Eur. J. Med. Chem. 35 (2000) 931–940

PII: S0223-5234(00)01167-3/FLA

Original article

6-Substituted 1*H*-quinolin-2-ones and 2-methoxy-quinolines: synthesis and evaluation as inhibitors of steroid 5α reductases types 1 and 2

Eckhard Baston, Anja Palusczak, Rolf W. Hartmann*

Fachrichtung 8.5 Pharmazeutische und Medizinische Chemie, Universität des Saarlandes, P.O. Box 15 11 50, D-66041 Saarbrücken, Germany

Received 25 January 2000; revised 20 April 2000; accepted 26 April 2000

Abstract – A Negishi-type coupling reaction between 6-bromo-2-methoxyquinoline (**1a**) and various 4-bromo-*N*,*N*-dialkyl-benzamides gave access to 6-substituted 2-methoxy-quinolines **1**-**3** and 1*H*-quinolin-2-ones **4**–**12**. Most of these compound proved to be inhibitors of steroid 5α reductases with activity and selectivity both being strongly dependent on the features of the heterocycle and the size of the *N*,*N*-dialkylamide substituent. The most active inhibitor for the human type 2 isozyme was 6-[4-(*N*,*N*-diisopropylcarbamoyl)phenyl]-1*H*-quinolin-2-one **4** (K_i 800 ± 85 nM), showing mostly competitive inhibitory patterns. A type 1 selective inhibitor could be identified with 6-[4-(*N*,*N*-diisopropylcarbamoyl)phenyl]-*N*-methyl-quinolin-2-one (**5**, IC_{50} 510 nM). © 2000 Éditions scientifiques et médicales Elsevier SAS

1*H*-quinolin-2-ones / methoxyquinolines / nonsteroidal inhibitors / 5α reductase isozymes 1 and 2

1. Introduction

Recognition of the importance of 5α dihydrotestosterone (DHT) in many endocrine diseases such as benign prostatic hyperplasia (BPH) [1, 2], male pattern baldness [3] or cystic acne [4] has stimulated efforts to synthesize inhibitors of steroid 5α reductase (EC 1.3.99.5) [5]. This enzyme catalyzes the irreversible reduction of testosterone (T) to the more potent tissue-specific androgen DHT [6]. On the basis of pathological studies of males genetically deficient in this enzyme, selective blockade of DHT biosynthesis is expected to provide a potential treatment for the aforementioned disorders [7, 8].

The discovery of two isozymes (named type 1 and type 2) and the determination of their tissue distribution has enhanced the prospects for the development of more specific drugs for certain diseases [9]. Dual acting inhibitors are now considered to be advantageous for the treatment of BPH and possibly prostate cancer and type 1 selective compounds for the systemic and topical therapy of skin disorders.

Several categories of steroidal and nonsteroidal compounds that inhibit 5α reductase have been reported, among them Finasteride (Proscar®), Epristeride or LY 191704 [4, 10, 11]. All these compounds have structural features in common, which enable them to interfere with the enzyme's electrophilic residue, that promotes enolization of T and thus permits the transfer of an hydride into the 5α position of the substrate. Finasteride and LY 191704, both possessing a lactam moiety as A-ring, can compete with the natural substrate via the enol form of this carboxamide structure.

Our attention has been drawn to nonsteroidal inhibitors in view of the possible side effects of steroidal compounds. Based on the transition state paradigm, that besides Finasteride also led to the development of 4-MA [5] as at least in vitro very potent inhibitor of steroid 5α reductase, we opted for the synthesis and biochemical evaluation of 6-aryl substituted 1*H*-

* Correspondence and reprints:
E-mail address: rwh@rz.uni-sb.de (R.W. Hartmann).

quinolin-2-ones and 2-methoxy-quinolines (*figure 1*). These structures can be considered as conformationally restricted analogues of pyridone derivatives of type **A**, that were recently reported by our group to be mediocre inhibitors of the human type 2 isozyme (**A**, IC_{50} 13 μM) [12].

2. Chemistry

6-Bromo-2-methoxyquinoline (**1a**) was chosen as key intermediate (*figure 2*). This compound seemed very attractive as a starting point, as it also allowed the derivatization to *N*-nonalkylated and hydro-

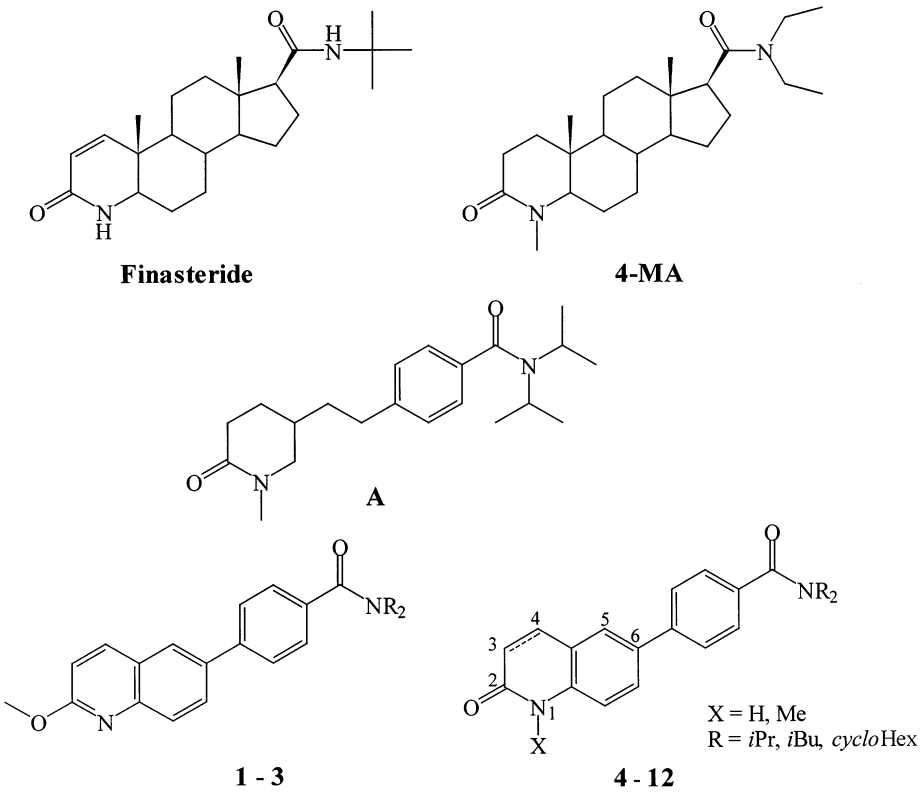


Figure 1. 5α-Reductase inhibitors and general structure of title compounds.

