Synthesis and biological evaluation of 3-(4-fluorophenyl)-1*H*-pyrazole derivatives as androgen receptor antagonists

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A novel series of 3-(4-fluorophenyl)-1*H*-pyrazole derivatives were synthesized and evaluated for their antiproliferative activity against two prostate cancer cell lines (LNCaP and PC-3) and androgen receptor target gene prostate-specific antigen (PSA) inhibitory activity in LNCaP cells. Several compounds showed potent antiproliferative activity against LNCaP cells and showed a promising PSA downregulation rate. Among these, compound 10e selectively inhibited LNCaP cell growth with an IC_{50} value of 18 μmol/l and showed a PSA downregulation rate of 46%, which was better than the lead compound T3. *Anti-Cancer Drugs* 27:278–285

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Introduction

Prostate cancer (PC) is one of the most frequently diagnosed cancers in men in the USA [1,2]. Androgen receptor (AR) plays a vital role in PC, which regulates the transcription and translation of downstream genes for the survival of PC cells when activated by androgens such as testosterone and dihydrotestosterone [3]. Blocking the androgen signaling pathway by blocking the production of testicular androgen through chemical or surgical castration [androgen deprivation therapy (ADT)] has been the standard method for the treatment of PC for decades. However, after several months of ADT, there may be a relapse of PC and progression to a new stage called castration-resistant/recurrent prostate cancer (CRPC) [4,5]. Once CRPC occurs, ADT will no longer work, but the survival of CRPC still relies on the androgen signaling pathway even after castration [6–8]. Therefore, use of AR antagonists to block the binding of androgens to AR remains an attractive strategy to treat CRPC [1].

Our study began with compound **T3** (Fig. 1), a 3-(4-fluorophenyl)-1*H*-pyrazole derivative, which was reported in our previous work [5]. **T3** was tested for antiproliferative activity against the LNCaP cell line, which expresses mutant AR, and the PC-3 cell line, which is AR negative, and showed an IC_{50} of 17 and 57 μmol/l respectively, suggesting a certain degree of selectivity against the LNCaP cell line. The inhibition of prostate-specific antigen (PSA) expression was also investigated, with an inhibition rate of 60.7% at the concentration of 10 μmol/l. The binding affinity of **T3**, evaluated by measuring the ability to displace a potent fluorescently labeled androgen (fluorophore) from the active site of AR ligand binding domain, was 58.1% at the concentration of 10 μmol/l. In our efforts to improve the selectivity to inhibit cell growth of LNCaP cell and the ability to inhibit the expression of PSA, we performed structure modification of **T3**. First, we replaced the propyl chain of **T3** to methylene to shorten the distance between the pyrazole ring and the aromatic ring and removed the methyl group of amino to increase the flexibility of the heteroaromatic ring, generating compound **10**. Second, we changed the position of amino and methylene of compound **10**, obtaining compound **11**. Third, replacement of methylene of compound **11** to the carbonyl group yielded compound **12** (Fig. 1).

Materials and methods

Reagents and materials

All materials were commercially available and used without further purification. Thin-layer chromatography (TLC) was used to monitor reaction in SGF254 plates. Column chromatography was performed on a silica gel (60 Å, 200–300 mesh). Melting points were determined on a Büchi capillary melting point apparatus (Buchi Labortechnik AG, Flawill, Switzerland) without correction. The ^{1}H NMR spectra were recorded on a Bruker Avance DRX-600 Spectrometer (Bruker, Rheinstetten, Germany) or a Bruker Avance DRX-400 Spectrometer. The ^{13}C NMR spectra were assessed on a Bruker Avance DRX-400 spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) and the following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), and quartet of doublets (qd). The purity data were obtained

