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DESIGN, SYNTHESIS AND CHARACTERIZATION OF FLUORESCENT PROBES BASED ON SCHIFF BASES

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Abstract:

Schiff bases of fluorescein were synthesized from fluorescein hydrazide using different aromatic aldehydes. These compounds show bright fluorescence different from that of starting material, fluorescein. The compounds were characterised by spectroscopic methods. Absorption and fluorescence spectra of the compounds were also recorded.

Keywords: *Fluorescein, Schiff bases, Fluorescein hydrazide, fluorescent probes, fluorescence*

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INTRODUCTION:

Fluorescence techniques have proved to be significant in the advancement of chemical and molecular biology and have achieved rapid development [1]. Fluorescent detection has the advantages of being safe, sensitive, selective, reproducible and applicable in high-throughput screening applications [2]. The applications of fluorescent probes also extend to fields of cellular biology to determine the concentrations of ions in cells, protein-protein interactions, DNA sequencing and immunoassay among many other applications [3]. Fluorescent probes are also utilized greatly *in vivo* labelling for various applications in cell biology has been an emerging area of research in the recent few years.

Fluorescein possesses a rigid coplanar tricyclic structure, a high molar absorptivity (88,000 $cm^{-1}M^{-1}$ at pH 9) and high fluorescence quantum yield (0.92 at pH> 8) [4]. Fluorescein provides for a modifiable molecule that can be derived into adaptable fluorescent reagents comprising of various reactive functional groups [5]. Therefore, fluorescein probes have emerged to be one of the most noteworthy and useful classes of fluorescent probes. Even though there are wide applications of fluorescein, there is always a requirement for the synthesis of new analogues to overcome other shortcomings of fluorescein such as photobleaching, pH dependence and instability of other derivatives [6]. Moreover, fluorescein dyes available commercially are often very expensive and their protocols for labeling with bioactive molecules frequently involve further optimization [7]. Therefore, new scale-up synthesis routes and new fluorescein dye syntheses are of a current research interest. Special investigation has been carried out to introduce special terminal functional groups that can be modified for coupling with biomolecules [8].

Fluorescein was first synthesized by von Bayer in 1871 with resorcinol and phthalic anhydride via Friedel-Crafts acylation/cyclodehydration [9]. Fluorescein itself does not have a coupling group, and thus, focus has been the syntheses of its derivatives with diverse linkers at different positions [10]. This work presents the synthesis of fluorescent probes using fluorescein after conversion to a non fluorescent fluorescein hydrazide that provides a modifiable functional group for generation of new probes.

MATERIALS AND METHODS

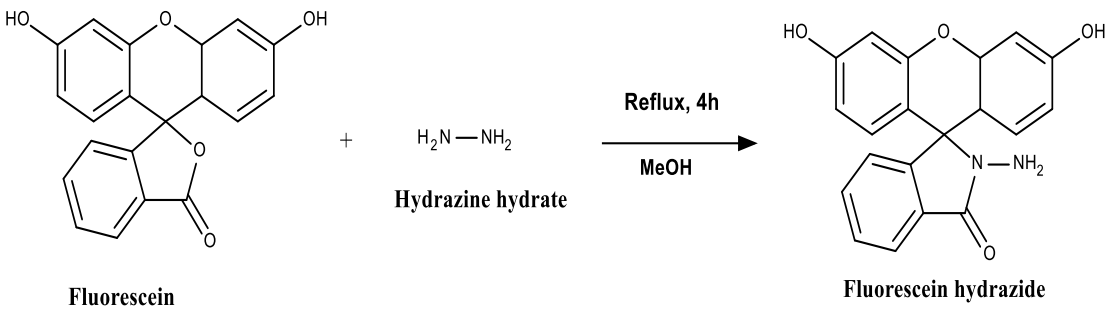
General

All melting points (m.p.) were determined in open capillaries on a Veego apparatus and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck, Darmstadt Germany). Spectroscopic data were recorded with the following instruments: Fluorescence emission spectrawere recorded on a JASCO FP-8000 Series Fluorescence Spectrofluorometer (Tokyo, Japan). UV-Vis spectra were obtained on a Perkin Elmer Lambda 25 UV-Vis spectrophotometer (Waltham, MA). FT-IR spectra were recorded on a Perkin Elmer RX1 instrument (Waltham, MA). Mass spectra (MS) were recorded on a Shimadzu 8040LC-MS/MS system (Japan). HPLC analysis was performed on Agilent 1220 Infinity system (USA). An isocratic mobile phase system consisting of (A) Acetonitrileand (B) 0.05 % orthophosphoric acid (80:20, v/v) was used with a C18 Kromasil column (25 cm × 4.6 mm, 5 µm particle size, 100 Å pore size) and fluorescence detection at 390 nm as excitation wavelength.

