Egypt. J. Chem. **Vol. 64,** No. 4 pp. 2113 - 2126 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Using organic compounds for Fluorescence Photoactivation

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Abstract

Fluorescence microscopy is an essential analytical tool in the biomedical laboratory to investigate cells and tissues. Diffraction, however, limits the resolution of this imaging technique to the submicron scale. As a result, the factors regulating biological processes and structures at the molecular level cannot be appreciated with conventional fluorescence microscopy. The photoswitchable luminescent probes offer the opportunity to overcome this limitation and permit the visualization of biological samples with a resolution at the nanoscale. Our review article highlights some advanced organic molecules that could be utilized as invaluable molecular probes for super-resolution imaging and had a significant long-term impact on biomedical research in addition to other promising applications such as colorimetric identification of cyanide, photoactivatable barcodes, and saving papers with switchable ink.

Kewords: photochromism; fluorescence; emission; fluorophores; photoswitchable

Contents

1. Introduction

Certain organic molecules emit light in the form of fluorescence upon excitation at an appropriate wavelength [1]. This unique property offers the opportunity of using optical methods to probe biological structures and processes [2]. In fact, the wide visualization of specimens in real time can be attained through labeling strategies to introduce fluorescent probes within biological samples, and this is achieved in combination with microscopic techniques to excite the labels and collect their fluorescence [3,4]. It should be noticed that the basic operating principles of a fluorescence microscope involve using an excitation source to illuminate a labeled specimen through an objective lens. The radiations focused on the sample excite the many fluorescent labels from their ground state (S_0 in Figure 1) to one of their excited singlet states (e.g., S_2). The excited species relax thermally to the first singlet excited state (S_1) and then radiatively to S_0. Collection of the emitted light on the detector was achieved using the same objective lens and this offer

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Receive Date: 12 January 2021, Revise Date: 26 January 2021, Accept Date: 01 February 2021
DOI: 10.21608/EJCHEM.2021.57807.3242

the opportunity to reconstruct an image of the labeled sample with micrometer resolution.

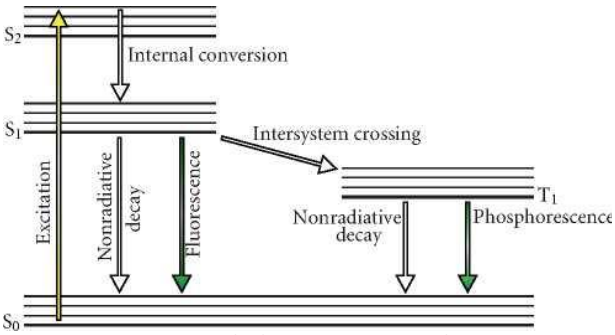


Figure 1. The excitation of a fluorescent molecule from the ground state (S_0) to the second singlet excited state (S_2) is followed by internal conversion to the first singlet excited state (S_1).

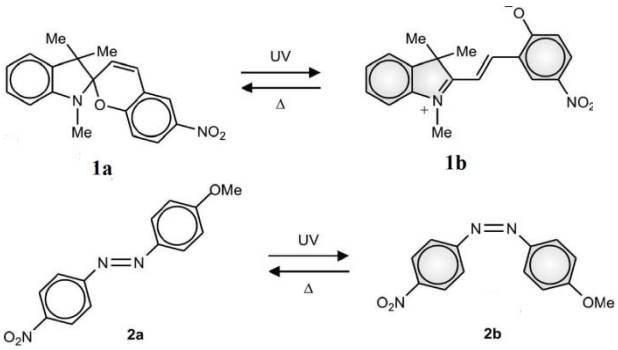
2. Photochromism

2.1. History and Definitions

The term "photochromism" was back to 1950s to indicate the photoinduced and reversible color change of certain compounds. The origin of the term "photochromism" comes from the Greek words "phos" and "chroma", that mean light and color, respectively and literally indicates a photoinduced change in color [5]. The basis of photochromic processes involve the photoinduced interconversion of a colorless form into and colored one. It is less common for the photoinduced transformation of a colored form into a colorless one. In fact, two types of photochromic compounds are present according to the nature of the reverse process. Thermally reversible photochromic compounds: storing this kind of compounds in dark, switch them back to the original state. While subjecting thermally irreversible photochromic compounds to irradiation revert them to the original form.

2.2. Properties of Photochromic Compounds

The photochromic compound can be photochemically interconverted from one state into another and this is significantly altering the structural and electronic behavior [6-10]. It is shown from the example outlined below that compound **1a** (Scheme 1) which incorporate an indole and benzopyran appendage within its molecular skeleton is colorless [11]. Generation of the colored isomer **1b** was performed through the ultraviolet excitation of **1a**, after some structural and electronic transformation including cleavage of the [C–O] bond at the spirocenter and, *cis→trans* isomerization of the adjacent double bond. Another good representative involves the effect of ultraviolet irradiation in changing the colorless *trans*-azobenzene **2a** into the colored *cis* isomer **2b**.

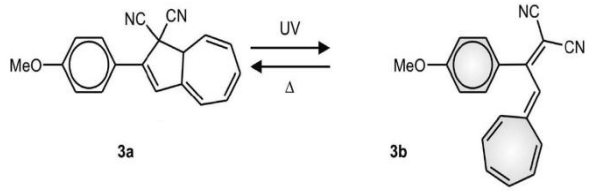


Scheme 1. Photoinduced interconversion of chromophores **1,2**

3. Intramolecular Fluorescence Modulation

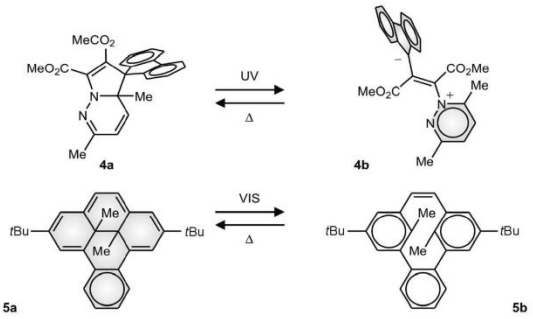
3.1 Fluorescent and photochromic compounds

The structural and electronic modifications that result from the interconversion of photochromic compounds can be exploited in significant changes in fluorescence quantum yield [12,13]. Here are some representatives: the spiropyran **1a** (Scheme 1) and the partially substituted azulene **3a** (Scheme 2) do not emit electromagnetic radiations [14,15]. However, in the visible region, their photogenerated isomers **1b** and **3b** have a good fluorescence.



