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Original article

Heteroatom insertion into 3,4-dihydro-1*H*-quinolin-2-ones leads to potent and selective inhibitors of human and rat aldosterone synthase

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ABSTRACT

Aldosterone synthase (CYP11B2) catalyzes the conversion of 11-deoxycorticosterone to aldosterone via corticosterone and 18-hydroxycorticosterone. CYP11B2 is regarded as a new target for several cardiovascular diseases which are associated with chronically elevated aldosterone levels such as hypertension, congestive heart failure and myocardial fibrosis. In this paper, we optimized heterocycle substituted 3,4-dihydropyridin-2(1*H*)-ones as CYP11B inhibitors by systematic introduction of heteroatoms and by bioisosteric exchange of the lactame moiety by a sultame moiety. The most promising compounds regarding inhibition of human CYP11B2 and selectivity versus human enzymes CYP11B1, CYP17, and CYP19 were tested for inhibition of rat CYP11B2. Thus, we discovered compounds **4** and **9** which show potent inhibition of *h*CYP11B2 (IC_{50} < 1 nM) and the corresponding rat enzyme (**4**: 64%, **9**: 51% inhibition, at 2 μM).

1. Introduction

Aldosterone synthase (CYP11B2) is the key enzyme in the biosynthesis of the major mineralocorticoid aldosterone. Located in the zona glomerulosa of the adrenal cortex, this enzyme catalyzes the last three steps of aldosterone biosynthesis, i.e. the conversion of 11-deoxycorticosterone to aldosterone via corticosterone and 18-hydroxycorticosterone [1]. Aldosterone is mainly regulated by the renin-angiotensin-aldosterone system (RAAS) and the plasma potassium concentration. It plays a major role in the regulation of sodium and water homeostasis and therefore in the regulation of blood pressure. Chronically elevated plasma aldosterone levels are associated with certain cardiovascular diseases such as hypertension, congestive heart failure and myocardial fibrosis [2]. Currently, the most common pharmaco-therapies interfere with the RAAS, e.g. by inhibition of the angiotensin-converting enzyme or blockade of the angiotensin-II receptor. Initially a reduction of plasma aldosterone levels can be observed. However, after several months, aldosterone plasma concentrations increase again, a phenomenon referred to as aldosterone escape in literature [3]. Another therapeutical option that blocks aldosterone action is the administration of mineralocorticoid receptor (MR) antagonists. In clinical studies, it has been shown that the MR antagonists Spironolactone and Eplerenone reduce hospitalizations and mortality rates of patients diagnosed with congestive heart failure and post myocardial infarction [4,5]. However, due to the steroidal structure of these compounds, severe side effects such as gynecomastia or dysmenorrhea occurred [4], and the increase in prescriptions rates for Spironolactone was followed by a rise in cases of life-threatening hyperkalemia [6]. Furthermore, the unaffected elevated plasma aldosterone levels can lead to an up-regulation of mineralocorticoid receptor expression [7] and to nongenomic aldosterone effects [8]. As a novel promising approach, inhibition of aldosterone biosynthesis by selective inhibition of CYP11B2 was proposed by our group [9].

Utilizing the long experience gained in the development of inhibitors of different CYP enzymes, such as *h*CYP17 [10–18], *h*CYP19

Abbreviations: CYP, cytochrome P450; CYP11B1, steroid 11β-hydroxylase; CYP11B2, aldosterone synthase; CYP17, 17α-hydroxylase-17,20-lyase; CYP19, aromatase; DMF, dimethylformamide; HPLC, high performance liquid chromatography; MR, mineralocorticoid receptor; NBS, *N*-bromosuccinimide; RAAS, renin-angiotensin-aldosterone system; sf, selectivity factor = $IC_{50\ CYP11B1}/IC_{50\ CYP11B2}$.

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[19–22] and *h*CYP11B1 [23–25], assay development and biological screening of a library of CYP inhibitors yielded the first hit compounds [26]. Subsequent optimization resulted in several classes of nonsteroidal highly potent *h*CYP11B2 inhibitors, i.e. imidazolyl- and pyridylmethylenetetrahydronaphthalenes and -indanes [27,28], heterocycle substituted naphthalenes, dihydronaphthalenes [29–32], pyridinyl substituted aliphatic cycles [33], dihydroquinolinones [34], indolines [35], tetrahydropyrroloquinolinone [36,37], and phenylsulfinyl naphthalenol [38]. Recently, several compound classes derived from the anesthetic *R*-etomidate or the CYP19 inhibitor Fadrozole (Chart 1) were described as inhibitors of *h*CYP11B2. For example, Hermans et al. describe a class of *N*-benzyl-1*H*-imidazoles [39], and several patents were filed by *Speedel* and *Novartis* comprising, for example, imidazo[1,5]pyridine [40] or heterocyclic spiro-compounds [41]. One of these compounds named LCI699 (Chart 1) has already been investigated in clinical trials for use in primary hyperaldosteronism and essential hypertension, yet was discontinued in Phase II [42]. Other classes include xanthones [43], dihydrotriazoloquinolines [44] and benzimidazoles [45] that are also promising.

The preceding study was focused on the development of a new core structure, i.e. the class of 3,4-dihydro-1*H*-quinolinones (Chart 1) was developed starting from naphthalene compounds by subsequent reduction of aromaticity and planarity. This strategy aimed to minimize the inhibition of hepatic CYP enzymes, especially CYP1A2 [34]. Heterocycle substituted 3,4-dihydro-1*H*-quinolinones exert a strong influence on the target enzyme (IC_{50} = 0.1–64 nM) and excellent selectivities versus the highly homologous (homology > 93%) [46] *h*CYP11B1 (sf = 44–440) and versus other CYP enzymes [34]. However, these compounds showed little inhibition against rat CYP11B2 and thus made it difficult to perform *in vivo* studies in this well established animal model.

In the present study, we focused on the further improvement of this core structure by bioisosteric exchange of a methylene unit by heteroatoms in the 3,4-dihydropyridin-2(1*H*)-one ring of the 3,4-dihydro-1*H*-quinolinones core structure and substitution of the lactame moiety by a sultame moiety (Chart 2). The aim is to increase inhibition of *r*CYP11B2 while maintaining the potency and selectivity toward human enzymes. First trials to test this approach for the class of dihydroquinolinone type CYP11B2 inhibitors were performed by us recently [34] By introduction of sulfur instead of the methylene bridge in position 4 (compound **II**), inhibitory potency for *h*CYP11B2 was enhanced by a factor of two, thus suggesting that the introduced heteroatom is able to undergo interactions with the binding site of the enzyme. To avoid potential

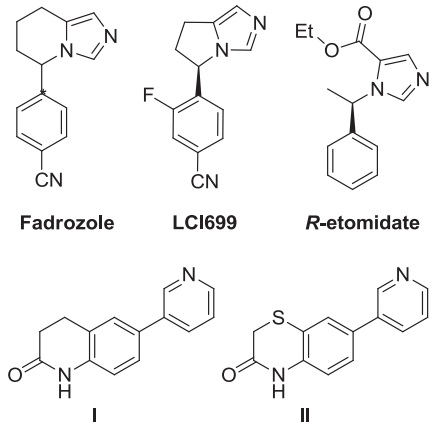


Chart 1. The structures of typical CYP11B2 inhibitors (fadrozole, LCI699 and *R*-etomidate) and lead compounds **I** and **II**.

