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Synthesis and activity of 8-substituted benzo[*c*]quinolizin-3-ones as dual inhibitors of human 5α-reductases 1 and 2

Alessandro Ferrali,[a] Gloria Menchi,[a] Ernesto G. Occhiato,[a] Giovanna Danza,[b] Rosa Mancina,[b] Mario Serio[b] and Antonio Guarna[a,*]

[a]*Dipartimento di Chimica Organica 'Ugo Schiff', Polo Scientifico Università di Firenze, Via della Lastruccia 13, I-50019 Sesto Fiorentino, Italy*

[b]*Dipartimento di Fisiopatologia Clinica, Unità di Endocrinologia, Università di Firenze, Viale Pieraccini, 6, I-50139 Firenze, Italy*

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Abstract—Some potent dual inhibitors of 5α-reductases 1 and 2, based on the benzo[*c*]quinolizin-3-one structure and with IC_{50} values ranging between 93 and 166 nM for both isozymes, were found. The presence of the F atom on the ester moiety at the position 8 was crucial. This result can help in the design of other potent, dual inhibitors to be developed as drugs in the treatment of 5α-reductase related diseases.

Dihydrotestosterone (DHT) is produced by the NADPH-dependent 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, is a therapeutic target for the treatment of benign prostate hyperplasia (BPH), androgenic alopecia, and acne in men, and hirsutism in women.[9,10] Two different DNA-encoded isoenzymes of 5α-reductase, named type 1 and type 2 (5αR-1 and 5αR-2), transform T into DHT with different efficacy, which are not equally distributed in the human tissues, 5αR-1 being present mainly in scalp, skin, and liver, and 5αR-2 in the prostate. 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 introduction on the market by Merck of finasteride (Fig. 1).[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 dual 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 azasteroid dutasteride (Fig. 1), a dual inhibitor which was approved by FDA on 2002 for the treatment of BPH.[13]

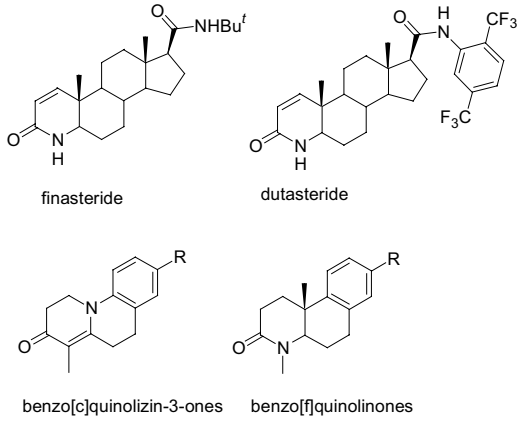

Figure 1.

As part of our studies on 5α-reductase inhibitors,[14] we recently reported on the synthesis and inhibitory activity of benzo[*c*]quinolizin-3-ones (Fig. 1) as potent and selective nonsteroidal inhibitors of 5α-reductase type 1

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* Corresponding author. Tel.: +39 055 4573481; fax: +39 055 4573531; e-mail: antonio.guarna@unifi.it

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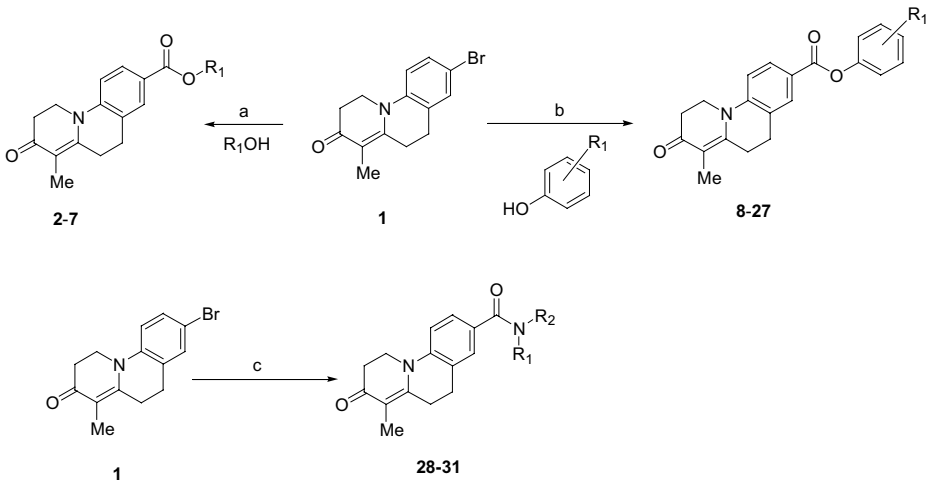

