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New Photochemical Properties of Azidoaniline and Ciprofloxacin †

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Abstract: There is a trend to find new ways of using photocatalysis in order to synthesize valuable products or to control or track live processes with special fluorescence-based molecular probes. The paper presents some results concerning new photochemical properties of azidoaniline, its 7-nitrobenzofurazan (NBD-) derivative and ciprofloxacin derivatives.

Keywords: photochemistry; fluorescence; 7-nitrobenzofurazan (NBD); azidoaniline; ciprofloxacin

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1. Introduction

Over the last few decades researchers studied incorporation of antibacterial agents into composite polymeric materials in order to achieve effective inhibition of bacterial growth. Ciprofloxacin is a broad-spectrum antibiotic effective against plenty of the Gram-positive and the Gram-negative bacteria and it has many functional groups which can be modified. Its hydrophobic derivatives can be used as fluorescent photo-initiators showing antimicrobial properties [1–3]. In this work the photopolymerization of acrylamide using a hydrophobic CPF-derivative as initiator was performed.

NBD-compounds can give off fluorescence, consequently they are employed as molecular probes and potentially can be utilized in disease diagnostics, however their photochemistry wasn't sufficiently described. In biochemical studies a combination of fluorescent properties with different effects of functional groups which may modify characteristics of fluorescence is a promising way for drug design. Here different properties of para-azidoaniline and its NBD derivative were discovered in order to elucidate its potential as a molecular probe for protein studies.

Moreover NBD-compounds can be applied as prodrugs, fluorescent probes for enzyme analysis [4]. An interaction of NBD-derivatives with plasma proteins is being examined for its potential to bind with these proteins in order to transfer through the organism tissues and living systems with the help of blood plasma [5].

The recent studies show that NBD-compounds are potentially able to cross biological membranes, while they can be linked to such important for metabolism proteins as cytochrome P450 [6].

2. Materials and Methods

The following methods and tools were used: UV light source (365 nm), Blue LED light source (450 nm), Gaussian 09W software (HF/STO-3G and b3lyp/6-31+g(d,p) theory levels), Autodock Vina [7], FYTdock [8], spectrofluorimeter (CM 2203, Solar, Belarus), spectrophotometer (PB 2201, Solar, Belarus), thin-layer chromatography (Sorbfil), mass-spectrometry (LCMS 2020, Shimadzu), pharmaceutical substance of CPF hydrochloride (Zhejiang LangHua Pharmaceutical Co LTD), hexanoyl acid anhydride, triethylamine (Sigma-Aldrich, USA), methanol and acetonitrile (HPLC quality, Merck, Germany), chloroform, DMSO, acrylamide, N,N′-methylene-bis-acrylamide, DCC, NBD-chloride, $NaHCO_3$, 97% hexyne-1, para-azidoaniline, CuI. Synthesis of hexanoyl derivative of CPF was performed according to [3].

3. Results and Discussion

Ciprofloxacin (CPF) is known to emit blue fluorescence as well as to generate free radicals during photoexcitation due to the presence of acetophenone fragment. In this way CPF and its derivatives can be used as initiators for radical photopolymerization [9,10]. CPF is very soluble in water, therefore its modification with hydrophobic groups allows to produce amphiphilic fluorescent initiator of photopolymerizations. CPF, CPF-Hex and CPF-DCC (Figure 1) were synthesized using hexanoic anhydride and dicyclohexyl carbodiimide, respectively, with yields 90 % and 30 %, respectively. TLC Rf values amounted to 0.7 for CPF-DCC and 0.8 for CPF-Hex, 5:1 acetone/acetic acid solvent was used as eluent.

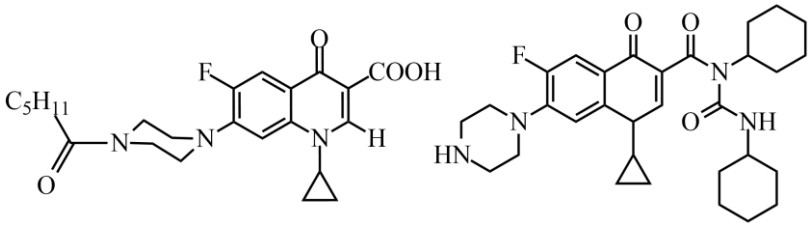

Figure 1. CPF-Hex (on the left) and CPF-DCC (on the right) structures.

