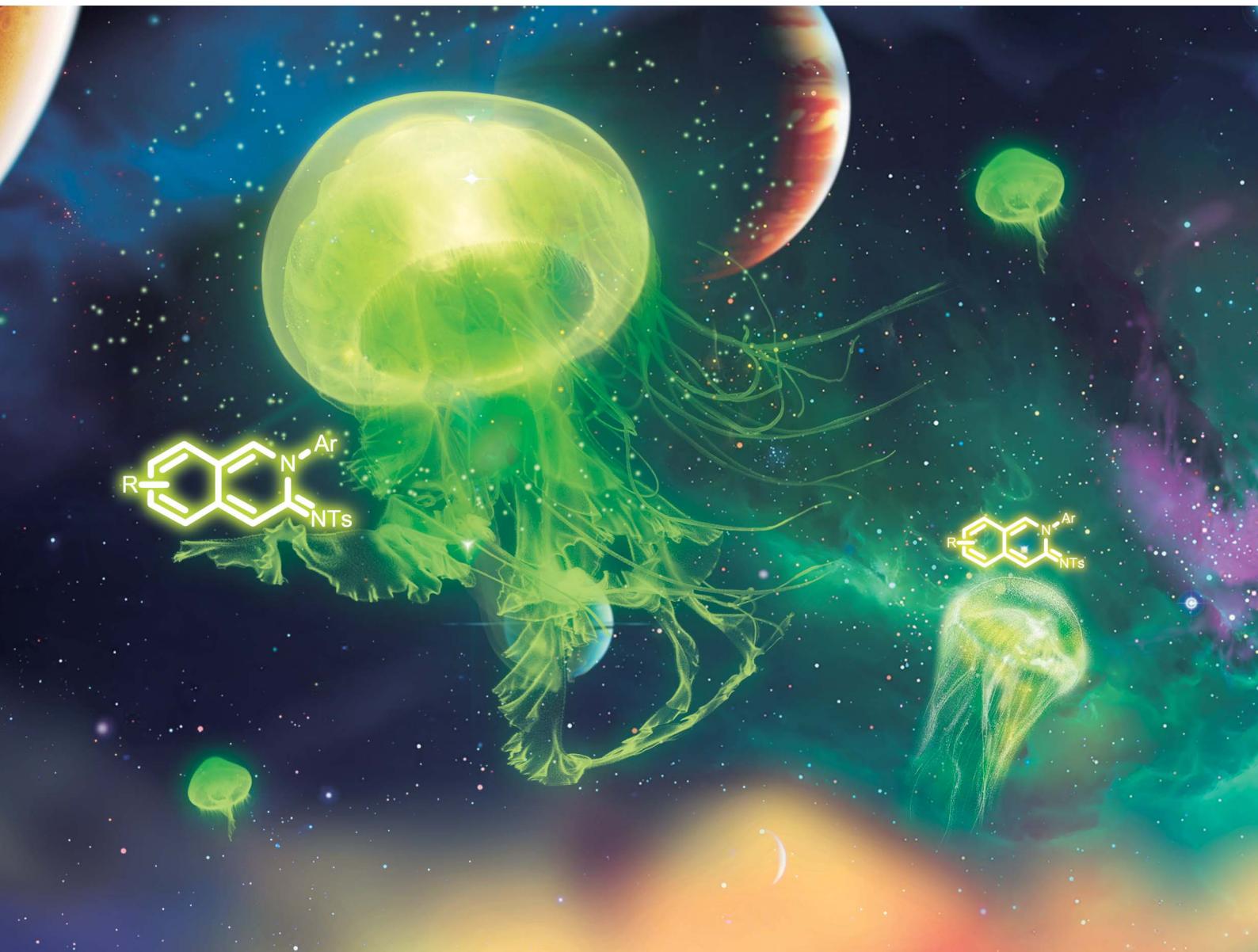


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Introduction

Small organic fluorophores have been used widely in biological science and drug discovery due to their easy handling, high sensitivity and minimal disruption to living systems.¹ Although a large variety of fluorescent small molecules have been developed, their core scaffolds are still limited to several types such as prodan, fluorescein, coumarin *etc.*,² most of which usually possess a π -conjugated system with an electron-donating and an electron-withdrawing group located at the opposite position of the fluorescent moiety (Fig. 1).³ On the other hand, the ideal fluorophores for living systems with good photophysical properties including a large Stokes shift, an applicable pH environment, good stability and low biological toxicity are in high demand. Consequently, the development of small organic fluorophores with a new framework and good photophysical properties is an appealing and challenging task.⁴

To develop a new fluorescent framework, we wondered whether it is possible to incorporate an electron-donating and an electron-withdrawing group at the adjacent position of a π -system. Considering the particularity of this structure, we tried

to choose π -system cyclic amidines as the core structure of novel fluorophores, not only due to their potent fluorescence properties,⁵ but also due to the electronic specificity of amidines (Fig. 1). However, how to incorporate an amidine into a π -system is a challenge, since the synthesis of such a scaffold is limited and difficult. Moreover, it usually needs a variety of cyclic amidines with structural diversity to study the fluorescence–structure relationship. Thus, it is necessary yet challenging to develop a simple and short synthetic route to access various cyclic amidines.

