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Bioorganic & Medicinal Chemistry Letters 17 (2007) 439–443
Bioorganic & Medicinal Chemistry Letters

Serendipitous discovery of novel imidazolopyrazole scaffold as selective androgen receptor modulators

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Received 24 August 2006; revised 10 October 2006; accepted 12 October 2006
Available online 17 October 2006

Abstract—A novel imidazolopyrazole derivative has been fortuitously discovered as potent selective androgen receptor modulator with in vivo efficacy.

Androgens control sexual function in males and are central to the anabolic processes that underlie the development of male sexual and physiological characteristics. Natural androgen testosterone (T) and its metabolite-dihydrotestosterone (DHT) act via the androgen receptor (AR), which is a member of the nuclear receptor superfamily. While T and its esters were approved for a limited number of therapeutic applications, including primary or hypogonadotropic hypogonadism and delayed puberty, fear of exacerbating nascent prostate disease has limited more extensive legitimate use of these natural ligands.[1] The successful marketing of drugs such as raloxifene, a selective estrogen receptor modulator (SERM), has raised the possibility of generating non-steroidal AR ligands with tissue selectivity.[2] The term selective androgen receptor modulators (SARMs) is chosen after the terminology used for similar ligands targeting estrogen receptor. An ideal SARM is an antagonist or weak agonist in the prostate, but an agonist in muscle, which has potentially less side effects compared to T. Therefore, SARMs have potential for treating muscle wasting, hypogonadism of aging, osteoporosis, and female sexual dysfunction.[3]

Progress has been made in identifying novel pharmacophores of non-steroidal SARMs by structural modification of non-steroidal antiandrogens (Fig. 1).[4] S-4 (**I**) and BMS-564929 (**II**) are two leading SARMs that can prevent castration caused tissue weight loss and behave as partial agonists in the prostate but full agonists in the levator ani. muscle in the castrated rat model. Here we describe our discovery of a novel imidazolopyrazole scaffold as SARM. The lead compound **10c** is a tissue-selective non-steroidal androgen receptor ligand with agonist activity in rat muscle and mixed agonist and antagonist activity in the rat prostate. The synthesis and in vivo SAR studies of this novel series of bicyclic structures are presented.

Recently, we have identified a novel series of pyrazolines (**III**) as SARMs.[5] The potential of metabolic instability of the amide bond in structure **III** led us to consider replacement with an amidate linkage as effective bioisostere. The goal of this design was to probe the influence of the amidate portion of the molecule and identify those features that could improve metabolic stability, while maintaining the efficacy as seen in structure **III**. The syntheses of amidates **8** are described in Scheme 1. Preparation of **5** utilized an efficient 1,3-dipolar cycloaddition between the α,β-unsaturated alkenes **3** and the hydrazones **4** ($R^5CH{=}NNHTs$) in the presence of NaH to afford pyrazolines **5** in moderate to good yields. The alkene precursors **3** were obtained by coupling of methylacryl acid **2** with the corresponding anilines **1** in the presence of thionyl chloride. Compounds **5** were then transformed into their thio-amide analogs **6** by treatment with Lawesson's reagent. Alkylations of **6** with EtI in the presence of K_2CO_3 afforded the corresponding thio-imidates **7**. Nucleophilic displacements of SEt group with nitrogen-containing nucleophiles

Keywords: SARMs; Imidazolopyrazole; Tissue selectivity.
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doi:10.1016/j.bmcl.2006.10.035

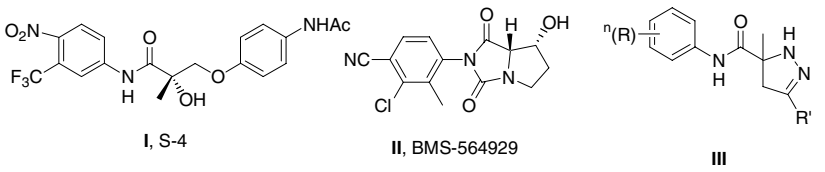
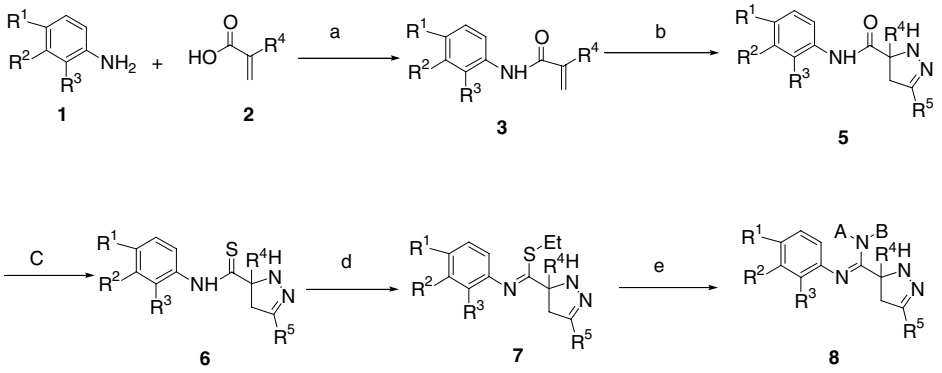
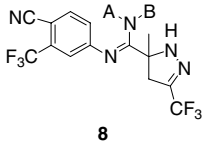


