Novel functionalized 5-(phenoxymethyl)-1,3-dioxane analogs exhibiting cytochrome P450 inhibition: a patent evaluation WO2015048311 (A1)

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Abstract

Cytochrome P450's (CYP's) constitute a diverse group of over 500 monooxygenase hemoproteins, catalyzing transformations that involve xenobiotic metabolism, steroidogenesis and other metabolic processes. Over-production of the steroid hormone cortisol is implicated in the progression of diseases such as diabetes, heart failure and hypertension, stroke, Cushing's syndrome, obesity and renal failure, among others. The biosynthesis of cortisol involves a cascade of cholesterol metabolizing reactions regulated through three major CYP proteins: 17α–hydroxylase-C17/20-lyase (CYP17), 21-hydroxylase (CYP21), and 11β-hydroxylase (CYP11B1). Excess activities of these enzymes are linked to the progression of malignancies including prostate, breast, ovarian, and uterine cancers. A series of novel functionalized dioxane analogs have been developed and recently patented as CYP17, CYP21, and CYP11B1 inhibitors, which lead to the modulation of cortisol production as a method for treating, delaying, slowing, and inhibiting the implicated diseases. The findings disclosed in this patent have been analyzed and compared with the literature data on inhibitors of CYP17, CYP21, and CYP11B1. The compiled data provide insight into the novel functionality of the compounds described in the patent. In this regard, an objective opinion on the effectiveness and novel biochemistry of these compounds in comparison to current CYP inhibitors used in the treatment of cortisol-related diseases is presented in this paper.

Keywords

cortisol; CYP11; CYP17; CYP21; cytochrome P450; P450 inhibition

1. Introduction

Cortisol is a glucocorticoid class steroid hormone that plays an important role in the regulation of carbohydrate, protein and lipid metabolism.[1] It is also known to assist in regulating blood pressure and cardiovascular maintenance, as well as inducing insulin resistance.[2,3] In humans, cortisol is biosynthesized through the androgen- and progestragen-catalyzed reactions during the metabolism of cholesterol. Cortisol dysregulation has been associated with diseases such as diabetes, heart failure, hypertension, stroke, obesity, renal failure, prostate cancer, breast cancer, uterine cancer and ovarian cancer, among others.[4–7]

In some cases, an excess amount of circulating cortisol is produced. This condition, sometimes classified as Cushing's syndrome, is known as hypercortisolemia. This excess production of cortisol may be due to a number of reasons including abnormalities due to hypothalamic, pituitary or adrenal axis impairment, or from the use of corticosteroid medications.[8] Another common disorder linked to the overproduction of cortisol is metabolic syndrome.[9] Also known as syndrome X or insulin-resistance syndrome, this condition involves abnormal energy storage and utilization issues, and is diagnosed by having a combination of the following risk factors: abdominal obesity, high blood pressure, elevated fasting plasma glucose, high serum triglycerides and low high-density lipoprotein (HDL) levels.[10]

Cortisol production is primarily regulated through cytochrome P450 (CYP) enzymes. These enzymes comprise a superfamily responsible for catalyzing numerous reactions including xenobiotic metabolism, hormone synthesis/break-down, bile acid synthesis, fatty acid metabolism and other important metabolic regulations.[11] The active site of CYP enzymes features a heme prosthetic group that uses the electron transport properties of NAD(P)H and molecular oxygen to generate important metabolites in the excretion and signaling pathways of exogenous and endogenous compounds.[12] Although the steroidogenic reactions use the same electron transport and oxidative functionality as xenobiotic metabolism, these reactions produce the progestragens and androgens, which are important hormones in metabolic and signaling pathways. These enzymes are prominently found in the inner mitochondrial membrane and endoplasmic reticulum of liver and adrenal cells as well as gonadal and surrounding tissue.[13]

While steroid production occurs primarily in the adrenal cortex and gonads, cortisol production is localized mainly in the zona fasiculata region of the adrenal cortex. The production of cortisol is regulated through three major CYP proteins, 17α–hydroxylase-C-17/20-lyase (CYP17), 21-hydroxylase (CYP21) and 11β-hydroxylase (CYP11B1) in the zona glomurulosa and zona fasiculata (Figure 1).[14] The bifunctional CYP17 enzyme contains both C-17 hydroxylation and C-17/C-20 lyase functionality. The latter of the functions is responsible for the production of androgenic compounds and does not play a role in the production of cortisol.

