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General review

Selective non-steroidal inhibitors of 5α-reductase type 1

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Abstract

The enzyme 5α-reductase (5αR) catalyses the reduction of testosterone (T) into the more potent androgen dihydrotestosterone (DHT). The abnormal production of DHT is associated to pathologies of the main target organs of this hormone: the prostate and the skin. Benign prostatic hyperplasia (BPH), prostate cancer, acne, androgenetic alopecia in men, and hirsutism in women appear related to the DHT production. Two isozymes of 5α-reductase have been cloned, expressed and characterized (5αR-1 and 5αR-2). They share a poor homology, have different chromosomal localization, enzyme kinetic parameters, and tissue expression patterns. Since 5αR-1 and 5αR-2 are differently distributed in the androgen target organs, a different involvement of the two isozymes in the pathogenesis of prostate and skin disorders can be hypothesized. High interest has been paid to the synthesis of inhibitors of 5α-reductase for the treatment of DHT related pathologies, and the selective inhibition of any single isozyme represents a great challenge for medical and pharmaceutical research in order to have more specific drugs. At present, no 5αR-1 inhibitor is marketed for the treatment of 5αR-1 related pathologies but pharmaceutical research is very active in this field. This paper will review the major classes of 5αR inhibitors focusing in particular on non-steroidal inhibitors and on structural features that enhance the selectivity versus the type 1 isozyme. Biological tests to assess the inhibitory activity towards the two 5αR isozymes will be also discussed.

Keywords: 5α-Reductase; Steroid; Androgens; Inhibitors; Acne; Hirsutism; Alopecia

1. Introduction

The control of the biological action of a single steroid, through the inhibition of specific enzymes involved in its biosynthesis and metabolism without significant changes in the overall profile of the other hormones, has represented an attractive major pharmaceutical target during the last 20 years. The metabolic pathway of steroids is very complex and the possibility of modifying selectively any single point of the steroid metabolism without affecting the others represents a great challenge for medical and pharmaceutical research. In many cases weak precursor hormones are peripherally converted into more potent metabolites by specific enzymes in the target organs.

This is the case of testosterone (T) that is converted into the more potent androgen dihydrotestosterone (DHT) by the enzyme 5α-reductase (5αR). DHT is the androgen with the highest affinity for the androgen receptor and is primarily responsible for the effect of androgens in the end-organs.

Recently high interest has been paid to the synthesis of inhibitors of 5αR, since several androgen-dependent disorders and diseases such as benign prostatic hyperplasia (BPH), prostate cancer, acne, androgenetic alopecia in men, and hirsutism in women appear related to the DHT production [1–7].

1.1. The two 5α-reductase isozymes

Steroid 5α-reductase (EC 1.3.99.5) is a membrane bound, NADPH-dependent enzyme which catalyzes the selective, irreversible reduction of 4-ene-3-oxosteroids to the corresponding 5α-H 3-oxosteroids (Scheme 1). Two isozymes of 5α-reductase have been cloned, expressed and characterized (5αR-1 and 5αR-2) [8–10]. The homology between the two 5αR isozymes is poor (50% ca.) and they have different chromosomal localization, enzyme kinetic parameters, and tissue expression patterns. The most important substrate, testosterone, has an high affinity for the type 2 isozyme (Km = 4–50 nM) while the affinity for the type 1

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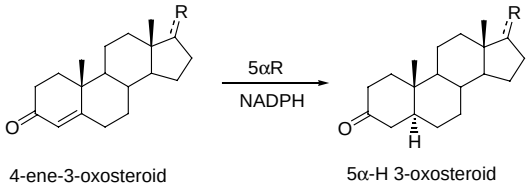


Scheme 1.

5αR is considerably lower (Km = 1–5 μM). The different response to inhibitors reported for the two 5αR isozymes suggests that sequence differences are localized also in the binding domains.

The tissue localization of 5αR-1 and 5αR-2 is at now a very debated question. At the beginning of 1990 decade, when the two isozymes were cloned, the prostate was supposed to express only 5αR-2 while 5αR-1 was found in skin and liver. Successively the increase of reliability and sensibility of the techniques employed to detect the proteins or their m-RNAs has allowed the individuation of both isozymes in almost all the target tissues with different relative ratios and a peculiar distribution within each target organ. Type 2 isozyme is found predominantly in the prostate, genital skin, seminal vesicles and in the dermal papilla; while type 1 isozyme occurs predominantly in non genital skin, in the scalp, in the sebaceous gland, in the liver and in the brain [11–16].

2. DHT-related pathologies

The physiologic role of T and DHT is quite different. In the embryo T stimulates the transformation of Woolfian ducts in epididymis, deferent ducts and seminal vesicles and activates the expression of 5αR with the subsequent production of DHT. The effect of DHT in the embryo is determinant for the sexual differentiation of the male foetus organs with formation of external genitalia, urethra and prostate. After the puberty T determines in males the modification of external genitalia, increases of muscle mass, deepening of voice, spermatogenesis, sexual potency, and male sexual behavior. The DHT formation is related in male puberty with the increase of body hair and facial hair and the enlargement of prostate.

The abnormal production of DHT is associated to pathologies of the main target organs of this hormone, the prostate and the skin. Since 5αR-1 and 5αR-2 are differently distributed in these organs, a different involvement of the two isozymes in the pathogenesis of prostate and skin disorders can be hypothesized.

Moreover other important information on the role of the two 5α–R isozymes in the pathogenesis of DHT-related disorders comes from the clinical evidences of 5αR-2 deficiency. Male pseudohermaphroditism, in which total or partial deficiency of 5αR-2 has been found, demonstrates that the type 2 isozyme is essential for differentiation of male external genitalia during foetal life [9]. In these individuals the prostate remains undeveloped, facial and body hair growth patterns are more feminine in character, and temporal regression of the hair line is significantly reduced, sebum production rate is instead unchanged respect to normal individuals indicating that 5αR-2 is involved in prostate diseases and to some extent in androgenetic alopecia but probably not in acne [17]. For this reason the development of isozyme specific inhibitors became an important pharmacological target for the treatment of DHT related pathologies.

2.1. Prostatic diseases

The main 5αR isozyme expressed in the prostate is 5αR-2. For this reason the first 5αR inhibitor developed, finasteride, that is specific for 5αR-2, found its main application in the treatment of benign prostatic hyperplasia. Finasteride 5 mg per die is able to reduce about 70% of the serum DHT concentration, to reduce the total gland size of 15–25% and to ameliorate the urodinamic symptoms of this pathology [18–20]. Due to the presence of small amount of 5αR-1 in the prostate gland the use of a dual inhibitor was supposed to have a better therapeutic effect. Recently a potent 5αR dual inhibitor, dutasteride, was marketed by Glaxo and the first report of its use in BPH treatment indicates a 90% reduction of serum DHT with a 25% gland size reduction, very similar to that obtained with finasteride [21]. This result demonstrates that the type 2 isozyme is predominantly involved in this prostate pathology. The role of the two 5αR isozymes in prostate cancer is instead not yet completely understood. Prostate cancer is an endocrine-responsive tumor and normal and aberrant prostate cellular function is governed chiefly by androgens. Long-term androgenic stimulation has been suggested as a cause of prostate cancer. From these facts the use of 5αR inhibitors in prostate cancer prevention can be proposed. Moreover the PSA dosage is an easy screening that allows an early detection of prostate cancer and this has increased the number of relatively young and fertile patients that need to be treated. A drug designed for prevention or treatment of young subjects in the early stage of the disease must have low toxicity in order to be clinically useful. 5αR-1 isozyme seems to be the only one expressed in human established prostate cancer cell lines and to predominate in prostate cancer tissue. These observations indicate the possibility of testing dual 5αR inhibitors or specific type 1 inhibitors in prostate cancer prevention or in the early treatment of the disease. The preventive action of finasteride in prostate cancer has been evaluated in a large clinical trial indicating that this drug prevents or delays the appearance of prostate cancer but increases the risk of developing an high grade tumor [22].