Three dimensional grid structure forming stable aqua gel with UV-induced blue fluorescence of CPF-Hex was produced as a result of UV light exposure (λ = 365 nm) of acrylamide and N,N′-methylene-bis-acrylamide mixture laced with 1 % hexanoyl CPF derivative (CPF-Hex) in water-methanol solution proving ability of CPF-Hex to be a polymerization photo-initiator.

Similar conversions were monitored for CPF-DCC and was also reported for methylmethacrylates [3]. The 0.5% pyridine or triethylamine were essential as co-initiators. Polyacrylamide gels are formed during polymerization of 0.1 g/mL acrylamide aqueous solution with the presence of tiny amounts of bifunctional crosslinking agent (0.005 g/mL N,N′-methylene-bis-acrylamide). An example of such gel formation is shown on Figure 2 (a).

Multiple washing with equal volumes of water permitted to construct a CPF-Hex release from gel curve which shows that mostly CPF-Hex is quickly released, hence it is not covalently linked to the matrix (Figure 2).

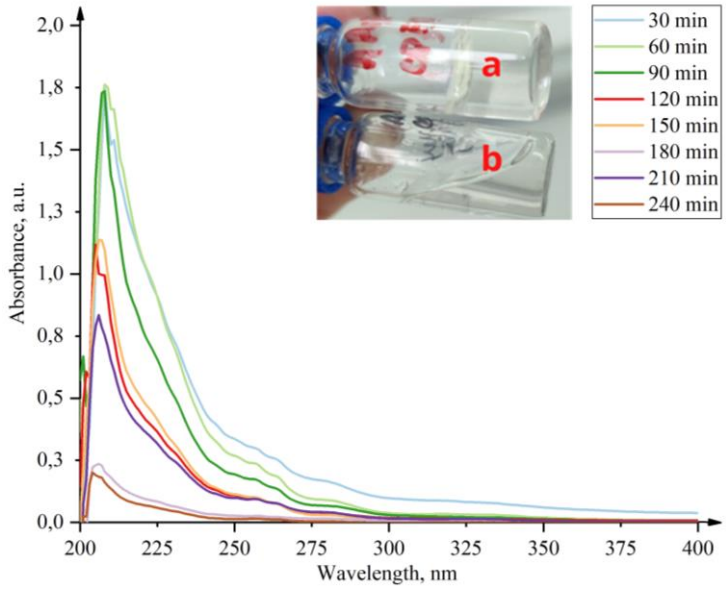

Figure 2. Absorbance spectrum of CPF-Hex transferred into aqueous solution after 8 washing within 240 min (incubation time was 30 min), a—gel formed after UV light exposure of the acrylamide and N,N′-methylene-bis-acrylamide mixture laced with CPF and CPF-DCC initiators in water, b—the mixture under consideration without UV light exposure.

On the other hand, gel showed trace blue fluorescence, so it was suggested that CPF-Hex retained inside of the gel partially, but, strongly, due to, e.g., covalent bond formation at an initial photopolymerization step.

Dealing with 7-nitrobenzofurazan p-azidoaniline (NBD-AzAn) derivative, reactions between the compound and hexyne-1 were done using both CuI as catalyst ([3+3] azide-alkyne cycloaddition, classical "click chemistry" reaction) without UV light exposure and vice versa (based on known photo-crosslinking properties of phenylazide derivatives). NBD-AzAn synthesis was performed at room temperature by mixing para-azidoaniline with NBD-chloride laced with $NaHCO_3$ in the solution of acetonitrile-methanol (2:1) followed by SiO_2 column chromatography. Synthesis of triazole (Figure 3, 2) was performed by mixing NBD-AzAn (Figure 3, 1) with an excess of hexyne-1 using CuI as a catalyst followed by TLC analysis confirming formation of the triazole (Figure 3, 2) as a single product.

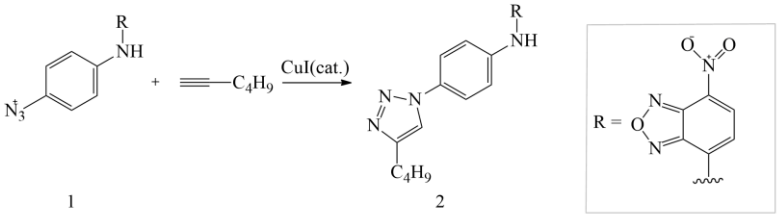

Figure 3. Click modification of NBD-AzAn proving azide group in its structure and possibility for further functionalization using CuAAC.

Photoinduced process in the mixture of NBD-AzAn and hexyne-1 yielded a yellow fluorescent product under 365 nm UV light, whereas copper-catalyzed reaction gave a purple compound (Figure 3). Changes in absorbance and fluorescence spectra in various solvents were estimated (absorbance maximum in methanol is at 478 nm).

