Isopropylidene Substitution Increases Activity and Selectivity of Biphenylmethylene 4-Pyridine Type CYP17 Inhibitors

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CYP17 inhibition is a promising therapy for prostate cancer (PC) because proliferation of 80% of PC depends on androgen stimulation. Introduction of isopropylidene substituents onto the linker of biphenylmethylene 4-pyridines resulted in several strong CYP17 inhibitors, which were more potent and selective, regarding CYP 11B1, 11B2, 19 and 3A4, than the drug candidate abiraterone.

Introduction

Prostate cancer (PC[a]) is the carcinoma with the highest incidence in male, and it accounts for a quarter of cancer related deaths each year. Few patients in early stages can be cured by local therapy, like prostatectomy or radiotherapy. Most, especially the ones with metastases, are treated with hormone therapy. Because of severe side effects, chemotherapy is usually reserved as the last choice. Hormone therapy is based on the finding that up to 80% of PCs depend on androgen stimulation for proliferation. Early attempts to suppress androgen production by estrogen application were soon replaced by orchidectomy and later by administration of gonadotropin-releasing hormone (GnRH) analogues.[1] GnRH analogues unfold their activity via the hypothalamic, pituitary, and gonadal axis, resulting in an annihilation of testicular androgen production. However, adrenal androgen formation is not affected. Although ~90% of androgens are no longer produced and the plasma testosterone concentration is reduced below 50 ng/dL, androgen levels (testosterone and subsequently dihydrotestosterone, DHT) in the prostate are higher and maintain cancer cell growth.[2,3] This accumulation is due to the presence of androgen receptor (AR) and steroidogenic enzymes that convert adrenal steroids into testosterone and DHT.[3,4] Hence, AR antagonists (anti-androgens) are employed in combination with GnRH analogues to prevent adrenal androgens from unfolding activity.[5] This is the current standard therapy, the so-called "combined androgen blockade" (CAB). However, long-term application of antagonists induces mutations of AR that render the receptor to be activated by the anti-androgenic drug[6] or by endogenous glucocorticoids,[7] resulting in resistance to CAB.

To avoid the stimulation, the inhibition of 17α-hydroxylase-17,20-lyase (CYP17) was proposed as a superior alternative to CAB. CYP17 catalyzes not only the testicular but also the adrenal conversion of pregnenolone and progesterone to the weak androgens DHEA and androstenedione, respectively. Moreover, recent observations suggest that there is also CYP17 activity in prostate cancer cells.[6] Testosterone subsequently formed from these two weak androgens is in the prostate converted to DHT, which is the most potent androgen. This final step of androgen activation can be inhibited by 5α-reductase (5αR) inhibitors.[8] However, CYP17 inhibition should be a better strategy than 5αR inhibition, as it totally blocks not only androgen biosynthesis in testes and adrenals but also intracellular androgen formation in the cancer cell.
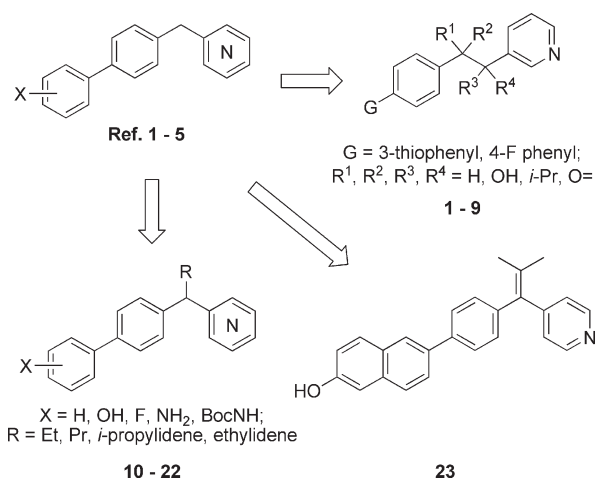
The benefit of PC treatment via CYP17 inhibition was shown by the off-label administration of the antimycotic ketoconazole.[9] However, ketoconazole had to be withdrawn because of severe hepatic toxicity resulting from its nonselective inhibition of other CYP enzymes. Nevertheless, as it has been demonstrated by the success of aromatase (CYP19)[10] and more recently aldosterone synthase (CYP11B2) inhibitors,[11] high selectivity can be achieved. As for CYP17, abiraterone[12] is an outstanding example of a potent and selective inhibitor among the steroidal compounds synthesized.[13] This compound exhibited significant antitumor effects in patients diagnosed as "castration resistant" in phases II and III clinical trials.[12b] However, the potential affinity of steroidal scaffolds for steroid receptors, which often results in side effects whether acting as agonists or antagonists, prompted the development of nonsteroidal CYP17 inhibitors.[14]

