Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 1385–1393

BIOORGANIC & MEDICINAL CHEMISTRY

Effect of C-ring Modifications in Benzo[*c*]quinolizin-3-ones, New Selective Inhibitors of Human 5α-reductase 1

Antonio Guarna,[a,*] Ernesto G. Occhiato,[a] Fabrizio Machetti,[a] Andrea Trabocchi,[a] Dina Scarpi,[a] Giovanna Danza,[b] Rosa Mancina,[b] Alessandra Comerci[b] and Mario Serio[b]

[a]*Dipartimento di Chimica Organica 'U. Schiff' and Centro di Studio sulla Chimica e la Struttura dei Composti Eterociclici e loro Applicazioni, C.N.R., Università di Firenze, Via G. Capponi 9, I-50121 Firenze, Italy*

[b]*Dipartimento di Fisiopatologia Clinica, Unità di Endocrinologia, Università di Firenze, Viale Pieraccini 6, I-50134 Firenze, Italy*

Received 13 November 2000; accepted 5 January 2001

Abstract—The synthesis and the inhibition potency of octahydro- and decahydrobenzo[*c*]quinolizin-3-one derivatives **3**–**7**, as new non-steroidal selective inhibitors of human enzyme 5α-reductase type 1, are reported. These compounds differ from the recently reported benzo[*c*]quinolizin-3-one inhibitors **2** by the presence of a fully or partially saturated C-ring. Compounds **3** and **4,** with a double bond in the C-ring, were prepared by sequential rearrangement-annulation of isoxazolines **19** and **20**. C-ring saturated compounds **5**–**7** were prepared by the Lewis acid-promoted Mannich-Michael tandem reaction of Danishefsky diene with the appropriate *N*-*t*-Boc iminium ion. Inhibition experiments were carried out on 5αR-1 and 5αR-2 expressed by CHO cells. Among the prepared compounds, octahydrobenzo[*c*]quinolizin-3-one **3**, with a double bond at the position 6a–10a, was a potent and selective inhibitor of human 5αR-1 (IC_{50} = 58 nM). The introduction of a *tert*-butylcarboxyamide at the position 8 (compound **4**) was deleterious for the inhibition activity. The lack of the double bond in the C-ring reduced strongly the inhibition activity of compounds **5**–**7**. The extended planarity of the most potent benzo[*c*]quinolizin-3-ones as well as favorable interactions of the C-ring unsaturation with the enzyme active site could account for the inhibition activity of these compounds.

Introduction

Steroid 5α-reductase (EC 1.3.99.5) is a system of two isoenzymes (5αR-1 and 5αR-2) involved in the NADPH-dependent conversion of testosterone to the more potent androgen dihydrotestosterone (DHT).[1] Human 5α-reductases 1 and 2 are differently expressed in various tissues: type 1 is found mainly in liver, skin and scalp; type 2 in prostate, seminal vesicle and epididymis.[1] It is now well established that the formation of DHT in these tissues is related to several human endocrine diseases and skin disorders such as acne, androgenic alopecia, benign prostatic hyperplasia (BPH) and prostatic carcinoma in men, and hirsutism in women.[2–8]

The role of DHT in the maintenance of these pathologies has prompted several synthetic efforts toward potent and selective 5α-reductases inhibitors which, blocking the formation of DHT without testosterone deprivation, could be used in a pharmacological treatment of DHT-related diseases.[9] Some potent inhibitors are based on the steroidal structure of testosterone, modified with the introduction of a nitrogen atom in the A or B ring as in 4-azasteroid and 6-azasteroids. Recently, we have described the synthesis and the biological evaluation of a novel class of azasteroids having as a new feature the nitrogen atom at position 10 of the steroidal skeleton,[10,11] with 19-nor-10-azasteroid **1** (as a 9:1 mixture of $\Delta^{9(11)}$ and $\Delta^{8(9)}$ isomers) (Fig. 1) being a potent dual inhibitor of the isoenzymes 5αR-1 and 5αR-2. Since the presence of all the four fused A–D steroidal rings in an inhibitor seems to be not essential for the activity toward this enzyme,[12,13] we have recently prepared a novel class of non-steroidal compounds having the benzo[*c*]quinolizin-3-one skeleton **2** which resulted in very potent and selective inhibitors of 5αR-1 isoenzyme.[14]

Since there is now clinical evidence that dual inhibitors could give great benefit in the treatment of BPH,[15] and because only few examples of non-steroidal dual inhibitors of 5αR have been reported so far,[1c,9] we decided to

*Corresponding author. Tel.: +39-55-2757611; fax: +39-55-2476964; e-mail: guarna@chimorg.unifi.it

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00012-8

investigate if non-steroidal octahydro-(1*H*)-benzo[*c*]quinolizin-3-ones **3** and **4** (Fig. 1) and decahydrobenzo [*c*]quinolizin-3-ones **5**–**7**, which differ from the previous series of tetrahydrobenzo[*c*]quinolizin-3-one **2** by the presence of an aliphatic C-ring, could maintain the inhibitory potency toward 5αR-1 typically associated to the tricyclic structure **2** while displaying at the same time some activity toward the other isozyme. In this paper we therefore describe the synthesis and the evaluation of the inhibitory activity of compounds **3**–**7** against human 5αR-1 and 5αR-2 expressed in recombinant CHO cells.

Chemistry

All compounds were synthesized starting from racemic material. The common key step in the synthesis of octahydro-(1*H*)-benzo[*c*]quinolizin-3-ones **3**–**4** is the sequential rearrangement-annulation of isoxazoline-5-spirocyclopropanes **19** and **20** (Scheme 1)[16] prepared starting from esters **8** and **9**, respectively, the latter as a 1:1 mixture of diastereoisomers. The transformation of ester **8** into the corresponding *E*/*Z* mixture of oxime **15** was achieved, after protection of the carbonyl group as a ketal, by partial reduction of **10** with DIBAL in toluene at −78 °C, which provided aldehyde **12** in 48% yield, followed by treatment with $NH_2OH{\cdot}HCl$ in pyridine. Due to the low yield obtained in the preparation of aldehyde **12** by partial reduction of the ester group with DIBAL, an excess of this reagent was used at −78 °C in order to have complete reduction of ester **11** to alcohol **13**. However, in these conditions, reduction of the amide to amine also occurred to some extent (25%). Alcohol **13** was oxidized to aldehyde **14** by Swern reaction and the crude aldehyde was finally converted to oxime under the usual conditions providing compound **16** in 35% overall yield after chromatographic purification.

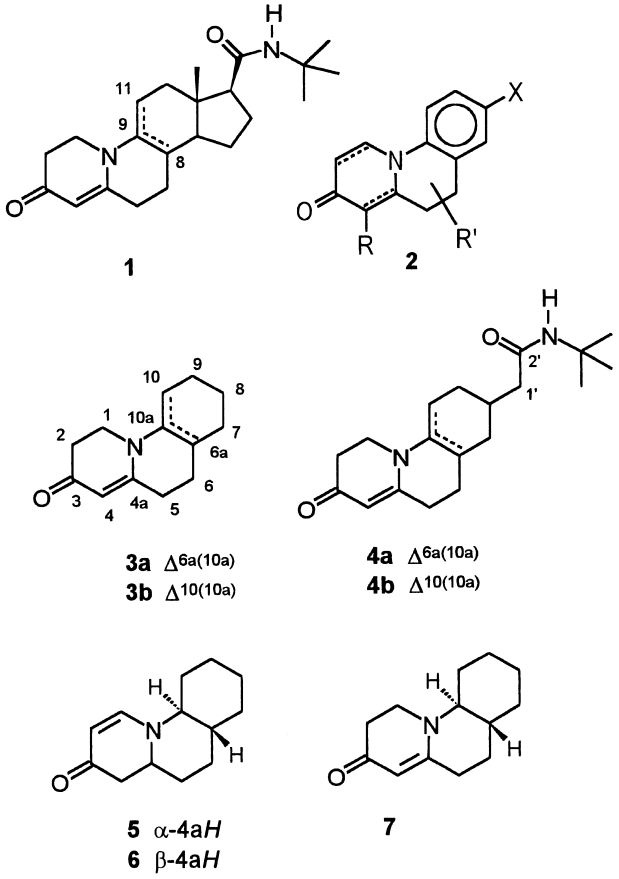


Figure 1.

