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Novel 5α-reductase inhibitors. Synthesis and structure–activity studies of 5-substituted 1-methyl-2-pyridones and 1-methyl-2-piperidones

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Summary — In search for nonsteroidal inhibitors of 5α-reductase for the treatment of benign prostatic hyperplasia (BPH) and possibly prostate cancer, substrate mimicks were synthesized comprising of a 1-methyl-2-pyridone (**2**, **4–16**) or 1-methyl-2-piperidone (**1**, **3**, **17–22**) moiety (mimicking steroidal ring A) and a diisopropyl (**1**, **2**, ***(E)*-5-*(E)*-7**, ***(Z)*-5-*(Z)*-7**, **11–13**, **17–19**) or a *tert*-butyl (**3**, **4**, ***(E)*-8-*(E)*-10**, ***(Z)*-8-*(Z)*-10**, **14–16**, **20–22**) benzamide (mimicking steroidal ring D). The bridge connecting the 5 and 4 positions of the rings consisted of amide (CONH: **1–4**), ethenyl (CH=CH, CCH_3=CH, CH=CH_3: ***(E)*-5-*(E)*-10**, ***(Z)*-5-*(Z)*-10**) or ethylene groups (CH_2CH_2, $CHCH_3CH_2$, CH_2CHCH_3: **11–22**). The amides **1–4** were obtained by amidation of the carboxylic acid chlorides with the 4-amino-*N*-substituted benzamides. The ethenyl compounds ***(E)*-5-*(E)*-10** and ***(Z)*-5-*(Z)*-10** were synthesized by Wittig reaction of the carbonyl compounds and the corresponding triphenylphosphonium salts and subsequent separation of the stereoisomers. Depending on the time of reaction, catalytic hydrogenation of the ethenyl isomers ***(E)*-5-*(E)*-10** led to the pyridone-substituted ethylene compounds **11–16** as well as to the piperidone-substituted ethylene compounds **17–22**. The 5α-reductase inhibitory properties were determined using rat ventral prostate, as well as human BPH and prostate cancer as source, 1β,2β-[^{3}H]testosterone as substrate and a HPLC procedure for the separation of dihydrotestosterone (DHT). Tested at a concentration of 100 μM, the inhibition values of **1–22** ranged from 0–99%. Significant differences were observed between rat and human enzyme. The most active compound was *N*,*N*-diisopropyl-4-[2-(1-methyl-2-oxo-piperidine-5-yl)ethylene]benzamide **17** (IC_{50}: 13 μM).

nonsteroidal 5α-reductase inhibitors / 5-substituted 1-methyl-2-pyridones / 5-substituted 1-methyl-2-piperidones / benign prostatic hyperplasia (BPH) / prostate cancer

Introduction

The prostate is the organ most commonly involved in urological problems. It is the site of the most often diagnosed cancer in males [1], as well as the most common site of benign disease in males [2]. More than 50% of all men above the age of 50 have benign prostatic hyperplasia (BPH). Though the etiology of neither disease is known, it is generally believed that 5α-dihydrotestosterone (DHT) has at least a permissive role, if not an inductive one. A large variety of studies have demonstrated that DHT and not testosterone is the major androgen regulating prostatic cell proliferation [3]. Compounds that inhibit the prostatic conversion of testosterone to DHT, *ie* 5α-reductase (5α-R) inhibitors, have been shown to specifically lower the DHT content in BPH tissue without lowering the systemic level of serum testosterone [3]. The chronic depression in the DHT level should inhibit prostatic cell proliferation and thus decrease the amount of BPH tissue. Since testosterone levels are not decreased, this medication should not affect libido, muscle mass and potency.

Recently 2 types of human 5α-R, named type 1 and type 2, have been identified from human prostate cDNA library [4–6]. Type 1 is found in skin and liver, whereas type 2 was identified in prostate, seminal vesicle, liver and epididymis. A number of highly active steroidal inhibitors of 5α-R have been developed [7–10]. The 4-azasteroid 4-MA exhibits K_i values of approximately 6 nM for both isoenzymes. Another competitive 4-azasteroid, finasteride (fig 1), inhibits type 2 about 30 times more (K_i 12 nM) than type 1 (K_i 325 nM) [11]. Finasteride has recently been introduced into the US market for BPH treatment [12]. The 'dead end' inhibitor SK&F 105657, also named epristeride, is highly type-2 selective showing a K_i value of 7 nM for type 2 [13], whereas its K_i for type 1 exceeds 5000 nM [10]. Type 1 selective inhibitors are expected to be useful in the treatment of skin disorders such as acne, male pattern baldness and hirsutism. Nonsteroidal [11, 14, 15] and steroidal [16] selective type

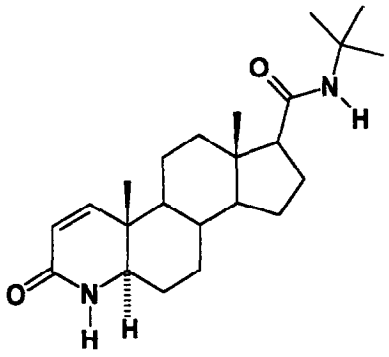


Fig 1. Structure of finasteride.

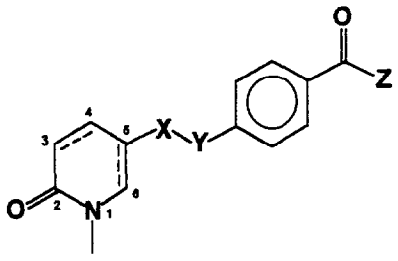


Chart 1. Structures of compounds **1–22**. X-Y: CO-NH, CH=CH (*E*/*Z*), CCH_3=CH (*E*/*Z*), CH=CCH_3 (*E*/*Z*), CH_2CH_2, $CHCH_3CH_2$, CH_2CHCH_3; Z: N(*i*-Pr)$_2$, NH(*t*-Bu).

1 inhibitors have been described very recently. The nonsteroidal compound LY 191704 has a K_i of 3 nM for type 1, and a K_i of 20 000 nM for type 2 [11].

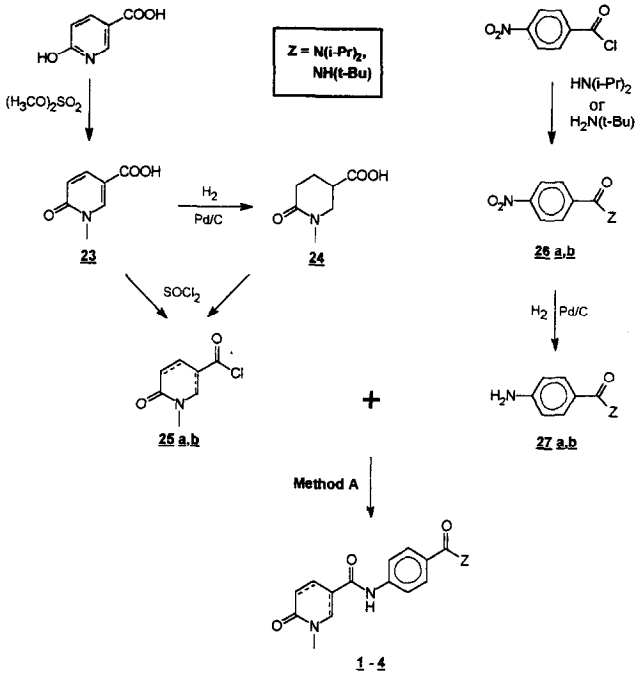
As it cannot be excluded that steroidal inhibitors cause side effects due to their steroidal structure (*eg* by interactions with steroid receptors), it is the aim of our group to develop nonsteroidal inhibitors. In the following the synthesis of 5-substituted 1-methyl-2-pyridones and 1-methyl-2-piperidones (chart 1) will be described and their 5α-R inhibitory properties will be reported using rat prostate and human prostatic carcinoma and BPH enzyme.

Chemistry

The 4-[(1-methyl-2-oxopiperidin-5-yl)amido]- and 4-[(1,2-dihydro-1-methyl-2-oxopyridin-5-yl)amido]-substituted benzamides[1] **1–4** (table I) were obtained by amidation of the carboxylic acid chlorides **25a, b** with the 4-amino-*N*-substituted benzamides **27a, b** (*Method A*, scheme 1).

For the preparation of the carboxylic acid chlorides **25a, b** 6-hydroxynicotinic acid was *N*-methylated to yield compound **23**. Treatment with $SOCl_2$ gave **25a**. For the synthesis of **25b** compound **23** was first hydrogenated using palladium on charcoal as a catalyst to yield the saturated carboxylic acid **24** and then treated with $SOCl_2$. The 4-amino-*N*-substituted benzamides **27a, b** were obtained by amidation of 4-nitrobenzoyl chloride with diisopropylamine and *tert*-butylamine, respectively, followed by catalytic hydrogenation using palladium on charcoal (scheme 1, table II).

For the preparation of the C-C-linked compounds **5–22** a convergent synthesis strategy was used. The key step was a Wittig-reaction of an aldehyde **30a** or ketone **30b** with the triphenylphosphonium salts **33a–d** (*Method B*, scheme 2). The obtained mixture of *E*/*Z*-isomers was separated to yield ***(E)*-5–*(E)*-10** and



Scheme 1.

[1]For didactic reasons compounds **1–4**, which are derivatives of 3-piperidinecarboxylic acid and 3-pyridinecarboxylic acid, respectively, are called substituted benzamides. The correct names are: *N*-[4-(*N*,*N*-diisopropylcarbamoyl)phenyl]-1-methyl-6-oxo-3-piperidinecarboxamide (**1**), *N*-[4-(*N*,*N*-diisopropylcarbamoyl)phenyl]-1,6-dihydro-1-methyl-6-oxo-3-pyridinecarboxamide (**2**), *N*-[4-(*N*-*tert*-butylcarbamoyl)phenyl]-1-methyl-6-oxo-3-piperidinecarboxamide (**3**) and N-[4-(*N*-*tert*-butylcarbamoyl)phenyl]-1,6-dihydro-1-methyl-6-oxo-3-pyridinecarboxamide (**4**).

