Tetrahydronaphthalenes: Influence of Heterocyclic Substituents on Inhibition of Steroidogenic Enzymes P450 arom and P450 17

Gerald A. Wächter, Rolf W. Hartmann,* Tom Sergejew, Gertrud L. Grün, and Dorothea Ledergerber

Fachrichtung 12.1 Pharmazeutische Chemie, Universität des Saarlandes, P.O. Box 15 11 50, D-66041 Saarbrücken, Germany

In search of new leads for selective inhibition of estrogen and androgen biosynthesis, respectively, heterocyclic substituted 2-(arylmethylene)-1-tetralones (**1−4**, **9−17**), 2-(aryl-hydroxymethyl)-1-tetralones (**5−8**), *exo*-1a,2,3,7b-tetrahydro-*1H*-cyclopropa[*a*]naphthalenes (**18−24**), and 3-alkyl substituted 4,5-dihydronaphtho[1,2-*c*]pyrazoles (**25−27**) were synthesized and tested for inhibitory activity toward four steroidogenic enzymes, P450 arom, P450 17, P450 18, and P450 scc, as well as another P450 enzyme, thromboxane A_2 (TXA$_2$) synthase. The test compounds inhibited human placental P450 arom, showing a wide range of inhibitory potencies. (*Z*)-4-Imidazolyl compound **17** was the most potent inhibitor, with a relative potency (rp) of 110 [rp of aminoglutethimide (AG) ≡ 1, rp of fadrozole = 359]. A competitive type of inhibition was shown by the (*E*)-4-imidazolyl compound **16** (rp = 71). On the other hand some of these compounds inhibited rat testicular P450 17. Maximum activity was shown by the 3-pyridyl compound **20** (rp = 10, rp of ketoconazole ≡ 1). **20** was the only compound which exhibited a marked inhibition of TXA$_2$ synthase (IC$_{50}$ = 14.5 μM; IC$_{50}$ of dazoxiben = 1.1 μM). Regarding selectivity toward the steroidogenic enzymes, compound **16** was relatively selective toward P450 arom, whereas compound **20** was relatively selective toward P450 17. (P450 arom: K_m testosterone = 42 nM, K_i **16** = 33 nM, K_i **20** = 3 μM. P450 17: K_m progesterone = 7 μM, K_i **16** = 9 μM, K_i **20** = 80 nM). **17** and **24** were not selective since they showed strong inhibition of P450 arom (K_i **17** = 26 nM, K_i **24** = 0.12 μM) and P450 17 (K_i **17** = 0.7 μM, K_i **24** = 0.11 μM).

In the last few years several publications have appeared dealing with the development of nonsteroidal inhibitors of aromatase (P450 arom, CYP 19). Inhibitors of this enzyme are potential therapeutics for the treatment of estrogen dependent diseases, such as breast cancer.[1] Several structure−activity studies have resulted in highly active inhibitors,[2−19] some of which are presently under clinical evaluation (fadrozole, vorozole, letrozole, and anastrozole).[20,21] An important structural feature of potent nonsteroidal inhibitors is an accessible heterocyclic nitrogen atom (N). Although showing wide variety in their structures, highly active compounds interact with the enzyme in an identical or at least very similar fashion. As shown by difference spectroscopic experiments,[18,22,23] they complex the heme iron of the enzyme by means of their heterocyclic N, while the rest of the molecule interacts with the apoprotein moiety of the active site.

Structure−activity studies of our group with 4-pyridyl-substituted benzocycloalkenes of the types shown in Chart 1, also led to highly active inhibitors of P450 arom.[9,18,19,24]

Some of the compounds turned out to be selective inhibitors of P450 arom, since they did not inhibit other steroidogenic P450 enzymes.[19] On the other hand, some compounds showed inhibition of 17α-hydroxylase/C17,20-lyase (P450 17, CYP 17) as well.[25] In contrast to P450 arom, this enzyme has not received much attention as a therapeutic target. Most azole-type antimycotics, i.e. 14α-demethylase inhibitors, show a more or less pronounced inhibition of P450 17.[26,27] Only few other nonsteroidal compounds are known to inhibit the enzyme.[25,28−32] Inhibitors of P450 17, however, might

Chart 1

X: H, OH, OCH$_3$　　　Y: H, H; =O　　　n = 0,1
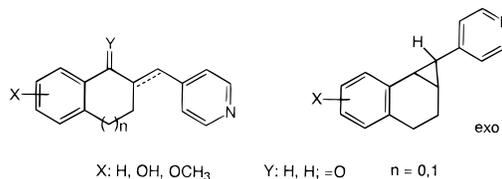
be an alternative to antiandrogens in the treatment of androgen dependent diseases such as prostate cancer.[27,33] The antimycotic ketoconazole, which is also an inhibitor of P450 17,[26] has been useful in the treatment of prostate cancer in men.[34] Because of its low selectivity,[35] it is not commonly accepted for wide use.

The benzocycloalkenes might be appropriate tools to elucidate the structural requirements for selective inhibition of both P450 arom and P450 17. In the present paper the influence of different heterocyclic substituents on P450 arom and P450 17 inhibition of tetrahydronaphthalenes is examined. In the following paragraphs the syntheses of compounds **1−24** (Chart 2) and **25−27** and their inhibitory activities toward the title enzymes (P450 arom, P450 17) and other steroidogenic P450 enzymes (namely cholesterol side chain cleavage enzyme (P450 scc, CYP 11A1), 18-hydroxylase (P450 18, CYP 11B2)) as well as the P450 enzyme thromboxane A_2 (TXA$_2$) synthase will be described.
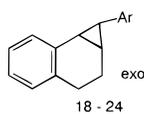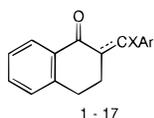
Chemistry

The substituted (*E*)-2-methylene-1-tetralones **1−4** and **9−16** were synthesized by condensation of 1-tetralone with the respective aromatic aldehydes which were either purchased or prepared according to described methods (Scheme 1, Table 1).