The 1,3-dipolar cycloadditions were performed in one-pot converting oximes **15** and **16** into the corresponding α-chloro oximes by adding NaClO to an ice-cooled solution of the oximes and Et_3N. The nitrile oxides, formed under these conditions by HCl elimination, were trapped in situ by an excess of methylenecyclopropane, providing isoxazoline-5-spirocyclopropanes **17** (73%) and **18** (58%). After deprotection, the thermal rerrangement of **19** and **20** was performed in refluxing DMF. According to the proposed mechanism,[10,16] along with target octahydro-(1*H*)-benzo[*c*]quinolizin-3-ones **3** and **4**, open chain compounds **21** and **22**, as well as benzoquinolinone derivatives **23** and **24**, could be obtained in the thermal processes, all as mixtures of two regioisomers.

In the rearrangement of **19** only compounds **3** (37%) and **21** (28%) were formed. The ratio between isomers **3a** and **3b** seen in the ^1H NMR spectrum was 10:1. Compound **3a**, with the double bond at 6a–10a 'internal' position, was the major regioisomer. In the thermal rearrangement of isoxazoline **20**, a mixture of products **4**, **22** and **24** was obtained, with low yields in isolated compounds **4** and **22** (18 and 21%, respectively). Compound **4** was obtained, as in the previous case, as 10:1 mixtures of regioisomers, with isomers **4a** being the major one. In similar rearrangements producing 10-azasteroids,[10] compounds having 'external' C9–C11 double bond were predominant with respect to the corresponding regioisomers having 'internal' C8–C9 double bond (9:1 ratio). Ab initio HF-STO3-21G* calculations[17] performed on compounds **3a**,**b** indicate **3a** as the thermodynamically favored isomer (by 1.25 kcal/mol). Because all the efforts to separate the regioisomers by chromatography failed, racemic products **3** and **4** were isolated, characterized and tested as 10:1 regioisomeric mixtures.

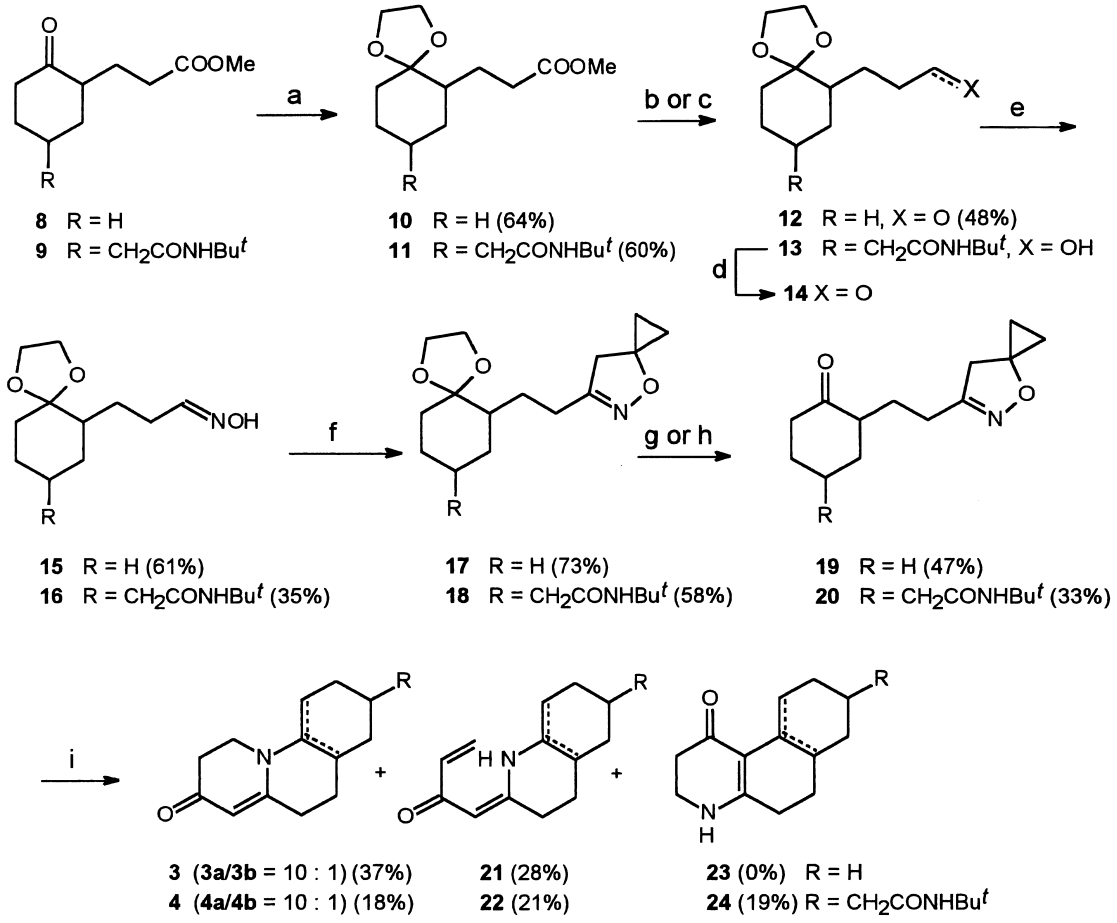
Racemic decahydro-(4a*H*)-benzo[*c*]quinolizin-3-ones **5** and **6** (Scheme 2) were prepared by the tandem Mannich-Michael addition reaction of Danishefsky's diene with the *N*-*t*-Boc iminium ion derived from its precursor **27**, in analogy to the synthesis of 19-nor-10-azasteroids recently reported by us.[18] Treatment of **27** with $TiCl_4$ and Danishefsky's diene thus gave compound **5** having 5α relative stereochemistry, whereas its 5β isomer **6** was obtained through the use of TMSOTf to promote the iminium ion formation. Complete hydrogenation of **5** over Pd/C in MeOH, followed by sequential Jones and $Hg(OAc)_2$ oxidations afforded compound **7** in 16% overall yield.

IC_{50} determination

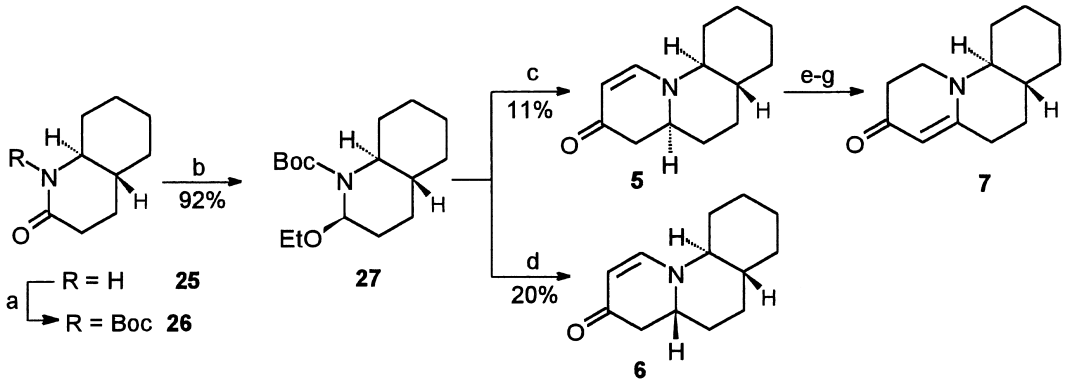
The inhibition potency of compounds **3**–**7** was evaluated on CHO cells expressing human 5αR-1 and 5αR-2

according to the reported methods,[14c,19] using tritiated testosterone as substrate of the enzyme and NADPH as cofactor. In Table 1 the IC_{50} values of **3–7** are reported.

