patients when used in conjunction with ACEI (RALES trial), and to improve survival and reduce hospitalisation in patients with post-myocardial infarction heart failure when added to the standard treatment regimen (EPHESUS trial).[8,9] However, a suppression of the plasma aldosterone level by an MR antagonist, after a prolonged usage, would induce an increased aldosterone level by a compensatory mechanism and therefore limit its efficacy. Therefore, blocking the biosynthesis of aldosterone by an aldosterone synthase inhibitor (ASI) has been explored as an alternative approach in recent years.[10-12] Additionally, an ASI is expected to block MR-independent

genomic and non-genomic effects and it may provide even better protection against the aforementioned diseases.[13]

Aldosterone is a steroid hormone synthesized from cholesterol via a cascade of enzymatic reactions by a number of cytochrome P450s. Located mainly in adrenal gland zona glomerulosa, aldosterone synthase (encoded by the CYP11B2 gene) is the enzyme responsible for the last three steps of reactions by converting 11-deoxycorticosterone to aldosterone via corticosterone and 18-hydroxy-corticosterone intermediates (**Figure 1**).[10] Since in human, the aldosterone synthase shows a high sequence identity (~93%) to cortisol synthase (encoded by the CYP11B1 gene, mainly present in the zona fasciculate/reticularis),[14] the development of a highly selective aldosterone synthase inhibitor has been challenging over the past several years.[10-12]

The proof-of-concept efficacy study was first demonstrated by Novartis ASI LCI699 in patients with primary hyperaldosteronism. The oral administration of LCI699 first at 0.5 mg *b.i.d.* for 2 weeks, followed by 1.0 mg *b.i.d.* for another 2 weeks, significantly reduced levels of aldosterone in both plasma and urine, leading to a rapid correction of hypokalemia, and a modest decrease in blood pressure. However, due to the low selectivity of LCI699 (CYP11B1 IC_{50}/CYP11B2 IC_{50} = 3.6-fold), a blunted cortisol response to adrenocorticotropic hormone (ACTH) was observed at dose 1 mg/d or greater in the safety evaluation.[15,16] Good selectivity against CYP11B1 is critical in order to prevent undesired side-effects related to the hypothalamic–pituitary–adrenal (HPA) axis. We recently reported our own clinical study of a highly selective ASI RO6836191 (CYP11B1 IC_{50}/CYP11B2 IC_{50} = 100-fold). In a phase I SAD study (0-360 mg/d), RO6836191 achieved high CYP11B1/CYP11B2 selectivity with maximum suppression of aldosterone production at 10 mg/d, and no change of cortisol level up to 360 mg/d. Such good selectivity was also reflected by increase of precursors 11-deoxycorticosterone and 11-deoxycortisol only at ≥ 90 mg.[17]

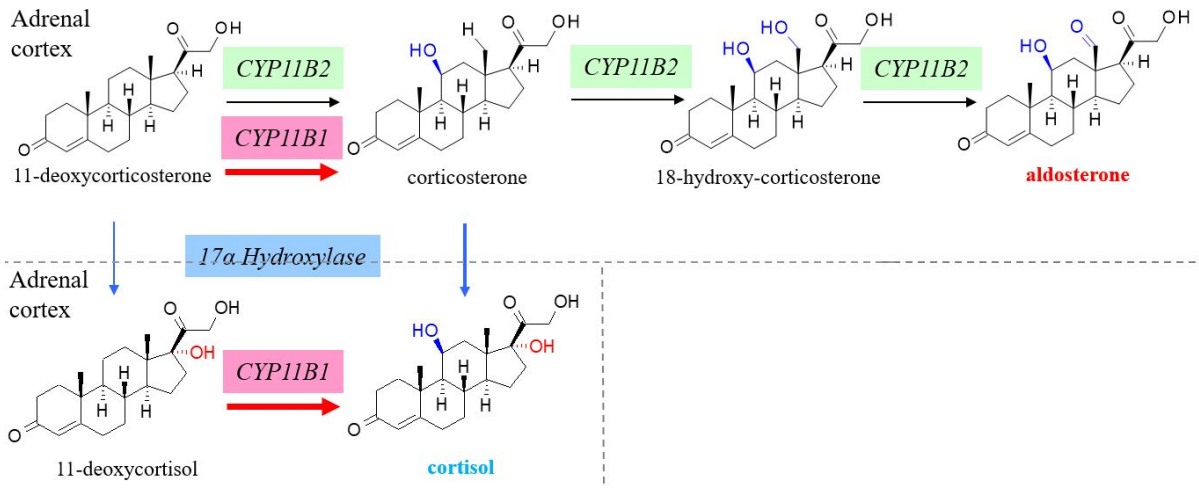


Figure 1. Terminal steroidogenesis pathways leading to aldosterone and cortisol.

Herein, we report a new type of ASIs with even superior CYP11B1/CYP11B2 selectivity both *in vitro* and *in vivo*.

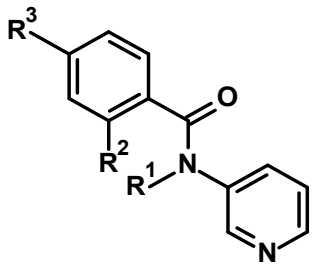
RESULT AND DISCUSSION

SAR and Lead Optimization Strategy

To identify novel ASIs we performed a focused screen of a subset of the Roche library. First selection criterion for this library was the presence of a nitrogen lone pair in a heteroaromatic ring without substitution *alpha* to the nitrogen atom to enable potential heme-iron binding in CYP11B2. This initial list was further reduced to a final set of 2'000 compounds by 2D fingerprint and 3D shape similarity to several selected reference inhibitors (SI, **Figure S1**),[18-21] molecular property filtering, and structure clustering. Screening of this library against human CYP11B2 yielded a significant number of hits, several of them with very high ligand efficiencies. However, only few compounds of the hit list showed initial selectivity against human CYP11B1. This is not surprising as the active site residues are fully conserved between the two isoforms.[22] Interestingly, despite the presence of unshielded nitrogen lone pairs in our hit structures, which are privileged motifs for iron-heme binding, we did not find strong cross-reactivities against the drug-metabolizing CYP isoforms 3A4, 2C9, 2D6, 2C19, and 1A2.

Among the promising hits, a class of *N*-(3-pyridyl)benzamides (**1-6**)[23] was selected as our chemistry starting point. These initial hits showed sub-micromolar CYP11B2 IC_{50} values and some selectivity against human CYP11B1 in in-house developed cellular assays (**Table 1**). Initial structure-activity relationship (SAR) indicated that a chlorine (**1**) or nitrile (**2**) substitution at the para-position of the benzamide was favored for better potency. In addition, in the ortho-position small substituents like fluorine (**5**) or methyl (**6**) slightly improved the potency. Methylation at the benzamide nitrogen led to almost inactive compounds (**7-9**), presumably because of the drastic conformational change compared to the unsubstituted analogs.

Table 1. Preliminary SAR for initial *N*-(3-pyridyl)benzamide hits, **1-9**.

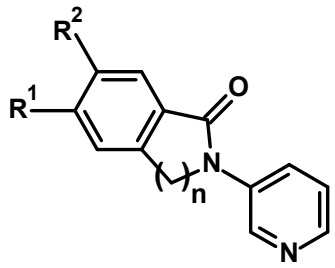


Cmpd	Structure			IC_{50} (μM)[a]		SF[b]
	R^1	R^2	R^3	CYP11B2	CYP11B1	
1	H	H	Cl	0.42	> 3	> 7
2	H	H	CN	0.454	> 3	> 7
3	H	H	Me	> 3	> 3	na
4	H	Cl	H	0.967	> 3	> 3
5	H	F	Cl	0.297	> 3	> 10
6	H	Me	Cl	0.277	> 3	> 11
7	Me	F	Cl	> 3	> 3	na
8	Me	Me	Cl	> 3	> 3	na
9	Me	H	CN	> 3	> 3	na

[a]IC_{50}: the concentration of compound that inhibited 50% enzyme activity; [b]SF: the selective factor defined as the ratio between the IC_{50} values of human CYP11B1 and CYP11B2.

Subsequent conformational restriction of 4-chloro-*N*-(3-pyridyl)benzamide **1** via cyclization gave either 6-membered lactam, 3,4-dihydroisoquinolin-1-one derivative **10** (IC_{50} = 61 nM) or 5-membered lactam, isoindolin-1-one derivative **13** (IC_{50} = 179 nM), which showed 7- and 2-fold CYP11B2 potency improvements, respectively. Additional substitution on the phenyl ring, such as **11**, did not gain further potency. The CN substituted isoindolin-1-one analog **14** (IC_{50} = 232 nM), despite a two-fold potency improvement (vs. **2**), was still inferior to a chlorine substitution. Apart from their better potencies, the cyclized lactams also showed encouraging double-digit selectivity factors against CYP11B1 (**Table 2**).

Table 2. Potency, selectivity, and microsomal stability of 3,4-dihydroisoquinolin-1-one and isoindolin-1-one derivatives, **10-14**.



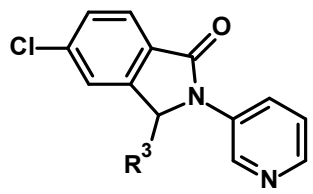
Cmpd	Structure			IC_{50} (μM)[a]		SF[b]	LM[c] (mL/min/kg)	
	n	R^1	R^2	CYP11B2	CYP11B1		human	mouse
10	2	Cl	H	0.061	5.002	82	< 3.5	< 20.2
11	2	Cl	F	0.104	3.541	34	< 3.5	30.9
12	1	H	H	1.12	> 3	> 3	nd	nd
13	1	Cl	H	0.179	10.74	60	12.8	76.5
14	1	CN	H	0.232	15.544	67	8.7	45.6

[a]IC_{50}: the concentration of compound that inhibited 50% enzyme activity; [b]SF: the selective factor defined as the ratio between the IC_{50} values of human CYP11B1 and CYP11B2; [c]LM: scaled intrinsic clearance of compound in liver microsomes.

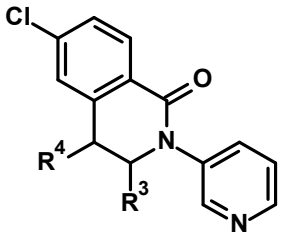
Compared to the 3,4-dihydroisoquinolin-1-one analog **10** (logD = 2.25), the slightly more lipophilic isoindolin-1-one analog **13** (logD = 2.52) was much less stable in liver microsomes, likely due to oxidative metabolism at the benzylic C-H position. Although metabolic stability could be

improved by lowering the lipophilicity, as demonstrated by analog **14** (logD = 1.35), we also envisioned that increasing the steric hindrance at the benzylic carbon could reduce metabolic oxidation. As shown in **Table 3**, a number of such analogs were prepared as either a racemic mixture or an achiral compound. Indeed, benzylic substitutions (Me, Et, and cyclopropyl) did improve the metabolic stability (**15**, **16**, and **19**), and gem-dimethyl (**17**) as well as spirocyclopropyl (**18**) analogs showed the most significant improvement in liver microsomal stability. Except for the benzyl substituted **20**, all analogs (**15-19**) substituted at the benzylic position retained comparable CYP11B2 potency and selectivity against CYP11B1. Encouraging, but difficult to rationalize, a surprising 202-fold of CYP11B1 selectivity was observed for analog **19** (IC_{50} = 161 nM). On the other hand, analogs derived from derivatization at the 3- or 4- position of 3,4-dihydroisoquinolin-1-one core such as **21-22** (Me) and **23** (gem-dimethyl), either decreased potency, selectivity or had reduced microsomal stability (vs. **10**). These new types of compounds also showed excellent selectivity against a common cytochrome P450 screening panel (3A4, 2D6, and 2C9) with IC_{50} values generally greater than 50 μM.

Table 3. Potency, selectivity, and microsomal stability of 3,4-dihydroisoquinolin-1-one and isoindolin-1-one derivatives, **15-23**.



15-20



21-23

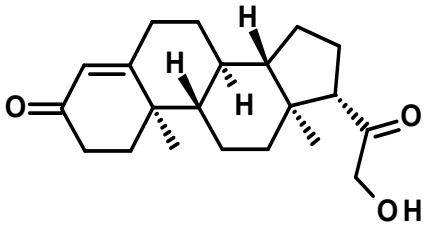
Cmpd	Structure	IC_{50} (μM)[a]		SF[b]	LM[c] (mL/min/kg)	
	R^3/R^4	CYP11B2	CYP11B1		human	mouse
15	Me	0.123	1.248	10	12.2	66.5
16	Et	0.121	3.341	28	6.6	65.9
17	gem-dimethyl	0.108	4.276	40	< 3.5	25
18	spirocyclopropyl	0.120	4.450	37	3.5	nd
19	cyclopropyl	0.161	32.522	202	9.4	nd
20	Bn	1.07	0.214	0.2	nd	nd
21	Me / -	0.761	>10	>13	nd	nd

22	- / Me	0.164	2.588	16	6.6	45
23	- / gem-dimethyl	1.538	> 10	> 7	nd	nd

[a]IC_{50}: the concentration of compound that inhibited 50% enzyme activity; [b]SF: the selective factor defined as the ratio between the IC_{50} values of human CYP11B1 and CYP11B2; [c]LM: scaled intrinsic clearance of compound in liver microsomes.

Having optimized the pyridine left-hand side moiety, in particular the isoindolin-1-one core for potency, selectivity, and liver microsomal stability, the lead optimization effort then focused onto the central pyridine. An overlay between the modeled binding mode of compound **13** in the X-ray complex structure of deoxycorticosterone with CYP11B2 indicated room for extension at the steroid D ring and its polar substituent (**Figure 2**). We first tried to mimic this motif with a bicyclic tetrahydroisoquinoline core. Thus, a number of 5,6,7,8-tetrahydroisoquinolin-8-amine derived analogs were prepared with a small set of amide or sulfonamide modifications to both mimic the deoxycorticosterone hydroxymethyl ketone and as a handle for physico-chemical property modulation (**Table 4**). Similar to previously reported examples,[24] the chirality of C-8 was found to be critical for CYP11B2 potency, with the (*R*)-configuration enantiomer being the preferred one (**25**, IC_{50} = 44 nM vs. **24**, IC_{50} = 5.14 μM). In general, amide substitutions were more potent than sulfonamide (**25** vs. **27**, and **26** vs. **28**), and bigger substitutions were better tolerated than small ones (e.g., propyl amide **26** vs. acetyl amide **25**, and ethyl sulfonamide **28** vs. methyl sulfonamide **27**). Interestingly, for this new series of compounds, even without benzylic substitution at the isoindolin-1-one core, all analogs were quite stable in liver microsomes. The most potent propyl amide analog **26** showed a CYP11B2 IC_{50} of 28 nM and a 186-fold selectivity vs. CYP11B1, together with a very good physico-chemical profile (lipophilicity, logD = 2.14, permeability, Pampa Pe = 3.63 x 10^{-6} cm/s, and solubility, LYSA > 460 μg/mL). Subsequent hybrids with isoindolin-1-one derivatives having small alkyl substitutions at the benzylic position did result in further potency improvements and single digit nanomolar IC_{50} values. Unfortunately, several of the most potent analogs (**30**, IC_{50} = 4 nM, **31**, IC_{50} = 7 nM, and **34**, IC_{50} = 6 nM) did not pass the 100-fold CYP11B1 selectivity threshold that we had set to achieve. The combination with 3,4-dihydroisoquinolin-1-one core (**39-41**) also did not yield better candidates for further development.

The relative configuration of compounds **29-38** were tentatively assigned based on optical rotation and logD values correlation between each pair of diasteromers, in which (*3R,8R*) vs. (*3S,8R*) has consistent trend (SI, Table S6). Corresponding gem-dimethyl and spirocyclopropyl hybrid analogs could not be synthesized via the Ullman coupling reaction with the 5,6,7,8-tetrahydroisoquinolin-8-amine building block due to significant steric hindrance at the *alpha* position of the lactam nitrogen.



deoxycorticosterone

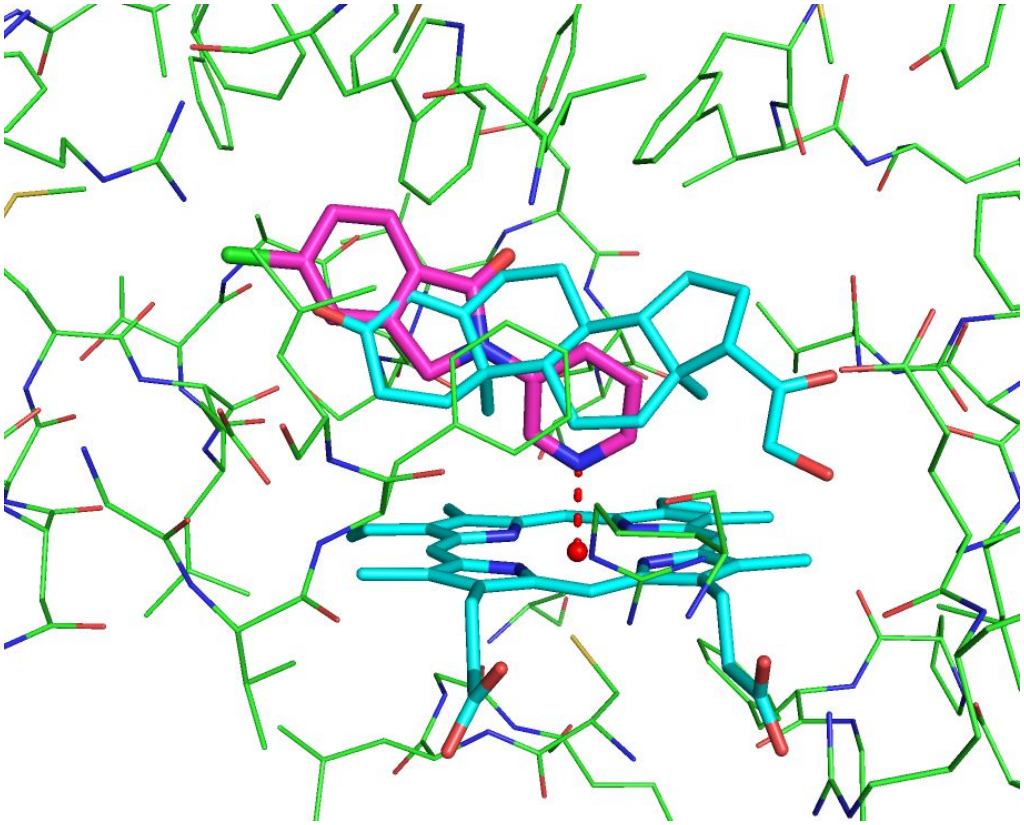
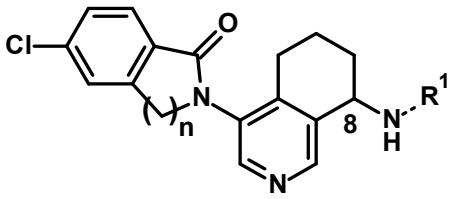
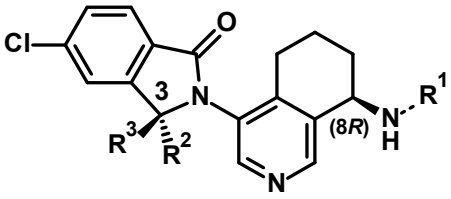


Figure 2. Overlay of modeled binding mode of compound **13** (magenta) with the X-ray co-crystal structure of human CYP11B2 with deoxycorticosterone (PDB code: 4DVQ, protein in green, heme and steroid ligand in cyan). In the model, the pyridine nitrogen atom engages in a short contact with the heme iron atom (dashed line).