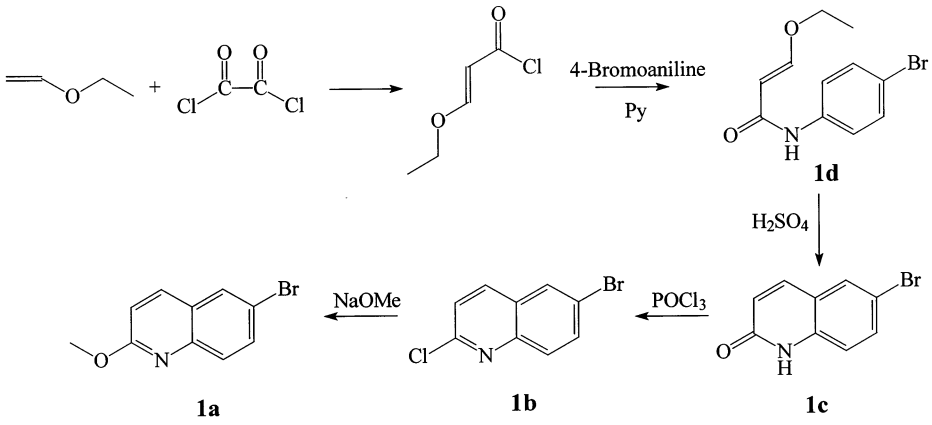


Figure 2. Synthesis of key intermediate **1a**.

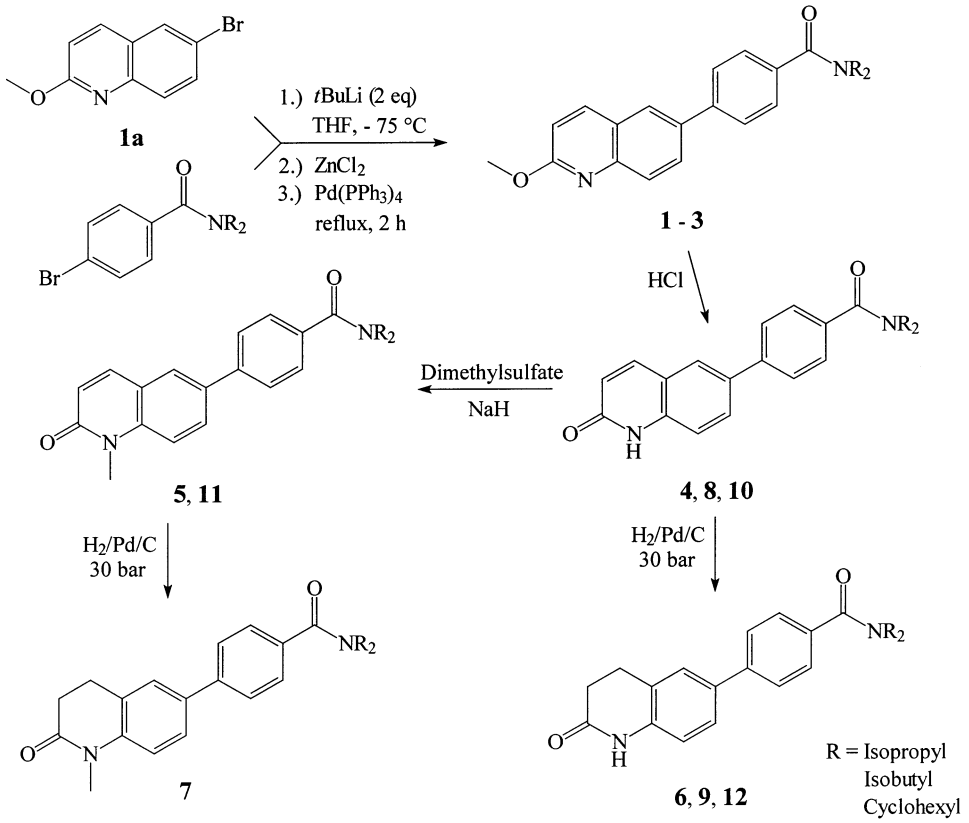


Figure 3. Synthesis of the title compounds **1–12.**

genated analogues, that cannot easily be obtained directly from quinoline derivatives.

E-Ethoxyacryloyl chloride, which was prepared in excellent yield according to a method of Tietze et al. [13] from ethylvinylether and oxalyl dichloride, was condensed with 4-bromoaniline to give amide **1d**. This was cyclized using concentrated sulfuric acid, yielding 6-bromo-1*H*-quinolin-2-one (**1c**). Treatment of **1c** with phosphorusoxolylchloride and replacement of the chlorine substituent in **1b** by a methoxy group using sodium methanolate, gave the desired 6-bromo-2-methoxyquinoline (**1a**) in excellent overall yield (43 %, 4 steps).

From **1a** a Negishi-type coupling reaction [14, 15] with various 4-bromobenzamides was performed and gave the 6-aryl-substituted 2-methoxyquinolines **1**–**3** in high yield (*figure 3*). The required benzamides were synthesized according to a general procedure using 4-bromobenzoic acid and the corresponding amines [16]. Subsequent hydrolysis with hydrochloric acid (6 M) afforded 1*H*-quinolin-2-ones **4**, **8**, **10** that were either methylated using dimethylsulfate (**5**, **11**) or hydrogenated under pressure (**6**, **9**, **12**). Hydrogenation of the methyl compound **5** resulted in compound **7**.