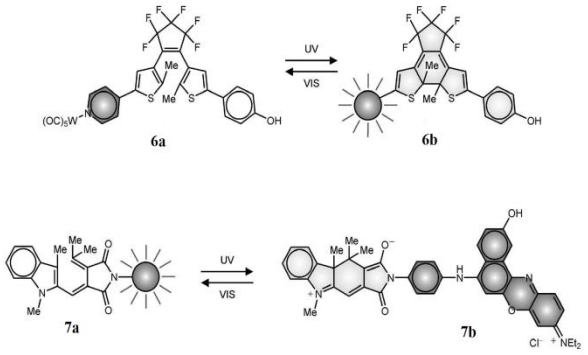
Scheme 2. The photoinduced (UV and VIS) and thermal (Δ) interconversion of some **3a**.

In a similar manner, the spiroindolizine **4a** and the dihydropyran **5a** fluoresce, while their photo-generated isomers **4b** and **5b** are not fluorescent (Scheme 3) [16-18]. Consequently, the photochemical and reversible transformation of these four isomers can be utilized in the modulation of their fluorescence intensity. Another method for using photochromic compounds to polish fluorescence depends on the integration of fluorescent and photochromic components within the same molecular skeleton. Thus, with these systems, the photochemically isomerization of one component assist the regulation of the emissive behavior of the other. Many other mechanisms can be invented to implement these operating principles. Some representatives for these include changes in either the conjugation or the polarity of the photochromic switch which can affect the fluorescence quantum yield [19-28].



Scheme 3. The photoinduced (UV and VIS) and thermal (Δ) interconversion of some photochromic compounds.

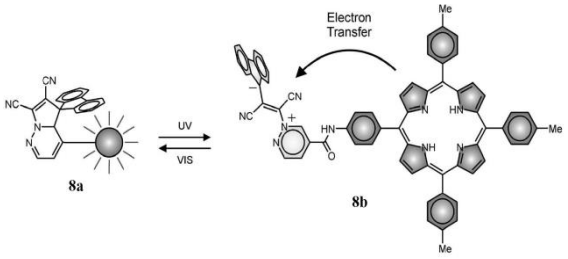
The photochromic component ring closes upon ultraviolet irradiation. While reversing the process can be performed by visible light irradiation. The photoinduced and reversible interconversion between **6a** and **6b** and between **7a** and **7b** (Scheme 4) can lead to the modulation of their luminescence quantum yield [20-24].



Scheme 4. The photoinduced and reversible interconversion between **6a** and **6b** and between **7a** and **7b**.

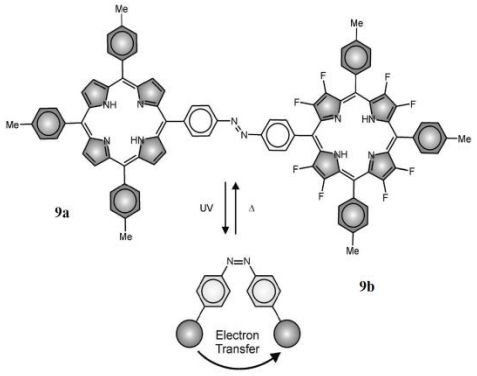
3.2. Electron Transfer

The emissive attitude of a fluorescent partner attached to a photochromic switch can also be amended on the basis of photoinduced electron transfer [12,29]. Indeed, there is a difference in the oxidation and reduction potentials of the two states of a photochrome. Thus, the photoinduced and reversible change in redox potentials of the photochromic switch can be utilized to activate or suppress intramolecular electron transfer pathways in fluorophore–photochrome dyads [30-33]. For example, the molecular dyad **8a** (Scheme 5) forges a spiroindolizine as a photochrome and porphyrin as a fluorophores [31]. After ultraviolet irradiation, opening of the spiroindolizine photochrome ring occurs to furnish the corresponding zwitterionic isomer. Then, electrons can be transferred from the excited porphyrin to the photochrome and this will be favored in terms of thermodynamics and induce the fluorescence quenching of the porphyrin. The initial fluorescence intensity can be restored using visible irradiation, where the zwitterionic form of the photochrome reverts back to the original form, inhibiting the electron transfer pathway. As a result, modulation of the emission of one component can be realized by operating the other with ultraviolet and visible inputs.



Scheme 5. The photoinduced interconversion of **8a** into **8b** activates an electron-transfer pathway quenching the fluorescence of the porphyrin appendage.

The photoinduced and reversible interconversion of a photochromic component can also be utilized to adjust the physical separation between an electron donor and a complementary acceptor [34-39]. A promising example was shown in (Scheme 6), the triad **9a** forge two porphyrin fluorophores and an azobenzene photochrome [36]. In this switch, the fluorinated porphyrin can donate electrons to its nonfluorinated counterpart upon excitation. Particularly, the photoinduced conversion of the azobenzene from a *trans* to a cis configuration minimize the physical separation between the electron donor and the acceptor. Achieving the re-isomerization of the *cis*-azobenzene to the *trans* state can be thermally performed, and the original distance between acceptor and donor is restored with the accompanied recovery of the initial emission intensity.



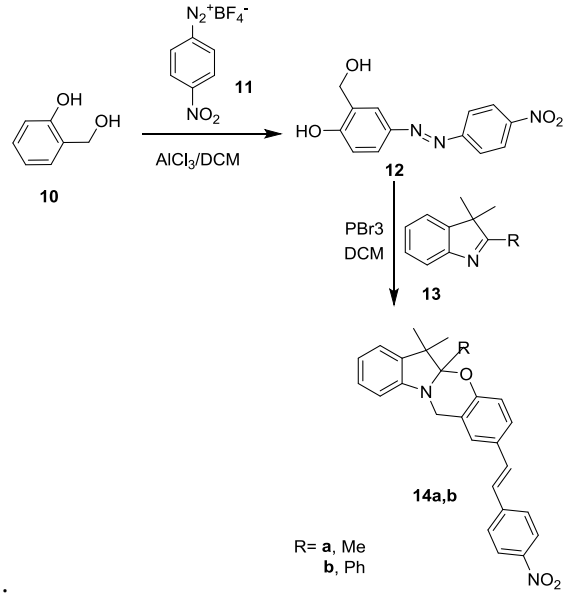
Scheme 6. The photoinduced interconversion of **9a** into **9b** suppresses the emission of the porphyrin fluorophores through activating an electron-transfer pathway.

