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Short communication

Novel naftopidil-related derivatives and their biological effects as alpha$_1$-adrenoceptors antagonists and antiproliferative agents

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ABSTRACT

Eleven novel naftopidil-related compounds that contain amide and indole groups were designed and synthesized. The biological effects of these compounds on three α_1-adrenoceptor subtypes and cancerous human prostate cell lines (PC-3, DU-145, and LNCaP) were determined. Compounds **2**, **3**, **5**, **11**, and **12** exhibited an α_1-adrenoceptor antagonistic activity, whereas compounds **9**, **10**, and **12** displayed moderate antiproliferative activities. Compound **3** exhibited a significant $\alpha_{1D/1A}$ blocking activity in isolated rat tissues (97.7- and 64.6-fold selective for α_{1D} and α_{1A} compared with α_{1B}) but not a relevant cytotoxic activity. Compound **12** demonstrated a potent and selective $\alpha_{1D/1A}$ antagonistic activity (47.9- and 19.1-fold for α_{1D} and α_{1A} compared with α_{1B}) and a potent antiproliferative activity in PC-3 cells (IC_{50} = 15.70 μM). Further testing confirmed that compound **12** inhibited the growth of PC-3 cells by inducing apoptosis and G0/G1 cell cycle arrest, which was mediated by α_1-adrenoceptor. Therefore, compound **12** is a potential multipotent agent that can act as an effective α_1-adrenoceptor subtype antagonist for treating benign prostatic hyperplasia and a preventive medication against human prostate cancer.

1. Introduction

Benign prostatic hyperplasia (BPH) is a noncancerous enlargement of the prostate that can cause urinary symptoms identical to prostate cancer (PCa). Both diseases start in the human prostate gland and display similarities; that is, the incidence and prevalence of both diseases increase with age, and both diseases require androgens for development and growth [1]. These diseases often coexist [2], and PCa usually arises in the prostate concomitant with BPH elsewhere [3]. Therefore, the development of drugs with both alpha$_1$-adrenoceptor (α_1-AR) antagonistic and anticancer activities is important to treat BPH and prevent PCa.

α_1-AR antagonists relax prostate smooth muscle and are the first-line medical treatment for lower urinary tract symptoms (LUTS) associated with BPH [4,5]. Molecular and pharmacological studies have revealed three α_1-AR subtypes: α_{1A}-, α_{1B}-, and α_{1D}-AR [6]. A fourth subtype, α_{1L}-AR, displays low affinity for prazosin and represents a functional phenotype of α_{1A}-AR [7]. The three α_1-AR subtypes exert markedly different functions; hence, selecting the appropriate α_1-AR subtype antagonist, particularly α_{1A}- and α_{1D}-ARs, is critical to decrease the severity of side effects on blood pressure and to improve the therapeutic effect (relieving obstructive and irritative symptoms) in BPH/LUTS patients [8,9].

α_1-AR antagonists exert antiproliferative effects on human PCa cell lines. In 2000, Benning and Kyprianou [10] reported for the first time that the quinazoline-derived α_1-AR antagonists doxazosin and terazosin suppress PCa growth by inducing apoptosis but methoxysulfonamide-derived tamsulosin exhibits no effect on prostate cell growth. Since then, several studies have investigated α_1-AR antagonists (subtype nonselective: doxazosin and terazosin; subtype selective: naftopidil **1**) and antiprostate tumor growth. The

Abbreviations: BPH, benign prostatic hyperplasia; LUTS, lower urinary tract symptoms; PCa, prostate cancer; α_1-AR, alpha$_1$-adrenoceptor.

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apoptotic activities of doxazosin and terazosin against PCa cells are independent of their capacity to antagonize α_1-ARs [11] but involve the activation of transforming growth factor-beta 1 (TGF-β1) signaling [12,13]. The arylpiperazine-derived $\alpha_{1D/1A}$-AR antagonist naftopidil **1** is a therapeutic agent for LUTS/BPH; this agent does not markedly affect blood pressure or the vascular system and exhibits high selectivity to the prostate [14]. Kanda et al. [15] reported that naftopidil **1** exhibited a growth inhibitory effect as it causes an arrest of the G0/G1 cell cycle in androgen-sensitive (LNCaP) and -insensitive (PC-3) human prostate cancer cell lines and inhibited PC-3 tumors growth in nude mice models. Hori et al. [3] investigated the effects of three subtype-selective α_1-AR antagonists (naftopidil **1**, tamsulosin, and silodosin) on prostate tumor growth. They found that orally administering naftopidil **1** suppresses human PCa tumor growth by altering tumor–stroma interactions. These reports suggest that naftopidil **1** is useful in PCa chemoprevention and PCa treatment.

The present study modified the lead compound **1** by combining multiple biological activities in the same molecule to expand the biological profile of **1**-related compounds. The amide or lactam group might be an effective moiety in phenylpiperazine derivatives: α_{1D} subtype antagonists BMY 7378 and SNAP-8719 [16,17]; α_{1A} subtype antagonists RWJ-38063, RWJ-69736, Rec-15/2739, and silodosin [18] (Fig. 1). Therefore, we replaced the 2-hydroxypropane moiety of **1** with an amide structure to improve the $\alpha_{1D/1A}$ binding affinity of the compound. We introduced the indole substituent and its derivative groups to enhance the anticancer property of the compound. Indole-3-carbinol (I3C) is endogenously produced from naturally occurring glucosinolates contained in various plant food substances; this compound suppresses the proliferation of various tumor cells including PCa and induces G0/G1 cell cycle arrest and apoptosis [19,20]. New derivatives (**2**–**12**) of naftopidil (Fig. 2) were designed and synthesized in the current study to evaluate both α_1-ARs and malignant human prostate cells (PC-3, DU145, and LNCaP). Results showed that compound **12**, as a novel strong $\alpha_{1D/1A}$ subtype blocker, can suppress PC-3 cancer cell growth by inducing cell apoptosis and G0/G1 cell cycle arrest. Overall, compound **12** is a potential agent for PCa prevention.