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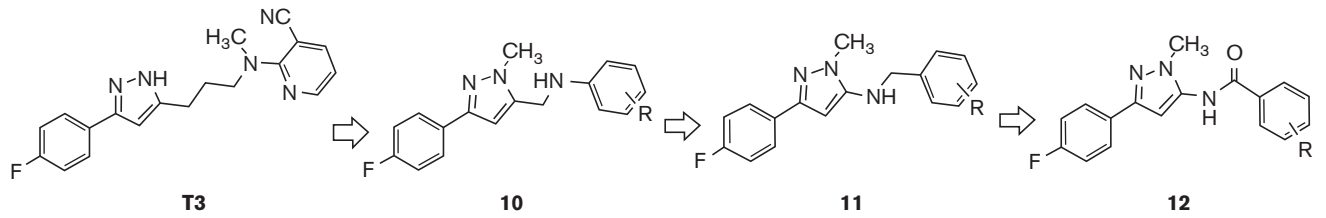
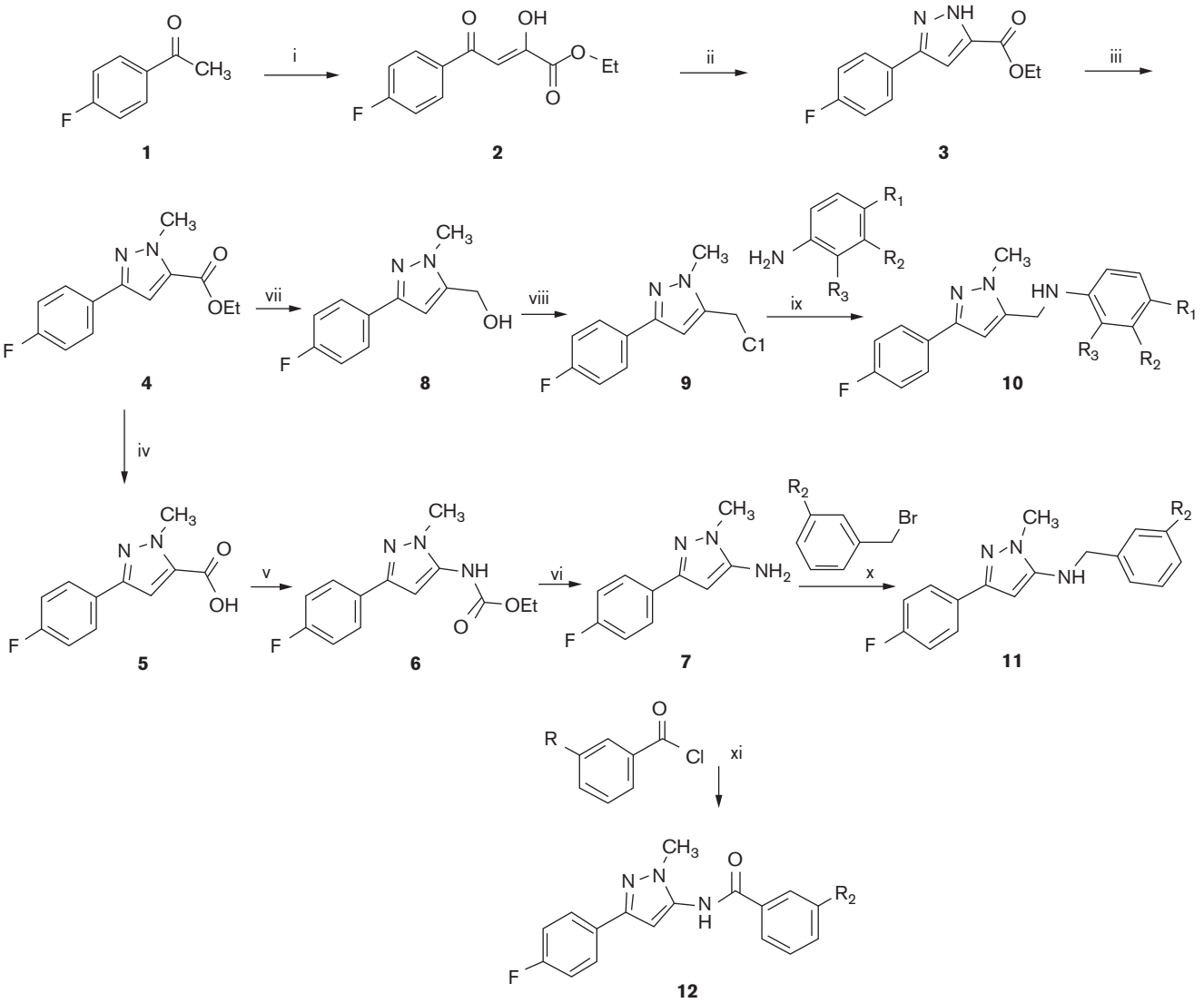
Fig. 1



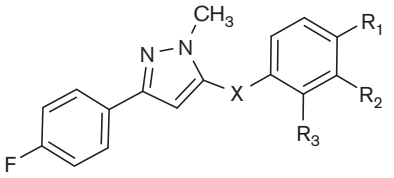
Design strategy for target compounds **10**, **11**, and **12**.

Scheme 1



Synthesis of target compounds **10**, **11**, and **12**. The reagents and conditions were as follows: (i) EtONa, diethyl oxalate, EtOH, reflux, 2 h; (ii) hydrazine hydrate, acetic acid, EtOH, reflux, 3 h; (iii) dimethyl sulfate, NaH, DMF, 0°C; (iv) KOH, EtOH, reflux, 5 h; (v) DPPA, Et_3N,1,4-dioxane, EtOH, reflux, 7 h; (vi) NaOH, EtOH, reflux, 5 h; (vii) $LiAlH_4$, THF, 0°C, 15 min; (viii) $SOCl_2$, CH_2Cl_2, rt, 1 h; (ix) K_2CO_3, KI, acetone, rt, 8 h; (x) NaH, DMF, rt, 16 h; and (xi) pyridine, THF, rt, 2 h. DMF, *N*,*N*-dimethylformamide; DPPA, diphenylphosphoryl azide; THF, tetrahydrofuran.

Table 1 Abilities of target compounds to inhibit prostate-specific antigen expression and growth of prostate cancer cell lines



Codes	X	R_1	R_2	R_3	PSA expression inhibition rate (%) (5 μmol/l)	Cell growth inhibition (IC_{50}/μmol/l)	
						LNCaP	PC-3
Enzalutamide	–	–	–	–	67.7	32.1 ± 6.5	> 80
T3	–	–	–	–	36.0	26.9 ± 7.9	48.4 ± 8.0
10a	CH_2NH	H	Br	H	33.8	26.2 ± 8.7	38.7 ± 2.1
10b	CH_2NH	H	CF_3	H	11.9	20.4 ± 3.8	21.6 ± 3.3
10c	CH_2NH	CN	CN	H	46.8	57.1 ± 2.7	> 80
10d	CH_2NH	H	CN	H	39.8	27.9 ± 7.0	> 80
10e	CH_2NH	F	H	H	46.3	17.8 ± 0.1	> 80
10f	CH_2NH	H	Cl	H	41.3	20.1 ± 5.8	51.6 ± 1.6
10g	CH_2NH	Cl	H	H	36.3	27.9 ± 1.8	60.3 ± 7.3
10h	CH_2NH	CN	H	H	63.6	61.8 ± 2.9	> 80
10i	CH_2NH	Br	H	H	30.3	26.6 ± 2.9	38.7 ± 2.8
10j	CH_2NH	CF_3	H	H	43.2	30.7 ± 9.9	26.0 ± 9.9
10k	CH_2NH	Cl	CF_3	H	24.5	25.0 ± 1.8	36.6 ± 5.3
10l	CH_2NH	H	F	Br	37.5	31.6 ± 9.4	68.4 ± 5.8
10m	CH_2NH	H	Cl	F	27.8	41.1 ± 9.5	48.3 ± 3.0
10n	CH_2NH	H	Br	CH_3	33.0	25.4 ± 2.1	37.4 ± 6.4
10o	CH_2NH	F	Cl	H	54.7	37.2 ± 3.5	64.9 ± 5.2
12a	NHCO	H	Br	H	6.1	43.6 ± 9.0	> 80
12b	NHCO	H	CF_3	H	2.6	30.8 ± 7.6	> 80
11a	$NHCH_2$	H	Br	H	1.4	30.0 ± 5.1	26.9 ±3.3

PSA, prostate-specific antigen.

in a Shimadzu LC-20AT High-performance Liquid Chromatograph (Shimadzu Corporation, Tokyo, Japan).

