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Novel naftopidil derivatives containing methyl phenylacetate and their blocking effects on $\alpha_{1D/1A}$-adrenoreceptor subtypes

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ABSTRACT

α_1-Adrenoceptor (α_1-AR) antagonists are considered to be the most effective monotherapy agents for lower urinary tract symptoms associated with benign prostatic hyperplasia (LUTS/BPH). In this study, we synthesized compounds **2**–**17**, which are novel piperazine derivatives that contain methyl phenylacetate. We then evaluated the vasodilatory activities of these compounds. Among them, we found that compounds **2**, **7**, **12**, which contain 2-OCH_3, 2-CH_3 or 2, 5-CH_3, respectively, exhibited potent α_1-blocking activity similar to protype drug naftopidil (**1**). The antagonistic effects of **2**, **7**, and **12** on the (−)-noradrenaline-induced contractile response of isolated rat prostatic vas deferens (α_{1A}), spleen (α_{1B}) and thoracic aorta (α_{1D}) were further characterized to assess the sub receptor selectivity. Compared with naftopidil (**1**) and terazosin, compound **12** showed the most desirable $\alpha_{1D/1A}$ subtype selectivity, especially improved α_{1A} subtype selectivity, and the ratios pA_2 (α_{1D})/pA_2 (α_{1B}) and pA_2 (α_{1A})/pA_2 (α_{1B}) were 17.0- and 19.5-fold, respectively, indicating less cardiovascular side effects when used to treat LUTS/BPH. Finally, we investigated the chiral pharmacology of **12**. We found, however, that the activity of enantiomers (*R*)-**12** and (*S*)-**12** are not significantly different from that of *rac*-**12**.

Lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH) are highly prevalent in aging men.[1,2] The American and European Urological Associations recommend the use of α_1-AR antagonists as prostatic smooth muscle relaxants for the first-line medical treatment for patients with LUTS/BPH.[3] α_1-AR is linked to $G_{q/11}$ protein involving phospholipase C activation, and further divided into α_{1A}, α_{1B}, and α_{1D}-subtypes.[4] As the three subtypes having distinct pharmacology and tissue expression[5], recent studies have shown that treatment with antagonists with better selectivities for α_{1A}-AR and α_{1D}-AR over α_{1B}-AR may efficiently alleviate BPH symptoms without causing significant cardiovascular side effects.[6,7]

Naftopidil (NAF, Fig. 1) is a *N*-aryl piperazine derivative and a specific α_{1D}-AR antagonist. It has been marketed in Japan since 1999 for the treatment of LUTS/BPH and proven to be more effective than tamsulosin, a major α_{1A} blocker, in treating storage symptoms.[8,9] Recent reports indicate that NAF modulates sensory transmission in the substantia gelatinosa by enhancing the release of GABA and glycine via a receptor other than an α_1 adrenoceptor.[10] And NAF is also effective for reduced the spinal cord injury-related tissue remodeling in the bladder.[11]

However, NAF has some disadvantages, such as low subtype selectivity, high required dosage, and low bioavailability.[12] *N*-hetero arylpiperazine derivatives have been structurally modified to improve their affinity and selectivity.[6,13,14] Research of NAF and similar α_1 subtype blockers has been ongoing for years in our lab. The present study is a continuation of our previous work on the synthesis of heterocyclic compounds containing acetophenone.[15] The proposed ligand-based pharmacophore model for α_1-AR antagonists is mainly consisted of positive ionisable (PI), hydrophobic features (HY) and hydrogen bond acceptor (HBA).[16–18] Our docking study of two NAF enantiomers towards α_{1D} showed that the right arylpiperazine part was placed on the entrance of hydrophobic pocket, and the left naphthalene moiety entered into a deep hydrophobic region.[19] We changed naphthalene to methyl phenylacetate in order to improve the hydrophobic property and thus novel derivatives **2**–**17** (Scheme 1) were designed and synthesized. All new compounds were subjected to preliminary pharmacological screening for their vasodilatory effects. Finally, a functional

