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Imidazole Substituted Biphenyls: A New Class of Highly Potent and In Vivo Active Inhibitors of P450 17 as Potential Therapeutics for Treatment of Prostate Cancer

Bertil G. Wachall, Markus Hector, Yan Zhuang and Rolf W. Hartmann*

University of the Saarland, FR 12.1 Pharmaceutical and Medicinal Chemistry, P.O. Box 151150, D-66041 Saarbrücken, Germany

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Abstract—3- And 4-imidazol-1-yl-methyl substituted biphenyl compounds (named as *meta*- and *para*-substituted compounds) were synthesized bearing additional substituents in 3′-/4′-position as inhibitors of P450 17 (17α-hydroxylase-C17,20-lyase). P450 17 is the key enzyme of androgen biosynthesis. Its inhibition is a novel therapeutic strategy for treatment of prostate cancer (PC). Twenty-nine compounds were synthesized by Ar-Mg-Br, Negishi or Suzuki aryl–aryl cross coupling and tested toward human and rat enzyme. Most of the compounds showed moderate to excellent activity against one of the enzymes ($0.087\,\mu M \leq IC_{50} \leq 7.7\,\mu M$ (ketoconazole: 0.74 μM) for the human enzyme, $0.63\,\mu M \leq IC_{50} \leq 32\,\mu M$ (ketoconazole: 67 μM) for the rat enzyme). Interestingly, strong species differences were observed. In addition compounds were tested for inhibition toward P450 arom. The 3-imidazol-1-yl-methyl substituted compounds showed good inhibitory activity of P450 arom, while for the 4-substituted compounds negligible inhibition was found. For the most active group of P450 17 inhibitors, (i.e. the 4-imidazol-1-yl-methyl substituted compounds) a QSAR study was performed for inhibition of the human enzyme leading to the result that a hydrophilic substituent in 3′-/4′-position is very important. The most promising compounds (with respect to activity toward both enzymes) were tested in vivo using SD-rats for reduction of plasma testosterone concentrations 2 and 6 h after single ip application. The fluorine substituted compound **8c** decreased the testosterone plasma concentration to castration level (after 2 h; 5 mg/kg) showing a biological half live of about 6 h.

Introduction

17α-Hydroxylase-C17,20-lyase (P450 17), a cytochrome P450 monooxygenase, is the key enzyme of the androgen biosynthesis. It catalyzes the hydroxylation of progesterone and pregnenolone into the corresponding 17α-products, as well as the cleavage of the C17–C20 bond to yield androstenedione and DHEA (dehydroepiandrosterone). These steroids are weak androgens which subsequently are converted by other enzymes (17βHSD, 3βHSD, 5α-reductase) to the most potent androgens testosterone and DHT (dihydrotestosterone).

As androgens have been implicated in the development and progression of prostatic cancer, a promising alternative to treatment with antiandrogens and GnRH analogues might be the use of inhibitors of P450 17.[1–5] For that reason P450 17 attracted attention as a therapeutic target and attempts were made to obtain specific steroidal[6–10] as well as nonsteroidal[1–3,11–17] inhibitors.

For the development of nonsteroidal inhibitors an iron complexing group, mostly a nitrogen bearing heterocycle, was combined with a lipophilic moiety.[4,5,11,13,16,17] In some cases an additional substituent[1,12,14,15,18] was introduced to mimic the hydrophilic C3 group of the substrate. As a substitute of the steroidal nucleus different strategies were used. While other groups pursued A-ring[12] or B-(D)-ring[11,13,18] mimetic approaches, our group developed A-B-ring type[1,2,14,16] inhibitors.

Bifonazole (**I**, Chart 1), a long known antimycotic, which had shown weak rat P450 17 inhibitory activity,[19] is supposed to mimic the A- and C-ring of progesterone.[20] So far it has not received attention as a leading structure for P450 17 inhibitors. However, the highly potent steroidal inhibitor **II** (as well as its progesterone analogue), recently published by Njar et al.,[10] encouraged us to perform a systematic structure–activity study in the class of A-C-ring mimetics.

Key words: Imidazole-substituted biphenyls; 17α-hydroxylase-C17,20-lyase (P450 17); enzyme inhibitors; QSAR; theoretical calculations; antineoplastics.
* Corresponding author. Tel.: +49-681-302-3424; fax: +49-681-302-4386; e-mail: rwh@rz.uni-sb.de

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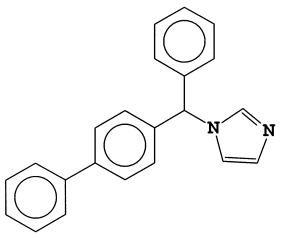
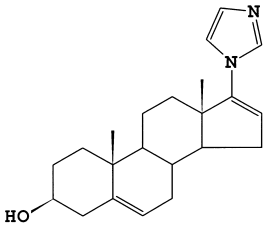


I (bifonazole)



II

Chart 1. Selected inhibitors of P450 17.

We report here the synthesis and the inhibition studies toward rat and human P450 17 (and P450 arom) of 29 imidazole substituted biphenyls (Chart 2). We present the first QSAR study for P450 17 inhibitors and describe the in vivo potency of selected compounds.

Chemistry

Formation of **1**[21] and **2**[21] was carried out by S_N reaction with imidazole, $18K_6$ and K_2CO_3 starting from the corresponding bromobenzylbromides according to Baggaley et al.[22] The synthesis of the biphenyls was performed by Suzuki reaction[23] (method A, Scheme 1) in case of available boronic acids. Otherwise a $Pd(PPh_3)_4$ catalyzed coupling of Grignard reagents was used[24] (method B, Scheme 1), starting from substituted bromobenzenes to give the biphenylic compounds **9a**, **9d**, **9e** and **12a** (Table 1). For the synthesis of **8f** an alternative synthetic route was used because of the lability of the nitrile group. The biphenyl structure was synthesized by Negishi reaction[25] (method G, Scheme 1). After NBS bromation (method H, Scheme 1), **8f** was obtained by reaction with imidazole (method I, Scheme 1).

The phenolic compounds were synthesized from the corresponding methoxy biphenyls by ether cleavage with BBr_3 (method C, Scheme 1).

To avoid polymerization acetal cleavage of **10a** was performed in HCl with resorcin[26] (method F, Scheme 1) to yield **10b**. Since the direct synthesis of the carboxy and amino biphenyls **8g**, **9l** and **12l** with aryl-Mg-Br in threefold excess with PdCl2(dppf)[27] failed, **8g**, **9l** and **12l** were synthesized by alternative routes. The amino compound **9l** was obtained by reduction of the nitro analogue **9i** under a 5 bar H_2 atmosphere (method D, Scheme 1). Because of the low yield of this reaction, for the synthesis of **12l** the acetylic group of **12k** was cleaved with H_2SO_4 (method E, Scheme 1). The carboxy compound **8g** was formed by hydrolyzing the nitrile **8f** (method K, Scheme 1).

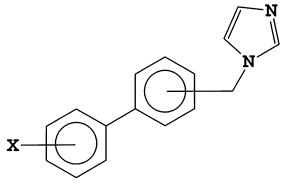


Chart 2. Structure of the title compounds.

Biological Results

The inhibitory activity towards P450 17 rat and human testicular enzymes was tested using microsomal fractions, progesterone as substrate and RP-HPLC with UV-detection. The rat assay was recently described by us.[1] It results in the formation of 17α-hydroxyprogesterone and androstenedione.

