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Highly Potent and Selective Nonsteroidal Dual Inhibitors of CYP17/CYP11B2 for the Treatment of Prostate Cancer To Reduce Risks of Cardiovascular Diseases

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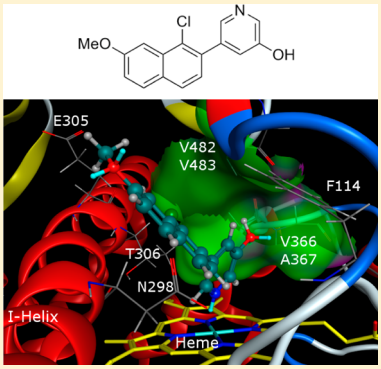
Supporting Information

ABSTRACT: Dual CYP17/CYP11B2 inhibitors are proposed as a novel strategy for the treatment of prostate cancer to reduce risks of cardiovascular diseases. Via a combination of ligand- and structure-based approaches, a series of dual inhibitors were designed leading to the 2-(3-pyridyl)naphthalenes **10** and **11** with strong inhibition of both enzymes (IC_{50} values around 20 nM) and excellent selectivities over CYP11B1, CYP19, and CYP3A4. These compounds are considered as promising candidates for further in vivo evaluation.



INTRODUCTION

Androgens stimulate the proliferation of prostate cancer (PCa) cells. The inhibition of androgen production and the blockage of their binding to androgen receptors are therefore effective approaches to tackle this lethal disease. Castration and gonadotropin-releasing hormone analogues were first employed to interrupt the biosynthesis of androgens in the testes. However, they have no effects on the minor amounts of androgens produced in the adrenals, not to mention on the intratumoral auto/paracrine production of androgens. In contrast, inhibition of 17α-hydroxylase-17,20-lyase (CYP17), which is the pivotal enzyme in the biosynthesis of androgens, can totally block androgen formation. The recently launched CYP17 inhibitor abiraterone not only improved the survival of PCa patients but also demonstrated curative effects in patients with castration-resistant prostate cancer,[1] which had been regarded as "androgen independent" by the time. However, similar to being observed with other androgen deprivation therapies (ADT),[2] CYP17 inhibition is associated with risks of cardiovascular complications.[1] This is not a surprise since testosterone can reduce cardiomyocyte apoptosis.[3a] In cardiomyocytes of congestive heart failure patients, the production of dehydroepiandrosterone is suppressed, whereas aldosterone is upregulated.[3b] The metabolic disorder of lipids caused by androgen deficiency has been proposed causative for cardiovascular diseases (CVD).[4a] However, the contribution of exorbitant aldosterone in this pathological process has been neglected. A systematic literature search reveals that elevation of aldosterone concentration is a consequence of androgen deficiency. It has been shown that testosterone inhibits the secretion of aldosterone with or without stimulation of adrenocorticotropic hormone and/or angiotensin II in rats.[5a] In rare cases of CYP17 absence due to genetic disorders, high plasma aldosterone concentrations were observed.[5b] Furthermore, CYP17 inhibition resulted in estrogen depletion[1b] and in accumulation of progesterone,[6a] leading to elevated aldosterone levels.[6b] Androgen deprivation also increased serum low- and high-density lipoprotein,[4b] which further promoted aldosterone secretion.[7a,b] The exorbitant aldosterone subsequently caused inflammation and activated multiple pathways, leading to CVD (reviewed in refs 6c and d). Interestingly, the concentrations of aldosterone in failing cardiac tissues are much higher than those in peripheral plasma,[8a] probably due to local overexpression of aldosterone synthase (CYP11B2),[8b] which is the pivotal enzyme catalyzing the last three steps in aldosterone biosynthesis. This makes it difficult to monitor CVD via plasma aldosterone determination, especially at the early stages of CVD, when cardiac aldosterone is high enough for damages, whereas its plasma levels are still in the normal range. This phenomenon together with the moderate inhibition of CYP11B2 by abiraterone (IC_{50} = 1750 nM)[9b] could be reasonable explanations for the apparent reduction of aldosterone plasma levels (1.5-fold)[1b] in the clinical trials of abiraterone, which is in contrast to the increased incidence of cardiac disorders.[1a] Therefore, we propose dual inhibition of CYP17/CYP11B2 as a novel strategy for the treatment of PCa to reduce CVD risks. These dual inhibitors should show

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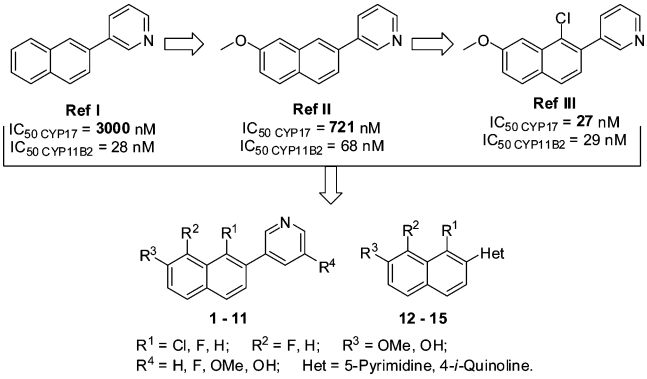
selectivity over other steroidogenic CYP enzymes, such as 11β-hydroxylase (CYP11B1) and aromatase (CYP19), to avoid side effects related to cortisol and estrogen deficiency. However, this aim is difficult to reach, especially for CYP11B1 because the homology between CYP11B1 and CYP11B2 is as high as 93%. For pursuing such a challenging project, recent progress in designing inhibitors of CYP17[9] and CYP11B1,[10] as well as other steroidogenic enzymes such as CYP11B2,[11] CYP19,[12] 5α-reductase,[13] and 17β-hydroxysteroid dehydrogenase type 1 and 2,[14] was very helpful.