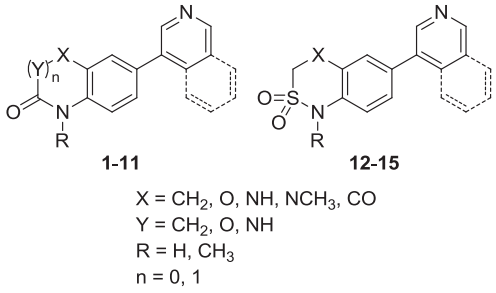


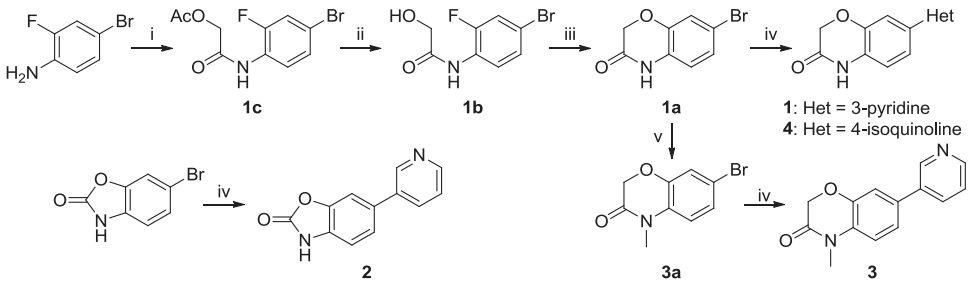
Chart 2. Title compounds.

metabolic oxidation of the sulfur bridge, we decided to focus on oxygen and nitrogen as heteroatoms in this study. The most promising compounds yielded regarding inhibitory potency for *h*CYP11B2 and selectivity versus *h*CYP11B1, *h*CYP17 and *h*CYP19 were further evaluated for their inhibition of *r*CYP11B2.

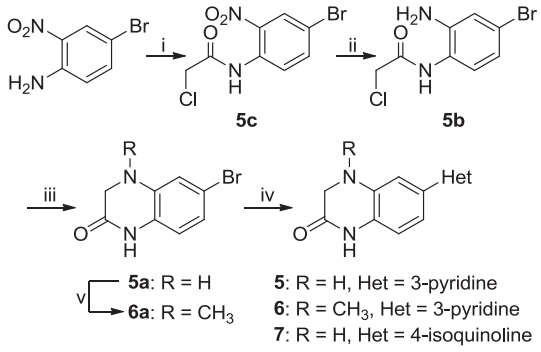
2. Results

2.1. Chemistry

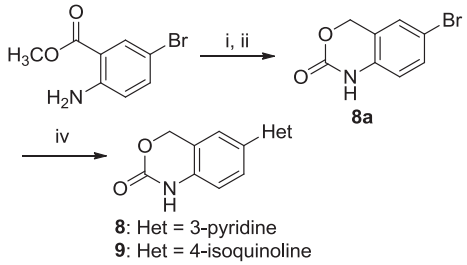
The synthetic procedures used to prepare the title compounds are shown in Schemes 1–6. Most of the final compounds were obtained via a Suzuki cross-coupling [47] between 3-pyridine- or 4-isoquinolineboronic acid and the corresponding brominated core, which were synthesized via different routes as described below. The 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one core **1a** (**1**, **3**, **4**, Scheme 1) was prepared from 4-bromo-2-fluoroaniline by the method of Varnes et al. (Scheme 1) with minor modifications [48]. Acetylation with acetoxyacetyl chloride, followed by cleavage of the acetoxy ester and subsequent substitution of fluorine by the resulting alcohol afforded **1a**. Methylation of lactame **1a** with iodomethane and potassium *tert*-butoxide yielded the methylated bromide **3a**. 3,4-Dihydroquinoxalin-2(1*H*)-ones **5**–**7** were synthesized on the basis of a procedure described by Li et al. [49] as depicted in Scheme 2. 4-Bromo-2-nitroaniline was acetylated with 2-chloroacetyl chloride. After reduction of the nitro group, cyclization under basic conditions yielded 6-bromo-3,4-dihydroquinoxalin-2(1*H*)-one (**5a**). Selective methylation of the amine in **5a** was carried out by reductive amination of formaldehyde. The *in situ* formed imine was reduced using sodium cyanoborohydride to provide methylated dihydroquinoxalinone **6a**. For preparation of 1*H*-benzo[*d*][1,3]oxazin-2(4*H*)-ones **8** and **9**, methyl 2-amino-5-bromobenzoate was reduced with lithium aluminum hydride to obtain (2-amino-5-bromophenyl)methanol as described by Snider et al. [50] The resulting alcohol was subsequently cyclized with triphosgene to yield **8a** (Scheme 3). The brominated 3,4-dihydroquinazolinone **10a** was prepared by cyclization of 2-(aminomethyl)aniline with triphosgene and subsequent bromination with NBS in DMF (Scheme 4). 1*H*,3*H*-4,2,1-Benzoxathiazine-2,2-dioxides **12**–**14** were prepared according to Scheme 5. *Ortho*-anisidine was sulfonated with chloromethanesulfonyl chloride. Subsequent cleavage of the methyl ether with boron tribromide followed by cyclization under basic conditions yielded benzoxathiazine-2,2-dioxide **12b**. Selective bromination in position 6 was achieved by treatment of **12b** with bromine in chloroform. To minimize the formation of 6,8-dibrominated byproduct and other possible isomers, bromine was added dropwise at 0 °C and the reaction mixture was subsequently stirred at the same temperature until the full consumption of starting material. Intermediate **12a** was thus obtained in good yield (92%) with only minor amounts of byproducts formed, which were removed by flash chromatography. Methylation of sultame **12a** was



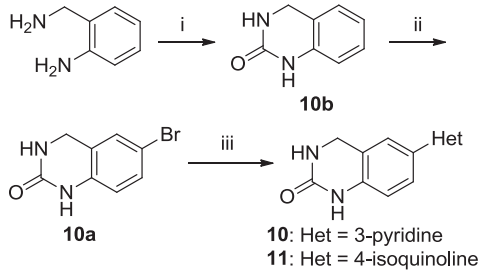
Scheme 1. Synthesis of compounds **1**–**4**. Reagents and conditions: (i) $ClCOCH_2OAc$, $CHCl_3$, room temperature; (ii) aq. NaOH, EtOH, 0 °C; (iii) NaH, DMF, 140 °C; (iv) heteroarylboronic acid, $Pd(PPh_3)_4$, aq. $NaHCO_3$, DMF, μW, 150 °C or 3-pyridineboronic acid, $Pd(PPh_3)_4$, aq. Cs_2CO_3, DME, reflux; (v) KO*t*Bu, CH_3I, DMF, room temperature.