Synthesis

(Z)-Ethyl 4-(4-fluorophenyl)-2-hydroxy-4-oxobut-2-enoate (2)

To a solution of 4-fluoroacetophenone **1** (3.00 g, 21.74 mmol) and diethyl oxalate (6.35 g, 43.48 mmol) in absolute anhydrous ethanol (10 ml) sodium ethoxide (2.96 g, 43.48 mmol) was added dropwise in absolute anhydrous ethanol (15 ml). The mixture was then heated to 80°C and stirred for 5–7 h. The reaction mixture was evaporated under reduced pressure and ice water was added to the residue obtained. The pH of the mixture was adjusted to 1 using 1 N HCl. The precipitation was filtered and washed with water to yield compound **2** as a yellowish white solid.

Ethyl 3-(4-fluorophenyl)-1H-pyrazole-5-carboxylate (3)

A solution of **2** (5.17 g, 21.72 mmol) in ethanol (15 ml) was added to hydrazine hydrate (1.36 g, 21.72 mmol), followed by the addition of glacial acetic acid (1 ml). The mixture was then heated to 90°C and refluxed for 3 h. The reaction mixture was concentrated under reduced pressure to yield compound **3** as a faint yellow solid (4.8 g, 93%). Mp: 161.0–163.0°C; MS (ESI) *m/z* found: 235.3 $[M + H]^+$.

Ethyl 3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-carboxylate (4)

To a solution of **3** (4.00 g, 17.1 mmol) in *N*,*N*-dimethylformamide (DMF) sodium hydride (1.03 g, 25.8 mmol) was added in an ice bath. Dimethyl sulfate (3.25 g, 25.8 mmol) was added dropwise after the solution became clear. The mixture was stirred for an additional 30 min then poured into 100 ml ice water. The aqueous phase was extracted with ethyl acetate (30 ml × 3). The organic layer was combined and washed with water (30 ml × 3) and brine (30 ml × 1), and dried with anhydrous sodium sulfate. The solution was filtered. The filtrate was purified by chromatography over a silica gel (90% petroleum ether : ethyl acetate) to yield **4** as a white solid (3.22 g, 76% yield). Mp: 54.0–56.0°C; MS (ESI) *m/z* found: 249.3 $[M + H]^+$, ^1H NMR (600 MHz, DMSO-d_6) δ (ppm): 7.900(dd, $J_1 = 9.0$ Hz, $J_2 = 5.4$ Hz, 2H), 7.354(s, 1H), 7.247(t, $J = 9.0$ Hz, 2H), 4.333(q, $J = 7.2$ Hz, 2H), 4.132(s, 3H), 1.334(t, $J = 7.2$ Hz, 3H).

3-(4-Fluorophenyl)-1-methyl-1H-pyrazole-5-carboxylic acid (5)

To a solution of compound **4** (1.98 g, 8 mmol) in ethyl alcohol (15 ml) a 10% potassium hydroxide aqueous solution was added (20 ml). The reaction mixture was refluxing for 5 h and monitored by TLC until no reactant was left. The reaction was cooled to room temperature

and then evaporated under reduced pressure to remove ethyl alcohol. The residue was neutralized with saturated citric acid solution until the pH was less than 2. The suspension was filtered and the filter cake was washed completely with water to yield compound **5** as a white solid (1.74 g, 99%). Mp: 212.0–214.5°C; MS (ESI) *m/z* found: 221.4 $[M+H]^+$, ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 13.473(s, 1H), 7.878(dd, $J_1=9.0$ Hz, $J_2=5.4$ Hz, 2H), 7.291(s, 1H), 7.243(t, $J=9.0$ Hz, 2H), 4.119(s, 3H).

Ethyl (3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl) carbamate (6)

Compound **5** (1.00 g, 4.55 mmol) was dissolved in absolute ethyl alcohol (15 ml) and anhydrous dioxane (15 ml). Diphenylphosphoryl azide (DPPA, 1.53 g, 5.9 mmol) and triethylamine (0.60 g, 5.9 mmol) were added and the solution was heated to reflux for 7 h and monitored by TLC. The reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue was then dissolved with ethyl acetate (30 ml) and water (30 ml). The organic layer was washed with saturated citric acid solution (30 ml × 3), aqueous sodium hydrogen carbonate (30 ml × 3), and brine (30 ml × 1), and dried with anhydrous sodium sulfate, filtered, and concentrated. The crude material was absorbed onto a silica gel and purified using 75% of petroleum ether in ethyl acetate to yield compound **6** as a white solid (0.9 g, 75% yield). Mp: 124–126°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 9.678(s, 1H), 7.774(dd, $J_1=8.4$ Hz, $J_2=6.0$ Hz, 2H), 7.206(t, $J=9.0$ Hz, 2H), 6.549(s, 1H), 4.154(q, $J=7.2$ Hz, 2H), 1.254(t, $J=7.2$ Hz, 3H).

3-(4-Fluorophenyl)-1-methyl-1H-pyrazole-5-amine (7)

Ten per cent sodium hydroxide aqueous solution (30 ml) was added to a solution of compound **6** (1.29 g, 4.9 mmol) in ethyl alcohol (30 ml). The reaction mixture was refluxed for 5 h and monitored by TLC. After completion of the reaction, ethyl alcohol was evaporated under reduced pressure. Solid was precipated after ice water was added. The the mixture was filtered to yield compound **7** as a white solid (0.75 g, 80% yield). Mp: 129–131°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 7.673(dd, $J_1=8.4$ Hz, $J_2=6.0$ Hz, 2H), 7.148(t, $J=9.0$ Hz, 2H), 5.563(s, 1H), 5.273(s, 2H), 3.553(s, 3H).

