Pharmacological Reports
https://doi.org/10.1007/s43440-019-00041-w

ARTICLE

Synthesis and pharmacological evaluation of naftopidil-based arylpiperazine derivatives containing the bromophenol moiety

Hong Chen[1,2] · Yuna Qian[3,4] · Huixia Jia[2] · Yuzhong Yu[1] · Haibo Zhang[1] · Shanchao Zhao[1] · Jianliang Shen[3,4]

Received: 7 July 2019 / Revised: 19 July 2019 / Accepted: 25 September 2019

Abstract

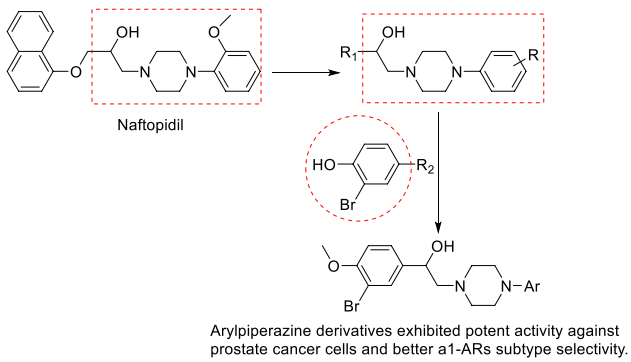
Background Prostate cancer (PCa) is the most common malignancy in men and in the absence of any effective treatments available.

Methods For the development of potential anticancer agents, 24 kinds of naftopidil-based arylpiperazine derivatives containing the bromophenol moiety were synthesized and characterized by using spectroscopic methods. Their pharmacological activities were evaluated against human PCa cell lines (PC-3 and LNCaP) and a_1-adrenergic receptors (a_1-ARs; α_{1a}, α_{1b}, and α_{1d}-ARs). The structure–activity relationship of these designed arylpiperazine derivatives was rationally explored and discussed.

Results Among these derivatives, **3c**, **3d**, **3h**, **3k**, **3o**, and **3s** exhibited the most potent activity against the tested cancer cells, and some derivatives with potent anticancer activities exhibited better a_1-AR subtype selectivity than others did (selectivity ratio > 10).

Conclusion This work provided a potential lead compound for the further development of anticancer agents for PCa therapy.

Graphic abstract



Keywords Prostate cancer · Synthesis · Arylpiperazine derivatives · Anticancer activity · Antagonistic activity

Introduction

Prostate cancer (PCa) is the most common malignancy and the second leading cause of cancer mortality in men [1]. In Europe, around 416,700 new PCa cases are diagnosed annually [2]. In the U.S. alone, 161,300 new cases of PCa and 26,730 deaths due to this disease were reported in 2017. Approximately one of six males in the U.S. may be afflicted with this type of cancer, and the risk is increased remarkably

Hong Chen and Yuna Qian have contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s43440-019-00041-w) contains supplementary material, which is available to authorized users.

✉ Shanchao Zhao
zhaoshanchao@263.net

✉ Jianliang Shen
shenjl@wibe.ac.cn

Extended author information available on the last page of the article

Published online: 23 January 2020

for older males. Genetics, age, race, diet, family history, and even lifestyle may contribute to the risk of PCa [3]. Current therapies (radical prostatectomy, chemotherapy, local radiotherapy, or hormonotherapy) are successful in treating localized diseases (androgen-dependent PCa) [4]. However, for nonorgan-confined diseases, especially metastatic PCa (androgen-independent PCa), no significantly effective therapies exist [5–8], and androgen ablation therapy has been the major therapeutic modality for advanced PCa [9]. Consequently, novel anticancer drugs are needed to stop the progression of PCa at later stages.

Piperazines and substituted piperazines are key pharmacophores that are crucial in many marketed drugs, such as the Merck HIV protease inhibitor Crixivan and other drugs under development [10]. Piperazine derivatives also exhibit receptor-blocking properties [11–15] and antiproliferative properties [16–23]. Naftopidil (Fig. 1), an arylpiperazine derivative, is an α_1-adrenoceptor blocker used for treating lower urinary tract symptoms associated with benign prostatic hyperplasia (BPH) [24], which is a widely used α_1-adrenergic receptor antagonist for the treatment of BPH in Japan. Other studies have demonstrated that naftopidil inhibits cell proliferation and causes cell cycle arrest in PC-3 and LNCaP cells [25]. It also induces apoptosis in malignant mesothelioma cell lines independent of α1-adrenoceptor blocking [26]. These findings indicate that naftopidil may be useful as an anticancer drug. Recently, we investigated compounds with a piperazine moiety as anticancer drugs for the site-directed chemotherapy of PCa. These new hybrids show a moderate to strong cytotoxic activity in PCa cell lines [27–31]. In vitro and in vivo evidence has shown that piperazine derivatives may be promising anticancer compounds. Hence, we further designed and synthesized a new naftopidil-based class of piperazine derivatives as potential anticancer agents.

Bromophenols, which are natural marine products isolated from marine organisms, such as algae, ascidian, sponges, jellyfish, and mollusks, possess various potent activities, including antioxidation; α-glucosidase inhibition; protein tyrosine kinase inhibition; protein tyrosine phosphatase 1B inhibition; antithrombosis; antimicrobial, anti-inflammatory, antibacterial, antifungal, and antiviral properties; free radical scavenging; aldose reductase inhibition; and anticancer activities [32–39]. Such products have been widely investigated in the fields of functional foods and pharmaceutical agents because of their potent activities and unique structures. Other studies have also shown that various bromophenols isolated from marine organisms, as well as synthesized derivatives from natural bromophenol, demonstrate an excellent anticancer activity against a panel of cancer cell lines [40–46].

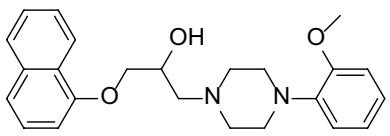


Fig. 1 Structures of naftopidil

Based on previous results, our current hypothesis is that the introduction of the bromophenol moiety into the piperazine skeleton might favor the pharmacological activity of piperazine derivatives. Herein, we designed and synthesized a series of novel naftopidil-based arylpiperazine derivatives containing the bromophenol moiety (Scheme 1). The design strategy of naftopidil-based arylpiperazine derivatives containing the bromophenol moiety is shown in Fig. 2. All of the derivatives were bioassayed against the PCa cell lines PC-3 and LNCaP and the normal prostate epithelial cell line WPMY-1. The antagonistic activities of the representative compounds with potent anticancer activities toward a_1-adrenergic receptors (a_1-ARs) were further evaluated through dual-luciferase reporter assays. A simple structure–activity relationship (SAR) study was also conducted to facilitate the further development of arylpiperazine derivatives. As expected, some arylpiperazine derivatives exhibited significant cytotoxic activities against the PC-3 and LNCaP cells. Some of these derivatives also showed a better a_1-AR subtype selectivity than others did.