All tested compounds were inactive towards 5αR-2 whereas they displayed inhibitory activity only towards 5αR-1, modulated by the number and position of the double bonds. Simple octahydro-(1*H*)-benzo[*c*]quinolizin-3-one **3** proved to be a potent and selective inhibitor of 5αR-1, its IC_{50} value being at the nanomolar level (58 nM). This inhibitory potency was comparable to that of the compounds belonging to the class of benzo[*c*]quinolinones inhibitors **2** recently reported by us (for example, the IC_{50} of compounds **28** depicted in Figure 2 was 298 nM).[14a–c] Instead, compound **4**, which was designed to be mimic of azasteroid **1**, was a very weak, although still selective, inhibitor of 5αR-1 (IC_{50} = 19 μM). Compared to its parent azasteroidal compound **1**, inhibitor **4** was about 150-fold less potent. Removing the double bond in the C ring caused a strong decrease



Scheme 1. (a) $(CH_2OH)_2$, *p*-TsOH, toluene; (b) DIBAL-H (1.9 equiv), toluene; (c) DIBAL-H (4.5 equiv), toluene; (d) Swern; (e) $NH_2OH{\cdot}HCl$, pyridine; (f) NaClO, Et_3N, methylenecyclopropane, CH_2Cl_2; (g) *p*-TsOH, acetone; (h) H_2SO_4, acetone; (i) DMF, reflux.



Scheme 2. (a) Boc_2O, Et_3N, DMAP, CH_2Cl_2, reflux, 6 days; (b) $LiEt_3BH$, THF, −78 °C, then 1 M HCl in EtOH; (c) 1-Methoxy-3-[(trimethylsilyl)oxy]-1,3-butadiene, $TiCl_4$, CH_2Cl_2, 0 °C, 1 h, then $NaHCO_3$ (satd) 30 min; (d) 1-Methoxy-3-(trimethylsilyl)oxy-1,3-butadiene, Et_3N, TMSOTf, CH_2Cl_2, 0 to 25 °C, then $NaHCO_3$ (satd) 48 h; (e) H_2, Pd/C (5%); (f) Jones oxidation; (g) $Hg(OAc)_2$, EDTA tetrasodium salt, 5% AcOH, H_2O, 85 °C, 2 h.

of potency: the IC_{50} of compound **7** toward 5αR-1 was only 4.78 μM. The same occurred with C-ring saturated compounds **5** and **6** which were very weak inhibitors and with IC_{50} values toward 5αR-1 in the 2–20 μM range.

In general, the selectivity observed for tricyclic inhibitors **3**–**7** is in accordance with the reported findings on benzo[*c*]quinolizinones of type **2** and other tricyclic inhibitors derived from 4-azasteroids which are selective inhibitors of isoenzyme 1.[13,14] It has been suggested that, for the these classes of inhibitors, the extended planarity of the molecular skeleton is an important feature to have a good inhibitory potency.[14c,20] Thus, the superposition of the minimized structures of **3**–**7** with that of compound **28** (Fig. 2), taken as a model of the benzo[*c*]quinolizinone inhibitors,[14] would provide us with a means of evaluating the overall planarity of the molecules.

After a Monte Carlo conformational analysis and AM1 geometry optimization,[21] six conformers for compound **3**–**7** and two conformers for **28** were found in the 0–3 kcal/mol range. After superposition of all the skeleton atoms of the global minimum conformers of **3a** and **28**, the resulting RMS fitting was 0.226 Å (Fig. 2). Therefore the two conformers are very close structures and the global minimum of **3a** could be considered almost as planar as **28**. As expected, the greater contribution to the RMS value arises from the presence of a half-twist C ring in **3a** instead of an aromatic one, whereas the A and B rings are almost coincident. The weak inhibitory activity of 8-*N*-(*tert*-butyl)acetamido substituted compound **4** toward 5αR-1 could be explained by the fact that, generally, lipophylic groups, such as a chlorine atom, at the position 8 of benzoquinoline and benzo[*c*]-quinolizin-3-one inhibitors, enhance the potency toward 5αR-1.[1c,13,14c] Therefore, the 8-*N*-(*tert*-butyl)acetamido group might be a 'wrong' substituent in a tricyclic inhibitor such as **4**. Moreover, due to the high number of conformers generated by the rotations of the C-8 substituent in **4**, a considerable entropy loss should occur in the binding, thus lowering the affinity of the inhibitor toward the enzyme. Concerning C-ring saturated compound **7**, the decrease of its inhibitory potency toward the enzyme appears consistent to, and even more marked than, the decrease of activity measured in 19-nor-10-azasteroids after reduction of the C-ring double bond.[10] The RMS fitting after superposition of the global minimum conformer of **7** with that of **28** was 0.235 Å, a value not significantly different from that measured after superposition of **3a** and **28** (Fig. 2). Therefore, the decrease of inhibition activity of compound **7** can be only in part accounted for on the basis of the flatness of the skeleton, and thus favorable interactions between the C-ring unsaturation in **3a** and the enzyme active site could have an important role in increasing the inhibitory potency. While in benzo[*c*]quinolizin-3-ones **2** a higher activity toward 5αR-1 has been always reported for compounds having the double bond at the 4–4a position, for compounds **5**–**7** the different position of the double bond in the A ring seems instead to have no effect on the inhibitory potency.[14] The distorted planarity of compounds **5** and **6** with respect to **28** (RMS values greater than 0.32 Å) and the lack of the C-double bond could both contribute to the low activity displayed by these two inhibitors.

Table 1. IC_{50} values for compounds **1**–**7** toward human 5αR-1 and 5αR-2

Compound	IC_{50} (nM)	
	5αR-1[a]	5αR-2[b]
1	127 ± 12[c]	122 ± 37 nM
3[d]	58 ± 15	n.i.
4[d]	19,000 ± 1000	n.i.
5	20,000 ± 400	n.i.
6	2400 ± 400	n.i.
7	4780 ± 458	n.i.

[a]Determined using CHO 1827 cells.
[b]Determined using CHO 1829 cells.
[c]Determined on DU-145 cells.

Conclusion

In conclusion, non-steroidal octahydro- and decahydrobenzo[*c*]quinolizin-3-one inhibitors **3**–**7** failed to show dual inhibition activity toward 5αR-1 and 2, while they displayed an interesting selectivity toward human enzyme 5α-reductase type 1. In particular, inhibition experiments carried out toward 5αR-1 and 5αR-2

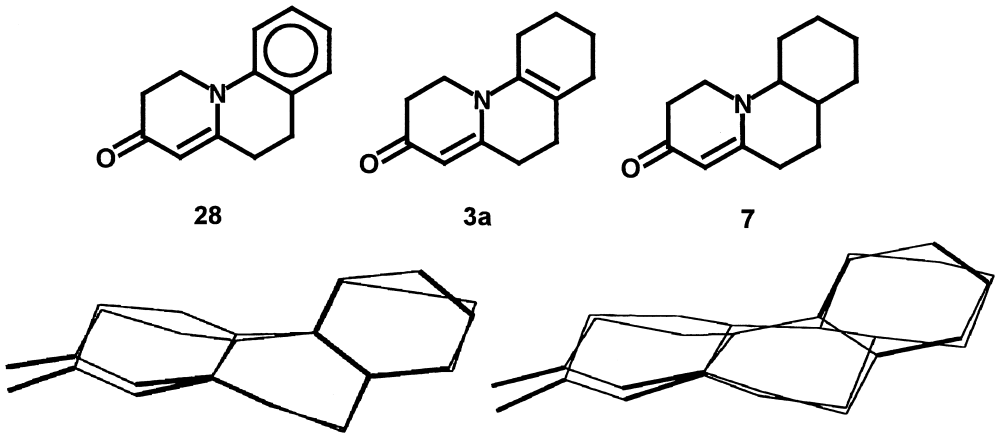


Figure 2. Left: superposition of global minimum conformers of compounds **3a** and **28**. Right: superposition of global minimum conformers of compounds **7** and **28**.

expressed by CHO cells showed that octahydrobenzo[*c*]-quinolizin-3-one **3**, with a double bond at the position 6a–10a, was a potent and selective inhibitor of human 5αR-1 ($IC_{50}=58$ nM), with a potency comparable to that of benzo[*c*]quinolizin-3-ones **2**. The lack of the double bond in the C-ring instead reduced strongly the activity whereas the introduction of a *tert*-butylcarboxyamide at the position 8, was deleterious for the inhibition activity. These results can be only in part explained on the basis of the extended planarity of the benzo[*c*]-quinolizin-3-one skeleton,[14c] but also favorable interactions between the C-ring unsaturation (e.g., in **3a**) and the enzyme active site should be taken into account.