N-Sulfonyl-1,2,3-triazoles have become an important class of intermediates for accessing a wide variety of complex molecular scaffolds.⁶ Metal-bound imino carbenes, readily generated from *N*-sulfonyl 1,2,3-triazoles, have found wide application in many useful transformations, including cycloaddition, X–H insertion, alkyl migration, sigmatropic rearrangement and some other carbene induced reactions.^{6,7} In addition to imino carbenes, *N*-sulfonyl-1,2,3-triazoles could also form active ketenimine intermediates, which would readily undergo nucleophilic additions at C2.⁸ In particular, the ketenimines containing

^aGuangdong Key Laboratory of Chiral Molecular and Drug Discovery, School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510006, China. E-mail: limin65@mail.sysu.edu.cn; hongliang@sysu.edu.cn

^bKey Laboratory of Preclinical Study for New Drugs of Gansu Province, School of Basic Medical Sciences, Lanzhou University, Lanzhou 730000, China

^cGuangdong Key Lab of Nano-Micro Material Research, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen 518055, China

† Electronic supplementary information (ESI) available: Details of the experimental procedure, structural characterization and spectral data of all new compounds and crystallographic data (CIF). CCDC 1942349. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d0sc00798f

‡ These authors contributed equally to this work.

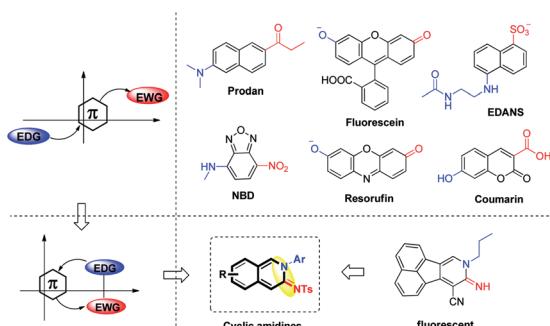
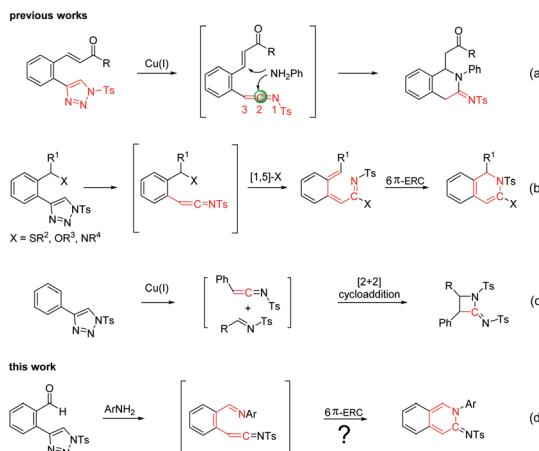


Fig. 1 Structural characteristics of traditional fluorophores and amidines.