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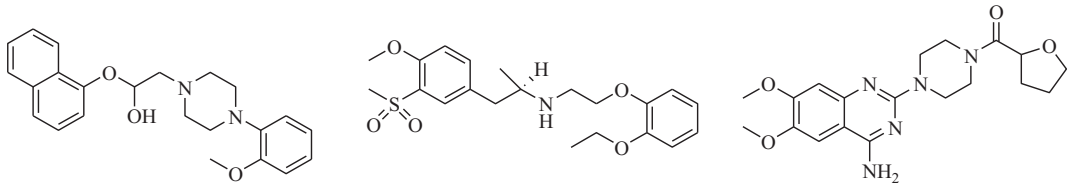
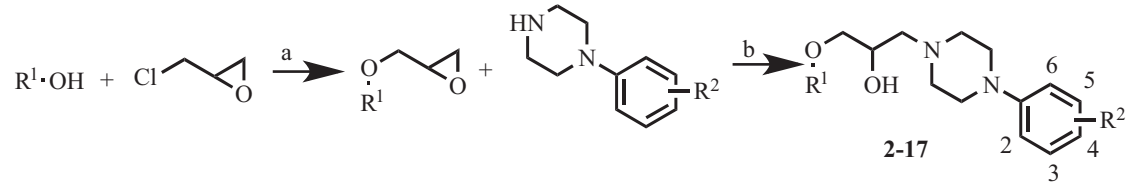
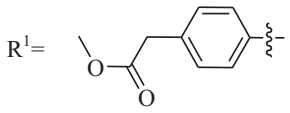


Fig. 1. Marketed drugs for treating LUTS/BPH.





2: R^2=2-OCH_3 **8:** R^2=3- CH_3 **14:** R^2=3,5-CH_3

3: R^2=3-OCH_3 **9:** R^2=4- CH_3 **15:** R^2=4-CH_3-5-Cl

4: R^2=4-OCH_3 **10:** R^2=2,3- CH_3 **16:** R^2=2-Cl

5: R^2=2, 4-OCH_3-5-Cl **11:** R^2=2,4- CH_3 **17:** R^2=4-Br

6: R^2=3-OCH_2CH_3 **12:** R^2=2,5- CH_3

7: R^2=2-CH_3 **13:** R^2=3,4-CH_3

Scheme 1. Synthetic route of target compounds **2**–**17**. Reagents and conditions: a) NaOH, 0 ~ 60 °C; b) 1-(substituted phenyl)-piperazine, *2*-propanol, reflux.

α_1-AR subtype selectivity study of selected compounds (**2**, **7**, and **12**) was performed. Results showed that methyl phenylacetate derivative with 2, 5-CH_3 group (**12**) was a moderately selective $\alpha_{1D/1A}$ blocker with lower antagonist potency against rat spleen α_{1B}-AR than NAF (pA_2 of 6.12 for **12** *vas* 6.75 for NAF).

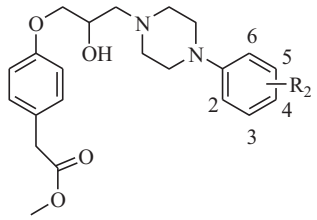
To begin with, compounds **2–17** were synthesized through the routes outlined in Scheme 1. Substituted phenols were converted into epoxide through nucleophilic substitution with epichlorohydrin, followed by substitution with piperazine in *2*-propanol to yield the target compounds **2–17**.[15] The derivatives were characterized on the basis of HRESI-MS, ^{1}H NMR and ^{13}C NMR spectral data, which were fully consistent with their depicted structures.

