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RESEARCH ARTICLE

# Fluorescein Derivative: Synthesis and Significance of 2-(7,8-Dihydro-10-Methyl-3,8-Dioxo-3H-Benzo[B]Xanthen-12-Yl) Benzoic Acid

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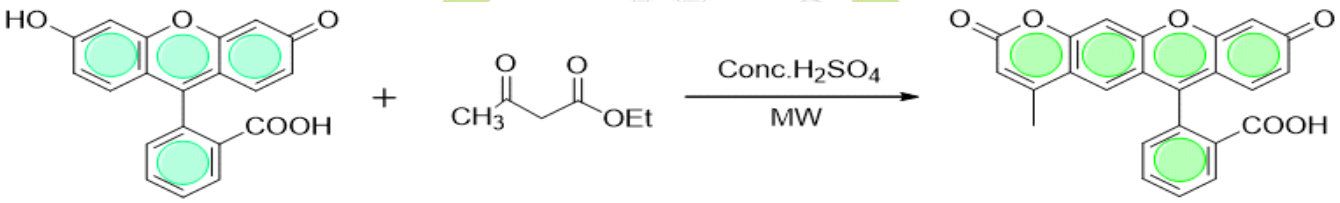
## ABSTRACT

Many fluorescein derivatives have functional groups that are suitable for reaction with other molecules, and can therefore serve as labels in a variety of analytical applications ranging from probing cell functions to monitoring the level of drugs in human fluids via immunoassays. We have tried to synthesize new derivative of Fluorescein by adding substituent on the fluorescein molecule. In present methodology Fluorescein was synthesized from resorcinol and Phthalic anhydride. From Fluorescein by adding ethyl acetoacetate and sulphuric acid, in ethanol and water under microwave irradiation 2-(7,8-dihydro-10-methyl-3,8-dioxo-3H-benzo[b]xanthen-12-yl)benzoic acid (P) was synthesized. This fluorescein derivative shows Minimum Inhibition Growth (MIC) against *Proteus mirabilis* pathogen.




## KEYWORDS

Fluorescein**,** Resorcinol, Phallic Anhydride, Ethyl Acetoacetate

## INTRODUCTION

Fluorescein is a synthetic organic compound available as a dark orange/red powder soluble in water and alcohol. It is widely used as a fluorescent tracer for many applications. Fluorescein[1,2] is a fluorophore commonly used in microscopy, in forensics and serology to detect latent blood stains and in dye tracing. There are many fluorescein derivatives.[3,4,5]

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For example, fluorescein isothiocyanate, often abbreviated as FITC, is the original fluorescein molecule functionalized with an isothiocyanate group (-N=C=S), replacing a hydrogen atom on the bottom ring of the structure. Many fluorescein[6] derivatives have functional groups that are suitable for reaction with other molecules, and can therefore serve as labels in a variety of analytical applications ranging from probing cell functions to monitoring the level of drugs in human fluids via immunoassays. Commonly used derivatives include 5- and 6-carboxyfluorescein[7] 5- and 6-

aminofluorescein[8] 5- and 6-fluorescein isothiocyanate and 4'-(aminomethy1) fluorescein (4'-AMF) Fluorescein[9,10] is a highly fluorescent molecule in water, and Fluoresce in dyes have been widely employed to develop useful fluorescence probes for important biomolecules since fluorescence imaging is the most powerful technique currently available for continuous observation of the dynamic intracellular events of living cells. Intravenous or oral fluorescein[11,12] is used in fluorescein angiography in research and to diagnose and categorize vascular disorders in e.g. legs, including retinal disease macular degeneration, diabetic retinopathy, inflammatory intraocular conditions, and intraocular tumors and increasingly, during surgery for brain tumours. Other uses of fluorescein[13] include using it as a water-soluble dye added to rainwater in environmental testing simulations to aid in locating and analyzing any water leaks.

As different derivatives of fluorescein are having their own importance, we had attempted this work to synthesize new fluorescein derivative.

