Synthesis and Evaluation of Imidazolylmethylenetetrahydronaphthalenes and Imidazolylmethyleneindanes: Potent Inhibitors of Aldosterone Synthase

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Elevated plasma aldosterone levels play a detrimental role in certain forms of congestive heart failure and myocardial fibrosis. We proposed aldosterone synthase (CYP11B2) as a novel target for the treatment of these diseases. In this study, the synthesis and biological evaluation of substituted *E*- and *Z*-imidazolylmethylenetetrahydronaphthalenes and *E*- and *Z*-imidazolylmethyleneindanes (compounds **1a**,**b**−**9a**,**b**) is described. The compounds were prepared by a Wittig-like reaction. They were tested for activity using bovine CYP11B and human CYP11B2 expressed in fission yeast and V79 MZh cells. Selectivity was determined toward human CYP11B1, CYP19, and CYP17. Especially in the case of CYP11B1 (steroid 11β-hydroxylase), selectivity is a crucial issue, since sequence homology between this enzyme and the target enzyme is very high (93%). On the basis of the X-ray structure of human CYP2C9, a protein model of CYP11B2 was developed and docking experiments with the title compounds were performed. The biological results revealed highly potent inhibitors of CYP11B2 (IC_{50} = 4−93 nM). The *Z*-isomers usually were more active than the corresponding *E*-isomers. Different inhibitory profiles could be observed: rather selective inhibitors of CYP11B1, dual inhibitors of both enzymes, and rather selective inhibitors of CYP11B2. The chloro derivative **8b** was found to be a highly potent CYP11B2 inhibitor (IC_{50} = 4 nM) showing a 5-fold selectivity for CYP11B1 (IC_{50} = 20 nM). This compound could be an interesting lead for further optimization as a therapeutic agent. It also could be used as well as the CYP11B1 selective compounds as a pharmacological tool.

Introduction

Aldosterone synthase (CYP11B2), the key enzyme of mineralocorticoid biosynthesis, catalyzes the conversion of its steroidal substrate 11-deoxycorticosterone to the most potent mineralocorticoid aldosterone.[1] It is localized at the inner mitochondrial membrane, predominantly in adrenal cells and to a minor extent in the heart, brain, and vascular smooth muscle cells. The adrenal aldosterone synthesis is regulated by several physiological parameters such as the renin−angiotensin−aldosterone system (RAAS) and the plasma potassium concentration. Elevations in plasma aldosterone levels increase blood pressure and play an important role in the pathophysiology of certain forms of myocardial fibrosis and congestive heart failure.[2] The RAAS is pathologically activated by an insufficient renal flow that leads to an excessive release of aldosterone. Chronic elevations in plasma aldosterone increase the blood volume and, as a consequence of this, elevate the blood pressure and are known to stimulate cardiac fibroblasts, resulting in cardiac hypertrophy, myocardial fibrosis, and ventricular arrhythmia.[3−5] The RALES trial (randomized aldactone evaluation study, 1999)[6] showed that spironolactone[7,8] (Chart 1), functionally acting as a competitive antagonist of aldosterone at the mineralocorticoid receptor, reduces mortality in heart failure patients by 30%. However, its use is accompanied by progestational and antiandrogenic side effects due to its steroidal structure.[6,9] Recently the aldosterone antagonist eplerenone showed a reduced mortality in acute myocardial infarction of 15% and a reduction of sudden death by 21% (EPHESUS study, 2003).[10] Because increased plasma aldosterone levels do not result in a down-regulation but in an up-regulation of the aldosterone receptor,[11] long-term therapy with aldosterone antagonists might be ineffective for myocardial fibrosis. A new pharmacological approach for the treatment of hyperaldosteronism and congestive heart failure and a better strategy for the treatment of myocardial fibrosis should be the blockade of aldosterone formation, preferably by inhibition of CYP11B2. Nonsteroidal, selective inhibitors are to be preferred because they can be expected to have fewer side effects on the endocrine system. The inhibitors must not affect other P450 (CYP) enzymes. In the case of 11β-hydroxylase (key enzyme of glucocorticoid biosynthesis, CYP11B1), this is supposed to be very difficult to achieve, since it has a sequence homology of more than 93% compared to CYP11B2.[1,12] To date, the only described compound affecting corticoid formation in humans is the aromatase

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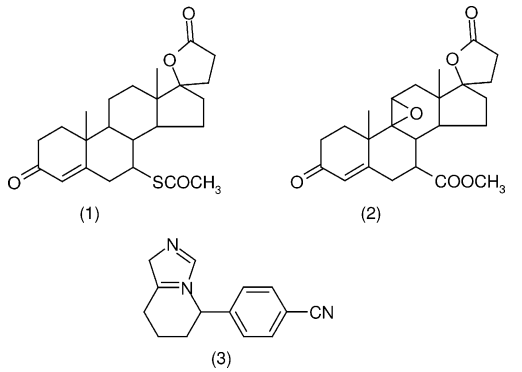
Chart 1. Chemical Structures of Spironolactone (**1**), Eplerenone (**2**), and Fadrozole (**3**)

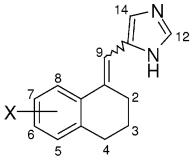


(estrogen synthase, CYP19) inhibitor fadrozole, which is used in the therapy of breast cancer. It suppresses aldosterone and cortisol levels after application of a dose 10-fold higher than the therapeutical dose.[13] For the discovery and structural optimization of CYP11B2 inhibitors, we recently have described a screening procedure.[14] Bovine adrenal mitochondria consisting of 18-hydroxylase (CYP18, CYP11B)[15] are used for preselection.[14] Because this enzyme (which catalyzes the synthesis of gluco- and mineralocorticoids[16]) shows a greater evolutionary distance to CYP11B2 than CYP11B1 to CYP11B2, human CYP11B2 expressed in fission yeast (*S. pombe*)[17] is used in the next screening step.[18] To ascertain the selectivity of potent compounds, an assay was developed[18] with V79 MZh cells (hamster lung fibroblasts) expressing human CYP11B1 and CYP11B2,[19] which is applied at the subsequent level. From a screeening of our compound library consisting of substances synthesized as (CYP) inhibitors, several hits were obtained.[14] This initial search also resulted in the *E*- and *Z*-isomers **1a**,**b** and **2a**,**b**. In the present study, the syntheses of a series of imidazolylmethylenetetrahydronaphthalenes and imidazolylmethyleneindanes is described (Chart 2), as well as the determination of their biological activity regarding inhibition of bovine CYP11B and human CYP11B2 for potency and human CYP11B1, human CYP17 (17α-hydroxylase-C17,20-lyase, key enzyme responsible for androgen biosynthesis), and CYP19 for selectivity.

