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Concerning the Absorption and Photochemical Properties of an ω-4-Dimethylaminobenzal Hypericin Derivative

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Summary. A hypericin derivative containing ω,ω'-4-dimethylaminobenzal residues was shown to undergo an intramolecular [2 + 2] cycloaddition upon irradiation leading to a cyclobutane derivative whose main absorption band is hardly shifted as compared to hypericin. The corresponding ω-substituted derivative displayed a 34 nm bathochromic shift and a strongly reduced fluorescence quantum yield rendering it a nice candidate for a photodynamic therapy agent. Unfortunately, however, it produced virtually no photosensitized active oxygen species, making it thus unsuited for this purpose.

Keywords. Photodynamic therapy; Bilirubin photooxidation; Bathochromic shift; Fluorescence; Intramolecular cycloaddition.

Introduction

Hypericin (**1**) is a photosensitizing pigment isolated from the weed St. John's wort (*Hypericum perforatum* L.). It has been shown to constitute a promising photodynamic agent for the therapy of certain viral infections and tumors [1]. However, a serious drawback for its use is its main absorption band situated slightly below 600 nm, which is just outside of the 640–660 nm wavelength of commercial medicinal lasers. Therefore, efforts have been undertaken to shift the absorption band of **1** to longer wavelengths, but concomitantly retaining its main functional outfit. Thus, restricting the derivatization of **1** to its carbon atoms, derivatives like 2,5-diiodo-hypericin or the enhanced conjugation derivative **2** have been synthesized [2]. However, upon irradiation with visible light **2** rapidly underwent an intramolecular [2 + 2] cycloaddition to **3**, thereby destroying the 36 nm bathochromic shift of its long-wavelength absorption band as compared to **1**.

To overcome this problem we investigated if substitution of the benzene rings of **2** with an electron donating auxochromic group would perhaps suppress the [2 + 2] cycloaddition on the one hand; on the other hand, it seemed of interest if such a substitution would sufficiently shift the long-wavelength band if only one methyl group of the hypericin moiety is substituted with a benzal moiety.

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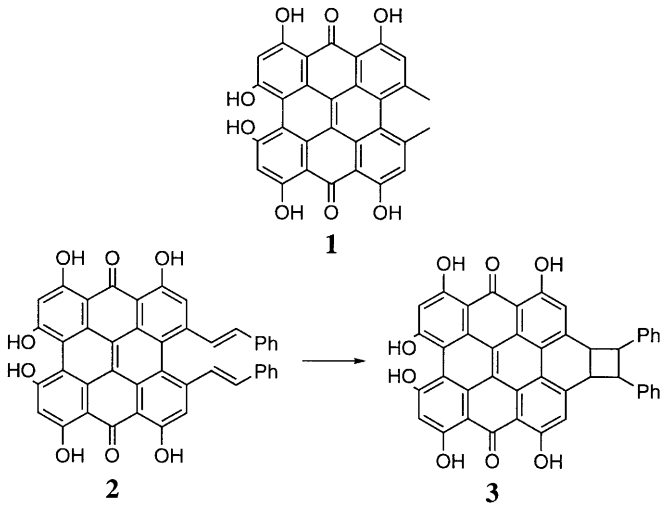
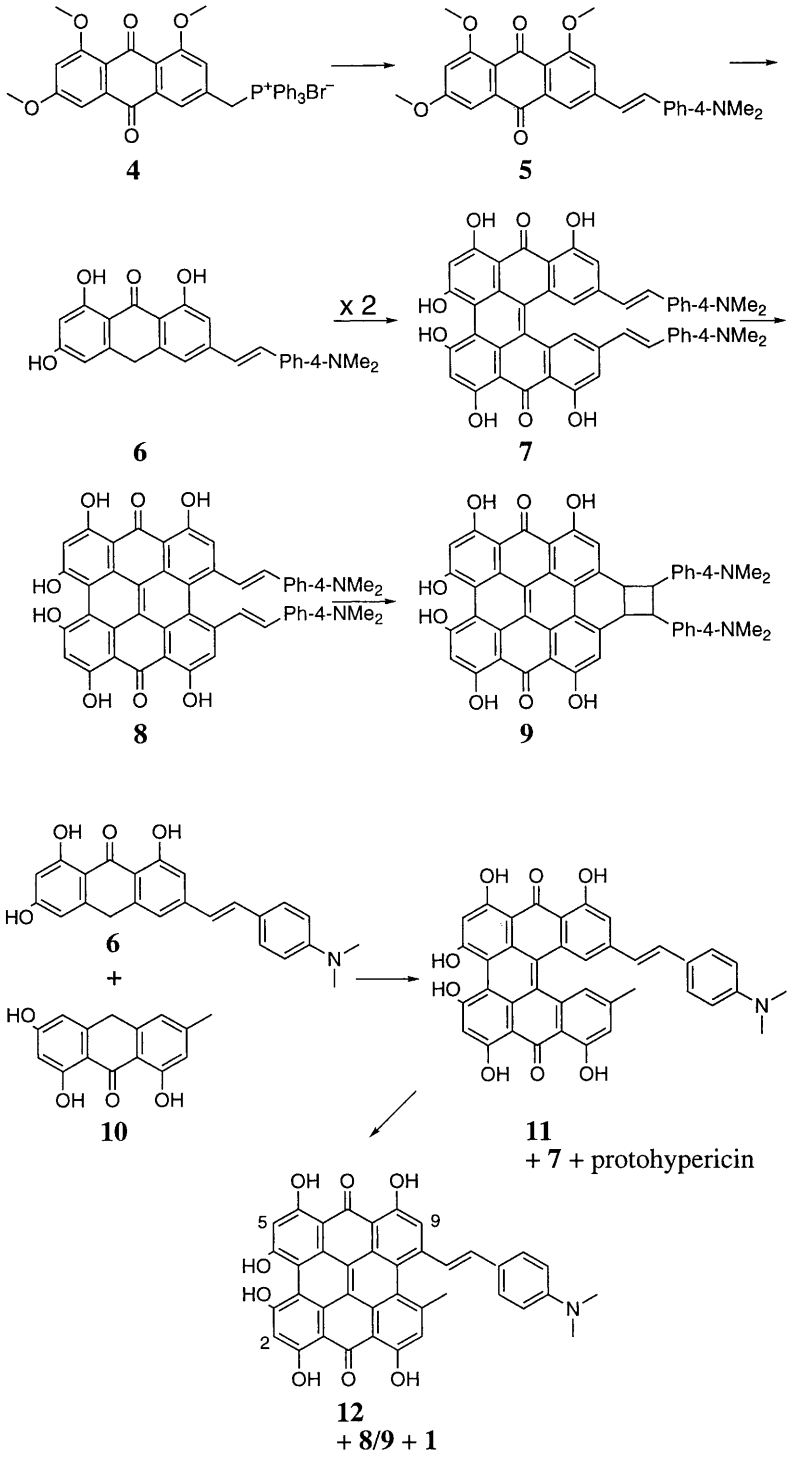