3. Pharmacology

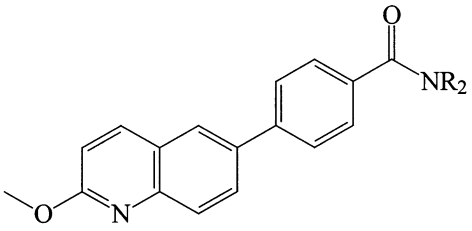
All newly synthesized compounds were tested for inhibitory potency versus 5α reductases using prostate homogenates (pH 5.5, human type 2), the DU 145 cell line (human type 1) and rat ventral prostate (pH 6.6, type 1). The percentage inhibition values of the compounds at a concentration of 10 μM are presented, in case of highly active compounds IC_{50} values (*table I*). Experiments using the steroidal compounds Finasteride and 4-MA have been performed in parallel. The data obtained are also presented in *table I*.

4. Results and discussion

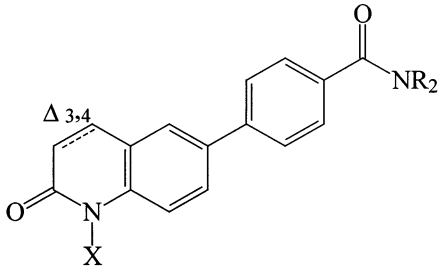
2-Methoxyquinoline derivatives **1**–**3** (*table I*), that can be considered as 'frozen' enol tautomers of e.g. compound **5** do not display any inhibitory potency. This result might be ascribed to a steric hindrance caused by the methyl group, that prevents the oxygen lone pair electrons from interacting with the enzyme's electrophilic residue.

Quinoline derivative **4** shows a good inhibitory potency for the human type 2 isozyme, without displaying considerable inhibition of the human type 1 isoform. Surprisingly *N*-methylation of **4** leads to a complete loss of type 2 activity but a strong increase

Table I. Inhibition of human and rat steroid 5α reductases by 6-substituted 2-methoxy quinolines **1**–**3** and 6-substituted 1*H*-quinolin-2-ones **4**–**12**.



1 - 3

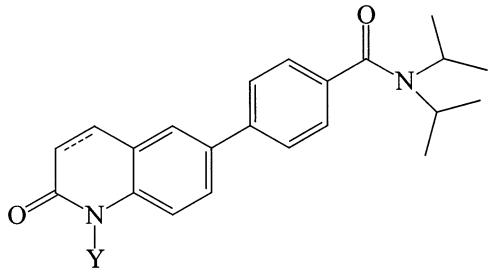


4 - 12

Compound	X	Δ	R	Human BPH[a,c] type 2 % inh. (10 μM) [iC_{50} (nM)][e]	Human DU 145[b] type 1 % inh. (10 μM) [iC_{50} (nM)][e]	Rat[c,d] % inh (10 μM) [iC_{50} (nM)][e]
1	–	–	isopropyl	n.i.[f]	n.d.[g]	n.i.
2	–	–	isobutyl	n.i.	n.d.	n.i.
3	–	–	cyclohexyl	n.i.	n.d.	n.i.
4	H	$\Delta_{3,4}$	isopropyl	83 [1700]	27	65
5	Me	$\Delta_{3,4}$	isopropyl	9	87 [510]	55
6	H		isopropyl	35	28	55
7	Me		isopropyl	n.i.	30	28
8	H	$\Delta_{3,4}$	isobutyl	70 [4850]	73	79
9	H		isobutyl	52	47	74
10	H	$\Delta_{3,4}$	cyclohexyl	63 [7660]	82 [2160]	95 [1470]
11	Me	$\Delta_{3,4}$	cyclohexyl	n.i.	36	6
12	H		cyclohexyl	30	78 [2740]	93 [1320]
4 MA				[4]	[7]	[16]
Finasteride	–	–		[3]	[45]	[11]

[a] Human prostate homogenates, pH 5.5: mainly type 2 isozyme is active.
[b] Human prostatic carcinoma of the brain (DU 145 cell line expressing type 1 isozyme), substrate ^{3}H androstenedione 5 nM.
[c] Substrate 1β2β ^{3}H testosterone, 210 nM.
[d] Rat ventral prostate, pH 6.6.
[e] The indicated values represent the mean of at least two experiments; relative standard deviation for IC_{50}-values <20 %; absolute standard deviation for percent inhibition <10 %.
[f] No inhibition.
[g] Not determined.

Table II. ^{13}C-NMR chemical shifts of C-2 of 6-[4-(*N*,*N*-diisopropylcarbamoyl) phenyl] -substituted quinolinones **4**–**7**[a].



Compound	Δ	Y	δ C-2 (ppm)
4	$\Delta_{3,4}$	H	164.4
5	$\Delta_{3,4}$	CH_3	162.1
6		H	170.8
7		CH_3	170.1

[a] Solvent: $CDCl_3$; concentration 25 mg/mL.

of type 1 activity (compound **5** type 1: IC_{50} 510 nM). However, no compound reaches the activity of the steroidal references which are approximately two orders of magnitude more potent than **4** and **5**. The corresponding hydrogenated analogues **6** and **7** are less active for both isoforms than the parent compounds. Although these findings do not correlate well with results obtained from steroidal inhibitors [17], this observation supports the important role of the carboxamide function for enzyme inhibitory activity. Alkylation of the nitrogen can either disrupt or enhance this interaction probably due to additional steric effects. On the basis of calculated electron populations for the heterocycles quinolone and *N*-methylquinolone [18], electronic changes caused by *N*-methylation, that could disfavour amide resonance, can be excluded. This is in accordance with the small observed changes in ^{13}C-NMR chemical shifts at C-2 for compounds **4**–**7** (*table II*).

In the series of compounds **8**–**12** we then examined the influence of altering the size of the amide substituent. Compounds **8** and **10** with the bulkier diisobutylamide or dicyclohexylamide substituent are more active for the human type 1 isoform in comparison to compound **4** with the smaller diisopropylamide group. On the other hand, these structural modifications lead to a decrease of activity for the type 2 isoform. Very surprisingly *N*-methylation of **10** leads not only to a reduction of activity for the type 2 isozyme, but also for the type 1 isozyme. In comparison to compounds **4** and **5** an increase in activity was expected, but with **11** no potent inhibitor was obtained.