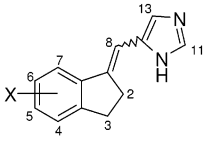
Chemistry

Our retrosynthetic analysis of compounds **1**−**9** demonstrates that the key step for the synthesis of the target molecules could be a Wittig reaction. This reaction has already been studied by Reimann et al.[20] on similar isochinolines, and they concluded that the phosphonium salt should be prepared from the bicyclic component. The various alcohol intermediates **10**−**18** were obtained from the corresponding ketones. Compounds **1**, **2**, and **7**−**9** were synthesized from commercially available tetralones and indanones. Compounds **3**−**5**, substituted with a nitrile function at different positions on the aromatic ring, required the synthesis of the corresponding ketones **3i**, **4i**, and **5i** (Scheme 1). The hydroxylated compounds **3iii**, **4iii**, and **5iii** have been prepared following the procedure described by Woo et al.[21] These intermediates were transformed into trifluoromethane sulfonates **3ii, 4ii, 5ii** followed by palladium-catalyzed cross coupling to yield the nitriles **3i, 4i, 5i** as described by Almansa et al. for similar compounds.[22] A novel synthetic pathway was pursued for the synthesis of the ketone **6i**:[23] a Friedel−Crafts acylation of chlorobenzene with succinic anhydride gave 3-(4-chlorobenzoyl)propionic acid,[24] which was transformed to 4-(4-chlorophenyl)butyric acid by a modified Clemmensen reduction[25] and then cyclized with polyphosphoric acid to the ketone[26] (Scheme 2). The ketones were reduced with $NaBH_4$,[27] and the obtained alcohols **10**−**18** were transformed into the phosphonium salts using triphenylphosphonium bromide in benzene.[20] The reaction was slow in benzene, but the phosphonium salts precipitated easily. Sodium ethanolate deprotonated the 4(5)-imidazolecarboxaldehyde, and the newly formed imidazolid anion deprotonated the phosphonium salt, which subsequently reacted with the imidazole-

Chart 2. Title Compounds



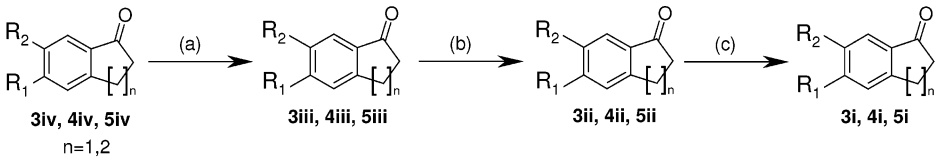
1a, 1b, 3a, 3b, 4b, 6a



2a, 2b, 5a, 5b, 7a, 7b, 8a, 8b, 9a, 9b

no	X	Isomer	no	X	Isomer	no	X	Isomer
1a	H	*E*	**3b**	7-CN	*Z*	**7a**	5-F	*E*
1b	H	*Z*	**4b**	6-CN	*Z*	**7b**	5-F	*Z*
2a	H	*E*	**5a**	5-CN	*E*	**8a**	5-Cl	*E*
2b	H	*Z*	**5b**	5-CN	*Z*	**8b**	5-Cl	*Z*
3a	7-CN	*E*	**6a**	7-Cl	*E*	**9a**	5-Br	*E*
						9b	5-Br	*Z*

Scheme 1. Synthesis of Compounds **3i**, **4i**, and **5i**[a]



	R_1	R_2	n
3iv	H	OMe	2
4iv	OMe	H	2
5iv	OMe	H	1

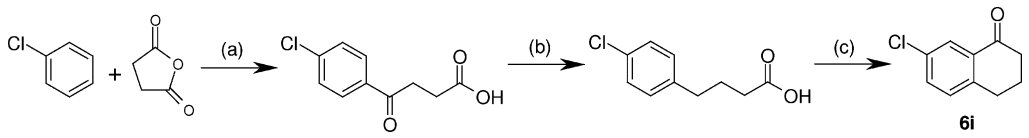
	R_1	R_2	n
3iii	H	OH	2
4iii	OH	H	2
5iii	OH	H	1

	R_1	R_2	n
3ii	H	OTf	2
4ii	OTf	H	2
5ii	OTf	H	1

	R_1	R_2	n
3i	H	CN	2
4i	CN	H	2
5i	CN	H	1

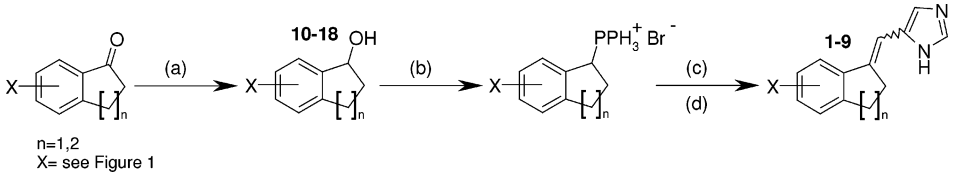
[a] Conditions: (a) $AlCl_3$, benzene, 3 h, reflux; (b) trifluoromethanesulfonic anhydride, dry pyridine, 15 min at 0−5 °C, then 2 h at room temp; (c) Zn, PPh_3, KCN, $Ni(PPh_3)_2Cl_2$, MeCN, 2 h, 60°C.

Scheme 2. Synthesis of Compound **6i**[a]



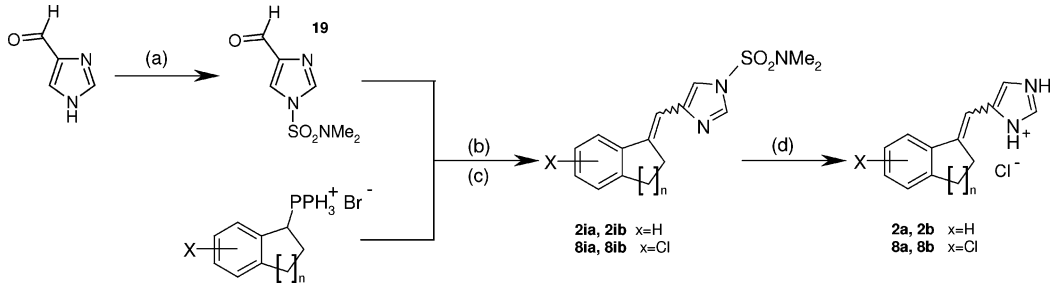
[a] Conditions: (a) $AlCl_3$, 40−50 °C; (b) $HgCl_2$/Zn, H_2O, toluene, 24 h, reflux, addition of HCl every 6 h; (c) PPA, 40 min, 70°C.

Scheme 3. Synthesis of Compounds **1**−**9**[a]



[a] Conditions: (a) $NaBH_4$, $MeOH/CH_2Cl_2$, 15 min at 0°C, 1 h at room temp; (b) PPh_3·HBr, benzene, 12 h, reflux; (c) EtONa, 4(5)-imidazolecarboxaldehyde, N_2, 12 h, reflux; (d) separation of the isomers by flash column chromatography.

Scheme 4. Alternative Synthesis of Compounds **2a**,**b** and **8a**,**b**[a]



[a] Conditions: (a) Me_2NSO_2Cl, NEt_3, CH_2Cl_2, 12 h at room temp; (b) K_2CO_3, 18-crown-6, CH_2Cl_2, 12 h, reflux; (c) separation of the isomers by flash column chromatography; (d) HCl (4 N), dioxane, 12 h, reflux.

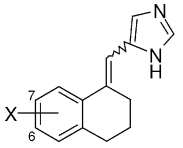
carboxaldehyde (Scheme 3). After the Wittig reaction, the mixture of *E*- and *Z*-isomers was separated by flash column chromatography. The obtained *E*- and *Z*-isomers could be distinguished by NMR. According to an incremental analysis,[28] the vinyl-H of the E-isomer should display a chemical shift of 0.37 ppm downfield (~6.71 ppm) compared to the *Z*-isomer (~6.34 ppm). These theoretical values could be confirmed experimentally.

An alternative synthetic route was used to synthesize compounds **2a**, **2b**, **8a**, and **8b**. To protect the imidazolecarboxaldehyde, dimethylsulfamoyl chloride was employed as described.[29,30] This sulfonamide (**19**) was subsequently used in the Wittig reaction with K_2CO_3 as base (Scheme 4) to form the intermediates **2ia, 2ib, 8ia**, and **8ib**. By use of hydrochloric acid, the protecting group was removed and the HCl salts were formed. With this method, the overall yield of the isomeric mixture could be enhanced by about 15−20%.

