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Brief Article

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Novel Imidazol-1-ylmethyl Substituted 1,2,5,6-Tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-ones as Potent and Selective CYP11B1 Inhibitors for the Treatment of Cushing's Syndrome

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Supporting Information

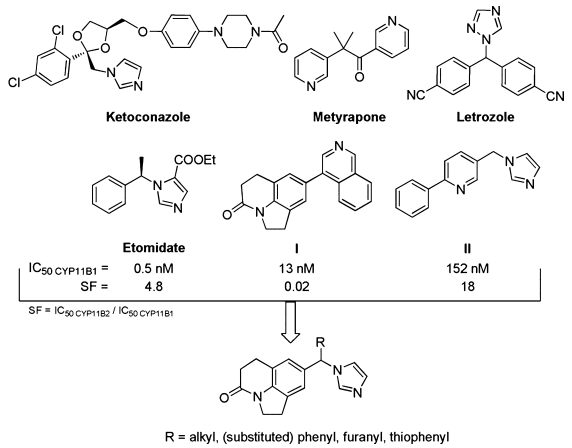
ABSTRACT: CYP11B1 inhibition is a promising therapy for Cushing's syndrome. Starting from etomidate, references **I** and **II**, the title compounds were designed and synthesized. Cyclopropyl analogue **4** was identified as a CYP11B1 inhibitor more potent (IC_{50} = 2.2 nM) than leads and more selective (SF = 11) than **I** and metyrapone. Since it also showed potent inhibition of rat CYP11B1 and good selectivity over human CYP17 and CYP19, it is a promising candidate for further development.

INTRODUCTION

Cortisol is a principal human glucocorticoid exhibiting many important physiological functions. It is involved in the regulation of the metabolism of proteins, carbohydrates, and fats; it counteracts insulin, maintains blood pressure and cardiovascular function, and suppresses the immune system's inflammatory response. Cortisol is biosynthesized in the adrenal cortex with the final conversion from 11-deoxycortisol catalyzed by steroid 11β-hydroxylase (CYP11B1) (steroidogenesis cascade is detailed in ref 6c). Normally, the production and secretion of cortisol are precisely controlled by adrenocorticotropic hormone within the negative feedback cycle of hypothalamic−pituitary−adrenal axis. However, pathological changes in adrenal and the upstream regulating switches can cause an overproduction of cortisol, which is known as Cushing's syndrome (also termed hypercortisolism). In addition to symptoms like central obesity, headache, and depression in patients with hypercortisolism, overproduction of cortisol is associated with hypertension and diabetes mellitus type II,[1] which consequently leads to increased mortality.

CYP11B1 inhibition as the pharmacological approach to block cortisol biosynthesis is a promising alternative to the surgical removal of adrenal or pituitary tumors for the treatment of hypercortisolism. Currently, inhibitors of adrenal steroidogenesis employed in clinics include ketoconazole, etomidate, and metyrapone (Chart 1). Among them, the antifungal agent ketoconazole (inhibiting CYP11B1, CYP11B2, and CYP17) and the anesthetic etomidate (inhibiting CYP11B1 and CYP11B2) are used as multiple enzyme inhibitors. Thus, their use is accompanied by severe side effects,[2] whereas metyrapone is the only drug being reported as a relatively selective CYP11B1 inhibitor.[2] Studies have demonstrated that metyrapone can effectively reduce cortisol levels in patients with all types of Cushing's syndrome.[3] However, long-term administration of metyrapone results in nausea, dizziness, skin rash, edema, acne, and hirsutism.[4] This absence of effective and safe therapy for high cortisol levels encourages scientists to design novel CYP11B1 inhibitors that are more potent and selective.

Chart 1. Structures of Ketoconazole, Metyrapone, Letrozole, and Etomiate and Drug Design Conception



Many reversible CYP inhibitors have been successfully developed based on a sp^2 hybrid nitrogen coordinating to the heme iron (e.g., inhibitors of CYP11B2,[5] CYP17,[6] and CYP19[7]). This mode of action is an important contributor to high potency, impairing both substrate binding and oxygen coordinate to the active site. However, the selectivity is a challenging goal to achieve, especially in the case of CYP11B2, which is crucial in aldosterone production, because of the high sequence homology of more than 93% shared between CYP11B1 and CYP11B2.[5c] The inhibition of other important steroidogenic enzymes, such as CYP17 and CYP19, should also

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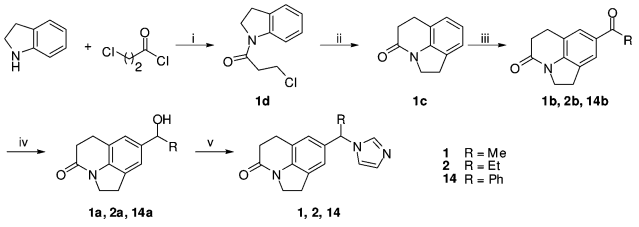
be avoided because this would result in a decrease of androgen or estrogen, respectively, leading to severe undesired effects. Recently compounds originating from etomidate were reported by Zolle et al.[8a] and us[9] to be potent CYP11B1 inhibitors.

