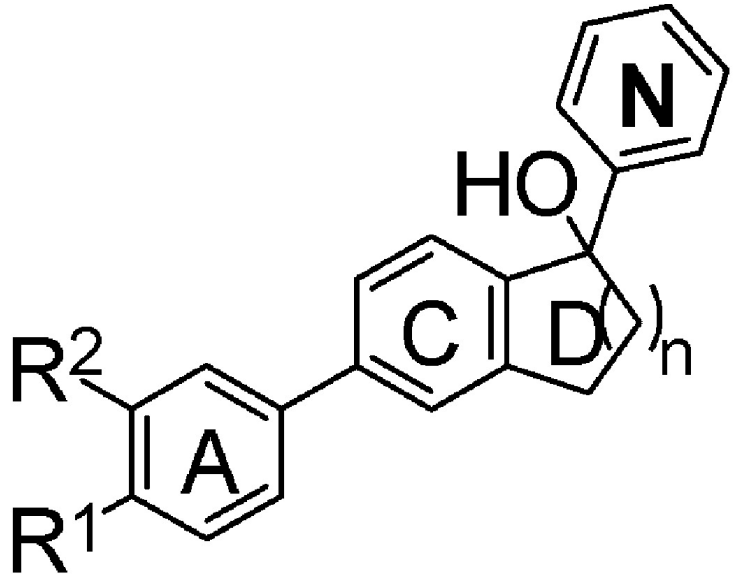
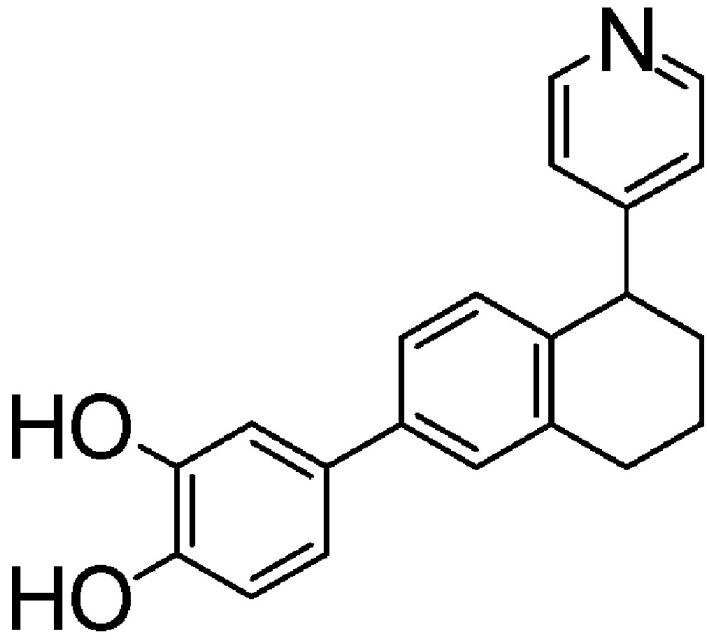
Journal of Medicinal Chemistry



abiraterone analogs



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Synthesis, Biological Evaluation, and Molecular Modeling of Abiraterone Analogues: Novel CYP17 Inhibitors for the Treatment of Prostate Cancer

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Abiraterone, a steroidal cytochrome P450 17α-hydroxylase-17,20-lyase inhibitor (CYP17), is currently undergoing phase II clinical trials as a potential drug for the treatment of androgen-dependent prostate cancer. Since steroidal compounds often show side effects attributable to their structure, we have tried to replace the sterane scaffold by nonsteroidal core structures. The design and synthesis of 20 new abiraterone mimetics are described. Their activities have been tested with recombinant human CYP17 expressed in *E. coli*. Promising compounds were further evaluated for selectivity against CYP11B1, CYP11B2, and the hepatic CYP3A4. Compounds **19** and **20** showed comparable activity to abiraterone (IC_{50} values of 144 and 64 nM vs 72 nM) and similar or even better selectivity against the other CYP enzymes. Selected compounds were also docked into our homology model, and the same binding modes as for abiraterone were found.

Introduction

Prostate cancer (PC[a]) is the most common tumor and age-related cause of death in elder men worldwide.[1] Because of the advanced age of the patients, less invasive approaches are needed. Accordingly, the treatment of choice is "watchful waiting",[2] followed by radiation therapy only when it is necessary. Since PC is androgen dependent in over 80% of the cases, another current standard treatment is orchiectomy, the surgical removal of the testes, usually applied to patients under 70 years old. The reduction of testicular androgen production by gonadotropin-releasing hormone (GnRH) analogues,[3] a "medical castration", is often preferred over the surgical one and can also be used for treating older patients. Nevertheless, castration whether surgical or medical reduces maximally 90−95% of the daily testosterone production, which is often not enough to stop the tumor from growing, since prostate levels of testosterone and dihydrotestosterone are still about 25% and 10%, respectively, even after three months of treatment with a GnRH agonist.[4]

The remaining 5−10% of the androgens are produced in the adrenals. In the 1980s, Labrie[5] hypothesized that additionally counteracting adrenal androgens by application of antiandrogens would further inhibit tumor growth. This approach, known as "combined androgen blockade" (CAB), has been widely used in the past. The results have been partially positive, especially in patients with minimal disease and good performance status. However, it must be mentioned that antiandrogen therapy is associated with notable side effects.[6] Another drawback of CAB is that in refractory PC regression is observed after discontinuation of antiandrogen administration. This led to the hypothesis that under antiandrogen treatment androgen receptor mutations occur causing PC cells to recognize antagonists as agonists.[7]

An alternative target proposed in the recent past is the cytochrome P450 enzyme 17α-hydroxylase-17,20-lyase (CYP17). This enzyme is localized in the endoplasmic reticulum in the testes as well as in the adrenals and is the key enzyme for androgen biosynthesis. Its inhibition should stop the production of androgens both in the testes and in the adrenals, and therefore, inhibitors of CYP17 should be more effective for treating androgen-dependent PC than GnRH analogues. Proof of principle was achieved by the unspecific CYP inhibitor ketoconazole, which clinically turned out to be a good adjuvant therapeutic capable of reducing testosterone levels.[8] Nevertheless, the side effects shown by this antimycotic compound are the reason that it is not used anymore.[9]

CYP17 catalyzes two reactions, the 17α-hydroxylation of pregnenolone and progesterone to the corresponding 17α-alcohols and the subsequent 17,20-lyase reaction cleaving the C17−C20 bond. This yields the 17-keto androgens androstendione and dehydroepiandrosterone, precursors of all other androgens, including testosterone (Chart 1).

The side effects of ketoconazole caused others[10] and our group to develop steroidal[11] and nonsteroidal[12,13] inhibitors. One compound, the steroidal CYP17 inhibitor abiraterone (Chart 2), is currently undergoing phase II clinical trials showing high activity in postdocetaxel castration refractory PC patients. In contrast to ketoconazole, it seems to have no dose-limiting toxicity.[14]

Because steroidal compounds often show side effects due to interactions with steroid receptors,[15] it is our aim to develop nonsteroidal compounds. In this work, we present the design, synthesis, in vitro evaluation regarding activity and selectivity and modeling studies of nonsteroidal analogues of abiraterone (Chart 3).

