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Design, synthesis and α_1-adrenoreceptor blocking activity of new arylpiperazines containing acetophenone substituents

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α_1-AR antagonists are currently first-line therapy for lower urinary tract symptoms associated with benign prostatic hyperplasia (LUTS/BPH). In this study, we report the synthesis of a new series of arylpiperazine derivatives containing acetophenone (**3-17**) which possess α_1-adrenoreceptor blocking activity. The *in vitro* α_1-adrenoreceptor blocking activity of each derivative was first screened using rabbit thoracic aortic rings by measuring the relaxation response (%) activated by (–)-noradrenaline (3 μM). Compounds **6** and **7** with 2,5-dimethoxy and 2-ethoxy substituent were found to have significant vasodilatory effect. Since the presence of a chiral carbon in the structure, **6** and **7** together with their enantiomers **14-17** were further evaluated by testing diastolic effect on rabbit thoracic aorta, prostate and bladder smooth muscle. The *S*-enantiomer was found to have more potent diastolic activity than the *R*-enantiomer and racemate, **17** being the most effective α_1-adrenoreceptor antagonist. In order to assess tissue selectivity, the antagonistic effect of **17** on the (-)-noradrenaline induced contractile response of isolated rat vas deferens (α_{1A}), spleen (α_{1B}) and aorta (α_{1D}) was characterized finally. Compared with naftopidil (**1**)and terazosin, compound **17** exhibited higher selectivity (18-fold) for the α_{1D}-adrenoceptor subtype as compared to the α_{1B}-adrenoceptor subtype, indicating less cardiovascular side effects for the treatment of LUTS/BPH. These data suggest that the acetophenone is a new effective adrenergic receptor ligand as well as incentivizes further research regarding pharmacological properties of chiral molecules.

1. Introduction

Benign prostatic hyperplasia is a common enlargement of the prostate gland that may lead to bladder outlet obstruction, lower urinary tract symptoms and reduced quality of life. LUTS comprise storage symptoms, often related to detrusor overactivity, and voiding symptoms (Berry et al. 1984; Thorpe and Neal 2003). LUTS/BPH is highly prevalent in aging men. α_1-Adrenoceptor (α_1-AR) antagonists (e. g. tamsulosin, terazosin, naftopidil, **1**) are considered to be the most effective monotherapy for LUTS associated with BPH (Kojima et al. 2009). They improve both symptom score and urinary flow in that condition. It is worth to note that selective urinary $\alpha_{1A/1D}$-AR blockers have become important tools in the treatment of LUTS/BPH. Distribution studies suggest that α_{1A}-AR selective blockers relieve obstructive outflows symptoms and improve urine flow *via* relaxation of prostate smooth muscle, and α_{1D}-AR blockers relieve bladder symptoms. However, α_{1B}-AR blockers have little benefit with respect to LUTS and may promote blood pressure related side effects (Schwinn 2008; Nichel 2003).

α_1-AR antagonists were divided into nine categories according their chemical structures (Jain et al. 2008). However, all the compounds possess a central basic center flanked on at least one side by aromatic systems. The presence of a protonated form of the molecule at physiological pH appears to be a vital feature for α_1-AR antagonists. Most of the α_1-AR antagonists - terazosin, doxazosin and naftopidil, which have a good effect for the treatment of LUTS/BPH, possess a structural fragment of piperazine (Marona et al. 2011). Naftopidil (**1**) is an $\alpha_{1D/1A}$ selective drug, belonging to the *N*-aryl piperazine derivatives. It has been marketed in Japan since 1999 for the treatment of LUTS/BPH and proven to be more effective than tamsulosin in treating storage symptoms (Takei et al. 1999; Nishino et al. 2006). Structural modifications of naftopidil have been reported recently. A series of naftopidil derivatives linked with a benzothiazine nucleus were synthesized and some compounds showed high α_1-AR and β_1-AR affinity (Cecchetti et al. 2000). Some rigid analogues of naftopidil with 1,3-dioxolane based ligands

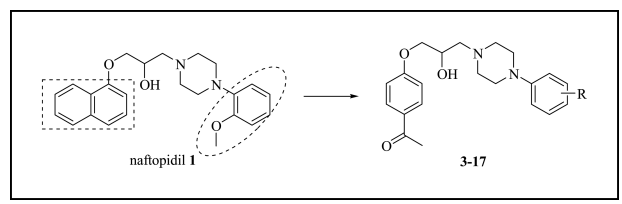


Fig. 1: Presentation of designed novel structures **3-17**.

Table 1: Percentage relaxation response of compounds 3-13 at 10 μM in rabbit thoracic aorta, previously contracted with NE (3 μM)

Compd.	% Relaxation Response ± SEM (n = 5)
3	61.6 ± 3.4
4	69.0 ± 7.8
5	14.3 ± 5.2
6	89.4 ± 4.9
7	90.6 ± 3.0
8	86.2 ± 3.6
9	73.9 ± 6.2
10	18.0 ± 9.1
11	45.5 ± 10.5
12	5.1 ± 2.1
13	30.7 ± 8.3
1	97.8 ± 1.5

Percentage relaxation was calculated according to the formula: (1-contraction induced by NE after compound (10 μM) incubation /contraction induced by NE (3 μM)) × 100

were found to be useful for the selectivity of α_1-AR and 5-HT_{1A} receptor systems (Sorbi et al. 2009).

We decided to replace the naphthalene moiety of **1** with acetophenone, in order to study the effect of a heterocyclic ring on the pharmacological profile (Fig. 1), which has never been reported. The substituent on the phenylpiperazine moiety was also changed. The designed structures **3-17** were synthesized and evaluated on functional α_1-AR blocking assays including vasodilatory effect and smooth muscle (rabbit prostate and bladder trigone) diastolic effect. Finally, a functional α_1-AR subtype selectivity study of the selected compound (**17**) was performed.

