# FLUOROPHORE BASED ON (*E*)-4-ARYL-4-OXO-2-BUTENOIC ACIDS

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## Abstract

In this manuscript synthesis and properties of six alkyl-, halo- and nitro-phenyl substituted (E)-4-phenyl-4-oxo-2-butenoic acids are described. Since aroylacrylic acids show biological activity their fluorescent analogues offer possibility to investigate mechanism and kinetics of their interactions within the cell, without additional modification of molecule [e.g. fluorescent labeling].

## Introduction

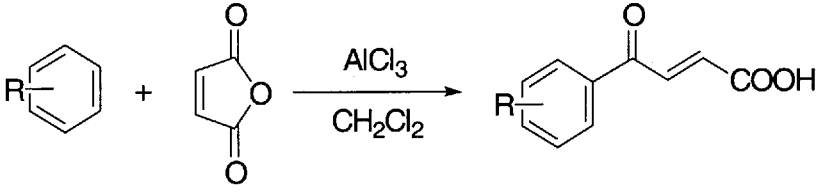
(*E*)-4-Aryl-4-oxo-2-butenoic acids exert antibacterial [1], antiviral [2] and antiproliferative action [3]. Ketovinyl fragment -C- (O)-C=C- as a part of (*E*)-4-aryl-4-oxo-2-butenoic acids is Michael acceptor suitable for addition of nucleofiles, thiol and amino group of biomolecules. It is suggested that biological activity of aroylacrylic acids could be attributed to this type of action.

On the other side (*E*)-4-Aryl-4-oxo-2-butenoic acids are convenient for the fluorescent investigation due to the presence of the system of conjugated double bonds, from a phenyl ring to the carboxylic group.

The aim of our research is to use the fluorescent properties of (*E*)-4-aryl-4-oxo-2-butenoic acids for monitoring biological tests. It is important to noticed that, for good monitoring fluorescence, emission should be between 450-620 nm in order to distinguished emission of probe from autoflorescence of the cell [4].

## Experimental

Title compounds were prepared by the modification of Friedel-Crafts reaction (*Scheme 1*). Aromatic substrates were added to the solution of maleic anhydride and anhydrous aluminium-chloride (molar ratio 1:2) in methylene chloride at the room temperature. The structures of acids were confirmed by microanalysis, $^{1H}$NMR, $^{13C}$NMR and IR spectrometry. Crystal structure of (*E*)-4-phenyl-4-oxo-2-butenoic acid was determinate as well [5].




R- = H- (1), 2,5-di-*i*-Pr- (2), 3-$NO_2$-4-Me- (3), 2-Cl-4-Me- (4), 2,4-di-Cl- (5), 4-Br- (6)

**Scheme 1.**

Fluorescence spectra were recorded on *Spex Fluoromax-3* spectrofluorometer (Jobin Yvon, Edison, NJ, USA) in a 150 μl quartz cuvette (Hellma).

## Result and Discussion

All title compounds were investigated under the same condition. Emission and excitation spectra were recorded from $CHCl_3$ solution at concentration of $2 \cdot 10^{-5}$ M at 25 °C. Excitation spectra show maxima between 340-440 nm. It is possible to assume that this peak is caused by charge transfer between electron donor-acceptor pair *via* conjugate unit [6,7]. Intensity of this peak is much more higher, related to the peak between 260-320 nm which correspond to absorption of phenyl moiety. Since absorption of the charge transfer is dominant, molecules were excited with the wavelength of the CT maxima and corresponding emission were recorded. In *Table 1* emission and excitation maxima are summarized.

**Table 1.** Fluorescence and excitation maxima (nm)

| Compounds № | Emission max. | Excitation max. | Stokes shift |
|---|---|---|---|
| **1** | 400 | 270 | 130 |
| **2** | 450 | 430 | 20 |
| **3** | 470 | 425 | 40 |
| **4** | 470 | 410 | 60 |
| **5** | 490 | 425 | 65 |
| **6** | 470 | 420 | 50 |

Emission and excitation spectra of compounds **2** and **6** are presented in Figure 1.

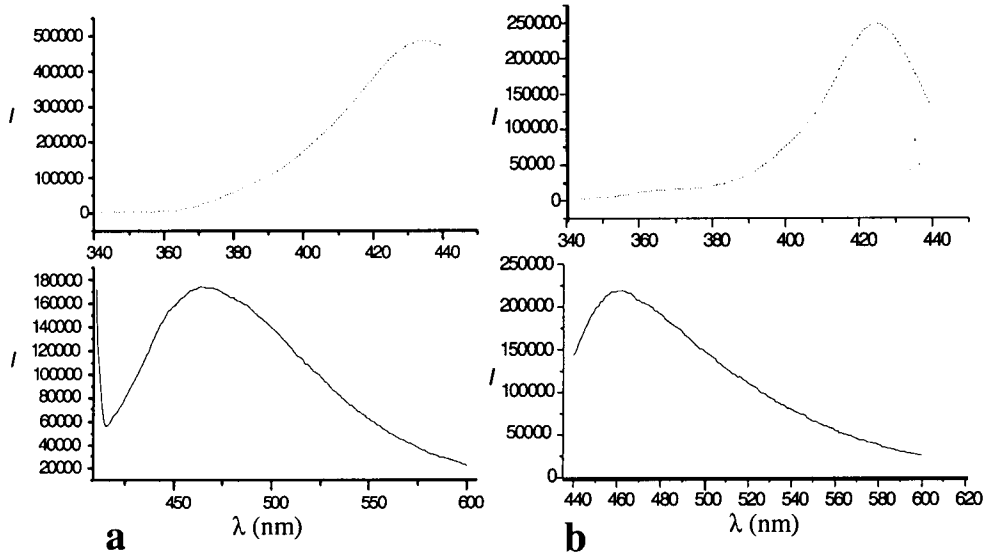



**Figure 1.** Excitation (···) and emission (—) spectra of compounds **2** (a) and **6** (b)

Results clearly show that halogenation and nitration of aryl moiety cause shift of fluorescence into red region (batochromic shift). Generally batochromic shift is caused by additional stabilization of polar excited state ($S^1$) in comparison to the ground state. Similar shifting of fluorescence emission due to halogenation of molecules was recently published for different compounds [8]. Stokes shifts (*Table 1*) represent difference between absorption and emission maximum and is a measure of change in geometry between excited and ground states between which transitions occurs. Substitution of phenyl moiety increases Stokes shift due to stabilization of excited states.

Presented synthetic approach offer possibility for designing a small fluorophore, which can be used for monitoring different biological processes. One of the most interesting outcomes of this research is to introduce these molecules as non-natural analogue of amino acid into peptide chain *via* solid phase method. Kinetics and dynamics of these non-natural peptides or proteins could be monitored by fluorescence techniques.

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