4. Chromophores platforms

4.1. Oxazine chromophores

4.1.1. Chromogenic Oxazines for Cyanide Detection

Cyanide is extremely toxic and even relatively small quantities of this species are fatal to humans [40, 41]. In fact, most of cyanide detections strategies require either multistep procedures with tiresome sample pretreatments or complicated instrumentation [42]. Two heterocyclic compounds for the colorimetric detection of cyanide designed. The two molecules have a skeleton of a benzooxazine ring fused to an indoline fragment and can be synthesized in three steps starting from commercial precursors [43]. Specifically, two [1,3]oxazines **14a,b** were synthesized in two steps (Scheme 7). The reaction of 2-hydroxymethylphenol (**10**) with 4-nitrobenzene-diazonium tetrafluoroborate (**11**) in aqueous NaOH affords the 4-nitrophenylazophenol **12** which was treated with PBr_3 and the reaction then subjected to an excess of the indoles **13a,b** in situ to furnish the targeted [1,3]oxazine **14a,b.**



Scheme 7. Synthesis of **14a,b**

Two different substituents (a methyl or a phenyl group) are present on the carbon atom at the junction of the fused heterocycles. In the presence of cyanide, the two molecules are quantitatively converted into cyanoamines with the appearance of an intense band in the absorption spectrum at the visible region. The reason for the developing absorption is due to the ring-opening of the benzooxazine with the generation of a 4-nitrophenylazophenolate chromophore [43].

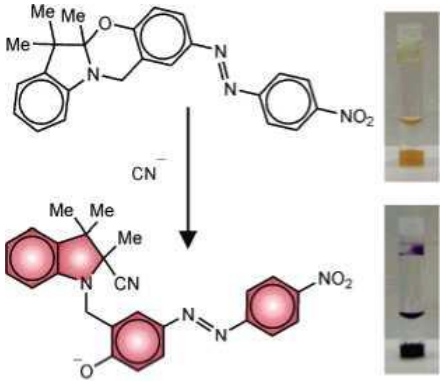


Figure 2. Colour change accompanied the CN detection

For the methyl- substituted oxazine, the fast chromogenic process could be exploited to detect micromolar concentrations of cyanide in water (Figure 2). Additionally, the colorimetric response of this compound to cyanide does not have the interference problem of the halide anions, which instead are common to make the detection of cyanide in conventional sensing protocols are complicated. Thus, this new mechanism and compounds for the colorimetric identification of cyanide can lead to the development of practical strategies for the convenient

determination of this toxic anion in aqueous environments [43].

As shown in figure 3, solutions of **14a** in dichloromethane (DCM) change colour when subjected to aqueous solutions containing micromolar concentrations of cyanide (a-c in Figure 3).

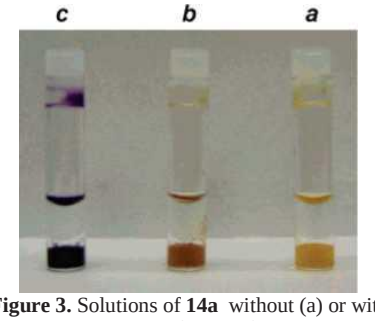
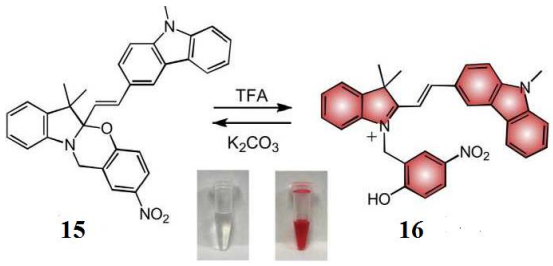


Figure 3. Solutions of **14a** without (a) or with 10 (b) or 100 fM (c) of NaCN.

4.1.2. Switchable inks for saving paper

The world consumed about 400 million tons of paper per year [44]. This huge figure causes serious environmental problems and is mainly a consequence of printing and writing. To alleviate the high demand for paper, and its raw sources, the development of rewritable inks emerged as an applicable strategy. The development of pigments that could easily be removed, or even decolored, from the surface of conventional paper would assist the recycle process of the sheets many times and effectively reduce the consumption. In this context, the development of switchable organic dyes [45] would be particularly valuable. To ensure the rewritable character required for recycling, development of operating principles to erase the color of their constituent components with appropriate stimulations, after printing.



Scheme 8. Reversible interconversion of **15** and **16** under the influence of acid and base, and photos of the corresponding MeCN solutions.

Raymo et al. demonstrated the possibility of developing switchable inks based on the opening and closing of oxazine rings under the action of acid and base respectively [45]. The molecular switch (**15** in Scheme 8) which incorporates an oxazine auxochrome and a carbazole chromophore within its covalent skeleton was designed and prepared in a single synthetic step. Upon addition of acid, opening the oxazine ring of **15** occurred to form **16** and get the carbazole moiety in conjugation with a 3*H*-indolium cation. Due to this structural transformation, a significant change in the visible region of the absorption spectrum occurred and an intense red color appeared (Scheme 8). Addition of base can reverse the process and restore the original and colorless state.

The colour change associated with the reversible interconversion of **15** and **16** could be exploited to develop switchable inks. Specifically, a solution of the colored species could be used for printing a given pattern on conventional paper. Basic treatment can then switch the colored form to the colorless one and in turn, erase the printed information. Consequently, the same sheet of paper can be recycled for further printing. Fortunately, the coloration and decoloration steps can be repeated many times without causing damage of printed paper. Thus, such a switchable ink offers the opportunity to recycle the same sheet for multiple printing and erasing steps and, would permit to significantly save the amount of used papers (Figure 4) [46].

Thus, we can expect that these findings would assist the realization of series of rewritable inks and, possibly, enable advanced printing technologies for the significant reduction of paper consumption.

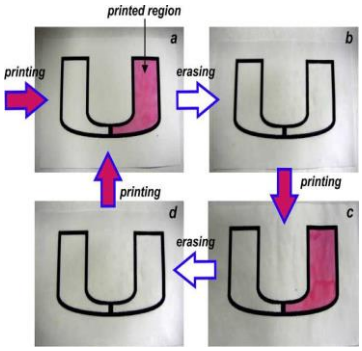
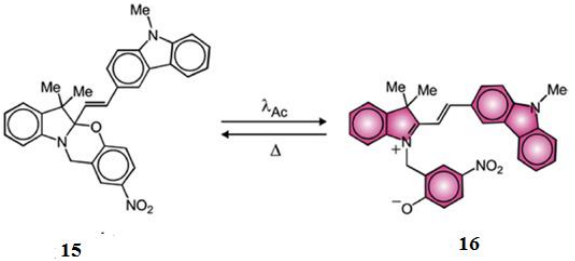


Figure 4. Photos of a paper sheet after treating with **16** in the indicated region (**a** and **c**) and treating the entire sheet with aq. K_2CO_3 for 15 min and drying for 5 min (**b** and **d**).