DESIGN CONCEPT FOR DUAL INHIBITORS

During the development of 3-pyridyl substituted naphthalenes as CYP11B2 inhibitors, it has been observed that some substituents on the naphthalene core showed little impact on CYP11B2 inhibition yet profound influence on CYP17. 2-(3-Pyridyl)naphthalene (**ref I**;[11a] Chart 1) exhibited a much

Chart 1. Design Concept for the Title Compounds



weaker inhibition of CYP17 (IC_{50} = 3000 nM) compared to its 7-OMe derivative (**ref II**;[11a] Chart 1, IC_{50} = 721 nM). Further introduction of a Cl at the 1-position (**ref III**;[11a] Chart 1) dramatically boosted CYP17 inhibition to 27 nM. In contrast, the inhibition of these compounds toward CYP11B2 remained constantly strong (around 30 nM for **ref I** and **ref III**, and 68 nM for **ref II**). This intriguing observation directed our attention to the interactions of these substituents with the CYP17 active site and inspired the design of dual inhibitors of CYP17/CYP11B2. **ref III** was therefore docked into the CYP17 (PDB ID: 3RUK)[15a] and CYP11B2 (PDB ID: 4DVQ)[15b] structures. With the resulting predominant binding mode in CYP17 (Figure 1A), further evidence about the importance of the 7-OMe and 1-Cl substituents was revealed. The compound coordinates perpendicularly to the heme iron with the pyridyl sp^2 hybrid N. The naphthalene core leans against the I-helix and forms $\pi-\pi$ interactions with both Phe114 and the π-systems of the amino acid backbones in the I-helix in parallel orientation. The 7-OMe acting as an H-bond acceptor interacts with the side chains of Glu305. Furthermore, besides the possible contribution of 1-Cl in inducing the bioactive conformation, the vicinity of the 1-Cl group to the carbonyl oxygens of Gly301 and Ala302 (distances of 3.1 Å and 3.2 Å, respectively) indicates the existence of halogen bonds.[15c] Interestingly, such interactions of 1-Cl and 7-OMe are not present in the binding of **ref III** to CYP11B2, where the compound adopts a binding mode similar to that of the natural substrate deoxycorticosterone (Figure 2), which is totally different from its pose in CYP17. In CYP11B2 it is oriented to

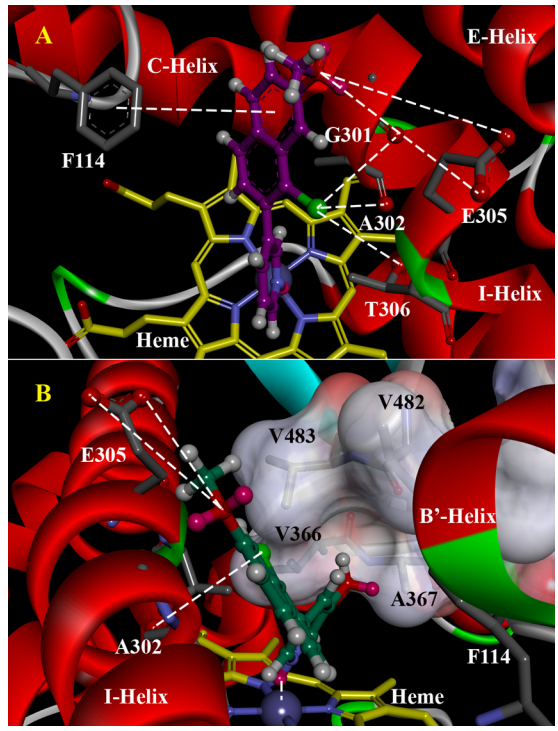


Figure 1. Docking of **ref III** (**A**, depicted in purple) and compound **10** (**B**, depicted in celadon green) in CYP17 (PDB ID: 3RUK).

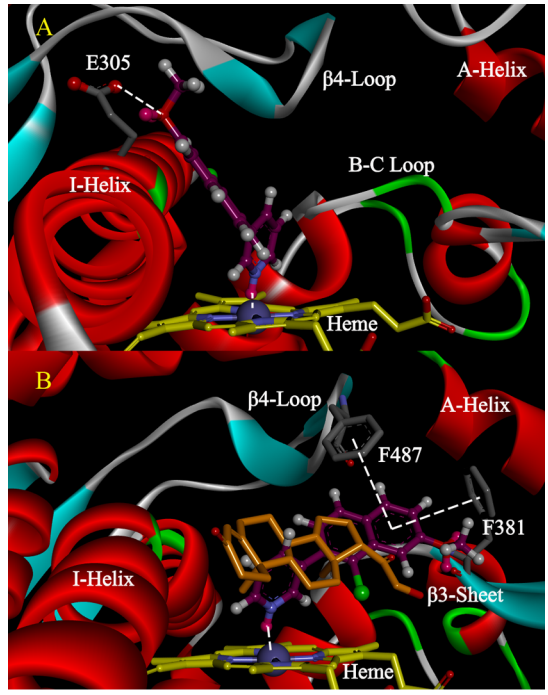


Figure 2. Comparison between different binding modes of **ref III** in CYP17 (**A**, PDB ID: 3RUK) and CYP11B2 (**B**, PDB ID: 4DVQ). **ref III** is depicted in purple, while deoxycorticosterone is in orange.

the heme in a nearly parallel way and stretches to the β3-sheet instead of leaning against the I-helix, as observed in CYP17. The naphthalene core forms $\pi-\pi$ interactions with Phe381 and Phe487 in perpendicular and parallel manners, respectively. The different roles of 1-Cl and 7-OMe in the binding to CYP17 and CYP11B2 are accordant with their different influence on

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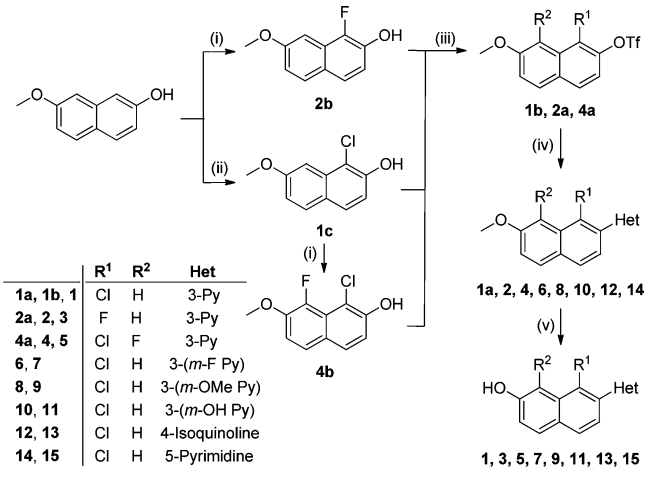
the inhibition of the corresponding enzymes. This comparison of the binding modes also provides further proof for the existence and importance of the H- and halogen bonds they formed in the CYP17 active site. Therefore, in the design of dual inhibitors, the OMe group was sustained or replaced by OH to form new H-bonds and to probe the possibly different protonation states of Glu305 (Chart 1). Since the core is close enough to the I-helix for F to form orthogonal multipolar interactions[15d] with the carbonyl of Gly301 and Ala302, as well as to form H-bonds with NH of Gly301 and Ala302 and probably OH of Thr306, F was considered as an alternative to Cl. Several modifications were therefore performed with Cl being maintained or replaced by F and further introduction of F into the 8-position (Chart 1). Moreover, the *m*-position of pyridyl points to a shallow cavity confined by Ala367, Val366, Val482, and Val483 (Figure 1B, where compound **10** exhibits the same binding mode as **ref III**). Since the backbones of these amino acid residues are accessible, F, OMe, OH, or an additional N was introduced into the *m*-position of the pyridyl moiety (Chart 1) to form H-bonds. A benzene nucleus was also fused to the pyridine to explore whether isoquinoline is tolerated. These efforts led to compounds **1**–**15**, which were tested for inhibition of CYP17 and CYP11B2, as well as for selectivity regarding the steroidogenic enzymes CYP11B1, CYP19, and the hepatic CYP3A4.

