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Highlights.

- A semisquaraine derived from 3-methylimidazo[1,5-a]pyridine nuclei was obtained.
- New unsymmetrical squaraine dyes containing the imidazo[1,5-a]pyridine ring were synthesized.
- Quantum-chemical calculations were performed for the dyes prepared.
- The photostability of the dyes was assessed by simple fading tests.
- All dyes possess inherent singlet oxygen generation ability.

New unsymmetrical squaraine dyes derived from imidazo[1,5-a]pyridine

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Abstract

The reaction of squaric acid with excess 3-methylimidazo[1,5-a]pyridine afforded almost exclusively the corresponding semisquaraine. The later underwent condensation with several heterocyclic compounds possessing active methyl groups, forming a novel class of unsymmetrical squaraines. The spectral properties of the obtained dyes were investigated and quantum-chemical calculations were performed to examine the dependence of the electronic structure and electron transitions on the molecular constitution of the dyes. The oxygen generation ability of the new dyes was accessed by the 1,3-diphenylisobenzofuran (DPBF) quenching method, envisioning their potential use as sensitizers for photodynamic therapy (PDT).

Keywords: unsymmetrical squaraines, imidazo[1,5-a]pyridine, semisquaraines, singlet oxygen, PDT

1. Introduction

Squarylium dyes, also called squaraines, are zwitterionic donor-acceptor-donor (D-A-D) compounds bearing a central acceptor squaric ring 1,3-disubstituted with electron-rich aromatic or heteroaromatic nuclei. Compounds from this class of dyes typically display sharp and intense absorption bands ($\epsilon \sim 10^5 \text{ M}^{-1}\text{cm}^{-1}$) in the red to near-infrared region [1-3]. Their unique photophysical and photochemical properties turns them highly appropriated for a number of applications [2, 3], both in the technological (*e.g.* photoreceptors in copiers [4], photoconductors in organic solar cells [5-7], IR absorbers in organic optical disks [8]) and biological fields (*e.g.* metal ion sensors [9], long wavelength fluorescent labels [10-12], reagents for the detection of thiols

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[13]). The intense absorption in the so-called phototherapeutic window (600-850 nm), due to the intramolecular charge-transfer (CT) character of the S0-S1 electronic transition, combined with an extended conjugated π -electron network, also makes squaraines promising sensitizers for photodynamic therapy (PDT) [14].

The imidazo[1,5-a]pyridines are an important class of 10π -electron fused nitrogencontaining heterocyclic compounds due to their biological activity [15-18] and special photophysical properties. The later have turned them, for example, potential materials for organic light-emitting diodes (OLED) [19] and organic field-effect transistors (OFET) [20]. Their use as metal ligands [21, 22] and as precursors of *N*-heterocyclic carbenes [23, 24] has also been reported.

To the best of our knowledge neither polymethine nor squaraine dyes based on this heterocyclic system have hitherto been described. Earlier, Pagani and co-workers reported the synthesis of squaraine **1** (Fig. 1) based on the indolizine (pyrrolo[1,2-a]pyridine) ring, a structural analogue of the imidazo[1,5-a]pyridine system, and find it to be a potential candidate as non-porphyrinic photosensitizer for PDT [25]. We anticipated that imidazo[1,5-a]pyridine-derived squaraines may likewise exhibit interesting singlet oxygen generation ability and envisioned the synthesis of several squaraine dyes starting from 3-methylimidazo[1,5-a]pyridine.

Figure 1

2. Experimental

2.1. General

All reagents were purchased from Aldrich and used as received. Solvents were of analytical grade. Acetic anhydride was distilled prior to use. The quaternary heterocyclic slats were prepared according to the usual methods described in the literature [26]. All reactions were monitored by thin-layer chromatography on aluminium plates coated with Merck silica gel 60 F_{254} (0.25 mm). Column chromatography was performed on Merck silica gel 60 (70-230 mesh). ¹H and ¹³C NMR spectra were recorded at 298 K on a Bruker ARX400 spectrometer (at 400.13 and 100.62 MHz).

Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. IR spectra were obtained on a Unicam Research Series FTIR spectrometer using KBr pellets. Wavenumbers (v_{max}) are reported in cm⁻¹. UV-Vis spectra were recorded on a Perkin-Elmer Lambda 25 spectrophotometer in spectral grade acetonitrile and dichloromethane. High resolution electrospray ionization time-offlight (ESI-TOF) mass spectra and electron impact time-of-flight (EI-TOF) mass spectra were measured with a VG AutoSpec M spectrometer. Melting points were determined in open capillary tubes in a Buchi 530 melting point apparatus and are uncorrected. Light fluence rate was measured with an International Light IL 1400 radiometer equipped with a SEL033 probe.

2.2. Synthesis of compounds

2.2.1. 3-Methylimidazo[1,5-a]pyridine (3).