Experimental

All the reactions requiring dry conditions were performed under nitrogen and anhydrous solvents. Chromatographic separations were performed under pressure on silica gel using flash-column techniques; R_f values refer to TLC carried out on 25-mm silica gel plates (Merck F254), with the same eluant indicated for the column chromatography. Melting points (mp) are uncorrect. IR spectra were recorded on a Perkin–Elmer 881 spectrophotometer in $CDCl_3$ solution. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a Varian XL 200 instrument in $CDCl_3$ solution. Mass spectra were carried out in EI at 70 eV on 5790A-5970A Hewlett-Packard and QMD 1000 Carlo Erba instruments. Microanalyses were carried out with a Perkin–Elmer 240C elemental analyzer. All chiral compounds were prepared in racemic form.

Methyl 3-(2-Oxocyclohexyl)propanoate (8). In a flask provided with a Dean-Stark apparatus, cyclohexanone (21.2 g, 216 mmol) and pyrrolidine (19 mL, 227 mmol) were dissolved under stirring in benzene (65 mL). The solution was refluxed for 5.5 h, the solvent and the excess of pyrrolidine were then distilled off and the residual crude oil was dissolved in dioxane (77 mL). Fresh distilled methyl acrylate (29.2 mL, 324 mmol) was added under stirring and the resulting solution was refluxed for 3 h. Water was then added (10 mL) and the mixture heated again under reflux. After 1 h, the product was extracted with Et_2O and the organic layer washed with diluted HCl and dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude oil obtained was purified by distillation under reduced pressure, affording pure **8** (20.0 g, 50%).

8: oil; bp 115–117 °C (1 mbar); ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 3H), 2.40–2.22 (m, 5H), 2.14–1.94 (m, 3H), 1.88–1.76 (m, 1H), 1.74–1.56 (m, 2H), 1.56–1.44 (m, 1H), 1.42–1.22 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 212.2 (s), 173.8 (s), 51.2 (q), 49.5 (t), 41.9 (d), 33.9 (t), 31.4 (t), 27.8 (t), 24.8 (t), 24.6 (t); MS *m*/*z* (rel. intensity) 184 (M^+, 1), 151 (70), 124 (48), 54 (100); IR ($CDCl_3$) 1742, 1709 cm^{-1}.

Methyl 3-[2-(1,3-Dioxolan-2-yl)cyclohexyl]propanoate (10). In a flask provided with a Dean-Stark apparatus, methyl ester **8** (20.0 g, 109 mmol), ethylene glycol (60 mL, 1.08 mol) and *p*-TsOH (0.8 g, 5 mmol) were dissolved in toluene (550 mL) and the resulting solution was heated under reflux. After 4 h the reaction was complete and the mixture was washed with 2 N $NaHCO_3$, water and dried over Na_2SO_4. After filtration and evaporation of the solvent, a crude yellow oil was obtained. This was purified by distillation under reduced pressure, affording pure **10** (15.9 g, 64%).

10: oil; bp 127–130 °C (2 mbar); ^{1}H NMR ($CDCl_3$) δ 3.95–3.88 (m, 4H), 3.63 (s, 3H), 2.42–2.25 (m, 2H), 1.96–1.88 (m, 1H), 1.76–1.70 (m, 2H), 1.63–1.18 (m, 8H); MS *m*/*z* (rel. intensity) 228 (M^+, 0.5), 154 (16), 98 (100); IR ($CDCl_3$) 1739 cm^{-1}.

3-[2-(1,3-Dioxolan-2-yl)cyclohexyl]propanal (12). To a solution of **10** (15.7 g, 69.1 mmol) in toluene (220 mL) cooled at −78 °C, DIBAL-H (1.2 M solution in toluene, 116 mL, 135 mmol) was slowly added during 3 h. After 3 h of stirring, the mixture was poured into water (110 mL) and allowed to warm to room temperature. After filtration on a Celite layer, the organic phase was dried over Na_2SO_4. After filtration and evaporation of the solvent the residual crude oil was purified by chromatography (petroleum ether/EtOAc, 2:1, R_f 0.30), affording pure aldehyde **12** (6.6 g, 48%).

12: oil; ^{1}H NMR ($CDCl_3$) δ 9.75 (t, $J=1.8$ Hz, 1H), 3.99–3.90 (m, 4H), 2.50–2.27 (m, 2H), 2.02–1.91 (m, 1H), 1.80–1.19 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 202.8 (d), 110.4 (s), 64.6 (t), 64.4 (t), 43.9 (d), 42.2 (t), 34.4 (t), 29.1 (t), 24.4 (t), 23.6 (t), 20.8 (t); MS *m*/*z* (rel. intensity) 198 (M^+, 0.1), 155 (22), 99 (100); IR ($CDCl_3$) 2940, 1723 cm^{-1}. Anal. calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15. Found: C, 65.69; H, 9.25.

3-[2-(1,3-Dioxolan-2-yl)cyclohexyl]propanal oxime (15). A solution of aldehyde **12** (6.12 g, 31.0 mmol) and $NH_2OH{\cdot}HCl$ (2.76 g, 40.0 mmol) in pyridine (120 mL) was stirred for 2 h at room temperature. The mixture was extracted with Et_2O and the organic layer washed with water and dried over Na_2SO_4. After filtration and evaporation of the solvent the crude oil obtained was purified by chromatography (petroleum ether/EtOAc, 1.5:1, R_f 0.5). Recrystallization from Et_2O-petroleum ether gave pure oxime **15** (4.02 g, 61%) as a 1:1 mixture of diastereoisomers.

15: mp 74–75 °C; ^{1}H NMR ($CDCl_3$) δ 7.50 (s, 1H, *E*), 7.39 (t, $J=6.0$ Hz, 1H, *Z*), 7.17 (s, 1H, *Z*), 6.69 (t, $J=5.4$ Hz, 1H, *E*), 3.91 (m, 4H), 2.48–2.08 (m, 2H), 1.90–1.18 (m, 11H); ^{13}C NMR ($CDCl_3$) δ 153.2 (d, *Z*), 152.4 (d, *E*), 110.6 (s), 64.6 (t), 64.5 (t), 44.2 and 43.9 (d), 34.5 (t), 28.8 and 28.7 (t), 27.5 and 25.1 (t), 24.5 and 24.4 (t), 24.2 (t), 23.6 and 22.9 (t); MS *m*/*z* (rel. intensity) 213 (M^+, 2), 155 (40), 99 (100); IR ($CDCl_3$) 3587, 3321, 1602 cm^{-1}. Anal. calcd for $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57. Found: C, 62.31; H, 9.28; N, 6.34.

6-[2-[2-(1,3-Dioxolan-2-yl)cyclohexyl]ethyl]-4-oxa-5-aza-spiro[2.4]hept-5-ene (17). Liquid methylenecyclopropane (5 mL) was transferred by a double-tipped needle into a

solution of oxime **15** (4.02 g, 18.8 mmol) and Et_3N (226 mg, 2.23 mmol) in CH_2Cl_2 (35 mL) cooled at −60 °C. The mixture was allowed to warm to 0 °C and NaClO (8% solution, 54 mL) was slowly added in 3.5 h. The solution was stirred for 21 h, then the phases were separated, the aqueous layer was extracted with CH_2Cl_2 (3×25 mL) and the combined organic layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, crude **17** (4.89 g, 73%) was obtained and used without purification in the next reaction.

17: ^{1}H NMR ($CDCl_3$) δ 3.90 (m, 4H), 2.98 (m, 2H), 2.35 (m, 2H), 1.99–1.12 (m, 11H), 1.09 (m, 2H), 0.67 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 160.1 (s), 110.5 (s), 64.6 (s), 64.6 (s), 64.5 (t), 43.9 (d), 41.9 (t), 34.4 (t), 28.9 (t), 26.3 (t), 24.8 (t), 24.3 (t), 23.6 (t), 11.4 (t, 2 C); MS *m*/*z* (rel. intensity) 265 (M^+, 10), 155 (41), 99 (95), 55 (100); IR ($CDCl_3$) 1617 cm^{-1}.