(3-(4-Fluorophenyl)-1-methyl-1H-pyrazole-5-yl) methanol (8)

To a solution of compound **4** (2.98 g, 12 mmol) in tetrahydrofuran (THF) (40 ml), lithium aluminium hydride (1.37 g, 36 mmol) was added under ice-cold conditions. The mixture was stirred at room temperature for 15 min. The reaction was quenched with ice water (50 ml) that was added dropwise under ice-cold conditions. THF was then removed under vacuum. The residue liquid was neutralized with 1 N hydrochloric acid until the pH was less than 1 and extracted with ethyl acetate (50 ml × 3). Organic layers were combined and washed with brine (50 ml × 1), dried with anhydrous sodium sulfate, filtered, and concentrated to yield compound **8** as a white solid (2.47 g, 100% yield). Mp: 112–114°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 7.783(dd, $J_1=8.4$ Hz, $J_2=5.4$ Hz, 2H), 7.203(t, $J=9.0$ Hz, 2H), 6.597(s, 1H), 5.323(t, $J=5.4$ Hz, 1H), 4.518(d, $J=5.4$ Hz, 2H), 3.824 (s, 3H).

5-(Chloromethyl)-3-(4-fluorophenyl)-1-methyl-1H-pyrazole (9)

A solution of compound **8** (165 mg, 0.8 mmol) in dichloromethane was added to thionyl chloride (190 mg, 1.6 mmol) and stirred at room temperature for 1 h. The solvent was removed under vacuum to yield compound **9** as a faint yellow solid (180 mg, 100% yield). Mp: 124–126°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.799(t, $J=7.2$ Hz, 2H), 7.227(t, $J=7.2$ Hz, 2H), 6.800(s, 1H), 4.953(s, 2H), 3.891(s, 3H).

General procedure for the preparation of 10a–10o

A mixture of substituted aniline (0.8 mmol), potassium carbonate (553 mg, 4 mmol), and potassium iodide (13 mg, 0.08 mmol) in acetone (12 ml) was sitrred at room temperature. To the suspension, a solution of compound **9** (179 mg, 0.8 mmol) was added in acetone (6 ml) dropwise. The reaction was stirred for 8 h and monitored by TLC. Solvent was removed under vaccum, followed by the addition of ethyl acetate (40 ml) and water (30 ml). The organic layer was washed with water (30 ml × 1) and brine (30 ml × 1), dried with anhydrous sodium sulfate, filtered, and concentrated. The crude material was absorbed onto silica gel and purified using 90% of petroleum ether in ethyl acetate to yield title compound **10**.

3-Bromo-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)methyl)aniline (10a)

White solid, 115 mg, yield 40%. Mp: 85–87°C, purity: 97.1%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.759 (dd, $J_1=8.8$ Hz, $J_2=5.6$ Hz, 2H), 7.196(t, $J=8.8$ Hz, 2H), 7.030(t, $J=8.0$ Hz, 1H), 6.844(t, $J=1.6$ Hz, 1H), 6.703 (dd, $J_1=7.6$ Hz, $J_2=1.2$ Hz, 1H), 6.669(dd, $J_1=8.4$ Hz, $J_2=2.0$ Hz, 1H), 6.621(s, 1H), 6.447(t, $J=5.6$ Hz, 1H), 4.324(d, $J=5.6$ Hz, 2 H), 3.839(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.02(d, $J=242$ Hz), 150.36, 147.99, 142.31, 131.21, 130.41(d, $J=3$ Hz), 127.23(d, $J=8$ Hz), 122.77, 119.06, 115.93(d, $J=22$ Hz), 114.99, 111.80, 103.00, 38.35, 36.95.

N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl) methyl)-3-(trifluoromethyl)aniline (10b)

White solid, 95 mg, yield 34%. Mp: 85–87°C, purity: 97.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.755 (dd, $J_1=8.4$ Hz, $J_2=5.6$ Hz, 2H), 7.299(t, $J=8.0$ Hz, 1H), 7.193(t, $J=8.8$ Hz, 2H), 6.959(s, 1H), 6.938(d, $J=8.8$ Hz, 1H), 6.858(d, $J=7.6$ Hz, 1H), 6.636(m, 2H), 4.385(s, 2H), 3.852(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.04(d, $J=242$ Hz), 149.16, 148.02, 142.15, 130.42(d, $J=3$ Hz),

130.44(q, $J = 31$ Hz), 130.32, 127.22(d, $J = 8$ Hz), 124.98 (q, $J = 271$ Hz), 116.13, 115.92(d, $J = 21$ Hz), 112.65(q, $J = 4$ Hz), 108.81(q, $J = 4$ Hz), 103.07, 38.33, 36.95.

4-(((3-(4-Fluorophenyl)-1-methyl-1H-pyrazole-5-yl) methyl)amino)phthalonitrile (10c)

White solid, 45 mg, yield 17%. Mp: 224–226°C, purity: 97.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.786 ~ 7.712(m, 4H), 7.262(d, $J = 2.4$ Hz, 1H), 7.203(t, $J = 8.8$ Hz, 2H), 7.042(dd, $J_1 = 8.8$ Hz, $J_2 = 2.4$ Hz, 1H), 6.646(s, 1H), 4.500(d, $J = 5.6$ Hz, 2H), 3.843(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.07(d, $J = 242$ Hz), 152.09, 148.15, 140.95, 135.24, 130.28(d, $J = 3$ Hz), 127.27 (d, $J = 8$ Hz), 117.86, 116.94, 115.94(d, $J = 21$ Hz), 103.13, 99.23, 37.82, 37.01.