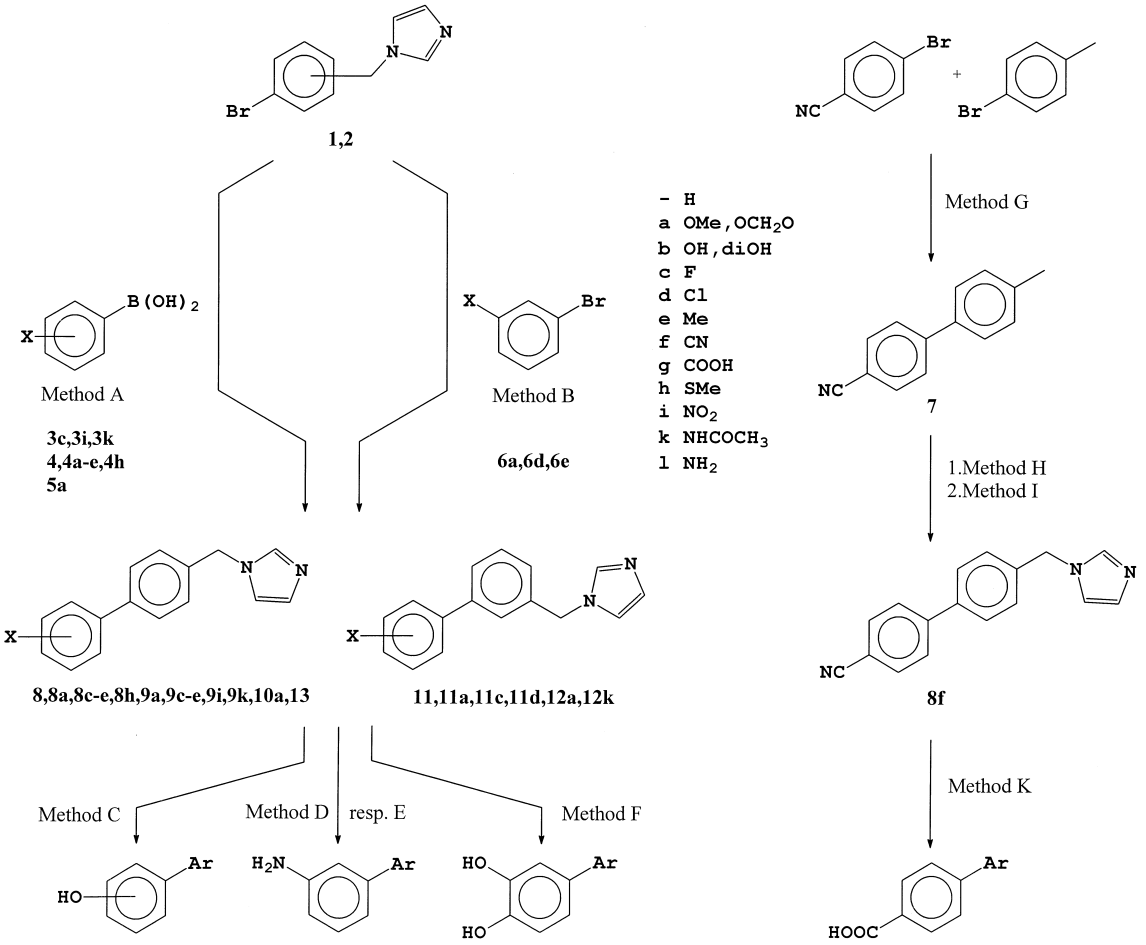
For the human enzyme assay we have applied the same procedure, as it gets along without radiolabelled substrate (tritiated pregnenolone is commonly used[29]). In contrast to the rat enzyme 16α- and 17α-hydroxyprogesterone are formed in a ratio of 3:10 as it is described by others.[30]

Table 2 shows the IC_{50} values and the inhibitory potencies of the compounds relative to the long known P450 17 inhibitor ketoconazole (KTZ; rp values). KTZ, an antimycotic (inhibitor of fungal lanosterol-14α-demethylase), had shown benefit in the treatment of prostate cancer in men, but because of its short biological half life, its poor selectivity (i.e. its considerable ability to inhibit other steroidogenic P450 enzymes as well) and its gastro-intestinal effects, it is not commonly accepted for wide use in patients.[31]

In general no correlation between the inhibition data of the two species could be observed ($r_{human,rat} = 0.24$). While KTZ is only a moderate inhibitor against the rat enzyme, its potency increases more than 90-fold to be a strong inhibitor toward the human enzyme. On the other hand, some compounds showed a decreased inhibition of the human enzyme compared to the rat data (e.g. **8d**, by a factor of four).

The compounds showed moderate to excellent activity against the rat enzyme, the potency of the *p*-substituted biphenyls being clearly enhanced compared to the *m*-substituted. In case of the latter compounds inhibitory activity of the unsubstituted substance was only increased by one substituent, the 4′-OH group (compound **11b**).

In the group of the *p*-substituted biphenyls compounds bearing their substituent in 4′-position (compounds **8**–**8h**) were, with the exception of **9b**, superior to the 3′-substituted compounds. Lipophilic substituents in 4′-position enhanced activity leading to rp values of more than 50. Contrarily substitution in 3′-position by



Scheme 1. Preparation of compounds. Reagents: Method A: $Pd(PPh_3)_4$, Na_2CO_3, toluene; Method B: Mg, $Pd(PPh_3)_4$, THF; Method C: BBr_3, CH_2Cl_2 MeOH; Method D: Pd/C, H_2, p; Method E: H_2SO_4, rflx; Method F: HCl, resorcin; Method G: BuLi, $ZnCl_2$, $Pd(PPh_3)_4$; Method H: NBS, CCl_4; Method I: imidazole, 18-K-6, K_2CO_3, acetone; Method K: H_2SO_4, HOAc.

lipophilic substituents led to a loss of activity. The 3′-OH compound **9b** was the most potent inhibitor of the rat enzyme in this study (rp = 106). Neither the same substitutent in 4′-position (**8b**) nor other hydrophilic substituents in 3′- or 3′- and 4′-position (**9k**, **9l** and **10b**) could enhance activity.

Looking at the human enzyme data different structure activity relationships were observed. With few exceptions the compounds showed a moderately to strongly enhanced inhibitory activity (up to 36 times for **10b**) compared to the rat enzyme. As seen for the rat enzyme, *m*-substituted biphenyls (compounds **11**–**12**) were less active than the para-substituted analogues (compound **8**–**10**). An increase in activity could only be obtained by a hydroxy substituent (compounds **11b** and **12b**). In contrast to the rat enzyme lipophilic substituents in the 4′-position of the para-substituted biphenyls decreased inhibitory activity, whereas the hydroxy group enhanced activity (**8b**, rp = 2.4). The 3′-substituted compounds (**9a**–**9l**) turned out to be superior to 4′-substituted derivatives (**8**–**8h**), the hydrophilic compounds **9b**, **9k** and **9l** showing maximum activity (OH compound **9b**, rp = 5.7).

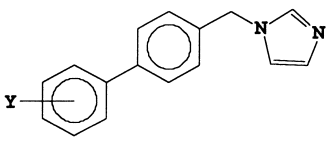
A further increase in activity could be achieved by introducing two OH groups in 3′- and 4′-position. Compound **10b** is the most active compound of this study against the human enzyme with an rp of 8.5. The thiophene bearing inhibitor **13** showed, in contrast to the rat enzyme, a very good inhibition of the human enzyme.

In addition, selected compounds were tested for inhibition of P450 arom (Table 3). While the *m*-substituted compounds showed a good inhibition potency for P450 arom, the most active P450 17 inhibitors, that is, the *p*-group compounds showed poor inhibitory activity for this enzyme. Furthermore compounds **8a** and **11d** were tested for inhibition of P450 TxA_2. With IC_{50} values of 17 μM (**8a**) and 12 μM (**11d**), respectively, they showed only weak activity compared to the reference compound dazoxiben (IC_{50} = 1.1 μM).

QSAR

The good correlation between the human IC_{50}s of the *p*-substituted compounds and physicochemical parameters

Table 1. Structures, synthetic methods, and analytic data



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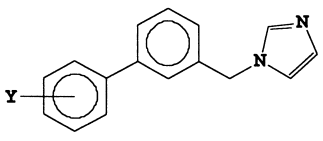

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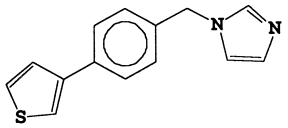

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Compound	Y	Formula	mp (°C)	Method[a]	Recrystallization	Yield[b]
8	H	$C_{16}N_{14}N_2$	141–143[c]	A	—[f]	73
8a	4′-OMe	$C_{17}H_{16}N_2O$	170–171	A	*i*-propanol/MeOH	90
8b	4′-OH	$C_{16}H_{14}N_2O$	260–262	C	*i*-propanol/MeOH	67
8c	4′-F	$C_{16}H_{13}N_2F$	139–141	A	*i*-propanol	87
8d	4′-Cl	$C_{16}H_{13}N_2Cl$	94–96	A	—[f]	58
8e	4′-Me	$C_{17}H_{16}N_2$	138–140	A	*n*-hexane/ethyl acetate	65
8f	4′-CN	$C_{17}H_{13}N_3$	49–51	I	*n*-hexane-acetone	92
8g	4′-COOH	$C_{17}H_{14}N_2O_2$	308–310	K	—[f]	50
8h	4′-SMe	$C_{17}H_{16}N_2S$[d]	248–250	A	*i*-propanol/MeOH	44
9a	3′-OME	$C_{17}H_{16}N_2O$	90–91	B	*n*-hexane/ethyl acetate	89
9b	3′-OH	$C_{16}H_{14}N_2O$	194–196	C	*i*-propanol	40
9c	3′-F	$C_{16}H_{13}N_2F$	oil	A	—[g]	45
9d	3′-Cl	$C_{16}H_{13}N_2Cl$	88–89	B	—[h]	65
9e	3′-Me	$C_{17}H_{16}N_2$	65–66	B	—[h]	56
9i	3′-NO_2	$C_{16}H_{13}N_3O_2$	97–98	A	*n*-hexane/ethyl acetate	78
9k	3′-$NHCOCH_3$	$C_{18}H_{17}N_3O$	171–173	A	*n*-hexane *i*-propanol	34
9l	3′-NH_2	$C_{16}H_{15}N_3$	159–160	D	*n*-hexane/MeOH	35
10a	3′,4′-OCH_2O-	$C_{17}H_{14}N_2O_2$	134–135	A	*i*-propanol	68
10b	3′,4′-diOH	$C_{16}H_{14}N_2O_2$	201–203	F	ethy acetate/MeOH	27
11	H	$C_{16}H_{14}N_2$	oil	A	—[h]	47
11a	4′-OMe	$C_{17}H_{16}N_2O$[d]	146–148	A	*i*-propanol/MeOH	71
11b	4′-OH	$C_{16}H_{14}N_2O$	192–194	C	*i*-propanol	90
11c	4′-F	$C_{16}H_{13}N_2F$	200[e]	A	—[f]	62
11d	4′-Cl	$C_{16}H_{13}N_2Cl$	59–60	A	*i*-propanol	70
12a	3′-OME	$C_{17}H_{16}N_2O$	oil	B	—[i]	72
12b	3′-OH	$C_{16}H_{14}N_2O$	194–195	C	MeOH	99
12k	3′-$NHCOCH_3$	$C_{18}H_{17}N_3O$	160–161	A	—[f]	71
12l	3′-NH_2	$C_{16}H_{15}N_3$	68–69	E	*n*-hexane/ethyl acetate	95
13		$C_{14}H_{12}N_2S$	144–145	A	*i*-propanol/MeOH	79