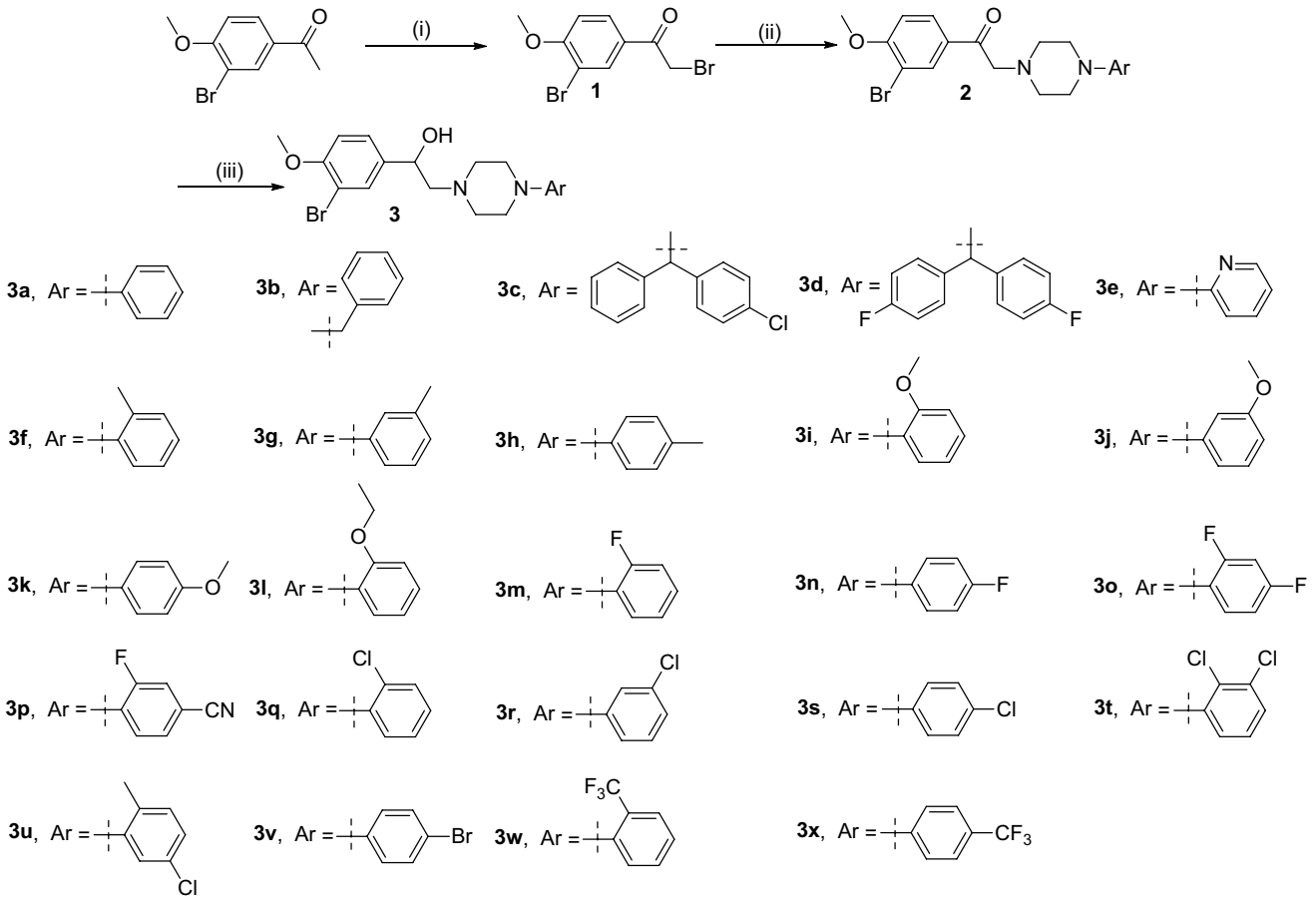
Materials and methods

Apparatus and analysis

All chemicals and reagents used in the current study were of analytical grade. Melting points (uncorrected) were measured on a SGW X-4 micro melting point apparatus. NMR spectra were obtained on a Bruker AVANCE-400 spectrometer in $CDCl_3$, with TMS as an internal standard, and chemical shift values were reported in δ (ppm) and coupling constants in Hertz. HRMS spectra were recorded on the AB Sciex X500R QTOF mass spectrometer (Foster, CA, USA). The completion of all reactions was monitored by TLC on precoated silica-gel 60 F_{254} TLC plates (VWR). The chromatograms were viewed under UV light at 254 and/or 365 nm.

Synthesis of 2-bromo-1-(3-bromo-4-methoxyphenyl) ethan-1-one (1) [47]

To a stirred solution of acetophenones 3-bromo-4-methoxyacetophenone (5 g, 21.9 mmol) in THF (80 mL) was added trimethylphenylammonium tribromide (9.07 g, 24.1 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 12 h. The solid was filtered, and to the filtrate was



Scheme 1 Reagents and conditions are as follows: (i) 1.1 times of $PhN^{+}Me^{3}\cdot Br_{3}^{-}$, THF, rt. (ii) 1.2 times of arylpiperazines, 4.0 times of K_2CO_3, CH_3CN, reflux; (iii) 2.0 times of $NaBH_4$, EtOH, rt

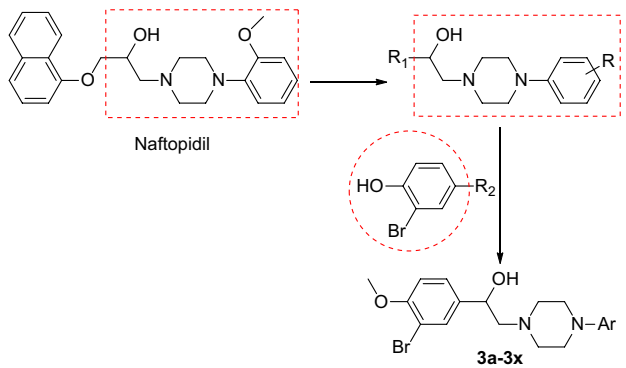


Fig. 2 Design strategy for naftopidil-based arylpiperazine derivatives containing the bromophenol moiety **3a**–**3x**

added EtOAc (150 mL). The organic layer was washed successively with H_2O (50 mL) and brine (50 mL). The organic layer was then dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was then purified by chromatography on silica-gel column (petroleum ether: ethyl acetate = 10:1, v/v) to obtain the intermediates **1**. White solid (ethanol); Yield: 82%; Mp 112–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 2.2 Hz, 1H), 7.94 (dd, J = 8.6, 2.2 Hz, 1H), 6.95 (d, J = 8.6 Hz, 1H), 4.37 (s, 2H), 3.98 (s, 3H). HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_9H_9Br_2O_2$, 306.8964, found, 306.8962.

General synthetic procedure of intermediates (2)

Arylpiperazines (1.2 equiv) and potassium carbonate (4.0 equiv) were added to a solution of **1** (1 equiv) in acetonitrile (CH_3CN, 20 mL). The reaction mixture was heated to 85 °C and stirred for 12 h. Afterward the mixture was cooled to room temperature. The reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was extracted with ethyl acetate (60 mL) and water (20 mL). After drying the organic layer with anhydrous Na_2SO_4 and evaporating the solvent under reduced pressure, a solid was appeared. The solid was recyrstallized from ethanol to obtain intermediates **2**.

General synthetic procedure of target compounds 3a–3x

$NaBH_4$ (2 equiv) was added to a stirred solution of the intermediates **2** (1 equiv) in ethanol (20 mL). The reaction mixture was left overnight at room temperature. The solvent was removed *in vacuo*. Then the residue was purified by chromatography on silica-gel column (petroleum ether: ethyl acetate = 1:1, v/v) to obtain the corresponding products **3a–3x**, and the solid was further recyrstallized from ethanol.

1-(3-Bromo-4-methoxyphenyl)-2-(4-phenylpiperazin-1-yl) ethan-1-ol (3a) White solid (ethanol); Yield: 85%; Mp 147–148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.36–7.27 (m, 3H), 6.95 (d, J = 7.9 Hz, 2H), 6.88 (dd, J = 7.9, 5.0 Hz, 2H), 4.71 (dd, J = 10.0, 3.9 Hz, 1H), 3.90 (s, 3H), 3.35–3.11 (m, 4H), 3.06–2.83 (m, 2H), 2.70–2.59 (m, 2H), 2.57–2.45 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.12, 150.97, 135.41, 130.75, 128.99, 125.88, 119.80, 116.01, 111.59, 111.50, 67.64, 65.91, 56.14, 52.85, 49.16; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{19}H_{24}BrN_2O_2$, 391.1016, found, 391.1020.

2-(4-Benzylpiperazin-1-yl)-1-(3-Bromo-4-methoxyphenyl) ethan-1-ol (3b) White solid (ethanol); Yield: 82%; Mp 107–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, J = 2.0 Hz, 1H), 7.32 (d, J = 4.4 Hz, 4H), 7.29–7.25 (m, 2H), 6.86 (d, J = 8.5 Hz, 1H), 4.64 (dd, J = 10.4, 3.7 Hz, 1H), 3.88 (s, 3H), 3.53 (d, J = 2.5 Hz, 2H), 2.77 (br s, 2H), 2.59–2.40 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.23, 138.00, 135.79, 130.92, 129.21, 128.27, 127.13, 126.06, 111.75, 111.64, 67.73, 66.02, 63.01, 56.31, 53.13, 29.72; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{26}BrN_2O_2$, 405.1172, found, 405.1147.