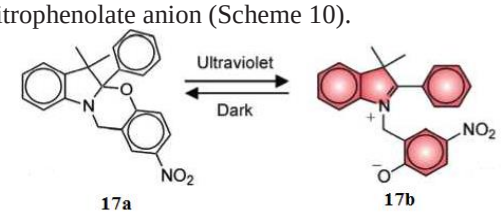
An alternative method for fluorescence photo-activation under the optical control was shown in Scheme 9. Thus, illumination of compound **15** using ultraviolet irradiation opens the oxazine ring and induces switching **15** into **16**. Upon heating the zwitterionic isomer **16** (fluorescent compound), it reverts to the original compound **15** (nonfluorescent).



Scheme 9. Photoinduced and reversible interconversion of the ring-closed (**15**) and –open isomer **(16)**.

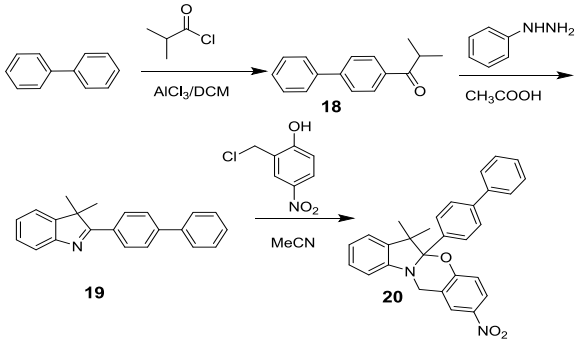
4.1.3. Bichromophoric Photochromes

A new design of photochromic transformation can be regulated to produce a pair of distinct chromophores upon the absorption of only one photon. Opening of the [1,3]-oxazine ring of **17a** was photochemically induced to produce a 4-nitrophenolate chromophore that absorbs in the visible region [47]. Moreover, breaking down of the [C-O] bond at the junction of the two heterocyclic moieties of **17a** merge the adjacent phenyl ring in conjugation with the 3*H*-indolium cation of **17b**. This transformation is utilized to produce additional chromophore capable of absorbing in the visible region in addition to the 4-nitrophenolate anion (Scheme 10).



Scheme 10. Photoinduced and reversible interconversion of the [1,3]oxazine **17a** and zwitterion **17b**.

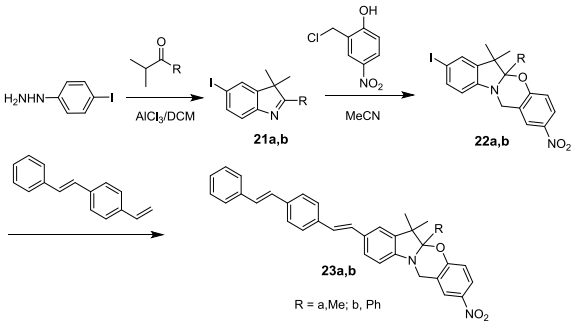
The product **20** was prepared (Scheme 11) in three steps, starting from biphenyl which was acylated with isobutyryl chloride in the presence of aluminium chloride to form the ketone **18** which condensed with phenyl hydrazine. Then, the indole **19** was treated with the phenol derivative to deliver the product **20**.



Scheme 11. Synthesis of the [1,3]oxazine **20**.

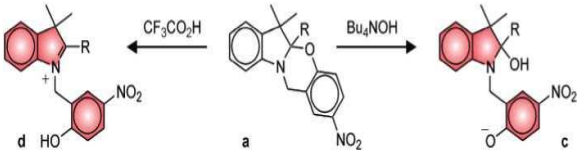
In an attempt to extend the conjugation, **23a,b** were synthesized in three steps from isopropyl methyl ketone and isopropyl phenyl ketone, respectively (Scheme 12). Both ketones condensed with 4-iodophenyl hydrazine to generate the 3*H*-indoles **21a,b** which treated with 4-nitro-2-chloromethylphenol to forge the oxazine platforms **22a,b**. In the last step, these two compounds were coupled with 1-vinyl-*trans*-stilbene in the presence of triethylamine to deliver the target oxazines **23a,b**.

During studying photochemistry of **25a**, a big difference was noticed, where the behavior of **25** does not resemble that of **17a**. Indeed, opening of the [1,3]oxazine ring did not occur upon the laser excitation but intersystem crossing was noticed instead [48].



Scheme 12. Synthesis of the [1,3]oxazines **23a,b**.

Indeed, the acid-base action on the oxazine ring is different. Thus, tetrabutylammonium hydroxide induces opening of the [1,3]oxazine ring of **17a**, to form the anionic hemiaminals **17c**, (Scheme 13). While, trifluoracetic acid induce opening the [1,3]oxazine ring of **17a**, to form the 3H-indolium cations **17d**.



Scheme 13. Opening of the [1,3]oxazine ring of **a** to form either the anionic hemiaminal **c** or the cationic 3*H*-indolium **d**.

4.2. Photoactivatable Anthracenes

The skeleton of anthracene is considered as one of the promising building block for the construction of photoactivatable fluorophores (Figure 5).

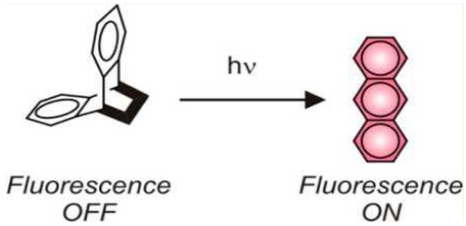
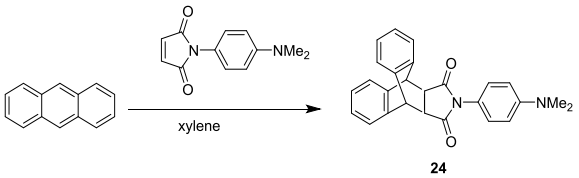


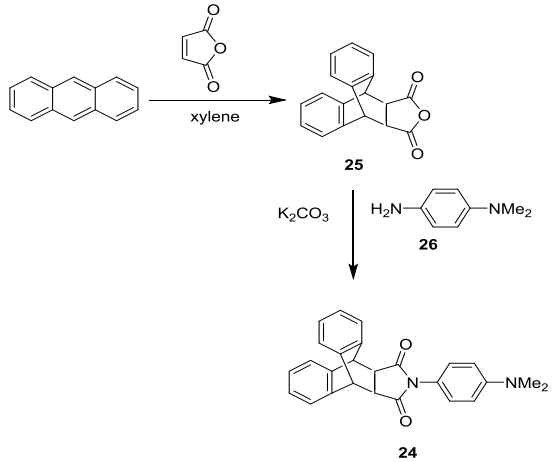
Figure 5. Fluorescence photoactivation of anthracene

It was noticed that substitution on 9,10 positions of the anthracene skeleton with a maleimide bridge electronically isolates its peripheral phenylene rings and kills the characteristic fluorescence. For this study, 9,10-substituted cycloadduct **24** was synthesized through the cycloaddition of maleimide on the central ring of anthracene (Scheme 14) [50]. This method can be generally applied for the synthesis of an entire family of anthracene cycloadducts with different bridging units.



