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Article

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Design, Synthesis and Biological Evaluation of Novel Tetrahydroprotoberberine Derivatives (THPBs) as Selective α_{1A}-Adrenoceptor Antagonists

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Abstract

A novel series of tetrahydroprotoberberine derivatives (THPBs) were designed, synthesized and evaluated as selective α_{1A}-adrenergic receptors (AR) antagonists for the treatment of benign prostatic hyperplasia. Based on the pharmacophore model of the marketed drug silodosin, THPBs were modified by introducing an indole segment into their core scaffolds. In calcium assays, 7 out of 32 compounds displayed excellent antagonistic activities against α_{1A}-ARs, with IC_{50s} less than 250 nM. Among them, compound **(*S*)-27** had the most potent biological activity; its IC_{50} toward α_{1A}-AR was 12.8 ± 2.2 nM, which is 781 and 20 times more selective than that toward α_{1B}- and α_{1D}-AR, respectively. In the functional assay using isolated rat tissues, compound **(*S*)-27** inhibited norepinephrine-induced urethra smooth muscle contraction potently (IC_{50} = 0.5 ± 0.3 nM), without inhibiting the aortic contraction (IC_{50} > 1000 nM), displaying a better tissue selectivity than the marketed drug silodosin. Additional results of preliminary safety studies (acute toxicity and hERG inhibition) and pharmacokinetics studies indicated the potential druggability for compound **(*S*)-27** which is a promising lead for the development of selective α_{1A}-AR antagonists for the treatment of BPH.

1. Introduction

Benign prostatic hyperplasia (BPH) is a benign increase in the size of the prostate that leads to urinary hesitancy, frequent urination, dysuria and increased risk of lower urinary tract symptoms (LUTS).[1,2] An estimated 50% of men have histological evidence of BPH by the age of 50 years and that number increases to 75% by the age

of 80 years. As life expectancy rises, so does the occurrence of BPH.[3] There are two components of BPH/LUTS, namely increased size and elevated muscle tone of the gland. Therefore, medications are also divided into two categories: those that decrease the gland size and those that relax the urethra smooth muscle.

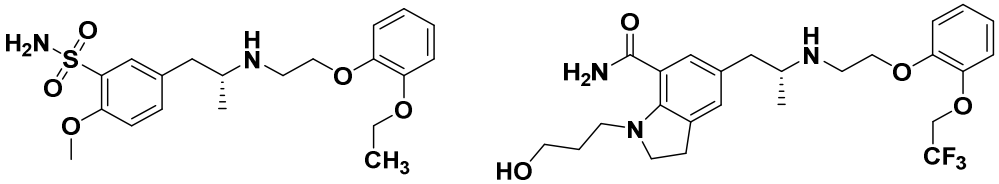
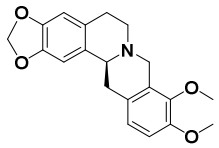
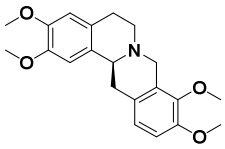
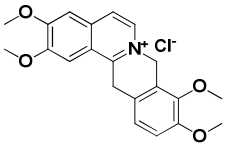
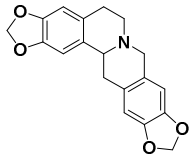


Figure 1. Marketed α_{1A}-AR selective antagonists, tamsulosin and silodosin.

α_1-Adrenergic receptors (α_1-adrenoceptors, α_1-ARs) belong to the G protein-coupled receptor (GPCR) superfamily, and regulate the contraction of smooth muscle by activating phospholipase C, followed by the increase of intracellular calcium levels.[4,5] α_1-ARs are divided into three subtypes, α_{1A}, α_{1B} and α_{1D}-AR.[6-8] The α_{1A}-AR, expressed mainly in the prostate, bladder and urethra, is considered to play a major role in regulating prostatic muscle contraction, while α_{1D}-AR has a minor contribution.[4,9] On the other hand, α_{1B}-AR, expressed predominantly in the heart and vascular smooth muscle, is considered a drug target for treating hypertension.[10,11] α_{1A} antagonists are used as anti-BPH agents; however, antagonism of the α_{1B} subtype will lead to cardiovascular side effects, such as hypotension.[12,13] The α_{1D} subtype is predominant and functional in human epicardial coronary arteries, and its inhibition might result in coronary vasodilation.[14] In the last two decades, α_{1A}-AR selective antagonists, such as the marketed drugs tamsulosin (**1**) and silodosin (**2**), have been developed to treat BPH/LUTS (Figure 1). These agents have relatively high α_{1A}-AR

subtype selectivity and effectively relieve the symptoms of BPH, with reduced side effects.[15-18] However, the cardiovascular adverse effects can still be observed and impact blood pressure. Clinical trial results showed that tamsulosin treatment results in a significant decrease in mean systolic blood pressure.[19] Marks et al reported the incidence of orthostatic hypotension caused by silodosin is 2.6%.[20] Recently, increasing efforts have been made to identify novel small molecule α_{1A}-AR selective antagonists based on the scaffolds of the molecules mentioned above.[21-32]

Table 1. The predicted target of THPB analogs by SEA, ChemMapper and calcium assay evaluation

Compd.	Structure	Predicted Target	*E*-value [a]	Similarity Score[b]	Calcium Assay (IC_{50})
3		α_{1A}-AR	3.75e-8	1.9	5.4 μM
4		α_{1A}-AR	-	-	41.2% inhibition @ 10 μM
5		α_{1A}-AR	-	-	7.6 μM
6		α_{1A}-AR	-	-	462 nM

[a] SEA E-values against all 246 MDDR activity classes. [b] ChemMapper similarity score against the ChEMBL database.

As a rapid complementary approach to experimental methods, *in silico* target prediction methods had been approved to identify the pharmacological effects of a drug.[33,34] Tetrahydroprotoberberines (THPBs) extracted from the Chinese herb Corydalis ambigua, are important and intriguing scaffolds. Herein, to find their potential targets, we have used the similarity ensemble approach (SEA)[35] and ChemMapper[36,37] to predict their potential targets.[33,34] Through comparison of the results derived from the above two methods, adrenergic receptor is an interesting unreported target for these compounds, which were therefore selected for further biological evaluation. The calcium assay results indicated that THPBs presented moderate antagonism against α_{1A}-AR, with sub-micromolar affinities (Table 1, compounds **3-6**).

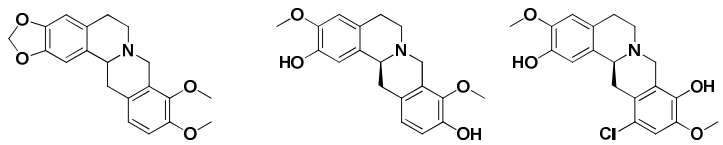


Figure 2. THPB analogs and their binding affinities (pK_i) on α_1-AR.

With the above results in mind, a literature research was carried out and evidence that some THPBs including tetrahydroberberines (THB, **7**), *l*-tetrahydropalmatine (*l*-THP, **4**), *l*- stepholidine (*l*-SPD, **8**) and *l*-chlorosoulerine (*l*-CSL, **9**), were observed to possess weak α_1-AR binding affinity and to block phenylephrine-induced vasocontraction (Figure 2).[35]

Based on these findings, we believed that THPB derivatives should be explored as a novel class of α_{1A}-AR antagonists for the treatment of BPH/LUTS. Therefore, we report the design and synthesis of a series of novel indole-containing THPB

derivatives as selective α_{1A}-AR antagonists, as well as the detailed structure-activity relationship analysis, in vitro and in vivo biological evaluation, preliminary toxicity and pharmacokinetic studies.

2. Chemistry

2.1 Design of Target Compounds

In addition to a chemical similarity approach, pharmacophore models and quantitative structure–activity relationship (QSAR) studies have been used frequently to design novel α_{1A}-AR antagonists in recent years. Meaningful pharmacophore and QSAR models have been established, and several key components of pharmacophores were identified in various scaffolds, including the marketed drugs tamsulosin and silodosin. [36-40]

To design our compounds, silodosin was treated as a model molecule. Interestingly, we found that THB has a pharmacophore similar to silodosin. The positive ionizable (PI) nitrogens in the center of both molecules are probably crucial for recognition between the compound and the receptor. In addition, the two aromatic ring segments (Ar) at both ends of the two compounds represent evidence of hydrophobic interaction. The alkoxy groups on the aromatic rings are potential hydrogen bond sites (HBS) that form interactions with α_1-AR. Note that both molecules have an chiral carbon located next to the PI nitrogen. These common pharmacophore patterns of the two molecules provide a reasonable explanation for the interaction between THB and α_1-AR (Figure 3).

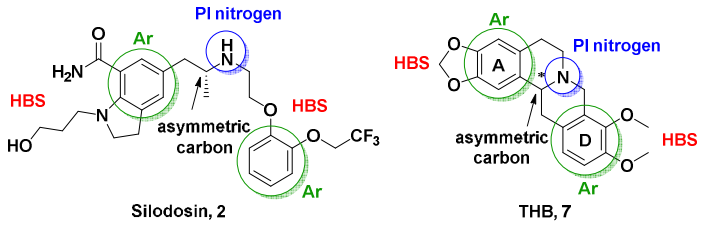


Figure 3. Similar pharmacophore models of silodosin and THB.

Based on these findings, we hypothesized that THB could be modified into α_{1A}-AR antagonists with higher affinity and selectivity. In our previous studies, a class of indole-containing polycyclic compounds obtained by the gold catalyzed cascade reaction was identified as selective α_{1A}-AR blockers with moderate activities.[41] Other indole-based scaffolds have also been reported to exhibit good bioactivities toward ARs.[42-46] Thus, an indole fragment was introduced into THB on its D-ring, such that a series of indole-containing THB derivatives were designed and synthesized (Figure 4). All synthesized compounds (**13-44**) were further evaluated for their biological activities using calcium mobilization assays. In addition, chiral compounds **(*S*)-18**, **(*S*)-27** and **(*R*)-27** were also synthesized to explore the influence of the molecular configuration (Scheme 2).

