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LETTER

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Optimization of the First Selective Steroid-11β-hydroxylase (CYP11B1) Inhibitors for the Treatment of Cortisol Dependent Diseases

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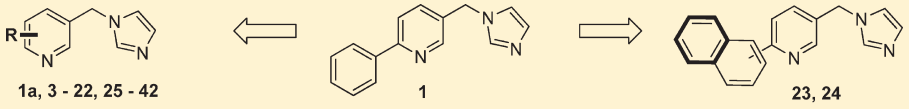
Supporting Information

ABSTRACT:



CYP11B1 is the key enzyme in cortisol biosynthesis, and its inhibition with selective compounds is a promising strategy for the treatment of diseases associated with elevated cortisol levels, such as Cushing's syndrome or metabolic disease. Expanding on a previous study from our group resulting in the first potent and rather selective inhibitor described so far (**1**, IC_{50} = 152 nM), we herein describe further optimizations of the imidazolylmethyl pyridine core. Five compounds among the 42 substances synthesized showed IC_{50} values below 50 nM. Most interesting was the naphth-1-yl compound **23** (IC_{50} = 42 nM), showing a 49-fold selectivity toward the highly homologous CYP11B2 (**1**: 18-fold) as well as selectivity toward the androgen and estrogen forming enzymes CYP17 and CYP19, respectively.

KEYWORDS: Cushing's syndrome, metabolic syndrome, steroid hormone biosynthesis, steroid-11β-hydroxylase (CYP11B1), CYP11B2, selective inhibitors

Endogenous Cushing's syndrome is a hormonal disorder caused by prolonged exposure to excessive levels of circulating glucocorticoids; therefore, it is also called hypercortisolism. Most people develop central obesity and often diabetes and hypertension. In many cases, hypersecretion of ACTH is observed, which is caused by a pituitary adenoma (Cushing's disease).[1] Adrenocortical tumors are common reasons for ACTH-independent hypercortisolism. In many cases, surgical removal of the tumor or radiation therapy cannot be applied and patients require temporary or permanent medication.[2] However, application of the glucocorticoid receptor antagonist mifepristone triggers a massive secretion of cortisol which is probably caused by the hypothalamic pituitary feedback mechanism.[3] A decrease of glucocorticoid formation should be a better therapeutic option. The best target for such an approach is steroid-11β-hydroxylase (CYP11B1), an adrenal CYP enzyme catalyzing the last step in cortisol production, the hydroxylation of deoxycortisol in the 11β-position (Scheme 1). There are inhibitors of cortisol biosynthesis such as ketoconazole, etomidate, and metyrapone in clinical use.[4] However, all of them show severe side effects due to the fact that they are unselective.

A challenge in the development of CYP enzyme inhibitors is the selectivity versus other CYP enzymes. In the past, we and others have developed selective inhibitors of steroidogenic CYP enzymes. Aromatase (estrogen synthase, CYP19)[5−8] and 17α-hydroxylase-C17,20-lyase (CYP17) inhibitors[9−12] are first line drugs for the treatment of breast cancer and upcoming therapeutics for castration refractory prostate cancer, respectively. In the case of adrenal CYP11B enzymes, selectivity is very difficult to achieve, as the homology between CYP11B1 and CYP11B2 (aldosterone synthase, Scheme 1) is very high (93%),[13] and for a long time it was considered impossible to obtain selective inhibitors. Recently, however, we succeeded in the development of highly active and selective CYP11B2 inhibitors[14−18] with *in vivo* activity.[19,20] Regarding CYP11B1 inhibition, the hypnotic and unselective CYP inhibitor etomidate was used as starting point for three investigations: Roumen et al. discovered selective CYP11B2 inhibitors,[21] Zolle et al. described CYP11B1 inhibitors without investigating selectivity,[22] and we discovered the first rather selective CYP11B1 inhibitors.[23] The best compound identified (**1**, Scheme 2) shows an activity comparable to the clinically administered ketoconazole but strongly exceeds its selectivity (IC_{50} = 152 nM, a fairly good selectivity toward CYP11B2 (selectivity factor, sf =18) and no inhibition of CYP17 and CYP19). For improving activity and selectivity, we describe here structural modifications of **1** leading to 42 novel compounds (Scheme 2). Either the phenyl ring was replaced by small substituents (**1a**, **3**−**6**), or substituents were introduced into the phenyl ring (**7**− **22**), and a benzene was annulated to the phenyl moiety (**23** and **24**). Finally, the phenyl ring was