2. Investigations and results

We explored the potential of acetophenone ligand in affecting the α_1 blocking activity. Various new acetophenone derivatives have been synthesized using conventional synthetic methods and were screened for their α_1 blocking activity.

2.1. Synthesis of the compounds

All the racemic compounds **3-13** were generally prepared by the following procedures (Scheme): 4'-Hydroxyacetophenone was converted into the epoxide **2** using the epoxidation reaction with epichlorohydrine (Rauls and Baker 1979), followed by substitution with various piperazines in 2-propanol to provide the target compounds **3-13** (Pollard and Christie 1958). Optically active compounds were prepared starting from the optically active (*2R*) - or (*2S*) -glycidyltosylate in DMF to afford chiral epoxide (*R*)-**2** and (*S*)-**2** (Klunder et al. 1986), followed by substitution with piperazine in 2-propanol to provide the target enantiomers **14-17**. The structures of compounds **3-17** were determined by ESIMS, HRESIMS and ^{1}H NMR spectral data, and their absolute configurations were determined by Mosher's method (Piao et al. 2011; Mojid Mondol et al. 2011). The *ee* values of enantiomers were accomplished using a Chiralpak AD-H column (250 × 4.6 mm, 5 μm, Daicel, Japan) with an agilent 1200 HPLC (≥ 99 %).

2.2. Pharmacological results

*2.2.1. Vasodilatory activity of compounds **3-13** in rabbit thoracic aorta*

In preliminary screening, racemic compounds **3-13** were tested for their vasodilatory activity at a concentration of 10 μM on rabbit thoracic aortic rings precontracted with (–)-noradrenaline (NE) where β- and α_2-adrenoceptors were inhibited (Kava et al. 1998; Fagura et al. 1997). The percentage response produced by various compounds is shown Table 1.

Compound **1** exhibited strong vasodilatory activity (97.8 ± 1.5 %), thus was initially used as an antihypertensive drug. Substitution of acetophenone showed slightly weaker activity than that of protype **1**, and some of them (**5**, **10**, **12**) showed extremely weak activities. Meanwhile, substitution at *meta*-, *para*- and *ortho*- positions of the phenylpiperazine moiety led to differing levels of pharmacological activity. Compounds with *ortho*- or *meta*- alkyl and alkoxy substituted phenylpiperazine moiety (**6**, **7**, **8**) exhibited more potent α_1-adrenoceptor antagonistic activity than *para*-substituted derivatives (**3**, **4**, **5, 9, 10, 11, 12**). *Ortho*- bromine substituted phenylpiperazine (**13**) also showed disappointingly low vasodilatory activity (30.7 ± 8.3%), however, more potent than *para*- bromine substitution (**12**). Among all acetophenone derived arylpiperazines, **6** and **7** with 2,5 dimethoxy and 2-ethoxy moiety exhibited the best vasodilatory activity and a similar degree effect compared to that of **1** and were further studied for α_1 blocking activity. Moreover, **6** and **7** are racemic compounds; therefore, it is of special interest to investigate if the stereoisomers differ in their pharmacological properties.

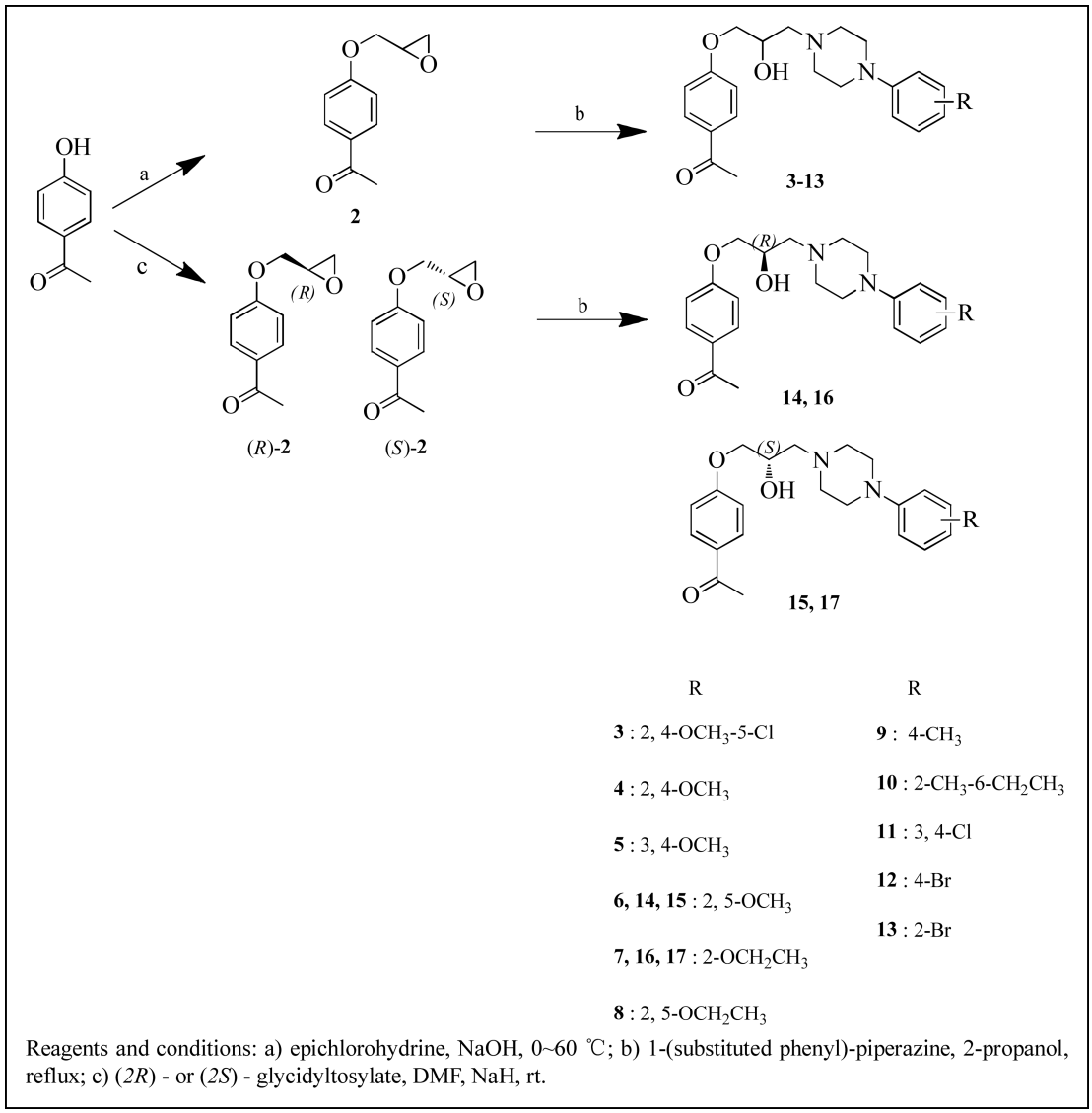
Table 2: Maximal relaxant effect (E_{max}) to inhibit the contractions induced by NE on rabbit aortic rings (3 μM), prostate (30 μM) and bladder strips (10 μM)

