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LETTERS TO THE EDITOR

Synthesis and Optical Properties of the Conformationally Locked Diarylmethene Derivative of the GFP Chromophore

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Abstract—We report a novel derivative of the conformationally locked derivative of the green fluorescent protein chromophore, (*Z*)-5-((2-(difluoroboryl)-4-(dimethylamino)phenyl)(4-(dimethylamino)phenyl)methylene)-2-propyl-3,5-dihydro-4*H*-imidazol-4-on. The presence of an additional aryl substituent in the structure causes a decrease in the fluorescence intensity and does not lead to absorption and emission spectra shifts. A promising direction of research is the replacement of this substituent with the electron-withdrawing groups, such as the nitrile or CF_3 group, capable of more efficient conjugation with the π-system of the molecule.

Keywords: imidazolones, chromophores, fluorescence
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INTRODUCTION

Studies in recent decades show that fluorescent labels are of high practical importance for visualization and study of biological processes [1]. Synthetic fluorophores include various benzylidene imidazolones, derivatives of the chromophore of the green fluorescent protein GFP [2]. This class of substances is represented by a large number of compounds that have different chemical structures and, as a result, are characterized by different properties. A number of well-developed methods for the chemical synthesis of these compounds are described in the literature [3].

Important properties of fluorophores include the ability to absorb and emit light in the far-red region of the spectrum and a high quantum yield of fluorescence. It is well known that the GFP chromophore and other benzylidene imidazolones weakly fluoresce due to the mobility of the benzylidene fragment of the molecule [4]. However, it was shown that this fragment can be rigidly fixed, for example, using a difluoroboryl bridge (for example, the compound (**Ia**) in Fig. 1) or other fixing methods. Such modifications made it possible to multiply the fluorescence quantum yield [5–7]. "Red" benzylidene imidazolones can be obtained by increasing the conjugated π-system (introducing styrene or arylacetylene substituents) [8–11] or by expanding the aromatic system by annulating the benzylidene moiety [12–14].

Abbreviation: GFP, green fluorescent protein.

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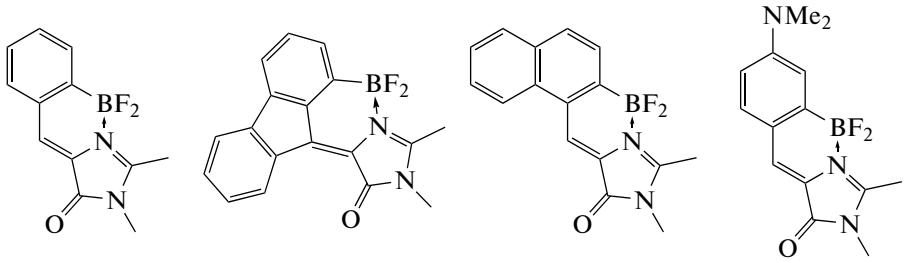
Previously, we synthesized a pair of annulated derivatives (Fig. 1, compounds (**Ib**) and (**Ic**)) [12, 15]. It was found that an increase in the aromatic system led to a bathochromic shift of the absorption maxima by 54–65 nm and emission by 50–77 nm relative to the unmodified derivative (**Ia**). However, it also turned out that fluorescence is retained only for the compound (**Ic**), while the fluorescence quantum yield of the derivative (**Ib**) does not exceed 0.2% (Fig. 1). We found that this behavior is caused by the presence of a covalent bond between two phenyl substituents in the derivative (**Ib**), due to which an additional free molecular orbital arises, fluorescence from which is impossible. It is likely that the removal of this bond can solve this problem and lead to an increase in fluorescence.

The purpose of this work is the synthesis the corresponding diarylmethylene-imidazolone, in which the aryl substituents are not linked to each other by a covalent bond, and the study of the optical properties of this compound.

RESULTS AND DISCUSSION

As a model, we chose the compound (**II**) (Fig. 1) [16]. This compound and its closest analogs act as substrates for lipocalin-based fluorogen-activating proteins and can be used in genetically encoded labeling [17, 18].