Figure 1.



Scheme 1. Reagents and conditions: (a) $SOCl_2$, 0 °C–rt, 2–4 h; (b) $R^5CH{=}NNHTs$ (**4**), NaH, 0 °C, 30 min then 70 °C 4–6 h, 55–70%; (c) Lawesson reagent, 120 °C, 6–10 h, 42–60%; (d) K_2CO_3, EtI, acetone, 50–70 °C, 1–2 h, 75–85%; (e) A–NH–B (extra), dioxane in seal tube, 100 °C or A–NH–B, DMF, K_2CO_3, rt, or A–NH–B, K_2CO_3, dioxane, 80 °C, 1–4 h, 35–80%. All compounds were characterized by ^{1}H NMR and LC–MS. LC purity is >95%.

Table 1. SAR at substitutions A and B of structure **8**[a]

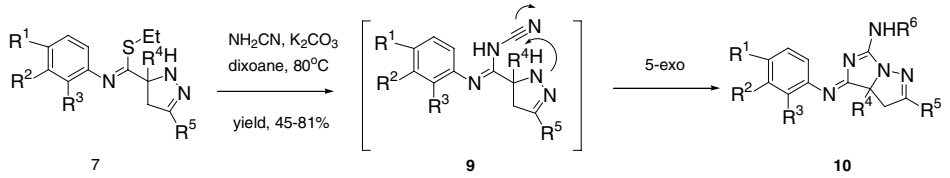


Compound	A	B	Levator ani. stimulation (%)	Prostate stimulation (%)	Prostate inhibition[e] (%)
TP[b]			100	100	
Bicalutamide[c]					70
8a	H	H	76	39	20
8b	H	Me	17	<10[d]	15
8c	H	Et	50	<10	18
8d	H	OMe	23	14	<10
8e	H	OH	<10	<10	<10
8f	H	SO_2Me	<10	<10	<10
8g	Et	Et	32	22	<10
8h	$-(CH_2)_4-$		<10	<10	<10

[a] All compounds were administered via po (vehicle: 20% cyclodextrin) once daily at a dose rate of 2 mg/day for 5 days. The data were normalized to control group administer with vehicle (N = 3/group).
[b] Testosterone propionate was administered subcutaneously by injection at the nape of the neck at 5 mg/kg, in a volume of 0.1 mL in sesame oil.
[c] Average value based on 10 tests.
[d] Inhibition at a dose rate of 2 mg/day.
[e] Compounds were dosed via po in the presence of 0.1 mg/day (approximately 1.3 mg/kg) testosterone propionate (subcutaneous dosing, vehicle: sesame oil).

(A–NH–B) under the conditions described in Scheme 1 led to a series of amidates **8**.

As described in our recent communication, a modified Hersherberger assay as an in vivo screening protocol was utilized to evaluate all compounds.[6] We found this strategy provided us with fast turnaround time, reasonable compound amount (~40 mg for each test), and simplified data analysis. The data indicate the combination of compound's ADME properties and intrinsic efficacy, which allows us to advance potent compound into mature rat efficacy model. Thus, all compounds were screened in immature castrated male Sprague–Dawley rats agonist and antagonist assays. In these studies, the



Scheme 2.