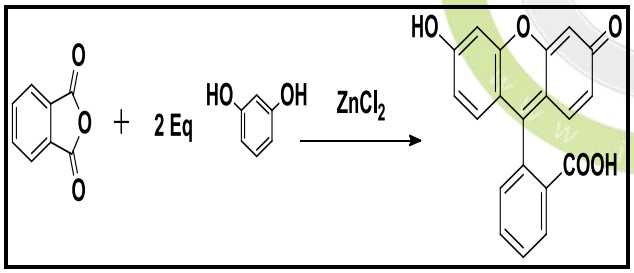


Figure1: Synthesis of Fluorescein

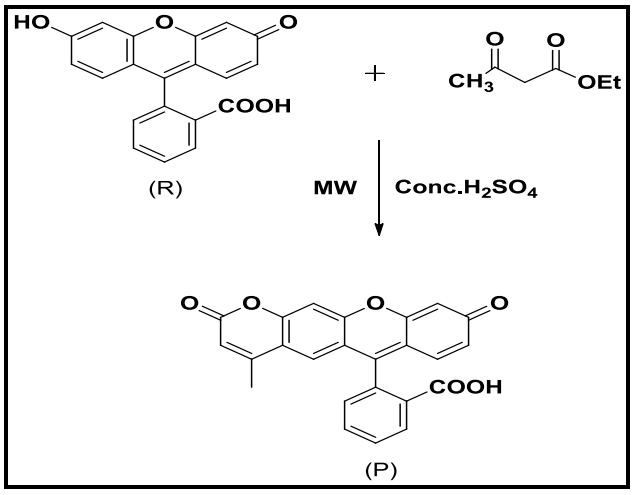


Figure2: Synthesis of fluorescein derivative

## MATERIALS AND METHOD

### Synthesis of Fluorescein Derivative

2-(7,8-dihydro-10-methyl-3,8-dioxo-3H-benzo[b]xanthen-12-yl)benzoic acid.

To the 1gm of fluorescein 1.34 gm of ethyl acetoacetate was added in a round bottom then 14 ml of Conc. $H_2SO_4$ was added to the above mixture with constant stirring. After few minutes 25 ml ethanol and 10 ml water were added in reaction mixture. Reaction mixture was irradiated under microwave. The reaction is monitored by TLC.

IR : 3434 (b)-OH Stretch,1632 -C=O Stretch, 1108 C-O-C Stretch, 989 C-O-C Stretch.

$^1$H NMR: δ2.14 (S, 3H), δ6.57-6.52(m, 4H), δ6.83-6.80(d, 1H), δ7.36-7.34(d, 1H), δ7.73-7.70 (t, 1H), δ7.81-7.77(t, 1H), δ7.93 (bs, 1H), δ8.21-8.19(d, 1H)

## RESULTS AND DISCUSSION

Synthesis of Fluorescein has been carried out by known method using Phthalic anhydride and resorcinol in presence of zinc chloride[14].

### Ultra-Violet Spectrum

Comparative UV spectrum of Reactant (R) and Product (P) was given in the figure: 3. UV spectrum reveals that there is small difference in the absorption spectrum of (R) and (P). Though there is addition of one more ring in the structure of product but the absorption in UV region has been decrease which means product is less conjugated than the reactant.

### Antimicrobial Activity

Minimum Inhibition Growth (MIC) of fluorescein derivative on different pathogenic species was carried out. All the microorganisms were inoculated in nutrient broth and incubated for overnight to observe their growth. The optical density (O.D.) was maintained at 1.0 (600 nm). The petri plates were prepared with the selective agar media for each culture of the species. The plates were spread with the respective culture of each species. These plates were incubated at room temperature for half an hour.

The wells were made using a borer. The wells were filled with the 2-(7,8-dihydro-10-methyl-3,8-dioxo-3H-benzo[b]xanthen-12-yl)benzoic acid (P), volume 10µl, 20µl and 30µl to check the antimicrobial activity.

As (P) has showed antimicrobial activity were further examined to find out their MIC value. The same procedure is followed to find out their MIC value only the volumes were taken to be 5µl - 30µl depending upon the zone in which the specific chemical showed antimicrobial activity. Sterile conditions were strictly maintained to avoid any kind of contamination.