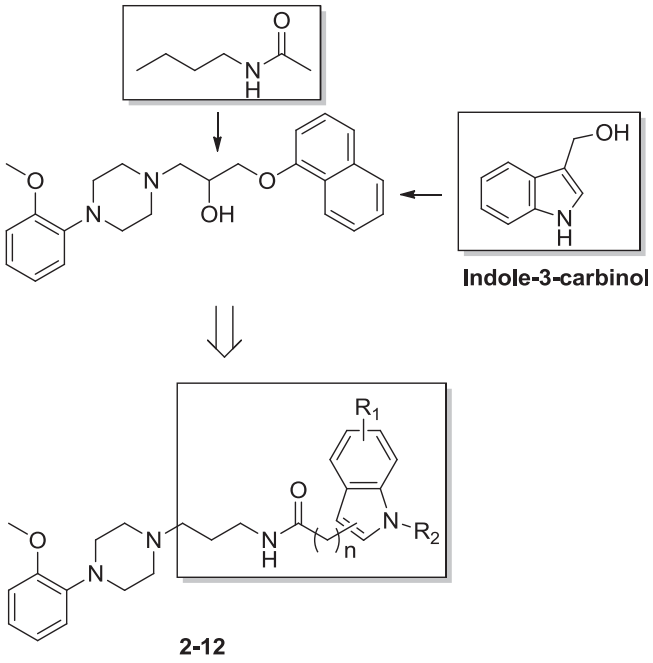


Fig. 2. Structures of newly designed compounds **2**–**12** related to naftopidil **1**.

2. Results

2.1. Chemistry

Target compounds **2**–**12** were synthesized (Scheme 1) and characterized by ^{1}H NMR, ^{13}C NMR, and HRESI-MS. The amino group of 3-bromopropylamine hydrobromide was protected as the Boc-derivative and then reacted with *o*-methoxyphenyl piperazine to produce the *N*-Boc-protected piperazine **13**. Removal of the protecting group resulted in the creation of **14**, followed by the

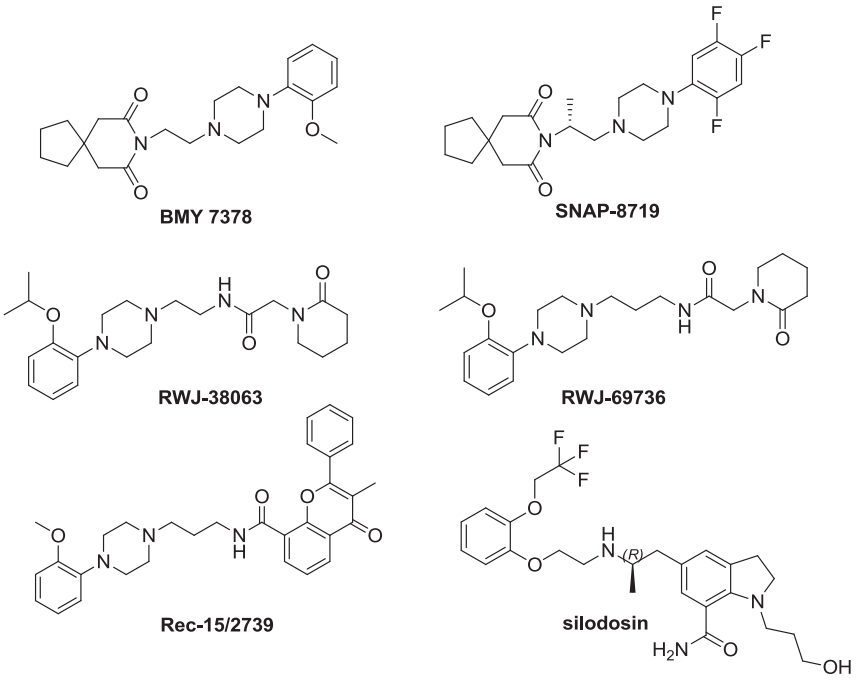
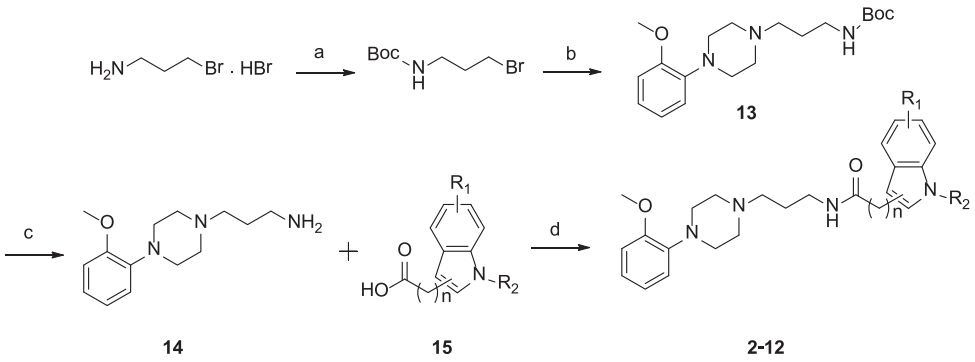


Fig. 1. Chemical structures of α_{1D}-AR selective antagonists: BMY 7378, SNAP-8719; α_{1A}-AR selective antagonists: RWJ-38063, RWJ-69736, Rec-15/2739, and silodosin.



Comp.	Position	n	R_1	R_2
2	5	0	H	H
3	3	1	H	H
4	3	2	H	H
5	3	3	H	H
6	2	0	5-OCH_3	H
7	2	0	5-OH	H
8	2	0	7-NO_2	H
9	2	0	6-Br	H
10	2	0	5-Cl	H
11	3	0	H	CH_3
12	2	0	H	CH_2-C_6H_5

a) $(BOC)_2O$, DMAP, TEA, CH_2Cl_2, 1 h, rt, then 0.5 M HCl; b) NaI, *o*-methoxyphenyl piperazine, CH_2Cl_2, 6 h, rt; c) TFA, CH_2Cl_2, saturated Na_2CO_3 aq.; d) HATU, DIPEA, N_2, rt, 12–24 h.

Scheme 1. Synthetic route of target compounds **2**–**12**.

coupling reaction with various indole carboxylic acids **15**, eventually gave rise to **2**–**12**.

2.2. α_1-Adrenoceptor antagonistic activity

Table 1 compares the antagonistic effects (pA_2 values) of target compounds **2**–**12**, lead compound **1**, tamsulosin, and terazosin on three α_1-AR subtypes from rat isolated prostatic vas deferens (α_{1A}), spleen (α_{1B}), and thoracic aorta (α_{1D}) [21]. All compounds exhibited moderate to excellent antagonistic activity and selectivity to α_1-AR subtypes.