(Z)-**5**–*(Z)*-**10** (table I). The configuration was determined by ^{1}H-NMR spectroscopy (in the case of compounds **5** and **8** by determination of the coupling constants of the vinyl protons, in the case of **6** and **9** by NOE experiments and in the case of **7** and **10** by increment system calculations [21]). In all cases the ethenyl proton(s), the heterocyclic ring protons and the protons of the *N*-methyl group of the *E*-isomers gave signals at a greater chemical shift than the corresponding protons of the *Z*-isomers (table III).

The oxo compounds **30a, b** were obtained from the corresponding protected pyridine derivatives **28a, b** by methylation using dimethyl sulfate and subsequent oxidation with $K_3[Fe(CN)_6]$ to yield **29a, b**. The latter compounds were treated with diluted HCl to cleave the protective group (scheme 2, table II). The oxidation of **28a** yielded **29a** and its isomer 1,2-dihydro-3-(ethylenedioxymethyl)-1-methyl-2(1*H*)-pyridone. From the mixture of isomers **29a** was separated by fractional crystallization.

The triphenylphosphonium salts **33a–d** were prepared from the *N*-substituted 4-alkyl-benzamides **31a–d** by bromination using *N*-bromosuccinimide and subsequent treatment of the resulting 4-(1-bromoalkyl) derivatives **32a–d** with triphenylphosphine (scheme 2, table II).

Depending on the time of reaction catalytic hydrogenation of the *E*-isomers *(E)*-**5**–*(E)*-**10** led to the 4-[2-(1,2-dihydro-1-methyl-2-oxo-pyridine-5-yl)ethylene]-substituted benzamides **11–16** (30–60 min, *Method C*) and to the 4-[2-(1-methyl-2-oxo-piperidine-5-yl)ethylene]-substituted benzamides **17–22** (24 h, *Method D*, scheme 2, table I).

Biological results and discussion

For the evaluation of the 5α-R inhibiting activity of the synthesized compounds an enzyme inhibition assay was established on the basis of a previously described procedure [22]. Since the prostatic enzyme preparation not only contained 5α-R, but also 17β-hydroxysteroid-dehydrogenase and 3α-ketosteroid-reductase, androstenedione (AD) and androstanediol (A) were formed in addition to dihydrotestosterone (DHT) during incubation of [1,2-^{3}H]testosterone (T) [23].

For the quantification of the radiolabelled steroids a reversed-phase HPLC separation procedure was performed similar to that described previously [24]. The approximate retention times were 7.5 min (AD), 10.0 min (T), 14.5 min (DHT), 19.5 min (A) and were highly reproducible.

As has been described [25], the formation of A depends on the NADPH concentration used in the assay. At high NADPH concentration, A is formed from DHT (fig 2A). At low NADPH concentration (50–200 μM), no A is formed (fig 2B). Figure 2C shows the reduction of DHT formation by 4-MA. This cornpound was used as standard 5α-R inhibitor in all incubations. 4-MA inhibited the rat enzyme by 50 ± 5% and the human enzyme by 80 ± 5% in a concentration of 20 nM.

In table IV, the % inhibition values of the test compounds are given. Only a limited number of compounds were tested using enzyme preparation from BPH, because the tissue is difficult to obtain. With the exception of compound *(Z)*-**5**, however, the values obtained with the two human sources of enzyme preparation are very similar.

On the other hand, there are a number of marked differences between inhibition values from enzyme

Table I. Physical and chemical data of the 4-substituted benzamides **1–22**, see chart 1 for structure.

No	unsat.	X-Y	Z	Formula[a]	MW	mp (°C)	Recryst. solvent[b]	Yield (%)
1		CO-NH	N(i-Pr)$_2$	$C_{20}H_{25}N_3O_3$	359.47	192-195	A	89
2	3-4, 5-6	CO-NH	N(i-Pr)$_2$	$C_{20}H_{25}N_3O_3$	355.44	305-306	B	46
3		CO-NH	NH(t-Bu)	$C_{18}H_{25}N_3O_3$	331.42	220-223	A	37
4	3-4, 5-6	CO-NH	NH(t-Bu)	$C_{18}H_{21}N_3O_3$	327.38	285-286	A	56
(E)-5	3-4, 5-6	CH=CH	N(i-Pr)$_2$	$C_{21}H_{26}N_2O_2$	338.45	179-181	A	19
(E)-6	3-4, 5-6	CCH$_3$=CH	N(i-Pr)$_2$	$C_{22}H_{28}N_2O_2$	352.48	184-185	A	26
(E)-7	3-4, 5-6	CH=CCH$_3$	N(i-Pr)$_2$	$C_{22}H_{28}N_2O_2$	352.48	137-140	C	10
(E)-8	3-4, 5-6	CH=CH	NH(t-Bu)	$C_{19}H_{22}N_2O_2$	310.40	268-270	C	26
(E)-9	3-4, 5-6	CCH$_3$=CH	NH(t-Bu)	$C_{20}H_{24}N_2O_2$	324.42	178-180	A	28
(E)-10	3-4, 5-6	CH=CCH$_3$	NH(t-Bu)	$C_{20}H_{24}N_2O_2$	324.42	200-203	B	59
(Z)-5	3-4, 5-6	CH=CH	N(i-Pr)$_2$	$C_{21}H_{26}N_2O_2$	338.45	134-136	A	22
(Z)-6	3-4, 5-6	CCH$_3$=CH	N(i-Pr)$_2$	$C_{22}H_{28}N_2O_2$	352.48	187-188	A	26
(Z)-7	3-4, 5-6	CH=CCH$_3$	N(i-Pr)$_2$	$C_{22}H_{28}N_2O_2$	352.48	167-170	C	5
(Z)-8	3-4, 5-6	CH=CH	NH(t-Bu)	$C_{19}H_{22}N_2O_2$	310.40	176-178	A	23
(Z)-9	3-4, 5-6	CCH$_3$=CH	NH(t-Bu)	$C_{20}H_{24}N_2O_2$	324.42	188.5-190	A	10
(Z)-10	3-4, 5-6	CH=CCH$_3$	NH(t-Bu)	$C_{20}H_{24}N_2O_2$	324.42	187-190	B	7
11	3-4, 5-6	CH$_2$-CH$_2$	N(i-Pr)$_2$	$C_{21}H_{28}N_2O_2$	340.47	110-112	-[c]	83
12	3-4, 5-6	CHCH$_3$-CH$_2$	N(i-Pr)$_2$	$C_{22}H_{30}N_2O_2$	354.49	99-101	-[c]	65
13	3-4, 5-6	CH$_2$-CHCH$_3$	N(i-Pr)$_2$	$C_{22}H_{30}N_2O_2$	354.49	oil	-[c]	73
14	3-4, 5-6	CH$_2$-CH$_2$	NH(t-Bu)	$C_{19}H_{24}N_2O_2$	312.41	140-143	-[c]	73
15	3-4, 5-6	CHCH$_3$-CH$_2$	NH(t-Bu)	$C_{20}H_{26}N_2O_2$	326.44	149-152	-[c]	77
16	3-4, 5-6	CH$_2$-CHCH$_3$	NH(t-Bu)	$C_{20}H_{26}N_2O_2$	326.44	136-139	-[c]	75
17		CH$_2$-CH$_2$	N(i-Pr)$_2$	$C_{21}H_{32}N_2O_2$	344.50	117-120	A	80
18		CHCH$_3$-CH$_2$	N(i-Pr)$_2$	$C_{22}H_{34}N_2O_2$	358.53	oil[d]	-	83
19		CH$_2$-CHCH$_3$	N(i-Pr)$_2$	$C_{22}H_{34}N_2O_2$	358.53	oil[d]	-	90
20		CH$_2$-CH$_2$	NH(t-Bu)	$C_{19}H_{28}N_2O_2$	316.45	128-130	A	57
21		CHCH$_3$-CH$_2$	NH(t-Bu)	$C_{20}H_{30}N_2O_2$	330.47	135-160[d]	A	59
22		CH$_2$-CHCH$_3$	NH(t-Bu)	$C_{20}H_{30}N_2O_2$	330.47	40-85[d]	A	89

[a]C, H and N analyses were within ± 0.4% of the theoretical values; oils were not analyzed; [b]A: ethyl acetate, B: acetone, C: petroleum ether (40–60°C)/acetone (1:2); [c]purified by chromatography on a silica-gel column using petroleum ether (40–60°C)/acetone (3:1) as eluent; [d]mixture of diastereomers.

Table II. Physical and chemical data of compounds **23**, **24**, **26**, **27**, **29**, **30**, **32** and **33**.

