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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and biological evaluation of 2-(5-methyl-1*H*-pyrazol-1-yl) acetamide derivatives as androgen receptor antagonists

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Abstract
Androgen receptor (AR) signaling is often activated in prostate cancer (PCa) cells, and blockage of this signaling by AR antagonists is an important strategy in PCa therapy. In this study, we designed and synthesized a series of 2-(5-methyl-1*H*-pyrazol-1-yl) acetamide derivatives, and evaluated their biological activities. AR luciferase reporter assay revealed compound **6f** (59.7%) as a potent AR antagonist. Some compounds in this series showed higher anti-proliferative activity against LNCaP cells than Bicalutamide ($IC_{50} = 35.0\ \mu M$), especially **6g** with IC_{50} value of 13.6 μM.

Keywords Androgen receptor · Prostate cancer · Antagonists · Pyrazole derivatives

Introduction

Early prostate cancer (PCa) can be effectively remitted by androgen deprivation therapy (ADT), which is a combination of surgical or medical castration with administration of androgen receptor (AR) antagonist (Mercader 2007; Harris et al. 2009). However, the disease often relapses after a period of ADT treatment and progresses to a new stage termed castration-resistant prostate cancer (CRPC) (Katzenwadel and Wolf 2015). Multiple mechanisms for the relapse have been proposed, most of them suggesting reactivation of the AR signaling pathway, including over-expression or mutations of AR, and intratumoral *de novo* steroidogenesis (Locke et al. 2008; Montgomery 2008; Mostaghel 2007). This indicates that AR signaling is a critical driver for the development of CRPC (Taplin 2007). Research of drugs targeting CRPC is mainly divided into two directions, one is blockage of endogenous synthesis of androgen by the CYP17A inhibitor Abiraterone (De Bono et al. 2011; Reid 2008) and the other is to combat drug resistance that emerges after prolonged usage of first-generation AR antagonists (e.g., Bicalutamide, Fig. 1). The latter has led to the development of the second-generation AR antagonists such as Enzalutamide (MDV3100, Fig. 1) (Tran et al. 2009; Oudard 2013).

Our previous work focused on the pharmacological characterization of molecules based on a pyrazole scaffold as potential anti-PCa agents. Among the pyrazole derivatives, compound **10e** showed potent anti-proliferative activity in LNCaP cells with an IC_{50} value of 17.8 μM (Guo et al. 2016). However, in a later study, **10e** was found to be rapidly metabolized by human liver microsomes (HLMs) with a half-life ($T_{1/2}$) of ~30 min. The methylene amino-linker bridging the pyrazole core and terminal phenyl ring was a possible metabolism site, as the amino group could undergo de-alkylation or oxidation via the catalysis of cytochrome P450 that generally existed in HLMs. In this work, structural modification focused on this methylene amino-linker motif, which was replaced by an acetamido structure (Fig. 2). Therefore a novel series of 2-(5-methyl-1*H*-pyrazol-1-yl) acetamide derivatives was designed and synthesized.

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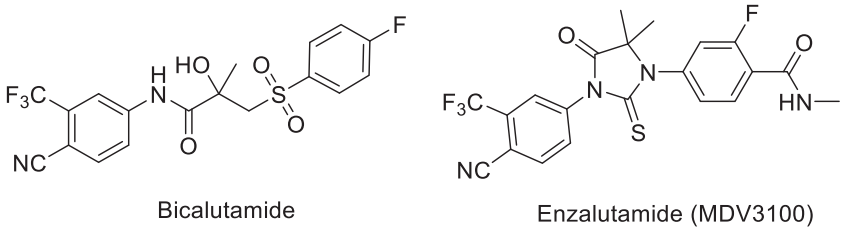


Fig. 1 Representative AR antagonists in clinical application

Results and discussion

Chemistry

Synthesis of novel compounds was performed following the route depicted in Scheme 1. As the starting material, a *p*-substituted benzaldehyde (**1**) was condensed with chloroacetyl chloride to obtain the substituted (*E*)-4-phenylbut-3-en-2-one as intermediate **2** via an aldol condensation. Cyclization of intermediate **2** with *p*-toluenesulfonyl hydrozide under alkaline conditions generated the substituted 5-methyl-3-phenyl-1*H*-pyrazole as intermediate **3**. A substituted aniline (**4**) was utilized as an additional starting material, which when condensed with chloroacetyl chloride resulted in substituted 2-chloro-*N*-phenylacetamide as intermediate **5**. Finally, a substitution of intermediates **3** and **5** under alkaline condition afforded novel compounds **6**.

Biological activity

AR antagonistic activity

AR antagonistic activity of the new compounds was quantified via an AR luciferase reporter assay in MDA-Kb2 cells (Fig. 3). The MDA-Kb2 cell line stably expresses an androgen-responsive reporter, and exposure to compounds with AR antagonistic activity will result in a reduction of the luciferase reporter levels. All the compounds in this series were tested at 1 μM concentration. Among them, five compounds, **6a**, **6f**, **6h**, **6l**, and **6n**, exhibited AR antagonistic activities with inhibitory rates in a range of 34.7–59.7%, which was higher than that of bicalutamide (32.0% inhibition).