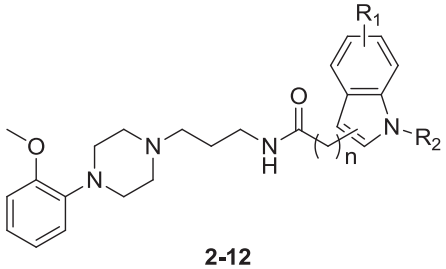
Table 1 clearly suggest that antagonistic activity is modulated by a) the length of chain between the amide and indole moieties, b) the presence and position of substituents on the indole moiety (R_1 group), and c) the substituent of hydrogen in the *N*-position of the indole moiety (R_2 group).

Compounds **2**–**5** are characterized by the presence of an indole system separated from the piperazine moiety by a spacer of different length. Modifications to the length of this spacer chain greatly influence the pharmacological profile of the synthesized compounds. The antagonistic activity against α_{1D}- and α_{1A}-AR subtypes was **3** ≥ **5** > **2** > **4**. Compound **3**, in which the spacer length was decreased to one methylene, displayed the lowest affinity to α_{1B} ($pA_2 = 6.29$) and the highest affinity to α_{1D}- and α_{1A}-ARs ($pA_2 = 8.28, 8.10$). Therefore, **3** showed the highest selectivity to the α_{1D}- and α_{1A}-AR subtypes (97.7- and 64.6-fold, respectively) with respect to α_{1B}-AR. Compound **4**, which contains ethylene, showed the lowest potency and selectivity to the three α_1-AR subtypes. Derivative **5** possessed the longest chain that exhibited several interesting traits; this compound showed high affinity to α_{1D}- and α_{1A}-ARs ($pA_2 = 8.29, 7.90$) at levels close to **3** but demonstrated higher potency toward α_{1B}-AR ($pA_2 > 7$), thereby resulting in poor selectivity on $\alpha_{1D/1A}$-ARs. Basing on these results, we hypothesized that the receptor possesses a definite binding area, probably methylene, in a precise region of the receptor; the indole system possibly interacts with this receptor site only at an appropriate distance from the piperazine ring [22].

A dividing line can be drawn between the electron-donating group (**6** and **7**) and the electron-withdrawing group derivatives (**8**–**10**). The 5-methoxy-2-indole derivative **6** showed the lowest blocking effect on the three α_1-AR subtypes ($pA_2 = 7.11, 5.84, 7.23$). Formed by replacing the methoxy with a hydroxy group, the 5-hydroxy-2-indole derivative **7** demonstrated improved blocking activity, particularly at the α_{1B}-subtype (from 5.84 to 6.81), but showed no subtype selectivity. The introduction of the hydroxy group could have made the compound suitable for binding to the α_{1B}-subtype. Cases of introducing the electron-withdrawing group also failed. The blocking activity at α_{1A}- and α_{1D}-ARs of the derivatives added with an electron-withdrawing group remained unsatisfactory ($pA_2 < 8$). This result suggests that modifying the R_1 group has no significant effect on the α_1-ARs.

We also studied the effect of adding groups to the indole-*N* atom on activity toward the three α_1-ARs. The *N*-methyl derivative **11** exerted an α_{1D}-antagonistic effect with excellent subtype selectivity on α_{1D}-AR (30.2-fold of $\alpha_{1D/1B}$). Formed by adding a large benzyl group on the indole-*N* atom, compound **12** exhibited high affinity to α_{1A}- and α_{1D}-ARs ($pA_2 = 8.13, 8.56$) and low potency at α_{1B}-AR ($pA_2 = 6.85$) (Fig. 3). The subtype selectivity of **12** (47.9- and 19.1-fold) was not as strong as that of compound **3**. However, the blocking activity of **12** against $\alpha_{1D/1A}$-AR was slightly more potent than that of **3**. The introduced benzyl group at the *N*-position exhibited high flexibility of the ligand, which may be buried in the

Table 1
Antagonist Affinities of Compounds **2**–**12**, Expressed as pA_2, at α_{1A}-, α_{1B}-, and α_{1D}-AR on Isolated Rat Tissues.



2-12

Comp.	P	n	R_1	R_2	pA_2[a] (slope)			Affinity ratio[b]		
					α_{1A}	α_{1B}	α_{1D}	α_{1D}/α_{1B}	α_{1A}/α_{1B}	α_{1D}/α_{1A}
					Prostatic vas deferens	Spleen	Thoracic aorta			
2	5	0	H	H	7.58 ± 0.05 (1.08)	6.91 ± 0.11 (1.14)	7.93 ± 0.02 (1.05)	10.5	4.7	2.2
3	3	1	H	H	8.10 ± 0.14 (1.06)	6.29 ± 0.03 (1.00)	8.28 ± 0.09 (0.94)	97.7	64.6	1.5
4	3	2	H	H	6.71 ± 0.11 (1.23)	6.66 ± 0.15 (0.94)	7.46 ± 0.03 (1.00)	6.3	1.1	5.6
5	3	3	H	H	7.90 ± 0.14 (1.09)	7.02 ± 0.06 (1.08)	8.29 ± 0.23 (0.98)	18.6	7.6	2.5
6	2	0	5-OCH_3	H	7.11 ± 0.07 (0.91)	5.84 ± 0.08 (0.92)	7.23 ± 0.12 (1.03)	24.5	18.6	1.3
7	2	0	5-OH	H	7.26 ± 0.01 (1.04)	6.81 ± 0.10 (1.03)	7.57 ± 0.02 (1.11)	5.6	2.8	2.0
8	2	0	7-NO_2	H	7.23 ± 0.02 (1.05)	6.19 ± 0.09 (0.98)	7.55 ± 0.04 (1.05)	22.9	11.0	2.1
9	2	0	6-Br	H	7.16 ± 0.14 (1.01)	6.68 ± 0.03 (0.92)	7.82 ± 0.10 (1.12)	13.8	3.0	4.6
10	2	0	5-Cl	H	7.32 ± 0.15 (0.89)	6.59 ± 0.09 (0.92)	7.71 ± 0.11 (1.02)	13.2	5.4	2.5
11	3	0	H	CH_3	7.28 ± 0.06 (1.18)	6.78 ± 0.12 (1.00)	8.26 ± 0.01 (0.99)	30.2	3.2	9.6
12	2	0	H	CH_2–C_6H_5	8.13 ± 0.01 (1.13)	6.85 ± 0.01 (0.98)	8.56 ± 0.06 (0.95)	47.9	19.1	2.6
1[c]					7.48 ± 0.30	6.75 ± 0.01	7.93 ± 0.13	15.1	5.4	2.8
Tamsulosin [24]					9.46 ± 0.13	9.30 ± 0.08	10.00 ± 0.10	5.0	1.4	3.5
Terazosin[d]					7.90 ± 0.15	8.59 ± 0.08	8.83 ± 0.17	1.7	0.2	8.5