No	R_1	R_2	Z	Formula	MW	mp (°C)	Yield (%)	Lit.
23	-	-	-	$C_7H_7NO_3$	153.14	236-238	62	[17]
24	-	-	-	$C_7H_{11}NO_3$	157.17	191-193	70	[18]
26 a	-	-	N(i-Pr)$_2$	$C_{13}H_{18}N_2O_3$	250.30	144-144.5	76	
26 b	-	-	NH(t-Bu)	$C_{11}H_{14}N_2O_3$	222.24	158-161	87	[19]
27 a	-	-	N(i-Pr)$_2$	$C_{13}H_{20}N_2O$	220.32	169-172	70	
27 b	-	-	NH(t-Bu)	$C_{11}H_{16}N_2O$	192.26	128-129	90	[19]
29 a	H	-	-	$C_9H_{11}NO_3$	181.19	75-78	61	
29 b	CH_3	-	-	$C_{10}H_{13}NO_3$	196.22	91-93.5	78	[20]
30 a	H	-	-	$C_7H_7NO_2$	137.14	119-120	87	
30 b	CH_3	-	-	$C_8H_9NO_2$	163.18	147.5-148.5	90	[20]
32 a	-	H	N(i-Pr)$_2$	$C_{14}H_{20}BrNO$	298.23	101-103	34	
32 b	-	H	NH(t-Bu)	$C_{12}H_{16}BrNO$	270.18	144.5-146	33	
32 c	-	CH_3	N(i-Pr)$_2$	$C_{15}H_{22}BrNO$	312.25	55-58	86	
32 d	-	CH_3	NH(t-Bu)	$C_{13}H_{18}BrNO$	284.20	147-148	83	
33 a	-	H	N(i-Pr)$_2$	$C_{32}H_{33}BrNOP$	560.52	262-265	89	
33 b	-	H	NH(t-Bu)	$C_{30}H_{31}BrNOP$	532.47	> 300	84	
33 c	-	CH_3	N(i-Pr)$_2$	$C_{33}H_{37}BrNOP$	574.54	195-198	79	
33 d	-	CH_3	NH(t-Bu)	$C_{31}H_{33}BrNOP$	546.49	287-290	69	

preparations of rat and human origin: compounds ***(E)*-5**, ***(E)*-7**, ***(E)*-10**, ***(Z)*-5**, ***(Z)*-7**, **11–14**, **17** and **19** inhibited rat enzyme less than human enzyme, whereas compounds ***(Z)*-8** and **21** inhibited rat enzyme much more than human enzyme. This finding is in accordance with observations of Holt *et al* and Liang *et al* [13, 22]. Using 4-azasteroids as enzyme inhibitors, they reported differing 5α-R inhibitory activities depending on the source of enzyme preparation.

The test compounds show inhibition values from 0 to 99%. The amides **1–4** turned out to be inactive or exhibited only marginal activity (compound **2**; source: prostate carcinoma).

In the case of the unsaturated C-C linked compounds **5–10** the *E* configurated compounds ***(E)*-5–*(E)*-10** are generally stronger inhibitors than the corresponding *Z* isomers. Showing inhibition values below 30%, compound ***(E)*-8**, however, appeared to be a relatively weak inhibitor. In the case of rat prostatic enzyme, this compound is less active than ***(Z)*-8**. The diisopropyl compounds ***(E)*-5–*(E)*-7** and ***(Z)*-5–*(Z)*-7** are more active than the corresponding *tert*-butyl compounds ***(E)*-8–*(E)*-10** and ***(Z)*-8–*(Z)*-10**. With the exception of compound ***(E)*-8**, exchange of a vinyl hydrogen by a methyl group does not lead to an increase in inhibitory activity.

In the case of the saturated C-C linked compounds **11–22**, the pyridinones **11–16** are less active than the piperidinones **17–22**. In this class of compounds, the diisopropyl amides **11–13** and **17–19** are also superior to the *tert*-butyl amides **14–16** and **20–22**. With respect to the enzyme preparation from human prostate carcinoma, the introduction of a methyl group into the ethylene bridge decreases activity, whereas the same structural modification enhances inhibition of rat prostatic enzyme.

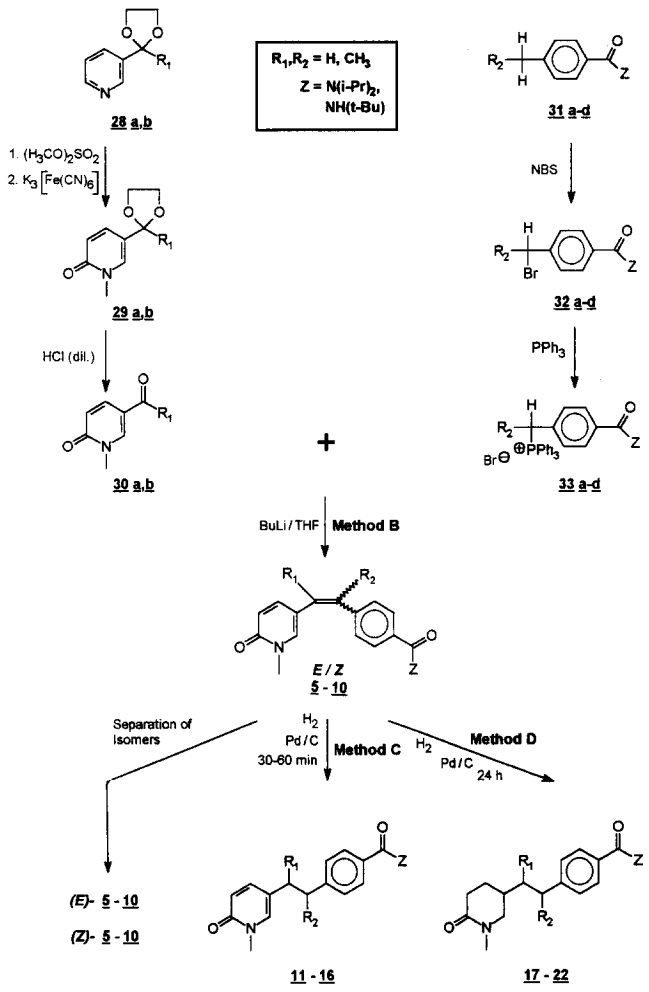
Summarizing, of the compounds described in this paper, the diisopropyl benzamides ***(E)*-5–*(E)*-7**, **17** and **18** are the most active inhibitors of the human enzyme, exhibiting inhibition values exceeding 85%. Their IC_{50} values are in the range of approximately 20 μM: **17** exhibits an IC_{50} value of 13 μM, ***(E)*-6** 19 μM, ***(E)*-5** 25 μM, ***(E)*-7** 26 μM and **18** 27 μM.

These compounds, however, are more than a 1000 times weaker inhibitors than the highly active ster-

Table III. Chemical shift of selected protons of the *E*/*Z*-isomers ***(E)*-5–*(E)*-10** and ***(Z)*-5–*(Z)*-10**[a].

Compound	*C_3-H*	*C_4-H*	*C_6-H*	*Vinyl-H*	*-N(CH_3)-*
***(E)*-5**	6.63	7.69	7.36	6.79 and 6.81	3.58
***(E)*-6**	6.61	7.65	7.40	6.70	3.60
***(E)*-7**	6.59	7.42	~7.30	6.46	3.58
***(E)*-8**	6.62	7.68	7.37	6.87 and 6.79	3.57
***(E)*-9**	6.61	7.64	7.41	6.72	3.60
***(E)*-10**	6.60	7.44	7.32	6.50	3.59
***(Z)*-5**	6.40	7.2–7.3	7.2–7.3	6.28 and 6.51	3.48
***(Z)*-6**	6.48	7.08–7.19	7.08–7.19	6.45	3.47
***(Z)*-7**	6.28	6.87	6.93	6.15	3.39
***(Z)*-8**	6.38	7.14	7.22	6.31 and 6.53	3.49
***(Z)*-9**	6.46	7.12	7.12	6.47	3.47
***(Z)*-10**	6.27	6.81	6.96	6.17	3.41

[a]δ: ppm (400 MHz, $CDCl_3$).



Scheme 2.

oidal inhibitors 4-MA and finasteride, which in our assay showed IC_{50} values of 5 and 3 nM, respectively, and are also weaker than the nonsteroidal compound ONO 3805 (IC_{50} 0.12 μM) [26]. Therefore no attempts to separate the enantiomers of compounds **11–22** were made. For the same reason we did not try to separate the mixture of two diastereomers in the case of **18**, **19**, **21** and **22**.

Because of the disappointing enzyme inhibitory activities we also refrained from determining the steroid receptor affinities of the test compounds.

One might suppose that a *meta*-substitution of the aromatic ring would more closely mimic the 17β-carboxamide substituents of the steroidal inhibitors mentioned above. The question is currently elucidated by evaluating the corresponding *meta*-substituted compounds. In the case of synthetic estrogens, however, it has been known for a long time that *para*-substitution results in a higher affinity for the estrogen receptor than *meta*-substitution (*meso*-hexestrol *vs* its *meta*-substituted analogue)[27].

One more interesting question remains to be clarified. The compounds described in this paper might show a stronger inhibition of type 1 5α-R compared with type 2 5α-R.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus (Reichert, Wien) and are uncorrected. Elemental analyses were performed by the section of Physical Chemistry, University of Saarland and were within ± 0.4% of the calculated values. ^{1}H-NMR spectra were measured on Bruker AW-80 (80 MHz) and Bruker AM-400 (400 MHz) spectrometer, using Me_4Si as

Table IV. Inhibition of 5α-reductase of different origins by test compounds **1–22** (PC: human prostatic carcinoma; BPH: benign prostatic hyperplasia; RVP: rat ventral prostate)[a].