RESULTS AND DISCUSSION

Chemistry. The syntheses of compounds **1**–**15** followed the route shown in Scheme 1. The corresponding phenols **1c**, **2b**, and **4b** were treated with trifluoromethanesulfonic anhydride to give triflates **1b**, **2a**, and **4a**, which subsequently underwent Suzuki coupling with the corresponding boronic acids[16] to introduce the N containing heterocycles into the molecules, yielding compounds **2**, **4**, **6**, **8**, **10**, **12**, and **14**. The methoxy groups were then cleaved with boron tribromide to give the corresponding naphthols **1**, **3**, **5**, **7**, **9**, **11**, **13**, and **15**.

Scheme 1[a].



	R^1	R^2	Het
1a, 1b, 1	Cl	H	3-Py
2a, 2, 3	F	H	3-Py
4a, 4, 5	Cl	F	3-Py
6, 7	Cl	H	3-(*m*-F Py)
8, 9	Cl	H	3-(*m*-OMe Py)
10, 11	Cl	H	3-(*m*-OH Py)
12, 13	Cl	H	4-Isoquinoline
14, 15	Cl	H	5-Pyrimidine

[a]Reagents and conditions: (i) Method A: Selectfluor, acetonitrile, rt, 16 h; (ii) *N*-chlorosuccinimide, 1,2-dimethoxyethane, reflux, 3 h; (iii) Method B: pyridine, Tf_2O, dichloromethane, 0 °C to rt, 3 h; (iv) Method C: corresponding boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, H_2O, reflux, 8 h; (v) Method D: BBr_3, dichloromethane, −78 °C − rt, 16 h.

Cl or F substituents were site-selectively inserted at the start of the syntheses with either *N*-chlorosuccinimide or Selectfluor.

Inhibition of Human CYP17. The synthesized compounds were evaluated for inhibition of CYP17 using the 50,000*g* sediment of *E. coli* expressing human CYP17.[9a,17a,b] IC_{50} values are presented in comparison to abiraterone in Table 1. It is apparent that the replacement of 1-Cl by F led to a reduction of inhibitory potency. The Cl analogue **ref III** with a 7-OMe group exhibited an IC_{50} value of 27 nM, whereas the corresponding F compound **2** is 4-times weaker (IC_{50} = 106 nM). An additional F at the 8-position did not promote CYP17 inhibition either (compound **4**, IC_{50} = 147 nM). Similar results were observed for their 7-OH analogues **1**, **3**, and **5** (IC_{50} values of 64, 523, and 375 nM, respectively). This is probably due to the electron withdrawing effect of F reducing the electron density on the sp^2 hybrid N that is supposed to coordinate with the heme.

Moreover, substituents on the pyridyl ring showed significant influence on CYP17 inhibition as well. Although F, OMe, and OH at the *m*-position can probably form H-bonds and multipolar interactions with the backbones of the cavity, their different electrostatic and steric properties result in different inhibitory potencies. *m*-F substitution (**6**) decreased the inhibitory potency to 454 nM compared to the nonsubstituted **ref III** (IC_{50} = 27 nM), which might be due to its electron withdrawing effects reducing the electron density of the sp^2 hybrid N. In contrast, the electron donating group OH (**10**) increased the inhibition to 11 nM, whereas the OMe analogue **8** showed an IC_{50} value of 94 nM, which might be caused by steric effects in the shallow cavity. Since the electron density at the coordinating N is reduced by the additional N in the pyrimidine compound **12**, it is not surprising to observe a reduced potency of 632 nM. As for the isoquinoline analogue **14**, the additional benzene nucleus fused to the pyridine ring increases the electron density of the sp^2 hybrid N. However, the moderate activity (IC_{50} = 294 nM) indicates clashes with the binding cavity. The same structure activity relationship (SAR) was observed for the corresponding 7-OH analogues **7**, **9**, **11**, **13**, and **15**.

Furthermore, it is obvious that the 7-OMe compounds are more potent than the corresponding 7-OH analogues, e.g. **ref III** (IC_{50} = 27 nM) vs compound **1** (IC_{50} = 64 nM). The differences in potency can be as high as 5-fold (IC_{50} of 106 nM for compound **2** vs 523 nM for compound **3**). The *m*-OH pyridyl compound **11** with 7-OH, however, exhibited a similar potency as the 7-OMe analogue **10** (IC_{50} values of 16 and 11 nM, respectively). Both are more potent than abiraterone (IC_{50} = 72 nM). It is notable that the introduction of OH groups decreased lipophilicity by reducing clogP values from 4.93 (**ref III**) to 4.19 (compound **10**) and 3.26 (compound **11**).