[a] pA_2 values ± SEM (n = 5–8) were calculated from Schild plots, pA_2 is the positive value of the line intercept derived through plotting log (Dr-1) *vs.* log [antagonist].
[b] Antilog of ΔpA_2.
[c] The corresponding pK_i values were 8.43 ± 0.06, 7.70 ± 0.02, and 8.92 ± 0.00, as obtained in binding experiments [14].
[d] The reference data were 8.04, 8.60, and 8.65 [25].

core of the helical bundle receptors of the transmembrane domain, inside an area sterically accessible to bind α_{1A}- and α_{1D}-ARs [23]. The significant hydrophobic property of the benzyl may also be responsible for the binding energy on α_{1A}- and α_{1D}-ARs evoked by **12**.

2.3. *In vitro cytotoxic activity*

The antiproliferative effects of **1**–**12** on PC-3, DU-145, and LNCaP human PCa cell lines were evaluated *in vitro* (Table 2). Kanda [15] reported that naftopidil inhibits cell growth in PC-3 and DU145 cell lines with IC_{50} values of 33.2 and 22.2 μM, respectively. However, naftopidil **1** in the present study showed poor cytotoxic effects (IC_{50} > 50 μM) on the three human PCa cell lines. This result may be ascribed to the different incubation times used in the previous study (3–4 d) and in the present study (2 d). Doxazosin displayed moderate antiproliferative effects on the DU145 and LNCaP cell lines with IC_{50} values of 37.67 and 27.89 μM, respectively. Tamsulosin and terazosin displayed no activity on cell proliferation (data not shown). This finding is consistent with previous reports [10].

Compounds **2**–**5** did not affect the proliferation of PC-3 and DU145 cells (IC_{50} > 100 μM) regardless of the carbon chain length. Interestingly, **5** inhibited the proliferation of LNCaP cells with an IC_{50} value of 52.31 μM compared with the minimal effects of **2**–**4** (IC_{50} > 100 μM). This result may be attributed to the effect of molecular polarity on cell membrane permeability. That is, high lipophilicity increases the membrane permeability and thus the cytotoxic effect of a compound.

Compounds **9**–**12** displayed moderate antiproliferative effects on all tested PCa cell lines. These results suggest that compounds with halogen atoms or with large hydrophobic groups may have improved cytotoxic activity. Compound **12** showed the highest cytotoxic activity and displayed a promising antitumor activity against the PC-3, Du145, and LNCaP cell lines with IC_{50} values of 15.70, 33.33, and 25.54 μM, respectively (Fig. 4). This compound also possessed a slightly greater potency than doxazosin. Given its α_1-AR blocking activity in rat isolated tissues and antiproliferative ability against the three cancer cell lines, the benzyl derivative **12** was deemed the most promising among all the tested and derived compounds because of its multitarget activities.

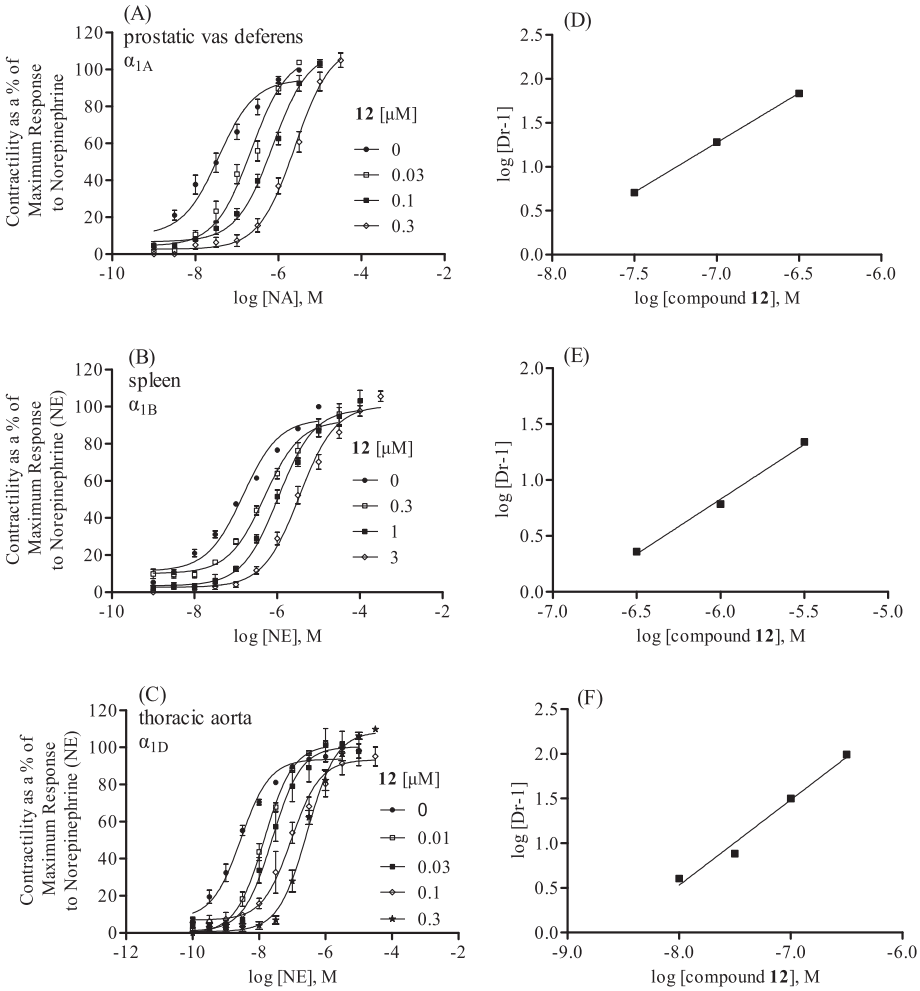


Fig. 3. Left part: Concentration–response curves of NE-induced contractions in rat isolated prostatic vas deferens (A), spleen (B), and thoracic aorta (C) in compound **12** treatment at different concentrations. Data are the mean ± SEM of five to eight separate experiments. Compound **12** produced a rightward shift in the NE concentration response curve and no reduction of maximal agonist-induced contractile responses with the increasing concentration of **12**. Right part: Schild plots for the antagonism to NE response through compound **12** in rat isolated prostatic vas deferens (D), spleen (E), and aorta (F), whose slopes were not significantly different from unity.