The patent WO2015048311 describes the design, syntheses and formulations of the 5-phenoxymethyl-1,3-dioxane analogs.[15] The *in vitro* inhibition of CYP17, CYP21 and

CYP11B1 is also described in this patent. The i*n vitro* goal of this class of compounds is an IC_{50} value of <100 nM for CYP17, CYP21 and CYP11B1, with lower potency for off-target CYP19 and CYP3A4. The effect of these analogs on the liver was also estimated by analyzing the inhibition of bile acid synthesis followed by pharmacokinetic studies in the guinea pig.

2. Cortisol production

In the biosynthesis of cortisol, pregnenolone and progesterone are both hydroxylated at the C-17 position by CYP17 (hydroxylase) activity in the zona fasiculata producing 17α-hydroxypregnenolone and 17α-hydroxyprogesterone, respectively. Additionally, pregnenolone can be converted to progesterone through the non-P450-catalyzed oxido-reductase 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme. This enzyme also catalyzes the conversion of 17α-hydroxypregnenolone to 17α-hydroxyprogesterone, which is then hydroxylated to 11-deoxycortisol at the C-21 position by CYP21 activity. The last step in the biosynthesis of cortisol involves additional hydroxylation at the C-11 position, which is catalyzed by CYP11B1.

3. Cortisol inhibitors

Compounds shown to inhibit enzymatic activities of CYP17, CYP21 and CYP11B1 lead to a decreased amount of cortisol production and provide the most effective strategy in treating diseases caused by cortisol overproduction.[16] There are many CYP17 and selected CYP11 inhibitors, but the literature on CYP21 inhibitors is not as prevalent. Since many CYP inhibitors have been developed, the most widely known and used inhibitors are briefly reviewed below.

3.1. Abiraterone acetate (CB 7630)

Abiraterone acetate is a potent CYP17 inhibitor derived from naturally occurring endogenous substrates (Figure 2).[17] Because of its poor bioavailability, the acetylated pro-drug was developed and found to inhibit enzymatic activities of both CYP17 and CYP11, leading to noted antitumoral effects.[18] Abiraterone acetate irreversibly binds to the iron heme complex through *N*-pyridyl binding at the C-23 position, causing a decreased production of cortisol and upregulation of adrenocorticotropic hormone production (ACTH). [19,20] Abiraterone acetate has also demonstrated increased survival rates in patients with metastatic castration-resistant prostate cancer (mCRPC) and is currently undergoing Phase II trials.[21]

3.2. Galeterone (VN/124-1,TOK-001)

Galeterone is an azolyl steroid inhibitor shown to inhibit CYP17 hydroxylase activity with increased 17,20 lyase selectivity.[22] It was also shown to competitively inhibit androgen receptor activity.[23] While galeterone exhibited decreased cortisol suppression efficacy compared to other drugs, its anti-tumoral effects were significant in CYP17 overexpressed malignancies.[23] Contrary to other CYP17 inhibitors, patients given galeterone show no signs of hepatotoxicity, which has aided galeterone in entering Phase III trials.[24]

3.3. Ketoconazole

Ketoconazole is a broad-spectrum imidazole antifungal drug that inhibits steroidogensesis through CYP17 hydroxylase and CYP11A1 cholesterol side chain cleavage inhibition. [25,26] High doses have shown to exhibit selective CYP17 hydroxylase inhibition.[27] In patients with androgen-independent diseases such as metastatic prostatic carcinoma, ketoconazole exhibited response rates in the range of 11 – 14%.[28] However, alternatives to ketoconazole have been investigated due to the development of resistances and adverse side effects such as hepatotoxicity, as well as neurological and respiratory associated complications.[26,29]