Design

The three most important structural features of abiraterone are the aromatic nitrogen-containing heterocycle, the hydro-

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[a] Abbreviations: CYP, cytochrome P450; CYP17, cytochrome P450 17α-hydroxylase-17,20-lyase; PC, prostate cancer; CYP11B1, cytochrome P450 steroid-11-β-hydroxylase; CYP11B2, cytochrome P450 18-hydroxylase; GnRH, gonadotropin-releasing hormone; CAB, combined androgen blockade; PDB, Protein Data Bank; TMS, trimethylsilane; ESI, electrospray ionization; ATR, attenuated total reflection; GA, genetic algorithm.

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Chart 1. Role of CYP17 in Androgen Biosynthesis

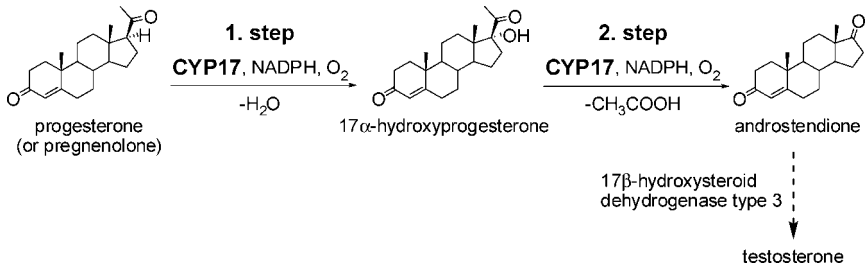


Chart 2. Substrate, Abiraterone, and Synthesized Mimetics

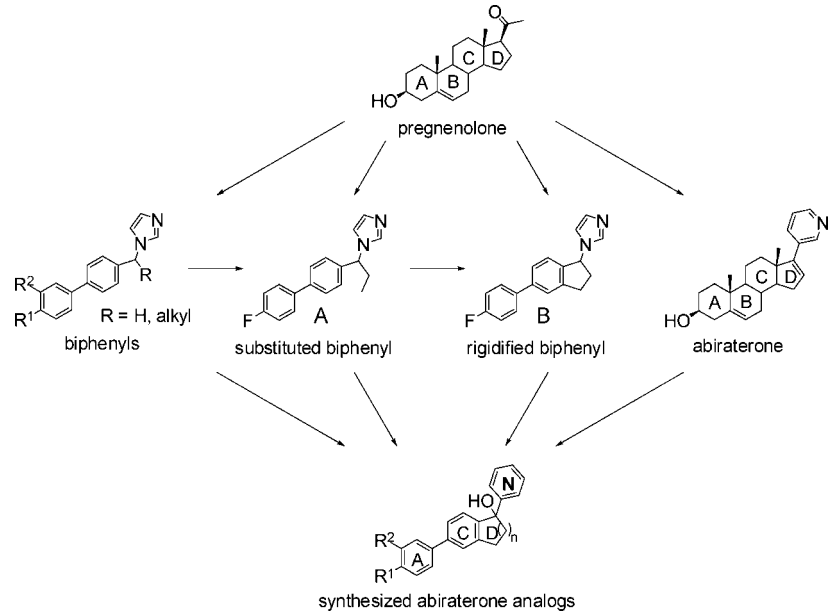
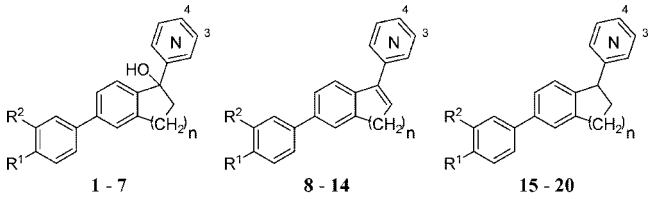


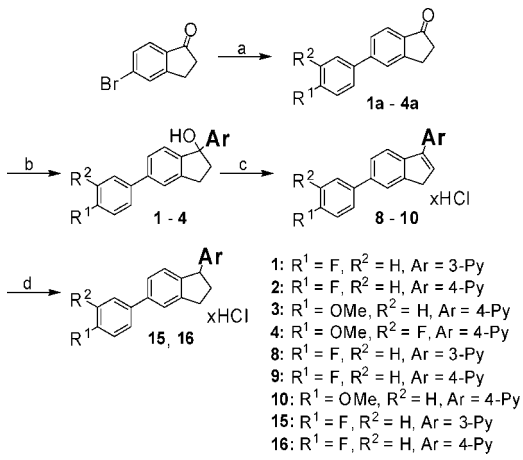
Chart 3. List of Synthesized Compounds **1**−**20**



Compound	R^1	R^2	n	N
1	F	H	1	3
2	F	H	1	4
3	OMe	H	1	4
4	OMe	F	1	4
5	F	H	2	4
6	F	F	2	4
7	OMe	F	2	4
8	F	H	1	3
9	F	H	1	4
10	OMe	H	1	4
11	F	H	2	4
12	F	F	2	4
13	OH	OH	2	4
14	OH	F	2	4
15	F	H	1	3
16	F	H	1	4
17	F	H	2	4
18	F	F	2	4
19	OH	OH	2	4
20	OH	F	2	4

phobic steroidal core, and the hydroxy group mimicking the oxygen at the 3-position of the substrates. All of them are similarly important for a high affinity to the enzyme. CYP enzymes have the particularity of bearing a heme moiety in their binding site. The complexation of the Fe^{2+} of the heme with an sp^2 hybridized nitrogen as in pyridyl and imidazolyl is widely described.[16] In recent studies[13] we showed that a biphenyl moiety can replace the steroidal core AC-rings and that a biphenyl moiety furnished with an imidazolyl-methylene group ensures high CYP17 inhibitory activity (Chart 2). Introduction of alkyl substituents at the methylene bridge[13b,f] (for example, compound **A**[13f]) and first attempts to rigidify the structures resulting in indane compounds (like compound **B**[13b]) increased activity. Interestingly, 4-fluoro substituted compounds in general showed very high inhibitory activity. Compound **A**, the indane ring opened analogue to compound **B**, was more active in the rat and showed a longer plasma half-life and higher bioavailability compared to abiraterone, while its in vitro activity with regard to potency and selectivity was not as outstanding as that of the steroidal compound. In this work we want to elucidate whether it is possible to further increase activity and especially selectivity by additional attempts to rigidify the biphenyl structure and to exchange the heterocycle. The resulting compounds are mimics of the steroidal compound abiraterone. For the design of the synthesized compounds, the steroidal core was replaced by an indane (mimicking the steroidal C- and D-rings) or tetrahydronaphthalene scaffold (for expansion of the D-ring) connected to an A-ring mimicking phenyl moiety. Electron donating and withdrawing substituents (including hydroxy and fluoro) were introduced in different positions at the phenyl ring. The iron-complexing aromatic nitrogen was represented by a 3- or 4-pyridine moiety.

Scheme 1. Synthesis of Indane Derivatives[a]



[a] Reagents and conditions. (a) Method A: Na_2CO_3, $R^1R^2C_6H_3B(OH)_2$, $Pd(PPh_3)_4$, toluene, H_2O, reflux, 8 h. (b) Method B: 3-iodopyridine or 4-bromopyridine, *n*-BuLi, THF, Et_2O, −78 °C to room temp, 3 h. (c) Method C: HCl in isopropanol, 80 °C, 2 h. (d) Method D: $Pd(OH)_2$, ethanol, THF, H_2, room temp, 3 h.

Three classes of mimetically relevant compounds were synthesized. Compounds **1**−**7**, hydroxylated at the C17 mimicking position, are analogues of the steroidal substrates for the second enzymatic step (lyase reaction). Compounds **7**−**14** bearing a double bond are structurally analogous to abiraterone. The saturated alkanes **15**−**20** show the highest similarity to the first-step (hydroxylase reaction) substrates.