A solution of 2-picolylamine (2) (5.00 g, 4.63 mmol) and *p*-toluenesulfonic acid monohydrate (8.34 g, 4.63 mmol) in acetic anhydride (25 mL) was heated at 100 °C for 3h. After cooling to room temperature, the reaction mixture was poured into water (100 mL), made alkaline with 20% aqueous NaOH and the resulting mixture was extracted with CH₂Cl₂ (3x35 mL). The combined organic layers were washed with water (50 mL), dried over anhydrous Na₂SO₄ and evaporated to dryness under reduced pressure to afford **3** (5.54 g, 91%), as a sticky yellow-greenish residue which solidified on standing. Mp 54-56 °C (dec.). IR v_{max} : 663, 732, 782, 1000, 1359, 1399, 1489, 1640. ¹H NMR (CDCl₃) δ : 2.54 (3H, s, CH₃), 6.39–6.43 (1H, m, Ar*H*), 6.51–6.55 (1H, m, Ar*H*), 7.25–7.30 (2H, m, Ar*H*), 7.53 (1H, d, *J* = 7.1, Ar*H*). ¹³C NMR (CDCl₃) δ : 12.2, 111.8, 117.1, 117.9, 118.0, 120.2, 129.9, 134.5. MS (EI-TOF) *m/z* (%BPI): 64 (13), 104 (7), 127 (5), 132 (100, M⁺).

2.2.2. 3-Hydroxy-4-(3-methylimidazo[1,5-a]pyridin-1-yl)cyclobut-3-ene-1,2-dione (5).

Squaric acid (0.43 g, 3.8 mmol) in a mixture of butan-1-ol/toluene (1/1, v/v) (25 mL) was heated under reflux, with azeotropical removal of water, for 30 min. Then a solution of **3** (1.00 g, 7.6 mmol) in butan-1-ol/toluene (1/1, v/v) was added and the reaction mixture was maintained at 100 °C for 5 h. The solid residue formed on cooling was filtered off and washed with Et₂O (15 mL) to

afford **5** (0.30 g, 40%), as a yellow solid. Mp > 300 °C. IR v_{max} : 755, 863, 1091, 1226, 1590, 1775, 3036, 3417. ¹H NMR (DMSO-*d*₆) δ : 2.86 (3H, s, C*H*₃), 7.15 (1H, t, *J* = 6.5, Ar*H*), 7.20-7.24 (1H, m, Ar*H*), 8.37 (1H, d, *J* = 7.2, Ar*H*), 8.67 (1H, d, *J* = 9.2, Ar*H*). ¹³C NMR (DMSO-*d*₆) δ : 10.6, 114.7, 117.5, 121.3, 123.3, 123.7, 126.3, 137.2, 164.3, 193.3, 212.0. HRMS (ESI-TOF) *m/z*: 229.06011 ([M+H]⁺; calc. for C₁₂H₉N₂O₃: 229.06077).

2.2.3. 4-[(1,3,3-Trimethylindol-1-ium-2-yl)methylidene]-2-(3-methylimidazo[1,5-a]pyridin-1-yl)-3-oxocyclobut-1-en-1-olate (**6a**).

A solution of **5** (0.034 g, 0.15 mmol) and 1,2,3,3-tetramethylindolium iodide (0.045 g, 0.15 mmol) in a mixture of butan-1-ol/pyridine (10/1, v/v) (10 mL) was heated under reflux for 5 h. After cooling to room temperature, the mixture was evaporated to dryness under reduced pressure. The resulting residue was purified by column chromatography (CHCl₃/MeOH, 9/1, v/v) to afford **6a** (0.038 g, 67%), as a blue solid. Mp 254-256 °C. IR v_{max} : 632, 756, 796, 972, 1059, 1258, 1444, 1521, 1602, 3415. ¹H NMR (CDCl₃) δ : 1.82 (6H, s, C(CH₃)₂), 2.74 (3H, s, CH₃), 3.73 (3H, s, NCH₃), 6.17 (1H, s, CH=C), 6.87 (1H, t, *J* = 6.5, Ar*H*), 7.13-7.16 (2H, m, Ar*H*), 7.25-7.28 (1H, m, Ar*H*), 7.36-7.42 (2H, m, Ar*H*), 7.83 (1H, d, *J* = 7.0, Ar*H*), 9.18 (1H, d, *J* = 9.0, Ar*H*). ¹³C NMR (CDCl₃) δ : 12.8, 26.5, 29.6, 31.8, 50.5, 89.3, 110.5, 115.4, 122.1, 122.4, 123.1, 123.7, 124.3, 125.5, 128.1, 136.1, 141.2, 142.3, 142.4, 170.7, 175.3, 187.5. HRMS (ESI-TOF) *m*/*z*: 384.17053 ([M+H]⁺; calc. for C₂₄H₂₂N₃O₂: 384.17065).

2.2.4. 4-[(3-Ethylbenzothiazol-3-ium-2-yl)methylidene]-2-(3-methylimidazo[1,5-a]pyridin-1-yl)-3-oxocyclobut-1-en-1-olate (**6b**).

