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«Fluorescent Switchable Dyes for Monitoring of Targeted Drug Delivery»

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Бондар Катерина Вікторівна

Керівник: д.х.н., проф. Анікін В.Ф. ______
(підпис)
к.х.н., проф. Паценкер Л.Д. ______
(підпис)

Рецензент: к.х.н., доц. Щербакова Т.М. ______
(підпис)

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Завідувач кафедри:

______ д.х.н., проф. Ішков Ю.В.
(підпис)

Голова екзаменаційної комісії:

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Odessa I.I. Mechnikov National University
Faculty of Chemistry
Department of Organic Chemistry

Master Thesis

Fluorescent Switchable Dyes for Monitoring of Targeted Drug Delivery

Performed by: full-time student
Specialty 8.04010101 Chemistry
Bondar Kateryna

Supervisor: Prof. Valeriy F. Anikin ______
(signature)

Prof. Leonid D. Patsenker ______
(signature)

Reviewer: Ph.D Tatyana M. Shcherbakova ______
(signature)

Recommended for defense:
Protocol of the meeting
of the department:
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The head of the department:

______ Prof. Yuriy Ishkov
(signature)

The head of the examination committee:

___________ ______________________
(signature) (name, surname)

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ABSTRACT

Targeted drug delivery (TDD) including monitoring of drug release improves the quality of anti-cancer treatment. A basic drug delivery system (conjugate) consists of a turn-on switchable fluorescent reporter bound to a drug and to a targeting carrier that increases selectivity of drug delivery to the treated cells or tissue. When drug is released, the fluorescence intensity increases signaling the drug delivery event. At the moment there is a pronounced lack of fluorescent dyes suitable as the switchable reporters for TDD while the exciting switchable reporters have certain shortcomings such as poor dynamic range of the fluorescence intensity increase upon the drug release, insufficient brightness within the long-wavelength spectral region, and/or an increased toxicity. These drawbacks decrease efficiency of anti-cancer treatment.

In this work, we synthesize novel, switchable TDD reporters based on the styryl and oxonol dyes, investigate spectral properties of these new dyes, and evaluate these reporters and their conjugates with a drug simulating compound. In particular, we develop synthetic approaches for the new, indolenine based, hydrophilic 4-hydroxy-styryl- and (4-hydroxyphenyl)buta-1,3-dienyl styryl dyes and pentamethine oxonol dye containing terminal 1,3-diphenyl-1*H*-pyrazol-5(4*H*)-one terminal moieties and synthesize these dyes using the developed approaches. Hydroxylic function of the obtained styryl dyes is derivatized by acetyl group that simulate a potential drug moiety. Spectral properties of the dyes and the acetyl group cleavage profiles are investigated and it is evidenced that these dyes have a great potential as the basic structures for further the development of switchable reporters for TDD monitoring.

The obtained results are promising and indicate that further research in the field of styryl and oxonol based switchable reporters may help to succeed in the development of efficient fluorescence based systems for targeted drug delivery.

Keywords: styryl dye, oxonol dye, switchable dye, reporter, drug delivery platform, targeted drug delivery, anti-cancer treatment, fluorescent monitoring.

The master thesis consists of 60 pages, 17 figures, 29 schemes and 112 literature sources.

CONTENT

ABBREVIATIONS

ε	Extinction coefficient
Φ_F	Fluorescence quantum yield
λ	Wavelength
λ^*	Fluorescence excitation wavelength
$\tau_{1/2}$	Half-life of a chemical process
ACN	Acetonitrile
aq.	Aqueous
DCM	Dichloromethane
LC-MS	Liquid chromatography — mass spectrometry method
LDA	Lithium diisopropylamide, $[(CH_3)_2CH]_2NLi$
MTBE	2-Methoxy-2-methylpropane (methyl *tert*-butyl ether)
NIR	Near-infrared
NMR	Nuclear magnetic resonance
RT	Room temperature (25±2 ºC)
TDD	Targeted drug delivery
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

INTRODUCTION

Targeted drug delivery (TDD) including monitoring of drug release increases the quality of medical treatment including anti-cancer therapy [1–3]. A basic drug delivery platform (TDD system or TDD conjugate) comprises a turn-on switchable *fluorescent reporter* bound to an anti-cancer *drug* by means of a biodegradable *cleavable linker*, and to a targeting *carrier* by means of a *non-cleavable linker* and, together, form the drug delivery conjugate [4,5].