Scheme 14. Synthesis of 9,10-substituted cycloadduct **24**.

Compound **24** can be accessed by an alternative method of preparation. Thus, reaction of maleic anhydride with anthracene produces anhydrides **25**. Reaction of this compound with **26**, under the action of potassium carbonate, produces **24** (Scheme 15).



Scheme 15. Alternative method for synthesis of **24**.

Upon ultraviolet illumination, the corresponding oligoacenes with a 4-(NMe_2)-phenyl group on the maleimide nitrogen atom can be subjected to retro-cycloaddition (Figure 6). This structural conversion recovers the aromatic behaviour of the central ring of chromophore and activates its emission with high fluorescence quantum yields. Thus, these anthracene cycloadducts can be used to as valuable materials to translate into viable operating principles for fluorescence activation and, particularly, realize valuable photoactivatable fluorophores for imaging applications [49].

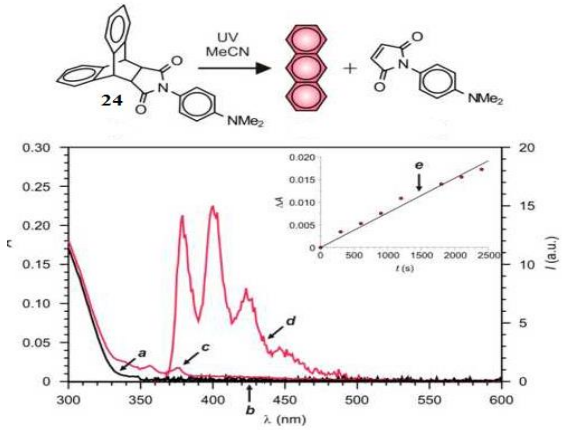


Figure 6. Absorption and emission spectra of **24** before (a and b) and after (c and d) ultraviolet (UV) irradiation

Substitution in 9,10-positions in anthracene with phenylethynyl moiety lead to the formation of the highly fluorescent chromophore **28** [51]. Emission activation of this molecule can be achieved under photochemical control by introducing an α-diketone bridge across 9,10-positions of the anthracene scaffold like the cycloadduct **27**. Illumination of this compound at λ_{Ac} (Figure 7) results in the cleavage of the α-diketone bridge with the liberation of two molecules of carbon monoxide (2CO) and the corresponding anthracene chromophore **28** was reproduced with its characteristic band in the absorption spectrum [51]. Upon illumination with an excitation wavelength of 390 nm, an intense emission of the photoproduct appears as a result of this photochemical transformation, (d in Figure 7).

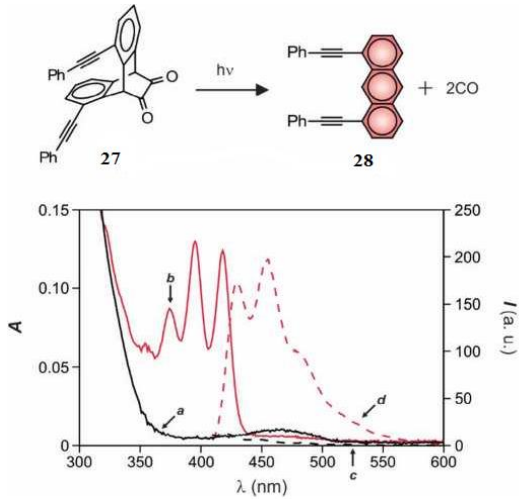


Figure 7. Absorption and emission spectra of **27** before (**a** and **b**) and after (**c** and **d**) ultraviolet (UV) irradiation (254 nm, 40 min)

BODIPY chromophores

The borondipyrromethene (BODIPY) skeleton emerged as a promising choice which offers outstanding photophysical properties along with synthetic accessibility [52-53]. Therefore, the identification of structural designs to activate the fluorescence of this versatile organic chromophore can be exploited to realize valuable photoactivatable probes for a variety of imaging applications. Indeed, structural modifications can be achieved through the substitution on the two pyrrole rings, the boron, or the carbon atoms connecting the two heterocycles and this can be utilized to regulate the spectroscopic signature of the BODIPY chromophore and provide molecules able to emit light at the visible region with super quantum efficiencies (Figure. 8). Hence, BODIPY chromophores have become valuable building blocks for the assembly of laser dyes, fluorescent probes, electroluminescent materials, light harvesters and sensitizing agents [54].

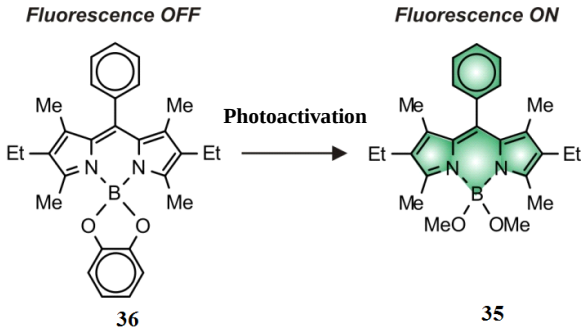
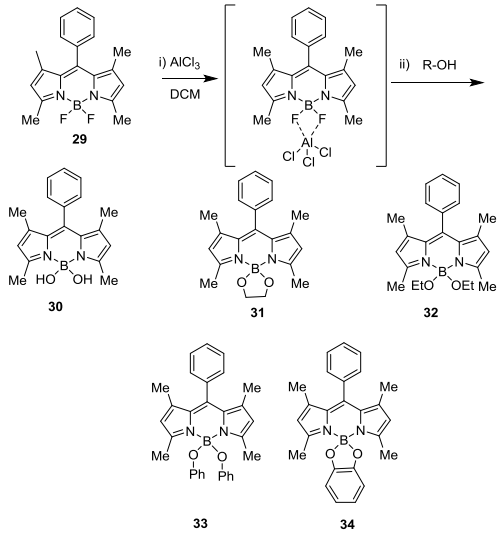


Figure 8. Photoactivation of BODIPY fluorophore

4.3.1. *Meso*-Phenyl-BODIPY

Reaction of BODIPY **29** with catechol and salicylic derivatives, in the presence of aluminum chloride, results in the displacement of the fluoride ligands around the boron center of the BODIPY platform to produce **30**-**34** (Scheme 16).