Substituents on both terminal phenyl rings affected the AR antagonistic activity. Generally, most of compounds with a *p*-fluoro group on the phenyl ring at 3-position of the pyrazole core showed higher AR antagonistic activity than a *p*-cyano substitution at the same position (**6a** vs. **6h**, **6c** vs. **6j**). However, if there was a *p*-substitution at the R_2-position, opposite effects were observed (**6d** vs. **6k**, **6e** vs. **6l**, and **6g** vs. **6n**). In this series, compound **6f** showed the highest AR antagonistic activity (59.7%), which was nearly twofold higher than bicalutamide (32.0%).

Anti-proliferative activity in PCa cell lines

The anti-proliferative property of the new compounds was tested by using MTT assay in two PCa cell lines, LNCaP (with a T877A mutation in AR) and PC-3 (with wild-type AR) (Table 1). Most compounds showed good responses in LNCaP cells, and exhibited more potent anti-proliferative activity in LNCaP cells than the positive control, bicalutamide ($IC_{50} = 35.0$ μM). It was worth noticing that compounds (**6h–6n**) with a *p*-cyano substitution at the R_1-position showed high selection for LNCaP cells. Analysis of structure–activity relationship showed that a *p*-fluoro group on the 3-phenyl ring of the pyrazole core favored improved growth inhibition in PC-3 cells. In this series, the most potent compound, **6g** ($IC_{50} =$ 13.6 μM), showed a more than twofold higher anti-proliferative activity in LNCaP cells than bicalutamide ($IC_{50} = 35.0$ μM), as well as a more than threefold higher activity ($IC_{50} = 20.2$ μM) compared with bicalutamide ($IC_{50} = 63.1$ μM) in PC-3 cells. The most potent AR antagonist, **6f**, also showed superior anti-proliferative activity in both LNCaP and PC-3 cells with IC_{50} values of 28.2 and 28.6 μM, respectively.

Materials and methods

All materials and reagents were commercially available and used without further purification. Thin-layer chromatography (TLC) performed on silica gel HSGF254 plates was used to monitor the reaction. Column chromatography was performed using silica gel (60 Å, 200–300 mesh). Melting points were determined in a capillary melting point apparatus and are uncorrected. All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX-400 Spectrometer (Bruker, Rheinstetten, Germany) with tetramethyl-silane (TMS) as the internal standard, and compounds were dissolved in deuterated dimethylsulfoxide (DMSO-d_6). The high-resolution mass spectra (HRMS) were measured with $X500_R$ QTOF (AB SCIEX, USA). All tested compounds were more than 95% pure on the HPLC analysis. The method for HPLC analysis and a table of the data for all tested compounds are in Supporting Information.

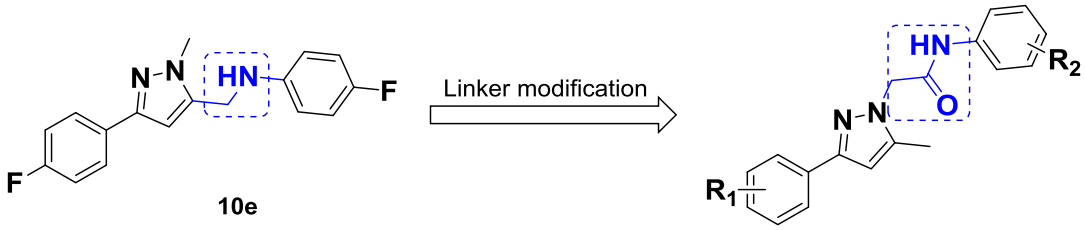


Fig. 2 Design of novel 2-(5-methyl-1*H*-pyrazol-1-yl) acetamide derivatives

General synthetic procedure of intermediate 2

A mixture of substituted benzaldehyde (20.0 mmol) and morpholine trifluoroacetate (0.8 g, 4 mmol) in acetone (50 mL) was sealed in a tube and refluxed at 80–90 °C for 72 h. It was then cooled down, and excess acetone was evaporated in vacuo. The residue was dissolved in ethyl acetate (EA, 50 mL), washed with H_2O (20 mL), brine (20 mL), dried over Na_2SO_4, filtered, and concentrated to afford intermediate **2**, which was directly used for the next step without further purification.

(E)-4-(3-oxobut-1-en-1-yl)benzonitrile (2a)

Light yellow solid; yield: 80%; mp: 93–96 °C .^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93–7.89 (m, 4H, Ar–H), 7.68 (d, $J = 16.4$ Hz, 1H, C_1–H), 6.97 (d, $J = 16.4$ Hz, 1H, C_2–H), 2.37 (s, 3H, CH_3).

General synthetic procedure of intermediate 3

A mixture of intermediate **2** (20.0 mmol) and 4-toluenesulfonyl hydrazide (4.1 g, 22.0 mmol) in acetonitrile (50 mL) was stirred at 40 °C for 3 h. NaOH (1.2 g, 30.0 mmol) was added, and the reaction mixture was refluxed for 12 more hours. It was then cooled down, and EA (50 mL) was added to the solution, washed with water (20 mL × 3), and dried over Na_2SO_4. The solvent was removed in vacuo to afford intermediate **3**.

