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Original article

Discovery of naphthalimide conjugates as fluorescent probes for α_1-adrenoceptors

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ABSTRACT

α_1-Adrenoceptors (α_1-ARs), including at least three subtypes, α_{1A}, α_{1B} and α_{1D}, which play essential roles in G protein-coupled receptors (GPCRs), can convey multiple pivotal extracellular signals in varied tissues and organs. In this research, a series of napthalimide-based small-molecule fluorescent probes (**1a–1f**) for α_1-ARs, including two parts, a pharmacophore (quinazoline and phenylpiperazine) for α_1-AR recognition and a fluorophore (naphthalimide) for visualization, were designed and synthesized successfully. These compounds display excellent fluorescence property and high affinity to receptors, which were used successfully for *in vitro* visualization of α_1-adrenoceptors.

1. Introduction

As one of the essential members of G protein-coupled receptors (GPCRs), α_1-adrenoceptors (α_1-ARs), distributing in various cells, tissues and organs, can convey multiple pivotal extracellular signals. These receptors are categorized into at least three subtypes (α_{1A}, α_{1B}, and α_{1D}) based on their diversities on the biological structure, pharmacological properties, tissue distributions, and signaling pathways [1–3].

It has been confirmed that α_1-ARs are bound up with hypertension, benign prostatic hyperplasia (BPH), and other diseases [4–6]. In order to prevent and treat diseases connected with α_1-ARs anomalously expressed, numerous α_1-ARs antagonists have been discovered, such as quinazoline or phenylpiperazine derivatives [7]. Nevertheless, we still face many challenges, which become the stumbling obstacle to studying the biological and pharmacological characteristics of α_1-ARs, due to the lack of the three-dimensional crystal structures and tissue-selective antagonists.

Fortunately, with the speedy growth of fluorescence technology, small-molecule fluorescent probes have many merits such as high sensitivity and selectivity for the detection of proteins, enzymes, *etc.* [8–10]. Small-molecule fluorescent probes are normally constitutive of two portions, the pharmacophore moiety which could bind to the targets through the receptor–ligand interaction, and the fluorophore which is used to trace the targets by emitting fluorescence signals.

According to our previous work [11–15], a varied of fluorescent probes for α_1-ARs based on naphthalimide were well designed and synthesized (Fig. 1). In this instance, the quinazoline and phenylpiperazine moiety are chosen as the pharmacophore for their high affinity to α_1-ARs, and naphthalimide is selected as the fluorophore. With the help of the biological evaluation, we find that our probes showed off the high affinities to α_1-ARs and acceptable cell fluorescence imaging potential. It can be expected that these probes could be utilized as useful tools for nowadays high throughput screening of fluorescent competitive substrates in α_1-ARs.

2. Experimental

2.1. Materials and instruments

All materials were purchased from commercial companies (Aladdin and J&K Scientific) and used without further purification. Twice-distilled water was used throughout all experiments. Mass spectra were performed by the analytical and the mass spectrometry facilities in Drug Analysis Center at Shandong University on Agilent Technologies 1100 infinity HPLC, Applied Biosystems API4000. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker 300 MHz NMR spectrometer.

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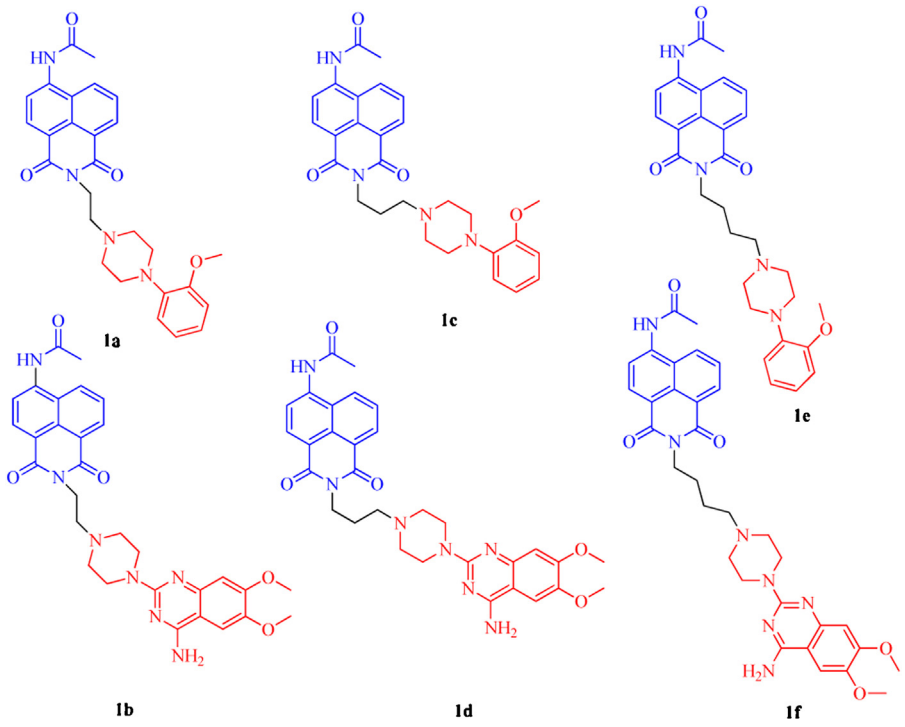


