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Novel dyes derived from benzo[*a*]phenoxazine and 3-formyl-2(1*H*)-quinolone – synthesis, properties and application as sensors for thiols

Abstract

In the past decades, the design, synthesis and application of colorimetric and fluorescent probes that are selective and exhibit specific response in the presence of various analytes have attracted significant attention.

Alterations in the levels of biothiols are connected with some neurodegenerative and cardiovascular diseases. The detection and determination of mercapto biomolecules, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), is very important because this compounds play crucial roles in biological systems. The optical detection of small molecules such as biothiols has proven to be an excellent method among the various techniques available. The use of the colorimetric and fluorescent probes as a tool for the detection of thiols is particularly attractive owing to their high sensitivity, selectivity and simplicity. In addition, optical probes do not require any sophisticated instruments or complicated procedures.

The main goal of this thesis was to synthesize novel dyes based on a benzo[*a*]phenoxazine and a 3-formyl-2(1*H*)-quinolone skeleton, and to investigate their spectroscopic properties and the evaluation of their potential application as optical chemosensors for the detection of thiols.

The first stage of the study was a multi-step synthesis of dyes based on 2-quinolone skeleton. Among these fourteen synthesized novel dyes, the eight compounds contain dicyanoethylene moiety and the six dyes possess 3-methylbenzothiazoliumethylene moiety. In the next stage, the three benzo[*a*]phenoxazine dyes containing a maleimide moiety were prepared in a four-step synthesis. All compounds were synthesized in very good or moderate

yields, and their chemical structures were verified by nuclear magnetic resonance spectroscopy and mass spectrometry.

It was found that the basic spectroscopic and photophysical parameters of the examined dyes, such as the position of the absorption and emission band, molar absorption coefficients, fluorescence quantum yields and singlet lifetimes, depend on the chemical structure of dye and a partly depend on the solvent polarity.

The obtained dyes contain various moieties in the structure, which can act as the acceptors in nucleophilic addition.

It was demonstrated that the addition of L-cysteine to solution of dyes with dicyanoethylene moiety at a pH of 7.4 results in blue shifts of their absorption bands and quenches their fluorescence totally. On the other hand, the addition of thiol amino acids, such as L-cysteine, N-acetyl-L-cysteine and L-glutathione to solution of dyes containing 3-methylbenzothiazoliumethylene moiety causes a hypsochromic shift of their absorption bands. While, in the presence of L-cysteine, the fluorescence intensity of dyes derived from benzo[*a*]phenoxazine is greatly enhanced and a red shift of their absorption bands was observed.

It was also shown that the first-order kinetic model describe the reaction of L-cysteine with the studied dyes possessing dicyanoethylene moiety. Moreover, the reaction of thiols with dyes containing 3-methylbenzothiazoliumethylene moiety was completed after a much longer time by comparison with other dyes. It is apparent that the steric effect overcame the electronic effects of the substituents in activation of the double bond to nucleophilic addition.

The investigation of reactivity of the tested dyes towards L-Cys at different pH values were revealed that the synthesized dyes exhibit a optical response to this amino acid under physiological conditions.

The lowest concentration of L-Cys that can be determined by the presented compounds is comparable with other reported chemosensors.

The synthesis, mass spectral and NMR analyses of a product obtained from the reaction of dye possessing dicyanoethylene moiety with 2-mercaptoethanol were confirmed the thiol addition to the double bond. Whereas, UPLC and mass spectral analyses of products obtained in the reactions dyes derived from benzo[*a*]phenoxazine with L-Cys suggest that reactions

may proceed by a different mechanism to Michael addition and L-Cys can be attached to a dye molecule at two different positions.

It was found that the obtained dyes exhibit low cytotoxic effect against human neuroblastoma cells (SH-SY5Y) in MTT-based assay.

The obtained results clearly indicate that the synthesized novel dyes may by candidates for the potential applications as optical chemosensors for the detection of thiols.

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