6-[2-(2-Oxocyclohexyl)ethyl]-4-oxa-5-azaspiro[2.4]hept-5-ene (19). Isoxazoline **17** (3.64 g, 13.7 mmol) and *p*-TsOH (392 mg, 2.23 mmol) were dissolved in acetone (90 mL) and water (30 mL) and the resulting solution was stirred at room temperature for 7 days. The product was extracted with CH_2Cl_2, the organic phase washed with 2 N $NaHCO_3$ and dried over Na_2SO_4. After filtration and evaporation of the solvent, a yellow crude oil (2.36 g) was obtained. This was purified first by chromatography (CH_2Cl_2/EtOAc, 12.5:1, R_f 0.35) and then by recrystallization from Et_2O-petroleum ether, affording pure isoxazoline **19** (1.43 g, 47%).

19: mp 109 °C; ^{1}H NMR ($CDCl_3$) δ 2.98 (s, 2H), 2.35 (m, 3H), 2.20–1.30 (m, 10H), 1.09 (m, 2H), 0.68 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 213.2 (s), 159.9 (s), 64.8 (s), 49.7 (d), 42.1 (t), 42.0 (t), 34.0 (t), 27.9 (t), 25.9 (t), 25.8 (t), 24.9 (t), 11.4 (t, 2 C); MS *m*/*z* (rel. intensity) 221 (M^+, 30), 55 (100); IR ($CDCl_3$) 1704, 1617 cm^{-1}. Anal. calcd for $C_{13}H_{19}NO_2$: C, 70.56; H, 8.65; N, 6.33. Found: C, 70.59; H, 8.90; N, 6.36.

2,3,5,6,7,8,9,10- and 2,3,5,6,6a,7,8,9-Octahydro-(1*H*)-benzo[*c*]quinolizin-3-one (3a and 3b). Isoxazoline **19** (476 mg, 2.15 mmol) dissolved in dry DMF (50 mL) was heated under reflux for 3 h. After distillation of the solvent, a yellow crude oil (470 mg) was obtained, containing a mixture of products **3** and **21**. This oil was purified by chromatography ($CHCl_3$/MeOH, 30:1) affording pure **3** (163 mg, 37%, R_f 0.27) as an orange solid and **21** (122 mg, 28%, R_f 0.48), both as mixtures of two isomers (**3a**/**3b**, 10:1; **21a**/**21b**, 10:1).

3: mp 85–86 °C; ^{1}H NMR ($CDCl_3$) δ 5.12 (br s, 1H, **3b**), 4.95 (s, 1H, **3a**), 4.92 (s, 1H, **3b**), 3.65 (t, J = 7.5 Hz, 2H), 2.45 (m, 4H), 2.14 (m, 2H), 2.00 (m, 4H), 1.71 (m, 2H), 1.57 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 191.5 (s), 159.5 (s), 130.4 (s), 115.8 (s), 99.6 (d), 43.5 (t), 35.8 (t), 29.5 (t), 29.1 (t), 25.5 (t), 24.3 (t), 23.0 (t), 22.0 (t); MS *m*/*z* (rel. intensity) 203 (M^+, 100), 174 (56), 147 (59); IR ($CDCl_3$) 1619, 1551, 1470 cm^{-1}. Anal. calcd for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 76.72; H, 8.65; N, 6.78.

21: oil; ^{1}H NMR ($CDCl_3$) δ 11.80 (s, 1H), 6.31 (dd, J = 9.9, 17.2 Hz, 1H, **21a**), 6.28 (dd, 1H, **21b**), 6.10 (dd, J = 2.2, 17.2 Hz, 1H, **21a**), 6.08 (dd, 1H, **21b**), 5.48 (dd, J = 2.2, 9.9 Hz, 1H, **21a**), 5.45 (dd, 1H, **21b**), 5.14 (s, 1H, **21b**), 5.07 (s, 1H, **21a**), 4.99 (s, 1H, **21b**), 2.51 (t, 2H), 2.05 (m, 6H), 1.63 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 186.6 (s), 158.7 (s), 137.6 (d), 128.0 (s), 122.7 (t), 113.0 (s), 94.7 (d), 28.4 (t), 28.0 (t), 26.3 (t), 24.1 (t), 22.5 (t), 22.4 (t); MS *m*/*z* (rel. intensity) 203 (M^+, 43), 202 (100), 147 (14); IR ($CDCl_3$): 3692, 1690, 1589, 1543 cm^{-1}. Anal. calcd for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 76.64; H, 8.21; N, 7.00.

Methyl 3-[[2-(1,3-Dioxolan-2-yl)-5-(*N*-*t*-butyl)acetamido]-cyclohexyl]]propanoate (11). Prepared as compound **10**. Starting from **9** (32.14 g, 108 mmol), crude ketal **11** (22.2 g, 60%) was obtained as an oil. A portion (100 mg) of this crude oil was purified by chromatography (CH_2Cl_2/MeOH, 30:1, 1% Et_3N, R_f 0.31), affording **11** as a 5:1 mixture of *cis* and *trans* isomers. Spectroscopic data refer to the major isomer.

11: oil; ^{1}H NMR ($CDCl_3$) δ 5.27 (br s, 1H), 3.88 (m, 4H), 3.60 (s, 3H), 2.40–2.20 (m, 2H), 2.00–1.81 (m, 5H), 1.76–1.43 (m, 6H), 1.28 (s, 9H), 1.16–0.91 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 174.4 (s), 171.3 (s), 110.2 (s), 64.7 (t), 64.6 (t), 51.4 (s), 51.1 (q), 44.7 (d), 43.6 (t), 35.6 (t), 34.5 (t), 34.1 (d), 32.2 (t), 29.7 (t), 28.8 (q, 3 C), 23.7 (t); MS *m*/*z* (rel. intensity) 341 (M^+, 6), 310 (5), 185 (57), 99 (100); IR ($CDCl_3$) 3439, 1725, 1660, 1502 cm^{-1}. Anal. calcd for $C_{18}H_{31}NO_5$: C, 63.32; H, 9.15; N, 4.10. Found: C, 63.01; H, 9.42; N, 3.99.

3-[[2-(1,3-Dioxolan-2-yl)-5-(*N*-*t*-butyl)acetamido]cyclohexyl]]propanal Oxime (16). A solution of **11** (22.1 g, 64.7 mmol) in toluene (500 mL) was cooled at −78 °C; DIBAL-H (solution 1 M in toluene, 288 mL) was then slowly added in 4 h and the resulting solution was stirred for 3 h. After addition of water (260 mL), the mixture was allowed to warm to room temperature, extracted with CH_2Cl_2 (4×200 mL) and the organic layer dried over Na_2SO_4. After filtration and evaporation of the solvent a crude oil (17.2 g) was obtained (**13**) which was used without purification in the next reaction. ^{1}H NMR ($CDCl_3$) δ 5.20 (br s, 1H), 3.94 (m, 4H), 3.62 (m, 2H), 2.10–1.10 (m, 14H), 1.32 (s, 9H).

Then, under stirring, to a solution of distilled oxalyl chloride (10.9 mL, 125 mmol) in CH_2Cl_2 (270 mL), cooled at −60 °C, DMSO (15 mL, 211 mmol) was added, followed by slow addition (25 min) of a solution of the above crude oil in CH_2Cl_2 (260 mL). After 15 min, Et_3N (56 mL) was slowly added in 15 min. After 5 min stirring, the mixture was warmed to room temperature and washed with water (535 mL); after separation of the phases, the aqueous one was extracted with CH_2Cl_2 (3×250 mL) and the combined organic layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, crude aldehyde **14** (14.6 g) was obtained, which was used without purification in the next reaction. ^{1}H NMR ($CDCl_3$) δ 9.74 (s, 1H), 5.20 (br s, 1H), 3.92 (m, 4H), 2.55 (m, 2H), 2.10–1.10 (m, 12H), 1.32 (s, 9H).

A solution of this crude oil (14.6 g) in pyridine (210 mL) was added to a solution of $NH_2OH{\cdot}HCl$ (13.7 g, 196.9 mmol) in pyridine (107 mL) and the resulting mixture was stirred at room temperature for 20 h. The mixture was poured into CH_2Cl_2 (800 mL) and washed with water; after separation of the phases, the aqueous one was extracted with CH_2Cl_2 (3×200 mL) and the combined organic layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, crude **16** (11.3 g) was obtained. This was purified by chromatography eluting with $CHCl_3$/MeOH, 50:1, 1% Et_3N, and then with $CHCl_3$/MeOH, 3:1, 1% Et_3N (R_f 0.32), affording oxime **16** (7.41 g, 35%) as a 1:1 mixture of *E*/*Z* diastereoisomers. Spectral data refer to the major *E* and *Z* diastereoisomers.