Scheme 2. Synthesis of compounds **5**–**7**. Reagents and conditions: (i) $ClCH_2COCl$, toluene, reflux; (ii) Fe, NH_4Cl, CH_3COOH, H_2O, DMF, 50 °C; (iii) NaI, Na_2CO_3, acetonitrile, reflux; (iv) heteroarylboronic acid, $Pd(PPh_3)_4$, aq. $NaHCO_3$, DMF, μW, 150 °C; (v) 1. aqueous formaldehyde (37%), CH_3COOH, MeOH; 2. $NaBH_3CN$, MeOH.



Scheme 3. Synthesis of compounds **8** and **9**. Reagents and conditions: (i) $LiAlH_4$, THF, 0 °C; (ii) triphosgene, THF, room temperature; (iii) heteroarylboronic acid, $Pd(PPh_3)_4$, aq. Na_2CO_3, DME, reflux.



Scheme 4. Synthesis of compounds **10** and **11**. Reagents and conditions: (i) triphosgene, THF, room temperature; (ii) NBS, DMF, 0 °C; (iii) heteroarylboronic acid, $Pd(PPh_3)_4$, aq. Na_2CO_3, DME, reflux.

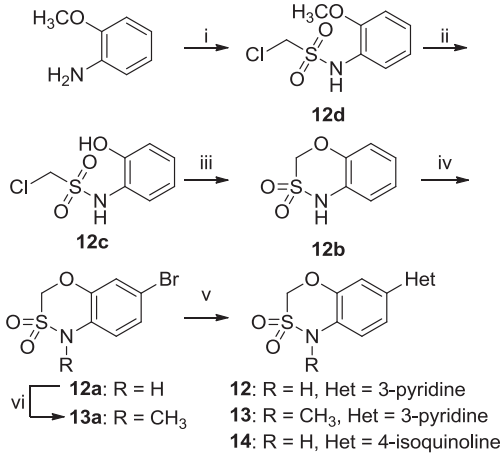
carried out with potassium carbonate and iodomethane to afford benzoxathiazinedioxide **13a**. The synthesis of 6-(pyridin-3-yl)-3,4-dihydrobenzothiazin-4-one-2,2-dioxide (**15**) is depicted in Scheme 6. The benzyl protected benzothiazinonedioxide **15b** was synthesized according to a reaction sequence previously described for similar compounds by Volovenko et al. [51] Suzuki coupling and subsequent reductive deprotection of the benzyl group provided compound **15**.

2.2. Biological results

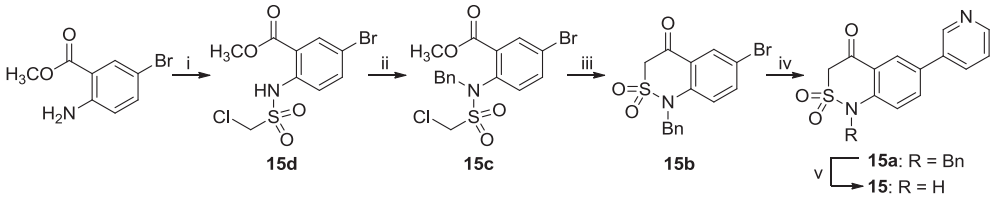
2.2.1. Inhibition of human CYP11B2 and CYP11B1

The inhibition of human adrenal corticoid producing enzymes CYP11B2 and CYP11B1 (Table 1) was determined in V79MZ cells stably expressing either *h*CYP11B2 or *h*CYP11B1 with [^{3}H]-11-deoxycorticosterone as substrate [26]. The product formation was monitored by HPLC using a radio flow detector. CYP11B2 inhibitor LCI699 and Fadrozole, which is an aromatase (CYP19) inhibitor reducing aldosterone levels in humans [52], were used as reference compounds.

In the class of 3-pyridine substituted dihydroquinolinones, exchange of methylene in position 4 by oxygen, as accomplished in benzo[1,4]oxazinone **1**, leads to a slightly decreased inhibitory potency (IC_{50} = 41 nM, versus lead compound **I**: IC_{50} = 28 nM). However, selectivity toward *h*CYP11B1 is considerably reduced (**1**: sf = 32, versus **I**: sf = 241). Ring contraction leads to a significant reduction of inhibitory potency (**2**, 39% inhibition at 500 nM). In contrast , methylation of the lactame increases inhibitory potency (**3**, IC_{50} = 11 nM) and also selectivity toward *h*CYP11B1 (sf = 73). Introduction of an NH or NMe results in a decreased inhibition of *h*CYP11B2 (**5**: IC_{50} = 366 nM, **6**: IC_{50} = 89 nM) as well as a reduction

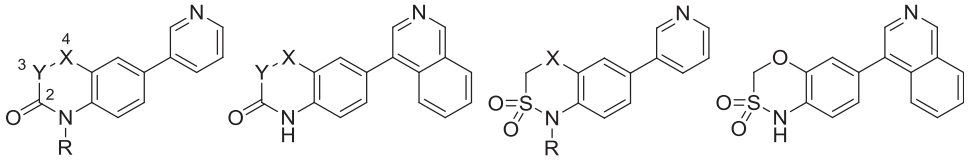


Scheme 5. Synthesis of compounds **12**–**14**. Reagents and conditions: (i) $ClCH_2SO_2Cl$, NEt_3, CH_2Cl_2, 0 °C; (ii) BBr_3, CH_2Cl_2, 0 °C; (iii) aq. NaOH, MeOH, 50 °C; (iv) Br_2, $CHCl_3$, 0 °C; (v) heteroarylboronic acid, $Pd(PPh_3)_4$, aq. Na_2CO_3, DME, reflux; (vi) CH_3I, K_2CO_3, DMF, 50 °C.



Scheme 6. Synthesis of compound **15**. Reagents and conditions: (i) $ClCH_3SO_2Cl$, pyridine, CH_2Cl_2, 0 °C; (ii) BnBr, K_2CO_3, DMF, 50 °C; (iii) NaH, DMF, room temperature; (iv) 3-pyridineboronic acid, $Pd(PPh_3)_4$, aq. Na_2CO_3, toluene/ethanol (4/1 v/v), reflux; (v) H_2, 10% Pd/C, methanol, reflux.

Table 1
Inhibition of *h*CYP11B2 and *h*CYP11B1 by compounds **I**, **II**, and **1**–**15**.