Inhibition of Human CYP11B2. The synthesized compounds were also evaluated for their inhibitory activities in V79MZh cells expressing either human CYP11B1[10,17c] or CYP11B2,[11b,17c] and the results are presented in Table 1 with fadrozole as a reference. All compounds are very potent against CYP11B2, as expected (IC_{50} values ranging from 13 to 72 nM), with the different substituents showing little influence on CYP11B2 inhibition. This can be explained by the deoxycorticosterone-like binding of these compounds (the same binding mode as that of **ref III**), in which π–π interactions between pyridyl and Phe130 as well as between the naphthaline core and Phe381 and Phe487 play prominent roles (Figure 3, illustrated with compound **11**). Although the

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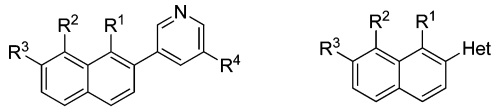
Table 1. Inhibition of CYP17, CYP11B2, CYP11B1, and CYP3A4 by Compounds Ref I–Ref III and 1–15



Ref I - Ref III, 1 - 11 12 - 15

	structures					IC_{50} (nM)[d]				
compd	R^1	R^2	R^3	R^4	Het	CYP17[a]	CYP11B2[b]	CYP11B1[c]	SF[f]	CYP3A4
ref I	H	H	H	H		3000 ± 159	28 ± 5	5826 ± 374	208	n.d.[f]
ref II	H	H	OMe	H		721 ± 46	68 ± 9	n.d.[f]	n.d.[f]	n.d.[f]
ref III	Cl	H	OMe	H		27 ± 3	29 ± 6	2724 ± 347	94	3560 ± 518
1	Cl	H	OH	H		64 ± 2	26 ± 3	235 ± 19	9	1759 ± 93
2	F	H	OMe	H		106 ± 13	17 ± 2	1609 ± 82	95	≫10000
3	F	H	OH	H		523 ± 21	30 ± 5	1159 ± 59	39	1262 ± 57
4	Cl	F	OMe	H		147 ± 11	28 ± 6	2935 ± 123	104	1009 ± 66
5	Cl	F	OH	H		375 ± 25	25 ± 3	224 ± 22	9	>10000
6	Cl	H	OMe	F		454 ± 39	38 ± 4	1415 ± 99	37	≫10000
7	Cl	H	OH	F		525 ± 36	33 ± 7	450 ± 29	15	3581 ± 269
8	Cl	H	OMe	OMe		94 ± 6	24 ± 2	447 ± 32	19	>10000
9	Cl	H	OH	OMe		185 ± 27	15 ± 3	245 ± 25	16	4196 ± 357
10	Cl	H	OMe	OH		11 ± 3	13 ± 2	7099 ± 331	546	6720 ± 519
11	Cl	H	OH	OH		16 ± 3	27 ± 4	2824 ± 153	104	5046 ± 375
12	Cl	H	OMe		5-Pyrim[f]	632 ± 57	72 ± 7	12286 ± 868	170	>10000
13	Cl	H	OH		5-Pyrim[f]	1520 ± 63	22 ± 3	2426 ± 109	110	2145 ± 197
14	Cl	H	OMe		4-Isoqu[f]	294 ± 27	54 ± 6	362 ± 33	7	>10000
15	Cl	H	OH		4-Isoqu[f]	1028 ± 53	66 ± 4	238 ± 19	4	1739 ± 153
ABT[f]						72 ± 6	1750 ± 136	1610 ± 125	1	2700 ± 207
FAD[f]						n.d.[e]	0.8 ± 0.2	6.3 ± 0.5	8	n.d.[f]

[a] *E. coli* expressing human CYP17; substrate: progesterone, 25 μM. [b] Hamster fibroblasts expressing human CYP11B2; substrate: deoxycorticosterone, 100 nM. [c] Hamster fibroblasts expressing human CYP11B1; substrate: deoxycorticosterone, 100 nM. [d] Mean value of at least three experiments, relative standard deviation less than 25%, $P < 0.001$. [e] less than 5% inhibition at 2000 nM. [f] n.d.: not determined; Pyrim: pyrimidine; Isoqu: isoquinoline; ABT: abiraterone; FAD: fadrozole; SF: selectivity factor = $IC_{50\ CYP11B1}/IC_{50\ CYP11B2}$.

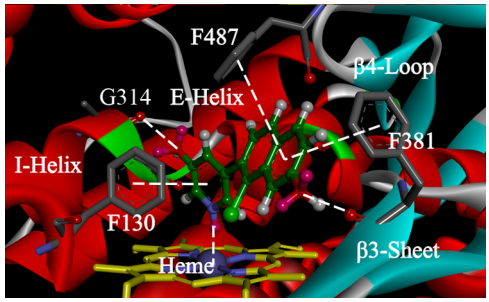


Figure 3. Binding of compound **11** in CYP11B2 (PDB ID: 4DVQ) and the interactions formed.

OH groups in compound **11** can still form H-bonds with the carbonyl oxygens of Gly314 and Phe381, they are less important than in CYP 17.

Dual Inhibitors of CYP17 and CYP11B2. This study was successful, leading to the identification of compounds **10** and **11** as potent dual inhibitors of CYP17/CYP11B2 with IC_{50} values of 11 ± 3/13 ± 2 nM and 16 ± 3/27 ± 4 nM, respectively, which are more potent than that of the parent compound **ref III** (27 ± 3/29 ± 6 nM).

Selectivity: Inhibition of Human CYP11B1. In contrast to CYP11B2 inhibition, the modifications had a strong impact on CYP11B1 inhibition, leading to huge differences in selectivity factors (SFs), ranging from 4 to 546. Contrary to the results observed with CYP17 inhibition, OH analogues are more potent than the corresponding OMe compounds toward CYP11B1. Furthermore, the replacement or addition of F on the core (**2**–**5**) did not reduce CYP11B1 inhibition. Neither did F or OMe substitution on the pyridyl moiety (**6**–**9**). In contrast, the *m*-OH group in compounds **10** and **11** strongly decreased CYP11B1 inhibition. As both compounds showed very strong CYP11B2 inhibition, excellent SFs of 546 and 104 have been achieved, which are clearly superior to that of fadrozole (SF = 8). Moreover, pyrimidine (**12** and **13**) reduced the inhibition of CYP11B1, whereas isoquinoline (**14** and **15**) enhanced it.

Selectivity: Inhibition of Human CYP19 and Hepatic CYP3A4. All compounds showed IC_{50} values of more than 2000 nM toward CYP19, in contrast to their potent inhibition of CYP17 and CYP11B2. Furthermore, most of the compounds showed no inhibition of CYP3A4 (IC_{50} > 5000 nM). The most potent dual inhibitors **10** and **11** exhibited IC_{50} values of 6720 and 5046 nM, respectively, thus showing a better profile than abiraterone (IC_{50} = 2700 nM) and **ref III** (IC_{50} = 3560 nM).

