

A REVIEW ON FLUORESCEIN, FLUORESCEIN DERIVATIVES AND THEIR APPLICATIONS

¹S. Janet Beula*, ¹ Dr. R. Suthakaran, ² Rajaram Das, ²Laxmidar Sahoo, and ³Dr. J. Muthu Mohammed.

¹Vijaya college of Pharmacy, Associate professor, Munuganoor, RR district, India.

²Roland institute of pharmaceutical sciences, Assistant professor, Berhampur, Odisha, India.

³Fathima college of pharmacy, Associate professor, Kadapa, AP, India.

ABSTRACT:

Fluorescein is a manufactured organic compound and dye. It is available as a dark orange or red powder slightly soluble in water and alcohol. It is widely used as a fluorescent tracer for many applications. The IUPAC name of fluorescein is 3',6'-Dihydroxy-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one. It is prepared by heating phthalic anhydride and resorcinol over a zinc catalyst, and it crystallizes as a deep red powder. Now a days fluorescein and its derivatives are used as an Antioxidant, Antibiotics, Anti-inflammatory, Anti cancer, Anti fungal, detection of skin ulcers and detection of ophthalmic infections etc. The aim of this review is to provide the recent efforts of scientists in applications of fluorescein, important and synthesis of fluorescein derivatives, pharmacological action of fluorescein and their biological activity.

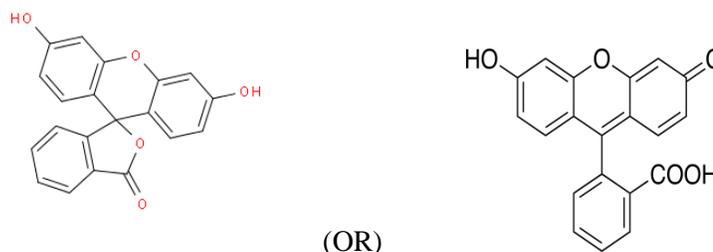
Key words:

Fluorescein, Anticancer, Antibiotics, Insecticidal, Anti-inflammatory, Anti fungal.

Introduction:

Fluorescein is a fluorophore commonly used in microscopy, in a type of dye laser as the gain medium, in forensics and serology to detect latent blood stains, and in dye tracing. Fluorescein has an absorption maximum at 494 nm and emission maximum of 512 nm (in water). Fluorescein is now sometimes used for determination of circulation time, adequacy of blood supply, and viability of tissue¹. Fluorescein sodium, the sodium salt of fluorescein, is used extensively as a diagnostic tool in the field of ophthalmology and optometry, where topical fluorescein is used in the diagnosis of corneal abrasions, corneal ulcers and herpetic corneal infections. It is also used in rigid gas permeable contact lens fitting to evaluate the tear layer under the lens. It is available as sterile single-use sachets containing lint-free paper applicators soaked in fluorescein sodium². Intravenous or oral fluorescein is used in fluorescein angiography in research and to diagnose and categorize vascular disorders including retinal disease macular degeneration, diabetic retinopathy, inflammatory intraocular conditions, and intraocular tumors. It is also being used increasingly during surgery for brain tumors. Diluted fluorescein dye has been used to localise multiple muscular ventricular septal defects during open heart surgery and confirm the presence of any residual

defects³. It is prepared by heating phthalic anhydride and resorcinol over a zinc catalyst, and it crystallizes as a deep red powder⁴.



Structure of fluorescein

Applications of fluorescein and its derivatives:

1. Antioxidant activity⁵:

Mani Rajasekar *etal* has been reported the one-pot synthesis of fluorescein based β -aminoglycosylketones and their biological and material applications. Facile one-pot synthesis of fluorescein based β -aminoglycosylketones was carried out using 4, 6-*O*-protected-*C*-glycoside, fluorescein-monoaldehyde and aromatic amines in the presence of potassium carbonate as a catalyst. Studies reveal that the use of potassium carbonate resulted in good yields. All these compounds were characterized using different spectral techniques. The gelation properties of these compounds were studied in regard to their molecular structure by HRTEM, DSC and powder XRD techniques. Fluorescein based β -aminoglycosylketones show moderate antioxidant activities with maximum inhibitory activity.

2. Anti microbial activity⁶:

P. N. Patle *etal* has been reported the fluorescein diacetate (FDA) measure of total microbial activity and as indicator of soil quality. Soil is crucial to life on earth. Enormous agriculture practices may emerge a numerous problem like reduction in soil fertility, erosion, ground water contamination which has direct impact on the soil health. It is indispensable to study overall microbial activity potential and different changes in soil biological properties for maintaining soil health and its quality. Soil enzyme is essential to play important role in maintaining soil health and to asses it quality. The fluorescein diacetate (FDA) hydrolysis is one of the important to measures the enzyme activity of microbial populations and can provide an assessment of microbial activity in soil. Assessment of Hydrolysis of fluorescein diacetate [3', 6'- diacetylfluorescein (FDA)] has been suggested as prospective method for determination of total microbial activity because it includes several enzyme classes including lipases, esterases, and proteases. Spectrophotometric determination of the hydrolysis of fluorescein diacetate (FDA) was shown to be a simple, sensitive, and rapid method for determining microbial activity in soil. The assessment of FDA in soil is done by incubating the soil sample with buffer and FDA for 1 – 2 hours. The amount of fluorescent color formation during the incubation is indicative of the enzymatic activity of the microbial community in the sample. The intensity of the color will be measured by a spectrophotometer and compared to a standard curve to determine the relative microbial

activity in your sample. The main intendment of this review paper to define the role of FDA hydrolysis in soil environment as well as method to estimate FDA enzyme activity in soil. Paper also illustrate that FDA enzyme activity serves as microbial indicator and measure of total microbial activity to asses soil health and is quality.

