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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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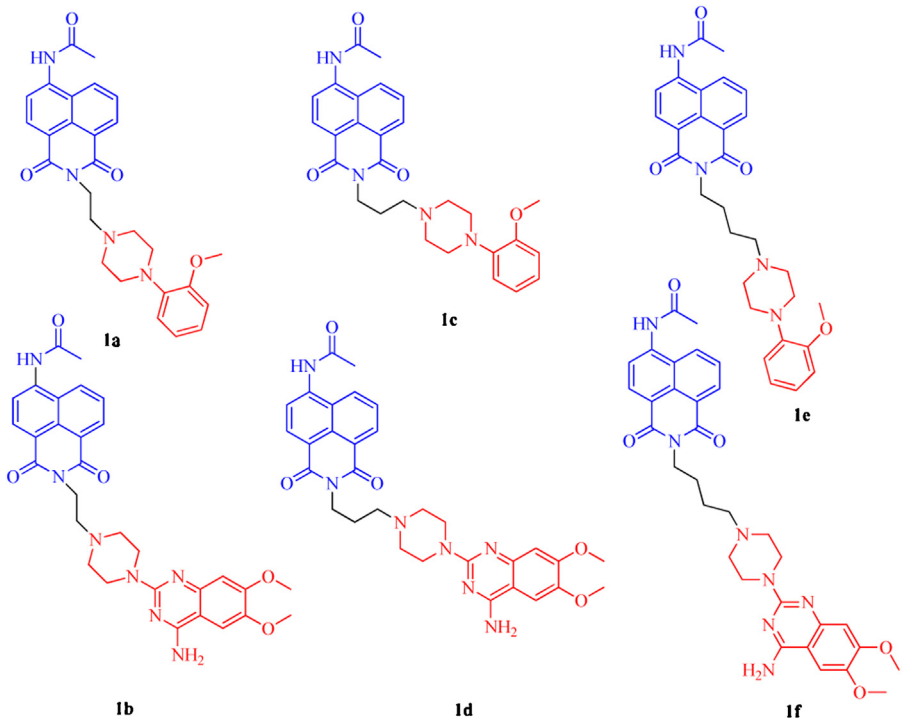


Fig. 1. Designed fluorescent probes based on quinazoline and phenylpiperazine for α_1-ARs.

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.

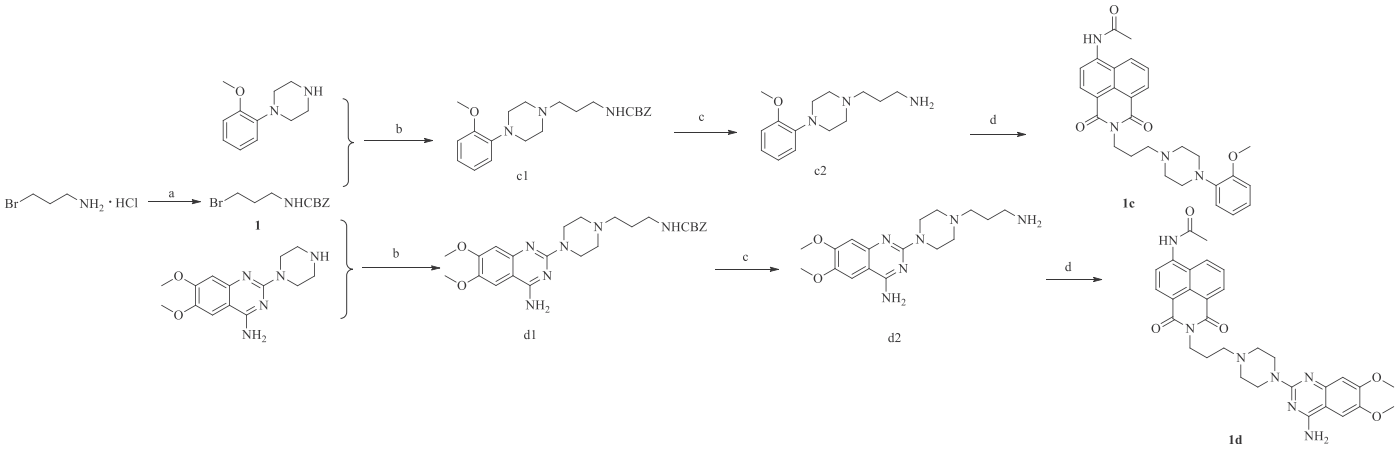
3. Results and discussion

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%.