2.2. Skin-related diseases

The pilosebaceous unit (PSU), that is composed by the hair follicle and the sebaceous gland, can be considered a

skin component under endocrine control. The PSU development and function is androgen dependent and androgens are the key hormones involved in the main PSU disorders: androgenetic alopecia, hirsutism and acne [23]. Recently it has been established that the DHT production rate is higher in bald men than in non-bald ones but the in situ production of this hormone in the hair follicle can have a considerable weight [24]. The local production of DHT is dependent on the distribution of the two 5αR isozymes through the hair follicle, that is currently poorly understood: some authors have localized the type 2 isozyme in the inner root sheath and in the dermal papilla, while others have found the type 1 isozyme in the outer root sheath and in the dermal papilla. However the dermal papilla is considered the main site of androgen action. The binding of androgens to their receptor causes, in the dermal papilla, an alteration of the production of paracrine factors that act on other target cells such as keratinocytes. After puberty androgens promote transformation of vellus follicles to terminal ones, producing larger and pigmented hairs, in ambosexual areas e.g. axilla and pubis. Androgens have no effect in other areas like the eyelashes but cause the opposite transformation from terminal to vellus hairs in balding prone areas. This paradoxical behavior appears to be an unique hormonal effect [25]. The treatment of balding men with finasteride has been shown to be effective producing visible hair growth in up to 66% of subjects with mild to moderate alopecia and stopping hair loss [26]. This clinical data highlight the involvement of 5αR-2 in this pathology. However the effect of inhibiting also the type 1 isozyme is still unknown and the use of a specific type 1 inhibitor could be interesting and likely at low risk of side effects. Different considerations can be made for hirsutism: hirsute women are generally treated with antiandrogens, finasteride has been used with results sometimes comparable with antiandrogens indicating the involvement of 5αR-2 also in this pathology [27]. However contraception is mandatory during the treatment of fertile women with these drugs to avoid the risk of feminilization of the male foetus. For this reason the use of pure 5αR-1 inhibitors could be a suitable alternative if they are proven to be efficacious and without teratogenic effects. In female mice with an induced null mutation of the 5αR-1 gene the oestrus cycle and the early stages of pregnancy are normal [28], and these data support the hypothesis that specific 5αR-1 inhibitors could not have serious side effects in fertile women.

As regard the second component of the pilosebaceous unit, the sebaceous gland, literature data are more concordant indicating 5αR-1 as the only expressed isozyme [13,29]. In acne prone areas androgens cause the prepubertal vellus follicle to develop into a sebaceous follicle in which the hair remains vellus and the sebaceous gland enlarges tremendously. DHT is responsible for sebum hyperproduction and 5αR-1 specific inhibitors could be the elective drugs for the treatment of this pathology that need long-lasting treatment and, since patients are generally young people, low risk of side effects.

At present, no 5αR-1 inhibitor is marketed for the treatment of 5αR-1 related pathologies but pharmaceutical research is very active in this field. This paper will review the major classes of 5αR inhibitors focusing in particular on non-steroidal inhibitors and on structural features that enhance the selectivity versus the type 1 isozyme.

3. 5αR inhibitors

3.1. Steroidal inhibitors

An important point that affects the discovery of novel inhibitors is the absence of information on the active site structure of the two 5αR isozymes. Nowadays, they are not yet isolated and purified from tissues or cells, and the only information available is the primary sequence estimated from their c-DNAs.

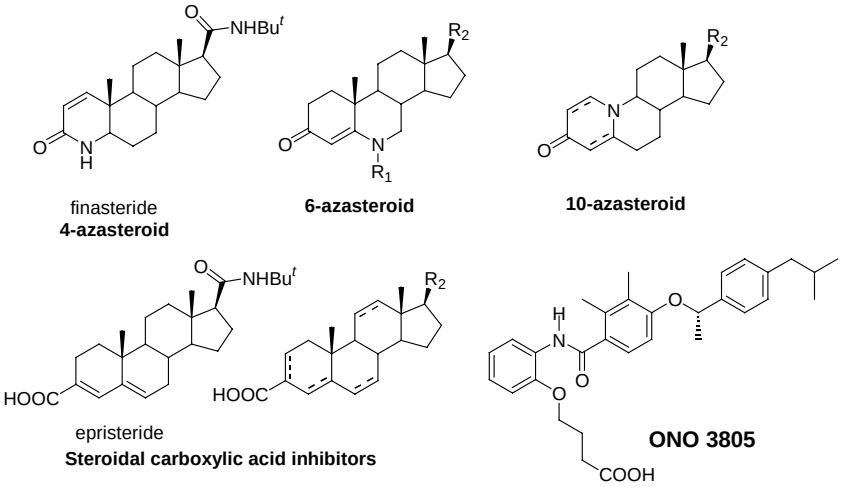
The first inhibitors have been therefore designed by modifying the structure of natural substrates, including the substitution of one carbon atom of the A or B ring of the steroids by an heteroatom, leading to the discovery of potent inhibitors of human 5α-reductase such as 4-azasteroids (among which finasteride, marketed for the treatment of BPH) [30–32], 6-azasteroids [33,34], 10-azasteroids [35–39], and steroidal carboxylic acid inhibitors [40–43] (Scheme 2). The kinetic mechanism by which T is reduced to DHT is believed to proceed via a preferentially ordered binding of the substrate and release of DHT from the enzyme.

The proposed mechanism of T reduction to DHT by 5αR catalysis (Scheme 3), based on the known regio and stereochemistry of the reduction, involves the formation of a binary complex between the enzyme and NADPH, followed by the formation of a ternary complex with the substrate T.

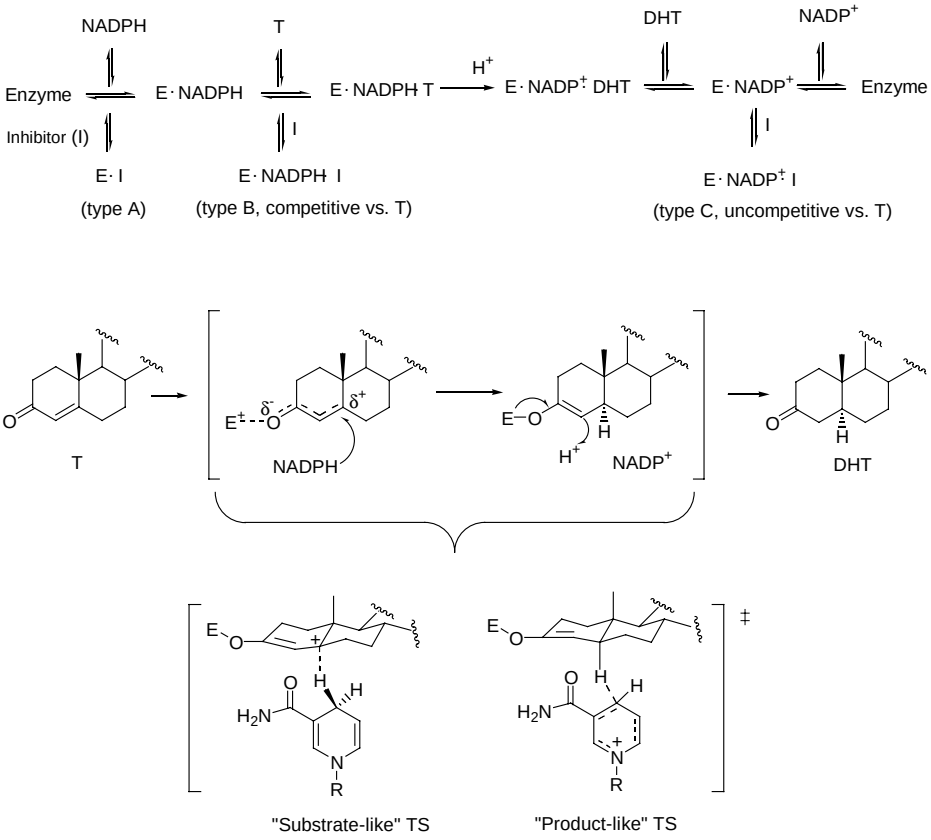
Once this complex is formed the activation of the enone system by a strong interaction with an electrophilic residue (E^+ = proton, positively charged group, proton donor) present in the active site gives the delocalized carbocation which is reduced selectively at C-5, on the α face, by a direct hydride transfer from NADPH, leading to the formation of the enolate of DHT. This intermediate, which is presumably coordinated with $NADP^+$ on the α face, is attacked by a proton on the β-face at C-4 giving the ternary complex E–$NADP^+$–DHT. Then, the departure of DHT gives the binary $NADP^+$–enzyme complex, and finally the release of $NADP^+$ leaves the enzyme free for further catalytic cycles.