3-(((3-(4-Fluorophenyl)-1-methyl-1H-pyrazole-5-yl) methyl)amino)benzonitrile (10d)

White solid, 196 mg, yield 80%. Mp: 129–131°C, purity: 97.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.759 (dd, $J_1 = 8.4$ Hz, $J_2 = 5.6$ Hz, 2H), 7.275(d, $J = 8.0$ Hz, 1H), 7.193(t, $J = 8.8$ Hz, 2H), 7.016(s, 1H), 7.005(d, $J = 8.8$ Hz, 1H), 6.964(d, $J = 7.2$ Hz, 1H), 6.662(t, $J = 7.6$ Hz, 1H), 6.628(s, 1H), 4.374(d, $J = 7.6$ Hz, 2H), 3.845(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.03(d, $J = 242$ Hz), 149.22, 148.01, 142.01, 130.52, 130.45(d, $J = 3$ Hz), 127.22(d, $J = 8$ Hz), 119.94, 117.84, 115.92(d, $J = 22$ Hz), 114.69, 112.17, 103.03, 38.23, 37.00.

4-Fluoro-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)methyl)aniline (10e)

White solid, 96 mg, yield 50%. Mp: 115–117°C, purity: 97.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.759 (dd, $J_1 = 8.8$ Hz, $J_2 = 5.6$ Hz, 2H), 7.199(t, $J = 8.8$ Hz, 2H), 6.939(t, $J = 8.8$ Hz, 2H), 6.671(dd, $J_1 = 8.8$ Hz, $J_2 = 4.8$ Hz, 2H), 6.626(s, 1H), 6.056(t, $J = 6.0$ Hz, 1H), 4.286(d, $J = 6.0$ Hz, 2H), 3.848(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.01(d, $J = 242$ Hz), 155.08(d, $J = 230$ Hz), 147.95, 145.41(d, $J = 1$ Hz), 142.74, 130.46(d, $J = 3$ Hz), 127.21(d, $J = 8$ Hz), 115.91(d, $J = 21$ Hz, 115.71 (d, $J = 22$ Hz), 113.74(d, $J = 7$ Hz), 103.01, 39.12, 36.91.

3-Chloro-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)methyl)aniline (10f)

White solid, 159 mg, yield 63%. Mp: 77–79°C, purity: 97.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.759 (dd, $J_1 = 8.4$ Hz, $J_2 = 7.2$ Hz, 2H), 7.201(t, $J = 8.8$ Hz, 2H), 7.095(t, $J = 8.0$ Hz, 1H), 6.703(t, $J = 2.0$ Hz, 1H), 6.648(d, $J = 1.6$ Hz, 1H), 6.629(s, 1H), 6.578(dd, $J_1 = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H), 6.467(t, $J = 5.6$ Hz, 1H), 4.326(d, $J = 5.6$ Hz, 2H), 3.846(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.03(d, $J = 242$ Hz), 150.25, 147.99, 142.77, 134.11, 130.84, 130.47(d, $J = 2$ Hz), 127.22(d, $J = 8$ Hz), 116.15, 115.92(d, $J = 21$ Hz), 112.08, 111.53, 103.02, 38.42, 36.98.

4-Chloro-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)methyl)aniline (10g)

White solid, 119 mg, yield 47%. Mp: 106–108°C, purity: 96.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.758 (dd, $J_1 = 8.8$ Hz, $J_2 = 5.6$ Hz, 2H), 7.197(t, $J = 8.8$ Hz, 2H), 7.114(d, $J = 8.8$ Hz, 2H), 6.684(d, $J = 8.8$ Hz, 2H), 6.618(s, 1H), 6.344(t, $J = 5.6$ Hz, 1H), 4.310(d, $J = 6.0$ Hz, 2H), 3.843(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.01(d, $J = 242$ Hz), 147.95, 147.61, 142.46, 130.43(d, $J = 3$ Hz), 129.04, 127.22(d, $J = 9$ Hz), 120.06, 115.91(d, $J = 22$ Hz), 114.26, 103.02, 38.60, 36.93.

4-(((3-(4-Fluorophenyl)-1-methyl-1H-pyrazole-5-yl) methyl)amino)benzonitrile (10h)

White solid, 169 mg, yield 69%. Mp: 194–196°C, purity: 94.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.763 (dd, $J_1 = 9.2$ Hz, $J_2 = 5.6$ Hz, 2H), 7.491(d, $J = 8.8$ Hz, 2H), 7.199(t, $J = 9.2$ Hz, 2H), 6.763(d, $J = 8.8$ Hz, 2H), 6.636(s, 1H), 4.420(d, $J = 6.0$ Hz, 1H), 3.846(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.04(d, $J = 242$ Hz, 152.14, 148.06, 141.70, 133.84, 130.34(d, $J = 3$ Hz), 127.25(d, $J = 8$ Hz), 120.93, 115.93(d, $J = 21$ Hz), 112.75, 103.13, 97.00, 37.86, 36.98.

4-Bromo-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)methyl)aniline (10i)

White solid, 182 mg, yield 63%. Mp: 117–119°C, purity: 97.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.751 (dd, $J_1 = 8.8$ Hz, $J_2 = 5.6$ Hz, 2H), 7.208(t, $J = 8.8$ Hz, 2H), 7.190(d, $J = 8.8$ Hz, 2H), 6.454(d, $J = 8.8$ Hz, 2H), 6.607(s, 1H), 6.361(t, $J = 5.6$ Hz, 1H), 4.301(d, $J = 6.0$ Hz, 2H), 3.835(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.04(d, $J = 243$ Hz), 148.06, 147.80, 142.59, 131.88, 130.19(d, $J = 3$ Hz), 127.28(d, $J = 8$ Hz), 115.94(d, $J = 22$ Hz), 114.89, 107.56, 103.02, 38.39, 36.80.