Syntheses

Synthesis of Fluorescein hydrazide

Fluorescein was synthesized from resorcinol and phthalic anhydride using reported methods in literature [11]. Fluorescein was treated with hydrazine hydrate to give fluorescein hydrazide (Scheme 1).

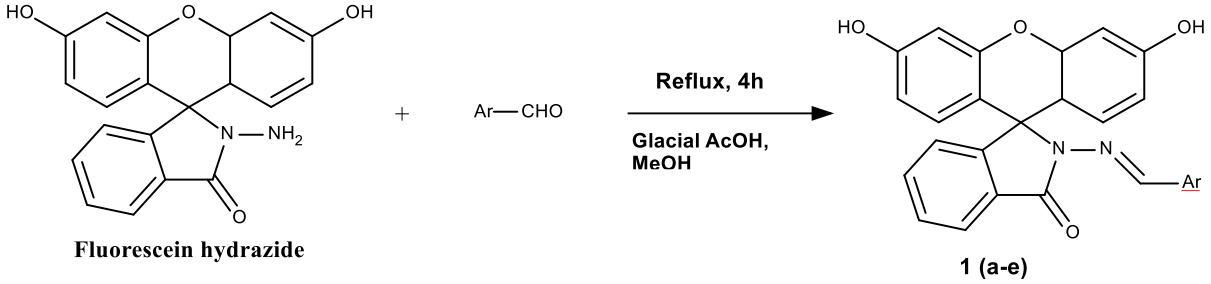


Scheme 1. Synthesis of Fluorescein Hydrazide

Fluorescein (3.32 g, 0.01 mol) was dissolved in 15 mL of methanol, followed by the addition of hydrazine hydrate (excess, 2 mL). The mixture was refluxed for 4 h until the fluorescence of the solution disappeared. The reaction mixture was brought to room temperature and was allowed to stand overnight. The product was obtained as white precipitated, which was filtered and washed with cold methanol. The crude product was recrystallized from methanol to give 2 (3.35 g, 99%) as white powder; Rf = 0.55 (CH_2Cl_2: MeOH= 9:1); mp 272−273 °C. ^{1}H NMR (400 MHz): δ, 7.89 (d, J = 5 Hz, 1H), 7.58−7.52 (m, 2H), 7.05 (d, J = 8 Hz, 1H), 6.65 (s, 2H), 6.48 (s, 4H) ppm. ESI mass (m/z) 347.0796 $[M + H]^+$.

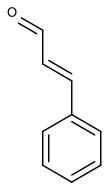
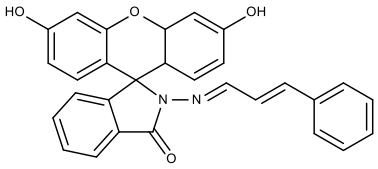
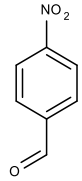
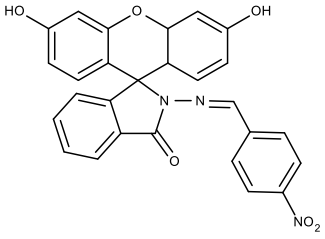
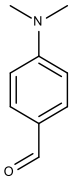
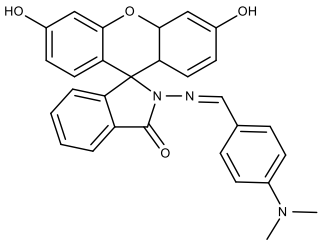
General Procedure for the Synthesis of 1a−1e (Scheme 2).

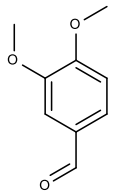
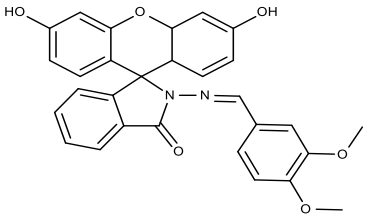
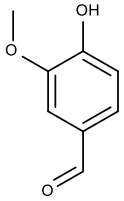
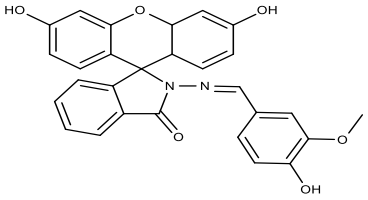
To a mixture of fluorescein hydrazide (0.3 mmol) and substituted aromatic aldehyde (0.3 mmol) in 10 mL of methanol, was added a drop of glacial acetic acid, and the reaction mixture was refluxed 4 h. The precipitated solid was filtered, washed with cold methanol, and air-dried to obtain **1a-1e**, which was further purified by recrystallization using methanol.