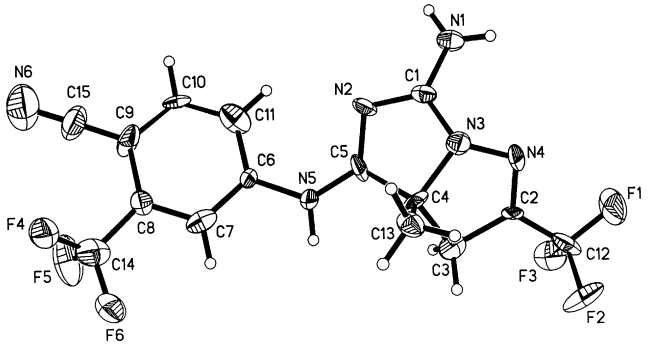
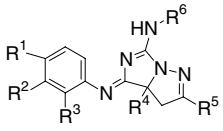


Figure 2. X-ray crystal structure of **10c**.

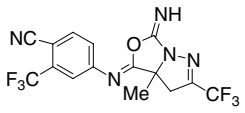
weights of the ventral prostate and seminal vesicle were used as the indicators of androgenic activity, while the weight of levator ani. muscle was used as the indicator of anabolic activity. Table 1 summarizes the in vivo anabolic and antiandrogenic activities for compounds **8** containing amidate functionality. In castrated immature rat agonist assay, compound **8a** showed tissue-selective in vivo pharmacologic activity at a po dose of 2 mg/day, with anabolic activity on levator ani. muscle weight stimulation to 76% and androgenic activity on ventral prostate weight stimulation to 39%. Interestingly, when further tested in an immature rat antagonist assay, **8a** presented weak inhibition on the ventral prostate weight to 20% in TP treated immature rat, which secured its selectivity between anabolic and androgenic tissues. SAR around the amidate structure suggested that the size of the amidate as a bioisostere was critical for activity, as indicated by the data in Table 1. Any modifications on substituent B by adding size or hydrophilicity resulted in either partial or complete loss of the agonist activity on levator ani. and prostate (**8b**–**8f**). Moreover, bis-substitutions on A and B were detrimental to the activity and led to inefficacious compounds **8g** and **8h**.

Table 2. SAR at substitutions R^1–R^6 of structures **10**[a]



10

Compound	R^1	R^2	R^3	R^4	R^5	R^6	Levator ani. stimulation (%)	Prostate stimulation (%)	Prostate inhibition (%)
TP							100	100	
Bicalutamide[b]									70
10a	CN	CF_3	H	Me	H	H	<10	<10	<10
10b	CN	CF_3	H	Me	CO_2Et	H	<10	<10	<10
10c (*R*)	CN	CF_3	H	Me	CF_3	H	91[d]	36[c]	30
10d (*S*)	CN	CF_3	H	Me	CF_3	H	72[d]	29[c]	<10
10e	CN	CF_3	Et	Me	CF_3	H	19	<10	<10
10f	CN	CF_3	H	Me	per-F–Ph	H	21	<10	<10
10g	CN	CF_3	H	Et	CF_3	H	16	<10	<10
10h	CN	CF_3	H	Me	CF_3	CF_3CO	61	55	31
10i	Cl	Cl	H	Me	CF_3	H	<10	<10	31
10j	Br	CF_3	H	Me	CF_3	H	<10	<10	<10
10k	NO_2	CF_3	H	Me	CF_3	H	<10	<10	<10
10l							29	55	<10



[a] All footnotes in Table 1 apply in this table.
[b] Bicalutamide, average value based on 10 tests.
[c] stimulation at the dose of 3 mg/day.
[d] **10c** and **10d** were followed up with a five point dose-dependent study at doses from 0.03, 0.1, 0.3, 1 to 3 mg/day to generate ED_{50} value on levator ani.: ED_{50} for **10c**, 0.07 mg/day; ED_{50} for **10d**, 0.85 mg/day.

