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Bioorganic & Medicinal Chemistry Letters 16 (2006) 3233–3237
Bioorganic & Medicinal Chemistry Letters

Discovery of indole-containing tetracycles as a new scaffold for androgen receptor ligands

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Received 1 February 2006; revised 10 March 2006; accepted 14 March 2006
Available online 5 April 2006

Abstract—A novel series of tetracyclic indoles have been designed, synthesized and evaluated as androgen receptor (AR) ligands. Studies of structure–activity relationships (SARs) were investigated, which led to some compounds in this series as strong binders to androgen receptors.

The androgen receptor (AR) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors.[1] It plays a critical role in numerous physiological processes, including the development and maintenance of male secondary sexual characteristics such as muscle, hair, bone mass, prostate growth, and spermatogenesis. Two endogenous androgens most active in promoting these effects are, testosterone and nonaromatizable 5α-dihydrotestosterone (5α-DHT) (Fig. 1). The primary focus for drug design has been the synthesis of chemicals to regulate the transcriptional activity of AR based upon the structural, steroidal or nonsteroidal, and functional androgenic, antiandrogenic, or anabolic properties of ligands.[2] Nonsteroidal androgens can be designed and synthesized that will mimic the pharmacological effects of testosterone, and would likely avoid many of the undesired physicochemical and pharmacokinetic properties of their steroidal counterparts, including poor oral bioavailability, rapid hepatic metabolism, and activation of other steroid receptors.[3] The AR agonists and antagonists are useful in the treatment of a variety of disorders and diseases.[4] More particularly, antagonists of the androgen receptor could be employed in the treatment of prostate cancer, benign prostate hyperplasia, hirsutism in women, alopecia, anorexia nervosa, breast cancer, and acne. Agonists of the androgen receptor could be employed in male contraception, male performance enhancement, as well as in the treatment of cancer, AIDS, cachexia, and other disorders. In our efforts directed at identifying novel AR ligands, we have examined various chemical scaffolds as core structural elements.[5] Herein, we wish to present our design and synthesis of a novel series of nonsteroidal tetracycles (**I**) that incorporate indole moiety as the core to mimic the tetracyclic alignment of testosterone structure. SAR studies based on the binding affinity and the potency in cell functional assay will be described.

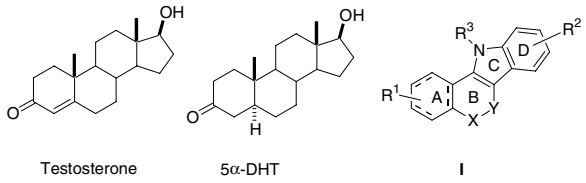

Figure 1.

The indole core present in the target structures was most conveniently introduced by a Fisher-indole type of reaction between various substituted phenyl hydrazine HCl salts (**1**) and ketones (**2**) via acid catalysis (Scheme 1).[6] Indoles **3** were then N-alkylated or acylated to afford the corresponding protected adducts **4**. Compound **4i** was prepared from **4h** by treatment with CuCN in DMF. The target compounds **4j–t**, **4v**, **5c–h**, and **6a–g** were obtained in accepted yields after de-methylation under pyridine HCl or $AlCl_3$ and EtSH condition. Sulfoxide **5a** and sulfone **5b** were synthesized through oxone oxidation of the sulfide **4d** followed by de-methylation under pyridine HCl condition.

Keyword: Androgen receptor ligand.
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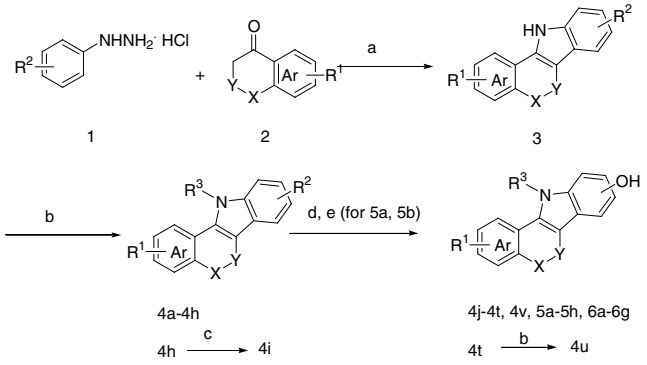
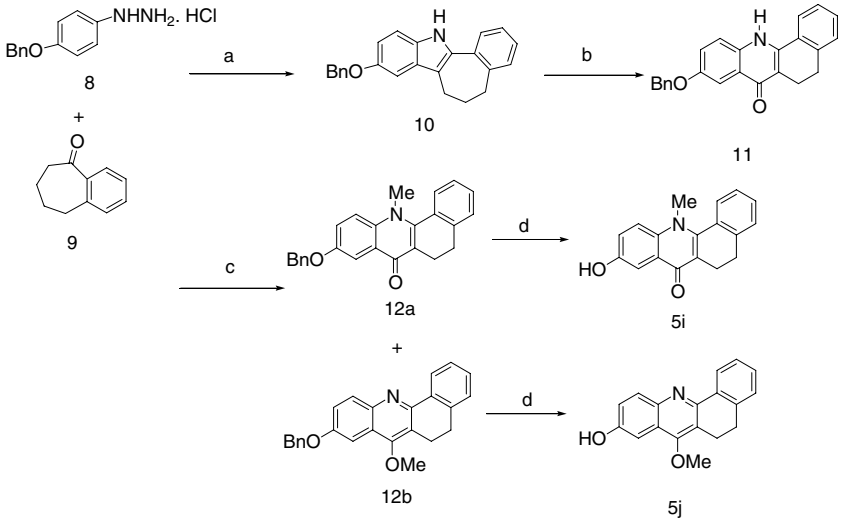
doi:10.1016/j.bmcl.2006.03.047



