Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 353–356

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of 8-Chloro-benzo[*c*]quinolizin-3-ones as Potent and Selective Inhibitors of Human Steroid 5α-Reductase 1

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Received 24 September 1999; accepted 14 December 1999

Abstract—The synthesis of a series of differently substituted 8-chloro-benzo[*c*]quinolizin-3-ones, as potent and selective human steroid 5α-reductase type 1 inhibitors, has been accomplished by a four-step procedure based on the $TiCl_4$-promoted tandem Mannich–Michael cyclization of 2-silyloxy-1,3-butadienes with *N*-*t*-Boc iminium ions from quinolin-2-ones. The presence on the benzo[*c*]quinolizinone nucleus of a methyl group and a double bond at positions 6 and 4-4a, respectively, as in compound **1d**, gave rise to one of the most potent non-steroidal 5αR-1 inhibitors reported so far (IC_{50} = 14 nM).

Human steroid 5α-reductase (5αR) is a family of two isozymes (types 1 and 2) that convert testosterone to the more potent androgen dihydrotestosterone (DHT). Selective inhibition of 5αR-1 is currently investigated as a potential therapeutic tool for the treatment of DHT-related skin disorders, such as acne, alopecia, male baldness, and hirsutism.[1] Some potent steroidal and non-steroidal inhibitors of 5αR-1 (Fig. 1) have been described in recent years,[2] and one of them (LY191704) has been progressed to human clinical trials. As part of our studies on 5α-reductase inhibitors,[3–6] we recently reported on the synthesis and inhibitory activity of benzo[*c*]quinolizin-3-ones **1a** and **2a** (Fig. 1) as selective, although weak, non-steroidal inhibitors of 5α-reductase type 1 isozyme.[7]

Since the potency toward 5αR-1 of Eli Lilly inhibitors (Fig. 1) has been reported to increase noticeably after the introduction of the chlorine atom on the benzene ring,[8] we decided to investigate the effect of the 8-chloro substituent on the inhibitory potency of our benzo[*c*]-quinolizinones **1** and **2**. Moreover, it has been demonstrated that small alkyl groups at the position 7 of some 4-azasteroids (Fig. 1) had a positive effect on the potency toward 5α-reductase 1.[9] In 6-azasteroids, the presence of small alkyl groups at the position 6 increased the potency toward both isozymes.[10]

Thus, we considered it useful to introduce also a methyl group at the position 5 or 6 of **1** and **2** (corresponding to the positions 6 and 7 of steroids) to evaluate its influence on the selectivity and potency.

We based the synthesis of compounds **1a** and **2a** on the TMSOTf-promoted tandem Mannich–Michael reaction[11] (Scheme 1) of *N*-*t*-Boc iminium ion **4** (R, X = H), generated in situ from *N*-*t*-Boc 2-ethoxy derivative **5**, with two different 2-silyloxy-1,3-butadienes **3** (R′ = H, OMe).[7] However, when we applied this strategy to 8-chloro substituted 2-ethoxy derivatives **5** (X = Cl, R = H, Me) and using TMSOTf as a Lewis acid, the yield of the cyclizations was very low (<18%) due to the rapid degradation of **4** (or **5**) to the corresponding quinolines.

We employed other Lewis acids [$TiCl_4$, $SnCl_4$, $AlCl_3$, $TiCl_2(O\text{-}i\text{-}pr)_2$] to promote the cyclization reaction and, studying in particular the reaction between **5a** (X = Cl, R = H), prepared as shown in Scheme 2 and **3a**[12] (R′ = H), we found that only $TiCl_4$ promoted the iminium ion formation and the cyclization step better than TMSOTf, providing **2b**[13] (X = Cl, R = H, see Scheme 3 for the structure) in 35% yield.[14]

The $TiCl_4$ procedure was then applied to the synthesis of compounds **2c–j** (Scheme 2) from 3-methyl- and 4-methyl substituted 2-ethoxy carbamates (**5b**) and (**5c**), using diene **3a** or Danishefsky's diene **3b** (R′ = OMe) as partners in the cyclization reactions.