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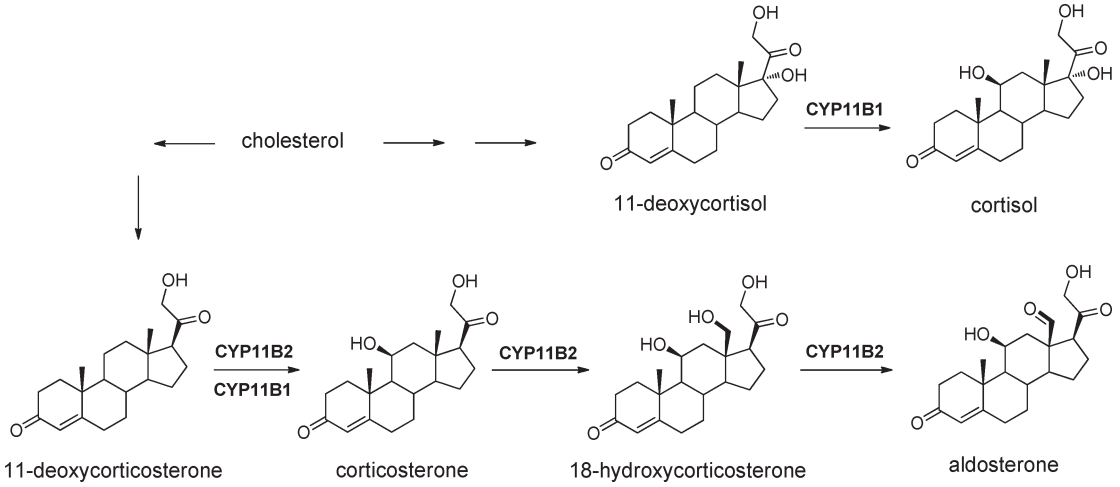
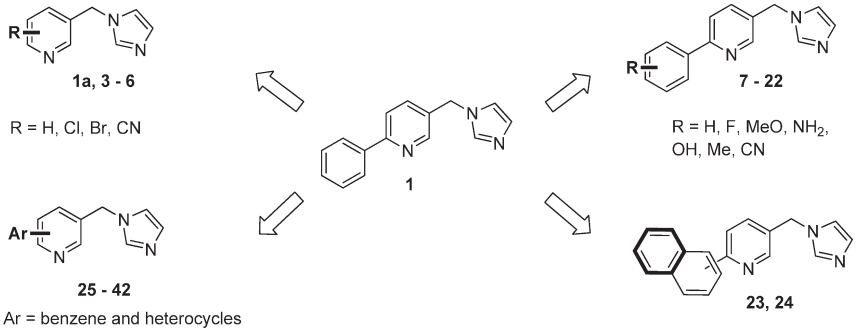
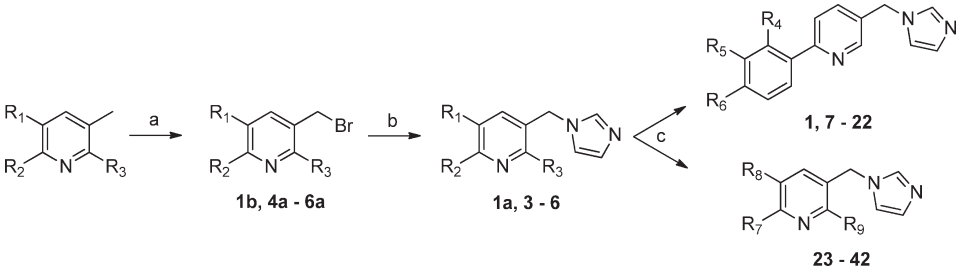
Scheme 1. Role of CYP11B1 and CYP11B2 in Cortisol and Aldosterone Biosynthesis



Scheme 2. Synthesized Inhibitors



Scheme 3. Synthesis of Compounds 1, 1a, and 3−42[a]



[a] Conditions: (a) Method A: NBS, DBPO, CCl_4, 90 °C, 12 h. (b) Method B: imidazole, K_2CO_3, acetonitrile, 90 °C, 2 h. (c) Method C: boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/MeOH/H_2O, reflux, 5 h.

exchanged by several heterocycles (**25**−**42**). All compounds were tested for inhibition of human CYP11B1 and CYP11B2 and selected compounds for CYP19 and CYP17 inhibition.