Chart 2

compd	C–C	X	Ar	compd	C–C	X	Ar
1	(E) C=C	H	phenyl	13	(E) C=C	H	5-(2-NH$_2$)thiazolyl
2	(E) C=C	H	2-pyridyl	14	(E) C=C	H	5-thiazolyl
3	(E) C=C	H	3-pyridyl	15	(E) C=C	H	3-pyrazolyl
4	(E) C=C	H	4-pyridyl	16	(E) C=C	H	4-imidazolyl
5	CH–CH	OH	2-pyrazinyl	17	(Z) C=C	H	4-imidazolyl
6	CH–CH	OH	3-pyridazinyl	18			phenyl
7	CH–CH	OH	4-pyridazinyl	19			2-pyridyl
8	CH–CH	OH	4-pyrimidinyl	20			3-pyridyl
9	(E) C=C	H	2-pyrazinyl	21			4-pyridyl
10	(E) C=C	H	3-pyridazinyl	22			3-thienyl
11	(E) C=C	H	4-pyridazinyl	23			3-pyrazolyl
12	(E) C=C	H	3-thienyl	24			4-imidazolyl

Scheme 1
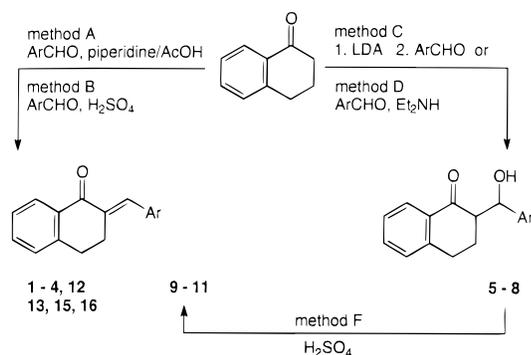
The condensation of benzaldehyde, 3-thiophenecarboxaldehyde, and the 2-, 3-, and 4-pyridinecarboxaldehydes to 1-tetralone was carried out using piperidine/acetic acid as a catalyst, yielding the substituted (*E*)-2-methylene-1-tetralones **1**−**4**, and **12** as described previously (method A).[9,36] 4-Imidazolecarboxaldehyde,[37,38] 3-pyrazolecarboxaldehyde,[39] and 2-amino-5-thiazolecarboxaldehyde[40] were condensed to 1-tetralone in sulfuric acid and gave the substituted (*E*)-2-methylene-1-tetralones **13**, **15**, and **16** (method B). 2-Pyrazinecarboxaldehyde,[41] 3-pyridazinecarboxaldehyde,[42,43] and 4-pyrimidinecarboxaldehyde[39,44] gave the substituted 2-(hydroxymethyl)-1-tetralones **5**, **6**, and **8** when reacted with the lithium enolate of 1-tetralone (method C). The 2-[hydroxy(4-pyridazinyl)methyl]-1-tetralone **7** was obtained by the diethylamine-catalyzed reaction of 4-pyridazinecarboxaldehyde[43,45] with 1-tetralone (method D). Only one diastereomer (racemate) was obtained in case of **5**−**8**. This became apparent from the analytical data (mp, ¹H-NMR) and TLC experiments. The configuration was not determined because the compounds were either inactive or showed only poor activity (see below). Treatment of the 2-(hydroxymethyl)-1-tetralones **5**−**7** with sulfuric acid yielded the (*E*)-2-methylene-1-tetralones **9**−**11** (method F). In case of 2-[hydroxy(4-pyrimidinyl)methyl]-1-tetralone **8**, the same reaction resulted in decomposition. (*E*)-2-[5-(2-Aminothiazolyl)methylene]-1-tetralone **13** was deaminated with isopentylnitrite in tetrahydrofuran,[46] yielding the (*E*)-2-(5-thiazolylmethylene)-1-tetralone **14** (method E).

The *E*-configuration of the compounds was determined by ¹H-NMR spectroscopy according to the method previously reported by us[9,18] (irradiation with UV light

results in a shift of the vinyl-H singlets by approximately 0.9 ppm, from 8.04−7.62 to 7.11−6.73, indicating formation of the corresponding *Z*-isomers). After irradiation of (*E*)-2-(4-imidazolylmethylene)-1-tetralone **16** the newly formed *Z*-2-(4-imidazolylmethylene)-1-tetralone **17** was isolated (method G, Table 1).

