Benzo[c]quinolizin-3-ones: a novel class of potent and selective nonsteroidal inhibitors of human steroid 5alpha-reductase 1

Benzo[*c*]quinolizin-3-ones: A Novel Class of Potent and Selective Nonsteroidal Inhibitors of Human Steroid 5α-Reductase 1

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The synthesis and biological evaluation of a series of novel, selective inhibitors of isoenzyme 1 of human 5α-reductase (5αR) (EC 1.3.99.5) are reported. The inhibitors are 4a*H*- (**19**−**29**) or 1*H*-tetrahydrobenzo[*c*]quinolizin-3-ones (**35**−**47**) bearing at positions 1, 4, 5, and 6 a methyl group and at position 8 a hydrogen, methyl group, or chlorine atom. All these compounds were tested toward 5αR-1 and 5αR-2 expressed in CHO cells (CHO 1827 and CHO 1829, respectively) resulting in selective inhibitors of the type 1 isoenzyme, with inhibitory potencies (IC_{50}) ranging from 7.6 to 9100 nM. The inhibitors of the 4a*H*-series, having a double bond at position 1,2, were generally less active than the corresponding inhibitors of the 1*H*-series having the double bond at position 4,4a on the A ring. The presence of a methyl group at position 4 (as in compounds **39**−**40** and **45**−**47**), associated with a substituent at position 8, determined the highest inhibition potency (IC_{50} from 7.6 to 20 nM). Compounds **39** and **40**, having K_i values of 5.8 ± 1.8 and 2.7 ± 0.6 nM, respectively, toward 5αR-1 expressed in CHO cells, were also tested toward native 5αR-1 in human scalp and 5αR-2 in human prostate homogenates, in comparison with finasteride and the known 5αR-1-selective inhibitor LY191704, and their mechanism of inhibition was determined. They both inhibited the enzyme through a reversible competitive mechanism and again were selective inhibitors of 5αR-1 with IC_{50} values of 41 nM. These specific features make these inhibitors suitable candidates for further development as drugs in the treatment of DHT-dependent disorders such as acne and androgenic alopecia in men and hirsutism in women.

Introduction

Dihydrotestosterone (DHT), the most potent circulating androgen hormone, is produced by the NADPH-dependent, stereoselective reduction of testosterone (T) under catalysis of the enzyme steroid 5α-reductase (5αR) (EC 1.3.99.5).[1,2] The DHT production is in many cases related to the maintenance of some pathological human diseases and endocrine disorders,[3−8] so that the use of 5αR inhibitors for the possible control or suppression of DHT formation, without significant changes in the circulating testosterone, became some years ago a therapeutic target for the treatment of benign prostate hyperplasia (BPH), androgenic alopecia, and acne in men and hirsutism in women.[9,10]

Because of the discovery that two different DNA-encoded isoenzymes of 5αR, named type 1 and type 2 (5αR-1 and 5αR-2), transform T into DHT with different efficacy and that these two isozymes are not equally distributed in the human tissues, 5αR-1 being present mainly in scalp, skin, and liver, and 5αR-2 in the prostate, different therapeutic approaches were later developed. The synthesis and use of selective 5αR-2 inhibitors was initially envisioned for the specific treatment of a prostate disease such as BPH, culminating with the discovery of the first class of steroidal 5αR inhibitors, the 4-azasteroids, and the introduction on the market by Merck of finasteride (Proscar) (Figure 1), the first drug for BPH treatment based on the concept of 5αR inhibition.[11] However, after the observation that finasteride was not equally efficacious in all treated patients and that only in 30−40% of the treated cases the circulating level of DHT decreases up to 20% of the basal level,[12] the synthesis and use of double 5αR-1 and 5αR-2 inhibitors became a therapeutic model to completely reducing the circulating DHT. This new approach has brought to development by Glaxo of dutasteride (Figure 1), a double inhibitor which entered phase III clinical trials in 1997 and presumably will be on the market for the treatment of BPH in 2000−2001.[13]

Although the role of 5αR-1 is not completely clear, its diffuse presence in the scalp and skin of men suffering from alopecia or acne and skin of women suffering from hirsutism and polycistic ovarian syn-

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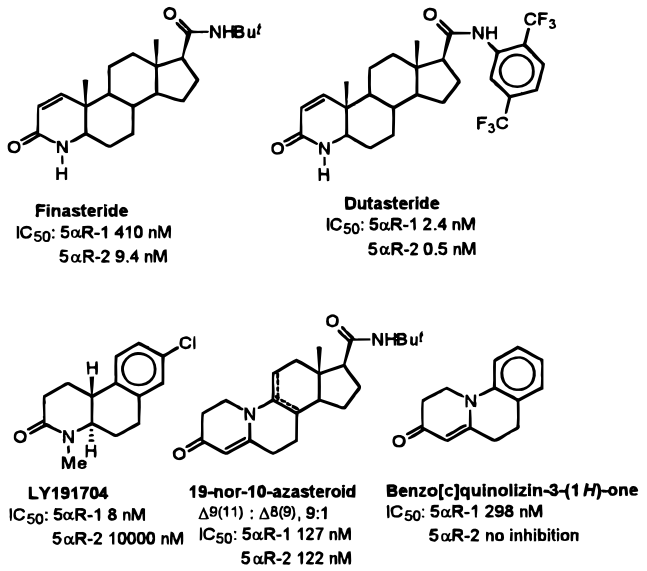


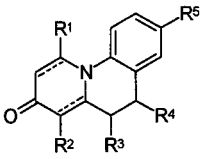
Figure 1.

drome (PCOS) suggested a strong implication of 5αR-1 in these disorders.[14] Therefore, the possibility of reducing the DHT level in those tissues by using selective 5αR-1 inhibitors (not affecting 5αR-2, mainly located in prostate tissue) could be a new therapeutic approach for the treatment of the above skin disorders.[15] In particular, the use of a "pure" 5αR-1 inhibitor is essential for the treatment of hirsutism and PCOS because of the well-known risks of pseudohermafroditism for the male fetus associated with 5αR-2 blockade in pregnant women.[16] To date, few compounds (one of them, a nonsteroidal benzoquinoline synthesized by Eli-Lilly, LY191704, is reported in Figure 1) have been reported to have this selectivity but their clinical development has not yet reached completion.[17]

On the basis of the above considerations, we started some years ago a project aimed at the design and synthesis of new inhibitors of 5αR-1 and 5αR-2,[18−22] focusing in particular on the development of selective 5αR-1 inhibitors. We thus discovered that 19-nor-10-azasteroids (Figure 1), designed by us as possible mimics of a substrate-like transition state involved in the T to DHT reduction, were dual inhibitors of 5αR, with a potency tightly dependent on the presence, position, and number of unsaturations on the A−C rings, as well as the type of substituent at position 17.[18]

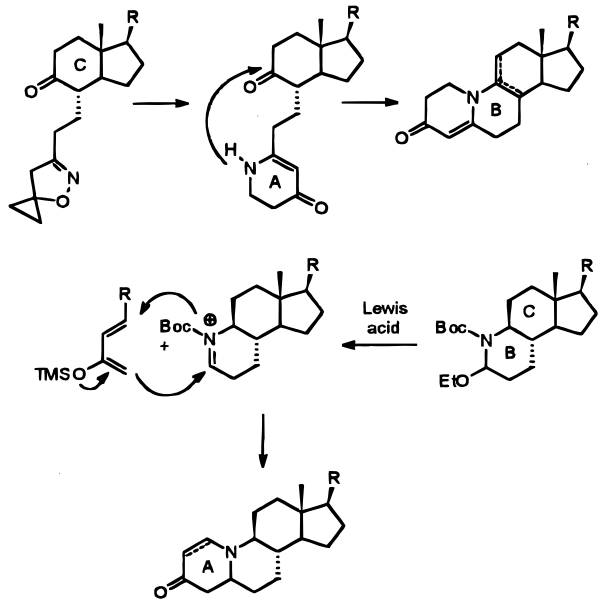
The observation that an increase of potency could be associated with the presence of unsaturations on the C ring, which determined a certain flatness of the azasteroidal skeleton, prompted us to design a novel class of nonsteroidal inhibitors based on the benzo[*c*]quinolizin-3-ones structure (Figure 1).[23,24] These compounds, while maintaining the A ring enaminone moiety as an essential feature of the 19-nor-10-azasteroids, lacked the D ring and incorporated a benzene ring in place of the C ring to have a more planar overall structure. In analogy to Eli Lilly compounds and on the basis of the above observations on 19-nor-10-azasteroids, we anticipated that these novel compounds could be selective inhibitors of 5αR-1. In the present paper we therefore describe the synthesis and structure−activity relationship (SAR) of a series of benzo[*c*]quinolizin-3-ones **1**−**47** (Chart 1), differentiated by the presence and position

Chart 1. Compounds of the 1*H*- and 4a*H*-Series



Compound	Unsaturation	R^1	R^2	R^3	R^4	R^5
1		H	H	H	H	H
2		H	H	H	H	Me
3		H	H	H	H	Cl
4		H	Me (α)	H	H	H
5		H	Me (β)	H	H	H
6		H	Me (α)	H	H	Me
7		H	Me (β)	H	H	Me
8		H	Me (α)	H	H	Cl
9		H	Me (β)	H	H	Cl
10		H	H	Me (α)	H	Cl
11		H	H	H	Me (α)	Me
12		H	H	H	Me (α)	Cl
13		Me (α)	H	H	H	Cl
14		Me (β)	H	H	H	Cl
15		H	Me (α)	Me (α)	H	Cl
16		H	Me (β)	Me (α)	H	Cl
17		H	Me (α)	H	Me (α)	Me
18		H	Me (α)	H	Me (α)	Cl
19	1,2	H	H	H	H	H
20	1,2	H	H	H	H	Me
21	1,2	H	H	H	H	Cl
22	1,2	H	Me (α)	H	H	H
23	1,2	H	Me (β)	H	H	H
24	1,2	H	Me (α)	H	H	Me
25	1,2	H	Me (β)	H	H	Me
26	1,2	H	Me (β)	H	H	Cl
27	1,2	H	H	Me (α)	H	Cl
28	1,2	H	H	H	Me (α)	Me
29	1,2	H	H	H	Me (α)	Cl
30	1,2	Me	H	H	H	Cl
31	1,2	H	Me (α)	Me (α)	H	Cl
32	1,2	H	Me (β)	Me (α)	H	Cl
33	1,2	H	Me (α)	H	Me (α)	Me
34	1,2	H	Me (α)	H	Me (α)	Cl
35	4,5	H	H	H	H	H
36	4,5	H	H	H	H	Me
37	4,5	H	H	H	H	Cl
38	4,5	H	Me	H	H	H
39	4,5	H	Me	H	H	Me
40	4,5	H	Me	H	H	Cl
41	4,5	H	H	Me	H	Cl
42	4,5	H	H	H	Me	Me
43	4,5	H	H	H	Me	Cl
44	4,5	Me	H	H	H	Cl
45	4,5	H	Me	Me	H	Cl
46	4,5	H	Me	H	Me	Me
47	4,5	H	Me	H	Me	Cl

Scheme 1



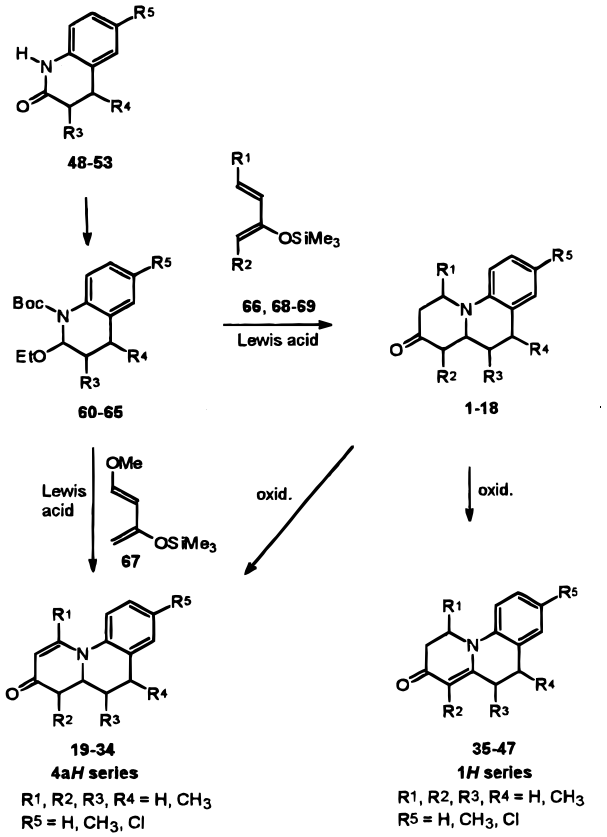
of double bonds on the A ring and the type and number of substituents introduced at positions 1, 4, 5, 6, and 8, which resulted in potent and selective 5αR-1 inhibitors.

Chemistry

The preparation of the new class of benzo[*c*]quinolizin-3-one inhibitors **1**−**47** required the study and development of a synthetic strategy different from that employed in the synthesis of 19-nor-10-azasteroids. The latter were in fact prepared through the tandem thermal rearrangement−annulation of suitably functionalized isoxazoline-5-spirocyclopropanes,[18,25] a methodology developed in our laboratory which allows the sequential construction of the A and B rings of the azasteroids (Scheme 1). However it is a matter of course that this procedure is inapplicable to the synthesis of benzo[*c*]-quinolizin-3-ones due to the presence of an aromatic C ring in the structure.

As an alternative to the isoxazoline methodology, we recently reported a different synthesis of 19-nor-10-azasteroids based on the TMSOTf-promoted tandem Mannich−Michael reaction of 2-silyloxy-1,3-dienes with *N*-(acyloxy)iminium ions generated in situ from the corresponding *N*-Boc-α-ethoxy derivatives[20−22] (Scheme 1). If applied to the synthesis of benzo[*c*]quinolizinones **1**−**47** (Scheme 2), this methodology would require the preparation, from lactams **48**−**53**, of bicyclic *N*-Boc-α-ethoxy quinolines **60**−**65** to be reacted in turn with the suitable silyloxydienes **66**−**69** in the presence of a Lewis acid. This would generate the corresponding iminium ion which undergoes tandem Mannich−Michael attack by the silyloxydiene. In the case Danishefsky's diene **67** (R^1 = OMe, R^2 = H) the reaction would lead directly to 1,2-unsaturated compounds **19**−**34** (wherein R^1 = H, R^2 = H); otherwise oxidation of intermediates **1**−**18** is necessary to introduce a double bond that conjugates the bridgehead N and the 3-oxo group. As we have already found in 19-nor-10-azasteroids, the lack of conjugation between N and C=O causes a loss of inhibitory potency, and this, if the inhibition mechanism is similar for the two classes of compounds, is likely to occur also in benzo[*c*]quinolizinones **1**−**18**.

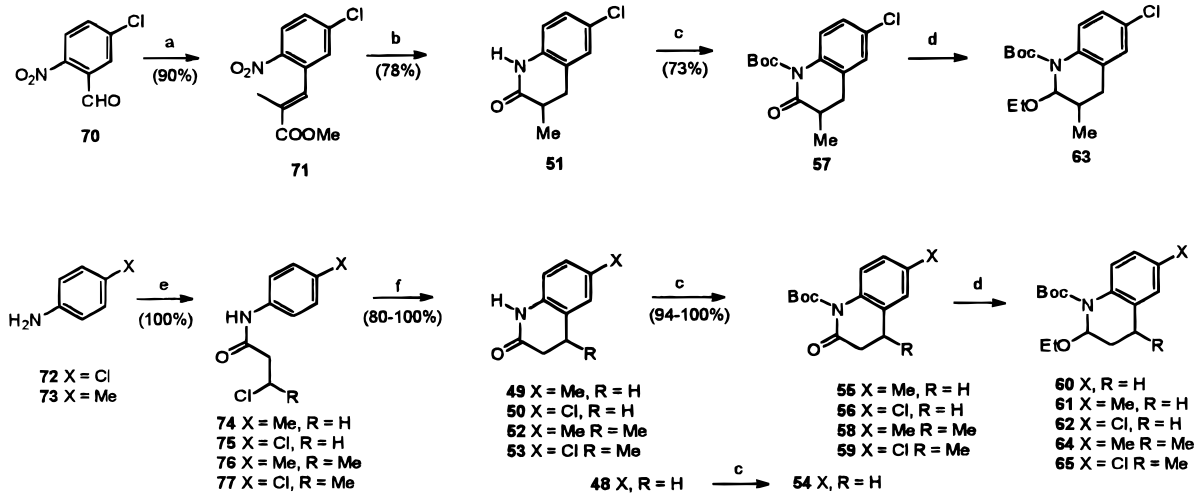
Scheme 2



The synthesis of *N*-Boc-α-ethoxyquinolines **60**−**65** is reported in Scheme 3. Lactams **49**−**53** were prepared according to known procedures,[26] whereas lactam **48** (R^3,R^4,R^5 = H) is commercially available. Aldehyde **70** (Scheme 3), treated with 2-(triphenylphosphanylidene)-propionic acid methyl ester[27] gave cynnamate **71** as a mixture of diastereoisomers in 90% yield, which was converted into 3-methyl-substituted lactam **51** by hydrogenation over PtO_2 in AcOH. 4-Substituted anilines **72**−**73** were reacted with 3-chloropropanoyl or 3-chlorobutanoyl chlorides to give the corresponding *β*-chloroamides **74**−**77** in quantitative yield. Lactams **49**−**50** and **52**−**53** were obtained after the intramolecular Friedel−Craft alkylation that amides **74**−**77** underwent in the presence of $AlCl_3$ at 120−130 °C. In the case of 6-methyl-substituted amides **74** and **76**, a strict control of the temperature was necessary to avoid migration of the methyl group on the aromatic ring and thus the formation of isomers. After protection of N atom as *N*-Boc to give compounds **54**−**59**, reduction of the 2-oxo group by $NaBH_4$ in ethanol at −25 °C, followed by acidic quench with 2 N HCl in ethanol, afforded *N*-Boc-2-ethoxy derivatives **60**−**65** in 94−100% overall yield. In the case of 3- and 4-methyl-substituted derivatives **63**−**65**, these compounds were obtained as 1:1 mixtures of diastereoisomers.

