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Synthesis and cytotoxicity study of novel 3-(triazolyl)coumarins based fluorescent scaffolds

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ABSTRACT

Recently a choice of fluorescent bioimaging probes have been developed as medical diagnostic tools. Herein, we have introduced a series of coumarin-based target specific probes for cancer theranostic application which play a dual role in the field of both diagnosis and therapy. A fluorogenic version of 1,3-dipolar cycloaddition between azides and alkynes (DBCO) has been introduced to develop the triazolylcoumarin based fluorescent scaffolds. These scaffolds were screened for their anticancer activity against breast cancer (MCF7) and human epitheloid cervix carcinoma (HeLa) cell line. It was established that triazolylcoumarins (**5c** and **5d**) are having electronegative substitution in the benzene ring displayed most effective anticancer profile in both the cell lines. Compounds **5a** and **5d** exhibited maximum quantum yield and strong cellular uptake in the MCF-7 cell line.

The term 'theranostic' signifies a specific combination of the diagnostic and therapeutic capabilities into a single agent (called 'companion diagnostic'). This includes the use of diagnostic tests to identify a particular disease, select a treatment regimen for it and monitor the patient compliance. The diagnostic tests are used to identify a biomarker which allows the use of the new drug.[1] Theranostic promises significantly in the area of personalized medicine which customizes healthcare to individual patients and for better patient compliance. The efficient process of theranostic development is quite complex as it requires a stepwise phasing from development to remuneration. All the stages of the system should be harmonized to process simultaneous availability and accessibility of the drugs. In spite of this, it is essential to simplify the rules and regulations to create a better modification of theranostic towards the robustness of invention.[2] The execution of theranostic is challenging since it possess considerable economic disputes for industrial organisations as well as the process and timelines for drug and test development are different from one another.

'Click chemistry' is a selective, quantitative, cost effective and pH-insensitive green approach,[3,4] and hence this approach is useful for the synthesis of a variety of bio conjugates including peptides,[5] proteins,[6] polysaccharides,[7] and even entire viruses[8] and cells.[9] However, the conventional 'Click Chemistry' requires Cu(I) catalyst for 1,3-dipolar cycloaddition between azides and terminal alkynes which is toxic to most organisms[10] and thus, prevents its use in many biological systems. The novel Copper-free Click Chemistry is based on the reaction of a cyclooctyne (DBCO) moiety with an azide-labelled reaction partner, known as strain-promoted alkyne azide cycloaddition (SPAAC).[11] SPAAC has several benefits over the conventional click technique as it is extremely fast at room temperature, biocompatible, bioorthogonal along with highly specific and stable in nature.[12]

3-Azido coumarin, a profluorophore, reacts with terminal alkynes to generate highly fluorescent triazole compounds which are very useful for biological applications, especially for in vivo labelling.[13] Furthermore, substitutions at the 3- and 7-positions of coumarin dyes are known to have a strong impact on their fluorescence properties.[14] Coumarin derivatives have also been developed as an anti-tumour agents and their metabolite 7-hydroxycoumarin was tested in several human tumour cell lines.[15] However, the biological actions of coumarins are greatly enhanced by attaching the triazole rings with these moieties. These unique templates have been associated with anti-viral, anti-fungal, anti-tumour, anti-bacterial, anti-inflammatory and also CNS activities.[16] These scaffolds were also reported as anti-microbial, anti-inflammatory, anti-tubercular, anti-HIV, anti-malarial, cardiovascular and diuretic agents.[17–20] In light of these research works going on, we have successfully developed a series of azido coumarins which on further treatment with DBCO provide a class of fluorescent triazoles for theranostic application.