Reduction of the (*E*)-2-phenylmethylene-1-tetralone **1** with hydrazine hydrate and KOH is known to yield the 1a,2,3,7b-tetrahydro-1-phenyl-1*H*-cyclopropa[*a*]naphthalene **18** (Scheme 2).[47] We have described the application of this method to the (*E*)-2-(4-pyridylmethylene)-1-tetralone **4** recently.[19] The configuration of the resulting 1a,2,3,7b-tetrahydro-1-phenyl-1*H*-cyclopropa[*a*]naphthalene **21** has been determined to be exo by ¹H- and ¹³C-NMR spectroscopy and by X-ray analysis.[19] We extended the use of this reaction to compounds **2**, **3**, **12**, **15**, and **16**, yielding the 2- and 3-pyridyl-, 3-thienyl-, 3-pyrazolyl-, and 4-imidazolyl-substituted 1a,2,3,7b-tetrahydro-1*H*-cyclopropa[*a*]naphthalenes **19**, **20**, **22**, **23**, and **24** (method H, Scheme 2, Table 1). As observed with **21**, only one diastereomer was obtained in each reaction which also has to be assigned the exo configuration (pyridyl substituent exo corresponding to the tetrahydronaphthalene moiety) as shown by comparison of the ¹H-NMR data. However, reaction of the diazines **9**−**11** and the thiazoles **13** and **14** with hydrazine hydrate and KOH gave the 3-alkyl-substituted 4,5-dihydronaphtho[1,2-*c*]pyrazoles **25**−**27** (method H, Scheme 2, Table 1). Obviously the 2-pyrazoline intermediates formed by addition of the hydrazone nitrogen to the double bond aromatize to yield the heterocyclic substituted pyrazoles (Scheme 3). Under the conditions of the reaction (high temperature and excess reductive agent) the latter compounds decompose, leading to the production of the corresponding alkyl-substituted pyrazoles. Depending on the heterocyclic substituent, the 2-pyrazoline intermediates can also isomerize to the corresponding 1-pyrazoline compounds. The cleavage of molecular nitrogen from the latter results in cyclopropane formation (Scheme 3).

Biological Properties

Inhibition of P450 arom. The method of Thompson and Siiteri, i.e. human placental microsomes and [1*β*,2*β*-³H]testosterone,[49] was used for the determination of inhibitory activities. Table 2 shows the IC$_{50}$ values and the inhibitory potencies of the compounds relative to aminoglutethimide (AG; rp values).

In the case of the (*E*)-2-arylmethylene-substituted 1-tetralones, exchange of the 4-pyridyl group of compound **4** (rp = 4[9]) by a 5-thiazolyl group (**14**, rp = 17) and a 4-imidazolyl substituent (**16**, rp = 71, K_i = 33 nM, K_m for testosterone = 42 nM) enhanced P450 arom inhibitory activity by factors of 4 and 18, respectively. The change of the configuration of compound **16** led to a further increase: the corresponding (*Z*)-4-imidazolylmethylene compound **17** showed a relative potency of 110 (K_i = 26 nM). It reaches about 30% of the activity shown by the well-known aromatase inhibitor fadrozole (rp = 359[19]). The other compounds with a five-membered aryl substituent were either inactive [4-thienyl (**12**), 5-(2-NH$_2$)thiazolyl (**13**)] or showed only a moderate activity [4-pyrazolyl (**14**)]. The six-membered aryl substituents, 3-pyridyl, 2-pyrazinyl, and 4-pyrid-

Table 1. Analytic Data for Heterocyclic Substituted 2-Methylene-1-tetralones (**1–4, 9–17**), 2-(Hydroxymethyl)-1-tetralones (**5–8**), 1a,2,3,7b-Tetrahydro-1*H*-cyclopropa[*a*]naphthalenes (**18–24**), and 4,5-Dihydronaphtho[1,2-*c*]pyrazoles (**25–27**)
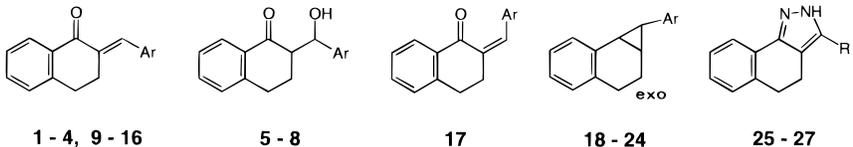
compd	Ar/R	mp, °C	formula[a]	method of prpn	recryst solvent	yield, %
1[c]	phenyl	106–107	$C_{17}H_{14}O$	A	petroleum ether	44
2[d]	2-pyridyl	121–122	$C_{16}H_{13}NO$	A	2-propanol	56
3[d]	3-pyridyl	77–78	$C_{16}H_{13}NO$	A	2-propanol	45
4[e]	4-pyridyl	113–115	$C_{16}H_{13}NO$	A	2-propanol	75
5[f]	2-pyrazinyl	107–108	$C_{15}H_{14}N_2O_2$	C	acetone/petroleum ether	54
6[f]	3-pyridazinyl	158–159	$C_{15}H_{14}N_2O_2$	C	acetone/petroleum ether	43
7[f]	4-pyridazinyl	124–126	$C_{15}H_{14}N_2O_2$	D	acetone/petroleum ether	54
8[f]	4-pyrimidinyl	132–133	$C_{15}H_{14}N_2O_2$	C	acetone/petroleum ether	62
9	2-pyrazinyl	144–148	$C_{15}H_{12}N_2O$	F	acetone	62
10	3-pyridazinyl	112–114	$C_{15}H_{12}N_2O$	F	acetone/petroleum ether	45
11	4-pyridazinyl	135–139	$C_{15}H_{12}N_2O$	F	2-propanol/petroleum ether	68
12	3-thienyl	97–99	$C_{15}H_{12}SO$	A	2-propanol	65
13	5-(2-NH₂)thiazolyl	dec >200	$C_{14}H_{12}N_2OS$	B	tetrahydrofuran/petroleum ether	68
14	5-thiazolyl	132–133	$C_{14}H_{11}NOS$	E	acetone	12
15	3-pyrazolyl	160–162	$C_{14}H_{12}N_2O$	B	acetone	46
16	4-imidazolyl	135–137	$C_{14}H_{12}N_2O$	B	acetone/petroleum ether	57
17	4-imidazolyl	120–124	$C_{14}H_{12}N_2O$	G	acetone	29
18[g]	phenyl	58–60	$C_{17}H_{16}$	H	*i*	85
19	2-pyridyl	80–82	$C_{16}H_{15}N$	H	petroleum ether	72
20	3-pyridyl	61–64	$C_{16}H_{15}N$	H	petroleum ether	68
21[h]	4-pyridyl	79–80	$C_{16}H_{15}N$	H	petroleum ether	60
22	3-thienyl	53–54	$C_{15}H_{14}S$	H	*k*	56
23	3-pyrazolyl	dec >150	$C_{14}H_{14}N_2$·HBr	H	*k, l*	57
24	4-imidazolyl	glass	$C_{14}H_{14}N_2$	H	*k*	82
25	CH_2CH_3	oil	$C_{12}H_{14}N_2$[b]	H	*k*	44
26	$(CH_2)_3CH_3$	oil	$C_{15}H_{18}N_2$[b]	H	*k*	66
27	$CH(CH_3)CH_2CH_3$	oil	$C_{15}H_{18}N_2$[b]	H	*k*	7