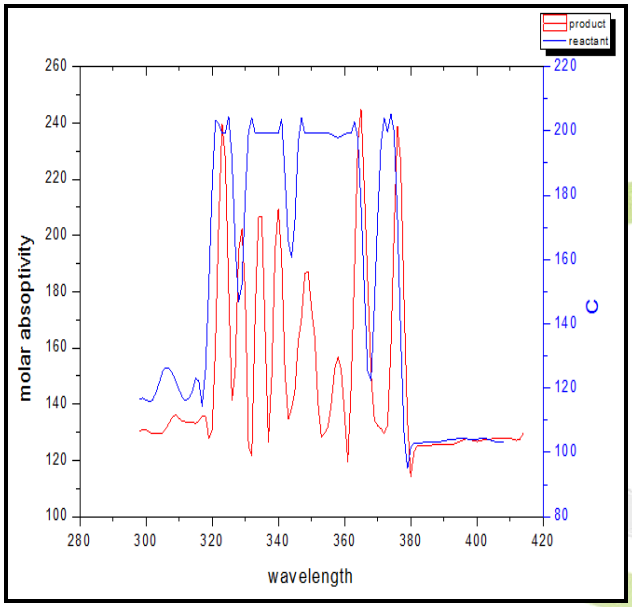


Figure 3: UV absorption spectrum of Reactant (R) and Product (P)

Table 1: Inhibition activity of (P) against different pathogens

| Sr. no. | Species | MIC value (in µl) |
|---|---|---|
| 1. | Staphylococcus aureus | -- |
| 2. | Escherechia coli | -- |
| 3. | Proteus mirabilis | 09 |
| 4. | Shiegella flexineri | -- |
| 5. | Salmonella typhi | -- |
| 6. | Salmonella paratyphi | -- |
| 7. | Klebleshiella pnemoniae | -- |
| 8. | Aspergillus niger | -- |
| 9. | Candida albicans | -- |

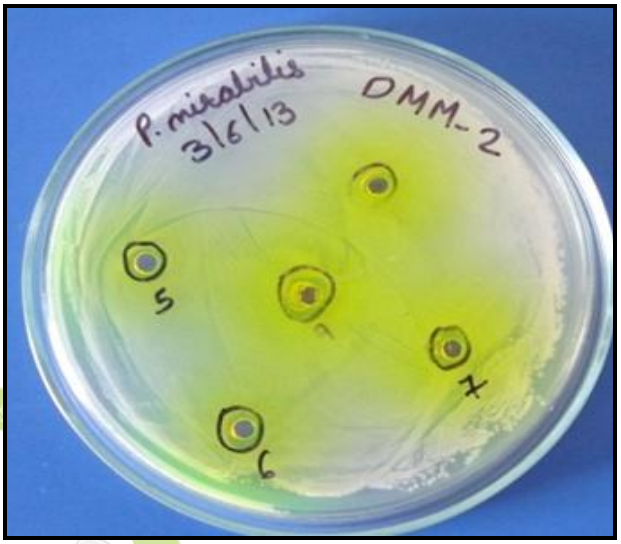


Figure3: (P) showing Inhibition of *Proteus mirabilis* bacterium

It was observed that 2-(7,8-dihydro-10-methyl-3,8-dioxo-3H-benzo[b]xanthen-12-yl)benzoic acid showed minimum inhibition growth (MIC 9 µl) for *Proteus mirabilis* pathogen.

This rod shaped bacterium has the ability to produce high levels of urease which makes the urine more alkaline. If left untreated, the increased alkalinity can lead to the formation of crystals of struvite, calcium carbonate. The bacteria can be found throughout the stones, and these bacteria lurking in the kidney stones. Once the stones develop, over time they may grow large enough to cause obstruction and renal failure.

## CONCLUSION

A new derivative of fluorescein is accessible through reaction with ethyl acetoacetate in presence of sulphuric acid. The reaction proceeds in ethanol and water under microwave irradiation. A fluorescein derivative shows inhibition for *Proteus mirabilis* bacterium.

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