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Development of a chalcone–triazine fusion library: combination of a fluorophore and biophore

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ABSTRACT

A chalcone–triazine fusion library has been developed. We designed fused chalcone–triazine structure by combining a fluorophore and a biophore for imaging and biological probe development. Through solid support chemistry, 80 compounds with amine building blocks were synthesized. Spectroscopic studies and cell image testing of the compounds showed their potential to be used as probes for bio-imaging.

The chalcone[1] moiety is a well-known biologically active motif, however, it has not been explored in detail as a fluorescent probe. We previously reported the first construction of a fluorescent chalcone library and its application in stem cell probe development, which successfully demonstrated the potential of chalcone as a bio-imaging probe.[2] With a significant progress in the development of this chalcone bio-imaging probe, we explored the design of a chalcone library based on combination of a biophore and fluorophore (Fig. 1).

1,3,5-Triazine (*s*-triazine) derivatives are applied widely as biophores, due to their structural similarity with naturally abundant heteroaromatic components (e.g., the bases in nucleosides and nucleotides). Among interest devoted to triazine derivatives, we have reported a variety of their biological activities, including biofuel production, HSP90 interruption, insulin mimetics, neuroblastoma inhibition, antimicrobial, tyrosinase inhibition, and microtubule inhibition.[3] The 1,3,5-triazine scaffold is also of particular interest in combinatorial chemistry approaches because of its synthetic accessibility;[4] using 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride), three chlorides can be replaced by amine, alcohol, sulfur, and Grignard reagents.[5] The convenience of manipulating the triazine scaffold and the diverse biological properties inspired us to design a fusion library by combining a fluorophore and biophore utilizing chalcone and triazines, respectively (Fig. 1). To facilitate synthesis on a solid support, we introduced a 2-hydroxyethyl(methyl)amino group on the chalcone side, and two amino groups on each side of the chalcone scaffold were retained for their strong fluorescence properties.[2] This novel fusion library was named **CT** (Chalcone Triazine), and we expect to discover useful imaging and biological probes from it.

The overall synthetic procedure for the **CT** library is shown in Scheme 1. The preparation of intermediate **4** was approached through two different pathways, routes A and B. In route A, nitro reduction and coupling of cyanuric chloride followed after loading nitrochalcone compound **1** on to a solid support resin. In route B,[6] two steps of the solid phase reaction were removed and the triazine-coupled chalcone was directly loaded onto 2-chlorotrityl resin. Both routes A and B were practical for the synthesis of the **CT** library, but we elected to follow route A to save on the workload involved in the extra work-up procedure. However, route B remains an optional procedure for individual compound scale-up synthesis.

The key step in the solid supported **CT** library generation is amine substitution. Synthetic methods for 1,3,5-triazine substitu-

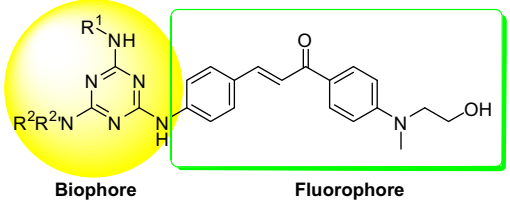

Figure 1. The design of the **CT** library.

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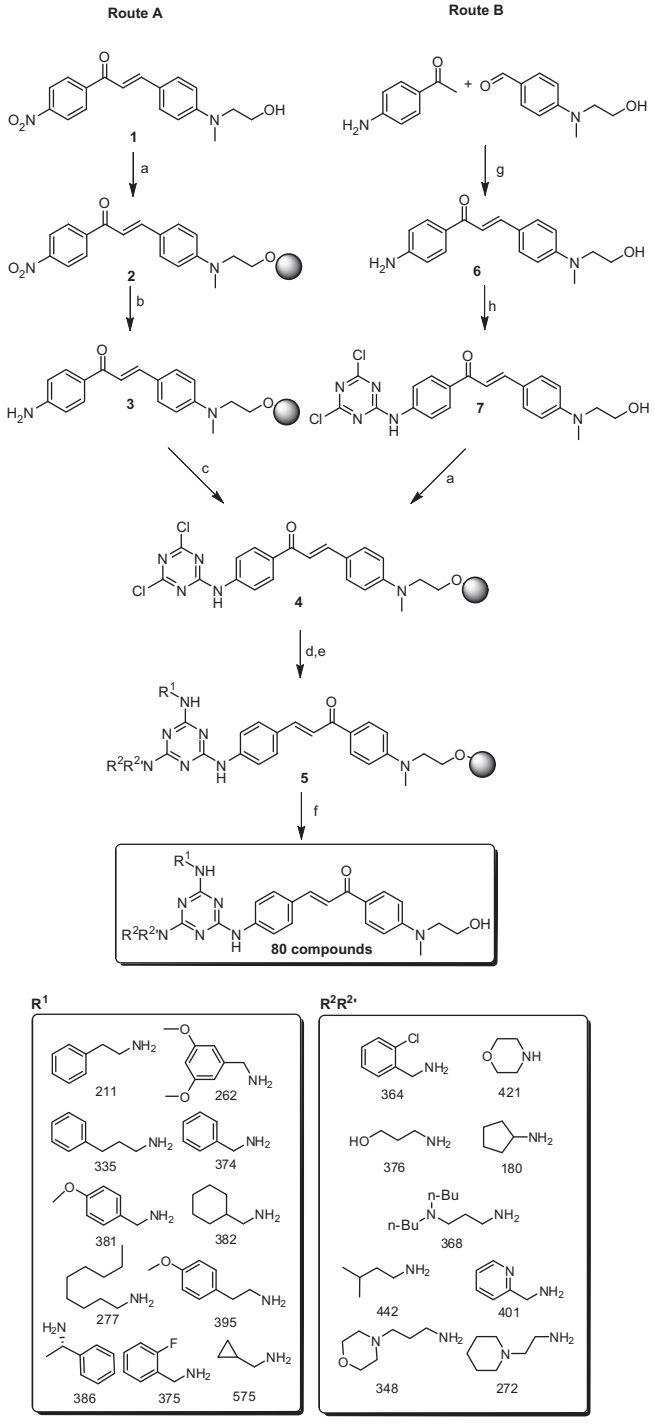