CONCLUSION

Although abiraterone as a CYP17 inhibitor significantly improves the survival of PCa patients, it is associated with CVD risks, which are probably caused by exorbitant aldosterone in cardiomyocytes as a consequence of androgen deficiency. We therefore propose dual inhibition of CYP17/CYP11B2 as a novel strategy to reduce CVD comorbidity and thus to further improve the quality of life and survival of PCa patients. Such multitargeting strategies have been proposed for other steroidogenic CYP enzymes to reduce CVD risks in

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breast cancer patients by dual inhibition of CYP19/CYP11B2[6c,d] and to prevent or delay relapse in PCa by dual inhibition of CYP17/CYP11B1.[9e] Administration of dual inhibitors is regarded to be advantageous compared to the application of two drugs in combination, as there is no risk of drug−drug interactions and a better compliance for the patients. On the basis of the observation that 1-Cl and 7-OMe groups in CYP11B2 inhibitors of the 2-(3-pyridyl)-naphthalene type are crucial for CYP17 inhibition, a combination of ligand- and structure-based approaches was employed, leading to the identification of novel dual CYP17/CYP11B2 inhibitors **10** and **11**. These compounds showed strong inhibition of both enzymes (IC_{50} values around 20 nM) and excellent selectivity over CYP11B1 (SFs of 546 and 104, respectively), CYP19, and CYP3A4. These dual inhibitors are more potent regarding CYP17 inhibition and more selective (CYP11B1 and CYP3A4) than the clinically used compound abiraterone, and thus, they may be devoid of some unwanted effects seen with the steroidal drug.

EXPERIMENTAL SECTION

Biological Tests. *CYP17 Preparation and Assay.* Human CYP17 and NADPH-P450 reductase were coexpressed in *E. coli*, and the assay was performed according to the previously described method with progesterone (25 μM) as the substrate and NADPH as the cofactor.[17a]

Inhibition of CYP11B1 and CYP11B2. V79MZh cells expressing human CYP11B1 or CYP11B2 were incubated with [1,2-^{3}H]-11-deoxycorticosterone (100 nM) as the substrate and the inhibitor at different concentrations. The assay was performed as previously described.[17b]

CYP19 Preparation and Assay. Human CYP19 was obtained from microsomal preparations of human placenta, and the assay was performed using the ^3H_2O-method as previously described with [1β-^{3}H]androstenedione (500 nM) as the substrate.[17c]

Inhibition of CYP3A4. The recombinantly expressed CYP3A4 enzyme from baculovirus-infected insect microsomes (Supersomes) was used, and the assay was performed according to the manufacturer's instructions (www.gentest.com).

Chemistry. *General Methods.* Melting points were determined on a Mettler FP1 melting point apparatus, and the values are uncorrected. ^{1}H NMR and ^{13}C 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. All coupling constants (J) are given in hertz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. The purities of the final compounds were controlled with a Surveyor-LC-system. Purities were greater than 95%. Column chromatography was performed using silica-gel 60 (50−200 μm), and reaction progress was monitored by TLC analysis on Alugram SIL G/UV254 (Macherey-Nagel). Commercially available reagents and solvents were used directly without further purification.

*1-Chloro-7-methoxynaphthalen-2-ol (**1c**).* The suspension of *N*-chlorosuccinimide (7.67 g, 56.26 mmol) and 7-methoxynaphthalen-2-ol (10.0 g, 56.26 mmol) in 1,2-dimethoxyethane (100 mL) was refluxed under N_2 for 3 h. After cooling down to room temperature, the solvent was removed under reduced pressure. The residue was redissolved in EtOAc (50 mL), washed with HCl (1 N, aq) for 3 times, dried over Na_2SO_4, and concentrated to yield the crude product. No further purification was performed. Yield: 8.02 g (67%); δ_H ($CDCl_3$, 500 MHz) 3.87 (s, 3H), 5.79 (s, 1H), 6.95 (dd, J = 2.5, 8.8 Hz, 1H), 7.01 (d, J = 8.8 Hz, 1H), 7.23 (d, J = 2.5 Hz, 1H), 7.52 (d, J = 8.8 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 55.4, 101.6, 112.5, 114.5, 116.7, 124.7, 128.1, 129.9, 132.5, 149.9, 159.3.

Method C: Suzuki-Coupling. The corresponding naphthalene triflate (1 equiv), boronic acid (1.5 equiv), and Na_2CO_3 (3 equiv) were suspended in toluene (20 mL) and H_2O (5 mL). The mixture was degassed under reduced pressure and flushed with N_2 before $Pd(PPh_3)_4$ (5 mol %) was added. The reaction mixture was heated under reflux for 8 h. After cooling down to room temperature, the phases were separated and the water phase was extracted twice with EtOAc. Then the combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give the crude products, which were subsequently purified with silica gel flash-chromatography.

*5-(1-Chloro-7-methoxynaphthalen-2-yl)pyridin-3-ol (**10**).* Synthesized from **1b** (1.50 g, 4.40 mmol) and 5-hydroxy-3-pyridinylboronic acid (1.01 g, 6.61 mmol) according to Method C; yield: 0.57 g (45%); white solid: mp 242−243 °C; R_f = 0.23 (DCM/MeOH, 20:1); ^{1}H NMR δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 3.91 (s, 3H), 7.16 (dd, J = 2.4, 8.9 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 2.4 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 8.9 Hz, 1H), 8.09 (d, J = 2.3 Hz, 2H); ^{13}C NMR δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 58.5, 106.1, 106.4, 122.9, 127.8, 127.8, 128.6, 129.9, 132.6, 132.9, 135.5, 137.4, 139.3, 143.7, 162.3; MS (ESI): m/z = 285.15 [M^+ + H].

Method D: Ether Cleavage with BBr_3. To a solution of the corresponding ether (0.5 mmol) in dichloromethane (5 mL), borontribromide in dichloromethane (1 M, 25 mmol) was added dropwise at −78 °C. After being warmed to room temperature, it was stirred overnight before quench with water. The resulted emulsion was stirred for a further 30 min before it was extracted with EtOAc for 3 times. The combined organic layers were subsequently washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give the crude product, which was further purified with flash-chromatography on silica gel.