3. Anti-inflammatory activity⁷:

Refilwe G *etal* has been reported the anti-inflammatory effect of two medicinal plant species. This study was aimed at investigating the possible anti-inflammatory effect of selected medicinal plants based on their traditional usage. The ability of *Dombeya rotundifolia* and *Schkuhria pinnata* extracts to inhibit the production of reactive oxygen species (ROS) in LPS induced RAW 264.7 macrophage cells was evaluated using Dichloro-dihydro-fluorescein diacetate (H₂DCF-DA) assay to determine anti-inflammatory potential and the toxicity on African green monkey kidney (Vero) cells was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. *Rotundifolia* demonstrated anti-inflammatory potential by inhibiting the production of ROS in a dose dependant manner. The cytotoxicity of the plants (LC₅₀ values) ranged from < 25.0 to 466.1 µg/mL. *S. pinnata* extract was the most toxic with the lowest LC₅₀ value of < 25.0 µg/mL. The synergistic interaction observed indicates that combinational therapy may improve biological activity. This report highlights the anti-inflammatory potential of *S. pinnata* and *D. rotundifolia*. **4. Detection of cancer⁸:**

Ahmed A.H *etal* has been reported the Octreotide labeled fluorescein isothiocyanate for identification of somatostatin receptor Subtype 2. Cancer can be detected by certain signs and symptoms, it is can be investigated by medical imaging and confirmed by biopsy. Many tumor cells express some kind of receptor, e.g., somatostatin receptors. Somatostatin receptor-positive human tumors can be detected using labeled analogues of somatostatin. In vitro labeled octreotide (OCT) has been successfully used in the visualization of somatostatin receptor-positive tumors by flow cytometry. OCT was labeled with fluorescein isothiocyanate (FITC) to easy identify the somatostatin receptor subtype 2 (SSTR 2) in different living cell lines. The labeling of OCT was proceeded depending on the difference of pKa of amino groups of OCT. The reaction was preceded at pH 6 as the amino group phenylalanine has pKa 7.1 and the amino group of lysine has pKa 10.1. The labeling of OCT was confirmed by high performance liquid chromatography and mass spectrometry. The

expression of somatostatin receptor in tumor cells was confirmed by incubation with FITC-OCT. All cells were positively expressed with SSTR2 in comparison with standard positive SSTR2. The labeled FITC-OCT is a promising model for identification of SSTR2. The tumor cell lines were chosen in this study were completely positive SSTR2.

5. Anti fungal activity⁹:

Lestan D *etal* has been reported the biological potential of fungal inocula for bioaugmentation of contaminated soils. The suitability of the fluorescein diacetate hydrolyzing activity (FDA) assay for determining the biological potential (ie fungal biomass produced per unit of substrate) of solid pelleted fungal inoculums intended for use in the bioaugmentation of contaminated soils with white-rot fungi, was evaluated. FDA activity of the white-rot fungus *Phanerochaete chrysosporium* grown on pelleted substrates and on agar was found to be proportional to quantities of fungal ergosterol and fungal dry matter, respectively. Inoculum biological potential was found to be greatly influenced by substrate formulation and structure, and temperature. Biological potential and the type of carrier influenced the ability of *P. chrysosporium* to tolerate pentachlorophenol (PCP). *Phanerochaete chrysosporium* and *Trametes versicolor* introduced into PCP-contaminated soil on pellets with higher biological potential and higher nitrogen content (C: N ratio of 50:1), did not remove PCP more efficiently than when the fungi were introduced on pellets with a lower biological potential (C: N ratio of 309:1). However, under the latter conditions most of the PCP was transformed to pentachloroanisole (PCA). In soil inoculated with *T. versicolor* on pellets with high biological potential, higher manganese peroxidase activity was detected compared to soil inoculated with pellets with a lower biological potential. Keywords: fungal inoculums; bioaugmentation; biological potential; pentachlorophenol.