In a previous communication[23] we have reported that TMSOTf catalyzes the tandem Mannich−Michael cyclization of unsubstituted *N*-*t*-Boc derivative **60** (X,R = H) with silyloxydienes **66** (R^1 = R^2 = H) and **67** (R^1 = OMe, R^2 = H) providing benzo[*c*]quinolizin-3-ones **1** and **19** in moderate yields. To extend this methodology to *N*-Boc-α-ethoxy derivatives such as **62**, **63,** or **65**, bearing a chlorine atom on the benzene ring, we studied

Scheme 3[a]



[a] (a) $Ph_3P{=}C(Me)COOMe$, toluene, 80 °C, 3 h; (b) H_2, 10 atm, PtO_2, AcOH, 60 °C, 14 h; (c) $(BOC)_2O$, Et_3N, DMAP, CH_2Cl_2, 18 h, 25 °C; (d) $NaBH_4$, EtOH, −25 °C, 2−5 h, then 2 N HCl in EtOH, pH 3−4, −25 → 0 °C, 1.5−7 h; (e) $RCH(Cl)CH_2COCl$, acetone, reflux, 1 h; (f) $AlCl_3$, 120−160 °C, 3−24 h.

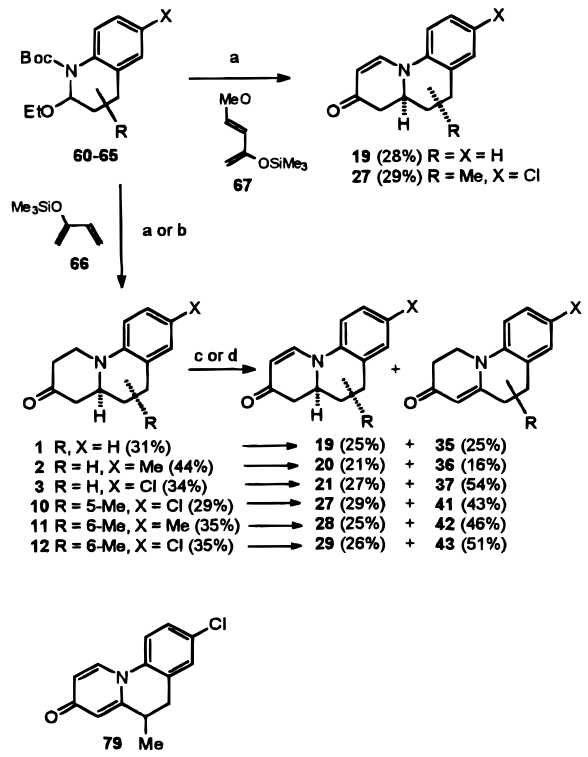
the use of different Lewis acids to promote the formation of *N*-(acyloxy)iminium ions, finding out that $TiCl_4$ promoted the iminium ion formation and the succeeding cyclization step better than TMSOTf. The new procedure applied to *N*-Boc-α-ethoxy derivative **62** (X = Cl, R = H) and diene **66** afforded **3** (X = Cl, R = H) in 34% yield, whereas the same compound was obtained in 17% yield employing TMSOTf as a Lewis acid.[24]

The $TiCl_4$ methodology was applied to the synthesis of 8-H-, 8-Cl-, and 8-CH_3-substituted benzo[*c*]quinolizinones **1**−**3**, **10**−**12**, **19,** and **27** (Scheme 4), bearing no substituents on the A ring and therefore deriving from cyclization of **60**−**65** with Danishefky diene **67** and 2-trimethylsilyloxy-1,3-butadiene **(66)**. Only in the case of compound **2** the TMSOTf procedure furnished higher yield (44%) than $TiCl_4$.

The reaction of 3- and 4-substituted iminium ions from **63**−**65** with diene **66** was stereoselective, providing the isomer with the methyl group and the bridgehead 4a proton in a cis relative position as the major product. These (and the following) stereochemical assignments were possible by inspection of the coupling constants in the ^1H NMR spectrum of the saturated and, then, the corresponding Δ^1- or Δ^4-oxidized derivatives. A complete discussion on the attribution of the relative stereochemistry is reported in the Supporting Information. Compounds **10**−**12** were isolated in 29−35% yield after chromatographic purification, while their trans isomers were only detected (<3%) by ^1H NMR analysis of the crude reaction mixtures. The reactions of **63** and **64** with Danishefsky's diene **67**, which afforded Δ^1-unsaturated compounds **27** (29%) and **19** (28%), occurred with similar stereoselectivity. Since strong Lewis acids are able to remove the *N*-*t*-Boc protection, the stereochemical outcome of these reactions could be explained by the formation, after the addition of $TiCl_4$ to the α-ethoxy carbamate, of a planar imine in which the N atom coordinates a titanium complex.[21] The methyl group at position 3 or 4 then leads to a preferred less hindered anti attack by the dienes.

The oxidation of compounds **1**−**3** and **10**−**12** to the 1,2- or 4,4a-unsaturated analogues (Scheme 4) was

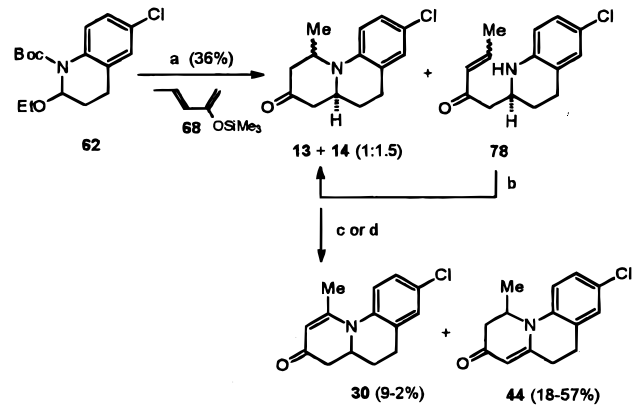
Scheme 4[a]



[a] (a) 1 M $TiCl_4$ in CH_2Cl_2, −30 °C, 10 min, then **66** or **67**, −30 → 25 °C, 30 min; then $NaHCO_3$ (satd), 45 min; (b) methyl vinyl ketone, TMSOTf, Et_3N, CH_2Cl_2, 0 °C, 30 min; then **60**−**65**, TMSOTf, 0 → 25 °C, 45 min; then $NaHCO_3$, 25 °C, 36 h; (c) $Hg(OAc)_2$, EDTA tetrasodium salt, 5−50% CH_3COOH (aq), 90 °C, 2 h; (d) LDA, Me_3SiCl, THF, −78 → 25 °C; then DDQ, 25 °C, 18 h.

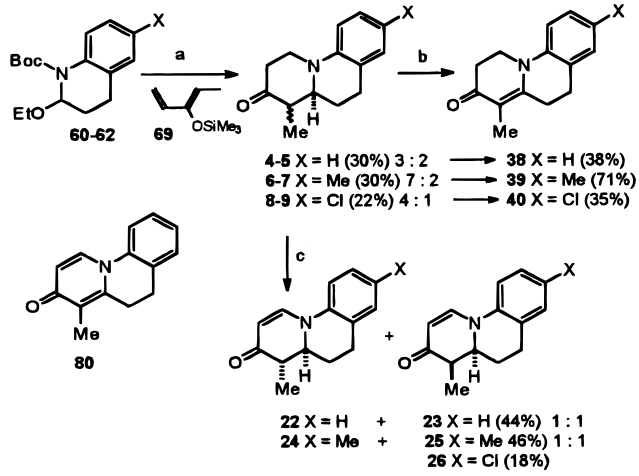
performed by treatment with $Hg(AcO)_2$ as already reported for the 19-nor-10-azasteroid synthesis.[28] As an alternative, after formation of the corresponding silyl enol ethers by treatment with LDA and TMSCl in THF at −78 °C, oxidation can be achieved by employing DDQ at room temperature.[29] Usually, the latter conditions afforded almost equimolar amounts of Δ^1- and Δ^4-isomers (as in the oxidation of **1** and **2**) and lower yields than $Hg(OAc)_2$, which instead provided always mixtures containing compounds of the 1*H*-series (**35**, **37**, **41**−**43**)

Scheme 5[a]



[a] (a) 3-Penten-2-one, TMSOTf, Et_3N, CH_2Cl_2, 0 °C, 30 min, then **62**, TMSOTf, 0 → 25 °C, 45 min, then $NaHCO_3$, 25 °C, 36 h; (b) 1 M $TiCl_4$ in CH_2Cl_2, rt, 3 h, then $NaHCO_3$(satd), 45 min; (c) $Hg(OAc)_2$, EDTA tetrasodium salt, 45% CH_3COOH(aq), 90 °C, 2 h; (d) LDA, Me_3SiCl, THF, −78 → 25 °C, then DDQ, 25 °C, 18 h.

Scheme 6[a]



[a] (a) 1-Penten-3-one, TMSOTf, Et_3N, CH_2Cl_2, 0 °C, 30 min, then **60**−**62**, TMSOTf, 0 → 25 °C, 45 min, then $NaHCO_3$, 25 °C, 36 h; (b) LDA, Me_3SiCl, THF, −78 → 25 °C, then DDQ, 25 °C, 18 h; (c) $Hg(OAc)_2$, EDTA tetrasodium salt, 5−8% CH_3COOH(aq), 90 °C, 2 h.

as the major isomers. On the other hand, complete oxidation of the A ring was in some cases observed (oxidation of **10** and the **4**−**5** mixture) under the more drastic conditions required with mercuric acetate, and compounds **79** (Scheme 4) and **80** (Scheme 6) were obtained in 16 and 12% yield.

While employing $TiCl_4$ as a Lewis acid requires the addition of a pre-formed pure diene to the reaction mixture, in the TMSOTf-promoted Mannich−Michael cyclization process the reacting silyloxydiene may be generated in situ using the corresponding alkyl vinyl ketone. Thus, we found more convenient to employ the TMSOTf procedure in the preparation of 1- and 4-methyl-substituted benzo[*c*]quinolizin-3-ones (Schemes 5 and 6) by the in situ generation of 4-methyl-2-(trimethylsilyloxy)-1,3-butadiene **(68)** from 3-penten-2-one with the TMSOTf/Et_3N system in dichloromethane. The addition of **62** and a further amount of TMSOTf (Scheme 5) to the solution of **68**, afforded an approximatively 1:1 mixture of 1-methyl-substituted compounds **13** and **14**, together with a smaller amount of open chain product **78**. Treatment of this mixture with $TiCl_4$ caused complete cyclization of **78** to a mixture of **13** and **14**. Oxidation of **14** was carried out by $Hg(OAc)_2$, providing Δ^4-compound **44** in 96% yield and its Δ^1-isomer **30** in 4% yield (18% and 9% yield, respectively, by the DDQ oxidation).

With a similar procedure, α-ethoxy carbamates **60**−**62** were reacted with silyloxydiene **69** derived from 1-penten-3-one (Scheme 6), yielding 4-methyl-substituted compounds **4**−**5**, **6**−**7**, and **8**−**9** in 22−30% yield as 4α/*β*-variable mixtures. Only in the case of the **6**−**7** mixture were we able to obtain, after chromatograaphy, pure *β*-isomer **7**. A portion of each mixtures was then oxidized by $Hg(OAc)_2$, affording Δ^4-unsaturated compounds **38**−**40** (35−71%), whereas another portion was subjected to the DDQ procedure, obtaining only Δ^1-unsaturated compounds **22**−**25** in 44−46% yield as epimeric 4α/*β*-mixtures. Compound **26** was obtained as a single 4*β*-isomer, but taking into account the very low final yield (18%), the other isomer might have been lost during the chromatographic purification.

Diene **69** was employed for the synthesis of 4,5- and 4,6-dimethyl-substituted benzo[*c*]quinolizinones **15**−**18** (Scheme 7) according to the TMSOTf procedure. However, very complex isomeric mixtures were obtained after reaction of **69** with 3- and 4-methyl-substituted carbamates **63**−**65**. A careful chromatographic separation allowed the recovery of the 1:2 mixture of epimers **15** and **16**, having the methyl at C5 in a cis relative position with the 4a-H proton, but in low yield (20%). Both 4,6-dimethyl derivatives **17** (X = Me) and **18** (X = Cl) were obtained in 14% yield as mixtures in which the diastereoisomer having both the methyl groups α orientated with respect to the 4a-H hydrogen was prevailing. Usual oxidation of the **15**/**16** mixture by Hg(OAc)$_2$ yielded Δ^4-compound **45** (14%) besides the 1:2 mixture of Δ^1-isomers **31** and **32** (11%) which were separated after repeated chromatographies. Similarly, oxidation of **17** and **18** gave rise to Δ^4-compounds **46** (27%) and **47** (34%), respectively, together with Δ^1-isomers **33** and **34**.

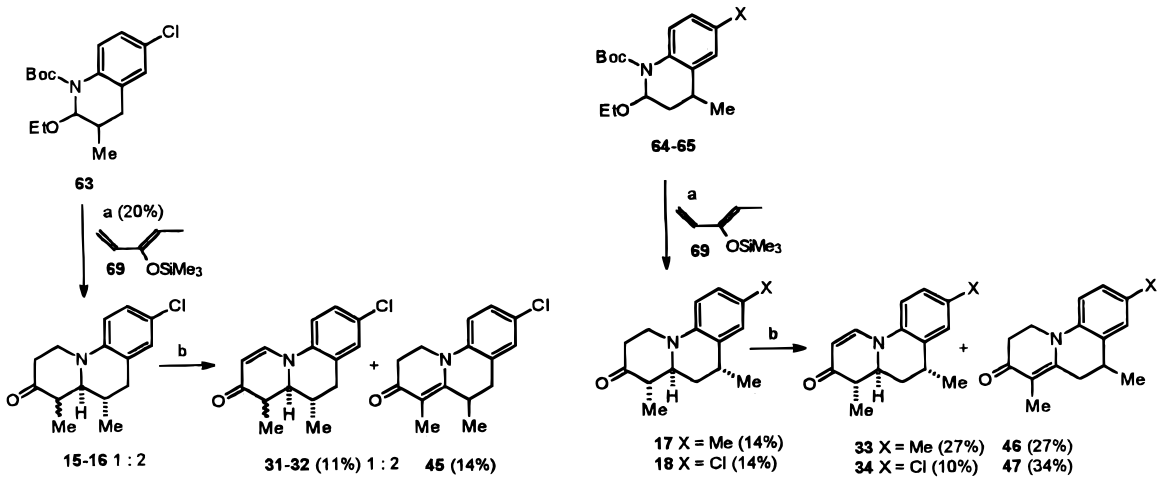
The stereochemical outcome of the reaction of **69** with the iminium ions generated from **63** seems to be in accordance with the initial anti approach of the diene as already discussed, as well as the formation of compounds **17** and **18**, which apparently derive from the anti approach of **69** to the iminium ions from **64** and **65**.

Finally, also the HCl salts of two unsaturated benzo[*c*]quinolizin-3-ones were prepared by dissolving compounds **38** and **40** in a solution of HCl in anhydrous methanol, obtaining after evaporation of the solvent salts **81** and **82**, both tested toward 5αR-1 and 5αR-2.

Results

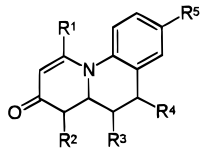
Inhibition toward Human Recombinant 5αR-1 and 5αR-2 in CHO Cells. Most of the synthesized compounds were tested toward the two human recombinant isozymes of 5αR to evaluate the inhibitory potency and isozyme selectivity using finasteride as a control (Table 1). The assays were performed using stably transfected CHO cells (CHO 1827 for 5αR-1 and CHO 1829 for 5αR-2, respectively)[30] incubated for 30 min with [^{3}H]testosterone at the K_m concentration (2

Scheme 7[a]

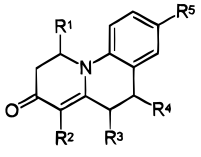


[a] (a) 1-Penten-3-one, TMSOTf, Et_3N, CH_2Cl_2, 0 °C, 30 min, then **63**−**65**, TMSOTf, 0 → 25 °C, 45 min, then $NaHCO_3$, 25 °C, 36 h; (b) $Hg(OAc)_2$, EDTA tetrasodium salt, 50% CH_3COOH(aq), 90 °C, 2 h.

Table 1. Inhibition of Compounds of the 1*H*- and 4a*H*-Series toward Recombinant 5αR-1 Expressed in CHO Cells



4a*H* series



1*H* series

compd	R^1	R^2	R^3	R^4	R^5	IC_{50} (nM)	compd	R^1	R^2	R^3	R^4	R^5	IC_{50} (nM)
19	H	H	H	H	H	5130 ± 130	**35**	H	H	H	H	H	298 ± 75
20	H	H	H	H	Me	176 ± 17	**36**	H	H	H	H	Me	376 ± 185
21	H	H	H	H	Cl	459 ± 118	**37**	H	H	H	H	Cl	49 ± 19
22	H	Me (α)	H	H	H	2700 ± 300	**38**	H	Me	H	H	H	185 ± 62
23	H	Me (*β*)	H	H	H	4300 ± 400	**39**	H	Me	H	H	Me	20 ± 8 5.8 ± 1.8[a]
24	H	Me (α)	H	H	Me	137 ± 58	**40**	H	Me	H	H	Cl	7.6 ± 0.9 2.7 ± 0.6[a]
25	H	Me (*β*)	H	H	Me	312 ± 23	**41**	H	H	Me	H	Cl	346 ± 185
26	H	Me (*β*)	H	H	Cl	141 ± 24	**42**	H	H	H	Me	Me	14.3 ± 5.9
27	H	H	Me (α)	H	Cl	9100 ± 500	**43**	H	H	H	Me	Cl	14.4 ± 3.4
29	H	H	H	Me (α)	Cl	188 ± 42	**44**	Me	H	H	H	Cl	204 ± 49
							45	H	Me	Me	H	Cl	15.6 ± 4.0
							46	H	Me	H	Me	Me	15.8 ± 4.6
							47	H	Me	H	Me	Cl	8.5 ± 2.1

[a] This is a K_i value.

