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N-(Pyridin-3-yl)benzamides as selective inhibitors of human aldosterone synthase (CYP11B2)

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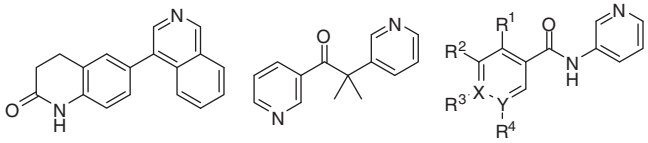
ABSTRACT

A series of 23 *N*-(Pyridin-3-yl)benzamides was synthesized and evaluated for their potential to inhibit human steroid-11β-hydroxylase (CYP11B1) and human aldosterone synthase (CYP11B2). The most potent and selective CYP11B2 inhibitors (IC_{50} values 53–166 nM) were further evaluated for their potential to inhibit human CYP17 and CYP19, and no inhibition was observed. Clear evidence was shown for *N*-(Pyridin-3-yl)benzamides to be a highly selective class of CYP11B2 inhibitors in vitro.

Aldosterone synthase (CYP11B2) is the key enzyme in the human biosynthesis of mineralocorticoids, catalysing the three-step interconversion of 11-deoxycorticosterone to aldosterone via corticosterone and 18-hydroxycorticosterone.[1] Due to its pivotal role, CYP11B2 is claimed as an useful target for the treatment of hyperaldosteronism, myocardial fibrosis and congestive heart failure.[2] After having successfully developed selective inhibitors of crucial steroidogenic enzymes involved in the endocrine (CYP19[3–5] and CYP17[6–8]) as well as intracrine (5α-reductase[9–11] and 17β-HSD1[12–14]) stimulation of hormone dependent diseases, we more recently have been concerned with the development of selective CYP11B2 inhibitors.[2,15–21] Thus, we have discovered compound **1** to be a highly potent and selective inhibitor of CYP11B2.[15] We were able to verify the lowering of plasma aldosterone levels by **1** in rats, which can be considered as validation of the target and *proof-of-principle*.[22]



1 **2** Metyrapone **3**

In an effort to develop and evaluate new classes of compounds as CYP inhibitors, we based our investigations on the structure of the well-known CYP inhibitor Metyrapone **2**, which has been used for the treatment of hypercortisolism and *Cushing's* syndrome for several decades.[23–27] We decided to substitute the bridging 2,2-dimethylethanone moiety in **2** by a much easier accessible amide linker to generate *N*-(Pyridin-3-yl)benzamides **3**. In addition—and with respect to activity studies carried out with the bovine CYP11B enzyme by Hays et al.[28] resp. Tobes et al.[29]—we also investigated the exchange of the keto-substituted pyridine system of Metyrapone **2** against substituted phenyl moieties in order to improve the potency of the compounds. We were very pleased by the activity and astonishingly high CYP11B2 selectivity of these small and structurally quite simple compounds in vitro, and we would like to communicate our detailed observations in this Letter.

Compounds of type **3** were prepared by a routine amide coupling procedure using commercially available acid chlorides **4** and 3-aminopyridine **5** in pyridine at room temperature or with application of gentle heating (Scheme 1). The products were obtained as solids in moderate to good yields.

Routinely, all synthesized compounds **3** were evaluated for their potential to inhibit human CYP11B2 and CYP11B1 at an inhibitor concentration of c = 500 nM. Tests were carried out using our established comparative *in-house* test system (V79 Chinese hamster cells stably transfected with either human CYP11B2 or CYP11B1; substrate: 11-deoxycorticosterone for both enzymes; substrate concentration: c = 100 nM).[30] The results are shown in Table 1. As can be seen from data, none of compounds **3** showed significant inhibition

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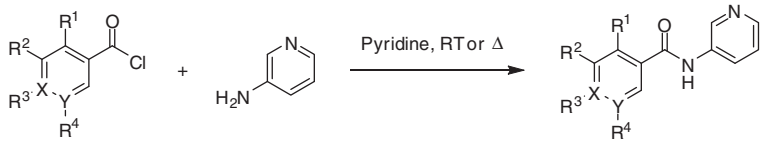


4 5 3

Scheme 1. Synthesis of *N*-(Pyridin-3-yl)benzamides **3**.