1-(3-Bromo-4-methoxyphenyl)-2-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethan-1-ol (3c) Colorless oil liquid; Yield: 76%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.39–7.33 (m, 4H), 7.32–7.26 (m, 3H), 7.25–7.17 (m, 3H), 6.86 (d, J = 8.5 Hz, 1H), 4.62 (dt, J = 10.0, 3.0 Hz, 1H), 4.22 (s, 1H), 3.88 (s, 3H), 2.75 (br s, 2H), 2.59–2.33 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.24, 142.05, 141.28, 135.76, 132.64, 130.92, 129.18, 128.73, 128.66, 127.82, 127.25, 126.06, 111.75, 111.64, 75.44, 67.73, 65.97, 56.31, 51.89, 29.72; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{26}H_{29}BrClN_2O_2$, 515.1095, found, 515.1097.

2-(4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)-1-(3-bromo-4-methoxyphenyl)ethan-1-ol (3d) Colorless oil liquid; Yield: 78%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, J = 2.0 Hz, 1H), 7.37–7.33 (m, 4H), 7.25 (dd, J = 7.9, 2.0 Hz, 1H), 7.00–6.95 (m, 4H), 6.86 (d, J = 8.5 Hz, 1H), 4.62 (dd, J = 10.1, 3.7 Hz, 1H), 4.23 (s, 1H), 3.87 (s, 3H), 2.75 (br s, 2H), 2.57–2.32 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.07, 160.63, 155.25, 138.14, 135.75, 130.91, 129.27, 129.20, 126.06, 115.57, 115.36, 111.75, 111.65, 74.48, 67.75, 65.96, 56.31, 51.82, 29.72; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{26}H_{28}BrF_2N_2O_2$, 517.1297, found, 517.1295.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(pyridin-2-yl)piperazin-1-yl)ethan-1-ol (3e) White solid (ethanol); Yield: 68%; Mp 101–102 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (dd, J = 4.8, 1.2 Hz, 1H), 7.59 (d, J = 2.0 Hz, 1H), 7.49 (ddd, J = 8.8, 7.2, 2.0 Hz, 1H), 7.29 (dd, J = 8.4, 2.0 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.76–6.54 (m, 2H), 4.73 (dd, J = 9.9, 3.6 Hz, 1H), 3.89 (s, 3H), 3.68–3.51 (m, 4H), 2.97–2.78 (m, 2H), 2.62–2.54 (m, 2H), 2.55–2.49 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.22, 155.11, 147.80, 137.36, 135.37, 130.74, 125.88, 113.38, 111.58, 111.48, 106.96, 67.65, 65.99, 56.12, 52.66, 45.14; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{18}H_{23}BrN_3O_2$, 392.0968, found, 392.0970.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(o-tolyl)piperazin-1-yl) ethan-1-ol (3f) White solid (ethanol); Yield: 80%; Mp 116–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.4, 2.0 Hz, 1H), 7.18 (t, J = 6.8 Hz, 2H), 7.07–6.96 (m, 2H), 6.89 (d, J = 8.4 Hz, 1H), 4.71 (dd, J = 10.4, 3.5 Hz, 1H), 3.89 (s, 3H), 3.13–2.79 (m, 6H), 2.67–2.48 (m, 4H), 2.31 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.28, 151.29, 135.76, 132.61, 131.12, 130.95, 126.62, 126.08, 123.31, 119.01, 111.79, 111.68, 67.79, 66.17, 56.33, 51.84, 29.72, 17.89; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{26}BrN_2O_2$, 405.1172, found, 405.1178.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(m-tolyl)piperazin-1-yl) ethan-1-ol (3g) Colorless oil liquid; Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.4, 2.0 Hz, 1H), 7.17 (t, J = 7.7 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.80–6.66 (m, 3H), 4.74 (dd, J = 9.4, 3.8 Hz, 1H), 3.89 (s, 3H), 3.36–3.13 (m, 4H), 3.03–2.81 (m, 2H), 2.74–2.60 (m, 2H), 2.61–2.48 (m, 2H), 2.33 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.33, 151.14, 138.90, 130.93, 129.03, 126.07, 120.99, 117.12, 113.39, 111.79, 67.81, 66.04, 56.33, 53.09, 49.33, 29.72, 21.79; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{26}BrN_2O_2$, 405.1172, found, 405.1176.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(p-tolyl)piperazin-1-yl) ethan-1-ol (3h) White solid (ethanol); Yield: 82%; Mp 146–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.4, 2.0 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 6.87 (t, J = 8.6 Hz, 3H), 4.71 (dd, J = 10.1, 3.8 Hz, 1H), 3.89 (s, 3H), 3.31–3.09 (m, 4H), 3.02–2.82 (m, 2H), 2.78–2.58 (m, 2H), 2.58–2.44 (m, 2H), 2.28 (s,

3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.30, 149.07, 135.65, 130.94, 129.70, 129.53, 126.07, 116.54, 111.78, 111.68, 67.83, 66.10, 56.33, 53.07, 49.91, 20.46; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{26}BrN_2O_2$, 405.1172, found, 405.1175.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(2-methoxyphenyl)piperazin-1-yl)ethan-1-ol (3i) Colorless oil liquid; Yield: 74%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 1.9 Hz, 1H), 7.29 (dd, J = 8.4, 1.9 Hz, 1H), 7.06–6.99 (m, 1H), 6.99–6.92 (m, 2H), 6.93–6.85 (m, 2H), 4.71 (dd, J = 10.2, 3.5 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.14 (br s, 4H), 3.02–2.89 (m, 2H), 2.71–2.63 (m, 2H), 2.62–2.44 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.40, 152.39, 141.21, 135.87, 131.07, 126.22, 123.27, 121.16, 118.38, 111.91, 111.80, 111.34, 67.89, 66.27, 56.45, 55.52, 50.88; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{26}BrN_2O_3$, 421.1121, found, 421.1118

1-(3-Bromo-4-methoxyphenyl)-2-(4-(3-methoxyphenyl) piperazin-1-yl)ethan-1-ol (3j) White solid (ethanol); Yield: 82%; Mp 133–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.5, 2.0 Hz, 1H), 7.19 (t, J = 8.2 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.55 (dd, J = 8.2, 2.0 Hz, 1H), 6.48 (t, J = 2.2 Hz, 1H), 6.44 (dd, J = 8.1, 2.1 Hz, 1H), 4.71 (dd, J = 9.7, 3.6 Hz, 1H), 3.89 (s, 3H), 3.80 (s, 3H), 3.39–3.10 (m, 4H), 3.03–2.85 (m, 2H), 2.66–2.58 (m, 2H), 2.58–2.45 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.62, 155.31, 152.53, 135.59, 130.93, 129.86, 126.07, 111.79, 111.69, 108.97, 104.67, 102.67, 67.84, 66.08, 56.33, 55.22, 52.99, 49.22; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{26}BrN_2O_3$, 421.1121, found, 421.1120

1-(3-Bromo-4-methoxyphenyl)-2-(4-(4-methoxyphenyl)piperazin-1-yl)ethan-1-ol (3k) White solid (ethanol); Yield: 76%; Mp 148–149 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.5, 2.0 Hz, 1H), 6.96–6.85 (m, 5H), 4.70 (dd, J = 10.0, 3.6 Hz, 2H), 3.90 (s, 3H), 3.78 (s, 3H), 3.17–3.12 (m, 4H), 2.95–2.85 (m, 2H), 2.69–2.59 (m, 2H), 2.59–2.49 (m, 2H); HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{26}BrN_2O_3$, 421.1121, found, 421.1116.

