Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Selective Dual Inhibitors of CYP19 and CYP11B2: Targeting Cardiovascular Diseases Hiding in the Shadow of Breast Cancer

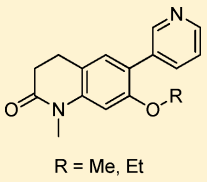
Qingzhong Hu,*[,†] Lina Yin,[†,‡] and Rolf W. Hartmann*[,†]

[†]Pharmaceutical and Medicinal Chemistry, Saarland University & Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus C2-3, P.O. Box 151150, D-66123 Saarbrücken, Germany

[‡]ElexoPharm GmbH, Campus A1, D-66123 Saarbrücken, Germany

Supporting Information

ABSTRACT: Postmenopausal women are at high risk for cardiovascular diseases because of the estrogen deficiency. As for postmenopausal breast cancer patients, this risk is even higher due to inhibition of estrogens biosyntheses in peripheral tissue by the aromatase (CYP19) inhibitors applied. Because estrogen deficiency results in significantly elevated aldosterone levels, which are a major cause of cardiovascular diseases, dual inhibition of CYP19 and CYP11B2 (aldosterone synthase) is a promising treatment for breast cancer and the coinstantaneous cardiovascular diseases. By combination of important structural features of known CYP19 and CYP11B2 inhibitors, we succeeded in obtaining compounds **3** and **5** as selective dual inhibitors with IC_{50} values around 50 and 20 nM toward CYP19 and CYP11B2, respectively. These compounds showed also good selectivity toward CYP11B1 (selectivity factors ($IC_{50\ CYP11B1}/IC_{50\ CYP11B2}$) around 50) and CYP17 (no inhibition).



R = Me, Et

INTRODUCTION

Breast cancer (BC) is the carcinoma with the highest morbidity in females in the western countries. Although BC is still the second leading cause of death, the mortality is significantly reduced because of the cancer screening to identify cases in early stages[1] and, more importantly, the employment of adjuvant endocrine therapy. Endocrine therapy is based on the fact that estrogen stimulates the growth of "hormone sensitive" breast cancer, in which estrogen receptor (ER) and/or progesterone receptor (PgR) are expressed.[2] Therefore, deprivation of estrogens is a feasible treatment for the hormone sensitive BC, which accounts for more than 60% of all cases. Since the late 1970s, selective estrogen receptor modulators (SERMs),[3] such as Tamoxifen and Raloxifen (Chart 1), were introduced into the clinic. These SERMs competitively bind to ER inside breast cancer tissue, inhibiting transcription and the subsequent mitogenic effects. However, the unsatisfying risk/benefit profiles prevented Tamoxifen from being applied for more than five years, and severe toxicities such as endometrial cancer and thrombosis were observed.[4] On the contrary, the third-generation aromatase inhibitors (AIs), for example, Anastrozole, Letrozole, and Exemestane (Chart 1), exhibited better efficacy and tolerability compared to Tamoxifen, which rendered AIs to become the first choice as adjuvant therapeutics for postmenopausal women, the majority of breast cancer patients. Aromatase (CYP19) is the crucial enzyme catalyzing the final aromatization of the steroidal A-ring in the biosynthesis of estrogens from corresponding androgen precursors: testosterone and androstenedione. Inhibition of CYP19 can totally block estrogen production and consequently prevent BC cells from proliferation. After administration of the third-generation AIs, plasma estrogen concentration can be reduced to undetectable level.[5] Several clinical trials demonstrated AIs as adjuvant therapeutics, significantly improving disease-free and relapse-free survival with the overall survival increased accordingly.[6]

However, it has been unveiled that only around 40% of BC patients' deaths are eventually caused by BC.[7] A lot of people survive BC but die of other medical conditions, especially cardiovascular diseases (CVD).[7] Therefore, there is an urgent need to manage CVD in order to further improve the overall survival.

Recently, estrogens have been proven to exhibit protective effects on heart[8] and kidney.[9] The administration of estrogens prevents the development of heart failure postmyocardial infarction[8b] and attenuates ventricular hypertrophy and remodelling.[8c,d] Moreover, the incidences of CVD in postmenopausal women triple those of premenopausal women at the same age,[10] indicating that low estrogen levels are closely correlated with CVD. Regarding postmenopausal BC patients under endocrine therapy, AIs further decrease estrogen formation, leading to an even higher risk of CVD.[11] The ischemic side effects observed in clinical trials with AIs were considered as results of lipid metabolism dysfunction caused by estrogen deficiency[11c] and can be managed with antihyperlipidemic agents. However, the up-regulation of the renin−angiotensin−aldosterone system (RAAS) during this pathological procedure, especially aldosterone, was still neglected. It has been showed that the depletion of estrogens not only directly increases circulating aldosterone levels, but also augments the concentrations of other RAAS components[12] (Chart 2). Elevated levels of renin, angiotensin II (Ang II), angiotensin converting enzyme (ACE), and angiotensin type 1

Received: April 3, 2012
Published: August 3, 2012

Chart 1. Structures of Selective Estrogen Receptor Modulators (Tamoxifen and Raloxifen) and Aromatase Inhibitors (Anastrozole, Letrozole, Fadrozole, and Exemestane)

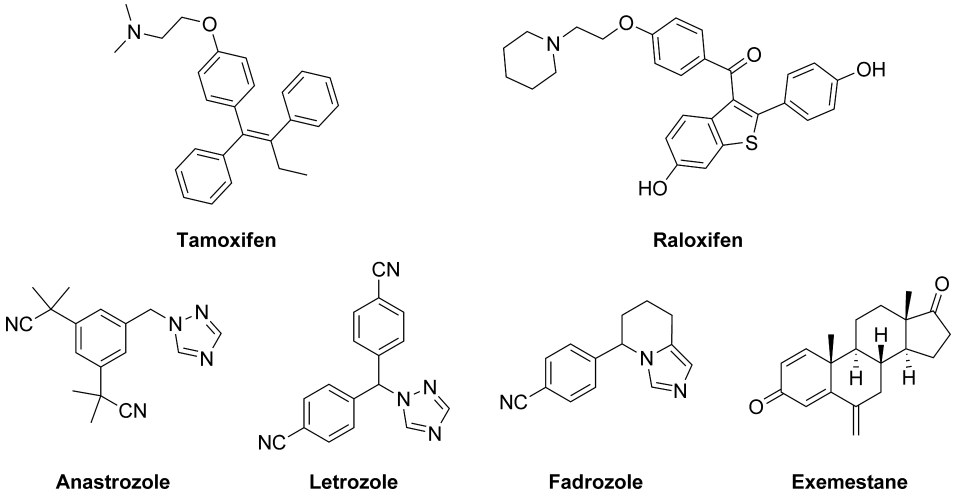
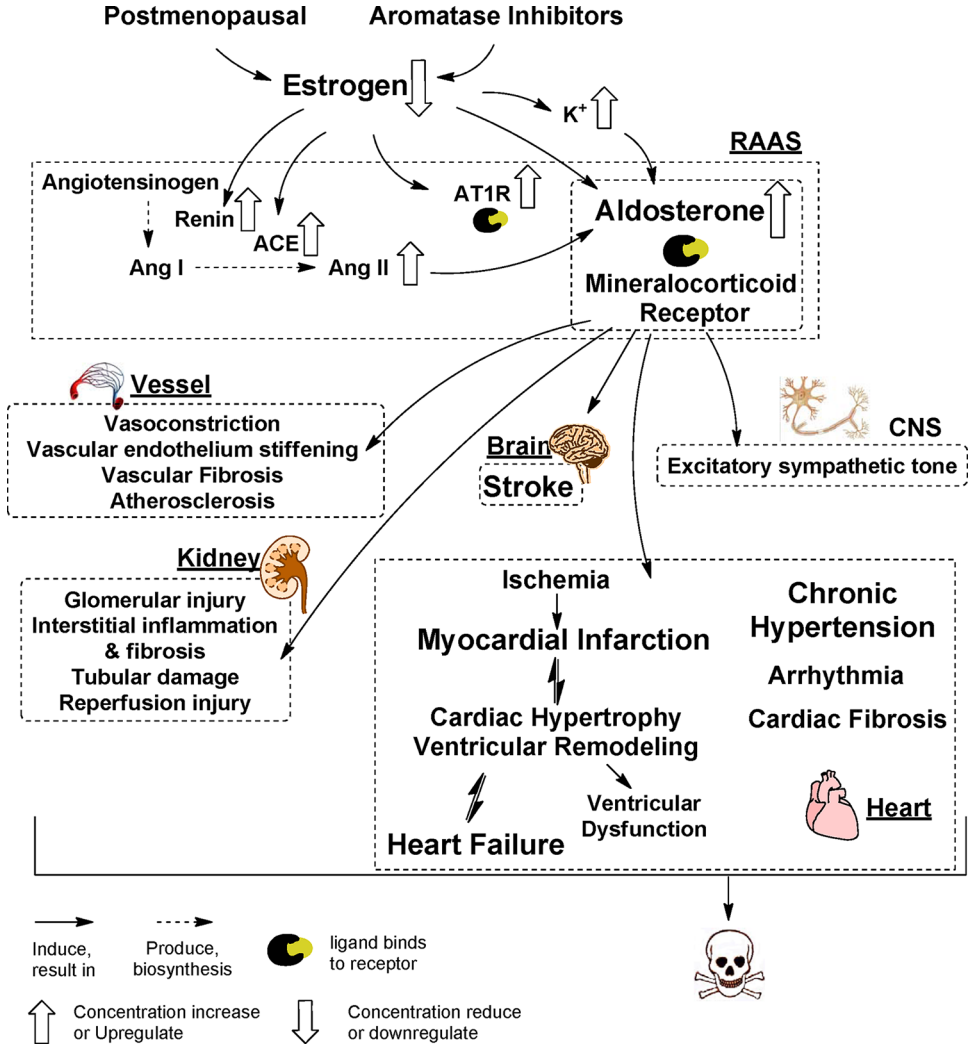