Our group has designed and synthesized several series of biphenylmethylene heterocycles[15] as CYP17 inhibitors, in which some imidazoles were found to be very potent.[15a–f] During further optimization, it was revealed that the replacement of imidazolyl by 4-pyridyl significantly improved potency and selectivity, whereas 3-pyridyl analogues exhibited similar activity as imidazoles.[15g] For the imidazoles, we observed that small alkyl groups, especially ethyl, significantly increase inhibitory potency, while bulkier substituents reduce activity.[15c] This observation inspired us to perform a thorough

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[a] Abbreviations: CYP, cytochrome P450; PC, prostate cancer; CAB, combined androgen blockade; GnRH, gonadotropin-releasing hormone; AR, androgen receptor; CYP17, 17α-hydroxylase-17,20-lyase; 5αR, 5α-reductase; CPY19, aromatase, estrogen synthase; CYP11B2, aldosterone synthase; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.

Chart 1. Design of Synthesized Compounds **1−23**

Scheme 1[a]

Comp	R[a]	R[b]	R[c]	py
10	*p*-OMe	H	*p*-OH	3
11	*m*-OMe	H	*m*-OH	4
12	*m*-OMe	Me	*m*-OH	4
13	*m*-OMe	Me	*m*-OH	4
14	*m*-F, *p*-OMe	H	*m*-F, *p*-OH	4
15	*m*-F, *p*-OMe	Me	*m*-F, *p*-OH	4
16	*m, p*-di OMe	Me	*m, p*-di OH	4

Compound **10** is an E/Z mixture

[a] Reagents and conditions: Compounds were synthesized from the corresponding **a**, **b**, **c**, or **d** intermediate unless annotated otherwise. (i) Method A: Pd(OAc)$_2$, corresponding boronic acid, TBAB, EtOH, Na$_2$CO$_3$ aq, toluene, 110 °C, 4 h. (ii) Method B: *i*-PrMgCl or EtMgCl, THF, room temp, 8 h. (iii) Method D: HCOOH, Pd(OAc)$_2$, reflux, 16 h. (iv) Method E: H$_2$, Pd/C, THF, rt, 3 h. (v) Method F: BBr$_3$, DCM. (vi) Method G: HBr, reflux, 4 h.
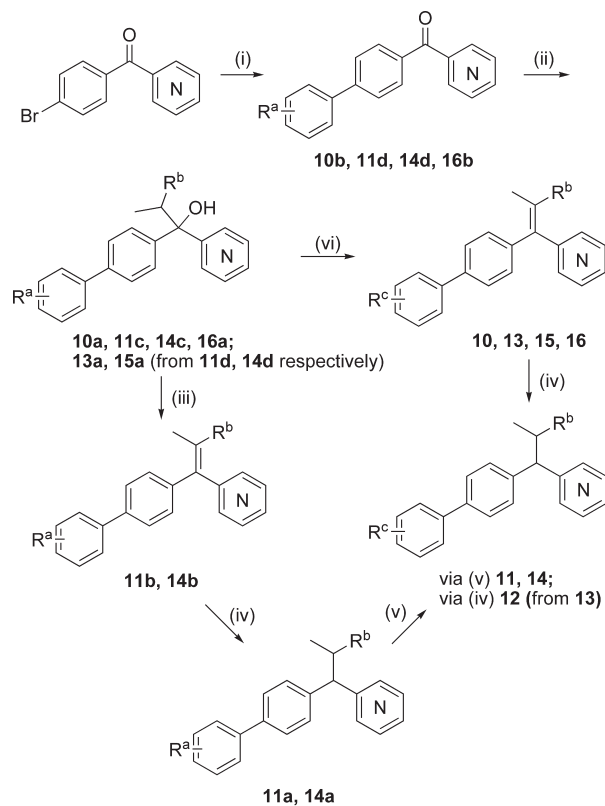
study on the influence of the linker between the biphenyl and pyridyl moieties, which is described in this article. Besides freely rotable alkyl groups, ethylidene and isopropylidene, which rigidify the conformation of the whole molecule, were also inserted onto the methylene bridge (**10−22**, Chart 1). Because different interaction angles between sp^2 hybrid N and heme Fe might account for the disparity in potency between 3- and 4-pyridines, further modifications, such as prolonging the bridge and inserting various substitutions onto the bridge, were performed on the 3-pyridyl scaffold (**1−9**, Chart 1). Furthermore, optimizations of the A-ring by exchanging phenyl to naphthyl or thiophenyl were also done, resulting in, for example, compound **23**. In the following, we also describe the selectivity of selected compounds against CYP11B1, CYP11B2, CYP19, and hepatic CYP enzyme 3A4 to evaluate safety.