To evaluate biological properties of the compound we used an inverse high-throughput virtual screening using Autodock Vina [7] and a helper tool FYTdock [8]. 450 randomly chosen PDB structures of cytochromes P450 were used because the enzymes are known to reduce organic azides in hypoxic conditions and our experience to use dockings

to evaluate protein ligand interactions [5]. Docking results revealed 55 hits with binding energy values from −11.3 to −9.9 kcal/mol and complexes with structures PDB 6DWN, 6UDM (CYP1A1), 6CIZ, 6WW (CYP17), 3TDA (CYP2D6).

The investigation of NBD-AzAn photochemistry (Figure 4) was performed in 97% hexyne-1 solution using blue LED light exposure. The reference methanol sample exposed to UV-light after 30 min of the experiment did not gain any fluorescent properties (Figure 5a). 160 mkM NBD-AzAn solution in 97% hexyne-1 was studied (Figure 5b), the stock solution of 1 mg/mL NBD-AzAn was prepared by dissolving dry pure substance in acetonitrile. 20 mkL of the stock solution were added to the 400 mkL of hexyne-1. Methanol solution of the substance which was prepared the same way from the same stock solution as described above was used as a reference sample (Figure 5a). Also extra test was performed for NBD-AzAn in 97% hexyne-1, in another vial the solution was placed to the place with no light exposure. In the first 5–7 min yellow fluorescence of the test solution exposed to UV-light was increasing (Figure 5b). Experiment lasted for 30 min. The reference unexposed to UV-light sample in 97% hexyne-1 was left for 24 h, no fluorescence in blue light was observed.

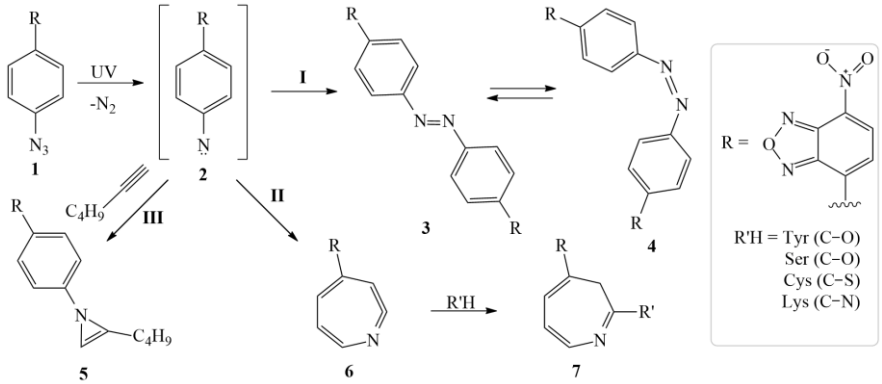

Figure 4. The scheme of a set of theoretically-possible photoinduced reactions for azidoaniline and its NBD-derivative.

Another reference test was held when the same preparation and exposure scheme was used for NBD-ethynylaniline (Figure 5d,e). None of the three samples showed fluorescence by the end of the experiment. Moreover, 97% hexyne-1 isn't fluorescent after visible light exposure (Figure 5c).

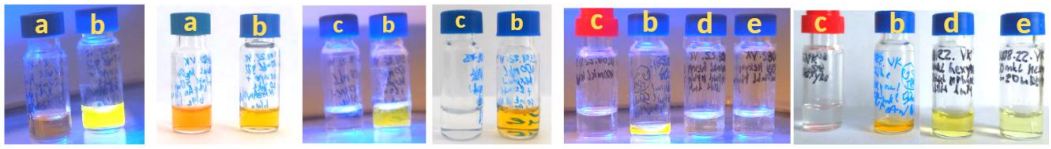

Figure 5. The samples before blue light exposure (450 nm) (on the right) and after (on the left): a—methanol and NBD-AzAn, b—hexyne-1 and NBD-AzAn, c—hexyne-1, d—hexyne-1 and NBD-ethynylaniline, e—hexyne-1 and 3-ethynylaniline.

In addition to the photoinduced radical processes with nitrene intermediate [11] it is highly possible that click-reactions similar to the Copper-catalysed ones (Figure 3) may occur under the light influence.

Table 1. The Gibbs reaction energy values (a.u.) of the formation of the products from the reactants designated in Figure 4.