[a] Capital letters refer to synthetic methods A–K in Experimental.
[b] No efforts were made to optimize yields.
[c] Cuberes et al.[28]: 137–139°C.
[d] ×0.5 H_2SO_4.
[e] Sublimation.
[f] No further purification was neceaasry.
[g] HPLC, RP18, H_2O/MeOH (6/4).
[h] Column chromatography, ammonia saturated ethyl acetate:MeOH (5:1).
[i] Column chromatography, ammonia saturated CH_2Cl_2:EtOH (9:1).

from a qualitative point of view, led to a QSAR study. Chosen for QSAR were all *p*-substituted compounds (**8**–**10** and **13**) with exception of the totally inactive COOH compound **8g**.

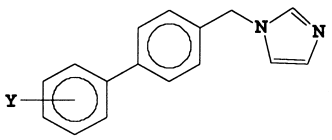
For some parameters no data were available for compounds **10** and **13**. Hence these compounds were not implemented in these cases (not implemented: **10**, **13**:*). Stepwise multiple regression of pIC_{50}s against various hydrophobic, steric and electronic parameters was performed for the 3′- and 4′-substituted groups individually.

The 3′-substituted compounds (**9a**–**9k**) had no correlations with electronic parameters (σ (Hammett)[33]: $r = 0.53$) and only weak to moderate correlations with steric or lipophilic parameters (B_3 (Sterimol[33]): $r = 0.60$; f_R(revised Rekker substituent lipophilicity[32]): $r = 0.76$). To get a better correlation, RM_W values were determined (for all compounds; Table 4) in a MeOH-buffer system ($r(f_R; RM_W) = 0.91$, $s = 0.26$, $F = 96.5$; $r(f_R; RM_W) = 0.96$*, $s = 0.26$, $F = 147.0$) and regressed against $pK(IC_{50})$s to give an improved correlation ($r = 0.88$, $s = 0.22$, $F = 19.7$). It was found, that the addition of a second variable (Table 5) increased correlation significantly.

Beside combination of RM_W with an electronic parameter, that is, σ ($pIC_{50} = -0.36$ (± 0.07) $RM_W - 0.53$ (± 0.25) $\sigma + 1.18$ (± 0.17), $n = 8$, $r = 0.94$, $s = 0.17$, $F = 18.2$) best results (eq. 1) were obtained by combination with the steric parameter B_3:

$$pIC_{50} = -0.37(\pm 0.05)RM_W - 0.51(\pm 0.13)B_3 + 2.02(\pm 0.26) \quad (1)$$
$$n = 8,\ r = 0.97,\ s = 0.12,\ F = 39.8$$

Table 2. P450 17 inhibition data



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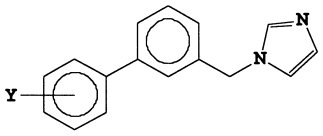

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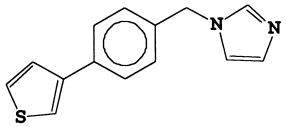

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Compound	Y	Imidazolyl-methyl	IC_{50} rat (μM)	rp rat[a]	IC_{50}[c] human (μM)	rp human[a]
8	H	p	2.0	34	0.98	0.76
8a	4′-OMe	p	7.9	9	3.7	0.21
8b	4′-OH	p	2.6	26	0.31	2.4
8c	4′-F	p	0.98	68	0.96	0.77
8d	4′-Cl	p	1.4	48	5.8	0.13
8e	4′-Me	p	1.3	52	4.2	0.18
8f	4′-CN	p	1.2	56	2.5	0.30
8g	4′-COOH	p	n.i.	—	n.i.[b]	—
8h	4′-SMe	p	8.5	9	7.7	0.10
9a	3′-OME	p	6.5	10	0.59	1.3
9b	3′-OH	p	0.63	106	0.13	5.7
9c	3′-F	p	4.9	14	0.66	1.1
9d	3′-Cl	p	11	6	1.3	0.57
9e	3′-Me	p	7.6	9	1.3	0.57
9i	3′-NO_2	p	4.6	15	1.8	0.41
9k	3′-$NHCOCH_3$	p	3.0	22	0.23	3.2
9l	3′-NH_2	p	6.0	11	0.21	3.5
10a	3′,4′-OCH_2O-	p	20	3	3.1	0.24
10b	3′,4′-diOH	p	3.1	22	0.087	8.5
11	H	m	5.3	13	2.2	0.34
11a	4′-OMe	m	32	2	4.5	0.16
11b	4′-OH	m	2.9	23	0.86	0.86
11c	4′-F	m	6.1	11	2.9	0.26
11d	4′-Cl	m	5.0	13	13	0.07
12a	3′-OME	m	16	4	3.7	0.20
12b	3′-OH	m	5.1	13	1.2	0.62
12k	3′-$NHCOCH_3$	m	13	5	2.1	0.35
12l	3′-NH_2	m	27	2	4.2	0.18
13		p	2.2	30	0.33	2.2
KTZ			67	1	0.74	1.0

[a] rp: Relative potency (against ketoconazole (KTZ), rp ketoconazole = 1).
[b] n.i. = No inhibition (at 125 μM for the rat and 2.5 μM for the human enzyme, respectively).
[c] IC_{50} of pregnenolone = 23 μM.

The 4′-substituted compounds (**8**–**8h**) showed better correlation against electronic (Pol (polarizability): $r = 0.79$, $s = 0.32$, $F = 9.9$), steric (against B_3: $r = 0.81$, $s = 0.30$, $F = 11.8$) and mixed parameters (MR (molar refractivity[33]): $r = 0.78$, $s = 0.32$, $F = 9.2$). Although an enhanced correlation factor was also achieved for f_R ($r = 0.78$, $s = 0.33$, $F = 3.9$), only a minor improvement could be found for replacing f_R by RM_W ($r = 0.78$, $s = 0.32$, $F = 9.1$). Again increasing correlations were obtained by adding a further variable (Table 6).

Combination of lipophilic and electronic as well as steric parameters gave improved correlations: $pIC_{50} = -0.53\ (\pm 0.22)\ f_R - 0.17\ (\pm 0.06)$ Pol $+ 4.90\ (\pm 1.87)$, $n = 8$, $r = 0.91$, $s = 0.23$, $F = 12.0$; $pIC_{50} = -0.23\ (\pm 0.10)\ RM_W - 0.80\ (\pm 0.30)\ B_3 + 1.16\ (\pm 0.42)$, $n = 8$, $r = 0.91$, $s = 0.23$, $F = 12.7$. However, the following equation (eq. 2) describes the variables which were best correlated to the inhibition data:

$$pIC_{50} = -0.26(\pm 0.08)RM_W - 0.06(\pm 0.02)MR + 0.74(\pm 0.21) \quad (2)$$
$$n = 8, r = 0.94, s = 0.20, F = 18.2$$

For the 3′- and 4′-substituted compounds **8**–**10b** (**+13**) a correlation could only be found with lipophilicity; again with RM_W superior to f_R ($r(f_R) = 0.70$, $s = 0.42$, $F = 16.5$; (RM_W) $= 0.82$, $s = 0.34$, $F = 33.9$). Although an improvement was observed for group **8** as well as for **9**, adding of B_3* as second variable (Table 7) gave no significantly enhanced correlation.