Accordingly to the kinetic mechanism of testosterone reduction three different type of inhibitors could be conceived: inhibitors competitive with the cofactor (NADPH) and the substrate (T) (type A) should interact with the free enzyme; inhibitors competitive with the substrate (type B), should fit the enzyme–NADPH complex, and inhibitors fitting the enzyme–$NADP^+$ complex, should be uncompetitive versus the substrate (type C).

The concept of transition state analogue states that the enzyme binding, and so inhibition, should be greater



Scheme 2.



Scheme 3.

for molecules being mimics of the transition state of the enzymatic process. Therefore, considering the substrate-competitive inhibitors (type B), two possible transition states (TS) have been postulated [35] (Scheme 3): the 'substrate-like' TS in which the C-5 has not yet changed its sp^2 hybridization where the structures of C-3, C-4 and C-5 are similar to those of the intermediate carbocation, and the 'product-like' TS, in which the C-5 has assumed the final sp^3 hybridization and the structures of the C-3, C-4, and C-5 are similar to those of the enol form of DHT. Thus, 4-azasteroids (Scheme 2) which are competitive versus T act as 'product like' TS analogues being similar to the DHT enol [44].[1] 6-Azasteroids and 10-azasteroids, which have

[1] Recent work by both Glaxo and Merck indicates that finasteride and other related Δ 1,2 4-azasteroids like, behave essentially as irreversible inhibitors caused by the alkylation by $NADP^+$ of the enolate formed after 1,4 reduction of the Δ 1 A-ring of finasteride. The resulting

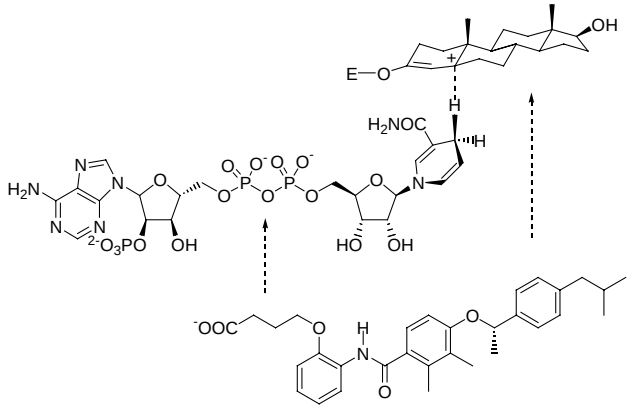
a sp^2 C-5 atom are competitive inhibitors which can be thought as 'substrate-like' TS analogue.

The androstenecarboxylic acids are designed as mimics of the enolate intermediate where the anionic carboxylate ion serves as a mimic for the enolate oxygen in binding to the active site. These charged carboxylates display uncompetitive kinetics and bind to the E–$NADP^+$ complex. This type of inhibitors could have advantages in vivo, as an uncompetitive inhibitor cannot be directly displaced by an increased substrate concentration. Epristeride (Scheme 2) is one of the more studied compounds of this class.

Concerning the competitive inhibitors, the C-5 hybridization has no effect on the selectivity versus one of the two 5αR isozymes and, in general, a high nucleophilicity of the C-3 substituent is required in the A ring structure for an effective inhibitory activity versus both the isozymes. This indicates that the part of the enzymatic cavity that interacts with the A ring of the steroid and is responsible for the molecular recognition of substrates or inhibitors is similar for both the isozymes, and the two isozymes are supposed to have the same mechanism of reduction.

The modulation of both activity and selectivity, towards the 5αR-1 isozyme has been realized changing the substituent at the position 17. This part of the steroid is supposed to interact with a flexible hydrophobic pocket that is different in the two isozymes. In 4-azasteroids it is the increase of lipophilicity of the 17-substituent that addresses selectivity toward 5αR-1. Compound MK386 (not shown), with the cholesterol side chain at position 17 has been the first 5αR-1 inhibitor reported in literature (although abandoned for its epatotoxicity). Moreover, the introduction of a small lipophilic group at position 7 of the B ring caused an increase of potency and selectivity towards 5αR-1 [45].

Still concerning the mechanism of action, a bi-substrate inhibitor (type A) could have some additional advantages either with respect to an inhibitor competitive versus substrate (because further positive stabilizing interactions could be developed within the cofactor sub-site), or with respect to an inhibitor competitive versus NADPH (because it could be more selective towards other enzymes NADPH-dependent). To this class of inhibitors belong some non-steroidal compounds such as ONO 3805 (Scheme 2), an early lead originally prepared as a leukotriene inhibitor [46–48], in which the butanoic acid moiety is thought to be localised in the region of the phosphate group of NADPH and the lipophilic part could be orientated in the region of the steroidal C and D ring, thus occupying the hydrophobic pocket of the enzyme (Scheme 4). The fact that this compound acts as non-competitive inhibitor (versus T) and not as uncompetitive one, supports this hypothesis.



Scheme 4.