6. Detection of skin ulcers¹⁰:

Mai D.Ibrahem *etal* has been reported the determining the safety and suitability of fluorescein dye for characterization of skin ulcerations in cultured Nile tilapia (*Oreochromis niloticus*) and African sharp-tooth catfish (*Clarias gariepinus*). There is a need to identify the presence of lesions in fish skin as soon as they erupt. Fish skin lesions are either macroscopic (can be visualized by the naked eye) or microscopic (difficult to detect with the naked eye). Skin wounds resulting in loss of the epithelium (superficial or deep ulcers) are serious as they may interfere with osmoregulation and open portals for

opportunistic pathogens. Herein, we report on the use of a fluorescein dye for the detection of skin ulcers that cannot be seen by the naked eye. Due to their importance in aquaculture endeavors in Egypt, this study focused on two indigenous species, the Nile tilapia (*Oreochromis niloticus*) and the scale-less African sharptooth catfish (*Clarias gariepinus*). Fluorescein dye was tested for safety to fish without interfering with microbiological analysis. Parallel to the use of the fluorescein dye, the detected ulcers were examined for the presence of bacteria or tissue alterations. Further, we experimentally induced the formation of skin ulcers in *O. niloticus* physically or by injecting *Aeromonas hydrophila*, and then assessed the utility of fluorescein dye in detecting the induced skin lesions. Results obtained in this study demonstrated that fluorescein dye application is harmless to Nile tilapia at concentrations up to 0.5 mg fluorescein/ml water for up to 15 min. Indeed, a low dose of fluorescein (0.10 mg/ml for 5 min) could identify very minute skin abrasions. We highly recommend the use of fluorescein dye for the evaluation of skin health in farmed fish species and the visualization of minute skin abrasions.

7. Earth sciences^{11,12}:

Kass.W *etal* has been reported the fluorescein is used as a rather conservative flow tracer in hydrological tracer tests to help in understanding of water flow of both surface waters and groundwater. The dye can also be added to rainwater in environmental testing simulations to aid in locating and analyzing any water leaks, and in Australia and New Zealand as a methylated spirit dye. As fluorescein solution changes its color depending on concentration it has been used as a tracer in evaporation experiments. One of its more recognizable uses was in the Chicago River, where fluorescein was the first substance used to dye the river green on St. Patrick's Day in 1962. In 1966, environmentalists forced a change to a vegetable-based dye to protect local wildlife

8. Detection of ophthalmic infections¹³:

US national institute of health and science has been reported the Fluorescein sodium is used extensively as a diagnostic tool in the field of ophthalmology. Fluorescein is a fluorescent compound or fluorophore having a maximum absorbance of 494 nm and an emission maximum of 521 nm. The yellowish-green fluorescence of the compound can be used to demarcate the vascular area under observation, distinguishing it from adjacent areas. It is applied topically in the form of a drop or it can be injected intravenously to produce a fluorescein angiogram. Topical fluorescein is a useful tool in the diagnosis of corneal abrasions, corneal ulcers, herpetic corneal infections, and dry eye. Fluorescein angiography is used to diagnose and categorize macular degeneration, diabetic retinopathy, inflammatory intraocular conditions, and intraocular tumors

9. Ureteral jet detection¹⁴:

Lea.D *etal* has been reported the background and objectives laparoscopic hysterectomy poses the risk of lower urinary tract injury, and intraoperative recognition of urinary tract injury is often difficult. We sought to evaluate sodium fluorescein coloration of ureteral jets during cystoscopy after laparoscopic hysterectomy and to evaluate surgeons'

satisfaction with this method of injury detection. Methods this was an observational study, in which data were collected prospectively. A total of 30 women who underwent laparoscopic hysterectomy from August 2016 to February 2017 at the Centre Hospitalier de l'Université Laval (CHUL) de Québec. Twenty-five milligrams of 10% intravenous sodium fluorescein was administered after vaginal cuff closure. Cystoscopy was then systematically performed, and the time to visualization (time from injection to coloration of ureteral jet), the quality of coloration, and the duration of cystoscopy were recorded. Side effects were systematically recorded and rigorously analyzed. Results Most frequent indications for hysterectomy were fibroids and endometriosis with uterine weight from 76 to 885 g. Ninety-three percent of patients (n = 28) had bilateral ureteral jet coloration, whereas 1 patient had no coloration bilaterally, and another patient had a complete unilateral obstruction immediately diagnosed and resolved intraoperatively after removal of the vaginal sutures. In 90% of cases (n = 27), surgeons were highly satisfied with the use of sodium fluorescein. The average time between visualization of both left and right ureteral jets was 1 minute. Side effects included hypotension (n = 3) and yellow coloration of urine (n = 15). Conclusion our study suggests that sodium fluorescein is an effective dye for documenting ureteral patency and improving visualization of ureteral jets during cystoscopy upon completion of laparoscopic hysterectomy.

10. Other diagnostic agents¹⁵:

Mathew L.S *etal* has been reported the fluorescein may be administered as a diagnostic agent in the eye, orally and intravenously (angiography). A case series with over 100 pregnant women who had angiography with fluorescein failed to demonstrate any unwanted fetal effects (Halperin 1990). Animal experiments also showed no teratogenic effects. The substance was retrieved in the amniotic fluid of a pregnant woman after application in the eye.

Conclusion:

In the study of fluorescein and fluorescein derivative compounds having different applications. The fluorescein derivatives used for the detection of various disease like Cancer, Ophthalmic infections, Skin ulcers and it is act as an Anti fungal, Anti-inflammatory and Antioxidant etc. The study of fluorescein molecule find most effective treatment of multi diseases. This review is expected to be a comprehensive, authoritative and critical as a of the fluorescein template to the chemistry country.

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