To solve this problem an indicator variable[34] m* was introduced: m was set 0 for 4′-substituted and 1 for 3′-substituted compounds. This structure information describing parameter led to the final QSAR description (eq. 3) of the human P450 17 inhibitor activity for the

Table 3. P450 arom inhibition data

Compound	Imidazolyl-methyl	IC_{50} P450 arom [μM]
8	p	66%[a]
8b	p	31%[a]
8e	p	51%[a]
9a	p	55%[a]
9b	p	57%[a]
9d	p	65%[a]
9i	p	70%[a]
10a	p	48%[a]
11	m	0.60
11a	m	0.56
11b	m	0.57
11c	m	0.37
11d	m	0.20
12a	m	0.25
12b	m	0.49
12k	m	2.5
12l	m	1.9

[a] % Inhibition at 25 μM (aminogluethimide: $IC_{50} = 18.5$ μM).

p-substituted compounds with an strongly improved correlation:

$$\begin{aligned} pIC_{50} = &-0.31(\pm 0.05)RM_W - 0.60(\pm 0.15)B_3 \\ &+ 0.58(\pm 0.10)m + 1.47(\pm 0.25) \\ n = 16^*, &\; r = 0.95, \; s = 0.18, \; F = 39.1 \end{aligned} \quad (3)$$

In vivo

Some of the most potent inhibitors of the human enzyme showed too poor rat activity to be tested in vivo (e.g. compounds **10b** and **13**). Compounds **8c** and **9b** were evaluated for reduction of plasma testosterone concentration in male Sprague–Dawley rats (Table 8). Compounds were applied intraperitoneally equimolar to 10 mg/kg body weight KTZ. Testosterone concentrations were determined from blood samples, taken by cardiac puncture after 2 and 6 h.

Table 4. RM_W values

Compound	RM_w	f_R subst.[a]	f_R tot.[b]
8	2.48	0.204	4.29
8a	2.55	0.274	4.36
8b	1.04	−0.353	3.73
8c	4.20	0.933	5.02
8d	2.90	0.444	4.53
8e	3.54	0.823	4.91
8f	1.78	−0.155	3.93
8h	3.50	0.724	4.81
9a	2.52	0.274	4.36
9b	1.14	−0.353	3.73
9c	3.38	0.933	5.02
9d	2.73	0.444	4.53
9e	3.40	0.724	4.81
9i	2.34	−0.039	4.05
9k	1.22	−0.902	3.18
9l	1.20	−0.616	3.47
10a	2.38	−0.585	3.50
10b	0.64	−0.910	3.18
13	2.00	−0.603	3.48

[a] subst.: Substituent, revised Rekker lipophilicity.[32]
[b] tot.: Molecule, revised Rekker lipophilicity.[32]

Table 5. Correlation matrix for group **8**

Parameter	B_3	RM_W	σ
B_3	1	0.13	0.47
RM_W		1	0.23
σ			1

Table 6. Correlation matrix for group **9**

Parameter	B_3	f_R	MR	Pol	RM_W
B_3	1	0.54	0.79	0.80	0.52
f_R		1	0.43	0.46	0.99
MR			1	0.99	0.37
Pol				1	0.23
RM_W					1

KTZ slightly reduced the testosterone plasma level after 2 h. After 6 h, however, a stimulation of the testosterone concentration above control (threefold), was observed. In contrast **9b** showed enhanced (but not significant) inhibitory activity after 2 h, combined with a moderate increase after 6 h. The most active fluoro compound **8c** totally blocked androgen biosynthesis 2 h after application, and showed a 50% inhibition of the testosterone concentraton after 6 h.

Discussion and Conclusion

The synthesized imidazole substituted biphenyls demonstrate that an A-C-ring mimetic approach is a reasonable strategy for developing potent inhibitors of P450 17. Compared to KTZ or bifonazole (similar activity as KTZ toward rat enzyme[20]) the best compounds showed excellent inhibitory activity against rat (**9b**: rp 106) and human enzyme (**10b**: rp 8.5). They almost reached the activity of the powerful steroidal inhibitor **II**[10] (IC_{50} rat: 0.18 μM; IC_{50} human: 0.04 μM in our test system).

Table 7. Correlation matrix for group **8** and **9**

Parameter	B_3	m	RM_W
B_3	1	0.32	0.21
m		1	0.26
RM_W			1

Table 8. Reduction of the plasma testosterone concentrations in SD-rats

Compound[a]	Plasma testosterone level [ng/mL][b]	
	2 h	6 h
Control	3.31 ± 2.07	0.52 ± 0.26
KTZ	1.66 ± 0.74***	1.43 ± 0.61*
Control	2.15 ± 2.01	0.29 ± 0.08
8c	0.06 ± 0.06*	0.15 ± 0.11**
9b	0.83 ± 0.28[ns]	0.37 ± 0.07**

[a] All compounds were applied equimolar to 10 mg/kg **KTZ**.
[b] Significance (Wilcoxon): * $p < 0.01$; ** $p < 0.025$; *** $p < 0.05$; ns: not significant: $p > 0.05$.

In both species the *para* substituted compounds were superior to the meta substituted. Nevertheless large species discrepancies were observed ($r_{human,rat} = 0.24$), as found by others.[9,10] For the rat enzyme best results were obtained with lipophilic substituent in 4′-position (**10c**–**f**) or an OH substituent in 3′-position (**9b**).

For the human enzyme different correlations were found: The first QSAR study for P450 17 human led to eq. 3, which demonstrates the superiority of the 3′-substituted compounds (positive coefficient of *m*), and the similar binding mode of the 3′- and 4′-substituted inhibitors (correlation with *m*) as well as the steric hindrance for substituents with too big width (negative coefficient of B_3). In addition a strong correlation with substituent hydrophilicity was found (negative coefficient of RM_W). This correlation is the first QSAR proof for the hydrophilic residues at the ends of the substrate binding loops, predicted for P450 17 in different molecular modeling studies.[35–37]

Looking at inhibition potency against other P450 enzymes, P450 arom plays an important role. Since testosterone is accumulated by blocking of P450 arom, more testosterone (already a potent androgen) will be converted into the most potent androgen DHT. To avoid androgen stimulation, inhibitory activity against P450 arom has to be estimated as an unwanted side effect. Hence the good selectivity toward P450 arom of the most active, *para* substituted compounds, is of great importance in the development of P450 17 inhibitors for the treatment of PC. On the other hand the *meta* substituted compounds might be a starting point for developing specific P450 arom inhibitors as potential therapeutics for breast cancer.

For the in vivo study only compounds with a high potency toward human and rat enzyme could be taken in consideration. Compounds with high activity toward the human enzyme and low activity against the rat enzyme could not be tested. They might of course be promising drug candidates (e.g. **9k**,**l** or the most active compound **10b**, as well as the *F*-compound **9c**, and the thiophene compound **13**, with a possibly enhanced metabolic stability).

The moderate activity of the OH compound **9b** is probably due to fast phase 2 metabolism (e.g. glucuronidation or sulfatation). The possibly metabolically more stable, fluorine substituted compound **8c** has decreased the testosterone plasma concentration to castration level (after 2 h; 5 mg/kg) and might be a promising candidate for treatment of hormone sensitive PC.

Experimental

Chemistry

General procedures. Melting points were measured on a Kofler melting point Thermopan apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 398 infrared spectrometer as KBr disks or films as indicated. ^{1}H NMR spectra were recorded on a Bruker AM-400 instrument (400 MHz). Chemical shifts are given in parts per million, and TMS was used as internal standard for spectra obtained in DMSO-d_6 and $CDCl_3$. All *J* values are given in Hz. Purity was checked by GC-MS on a HP G1800A GCD-system. Reagents and solvents were used as obtained from commercial suppliers without further purification. 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). Boronic acids and bromoaryls used as starting materials were commercially obtained (Lancaster, Fluka, Acros).

Method A

4-(Imidazol-1-yl-methyl)biphenyl (8). A 50 mL-flask was charged with $Pd(PPh_3)_4$ (0.076 g, 0.066 mmol), toluene (10 mL), *N*-(4-bromobenzyl)imidazole **(1)** (0.37 g, 1.6 mmol), and an aqueous solution of Na_2CO_3 (5 mL of a 2 M solution) under nitrogen atmosphere, and then phenyl boronic acid (0.38 g, 3.1 mmol) in ethanol was added. The mixture was refluxed for 2.5 h under vigorous stirring. After the reaction was complete, the residual boronic acid was oxidized with 30% H_2O_2 (0.5 mL) at room temperature for 1 h. The product was extracted with CH_2Cl_2/ethanol (9/1), and washed with saturated NaCl solution. The organic layer was extracted with 0.1 N HCl. The aqueous layer was washed with ethyl acetate and alkalized with saturated Na_2CO_3 until the product precipitated. The crude product was washed with water and diethyl ether to give **8** (0.135 g, 73%) as a white solid: mp 141–143°C (Cuberes et al.[29]: 137–139°C); IR 3100, 3030, 1510, 1485, 1440, 1235, 1065, 825, 665 cm^{-1}; ^{1}H NMR ($CDCl_3$) 5.16 (s, 2H, -CH_2-), 6.94 (s, 1H, Im H4′), 7.11 (s, 1H, Im H5′), 7.22 (d, $J = 8.0$ Hz, 2H, Ar H3,H5), 7.34–7.38 (m, 1H, Ar H4′), 7.42–7.46 (m, 2H, Ar H3′,H5′), 7.56–7.58 (m, 5H, Ar H2,H6,H2′,H6′, Im H2′); GC–MS *m*/*z* (M^+): 234.1 (calcd 234.1).