Compd.	E_{max} (%)		
	Thoracic aorta	Prostate smooth muscle	Bladder smooth muscle
6	89.4 ± 4.9	80.0 ± 3.5	73.5 ± 6.7
14 (*R*-**6**)	79.7 ± 6.4	57.6 ± 1.5	70.6 ± 4.2
15 (*S*-**6**)	92.6 ± 2.0	90.9 ± 2.5	81.6 ± 2.6
7	90.6 ± 3.0	98.3 ± 5.1	70.7 ± 9.1
16 (*R*-**7**)	83.2 ± 5.5	73.4 ± 7.4	62.0 ± 9.6
17 (*S*-**7**)	99.0 ± 2.0	106.4 ± 6.9	99.0 ± 10.9
1	97.8 ± 1.5	60.7 ± 3.4	72.7 ± 0.8

Percentage relaxation was calculated according to the formula: (1-contraction induced by NE after compound incubation /contraction induced by NE without compound) × 100

*2.2.2. α_1-AR blocking assays results of **14-17** including vasodilatory effect and smooth muscle (rabbit prostate and bladder trigone) diastolic effect*

Table 2 reports the relaxant experiments, expressed as E_{max} values of the enantiomeric pairs of **6-7** from rabbit isolated aortic rings, prostate and trigone of the bladder (Azuma et al. 1989; Oger et al. 2010). Compounds **6**, **7** and their enantiomers **14-17** (at a concentration of 0.001 μM-10 μM) antagonized the contractile responses to NE and produced concentration-dependent relaxations. Compound **1** produced E_{max} of 97.8 ± 1.5 %, 60.7 ± 3.4 %, 72.7 ± 0.8 % on aorta, prostate and bladder tissues, respectively. Concentration-response curves to acetophenone derivatives **6**, **14** (*R*-**6**), **15** (*S*-**6**), **7**, **16** (*R*-**7**) and **17** (*S*-**7**) in the three tissues are shown in Fig. 2. In terms of diastereoselectivity, α_1-AR blocking activity was found as *S*-configuration > racemate > *R*-configuration in the three tissues. In the rabbit aorta, **14** (*R*-**6**) showed the worst relaxation activity ($p < 0.05$ compared with **1**). Substitution of the 2-ethoxy moiety (**7**, **16**, and **17**) seems slightly more potent than 2,5-dimethoxy substitution (**6**, **14**, and **15**). An almost complete vasodilatory activity (E_{max} 99.0 ± 2.0%) was observed on compound **17**, although no significant differences were found between **17** and **1**. Compounds **6** and **7** showed a slightly weaker vasodilatory activity than that of **1**, however, they had more potent α_1-AR



Reagents and conditions: a) epichlorohydrine, NaOH, 0~60 ℃; b) 1-(substituted phenyl)-piperazine, 2-propanol, reflux; c) (*2R*) - or (*2S*) - glycidyltosylate, DMF, NaH, rt.

Scheme: Synthetic route to target compounds **3-17**.

blocking activity in prostate than **1** and their *R*-configuration ($p < 0.05$), indicating more selectivity on the lower urinary tract than on the blood vessels.

Compounds **14** (*R*-**6**) and **16** (*R*-**7**) showed no differences on prostate relaxation compared with **1** ($p > 0.05$), however, the *S*-enantiomers **15** and **17** elicited a significant increase on prostate than that of **1** ($p < 0.01$). **17** was the most effective compound in rabbit prostate relaxation assay (E_{max} 106.4 ± 6.9 %), and also induced the strongest relaxation effect on rabbit trigone of the isolated bladder (E_{max} 99.0 ± 10.9 %).