Scheme 1. Synthesis of the **CT** library. Reagents and conditions: (a) 2-ClTr chloride resin, pyridine, CH_2Cl_2, 5 h, RT; (b) $SnCl_2$, 6% H_2O in DMF, rt, overnight; (c) 3 equiv cyanuric chloride, DIEA, THF/CH_2Cl_2, 0 °C, 2 h; (d) 3 equiv R^1-NH_2 in THF at rt, 1 h; (e) R^2 $R^{2\prime}$NH in DMF at 60 °C, 3 h; (f) 5% TFA in CH_2Cl_2; (g) 4 M NaOH, EtOH, reflux, overnight; and (h) 1.2 equiv cyanuric chloride, DIEA, THF 0 °C, 1 h. The R^1, $R^2R^{2\prime}$ amine numbers originate from in-house building block code numbers.

tion have been well studied, mostly based on derivatization of cyanuric chloride.[7] The decreasing reactivity of cyanuric chloride with increasing numbers of substituents is an advantage for controlling nucleophilic substitution of each chloride. The first reactive chloride of cyanuric chloride was successfully substituted by the aniline of resin-loaded chalcone (intermediate **3**) at 0 °C in tetrahydrofuran (THF)/dichloromethane (CH_2Cl_2) co-solvent, which is a slight modification from the reported conditions.[3e] Incorporation of R^1-NH_2 and $R^2R^{2\prime}$NH (1° or 2° amine) was controlled by the temperature and solvent. Conjugation of the R^1 amine successfully occurred at room temperature in THF, and even with 3–5 equiv of the R^1 amine, no further substitution of chloride was observed. In total, 11 commercially available primary amines were loaded as the R^1 substituents. The second substitution was carried out at a higher temperature (60 °C) with a more polar solvent (DMF) and another nine different $R^2R^{2\prime}$ amines were incorporated.

Table 1
Spectroscopic summary of representative compounds

CT {R^1, $R^2R^{2\prime}$}	MW	λ_{abs} (nm)	λ_{em}[a] (nm)	ε (M^{-1} cm^{-1})	Φ[b]
{335, 180}	571.8	427	545	14.333	0.37
{382, 180}	591.8	428	544	15.000	0.40
{211, 376}	567.7	428	548	17.666	0.38
{211, 421}	579.7	428	547	18.666	0.36
{374, 376}	553.7	428	545	23.333	0.38
{374, 421}	565.7	428	547	15.666	0.36

MW: molecular weight, λ_{abs}: absorption maxima, ε: molar extinction coefficient, λ_{em}: fluorescence emission maxima, Φ: quantum yield.
[a] Excited at 430 nm.
[b] Quantum yield was calculated using the known reference compound, dansyl amide at 10 μM in DMSO.

The products were then cleaved from the resin under mild acidic conditions. We used 1%, 2%, and 5% trifluoroacetic acid (TFA) in CH_2Cl_2 and found that 5% TFA in CH_2Cl_2 afforded the product free from the polymer resin within 10 min. The cleaved products were simply filtered through a short silica plug to give 99 library compounds. By removing 19 impure compounds, 80 relatively pure compounds (see Supplementary data, Tables S1 and S2) were collected as the final **CT** library (average purity was 85% at 430 nm by LC/MS).

All the compounds showed similar absorption and emission profiles (i.e., λ_{abs}: 424–429 nm; λ_{em}: 549–553 nm) with an average quantum yield of 0.35. Representative compounds from the library and their absorption and emission spectra are shown in Figure 2 and Table 1. The Stokes shifts of the **CT** compounds are around 125 nm, which are larger than those of typical fluorescent dyes, such as BODIPY, rhodamine, and fluorescein, which have Stokes shift ranging from 15 to 60 nm. The full width at half maximum peak height absorptions and emissions of the **CT** compounds ranged from 60 to 80 nm.

To gain preliminary proof of our concept of designing a fusion library, we selected two **CT** compounds (**CT** {335, 180} and **CT** {382, 180}) and tested them in normal and cancer liver cells (Chang and HepG2). The compounds were incubated for one hour, and the images were acquired using the fluorescein isothiocyanate (FITC) filter set (ex: 450–490 nm, em: 515 nm).

The two **CT** compounds clearly stained cells in the cytosol with low background, implying that they are cell permeable and have the potential to be useful tools for bio-imaging studies (Fig. 3). The unique fluorescent properties of the **CT**s would facilitate investigation of cellular mechanism and target identification.

In summary, we have designed and synthesized a novel **CT** fusion library, utilizing the advantages of chalcone and 1,3,5-triazine as a fluorophore and biophore, respectively. The **CT** library contains 80 compounds with structures of two-dimensional amine building blocks, and their spectroscopic properties showed the potential for study as bio-imaging probes. Furthermore, the **CT** compounds can penetrate cell membranes and clearly stain cells in the cytosol, indicating that they should be useful imaging tools for studying cellular function. Their biological applications will be reported in due course.