A solution of **5** (0.15 g, 0.68 mmol) and 3-ethyl-2-methylbenzothiazolium iodide (0.23 g, 0.68 mmol) in a mixture of butan-1-ol/pyridine (10/1, v/v) (25 mL) was heated under reflux for 5 h. After cooling to room temperature the mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography (CHCl₃/MeOH, 9/1, v/v) to afford **6b** (0.13 g, 38%), as a greenish-blue solid. Mp 273-275 °C. IR ν_{max} : 750, 1082, 1264, 1449, 1516, 1602, 3426.

¹H NMR (DMSO- d_6) δ : 1.37 (3H, t, J = 7.2, NCH₂CH₃), 2.64 (3H, s, CH₃), 4.61 (2H, brq, J = 7.2, NCH₂CH₃), 6.32 (1H, s, CH=C), 6.90 (1H, t, J = 6.9), 7.09 (1H, t, J = 6.6, ArH), 7.51 (1H, t, J = 7.8, ArH), 7.64 (1H, t, J = 8.3, ArH), 7.92 (1H, d, J = 8.5, ArH), 8.16 (1H, d, J = 7.9, ArH), 8.22 (1H, d, J = 7.1, ArH), 8.92 (1H, d, J = 9.1, ArH). ¹³C NMR (DMSO- d_6) δ : 12.3, 12.9, 42.2, 89.5, 114.1, 114.3, 121.2, 122.3, 122.6, 123.3, 125.9, 128.1, 128.9, 132.7, 139.6, 140.0, 161.8, 164.4, 178.2, 179.1, 184.4. HRMS (ESI-TOF) m/z: 388.11085 ([M+H]⁺; calc. for C₂₂H₁₈N₃O₂S: 388.11142).

2.2.5. 4-[(3-Ethylbenzoselenazol-3-ium-2-yl)methylidene]-2-(3-methylimidazo[1,5-a]pyridin-1-yl)-3-oxocyclobut-1-en-1-olate (**6c**).

A solution of **5** (0.10 g, 0.44 mmol) and 3-ethyl-2-methylbenzoselenazolium iodide (0.17 g, 0.44 mmol) in a mixture of butan-1-ol/pyridine (10/1, v/v) (25 mL) was heated under reflux for 5h. After cooling to room temperature, the mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography (CHCl₃/MeOH, 9/1, v/v) to afford **6c** (0.11 g, 58%), as a greenish-blue solid. Mp 194-196 °C. IR v_{max}: 746, 978, 1076, 1258, 1313, 1444, 1516, 1590, 3412. ¹H NMR (DMSO-*d*₆) δ : 1.36 (3H, t, *J* = 7.0, NCH₂CH₃), 2.64 (3H, s, CH₃), 4.59 (2H, q, *J* = 7.0, NCH₂CH₃), 6.39 (1H, s, CH=C), 6.90 (1H, t, *J* = 6.4, ArH), 7.09-7.13 (1H, m, ArH), 7.41 (1H, t, *J* = 7.6, ArH), 7.58 (1H, t, *J* = 7.3, ArH), 7.84 (1H, d, *J* = 8.4, ArH), 8.18 (1H, d, *J* = 7.3, ArH), 8.23 (1H, d, *J* = 7.0, ArH), 8.90 (1H, d, *J* = 9.0, ArH). ¹³C NMR (DMSO-*d*₆) δ : 12.1, 12.7, 42.8, 92.9, 114.1, 115.6, 121.1, 122.3, 122.6, 123.2, 125.5, 126.3, 127.7, 130.6, 132.8, 139.6, 141.4, 162.0, 169.7, 178.8, 179.1, 184.8. HRMS (ESI-TOF) *m*/*z*: 436.05478 ([M+H]⁺; calc. for C₂₂H₁₈N₃O₂Se: 436.05597).

2.2.6. 4-[(3-Ethylquinolin-3-ium-4-yl)methylidene]-2-(3-methylimidazo[1,5-a]pyridin-1-yl)-3oxocyclobut-1-en-1-olate (**6d**).

A solution of **5** (0.10 g, 0.44 mmol) and 1-ethyl-4-methylquinolinium iodide (0.13 g, 0.44 mmol) in a mixture of butan-1-ol/pyridine (10/1, v/v) (25 mL) was heated under reflux for 5h. After cooling