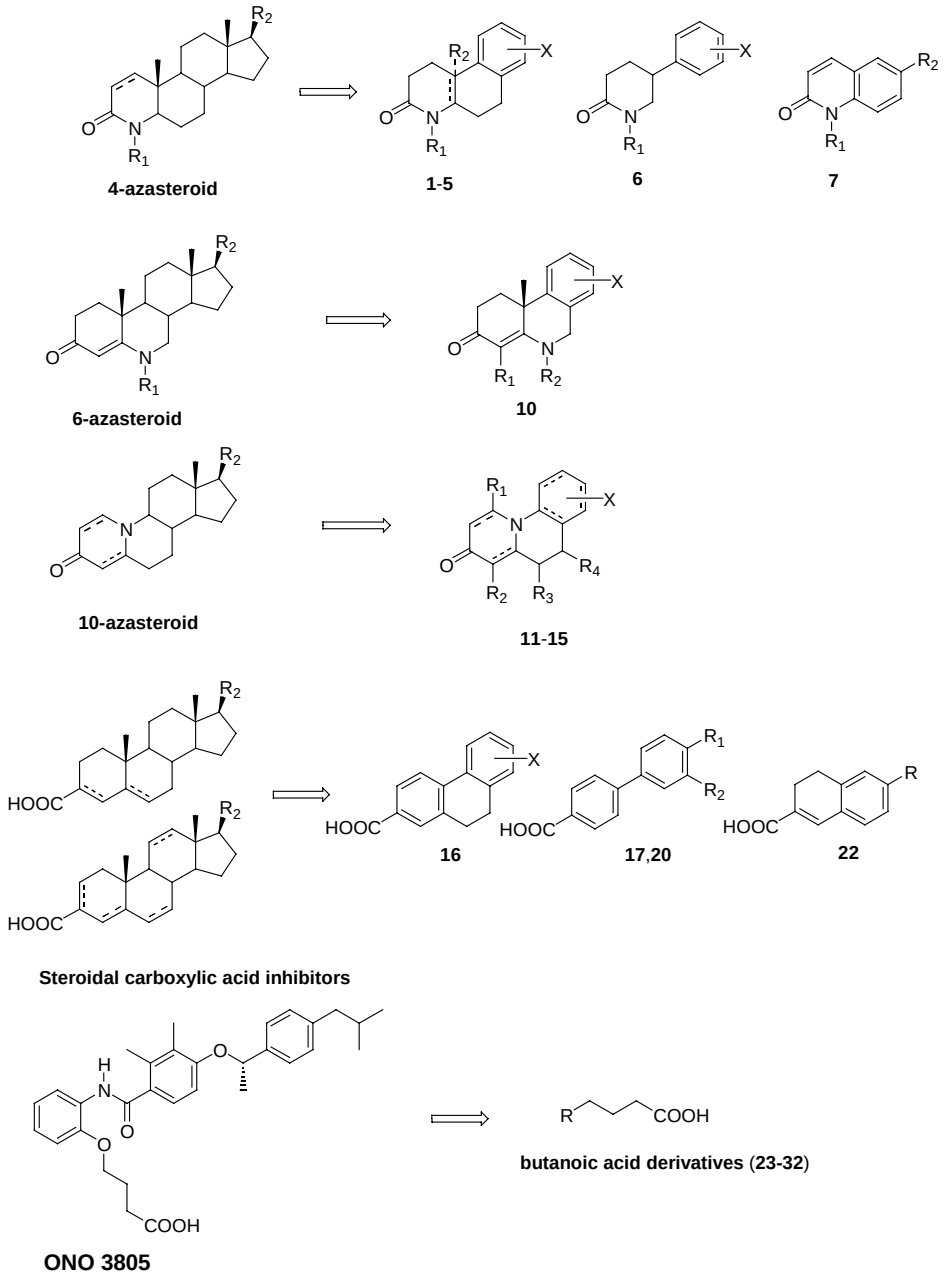
3.2. Non-steroidal inhibitors

Due to the potential undesired hormonal action exhibited by steroidal compounds, the research toward the discovery of non-steroidal inhibitors has gained great importance in the last years, and several pharmaceutical and academic groups have pursued the discovery of non-steroidal compounds that inhibit human 5α-reductases. Non-steroidal inhibitors reported so far can be classified according to their structure. They have in fact emerged from the design of compounds mimic of (aza)steroidal inhibitors, generally by the formal removing of one or more rings from the (aza)steroidal structure. These compounds are generally thought to act all as competitive inhibitors vs. testosterone with exception of epristeride analogues which are uncompetitive inhibitors. They include benzo[f]quinolinones, pyridones and quinolinones mimics of 4-azasteroid inhibitors, benzo[c]quinolinones mimics of 6-azasteroids, and benzo[c]quinolizinones mimics of 10-azasteroids (Scheme 5). The most potent and selective inhibitors of human 5αR-1 are found among these classes of compounds. Almost all the other non-steroidal inhibitors can be grouped as carboxylic acid (generally butanoic acid) derivatives which are thought to act as non-competitive inhibitors versus testosterone in analogy to ONO 3805.

3.3. Benzo[f]quinolinones

The first non-steroidal inhibitors mimicking steroid inhibitors were prepared by Lilly's researchers, who published a series of benzo[f]quinolinones (Table 1) formally derived from the removal of the D ring from 4-azasteroids and the substitution of the C ring with an aromatic one [49]. Almost all of these compounds are type 1 selective, although dual inhibitors can be obtained if an appropriate substitution is present at the position 8 on the aromatic ring. Two main classes of benzo[f]quinolinones have been described,

enzyme–NADP–dihydrofinasteride complex is extremely stable and, having an apparent dissociation constant $K_i \leq 1 \times 10^{-13}$ M, ranks among the most potent non covalently-bound inhibitors known for any enzyme. This explains the exceptional potency and selectivity of finasteride which is converted in vitro and in vivo to the more potent NADP-dihydrofinasteride adduct. The exceptional affinity is due to the action of this complex as a potent bi-substrate inhibitor having a structure fitting with both the cavity sites of the enzyme usually occupied by the substrate and the cofactor.

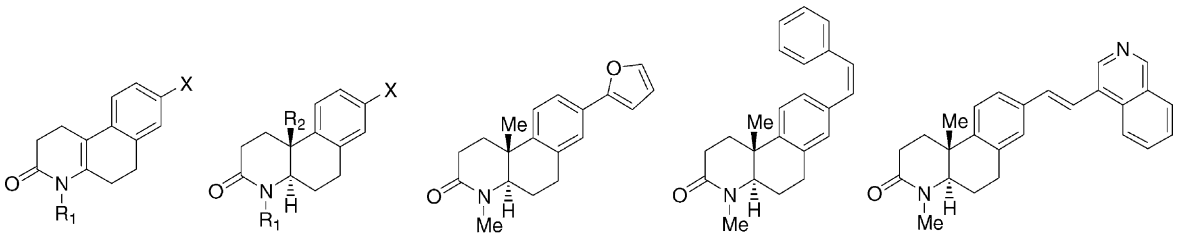


Scheme 5.

the hexahydro derivative **1**, which have an unsaturation at potition 4a–10a, and the octahydro derivatives **2**. A thorough exploration of the effect of various substituents on the different positions of the benzo[f]quinolinone ring has not been reported for these compounds, with exceptions for position 4 (N substituent), 8 (on the aromatic ring), and 10a. In general octahydro derivatives **2** are more potent inhibitors than the corresponding 4a–10a unsaturated compounds **1**, and in both series the potency toward 5αR-1 increases if an halogen atom is present at position 8 (in particular a Cl atom) and a methyl group at position 4; in fact the most potent inhibitor of the series is compound **2d** (LY191704) with $IC_{50} = 8$ nM. The increase of potency associated to the presence of a 4-methyl group is in analogy with the corresponding 4-methyl substitution in the steroid 5αR-1 inhibitor MK-386 by Merck. In this 4-azasteroid a methyl at position 7 also appears to increase the potency toward 5αR-1, but the corresponding 6-substitution in benzo[f]quinolinones has not been yet reported.

It is worth also to note the deleterious effect on the inhibition potency of the angular methyl group in compound **2b** ($IC_{50} = 1800$ nM) compared to **2a** ($IC_{50} = 60$ nM). The potency is restored with the 4-Me group in **2c** ($IC_{50} = 17$ nM). A QSAR study on these compounds has focused on the effect of the 8-substituent on the aromatic ring, which can be accounted for by its lipophilic character [50]. Since the

Table 1
Benzo[f]quinolinones mimics of 4-azasteroid inhibitors



1a-e **2a-i** **3** **4** **5**

Number	R_1	X	IC_{50} (nM) (5αR-1)	IC_{50} (nM) (5αR-2)	Number	R_1	R_2	X	IC_{50} (nM) (5αR-1)[a]	IC_{50} (nM) (5αR-2)[b]
1a	H	H	6500	–	**2a**	H	H	Cl	60	
1b	H	F	600	–	**2b**	H	Me	Cl	1800	
1c	H	Cl	460	–	(±)-**2c**	Me	Me	Cl	17	
1d	Me	Cl	30	–	(+)-**2c**	Me	Me	Cl	9 (K_i)	>1000
1e	Me	Br	60	–	(−)-**2c**	Me	Me	Cl	10 (K_i)	>1000
					(±)-**2d**	Me	H	Cl	8	10000
					(+)-**2d**	Me	H	Cl	6 (K_i)	1000
					(−)-**2d**	Me	H	Cl	4 (K_i)	1200
					2e	Me	H	Me	11	–
					2f	Me	H	Br	35	–
					2g	Me	H	F	35	–
					2h	Me	H	MeO	120	–
					2i	Me	H	H	560	
					3				59[c]	>10
					4				6[c]	1400
					5				6[c]	1340

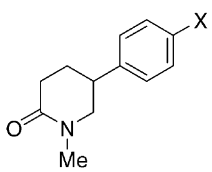
[a] 5αR-1 in cultured Hs68 human foreskin fibrobrast cells.
[b] 5αR-2 from human prostate homogenates.
[c] Native 5αR-1 in nuclear membrane preparation from human scalp.