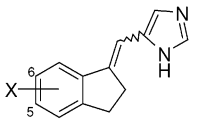
Biological Results

Inhibition of Biosynthesis of Adrenal Corticoids in Vitro (Table 1). The percent inhibition values of the compounds toward CYP18 were determined in vitro using enzyme from bovine adrenal mitochondria and corticosterone as substrate according to Hartmann et al.[15] The compounds showed a high inhibitory activity in the bovine enzyme at an inhibitor concentration of 1 μM compared to the reference ketoconazole (78%), which is a nonselective CYP inhibitor (Table 1). A lot of compounds exceeded the reference (**1b**, **2b**, **3b**, **4b**, **5a**, **5b**, **8b**, **9b**), showing inhibition values in the range of 90%. Only the unsubstituted tetraline derivative **1a** and the 5-chlorinated indane derivative **8a** exhibited low inhibition values of about 40%. It is striking that the *Z*-isomers were more potent than the corresponding *E*-isomers. The only exception were the fluorinated compounds **7a** and **7b**, exhibiting similar inhibition values of about 65%. Introduction of a nitrile group at

Table 1. Inhibition of Biosynthesis of Adrenal CYP11B Enzymes in Vitro



1a, 1b, 3a, 3b, 4b, 6a



2a, 2b, 5a, 5b, 7a, 7b, 8a, 8b, 9a, 9b

			% inhibition[a]		IC_{50} (nM)[d]	
compd	X	isomer	bovine[b] bCYP11B	human[c] hCYP11B2	V79 11B1[e] hCYP11B1	V79 11B2[f] hCYP11B2
1a	H	*E*	40	80	31.4	24.8
1b	H	*Z*	95	82	3.3	9.6
2a	H	*E*	62	77	25.9	41.0
2b	H	*Z*	94	81	6.1	11.0
3a	7-CN	*E*	62	8	nd	nd
3b	7-CN	*Z*	97	23	nd	nd
4b	6-CN	*Z*	97	54	6.90	22.7
5a	5-CN	*E*	81	49	15.0	35.9
5b	5-CN	*Z*	100	65	12.3	35.7
6a	7-Cl	*E*	66	48	18.7	47.3
7a	5-F	*E*	67	59	20.6	16.7
7b	5-F	*Z*	64	58	11.17	13.9
8a	5-Cl	*E*	45	79	28.7	88.8
8b	5-Cl	*Z*	87	80	19.5	3.7
9a	5-Br	*E*	63	68	26.2	92.8
9b	5-Br	*Z*	88	76	23.5	10.3
fadrozole			nd	68	9.7	1.0
ketoconazole			78	nd	nd	nd

[a] Mean value of four determinations, standard deviation less than 10%. nd = not determined. [b] Bovine adrenal mitochondria, 1 mg/mL of protein; substrate corticosterone, 200 μM; inhibitor, 1 μM. [c] *S. pombe* expressing human CYP11B2; substrate deoxycorticosterone, 100 nM; inhibitor, 500 nM. [d] Mean value of four determinations, standard deviation less than 20%. nd = not determined. [e] Hamster fibroblasts expressing human CYP11B1; substrate deoxycorticosterone, 100 nM. [f] Hamster fibroblasts expressing human CYP11B2; substrate deoxycorticosterone, 100 nM.

the benzene nucleus enhanced the potency of the compounds, while substitution with halogen reduced the inhibitory activity. When the skeletal structures were compared, no difference in potency was observed between indane and tetraline derivatives.

For the determination of the inhibitory activity of the compounds toward human CYP11B2, a screening assay was used as described by us.[18] CYP11B2 expressing fission yeast was incubated with inhibitor and [^{14}C]-deoxycorticosterone as substrate, and the product formation was monitored by HPTLC using a phosphoimager. Most of the compounds showed a higher inhibitory activity than the reference fadrozole (68%) at an inhibitor concentration of 500 nM (Table 1). Only the 7-CN substituted tetralines **3a** and **3b** exhibited a low activity with inhibition values of 8% and 23%, respectively. The unsubstituted compounds **1a**, **1b**, and **2b** and the chloro derivative **8b** were the most active compounds. As observed with the bovine enzyme, in human CYP11B2 the *Z*-isomers were more potent than the corresponding *E*-isomers. Substitution at the 7-position at the benzene nucleus of the tetraline derivatives diminished potency (**3a**, **3b**, **6a**). In contrast to the bovine enzyme, substitution with a nitrile group reduced the inhibitory activity of the compounds in human CYP11B2, while introduction of halogen did not show a significant effect. Surprisingly, there was little to no correlation between the inhibition data for the bovine and the human enzyme. In the case of compounds **1a**, **3a**, and **3b** a reversal of potency was observed for the two enzymes. While compound **1a** showed low activity in CYP11B, it displayed a high inhibition value in human CYP11B2 (40% → 80%). For compounds **3a** and **3b** a strong reduction of potency from bovine to human enzyme was observed (62% → 8%; 97% → 23%).

The most active compounds, showing more than 50% inhibition in *S. pombe*, were tested for activity and selectivity in V79 MZh cells (hamster lung fibroblasts) expressing either CYP11B1 or CYP11B2 as described recently.[18] [^{14}C]deoxycorticosterone was used as substrate for V79 MZh 11B1 and V79 MZh 11B2, and the products were monitored as in the yeast assay. In Table 1 the IC_{50} values are presented. All compounds showed a strong inhibition of CYP11B1, the corresponding IC_{50} values differing by 1 order of magnitude (IC_{50} CYP11B1, 3−31 nM). In the case of CYP11B2, the compounds exhibited very high activity too (IC_{50} CYP11B2, 4−93 nM). As observed for the bovine CYP11B and the human CYP11B2 expressed in yeast, the *Z*-isomers showed a higher potency for the human CYP11B1 and CYP11B2 expressed in V79 cells compared to the *E*-isomers. In particular for the chlorinated and brominated compounds **8a**,**b** and **9a**,**b**, this observation was very prominent. Regarding the selectivity of the compounds, it is striking that **1b**, **2b**, **4b**, **5a**, **5b**, **6a**, **8a**, and **9a** exhibited a slight selectivity for CYP11B1 compared to CYP11B2 with a 2- to 3-fold lower IC_{50} for the 11β-hydroxylase. The inhibitors **1a**, **2a**, **7a**, and **7b** did not show any selectivity for one of the two enzymes. Compounds **8b**, **9b** and the reference fadrozole displayed a stronger inhibition for CYP11B2.