4′-Methoxy-4-(imidazol-1-yl-methyl)-biphenyl (8a). Colorless needles: mp 170–171°C; IR 3100, 2960, 1610, 1500, 1255, 1035, 815 cm^{-1}; ^{1}H NMR ($CDCl_3$) 3.84 (s, 3H, -OCH_3), 5.14 (s, 2H, -CH_2-), 6.93 (s, 1H, Im H4′), 6.98 (d, $J = 8.8$ Hz, 2H, Ar H3′,H5′), 7.10 (s, 1H, Im H5′), 7.20 (d, $J = 8.0$ Hz, 2H, Ar H3,H5), 7.50 (d, $J = 8.8$ Hz, 2H, Ar H2′,H6′), 7.53 (d, $J = 8.0$ Hz, 2H, Ar H2,H6), 7.57 (s, 1H, Im H2′); GC–MS *m*/*z* (M^+): 264.1 (calcd 264.1).

4′-Fluoro-4-(imidazol-1-yl-methyl)-biphenyl (8c). White crystals: mp 139–141°C; IR 3100, 1605, 1500, 1230, 1080, 825 cm^{-1}; ^{1}H NMR ($CDCl_3$) 5.17 (s, 2H, -CH_2-), 6.96 (s, 1H, Im H4′), 7.10–7.14 (m, 3H, Ar H3′,H5′, Im H5′), 7.23 (d, $J = 8.0$ Hz, 2H, Ar H3,H5), 7.50–7.54 (m, 4H, Ar H2,H6,H2′,H6′), 7.65 (s, 1H, Im H2′); GC–MS *m*/*z* (M^+): 252.1 (calcd 252.1).

4′-Chloro-4-(imidazol-1-yl-methyl)-biphenyl (8d). White crystals: mp 94–96°C; IR 3090, 1500, 1485, 1390, 1235, 1070, 815 cm^{-1}; ^{1}H NMR ($CDCl_3$) 5.17 (s, 2H, -CH_2-), 6.95 (s, 1H, Im H4′), 7.13 (s, 1H, Im H4′), 7.23 (d,

J = 8.0 Hz, 2H, Ar H3,H5), 7.41 (d, J = 8.4 Hz, 2H, Ar H3′,H5′), 7.49 (d, J = 8.4 Hz, 2H, Ar H2′,H6′), 7.54 (d, J = 8.0 Hz, 2H, Ar H2,H6), 7.64 (s, 1H, Im H2′); GC–MS m/z (M^+): 268.1 (calcd 268.1).

4′-Methyl-4-(imidazol-1-yl-methyl)-biphenyl (8e). White needles: mp 138–140°C; IR 3110, 3015, 1500, 1235, 1115, 1075, 820 cm^{-1}; ^1H NMR (DMSO-d_6) 2.33 (s, 3H, -CH_3), 5.22 (s, 2H, -CH_2-), 6.92 (s, 1H, Im H4′), 7.21 (s, 1H, Im H5′), 7.23 (d, J = 8.0 Hz, 2H, Ar H3′,H5′), 7.32 (d, J = 8.0 Hz, 2H, Ar H3,H5), 7.54 (d, J = 8.0 Hz, 2H, Ar H2′,H6′), 7.62 (d, J = 8.4 Hz, 2H, Ar H2,H6), 7.78 (d, J = 8.0 Hz, Ar H2,H6), 7.64 (s, 1H, Im H2′); GC–MS m/z (M^+): 248.2 (calcd 248.1).

4′-Thiomethyl-4-(imidazol-1-yl-methyl)-biphenyl (8h)×0.5 H_2SO_4. Yellow crystals: mp 248–250°C; IR 3125, 3040, 1570, 1485, 1190, 1115, 1000, 805 cm^{-1}; ^1H NMR (DMSO-d_6) 2.51 (s, 3H, -CH_3), 5.40 (s, 2H, -CH_2-), 7.35 (d, J = 8.4 Hz, 2H, Ar H3′,H5′), 7.44–7.46 (m, 3H, Ar H3,H5, Im H4′), 7.62 (d, J = 8.4 Hz, 2H, Ar H2′,H6′), 7.64 (s, 1H, Im H5′), 7.69 (d, J = 8.4 Hz, 2H, Ar H2,H6), 8.83 (s, 1H, Im H2′); GC–MS m/z (M^+): 280.1 (calcd 280.1).

3′-Fluoro-4-(imidazol-1-yl-methyl)-biphenyl (9c). Slightly-yellow oil: IR 3105, 3070, 2930, 1620, 1590, 1480, 1240, 1185, 795 cm^{-1}; ^1H NMR ($CDCl_3$) 5.18 (s, 2H, -CH_2-), 6.94 (s, 1H, Im H4′), 7.06–7.08 (m, 1H, Ar H4′), 7.14 (s, 1H, Im H5′), 7.23–7.27 (m, 3H, Ar H2′,H3,H5), 7.34 (d, J = 8.0 Hz, 1H, Ar H6′), 7.38–7.42 (m, 1H, Ar H5′), 7.56 (d, J = 8.4 Hz, 2H, Ar H2,H6), 7.73 (s, 1H, Im H2′); GC–MS m/z (M^+): 252.1 (calcd 252.1).

3′-Nitro-4-(imidazol-1-yl-methyl)-biphenyl (9i). Yellow needles: mp 97–98°C; IR 3090, 3020, 1510, 1350, 1230, 1070, 850, 800, 740 cm^{-1}; ^1H NMR ($CDCl_3$) 5.21 (s, 2H, -CH_2-), 6.95 (s, 1H, Im H4′), 7.14 (s, 1H, Im H5′), 7.29 (d, J = 8.0 Hz, 2H, Ar H3,H5), 7.60–7.66 (m, 4H, Ar H2,H6,H5′, Im H2′), 7.89 (d, 8.0 Hz, 1H, Ar H6′), 8.22 (dd, J = 2.2, 7.5 Hz, 1H, Ar H4′), 8.44 (d, J = 2.2, 1H, Ar H2′); GC–MS m/z (M^+): 279.1 (calcd 279.1).

3′-Acetamido-4-(imidazol-1-yl-methyl)-biphenyl (9k). White needles: mp 171–173°C; IR 3140, 3120, 3020, 2920, 1680, 1620, 1555, 1480, 1320, 920, 830 cm^{-1}; ^1H NMR (DMSO-d_6) 2.06 (s, 3H, -CH_3), 5.24 (s, 2H, -CH_2-), 6.92 (s, 1H, Im H4′), 7.22 (s, 1H, Im H5′), 7.29–7.40 (m, 4H, Ar H3,H5,H4′,H5′), 7.53–7.56 (m, 3H, Ar H2,H6,H6′), 7.78 (s, 1H, Im H2′), 7.87 (s, 1H, Ar H2′), 10.02 (s, 1H, NH); GC–MS m/z (M^+): 291.2 (calcd 291.1).

3′,4′-Methylene dioxy-4-(imidazol-1-yl-methyl)biphenyl (10a). White powder: mp 134–135°C; IR 3100, 2905, 1480, 1450, 1240, 1040, 920, 810, 750 cm^{-1}; ^1H NMR ($CDCl_3$) 5.15 (s, 2H, -CH_2-), 6.00 (s, 2H, -O-CH_2-O-), 6.87 (d, J = 8.8 Hz, 1H, Ar H5′), 6.94 (s, 1H, Im H4′), 7.01–7.05 (m, 2H, Ar H2′,H6′), 7.12 (s, 1H, Im H5′), 7.19 (d, J = 8.4 Hz, 2H, Ar H3,H5), 7.49 (d, J = 8.4 Hz, 2H, Ar H2,H6), 7.61 (s, 1H, Im H2′); GC–MS m/z (M^+): 278.1 (calcd 278.1).

3-(Imidazol-1-yl-methyl)biphenyl (11). Colorless oil: IR 3100, 3030, 1600, 1505, 1230, 1080, 910 cm^{-1}; ^1H NMR ($CDCl_3$) 5.19 (s, 2H, -CH_2-), 6.95 (s, 1H, Im H4′), 7.13 (m, 2H, Ar H4, Im H5′), 7.34–7.45, 7.51–7.56 (m, 8H, Ar H2,H5,H6,H2′,H3′, H4′,H5′,H6′), 7.64 (s, 1H, Im H2′); GC–MS m/z (M^+): 234.1 (calcd 234.1).