Reactant	Product	R = H	R = NBD
1	2, N_2	−0.015322	−0.00332

2	3	−0.201323	−0.232164
	4	−0.187801	−0.210173
2, hexyne-1	5	−0.065421	−0.078967
2	6	−0.647449	−0.837335
6, Tyr	7, Tyr (C-O)	39.149172	39.28373
6, Lys	7, Lys (C-N)	0.564235	0.836605
6, Ser	7, Ser (C-O)	0.54942	0.824268
6, Cys	7, Cys (C-S)	0.558844	0.73701

DFT calculations analysis demonstrated that presence of the NBD-fragment had almost no influence on the Gibbs reaction energy values. DFT calculations were performed using b3lyp/6-31+g(d,p) theory level, the Gibbs energy value obtained for S0 state amounted to −1070.090218 a.u for NBD-AzAn and −451.144638 a.u. for para-azidoaniline. Theoretical Gibbs energy values calculated using HF/STO-3G theory level in vacuum for the click-interactions with para-azidoaniline and its NBD-derivative (Figure 3) amounted to −0.162339 и −0.163499 a.u., consequently, thus NBD-derivative has almost no influence on the Gibbs reaction energy in this particular case. The click-chemistry of NBD-AzAn and para-azidoaniline can be successfully applied in the field of medicinal chemistry where this approach is used for design of the triazole functional compounds frequently playing pharmacophore role in drugs. Besides click-interactions are applied in lead-compound search, in bioconjugation proteomic strategies and in DNA studies [12]. Click-reactions, honored by Nobel Price 2022, are held in physiological conditions, despite copper toxicity might limit their usage [13].

Azidoaniline photolysis was studied in acetonitrile and methanol using UV-Vis spectroscopy (Figure 6) resulted in monitoring of a new red fluorescent product formation in the first case (Figure 6, I, II).

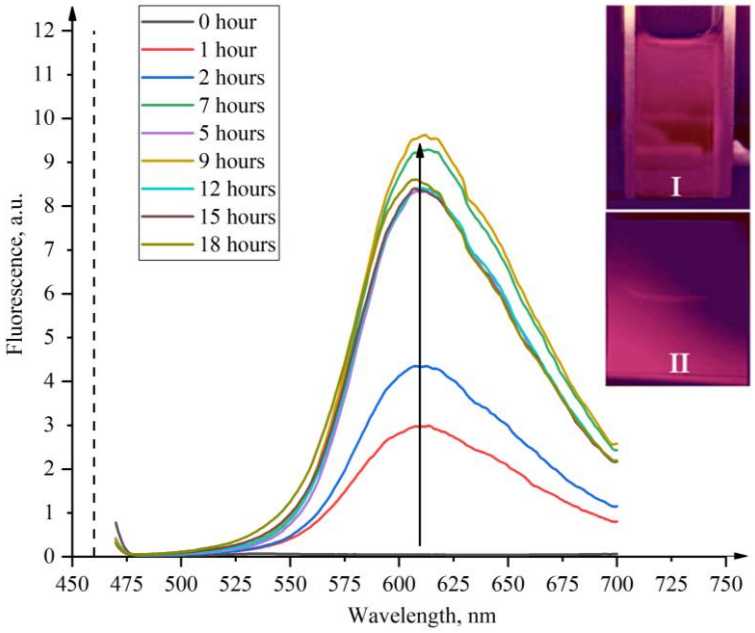

Figure 6. The fluorescence emission spectra of para-azidoaniline sample in progress during 18 h of its UV-driven photolysis in methanol (excitation at 460 nm), I—red fluorescence of the test solution in UV light, II—TLC of the test solution in UV light.

4. Conclusions

It was found out that the CPF itself, N-hexanoyl (CPF-Hex) and some other derivatives of ciprofloxacin exhibit the properties of a photo-initiator of polymerization under

UV light (365 nm) on the model system of aqueous alcoholic solutions of acrylamide and N,N′-methylene-bis-acrylamide, and that part of CPF-Hex molecule is capable to bind tightly with the polymer gel.

A new visible light induced interaction and the click-process yielding purple product between alkyne and azide was performed. Revealed transformations were partially characterized with quantum-chemical calculations and photometry. The photolysis of para-azidoaniline in methanol was performed and resulted in a red fluorescent product. Clarification of the molecular basis of these processes may allow to design new molecular probes or agents for the photodynamic therapy.

Author Contributions: Conceptualization, Y.V.F., V.S.K., L.I.G., K.N.B. and Y.A.P.; writing—original draft preparation, V.S.K. and L.I.G.; writing—review and editing, Y.V.F.; supervision, V.M.S. and S.V.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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