Compounds **2–17** were then preliminarily screened at concentrations of 0.1, 1, and 10 μM for their vasodilatory activity on rabbit thoracic aortic rings precontracted with (−)-noradrenaline (NE) where β- and α_2-ARs were inhibited. NAF was used as a reference compound. The percentage response produced by various compounds is shown Table 1. NAF (**1**) was initially used as an antihypertensive drug given its strong vasodilatory activity (1 μM, 86.9 ± 3.3%; 10 μM, 97.8 ± 1.5%).[20] The replacement of methyl phenylacetate resulted in compounds with slightly weaker activity than prototype **1**, and some compounds, such as **4** and **6**, exhibited weak performances. Different substituent groups, including alkoxy, alkyl and halogen, were designed on the phenylpiperazine moiety to verify the role of different groups. Meanwhile, substitution at the *meta*-, *para*- and *ortho*-positions conferred differing levels of pharmacological activity to the derivatives. In compounds **2**–**4**, *ortho*-substitution (**2**) exhibited the best α_1-blocking activity, as well as compounds **7** and **16** (relaxation response >90%). Substitution on position *3*-alone (**3**, **6**, and **8**) resulted in compounds with disappointingly low vasodilatory activity. Out of all derived compounds, **6** with *3*-OCH_2CH_3 showed the worst α_1-blocking activity. Its poor performance could be attributed to large steric hindrance. *Para*-substituted derivatives (**4**, **9**, **15**, and **17**) also exhibited poor pharmacological effects. Among the disubstituted derivatives **11–14**, *2, 5*-disubstituted compounds exhibited preferable activity second only to *2*-substituted compounds. For example, the activity of compound **12** was more potent than those of other disubstituted derivatives. Among compounds **7–14**, which were derived through one or more methyl substitution, *2*-CH_3 (**7**) and *2, 5*-CH_3 (**12**) exhibited most desirable vasodilatory activity.

Among all methyl phenylacetate-derived arylpiperazines, **2**, **7**, and **12** substituted with 2-OCH_3, 2-CH_3 or 2, 5-CH_3, producing relaxation response more than 60% at 1.0 μM on rabbit thoracic aortic rings, exhibited the best vasodilatory activity and a degree of effect compared with **1**. Thus, the α_1-subtype blocking activity of these derivatives was further studied.

It has been suggested that contractions of rat vas deferens and spleen to exogenous NE are mediated predominantly by α_{1A} and α_{1B} adrenoceptor, whereas contractions to NE in rat aorta are mediated predominantly by α_{1D} adrenoceptor.[21,22] Thus, contractions of these three tissues to NE have been used as a functional model for α_{1A}, α_{1B}, α_{1D} adrenoceptors.[23] The antagonistic effects of compounds **2**, **7**, and **12** on Sprague-Dawley rat prostatic vas deferens (α_{1A}), spleen (α_{1B}), and thoracic aorta (α_{1D}) were characterized to assess the sub receptor selectivity of the compounds (Table 2). NAF and terazosin were used as reference compounds. All three compounds appeared to be competitive antagonists at each sub receptor, because the slopes of the Schild plots were not significantly different from unity on the basis of parallel shifts

Table 1
Percentage relaxation response of compounds **2**–**17** at 0.1, 1 and 10 μM in rabbit thoracic aorta, previously contracted with NE (3 μM).



Comp.	R^2	% Relaxation Response ± SEM (n = 5)		
		0.1 μM	1 μM	10 μM
2	2-OCH_3	27.6 ± 4.8	71.1 ± 3.5	100.0 ± 6.6
3	3-OCH_3	13.0 ± 3.1	23.7 ± 2.0	39.8 ± 2.8
4	4-OCH_3	6.3 ± 1.4	7.0 ± 3.7	15.8 ± 2.9
5	2,4-OCH_3-5-Cl	8.2 ± 2.9	20.4 ± 5.0	46.1 ± 9.0
6	3-OCH_2CH_3	6.1 ± 0.8	10.2 ± 2.5	14.9 ± 1.6
7	2-CH_3	27.3 ± 5.4	62.3 ± 7.4	99.3 ± 9.5
8	3-CH_3	14.8 ± 1.1	36.9 ± 3.2	63.5 ± 2.8
9	4-CH_3	15.3 ± 2.5	28.7 ± 3.4	56.7 ± 6.2
10	2,3-CH_3	12.5 ± 2.0	43.3 ± 6.8	81.7 ± 8.8
11	2,4-CH_3	13.7 ± 1.9	43.3 ± 5.0	92.7 ± 4.4
12	2,5-CH_3	24.3 ± 4.5	66.2 ± 6.9	100.0 ± 6.3
13	3,4-CH_3	8.9 ± 1.3	28.4 ± 3.2	70.2 ± 5.8
14	3,5-CH_3	5.8 ± 0.5	18.6 ± 1.4	61.4 ± 2.1
15	4-CH_3-5-Cl	19.5 ± 4.8	25.9 ± 3.9	38.7 ± 5.1
16	2-Cl	29.0 ± 2.4	58.7 ± 1.9	98.3 ± 3.6
17	4-Br	7.9 ± 0.9	16.6 ± 4.2	43.5 ± 2.4
1	–	25.1 ± 1.4	86.9 ± 3.3	97.8 ± 1.5