No.	*unsat.*	*X-Y*	*Z*	*PC* [b]	*BPH* [c]	*RVP* [c]
				% inhibition [d]	*% inhibition*	*% inhibition*
1		CO-NH	N(i-Pr)$_2$	n.I.[e]		n.I.
2	3-4, 5-6	CO-NH	N(i-Pr)$_2$	8		n.I.
3		CO-NH	NH(t-Bu)	n.I.		n.I.
4	3-4, 5-6	CO-NH	NH(t-Bu)	n.I.		n.I.
(*E*)-5	3-4, 5-6	CH=CH	N(i-Pr)$_2$	90	89	62
(*E*)-6	3-4, 5-6	CCH$_3$=CH	N(i-Pr)$_2$	86	85	88
(*E*)-7	3-4, 5-6	CH=CCH$_3$	N(i-Pr)$_2$	85		52
(*E*)-8	3-4, 5-6	CH=CH	NH(t-Bu)	27	22	28
(*E*)-9	3-4, 5-6	CCH$_3$=CH	NH(t-Bu)	75	79	75
(*E*)-10	3-4, 5-6	CH=CCH$_3$	NH(t-Bu)	44		30
(*Z*)-5	3-4, 5-6	CH=CH	N(i-Pr)$_2$	34	46	24
(*Z*)-6	3-4, 5-6	CCH$_3$=CH	N(i-Pr)$_2$	22	19	21
(*Z*)-7	3-4, 5-6	CH=CCH$_3$	N(i-Pr)$_2$	37		22
(*Z*)-8	3-4, 5-6	CH=CH	NH(t-Bu)	20	19	56
(*Z*)-9	3-4, 5-6	CCH$_3$=CH	NH(t-Bu)	18	22	27
(*Z*)-10	3-4, 5-6	CH=CCH$_3$	NH(t-Bu)	27		28
11	3-4, 5-6	CH$_2$-CH$_2$	N(i-Pr)$_2$	82		31
12	3-4, 5-6	CHCH$_3$-CH$_2$	N(i-Pr)$_2$	70		44
13	3-4, 5-6	CH$_2$-CHCH$_3$	N(i-Pr)$_2$	59		37
14	3-4, 5-6	CH$_2$-CH$_2$	NH(t-Bu)	45		28
15	3-4, 5-6	CHCH$_3$-CH$_2$	NH(t-Bu)	23		31
16	3-4, 5-6	CH$_2$-CHCH$_3$	NH(t-Bu)	31		26
17		CH$_2$-CH$_2$	N(i-Pr)$_2$	92	99	83
18		CHCH$_3$-CH$_2$	N(i-Pr)$_2$	86	86	89
19		CH$_2$-CHCH$_3$	N(i-Pr)$_2$	56		44
20		CH$_2$-CH$_2$	NH(t-Bu)	57		50
21		CHCH$_3$-CH$_2$	NH(t-Bu)	50		72
22		CH$_2$-CHCH$_3$	NH(t-Bu)	24		17

[a]Concentration of inhibitor 100 μM; [b]mean value of 2–8 determinations. Each determination was run in duplicate (standard deviation < 10%); [c]each determination was run in duplicate; [d]calculation: see *Experimental protocols*; [e]nI: no inhibition, *ie* inhibition < 5%.

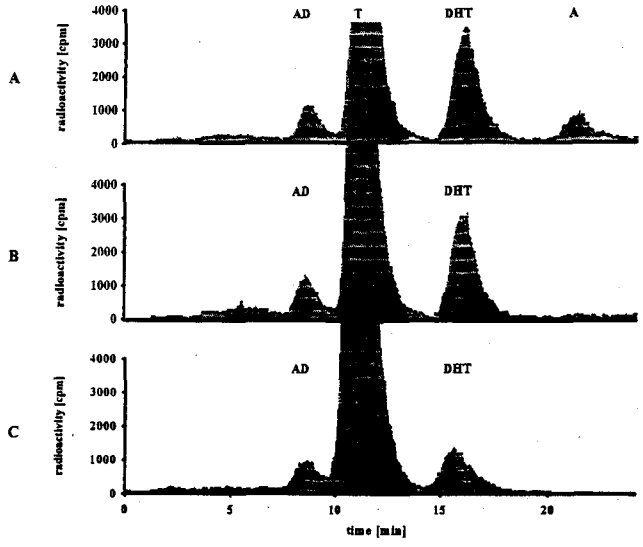


Fig 2. Conversion of [1,2-^{3}H]testosterone (T) by rat prostatic enzyme preparation: A) at high NADPH concentration (400 µM); B) at low NADPH concentration (50 µM); and C) in the presence of 5α-reductase inhibitor 4-MA (20 nM; NADPH 50 µM). AD: androstenedione, DHT: dihydrotestosterone, A: androstanediol. 1000 cpm is equivalent to approximately 11 fmol steroid/mg protein/min.

the internal standard. IR spectra were recorded as KBr pellets, as liquid films or as paraffin oil and poly(chlorotrifluoroethylene) oil films on a Perkin-Elmer infrared spectrometer 398; the values are expressed in cm^{-1}. Analytical TLC was carried out on 0.25 mm layer silica-gel plates (Macherey-Nagel, Alugram Sil G/UV_{254}, silica-gel 60) containing a fluorescent indicator; spots were detected under UV light (254 nm). The purification by column chromatography was performed on Macherey-Nagel silica-gel 60 (50–200 µm).

Method A. General procedure for the preparation of 4-[(1-methyl-2-oxopiperidine-5-yl)amido]- and 4-[(1,2 dihydro-1-methyl-2-oxopyridine-5-yl)amido]-substituted benzamides ***1–4***
The carboxylic acid chlorides **25a** and **25b**, prepared from 6 mmol of the carboxylic acids **23** and **24**, respectively, were dissolved in CH_2Cl_2 (30 ml) and slowly dropped into a solution of the amino derivative (6 mmol) in CH_2Cl_2 (30 ml). After completion, the mixture was stirred for 15 min at room temperature. Water (50 ml) was added and stirring continued for further 15 min. The layers were separated; the organic layer was washed with KOH (10%) and dried (anhydrous Na_2SO_4). The solvent was evaporated to dryness and the residue obtained was recrystallized from ethyl acetate.

N,N-Diisopropyl-4-[(1-methyl-2-oxopiperidine-5-yl)amido]benzamide ***1***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.33 (m; broad; 12H, 2 -CH(CH_3)$_2$), 2.00–2.18 (m; 2H, -CO-CH_2-CH_2-), 2.28–2.40 and 2.47–2.57 (2m; each 1H, -CO-CH_2-), 2.84–2.96 (m; 1H, C_5-H), 2.96 (s; 3H, -N(CH_3)-), 3.35–3.45 and 3.58–3.71 (2m; each 1H, -N(CH_3)-CH_2-), 3.70 (m; broad; 2H, 2 -CH(CH_3)$_2$), 7.08 and 7.26 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8.5 Hz), 9.71 (s; 1H, -NH-). IR (KBr): ν = 3250, 3180, 3090, 3040, 2960, 2930, 2880, 1690, 1620, 1610, 1600, 1525, 1440, 1400, 1380, 1370, 1340, 1035, 850, 840.

N,N-Diisopropyl-4-[(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)amido]benzamide ***2***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.32 (m; broad; 12H, 2 -CH(CH_3)$_2$), 3.63 (s; 3H, -N(CH_3)-), 3.72 (m; broad; 2H, 2 -CH(CH_3)$_2$), 5.56 (d; 1H, C_3-H, ^3J = 9.5 Hz), 7.00 and 7.19 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 7.5 Hz), 8.20 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz); 8.32 (d; 1H, C_6-H, ^4J = 2.5 Hz), 9.69 (s; 1H, -NH-). IR (KBr): ν = 3250, 3070, 2980, 2940, 1685, 1645, 1620, 1590, 1520, 1460, 1380, 1370, 1355, 1315, 1300, 1045, 840, 825.

N-tert-Butyl-4-[(1-methyl-2-oxopiperidine-5-yl)amido]benzamide ***3***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.47 (s; 9H, -C(CH_3)$_3$), 1.95–2.17 (m; 2H, -CO-CH_2-CH_2-), 2.19–2.34 and 2.42–2.55 (2m; each 1H, -CO-CH_2-), 2.82–3.01 (m; 1H, C_5-H), 2.93 (s; 3H, -N(CH_3)-), 3.35–3.45 and 3.60–3.72 (2m; each 1H, -N(CH_3)-CH_2-), 6.10 (s; 1H, -NH-C(CH_3)$_3$), 7.61 (s; 4H, 1,4-disubst arom H), 9.33 (s; 1H, -NH-C_6H_4-). IR (KBr): ν = 3250, 3180, 3095, 3040, 2960, 2920, 2860, 1685, 1630, 1620, 1600,1530, 1505, 1405, 1385, 1315, 850, 770.

N-tert-Butyl-4-[(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)amido]benzamide ***4***. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.38 (s; 9H, -C(CH_3)$_3$), 3.53 (s; 3H, -N(CH_3)-), 6.48 (d; 1H, C_3-H, ^3J = 9.5 Hz), 7.63 (s; 1H, -NH-C(CH_3)$_3$), 7.76 and 7.81 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 9 Hz), 7.98 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz); 8.55 (d; 1H, C_6-H, ^4J = 2.5 Hz), 10.12 (s; 1H, -NH-C_6H_4-). IR (KBr): ν = 3350, 3050, 3020, 2950, 2920, 1660, 1635, 1610, 1595, 1525, 1440, 1405, 1325, 835, 770.

Method B. General procedure for the preparation of 4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)ethenyl]-substituted benzamides ***(E) 5–10*** *and* ***(Z) 5–10***
Butyllithium (12 mmol, 0.77 g) was added to a suspension of triphenylphosphonium salt **33a–d** (11 mmol) in dry THF (50 ml) and the mixture was stirred for 30 min at room temperature. The aldehyde **30a** or ketone **30b** (11 mmol) dissolved in dry THF (50 ml) was dropped into the mixture and stirring was continued for 1 h. The solvent was evaporated and the residue was dissolved in a small volume of acetone. In some cases a sparingly soluble residue of *E*-isomer was left, which was recrystallized from ethyl acetate. The *E*/*Z*-isomers were separated by column chromatography using petroleum ether/acetone (1:1) as eluent and finally recrystallized from ethyl acetate.

(E)-N,N-Diisopropyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)ethenyl]benzamide ***(E)-5***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.98–1.69 (m; 12H, 2 -CH(CH_3)$_2$), 3.58 (s; 3H, -N(CH_3)-), 3.39–3.95 (m; 2H, 2 -CH(CH_3)$_2$), 6.63 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.79 and 6.81 (2d, 2H, vinyl-H, ^3J = 16.5 Hz), 7.30 and 7.43 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz), 7.36 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.69 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz). IR (KBr): ν = 2990, 2960, 2930, 1665, 1610, 1535, 1440, 1370, 1340, 1150, 830.