3.4. Metyrapone

Metyrapone is a pyridyl derivative that suppresses steroidogenesis through selective inhibition of CYP11B activity, causing a reduction of both cortisol and aldosterone.[30,31] Although frequently used for the treatment of Cushing's disease, inhibition using metyrapone is reversible and induces a negative feedback response due to low levels of cortisol.[31] This response drives the production of ACTH and allows for continued steroidogenic function.[32] It has been suggested for use as a combinatorial inhibitor in conjunction with other drugs such as ketoconazole and mitotane to increase efficacy and limit associated toxicities.[33] Adverse effects are mild, but in rare cases hypokalemia may occur.[34]

4. 5-(phenoxymethy)-1,3-dioxane analogs described in the patent WO2015048311

The patented compounds are novel dioxane inhibitors showing enzymatic inhibition of CYP17, CYP11 and CYP21 (Figure 3).[15] Target compounds were considered highly active if they met the *in vitro* target potency activity of $IC_{50} < 100$ nM (CYP17, CYP11 and CYP21), as well as decreased selectivity for specified off-target enzymatic activity and bile acid synthesis inhibition. By selecting these off-target enzymes, it was estimated that no potential harmful liver effects would occur *in vivo* as a result.

Of the compounds tested *in vitro*, 13 showed CYP17 IC_{50} values < 100 nM, 11 showed CYP11 IC_{50} values < 100 nM and only 1 met the targeted potency activity for CYP21, exhibiting an IC_{50} value < 100 nM (Table 1).

4.1. *In vivo* pharmacokinetic studies

Over 200 compounds were initially screened for *in vitro* inhibitory activity. Thirteen compounds showed an inhibition potency of <100 nM for CYP17. The patent describes the achieved target goals for the representative compound COR-500015, the most potent inhibitor (Figure 4). COR-500015 showed high enzymatic activity in CYP17 ($IC_{50} = 8$ nM) and CYP11 ($IC_{50} = 12$ nM), with moderate activity in CYP21 ($IC_{50} = 208$ nM) (Table 2). This compound was chosen as the lead compound for *in vivo* studies where pharmacokinetic evaluations were performed using the guinea pig with a 1 mg/kg IV dosing (20% dimethacrylate (DMA), 40% triethylene glycol (TEG), 40% water) and 10 mg/kg oral dosing (2% Tween-80, 97% hydroxypropyl methylcellulose, 1% water). Maximum drug

serum concentration was 1018 ng/ml (C_{max}) at 3.0 h (T_{max}), and the half-life was determined at 6.0 h ($t_{1/2}$). Total drug exposure over time was 14,891 ng.h/ml ($AUC_{0\text{-inf}}$).

5. Conclusion

The most potent inhibitors contain a 2,4-dichlorophenyl group in the C-2 position. Some less active inhibitors, which contain a 2-chlorophenyl group in the C-2 position, showed moderate activity in CYP17, but much higher, or even no activity in some cases in CYP11 and CYP21. A few of the compounds showed some selectivity toward CYP17 or CYP11, but very few compounds exhibited high inhibition potency for CYP21. It is important to note the structural similarities between ketoconazole and the lead compound used for *in vivo* studies. The compounds share the same scaffold and stereochemical features with the exception of dioxane (patent compound) in place of the dioxolane (ketoconazole) and an oxygen substitution on the adjacent carbon position, shown in box (Figure 5). The most remarkable feature of these compounds is that the stereocenter at C-2 of the dioxane ring seems to have enormous impact on the potency of these compounds against CYP17, CYP11B and CYP21. For example, COR-500015 and COR-500019 are enatiomers. While COR-500015 has an inhibition profile of 8, 12 and 208 nM for CYP17, CYP11B and CYP21, COR-500019 exhibits a profile of 10,000, 5800 and 10,000 nM for the same enzymes. Glucocorticoids are locally synthesized by enzymes such as CYP17 (androgens, estrogens and cortisone), CYP21 (11-deoxycorticosterone) and CYP11B (corticosterone and aldosterone) and these regulate metabolic activity and immune function with high spatial specificity. Inhibition of CYP17 alone can lead to elevated levels of corticosterone. Since COR-500015 is an equipotent inhibitor of CYP11B and CYP17 and a less potent inhibitor CYP21, it will also inhibit the synthesis of corticosterone. Inhibition of all three enzymes (CYP17, CYP21 and CYP11B) will potentially result in elevated levels of pregnolone. This can lead to lower levels of the glucocorticoids and an accumulation of pregnolone which can lead to other side effects as pregnolone interacts with progestin, testosterone and estrogen.