Table 2
Functional antagonistic potency of **2**, **7**, and **12** together with its enantiomers, expressed as pA_2, at α_1-adrenoceptor subtypes of SD rat isolated tissues: prostatic vas deferens (α_{1A}), spleen (α_{1B}) and thoracic aorta (α_{1D}).

Compound	pA_2[a](slope)			Selectivity ratio		
	α_{1A}	α_{1B}	α_{1D}	α_{1D}/α_{1A}	α_{1D}/α_{1B}	α_{1A}/α_{1B}
2	6.83 ± 0.07 (1.09 ± 0.03)	6.22 ± 0.07 (1.03 ± 0.15)	7.11 ± 0.03 (1.22 ± 0.20)	1.91	7.76	4.07
7	6.31 ± 0.09 (1.17 ± 0.15)	6.49 ± 0.14 (0.86 ± 0.07)	7.70 ± 0.12 (1.12 ± 0.04)	24.55	16.22	0.66
12	7.41 ± 0.21 (1.06 ± 0.02)	6.12 ± 0.34 (0.84 ± 0.10)	7.35 ± 0.14 (1.14 ± 0.05)	0.87	17.00	19.50
(*R*)-12	7.20 ± 0.28 (1.00 ± 0.04)	6.35 ± 0.22 (0.92 ± 0.10)	7.42 ± 0.18 (0.99 ± 0.12)	1.66	11.75	7.08
(*S*)-12	7.52 ± 0.06 (1.20 ± 0.18)	6.57 ± 0.21 (1.06 ± 0.07)	7.81 ± 0.25 (1.15 ± 0.02)	1.95	17.38	8.91
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 corresponding pK_i values were 8.43 ± 0.06, 7.70 ± 0.02, 8.92 ± 0.00, obtained in binding experiment.[8]
[c] The reference data was 8.04, 8.60, 8.65.[24]

to concentration-response curves. In addition, treatment with increasing concentrations of these compounds did not decrease the maximum agonist-induced contractile response (Fig. 2).

The blocking activities of the test compounds on the three α_1 subtypes were less effective than that of NAF (**1**). The pA_2 values of compounds **2** and **7** for α_{1A} were 6.83 and 6.31 (<7); these values are considerably lower than that of **1** (pA_2 = 7.48). Similar to **1**, the selectivity of all compounds for α_{1D} were more potent than that for α_{1A}. However, the compounds had different subtype selectivity. As we can see, the selectivity ratios pA_2 (α_{1D})/pA_2 (α_{1B}) and pA_2 (α_{1A})/pA_2 (α_{1B}) of **1** were approximately 15- and 5-fold, respectively. Terazosin showed no subtype selectivity at all. Introduction of methyl moiety as in **7** and **12** gave rise to the antagonistic activity of α_{1D}. Compared with **1** and terazosin, **12** exhibited higher selectivity for α_{1D} and α_{1A}, with selectivity ratios of approximately 17- and 19.5-fold. Given its low potency for α_{1B}, **12** may be an effective agent for improving symptoms related to bladder filling in BPH.