Compd.	X	Y	R	% Inhibition[a]	IC_{50} (nM)[b]		sf[e]
				*h*CYP11B2[c]	*h*CYP11B2[c]	*h*CYP11B1[d]	
I	CH_2	CH_2	H		28 ± 1	6746 ± 807	241
II	S	CH_2	H		12 ± 0.3	525 ± 147	44
1	O	CH_2	H	87	41 ± 2	1312 ± 22	32
2	O	–	H	39	nd	nd	nd
3	O	CH_2	CH_3	98	11 ± 2	776 ± 47	73
4	O	CH_2		100	0.5 ± 0.1	28 ± 5	58
5	NH	CH_2	H	57	366 ± 21	3451 ± 814	10
6	NCH_3	CH_2	H	80	89 ± 11	796 ± 40	9
7	NH	CH_2		100	1.1 ± 0.1	54 ± 11	48
8	CH_2	O	H	95	24 ± 6	1692 ± 382	70
9	CH_2	O		100	0.3 ± 0.1	15 ± 2.7	54
10	CH_2	NH	H	72	228 ± 21	120 ± 5	0.5
11	CH_2	NH		100	2.2 ± 0.3	4.5 ± 0.5	2
12	O		H	<5	nd	nd	nd
13	O		CH_3	60	193 ± 16	7483 ± 1425	39
14				16	nd	nd	nd
15	CO		H	<5	nd	nd	nd
Fadrozole					0.8 ± 0.1	6.3 ± 0.7	8
LCI699					0.2 ± 0.1	2.9 ± 0.2	15

[a] Mean value of at least two experiments; inhibitor, 500 nM.
[b] Mean value of at least three experiments, standard deviation less than 25%.
[c] Hamster fibroblasts expressing *h*CYP11B2; substrate deoxycorticosterone, 100 nM.
[d] Hamster fibroblasts expressing *h*CYP11B1; substrate deoxycorticosterone, 100 nM.
[e] $IC_{50\ CYP11B1}/IC_{50\ CYP11B2}$.

of the selectivity toward *h*CYP11B1 (**5**: sf = 10, **6**: sf = 9). The introduction of heteroatoms in position 3 leads to more potent inhibitors than their corresponding analogs with heteroatoms in position 4. Benzo[1,3]oxazinone **8** exhibits an IC_{50} of 24 nM, which is not only twice potent as the 4-O compound **1** (IC_{50} = 41 nM), but also similar to lead **I** (IC_{50} = 28 nM). As for NH insertion, the 3-NH compound **10** is a stronger inhibitor than the 4-NH one (IC_{50} of 228 versus 366 nM), it is much weaker than lead **I**, and, notably, a preference of CYP11B1 inhibition is observed. Exchange of lactame by sultame results in inactive compounds (**12** and **15**), however, methylation of amide (**13**) leads to a moderate IC_{50} of 193 nM and a selectivity factor of 39.

The isoquinoline compounds generally exhibit much more potent CYP11B2 inhibition than the pyridine analogs. The 3- and 4-oxygen substituted compounds, i.e. benzo[1,4]oxazinone **4** and benzo[1,3]oxazinone **9**, demonstrate IC_{50} values in the sub-nanomolar range (**4**: IC_{50} = 0.5 nM, **9**: IC_{50} = 0.3 nM) and moderate *h*CYP11B1 selectivities (**4**: sf = 58, **9**: sf = 54). The nitrogen substituted compounds display IC_{50} values in the low nanomolar

Table 2
Inhibition of *h*CYP17 and *h*CYP19.

Compd.	% Inhibition[a]	
	CYP17[b]	CYP19[c]
I	20	31
1	22	<10
3	<10	20
4	24	<10
7	18	<10
8	<10	31
9	15	<10

[a] Mean value of three experiments, standard deviation less than 10%.
[b] *E. coli* expressing human CYP17; substrate progesterone, 25 μM; inhibitor, 2.0 μM; ketoconazole, IC_{50} = 2780 nM.
[c] Human placental CYP19; substrate androstenedione, 500 nM; inhibitor, 500 nM; Fadrozole, IC_{50} = 30 nM.

range (**7**: IC_{50} = 1.1 nM, **11**: IC_{50} = 2.2 nM) and moderate to low selectivities toward *h*CYP11B1 (**7**: sf = 48; **11**: sf = 2). Interestingly, isoquinolinyl in 1*H*-benzo[e][1,3,4]oxathiazine 2,2-dioxide (**14**) hardly increases the potency (16% inhibition at 500 nM) compared to the pyridyl compound **12** (5% inhibition at 500 nM).

To determine whether the reduction in inhibitory potency of the sultams results from permeation or efflux problems due to the use of a cellular assay, CYP11B2 inhibition of compounds **12** and **14** was evaluated using a V79MZ*h*CYP11B2 cell homogenate. Compound **12** inhibits 31% and **14** 70% of product formation (at 500 nM) in contrast to the 5% and 16% inhibition in the cellular assay.

2.2.2. Inhibition of human CYP17 and CYP19

For potent *h*CYP11B2 inhibitors, selectivities versus the steroidogenic enzymes *h*CYP17 and *h*CYP19 were determined. Investigation of *h*CYP17 inhibition was carried out using the 50,000 g sediment of an *Escherichia coli* homogenate recombinantly expressing *h*CYP17. Progesterone (25 μM) was used as a substrate [22]. All tested compounds demonstrate an inhibition of less than 25% at a concentration of 2 μM. Inhibition of *h*CYP19 was evaluated *in vitro* with human placental microsomes using [1β-^{3}H]androstenedione (500 nM) as a substrate [53]. All evaluated compounds (**1**, **4**, **7**, and **9**) display almost no *h*CYP19 activity (<10%) at a concentration of 0.5 μM (Table 2), except compounds **8** and **3**, for which weak *h*CYP19 inhibition was observed (**8**: 31%, **3**: 20%). Thus, it was shown, that the title compounds do not interfere with the steroidogenic CYP enzymes *h*CYP17 and *h*CYP19.

2.2.3. Inhibition of rat CYP11B2

Selected compounds were evaluated in V79MZ cells expressing *r*CYP11B2 [54]. At an inhibitor concentration of 2 μM the pyridine compound **8** as well as the reference **I** [54] show no significant inhibition of the rat enzyme (Table 3). In contrast, the isoquinoline substituted dihydroquinoxalinone **7** exhibits 34% inhibition, while compounds **4** and **9** strongly inhibit *r*CYP11B2 (64% and 51%, respectively).

3. Discussion and conclusion

Recently, we reported heterocycle substituted dihydroquinolinones as a new class of CYP11B2 inhibitors with good selectivity profiles toward several steroidogenic and hepatic CYP enzymes [34]. Further modifications in this compound class were performed in this study in order to find new compounds suitable for *in vivo* tests in the rat.

The title substances (Chart 2) can be divided into two classes: compounds containing a 3-pyridine as heme complexing group and those bearing a 4-isoquinoline moiety. The pyridine series shows clearly that introduction of a pure hydrogen bond acceptor like O or NMe (compounds **1**, **6** and **8**) in position 3 or 4 is tolerated regarding *h*CYP11B2 inhibitory potency but results in a reduced selectivity toward *h*CYP11B1. Introduction of a hydrogen bond donor like NH (compounds **5** and **10**), leads to a considerably decreased *h*CYP11B2 inhibition, which is attenuated by transforming the mixed hydrogen bond donor/acceptor capability into a pure hydrogen acceptor by methylation of the NH (compound **6**), thus showing that hydrogen bond donors in position 3 or 4 are prohibited by the enzyme. Interestingly, dihydroquinazolinone **10** showed a slight selectivity for *h*CYP11B1 (sf = 0.5). Therefore, it could serve as a starting point in the search for *h*CYP11B1 selective compounds as *h*CYP11B1 is a target for the treatment of cortisol dependent diseases such as Cushing's disease [24] and wound healing [25].