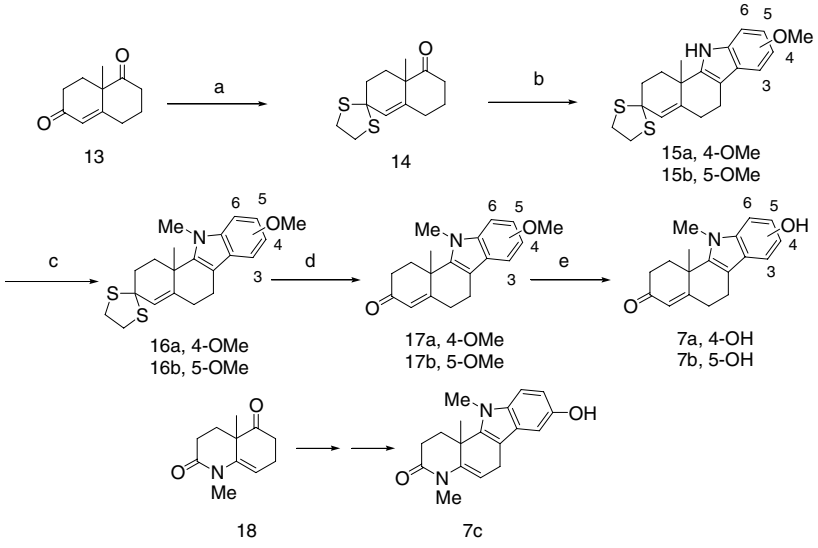
Scheme 1. Reagents and conditions: (a) HCl, EtOH, reflux, 4 h, 35–73%; (b) NaH, R_3I [MeI, EtI, and $Cl(CH_2)_2NMe_2$ + KI (cat.)], DMF, 0–50 °C, 1 h, 71–87% or Et_3N, AcCl, CH_2Cl_2, 0 °C to rt, 2 h, 54–71%; (c) CuCN, DMF, 160 °C, 6 h, 45%; (d) pyridine hydrochloride, neat, 200 °C, 30 min, 25–65% or $AlCl_3$, EtSH, 0 °C to rt, 2 h, 50–75%; (e) oxone, MeOH–H_2O, rt, 6 h, 25% (the sulfoxide), 45% (the sulfone) for preparations of **5a** and **5b**.

To prepare quinolone **11**, we employed a Winterfeldt oxidation procedure (*t*-BuOK and O_2) to achieve the indole-quinolone conversion in good yield (Scheme 2).[7] The required tetracyclic indole **10** for this reaction was prepared by Fisher indole synthesis from hydrazine **8** and ketone **9**. Compound **11** was then N- or O-methylated by treatment with K_2CO_3 and MeI to afford **12a** and **12b**, which were then converted into the phenols **5i** and **5j** after reductive de-benzylations.

The synthetic approaches to the indole analogues with non-aromatized A-ring (**7a** and **7b**) were initiated from Wieland–Miescher ketone **13** (Scheme 3). Selective protection of the conjugated ketone group of **13** by 1,2-bis-trimethylsilanylsulfanyl-ethane and ZnI_2 gave **14**. Condensation of **14** with phenyl hydrazines via Fisher-indole synthesis afforded the corresponding indoles **15** in acceptable yields. Compound **15** was then methylated and the thio-ketal protective group was removed by



Scheme 2. Reagents and conditions: (a) HCl, EtOH, reflux, 4 h, 52%; (b) *t*-BuOK, O_2, rt, 10 h, 55%; (c) K_2CO_3, MeI, DMF, rt, 2 h, 35% for **12a**, 30% for **12b**; (d) H_2, Pd–C, rt, 2 h, 75% for **5i**, 79% for **5j**.