*5-(1-Chloro-7-hydroxynaphthalen-2-yl)pyridin-3-ol (**11**).* Synthesized from **10** (2.5 g, 8.75 mmol) according to Method D; yield: 1.95 g (82%); white solid: mp 389−390 °C; R_f = 0.25 (DCM/MeOH, 10:1); δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 7.06 (d, J = 9.3 Hz, 1H), 7.08 (dd, J = 2.5, 8.8 Hz, 1H), 7.30−7.31 (m, 1H), 7.54 (d, J = 2.2 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.63 (d, J = 8.8 Hz, 1H), 8.09 (d, J = 1.5 Hz, 1H), 8.10 (d, J = 2.5 Hz, 1H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 106.6, 119.5, 124.6, 125.7, 126.9, 127.9, 128.8, 129.9, 132.6, 133.4, 135.7, 137.9, 140.4, 153.9, 156.6; MS (ESI): m/z = 271.93 [M^+ + H].

ASSOCIATED CONTENT

Supporting Information

The experimental details and characterization of the remaining and final products, IR data of compound **10** and **11**, HPLC purities of all final compounds, as well as docking studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

PCa, prostate cancer; ADT, androgen deprivation therapy; CVD, cardiovascular diseases; CYP, cytochrome P450; ref,

reference compound; CYP11B1, 11β-hydroxylase; CYP11B2, aldosterone synthase; CYP17, 17α-hydroxylase-17,20-lyase; CYP19, aromatase; SF, selectivity factor; EtOAc, ethyl acetate; equiv, equivalent

REFERENCES

(1) (a) Reid, A. H. M.; Attard, G.; Danila, D. C.; Oommen, N. B.; Olmos, D.; Fong, P. C.; Molife, L. R.; Hunt, J.; Messiou, C.; Parker, C.; Dearnaley, D.; Wennenhuis, J. F.; Terstappen, L. W. M. M.; Lee, G.; Kheoh, T.; Molina, A.; Ryan, C. J.; Small, E.; Scher, H. I.; de Bono, J. S. Significant and sustained antitumor activity in postdocetaxel, castration-resistant prostate cancer with the CYP17 inhibitor abiraterone acetate. *J. Clin. Oncol.* **2010**, *28*, 1489−1495. (b) Attard, G.; Reid, A. H. M.; Yap, T. A.; Raynaud, F.; Dowsett, M.; Settatree, S.; Barrett, M.; Parker, C.; Martins, V.; Folkerd, E.; Clark, J.; Cooper, C. S.; Kaye, S. B.; Dearnaley, D.; Lee, G.; de Bono, J. S. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. *J. Clin. Oncol.* **2008**, *26*, 4563−4571. (c) Yin, L.; Hu, Q. CYP17 inhibitors: from promiscuous abiraterone to selective C17-20 lyase inhibitors and multi-targeting agents. *Nat. Rev. Urol.* **2013**, in press. (d) Yin, L.; Hu, Q.; Hartmann, R. W. Recent progress in pharmaceutical therapy for castration-resistant prostate cancer. *Int. J. Mol. Sci.* **2013**, *14*, 13958−13978.

(2) Schwandt, A.; Garcia, J. A. Complications of androgen deprivation therapy in prostate cancer. *Curr. Opin. Urol.* **2009**, *19*, 322−326.

(3) (a) Sánchez-Más, J.; Turpín, M. C.; Lax, A.; Ruipérez, J. A.; Chávarri, M. V.; Pascual-Figal, D. A. Differential actions of eplerenone and spironolactone on the protective effect of testosterone against cardiomyocyte apoptosis in vitro. *Rev. Esp. Cardiol.* **2010**, *63*, 779−787. (b) Nakamura, S.; Yoshimura, M.; Nakayama, M.; Ito, T.; Mizuno, Y.; Harada, E.; Sakamoto, T.; Saito, Y.; Nakao, K.; Yasue, H.; Ogawa, H. Possible association of heart failure status with synthetic balance between aldosterone and dehydroepiandrosterone in human heart. *Circulation* **2004**, *110*, 1787−1793.

(4) (a) Traish, A. M.; Abdou, A.; Kypreos, K. E. Androgen deficiency and atherosclerosis: The lipid link. *Vascul. Pharmacol.* **2009**, *51*, 303−313. (b) Smith, M. R.; Finkelstein, J. S.; Mcgovern, F. J.; Zietman, A. L.; Fallon, M. A.; Schoenfeld, D. A.; Kantoff, P. W. Changes in body composition during androgen deprivation therapy for prostate cancer. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 599−603.

(5) (a) Kau, M.; Lo, M.; Wang, S.; Tsai, S.; Chen, J.; Chiao, Y.; Yeh, J.; Lin, H.; Shum, A. Y.; Fang, V. S.; Ho, L.; Wang, P. S. Inhibition of aldosterone production by testosterone in male rats. *Metabolism* **1999**, *48*, 1108−1114. (b) Shima, H.; Kawanaka, H.; Yabumoto, Y.; Okamoto, E.; Ikoma, F. A case of 17 alpha-hydroxylase deficiency with chromosomal karyotype 46, XY and high plasma aldosterone concentration. *Int. Urol. Nephrol.* **1991**, *23*, 611−618.

(6) (a) De Coster, R.; Mahler, C.; Denis, L.; Coene, M. C.; Caers, I.; Amery, W.; Haelterman, C.; Beerens, D. Effects of high-dose ketoconazole and dexamethasone on ACTH-stimulated adrenal steroidogenesis in orchiectomized prostatic cancer patients. *Acta Endocrinol.* **1987**, *115*, 265−271. (b) Braley, L. M.; Menachery, A. I.; Yao, T.; Mortensen, R. M.; Willams, G. H. Effect of progesterone on aldosterone secretion in rats. *Endocrinology* **1996**, *137*, 4773−4778. (c) Hu, Q.; Yin, L.; Hartmann, R. W. Selective dual inhibitors of CYP19 and CYP11B2: targeting cardiovascular diseases hiding in the shadow of breast cancer. *J. Med. Chem.* **2012**, *55*, 7080−7089. (d) Yin, L.; Hu, Q.; Hartmann, R. W. Tetrahydropyrroloquinolinone type dual inhibitors of aromatase/aldosterone synthase as a novel strategy for breast cancer patients with elevated cardiovascular risks. *J. Med. Chem.* **2013**, *56*, 460−470.

(7) (a) Nishikawa, T.; Suematsu, S.; Saito, J.; Soyama, A.; Ito, H.; Kino, T.; Chrousos, G. Human renal mesangial cells produce aldosterone in response to low-density lipoprotein (LDL). *J. Steroid Biochem. Mol. Biol.* **2005**, *96*, 309−316. (b) Saha, S.; Graessler, J.; Schwarz, P. E. H.; Goettsch, C.; Bornstein, S. R.; Kopprasch, S. Modified high-density lipoprotein modulates aldosterone release through scavenger receptors via extra cellular signal-regulated kinase and Janus kinase-dependent pathways. *Mol. Cell. Biochem.* **2012**, *366*, 1−10.

