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FLUORESCENT BODIPY DYES AS LIGANDS FOR MAJOR STEROIDOGENIC PROTEINS: *in silico* EVALUATION

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In silico docking simulations of interactions between BODIPY-based fluorescent compounds and mammalian proteins, responsible for steroidogenesis, namely, cytochromes P450: CYP11A1, CYP17A1, CYP19A1, CYP7A1 and STARD1 transport protein, have been performed. Experimental results show a high affinity of some tested compounds to the active centers of the selected proteins (binding energies range of –6.7 to –12.4 kcal/mol), indicating possible impact of BODIPY fluorescent tags into whole labeled molecules affinity towards the selected proteins and perspectives of the experimental testing of the predicted interactions. For the last purpose different BODIPY dyes were obtained using validated methodologies and characterized using spectrofluorimetry. A strategy of quantum chemical calculation, allowing us to predict the absorption spectra of the compounds under consideration, has been also developed. Our results demonstrate suitability of the calculations used for estimation of physical and biological properties of BODIPY-based compounds as a part of rational design of novel biological probes. Synthesized dyes have a potential as fluorescent probes for the biomedical studies.

Key words: BODIPY; fluorescence; cytochrome P450; STARD1; docking; quantum chemical calculations.

In silico ИССЛЕДОВАНИЕ ФЛУОРЕСЦЕНТНЫХ BODIPY-КРАСИТЕЛЕЙ КАК ЛИГАНДОВ СТЕРОИДОГЕННЫХ БЕЛКОВ

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Проведено моделирование взаимодействия флуоресцентных соединений на основе BODIPY с белками млекопитающих, ответственными за стероидогенез, а именно цитохромами P450: CYP11A1, CYP17A1, CYP19A1, CYP7A1 и транспортным белком STARD1. Результаты расчетов демонстрируют высокую аффинность связывания выбранных соединений в активных центрах данных белков (теоретически рассчитанные энергии связывания в диапазоне от –6,7 до –12,4 ккал/моль), что свидетельствует о перспективности экспериментального тестирования ряда BODIPY-производных с указанными белками-мишенями. Для этого были получены и охарактеризованы методом спектрофлуориметрии различные BODIPY-красители. Представлены методы квантово-химических расчетов,

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предсказывающие спектры поглощения выбранных соединений. Результаты позволили сделать вывод о приемлемости использования расчетов для оценки физико-химических свойств соединений на основе BODIPY в целях создания новых соединений для медико-биологических исследований.

Ключевые слова: BODIPY; флуоресценция; цитохром P450; STARD1; докинг; квантово-химические расчеты.

Introduction

4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) has become a popular fluorophore as a result of its valuable properties such as relatively small size, non-polarity, photochemical stability, exceptional spectral properties (extinction coefficients range of ~ 70 000–700 000 [$mol^{-1} \cdot cm^{-1}$] at $\lambda_{max} \geq 500$ up to 630 nm) [1; 2]. Various BODIPY derivatives are used as fluorescent probes in biological studies. In particular, due to lipophilic nature of the fluorophore it has been used to create lipid droplet marker (BODIPY 493/503 or 1,3,5,7,8-pentamethyl-BODIPY) [3] and fluorescently-labeled analogues of cholesterol and other lipids [4; 5]. For instance, BODIPY-labeled TopFluor™-cholesterol is a fluorescent probe, widely used for analysis of cholesterol trafficking and metabolism in mammalian cells [4–6] and pathogenic bacteria [7–9]. In spite of this, to the best of our knowledge, there is a lack of data describing direct interactions of BODIPY-labeled steroids and the BODIPY dyes themself with proteins, realizing traffic and bioconversions of steroids, even at *in silico* level. ABC1-transporter-related BODIPY-cholesterol efflux from mammalian macrophages [6] could mean the molecule is a potential ligand for the protein, but direct interaction and binding site was not described. STARD1-dependent transport of BODIPY-cholesterol from phospholipid vesicles were mentioned [10], but the binding site was not determined or proposed. BODIPY core positions were discussed for steroid-converting CYP51 of *Mycobacterium tuberculosis*, covalently-labeled with the cysteine-selective BODIPY maleimide [11]. Few new publications describe BODIPY derivatives for covalent labeling of bovine serum albumin (BSA) as a model protein [12–14], but non-covalent interactions of similar dyes with the hydrophobic protein were not rationalized even *in silico* excepting the last work mentioned in the sentence. It should be also noted that BODIPY-labeled molecules could have more pronounce affinity to lipophilic subcellular bioobjects (membranes, lipid droplets, proteins) in comparison with correspondent parent compounds, providing the conjugates novel biological properties. For example, extra lipid droplets staining was reported for dialkynylcarbinols, labeled with BODIPY vs. labeled with coumarin and NBD-fluorophores [15].