4′-Methoxy-3-(imidazol-1-yl-methyl)-biphenyl (11a)×0.5 H_2SO_4. White solid: mp 146–148°C; IR 3120, 3020, 2810, 1610, 1565, 1445, 1250, 1185, 1025, 845 cm^{-1}; ^1H NMR (DMSO-d_6) 3.80 (s, 3H, -OCH_3), 5.42 (s, 2H, -CH_2-), 6.93 (s, 1H, Im H4′), 7.02–7.05 (m, 2H, Ar H3′,H5′), 7.31 (d, J = 6.6 Hz, 1H, Ar H4), 7.42–7.50 (m, 2H, Ar H5, Im H4′), 7.58–7.63 (m, 3H, Ar H2,H6, Im H5′), 7.69 (d, J = 10.6 Hz, 2H, Ar H2′,H6′), 8.92 (s, 1H, Im H2′); GC–MS m/z (M^+): 264.1 (calcd 264.1).

4′-Fluoro-3-(imidazol-1-yl-methyl)-biphenyl (11c). White powder: mp 200°C (sublimation); IR 3120, 1610, 1515, 1485, 1250, 1185, 1025, 845 cm^{-1}; ^1H NMR (DMSO-d_6) 5.26 (s, 2H, -CH_2-), 6.91 (s, 1H, Im H4′), 7.22–7.31, 7.42–7.46, 7.55–7.70 (m, 9H, Ar H2,H4,H5,H6,H2′, H3′,H5′,H6′, Im H4′), 7.81 (s, 1H, Im H2′); GC–MS m/z (M^+): 252.1 (calcd 252.1).

4′-Chloro-3-(imidazol-1-yl-methyl)-biphenyl (11d). Colorless crystals: mp 59–60°C; IR 3110, 3040, 1610, 1590, 1480, 1090, 1010, 810 cm^{-1}; ^1H NMR (DMSO-d_6, TFA) 5.38 (s, 2H, -CH_2-), 7.30 (s, 1H, Im H4′), 7.36 (d, J = 7.5 Hz, 1H, Ar H4), 7.46–7.51 (m, 1H, Ar H5), 7.52–7.57 (m, 3H, Ar H3′,H5′, Im H5′), 7.65 (d, J = 8.0 Hz, 1H, Ar H6), 7.68–7.71 (m, 3H, Ar H2,H2′,H6′), 8.59 (s, 1H, Im H2′); GC–MS m/z (M^+): 268.1 (calcd 268.1).

3′-Acetamido-3-(imidazol-1-yl-methyl)-biphenyl (12k). White needles: mp 160–161°C; IR 3120, 3040, 2970, 2960, 1680, 1560, 1410, 1290, 1090, 835, 780 cm^{-1}; ^1H NMR ($CDCl_3$) 2.19 (s, 3H, -CH_3), 5.15 (s, 2H, -CH_2-), 6.95 (s, 1H, Im H4′), 7.09–7.12 (m, 2H, Ar H4′, Im H5′), 7.23–7.27 (m, 1H, Ar H4), 7.31–7.41 (m, 3H, Ar H2,H5,H5′), 7.48–7.53 (m, 2H, Ar H6,H6′), 7.59 (s, 1H, Im H2′), 7.75 (s, 1H, Ar H2′), 8.06 (s, 1H, NH); GC–MS m/z (M^+): 291.2 (calcd 291.1).

3-(4-(Imidazol-1-yl-methyl)phenyl)-thiophene (13). Slightly-yellow small leafs: mp 144–145°C; IR 3100, 1510, 1440, 1235, 1110, 1070, 745 cm^{-1}; ^1H NMR ($CDCl_3$) 5.13 (s, 2H, -CH_2-), 6.92 (s, 1H, Im H4′), 7.10 (s, 1H, Im H5′), 7.18 (d, J = 8.0 Hz, 2H, Ar H3,H5), 7.36–7.41, 7.44–7.46 (m, 3H, Thiophene H2′,H4′,H5′); 7.55–7.59 (m, 3H, Ar H2, H6, Im H2′); GC–MS m/z (M^+): 240.1 (calcd 240.1).

Method B

3′-Methoxy-4-(imidazol-1-yl-methyl)-biphenyl (9a). To a solution of 3-methoxy phenyl magnesium bromide under nitrogen (prepared with Mg turnings (0.31 g, 12.6 mmol) in dry ether (10 mL) and 3-bromomethoxy-benzene (1.4 g, 7.6 mmol) in dry THF (15 mL)) was added $Pd(PPh_3)_4$ (0.25 g, 0.22 mmol), and *N*-(4-bromo-benzyl)imidazole **(1)** (1.2 g, 5.1 mmol) in dry THF (12 mL). The mixture was refluxed for 1 h, and the resulting suspension was quenched with water (10 mL) and 1 N HCl (8 mL). The aqueous layer was washed

with ethyl acetate, alkalized with a saturated Na_2CO_3 solution, and extracted with ethyl acetate-MeOH (5:1). The combined extracts were washed with water and dried over Na_2SO_4. After evaporation of the solvent, the obtained yellow oil was purified by column chromatography (ammonia saturated CH_2Cl_2/EtOH, 9/1), and the resulting solid was recrystallized from *n*-hexane-ethyl acetate to give **9a** (1.2 g, 89%) as white needles: mp 90–91°C; IR 3100, 2960, 1610, 1590, 1480, 1300, 1230, 845, 760 cm^{-1}; ^1H NMR ($CDCl_3$) 3.86 (s, 3H, -OCH_3), 5.16 (s, 2H, -CH_2-), 6.91 (dd, $J=2.2$, 8.4 Hz, 1H, Ar H4′), 6.94 (s, 1H, Im H4′), 7.09 (t, $J=2.2$ Hz, 1H, Ar H2′), 7.12 (s, 1H, Im H5′), 7.15 (d, $J=7.5$ Hz, 1H, Ar H6′), 7.22 (d, $J=8.4$ Hz, 2H, Ar H3,H5), 7.36 (t, $J=8.0$ Hz, 1H, Ar H5′), 7.57 (d, 2H, Ar H2,H6), 7.62 (s, 1H, Im H2′); GC–MS *m*/*z* (M^+): 264.1 (calcd 264.1).

3′-Chloro-4-(imidazol-1-yl-methyl)-biphenyl (9d). Colorless crystals: mp 88–89°C; IR 3140, 3100, 1620, 1530, 1470, 1420, 1260, 1090, 820, 795, 755 cm^{-1}; ^1H NMR ($CDCl_3$) 5.20 (s, 2H, -CH_2-), 6.96 (s, 1H, Im H4′), 7.15 (s, 1H, Im H5′), 7.25 (d, $J=8.0$ Hz, 2H, Ar H3,H5), 7.32–7.45 (m, 3H, Ar H4′,H5′,H6′), 7.53–7.57 (m, 3H, Ar H2,H6,H2′), 7.75 (s, 1H, Im H2′); GC–MS *m*/*z* (M^+): 268.1 (calcd 268.1).

3′-Methyl-4-(imidazol-1-yl-methyl)-biphenyl (9e). Slightly-yellow solid: mp 65–66°C; IR 3100, 2930, 1605, 1500, 1230, 1025, 790, 765 cm^{-1}; ^1H NMR ($CDCl_3$) 2.42 (s, 3H, -CH_3), 5.16 (s, 2H, -CH_2-), 6.94 (s, 1H, Im H4′), 7.13 (s, 1H, Im H5′), 7.16–7.24 (m, 3H, Ar H3,H5,H4′), 7.31–7.38 (m, 3H, Ar H2′,H5′,H6′), 7.57 (d, $J=8.4$ Hz, 3H, Ar H2,H6), 7.71 (s, 1H, Im H2′); GC–MS *m*/*z* (M^+): 248.2 (calcd 248.1).

3′-Methoxy-3-(imidazol-1-yl-methyl)-biphenyl (12a). Colorless oil: IR 3100, 2940, 2840, 1605, 1580, 1480, 1230, 1040, 740 cm^{-1}; ^1H NMR ($CDCl_3$) 3.86 (s, 3H, -OCH_3), 5.18 (s, 2H, -CH_2-), 6.91 (dd, $J=1.8$, 8.0 Hz, 1H, Ar H4′), 6.94 (s, 1H, Im H4′), 7.06 (t, $J=1.8$ Hz, 1H, Ar H2′), 7.10–7.15 (m, 3H, Ar H4,H5′, Im H5′), 7.33–7.36 (m, 2H, Ar H2,H6′), 7.42 (t, $J=7.5$ Hz, 1H, Ar H5), 7.54 (d, $J=8.0$ Hz, 1H, Ar H6), 7.62 (s, 1H, Im H2′); GC–MS *m*/*z* (M^+): 264.1 (calcd 264.1).