Table 4. Potency, selectivity, and microsomal stability of 5,6,7,8-tetrahydroisoquinolin-8-amine derived analogs, **24-41**.



n = 1, 24-28
n = 2, 39-41



29-38

Cmpd	Structure				IC_{50} (μM)[a]		SF[b]	LM[c] (mL/min/kg)	
	C8	R^1	R^2	R^3	CYP11B2	CYP11B1		human	mouse
24	*(S)*		---	---	5.141	39.623	8	nd	nd
25	*(R)*		---	---	0.044	2.332	53	< 3.5	< 20.2
26	*(R)*		---	---	0.028	5.208	186	< 3.5	< 20.2
27	*(R)*		---	---	0.998	9.352	9	< 3.5	< 20.2
28	*(R)*		---	---	0.040	1.788	45	< 3.5	27
29	*(R)*		Me	H	0.130	5.137	40	< 3.5	43.5
30	*(R)*		H	Me	0.004	0.081	20	< 3.5	< 20.2
31	*(R)*		Et	H	0.007	0.334	48	< 3.5	< 20.2
32	*(R)*		H	Et	0.097	3.634	37	< 3.5	< 20.2
33	*(R)*		Me	H	0.066	9.414	143	< 3.5	45.6
34	*(R)*		H	Me	0.006	0.414	69	< 3.5	< 20.2
35	*(R)*		Et	H	0.090	10.246	114	< 3.5	44.9
36	*(R)*		H	Et	0.012	0.345	29	< 3.5	< 20.2
37	*(R)*		cyclo-propyl	H	0.1	22.8	228	nd	nd
38	*(R)*		H	cyclo-propyl	0.354	>30	>85	nd	nd
39	*(R)*		---	---	0.054	3.921	73	4.3	< 20.2[d]

40	*(R)*		---	---	0.119	3.326	78	< 3.5	< 20.2[d]
41	*(R)*		---	---	0.076	5.148	68	< 3.5	< 20.2[d]

[a]IC_{50}: the concentration of compound that inhibited 50% enzyme activity; [b]SF: the selective factor defined as the ratio between the IC_{50} values of human CYP11B1 and CYP11B2; [c]LM: scaled intrinsic clearance of compound in liver microsomes using well-stirred liver model: [d]LM measured in rat.

The structural model in **Figure 2** suggests that extension in the meta-position at the right-hand side of the central pyridyl ring could be a good direction to further grow into the CYP11B2 binding site. Consequently, a number of 5-pyridyl substituted analogs with different size and polarity were synthesized while keeping the 5-chloro-3,3-dimethyl-2-(3-pyridyl)isoindolin-1-one constant at the left-hand side (**Table 5**). Based on the assumption that electron-donating substitution might help to improve potency via enhancing pyridine nitrogen binding affinity to the heme-iron, we decided to limit substitutions to carbon, oxygen, and nitrogen linked attachments. Various types of linkers with variable length were explored and a terminal amine group was specifically chosen for further derivatization with either amides or sulfonamides. As shown in **Table 5**, the majority of analogs showed improved CYP11B2 potencies compared to the unsubstituted analog **17** (IC_{50} = 108 nM). For a few methylene amine-linked compounds (**42-47**), either the installation of an (*R*)-methyl at the methylene carbon (**45**, IC_{50} = 8 nM) or a 3-methylpyridyl amide substitution (**47**, IC_{50} = 9 nM) resulted in single digit nanomolar potencies. While the binding affinity towards CYP11B2 could be improved >10-fold in this way, it is worth mentioning that the ligand efficiency (LE) is significantly reduced (**17**: LE = 0.50, **45**: LE = 0.43, **47**: LE = 0.37). This indicates that protein interactions made at the right-hand side of the scaffold contribute less binding energy per atom compared to the rest of the molecule.

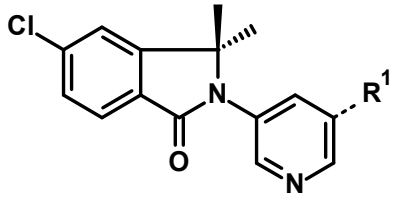
To further guide our inhibitor design efforts, we determined the X-ray structure of human CYP11B2 in complex with compound **45** (**Figure 3**). Despite the limited resolution of 3.0 Å, the structure clearly confirmed our proposed binding mode with the central pyridine nitrogen in close contact with the heme iron atom (d = 2.2 Å) and the isoindolin-1-one core positioned at the left-hand side of the binding pocket. Pyridine and isoindolin-1-one ring systems are rotated against each other by ~60° and the gem-dimethyl group is engaged in a number of van der Waals interactions with Phe 130

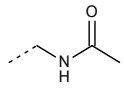
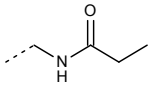
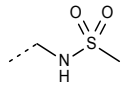
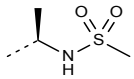
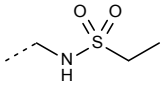
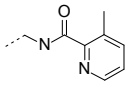
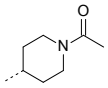
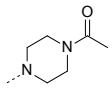
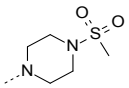
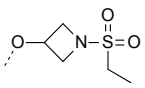
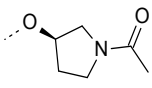
and Trp 116, respectively. Several additional protein interactions are made around the isoindolin-1-one moiety. In contrast, only few short protein contacts are visible around the ethylmethanesulfonamide substituent, which fills the right-hand side of the binding pocket only partly. This is in line with the generally reduced ligand efficiency when substituting at the 5-pyridyl position.

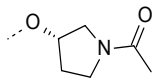
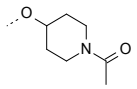
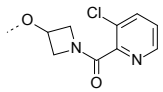
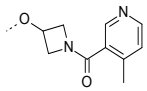
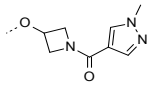
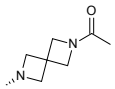
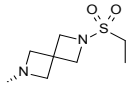
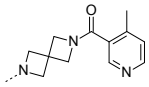
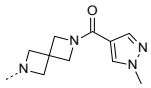
Several factors likely contribute to the enhanced potency of the substitutions displayed in **Table 5**. First, electron-donating substituents might increase the interaction strength of the central pyridine nitrogen atom with the heme iron. Second, additional favorable protein interactions can be formed in some cases, as in the matched pair of compound **44** (IC_{50} = 33 nM) with the methylated analogue **45** (IC_{50} = 8 nM). In this example, the extra methyl group in **45** engages in a short dispersion interaction with the side chain of Ile 488 (**Figure 3**), which likely contributes to its 4-fold potency improvement. On the other hand, suboptimal interactions for some substitutions, as exemplified by the amide and sulfonamide pairs **42** vs. **43** and **44** vs. **46**, respectively, can lead to reduced CYP11B2 binding. In **Figure 3**, the terminal methyl group of compound **46** would point to a rather polar region of the binding site, and the resulting desolvation penalty might explain the loss of binding affinity.

Unfortunately, none of the methylene amine-linked analogs showed a selectivity against CYP11B1 greater than 100-fold. Therefore, linkers with different shape and length including piperidine (**48-49**), piperazine (**50-51**), 2,6-diazaspiro[3.3]heptane (**59-62**), oxygen-linked azetidine (**52**, and **56-58**), pyrrolidine (**53-54**), and piperidine (**55**) were prepared. Notably, now we found analogs for each of these types of linkers (**48-62**) which had superior CYP11B2 activities (< 10 nM), revealing that quite diverse substituents are well tolerated in this region of the binding site. It suggests that this part of the binding site is quite flexible, to be able to accommodate such a variety of substitutions. One would expect an entropic penalty for binding to such a region, which is supported by the generally reduced ligand efficiency for 5-pyridyl substituents. For some substitutions at the terminal nitrogen, we finally achieved very good CYP11B1 selectivities (> 100-fold). In particular, analogs containing the unique heteroaryl amide substitution patterns in **57, 58,** and **62** gave superior CYP11B1 selectivity even over 500-fold while maintaining excellent CYP11B2 potency.

Table 5. Potency and selectivity of 5-chloro-3,3-dimethyl-2(3-pyridyl)isoindolin-1-one derivatives, **42-62**.



Cmpd	Structure R[1]	IC_{50} (μM)[a] *h*CYP11B2	***h*SF**[b]	IC_{50} (μM)[a] *cy*CYP11B2	***cy*SF**[c]
42		0.059	47	0.014	333
43		0.282	28	na	na
44		0.033	49	0.01	179
45		0.008	71	0.004	93
46		0.092	31	na	na
47		0.009	89	0.009	191
48		0.005	77	0.002	466
49		0.003	106	0.0004	430
50		0.009	80	0.003	236
51		0.002	146	0.0002	170
52		0.004	162	0.002	664
53		0.011	113	0.007	643

54		0.007	81	0.005	193
55		0.004	61	0.002	225
56		0.005	174	0.0005	1120
57		0.004	553	0.0009	1772
58		0.0005	4164	na	na
59		0.023	640	na	na
60		0.016	855	0.001	19699
61		0.014	1870	na	na
62		0.002	5683	na	na

[a]IC_{50}: the concentration of compound that inhibited 50% enzyme activity; [b]*h*SF: the selective factor defined as the ratio between the IC_{50} values of human CYP11B1 and CYP11B2; [c]*cy*SF: the selective factor defined as the ratio between the IC_{50} values of cynomolgus monkey CYP11B1 and CYP11B2.

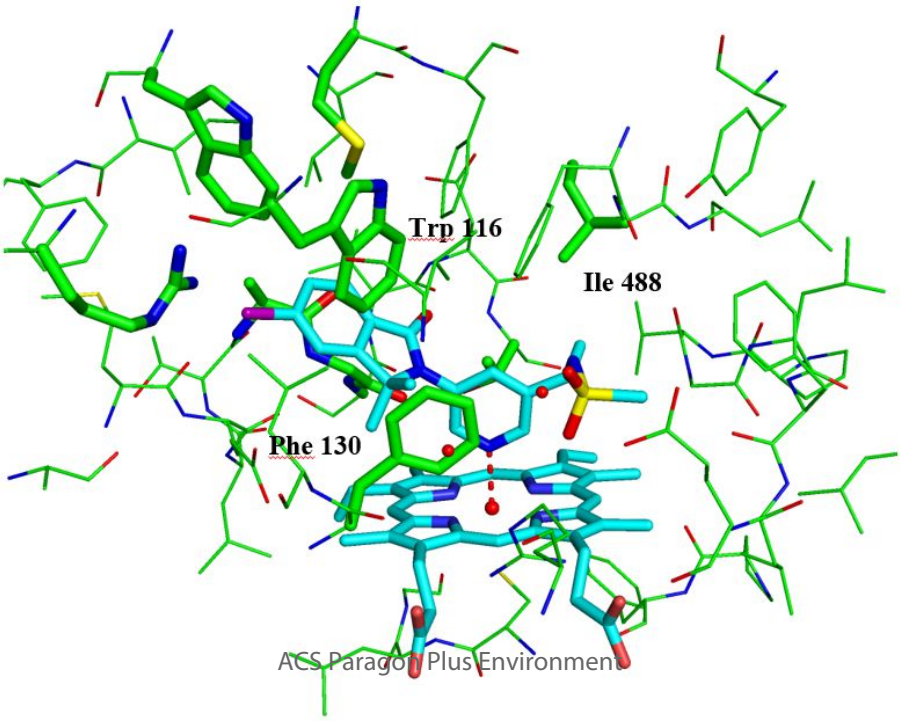


Figure 3. X-ray structure of human CYP11B2 in complex with compound **45** (PDB code: 6XZ8). The protein is displayed in green, and the heme cofactor and compound **45** are shown as cyan sticks. Protein residues with short contacts to the ligand (d < 4.0 Å) are highlighted as green sticks.

We were able to determine the X-ray co-crystal structure of the highly selective compound **58**, revealing particular protein interactions of the terminal heterocycle (**Figure 4**). Apart from a hydrogen bond of the pyrazole nitrogen atom with the sidechain of Tyr 485, we observe significant protein induced fit around Phe 381 and Phe 406. Interestingly, there is one amino acid difference between CYP11B2 and B1 in a 5 Å environment around the 5-pyridyl substituent, which is at position 404 (B2: Gln vs. B1: Arg). We hypothesize that the increased selectivity for **58** and related compounds might be a consequence of a) different free energy costs for CYP11B2 and B1 to accommodate the terminal heterocycle in the induced fit pocket, and b) potentially different positions of the side chain of Tyr 485 leading to different hydrogen bond interactions. Co-crystal structures of these compounds with CYP11B1 would be highly valuable to shed further light on the reasons for selectivity.

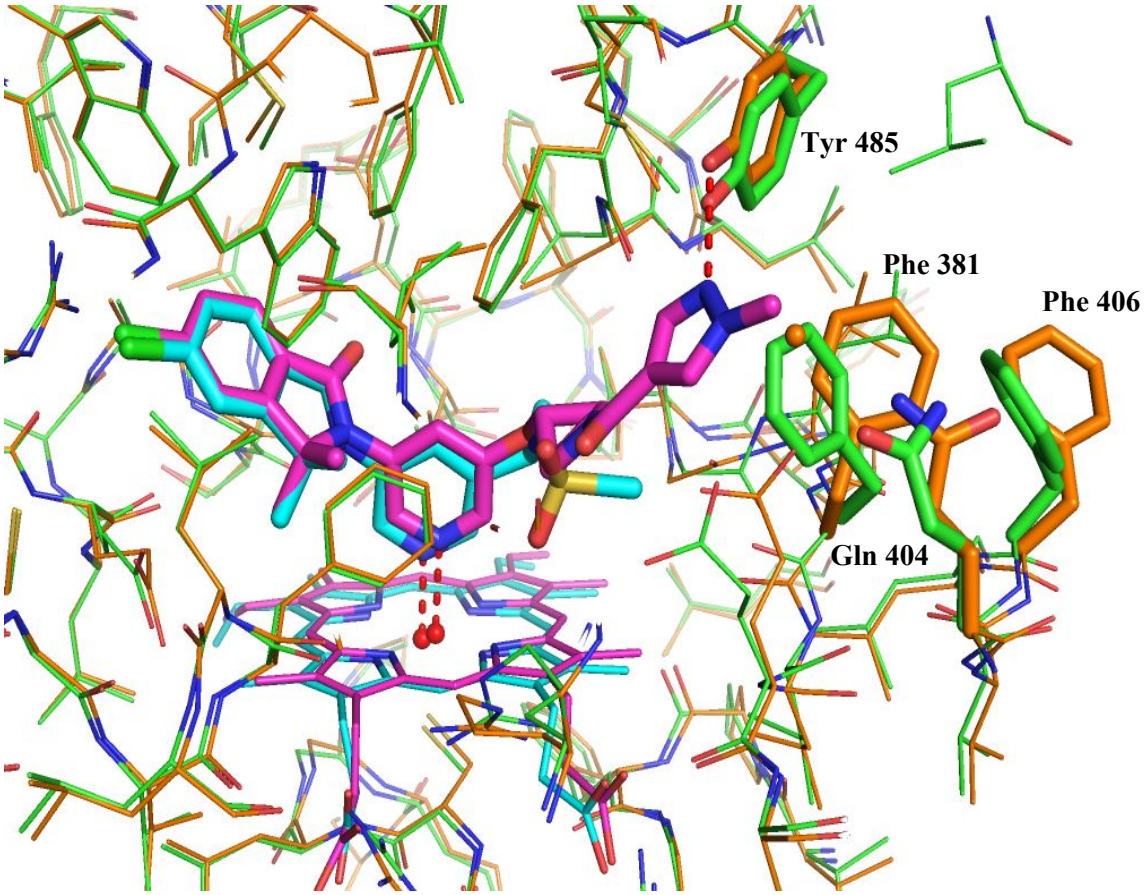


Figure 4. Overlay of X-ray structures of human CYP11B2 with compounds **45** (PDB code: 6XZ8; protein: green, heme and ligand: cyan) and **58** (PDB code: 6XZ9; protein: orange, heme and ligand: magenta) illustrating protein induced fit around the pyrazole tail. The heme iron…pyridine nitrogen contact and the hydrogen bond between the ligand pyrazole and Tyr 485 are shown as dashed lines. At position 404, the Gln residue CYP11B2 is mutated to Arg in CYP11B1.

One of the challenges to identify selective ASIs and to validate them in *in vivo* studies is the fact that the primary structures between human and rodent CYP11B2 and CYP11B1 enzymes is relatively poorly conserved (68% for human and rodent CYP11B2, and 63% for human and rodent CYP11B1). Moreover, rodents produce corticosterone as their major glucocorticoid rather than cortisol observed in man and monkeys.[10] As such, pharmacodynamic characterization of a selective ASI in rodent animal model can not be easily translated into a human situation. Therefore, in recent years, due to higher species homology between human and monkey (93% for both CYP11B2 and CYP11B1), functional animal models in non-human primates were developed to support the discovery and

development of selective ASIs.[24-28] To validate candidate molecule in cynomolgus monkey, good *in vitro* potency and selectivity towards the cynomolgus monkey derived enzymes was assessed. **Table 5** summarizes the potency and selectivity results of compounds **42-62** using cell line G401 expressing the corresponding monkey enzymes. In general, this series of analogs showed more potent cyCYP11B2 activities (1~16-fold of increase) and a higher cyCYP11B1 selectivity. For analogs that already achieved > 100-fold of CYP11B1/CYP11B2 selectivity in human enzymes, their selectivity values for the monkey enzymes were even higher (**49**, **51**, **52**, **56**, and **57**).

In Vivo Rat and Cynomolgus Monkey Pharmacokinetics Studies

Compounds with sub-nanomolar human CYP11B2 potency (< 10 nM) and > 100-fold human CYP11B1 selectivity were evaluated in rat SDPK study first (**Table 6**). A good *in vitro* and *in vivo* clearance correlation was observed for this series of compounds, as the *in vivo* clearance could be well predicted from rat liver microsomal clearance. Meanwhile, due to acceptable solubility and permeability, these analogs also showed good to excellent oral bioavailability. Among three sulfonamides (**49**, **51**, and **52**), the oxygen-linked azetidine analog **52** showed a better metabolic stability than those of piperidine (**49**) and piperazine (**51**) analogs. Therefore, it achieved a higher oral exposure with a bioavailability of 45%. A similar or better PK profile was also maintained for the two heteroaryl amides that were derived from the same linker (**56** and **58**). A spiro analog **62**, which was designed to address the metabolic liability of the piperazine linker, also gave good oral exposure due to the decreased metabolism of 2,6-diazaspiro[3.3]heptane ring (e.g., rat LM for compounds **50**, **51**, **59**, and **60** are 17.7 and 16.8 vs. 3.71, and 0.87 mL/min/kg, respectively).

Compounds with a good oral exposure in rats were subsequently evaluated in cynomolgus monkey SDPK study. As shown in **Table 7**, although low *in vivo* clearances were maintained, lower oral bioavailabilities were observed for both compounds **52** and **62**. Portal vein cannulated cynomolgus monkey PK study later suggested that for these analogs high intestinal CYP3A4 metabolism could be the cause for the lower bioavailability observed in cynomolgus monkey PK studies.

Table 6. Physico-chemical property and single-dose pharmacokinetic evaluation of representative compounds in rat.

Cmpd	LYSA[a] (μg/mL)	PAMPA[b] (10^{-6} cm/s)	RLM[c] (mL/min/kg)	Cl_{iv}[d] (mL/min/kg)	$AUC_{(0-\infty)}$[e] po (hr*ng/mL)	F[f] (%)
49	39	4.01	35.1	29.7	1465	87
51	91	6.03	16.8	16.6	1640	54
52	78	4.17	5.0	3.8	6370	45
56	20	3.32	0	3.1	10818	58
57	290	5.4	10.4	15.8	1020	40
58	310	3.45	6.4	3.8	7880	60
62	44	3.63	6.4	6.4	5660	69

[a]LYSA: Lyophilisation solubility assay; [b]PAMPA: parallel artificial membrane permeability assay; [c]RLM: scaled intrinsic clearance of compound in rat liver microsomes; [d]Cl_{iv}: plasma clearance; [e]AUC: area under the curve; [f]F: oral bioavailability; intravenous dosing (iv) at 1 mg/kg, oral dosing (po) at 3 mg/kg.