[a] C, H, and N analyses were within ±0.4% of the theoretical value except footnote b. [b] Compounds were checked for purity using HPLC. CI mass spectra show M + 1 peaks at m/z = 199.1, 227.2, and 227.2 for compounds **25**, **26**, and **27**, respectively (calculated 199.3, 227.3, and 227.3). [c] See ref 48. [d] See ref 36. [e] See refs 36 and 9. [f] One diastereomer. [g] See ref 47. [h] See ref 19. [i] Purified by bulb tube distillation. [k] Purified by column chromatography on silica gel with mixtures of petroleum ether and ethyl acetate. [l] HBr was bubbled through a solution of the crude product in dry diethyl ether to yield the hydrobromide.

Scheme 2
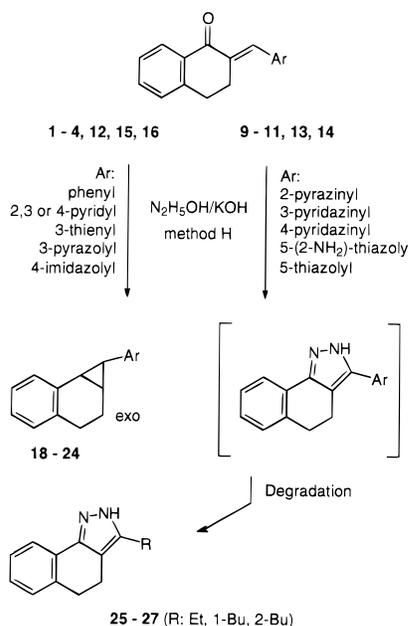
Scheme 3
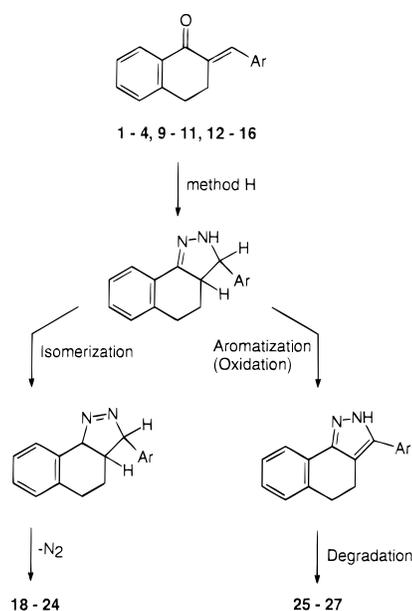
azinyl, led to compounds (**3**, **9**, and **11**) which were less active (rp values of 2, 0.6, and 0.18, respectively) than the parent 4-pyridyl compound **4**. The phenyl, 2-pyridyl, and 3-pyridazinyl compounds (**1**, **2**, and **10**) were inactive.

With the exception of the 4-pyrimidinyl compound **8**, which showed little activity, the aryl-substituted 2-(hydroxymethyl)-1-tetralones were inactive.

In the case of the tetrahydrocyclopropanaphthalenes, the exchange of the 4-pyridyl group of **21** (rp value of 99; K_i = 29 nM) by other heterocycles led to a strong

Table 2. Inhibition of Steroidogenic P450 enzymes (P450 arom, P450 17, and P450 18) by Heterocyclic Substituted 2-Methylene-1-tetralones (**1**−**4**, **9**−**17**), 2-(Hydroxymethyl)-1-tetralones (**5**−**8**), and 1a,2,3,7b-Tetrahydro-1*H*-cyclopropa[*a*]naphthalenes (**18**−**24**)