Modification of the core structure by bioisosteric exchange of the lactame by a sultame resulted in a significantly reduced biological activity. Obviously, the planar geometry of the lactames is preferred for interaction with the enzyme compared to the tetrahedral of the sultame. Whether this is due to steric hindrance or inappropriate electronic properties remain to be clarified. Furthermore, the increased acidity of the sultame NH might also play a role. This theory is supported by the fact, that CYP11B2 inhibitory potency increases moderately when the sulfonamide moiety is protected with a methyl group (compound **13** 60% inhibition versus **12** < 5% inhibition). The change in polarity and acidity might also influence transport properties. Especially, membrane permeability or efflux problems might be responsible for the observed reduced activities in the cellular assay compared to the lactame analogs as was confirmed by increased activities in a cell free system (for compound **12**, 5% versus 31%, and for compound **14**, 16% versus 70%, at 500 nM in cellular and homogenate assays, respectively).

Substitution of 3-pyridine by 4-isoquinoline has been shown to improve *h*CYP11B2 inhibition in several compound classes, e.g. naphthalenes [30], dihydronaphthalenes [31] and dihydroquinolinones [34]. In this study, all 4-isoquinoline substituted compounds also show stronger *h*CYP11B2 inhibitory activity than the parent pyridine compounds. Thus, it can be concluded that substitution of the pyridine by isoquinoline as heme-complexing group leads to a considerable improvement in *h*CYP11B2 inhibition without affecting selectivity toward *h*CYP11B1. This trend is especially pronounced for the dihydroquinoxalinone compound **7** which shows an improvement of inhibitory potency by more than 300-fold compared to the corresponding pyridine analog **5**. Possibly, the annulated benzene ring enhances binding of the dihydroquinolinone type inhibitors to *h*CYP11B2 in two ways. First, the difference in electronic density of the pyridine and isoquinoline nitrogen might influence the coordination to the heme iron. Second, there might exist further interactions between the annulated benzene ring and the enzyme.

For our objective to discover new compounds suitable for *in vivo* studies preferably in rats, the most promising compounds regarding potency and selectivity were evaluated for inhibition of *r*CYP11B2 *in vitro*. A prediction of CYP11B2 inhibitory potency in rats based on human enzyme data is not reliable due to interspecies differences reflected in the rather low homology between human and rat CYP11B2 of about 70% [54]. The results of this study suggest a rather clear-cut structure activity relationship regarding the heme complexing moiety of the compounds under investigation. Pyridine compounds show no inhibitory activity of *r*CYP11B2, whereas isoquinoline substituted compounds inhibit both, the human and the rat CYP11B2. Furthermore, inhibition of the rat enzyme is not influenced by exchange of one core methylene by a hydrogen bond acceptor (compounds **4** and **9**), but, as observed for the human enzyme, introduction of an amine function results in a less potent

Table 3
Inhibition of *r*CYP11B2.

Compd.	% Inhibition[a]
	*r*CYP11B2[b]
I	<5
4	64
7	34
8	<5
9	51
Fadrozole	67 [0.5 μM]

[a] Mean value of at least three experiments; standard deviation less than 25%, inhibitor, 2 μM.
[b] Hamster fibroblasts expressing *r*CYP11B2; substrate deoxycorticosterone, 500 nM.

inhibitor (compound **7**).

In summary, we were able to discover highly active and selective compounds that inhibit human as well as *r*CYP11B2, like compounds **4** (*h*CYP11B2: IC_{50} = 0.5 nM, *r*CYP11B2: 64% inhibition, c = 2 μM) and **9** (*h*CYP11B2: IC_{50} = 0.3 nM, *r*CYP11B2: 51% inhibition, c = 2 μM). Future research will focus on the investigation of pharmacokinetic parameters *in vitro* and *in vivo* and on proof of concept studies in rats to determine the compounds' capability to improve the outcome of diseases, which are associated with elevated aldosterone concentrations in the plasma, such as hypertension, congestive heart failure or myocardial fibrosis.

4. Experimental section

4.1. Chemistry

4.1.1. Chemical and analytical methods

Melting points were measured on a Mettler FP1 melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker AM500 spectrometer at 500 MHz and 125 MHz, respectively, at 300 K. Chemical shifts are reported in parts per million (ppm), by reference to the hydrogenated residues of the deuterated solvent as internal standard. All coupling constants (J) are given in Hertz (Hz). Mass spectra (LC/UV/MS: ESI) were recorded on a SpectraSystem/MSQ Plus (ThermoFinnigan) instrument with an RP18-100-5 column (Macherey–Nagel). A water/acetonitrile gradient was used as eluent system. All compounds are >95% chemical pure as measured by LC/UV trace at 254 nm. Reagents were used as obtained from commercial suppliers without further purification. Solvents were distilled before use. If necessary, solvents were dried by distillation from appropriate drying reagents prior to use. Flash chromatography was performed on silica gel 40 (35/40–63/70 μM) with petroleum ether/ethyl acetate or CH_2Cl_2/methanol mixtures as eluents. Reaction progress was monitored by thin-layer chromatography (TLC) on TLC Silica Gel 60 F_{254} (Merck KGaA). Visualization was accomplished with UV light. Microwave irradiation experiments were carried out in a CEM-Discover monomode microwave apparatus.

4.1.2. General procedure A

Heteroarylboronic acid (1.3 equivalents), aryl bromide (1 equivalent), and tetrakis(triphenylphosphine) palladium(0) (5 mol %) were suspended in 1.5 ml DMF in a 10 ml septum-capped tube containing a stirring magnet. A solution of sodium hydrogen carbonate (3 equivalents) in 1.5 ml water was added. The tube was flushed with nitrogen and the vial was sealed tightly with a Teflon crimp top. The mixture was irradiated for 15 min at a temperature of 150 °C with an initial irradiation power of 100 W. After cooling to room temperature, the crude mixture was partitioned between ethyl acetate and water and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over anhydrous $MgSO_4$, concentrated and purified by flash chromatography and if necessary crystallization.

4.1.3. General procedure B

Heteroarylboronic acid (1.3 equivalents), aryl bromide (1 equivalent), and tetrakis(triphenylphosphine) palladium(0) (5 mol %) were suspended in DME to give a 0.07–0.1 M solution of boronic acid under nitrogen atmosphere. A 0.5 M aqueous solution of sodium carbonate (6 equivalents) was added. The mixture was refluxed for 3.5–14 h, cooled to room temperature, diluted with water and extracted several times with ethyl acetate. The combined extracts were dried over anhydrous $MgSO_4$, concentrated and purified by flash chromatography and were if necessary recrystallized.