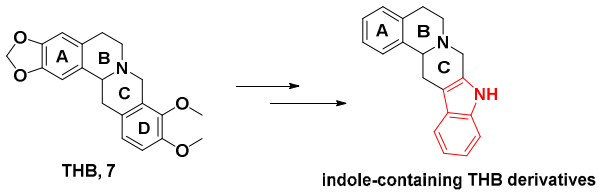


Figure 4. The structural optimization for THB

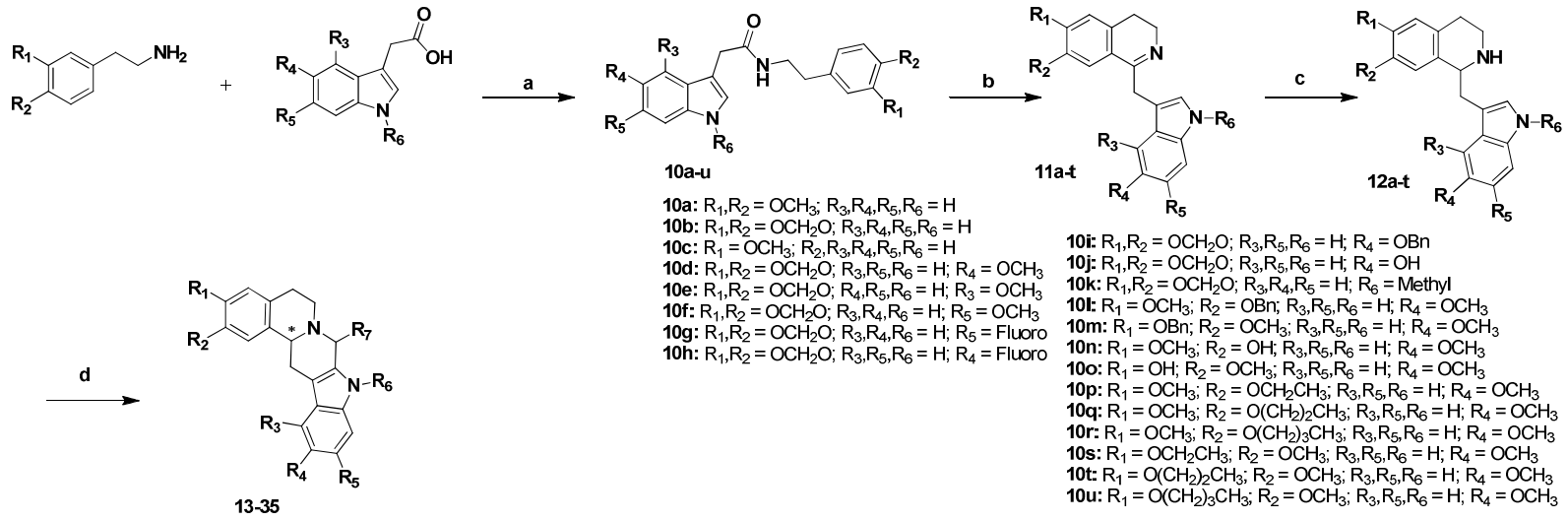
2.2 Synthetic Procedures of Target Compounds

The designed compounds were synthesized via the procedures shown in Schemes 1-3. Condensation of commercially available phenylethanamines and indole-3-acetic acids generated amides **10a-u**, which were further cyclized under the presence of

phosphoryltrichloride ($POCl_3$) to give imines **11a-u** in excellent yields, according to the procedures of the Bischler–Napieralski reaction. Reduction of the resulting imines **11a-u** with sodium borohydride produced the key amine intermediates **12a-u**. Cyclization of amines **12a-u** via the Pictect–Spengler reaction with various aldehydes resulted in the target products **13-35** (Scheme 1). Further deprotection of groups of products **32**, **34** and **35** gave compounds **36-38,** respectively. Compounds **39-44** were synthesized by substitution reactions of **37** and **38** with different alkyl bromides (Scheme 2).

Additionally, chiral compounds **(*S*)-18**, **(*S*)-27** and **(*R*)-27** were prepared according to the procedure outlined in Scheme 3. Asymmetric hydrogenation of imines **11b** and **11d,** catalyzed by a chiral Ru-(II) complex (Noyori's catalyst)[47-49] produced chiral amines **(*S*)-12b, d** and **(*R*)-12d**, followed by cyclization with formaldehyde to give **(*S*)-18**, **(*S*)-27** and **(*R*)-27**. All target compounds were characterized by ^{1}H-NMR, ^{13}C-NMR and MS (see Experimental Section).

Scheme 1. Synthesis of compounds **13-35**.[a]

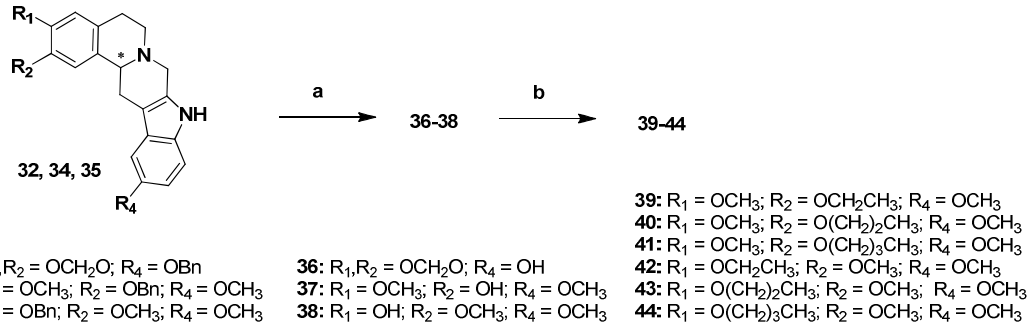


10a: R_1,R_2 = OCH_3; R_3,R_4,R_5,R_6 = H
10b: R_1,R_2 = OCH_2O; R_3,R_4,R_5,R_6 = H
10c: R_1 = OCH_3; R_2,R_3,R_4,R_5,R_6 = H
10d: R_1,R_2 = OCH_2O; R_3,R_5,R_6 = H; R_4 = OCH_3
10e: R_1,R_2 = OCH_2O; R_4,R_5,R_6 = H; R_3 = OCH_3
10f: R_1,R_2 = OCH_2O; R_3,R_4,R_6 = H; R_5 = OCH_3
10g: R_1,R_2 = OCH_2O; R_3,R_4,R_6 = H; R_5 = Fluoro
10h: R_1,R_2 = OCH_2O; R_3,R_5,R_6 = H; R_4 = Fluoro
10i: R_1,R_2 = OCH_2O; R_3,R_5,R_6 = H; R_4 = OBn
10j: R_1,R_2 = OCH_2O; R_3,R_5,R_6 = H; R_4 = OH
10k: R_1,R_2 = OCH_2O; R_3,R_4,R_5 = H; R_6 = Methyl
10l: R_1 = OCH_3; R_2 = OBn; R_3,R_5,R_6 = H; R_4 = OCH_3
10m: R_1 = OBn; R_2 = OCH_3; R_3,R_5,R_6 = H; R_4 = OCH_3
10n: R_1 = OCH_3; R_2 = OH; R_3,R_5,R_6 = H; R_4 = OCH_3
10o: R_1 = OH; R_2 = OCH_3; R_3,R_5,R_6 = H; R_4 = OCH_3
10p: R_1 = OCH_3; R_2 = OCH_2CH_3; R_3,R_5,R_6 = H; R_4 = OCH_3
10q: R_1 = OCH_3; R_2 = $O(CH_2)_2CH_3$; R_3,R_5,R_6 = H; R_4 = OCH_3
10r: R_1 = OCH_3; R_2 = $O(CH_2)_3CH_3$; R_3,R_5,R_6 = H; R_4 = OCH_3
10s: R_1 = OCH_2CH_3; R_2 = OCH_3; R_3,R_5,R_6 = H; R_4 = OCH_3
10t: R_1 = $O(CH_2)_2CH_3$; R_2 = OCH_3; R_3,R_5,R_6 = H; R_4 = OCH_3
10u: R_1 = $O(CH_2)_3CH_3$; R_2 = OCH_3; R_3,R_5,R_6 = H; R_4 = OCH_3

13: R_1,R_2 = OCH_3; R_3,R_4,R_5,R_6,R_7 = H
14: R_1,R_2 = OCH_3; R_3,R_4,R_5,R_6 = H; R_7 = isobutyl
15: R_1,R_2 = OCH_3; R_3,R_4,R_5,R_6 = H; R_7 = Ph
16: R_1,R_2 = OCH_3; R_3,R_4,R_5,R_6 = H; R_7 = 4-FluoroPh
17: R_1,R_2 = OCH_3; R_3,R_4,R_5,R_6 = H; R_7 = 4-MethoxyPh
18: R_1,R_2 = OCH_2O; R_3,R_4,R_5,R_6,R_7 = H
19: R_1,R_2 = OCH_2O; R_3,R_4,R_5,R_6 = H; R_7 = ethyl
20: R_1,R_2 = OCH_2O; R_3,R_4,R_5,R_6 = H; R_7 = isobutyl
21: R_1,R_2 = OCH_2O; R_3,R_4,R_5,R_6 = H; R_7 = 4-FluoroPh
22: R_1,R_2 = OCH_2O; R_3,R_4,R_5,R_6 = H; R_7 = 4-MethoxyPh
23: R_1 = OCH_3; R_2,R_3,R_4,R_5,R_6,R_7 = H
24: R_1 = OCH_3; R_2,R_3,R_4,R_5,R_6 = H; R_7 = Methyl
25: R_1 = OCH_3; R_2,R_3,R_4,R_5,R_6 = H; R_7 = cyclopropyl
26: R_1 = OCH_3; R_2,R_3,R_4,R_5,R_6 = H; R_7 = 4-MethoxyPh
27: R_1,R_2 = OCH_2O; R_3,R_5,R_6,R_7 = H; R_4 = OCH_3
28: R_1,R_2 = OCH_2O; R_4,R_5,R_6,R_7 = H; R_3 = OCH_3
29: R_1,R_2 = OCH_2O; R_3,R_4,R_6,R_7 = H; R_5 = OCH_3
30: R_1,R_2 = OCH_2O; R_3,R_4,R_6,R_7 = H; R_5 = Fluoro
31: R_1,R_2 = OCH_2O; R_3,R_5,R_6,R_7 = H; R_4 = Fluoro
32: R_1,R_2 = OCH_2O; R_3,R_5,R_6,R_7 = H; R_4 = OBn
33: R_1,R_2 = OCH_2O; R_3,R_4,R_5,R_7 = H; R_6 = Methyl
34: R_1 = OCH_3; R_2 = OBn; R_3,R_5,R_6,R_7 = H; R_4 = OCH_3
35: R_1 = OBn; R_2 = OCH_3; R_3,R_5,R_6,R_7 = H; R_4 = OCH_3

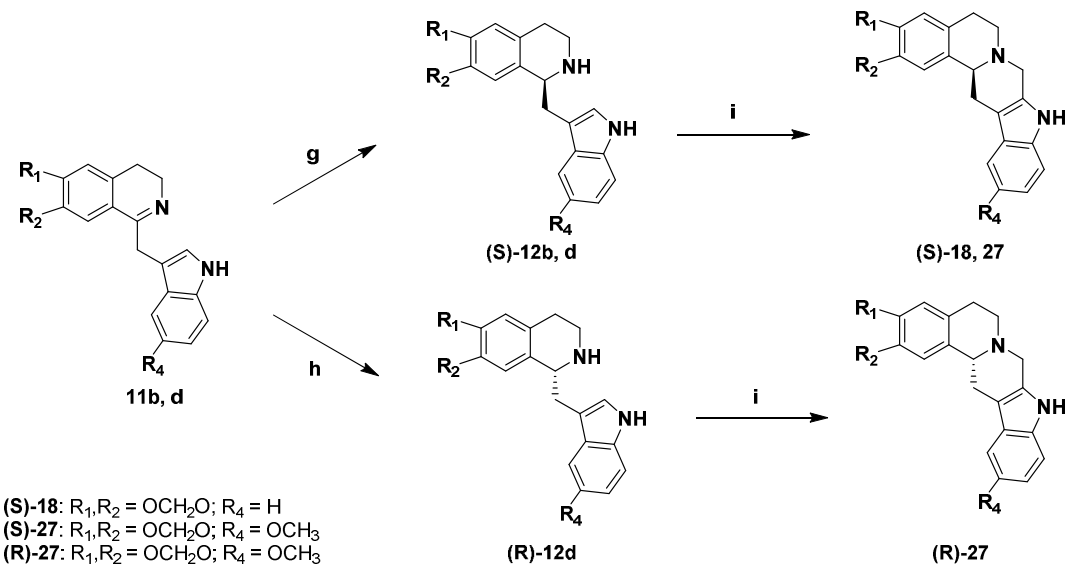
[a]Reagents and conditions: (a) EDCI, Et_3N, CH_2Cl_2, rt, 8 h; (b) $POCl_3$, CH_3CN, reflux; (c) $NaBH_4$, methanol, rt, 8 h; (d) HCOOH, R_7CHO, 25–90°C, 2 h.