Scheme 1. Reagents and conditions: (a) R_1OH, CO, $PdCl_2$, Et_3N, benzene, 115 °C, 24 h, 50 bar; (b) phenol, CO, $PdCl_2$, Et_3N, benzene, 115 °C, 24 h, 50 bar; (c) amine, CO, $PdCl_2$, Et_3N, benzene, 115 °C, 24 h, 50 bar.

isozyme. We have shown that the potency of these compounds can be modulated by the substituent at the position 8 on the aromatic ring. Similar findings have been reported on Ely Lilly benzo[*f*]quinolinone compounds (Fig. 1), which, if bearing particular groups on the aromatic ring, showed even a weak to fair activity toward 5αR-2.[15] Aimed at discovering new nonsteroidal, dual inhibitors of 5α-reductases 1 and 2, having possible therapeutic applications, we started a program based on the synthesis and the evaluation of a series of benzo[*c*]quinolizin-3-ones bearing diverse substituents at position 8.

By a modification of the aza-Robinson annulation, involving lactams as starting materials, we prepared, as recently reported,[16] a bulk quantity of 8-bromo-4-methyl-benzo[*c*]quinolizin-3-one **1** (Scheme 1), a common intermediate which can be easily, and possibly in a single step, transformed into the target compounds by Pd-catalyzed cross-coupling reactions. A random screening of various benzo[*c*]quinolizinones prepared starting from **1**, allowed us to observe for the first time a dual inhibition (although inhibition of 5α-reductase 2 was still weak) when a carbomethoxy group was inserted in place of the bromine atom (compound **2**, Table 1, entry 1). Other compounds, lacking the ester moiety, were inactive toward 5αR-2. Based on this observation we synthesized a series of esters of aliphatic alcohols **3**–**7** and phenols **8**–**27**. The synthesis of these compounds was realized by Pd-catalyzed carbonylation of **1** in the presence of the suitable alcohol or phenol as depicted in Scheme 1.[17] The introduction of F and CF_3 groups in our inhibitors was dictated by the knowledge that H–C(α)–C=O fragments in an enzyme active site provide a pronounced fluorophilic environment due to occurrence of C–F·C=O contacts that are best described in terms of multipolar interactions between the intrinsically polar C–F and C=O units. Such F-interactions could be effectively exploited for enhancing ligand affinity or selectivity in structure based design.[18] Dutasteride (Fig. 1), indeed, possesses two CF_3 groups on the phenyl ring of the 17-amide moiety. We prepared and tested also a series amides **28**–**31**.[17] The synthesis of these amides was realized as described above by using the correspondingly substituted amine as the nucleophile. All of the esters showed weak activity toward 5αR-2 (Table 1, entries 1–6), with the exception of compound **8** (entry 7), which displayed a fair activity toward this isozyme (295 nM), and the other substituted phenol esters.

All of esters **9**–**27** were tested toward 5αR-1 and 2 expressed by CHO 1827 and CHO 1829, respectively, as already reported.[14b] They maintained activity toward 5αR-1, with IC_{50} values always below 1 μM (one exception only, entry 12), some of them being potent inhibitors of this isozyme. In particular, among the most potent were those having on the phenol moiety a small lipophilic group: compound **9**, bearing a *p*-methyl group on the phenol moiety, and compounds **11** (*p*-OMe substituted), **14** (*p*-F substituted), **17** (*p*-CF_3 substituted), and **22** (*p*-COOMe) all displayed inhibition toward 5αR-1 with IC_{50} in the 93–149 nM range. An increase in steric bulk (compare entries 8 and 10 to entries 9 and 11) determined an appreciable decrease of activity. As for the position of the substituent on the phenol moiety, a group in *meta* appears to be less tolerated: among the series of F-, CF_3-, and COOMe-substituted compounds (entries 13–18 and 21–22), *para*- and *ortho*-substituted derivatives were the most potent inhibitors. In particular for *o*-CF_3 derivative **19** (entry 18) the IC_{50} value was 42 nM, that is, close to the inhibition value of the most potent 5αR-1 inhibitor belonging to the benzo[*c*]quinolizinone series, that is, 8-Cl-benzo[*c*]quinolizin-3-one (IC_{50} = 7.6 nM).[14b] Substitution with a *p*-amino group (entries 19–20) lead to a general decrease of activity compared to the *p*-F or *p*-CF_3 substituted compounds. Also, positioning a *p*-amide group on the phenol moiety (entries 23–26) did not lead to better inhibitors and in one case (entry 26) inhibition toward 5αR-2 was quite low.