Fig. 1. Designed fluorescent probes based on quinazoline and phenylpiperazine for α_1-ARs.

2.2. Synthesis of the probes

The two synthetic routes of these probes were shown in Schemes 1 and 2. The synthesis of key intermediates **c2** and **d2** began with the CBZ protection of 3-bromopropan-1-amine, and then the nucleophilic substitution and deprotection reactions were conducted (Scheme 1). The other key intermediates **a2**, **e2**, **b2** and **f2** were synthesized through the Gabriel reactions (Scheme 2). All final compounds (**1a** to **1f**) were obtained from the acylation reactions of the key intermediates with a 1,8-naphthalic anhydride.

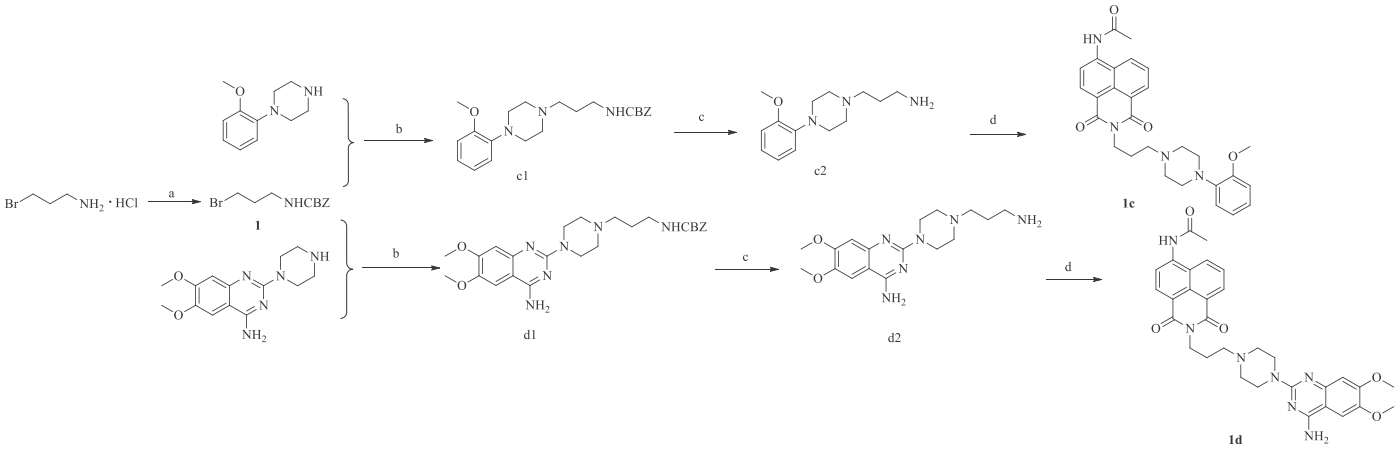
3. Results and discussion

3.1. Optical property

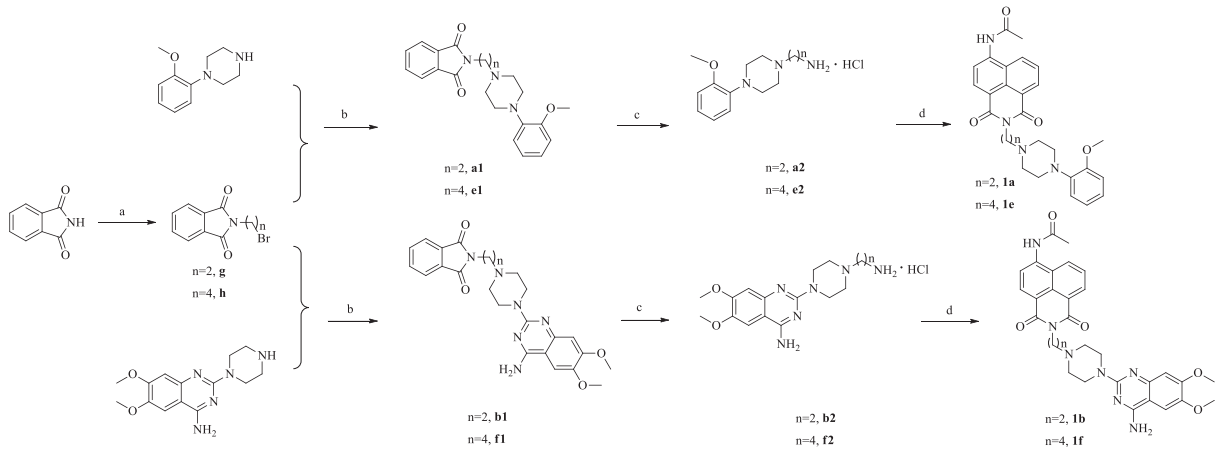
It is important that the rational fluorescent probes should possess the ideal optical property. After the optical properties of those compounds are measured, the consequences validated that most of the probes owned reasonable optical properties (Table 1). The optical properties were performed on a Thermo-Fisher Varioskan microplate reader by dissolving the probes in 50 mmol/L PBS, pH 7.4. As we can see in Table 1, the maximum absorption wavelength, the excitation wavelength and the fluorescence emission wavelength of all target compounds (**1a**–**1f**) are approximately 355 nm, 355 nm and 470 nm, respectively.

3.2. Affinity to α_1-ARs