Scheme 2. Synthesis of compounds **36-44**.[a]



[a]Reagents and conditions: (a) H_2, Pd/C, rt, 8h; (b) RBr, K_2CO_3, acetone, reflux, 2 h.

Scheme 3. Synthesis of compounds **(*S*)-18**, **(*S*)-27** and **(*R*)-27**.[a]



[a]Reagents and conditions: (g) (R,R)-Noyori's catalyst, HCOONa, $AgSbF_6$, $La(OTf)_3$, CTAB, H_2O, 40 °C, 12 h; (h) (S,S)-Noyori's catalyst, HCOONa, $AgSbF_6$, $La(OTf)_3$, CTAB, H_2O, 40 °C, 12 h; (i) HCOOH, 40% HCHO, 25–90 °C, 2 h.

3 Results and Discussion

3.1 Chemistry

On the basis of the structure features of THB, an indole fragment was introduced on its D-ring, and 32 new indole-containing compounds were designed and synthesized. Their synthetic routes and chemical structures (**13-44**) are shown in Scheme 1. In addition, chiral compounds **(*S*)-18**, **(*S*)-27** and **(*R*)-27** were also synthesized to explore the influence of their configuration (Scheme 2). The details of the synthetic procedures and structural characterizations are described in the Experimental Section.

3.2 Structure-Activity Relationship for All Compounds

All target compounds (**13-44, (*S*)-18**, **(*S*)-27** and **(*R*)-27**) were evaluated for their biological activities toward α_1-AR using calcium mobilization assays. The initial screening was carried out at a concentration of 10 μM for each compound, and compounds that displayed >80% inhibition were further evaluated for their IC_{50}s. The results are summarized in Table 2, and the details of the bioassay procedures are described in the Experimental Section. As shown in Table 2, twenty compounds demonstrated good inhibitory activities, with >80% inhibition at a concentration of 10 uM for each compound. Further analysis showed that introducing substituents (alkyl or aryl groups) into the R7 position was detrimental to the inhibitory potency when R1 and R2 were replaced by a methoxyl group (*see* compounds **13-17**, as shown in Table 2 and Scheme 1), respectively. However, the introduction of a methylenedioxy group into the R1 and R2 position of the scaffold of compound **13** afforded the new compound **18**, which displayed an excellent

inhibitory effect toward α_{1A}-AR, its IC_{50} value was 125.0 ± 20.4 nM. Based on these positive results, several bulky alkyl and aryl substituents were introduced into R7 position of the scaffold of **18** to form compounds **19-22**; unfortunately, the introduction of these bulky groups caused a substantial loss in inhibitory activity, which suggested that the bulky steric hindrance at R7 position might decrease their activity. Thereafter, we attempted to remove the R2 group (-OCH_3) from the scaffold of compound **13** to prepare compound **23,** and the bioassay showed a moderate inhibitory potency against α_{1A}-AR, with an IC_{50} of 552.3 ± 67.9 nM. Based on this finding, we further substituted R7 position with methyl, cyclopropyl and 4-methoxyphenyl to form compounds **24-26**, which have hardly any inhibitory potency against α_{1A}-AR.

Table 2. Inhibition ratio and IC_{50} values of all synthesized compounds for α_{1A}-AR.[a]

Compd.	IR[b] (10 μM)	IC_{50} (Means ± SEM) on calcium assays, nM			Selectivity	
		α_{1A}	α_{1B}	α_{1D}	α_{1B}/α_{1A}	α_{1D}/α_{1A}
13	60%	/	/	/	/	/
14	72%	/	/	/	/	/
15	0%	/	/	/	/	/
16	0%	/	/	/	/	/
17	21%	/	/	/	/	/
18	100%	125.0±20.4	> 10000	> 10000	>80.0	>80.0
19	82%	> 10000	/	/	/	/
20	0%	/	/	/	/	/

21	0%	/	/	/	/	/
22	0%	/	/	/	/	/
23	100%	552.3±67.9	/	/	/	/
24	90%	8669±625.6	/	/	/	/
25	31%	/	/	/	/	/
26	80%	> 10000	/	/	/	/
27	99%	17.6±2.1	> 10000	4038±1725	>568.2	229.4
28	100%	202.3±32.5	> 10000	> 10000	>19.4	>19.4
29	100%	594.0±147.0	> 10000	> 10000	>16.8	>16.8
30	100%	2057±183.0	/	/	/	/
31	100%	253.8±32.6	> 10000	8392±526.4	>39.4	33.1
32	100%	2880±77.5	/	/	/	/
33	100%	1474±138.8	/	/	/	/
34	100%	124.7±7.1	7928±250.3	640.6±95.7	53.6	5.1
35	97%	> 10000	/	/	/	/
36	100%	402.9±53.6	9170±1133	1456±68.6	22.8	3.6
37	56%	/	/	/	/	/
38	0%	/	/	/	/	/
39	75%	/	/	/	/	/
40	100%	225.3±35.2	> 10000	> 10000	>44.4	>44.4
41	98%	2286±429.3	/	/	/	/

42	17%	/	/	/	/	/
43	21%	/	/	/	/	/
44	63%	/	/	/	/	/
(*S*)-18	99%	57.4±10.2	> 10000	> 10000	>174.0	>174.0
(*S*)-27	100%	12.8±2.2	> 10000	250.7±40.7	>780.6	19.6
(*R*)-27	100%	3187±149.4	/	/	/	/
tamsulosin[c]	100%	2.2±0.3	4.8±0.9	1.4±0.2	2.2	0.6
silodosin[c]	100%	1.8±0.1	116.0±12.5	6.3±1.0	66.0	3.6

[a]The initial screening was carried out at a concentration of 10 μM for each compound and IC_{50} were measured for compounds that displayed >80% inhibition of α_{1A}-AR, and "/" means that no experiment was conducted. [b]IR represents inhibition ratio. [c]The reference drug.

On the basis of above results, we held the methylenedioxy group at R1 and R2 position on the molecular scaffold of compounds **19-22**, and further explored the influence of different substituents on the indole ring on inhibitory potency of α_{1A}-AR, namely that introducing different substituents into R3, R4, R5 and R6 position, respectively. The results demonstrated that introducing a methoxy group into the R4 position of the indole ring can produce the highest inhibitory activity against α_{1A}-AR, with an IC_{50} of 17.6 ± 2.1 nM (**27**). However, moving this methoxy group from R4 to R3 or R5 position of the indole ring brought about a decrease in inhibitory activity against α_{1A}-AR (**28** and **29**). The introduction of a fluorine atom (**30** and **31**) and an electron-donating group (**32** and **33**) into R4, R5 or R6 position of the scaffold of the indole could not increase the inhibitory

potency. It follows that substituting R4 with a methoxy group was favorable to retain the inhibitory effects against α_{1A}-AR. Therefore, in the subsequent structural modification, we kept a methoxy group on the R4 position of indole ring and further investigated the influences of different R1 and R2 substituents (**34, 35, 37-44**). The results displayed that a methoxy group in the R1 position is important to maintain the inhibitory potency of the compounds toward α_{1A}-AR (**34, 40**). Removal of the methoxy group from the R1 position almost completely ablated their activities (**38, 42-44**).

For some natural products, such as *l*-SPD, the *R*-configuration exhibited worse α_{1A}-AR antagonistic activity compared to its *S*-configured counterparts.[50-52] Therefore, we also synthesized the chiral compounds **(*S*)-18**, **(*S*)-27** and **(*R*)-27**, and further evaluated their biological activities toward α_{1A}-AR. The results showed that the *S*-configuration is an important determinant of the α_{1A}-AR inhibitory activity. As shown in Table 2, the *S*-configured enantiomer **(*S*)-18** (IC_{50} = 57.4 ± 10.2 nM) offered almost two-fold higher potency than the racemate **18** (IC_{50} = 125.0 ± 20.4 nM). The *S*-configured enantiomer **(*S*)-27** exhibited the most potent α_{1A}-AR antagonistic activity; its IC_{50} reached 12.8 ± 2.2 nM, which was much more effective than its racemate (**27**, IC_{50} = 17.6±2.1 nM) and *R*-configured compound ((**(*S*)-27**, IC_{50} = 3187±149.4 nM). These data demonstrated that the stereochemical configuration has an important influence on the α_{1A}-AR antagonistic activity of these compounds.

3.2 Evaluation of α_1-AR Subtype Selectivity for Selected Compounds

Previous studies indicated that the α_{1B} subtype is found widely in vascular smooth

muscle, and blocking it can cause orthostatic hypotension.[53] The α_{1D} subtype is predominant and functional in human epicardial coronary arteries, and its inhibition might mediate coronary vasodilation.[54] Therefore, to characterize α_1-AR subtype selectivity, representative compounds (**18, 27-29, 31, 33, 35, 40, (*S*)-18** and **(*S*)-27**) were selected to determine their selectivities using a calcium mobilization assay. The results are summarized in Table 2. In general, most of these compounds displayed moderate to high antagonist activity toward α_{1A}-AR. However, none of them displayed a significant inhibitory effect toward α_{1B}-AR. Only a few compounds had measurable antagonist activity on α_{1D}-AR. All compounds displayed much better α_{1A}-AR selectivity compared with the reference drug tamsulosin. Although compounds **27** and **(*S*)-27** showed slightly less potent antagonistic activity than silodosin against α_{1A}-AR, both demonstrated much higher selectivities than silodosin and tamsulosin. **(*S*)-27** showed higher selectivity for α_{1A}-AR (IC_{50} = 12.8 nM) compared with α_{1B}-AR (IC_{50}>10 μM, α_{1B}/α_{1A}> 780) and α_{1D}-AR (IC_{50} = 250.7 nM, α_{1D}/α_{1A} =19.6), which was much better than tamsulosin (α_{1B}/α_{1A} = 2.2, α_{1D}/α_{1A} = 0.6) and silodosin (α_{1B}/α_{1A} = 66.0, α_{1D}/α_{1A} = 3.6).