*μ*M for 5αR-1 and 0.2 *μ*M for 5αR-2) and each inhibitor in the 10^{-9}−10^{-5} M concentration range. Data were processed with the program ALLFIT[31] using the four parameter logistic equation to calculate the IC_{50} values. The interassay reproducibility of the method was good as assessed by calculating the mean IC_{50} of finasteride: 911 ± 85 nM (CV% = 9.4, n = 15) and 21 ± 1.8 nM (CV% = 8.6, n = 12) for 5αR-1 and 5αR-2, respectively, consistent with the established selectivity reported for the inhibitor.[15a] All the tested molecules resulted as selective inhibitors of 5αR-1 having IC_{50} values (Table 1) ranging from 7.6 nM to 9.1 *μ*M, whereas they displayed very poor or no inhibition toward 5αR-2.

The inhibitors belonging to the 1*H*-series were more potent than those of the 4a*H*-series. For further studies we selected the very potent compounds **39** and **40**, belonging to the 1*H*-series, which being achiral compounds do not require a separation of enantiomers as for many of the other inhibitors. The K_i values of **40** and **39** were then determined using again CHO 1827 intact cells, and a competitive mechanism of action was demonstrated. Also for these experiments finasteride was used as a control inhibitor, resulting in a competitive inhibitor. The K_i values were 2.7 ± 0.6 and 5.8 ± 1.8 nM, respectively, for **40** and **39** and 366 ± 186 nM for finasteride, the latter in good agreement with the reported value.[30]

The mechanism of action of the most potent inhibitor **40** was studied on recombinant 5αR-1 isozyme using intact CHO 1827 transfected cells. To demonstrate the reversibility of inhibition, we applied to our cell system a method recently described by Azzolina et al.[32] for the elucidation of the mechanism of action of finasteride on the rat 5αR-1 and 5αR-2 isozymes. Intact cells were maintained in contact with the inhibitor at the IC_{50} concentration, and the reduction of enzymatic activity was monitored at different times versus a control. After 1 h the medium containing the inhibitor was removed; cells were washed with phosphate-buffered saline (PBS) solution and incubated without the inhibitor. If the enzymatic activity is restored at the control level the inhibitor is considered reversible. Finasteride, used as

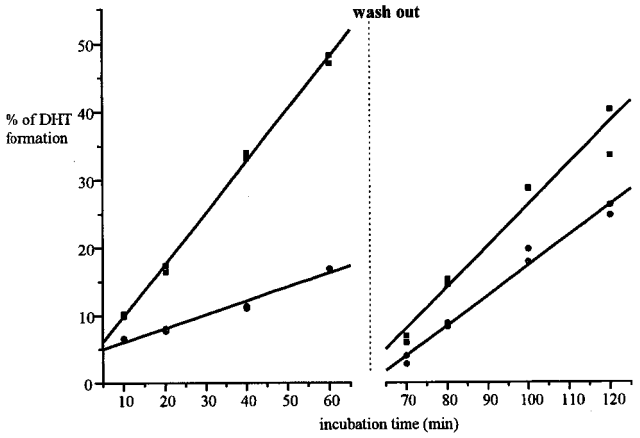


Figure 2. Determination of the mechanism of inhibition for compound **40**: top lines, control; bottom lines, experiments with inhibitor. Concentration of **40** was 10 nM.

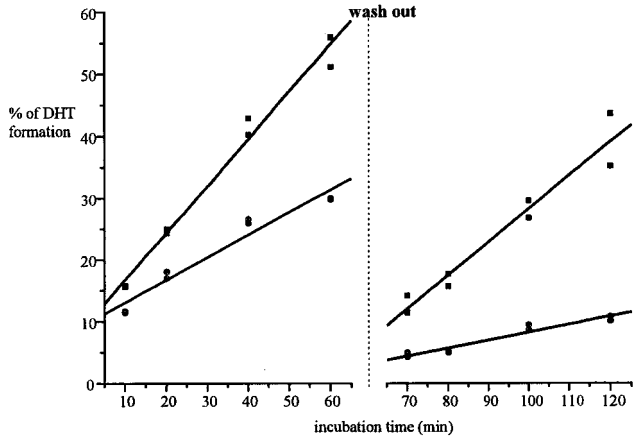


Figure 3. Determination of the mechanism of inhibition for finasteride: top lines, control; bottom lines, experiments with inhibitor. Concentration of finasteride was 700 nM.

Table 2. Inhibition Activity toward Native 5αR-1 and 5αR-2

	inhibition	
compd	5αR-1[a]	5αR-2[b]
finasteride		2.2 ± 0.2 nM[c]
LY191704	2.3 ± 1.4 nM[c,d]	19% at 10 μM
39	41 ± 22 nM[c,d]	20% at 10 μM
40	41 ± 17 nM[c,d]	20% at 10 μM

[a] From human scalp homogenates. [b] From human prostate homogenate. [c] IC_{50} value. [d] In these experiments 10 nM finasteride was used to block 5αR-2 present in the scalp tissue.

a control inhibitor, showed an irreversible behavior as expected under these conditions, whereas **40** displayed a reversible mechanism of action. In Figures 2 and 3 are reported the graphs of the experiments conducted with **40** and finasteride.

Inhibitory Activity toward Native 5αR-1 and 5αR-2. To confirm the complete selectivity observed for these compounds toward the recombinant enzymes, we selected **39** and **40** for determining their inhibitory activity and selectivity versus native 5αR-1 and 5αR-2 using human scalp and human prostate tissues, respectively (Table 2).

To have an in vitro system representative of the in vivo situation, we used the total tissue homogenate without any fractionation. It is known that 5αR-2 is the prevalent isozyme in prostate tissue, while 5αR-1 is mainly expressed in scalp skin. However, the presence of both 5αR isozymes in prostate tissue and also in scalp skin has been recently documented (mRNA, expressed protein, and functional protein).[33] A major problem arises from the lack of knowledge on the exact ratio of the two isoenzymes within the different parts of the tissues. For this reason, aimed at evaluating the specific inhibition for each enzyme, we set the experimental conditions to measure the activity of desired target isozyme avoiding any possible interference due to the presence of the other isoenzyme. As a control of the assay selectivity, we tested finasteride which is a quite selective 5αR-2 inhibitor and LY191704 (Figure 1) as a completely selective 5αR-1 inhibitor.

The inhibition test against 5αR-2 in human prostate homogenates was performed using a 50 nM testosterone concentration; only 5αR-2 should be active at this concentration because of the different K_m values of the two isozymes for the substrate (1.7 μM for 5αR-1 and 0.2 μM for 5αR-2). Under these experimental conditions the IC_{50} value obtained for finasteride was 2.2 ± 0.2 nM, while LY191704 resulted inactive (only 19% inhibition at 10 μM). These results are in good agreement with the data reported in the literature for 5αR-2 inhibition of both compounds,[11,17] assessing that in the above assay only the type 2 isoenzyme was activated. Also compounds **39** and **40** resulted inactive versus 5αR-2, displaying only a 20% inhibition at 10 μM concentration.

By contrast, when performing the inhibition tests with human scalp homogenates to measure the 5αR-1 inhibition, it is not possible to find a testosterone concentration where only the 5αR-1 is active. Indeed, at the micromolar concentration of testosterone required for the activation 5αR-1, also 5αR-2 is active and works at its maximum velocity. However, by adding finasteride (10 nM) in all inhibition experiments, we were able to selectively block the 5αR-2 activity without interfering with the 5αR-1 activity. Thus, in this assay, the reference compound LY1917104 resulted active with an IC_{50} of 2.3 ± 1.4 nM, in good agreement with the value reported for its 5αR-1 inhibition.[17] Benzo[*c*]quinolizinones **39** and **40** both displayed an IC_{50} value of 41 nM, assessing that these compounds are therefore selective and potent inhibitors of the native 5αR-1 present in the scalp tissue.

Discussion

We have already reported in a previous communication[23] on the inhibitory activity of two simple unsubstituted benzo[*c*]quinolizinone compounds **(35** and **19)** toward both 5αR-1, expressed by transfected CHO cells, and native 5αR-2, in human prostate homogenates. Both compounds were selective 5αR-1 inhibitors with IC_{50} values of 298 and 5130 nM, respectively, with Δ^4-compound **35** being about 17-fold more active than its Δ^1-isomer **19**.

The introduction of substituents on different positions of the benzo[*c*]quinolizinone skeleton of **35** and **19** allowed us to obtain two classes (1*H*- and 4a*H*-series) of compounds differing by the position of the double bond on the A ring. In Table 1 the inhibition values of Δ^1-compounds **19**−**29** and Δ^4-compounds **35**−**47** toward 5αR-1 expressed in recombinant CHO cells are reported. All of them were inactive toward 5αR-2 in CHO cells.

In general, the compounds of the 1*H*-series resulted significantly more active than those of the 4a*H*-series,

the IC_{50} values of the latter being approximately 10-fold higher. In fact the inhibition values ranged from 137 to 9100 nM for the 4a*H*-compounds and from 7.6 to 376 nM for the 1*H*-compounds.

The presence of a substituent at position 8, for instance a chlorine or methyl group, generally increased the potency of the inhibitors in both series. Thus, in the 4a*H*-series compounds **20** (IC_{50} 176 nM) and **21** (IC_{50} 459 nM), bearing an 8-methyl and 8-chlorine, respectively, were significantly more active than unsubstituted compound **19** (IC_{50} 5130 nM). Analogously, in the 1*H*-series the chlorine atom at position 8, either alone or in the presence of other substituents on the two aliphatic rings, increased noticeably the potency toward 5αR-1. Thus 8-Cl-substituted compounds **37** (IC_{50} 49 nM) and **40** (IC_{50} 7.6 nM) were significantly more active than unsubstituted compound **35** (IC_{50} 298 nM) and **38** (IC_{50} 185 nM), respectively. Instead, the methyl group at position 8 of compounds of the 1*H*-series was ineffective when alone, while it increased the potency if combined with one or two methyl groups on the two aliphatic rings. In fact, 8-methyl-substituted compound **36** (IC_{50} 376 nM) was approximately as potent as the unsubstituted compound **35** (IC_{50} 298 nM), while the modification of compound **38** (IC_{50} 185 nM) by introducing a methyl group at position 8 led to the more potent compound **39** having an IC_{50} of 20 nM.

The introduction of a methyl group at position 4 in both series was effective in increasing the inhibitory potency. The extent of the increase is more slight in the 4a*H*-series and higher in the 1*H*-series. In the 1*H*-series the introduction of a methyl at 4 position determines a strong increase of potency, in particular when the 8-position is substituted with a chlorine or methyl. Thus, whereas 4-methyl derivative **38** displayed an inhibition activity (IC_{50} 185 nM) not too significantly different from the unsubstituted compound **35** (IC_{50} 298 nM), a very strong increase of potency is observed in 8-chloro-4-methyl derivative **40** (IC_{50} 7.6 nM) and 4,8-dimethyl derivative **39** (IC_{50} 20 nM) with respect to the 4-methyl-unsubstituted compounds **37** (IC_{50} 49 nM) and **36** (IC_{50} 376 nM), respectively.

The substitution with a methyl group at position 6 positively affected the potency of the inhibitors, although more markedly in the 1*H*-series than in the 4a*H*-series. So compounds **42** (IC_{50} 14.3 nM) and **43** (IC_{50} 14.4 nM) were significantly more active than the corresponding compounds **36** and **37**, not substituted at position 6 (compare also compounds **29** and **21** of the 4a*H*-series). The further substitution with a methyl group at position 4 in trisubstituted compounds **46** (IC_{50} 15.8 nM) and **47** (IC_{50} 8.5 nM) maintained the inhibitory activity compared to 6,8-disubstituted compounds **42** and **43**.

The results discussed so far are consistent with those reported for structurally related compounds,[17] whose potency toward 5αR-1 increased noticeably by introducing a methyl group at position 4 of the skeleton and a chlorine at position 8. Also, the beneficial effect of the methyl at position 6 seems consistent with the observation that the introduction of the same group on the corresponding position 7 in 4-azasteroids increased their 5αR-1 selectivity.[34] By contrast, the presence of a methyl group at position 5 in general reduced the potency

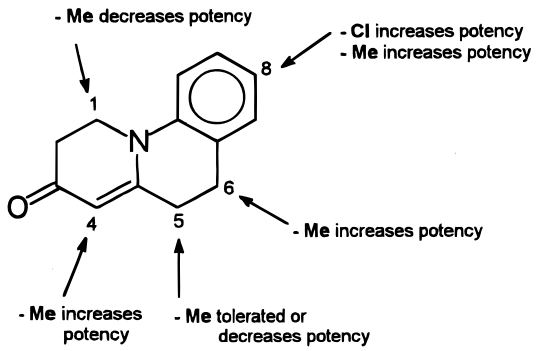


Figure 4. Qualitative structure−activity relationship for Δ^4-benzo[*c*]quinolizin-3-ones as 5αR-1 inhibitors.

unless it was associated to another methyl group at the position 4. Finally, the introduction of a methyl at the position 1 in 8-Cl-substituted compound **44** (IC_{50} 204 nM) slightly decreased the activity toward 5αR-1 in comparison with the homologous derivative **37** (IC_{50} 49 nM).

In conclusion, the contemporaneous presence of the methyl or chlorine substituent at position 8 and a methyl group at the 4 or 6 position appears to be the best combination for fitting the active site of the enzyme. This is in accordance with the inhibition values obtained in the Eli Lilly series in which the most potent inhibitor bears a methyl on position 4 and a chlorine on position 8 (LY191704, Figure 1) and with the observation that in the 4-azasteroid class the introduction of a methyl at position 4 or 7 (equivalent to the 6 position of benzoquinolizines) always increases the inhibition against 5αR-1. In Figure 4 a qualitative SAR analysis for the inhibitors of the 1*H*-series is reported.

Among the saturated compounds which were intermediates in the synthesis of the 1*H*- and 4a*H*-inhibitors, we decided to evaluate the inhibitory potency of compound **8** bearing a methyl on position 4 and a chlorine on position 8 since these are the substituents leading to the best inhibitors in both 1*H*- and 4a*H*-series. As we have already observed for the 10-azasteroids, the presence of a double bond allowing the conjugation between the carbonyl group and the nitrogen atom is an essential feature for having good inhibition values. In fact, compound **8** had an IC_{50} of 478 ± 95 nM toward 5αR-1, with potency significantly lower than those obtained for compounds **40** and **26**.

In some of the oxidation reactions leading to the 1*H*-series we have isolated also the 1,2- and 4,4a-unsaturated compounds, in particular 4-Me-substituted compound **80** and 8-Cl,5-Me-substituted compound **79**. These were tested toward both isozymes resulting as selective, although very weak, inhibitors of 5αR-1 (**80**, IC_{50} 1.4 ± 0.2 μM; **79**, 1.88 ± 0.3 μM). Also the HCl salts of **38** and **40** were tested toward 5αR-1, but they did not show any significant difference in the inhibitory activity compared to their parent compounds.

To determine a possible model accounting for the observed potency of benzo[*c*]quinolizin-3-ones, we performed a complete conformational analysis on compounds LY191704, **40,** and **24** as already reported for 19-nor-10-azasteroids,[19] i.e., a first exhaustive Monte Carlo conformational search (MM2* force field, MacroModel software)[35] followed by AM1 geometry optimiza-

Table 3. Predicted Conformers for LY191704, **40**, and **24** after Monte Carlo Conformational Search (MM2* force field) and AM1 Geometry Optimization

conformer	heat of formation (kcal/mol)	A ring	B ring	C3−C2−C1−N(C10a) dihedral angle (deg)	C4a−C5−C6−C6a dihedral angle (deg)	index of planarity (Å)[a]
LY191704_I	−30.70			−46.3	+45.9	0.178
LY191704_II	−30.04			+27.2	+50.2	0.253
40_I	−8.07	1β,2α	6α-sofa	+45.8	+45.6	0.204
40_II	−6.86	1α,2β	6α-sofa	−48.3	+48.1	0.182
24_I	−3.12				+55.1	0.297
24_II	−3.07				+38.6	0.415

[a] Averaged distance of all skeleton atoms from the mean plane of the molecule.

tion (Spartan software).[36] The AM1 calculation produced two thermally accessible conformers for each compound, having relative energies comprised in a range of only 1.2 kcal/mol with respect to the global minimum (Table 3). A third conformer of **24** was discarded because of its higher energy (>3 kcal/mol with respect to the global minimum). The conformers of each compound of the 1*H*-series differed by the conformation of the A ring, which was 1β,2α or 1α,2β, while the B ring had a fixed 6α-sofa conformation (referring to the C3−C4−C4a−N11 mean plane).[19] The interconversion barriers among the conformers were also calculated as already described.[19] The low values found (<3 kcal/mol) suggest that, similarly to the 19-nor-10-azasteroids, **40** and **24** are very flexible molecules in the A ring portion. The same low conformational barrier was found for the Eli Lilly compound. Eli Lilly's researchers suggested that the high inhibitory activity toward 5αR-1 isoenzyme of their benzoquinoline compounds, such as LY191704, could be associated to their extended planarity.[37] We had also already observed that among the 19-nor-10-azasteroid inhibitors, those having a double bond at position 8(9), i.e., between rings A and B, were more potent than the corresponding compounds with the double bond at position 9(11).[18] This result appears to be in accordance with the Eli Lilly's researcher assumption, since the 8(9) double bond forces the molecule to assume a more planar structure.

An index of the planarity of the above inhibitors could be obtained by calculating the average distance of all the skeleton atoms from the mean plane of the molecule. The global minimun conformer LY191704_I resulted as the most planar, with an average distance of 0.178 Å. Therefore, because of the highest potency and selectivity of LY191704 toward 5αR-1 among the Eli Lilly inhibitors, we may take conformer LY191704_I as a reference structure and compare our benzo[*c*]quinolizin-3-ones with it.