Scheme 1 Related research and this work.

another electrophilic site would undergo double nucleophilic addition with amines to give cyclic amidines (Scheme 1a).⁹ Moreover, ketenimines could also in whole or in part participate in some cyclization reactions. For example, in the [1,5]-X sigmatropic shift/6π-electrocyclic ring closure (6π-ERC), the whole ketenimines (C=C=NTs) take part in the reaction to give dihydroisoquinolines (Scheme 1b),^{8f,10} while part of the ketenimines (C=C) in the [2 + 2] cycloadditions reacts to give amidines (Scheme 1c).¹¹ From these studies, we found that cyclic amidines could be constructed by the intramolecular conjugation of a nitrogen atom to the C2 of the ketenimine. Accordingly, we designed the intermediate **A** functionalized with an adjacent imine and ketenimine. The imine bond (C=NAr) was introduced for the formation of 6π-substrates suitable for ERC and might react with part of the ketenimines to give cyclic amidine products (Scheme 1d).

Results and discussion

With this reaction design in mind, we first investigated the reaction of *N*-sulfonyl triazoles **1a** with phenylamine **2a** as the model reaction in the presence of a catalyst at 120 °C in a sealed tube for 2 h (Table 1). A low yield of 17% was observed in the presence of a CuI catalyst (entry 1). We also examined some other catalysts including Cu(OAc)₂, CuTc, Rh₂(OAc)₄, Rh₂(Oct)₄ and Pd(OAc)₂, and they gave yields ranging from 12% to 54% (entries 2–6). Interestingly, when the reaction was conducted in the absence of any catalyst, the reaction could give a significant improvement of the yield to 83% (entry 7). Increasing the reaction time to 4 h could further improve the yield to 91% (entry 8), but further increasing the reaction time to 10 h reduced the yield to 86% (entry 9). After screening several solvents, CHCl₃ was found to be the optimum solvent for this reaction (entries 8–13).

Under optimal conditions, the scopes of the reaction were examined as shown in Scheme 2. We first examined the scope of benzenamines with various substituents at different positions. The reaction proceeded efficiently to afford the corresponding cyclic amidines **3aa**–**3ar** in good to excellent yields, though

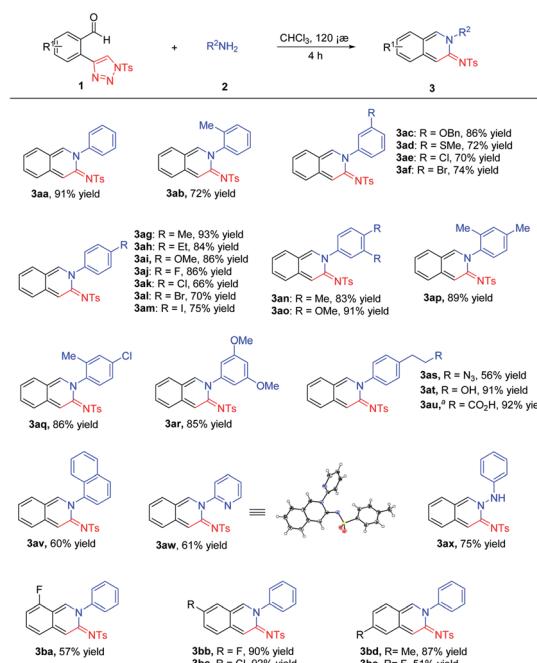
Table 1 Optimization of the reaction conditions^a

Entry	Cat.	Solvent	<i>t</i> (h)	Yield ^b (%)
1	CuI	CHCl ₃	2	17
2	Cu(OAc) ₂	CHCl ₃	2	26
3	CuTc	CHCl ₃	2	46
4	Rh ₂ (OAc) ₄	CHCl ₃	2	23
5	Rh ₂ (Oct) ₄	CHCl ₃	2	54
6	Pd(OAc) ₂	CHCl ₃	2	54
7	—	CHCl ₃	2	83
8	—	CHCl ₃	4	91
9	—	CHCl ₃	10	86
10	—	Toluene	4	40
11	—	DCE	4	77
12	—	THF	4	30
13	—	1,4-Dioxane	4	44

^a Conditions: **1a** (0.10 mmol, 1.0 equiv.), **2a** (0.12 mmol, 1.2 equiv.), the catalyst (0.005 mmol, 5 mol%), and solvent (2.0 mL) in a sealed tube.