In the present study, three leads were employed to design novel inhibitors (Chart 1) including potent CYP11B1 inhibitor etomidate (IC_{50} = 0.5 nM [9a]) and reference compound **I** (IC_{50} = 13 nM), which we have described previously as a CYP11B2 inhibitor,[5e] and a modest (IC_{50} = 152 nM) but selective (selectivity factor SF = 18 = $IC_{50\ CYP11B2}/IC_{50\ CYP11B1}$) CYP11B1 inhibitor reference compound **II**.[9a] The replacement of phenyl moiety of the etomidate scaffold by tetrahydropyrroloquinolinone group from reference **I** led to a series of imidazol-1-ylmethyl substituted 1,2,5,6-tetrahydropyrrolo-[3,2,1-*ij*]quinolin-4-ones. Not only would this hybrid inherit the potent inhibition of CYP11B1 but also the replacement of phenyl by tetrahydropyrroloquinolinone, which is more bulky, should make the molecule difficult to permeate through the blood–brain barrier, thereby avoiding undesired anesthetic effects, which is one of the bioactivities of etomidate. The introduction of the methylene bridge between the core and N-containing heterocycle, as present in etomidate and reference **II**, was deemed to be advantageous for the selectivity over CYP11B2, as we have shown in the class of imidazolyl methylene phenylpyridines.[9] In contrast, the ester group from etomidate was removed because it has been demonstrated to be deleterious for that selectivity.[8b] After optimization of the substituents at the methylene bridge with various functional groups differing in electronic properties and bulkiness, such as aliphatic groups, substituted phenyls, and heteroaryls, the selectivity should be further improved. Besides evaluation of CYP11B1 inhibition and selectivity toward CYP11B2, selected potent and selective compounds were further tested for CYP17 and CYP19 inhibition. Furthermore, biological activities of the enantiomers of **4** were also investigated.

RESULTS AND DISCUSSIONS

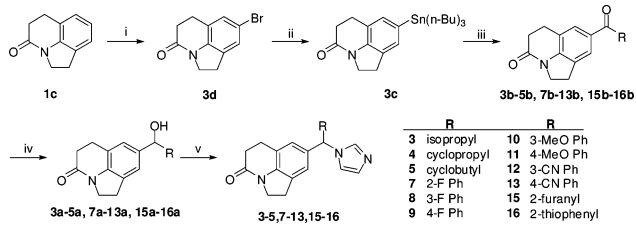
Chemistry. The syntheses of **1**–**17** are shown in Schemes 1–3. A common synthetic route was exploited for the final compounds **1**–**5** and **7**–**16** (Schemes 1 and 2). The tetrahydropyrroloquinolinone core **1c**, as the common intermediate, was synthesized from commercially available 2,3-dihydro-1*H*-indole via N-acylation with 3-chloropropionyl chloride and subsequent cyclization with $AlCl_3$ under molten conditions. **1c** was subsequently converted into substituted ketone intermediates, from which the final compounds were yielded after further reduction using sodium borohydride and thereafter the introduction of imidazoyl with 1,1′-sulfonyldiimidazole in THF. The ketone intermediates were prepared via two different approaches. The introduction of acetyl (**1b**), propanoyl (**2b**), and benzoyl (**14b**) was accomplisheded with Friedel–Crafts acylation (Scheme 1). For the other ketones the core was first brominated with NBS and subsequently reacted with bis(tributyltin) to afford key intermediate **3c**, which was subsequently coupled with various acyl chlorides to give the corresponding ketones **3b**–**5b**, **7b**–**13b**, and **15b**–**16b** (Scheme 2). To abrogate the chiral center, **1b** was reacted with excess 1,1′-sulfonyldiimidazole to yield the vinyl analogue **6** as the minor product and the diimidazolyl **17** as the major one.

