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Design and Synthesis of New 1,8-Naphthalimide Derivatives and Their Application in Nanotechnology and Fluorescent Bioimaging

Abstract

The development of new, effective fluorophores suitable for targeted drug delivery systems is a pressing challenge in modern organic chemistry and nanotechnology. Fluorescence imaging enables real-time studies of the biodistribution and release kinetics of drugs both *in vitro* and *in vivo*, and it also opens up opportunities for intraoperative monitoring, providing visual support for surgical interventions.

Among all the diverse organic fluorophores, 1,8-naphthalimide derivatives stand out due to their unique combination of properties: chemical reactivity (relative ease of functionalization), high spectral characteristics and a favorable biological profile (low toxicity and biological activity). These features are driving the intensive development of the chemistry of this class of compounds, particularly in the direction of creating theranostic systems that combine diagnostic and therapeutic functions. Varying the substituents in the 4^{th} position of the aromatic ring (introduction of *O*-, *S*- or *N*-containing groups) makes it possible to obtain fluorophores that luminesce in a wide range of the optical spectrum. The introduction of reactive functional groups into the 1,8-naphthalimide structure opens up the possibility of their covalent modification for the subsequent development of targeted delivery systems, enabling their reliable visualization using advanced fluorescence microscopy methods.

Polymers of both natural and synthetic origin are widely used as drug carriers. In this study, human serum albumin (HSA) and poly(lactic-*co*-glycolic acid) (PLGA) were selected, both of which have been used to develop and introduce more than 30 drugs into clinical practice.

To ensure selective targeting of cellular structures, polymers are functionalized with vector groups. To create osteotropic systems, an α-hydroxybisphosphonate

fragment, which has a high affinity for hydroxyapatite and bone tissue, is used. Thus, the presence of a vector group and a functional substituent for covalent binding to the polymer in the fluorophore structure enables the creation of targeted delivery systems capable of fluorescence imaging, where labeling does not significantly affect the physicochemical properties of the carrier or the release profile of the pharmaceutical substance.

In this work, the synthesis of new derivatives of 1,8-naphthalimide was carried out, which can be considered as an affordable alternative to foreign analogues in conducting medical and biological research.

The aim of the study was to develop methods for the synthesis of new 1,8-naphthalimide derivatives that fluoresce in a wide range of the optical spectrum, and to create polymer systems based on them for targeted delivery of pharmaceutical substances with the possibility of fluorescent biovisualization.

Objectives:

1. Synthesis of *O*-, *S*-, *N*-substituted 1,8-naphthalimide derivatives containing amino-, carboxy-, hydroxy-, and maleimide functional groups for subsequent covalent modification of natural and synthetic polymers.

2. Synthesis of *O*-, *S*-, *N*-substituted 1,8-naphthalimides with an α-hydroxybisphosphonate moiety for visualization of hydroxyapatite and the study of osteotropic targeted delivery systems based on them.

3. Covalent modification of HSA and PLGA with synthesized 1,8-naphthalimide derivatives to obtain fluorescent targeted delivery systems for biologically active compounds.

4. Characterization of the spectral and luminescent properties of the obtained fluorophores, their polymer conjugates, and nanoparticles, including determination of fluorescence quantum yields, brightness, and photostability.

5. Evaluation of the ability of the synthesized fluorophores and polymer systems to enable intracellular localization and *in vitro* visualization in cell cultures using fluorescence microscopy.

Scientific novelty.

In this study, 23 new compounds were synthesized, including 2 new 4-(2-hydroxyethylthio)-1,8-naphthalimide derivatives containing 4-(2-aminoethyl)-morpholine, N-tosylethylenediamine, N,N-dimethylethylenediamine, ethylene-diamine, hexamethylenediamine, and propylcarboxyl substituents at the imide nitrogen atom. These compounds were used to create fluorophores for the covalent modification of PLGA. The introduction of vector groups into the fluorophore structure potentially enables their use for selective labeling of cellular organelles such as lysosomes, the endoplasmic reticulum, and mitochondria.