^b Isolated yield.

higher reactivity was observed with electron-donating groups. Significantly, the benzenamines with functional groups such as azide, hydroxy and carboxyl groups were well tolerated to give **3as**–**3au**, which could be modified easily for the purpose of bioconjugate chemistry. Some other amines, including naphthyl amine, 2-aminopyridine and phenylhydrazine were also



Scheme 2 Scope of the reaction. Conditions: **1** (0.10 mmol, 1.0 equiv.), **2** (0.12 mmol, 1.2 equiv.) and the solvent (2.0 mL) in a sealed tube for 4 h at 120 °C. ^a1 mmol scale.

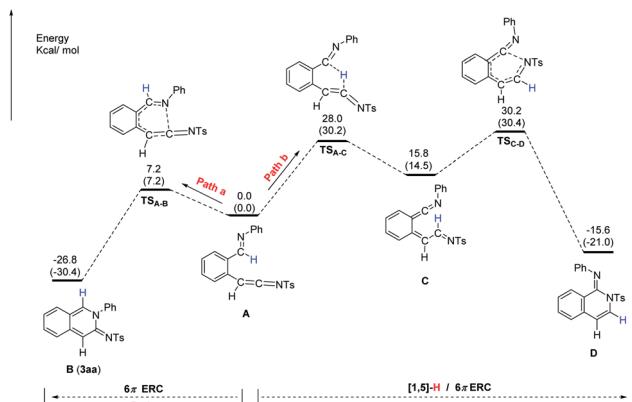


Fig. 2 DFT calculations for the transformation of ketenimine A.

suitable substrates for the reaction, leading to the desired products **3av–3ax** in moderate yields. The structure of **3aw** was determined by X-ray crystallography.¹² The scope of *N*-sulfonyl-1,2,3-triazoles was subsequently investigated. The reaction took place with moderate to excellent yields with substrates bearing different substituents **3ba–3be**.

Table 2 Spectral properties of fluorophores

Compound	λ_{max}^a (nm)	λ_{em}^a (nm)	ε^b ($\text{M}^{-1} \text{cm}^{-1}$)	Stokes shift (nm)	Φ^c (%)
Coumarin ^{13d}	386	448	36 700	62	70
Fluorescein ^{3bd}	490	512	93 000	22	95
3aa	433	535	2110	102	17
3ab	432	527	3066	95	23
3ac	434	542	3613	108	14
3ad	434	538	3790	104	13
3ae	436	543	3588	107	11
3af	437	545	3440	108	12
3ag	434	538	3558	104	16
3ah	434	541	3430	107	17
3ai	434	535	3312	101	19
3aj	435	536	2770	101	15
3ak	435	540	3209	105	14
3al	436	542	3786	106	14
3am	435	543	3418	108	12
3an	433	536	4364	103	19
3ao	434	537	3874	103	18
3ap	432	525	2804	93	22
3aq	434	530	3917	96	18
3ar	433	539	3578	106	14
3as	435	535	2915	100	18
3at	434	538	3250	104	18
3au	434	536	2935	102	17
3av	434	532	3170	98	18
3aw	437	552	3099	115	1
3ax	425	528	3865	103	16
3ba	440	542	2285	102	09
3bb	443	550	3771	107	09
3bc	443	547	2796	104	10
3bd	427	536	3853	109	17
3be	424	535	2458	111	12

^a Measured in CH_3CN at 200 μM . ^b Molar extinction coefficient. ^c Absolute fluorescence quantum yield determined with an integrating sphere system. ^d The structures of coumarin and fluorescein are shown in Fig. 1.