(8) (a) Mizuno, Y.; Yoshimura, M.; Yasue, H.; Sakamoto, T.; Ogawa, H.; Kugiyama, K.; Harada, E.; Nakayama, M.; Nakamura, S.; Ito, T.; Shimasaki, Y.; Saito, Y.; Nakao, K. Aldosterone production is activated in failing ventricle in humans. *Circulation* **2001**, *103*, 72−77. (b) Young, M. J.; Clyne, C. D.; Cole, T. J.; Funder, J. W. Cardiac steroidogenesis in the normal and failing heart. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 5121−5126.

(9) (a) Hu, Q.; Negri, M.; Olgen, S.; Hartmann, R. W. The role of fluorine substitution in biphenyl methylene imidazole type CYP17 inhibitors for the treatment of prostate carcinoma. *ChemMedChem* **2010**, *5*, 899−910. (b) Hu, Q.; Yin, L.; Jagusch, C.; Hille, U. E.; Hartmann, R. W. Isopropylidene substitution increases activity and selectivity of biphenyl methylene 4-pyridine type CYP17 inhibitors. *J. Med. Chem.* **2010**, *53*, 5049−5053. (c) Hu, Q.; Negri, M.; Jahn-Hoffmann, K.; Zhuang, Y.; Olgen, S.; Bartels, M.; Müller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. Synthesis, biological evaluation, and molecular modeling studies of methylene imidazole substituted biaryls as inhibitors of human 17α-hydroxylase-17,20-lyase (CYP17)-Part II: Core rigidification and influence of substituents at the methylene bridge. *Bioorg. Med. Chem.* **2008**, *16*, 7715−7727. (d) Hille, U. E.; Hu, Q.; Vock, C.; Negri, M.; Bartels, M.; Mueller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. Novel CYP17 inhibitors: Synthesis, biological evaluation, structure-activity relationships and modeling of methoxy- and hydroxy-substituted methyleneimidazolyl biphenyls. *Eur. J. Med. Chem.* **2009**, *44*, 2765−2775. (e) Hu, Q.; Jagusch, C.; Hille, U. E.; Haupenthal, J.; Hartmann, R. W. Replacement of imidazolyl by pyridyl in biphenyl methylenes results in selective CYP17 and dual CYP17/CYP11B1 inhibitors for the treatment of prostate cancer. *J. Med. Chem.* **2010**, *53*, 5749−5758. (f) Jagusch, C.; Negri, M.; Hille, U. E.; Hu, Q.; Bartels, M.; Jahn-Hoffmann, K.; Pinto-Bazurco Mendieta, M. A. E.; Rodenwaldt, B.; Müller-Vieira, U.; Schmidt, D.; Lauterbach, T.; Recanatini, M.; Cavalli, A.; Hartmann, R. W. Synthesis, biological evaluation and molecular modeling studies of methyleneimidazole substituted biaryls as inhibitors of human 17α-hydroxylase-17,20-lyase (CYP17)—Part I: heterocyclic modifications of the core structure. *Bioorg. Med. Chem.* **2008**, *16*, 1992−2010. (g) Pinto-Bazurco Mendieta, M. A. E.; Negri, M.; Hu, Q.; Hille, U. E.; Jagusch, C.; Jahn-Hoffmann, K.; Müller-Vieira, U.; Schmidt, D.; Lauterbach, T.; Hartmann, R. W. CYP17 inhibitors. Annulations of additional rings in methylene imidazole substituted biphenyls: synthesis, biological evaluation and molecular modeling. *Arch. Pharm. (Weinheim, Ger.)* **2008**, *341*, 597−609. (h) Hille, U. E.; Hu, Q.; Pinto-Bazurco Mendieta, M. A. E.; Bartels, M.; Vock, C. A.; Lauterbach, T.; Hartmann, R. W. Steroidogenic cytochrome P450 (CYP) enzymes as drug targets: Combining substructures of known CYP inhibitors leads to compounds with different inhibitory profile. *C. R. Chim.* **2009**, *12*, 1117−1126.

(10) (a) Yin, L.; Lucas, S.; Maurer, F.; Kazmaier, U.; Hu, Q.; Hartmann, R. W. Novel imidazol-1-ylmethyl substituted 1,2,5,6-tetrahydro-pyrrolo[3,2,1-ij]quinolin-4-ones as potent and selective CYP11B1 inhibitors for the treatment of Cushing's syndrome. *J. Med. Chem.* **2012**, *55*, 6629−6633. (b) Emmerich, J.; Hu, Q.; Hanke, N.; Hartmann, R. W. Cushing's syndrome: Development of highly potent and selective CYP11B1 inhibitors of the (pyridylmethyl)-pyridine type. *J. Med. Chem.* **2013**, DOI: 10.1021/jm400240r.

(11) (a) Voets, M.; Antes, I.; Scherer, C.; Müller-Vieira, U.; Biemel, K.; Barassin, C.; Marchais-Oberwinkler, S.; Hartmann, R. W. Heteroaryl-substituted naphthalenes and structurally modified derivatives: selective inhibitors of CYP11B2 for the treatment of congestive heart failure and myocardial fibrosis. *J. Med. Chem.* **2005**, *48*, 6632−6642. (b) Lucas, S.; Heim, R.; Ries, C.; Schewe, K. E.; Birk, B.; Hartmann, R. W. In vivo active aldosterone synthase inhibitors with improved selectivity: lead optimization providing a series of pyridine substituted 3,4-dihydro-1H-quinolin-2-one derivatives. *J. Med. Chem.* **2008**, *51*, 8077−8087. (c) Lucas, S.; Heim, R.; Negri, M.; Antes, I.;

