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9,12-Dibenzothiazolylhypericin and 10,11-Dibenzothiazolyl-10,11-didemethylhypericin: Photochemical Properties of Hypericin Derivatives Depending on the Substitution Site

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Summary. Investigating the properties of similar but regioselectively differently substituted hypericin derivatives, 9,12-dibenzothiazolylhypericin was synthesized and compared with the recently prepared 10,11-analogue. A significant difference in the ability to generate singlet oxygen and/or reactive oxygen species and different absorption spectra of these two derivatives were observed.

Keywords. Phenanthroperylenequinones; Heterocycles; Photosensitizer; Anthraquinones; Photodynamic Therapy.

Introduction

Hypericin (**1**) is a natural organic compound occurring in a variety of organisms with *Hypericum* species as the main source [1]. To obtain further information about the photosensitizing processes, which are essential for the applicability of **1** and its derivatives in photodynamic therapy (PDT), it seems worth to know influence and effect of the substitution site on the spectrophotochemical properties. Several new and promising 10,11-disubstituted didemethylhypericin derivatives have been reported over the last years [2–4] with the heterocyclically substituted 10,11-dibenzothiazolyl-10,11-didemethylhypericin (**2**) [2] being one of the most interesting ones among them. Since **2** fulfils the three main demands for second generation photodynamically active hypericin derivatives (better solubility, enhanced ability

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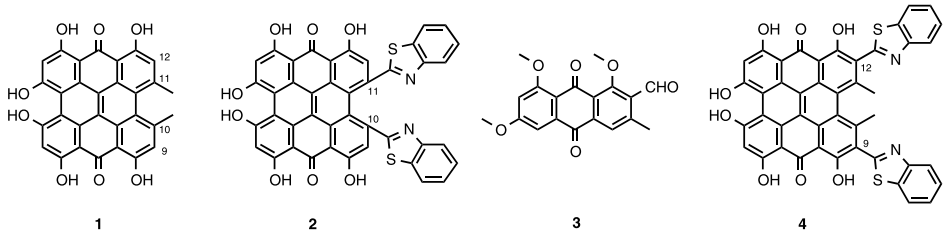


Fig. 1. Constitutions of hypericin (**1**), 10,11-dibenzothiazolyl-10,11-didemethylhypericin (**2**), 1,6,8-trimethoxy-2-formyl-3-methyl-9,10-anthraquinone (**3**), and 9,12-dibenzothiazolylhypericin (**4**)

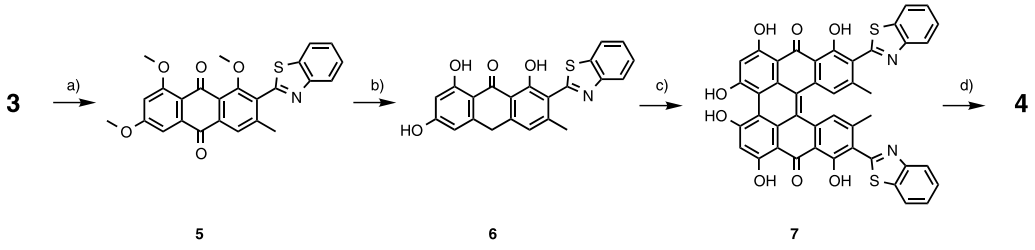
for the generation of singlet oxygen and/or reactive oxygen species, and bathochromically shifted absorption maximum), the benzothiazole substituent seems to be ideal for investigations concerning the chemical and photochemical properties of new hypericin derivatives with respect to their substitution site. As the recently prepared emodin-2-carbaldehyde (**3**) [5] provides a useful synthon for the synthesis of a new class of 9,12-disubstituted hypericin derivatives, we have now investigated synthesis and properties of 9,12-dibenzothiazolylhypericin (**4**), thus allowing a direct comparison with the 10,11-analogue **2** (Fig. 1).