A good fluorophore for living systems has good properties including a remarkable Stokes shift, an applicable pH environment, high photostability and low biological toxicity. Keeping this in mind, we further examined these photophysical properties (Fig. 3). Based on the absorption and emission wavelengths in Table 2, all cyclic amidines exhibited remarkable Stokes shifts of ~ 100 nm, which could minimize self-absorption and provide better fluorescence imaging. We next studied the effect of pH on cyclic amidine **3aa**. The results showed that the pH value of the environment ranging from 2.8–11.4 had no effect on the emissive properties (Fig. 3c). In addition, amidine **3aa** showed excellent resistance to photo-bleaching with only 3.4% fluorescence lost after one hour at the wavelength of maximum excitation (Fig. 3d). Subsequently, the impact of different solvent environments was investigated. As shown in Fig. 3e, **3aa** worked well in aqueous environments, indicating that it could be used as a polarity probe. In addition, the emission intensity could be maintained in aqueous solution without loss after 24 h (see the ESI†). Moreover, cytotoxicity was another important factor for its application in living systems. We evaluated the cytotoxicity of **3aa** by using CCK-8 assays for HeLa cells, and it exhibited no significant cytotoxicity at concentrations of up to 100 μ M (Fig. 3f). These results demonstrated that the novel amidine fluorophore has good properties of a large Stokes shift, pH insensitivity, low cytotoxicity and good photostability, which has great potential for biological imaging.

Ligand-based probes¹⁵ have received extensive attention and have extensive applications due to their high selectivity and

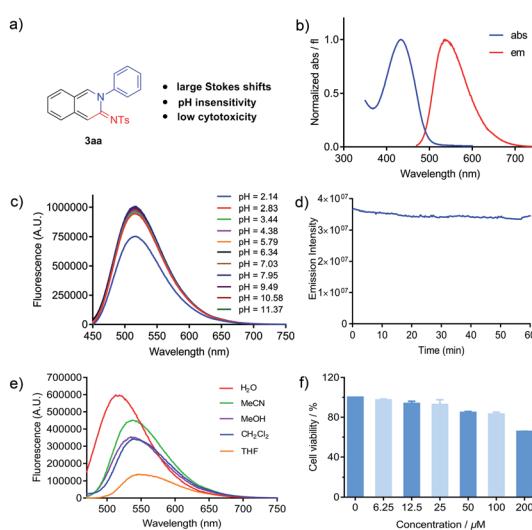


Fig. 3 (a) The photophysical properties was examined using **3aa** as a representative. (b) Normalized absorbance (abs) and emission (em) spectra of **3aa** in CH_3CN . (c) **3aa** was prepared as a 1 mM stock solution in DMSO and then diluted to a concentration of 10 μM with phosphate buffers of different pH values prepared in advance. Emission spectra were measured at 433 nm excitation. (d) Test of the photostability of **3aa** ($\lambda_{\text{ex}} = 433$ nm) in CH_3CN at 100.0 μM . (e) Emission spectra of **3aa** in various solvents at 20 μM . (f) Cell viability (%) was measured by using CCK-8 assays, treated in the presence of 6.25–200 μM of **3aa** using WT HeLa cells for 24 h at 37 °C.

affinity in the visualization of receptor–ligand interactions and drug evaluation. In order to evaluate the potential of cyclic amidines for cell imaging, we synthesized a fluorescent probe **4** by conjugating cyclic amidine **3au** with the hemokinin-1 (HK-1) peptide (TGKASQFFGLM-NH₂), which was highly selective to the NK-1 receptor (Fig. 4a).¹⁶ The neurokinin-1 (NK-1) receptor, as a member of the G-protein-coupled receptor (GPCR) family, is located at the cell membrane. If a fluorescent ligand binds to the NK-1 receptor, it would give fluorescence signals at the cell membrane. To test whether fluorescent probe **4** could bind to the NK-1 receptor, WT 22RV1 and NK-1-overexpressing 22RV1 cells were treated with **4** respectively. As Fig. 4 shows, green fluorescence could only be observed in the cell membrane of NK-1-overexpressing 22RV1, not in the cell membrane of WT 22RV1. Furthermore, the fluorescence could be blocked with

