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Identification of 4-(4-nitro-2-phenethoxyphenyl)pyridine as a promising new lead for discovering inhibitors of both human and rat 11β-Hydroxylase

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ABSTRACT

The inhibition of 11β-hydroxylase is a promising strategy for the treatment of Cushing's syndrome, in particular for the recurrent and subclinical cases. To achieve proof of concept in rats, efforts were paid to identify novel lead compounds inhibiting both human and rat CYP11B1. Modifications on a potent promiscuous inhibitor of *h*CYP11B1, *h*CYP11B2 and *h*CYP19 (compound **IV**) that exhibited moderate *r*CYP11B1 inhibition led to compound **8** as a new promising lead compound. Significant improvements compared to starting point **IV** were achieved regarding inhibitory potency against both human and rat CYP11B1 (IC_{50} values of 2 and 163 nM, respectively) as well as selectivity over *h*CYP19 (IC_{50} = 1900 nM). Accordingly, compound **8** was around 7- and 28-fold more potent than metyrapone regarding the inhibition of human and rat CYP11B1 and exhibited a comparable selectivity over *h*CYP11B2 (SF of 3.5 vs 4.9). With further optimizations on this new lead compound **8**, drug candidates with satisfying profiles are expected to be discovered.

1. Introduction

Although endogenous Cushing's syndrome is a rare disease with an annual incidence as low as 2–3 cases per million of the population [1], it leads to a high mortality via co-morbidities including cardio- and cerebrovascular diseases [1,2]. As for the majority of this syndrome that are caused by pituitary adenomas and termed Cushing's disease, the 5 years' survival is only about 50% if without effective treatments [3]. Despite of the fact that the surgical removal of tumors leads to cure or remission in 65–85% of patients, recurrences are observed in up to 20% of cases [1]. Furthermore, investigations indicate a high prevalence of undiagnosed subclinical Cushing's syndrome in particular in patients with type II diabetes and osteoporosis [4,5]. Although no evident symptoms other than high levels of cortisol in plasma are observed in these patients, this syndrome is considered to exacerbate concomitant diseases. More severe is that subclinical Cushing's syndrome is deemed to be associated with metabolic dementia and to promote the progression of Alzheimer disease [6]. Apparently, for these recurrent and subclinical cases, pharmacotherapy via the inhibition of steroid 11β-hydroxylase (*h*CYP11B1), which catalyzes the hydroxylation of 11-deoxycortisol to cortisol, to reduce the circulating cortisol levels is a superior approach. Metyrapone (IC_{50} = 15 nM, Chart 1) as an inhibitor of adrenal steroidogenesis has been employed to relieve patients' symptoms before surgery [7]; and its long-term applications were also reported to successfully control cortisol levels and psychiatric manifestations [8,9]. In a phase I clinical study, an experimental *h*CYP11B1 inhibitor LCI699 (IC_{50} = 2.9 nM, Chart 1) normalized urinary free cortisol levels or reduced its concentrations by more than half from baseline in patients with moderate-to-severe Cushing's disease [10]. However, simultaneously, the plasma aldosterone levels were significantly decreased by around 3-fold from 4.2 to 1.3 ng/dL because of the potent inhibition of aldosterone synthase (*h*CYP11B2) by LCI699 (IC_{50} = 0.2 nM). Although this inhibition contributed to the reduction of both systolic and diastolic blood pressures (10.0 and 6.0 mmHg, respectively), it, together with the twofold elevated adrenocorticotropic hormone in plasma that

Abbreviations: CYP, cytochrome P450; *h*CYP11B1, steroid 11β-hydroxylase; *h*CYP11B2, aldosterone synthase; *h*CYP17, 17α-hydroxylase-17,20-lyase; *h*CYP19, aromatase; SF, selectivity factor = $IC_{50\ hCYP11B2}/IC_{50\ hCYP11B1}$.

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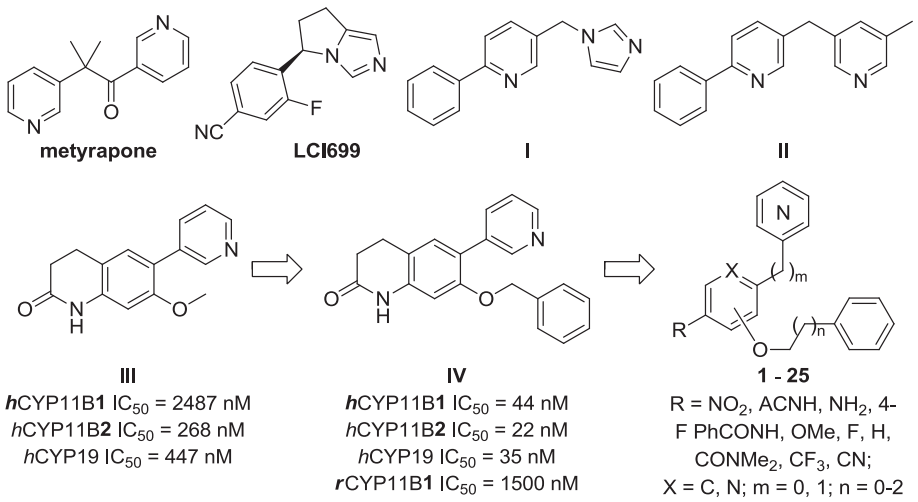

Chart 1. The structures of typical *h*CYP11B1 inhibitors (Metyrapone, LCI699, compounds **I** and **II**) as well as lead compounds **III**, **IV** and designed compounds **1**–**25**.