By the superposition of all the skeleton atoms of LY191704_I and the predicted conformers of our benzo-[*c*]quinolizin-3-one inhibitors, we found that **40**_II was the closest (rms = 0.223) to LY191704_I (Figure 5). In this case it was possible to observe a fair spatial correspondence of skeleton atoms C2, C3, and C4 and, as a consequence, of the carbonyl and methyl groups of the two molecules, of atoms C5 and C6 of the B ring, and of all atoms (included the chlorine one) of the benzene ring. Incidentally, the two conformers of the Δ^4-compound **40** were also the most planar among the benzo[*c*]quinolizin-3-ones compounds (see Table 3). Greater deviations were instead observed with the compound of the 4a*H*-series when superimposing both the conformers of **24** with LY191704_I (rms = 0.270 and 0.300) (Figure 5). Therefore, the low potency of the 4a*H*-inhibitors, if compared to the corresponding 1*H*-compounds, could be explained on the basis of their less extended planarity.[38]

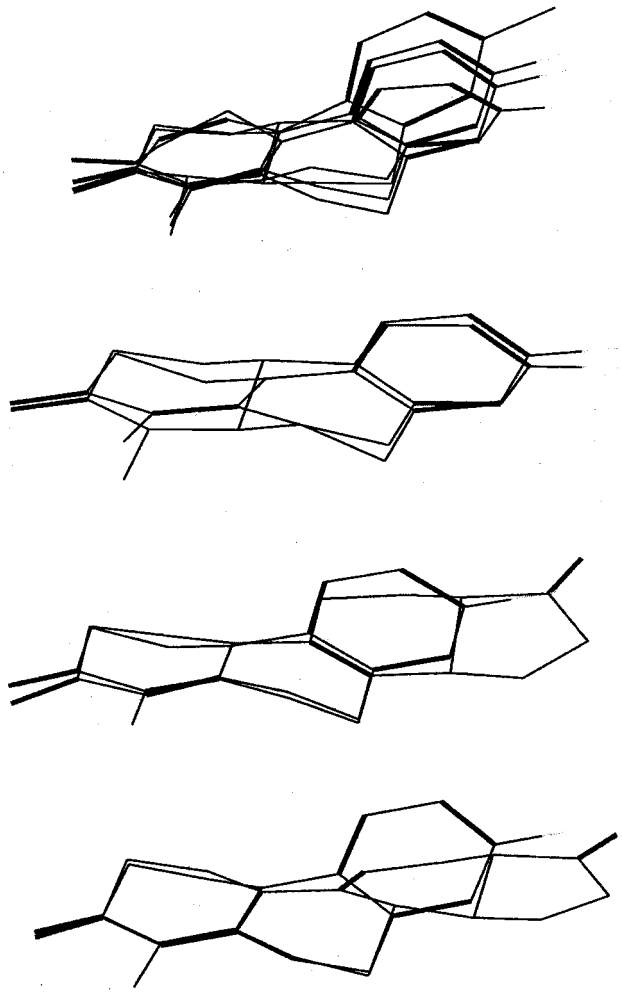


Figure 5. From the top: (a) superposition of the A rings of conformers LY191704_I, **40**_I, **40**_II, and **24**_I, showing a remarkable deviation of **24**_I from the reference conformer LY191704_I; (b) superposition of all skeleton atoms of conformers LY191704_I and **40**_II; (c) superposition of the A and B rings of **40**_II and the corresponding conformer of 10-azaestra-4,8(9)-diene-3,17-dione; (d) superposition of the A and B rings of **40**_II and the corresponding conformer of 10-azaestra-4,9(11)-diene-3,17-dione.

If we now take **40**_II as a reference conformer for the inhibitors of 5αR-1, we can compare $\Delta^{8(9)}$- and $\Delta^{9(11)}$-19-nor-10-azasteroids with that in order to find the closest one in terms of extended planarity. The superposition of the A and B rings of $\Delta^{8(9)}$- and $\Delta^{9(11)}$-10-aza-3,17-androstenedione[39] with the corresponding rings of **40**_II (Figure 3) clearly resulted in a greater deviation of the C ring of the $\Delta^{9(11)}$-azasteroids from the aromatic

Table 4. HF-STO 3-21G(*) Partial Charges Calculated for Benzo[*c*]quinolizin-3-ones

	charge				
compd	N	C4a	C4	C3	O3
35	−0.26	0.41	−0.90	0.98	−0.62
37	−0.35	0.40	−0.87	0.92	−0.62
38	−0.30	0.17	−0.41	0.80	−0.59
40	−0.37	0.20	−0.35	0.73	−0.58

ring of the benzo[*c*]quinolizinone conformer. This can be quantified by calculating the average distances between the corresponding pair of atoms C7/C14, C8/C13, C9/C12, and C10/C11, which resulted in 0.856 and 0.404 Å for the $\Delta^{9(11)}$- and $\Delta^{8(9)}$-isomers, respectively.

The introduction of the 4-methyl group increases dramatically the potency of both LY191704 and **40** with respect to the 4-unsubstituted compound. It is possible that a small hydrophobic pocket in the enzyme active site accommodates this substituent. However, also electronic effects could be taken into account to explain the increase of potency. We have shown in a previous work on 10-azasteroids[18] that a growth of potency toward both isoenzymes could be associated with the increase of the electrostatic charge on the oxygen atom at C3, which would favor the initial interaction with an H-bond donor residue of the active site. The partial charges of benzo[*c*]quinolizin-3-ones were determined by ab initio HF-STO 3-21G(*) calculations. The substitution of the C ring of the azasteroids with an aromatic ring did not affect the negative partial charges on the oxygen which was about −0.62 for compounds **35** and **37** unsubstituted at position 4 (the charge was −0.63 in $\Delta^{4(5)}$-10-azasteroids). When a methyl group was introduced at position 4 the negative charge decreased to −0.58 and −0.59 (Table 4), very close to the charge of the oxygen atom in saturated 10-azasteroids. These last were poor inhibitors of 5αR, and we justified this observation with the decrease of negative charge on the oxygen. On the basis of the current results, however, it seems that the steric effects of the 4-methyl group in the benzo[*c*]quinolizinones series are by far more important than the electronic ones, and the presence of a hydrophobic pocket in the active site of the enzyme which accommodates small alkyl groups appears consequently possible.

Conclusion

In this paper we have reported on the synthesis and biological evaluation of a series of potent and selective inhibitors of 5αR-1, the isozyme involved in the transformation of testosterone to dihydrotestosterone mainly in human skin and scalp tissues and thus possibly causing the development and maintenance of disorders such as acne and androgenic alopecia in men and hirsutism in women. The synthesized inhibitors are benzo[*c*]quinolizin-3-ones derivatives bearing at position 1, 4, 5, and/or 6 a methyl group and at position 8, i.e., on the aromatic ring, a hydrogen, methyl group, or chlorine atom. Depending on the position of the double bond on the A ring, two classes of inhibitors can be identified: i.e., those having the double bond at position 1,2 (4a*H*-series) and those having the double bond at position 4,4a (1*H*-series). All these compounds were tested toward 5αR-1 and 5αR-2 expressed in CHO cells (CHO 1827 and CHO 1829, respectively) resulting in selective inhibitors of the type 1 isoenzyme, with inhibitory potencies (IC_{50}) ranging from 7.6 to 9100 nM. The inhibitors of the 4a*H*-series were generally less active than the corresponding inhibitors of the 1*H*-series, which have the double bond at position 4,4a on the A ring, and this could be associated with the higher extended planarity of the compounds belonging to the latter series, as evidenced by a molecular modeling study carried out in comparison with the known 5αR-1-selective inhibitors LY191704. The most potent inhibitors had a chlorine atom (or a methyl group) at position 8, although it was the presence of a methyl group at position 4 (as in compounds **39**−**40** and **45**−**47**), associated with the substitution at position 8, that determined the highest inhibition potency (IC_{50} from 7.6 to 20 nM). This suggests the presence of a small hydrophobic pocket in the enzyme active site accommodating the methyl group at position 4 (the same substitution is present in LY191704). The two not chiral 4-methyl-(1*H*)-2,3,5,6-tetrahydrobenzo[*c*]quinolizin-3-ones **39** and **40**, bearing at the 8 position a methyl group or chlorine, respectively, were selected among the most potent inhibitors as possible candidate for a lead development. They both selectively inhibited 5αR-1 expressed in CHO 1827 cells through a reversible competitive mechanism displaying K_i values of 5.8 and 2.7 nM for **39** and **40**, respectively. When they were tested toward native 5αR-1 in scalp homogenates and 5αR-2 in prostate homogenates, in comparison with finasteride and the known 5αR-1-selective inhibitor LY191704, both resulted as selective inhibitors of 5αR-1 in scalp with IC_{50} values of 41 nM, being inactive toward 5αR-2 present in human prostate tissue. Therefore, on the basis of these results, these two new potent and selective 5αR-1 inhibitors are the best candidates for the development of a drug for the treatment of acne and androgenic alopecia in men and hirsutism and polycystic ovarian syndrome in women.

Experimental Section

All the reactions were performed under nitrogen, unless otherwise stated. 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. IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer in $CDCl_3$ solution. ^{1}H NMR (200 MHz) and ^{13}C NMR (50.33 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 a 5790A-5970A Hewlett-Packard and QMD 1000 Carlo Erba instruments. Microanalyses were carried out with a Perkin-Elmer 240C elemental analyzer. 2-(Triphenylphosphanylidene)propionic acid methyl ester was prepared as reported.[27] Diene **66** was prepared as reported.[40]

6-Methyl-3,4-dihydroquinolin-2(1*H*)-one (49).[26] To a refluxing solution of *p*-toluidine **73** (10.7 g, 100 mmol) in acetone (20 mL) was slowly added a solution of 3-chloropropanoyl chloride (5 mL, 50 mmol) in acetone (10 mL). Then the solution was refluxed 1 h and finally cooled to room temperature. The resulting suspension was transferred into a flask containing a solution of 6 N HCl in water (40 mL) achieving the complete precipitation of pure amide **74** (9.87 g, 100%) as a white solid: mp 115 °C (lit. mp 121 °C).[26] A flask equipped with a mechanical stirrer and containing compound **74** (7.5 g, 38 mmol) was then put in an oil bath heated at 120−130 °C and, after complete melting of **74**, $AlCl_3$ (5.56 g, 41.7 mmol)

was added in small portions and the mixure left under stirring at 120 °C for 24 h. After cooling to room temperature, 10% HCl (100 mL) was added dropwise and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, water, and dried over Na_2SO_4. After concentration, lactam **49** (5.63 g, 92%) was obtained as a brown solid sufficiently pure for the next step: ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1 H), 7.05−6.98 (m, 2 H), 6.70−6.66 (m, 1 H), 2.98−2.91 (m, 2 H), 2.67−2.59 (m, 2 H), 2.31 (s, 3 H).

6-Chloro-3,4-dihydroquinolin-2(1*H*)-one (50).[26] It was prepared as reported for **49**. Starting from *p*-chloroaniline **72** (12.76 g, 100 mmol), amide **75**[26] (10.9 g) was obtained in 100% yield after recrystallization from MeOH−H_2O, 1:1 (mp 120−122 °C). The cyclization of **75** (7.3 g, 33.5 mmol) to the corresponding lactam was carried out by heating at 140 °C and using 2 equiv of $AlCl_3$ (8.9 g, 66.9 mmol), obtaining **50** (5.70 g, 94%) as a pale yellow solid sufficiently pure for the next step: mp 106 °C; ^{1}H NMR ($CDCl_3$) δ 8.52 (s, 1 H), 7.17−7.10 (m, 2 H), 6.76−6.71 (m, 1 H), 3.00−2.92 (m, 2 H), 2.67−2.60 (m, 2 H).

6-Chloro-3-methyl-3,4-dihydroquinolin-2(1*H*)-one (51). A solution of aldehyde **70** (3.104 g, 16.7 mmol) and 2-(triphenylphosphanylidene)propionic acid methyl ester[27] (8.28 g, 23.77 mmol) in toluene (170 mL), heated at 80 °C, was stirred for 3 h. The reaction mixture was then concentrated, the crude oil redissolved in Et_2O (250 mL) and kept under vigorous stirring for 1 h. The resulting suspension was filtered, concentrated and the residual oil chromatographed (EtOAc−petroleum ether, 1:4, R_f 0.52), affording cynnamate **71** (4.20 g, 90%) as an oil: ^{1}H NMR ($CDCl_3$) (major diastereoisomer) δ 8.12 (d, J = 8.7 Hz, 1 H), 7.85 (s, 1 H), 7.49 (dd, J = 8.7, 2.5 Hz, 1 H), 7.34 (d, J = 2.5 Hz, 1 H), 3.85 (s, 3 H), 1.92 (s, 3 H).

The hydrogenation of **71** was then carried out in a magnetically stirred 150 mL stainless steel autoclave. PtO_2 (160 mg) was added to a solution of substrate **71** (4 g, 14.4 mmol) in AcOH (60 mL), then the reactor was purged with nitrogen, pressurized with H_2 (10 atm) and finally heated in a thermostated bath at 60 °C for 14 h. The catalyst was then filtered, the solution concentrated under vacuum and adjusted to pH 8 by addition of 0.5 N NaOH. The mixture was extracted with $CHCl_3$, dried over Na_2SO_4 and concentrated to give crude **51**. Pure **51** (2.190 g, 78%) was obtained by recrystallization from *i*-Pr_2O: ^{1}H NMR ($CDCl_3$) δ 9.82 (br s, 1 H), 7.14−7.11 (m, 2 H), 6.82 (d, J = 9.1 Hz, 1 H), 3.00−2.83 (m, 1 H), 2.79−2.64 (m, 2 H), 1.28 (d, J = 6.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 175.0 (s), 135.9 (s), 127.9 (d), 127.7 (s), 127.3 (d), 125.0 (s), 116.5 (d), 34.5 (d), 33.0 (t), 15.2 (q); MS *m*/*z* (%) 195 (M^+, 90), 166 (100).

4,6-Dimethyl-3,4-dihydroquinolin-2(1*H*)-one (52).[26] It was prepared as reported for **49**. Starting from *p*-toluidine **73** (7.08 g, 66.0 mmol) and 3-chlorobutanoyl cholride (3.8 mL, 33 mmol), amide **76** (6.0 g) was obtained in 86% yield: ^{1}H NMR ($CDCl_3$) δ 7.40 (d, J = 8.2 Hz, 2 H), 7.21 (d, J = 8.2 Hz, 2 H), 4.56 (m, 1 H), 2.75 (d, J = 6.6 Hz, 2 H), 2.32 (s, 3 H), 1.62 (d, J = 6.6 Hz, 3 H). The cyclization of **76** (5.43 g, 25.6 mmol) to the corresponding lactam was carried out by heating at 130 °C for 5 h and using 1 equiv of $AlCl_3$ (3.50 g, 25.6 mmol), obtaining **52** (4.47 g, 100%) as a pale brown solid sufficiently pure for the next step: ^{1}H NMR ($CDCl_3$) δ 10.11 (br s, 1 H), 6.99−6.81 (m, 3 H), 3.07 (m, 1 H), 2.72 (dd, J = 16.1, 5.8 Hz, 1 H), 2.41 (dd, J = 16.1, 7.0 Hz, 1 H), 2.30 (s, 3 H), 1.29 (d, J = 6.9 Hz, 3 H).

6-Chloro-4-methyl-3,4-dihydroquinolin-2(1*H*)-one (53).[26] It was prepared as reported for **49**. Starting from *p*-chloroaniline **72** (8.42 g, 66.0 mmol) and 3-chlorobutanoyl chloride (3.8 mL, 33 mmol), amide **77** (7.66 g) was obtained in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 7.56 (br s, 1 H), 7.47 (d, J = 8.7 Hz, 2 H), 7.28 (d, J = 8.7 Hz, 2 H), 4.55 (m, 1 H), 2.75 (m, 2 H), 1.61 (d, J = 6.6 Hz, 3 H). The cyclization of **77** (4.10 g, 17.7 mmol) to the corresponding lactam was carried out by heating at 160 °C for 3 h and using 2 equiv of $AlCl_3$ (4.80 g, 35.3 mmol), obtaining **53** (3.46 g, 100%) as a pale yellow solid sufficiently pure for the next step: ^{1}H NMR ($CDCl_3$) δ 9.51 (br s, 1 H), 7.26−7.17 (m, 2 H), 6.81 (d, J = 8.0 Hz, 1 H), 3.11 (m, 1 H), 2.72 (dd, J = 16.4, 5.8 Hz, 1 H), 2.49−2.35 (m, 1 H), 1.31 (d, J = 7.0 Hz, 3 H).

***N*-(*tert*-Butoxycarbonyl)-3,4-dihydroquinolin-2(1*H*)-one (54).** To a solution of **48** (5 g, 34 mmol) in anhydrous CH_2Cl_2 (110 mL) were added Et_3N (4.7 mL, 34 mmol), $(BOC)_2O$ (8.9 g, 40.8 mmol), and DMAP (410 mg, 3.4 mmol). After stirring for 18 h at room temperature, the solvent was evaporated and water (200 mL) was added. The resulting mixture was extracted with Et_2O (3 × 150 mL), the combined organic layers were washed with 1 M $KHSO_4$, $NaHCO_3$(satd), brine, and dried over Na_2SO_4. After filtration and evaporation of the solvent *N*-Boc derivative **54** (8.23 g, 98%) was obtained as a white solid: mp 68−69 °C; ^{1}H NMR ($CDCl_3$) δ 7.22−6.93 (m, 4 H), 2.97 (t, J = 7.0 Hz, 2 H), 2.67 (t, J = 7.0 Hz, 2 H), 1.62 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 169.3 (s), 151.7 (s), 137.5 (s), 128.0 (d), 127.3 (d), 125.9 (s), 124.1 (d), 116.9 (d), 85.0 (s), 32.3 (t), 27.7 (q, 3 C), 25.5 (t); MS *m*/*z* (%) 247 (M^+, 4), 147 (100), 57 (100); IR ($CDCl_3$) 1767, 1691 cm^{-1}.

***N*-(*tert*-Butoxycarbonyl)-6-methyl-3,4-dihydroquinolin-2(1*H*)-one (55).** It was prepared as reported for **54**. Starting from **49** (5.5 g, 34 mmol), pure **55** (8.3 g, 94%) was obtained after chromatography ($CHCl_3$, R_f 0.50) as a gummy solid: ^{1}H NMR ($CDCl_3$) δ 7.20 (m, 2 H), 6.84 (d, J = 8.0 Hz, 1 H), 2.89 (m, 2 H), 2.64 (m, 2 H), 2.29 (s, 3 H), 1.59 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 168.7 (s), 151.2 (s), 134.1 (s), 133.1 (s), 128.0 (d), 127.2 (s), 125.3 (d), 116.4 (d), 84.1 (s), 31.9 (t), 27.1 (q, 3 C), 24.8 (t), 20.1 (q); MS *m*/*z* (%) 261 (M^+, 2), 161 (100); IR ($CDCl_3$) 1760, 1680 cm^{-1}.