| compd | Ar | P450 arom[a] | | P450 17[f] % inhibn (IC_{50}, μM) | P450 18[h] % inhibn (IC_{50}, μM) |
		IC_{50}, μM[b]	rp[c]		
		2-Methylene-1-tetralones			
1	phenyl	>250		<10	<10
2	2-pyridyl	>250		<10	<10
3	3-pyridyl	9.2	2	48	<10
4	4-pyridyl	4.6	4[d]	37	12
9	2-pyrazinyl	38	0.6	nd[g]	10
10	3-pyridazinyl	>250		<10	21
11	4-pyridazinyl	103	0.18	<10	<10
12	3-thienyl	>250		<10	<10
13	5-(2-NH$_2$)thiazolyl	>250		nd	<10
14	5-thiazolyl	1.1	17	21	<10
15	3-pyrazolyl	18	1	<10	20
16	4-imidazolyl	0.26	71	31	43
17	4-imidazolyl	0.17	110	74	73
		2-(Hydroxymethyl)-1-tetralones			
5	2-pyrazinyl	>250		<10	<10
6	3-pyridazinyl	>250		<10	<10
7	4-pyridazinyl	>250		<10	<10
8	4-pyrimidinyl	27	0.7	<10	<10
		1a,2,3,7b-Tetrahydro-1*H*-cyclopropa[*a*]naphthalenes			
18	phenyl	>250		<10	14
19	2-pyridyl	>250		<10	<10
20	3-pyridyl	13	1.4	87 (6.3)	24
21	4-pyridyl	0.37	99[e]	36	55
22	3-thienyl	>250	´o	<10	<10
23	3-pyrazolyl	51	0.36	nd	19
24	4-imidazolyl	0.6	31	90 (13)	90 (0.34)

^{a} Human placental microsomes. ^{b} Concentration of inhibitor required to give 50% inhibition. Concentration of testosterone (substrate), 2.5 μM; [1β,2β-^{3}H]testosterone, 0.225 μCi. The given values are mean values of at least three experiments. The deviations were within ±5%. ^{c} Relative potency, calculated from the IC_{50} values and related to AG (IC_{50} of AG = 18.5 μM). ^{d} See ref 9. ^{e} See ref 19. ^{f} Rat testicular microsomes. Concentration of inhibitor, 125 μM; concentration of progesterone (substrate), 25 μM. Inhibition shown by ketoconazole, 62% under identical conditions (IC_{50} = 65 μM). The given values are mean values of at least two experiments. The deviations were within ±5%. ^{g} nd, not determined. ^{h} Bovine adrenal mitochondria. Concentration of inhibitor, 1 μM; concentration of corticosterone (substrate), 200 μM. Inhibition shown by ketoconazole, 78% under identical conditions. The given values are mean values of at least two experiments. The deviations were within ±5%.

decrease in activity: The 4-imidazolyl compound (**24**) showed modest activity (rp = 31; 0.12 μM), the 3-pyridyl (**20**) and the 3-pyrazolyl compound (**23**) were only slightly active [rp values of 1.4 (K_i = 3 μM) and 0.36], and the other compounds were inactive. The 3-alkyl-substituted 4,5-dihydronaphtho[1,2-*c*]pyrazoles **25**−**27** were void of inhibitory activity toward P450 arom (as well as toward the other P450 enzymes, see below; data not given).

To get an insight into the mode of aromatase inhibition, difference spectroscopy experiments were performed with **20**. A type II difference spectrum was found (minimum at 394 nm, maximum at 425 nm, data not given), which is typical of the interaction of the heterocyclic nitrogen with the central iron ion of the cytochrome P450 component.[22]

Inhibition of P450 17. The assay was performed as recently described by us using rat testicular microsomes as source of the enzyme and nonlabeled progesterone as substrate.[25] The inhibition values at the high inhibitor concentration of 125 μM are given in Table 2. The aryl-substituted 2-methylene-1-tetralones showed either no or slight inhibition. The only exception was the *Z*-isomer **17**, which inhibited the enzyme by 74% (K_i = 0.7 μM, K_m for progesterone = 7 μM). Interestingly, the exchange of the 4-pyridyl group of compound **4** by a 4-imidazolyl substituent (compound **16**) further decreased the moderate inhibition of P450 17 slightly [inhibition values of 37% (±4, n = 4; K_i = 4 μM) and 31% (±3, n = 4; K_i = 9 μM); the same structural

modification increased inhibition of P450 arom strongly; see above].

In the case of the tetrahydrocyclopropanaphthalenes, compound **21**, which was the strongest inhibitor of P450 arom, exhibited only modest inhibition of P450 17 (K_i = 3 μM). Strong inhibitors of this enzyme are the 3-pyridyl compound **20** and the 4-imidazolyl compound **24** [IC_{50} values of 6.3 and 13 μM corresponding to relative potencies (rp) of 10 and 5; rp ketoconazole ≡ 1; K_i **20** = 80 nM; K_i **24** = 0.11 μM], which are weak or moderate inhibitors of P450 arom, respectively. The other cyclopropyl compounds and the aryl substituted 2-(hydroxymethyl)-1-tetralones showed no activity.

Inhibition of Other Steroidogenic P450 Enzymes. An essential prerequisite for an inhibitor of sex hormone biosynthesis to be used as a drug is selectivity of inhibition, i.e. other steroidogenic P450 enzymes except the target enzymes must not be inhibited.