Scheme 16. Synthesis of BODIPY derivatives **30**-**34**

Some important calculated studies (Figure 9) demonstrated the exclusive presence of an intramolecular charge-transfer (ICT) state in the case of **36**. Despite this state is forbidden, but it can be possible from S_1 after excitation. This will provide a nonradiative decay of the excited state and that makes BODIPY chromophore fluorescent. In the case of **35**, such pathway is not available, instead, this compound radiatively relaxed to S_0 after excitation to S_1 (Figure 9) [55].

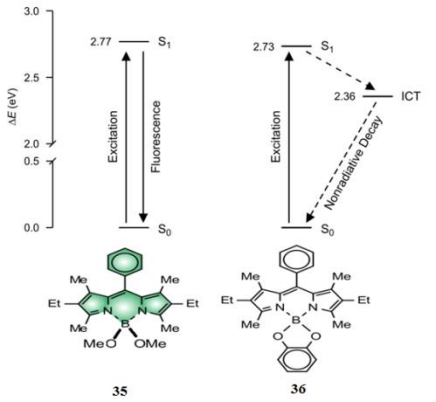


Figure 9. Excitation dynamics of **35** and **36** with B3LYP energies of the associated electronic states.

The characteristic band of the BODIPY chromophore is shown at a λ_{Ab} of 528 nm in the absorption spectrum (***a*** in Figure 10), while no fluorescence appears in the emission spectrum (***b*** in Figure 10). Addition of just one equivalent of trifluoroacetic acid (TFA) is sufficient to induce the appearance of an intense band at a λ_{Em} of 550 nm (***c*** in Figure 10).

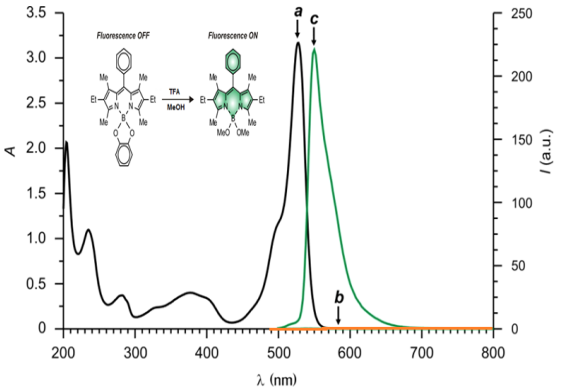


Figure 10. Absorption spectrum (***a***) of a MeOH solution of **36**. Emission spectra (λ_{Ex} = 490 nm) of a MeOH solution of **36**, recorded before (***b***) and (***c***) after the addition of TFA (1 eq.).

It is evident that replacing the catechol chelator of the nonemissive compound **36** with two methoxy ligands was achieved under acidic conditions in methanol to form the emissive BODIPY **35**.

It was confirmed that the photoacid generator is responsible for emission activation. Figure 11 shows Emission spectra alone for photolysis of bodipycatechol **36** (10^{-5} M) in MeOH with photoacid generator **37** (1: 1 eq).

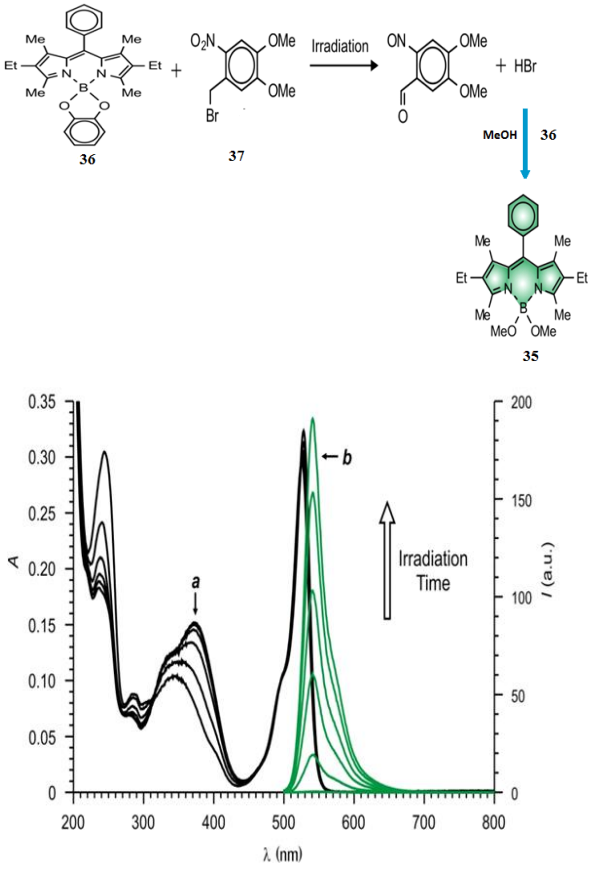
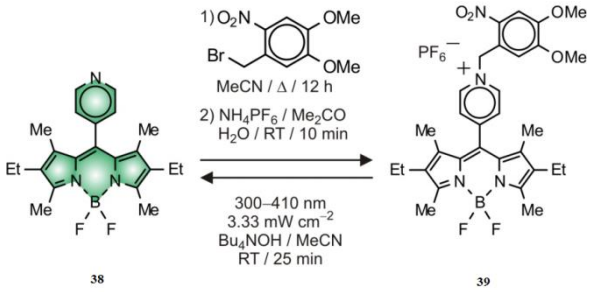


Figure 11. Absorption (***a***) and emission (***b***) spectra of equimolar MeOH solutions of **36** and **37**, before and during irradiation

4.3.2 *Meso*-Pyridinyl-BODIPY

The 2-nitrobenzyl group of **39** can be photochemically transformed into the corresponding 2-nitrosobenzaldehyde and a proton is released. In turn, the emission of the adjacent BODIPY fluorophore is quenched if the pyrid-4-yl moiety of **38** is protonated [56]. To study this technique, N-alkylation of pyrid-4-yl-BODIPY **38** with (1-bromomethyl)-4,5-dimethoxy-2-nitrobenzene (Scheme 17) was realized to form **39**. Isolation of the hexafluorophosphate salt of the corresponding pyridinium cation **39** was achieved after counterion exchange.



Scheme 17. Synthesis of the hexafluorophosphate salt of **39** from **38** and photoinduced generation of the latter from the former

The absorption spectrum of the hexafluorophosphate salt of **39** showed the appearance of an absorption band at 534 nm which is characteristic of BODIPY chromophore. After the ultraviolet illumination of **39**, the characteristic emission band (c–g in Figure 12) of **38** appeared [57].

.