4.1.4. 7-(Pyridin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**1**)

Compound **1** was obtained according to general procedure A using **1a** (342 mg, 1.5 mmol) and 3-pyridineboronic acid (242 mg, 1.95 mmol) after flash chromatography (CH_2Cl_2/methanol 95/5) as pale yellow solid (121 mg, 0.53 mmol, 36%), mp 256 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 4.63 (s, 2H), 7.00 (d, ^3J = 8.2 Hz, 1H), 7.32–7.34 (m, 2H), 7.44 (ddd, ^3J = 7.9 Hz, ^3J = 4.7 Hz, ^5J = 0.8 Hz, 1H), 8.00–8.03 (m, 1H), 8.52 (dd, ^3J = 4.7 Hz, ^4J = 1.6 Hz, 1H), 8.85 (d, ^4J = 2.5 Hz, 1H), 10.82 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 66.7, 114.3, 116.3, 120.7, 123.7, 127.2, 131.9, 133.5, 134.6, 143.7, 147.2, 148.1, 164.6. MS m/z 227.7 (MH^+).

4.1.5. 6-(Pyridin-3-yl)benzo[d]oxazol-2(3H)-one (**2**)

Compound **2** was obtained according to general procedure B using 6-bromobenzo[*d*]oxazol-2(3*H*)-one (428 mg, 2.0 mmol) and 3-pyridineboronic acid (320 mg, 2.6 mmol) with cesium carbonate as base instead of sodium carbonate after flash chromatography (CH_2Cl_2/methanol 95/5) and flash chromatography (ethyl acetate) as colorless solid (99 mg, 0.47 mmol, 23%), mp 265 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.20 (d, ^3J = 8.2 Hz, 1H), 7.46 (ddd, ^3J = 7.9 Hz, ^3J = 4.7 Hz, ^5J = 0.6 Hz, 1H), 7.51 (dd, ^3J = 8.0 Hz, ^4J = 1.7 Hz, 1H), 7.71 (d, ^4J = 1.9 Hz, 1H), 8.04–8.07 (m, 1H), 8.54 (dd, ^3J = 4.7 Hz, ^4J = 1.6 Hz, 1H), 8.88 (d, ^4J = 2.5 Hz, 1H), 11.73 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 108.0, 110.1, 122.5, 123.7, 130.4, 131.1, 133.8, 135.1, 144.0, 147.4, 148.1, 154.3. MS m/z 212.97 (MH^+).

4.1.6. 4-Methyl-7-(pyridin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**3**)

Compound **3** was obtained according to general procedure B using **3a** (225 mg, 0.93 mmol) and 3-pyridineboronic acid (149 mg, 1.21 mmol) after flash chromatography (CH_2Cl_2/methanol 99/1) as white solid (70 mg, 0.29 mmol, 31%), mp 149–150 °C. Anal. $C_{14}H_{12}N_2O_2$ (C, H, N, O). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.41 (s, 3H), 4.67 (s, 2H), 7.07 (d, ^3J = 8.2 Hz, 1H), 7.22 (d, ^4J = 2.0 Hz, 1H), 7.27 (dd, ^3J = 8.5 Hz, ^4J = 1.9 Hz, 1H), 7.35 (ddd, ^3J = 7.9 Hz, ^3J = 4.7 Hz, ^5J = 0.8 Hz, 1H), 7.83 (ddd, ^3J = 7.9 Hz, ^4J = 2.5 Hz, ^4J = 1.6 Hz, 1H), 8.59 (dd, ^3J = 4.7 Hz, ^4J = 1.6 Hz, 1H), 8.82 (d, ^4J = 1.9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.1, 67.6, 115.32, 115.35, 131.3, 123.6, 129.4, 133.7, 133.9, 135.3, 145.6, 148.0, 148.6, 164.3. MS m/z 241.20 (MH^+).

4.1.7. 7-(Isoquinolin-4-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**4**)

Compound **4** was obtained according to general procedure A using **1a** (242 mg, 1.95 mmol) and 4-isoquinolineboronic acid (337 mg, 1.95 mmol) after flash chromatography (CH_2Cl_2/methanol 97/3) as off-white solid (177 mg, 0.64 mmol, 43%), mp 266 °C. Anal. $C_{17}H_{12}N_2O_2$ (C, H, N, O). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 4.66 (s, 2H), 7.08 (d, ^3J = 8.5 Hz, 1H), 7.12–7.13 (m, 2H), 7.71–7.74 (m, 1H), 7.80–7.81 (m, 1H), 7.89 (d, ^3J = 8.5 Hz, 1H), 8.21 (d, ^3J = 8.2 Hz, 1H), 8.41 (s, 1H), 9.32 (s, 1H), 10.88 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 66.7, 115.9, 117.4, 123.9, 124.0, 127.1, 127.4, 127.9, 128.0, 131.0, 131.1, 131.7, 133.1, 142.3, 143.3, 151.7, 164.7. MS m/z 276.6 (MH^+).

4.1.8. 6-(Pyridin-3-yl)-3,4-dihydroquinoxalin-2(1H)-one (**5**)

Dihydroquinoxalinone **5a** (340 mg, 1.50 mmol) and 3-pyridineboronic acid (240 mg, 1.95 mmol) were coupled according to general procedure A. Hydration over 10% Pd/C at room temperature overnight and purification by flash chromatography (CH_2Cl_2/methanol 95/5) afforded **5** as yellow solid (74 mg, 0.33 mmol, 22%), mp (decomp.) 182 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.77 (s, 2H), 6.08 (s, 1H), 6.83 (d, ^3J = 8.2 Hz, 1H), 6.95 (dd, ^3J = 8.0 Hz, ^4J = 2.0 Hz, 1H), 6.97 (d, ^4J = 1.9 Hz, 1H), 7.43 (dd, ^3J = 7.7 Hz, ^3J = 4.6 Hz, 1H), 7.91 (dt, ^3J = 7.9 Hz, ^4J = 2.0 Hz, 1H), 8.50 (dd, ^3J = 4.6 Hz, ^4J = 1.1 Hz, 1H), 8.75 (d, ^4J = 2.2 Hz, 1H), 10.34 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 46.1, 111.2, 115.4, 116.3, 123.7,

126.2, 131.5, 133.3, 135.3, 135.7, 147.0, 147.7, 165.8. MS *m*/*z* 226.15 (MH^+).