4-(5-methyl-1H-pyrazol-3-yl)benzonitrile (3a)

Light yellow solid; yield: 89%; mp: 130–133 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.84 (s, 1H, NH), 7.95 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.83 (d, $J = 8.2$ Hz, 2H, Ar–H), 6.60 (s, 1H, C_4–H), 2.28 (s, 3H, CH_3).

General synthetic procedure of intermediate 5

A substituted aniline (5.0 mmol) and triethylamine (1.87 g, 18.5 mmol) were dissolved by dichloromethane (10 mL), and stirred under an ice–water bath, to which chloroacetyl chloride (1.29 g, 11.5 mmol) was added dropwise. After addition, the reaction mixture was stirred for one more hour at room temperature, then EA (30 mL) and H_2O (30 mL) were added for an extraction. The organic contract was washed with water (15 mL × 3) and saturated brine (15 mL), dried over Na_2SO_4 and evaporated under reduced pressure to afford intermediate **5**.

2-chloro-N-(3-cyanophenyl)acetamide (5a)

White solid; yield: 73%; mp: 153–155 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H, NH), 8.08 (s, 1H, Ar–H), 7.82–7.79 (m, 1H, Ar–H), 7.57–7.56 (m, 2H, Ar–H), 4.09 (s, 2H, CH_2).

2-chloro-N-(4-cyano-3-(trifluoromethyl)phenyl)acetamide (5b)

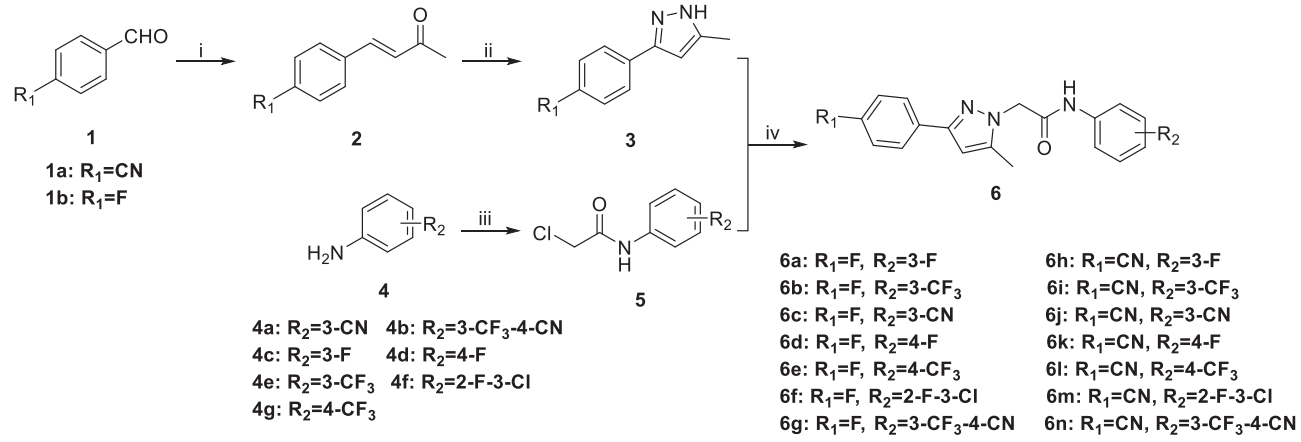
White solid; yield: 76%; mp: 127–130 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s, 1H, NH), 8.25 (d, $J = 1.5$ Hz, 1H, Ar–H), 8.14 (d, $J = 8.5$ Hz, 1H, Ar–H), 7.99 (dd, $J =$ 8.5, 1.6 Hz, 1H, Ar–H), 4.13 (s, 2H, CH_2).

General synthetic procedure of target compounds

NaH (60%, 120 mg, 3.0 mmol) was added slowly into a solution of intermediate **3** (1.0 mmol) in *N*, *N*-dimethylformamide (DMF, 2.5 mL) and stirred for 30 min at room temperature. Then intermediate **5** (1.0 mmol) was added and the reaction mixture was stirred for another 12 h. It was then poured into ice-cold water (35 mL) to form precipitate that was collected by filtration, washed with water, and dried. The crude product was dissolved with DCM and purified using column chromatography (petroleum ether: EA = 3:1) on silica gel to obtain target compounds.

N-(3-fluorophenyl)-2-(3-(4-fluorophenyl)-5-methyl-1H-pyrazol-1-yl)acetamide (6a)

White solid; yield: 30%; mp: 167–169 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1H, CONH), 7.84 – 7.72 (m, 2H, Ar–H), 7.58 (dt, $J = 11.5$, 2.0 Hz, 1H, Ar–H), 7.35 (dt, $J = 17.8$, 8.3 Hz, 2H, Ar–H), 7.20 (t, $J = 8.9$ Hz, 2H, Ar–H), 6.91 (td, $J = 8.5$, 2.0 Hz, 1H, Ar–H), 6.52 (s, 1H, C_4–



Scheme 1 Reagents and conditions: (i) morpholine trifluoroacetate, acetone, 85 °C; (ii) *p*-toluenesulfonyl hydrazide, NaOH, acetonitrile, 50 °C; (iii) chloroacetyl chloride, triethylamine, CH_2Cl_2, r.t.; (iv) NaH, DMF, r.t