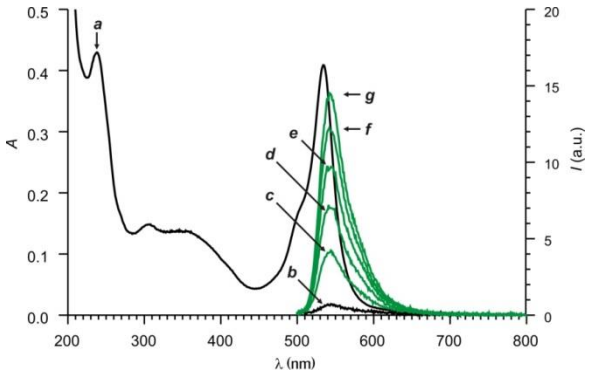
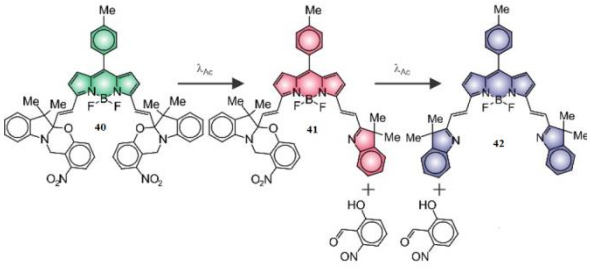


Figure 12. Absorption (***a***) and emission (***b***) spectra of an equimolar solution of the hexafluorophosphate salt of **39** and Bu_4NOH. Emission spectra of the same solution after irradiation (300–410 nm)

The photoinduced conversion of a pyridinium cation into a pyridine moiety prevent the electron transfer process occurred from the BODIPY fluorophore upon excitation and induce the activation of the emission of BODIPY [57].

Recently, a promising application of photoactivatable fluorophore was reported [58]. Specifically, under mild visible illumination, two photocleavable oxazines are successively cleaved of from the same BODIPY, producing mixtures of three fluorophores with resolved emissions inside polymer beads (Scheme 18). Controlling illumination conditions assisted the precise regulation of amounts of the three emissive molecules which enable imprinting barcodes, each consisting of three fluorescence signals, within distinctive beads. Furthermore, using unique and detectable codes to mark a wide range of beads would allow their individual tracking in time. By these excellent conditions (visible illumination and moderate intensities), it is easy to photochemically imprint barcodes also in living worms [58].

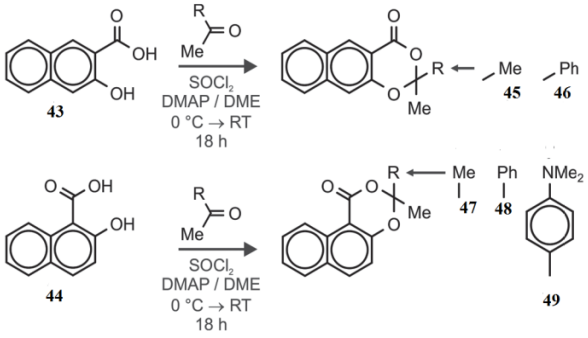


Scheme 18. Photoinduced transformation of **40** into **41**, **42**, and nitrosoaldehyde.

4.4. Dioxinone chromophore (A pH-Gated Photocage)

Photoinduced electron transfer (PET) [59] mechanisms to activate fluorescence under chemical control could be utilized to gate photochemical reactions instead. Particularly, covalent connecting a chromophore capable of processing a certain photochemical transformation to an electron donor, in the shape of an amino group. By this pathway, an electron is transferred from the amino group to the excited chromophore and this precedes a nonradiative deactivation of the latter and blocks the photochemical reaction. The amino group is protonated and this can then be utilized to inhibit the PET process and activate the photochemical reaction. In fact, dioxinone heterocycles emerged as a promising photocleavable protecting groups [60] for carbonyl compounds that, upon excitation, generate reactive ketenes, [60,61] which can be trapped in the form of esters, and either aldehydes or ketones [61-64] .

Dioxinones **45-49** were simply synthesized (Scheme 19) from the commercially available compounds **43,44** through treating a dimethoxyethane solution of these compounds with an thionyl chloride and either acetone or acetophenone, using catalytic amounts of 4-dimethylaminopyridine [65] .



Scheme 19. Synthesis of dioxinones **45-49**

Particularly, the fluorescence quantum yields of **48** and **49** are very low. Protonation of the dimethylamino group using one equivalent of trifluoracetic acid (CF_3CO_2H), however, block its electron donation and a significant increase in the fluorescence of the naphthalene chromophore was noticed. Specifically, a new absorption band characteristic of 1-[4-(dimethylamino)-phenyl]-ethanone was developed in the absorption spectrum (b and *c* in Figure 13), and that reveal the photoinduced cleavage of the dioxinone ring and the production of this particular ketone.

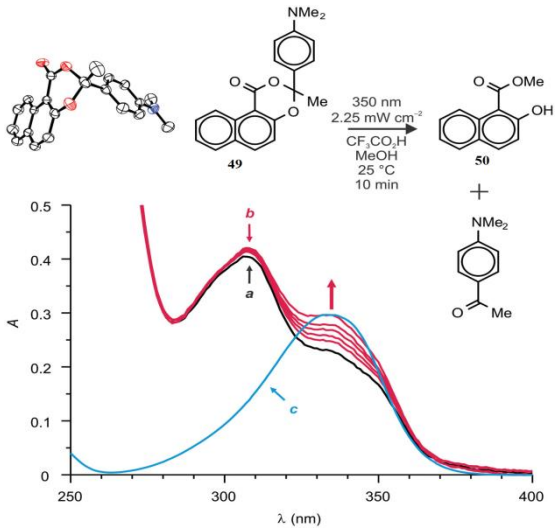


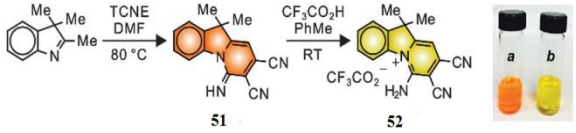
Figure 13. Absorption spectra of a solution of **49** and CF_3CO_2H recorded before (a) and during (b) irradiation together with the normalized absorption spectrum (c) of 1-[4-(dimethylamino) phenyl]ethanone

4.5. Indolizine switch

Halochromism is a phenomenon by which certain organic chromophores reversibly change, upon acidification, their ability to absorb photons in the visible region of the electromagnetic spectrum [66].

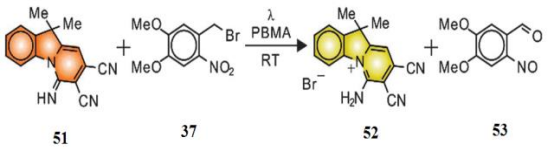
This causes drastic changes in color and is the basis the operating principles of pH indicators [67] and smart inks [68-70].