The inhibitory data for the rat enzyme neither shows a good correlation with human type 1 nor type 2 enzyme. Especially compound **5**, which was a rather

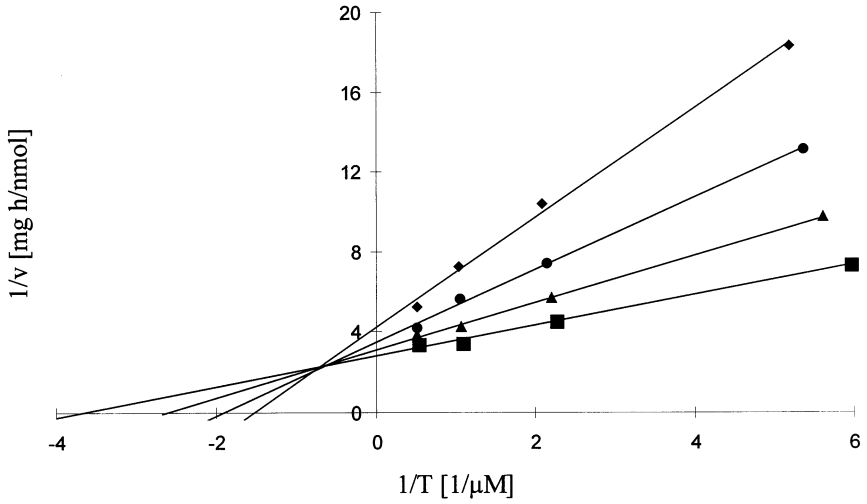


Figure 4. Determination of the type of inhibition of 6-[4-(*N*,*N*-diisopropylcarbamoyl) phenyl]-1*H*-quinolin-2-one **(4)** versus human type 2 5α-reductase. For details concerning the determination of the K_i-value and the type of inhibition, see experimental section (K_m Testosterone: 800–1000 nM, K_i, competitive (**4**): 800 ± 85 nM, α = 5.2). The indicated data points represent the mean of at least 3 experiments. Inhibitor concentrations: --◆-- 2000 nM, --●-- 1000 nM, --▲-- 500 nM, --■-- control; testosterone concentrations: 0.21 μM, 0.51 μM, 1.01 μM, 2.01 μM.

potent inhibitor of the human type 1 isozyme (IC_{50} 510 nM) but exhibits only moderate activity for the rat enzyme ($IC_{50} \approx 10$ μM). These species differences reveal the difficulties using rat enzyme for an initial inhibitor evaluation. The inhibitory nature of this series of compounds for human type 2 5α reductase was further evaluated. Quinolinone derivative **4**, the most potent inhibitor for this isozyme shows a mixed type of inhibition (competitive/noncompetitive) with predominant competitive inhibitory pattern ($K_i = 800 \pm 85$ nM, K_m (testosterone): 800–1000 nM, *figure 4*) in analogy to the 4-azasteroids [17, 19].

Physicochemical measurements with **4** reveal a pK_s value of > 12, confirming that at physiological conditions no ionization takes place [20].

5. Conclusions

In conclusion, the present paper presents a new class of nonsteroidal inhibitors of 5α reductase isozymes 1 and 2. Furthermore, it was shown that the results obtained with steroidal inhibitors (especially with respect to modifications of the steroidal A-ring) cannot be directly translated into this series of quinoline derivatives. With compound **5** we identified a new highly active type 1 selective inhibitor of steroid 5α reductase, which might be a lead for drug development.

6. Experimental protocols

6.1. Chemistry

Unless otherwise indicated, materials obtained from commercial suppliers were used without further purification. Solvents for reactions under anhydrous conditions were dried according to standard procedures [21]. All reactions, except those involving water as a reagent, were conducted under nitrogen atmosphere. Melting points were measured on a Reichert thermometer hot stage microscope and are uncorrected. Silica gel TLC and column chromatography were performed on a Merck TLC 60F-254 (0.25 mm) and a Merck Kieselgel 60. IR spectra were determined with a Perkin Elmer infrared spectrometer 398 (KBr). ^{1}H-NMR spectra were determined on a Bruker AM 400 at 400 MHz in d_6-Me_2SO (DMSO) solution and chemical shifts are reported in δ parts per million downfield from tetramethylsilane. Elemental analysis indicated by the symbols of the elements were within ± 0.4 % of the theoretical values and were performed in the Institut für Anorganische Chemie, Universität des Saarlandes, Saarbrücken.

6.1.1. *N-(4-Bromophenyl)-(E)-3-ethoxyacrylamide* ***(1d)***

(*E*)-3-Ethoxyacryloyl chloride (47 g, 0.35 mol) was slowly added to a solution of 4-bromoaniline (51.6 g, 0.3 mol) in dichloromethane (400 mL) and pyridine (50 mL) and stirring was continued for 1.5 h at 25 °C. The precipitated yellow solid was filtered off and repeatedly washed with water (3 × 100 mL). Upon evaporation of the mother liquids further product fractions of varying purity were obtained, that were recrystallized from petroleum ether (40–60 °C)/acetone of increasing polarity. Yield: 81 %, pale yellow needles, mp: 165–166 °C. ^{1}H-NMR: δ 1.27 (t; 3H, $^3J = 7.08$ Hz, $\underline{CH_3}CH_2$); 3.95 (q; 2H, $^3J = 7.08$ Hz, $CH_3\underline{CH_2}$); 5.50 (d; $\overline{1H}$, $^3J = 12.40$ Hz, O–$\underline{CH}$=CH); 7.49 (d; 1H, $^3J = 12.40$ Hz, O–CH=$\underline{CH}$); 7.46 u. 7.58 (AA′BB′; 4H, $^3J = 8.84$ Hz, aromat. H).

6.1.2. *6-Bromo-1H-quinolin-2-one* ***(1c)***

N-(4-Bromophenyl)-(*E*)-3-ethoxyacrylamide (**1d**) (54 g, 0.2 mol) was gradually added to concentrated sulfuric acid (550 mL). After stirring at 25 °C for 3 h, the mixture was cautiously poured onto crushed ice (1.5 kg). The precipitated product was filtered off, washed with water (5 × 100 mL) and recrystallized from ethylacetate/methanol (95/5). Yield: 83 %, colourless crystals, mp: 275 °C (Lit. [22]: mp: 269–271 °C). ^{1}H-NMR: δ 6.55 (d; 1H, $^3J = 9.32$ Hz, H-3); 7.25 (d; 1H, $^3J = 8.84$ Hz, H-8); 7.63 (dd; 1H, $^3J = 8.62$ Hz, $^4J = 1.76$ Hz, H-7); 7.87 (d; 1H, $^3J = 9.72$ Hz, H-4); 7.92 (d; 1H, $^4J = 1.76$ Hz, H-5); 11.84 (s; 1H, NH). Anal. C_9H_6BrNO (C, H, N).