Table 2
In vitro antiproliferative activity (IC_{50}) of **2**–**12** in PC-3, DU-145, and LNCaP human prostate cancer cells.

Compound[b]	IC_{50} (μM)[a]		
	PC-3	DU-145	LNCaP
5	>100	>100	52.31 ± 0.92
9	31.20 ± 1.11	39.31 ± 1.06	26.73 ± 1.36
10	36.88 ± 1.14	46.96 ± 1.01	28.44 ± 1.08
12	15.70 ± 1.03	33.33 ± 1.02	25.54 ± 1.31
1	>50	>50	>50
Doxazosin	>50[c]	37.67 ± 1.13	27.89 ± 1.07

[a] The cell proliferation inhibition of three cancer cells after treatment with different concentrations of **1**–**12** for 48 h was measured using WST-8 cell proliferation assay. The data, expressed as inhibition percentage of control, are reported as IC_{50} values, which represent the concentration (μM) of test compounds required to inhibit 50% of cell growth.

[b] Compounds **1**–**4**, **6**, **7**, **8**, and **11** showed no relevant antiproliferative activity (IC_{50} > 50 μM).

[c] The IC_{50} value of doxazosin was 38.60 μM in reported literature [26], with an incubation time of 72 h.

*2.4. Induction of cell cycle arrest at the G0/G1 phase by **12** in PC-3 cells*

To examine the mechanism by which **12** inhibits cell proliferation, we evaluated the effect of **12** on cell cycle progression. PC-3 cells were not only treated with DMSO (0.1%) alone as controls but were also treated with different concentrations (5, 10, 15, 20, and 30 μM) of **12** for 24 h and then stained with propidium iodide (PI). The effects of **12** on the cell cycle distribution of PC-3 were evaluated by flow cytometry [15]. Representative flow histograms are shown in Fig. 5. Treatment with **12** at 10 μM (58.52%), 15 μM (70.60%), and 20 μM (61.58%) for 24 h significantly increased the number of PC-3 cells in the G0/G1 phase compared with vehicle-treated controls (39.20%). Interestingly, the cell numbers in the G0/G1 phase were significantly lower while those in the G2/M phase were significantly higher in the 30 μM **12**-treated cells than in the 20 μM-treated cells. The cell numbers in the G2/M phase were also significantly higher in the 30 μM **12**-treated cells than in the vehicle-treated controls. Considering that various concentrations of **12** treatment may induce inhibition at different cell cycle phases, we hypothesized that **12** has more than one mechanism in inhibiting cancer cell proliferation.

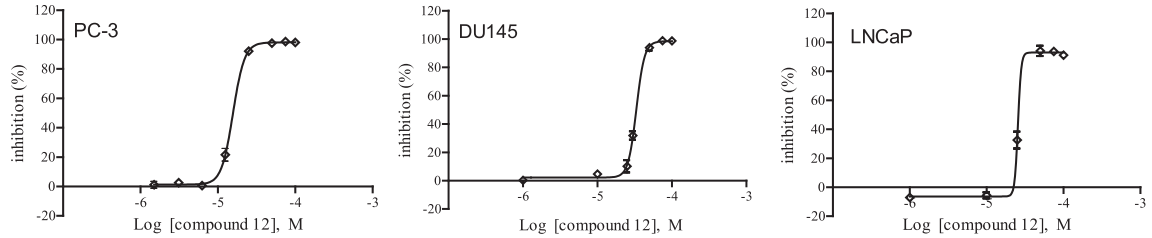


Fig. 4. Effect of **12** on cell proliferation inhibition against PC-3, DU145, and LNCaP cell lines. The cell proliferation inhibition (%) of three cancer cells after treatment with different concentrations of **12** for 48 h was measured using WST-8 cell proliferation assay.