Thus, we decided to perform computer-aided simulations of interactions of BODIPY-cholesterol, its new analogue (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene-propionyl)-20-amino-pregn-5-en-3-beta-ol (BDP20AP), and six non-steroidal BODIPY-derivatives (fig. 1) with mammalian proteins, responsible for key steroidogenesis process, namely, transport protein STARD1 and cytochromes P450 CYP11A1, CYP17A1, CYP19A1, CYP7A1; three of non-steroidal BODIPY ligands (see fig. 1, 1a–1c) as well as BDP20AP (see fig. 1, 3a) were synthesized in our laboratory for further experimental evaluation.

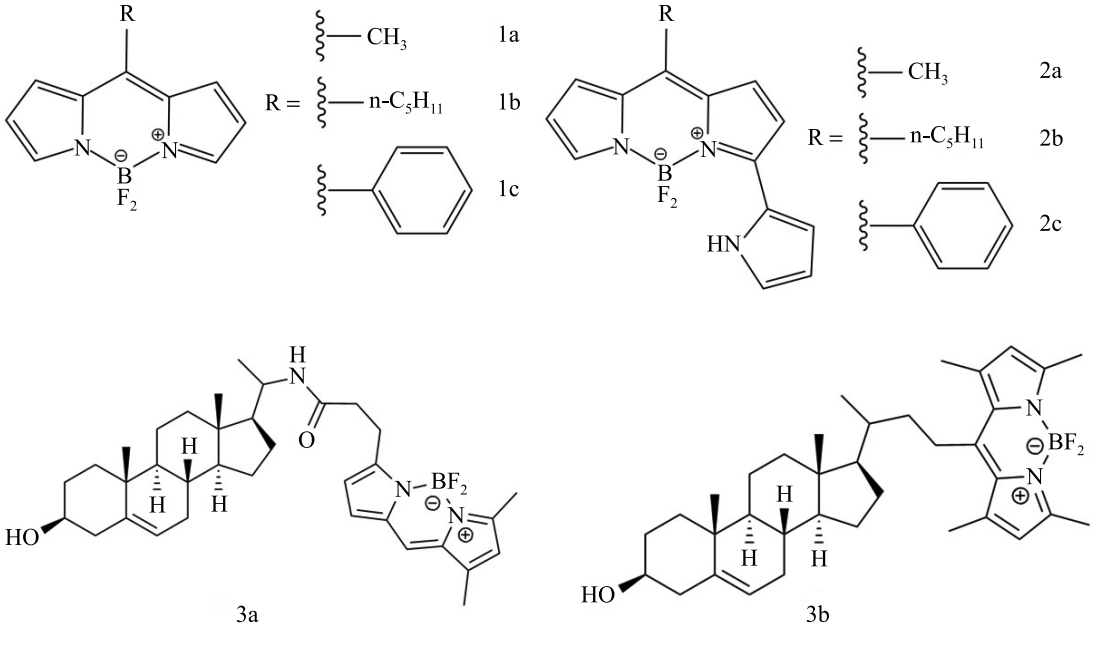

Fig. 1. BODIPY derivatives under investigation

Experimental section

For *in silico* work protein structures were taken from the on-line service RCSB Protein Data Bank. Ligand structures for molecular docking were made using ChemOffice (CambrigeSoft, USA) with subsequent PM6 ground state geometry optimization in Gaussian09 (Gaussian, Inc., USA) software. Simulation of ligand-protein interaction was performed *via* AutodockVina and AutoDock Tools (Graphics Laboratory, Scripps Research Institute, USA). Quantum-chemical calculations of UV-visible absorption spectra were performed in Gaussian09 using the time-dependent density functional theory TD-B3LYP, 6-311+G(d, p) basis set, CPCM – solvation model, solvent – ethanol [16]. Molecular structures for TD-DFT calculations were optimized in Gaussian09 using B3LYP functional, 6-311+G(d, p) basis set and CPCM – solvation model, solvent – ethanol. For experimental work all reagents and solvents were received from Sigma Aldrich (USA). Pyrrole and dichloromethane were purified in advance. All reactions were performed in oven-dried glassware. UV-visible absorption spectra and fluorescence emission spectra were recorded on a commercial spectrophotofluorometer (SOLAR CM2203) with 5 nm gap in ethanol. General procedure for non-steroidal BODIPY ligands synthesis was according to [17] (fig. 2).