Table 7. Single-dose pharmacokinetic parameters comparison between rat and cynomolgus monkey for compounds **52** and **62.**

Cmpd	Species	fu[a] (%)	Hepa Cl[b] (mL/min/kg)	Cl_{iv}[c] (mL/min/kg)	AUC_Dose[d] (hr*kg*ng/mL/mg)	F[e] (%)
52	Rat	5.2	26.7	3.8	2123	45
	Cynomolgus monkey	13.8	7.2	4.6	1085	19
62	Rat	6.6	< 4.4	6.4	1889	69
	Cynomolgus monkey	3.6	< 3.0	2.1	3111	37

[a]fu: percentage of compound free fraction in a plasma protein binding assay; [b]Hepa Cl: scaled intrinsic clearance of compound in liver hepatocytes; [c]Cl_{iv}: plasma clearance; [d]AUC_Dose: dose normalized area under the curve; [e]F: oral bioavailability.

In Vivo Evaluation of ASI in Cynomolgus Monkey ACTH Model

In clinical studies, adrenocorticotropic hormone (ACTH, Synacthen®) stimulation test is used as a diagnostic tool to assess adrenocortical function. Plasma aldosterone and cortisol both increase in response to an ACTH stimulation. This test can be used to measure quantitatively the aldosterone (potency) / cortisol (selectivity) production change as a consequence of CYP11B2/B1 inhibition.[29] As previously described, we chose to adapt this test to cynomolgus monkeys and developed an acute Synacthen® challenge animal model for PKPD profiling of ASI in-house.[24] To measure steroid levels reliably in monkey requires a lot of skill and the right cooperative animals together with well trained animal keepers. Indeed, in the cohort that we were using only selected animals that displayed robust ACTH induced steroid responses; and that were cooperative and voluntarily donated blood samples were selected. For animal welfare reasons we had to limit the number of primates used in this study to an n=2 and the number of compounds tested to an absolute minimum.

Using compound **52** as an example, both plasma exposure and several steroids profile changes in cynomologus monkeys (n =2) were recorded over a period of 24 hours following the oral doses of isoindolin-one compound **52** at 0.01, 0.1, 1, and 10 mg/kg (t = 0 min) and an ACTH challenge (t = 1 hr, time point close to the Cmax of **52**). Highly sensitive LC/MS-MS analysis was applied to guarantee each hormone level's accurate measurement. 60 min post ACTH stimulation was selected in agreement with the human protocol for the Synacthen test and our experience of achieving stable and maximal steroid induction. **Figure 5** shows the levels of 5 steroids 1 hour after the ACTH challenge. Compound **52** reduced the stimulated aldosterone level below basal levels with 3 out of the 4 tested doses (0.1, 1, and 10 mg/kg). The corresponding CYP11B2-dependent steroid precursors, corticosterone and 11-deoxycorticosterone remained comparable to vehicle treated animals displaying a natural variability. Concomitantly, selectivity against CYP11B1 was investigated independently by assessing both the precursor level (11-deoxycortisol) and the product level (cortisol) of the CYP11B1 enzyme. There were no or minimum cortisol changes at doses 0.01, 0.1, and 1 mg/kg. At the top dose of 10 mg/kg, cortisol levels were observed below average pre-treatment levels (vehicle t = 0 min); importantly the precursor 11-deoxycortisol changed minimally or not at all. This in marked contrast to LCI699 that led to a dramatic increase in the precursors of deoxycorticosterone and deoxycortiosl. This phenomenon has

been reported in clinical studies as well when another type of ASI was tested at high, non-selective doses.[17] Selectivity in enzyme inhibition at the 10 mg/kg dose is also suggested based on simple PK/PD modeling (**Figure 6**). Four different doses were applied to cynomolgus monkey. For each dose, the average free exposure was calculated from $AUC_{0\text{-}7hr}$ and corrected for plasma protein binding. There is a good linear correlation between applied doses and average free drug concentrations. When comparing exposure data with measured cellular IC_{50} values, we predict that the compound **52** is efficacious and selective at doses between ~0.1-72 mg/kg, this modeling data is in agreement with data shown in **Figure 5**. For a definitive answer, doses up to 100 mg/kg would have to be tested, which are expected to result in free exposures clearly above the IC_{50} of CYP11B1.

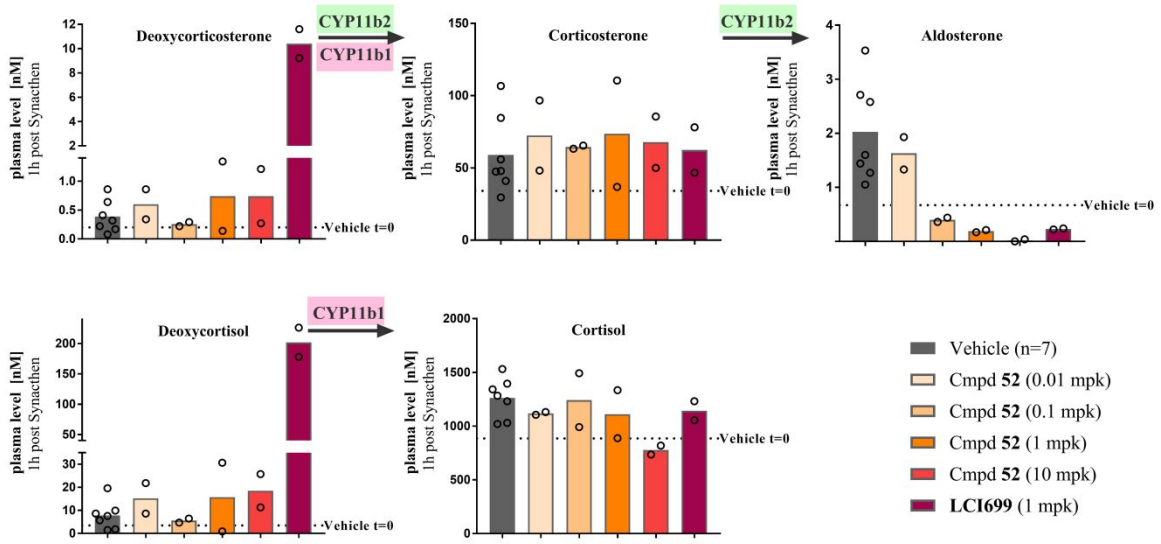


Figure 5. Steroid levels in cynomologus monkey 60 min post ACTH challenge and treatment with compound **52** are compared to the changes of steroid levels induced by LCI699, a less selective ASI. Note the 3 highest doses of compound **52** lowered the aldosterone clearly below basal levels (vehicle t = 0 min). **LCI699** treatment led to a dramatic increase in deoxycorticosterone and deoxycortisol levels. Enzymatic step mediated by CYP11B1 or CYP11B2 are indicated.

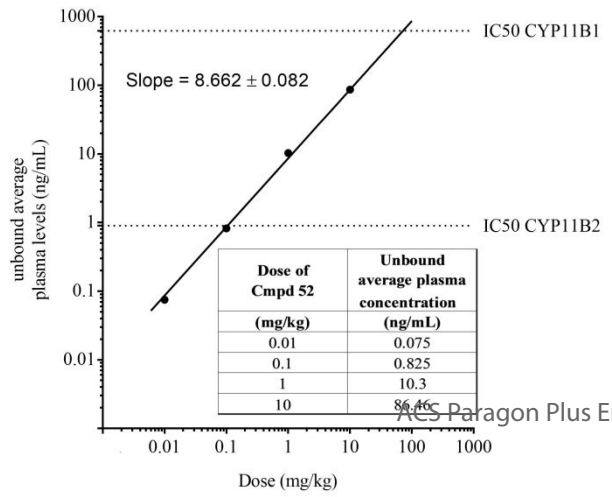


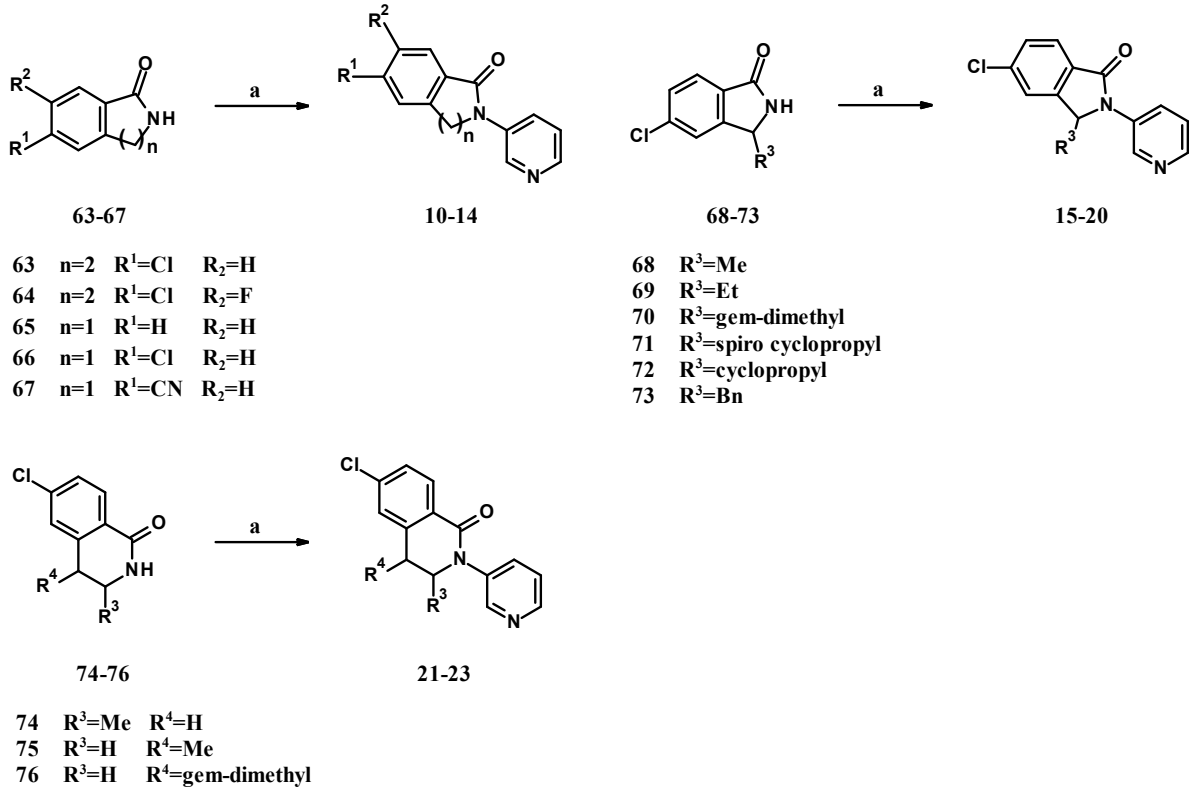
Figure 6. PK/PD prediction in cynomolgus monkeys. Different doses of compound **52** were applied and the PK profile was determined (see also SI, **Table S8**). For each dose, the average free exposure was calculated from $AUC_{0\text{-}7hr}$ and corrected for plasma protein binding. Overall, a good linear correlation between applied doses and achieved free plasma exposures was observed. Horizontal crosslines mark the IC_{50} values for CYP11B2 and CYP11B1. This predicts that the doses of 0.1, 1 and 10 mg/kg display significant and selective inhibition of aldosterone.

Additionally, selectivity against CYP11B1 is not the only concern. For example, an early ASI FAD286 was derived from a known aromatase (CYP19A1) inhibitor Fadrozole. To exclude such un-selectivity, we studied steroid production in the human adrenocarcinoma cell line H295R. A similar assay was recently published as part of the ToxCast program that includes measurement of steroid hormones, including progestagens, corticosteroids, androgens, and estrogens.[30] Specifically we induced steroidogenesis with 16 mM potassium chloride for 48 hours in the presence of various amounts of inhibitors (0.001-20 μM) and analyzed 9 different steroids (deoxycorticosterone, corticosterone, 18-hyroxycorticosterone, aldosterone, deoxycortisol, cortisol, 17-hydroxyprogesterone, testosterone and estrone). Using non-linear 4 parameter fitting (GraphPad Prism 7.04), the concentration that led to a 50% reduction of steroid levels was calculated. In this analysis, aldosterone and 18-hydroxycorticosterone were the only two steroids inhibited by compound **52** at less than 1 nM drug concentrations (SI, **Table S8**).

CHEMISTRY

The syntheses of compounds **10-23** are depicted in **Scheme 1**. These analogs were generally prepared through copper(I)-catalyzed Ullman coupling reactions of substituted 3,4-dihydroisoquinolin-1-ones or isoindolin-ones intermediates with 3-bromo or 3-iodopyridine.[31] For commercially not available intermediates, their syntheses are described in **Schemes 2-5**. For example, the 3,4-dihydroisoquinolin-1-one **63** was obtained via PPA catalyzed ring cyclization of methyl phenethylcarbamate **63b** (**Scheme 2**). Similar syntheses were also applied for the preparation of 3,4-dihydroisoquinolin-1-ones **74-76**. In another route, the 3,4-dihydroisoquinolin-1-one **64** was prepared by first cyclization of *N*-trifluoroacylated phenethylamine **64e** in the presence of paraformaldehyde and acetic-sulfuric acid to provide tetrahydroisoquinoline **64f**, followed by protecting group removal and iodosylbenzene oxidation (**Scheme 2**). For the isoindolin-one intermediate **67**, it started from the ester formation of 4-bromo-2-methyl-benzoic acid to yield **67b**, followed by nucleophilic aromatic substitution with CuCN to give **67c**. Bromination of **67c** with NBS/BPO furnished **67d**, which further reacted with $PMBNH_2$ to give an in situ cyclized product, isoindolin-one **67e**. After removal of PMB protecting group by the treatment with CAN then afforded intermediate **67** (**Scheme 3**).

Scheme 1. General syntheses of aldosterone synthase inhibitors, **10-23.**



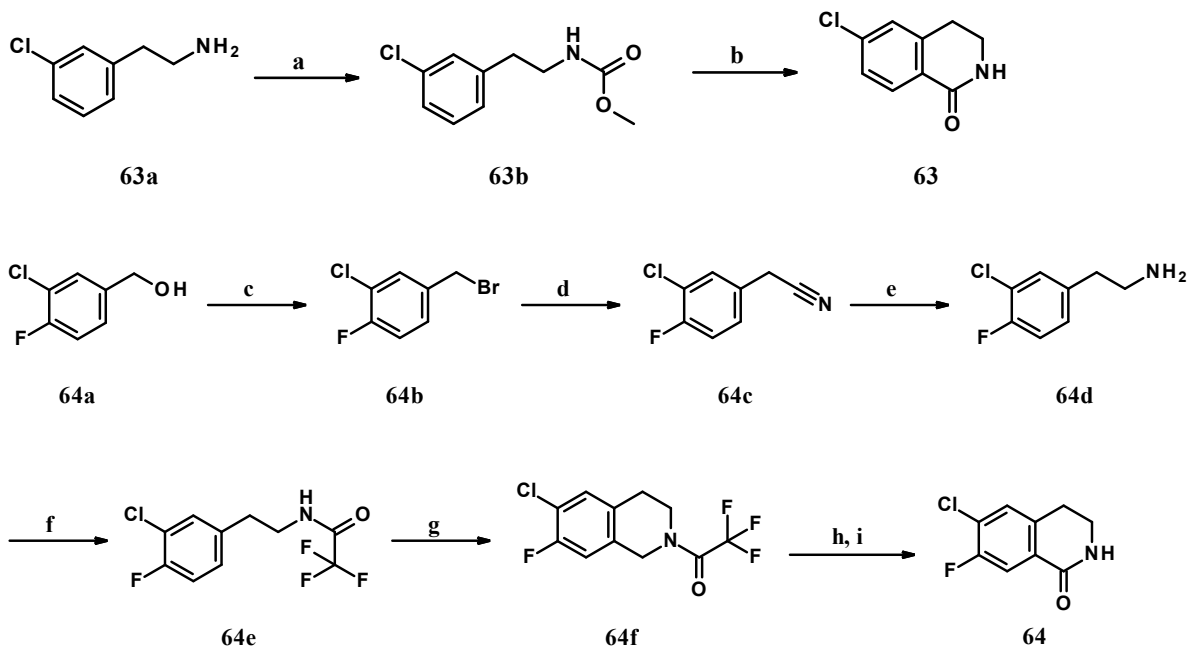
63 n=2 R^1=Cl R_2=H
64 n=2 R^1=Cl R_2=F
65 n=1 R^1=H R_2=H
66 n=1 R^1=Cl R_2=H
67 n=1 R^1=CN R_2=H

68 R^3=Me
69 R^3=Et
70 R^3=gem-dimethyl
71 R^3=spiro cyclopropyl
72 R^3=cyclopropyl
73 R^3=Bn

74 R^3=Me R^4=H
75 R^3=H R^4=Me
76 R^3=H R^4=gem-dimethyl

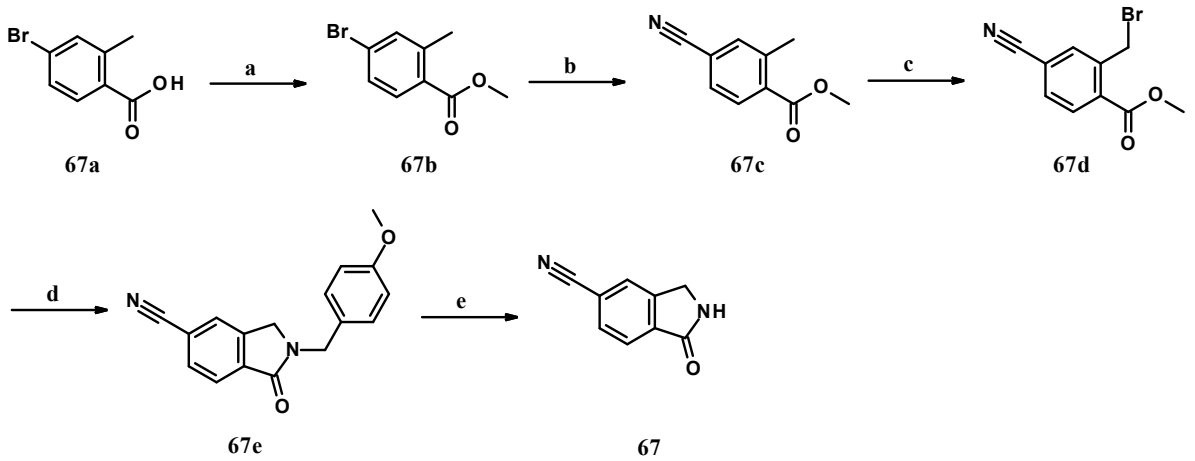
Reagents and conditions: a) 3-bromopyridine, CuI, K_2CO_3 (or Cs_2CO_3), *N,N'*-dimethylethylenediamine (or (+)-(*S,S*)-1,2-diaminocyclohexane), 1,4-dioxane, 110~150 °C.

Scheme 2. Syntheses of 3,4-dihydroisoquinolin-1-one intermediates, **63** and **64.**



Reagents and conditions: a) methyl chloroformate, Et_3N, DCM, rt; b) neat PPA, 120 ^oC; c) PBr_3, DCM, 0 ^oC; d) trimethylsilyl cyanide, TBAF, CH_3CN, reflux; e) BH_3-THF, reflux; f) trifluoroacetic anhydride, Et_3N, DCM, rt; g) paraformaldehyde, AcOH, H_2SO_4, rt; h) K_2CO_3, MeOH-H_2O, rt; i) KBr, iodosylbenzene, H_2O, rt.