H), 5.01 (s, 2H, CH_2), 2.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.40 (CO), 162.61 (d, $^1J_{CF} = 240.0$ Hz), 162.06 (d, $^1J_{CF} = 242.0$ Hz), 148.79 (C-3), 141.98 (C-5), 140.76 (d, $^3J_{CF} = 11.0$ Hz), 131.03 (d, $^3J_{CF} = 10.0$ Hz), 130.48 (d, $^4J_{CF} = 3.0$ Hz), 127.32 (d, 2C, $^3J_{CF} = 8.0$ Hz), 115.88 (d, 2C, $^2J_{CF} = 21.0$ Hz), 115.43 (d, $^4J_{CF} = 2.0$ Hz), 110.59 (d, $^2J_{CF} = 21.0$ Hz), 106.50 (d, $^2J_{CF} = 27.0$ Hz), 102.96 (C-4), 52.68 (CH_2), 11.32 (CH_3). HRMS (ESI+) *m/z* $C_{18}H_{15}ON_3F_2$ ($M+H^+$) calcd 328.1256, obsd 328.1250.

2-(3-(4-fluorophenyl)-5-methyl-1H-pyrazol-1-yl)-N-(3-(trifluoromethyl)phenyl) acetamide (6b)

White solid; yield: 26%; mp: 145–147 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (s, 1H, NH), 8.11 (s, 1H, Ar–H), 7.88 – 7.69 (m, 3H, Ar–H), 7.59 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.44 (d, $J = 7.8$ Hz, 1H, Ar–H), 7.20 (t, $J = 8.9$ Hz, 2H, Ar–H), 6.53 (s, 1H, C_4–H), 5.04 (s, 2H, CH_2), 2.30 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.67 (CO), 162.08 (d, $^1J_{CF} = 242.0$ Hz), 148.86 (C-3), 142.00 (Ar–C), 139.82 (C-5), 130.60 (Ar–C), 130.49 (d, $^4J_{CF} = 3.0$ Hz), 130.06 (q, $^2J_{CF} = 31.7$ Hz), 127.32 (d, 2C, $^3J_{CF} = 8.1$ Hz), 124.51 (q, $^1J_{CF} = 271.0$ Hz), 123.25 (Ar–C), 120.41 (q, $^3J_{CF} = 4.0$ Hz), 115.86 (d, 2C, $^2J_{CF} = 22.0$ Hz), 115.77 (q, $^3J_{CF} = 4.0$ Hz), 102.96 (C-4), 52.69 (CH_2), 11.30 (CH_3). HRMS (ESI+) *m/z* $C_{19}H_{15}ON_3F_4$ ($M+H^+$) calcd 378.1224, obsd 378.1208.

N-(3-cyanophenyl)-2-(3-(4-fluorophenyl)-5-methyl-1H-pyrazol-1-yl)acetamide (6c)

White solid; yield: 26%; mp: 180–182 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 1H, NH), 8.08 (d, $J = 1.1$ Hz, 1H, Ar–H), 7.87 – 7.69 (m, 3H, Ar–H), 7.62 – 7.50 (m, 2H, Ar–H), 7.20 (t, $J = 8.9$ Hz, 2H, Ar–H), 6.53 (s, 1H, C_4–H), 5.04 (s, 2H, CH_2), 2.32 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.71 (CO), 162.07 (d, $^1J_{CF} = 242.0$ Hz), 148.85 (C-3), 142.01 (Ar–C), 139.82 (C-5), 13.87 (Ar–C), 130.46 (d, $^4J_{CF} = 3.0$ Hz), 127.70 (CN), 127.33 (d, 2C, $^3J_{CF} = 8.0$ Hz), 124.30 (Ar–C), 122.41 (Ar–C), 119.05 (Ar–C), 115.88 (d, 2C, $^2J_{CF} = 22.0$ Hz), 112.19 (Ar–C), 103.00 (C-4), 52.67 (CH_2), 11.31 (CH_3). HRMS (ESI+) *m/z* $C_{19}H_{15}ON_4F$ ($M+H^+$) calcd 335.1303, obsd 335.1288.

N-(4-fluorophenyl)-2-(3-(4-fluorophenyl)-5-methyl-1H-pyrazol-1-yl)acetamide (6d)

White solid; yield: 27%; mp: 181–183 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H, NH), 7.81 – 7.73 (m, 2H, Ar–H), 7.62 (ddd, $J = 7.1$, 5.3, 2.8 Hz, 2H, Ar–H), 7.19 (dt, $J = 12.5$, 9.0 Hz, 4H, Ar–H), 6.52 (s, 1H, C_4–H), 4.99 (s, 2H, CH_2), 2.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.91 (CO), 162.05 (d, $^1J_{CF} = 242.0$ Hz), 158.65 (d, $^1J_{CF} = 239.0$ Hz), 148.73 (C-3), 141.93 (C-5), 135.46 (d, $^4J_{CF} = 3.0$ Hz), 130.51 (d, $^4J_{CF} = 3.0$ Hz), 127.31 (d, 2C, $^3J_{CF} = 8.0$ Hz), 121.47 (d, 2C, $^3J_{CF} = 8.0$ Hz), 115.92 (d, 2C, $^2J_{CF} = 22.0$ Hz), 115.87 (d, 2C, $^2J_{CF} = 21.0$ Hz), 102.94 (C-4), 52.63 (CH_2), 11.21 (CH_3). HRMS (ESI+) *m/z* $C_{18}H_{15}ON_3F_2$ ($M+H^+$) calcd 328.1256, obsd 328.1253.