6.1.3. *6-Bromo-2-chloroquinoline* ***(1b)***

6-Bromo-1*H*-quinoline-2-one (**1c**) (34 g, 0.15 mol) was suspended in phosphorusoxylchloride (120 mL) and heated under reflux for 1 h. After cooling to 25 °C, the solvent was evaporated and the remaining residue dissolved in chloroform (200 mL) and poured onto crushed ice (500 g). The mixture was neutralized with a saturated ammonia solution, the phases were separated and the aqueous phase was extracted with chloroform (2 × 150 mL). The combined organic layers were dried over magnesium sulfate and the solvent was evaporated to give a solid, that was recrystallized from hexane/ethylacetate of increasing polarity. Yield: 83 %, colourless crys-

tals, mp: 165 °C (Lit. [23]: mp: 157 °C). ^{1}H-NMR: δ 7.66 (d; 1H, $^3J = 8.40$ Hz, H-3); 7.90 (d; 1H, $^3J = 8.84$ Hz, H-8); 7.95 (dd; 1H, $^3J = 9.06$ Hz, $^4J = 2.20$ Hz, H-7); 8.36 (d; 1H, $^4J = 2.20$ Hz, H-5); 8.44 (d; 1H, $^3J = 8.40$ Hz, H-4). Anal. C_9H_5BrClN (C, H, N).

*6.1.4. 6-Bromo-2-methoxyquinoline (**1a**)*

Sodium (0.5 g, 22 mmol) was dissolved in dry methanol (20 mL) and then a suspension of 6-bromo-2-chloroquinoline (**1b**) (4 g, 16.5 mmol) in methanol (20 mL) was added within 10 min. The mixture was refluxed for 17 h and, after cooling to 25 °C, the solvent was distilled off. The remaining residue was treated with water (50 mL) and chloroform (120 mL), the phases were separated and the aqueous layer was extracted with chloroform (2 × 50 mL). The combined organic extracts were dried over magnesium sulfate, the solvent was evaporated and the remaining solid was recrystallized from petroleum ether (40–60 °C). Yield: 77 %, colourless crystals, mp: 98 °C. IR: ν 3140, 2880, 1695, 1680, 1425, 1280, 1255, 1200, 930, 890, 810. ^{1}H-NMR δ 3.99 (s; 3H, OCH_3); 7.08 (d; 1H, $^3J = 8.84$ Hz, H-3); 7.71 (d; 1H, $^3J = 8.84$ Hz, H-8); 7.78 (dd; 1H, $^3J = 9.06$ Hz, $^4J = 1.76$ Hz, H-7); 8.17 (d; 1H, $^4J = 1.76$ Hz, H-5); 8.22 (d; 1H, $^3J = 8.84$ Hz, H-4). Anal. $C_{10}H_8BrNO$ (C, H, N).

*6.1.4.1. 6-[4-(N,N-Diisopropylcarbamoyl) phenyl]-2-methoxy-quinoline (**1**)*

6-Bromo-2-methoxy-quinoline (**1a**) was dissolved in tetrahydrofuran (40 mL) and cooled to −75 °C. Then *t*-butyllithium (14 mmol, 8.75 mL of a 1.6 M solution in pentane) was injected via a syringe within 5 min. After 10 min carefully dried zinc(II)chloride (0.94 g, 7 mmol) was added and the mixture was warmed to 25 °C. 4-Bromo-*N*,*N*-diisopropylbenzamide (1.99 g, 7 mmol) and tetrakis(triphenylphosphine)palladium (0) (100 mg) were added and the mixture was heated under reflux for 2 h. After cooling, the solvent was distilled off and the residue was treated with water (50 mL) and chloroform (100 mL). The phases were separated and the aqueous phase was extracted with chloroform (2 × 50 mL). The combined organic layers were dried over magnesium sulfate, the solvents were evaporated and the crude product obtained was purified by column chromatography using hexane/ethylacetate of increasing polarity. Yield: 63 %, white powder, mp: 136–138 °C. IR: ν 2960, 1630, 1610, 1485, 1440, 1395, 1380, 1340, 1285, 1245, 1215, 1040, 1020, 900, 860, 840, 830, 770. ^{1}H-NMR: δ 1.31 (m; 12H, $CH(\underline{CH_3})_2$); 3.69 (s; 2H, $\underline{CH}(CH_3)_2$); 4.01 (s; 3H, OCH_3); 7.06 (d; 1H, $^3J = 8.84$ Hz, H-3); 7.40 (d; 2H, $^3J = 7.96$ Hz, 2H of AA′BB′ system); 7.85 (m; 3H, H-8 and 2H of AA′BB′ system); 8.02 (d; 1H, $^3J = 8.40$ Hz, H-7); 8.24 (s; 1H, H-5); 8.30 (d; 1H, $^3J = 8.84$ Hz, H-4). Anal. $C_{23}H_{26}N_2O_2$ (C, H, N).

*6.1.4.2. 6-[4-(N,N-Diisobutylcarbamoyl) phenyl]-2-methoxy-quinoline (**2**)*

Prepared in a similar way as described for **1** from 6-bromo-2-methoxyquinoline (**1a**) and 4-bromo-*N*,*N*-diisobutylbenzamide. The compound was purified by a double recrystallization from hexane. Yield: 52 %, colourless crystals, mp: 122 °C. IR: ν 2960, 2870, 1625, 1610, 1480, 1420, 1395, 1285, 1265, 1110, 1020, 830. ^{1}H-NMR: δ 0.70 and 0.95 (2s; 12H, $CH(\underline{CH_3})_2$); 1.84 and 2.07 (2s; 2H, $\underline{CH}(CH_3)_2$); 3.14 and 3.32 (2s; 4H, $\underline{CH_2}CH(CH_3)_2$); 4.01 (s; 3H, OCH_3); 7.07 (d; 1H, $^3J = 8.84$ Hz, H-3); 7.44 (d; 2H, $^3J = 7.96$ Hz, 2H of AA′BB′ system); 7.86 (m; 3H, H-5 and 2H of AA′BB′ system); 8.03 (d; 1H, $^3J = 8.40$ Hz, H-7); 8.26 (s; 1H, H-8); 8.31 (d; 1H, $^3J = 8.84$ Hz, H-4). Anal. $C_{25}H_{30}N_2O_2$ (C, H, N).