Chart 2. Deleterious Effects of High Aldosterone Levels Resulting from Estrogen Deficiency

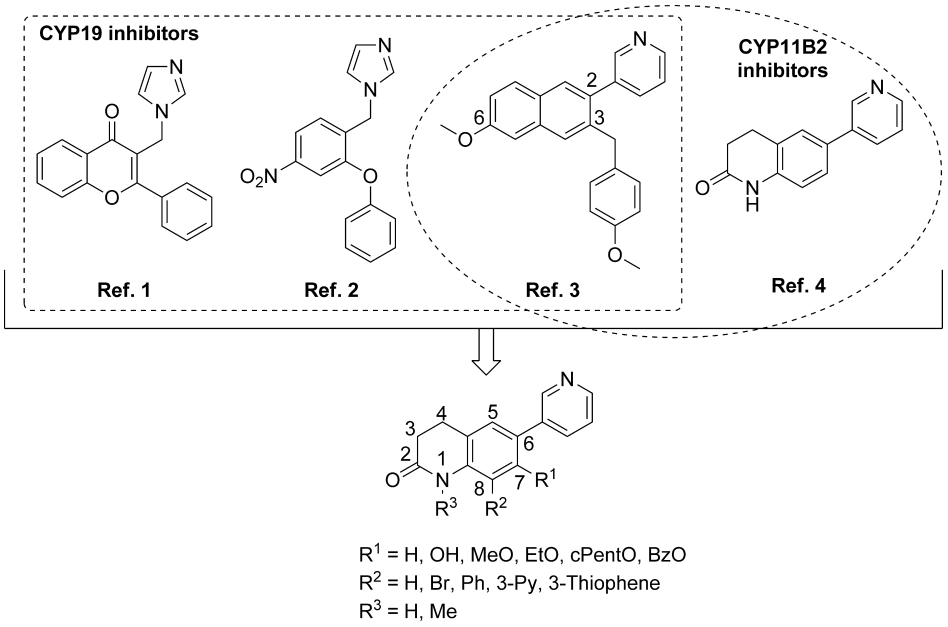


receptor (AT1R) further stimulate aldosterone biosynthesis.[13] Moreover, estrogen deficiency also increases the potassium plasma concentration, resulting in an enhancement of aldosterone secretion.[14]

The resulting high aldosterone levels exhibit deleterious effects on kidney, vessels, brain, and most severe on heart[15] (Chart 2, for detailed description and the corresponding references, see Supporting Information). Therefore, reducing

dx.doi.org/10.1021/jm3004637 | J. Med. Chem. 2012, 55, 7080−7089

Chart 3. Design of Heterocyclic Dihydroquinolinones As Dual CYP19/CYP11B2 Inhibitors



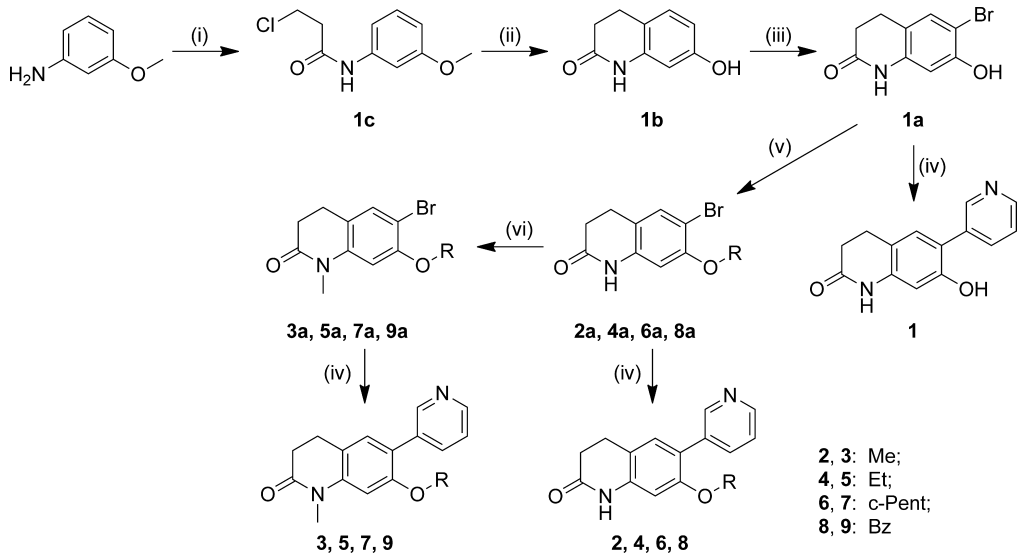
the high plasma aldosterone concentration rendered by estrogen deficiency should be an effective treatment for cardiovascular conditions in BC patients. Although ACE inhibitors and/or MR antagonists, which have been implemented in the clinic for a long time, could be options for combinatory application with AIs to meet this need, they are not the best choice due to the side effects observed and the phenomenon of "aldosterone escape". Because aldosterone synthase (CYP11B2) catalyzing the conversion of 11-deoxycorticosterone to aldosterone is the key enzyme in aldosterone biosynthesis, the inhibition of CYP11B2 is the method of choice to reduce aldosterone levels. Because AIs as adjuvant therapeutics have to be applied for at least five years and there are no selective CYP11B2 inhibitors in clinical use until now, it is our aim to design dual inhibitors of CYP19 and CYP11B2, which are selective to other steroidogenic CYP enzymes like CYP17 and CYP11B1. It is not possible to adjust individually the suppression degree of estrogens and aldosterone productions with dual inhibitors, which projects the demands of careful balancing on the inhibition toward both enzymes for the drug candidates. When doing so, the exposure of the inhibitors to both enzymes should also be taken into consideration because CYP19 is expressed in endoplasmic reticulum whereas CYP11B2 is located in mitochondria. Despite the potential issues to be addressed, the benefits of dual CYP19 and CYP11B2 inhibitors are apparent. This kind of multitarget-directed agents[16,17] should not only improve patients' compliance but should also avoid possible drug–drug interactions caused by combinative application. Furthermore, accurate designation of the suitable patient cohort at the drug design stage by narrowing down the patient scope from whole population to the most promising ones to response can probably improve the performances and outcomes of the drug candidates in clinical trials. In this study, important structural features of CYP19 inhibitors were incorporated into CYP11B2 inhibitors. Thus, a series of pyridyl dihydroquinolinone derivatives were designed and synthesized, leading to compounds **1**–**13**. The inhibition of these compounds toward CYP11B2 and CYP19 are presented with the unselective CYP19 inhibitor Fadrozole (Chart 1) as a reference. Furthermore, the selectivity of these compounds against 11β-hydroxylase (CYP11B1) and 17α-hydroxylase-17,20-lyase (CYP17), which are the crucial enzymes in the biosynthesis of glucocorticoids and androgens, respectively, were also determined as safety criteria.

Design Conception for Dual Inhibitors. Besides Anastrozole and Letrozole, the CYP19 inhibitors in clinical use, a lot of potent nonsteroidal CYP19 inhibitors have been designed and synthesized.[18] Almost all of these inhibitors coordinate with their sp^2 hybrid nitrogen to the heme iron, leading to tight binding and reversible inhibition of this enzyme.[18a] Because all CYP enzymes contain a heme iron as the catalytic center, inhibitors toward other steroidogenic enzymes, such as CYP17,[19] CYP11B2,[20] and CYP11B1,[21] have also been designed based on this mechanism.