Scheme 3. Synthesis of isoindolin-one intermediate, **67.**

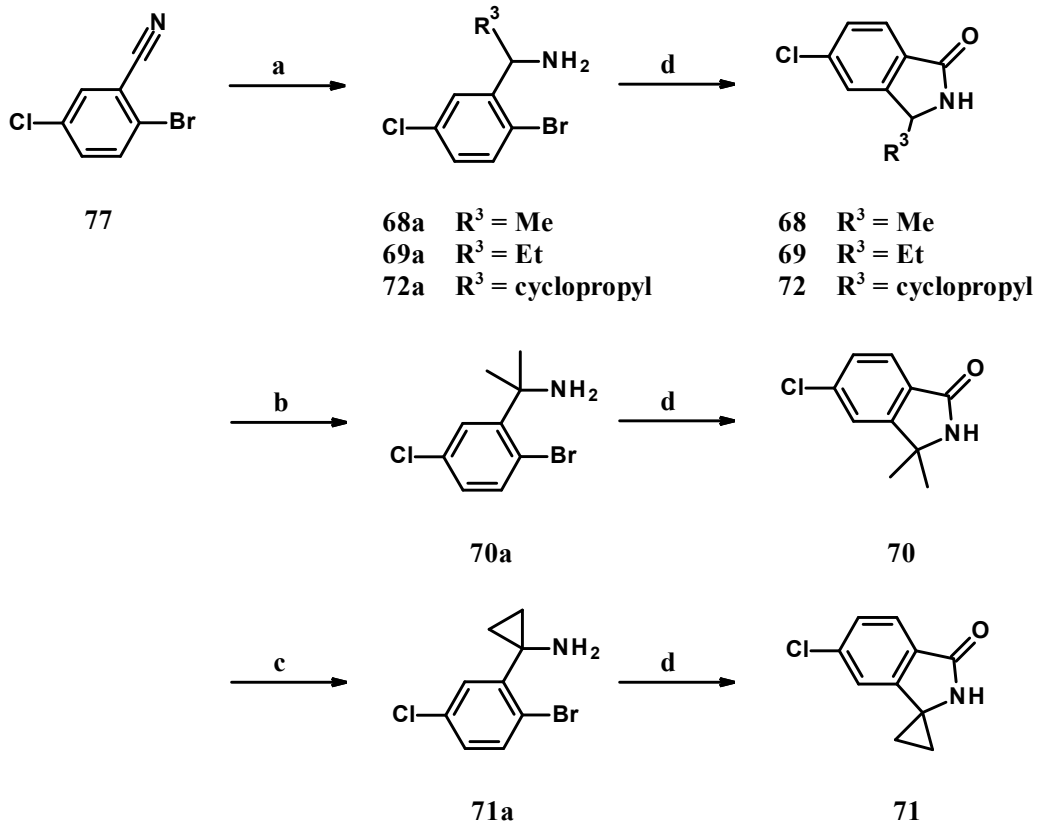


Reagents and conditions: a) $SOCl_2$, MeOH, 70 °C; b) CuCN, 1-methyl-pyrrolidin-2-one, 180 °C; c) NBS, BPO, CCl_4, reflux; d) $PMBNH_2$, THF, rt; e) CAN, CH_3CN, 0 °C.

For the several 3-substituted 5-chloro-isoindolin-1-one intermediates **68-72**, an efficient two-step synthesis was developed as outlined in **Scheme 4**. The first step involved the reaction of 2-bromo-5-chloro-benzonitrile with corresponding Grignard reagents under the conditions of a) sodium borohydride reduction; b) Ti(O*i*-Pr)$_4$-mediated addition; and c) Lewis acid $BF_3.OEt_2$ catalyzed addition to give benzylic substituted phenylmethanamine intermediates **68a-72a**, respectively. These intermediates then underwent palladium catalyzed carbonylation and in situ cyclization to give 3-substituted 5-chloro-isoindolin-1-ones **68-72**. Alternatively, a 3-substituted 5-chloro-isoindolin-one intermediate, such as **73** could also be synthesized via reacting 5-chloro-isoindole-1,3-diones with benzylmagnesium chloride followed by Et_3SiH reduction; although under this route, a mixture of two regioisomeric products were produced with only the desired one shown in **Scheme 5**. Additionally, when there is a chiral center involved, chiral column separation was applied to obtain each individual

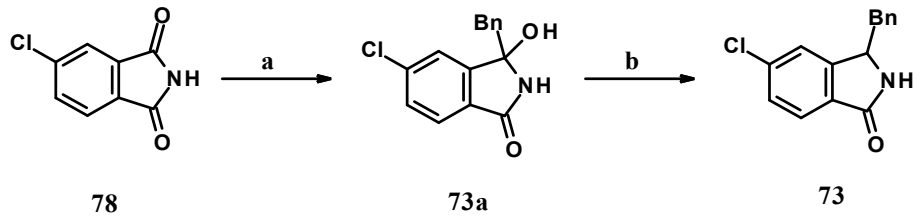
enantiomeric intermediate. Based on the optical rotation measurement, the relative configuration of an enantiomer was assigned as either *R* (negative sign) or *S* (positive sign) accordingly.

Scheme 4. Syntheses of isoindolin-one intermediates, **68-72**.



Reagents and conditions: a) MeMgBr (or EtMgBr, or cyclopropyl MgBr), THF, 0 °C then $NaBH_4$, rt; b) MeMgBr, THF, 0 °C then $Ti(Oi\text{-}Pr)_4$, rt; c) $Ti(Oi\text{-}Pr)_4$, EtMgBr, -78 °C to rt then $BF_3\text{-}Et_2O$; d) $Pd(dppf)Cl_2$, DIPEA, CO, 130 °C.

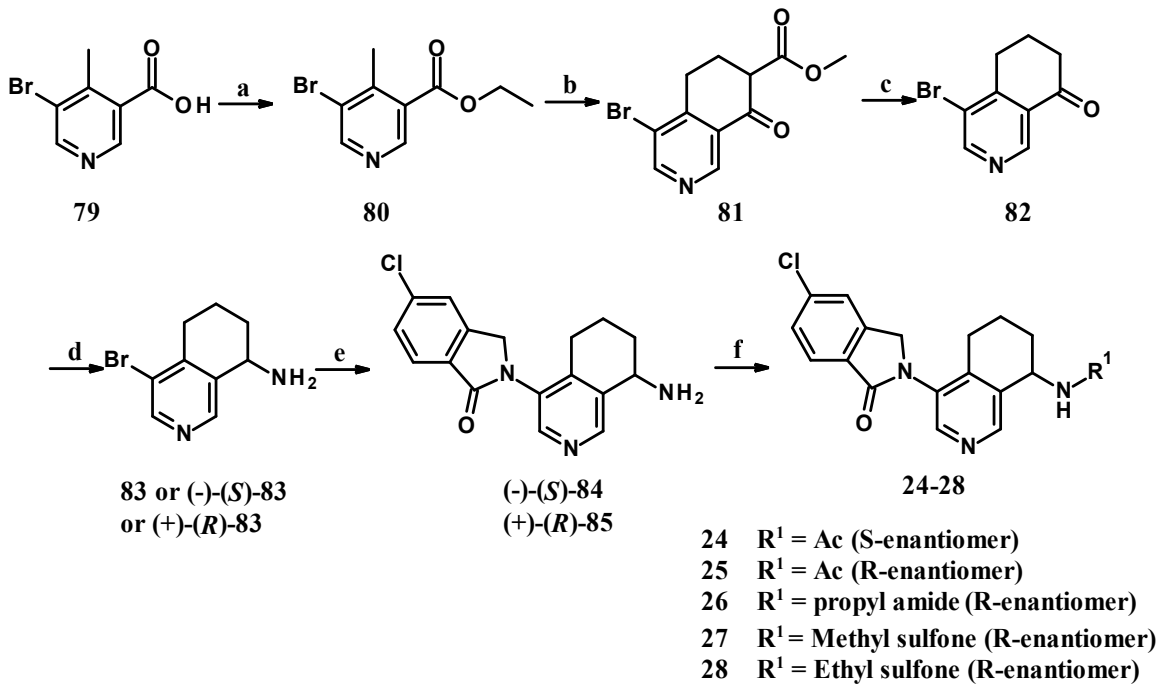
Scheme 5. Synthesis of isoindolin-one intermediate, **73**.



Reagents and conditions: a) benzylmagnesium chloride, DCM, 0 °C; b) Et_3SiH, TFA, rt.

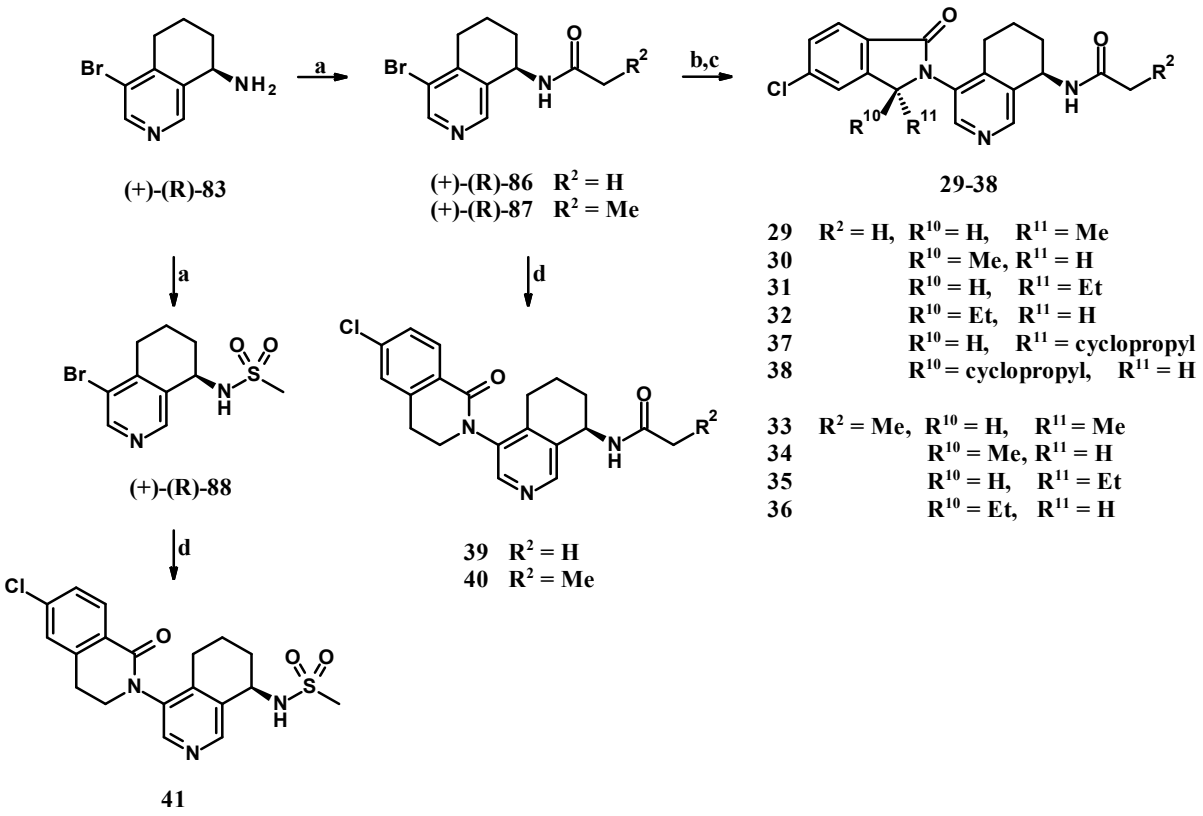
The syntheses of bicyclic tetrahydroisoquinoline series of isoindolin-one compounds **24-41** are depicted in **Schemes 6** and **7**. The key intermediate **83** was prepared as previously disclosed.[24] Esterification of commercially available 5-bromo-4-methyl nicotinic acid with ethanol under EDCI / DMAP activation provided ethyl ester **80**. Deprotonation of the methyl group with freshly prepared lithium diisopropylamide (LDA) followed by addition of methyl acrylate gave access to intermediate **80** via a two-step cascade reaction. The crude keto ester **81** was then engaged in a Krapcho decarboxylation using 6M aq. HCl to provide ketone **82**. A reductive amination of ketone **82** with ammonia in methanol in the presence of titanium(IV) isopropoxide and treatment with sodium borohydride furnished key building block **83**. After chiral column separation, the obtained individual enantiomer **(-)-(*S*)-83** and **(+)-(*R*)-83** were applied in the subsequent Ullman coupling reactions with 5-chloroisoindolin-1-one to give intermediates **(-)-(*S*)-84** and **(+)-(*R*)-85**, which were further reacted with either acid chlorides or sulfonyl chlorides to give compounds **24-28** (**Scheme 6**). Similarly, compounds **29-41** were prepared by first reacting **(+)-(*R*)-83** with acetyl chloride, propanoyl propanoate, and methane sulfonyl chloride to give intermediates **(+)-(*R*)-86**, **(+)-(*R*)-87**, and **(+)-(R)-88**, respectively. Subsequent Ullman coupling reaction with appropriate 3-substituted isoindolin-ones or 3,4-dihydroisoquinolin-1-one then afforded compounds **29-41** (**Scheme 7**).

Scheme 6. Syntheses of bicyclic tetrahydroisoquinoline series of isoindolin-one aldosterone synthase inhibitors, **24-28**.



Reagents and conditions: a) EtOH, EDCI, DMAP, DCM, rt; b) LDA, methyl acrylate, THF then 10% AcOH; c) 6M HCl, reflux; d) $Ti(Oi\text{-}Pr)_4$, NH_3-MeOH, 0 °C then $NaBH_4$; e) CuI, (*1S,2S*)-cyclohexane-1,2-diamine, Cs_2CO_3, **(-)-(*S*)-83** (or **(+)-(*R*)-83**), 1,4-dioxane, microwave 150 °C; f) acetyl chloride (or propanoyl propanoate, methanesulfonyl chloride, ethanesulfonyl chloride), Et_3N, DCM, 0 °C.

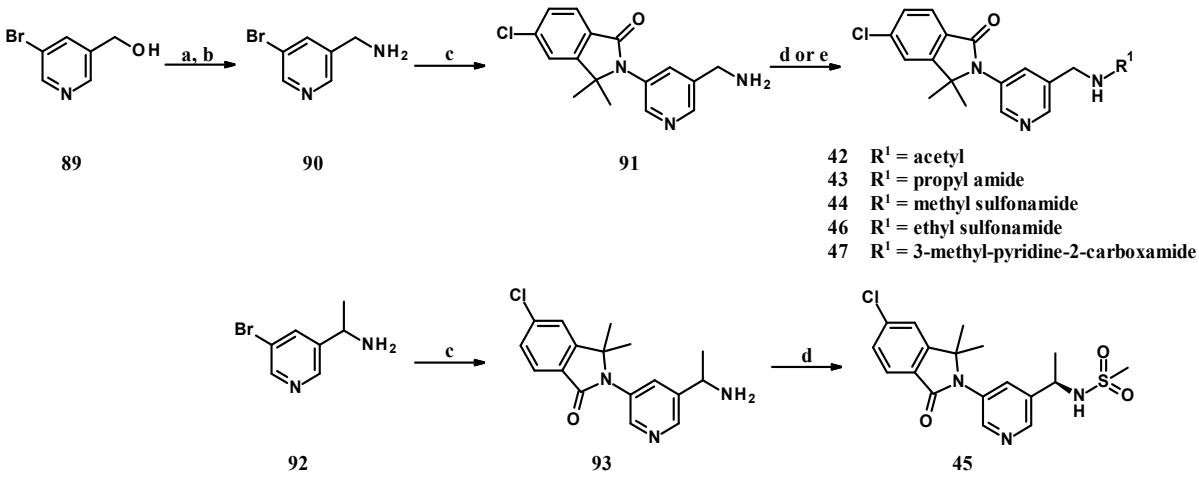
Scheme 7. Syntheses of bicyclic tetrahydroisoquinoline series of isoindolin-one aldosterone synthase inhibitors, **29-41**.



Reagents and conditions: a) acetyl chloride (or propanoyl propanoate, methanesulfonyl chloride), Et_3N, DCM, 0 ^oC; b) **68 (or 69, 72)**, CuI, (*1S,2S*)-cyclohexane-1,2-diamine, Cs_2CO_3, 1,4-dioxane, microwave 150 ^oC; c) chiral column separation; d) **63**, CuI, (*1S,2S*)-cyclohexane-1,2-diamine, Cs_2CO_3, 1,4-dioxane, microwave 150 ^oC.

The syntheses of 5-chloro-3,3-dimethyl-isoindolin-1-one derivatized compounds **42-47** are described in **Schemes 8**. Starting from (5-bromo-3-pyridyl)methanol, treatment with thionyl chloride followed by quenching with ammonia in methanol, afforded 3-pyridylmethanamine **90**. Subsequent Ullman coupling reaction with 5-chloro-3,3-dimethyl-isoindolin-1-one then provided compound **91**, which further reacted with various acid chlorides and sulfonyl chlorides to give compounds **42-47**, respectively. Compound **45** was obtained in a similar manner when 1-(5-bromo-3-pyridyl)ethanamine **92** was used as the corresponding starting material.

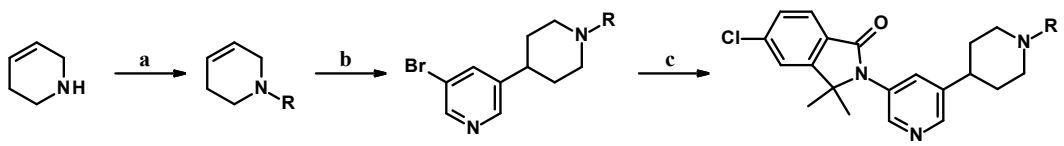
Scheme 8. Syntheses of 5-chloro-3,3-dimethyl-isoindolin-1-one derived aldosterone synthase inhibitors, **42-47**.



Reagents and conditions: a) $SOCl_2$, DCM, rt; b) NH_3-MeOH, 60 ^oC; c) intermediate **62**, CuI, Cs_2CO_3, (+)-(*S,S*)-1,2-diaminocyclohexane, 1,4-dioxane, 150 ^oC; d) acetyl chloride (or propanoyl propanoate**,** methanesulfonyl chloride, ethanesulfonyl chloride**)**, Et_3N, DCM, 0 ^oC; e) 3-methylpyridine-2-carboxylic acid, HATU, DIPEA, DCM, rt.

Scheme 9 outlines the syntheses of compounds **48** and **49**, which contain a piperdinyl linker. Commercial available 1,2,3,6-tetrahydropyridine was first protected with acetyl chloride or ethyl sulfonyl chloride. Arylation with 3-bromo-5-iodopyridine under the reductive Heck coupling condition gave compounds **97** and **98**. The subsequent Ullman coupling reaction with 5-chloro-3,3-dimethyl-isoindolin-1-one then afforded compounds **48** and **49**, respectively. Compounds **50** and **51** that utilized piperazine as the linker were prepared as depicted in **Scheme 10**. The Buchwald coupling reaction between 3,5-dibromopyridine and piperazine-1-carboxylic acid *tert*-butyl ester gave compound **100**, which was further reacted with 5-chloro-3,3-dimethyl-isoindolin-1-one under the Ullman coupling condition to afford compound **101**. After removal of the *N*-Boc-protecting group, the resulting intermediate was reacted with acetyl chloride and methyl sulfonyl chloride to give compounds **50** and **51**, respectively.

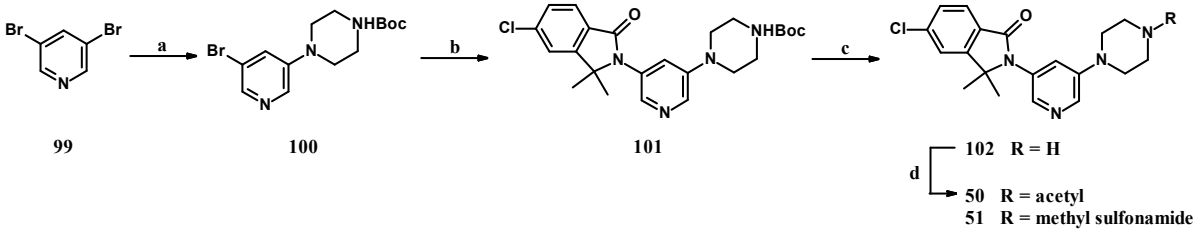
Scheme 9. Syntheses of 5-chloro-3,3-dimethyl-isoindolin-1-one derived aldosterone synthase inhibitors, **48-49**.