*6.1.4.3. 6-[4-(N,N-Dicyclohexylcarbamoyl) phenyl]-2-methoxy-quinoline (**3**)*

Prepared in a similar way as described for **1** from 6-bromo-2-methoxyquinoline (**1a**) and 4-bromo-*N*,*N*-dicyclohexylbenzamide. The compound was purified by recrystallization from hexane. Yield: 58 %, colourless solid, mp: 195 °C. IR: ν 2860, 2830, 1640, 1610, 1480, 1435, 1370, 1250, 1130, 1030, 900, 840. ^{1}H-NMR: δ 1.08–1.71 (m; 20H, Cyclohexyl H); 3.30 (s; 2H, $\underline{CH}(CH_2)_2$); 4.01 (s; 3H, OCH_3); 7.07 (d; 1H, $^3J = 8.84$ Hz, H-3); 7.39 (d; 2H, $^3J = 7.96$ Hz, 2H of AA′BB′ system); 7.85 (m; 3H, H-8, 2H of AA′BB′-system); 8.04 (d; 1H, $^3J = 8.84$ Hz, H-7); 8.27 (s; 1H, H-5); 8.31 (d; 1H, $^3J = 9.28$ Hz, H-4). Anal. $C_{29}H_{34}N_2O_2$ (C, H, N).

*6.1.4.4. 6-[4-(N,N-Diisopropylcarbamoyl) phenyl]-1H-quinolin-2-one (**4**)*

6-[4-(*N*,*N*-Diisopropylcarbamoyl)phenyl]-2-methoxy-quinoline (**1**) (1.0 g, 2.8 mmol) in hydrochloric acid (6 M, 10 mL) was heated under reflux for 5 h. After being cooled to 25 °C, water (50 mL) was added and the pH adjusted to 9 with sodium hydroxide. The mixture was extracted with chloroform (3 × 50 mL) and the combined organic layers were dried over magnesium sulfate. Evaporation of the solvent gave a residue, that was purified by recrystallization from ethylacetate. Yield: 41 %, pale yellow crystals, mp: 246–247 °C. IR: ν 3140,

2960, 1660, 1620, 1430, 1375, 1335, 1260, 1210, 820, 765, 540, 520. ^{1}H-NMR δ 1.39 (m; 12H, CH($\underline{CH_3}$)$_2$); 3.68 (s; 2H, $\underline{CH}$(CH$_3$)$_2$); 6.55 (d; 1H, $^3J = 9.76$ Hz, H-3); 7.39 (m; 3H, H-8 und 2H of AA′BB′ system); 7.74 (d; 2H, $^3J = 8.40$ Hz, 2H of AA′BB′ system); 7.87 (dd; 1H, $^3J = 8.84$ Hz, $^4J = 1.76$ Hz, H-7); 7.97 (d; 1H, $^3J = 9.72$ Hz, H-4); 8.03 (s; 1H, H-5); 11.85 (s; 1H, NH). Anal. $C_{22}H_{24}N_2O_2$ (C, H, N).

6.1.4.5. 6-[4-(N,N-Diisobutylcarbamoyl) phenyl]-1H-quinolin-2-one ***(8)***

Prepared from 6-[4-(*N*,*N*-diisobutylcarbamoyl)-phenyl]-2-methoxy-quinoline (**2**) in a similar way, as described for compound **4**. The mixture was refluxed for 24 h and the target compound was purified by recrystallization from acetone/methanol (12/1). Yield: 65 %, colourless solid, mp: 250 °C. IR: *v* 3140, 2960, 1655, 1640, 1620, 1570, 1470, 1430, 1260, 1200, 1100, 935, 820. ^{1}H-NMR: δ 0.69 and 0.95 (2s; 12H, CH($\underline{CH_3}$)$_2$); 1.83 and 2.08 (2s; 2H, $\underline{CH}$(CH$_3$)$_2$); 3.13 and 3.32 (2s; 4H, $\underline{CH_2}$CH(CH$_3$)$_2$); 6.55 (d; 1H, $^3J = 9.72$ Hz, H-3); 7.41 (m; 3H, H-8 and 2H of AA′BB′-system); 7.76 (d; 2H, $^3J = 7.96$ Hz, 2H of AA′BB′-system); 7.88 (d; 1H, $^3J =$ 8.84 Hz, H-7); 7.98 (d; 1H, $^3J = 9.72$ Hz, H-4); 8.05 (s; 1H, H-5); 11.84 (s; 1H, NH). Anal. $C_{24}H_{28}N_2O_2$ (C, H, N).

6.1.4.6. 6-[4-(N,N-Dicyclohexylcarbamoyl) phenyl]-1H-quinolin-2-one ***(10)***

Prepared from 6-[4-(*N*,*N*-dicyclohexylcarbamoyl)-phenyl]-2-methoxy-quinoline (**3**) in a similar way, as described for compound **4**. The mixture was refluxed for 24 h in hydrochloric acid (6 M)/methanol (8/2) and the target compound was purified by recrystallization from ethylacetate and subsequent digestion with hexane. Yield: 60 %, colourless solid, mp: 273–274 °C. IR *v* 2930, 2850, 1680, 1630, 1435, 1370, 1320, 1250, 1130, 830. ^{1}H-NMR: δ 0.83–1.71 (2 m; 20H, Cyclohexyl H); 3.20 (m; 2H, $\underline{CH}$(CH$_2$)$_2$); 6.54 (d; 1H, $^3J = 9.72$ Hz, H-3); 7.39 (d; 1H, $^3J = 8.40$ Hz, H-8); 7.35 and 7.75 (AA′BB′; 4H, $^3J = 8.40$ Hz); 7.88 (d; 1H, $^3J = 8.40$ Hz, H-7); 7.98 (d; 1H, $^3J = 9.72$ Hz, H-4); 8.05 (s; 1H, H-5); 11.84 (s; 1H, NH). Anal. $C_{28}H_{32}N_2O_2$ (C, H, N).