(E)-N,N-Diisopropyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-2-methylethenyl]benzamide ***(E)-6***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.87–1.80 (m; 12H, 2 -CH(CH_3)$_2$), 2.15 (s; 3H, vinyl CH_3), 3.30–4.03 (m; 2H, 2 -CH(CH_3)$_2$), 3.60 (s; 3H, -N(CH_3)-), 6.61 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.70 (s; 1H, vinyl-H), 7.31 (s; 4H, 1,4 disubst arom H), 7.40 (d, 1H, C_6-H, ^4J = 1.5 Hz), 7.65 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 1.5 Hz). IR

(KBr): ν = 3000, 2960, 2930, 1670, 1620, 1605, 1540, 1465, 1445, 1365, 1345, 1160, 1040, 865, 835.

(E)-N,N-Diisopropyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-1-methylethenyl]benzamide ***(E)-7***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.93–1.73 (m; 12H, 2 -CH(CH_3)$_2$), 2.22 (d; 3H, vinyl-CH_3, ^4J = 1 Hz), 3.38–3.98 (m; 2H, 2 -CH(CH_3)$_2$), 3.58 (s; 3H, -N(CH_3)-), 6.46 (s; 1H, vinyl-H), 6.59 (d; 1H, C_3-H, ^3J = 9.5 Hz), 7.25–7.44 (m; 6H, 1,4-disubst arom H, ^3J = 6.5 Hz, C_4-H, ^4J = 2.5 Hz, C_6-H). IR (KBr): ν = 3000, 2960, 2930, 1660, 1625, 1600, 1535, 1450, 1440, 1375, 1350, 1155, 1065, 850, 835, 825.

(E)-N-tert-Butyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)ethenyl]benzamide ***(E)-8***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.48 (s; 9H, -C(CH_3)$_3$), 3.57 (s; 3H, -N(CH_3)-), 6.00 (s; 1H, -NH-), 6.62 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.79 (d, 1H, vinyl-H, ^3J = 16 Hz), 6.85 (d; 1H, vinyl-H, ^3J = 16 Hz), 7.37 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.45 and 7.70 (AA'BB'; 4H, 1,4 disubst arom H, ^3J = 8 Hz), 7.69 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz). IR (KBr): ν = 3310, 3040, 2980, 2925, 1670, 1660, 1600, 1540, 1450, 950, 845, 835.

(E)-N-tert-Butyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-2-methylethenyl]benzamide ***(E)-9***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.49 (s; 9H, -C(CH_3)$_3$), 2.14 (d; 3H, vinyl-CH_3, ^4J = 1 Hz), 3.60 (s; 3H, -N(CH_3)-), 5.99 (s; 1H, -NH-), 6.61 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.72 (s; 1H, vinyl-H), 7.34 and 7.73 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz), 7.41 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.65 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz). IR (KBr): ν = 3300, 2960, 2910, 1660, 1605, 1590, 1540, 1440, 1315, 1155, 865, 825.

(E)-N-tert-Butyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-1-methylethenyl]benzamide ***(E)-10***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.48 (s; 9H, -C(CH_3)$_3$), 2.24 (d; 3H, vinyl-CH_3, ^4J = 1 Hz), 3.59 (s; 3H, -N(CH_3-), 5.98 (s; 1H, -NH-), 6.50 (s; 1H, vinyl-H), 6.60 (d; 1H, C_3-H, ^3J = 9.5 Hz), 7.32 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.44 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz), 7.48 and 7.72 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3280, 2960, 2930, 1660, 1640, 1590, 1535, 1310, 845, 830.

(Z)-N,N-Diisopropyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)ethenyl]benzamide ***(Z)-5***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.92–1.77 (m; 12H, 2 -CH(CH_3)$_2$), 3.48 (s; 3H, -N(CH_3)-), 3.38–3.95 (m; 2H, 2 -CH(CH_3)$_2$), 6.28 and 6.51 (2d, 2H, vinyl-H, ^3J = 12 Hz), 6.40 (d; 1H, C_3-H, ^3J = 10 Hz), 7.20–7.30 (m; 6H, 1,4-disubst arom H, C_4-H, C_6-H,). IR (KBr): ν = 2990, 2970, 2920, 1675, 1630, 1595, 1530, 1440, 1370, 1340, 1135, 835.

(Z)-N,N-Diisopropyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-2-methylethenyl]benzamide ***(Z)-6***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.96–1.75 (m; 12H, 2 -CH(CH_3)$_2$), 2.13 (d; 3H, vinyl-CH_3, ^4J = 1.5 Hz), 3.40–3.96 (m; 2H, 2 -CH(CH_3)$_2$), 3.47 (s; 3H, -N(CH_3)-), 6.45 (s; 1H, vinyl-H), 6.48 (d; 1H, C_3-H, ^3J = 9.5 Hz), 7.08–7.19 (m; 6H, 1,4-disubst arom H, C_4-H, C_6-H). IR (KBr): ν = 2990, 2960, 2920, 1660, 1625, 1600, 1525, 1435, 1340, 1155, 1030, 895, 835, 815.

(Z)-N,N-Diisopropyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-1-methylethenyl]benzamide ***(Z)-7***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.96–1.76 (m; 12H, 2 -CH(CH_3)$_3$), 2.16 (d; 3H, vinyl-CH_3, ^4J = 1 Hz), 3.39 (s; 3H, -N(CH_3)-), 3.45–3.95 (m; 2H, 2 -CH(CH_3)$_2$), 6.15 (s; 1H, vinyl-H), 6.28 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.87 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz), 7.20–7.28 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 2970, 2930, 1670, 1625, 1535, 1450, 1440, 1350, 835, 830.

(Z)-N-tert-Butyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)ethenyl]benzamide ***(Z)-8***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.48 (s; 9H, -C(CH_3)$_3$), 3.49 (s; 3H, -N(CH_3)-), 6.60 (s; 1H, -NH-), 6.31 (d, 1H, vinyl-H, ^3J = 12 Hz), 6.38 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.53 (d; 1H, vinyl-H, ^3J = 12 Hz), 7.14 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz), 7.22 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.30 and 7.66 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3280, 3050, 2970, 2920, 1660, 1630, 1595, 1540, 1440, 1315, 880, 830.

(Z)-N-tert-Butyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-2-methylethenyl]benzamide ***(Z)-9***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.46 (s; 9H, -C(CH_3)$_3$), 2.14 (d; 3H, vinyl-CH_3, ^4J = 1.5 Hz), 3.47 (s; 3H, -N(CH_3)-), 5.93 (s; 1H, -NH-), 6.46 (d; 1H, C_3-H, ^3J = 10 Hz), 6.47 (s; 1H, vinyl-H), 7.11–7.13 (m; 4H, AA'BB' (2H), 1,4-disubst arom H, C_4-H, C_6-H), 7.56 (d; 2H, AA'BB', 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3320, 2980, 2960, 2910, 1660, 1645, 1590, 1530, 1310, 1220, 835.

(Z)-N-tert-Butyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-1-methylethenyl]benzamide ***(Z)-10***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.48 (s; 9H, -C(CH_3)$_3$), 2.15 (d; 3H, vinyl-CH_3, ^4J = 1.5 Hz), 3.41 (s; 3H, -N(CH_3)-), 5.98 (s; 1H, -NH-), 6.17 (s; 1H, vinyl-H), 6.27 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.81 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz), 6.96 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.23 and 7.69 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3050, 2990, 2950, 2900, 1660, 1640, 1590, 1535, 1440, 1310, 885, 830.

Method C. General procedure for the prepration of 4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)ethylene]-substituted benzamides ***11–16***

The alkenes ***(E)*** **5–(*E*) 10** (1 mmol) were dissolved in ethanol (20 ml), mixed with palladium on charcoal (5%, 100 mg) as a catalyst and hydrogenated for 30–60 min at room temperature and atmospheric pressure. After filtration, the solvent was evaporated to dryness and the residue was purified by column chromatography with petroleum ether/acetone (3:1) as an eluent.

N,N-Diisopropyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)ethylene]benzamide ***11***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.93–1.78 (m; 12H, 2 -CH(CH_3)$_2$), 2.65 (t; 2H, -CH_2-, ^3J = 7.5 Hz), 2.83 (t; 2H, -CH_2-, ^3J = 7.5 Hz), 3.35–3.98 (m; 2H, 2 -CH(CH_3)$_2$), 3.47 (s; 3H, -N(CH_3)-), 6.54 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.93 (d, 1H, C_6-H, ^4J = 2,5 Hz), 7.12 and 7.24 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz), 7.20 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz). IR (KBr): ν = 3060, 2970, 2920, 1665, 1630, 1595, 1530, 1435, 1345, 1140, 825.

N,N-Diisopropyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-2-methylethylene]benzamide ***12***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.95–1.77 (m; 12H, 2 -CH(CH_3)$_2$), 1.20 (d; 3H, -CHCH_3-, ^3J = 6.5 Hz), 2.67–2.80 (m; 3H, -CH_2-, -$CH$$CH_3$-), 3.32–3.95 (m; 2H, 2 -CH(CH_3)$_2$), 3.46 (s; 3H, -N(CH_3)-), 6.56 (d; 1H, C_3-H, ^3J = 9 Hz), 6.86 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.03 and 7.20 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz), 7.27 (dd; 1H, C_4-H, ^3J = 9 Hz, ^4J = 2.5 Hz). IR (liquid film; ν = 4000–1320 in poly(chlorotrifluoroethylene) oil and ν = 1320–400 in paraffin): ν = 3040, 2970, 2930, 2920, 1670, 1655, 1615, 1595, 1540, 1440, 1350, 1135, 1035, 830.