Results and Discussions

Syntheses

The synthesis of **4** was carried out in four steps starting from the aldehyde **3** in a similar way as in the case of **2** [2, 6] (Scheme 1). First, reaction of **3** (prepared according to Ref. [5]) with *o*-aminothiophenol under conventional means (22 h reflux in toluene) yielded the 2-benzothiazolyl-emodin derivative **5** (75%). It is noteworthy that microwave assisted synthesis, as described in the case of **2** [2] or in the case of other heterocycles [7, 8] turned out to be less efficient and less reproducible than the conventional liquid phase synthesis described herein.

The dimerization to the corresponding hypericin derivative was carried out in three steps. First, a combined deprotection/reduction with HBr (47%)/*Ac*OH and $SnCl_2 \cdot H_2O$ under reflux (carried out in analogy to Ref. [9]) provided the anthron **6** (85%). Compounds **5** and **6** were fully characterized on basis of their IR, UV-Vis,



a) *o*-aminothiophenol/toluene, reflux; b) $SnCl_2 \cdot 2H_2O$/HBr (47%)/*Ac*OH, reflux;
c) $FeSO_4 \cdot 7H_2O$/pyridine-*N*-oxide/pyridine/piperidine; d) *h*ν, acetone

Scheme 1

mass, and NMR spectra, in particular by 2D NMR experiments. Subsequent treatment of **6** with $FeSO_4 \cdot 7H_2O$, pyridine-*N*-oxide, pyridine, and piperidine (following Ref. [10]) yielded the light-sensitive protohypericin **7** (87%). Due to its instability, in particular in solution, **7** was directly used without purification for the photocyclization to the desired 9,12-disubstituted hypericin derivative **4** (63% after column chromatography). Similarly to **2**, the ^{1}H NMR spectrum of **4** showed no single species, which is due to a web of tautomerism, internal salt formation, association, rotational phenomena, and protonation-deprotonation behaviour [2, 3]. Therefore, **4** was mainly characterized by means of its IR, UV-Vis, and mass spectra. In conclusion, **4** was obtained from **3** in a four step synthesis with an overall yield of 35%. Thus, in comparison to the 10,11-analogue **2** which was obtained in 39% [2] from the tri-*O*-methyl protected emodin-3-carbaldehyde [11], synthesis of **4** turned out to be similarly efficient.

Chemical and Photochemical Properties

It was already mentioned that the main interest in this work lies on the direct comparison of the photochemical properties of **2** and **4**. As **2** fulfilled the three main demands for a second generation photosensitizer (bathochromic shift of its main absorption wavelength of $\geq$20 nm, an enhanced ability for the generation of singlet oxygen and/or reactive oxygen species as compared to **1**, and a better solubility [2]), such a comparison should give a representative illustration of the influence of the substitution site on the photosensitizing processes.

A bathochromically shifted long wavelength absorption maximum ($\lambda_{max} \geq$ 620 nm) is of major interest as the incorporation depth of the light source increases with the wavelength and allows the use of commercially available standard medicinal lasers. In the case of **4** a bathochromic shift of $\sim$15 nm in comparison to **1** was observed, thus giving an absorption maximum in the range of 605–615 nm with a molar extinction coefficient between 8000–10000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ depending on the solvent. Concerning the *pH* dependent protonation-deprotonation behaviour of **4**, similar results as in the case of **2** [2] were observed. In comparison to **2**, the bathochromic shift of **4** is $\sim$5 nm lower and also the molar extinction coefficient of its long wavelength absorption maximum is only 50–60% of the one of **2** [2], thus making the 10,11-analogue **2** more suitable in this respect for a possible application in PDT.

To investigate the ability for the generation of singlet oxygen and/or reactive oxygen species the hypericin sensitized destruction of bilirubin has been established as a rapid experiment to assess the sensitized production of singlet oxygen and/or reactive oxygen species [12, 13]. Accordingly, the sensitized destruction of bilirubin by **4** was compared with that of the unsubstituted parent compound **1** and the 10,11-disubstituted **2** (Fig. 2).