is the response to the drop of cortisol levels, boosted the concentrations of its precursor 11-deoxycorticosterone by 42-fold from 3.5 to 147.5 ng/dL. The binding of these over-produced 11-deoxycorticosterone to mineralocorticoid receptors induced around 8% reduction of the plasma potassium levels and consequently hypokalemia in one-third (4 out of 12) of patients. As evidenced by the decreased plasma renin levels (13–8 ng/L), which remained suppressed two weeks after the treatment discontinuation, the renin–angiotensin–aldosterone system was impaired. Therefore, the selectivity over *h*CYP11B2 is considered to be a crucial criterion for safety when treating Cushing's syndrome via *h*CYP11B1 inhibition. Besides this indication, topical application of *h*CYP11B1 inhibitors has also been proposed as a novel strategy to promote the healing of chronic wounds [11] based on the observation that cutaneously over-expressed cortisol impairs fibroblast proliferation and collagen synthesis, and thus induces chronic wounds and ulcers [12]. Cortisol also stimulates the proliferation of prostate cancer cells via certain mutated androgen receptors [13], therefore the combinatory application of *h*CYP11B1 inhibitors [14] or dual inhibitors of 17α-hydroxylase-17,20-lyase (*h*CYP17) and *h*CYP11B1 [15] could be superior approaches to improve the survival of prostate cancer patients. Since several other steroidogenic cytochrome P450 (CYP) enzymes including *h*CYP11B2, *h*CYP17 and aromatase (*h*CYP19) catalyze the biosynthesis of important steroidal hormones (mineralocorticoids, androgens and estrogens, respectively), drug candidates inhibiting *h*CYP11B1 ought to show adequate selectivity over these enzymes, in particular *h*CYP11B2 (as indicated by the results of the clinical trial of LCI699). The selectivity between *h*CYP11B1 and *h*CYP11B2 is especially challenging to achieve because of the high homology (>93%) between these two enzymes. Despite of this difficulty, potent and selective inhibitors of *h*CYP11B2 [16–21] were discovered recently. Aided by our broad experience in developing selective inhibitors of steroidogenic CYP enzymes, including *h*CYP11B2 [22–26], *h*CYP17 [27–33] and *h*CYP19 [34–38], several classes of potent *h*CYP11B1 inhibitors were identified [39–41]. Examples like compounds **I** (IC_{50} = 107 nM) [45] and **II** (IC_{50} = 2 nM) [41] exhibited both potent inhibition of *h*CYP11B1 (Chart 1 & Table 1) and promising selectivity over other CYP enzymes (for *h*CYP11B2, selectivity factors ($SF = IC_{50\ hCYP11B2}/IC_{50\ hCYP11B1}$) around 15 were observed). However, these compounds showed no (**I**, IC_{50} > 10,000 nM) to low (**II**, IC_{50} = 2440 nM) inhibition toward rat CYP11B1 (Table 1), which makes it difficult to prove the concept in rats. Therefore, discovery of compounds with novel structures inhibiting both human and rat CYP11B1 is necessary and urgent. In this study, we describe our efforts to identify new lead compounds exploiting previously acquired knowledge on developing selective inhibitors of steroidogenic CYP enzymes.

2. Results

2.1. Design concept

In our previous investigation of pyridyl substituted 3,4-dihydroquinolin-2(*1H*)-ones as potent inhibitors of *h*CYP11B2, an interesting SAR was observed that the bulkiness augment of the alkoxy substituent at the 7-position largely increased the *h*CYP11B1 inhibition [42]. The benzyloxy compound **IV** exhibited a 56-fold stronger *h*CYP11B1 inhibition than the corresponding methoxy analog **III** (IC_{50} = 2487 nM) with an IC_{50} value of 44 nM (Chart 1). Although the inhibition of *h*CYP11B2 was accordingly enhanced to 22 nM thus only showing an inadequate selectivity factor of 0.5 for *h*CYP11B1, a trend of reverse preference between *h*CYP11B1 and *h*CYP11B2 inhibition was anticipated when comparing it to that of compound **III** (SF = 0.1). More important is that compound **IV** showed a moderate inhibition of *r*CYP11B1 (IC_{50} = 1500 nM), which is apparently superior to the clinical used drug metyrapone (IC_{50} = 4607 nM) and the previously identified potent *h*CYP11B1 inhibitors **I** (IC_{50} > 10,000 nM) and **II** (IC_{50} = 2440 nM) (Chart 1 & Table 1). Due to the difficulty of identifying inhibitors of both human and rat CYP11B1 to facilitate the proof of concept in rats, compound **IV** was considered as a promising starting point for further modifications. However, as compound **IV** actually originated from a series of dual inhibitors of *h*CYP11B2 and *h*CYP19 [42,43], it showed significant inhibition of *h*CYP19 as well (IC_{50} = 35 nM). Therefore, besides elevating the potencies against human and rat CYP11B1, improvement of the selectivity over both *h*CYP11B2 and *h*CYP19 is another challenge encountered. To solve these problems, modifications were performed as shown in Chart 1. Since the 3,4-dihydroquinolin-2(*1H*)-one core was regarded as a privileged structure for *h*CYP11B2 inhibition [44], the lactam ring was opened and simplified into a phenyl substituted by various groups. As the insertion of a nitrogen atom into the core was demonstrated to be beneficial for the selectivity over *h*CYP11B2 [45], a pyridyl was also attempted replacing the benzene core. Based on the comparison between compounds **III** and **IV**, the