***N*-(*tert*-Butoxycarbonyl)-6-chloro-3,4-dihydroquinolin-2(1*H*)-one (56).** It was prepared as reported for **54**. Starting from **50** (6.59 g, 36.3 mmol), pure **56** (9.45 g, 92%) was obtained after chromatography (CH_2Cl_2−MeOH, 30:1, R_f 0.63) as a pale yellow solid: mp 95 °C; ^{1}H NMR ($CDCl_3$) δ 7.21 (m, 2 H), 6.91 (d, J = 9.2 Hz, 1 H), 2.93 (m, 2 H), 2.66 (m, 2 H), 1.61 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 168.8 (s), 151.3 (s), 135.6 (s), 133.1 (s), 129.2 (d), 127.8 (s), 127.2 (d), 118.4 (s), 112.2 (d), 85.2 (s), 32.0 (t), 27.6 (q, 3 C), 25.2 (t); MS *m*/*z* (%) 181 (M^+ − 100, 100); IR ($CDCl_3$) 1762, 1692 cm^{-1}.

***N*-(*tert*-Butoxycarbonyl)-6-chloro-3-methyl-3,4-dihydroquinolin-2(1*H*)-one (57).** It was prepared as reported for **54**. Starting from **51** (1.0 g, 6.22 mmol), crude **57** (1.62 g, 88%) was obtained sufficiently pure for the next step as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.20−7.15 (m, 2 H), 6.85 (d, J = 9.1 Hz, 1 H), 2.85−2.62 (m, 3 H), 1.59 (s, 9 H), 1.26 (d, J = 6.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 171.5 (s), 151.7 (s), 135.5 (s), 129.1 (s), 127.9 (d), 127.3 (s), 127.2 (d), 118.1 (d), 85.2 (s), 35.7 (d), 33.1 (t), 27.5 (q, 3 C), 14.7 (q).

***N*-(*tert*-Butoxycarbonyl)-4,6-dimethyl-3,4-dihydroquinolin-2(1*H*)-one (58).** It was prepared as reported for **54**. Starting from **52** (1.0 g, 5.23 mmol), crude **58** (1.44 g, 100%) was obtained sufficiently pure for the next step as an oil: ^{1}H NMR ($CDCl_3$) δ 7.04−6.87 (m, 2 H), 6.84−6.82 (m, 1 H), 3.07 (m, 1 H), 2.72 (dd, J = 15.3, 5.1 Hz, 1 H), 2.44 (dd, J = 15.3, 6.9 Hz, 1 H), 2.33 (s, 3 H), 1.60 (s, 9 H), 1.30 (d, J = 6.9 Hz, 3 H).

***N*-(*tert*-Butoxycarbonyl)-6-chloro-4-methyl-3,4-dihydroquinolin-2(1*H*)-one (59).** It was prepared as reported for **54**. Starting from **53** (4.0 g, 20.4 mmol), crude **59** (6.01 g, 100%) was obtained sufficiently pure for the next step as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.22−7.16 (m, 2 H), 6.92−6.88 (m, 1 H), 3.09 (m, 1 H), 2.72 (dd, J = 15.4, 5.1 Hz, 1 H), 2.44 (dd, J = 15.4, 7.7 Hz, 1 H), 1.60 (s, 9 H), 1.31 (d, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 167.9 (s), 151.2 (s), 134.7 (s), 132.5 (s), 129.3 (s), 127.0 (d), 126.3 (d), 118.4 (d), 85.0 (s), 39.2 (d), 30.5 (t), 27.5 (q, 3 C), 18.9 (q); MS *m*/*z* (%) 195 (M^+ − 100, 89), 180 (100), 152 (73), 117 (81), 89 (46).

***N*-(*tert*-Butoxycarbonyl)-2-ethoxy-1,2,3,4-tetrahydroquinoline (60).** To a solution of *N*-Boc lactam **54** (4.35 g, 17.6 mmol) in absolute ethanol (140 mL) cooled at −25 °C and under stirring, was added $NaBH_4$ (2.66 g, 70.4 mmol) in 6 portions during 1 h. After 4 h, the solution was adjusted to pH 3−4 by adding slowly a 2 N HCl solution in absolute EtOH and the resulting suspension was left under stirring at 0 °C for 1.5 h. Then water (100 mL) was added, the mixture was

extracted with CH_2Cl_2 and the organic layer washed with $NaHCO_3$(satd), brine, and dried over Na_2SO_4. After evaporation of the solvent pure compound **60** (4.74 g, 96%) was obtained as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.55 (d, J = 8 Hz, 1 H), 7.20−6.99 (m, 3 H), 5.84 (t, J = 4.0 Hz, 1 H), 3.59 (qd, J = 7.0, 3.3 Hz, 2 H), 2.96−2.80 (m, 1 H), 2.74−2.60 (m, 1 H), 2.25−2.11 (m, 1 H), 2.07−1.99 (m, 1 H), 1.53 (s, 9 H); 1.13 (t, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 154.3 (s), 136.6 (s), 130.8 (s), 128.8 (d), 126.3 (d), 125.6 (d), 124.4 (d), 81.9 (d), 81.7 (s), 63.3 (t), 29.8 (t), 29.0 (q, 3 C), 23.9 (t), 15.7 (q); MS *m*/*z* (%) 277 (M^+, 8), 131 (100); IR ($CDCl_3$) 1685 cm^{-1}.

***N*-(*tert*-Butoxycarbonyl)-2-ethoxy-6-methyl-1,2,3,4-tetrahydroquinoline (61).** It was prepared as reported for **60**, starting from **55** (2 g, 7.65 mmol) and $NaBH_4$ (1.74 g, 45.9 mmol), but the reaction was quenched with 2 N HCl in ethanol after 2 h and left under stirring at 0 °C for 2.5 h before the addition of water and extraction. Pure **61** (1.37 g, 62%) was obtained by chromatography (EtOAc−petroleum ether, 1:10, R_f 0.45) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.43 (d, J = 8.4 Hz, 1 H), 6.95 (m, 2 H), 5.83 (t, J = 4.0 Hz, 1 H), 3.58 (m, 2 H), 2.84 (m, 1 H), 2.67 (m, 1 H), 2.29 (s, 3 H), 2.05 (m, 2 H), 1.53 (s, 9 H), 1.13 (t, J = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 153.5 (s), 133.2 (s), 132.7 (s), 129.7 (s), 128.5 (d), 126.2 (d), 124.6 (d), 81.1 (d), 80.7 (s), 62.4 (t), 29.0 (t), 28.1 (q, 3 C), 23.0 (t), 20.6 (q), 14.8 (q); MS *m*/*z* (%) 291 (M^+, 7), 145 (100); IR ($CDCl_3$) 1709 cm^{-1}.

***N*-(*tert*-Butoxycarbonyl)-6-chloro-2-ethoxy-1,2,3,4-tetrahydroquinoline (62).** It was prepared as reported for **60**, starting from **56** (2.9 g, 10.65 mmol) and $NaBH_4$ (2.42 g, 63.9 mmol), but the reaction was quenched with 2 N HCl in ethanol after 2.5 h and left under stirring at 0 °C for 2.5 h before the addition of water and extraction. Pure **62** (2.44 g, 68%) was obtained by chromatography (EtOAc−petroleum ether, 1:10, R_f 0.47) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.52 (d, J = 8.1 Hz, 1 H), 7.10 (m, 2 H), 5.81 (t, J = 2.5 Hz, 1 H), 3.55 (m, 2 H), 2.84 (m, 1 H), 2.66 (m, 1 H), 2.11−2.01 (m, 2 H), 1.53 (s, 9 H), 1.12 (t, J = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 153.3 (s), 134.5 (s), 131.6 (s), 128.9 (s), 128.0 (d), 126.0 (d), 125.7 (d), 81.4 (d), 81.0 (s), 62.7 (t), 28.5 (t), 28.3 (q, 3 C), 22.9 (t), 14.9 (q); MS *m*/*z* (%) 311 (M^+, 6), 185 (100); IR ($CDCl_3$) 1685 cm^{-1}.

***N*-(*tert*-Butoxycarbonyl)-6-chloro-2-ethoxy-3-methyl-1,2,3,4-tetrahydroquinoline (63).** It was prepared as reported for **60**, starting from **57** (239 mg, 0.81 mmol) and $NaBH_4$ (200 mg, 5.3 mmol), but the reaction was quenched with 2 N HCl in ethanol after 3 h and left under stirring at 0 °C for 7 h before the addition of water and extraction. Compound **63** (240 mg, 91%) was obtained as a colorless oil sufficiently pure for the next step as a 1:1 mixture of diastereoisomers: ^{1}H NMR ($CDCl_3$) δ 7.56 (d, J = 8.8 Hz, 1 H), 7.49 (d, J = 8.8 Hz, 1 H), 7.13−7.01 (m, 2 H + 2 H), 5.51 (d, J = 2.5 Hz, 1 H), 5.47 (d, J = 3.3 Hz, 1 H), 3.65−3.42 (m, 2 H + 2 H), 2.95 (dd, J = 16.5, 6.6 Hz, 1 H), 2.69−2.61 (m, 2 H + 2 H), 2.40−2.33 (m, 1 H), 2.18−1.94 (m, 1 H), 1.57 (s, 9 H), 1.56 (s, 9 H), 1.09 (t, J = 7.0 Hz, 3 H), 1.07 (d, J = 5.5 Hz, 3 H), 1.06 (t, J = 7.2 Hz, 3 H), 0.97 (d, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 153.9 (s), 153.1 (s), 134.0 (s), 133.8 (s), 130.4 (s), 130.3 (s), 129.4 (s), 128.7 (s), 128.4 (d), 128.2 (d), 125.9 (d), 125.7 (d), 125.5 (d, 2 C), 86.3 (d), 84.4 (d), 81.5 (s), 81.3 (s), 62.9 (t), 62.8 (t), 32.8 (d), 32.0 (d), 30.9 (t), 30.6 (t), 28.3 (q, 3 C), 28.2 (q, 3 C), 17.5 (q), 17.2 (q), 14.9 (q), 14.7 (q).

***N*-(*tert*-Butoxycarbonyl)-4,6-dimethyl-2-ethoxy-1,2,3,4-tetrahydroquinoline (64).** It was prepared as reported for **60**, starting from **58** (1.44 g, 5.22 mmol) and $NaBH_4$ (1.50 g, 39.5 mmol), but the reaction was quenched with 2 N HCl in ethanol after 5 h and left under stirring at 0 °C for 3.5 h before the addition of water and extraction. Compound **64** (1.49 g, 94%) was obtained as a colorless oil sufficiently pure for the next step as a 1:1 mixture of diastereisomers: ^{1}H NMR ($CDCl_3$) δ 7.42 (d, J = 8.1 Hz, 1 H), 7.25−7.20 (m, 1 H), 7.03−6.94 (m, 2 H + 2 H), 5.85 (dd, J = 7.7, 5.5 Hz, 1 H), 5.78 (t, J = 4.1 Hz, 1 H), 3.78−3.47 (m, 2 H + 2 H), 3.11−2.97 (m, 1 H), 2.62−2.43 (m, 1 H + 1 H), 2.34 (s, 3 H), 2.30 (s, 3 H), 2.13 (ddd, J = 10.3, 6.6, 3.3 Hz, 1 H), 1.81 (ddd, J = 13.9, 9.9, 4.0 Hz, 1 H), 1.52 (s, 9 H), 1.60−1.50 (m, 1 H), 1.50 (s, 9 H), 1.31 (d, J = 6.6 Hz, 3 H), 1.29 (d, J = 7.3 Hz, 3 H), 1.14 (t, J = 7.0 Hz, 3 H), 1.11 (t, J = 6.9 Hz, 3 H).

***N*-(*tert*-Butoxycarbonyl)-6-chloro-2-ethoxy-4-methyl-1,2,3,4-tetrahydroquinoline (65).** It was prepared as reported for **60**, starting from **59** (2.00 g, 6.77 mmol) and $NaBH_4$ (1.07 g, 28 mmol), but the reaction was quenched with 2 N HCl in ethanol after 5 h and left under stirring at 0 °C for 3 h before the addition of water and extraction. Compound **65** (1.99 g, 91%) was obtained as a colorless oil sufficiently pure for the next step as a 1:1 mixture of diastereisomers: ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 8.0 Hz, 1 H), 7.31−7.26 (m, 1 H), 7.20−7.07 (m, 2 H + 2 H), 5.81 (dd, J = 7.8, 5.3 Hz, 1 H), 5.72 (t, J = 3.9 Hz, 1 H), 3.68−3.47 (m, 2 H + 2 H), 3.14−3.03 (m, 2 H + 2 H), 2.64−2.46 (m, 2 H), 2.16 (ddd, J = 13.9, 6.9, 3.6 Hz, 1 H), 1.77 (ddd, J = 13.9, 10.3, 3.7 Hz, 1 H), 1.53 (s, 9 H), 1.50 (s, 9 H), 1.31 (d, J = 6.6 Hz, 3 H), 1.29 (d, J = 7.0 Hz, 3 H), 1.18−1.03 (m, 3 H + 3 H).

General Procedure for the $TiCl_4$-Promoted Cyclizations. Synthesis of 1,2,3,4,5,6-Hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (1). A 1 M solution of $TiCl_4$ in CH_2Cl_2 (28.84 mL, 28.84 mmol) was added dropwise in 10 min to a solution of **60** (4 g, 14.42 mmol) in CH_2Cl_2 (72 mL) cooled at −30 °C and under argon atmosphere, leaving under stirring for 10 min. To the resulting dark solution were then added diene **66** (3.15 mL, 17.29 mmol) and, after 30 min at room temperature, a saturated solution of $NaHCO_3$ (70 mL, very carefully at the beginning). The mixture was vigorously stirred for 40 min, then it was filtered through a Celite layer, washing with CH_2Cl_2, the two layers separated and the aqueous one extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and concentrated, obtaining a dark crude oil. Pure **1** (895 mg, 31%) was obtained after chromatography (EtOAc−petroleum ether, 1:4, R_f 0.32) as a pale yellow solid: mp 53−54 °C; ^{1}H NMR ($CDCl_3$) δ 7.19−7.02 (m, 2 H), 6.87 (d, J = 8.1 Hz, 1 H), 6.74 (td, J = 7.3, 1.1 Hz, 1 H), 4.22 (ddd, J = 13.6, 6.2, 3.3 Hz, 1 H), 3.48 (m, 1 H), 3.17 (ddd, J = 13.6, 11.4, 3.7 Hz, 1 H), 2.84−2.36 (m, 6 H), 2.21−2.06 (m, 1 H), 1.91−1.75 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 208.5 (s), 144.0 (s), 129.3 (d), 127.4 (d), 124.4 (s), 118.3 (d), 112.9 (d), 55.8 (d), 47.4 (t), 47.0 (t), 40.5 (t), 28.6 (t), 25.4 (t); MS *m*/*z* (%) 201 (M^+, 100); IR ($CDCl_3$) 1710 cm^{-1}.

General Procedure for the TMSOTf-Promoted Cyclizations. Synthesis of 8-Methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (2). To a solution of methyl vinyl ketone (730 mL, 9.05 mmol) in CH_2Cl_2 (25 mL), cooled at 0 °C and under argon atmosphere, were added Et_3N (1.79 mL, 12.82 mmol) and, slowly, TMSOTf (2.2 mL, 11.3 mmol). After stirring 30 min, compound **61** (1.1 g, 3.77 mmol), dissolved in CH_2Cl_2 (25 mL), was added followed by further TMSOTf (0.73 mL, 3.77 mmol). The solution was allowed to warm to room temperature and left under stirring 45 min. Then $NaHCO_3$(satd) (50 mL) was added and the mixture stirred 36 h. Then water (50 mL) and CH_2Cl_2 (50 mL) were added, the layers separated and the aqueous one extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and concentrated, obtaining a dark crude oil. Pure **2** (353 mg, 44%) was obtained after chromatography (CH_2Cl_2, 0.5% Et_3N, R_f 0.12) as a pale yellow solid: mp 85−86 °C; ^{1}H NMR ($CDCl_3$) δ 7.00−6.75 (m, 3 H), 4.20 (ddd, J = 13.2, 6.8, 2.7 Hz, 1 H), 3.41 (m, 1 H), 3.10 (ddd, J = 15.5, 11.8, 3.6 Hz, 1 H), 2.85−2.00 (m, 7 H), 2.25 (s, 3 H), 1.82 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 208.4 (s), 141.8 (s), 129.9 (d), 127.7 (d), 127.5 (s), 124.2 (s), 113.1 (d), 56.1 (d), 47.3 (t), 47.1 (t), 40.4 (t), 29.6 (t), 25.3 (t), 20.1 (q); MS *m*/*z* (%) 215 (100); IR ($CDCl_3$) 1710 cm^{-1}.

8-Chloro-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3-(4a*H*)-one (3). It was prepared as reported for **1**. Starting from **62** (500 mg, 1.6 mmol), pure **3** (128 mg, 34%) was obtained after chromatography (CH_2Cl_2, 0.5% Et_3N, R_f 0.28) as a pale yellow gummy solid: ^{1}H NMR ($CDCl_3$) δ 7.09−7.01 (m, 2 H), 6.76 (d, J = 8.8 Hz, 1 H), 4.12 (ddd, J = 13.2, 6.5, 3.6 Hz, 1 H), 3.46 (m, 1 H), 3.17 (ddd, J = 15.5, 11.0, 4.4 Hz, 1 H), 2.80−2.37 (m, 6 H), 2.10 (m, 1 H), 1.80 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 207.9 (s), 142.0 (s), 127.8 (d), 126.1 (d), 125.1 (s), 121.7 (s),

113.0 (d), 55.6 (d), 46.2 (t), 45.9 (t), 39.3 (t), 27.4 (t), 25.3 (t); MS *m*/*z* (%) 235 (100); IR ($CDCl_3$) 1709 cm^{-1}.