N,N-Diisopropyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-2-methylethylene]benzamide ***13***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.97–1.76 (m; 12H, 2 -CH(CH_3)$_2$), 1.28 (d; 3H, -CHCH_3-, ^3J = 7 Hz), 2.52–2.66 (m; 2H, -CH_2-, ^3J = 7 Hz), 2.89 (sextet; 1H, -CHCH$_3$-, ^3J = 7 Hz), 3.39–4.02 (m; 2H, 2 -CH(CH$_3$)$_2$), 3.44 (s; 3H, -N(CH$_3$)-), 6.47 (d; 1H, C_3-H, ^3J = 9 Hz), 6.81 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.07 (dd; 1H, C_4-H, ^3J = 9 Hz, ^4J = 2.5 Hz), 7.12–7.23 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (liquid film; ν = 4000–1320 in poly(chlorotrifluoroethylene) oil and ν = 1320–400 in paraffin): ν = 3050, 2960, 2915, 1655, 1615, 1595, 1535, 1435, 1340, 1145, 1040, 835.

N-tert-Butyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl), ethylene]benzamide ***14***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.47 (s; 9H, -C(CH$_3$)$_3$), 2.66 (t; 2H, -CH_2-, ^3J = 7.5 Hz), 2.86 (t; 2H, -CH_2-, ^3J = 7.5 Hz), 3.46 (s; 3H, -N(CH$_3$)-), 5.96 (s; 1H, -NH-), 6.52 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.92 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.12–7.20 (m; 3H, 2H of a AA'BB'-system, 1,4-disubst arom H, ^3J = 8 Hz, C_4-H), 7.64 (d; 2H, 2H of an AA'BB'-system, ^3J = 8 Hz). IR (KBr): ν = 3310, 2970, 2930, 1665,1650, 1600, 1540, 1450, 1315, 1225, 840.

N-tert-Butyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-2-methylethylene]benzamide ***15***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.20 (d; 3H, -CHCH_3-, ^3J = 6 Hz), 1.47 (s; 9H, -C(CH$_3$)$_3$), 3.44 (s; 3H, -N(CH$_3$)-), 3.71–3.85 (m; 3H, -CH_2-, -CHCH$_3$-), 5.94 (s; 1H, -NH-), 6.55 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.84 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.06 and 7.60 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz), 7.24 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz). IR (KBr): ν = 3300, 3020, 2950, 2910, 1665, 1650, 1600, 1540, 1440, 1315, 1220, 1150, 840.

N-tert-Butyl-4-[2-(1,2-dihydro-1-methyl-2-oxopyridine-5-yl)-1-methylethylene]benzamide ***16***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.20 (d; 3H, -CHCH_3-, ^3J = 7 Hz), 1.47 (s; 9H, -C(CH$_3$)$_3$), 2.59 (d; 2H, -CH_2, ^3J = 7.4 Hz), 2.93 (sextet; 1H, -CHCH$_3$-, ^3J = 7 Hz) 3.43 (s; 3H, -N(CH$_3$)-), 5.94 (s; 1H, -NH-), 6.46 (d; 1H, C_3-H, ^3J = 9.5 Hz), 6.82 (d, 1H, C_6-H, ^4J = 2.5 Hz), 7.04 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz), 7.15 and 7.64 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3300, 3040, 2970, 2930, 1665, 1595, 1540, 1445, 1310, 1235, 1155, 860, 830.

Method D. General procedure for the preparation of 4-[2-(1-methyl-2-oxopiperidine-5-yl)ethylene-substituted benzamides ***17–22***

The alkenes *(E)* **5**–*(E)* **10** (1 mmol) were dissolved in ethanol (20 ml), mixed with palladium on charcoal (5%, 100 mg) as a catalyst and hydrogenated for 24 h at room temperature and atmospheric pressure. After filtration, the solvent was evaporated to dryness and the residue was recrystallized from ethyl acetate. After hydrogenation, a mixture of diastereomers was obtained in the ratio of 1:1 (**18**, **19**, **22**) and 1:2 (**21**).

N,N-Diisopropyl-4-[2-(1-methyl-2-oxopiperidine-5-yl)ethylene]benzamide ***17***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.74–1.77 (m; 15H, 2 -CH(CH_3)$_2$, -CH_2-CH_2-Ph, -CH_2-CH-CH_2-), 1.80–2.00 (m; 2H, -CO-CH_2-CH_2-), 2.27–2.38 and 2.42–2.52 (2m; 2H, -COCH_2-), 2.59–2.78 (m; 2H, CH_2-Ph-), 2.93 (s; 3H, -N(CH$_3$)-), 3.00 and 3.25–3.33 (t; 1H, ^3J = 10 Hz, and m; 1H, -N(CH$_3$)-CH_2-), 3.36–4.02 (m; 2H, 2 -CH(CH$_3$)$_2$), 7.18 and 7.25 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 2960, 2930, 2900, 1640, 1625, 1450, 1370, 1345, 1225, 1215, 1040, 850, 800.

N,N-Diisopropyl-4-[2-(1-methyl-2-oxopiperidine-5-yl)-2-methylethylene]benzamide ***18***. ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of diastereomers): δ = 0.86 and 0.87 (d; 3H, -CHCH_3-CH_2-Ph-, ^3J = 6 Hz), 0.92–1.70 (m; 13H, 2 -CH(CH$_3$)$_2$), -N(CH$_3$)-CH$_2$-CH-), 1.71–1.98 (m; 3H, -CO-CH_2-CH_2-, -CHCH$_3$-CH_2-Ph-), 2.25–2.47 (m; 2H, -CO-CH_2-), 2.49–2.63 and 2.67–2.86 (2m; 2H, -CH_2-Ph-), 2.95 and 2.97 (s; 3H, -N(CH$_3$)), 3.08–3.32 (m; 2H, -N(CH$_3$)-CH_2-), 3.36–4.07(m; 2H, 2 -CH(CH$_3$)$_2$), 7.14 and 7.25 (AA'BB'; 4H, 1,4-disubst arom H, ^3J ~ 8 Hz). IR KBr): ν = 3030, 2960, 2920, 2870, 1640, 1630, 1440, 1370, 1335, 1135, 1035, 800.

N,N-Diisopropyl-4-[2-(1-methyl-2-oxopiperidine-5-yl)-1-methylethylene]benzamide ***19***. ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of diastereomers): δ = 0.87–1.97 (m; 20H, 2 -CH(CH_3)$_2$, -N(CH$_3$)-CH$_2$-CH-, -CO-CH_2-CH_2-, -CH_2CH$_3$-Ph-, at 1.25 and 1.27 d; -CHCH_3-Ph-, ^3J = 7 Hz), 2.19–2.32 and 2.38–2.47 (2m; 2H, -CO-CH_2-), 2.72–2.85 (m; 1H, -CHCH$_3$-Ph-), 2.85–3.02, 3.07–3.15 and 3.23–3.31 (3m; 2H, -N(CH$_3$)-CH_2-), 2.87 and 2.91, (s; 3H, -N(CH$_3$)-), 3.46–4.05 (m; 2H, 2 -CH(CH$_3$)$_2$), 7.17 and 7.27 (2m; 4H, 1,4-disubst arom H). IR (liquid film; ν = 4000–1320 in poly(chlorotrifluoroethylene) oil and ν = 1320–400 in paraffin): ν = 2970, 2930, 1650, 1635, 1445, 1380, 1340, 1210, 1135, 1035, 830.

N-tert-Butyl-4-[2-(1-methyl-2-oxopiperidine-5-yl)ethylene]-benzamide ***20***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.47 (s; 9H, -C(CH$_3$)$_3$), 1.43–1.56 (m; 1H, -N(CH$_3$)-CH$_2$-CH-), 1.65 (q; 2H, -CH_2-CH_2-Ph-, ^3J = 8 Hz), 1.78–1.99 (m; 2H, -CO-CH_2-CH_2-), 2.25–2.36 and 2.42–2.52 (2m; 2H, -CO-CH_2-), 2.63–2.78 (m; 2H, -CH_2-Ph-), 2.92 (s; 3H, -N(CH$_3$)-), 2.97–3.02 and 3.25–3.30 (2m; 2H, -N(CH$_3$)-CH_2-), 5.93 (s; 1H, -NH-), 7.21 and 7.66 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3270, 3060, 2970, 2930, 2900, 1660, 1630, 1555, 1450, 1320, 1225, 855, 845, 775, 675.

N-tert-Butyl-4-[2-(1-methyl-2-oxopiperidine-5-yl)-2-methylethylene]benzamide ***21***. ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of diastereomers): δ = 0.84–0.86 (d, 3H, -CHCH_3-CH_2-Ph-), 1.42–1.68 (m; 1H, -N(CH$_3$)-CH$_2$-CH-), 1.47 (s; 9H, -C(CH$_3$)$_3$), 1.72–1.97 (m; 2H, -CO-CH_2-CH_2-), 2.22–2.56 and 2.72–2.88 (2m, 4H, -CO-CH_2-, -CH_2-Ph-), 2.92 and 2.95 (s; 3H, -N(CH$_3$)-), 3.09–3.29 (m; 2H, -N(CH$_3$)-CH_2-), 5.93 (s; 1H, -NH-), 7.18 and 7.66 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3330, 2960, 2920, 1655, 1630, 1535, 1500, 1450, 1305, 1220, 875, 745.

N-tert-Butyl-4-[2-(1-methyl-2-oxopiperidine-5-yl)-1-methylethylene]benzamide ***22***. ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of diastereomers): δ = 1.25 and 1.27, (d; 3H, -CHCH_3-Ph-), 1.37–1.52 (m; 1H, -N(CH$_3$)-CH$_2$-CH-), 1.47 (s; 9H, -C(CH$_3$)$_3$), 1.52–1.80 and 1.88–1.97 (m; 4H, -CH_2-CHCH$_3$-Ph-, -CO-CH_2-CH_2-), 2.16–2.47 (m; 2H, -CO-CH_2-), 2.75–3.01, 3.06–3.14 and 3.22–3.30, (3m; 3H, -CHCH$_3$-Ph-, -N(CH$_3$)-CH_2-), 2.86 and 2.90 (s; 3H, -N(CH$_3$)-), 5.96 (s; 1H, -NH-), 7.19 and 7.68 (m and d (AA'BB'); 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (liquid film) ν = 3320, 3030, 2950, 2910, 1655, 1640, 1630, 1530, 1500, 1450, 1300, 1220, 850, 770.