The effects of the title compounds on inhibition of P450 18, which catalyzes the last step in aldosterone biosynthesis, are also shown in Table 2. Bovine adrenal mitochondria as the source of the enzyme and corticosterone as the substrate were used, and the procedure recently described[19] was applied. The 2-methylene-1-tetralones **1**−**4** and **9**−**15** showed either no or only slight inhibition of P450 18. Somewhat more active was the (*E*)-4-imidazolyl compound **16**, whereas the corresponding *Z*-compound **17** showed a marked inhibition (inhibition values of 43% and 73%, respectively). The 2-(hydroxymethyl)-1-tetralones **5**−**8** were inactive toward

Table 3. Inhibition of Thromboxane A_2 Synthase by Select Heterocyclic Substituted 2-Methylene-1-tetralones and 1a,2,3,7b-Tetrahydro-1H-cyclopropa[a]naphthalenes[a,b]

compd	% inhibn[c]	compd	% inhibn	IC$_{50}$, μM
1	45	**16**	<10	
2	31	**17**	26	
3	36	**18**	23	
4	13	**19**	40	
9	21	**20**	98	14.5[d]
10	<10	**21**	42	
11	<10	**23**	38	
15	21	**24**	17	

[a] Human whole blood; collagen challenge (53.6 μg/mL). [b] TxA$_2$ synthase-catalyzed malondialdehyde formation determined by a thiobarbituric acid assay (ref 53). [c] Concentration of inhibitor, 50 μM. The given values are mean values of at least two experiments. The deviations were within ±5%. [d] Mean value of three experiments (IC$_{50}$ value).

this enzyme, as they were toward P450 arom and P450 17. The tetrahydrocyclopropanaphthalenes **18**−**24** showed no (**19**, **22**), slight (**18**, **20**, **23**), or moderate (**21**) inhibition. Only compound **24**, which had shown strong inhibition of P450 17, exhibited a strong inhibition of P450 18 as well (IC$_{50}$ = 0.34 μM).

The effects of select tetrahydronaphthalenes on corticosterone (**24**) and aldosterone biosynthesis (**16**, **21**, **24**) were determined using the method of Häusler et al.,[50] i.e. ACTH stimulated rat adrenal fragments in potassium containing buffer. Compound **24** inhibited corticosterone formation, showing an IC$_{50}$ value of 48 μM (IC$_{50}$ values: AG, 50 μM; fadrozole, 80 μM). Compounds **16**, **21**, and **24** inhibited aldosterone formation, showing IC$_{50}$ values of 6, 3, and 0.7 μM, respectively (fadrozole, 0.6 μM). This data corresponds very well to the inhibition values toward P450 18 (**16**, 43%; **21**, 55%; and **24**, 90%; Table 2).

The key enzyme of steroid hormone biosynthesis is the cholesterol side chain cleavage enzyme (P450 scc), which is responsible for the conversion of cholesterol into pregnenolone. By using bovine adrenal mitochondria and [26-^{14}C]cholesterol (3 μM) and applying the procedure of Hochberg et al.[51] select compounds were tested at a relatively high concentration of 25 μM. The 4-pyridyl-substituted compounds **4** and **21** did not inhibit the enzyme, whereas the 4-imidazolyl compounds **16** and **24** showed a weak inhibition (21% for each of the compounds). In contrast to the tetrahydronaphthalenes, the reference compound AG inhibited the enzyme strongly (53%).

Inhibition of P450 TXA$_2$. It has been known for some time that 1-imidazolyl-substituted tetrahydronaphthalenes are strong inhibitors of thromboxane A$_2$ (TXA$_2$) synthase, depending on the substituent at the benzene nucleus.[52] Since inhibition of this enzyme, which contains a P450 moiety, might influence haemostatic function, select compounds were tested for inhibition of TXA$_2$ synthase (Table 3). A high concentration of inhibitor of 50 μM was used. With the exception of compound **20**, the compounds showed inhibition values below 50% (IC$_{50}$ > 50 μM), i.e. they are only slightly active. Only the 3-pyridyl-substituted tetrahydrocyclopropanaphthalene **20** showed a strong inhibition of TXA$_2$ synthase (98% inhibition, IC$_{50}$ = 14.5 μM), being only 1 order of magnitude less active than the well-known inhibitor dazoxiben (IC$_{50}$ = 1.1 μM).

Discussion

The 2-(arylmethylene)-1-tetralone and the 1-aryl-tetrahydrocyclopropanaphthalene basic structures and the variation of the heterocyclic substituents turned out to be appropriate means for the discovery of new leads for selective inhibitors of both P450 arom and P450 17.

In the case of the (E)-2-(arylmethylene)-1-tetralones, the aromatase inhibitory properties of the 4-pyridyl compound **4**[9] could be markedly enhanced. The 4-imidazolyl compound **16** is a strong inhibitor of P450 arom, which affects the other steroidogenic P450 enzymes only in high concentrations. The corresponding Z-compound **17** is a strong inhibitor of P450 arom and also inhibits P450 17 and P450 18 markedly. Compounds of this kind have to be excluded as potential therapeutics, but they might be important scientific tools for the elucidation of the topography of the corresponding steroidogenic P450 enzymes.

Unlike the results shown by the arylmethylenetetralones, the tetrahydrocyclopropanaphthalenes exhibit decreased inhibition of P450 arom but increased inhibition of P450 17 upon exchange of the 4-pyridyl group of **21**[19] by other heterocycles. Compound **20** turned out to be a strong inhibitor of P450 17, which shows only minor effects on the other steroidogenic P450 enzymes. **20** is a 3-pyridyl compound just like another very potent inhibitor of P450 17, 17-(3-pyridyl)androsta-5,16-dien-3β-ol.[54] A compound showing interestingly strong inhibition of P450 arom, P450 17, and P450 18 is the 4-imidazolyl compound **24**.