*2.2.3. Functional bioassays results of compound **17***

In order to assess tissue selectivity, the antagonistic effect of compound **17** on Sprague-Dawley rat vas deferens (α_{1A}), rat spleen (α_{1B}) and thoracic aorta (α_{1D}) was characterized (Table 3). Compound **17** appeared to be a competitive antagonist at each sub-receptor, because the slopes of the schild plots were not significantly different from unity on the basis of parallel shifts to concentration-response curves, and no reduction of maximal agonist-induced contractile responses with increasing concentration of **17**. As expected, **17** was a potent α_1 antagonist with pA_2 values of 7.76 (α_{1A}), 6.83 (α_{1B}), 8.10 (α_{1D}). Moreover, **17** exhibited a higher selectivity on α_{1A} and α_{1D} subtypes compared with **1** and terazosin, the selectivity ratios pA_2 (α_{1D})/pA_2 (α_{1B}) and pA_2 (α_{1A})/pA_2 (α_{1B}) were about -18 and -8 fold. It is worth to note that the affinity from the functional assay for **17** was in the same concentration range as determined in rabbit diastolic assay.

3. Discussion

The present study provides experimental support to a new effective α_1-AR ligand-acetophenone. We demonstrated a new series of naftopidil derivatives and their α_1-AR blocking activities using diastolic effect assays on rabbit isolated tissues and rat functional assays.

The expressions of α_1-AR in the prostate, urethra, spinal cord and bladder are important in the development of LUTS (Schwinn and Roehrborn 2008). In rabbit isolated aorta and bladder trigone, the activation of postsynaptic α_1-adrenoceptors may cause NE, phenylephrine and clonidine-elicited concentration-dependent contractions. α_1-AR can also mediate contraction of the smooth muscle of rabbit prostate. A preliminary vasodilatory effect assay was proceeded to choose some promising derivatives for further experimental study. *Ortho*-substitution (**7**) and *ortho*, *meta*-substitution (**6**) showed much better α_1-AR blocking activity in rabbit aorta than the *para*-substitution derivatives. However, in the following diastolic experiment of rabbit isolated prostate and bladder, the 1- (*o*-ethoxyphenyl)-piperazine moiety

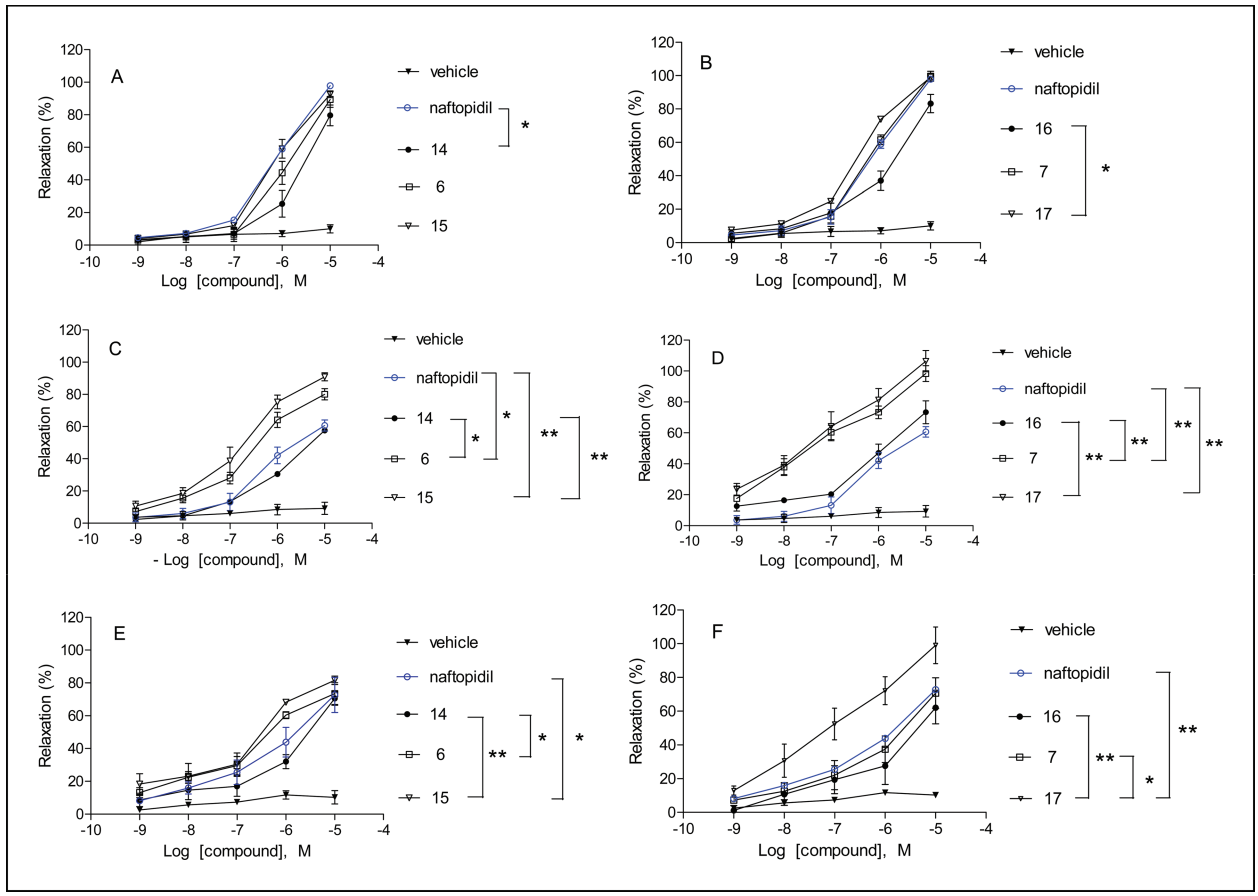


Fig. 2: Concentration-response curves to compounds **6**, **14** (*R*-**6**), **15** (*S*-**6**) and **7**, **16** (*R*-**7**), **17** (*S*-**7**) in rabbit aorta (A, B), prostate (C, D) and bladder (E, F). Data are mean ± S.E.M. for n = 6-8. $^{*}p < 0.05$, $^{**}p < 0.01$, two-way analysis of variance (ANOVA) followed by post-hoc-tests.

