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First Selective CYP11B1 Inhibitors for the Treatment of Cortisol-Dependent Diseases

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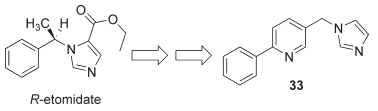
ABSTRACT Outgoing from an etomidate-based design concept, we succeeded in the development of a series of highly active and selective inhibitors of CYP11B1, the key enzyme of cortisol biosynthesis, as potential drugs for the treatment of Cushing's syndrome and related diseases. Thus, compound **33** (IC_{50} = 152 nM) is the first CYP11B1 inhibitor showing a rather good selectivity toward the most important steroidogenic CYP enzymes aldosterone synthase (CYP11B2), the androgen-forming CYP17, and aromatase (estrogen synthase, CYP19).

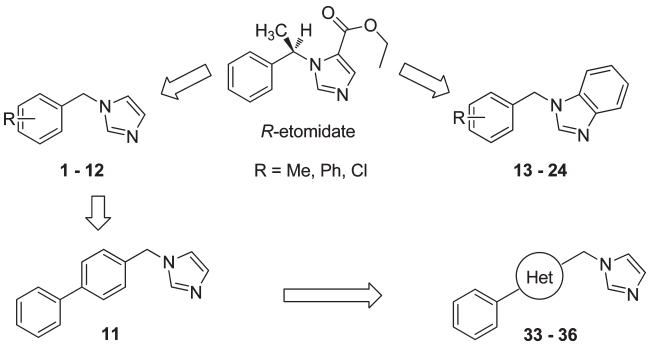
KEYWORDS Cushing's syndrome, steroid hormone biosynthesis, steroid-11β-hydroxylase, CYP11B1 inhibitor, CYP17, CYP19, CYP11B2, etomidate



It is well-known that steroid hormones are essential for a large number of vitally important physiological processes. However, they are also associated with life-threatening diseases. Application of hormone receptor antagonists or biosynthesis inhibitors are regarded as therapeutic methods of choice. The biosynthetic pathways contain several established and potential drug targets. In the last decades, aromatase (CYP19) inhibitors were developed and continuously improved.[1−4] Nowadays, second and third generation inhibitors are used as first line therapeutics for hormone-dependent breast cancer.[5] Some 15 years ago, the first selective androgen synthase (CYP17) inhibitors were described,[6−8] and recently, their benefits for the treatment of castration refractory prostate cancer were demonstrated.[9] Research was not only focused on the formation of steroid hormones in their endocrine glands but also on their activation in the target cell. Inhibition of steroid 5α-reductase is clinically well-established for androgen-dependent diseases.[10−13] Experimental results with hydroxysteroid dehydrogenase (HSD) inhibitors are very encouraging for estrogen- and glucocorticoid-dependent diseases.[14−18]

Until some years ago, selective inhibitors of mineralo- and glucocorticoids were not in the focus of research efforts. This was due to the fact that the sequence identity between aldosterone synthase (CYP11B2) and cortisol synthase (steroid-11β-hydroxylase, CYP11B1) is very high (93%),[19] and it was considered impossible to obtain selective inhibitors of one enzyme versus the other. Recently, however, we have been able to demonstrate that it is possible to selectively inhibit CYP11B2.[20−24] Further structural optimizations resulted in in vivo active compounds with selectivity factors (sfs) reaching 1000 with regard to CYP11B1.[25,26] They could be candidates for the treatment of hyperaldosteronism, congestive heart failure, and myocardial fibrosis. Although there is a high medical need for drugs interfering with excessive glucocorticoid formation resulting in Cushing's syndrome, there are only a few inhibitors of CYP11B1 described so far.[27] Because of their unselective action, their application is associated with severe side effects: The CYP19 inhibitor aminoglutethimide, metyrapone, the antimycotics ketoconazole and fluconazole, and the hypnotic etomidate are also inhibitors of other adrenal and gonadal cytochrome P450 (CYP) enzymes, and trilostane is a 3βHSD inhibitor.[28] In the present work, we report about the design, synthesis, and biological evaluation of the first selective (regarding CYP11B2, CYP17, and CYP19) inhibitors of human CYP11B1.

Scheme 1. Inhibitor Design Concept



As the lead for the design of the compounds, the highly active CYP11B1 inhibitor *R*-etomidate was used (Scheme 1), in spite of the fact that it shows a stronger inhibition of CYP11B2. Indeed, this compound was one of the starting points recently used for the development of novel CYP11B2 inhibitors.[29] Therein, the authors have shown that modifications of the ester group by conformationally flexible substituents resulted in CYP11B2 selective compounds, while eliminating the ester led to moderate CYP11B1 selectivity.