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PII: S0960-894X(99)00698-8

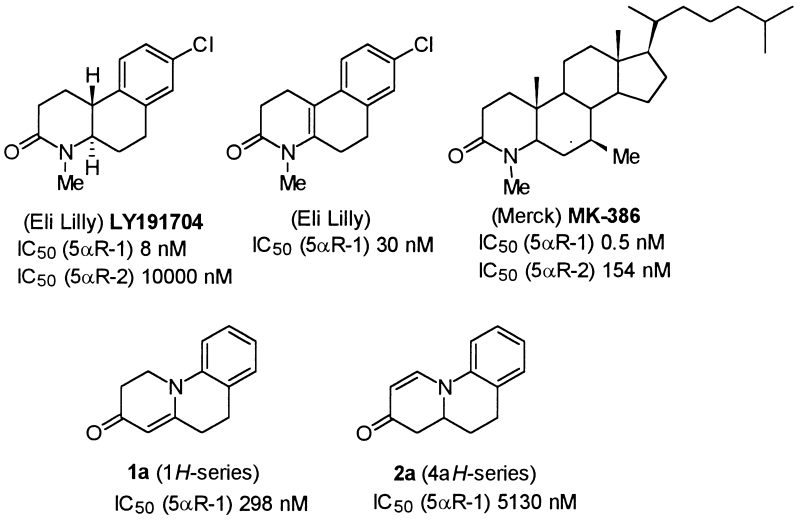
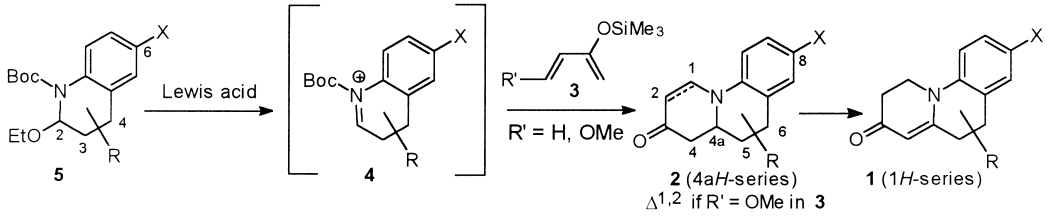
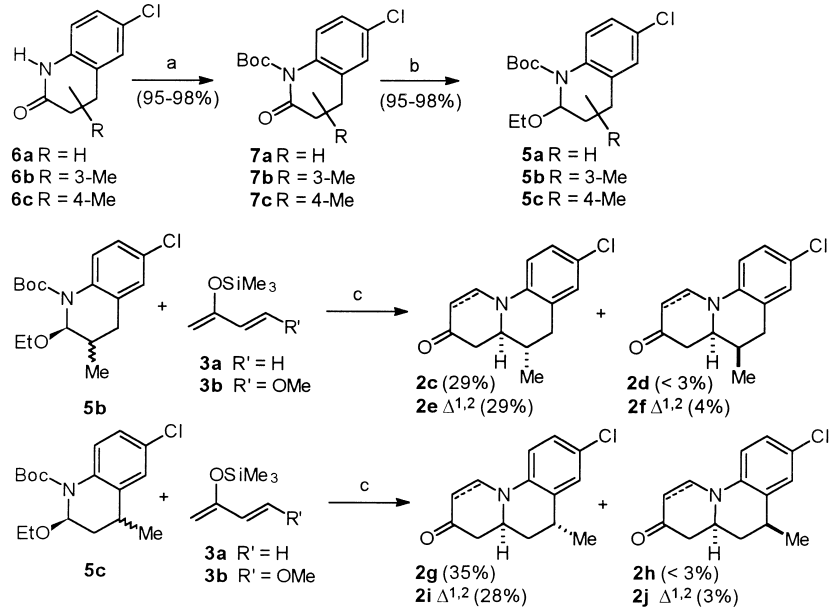


Figure 1.



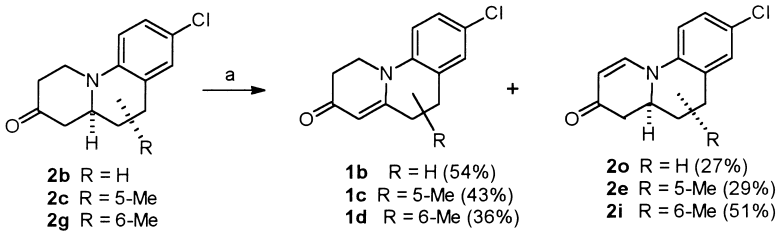
Scheme 1.



Scheme 2. (a) Boc_2O, Et_3N, DMAP, CH_2Cl_2, 25 °C, 18 h; (b) 6 equiv $NaBH_4$, EtOH, −25 °C, 6 h, then 2 N HCl in EtOH, −25 °C→25 °C, 2–4 h; (c) 1 M $TiCl_4$ in CH_2Cl_2, −30 °C, 10 min, then **3a** or **3b**, −30→25 °C, 30 min; then $NaHCO_3$ (satd), 40 min.

Lactams **6a–c** were known or prepared according to procedures already described for similar compounds.[15,16] After protection of the lactams as *N*-*t*-Boc derivatives **7a–c**, these were transformed as already reported[7] into the iminium ion precursors **5a–c** in 90–96% overall yield.

The reaction of iminium ions from **5b**,**c** with diene **3a** was stereoselective, providing as the major products the isomers **2c** and **2g** having the methyl group and the bridgehead 4a proton in *cis* relative position. Thus, compounds **2c** and **2g** were isolated in 29 and 35% yield, respectively, after chromatographic purification



Scheme 3. (a) $Hg(OAc)_2$, EDTA tetrasodium salt, 5% AcOH (aq), 90 °C, 2 h.