6.1.4.7. 6-[4-(N,N-Diisopropylcarbamoyl) phenyl]-N-methylquinolin-2-one ***(5)***

To a solution of 6-[4-(*N*,*N*-diisopropylcarbamoyl)-phenyl]-1*H*-quinolin-2-one (**4**) (0.98 g, 2.8 mmol) in dry *N*,*N*-dimethylformamide (5 mL) was added sodium hydride (0.13 g, 3.2 mmol, 60 % solution in mineral oil) at 25 °C. After 1 h dimethylsulfate (150 µl, 1.5 mmol) was injected with a syringe and stirring was continued for 1.5 h. The solvent was evaporated in vacuo, water (20 mL) was added and the mixture was extracted with ethylacetate (3 × 30 mL). The combined extracts were dried over magnesium sulfate and the solvent was evaporated. The compound was purified by column chromatography (silica gel) using ethylacetate as eluent. Yield: 45 %, pale yellow crystals, mp: 284 °C. IR: ν 2960, 1665, 1615, 1440, 1345, 1210, 1120, 855, 820, 770. ^{1}H-NMR: δ 1.23 (s; 12H, CH($\underline{CH_3}$)$_2$); 3.66 (m; 5H, $\underline{CH}$(CH$_3$)$_2$ and N-CH$_3$); 6.67 (d; 1H, $^3J = 9.28$ Hz, H-3); 7.39 and 7.79 (AA′BB′; 4H, $^3J = 8.40$ Hz); 7.63 (d; 1H, $^3J = 8.84$ Hz, H-8); 7.99 (2 d; 2H, H-4 and H-7); 8.12 (s; 1H, H-5). Anal. $C_{23}H_{26}N_2O_2$ (C, H, N).

6.1.4.8. 6-[4-(N,N-Dicyclohexylcarbamoyl) phenyl]-N-methylquinolin-2-one ***(11)***

Prepared from 6-[4-(*N*,*N*-dicyclohexylcarbamoyl)-phenyl]-1*H*-quinolin-2-one (**10**) in a similar way as described for compound **5**. Yield: 38 %, colourless solid, mp: 191 °C. IR: *v* 2930, 2850, 1640, 1605, 1480, 1435, 1370, 1270, 1250, 1190, 1130, 1030, 900, 840. ^{1}H-NMR: δ 0.77–1.72 (m; 20H, Cyclohexyl H); 3.20 (s; 2H, $\underline{CH}$(CH$_2$)$_2$); 4.01 (s; 3H, N–CH$_3$); 7.07 (d; 1H, $^3J = 8.40$ Hz, H-3); 7.39 (d; 2H, $^3J = 7.52$ Hz, 2H of AA′BB′-system); 7.85 (m; 3H, H-8 and 2H of AA′BB′-system); 8.04 (d; 1H, $^3J = 7.52$ Hz, H-7); 8.27 (s; 1H, H-5); 8.31 (d; 1H, $^3J = 8.40$ Hz, H-4). Anal. $C_{29}H_{34}N_2O_2$.0.6 H_2O (C, H, N).

6.1.4.9. 6-[4-(N,N-Diisopropylcarbamoyl) phenyl]-1H-3,4-dihydroquinolin-2-one ***(6)***

6-[4-(*N*,*N*-Diisopropylcarbamoyl)phenyl]-1*H*-quinolin-2-one (**4**) (0.5 g, 1.38 mmol) was dissolved in ethanol (150 mL) and palladium (5 %) on charcoal (50 mg) was added. The mixture was hydrogenated for 20 h at a pressure of 30 bars. The catalyst was filtered off and the solvent was evaporated in vacuo. The remaining oil crystallized after 1 day at 4 °C and was recrystallized from ethylacetate. Yield: 72 %, colourless crystals, mp: 251–252 °C. IR: *v* 3230, 2980, 1690, 1610, 1520, 1495, 1445, 1340, 1240, 1200, 1035, 820. ^{1}H-NMR δ 1.50 (s; 12H, CH($\underline{CH_3}$)$_2$); 2.69 (t; 2H, $^3J = 7.52$ Hz, H-3); 3.04 (t; 2H, $^3J = 7.52$ Hz, H-4); 3.70 (s; 2H, $\underline{CH}$(CH$_3$)$_2$); 6.94 (d; 1H, $^3J = 8.40$ Hz, H-8); 7.32 and 7.65 (AA′BB′; 4H, $^3J = 8.18$ Hz); 7.48 (d; 1H, $^3J = 7.96$ Hz); 7.53 (s; 1H, H-5); 10.18 (s; 1H, NH). Anal. $C_{22}H_{26}N_2O_2$ (C, H, N).

6.1.4.10. 6-[4-(N,N-Diisopropylcarbamoyl) phenyl]-N-methyl-3,4-dihydroquinolin-2-one (7)

Prepared from 6-[4-(*N*,*N*-diisopropylcarbamoyl)-phenyl]-*N*-methylquinolin-2-one (**5**) in a similar way as described for compound **6**. Yield: 68 %, colourless crystals, mp: 266 °C. IR: *v* 2960, 1680, 1620, 1500, 1470, 1435, 1365, 1340, 1130, 855, 820, 770. ^{1}H-NMR δ 1.40 (m; 12H, CH($\underline{CH_3}$)$_2$); 2.70 (t; 2H, $^3J = 7.28$ Hz, H-3); 2.98 (t; 2H, $^3J = 7.52$ Hz, H-4); 3.40 (s; 3H, N-CH_3); 3.57 (s; 2H, $\underline{CH}(CH_3)_2$); 7.05 (d; 1H, $^3J = 8.40$ Hz, H-8); 7.38 and 7.57 (AA′BB′; 4H, $^3J = 8.18$ Hz); 7.40 (d; 1H, $^4J = 1.76$ Hz, H-5); 7.48 (dd; 1H, $^3J = 8.40$ Hz, $^4J = 1.76$ Hz, H-7). Anal. $C_{23}H_{28}N_2O_2$ (C, H, N).