Table 3: Functional antagonistic potency of 17, expressed as pA_2, at α_1-adrenoceptor subtypes of SD rat isolated tissues: vas deferens (α_{1A}), spleen (α_{1B}) and thoracic aorta (α_{1D})

Compd.	pA_2 [a] (slope)			Selectivity ratio		
	α_{1A}	α_{1B}	α_{1D}	α_{1D}/α_{1A}	α_{1D}/α_{1B}	α_{1A}/α_{1B}
17	7.76 ± 0.07 (1.05 ± 0.03)	6.83 ± 0.07 (0.99 ± 0.15)	8.10 ± 0.03 (1.12 ± 0.20)	2.19	18.62	8.51
1 [b]	7.48 ± 0.07 (1.00 ± 0.11)	6.75 ± 0.11 (1.22 ± 0.14)	7.93 ± 0.11 (1.10 ± 0.04)	2.82	15.14	5.37
Terazosin [c]	7.90 ± 0.15 (1.13 ± 0.07)	8.59 ± 0.08 (0.99 ± 0.12)	8.83 ± 0.17 (1.06 ± 0.19)	8.51	1.74	0.20

[a] pA_2 values, expressed as Mean ± S.E.M. of three different concentrations, each tested at least four times.
[b] the correponding pK_i values were 8.43 ± 0.06, 7.70 ± 0.02, 8.92 ± 0.00, obtained in binding experiment (Takei 1999).
[c] the reference data was 8.04, 8.60, 8.65 (Hancock 2002).

provides compound **17** with the highest α_1-AR affinity, which is in agreement with a previous SAR study (Marona et al. 2011). Substitution at *meta*- and *para*- positions of the phenylpiperazine moiety did not lead to good α_1-AR blocking activities.

α_1-AR have a crucial role in conveying human prostate smooth muscle and bladder contraction in response to NE (Oger 2010). By reducing the adrenergic tone of the prostate and decreasing bladder contractile tone, the promising compound **17** may have the potential to relieve LUTS/BPH symptoms. Combined with the studies on tissue distribution of α_1-AR subtypes, the contraction in the rabbit prostate may be mediated by a subtype corresponding to the α_{1A} (Delaflotte et al. 1996; Palea et al. 2008) and α_{1D}-AR (Suzuki et al. 1997), whereas in the rabbit aorta, α_{1B}-AR activation seems to be responsible for contraction (Marucci et al. 2005). α_1-AR mediated contraction in rabbit trigone resembles the α_{1A}-AR (Michel and Vrydag 2006). These facts explain the reason why three rabbit isolated tissues (thoracic aorta, prostate, bladder smooth muscle) were used in this study. Compound **17** increases the blocking activity of rabbit prostate and trigone bladder better than **1**, indicating that **17** may improve uro-selectivity as an $\alpha_{1A/1D}$-AR antagonist that may be useful for the treatment of LUTS/BPH. Taking into account three sub-receptor of α_1-AR, functional assays of rat isolated tissues on **17** were done to confirm the selectivity and blocking activity of α_{1A}, α_{1B}, α_{1D}-subtypes. Compound **17** was a promising $\alpha_{1A/1D}$ antagonist with selectivity ratios of 18 ($\alpha_{1D/1B}$) and 8 ($\alpha_{1D/1A}$), suggesting that it may be an effective agent for improving symptoms related to bladder filling in the treatment of BPH.

In conclusion, a new class of α_1-AR ligands bearing an acetophenone structure has been reported. These fifteen derivatives display moderate to excellent α_1-AR blocking activity in the rabbit functional assays. The 1-(2-ethoxyphenyl)-piperazine moiety as well as the *S*-configuration provides compound **17** with the highest α_1-AR blocking activity. Importantly, **17** shows high selectivity toward α_{1A}, α_{1D}-subtypes over α_{1B}. Moreover, studies of chiral pharmacology reveals differences between the configurations, and *S*-enantiomers are always more potent than

racemates and *R*-enantiomers. However, a greater number of derivatives is needed to confirm this hypothesis. The results from the study increase the molecular diversity of selective $\alpha_{1A/1D}$-AR antagonists and provide new information for the development of chiral drugs used in the treatment of LUTS/BPH.

4. Experimental

4.1. Chemistry

Melting points were determined with an X-4 apparatus and the temperature was uncorrected. ^{1}H NMR (500 MHz) spectra were recorded in $CDCl_3$ or DMSO-d_6 on a BrukerAvance spectrometer using tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI) Mass spectra (electron ionization (EI), 70 eV) were recorded on an agilent 6330 ion trap LC/MS system. HRESIMS spectra were recorded on Shimadzu LCMS-IT-TOF Mass Spectrometer. Thin-layer chromatography (TLC) was performed on an aluminum plate pre-coated with silica gel and a fluorescence indicator (Merck). Detection on TLC was made by UV (254 nm). Detection of *ee* values of enantiomers was using a Chiralpak AD-H column (250 × 4.6 mm, 5 μm, DAICEL, Japan) with an agilent 1200 HPLC. All the other reagents and chemicals were obtained from commercial sources and used as received unless otherwise stated.

*4.1.1. General procedure for the synthesis of racemic derivatives **3-13***

NaOH aq. (40 %, 3 mL) was added dropwise to an ice-cooled solution of 4'-hydroxyacetophenone (2.7 g, 20 mmol) and epichlorohydrine (2.2 g, 24 mmol). The reaction mixture was further stirred at 60 °C for 6 h and checked by TLC. After completed, the reaction mixture was diluted with water (100 mL) and extracted with ethyl acetate (100 mL × 3). The combined organic layer was washed with water and brine, dried over anhydrous sodium sulfate and was evaporated under reduced pressure. The crude product **2** was then refluxed with various piperazine (20 mmol) in 2-propanol (100 mL) for 6 h. The mixture was evaporated under reduced pressure and then purified by silica gel chromotography using ethyl acetate and petroleum ether as eluent to afford target compounds **3-13**.