In the following, the synthesis, determination of activity, and selectivity and molecular modeling studies of the synthesized abiraterone analogues are presented. Besides CYP17 inhibitory activity, inhibition of other CYP enzymes was examined to exclude possible side effects due to unspecific heme iron complexation. Thus, selectivity toward CYP11B1 and CYP11B2 was determined, since their biological relevance relies on the fact that CYP11B1 is involved in glucocorticoid biosynthesis while CYP11B2 catalyzes the last step in mineralocorticoid formation. Special attention was given to the most crucial hepatic enzyme CYP3A4, since it is involved in the metabolism of over 50% of all prescription drugs.[17] The most interesting compounds were docked into our homology-approach derived protein model and the binding modes in the active site discussed.

Chemistry

The syntheses of compounds **1**−**20** are shown in Schemes 1 and 2. In our aim to rigidify our biphenylic core structure by connecting the methylene bridge with the C-ring mimicking phenyl moiety, we synthesized indane derivatives (Scheme 1), which can be considered abiraterone analogues, and expanded the nonaromatic ring by one methylene group yielding tetrahydronaphthalene derivatives (Scheme 2). Different substitution patterns at the A-ring mimicking phenyl substituent were used. Suzuki coupling[18] (method A) using 5-bromoindanone for the abiraterone analogues and 1-tetralon-6-yl trifluoromethanesulfonate, synthesized from the respective alcohol, for the tetrahydronaphthalenes yielded the indanones **1a**−**4a** and the tetralones **5a**−**7a**. The pyridine moiety was introduced by means of a nucleophilic addition (method B) with the corresponding lithium pyridinyl salt to yield the tertiary alcohols **1**−**7** and **13a**. The condensation to the corresponding alkenes was performed in HCl/isopropanol (**8**−**12**, method C) or in HBr/H_2O (**13** and **14**, method E). The latter method was applied when a simultaneous ether cleavage was desired. The hydration to the saturated rings was carried out with Pearlman's catalyst ($Pd(OH)_2$), resulting in compounds **15**−**20** (method D).

Results

Biological Results. Inhibition of human CYP17 was determined by performing our previously described assay.[13d] For the source of human CYP17, *E. coli*[19] coexpressing human CYP17 and NADPH-P450 reductase were used. After homogenization, the 50 000 g sediment was incubated with progesterone (25 μM) and the inhibitor.[12a] Separation of the product was performed by HPLC using UV detection. The IC_{50} values determined for compounds **1**−**20** are shown in Table 1.

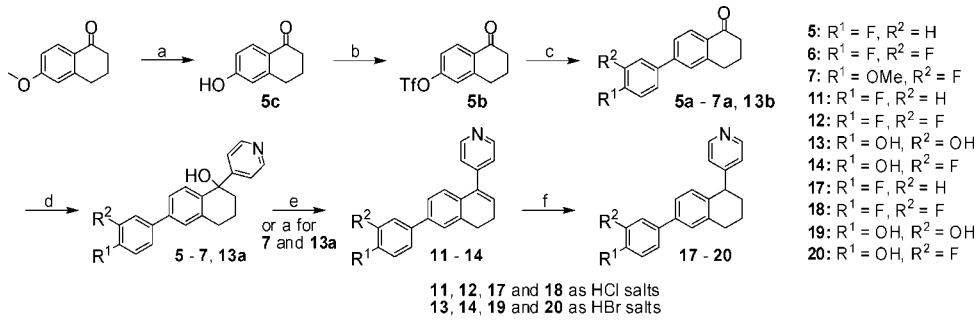
The compounds can be divided into two classes according to the CD-ring mimicking moiety, namely in indane and hydronaphthalene derivatives, and also into three classes regarding the pyridyl bearing C atom, that is in alcohols, alkenes, and alkanes. Most of the indane derivatives, either bearing a 3-pyridyl or a 4-pyridyl substituent, showed no or little CYP17 inhibition. Only compound **2**, with a moderate inhibition of 333 nM, and compound **16** (IC_{50} = 233 nM) were active. Interestingly, both compounds contain a 4-pyridyl group as iron-complexing heterocycle and a fluorine at R^1.

The hydronaphthalenes were more potent than the indanes, showing a broad range of activity. The alkenes (**11**−**14**) were weaker inhibitors than the corresponding compounds with a single bond, the alcohols (**5**−**7**) and tetrahydronaphthalenes (**17**−**20**). The inhibition values range from about 200 to over 5000 nM, while for the single bond compounds the values range mostly from around 100 to 500 nM. An important condition for inhibitory activity in the class of the alkanes is a 4-pyridyl substituent. In the 4-position of the phenyl ring (R^1) a F or OH group and in 3-position (R^2) H, F, or OH led to highly active compounds. Interestingly, the 3,4-di-F-substituted compound **18** only showed moderate activity. One explanation for this finding might be the electron withdrawing effect of the two F atoms at the phenyl ring. The most potent compounds were the 4-OH compounds **19** and **20** (IC_{50} = 144 and 64 nM). A hydroxy substituent in 4-position of the phenyl ring also makes the alkenes from the 4-pyridyl substituted dihydronaphthalene type potent inhibitors (compounds **13** and **14**). In the class of the indenes, 3-pyridyl substitution, as in abiraterone, led to an active compound (**8**) whereas 4-pyridyl substitution does not (**9**). It is striking, that 11 out of the 20 synthesized compounds were more active than ketoconazole. The most potent compound **20** showed a slightly higher inhibition compared to abiraterone (IC_{50} = 72 nM). Our seven most potent compounds from this work were more active than compound **A** (345 nM), and regarding compound **B** (670 nM), nine showed significantly higher inhibitory activities for CYP17. In the whole cell assay, i.e., *E. coli* coexpressing human CYP17 and NADPH-P450 reductase,[12f] the most active compound of this series, **20**, was also highly active (IC_{50} < 200 nM).

Since the target enzyme contains a heme in its binding site, hence CYP/P450 enzyme, which is crucial for the enzymatic reaction and for binding the iron-complexing nitrogen containing inhibitors, the latter compounds were tested for selectivity against other CYP enzymes. Accordingly, selected compounds were tested for inhibitory activity on the steroidogenic CYP enzymes CYP11B1 and CYP11B2, key enzymes in glucocorticoid and mineralocorticoid biosynthesis. For the assay,[20] V79MZh11B1 cells expressing human CYP11B1 and V79MZh11B2 cells expressing human CYP11B2 were used. The IC_{50} values determined for selected compounds are shown in Table 2.

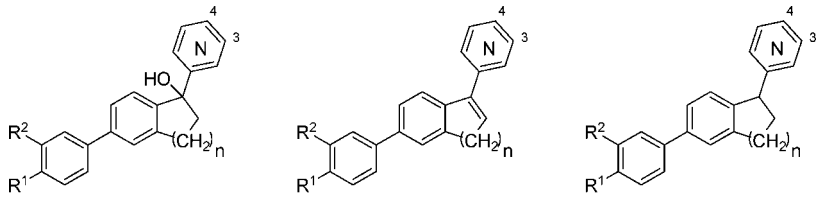
With the exception of compounds **4** and **7** showing IC_{50} values of 291 and 686 nM toward CYP11B1, respectively, the

Scheme 2. Synthesis of Tetrahydronaphthalene Derivatives[a]



[a] Reagents and conditions. (a) Method E: HBr, reflux, 16 h. (b) Pyridine, Tf_2O, DCM, 0 °C to room temp, 3 h. (c) Method A: Na_2CO_3, $R^1R^2C_6H_3B(OH)_2$, $Pd(PPh_3)_4$, toluene, H_2O, reflux, 8 h. (d) Method B: 3-iodopyridine or 4-bromopyridine, *n*-BuLi, THF, Et_2O, −78 °C to room temp, 3 h. (e) Method C: HCl in isopropanol, 80 °C, 2 h. (f) Method D: $Pd(OH)_2$, ethanol, THF, H_2, room temp, 3 h.