N-((3-(4-Fluorophenyl)-1-methyl-1H-pyrazole-5-yl) methyl)-4-(trifluoromethyl)aniline (10j)

White solid, 81 mg, yield 29%. Mp: 109–111°C, purity: 91.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.759 (dd, $J_1 = 9.2$ Hz, $J_2 = 5.6$ Hz, 2H), 7.399(d, $J = 8.8$ Hz, 2H), 7.191(t, $J = 9.2$ Hz, 2H), 6.893(s, 1H), 6.785(d, J = 8.8 Hz, 2H), 6.634(s, 1H), 4.393(s, 2H), 3.849(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.03(d, $J = 242$ Hz), 151.67, 148.02, 142.03, 130.43(d, $J = 3$ Hz), 127.23(d, J = 8 Hz), 126.68(q, $J = 4$ Hz), 125.77(q, $J = 268$ Hz), 116.41(q, $J = 32$ Hz), 115.90(d, $J = 22$ Hz), 112.23, 103.09, 38.12, 36.98.

4-Chloro-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)methyl)-3-(trifluoromethyl)aniline (10k)

White solid, 25 mg, yield 8%. Mp: 124–126°C, purity: 87.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.758 (dd, $J_1 = 8.8$ Hz, $J_2 = 5.6$ Hz, 2H), 7.372(d, $J = 8.8$ Hz, 1H), 7.196(t, $J = 8.8$ Hz, 2H), 7.096(d, $J = 2.8$ Hz, 1H), 6.911(dd, $J_1 = 8.8$ Hz, $J_2 = 2.8$ Hz, 1H), 6.814(t, $J = 5.2$ Hz, 1H), 6.629(s, 1H), 4.393(d, $J = 5.2$ Hz, 2H),

3.844(s, 3H), ^{13}C NMR (100 MHz, DMSO) δ: 162.08(d, J = 243 Hz), 148.19, 147.57, 142.03, 132.43, 130.02(d, J = 3 Hz), 127.33(q, J = 30 Hz), 127.30(d, J = 8 Hz), 123.46(q, J = 271 Hz), 116.83, 116.55, 115.97(d, J = 21 Hz), 111.63(q, J = 5 Hz), 103.08, 38.16, 36.77.

3-Bromo-4-fluoro-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)methyl)aniline (10l)

White solid, 42 mg, yield 14%. Mp: 101–103°C, purity: 97.1%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.757 (dd, J_1 = 8.8 Hz, J_2 = 5.6 Hz, 2H), 7.195(t, J = 8.8 Hz, 2H), 7.012(t, J = 8.8 Hz, 1H), 6.922(dd, J_1 = 5.6 Hz, J2 = 2.8 Hz, 1H), 6.932 ~ 6.911(m, 1H), 6.616(s, 1H), 6.300(t, J = 5.6 Hz, 1H), 4.307(d, J = 6.0 Hz, 2H), 3.836(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.03(d, J = 242 Hz), 150.91(d, J = 231 Hz), 148.00, 146.52(d, J = 2 Hz), 142.27, 130.41(d, J = 2 Hz), 127.23(d, J = 6 Hz), 117.15(d, J = 23 Hz), 116.00(d, J = 7 Hz), 115.82, 113.24 (d, J = 7 Hz), 108.46(d. J = 21 Hz), 103.00, 38.82, 36.95.

3-Chloro-2-fluoro-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)methyl)aniline (10m)

White solid, 91 mg, yield 34%. Mp: 87–89°C, purity: 98.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.749 (dd, J_1 = 8.8 Hz, J_2 = 5.6 Hz, 2H), 7.178(t, J = 8.8 Hz, 2H), 6.950(td, J_1 = 8.0, J_2 = 1.6 Hz, 1H), 7.758(td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H), 6.688(td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H), 6.599(s, 1H), 6.453(td, J_1 = 6.0 Hz, J_2 = 1.2 Hz, 1H), 4.416 (d, J = 6.0 Hz, 2H), 3.858(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.00(d, J = 242 Hz), 147.95, 146.72(d, J = 239 Hz), 142.24, 138.00(d, J = 11 Hz), 130.43(d, J = 3 Hz), 127.22(d, J = 8 Hz), 125.60(d, J = 4 Hz), 119.67 (d, J = 14 Hz), 116.79, 115.87(d, J = 22 Hz), 111.65(d, J = 3 Hz), 102.94, 38.29, 36.99.

3-Bromo-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)methyl)-2-methylaniline (10n)

White solid, 204 mg, yield 68%. Mp: 108–110°C, purity: 98.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.751 (dd, J_1 = 8.4 Hz, J_2 = 5.6 Hz, 2H), 7.179(t, J = 8.8 Hz, 2H), 6.903(t, J = 8.0 Hz, 1H), 6.825(d, J = 8.0 Hz, 1H), 6.606(d, J = 8.0 Hz, 1H), 5.874(t, J = 4.8 Hz, 1H), 4.393(d, J = 4.8 Hz, 1H), 3.867(s, 2H), 2.264(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 161.99(d, J = 242 Hz), 147.90, 147.75, 142.60, 130.52(d, J = 3 Hz), 128.21, 127.21(d, J = 8 Hz), 125.32, 121.63, 120.48, 115.87(d, J = 21 Hz), 109.64, 102.96, 38.98, 37.03, 17.56.

3-Chloro-4-fluoro-N-((3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)methyl)aniline (10o)

White solid, 150 mg, yield 56%. Mp: 121–123°C, purity: 99.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.759 (dd, J_1 = 8.4 Hz, J_2 = 5.6 Hz, 2H), 7.198(t, J = 8.8 Hz, 2H), 7.134(t, J = 9.2 Hz, 1H), 6.791(dd, J_1 = 6.0 Hz, J_2 = 2.4 Hz, 1H), 6.661 ~ 6.623(m, 2H), 6.337(t, J = 5.2 Hz, 1H), 4.309(d, J = 5.6 Hz, 2H), 3.837(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 162.01(d, J = 242 Hz), 149.83(d, J = 133 Hz), 147.98, 146.31(d, J = 2 Hz), 142.22, 130.47(d, J = 3 Hz), 127.21(d, J = 8 Hz), 119.87(d, J = 18 Hz), 117.37(d, J = 21 Hz), 115.92(d, J = 21 Hz), 113.16, 112.63(d, J = 6 Hz), 103.01, 38.84, 36.98.