4-(4-(2,4-Difluorophenyl)piperazin-1-yl)-2H-benzo[h] chromen-2-one (3l) Colorless oil liquid; Yield: 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.4, 2.0 Hz, 1H), 7.02–6.96 (m, 1H), 6.95–6.84 (m, 4H), 4.72 (dd, J = 10.4, 3.5 Hz, 1H), 4.08 (q, J = 7.0 Hz, 2H), 3.89 (s, 3H), 3.16 (br s, 4H), 3.07–2.89 (m, 2H), 2.82–2.63 (m, 2H), 2.60–2.42 (m, 2H), 1.46 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.27, 151.57, 141.11, 135.77, 130.94, 126.07, 122.95, 121.02, 118.21, 112.47, 111.78, 111.67, 67.77, 66.18, 63.58, 56.32, 50.64, 29.72, 14.96; HRMS (ESI) m/z $[M+1]+$: Calcd for $C_{21}H_{28}BrN_2O_3$, 435.1278, found, 435.1276.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(2-fluorophenyl)piperazin-1-yl)ethan-1-ol (3m) White solid (ethanol); Yield: 78%; Mp 105–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.5, 2.0 Hz, 1H), 7.13–6.92 (m, 4H), 6.88 (d, J = 8.5 Hz, 1H), 4.71 (dd, J = 10.4, 3.5 Hz, 1H), 3.89 (s, 3H), 3.23–3.09 (m, 4H), 3.03–2.88 (m, 2H), 2.70–2.61 (m, 2H), 2.61–2.47 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.97, 155.31, 139.98, 139.89, 135.62, 130.94, 126.08, 124.53, 124.49, 122.70, 122.62, 118.99, 118.96, 116.28, 116.07, 111.78, 111.70, 67.80, 66.10, 56.33, 53.10, 50.67, 50.64; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{19}H_{23}BrFN_2O_2$, 409.0921, found, 409.0925.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(4-fluorophenyl)piperazin-1-yl)ethan-1-ol (3n) White solid (ethanol); Yield: 86%; Mp 126–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.4, 2.0 Hz, 1H), 7.04–6.93 (m, 2H), 6.91–6.85 (m, 3H), 4.71 (dd, J = 9.9, 3.6 Hz, 1H), 3.89 (s, 3H), 3.25–3.03 (m, 4H), 3.02–2.85 (m, 2H), 2.74–2.59 (m, 2H), 2.59–2.44 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.56, 155.76, 148.23, 136.00, 131.36, 126.50, 118.45, 118.38, 116.14, 115.92, 112.23, 112.13, 68.28, 66.47, 56.76, 53.47, 50.77; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{19}H_{23}BrFN_2O_2$, 409.0921, found, 409.0917.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(2,4-difluorophenyl)piperazin-1-yl)ethan-1-ol (3o) White solid (ethanol); Yield: 75%; Mp 103–104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.1 Hz, 1H), 7.28 (dd, J = 8.5, 2.0 Hz, 1H), 7.02–6.86 (m, 2H), 6.87–6.76 (m, 2H), 4.70 (dd, J = 10.4, 3.6 Hz, 1H), 3.89 (s, 3H), 3.34–3.00 (m, 4H), 3.06–2.84 (m, 2H), 2.70–2.60 (m, 2H), 2.59–2.45 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.32, 135.57, 130.93, 126.07, 119.44, 111.78, 111.70, 110.87, 110.62, 105.02, 104.76, 104.52, 67.81, 66.06, 56.33, 53.09, 51.02, 50.99; HRMS(ESI)m/z$[M+1]^+$: Calcd for $C_{19}H_{22}BrF_2N_2O_2$, 427.0827, found, 427.0825.

4-(4-(2-(3-Bromo-4-methoxyphenyl)-2-hydroxyethyl)piperazin-1-yl)-3-fluorobenzonitrile (3p) White solid (ethanol); Yield: 67%; Mp 132–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 1.9 Hz, 1H), 7.38 (dd, J = 8.4, 1.2 Hz, 1H), 7.30 (dd, J = 3.9, 1.9 Hz, 1H), 7.27 (d, J = 2.0 Hz, 1H), 6.98–6.84 (m, 2H), 4.71 (dd, J = 10.1, 3.7 Hz, 1H), 3.90 (s, 3H), 3.38–3.18 (m, 4H), 3.06–2.87 (m, 2H), 2.77–2.61 (m, 2H), 2.61–2.43 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.38, 155.26, 152.79, 143.90, 135.37, 130.92, 129.47, 126.06, 119.88, 119.64, 118.78, 118.74, 118.37, 118.35, 111.80, 111.73, 103.97, 67.87, 66.02, 56.33, 52.77, 49.75, 49.70, 29.71; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{22}BrFN_3O_2$, 434.0874, found, 434.0879.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(2-chlorophenyl)piperazin-1-yl)ethan-1-ol (3q) White solid (ethanol); Yield:

80%; Mp 105–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 2.0 Hz, 1H), 7.37 (dd, J = 7.9, 1.5 Hz, 1H), 7.29 (dd, J = 8.5, 2.0 Hz, 1H), 7.26–7.19 (m, 1H), 7.06 (dd, J = 8.0, 1.4 Hz, 1H), 6.99 (td, J = 7.8, 1.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 4.71 (dd, J = 10.5, 3.5 Hz, 1H), 3.89 (s, 3H), 3.12 (br s, 4H), 3.01–2.87 (m, 2H), 2.75–2.62 (m, 2H), 2.60–2.45 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.41, 149.18, 135.79, 131.05, 130.81, 128.91, 127.76, 126.20, 123.98, 120.52, 111.90, 111.81, 67.90, 66.19, 56.44, 51.42; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{19}H_{23}BrClN_2O_2$, 425.0626, found, 425.0628.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(3-chlorophenyl)piperazin-1-yl)ethan-1-ol (3r) White solid (ethanol); Yield: 73%; Mp 107–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.28 (dd, J = 8.4, 2.0 Hz, 1H), 7.17 (t, J = 8.1 Hz, 1H), 6.88 (dd, J = 5.4, 3.0 Hz, 2H), 6.84–6.78 (m, 2H), 4.71 (dd, J = 9.6, 3.7 Hz, 1H), 3.89 (s, 3H), 3.33–3.12 (m, 4H), 2.99–2.80 (m, 2H), 2.70–2.57 (m, 2H), 2.57–2.46 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.34, 152.17, 135.49, 135.01, 130.93, 130.09, 126.07, 119.57, 115.93, 114.01, 111.79, 111.71, 67.87, 66.04, 56.33, 52.85, 48.86; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{19}H_{23}BrClN_2O_2$, 425.0626, found, 425.0616.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(4-chlorophenyl)piperazin-1-yl)ethan-1-ol (3s) White solid (ethanol); Yield: 78%; Mp 126–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.5, 2.0 Hz, 1H), 7.24–7.18 (m, 2H), 6.95–6.80 (m, 3H), 4.71 (dd, J = 9.8, 3.8 Hz, 1H), 3.89 (s, 3H), 3.30–3.10 (m, 4H), 2.96–2.84 (m, 2H), 2.68–2.58 (m, 2H), 2.59–2.46 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.34, 149.75, 135.49, 130.92, 129.02, 126.06, 124.84, 117.38, 111.79, 111.71, 67.85, 66.03, 56.33, 52.90, 49.33; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{19}H_{23}BrClN_2O_2$, 425.0626, found, 425.0608.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)ethan-1-ol (3t) White solid (ethanol); Yield: 68%; Mp 128–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 2.0 Hz, 1H), 7.28 (dd, J = 8.5, 2.0 Hz, 1H), 7.22–7.13 (m, 2H), 6.97 (dd, J = 6.6, 3.0 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 4.71 (dd, J = 10.5, 3.5 Hz, 1H), 3.89 (s, 3H), 3.11 (br s, 4H), 2.93 (d, J = 4.3 Hz, 2H), 2.66 (d, J = 5.9 Hz, 1H), 2.61–2.46 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.32, 151.05, 135.61, 134.12, 130.94, 127.57, 127.51, 126.08, 124.79, 118.64, 111.79, 111.71, 67.81, 66.05, 56.33, 51.43; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{19}H_{22}BrCl_2N_2O_2$, 459.0236, found, 459.0232.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(5-chloro-2-methylphenyl)piperazin-1-yl)ethan-1-ol (3u) Colorless oil liquid; Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 2.0 Hz, 1H), 7.22 (dd, J = 8.4, 2.0 Hz, 1H), 7.02 (d, J = 8.0 Hz, 1H), 6.95–6.85 (m, 2H), 6.81 (d, J = 8.5 Hz, 1H), 4.63 (dd, J = 10.4, 3.6 Hz, 1H), 3.82 (s, 3H), 3.03–2.70 (m, 6H), 2.65–2.33 (m, 4H), 2.18 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.30, 152.35, 135.66, 132.00, 131.78, 130.94, 130.82, 126.08, 123.14, 119.54, 111.79, 111.69, 67.80, 66.11, 56.33, 51.66, 29.72, 17.50. HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{25}BrClN_2O_2$, 439.0782, found, 439.0776.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(4-bromophenyl)piperazin-1-yl)ethan-1-ol (3v) White solid (ethanol); Yield: 73%; Mp 131–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.44–7.32 (m, 2H), 7.28 (dd, J = 8.5, 2.0 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.85–6.76 (m, 2H), 4.71 (dd, J = 9.5, 3.8 Hz, 1H), 3.89 (s, 3H), 3.33–3.11 (m, 4H), 3.01–2.82 (m, 2H), 2.72–2.59 (m, 2H), 2.57–2.45 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.33, 150.15, 135.50, 131.93, 130.92, 126.06, 117.76, 112.12, 111.79, 111.70, 67.87, 66.03, 56.33, 52.86, 49.14; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{19}H_{23}Br_2N_2O_2$, 469.0121, found, 469.0115.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)ethan-1-ol (3w) Colorless oil liquid; Yield: 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 7.9 Hz, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.53 (t, J = 7.4 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.28 (dd, J = 8.4, 2.0 Hz, 1H), 7.23 (t, J = 7.7 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 4.70 (dd, J = 10.5, 3.6 Hz, 1H), 3.89 (s, 3H), 3.07–2.93 (m, 4H), 2.90 (d, J = 5.1 Hz, 2H), 2.60 (d, J = 3.5 Hz, 2H), 2.59–2.41 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.28, 152.33, 135.74, 132.79, 130.93, 127.28, 127.23, 126.08, 124.94, 123.99, 111.78, 111.70, 67.78, 66.08, 56.32, 53.52; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{23}BrF_3N_2O_2$, 459.0890, found, 459.0888.