2-(3-(4-fluorophenyl)-5-methyl-1H-pyrazol-1-yl)-N-(4-(trifluoromethyl)phenyl) acetamide (6e)

White solid; yield: 37%; mp: 150–152 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (s, 1H, NH), 7.86 – 7.74 (m, 4H, Ar–H), 7.70 (d, $J = 8.7$ Hz, 2H, Ar–H), 7.20 (t, $J = 8.9$ Hz, 2H, Ar–H), 6.53 (s, 1H, C_4–H), 5.05 (s, 2H, CH_2), 2.30 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.69 (CO), 162.08 (d, $^1J_{CF} = 242.0$ Hz), 148.85 (C-3), 142.62 (Ar–C), 141.99 (C-5), 130.47 (d, $^4J_{CF} = 3.0$ Hz), 127.32 (d, 2C,

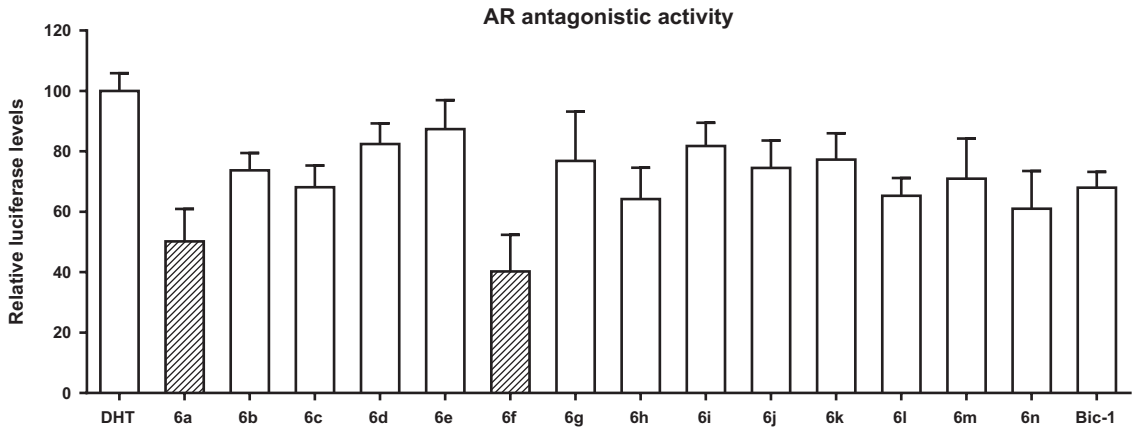
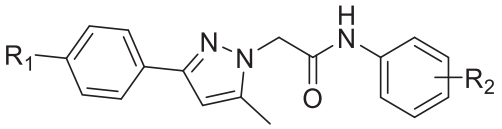


Fig. 3 AR antagonistic activity of compounds at the concentration of 1 μM (DHT: DHT at 10 nM, Bic-1 bicalutamide at 1 μM)

Table 1 Anti-proliferative activity in LNCaP and PC-3 cell lines



Code	Structure		Anti-proliferative activity (IC_{50}, μM)	
	R_1	R_2	LNCaP	PC-3
6a	F	3-F	38.66 ± 0.50	>80
6b	F	3-CF_3	25.45 ± 19.33	19.03 ± 0.33
6c	F	3-CN	51.10 ± 5.92	>80
6d	F	4-F	41.20 ± 17.68	>80
6e	F	4-CF_3	21.52 ± 11.16	34.92 ± 1.45
6f	F	2-F-3-Cl	28.20 ± 13.30	28.58 ± 4.39
6g	F	3-CF_3-4-CN	13.58 ± 3.18	20.21 ± 1.06
6h	CN	3-F	40.85 ± 18.07	>80
6i	CN	3-CF_3	21.09 ± 21.17	>80
6j	CN	3-CN	28.18 ± 12.01	>80
6k	CN	4-F	13.86 ± 3.33	>80
6l	CN	4-CF_3	32.55 ± 8.02	>80
6m	CN	2-F-3-Cl	24.84 ± 7.32	>80
6n	CN	3-CF_3-4-CN	52.35 ± 17.93	>80
Bicalutamide			34.96 ± 4.45	63.13 ± 2.49

$^3J_{CF} = 8.0$ Hz), 126.64(q, 2C, $^3J_{CF3} = 3.3$ Hz), 124.78 ((q, $^1J_{CF} = 269.7$ Hz), 124.16(q, $^2J_{CF} = 31.7$ Hz), 119.64 (2C, Ar–C), 115.86 (d, 2C, $^2J_{CF} = 21.0$ Hz), 102.97 (C-4), 52.75 (CH_2), 11.29 (CH_3). HRMS (ESI+) *m/z* $C_{19}H_{15}ON_3F_4$ ($M+H^+$) calcd 378.1224, obsd 378.1212.