Method C

4′-Hydroxy-4-(imidazol-1-yl-methyl)-biphenyl (8b). A solution of 4′-hydroxy-4-(imidazol-1-yl-methyl)biphenyl (**8a**) (0.5 g, 1.9 mmol) in dry CH_2Cl_2 (54 mL) was cooled at −78°C. Under an atmosphere of nitrogen, BBr_3 (0.43 mL, 4.2 mmol) was added slowly. After further stirring 30 min at −78°C and 3 h at room temperature, 2.3 mL methanol was added dropwise.

The hydrobromide precipitated out of solution after the volume was reduced to 1/4. The solid was filtered off and after dissolving the precipitate in 1 M H_2SO_4, it was neutralized with saturated $NaHCO_3$. (If no hydrobromide precipitated, the solution was evaporated to dryness and the resulting residue was suspended several times with 1 M sulfuric acid. The collected aqueous layers were neutralized with saturated $NaHCO_3$). The precipitating crude product was filtered and purified by recrystallisation from *i*-propanol-MeOH to give **8b** (0.27 g, 67%) as colorless needles: mp 260–262°C; IR 3120, 3020, 2940, 2550, 1600, 1500, 1435, 1390, 1280, 1110, 930, 820, 665 cm^{-1}; ^1H NMR (DMSO-d_6) 5.20 (s, 2H, -CH_2-), 6.83 (d, $J=8.4$ Hz, 2H, Ar H3′,H5′), 6.91 (s, 1H, Im H4′), 7.20 (s, 1H, Im H5′), 7.28 (d, $J=8.0$ Hz, 2H, Ar H3′,H5′), 7.45 (d, $J=8.4$ Hz, 2H, Ar H2′,H6′), 7.55 (d, $J=8.0$ Hz, 2H, Ar H2,H6), 7.77 (s, 1H, Im H2′), 9.74 (s, 1H, -OH); GC–MS *m*/*z* (M^+): 250.1 (calcd 250.1).

3′-Hydroxy-4-(imidazol-1-yl-methyl)-biphenyl (9b). White needles: mp 194–196°C; IR 3110, 3020, 2930, 2800, 2660, 1620, 1575, 1480, 1310, 1080, 790 cm^{-1}; ^1H NMR (DMSO-d_6) 5.22 (s, 2H, -CH_2-), 6.84 (d, $J=8.4$ Hz, 2H, Ar H3,H5), 6.90 (s, 1H, Im H4′), 7.15 (d, $J=7.5$ Hz, 1H, Ar H4′), 7.23 (s, 1H, Im H5′), 7.38 (t, $J=7.5$ Hz, 1H, Ar H5′), 7.43–7.50 (m, 4H, Ar H2,H6,H2′,H6′), 7.79 (s, 1H, Im H2′), 9.56 (s, 1H, -OH); GC–MS *m*/*z* (M^+): 250.0 (calcd 250.1).

4′-Hydroxy-3-(imidazol-1-yl-methyl)-biphenyl (11b). Colorless needles: mp 192–194°C; IR 3120, 3020, 3040, 2910, 2580, 1610, 1520, 1485, 1280, 1110, 790 cm^{-1}; ^1H NMR (DMSO-d_6) 5.22 (s, 2H, -CH_2-), 6.75 (d, $J=8.0$ Hz, 1H, Ar H4), 6.91 (s, 1H, Im H4′), 6.99 (s, 1H, Ar H2), 7.04 (d, $J=7.5$ Hz, 1H, Ar H6), 7.19–7.25 (m, 2H, Ar H5, Im H5′), 7.31 (d, $J=8.0$ Hz, 2H, Ar H3′,H5′), 7.57 (d, $J=8.0$ Hz, 2H, Ar H2′,H6′), 7.77 (s, 1H, Im H2′); 9.48 (s, 1H, -OH); GC–MS *m*/*z* (M^+): 250.0 (calcd 250.1).

3′-Hydroxy-3-(imidazol-1-yl-methyl)-biphenyl (12b). Colorless needles: mp 194–195°C; IR 3160, 3100, 2940, 2680, 2560(l), 1600, 1515, 1450, 1310, 1090, 785, 750 cm^{-1}; ^1H NMR (DMSO-d_6) 5.26 (s, 2H, -CH_2-), 6.77 (dd, $J=1.8$, 8.4 Hz, 1H, Ar H4′), 6.92 (s, 1H, Im H4′), 6.97 (s, 1H, Ar H2′), 7.03 (d, $J=8.0$ Hz, 1H, Ar H4), 7.22–7.26 (m, 3H, Ar H5′,H6′, Im H5′), 7.42 (t, $J=8.0$ Hz, 1H, Ar H5), 7.49 (s, 1H, Ar H2), 7.52 (d, $J=7.5$ Hz, 1H, Ar H6), 7.81 (s, 1H, Im H2′), 9.57 (s, 1H, -OH); GC–MS *m*/*z* (M^+): 250.0 (calcd 250.1).

Method D

3′-Amino-4-(imidazol-1-yl-methyl)-biphenyl (9l). To a solution of 3′-nitro-4-(imidazol-1-yl-methyl)biphenyl (**9i**) (0.1 g, 0.4 mmol) was added Pd on charcoal (10%, 0.01 g). The flask was evaporated and the solution was stirred under an atmosphere of 5 bar hydrogen for 3 days.

After removing the catalyst by filtration and drying (Na_2SO_4), the solvent was evaporated. The crude product was purified by column chromatography (ammonia saturated CH_2Cl_2/EtOH, 9/1), and the resulting solid was recrystallized from *n*-hexane-MeOH to give **9l** (0.035 g, 35%) as slightly-yellow small leafs: mp 159–160°C; IR 3410, 3340, 3220, 3120, 3100, 1645, 1605, 1230, 1080, 740 cm^{-1}; ^1H NMR (DMSO-d_6) 5.14 (s, 2H, -NH_2), 5.22 (s, 2H, -CH_2-), 6.55 (d, $J=8.0$ Hz, 1H, Ar H4′), 6.75 (d, $J=7.5$ Hz, 1H, Ar H6′), 6.81 (s, 1H, Ar H2′), 6.91 (s, 1H, Im H5′), 7.08 (t, $J=7.5$, 8.0 Hz, 1H, Ar H5′), 7.21 (s, 1H, Im H5′), 7.29 (d, $J=8.0$ Hz, 2H, Ar

H3,H5), 7.53 (d, $J=8.4$ Hz, 2H, Ar H2,H6), 7.77 (s, 1H, Im H2′); GC–MS m/z (M^+): 249.1 (calcd 249.1).

Method E

3′-Amino-3-(imidazol-1-yl-methyl)-biphenyl (12l). A solution of 3′-acetamido-3-(imidazol-1-yl-methyl)-biphenyl **(12k)** (0.45 g, 1.5 mmol) in 50% H_2SO_4 (45 mL) was refluxed for 18 h and slowly neutralized with saturated $NaHCO_3$. The mixture was extracted with ethyl acetate/MeOH (5/1). After drying (Na_2SO_4) and evaporating the solvent, the crude product was recrystallized from *n*-hexane-ethyl acetate to give **12l** (0.35 g, 95%) as slightly-brown crystals: mp 68–69°C; IR 3450, 3420, 3370, 3180, 1645, 1620, 1480, 1235, 1110, 785 cm^{-1}; ^1H NMR (DMSO-d_6) 5.18 (s, 2H, -NH_2), 5.25 (s, 2H, -CH_2-), 6.56 (d, $J=7.9$ Hz, 1H, Ar H4′), 6.72 (d, $J=7.4$ Hz, 1H, Ar H6′), 6.79 (s, 1H, Ar H2′), 6.92 (s, 1H, Im H4′), 7.09 (t, $J=7.9$ Hz, 1H, Ar H5′), 7.20 (d, $J=8.0$ Hz, 1H, Ar H4), 7.23 (s, 1H, Im H5′), 7.38–7.48 (m, 3H, Ar H2,H5,H6), 7.80 (s, 1H, Im H2′); GC–MS m/z (M^+): 249.1 (calcd 249.1).