Another pivotal characteristic for fluorescent probes is the affinity to the targets (α_1-ARs) besides the optical properties. For this reason, the radioligand binding assay for evaluating the affinity of these probes to three different adrenergic receptor subtypes (α_{1A}-, α_{1B}- and α_{1D}-AR) was carried out, in which phentolamine serves as the positive control and atropine served as



Scheme 1. Reagents and conditions: (a) Cbz-Cl, 3 mol/L NaOH, $CHCl_3$, overnight; (b) K_2CO_3, CH_3CN, 80 °C, 5 h, 92%; (c) H_2, Pd/C, 30 °C, overnight, 95%; (d) 4-acetamino-1,8-naphthalic anhydride, CH_3CH_2OH, 85 °C, 3 h, 39%–87%.



Scheme 2. Reagents and conditions: (a) K_2CO_3, DMF, 30 °C, overnight; (b) triethylamine, CH_3CN, 85 °C, 6 h, 60%–88%; (c) (i) hydrazine hydrate, EtOH, 85 °C, 3 h, (ii) HCl/EtOH; (d) 4-acetamino-1,8-naphthalic anhydride, EtOH, 85 °C, 3 h, 19%–38%.

Table 1
Optical properties of compounds **1a–1f**.

Compound	UV λ_{max} (nm)	λ_{ex} (nm)	λ_{em} (nm)
1a	355	355	470
1b	355	355	470
1c	355	355	470
1d	355	355	470
1e	355	355	470
1f	350	350	470

Table 2
The affinity of probes to α_1-ARs.

Compound	K_i[a] (nmol/L)			IC_{50} (nmol/L)		
	α_{1A}	α_{1B}	α_{1D}	α_{1A}	α_{1B}	α_{1D}
Phentolamine	0.8	7.7	11.1	1.4	19.2	18.0
Atropine	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]
1a	0.09	0.33	0.34	0.2	1.97	0.75
1b	8.1	10.7	81.4	17.5	64.0	183
1c	0.65	6.8	4.3	1.4	40.8	9.8
1d	4.3	6.4	32.5	9.4	38.4	73.1
1e	0.2	8.7	0.97	0.5	52.1	2.2
1f	39.1	25.5	148	85.2	153	333

[a] K_i was calculated from IC_{50} using the Cheng–Prusoff equation.
[b] Not available.