to room temperature the mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography, eluting sequentially with CHCl₃/MeOH, 9/1 (v/v), CHCl₃/MeOH, 2/1 (v/v) and finally CHCl₃/MeOH, 1/1 (v/v), to afford **6d** (0.060 g, 36%), as a green solid. Mp > 300 °C. IR v_{max}: 678, 755, 854, 1076, 1168, 1221, 1249, 1380, 1435, 1527, 1576, 3425. ¹H NMR (DMSO-*d*₆) δ : 1.50 (3H, t, *J* = 7.1, NCH₂CH₃), 2.63 (3H, s, CH₃), 4.71 (2H, q, *J* = 7.1, NCH₂CH₃), 6.72 (1H, s, CH=C), 6.83 (1H, t, *J* = 7.3, ArH), 6.96-7.00 (1H, m, ArH), 7.77 (1H, t, *J* = 7.6, ArH), 8.02 (1H, t, *J* = 8.6, ArH), 8.13 (1H, d, *J* = 7.1, ArH), 8.22 (1H, d, *J* = 8.8, ArH), 8.63 (1H, d, *J* = 8.0, ArH), 8.76 (1H, d, *J* = 7.0, ArH), 8.88 (1H, d, *J* = 9.0, ArH), 9.33 (1H, d, *J* = 6.9, ArH). ¹³C NMR (DMSO-*d*₆) δ : 12.2, 14.8, 50.1, 99.1, 113.8, 118.1, 120.8, 121.2, 122.8, 123.1, 125.3, 126.0, 127.4, 131.2, 133.6, 137.6, 138.7, 143.5, 150.8, 155.7, 180.0, 181.2. HRMS (ESI-TOF) *m/z*: 382.15552 ([M+H]⁺; calc. for C₂₄H₂₀N₃O₂: 382.15500).

2.3. Evaluation of the singlet oxygen generation ability

Solutions of DPBF (5·10⁻⁵ M) and dyes **6a-c** (5·10⁻⁶ M) in dichloromethane were prepared and saturated with oxygen for 3 min. Each oxygenated solution was placed into a 1cm quartz cell and irradiated under gentle magnetic stirring with a 24V, 250 W halogen lamp (Osram) for different time intervals. The light was filtered with an Oriel 59494 optical filter ($\lambda > 515$ nm) and the fluence rate at the surface of the solution was adjusted to 6.3-6.8 mWcm⁻².

3. Results and discussion

3.1. Synthesis

The starting 3-methylimidazo[1,5-a]pyridine (**3**) was readily obtained by cyclocondensation of commercially available 2-picolylamine (**2**) with acetic anhydride, in the presence of an equimolar amount of *p*-toluenesulfonic acid monohydrate [27].

Aiming at obtaining squaraine dye 4, the condensation of 3 with half molar equivalent of squaric acid was carried under the reaction conditions commonly employed for the preparation of symmetrical squaraine dyes (*e.g.* reflux in butan-1-ol/toluene or butan-1-ol/pyridine) [3].

Unexpectedly, all attempts to obtain dye **4** resulted in the formation of semisquaraine **5** as the major reaction product, while the desired compound was detected only in trace amounts (Scheme1).

Scheme 1

The usual synthesis of symmetrical squarylium dyes involves the one-pot condensation of electron-rich aromatic or heterocyclic compounds with squaric acid in a solvent mixture containing butan-1-ol. Butylsquarate (3-butoxy-4-hydroxy-1,2-dioxocyclobut-3-ene) was proposed as the reactive intermediate [28, 29], firstly reacting with one molecule of the (hetero)aromatic compound to give an intermediate semisquaraine that subsequently undergoes a second condensation with another molecule of the same (hetero)aromatic compound to yield the final squaraine dye. The success of the reaction depends primarily on the nucleophilicity of the (hetero)aromatic species [30, 31]. In the present case the electron-donating ability of the saturated nitrogen atom in the imidazo[1,5-a]pyridine nucleus rends the squaric ring less reactive towards an additional molecule of **3**, which, in turn, is not nucleophilic enough to complete the reaction, that consequently stops at the semisquaraine formation. On the basis of the possible resonanse structures for the imidazo[1,5-a]pyridine nucleus, a discrete electron density is expected both on the imidazolic nitrogen and on the carbon close to it, but only the constitutional isomer **3** was isolated and unambiguously confirmed structurally.

A similar failure in obtaining a squaraine dye under the usual conditions employed for the synthesis of symmetrical squaraines has been observed in the reaction of 5-hydroxy- and 5-ethoxyquinaldinium iodides [32] with squaric acid. However, therein the semisquaraine was isolated as the butoxy-substituted 1,3-regioisomer [33]. The isolation of a simple unsubstituted semisquaraine intermediate under the reaction conditions used for the preparation of symmetrical squaraine dyes is very uncommon and, to the best of our knowledge, has only been briefly mentioned in the condensation of antipyrine and 3-aminopyridine with squaric acid [28]. Such type of intermediate is found in the conventional procedure for the synthesis of unsymmetrical squaraine

dyes, resulting from the deliberated basic hydrolysis of the intermediate semisquaraine alcoxysubstituted 1,2-regioisomer, obtained from the addition of one molar equivalent of the aromatic nucleophile to a starting dialkylsquarate [34, 35].

Semisquaraine **5** was found to react easily with the nucleophilic enamines generated from 2,3,3-trimethylindolinium, 2-methylbenzothiazolium, 2-methylbenzoselenazolium and 4-methylquinolinium iodides in butan-1-ol/pyridine - 10/1 (v/v), forming the corresponding unsymmetrical dyes **6a-d**, which were isolated in good to moderate yields (Scheme 1).