6.1.4.11. 6-[4-(N,N-Diisobutylcarbamoyl) phenyl]-1H-3,4-dihydroquinolin-2-one (9)

Prepared from 6-[4-(*N*,*N*-diisobutylcarbamoyl)-phenyl]-1*H*-quinolin-2-one (**8**) in a similar way as described for compound **6**. Yield: 67 %, colourless solid, mp: 208 °C. IR: *v* 3200, 3050, 2960, 1680, 1635, 1500, 1465, 1435, 1375, 1275, 1100, 825. ^{1}H-NMR δ 0.69 and 0.94 (2s; 12H, CH($\underline{CH_3}$)$_2$); 1.82 and 2.06 (2s; 2H, $\underline{CH}(CH_3)_2$); 2.50 (t; 2H, H-3); 2.96 (t; 2H, $^3J = 7.52$ Hz, H-4); 3.12 and 3.27 (2s; 4H, $\underline{CH_2}CH$); 6.94 (d; 1H, $^3J = 7.96$ Hz, H-8); 7.36 and 7.67 (AA′BB′; 4H, $^3J = 8.18$ Hz); 7.50 (d; 1H, $^3J = 7.96$ Hz, H-7); 7.55 (s; 1H, H-5). Anal. $C_{24}H_{30}N_2O_2$ (C, H, N).

6.1.4.12. 6-[4-(N,N-Dicyclohexylcarbamoyl) phenyl]-1H-3,4-dihydroquinolin-2-one (12)

Prepared from 6-[4-(*N*,*N*-dicyclohexylcarbamoyl)-phenyl]-1*H*-quinolin-2-one (**10**) in a similar way as described for compound **6**. Yield: 68 %, colourless solid, mp: 236–237 °C. IR: *v* 3200, 3060, 2930, 2850, 1680, 1640, 1500, 1435, 1365, 1315, 1250, 1185, 1130, 1000, 835. ^{1}H-NMR: δ 0.81–1.70 (m; 20H, Cyclohexyl H); 2.69 (t; 2H, $^3J = 7.52$ Hz, H-3); 2.95 (t; 2H, $^3J = 7.48$ Hz, H-4); 3.20 (s; 2H, $\underline{CH}(CH_2)_2$); 6.94 (d; 1H, $^3J = 8.40$ Hz, H-8); 7.30 and 7.66 (AA′BB′; 4H, $^3J = 7.96$ Hz); 7.50 (d; 1H, $^3J = 8.40$ Hz, H-7); 7.55 (s; 1H, H-5); 10.19 (s; 1H, NH). Anal. $C_{28}H_{34}N_2O_2$ (C, H, N).

6.2. Enzyme inhibitor evaluation

6.2.1. Prostatic homogenate assays

Rat ventral prostate and human prostatic tissue from BPH patients served as enzyme source and were homogenized as previously reported [12]. The enzyme preparation contained nuclei, mitochondria and microsomes. Each incubation mixture contained homogenate equivalent to 200–300 μg human protein or 200–250 μg rat protein, NADPH (100 μM for human and 200 μM for rat enzyme) and testosterone (0.21 μM including 100 nCi 1β2β ^{3}H T) in a final volume of 250 μl. The test compounds were dissolved in DMSO (2 % per incubation). After preincubation for 5 min, the enzymatic reaction was started by addition of testosterone and carried out at 37 °C for 30 min using sodium phosphate buffer (40 mM, pH 6.6, rat enzyme) or sodium citrate buffer (40 mM, pH 5.5, human enzyme). The reaction was stopped by addition of 50 μL sodium hydroxide solution (10 M) and the steroids were extracted with diethylether (500 μL). Steroid separation by RP-HPLC was performed as reported [12].

For the determination of K_i values the inhibitor was tested at 3 concentrations near its IC_{50} value with variable substrate concentrations (0.21, 0.51, 1.01 and 2.02 μM T). The kinetic parameters were calculated using the following equations [19]:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}} \quad (1)$$

$$\frac{1}{V} = \frac{K_m}{V_{max}}\left(1 + \frac{I}{K_i}\right)\frac{1}{S} + \frac{1}{V_{max}}\left(1 + \frac{I}{\alpha K_i}\right) \quad (2)$$

The first equation represents a modification of the Lineweaver–Burk relation according to Lee and Wilson [24] with V (reaction velocity), V_{max} (maximum velocity), K_m (Michaelis–Menten constant) and S (mean substrate concentration). Equation (2) indicates the special case of a mixed type (competitive/non competitive) inhibitor ($\alpha = \infty$, pure competitve inhibitor; $\alpha = 1$, pure non competitive inhibitor).

6.2.2. DU145 cell assay

Intact human prostatic carcinoma DU145 cells are used as the source of type 1 5α-reductase [25, 26]. The inhibitory potency of the compounds was determined similar to [27] by monitoring the conversion of the tritiated substrate androstenedione during an incubation period of 6 h (RPMI 1640 medium containing 10 % fetal calf serum; pH 7.2) A day before the experiment DU145 cells were seeded in a 24-multiwell-plate at a density of 200 000 cells/well and allowed to become adherent overnight. Compounds to be tested were dissolved in DMSO and 5 μL of each were added to the cells in a final volume of 0.5 mL complete medium. As control of conversion (typically about 35 % under these conditions) served a triplicate of wells without inhibitors and as positive control for inhibition a steroidal reference (e.g.

Finasteride: 80, 60, 40, 20 nM) was used. After the 6 h incubation period in 5 % CO_2 at 37 °C the medium samples were extracted twice with 1 mL diethylether and the steroids were separated by HPLC [12].

Acknowledgements

Thanks are due to the Deutsche Forschungsgemeinschaft (DFG) and to the Fonds der Chemischen Industrie for financial support. We thank Wolfgang Reichert for performing the DU 145 cell culture assay and Dr Martina Kotthaus for fruitful discussions.

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