94 **95** R = acetyl; **96** R = ethyl sulfonamide **97** R = acetyl; **98** R = ethyl sulfonamide **48** R = acetyl; **49** R = ethyl sulfonamide

Reagents and conditions: a) Et_3N, ethanesulfonyl chloride (or acetyl chloride), DCM, 0 °C; b) 3-bromo-5-iodo-pyrdine, palladium(II)bis(triphenylphosphine) dichloride, Et_3N, formic acid, DMF, 90 °C; c) intermediate 62, CuI, Cs_2CO_3, *trans*-cyclohexane-1,2-diamine, 1,4-dioxane, 150 °C.

Scheme 10. Syntheses of 5-chloro-3,3-dimethyl-isoindolin-1-one derived aldosterone synthase inhibitors, **50-51**.

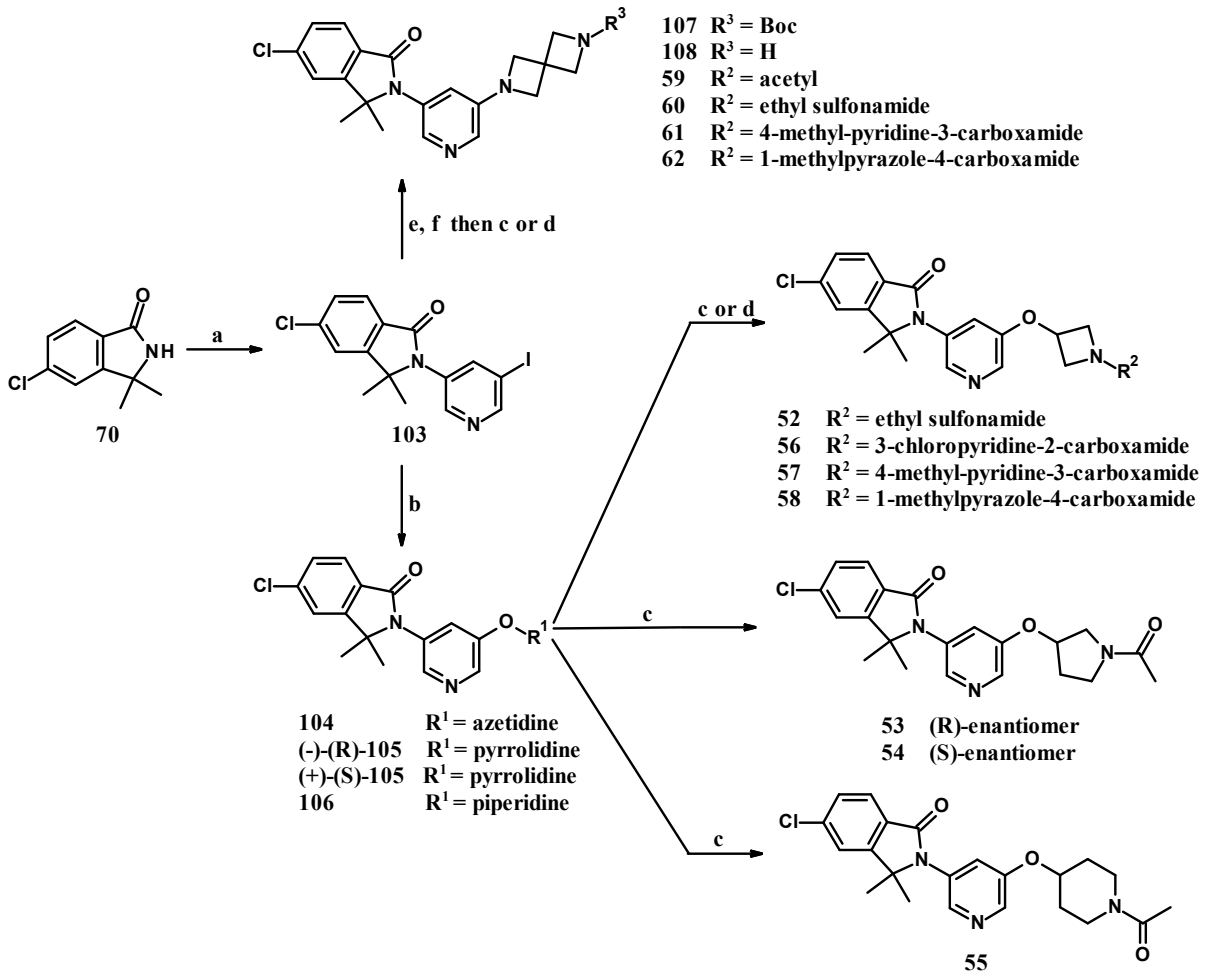


99 **100** **101** **102** R = H; **50** R = acetyl; **51** R = methyl sulfonamide

Reagents and conditions: a) piperazine-1-carboxylic acid *tert*-butyl ester, palladium(II) acetate, XPhos, sodium *tert*-butoxide, 1,4-dioxane, microwave, 130 °C; c) TFA, DCM, rt; d) acetyl chloride (or methanesulfonyl chloride), Et_3N, DCM, 0 °C.

Finally, for the syntheses of compounds **52-58** (**Scheme 11**), the key intermediate **103**, was firstly prepared by reacting 5-chloro-3,3-dimethyl-isoindolin-1-one with 3,5-diiodo-pyridine under the Ullman coupling condition. The oxygen-linked compounds **52-58** were prepared via CuI-mediated nucleophilic aromatic displacement of iodine with various alcohols. The resulting amino intermediates were then capped with various acylating reagents to give compounds **52-58**. Compounds **59-62** that contained a 2,6-diazaspiro[3.3]heptane as the linker were prepared in a similar manner when corresponding starting materials were applied.

Scheme 11. Syntheses of 5-chloro-3,3-dimethyl-isoindolin-1-one derived aldosterone synthase inhibitors, **52-62**.



Reagents and conditions: a) 3,5-diiodo-pyridine, CuI, (*1S,2S*)-cyclohexane-1,2-diamine, K_3PO_4, 1,4-dioxane, 110 ºC; b) CuI, 1,10-phenanthroline, Cs_2CO_3, *tert*-butyl 3-hydroxyazetidine-1-carboxylate (or (*R*)-3-hydroxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester, (*S*)-3-hydroxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester, *tert*-butyl 4-hydroxypiperidine-1-carboxylate), toluene, 110 ºC; then TFA, DCM, rt; c) Et_3N, DCM, ethanesulfonyl chloride (or acetyl chloride); d) 3-chloropyridine-2-carboxylic acid (or 4-methylpyridine-3-carboxylic acid, 1-methylpyrazole-4-carboxylic acid), HATU, DIPEA, DCM, 0 ºC; e) *tert*-butyl 2,6-diazaspiro[3.3]heptane-2-carboxylate oxalate, Pd_2dba_3, BINAP, ^tBuONa, Et_3N, toluene, 85 ºC; f) TFA, DCM, rt.

CONCLUSION

Achieving high selectivity against highly homologous CYP11B1 enzyme is necessary for the development of an effective and safe aldosterone synthase (CYP11B2) inhibitor, but challenging due to the high sequence conservation in the substrate binding site. Starting from a focused screening

benzamide pyrimidine hit, we identified a series of isoindoline-1-one derivatives as potent and selective CYP11B2 inhibitors. The right-hand side modification on the central pyridine core not only provided a handle for potency and selectivity improvements, but was also effective in modulating the physical chemical property profile for good PK oral exposure. Through the systematic exploration of a pocket identified from biostructure analysis, we found unique structural features for achieving high CYP11B1 selectivity. We speculate that this is due to differences in protein flexibility and hydrogen bonding geometry between both proteins. Representative selective compounds were evaluated in an acute ACTH challenged animal model and demonstrated both excellent *in vivo* efficacy of aldosterone lowering effect as well as superior selectivity impact of minimum cortisol change. We hope our findings will be a useful knowledge in designing the next generation of potent and highly selective aldosterone synthase inhibitor to enter into clinical testing.

EXPERIMENTAL SECTION

General Methods for Chemistry. Reactions involving air-sensitive reagents were carried out under an atmosphere of nitrogen or argon. The microwave assisted reactions were carried out in a Biotage Initiator Sixty. Solvents and reagents were obtained from commercial sources and were used without further purification unless otherwise noted. All reactions were monitored by thin-layer chromatography (TLC) on Merck silica gel 60 F254 TLC glass plates (visualized by UV fluorescence at λ = 254 nm) or analytical LC-MS. All intermediates and final compounds were purified by either silica gel flash chromatography or preparative HPLC (prep-HPLC) using one of the following instruments: (i) Biotage SP1 system and the Quad 12/25 cartridge module; (ii) ISCO Combi-flash chromatography instrument. Silica gel brand and pore size: (a) KP-SIL 60 Å, particle size 40–60 μm; (b) CAS registry no., silica gel, 63231-67-4, particle size 47–60 μm silica gel; (c) ZCX from Qingdao Haiyang Chemical Co., Ltd., pore 200–300 or 300–400; (iii) prep-HPLC on a reverse-phase column using a Waters XBridge OBD Phenyl (30 mm x 100 mm, 5 μm) or OBD RP18 (30 mm x 100 mm, 5 μm) column under acidic conditions (A, 0.1% formic acid in H_2O; B, 0.1% formic acid in acetonitrile) or basic conditions (A, 0.01% ammonia in H_2O; B, acetonitrile). For SFC chiral separation, either

intermediates or final compounds were separated using a chiral column (Daicel Chiralpak IC, 30 mm x 250 mm, 5 μm) on a Mettler Teledo SFC-Multigram system (solvent system of 95% CO_2 and 5% IPA (0.5% Et_3N in IPA), backpressure of 100 bar, UV detection at 254 nm). Optical rotation was measured using a Rudolph Autopol V automatic polarimeter at a wavelength of 589 nm. The purity of final compounds as measured by LC-MS was ≥95%. LC-MS spectra were obtained using UPLC coupled with single quadrupole mass detector (Waters Acquity UPLC-3100 Mass Detector, Waters Acquity UPLC-SQ Detector, Waters Acquity UPLC-SQ Detector 2). Standard LC-MS conditions were as follows. Columns: Waters Acquity BEH C18, 2.1 mm × 50 mm x 1.7 μm and Waters Acquity CSH C18 column, 2.1 mm × 50 mm x 1.7 μm. Flow rate: 0.8 mL/min. Gradient: 5-95% eluent B over 3 min under mobile phase conditions: 1) acidic condition: A, 0.1% formic acid in H_2O; B, 0.1% formic acid in acetonitrile; or 2) basic condition: A, 0.05% $NH_3 \cdot H_2O$ in H_2O; B, acetonitrile. Mass spectra (MS): Generally only ions which indicate the parent mass are reported, and unless otherwise stated the mass ion quoted is the positive mass ion $(M+H)^+$. NMR spectra were obtained using Bruker Avance 400 MHz spectrometer, operating at 400.13 MHz (^1H) and 100.62 MHz (^{13}C). High-resolution mass spectra were obtained on Xevo G2-XS-QTOF mass spectrameter equipped with an electrospray ionization source. All of the reported yields are for isolated products and not optimized.

Synthetic procedure for the synthesis of 6-chloro-2-(3-pyridyl)-3,4-dihydroisoquinolin-1-one (10). In a sealed tube, 3-bromopyridine (0.1 g, 0.633 mmol) was combined with 6-chloro-3,4-dihydro-2*H*-isoquinolin-1-one (intermediate **63**, 0.115 g, 633 mmol), copper (I) iodide (0.012 g, 0.063 mmol), potassium carbonate (0.175 g, 1.27 mmol), and *N,N'*-dimethylethylenediamine (0.013 g, 0.127 mmol) in 1,4-dioxane (2 mL). The reaction mixture was heated at 110 °C overnight. The mixture was cooled to room temperature, filtered through dicalite and washed with DCM. The residue was purified by silica gel flash chromatography eluting with a 5 to 100% EtOAc-heptane gradient to give the title compound (0.107 g, 65 % yield) as a white solid. MS obsd (ESI+) $[(M+H)^+]$ 259.1. ^1H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 8.64-8.70 (m, 1H), 8.47-8.54 (m, 1H), 8.09 (d, J=8.31 Hz, 1H), 7.75-7.82 (m, 1H), 7.33-7.41

(m, 2H), 7.27-7.29 (m, 1H), 4.00-4.08 (m, 2H), 3.11-3.22 (m, 2H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 163.5, 147.2, 146.1, 139.9, 139.3, 138.6, 132.7, 130.5, 127.8, 127.5, 127.1, 123.3, 49.0, 28.3.

For the following compounds, except those described specifically, all the analogs were prepared in analogy to compound **10** from either commercially available reagents or in-house prepared intermediates.

6-Chloro-7-fluoro-2-(3-pyridyl)-3,4-dihydroisoquinolin-1-one (11). LC-MS: tR 1.19 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 277.1. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.66-8.74 (m, 1H), 8.40-8.53 (m, 1H), 7.90-8.00 (m, 1H), 7.78-7.89 (m, 1H), 7.50-7.61 (m, 2H), 4.01-4.14 (m, 2H), 3.16-3.27 (m, 2H).

2-(3-Pyridyl)isoindolin-1-one (12). MS obsd (ESI+) [(M+H)$^+$] 211.1. HRMS calcd [(M+H)$^+$] 211.0871, measured [(M + H)$^+$] 211.0874. ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 8.89-9.09 (m, 1H), 8.52-8.58 (m, 1H), 8.40-8.51 (m, 1H), 7.91-7.96 (m, 1H), 7.60-7.68 (m, 1H), 7.50-7.59 (m, 2H), 7.33-7.45 (m, 1H), 4.91 (s, 2H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 167.9, 145.4, 140.0, 140.0, 132.6, 132.4, 128.7, 126.5, 124.3, 122.8, 50.0.

5-Chloro-2-(3-pyridyl)isoindolin-1-one (13). MS obsd (ESI+) [(M+H)$^+$] 245.1. ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 8.85-8.96 (m, 1H), 8.40-8.54 (m, 2H), 7.82-7.91 (m, 1H), 7.47-7.59 (m, 2H), 7.33-7.42 (m, 1H), 4.81-4.94 (m, 2H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 166.8, 145.7, 141.5, 140.0, 139.1, 131.0, 129.4, 126.6, 125.6, 123.8, 123.3, 49.6.

1-Oxo-2-(3-pyridyl)isoindoline-5-carbonitrile (14). MS obsd (ESI+) [(M+H)$^+$] 236.1. HRMS calcd [(M+H)$^+$] 236.0824, measured [(M + H)$^+$] 236.0816. ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 8.92-8.98 (m, 1H), 8.44-8.54 (m, 2H), 8.03-8.11 (m, 1H), 7.77-7.92 (m, 2H), 7.37-7.44 (m, 1H), 4.97-5.00 (m, 2H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 165.9, 146.3, 140.4, 140.3, 136.2, 135.5, 132.6, 126.9, 125.2, 123.9, 117.9, 116.1, 49.8.

5-Chloro-3-methyl-2-(3-pyridyl)isoindolin-1-one (15). LC-MS: tR 1.14 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 259.1. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.93 (d, J=2.53 Hz, 1H), 8.46

(dd,J=1.52, 4.80 Hz, 1H), 8.15 (ddd, J=1.39, 2.65, 8.34 Hz, 1H), 7.86 (d,J=8.08Hz,1H), 7.77 (s,1H),7.55-7.64 (m, 2H), 5.47-5.55 (m, 1H), 1.49 (d, J=6.57 Hz, 3H).

5-Chloro-3-ethyl-2-(3-pyridyl)isoindolin-1-one (16). LC-MS: tR 1.45 min, 100% (254 nm). MS obsd (ESI+) [$(M+H)^+$] 273.2. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.89 (d, J=2.02 Hz, 1H), 8.43 (dd, J=1.52,4.80 Hz, 1H), 8.13 (d, J=8.54 Hz, 1H), 7.84 (d, J=8.34Hz, 1H),7.72 (s,1H), 7.53-7.61(m,2H), 5.59 (dd, J=2.78, 5.05 Hz, 1H), 2.05-2.15 (m, 1H), 1.92-2.04 (m, 1H), 0.45 (t, J=7.33 Hz, 3H).

5-Chloro-3,3-dimethyl-2-(3-pyridyl)isoindolin-1-one (17). LC-MS: tR 1.36 min, 100% (254 nm). MS obsd (ESI+) [$(M+H)^+$] 273.1. HRMS calcd [$(M+H)^+$] 273.0795, measured [$(M + H)^+$] 273.0794. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.67 (dd, J=1.52, 4.80 Hz, 1H), 8.56 (dd, J= 0.76, 2.53 Hz, 1H), 7.81-7.91 (m, 3H), 7.59-7.67 (m, 2H), 1.59 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 166.9, 153.1, 150.4, 149.4, 138.9, 137.2, 132.5, 129.1, 128.4, 125.7, 124.1, 121.7, 64.4, 26.8.

5'-Chloro-2'-(3-pyridyl)spiro[cyclopropane-1,3'-isoindoline]-1'-one (18). LC-MS: tR 1.30 min, 100% (254 nm). MS obsd (ESI+) [$(M+H)^+$] 271.2. HRMS calcd [$(M+H)^+$] 271.0638, measured [$(M + H)^+$] 271.0638. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.68 (dd, J=1.52, 5.05Hz,1H), 8.53 (d, J=2.53 Hz, 1H), 7.88 (d, J=7.99 Hz, 1H), 7.85 (d, J=7.78 Hz, 1H), 7.54-7.69 (m, 2H), 7.47 (d, J=1.77 Hz, 1H), 1.52-1.67 (m, 2H), 1.34-1.48 (m, 2H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 167.3, 150.6, 149.9, 149.0, 138.8, 137.4, 130.8, 129.3, 128.3, 125.5, 124.2, 118.5, 46.5, 11.6.

5-Chloro-3-cyclopropyl-2-(3-pyridyl)isoindolin-1-one (19). MS obsd (ESI+) [$(M+H)^+$] 285.1. HRMS calcd [$(M+H)^+$] 285.0794, measured [$(M + H)^+$] 285.0790. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.79 (d, J=2.51 Hz, 1H), 8.52 (dd, J=1.38, 4.89 Hz, 1H), 8.06 (d, J=8.31 Hz, 1H), 7.85 (d, J=8.20 Hz, 1H), 7.81(s, 1H),7.63 (d, J=7.78 Hz, 1H), 7.57-7.61 (m, 1H), 4.78 (d, J=9.54 Hz, 1H), 0.85-0.95 (m, 1H), 0.62-0.73 (m, 2H), 0.43-0.52 (m, 1H), 0.34 (td, J=4.77, 6.02 Hz, 1H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 165.5, 146.5, 145.8, 137.8, 133.2, 132.3, 128.8, 128.5, 124.5, 122.8, 122.4, 64.7, 13.7, 3.7.

3-Benzyl-5-chloro-2-(3-pyridyl)isoindolin-1-one (20). LC-MS: tR 1.66 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 335.1. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.93 (d, J=2.53 Hz, 1H), 8.46 (dd, J=1.52, 4.80 Hz, 1H), 8.16 (d, J=7.99 Hz, 1H), 7.72 (d, J=7.62 Hz, 1H),7.52-7.61 (m, 3H),7.05-7.17 (m, 3H), 6.75 (d, J=6.58 Hz, 2H), 5.88 (dd, J=4.17, 6.19 Hz, 1H), 3.39 (dd, J=4.04, 14.15 Hz, 1H), 3.17 (dd, J=6.19, 14.27 Hz, 1H).