Two potent CYP19 inhibitors have been employed in the design of the compounds presented in this investigation (Chart 3), Ref. **1** ($IC_{50\ CYP19}$ = 71 nM)[18b] with a phenyl group furnishing the flavone scaffold, and Ref. **2** ($IC_{50\ CYP19}$ = 12 nM),[18c] with a phenyl core substituted by a phenoxy group. Both compounds possess an imidazolylmethyl moiety adjacent to the bulky phenyl or phenoxy substituents. Three important structural features can be noticed in these two potent CYP19 inhibitors: a heterocycle providing a sp^2 hybrid N (imidazolyl), an aromatic core (chromone or phenyl), and a bulky hydrophobic substituent nearby. Besides, the groups furnishing the core are also important for CYP19 inhibition. This was demonstrated by Ref. **3** (Chart 3),[20b] which is the only compound exhibiting potent CYP19 inhibition ($IC_{50\ CYP19}$ = 38 nM) in a series of CYP11B2 inhibitors, despite all compounds in that series containing the structural features described above. This inhibition is considered to be rendered by the methoxy substitution at the 6 position of the naphthalene core.[20b] Accordingly, we selected 6-(pyridin-3-yl)-3,4-dihydroquinolin-2(1*H*)-one (Ref. **4**, Chart 3)[20a] as the template for CYP11B2 inhibition to design dual inhibitors of CYP19 and CYP11B2, because Ref. **4** is already composed of two structural features important for CYP19 inhibition: the 3-pyridyl as N containing

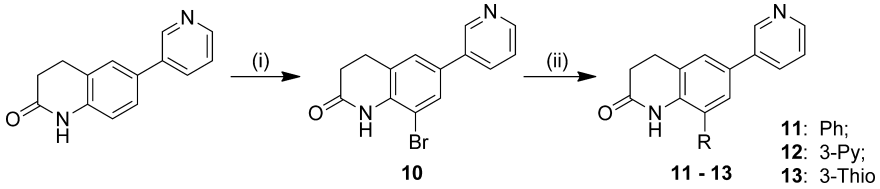
dx.doi.org/10.1021/jm3004637 | J. Med. Chem. 2012, 55, 7080–7089

Scheme 1[a]



[a]Reagents and conditions: (i) method A, 3-chloropropanoyl chloride, pyridine, THF, rt; (ii) method B, $AlCl_3$, DMA, 140 °C, 4 h; (iii) method C,: NBS, DMF, 0 °C, 9 h; (iv) method D, $Pd(OAc)_2$, corresponding boronic acid, Na_2CO_3, TBAB, toluene, H_2O, ethanol, reflux, 6 h; (v) method E, corresponding halide, K_2CO_3, KI, EtOH, 70 °C, overnight; (vi) method F, MeI, KOt-Bu, DMF, 50 °C, overnight.

Scheme 2[a]



[a]Reagents and conditions: (i) method G, NBS, DMF, 65 °C, 6 h; (ii) method D, $Pd(OAc)_2$, corresponding boronic acid, Na_2CO_3, TBAB, toluene, H_2O, ethanol, reflux, 6 h.

heterocycle and the dihydroquinolinone as aromatic core. Moreover, the presence of a ketone group mimicking the 6-methoxy substituent of Ref. **3** as hydrogen bond acceptor fulfilled another precondition of potent CYP19 inhibition. Because Ref. **4** is a highly potent and selective CYP11B2 inhibitor ($IC_{50\ CYP11B2}$ = 28 nM) showing no inhibition of CYP19, the desired CYP19 inhibition could probably be acquired after introduction of the third structural feature into this template while its CYP11B2 activity and selectivity over CYP11B1 and CYP17 should be maintained. Therefore, hydrophobic groups, such as alkoxyl, halogen, and aryl in various sizes, were introduced into the 7- or 8-position of the core. For substituents at the 7-position, an oxygen was exploited as a linker, similar to that of Ref. **2**, because of our previous observation that bulky groups adjacent to the pyridyl substituent significantly reduce CYP11B2 inhibition.[20a] Furthermore, methylation of the amide was also performed to explore the role of the NH proton.

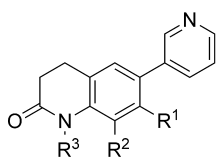
RESULTS AND DISCUSSION

Chemistry. The synthesis of compounds **1**−**13** is shown in Schemes 1−2. First, the substituted 3,4-dihydroquinolin-2(1*H*)-one core **1b** was built from the corresponding methoxy aniline after amidation with 3-chloropropanoyl chloride, followed by cyclization via Friedel−Craft alkylation in the presence of *N*,*N*-dimethylacetamide (DMA) to suppress side reactions. During the Friedel−Craft alkylation, the methoxy group was cleaved simultaneously to afford the hydroxy substituent. Second, 6-position specific bromination was achieved with *N*-bromosuccinimide (NBS), which was followed by Suzuki coupling with 3-pyridyl boronic acids, leading to compound **1**. Further derivatives **2**−**9** were obtained after alkylation of the phenol or the amide, which were performed before the final step of Suzuki coupling. As for 8-substituted analogues, 6-(pyridin-3-yl)-3,4-dihydroquinolin-2(1*H*)-one was prepared similarly as described above as a common intermediate, which after 8-position specific bromination gave compound **10**. Further aryl groups were subsequently introduced via another Suzuki coupling reaction resulting in the desired compounds **11**−**13**.

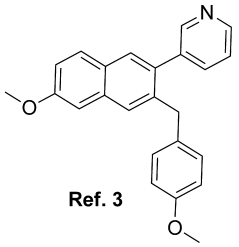
Inhibition of CYP19, CYP11B2, and CYP11B1. Inhibitory activity of the synthesized compounds toward CYP19 was determined using human placental microsomes with [1β-^{3}H]-androstenedione as substrate,[22d] whereas V79MZh cells expressing human CYP11B2 were employed for the CYP11B2 assay with [^{14}C]-11-deoxycorticosterone as substrate.[22c] Because CYP11B1 and CYP11B2 exhibit more than 93% of homology and the inhibition of CYP11B1 results in cortisol deficiency, the selectivity toward CYP11B1 was also examined for safety evaluation using a similar procedure as described for CYP11B2, except for V79MZh cells expressing human CYP11B1 as source of enzyme.[22c] The IC_{50} values of the synthesized compounds toward these three enzymes are presented in Table 1 with the reference Fadrozole.

dx.doi.org/10.1021/jm3004637 | J. Med. Chem. 2012, 55, 7080−7089

Table 1. Inhibition of CYP11B1, CYP11B2, and CYP19 by Compounds 1−13



1 - 13, Ref. 4, Ref. 5



Ref. 3

compd	R^1	R^2	R^3	CYP19 IC_{50} nM[b,d]	CYP11B2 IC_{50} nM[b,c]	CYP11B1 IC_{50} nM[b,c]	SF[e]
Ref. **4**	H	H	H	>5000	28	6747	241
Ref. **5**[20a]	H	H	Me	426	2.6	742	289
1	OH	H	H	3073	312	11940	38
2	OMe	H	H	447	268	2867	9
3	OMe	H	Me	49	19	1098	59
4	OEt	H	H	488	674	3737	5.5
5	OEt	H	Me	48	19	790	41
6	O-*c*-Pent	H	H	162	1703	1077	0.6
7	O-*c*-Pent	H	Me	22	759	1167	1.5
8	OBz	H	H	35	22	44	2
9	OBz	H	Me	11	178	139	0.8
10	H	Br	H	2901	12	1422	120
11	H	Ph	H	394	1167	16170	13.9
12	H	3-Py	H	954	1097	4087	3.7
13	H	3-Thienyl	H	275	208	4589	22
Ref. **3**				38	11	4329	394
FDZ[a]				52	0.8	6.3	8.3

[a]FDZ: Fadrozole. [b]Concentration of inhibitors required to give 50% inhibition. Standard deviations were within < ±5%; all the data are the mean values of at least three tests. [c]Hamster fibroblasts expressing human CYP11B1 or CYP11B2 are used with deoxycorticosterone as the substrate at a concentration of 100 nM. [d]Human placental CYP19 is used with androstenedione as the substrate at a concentration of 500 nM. [e]SF: selective factor = $IC_{50\ CYP11B1}/IC_{50\ CYP11B2}$.