Inhibition of CYP19 and CYP17 in Vitro (Table 2). The inhibitory activities of the compounds toward CYP19 were determined in vitro using human placental microsomes and [1β,2β-^{3}H]testosterone as substrate as described by Thompson and Siterii [31] using our modi-

Table 2. Inhibition of CYP17 and CYP19 in Vitro

compd	X	isomer	% inhibition of CYP17[a]	IC_{50} (μM) of CYP19[b]
1a	H	*E*	13	0.226
1b	H	*Z*	13	0.190
2a	H	*E*	11	0.955
2b	H	*Z*	1	0.130
3a	7-CN	*E*	2	0.970
3b	7-CN	*Z*	3	0.810
4b	6-CN	*Z*	4	0.125
5a	5-CN	*E*	21	0.119
5b	5-CN	*Z*	4	0.015
6a	7-Cl	*E*	24	0.120
7a	5-F	*E*	16	0.218
7b	5-F	*Z*	11	0.020
8a	5-Cl	*E*	37	0.330
8b	5-Cl	*Z*	26	0.039
9a	5-Br	*E*	13	0.027
9b	5-Br	*Z*	15	0.100
fadrozole			7	0.005[c]
ketoconazole			40	nd

[a] Mean value of four determinations, standard deviation less than 10%; *E. coli* expressing human CYP17; 5 mg/mL of protein; substrate progesterone, 2.5 μM; inhibitor, 2.5 μM. [b] Mean value of four determinations, standard deviation less than 5%; human placental CYP19, 1 mg/mL of protein; substrate testosterone, 2.5 μM. nd = not determined. [c] See ref 33.

fication.[32] Several compounds turned out to be potent inhibitors of CYP19 (IC_{50} values: **5b**, 15 nM; **7b**, 20 nM; **9a**, 21 nM) but were less active than the reference compound fadrozole (IC_{50} = 5.0 nM).[33] The chloro derivative **8b**, the strongest inhibitor of CYP11B2, was less active an inhibitor of CYP19 (IC_{50} = 39 nM).

The percent inhibition values of the compounds toward CYP17 were determined in vitro using microsomes from *E. coli* recombinantly expressing CYP17 and progesterone as substrate as described by us.[34] All compounds showed no or only weak inhibition (<25%) at an inhibitor concentration of 2.5 μM compared to the reference ketoconazole (40%).

Protein Modeling and Docking. To understand the experimental results on a structural basis, molecular docking studies for several inhibitors toward CYP11B2 had to be performed. Because there are no X-ray structures available for CYP11B2 because of its membrane-bound nature, a 3D structural model for CYP11B2 was used, which had been built on the basis of various CYP structures from different microorganisms.[35] Because docking calculations for several inhibitors and noninhibitors using the software FlexX-Pharm,[36] using the above-mentioned model, did not result in sufficient selectivity between binding and nonbinding compounds, a new model was built. For this purpose a multiple sequence alignment was performed and the human CYP2C9 was identified as the best template structure. Since the X-ray structure of CYP2C9 was not available at the time the previous model was built, it was not included there. Because it was shown in a recent study that the use of mammalian cytochrome P450 structures as templates improved the quality of the resulting 3D structural models of CYP2D6 considerably,[37] we decided to use CYP2C9 as template.[38] For the new model the same docking calculations were performed as for the original one. The new model showed a considerably increased selectivity and was therefore chosen for further studies. During the docking process the model was refined through energy minimizations and simulated annealing simulations. The position of the Phe106 phenyl ring changed considerably during these refinements and was found to be crucial for proper inhibitor binding. In Figure 1 the docking solution with the lowest energy score for the CYP11B2 complex with the inhibitor **8b** is shown. Analysis of the protein−ligand interactions revealed that the inhibitor is predominantly bound through hydrophobic interactions except for the nitrogen−metal coordination with the heme iron. The strongest interactions are formed with residues of the α-helix I (Ala290, Gly291, and Thr295) and Phe106.

Discussion and Conclusion

Until recently,[14] inhibition of aldosterone formation was monitored to assess side effects of drugs aiming at the selective inhibition of steroidogenic CYP enzymes. In the case of CYP19, the potent inhibitor fadrozole, which is in clinical use for breast cancer, was extensively evaluated. In typical in vitro assays the formation of aldosterone and corticosterone was determined using rat adrenal slices.[39,40] In vivo the plasma concentration of aldosterone and cortisol in breast cancer patients was monitored.[13,41,42] In the present paper, we discovered that the reduction of aldosterone formation caused by fadrozole is definitely due to inhibition of CYP11B2.

The idea that CYP11B2 can be used as a novel target in the field of cardiovascular research is new and has been suggested by us in a recent paper.[14,18] Potent and selective inhibitors are likely to show benefit in such severe diseases as congestive heart failure, myocardial fibrosis, and hyperaldosteronism.

In this study, we describe the optimization of the imidazolylmethylenetetrahydronaphthalenes and imidazolylmethyleneindanes **1a**,**b** and **2a**,**b**, which we formerly had discovered by compound library screening.

The structure−activity relationships obtained demonstrate that it is possible to inhibit CYP11B2 and CYP11B1 strongly. More important is the finding that a rather selective inhibition of CYP11B2 versus CYP11B1 and vice versa is possible, although the homology between these two enzymes is very high.[1,12] Thus, we discovered rather selective inhibitors of CYP11B2, dual inhibitors of both enzymes, and rather selective inhibitors of CYP11B1. They might be pharmacological tools for the elucidation of so far unknown physiological effects of gluco- and/or mineralocorticoids. It is noteworthy to state that only part of the corticoid effects can be studied using the corresponding receptor antagonists. As already mentioned, selective inhibitors of CYP11B2 could also be used as drugs for the treatment of certain cardiovascular diseases. A promising candidate as a lead for further optimization might be compound **8b**, which exhibited a 5-fold selectivity toward the aldosterone synthase and a very low IC_{50} value (IC_{50} CYP11B2, 4 nM; IC_{50} CYP11B1, 20 nM).

The data presented in Table 1 showed that the *Z*-isomers as a rule were more active CYP11B2 inhibitors than the corresponding *E*-isomers. Obviously the *Z*-isomers fit better into the hydrophobic pocket next to the α-helix I at the active site of CYP11B2 than the *E*-isomers (Figure 1). This hypothesis is confirmed by our protein model. The docked *Z*-isomers are energetically more favorable (about 2−3 kcal/mol). The reason for this is the limited height of the binding pocket above

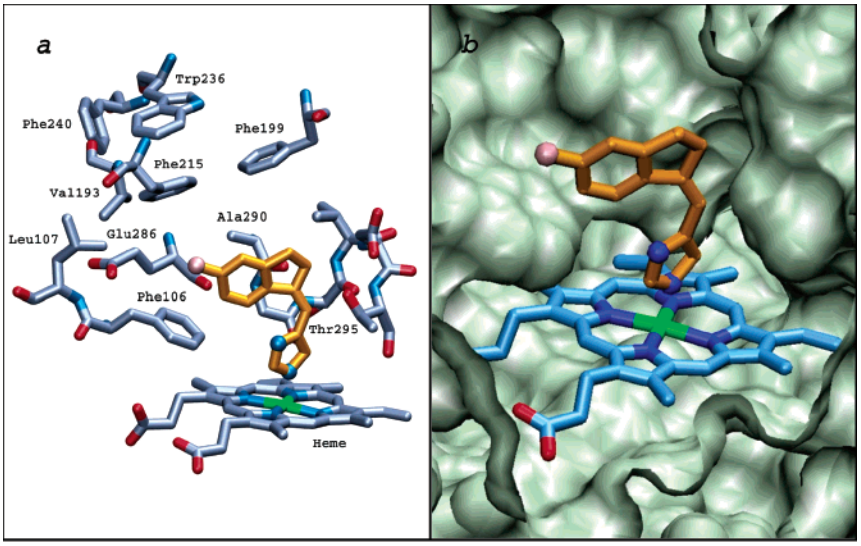


Figure 1. Structure of the CYP11B2-**8b** complex: (a) details of the active site, showing inhibitor, heme cofactor, interacting residues, and residues of the hydrophobic region between Ala290 and Phe106; (b) surface of the binding pocket surrounding the inhibitor and the heme cofactor (docking result with the lowest energy score).

the heme moiety. Therefore, the more compact *Z*-isomers show a better fitting compared to the long stretched *E*-isomers.