6-Chloro-3-methyl-2-(3-pyridyl)-3,4-dihydroisoquinolin-1-one (21). LC-MS: tR 1.38 min, 98% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 273.1. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.64 (s, 1H), 8.54 (dd, 1H), 8.04-7.90 (m, 2H), 7.63-7.54 (m, 1H), 7.48-7.42 (m, 2H), 4.39-4.28 (m, 1H), 3.63 (dd, 1H), 3.01 (dd, 1H), 1.24 (d, 3H).

6-Chloro-4-methyl-2-(3-pyridyl)-3,4-dihydroisoquinolin-1-one (22). LC-MS: tR 1.44 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 273.1. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.69 (d, 1H), 8.48 (dd, 1H), 8.03 (d, 1H), 7.97-7.90 (m, 1H), 7.60-7.51 (m, 1H), 7.47 (d, 1H), 7.44 (dd, 1H), 4.21 (dd, 1H), 3.81 (dd, 1H), 3.41-3.35 (m, 1H), 1.46 (d, 3H).

6-Chloro-4,4-dimethyl-2-(3-pyridyl)-3H-isoquinolin-1-one (23). LC-MS: tR 1.56 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 287.2. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.70 (dd, 1H), 8.49 (dd, 1H), 8.05 (d, 1H), 7.95 (ddd, 1H), 7.58-7.53 (m, 2H), 7.44 (dd, 1H), 3.89 (s, 2H), 1.48 (s, 6H).

Synthetic procedure for the synthesis of (-)-*N*-[(8*S*)-4-(5-chloro-1-oxo-isoindolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (24).

(-)-2-[(8*S*)-8-Amino-5,6,7,8-tetrahydroisoquinolin-4-yl]-5-chloro-isoindolin-1-one ((-)-(*S*)-84). 5-Chloroisoindolin-1-one (501 mg, 3 mmol), (-)-(*S*)-4-bromo-5,6,7,8-tetrahydroisoquinolin-8-amine (intermediate **(-)-(*S*)-83**, 703 mg, 3.1 mmol), CuI (171 mg, 0.9 mmol), (1*S*,2*S*)-cyclohexane-1,2-diamine (69 mg, 0.6 mmol) and Cs_2CO_3 (1.95 g, 6 mmol) were dissolved in 1,4-dioxane (45 mL). The reaction mixture was subjected to microwave reaction at 150 ºC for 2.5 hours before it was poured into H_2O (50 mL) and extracted with EtOAc (25 mL x 2). Combined organics were washed with brine, dried

over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to afford the title compound (800 mg, 85.2% yield) as a yellow solid. MS obsd (ESI+) [$(M+H)^+$] 314.1.

(-)-*N*-[(8*S*)-4-(5-Chloro-1-oxo-isoindolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (24). To a stirred solution of (-)-2-[(8*S*)-8-amino-5,6,7,8-tetrahydroisoquinolin-4-yl]-5-chloro-isoindolin-1-one (intermediate **(-)-(*S*)-84**, 438 mg, 1.4 mmol) and Et_3N (3.0 mL) in DCM (10 mL) was added acetyl chloride (0.106 mL, 1.4 mmol) at 0 °C and stirring was continued at 0 °C for 1 hour. The resulting mixture was extracted with EtOAc (100 mL x 2). Combined organics were washed with brine, dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to afford a crude product, which was purified by prep-HPLC to yield the title compound (99.4 mg, 20% yield) as a white solid. LC-MS: tR 1.06 min, 100% (254 nm). MS obsd (ESI+) [$(M+H)^+$] 356.1. HRMS calcd [$(M+H)^+$] 356.1166, measured [$(M+H)^+$] 356.1166. Optical rotation: $[\alpha]_D^{20}$ = -47.6 (1.8 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.43-8.51 (m, 2H), 7.83-7.90 (m, 1H), 7.71-7.77 (m, 1H), 7.59-7.67 (m, 1H), 5.22-5.28 (m, 1H), 4.93 (s, 2H), 2.60-2.81 (m, 2H), 2.05 (s, 3H), 1.79-2.01 (m, 4H).

Synthetic procedure for the synthesis of (+)-*N*-[(8*R*)-4-(5-chloro-1-oxo-isoindolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (25).

(+)-2-[(8*R*)-8-amino-5,6,7,8-tetrahydroisoquinolin-4-yl]-5-chloro-isoindolin-1-one ((+)-(*R*)-85). 5-Chloroisoindolin-1-one (1.02 g, 6 mmol), (+)-(*R*)-4-Bromo-5,6,7,8-tetrahydroisoquinolin-8-amine (intermediate **(+)-(*R*)-83**, 1.4 g, 6.2 mmol), CuI (342 mg, 1.8 mmol), (*1S,2S*)-cyclohexane-1,2-diamine (140 mg, 1.2 mmol), and Cs_2CO_3 (3.9 g, 12 mmol) were dissolved in 1,4-dioxane (100 mL). The reaction mixture was subjected to microwave reaction at 150 ºC for 2.5 hours before it was poured into H_2O (50 mL) and extracted with EtOAc (25 mL x 2). Combined organics were washed with brine, dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to afford the title compound (1.5 g, 80% yield) as a yellow solid. MS obsd (ESI+) [$(M+H)^+$] 314.1.

(+)-*N*-[(8*R*)-4-(5-Chloro-1-oxo-isoindolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (25). To a stirred solution of (+)-2-[(8*R*)-8-amino-5,6,7,8-tetrahydroisoquinolin-4-yl]-5-chloro-isoindolin-1-one (intermediate **(+)-(*R*)-85**, 438 mg, 1.4 mmol) and Et_3N (3 mL) in DCM (10 mL) was added acetyl chloride (0.106 mL, 1.4 mmol) at 0 °C and stirring was continued at 0 °C for 1 hour. The resulting mixture was extracted with EtOAc (100 mL x 2). Combined organics were washed with brine, dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to afford a crude product, which was purified by prep-HPLC to yield the title compound (64.6 mg, 13% yield) as a white solid. LC-MS: tR 1.06 min, 100% (254 nm). MS obsd (ESI+) [$(M+H)^+$] 356.1. Optical rotation: $[\alpha]_D^{20}$ = +37.8 (1.8 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.40-8.52 (m, 2H), 7.81-7.91 (m, 1H), 7.71-7.78 (m, 1H), 7.59-7.66 (m, 1H), 5.20-5.31 (m, 1H), 4.92 (s, 2H), 2.61-2.81 (m, 2H), 2.01 (s, 3H), 1.77-1.98 (m, 4H).

For the following compounds, except those described specifically, all the analogs were prepared in analogy to compound **25** from either commercially available reagents or in-house prepared intermediates.

(+)-*N*-[(8*R*)-4-(5-Chloro-1-oxo-isoindolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]propanamide (26). LC-MS: tR 1.14 min, 100% (254 nm). MS obsd (ESI+) [$(M+H)^+$] 370.1. HRMS calcd [$(M+H)^+$] 370.1322, measured [$(M + H)^+$] 370.1317. Optical rotation: $[\alpha]_D^{20}$ = +54.3 (1.6 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.43-8.47 (m, 2H), 7.83-7.89 (m, 1H), 7.72-7.75 (m, 1H), 7.59-7.67 (m, 1H), 5.21-5.30 (m, 1H), 4.97 (s, 2H), 2.68-2.80 (m, 2H), 2.24-2.39 (m, 2H), 1.94-2.09 (m, 2H), 1.84-1.93 (m, 2H), 1.17-1.27 (m, 3H).

(+)-*N*-[(8*R*)-4-(5-Chloro-1-oxo-isoindolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]methanesulfonamide (27). LC-MS: tR 1.23 min, 100% (254 nm). MS obsd (ESI+) [$(M+H)^+$] 392.1. HRMS calcd [$(M+H)^+$] 392.0836, measured [$(M + H)^+$] 392.0847. Optical rotation: $[\alpha]_D^{20}$ = +7.7 (1 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.72-8.77 (m, 1H), 8.43-8.49 (m, 1H), 7.82-7.90 (m, 1H), 7.71-7.76 (m, 1H), 7.60-7.66 (m, 1H), 5.20-5.30 (m, 1H), 4.97 (s, 2H), 3.18 (s, 3H), 2.60-2.79 (m, 2H), 2.11-2.30 (m, 2H), 1.91-2.06 (m, 2H).

(+)-*N*-[(8*R*)-4-(5-Chloro-1-oxo-isoindolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]ethanesulfonamide (28). LC-MS: tR 1.23 min, 98% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 406.1. HRMS calcd [(M+H)$^+$] 406.0992, measured [(M + H)$^+$] 406.0993. Optical rotation: $[\alpha]_D^{20}$ = +28.7 (1.4 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.73-8.78 (m, 1H), 8.43-8.48 (m, 1H), 7.83-7.89 (m, 1H), 7.70-7.77 (m, 1H), 7.59-7.66 (m, 1H), 4.96 (s, 2H), 4.72-4.81 (m, 1H),3.13-3.20 (m, 2H), 2.61-2.75 (m, 2H), 2.08-2.26 (m, 2H), 1.92-2.08 (m, 2H),1.38-1.46 (m, 3H).

(+)-*N*-[(8*R*)-4-(6-Chloro-1-oxo-3,4-dihydroisoquinolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (39). MS obsd (ESI+) [(M+H)$^+$] 370.0. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.42 (d, 1H), 8.37 (d, 1H), 7.99 (d, 1H), 7.41-7.47 (m, 2H), 5.19-5.27 (m, 1H), 3.95-4.13 (m, 1H), 3.76-3.88 (m, 1H), 3.20-3.38 (m, 3H), 2.64-2.85 (m, 2H), 1.82-2.09 (m, 5H), 1.78-2.18 (m, 1H), 1.78-2.22 (m, 1H).

(+)-*N*-[(8R)-4-(6-chloro-1-oxo-3,4-dihydroisoquinolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]propanamide (40). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.50-8.31 (m, 2H), 8.01 (d, 1H), 7.46 (s, 1H), 7.45 (d, 2H), 5.23 (br t,1H), 4.18-3.95 (m, 1H), 3.92 - 3.73 (m, 1H), 3.57-3.35 (m, 1H), 3.30-3.08 (m, 2H), 2.75 (br t, 2H), 2.39-2.21 (m, 2H), 2.08-1.81 (m, 4H), 1.31-1.14 (m, 3H).

(+)-*N*-[(8R)-4-(6-chloro-1-oxo-3,4-dihydroisoquinolin-2-yl)-5,6,7,8-tetrahydroisoquinolin-8-yl]methanesulfonamide (41). LC-MS: tR 1.19 min, 98% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 406.0. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.74-8.65 (m, 1H), 8.38 (s, 1H), 8.00 (d, 1H), 7.46 (s, 1H), 7.45 (d, 2H), 4.84-4.70 (m, 1H), 4.15-3.94 (m, 1H), 3.90-3.70 (m, 1H), 3.56-3.35 (m, 1H), 3.31-3.18 (m, 2H), 3.13 (d, 3H), 2.91-2.63 (m, 2H), 2.29-2.09 (m, 1H), 2.08-1.82 (m, 3H).

Synthetic procedure for the syntheses of (+)-*N*-[(8*R*)-4-[(3*R*)-5-chloro-3-ethyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (31) and (+)-*N*-[(8*R*)-4-[(3*S*)-5-chloro-3-ethyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (32).

5-Chloro-3-ethyl-isoindolin-1-one (intermediate **69**, 900 mg, 4.62 mmol), (+)-*N*-[(8*R*)-4-bromo-5,6,7,8-tetrahydro-isoquinolin-8-yl)-acetamide (intermediate **(+)-(*R*)-86**), 1.2 g, 4.62 mmol), CuI (263 mg, 1.38 mmol), (1*S*,2*S*)-cyclohexane-1,2-diamine (333 mg, 2.7 mmol) and Cs_2CO_3 (3 g, 9.24 mmol)

were dissolved in 1,4-dioxane (45 mL). The reaction mixture was subjected to microwave reaction at 150 ºC for 2.5 hours before it was poured into H_2O (50 mL) and extracted with EtOAc (25 mL x 2). The combined organics were washed with brine, dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product, which was purified by silica gel flash chromatography eluting with a 0 to 30 % MeOH-EtOAc gradient to give racemic title compound (707.8 mg, 40% yield) as a white solid. MS obsd (ESI+) [(M+H)$^+$] 384.1.

This racemic mixture was then separated by chiral HPLC to afford enantiomeric compound **31** (45 mg), LC-MS: tR 1.27 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 384.1. HRMS calcd [(M+H)$^+$] 384.1478, measured [(M + H)$^+$] 384.1476. Optical rotation: $[\alpha]_D^{20}$ = +147.6 (3.2 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.26-8.58 (m, 2H), 7.80-7.96 (m, 1H), 7.70-7.80 (m, 1H), 7.56-7.70 (m, 1H), 5.06-5.49 (m, 2H),2.48-2.75 (m, 2H), 1.90-2.17 (m, 7H), 1.72-1.91 (m, 2H), 0.66-0.84 (m, 3H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 169.4, 150.8, 148.9, 146.7, 144.4, 138.8, 134.1, 129.2, 125.6, 122.9, 62.2, 44.6, 28.9, 24.7, 24.1, 23.3, 18.3, 7.0 and enantiomeric compound **32** (53 mg), LC-MS: tR 1.27 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 384.1. HRMS calcd [(M+H)$^+$] 384.1478, measured [(M + H)$^+$] 384.1474. Optical rotation: $[\alpha]_D^{20}$ = +37.8 (2.3 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.27-8.56 (m, 2H), 7.79-7.98 (m, 1H), 7.71-7.81 (m, 1H), 7.56-7.70 (m, 1H), 5.06-5.51 (m, 2H), 2.51-2.78 (m, 2H), 1.71-2.21 (m, 9H), 0.62-0.92 (m, 3H).

For the following compounds, except those described specifically, all the analogs were prepared in analogy to compounds **31** and **32** from either commercially available reagents or in-house prepared intermediates.

(+)-*N*-[(8*R*)-4-[(3*R*)-5-Chloro-3-methyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (29) and (+)-*N*-[(8*R*)-4-[(3*S*)-5-chloro-3-methyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (30). Enantiomeric compound **29**, LC-MS: tR 1.29 min, 95% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 370.1. Optical rotation: $[\alpha]_D^{20}$ = +90.0 (1 mg/mL, MeOH). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.26-8.54 (m, 2H), 7.85 (br d, J=7.78 Hz, 1H), 7.70-7.80 (m, 1H), 7.63 (dd, J=1.76, 8.28 Hz, 1H), 5.22-5.43 (m, 2H), 2.54-2.69 (m, 2 H), 1.80-2.07 (m, 6 H), 1.50 (br d, J=6.27 Hz, 2H), 1.41 (br d, J=6.27 Hz, 1H), 1.23-1.37 (m, 1H). Enantiomeric compound **30**, LC-MS:

tR 1.27 min, 95% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 370.1. Optical rotation: $[\alpha]_D^{20}$ = +16.0 (1 mg/mL, MeOH). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.41-8.62 (m, 2H), 7.85 (br d, J=8.28Hz,1H), 7.77 (s, 1H), 7.63 (dd, J=1.76, 8.28 Hz, 1H), 5.25-5.36 (m, 2H), 2.91 (br d, J=1.51 Hz, 1H), 2.52-2.76 (m, 1H), 1.83-2.14 (m,6H), 1.66-1.94 (m, 2H), 1.41-1.48 (m, 2H), 1.23-1.35 (m, 1H).

(+)-*N*-[(8*R*)-4-[(3*R*)-5-Chloro-3-methyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]propanamide (33) and (+)-*N*-[(8*R*)-4-[(3*S*)-5-chloro-3-methyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]propanamide (34). Enantiomeric compound **33**, LC-MS: tR 1.36 min, 98% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 374.1. HRMS calcd [(M+H)$^+$] 384.1478, measured [(M + H)$^+$] 384.1476. Optical rotation: $[\alpha]_D^{20}$ = +100.0 (1 mg/mL, MeOH). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.38-8.50 (m, 2H), 7.84-7.86 (m, 1H), 7.76-7.78 (m, 1H), 7.62-7.65 (m, 1H), 5.27-5.36 (m, 2H), 2.55-2.70 (m, 1H), 2.15-2.38 (m, 2H), 1.70-1.96 (m, 3H), 1.50 (m, 1H), 1.42 (m, 1H), 1.26-1.36 (m, 2H), 1.06-1.25 (m, 3H), 0.82-1.05 (m, 1H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 173.3, 150.9, 149.2, 148.6, 146.1, 145.8, 138.8, 129.3, 125.5, 122.9, 59.3, 57.3, 44.5, 32.0, 29.9, 29.7, 29.1, 24.4, 19.1, 18.4, 9.8. Enantiomeric compound **34**, LC-MS: tR 1.36 min, 98% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 384.1. HRMS calcd [(M+H)$^+$] 384.1479, measured [(M + H)$^+$] 384.1480. Optical rotation: $[\alpha]_D^{20}$ = +20.0 (1 mg/mL, MeOH). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.31-8.50 (m, 2H), 7.84 (br d, J=8.28 Hz, 1H), 7.77 (s, 1H), 7.58-7.66 (m, 1H), 5.32-5.47 (m, 1H), 5.26 (m, 1H), 2.94 (m, 1H), 2.58 (m, 1H), 2.32 (q, J=7.19 Hz, 2H), 2.08 (br s, 1H), 1.93-2.04 (m, 1H), 1.84 (br d, J=8.03 Hz, 1H), 1.48 (br d, J=6.53 Hz, 1H), 1.41 (br d, J=6.78 Hz, 2H), 1.28-1.35 (m, 2H), 1.15-1.26 (m, 3H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 173.4, 165.8, 149.8, 148.6, 146.1, 138.8, 134.3, 131.7, 129.3, 125.4, 122.7, 59.5, 45.3, 29.9, 29.7, 29.5, 24.9, 24.9, 19.5, 18.0, 9.9.

(+)-*N*-[(8*R*)-4-[(3*R*)-5-Chloro-3-ethyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]propanamide (35) and (+)-*N*-[(8*R*)-4-[(3*S*)-5-chloro-3-ethyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]propanamide (36). Enantiomeric compound **35**, LC-MS: tR 1.45 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 398.1. Optical rotation: $[\alpha]_D^{20}$ = +110.6 (1.2 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.24-8.54 (m, 2H), 7.78-7.94 (m, 1H), 7.71-7.82 (m, 1H), 7.56-7.66 (m, 1H), 5.05-5.52 (m, 2H), 2.53-2.76 (m, 2H), 2.22-2.38 (m, 2H), 1.73-2.18 (m, 6H), 1.12-1.29

(m, 3H), 0.62-0.89 (m, 3H). Enantiomeric compound **36**, LC-MS: tR 1.45 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 398.1. Optical rotation: $[\alpha]_D^{20}$ = +25.1 (1.3 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.29-8.53 (m, 2H), 7.80-7.96 (m, 1H), 7.71-7.81 (m, 1H), 7.56-7.68 (m, 1H), 5.02-5.47 (m, 2H), 2.49-2.70 (m, 1H), 2.23-2.40 (m, 2H), 1.71-2.18 (m, 7H), 1.13-1.28 (m, 3H), 0.65-0.87 (m, 3H).