3.3 Functional Assay in Isolated Rat Tissues

It is desirable to develop α_1-AR antagonists that can selectively suppress the tone of the lower urinary tract, without vascular effects, to treat urinary outlet obstruction problems in patients with BPH.[54] Therefore, we selected the most effective compounds, **27** and **(*S*)-27,** and evaluated their antagonist effect on smooth muscle contraction of rat urethras and aortas. As shown in Table 3, both **27** and **(*S*)-27** showed strong

anti-contractile activity on urethra smooth muscle stimulated with norepinephrine. Compound **(*S*)-27** (IC_{50} = 0.5±0.3 nM) was slightly more potent than the marketed drug silodosin (IC_{50} = 0.8±0.03 nM). Encouragingly, neither compound had a significant effect on norepinephrine-induced contraction of aortic smooth muscle (IC_{50} >1000 nM), while the control compound silodosin potently inhibited aortic contraction (IC_{50} = 90.1±7.6 nM). The uroselectivity of compound **(*S*)-27** was better than silodosin, indicating that **(*S*)-27** might have fewer vascular side effects than silodosin.

Table 3. Antagonist effect on smooth muscle contraction of rat urethra and aorta.

Compd.	IC_{50} (nM)		Selectivity
	Urethra	Aorta	Urethra/ Aorta
27	45.4±8.8	>1000	>22.02
(*S*)-27	0.5±0.3	>1000	>2083
silodosin[a]	0.8±0.03	90.1±7.6	112.6

[a] The reference drug.

3.4 Micturition Behavior in BPH Model Rats.

The BPH model rats have higher micturition frequency (Figure 5A) and lower mean voided volume (Figure 5B) than the sham rats. In our studies, we found that silodosin can dose-dependently reduce the urinary frequency and increase the voided volume (Figure 5A and 5B). Our compounds **27** and (***S***)-**27** can also offer improved effects in the micturition behavior of BHP rats. The minimal effective dose of reducing micturition frequency is 10 mg/kg and 3 mg/kg (Figure 5A), respectively; and the minimal effective dose of increasing

the mean voided volume is 3 mg/kg (Figure 5B). The most optimal dose of 27 and (S)-27 in this study is 10 mg/kg, which led the maximum the urinary frequency reduction and voided volume increase. Although compounds (***S***)-**27** shows a slightly weaker activity in BPH rats than silodosin, it can effectively alleviate voiding symptoms of BPH rats, which is worthy of further investigation.

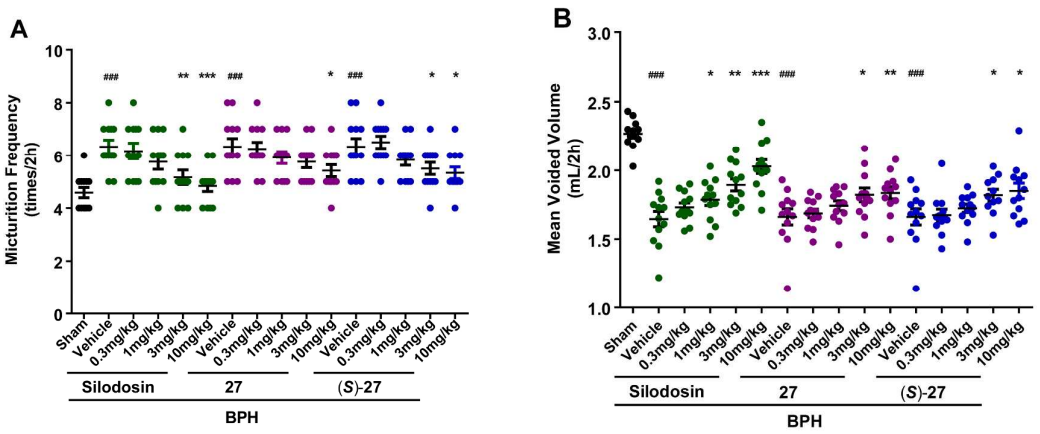


Figure 5. Effects of **27** and (***S***)-**27** on the micturition parameters in BPH rats. (A) Micturition frequency and (B) mean voided volume were measured in a metabolic cage. $^{###}P < 0.001$, versus sham control. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle control.

3.5 Safety Evaluation: Inhibitory Potency against hERG Potassium Ion Channel

In view of excellent biological activities of compound **(*S*)-27**, we further evaluated its hERG potassium ion channel inhibition profiles. As shown in Table 4, these results displayed that **(*S*)-27** and its racemic compound **27** have lower hERG potassium ion channel inhibition than the marketed drug silodosin and dofetilide.

Table 4. Inhibition assay of the hERG potassium ion channel

Compound	hERG inhibition
	IC_{50} (μM)
27	16.5
(*S*)-27	13.2
silodosin[a]	8.2
dofetilide[a]	0.2

[a] The reference drug

3.6 Safety Evaluation: Acute Toxicity for Compound (*S*)-27

We performed acute toxicity tests in Kunming mice. Compound **(*S*)-27** was given orally in a single-dosing experiment at 500 mg/kg. The animals were closely monitored, and no animal died within 7 days after treatment. Body weights were not affected and the animals had shining fur. In addition, the behavior of the mice was unaffected by the single-dose administration of **(*S*)-27** at 500 mg/kg.

3.7 Preliminary Pharmacokinetic Evaluation for Compound (*S*)-27.

Compound **(*S*)-27** was further evaluated for its preliminary pharmacokinetic profile in rat. The results showed that compound **(*S*)-27** had a good pharmacokinetic profile, with 60.9% oral bioavailability, an AUC_{0-t} (area under the plasma concentration-time curve from zero (0) hours to time (t)) of 2274 ng/mL*h, and had a good half life (Table 5). We evaluated the plasma protein binding of this compound in rat. The results showed that compound **(*S*)-27** present a high plasma protein binding, which is about 97%.

Table 5. Preliminary pharmacokinetic parameters for compound **(*S*)-27**.

	Dose	T_{max}	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$	MRT	$t_{1/2}$	CLz	F
	mg/kg	h	ng/mL	ng/mL*h	ng/mL*h	h	h	L/h/kg	%
ig[a]	20	1.0	684	2274	2277	2.52	1.18	/	60.9
iv[b]	10	0.25	855	1867	1877	1.79	0.98	5.79	/

[a]Intragastric administration (oral gavage). [b]Intravenous injection.

4. Conclusions

A class of novel indole-containing THPB derivatives were designed and synthesized, and their antagonistic activities against α_{1A}-ARs were evaluated using calcium mobilization assays. Among them, seven compounds displayed excellent antagonistic activities against α_{1A}-ARs, with $IC_{50s} < 250$ nM. Compound **(*S*)-27** showed the best biological activity, with an IC_{50} of 12.8 nM. More importantly, **(*S*)-27** showed less inhibition against α_{1B}- and α_{1D}-ARs and excellent selectivity towards α_{1A}-AR, which is superior to silodosin. Compound **(*S*)-27** potently inhibited norepinephrine-induced urethra smooth muscle contraction without inhibiting the aortic contraction, displaying better uroselectivity than the control drug silodosin. Additionally, compound **(*S*)-27** has lower hERG potassium ion channel inhibition than the marketed drug silodosin and dofetilide. Preliminary pharmacokinetics studies in rats indicated that compound **(*S*)-27** has a good pharmacokinetic profile. In summary, on the basis of the excellent antagonistic activities and selectivities against α_{1A}-ARs, these novel indole-containing THPB derivatives, especially compound **(*S*)-27**, have promising potential as candidate selective α_{1A}-AR

antagonist drugs for the treatment of BPH.

5. Experimental Section

5.1 Chemistry. Chemicals and solvents were purchased from commercial sources and used without further purification. Analytical thin layer chromatography (TLC) was HSGF 254 (0.15-0.2 mm thickness, YantaiHuiyou Company, China). Column chromatography was performed with CombiFlash® Companion system (Teledyne Isco, Inc.). All target products were characterized by ^{1}H NMR and LC-MS (ESI), and some products were also characterized by ^{13}C NMR. ^{1}H NMR spectra were recorded on a Brucker AMX 300 or 400 MHz instrument (TMS as IS). ^{13}C NMR spectra were recorded on a Brucker AMX 100 MHz instrument (TMS as IS). Chemical shifts were reported in parts per million (ppm). Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). In addition, the purity of all tested compound was determined in the system condition of CH_3OH/H_2O which CH_3OH gradient changed from 70%(v/v) to 85%(v/v) by Agilent 1260 with binary pump, photodiode array detector (DAD), Agilent Eclipse XDB-C18 (4.6×150mm, 5 μm particle size). The percentage of purity of all products were more than 96%.

General Synthetic Procedures for the Target Compounds 13-32 and 34-36 (Compound 13 as the example)

2-(3,4-dimethoxyphenyl)ethan-1-amine (1.5 g, 8.562 mmol), 2-(1H-indol-3-yl)acetic acid (1.5 g, 8.276 mmol, 1 equiv) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.8 g, 14.583 mmol, 1.7 equiv) were dissolved in 30 mL of

dichloromethane. Triethylamine (2.36 mL, 17 mmol, 2 equiv) was added dropwise to the solution and the mixture was stirred for 12 h at rt. The reaction mixture diluted with water. The organic layer was separated and washed with water (30 mL), dried (Na_2SO_4). The combined organic phase was evaporated under reduced pressure to get the crude product (**10a**) and used for the next step without further purification. ESI-MS m/z: 339 $[M+H]^+$.

N-(3,4-dimethoxyphenethyl)-2-(1H-indol-3-yl)acetamide (**10a**) (2.1 g, 6.206 mmol) was dissolved in 30 mL of acetonitrile and added $POCl_3$ (2.1 mL, 13.2 mmol, 2 equiv). The solution was heated to reflux under argon for 1.5 h. The solvents were evaporated under reduced pressure. The pH of the mixture was adjusted to alkalinity with the addition of saturated $NaHCO_3$. The organic layer was separated and washed with water. The combined organic phase was evaporated under reduced pressure to get the crude product (**11a**) and used for the next step without further purification. ESI-MS m/z: 321 $[M+H]^+$.

The intermediate **11a** was dissolved in 20 mL of methanol, and $NaBH_4$ (2.5 g, 66 mmol, 10 equiv) was added in batches at 0 °C. The mixture was stirred for 10 h at rt. The reaction mixture was quenched with water and extracted with ethylacetate. The organic layer was washed with satd brine, and the combined organic phase was evaporated under reduced pressure to get the crude product, which was purified by flash chromatography on silica gel to get key intermediate **12a** (1.5 g, 4.658 mmol, 80% over two steps). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.66 (br, 1H), 7.69-7.67 (m, 1H), 7.34-7.26 (m, 1H), 7.23-7.11 (m, 2H), 7.17-7.12 (m, 1H), 7.00-6.99 (m, 1H), 6.80 (s, 1H), 6.62 (s, 1H), 4.32-4.28 (m, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.45-3.39 (m, 2H), 3.23-3.19 (m, 1H), 3.13-3.05 (m, 1H),

2.91-2.85 (m, 1H), 2.79-2.73 (m, 1H), 2.23 (br, 1H). ESI-MS m/z: 323 $[M+H]^+$.