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Therefore, we decided to either remove the ethyl-ester or to replace it by a rigid benzene nucleus. The chiral core was abolished by eliminating the methyl group as it has been shown that the alkyl group at the methylene bridge is a prerequisite for the hypnotic activity of etomidate derivatives.[30] As polar substituents at the phenyl moiety were shown to result in poor CYP11B1 inhibition,[29] methyl, chloro, and additional phenyl substituents were introduced (**2**−**24**). The most selective inhibitor obtained, **11**, was further optimized regarding selectivity versus CYP17 by exchange of one benzene ring by pyridine, furane, or thiophene (**33**−**36**).

The syntheses of **2**−**36** are shown in Schemes 2 and 3. The starting point was commercially available *N*-benzylimidazole **1**. The preparation of the *N*-benzylimidazoles **2**−**12** and **33** and -benzimidazoles **13**−**24** was carried out via S_N reaction of the corresponding benzyl-halogenides. For the syntheses of **12**, **24**, and **33**, palladium-catalyzed Suzuki coupling and Wohl−Ziegler bromination were used. Preparation of the adamantanes **25** and **26** included tosylation of adamantan-1-yl-methanol followed by S_N reaction with imidazole or benzimidazole. S_N reaction of tritylchloride with imidazole or benzimidazole afforded **27** and **30**, carrying two additional phenyls at the methylene bridge (not shown). For the synthesis of **28**, **29**, **31**, and **32**, the corresponding carbonic acids were reduced to the alcohols with $LiAlH_4$, and the products were further processed as described above. Compounds **34**−**36** were obtained by reduction of the aldehydes or carbonic acids to the primary alcohols with $NaBH_4$ or $LiAlH_4$ and a subsequent CDI-assisted S_Nt reaction.

For the determination of CYP11B1 and CYP11B2 inhibition, V79MZ cells expressing either human CYP11B1 or CYP11B2 were used, and [^{3}H]-labeled 11-deoxycorticosterone was used as the substrate.[31,32] Metyrapone, etomidate, and ketoconazole served as references. The IC_{50} values determined for **1**−**36** are shown in Tables 1−3. All imidazoles **1**−**12**, **25**, **27**−**29**, and **33**−**36** strongly inhibited CYP11B1, mostly showing IC_{50} values below 100 nM, and compounds **8**, **25**, **27**, and **28** even reached values below 10 nM. Regarding the benzimidazoles, lower CYP11B1 inhibition than for the corresponding imidazoles was observed, some compounds showing IC_{50} values above 1000 nM. Most imidazoles exhibited inhibitory activity toward CYP11B2, while only some benzimidazoles (**14**−**18**; IC_{50} values, 107−632 nM) showed marked inhibition of this enzyme.

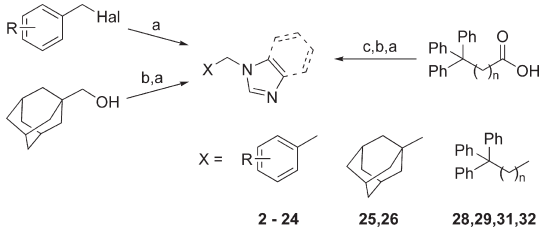
Inhibition of CYP17 was investigated using a homogenate of *Escherichia coli* recombinantly expressing human CYP17 and progesterone as substrate.[8,33] At a concentration of 2000 nM, only **11** and **12** showed a marked inhibition of 40 and 52%, while **25**, **35**, and **36** exhibited only weak effects with inhibition values around 20%. All other substances showed no inhibition (data not shown). Inhibitory effects toward CYP19 were determined using human placental microsomes and [1β-^{3}H]androstenedione as substrate.[8] At a concentration of 500 nM, compounds **6**, **9**, **10**, **27**−**29**, **34**, and **36** showed inhibition values above 38%, while compounds **3**, **4**, **8**, **12**, **25**, **26**, and **35** exhibited little activity (12−28%), and all other substances showed no inhibition (data not shown).

In the last decades, we and others have demonstrated that the concept of heme complexation is appropriate for the development of highly active inhibitors of CYP enzymes. Furthermore, high selectivity could be obtained by modifying the corresponding molecules using ligand- and structure-based medicinal chemistry strategies. In the present study, etomidate was used as a starting point to develop highly potent and selective CYP11B1 inhibitors that were superior to the currently used drugs ketoconazole, metyrapone, and etomidate. The latter show a broad range of adverse effects, which are mainly due to inhibition of other CYPs. Therefore, selectivity studies regarding the most important steroidogenic CYPs, CYP11B2, CYP17, and CYP19, were performed.