1-(3-Bromo-4-methoxyphenyl)-2-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)ethan-1-ol (3x) White solid (ethanol); Yield: 83%; Mp 125–126 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 2.0 Hz, 1H), 7.49 (d, J = 8.7 Hz, 2H), 7.29 (dd, J = 8.5, 2.0 Hz, 1H), 6.94 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 1H), 4.72 (dd, J = 9.3, 3.9 Hz, 1H), 3.89 (s, 3H), 3.41–3.24 (m, 4H), 2.95–2.84 (m, 2H), 2.67–2.59 (m, 2H), 2.58–2.47 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.36, 153.14, 135.44, 130.93, 126.47, 126.43, 126.07, 114.69, 111.80, 111.72, 67.90, 66.05, 56.33, 52.76, 48.16; HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{20}H_{23}BrF_3N_2O_2$, 459.0890, found, 459.0892.

Biological assays

In vitro cytotoxic assay

Cell culture PC-3 and WPMY-1 cells were cultured in Dulbecco's modification Eagle's medium (DMEM, Invitrogen,

Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), 100 U/mL penicillin and 0.1 mg/mL streptomycin (Invitrogen). LNCaP cells were cultured in F12 media supplemented with 10% fetal bovine serum (FBS, Hyclone), 100 U/mL penicillin and 0.1 mg/mL streptomycin (Invitrogen). The cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

Assessment of antitumor activity by CCK-8 assay Cell proliferation was measured with the Cell Counting Kit-8 (CCK-8) assay kit (Dojindo Corp., Kumamoto, Japan). Cells were harvested during logarithmic growth phase and seeded in 96-well plates at a density of 1×10^5 cells/mL, and cultured at 37 °C in a humidified incubator (5% CO_2) for 24 h, followed by exposure to various concentrations of compounds tested for 24 h. Subsequently 10 µL of CCK-8 (Dojindo) was added to each well, the cells were then incubated for an additional 1 h at 37 °C to convert WST-8 into formazan. Cell growth inhibition was determined by measuring the absorbance (Abs) at $\lambda = 450$ nm using amicroplate reader. Three independent experiments were performed. Cell growth inhibition was calculated according to the following equation:

$$\text{Growth inhibition} = (1 - \text{OD of treated cells/OD of control cells}) \times 100\%.$$

The half maximal inhibitory concentrations (IC_{50}) were obtained from liner regression analysis of the concentration–response curves plotted for each tested compound.

Antagonistic activity in a_1-ARs by dual-luciferase reporter gene assay [48] Firefly and Renilla luciferase activities, which are indicated as RLUs, were determined using Dual-Glo luciferase assay kits (Promega) according to the manufacturer's instructions. RLUs were measured using a luminometer (GloMaxTM 96-Microplate Luminometer, Promega) and are reported as the mean ± SEM of three individual experiments. For agonists, fold of induction = $LU_{induced}$/$RLU_{uninduced}$. For antagonists, % of control = 100 × RLU (agonist + antagonist)/RLU (agonist alone). All RLUs were normalized against firefly RLUs/Renilla RLUs. Data are expressed as EC_{50}/IC_{50} values in µM, and the IC_{50} of phenylephrine (µM) was calculated by plotting the data using nonlinear regression analysis in Graph-Pad Prism 5 software.

Results

Chemistry

The synthetic route of compounds **3a**–**3x** followed the general pathway outlined in Scheme 1. These compounds were prepared in three steps. First, α-bromination reaction between 3-bromo-4-methoxyacetophenone and trimethylphenylammonium tribromide was carried out by using THF as the solvent to obtain intermediate **1**. Second, intermediate **2** was obtained by applying the nucleophilic substitution reaction between intermediate **1** and arylpiperazines, adding 4 times of K_2CO_3 in acetonitrile. Lastly, the reduction of intermediate **2** with $NaBH_4$ in ethanol led to the formation of the final arylpiperazine derivatives containing the bromophenol moiety. All of the synthesized arylpiperazine derivatives were confirmed via ^{1}H-NMR, ^{13}C-NMR, and HRMS.

Biological evaluation

Antitumor activity

All of the target compounds were screened in terms of their in vitro cytotoxicity against PC-3 and LNCaP, and compared with their effects on WPMY-1 via the CCK-8 assay [28, 29]. Naftopidil and finasteride [49] were used as reference compounds, and the results were reported in terms of IC_{50}. The results are summarized in Table 1.

As shown in Table 1, the cytotoxic activities of all the derivatives ($IC_{50} < 10$ µM) except **3p**, **3t**, and **3u** against PC-3 and LNCaP cells were strong, and their activities were also higher than those of finasteride. Among these compounds, **3c**, **3d**, **3h**, **3k**, **3o** and **3s** exhibited the most potent activity against PC-3 cells with IC_{50} of 0.67, 0.25, 0.16, 0.08, 0.79, and 0.55 µM, which were 26-, 71-, 111-, 222-, 22-, and 356-fold more active than finasteride, respectively. They also exhibited a weak cytotoxic effect on normal human prostate epithelial cell (WPMY-1) with IC_{50} of > 50 µM.

Antagonistic activity in a_1-ARs (α_{1a}, α_{1b}, and α_{1d})

PCa and BPH are common diseases in elderly males, and studies have shown that androgen receptor-mediated androgen affects the incidence of BPH and PCa, and derivatives with piperazine moiety may act as potential α_{1a}-AR- and/or α_{1a}-AR + α_{1d}-AR-selective ligands for the treatment of BPH [48, 50–52]. Therefore, to evaluate antagonistic action of arylpiperazine derivatives with bromophenol moiety on a_1-ARs (α_{1a}, α_{1b}, and α_{1d}), the derivatives with potent anticancer activities were selected for further investigating their antagonistic activities using dual-luciferase reporter assays [48] to identify a_1-AR subselective antagonist candidates to treat BPH from arylpiperazine derivatives. The results are shown in Table 2.