6. Expert opinion

Abiraterone acetate is a more potent inhibitor of CYP17 than ketoconazole. When the effectiveness of these agents was compared in docetaxel refractory, metastatic castrate-resistant prostate cancer (mCRPC), abiraterone treatment was found to be much more effective.[35,36] Ketoconazole also inhibits CYP3A and CYP24A1, while abiraterone is a highly selective inhibitor of CYP17. This may reduce the many adverse effects associated with ketoconazole treatment. The illustrated compound COR-500015 in the patent WO2015048311 is inhibiting CYP3A4 with equal potency and CYP19 with slightly lower potency. This might cause these agents to have similar adverse side effects as ketoconazole. There are three isoenzymes of CYP11: (1) CYP11A1 which converts cholesterol to pregnenolone, is the first and rate-limiting enzyme in all steroid biosynthesis and its deficiency leads to congenital adrenal hyperplasia (CAH); (2) CYP11B1 which is the enzyme that makes cortisol from 11-deoxycortisol, and its deficiency leads to CAH and hypertension; and (3) CYP11B2 which is a aldosterone synthase and hydroxylates corticosterone is known to have angiogenic effects. The CYP11B2 (−344 C) allele in the promoter region greatly increases the risk of nonfatal myocardial infarction in those with

smoking and dyslipidemia. The present patent describes the biological effect of the inhibition of CYP11B1. While describing the CYP11 inhibition assays, the isozyme of CYP11 analyzed is not specified. If the authors have used a mixture of CYP11 isozymes, then the side effects of inhibiting the other isozymes of CYP11 need to be considered. These compounds have a huge therapeutic potential for treating diseases associated with overproduction of cortisol given their high inhibition potency for CYP17, CYP11 and CYP21. These compounds are capable of exhibiting therapeutic potential that is comparable and possibly better than the existing drug candidates such as ketoconazole, metyrapone and abiraterone to treat such disease conditions.

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(•) or of considerable interest

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Article highlights

● The biosynthesis of cortisol involves several steps regulated by many enzymes including the three major CYP proteins, 17α–hydroxylase-C17/20-lyase (CYP17), 21-hydroxylase (CYP21) and 11β-hydroxylase (CYP11B1).

● Cortisol dysregulation has been associated with diseases such as diabetes, heart failure, hypertension, stroke, obesity, renal failure, prostate cancer, breast cancer, uterine cancer, ovarian cancer and so on.

● The patent evaluated in this article WO2015048311 describes the development of ~200 analogs of 5-(phenoxymethyl)-1,3-dioxane that inhibit the CYP proteins CYP17, CYP21 and CYP11B1 with IC_{50} values of <100 nM.

● Of the compounds tested *in vitro*, 13 showed CYP17 IC_{50} values < 100 nM, 11 showed CYP11 IC_{50} values < 100 nM and only 1 met the targeted potency activity for CYP21, exhibiting an IC_{50} value <100 nM.

● Pharmacokinetic studies were performed *in vivo* using the guinea pig and COR-500015 satisfied all required criteria.