***N*-[(8*R*)-4-[(3*R*)-5-Chloro-3-cyclopropyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (37) and *N*-[(8*R*)-4-[(3*S*)-5-chloro-3-cyclopropyl-1-oxo-isoindolin-2-yl]-5,6,7,8-tetrahydroisoquinolin-8-yl]acetamide (38).** Enantiomeric compound **37**, LC-MS: tR 1.37 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 396.7. Optical rotation: $[\alpha]_D^{20}$ = +370.0 (1 mg/mL, MeOH). ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm 8.48 (br d, 0.5H), 8.30-8.42 (m, 1.5H), 7.72-7.92 (m, 2H), 7.66 (d, 1H), 4.99-5.19 (m, 1H), 4.57 (d, 0.5H), 4.17 (d, 0.5H), 2.40-2.80 (m, 4H), 1.80-1.95 (m, 5H), 1.66 (bs s, 2H), 1.25 (b, 1H), 0.77-1.03 (m, 1H), 0.49-0.74 (m, 1H), 0.24 (br s, 1H), -0.15 (dt, 1H). Eantiomeric compound **38**, LC-MS: tR 1.37 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 396.7. Optical rotation: $[\alpha]_D^{20}$ = -24.0 (1 mg/mL, MeOH). ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm 8.31-8.47 (m, 2H). 7.78-7.83 (m, 2H), 7.66 (dd, 1H), 5.05-5.14 (m, 1H), 4.57 (d, 0.6H), 4.17 (d, 0.4H), 2.37-2.80 (m, 4H), 1.80-1.96 (m, 5H), 1.65 (br d, 2H), 0.81-1.04 (m, 1H), 0.47-0.75 (m, 2H), 0.20-0.36 (m, 1H), -0.14 (dq, 1H).

Synthetic procedure for the synthesis of *N*-[[5-(6-chloro-1,1-dimethyl-3-oxo-isoindolin-2-yl)-3-pyridyl]methyl]acetamide (42).

To a stirred solution of 2-[5-(aminomethyl)-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (intermediate **91**, 210 mg, 0.7 mmol) and Et_3N (2.0 mL) in DCM (10 mL) was added acetyl chloride (0.051 mL, 0.7 mmol) at 0 ^{o}C and stirring was continued at 0 ^{o}C for 1 hour. The resulting mixture was extracted with EtOAc (100 mL x 2). Combined organics were washed with brine, dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product, which was purified by prep-HPLC to afford the title compound (36 mg, 15% yield) as a white foam. LC-MS: tR 1.15 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 344.2. HRMS calcd [(M+H)$^+$] 344.1165, measured [(M + H)$^+$]

344.1164. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.28-8.75 (m, 2H), 7.75-7.89 (m, 3H), 7.54-7.63 (m, 1H),4.38-4.56 (m, 2H),1.91-2.11 (m, 3H), 1.47-1.67 (m, 6H).

For the following compounds, except those described specifically, all the analogs were prepared in analogy to compound **42** from either commercially available reagents or in-house prepared intermediates.

***N*-[[5-(6-Chloro-1,1-dimethyl-3-oxo-isoindolin-2-yl)-3-pyridyl]methyl]propanamide (43).** LC-MS: tR 1.22 min, 95% (254 nm). MS obsd (ESI+) [(M+H)$^{+}$] 358.1. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.60 (d, *J*=2.01 Hz, 1H), 8.44-8.47 (m, 1H),7.82-7.86 (m, 2H),7.80-7.82 (m, 1H),7.58-7.63 (m, 1H), 4.46 (s, 2H), 2.64-2.72 (m, 2H),1.55-1.61 (m, 6H), 1.10-1.22 (m, 3H).

***N*-[[5-(6-Chloro-1,1-dimethyl-3-oxo-isoindolin-2-yl)-3-pyridyl]methyl]methanesulfonamide (44).** LC-MS: tR 1.26 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^{+}$] 380.1. HRMS calcd [(M+H)$^{+}$] 380.0836, measured [(M + H)$^{+}$] 380.0835. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.61-8.76 (m, 1H), 8.44-8.54 (m, 1H), 7.87-7.97 (m, 1H), 7.79-7.86 (m, 2H), 7.57-7.63 (m, 1H), 4.32-4.51 (m, 2H), 3.00 (s, 3H), 1.60 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 167.1, 153.2, 149.5, 148.7, 139.1, 136.7, 133.9, 132.6, 129.2, 128.1, 125.6, 121.8, 64.7, 44.1, 41.2, 26.8.

***N*-[[5-(6-Chloro-1,1-dimethyl-3-oxo-isoindolin-2-yl)-3-pyridyl]methyl]ethanesulfonamide (46).** LC-MS: tR 1.33 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^{+}$] 394.1. HRMS calcd [(M+H)$^{+}$] 394.0992, measured [(M + H)$^{+}$] 394.0988. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.65-8.69 (m, 1H), 8.47-8.51 (m, 1H), 7.89-7.93 (m, 1H), 7.79-7.86 (m, 2H), 7.58-7.64 (m, 1H), 4.43 (s, 2H), 3.06-3.15 (m, 2H), 1.63 (s, 6H), 1.30-1.40 (m, 3H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 166.9, 153.2, 149.5, 148.7, 139.1, 136.7, 134.1, 132.5, 129.1, 125.7, 121.7, 64.6, 47.8, 44.2, 27.7, 26.8, 8.3.

Synthetic procedure for the synthesis of (+)-*N*-[(1R)-1-[5-(6-chloro-1,1-dimethyl-3-oxo-isoindolin-2-yl)-3-pyridyl]ethyl]methanesulfonamide (45).

To a stirred solution of 2-[5-(1-aminoethyl)-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (intermediate **92**, 360 mg, 1.14 mmol) and Et_3N (477 μL, 3.42 mmol) in DCM (15 mL) was added

methanesulfonyl chloride (89.4 μL, 1.14 mmol) at 0 °C and stirring continued at 0 °C for 1 hour. The resulting mixture was extracted with EtOAc (100 mL x 2). Combined organics were washed with brine, dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography eluting with a 0 to 10 % MeOH-EtOAc gradient to give a racemic mixture of title compound (160 mg, 34 %) as a light yellow solid. MS obsd (ESI+) [$(M+H)^+$] 394.1.

This racemic mixture was then subject to chiral HPLC separation to afford the enantiomeric compound **45** (36 mg) as a light yellow solid, Optical rotation: $[\alpha]_D^{20}$ = +6.1 (1 mg/mL, DCM). LC-MS: tR 1.30 min, 100% (254 nm). MS obsd (ESI+) [$(M+H)^+$] 394.1. HRMS calcd [$(M+H)^+$] 394.0992, measured [$(M + H)^+$] 394.0993. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.67-8.73 (m, 1H), 8.45-8.50 (m, 1H), 7.89-7.93 (m, 1H), 7.80-7.86 (m, 2H), 7.58-7.64 (m, 1H), 4.77-4.84 (m, 1H), 2.93 (s, 3H), 1.60 (d, *J*=6.90 Hz, 9H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 167.0, 153.1, 149.2, 147.6, 139.1, 135.2, 132.6, 129.2, 128.2, 125.6, 121.7, 64.7, 51.0, 42.1, 26.9, 26.8, 23.5.

Synthetic procedure for the synthesis of *N*-[[5-(6-chloro-1,1-dimethyl-3-oxo-isoindolin-2-yl)-3-pyridyl]methyl]-3-methyl-pyridine-2-carboxamide (47).

To a solution of 2-[5-(aminomethyl)-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (intermediate **91**, 210 mg, 0.7 mmol) and 3-methylpyridine-2-carboxylic acid (120 mg, 0.87 mmol) in DCM was added HATU (400 mg, 1.05mmol) and DIPEA (2 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred overnight. Brine was added to quench the reaction and the mixture was extracted with DCM twice. The organic layers were combined, washed with brine, dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to give yellowish oil which was purified by prep-HPLC to give the title compound (29.4 mg, 10% yield) as a white foam. LC-MS: tR 1.49 min, 100% (254 nm). MS obsd (ESI+) [$(M+H)^+$] 421.1. HRMS calcd [$(M+H)^+$] 421.1431, measured [$(M + H)^+$] 421.1429. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.64-8.74 (m, 1H), 8.41-8.52 (m, 2H), 7.88-7.98 (m, 1H), 7.81-7.85 (m, 1H), 7.79-7.81 (m, 1H), 7.73-7.77 (m, 1H), 7.56-7.62 (m, 1H), 7.41-7.46 (m, 1H), 4.72 (s, 2H), 2.61 (s, 3H), 1.58 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 166.8, 166.2,

153.2, 149.2, 148.9, 146.6, 145.6, 140.9, 138.9, 136.5, 135.7, 135.3, 132.4, 129.0, 128.4, 126.0, 125.7, 121.7, 64.5, 40.4, 26.8, 20.5.

Synthetic procedure for the synthesis of 5-chloro-2-[5-(1-ethylsulfonyl-4-piperidyl)-3-pyridyl]-3,3-dimethyl-isoindolin-1-one (49).

A mixture of 3-bromo-5-(1-ethylsulfonyl-4-piperidyl)pyridine (intermediate **98**, 200 mg, 0.6 mmol), 5-chloro-3,3-dimethyl-isoindolin-1-one (intermediate **70**, 130 mg, 0.67 mmol), copper(I) iodide (34 mg, 0.18 mmol), *trans*-cyclohexane-1,2-diamine (41 mg, 0.36 mmol), and cesium carbonate (422 mg, 1.3 mmol) in 1,4-dioxane (5 mL) was heated at 150 °C for 2 hours. After the reaction mixture was cooled back to room temperature, it was filtered and the filtrate was concentrated *in vacuo* to give a crude product, which was purified by prep-HPLC to give the title compound (10 mg, 4% yield) as a white solid. MS obsd (ESI+) [(M+H)$^+$] 448.1. HRMS calcd [(M+H)$^+$] 448.1462, measured [(M + H)$^+$] 448.1463. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.61 (s, 1H), 8.42 (s, 1H), 7.75-7.88 (m, 3H), 7.60 (dd, J=1.88, 8.16 Hz, 1H), 3.89-3.96 (m, 2H), 3.10 (q, J=7.28 Hz, 2H), 3.02 (dt, J=2.51, 12.30 Hz, 2H), 2.93 (tt, J=3.51, 12.17 Hz, 1H), 2.03 (br d, J=11.04 Hz, 2H), 1.85 (dq, J=4.02, 12.55 Hz, 2H), 1.59 (s, 6H), 1.36 (t, J=7.40 Hz, 3H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 166.9, 153.1, 148.5, 148.3, 140.9, 139.0, 135.2, 132.5, 129.1, 128.4, 125.7, 121.7, 64.4, 46.3, 44.4, 39.4, 32.9, 26.8, 7.9.

For the following compounds, except those described specifically, all the analogs were prepared in analogy to compound **49** from either commercially available reagents or in-house prepared intermediates.

2-[5-(1-Acetyl-4-piperidyl)-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (48). LC-MS: tR 1.34 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 398.1. HRMS calcd [(M+H)$^+$] 398.1635, measured [(M + H)$^+$] 398.1628. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.61 (d, J=2.01 Hz, 1H), 8.41 (d, J=2.26 Hz, 1H), 7.76-7.85 (m, 3H), 7.60 (dd, J=1.76, 8.03 Hz, 1H), 4.65-4.83 (m, 1H), 4.09 (td, J=1.95, 13.68 Hz, 1H), 3.25-3.31 (m, 1H), 2.95-3.16 (m, 1H), 2.77 (dt, J=2.64, 12.99 Hz, 1H), 2.16 (s,

3H), 1.91-2.06 (m, 2H), 1.80 (dd, J=4.27, 12.55 Hz, 1H),1.68 (dd, J=4.27, 12.80 Hz, 1H), 1.58 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 168.9, 166.7, 153.1, 148.4, 141.2, 139.0, 135.3, 132.3, 129.0, 128.4, 125.7, 121.7, 64.4, 46.8, 41.9, 39.9, 33.4, 32.5, 26.8, 21.5.

Synthetic procedure for the synthesis of 2-[5-(4-acetylpiperazin-1-yl)-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (50).

To a solution of 5-chloro-3,3-dimethyl-2-(5-piperazin-1-yl-pyridin-3-yl)-2,3-dihydro-isoindol-1-one (intermediate **102**, 52 mg, 0.146 mmol) and Et_3N (29.5 mg, 0.292 mmol) in DCM (5 mL) was added acetyl chloride (17.2 mg, 0.219 mmol) dropwise at 0 ^{o}C. After stirring at room temperature for 1 hour, the mixture was treated with water and extracted with DCM. The organic layer was dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product, which was purified by prep-HPLC to afford the title compound (23 mg, 40% yield) as a white solid. LC-MS: tR 1.30 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 399.1. HRMS calcd [(M+H)$^+$] 399.1588, measured [(M + H)$^+$] 399.1591. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.39 (d, J=2.51 Hz, 1H), 7.98 (d, J=1.76 Hz,1H), 7.80 (s, 1H), 7.81 (d, J=9.51 Hz, 1H), 7.59 (dd, J=1.76,8.03 Hz,1H), 7.40 (t, J=2.38 Hz,1H), 3.70-3.82 (m, 4H), 3.35-3.50 (m, 4H), 2.17 (s, 3H), 1.59 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 169.0, 166.9, 153.2, 140.8, 138.9, 138.1, 129.1, 128.3, 125.6, 123.7, 121.7, 64.5, 48.6, 48.3, 45.8, 40.9, 26.9, 21.2.

For the following compounds, except those described specifically, all the analogs are prepared in analogy to compound **50** from either commercially available reagents or in-house prepared intermediates.

5-Chloro-3,3-dimethyl-2-[5-(4-methylsulfonylpiperazin-1-yl)-3-pyridyl]isoindolin-1-one (51). LC-MS: tR 1.43 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 435.1. HRMS calcd [(M+H)$^+$] 435.1258, measured [(M + H)$^+$] 435.1259. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.41 (d, J=2.76 Hz, 1H), 8.00 (d, J=1.76 Hz, 1H),7.82 (d, J=10.01 Hz, 1H), 7.80 (s, 1H), 7.59 (dd, J=1.63, 8.16 Hz, 1H), 7.42 (t, J=2.38 Hz, 1H), 3.35-3.52 (m, 8H), 2.91 (s, 3H), 1.59 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*)

δ ppm 166.8, 152.9, 146.9, 141.1, 139.0, 138.5, 132.7, 129.1, 128.4, 125.6, 124.0, 121.7, 64.5, 48.4, 45.5, 34.8, 26.9.

Synthetic procedure for the synthesis of 5-chloro-2-[5-(1-ethylsulfonylazetidin-3-yl)oxy-3-pyridyl]-3,3-dimethyl-isoindolin-1-one (52).

To a mixture of 2-[5-(azetidin-3-yloxy)-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (intermediate **104**, 35 mg, 0.1 mmol) and Et_3N (200 mg, 2.0 mmol) in DCM (5 mL) was added ethanesulfonyl chloride (50 mg, 0.4 mmol) at 0 °C and resulting mixture was stirred at room temperature for 3 hours. The mixture was then washed with water, and the separated organic layer was concentrated *in vacuo* to give a crude product, which was purified by prep-HPLC to afford the title compound (11 mg, 25.2% yield) as a white solid. LC-MS: tR 1.52 min, 100% (254 nm). MS obsd (ESI+) $[(M+H)^+]$ 436.1. HRMS calcd $[(M+H)^+]$ 436.1097, measured $[(M + H)^+]$ 436.1092. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.35 (br d, J=2.26 Hz, 1H), 8.23 (s, 1H), 7.80-7.85 (m, 2H), 7.61 (d, J=1.76 Hz, 1H), 7.37-7.41 (m, 1H), 5.15-5.25 (m, 1H), 4.37 (dd, J=6.40, 9.66 Hz, 2H), 4.10 (dd, J=4.52, 9.54 Hz, 2H), 3.10-3.18 (m, 2H), 1.50-1.66 (s, 6H), 1.36 (t, J=7.40 Hz, 3H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 166.9, 153.1, 152.9, 143.2, 139.1, 136.7, 133.3, 129.2, 128.2, 125.7, 123.1, 121.7, 65.5, 64.6, 56.8, 46.4, 26.9, 7.9.

For the following compounds, except those described specifically, all the analogs are prepared in analogy to compound **52** from either commercially available reagents or in-house prepared intermediates.

(-)-2-[5-[(3*R*)-1-Acetylpyrrolidin-3-yl]oxy-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (53). MS obsd (ESI+) $[(M+H)^+]$ 400.2. HRMS calcd $[(M+H)^+]$ 400.1427, measured $[(M + H)^+]$ 400.1422. Optical rotation: $[\alpha]_D^{20}$ = -23.01 (3 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.37-8.44 (m, 1H), 8.16-8.21 (m, 1H), 7.77-7.88 (m, 2H), 7.57-7.63 (m, 1H), 7.50-7.54 (m, 1H), 5.18-5.29 (m, 1H), 3.48-3.84 (m, 4H), 2.22-2.39 (m, 2H), 2.15 (s, 3H), 1.61 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*)

δ ppm 169.5, 166.9, 153.2, 142.6, 142.4, 139.0, 137.8, 129.1, 125.6, 123.7, 123.4, 121.7, 64.6, 52.8, 51.2, 45.2, 43.5, 31.7, 30.1, 22.3.

(+)-2-[5-[(3*S*)-1-Acetylpyrrolidin-3-yl]oxy-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (54). LC-MS: tR 1.30 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 400.2. HRMS calcd [(M+H)$^+$] 400.1428, measured [(M + H)$^+$] 400.1433. Optical rotation: $[\alpha]_D^{20}$ = +19.42 (3.5 mg/mL, DCM). ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.36-8.43 (m, 1H), 8.17-8.23 (m, 1H), 7.79-7.86 (m, 2H), 7.58-7.63 (m, 1H), 7.49-7.54 (m, 1H), 5.18-5.31 (m, 1H), 3.47-3.85 (m, 4H), 2.22-2.40 (m, 2H), 2.16 (s, 3H), 1.64 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 169.5, 166.9, 153.5, 153.2, 142.5, 139.0, 137.8, 137.6, 133.1, 129.1, 128.3, 125.7, 121.7, 64.7, 52.7, 51.2, 45.2, 43.5, 31.7, 29.9, 22.3.

2-[5-[(1-Acetyl-4-piperidyl)oxy]-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (55). LC-MS: tR 1.39 min, 100% (254 nm). MS obsd (ESI+) [(M+H)$^+$] 414.2. HRMS calcd [(M+H)$^+$] 414.1584, measured [(M + H)$^+$] 414.1583. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.60-8.63 (m, 1H), 8.46-8.52 (m, 1H), 7.81 (s, 3H), 7.57-7.63 (m, 1H), 3.35-3.44 (m, 4H), 3.30-3.35 (m, 5H), 2.82 (s, 3H), 1.59 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 169.0, 166.9, 153.1, 142.2, 139.0, 138.6, 129.1, 128.3, 125.7, 123.8, 121.7, 72.7, 64.6, 42.9, 38.0, 30.8, 30.0, 26.9, 26.8, 21.4.

Synthetic procedure for the synthesis of 5-chloro-2-[5-[1-(3-chloropyridine-2-carbonyl)azetidin-3-yl]oxy-3-pyridyl]-3,3-dimethyl-isoindolin-1-one (56).

To a solution of 2-[5-(azetidin-3-yloxy)-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (intermediate **104**, 35 mg, 0.1 mmol) and 3-chloropyridine-2-carboxylic acid (20 mg, 0.15 mmol) in DCM was added HATU (76 mg, 0.2 mmol) and DIPEA (2 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred overnight. Brine was added to quench the reaction and the mixture was extracted with DCM twice. Combined organics were washed with brine, dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to give yellow oil, which was purified by prep-HPLC to give the title compound (6.4 mg, 13% yield) as a white solid. MS obsd (ESI+) [(M+H)$^+$] 483.1.