16: oil; ^{1}H NMR ($CDCl_3$) δ 8.80 (br s, 1H, *E*), 8.40 (br s, 1H, *Z*), 7.36 (t, J = 5.8 Hz, 1H, *Z*), 6.64 (m, 1H, *E*), 5.29 (br s, 1H), 3.89 (m, 4H), 2.34–2.08 (m, 2H), 2.01–1.61 (m, 8H), 1.50–1.12 (m, 3H), 1.30 (s, 9H), 1.03–0.84 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 171.6 (s), 152.7 (d) and 152.0 (d), 110.3 (s), 64.8 (t), 64.7 (t), 51.2 (s), 44.8 and 43.6 (d), 35.4 and 35.6 (t), 34.2 (d), 32.7 (t), 29.7 (t), 28.8 (q, 3 C), 27.4 (t), 25.1 (t), 22.7 (t); MS *m*/*z* (rel. intensity) 326 (M^+, 2), 309 (13), 268 (4), 99 (100); IR ($CDCl_3$) 3588, 3550–3000 (br), 3443, 1657, 1508 cm^{-1}. Anal. calcd for $C_{17}H_{30}N_2O_4$: C, 62.55; H, 9.26; N, 8.58. Found: C, 62.27; H, 9.20; N, 8.30.

6-[2-[2-(1,3-Dioxolan-2-yl)-5-(*N*-*t*-butyl)acetamido]cyclohexyl]ethyl]-4-oxa-5-azaspiro[2.4]hept-5-ene (18). Prepared as compound **17**. Starting from oxime **16** (7.40 g, 22.6 mmol), isoxazoline **18** (4.96 g, 58%) was obtained as a crude oil used without purification in the next reaction.

18: ^{1}H NMR ($CDCl_3$) δ 5.31 (br s, 1H), 3.86 (m, 4H), 2.92 (br s, 2H), 2.40–2.17 (m, 2H), 1.97–1.47 (m, 8H), 1.40–0.92 (m, 6H), 1.27 (s, 9H), 0.64 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 171.3 (s), 160.1 (s), 110.2 (s), 64.7 (s), 64.1 (t, 2 C), 51.1 (s), 44.6 (d), 43.7 (t), 42.0 (t), 35.6 (t), 34.4 (t), 34.1 (d), 29.6 (t), 28.7 (q, 3 C), 26.3 (t), 24.8 (t), 11.5 (t, 2 C).

6-[2-[2-Oxo-5-[(*N*-*t*-butyl)acetamido]cyclohexyl]ethyl]-4-oxa-5-azaspiro[2.4]hept-5-ene (20). Crude isoxazoline **18** (4.92 g, 13.1 mmol) was dissolved in acetone (150 mL) and H_2SO_4 (1.7 M solution in acetone, 9.8 mL) was slowly added, under vigorous stirring, at room temperature. When the reaction was complete, Na_2CO_3 was added up to pH 7; after filtration and evaporation of the solvent, crude **20** was obtained. This was purified by chromatography, eluting with CH_2Cl_2/MeOH, 60:1 and then 20:1 (R_f 0.28), affording pure **20** (1.45 g, 33%) as a mixture of *cis* and *trans* isomers. Spectroscopic data refer to the major isomer.

20: oil; ^{1}H NMR ($CDCl_3$) δ 5.25 (br s, 1H), 2.98 (br s, 2H), 2.45–2.34 (m, 5H), 2.18–1.93 (m, 4H), 1.80–1.66 (m, 2H), 1.45–1.01 (m, 3H), 1.33 (s, 9H), 1.09 (m, 2H), 0.68 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 212.0 (s), 170.7 (s), 159.5 (s), 64.6 (s), 51.1 (s), 48.4 (d), 43.4 (t), 42.0 (t), 41.2 (t), 39.6 (t), 34.0 (d), 33.3 (t), 28.6 (q, 3 C), 25.8 (t), 25.6 (t), 11.4 (t, 2 C); MS *m*/*z* (rel. intensity) 334 (M^+, 22), 277 (15), 55 (100); IR ($CDCl_3$) 3440, 1707, 1665, 1505 cm^{-1}. Anal. calcd for $C_{19}H_{30}N_2O_3$: C, 68.23; H, 9.04; N, 8.38. Found: C, 68.21; H, 9.00; N, 8.60.

2,3,5,6,7,8,9,10- and 2,3,5,6,6a,7,8,9-Octahydro-(1*H*)-8-(*N*-*t*-Butyl)acetamido-benzo[*c*]quinolizin-3-one (4a and 4b). A solution of isoxazoline **20** (947 mg, 2.83 mmol) in DMF (109 mL) was heated under reflux for 3 h. After distillation under reduced pressure of the solvent, a crude oil containing a mixture of **4**, **22** and **24** was obtained. Chromatographic separation (CH_2Cl_2/MeOH, 25:1, 1% NH_3) afforded pure **4** (161 mg, 18%, R_f 0.32) and **22** (188 mg, 21%, R_f 0.43) both as mixtures of two isomers (**4a**/**4b**, 10:1, and **22a**/**22b**).

4: oil; ^{1}H NMR ($CDCl_3$) δ 5.29 (br s, 1H), 4.94 (s, 1H, **4a**), 4.91 (s, 1H, **4b**), 3.67 (m, 2H), 2.59–2.38 (m, 4H), 2.30–1.70 (m, 11H), 1.33 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 191.7 (s), 171.1 (s), 159.6 (s), 130.2 (s), 114.7 (s), 99.9 (d), 51.3 (s), 43.7 (t), 35.8 (t), 35.4 (t), 30.8 (d), 29.6 (t), 28.9 (t), 28.8 (t), 28.8 (q, 3 C), 25.4 (t), 24.3 (t); MS *m*/*z* (rel. intensity) 316 (M^+, 92), 315 (41), 259 (12), 244 (17), 202 (98), 201 (100); IR ($CDCl_3$): 3441, 1662, 1621, 1551, 1509 cm^{-1}. Anal. calcd for $C_{19}H_{28}N_2O_2$: C, 72.12; H, 8.92; N, 8.85. Found: C, 72.01; H, 8.70; N, 8.90.

22: oil; ^{1}H NMR ($CDCl_3$) δ 11.78 (br s, 1H), 6.26 (dd, J = 15.4, 9.9 Hz, 1H), 6.08 (dd, J = 15.4, 2.2 Hz, 1H), 5.45 (dd, J = 9.9, 2.2 Hz, 1H), 5.36 (br s, 1H), 5.07 (s, 1H), 2.80–1.62 (m, 13H), 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 181.8 (s), 171.2 (s), 158.8 (s), 137.7 (s), 126.6 (d), 123.0 (t), 111.9 (s), 94.9 (d), 51.3 (s), 34.5 (d), 34.2 (t), 32.4 (t), 31.3 (t), 28.7 (q, 3 C), 28.7 (t), 25.9 (t), 24.0 (t); MS *m*/*z* (rel. intensity) 316 (M^+, 41), 315 (68), 259 (17), 202 (81), 201 (100); IR ($CDCl_3$) 3437, 1705, 1660, 1588, 1504 cm^{-1}. Anal. calcd for $C_{19}H_{28}N_2O_2$: C, 72.12; H, 8.92; N, 8.85. Found: C, 72.07; H, 8.90; N, 9.00.

24: ^{1}H NMR ($CDCl_3$) δ 7.30 (br s, 1H); 5.30 (br s, 1H); 3.60–3.40 (m, 2H); 2.60–1.50 (m, 15H); 1.31 (s, 9H).

