Design and studies of o-keto benzaldehyde derivatives as a probe for efficient fluorescent lysine modification

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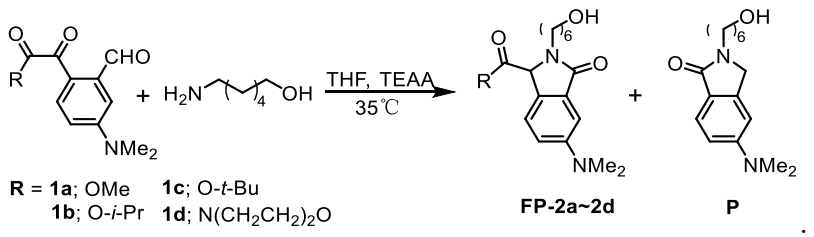
Fluorescent labeling is a powerful method to study biomolecules. We have developed a series of o-keto benzaldehyde derivatives, which convert lysine residues of proteins to fluorophore achieving turn-on fluorescent protein modification. In this study, we designed benzaldehyde derivatives containing o-ketoester or o-ketoamide groups, to facilitate rapid fluorescent lysine modification by electron withdrawing properties of ester and amide. Ligands for selective targeting can be also connected through ester or amide linkages.

A series of benzaldehydes containing o-ketoester or o-ketoamide groups were synthesized and their reactions with amine in a 1:1 mixture of THF and TEAA buffer (100 mM, pH 7) were studied as a model for lysine modification. Reaction of methyl ester **1a** (10 mM) with 1 equiv. of amine afforded "non-fluorescence product" (**P**) and "fluorescence product" (**FP-2a**) in 33 and 30 %, respectively. Reaction of isopropyl ester **1b** gave similar results. However, *t*-butyl ester **1c** afforded **FP-2c** exclusively in 90 % yield and reaction rate was faster than other esters under the same conditions. Compared to o-ketoesters, o-ketoamides reacted slowly. Overnight-reaction of morpholine amide **1d** yielded **FP-2d** in 76%**.** Studies in detail indicated that **FP-2c** formation from *t*-butyl ester **1c** was rapid and efficient even at 50 μM with 3 equiv. of amine.

In addition, we found that FPs were solvatochromic fluorophores exhibiting linear correlation between solvent polarity and fluorescence properties such as intensity and λ_{emmax}.

Obtained data indicated that benzaldehyde derivatives with o-ketoester designed in this study were identified as promising molecules for lysine modification. Preparation of fluorescent antibody-drug conjugate is an attractive application and incorporation of ligand for targeting and drug release system will be studied.



R = **1a**; OMe **1c**; O-*t*-Bu
1b; O-*i*-Pr **1d**; $N(CH_2CH_2)_2O$

FP-2a~2d **P**