As for the 5αR-2 inhibition, this seems directly dependent on the size of the *para* substituent: *p*-*t*-butyl substituted compound **10** (entry 9) showed low activity; *p*-EtO substituted compound **12** (IC_{50} 3300 nM) was less

Table 1. Inhibition activity of compounds **2**–**31**

Entry	Compound	R_1	R_2	5αR-1[a] IC_{50} (nM)[c]	5αR-2[b] IC_{50} (nM)[c]
1	**2**	Me	—	117	2100
2	**3**	Et	—	221	4800
3	**4**	*i*-Pr	—	731	Low activity[d]
4	**5**	*t*-Bu	—	1000	Low activity
5	**6**	Bn	—	97	9500
6	**7**	C_3H_6OH	—	6990	Low activity
7	**8**	H	—	942	295
8	**9**	*p*-Me	—	102	553
9	**10**	*p*-(*t*-Bu)	—	560	Low activity
10	**11**	*p*-OMe	—	116	1200
11	**12**	*p*-OEt	—	209	3300
12	**13**	*p*-Cl	—	1400	Low activity
13	**14**	*p*-F	—	93	119
14	**15**	*m*-F	—	160	134
15	**16**	*o*-F	—	138	166
16	**17**	*p*-CF_3	—	149	1200
17	**18**	*m*-CF_3	—	271	1000
18	**19**	*o*-CF_3	—	42	368
19	**20**	*p*-NH_2	—	272	801
20	**21**	*p*-NHAc	—	707	255
21	**22**	*p*-CO_2Me		129	584
22	**23**	*m*-CO_2Me		965	467
23	**24**	*p*-$CONH_2$	—	361	308
24	**25**	*p*-CONHPr	—	268	665
25	**26**	*p*-$CONEt_2$	—	463	372
26	**27**	*p*-CONH-*t*-Bu	—	225	Low activity
27	**28**	H	*p*-Tol	1000	Low activity
28	**29**	Et	Et	Low activity	Low activity
29	**30**	*i*-Pr	*i*-Pr	Low activity	Low activity
30	**31**	H	*t*-Bu	Low activity	Low activity

[a] Isozyme expressed by CHO 1827 cells.
[b] Isozyme expressed by CHO 1829 cells.
[c] Error in the 8–20% range.
[d] % Inhibition <50% at 10 μM.

active than *p*-methoxy and *p*-CF_3 substituted compounds **11** and **17** (1200 nM), in turn less potent than *p*-CH_3 compound **9** (553 nM). Based on our initial considerations, we were pleased to find that the most potent inhibitor was again a compound with the F atom on the phenol moiety, that is, *p*-F substituted compound **14**, with an IC_{50} value of 119 nM. This was in particular the best dual inhibitor of the series, with IC_{50} values of about 100 nM for both isozyme. Also *meta*- and *ortho*-F-substituted derivatives **15** and **16** showed good activity toward 5αR-2. It is interesting the comparison with the corresponding CF_3 substituted derivatives **17**–**19**, in which the position of the substituent strongly affect the potency and in a opposite way than the F group (*o*-CF_3 compound **19** being the most potent of the series). As shown in Table 1, no other substitution on the phenol moiety of the ester group led to better results. Amides **28**–**31** (Table 1) were instead poor inhibitors of both isozyme, irrespective of the N substitution.

In conclusion, some potent dual inhibitors of 5α-reductase, with IC_{50} ranging between 93 and 166 nM for both isozymes, were found when the F atom was introduced on the phenol moiety of these esters. This result can help in the design of other potent, dual inhibitors to be developed as drugs in the treatment of 5α-reductase related diseases.

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