Scheme 2. Synthesis of Schiff bases derivatives (1a-1e)

Table 1:Physical characterization data of fluorescein Schiff bases

Name	Ar	Product	M.W.	HPLC Purity (%)
1a			462.49	98
1b			481.45	97
1c			479.52	97

1d			496.51	99
1e			482.48	99

3',6'-dimethyl-2-{[(2E)-3-phenylprop-2-en-1-ylidene]amino}-2,3-dihydrospiro[isoindole-1,9'-xanthene]-3-one ***(1a)*** Brown solid; yield: 84%; R_f: 0.75; mp: 210-212 ^oC; purity (HPLC): 98%; MS (ESI) $(M+H)^+$: 463.20; IR (KBr pellets) cm^{-1}: 3400 (C-OH *str*), 1597 (Aromatic C-C *str*), 1094 (C-N *str*), 737 (Aromatic C-H *str*)

3',6'-dimethyl-2-{[(4-nitrophenyl)methylidene]amino}-2,3-dihydrospiro[isoindole-1,9'-xanthene]-3-one ***(1b)*** Bright Yellow solid; yield: 87%; R_f: 0.72; mp: 201-204 ^oC; purity (HPLC): 97%; MS (ESI) $(M+H)^+$: 482.20; IR (KBr pellets) cm^{-1}: 3400 (C-OH *str*), 1595 (Aromatic C-C *str*), 1327 (N-O symmetric *str*), 1098 (Aliphatic C-N *str*).

2-({[4-(dimethylamino)phenyl]methylidene}amino)-3',6'-dimethyl-2,3-dihydrospiro [isoindole-1,9'-xanthene]-3-one ***(1c)*** White solid; yield: 82%; R_f: 0.85; mp: 233-235 ^oC; purity (HPLC): 97%; MS (ESI) $(M+H)^+$: 480.90; IR (KBr pellets) cm^{-1} : 3428 (C-OH *str*), 1598 (Aromatic C-C *str*), 1096 (C-N *str*).

2-{[(3,4-dimethoxyphenyl)methylidene]amino}-3',6'-dimethyl-2,3-dihydrospiro[isoindole-1,9'-xanthene]-3-one ***(1d)*** White solid; yield: 88%; R_f: 0.70; mp: 254-256 ^oC; purity (HPLC): 99%; MS (ESI) $(M+H)^+$:497.06; IR (KBr pellets) cm^{-1} : 3468 (C-OH *str*), 1595 (Aromatic C-C *str*), 1501 (Aromatic C-C *str*), 1452 (Aromatic C-C *str*), 1323 (C-N *str*), 1254 (C-O *str* of ethers), 1163 (C-O *str* of ethers), 1006 (C-N *str*).

2-{[(4-hydroxy-3-methoxyphenyl)methylidene]amino}-3',6'-dimethyl-2,3-dihydrospiro [isoindole-1,9'-xanthene]-3-one ***(1e)*** Off-White solid; yield: 84%; R_f: 0.77; mp: 226-228 ^oC; purity (HPLC): 99%; MS (ESI) $(M+H)^+$: 483.76; IR (KBr pellets) cm^{-1} : 3382 (C-OH *str*), 1666 (C=O *str*), 1598 (Aromatic C-C *str*), 1495 (Aromatic C-C *str*), 1453 (Aromatic C-C *str*), 1018 (C-O *str* of ethers).

RESULTS AND DISCUSSION:

Chemistry.

Fluorescein hydrazide is a non fluorescent molecule prepared directly from fluorescein by refluxing in the presence of excess amount of hydrazine hydrate in methanol to give the product in 80−90% yield (Scheme 1). The design strategy behind this synthesis was to provide a $–NH_2$ handle to the fluorophore that can be modified in many ways to generate newer fluorescent probes [12]. Modification of fluorescein hydrazide by making Schiff bases yielded highly fluorescent molecules that could be further used in various biological applications [13]. Synthesis of 1a−1e was straightforward as shown in Scheme 2. These reactions were carried out with aromatic substituted benzaldehydes in the presence of a catalytic amount of acetic acid in methanol under refluxing conditions to afford the corresponding **1a−1e**. It was observed that condensation of fluorescein hydrazide with substituted benzaldehydes in methanol without acetic acid formed no products and/or byproducts even in refluxing condition for 2 days. After addition of a catalytic amount (1−3 drops) of glacial acetic acid, we obtained excellent yields (80− 90%). All the synthesized compounds are listed in Table 1.