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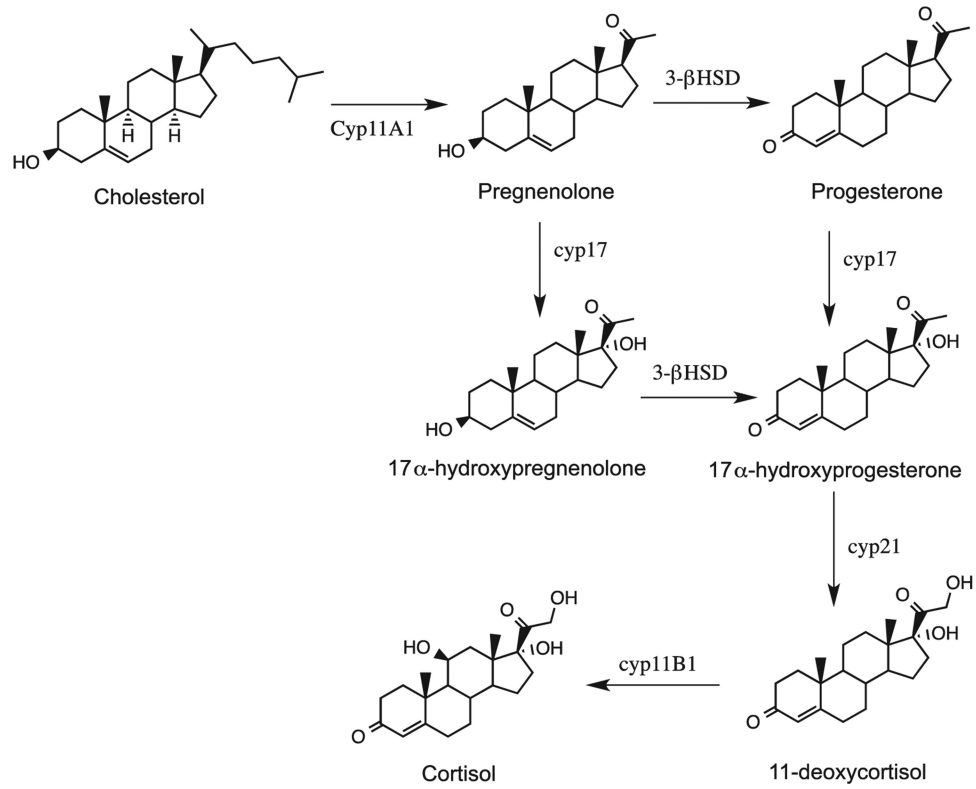
Figure 1.
Cholesterol metabolizing reactions in the cortisol biosynthesis pathway.