authors of this study were unable to define a relationship between the potency and the calculated octanol–water partition coefficient (clogP, describing the lipophilic property of the whole molecule), they postulated that the substituent in position 8 interacts with a local lipophilic pocket in the enzyme. From the screening of 100 substituent using the appropriate QSAR equation they found that the optimum activity may reside in the property space around the chlorine substituent. The substitution of the 8-Cl atom by a F (compound **2g**) or a Br atom (compound **2f**) decreased very slightly the potency (IC_{50} about 35 nM for both compounds). Besides this relationship between potency and substituent lipophilicity, that involves a localized feature of the structure around the substituent on the aromatic ring, the authors also found a relationship between potency and energy of the HOMO orbital (E_{homo}), this being now a global molecular feature. In particular, they found that all the considered compounds in the more active series **2** have a higher E_{homo} than the corresponding 4a–10a-unsaturated compounds of series **1**, but they do not give a clear explanation of the reasons behind this relationship. However, the most important study that has been carried out on benzo[f]quinolinones **1** and **2** involved the separation of the enantiomers of compounds **2c** and **2d**, among others, and the subsequent inhibition potency evaluation of the single enantiomers [51]. As reported in Table 1, the two enantiomers of both **2c** and **2d** have practically the same K_i values (9 and 10 nM for the enantiomers of **2c**, and 6 and 4 nM for those of **2d**). This important result has been explained, after a complete conformational analysis, on the basis of the extended planar structure of these 4a–10a *trans*-fused compounds, which permits an excellent overlay of each enantiomeric pair.

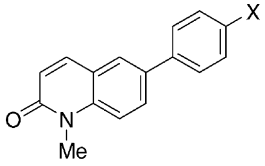
In the corresponding 4a–10a *cis*-fused compounds (not reported in Table 1) the disruption of the planarity can be tolerated without the loss of inhibitory activity as long as the angular 10a position is unsubstituted (IC_{50} = 15 nM for both the enantiomers) but the bowl shaped structure that derives from the *cis*-fusion dramatically reduces type 1 potency if the angular methyl is present at the position 10a (IC_{50} = 4000 and 7000 nM for the two enantiomers).

Finally, several kinds of substituents, including complex aromatic groups, were introduced at the position 8 [52], with a modulation of the selectivity towards the two 5αR isozymes. In fact some potent dual inhibitors have been discovered, but also some potent 5αR-1 selective inhibitors such as compounds **3**, **4**, and **5** (Table 1). It is interesting to notice the difference in selectivity between compound **4**, with a *cis* 8-styryl group, a potent and selective type 1 inhibitor (Table 1) and the corresponding *trans* 8-styryl group (not shown in Table 1) which is instead a dual inhibitor

Table 2
Piperidones and quinolinones mimics of 4-azasteroid inhibitors



6a-b



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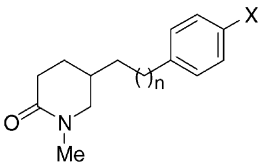

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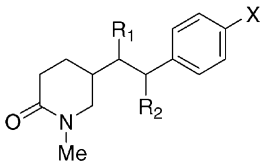


9

Number	X	IC_{50} (nM) (5αR-1)[a]	IC_{50} (nM) (5αR-2)[b] or percent inhibition
6a	H	2477	13.5% at 40 μM
6b	Cl	1690	12350
7	$CON(i\text{-}Pr)_2$	510[c]	9% at 10 μM

[a] 5αR-1 isolated from human scalp.
[b] 5αR-2 from human prostate tissues.
[c] Human 5αR-1 expressed in DU145 cells.

with IC_{50} = 23 nM towards 5αR-1 and IC_{50} = 180 nM towards 5αR-2 [52]. Of all compounds belonging to the benzo[f]quinolinone series, LY191704 (**2d**) has been progressed to human clinical trials.

3.4. Piperidones, quinolinones, and pyridones

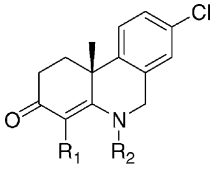
While the formal removal of the D ring from 4-azasteroids resulted in an increased selectivity (and potency) towards 5αR-1, removal of two or more rings resulted in a strong decrease of potency. For example, compounds **6a**–**b** (Table 2), lacking the B and D steroidal rings, were only poor 5αR-1 inhibitors, with the highest potency associated to the presence of the Cl atom on the aromatic ring of **6b** (IC_{50} = 1690 nM) [53]. Similarly, pyridones **8** and **9** (n = 1,2; X = $CON(i\text{-}Pr)_2$; R_1, R_2 = H,Me) where the B and C rings of the steroid system have been replaced by an acyclic linker display relatively weak activity versus both 5α–R isozymes [54–56]. Removal of the C ring led to decrease of potency, too, although the appropriate substituent in **7** afforded a selective, but still weak 5αR-1 inhibitor (Table 2) [57].

Finally it is worth to mention that attempts at modifying the A ring structure of Eli Lilly compounds, by removing or adding a methylene group, resulted in loss of inhibitory potency and the research in this field has been abandoned [58].

3.5. Benzo[c]quinolinones

The series of tricyclic compounds reported in Table 3 and formally derived from the parent 6-azasteroidal Glaxo compounds, are weak, although selective, 5αR-1 inhibitors [59]. Although structurally close to Eli Lilly inhibitors **2**, and with a Cl atom on the aromatic C ring, these compounds failed to show a promising inhibitory activity. The increase of potency from compound **10a** to **10b** and **10c** is due to the methyl group on the A ring corresponding to the 4-Me of Eli Lilly inhibitors. The same effect, due to a methyl group at position 4, will be found also in benzo[c]quinolizinones (see below), formally derived from 10-azasteroids. This effect can be accounted for by the presence in the active site of the enzyme of a hydrophobic pocket able to accommodate a small alkyl group located at the position 4 of the A ring of all these tricyclic inhibitors.

Table 3
Phenanthridin-3-ones mimics of 6-azasteroid inhibitors



10a-c

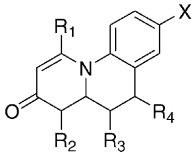
Number	R_1	R_2	$K_{i,app}$ (nM) (5αR-1)[a]	Percent inhibition (5αR-2)[b]
10a	H	Me	≫10000	42 at 30 μM
10b	Me	Me	1100	57 at 29 μM
10c	Me	H	920	49 at 20 μM

[a] Recombinant human type 1 5α-reductase.
[b] Recombinant human type 2 5α-reductase.

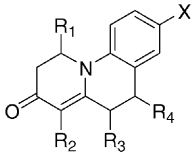
3.6. Benzo[c]quinolizinones

Benzo[c]quinolizinones are the last class of non-steroidal inhibitors derived from azasteroids that appeared in literature and some very potent, type 1 selective inhibitors can be found among them (Tables 4 and 5) [60–62]. This novel class of compounds has a reversible mechanism of inhibition and the efficacy and selectivity of the compounds have been demonstrated on recombinant human 5αR-1 and 5αR-2 expressed in CHO cells and on the native isozymes contained in human scalp and prostate [60]. Two major series of benzo[c]quinolizin-3-ones have been described: the 4a*H*-series (compound **11a**–**j**), with a double bond between the positions 1 and 2, and 1*H*-series (compound **12a**–**m**), with the double bond between the positions 4 and 4a. 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: as we have already observed for the 10-azasteroids [35]. Removing, as an example, the double bond from compound **12f** (IC_{50} = 7.6 nM) lead to a molecule (not shown in Table 4) with a marked decrease of the inhibition activity (IC_{50} value of 478 nM toward the 5αR-1).