1.1. UV-Vis and Fluorescence Measurements

The compounds (**1a-1e**) were subjected to spectroscopic measurements to study their absorption and fluorescence characteristics. UV-Vis data shows a sharp peak at around 390 nm for the compounds. Representative absorption spectrum of 1a is shown in Figure 1. At the excitation wavelength of 390 nm, the emission wavelength was observed at 480 nm. A representative fluorescence spectrum of **1a** is shown in Figure 2. The spectroscopic data for these fluorescent compounds are depicted in Table 2.

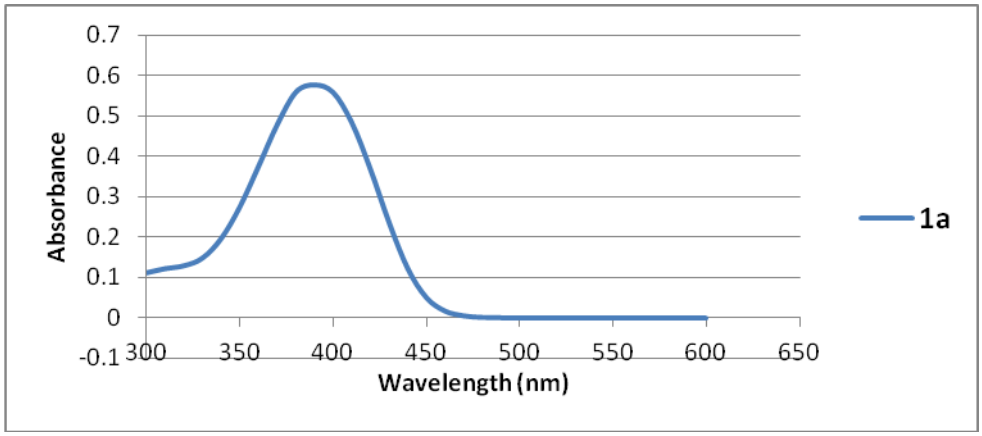


Fig 1: UV-Vis absorption spectra of 1a in methanol (10µM). λ_{max}= 390 nm

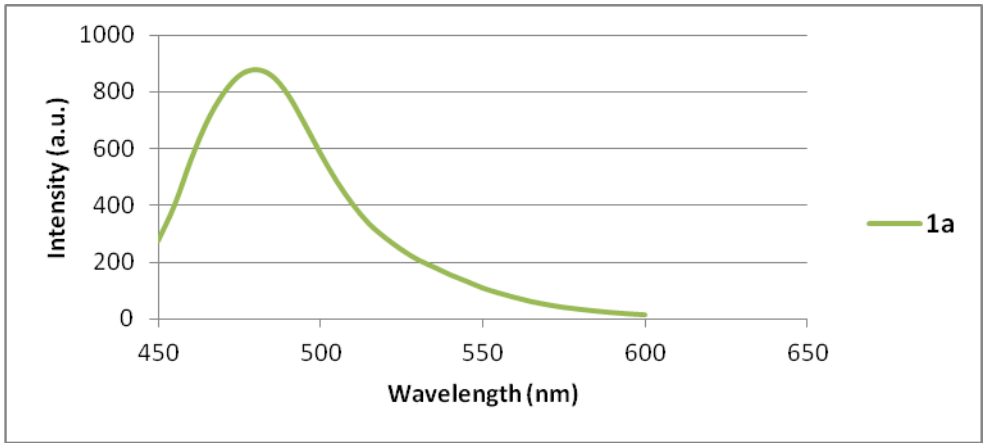


Fig 2: Fluorescence emission spectra of 1a in methanol (1µM). λ_{em}= 480 nm

Table 2: Spectroscopic data for fluorescent compounds

Compound Name	UV-Vis	Fluorescence	
	λ_{max} (nm)	λ_{ex} (nm)	λ_{em} (nm)
1a	390	390	480
1b	392	392	482
1c	390	390	481
1d	389	389	480
1e	389	389	480

CONCLUSION:

Fluorescent probes play a significant role in life and environmental sciences. Fluorescein-derived probes are a common set of fluorescent dyes and they fit well into various chemical and biological property requirements. They have proved to be a selective and sensitive analytical tool in biological sciences and are a major replacement of radioisotopes. Since the advent of fluorescein in 1871, several fluorescein-based dyes have been designed and synthesized via modification of existing fluorophores via linkers or introduction of linkers at different positions. These fluorophores have been adapted depending on the desired applications by altering the chemical, fluorescent and biological properties of fluorescein-based dyes.

As the exciting applications of fluorescein probes continue to rise, design and synthesis of novel fluorescein probes will certainly attract more attention, in addition to their high yield synthesis and efficient separation.

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