Abiraterone acetate

Galeterone

Metyrparone

Ketoconazole
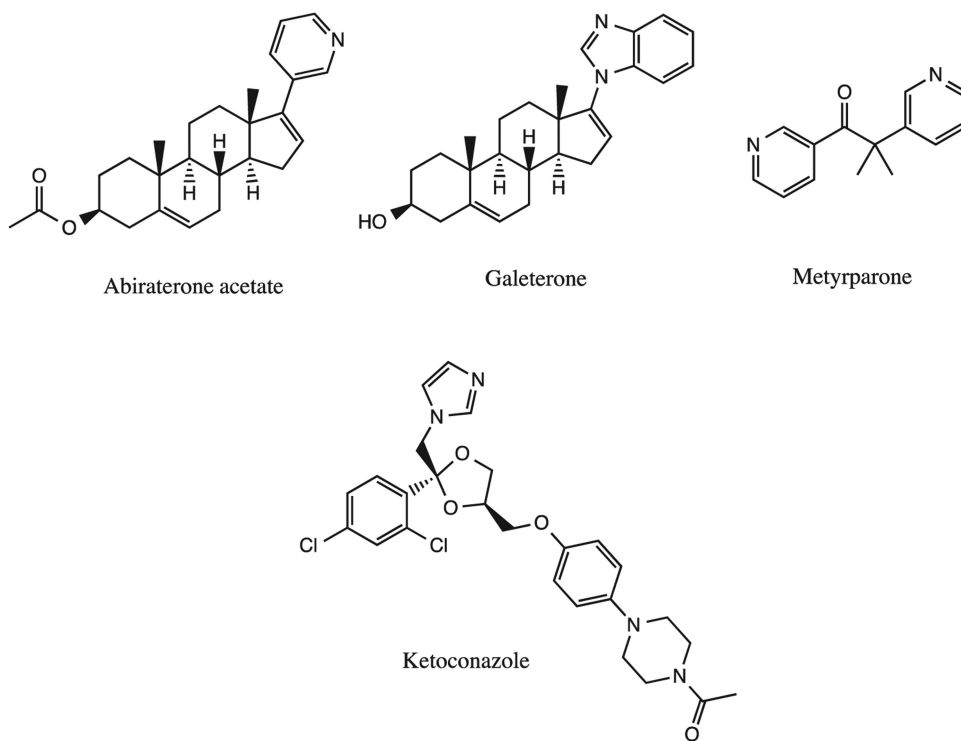
Figure 2.
Widely known CYP inhibitors known to decrease cortisol production.
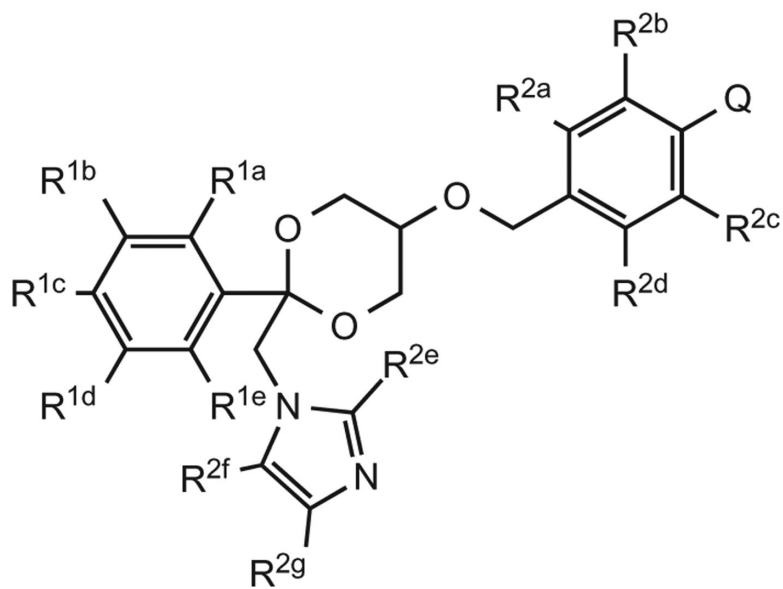
Figure 3.
Novel dioxane analogs claimed in patent.
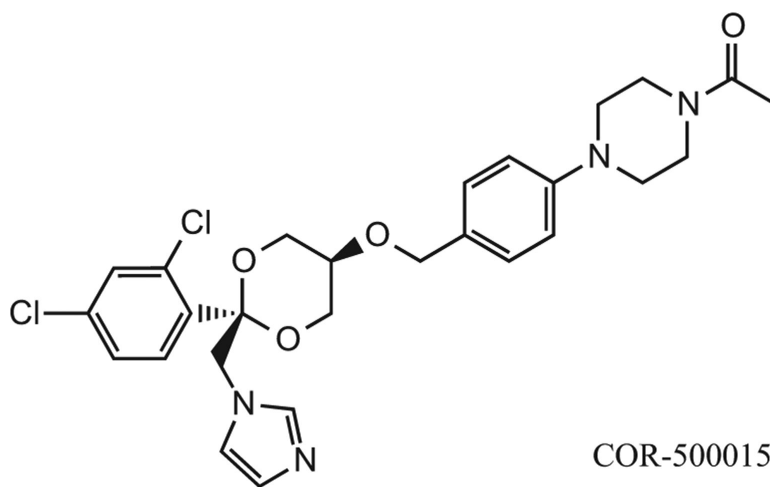
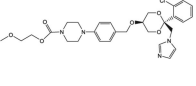COR-500015