4α- and 4β-Methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (4 and 5). Compounds **4** and **5** were prepared as reported for **2**, starting from **60** (1.75 g, 6.31 mmol) and employing 1-penten-3-one (1.49 mL, 15.1 mmol) for the generation in situ of silyloxydiene **69**. The 3:2 mixture (the same ratio in the crude) of **4** and **5** (407 mg, 30%) was obtained after chromatography (CH_2Cl_2, 0.5% Et_3N, R_f 0.40) as an oil. Attempts at separating the two diastereoisomers by chromatography failed: ^1H NMR ($CDCl_3$) (mixture) δ 7.21−6.57 (m, 4 H + 4 H), 4.27 (ddd, *J* = 13.5, 5.8, 2.5 Hz, 1 H, **4**), 4.04 (m, 1 H, **5**), 3.61 (t, *J* = 7.0 Hz, 1 H, **5**), 3.51 (ddd, *J* = 9.1, 4.8, 3.3 Hz, 1 H, **5**), 3.33−3.15 (m, 2 H, **4**), 2.94−2.43 (m, 5 H + 5 H), 2.39−2.16 (m, 1 H), 2.06−1.81 (m, 2 H + 1 H), 1.24 (d, *J* = 7 Hz, 3 H, **5**), 1.12 (d, *J* = 7 Hz, 3 H, **4**).

4α- and 4β,8-Dimethyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (6 and 7). Compounds **6** and **7** were prepared as reported for **2**, starting from **61** (2.00 g, 7.05 mmol) and employing 1-penten-3-one (1.67 mL, 16.92 mmol) for the generation in situ of silyloxydiene **69**. The 7:2 (the same ratio in the crude) mixture of **6** and **7** (485 mg, 30%) was obtained after chromatography (CH_2Cl_2, 0.5% Et_3N, R_f 0.20) as an oil. Another chromatography of a small amount of the 7:2 mixture, with the same eluant as above, provided 4α-compound **6** in pure form as a thick oil: ^1H NMR ($CDCl_3$) δ 6.92 (m, 2 H), 6.79 (d, *J* = 8.4 Hz, 1 H), 4.29 (ddd, *J* = 13.0, 5.8, 2.5 Hz, 1 H), 3.15 (m, 2 H), 2.82−2.09 (m, 6 H), 2.25 (s, 3 H), 1.97 (m, 1 H), 1.08 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 209.8 (s), 141.4 (s), 130.0 (d), 127.7 (d), 127.7 (s), 124.0 (s), 112.6 (d), 62.2 (d), 47.9 (d), 47.6 (t), 39.9 (t), 25.4 (t), 24.3 (t), 20.2 (q), 9.5 (q); MS *m*/*z* (%) 229 (100); IR ($CDCl_3$) 1710 cm^{-1}.

8-Chloro-4α- and 4β-methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (8 and 9). Compounds **8** and **9** were prepared as reported for **2**, starting from **62** (4.20 g, 13.47 mmol) and employing 1-penten-3-one (3.22 mL, 32.33 mmol) for the generation in situ of silyloxydiene **69**. The 4:1 (the same ratio in the crude) mixture of **8** and **9** (731 mg, 22%) was obtained after chromatography (CH_2Cl_2, 0.5% Et_3N, R_f 0.11) as pale yellow solid. Attempts of further separation by chromatography failed: ^1H NMR ($CDCl_3$) δ 7.00 (m, 2 H + 2 H), 6.75 (m, 1 H + 1 H), 4.29 (ddd, *J* = 13.5, 5.1, 2.5 Hz, 1 H, **8**), 3.91 (m, 1 H, **9**), 3.55−3.30 (m, 2 H, **9**), 3.20 (m, 2 H, **9**), 2.90−1.80 (m, 6 H + 6 H), 1.20 (d, *J* = 7.2 Hz, 3 H, **9**), 1.08 (d, *J* = 7.0 Hz, 3 H, **8**); ^{13}C NMR ($CDCl_3$) (major isomer **8**) δ 209.3 (s), 142.5 (s), 128.8 (d), 127.1 (d), 125.8 (s), 122.5 (s), 113.5 (d), 61.7 (d), 48.2 (d), 47.3 (t), 39.8 (t), 25.2 (t), 24.4 (t), 9.6 (q); MS *m*/*z* (%) 249 (100); IR ($CDCl_3$) 1713 cm^{-1}.

8-Chloro-5α-methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (10). It was prepared as described for **1**. Starting from **63** (953 mg, 2.92 mmol), pure **10** (210 mg, 29%) was obtained after chromatography (CH_2Cl_2, 0.5% Et_3N, R_f 0.39) of the crude reaction mixture as a thick oil: ^1H NMR ($CDCl_3$) δ 7.07 (dd, *J* = 8.8, 2.6 Hz, 1 H), 6.98 (d, *J* = 2.5 Hz, 1 H), 6.74 (d, *J* = 8.8 Hz, 1 H), 4.15 (ddd, *J* = 13.5, 5.9, 3.3 Hz, 1 H), 3.28−3.07 (m, 2 H), 2.79 (dd, *J* = 15.8, 4.8 Hz, 1 H), 2.69−2.21 (m, 5 H), 1.85 (m, 1 H), 1.06 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 208.0 (s), 142.1 (s), 129.0 (d), 127.1 (d), 125.1 (s), 122.6 (s), 113.5 (d), 61.9 (d), 47.0 (t), 46.8 (t), 40.2 (t), 33.1 (t), 32.8 (d), 24.4 (t), 19.0 (q); MS *m*/*z* (%) 249 (M^+, 100); IR ($CDCl_3$) 1715 cm^{-1}.

6α,8-Dimethyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (11). It was prepared as described for **1**. Starting from **64** (720 mg, 2.35 mmol), pure **11** (190 mg, 35%) was obtained after chromatography (CH_2Cl_2, 0.5% Et_3N, R_f 0.40) of the crude reaction mixture as an oil: ^1H NMR ($CDCl_3$) δ 7.01−6.98 (m, 1 H), 6.94 (s, 1 H), 6.81−6.77 (m, 1 H), 4.18 (ddd, *J* = 12.8, 6.5, 2.9 Hz, 1 H), 3.41 (m, 1 H), 3.09 (td, *J* = 12.1, 4.4 Hz, 1 H), 2.90 (m, 1 H), 2.74−2.60 (m, 1 H), 2.59−2.33 (m, 3 H), 2.27 (s, 3 H), 1.96−1.71 (m, 2 H), 1.33 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 208.6 (s), 141.7 (s), 129.8 (s), 128.6 (d), 127.7 (d), 127.6 (s), 113.2 (d), 53.1 (d), 47.9 (t), 47.2 (t), 40.7 (t), 36.8 (t), 28.9 (d), 22.2 (q); MS *m*/*z* (%) 229 (100); IR ($CDCl_3$) 1712 cm^{-1}.

8-Chloro-6α-methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (12). It was prepared as described for **1**. Starting from **65** (950 mg, 2.90 mmol), pure **12** (193 mg, 27%) was obtained after chromatography (CH_2Cl_2, 0.5% Et_3N, R_f 0.55) of the crude reaction mixture as a thick oil: ^1H NMR ($CDCl_3$) δ 7.09−7.06 (m, 2 H), 6.77−6.71 (m, 1 H), 4.10 (ddd, *J* = 12.9, 6.3, 3.7 Hz, 1 H), 3.51−3.40 (m, 1 H), 3.14 (ddd, *J* = 15.4, 11.0, 4.4 Hz, 1 H), 2.88 (m, 1 H), 2.72−2.38 (m, 4 H), 1.81 (m, 2 H), 1.31 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 208.0 (s), 142.7 (s), 131.6 (s), 127.4 (d), 126.9 (d), 125.9 (s), 114.0 (d), 52.7 (d), 47.8 (t), 46.7 (t), 40.5 (t), 36.4 (t), 28.9 (d), 21.6 (q); MS *m*/*z* (%) 249 (M^+, 100); IR ($CDCl_3$) 1715 cm^{-1}.

8-Chloro-1α- and 1β-methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (13 and 14). Compounds **13** and **14** were prepared as reported for **2**, starting from **62** (2.99 g, 9.62 mmol) and employing 3-penten-2-one (2.25 mL, 23.1 mmol) for the generation in situ of silyloxydiene **68**. The 1:1.5:2 crude mixture of **13**, **14**, and **78**, was dissolved in CH_2Cl_2 (34 mL) and treated with 1 M $TiCl_4$ (3.97 mL) in CH_2Cl_2 at room temperature for 3 h, obtaining after usual $NaHCO_3$(satd) quench (45 min) a crude oil. This was chromatographed eluting with CH_2Cl_2−petroleum ether, 1:1, 0.5% Et_3N. The fractions having R_f 0.23 (864 mg, 36%) contained a complex mixture of products in which **13** and **14** were prevailing. Further attempts of purification failed and the above mixture was thus used directly for the next oxidative step: ^1H NMR ($CDCl_3$) δ 7.00−6.51 (m, 3 H + 3 H), 4.60−4.40 (m, 1 H), 3.85−3.50 (m, 1 H + 1 H), 2.80−1.60 (m, 9 H + 8 H), 1.17 (d, *J* = 7.1 Hz, 3 H), 1.00 (d, *J* = 7.1 Hz, 3 H).

8-Chloro-4α,5α- and 4β,5α-dimethyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (15 and 16). Compounds **15** and **16** were prepared as reported for **2**, starting from **63** (2.20 g, 6.75 mmol) and employing 1-penten-3-one (1.68 mL, 16.87 mmol) for the generation in situ of silyloxydiene **69**. The complex crude reaction mixture obtained was chromatographed (EtOAc−petroleum ether, 1:10, 0.5% Et_3N, R_f 0.23) providing a 1:2 mixture of **15** and **16** (356 mg, 20%) as an oil used directly in the next oxidative step: ^1H NMR ($CDCl_3$) δ 7.12−7.01 (m, 2 H), 6.97−6.36 (m, 1 H + 1 H), 6.80 (d, *J* = 8.8 Hz, 1 H), 6.70 (d, *J* = 8.8 Hz, 1 H), 4.27 (ddd, *J* = 14.3, 5.9, 1.8 Hz, 1 H, **15**), 3.96 (m, 1 H, **16**), 3.30−1.85 (m, 8 H + 8 H), 1.12 (d, *J* = 7.3 Hz, 3 H), 1.06 (d, *J* = 7.1 Hz, 3 H), 1.05 (d, *J* = 7.0 Hz, 3 H), 1.04 (d, *J* = 7.0 Hz, 3 H).

A pure sample of 4α,5α-compound **15** was recovered after $Hg(OAc)_2$ oxidation: ^1H NMR ($CDCl_3$) δ 7.11−7.04 (m, 2), 6.79 (d, *J* = 8.8 Hz, 1 H), 4.27 (ddd, *J* = 14.3, 5.9, 1.8 Hz, 1 H), 3.17 (ddd, *J* = 14.3, 12.5, 3.3 Hz, 1 H, 1 H), 2.98 (dd, *J* = 16.5, 5.5 Hz, 1 H), 2.87 (dd, *J* = 11.0, 1.8 Hz, 1 H), 2.66−2.47 (m, 1 H), 2.45−2.16 (m, 4 H), 1.05 (d, *J* = 7.0 Hz, 3 H), 1.04 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 209.0 (s), 140.7 (s), 129.9 (d), 127.0 (d), 123.6 (s), 122.5 (s), 112.7 (d), 67.2 (d), 48.3 (d), 47.0 (t), 39.4 (t), 29.6 (t), 26.8 (d), 19.4 (q), 8.8 (q); MS *m*/*z* (%) 263 (M^+, 73), 192 (100); IR ($CDCl_3$) 1701 cm^{-1}.

4α,6α,8-Trimethyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (17). It was prepared as reported for **2**, starting from **64** (1.5 g, 4.91 mmol) and employing 1-penten-3-one (1.17 mL, 11.8 mmol) for the generation in situ of silyloxydiene **69**. The crude reaction mixture obtained was chromatographed (EtOAc−petroleum ether, 1:10, 0.5% Et_3N, R_f 0.24) providing **17** (170 mg, 14%), in mixture with a minor amount of its 4β-epimer, as an oil used directly in the next oxidative step: ^1H NMR ($CDCl_3$) δ 7.00−6.83 (m, 2 H), 6.81−6.75 (d, *J* = 7.9 Hz, 1 H), 4.16 (ddd, *J* = 14.4, 5.5, 2.0 Hz, 1 H), 3.10 (m, 2 H), 3.00−2.19 (m, 5 H), 2.29 (s, 3 H), 2.00 (m, 1 H), 1.34 (d, *J* = 8.0 Hz, 3 H), 1.11 (d, *J* = 6.5 Hz, 3 H).

8-Chloro-4α,6α-dimethyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (18). It was prepared as reported for **2**, starting from **65** (1.8 g, 5.5 mmol) and employing 1-penten-3-one (1.36 mL, 13.7 mmol) for the generation in situ of silyloxydiene **69**. The crude reaction mixture obtained was chromatographed (CH_2Cl_2−petroleum ether, 2:1, 0.5% Et_3N, R_f 0.47) providing **18** (200 mg, 14%), in mixture with a minor amount of its 4β-epimer, as an oil used directly in the next oxidation step: ^1H NMR ($CDCl_3$) δ 7.09−7.01 (m, 2 H + 2 H),

6.77−6.69 (m, 1 H + 1 H), 4.31−4.04 (ddd, J = 14.2, 5.7, 2.3 Hz, 1 H), 3.98−3.87 (m, 1 H), 3.50 (m, 1 H), 3.3.30−3.00 (m, 2 H + 1 H), 2.96−2.10 (m, 5 H + 3 H), 2.01−1.87 (m, 1 H + 1 H), 1.60−1.42 (m, 1 H + 1 H), 1.31 (d, J = 6.6 Hz, 3 H), 1.28 (d, J = 6.2 Hz, 3 H), 1.09 (d, J = 6.2 Hz, 3 H), 1.08 (d, J = 6.6 Hz, 3 H).

3,4,5,6-Tetrahydrobenzo[*c*]quinolizin-3(4a*H*)-one (19). It was prepared as reported for **1**, starting from **60** (4 g, 14.4 mmol) and employing Danishefsky's diene **67** (3.29 mL, 17.3 mmol). Pure **19** (820 mg, 28%) was obtained after chromatography (EtOAc−petroleum ether, 4:1, R_f 0.35) as a solid: mp 135−136 °C; ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J = 7.7 Hz, 1 H), 7.23−6.95 (m, 4 H), 5.38 (d, J = 7.7 Hz, 1 H), 4.10−3.92 (m, 1 H), 2.83 (m 2 H), 2.58−2.52 (m, 2 H), 2.27−2.15 (m, 1 H), 1.87−1.75 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 193.3 (s), 146.2 (d), 138.5 (s), 129.4 (d), 127.8 (d), 126.5 (s), 122.6 (d), 114.1 (d), 104.0 (d), 57.3 (d), 43.1 (t), 28.8 (t), 27.5 (t); MS m/z (%) 199 (M^+, 100); IR ($CDCl_3$) 1638, 1565 cm^{-1}.

8-Chloro-5α-methyl-3,4,5,6-tetrahydrobenzo[*c*]quinolizin-3(4a*H*)-one (27). It was prepared as reported for **1**, starting from **63** (120 mg, 0.37 mmol) and employing Danishefsky's diene **67** (85 μL, 0.45 mmol). Pure **27** (27 mg, 29%) was obtained after chromatography (EtOAc−petroleum ether, 2:1, R_f 0.23) as a thick oil: ^{1}H NMR ($CDCl_3$) δ 7.63 (d, J = 7.6 Hz, 1 H), 7.17 (dd, J = 8.7, 2.5 Hz, 1 H), 7.08 (s, 1 H), 6.92 (d, J = 8.7 Hz, 1 H), 5.38 (d, J = 7.6 Hz, 1 H), 3.61 (ddd, J = 15.6, 10.9, 3.4 Hz, 1 H), 2.78−2.51 (m, 2 H), 2.60−2.20 (m, 2 H), 2.00−1.76 (m, 1 H), 1.15 (d, J = 6.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 193.4 (s), 146.1 (d), 137.3 (s), 128.7 (d), 128.0 (s), 127.7 (d), 127.6 (s), 115.4 (d), 104.5 (d), 63.2 (d), 41.0 (t), 36.0 (t), 33.4 (d), 17.8 (q); MS m/z (%) 247 (M^+, 100); IR ($CDCl_3$) 1645, 1570 cm^{-1}.

Oxidation of 1,2,3,4,5,6-Hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (1). Method A: To a solution of **1** (60 mg, 0.3 mmol) in 5% AcOH (7.5 mL) were added EDTA tetrasodium salt (500 mg, 1.2 mmol) and $Hg(OAc)_2$ (382 mg, 1.2 mmol) under stirring and nitrogen atmosphere. The resulting mixture was heated at 90 °C for 2 h. Then it was cooled to room temperature and diluted with water (7.5 mL), extracted with chloroform, washed with $NaHCO_3$(satd), brine and dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude obtained was chromatographed (EtOAc−petroleum ether, 4:1, 0.5% ET_3N) affording pure **19** (R_f 0.38, 7 mg, 10%) and **35** (R_f 0.22, 14 mg, 20%).

Method B: To a solution of freshly prepared LDA (0.68 mmol) in THF (3.5 mL), cooled at −78 °C, was added dropwise a solution of **1** (130 mg, 0.64 mmol) in THF (3.5 mL) followed by TMSCl (139 μL, 1.09 mmol). The reaction mixture was allowed to warm to room temperature and, after 1 h, DDQ (147 mg, 0.64 mmol) was added and the mixture stirred 18 h. Then, a saturated solution of $NaHCO_3$ was added, the white precipitate was filtered and the resulting solution extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The crude was chromatographed as described above, providing isomers **19** (32 mg, 25%) and **35** (33 mg, 25%) in 1:1 ratio.

35: mp 53 °C; ^{1}H NMR ($CDCl_3$) δ 7.23−6.95 (m, 4 H), 5.13 (s, 1 H), 4.03 (t, J = 7.7 Hz, 2 H), 2.85−2.75 (m, 2 H), 2.70−2.60 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 191.6 (s), 159.3 (s), 139.5 (s), 128.1 (d), 127.8 (d), 127.1 (s), 122.5 (d), 113.5 (d), 100.8 (d), 45.4 (t), 35.6 (t), 30.1 (t), 24.9 (t); MS m/z (%) 199 (M^+, 100); IR ($CDCl_3$) 1630 cm^{-1}.