Method F

3′4′-Dihydroxy-4-(imidazol-1-yl-methyl)biphenyl (10b). A solution of 3′,4′-methylendioxy-4-(imidazol-1-yl-methyl)-biphenyl (**10a**) (0.15 g, 0.56 mmol) in concentrated HCl (3 mL) was refluxed with 1,3-benzenediol (0.15 g, 1.4 mmol) for 1.5 h. The mixture was slowly cooled to room temperature, diluted with water (20 mL), and extracted with 1 N HCl. The combined aqueous layers were neutralized with saturated $NaHCO_3$, extracted with ethyl acetate/MeOH (5/1). After drying (Na_2SO_4) and evaporating the solvent, the crude product was washed with diethyl ether, recrystallized from ethyl acetate/MeOH to give **10b** (0.04 g, 27%) as slightly-pink solid: mp 260–262°C; IR 3120, 3020, 2940, 2560(l), 1605, 1510, 1415, 1290, 1080, 820 cm^{-1}; ^1H NMR (DMSO-d_6) 5.21 (s, 2H, -CH_2-), 6.79 (d, $J=8.4$ Hz, 1H, Ar H5′), 6.91 (d, $J=8.0$ Hz, 1H, Ar H6′), 6.94 (s, 1H, Im H4′), 7.00 (s, 1H, Ar H2′), 7.23 (s, 1H, Im H5′), 7.27 (d, $J=8.4$ Hz, 2H, Ar H3,H5), 7.49 (d, $J=8.0$ Hz, 2H, Ar H2,H6), 7.83 (s, 1H, Im H2′), 9.02, 9.07 (s, 2H, -OH); GC–MS m/z (M^+): 266.1 (calcd 266.1).

Method G

4′-Cyano-4-methylbiphenyl (7). To a solution of 4-bromotoluene (4.3 g, 25 mmol) in dry THF (350 mL) under nitrogen atmosphere at −78°C was added slowly *t*-BuLi (33 mL of a 1.5 M solution in *n*-pentane, 50 mmol). After the addition of anhydrous $ZnCl_2$ (3.4 g, 25.5 mmol), the cooling bath was removed. $Pd(PPh_3)_4$ (0.5 g, 0.43 mmol) and 4-bromobenzonitrile (1.8 g, 10.0 mmol) were added and the mixture was refluxed for 2.5 h and cooled to 0°C. The mixture was quenched with water (250 mL) and 0.1 N HCl (250 mL) and extracted with ethyl acetate. After drying (Na_2SO_4) and evaporating the solvent the crude product was recrystallized from *i*-propanol to give **7** (1.6 g, 83%) as slightly-ocherous needles: mp 104–107°C (Gray and Mosley:[38] 109°C); IR 3040, 2960, 2930, 2240, 1610, 1500, 1010, 820 cm^{-1}; ^1H NMR ($CDCl_3$) 2.41 (s, 3H, -CH_3), 7.29 (d, $J=8.0$ Hz, 2H, Ar H3,H5), 7.49 (d, $J=8.0$ Hz, 2H, Ar H2,H6), 7.65–7.71 (m, 4H, Ar H2′, H3′,H5′,H6′).

Method H

4′-Cyano-4-bromomethylbiphenyl. To a solution of 4′-cyano-4-methylbiphenyl (**7**) (0.75 g, 3.9 mmol) in dry CCl_4 (4 mL) was added *N*-bromo succinimide (NBS; 0.69 g, 3.9 mmol) and dibenzoyl peroxide (DBPO; 0.034 g, 0.14 mmol). The mixture was stirred and slowly heated to 60°C. After the exogenous reaction had begun, the mixture was refluxed for 2.5 h. After cooling, it was filtered and washed with CCl_4. The organic layer was concentrated to half of the volume the crude product precipitated out of solution and was recrystallized from CCl_4 to give the title compound (0.45 g, 52%) as slightly ocherous crystals: mp 97–98°C (Patent Sandoz:[39] 106–107°C); IR 3060, 3040, 2240, 1610, 1500, 1010, 825 cm^{-1}; ^1H NMR ($CDCl_3$) 4.54 (s, 2H, -CH_2Br), 7.51 (d, $J=8.4$ Hz, 2H, Ar H3,H5), 7.57 (d, $J=8.4$ Hz, 2H, Ar H2,H6), 7.69 (d, $J=8.4$ Hz, 2H, Ar H3′,H5′), 7.73 (d, $J=8.4$ Hz, 2H, Ar H2′,H6′).

Method I

4′-Cyano-4-(imidazol-1-yl-methyl)-biphenyl (8f). Imidazole (0.4 g, 5.9 mmol), 4′-cyano-4-bromomethylbiphenyl **(7f)** (0.4 g, 1.5 mmol), 18-K-6 (0.01 g, 0.04 mmol) and anhydrous K_2CO_3 (1.7 g, 11.8 mmol) in dry acetone (10 mL) were refluxed for 2 h. The mixture was cooled, extracted with ethyl acetate/MeOH (5/1), washed with water, acidified, and extracted with 0.1 N HCl. The combined aqueous layers were alkalized with saturated Na_2CO_3 and again extracted with ethyl acetate/MeOH (5:1). After drying (Na_2SO_4) and evaporating the solvent, the crude product was purified by column chromatography (ammonia saturated ethyl acetate/MeOH, 9/1), and the resulting solid was recrystallized from *n*-hexane/acetone to give **8** (0.35 g, 92%) as colorless needles: mp 49–51°C; IR 3390, 3300, 3120, 2240, 1610, 1510, 1500, 1080, 820 cm^{-1}; ^1H NMR ($CDCl_3$) 5.20 (s, 2H, -CH_2-), 6.94 (s, 1H, Im H4′), 7.14 (s, 1H, Im H5′), 7.27 (d, $J=8.0$ Hz, 2H, Ar H3,H5), 7.58 (d, $J=8.0$ Hz, 2H, Ar H2,H6), 7.65–7.67 (m, 3H, Ar H3′,H5′, Im H2′), 7.74 (d, $J=8.4$ Hz, 2H, Ar H2′,H6′); GC–MS m/z (M^+): 259.1 (calcd 259.1).

Method K

4′-Carboxy-4-(imidazol-1-yl-methyl)-biphenyl (8g). A solution of 4′-cyano-4-(imidazol-1-yl-methyl)biphenyl **(8f)** (0.15 g, 0.58 mmol) in 50% H_2SO_4 (2 mL) and acetic acid (1 mL) was refluxed for 3.5 h, diluted with water (7 mL), washed with ethyl acetate, alkalized with saturated Na_2CO_3, and extracted with ethyl acetate/MeOH (5/1). 0.1 N HCl was added slowly until **8g** started to precipitate as colorless crystals (0.80 g, 50%): mp 308–310°C; IR 3140, 3040, 2440(l), 1700, 1610, 1400, 1290(l), 1110, 1010, 740 cm^{-1}; ^1H NMR ($CDCl_3$) 5.26 (s, 2H, -CH_2-), 6.94 (s, 1H, Im H4′), 7.25 (s, 1H, Im H5′), 7.37 (d, $J=8.0$ Hz, 2H, Ar H3,H5), 7.73 (d, $J=8.0$ Hz, 2H, Ar H2,H6), 7.77–7.80 (m, 3H, Ar H3′,H5′, Im H2′), 8.01

(d, $J = 8.0$ Hz, 2H, Ar H2′,H6′); GC–MS m/z ($M^+_{\text{methylester}}$): 292.1 (calcd 292.1).

Biological activity

Enzyme preparations. The enzymes were prepared according to described methods: human and rat testicular P450 17[1] and human placental P450 arom.[40] For the P450 TxA_2 assay citrated human whole blood was used.[4]

Enzyme assays. The enzyme assays were performed as described: rat P450 17[1], P450 arom[40] and P450 TxA_2[4]. For determination of human P450 17 inhibition 50 µl of the microsomal enzyme fraction (800 µg protein) and a solution of 165 µL phosphate buffer (50 mM sodium phosphate, 1 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, pH 7.4) with 6.25 nmol progesterone (in 5 µL methanol), 150 nmol NADPH (in 25 µL of the above mentioned phosphate buffer) and inhibitor (in 5 µL DMSO) were preincubated separately at 37°C for 5 min. The reaction was started by addition of the enzyme and stopped after 40 min incubation (37°C) by adding 50 µL 1 N HCl. Steroid extraction and HPLC procedure was performed according to a described method.[1]

Determination of the plasma testosterone concentration. Tests were performed with adult male Sprague–Dawley rats (each group consisted of 6–8 animals). All compounds were dissolved in 0.1 N HCl and administered one time ip equimolar to 10 mg/kg ketoconazole. Blood samples were taken by cardiac puncture under diethyl ether anesthesia after 2 and 6 h. Plasma testosterone values were determined by commercially available RIA and are given in ng/mL plasma ± standard deviation.

QSAR

***RM*$_W$ Determination.** RM_W values were determined by measuring R_m values of different solvent mixtures (phosphate buffer pH 7.4/MeOH = 0/100, 10/90, 15/85, 20/80, 30/70, 40/60, 50/50) and extrapolation to pure aqueous phase. R_f values were obtained using thin layer chromatography on RP-18 F_{254S}.

Other QSAR parameter. Other QSAR parameters, except molar refractivity (MR) and polarizibility (Pol), which were calculated by ChemPlus™, extention for Hyperchem® 5.0, were taken (or calculated:[32] f_R total) from literature.[33]

Regression analysis. Regression and multiple regression analysis were performed by MS Excel 97®.

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