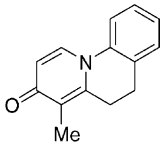
Table 4
Benzo[c]quinolozin-3-ones mimics of 10-azasteroid inhibitors



11a-j



12a-m



13

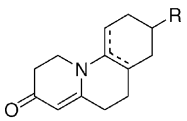
Number	R_1	R_2	R_3	R_4	X	IC_{50} (nM)[a]	Number	R_1	R_2	R_3	R_4	X	IC_{50} (nM)[a]
11a	H	H	H	H	H	5130 ± 130	**12a**	H	H	H	H	H	298 ± 75
11b	H	H	H	H	Me	176 ± 17	**12b**	H	H	H	H	Me	376 ± 185
11c	H	H	H	H	Cl	459 ± 118	**12c**	H	H	H	H	Cl	49 ± 19
11d	H	Me (α)	H	H	H	2700 ± 300	**12d**	H	Me	H	H	H	185 ± 62
11e	H	Me (β)	H	H	H	4300 ± 400	**12e**	H	Me	H	H	Me	20 ± 8 5.8 ± 1.8[b]
11f	H	Me (α)	H	H	Me	137 ± 58	**12f**	H	Me	H	H	Cl	7.6 ± 0.9 2.7 ± 0.6[b]
11g	H	Me (β)	H	H	Me	312 ± 23	**12g**	H	H	Me	H	Cl	346 ± 185
11h	H	Me (β)	H	H	Cl	141 ± 24	**12h**	H	H	H	Me	Me	14.3 ± 5.9
11I	H	H	Me (α)	H	Cl	9100 ± 500	**12i**	H	H	H	Me	Cl	14.4 ± 3.4
11j	H	H	H	Me (α)	Cl	188 ± 42	**12j**	Me	H	H	H	Cl	204 ± 49
							12k	H	Me	Me	H	Cl	15.6 ± 4.0
							12l	H	Me	H	Me	Me	15.8 ± 4.6
							12m	H	Me	H	Me	Cl	8.5 ± 2.1
							13						1400 ± 200

[a] Recombinant human 5αR-1 expressed in CHO 1827 cells.
[b] This is a K_i.

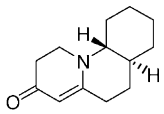
In general, the compounds of the 1*H*-series resulted significantly more active than those of the 4a*H*-series, the IC_{50} values of the latter being approximately 10-fold higher. The inhibition values ranged from 137 to 9100 nM for compounds **11a–j** and from 7.6 to 376 nM for compounds **12a–m**.

An exploration of the effects of substituents on the different positions of the tricyclic system, has been carried out on this class of inhibitors using a methyl group in positions 1, 4, 5, 6, and 8. In analogy with Eli Lilly benzo[f]quinolinones a Cl atom was also used in position 8. A graphical representation of the SAR for the most active series **12a–m** is reported in Fig. 1.

Table 5
Benzo[c]quinolozin-3-ones mimics of 10-azasteroid inhibitors



14



15

Number	R	IC_{50} (nM) 5αR-1[a]	IC_{50} (nM) 5αR-2[b]
14[c]	H	58 ± 15	No inhibition
15		20000 ± 400	No inhibition

[a] Determined with human 5αR-1 expressed in CHO 1827 cells.
[b] Determined with human 5αR-2 expressed CHO 1829 cells.
[c] Mixture of $\Delta^{6a(10a)}/\Delta^{10(10a)}$ isomers in 10:1 ratio.

Position 8. The presence of a substituent at position 8, generally increases the potency of the inhibitors in both series, in analogy to the previous two classes of tricyclic inhibitors, derived from 4- and 6-azsteroids, seen so far. In the 1*H*-series the chlorine atom increases noticeably the potency toward 5αR-1, either alone or in presence of other substituents on the two aliphatic rings. Thus, 8-Cl substituted compounds **12c** (IC_{50} = 49 nM) and **12f** (IC_{50} = 7.6 nM) were significantly more active than unsubstituted compound **12a** (IC_{50} = 298 nM) and **12d** (IC_{50} = 185 nM), respectively. Instead, the methyl group at position 8 of compounds

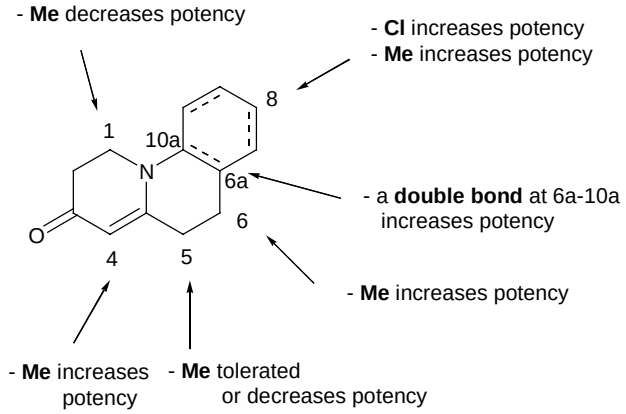


Fig. 1. Structure–activity relationships for benzo[c]quinolizinones.

of 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 **12b** (IC_{50} = 376 nM) is approximately as potent as the unsubstituted compound **12a** (IC_{50} = 298 nM), while the modification of compound **12d** (IC_{50} = 185 nM) by introducing a methyl group at position 8 leads to the more potent compound **12e** having an IC_{50} of 20 nM.

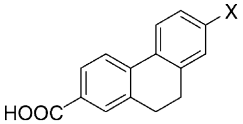
Position 4. The introduction of a methyl group at position 4 in both series gives rise to an increase of the inhibitory potency, again in analogy to the effects observed on benzo[f]quinolinones **2** and phenanthridin-3-one derivatives **10**. The extent of this increase is highest in the 1*H*-series, especially when the 8-position is substituted with a chlorine or methyl. Thus, whereas 4-methyl derivative **12d** displayed an inhibition activity (IC_{50} = 185 nM) only moderately higher than the unsubstituted compound **12a** (IC_{50} = 298 nM), a very strong increase of potency is observed comparing the 8-Cl substituted compound **12c** (IC_{50} = 49 nM) and the 8-Me substituted compound **12b** (IC_{50} = 376 nM) with the corresponding 8-chloro-4-methyl derivative **12f** (IC_{50} = 7.6 nM) and 4,8-dimethyl derivative **12e** (IC_{50} = 20 nM), respectively.

Position 6. The substitution with a methyl group at position 6 positively affected the potency of the inhibitors, although more markedly in the 1*H*- than in the 4a*H*-series. So compound **12h** (IC_{50} = 14.3 nM) and **12i** (IC_{50} = 14.4 nM) were significantly more active than the corresponding compounds **12b** and **12c**, not substituted at position **6**. The further substitution with a methyl group at position 4 in trisubstituted compounds **12l** (IC_{50} = 15.8 nM) and **12m** (IC_{50} = 8.5 nM) maintained the inhibitory activity. This 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 [63].

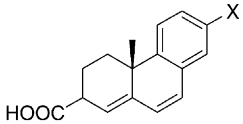
Position 5. By contrast, the presence of a methyl group at position 5 in general reduced the potency (unless it is associated to another methyl group at the position 4) an effect which has already been seen with the analogous substitution on tricyclic inhibitors derived from 6-azasteroids **10**.

Position 1. Finally, the introduction of a methyl at the position 1 in 8-Cl substituted compound **12j** (IC_{50} = 204 nM) decreased the activity toward 5αR-1 in comparison with the homologous derivative **12c** (IC_{50} = 49 nM). In analogy to Eli Lilly compounds **2**, the higher inhibitory activity toward 5αR-1 displayed by benzo[c]quinolizin-3-ones **12a**–**m** respect to the inhibitors of the 4a*H*-series **11a**–**j** has been associated to their more extended planarity [60]. However, very recent results indicate that the molecular planarity is not the only feature to take into account in the evaluation of the inhibition data. As shown in Table 5, a benzo[c]quinolizin-3-one inhibitor lacking the aromatic C ring but with a double bond at 6a–10a (compound **14**) displayed an inhibitory potency 345-fold higher than that of the corresponding 6a–10a saturated, *trans*-fused compound **15** [64]. A molecular modeling study showed that there is not a significant difference between the two compounds in terms of overall extended planarity, thus this enormous difference in the inhibitory activity of compounds **14** and **15** must be ascribed to some important interaction between the 6a–10a double bond (and for extension, the aromatic ring in compounds **11** and **12**) with an aromatic amino acid side chain in the active site of the enzyme. The recent report that the sequence AVFA (residue 26-29) in 5αR-1, containing an aromatic residue, is involved in inhibitor/substrate binding is in accordance with this hypothesis [65].