It can be seen that compared to Ref. **4** ($IC_{50\ CYP11B2}$ = 28 nM and $IC_{50\ CYP19}$ > 5000 nM), the hydroxy substitution at the 7-position (compound **1**) slightly improved CYP19 inhibition but decreased CYP11B2 inhibitory potency to about 300 nM and selectivity factor over CYP11B1 (SF, $IC_{50\ CYP11B1}/IC_{50\ CYP11B2}$) to 38. After alkylation of the hydroxy group with subnstituents of different sizes, strong effects were observed. With increasing bulkiness of the substituents, the inhibition of the corresponding compounds toward CYP19 rose from 450−500 nM (compounds **2** and **4** with methoxy and ethoxy, respectively) to 162 nM (compound **6** with cyclopentyloxy), and finally to 35 nM (compound **8** with benzyloxy). In parallel, the inhibitory activities of these compounds toward CYP11B2 decreased, the methoxy compound **2** exhibiting an IC_{50} value of 268 nM, while 673 nM for the ethoxy compound **4** and 1700 nM for the cyclopentyloxy compound **6**. Surprisingly, the benzyloxy analogue **8** turned out to be a potent CYP11B2 inhibitor with an IC_{50} value of 22 nM, probably due to a different binding mode. The inhibition of CYP11B1 followed the same trend. However, it seemed that CYP11B1 is less sensitive to an increase in bulkiness compared to CYP11B2. Accordingly, significant reduction of selectivity was observed with SFs of less than 10 for these compounds. However, methylation of the amide N dramatically improved the inhibition of CYP19 and CYP11B2 as well as slightly increased CYP11B1 inhibition. It is apparent that after amide methylation, the methoxy (**3**) and ethoxy (**5**) analogues exhibited 10-fold more potent inhibition of CYP19 than their parent compounds (**2** and **4**), with IC_{50} values around 50 nM. The inhibition of CYP11B2 was also strongly increased to IC_{50} values below 20 nM. It is important that the selectivity over CYP11B1 of these compounds was enhanced to 59 and 41, respectively. Accordingly, by a combination of introducing small alkoxy groups into the 7-position and amide methylation, the desired dual inhibitors of CYP19/11B2 were successfully obtained with compounds **3** and **5**, showing good selectivity over CYP11B1. As for more bulky substituents like cyclopentyloxy (compound **7**), the promotion of CYP11B2 inhibition rendered by amide methylation could not neutralize the deterioration from the increase of bulkiness. As the inhibition of CYP19 was increased to 22 nM, this compound turned out to be a selective CYP19 inhibitor with selectivity of 35 and 53 ($IC_{50\ CYP19}/IC_{50\ CYP11B2\ or\ CYP11B1}$) over CYP11B2 and CYP11B1, respectively. As for the benzyloxy analogues **8** and **9**, which were believed to bind in a different mode to the two CYP11Bs, the amide methylation significantly decreased CYP11B inhibition to around 150 nM. Because compound **9** showed potent inhibition of CYP19 (IC_{50} = 11 nM), it has to be considered as a selective CYP19 inhibitor, showing selectivities around 15-fold toward CYP11B2 and CYP11B1.

Furthermore, the influence of substituents at the 8-position was also investigated. The bromo analogue **10** turned out to be a potent and selective CYP11B2 inhibitor, exhibiting an IC_{50} value of 11 nM and a SF of 120 over CYP11B1. However, this compound showed only weak inhibition of CYP19 (IC_{50} = 2900 nM). The introduction of aryl groups (**17** with phenyl and **18** with 3-pyridyl) increased CYP19 inhibition, with the potency toward CYP11B2 reduced accordingly. It is notable

dx.doi.org/10.1021/jm3004637 | J. Med. Chem. 2012, 55, 7080−7089

that unlike its bioisostere phenyl, the 3-thienyl group with its smaller size rendered compound **19** as a modest dual inhibitor of CYP19/11B2 (IC_{50} values of 275 and 208 nM, respectively), with a fair SF of 22 over CYP11B1.

Selectivity over CYP17. The inhibition of CYP17 by the synthesized compounds was also determined as a criterion to evaluate safety because this enzyme is crucial in the biosynthesis of androgens. It turned out that all compounds exhibited IC_{50} values of more than 10000 nM (data not shown), indicating no inhibition of CYP17.

Selectivity over Hepatic CYP3A4. Because CYP3A4 is responsible for the metabolism of a large percentage of drugs and xenobiotics and therefore is a key factor in drug−drug interactions, compounds **3** and **5** were tested for the inhibition against CYP3A4. Both compounds showed IC_{50} values around 90 μM, which grants the compounds a wide safety window.

Solubility in Water. Solubility is always an important issue in drug discovery and development because low solubility not only limits oral absorption and reduces bioavailability but also is an obstacle to formulation. Therefore, the solubility of selected compounds **3**, **5**, **7**, and **10** in water with 2% of DMSO were determined at 25 °C. Compounds **3**, **5**, and **10** showed solubility of 145.5, 136.7, and 177.3 μM, respectively, whereas the cyclopentyloxy analogue **7** is less soluble (76.8 μM). The solubility can be further improved simply by forming salts due to the presence of pyridine moiety.

CONCLUSIONS

Although AIs as BC therapeutics are successful by reducing estrogen concentrations to prevent BC cell proliferation, the estrogen deficiency they result in together with the postmenopausal status of the patients results in elevations of aldosterone levels, which sometimes are not detected in the plasma but are intracellularly in the myocardia and other target cells. The abnormally high aldosterone levels exert deleterious effects on kidney, vessels, brain, and most severe on the myocardia. These damages result in asymptomatic left ventricular dysfunction and may exist long before the clinical syndrome appears and sudden death occurs. Therefore, it is an urgent need to find an appropriate way to cope with BC and the coinstantaneous cardiovascular diseases. Because AIs as adjuvant therapeutics have to be administered for a long time, the compliance of the patients is another advantage of dual inhibitors of CYP19 and CYP11B2, besides the reduction of drug−drug interactions risks, which possibly happen when administrating in combination.

The approach of combining important structural features of CYP19 and CYP11B2 inhibitors to design dual inhibitors has been demonstrated to be successful in this study. Structural modifications exhibit different effects on the SARs toward the different target enzymes. It has been found that the increase of bulkiness of the alkoxy groups at the 7-position strongly elevated CYP19 inhibition. However, it also reduced CYP11B2 inhibition and selectivity over CYP11B1. On the contrary, methylation of the amide N led to an increase of inhibition toward all of these three enzymes. After balancing the different effects of the substituents on the SARs, compounds **3** and **5** were obtained as selective dual inhibitors with IC_{50} values around 50 and 20 nM toward CYP19 and CYP11B2, respectively, similar to that of Fadrozole. These compounds also showed a better selectivity compared to Fadrozole against CYP11B1, with selectivity factors around 50 and no inhibition of CYP17. Also, in this study, compound **7** was identified as a selective CYP19 inhibitor with an IC_{50} value of 22 nM and selectivity factors of more than 35 for other enzymes, as well as compound **10** as a selective CYP11B2 inhibitor (IC_{50} = 12 nM), with selectivity factors of more than 120 over other enzymes. The dual inhibitors **3** and **5** are promising lead compounds to be further developed for treating BC patients with risk of CVD.

EXPERIMENTAL SECTION

Inhibition of CYP11B1 and CYP11B2. V79MZh cells expressing human CYP11B1 or CYP11B2[22e] were incubated with [^{14}C]-11-deoxycorticosterone as substrate. The assay was performed as previously described.[22c]

Inhibition of CYP19. The inhibition of CYP19 was determined in vitro using human placental microsomes with [1β-^{3}H]androstenedione as substrate.[22d]

Inhibition of CYP17. Human CYP17 was expressed in *Escherichia coli*[22b] (coexpressing human CYP17 and NADPH-P450 reductase), and the assay was performed with progesterone as substrate at high concentrations of 25 μM as previously described.[22a]

Chemistry. *General.* Melting points were determined on a Mettler FP1 melting point apparatus and are uncorrected. IR spectra were recorded neat on a Bruker Vector 33FT-infrared spectrometer. ^{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 Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. The purities of the final compounds were controlled by a Surveyor-LC system. Purities were greater than 95%. 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).

Method A: Amidation. To the corresponding aniline (1.0 equiv) solution in dry THF (10 mL/mmmol) was added pyridine (1.5 equiv) before the mixture was cooled to 0 °C in an ice bath. Then 3-chloropropanoyl chloride (1.1 equiv) was dropped in carefully. After the addition, the reaction mixture was warmed to ambient temperature and stirred overnight. THF was then removed by reduced pressure, and the residues were taken up with ethyl acetate (10 mL) and water (10 mL). After extraction of the water phase three times with ethyl acetate (10 mL), the organic phases were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give the crude product. The compounds were then purified by flash chromatography on silica gel.

*3-Chloro-N-(3-methoxy-phenyl)-propionamide (**1c**).* Synthesized according to method A using 3-methoxyaniline (0.50 g, 4.06 mmol) and 3-chloropropanoyl chloride (0.62 g, 4.87 mmol). This intermediate was used directly in the next step without further purification and characterization

Method B: Friedel−Craft alkylation in DMA. To the cold mixture of corresponding 3-chloro-*N*-phenylpropanamides (1.0 equiv) and $AlCl_3$ (5.0 equiv) was dropped in DMA (1.0 equiv). Then the reaction mixture was heated to 140 °C for 4 h. After cooling down to ambient temperature, the black mixture was worked up with ethyl acetate (20 mL) and ice water (20 mL). After extraction of the water phase three times with ethyl acetate (10 mL), the organic phases were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give the crude product. The compounds were then purified by flash chromatography on silica gel.