Scheme 1[a]



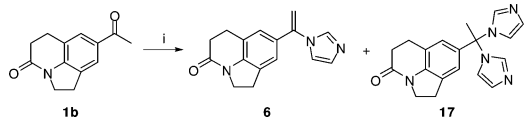
[a]Reagents and conditions: (i) pyridine, THF, 0 °C; (ii) $AlCl_3$, 140 °C. (iii) Method A: RCOCl, $AlCl_3$, CH_2Cl_2, reflux, then 6 N aq HCl. (iv) Method B: $NaBH_4$, MeOH, 0 °C. (v) Method C: imidazole, $SOCl_2$, THF, room temp.

Scheme 2[a]



[a]Reagents and conditions: (i) NBS, DMF, 0 °C; (ii) Sn_2Bu_6, $Pd(PPh_3)_4$, toluene. (iii) Method D: RCOCl, $PdCl_2(PPh_3)_2$, toluene. (iv) Method B: $NaBH_4$, MeOH, 0 °C. (v) Method C: imidazole, $SOCl_2$, THF, room temp.

Scheme 3[a]



[a]Reagents and conditions: (i) imidazole, $SOCl_2$, CH_2Cl_2, room temp.

Inhibition of Human CYP11B1 and CYP11B2. The synthesized compounds were evaluated for their inhibitory activities in V79 MZh cells expressing human CYP11B1 or CYP11B2.[10] IC_{50} values are presented in comparison to leads **I** and **II**, etomidate, and another CYP11B1 inhibitor metyrapone (Table 1).

Most of the compounds showed strong inhibition of CYP11B1, with IC_{50} ranging from 1.4 to 50 nM. It is apparent that analogues with aliphatic chains substituting at the methylene bridge (**1**–**6**, IC_{50} < 20 nM) exhibited more potent inhibition than compounds substituted with aromatic rings (**7**–**17**, IC_{50} of 13–144 nM). All aliphatic analogues **1**–**5** showed IC_{50} of less than 10 nM except for **1** (IC_{50} = 17 nM). A trend can be observed despite of feeble potency difference that as the bulkiness of aliphatic substituents increased (Me < Et < cyclopropyl < cyclobutyl < isopropyl),[10] the potency of corresponding compounds increased accordingly. Thus, the isopropyl **3** turned out to be the most potent CYP11B1 inhibitor in this study, being 10-fold more potent than metyrapone (IC_{50} = 15 nM). However, **3** also exhibited strong inhibition of CYP11B2 (IC_{50} = 3.8 nM), thereby leading to poor selectivity (SF = 2.7). In contrast, **4** exceeded metyrapone in terms of selectivity (SF of 11 and 4.8 for **4** and metyrapone, respectively) and inhibitory potency (IC_{50} = 2.2 nM). After the modification from methyl group to methylidene (**6**), similar potency was observed with improved selectivity (SF = 5.3 vs 0.9).

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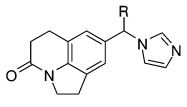
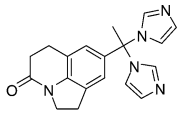
Table 1. Inhibition of CYP11B1 and CYP11B2 by 1−17



1-16



17

compd	R	IC_{50} [a] (nM) CYP11B1[b]	IC_{50} [a] (nM) CYP11B2[c]	SF [d]
1	Me	17	15	0.9
2	Et	6.5	5.8	0.9
3	*i*-propyl	1.4	3.8	2.7
4	*c*-propyl	2.2	24	11
(+)-**4**		10.3		
(−)-**4**		1.5		
5	*c*-butyl	3.4	4.2	1.2
6	$=CH_2$	19	100	5.3
7	2-F Ph	40	19	0.5
8	3-F Ph	29	18	0.6
9	4-F Ph	27	29	1.1
10	3-MeO Ph	110	88	0.8
11	4-MeO Ph	40	104	2.6
12	3-CN Ph	144	304	2.1
13	4-CN Ph	70	308	4.4.
14	Ph	50	33	0.7
15	2-furanyl	20	47	2.4
16	2-thienyl	13	18	1.4
17		438	851	1.9
I		13	0.2	0.02
II		152	2768	18
etomidate		0.5	0.1	0.5
metyrapon		15	72	4.8

[a]Mean value of at least three experiments, relative standard deviation less than 25%. Inhibition curves show no significant difference from unity. [b]Hamster fibroblasts expressing human CYP11B1. Substrate: deoxycorticosterone, 100 nM. [c]Hamster fibroblasts expressing human CYP11B2. Substrate deoxycorticosterone, 100 nM. [d]$IC_{50\ CYP11B2}/IC_{50\ CYP11B1}$.