Since **2** showed a slightly enhanced ability for the generation of singlet oxygen and/or reactive oxygen species [2] it was quite surprising that bilirubin destruction by **4** turned out to be about 50% less efficient than in the case of the reference experiment. This result clearly indicates that the heterocycles introduced in positions 9 and 12 interfere with the sensitization processes. As this phenomenon was not observed in two recently prepared 9,12-dicarbonylsubstitued hypericin deriva-

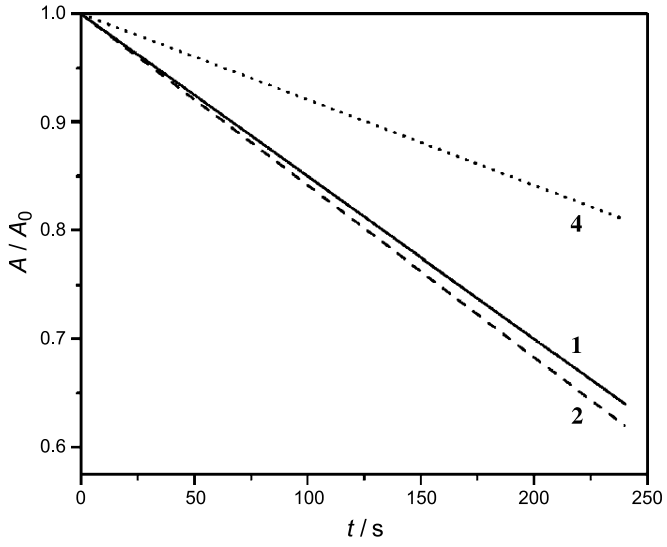


Fig. 2. Hypericin derivative sensitized photooxidation of bilirubin IXα in aerated 80% aqueous ethanol upon irradiation at $\lambda > 570$ nm; normalized absorption (A/A_0) at 460 nm *vs*. time of solutions of disodium bilirubinate IXα with **1**, **2**, or **4**

tives [14], this effect might originate from the much larger heterocycles next to the 8,13-*peri*-hydroxyl groups. The carbonyl groups and the close-by *peri*-hydroxyl groups are supposed to play an essential role in the photosensitizing processes occurring in perylenequinoid photosensitizers [15–17]. The results obtained herein suggest that these key functionalities seem to be affected by larger or electronically active substituents in their surrounding leading to a limited ability for the generation of singlet oxygen and/or reactive oxygen species. This decreased photosensitizing ability is also corroborated by the observed higher fluorescence quantum yield of **4** ($\Phi_f = 0.14$) in comparison to the very low fluorescence quantum yield of the more efficient photosensitizer **2** ($\Phi_f \sim 0.02$–0.03 [2]). Thus, the reason for the less efficient singlet oxygen sensitization might originate from a less efficient intersystem crossing and might not depend on the better triplet stabilization derived for the *peri*-hydroxyl groups in position 1 and 6 [17].

Similarly to **2**, **4** showed a rather good solubility in common organic solvents (*e.g.*, *Me*OH, *Et*OH, acetone, $CHCl_3$, *DMSO*, ether), proving that the solubility is not affected by the substitution site.

Conclusion

It was shown, that the synthesis of 9,12-dibenzothiazolylhypericin (**4**) is possible in a yield comparable to the case of 10,11-dibenzothiazolyl-10,11-didemethylhypericin (**2**) [2]. The photochemical properties of these two derivatives mainly differ with respect to the substitution site showing a decreased ability for the generation of singlet oxygen and/or reactive oxygen species and a slightly less bathochromically shifted long wavelength absorption maximum in case of the 9,12-disubstituted **4**. Thus, a significant influence of large, electron rich substituents in neighbourhood of the 8,13-*peri*-hydroxyl groups (which are supposed to play a

major role in the photosensitization processes) may be assumed. Accordingly, with respect to the substitution site, 10,11-heterocyclically substituted hypericin derivatives seem to be superior for a possible application in photodynamic therapy (PDT) than their 9,12-substituted analogues.