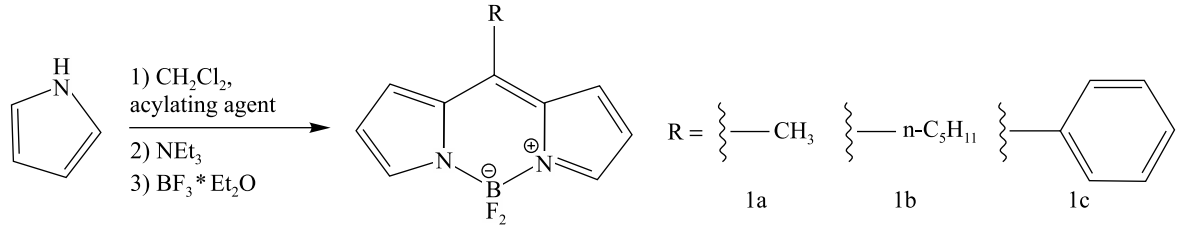

Fig. 2. A scheme of non-steroidal BODIPY synthesis

Briefly, to freshly purified pyrrole in anhydrous dichloromethane (10 ml) corresponding acylating agent (acetyl chloride, hexanoic anhydride and benzoyl chloride for 1a, 1b and 1c, respectively) was added. The reaction mixture was stirred at room temperature 6 h is the case of 1a and 1b as well as 12 h in the case of 1c. Triethylamine and $BF_3 * Et_2O$ were then added. After 1 h the reaction mixture was washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under vacuum. The crude product was purified by silica-based column chromatography (hexane/ethyl acetate is equal 10/0.5, v/v). Detailed information about the reagents amount is shown in table 1.

Table 1

Reagents amount taken for BODIPY synthesis

Substance	Pyrrole, μmol	Acylating agent, μmol	NEt_3, μmol	$BF_3 * Et_2O$, mmol
1a	485	255	926	1.94
1b	382	200	730	1.53
1c	374	196	746	1.5

Results and discussion

UV-Visible absorption spectra of compounds 1a – 1c were theoretically calculated. The results and transition types are shown in table 2.

Table 2

Calculated and experimentally-determined spectral properties for 1a – 1c and transition types

Compound	λ abs TD-DFT, nm	λ abs in ethanol, nm	λ em in ethanol, nm
1a	419	492	502
1b	421	496	506
1c	427	497	530

Excitation process corresponds to HOMO → LUMO transitions ($S_0 \rightarrow S_1$ excited states) for all selected compounds. The calculation strategy used has shown good correlation with the experimental data. The results could be evaluated as positive and this calculation method could be used to predict spectral properties of BODIPY dyes.

For estimation of considerated BODIPY's ability to interact with steroidogenic proteins, docking simulations were performed [18]. Six non-steroidal BODIPY and two steroidal BODIPY derivatives, depicted on fig. 1, were chosen as ligands for main steroidogenic proteins. Calculated free binding energies (kcal/mol) of selected compounds and native ligands (cholesterol, pregnenolone and estrone) are shown in table 3. Localizations with highest affinity are illustrated on fig. 3.