Results and Discussion

Chemistry. The synthetic routes for the preparation of **1−23** are shown in Scheme 1−4. For the syntheses of the compounds with the two-membered linker between the biphenyl and pyridine moieties, the corresponding 4-bromo-phenylpyridin-3-yl ethanones **2a** and **5a** were prepared as building blocks. These bromo compounds were coupled with the corresponding boronic acids via Suzuki coupling to yield the ketones **2**, **5**, and **7**, which were then converted to the alcohols **3**, **4**, **6**, **8**, and **9** via Grignard reaction or reduced by Wolff−Kishner reduction to give **1** (Schemes 3 and 4, see Supporting Information).

Similarly, the syntheses of the biphenylmethylene pyridines started from (4-bromophenyl)pyridyl methanones, which were subjected to Suzuki coupling and Grignard reaction to form the corresponding alcohol intermediates. After elimination of a H$_2$O molecule under acidic conditions, the alcohols were converted to the enylpyridines **10**, **13**, **15**, and **16**. The hydrogenation of the double bond led to saturated analogues **11**, **12**, and **14** (Scheme 1). Subsequently, a more efficient strategy was employed by synthesizing 4-(1-(4-bromophenyl)-2-methylprop-1-enyl)pyridine **17a** as a common building block, followed by introduction of the aryl ring via Suzuki reaction. Via this route, the final compounds **17−23** were conveniently obtained (Scheme 2).

CYP17 Inhibitory Activity. Inhibition of CYP17 by the synthesized compounds was determined using the 50 000 sediment after homogenation of *E. coli* coexpressing human

CYP17 and cytochrome P450 reductase.[16] The assay was run with progesterone (25 μM) as substrate and NADPH as cofactor.[15a] Separation of substrate and product was accomplished by HPLC using UV detection. IC$_{50}$ values are presented in comparison to ketoconazole, abiraterone, and reference compounds **24−28**[15g] in Table 1. All 3-pyridyl compounds (detailed structures can be found in Schemes 3 and 4, see Supporting Information) were only weakly active (IC$_{50}$ > 5000 nM), whether furnished with a two-membered linker (**1−9**) or ethylidene substituted methylene (**10**).

In contrast, the 4-pyridyl compounds were potent CYP17 inhibitors and the substituents on the methylene bridge showed profound influence on the inhibitory potency. It is apparent that for the A-ring *m*-OH analogues, the introduction of an ethyl (**11**) and a propyl (**12**) group decreased activities to 189 and 783 nM, respectively, compared to the corresponding nonsubstituted reference compound **24** (IC$_{50}$ = 97 nM). However, the isopropylidene substitution increased activity to 56 nM. A similar observation can be made

Scheme 2[a]
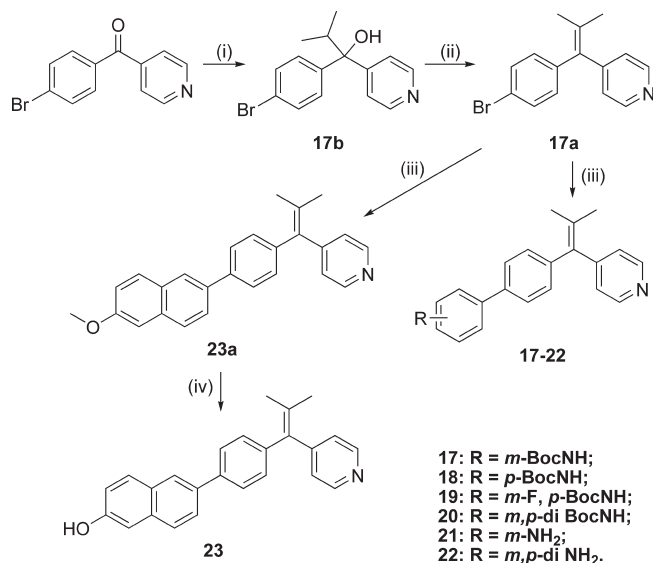
[a] Reagents and conditions. (i) Method B: *i*-PrMgCl, THF, room temp, 8 h. (ii) Method G: HBr, reflux, 4 h. (iii) Method A: Pd(OAc)$_2$, corresponding boronic acid, TBAB, EtOH, Na$_2$CO$_3$ aq, toluene, 110 °C, 4 h. (iv) Method F: BBr$_3$, DCM.