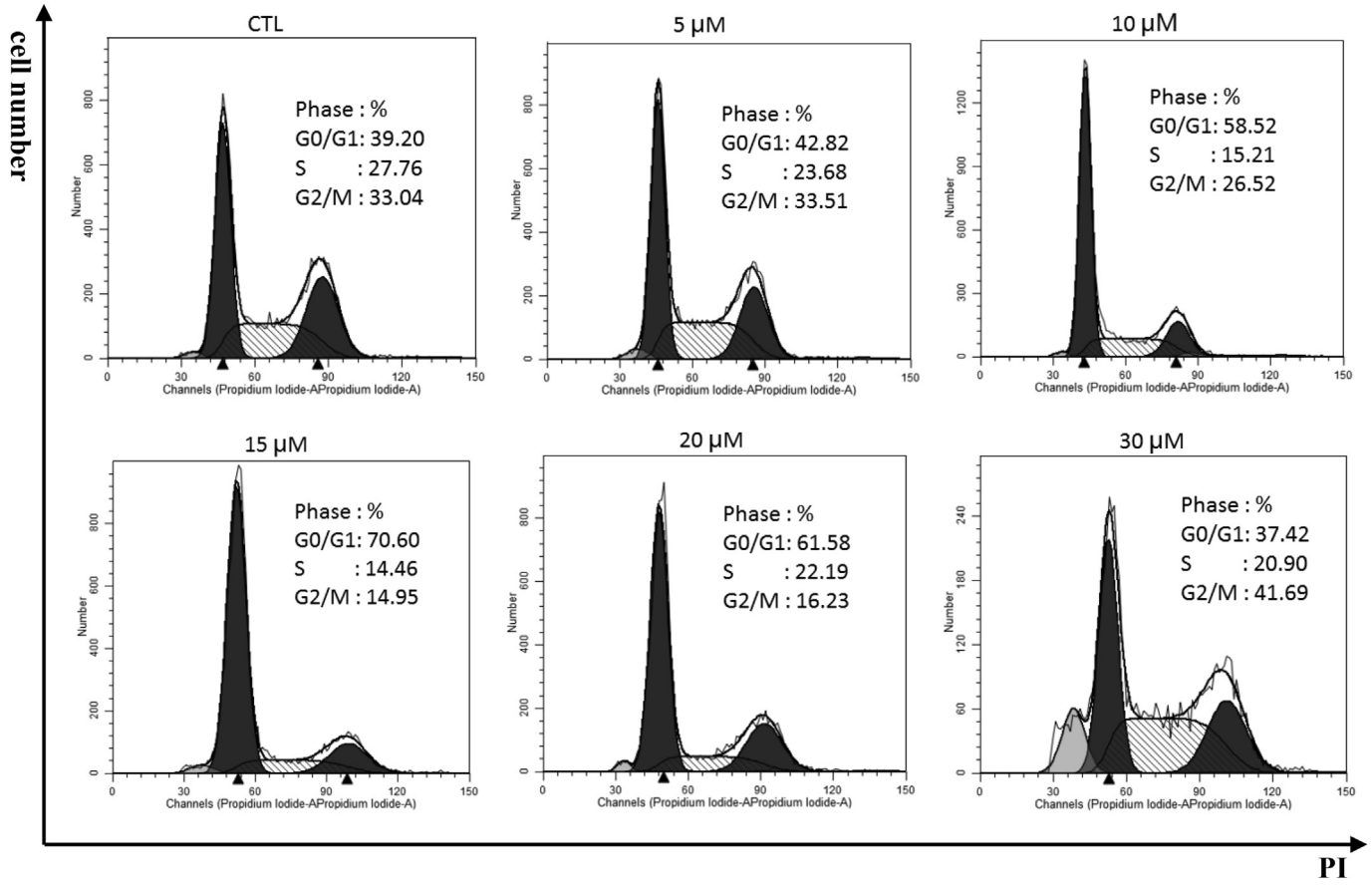


Fig. 5. Cell cycle analysis of PC-3 cells treated with **12** (0, 5, 10, 15, 20, and 30 μM) for 24 h through flow cytometry. The percentages of cells in the G0/G1, S, and G2/M phases of the cell cycle were further analyzed using MultiCycle AV software.

2.5. Induction of apoptosis by **12** in PC-3 cells

Further experiments were conducted to determine whether or not the antiproliferative effect of **12** on PC-3 cell viability is closely associated with apoptosis. Quantification by Alexa Fluor® 488 Annexin V/PI double staining assay showed that **12** increased the percentage of apoptotic cells in a dose-dependent manner in PC-3 cells (Fig. 6). The lower right quadrant represents early apoptotic cells, which are positive for AV binding and negative for PI uptake (AV^+/PI^-). The upper right quadrant represents late apoptotic cells or dead cells, which are positive for PI uptake with AV fluorescence (AV^+/PI^+). Treatment with **12** for 24 h significantly increased the number of apoptotic PC-3 cells at both early- and late-stage apoptosis. Statistical analysis indicated that the apoptotic rates were approximately 23.7%, 51.1%, 62.7%, and 83.0% after treatment with **12** at 5, 10, 20, and 30 μM, respectively. Western blotting indicated compound **12** (1–30 μM) markedly up-regulated Bax/Bcl-2 values and the levels of activated caspase-3 in a dose-dependent manner at 24 h post-incubation in PC-3 cells (Fig. 7).

2.6. Effects of **12** on NE-stimulated cell proliferation

Previous studies demonstrated that α_1-ARs are expressed in PC-3 cells. The catecholamine neurotransmitter NE binds to α_1-ARs located on the cell membrane and activates phospholipase, thereby generating a second messenger that ultimately results in smooth muscle contraction and promoting proliferative responses, such as DNA synthesis, probably through the activation of mitogen-activated protein kinases in vascular smooth muscle cells and the modulation of cytoskeletal proteins in prostate smooth muscle cells [10,27–31]. To determine whether or not the cell growth inhibitory ability of **12** is associated with its antagonistic activity against α_1-