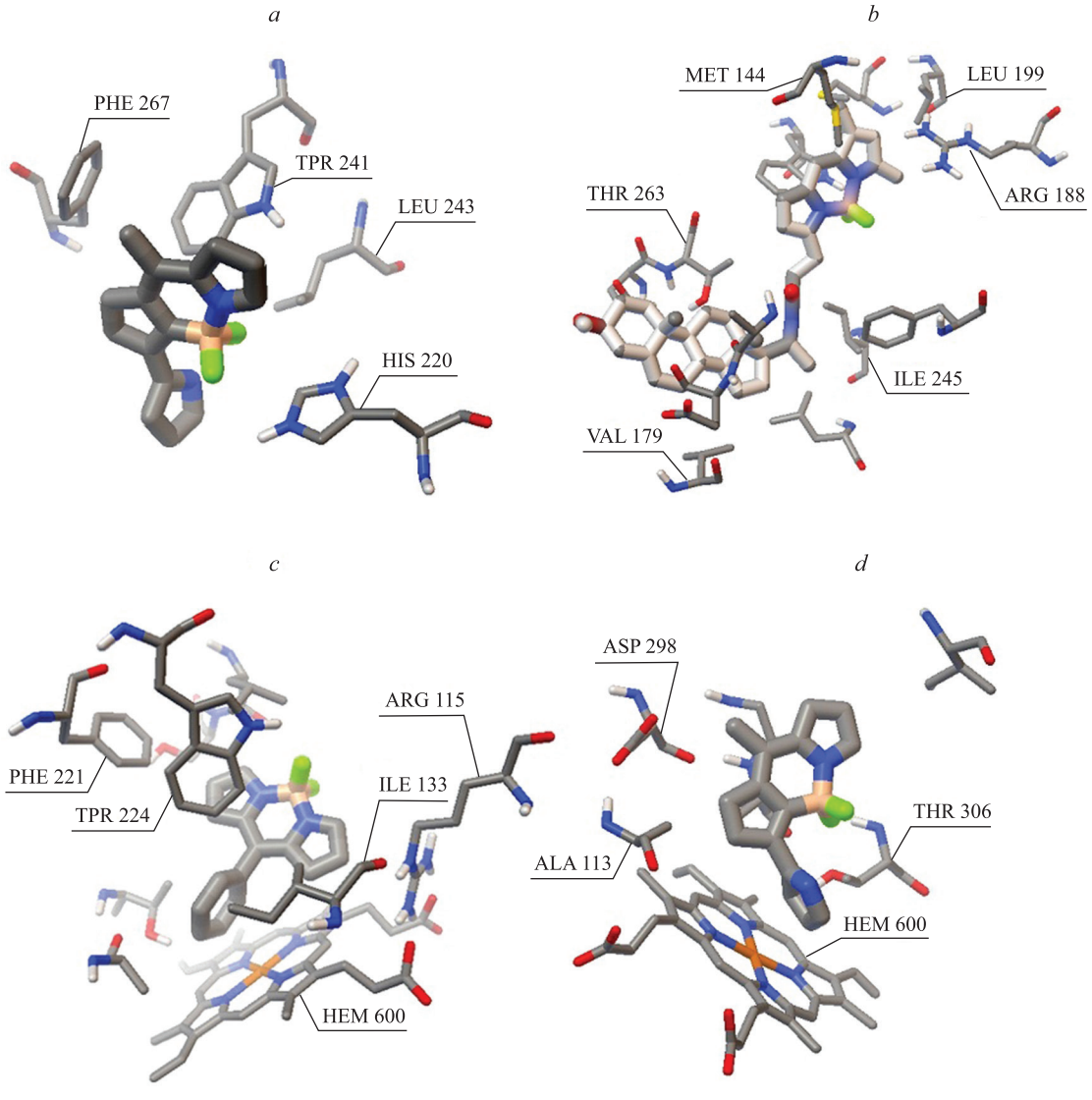

Fig. 3. Computed localizations of the BODIPY compounds with proteins of steroidogenesis, showing the highest affinities: *a* – 2a with STARD1 protein (3p0l); *b* – 3a with STARD1 protein; *c* – 1c with CYP19A1 (5jkw); *d* – 2a with CYP17A1 (3ruk)

Table 3

Calculated free binding energy (kcal/mol) of ligands under investigation

Ligand	3dax*	3v8d	3sn5	3mzs	3p0l	3ruk	5jkw
1a	–6.7	–8	–8.2	–8.2	–7.2	–7.5	–8.5
1b	–7.6	–8.1	–7.9	–8.9	–7	–8.1	–8.7
1c	–8.9	–7.8	–9.1	–9.8	–8.4	–9.4	–10.2
2a	–8.6	–10.3	–10.3	–9.4	–8.8	–9.7	–9.9