However, further modification by introducing a phenyl group onto the methylene bridge (**14**) led to a slight decrease in inhibition (IC_{50} = 50 nM). Moreover, F substitution of the phenyl ring (**7**−**9**) led to a slight improvement of inhibitory potency (IC_{50} from 27 to 40 nM) compared to nonsubstituted **14**, whereas analogues substituted with MeO or CN (**10**−**13**) exhibited similar or reduced inhibitory activity (IC_{50} from 40 to 144 nM). Furthermore, the position of the substituents is also a determinant of inhibitory potency. Analogues with para-substituted phenyl are more potent than the ones with meta-substitution, especially for MeO and CN, whereby para-substituted analogues **11** and **13** (IC_{50} of 40 and 70 nM, respectively) are twice as potent as the corresponding meta-substituted **10** and **12** (IC_{50} of 110 and 144 nM, respectively).

Interestingly, replacement of the phenyl group by its less bulky bioisosteres 2-furanyl (**15**) and 2-thienyl (**16**) elevated the inhibition of CYP11B1 to 20 and 13 nM, respectively. However, analogue **17** with a methyl and an additional imidazolyl group at the methylene bridge exhibited a dramatic decrease in inhibitory activity (IC_{50} = 438 nM).

Compound **4** as the most selective one (SF = 11) in this study is more potent and selective toward CYP11B1 than parent reference **I** (SF = 0.02). Although **4** is slightly less selective than reference **II** (SF = 18), it is much more potent (IC_{50} of 2.2 nM vs 152 nM). This can probably be attributed to the introduction of the methylene bridge between the tetrahydropyrroloquinolinone core and the N-containing heterocycle and also probably to the replacement of isoquinolinyl by imidazolyl.

Biological Difference between Enantiomers. To investigate the possible influence that different configurations may show on the inhibitory potency, racemic **4** was resolved by chiral HPLC. It turned out that enantiomer (−)-**4** is about 6-fold more potent than (+)-**4** toward CYP11B1 (IC_{50} of 1.5 nM vs 10.3 nM), which indicates the presence of a hydrophobic cavity close to the heme that is large enough to accommodate the *c*-propyl group of both configurations. The presence of such hydrophobic subpockets in both CYP11B isoforms is supported by pharmacophore and homology models.[5]

Selectivity against Human CYP17 and CYP19. Since CYP17 and CYP19 are crucial enzymes involved in androgen and estrogen biosynthesis, respectively, the selectivities for four of the most potent and/or selective compounds against these two enzymes were tested as criteria to evaluate safety (Table 2).

Table 2. Inhibition of CYP17 and CYP19 by Selected Compounds

compd	CYP17[a] IC_{50} [c] (nM)	SF [d] over CYP17	CYP19[b] IC_{50} [c] (nM)	SF [e] over CYP19
3	1550	1107	>500	>357
4	2880	1309	228	104
6	>5000	>260	17	0.9
11	>5000	>125	43	1.1

[a]*E. coli* expressing human CYP17; substrate progesterone, 25 μM; abiraterone, IC_{50} = 72 nM. [b]Human placental CYP19; substrate androstenedione, 500 nM; inhibitor concentration 500 nM; fadrozole IC_{50} = 41 nM. [c]Mean value of at least three experiments, standard deviation less than 10%. [d]$IC_{50\ CYP17}/IC_{50\ CYP11B1}$. [e]$IC_{50\ CYP19}/IC_{50\ CYP11B1}$.

As can be seen, **3** and **4** exhibited weak CYP17 inhibition with IC_{50} values over 1500 nM, whereas **6** and **11** were even less active (IC_{50} > 5000 nM). Moreover, because of the structural similarity of the synthesized compounds to the CYP19 inhibitors, like letrozole (Chart 1), the inhibition of CYP19 is not a surprise. Compounds **6** and **11** showed strong inhibition with IC_{50} of 17 and 43 nM (Table 2), respectively. In contrast, **3** and **4** with alkyl groups exhibited only modest inhibition of CYP19 (IC_{50} > 200 nM, Table 2) resulting in good selectivity factors ($IC_{50\ CYP19}/IC_{50\ CYP11B1}$) of more than 357 for **3** and 104 for **4**.

Inhibition of Rat CYP11B1 Enzyme. Compound **4** showed 91% inhibition of rat CYP11B1 at 2 μM, in comparison to 31% for reference **I**. This improvement of rat CYP11B1 inhibition facilitates further evaluation in vivo.