4.1.1.1. 1-(2,4-Dimethoxy-5-chlorophenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)- piperazine (**3**). Yield 68.2 % as a white solid, m.p. 174-176 °C. ^{1}H NMR (500 MHz, $CDCl_3$), (δ: ppm): 2.55 (s, 3H, -$COCH_3$), 2.63 (m, 4H, piperazinyl left H), 2.87 (brs, 2H, -CH_2-N), 3.03 (brs, 4H, piperazinyl right H), 3.88 (s, 6H, -OCH_3 × 2), 4.07 (m, 2H, -OCH_2-), 4.16 (m, 1H, -CH-OH), 6.53-7.93 (m, 6H, -ArH). ESIMS *m/z* 449.4 $(M+H)^+$, 471.3 $(M+Na)^+$. HRESIMS *m/z* 449.1830 (M^+ + H, calcd for $C_{23}H_{30}O_5N_2Cl$, 449.1824).

4.1.1.2. 1-(2,4-Dimethoxyphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**4**). Yield 77.8 % as a pink solid, m.p. 131-133 °C. ^{1}H NMR (500 MHz, $CDCl_3$), (δ: ppm): 2.48 (s, 3H, -$COCH_3$), 2.51-2.60 (m, 4H, piperazinyl left H), 2.82 (brs, 2H, -CH_2-N), 2.97 (brs, 4H, piperazinyl right H), 3.71, 3.77 (s, each 3H, -OCH_3 × 2), 3.97-4.03 (m, 2H, -OCH_2-), 4.08 (m, 1H, -CH-OH), 6.35-7.87 (m, 7H, -ArH). ESIMS *m/z* 415.4 $(M+H)^+$, 437.3 $(M+Na)^+$. HRESIMS *m/z* 415.2235 (M^+ + H, calcd for $C_{23}H_{31}O_5N_2$, 415.2228).

4.1.1.3. 1-(3,4-Dimethoxyphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**5**). Yield 78.0 % as a white solid, m.p. 130-132 °C. ^{1}H NMR (500 MHz, $CDCl_3$), (δ: ppm): 2.56 (s, 3H, -$COCH_3$), 2.59-2.69 (m, 4H, piperazinyl left H), 2.87 (m, 2H, -CH_2-N), 3.11-3.17 (brs, 4H, piperazinyl right H), 3.84, 3.88 (s, each 3H, -OCH_3 × 2), 4.09 (m, 2H, -OCH_2-), 4.16-4.19 (m, 1H, -CH-OH), 6.46-7.95 (m, 7H, -ArH). ESIMS *m/z* 415.3 $(M+H)^+$, 437.3 $(M+Na)^+$. HRESIMS *m/z* 415.2231 (M^+ + H, calcd for $C_{23}H_{31}O_5N_2$, 415.2228).

4.1.1.4. 1-(2,5-Dimethoxyphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**6**). Yield 56.2 % as a white solid, m.p. 129-130 °C. ^{1}H NMR (500 MHz, $CDCl_3$), (δ: ppm): 2.56 (s, 3H, -$COCH_3$), 2.59-2.68 (m, 4H, piperazinyl left H), 2.89 (m, 2H, -CH_2-N), 3.12 (brs, 4H, piperazinyl right H), 3.77, 3.83 (s, each 3H, -OCH_3 × 2), 4.06-4.11 (m, 2H, -OCH_2-), 4.14-4.18 (m, 1H, -CH-OH), 6.49-7.95 (m, 7H, -ArH). ESIMS *m/z* 415.4 $(M+H)^+$, 437.3 $(M+Na)^+$. HRESIMS *m/z* 415.2224 (M^+ + H, calcd for $C_{23}H_{31}O_5N_2$, 415.2228).

4.1.1.5. 1-(2-Ethoxyphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**7**). Yield 62.9 % as a white solid, m.p. 114-116 °C. ^{1}H NMR (500 MHz, DMSO-d_6), (δ: ppm): 1.47 (t, 3H, -OCH_2CH_3), 2.56 (s, 3H, -$COCH_3$), 2.64 (m, 4H, piperazinyl left H), 2.90 (m, 2H, -CH_2-N), 3.16 (brs, 4H, piperazinyl right H), 4.06-4.11 (m, 4H, -OCH_2, -OCH_2CH_3), 4.15-4.19 (m, 1H, -CH-OH), 6.86-7.95 (m, 8H, -ArH). ESIMS *m/z* 399.3 $(M+H)^+$, 421.3 $(M+Na)^+$. HRESIMS *m/z* 399.2278 (M^+ + H, calcd for $C_{23}H_{31}O_4N_2$, 399.2278).

4.1.1.6. 1-(2,5-Diethoxyphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**8**). Yield 61.4 % as a white solid, m.p. 143-145 °C. ^{1}H NMR (500 MHz, $CDCl_3$), (δ: ppm): 1.40, 1.42 (t, each 3H, -OCH_2CH_3 × 2), 2.57 (s, 3H, -$COCH_3$), 2.59-2.68 (m, 4H, piperazinyl left H), 2.89 (brs, 2H, -CH_2-N), 3.15 (brs, 4H, piperazinyl right H), 3.98, 4.02 (q, each 2H, -OCH_2CH_3 × 2), 4.06-4.11 (m, 2H, -OCH_2-), 4.15-4.20 (m, 1H, -CH-OH), 6.45-7.95 (m, 7H, -ArH). ESIMS *m/z* 443.4 $(M+H)^+$, 465.3 $(M+Na)^+$. HRESIMS *m/z* 443.2536 (M^+ + H, calcd for $C_{25}H_{35}O_5N_2$, 443.2541).