4.1.9. 4-Methyl-6-(pyridin-3-yl)-3,4-dihydroquinoxalin-2(1H)-one (**6**)

Compound **6** was obtained according to general procedure A using **6a** (342 mg, 1.42 mmol) and 3-pyridineboronic acid (229 mg, 1.86 mmol) after flash chromatography (CH_2Cl_2/methanol 97/3) and crystallization from acetone as pale yellow solid (55 mg, 0.23 mmol, 16%), mp > 300 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.87 (s, 3H), 3.70 (s, 2H), 6.90 (d, ^3J = 7.9 Hz, 1H), 6.98 (s, 1H), 7.06 (d, ^3J = 7.9 Hz, 1H), 7.43 (dd, ^3J = 7.6 Hz, ^3J = 5.0 Hz, 1H), 8.02 (dd, ^3J = 7.6 Hz, ^4J = 1.6 Hz, 1H), 8.51 (d, ^3J = 4.7 Hz, 1H), 8.86 (d, ^4J = 2.2 Hz, 1H), 10.52 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 36.8, 54.0, 109.7, 115.1, 117.0, 123.6, 127.4, 131.9, 133.6, 135.8, 136.7, 147.3, 147.7, 165.9. MS *m*/*z* 240.0 (MH^+).

4.1.10. 6-(Isoquinolin-4-yl)-3,4-dihydroquinoxalin-2(1H)-one (**7**)

Compound **7** was obtained according to general procedure A using **5a** (340 mg, 1.5 mmol) and 4-isoquinolineboronic acid (337 mg, 1.95 mmol) after flash chromatography (CH_2Cl_2/methanol 95/5) and crystallization from ethanol as yellow solid (148 mg, 0.54 mmol, 36%), mp > 300 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.82 (d, ^4J = 1.8 Hz, 2H), 6.14 (s, 1H), 6.75 (dd, ^3J = 7.9 Hz, ^4J = 1.6 Hz, 1H), 6.82 (d, ^4J = 1.8 Hz, 1H), 6.90 (d, ^3J = 7.9 Hz, 1H), 7.69–7.73 (m, 1H), 7.76–7.80 (m, 1H), 7.93 (d, ^3J = 8.5 Hz, 1H), 8.19 (d, ^3J = 8.2 Hz, 1H), 8.37 (s, 1H), 9.28 (s, 1H), 10.42 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 46.1, 114.4, 115.0, 119.2, 124.2, 126.0, 127.3, 127.9, 127.9, 130.7, 130.7, 132.7, 133.2, 134.9, 142.0, 151.4, 165.9. MS *m*/*z* 276.0 (MH^+).

4.1.11. 6-(Pyridin-3-yl)-1H-benzo[d][1,3]oxazin-2(4H)-one (**8**)

Compound **8** was obtained according to general procedure B using **8a** (342 mg, 1.50 mmol) and 3-pyridineboronic acid (240 mg, 1.95 mmol) after flash chromatography (CH_2Cl_2/methanol 97/3) and crystallization from acetone as colorless solid (85 mg, 0.38 mmol, 25%), mp 211 °C. Anal. $C_{13}H_{10}N_2O_2$ (C, H, N, O). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.35 (s, 2H), 7.00 (d, ^3J = 8.2 Hz, 1H), 7.46 (dd, ^3J = 8.2 Hz, ^3J = 4.7 Hz, 1H), 7.61 (s, 1H), 7.64 (dd, ^3J = 8.0 Hz, ^4J = 2.0 Hz, 1H), 8.00–8.02 (m, 1H), 8.53 (dd, ^3J = 4.7 Hz, ^4J = 1.6 Hz, 1H), 8.85 (d, ^4J = 1.9 Hz, 1H), 10.29 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 67.4, 114.2, 119.2, 122.9, 123.7, 125.1, 127.2, 131.0, 133.4, 136.4, 147.1, 148.0, 151.5. MS *m*/*z* 227.32 (MH^+).

4.1.12. 6-(Isoquinolin-4-yl)-1H-benzo[d][1,3]oxazin-2(4H)-one (**9**)

Compound **9** was obtained according to general procedure B using **8a** (255 mg, 1.12 mmol) and 4-isoquinolineboronic acid (232 mg, 1.34 mmol) after flash chromatography (CH_2Cl_2/methanol 98/2) and crystallization from acetone as colorless solid (74 mg, 0.27 mmol, 24%), mp 215 °C. Anal. $C_{17}H_{12}N_2O_2$ (C, H, N, O). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.38 (s, 2H), 7.08 (d, ^3J = 8.2 Hz, 1H), 7.41 (s, 1H), 7.44 (dd, ^3J = 8.2 Hz, ^4J = 1.9 Hz, 1H), 7.72–7.75 (m, 1H), 7.78–7.81 (m, 1H), 7.88 (d, ^3J = 8.5 Hz, 1H), 8.21 (d, ^3J = 8.2 Hz, 1H), 8.41 (s, 1H), 9.32 (s, 1H), 10.34 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 67.4, 113.8, 118.9, 124.0, 125.9, 127.4, 127.9, 128.0, 130.2, 130.2, 131.0, 131.8, 133.1, 136.3, 142.3, 151.6, 151.7. MS *m*/*z* 277.18 (MH^+).

4.1.13. 6-(Pyridin-3-yl)-3,4-dihydroquinazolin-2(1H)-one (**10**)

Compound **10** was obtained according to general procedure B using **10a** (341 mg, 1.50 mmol) and 3-pyridineboronic acid (221 mg, 1.80 mmol) after flash chromatography (CH_2Cl_2/methanol 93/7) as colorless solid (331 mg, 1.47 mmol, 98%), mp (decomp.) 170 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 4.39 (s, 2H), 6.86 (s, 1H), 6.88 (d, ^3J = 8.8 Hz, 1H), 7.43 (dd, ^3J = 7.7 Hz, ^3J = 4.9 Hz, 1H), 7.49–7.51 (m, 2H), 7.98 (d, ^3J = 8.2 Hz, 1H), 8.49 (dd, ^3J = 4.4 Hz, ^4J = 1.3 Hz, 1H), 8.82 (d, ^4J = 1.9 Hz, 1H), 9.14 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 42.5, 114.1, 118.9, 123.7, 124.2, 126.1, 129.6, 133.2, 135.1, 138.2, 147.0, 147.7, 154.3. MS *m*/*z* 226.16 (MH^+).

4.1.14. 6-(Isoquinolin-4-yl)-3,4-dihydroquinazolin-2(1H)-one (**11**)

Compound **11** was obtained according to general procedure B using **10a** (454 mg, 2.00 mmol) and 4-isoquinolineboronic acid (450 mg, 2.60 mmol) after flash chromatography (CH_2Cl_2/methanol 95/5) and crystallization from ethanol as yellow solid (345 mg, 1.25 mmol, 63%), mp (decomp.) 221 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 4.41 (s, 2H), 6.88 (s, 1H), 6.96 (d, ^3J = 8.2 Hz, 1H), 7.29–7.30 (m, 2H), 7.72 (dd, ^3J = ^3J = 7.4 Hz, 1H), 7.79 (dd, ^3J = ^3J = 7.4 Hz, 1H), 7.90 (d, ^3J = 8.5 Hz, 1H), 8.20 (d, ^3J = 8.2 Hz, 1H), 8.40 (s, 1H), 9.20 (s, 1H), 9.30 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 42.4, 113.7, 118.6, 124.1, 127.1, 127.3, 127.9, 128.0, 129.2, 133.2, 138.0, 141.4, 142.2, 147.0, 151.4, 151.5, 154.4. MS *m*/*z* 276.05 (MH^+).