for the A-ring *m*-F, *p*-OH series: the ethyl compound **14** is less potent than the nonsubstituted reference compound **25** (IC$_{50}$ of 343 nM vs 186 nM), whereas the isopropylidene **15** once again was the most active one exhibiting an IC$_{50}$ of 75 nM. More examples for the enhancement of inhibitory potency rendered by isopropylidene were observed with the remaining couples: the di-OH analogues (reference compounds **26** and **16**, IC$_{50}$ of 52 nM vs 37 nM), the amines (reference compounds **27** and **21**, IC$_{50}$ of 226 nM vs 38 nM), and the diamino compounds (reference compounds **28** and **22**, IC$_{50}$ of 337 nM vs 75 nM). The change in geometry from sp^3 to sp^2 leading to a planar compound with less conformational flexibility is a plausible explanation for the observed increase in activity. Moreover, the conjugation of the biphenyl moiety and the pyridyl ring facilitated by isopropylidene increases the electron density in the heteroaromat, which might additionally contribute to the enhanced affinity to the enzyme. In our previous studies on biphenylmethylene imidazoles, introduction of an ethyl group into the methylene bridge was found to significantly increase activity.[15c] The opposite impact of ethyl exhibited with the pyridines presented in this paper compared to those imidazoles is probably due to the longer distance between the sp^2 hybrid N and the methylene group in the pyridines, which might prevent the ethyl group from binding into the hydrophobic pocket.[15c] Furthermore, Boc-amido was again[15g] proven to be not tolerated: the corresponding analogues **17−20** showed modest to no inhibition of CYP17 (IC$_{50}$ ranging from 493 nM to more than 10 000 nM). Besides the introduction of isopropylidene, the exchange of the A-ring from phenyl to 6-OH naphthyl also led to a highly potent CYP17 inhibitor, **23** (IC$_{50}$ = 62 nM).

Selectivity. As a criterion to evaluate safety, the inhibition values of the most potent CYP17 inhibitors (all of them isopropylidene compounds) toward CYP11B1, CYP11B2, and CYP19 were determined (Table 2). CYP11B1 and CYP11B2 catalyze the crucial final steps in cortisol and aldosterone biosynthesis, respectively. Inhibition of these enzymes could lead to hyponatremia, hyperkalemia, adrenal

Table 1. Inhibition of CYP17 by **11−23** and Reference Compounds **24−28**

compd	X	R	IC$_{50}$ (nM)[b]
24[c]	*m*-OH	H	97
11	*m*-OH	Et	189
12	*m*-OH	*i*-Pr	783
13	*m*-OH	isopropylidene	56
25[c]	*m*-F, *p*-OH	H	186
14	*m*-F, *p*-OH	Et	343
15	*m*-F, *p*-OH	isopropylidene	75
26[c]	*m, p*-di-OH	H	52
16	*m, p*-di-OH	isopropylidene	37
17	*m*-BocNH	isopropylidene	1458
18	*p*-BocNH	isopropylidene	493
19	*m*-F, *p*-BocNH	isopropylidene	852
20	*m, p*-di-BocNH	isopropylidene	>10 000
27[c]	*m*-NH$_2$	H	226
21	*m*-NH$_2$	isopropylidene	38
28[c]	*m, p*-di-NH$_2$	H	337
22	*m, p*-di-NH$_2$	isopropylidene	75
23			62
KTZ[a]			2780
ABT[a]			72

[a] KTZ: ketoconazole. ABT: abiraterone. [b] Concentration of inhibitors required to give 50% inhibition. The given values are mean values of at least three experiments. The deviations were within ±10%. The assay was run with human CYP17 expressed in *E. coli* using progesterone as substrate (25 μM). [c] Reference compounds **24−28** were taken from ref 15g.