1,6-Dihydro-1-methyl-6-oxopyridine-3-carboxylic acid ***23*** *and 1-methyl-6-oxopiperidine-3-carboxylic acid* ***24***

Compounds **23** and **24** were prepared according to previously described methods [17, 18].

1,6-Dihydro-1-methyl-6-oxopyridine-3-carboxylic acid chloride ***25a*** *and 1-methyl-6-oxopiperidine-3-carboxylic acid chloride* ***25b***

Compound **23** or **24** (65 mmol) was stirred with 3 ml (42 mmol, 5 g) $SOCl_2$ (for **25a**: 60 min, reflux; for **25b**: 10 min, room

temperature). Excess $SOCl_2$ was removed by distillation under reduced pressure. The solid carboxylic acid chlorides were used without further purification or characterization.

N-Substituted 4-nitrobenzamides ***26a, b***
A solution of the amine (32 mmol) in benzene (30 ml) was added dropwise to the solution of 4-nitrobenzoyl chloride (16 mmol, 3 g) in benzene (30 ml). After stirring for 3 h, 50 ml H_2O was added and the mixture was stirred for further 30 min. The layers were separated; the organic layer was washed with KOH (10%), dried (anhydrous Na_2SO_4) and the solvent was evaporated to dryness. The crude product was recrystallized from petroleum ether.

N,N-Diisopropyl-4-nitrobenzamide ***26a***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.34 (d; 12H, 2 -CH(CH_3)$_2$, ^3J = 7 Hz), 3.61 (sept; 2H, 2 -C*H*(CH_3)$_2$, ^3J = 7 Hz), 7.48 and 8.25 (AA'BB', 4H, 1,4-disubst arom H, ^3J = 9 Hz). IR (KBr): ν = 3040, 2980, 2940, 1635, 1605, 1525, 1455, 1445, 1370, 1350, 1040, 835, 720.

N-tert-Butyl-4-nitrobenzamide ***26b***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.45 (s; 9H, $(CH_3)_3$), 6.05 (s, broad; 1H, NH), 7.81 and 8.11 (AA'BB', 4H, 1,4-disubst arom H, ^3J = 9 Hz). IR (KBr): ν = 3320, 3070, 2970, 2940, 2880, 1670, 1645, 1605, 1550, 1530, 1370, 1350, 1330, 1315, 1225, 870, 850, 730.

4-Amino-N-substituted benzamides ***27a, b***
Palladium on charcoal (5%, 200 mg) was added to a solution of the amide **26** (4 mmol) in ethanol (25 ml). The mixture was hydrogenated for 24 h at room temperature. The catalyst was removed by filtration and the solvent evaporated to dryness. The crude product was recrystallized from petroleum ether.

4-Amino-N,N-diisopropylbenzamide ***27a***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.30 (d; 12H, 2 -CH(CH_3)$_2$, ^3J = 7 Hz), 3.42–4.02 (m; 4H, -NH_2 and 2 C*H*(CH_3)$_2$, ^3J = 7 Hz), 6.59 and 7.13 (AA'BB', 4H, 1,4-disubst arom H, ^3J = 9 Hz). IR (KBr): ν = 3460, 3350, 3210, 3030, 2970, 2950, 2930, 1630, 1615, 1590, 1450, 1380, 1370, 1345, 1300, 1035, 840, 590.

4-Amino-N-tert-butylbenzamide ***27b***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.44 (s; 9H, -C(CH_3)$_3$), 4.02 (s, broad; 2H, NH_2), 5.81 (s, broad; 1H, -NH-), 6.60 and 7.52 (AA'BB', 4H, 1,4-disubst arom H, ^3J = 9 Hz). IR (KBr): ν = 3340, 3220, 3060, 3030, 2960, 2920, 1710, 1640, 1605, 1550, 1450, 1390, 1365, 1295, 1220, 1185, 840, 770.

1,2-Dihydro-5-[(ethylenedioxy)methyl]-1-methyl-2(1H)-pyridone ***29a*** *and 1,2-dihydro-5-(ethylenedioxy)-ethyl]-1-methyl-2(1H)-pyridone* ***29b***
Dimethyl sulfate (0.42 mmol, 53 g) was slowly added dropwise to 0.42 mmol 3-(ethylenedioxymethyl)pyridine **28a** (63.5 g) or 3-(ethylenedioxyethyl)pyridine (69.4 g) **28b**, and heated for 1 h on a steam bath. The product was dissolved in H_2O (150 ml) and oxidized by adding an aqueous solution of $K_3[Fe(CN)_6]$ (1 mol, 329.3 g) in H_2O (1000 ml) under stirring and cooling. KOH pellets (3.36 mol, 188.5 g) were added slowly, keeping the temperature at 5–10°C. After adding CH_2Cl_2 (250 ml), the mixture was stirred for 30 min, before additional portions of $K_3[Fe(CN)_6]$ (0.45 mol, 148 g) in H_2O (450 ml) and KOH pellets (1.6 mol, 90 g) were added at room temperature. The mixture was left standing overnight. Filtered from the solid, the layers were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried (anhydrous Na_2SO_4), filtered and evaporated to dryness. In case of **29a** an oily residue was obtained, which was a mixture of **29a** (72%) and 1,2-dihydro-3-(ethylenedioxymethyl)-1-methyl-2(1*H*)pyridone (28%). The oil was crystallized and the isomers separated by actional crystallization from petroleum ether/acetone (1:1). In the case of the synthesis of **29b** only one product was obtained as an oil which was crystallized and purified by recrystallization from *n*-hexane.

1,2-Dihydro-5-[ethylenedioxy)methyl]-1-methyl-2(1H)-pyridone ***29a***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.54 (s; 3H, -N(CH_3)-), 3.98–4.12 (m; 4H, -CH_2-CH_2-), 5.57 (s; 1H, -C*H*(-O-CH_2-CH_2-O-)-), 6.59 (d; 1H, C_3-H, ^3J= 10 Hz), 7.41–7.44 (m; 2H, C_4-H, C_6-H). IR (KBr): ν = 3080, 2980, 2905, 2870, 1670, 1630, 1595, 1550, 1440, 1270, 1095, 1045, 950, 915, 845.

1,2-Dihydro-5-[(1,1-ethylenedioxy)ethyl]-1-methyl-2(1H)-pyridone ***29b***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.57 (s; 3H, -C(CH_3)-), 3.52 (s; 3H, -N(CH_3)-), 3.68–4.15 (m; 4H -CH_2-CH_2-), 6.52 (d; 1H C_3-H, ^3J = 10 Hz), 7.30–7.48 (m; 2H C_4-H, C_6-H). IR (KBr): ν = 3080, 3040, 2980, 2950, 2895, 1670, 1610, 1545, 1435, 1280, 1225, 1210, 1030, 875, 850.

5-Formyl-1,2-dihydro-1-methyl-2(1H)-pyridone ***30a*** *and 5-acetyl-1,2-dihydro-1-methyl-2(1H)-pyridone* ***30b***
Compound **29a** or **29b** (120 mmol) was heated in dilute HCl (3%, 250 ml) on a steam bath for 5 h. After cooling, the solution was extracted with CH_2Cl_2. The organic layer was dried (anhydrous Na_2SO_4), filtered and the solvent was evaporated to dryness. The crude product was recrystallized from ethyl acetate.

5-Formyl-1,2-dihydro-1-methyl-2(1H)-pyridone ***30a***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 3.63 (s; 3H -N(CH_3)-), 6.60 (d; 1H, C_3-H, ^3J = 9 Hz), 7.82 (dd; 1H, C_4-H, ^3J = 9 Hz, ^4J = 2.5 Hz), 7.94 (d; 1H, C_6-H, ^4J = 2.5 Hz), 9.62 s; 1H -CHO). IR (KBr): ν = 3070, 3040, 2830, 1665, 1615, 1545, 1450, 1395, 1330, 1260, 1150, 935, 830, 640, 455.

5-Acetyl-1,2-dihydro-1-methyl-2(1H)-pyridone ***30b***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 2.43 (s; 3H -$COCH_3$), 3.60 (s; 3H, -N(CH_3)-), 6.53 (d; 1H, C_3-H, ^3J = 9.5 Hz), 7.86 (dd; 1H, C_4-H, ^3J = 9.5 Hz, ^4J = 2.5 Hz), 8.13 (d; 1H, C_6-H, ^4J = 2.5 Hz). IR (KBr) ν = 3060, 3030, 2960, 2920 1660, 1615, 1595, 1545, 1440, 1305, 1155, 1115, 960, 845, 595.

4-(1-Bromoalkyl)-N-substituted-benzamides ***32a–d***
The 4-alkyl-*N*-substituted-benzamides **31a–d** (50 mmol), *N*-bromosuccinimide (50 mmol, 8.9 g) and a small amount of dibenzoylperoxide were heated under reflux in CCl_4 (150 ml) for 30–60 min under stirring. After cooling, H_2O (150 ml) was added and the mixture was stirred for additional 30 min. The layers were separated, the organic layer was dried (anhydrous Na_2SO_4), filtered and the solvent evaporated to dryness. The oily residue was crystallized and purified by recrystallization from petroleum ether.

4-Bromomethyl-N,N-diisopropylbenzamide ***32a***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.32 (d, 12H, 2 -CH(C*H*$_3$)$_2$, ^3J = 7 Hz), 3.68 (septet; 2H, 2 -C*H*(CH_3)$_2$, ^3J = 7 Hz), 4.48 (s; 2H, Br-C*H*$_2$-), 7.26 and 7.41 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 7 Hz). IR (KBr): ν = 2970, 2930, 2870, 1625, 1445, 1370, 1340, 1035, 845, 800, 595, 560.