Table 1. Inhibition of CYP17 by Alcohols **1**−**7**, Alkenes **8**−**4**, and Alkanes **15**−**20**



structures[a]									
R^1	R^2	n	N	compd[a]	alcohol, CYP17 IC_{50} (nM)[b]	compd[a]	alkene, CYP17 IC_{50} (nM)[b]	compd[a]	alkane, CYP17 IC_{50} (nM)[b]
						indanes			
F	H	1	3	**1**	>20000	**8**	2346	**15**	>20000
F	H	1	4	**2**	333	**9**	>20000	**16**	233
OMe	H	1	4	**3**	>20000	**10**	>5000		
OMe	F	1	4	**4**	>10000				
						hydronaphthalenes			
F	H	2	4	**5**	587	**11**	>5000	**17**	163
F	F	2	4	**6**	423	**12**	>5000	**18**	1222
OMe	F	2	4	**7**	>5000				
OH	OH	2	4			**13**	307	**19**	144
OH	F	2	4			**14**	188	**20**	64
		KTZ[c]			2780				
		ABT[d]			72				

[a] Compounds **8**−**12** and **15**−**18** were tested as HCl salts and **13**, **14**, **19** and **20** as HBr salts. [b] Data shown were obtained by performing the tests in duplicate. The deviations were within ±5%. Concentration of progesterone (substrate) was 25 μM. [c] KTZ: ketoconazole. [d] ABT: abiraterone.

other tested compounds exhibited IC_{50} values above 1 μM. Most of the compounds (**2**, **6**, **11**−**14**, **17**−**19**) turned out to be more selective than abiraterone (IC_{50} = 1608 nM), and all compounds were more selective compared to ketoconazole (127 nM) regarding the cortisol forming enzyme. Concerning compound **A** (66% inhibition at 0.2 μM), all compounds from this study showed much higher selectivity.

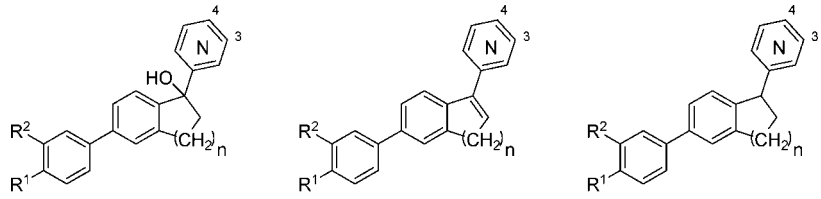
The inhibition of CYP11B2 observed with the evaluated compounds was a little higher showing IC_{50} values between 121 and 2324 nM. Compared to abiraterone (IC_{50} = 1751 nM), compounds **6** and **13** exhibited a similar selectivity whereas compound **19** (IC_{50} = 2324 nM) was more selective than the reference. Compared to ketoconazole (IC_{50} = 67 nM) and compound **A** (66% inhibition at 0.2 μM), all compounds were more selective with the exception of compound **11** (IC_{50} = 121 nM).

The synthesized compounds were also evaluated for inhibition of the most crucial hepatic CYP enzyme CYP3A4 in regard to its important role in drug metabolism. Except for compound **9** (IC_{50} = 159 nM) and compound **13** (IC_{50} = 357 nM), the IC_{50} values for compounds **1**−**20** were between 632 and >20000 nM. These compounds were more selective than ketoconazole (72 nM) and compound **A** (88% at 1 μM). Most of the compounds were even more selective than abiraterone (IC_{50} = 2704 nM).

Molecular Modeling. Since there is no crystal structure of CYP17 available, we recently built a homology model using the X-ray structure of human CYP2C9 (PDB ID 1r9o) as template.[13e] Docking simulations with energy minimized compounds were carried out by means of the GOLD 3.2 software running Linux CentOS 5.1 on an Intel P4 CPU 3.00 GHz computer, using a slightly modified GOLDSCORE function with goldscore.P450_pdb.parameters, for a better evaluation of hydrophobic interactions. From every class, the most potent compounds were docked into this protein model, and in the case of chiral compounds, both enantiomers were docked.

In Figure 1 two binding modes of the substrate pregnenolone are shown: one already described by others[21] (SM1 mode) and an additional one (SM2). Poses in SM1 occur more often (approximately 7:3) with an overall better scoring with respect to those in SM2. Furthermore, pregnenolone was also docked with CHEMSCORE, a scoring function with a higher assessment of the hydrophobic interactions, showing poses mostly in SM1 (>9:1). Interestingly, great similarities between SM2 and the

Table 2. Inhibition of CYP11B1, CYP11B2, and CYP3A4 by Compounds **1**−**20**



structures					IC_{50} (nM)		
compd[a]	R^1	R^2	n	N	CYP11B1[b]	CYP11B2[b]	CYP3A4[b]
1	F	H	1	3	nd[c]	nd[c]	>20000
2	F	H	1	4	>10000	816	3231
3	OMe	H	1	4	nd[c]	nd[c]	3291
4	OMe	F	1	4	291	436	3364
5	F	H	2	4	1159	840	8757
6	F	F	2	4	3109	1676	13841
7	OMe	F	2	4	686	945	15190
8	F	H	1	3	nd[c]	nd[c]	6947
9	F	H	1	4	nd[c]	nd[c]	159
10	OMe	H	1	4	nd[c]	nd[c]	2394
11	F	H	2	4	>10000	121	4199
12	F	F	2	4	>20000	311	3643
13	OH	OH	2	4	>10000	1492	357
14	OH	F	2	4	2748	991	2114
15	F	H	1	3	nd[c]	nd[c]	1697
16	F	H	1	4	1076	543	1093
17	F	H	2	4	>5000	518	2173
18	F	F	2	4	>20000	567	2300
19	OH	OH	2	4	2135	2324	632
20	OH	F	2	4	1370	587	3386
	KTZ[d]				127	67	72
	ABT[e]				1608	1751	2704

[a] Compounds **8**− **12** and **15**−**18** were tested as HCl salts and **13**, **14**, **19** and **20** as HBr salts. [b] Data shown were obtained by performing the tests in duplicate. The deviations were within ±5%. Concentration of progesterone (substrate) was 25 μM. [c] nd: not determined. [d] KTZ: ketoconazole. [e] ABT: abiraterone.