Benzylidene-imidazolone (**V**) was synthesized using the ketone condensation reaction (**III**) and saturated imidazolone (**IV**) in pyridine (Scheme 1). The conformationally fixed derivative (**VI**) was obtained by

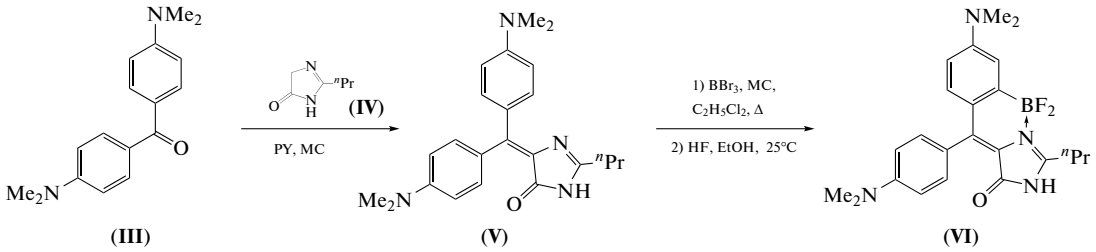


	(Ia)	(Ib)	(Ic)	(II)
Absorption maximum	360 nm	414 nm	425 nm	485 nm
Emission maximum	420 nm	~470 nm	497 nm	552 nm
Fluorescence quantum yield	55%	<0.2%	86%	48%

Fig. 1. Previously synthesized difluoroboryl derivatives (**I**), (**II**) and their optical properties in acetonitrile.

the action of boron tribromide on benzylidene-imidazolone (**V**) in dichloroethane in the presence of molecular sieves (Scheme 1).

The study of optical properties showed that the new derivative (**VI**), as well as the model connection (**II**), absorbs light in the region of 450–480 nm, and fluoresces in the region of 530–555 nm (Fig. 2, Table 1). It is likely that the absence of a significant difference is due to incomplete conjugation of the second aryl substituent with the π-system of the molecule.



Scheme 1. Scheme for the synthesis of compounds (**V**) and (**VI**).

The mobility of the second aryl substituent in the structure of compound (**VI**) also had a negative effect on the fluorescence intensity. It was found that the fluorescence quantum yield of this substance in acetonitrile does not exceed 0.5%, while the quantum yield of compound (**II**) is 48% (Fig. 1).

The results obtained indicate that a promising direction for further research may be the introduction

Table 1. Optical properties of compounds (**VI**) and (**II**) in different solvents

Solvent	Compound	Absorption maximum, nm	Emission maximum, nm
Ethanol	**(VI)**	449	528
	(II)	496	555
Acetonitrile	**(VI)**	478	558
	(II)	485	552
Ethyl acetate	**(VI)**	477	541
	(II)	484	538
Dioxane	**(VI)**	484	540
	(II)	489	532

Data on compound (**II**) are taken from Baranov et al. [16].

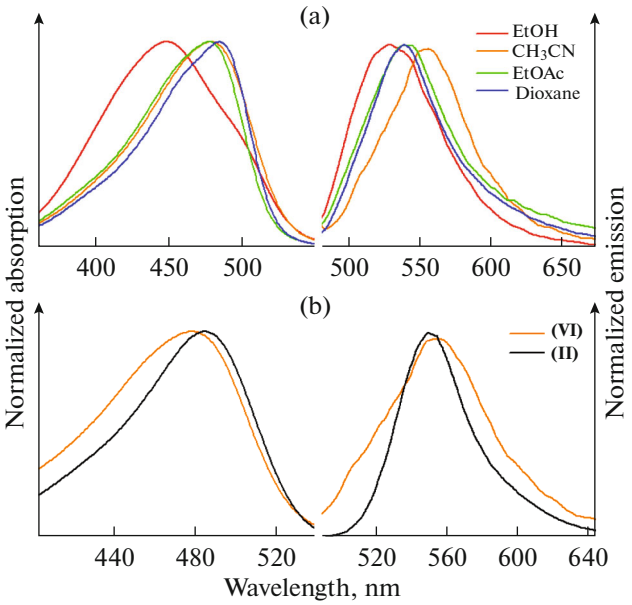


Fig. 2. (a) The normalized absorption and emission spectra of the compound (**VI**) in different solvents; (b) normalized absorption and emission spectra of compounds (**VI**) and (**II**) in acetonitrile.

other groups more capable of effective conjugation with the π-system of the molecule, for example, the introduction of not the second aryl substituent, as in the case of the compound (**VI**), but with various electron-acceptor groups, such as a nitrile or CF_3 group.

EXPERIMENTAL

Equipment. NMR spectra (δ, ppm; *J*, Hz) were recorded on an Avance spectrometer III NMR (700 MHz; Bruker, United States) at 303 K in DMSO-d_6 (internal standard Me_4Si), absorption spectra on a Cary 100 Bio spectrophotometer (Varian, United States), and fluorescence spectra on a Cary Eclipse spectrofluorimeter (Varian, United States). Melting points were determined on an SMP 30 instrument (Stuart Scientific, UK) and were not corrected. High-resolution mass spectra were recorded on a micrOTOF II instrument (Bruker, Germany), electrospray ionization.