Ending table 3

Ligand	3dax*	3v8d	3sn5	3mzs	3p0l	3ruk	5jkw
2b	–7.6	–9.7	–9	–9.9	–8	–9.4	–6.5
2c	–8.8	–8.4	–9.9	–10	–7.5	–9	–7.1
3a	–9.4	–10.2	–12.4	–14.4	–12.2	–7.9	–10.1
3b	–10.6	–10.1	–10.2	–9.9	–9.3	–8	–8.9
Chol	–7.9	–12.2	–12	–11.9	–9.8	–	–
Preg	–	–	–	–	–	–11.4	–
Estrone	–	–	–	–	–	–	–11.8

*PDB proteins codes 3dax, 3v8d and 3sn5 for CYP7A1, 3mzs for CYP11A1, 3p0l for STARD1, 3ruk for CYP17A1, and 5jkw for CYP19A1.

According to our calculation results BODIPY-based compounds could interact with cytochromes P450 CYP11A1, CYP17A1, CYP19A1, CYP7A1 and STARD1 protein. Interacting energies for the majority of computed ligand-protein pair interactions are from –8 up to –10 kcal/mol. It should be mentioned that BODIPY ligands have relatively worse affinity to selected proteins in comparison with the BODIPY labeled steroids. This might be due to similarity with natural substrates and impact of both BODIPY and steroidal part in realization of hydrophobic interactions with the proteins.

For the STARD1 lipid transfer protein the best affinity was shown with 2a (–8.8 kcal/mol) and 3b (–12.2 kcal/mol) ligands. For the CYP19A1 protein the best affinity was shown with 1c (–10.2 kcal/mol) and 3a (–10.1 kcal/mol) ligands. For the CYP11A1 protein the best affinity was shown with 2c (–10 kcal/mol) and 3a (–14.4 kcal/mol) ligands. For the CYP17A1protein the best affinity was shown with 2a (–9.7 kcal/mol) and 3b (–8 kcal/mol) ligands. For the CYP7A1 the best affinity was shown with 2a (–10.3 kcal/mol) and 3a (–12.4 kcal/mol) ligands. For the CYP7A1 the best affinity was shown with 2a (–10.3 kcal/mol) and 3a (–10.2 kcal/mol) ligands. For the CYP7A1 lipid transfer protein the best affinity was shown with 1c (–8.9 kcal/mol) and 3b (–10.6 kcal/mol) ligands.

The results indicate on interactions possibility. Most of calculated ligands possess even better binding affinities comparing with primitive steroid ligands. Some of the most promising docking results are shown on fig. 3.

Conclusion

Using *in silico* docking simulations we have demonstrated a possibility of some BODIPY dyes to bind into active sites of five proteins of steroidogenesis, namely, cytochromes P450 CYP11A1, CYP17A1, CYP19A1, CYP7A1 and STARD1 transport protein. Thus, possible impact of BODIPY fluorescent tags into compositions of fluorescently labeled molecules affinity towards the enzymes should be taken into consideration. A suitable strategy of quantum chemical calculation has allowed us to predict the absorption spectra of the compounds under consideration. The BODIPY dyes have a potential as fluorescent probes for the druggable proteins under consideration. The new BODIPY-labeled steroid might be used alternatively to common BODIPY-cholesterol for some steroidogenic proteins studies. For the purpose of experimental evaluation of the *in silico* found interactions different BODIPY dyes were obtained using validated methodologies and characterized using spectrofluorimetry.

References

1. Heisig F., Gollos S., Freudenthal S. J., et al. Synthesis of BODIPY derivatives substituted with various bioconjugatable linker groups: a construction kit for fluorescent labeling of receptor ligands. *J. Fluoresc.* 2014. Vol. 24, issue 1. P. 213–230. DOI: 10.1007/s10895-013-1289-4.

2. Guzow K., Kornowska K., Wiczk W. Synthesis and photophysical properties of a new amino acid possessing a BODIPY moiety. *Tetrahedron Lett.* 2009. Vol. 50. P. 2908–2910. DOI: 10.1016/j.tetlet.2009.03.195.

3. Gocze P. M., Freeman D. A. Factors underlying the variability of lipid droplet fluorescence in MA-10 Leydig tumor cells. *Cytometry. Part A.* 1994. Vol. 17, issue 2. P. 151–158. DOI: 10.1002/cyto.990170207.