Table 2. Selectivity Profiles of Selected Compounds toward CYP11B1, CYP11B2, CYP19, and CYP3A4

	IC$_{50}$ (nM)[b]			
compd	CYP11B1[c]	CYP11B2[c]	CYP19[d]	CYP3A4[e]
24[f]	342	261	663	538
13	3100	3450	12000	730
25[f]	351	110	1670	896
15	2000	1870	5750	17650
26[f]	1400	948	2440	7580
16	37470	974	9500	464
27[f]	287	921	2830	1520
21	7300	3830	5060	1330
28[f]	502	368	24560	nd[g]
22	7180	1130	49500	1770
23	5060	49500	48700	nd[g]
KTZ[a]	127	67	>50000	57
ABT[a]	1610	1750	>50000	2700

[a] KTZ: ketoconazole. ABT: abiraterone. [b] Standard deviations were within ±5%. All the data are mean values of at least three tests. [c] Hamster fibroblasts expressing human CYP11B1 or CYP11B2 are used with deoxycorticosterone as the substrate at 100 nM. [d] Human placental CYP19 is used with androstenedione as the substrate at 500 nM. [e] Microsomal fraction of recombinantly expressed enzyme from baculovirus-infected insect is used with 7-benzoyloxytrifluoromethyl coumarin as the substrate at 50 μM. [f] Reference compounds **24−28** were taken from reference 15g. [g] n. d.: not determined.

hyperplasia, and hypovolemic shock.[17a] CYP19 catalyzes the formation of estrogens, which have been proven to be capable of reducing the incidence of heart disease.[17b]

Furthermore, estrogen deficiency resulting from CYP19 inhibition causes osteoporosis, increased fracture risk, and memory loss.[17c] It can be seen that all the isopropylidene analogues tested are much more selective compared to the corresponding nonsubstituted compounds. For CYP11B1, only weak inhibition (IC_{50} of around 2000−3000 nM for **13** and **15**) or no inhibition (IC_{50} of more than 5000 nM for **16**, **21**−**23**) was observed, which makes these compounds superior to abiraterone (IC_{50} = 1610 nM). A similar selectivity pattern was achieved for CYP11B2: most of the tested compounds did not interfere with this enzyme with IC_{50} of more than 3000 nM (**13**, **21**, and **23**). The only exception was **15** exhibiting weak inhibition with an IC_{50} of 1870 nM, which is comparable to that of abiraterone (IC_{50} = 1750 nM). Compounds **16** and **22** were less selective with IC_{50} of ∼1000 nM. Regarding CYP19, all of the tested compounds **13**, **15**, **16**, and **21**−**23** showed no inhibition with IC_{50} of more than 5000 nM. Furthermore, the inhibition of the hepatic CYP enzyme 3A4 was also determined because it accounts for most of the drug metabolism and is therefore to a large extent involved in drug−drug interactions. Although **13** and **16** were less selective, showing IC_{50} of around 500−700 nM, **21** and **22** exhibited only weak inhibition with IC_{50} of ∼1500 nM. The most selective compound **15** exhibited almost no inhibition of CYP3A4 with an IC_{50} of more than 17 600 nM, which is much better than the drug candidate abiraterone (IC_{50} = 2700 nM).

Conclusion

CYP17 inhibition is a promising therapy for prostate cancer because it blocks not only the androgen biosyntheses in testes and adrenals but also intracellular androgen formation in the cancer cell.[3,4] Biphenylmethylene heterocycles, especially pyridines, have been proven to be potent CYP17 inhibitors.[15] In the present study, modifications of the linker between biphenyl and pyridine moieties were described. Variations on the 3-pyridyl scaffold, such as prolongation of the linker and introduction of different substituents onto the bridge, resulted in only weak inhibitors. In contrast, modifications of 4-pyridyl analogues led to potent and selective CYP17 inhibitors. The differing substituents on the methylene bridge had a profound influence on the inhibitory potency: flexible alkyl groups reduced activity, whereas conformation rigidifying isopropylidene groups significantly improved activity and selectivity. Among the nine isopropylidene substituted biphenylmethylene 4-pyridines synthesized, six compounds (**13**, **15**, **16**, **21**, **22**, and **23**, IC_{50} between 37 and 75 nM) were more potent than or comparable to the drug candidate abiraterone (IC_{50} = 72 nM). Most of these potent compounds also exhibited better selectivity profiles toward CYP11B1, CYP11B2, CYP19, and hepatic CYP3A4 than the parent compounds and abiraterone. Thus, this study presents an example that a single substituent can be the key for selectivity among several CYP enzymes. Several compounds of this investigation can be considered as promising drug candidates after further validation in vivo.

Experimental Section

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 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. All coupling constants (J) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corp.) 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: Suzuki Coupling. See Supporting Information for details.