N-(3-bromobenzyl)-3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-amine (11a)

To a solution of compound **7** (132 mg, 0.69 mmol) in DMF (15 ml) sodium hydride 60% (51 mg, 1.265 mmol) was added. The mixture was stirred till no hydrogen was generated. Then, a solution of 1-bromo-3-(bromomethyl) benzene (190 mg, 0.76 mmol) in DMF (5 ml) was added dropwise. The reaction was stirred for 16 h. The mixture was poured into water and extracted with ethyl acetate (30 ml × 3). The organic layer was combined and washed with brine (30 ml × 1), dried with anhydrous sodium sulfate, filtered, and concentrated. The crude material was absorbed onto silica gel and purified using 75% of petroleum ether in ethyl acetate to yield the title compound as a faint yellow solid (67 mg, 27% yield). Mp: 117–119°C, purity: 97.8%; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 7.661(dd, J_1 = 8.4 Hz, J_2 = 5.4 Hz, 2H), 7.617 (s, 1H), 7.432(t, J = 8.4 Hz, 2H), 7.305(t, J = 7.8 Hz, 1H), 7.145(t, J = 8.4 Hz, 2H), 6.261(t, J = 6.0 Hz, 1H), 5.730(s, 1H), 4.261(d, J = 6.0 Hz, 2H), 3.621(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 161.83(d, J = 242 Hz), 149.93, 147.40, 143.27, 131.13(d, J = 3 Hz), 130.88, 130.46, 130.15, 126.94, 126.90(d, J = 8 Hz), 122.16, 115.54(d, J = 2 Hz), 84.42, 48.26.

General procedure for the preparation of 12a–12b

To a solution of compound **7** (153 mg, 0.8 mmol) in THF (15 ml) anhydrous pyridine (1 ml) was added, followed by the addition of substituted benzoyl chloride (1 mmol) in THF (20 ml) dropwise under an ice bath. The ice bath was removed after addition was complete. The reaction was stirred at room temperature for 2 h and monitored by TLC. The sovent was removed under reduced pressure and ethyl acetate was added. The organic layer was washed completely with water (30 ml × 3) and brine (30 ml × 1), dried with anhydrous sodium sulfate, filtered, and concentrated. The crude material was absorbed onto silica gel and purified using 85% of petroleum ether in ethyl acetate to yield title compound **12**.

3-Bromo-N-(3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)benzamide (12a)

White solid, 162 mg, yield 54%. Mp: 156–158°C, purity: 97.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.545 (s, 1H), 8.185(t, J = 1.6 Hz, 1H), 7.994(d, J = 8.0 Hz, 1H), 7.966 ~ 7.806(m, 3H), 7.539(t, J = 8.0 Hz, 1H), 7.230(t, J = 8.8 Hz, 2H), 6.720(s, 1H), 3.758(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 164.90, 162.17(d, J = 242 Hz), 147.92, 137.81, 135.86, 135.40, 131.31, 130.97, 130.29(d, J = 3 Hz), 127.50, 127.24(d, J = 8 Hz), 122.27, 115.97(d, J = 21 Hz), 98.27, 36.34.

N-(3-(4-fluorophenyl)-1-methyl-1H-pyrazole-5-yl)-3-(trifluoromethyl)benzamide (12b)

White solid, 131 mg, yield 45%. Mp: 173–175°C, purity: 99.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.694 (s, 1H), 8.341(s, 1H), 8.305(d, J = 7.6 Hz, 1H), 8.027(d, J = 7.6 Hz, 1H), 7.848 ~ 7.214(m, J = 3H), 7.236(t, J = 8.8 Hz, 2H), 6.7240(s, 1H), 3.768(s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 164.83, 162.17(d, J = 242 Hz), 147.89, 137.75, 134.70, 132.54, 130.39, 129.82(q, J = 2 Hz), 129.19(q, J = 3 Hz), 127.22(d, J = 8 Hz), 125.01 (q, J = 4 Hz), 124.39(q, J = 271 Hz), 115.95(d, J = 21 Hz), 98.29, 36.44.

Antiproliferative activity

All target compounds were evaluated for their in-vitro antiproliferative activity against two PC cell lines LNCaP (androgen dependent) and PC-3 (androgen independent) on the basis of the standard MTT assay methodology. Cell lines were obtained from the American Type Culture Collection (Manassas, Virginia, USA). Cells were cultured in medium RPMI 1640 (Gibco, Carlsbad, California, USA) containing 10% fetal bovine serum (Gibco), 100 IU/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere with 5% CO_2 at 37°C. Cells were seeded in 96-well plates at a density of 4×10^3 cells/well. Compounds were dissolved in DMSO and added to plates when cells were attached to the bottom. The final concentrations of compounds were 5, 10, 20, 40, and 80 μmol/l and that of DMSO was less than 0.5%. After 96 h of treatment with compounds, MTT (20 μl, 5 mg/ml) was added, followed by another 4 h of incubation; the absorbance at 570 and 630 nm was measured using an ELX800 Microplate Reader (BioTek, Winooski, Vermont, USA) three times for each plate.

Inhibition of prostate-specific antigen expression measured by ELISA

The human PSA ELISA kit (catalog no. 1500) purchased from Alpha Diagnostic International (San Antonio, Texas, USA) was used to determine the PSA level in medium according to the procedure of the manufacturer. LNCaP cells (4×10^3/well), cultured in medium RPMI 1640 containing 10% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin, were seeded in 96-well plates and incubated until cells attached to the bottom. Then, the compounds were added at a final concentration of 5 μmol/l. After 96 h treatment, 25 μl supernatant, PSA, standards, and control were added to appropriate wells of an ELISA plate. Then, 100 μl of assay buffer was added to each well and incubated on a plate shaker for 60 min at room temperature. The wells were washed three times with 300 μl of 1× wash buffer, followed by the addition of 100 μl Ab–enzyme conjugate buffer. After incubation and shaking for 30 min, the wells were washed another three times with 1× wash buffer. 100 μl 3,3′,5,5′-tetramethylbenzidine substrate was then aspirated into each well. The reaction was stopped after 15 min by adding 50 μl of stopping solution to all wells. The absorption was measured at 450 nm using the ELX800 Microplate Reader.