The synthesis of compounds **1a** and **3**−**42** is shown in Scheme 3. The reaction sequence was basically as already described.[23] The key reaction leading to the final compounds **7**−**42** was a Suzuki coupling with the corresponding boronic acids and compounds **5** (for the synthesis of **36**), **6** (for **25**, **35**, and **42**) and **1a** (for all other compounds). Compounds **5** and **6** were obtained from 2-bromo-3-methylpyridine or 3-bromo-2-chloro-5-methylpyridine as starting materials. Interestingly, Suzuki coupling of **6** with phenylboronic acid and furan-2-ylboronic acid led to bis-substituted compounds **25** and **42**, while reaction with thiophen-2-ylboronic acid only replaced the bromine, not the chlorine in **6** leading to **35**. The unsubstituted pyridines **2** and **3** were obtained via S_N reaction from the commercially available bromomethylpyridines. Compound **4** was obtained from 3-methylpicolinonitrile via Wohl−Ziegler bromination and subsequent S_N reaction with imidazole.

For the determination of CYP11B1 and CYP11B2 inhibition, V79 MZ cells expressing either human CYP11B1 or CYP11B2 were used and [^{3}H]-labeled 11-deoxycorticosterone was used as

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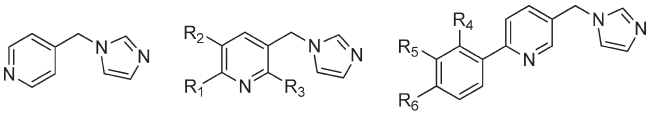
Table 1. Inhibition of CYP11B2 and CYP11B1 by Compounds 2−22



2 **1a, 3 - 6** **1, 7 - 22**

	structure			IC_{50} value (nM)[a,b]		
no.	R_1	R_2	R_3	CYP11B1	CYP11B2	sf[c]
2				663	>1000	
3[d]				816	>1000	
4			CN	971	>1000	
1a	Br			500	>1000	
5			Br	61	911	15
6	Cl	Br		168	576	3.4
	R_4	R_5	R_6			
7	F			72	1736	24
8		F		320	>1000	
9[h]			F	213	2153	10
10[h]		F	F	329	1665	5
11		F	OH	17	237	14
12[h]	MeO			167	4391	26
13[h]			MeO	782	>1000	
14[h]		MeO	MeO	>1000	>1000	
15	NH_2			101	2114	21
16[h]		NH_2		110	3407	31
17			NH_2	106	528	5
18		NH_2	Me	542	>1000	
19		CN		409	>1000	
20			CN	782	>1000	
21			CHO	246	>1000	
22			di-Ph-N	611	n.i.[e]	
1[f,h]				152	2768	18
MTP[g]				15	72	4.8
ETO[g]				0.5	0.1	0.2
KTZ[g]				127	67	0.5

[a] Mean value of at least three experiments. The deviations were within < ±25%. [b] Hamster fibroblasts expressing human CYP11B1 or CYP11B2; substrate 11-deoxycorticosterone, 100 nM. [c] sf: selectivity factor: IC_{50} (CYP11B2)/IC_{50} (CYP11B1). [d] Compound described in ref 22. [e] n.i.: no inhibition at an inhibitor concentration of 500 nM. [f] Compound described in ref 23. [g] MTP, metyrapone; ETO, etomidate; KTZ, ketoconazole. [h] Compound described in ref 31.

substrate.[24,25] Metyrapone, etomidate, ketoconazole, and **1** served as references. Compounds **2** and **3**, bearing the unsubstituted pyridine ring, showed moderate inhibitory activity (Table 1). Introduction of different substituents into **3** led to the highly active *o*-Br compound **5** (IC_{50} = 61 nM) with a good selectivity toward CYP11B2 (sf = 15). While introduction of *o*-CN and *p*-Br did not change activity strongly, the *p*-Cl,*m*-Br compound **6** (IC_{50} = 168 nM) showed good activity but poor selectivity.