N-(3-chloro-2-fluorophenyl)-2-(3-(4-fluorophenyl)-5-methyl-1H-pyrazol-1-yl) acetamide (6f)

White solid; yield: 30%; mp: 157–159 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H, NH), 7.58–7.49 (m, 2H, Ar–H), 7.34–7.27 (m, 2H, Ar–H), 7.25–7.16 (m, 3H, Ar–H), 6.21 (s, 1H, C_4–H), 4.95 (s, 2H, CH_2), 2.19 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.20 (CO), 162.65 (d, $^1J_{CF} = 245.0$ Hz), 162.07 (d, $^1J_{CF} = 243.0$ Hz), 147.63 (C-3), 141.97 (C-5), 131.07 (d, 2C, $^3J_{CF} = 8.0$ Hz), 130.45 (d, $^4J_{CF} = 2.0$ Hz), 127.32 (d, $^3J_{CF} = 8.0$ Hz), 126.32 (d, $^4J_{CF} = 2.0$ Hz), 125.55 (d, $^3J_{CF} = 4.0$ Hz), 120.36 (d, $^2J_{CF} = 16.0$ Hz), 116.23 (d, 2C, $^2J_{CF} = 21.0$ Hz), 115.90 (d, $^2J_{CF} = 22.0$ Hz), 106.29(C-4), 52.41 (CH_2), 13.72 (CH_3). HRMS (ESI+) *m/z* $C_{18}H_{14}ON_3F_2Cl$ ($M+H^+$) calcd 362.0866, obsd 362.0855.

N-(4-cyano-3-(trifluoromethyl)phenyl)-2-(3-(4-fluorophenyl)-5-methyl-1H-pyrazol-1-yl) acetamide (6g)

White solid; yield: 44%; mp: 150–152 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17 (s, 1H, NH), 8.28 (s, 1H, Ar–H), 8.13 (d, $J = 8.6$ Hz, 1H, Ar–H), 7.97 (dd, $J = 8.5$, 1.8 Hz, 1H, Ar–H), 7.77 (dd, $J = 8.8$, 5.6 Hz, 2H, Ar–H), 7.20 (t, $J = 8.9$ Hz, 2H, Ar–H), 6.54 (s, 1H, C_4–H), 5.09 (s, 2H, CH_2), 2.25 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.49 (CO), 162.10 (d, $^1J_{CF} = 243.0$ Hz), 149.00 (C-3), 143.57 (Ar–C), 142.05 (Ar–C), 137.05 (C-5), 132.30 (q, $^2J_{CF} = 31.3$ Hz), 130.39 (d, $^4J_{CF} = 3.0$ Hz), 127.32 (d, 2C, $^3J_{CF} = 8.0$ Hz), 124.20 (CN), 122.84 (q, $^1J_{CF} = 272.0$ Hz), 117.02 (q, $^3J_{CF} = 5.0$ Hz), 116.13 (Ar–C), 115.85 (d, 2C, $^2J_{CF} = 21.0$ Hz), 103.04 (Ar–C), 102.54 (C-4), 52.79 (CH_2), 11.24 (CH_3). HRMS (ESI+) *m/z* $C_{20}H_{14}ON_4F_4$ ($M+H^+$) calcd 403.1176, obsd 403.1188.

2-(3-(4-cyanophenyl)-5-methyl-1H-pyrazol-1-yl)-N-(3-fluorophenyl)acetamide (6h)

White solid; yield: 11%; mp: 218–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H, NH), 7.93 (d, $J = 8.5$ Hz, 2H, Ar–H), 7.83 (d, $J = 8.5$ Hz, 2H, Ar–H), 7.62 – 7.53 (m, 1H, Ar–H), 7.35 (dt, $J = 19.8$, 8.4 Hz, 2H, Ar–H), 6.92 (td,

$J = 8.5$, 2.8 Hz, 1H, Ar–H), 6.70 (s, 1H, C_4-H), 5.07 (s, 2H, CH_2), 2.31 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.16 (CO), 162.61 (d, $^1J_{CF} = 240.0$ Hz), 148.02 (C-3), 142.53 (Ar–C), 140.71 (d, $^3J_{CF} = 11.0$ Hz), 138.28 (C-5), 133.16 (2C, Ar–C), 131.04 (d, $^3J_{CF} = 10.0$ Hz), 125.97 (2C, Ar–C), 119.47 (CN), 115.46 (d, $^4J_{CF} = 2.0$ Hz), 110.64 (d, $^2J_{CF} = 21.0$ Hz), 110.02 (Ar–C), 106.54 (d, $^2J_{CF} = 26.0$ Hz), 104.08 (C-4), 52.91 (CH_2), 11.34 (CH_3). HRMS (ESI+) *m/z* $C_{19}H_{15}ON_4F$ ($M+H^+$) calcd 335.1303, obsd 335.1303.