The starting point was the unsubstituted *N*-benzylimidazole **1**[29] and the corresponding benzimidazole **13**. Both showed a good inhibition of the target enzyme (IC_{50} = 135 and 246 nM) and reasonable sfs of 3.4 and 3.5 toward CYP11B2. Interestingly, the introduction of a chloro substituent into the phenyl ring led to an inversion of the selectivity and resulted in CYP11B2 inhibitors (**2** and **14**). As we hypothesized from the results of Roumen et al.,[29] the introduction of apolar substituents increased the inhibitory activity of the imidazoles as can be seen for the methyl-substituted compounds **3**−**8**. The penta methyl compound **8** was the most active compound of this series (IC_{50} = 5 nM). As a similar structure−activity relationship (SAR) was observed for inhibition of CYP11B2, only a slight enhancement of the sf was found for **8**. In contrast, increasing the number of methyl groups at the benzimidazoles was not tolerated (**19** and **20**).

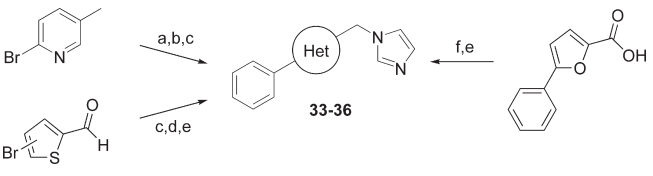
Remarkable SARs were found for the phenyl-substituted compounds. In the case of the *N*-benzylimidazoles, **9**−**11** strongly inhibited cortisol formation (IC_{50} = 15−46 nM). A

Scheme 2. Synthesis of Compounds **2**−**26** and **28**, **29**, **31**, and **32**[a]



[a] Conditions: (a) Imidazole or benzimidazole, K_2CO_3, DMF, 120 °C, 2 h. (b) Trifluoromenthanesulfonic anhydride, pyridine, 0 °C to room temperature, 3 h. (c) $LiAlH_4$, 0 °C to room temperature, overnight.

Scheme 3. Synthesis of Compounds **33**−**36**[a]



[a] Conditions: (a) NBS, DBPO, CCl_4, 90 °C, 12 h. (b) Imidazole, K_2CO_3, acetonitrile, 90 °C, 2 h. (c) Phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/MeOH/H_2O, reflux, 5 h. (d) $NaBH_4$, MeOH, 2 h, room temperature. (e) CDI, acetonitrile, reflux, 8 h. (f) $LiAlH_4$, 0 °C to room temperature, overnight.

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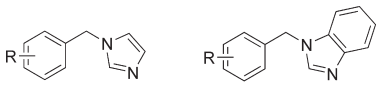
Table 1. Inhibition of CYP11B1 and CYP11B2 by Compounds **1**−**24**

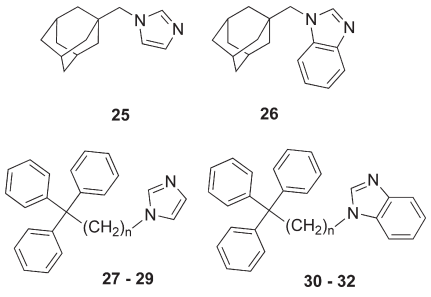


1 - 12 **13 - 24**

structure		IC_{50} value (nM)[a,b]				IC_{50} (nM)[a,b]		
R	no.	CYP11B1	CYP11B2	sf[c]	no.	CYP11B1	CYP11B2	sf[c]
H	**1**[d]	135	456	3.4	**13**	246	865	3.5
4-Cl	**2**[d]	140	46	0.3	**14**	635	107	0.2
2-Me	**3**	61	62	1.0	**15**	779	262	0.3
3-Me	**4**	48	110	2.3	**16**	194	309	1.6
4-Me	**5**	258	320	1.2	**17**	500	520	1.0
3,5-di-Me	**6**	32	77	2.4	**18**	188	632	3.4
2,4,6-tri-Me	**7**	24	30	1.3	**19**	> 1000	> 1000	
2,3,4,5,6-penta-Me	**8**	5	23	4.6	**20**	> 1000	> 1000	
2-Ph	**9**	15	39	2.6	**21**	> 1000	> 1000	
3-Ph	**10**	46	265	5.8	**22**	369	2226	6.0
4-Ph	**11**	32	637	20	**23**	197	1903	10
3,5-di-Ph	**12**	128	332	2.6	**24**	> 1000	> 1000	
MTP[e]		15	72	4.8				
ETO[e]		0.5	0.1	0.5				
KTZ[e]		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: IC_{50} (CYP11B2)/IC_{50} (CYP11B1). [d] See ref 29. [e] MTP, metyrapone; ETO, etomidate; and KTZ, ketoconazole.