4.1.1.7. 1-(4-Methylphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**9**). Yield 60.1 % as a white solid, m.p. 153-155 °C. ^{1}H NMR (500 MHz, $CDCl_3$), (δ: ppm): 2.14 (s, 3H, -CH_3), 2.57 (s, 3H, -$COCH_3$), 2.59-2.69 (m, 4H, piperazinyl left H), 2.87 (m, 2H, -CH_2-N), 3.18 (t, 4H, piperazinyl right H), 4.06-4.11 (m, 2H, -OCH_2-), 4.16-4.20 (m, 1H, -CH-OH), 6.85-7.96 (m, 8H, -ArH). ESIMS *m/z* 369.4 $(M+H)^+$, 391.3 $(M+Na)^+$. HRESIMS *m/z* 369.2183 (M^+ + H, calcd for $C_{22}H_{29}O_3N_2$, 369.2173).

4.1.1.8. 1-(2-Methyl-6-ethylphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**10**). Yield 64.8 % as a white solid, m.p. 98-100 °C. ^{1}H NMR (500 MHz, DMSO-d_6), (δ: ppm): 1.14 (t, 3H, -CH_2CH_3), 2.28 (s, 3H, -CH_3), 2.43-2.48 (m, 4H, piperazinyl left H), 2.61-2.67 (m, 4H, piperazinyl right H), 3.11-3.16 (m, 2H, -CH_2-N), 3.30 (s, 3H, -$COCH_3$), 4.00-4.02 (m, 2H, -OCH_2-), 4.12-4.14 (m, 1H, -CH-OH), 6.95-7.94 (m, 7H, -ArH). ESIMS *m/z* 397.4 $(M+H)^+$, 419.3 $(M+Na)^+$. HRESIMS *m/z* 397.2483 (M^+ + H, calcd for $C_{24}H_{33}O_3N_2$, 397.2486).

4.1.1.9. 1-(3,4-Dichlorophenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**11**). Yield 71.2 % as a white solid, m.p. 135-136 °C. ^{1}H NMR (500 MHz, $CDCl_3$), (δ: ppm): 2.57 (s, 3H, -$COCH_3$), 2.59-2.70 (m, 4H, piperazinyl left H), 2.85 (m, 2H, -CH_2-N), 3.17-3.25 (m, 4H, piperazinyl right H), 4.06-4.11 (m, 2H, -OCH_2-), 4.15-4.19 (m, 1H, -CH-OH), 6.74-7.95 (m, 7H, -ArH). ESIMS *m/z* 445.4 $(M+Na)^+$. HRESIMS *m/z* 423.1233 (M^+ + H, calcd for $C_{21}H_{25}O_3N_2Cl_2$, 423.1237).

4.1.1.10. 1-(4-Bromophenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**12**). Yield 78.2 % as a white solid, m.p. 186-188 °C. ^{1}H NMR (500 MHz, $CDCl_3$), (δ: ppm): 2.56 (s, 3H, -$COCH_3$), 2.58-2.68 (m, 4H, piperazinyl left H), 2.82-2.86 (m, 2H, -CH_2-N), 3.16-3.22 (m, 4H, piperazinyl right H), 4.08-4.10 (m, 2H, -OCH_2-), 4.14-4.19 (m, 1H, -CH-OH), 6.78-7.94 (m, 8H, -ArH). ESIMS *m/z* 455.5 $(M+Na)^+$. HRESIMS *m/z* 433.1134 (M^+ + H, calcd for $C_{21}H_{26}O_3N_2Br$, 433.1121).

4.1.1.11. 1-(2-Bromophenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**13**). Yield 45.8 % as a white solid, m.p. 166-168 °C. ^{1}H NMR (500 MHz, $CDCl_3$), (δ: ppm): 2.56 (s, 3H, -$COCH_3$), 2.61-2.67 (m, 4H, piperazinyl left H), 2.83-2.87 (m, 2H, -CH_2-N), 3.18-3.24 (m, 4H, piperazinyl right H), 4.07-4.10 (m, 2H, -OCH_2-), 4.15-4.19 (m, 1H, -CH-OH), 6.80-7.95 (m, 8H, -ArH). ESIMS *m/z* 457.3 $(M+Na)^+$. HRESIMS *m/z* 433.1121 (M^+ + H, calcd for $C_{21}H_{26}O_3N_2Br$, 433.1121).

*4.1.2. General procedure for the synthesis of symmetric derivatives **14-17***

A mixture of sodium hydride (0.96 g, 24 mmol) and DMF (50 mL) was stirred at room temperature. 4'-Hydroxyacetophenone (2.7 g, 20 mmol, dissolved in 30 mL DMF), was dropwise added to the mixture. One hour after addition was completed, (2*R* or 2*S*)-glycidyl tosylate (4.5 g, 20 mmol, dissolved in 30 mL DMF) was then added dropwise. After that, the reaction was stirred at room temperature for 6 h and checked by TLC. The reaction was ended by adding water (100 mL). The mixture was extracted with ethyl acetate (100 mL × 3), and the combined organic phase was washed with water and brine, and then concentrated under reduced pressure. The obtained residue (*R*)-**2** or (*S*)-**2** was then refluxed with various piperazines (20 mmol) in 2-propanol (100 mL) for 6 h. The mixture was concentrated under reduced pressure. The obtained residue was purified by column chromatography (petroleum ether/ethyl acetate) on silica gel. The obtained solid was re-crystallized with 95% ethanol to give the target compounds **14-17**.