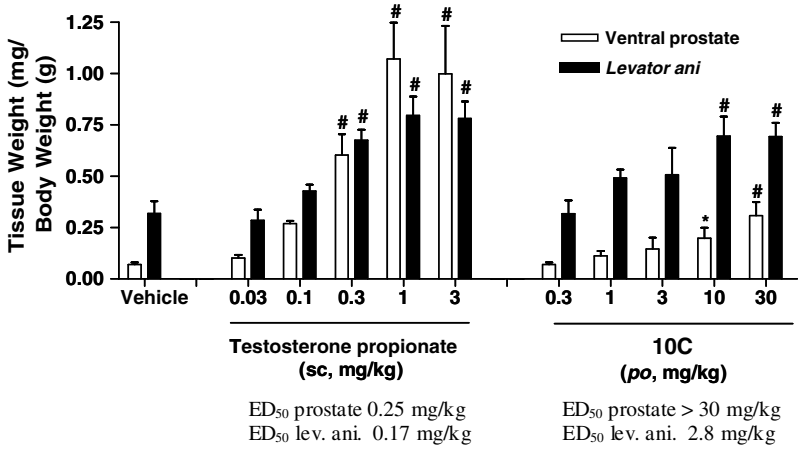


Figure 3. Effect of **10c** on muscle and prostate weights in castrated mature rats ($^{\#}p < 0.01$ and $^{*}p < 0.05$, means ± SD, $n = 3$, tissue weights relative to body weight).

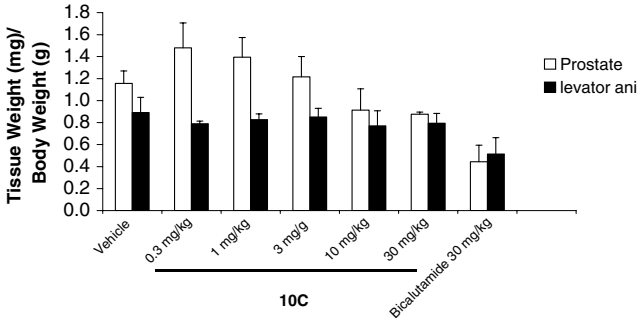


Figure 4. Effect of daily six-week treatment with **10c** on prostate and muscle wet weights in intact adult male rats (means ± SD, $n = 3$).

As a logic extension of the amidate series, the cyano amidate functionality was considered an effective replacement of the amide bond in structure **III**. A clean product originally assigned as structure **9** was obtained by reaction of compounds **7** and NH_2CN in the presence of K_2CO_3 (Scheme 2). Beyond our anticipation, displacement of the thio-imidate group of compounds **7** with cynamide followed by an intramolecular *5-exo* cyclization of the corresponding intermediates **9** occurred to afford the bicyclic imidazolopyrazole derivatives **10** in good yields. Structures **10** were differentiated from the amidate structures **8** initially by the spectroscopic data and later were confirmed by X-ray single crystallography of compound **10c**[7] (Fig. 2). Intramolecular *5-exo* cyclization of a nucleophile to cynamide was not unprecedented in the literature.[8] Compound **10c** (R configuration) was tested in castrated immature male rats agonist and antagonist assays (Table 2). It presented dose-dependent pharmacological responses in both anabolic (levator ani.) and androgenic (prostate) tissues. In the absence of testosterone, **10c** was a tissue-selective androgen agonist, with ED_{50} 0.07 mg/day on levator ani. muscle and 36% stimulation at 3 mg/day on prostate (Table 2). On the other hand, in the presence of exogenous testosterone, **10c** at a single dose of 2 mg/day inhibited stimulation of prostate weight by 30%, which exhibited its mixed agonist and antagonist activity on prostate. Interestingly, **10c** acted as the eutomer that contributed the observed in vivo efficacy, while its *S*-enantiomer-**10d** was a weaker agonist showing ED_{50} 0.85 mg/day on levator ani. muscle and 29% stimulation on prostate at the same dose in the castrated immature rat agonist assay. Brief survey of the substitutions of structure **10** revealed that this scaffold generally was not well tolerated for in vivo efficacy. Any modification on R^1, R^2, R^3, R^4, and R^5 seemed to be detrimental to the activity and led to inactive compounds, as exemplified by **10a–b**, **10e–g**, and **10i–k** in Table 1. Compound **10h** with trifluoro acetyl substitution on R^6 was an exception that maintained agonistic activity on levator ani muscle and prostate. Compound **10l**, O-analog of **10c** with a unique pyrazolooxazole structure, also showed strong potency on levator ani. muscle.

Compound **10c** was further tested in castrated mature male rats for its ability to stimulate prostate and muscle weights in the absence of endogenous testosterone (Fig. 3).[9] Compound **10c** had an ED_{50} on prostate of >30 mg/kg (31% stimulation at 30 mg/kg) and on levator ani. of 2.8 mg/kg. For comparison, TP had an ED_{50} on prostate of 0.25 mg/kg while it presented EC_{50} 0.17 mg/kg on levator ani. muscle. In the intact mature male rat assay (Fig. 4),[10] compound **10c** was able to reduce prostate weight dose-dependently, with 26% reduction at 30 mg/kg in the presence of endogenous testosterone. At the same time, there was no significant dose-dependent effect on muscle weight. For comparison, the androgen antagonist bicalutamide in the same assay reduced both prostate and levator ani weights by 60% at 30 mg/kg (Figure not shown here). These data strongly indicated that **10c** was a tissue-selective nonsteroidal androgen receptor ligand with agonist activity on rat muscle and mixed agonist and antagonist activity on the rat prostate.