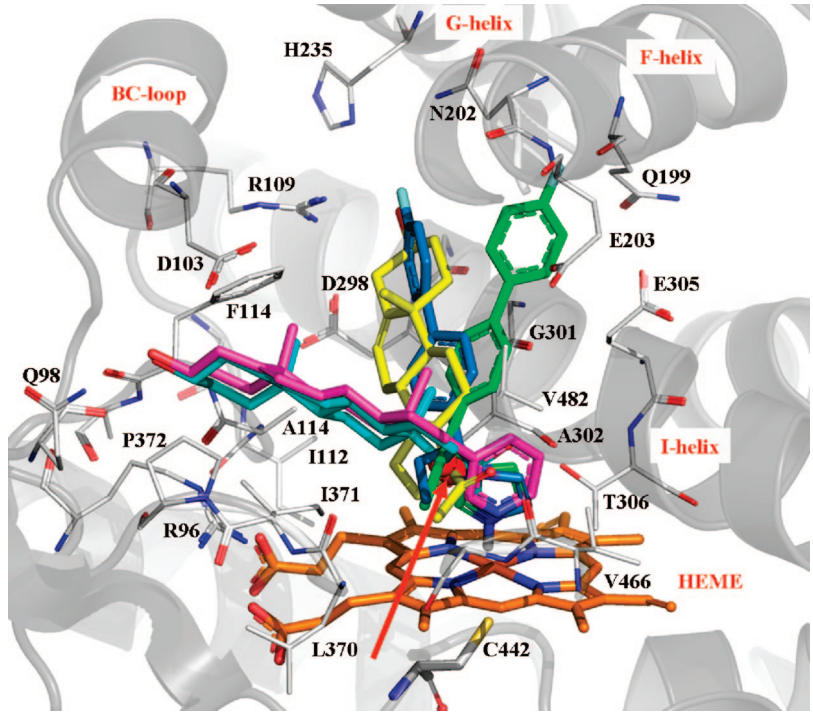


Figure 1. Both binding modes of the substrate pregnenolone (SM1, cyan, and SM2, yellow; the red arrow indicates the position of the C17 atoms, rendered as red spheres) are shown as docking complex with CYP17. For comparison, the two orientations of compound **A** (in the formerly described binding modes BM1, blue, and BM2, green[13f]) and abiraterone (magenta) are given. Heme, interacting residues, and ribbon rendered tertiary structure of the active site are also presented. Figures were generated with Pymol (http://www.pymol.org).

orientation of our previously described biphenyl compound **A** can be observed (there are actually two very similar modes described as BM1 and BM2[13e]). Like the substrate, the steroidal inhibitor abiraterone is also able to bind in both modes but

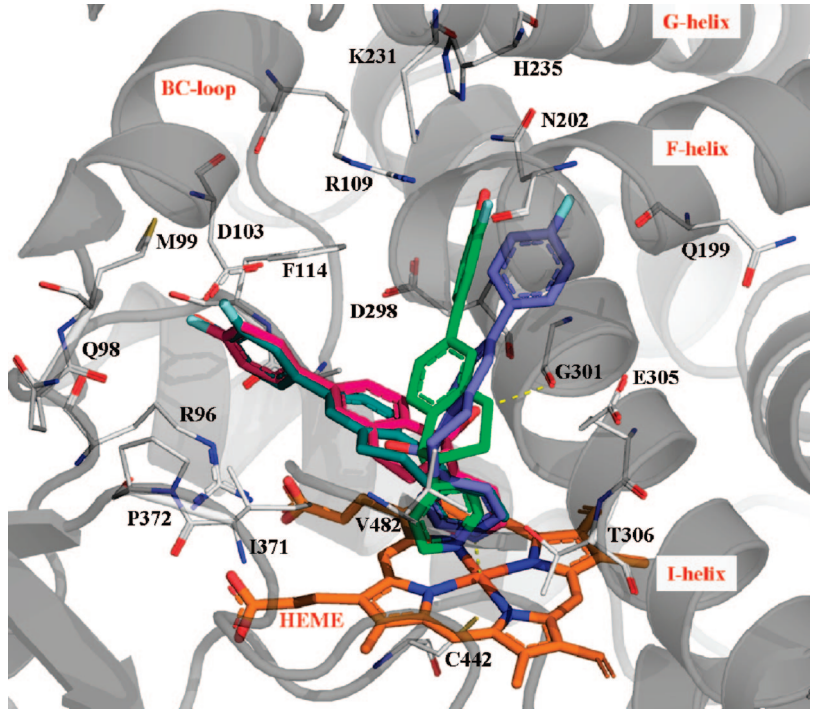


Figure 2. Docking complexes of CYP17 and compounds **5** (*R* enantiomer in SM1, blue-green; *S* enantiomer in SM2, violet) and **20** (*S* enantiomer in SM1, magenta; *R* enantiomer in SM2, green). A dashed yellow line is representing the H-bond between the OH in C17 and Gly301.

showing a good complexation with the heme iron only in SM1 (Figure 1). The same is true for compound **B**, the ring-closed analogue of compound **A** (not shown).

The *R* and *S* configurated abiraterone analogues of the alcohol and alkane class also bind in both modes. Interestingly, a correlation between the inhibitory activities of the compounds of each class and the score values could be observed. In the case of highly active compounds, the percentage of poses presenting a good iron−nitrogen coordination is higher than for weak inhibitors. For enantiomers of a given racemic compound, one enantiomer always shows a preference for poses in one binding mode while the other enantiomer prefers to dock in the alternative one. In the case of the alcohols, in Figure 2 exemplified by compound **5**, the *R* enantiomer binds predominantly in the SM1 mode (9:1) while the *S* enantiomer binds in SM2 (8:2). The opposite relation can be seen for the alkanes (Figure 2).

The most important interactions in SM1, besides heme iron complexation, are hydrophobic interactions with Ala113, Phe114, Ile371, Pro372, and Val482 and, depending on substituents R^1 and R^2, polar ones with the backbone of Glu98 and Met99 and the carboxyl group of Asp103. Furthermore, an H-bond formation between the OH group of R-alkohols and the backbone carbonile of Gly301 was observed, which could act as an additional stabilizing factor. Regarding SM2, the same interactions as described in refs 13e and 13f were found.

Low activity compounds did not show a real clustering in SM1 mode and were characterized by an unsuitable binding angle between the N lone electron pair and the heme plane (compound **12**, Figure 3). On the other hand, the highly active alkenes **13** and **14** bind preferentially in SM2 mode and present a good complexation of the heme iron and a strong binding due to their 3,4-di-OH or 3-F,4-OH substitution.

Discussion and Conclusion

In the class of the indanes the 3-pyridyl substituted unsaturated compound **8**, which is analogous to abiraterone, showed inhibitory activity in contrast to the respective alcohols and alkanes. In the case of the corresponding 4-pyridyl substituted compounds, it is the other way around: the alcohols and the alkanes were active, while the unsaturated compounds did not show activity. However, it became apparent that the five-membered ring, though analogous to the substrate and abiraterone, is not favorable for CYP17 inhibition. On the other hand, the expansion of this ring by one methylene group resulting in the hydronaphthalenes led to very potent and selective compounds. Summarizing, we have discovered nine very potent abiraterone analogues, four of them (**14**, **17**, **19**, and **20**) showing an IC_{50} value below 200 nM and one of them (**20**, IC_{50} = 64 nM) being slightly more active than abiraterone. These compounds showed low inhibition of the CYP enzymes considered for selectivity issues CYP11B1, CYP11B2 and the hepatic CYP3A4. Owing to their promising in vitro profile, presently compounds **20** and **19** are separated into their enantiomers and extensively evaluated for their in vivo activities and pharmacokinetics in the rat.