Table 1 In vitro cytotoxicity of arylpiperazine derivatives **3a**–**3x**

Compd.	IC_{50} (μM)[a]		
	PC-3[b]	LNCaP[b]	WPMY-1[b]
3a	3.05 ± 0.03	2.05 ± 0.12	49.2 ± 1.12
3b	1.23 ± 0.16	1.09 ± 0.15	> 50
3c	0.67 ± 0.12	0.56 ± 0.27	> 50
3d	0.25 ± 1.27	0.17 ± 0.54	> 50
3e	4.71 ± 0.16	3.62 ± 1.04	45.7 ± 0.46
3f	1.07 ± 0.32	5.73 ± 0.17	> 50
3g	0.89 ± 0.21	3.46 ± 0.26	> 50
3h	0.16 ± 0.02	1.04 ± 0.14	> 50
3i	2.73 ± 0.14	4.08 ± 1.10	32.2 ± 0.66
3j	1.45 ± 0.19	2.02 ± 0.53	42.8 ± 1.25
3k	0.08 ± 0.02	0.72 ± 0.22	> 50
3l	7.14 ± 0.13	5.47 ± 0.78	38.4 ± 1.07
3m	6.38 ± 1.09	5.42 ± 0.26	47.4 ± 0.65
3n	2.13 ± 0.15	3.42 ± 0.18	> 50
3o	0.79 ± 0.09	1.27 ± 0.34	> 50
3p	11.03 ± 0.78	9.27 ± 0.84	> 50
3q	4.03 ± 0.17	5.93 ± 0.32	37.7 ± 0.26
3r	1.32 ± 0.42	1.85 ± 0.18	> 50
3s	0.05 ± 0.04	0.18 ± 0.12	> 50
3t	15.31 ± 0.67	8.29 ± 1.04	> 50
3u	10.37 ± 0.54	12.57 ± 1.24	47.9 ± 1.05
3v	4.42 ± 0.15	5.77 ± 0.14	> 50
3w	7.38 ± 1.06	8.78 ± 0.65	> 50
3x	2.25 ± 0.15	3.25 ± 0.62	> 50
Naftopidil	42.10 ± 0.79	22.36 ± 0.61	> 50
Finasteride	17.83	14.53	–

[a]IC_{50} values are taken as mean ± standard deviation from three experiments

[b]PC-3, androgen-insensitive human prostate cancer cell line; LNCaP, androgen-sensitive human prostate cancer cell line; WPMY-1, normal non-cancer human prostate epithelial cell line

Table 2 Antagonistic activities (IC_{50}) on α_1-ARs (α_{1a}, α_{1b}, and α_{1d}) of arylpiperazine derivatives

Compd.	IC_{50} (nM)[a]			Selectivity ratio	
	α_{1a}	α_{1b}	α_{1d}	α_{1b}/α_{1a}	α_{1b}/α_{1d}
3b	412.12	926.31	765.26	2.2	1.2
3c	660.36	982.46	826.57	1.5	1.1
3d	393.85	764.43	472.98	1.9	1.6
3g	62.18	892.67	347.12	14.3	2.6
3h	467.63	1132.35	92.54	2.4	12.2
3j	77.84	972.05	873.27	12.5	1.1
3k	573.92	1045.63	87.36	1.8	11.9
3o	483.29	683.45	393.25	1.4	1.7
3r	92.64	963.72	673.56	10.4	1.4
3s	473.62	1053.35	87.47	2.2	12.0
Naftopidil	555	634	55.2	1.1	11.48

[a]IC_{50} values are taken as means from three experiments

Discussion

The SAR of these designed arylpiperazine derivatives was thoroughly discussed. With **3a** as the lead, the SAR investigation mainly focused on the variation in the phenyl group at the 4-position of the piperazine ring with other aryl groups and the substitute's type and position on the phenyl group as a required group for antitumor activities. (1) First, the resultant compound **3b** displayed an improved cytotoxic activity against PC-3 and LNCaP cells with IC_{50} values of 1.23 and 1.09 μM, respectively, after the phenyl group at the 4-position of the piperazine ring was replaced with the benzyl group. Especially, compounds **3c** and **3d** demonstrated potent activities against PC-3 and LNCaP cells. These results suggested that a larger group substituted at the 4-position of the piperazine ring was beneficial to the antitumor activity. (2) After the phenyl group was replaced with the pyridinyl group, the resultant compound **3e** displayed a comparable activity with that of **3a** against the tested cancer cells, and these compounds also elicited a weak cytotoxic effect on WPMY-1 with IC_{50} of > 50 μM. (3) The position of the substituent on the phenyl group also affected the cytotoxic activities. Among the compounds containing a methyl substituent, the order of the cytotoxic activities of compounds **3f** (2-CH_3), **3g** (3-CH_3), and **3h** (4-CH_3) against PC-3 and LNCaP cells could be placed as follows: **3h** > **3g** > **3f**. A similar order of antitumor activity was observed in **3i** (2-OCH_3), **3j** (3-OCH_3), **3k** (4-OCH_3), **3q** (2-Cl), **3r** (3-Cl), and **3s** (4-Cl). Namely, the activity of the *p*-substituted phenyl group derivatives against LNCaP and PC-3 cells was better than that of the substituted groups in other positions. In addition, the same order of antitumor activity against PC-3 and LNCaP cells was observed in **3m** (2-F) versus **3n** (4-F) and in **3w** (2-CF_3) versus **3x** (4-CF_3). (4) The effectiveness of the compounds with difluorosubstituents on the phenyl group was higher than that of the compounds with monofluorosubstituents. For example, the cytotoxic activity of **3o** (2,4-F_2, IC_{50} = 0.79 and 1.27 μM) against PC-3 and LNCaP cells was more effective than those of **3m** and **3n**. Moreover, the cytotoxic activity of **3o** against normal WPMY-1 was weak with IC_{50} of > 50 μM. However, the inhibitory activity of other disubstituted compounds **3p**, **3t**, and **3u** against cancer cells was relatively weak. (5) The cytotoxic activity of **3v** (4-Br) against PC-3 and LNCaP cells was relatively lower than those of **3n** (4-F) and **3s** (4-Cl). The activity profiles indicated that the introduction of a bromo group at the *p*-position in the phenyl group was inauspicious for anticancer activity. (6) The activity of **3k** (4-OCH_3) with electron-donating groups against LNCaP and PC-3 cells was relatively better than that of **3x** (4-CF_3)

with electron-withdrawing groups in the phenyl group. The activity profiles indicated that the introduction of electron-donating groups at the *p*-position in the phenyl group contributed to anticancer activities.

SAR studies revealed that a larger group substituted at the 4-position of the piperazine ring and the *p*-substituted phenyl group in the arylpiperazine derivatives displayed a relatively improved activity against the tested cancer cells.

As shown in Table 2, although arylpiperazine derivatives (**3b**, **3c**, and **3d**) with a larger group substituted at the 4-position of the piperazine ring exhibited strong cytotoxic activities against PC-3 and LNCaP cells, they demonstrated no a_1-ARs subtype selectivity. The *o*-substituted phenyl group arylpiperazine derivatives (**3g**, **3j**, and **3r**) showed potent cytotoxic activities against the tested cancer cells. Their a_{1a} subtype selectivity was better than a_{1b} subtype selectivity (a_{1b}/a_{1a} ratio > 10). By contrast, the a_{1d} subtype selectivity of *p*-substituted phenyl group arylpiperazine derivatives (**3h**, **3k**, and **3s**) with potent anticancer activities was better that the a_{1b} subtype (a1b/a1d ratio = 12.2, 11.9, and 12.0).