The key intermediate **12a** (1.5g, 4.658 mmol), 5 mL of formaldehyde and 1 mL of formic acid was dissolved in 30 mL of acetonitrile. The mixture was stirred for 2 h at 80~90 °C. The pH of the mixture was adjusted to alkalinity with the addition of satd $NaHCO_3$. The organic layer was separated and washed with water. The combined organic phase was evaporated under reduced pressure and then chromatographed on silica gel to give the target product **13** (1.28 g, 82%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.47-7.41 (m, 2H), 7.16-7.10 (m, 1H), 7.07-7.02 (m, 1H), 6.90-6.88 (m, 1H), 6.69 (s, 1H), 5.45 (m, 1H), 4.20-4.15 (m, 1H), 3.97-3.90 (m, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.67-3.63 (m, 1H), 3.43-3.37 (m, 1H), 3.22-3.19 (m, 1H), 3.07-2.96 (m, 1H), 2.82-2.62 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.68, 147.65, 136.48, 133.65, 130.67, 127.34, 126.83, 121.06, 119.46, 118.11, 112.11, 110.20, 110.16, 108.24, 65.75, 59.77, 56.25, 55.87, 51.70, 51.58, 29.58, 29.47. ESI-MS m/z: 335 $[M+H]^+$. EI-HRMS m/z calcd $C_{21}H_{22}N_2O_2$ (M^+) 334.1681, found 334.1674.

2,3-Dimethoxy-8-isobutyl-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (14). This compound was prepared by replacement of formaldehyde with 3-methylbutanal using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.81 (br, 1H), 7.50-7.48 (m, 1H), 7.36-7.32 (m, 1H), 7.20-7.08 (m, 2H), 6.72 (s, 1H), 6.66 (s, 1H), 4.30-4.25 (m, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 3.86-3.84 (m, 1H), 3.24-3.10 (m, 2H), 2.92-2.88 (m, 2H), 2.82-2.70 (m, 2H), 1.93-1.83 (m, 1H), 1.55-1.46 (m, 1H), 1.30-1.26 (m, 1H), 1.02-0.98 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.18, 146.73, 135.52, 126.61,

121.07, 118.92, 117.51, 111.22, 110.24, 109.18, 68.56, 55.57, 55.44, 51.13, 46.26, 43.78, 29.15, 25.77, 24.71, 22.65, 21.91. ESI-MS m/z: 391 $[M+H]^+$. EI-HRMS m/z calcd $C_{25}H_{30}N_2O_2$ (M^+) 390.2307, found 390.2296.

2,3-Dimethoxy-8-phenyl-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (15). This compound was prepared by replacement of formaldehyde with benzaldehyde using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.61-7.57 (m, 1H), 7.48-7.44 (m, 2H), 7.41-7.34 (m, 3H), 7.25-7.15 (m, 2H), 7.13-7.07 (m, 2H), 6.92 (s, 1H), 6.60 (s, 1H), 4.61 (s, 1H), 4.15-4.08 (m, 1H), 3.94 (s, 3H), 3.86 (s, 3H), 3.51-3.45 (m, 1H), 3.07-3.02 (m, 1H), 2.95-2.86 (m, 2H), 2.54-2.42 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.46, 140.44, 136.31, 134.04, 129.48, 127.95, 127.86, 127.20, 126.34, 126.11, 120.50, 118.38, 117.21, 110.16, 109.84, 107.99, 107.38, 66.20, 59.34, 55.16, 54.84, 47.43, 28.80, 28.72. ESI-MS m/z: 411 $[M+H]^+$. EI-HRMS calcd $C_{27}H_{26}N_2O_2$(M^+) 410.1994, found 410.1989.

2,3-Dimethoxy-8-(4-fluorophenyl)-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (16). This compound was prepared by replacement of formaldehyde with 4-fluorobenzaldehyde using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.61-7.58 (m, 1H), 7.45-7.40 (m, 1H), 7.25-7.18 (m, 2H), 7.15-7.03 (m, 4H), 6.91 (s, 1H), 6.60 (s, 1H), 4.61 (s, 1H), 4.02-3.98 (m, 1H), 3.94 (s, 3H), 3.86 (s, 3H), 3.50-3.44 (m, 1H), 3.04-2.98 (m, 1H), 2.94-2.85 (m, 2H), 2.56-2.41 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.84, 160.91, 147.54, 147.48, 137.29, 136.75, 133.51, 131.82, 131.24, 131.18, 126.95, 126.09, 121.31, 118.81, 118.32, 115.09, 114.92, 112.80, 111.52,

110.57, 108.14, 62.59, 56.04, 55.89, 51.11, 46.85, 29.83, 27.99. ESI-MS m/z: 429 $[M+H]^+$. EI-HRMS calcd $C_{27}H_{25}FN_2O_2(M^+)$ 428.1900, found 428.1897.

2,3-Dimethoxy-8-(4-methoxyphenyl)-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*] quinolizine (17). This compound was prepared by replacement of formaldehyde with 4-methoxybenzaldehyde using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.62-7.59 (m, 1H), 7.38-7.36 (m, 1H), 7.28-7.25 (m, 1H), 7.21-7.18 (m, 1H), 7.15-7.10 (m, 2H), 6.93-6.90 (m, 3H), 6.61 (s, 1H), 4.57 (s, 1H), 4.01-3.98 (m, 1H), 3.96 (s, 3H), 3.88 (s, 3H), 3.84 (s, 3H), 3.51-3.45 (m, 1H), 3.10-3.06 (m, 1H), 2.96-2.86 (m, 2H), 2.56-2.40 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.08, 147.01, 135.80, 129.67, 126.69, 121.01, 118.91, 117.73, 113.70, 110.70, 110.35, 108.52, 107.89, 66.06, 59.99, 55.70, 55.38, 54.82, 47.69, 29.24. ESI-MS m/z: 441 $[M+H]^+$. EI-HRMS calcd $C_{28}H_{28}N_2O_3(M^+)$ 440.2100, found 453.2451.

2,3-Methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (18). This compound was prepared by replacement of 2-(3,4-dimethoxyphenyl)ethan-1-amine with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine using a similar synthetic procedure of product **13**. ^{1}H NMR (CD_3OD, 400 MHz): δ7.42-7.39 (m, 1H), 7.27-7.17 (m, 2H), 7.08-7.00 (m, 1H), 6.83 (s, 1H), 6.57 (s, 1H), 5.89 (s, 2H), 5.45 (s, 1H), 4.20-4.03 (m, 2H), 3.74-3.64 (m, 2H), 3.22-3.04 (m, 2H), 2.77-2.62 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 145.72, 145.44, 136.00, 133.07, 131.40, 127.47, 126.84, 120.62, 119.02, 117.59, 109.70, 108.03, 107.65, 105.99, 100.54, 65.26, 59.48, 54.89, 51.10, 50.84, 29.38, 29.17. ESI-MS m/z: 319 $[M+H]^+$. EI-HRMS calcd $C_{20}H_{18}N_2O_2(M^+)$ 318.1368, found 318.1366.

(S)-2,3-Methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (S-18). This compound was prepared by replacement of 2-(3,4-dimethoxyphenyl)ethan-1-amine with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine using a similar synthetic procedure of product **13**. ^{1}H NMR (CD_3OD, 400 MHz): δ7.60-7.57 (m, 1H), 7.42-7.39 (m, 1H), 7.15-7.12 (m, 1H), 7.08-7.03 (m, 1H), 6.87 (s, 1H), 6.60 (s, 1H), 5.90 (s, 2H), 5.48 (s, 1H), 4.30-4.25 (m, 2H), 3.82-3.72 (m, 2H), 3.42-3.29 (m, 2H), 2.80-2.66 (m, 2H). ESI-MS m/z: 319 $[M+H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6) δ 145.72, 145.44, 136.00, 133.07, 131.40, 127.47, 126.84, 120.62, 119.02, 117.59, 109.70, 108.03, 107.65, 105.99, 100.54, 65.26, 59.48, 54.89, 51.10, 50.84, 29.38, 29.17. ESI-MS m/z: 319 $[M+H]^+$. EI-HRMS calcd $C_{20}H_{18}N_2O_2(M^+)$ 318.1368, found 318.1360.

2,3-Methylenedioxy-8-ethyl-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (19). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and propionaldehyde using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.88 (br, 1H), 7.49-7.45 (m, 1H), 7.34-7.31 (m, 1H), 7.19-7.07 (m, 2H), 6.68 (s, 1H), 6.62 (s, 1H), 5.93 (s, 2H), 4.28-4.23 (m, 1H), 3.70-3.65 (m, 1H), 3.21-3.07 (m, 2H), 2.95-2.70 (m, 4H), 1.93-1.75 (m, 2H), 1.19-1.12 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.55, 145.12, 135.10, 136.00, 132.50, 126.55, 121.01, 118.85, 117.52, 110.19, 108.22, 106.71, 106.18, 100.17, 62.24, 51.73, 46.15, 29.68, 27.51, 26.03, 17.92, 11.42. ESI-MS m/z: 347 $[M+H]^+$. EI-HRMS calcd $C_{22}H_{22}N_2O_2(M^+)$ 346.1681, found 346.1672.

2,3-Methylenedioxy-8-isobutyl-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinol

izine (20). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 3-methylbutanal using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85 (br, 1H), 7.49-7.46 (m, 1H), 7.34-7.31 (m, 1H), 7.20-7.08 (m, 2H), 6.69 (s, 1H), 6.63 (s, 1H), 5.93 (s, 2H), 4.23-4.14 (m, 1H), 3.87-3.82 (m, 1H), 3.21-3.09 (m, 2H), 2.89-2.85 (m, 2H), 2.78-2.69 (m, 2H), 1.83-1.75 (m, 1H), 1.31-1.22 (m, 2H), 1.07-1.03 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 145.49, 145.04, 136.41, 136.00, 133.37, 126.68, 120.36, 118.12, 117.44, 110.85, 108.35, 106.86, 105.10, 100.40, 58.30, 51.04, 45.78, 43.86, 29.92, 25.34, 24.74, 23.56, 21.83. ESI-MS m/z: 375 $[M+H]^+$. EI-HRMS calcd $C_{24}H_{26}N_2O_2(M^+)$ 374.1994, found 374.1992.

2,3-Methylenedioxy-8-(4-fluorophenyl)-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (21). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 4-fluorobenzaldehyde using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ7.72 (br, 1H), 7.58-7.55 (m, 1H), 7.30-7.27 (m, 1H), 7.22-7.14 (m, 4H), 7.01-6.94 (m, 2H), 6.69 (s, 1H), 6.56 (s, 1H), 5.89 (s, 2H), 5.20-5.18 (m, 1H), 4.16-4.11 (m, 1H), 3.27-3.21 (m, 2H), 2.97-3.85 (m, 3H), 2.72-2.65 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.51, 145.42, 135.89, 131.96, 131.45, 126.60, 126.46, 121.49, 119.07, 117.80, 114.62, 114.45, 110.45, 109.14, 108.01, 105.83, 100.22, 62.53, 51.35, 46.84, 29.37, 27.88. ESI-MS m/z: 413 $[M+H]^+$. EI-HRMS calcd $C_{26}H_{21}FN_2O_2$ (M^+) 412.1587, found 412.1581.