4-Bromomethyl-N-tert-butylbenzamide ***32b***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.45 (s; 9H, -C(CH_3)$_3$), 4.47 (s; 2H, Br-CH_2-), 5.92 (s, broad; 1H, -NH-), 7.40 and 7.68 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3330, 3060, 2970, 2930,

1645, 1635, 1545, 1500, 1445, 1360, 1325, 1310, 1225, 850, 590.

(±)-4-(1-Bromoethyl)-N,N-diisopropylbenzamide ***32c***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.33 (d, 12H, 2 -CH(CH_3)$_2$, ^3J = 7 Hz), 2.02 (d; 3H, H_3C-CHBr-, ^3J = 7 Hz), 3.68 (septet; 2H, 2 -C*H*(CH_3)$_2$, ^3J = 7 Hz), 5.19 (q; 1H, H_3C-C*H*Br-, ^3J = 7 Hz), 7.26 and 7.44 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3030, 2960, 2870, 1630, 1440, 1370, 1335, 1035, 830.

(±)-4-(1-Bromoethyl)-N-tert-butylbenzamide ***32d***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.44 (s; 9H -C(CH_3)$_3$), 2.01 (d; 3H, H_3C-CHBr-, ^3J = 7 Hz), 5.18 (q; 1H, H_3C-C*H*Br-, ^3J = 7 Hz), 5.94 (s, broad; 1H, -NH-), 7.43 and 7.68 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz). IR (KBr): ν = 3290, 2970, 2950, 2920, 2880, 1640, 1630, 1545, 1530, 1215, 850, 765.

Phosphonium salts [4-(N,N-diisopropylcarbamoyl)phenylmethyl]triphenylphosphonium bromide ***33a*** *and [4-(N-tert-butylcarbamoyl)phenylmethyl]triphenylphosphonium bromide* ***33b***

4-Bromomethylbenzamide **32a** or **32b** (30 mmol) and 30 mmol (7.9 g) of triphenylphosphine were heated in dry acetone under reflux for 2–3 h. The precipitated salt was removed by filtration and dried.

[4-(N,N-Diisopropylcarbamoyl)phenylmethyl]triphenylphosphonium bromide ***33a***. ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.28 (d; 12H, 2 -CH(CH_3)$_2$, ^3J = 7 Hz), 3.60 (septet; 2H, 2 -CH(CH_3)$_2$, ^3J = 7 Hz), 5.56 (d; 2H, -CH_2-, $^2J_{(PH)}$ = 14.5 Hz), 6.99–7.28 (m; 4H, 1,4-disubst arom H), 7.47–7.96 (m; 15H, -PPh_3). IR (KBr): ν = 3030, 2970, 2930, 2860, 2780, 1620, 1435, 1380, 1365, 1340, 1110, 860, 720, 680, 500.

[4-(N-tert-Butylcarbamoyl)phenylmethyl]triphenylphosphonium bromide ***33b***. ^{1}H-NMR (80 MHz, DMSO): δ = 1.42 (s; 9H, -C(CH_3)$_3$), 5.19 (d; 2H, -CH_3-, $^2J_{(PH)}$ = 15 Hz), 6.83 (s; 1H, -NH-), 7.09 (dd; 2H, AA'BB' 1,4-disubst arom H, ^3J = 9 Hz, ^4J = 2.5 Hz), 7.50–7.98 (m; 17H, AA'BB' (2H), 1,4-disubst arom H, -PPh_3). IR (KBr): ν = 3250, 3040, 3000, 2960, 2870, 2850, 1645, 1635, 1520, 1495, 1435, 1315, 1220, 1110, 850, 745, 715, 685, 620, 500, 490.

Phosphonium salts {1-[4-(N,N diisopropylcarbamoyl)phenyl]-ethyl}triphenylphosphonium bromide ***33c*** *and {1-4-(N-tert-butylcarbamoyl)phenyl]ethyl}triphenylphosphonium bromide* ***33d***

4-(1-Bromoethyl)benzamides **32c** or **32d** (30 mmol) and 30 mmol (7.9 g) of triphenylphosphine were melted together for 3–4 h. The cold substance was pulverized, washed several times with acetone and dried.

{1-[4-(N,N-Diisopropylcarbamoyl)phenyl]ethyl}triphenylphosphonium bromide ***33c***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.80–1.76 (m; 12H, 2 -CH(CH_3)$_2$), 1.84 (dd; 3H, H_3C-CH(PPh_3)-, $^3J_{(PCCH)}$ = 19 Hz, $^3J_{(HH)}$ = 7 Hz), 3.37–3.82 (m; 2H, 2 -C*H*(CH_3)$_2$), 6.91 (q; 1H, H_3C-C*H*(PPh_3)-, $^3J_{(HH)}$ = 7 Hz), 7.13–7.24 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz), 7.60–7.96 (m; 15H,-PPh_3). IR (KBr): ν = 3030, 2960, 2930, 2840, 1625, 1435, 1370, 1340, 1100, 755, 720, 690, 520.

{1-[4-(N-tert-Butylcarbamoyl)phenyl]ethyl}triphenylphosphonium bromide ***33d***. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.46 (s; 9H, -C(CH_3)$_3$), 1.78 (dd; 3H, H_3C-CH(PPh_3)-, $^3J_{(PCCH)}$ = 19 Hz, $^3J_{(HH)}$ = 7 Hz), 6.59 (q; 1H, H_3C-C*H*(PPh_3)-, $^3J_{(HH)}$ = 7 Hz), 6.65 (s; 1H, -NH-), 7.18 and 7.61 (AA'BB'; 4H, 1,4-disubst arom H, ^3J = 8 Hz, ^4J = 2 Hz), 7.65–7.94 (m; 15H, -PPh_3). IR (KBr): ν = 3270, 3250, 3040, 2980, 2960, 2830, 1645, 1635, 1610, 1525, 1500, 1430, 1300, 1215, 1105, 855, 745, 725, 700, 525, 515.

Enzyme inhibition test

Reagents

[1,2-^{3}H]Androstenedione (4-androstene-3,17-dione, AD), [1,2-^{3}H]testosterone (17β-hydoxy-4-androstene-3-one, T), [4-^{14}C]-dihydrotestosterone (17β-hydroxy-5α-androstane-3-one, DHT) and [1,2-^{3}H]androstanediol (5α-androstane-3α,17β-diol, A) were purchased from DuPont, Bad Homburg, Germany. Quickszint Flow 302 was fom Zinsser Analytic, Frankfurt, Germany. 4-MA (*N,N*-diethyl-4-methyl-3-oxo-4-aza-5α-androstane-17β-carboxamide) was a gift from Merck, Sharpe & Dome, Rahway, NJ, USA. All other reagents were of biochemical or analytical grade.

Methanol and water for HPLC were glass-distilled and degassed prior to use. HPLC was performed on a reversed-phase 125 x 3 mm ID column and 11 x 3 mm ID precolumn (Nukleosil C_8 3 µm, Macherey & Nagel, Düren, Germany).

Preparation of tissue

Rat prostatic enzyme preparation was obtained according to the procedure of Liang *et al* [22]. Male rats were sacrificed by ether overdose; the prostates were taken within 5 min of death and put in ice-cold 0.9% NaCl solution. All the following operations were performed at 0–4°C. The prostates were dissected free from fat and connective tissue, cut into pieces and weighed. Per 1g of tissue, 3 ml of 20 mM phosphate buffer pH 6.5 containing 0.32 mM sucrose and 1 mM DTT were added. The tissue was homogenized by ten 10-s strokes at 20 500 rpm of an ultraturrax (IKA) in 60-s intervals, filtered through cheesecloth and centrifuged for 60 min at 105 000 *g* in a Beckmann ultracentrifuge. The pellet obtained was resuspended in phosphate buffer. The centrifugation was repeated, the final pellet resuspended in a minimum volume of phosphate buffer and stored in 300-µl portions at –70°C. Human prostatic tissue from cancer or BPH patients was processed in the same way. The 105 000 *g* pellet suspension contains nuclei, mitochondria and microsomes and is referred to as the enzyme preparation. The protein content was determined by the method of Lowry *et al* [28] and was in the range of 15-25 mg/ml.

Incubation procedure

The assay was performed in the same way as the procedure of Liang *et al* [22]. All values were run in duplicate. The incubation was carried out for 30 min at 37°C in a total volume of 250 µl. In the case of rat enzyme preparation phosphate buffer (40 mM, pH 6.6) and in the case of human enzyme preparation citrate buffer (40 mM, pH 5.5) was used. The incubation mixture contained approximately 250 µg rat protein or 125 µg human protein, 50–200 µM NADPH, 0.2 µM T including 45nCi [1,2-^{3}H]-T, and 2% dimethyl sulfoxide with or without test compound (100 µM). The reaction was started by adding the prostatic enzyme preparation and stopped by addition of 50 µl $HgCl_2$-solution (5 mM). The steroids were extracted using 0.5 ml of diethylether. After centrifugation (5 min) the water layer was frozen and the ether layer was decanted in fresh tubes and evaporated to dryness.

HPLC procedure

Steroid separation was carried out similar to the method of Cook *et al* [24]. The steroids were dissolved in 25 µl methanol

and injected into the computer-controlled HPLC system, which was checked beforehand with the labelled reference compounds. Radioactivity was measured using a Benthold LB 506C monitor.

Using a methanol/water mixture (1:1, w/w) with a flow of 0.6 ml/min and an additive flow of 1.5 ml of scintillator, baseline-separation of AD, T, DHT and A was achieved within 24 min (fig 2).

Calculation procedure

The amount of DHT formed was calculated (% DHT). The zero value was subtracted from the control (cv) and inhibition (iv) value (cv_{corr} and iv_{corr}). Inhibition (I) was calculated using the following equation:

$$\%I = (1 - iv_{corr}/cv_{corr})100$$

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