Taking a closer look at the structure−activity relationships, it becomes apparent that for inhibition of each of the steroidogenic P450 enzymes, the heterocyclic nitrogen must be sterically accessible (i.e., in the case of a six-membered ring the N must be in the 3- or 4-position and in the case of a five-membered ring in the 3-position). Further, an additional N next to the heme iron complexing N is unfavorable. The decisive criterion as to what enzyme will be inhibited and how strongly it will be influenced is of course the three-dimensional structure of the compounds. As the present substances are very rigid, they are appropriate templates for the elucidation of the topography of the active site of the corresponding steroidogenic enzymes.

The fact that structurally related compounds are able to influence both P450 arom and P450 17 is not surprising. P450 arom hydroxylates the steroidal 19-methyl group, which is located in the 10β-position between ring A and B. Consequently, the heme has to be in a position above the steroid near the A-ring. Since the inhibitor complexes the heme iron by means of its N, as shown exemplarily in this as well as in other papers,[18,23] it has to be assumed that the rest of the inhibitor molecule is located in the area of the C- and D-rings, which means that the benzene moiety mimics the D-ring. P450 17 hydroxylates the steroid in 17α-position and subsequently attacks the 20-carbonyl C-atom by means of its reactive ferric peroxy species.[55,56] The heme must therefore be below the steroid near the D-ring. After complexing the heme iron, the rest of the inhibitor molecule is probably located in the area occupied by the A- and B-rings of the steroid in the course of the enzymatic reaction, the benzene nucleus probably mimicking the A-ring. Taking this into account, it becomes obvious that the distances between

the heme iron and the D-ring (P450 arom) or A-ring (P450 17) binding sites for the corresponding substrates might be very similar, and consequently, structurally related compounds can inhibit both P450 arom and P450 17. On the other hand, there are enough differences in the active sites, making a selective inhibition possible, as also shown in this paper.

Provided that the mode of interaction of the inhibitors with the active site is correct as suggested above, the introduction of substituents at the benzene nucleus should result in a further increase of activity and selectivity. Appropriate substituents could interact with the 17β-hydroxy or 17-keto (P450 arom) or with the 3-keto or 3β-hydroxy (P450 17) binding site of the corresponding steroidal substrates. In the case of the 4-pyridyl compounds **4** and **21**, we have shown that the introduction of OH or OCH_3 groups as a matter of fact increased activity.[9,19]

In conclusion, the present study resulted in new leads for selective inhibition of estrogen and androgen biosynthesis. Further optimization of the most interesting compounds, **16** and **20**, is presently under investigation.

Experimental Section

Melting points were determined on a Kofler microscope (Reichert; Vienna) and are not corrected. Elemental analyses were performed at the Physical Chemistry Department of the Universität des Saarlandes and were within ±0.4% of the calculated values, except where stated otherwise (Table 1). Mass spectra (CI) were carried out on a Finnigan MAT 90 using isobutane as reactant gas. ^{1}H-NMR spectra were measured on a Bruker AM 400 (400 MHz) and a Bruker AW 80 (80 MHz) and are consistent with the assigned structures. IR spectra of KBr disks were measured on a Perkin-Elmer Infrared Spectrometer 398. Silica gel 60 from Macherey & Nagel was used for column chromatography. Petroleum ether refers to petroleum ether (40−60 °C).

Method A. General Procedure for the Synthesis of the Substituted (*E*)-2-Methylene-1-tetralones 1−4 and 12. A mixture of 2.00 g (23.5 mmol) of piperidine, 2.00 g (33.3 mmol) of acetic acid, 14.6 g (100 mmol) of 1-tetralone, and 150 mmol of the respective aromatic aldehyde was heated at 130 °C for 1.5 h. After removal of the lower boiling materials under reduced pressure at temperatures not exceeding 130 °C, the residue was dissolved in CH_2Cl_2. For the isolation of the pyridine compounds **2**, **3**, and **4**, this solution was extracted with 2 N HCl. After neutralization of the acidic extract with saturated $NaHCO_3$ solution, the crude product was collected, washed with water, and dried *in vacuo*. For the nonbasic compounds **1** and **12**, the CH_2Cl_2 solution was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. Recrystallization from a suitable solvent (Table 1) was carried out in the absence of light.

Method B. General Procedure for the Synthesis of the Substituted (*E*)-2-Methylene-1-tetralones 13, 15, and 16. A 14.6 g (100 mmol) portion of 1-tetralone and 100 mmol of the respective aromatic aldehyde were suspended in 75 mL of 40% sulfuric acid. After heating at 90 °C for 20 h, the mixture was poured on ice and neutralized with concentrated NH_4OH. The precipitate was collected, washed with water, and dried *in vacuo*. The dried crude product was washed with petroleum ether. Recrystallization from a suitable solvent (Table 1) was carried out in the absence of light.

Method C. General Procedure for the Synthesis of the Substituted 2-(Hydroxymethyl)-1-tetralones 5, 6, and 8. A solution of lithium diisopropylamide prepared from 2.10 mL (15.0 mmol) of diisopropylamine in 10 mL of dry tetrahydrofuran and 10.0 mL (1.60 M, 16.0 mmol) of a butyllithium solution in hexane was cooled to −70 °C. A 2.00 mL (15.0 mmol) portion of 1-tetralone was added. After stirring for 15 min a solution of 15.0 mmol of the respective aromatic aldehyde in 25 mL of dry tetrahydrofuran was slowly added.

The temperature was allowed to rise to ambient temperature over a period of 4 h. The mixture was hydrolyzed with 100 mL of a saturated solution of NH_4Cl, alkalized with 2 N NH_4OH, and extracted three times with CH_2Cl_2. The combined organic phases were washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. Further purification was carried out by recrystallization from a suitable solvent (Table 1).