the negative control. According to the data in Table 2, all probes to α_1-ARs had a high affinity at the nanomolar level or even lower the affinity of phenylpiperazine derivatives to the α_1-ARs is much higher than that of quinazoline derivatives. However, some recent study indicated that phenylpiperazine derivatives may have high affinity to some subtypes of 5-HT besides α_1-ARs [16]. When the pharmacophore is either phenylpiperazine, the compound with a shorter linker shows the higher affinity for the targets $\left(K_{i_{1a}} < K_{i_{1c}} < K_{i_{1e}}\right)$, but for quinazoline pharmacophore, compound **1d** with the linker of three carbons shows highest affinity, which is

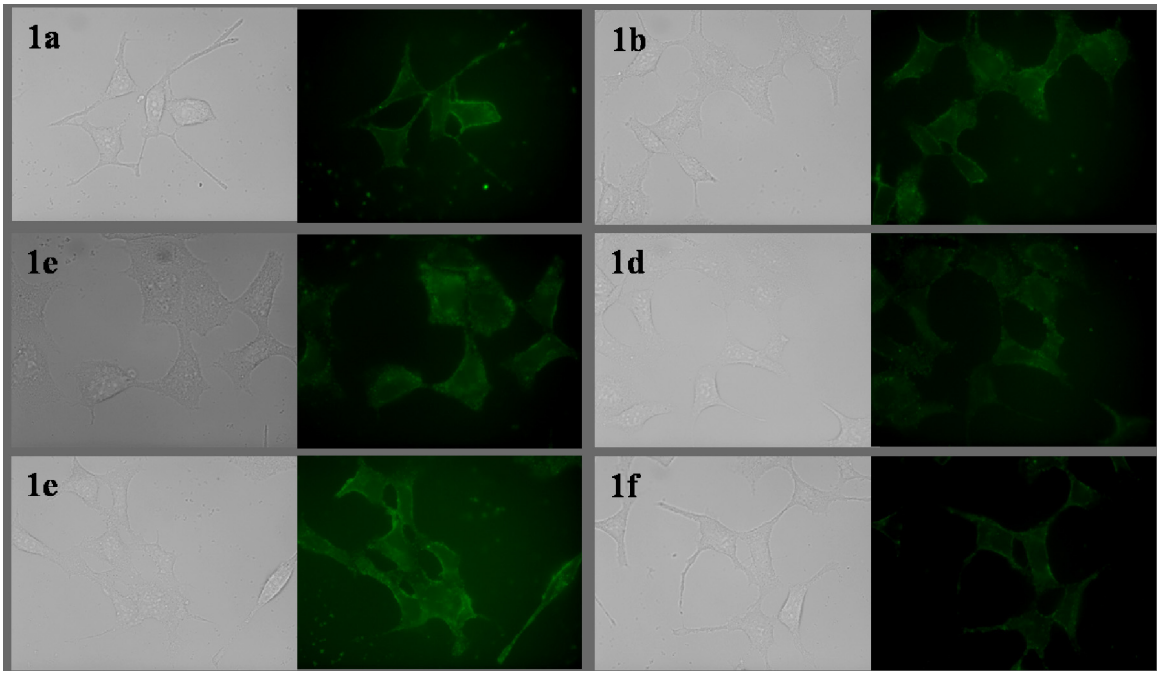


Fig. 2. The fluorescence images of probe **1a–1f** to HEK293A-α_{1A}-AR cells (left image is the bright field, right image is the fluorescence image), the concentration of each probe was 50 nmol/L.