In summary, the majority of the derivatives exhibited strong cytotoxic activities against PC-3 and LNCaP cells, and possessed higher activities than finasteride, and derivatives with potent anticancer activities exhibited better a_1-ARs subtype selectivity (selectivity ratio > 10) than others did. Overall, the results of this study suggested that these derivatives could serve as candidates for the treatment of PCa and BPH.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (No. 31800833), Zhejiang Provincial Natural Science of Foundation of China (Nos. LZ19H180001 and LQ19C100001), the Wenzhou Medical University and Wenzhou Institute of Biomaterials and Engineering (WIBEZD2017001-03), the Science and Technology Planning Projects of Guangdong Province (No. 2016A020215175), the Natural Science Foundation of Guangdong Province (No. 2016A030313583), the Medical Scientific Research Foundation of Guangdong Province (No. A2016555), the Outstanding Youths Development Scheme of Nanfang Hospital, Southern Medical University (No. 2015J005) and the Science and Technology Planning Project of Guangzhou (No. 201704020070).

Compliance with ethical standards

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

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA-Cancer J Clin. 2018;68(1):7–30.
2. Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, Gavin A, Visser O, Bray F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer. 2018;103:356–87.
3. Nelson WG, De Marzo AM, Isaacs WB. Mechanisms of disease. The molecular pathogenesis of prostate cancer: a new role for inflammation? New Engl J Med. 2003;349(4):366–81.
4. Frydenberg M, Stricker PD, Kaye KW. Prostate cancer diagnosis and management. Lancet. 1997;349(9066):1681–7.
5. Dorff TB, Glode LM. Current role of neoadjuvant and adjuvant systemic therapy for high-risk localized prostate cancer. Curr Opin Urol. 2013;23(4):366–71.
6. Loblaw DA, Walker-Dilks C, Winquist E, Hotte SJ, G.C.D.S.G. of C.C.O.P. Systemic therapy in men with metastatic castration-resistant prostate cancer: a systematic review. Clin Oncol (R Coll Riodiol). 2013;25(7):406–30.
7. Han JY, Zhu FQ, Xu X, Huang H, Huang WQ, Cui WH, Dai H, Jiang JX, Wang SL. Tetramethylpyrazine hydrochloride inhibits proliferation and apoptosis in human prostate cancer PC3 cells through Akt signaling pathway. J Third Mil Med Univ. 2013;35:105–8.
8. Zou C, Li X, Jiang R. The progress of molecular mechanism studies for Chinese traditional medicine on prostate cancer therapy. Chin J Androl. 2012;26(1):66–8.
9. Akduman B, Crawford ED. The management of high risk the prostate cancer. J Urol. 2003;169(6):1993–8.
10. Szkaradek N, Rapacz A, Pytka K, Filipek B, Siwek A, Cegła M, Marona H. Synthesis and preliminary evaluation of pharmacological properties of some piperazine derivatives of xanthone. Bioorg Med Chem. 2013;21(2):514–22.
11. Leopoldo M, Lacivita E, Passafiume E, Contino M, Colabufo NA, Berardi F, Perrone R. 4-[omega-[4-arylpiperazin-1-yl]alkoxy]phenyl)imidazo[1,2-a]pyridine derivatives: fluorescent high-affinity dopamine D3 receptor ligands as potential probes for receptor visualization. J Med Chem. 2007;50(20):5043–7.
12. Chen X, Sassano MF, Zheng L, Setola V, Chen M, Bai X, Frye SV, Wetsel WC, Roth BL, Jin J. Structure-functional selectivity relationship studies of β-arrestin-biased dopamine D_2 receptor agonists. J Med Chem. 2012;55(53):7141–53.
13. Romeiro LA, da Silva Ferreira M, da Silva LL, Castro HC, Miranda AL, Silva CL, Noël F, Nascimento JB, Araújo CV, Tibiriçá E, Barreiro EJ, Fraga CA. Discovery of LASSBio-772, a 1,3-benzodioxole *N*-phenylpiperazine derivative with potent alpha 1A/D-adrenergic receptor blocking properties. Eur J Med Chem. 2011;46(7):3000–12.
14. Baran M, Kepczyńska E, Zylewski M, Siwek A, Bednarski M, Cegła MT. Studies on novel pyridine and 2-pyridone derivatives of *N*-arylpiperazine as α-adrenoceptor ligands. Med Chem. 2014;10(2):144–53.
15. Ananthan S, Saini SK, Zhou G, Hobrath JV, Padmalayam I, Zhai L, Bostwick JR, Antonio T, Reith ME, McDowell S, Cho E, McAleer L, Taylor M, Luedtke RR. Design, synthesis, and structure-activity relationship studies of a series of [4-(4-carboxamidobutyl)]-1-arylpiperazines: insights into structural features contributing to dopamine D3 versus D2 receptor subtype selectivity. J Med Chem. 2014;57(16):7042–60.
16. Berardi F, Abate C, Ferorelli S, de Robertis AF, Leopoldo M, Colabufo NA, Niso M, Perrone R. Novel 4-(4-aryl)cyclohexyl-1-(2-pyridyl)piperazines as Delta(8)-Delta(7) sterol isomerase (emopamil binding protein) selective ligands with antiproliferative activity. J Med Chem. 2008;51(23):7523–31.
17. Abate C, Niso M, Contino M, Colabufo NA, Ferorelli S, Perrone R, Berardi F. 1-Cyclohexyl-4-(4-arylcyclohexyl) piperazines: Mixed σ and human Δ(8)–Δ(7) sterol isomerase ligands with antiproliferative and P-glycoprotein inhibitory activity. ChemMedChem. 2011;6(1):73–80.
18. Liu WH, Chang JX, Liu Y, Luo JW, Zhang JW. Design, synthesis and activities of novel benzothiazole derivatives containing arylpiperazine. Acta Pharmaceutica Sinica. 2013;48(8):1259–65.