2,3-Methylenedioxy-8-(4-methoxyphenyl)-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (22). This compound was prepared by replacement with

2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 4-methoxybenzaldehyde using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.78 (br, 1H), 7.58-7.55 (m, 1H), 7.26-7.24 (m, 1H), 7.19-7.10 (m, 4H), 6.83-6.80 (m, 2H), 6.69 (s, 1H), 6.56 (s, 1H), 5.89 (s, 2H), 5.06 (s, 1H), 4.16-4.09 (m, 1H), 3.76 (s, 3H), 3.26-3.13 (m, 2H), 2.97-2.88 (m, 1H), 2.85-2.78 (m, 2H), 2.67-2.61 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.81, 145.39, 135.88, 132.64, 130.12, 126.79, 126.53, 121.27, 118.91, 117.70, 113.02, 110.41, 108.94, 107.95, 105.82, 100.18, 62.71, 54.79, 51.48, 47.00, 29.43, 28.30.ESI-MS m/z: 425 $[M+H]^+$. EI-HRMS calcd $C_{27}H_{24}N_2O_3$ (M^+) 424.1787, found 424.1777.

3-Methoxy-5,8,14,14a- tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (23). This compound was prepared by replacement of 2-(3,4-dimethoxyphenyl)ethan-1-amine with 2-(4-methoxyphenyl)ethan-1-amine using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ7.49-7.46 (m, 1H), 7.42-7.39 (m, 1H), 7.29-7.13 (m, 3H), 6.84-6.80 (m, 1H), 6.70 (s, 1H), 5.48-5.38 (m, 1H), 3.97-3.88 (m, 1H), 3.83 (s, 3H), 3.72-3.61 (m, 1H), 3.37-3.19 (m, 2H), 3.05-3.01 (m, 1H), 2.79-2.68 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.44, 135.83, 134.94, 131.57, 129.48, 126.89, 121.06, 119.34, 117.56, 112.58, 112.19, 108.68, 108.59, 66.14, 59.08, 54.77, 51.13, 50.86, 29.22, 28.65. ESI-MS m/z: 305 $[M+H]^+$. EI-HRMS calcd $C_{20}H_{20}N_2O(M^+)$ 304.1576, found 304.1571.

3-Methoxy-8-methyl-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (24). This compound was prepared by replacement with 2-(4-methoxyphenyl)ethan-1-amine and acetaldehyde using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.50-7.47 (m, 1H), 7.37-7.28 (m, 1H), 7.18-7.04 (m, 3H), 6.80-6.78 (m, 1H), 6.66

(s, 1H), 3.96-3.90 (m, 1H), 3.89-3.85 (m, 1H), 3.80 (s, 3H), 3.20-3.12 (m, 3H), 2.85-2.63 (m, 3H), 1.30-1.25 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ157.87, 136.06, 135.44, 131.67, 127.54, 121.50, 119.36, 118.07, 113.37, 112.22, 110.72 108.24, 107.28, 60.08, 55.54, 55.24, 53.43, 51.44, 46.78, 30.36, 28.27. ESI-MS m/z: 319 $[M+H]^+$. ESI-HRMS calcd $C_{21}H_{22}N_2O$ ($M+H^+$) 319.1805, found 319.1804.

3-Methoxy-8-cyclopropyl-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (25). This compound was prepared by replacement with 2-(4-methoxyphenyl)ethan-1-amine and cyclopropanecarbaldehyde using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.21 (br, 1H), 7.52-7.47 (m, 1H), 7.38-7.32 (m, 1H), 7.26-7.23 (m, 1H), 7.19-7.09 (m, 2H), 6.82-6.79 (m, 1H), 6.69 (s, 1H), 3.92-3.85 (m, 2H), 3.82 (s, 3H), 3.46-3.38 (m, 1H),3.21-3.12 (m, 1H), 3.07-2.98 (m, 1H), 2.83-2.61 (m, 3H), 1.27-1.13 (m, 1H), 0.95-0.92 (m, 2H), 0.67-0.63 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.71, 136.42, 135.95, 127.92, 126.88, 120.93, 118.61, 118.01, 113.33, 112.60, 111.41, 106.42, 64.33, 55.43, 52.62, 47.29, 30.67, 28.99,13.63, 5.16, 2.05. ESI-MS m/z: 345 $[M+H]^+$. EI-HRMS calcd $C_{23}H_{24}N_2O$ (M^+) 344.1889, found 344.1871.

3-Methoxy-8-(4-methoxyphenyl)-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (26). This compound was prepared by replacement with 2-(4-methoxyphenyl)ethan-1-amine and 4-methoxybenzaldehyde using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.80 (br, 1H), 7.57-7.54 (m, 1H), 7.37-7.35 (m, 1H), 7.27-7.23 (m, 1H), 7.18-7.09 (m, 4H), 6.83-6.78 (m, 2H), 6.76-6.72 (m,

1H), 6.65-6.62 (m, 1H), 5.10 (s, 1H), 4.26-4.21 (m, 1H), 3.82-3.80 (m, 2H), 3.77 (s, 3H), 3.75 (s, 3H), 3.22-3.05 (m, 1H), 2.93-2.83 (m, 2H), 2.67-2.60 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ158.73, 157.22, 135.88, 135.05, 132.87, 131.04, 130.74, 130.10, 126.82, 121.17, 118.84, 117.70, 112.96, 112.76, 111.72, 110.42, 109.06, 62.70, 54.79, 54.75, 50.91, 46.92, 29.79, 28.31. ESI-MS m/z: 411 $[M+H]^+$. EI-HRMS calcd $C_{27}H_{26}N_2O_2$ (M^+) 410.1994, found 410.1989.

2,3-Methylenedioxy-12-methoxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (27). This compound was prepared by replacement with 2-(benzo[*d*][1,3]dioxol-5-yl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.27-7.24 (m, 1H), 6.88-6.87 (m, 1H), 6.83-6.77 (m, 2H), 6.60 (s, 1H), 5.93 (s, 2H), 5.37-5.34 (m, 1H), 3.83 (s, 3H), 3.70-3.63 (m, 2H), 3.23-3.08 (m, 2H), 2.72-2.61 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.35, 146.32, 146.14, 132.60, 131.38, 130.60, 127.81, 127.36, 111.14, 109.85, 108.84, 108.40, 105.77, 100.86, 100.46, 67.04, 59.97, 55.83, 51.65, 51.32, 29.49, 29.31. ESI-MS m/z: 349 $[M+H]^+$. EI-HRMS calcd $C_{21}H_{20}N_2O_3$ (M^+) 348.1474, found 348.1480.

(S)-2,3-Methylenedioxy-12-methoxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (*S*-27). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.27-7.24 (m, 1H), 6.91-6.87 (m, 1H), 6.83-6.79 (m, 2H), 6.60 (s, 1H), 5.93 (s, 2H),

5.38-5.34 (m, 1H), 3.83 (s, 3H), 3.70-3.63 (m, 2H), 3.23-3.08 (m, 2H), 2.72-2.61 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.32, 146.27, 146.12, 132.49, 131.31, 130.73, 127.78, 127.34, 111.08, 109.61, 108.75, 108.40, 105.78, 100.86, 100.41, 66.68, 59.82, 55.78, 51.65, 51.20, 29.40, 29.23. ESI-MS m/z: 349 $[M+H]^+$. EI-HRMS calcd $C_{21}H_{20}N_2O_3$ (M^+) 348.1474, found 348.1468.

(R)-2,3-Methylenedioxy-12-methoxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*] quinolizine (*R*-27). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 (br, 1H), 7.22-7.30 (m, 1H), 6.87-6.83 (m, 2H), 6.74 (s, 1H), 6.58 (s, 1H), 5.95 (s, 2H), 5.41 (s, 1H), 4.52-4.48 (m, 1H), 4.12-4.07 (m, 2H), 3.95-3.92 (m, 1H), 3.82 (s, 3H), 3.34-3.25 (m, 2H), 2.91-2.79 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.35, 146.33, 146.20, 132.26, 131.37, 130.43, 127.72, 127.21, 111.15, 109.79, 108.62, 108.40, 105.74, 100.89, 100.39, 66.64, 59.88, 55.79, 51.60, 51.19, 29.50, 29.28.. ESI-MS m/z: 349 $[M+H]^+$. EI-HRMS calcd $C_{21}H_{20}N_2O_3$ (M^+) 348.1474, found 348.1471.

2,3-Methylenedioxy-13-methoxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quin olizine (28). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 2-(4-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30-7.25 (m, 1H), 6.88-6.78 (m, 2H), 6.71-6.68 (m, 1H), 6.63 (s, 1H), 5.91 (s, 2H), 5.38 (s, 1H), 3.85 (s, 3H), 3.74-3.65 (m, 2H), 3.33-3.20 (m, 2H), 2.75-2.62 (m, 4H). ^{13}C NMR

(125 MHz, $CDCl_3$) δ 154.30, 146.25, 146.22, 132.39, 131.35, 130.55, 127.83, 127.30, 111.38, 109.71, 109.37, 108.41, 105.70, 100.97, 100.51, 66.71, 59.81, 55.77, 51.65, 51.20, 29.41, 29.24. ESI-MS m/z: 349 $[M+H]^+$. EI-HRMS calcd $C_{21}H_{20}N_2O_3$ (M^+) 348.1474, found 348.1481.

2,3-Methylenedioxy-11-methoxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (29). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 2-(6-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.31-7.28 (m, 1H), 6.93-6.92 (m, 1H), 6.78-6.75 (m, 2H), 6.60 (s, 1H), 5.94 (s, 2H), 5.44-5.43 (m, 1H), 3.85 (s, 3H), 3.82-3.76 (m, 1H), 3.66-3.63 (m, 1H), 3.48-3.46 (m, 1H), 3.27-3.23 (m, 2H), 2.80-2.64 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.31, 146.38, 146.35, 146.27, 132.18, 131.43, 130.25, 127.59, 127.08, 111.60, 111.17, 110.11, 108.38, 105.76, 100.91, 100.38, 66.80, 59.86, 55.85, 55.81, 51.55, 51.15, 29.72, 29.15. ESI-MS m/z: 349 $[M+H]^+$. EI-HRMS calcd $C_{21}H_{20}N_2O_3$ (M^+) 348.1474, found 348.1475.

2,3-Methylenedioxy-11-fluoro-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (30). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 2-(6-fluoro-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.13 (br, 1H), 7.37-7.34 (m, 1H), 6.97-6.87 (m, 2H), 6.85-6.79 (m, 1H), 6.61-6.60 (m, 1H), 5.94 (s, 2H), 4.13-4.00 (m, 1H), 3.81-3.76 (m, 2H), 3.28-3.10 (m, 3H), 2.74-2.71 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ159.60, 157.74, 145.75, 145.48, 136.12, 133.71, 131.34,

127.50, 123.62, 118.40, 111.17, 108.07, 107.85, 107.16, 106.97, 106.03, 100.58, 96.74, 96.53, 65.53, 59.42, 51.06, 50.81, 29.40, 29.09. ESI-MS m/z: 337 $[M+H]^+$. EI-HRMS calcd $C_{20}H_{17}FN_2O_2$ (M^+) 336.1274, found 336.1269.