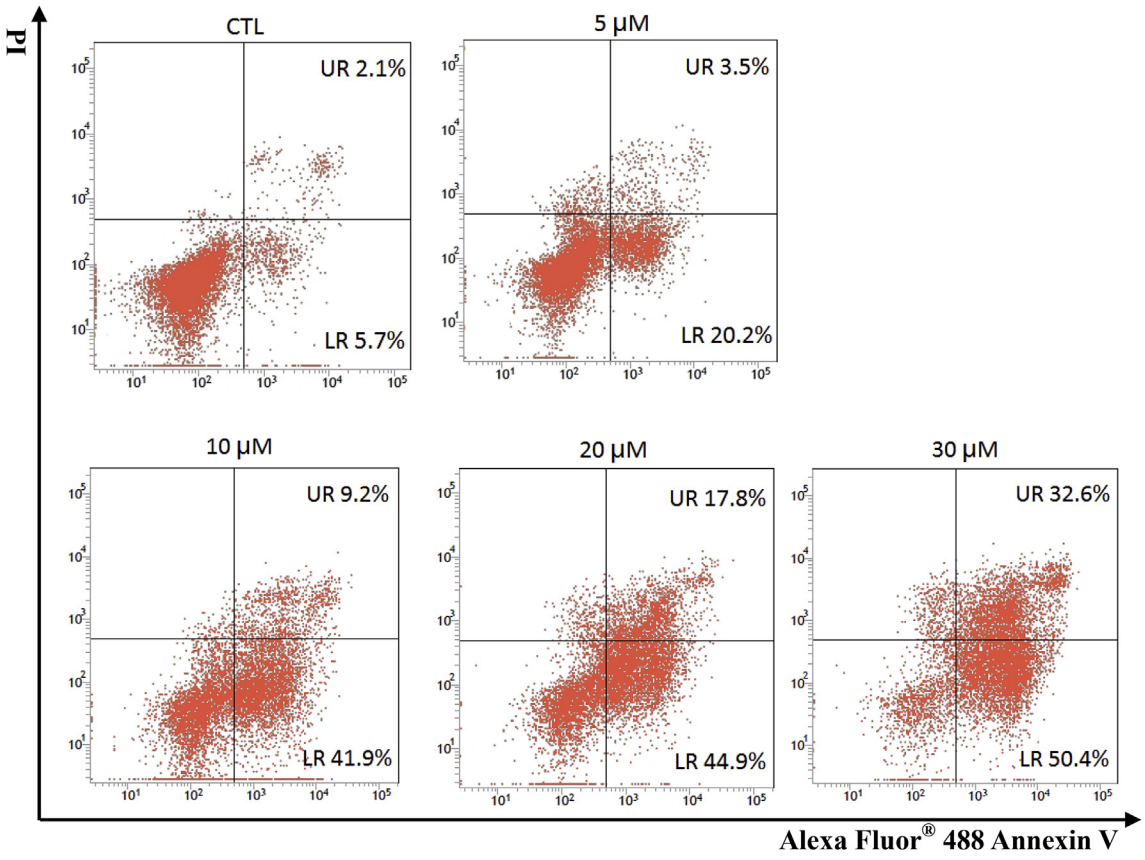


Fig. 6. Externalization of phosphatidylserine in PC-3 treated with **12** (0, 5, 10, 20, and 30 μM) for 24 h was detected through Alexa Fluor® 488 Annexin V/PI double staining assay. The cell population in the lower right quadrant (Annexin V^+/PI^-) represents early apoptotic cells, whereas the population in the upper right quadrant (Annexin V^+/PI^+) represents late apoptotic cells or dead cells.

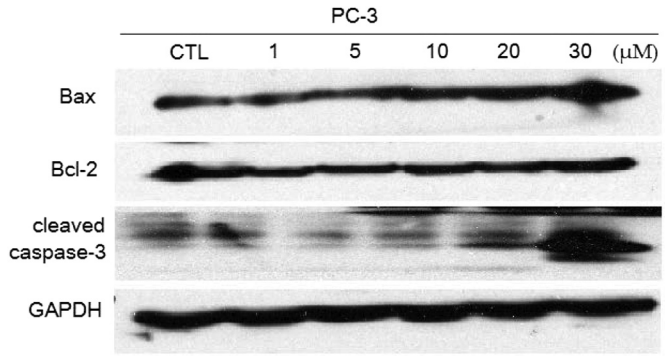


Fig. 7. Compound **12** causes an increase in Bax/Bcl-2 values and induces caspase-3 activation in PC-3 cells. PC-3 cells were exposed to **12** with various concentrations (0, 1, 5, 10, 20 and 30 μM) for 24 h.

ARs, we tested the ability of NE to interfere with the antigrowth action of **12** on PC-3 cells.

The endogenous agonist NE (0–50 μM) stimulated cell proliferation in a concentration-dependent manner (20 μM, cell growth increased by 23.55%; 50 μM, cell growth increased by 50.61%, $P < 0.01$, consistent with previous work [23]) (Fig. 8A). Treatment with NE at 20 μM did not affect the ability of **12** to inhibit the viability of PC-3 cells ($P > 0.05$, Fig. 8B). However, treatment with NE at 50 μM dose dependently reversed the **12**-induced reduction in PC-3 cell viability **12** ($P < 0.05$, Fig. 8B). Treatment with **12** also inhibited the NE-induced (50 μM) proliferation of PC-3 cells (Fig. 8C). This result indicates that the effect of **12** on these responses is mediated through α_1-ARs. Considering that catecholamines stimulate prostate growth, we attributed the effects of **12** to α_1-AR antagonism in the NE-stimulated models.

3. Discussion and conclusions

This research investigated the design and synthesis of eleven new amide derivatives (**2**–**12**) of naftopidil, the α_1-subtype antagonistic activity of compounds **2**–**12** by functional assays on isolated rat tissues, the cytotoxic activity of **2**–**12** on human prostate cancer cell lines, and the preliminary mechanism of **12** in inhibiting cancer cell growth.

The skeletal structure of compounds **2**–**12** in the present study has been reported by Kuo [32], in which des-hydroxy and hydroxy derivatives were both designed and synthesized as α_{1A}-subtype antagonists. Des-hydroxy compounds have lower α_{1A}-subtype selectivity than hydroxy compounds. However, K_i values show that the former significantly exhibits a more potent α_1-AR blocking activity than the latter. Our modification of the des-hydroxy skeletal structure was focused on the indole-related group and fixed the remaining (*o*-methoxyphenyl) piperazine moiety. In general, the blocking activity of these new derivatives was not as good as that of tamsulosin (pA_2 values of 7.0–8.0 *vs.* 9.0–10.0 of tamsulosin) but exhibited higher tissue selectivity compared with tamsulosin and terazosin. To improve α_{1D} blocking activity, it was involved with the spacer length ($n = 1$ or 3) and the substituent of the hydrogen in the *N*- position of indole. The corresponding compounds (**3**, **5**, **11**, and **12**) exhibited excellent α_{1D} blocking activity (pA_2 values > 8). The