Experimental

Solvents were of *p.a.* quality unless stated otherwise. Melting points were measured on a *Kofler* melting point microscope (Reichert, Vienna). NMR spectra were recorded on a Bruker Avance DRX 500 MHz spectrometer using a TXI cryoprobe with *z*-gradient coil. 2D NMR experiments were performed using standard pulse sequences as provided by the manufacturer. Typical 90° hard pulse durations were 8.2 µs (^{1}H) and 16.6 µs (^{13}C), 90° pulses in decoupling experiments were set to 67 µs. HSQC and HMBC experiments were optimized for coupling constants of 145 Hz for single quantum correlations and 10 Hz for multi-bond correlations. The N*O*ESY mixing time was set to 400 ms. IR, UV-Vis, and fluorescence spectra were recorded using a Bruker Tensor 27, a Varian Cary 100 Bio UV-Vis, and a Hitachi 4010F instrument. The fluorescence quantum yield was determined according to Ref. [18]. Mass spectra were recorded on a Hewlett Packard 59987 quadrupole instrument. Hypericin sensitized photooxidation of bilirubinate IXα was determined according to Ref. [13].

9,12-Bis-(benzo[d]thiazol-2-yl)-10,11-dimethyl-1,3,4,6,8,13-hexahydroxy-phenanthro[1,10,9,8-opqra]perylene-7,14-dione (**4**, $C_{44}H_{22}N_2O_8S_2$)
A solution of 55 mg crude **7** (0.071 mmol) in 1500 cm^3 of acetone was irradiated for 35 min by means of a 700 W Hg high pressure lamp with fluorescence screen (Philips) under stirring and air admission. After evaporation of the solvent and purification by column chromatography (*THF*:*Me*OH:petrolether = 7:1:1) 35 mg (0.045 mmol) **4** were obtained (63%). Mp > 350°C; TLC: $R_f = 0.85$ (*THF*:*Me*OH:petrolether = 7:1:1); IR (KBr): $\bar{\nu} = 2975$, 2925, 2853, 1737, 1585, 1436, 1363, 1247, 1235, 1133, 1081, 968 cm^{-1}; ESI-MS (*Me*OH + 1 vol-% NH_3, negative ion mode): $m/z = 769$ ([M–H]$^-$); UV-Vis (*Et*OH:H_2O = 4:1, $c = 3.6 \cdot 10^{-5}$ mol · dm^{-3}): λ_{max} (ε) = 560 (4400), 605 (8700) nm (dm^3 · mol^{-1} · cm^{-1}); UV-Vis (acetone, $c = 6.6 \cdot 10^{-5}$ mol · dm^{-3}): λ_{max} (ε) = 565 (6600), 611 (10400) nm (dm^3 · mol^{-1} · cm^{-1}); UV-Vis (*Me*OH, $c = 6.6 \cdot 10^{-5}$ mol · dm^{-3}): λ_{max} (ε) = 559 (5700), 603 (9000) nm (dm^3 · mol^{-1} · cm^{-1}); fluorescence (*Et*OH:H_2O = 4:1, $c = 6.8 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): λ_{em} (rel. int.) = 621 (100), 666 (39) nm, $\Phi_f = 0.14$; fluorescence (acetone, $c = 6.6 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): λ_{em} (rel. int.) = 630 (100), 679 (39) nm; fluorescence (*Me*OH, $c = 6.6 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): λ_{em} (rel. int.) = 620 (100), 668 (38) nm.