2,3-Methylenedioxy-12-fluoro-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (31). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 2-(5-fluoro-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.22-7.18 (m, 1H), 7.13-7.12 (m, 1H), 6.88-6.83 (m, 1H), 6.79 (s, 1H), 6.60 (s, 1H), 5.94 (s, 2H), 4.13-4.06 (m, 1H), 3.88-3.78 (m, 2H), 3.27-3.07 (m, 3H), 2.81-2.68 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.53, 156.69, 146.20, 145.94, 135.65, 133.10, 131.75, 127.94, 127.68, 111.17, 108.82, 108.52, 108.37, 106.44, 103.23, 103.04, 101.04, 65.95, 59.87, 51.56, 51.26, 29.82, 29.53.ESI-MS m/z: 337 $[M+H]^+$. EI-HRMS calcd $C_{20}H_{17}FN_2O_2$ (M^+) 336.1274, found 336.1272.

2,3-Methylenedioxy-12-benzyloxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (32). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 2-(5-(benzyloxy)-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.49-7.47 (m, 2H), 7.41-7.32 (m, 3H), 7.09 (s, 1H), 7.00 (s, 1H), 6.83-6.81 (m, 1H), 6.69 (s, 1H), 6.25-6.22 (m, 1H), 5.97 (s, 2H), 5.38-5.36 (m, 2H), 5.13-5.06 (m, 2H), 4.18-4.15 (m, 1H), 3.66-3.57 (m, 1H), 3.14-3.10 (m, 1H), 2.93-2.90 (m, 1H), 2.68-2.60 (m, 2H), 2.44-2.38 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.07, 145.16, 145.08, 136.48,

130.97, 130.70, 129.09, 126.78, 126.36, 126.09, 126.02, 125.63, 110.18, 108.38, 106.79, 106.52, 104.12, 100.65, 99.47, 76.48, 69.39, 64.10, 58.62, 49.92, 27.46. ESI-MS m/z: 425 $[M+H]^+$. EI-HRMS calcd $C_{27}H_{24}N_2O_3$ (M^+) 424.1787, found 424.1780.

2,3-Methylenedioxy-12-hydroxyl-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (33). This compound was prepared by reduction of compound **32** catalyzed by 10% Pd/C under hydrogen atmosphere for 8 h. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.70 (br, 1H), 7.23-7.21 (m, 1H), 6.99 (s, 1H), 7.72-7.66 (m, 2H), 6.59-6.56 (m, 1H), 6.15 (br, 1H), 5.95-5.93 (m, 2H), 5.31-5.30 (m, 2H), 4.13-4.09 (m, 1H), 3.64-3.53 (m, 1H), 3.22-3.07 (m, 1H), 2.90-2.87 (m, 1H), 2.65-2.57 (m, 2H), 2.38-2.32 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 151.28, 145.17, 145.86, 133.86, 131.94, 130.92, 128.02, 127.93, 110.70, 110.46, 108.48, 107.28, 106.50, 102.64, 100.99, 65.72, 60.08, 51.67, 51.32, 29.82. ESI-MS m/z: 335 $[M+H]^+$. EI-HRMS calcd $C_{20}H_{18}N_2O_3$ (M^+) 334.1317, found 334.1311.

2,3-Methylenedioxy-9-methyl-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (34). This compound was prepared by replacement with 2-(benzo[d][1,3]dioxol-5-yl)ethan-1-amine and 2-(1-methyl-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.52-7.50 (m, 1H), 7.30-7.26 (m, 1H), 7.21-7.17 (m, 1H), 7.12-7.09 (m, 1H), 6.83 (s, 1H), 6.65 (s, 1H), 5.94 (s, 2H), 4.14-4.10 (m, 1H), 3.80-3.71 (m, 2H), 3.64 (s, 3H), 3.35-3.31 (m, 1H), 3.24-3.11 (m, 2H), 2.82-2.71 (m, 3H).^{13}C NMR (125 MHz, $CDCl_3$) δ 146.22, 146.01, 137.18, 132.92, 131.31, 127.62, 126.81, 120.94, 118.98, 117.95, 108.72, 108.37, 107.47, 105.92, 100.82, 60.10, 52.17, 51.51, 29.90, 29.34. ESI-MS m/z: 333 $[M+H]^+$.

EI-HRMS calcd $C_{21}H_{20}N_2O_2$ (M^+) 332.1525, found 332.1527.

2-Benzyloxy-3,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (35). This compound was prepared by replacement with 2-(4-(benzyloxy)-3-methoxyphenyl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.31 (m, 5H), 6.86 – 6.84 (m, 2H), 6.74 (s, 1H), 6.62 (s, 1H), 5.40 (q, *J* = 11.7 Hz, 1H), 5.24 – 5.10 (m, 2H), 4.37 (d, *J* = 15.1 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.81 – 3.71 (m, 1H), 3.30 – 2.96 (m, 3H), 2.87 – 2.79 (m, 1H), 2.61 – 2.55 (m, 1H), 2.17 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.74, 148.15, 147.61, 137.09, 132.44, 128.48, 128.32, 128.16, 112.87, 112.44, 110.44, 110.36, 105.68, 102.70, 71.43, 61.92, 56.79, 56.04, 52.57, 51.14, 28.32, 28.28. ESI-MS m/z: 441 $[M+H]^+$. EI-HRMS calcd $C_{28}H_{28}N_2O_3$ (M^+) 440.2100, found 440.2101.

3-Benzyloxy-2,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (36). This compound was prepared by replacement with 2-(3-(benzyloxy)-4-methoxyphenyl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.23 (m, 5H), 6.88 – 6.81 (m, 2H), 6.77(s, 1H), 6.62 (s, 1H), 5.40 (q, *J* = 11.7 Hz, 1H), 5.24 – 5.10 (m, 2H), 4.37 (d, *J* = 15.1 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.81 – 3.71 (m, 1H), 3.30 – 2.96 (m, 3H), 2.87 – 2.79 (m, 1H), 2.61 – 2.55 (m, 1H), 2.17 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ153.57, 147.62, 146.07, 137.47, 133.77, 131.11, 130.17, 128.37, 127.95, 127.77, 127.24, 126.93, 111.88, 110.44, 110.09, 107.53, 99.87, 70.37, 65.39, 59.62,

55.38, 51.22, 29.10, 20.77, 14.10. ESI-MS m/z: 441 $[M+H]^+$. EI-HRMS calcd $C_{28}H_{28}N_2O_3$ (M^+) 440.2100, found 440.2101.

2-Hydroxyl-3,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (37). This compound was prepared by reduction of compound **35** catalyzed by 10% Pd/C under hydrogen atmosphere. ^{1}H NMR (400 MHz, MeOD) δ 7.35 (d, J = 8.9 Hz, 1H), 6.98 (d, J = 2.4 Hz, 1H), 6.84 (s, 1H), 6.80 (dd, J = 8.8, 2.5 Hz, 1H), 6.69 (s, 1H), 4.69 – 4.58 (m, 2H), 4.21 (d, J = 14.8 Hz, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.78 (d, J = 15.1 Hz, 1H), 3.70 (d, J = 7.1 Hz, 1H), 3.41 – 3.33 (m, 1H), 3.25 (dd, J = 15.3, 7.1 Hz, 1H), 3.12 (d, J = 11.4 Hz, 1H), 2.79 (dt, J = 25.4, 10.0 Hz, 2H), 2.67 – 2.60 (m, 1H).^{13}C NMR (125 MHz, DMSO-d_6) δ 153.74, 146.28, 145.81, 133.42, 132.44, 128.61, 128.30, 125.75, 112.16, 111.65, 110.36, 105.68, 102.70, 61.92, 56.79, 56.04, 52.57, 51.14, 28.32, 28.28. ESI-MS m/z: 351 $[M+H]^+$. EI-HRMS calcd $C_{21}H_{22}N_2O_3$ (M^+) 350.1630, found 350.1628.

3-Hydroxyl-2,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (38). This compound was prepared by reduction of compound **36** catalyzed by 10% Pd/C under hydrogen atmosphere. ^{1}H NMR (400 MHz, MeOD) δ 7.47 (t, J = 8.0 Hz, 2H), 7.39 (t, J = 7.2 Hz, 1H), 7.34 (d, J = 7.1 Hz, 1H), 7.13 (d, J = 5.3 Hz, 1H), 6.94 – 6.91 (m, 1H), 5.61 – 5.56 (m, 2H), 5.17 (s, 2H), 3.95 (s, 3H), 3.86 (s, 3H), 3.76 – 3.58 (m, 1H), 3.37 (s, 1H), 3.20 – 2.93 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.93, 147.43, 136.96, 131.53, 128.48, 127.96, 127.87, 126.30, 113.09, 111.69, 110.99, 109.63, 100.39, 69.91, 65.67, 60.25, 56.15, 55.47, 26.23, 25.37. ESI-MS m/z: 351 $[M+H]^+$. EI-HRMS calcd $C_{21}H_{22}N_2O_3$ (M^+) 350.1630, found 350.1628.

2-Ethoxy-3,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (39). This compound was prepared by reaction of compound **35** with bromoethane under K_2CO_3 as the base in acetone. ^{1}H NMR (400 MHz, CD_3OD) δ 7.17 (d, *J* = 8.7 Hz, 1H), 7.00 – 6.95 (m, 2H), 6.71 (dd, *J* = 8.8, 2.4 Hz, 2H), 4.13 – 4.03 (m, 2H), 3.82 (S, 6H), 3.76 (dd, *J* = 13.6, 8.8 Hz, 2H), 3.45 (dd, *J* = 15.1, 3.0 Hz, 1H), 3.30 (q, 7.0 Hz, 2H), 3.28 – 3.01 (m, 2H), 2.77 – 2.64 (m, 2H), 1.42 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 153.96, 147.89, 146.72, 132.19, 131.16, 129.95, 127.60, 126.53, 111.47, 111.41, 111.03, 110.60, 108.35, 100.15, 64.67, 59.76, 55.86, 52.96, 51.30, 29.66, 29.41, 29.26, 14.90. ESI-MS m/z: 379 $[M+H]^+$. EI-HRMS calcd $C_{23}H_{26}N_2O_3$ (M^+) 378.1943, found 378.1943.

2-propoxy-3,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (40). This compound was prepared by replacement with 2-(3-methoxy-4-propoxyphenyl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.17 (d, *J* = 8.7 Hz, 1H), 7.02 – 6.94 (m, 2H), 6.75 – 6.67 (m, 2H), 4.06 – 3.96 (m, 2H), 3.82 (d, *J* = 2.0 Hz, 6H), 3.77 (d, *J* = 16.7 Hz, 2H), 3.46 (dd, *J* = 14.6, 3.5 Hz, 1H), 3.30 (q, 7.4 Hz, 2H), 3.26 – 3.07 (m, 2H), 2.78 – 2.63 (m, 2H), 1.90 – 1.76 (m, 2H), 1.07 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.54, 147.63, 146.60, 131.67, 130.77, 129.47, 127.17, 126.03, 111.26, 111.03, 110.61, 110.40, 107.86, 99.78, 70.48, 59.33, 55.53, 55.46, 52.46, 50.81, 31.47, 29.25, 22.24, 22.17. ESI-MS m/z: 393 $[M+H]^+$. EI-HRMS calcd $C_{24}H_{28}N_2O_3$ (M^+) 392.2100, found 392.2106.