4′-[2-Methyl-1-(pyridin-4-yl)prop-1-enyl]biphenyl-3-amine (21). **21** was synthesized according to method A using **17a** (0.50 g, 1.74 mmol) and 3-aminophenylboronic acid (0.36 g, 2.60 mmol). Yield, 0.39 g (75%); white solid; mp 131−132 °C; R_f = 0.21 (DCM/MeOH, 20:1); δ_H (CDCl$_3$, 500 MHz) 1.85 (s, 3H, CH$_3$), 1.86 (s, 3H, CH$_3$), 3.73 (s, br, 2H, NH$_2$), 6.66 (dd, J = 2.1, 7.8 Hz, 1H), 6.90 (t, J = 2.0 Hz, 1H), 6.98 (dt, J = 1.4, 7.9 Hz, 1H), 7.09 (dd, J = 1.6, 6.1 Hz, 2H), 7.14 (d, J = 8.3 Hz, 2H), 7.21 (t, J = 7.8 Hz, 1H), 7.49 (d, J = 8.3 Hz, 2H), 8.51 (dd, J = 1.6, 6.0 Hz, 2H); MS (ESI), m/z = 301 [M$^+$ + H].

Method B: Grignard Reaction. See Supporting Information for details.

1-(4-Bromophenyl)-2-methyl-1-(pyridin-4-yl)propan-1-ol (17b). **17b** was synthesized according to method B using (4-bromophenyl)(pyridin-4-yl)methanone (2.00 g, 7.63 mmol) and 2.0 M isopropylmagnesium chloride solution in THF (4.20 mL, 8.39 mmol). Yield, 1.35 g (58%); amber oil; δ_H (CDCl$_3$, 500 MHz) 0.84 (d, J = 6.7 Hz, 3H, CH$_3$), 0.88 (d, J = 6.7 Hz, 3H, CH$_3$), 2.79 (q, J = 6.7 Hz, 1H, CH), 3.25 (s, br, 1H, OH), 7.36−7.42 (m, 6H), 8.41 (d, J = 6.1 Hz, 2H); MS (ESI), m/z = 307 [M$^+$ + H].

Method C: Reduction with NaBH$_4$. See Supporting Information for details.

Method D: Dehydroxylation with HCOOH. See Supporting Information for details.

Method E: Hydrogenation. See Supporting Information for details.

Method F: Ether cleavage with BBr$_3$. See Supporting Information for details.

6-[4-(2-Methyl-1-pyridin-4-yl-propenyl)phenyl]naphthalen-2-ol (23). **23** was synthesized according to method F using **23a** (0.25 g, 0.68 mmol) and 1 M BBr$_3$ in DCM (2.05 mL, 2.05 mmol). Yield, 0.18 g (73%); yellow oil; R_f = 0.52 (DCM/MeOH, 19:1); δ_H (DMSO-d_6, 500 MHz) 1.98 (s, 3H, CH$_3$), 2.02 (s, 3H, CH$_3$), 7.19 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.5 Hz, 1H), 7.65 (d, J = 6.4 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.72 (d, J = 8.5 Hz, 2H), 7.83 (dd, J = 1.8, 8.5 Hz, 1H), 8.00 (d, J = 1.8 Hz, 1H), 8.14 (d, J = 8.5 Hz, 1H), 8.60 (d, J = 6.4 Hz, 2H); MS (ESI), m/z = 352 [M$^+$ + H].

Method G: Dehydroxyltion and Ether Cleavage with HBr. See Supporting Information for details.

4-[1-(4-Bromophenyl)-2-methylprop-1-enyl]pyridine (17a). 17a was synthesized according to method G using **17b** (1.00 g, 3.27 mmol). Yield, 0.89 g (95%); white solid; R_f = 0.21 (DCM/MeOH, 10:1); δ_H (CDCl$_3$, 500 MHz) 1.85 (s, 3H, CH$_3$), 1.86 (s, 3H, CH$_3$), 7.09 (dd, J = 1.6, 6.1 Hz, 2H), 7.14 (d, J = 8.3 Hz, 2H), 7.49 (d, J = 8.3 Hz, 2H), 8.51 (dd, J = 1.6, 6.0 Hz, 2H); MS (ESI), m/z = 289 [M$^+$ + H].

Supporting Information Available: Synthetic procedures, characterization and HPLC purities of the intermediates and final compounds, and the biological assays for CYP17, CYP11B1, CYP11B2, CYP19, and CYP3A4. This material is available free of charge via the Internet at http://pubs.acs.org.

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