Dyes **6a-d** are the first representatives of a new type of skeleton of unsymmetrical squaraines. In addition to the unprecedented presence of an imidazo[1,5-a]pyridine end group, these dyes possess one unsaturated heterocyclic moiety directly connected to the central squaric ring whilst the other heterocyclic nucleus is bonded through an usual methyne bridge.

3.2. Structure and molecular modeling

Quantum-chemical calculations were performed for all the synthesized dyes in order to study the dependence of the electronic structure and electronic transitions on the molecular constitution. The equilibrium geometry of the dye molecules in the ground state was optimized by the nonempirical HF/6-31G(d,p) and DFT/6-31G(d,p)/B3LYP methods (package Gaussian 03 [36]). The electron transition characteristics were calculated by the TD DFT and semi-empirical ZINDO methods. Although there is not a complete concurrence between calculated and experimental data, it is adequate to a correct analysis of the nature of the electronic transitions. The calculations of the equilibrium molecular geometry showed the conjugated part of all molecules to be essentially planar, a typical feature of π -electron systems. Only the methyl groups of the indolenine fragment in dye **6a** are out of the plane of the main chromophore (Fig. 2). The optimized lengths of the C-C bonds in the central four-member ring are somewhat longer than those of the C-C bonds in the open chain.

Because of the unsymmetrical constitution of the dyes, the lengths of the bonds involving squaric C2 and C4 atoms and the adjacent carbons (bonds 1 and 2) differ from each other. According to the HF method, the difference between these bond lengths, Δl (1-2), increases upon increasing the end groups basicity in the order: indolenine (**6a**) > benzoselenazole (**6c**) > benzothiazole (**6b**) > quinoline (**6d**) (Table 1). The DFT method, by taking into consideration the electron correlation, gives lower values for Δl (1-2). Using this approach, the dependency of the asymmetry parameter Δl (1-2) on the basicity of the end nuclei is not confirmed. The two approximations (HF and DFT) show different trends for the lengths of the neighboring bonds 2 and 3, what points out to low asymmetry in the polymethine chain. It can be proposed that the difference in electronic transitions is mainly determined by the chemical constitution of the end groups and hence by their electron levels positions.

Table 1

3.3. Spectral properties and the nature of the electronic transitions

The absorption spectra of the obtained dyes were measured in acetonitrile and methylene chloride, an all exhibit intense bands in the range 600-700 nm, as typical for squaraines. When compared to the correspondent symmetrical analogues [34], compound **6a** displays absorption at approximately the same wavelength, while that of **6b** and **6c** is shifted hypsochromically (38 and 36 nm, respectively), a characteristic behavior of unsymmetrical squaraine dyes [34, 35] (Table 2).

Table 2

It was established earlier that end groups possessing highly localized orbitals produce the main contributions to the highly occupied MOs of cyanine dyes with relatively short polymethine chains [37-40]. Among short chain cationic cyanine dyes and their squaraine analogues, the local

MOs of the end groups are positioned higher than the highest delocalized orbital of the polymethine chain. It was ascertained that the two highest occupied MOs can be considered to be generated mainly from the highest MOs of both end residues conjugated with the delocalized π -electron system of the polymethine chain and, therefore, these MOs can be treated as donor orbitals [37-40]. The donor MOs ϕ (D₁) and ϕ (D₂) interact with each other to produce two new MOs resulting from the symmetrical (i) and unsymmetrical (ii) linear combinations:

$$\phi_1 = (2)^{-1/2} \{ \phi(D_1) + \phi(D_2) \} (i) \qquad \phi_2 = (2)^{-1/2} \{ \phi(D_1) - \phi(D_2) \} (ii)$$

Figure 3 shows schematically the splitting of the donor levels both in a symmetrical and an unsymmetrical dye. As the energies of the donor levels in unsymmetrical dyes should be dissimilar $(D_1 \neq D_2)$, the difference in the positions of the initial donor orbitals of the two end groups should determine the degree of asymmetry. Taking into consideration that an interaction of the energy levels is inversely proportional to the distance between them, the splitting energy (ΔE_{spl}) decreases in unsymmetrical dyes. In squaraines **6a-d**, the imidazo[1,5-a]pyridine nucleus is the less basic of the end groups and, consequently, its donor level is positioned lower (D₂).

Figure 3

The scheme in figure 3 is strongly simplified. The initial donor MOs interact with the delocalized MOs of both the open chain and the squaric ring. As a result, the direct quantum-chemical calculations for the unsymmetrical dyes gave considerable delocalization of both the highest occupied MO (HOMO) and the next orbital (HOMO-1). The increase of the basicity of the end groups is accompanied by shifting up of the splitting levels (HOMO and HOMO-1).