Experimental Section

Chemistry. IR spectra were recorded neat on a Bruker Vector 33FT-infrared spectrometer. ^{1}H NMR spectra were measured on a Bruker DRX-500 (500 MHz). Chemical shifts are given in parts per million (ppm), and TMS was used as an internal standard for spectra obtained in $CDCl_3$. All coupling constants (*J*) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. Column chromatography was performed using silica gel 60 (50−200 μm), and reaction progress was determined by TLC analysis on Alugram SIL G/UV_{254} (Macherey-Nagel). The purities of the final compounds were determined with the Surveyor LC system and were always greater than 98%. Boronic acids and bromoaryls used as starting materials were commercially obtained (CombiBlocks, Chempur, Aldrich, Acros).

Method A: Suzuki-Coupling. See Supporting Information.

Method B: Nucleophilic Addition of the Heterocycle. 3-Iodopyridine (4 mmol), or 4-bromopyridine (4 mmol) for the 4-pyridyl

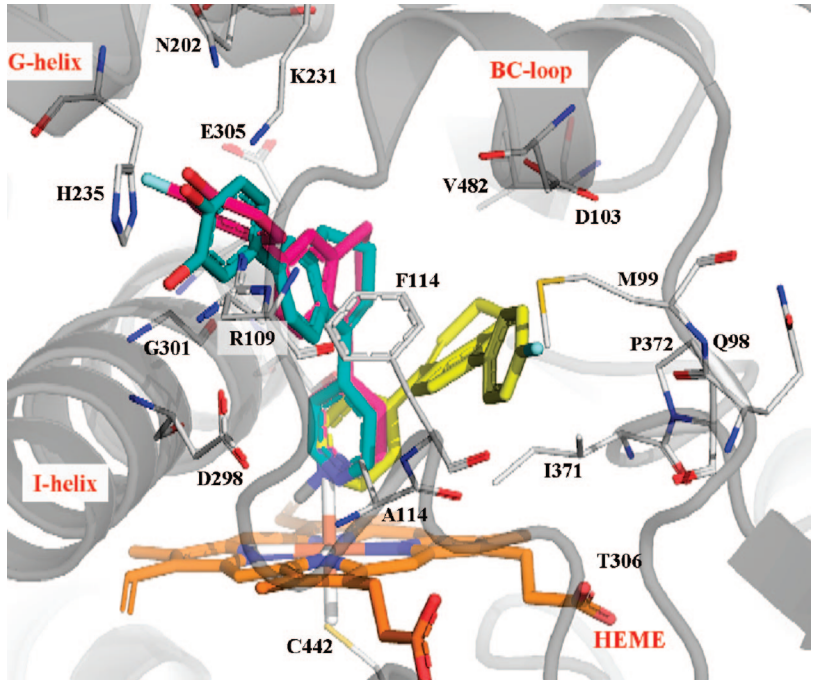


Figure 3. Docking complexes of CYP17 and compounds **11** (yellow; its lone electron pair at the nitrogen and the axis of the octahedral heme iron atom are shown), **13** (cyan), and **14** (magenta).

compounds, after basic extraction of its hydrochloride salt with Et_2O (20 mL) and $NaHCO_3$ (saturated, aqueous), followed by drying over Na_2SO_4 and concentration under reduced pressure, was prepared in Et_2O and THF (3:2, 50 mL) at −78 °C. Then *n*-BuLi in hexane (1.6 N, 4.4 mmol) was added, and after 5 s the corresponding ketone (4 mmol) in THF (20 mL) was added at once and the mixture was left stirring for an additional 3 h at room temperature. After neutralization with NH_4Cl (saturated, aqueous), the phases were separated, the aqueous layer was extracted two times with EtOAc, and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The purification was performed by silica gel flash chromatography.

5-(4-Fluorophenyl)-1-(pyridin-3-yl)-2,3-dihydro-1*H*-inden-1-ol (1). 1 was synthesized from **1a** and 3-iodopyridine according to method B: yield = 55%; δ_H ($CDCl_3$, 500 MHz) 2.36−2.47 (m, 2H), 2.85−2.89 (m, 1H), 3.08−3.14 (m, 1H), 6.98−7.04 (m, 3H), 7.14−7.16 (m, 1H), 7.24−7.26 (d, J = 7.9 Hz, 1H), 7.36 (s, 1H), 7.41−7.44 (m, 2H), 7.69 (d, J = 7.9 Hz, 1H), 8.29 (s, 1H), 8.48 (s, 1H); m/z = 305.17 [M^+ + H].

5-(4-Fluorophenyl)-1-(pyridin-4-yl)-2,3-dihydro-1*H*-inden-1-ol (2). 2 was synthesized from **2a** and 4-bromopyridine according to method B: yield = 50%; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 2.37−2.47 (m, 2H), 2.92−2.98 (m, 1H), 3.15−3.21 (m, 1H), 7.00 (d, J = 7.9 Hz, 1H), 7.04 (t, J = 8.6 Hz, 2H), 7.29−7.31 (m, 3H), 7.41 (s, 1H), 7.41−7.47 (m, 2H), 8.38 (d, J = 4.8 Hz, 2H); m/z = 306.21 [M^+ + H].

5-(4-Methoxyphenyl)-1-(pyridin-4-yl)-2,3-dihydro-1*H*-inden-1-ol (3). 3 was synthesized from **3a** and 4-bromopyridine according to method B: yield = 45%; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 2.34−2.46 (m, 2H), 2.91−2.97 (m, 1H), 3.12−3.20 (m, 1H), 3.77 (s, 3H), 6.90 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 7.9 Hz, 1H), 7.30−7.32 (m, 3H), 7.42 (s, 1H), 7.44 (d, J = 8.8 Hz, 2H), 8.38 (d, J = 6.2 Hz, 2H); m/z = 318.18 [M^+ + H].

5-(3-Fluoro-4-methoxyphenyl)-1-(pyridin-4-yl)-2,3-dihydro-1*H*-inden-1-ol (4). 4 was synthesized from **4a** and 4-bromopyridine according to method B: yield = 56%; δ_H ($CDCl_3$, 500 MHz) 2.39−2.48 (m, 2H), 2.93−2.98 (m, 1H), 3.14−3.19 (m, 1H), 3.85 (s, 3H), 6.93−6.98 (m, 2H), 7.21−7.30 (m, 5H), 7.40 (s, 1H), 8.40 (d, J = 6.2 Hz, 2H); m/z = 336.11 [M^+ + H].

6-(4-Fluorophenyl)-1-(pyridin-4-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (5). 5 was synthesized from **5a** and 4-bromopyridine according to method B: yield = 44%; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 1.75−1.82 (m, 1H), 2.00−2.08 (m, 2H), 2.09−2.16 (m, 1H), 2.86−3.00 (m, 2H), 6.98 (d, J = 8.1 Hz, 1H), 7.07 (t, J = 8.8 Hz, 2H), 7.27 (dd, J = 2.0 Hz, J = 8.1 Hz, 1H), 7.33−7.34 (m, 3H), 7.51 (dd, J = 5.4 Hz, J = 8.6 Hz, 2H), 8.40 (d, J = 6.3 Hz, 2H); m/z = 320.06 [M^+ + H].

6-(3,4-Difluorophenyl)-1-(pyridin-4-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (6). 6 was synthesized from **6a** and 4-bromopyridine according to method B: yield = 40%; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 1.81−1.89 (m, 1H), 1.99−2.07 (m, 2H), 2.12−2.18 (m, 1H), 2.89−3.00 (m, 2H), 6.99 (d, J = 8.1 Hz, 1H), 7.19 (q, J = 8.4 Hz, 1H), 7.24−7.26 (m, 2H), 7.30−7.37 (m, 4H), 8.48 (d, J = 6.0 Hz, 2H); m/z = 338.13 [M^+ + H].