Formation of **51** can be realized through heating a solution of 2,3,3-trimethyl-3*H*-indole and tetracyanoethylene (TCNE) in N,N-dimethyl-formamide (DMF) of at 80 °C for one hour (Scheme 20). Then, subjecting a mixture solution of 5**1** in toluene with CF_3CO_2H produced **52** and resulted in a color change from orange to yellow [71].



Scheme. 20 Synthesis of **51** and its conversion into **52** upon acidification together with a picture of PhMe solutions of **51** (a) and **52** (b).

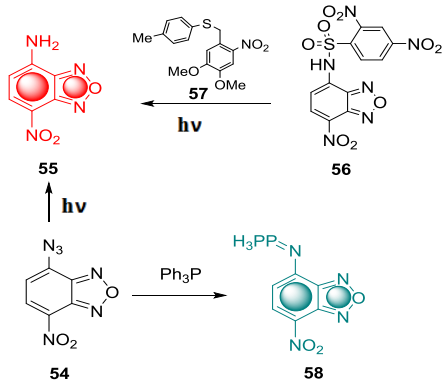
Using photoacid generators can enable operating halochromic molecular switches under the influence of optical stimulations [72]. Excitation of the photoacid generators results in the photochemically release of acid, which can then protonate the halochromic molecules. If the halochromic switch has a good emission in the protonated form, then it will be a fluorescent upon excitation. Thus, the illumination of **37** (Scheme 21) at an appropriate wavelength generates **53** with releasing hydrobromic acid [72]. In turn, the photogenerated acid realized switching **51** into **52** and allow the fluorescence activation of the latter species.



Scheme 21. Fluorescence activation of **51** with the aid of **37**

4.6. Furazan chromophores

It is well-known that amines are strong electron-donating substituents, while, the azides are weakly electron withdrawing. It is possible to exploit this information to recover fluorescence from aryl azides especially that they are photolabile. Additionally, nitrene intermediate can be stabilized by insertion of electron-withdrawing substituents on the aromatic ring and this will assist the generation of the amino substituent [73]. Because push–pull chromophores, like the furazan molecule **54** shown in Scheme 22, contain a strong electron withdrawing substituent, photoconversion of an azido push–pull molecule **54** to the fluorescent amino species **55** will be more facile upon irradiation with activating light [73]. Compound **55** can also be obtained from irradiation of mixture of **56** with **57,** which affected cleavage of sulphonamide to produce furazan **55** [74]. While the highly fluorescent compound **58** could be obtained upon treating **54** with triphenylphosphine [75].



Scheme 22. Photoconversion of an azido push–pull furazan to the fluorescent amino molecule

4. Conclusion

Great efforts were made to design functional chemical systems which combine luminescent and photochromic components within the same construct. The luminescent component emits electromagnetic radiations upon excitation at one wavelength (λ_{ON}). The photochromic component switches from a colorless state to a colored species upon excitation at another wavelength (λ_{OFF}). The photogenerated state of the photochrome is designed to quench the emission of the luminophore on the basis of intercomponent electron and/or energy transfer processes. Thus, the emission of these luminophore–photochrome constructs can repeatedly be switched off and on simply by turning on and off, respectively, an exciting beam at (λ_{OFF}), while irradiating the sample at λ_{ON}.

The unique photochemical and photophysical properties of photoactivatable fluorophores can be exploited also to solve the problem of diffraction which limitates the resolving power of fluorescence microscopes. And this assist to record images permits with spatial resolution at the nanometer level. Indeed, visualization of biological samples tagged with photoactivatable labels can be achieved with a resolution that is otherwise impossible to be attained with conventional fluorophores. The operating principles can be based on either the cleavage of a protecting group by photoinduction or the generation of an emissive chromophore by a photochromic transformation. In both cases, observation of significant fluorescence is noticed only upon the formation of the photochemical product.

Conflicts of interest

"There are no conflicts to declare".

5. References

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الملخــص العربي

استخدام المركبات العضوية في التنشيط الضوئى للتفلور (الوميض)

فى هذا البحث المرجعي تم تناول سلسلة من التحولات الضوءكيميائية المقترنة مع التفاعل الكيميائى التي تسمح بتنشيط الوميض الخاص بحاملات اللون تحت المتابعة البصرية. وهذه الميكانيكية ثنائية الجزئ لتنشيط الوميض أو التفلور يمكن ترجمتها الى استراتيجية عامة لانجاز مركبات مستجيبة للضوء بناءا على اقتران التحولات الضوئية بالتفاعلات الكيميائية.بالاضافة الى المزيج الرائع للخصائص الواعدة للمركبات فقد تم اكتشاف الاليات التى تعمل على التنشيط الضوئى الفلورى حيث أن هذه الجزيئات قادرة على أن تتحول من الحالة غير الانبعاثية الى الحالة الانبعاثية عند التعرض للضوء عند طول موجى منشط ويمكن أن تقدم امكانية التحول الفلورى حصريا داخل منطقة محددة من الفراغ فى فترة معينة من الزمن. ومثل هذا التحكم الفراغى المؤقت للتفلور قد تم ترجمته الى امكانية تتبع ورصد الأحداث الديناميكية فى الوقت الحقيقى لحدوثها وأيضا تسجيل صور عالية الدقة تصل تفاصيلها الى مستوى النانوميتر. كما تم أيضا دراسة دمج فلوروفور الكومارين وفوتوكروم الأوكسازين فى نفس الهيكل البنائى للجزئ الواحد بطريقة متكاملة كما تم اتصالهم مع الأجسام المضادة الثانوية عن طريق الترابط التساهمى. وعند إضاءة هذا المترافق (الأجسام المضادة - صبغي مزدوج) عند طول موجي تنشيطى مناسب يتم فتح حلقة الأوكسازين بطريقة انعكاسية. وعند إثارة الناتج الضوئي بطريقة انتقائية يتم ملاحظة وميض قوى، مما يسمح للكشف عن المترافقات الحيوية المنشطة في مستوى الجزيء الواحد. ويمكن استغلال هذه الأحداث الناتجة من تفعيل الوميض لعمل انحلال مؤقت للبواعث الفردية وإعادة بناء صور فائقة الدقة للخلايا المناعية المرقومة. وبالتالي، فإن هذه الجزيئات القادرة على التحول باستخدام الضوء, بالاشتراك مع امكانية وضع العلامات على الأجسام المضادة، يمكن الاستفادة منها فى تطوير مجسات قيمة للتصويرالضوئى الحيوى فائق الدقة. بالاضافة الي التطبيقات الواعدة الأخري مثل الحبر السري الفوسفوري وكواشف السيانيد السام وغيرها.