19. Lin HH, Wu WY, Cao SL, Liao J, Ma L, Gao M, Li ZF, Xu X. Synthesis and antiproliferative evaluation of piperazine-1-carbothiohydrazide derivatives of indolin-2-one. Bioorg Med Chem Lett. 2013;23(11):3304–7.
20. Cao SL, Han Y, Yuan CZ, Wang Y, Xiahou ZK, Liao J, Gao RT, Mao BB, Zhao BL, Li ZF, Xu X. Synthesis and antiproliferative activity of 4-substituted-piperazine-1-carbodithioate derivatives of 2,4-diaminoquinazoline. Eur J Med Chem. 2013;64(6):401–9.
21. Arnatt CK, Adams JL, Zhang Z, Haney KM, Li G, Zhang Y. Design, syntheses, and characterization of piperazine based chemokine receptor CCR5 antagonists as anti-prostate cancer agents. Bioorg Med Chem Lett. 2014;24(10):2319–23.
22. Lee YB, Gong YD, Yoon H, Ahn CH, Jeon MK, Kong JY. Synthesis and anticancer activity of new 1-[(5 or 6-substituted 2-alkoxyquinoxalin-3-yl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives. Bioorg Med Chem. 2010;18(22):7966–74.
23. Guo FJ, Sun J, Gao LL, Wang XY, Zhang Y, Qian SS, Zhu HL. Discovery of phenylpiperazine derivatives as IGF-1R inhibitor with potent antiproliferative properties in vitro. Bioorg Med Chem Lett. 2015;25(5):1067–71.
24. Castiglione F, Benigni F, Briganti A, Salonia A, Villa L, Nini A, Di Trapani E, Capitanio U, Hedlund P, Montorsi F. Naftopidil for the treatment of benign prostate hyperplasia: a systematic review. Curr Med Res Opin. 2014;30(4):719–32.
25. Kanda H, Ishii K, Ogura Y, Imamura T, Kanai M, Arima K, Sugimura Y. Naftopidil, a selective alpha-1 adrenoceptor antagonist, inhibits growth of human prostate cancer cells by G1 cell cycle arrest. Int J Cancer. 2008;122(2):444–51.
26. Masachika E, Kanno T, Nakano T, Gotoh A, Nishizaki T. Naftopidil induces apoptosis in malignant mesothelioma cell lines independently of α1-adrenoceptor blocking. Anticancer Res. 2013;33(3):887–94.
27. Chen H, Yang ZL, Sun T, Niu JX, Tian XM, Yuan M. Synthesis and biological evaluation of arylpiperazine derivatives as anticancer agents. Lett Org Chem. 2018;15(11):981–9.
28. Chen H, Liang X, Sun T, Qiao XG, Zhan Z, Li ZY, He CJ, Ya HY, Yuan M. Synthesis and biological evaluation of estrone 3-O-ether derivatives containing the piperazine moiety. Steroids. 2018;134:101–9.
29. Chen H, Wang CL, Sun T, Zhou Z, Niu JX, Tian XM, Yuan M. Synthesis, biological evaluation and SAR of naftopidil-based arylpiperazine derivatives. Bioorg Med Chem Lett. 2018;28(9):1534–9.
30. Chen H, Xu BB, Sun T, Zhou Z, Ya HY, Yuan M. Synthesis and Antitumor activity of novel arylpiperazine derivatives containing the saccharin moiety. Molecules. 2017;22(11):1857.
31. Chen H, Xu F, Xu BB, Xu JY, Shao BH, Huang BY, Yuan M. Design, synthesis and biological evaluation of novel arylpiperazine derivatives on human prostate cancer cell lines. Chin Chem Lett. 2016;27(1):277–82.
32. Liu M, Hansen PE, Lin X. Bromophenols in marine algae and their Bioactivities. Mar Drugs. 2011;9(7):1273–92.
33. Sun X, Xu N, Guo J, Yan X. Antitumor effects and the mechanism of two kinds of bromophenols from marine algae. China J Chin Mater Med. 2010;35(9):1173–6.
34. Lee JH, Park SE, Hossain MA, Kim MY, Kim M, Chung HY, Choi JS, Yoo Y. 2,3,6-Tribromo-4,5-dihydroxybenzyl methyl ether induces growth inhibition and apoptosis in MCF-7 human breast cancer cells. Arch Pharm Res. 2007;30(9):1132–7.
35. Liu M, Wang G, Xiao L, Xu X, Liu X, Xu P, Lin X. Bis(2,3-dibromo-4,5-dihydroxybenzyl) ether, a marine algae derived bromophenol, inhibits the growth of botrytis cinerea and interacts with DNA molecules. Mar Drugs. 2014;12(7):3838–51.
36. Liu M, Zhang W, Wei J, Qiu L, Lin X. Marine bromophenol bis(2,3-dibromo-4,5-dihydroxybenzyl) ether, induces mitochondrial apoptosis in k562 cells and inhibits topoisomerase I in vitro. Toxicol Lett. 2012;211(2):126–34.
37. Ma M, Zhao J, Wang S, Li S, Yang Y, Shi J, Fan X, He L. Bromophenols coupled with methyl gamma-ureidobutyrate and bromophenol sulfates from the red alga rhodomela confervoides. J Nat Prod. 2006;69(2):206–10.
38. Wang BG, Gloer JB, Ji NY, Zhao JC. Halogenated organic molecules of rhodomelaceae origin: chemistry and biology. Chem Rev. 2013;113(5):3632–85.
39. Wang LJ, Wang SY, Jiang B, Wu N, Li XQ, Wang BC, Luo J, Yang M, Jin SH, Shi DY. Design, synthesis and biological evaluation of novel bromophenol derivatives incorporating indolin-2-one moiety as potential anticancer agents. Mar Drugs. 2015;13(2):806–23.
40. Colon M, Guevara P, Gerwick WH, Ballantine D. 5′-Hydroxyisoavrainvilleol, a new diphenylmethane derivative from the tropical green-alga avrainvillea-nigricans. J Nat Prod. 1987;50(3):368–74.
41. Xu NJ, Fan X, Yan XJ, Tseng CK. Screening marine algae from China for their antitumor activities. J Appl Phycol. 2004;16(6):451–6.
42. Shi DY, Li J, Guo SJ, Su H, Fan X. The antitumor effect of bromophenol derivatives in vitro and Leathesia nana extract in vivo. Chin J Oceanol Limnol. 2009;27(2):277–82.
43. Shoeib NA, Bibby MC, Blunden G, Linley PA, Swaine DJ, Wheelhouse RT, Wright CW. In-vitro cytotoxic activities of the major bromophenols of the red alga Polysiphonia lanosa and some novel synthetic isomers. J Nat Prod. 2004;67(9):1445–9.
44. Liu M, Zhang W, Wei J, Lin X. Synthesis and alpha-glucosidase inhibitory mechanisms of bis(2,3-dibromo-4,5-dihydroxybenzyl) ether, a potential marine bromophenol alpha-glucosidase inhibitor. Mar Drugs. 2011;9(9):1554–65.
45. Pereira R, Benedetti R, Perez-Rodriguez S, Nebbioso A, Garcia-Rodriguez J, Carafa V, Stuhldreier M, Conte M, Rodriguez-Barrios F, Stunnenberg HG, Conte M, Rodríguez-Barrios F, Stunnenberg HG, Gronemeyer H, Altucci L, de Lera AR. Indole-derived psammaplin a analogues as epigenetic modulators with multiple inhibitory activities. J Med Chem. 2012;55(22):9467–91.
46. Guo CL, Wang L, Zhao Y, Liu H, Li XQ, Jiang B, Luo J, Guo SJ, Wu N, Shi DY. A novel bromophenol derivative BOS-102 induces cell cycle arrest and apoptosis in human A549 lung cancer cells via ROS-mediated PI3K/Akt and the MAPK signaling pathway. Mar Drugs. 2018;16(2):43.
47. Boulangé A, Parraga J, Galán A, Cabedo N, Leleu S, Sanz MJ, Cortes D, Franck X. Synthesis and antibacterial activities of cadiolides A, B and C and analogues. Bioorg Med Chem. 2015;23(13):3618–28.
48. Xu F, Chen H, Xu J, Liang X, He X, Shao B, Sun X, Li B, Deng X, Yuan M. Synthesis, structure-activity relationship and biological evaluation of novel arylpiperzines as $\alpha_{1A/1D}$-AR subselective antagonists for BPH. Bioorg Med Chem. 2015;23(24):7735–42.
49. Banday AH, Giri AK, Parveen R, Bashir N. Design and synthesis of D-ring steroidal isoxazolines and oxazolines as potential antiproliferative agents against LNCaP, PC-3 and DU-145 cells. Steroids. 2014;87:93–8.
50. Romeo G, Materia L, Modica MN, Pittalà V, Salerno L, Siracusa MA, Manetti F, Botta M, Minneman KP. Novel 4-phenylpiperidine-2,6-dione derivatives. Ligands for α_1-adrenoceptor subtypes. Eur J Med Chem. 2011;46(7):2676–90.
51. Awadallah FM, el-Eraky WI, Saleh DO. Synthesis, vasorelaxant activity, and molecular modeling study of some new phthalazine derivatives. Eur J Med Chem. 2012;52(6):14–21.

52. Prandi A, Franchini S, Manasieva LI, Fossa P, Cichero E, Marucci G, Buccioni M, Cilia A, Pirona L, Brasili L. Synthesis, biological evaluation, and docking studies of tetrahydrofuran–cyclopentanone- and cyclopentanol-based ligands acting at adrenergic α_1- and serotonine 5-HT1A receptors. J Med Chem. 2012;55(1):23–36.

Affiliations

Hong Chen[1,2] · Yuna Qian[3,4] · Huixia Jia[2] · Yuzhong Yu[1] · Haibo Zhang[1] · Shanchao Zhao[1] · Jianliang Shen[3,4]

[1] Department of Urology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China

[2] Luoyang Key Laboratory of Organic Functional Molecules, College of Food and Drug, Luoyang Normal University, Luoyang 471934, China

[3] State Key Laboratory of Ophthalmology, Optometry and Vision Science, School of Ophthalmology and Optometry, School of Biomedical Engineering, Wenzhou Medical University, Wenzhou 325035, China

[4] Wenzhou Institute of Biomaterials and Engineering, Chinese Academy of Science, Wenzhou 325001, China