HRMS calcd [(M+H)$^+$] 483.0991, measured [(M + H)$^+$] 483.0990. ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 8.53 (dd, J=1.25, 4.52 Hz, 1H), 8.29 (s, 1H), 8.23 (d, J=2.26 Hz, 1H), 7.79-7.90 (m, 2H), 7.46-7.56 (m, 2H), 7.36 (dd, J=4.52, 8.28 Hz, 1H), 7.16 (br s, 1H), 5.04-5.20 (m, 1H), 4.57-4.76 (m, 2H), 4.29-4.45 (m, 2H), 1.58 (d, J=5.77 Hz, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 166.8, 165.0, 153.1, 153.0, 149.6, 147.0, 143.2, 139.0, 138.5, 136.6, 133.3, 130.1, 129.1, 128.2, 125.7, 125.6, 123.0, 121.7, 66.8, 64.6, 58.5, 55.5, 26.9.

For the following compounds, except those described specifically, all the analogs are prepared in analogy to compound **56** from either commercially available reagents or in-house prepared intermediates.

5-Chloro-3,3-dimethyl-2-[5-[1-(4-methylpyridine-3-carbonyl)azetidin-3-yl]oxy-3-pyridyl]isoindolin-1-one (57). MS obsd (ESI+) [(M+H)$^+$] 463.1. HRMS calcd [(M+H)$^+$] 463.1536, measured [(M + H)$^+$] 463.1535. ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 8.56 (br s, 2H), 8.29 (br s, 1H), 8.23 (br s, 1H), 7.85 (br d, J=8.03 Hz, 1H), 7.42-7.56 (m, 2H), 7.22-7.35 (m, 1H), 7.13 (br s, 1H), 5.11 (br s, 1H), 4.66 (br s, 1H), 4.30-4.50 (m, 2H), 4.15 (br s, 1H), 2.53 (s, 3H), 1.58 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 168.8, 166.8, 153.1, 152.9, 150.9, 147.6, 145.9, 143.1, 139.1, 136.6, 133.4, 129.4, 129.2, 128.1, 126.0, 125.7, 123.0, 121.7, 66.4, 64.6, 58.4, 55.4, 26.9, 19.2.

5-Chloro-3,3-dimethyl-2-[5-[1-(1-methylpyrazole-4-carbonyl)azetidin-3-yl]oxy-3-pyridyl]isoindolin-1-one (58). MS obsd (ESI+) [(M+H)$^+$] 452.1. HRMS calcd [(M+H)$^+$] 452.1489, measured [(M + H)$^+$] 452.1487. ^{1}H NMR (400 MHz, MeOH-*d4*) δ ppm 8.37 (d, J=2.76 Hz, 1H), 8.24 (d, J=2.01 Hz, 1H), 8.09 (s, 1H), 7.79-7.89 (m, 3H), 7.60 (dd, J=1.76, 8.03 Hz, 1H), 7.37-7.45 (m, 1H), 5.25-5.34 (m, 1H), 4.91 (br s, 1H), 4.57 (br dd, J=8.53, 18.82 Hz, 2H), 4.19 (br d, J=9.79 Hz, 1H), 3.94 (s, 3H), 1.61 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 165.9, 162.8, 152.0, 142.0, 138.1, 138.0, 135.6, 132.3, 131.4, 128.1, 127.1, 124.7, 122.1, 120.7, 115.3, 66.0, 63.7, 38.3, 25.9.

Synthetic procedure for the synthesis of 2-[5-(2-Acetyl-2,6-diazaspiro[3.3]heptan-6-yl)-3-pyridyl]-5-chloro-3,3-dimethyl-isoindolin-1-one (59).

To a solution of 5-chloro-2-[5-(2,6-diazaspiro[3.3]heptan-2-yl)-3-pyridyl]-3,3-dimethyl-isoindolin-1-one (intermediate **108**, 40 mg, 0.1 mmol) was added Et_3N (1 mmol) and acetyl chloride (30 mg, 0.38 mmol) at 0 °C. The resulting reaction mixture was stirred at room temperature for 1 hour before it was poured into H_2O (1 mL) and extracted with EtOAc (15 mL x 2). Combined organics were washed with brine, dried over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product, which was purified by prep-HPLC to give the title compound (8 mg, 19.5% yield) as a white solid. MS obsd (ESI+) $[(M+H)^+]$ 411.2. HRMS calcd $[(M+H)^+]$ 411.1588, measured $[(M + H)^+]$ 411.1580. ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 8.00 (d, *J*=2.01 Hz, 1H), 7.91-7.96 (m, 1H), 7.82-7.88 (m, 1H), 7.45-7.53 (m, 2H), 6.64-6.72 (m, 1H), 4.31-4.37 (m, 2H), 4.19-4.26 (m, 2H), 4.07-4.19 (m, 4H), 1.91 (s, 3H), 1.56 (s, 6H).

For the following compounds, except those described specifically, all the analogs are prepared in analogy to compound **59** from either commercially available reagents or in-house prepared intermediates.

5-Chloro-2-[5-(2-ethylsulfonyl-2,6-diazaspiro[3.3]heptan-6-yl)-3-pyridyl]-3,3-dimethyl-isoindolin-1-one (60). MS obsd (ESI+) $[(M+H)^+]$ 461.1. HRMS calcd $[(M+H)^+]$ 461.1414, measured $[(M + H)^+]$ 461.1415. ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 8.00 (d, *J*=2.01 Hz, 1H), 7.94-7.98 (m, 1H), 7.83-7.89 (m, 1H), 7.48-7.59 (m, 2H), 6.66-6.78 (m, 1H), 4.31-4.39 (m, 2H), 4.22-4.3 (m, 2H), 4.00-4.21 (m, 4H), 3.06-3.15 (m, 2H), 1.63 (s, 6H), 1.30-1.40 (m, 3H).

Synthetic procedure for the synthesis of 5-chloro-3,3-dimethyl-2-[5-[2-(4-methylpyridine-3-carbonyl)-2,6-diazaspiro[3.3]heptan-6-yl]-3-pyridyl]isoindolin-1-one (61). To a solution of 5-chloro-2-[5-(2,6-diazaspiro[3.3]heptan-2-yl)-3-pyridyl]-3,3-dimethyl-isoindolin-1-one (intermediate **108**, 1.6 g, 4.3 mmol) and 4-methylpyridine-3-carboxylic acid (1.2 g, 8.7 mmol) in DCM was added HATU (3.2 g, 8.4 mmol) and DIPEA (2 g, 15.5 mmol) at 0 ^{o}C. The reaction mixture was allowed to warm up to room temperature and stirred overnight. Brine was added to quench the reaction and the mixture was extracted with DCM twice. The organic layers were combined, washed with brine, dried

over anhy. Na_2SO_4, filtered, and concentrated *in vacuo* to give a yellow oily residue, which was purified by silica gel flash chromatography to afford the title compound (732 mg, 35% yield) as a white foam. MS obsd (ESI+) [(M+H)$^+$] 488.1. HRMS calcd [(M+H)$^+$] 488.1853, measured [(M + H)$^+$] 488.1846. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.45-8.52 (m, 2H), 7.99 (d, *J*=1.51 Hz, 1H), 7.81-7.92 (m, 2H), 7.74 (s, 1H), 7.57-7.62 (m, 1H), 7.26-7.38 (m, 1H), 6.77-6.85 (m, 1H), 4.16-4.32 (m, 4H), 4.02-4.14 (m, 4H), 2.34 (s, 3H), 1.48 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 168.5, 166.9, 153.2, 150.7, 147.5, 147.0, 146.0, 139.3, 138.9, 134.3, 132.5, 129.5, 129.0, 128.5, 126.1, 125.6, 121.7, 119.3, 64.5, 62.2, 61.7, 58.4, 34.3, 26.7, 19.2.

For the following compounds, except those described specifically, all the analogs were prepared in analogy to compound **61** from either commercially available reagents or in-house prepared intermediates.

5-Chloro-3,3-dimethyl-2-[5-[2-(1-methylpyrazole-4-carbonyl)-2,6-diazaspiro[3.3]heptan-6-yl]-3-pyridyl]isoindolin-1-one (62). MS obsd (ESI+) [(M+H)$^+$] 477.1. HRMS calcd [(M+H)$^+$] 477.1805, measured [(M + H)$^+$] 477.1804. ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 7.98-8.03 (m, 1H), 7.92-7.96 (m, 1H), 7.82 (s, 2H), 7.71 (s, 1H), 7.44-7.53 (m, 2H), 6.65-6.71 (m, 1H), 4.31-4.67 (m, 4H), 4.16 (s, 4H), 3.95 (s, 3H), 1.56 (s, 6H). ^{13}C NMR (101 MHz, $CHCl_3$-*d*) δ ppm 166.9, 163.6, 153.2, 147.1, 139.3, 138.9, 138.8, 134.4, 132.5, 132.4, 129.0, 128.5, 125.6, 121.7, 119.3, 116.5, 64.5, 62.3, 39.3, 34.9, 29.6, 26.8.

***In vitro* Assay for the Determination of CYP11B2 and CYP11B1 Inhibition.** CYP11B1 and CYP11B2 proteins were expressed in a human renal leiomyoblastoma cell line (ATCC Number CRL-1440). qPCR revealed that these cells express FDXR and FDX, two proteins that are crucial for the enzymatic activity of CYP11B enzymes and importantly do not express detectable levels of CYP11B1 or CYP11B2 activity. The cells are grown in ATCC-formulated McCoy's 5a medium modified (catalog no. 30-2007) containing 10% fetal bovine serum. Stable cells expressing ectopically CYP11B1 or CYP11B2 from either human, cynomolgus monkey, or mouse were developed by transfecting

appropriate expression plasmids. The plasmids contain the cDNA (GeneCopoeia) for either CYP11B1 or CYP11B2 from the appropriate species (mouse, cynomolgus monkey, or man) under the control of a CMV promoter and the neomycin resistance marker. Using electroporation expression plasmid were transfected into cells, and these cells were then selected for expressing the given resistance markers. Individual cell-clones are then selected (400 µg/mL G-418; Geneticin) and assessed for displaying the desired enzymatic activity using 11-deoxycorticosterone (CYP11B2) or 11-deoxycortisol (CYP11B1) as a substrate. Cellular enzyme assays were performed in DMEM/F12 medium containing 2.5% charcoal treated FCS and appropriate concentration of substrate (1 µM 11-deoxycorticosterone or 11-deoxycortisol). For assaying enzymatic activity, cells were plated onto 96-well plates and incubated for 16 hr. An aliquot of the supernatant was then transferred and analyzed for the concentration of the expected product (aldosterone for CYP11B2; cortisol for CYP11B1). The concentrations of these steroids can be determined using HTRF (homogeneous time resolved fluorescence) assays from CisBio analyzing either aldosterone or cortisol. Inhibition of the release of produced steroids can be used as a measure of the respective enzyme inhibition by test compounds added during the cellular enzyme assay. The dose dependent inhibition of enzymatic activity by a compound is calculated by means of plotting added inhibitor concentrations (*x*-axes) vs. measured steroid/product level (*y*-axes). The inhibition is then calculated by fitting the following 4-parameter sigmoidal function [Morgan-Mercer-Flodin (MMF) model] to the raw data points using the least-squares method:

$$y = \frac{AB + Cx^D}{B + x^D}$$

Wherein, *A* is the maximum *y* value, *B* is the EC_{50} value determined using XLfit, *C* is the minimum *y* value and *D* is the slope value. The maximum value *A* corresponds to the amount of steroid produced in the absence of an inhibitor, and the value *C* corresponds to the amount of steroid detected when the enzyme is fully inhibited.

Microsomal Stability (LM). Each test compound was preincubated in an incubation mixture consisted of 0.5 mg of microsomal protein/mL liver microsomes, 1 mM NADP, 3 mM glucose 6-phosphate, 3

mM $MgCl_2$, and 0.05 mg/mL glucose 6-phosphate dehydrogenase in a total volume of 400 μL of potassium phosphate buffer (100 mM, pH 7.4) for 10 min at 37 °C. The reactions were initiated by the addition of NADPH regenerating system. At different time points (0, 3, 6, 9, 15, and 30 min), an aliquot (50 μL) sample was taken and quenched with 150 μL of acetonitrile containing an internal standard. Following precipitation and centrifugation, the supernatants were analyzed by LC/MS-MS.

log D Determination. Distribution coefficient of compounds was determined in a carrier mediated distribution system (CAMDIS, EP2005102211A) assay, as previously reported.[31]

Membrane Permeability. The permeability of compounds was determined via parallel artificial membrane permeation assay (PAMPA) using a "sandwich" construction. Sample stock solution (DMSO) was diluted to 150 μM (DMSO% < 2%) with donor buffer, and after the filtration the solution was added into donor plate. An acceptor plate coated with phospholipids (membrane) was placed onto the donor plate containing drug solution. Finally, the upper plate was filled with buffer solution. The donor concentration was determined at t-start as reference concentration. The drug concentrations in acceptor and donor plate were detected at t-end (~18 hr). Donor buffer is 0.05 M MOPSO buffer at pH 6.5 with 0.5% (w/v) glycocholic acid, acceptor buffer is 0.05 M MOPSO buffer at pH 6.5, and the membrane is 10% (w/v) egg lecithin with 0.5% (w/v) cholesterol in dodecane.

Solubility. The solubility of compounds was determined in a lyophilization solubility assay (LYSA). The test samples were prepared in duplicate from 10 mM DMSO stock solution. After evaporation of DMSO with a centrifugal vacuum evaporator, the compounds were redissolved in a phosphate buffer (0.05 M, pH 6.5). The mixture was stirred for 1 hr, shaken for 2 hr, and then allowed to stand overnight and filtered through a microtiter filter plate. The filtrate and its 1/10 dilution were analyzed by HPLC-UV. Solubility determination was determined from a four-point calibration curve; the percentage of sample measured in solution after evaporation divided by the calculated maximum of sample amount

was bigger than 80%; and the solubility data were reported as bigger than this value, with a unit of μg/mL.

Plasma Protein Binding. The percentage of unbound compound was determined using a 96-well Micro-Equilibrium dialysis device (HTDialysis, Gales Ferry, CT, USA) with molecular weight cutoff membrane of 12-14 kDa (HTDialysis, Gales Ferry, CT, USA) and using Diazepam as a positive control. Pooled rat and cynomolgus monkey plasma were purchased from Biopredic (Rennes, France). Compounds were measured in a cassette of 2-5 with an initial total concentration of 1 μM, and one of the cassette compounds is the positive control. The integrity of membranes was validated by confirming the unbound fraction values of the positive control. Equal volumes of blank dialysis buffer (Soerensen buffer at pH 7.4) and matrix samples containing substances were loaded into the acceptor and donor compartment, respectively. The HTD dialysis block was then sealed and kept in an incubator at 37 °C for 5 hr under 5% CO_2 environment. At the end of dialysis, the plasma and buffer samples were retrieved and the drug concentrations were quantified by LC/MS-MS.

Rat PK. All studies conducted were approved by the Institutional Animal Care and Use Committee (IACUC) of Roche Pharma Research and Early Development, Roche Innovation Center Shanghai. The single dose PK in male Wister-Han rats was performed to assess their pharmacokinetic properties. Compounds were dissolved in NMP:NS (v/v, 30/70) to yield a final concentration of 0.5 mg/mL for iv dose (1 mg/kg); and in a vehicle containing 2% Klucel and 0.1% Polysorbate 80 to yield a final concentration of 0.25 mg/mL for oral dose (3 mg/kg). Three rats in each group were used for blood collect at each time point. Blood samples were collected at eight time points (iv) and seven time points (po) post-dose into sodium heparin centrifuge tubes, and plasma samples were then isolated by centrifugation and stored at -20 °C prior to analysis. Plasma concentrations were determined by LC/MS-MS, and the data were analyzed by non-compartmental methods using WinNonlin® Pro (Pharsight Corp., Mountain View, CA, USA).

Experimental Details for Protein Crystallization and Structure Determination of CYP11B2 with compounds 45 and 58. CYP11B2 protein was overexpressed in *E. coli* and purified with nickel affinity and ion exchange chromatography. The ligand complex was co-crystallized by vapour diffusion in sitting drops with 10% PEG3350 in 90 mM ammonium citrate (pH 7.0). Thin plates grew after several days and were flash-frozen after addition of 20% ethylene glycol. X-ray diffraction images were collected at 100 K at the beam line X10SA (PXII) of the Swiss Light Source (SLS) using a Pilatus pixel detector.

Experimental Details for *in vivo* Cynomolgus Monkey Experiments. Animal work was performed according to Swiss federal law for animal protection and was approved by the Veterinary Office Basel. Before use, animal were provided an acclimation period of > 5 days under conventional hygienic conditions for housing and standard diet. To prepare for the in vivo experiment, non naive grouped male cynomolgus monkeys (*Macaca fascicularis*) were kept in cages on a 12 hr light-dark cycle with natural light in 50-60% humidity and 22.5 to 22.8 ^{o}C. Animals received the diet 3448 EM S12 (80 g 7 kg^{-1} day^{-1} animal^{-1}; Provimi-Kliba, Kaiseraugst, Switzerland) 2-3 times daily, on top of daily fruits, vegetables and nuts. Water access was ad libitum. Animals identified as good Synacthen (Novartis, Basel, Switzerland) responders participated in the study under the following conditions: at least, a 2-fold increase of plasma aldosterone, cortisol and corticosterone production should be measured 1 hr after intramuscular acute application of Synacthen. On the day of the experiment, the monkeys were moved from their group cages and individually housed in stainless-steel mesh cages (*H* 1.5 m x *L* 1.2 m x *W* 0.8 m) according to the Swiss regulation on animal welfare. For oral gavage (po) all samples were analyzed using routine protein precipitation technique followed by LC/MS-MS analysis. PK parameters were calculated using non-compartmental analysis (**Figure 6**). Oral treatment and Synacthen were given as follows: *T* = 0 min, oral dosing of 2 monkeys per group (vehicle or compound **52**) with compound administered as suspension in hydroxyethylcellulose, polysorbate 80, methyparaben and propylparaben vehicle using 2 mL/kg administration volume; *T* = 1 hr, intramuscular application of 14.5 µg/kg Synacthen. Blood samples were collected -5 min, 30 min, 55 min, 1.5 h, 2 h, 3 h, 5 h, 7 h,

and 24 h after compound or vehicle application. The selected animals were cooperative and trained to donate blood voluntarily. At each time point, 1.2 mL blood was collected in EDTA-coated tubes (BD Vacutainer K2 EDTA, Becton Dickinson, Franklin Lakes, NJ, USA) for plasma mineral corticoids and compound concentration measurements. Blood was centrifuged at 10 000 rpm for 15 min at 4 °C. Subsequently, plasma was separated and stored at -20 °C for further analysis. Plasma steroids, % inhibition of CYP11B1 and CYP11B2 and exposure data for compound **52** were analyzed from all time points by LC/MS-MS methodology (see SI).

ASSOCIATED CONTENT

Supporting Information

Structures of selected reference inhibitors; experimental procedures for the syntheses of key intermediates; ^{1}H NMR, ^{13}C NMR, and LC-MS spectra of compounds **52** and **62**; relative configuration assignment of compounds **29-38**; analytical method for the measurement of steroid concentrations in cynomolgus monkey plasma; selectivity assessment of steroid production in potassium induced Hek293 cells; molecular formula strings of compounds **1-62**. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The PDB codes for **45** and **58** are 6XZ8 and 6XZ9, respectively. Authors will release the atomic coordinates and experimental data upon article publication.

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Author Contributions

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

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

MR, mineralocorticoid receptor; *b.i.d.*, twice (two times) a day; SAD, single ascending dose; CYP, cytochrome P450 enzymes; PDB, protein data bank; Pe, permeability; SDPK, single-dose pharmacokinetics; PK, pharmacokinetics; PKPD, pharmacokinetic pharmacodynamic; PPA, polyphosphoric acid; NBS, N-bromosuccinimide; BPO, benzoyl peroxide; PMB, para-methoxylbenzyl; CAN, cerium ammonium nitrate; TBAF, tetrabutylammonium fluoride; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DMAP, 4-dimethylaminopyridine; LDA, Lithium diisopropylamide; HATU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate; XPhos, 2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl; TFA, trifluoroacetic acid; rt, room temperature.

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