As **12** has one chiral carbon center, we also synthesized two enantiomers of **12**, namely, (*R*)-**12** and (*S*)-**12** (Scheme 2). Optically active compounds were prepared with optically active (*2R*)- or (*2S*)-glycidyltosylate in DMF to obtain chiral epoxide[25], followed by substitution with piperazine in *2*-propanol to provide the target

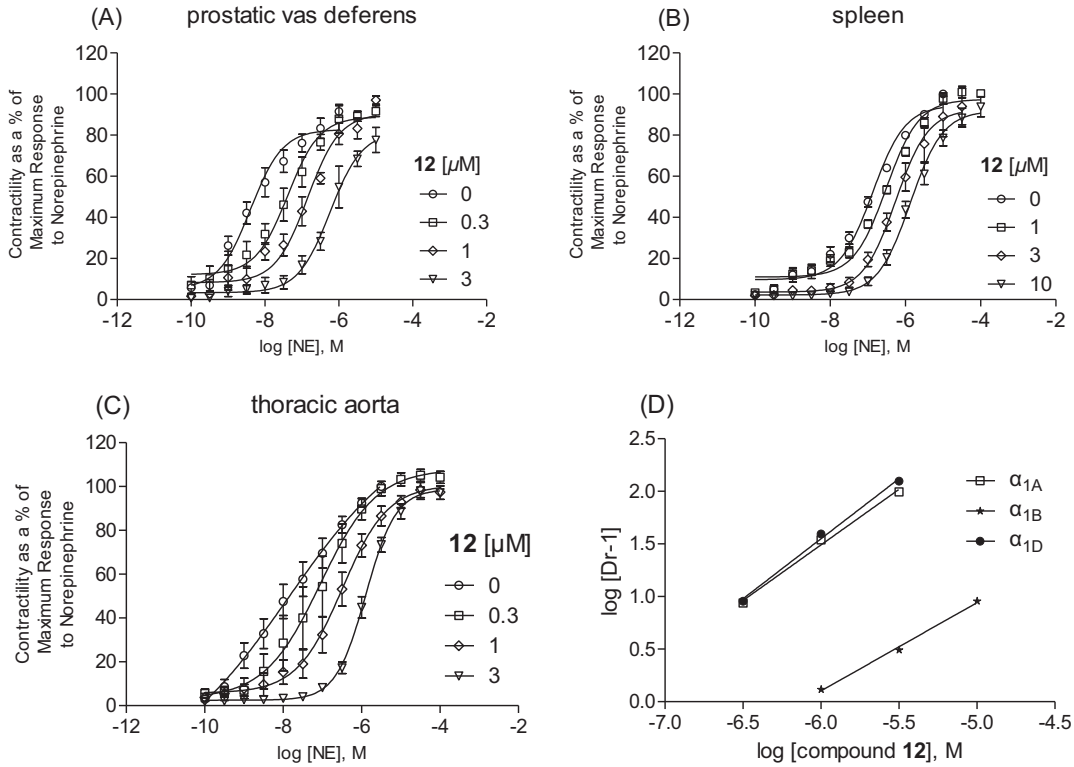
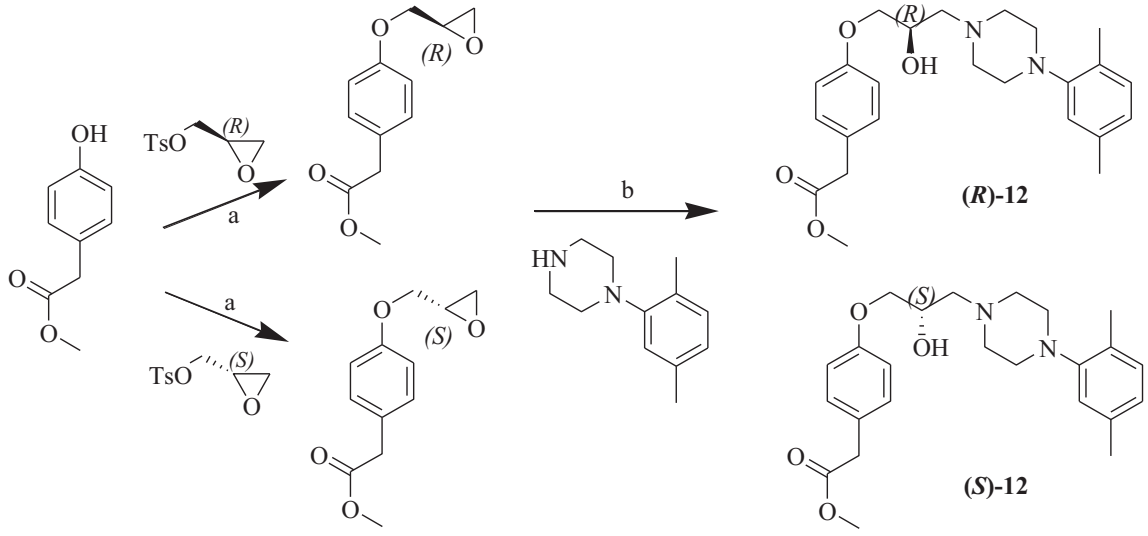