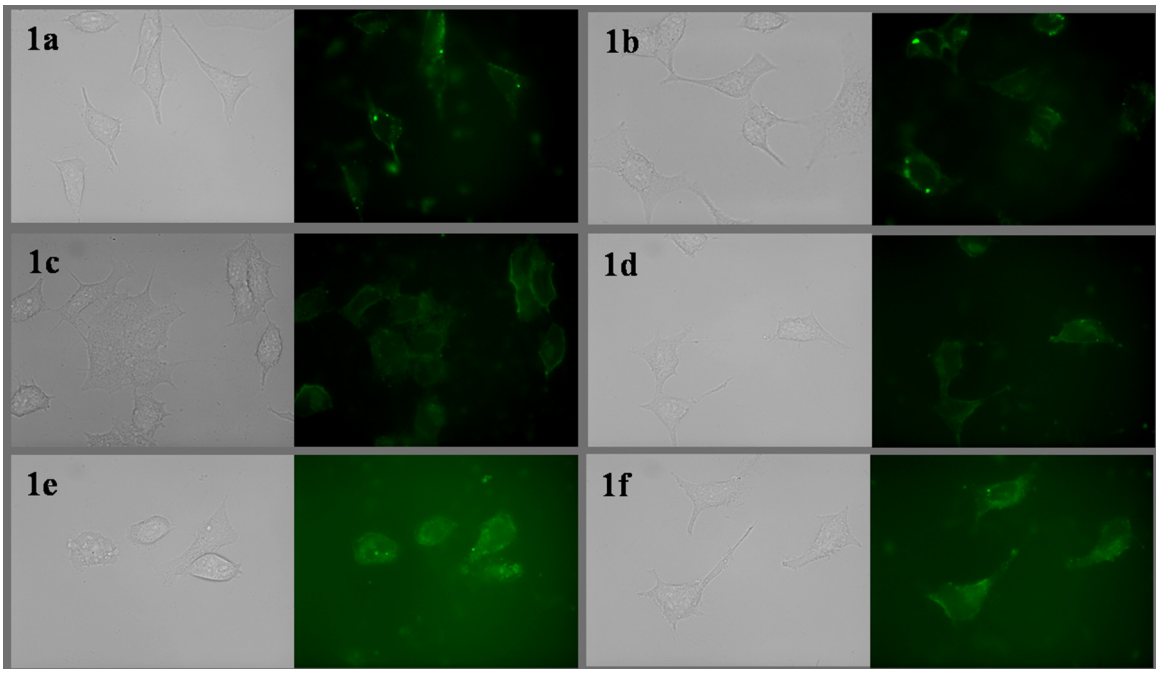


Fig. 3. The fluorescence images of probe **1a**–**1f** to HEK293A-α_{1D}-AR cells (Left image is the bright field, right image is the fluorescence image), the concentration of each probe was 50 nmol/L.

approximately 2-fold more potent than **1b**, and the affinity of compund **1f** with longer linker is lowest. Phenylpiperazine derivatives show higher affinity to α_{1A}-AR, while quinazoline derivatives show higher affinity to both α_{1A}-AR and α_{1B}-AR. Additionally, lengthening the linker may increase the affinity to α_{1D}-AR of phenylpiperazine derivatives.

3.3. Cell fluorescence imaging

These compounds showed no difference in fluorescence intensity when incubation with α_1-AR proteins, which indicated no "off-on" process happened. We incubated HEK293A cells transfected stably with α_{1A}-AR and α_{1D}-AR with the probes to gain the cell fluorescence imaging. Cells were incubated in DMEM medium complemented with 10% (v/v) fetal bovine serum under the condition of 5% CO_2 at 37 °C.

The cell lineages were reared in 35 mm glass bottom culture dishes (Mat Tek) at 37 °C for 24 h. Then cells were washed with DMEM medium and cultured in DMEM medium including the probes for 10 min at 37 °C. Fluorescence imaging was displayed on a Zeiss Axio Observer A1.

As a result, in Figs. 2 and 3, these cells highly expressing α_{1A}-AR and α_{1D}-AR could be highlighted by fluorescent probes at the nanomolar concentration, which would be an axis for exploiting new longer wavelength probes for α_1-adrenergic receptors.

4. Conclusion

In conclusion, we herein exploited a series of naphthalimide-based small-molecule fluorescent probes with high sensitivity, high affinity and reasonable optical properties for tracking the α_1-ARs in living cells. The interesting results laid a solid foundation for further structure-activity relationship optimization and activity screening and these fluorescent probes have been successfully used in visualization of α_1-AR in cell imaging. After several cycles of chemical optimization and activity screening, it is possible to pick up an ideal α_1-adrenergic receptor fluorescent probes that can be used to study pharmacological and biological characteristics of α_1-adrenergic receptor, accelerating the development of α_1-adrenergic receptor research. In addition, these probes can be synthesized easily from inexpensive starting materials. It is expected that these probes could be utilized as useful tools for nowadays high throughput screening as fluorescent competitive substrates in α_1-ARs.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2015.12.002.

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