Table 6
Tricyclic aryl acids mimics of steroid carboxylic acid inhibitors



16a-c



16d

Number	X	$K_{i,app}$ (nM) (5αR-1)[a]	$K_{i,app}$ (nM) (5αR-2)[b]
16a	H	315	>10000
16b	Cl	320	2500
16c	Br	26	10000
16d	Cl	1200	260

[a] Recombinant human type 1 5α-reductase.
[b] Recombinant human type 2 5α-reductase.

3.7. Non-steroidal aryl acids

The tricyclic non-steroidal aryl acids **16a**–**c** (Table 6), formally obtained by removal of the D ring from androstenecarboxylic acid inhibitors are, on the contrary of their parent steroid compounds, selective 5αR-1 inhibitors [66]. In contrast to the general observation in the other classes of tricyclic non-steroidal inhibitors the introduction of a chlorine atom at the position 7 of the aromatic ring in compound **16b** (IC_{50} = 320 nM) does not increase the potency respect to the unsubstituted compound **16a** (IC_{50} = 315 nM). This effect is obtained with a bromine atom in compound **16c** (IC_{50} = 26 nM). The different mechanism of action of these inhibitors, which are supposed to interact with the positively charged Enzyme–$NADP^+$ complex in a uncompetitive manner versus testosterone could be invoked to explain this experimental data. Moreover, it is interesting to notice that the introduction of a double bond on the B ring as in compound **16d** (formally obtained by removing the D ring from epristeride) the selectivity toward 5αR-1 is lost in favor of a increased potency toward 5αR-2 [66].

Several others aryl acids mimics of steroidal carboxylic acids have been reported (Fig. 2), which lack two or more rings compared to the parent steroid compounds [67–69]. However none of them can really be considered a true potent and selective human 5αR-1 inhibitor either because they do not display high inhibitory activity toward the human enzyme (such as compounds **22** [67], with best IC_{50} values

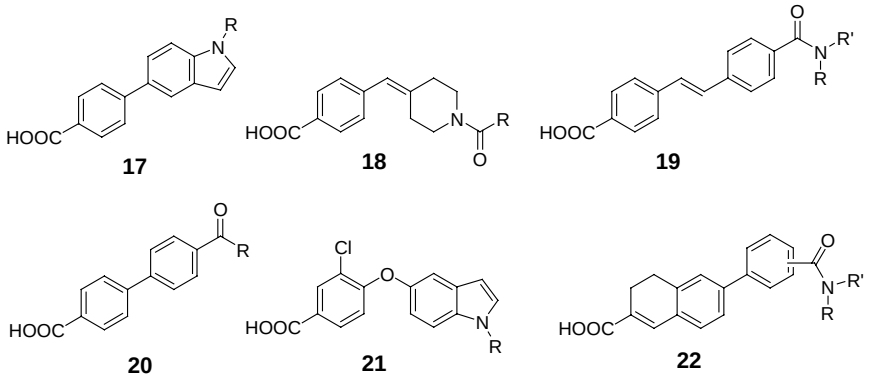


Fig. 2. Non-steroidal aryl acids.

of 680 nM) or selectivity toward the type 1 (such as compound **17** [68], with an $IC_{50} = 2.1\,\mu M$ toward 5αR-1 but also a 50% inhibition toward 5αR-2 at 10 μM) or because they were tested only against rat type 1 isozyme (from rat ventral prostate), such as compound **21** [69].

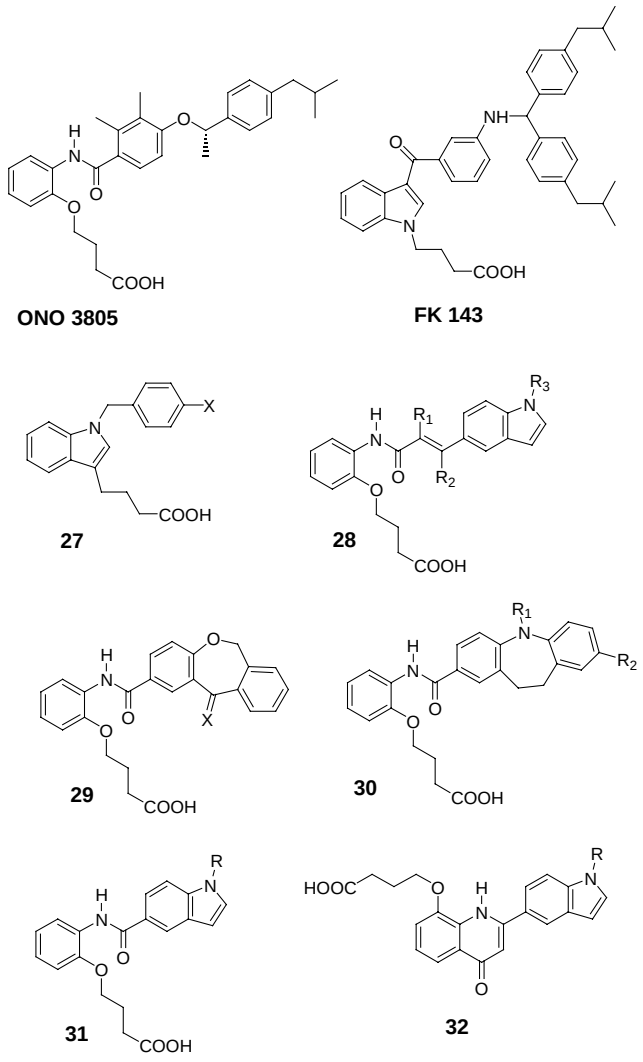


Fig. 3. Non-steroidal bi-substrate inhibitors.

3.8. Bi-substrate inhibitors

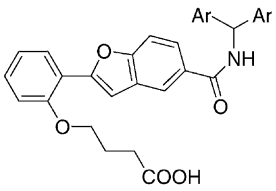
Non-steroidal inhibitors analogues of **ONO 3805** (Fig. 3) have furnished only a few true selective inhibitors of human type 1 isozyme (Table 7 and Fig. 3). All these compounds have in common an aromatic ring (generally a benzene or an indole) which bears the butanoic acid chain and, generally at the ortho position respect to this chain, the most different aromatic moieties substituted with lipophilic groups (see Fig. 3). The most selective, although not very potent, type 1 inhibitor, among the plethora of compounds reported so far (most of them, moreover, tested only versus rat enzymes) is compound **23** with $IC_{50} = 310$ nM towards type 1 isozyme and >100,000 towards type 2 [70,71]. Also compounds **24a–c** displayed some selectivity toward human 5αR-1, although the best structural combination is that of compound **26** from Pfizer [6,72] in which the methyl group on the indole ring forces the molecule to adopt only the '*trans*' conformation. The high potency and selectivity of this compound versus human 5αR-1 ($IC_{50} = 10$ nM toward 5αR-1, $IC_{50} = 6300$ nM toward 5αR-2) is thought to be associated to its particular conformation.

4. Biological tests

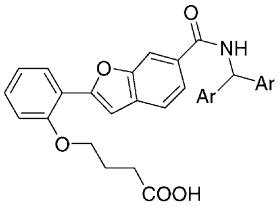
In the evaluation of a structure–activity relationship, the biological test that allows the determination of the activity is of paramount importance, since any assumption on the favorite molecular structure for the inhibition of an enzyme depends on the reliability and reproducibility of the biological test. This aspect, often undervalued, is probably the main source of errors in SAR based prediction of activity.