Results and discussion

Chemicals

Synthesis of target compounds started with commercially available 4-fluorophenylacetophenone **1** as shown in Scheme 1, which reacted with diethyl oxalate in the presence of sodium ethylate to yield β-keto ester as intermediate **2**, whose condensation with hydrazine hydrate catalyzed by acetic acid generated **3**. Methylation of **3** with dimethyl sulfate in DMF yielded intermediate **4**, which was subsequently hydrolyzed with potassium hydroxide to yield **5**. Then, Curtius rearrangement was performed in the presence of DPPA and triethylamine to transform acid **5** into carbamate **6**. Hydrolysis of **6** by sodium hydroxide yielded key intermediate **7**. Intermediate **8** was produced by a complete reduction of **4** by $LiAlH_4$ in THF, which was subsequently halogenated to **9** using thionyl chloride at room temperature. Then, a nucleophilic attack of substituted aniline to **9** yielded the target compound **10**. Compound **11** was obtained using **7** and substituted benzyl bromide in DMF in the presence of sodium hydride. Target compound **12** was obtained easily and completely by an acylation reaction of **7** using a combination of substituted benzoyl chloride and pyridine, which was used as an acid-binding agent.

Biological evaluation

All of the compounds were evaluated for their antiproliferative activity against two PC cell lines LNCaP and PC-3, and the ability to inhibit AR target gene PSA expression in LNCaP cells. The testing results are presented in Table 1. Most of the compounds from skeleton **10** showed increasing PSA expression inhibitory activity at 5 μmol/l compared with the lead compound **T3**, such as **10c**, **10d**, **10e**, **10f**, **10g**, **10h**, **10j**, **10l**, and **10o**. Among these, **10e** showed promising antiproliferative activity against the LNCaP cell line, with IC_{50} value of 18 μmol/l, and weak activity to the PC-3 cell line, which is better than **T3** in antiproliferative and PSA expression inhibitory activity, worth of further study. Compound **10h** has a PSA expression inhibitory rate of 63.6%, which is higher than any other compounds in the three skeletons, although a little weaker than enzalutamide, worthy of further structural modification.

Compounds **10a**, **10b**, **10i**, **10j**, **10k**, and **10n** showed good antiproliferative activity against both the cell lines, some of which showed better activity than enzalutamide and **T3**, such as **10b**, **10i**, **10k**, and **10n**. The good antiproliferative activity, combined with their PSA inhibitory activity, suggests that they may not only act on the AR pathway but also on other targets that exist in both PC-3 and LNCaP cells that can mediate apoptosis.

Inhibition to PSA expression decrease markedly when the position of amino and methylene was exchanged, compared with compound **10a** with **11a**, both of which were substituted by bromine on the meta-position of the benzene ring. The inhibition increased slightly on replacing methylene in the carbonyl group, and on comparing compound **11a** with **12a**.

Conclusion

In summary, a series of novel 3-(4-fluorophenyl)-1*H*-pyrazole derivatives were synthesized and evaluated for their antiproliferative activity against two PC cell lines LNCaP and PC-3, and the ability to inhibit AR target gene PSA expression in LNCaP cells. Some of the compounds showed promising antiproliferative activity against the androgen-dependent LNCaP cell line and a higher PSA inhibition rate, better than the lead compound **T3**. All compounds from skeleton **10** showed a higher PSA downregulation level than compounds from skeletons **11** and **12**. Compounds **10a**, **10b**, **10i**, **10j**, **10k**, and **10n** may not only act on the AR pathway, but on other targets that exist in both PC-3 and LNCaP cells. Compound **10e** selectively inhibited LNCaP cell growth and showed promising PSA expression inhibitory activity, better than **T3**, and this is worth further study.

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Conflicts of interest

There are no conflicts of interest.

References

1 Ahmed A, Ali S, Sarkar FH. Advances in androgen receptor targeted therapy for prostate cancer. *J Cell Physiol* 2014; **229**:271–276.
2 Hamann LG, Higuchi RI, Zhi L, Edwards JP, Wang XN, Marschke KB, *et al.* Synthesis and biological activity of a novel series of nonsteroidal, peripherally selective androgen receptor antagonists derived from 1,2-dihydropyridono [5,6-g]quinolines. *J Med Chem* 1998; **41**:623–639.
3 Caboni L, Kinsella GK, Blanco F, Fayne D, Jagoe WN, Carr M, *et al.* 'True' antiandrogens-selective non-ligand-binding pocket disruptors of androgen receptor-coactivator interactions: novel tools for prostate cancer. *J Med Chem* 2012; **55**:1635–1644.
4 Shen HC, Balk SP. Development of androgen receptor antagonists with promising activity in castration-resistant prostate cancer. *Cancer Cell* 2009; **15**:461–463.
5 Liu J, Liu B, Guo G, Jing Y, Zhao G. Discovery of novel androgen receptor antagonists: a hybrid approach of pharmacophore-based and docking-based virtual screening. *Anticancer Drugs* 2015; **26**:747–753.
6 Zhou J, Geng G, Shi Q, Sauriol F, Wu JH. Design and synthesis of androgen receptor antagonists with bulky side chains for overcoming antiandrogen resistance. *J Med Chem* 2009; **52**:5546–5550.
7 Bianchini D, Omlin A, Pezaro C, Lorente D, Ferraldeschi R, Mukherji D, *et al.* First-in-human phase I study of EZN-4176, a locked nucleic acid antisense oligonucleotide to exon 4 of the androgen receptor mRNA in patients with castration-resistant prostate cancer. *Br J Cancer* 2013; **109**:2579–2586.
8 Tian X, HeY, Zhou J. Progress in antiandrogen design targeting hormone binding pocket to circumvent mutation based resistance. *Front Pharmacol* 2015; **6**:57.