Introduction of F into the phenyl moiety of **1** led to interesting results. Compound **7** with *o*-F substitution showed an increase in potency and selectivity while the *m*-F, *p*-F, and *m*,*p*-di-F compounds **8**−**10** exhibited reduced inhibition and selectivity compared to **1**. Introduction of *p*-OH into **8** increased activity strongly (**11**, IC_{50} = 17 nM). Regarding MeO substitution, only the *o*-MeO compound **12** showed good CYP11B1 inhibition (IC_{50} = 167 nM) and a high selectivity (sf = 26). NH_2 substitution resulted in highly active compounds, no matter in which position the group is located (**15**−**17**, IC_{50} = 101−110 nM). Regarding selectivity, however, differences could be observed. Compound **16** turned out to be the most selective compound of this series (sf = 31). Introduction of a Me group into **16** strongly decreased inhibition (**18**, IC_{50} = 542 nM). Further substituents, such as CN (**19**, **20**), formyl (**21**), and diphenylamino (**22**), did not enhance the potency of **1**.

Annulation of an additional benzene ring yielding the naphthalenes **23** and **24** (Table 2) resulted in a remarkable finding. In the case of the 1-naphthyl compound **23**, a strong enhancement of activity and selectivity was observed (IC_{50} = 42 nM, sf = 49). In contrast, the 2-naphthyl compound **24** showed only moderate activity and low selectivity (IC_{50} = 246 nM, sf = 3).

Exchange of the phenyl moiety of **1** by nitrogen containing heterocycles (**26**−**30**) led only in the case of 4-pyridine (**27**, IC_{50} = 139 nM) and 4-isoquinoline (**30**, IC_{50} = 95 nM) to fairly active compounds. Contrarily, thiophene containing compounds were in most cases highly active, like the 2-thiophene **31** and the 3-thiophene **32** (IC_{50} = 75 and 126 nM). Introduction of 5-Cl or 5-formyl into the thiophene ring of **31** reduced activity or did not change it (**33**, **34**). Interestingly, annulation of a benzene ring onto the thiophene moiety reduced the activity of **31**, whereas for compound **32** it was increased (**37**, IC_{50} = 269 nM; **38**, IC_{50} = 40 nM). Shifting of the 2-thiophene group of **31** into other positions did not change the activity in the case of **35** (bearing an additional Cl-substituent) while in **36** the potency was strongly increased (IC_{50} = 16 nM). The furans **39** and **40** exhibited similar potencies to those of the thiophenes. As seen with the 2-thiophene **31**, annulation of a benzene ring onto the 2-furan ring of **39** decreased activity (**41**, IC_{50} = 500 nM).

The introduction of an additional ring, phenyl into compound **1** and 2-furanyl into compound **39**, again led to opposed results: Compound **25** (IC_{50} = 362 nM) showed a somewhat reduced activity compared to **1** whereas **42** (IC_{50} = 29 nM) turned out to be more potent than **39**.

The most interesting 24 compounds were tested for inhibition of CYP17 and CYP19 (see Supporting Information). Inhibition of CYP17 was investigated using recombinantly expressed human CYP17 and progesterone as substrate.[11,26] All substances showed no effect (inhibition values <10% at 2.0 μM). Inhibition of CYP19 was determined with human placental microsomes and [1β-^{3}H]androstenedione as substrate.[27] Only **33** and **34** exhibited little inhibition (30 and 19% at 0.5 μM, respectively), while all other substances showed no effect (values <10%).

Summarizing, we succeeded in the optimization of lead compound **1**. The activity was enhanced for a number of inhibitors showing IC_{50} values below 50 nM, and the selectivity toward the highly homologous CYP11B2 was increased. Most of the compounds exhibited no inhibition of CYP17 and CYP19. Interestingly, already small compounds such as the bromo substituted **5** exhibited high inhibitory activity, while being highly selective toward the other CYPs.