Table 1
Inhibition of human CYP11B2 and CYP11B1 enzymes by compounds **3**

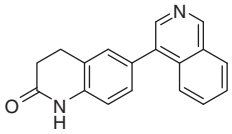
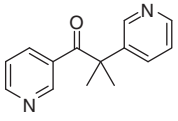
No.[a]	R^1	R^2	R^3	R^4	X	Y	CYP11B2 inhibition @ 500 nM[b] (%)	CYP11B1 inhibition @ 500 nM[b] (%)	log *P* (calcd)[c]
1	—	—	—	—	—	—	94 ± 2	91 ± 4	3.03
2	—	—	—	—	—	—	79 ± 4	94 ± 1	1.78
3a	H	H	H	—	C	N	8 ± 9	3 ± 2	0.81
3b	H	H	—	H	N	C	39 ± 7	4 ± 5	0.81
3c	H	Br	H	—	C	N	29 ± 3	0.4 ± 0.5	1.70
3d	H	H	H	H	C	C	30 ± 2	2 ± 2	1.98
3e	H	H	F	H	C	C	86 ± 3	7 ± 5	2.19
3f	H	H	Cl	H	C	C	88 ± 4	5 ± 7	2.76
3g	H	H	Br	H	C	C	64 ± 8	0	2.91
3h	H	H	OMe	H	C	C	19 ± 3	0	2.10
3i	H	H	OCF_3	H	C	C	3 ± 4	3 ± 3	3.21
3j	H	H	CF_3	H	C	C	1 ± 1	1 ± 1	2.99
3k	H	H	Me	H	C	C	25 ± 4	0	1.80
3l	H	H	Ph	H	C	C	6 ± 2	0	3.87
3m	H	H	CN	H	C	C	81 ± 5	9 ± 4	1.58
3n	H	H	NO_2	H	C	C	64 ± 3	0	1.88
3o	H	F	H	H	C	C	47 ± 11	1 ± 2	2.19
3p	H	Cl	H	H	C	C	26 ± 7	0	2.76
3q	F	H	H	H	C	C	34 ± 6	1 ± 2	1.78
3r	H	F	F	H	C	C	83 ± 2	6 ± 6	2.29
3s	H	OMe	OMe	H	C	C	2 ± 1	0.1 ± 0.1	1.75
3t	Cl	Cl	H	H	C	C	51 ± 3	4 ± 5	2.55
3u	F	H	F	F	C	C	70 ± 2	2 ± 2	2.03

[a] The purity of all tested compounds was ⩾95%.
[b] Test system: V79 Chinese hamster cells stably transfected with either human CYP11B2 or CYP11B1 enzyme; substrate: 11-deoxycorticosterone for both enzymes; substrate concentration: *c* = 100 nM.
[c] log *P* values were calculated using the add-on *ChemProp* implemented in *ChemDraw Ultra, Version 10.0*, CambridgeSoft.

of human CYP11B1 at *c* = 500 nM, while a number of derivatives strongly inhibit human CYP11B2. Concerning the two direct analogues **3a** and **3b** of Metyrapone **2**, isonicotinamide **3b** shows weak inhibition of CYP11B2, whereas nicotinamide **3a** is not active. Addition of a bromo substituent in *meta*-position of the nicotinamide moiety leads to the weakly active CYP11B2 inhibitor **3c**, but no improvement is achieved in comparison with **3b**. The unsubstituted benzamide **3d** is similarly active as **3b**. Introduction of halogeno substituents in *para*-position within the benzamide moiety (**3e**, **3f**, **3g**) leads to strong inhibitors of CYP11B2. Interestingly, similar activities could be observed with strongly electron-withdrawing substituents (-*M*-effect) like cyano derivative **3m** and nitro compound **3n**, whereas trifluoromethoxy-substituted **3i** and trifluoromethyl-substituted **3j** (-*I*-effect) showed no activity at all. Introduction of halogeno substituents in *meta*- (**3o**, **3p**) and *ortho*-position (**3q**) of the benzamide leads to selective CYP11B2 inhibitors with reduced activity in comparison with the *para*-substituted derivatives. The difluoro derivative **3r** shows similar activity to the monohalogenated compounds **3e**, **3f** and **3g**, whereas a slight drop of activity is observed for the trifluoro derivative **3u**. Compound **3t** bearing chloro substituents in *ortho*- and *meta*-position of the benzamide moiety is less active compared with **3u**.

For the seven most active inhibitors of CYP11B2 (**3e**, **3f**, **3g**, **3m**, **3n**, **3r**, **3u**) IC_{50} values were determined in order to quantify the

Table 2
Inhibition of human CYP11B2 and CYP11B1 enzymes (IC_{50} values) for selected compounds of type **3**

No.[a]	Structure	CYP11B2 IC_{50}[b] (nM)	CYP11B1 IC_{50}[b]	Selectivity factor IC_{50} (CYP11B1)/IC_{50} (CYP11B2)
1		0.20[15]	33 nM[15]	187[15]
2		72 ± 17	14.6 ± 2.1 nM	0.23

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