2-(3-(4-cyanophenyl)-5-methyl-1H-pyrazol-1-yl)-N-(3-(trifluoromethyl)phenyl) acetamide (6i)

White solid; yield: 29%; mp: 199–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H, NH), 8.10 (s, 1H, Ar–H), 7.94 (d, $J = 8.5$ Hz, 2H, Ar–H), 7.84 (d, $J = 8.5$ Hz, 2H, Ar–H), 7.77 (d, $J = 7.9$ Hz, 1H, Ar–H), 7.59 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.44 (d, $J = 7.8$ Hz, 1H, Ar–H), 6.71 (s, 1H, C_4–H), 5.09 (s, 2H, CH_2), 2.32 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.43 (CO), 148.08 (C-3), 142.55 (Ar–C), 139.76 (C-5), 138.28 (Ar–C), 133.15 (2C, Ar–C), 130.64 (Ar–C), 130.05 (q, $^2J_{CF} = 31.3$ Hz), 125.97 (2C, Ar–C), 124.50 (q, $^1J_{CF} = 271.0$ Hz), 123.28 (Ar–C), 120.47 (q, $^3J_{CF} = 4.0$ Hz), 119.45 (CN), 115.78 (q, $^3J_{CF} = 4.0$ Hz), 110.04 (Ar–C), 104.08 (C-4), 52.90 (CH_2), 11.33 (CH_3). HRMS (ESI+) *m/z* $C_{20}H_{15}ON_4F_3$ ($M+H^+$) calcd 385.1271, obsd 385.1269.

N-(3-cyanophenyl)-2-(3-(4-cyanophenyl)-5-methyl-1H-pyrazol-1-yl)acetamide (6j)

White solid; yield: 31%; mp: 219–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H, NH), 8.08 (s, 1H, Ar–H), 7.93 (d, $J = 8.5$ Hz, 2H, Ar–H), 7.87 – 7.80 (m, 3H, Ar–H), 7.56 (s, 2H, Ar–H), 6.71 (s, 1H, C_4–H), 5.09 (s, 2H, CH_2), 2.32 (s, 3H, CH_3).^{13}C NMR (100 MHz, DMSO-d_6) δ 166.48 (CO), 148.08 (C-3), 142.56 (Ar–C), 139.77 (C-5), 138.26 (Ar–C), 133.15 (2C, Ar–C), 130.87 (Ar–C), 127.74 (Ar–C), 125.97 (2C, Ar–C), 124.31 (Ar–C), 122.44 (Ar–C), 119.46 (CN), 119.04 (Ar–C), 112.20 (Ar–C), 110.04 (Ar–C), 104.10 (C-4), 52.89 (CH_2), 11.33 (CH_3). HRMS (ESI+) *m/z* $C_{20}H_{15}ON_5$ ($M+H^+$) calcd 342.1349, obsd 342.1343.

2-(3-(4-cyanophenyl)-5-methyl-1H-pyrazol-1-yl)-N-(4-fluorophenyl)acetamide (6k)

White solid; yield: 17%; mp: 247–250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H, NH), 7.93 (d, $J = 8.5$ Hz, 2H, Ar–H), 7.83 (d, $J = 8.5$ Hz, 2H, Ar–H), 7.61 (dd, $J =$ 9.1, 5.0 Hz, 2H, Ar–H), 7.17 (t, $J = 8.9$ Hz, 2H, Ar–H), 6.70 (s, 1H, C_4–H), 5.04 (s, 2H, CH_2), 2.31 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.67 (CO), 158.67 (d, $^1J_{CF} = 239.0$ Hz), 147.96 (C-3), 142.49 (Ar–C), 138.32 (C-5), 135.41 (d, $^4J_{CF} = 2.0$ Hz), 133.16 (2C, Ar–C), 125.96 (2C, Ar–C), 121.50 (d, 2C, $^3J_{CF} = 8.0$ Hz), 119.47 (CN), 115.94 (d, 2C, $^2J_{CF} = 23.0$ Hz), 110.00 (Ar–C), 104.06 (C-4), 52.85 (CH_2), 11.36 (CH_3). HRMS (ESI+) *m/z* $C_{19}H_{15}ON_4F$ ($M+H^+$) calcd 335.1303, obsd 335.1299.

2-(3-(4-cyanophenyl)-5-methyl-1H-pyrazol-1-yl)-N-(4-(trifluoromethyl)phenyl) acetamide (6l)

White solid; yield: 30%; mp: 230–231 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H, NH), 7.93 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.86 – 7.77 (m, 4H, Ar–H), 7.71 (d, $J = 8.7$ Hz, 2H, Ar–H), 6.71 (s, 1H, C_4–H), 5.10 (s, 2H, CH_2), 2.32 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.47 (CO), 148.05 (C-3), 142.54 (Ar–C), 138.26 (C-5), 133.15 (2C, Ar–C), 126.69 (q, 2C, $^3J_{CF} = 3.7$ Hz), 125.96 (2C, Ar–C), 124.77 (q, $^1J_{CF} = 269.7$ Hz), 124.16 (q, $^2J_{CF} = 32.0$ Hz), 123.42 (Ar–C), 119.65 (2C, Ar–C), 119.47 (CN), 110.03 (Ar–C), 104.09 (C-4), 52.95 (CH_2), 11.33 (CH_3). HRMS (ESI+) *m/z* $C_{20}H_{15}ON_4F_3$ ($M+H^+$) calcd 385.1271, obsd 385.1259.