Besides the delocalized MOs, local orbitals in the unsymmetrical squaraine dyes also exist. It is shown that the two n-orbitals (HOMO-2 and HOMO-4) are located nearer to the energy gap (these orbitals may take part in the electronic transitions responsible for the appearance of the longwavelength absorption bands) (Fig.4). The orbitals situated at the 4-membered ring (LUMO+5 in dye **6a** and LUMO+6 in dye **6d**) are positioned too high to be involved in the lowest electron

transitions. At the same time, the levels of the n-MOs are positioned not far from the energy gap and hence could be involved in electron transitions which are connected to the absorption spectra of dyes in the visible and/or UV.

It was shown that the lowest electron transitions in symmetrical and unsymmetrical polymethine dyes and squaraines possessing the typical Brooker's groups (indolenine, benzothiazole, pyridine, quinoline, etc.) are described mainly by single excited configurations [37-43]. The calculations confirmed this regularity for the unsymmetrical squaraines **6a-d**. Some lowest single excited transitions involving the frontier and nearest levels are presented in Figure 4 and Table 3. All lowest transitions are practically "pure", *e.i.*, they are described by one single excited configuration.

Figure 4

Table 3

There is a reasonable good agreement between the experimental position of the maximum of the longest wavelength absorption band, λ_{max} , and the calculated ZINDO value of λ for the first electron transitions, whereas the non-empirical TD DFT method shows considerable divergence between calculated and spectral data. The analysis of the agreement between calculated and experimental results for the higher transitions is turned difficult by the poorly defined bands in the short wavelength spectral region. Nevertheless, the spectra of dyes **6a** and **6d** (Fig. 5) demonstrate the considerable distance between the intense long-wavelength band (625 and 694 nm, respectively) and the weaker broad band in the short-wavelength region (approximately between 300 and 400 nm), what is confirmed by the calculations for the $\pi \rightarrow \pi^*$ transitions ($\Delta \lambda_{12} = \lambda_1 - \lambda_2$).

The ZINDO method gives $\Delta\lambda_{12} \approx 250\text{-}300$ nm, while the DFT method gives lower values: $\Delta\lambda_{12} \approx 140\text{-}180$ nm. Both approximations point out correctly the ratio of the oscillator strengths for the first and second $\pi \rightarrow \pi^*$ transitions, what agrees with the high intensity of the first spectral band and with the low intensity of the short-wavelength one.

An important result is connected to the fact that $n \rightarrow \pi^*$ electron transitions involve the lone electron pair of the oxygen atoms. The TD DFT method predicts the $n \rightarrow \pi^*$ transition energy to be even lower than the energy of the first $\pi \rightarrow \pi^*$ transition in dyes **6a**, **6b** and **6d**. In contrast, the semiempirical ZINDO method envisages that the energy of the $n \rightarrow \pi^*$ transition exceeds significantly the energy of the lowest $\pi \rightarrow \pi^*$ transition for all dyes, what should be more realistic because the non-empirical approximation points to a considerable divergence between the calculated energy of the first transition and the experimental value obtained from the absorption spectra. It was recently found that the increase of the n-MOs energies and hence the decrease of the $n \rightarrow \pi^*$ transitions energy, in squaraines, can be reached by the substitution of oxygen by sulfur atoms [43].

The calculations concerning the lowest electron transitions of dyes **6a-d** (Table 3) confirm the assumption about the comparatively high transition energies involving the MO localized in the π -system flanked by the oxygen atoms of the central squaric ring [S₀ \rightarrow S₅ transition in dyes **6a** and **6b**; S₀ \rightarrow S₆ transition in dye **6d** (ZINDO method)].

3.4. Photochemical stability

In order to be useful for a number of applications a dye must display good photochemical stability. Thus, the photostability of all prepared dyes in acetonitrile was investigated. The quinoline-derived dye **6d** showed to undergo decomposition quite rapidly (complete decomposition was achieved within 5 h) and, for that reason, the fading tests were performed only for dyes **6a-c**. The dyes absorption at the corresponding λ_{max} was measured after 60, 96 and 120 h of exposure to indirect sun light and it was compared to that of the unexposed dye (table 4). It was observed that the lightfastness of squaraines **6a-c** decreases in the order: indolenine (**6a**) > benzothiazole (**6b**) >

benzoselenazole (**6c**) >> quinoline (**6d**), which is in agreement with previously described data [34, 44].

Table 4

3.5. Evaluation of singlet oxygen generating ability

Having addressed the absorption and the photochemical stability issues, the evaluation of the singlet oxygen generation efficiency of the synthesized dyes was next undertaken using the 1,3-diphenylisobenzofuran (DPBF) quenching method. DPBF forms a colorless endoperoxide upon cycloaddition with singlet oxygen, resulting in the disappearance of its characteristic absorption band at 415 nm [45]. Thus, a solution of DPBF ($5 \cdot 10^{-5}$ M) and photosensitizers ($5 \cdot 10^{-6}$ M) in dichloromethane was irradiated with a filtered light ($\lambda > 515$ nm), for different time intervals, until a significant decrease of the quenchers and/or dyes absorption was attained.