In summary, we conducted SAR through modification on structure **III** by replacement of the amide bond with amidate structures. This led to the identification of a unique imidazolopyrazole derivative **10c** as potent SARMs with oral efficacy.

Acknowledgment

We thank Dr. Alexandra Shedlow and Dr. Derek Beauchamp for coordination on obtaining the X-ray crystallographic data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.10.035.

References and notes

1. (a) Andres, N.-V. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 3459; (b) Liu, P. Y.; Swerdloff, R. S.; Veldhuis, J. D. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 4789.
2. (a) Cosman, F.; Lindsay, R. *Endocr. Rev.* **1999**, *20*, 418; (b) Katzenellenbogen, J. A.; O'Malley, B. W.; Katzenellenbogen, B. S. *Mol. Endocrinol.* **1996**, *10*, 119; (c) Baracat, E.; Haidar, M.; Lopez, F. J. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 2020.
3. (a) Gao, W.; Bohl, C. E.; Dalton, J. T. *Chem. Rev.* **2005**, *105*, 3352; (b) Chen, J.; Kim, J.; Dalton, J. T. *Mol. Interventions* **2005**, *5*, 173; (c) Basaria, S.; Wahlstrom, J. T.; Dobs, A. S. *J. Clin. Endocrinol.* **2001**, *86*, 5108.
4. (a) Marhefka, C. A.; Gao, W.; Chung, K.; Kim, J.; He, Y.; Yin, D.; Bohl, C.; Dalton, J. T.; Miller, D. D. *J. Med. Chem.* **2004**, *47*, 993; (b) Hamann, L. G. *Natl. Meet. Am. Chem. Soc., Med. Chem. Div.* **2004**, *227*, MEDI-175.
5. Zhang, X.; Li, X.; Sui, Z. PCT Int. Appl. WO 2006055184, 2006; *Chem. Abstr.* **2006**, *495*, 991.
6. (a) Hershberger, L. G.; Shipley, E. G.; Meyer, R. K. *Proc. Soc. Exp. Biol. Med.* **1953**, *83*, 175; (b) Antagonist assay: Test compound activity was determined as the percent inhibition of testosterone-enhanced tissue weights, with a vehicle-treated control group set to 100% and a testosterone alone-treated control group set to 0%; (c) Agonist assay: Test compound activity was determined as the percent stimulation of tissue weight, with the vehicle-treated control group set to zero percent and the testosterone alone-treated control group set to 100%; Synthesis and SAR of novel hydantoin derivatives as selective androgen receptor modulators: (d) Zhang, X.; Allan, G. F.; Sbriscia, T.; Linton, O.; Lundeen, S. G.; Sui, Z. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5763; The discovery of a potent orally efficacious indole androgen receptor antagonist through in vivo screening: (e) Lanter, J. C.; Fiordeliso, J. J.; Jiang, W.; Allan, G. F.; Lai, M.-T.; Linton, O.; Hahn, D.-W.; Lundeen, S. G.; Sui, Z. *Bioorg. Med. Chem. Lett.* **2006**. doi:10.1016/j/bmcl.2006.09. 086.
7. Compound **10c** was prepared from its enantiomeric pure precursor of **6** obtained from Chiral Pak column separation. See the experimental section for spectroscopic data. X-ray crystallography data for **10c** (HCl salt) have been deposited with Cambridge Crystallographic Data Centre (CCDC). CCDC 623419 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
8. Varney, M. D.; Palmer, C. L.; Deal, J. G.; Webber, S.; Welsh, K. M.; Bartlett, C. A.; Morse, C. A.; Smith, W. W.; Janson, C. A. *J. Med. Chem.* **1995**, *38*, 1892.
9. Mature (150–200 g) castrated male Sprague–Dawley rats were treated once daily for two weeks with test compound in 20% cyclodextrin. The activity was determined following the same method with immature rat agonist assay.
10. Mature (150–200 g) intact male rats were treated once daily for six weeks with test compound.