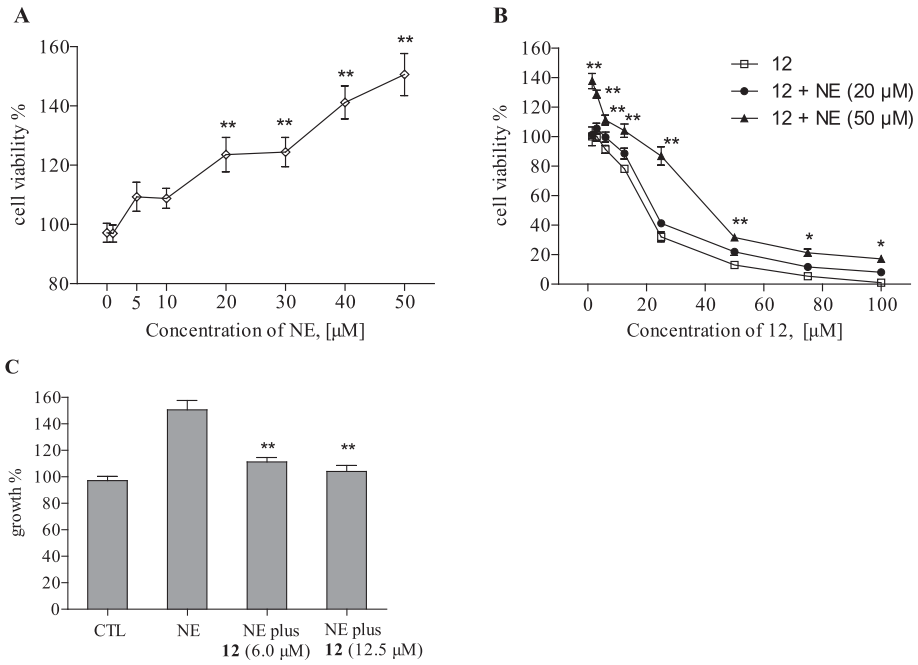


Fig. 8. NE protects PC-3 cells from **12**-induced antiproliferative ability. (A) NE stimulated PC-3 cell proliferation at different doses (0, 1, 5, 10, 20, 30, 40, and 50 μM) for 24 h. Data are the mean ± SEM, $^{*}P < 0.05$ and $^{**}P < 0.01$, one-way analysis of variance (ANOVA) followed by post-hoc tests. (B) The expression of the cell viability on PC-3 cells treated with **12** (100, 75, 50, 25, 12.5, 6.25, 3.0, and 1.5 μM), alone or in combination with different doses of NE (20 and 50 μM), was evaluated by WST-8 assays. Data are the mean ± SEM, $^{*}P < 0.05$ and $^{**}P < 0.01$, two-way analysis of variance (ANOVA) followed by post-hoc tests. (C) Compound **12** (6.0 and 12.5 μM) treatment inhibits NE (50 μM)-induced increase in PC-3 cell proliferation. Data are the mean ± SEM, $^{*}P < 0.05$ and $^{**}P < 0.01$, one-way analysis of variance (ANOVA) followed by post-hoc-tests. Columns, mean; error bars, SEM.

most potent α_{1B}-AR blocking activity was found to exhibit on compound **5**, which possessed the longest spacer length (pA_2 values > 7). Previous bladder construction studies on BPH suggested that the blockage of α_{1A}- and α_{1D}-ARs is necessary to achieve optimal clinical benefits [31]. Table 1 shows that compounds **3** and **12** preferentially bound with $\alpha_{1D/1A}$-ARs and displayed excellent levels of functional tissue selectivity. Since, the expression of α_{1B}-AR increases with age in the elderly population [33], therefore, the $\alpha_{1D/1A}$ -AR selective antagonists would possibly be less implicated in blood pressure regulation in elderly patients with spleen, suggesting it has a minimal effect on vascular tissues compared with prostatic tissue.

Previous studies have demonstrated that naftopidil **1** may be considered for the long-term prevention of PCa concomitant with BPH. Early detection and elimination of PCa cells are important to decrease PCa-related deaths. Thus, we examined the cytotoxic activity of naftopidil-related derivatives **2**–**12**. The moderate antiproliferative effects of **9** and **10** on the three cancer cell lines were attributed to their halogen group. Compound **12** exhibited the highest antigrowth activity probably because of the introduced benzyl moiety [23]. Naftopidil **1** was reported to suppress PC-3 cells growth via effecting G0/G1 phase arrest without inducing cell apoptosis, which could be yielded via its α_{1D}-AR antagonistic effect [15]. The present study demonstrated that compound **12** dose dependently inhibited PC-3 cell growth by inducing both G0/G1 cell cycle arrest and apoptosis. These effects indicate the potential potency of **12** in PCa prevention. Both compounds possessed a phenylpiperazine-based structure and arrested cells in the G0/G1 phase. The phenylpiperazine-based α_1-AR blockers may inhibit PCa cell growth through G0/G1 cell arrest, whereas the modifications of the substituent group can change the properties of the compound, resulting in other effects on PCa cells [3,34]. Combined with the expression of α_{1D}- and α_{1B}-AR subtypes in human androgen nonresponsive PC-3 prostate cancer cells [23], it is believed that the high α_1-AR antagonist potency, the more significant α_{1D}-selectivity and the higher lipophilic character with respect to naftopidil might be the factors responsible for the effects evoked by **12**. Targeting apoptosis to control prostatic growth has emerged as a potentially powerful therapeutic approach for the effective treatment of advanced PCa. The western blotting showed an increase in Bax/Bcl-2 values and caspase-3 expression following **12**-treatment in PC-3 cells, further confirming the apoptosis. Deep investigations on the mechanistic aspects of the antigrowth effect of **12** against prostatic tumors are currently in progress.

Most studies on α_1-AR antagonist and PCa focused on doxazosin. Quinazoline-derived subtype non-selective α_1-AR antagonist, doxazosin does active apoptosis in PCa cells via a mechanism independent of α_1-AR and androgens and without interfering with cell cycle progression. Treatment with NE (50 μM) does not affect the ability of doxazosin to inhibit cell viability and induce the apoptosis of PC-3 cells [23]. Moreover, NE does not influence the time-dependent inducing effect of doxazosin on caspase-3 activity in PCa cells, confirming the nonadrenergic nature of the phenomenon [13]. However, the results of the present study demonstrated that the antiproliferative effects of **12** are mediated by the α_1-ARs antagonism. Growing evidence supports the role of α_1-ARs in the direct mitogenic effect of catecholamines on prostate growth. As shown in Fig. 8, NE (20–50 μM) significantly increased in a concentration-dependent manner cell proliferation in PC-3 cells. NE (50 μM) exhibited the ability to inhibit PC-3 cell death induced by $\alpha_{1D/1A}$ -AR antagonist **12**. Moreover, Gonzalez-Cabera et al. [35] demonstrated that α_1-AR subtypes controlled proliferation by directly controlling the cell cycle; the α_{1A}- and α_{1D}-AR mediate G_1-S cell cycle arrest. This concept further confirmed the α_1-AR-dependent mechanism of compound **12**. The difference between the interaction of the novel antagonist **12** and doxazosin with NE is interesting and may be related to their structures.

In conclusion, we overlapped naftopidil **1**, the amide moiety, and

the I3C structure to develop new compounds with multipotent activities. We demonstrated the power of a multifunctional drug design strategy to discover drugs that combat various diseases. Compound **12** may represent a valid pharmacological tool and a promising lead to design new ligands for PCa prevention. These findings highlight the emerging therapeutic significance of using the class of phenylpiperazine-derived α_1-AR antagonists for BPH and PCa treatments. In addition to α_1-AR ligand, the methoxy phenylpiperazine scaffold might also be able to bind the 5-HT_{1A} and dopamine receptors [36–38]. A more comprehensive pharmacological evaluation and the mechanisms of **12** in BPH and PCa treatments will be studied in the future.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.04.005.

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