***N*-(*tert*-Butoxycarbonyl)-octahydroquinolin-2-one (26).** To a solution of lactam **25** (4.0 g, 26 mmol) in CH_2Cl_2 (90 mL) were added Et_3N (3.6 mL, 26 mmol), Boc_2O (34.6, g 156 mmol) and DMAP (3.17, 52 mmol) and the resulting solution was heated at reflux for 6 days. Water (90 mL) was added, and after separation of the phases, the aqueous layer was extracted with CH_2Cl_2 (3×30 mL). The combined organic phases were washed with 1 M $KHSO_4$ (30 mL), $NaHCO_3$ (satd) (30 mL), and brine (30 mL) and dried over Na_2SO_4. After filtration and evaporation of the solvent the crude residue was purified by flash chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH, 50:1) to give *N*-Boc-octahydroquinolin-2-one **26** (5.55g, 92%) as a clear oil.

26: ^{1}H NMR ($CDCl_3$) δ 3.24 (m, 1H), 2.46–2.40 (m, 2H), 1.96–1.89 (m, 1H), 1.76–1.63 (m, 5H), 1.41 (s, 9H), 1.27–1.24 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 170.7 (s), 153.9 (s), 83.4 (s), 61.9 (d), 40.0 (d), 33.5 (t), 31.6(t), 31.4 (t), 27.7 (t), 27.6 (q, 3 C), 25.4 (t), 24.4 (t); MS *m*/*z* (rel. intensity) 197 (42), 154 (100), 153 (84), 138 (72), 110

(96); IR ($CDCl_3$) 2937, 1735, 1660. Anal. calcd for $C_{14}H_{23}NO_3$: C, 66.37; H, 9.15; N, 5.53. Found: C, 66.07; H, 8.90; N, 5.36.

***N*-(*tert*-Butoxycarbonyl)-2-ethoxy-octahydroquinoline (27).** To a solution of *N*-Boc lactam **26** (2.32 g, 9.16 mmol) in anhydrous THF (25 mL) cooled at −78 °C was added dropwise a 1 M solution of $LiEt_3BH$ in THF (18 mL) over 5 min. The reaction mixture was stirred at −78 °C for 15 min and then 1 M HCl in EtOH was added dropwise until pH 3–4 was reached, immediately followed by the addition of 4 mL of EtOH. The mixture was allowed to warm to 0 °C, and after 30 min stirring, was diluted with CH_2Cl_2 (120 mL), the organic layer was washed with water (120 mL), $NaHCO_3$ (satd) (120 mL), brine (120 mL) and dried over Na_2SO_4. After filtration and evaporation of the solvent, **27** (2.5 g) was obtained as an oil that was sufficiently pure to be used in the next step.

27: ^{1}H NMR ($CDCl_3$) δ 5.38 (d, J = 6.2 Hz, 1H), 3.64–3.27 (m, 2H), 3.16 (m, 1H), 2.26–2.04 (m, 1H), 2.00–1.50 (m, 12H), 1.41 (s, 9H), 1.15 (t, J = 7.0 Hz, 3H); MS m/z (rel. intensity) 238 (M^+ - 45, 11), 181 (100), 136 (51), 57(91).

3,4, 5,6,6a,7,8,9,10,10a-Decahydro-(4aα*H*)-benzo[*c*]quinolizin-3-one (5). To a stirred solution of 1-methoxy-3-[(trimethylsilyl)oxy]-1,3-butadiene (276 μL, 1.42 mmol) and **27** (200 mg, 0.71 mmol) in CH_2Cl_2 (5 mL) cooled at 0 °C, $TiCl_4$ (155 μL, 1.42 mmol) was added drop wise under nitrogen atmosphere. After 60 min at 0 °C, $NaHCO_3$ (satd) (4 mL) was added and the resulting mixture stirred 30 min at room temperature. After separation of the phases, the product was extracted with CH_2Cl_2 (3×15 mL) and the combined organic layers dried over Na_2SO_4. After filtration and evaporation of the solvent, crude **5** was obtained. This was purified by chromatography, eluting with acetone (R_f = 0.43), affording pure **5** (16 mg, 11% from **26**).

5: ^{1}H NMR ($CDCl_3$) δ 7.16 (d, J = 7.8 Hz, 1H), 4.96 (d, J = 7.8 Hz, 1H), 3.43–3.37 (m, 1H), 2.72–2.60 (m, 2H), 2.33–2.21 (m, 2H), 2.14–1.41 (m, 6H), 1.37–1.09 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 191.7 (s), 148.5 (d), 98.2 (d), 65.5 (d), 59.2 (d), 42.9 (d), 42.3 (t), 32.7 (t), 31.8 (t), 30.9 (t), 30.3 (t), 29.2 (t), 25.4 (t); IR ($CDCl_3$) 2935, 1709, 1626, 1579 cm^{-1}; MS m/z (rel. intensity) 205 (M^+, 1), 162 (27), 110 (17), 86(64), 84 (100). Anal. calcd for $C_{13}H_{19}NO$: C, 76.06; H, 9.33; N, 6.82. Found: C, 75.99; H, 9.64; N, 6.44.

3,4,5,6,6a,7,8,9,10,10a-Decahydro-(4aβ*H*)-benzo[*c*]quinolizin-3-one (6). To a solution of **27** (500 mg, 1.76 mmol), 1-methoxy-3-(trimethylsilyl)oxy-1,3-butadiene (0.69 mL, 3.53 mmol), and TEA (0.110 mL, 0.79 mmol) in CH_2Cl_2 (3 mL) cooled at 0 °C was added dropwise TMSOTf (0.5 mL, 3.53 mmol). The mixture was allowed to warm to room temperature and kept under stirring for 45 min. Then $NaHCO_3$ (satd) was added (4 mL) and the resulting reaction mixture was stirred for 48 h at room temperature. After separation of the phases, the product was extracted with CH_2Cl_2 (3×30 mL), and the combined organic layers were dried over Na_2SO_4. After filtration the solution was concentrated under reduced pressure and the resulting residue was purified by chromatography (AcOEt, then AcOEt/MeOH 10:1, then AcOEt/MeOH 1:1) to give 42 mg (11% from **27**, R_f = 0.28) of **6** as a yellowish oil.

6: ^{1}H NMR ($CDCl_3$) δ 7.15 (d, J = 7.4 Hz, 1H) 4.89 (d, J = 7.4 Hz, 1H), 3.75 (m, 1H), 3.18–3.13 (m, 1H), 2.86–2.80 (m, 1H), 2.41–2.38 (m, 2H), 1.63–1.50 (m, 6H), 1.63–1.50 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 191.0 (s), 149.9 (d), 96.7 (d), 59.8 (d), 53.6 (d), 42.1 (d), 37.2 (t), 30.7 (t), 25.5 (t), 25.3 (t), 25.0 (t); IR ($CDCl_3$) 3050, 2937, 1709, 1571 cm^{-1}; MS m/z (rel. intensity) 205 (M^+, 1), 162 (27), 110 (17), 86 (64), 84 (100). Anal. calcd for $C_{13}H_{19}NO$: C, 76.06; H, 9.33; N, 6.82. Found: C, 76.16; H, 9.04; N, 6.90.

2,3,5,6,6a,7,8,9,10, 10a-Decahydro-(1*H*)-benzo[*c*]quinolizin-3-one (7). Compound **5** (130 mg, 0.63 mmol) was dissolved in EtOH (20 mL), Pd/C (5%) (170 mg) was added, the apparatus flushed three times with H_2 and the mixture stirred at 25 °C for 21 h under H_2 atmosphere. The mixture was filtered through a Celite layer and then concentrated under reduced pressure obtaining the corresponding satd alcohol which was sufficiently pure to be used in the next step. Thus, to a solution of the crude alcohol (130 mg) in acetone cooled at 0 °C was added dropwise a solution prepared dissolving CrO_3 (150 mg, 1.5 mmol) and 96% H_2SO_4 (0.28 mL) in water (2 mL) until the colour of the reaction mixture turned deep orange. After stirring the reaction mixture for 5 min, water (20 mL) was added and the mixture extracted with CH_2Cl_2. The organic phase was washed with 5% $NaHCO_3$ aqueous solution, brine and then dried over Na_2SO_4. After evaporation of the solvent the corresponding 3-oxo compound was obtained (103 mg) in sufficiently pure form to be used in the next step. ^{1}H NMR ($CDCl_3$) δ 3.60–3.42 (m,1H), 3.28 (dm, J = 6.4 Hz, 1H), 2.40–2.01 (m, 4H), 1.90–1.04 (m, 15H); MS m/z (rel. intensity) 207 (M^+, 8), 164 (100) IR ($CDCl_3$) 2935, 1712 cm^{-1}. The above compound (103 mg) was dissolved in 5% AcOH (12 mL) aqueous solution. EDTA (832 mg, 2.88 mmol) and $Hg(OAc)_2$ (637mg, 2.00 mmol) were added and the resulting solution was heated 2 h at 85 °C under vigorous stirring. After cooling to room temperature, the solution was filtered on a Celite layer and Na_2CO_3 (satd) was added up to pH 8. The product was extracted with CH_2Cl_2 (5×25 mL) and the organic layer washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude material was purified by chromatography, eluting with acetone, affording pure **7** (17 mg, 16%) as a pale yellow solid.