In the case of **6a** the absorption did not significantly changed within 60 min, while about half of DBPF was decomposed. Further irradiation led only to the slow simultaneous degradation of both the dye and DPBF. For dye **6b** irradiation over the same period of time resulted in a noticeable degradation of both squaraine and quencher; additional irradiation caused essentially the complete decomposition of the dye. Squaraine **6c** promoted the complete decay of DBPF absorption within 26 min, but a significant self degradation was also observed (Fig.6).

The decay of dyes absorption is likely to result from the reaction with the singlet oxygen produced upon irradiation, which turns out to be more probable as the sensitizers and DPBF concentrations become comparable.

Figure 6

The decrease of DPBF absorption at 415 nm as a function of the irradiation time for each dye compared to that promoted by 3,7-bis(N,N-dimethylamino)phenothiazin-5-ium chloride (Methylene

Blue), a well-known and very efficient long-wavelength singlet oxygen generator [46], shows that squaraines **6a-c** do possess singlet oxygen generation ability (Fig. 7). The later is more acquitted for the benzoselenazole-derived dye **6c**, due to the inherent heavy atom effect.

Figure 7

4. Conclusions

Attempts to obtain a symmetric squarylium dye derived from 3-methylimidazo[1,5a]pyridine led almost exclusively to the formation of the corresponding semisquaraine. This was found to undergo base-catalysed condensation with quaternary salts derived from 2,3,3trimethylindolenine, 2-methylbenzothiazole, 2-methylbenzoselenazole and 4-methylquinoline to yield the corresponding unsymmetrical squaraines, possessing a new skeleton among this family of dyes.

Quantum-chemical calculations of the obtained dyes shown that the asymmetry degree in the charge distribution and bond lengths is negligible due to the influence of the central squaraine (acceptor) fragment. The nature of the lowest two electron transitions is the same as in the correspondent cationic dicarbocyanines, both involving two HOMOs and the same LUMO.

By performing simple fading tests it was found that the photostability of the dyes in acetonitrile strongly depends on the end group, decreasing in the order: indolenine > benzothiazole > benzoselenazole >> quinoline.

The evaluation of the singlet oxygen sensitization ability of the dyes by the DBPF quenching method showed that all dyes are capable of producing singlet oxygen upon irradiation. The singlet oxygen generation efficiency is particularly interesting for dye **6c**, when compared to that of Methylene Blue.

The sharp and intense long-wavelength absorption bands and the inherent singlet oxygen generation capacity, make some of the synthezised dyes potential candidates as new non-porphyrinic photosensitizers for PDT.

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Table 1

Dye	Bond 1		Bond 2		Δl (1-2)		Bond 3		Δl (2-3)	
	HF	DFT	HF	DFT	HF	DFT	HF	DFT	HF	DFT
6a	1.419	1.407	1.391	1.395	0.018	0.012	1.382	1.388	0.009	0.007
6b	1.419	1.407	1.386	1.383	0.033	0.024	1.384	1.392	0.002	-0.009
6c	1.420	1.408	1.384	1.387	0.036	0.021	1.385	1.394	-0.001	-0.007
6d	1.424	1.408	1.381	1.388	0.043	0.020	1.395	1.405	-0.014	-0.017

Bond lengths in the polymethine chain of dyes **6a-d** (bond numeration showed in Fig. 2).

Table 2

Absorption maxima (λ_{max}) and molar extinction coefficient (ϵ) of squaraines **6a-d** ($c = 2 \cdot 10^{-5}$ M).

Dve	CH ₃ C	N	CH ₂ Cl ₂			
Dyc	λ_{max} (nm)	$\epsilon (M^{-1}cm^{-1})$	λ_{max} (nm)	$\epsilon (M^{-1}cm^{-1})$		
6a	578 (sh), 625	151000	584 (sh), 631 [637] ^a	157000		
6b	620	81000	632 [670] ^a	81500		
6c	632	89500	642 [678] ^a	72000		
6d	584 (sh), 668	43000	694 ^b	31500 ^b		

^a Absorption maxima of the parental symmetrical squaraines (based on indolenine, benzothiazole and benzoselenazole residues) [34]. ^b In $CH_2Cl_2/MeOH - 9/1$ (v/v) due to low solubility.

Table 3

Absorption maxima (λ_{max}) and oscillator strengths (f) of the lowest electron transitions of dyes **6a-d** calculated by the TD DFT and ZINDO methods.