Table 1. Inhibition of human 5α-reductase type 1 by benzo[c]quinolizinones

Compound	R	X	IC_{50} (nM)	Compound	R	X	IC_{50} (nM)
1a	H	H	298 ± 75	**2a**	H	H	5130 ± 130
1b	H	Cl	49 ± 19	**2o**	H	Cl	459 ± 118
1c	5-Me	Cl	346 ± 185	**2e**	5-Me	Cl	9100 ± 500
1d	6-Me	Cl	14.4 ± 3.4	**2i**	6-Me	Cl	188 ± 42

(CH_2Cl_2, 0.5% Et_3N), while their isomers **2d** and **2h** were only detected (<3%) by ^1H NMR analysis of the crude reaction mixtures. The reactions of **5b**,**c** with Danishefsky's diene **3b**, which afforded $\Delta^{1,2}$ unsaturated compounds **2e** and **2i**, occurred with similar stereoselectivity. Since strong Lewis acids are able to remove the *N*-*t*-Boc protection,[17] the stereochemical outcome of these reactions could be explained by the formation, after the addition of $TiCl_4$ (2 equiv) to α-ethoxy carbamate **5**, of a planar imine in which the N atom coordinates a titanium complex.[6] The methyl group at position 3 or 4 then leads to a preferred less hindered *anti* attack by the dienes.

Oxidation of compounds **2b**, **2c** and **2g** (Scheme 3), to prepare the 4-4a unsaturated compounds, was performed by $Hg(OAc)_2$ in 5% aqueous acetic acid,[7] obtaining **1b**–**d** in 36–54% yield, together with the corresponding compounds of the 4a*H*-series **2o**, **2e** and **2i** in 27–51%.

Only unsaturated compounds **1b**–**d**, **2i**, **2e** and **2o**, tested towards human recombinant 5αR type 1 (expressed in CHO cells), and homogenates of human prostate (expressing 5αR-2), according to the reported procedures,[3,7,18] resulted selective inhibitors of 5αR-1 (Table 1), while they displayed very poor or no inhibition against 5αR-2.[19] Saturated compounds **2b**–**c**,**g** were instead very weak inhibitors toward both isozymes. Compounds belonging to the 1*H*-series were more active than the corresponding ones of the 4a*H*-series, as already observed for the unsubstituted parent compounds **1a** and **2a**.

As we anticipated, the presence of a chlorine atom at position 8 of the benzo[*c*]quinolizinones **1b** and **2o** increased noticeably the inhibitory activity in both 1*H*- and 4a*H*-series with respect to the corresponding compounds **1a** and **1b**. Since the same effect is observed in Eli Lilly series,[8] this suggest that our benzo[*c*]quinolizinones and Eli Lilly's compounds could assume a similar arrangement in the enzyme active site. The presence of a methyl group at position 5 was instead not useful, since 5-methyl substituted compounds **2e** and **1c** were less active than **2o** and **1b**, respectively, and even than the completely unsubstituted parent compounds **2a** and **1a**. It is thus possible that the 5-methyl group occupies a sterically hindered region in the enzyme active site. Finally, the presence of the methyl group at position 6 gave rise to a strong increase of potency. This suggests that, since the same increase of potency toward 5αR-1 is observed in 4-azasteroidal inhibitors bearing a methyl group in the corresponding position 7,[9] at least the rings A and B of 4-azasteroids and our non-steroidal inhibitors could be similarly orientated in the enzyme active site. Thus, the best combination of substituent position and type of unsaturation was found in compound **1d** which, having an $IC_{50} = 14.4$ nM, resulted one of the most potent and selective non-steroidal 5αR-1 inhibitor reported so far. Finally, experiments carried out on some of the benzo[*c*]quinolizin-3-one compounds prepared by us revealed that they are reversible competitive inhibitors toward 5αR-1. Further details on these experiments will be published soon.

In conclusion we have demonstrated that $TiCl_4$ can be used to promote the tandem Mannich–Michael reaction of silyloxydienes with iminium ion from aromatic precursors with more efficiency than TMSOTf and considerably shorter reaction times. The described procedure afforded novel substituted benzo[*c*]quinolizin-3-ones as potent and selective 5α-reductase 1 inhibitors which could find application in the treatment of some skin disorders (acne, alopecia, baldness) related to the 5α-reductase type 1 activity.

Acknowledgements

The authors thank Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST), CNR (Target Project on Biotechnology, grants 97.01121.PF49 and 97.01221.PF49), and the Ares-Serono for financial support.

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13. All new compounds were fully characterized and gave satisfactory elemental analysis. The relative stereochemistry in compounds **2c**–**j** was attributed by analysis of the ^{1}H NMR coupling constant values and nOe studies.
14. The reaction was carried out under argon by adding dropwise a 1 M solution of $TiCl_4$ (2 equiv) in CH_2Cl_2 to a solution of **5a** in CH_2Cl_2 (5 mL/mmol) at $-30\,^{\circ}C$, leaving under stirring for 10 min and then adding to the resulting dark solution 1.2 equiv of diene **3a**. After 30 min at room temperature, $NaHCO_3$ (satd) (5 mL/mmol) was added and the mixture stirred for 40 min. The mixture was then filtered through a Celite® layer and extracted with CH_2Cl_2. Chromatography on silica gel (CH_2Cl_2, 0.5% Et_3N) afforded pure **2b**. The same compound was obtained in 17% yield by the usual TMSOTf procedure, which was, moreover, much longer due to the 36-hour $NaHCO_3$ quench.[11]
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