Fig. 2. Concentration-response curves of NE-induced contractions in rat isolated prostatic vas deferens (A), spleen (B), and thoracic aorta (C) in compound **12** treatment at different concentrations. Data are the mean ± SEM of five to eight separate experiments. (D) Schild plots for the antagonism to NE response through compound **12** in rat isolated rat prostatic vas deferens (α_{1A}), spleen (α_{1B}) and thoracic aorta (α_{1D}), whose slopes were not significantly different from unity.



Scheme 2. Enantiomeric synthesis of compound **12**. Reagents and conditions: a) DMF, NaH, rt; b) *2*-propanol, reflux.

enantiomers (*R*)-**12** and (*S*)-**12** (Scheme 2). These enantiomers did not exhibit substantially different blocking activities or sub receptor selectivity, as evidenced by the results of functional tests (Table 2). (*S*)-**12** exhibited slightly better blocking activity than the (*R*)-enantiomer and its racemate without increased subtype selectivity. Similar blocking effect on the α_{1A} subtype was observed among **12** and two enantiomers, however, the pA_2 (α_{1A})/pA_2 (α_{1B}) of **12** exhibited higher than the two enantiomers, as the low affinity on α_{1B} subtype (pA_2 of 6.12 for **12**). Chiral pharmacological differences were observable in some structures of NAF derivatives[15], however, both (*R*)-**NAF** and (*S*)-**NAF** displayed similar affinity[7], as well as compound **12** in current paper.

Compared with the previous acetophenone[15], carbazole and benzotriazole[17,26] derivatives, methyl phenylacetate series did not display a better antagonistic effect on α_1 subtypes, as their pA_2 values were all less than eight. It is worth noting that methyl phenylacetate derivative **12** has highly improved the subtype selectivity on the α_{1A}, that is, **12** exhibited 19.5-fold higher potency for the α_{1A} than for the α_{1B}. Moreover, this effect of higher α_{1A} subtype selectivity was reduced when using the enantiomers of **12**.

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In conclusion, we report the synthesis, structural characterization, and *in vitro* vasodilatory effect of novel piperazine derivatives. Compounds **2–17** were designed from naftopidil (**1**), an α_1-adrenoceptor subtype antagonist used for the treatment of LUTS/BPH. Compound **12** is a moderate therapeutic agent for BPH given its combined antagonistic effects against α_{1A} and α_{1D}. Moreover, no obvious differences among stereo configurations of **12** exist. Rac-**12** exhibits higher α_{1A} subtype selectivity than the two enantiomers. Our results show that the methyl phenylacetate ligand is a versatile class of compounds that provide a new starting point for the development of ligands with high selectivity for α_1 subtypes.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.01.068.

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