7: ^{1}H NMR ($CDCl_3$) δ 4.89 (s, 1H), 3.58 (m, 1H), 3.37 (dt, J = 11.3, 4.0 Hz), 3.14 (m, 1H), 2.10–0.90 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 191.6 (s), 128.2 (d), 100.0 (s), 64.0 (d), 45.2 (t), 41.6 (t), 35.9 (t), 32.6 (d), 31.6 (t), 28.5 (t), 27.1(t), 25.5 (t), 25.4 (t); MS m/z (rel. intensity) 205 (M^+, 42), 162 (100), 134 (38). IR ($CDCl_3$) 3050, 2920,1719,1560. Anal. calcd for $C_{13}H_{19}NO$: C, 76.06; H, 9.33; N, 6.82. Found: C, 76.26; H, 9.16; N, 6.64.

Biological assays

The IC_{50} determinations toward 5αR-1 expressed in CHO 1827 and 1829 cells were carried out as reported.[14c] In the inhibition experiments, the inhibitors were added in the range 10^{-9}–10^{-5} M for **3** and **5**–**7** and 10^{-7}–10^{-3} M for **4**. Each determination was performed in duplicate.

Acknowledgements

The authors thank Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST - COFIN 1998-2000), CNR (Target Project on Biotechnology, grants 99.00381.PF49 and 97.00482.PF49), and the Ares-Serono for financial support. Mr. Sandro Papaleo, Mrs. Brunella Innocenti and Mrs. Cristina Faggi are acknowledged for their technical assistance.

References and Notes

1. (a) Russell, D. W.; Wilson, J. D. *Annu. Rev. Biochem.* **1994**, *63*, 25. (b) Wilson, J. D.; Griffin, J. E.; Russell, D. W. *Endocrine Rev.* **1993**, *14*, 577–593. (c) Kenny, B.; Ballard, S.; Blagg, J.; Fox, D. *J. Med. Chem.* **1997**, *40*, 1293.
2. Rasmusson, G. H. *Annu. Rep. Med. Chem.* **1986**, *22*, 179.
3. Geller, J. *J. Am. Geriatr. Soc.* **1991**, *39*, 1208.
4. (a) Gormley, G. J. *Urol. Clin. North Am* **1991**, *18*, 93. (b) Brawley, O. W.; Ford, G. L.; Thompson, I.; Perlman, J. A.; Kramer, B. S. *Cancer Epidemiol. Biomarkers Prev* **1994**, *3*, 177.
5. Dallob, A. L.; Sadick, N. S.; Unger, W.; Lipert, S.; Geissler, L. A.; Gregoire, S. L.; Nguyen, H. H.; Moore, E. C.; Tanaka, W. K. *J. Clin. Endocrinol. Metab.* **1994**, *79*, 703.
6. Smith, L. S.; Tegler, J. J. *Annu. Rep. Med. Chem.* **1989**, *24*, 177.
7. (a) Price, V. H. *N. Engl. J. Med.* **1999**, *341*, 964 and references therein. (b) Gadwood, R. C.; Fiedler, V. C. *Annu. Rep. Med. Chem.* **1989**, *24*, 187.
8. Brooks, J. R. *Clin. Endocrinol. Metabol* **1986**, *15*, 391.
9. (a) Metcalf, B. W.; Levy, M. A.; Holt, D. A. *Trends Pharm. Sci.* **1989**, *10*, 491. (b) Holt, D. A.; Levy, M. A.; Metcalf, B. W. In *Advances in Medicinal Chemistry*, Maryanoff, B. E., Maryanoff, C. A., Eds.; JAI Press Inc: Greenwich USA, 1993, *2*, 1–29. (c) Abell, A. D.; Henderson, B. R. *Curr. Med. Chem.* **1995**, *2*, 583. (d) Li, X.; Chen, C.; Singh, S.; Labrie, F. *Steroids* **1995**, *60*, 430. (e) Frye, S. V. *Curr. Pharm. Des* **1996**, *2*, 59.
10. Guarna, A.; Belle, C.; Machetti, F.; Occhiato, E. G.; Payne, A. H.; Cassiani, C.; Comerci, A.; Danza, G.; De Bellis, A.; Dini, S.; Marrucci, A.; Serio, M. *J. Med. Chem.* **1997**, *40*, 1112.
11. Guarna, A.; Occhiato, E. G.; Machetti, F.; Marrucci, A.; Danza, G.; Serio, M.; Paoli, P. *J. Med. Chem.* **1997**, *40*, 3466.
12. Hartmann, R. W.; Göhring, S. *Eur. J. Med. Chem.* **1994**, *29*, 807.
13. (a) Jones, C. D.; Audia, J. E.; Lawhorn, D. E.; McQuaid, L. A.; Neubauer, B. L.; Pike, A. J.; Pennington, P. A.; Stamm, N. B.; Toomey, R. E.; Hirsch, K. S. *J. Med. Chem.* **1993**, *36*, 421. (b) Mook, R. A., Jr.; Lackey, K.; Bennet, C. *Tetrahedron Lett.* **1995**, *36*, 3969. (c) Abell, A. D.; Brandt, M.; Levy, M. A.; Holt, D. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2327. (d) Brandt, M.; Levy, M. A.; Holt, D. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 481.
14. (a) Guarna, A.; Occhiato, E. G.; Scarpi, D.; Tsai, R.; Danza, G.; Comerci, A.; Mancina, R.; Serio, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2871. (b) Guarna, A.; Occhiato, E. G.; Scarpi, D.; Zorn, C.; Danza, G.; Comerci, A.; Mancina, R.; Serio, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 353. (c) Guarna, A.; Machetti, F.; Occhiato, E. G.; Scarpi, D.; Comerci, A.; Danza, G.; Mancina, R.; Serio, M.; Hardy, K. *J. Med. Chem.* **2000**, *43*, 3718.
15. (a) Palomino, E. *Curr. Opin. Cent. Periph. Nerv. Syst. Invest. Drugs* **1999**, *1*, 253. (b) Tian, G.; Mook, R. J.; Moss, M. L.; Frye, S. L. *Biochemistry* **1995**, *34*, 13453.
16. Occhiato, E. G.; Guarna, A.; Brandi, A.; Goti, A.; De Sarlo, F. *J. Org. Chem.* **1992**, *57*, 4206.
17. Semiempirical and ab initio calculations were performed using the modules supported by SPARTAN SGI, Version 5.1.1, Wavefunction Inc., 18041 Von Karman Ave, Suite 370, Irvine, CA 92715.
18. (a) Guarna, A.; Occhiato, E. G.; Machetti, F.; Scarpi, D. *J. Org. Chem.* **1998**, *63*, 4111. (b) Occhiato, E. G.; Scarpi, D.; Machetti, F.; Guarna, A. *Tetrahedron* **1998**, *54*, 11589.
19. Thigpen, A. E.; Cala, K. M.; Russel, D. W. *J. Biol. Chem.* **1993**, *265*, 17404.
20. Abell, A. D.; Erhard, K. F.; Yen, H.-K.; Yamashita, D. S.; Brandt, M.; Mohammmed, H.; Levy, M. A.; Holt, D. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1365.
21. MacroModel (v 6.5) molecular modeling software, on a SGI IRIX 6.5 workstation, was used for the molecular mechanics calculations. Mohamadi, F.; Richards, N. G.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.