6-(3-Fluoro-4-methoxyphenyl)-1-(pyridin-4-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (7). 7 was synthesized from **7a** and 4-bromopyridine according to method B: yield = 64%; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 1.70−1.77 (m, 1H), 1.94−2.03 (m, 2H), 2.03−2.10 (m, 1H), 2.82−2.93 (m, 2H), 3.84 (s, 3H), 6.92 (dd, J = 2.7 Hz, J = 8.1 Hz, 2H), 6.96 (dt, J = 2.4 Hz, J = 8.4 Hz, 1H), 7.19−7.28 (m, 6H), 8.35−8.36 (m, 2H); m/z = 350.13 [M^+ + H].

Method C: Condensation with HCl. The corresponding alcohol (1 mmol) was refluxed in HCl in *i*-PrOH (10 mL, 3 N) for 2 h. Afterward, the resulting solution was concentrated under reduced pressure and washed three times with Et_2O. No further purification was necessary.

3-(5-(4-Fluorophenyl)-3*H*-inden-1-yl)pyridine Hydrochloride (8). 8 was synthesized from **1** according to method C: yield = 96%; δ_H ($CDCl_3$, 500 MHz) 3.53 (s, 2H), 6.61−6.62 (m, 1H), 7.06 (t, J = 8.7 Hz, 2H), 7.32 (dd, J = 4.8 Hz, J = 7.8 Hz, 1H), 7.44 (dd, J = 1.4 Hz, J = 7.8, 1H), 7.49−7.52 (m, 3H), 7.65 (s, 1H), 7.83−7.85 (m, 1H), 8.55−8.56 (d, J = 4.0 Hz, 1H), 8.81 (s, 1H); MS (ESI): m/z = 288.13 [M^+ + H].

4-(5-(4-Fluorophenyl)-3*H*-inden-1-yl)pyridine Hydrochloride (9). 9 was synthesized from **2** according to method C: yield = 100%; δ_H ($CDCl_3$, 500 MHz) 3.20−3.22 (m, 2H), 7.08 (t, J = 8.8 Hz, 2H), 7.30 (s, 1H), 7.54 (dd, J = 1.7 Hz, J = 8.1 Hz, 1H), 7.58 (dd, J = 5.4 Hz, J = 8.8 Hz, 2H), 7.69 (d, J = 8.1 Hz, 1H), 7.76 (d, J = 1.4 Hz, 1H), 8.30 (d, J = 6.8 Hz, 2H), 8.81 (d, J = 6.8 Hz, 2H); m/z = 287.96 [M^+ + H].

4-(5-(4-Methoxyphenyl)-3*H*-inden-1-yl)pyridine Hydrochloride (10). 10 was synthesized from **3** according to method C: yield = 99%; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 3.24−3.27 (m, 2H), 3.78

(s, 3H), 6.92 (d, $J = 8.8$ Hz, 2H), 7.43 (s, 1H), 7.50 (d, $J = 8.8$ Hz, 2H), 7.53 (dd, $J = 1.7$ Hz, $J = 8.1$ Hz, 1H), 7.60 (d, $J = 8.1$ Hz, 1H), 7.73 (d, $J = 1.7$ Hz, 1H), 8.23 (d, $J = 6.7$ Hz, 2H), 8.80 (d, $J = 6.7$ Hz, 2H); $m/z = 301.16$ [$M^+ + H$].

4-(6-(4-Fluorophenyl)-3,4-dihydronaphthalen-1-yl)pyridine Hydrochloride (11). 11 was synthesized from **4** according to method C: yield = 100%; δ_H ($CDCl_3$, 500 MHz) 2.58−2.61 (m, 2H), 2.94 (t, $J = 7.8$ Hz, 2H), 6.55 (t, $J = 4.7$ Hz, 1H), 6.95 (d, $J = 7.9$ Hz, 1H), 7.14 (t, $J = 8.7$ Hz, 2H), 7.38 (d, $J = 7.9$ Hz, 1H), 7.46 (s, 1H), 7.56 (dd, $J = 5.3$ Hz, $J = 8.7$ Hz, 2H), 7.93 (d, $J = 5.6$ Hz, 2H), 8.79 (d, $J = 5.6$ Hz, 2H); $m/z = 302.10$ [$M^+ + H$].

4-(6-(3,4-Difluorophenyl)-3,4-dihydronaphthalen-1-yl)pyridine Hydrochloride (12). 12 was synthesized from **5** according to method C: yield = 98%; δ_H ($CDCl_3$, 500 MHz) 2.57−2.61 (m, 2H), 2.94 (t, $J = 8.0$ Hz, 2H), 6.57 (t, $J = 4.8$ Hz, 1H), 6.96 (d, $J = 8.0$ Hz, 2H), 7.23 (q, $J = 8.5$ Hz, 1H), 7.29−7.31 (m, 1H), 7.36 (d, $J = 8.5$ Hz, 1H), 7.37−7.41 (m, 1H), 7.44 (s, 1H), 7.92 (s, 1H), 7.93 (s, 1H), 8.81 (s, 1H), 8.82 (s, 1H); $m/z = 320.11$ [$M^+ + H$].

Method D: Hydration with Pearlman's Catalyst. Pearlman's catalyst (10 mass %) and the corresponding alkene were prepared in EtOH and THF (2:1, 5 mL) under H_2 atmosphere. The mixture was left stirring for 3 h. Then the catalyst was filtered off three times and the solution concentrated under reduced pressure. The obtained solid was washed three times with Et_2O. No further purification was necessary.

3-(5-(4-Fluorophenyl)-2,3-dihydro-1*H*-inden-1-yl)pyridine Hydrochloride (15). 15 was synthesized from **8** according to method D: yield = 99%; δ_H ($CDCl_3$, 500 MHz) 1.92−1.98 (m, 1H), 2.65−2.71 (m, 1H), 2.95−3.06 (m, 2H), 4.51 (s, 1H), 6.84 (d, $J = 6.9$ Hz, 1H), 7.00 (t, $J = 8.6$ Hz, 2H), 7.24 (d, $J = 6.9$ Hz, 1H), 7.38−7.41 (m, 3H), 7.76 (s, 1H), 8.13 (d, $J = 5.0$ Hz, 1H), 8.49 (s, 1H), 8.55 (s, 1H); $m/z = 290.10$ [$M^+ + H$].

4-(5-(4-Fluorophenyl)-2,3-dihydro-1*H*-inden-1-yl)pyridine Hydrochloride (16). 16 was synthesized from **9** according to method D: yield = 100%; δ_H ($CDCl_3$, 500 MHz) 2.03−2.09 (m, 1H), 2.72−2.78 (m, 1H), 3.03−3.13 (m, 2H), 4.60 (s, 1H), 6.93 (d, $J = 7.1$ Hz, 1H), 7.06 (t, $J = 8.6$ Hz, 2H), 7.32 (d, $J = 6.7$ Hz, 1H), 7.45−7.47 (m, 3H), 7.68 (s, 2H), 8.71 (s, 2H); $m/z = 290.17$ [$M^+ + H$].