Synthesis of 5-(*bis*(4-(dimethylamino)phenyl)methylene)-2-propyl-3,5-dihydro-4*H*-imidazol-4-one (V). 4,4'-*Bis*(dimethylamino)benzophenone (2.5 g, 9 mmol), 2-propyl-3,5-dihydro-4*H*-imidazol-4-one (2.3 g, 18 mmol) and molecular sieves (1.2 g, 3 Å). The flask was evacuated, filled with argon, and 10 mL of pyridine was added. The resulting mixture was stirred at 120°C for 50 h. Then, the reaction mixture was cooled to room temperature and evaporated. The resulting product was purified using column chromatography (eluent, chloroform and ethanol, 50 : 1).

Orange powder (409 mg, 15%); m.p. 206–209°C. ^{1}H-NMR: 10.85 (s, 1H), 7.31 (d, J_2 9.0, 2H), 7.03 (d, J_2 8.8, 2H), 6.68 – 6.63 (m, 4H), 2.97 (s, 6H), 2.96 (s, 6H), 2.37 (t, J_2 7.5, 2H), 1.64 (sext, J_2 7.4, 2H), 0.94 (t, J_2 7.4, 3H). ^{13}C-NMR: 169.8, 159.3, 150.5, 150.4, 145.1, 134.0, 133.5, 132.4, 127.2, 125.2, 110.8, 110.6, 39.8, 39.7, 31.2, 19.2, 13.6. HRMS (ESI) *m/z*: found *M* 391.2466; calculated for $C_{24}H_{31}N_4O^+$, $[M + H]^+$ 391.2492.

Synthesis of (*Z*)-5-((2-(difluoroboryl)-4-(dimethylamino)phenyl)(4-(dimethylamino)phenyl)methylene)-2-propyl-3,5-dihydro-4*H*-imidazol-4-one (VI). The product of the first stage (**V**) (0.2 g, 0.5 mmol) was mixed with molecular sieves (2 g, 3 Å and 2 g, 4 Å) and boron tribromide (5 mmol) in 50 mL of dichloroethane. The resulting mixture was refluxed in an argon atmosphere for 6 h. The mixture was then cooled and filtered. The molecular sieves were washed with cold ethanol (2 × 10 mL) and chloroform (50 mL). HF solution (40%, 5 mL) was added to the resulting solution and stirred for 30 min. The mixture was diluted with EtOAc (100 mL), washed with saturated K_2CO_3 (2 × 50 mL), water (2 × 50 mL) and saturated NaCl solution (2 × 50 mL). Organic extracts were dried over anhydrous Na_2SO_4 and evaporated. The resulting product was purified by flash chromatography (eluent, chloroform and ethanol, 20 : 1).

Red powder (22 mg, 10%); m.p. ~250°C with decomposition. ^{1}H-NMR: 12.58 (s, 1H), 7.09 (d, J_2 8.6, 2H), 6.94–6.90 (m, 2H), 6.78 (d, J_2 8.6, 2H), 6.59 (dd, J_2 9.1, 2.9, 1H), 3.03 (s, 6H), 3.00–2.93 (m, 8H), 1.78 (sext, J_2 7.5, 2H), 0.98 (t, J_2 7.3, 3H). ^{13}C-NMR: 163.0, 162.7, 151.7, 150.2, 145.6, 132.2, 130.3, 123.7, 120.6, 119.0, 113.7, 111.3, 110.8, 110.8, 39.9, 39.8, 28.5, 19.9, 13.5. HRMS (ESI), *m/z*: found *M* 439.2480; calculated for $C_{24}H_{30}BF_2N_4O^+$, $[M + H]^+$ 439.2475.

CONCLUSIONS

In this work, a new derivative of the chromophore of the GFP protein, (*Z*)-5-((2-(difluoroboryl)-4-(dimethylamino)phenyl)(4-(dimethylamino)phenyl)methylene)-3-methyl-2-propyl-3,5-dihydro-4*H*-imidzol-4-one was synthesized. The optical properties of this compound have been studied and it was found that in the absence of rigid fixation of the second aryl substituent and the resulting incomplete conjugation with the π-system of the molecule leads to a sharp decrease in the intensity fluorescence of the new compound. It is likely that an effective modification aimed at increasing the fluorescence quantum yield of such compounds will be the replacement of an unfixed aryl substituent with various electron-acceptor groups, such as a nitrile or CF_3 group, capable of more efficient conjugation with the π-system of the molecule.

FUNDING

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans and animals as research objects.

Conflict of Interests

The authors declare they have no conflicts of interest.

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