Ries, C.; Schewe, K. E.; Bisi, A.; Gobbi, S.; Hartmann, R. W. Novel aldosterone synthase inhibitors with extended carbocyclic skeleton by a combined ligand-based and structure-based drug design approach. *J. Med. Chem.* **2008**, *51*, 6138−6149. (d) Gobbi, S.; Hu, Q.; Negri, M.; Zimmer, C.; Belluti, F.; Rampa, A.; Hartmann, R. W.; Bisi, A. Modulation of cytochromes P450 with xanthone-based molecules: from aromatase to aldosterone synthase and steroid 11β-hydroxylase inhibition. *J. Med. Chem.* **2013**, *56*, 1723−1729. (e) Roumen, L.; Peeters, J. W.; Emmen, J. M. A.; Beugels, I. P. E.; Custers, E. M. G.; de Gooyer, M.; Plate, R.; Pieterse, K.; Hilbers, P. A. J.; Smits, J. F. M.; Vekemans, J. A. J.; Leysen, D.; Ottenheijm, H. C. J.; Janssen, H. M.; Hermans, J. J. R. Synthesis, biological evaluation, and molecular modeling of 1-benzyl-1H-imidazoles as selective inhibitors of aldosterone synthase (CYP11B2). *J. Med. Chem.* **2010**, *53*, 1712−1725. (f) Heim, R.; Lucas, S.; Grombein, C. M.; Ries, C.; Schewe, K. E.; Negri, M.; Müller-Vieira, U.; Birk, B.; Hartmann, R. W. Overcoming undesirable CYP1A2 inhibition of pyridylnaphthalene-type aldosterone synthase inhibitors: influence of heteroaryl derivatization on potency and selectivity. *J. Med. Chem.* **2008**, *51*, 5064−5074. (g) Voets, M.; Antes, I.; Scherer, C.; Müller-Vieira, U.; Biemel, K.; Marchais-Oberwinkler, S.; Hartmann, R. W. Synthesis and evaluation of heteroaryl-substituted dihydronaphthalenes and indenes: potent and selective inhibitors of aldosterone synthase (CYP11B2) for the treatment of congestive heart failure and myocardial fibrosis. *J. Med. Chem.* **2006**, *49*, 2222−2231. (h) Yin, L.; Hu, Q.; Hartmann, R. W. 3-Pyridinyl substituted aliphatic cycles as CYP11B2 inhibitors: aromaticity abolishment of the core significantly increased selectivity over CYP1A2. *PLoS ONE* **2012**, *7* (11), e48048.

(12) (a) Gobbi, S.; Cavalli, A.; Negri, M.; Schewe, K. E.; Belluti, F.; Piazzi, L.; Hartmann, R. W.; Recanatini, M.; Bisi, A. Imidazolylmethylbenzophenones as highly potent aromatase inhibitors. *J. Med. Chem.* **2007**, *50*, 3420−3422. (b) Leze, M. P.; Le Borgne, M.; Pinson, P.; Palusczak, A.; Duflos, M.; Le Baut, G.; Hartmann, R. W. Synthesis and biological evaluation of 5-[(aryl)(1H-imidazol-1-yl)methyl]-1H-indoles: potent and selective aromatase inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1134−1137. (c) Abadi, A. H.; Abou-Seri, S. M.; Hu, Q.; Negri, M.; Hartmann, R. W. Synthesis and biological evaluation of imidazolylmethyl-acridones as cytochrome P-450 enzymes inhibitors. *MedChemComm* **2012**, *3*, 663−666. (d) Yin, L.; Hu, Q. Drug discovery for breast cancer and coinstantaneous cardiovascular disease: What is the future? *Future Med. Chem.* **2013**, *5*, 359−362.

(13) Picard, F.; Baston, E.; Reichert, W.; Hartmann, R. W. Synthesis of N-substituted piperidine-4-(benzylidene-4-carboxylic acids) and evaluation as inhibitors of steroid-5α-reductase type 1 and 2. *Bioorg. Med. Chem.* **2000**, *8*, 1479−1487.

(14) (a) Bey, E.; Marchais-Oberwinkler, S.; Negri, M.; Kruchten, P.; Oster, A.; Klein, T.; Spadaro, A.; Werth, R.; Frotscher, M.; Birk, B.; Hartmann, R. W. New Insights into the SAR and binding modes of bis(hydroxyphenyl)thiophenes and -benzenes: influence of additional substituents on 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) inhibitory activity and selectivity. *J. Med. Chem.* **2009**, *52*, 6724−6743. (b) Bey, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Werth, R.; Oster, A.; Algül, O.; Neugebauer, A.; Hartmann, R. W. Design, synthesis and biological evaluation of bis(hydroxyphenyl) azoles as potent and selective non-steroidal inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) for the treatment of estrogen-dependent diseases. *Bioorg. Med. Chem.* **2008**, *16*, 6423−6435.

(15) (a) DeVore, N. M.; Scott, E. E. Structures of cytochrome P450 17A1 with prostate cancer drugs abiraterone and TOK-001. *Nature* **2012**, *482*, 116−120. (b) Strushkevich, N.; Gilep, A. A.; Shen, L.; Arrowsmith, C. H.; Edwards, A. M.; Usanov, S. A.; Park, H. W. Structural insights into aldosterone synthase substrate specificity and targeted inhibition. *Mol. Endocrinol.* **2013**, *27*, 315−324. (c) Wilcken, R.; Zimmermann, M. O.; Lange, A.; Joerger, A. C.; Boeckler, F. M. Principles and applications of halogen bonding in medicinal chemistry and chemical biology. *J. Med. Chem.* **2013**, *56*, 1363−1388. (d) Müller, K.; Faeh, C.; Diederich, F. Fluorine in pharmaceuticals: looking beyond intuition. *Science* **2007**, *317*, 1881−1886.

(16) Ohe, T.; Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reaction of organoboron compounds with organic triflates. *J. Org. Chem.* **1993**, *58*, 2201−2208.

(17) (a) Ehmer, P. B.; Jose, J.; Hartmann, R. W. Development of a simple and rapid assay for the evaluation of inhibitors of human 17α-hydroxylase-C(17,20)-lyase (P450c17) by coexpression of P450c17 with NADPH-cytochrome-P450-reductase in Escherichia coli. *J. Steroid Biochem. Mol. Biol.* **2000**, *75*, 57−63. (b) Ehmer, P. B.; Bureik, M.; Bernhardt, R.; Müller, U.; Hartmann, R. W. Development of a test system synthase (CYP11B2): Screening in fission yeast and evaluation of selectivity in V79 cells. *J. Steroid Biochem. Mol. Biol.* **2002**, *81*, 173−179. (c) Hartmann, R. W.; Batzl, C. Aromatase inhibitors. Synthesis and evaluation of mammary tumor inhibiting activity of 3-alkylated 3-(4-aminophenyl)piperidine-2,6-diones. *J. Med. Chem.* **1986**, *29*, 1362−1369.