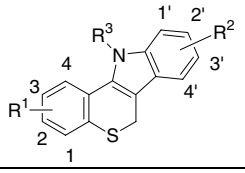
Scheme 3. Reagents and conditions: (a) $TMSSCH_2CH_2STMS$, ZnI_2, Et_2O, 10 h, 0 °C, 81%; (b) *p*-MeO–$PhNHNH_2$·HCl, HCl, EtOH, reflux, 4 h, 56–60%; (c) NaH, MeI, DMF, 0 °C, 1 h, 81%; (d) $Hg(ClO_4)_2$, MeOH, rt, 20 min, 61–65%; (e) pyridine hydrochloride, neat, 200 °C, 30 min, 50–72%.

the treatment of **16** with $Hg(ClO_4)_2$ in MeOH. Deprotection of the methoxy groups provided the targets **7a** and **7b**. Analogous synthesis from the bicyclic keto lactam **18** via the similar procedure afforded **7c**.

The lead optimization was primarily guided by a COS-7 whole-cell androgen receptor binding assay for assessing the AR binding affinities of the ligands.[8] Our initial SAR studies were focused on modifications of the tetacyclic core structure at R^1, R^2, and R^3 groups, as shown in Table 1. Exemplified by **4j**, one salient feature of the series was the phenol group at R^2 position. Any modification of the phenol group, including deletion (**4a**), replacement with halogen (**4c** and **4e–h**) or CN (**4i**) and masking as methyl ether (**4d**) or ester (**4u**), resulted in partial or total loss of activity. This indicates that the phenol group at D-ring might mimic 17β-OH of testosterone, which was crucial for hydrogen bonding with the receptor. 3′-OH group at R^3 position seemed to promote higher binding affinity than 1′-OH did, as illustrated by **4q** versus **4s**, albeit 2′-OH at R^3 position led to equally potent binder as its 3′-OH analogue did (**4v** vs **4j**). Another important requirement for strong binding affinity was having a methyl group at R^3 position. Significant loss in activity was seen for the compounds with more steric bulky group, such as Et, Ac, and dimethylaminoethyl groups (**4l–n**). It was not surprising to observe partial loss of activity in **4k** wherein H replaced methyl group at R^3 position. This might be due to the decreased lipophilicity of the ligand, which was critical for its fitting into the AR binding pocket. Additions of bulky (**4o** and **4p**) or hydrophilic groups (**4t**) at R^1 position were detrimental to activity. Replacements of H with F at R^1 position (**4q** and **4r**) were tolerated showing comparable binding affinities.

We next turned our attention to B- and C-ring modifications, as shown in Table 2. On the basis of the structure **4j**, B-ring was modified by various replacements based on steric bulkiness or functionality in order to define the SAR at this area. Attempts to replace sulfide of **4j** with some hydrophilic functionality such as sulfoxide (**5a**), sulfone (**5b**) or amino group (**5h**) resulted in total loss of activity. Surprisingly, switching the linkage of A- and C-ring from (ring A)–S–CH_2–(ring C) of **4j** to (ring A)–CH_2–S–(ring C) of **5c** abolished the binding affinity. This result indicated that the alignments of the tetracyclic cores in **4j** and **5c** resulted in different conformations, which led to total different binding affinities between these two regio-isomers. Steric bulkiness (**4t** vs **5d**) at Y seemed to be tolerated for comparable binding affinity. Compared with **4j**, the carbon analogue **5e** bound to the AR receptor with an improved affinity. Seven-membered B-ring analogues produced a detrimental effect, as presented by **5f** versus **5e** and **5g** versus **4j**. With regard to C-ring modification, replacement of

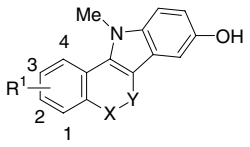
Table 1. SAR at the substitutions R_1, R_2, and R_3

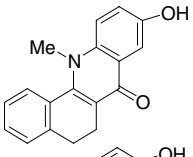
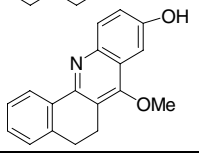


Compound	R^1	R^2	R^3	Rat AR COS-7 whole-cell binding[a] IC_{50} (μM)
R1881[d]				0.0015
Bicalutamide				0.85
4a	H	H	Me	—[b]
4b	H	3′-Me	Me	—[b]
4c	H	3′-Cl	Me	1.5
4d	H	3′-OMe	Me	—[b]
4e	H	3′-F	Me	3.7
4f	2-F	3′-F	Me	48%[c]
4g	2-CF_3	3′-F	Me	2.8
4h	H	3′-Br	Me	—[b]
4i	H	3′-CN	Me	—[b]
4j	H	3′-OH	Me	0.11
4k	H	3′-OH	H	4.5
4l	H	3′-OH	Et	2.3
4m	H	3′-OH	Ac	1.0
4n	H	3′-OH	$(CH_2)_2NMe_2$	—[b]
4o	2-OMe	3′-OH	Me	3.0
4p	2-Br	3′-OH	Me	2.8
4q	2-F	3′-OH	Me	0.63
4r	3-F	3′-OH	Me	0.11
4s	2-F	1′-OH	Me	3.8
4t	2-OH	3′-OH	Me	2.1
4u	H	3′-OAc	Me	0.88
4v	H	2′-OH	Me	0.13