	Transition	TD DFT				ZINDO			
Dye		λ_{max} (nm)	f	Main) (nm)	f	Main	
				co	onfiguration λ_{max} (IIII)		1	configuration	
6a	$S_0 \to S_1$	575 ^a	0.000 ^a	0.99	$H-1 \rightarrow L>^a$	644 (631) ^c	1.3334	0.96 H→L>	
	$S_0 \rightarrow S_2$	542	1.182	0.99	H→L>	424 ^a	0.0001 ^a	$0.96 \mid \text{H-2} \rightarrow \text{L}^{a}$	
	$S_0 \rightarrow S_3$	416	0.014	0.97	$ H\rightarrow L+1>$	387	0.0065	0.93 H→L+1>	
	$S_0 \rightarrow S_4$	393	0.0001	0.99	H-3→L>	374	0.0024	0.93 H-4→L>	
	$S_0 \rightarrow S_5$	357	0.166	0.85	H-2→L>	349 ^b	0.0002 ^b	$0.89 \mid \text{H-2} \rightarrow \text{L+6}^{\text{b}}$	
	$S_0 \rightarrow S_6$	331	0.000	0.98	H-1→L+1>	342	0.0706	0.68 H-1→L>	
6b	$S_0 \to S_1$	587 ^a	0.000 ^a	0.99	$H-1 \rightarrow L>^a$	652 (632) ^c	1.4019	0.96 H→L>	
	$S_0 \rightarrow S_2$	564	1.105	0.99	H→L>	433 ^a	0.0000 ^a	$0.97 \mid \text{H-2} \rightarrow \text{L}^{a}$	
	$S_0 \rightarrow S_3$	425	0.006	0.97	H→L+1>	386	0.0068	$0.92 \mid H \rightarrow L+1 >$	
	$S_0 \to S_4$	394	0.0002	0.99	H-3→L>	381	0.0033	0.92 H-4→L>	
	$S_0 \rightarrow S_5$	360	0.007	0.85	H-2→L>	352 ^b	0.0001 ^b	$0.89 \mid \text{H-2} \rightarrow \text{L+5}^{\text{b}}$	
	$S_0 \to S_6$	344	0.028	0.98	H-2→L+1>	345	0.0378	0.75 H-1→L>	
	$S_0 \mathop{\rightarrow} S_1$	568	1.062	0.98	H→L>	(642) ^c			
	$S_0 \rightarrow S_2$	560 ^a	0.006 ^a	0.99	H-1→L> ^a				
6 a ^d	$S_0 \rightarrow S_3$	424	0.002	0.97	$H \rightarrow L+1>$				
UC	$S_0 \to S_4$	385	0.0004	0.99	H-3→L>				
	$S_0 \rightarrow S_5$	351	0.010	0.93	H→L+2>				
	$S_0 \rightarrow S_6$	350	0.010	0.86	H-2→L>				
6d	$S_0 \mathop{\rightarrow} S_1$	623 ^a	0.000 ^a	0.99	$H-1 \rightarrow L>^a$	718 (694)	1.4780	0.96 H→L>	
	$S_0 \rightarrow S_2$	622	1.076	0.98	H→L>	433 ^a	0.0000 ^a	$0.95 \mid \text{H-2} \rightarrow \text{L}^{a}$	
	$S_0 \rightarrow S_3$	444	0.020	0.97	$H\rightarrow L+1>$	402	0.0220	$0.75 \mid H \rightarrow L+1 >$	
	$S_0 \rightarrow S_4$	412	0.0002	0.98	H-3→L>	380	0.0031	0.93 H-4→L>	
	$S_0 \rightarrow S_5$	406	0.002	0.91	H→L+2>	360	0.0223	0.85 H-1→L>	
	$S_0 \rightarrow S_6$	380	0.127	0.96	H→L+3>	357 ^b	0.0002 ^b	$0.71 \mid H \rightarrow L + 5 >^{b}$	

^a Data for $n \rightarrow \pi^*$ transitions. ^b Data for $\pi \rightarrow \pi^*$ (perpendicular system) transitions. ^c Experimental value of the dyes absorption maxima in CH₂Cl₂. ^d The semi-empirical ZINDO method does not operate with parameter for Se atom.

Table 4Remaining absorbance of dyes 6a-c in CH3CN after different exposure periods to ambient light.



Fig. 1. Indolizine-derived squaraine 1.



Fig. 2. Molecular geometry of dye 6a (bond lengths in Å) (HF method).



Fig. 3. Schematic disposition of delocalized and donor (D_1 and D_2) levels, as well as the first two electron transitions, in a symmetrical (a) and unsymmetrical dye (b) (ΔE_{spl} - splitting energy).



Fig. 4. Scheme of electron levels and electron transitions in dyes 6a and 6d (ZINDO approximation).



Fig.5. Absorption spectra of the dyes **6a** and **6d** in CH₃CN ($c = 2 \cdot 10^{-5}$ M).



Fig.6. Time evolution of the UV-Vis spectrum of DPBF ($c = 5 \cdot 10^{-5}$ M) and **6c** ($c = 5 \cdot 10^{-6}$ M) in CH₂Cl₂ solution.



Fig. 7. Comparative singlet oxygen generation ability of squaraines **6a-c** and Methylene Blue (**MB**) in CH₂Cl₂ solution ($c = 5 \cdot 10^{-6}$ M) (Abs₀ (DPBF) - absorbance of DPBF before irradiation, Abs_t (DPBF) - absorbance of DPBF after irradiation time *t*).