4-(6-(4-Fluorophenyl)-1,2,3,4-tetrahydronaphthalen-1-yl)pyridine Hydrochloride (17). 17 was synthesized from **11** according to method D: yield = 97%; δ_H ($CDCl_3$, 500 MHz) 1.82−1.90 (m, 3H), 2.33−2.37 (m, 1H), 2.94−3.00 (m, 2H), 4.46 (s, 1H), 6.78 (d, $J = 7.5$ Hz, 1H), 7.12 (t, $J = 8.6$ Hz, 2H), 7.30 (d, $J = 6.8$ Hz, 1H), 7.39 (s, 1H), 7.53 (dd, $J = 5.3$ Hz, $J = 8.6$ Hz, 2H), 7.67 (s, 2H), 8.71 (s, 2H); $m/z = 304.10$ [$M^+ + H$].

4-(6-(3,4-Difluorophenyl)-1,2,3,4-tetrahydronaphthalen-1-yl)pyridine Hydrochloride (18). 18 was synthesized from **12** according to method D: yield = 98%; δ_H ($CDCl_3$, 500 MHz) 1.82−1.90 (m, 3H), 2.33−2.37 (m, 1H), 2.94−3.00 (m, 2H), 4.46 (s, 1H), 6.79 (d, $J = 6.2$ Hz, 1H), 7.22 (q, $J = 8.4$ Hz, 2H), 7.26−7.28 (m, 2H), 7.34−7.38 (m, 2H), 7.66 (s, 2H), 8.72 (s, 2H); $m/z = 322.11$ [$M^+ + H$].

4-(5-(Pyridin-4-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)benzene-1,2-diol Hydrobromide (19). 19 was synthesized from **13** according to method D: yield = 98%; δ_H ($CDCl_3 + CD_3OD$, 500 MHz) 1.73−1.80 (m, 3H), 2.19−2.24 (m, 1H), 2.81−2.89 (m, 2H), 4.27−4.32 (m, 1H), 6.63−6.65 (m, 1H), 6.81 (d, $J = 8.2$ Hz, 1H), 6.88−6.90 (m, 1H), 7.00 (s, 1H), 7.17 (d, $J = 8.0$ Hz, 1H), 7.28 (s, 1H), 7.45−7.51 (m, 2H), 8.59 (s, 2H); $m/z = 318.65$ [$M^+ + H$].

2-Fluoro-4-(5-(pyridin-4-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)phenol Hydrobromide (20). 20 was synthesized from **14** according to method D: yield = 96%; δ_H ($CDCl_3 + CD_3OD$, 500 MHz) 1.84−1.92 (m, 3H), 2.34−2.40 (m, 1H), 2.92−3.00 (m, 2H), 4.48−4.50 (m, 1H), 6.76 (d, $J = 8.0$ Hz, 1H), 7.02 (t, $J = 8.8$ Hz, 1H), 7.22 (d, $J = 8.4$ Hz, 1H), 7.26−7.28 (m, 2H), 7.38 (s, 1H), 7.73 (s, 1H), 7.74 (s, 1H), 8.79 (s, 1H), 8.80 (s, 1H); $m/z = 320.08$ [$M^+ + H$].

Method E: Ether Cleavage and Condensation with HBr. The corresponding ether was refluxed overnight in 48% HBr_{aq} (100 mL for compound **5c**, 3 mL for compounds **13** and **14**). For compound **5c**, after reaction completion water (100 mL) was added and the mixture was cooled and filtered. The solid was washed three times with water, and no further purification was needed. For compounds **13** and **14**, after reaction completion the mixture was neutralized with solid $NaHCO_3$ and extracted three times with EtOAc. The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure, and purified by silica gel flash chromatography.

4-(5-(Pyridin-4-yl)-7,8-dihydronaphthalen-2-yl)benzene-1,2-diol Hydrobromide (13). 13 was synthesized from **13a** according to method E: yield = 65%; δ_H ($CDCl_3 + CD_3OD$, 500 MHz) 2.53−2.55 (m, 2H), 2.87 (t, $J = 7.8$ Hz, 2H), 6.53−6.55 (m, 1H), 6.83−6.89 (m, 2H), 6.94−6.96 (m, 1H), 7.28−7.31 (m, 1H), 7.40−7.41 (m, 1H), 7.94−7.96 (m, 2H), 8.77−8.79 (m, 2H); $m/z = 316.18$ [$M^+ + H$].

2-Fluoro-4-(5-(pyridin-4-yl)-7,8-dihydronaphthalen-2-yl)phenol Hydrobromide (14). 14 was synthesized from **7** according to method E: yield = 28%; δ_H ($CDCl_3 + CD_3OD$, 500 MHz) 2.49−2.53 (m, 2H), 2.94 (t, $J = 7.9$ Hz, 2H), 6.25−6.27 (m, 1H), 7.02 (q, $J = 8.4$ Hz, 2H), 7.26 (d, $J = 8.1$ Hz, 1H), 7.31 (s, 1H), 7.34 (d, $J = 5.8$ Hz, 1H), 7.39−7.42 (m, 3H), 8.58 (d, $J = 5.7$ Hz, 2H); $m/z = 318.08$ [$M^+ + H$].

Biological Assays. CYP17 Preparation and Assay. For the source of human CYP17, our *E. coli* system[19] (coexpressing human CYP17 and NADPH-P450 reductase) was used, and the assay was performed as previously described[12a] using unlabeled progesterone as substrate and applying HPLC with UV detection for separation.

Inhibition of CYP11B1 and CYP11B2. V79MZh11B1 or V79MZh11B2[22] cells expressing the respective human enzyme were used, and our assay procedure using [4-^{14}C]-11-deoxycorticosterone was performed.[20]

Inhibition of CYP3A4. The recombinantly expressed enzymes from baculovirus-infected insect microsomes and cytochrome b5 (BD supersomes) were used, and the manufacturer's instructions (www.gentest.com) were followed.

Molecular Modeling. Various inhibitors of Table 1 were docked into our CYP17 homology model by means of the GOLD 3.2 software[23] using GOLDSCORE and CHEMSCORE. Since the GOLD docking program allows flexible docking of the compounds, no conformational search was employed to the ligand structures. GOLD gave the best poses by a genetic algorithm (GA) search strategy, and then various molecular features were encoded as a chromosome.

The structures of the inhibitors were built with SYBYL 7.3.2 (Sybyl, Tripos Inc., St. Louis, MO) and energy-minimized in MMFF94s force-field[24] as implemented in Sybyl.

Ligands were docked in 50 independent GA runs using GOLD. Heme iron was chosen as active site origin, while its radius was set equal to 19 Å. The automatic active site detection was switched on. Furthermore, a distance constraint of a minimum of 1.9 Å and a maximum of 2.5 Å between the sp^2-hybridized nitrogen of the pyridine and the iron of the heme was set. Additionally, the goldscore.p450_pdb parameters were used and some of the GOLDSCORE parameters were modified to improve the weight of hydrophobic interaction and of the coordination between iron and nitrogen. The genetic algorithm default parameters were set as suggested by the GOLD authors.[23] On the other hand, the annealing parameters of fitness function were set at 3.5 Å for hydrogen bonding and 6.5 Å for van der Waals interactions.

All 50 poses for each compound were clustered with ACIAP,[25] and the representative structure of each significant cluster was selected. After the docking simulations and cluster analysis were performed, the quality of the docked representative poses was evaluated on the basis of visual inspection of the putative binding modes of the ligands. The latter compounds were further evaluated by means of Silver, version 3.1.1, the postprocessing tool of GOLD, and last by GOLDSCORE.

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Supporting Information Available: Physical data and synthetic procedures for **1a**, **3a**, **4a**, **5a**−**c**, **6a**, **7a**, and **13a**,**b** and purities for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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