*7-Hydroxy-3,4-dihydro-1H-quinolin-2-one (**1b**).* Synthesized according to method B using **1c** (0.55 g, 2.57 mmol). This intermediate was used directly in the next step without further purification and characterization.

Method C: Selective Bromination at 6-Position. To the solution of corresponding dihydroquinolinone (1.0 equiv) in DMF (5 mL/mmol) cooling in an ice bath was dropped in NBS (1.1 equiv) solution in DMF (2 mL/mmol). The addition lasted for 3 h, and then the reaction mixture was hold at 0 °C for another 6 h before extraction

dx.doi.org/10.1021/jm3004637 | J. Med. Chem. 2012, 55, 7080−7089

with ethyl acetate (20 mL) and water (20 mL). After extraction of the water phase three times with ethyl acetate (10 mL), the organic phases were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give the crude product. The compounds were then purified by flash chromatography on silica gel.

*6-Bromo-7-hydroxy-3,4-dihydro-1H-quinolin-2-one (**1a**).* Synthesized according to method C using **7b** (0.47 g, 2.88 mmol) and NBS (0.51 g, 2.88 mmol); yield 0.61 g (87%); white solid; R_f = 0.25 (DCM/MeOH, 20:1); δ_H ($CDCl_3$, 500 MHz) 2.38 (t, J = 7.5 Hz, 2H, CH_2), 2.75 (t, J = 7.7 Hz, 2H, CH_2), 6.53 (s, 1H), 7.24 (s, 1H), 10.05 (s, br, 1H, NH); MS (ESI): m/z = 242 [M^+ + H].

Method D: Suzuki Coupling. The corresponding brominated aromatic compound (1.0 equiv) was dissolved in toluene (7 mL/mmol), and an aqueous 2.0 M Na_2CO_3 solution (3.2 mL/mmol), an ethanolic solution (3.2 mL/mmol) of the corresponding boronic acid (1.5−2.0 equiv), and tetrabutylammonium bromide (1.0 equiv) were added. The mixture was deoxygenated under reduced pressure and flushed with nitrogen. After having repeated this cycle several times, $Pd(OAc)_2$ (5 mol %) was added and the resulting suspension was heated under reflux for 2−6 h. After cooling, ethyl acetate (10 mL) and water (10 mL) were added and the organic phase was separated. The water phase was extracted with ethyl acetate (2 × 10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered over a short plug of Celite, and evaporated under reduced pressure. The compounds were purified by flash chromatography on silica gel.

*7-Hydroxy-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**1**).* Synthesized according to method D using **1a** (0.35 g, 1.45 mmol) and pyridin-3-ylboronic acid (0.21 g, 1.74 mmol); yield 0.11 g (32%); white solid; mp 297−298 °C; R_f = 0.19 (DCM/MeOH, 20:1); δ_H (DMSO, 500 MHz) 2.44 (t, J = 7.9 Hz, 2H, CH_2), 2.81 (t, J = 7.9 Hz, 2H, CH_2), 6.55 (s, 1H), 7.13 (s, 1H), 7.37 (ddd, J = 0.7, 4.9, 8.0 Hz, 1H), 7.89 (dt, J = 2.2, 7.9 Hz, 1H), 8.43 (dd, J = 1.5, 4.7 Hz, 1H), 8.69 (d, J = 1.8 Hz, 1H), 9.68 (s, br, 1H, NH), 10.10 (s, br, 1H, OH); MS (ESI): m/z = 241 [M^+ + H].

*7-Methoxy-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**2**).* Synthesized according to method D using **2a** (0.35 g, 1.37 mmol) and pyridin-3-ylboronic acid (0.16 g, 1.64 mmol); yield 0.30 g (85%); white solid; mp 221−222 °C; R_f = 0.13 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 2.68 (t, J = 7.9 Hz, 2H, CH_2), 2.97 (t, J = 7.9 Hz, 2H, CH_2), 3.81 (s, 3H, OCH_3), 6.45 (s, 1H), 7.11 (s, 1H), 7.31 (ddd, J = 0.7, 5.4, 7.9 Hz, 1H), 7.81 (dt, J = 2.0, 7.9 Hz, 1H), 8.52 (dd, J = 1.6, 4.7 Hz, 1H), 8.63 (s, br, 1H, NH), 8.72 (d, J = 1.7 Hz, 1H); MS (ESI): m/z = 255 [M^+ + H].

*7-Methoxy-1-methyl-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**3**).* Synthesized according to method D using **3a** (0.50 g, 1.85 mmol) and pyridin-3-ylboronic acid (0.22 g, 2.22 mmol); yield 0.46 g (93%); white solid; mp 207−208 °C; R_f = 0.19 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 2.62 (t, J = 7.9 Hz, 2H, CH_2), 2.83 (t, J = 7.9 Hz, 2H, CH_2), 3.35 (s, 3H, NCH_3), 3.79 (s, 3H, OCH_3), 6.56 (s, 1H), 7.01 (s, 1H), 7.25 (ddd, J = 0.7, 5.3, 8.3 Hz, 1H), 7.76 (dt, J = 2.0, 8.0 Hz, 1H), 8.46 (dd, J = 1.6, 4.8 Hz, 1H), 8.68 (d, J = 1.7 Hz, 1H); MS (ESI): m/z = 269 [M^+ + H].

*7-Ethoxy-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**4**).* Synthesized according to method D using **4a** (0.35 g, 1.30 mmol) and pyridin-3-ylboronic acid (0.19 g, 1.55 mmol); yield 0.31 g (89%); white solid; mp 206−207 °C; R_f = 0.13 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 1.34 (t, J = 6.9 Hz, 3H, CH_3), 2.67 (t, J = 7.9 Hz, 2H, CH_2), 2.96 (t, J = 7.9 Hz, 2H, CH_2), 4.01 (q, J = 6.9 Hz, 2H, OCH_2), 3.35 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 6.49 (s, 1H), 7.10 (s, 1H), 7.31 (ddd, J = 0.7, 4.8, 7.8 Hz, 1H), 7.84 (dt, J = 1.8, 7.9 Hz, 1H), 8.51 (d, J = 3.8 Hz, 1H), 8.75 (s, 1H), 9.00 (s, br, 1H, NH); MS (ESI): m/z = 269 [M^+ + H].

*7-Ethoxy-1-methyl-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**5**).* Synthesized according to method D using **5a** (0.50 g, 1.76 mmol) and pyridin-3-ylboronic acid (0.26 g, 2.11 mmol); yield 0.43 g (86%); white solid; mp 217−218 °C; R_f = 0.20 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 1.35 (t, J = 6.9 Hz, 3H, CH_3), 2.66 (t, J = 7.9 Hz, 2H, CH_2), 2.88 (t, J = 7.9 Hz, 2H, CH_2), 3.38 (s, 3H, NCH_3), 4.04 (q, J = 6.9 Hz, 2H, OCH_2), 6.61 (s, 1H), 7.11 (s, 1H), 7.30 (ddd, J = 0.7, 4.9, 7.9 Hz, 1H), 7.84 (dt, J = 2.0, 7.9 Hz, 1H), 8.50 (dd, J = 1.6, 4.8 Hz, 1H), 8.76 (d, J = 1.7 Hz, 1H); MS (ESI): m/z = 283 [M^+ + H].

*7-Cyclopentyloxy-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**6**).* Synthesized according to method D using **6a** (0.35 g, 1.13 mmol) and pyridin-3-ylboronic acid (0.17 g, 1.35 mmol); yield 0.29 g (83%); white solid; mp 181−182 °C; R_f = 0.15 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 1.52−1.89 (m, 8H, c-Pent), 2.66 (t, J = 7.9 Hz, 2H, CH_2), 2.94 (t, J = 7.9 Hz, 2H, CH_2), 4.73 (sept, J = 2.8 Hz, 1H, OCH), 6.50 (s, 1H), 7.10 (s, 1H), 7.28 (ddd, J = 0.7, 4.7, 7.8 Hz, 1H), 7.90 (dt, J = 1.9, 7.9 Hz, 1H), 8.50 (d, J = 4.5 Hz, 1H), 8.71 (d, J = 1.4 Hz, 1H),8.98 (s, br, 1H, NH); MS (ESI): m/z = 309 [M^+ + H].