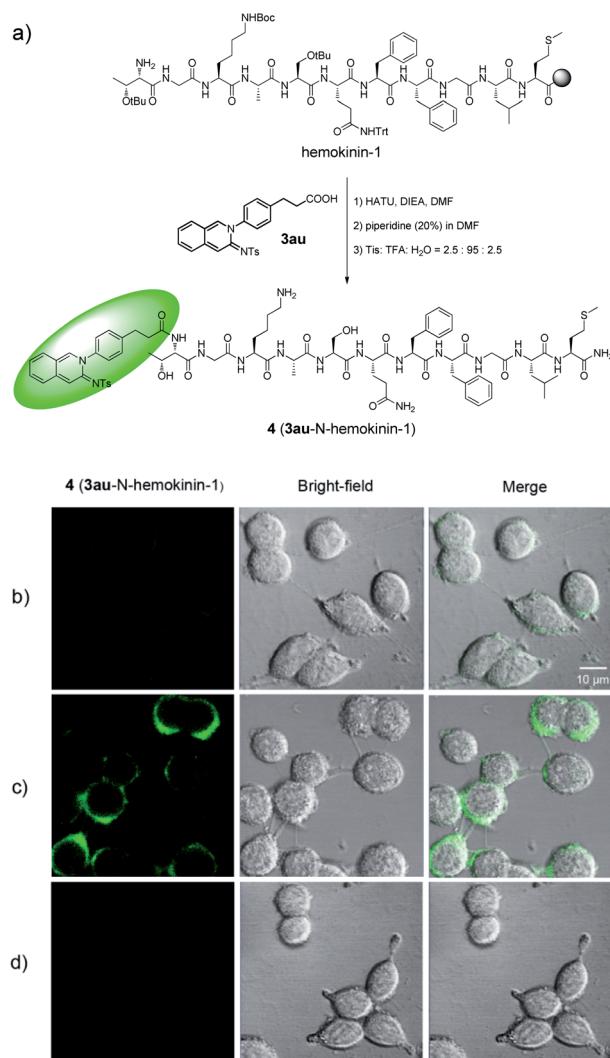


Fig. 4 (a) Synthesis and structure of fluorescent ligand **4** (3au-N-hemokinin-1). (b) WT 22RV1 cells and fluorescence images of 22RV1 cells treated with **4** (1 μM) at 37 °C for 30 min. (c) NK-1-overexpressing 22RV1 cells and fluorescence images of 22RV1 cells treated with **4** (1 μM) at 37 °C for 30 min. (d) NK-1-overexpressed 22RV1 cells treated with the NK1R inhibitor aprepitant (1 μM) at 37 °C for 30 min and then incubated with fluorescent ligand **4** (1 μM) for 30 min at 37 °C. Excitation wavelength 488 nm and emission wavelength 520 nm.



aprepitant, a selective NK-1 receptor antagonist, indicating that the fluorescent ligand **4** was specifically bound to the NK-1 receptor. These preliminary results demonstrated that amidine fluorophores could be used as potential bioprobes.

Conclusions

In summary, we have developed a general and efficient 6π -electrocyclic ring closure of ketenimine and imine starting from *N*-sulfonyl triazoles and arylamines. The method provides expeditious access to a variety of 10π -electron cyclic amidines in moderate to excellent yields. Through a fluorescence-structure relationship study, we found that cyclic amidine fluorophores have the advantages of large Stokes shifts, pH insensitivity, low cytotoxicity and higher photostability. Furthermore, they can be used efficiently in developing new fluorescent probes for imaging in living systems.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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