Biological tests which assess the activity of inhibitors versus the human 5αR system can be performed on the native isozymes (tissue homogenates, human cell lines) or on the recombinant isozymes (transfected cells). The use of tissue homogenates is complicated by the presence, in tissue preparations, of both the isozymes in different amounts. To assess the inhibitory efficacy of a compound versus the two 5αR

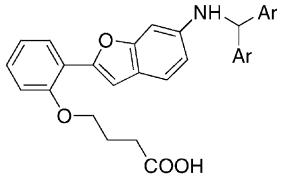
Table 7
Butanoic acid derivatives



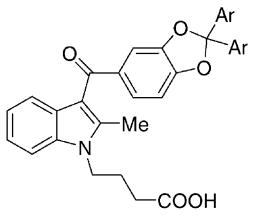
23



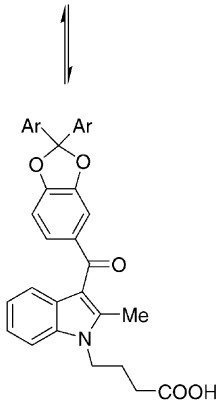
24a-c



25



26 "cis"



26 "trans"

Number	Ar	IC_{50} (nM) (5αR-1)[a]	IC_{50} (nM) (5αR-2)[b]
23	Ph	310	>100000
24a	Ph	62	270
24b	4-F-Ph	50	340
24c	4-MeO-Ph	130	930
25	4-MeO-Ph	42	480
26	4-Cl-Ph	10	6300

[a] Recombinant human type 1 5α-reductase in COS 1 cells.
[b] Recombinant human type 2 5α-reductase in COS 1 cells.

isozymes it is necessary to activate selectively one of the two isozymes in presence of the other. Some authors activate either 5αR-1 or 5αR-2 using different testosterone concentrations on the basis of the different affinity of this substrate (low nanomolar for 5αR-2 and micromolar for 5αR-1) [73]. However when testing 5αR-1 isozyme at micromolar concentration of testosterone in preparations containing also 5αR-2, this last isozyme is already active and working at its maximum velocity. Therefore, in this conditions correct results on 5αR-1 will be obtained only if the enzymatic activity of 5αR-2 in the tissue homogenate is negligible with respect to that of 5αR-1. This problem can be avoided by blocking 5αR-2 activity with a selective inhibitor, but completely selective 5αR-2 inhibitors are not available.

The use of human derived cell lines can be proposed to perform inhibition tests versus the type 1 isozyme. Cell lines expressing mainly or exclusively this isozyme are tumor cell lines of prostatic origin like PC3, DU145, and LnCaP [74–76], or skin derived cell lines like SZ95 (sebocytes) [77]. Tumor cell lines are generally to be avoided

in setting up routinary tests, moreover in many cases these cell lines are difficult to maintain in culture and have a low duplication rate.

The better choice in setting up and standardizing routinary inhibition tests is represented by the use of recombinant isozymes expressed in transfected cells. Cell lines stably expressing the two 5αR isozymes have been described and used to test 5αR inhibitors [78,60]. In this case the test is performed versus each single isozyme avoiding all the problems described for tissue homogenates. Since differences in the potency of the inhibitor between the native and the recombinant isozymes were sometimes described [79], results obtained with a recombinant 5αR isozyme should be confirmed on the native isozyme, especially if the inhibitor is a good candidate for clinical studies.

Frequently in the first evaluation of the activity of new compounds versus enzymes or receptors, animal tissues are used because they are readily accessible. In this case it is important to know if the enzyme or receptor of the species used is similar to the human counterpart. As regard the 5αR system many species, including mice, rats dogs and monkeys possess two 5αR genes [80–84]. The panspecific distribution and duplication of the enzyme in mammals underscore the need of DHT for androgen action. Studies on knock out mice lacking one or both the 5αR isozymes suggest that the conversion of testosterone to dihydrotestosterone represents a signal amplification mechanism common to all mammals. Unfortunately, to a similar function does not correspond a similar structure, in fact the homology of the human isozymes with the rat isozymes is about 50% and rises to 90% only with the cynomolgus monkey isozymes. The structural differences between species frequently cause a different response to the inhibitors. Considering as an example the rat versus the human isozymes (rat tissue is commonly used to test new molecules), many inhibitors, randomly distributed between all the above described classes, have a different potency versus rat 5αRs and human isozymes. This phenomenon is reported especially for 5αR-1. Among steroidal inhibitors, **Finasteride** is a weak inhibitor for human 5αR-1 ($K_i = 300$ nM) and a good inhibitor for the rat isozyme ($K_i = 5$ nM). However this difference is not characteristic of all the compounds of this class, in fact the activity of **MK386** is similar for the human and the rat isozymes. Similar differences are reported also in non-steroidal inhibitors, in fact **LY191704** while being a potent human 5αR-1 inhibitor, is a weak inhibitor of the rat enzyme. Similarly, benzo[c]quinolizinones that are potent human 5αR-1 inhibitors were inactive toward rat type 1 isozyme. Also the bi-substrate inhibitor **26** when tested against rat type 1 isozyme resulted almost 60-fold less active ($IC_{50} = 588$ nM) than versus human isozyme. These data demonstrate that only tests carried out with human enzymes give useful information for the discovery and processing of drugs candidate to the treatment of human pathologies.

Moreover another problem in the preclinical development of new 5αR inhibitors is the lack of animal models for the pathology of interest; in fact BPH, baldness, hirsutism and acne are human specific diseases. Therefore, in the choice of the animal species with which to perform in vivo studies for general toxicity and tolerability, the potency of the inhibitor versus its 5αRs should be taken into account; however only clinical trials with humans will establish the efficacy of the drug for the pathologies of interest.

5. Concluding remarks

The great incidence among population of DHT-related skin disorders such as acne, alopecia, and hirsutism has prompted in the recent years an active research by several pharmaceutical and academic groups towards the discovery of highly selective and potent 5αR-1 inhibitors as potential drugs.

The most important classes of potent and selective 5αR-1 inhibitors have a non-steroidal structure that derives from known (aza)steroidal inhibitors of 5αR by the formal removal of one ring from the steroidal structure. Among them, only two main classes of non-steroidal tricyclic compounds have really emerged, i.e. benzo[f]quinolinones, formally derived from 4-azasteroids, and benzo[c]quinolizin-3-ones, formally derived from 10-azasteroids. In both the classes, compounds displaying inhibition values at nanomolar level are found. The two general features that increase the potency of these inhibitors are the presence of an halogen atom such as Cl at the position 8 of their aromatic ring, and the presence of a small alkyl group such as a methyl group at position 4 of the A ring. This is thought to be accommodated inside a small hydrophobic pocket in the enzyme active site, while the lipophilic and space characteristics of Cl well fits another hydrophobic pocket in the D ring region usually occupied by the steroids. The extended planarity of these compounds has been suggested as another important feature for the potency and selectivity toward 5αR-1. Formal removal of more than one ring from steroidal inhibitors structure lead to weak inhibitors, whose potency is not modulated by the substituents introduced on the non-steroidal structure. Therefore benzo[f]quinolinones and benzo[c]quinolizin-3-ones appear the best candidates for the development of a drug for the treatment of DHT related pathologies in which 5αR-1 is involved.

An important point that we wish to stress is that special care should be taken in the choice of the biological test to assess the activity and selectivity of new inhibitors. The use of the human 5αR isozymes is, in our opinion, essential to establish the potency and selectivity of a new molecule. Moreover, due to the high species specificity of the 5αR system, the activity on animal species should be assessed before performing preclinical studies on animals. However, animal models for DHT related pathologies are not available and only clinical trials with humans will establish the real efficacy of 5αR-1 inhibitors in these diseases.

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