2-Butoxy-3,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinol

izine (41). This compound was prepared by replacement with 2-(4-butoxy-3-methoxyphenyl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.17 (d, *J* = 8.7 Hz, 1H), 7.02 – 6.92 (m, 2H), 6.75 – 6.69 (m, 2H), 4.01 (dd, *J* = 11.4, 4.8 Hz, 2H), 3.82 (d, *J* = 1.3 Hz, 6H), 3.77 (d, *J* = 16.6 Hz, 2H), 3.51 – 3.39 (m, 1H), 3.30 (q, 7.4 Hz, 2H), 3.26 – 3.06 (m, 2H), 2.77 – 2.63 (m, 2H), 1.85 – 1.72 (m, 2H), 1.55 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.01, 148.14, 147.13, 132.13, 131.26, 129.92, 127.63, 126.47, 111.76, 111.53, 111.07, 110.88, 108.29, 100.26, 69.17, 59.82, 56.01, 55.92, 52.94, 51.28, 31.94, 31.40, 29.71, 22.71, 19.29. ESI-MS m/z: 407 $[M+H]^+$. EI-HRMS calcd $C_{25}H_{30}N_2O_3$ (M^+) 406.2256, found 406.2258.

3-Ethoxy-2,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinol izine (42). This compound was prepared by replacement with 2-(3-ethoxy-4-methoxyphenyl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.95 (s, 1H), 6.80 (s, 1H), 6.77 (d, *J* = 8.6 Hz, 1H), 6.61 (s, 1H), 4.07 (dd, *J* = 13.5, 6.5 Hz, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.74 (d, *J* = 15.1 Hz, 2H), 3.29 (d, *J* = 14.5 Hz, 1H), 3.11 (t, *J* = 12.1 Hz, 2H), 2.73 – 2.67 (m, 2H), 2.01 – 1,80 (m, 2H), 1.45 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.98, 147.80, 132.13, 131.99, 131.28, 131.14, 129.93, 127.62, 112.74, 111.49, 111.44, 111.11, 108.26, 108.21, 100.23, 64.31, 59.84, 56.25, 55.93, 52.95, 51.30, 49.94, 49.76, 29.70. ESI-MS m/z: 379 $[M+H]^+$. EI-HRMS calcd $C_{23}H_{26}N_2O_3$ (M^+) 378.1943, found 378.1942.

3-Propoxy-2,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (43). This compound was prepared by replacement with 2-(4-methoxy-3-propoxyphenyl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 6.95 (s, 1H), 6.79 (d, *J* = 11.2 Hz, 2H), 6.62 (s, 1H), 3.95 (s, 4H), 3.89 (s, 4H), 3.84 (s, 2H), 3.73 (d, *J* = 12.1 Hz, 2H), 3.28 (d, *J* = 13.7 Hz, 1H), 3.10 (s, 2H), 2.71 (s, 2H), 1.86 (q, *J* = 6.8 Hz, 2H), 1.03 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.98, 147.87, 147.17, 132.26, 131.22, 129.94, 127.62, 126.49, 112.86, 111.59, 111.09, 109.40, 108.29, 100.14, 70.48, 59.89, 56.37, 55.92, 52.94, 51.37, 22.74, 22.52, 14.19, 10.52. ESI-MS m/z: 393 $[M+H]^+$. EI-HRMS calcd $C_{24}H_{28}N_2O_3$ (M^+) 392.2100, found 392.2108.

3-Butoxy-2,12-methylenedioxy-5,8,14,14a-tetrahydro-6*H*-benzo[*a*]indolo[2,3-*g*]quinolizine (44). This compound was prepared by replacement with 2-(3-butoxy-4-methoxyphenyl)ethan-1-amine and 2-(5-methoxy-1H-indol-3-yl)acetic acid using a similar synthetic procedure of product **13**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 6.95 (d, *J* = 2.1 Hz, 1H), 6.81 (s, 1H), 6.77 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.62 (s, 1H), 4.00 (t, *J* = 6.8 Hz, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.69 (d, *J* = 15.2 Hz, 2H), 3.32 – 3.24 (m, 1H), 3.10 (t, *J* = 12.0 Hz, 2H), 2.70 (s, 3H), 1.86 – 1.78 (m, 2H), 1.49 (dq, *J* = 14.8, 7.5 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.01, 147.90, 147.23, 132.38, 131.23, 130.08, 127.69, 126.60, 112.91, 111.50, 111.07, 109.52, 108.43, 100.21, 68.72, 59.89, 56.41, 55.92, 53.01, 51.42, 31.95, 31.29, 29.72,

22.72, 19.27. ESI-MS m/z: 407 $[M+H]^+$. EI-HRMS calcd $C_{25}H_{30}N_2O_3$ (M^+) 406.2256, found 406.2260.

5.2 Bioassay

5.2.1 Chemicals and Reagents. Silodosin and tamsulosin were purchased from J&K chemical (Shanghai, China), and phenylephrine was purchased from Tokoyokasei. Mammalian expression vectors encoding Gα16, α_{1A}-AR, α_{1B}-AR and α_{1D}-AR were purchased from the UMR cDNA Resource Center. Full-length cDNAs encoding human α_{1A}-AR, α_{1B}-AR or α_{1D}-AR were cloned into the pSNAP vector (Cisbio Bioassays) in-frame with SNAP-tag attached at the N terminus. The Tag-lite labeling medium, the Tb derivative of O6-benzylguanine (commercialized as SNAP-Lumi4-Tb) and the □α_1-AR antagonist (Prazosin) labeled with a d2 fluorescent probe was obtained from Cisbio Bioassays.

5.2.2 Cells Culture and Transfection. HEK293 cells obtained from American Type Culture Collection were maintained in Dulbecco's Modified Eagle's Medium(DMEM) supplemented with 10% fetal bovine serum(FBS), 100 mg/L penicillin, and 100 mg/L streptomycin at 37°C in a humidified atmosphere of 5% CO_2. HEK293 cells were cotransfected with plasmids encoding various α_1-ARs and Gα16 by electroporation. To generate stable cell lines, transfected cells were seeded onto 10-cm dishes and 1 mg/mL G418 and 40μg/mL blasticidin were added to the culture medium 24 h later. The selection medium was changed every 3 days until colonies formed. A single colony was isolated,

expanded, and tested with a calcium mobilization assay to confirm the expression and proper function of the transfected genes.

5.2.3 Calcium Mobilization Assay. Cells were seeded onto 96-well plates at a density of 3×10^4 cells/well and cultured overnight. Cells were then incubated with 2 μM Fluo-4 AM in HBSS (5.4 mM KCl, 0.3 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.6 mM $MgSO_4$, 137 mM NaCl, 5.6 mM D-glucose and 250 μM sulfinpyrazone, pH 7.4) at 37 ℃ for 45 min. After a thorough washing, 50 μL of HBSS containing either antagonists or 1% DMSO (negative control) were added. After incubation at room temperature for 10 min, 25μL of agonist were dispensed into the well using a FlexStation microplate reader (Molecular Devices), and intracellular calcium change was recorded at an excitation wavelength of 485 nm and an emission wavelength of 525 nm.

5.2.4 Rat isolated tissue functional assays. Freshly isolated male SD rat urethra or aorta were cleaned of adherent connective tissue and cut helically, and the endoethelium was removed by gentle rubbing. The tissue strips were then mounted vertically inan organ bath containing 20 mL of Krebs-Henseleit solution of the following composition (mM): NaCl, 118; KCl 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2, glucose 11.1. These tissues were then mounted in the buffer maintained at 37 ℃ and aerated with carbogen (95% oxygen and 5% carbon dioxide) during the entire length of experiment. Resting tension applied was 1 g for rat urethraor aorta, and the responses were recorded isometrically

through force-displacement transducers. The tissue strips noradrenaline cumulative concentration response curve was obtained in the absence or presence of compounds with different concentrations incubated for 20 min.

5.2.5 Data Analysis. Data were analyzed with GraphPad Prism software (GraphPad). Nonlinear regression analysis was performed to generate dose-response curves and calculate concentrations for 50% inhibition (IC_{50}) values. Means ± SEM were calculated using this software. The analyses were assessed by a Student *t* test. A p value < 0.05 was considered statistically significant.

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Abbreviations

THPBs, Tetrahydroprotoberberine derivatives; AR, α_{1A}-Adrenergic receptors; BPH, Benign prostatic hyperplasia; LUTS, Lower urinary tract symptoms; SEA, Similarity ensemble approach; PI, Positive ionizable.

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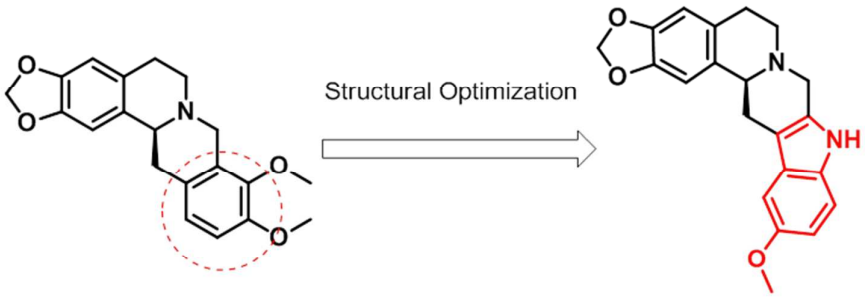
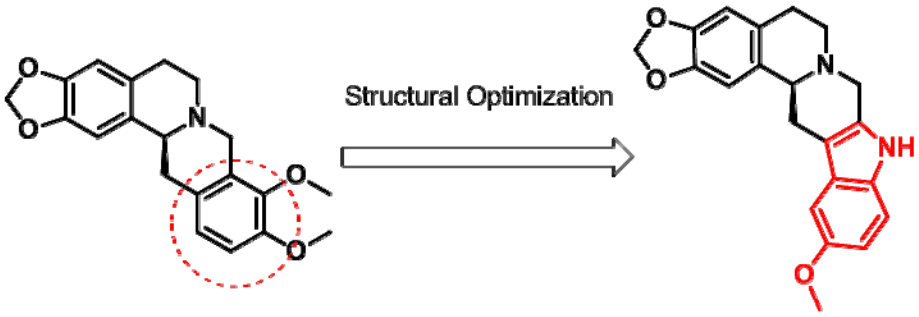
Table of Contents graphic



(*S*)-THB
α_{1A} IC_{50} = 5.4 μM
Poor bioavailability

(*S*)-27
α_{1A} IC_{50} = 12.8 ±2.2 nM
Selectivity of α_{1B}/α_{1A} > 780.6
Urethra IC_{50} = 0.5±0.3 nM
Selectivity of Urethra/Aorta > 2083
F = 60.9 %

Table of Contents graphic



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