The important role of the α-helix I for substrate and/or inhibitor binding, discovered for CYP11B2 in this study, is in agreement with results of phenylpropanoid CYP−substrate interactions performed by Rupasinghe et al.[43] In these studies the α-helix I was identified as a major region for CYP−substrate binding.

In a comparison of the results of the first screening step using bovine mitochondrial enzyme on one hand and the whole cell assay with recombinantly expressed CYP11B2 in *S. pombe* on the other hand, there was no or only poor correlation. From this, it can be concluded that the bovine enzyme assay is not suitable for preselection in general. This is not really a surprise, since the homology between bovine and human enzyme is not high (75%).[44] On the other hand, a good correlation was obtained for the inhibitory activities of the compounds in the yeast and the V79 CYP11B2 assays. The few outliers might be explained by different transport phenomena.

In summary, it can be concluded from our studies that, in principle, it is possible to inhibit rather selectively CYP11B2 and CYP11B1. However, it is a moot question whether the extent of selectivity obtained with the title compounds is pronounced enough to reduce aldosterone levels without affecting other steroids, especially glucocorticoids, in vivo. Fadrozole, for example, is known to reduce only the estrogen levels at therapeutic doses. Administered in 10-fold higher concentrations, aldosterone and cortisol production is also affected.[13,42] Presently in vivo studies are being performed with some of the title compounds to elucidate this issue. Nevertheless, further structural optimizations are required to increase the selectivity of lead compound **8b**.

Experimental Section

Chemical Methods. Melting points were measured on a Mettler FP1 melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 398 infrared spectrometer as KBr or NaCl disks or on a Bruker Vector 33FT infrared spectrometer as a powder. ^{1}H NMR spectra were recorded on a Bruker AW-80 (80 MHz), on an AM-400 (400 MHz), or on a DRX-500 (500 MHz) instrument. Chemical shifts are given in parts per million, and TMS was used as the internal standard for spectra obtained in DMSO-d_6 and $CDCl_3$. All coupling constants (J) are given in Hz. Elemental analyses were performed at the Inorganic Chemistry Department, Saarland University. Reagents and solvents were used as obtained from commercial suppliers without further purification. Flash column chromatography (FCC) was performed using silica gel 60 (40−63 μm), and reaction progress was determined by TLC analyses on ALUGRAM SIL G/UV$_{254}$ (Macherey-Nagel).

The following compounds were prepared according to previously described procedures: 7-hydroxy-1-tetralone (**3iii**),[21] 6-hydroxytetralone (**4iii**),[21] 5-hydroxyindan-1-one (**5iii**),[21] 8-oxo-5,6,7,8-tetrahydronaphthalen-2-yl trifluoromethanesulfonate (**3ii**),[22,45] 5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl trifluoromethanesulfonate (**4ii**),[22] 5-oxo-5,6,7,8-tetrahydronaphthalene-2-carbonitrile (**4i**),[22] 1-oxoindane-5-carbonitrile (**5i**),[22,46] 7-chloro-3,4-dihydro-2*H*-naphthalen-1-one (**6i**),[23−26] and 4-formyl-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfonamide (**18**).[29,30]

1-Oxo-2,3-dihydro-1*H*-inden-5-yl Trifluoromethanesulfonate (5ii). Purification: Kugelrohr distillation. Yield 82%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50−2.81 (m, 2H, H-*2*), 3.00−3.40 (m, 2H, H-*3*), 7.20−7.50 (m, 2H, H-*4*, H-*6*), 7.95 (d, ^3J = 8.5 Hz, 1H, H-*7*). IR (NaCl) cm^{-1}: ν_{max} 3060, 2920, 1720, 1610, 1590, 1210, 1140, 1090, 930.

8-Oxo-5,6,7,8-tetrahydronaphthalene-2-carbonitrile (3i). Purification: crystallization from ligroine. Yield 67%, yellow crystals, mp 154 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.16−2.22 (m, 2H, H-*3*), 2.71 (t, ^3J = 6.2 Hz, 2H, H-*4*), 3.05 (t, ^3J = 6.2 Hz, 2H, H-*2*), 7.42 (d, ^3J = 8.0 Hz, 1H, H-*5*), 7.71 (dd, ^3J = 8.0 Hz, ^4J = 1.8 Hz, 1H, H-*6*), 8.29 (d, ^4J = 1.8 Hz, 1H, H-*8*). IR (KBr) cm^{-1}: ν_{max} 3060, 3020, 2220, 1680, 1600, 1490, 1430, 1420, 1320, 1280, 1170, 1140, 1030, 920, 830.

General Procedure for the Synthesis of Compounds 1−9. In a mixture of methanol (110 mL) and dichloromethane (55 mL) 50 mmol of ketone were dissolved and 1.89 g of $NaBH_4$ (50 mmol) was added in portions while cooling the reaction mixture to 0 °C. After 15 min at 0 °C, the solution was stirred for 1 h at room temperature. The reaction mixture was diluted with water, and the product was extracted with diethyl ether. The organic phase was first washed with 1 N HCl, then with a saturated solution of $NaHCO_3$, and last with water. It was dried over $MgSO_4$, and the solvent was evaporated.

The resulting alcohol was transformed into the phosphonium salt. Amounts of 40 mmol of the alcohol and 13.7 g of triphenylphosphonium bromide (40 mmol) were suspended in 25 mL of benzene and refluxed for 12 h under nitrogen. The precipitate was filtered off and dried. The solid was suspended in dry diethyl ether and stirred for 10 min. The phosphonium salt was filtered off and washed with acetone.

A sodium ethanolate solution was prepared by dissolving 0.5 g of sodium (22 mmol) in 20 mL of ethanol. An amount of 2.1 g of imidazole-4(5)-carbaldehyde (22 mmol) was added to this solution. The solution was warmed for some minutes until it became clear. In a second flask, 20 mmol of the phosphonium salt were suspended in 14 mL of ethanol and refluxed under nitrogen atmosphere. The imidazolide solution was added in portions through a septum within 2 h, and the reaction mixture was refluxed for another 12 h. The mixture was cooled to room temperature, and the solid was filtered off and discarded. The solution was concentrated, and the residue was diluted with 100 mL of water and was extracted several times by diethyl ether. The combined organic phases were filtered through Celite, dried over $MgSO_4$, and evaporated. After purification, the free base was dissolved in acetone and an excess of oxalic acid in acetone was added to get the oxalate salt, or it was dissolved in dry diethyl ether and an excess of HCl in diethyl ether was added to get the hydrochloride.

5-[(*E*)-3,4-Dihydronaphthalen-1(2*H*)-ylidenemethyl]-1*H*-imidazole (1a). Purification: FCC (acetone) and recrystallization (acetone). Yield 14%, colorless crystals, mp 136−138 °C. ^{1}H NMR (400 MHz, DMSO-d_6, free base) δ 1.72−1.82 (m, 2H, H-*3*), 2.68−2.73 (m, 2H, H-*2*), 2.77−2.82 (m, 2H, H-*4*), 6.94 (s, 1H, H-*9*), 7.10−7.22 (m, 4H, H-*5*, H-*7*, H-*14*), 7.66 (d, ^3J = 7.8 Hz, 1H, H-*8*), 7.69 (s, 1H, H-*12*). IR (KBr, free base) cm^{-1}: ν_{max} 3110, 3055, 3020, 2935, 2865, 2830, 1666, 1621, 1597, 1481, 1453, 1441, 1430, 951, 943, 765. Anal. ($C_{14}H_{14}N_2$, free base) C, H, N.