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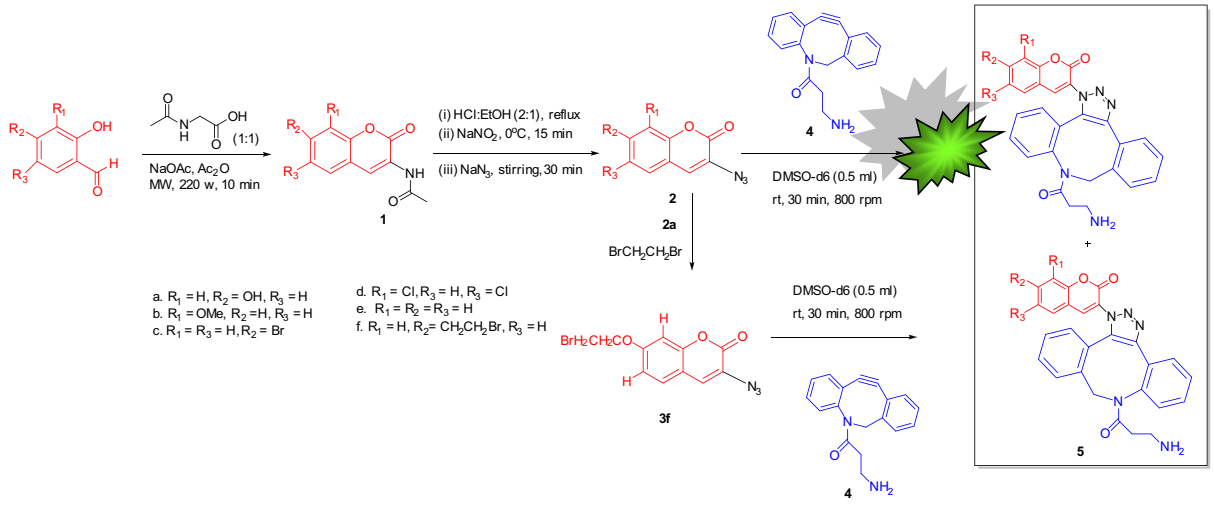
Our approach was to develop a new class of coumarin-based fluorogenic probes having cytotoxicity against various cancer cell lines by the application of 1,3-dipolar cycloaddition of azides and alkynes. Initially, 3-acetamido coumarin analogues (**1a–e**) were synthesized by using a choice of substituted salicylaldehyde and *N*-acetyl glycine in presence of acetic anhydride under microwave conditions. Microwave is an efficient tools for the construction of carbon–carbon building blocks with high reaction rate and yield.[21] These coumarins were then refluxed with HCl/Ethanol (1:1) mixture for 2 h and further treated with sodium nitrite followed by sodium azide to get the desired 3-azido coumarin derivatives (**2a–e**) (Scheme 1).[22] Subsequently, Compound **2a** was treated with dibromoethane for the formation of desired bromoalkoxyazidocoumarin (**3f**). Briefly, compound **2a** was dissolved in acetone, K_2CO_3 (1:10) was added to the reaction mixture and the resulting solution was refluxed for 30 min at 80 °C. Thereafter, dibromoethane (1:12) was added to it and the mixture was refluxed for 5 h. Progress of the reaction was monitored by TLC in hexane/ethyl acetate (3:1) solvent system. After complete conversion of the starting materials, the solution was filtered and the filtrate was evaporated to dryness to get the desired product (**3f**). The structure of compound **3f** was confirmed by ^{1}H NMR spectroscopy and ESI-MS. The characteristic peaks of two CH_2 exhibited at σ 3.65 and 4.35 as two distinct triplets. Subsequently, DBCO (**4**) was treated with 3-azidocoumarin analogues (**2a–e**, **3f**) in 0.5 ml of DMSO-d_6 at ambient temperature for 30 min. The progress of the reaction was monitored by TLC. The reaction resulted in the formation of two distinct regioisomers (1,4-triazole and 1,5-triazole regioisomer), which is a well known phenomenon for copper free click reaction. But in all the cases we could not isolate the regioisomer even after HPLC purification.[23] The structure of triazolylcoumarins (**5a**) was further confirmed by ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. The characteristic peaks of three CH_2 and one NH_2 were observed in the range of σ 1.86–1.88, 2.62, 3.18 and 5.04–5.07 ppm respectively. In the ^{13}C NMR spectra characteristic peaks of tertiary amide and ketone was observed at σ 169.8 and 159.0 ppm respectively. Likewise, three CH_2 peaks were appeared in the range of σ 33–54 ppm. The quantitative formation of all the 3-(triazolyl)coumarins derivatives (**5a–f**) were supported by NMR and Mass spectroscopy.[24]

The fluorescence spectra of compounds **5a–f** were determined at concentrations of 3×10^{-6} mol L^{-1} in water. Emission spectra of compounds **5a–f** are presented in Figure 1. The fluorescence excitation wavelengths (λ_{ex}/nm) at 315, 296, 315, 325, 325 and



Scheme 1. Plausible pathway for the synthesis of 3-(triazolyl)coumarin (**5a–f**).

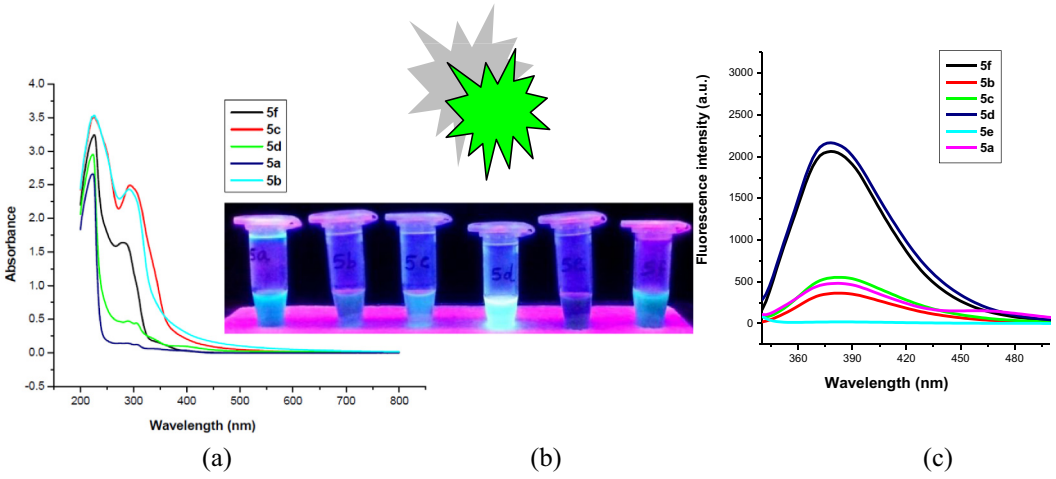


Figure 1. UV & Fluorescence emission spectra of 3-(triazolyl)coumarin (**5a–f**) in water.