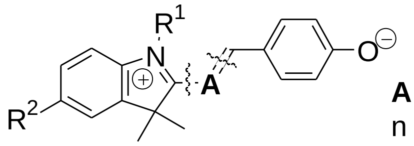
There are a lot of conventional fluorescent dyes available that do not change their spectral properties when bound—unbound covalently to other molecules. However, until now, there is a pronounced lack of **turn-on switchable** fluorescent reporters suitable for TDD monitoring. As compared to the conventional dyes the switchable dyes noticeably change their fluorescence (off-on) when bound—unbound to other molecules. The exciting switchable reporters have certain **drawbacks** such as insufficient brightness within the long-wavelength (red and NIR) spectral region that is more useful for detection in biological systems but also poor dynamic range in the fluorescence intensity change upon drug release. These shortcomings decrease the signal-to-noise ratio, sensitivity, accuracy, reliability, and robustness of fluorescence based TDD monitoring. This work aims to help to improve these drawbacks.

The main goal of the present study is to develop the novel, turn-on switchable reporters for fluorescence based monitoring of targeted drug delivery (TDD).

Objectives:

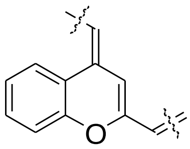
1. Synthesis of new turn-on switchable reporters.
2. Conjugation of these new reporters with drug-modeling compound in the example of acetyl group.
3. Spectral, photophysical and photochemical characterization of the new dyes and conjugates.
4. Spectrophotometric and spectrofluorometric determination of the model drug (acetyl group) cleavage profiles for the drug—reporter conjugates.

As the switchable reporters we intended to synthesize and evaluate *styryl* dyes of general formula **St** and *oxonol* dyes of general formula **Ox**:

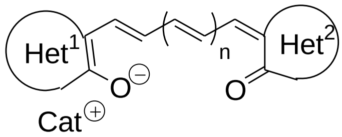


Styryl dyes (St)

A = (=CH-)$_n$
n = 0, 2



R^1 = $(CH_2)_5COOH$, $(CH_2)_3SO_3H$
R^2 = H, SO_3H, CH_2COOH



Oxonol dyes (Ox)

These dyes were chosen for the following reasons:

1. These dyes can potentially be bound with drugs by the oxygen atom to form non-fluorescent drug—dye conjugates (the dye is in the "off" form) but upon hydrolytic cleavage of the drug—dye bond the dye must turn into the fluorescent "on" form signaling the drug release event.

2. Due to the extended π-electron conjugated system and delocalized charge, these dyes must absorb and emit within the long-wavelength spectral region and therefore are useful for detection in deeper tissue. There is a possibility to further extend the conjugated chain to get even higher red-shift of the spectral bands.

3. The molecular structure of these dyes enables ease of their modification to introduce reactive groups for binding to a TDD carrier. It is possible also to introduce hydrophilic and hydrophobic groups to adjust an optimal balance of hydrophobic-hydrophilic properties, which is necessary for the transport of TDD conjugates with blood stream and their uptake by targeting cells and tissue.

4. These dyes belong to polymethines that are known to have low toxicity and are suitable therefore for *in vivo* applications.

5. These dyes are novel; they have never been synthesized and evaluated for drug delivery monitoring. At the same time, we anticipate that these dyes can be synthesized by known methods while their conjugation with drugs and carriers is supposed to be a challenging task.

A detailed explanation of these equipments is given in the next section.

CONCLUSIONS

1. Synthetic approaches to the novel, indolenine based, hydrophilic 4-hydroxystyryl- and (4-hydroxyphenyl)buta-1,3-dienyl styryl dyes and pentamethine oxonol dye containing terminal 1,3-diphenyl-1*H*-pyrazol-5(4*H*)-one terminal moieties were developed and the dyes were synthesized.
2. Hydroxyl function in the obtained styryl dyes was derivatized by acetyl group that simulated a drug moiety. Practical methods for the synthesis of these compounds were developed.
3. In the example of acetyl group, it was demonstrated that the introduction of substituents in the phenolic hydroxyl group of styryl dyes results in disappearing of the long-wavelength, highly-intensive absorption and emission bands. Simultaneously, a new, blue-shifted absorption band and a weak, blue-shifted fluorescence appear.
4. The half-life of the acetyl group cleavage in the hydroxy-styryl dye at pH 8.0 is about 2.5 h. Due to the reasonable cleavage half-life and pronounced increase in the fluorescence intensity, which accompanies the acetyl group cleavage, hydroxy-styryl dyes can be used for monitoring of targeted drug delivery.
5. Pentamethine-oxanol dye containing terminal 1,3-diphenylpyrazole-5-one moiety has intensive absorption and fluorescence within the red spectral region (639 nm / 681 nm). These bands significantly decrease with increasing the medium acidity due to the protonation of pyrazolone oxygen atom. This effect evidences that binding of the hydroxyl group to a drug molecule will enable application of pentamethine-oxonol based dyes for monitoring of targeted drug delivery.

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