2-(Benzo[d]thiazol-2-yl)-1,6,8-trimethoxy-3-methyl-9,10-anthraquinone (**5**, $C_{25}H_{19}NO_5S$)
A solution of 37 mg **3** (0.109 mmol) (prepared according to Ref. [5]) and 14 mm^3 of *o*-aminothiophenol (0.129 mmol) in 6 cm^3 of toluene was refluxed under Ar for 22 h. After cooling the solvent was evaporated and the residue purified by column chromatography ($CHCl_3$:*Et*OA*c* = 4:1), yielding 36 mg (0.081 mmol) **5** (75%). Mp 225–227°C; TLC: $R_f = 0.57$ ($CHCl_3$:*Et*OA*c* = 4:1); ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): $\delta = 2.43$ (s, ar-CH_3), 3.81 (s, 1-OCH_3), 3.98 (s, 6H, 6-OCH_3 and 8-OCH_3), 6.81 (d, $J = 2.14$ Hz, ar-H7), 7.38 (d, $J = 2.14$ Hz, ar-H5), 7.47 (m, 1H, ar-H5′ or ar-H6′), 7.55 (m, 1H, ar-H6′ or ar-H5′), 7.99 (m, 2H, ar-H4 and ar-H4′), 8.14 (s, ar-H7′) ppm; N*O*ESY ($CDCl_3$): ar-H4 ↔ ar-CH_3, 6-OCH_3 ↔ ar-H5 and ar-H7, 8-OCH_3 ↔ ar-H7; ^{13}C NMR (125 MHz, $CDCl_3$, 30°C): $\delta = 20.5$ (ar-CH_3), 55.4 (6-OCH_3 or 8-OCH_3), 56.1 (8-OCH_3 or 6-OCH_3), 62.7 (1-OCH_3), 101.8 (C5), 104.9 (C7), 117.6 (C8a), 121.1 (C4), 123.1 (C7′), 124.0 (C4′), 125.0 (C5′ or C6′), 125.4 (C9a), 125.5 (C6′ or C5′), 134.2 (C10a), 135.3 (C2), 135.9 (C4a), 136.0 (C7a′), 144.5 (C3), 152.5 (C3a′), 158.6 (C1), 161.5 (C8), 162.2 (C2′), 163.7 (C6), 180.1 (C9), 183.0 (C10) ppm; HMBC ($CDCl_3$): C1 ↔ 1-OCH_3, C3 ↔ ar-CH_3 and ar-H4, C4a ↔ ar-H4, C6 ↔ 6-OCH_3, ar-H5, and ar-H7, C8 ↔ ar-H7 and 8-OCH_3, C8a ↔ ar-H7 and ar-H5, C10 ↔ ar-H4 and ar-H5,

C10a ↔ ar-H4, C3a′ ↔ ar-H4′, C7a′ ↔ ar-H7′; HSQC data were according to structure; ESI-MS (*Me*OH:$CHCl_3$ = 1:1 + 1 vol-% HCOOH, positive ion mode): m/z = 446 ($[M+H]^+$); IR (KBr): $\bar{\nu}$ = 2957, 2924, 2854, 1744, 1671, 1595, 1461, 1260, 1093, 1017, 802 cm^{-1}; UV-Vis ($CHCl_3$): λ_{max} (rel. int.) = 281 (100), 348 (20), 384 (17) nm.