*7-Cyclopentyloxy-1-methyl-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**7**).* Synthesized according to method D using **7a** (0.50 g, 1.54 mmol) and pyridin-3-ylboronic acid (0.23 g, 1.85 mmol); yield 0.42 g (85%); colorless oil; R_f = 0.23 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 1.52−1.89 (m, 8H, c-Pent), 2.67 (t, J = 7.9 Hz, 2H, CH_2), 2.88 (t, J = 7.9 Hz, 2H, CH_2), 3.38 (s, 3H, NCH_3), 4.75 (sept, J = 2.8 Hz, 1H, OCH), 6.62 (s, 1H), 7.11 (s, 1H), 7.29 (ddd, J = 0.7, 5.3, 7.9 Hz, 1H), 7.81 (dt, J = 1.9, 7.9 Hz, 1H), 8.50 (dd, J = 1.5, 4.8 Hz, 1H), 8.72 (d, J = 1.8 Hz, 1H); MS (ESI): m/z = 322 [M^+ + H].

*7-Benzyloxy-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**8**).* Synthesized according to method D using **8a** (0.35 g, 1.05 mmol) and pyridin-3-ylboronic acid (0.16 g, 1.26 mmol); yield 0.30 g (86%); white solid; mp 189−190 °C; R_f = 0.17 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 2.67 (t, J = 7.9 Hz, 2H, CH_2), 2.97 (t, J = 7.9 Hz, 2H, CH_2), 5.06 (s, 2H, bz CH_2), 6.50 (s, 1H), 7.14 (s, 1H), 7.26−7.35 (m, 6H, bz), 7.86 (dt, J = 1.8, 8.0 Hz, 1H), 8.47 (s, br, 1H, NH), 8.50 (dd, J = 1.5, 4.8 Hz, 1H), 8.76 (d, J = 1.7 Hz, 1H); MS (ESI): m/z = 331 [M^+ + H].

*7-Benzyloxy-1-methyl-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**9**).* Synthesized according to method D using 9**a** (0.50 g, 1.44 mmol) and pyridin-3-ylboronic acid (0.21 g, 1.73 mmol); yield 0.45 g (90%); white solid; mp 209−210 °C; R_f = 0.23 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 2.67 (t, J = 7.9 Hz, 2H, CH_2), 2.89 (t, J = 7.9 Hz, 2H, CH_2), 3.33 (s, 3H, NCH_3), 5.09 (s, 2H, bz CH_2), 6.67 (s, 1H), 7.14 (s, 1H), 7.29−7.38 (m, 6H, bz), 7.87 (dt, J = 1.9, 8.0 Hz, 1H), 8.53 (dd, J = 1.6, 4.9 Hz, 1H), 8.78 (d, J = 1.8 Hz, 1H); MS (ESI): m/z = 345 [M^+ + H].

*8-Phenyl-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**11**).* Synthesized according to method D using **10** (0.35 g, 1.15 mmol) and phenylboronic acid (0.17 g, 1.38 mmol); yield 0.30 g (86%); white solid; mp 171−172 °C; R_f = 0.25 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 2.71 (t, J = 7.9 Hz, 2H, CH_2), 3.12 (t, J = 7.9 Hz, 2H, CH_2), 7.34−7.36 (m, 2H), 7.38−7.46 (m, 4H), 7.49−7.53 (m, 3H), 7.86 (dt, J = 1.9, 7.9 Hz, 1H), 8.57 (dd, J = 1.5, 4.7 Hz, 1H), 8.84 (d, J = 2.1 Hz, 1H); MS (ESI): m/z = 301 [M^+ + H].

*6,8-Dipyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**12**).* Synthesized according to method D using **10** (0.35 g, 1.15 mmol) and pyridin-3-ylboronic acid (0.17 g, 1.38 mmol); yield 0.29 g (83%); white solid; mp 177−178 °C; R_f = 0.19 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 2.73 (t, J = 7.9 Hz, 2H, CH_2), 3.14 (t, J = 7.9 Hz, 2H, CH_2), 7.33−7.38 (m, 2H), 7.44−7.47 (m, 2H), 7.74 (dt, J = 1.8, 7.9 Hz, 1H), 7.85 (dt, J = 2.2, 8.4 Hz, 1H), 8.59 (dd, J = 1.5, 4.8 Hz, 1H), 8.68 (d, J = 2.3 Hz, 1H), 8.71 (dd, J = 1.6, 4.8 Hz, 1H), 8.84 (d, J = 1.8 Hz, 1H); MS (ESI): m/z = 302 [M^+ + H].

*6-Pyridin-3-yl-8-thiophen-3-yl-3,4-dihydro-1H-quinolin-2-one (**13**).* Synthesized according to method D using **10** (0.47 g, 1.55 mmol) and thiophen-3-ylboronic acid (0.24 g, 1.86 mmol); yield 0.39 g (83%); white solid; mp 235−236 °C; R_f = 0.35 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 2.71 (t, J = 7.9 Hz, 2H, CH_2), 3.11 (t, J = 7.9 Hz, 2H, CH_2), 7.18 (dd, J = 1.2, 4.9 Hz, 1H), 7.34−7.38 (m, 2H), 7.40 (s, 2H), 7.52 (dd, J = 4.8, 4.9 Hz, 1H), 7.66 (s, br, 1H, NH), 7.85 (dt, J = 1.8, 8.0 Hz, 1H), 8.58 (dd, J = 1.5, 4.9 Hz, 1H), 8.83 (d, J = 2.1 Hz, 1H); MS (ESI): m/z = 307 [M^+ + H].

Method E: Alkylation of Phenol. The suspension of K_2CO_3 (2.0 equiv) and 6-bromo-7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (1.0 equiv) in dry ethanol (5 mL/mmol) was refluxed for 2 h before KI (0.05 equiv) and the corresponding halide (2.0 equiv) were added. Then the white suspension was boiled overnight. After cooling down

dx.doi.org/10.1021/jm3004637 | J. Med. Chem. 2012, 55, 7080−7089

to ambient temperature, ethanol was removed with reduced pressure and the residue was taken up with ethyl acetate (20 mL) and water (20 mL). After extraction of the water phase three times with ethyl acetate (10 mL), the organic phases were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give the crude product. The compounds were then purified by flash chromatography on silica gel.

Method F: Alkylation of Amide. The suspension of potassium *t*-butanolate (2.0 equiv) and corresponding dihydroquinolinone (1.0 equiv) in dry DMF (5 mL/mmol) was heated to 50 °C for 2 h before KI (0.05 equiv) and the iodomethane (2.0 equiv) were added. Then the white suspension was boiling overnight. After cooling down to ambient temperature, the reaction mixture was diluted with ethyl acetate (20 mL) and water (20 mL). After extraction of the water phase three times with ethyl acetate (10 mL), the organic phases were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give the crude product. The compounds were then purified by flash chromatography on silica gel.

Method G: Selective Bromination at 8-Position. To the solution of corresponding dihydroquinolinone (1.0 equiv) in DMF (5 mL/mmol) was dropped in NBS (1.1 equiv) solution in DMF (2 mL/mmol) at 65 °C. The addition lasted for 3 h, and then the reaction mixture was hold at 65 °C for another 3 h before extraction with ethyl acetate (20 mL) and water (20 mL). After extraction of the water phase three times with ethyl acetate (10 mL), the organic phases were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give the crude product. The compounds were then purified by flash chromatography on silica gel.

*8-Bromo-6-pyridin-3-yl-3,4-dihydro-1H-quinolin-2-one (**10**).* Synthesized according to method G using 6-(pyridin-3-yl)-3,4-dihydroquinolin-2(1H)-one (0.50 g, 2.23 mmol) and NBS (0.40 g, 2.23 mmol); yield 0.61 g (90%); white solid; mp 175−176 °C; R_f = 0.25 (DCM/MeOH, 50:1); δ_H ($CDCl_3$, 500 MHz) 2.70 (t, J = 7.9 Hz, 2H, CH_2), 3.09 (t, J = 7.9 Hz, 2H, CH_2), 7.35−7.37 (m, 2H), 7.64 (d, J = 1.8 Hz, 1H), 7.81 (dt, J = 1.8, 7.9 Hz, 1H), 7.84 (s, br, 1H, NH), 8.61 (dd, J = 1.5, 5.4 Hz, 1H), 8.79 (d, J = 1.8 Hz, 1H); MS (ESI): m/z = 303 [M^+ + H].

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The detailed description of deleterious effects of excessive aldosterone; the synthetic procedures and characterization of rest intermediates as well as the ^{13}C NMR spectra and HPLC purities of all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*For R.W.H.: phone, +(49) 681 302 70300; fax, +(49) 681 302 70308; E-mail, rolf.hartmann@helmholtz-hzi.de; web site, http://www.helmholtz-hzi.de/?id=3897. For Q.H.: E-mail, q.hu@mx.uni-saarland.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Dr. Christina Zimmer, Jeannine Jung, and Jannine Ludwig for the help in determination of bioactivity, and we thank Professor J. Hermans (Cardiovascular Research Institute, University of Maastricht, The Netherlands) for providing us with V79MZh11B cells expressing human CYP11B1.