5-[(*Z*)-3,4-Dihydronaphthalen-1(2*H*)-ylidenemethyl]-1*H*-imidazole Oxalate (1b). Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 5%, white solid, mp 187 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.87−1.94 (m, 2H, H-*3*), 2.46−2.50 (m, 2H, H-*2*), 2.81−2.84 (m, 2H, H-*4*), 6.24 (s, 1H, H-*9*), 7.00−7.20 (m, 4H, H-*5*, H-*6*, H-*7*, H-*14*), 7.37 (d, ^3J = 7.8 Hz, 1H, H-*8*), 8.31 (s, 1H, H-*12*). IR (KBr) cm^{-1}: ν_{max} 1610, 1480, 1450, 920, 850, 760. Anal. ($C_{14}H_{14}N_2 \cdot C_2H_2O_4$) C, H, N.

5-[(*E*)-2,3-Dihydro-1*H*-inden-1-ylidenemethyl]-1*H*-imidazole (2a). Purification: FCC (acetone). Yield 28%, white solid, mp 148−151 °C. ^{1}H NMR (400 MHz, DMSO-d_6, free base) δ 2.89−2.96 (m, 2H, H-*2*), 3.00−3.08 (m, 2H, H-*3*), 6.92 (t, ^4J = 2.5 Hz, 1H, H-*8*), 7.13−7.25 (m, 3H, H-*5*, H-*6*, H-*13*), 7.30 (d, ^3J = 6.5 Hz, 1H, H-*4*), 7.57 (d, ^3J = 6.8 Hz, 1H, H-*7*), 7.69 (s, 1H, H-*11*). IR (KBr, free base) cm^{-1}: ν_{max} 3055, 3020, 2960, 2920, 2840, 1643, 1601, 1460, 985, 757. Anal. ($C_{13}H_{12}N_2 \cdot C_2H_2O_4$) C, H, N.

5-[(*Z*)-2,3-Dihydro-1*H*-inden-1-ylidenemethyl]-1*H*-imidazole Oxalate (2b). Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 10%, white solid, mp 196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.85−2.94 (m, 4H, H-*2*, H-*3*), 6.36 (s, 1H, H-*8*), 7.14 (t, ^3J = 7.4 Hz, 1H, H-*5*), 7.24 (t, ^3J = 7.4 Hz, 1H, H-*6*), 7.31 (d, ^3J = 7.4 Hz, 1H, H-*4*), 7.37 (s, 1H, H-*13*), 8.13 (d, ^3J = 7.4 Hz, 1H, H-*7*), 8.30 (s, 1H, imidazole-H-*11*). IR (KBr) cm^{-1}: ν_{max} 1610, 1450, 750, 720. Anal. ($C_{13}H_{12}N_2 \cdot 0.75C_2H_2O_4$) C, H, N.

(8*E*)-8-(1*H*-Imidazol-5-ylmethylene)-5,6,7,8-tetrahydronaphthalene-2-carbonitrile Hydrochloride (3a). From compound **3i**. Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 11%, green crystals, mp 217 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.80−1.84 (m, 2H, H-*2*), 2.71−2.74 (m, 2H, H-*2*), 2.81−2.84 (m, 2H, H-*4*), 7.10 (s, 1H, H-*9*), 7.42 (d, ^3J = 7.9 Hz, 1H, H-*5*), 7.68 (d, ^3J = 7.9 Hz, 1H, H-*6*), 7.82 (s, 1H, H-*14*), 8.13 (s, 1H, H-*8*), 9.11 (s, 1H, H-*12*). IR (KBr, free base) cm^{-1}: ν_{max} 2940, 2220, 1620, 1600,1490, 1000, 900, 880, 840, 820. Anal. ($C_{15}H_{13}N_3$, free base) C, H, N.

(8*Z*)-8-(1*H*-Imidazol-5-ylmethylene)-5,6,7,8-tetrahydronaphthalene-2-carbonitrile Oxalate (3b). From compound **3i**. Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 3%, white solid, mp 197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.90−1.92 (m, 2H, H-*3*), 2.47−2.51 (m, 2H, H-*2*), 2.88−2.90 (m, 2H, H-*4*), 6.37 (s, 1H, H-*9*), 7.19 (s, 1H, H-*14*), 7.36 (d, ^3J = 8.0 Hz, 1H, H-*5*), 7.58 (dd, ^3J = 8.0 Hz, ^4J = 1.6 Hz, 1H, H-*6*), 7.95 (s, 1H, H-*8*), 8.13 (s, 1H, H-*12*). IR (KBr) cm^{-1}: ν_{max} 2840, 2240, 1600, 1600, 1000, 940, 930, 850, 820, 710. Anal. ($C_{15}H_{13}N_3 \cdot C_2H_2O_4$) C, H, N.

(5*Z*)-5-(1*H*-Imidazol-5-ylmethylene)-5,6,7,8-tetrahydronaphthalene-2-carbonitrile Oxalate (4b). From compound **4i**. Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 3%, white solid, mp 206 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.86−1.93 (m, 2H, H-*3*), 2.47−2.50 (m, 2H, H-*2*), 2.83−2.86 (m, 2H, H-*3*), 6.41 (s, 1H, H-*9*), 7.18 (s, 1H, H-*14*), 7.45 (dd, ^3J = 8.1 Hz, ^4J = 1.6 Hz, 1H, H-*7*), 7.66−7.68 (m, 2H, arom-H5, H-*8*), 8.12 (s, 1H, H-*12*). IR (KBr) cm^{-1}: ν_{max} 2220, 1610, 850, 710. Anal. ($C_{15}H_{13}N_3 \cdot C_2H_2O_4$) C, H, N.

(1*E*)-1-(1*H*-Imidazol-5-ylmethylene)indane-5-carbonitrile Oxalate (5a). From compound **5i**. Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 39%, white solid, mp 217 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.96−2.99 (m, 2H, H-*2*), 3.14−3.17 (m, 2H, H-*3*), 7.16 (s, 1H, H-*8*), 7.72−7.79 (m, 3H, H-*4,* H-*6*, H-*7*), 7.83 (s, 1H, H-*13*), 9.17 (s, 1H, H-*11*). IR (KBr) cm^{-1}: ν_{max} 3080, 2220, 1600, 830. Anal. ($C_{14}H_{12}N_3 \cdot$HCl) C, H. N: calcd, 16.31; found, 15.71.

(1*Z*)-1-(1*H*-Imidazol-5-ylmethylene)indane-5-carbonitrile Oxalate (5b). From compound **5i**. Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 10%, white solid, mp 207 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.89−2.99 (m, 4H, H-*2*, H-*3*), 6.57 (s, 1H, H-*8*), 7.36 (s, 1H, H-*4*), 7.60 (d, ^3J = 8.2 Hz, 1H, H-*6*), 7.72 (s, 1H, H-*13*), 8.02 (s, 1H, H-*11*), 9.13 (d, ^3J = 8.2 Hz, 1H, H-*7*). IR (KBr) cm^{-1}: ν_{max} 2220, 1620, 890, 830, 780, 720. Anal. ($C_{14}H_{12}N_3 \cdot 0.8C_2H_2O_4$) C, H, N.

5-[(*E*)-(6-Chloro-3,4-dihydronaphthalen-1(2*H*)-ylidene)-methyl]-1*H*-imidazole Hydrochloride (6a). Prepared via compound **6i**. Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 35%, pearly crystals, mp 203 °C. ^{1}H NMR (400 MHz, DMSO-d_6, free base) δ 1.73−1.80 (m, 2H, H-*3*), 2.67−2.70 (m, 2H, H-*2*), 2.80−2.84 (m, 2H, H-*4*), 6.99 (s, 1H, H-*9*), 7.16−7.25 (m, 3H, H-*5*, H-*6*, H-*14*), 7.67 (s, 1H, H-*8*), 7.76 (s, 1H, H-*12*). IR (KBr, free base) cm^{-1}: ν_{max} 3060, 2940, 2840, 1590, 1120, 950, 870, 850, 840, 800. Anal. ($C_{14}H_{13}ClN_2 \cdot 0.2H_2O$) C, H, N.