CONCLUSION

Starting from three leads (etomidate, references **I** and **II**), novel imidazol-1-ylmethyl substituted 1,2,5,6-tetrahydropyrrolo-[3,2,1-*ij*]quinolin-4-ones were designed and synthesized as potent and selective CYP11B1 inhibitors for the treatment of Cushing's syndrome. The replacement of the phenyl ring in the etomidate scaffold by tetrahydropyrroloquinolinone moiety from reference **I** sustained the potent inhibition of CYP11B1, while it decreased the CYP11B2 inhibition. It has been shown that the compounds with aliphatic substituents at the

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methylene bridge are more potent than phenyl analogues, while meta-substitution increased inhibition compared to para-substitution for these phenyl compounds. Furthermore, **4** was identified as a CYP11B1 inhibitor more potent (IC_{50} = 2.2 nM) than leads **I** and **II** and more selective (SF = 11) than reference **I** and metyrapone. Since **4** also showed improved inhibition of rat CYP11B1 and good selectivity over CYP17 and CYP19, it is considered to be a promising candidate for further evaluation in the rat.

EXPERIMENTAL SECTION

Chemistry. The purities of the final compounds were greater than 95%.

Method C: Introduction of Imidazoyl Moiety. A solution of the obtained alcohol (1.0 equiv) in dry THF was added to a solution of thionylbis(imidazole) (prepared previously by reaction of imidazole (16 equiv) with thionyl chloride (4.0 equiv) in dry THF and filtration to remove precipitated imidazole hydrochloride) at 0 °C. The mixture was stirred for 1 h at 0 °C and an additional 18−30 h at ambient temperature. Water was added and the mixture extracted with ethyl acetate three times. The combined organic extracts were washed with water and brine, and the solvent was evaporated in vacuo after drying over $MgSO_4$. The crude product was purified by flash chromatography to yield the corresponding product.

8-[Cyclopropyl(1*H*-imidazol-1-yl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (4). The title compound was synthesized according to method C using **4a** (0.56 g, 2.30 mmol), imidazole (1.88 g, 27.6 mmol), $SOCl_2$ (0.50 mL, 6.90 mmol), and dry THF (20 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 1:50) to yield white crystals (0.50 g, 74%), mp 146−147 °C, R_f = 0.14 ($MeOH/CH_2Cl_2$; 1:20). ^1H NMR (500 MHz, $CDCl_3$): δ 0.42−0.50 (m, 2H), 0.76−0.85 (m, 2H), 1.46−1.54 (m, 1H), 2.66 (t, J = 7.8 Hz, 2H), 2.93 (t, J = 7.8 Hz, 2H), 3.15 (t, J = 8.5 Hz, 2H), 4.08 (t, J = 8.5 Hz, 2H), 4.30 (d, J = 9.3 Hz, 1H), 6.82 (s, 1H), 6.90 (s, 1H), 6.95 (s, 1H), 7.07 (s, 1H), 7.67 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 4.8, 5.3, 16.6, 24.4, 27.7, 31.5, 45.4, 66.3, 118.3, 120.3, 121.8, 124.1, 129.3, 129.4, 135.6, 136.4, 141.4, 167.5. MS (ESI) m/z = 226 [M − imidazole]$^+$. HRMS (ESI): calcd for $C_{18}H_{19}N_3O$ [M + H]$^+$, 294.1562; found, 294.1558. The racemate **4** was separated by preparative HPLC on chiral stationary phase (45% hexane/ethanol, 1.0 mL/min) to yield (+)-**4** ($[\alpha]_D^{20}$ +3.5 (c 1.0, DMSO); 99.6% ee and t_R = 19.2 min by analytical HPLC using 30% hexane/ethanol) and (−)-**4** ($[\alpha]_D^{20}$ −4.0 (c 1.0, DMSO); 93.3% ee and t_R = 22.9 min by analytical HPLC using 30% hexane/ethanol).

ASSOCIATED CONTENT

Supporting Information

Experimental details and characterization of all intermediates, HPLC purities of all final compounds, ^1H and ^{13}C NMR of **1**−**3** and **5**−**17**, resolution of racemate **4**, and a description of biological tests. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

CYP, cytochrome P450; CYP11B1, 11β-hydroxylase; CYP11B2, aldosterone synthase; CYP17, 17α-hydroxylase-17,20-lyase; CYP19, aromatase; SF, selectivity factor; ee, enantiomeric excess

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