Oxidation of 8-Methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (2). It was carried out as reported for the oxidation of **1**, method B. Starting from **2** (254 mg, 1.18 mmol), pure **20** (52 mg, 21%) and **36** (40 mg, 16%) were obtained after chromatography, eluting first with EtOAc−petroleum ether, 2:1, 0.5% ET_3N, to recover compound **20** (R_f 0.26) and then with EtOAc−petroleum ether, 4:1, 0.5% ET_3N, to collect **36** (R_f 0.19).

20: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 7.7 Hz, 1 H), 7.00−6.94 (m, 3 H), 5.34 (d, J = 7.7 Hz, 1 H), 4.00 (m, 1 H), 2.79 (m, 2 H), 2.54 (m, 2 H), 2.30 (s, 3 H), 2.21 (m, 1 H), 1.78 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 193.1 (s), 146.1 (d), 132.1 (s), 130.0 (d), 129.9 (s), 128.3 (d), 126.3 (s), 113.9 (d), 103.3 (d), 57.2 (d), 43.0 (t), 28.9 (t), 27.4 (t), 20.4 (q); MS m/z (%) 213 (100); IR ($CDCl_3$) 1636, 1569 cm^{-1}.

36: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.15−7.05 (m, 1 H), 6.97−6.91 (m, 2 H), 5.11 (s, 1 H), 4.00 (t, J = 7.5 Hz, 1 H), 2.81−2.62 (m, 6 H), 2.31 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 191.5 (s), 159.4 (s), 137.2 (s), 132.1 (s), 128.8 (d), 128.1 (d), 126.7 (s), 113.5 (d), 100.3 (d), 45.4 (t), 35.3 (t), 30.2 (t), 24.9 (t), 20.5 (q); MS m/z (%) 213 (M^+, 100); IR ($CDCl_3$) 1627, 1559 cm^{-1}.

Oxidation of 8-Chloro-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (3). It was carried out as reported for the oxidation of **1**, method A, dissolving the starting material in 8% AcOH. Starting from **3** (498 mg, 0.698 mmol), pure **21** (R_f 0.37, 34 mg, 27%) and **37** (R_f 0.14, 78 mg, 54%) were obtained after chromatography (EtOAc−petroleum ether, 4:1, 0.5% ET_3N).

21: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.66 (d, J = 7.7 Hz, 1 H), 7.19−7.09 (m, 2 H), 6.95 (d, J = 8.5 Hz, 1 H), 5.37 (d, J = 7.7 Hz, 1 H), 4.10−3.96 (m, 1 H), 2.80 (m, 2 H), 2.56−2.51 (m, 2 H), 2.25−2.17 (m, 1 H), 1.87−1.72 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 193.1 (s), 145.7 (d), 137.2 (s), 129.1 (d), 128.1 (s), 127.7 (d), 115.2 (d), 104.5 (d), 57.2 (d), 43.0 (t), 28.6 (t), 27.4 (t); MS m/z (%) 233 (M^+, 100); IR ($CDCl_3$) 1650, 1575 cm^{-1}.

37: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.24−7.11 (m, 2 H), 6.91 (d, J = 8.4 Hz, 1 H), 5.14 (s, 1 H), 3.99 (t, J = 7.7 Hz, 2 H), 2.80−2.60 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 191.4 (s), 158.4 (s), 138.1 (s), 128.6 (s), 127.8 (d), 127.4 (d), 127.2 (s), 114.6 (d), 101.1 (d), 45.4 (t), 35.4 (t), 29.7 (t), 24.7 (t); MS m/z (%) 233 (M^+, 100); IR ($CDCl_3$) 1639, 1568 cm^{-1}.

Oxidation of 8-Chloro-5-methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (10). It was carried out as reported for the oxidation of **1**, method A, dissolving the starting material in 25% AcOH. Starting from **10** (200 mg, 0.81 mmol), pure **27** (58 mg, 29%) and **41** (86 mg, 43%) were obtained after chromatography eluting first with EtOAc−petroleum ether, 2:1, to collect **27** (R_f 0.32) and **41** (R_f 0.22), and then with EtOAc−MeOH, 5:1 to recover compound **79** (R_f 0.26, 16 mg, 8%).

41: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.16 (dd, J = 8.8, 2.3 Hz, 1 H), 7.05 (d, J = 2.3 Hz, 1 H), 6.87 (d, J = 8.8 Hz, 1 H), 5.15 (s, 1 H), 3.95 (t, J = 7.3 Hz, 1 H), 2.85 (dd, J = 15.0, 4.7 Hz, 1 H), 2.78−2.42 (m, 4 H), 1.05 (d, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 191.8 (s), 162.5 (s), 137.7 (s), 128.5 (d), 127.4 (d), 127.2 (s, 2 C), 114.3 (d), 99.6 (d), 45.6 (t), 35.3 (t), 33.2 (d), 31.9 (t), 17.5 (q); MS m/z (%) 247 (M^+, 100); IR ($CDCl_3$) 1636, 1561 cm^{-1}.

79: thick oil; ^{1}H NMR ($CDCl_3$) δ 8.03 (d, J = 7.7 Hz, 1 H), 7.38 (s, 2 H), 7.30 (s, 1 H), 6.79 (dd, J = 8.0, 2.5 Hz, 1 H), 6.61 (d, J = 2.9 Hz, 1 H), 3.18−2.92 (m, 2 H), 2.68 (dd, J = 15.0, 7.3 Hz, 1 H), 1.28 (d, J = 7.0 Hz, 3 H); MS m/z (%) 245 (M^+, 71), 202 (100).

Oxidation of 6,8-Dimethyl-1,2,3,4,5,6-hexahydrobenzo-[*c*]quinolizin-3(4a*H*)-one (11). It was carried out as reported for the oxidation of **1**, Method A, dissolving the starting material in 45% AcOH. Starting from **11** (160 mg, 0.70 mmol), pure **28** (R_f 0.37, 40 mg, 25%) and **42** (R_f 0.16, 74 mg, 46%) were obtained after chromatography eluting with EtOAc−petroleum ether, 3:2, 0.5% Et_3N.

28: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J = 7.7 Hz, 1 H), 7.00−6.93 (m, 3 H), 5.34 (d, J = 7.7 Hz, 1 H), 4.18−3.98 (m, 1 H), 3.07−2.89 (m, 1 H), 2.59−2.53 (m, 1 H), 2.50−2.42 (m, 2 H), 2.28 (s, 3 H), 1.94 (dd, J = 8.0, 4.6 Hz, 1 H), 1.80 (d, J = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 193.3 (s), 145.9 (d), 135.1 (s), 132.2 (s), 130.9 (s), 129.7 (d), 128.4 (s), 113.9 (d), 103.3 (d), 51.7 (d), 43.0 (t), 34.5 (t), 31.0 (d), 21.5 (q), 20.4 (q); MS m/z (%) 227 (100); IR ($CDCl_3$) 1632, 1569 cm^{-1}.

42: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.07−6.98 (m, 1 H), 6.95 (s, 1 H), 6.87 (d, J = 8.0 Hz, 1 H), 5.10 (s, 1 H), 3.94 (t, J = 7.7 Hz, 1 H), 2.86 (m, 1 H), 2.71−2.56 (m, 1 H), 2.62 (t, J = 7.6 Hz, 2 H), 2.34 (dd, J = 15.4, 7.3 Hz, 1 H), 2.28 (s, 3 H), 1.23 (d, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 191.5 (s), 158.4 (s), 136.4 (s), 132.3 (s), 131.7 (s), 128.0 (d), 127.3 (d), 113.8 (d), 101.2 (d), 45.4 (t), 37.4 (t), 35.6 (t), 29.4 (d), 20.6 (q), 18.7 (q); MS m/z (%) 227 (M^+, 100); IR ($CDCl_3$) 1628, 1551 cm^{-1}.

Oxidation of 8-Chloro-6-methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (12). It was carried out as reported for the oxidation of **1**, Method A, dissolving the starting material in 50% AcOH. Starting from **12** (133 mg, 0.53 mmol), pure **29** (R_f 0.22, 35 mg, 26%) and **43** (R_f 0.14, 65 mg, 51%) were obtained after chromatography eluting with EtOAc−petroleum ether, 3:1, 0.5% Et_3N.

29: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.68 (d, J = 7.7 Hz, 1 H), 7.19−7.09 (m, 2 H), 6.95 (d, J = 7.8 Hz, 1 H), 5.36 (d, J = 7.7 Hz, 1 H), 4.21−4.03 (m, 1 H), 3.08−2.97 (m, 1 H), 2.58−2.44 (m, 2 H), 1.97−1.89 (m, 2 H), 1.27 (d, J = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 193.1 (s), 145.3 (d), 136.2 (s), 132.6 (s), 128.8 (d), 127.6 (d), 127.4 (s), 115.2 (d), 104.5 (d), 51.6 (d), 43.0 (t), 34.2 (t), 30.9 (d), 21.3 (q); MS *m*/*z* (%) 247 (M^+, 100); IR ($CDCl_3$) 1646, 1568 cm^{-1}.

43: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.23−7.13 (m, 2 H), 6.91 (d, J = 8.8 Hz, 1 H), 5.14 (s, 1 H), 3.99 (t, J = 7.7 Hz, 2 H), 2.93 (m, 1 H), 2.71−2.68 (m, 1 H), 2.65 (t, J = 7.6 Hz, 2 H), 2.37 (dd, J = 15.0, 7.7 Hz, 1 H), 1.27 (d, J = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 191.4 (s), 157.5 (s), 137.4 (s), 133.5 (s), 127.6 (s), 127.4 (d), 126.5 (d), 114.9 (d), 102.0 (d), 45.5 (t), 37.0 (t), 35.5 (t), 29.4 (d), 18.4 (q); MS *m*/*z* (%) 247 (M^+, 100); IR ($CDCl_3$) 1640, 1567 cm^{-1}.

Oxidation of 8-Chloro-1-methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (13 and 14). It was carried out as reported for the oxidation of **1**, method B. Starting from the **13**−**14** mixture (687 mg, 2.75 mmol), pure **44** (R_f 0.42, 122 mg, 18%) was obtained after chromatography, eluting with EtOAc−petroleum ether, 2:1, 0.5% ET_3N, while compound **30** (R_f 0.55, 61 mg, 9%) was obtained in 1:1 mixture with **44**. Further attempts of separate **30** from **44** by chromatography failed.

The oxidation was also performed according to method A, dissolving the starting material in 45% AcOH. Starting from the **13**−**14** mixture (120 mg, 0.48 mmol), pure **44** (68 mg, 57%) was obtained after chromatography (the same eluant as above).

30: ^{1}H NMR ($CDCl_3$) (from the mixture with **44**) δ 7.26−7.13 (m, 2 H), 6.87 (d, J = 9.5 Hz, 1 H), 5.39 (s, 1 H), 4.10−3.90 (m, 1 H), 3.20−3.00 (m, 1 H), 2.75−2.56 (m, 3 H), 1.82−1.70 (m, 2 H), 1.26 (s, 3 H).

44: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.24−7.10 (m, 2 H), 6.95 (d, J = 8.2 Hz, 1 H), 5.13 (s, 1 H), 4.51 (m, 1 H), 3.05−2.93 (m, 1 H), 2.84−2.55 (m, 4 H), 2.38−2.30 (m, 1 H), 1.33 (d, J = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 190.9 (s), 155.7 (s), 137.3 (s), 128.8 (s), 128.7 (d), 127.7 (d), 123.5 (s), 114.7 (d), 100.5 (d), 51.5 (d), 41.9 (t), 30.00 (t), 25.1 (t), 15.0 (q); MS *m*/*z* (%) 247 (M^+, 100); IR ($CDCl_3$) 1637, 1567 cm^{-1}.

Oxidation of 4-Methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]-quinolizin-3(4a*H*)-one (4 and 5). It was performed as reported for the oxidation of **1**, method A, dissolving the starting material in 5% AcOH. Starting from the 3:2 mixture of **4** and **5** (79 mg, 0.37 mmol), pure **38** (R_f 0.29, 30 mg, 38%) was obtained after chromatography eluting with EtOAc−petroleum ether, 2:1, 0.5% Et_3N. A small amount of **80** (9 mg, 12%) was also obtained after chromatography by washing the silica gel with MeOH.

The oxidation was also performed according to method B. Starting from the 3:2 mixture of **4** and **5** (56 mg, 0.26 mmol), pure **22** (R_f 0.59, 13 mg, 22%) and **23** (R_f 0.36, 13 mg, 22%) were obtained after chromatography as thick oils, eluting with EtOAc−petroleum ether, 2:1, 0.5% ET_3N.

38: mp 88−89 °C; ^{1}H NMR ($CDCl_3$) δ 7.25−6.94 (m, 4 H), 3.93 (t, J = 7.7 Hz, 2 H), 2.80−2.63 (m, 6 H), 1.83 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 190.7 (s), 156.3 (s), 140.5 (s), 127.9 (d), 127.8 (d), 126.7 (s), 121.8 (d), 112.8 (d), 105.9 (s), 45.0 (t), 35.5 (t), 26.3 (t), 24.8 (t), 10.1 (q); MS *m*/*z* (%) 213 (M^+, 98), 212 (100); IR ($CDCl_3$) 1622, 1553 cm^{-1}.

22: ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 7.7 Hz, 1 H), 7.23−6.95 (m, 4 H), 5.40 (d, J = 7.7 Hz, 1 H), 3.63 (ddd, J = 12.4, 12.4, 4.4 Hz, 1 H), 2.89−2.77 (m, 2 H), 2.45−2.31 (m, 2 H), 1.82−1.61 (m, 1 H), 1.23 (d, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 195.3 (s), 145.8 (d), 139.2 (s), 129.0 (d), 127.8 (d), 126.8 (s), 122.5 (d), 114.5 (d), 103.4 (d), 62.7 (d), 45.1 (d), 27.5 (t, 2 C), 10.6 (q); MS *m*/*z* (%) 213 (M^+, 100); IR ($CDCl_3$) 1642, 1569 cm^{-1}.

23: ^{1}H NMR ($CDCl_3$) δ 7.67 (d, J = 7.7 Hz, 1 H), 7.24−6.97 (m, 4 H), 5.30 (dd, J = 7.7, 1.1 Hz, 1 H), 4.10−3.99 (dt, J = 12.4, 3.5 Hz, 1 H), 2.87−2.80 (m, 2 H), 2.42−2.36 (m, 1 H), 2.10−1.87 (m, 2 H), 1.04 (d, J = 7.3 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 195.2 (s), 145.7 (d), 139.5 (s), 129.2 (d), 127.9 (d), 127.3 (s), 122.7 (d), 114.5 (d), 102.2 (d), 60.2 (d), 44.3 (d), 27.7 (t), 25.3 (t), 10.1 (q); MS *m*/*z* (%) 213 (M^+, 100); IR ($CDCl_3$) 1634 cm^{-1}.

80: ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 7.6 Hz, 1 H), 7.40−7.00 (m, 4 H), 6.51 (d, J = 7.6 Hz, 1 H), 2.93 (m, 4 H), 2.18 (s, 3 H); MS *m*/*z* (%) 211 (M^+, 25), 210 (100).

Oxidation of 4,8-Dimethyl-1,2,3,4,5,6-hexahydrobenzo-[*c*]quinolizin-3(4a*H*)-one (6 and 7). It was carried out as reported for the oxidation of **1**, method A, dissolving the starting material in 8% AcOH. Starting from the 7:2 mixture of **6** and **7** (151 mg, 0.31 mmol), pure **39** (R_f 0.51, 50 mg, 71%) was obtained after chromatography eluting with EtOAc−petroleum ether, 2:1, 0.5% Et_3N.

The oxidation was also performed according to method B. Starting from the 7:2 mixture of **6** and **7** (243 mg, 1.06 mmol), pure **24** (R_f 0.60, 56 mg, 23%) and **25** (R_f 0.28, 56 mg, 23%) were obtained after chromatography, eluting with EtOAc−petroleum ether, 2:1, 0.5% ET_3N.

39: ^{1}H NMR ($CDCl_3$) δ 7.06−6.83 (m, 3 H), 3.91 (t, J = 7.7 Hz, 2 H), 2.73−2.61 (m, 6 H), 2.30 (s, 3 H), 1.82 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 190.3 (s), 156.8 (s), 138.1 (s), 131.4 (s), 128.4 (d), 128.0 (d), 126.6 (s), 112.8 (d), 105.3 (s), 45.0 (t), 35.3 (t), 26.4 (t), 24.7 (t), 20.4 (q), 10.0 (q); MS *m*/*z* (%) 227 (M^+, 100); IR ($CDCl_3$) 1652, 1573 cm^{-1}.

24: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.66 (d, J = 7.7 Hz, 1 H), 6.93 (m, 3 H), 5.35 (d, J = 7.7 Hz, 1 H), 3.60 (ddd, J = 12.4, 12.4, 4.4 Hz, 1 H), 2.78 (m, 2 H), 2.33 (m, 2 H), 2.29 (s, 3 H), 1.70 (m, 1 H), 1.21 (d, J = 7.3 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 195.1 (s), 145.8 (d), 136.6 (s), 132.0 (s), 129.5 (d), 128.2 (d), 126.6 (s), 114.3 (d), 102.6 (d), 62.5 (d), 44.8 (d), 27.4 (t), 27.3 (t), 20.4 (q), 10.6 (q); MS *m*/*z* 227 (M^+, 100); IR ($CDCl_3$) 1644, 1572 cm^{-1}.

25: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 7.4 Hz, 1 H), 6.99 (m, 3 H), 5.34 (d, J = 7.4 Hz, 1 H), 4.02 (dt, J = 12.7, 3.2 Hz, 1 H), 2.82 (m, 2 H), 2.40 (m, 1 H), 2.30 (s, 3 H), 2.03−1.85 (m, 2 H), 1.03 (d, J = 7.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 198.0 (s), 145.6 (d), 137.1 (s), 132.3 (s), 129.7 (d), 128.4 (d), 127.1 (s), 114.4 (d), 101.6 (d), 60.2 (d), 44.3 (d), 27.7 (t), 25.4 (t), 20.5 (q), 10.1 (q); MS *m*/*z* 227 (M^+, 100); IR ($CDCl_3$) 1636, 1501 cm^{-1}.