N-(3-chloro-2-fluorophenyl)-2-(3-(4-cyanophenyl)-5-methyl-1H-pyrazol-1-yl) acetamide (6m)

White solid; yield: 9%; mp: 165–167 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H, NH), 7.94 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.89 – 7.77 (m, 1H, Ar–H), 7.71 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.36 (t, $J = 7.5$ Hz, 1H, Ar–H), 7.19 (t, $J = 8.2$ Hz, 1H, Ar–H), 6.37 (s, 1H, C_4–H), 5.04 (s, 2H, CH_2), 2.20 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.03 (CO), 149.73 (d, $^1J_{CF} = 246.0$ Hz), 148.01 (C-3), 143.71 (Ar–C), 135.18 (C-5), 133.20 (2C, Ar–C), 129.48 (2C, Ar–C), 127.54 (d, $^3J_{CF} = 11.0$ Hz), 126.36 (Ar–C), 125.51 (d, $^4J_{CF} = 4.0$ Hz), 123.15 (Ar–C), 120.38 (d, $^2J_{CF} = 16.0$ Hz), 119.04 (CN), 111.45 (Ar–C), 107.18 (C-4), 52.81(CH_2), 13.65 (CH_3). HRMS (ESI+) *m/z* $C_{19}H_{14}ON_4FCl$ ($M+H^+$) calcd 369.0913, obsd 369.0906.

N-(4-cyano-3-(trifluoromethyl)phenyl)-2-(3-(4-cyanophenyl)-5-methyl-1H-pyrazol-1-yl) acetamide (6n)

White solid; yield: 29%; mp: 244–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H, NH), 8.28 (d, $J = 1.9$ Hz, 1H, Ar–H), 8.13 (d, $J = 8.6$ Hz, 1H, Ar–H), 7.99 – 7.90 (m, 3H, Ar–H), 7.84 (d, $J = 8.5$ Hz, 2H, Ar–H), 6.72 (s, 1H, C_4–H), 5.14 (s, 2H, CH_2), 2.31 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.29 (CO), 148.20 (C-3), 143.54 (Ar–C), 142.61 (Ar–C), 138.19 (C-5), 137.13 (Ar–C), 133.16 (2C, Ar–C), 132.30 (q, $^2J_{CF} = 31.7$ Hz), 125.98 (2C, Ar–C), 122.86 (q, $^1J_{CF} = 271.7$ Hz), 122.68 (CN), 119.44 (CN), 117.10 (q, $^3J_{CF} = 5.0$ Hz), 116.14 (Ar–C), 110.08 (Ar–C), 104.16 (Ar–C), 102.59 (C-4), 53.00 (CH_2), 11.29 (CH_3). HRMS (ESI+) *m/z* $C_{21}H_{14}ON_5F_3$ ($M+H^+$) calcd 410.1223, obsd 410.1235.

Biological analysis

Cell culture

MDA-Kb2, LNCaP, and PC-3 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). MDA-Kb2 cells were cultured in L15 media containing L-glutamine (Hyclone), Na pyruvate (Hyclone), penstrep (Hyclone), and 10% FBS (Hyclone). LNCaP cells were cultured in RPMI-1640 media (Gibco, with addition of $NaHCO_3$ 1.5 g/L, glucose 2.5 g/L, and sodium pyruvate 0.11 g/L) with 10% FBS (Gibco). PC-3 cells were cultured in F12 media (Hyclone) with 10% FBS (Hyclone). All cells were propagated in an incubator at 37 °C with 5% CO_2.

Luciferase reporter assay

MDA-Kb2 cells were seeded in 96-well plates (Nunc) at a density of 3×10^4 cells/well and incubated for 24 h. The cells were treated with the test compounds at 1 μM in combination with 10 nM DHT. Cells only exposed to 10 nM DHT were used as a control group. After 24 h of incubation, cells were lysed using reporter lysis buffer (Promega) and the luciferase levels were determined with luciferase assay kit (Promega) in a TD 20/20 luminometer (Turner Designs, USA). The antagonism rates of the synthesized compounds were calculated using the luciferase activities.

Cell proliferation assay

PC-3 cells (5000 cells/well) and LNCaP cells (3000 cells/well) were seeded in 96-well culture plates. The cells were cultured for 12 h (PC-3) or 36 h (LNCaP). The test compounds were added to determine their growth inhibitory effects at different concentrations. Bicalutamide was used as a positive control. Following incubation for 72 h (PC-3) or 96 h (LNCaP), 10 μL MTT solution (5 mg/ml) was added and the cells were incubated for another 4 h at 37 °C with 5% CO_2. Thereafter the medium was removed and 200 μL of DMSO was added to dissolve formazan. The absorbance was determined in an ELX800 microplate reader (BioTek, Winooski, VT, USA) at 570 nm.

Conclusion

In summary, a series of 2-(5-methyl-1*H*-pyrazol-1-yl) acetamide derivatives were developed via structural modification of a pyrazole based lead compound **10e**. We determined the AR antagonistic activity of the compounds and evaluated their growth inhibitory effects using the LNCaP and PCa cell lines. The AR luciferase reporter assay revealed that compound **6f** was a potent AR antagonist. Some compounds in this series also showed higher antiproliferative activity against LNCaP cells than Bicalutamide. These compounds can be used to discover novel potent AR antagonists after further optimization.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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