5-[(*E*)-(5-Fluoro-2,3-dihydro-1*H*-inden-1-ylidene)methyl]-1*H*-imidazole Hydrochloride (7a). Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 49%, beige crystals, mp 245 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.96−3.01 (m, 2H, H-*2*), 3.10−3.13 (m, 2H, H-*3*), 6.91 (s, 1H, H-*8*), 7.11−7.16 (m, 1H, H-*6*), 7.21 (d, ^3J(H,F) = 9.0 Hz, 1H, H-*4*), 7.64 (dd, ^3J = 8.5 Hz, ^4J(H,F) = 5.3 Hz, 1H, H-*7*), 7.69 (s, 1H, H-*13*), 9.14 (s, 1H, H-*11*). IR (KBr) cm^{-1}: ν_{max} 3080, 1600, 1590, 1090, 940, 870. Anal. ($C_{13}H_{11}FN_2 \cdot$HCl) C, H, N.

5-[(*Z*)-(5-Fluoro-2,3-dihydro-1*H*-inden-1-ylidene)methyl]-1*H*-imidazole Oxalate (7b). Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 15%, white solid, mp 205 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.88−2.93 (m, 4H, H-*2*, H-*3*), 6.33 (s, 1H, H-*8*), 6.98 (m, 1H, H-*6*), 7.14 (d, ^3J(H,F) = 9.1 Hz, 1H, H-*4*), 7.39 (s, 1H, H-*13*), 8.27−8.31 (m, 2H, H-*7*, H-*11*). IR (KBr) cm^{-1}: ν_{max} 1600, 1480, 1220, 930, 860, 830, 720. Anal. ($C_{13}H_{11}FN_2 \cdot C_2H_2O_4$) H, N. C: calcd, 59.21; found, 59.70.

5-[(*E*)-(5-Chloro-2,3-dihydro-1*H*-inden-1-ylidene)methyl]-1*H*-imidazole Hydrochloride (8a). Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 37%, white solid, mp 238 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.95−2.97 (m, 2H, H-*2*), 3.10−3.13 (m, 2H, H-*3*), 6.94 (s, 1H, H-*8*), 7.34 (d, ^3J = 8.3 Hz, 1H, H-*6*), 7.46 (s, 1H, H-*4*), 7.64 (d, ^3J = 8.3 Hz, 1H, H-*7*), 7.72 (s, 1H, H-*13*), 9.11 (s, 1H, H-*11*). IR (KBr) cm^{-1}: ν_{max} 3080, 1600, 1470, 1290, 1200, 1110, 1070, 830, 610. Anal. ($C_{13}H_{11}ClN_2 \cdot$HCl) C, H, N.

5-[(*Z*)-(5-Chloro-2,3-dihydro-1*H*-inden-1-ylidene)methyl]-1*H*-imidazole Oxalate (8b). Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 17%, white solid, mp 218 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.87−2.96 (m, 4H, H-*2*, H-*3*), 6.39 (s, 1H, H-*8*), 7.20 (dd, ^3J = 8.4 Hz, ^4J = 2.0 Hz, 1H, H-*6*), 7.37−7.38 (m, 2H, H-*4*, H-*13*), 8.24 (s, 1H, H-*11*), 8.50 (d, ^3J = 8.4 Hz, 1H, H-*7*). IR (KBr) cm^{-1}: ν_{max} 1600, 1470, 1210, 880, 860, 830, 710. Anal. ($C_{13}H_{11}ClN_2 \cdot C_2H_2O_4$) C, H, N.

5-[(*E*)-(5-Bromo-2,3-dihydro-1*H*-inden-1-ylidene)methyl]-1*H*-imidazole Hydrochloride (9a). Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 47%, white solid, mp 228 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.42−2.61 (m, 2H, H-*2*), 3.10−3.12 (m, 2H, H-*3*), 7.00 (s, 1H, H-*8*), 7.47 (d, ^3J = 8.3 Hz, 1H, H-*6*), 7.54−7.59 (m, 2H, H-*4*, H-*7*), 7.71 (s, 1H, H-*13*), 9.15 (s, 1H, H-*11*). IR (KBr) cm^{-1}: ν_{max} 3080, 1600, 1590, 1470, 830. Anal. ($C_{13}H_{11}BrN_2$·HCl) H, N. C: calcd, 50.11; found, 49.69.

5-[(*Z*)-(5-Bromo-2,3-dihydro-1*H*-inden-1-ylidene)methyl]-1*H*-imidazole Oxalate (9b). Purification: FCC ($CHCl_3$/DMF, 9:2). Yield 11%, white solid, mp 218 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.87−2.95 (m, 4H, H-*2*, H-*3*), 6.40 (s, 1H, H-*8*), 7.32−7.35 (m, 2H, H-*6*, H-*13*), 7.50 (s, 1H, H-*4*), 8.12 (s, 1H, H-*11*), 8.53 (d, ^3J = 8.4 Hz, 1H, H-*7*). IR (KBr) cm^{-1}: ν_{max} 1620, 1460, 1400, 1100, 1070, 820, 780, 720. Anal. ($C_{13}H_{11}BrN_2$·0.8$C_2H_2O_4$) C, H, N.

Alternative Procedure for the Synthesis of Compounds 2a, 2b, 8a, 8b. A suspension of 5 mmol of phosphonium salt (see general procedure section), 5 mmol of **19**, 50 mmol of K_2CO_3, and a few milligrams of 18-crown-6 in 25 mL of dry dichloromethane was refluxed for 12 h under nitrogen. The reaction mixture was poured into water and extracted several times with dichloromethane. The combined organic phases were dried over $MgSO_4$, and the solvent was evaporated. After purification, 2.5 mmol of the sulfonamide (**2ia**,**2ib** and **8ia**,**8ib**) were dissolved in a few milliliters of dioxane and 75 mL of 4 N HCl was added. The mixture was stirred under reflux overnight. When the mixture was cooled to room temperature, the hydrochloride precipitated and could be filtered off and washed with dry diethyl ether (yield almost quantitative).

5-[(*E*)-2,3-Dihydro-1*H*-inden-1-ylidenemethyl]-1*H*-imidazole-1-sulfonic Acid Dimethylamide (2ia). Purification: FCC (EtOAc/hexane, 3:2). Yield 43%, yellow solid, mp 122−123 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.92 (s, 6H, H-*methyl*), 3.12 (m, 4H, H-*2*, H-*3*), 6.97 (s, 1H, H-*8*), 7.28−7.32 (m, 2H, H-*4*, H-*6*), 7.38−7.40 (m, 1H, H-*5*), 7.61 (s, 1H, H-*13*), 7.70−7.73 (m, 1H, H-*7*), 8.28 (d, ^4J = 1.3 Hz, 1H, H-*11*). IR (powder) cm^{-1}: ν_{max} 3122, 2929, 2360, 1684, 1469, 1384, 1169, 1082, 724. Anal. ($C_{15}H_{17}N_3SO_2$) C, H, N.