■ ABBREVIATIONS USED

BC, breast cancer; CYP, cytochrome P450; ER, estrogen receptor; PgR, progesterone receptor; SERM, selective estrogen receptor modulator; AI, aromatase inhibitor; CPY19, aromatase, estrogen synthase; CVD, cardiovascular diseases; RAAS, renin−angiotensin−aldosterone system; Ang II, angiotensin II; ACE, angiotensin converting enzyme; AT1R, angiotensin type 1 receptor; CYP11B2, aldosterone synthase; MR, mineralocorticoid receptor; CYP17, steroid 17α-hydroxylase-17,20-lyase; CYP11B1, steroid 11β-hydroxylase; DMA, *N*, *N*-dimethylacetamide; NBS, *N*-bromosuccinimide; SF, selectivity factor

■ REFERENCES

(1) Puliti, D.; Miccinesi, G.; Collina, N.; De Lisi, V.; Federico, M.; Ferretti, S.; Finarelli, A. C.; Foca, F.; Mangone, L.; Naldoni, C.; Petrella, M.; Ponti, A.; Segnan, N.; Sigona1, A.; Zarcone, M.; Zorzi, M.; Zappa, M.; Paci, E. The IMPACT Working Group. Effectiveness of service screening: a case-control study to assess breast cancer mortality reduction. *Br. J. Cancer* **2008**, *99*, 423−427.

(2) Miller, W. R. Endocrine treatment for breast cancers: biological rationale and current progress. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 467−480.

(3) Wang, T.; You, Q.; Huang, F. S.; Xiang, H. Recent advances in selective estrogen receptor modulators for breast cancer. *Mini-Rev. Med. Chem.* **2009**, *9*, 1191−1201.

(4) Perez, E. A. Safety profiles of tamoxifen and the aromatase inhibitors in adjuvant therapy of hormone-responsive early breast cancer. *Ann. Oncol.* **2007**, *18*, viii26−viii35.

(5) (a) Jurgen, G.; Ben, H; Gun, A.; Mitch, D.; Per Eystein, L. Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study. *J. Clin. Oncol.* **2002**, *20*, 751−757. (b) Jurgen, G.; Nick, K.; Gun, A.; Giorgio, O.; Enrico, D. S.; Per Eystein, L.; Mitch, D. In vivo inhibition of aromatization by exemestane, a novel irreversible aromatase inhibitor, in postmenopausal breast cancer patients. *Clin. Cancer Res.* **1998**, *4*, 2089−2093.

(6) (a) Thurlimann, B.; Keshaviah, A.; Coates, A. S.; Mouridsen, H.; Mauriac, L.; Forbes, J. F.; Paridaens, R.; Castiglione-Gertsch, M.; Gelber, R. D.; Rabaglio, M.; Smith, I.; Wardley, A.; Price, K. N.; Goldhirsch, A. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *N. Engl. J. Med.* **2005**, *353*, 2747−2757. (b) Jakesz, R.; Jonat, W.; Gnant, M.; Mittlboeck, M.; Greil, R.; Tausch, C.; Hilfrich, J.; Kwasny, W.; Menzel, C.; Samonigg, H.; Seifert, M.; Gademann, G.; Kaufmann, M.; Wolfgang, J. Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial. *Lancet* **2005**, *366*, 455−462. (c) Goss, P. E.; Ingle, J. N.; Martino, S.; Robert, N. J.; Muss, H. B.; Piccart, M. J.; Castiglione, M.; Tu, D.; Shepherd, L. E.; Pritchard, K. I.; Livingston, R. B.; Davidson, N. E.; Norton, L.; Perez, E. A.; Abrams, J. S.; Cameron, D. A.; Palmer, M. J.; Pater, J. L. Efficacy of Letrozole extended adjuvant therapy according to estrogen receptor and progesterone receptor status of the primary tumor: National Cancer Institute of Canada Clinical Trials Group MA.17. *J. Clin. Oncol.* **2007**, *25*, 2006−2011.

(7) Chapman, J. W.; Meng, D.; Shepherd, L.; Parulekar, W.; Ingle, J. N.; Muss, H. B.; Palmer, M.; Yu, C.; Goss, P. E. Competing causes of death from a randomized trial of extended adjuvant endocrine therapy for breast cancer. *J. Natl. Cancer Inst.* **2008**, *100*, 252−260.

(8) (a) Wang, Y.; Wang, Q.; Zhao, Y.; Gong, D.; Wang, D.; Li, C.; Zhao, H. Protective effects of estrogen against reperfusion arrhythmias following severe myocardial ischemia in rats. *Circ. J.* **2010**, *74*, 634−643. (b) Beer, S.; Reincke, M.; Kral, M.; Callies, F.; Ströer, H.; Dienesch, C.; Steinhauer, S.; Ertl, G.; Allolio, B.; Neubauer, S. High-dose 17β-estradiol treatment prevents development of heart failure post-myocardial infarction in the rat. *Basic Res. Cardiol.* **2007**, *102*, 9−18. (c) Gardner, J. D.; Murray, D. B.; Voloshenyuk, T. G.; Brower, G. L.; Bradley, J. M.; Janicki, J. S. Estrogen attenuates chronic volume overload induced structural and functional remodeling in male rat hearts. *Am. J. Physiol., Heart Circ. Physiol.* **2010**, *298*, H497−H504.

(d) Donaldson, C.; Eder, S.; Baker, C.; Aronovitz, M. J.; Weiss, A. D.; Hall-Porter, M.; Wang, F.; Ackerman, A.; Karas, R. H.; Molkentin, J. D.; Patten, R. D. Estrogen attenuates left ventricular and cardiomyocyte hypertrophy by an estrogen receptor-dependent pathway that increases calcineurin degradation. *Circ. Res.* **2009**, *104*, 265−275.

(9) Arias-Loza, P. A.; Muehlfelder, M.; Elmore, S. A.; Maronpot, R.; Hu, K.; Blode, H.; Hegele-Hartung, C.; Fritzemeier, K. H.; Ertl, G.; Pelzer, T. Differential effects of 17β-estradiol and of synthetic progestins on aldosterone−salt-induced kidney disease. *Toxicol. Pathol.* **2009**, *37*, 969−982.

(10) (a) American Heart Association. *Heart Disease and Stroke Statistics: 2004 Update*; American Heart Association: Dallas, TX, 2003. (b) Witteman, J. C.; Grobbee, D. E.; Kok, F. J.; Hofman, A.; Valkenburg, H. A. Increased risk of atherosclerosis in women after the menopause. *Br. Med. J.* **1989**, *298*, 642−644.

(11) (a) Ewer, M. S.; Glück, S. A woman's heart. The impact of adjuvant endocrine therapy on cardiovascular health. *Cancer* **2009**, *115*, 1813−1826. (b) Healey Bird, B. R. J.; Swain, S. M. Cardiac toxicity in breast cancer survivors: review of potential cardiac problems. *Clin. Cancer Res.* **2008**, *14*, 14−24. (c) Bundred, N. J. The effects of aromatase inhibitors on lipids and thrombosis. *Br. J. Cancer* **2005**, *93*, S23−S27.

(12) (a) Macova, M.; Armando, I.; Zhou, J.; Baiardi, G.; Tyurmin, D.; Larrayoz-Roldan, I. M.; Saavedra, J. M. Estrogen reduces aldosterone, upregulates adrenal angiotensin II AT2 receptors and normalizes adrenomedullary Fra-2 in ovariectomized rats. *Neuroendocrinology* **2008**, *88*, 276−286. (b) Hoshi-Fukushima, R.; Nakamoto, H.; Imai, H.; Kanno, Y.; Ishida, Y.; Yamanouchi, Y.; Suzuki, H. Estrogen and angiotensin II interactions determine cardio-renal damage in dahl salt-sensitive rats with heart failure. *Am. J. Nephrol.* **2008**, *28*, 413−423.

(13) (a) Castelli, W. P. Cardiovascular disease in women. *Am. J. Obstet. Gynecol.* **1988**, *158*, 1553−1560. (b) Fischer, M.; Baessler, A.; Schunkert, H. Renin angiotensin system and gender differences in the cardiovascular system. *Cardiovasc. Res.* **2002**, *53*, 672−677. (c) Roesch, D. M.; Tian, Y.; Zheng, W.; Shi, M.; Verbalis, J. G.; Sandberg, K. Estradiol attenuates angiotensin-induced aldosterone secretion in ovariectomized rats. *Endocrinology* **2000**, *141*, 4629−4636. (d) Chappell, M. C.; Gallagher, P. E.; Averill, D. B.; Ferrario, C. M.; Brosnihan, K. B. Estrogen or the AT1 antagonist olmesartan reverses the development of profound hypertension in the congenic mRen2.Lewis rat. *Hypertension* **2003**, *42*, 781−786. (e) Harrison-Bernard, L. M.; Schulman, I. H.; Raij, L. Post-ovariectomy hypertension is linked to increased renal AT1 receptor and salt sensitivity. *Hypertension* **2003**, *42*, 1157−1163. (f) Krishnamurthi, K.; Verbalis, J. G.; Zheng, W.; Wu, Z.; Clerch, L. B.; Sandberg, K. Estrogen regulates angiotensin AT1 receptor expression via cytosolic proteins that bind to the 5′ leader sequence of the receptor mRNA. *Endocrinology* **1999**, *140*, 5435−5438.