2-(Benzo[d]thiazol-2-yl)-1,6,8-trihydroxy-3-methyl-10H-anthracen-9-one (**6**, $C_{22}H_{15}NO_4S$)
An Ar-flushed solution of 24 mg (0.054 mmol) **5** in 5 cm^3 glacial *Ac*OH was heated to reflux. Then 100 mg (0.48 mmol) $SnCl_2 \cdot 2H_2O$ dissolved in 2.25 cm^3 HBr (47%) were added and refluxed for 60 min. The solution was poured on ice/H_2O, centrifuged, and washed with H_2O giving 18 mg (0.046 mmol) **6** (85%). Mp ≥ 240°C (decomp.); TLC: R_f = 0.25 ($CHCl_3$:*Et*OA*c* = 4:1); ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): δ = 2.37 (s, ar-CH_3), 4.43 (s, ar-CH_2-ar), 6.27 (s, ar-H7), 6.49 (s, ar-H5), 7.04 (s, ar-H4), 7.50 (m, 1H, ar-H5′ or ar-H6′), 7.57 (m, 1H, ar-H6′ or ar-H5′), 8.10 (d, 1H, J = 7.63 Hz, ar-H4′ or ar-H7′), 8.17 (d, 1H, J = 7.63 Hz, ar-H7′ or ar-H4′), 10.92 (bs, 6-OH), 12.19 (s, 8-OH), 13.12 (s, 1-OH) ppm; N*O*ESY (*DMSO*-d_6): –CH_2– ↔ ar-H5 and ar-H4, ar-CH_3 ↔ ar-H4; ^{13}C NMR (125 MHz, *DMSO*-d_6, 30°C): δ = 21.2 (ar-CH_3), 32.4 (–CH_2–), 101.2 (C7), 107.7 (C5), 108.5 (C8a), 113.3 (C9a), 119.4 (C2), 121.2 (C4), 122.1 (C4′ or C7′), 123.0 (C7′ or C4′), 125.5 (C5′ or C6′), 126.2 (C6′ or C5′), 135.8 (C7a′), 143.8 (C4a or C10a), 145.0 (C10a or C4a), 146.1 (C3), 152.6 (C3a′), 160.2 (C1), 162.4 (C2′), 164.7 (C8), 165.5 (C6), 190.9 (C9) ppm; HMBC (*DMSO*-d_6): C1 ↔ 1-OH, C3 ↔ ar-CH_3 and ar-H4, C4a ↔ –CH_2–, C6 ↔ 6-OH, ar-H5, and ar-H7, C8 ↔ ar-H7 and 8-OH, C8a ↔ ar-H7, –CH_2–, and ar-H5, C9a ↔ 1-OH, ar-H4, and –CH_2–, C10a ↔ –CH_2–, C3a′ ↔ ar-H4′, C7a′ ↔ ar-H7′; HSQC data were according to structure; ESI-MS (*Me*OH + 1 vol-% HCOOH, positive ion mode): m/z = 390 ($[M+H]^+$); IR (KBr): $\bar{\nu}$ = 2954, 2924, 2854, 1737, 1603, 1460, 1377, 1287, 1261, 1119, 804, 722 cm^{-1}; UV-Vis (*Me*OH): λ_{max} (rel. int.) = 262 (100), 357 (96) nm.

9,14-Bis-(benzo[d]thiazol-2-yl)-10,13-dimethyl-1,3,4,6,8,15-hexahydroxy-dibenzo[ao]perylene-7,16-dione (**7**, $C_{44}H_{24}N_2O_8S_2$)
A mixture of 70 mg **6** (0.179 mmol), 3.3 mg $FeSO_4 \cdot 7H_2O$ (0.009 mmol), and 103.3 mg (1.09 mmol) of pyridine-*N*-oxide in 1.1 cm^3 of dry pyridine and 0.1 cm^3 of dry piperidine was stirred under Ar in the dark at 115°C for 1 h. After cooling to room temperature, the mixture was poured into 10 cm^3 2*N* HCl and stirred for further 30 min at room temperature (in the dark). After centrifugation the residue was washed three times with 3% HCl, three times with H_2O, and dried over P_2O_5 yielding 60 mg (0.078 mmol) **7** (87%) as a black solid. Mp > 350°C; ESI-MS (*Me*OH + 1 vol-% NH_3, negative ion mode): m/z = 771 ($[M-H]^-$); UV-Vis (acetone): λ_{max} (rel. int.) = 572 (100), 605 (93) nm. Due to its instability, crude **7** was directly used for the subsequent photocyclisation to **4** without further purification.

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