4. Maxfield F. R., Wüstner D. Analysis of cholesterol trafficking with fluorescent probes. *Methods Cell Biol.* 2012. Vol. 108. P. 367–393. DOI: 10.1016/B978-0-12-386487-1.00017-1.

5. Bandhuvula P., Li Z., Bittman R., et al. Sphingosine 1-phosphate lyase enzyme assay using a BODIPY-labeled substrate. *BBRC.* 2009. Vol. 380, issue 2. P. 366–370. DOI: 10.1016/j.bbrc.2009.01.106.

6. Mao M., Lei H., Liu Q., et al. Effects of miR-33a-5P on ABCA1/G1-mediated cholesterol efflux under inflammatory stress in THP-1 macrophages. *PLoS One.* 2014. Vol. 9, issue 10. Article ID: e109722. DOI: 10.1371/journal.pone.0109722.

7. Wunder C., Churin Y., Winau F., et al. Cholesterol glucosylation promotes immune evasion by Helicobacter pylori. *Nat. Med.* 2006. Vol. 12, issue 9. P. 1030–1038. DOI: 10.1038/nm1480.

8. Crowley J. T., Toledo A. M., LaRocca T. J., et al. Lipid exchange between *Borrelia burgdorferi* and Host cells. *PLoS Patog.* 2013. Vol. 9, issue 1. Article ID: e1003109. DOI: 10.1371/journal.ppat.1003109.

9. Faletrov Y., Brzostek A., Plocinska R., et al. Uptake and metabolism of fluorescent steroids by mycobacterial cells. *Steroids.* 2017. Vol. 117. P. 29–37. DOI: 10.1016/j.steroids.2016.10.001.

10. Baker B. Y., Epand R. F., Epand R. M., et al. Cholesterol binding does not predict activity of the steroidogenic acute regulatory protein, StAR. *J. Biol. Chem.* 2007. Vol. 282, issue 14. P. 10223–10232. DOI: 10.1074/jbc.M611221200.

11. Lepesheva G. I., Seliskar M., Knutson C. G., et al. Conformational dynamics in the F/G segment of CYP51 from *Mycobacterium tuberculosis* monitored by FRET. *Arch. Biochem. Biophys.* 2007. Vol. 464, issue 2. P. 221–227. DOI: 10.1016/j.abb.2007.05.017.

12. Kim D., Ma D., Kim M., et al. Fluorescent Labeling of protein using blue-emitting 8-amino-BODIPY derivatives. *J. Fluoresc.* 2017. Vol. 27, issue 6. P. 2231–2238. DOI: 10.1007/s10895-017-2164-5.

13. Cheng M. H. Y., Savoie H., Bryden F., et al. A convenient method for multicolour labeling of proteins with BODIPY fluorophores *via* tyrosine residues. *Photochem. Photobiol. Sci.* 2017. Vol. 16, issue 8. P. 1260–1267. DOI: 10.1039/c7pp00091j.

14. Zavadskaya O. A., Faletrov Y. V., Frolova N. S., et al. Preparation of fluorescent-labeled bovine serum albumin and determination of its spectral characteristics. *Vestnik BGU. Ser. 2, Khim. Biol. Geogr.* 2016. No. 2. P. 12–17 (in Russ.).

15. Listunov D., Mazéres S., Volovenko Y., et al. Fluorophore-tagged pharmacophores for antitumor cytoxicity: modified chiral lipidic dialkynylcarbinols for cell imaging. *Bioorg. Med. Chem. Lett.* 2015. Vol. 25, issue 20. P. 4652–4656. DOI: 10.1016/j.bmcl.2015.08.029.

16. He H., Si L., Zhong Y., et al. Iodized BODIPY dyes as long wavelength sensitizers for near-infrared emission of ytterbium(III) ion. *Chem. Commun.* 2012. Vol. 48, issue 13. P. 1886–1888. DOI: 10.1039/C2CC17037J.

17. Zhang M., Hao E., Xu Y., et al. One-pot efficient synthesis of pyrrolylBODIPY dyes from pyrrole and acyl chloride. *RSC Adv.* 2012. Vol. 2, issue 30. P. 11215–11218. DOI: 10.1039/C2RA22203E.

18. Trott O., Olson A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J. Comput. Chem.* 2010. Vol. 31, issue 2. P. 455–461. DOI: 10.1002/jcc.21334.

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