[a] IC_{50} values are representative of multiple determinations (N = 2–3).
[b] '—', not active (<20% and/or binding affinity >10 μM).
[c] Inhibition at 10 μM.
[d] Methyltrienolone.

Table 2. SAR at B- and C-rings



Compound	R^1	X	Y	Rat AR COS-7 whole-cell binding[a] IC_{50} (μM)
5a	H	SO	CH_2	—[b]
5b	H	SO_2	CH_2	—[b]
5c	H	CH_2	S	—[b]
5d	2-OH	S	$C(Me)_2$	3.4
5e	H	CH_2	CH_2	0.03
5f	H	CH_2	$(CH_2)_2$	0.92
5g	H	S	$(CH_2)_2$	5.0
5h	H	NMe	CH_2	—[b]
5i				—[b]
5j				—[b]

[a] IC_{50} values are representative of multiple determinations (N = 2–3).
[b] '—', not active (<20% and/or binding affinity >10 μM).

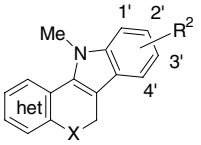
the indole with the quinolone core resulted in total loss of activity (**5i** and **5j**) (Table 3).

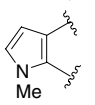
With the SAR of the compounds containing phenyl as A-ring understood, we then explored the effect of the heterocyclic A-ring analogues. These would potentially have the benefit of improved pharmacokinetic profile. As illustrated by **6b** and **6d** in Table 4, replacement of phenyl A-ring of **4j** or **5e** with 2-pyridinyl group displayed either comparable or better binding affinity. The best compound **6b** bound to the AR receptor with an IC_{50} of 29 nM. This result indicated that heteroaryl replacement was an effective strategy, albeit not all modifications presented here were rewarded by generating potent AR ligands (**6a**, **6c**, and **6e**–**g**).

The final group of compounds tested were nonaromatized A-ring analogues **7a**–**c**, which acted as a close mimic of testosterone structure. It was noted in these cases that the unsaturated A-ring seemed to have a detrimental effect for binding affinity.

Selected compounds showing high binding affinities were then evaluated in cell-based functional assay (Table 5). These compounds were somewhat less active in L929 AR mediated transcriptional assay.[9] However, it seemed that the ligands were able to cross the cell membrane and turn on the AR function. Nevertheless, these data provided us further confirmation to advance some lead compounds for in vivo evaluation later.

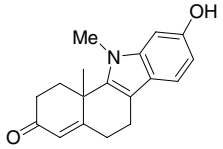
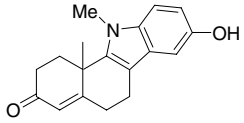
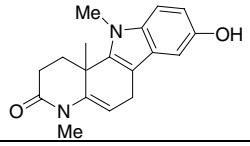
Table 3. SAR at heteroaryl A-ring



Compound	het	X	R^2	Rat AR COS-7 whole-cell binding[a] IC_{50} (μM)
6a		CH_2	3′-OH	0.93
6b		CH_2	3′-OH	0.029
6c		CH_2	2′-OH	29%[b]
6d		S	3′-OH	0.052
6e		CH_2	3′-OH	33%[b]
6f		CH_2	3′-OH	0.58
6g		CH_2	3′-OH	0.37

[a] IC_{50} values are representative of multiple determinations ($N = 2–3$).
[b] Inhibition at 3 μM.

Table 4. SAR at nonaromatized A ring

Compound	Structure	Rat AR Cos-7 whole-cell binding[a] IC_{50} (μM)
7a		—[b]
7b		42%
7c		—[b]

[a] IC_{50} values are representative of multiple determinations ($N = 2–3$).
[b] '—', not active (<20% and/or binding affinity >3 μM).

Table 5. Inhibitory activity toward L929 AR cells

Compound	L929 % of inhibition at 3 μM
4j	21
4q	87
4v	35
5e	43
6b	15
6d	18

In summary, we have developed and characterized a novel series of teracyclic indoles as AR ligands. These compounds were evaluated by AR binding and cell-based functional assays. Some of them demonstrated strong binding affinity. Further studies will be focused on transferring in vitro activity to in vivo efficacy.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.03.047.

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