4.1.15. 6-(Pyridin-3-yl)-1H-4,2,1-benzoxathiazine-2,2-dioxide (**12**)

Compound **12** was obtained according to general procedure B using **12a** (154 mg, 0.58 mmol) and 3-pyridineboronic acid (93 mg, 0.76 mmol) after flash chromatography (CH_2Cl_2/methanol 98/2) as colorless solid (53 mg, 0.20 mmol, 35%), mp (decomp.) 214 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.23 (s, 2H), 6.95 (d, ^3J = 8.5 Hz, 1H), 7.41–7.46 (m, 2H), 8.03 (d, ^3J = 7.9 Hz, 1H), 8.53 (dd, ^3J = 4.8 Hz, ^4J = 1.4 Hz, 1H), 8.85 (d, ^4J = 2.1 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 76.6, 116.5, 120.5, 122.1, 123.9, 127.4, 132.6, 133.9, 134.5, 142.5, 147.4, 148.4. MS *m*/*z* 262.92 (MH^+).

4.1.16. 1-Methyl-6-(pyridin-3-yl)-1H-4,2,1-benzoxathiazine-2,2-dioxide (**13**)

Compound **13** was obtained according to general procedure B using **13a** (46 mg, 0.52 mmol) and 3-pyridineboronic acid (84 mg, 0.68 mmol) after flash chromatography (CH_2Cl_2/methanol 99/1) as off-white solid (103 mg, 0.37 mmol, 72%), mp 128 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.34 (s, 3H), 5.01 (s, 2H), 7.06 (d, ^3J = 8.2 Hz, 1H), 7.29 (d, ^4J = 2.1 Hz, 1H), 7.33 (dd, ^3J = 8.4 Hz, ^4J = 2.0 Hz, 1H), 7.36 (ddd, ^3J = 7.9 Hz, ^3J = 4.9 Hz, ^5J = 0.6 Hz, 1H), 7.82 (d, ^3J = 7.9 Hz, 1H), 8.59 (dd, ^3J = 4.8 Hz, ^4J = 1.5 Hz, 1H), 8.80 (d, ^4J = 1.8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 31.6, 76.6, 117.1, 118.7, 122.5, 123.6, 129.9, 134.0, 134.2, 134.8, 143.4, 148.0, 148.8. MS *m*/*z* 276.93 (MH^+).

4.1.17. 6-(Isoquinolin-4-yl)-1H-4,2,1-benzoxathiazine-2,2-dioxide (**14**)

Compound **14** was obtained according to general procedure B using **12a** (169 mg, 0.64 mmol) and 4-isoquinolineboronic acid (144 mg, 0.83 mmol) after flash chromatography (CH_2Cl_2/methanol 98/2) as off-white solid (30 mg, 0.10 mmol, 15%), mp (decomp.) 213 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.30 (s, 2H), 7.02 (d, ^3J = 8.2 Hz, 1H), 7.23 (dd, ^3J = 8.0 Hz, ^4J = 2.0 Hz, 1H), 7.27 (d, ^4J = 1.6 Hz, 1H), 7.74 (ddd, ^3J = ^3J = 7.5 Hz, ^4J = 1.2 Hz, 1H), 7.81 (ddd, ^3J = ^3J = 7.6 Hz, ^4J = 1.5 Hz, 1H), 7.87 (dd, ^3J = 8.5 Hz, ^4J = 0.9 Hz, 1H), 8.22 (d, ^3J = 8.2 Hz, 1H), 8.42 (s, 1H), 9.34 (s, 1H), 10.84 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 76.4, 119.3, 119.9, 123.8, 125.1, 127.0, 127.4, 127.9, 128.0, 131.1, 131.2, 131.6, 133.0, 141.9, 142.3, 151.9. MS *m*/*z* 313.01 (MH^+).

4.1.18. 6-(Pyridin-3-yl)-3,4-dihydrobenzothiazin-4-one-2,2-dioxide (**15**)

Compound **15a** (273 mg, 0.75 mmol) and 10% Pd/C (273 mg) were refluxed in 10 ml methanol overnight under a hydrogen atmosphere. The suspension was filtered over charcoal and the solvent evaporated. Purification by flash chromatography (CH_2Cl_2/methanol 97/3) afforded **15** as colorless solid (33 mg, 0.12 mmol, 16%), mp (decomp.) 172 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 4.85

(s, 2H), 7.23 (d, ^3J = 8.5 Hz, 1H), 7.50 (ddd, ^3J = 7.9 Hz, ^3J = 4.7 Hz, ^5J = 0.9 Hz, 1H), 8.05 (dd, ^3J = 8.5 Hz, ^4J = 2.2 Hz, 1H), 8.08 (ddd, ^3J = 7.9 Hz, ^4J = 2.5 Hz, ^4J = 1.6 Hz, 1H), 8.16 (d, ^4J = 2.2 Hz, 1H), 8.59 (dd, ^3J = 4.7 Hz, ^4J = 1.6 Hz, 1H), 8.90 (d, ^4J = 1.6 Hz, 1H), 11.67 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 61.4, 119.4, 120.7, 123.9, 125.6, 131.5, 133.9, 134.0, 134.7, 142.3, 147.2, 148.5, 185.4. MS *m*/*z* 274.75 (MH^+).

4.2. Biology

4.2.1. Activity and selectivity assays using V79MZ cells

V79MZ*h*CYP11B1, V79MZ*h*CYP11B2 or V79MZ*r*CYP11B2 cells [26,54] were pre-incubated with 500 nM (*h*CYP11B) or 2 μM (*r*CYP11B2) of inhibitor for 1 hh at 37 °C. The reaction was started by addition of 100 nM (*h*CYP11B) or 500 nM (*r*CYP11B2) [^{3}H]-11-deoxycorticosterone as substrate. After incubation for 25 min (*h*CYP11B1), 45 min (*h*CYP11B2) or 7 h (*r*CYP11B2), the enzyme reactions were stopped by extracting the supernatant with ethyl acetate. Samples were centrifuged, and the ethyl acetate was separated [24]. The steroids were separated by HPLC and analyzed with radio flow detection.

4.2.2. Cell-free activity assay using V79MZhCYP11B2 homogenate

For preparation of recombinant enzyme, V79MZ*h*CYP11B2 cells were sonicated. The material thus obtained was centrifuged to remove unbroken cells and the supernatant was used for the evaluation of CYP11B2 inhibition. A solution containing buffer, cell homogenate and NADPH regenerating system was pre-incubated with inhibitor (c = 500 nM) for 10 min at 37 °C. The subsequent assay was carried out according to the procedure described in Chapter 4.2.1, but using an incubation time of 90 min.

4.2.3. Selectivity tests versus hCYP17 and hCYP19

*h*CYP17 [22] and *h*CYP19 [53] enzyme preparations and assay procedures were performed as previously described.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.12.022.

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