Table 2. Inhibition of CYP11B2 and CYP11B1 by Compounds **25**−**32**



25 **26**

27 - 29 **30 - 32**

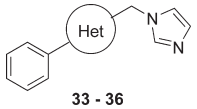
		IC_{50} value (nM)[a,b]				IC_{50} value (nM)[a,b]		
n	no.	CYP11B1	CYP11B2	sf[c]	no.	CYP11B1	CYP11B2	sf[c]
	25	5	30	5.9	**26**	75	677	9.0
0	**27**	3	11	3.4	**30**	> 1000	> 1000	
1	**28**	4	8	2.0	**31**	> 1000	> 1000	
2	**29**	80	290	3.6	**32**	> 1000	> 1000	
MTP[d]		15	72	4.8				
ETO[d]		0.5	0.1	0.2				
KTZ[d]		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: IC_{50} (CYP11B2)/IC_{50} (CYP11B1). [d] MTP, metyrapone; ETO, etomidate; and KTZ, ketoconazole.

strong decrease of CYP11B2 inhibition was observed in the order *ortho*, *meta*, and *para* (**9**, IC_{50} = 39 nM; **10**, 265 nM; and **11**, 637 nM). Further phenyl substituents at the *N*-benzyl moiety of benzimidazole **13** increased activity only in the case of **23**, a fairly selective compound (sf = 10).

Phenyl substitution at the methylene spacer and its elongation resulted in a loss of activity for the benzimidazoles **30**−**32**, while the corresponding imidazoles **27**−**29** showed very high inhibition values, especially **27** (IC_{50} = 3 nM).

Table 3. Inhibition of CYP11B1, CYP11B2, CYP17, and CYP19 by Compounds **33**−**36**



33 - 36

	Structure	IC_{50} value (nM)[a,b]		sf[c]	inhibition (%)	
Compound	Het	CYP11B1	CYP11B2		CYP17[a,f]	CYP19[a,g]
11	-	32	637	20	40	5
33[d]		152	2768	18	4	0
34		46	372	8.1	0	51
35		43	353	8.2	21	26
36[d]		19	277	15	21	72
MTP[e]		15	72	4.8		
ETO[e]		0.5	0.1	0.2		
KTZ[e]		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: IC_{50} (CYP11B2)/IC_{50} (CYP11B1). [d] Described in ref 34. [e] MTP, metyrapone; ETO, etomidate; and KTZ, ketoconazole. [f] *E. coli* expressing human CYP17; substrate progesterone, 25 μM; inhibitor concentration, 2.0 μM. [g] Human placental CYP19; substrate androstenedione, 500 nM; inhibitor concentration, 500 nM.

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However, these compounds are also highly potent CYP11B2 inhibitors. The replacement of the phenyl ring of **1** and **13** by an adamantane moiety led to the imidazole **25**, a highly potent (IC_{50} = 5 nM) and moderately selective (sf = 6) compound, and the corresponding benzimidazole **26**, showing a decreased activity (IC_{50} = 75 nM) but higher selectivity (sf = 9).

In the benzimidazole class, a series of highly selective compounds (**22**, **23**, and **26**) was found, demonstrating that this rigidification of the methyl ester group of etomidate was an appropriate optimization strategy. However, the compounds were less active than the imidazoles, especially in the case of the bulky core compounds **24** and **30**−**32** or the *ortho*-substituted phenyl compounds **15** and **19**−**21** with hindered rotation around the methylene bridge, presumably as they are not able to properly fit into the binding pocket.

As several compounds were observed to show some residual inhibition of CYP19 and CYP17, the most selective compound regarding CYP11B2, **11** was chosen for further modification, that is, exchange of the central phenyl moiety by different heterocycles.

The compounds obtained were highly potent CYP11B1 inhibitors with selectivity toward CYP11B2. The furan **34** showed no CYP17 but CYP19 inhibition. Both thiophenes **35** and **36** inhibited CYP17 to some extent but showed, especially **36**, enhanced CYP19 inhibition. The best selectivity, comparable to **11**, was achieved by introduction of a pyridine, resulting in **33** (IC_{50} = 152 nM, sf = 18), which, most importantly, did not affect CYP19 and CYP17. Regarding its activity, this compound is comparable to ketoconazole (IC_{50} = 127 nM), which is clinically used for the treatment of Cushing's syndrome, but highly exceeds ketoconazole (sf = 0.5) and the other clinically used compounds metyrapone (sf = 4.8) and etomidate (sf = 0.2).

Summarizing, we have discovered the first selective CYP11B1 inhibitors described so far. We regard them as novel leads for the development of drugs for the treatment of cortisol-dependent diseases. Thus, the design strategy starting from the CYP11B2 selective etomidate was successful. While Zolle et al. described chiral etomidate derivatives with a high affinity to rat adrenal membranes as well as strong inhibition of cortisol secretion without investigating selectivity issues,[27] the compounds described in this paper were examined for selectivity toward the most crucial steroidogenic CYP enzymes, and several were found to be selective.

SUPPORTING INFORMATION AVAILABLE

Synthetic experimental details, analytical 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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