(14) Ji, H.; Zheng, W.; Falconetti, C.; Roesch, D. M.; Mulroney, S. E.; Sandberg, K. 17β-Estradiol deficiency reduces potassium excretion in an angiotensin type 1 receptor-dependent manner. *Am. J. Physiol. Heart Circ. Physiol.* **2007**, *293*, H17−H22.

(15) See Supporting Information.

(16) (a) Woo, L. W.; Sutcliffe, O. B.; Bubert, C.; Grasso, A.; Chander, S. K.; Purohit, A.; Reed, M. J.; Potter, B. V. L. First dual aromatase-steroid sulfatase inhibitors. *J. Med. Chem.* **2003**, *46*, 3193−3196. (b) 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.

(17) (a) Richard, M.; Zoran, R. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* **2005**, *48*, 6523−6543. (b) Andrea, C.; Laura, B. M.; Anna, M.; Michela, R.; Vincenzo, T.; Maurizio, R.; Carlo, M. Multitarget-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.* **2008**, *51*, 347−372. (c) Richard, M. Selectively nonselective kinase inhibition: striking the right balance. *J. Med. Chem.* **2010**, *53*, 1413−1437.

(18) (a) Hartmann, R. W.; Bayer, H.; Grün, G. Aromatase inhibitors. Syntheses and structure−activity studies of novel pyridyl-substituted indanones, indans, and tetralins. *J. Med. Chem.* **1994**, *37*, 1275−1281. (b) Gobbi, S.; Cavalli, A.; Rampa, A.; Belluti, F.; Piazzi, L.; Paluszcak, A.; Hartmann, R. W.; Recanatini, M.; Bisi, A. Lead optimization providing a series of flavone derivatives as potent nonsteroidal inhibitors of the cytochrome P450 aromatase enzyme. *J. Med. Chem.* **2006**, *49*, 4777−4780. (c) 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)(1*H*-imidazol-1-yl)methyl]-1*H*-indoles: potent and selective aromatase inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1134−1137. (d) 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. (e) Leonetti, F.; Favia, A.; Rao, A.; Aliano, R.; Paluszcak, A.; Hartmann, R. W.; Carotti, A. Design, synthesis, and 3D QSAR of novel potent and selective aromatase inhibitors. *J. Med. Chem.* **2004**, *47*, 6792−6803. (f) Al-Soud, Y. A.; Heydel, M.; Hartmann, R. W. Design and synthesis of 1,3,5-trisubstituted 1,2,4-triazoles as CYP enzyme inhibitors. *Tetrahedron Lett.* **2011**, *52*, 6372−6375. (g) Abadi, A. H.; Abou-Seri, S. M.; Hu, Q.; Negri, M.; Hartmann, R. W. Synthesis and biological evaluation of imidazolylmethylacridones as cytochrome P-450 enzymes inhibitors. *Med. Chem. Commun.* **2012**, *3*, 663−666.

(19) (a) 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. (b) Hille, U. E.; Hu, Q.; Vock, C.; Negri, M.; Bartels, M.; Müller-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. (c) 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. (d) 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. (e) 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. (f) 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. (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. (i) Potter, G. A.; Barrie, S. E.; Jarman, M.; Rowlands, M. G. Novel steroidal inhibitors of human cytochrome P45017, (l7α-hydroxylase- $C_{17,20}$-lyase): potential agents for the treatment of prostatic cancer. *J. Med. Chem.* **1995**, *38*, 2463−2471. (j) Jarman, M.; Barrie, S. E.; Lera, J. M. The 16, 17-double bond is needed for irreversible inhibition of human cytochrome $P450_{17\alpha}$ by Abiraterone

(17-(3-pyridyl) androsta-5, 16-dien-3α-ol) and related steroidal inhibitors. *J. Med. Chem.* **1998**, *41*, 5375−5381. (k) Handratta, V. D.; Vasaitis, T. S.; Njar, V. C. O.; Gediya, L. K.; Kataria, R.; Chopra, P.; Newman, D., Jr.; Farquhar, R.; Guo, Z.; Qiu, Y.; Brodie, A. M. H. Novel C-17-Heteroaryl Steroidal CYP17 Inhibitors/Antiandrogens: Synthesis, in Vitro Biological Activity, Pharmacokinetics, and Antitumor Activity in the LAPC4 Human Prostate Cancer Xenograft Model. *J. Med. Chem.* **2005**, *48*, 2972−2984. (l) Kaku, T.; Hitaka, T.; Ojida, A.; Matsunaga, N.; Adachi, M.; Tanaka, T.; Hara, T.; Yamaoka, M.; Kusaka, M.; Okuda, T.; Asahi, S.; Furuya, S.; Tasaka, A. Discovery of orteronel (TAK-700), a naphthylmethylimidazole derivative, as a highly selective 17,20-lyase inhibitor with potential utility in the treatment of prostate cancer. *Bioorg. Med. Chem.* **2011**, *19*, 6383−6399.

(20) (a) 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-1*H*-quinolin-2-one derivatives. *J. Med. Chem.* **2008**, *51*, 8077−8087. (b) 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. (c) 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. (d) 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. (e) 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. (f) Ulmschneider, S.; Müller-Vieira, U.; Klein, C. D.; Antes, I.; Lengauer, T.; Hartmann, R. W. Synthesis and evaluation of (pyridylmethylene) tetrahydronaphthalenes/-indanes and structurally modified derivatives: potent and selective inhibitors of aldosterone synthase. *J. Med. Chem.* **2005**, *48*, 1563−1575. (g) Ulmschneider, S.; Müller-Vieira, U.; Mitrenga, M.; Hartmann, R. W.; Oberwinkler-Marchais, S.; Klein, C. D.; Bureik, M.; Bernhardt, R.; Antes, I.; Lengauer, T. Synthesis and evaluation of imidazolylmethylene tetrahydronaphthalenes and imidazolylmethyleneindanes: potent inhibitors of aldosterone synthase. *J. Med. Chem.* **2005**, *48*, 1796−1805. (h) 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-1*H*-imidazoles as selective inhibitors of aldosterone synthase (CYP11B2). *J. Med. Chem.* **2010**, *53*, 1712−1725.

(21) (a) Hille, U. E.; Zimmer, C.; Vock, C. A.; Hartmann, R. W. Discovery of the first selective steroid-11β-hydroxylase (CYP11B1) inhibitors for the treatment of cortisol dependent diseases. *ACS Med. Chem. Lett.* **2011**, *2*, 2−6. (b) Hille, U. E.; Zimmer, C.; Haupenthal, J.; Hartmann, R. W. Optimization of the first selective steroid-11β-hydroxylase (CYP11B1) inhibitors for the treatment of cortisol dependent diseases. *ACS Med. Chem. Lett.* **2011**, *2*, 559−564. (c) 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. (d) Zolle, I. M.; Berger, M. L.; Hammerschmidt, F.; Hahner, S.; Schirbel, A.; Peric-Simov, B. New selective inhibitors of steroid 11β-hydroxylation in the adrenal cortex: synthesis and structure−activity relationship of potent etomidate analogues. *J. Med. Chem.* **2008**, *51*, 2244−2253.

(22) (a) Ehmer, P. B.; Jose, J.; Hartmann, R. W. Development of a simple and rapid assay for the evaluation of inhibitors of human 17alpha-hydroxylase-C(17,20)-lyase (P450cl7) by coexpression of P450cl7 with NADPH-cytochrome-P450-reductase in *Escherichia coli*. *J. Steroid Biochem. Mol. Biol.* **2000**, *75*, 57−63. (b) Hutschenreuter, T. U.; Ehmer, P. E.; Hartmann, R. W. Synthesis of hydroxy derivatives of highly potent non-steroidal CYP 17 Inhibitors as potential metabolites and evaluation of their activity by a noncellular assay using recombinant human enzyme. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 17−32. (c) Ehmer, P. B.; Bureik, M.; Bernhardt, R.; Müller, U.; Hartmann, R. W. Development of a test system for inhibitors of human aldosterone synthase (CYP11B2): Screening in fission yeast and evaluation of selectivity in V79 cells. *J. Steroid Biochem. Mol. Biol.* **2002**, *81*, 173−179. (d) 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. (e) Denner, K.; Doehmer, J.; Bernhardt, R. Cloning of CYP11B1 and CYP11B2 from normal human adrenal and their functional expression in COS-7 and V79 Chinese hamster cells. *Endocr. Res.* **1995**, *21*, 443−448.