4.1.2.1. (R)-1-(2,5-Dimethoxyphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**14**). Yield 59.8 % as a white solid, m.p. 102-104 °C. (α)25 D = -6.875 (c = 0.175, CH_3OH), *ee* value 99 % determined by HPLC.

4.1.2.2. (S)-1-(2,5-Dimethoxyphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**15**). Yield 50.2 % as a white solid, m.p. 102-103 °C. (α)25 D = + 6.922 (c = 0.175, CH_3OH), *ee* value 99 % determined by HPLC.

4.1.2.3. (R)-1-(2-Ethoxyphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**16**). Yield 61.3 % as a white solid, m.p. 97-99 °C. (α)25 D = + 19.048 (c = 0.021, CH_3OH), *ee* value 99 % determined by HPLC.

4.1.2.4. (S)-1-(2-Ethoxyphenyl)-4-(3-(acetophenone-4-yl-oxy)-2-hydroxypropyl)-piperazine (**17**). Yield 56.8 % as a white solid, m.p. 97-99 °C. (α)25 D = -19.779 (c = 0.021, CH_3OH), *ee* value 99 % determined by HPLC.

4.2. Biological methods

4.2.1. Diastolic effect in isolated rabbit tissues (thoracic aorta, prostate and bladder trigone)

Male New Zealand White rabbits weighing 2-3 kg were killed by a blow on the head. The thoracic aorta, prostate tissue and urinary bladder tissue (from the trigone) were rapidly dissected and stored at 4 °C in a Kreb's solution of the following composition (mM) (Kava et al. 1998; Fagura et al. 1997): NaCl 120.0, KCl 5.5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25.0, Glucose 11.0, supplemented with 30 μM cocaine, 30 μM corticosterone, 100 μM ascorbate, 1 μM propranolol, 0.3 μM idazoxan, and 10 μM indomethacin, pH 7.4. After excess fat and connective tissue were removed, the aorta was cut intorings (3-4 mm in length). Prostatic samples (2 × 7 mm) and bladder samples (2 × 5 mm) were cut into sections. All samples were mounted under basal tension (Azuma et al. 1989; Oger et al. 2010) (aorta 2.0 g, prostate 1.0 g, bladder 1.0 g) in 10 ml organ baths containing Kreb's solution at 37 °C, continuously gassed with a mixture of 95% O_2 + 5% CO_2, and suspended between a metal hook and a isometric transducer. Isolated vascular ring strips were denuded of endothelium to avoid any complicating effects of endothelium-derived factors (Azuma et al. 1986). The isometric tension was recorded using a Powerlab 400^{TM} data acquisition system (software Chart, version 7.0, AD Instruments, MA, USA). The preparation was allowed to equilibrate for a minimum of 60 min prior to initiation of experimental procedures, and during this period, the incubation media was changed every 15 min. A single strip from each animal was used to construct individual experimental protocols. Therefore, numbers of strips reflect the number of animal used.

After equilibration, the three tissues were contracted twice by NE (aorta 3 μM, prostate 30 μM, bladder 10 μM). When the contractions were stable, test compounds were added in progressively- increasing cumulative concentrations (0.001 μM - 10 μM) at 30 min intervals. Compound induced relaxation of contracted muscle was expressed as a percentage of NE - induced responses. Concentration - response curves were analyzed using the Graph Pad Prism 5.0 software (GraphPad, San Diego, CA, USA), and the maximal relaxant effect (E_{max} %) was calculated. Data were expressed as mean ± S.E.M. The compounds were dissolved in DMSO. DMSO had no effect on NE-contractile response.

*4.2.2. Functional antagonism of **17** in isolated rat tissues*

The blocking activity (pA_2) of compound **17** was measured by using the methods similar to those described previously (Marucci et al. 2005; Buckner et al. 2002; Buccioni et al. 2009). Male Sprague-Dawley rats (SD rats; 200-225 g; Animal Center of Guangdong Province; China) were killed by cervical dislocation. The required organs were rapidly isolated and placed in Kreb's solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 1.9; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; NaH_2PO_4, 1.2; glucose, 11.7; again, desipramine hydrochloride (0.01 μM) and propranolol hydrochloride (1 μM) were added to prevent the neuronal uptake of NE and to block β-adrenoreceptors, respectively. After excess of fat and connective tissue was removed, three tissues were set up rapidly, under a suitable resting tension (vas deferens prostatic 0.5 g, spleen 1.0 g, thoracic aorta 1.0 g) in 10-mL organ baths containing Kreb's solution kept at 37 °C and aerated with 5% CO_2: 95% O_2 at PH 7.4. Isolated vascular ring strips were denuded of endothelium to avoid any complicating effects of endothelium-derived factors (Azuma et al. 1986).

After equilibration for 1 h in the Krebs solution, concentration-response curves were constructed by cumulative addition of agonist NE. The concentration of NE in the organ bath was increased approximately three-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. The first concentrations-response curve was the basic one, and the other three with NE were repeated by adding compound **17,** naftopidil (**1**) and terazosin, respectively. The individual tissues were exposed to only one concentration of test antagonist. The pA_2 values of each compound were calculated from the following equation: pA_2 = -log (B) + log (*r*-1), where (B) is the molar concentration of antagonists, and *r* is the ratio of agonist EC_{50} determined in the presence and absence of antagonist (Buckner et al. 2002).

4.3. Statistical analysis

The results are presented as mean ± S.E.M., and the statistical significance between the groups was analyzed by means of variance followed by two-way ANOVA test. P values less than 0.05 were considered significant.

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