5-[(*Z*)-2,3-Dihydro-1*H*-inden-1-ylidenemethyl]-1*H*-imidazole-1-sulfonic Acid Dimethylamide (2ib). Purification: FCC (EtOAc/hexane, 3:2). Yield 16%, yellow solid, mp 81 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.82 (s, 6H, H-*methyl*), 2.83−2.93 (m, 4H, H-*2*, H-*3*), 6.37 (s, 1H, H-*8*), 7.14−7.22 (m, 2H, H-*4*, H-*6*), 7.26 (m, 1H, H-*5*), 7.61 (s, 1H, H-*13*), 8.25 (s, 1H, H-*11*), 8.82 (d, ^3J = 7.6 Hz, 1H, H-*7*). IR (powder) cm^{-1}: ν_{max} 3122, 2929, 2361, 1461, 1388, 1176, 1081, 962, 725. Anal. ($C_{15}H_{17}N_3SO_2$) H, N. C: calcd, 59.38; found, 60.06.

5-[(*E*)-(5-Chloro-2,3-dihydro-1*H*-inden-1-ylidene)methyl]-1*H*-imidazole-1-sulfonic Acid Dimethylamide (8ia). Purification: FCC (EtOAc/hexane, 3:2). Yield 47%, yellow solid, mp 159 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.84 (s, 6H, H-*methyl*), 3.06 (m, 4H, H-*2*, H-*3*), 6.92 (s, 1H, H-*8*), 7.27 (dd, ^3J = 8.2 Hz, ^4J = 1.9 Hz, 1H, H-*6*), 7.39 (d, ^4J = 1.9 Hz, 1H, H-*4*), 7.56 (s, 1H, H-*13*), 7.66 (d, ^3J = 8.5 Hz, 1H, H-*7*), 8.22 (s, 1H, H-*11*). IR (powder) cm^{-1}: ν_{max} 3133, 2939, 2360, 1467, 1379, 1165, 1088, 726. Anal. ($C_{15}H_{16}N_3SO_2Cl$) C, H, N.

5-[(*Z*)-(5-Chloro-2,3-dihydro-1*H*-inden-1-ylidene)methyl]-1*H*-imidazole-1-sulfonic Acid Dimethylamide (8ib). Purification: FCC (EtOAc/hexane, 3:2). Yield 20%, yellow solid, mp 135 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.83 (s, 6H, H-*methyl*), 2.88−2.97 (m, 4H, H-*2*, H-*3*), 6.42 (s, 1H, H-*8*), 7.24 (dd, ^3J = 8.5 Hz, ^4J = 1.9 Hz, 1H, H-*6*), 7.36 (d, ^4J = 1.9 Hz, 1H, H-*4*), 7.66 (s, 1H, H-*13*), 8.28 (d, ^4J = 1.3 Hz, 1H, H-*11*), 9.00 (d, ^3J = 8.5 Hz, 1H, H-*7*). IR (powder) cm^{-1}: ν_{max} 3124, 2923, 2361, 1465, 1386, 1174, 1080, 962, 724. Anal. ($C_{15}H_{16}N_3SO_2Cl$) C, H, N.

Biological Methods. 1. Enzyme Preparations. The enzymes were prepared according to described methods: human CYP17 (expressed in *E. coli*),[34] human placental CYP19,[32] and bovine adrenal CYP18.[15]

2. Enzyme Assays. The following enzyme assays were performed as described: CYP17 assay,[34] CYP19 assay,[32] and CYP11B assay.[15]

3. Screening Assay in Fission Yeast. A fission yeast suspension (*S. pombe* PE1) with a cellular density of 3×10^7 cells/mL was prepared from a freshly grown culture using fresh EMMG (pH 7.4) as described by Ehmer et al.[18] with modifications. An amount of 492.5 μL of this cellular suspension was mixed with 5 μL of inhibitor solution (50 μM, dissolved in ethanol) and incubated for 15 min at 32 °C. Controls were mixed with 5 μL of ethanol. The enzyme reaction was started by addition of 2.5 μL of 11-deoxycorticosterone (20 μM, containing 6 nCi of [4-^{14}C]11-deoxycorticosterone, dissolved in ethanol) and then shaken horizontally at 32 °C for 6 h. The test was stopped by extracting the sample using ethyl acetate. After a centrifugation step (1000*g*, 5 min), the organic layer was pipetted into a fresh cup and evaporated to dryness. The residue was redissolved in 10 μL of chloroform, and the conversion of the substrate into corticosterone was analyzed by HPTLC as described in section 5.

4. Assay for Selective CYP11B2 Inhibitors. V79 MZh 11B1 and V79 MZh 11B2 cells (8×10^5 cells per well) were grown on 24-well cell culture plates with 1.9 cm^2 culture area per well (Nunc, Roskilde, Denmark) until confluence. Before testing, the DMEM culture medium was removed and 400 μL of fresh DMEM, containing the inhibitor in at least three different concentrations for determining the IC_{50} value, was added to each well. After a preincubation step of 60 min at 37 °C, the reaction was started by the addition of 100 μL of DMEM containing the substrate 11-deoxycorticosterone (20 μM, containing 6 nCi of [4-^{14}C]11-deoxycorticosterone, dissolved in ethanol). The V79 MZh 11B1 cells were incubated for 120 min, whereas the V79 MZh 11B2 cells were incubated for 40 min. Controls were treated in the same way without inhibitors. Enzyme reactions were stopped by extracting the supernatant with ethyl acetate. Samples were centrifuged (10000*g*, 10 min), and the solvent was pipetted into fresh cups. The solvent was evaporated, and the steroids were redissolved in 10 μL of chloroform and analyzed by HPTLC.

5. HPTLC Analysis and Phosphoimaging of Radiolabeled Steroids. The redissolved steroids were transferred onto an HPTLC plate (20 cm × 10 cm, silica gel 60F_{254}) with concentrating zone (Merck, Darmstadt, Germany) and developed two times using the solvent chloroform/methanol/water (300:20:1). Unlabeled 11-deoxycorticosterone and corticosterone were spotted on the HPTLC plate as reference compounds for the CYP11B1 reaction. For the CYP11B2 reaction 11-deoxycorticosterone, corticosterone, 18-hydroxycorticosterone, and aldosterone were used as reference. Subsequently imaging plates (BAS MS2340, for ^{14}C samples, Raytest, Straubenhardt, Germany) were exposed to the HPTLC plates for 48 h. The imaging plates were scanned using the phosphoimager system Fuji FLA 3000 (Raytest, Straubenhardt, Germany), and the steroids were quantified.

Computational Methods. By use of the recently resolved human cytochrome CYP2C9 structure (PDB code: 1OG5) as template,[38] a homology model was built for CYP11B2. For this purpose a multiple sequence alignment was performed using a variety of methods through the structure prediction meta-server 3D-Jury.[47] The results were critically compared and CYP2C9 was identified as the best template. On the basis of the above alignment, 3D models were created with Swiss-Model[48] and through the WHATIF server,[49] using CYP2C9 as our template structure. Both models were again critically compared. No significant differences were observed in proximity to the binding pocket. The final model was energy-minimized. Afterward several known inhibitors were docked and the binding pocket geometry was further refined through energy minimization and simulated annealing procedures using the GROMACS united atom force field and molecular dynamics program.[50] For our docking studies the docking program FlexX-Pharm was used.[36] Pharmacophore constraints were applied to ensure the right binding mode of the inhibitors. During the simulated annealing procedure the protein was

cooled from 400 to 0 K over 20 ps. A cutoff of 14 Å was used for the nonbonded interactions. The solvent was approximated through a dielectric constant of 4.0. This value was chosen because we are focusing on the conformational changes inside the binding pocket. Harmonic constraints were applied to all backbone atoms and the heme cofactor.

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Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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