Method D. Procedure for the Synthesis of 2-(Hydroxy(4-pyridazinyl)methyl-1-tetralone (7). Under warming 1.08 g (10.0 mmol) of 4-pyridazinecarboxaldehyde was dissolved in 2.0 mL of ethanol. After cooling to 4 °C, 3.00 mL (22.5 mmol) of 1-tetralone and 0.20 mL (1.92 mmol) of diethylamine were added. After refrigerating the mixture at 4 °C for 2 weeks, the product was precipitated with petroleum ether. The crude product was recrystallized from acetone/petroleum ether.

Method E. Procedure for the Synthesis of (*E*)-2-(5-Thiazolyl)methylene-1-tetralone (14). A 2.56 g (10.0 mmol) sample of (*E*)-2-(5-(2-aminothiazolyl)methylene)-1-tetralone (**13**) was suspended in 200 mL of dry tetrahydrofuran. After addition of 10.0 mL (75.3 mmol) of isopentylnitrite, the mixture was refluxed for 0.5 h. Evaporation of the solvent under reduced pressure gave a residue to which 100 mL of 2 N NH_4OH was added before extraction with three portions of 100 mL of CH_2Cl_2. After the combined organic phases were dried over Na_2SO_4, 200 g of silica gel was added. The solvent was removed under reduced pressure, and the silica gel was filled into a short column and extracted with 2 L of CH_2Cl_2. After evaporation of the solvent under reduced pressure, the product was purified by recrystallization from acetone (twice).

Method F. General Procedure for the Synthesis of the Substituted (*E*)-2-Methylene-1-tetralones 9 - 11. A solution of 20.0 mmol of the respective substituted 2-(hydroxymethyl)-1-tetralone was dissolved in 100 mL of 25% sulfuric acid and refluxed for 3 h. With intense cooling from an ice bath, the mixture was then alkalized with solid NaOH. The mixture was extracted three times with diethyl ether. The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. Further purification was carried out by recrystallization from a suitable solvent (Table 1).

Method G. Procedure for the Synthesis of (*Z*)-2-(4-Imidazolyl)methylene-1-tetralone (17). A solution of 224 mg (1.00 mmol) of (*E*)-2-(4-imidazolyl)methylene-1-tetralone (**16**) in 5.0 mL of methanol was irradiated with ultraviolet light ($\lambda = 200-600$ nm) for 20 h. The solvent was evaporated under reduced pressure. The residue was extracted three times with 20 mL portions of petroleum ether. Further purification of the residue obtained in this way was carried out by recrystallization from acetone in the absence of light.

Method H. General Procedure for the Synthesis of the Substituted *exo*-1a,2,3,7b-Tetrahydro-1*H*-cyclopropa[*a*]naphthalenes 18−24 and 4,5-Dihydronaphtho[1,2-*c*]-pyrazoles 25−27. A mixture of 18.0 g of KOH (322 mmol), 17.0 mL (340 mmol) of hydrazine hydrate, 300 mL of diethylene glycol, and 20.0 mmol of the respective (*E*)-2-methylene-1-tetralone was heated at 150 °C for 1.5 h. Water formed during the reaction and excess hydrazine hydrate were removed by distillation until the temperature reached 195 °C. The mixture was heated at this temperature for an additional 4 h. The mixture was poured on 600 g of ice and extracted three times with CH_2Cl_2. The combined organic phases were washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. The crude products were purified by column chromatography or recrystallization from a suitable solvent (Table 1).

Biological Methods. Enzyme Preparations. The enzymes were prepared according to described methods: human placental P450 arom,[3] rat testicular P450 17,[25] bovine adrenal P450 18,[57] and bovine adrenal P450 scc.[3]

Enzyme Assays. The enzyme assays were performed as described: P450 arom,[3] P450 17,[25] P450 18,[19] and P450 scc.[3] The K_i values and the difference spectrum were obtained according to procedures published recently.[23] The assay for

the determination of the corticoid formation was performed as described.[23]

For determination of P450 TXA$_2$ synthase inhibition, citrated human whole blood (0.5 mL) was preincubated with inhibitor [in 10 μL of ethanol/phosphate buffer, 0.01 M KH$_2$PO$_4$, 0.05 M Na$_2$HPO$_4$, pH 7.4 (1:1/v:v); control, vehicle; blank, dazoxiben HCl, 100 μM] for 10 min at 37 °C. A 50 μL portion of collagen suspension (final concentration, 53.6 μg/mL) was added and incubation continued for another 10 min at 37 °C. The reaction was terminated by the addition of 0.4 mL of trichloroacetic acid (20% in HCl, 0.6 M). After centrifugation (10 min, 4.400g) the supernatant (0.5 mL) was pipetted into 0.5 mL of thiobarbituric acid (TBA) solution (0.53% TBA in phosphate buffer, 0.01 M KH$_2$PO$_4$, 0.05 M Na$_2$HPO$_4$, pH 7.4). After heating for 30 min at 70 °C and cooling for another 30 min at ambient temperature, the samples were measured spectrofluorimetrically (λ excitation, 533 nm; λ emission, 550 nm).

Supporting Information Available: Mass spectral data (CI spectra) of **25**–**27** as well as ^{1}H NMR data of compounds **1**–**27** (Tables 5–10) (7 pages). Ordering information is given on any current masthead page.

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