Additionally, 6 new 1,8-naphthalimide derivatives containing a primary amino group and 2 previously undescribed fluorophores containing a carboxyl group were synthesized. Using compounds emitting in the 460-625 nm range, PLGA was covalently modified to yield 12 new conjugates, which were used to produce fluorescent nanoparticles.

5 new maleimide derivatives of 1,8-naphthalimide were synthesized for the covalent modification of human serum albumin (HSA).

This study also presents the synthesis of 4 new *O*-, *S*-, and *N*-substituted 1,8-naphthalimide derivatives containing a bisphosphonate moiety. For the first time, fluorescence imaging of a PLGA-based osteotropic targeted delivery system was performed using the osteosarcoma cell line *Saos-2*, opening up opportunities for the development of effective theranostic agents for the treatment of bone diseases.

Practical significance.

The synthesis and characterization of new 1,8-naphthalimide derivatives expands our fundamental understanding of the relationship between structure and photophysical properties in this series of heterocyclic fluorophores. The obtained data on the influence of the nature of substituents (*O*-, *S*-, *N*-containing, as well as maleimide and bisphosphonate moieties) on the spectral and luminescent characteristics of the compounds contribute to a better understanding of the mechanisms of photophysical processes, including the formation of TICT state, and provide a basis for the targeted design of fluorophores with desired properties.

This work resulted in the preparation of new 1,8-naphthalimide derivatives containing vector groups that ensure selective targeting to lysosomes, the endoplasmic reticulum, and mitochondria. Synthesized fluorophores with an α-hydroxybisphosphonate moiety can be used to visualize hydroxyapatite in soft tissues, as well as to study microcalcifications and metastatic bone lesions.

The developed maleimide derivatives of 1,8-naphthalimide enable covalent modification of human serum albumin, expanding the arsenal of fluorescent delivery systems based on natural polymers.

Covalent modification of poly(lactic-*co*-glycolic acid) (PLGA) with synthesized fluorophores represents one of the first examples of the creation of targeted delivery systems with fluorescence imaging capabilities based on this class of compounds. *In vitro* experiments demonstrated that the resulting PLGA–1,8-naphthalimide nanoparticles exhibit high photostability, comparable to that of particles based on commercially available markers. This enables long-term studies of the distribution of nanoobjects and their interactions with cells in complex biological environments.

The compounds obtained in this study can be considered an affordable Russian alternative to foreign fluorescent markers used in nanotechnology for visualization of targeted delivery systems, as well as in microscopy for studying biological objects.

The main provisions for the defense.

1. New methods have been developed for synthesizing *O*-, *S*-, and *N*-substituted 1,8-naphthalimide derivatives containing amino, carboxy, hydroxy, and maleimide functional groups. The resulting compounds can be used for covalent modification of natural (HSA) and synthetic (PLGA) polymers to create fluorescent targeted delivery systems.

2. The proposed approaches to synthesizing *O*-, *S*-, and *N*-substituted 1,8-naphthalimides containing an α-hydroxybisphosphonate moiety enable visualization of hydroxyapatite and provide the basis for the development of osteotropic targeted delivery systems.

3. Covalent binding of synthesized 1,8-naphthalimide derivatives to poly(lactic-co-glycolic acid) (PLGA) and human serum albumin (HSA) yields fluorescent conjugates that are promising for developing targeted delivery systems for biologically active compounds.

4. The spectral and luminescent properties of the synthesized fluorophores (position of absorption and emission maxima, quantum yields, and photostability) are retained after their covalent binding to polymers and during the formation of nanoparticles based on the resulting conjugates.

5. PLGA-based nanoparticles labeled with 1,8-naphthalimide derivatives are effectively internalized by 4T1 murine breast carcinoma cells and can be visualized by laser scanning confocal microscopy, confirming their suitability for studying intracellular distribution *in vitro*.

6. For the first time, the ability of an *S*-substituted 1,8-naphthalimide derivative with a bisphosphonate fragment and PLGA nanoparticles obtained from it and loaded with doxorubicin has been experimentally demonstrated to selectively bind to hydroxyapatite and visualize *Saos-2* osteosarcoma cells, which indicates the potential of these compounds as fluorescent probes for studying bone tissue and creating theranostic systems.