Figure 4.
Compound COR-500015 used for *in vivo* studies.
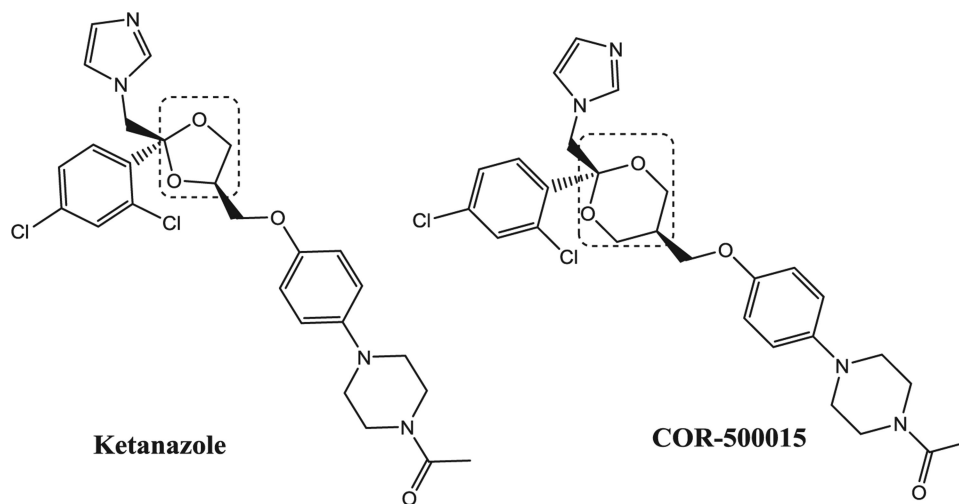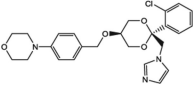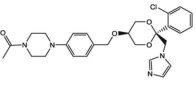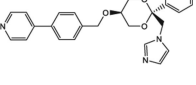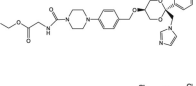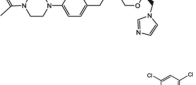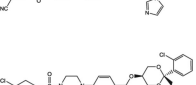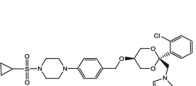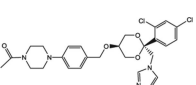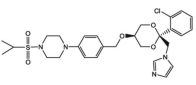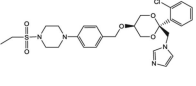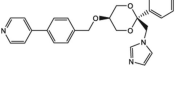
Figure 5.
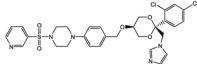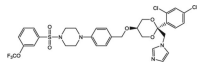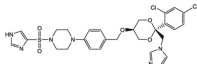Structural differences (shown in box) between COR-500015 and ketoconazole.

Table 1

Compounds meeting at least one of the targeted potency criteria ($IC_{50} < 100$ nM).

Compound	Structure	IC$_{50}$ (nM)		
		CYP17	CYP11	CYP21
COR-500012		**12**	100	1520
COR-510013		**27**	**70**	800
COR-510014		**20**	140	570
COR-510017		**42**	440	391
COR-510018		**40**	107	2800
COR-500015		**8**	**12**	208
COR-500016		**75**	**37**	415
COR-500017		106	**27**	1100
COR-500018		**11**	**6**	262
COR-500019		1100	**12**	10,000
COR-500020		**11**	**25**	110
COR-500021		**9**	200	130
COR-500024		**33**	**23**	**68**

Compound	Structure	IC$_{50}$ (nM)		
		CYP17	CYP11	CYP21
COR-500025		**13**	**12**	273
COR-500022		101	**13**	170
COR-500023		67	**14**	426

Table 2

Targeted inhibitor potency and profile goals observed for the selected representative compound, COR-500015, for *in vivo* trials.

Target activity		Activity of selected compound
CYP17	$IC_{50} < 100$ nM	$IC_{50} = 8$ nM
CYP11B1	$IC_{50} < 100$ nM	$IC_{50} = 12$ nM
CYP21	$IC_{50} < 100$ nM	$IC_{50} = 208$ nM
Off-target counter screens		
CYP19	$50\times$ CYP17	$IC_{50} = 350$ nM
Bile Acid (CYP7A surrogate)	$100\times$ CYP17	$IC_{50} = 880$ nM
CYP450 34A > 50% @ 1 mM	IC_{50} (nM)	$IC_{50} = 109$ nM
CYP450 2D6 > 50% @ 1 mM	>50% @ 1 µM	Data not given
CYP450 2C9 > 50% @ 1 mM	>50% @ 1 µM	Data not given
Pharmaceutical profiling/ADME		
Solubility >5 µM	µMolar	Data not given
Molecular Weight	<500 AMU	545 AMU
ClogP	<5	4.2 (logP)
TPSA	<140	69
Cytotoxicity in HerG2	10% @1 µM	Data not given
Caco-2-permeability (A-B, B-A)	>20 (10^{-6} cm/s)	Data not given
Guinea pig liver microsomal stability	>30 min	Data not given
Plasma stability	>30 min	Data not given
Plasma protein binding	>30 min	Data not given
Guinea pig bioavailability	>25%	Data not given
Guinea pig $t_{1/2}$	<4 h	6 h