Oxidation of 8-Chloro-4-methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (8 and 9). It was carried out as reported for the oxidation of **1**, method A, dissolving the starting material in 8% AcOH. Starting from the 4:1 mixture of **8** and **9** (212 mg, 0.85 mmol), pure **40** (R_f 0.18, 73 mg, 35%) was obtained after chromatography eluting with EtOAc−petroleum ether, 2:1, 0.5% Et_3N.

The oxidation was also performed according to method B. Starting from the 4:1 mixture of **8** and **9** (333 mg, 1.33 mmol), pure **26** (R_f 0.50, 47 mg, 18%) was obtained after chromatography, eluting with EtOAc−petroleum ether, 2:1, 0.5% ET_3N.

40: mp 108−110 °C; ^{1}H NMR ($CDCl_3$) δ 7.23−7.11 (m, 2 H), 6.87 (d, J = 8.8 Hz, 1 H), 3.90 (t, J = 7.5 Hz, 2 H), 2.76−2.64 (m, 6 H), 1.82 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 190.6 (s), 155.4 (s), 139.0 (s), 128.3 (s), 127.7 (d), 127.3 (d), 126.5 (s), 113.9 (d), 106.4 (s), 45.1 (t), 35.3 (t), 26.1 (t), 24.7 (t), 10.1 (q); MS *m*/*z* (%) 247 (M^+, 100); IR ($CDCl_3$) 1648, 1569 cm^{-1}.

26: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.60 (d, J = 7.3 Hz, 1 H), 7.26−7.11 (m, 2 H), 6.97 (d, J = 8.8 Hz, 1 H), 5.30 (dd, J = 7.7, 1.1 Hz, 1 H), 4.03 (ddd, J = 12.8, 12.8, 3.6 Hz, 1 H), 2.84−2.76 (m, 2 H), 2.42−2.36 (m, 1 H), 2.08−1.82 (m, 2 H), 1.02 (d, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 197.9 (s), 145.2 (d), 138.2 (s), 131.0 (s), 128.9 (d), 127.8 (d), 127.4 (s), 115.7 (d), 102.8 (d), 60.1 (d), 44.2 (d), 27.6 (t), 25.2 (t), 10.0 (q); MS *m*/*z* (%) 247 (M^+, 100); IR ($CDCl_3$) 1661, 1568 cm^{-1}.

Oxidation of 8-Chloro-4,5-dimethyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (15 and 16). It was performed as reported for the oxidation of **1**, method A, dissolving the starting material in 50% AcOH. Starting from

the 1:2 mixture of **15** and **16** (153 mg, 0.6 mmol), the crude was chromatographed eluting first with EtOAc−petroleum ether, 3:2, 0.1% Et_3N, obtaining a mixture of isomers **31** and **32** (R_f 0.39, 17 mg, 11%), and then with EtOAc−petroleum ether, 3:1, 0.1% Et_3N, affording compound **45** (R_f 0.32, 22 mg, 14%). Two successive chromatographies of the first fraction (EtOAc−petroleum ether, 2:1, 0.1% Et_3N) allowed the partial separation of **31** (4 mg, 3%) and **32** (8 mg, 6%), compound **31** and **32** being 98% and 75% pure, respectively, by GLC analysis.

45: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.20 (dd, J = 8.8, 2.6 Hz, 1 H), 7.11 (s, 1 H), 6.25 (d, J = 8.8 Hz, 1 H), 4.09−3.95 (m, 1 H), 3.86−3.62 (m, 1 H), 3.28 (ddd, J = 6.9, 4.7, 1.8 Hz, 1 H), 2.99 (dd, J = 15.0, 4.7 Hz, 1 H), 2.84−2.65 (m, 1 H), 2.60−2.47 (m, 2 H), 1.87 (s, 3 H), 0.90 (d, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 191.2 (s), 158.7 (s), 138.4 (s), 128.9 (d), 127.4 (d), 126.7 (s), 126.0 (s), 113.7 (d), 105.7 (s), 45.3 (t), 35.4 (t), 31.6 (d), 29.3 (t), 15.6 (q), 9.5 (q); MS m/z (%) 261 (M^+, 100); IR ($CDCl_3$) 1628, 1554 cm^{-1}.

31: oil; ^{1}H NMR ($CDCl_3$) δ 7.31 (d, J = 7.7 Hz, 1 H), 7.15 (m, 2 H), 7.00 (d, J = 8.8 Hz, 1 H), 5.24 (d, J = 7.7 Hz, 1 H), 3.14 (dd, J = 8.5, 8.4 Hz, 1 H), 2.90 (dd, J = 16.1, 5.1 Hz, 1 H), 2.61 (t, J = 7.3 Hz, 1 H), 2.54−2.48 (m, 1 H), 2.47−2.43 (m, 1 H), 1.31 (d, J = 7.0 Hz, 3 H), 1.03 (d, J = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 198.1 (s), 145.6 (d), 138.2 (s), 128.7 (s), 128.5 (d), 127.8 (d), 115.7 (d), 102.7 (d), 66.1 (d), 41.8 (d), 36.0 (t), 29.8 (d), 17.3 (q), 9.51 (q); MS m/z (%) 261 (M^+, 100); IR ($CDCl_3$) 1637, 1568 cm^{-1}.

32: oil; ^{1}H NMR ($CDCl_3$) δ 7.58 (d, J = 7.7 Hz, 1 H), 7.20 (dd, J = 8.5, 2.6 Hz, 1 H), 7.09 (s, 1 H), 6.95 (d, J = 8.5 Hz, 1 H), 5.31 (dd, J = 7.7, 1.1 Hz, 1 H), 3.65 (dd, J = 11.4, 3.0 Hz, 1 H), 2.72 (dd, J = 15.3, 3.6 Hz, 1 H), 2.59−2.44 (m, 2 H), 2.08−1.85 (m, 1 H), 1.14 (d, J = 6.6 Hz, 3 H), 0.98 (d, J = 7.3 Hz, 3 H).

Oxidation of 4,6,8-Trimethyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (17). It was performed as reported for the oxidation of **1**, method A, dissolving the starting material in 50% AcOH. Starting from **17** (170 mg, 0.7 mmol), pure **46** (R_f 0.21, 45 mg, 27%) was obtained after chromatography eluting with EtOAc−petroleum ether, 1:2, 1% Et_3N. By the same chromatography a fraction (R_f 0.37) containing compound **33** (46 mg, 27%) in mixture with minor amounts of some unidentified compounds was also obtained and further attempts of purification were not successful.

46: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.05−6.94 (m, 2 H), 6.87−6.83 (m, 1 H), 3.91 (t, J = 7.4 Hz, 2 H), 2.96−2.86 (m, 1 H), 2.74−2.64 (m, 1 H), 2.64 (t, J = 7.6 Hz, 2 H), 2.49 (dd, J = 15.4, 7.7 Hz, 1 H), 2.31 (s, 3 H), 1.82 (s, 3 H), 1.29 (d, J = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 190.4 (s), 155.5 (s), 137.3 (s), 131.4 (s), 131.1 (s), 127.7 (d), 126.6 (d), 112.9 (d), 106.0 (s), 45.0 (t), 35.4 (t), 33.7 (t), 29.1 (d), 20.5 (q), 18.4 (q), 10.0 (q); MS m/z (%) 241 (M^+, 91), 226 (100); IR ($CDCl_3$) 1628, 1561 cm^{-1}.

33: brown thick oil; ^{1}H NMR ($CDCl_3$) δ 7.68 (d, J = 7.7 Hz, 1 H), 7.25−6.80 (m, 3 H), 5.35 (d, J = 7.7 Hz, 1 H), 3.69 (ddd, J = 16.8, 13.2, 4.0 Hz, 1 H), 3.00 (m, 1 H), 2.80−2.00 (m, 2 H), 2.32 (s, 3 H), 1.80 (m, 1 H), 1.18 (d, J = 6.6 Hz, 3 H), 1.16 (d, J = 7.0 Hz, 3 H).

Oxidation of 8-Chloro-4,6-dimethyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one (18). It was performed as reported for the oxidation of **1**, method A, dissolving the starting material in 50% AcOH. Starting from **18** (170 mg, 0.7 mmol), pure **47** (R_f 0.21, 68 mg, 34%) was obtained after chromatography eluting with EtOAc−petroleum ether, 1:3, 1% Et_3N, together with a fraction containing compound **34** (R_f 0.29, 20 mg, 10%) in mixture with other unidentified compounds.

47: thick oil; ^{1}H NMR ($CDCl_3$) δ 7.19−7.09 (m, 2 H), 6.82 (d, J = 8.4 Hz, 1 H), 3.89 (t, J = 7.3 Hz, 2 H), 2.91 (m, 1 H), 2.70 (dd, J = 15.5, 4.4 Hz, 1 H), 2.65 (t, J = 7.3 Hz, 2 H), 2.45 (dd, J = 15.4, 8.0 Hz, 1 H), 1.80 (s, 3 H), 1.28 (d, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 190.5 (s), 154.5 (s), 138.3 (s), 132.9 (s), 127.1 (d), 126.8 (d), 125.8 (d), 114.1 (d), 107.0 (s), 45.0 (t), 35.4 (t), 33.4 (t), 29.1 (d), 18.1 (q), 10.0 (q); MS m/z (%) 261 (M^+, 74), 246 (100); IR ($CDCl_3$) 1633, 1564 cm^{-1}.

34: ^{1}H NMR ($CDCl_3$) δ 7.63 (d, J = 7.7 Hz, 1 H), 7.30−7.00 (m, 2 H), 6.93 (d, J = 8.7 Hz, 1 H), 5.39 (d, J = 7.7 Hz, 1 H), 3.73 (ddd, J = 16.5, 13.6, 4.4 Hz, 1 H), 3.04 (m, 1 H), 2.80−2.00 (m, 2 H), 1.85 (m, 1 H), 1.18 (d, J = 7.2 Hz, 3 H), 1.16 (d, J = 7.3 Hz, 3 H).

4-Methyl-2,3,5,6-tetrahydrobenzo[*c*]quinolizin-3(1*H*)-one·HCl Salt (81). Compound **38** (83 mg, 0.39 mmol) was dissolved in a HCl(g) saturated solution in anhydrous MeOH (5 mL), and after stirring 30 min, the solvent was evaporated and the yellow residue **81** (97 mg, 100%) dried under high vacuum: mp 139−145 °C; ^{1}H NMR ($CDCl_3$) δ 7.40−7.08 (m, 4 H), 4.08 (t, J = 8.2 Hz, 2 H), 2.80 (s, 4 H), 2.72 (t, J = 8.15 Hz, 2 H), 1.78 (s, 3 H).

8-Chloro-4-methyl-1,2,3,4,5,6-hexahydrobenzo[*c*]quinolizin-3(4a*H*)-one·HCl Salt (82). It was prepared as described for compound **81**. Starting from **40** (250 mg, 1 mmol) compound **82** (282 mg, 100%) was obtained as yellow solid: ^{1}H NMR (d_6-DMSO) δ 7.00 (m, 2 H), 6.80 (d, J = 7.8 Hz, 1 H), 3.79 (t, J = 8.0 Hz, 2 H), 2.81 (m, 4 H), 2.72 (t, J = 8.0 Hz, 2 H), 1.79 (s, 3 H).

Inhibition Tests toward Recombinant 5αR-1 and 5αR-2. Cell routinary treatment: CHO 1827 (transfected with 5αR-1) and CHO 1829 (transfected with 5αR-2) cells were maintained in Ham's nutrient mixture F12 supplemented with 5% of fetal bovine serum. Medium was replaced two or three times a week. At confluence, cells were washed with PBS (phosphate-buffered saline) without Ca^{2+} and Mg^{2+} and split with a solution of trypsin−EDTA. Cells were maintained in a fully humidified incubator with 95% air and 5% CO_2 at 37 °C. To set up the experiments CHO 1827 (5αR-1) and CHO 1829 (5αR-2) cells were trypsinized, counted by hemocytometer and seeded in 24-well plates at a density of 50 000 cells/well in Ham's nutrient mixture F-12 supplemented with 5% fetal bovine serum until the day after.

Stock and working solution of inhibitors: Stock solutions of the inhibitors were prepared at a concentration of 1 mg/mL in ethanol. To verify the stability and purity of our inhibitors, we tested the stock solutions by GC/MS. These solutions resulted unchanged after 1 year from the preparation. To avoid any possible alteration of the inhibitor solutions at the lowest concentrations, working solutions at concentration below 1 mg/mL were freshly prepared and used within 15 days.

Inhibition test: Cells were incubated for 30 min at 37 °C in a 5% CO_2 incubator in a medium containing 2 μM testosterone (T/[^{3}H]T = 100/1) for the 5αR-1 assay or 0.2 μM for the 5αR-2 assay and the inhibitor in a concentration range from 10^{-5} to 10^{-9} M. Substrate and inhibitor were added in ethanol (1% of the final volume). Medium was removed and extracted with 3 mL of ethyl acetate. After freezing of the aqueous layer, the organic phase was removed and evaporated. Samples were supplemented with T and DHT as carriers (20 μL of a solution containing 2 mg/mL of T and DHT) and steroids were separated on TLC silica plates using CH_2Cl_2−Et_2O 85/15 as eluant. Steroids were visible under UV light, [2-(*p*-toluidino)-naphthalene-6-sulfonic acid enhancer (TNS) was necessary to identify DHT]. Lanes corresponding to T and DHT were scraped and silica extracted with 2 mL of ethyl acetate. After 30 min ethyl acetate was counted in a β-counter with 5 mL of scintillation liquid. The percentage of conversion of T into DHT is calculated as follows: $C\%$ = [DHT counts/(T counts + DHT counts)] × 100. The percentage of conversion at each concentration of the inhibitor was normalized to the control (percent of conversion without the inhibitor) and data were processed with the program ALLFIT using the four-parameter logistic equation to calculate the IC_{50} values. This program allows the statistical comparison of different inhibition curves through the F-test giving a probability p value. With this method it was possible to compare the inhibitory potency of different inhibitors. Generally, two different compounds were tested simultaneously toward the two isozymes, using finasteride as a control inhibitor in each experiment. The total recovery of steroids was 90−100%, indicating that under the above

conditions T and DHT are no further metabolized by other enzymatic systems.

Inhibition Tests toward Native 5αR-1 and 5αR-2. Human prostate homogenate preparation: Human benign prostatic hyperplastic tissues were obtained from different patients undergoing surgical prostatectomy. Patients had not been previously treated with finasteride. Tissues were frozen in liquid nitrogen immediately after surgical asportation and stored at −80 °C until the assay. Prostatic homogenate was obtained from 4−5 surgical pieces using a Potter homogenizer in a 5:1 (v:w) buffer (50 mM Tris, pH 7.4, 61 mM NaCl, 1.5 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 1 mM fumaric acid, 15 mM nicotinamide, 0.25 mM sucrose, 1 mM PMSF) working at 4 °C. The homogenate was filtered through a nylon membrane, fractionated and stocked at −80 °C.

Human scalp homogenate preparation: Human scalp tissues were obtained from the neurosurgery division of our hospital. The human scalp homogenate was prepared as described for the prostate homogenate.

Experimental conditions of the assay: (a) Prostate tissue: Experimental conditions were: 0.1 mg of protein per tube, incubation time 30 min, NADPH 250 μM in a final volume of 1 mL. Testosterone concentration was 50 nM and inhibitors were tested in the range 10^{-9}−10^{-5} M. At the end of the incubation, samples were extracted with 3 mL of ethyl acetate and processed according to the method described in the previous paragraph

(b) Scalp tissue: Experimental conditions were: 0.1 mg of protein per tube, incubation time 30 min, NADPH 500 μM in a final volume of 0.1 mL. Testosterone concentration was 1 μM, a constant amount (10 nM) of finasteride was used to block 5αR-2, and inhibitors were tested in the range 10^{-9}−10^{-5} M. At the end of the incubation, samples were extracted with 3 mL of ethyl acetate and processed according to the method described previously.

Evaluation of the Mechanism of Action. The experiments were performed using two 24-well plates with 50 000 cells/well. In the first hour of the experiment the two plates were treated as follows: in control wells, cells were incubated with a medium containing the substrate testosterone 1 μM; and in test wells, the effect of the inhibitor was monitored using a medium supplemented with the substrate (1 μM) and the inhibitor at a concentration close to the calculated IC_{50}.

In the first plate the enzymatic activity was evaluated at different times (after 10, 20, 40 and 60 min, respectively) by withdrawing the medium, extracting it with ethyl acetate and determining the percent of conversion. After 60 min of incubation the medium of the second plate (treated as the first plate for the first 60 min) was withdrawn, cells were washed with medium and then fresh medium containing the substrate but not the inhibitor was added. The enzymatic activity was again monitored at different times after the wash out (after 10, 20, 40 and 60 min, respectively). If in the second phase of the experiment, when the medium without inhibitor was employed, the enzyme activity was restored to the control level, the mechanism of action of the inhibitor was considered reversible.

***K*$_i$ Determination.** CHO 1827 (5αR-1) cells were plated in 24-well plates at a density of 50 000 cells/well in Ham's nutrient mixture F-12 supplemented with 5% fetal bovine serum. Cells were incubated for 60 min at 37 °C in a 5% CO_2 incubator in a medium containing respectively testosterone and [^{3}H]testosterone 0.2−400 μM and inhibitors at three different concentrations close to the IC_{50} value. Medium was removed and extracted with 3 mL of ethyl acetate. After freezing of the aqueous layer, the organic phase was removed and evaporated. Samples were processed as described for the IC_{50} determination and the percent of conversion was calculated. Data were processed with the program GRAFIT[41] to calculate the K_i values. The Eadie−Hofstee transformation of the curves shows the mechanism of action.

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Supporting Information Available: Stereochemical assignments on substituted benzo[*c*]quinolizin-3-ones by ^{1}H NMR analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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(38) Double unsaturated compounds **79** and **80** (Schemes 4 and 6) are flat compounds (index of planarity about 0.2 Å) and, therefore, expected to be highly active inhibitors. However they should be also much more polar compounds than inhibitor of the 1*H*-series, their A ring being describable also by a *p*-oxypyridinium zwitterion structure with a negative charge on the oxygen and a positive charge on the nitrogen. Since 5αR is a membrane-bound enzyme and the IC_{50} values are measured using living systems, a possible explanation for the reduced inhibition activity of **79** and **80** is that their polar structure could affect their ability to cross the membranes, thus lowering the concentration in the cell.

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