In the class of the aryl-substituted imidazolylmethyl pyridines, important SARs have been obtained that permit the differentiation between CYP11B1 and CY11B2 inhibitors. It is striking that the *o*-substituted phenyl compounds **7**, **12**, and **15** show a higher activity and/or selectivity than the parent compound **1**. This can

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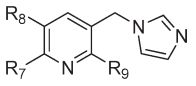
Table 2. Inhibition of CYP11B2 and CYP11B1 by Compounds 23−42



1, 23 - 42

	structure			IC_{50} value (nM)[a,b]		
no.	R_7	R_8	R_9	CYP11B1	CYP11B2	sf[c]
23	1-naphthalene			42	2075	49
24	2-naphthalene			246	782	3.2
25	Ph	Ph		362	851	2.4
26	3-pyridine			502	3955	8
27	4-pyridine			139	487	3.5
28	5-pyrimidine			971	n.i.[d]	
29	3-(6-methoxypyridine)			>1000	>1000	
30	4-isoquinoline			95	914	10
31	2-thiophene			75	1243	17
32	3-thiophene			126	3265	26
33	2-(5-chlorothiophene)			362	929	2.6
34	2-(5-formylthiophene)			62	968	16
35	Cl	2-thiophene		73	416	6
36			2-thiophene	16	251	16
37	2-benzo[*b*]thiophene			269	281	1.0
38	3-benzo[*b*]thiophene			40	1157	29
39	2-furan			167	5159	31
40	3-furan			76	2832	37
41	2-benzo[*b*]furan			500	>1000	
42	2-furan	2-furan		29	830	29
1[e]	Ph			152	2768	18
MTP[f]				15	72	4.8
ETO[f]				0.5	0.1	0.2
KTZ[f]				127	67	0.5

[a] Mean value of at least three experiments. The deviations were within < ±25%. [b] Hamster fibroblasts expressing human CYP11B1 or CYP11B2; substrate 11-deoxycorticosterone, 100 nM. [c] sf: selectivity factor: IC_{50} (CYP11B2)/IC_{50} (CYP11B1). [d] n.i.: no inhibition at an inhibitor concentration of 500 nM. [e] compound described in refs 23 and 31. [f] MTP, metyrapone; ETO, etomidate; KTZ, ketoconazole

also be observed with compounds **23** and **38**, with an *ortho*-position involved in benzene annulation, resulting in higher activity and/or selectivity compared to **1** and **32**. As *o*-substitution hinders rotation around the aryl−aryl bond, leading to nonplanarity and to an increase of "bulkiness" of the compound, it can be concluded that the active site of CYP11B1 favors a bulky structure whereas a flat compound geometry is preferred by the CYP11B2 binding site. Besides these important steric impacts, the paper shows that substituents at the phenyl group in the *meta*- or *para*-position or exchange of the phenyl by thiophenyl or furanyl are appropriate to modulate inhibition.

One of the most potent compounds of this series, **23**, is similarly active as the clinically used metyrapone. However, in contrast to metyrapone, **23** is much more selective: it is the most selective compound described so far and should be a promising candidate for further development. Such a potent and selective CYP11B1 inhibitor as **23** might not only be a good therapeutic for the treatment of Cushing's syndrome, it might also be beneficial for treating metabolic syndrome, as elevated cortisol levels play a central role in this disease.[28] A phase IIa study using the 2*S*,4*R* enantiomer of ketoconazole (DIO-902) with patients suffering from type 2 diabetes and other evidence of metabolic syndrome showed encouraging results. The levels of HbA1c and cholesterol were reduced, as well as weight loss and decreased blood pressure being observed.[29,30]

■ ASSOCIATED CONTENT

Supporting Information. Synthetic experimental details, analytical and further biological data of compounds, and biological assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

CYP, cytochrome P450; CYP11B1, steroid-11β-hydroxylase; CYP11B2, aldosterone synthase; CYP17, 17α-hydroxylase-17, 20-lyase; CYP19, aromatase; HbA1c, glycosylated hemoglobin; HSD, hydroxysteroid dehydrogenase; IC_{50}, concentration required for 50% inhibition; SAR, structure activity relationship; sf, selectivity factor; S_N, nucleophilic substitution

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