Results and Discussions

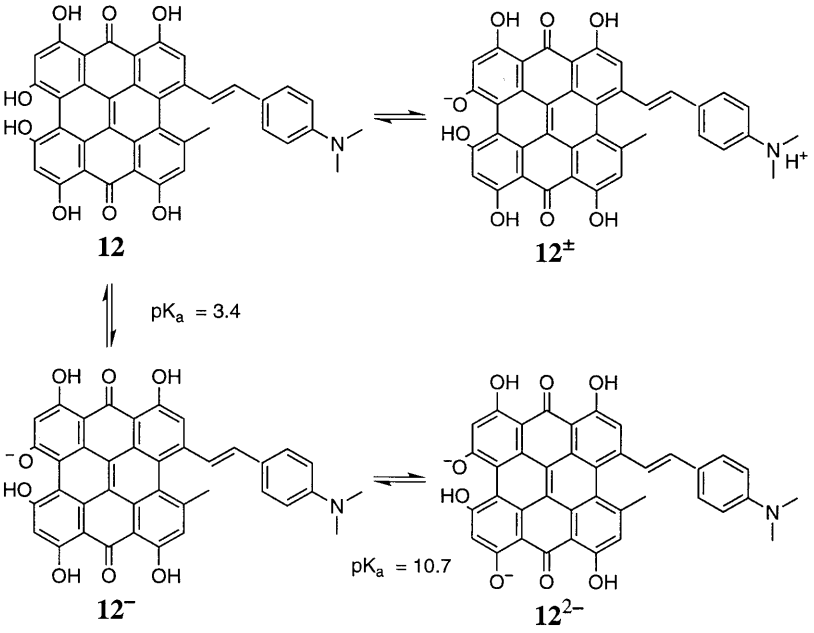
Following the well established route to functionalize the methyl group of hypericin [2, 3] the emodin phosphonium salt **4** was reacted with 4-dimethylamino-benzaldehyde to yield the (*E*)-anthraquinone derivative **5**. One-pot deprotection and reduction with $SnCl_2$/ HBr in acetic acid provided the anthrone derivative **6** in high yield. Dimerization of the latter in the conventional way [4] gave the protohypericin derivative **7**. Upon irradiation of **7**, the desired hypericin derivative **8** could only be traced by its bathochromically shifted long-wavelength absorption band which showed up at 655 nm. However, after being produced it immediately underwent a light-induced intramolecular [2 + 2] cycloaddition reaction to give the cyclobutane derivative **9** – even more efficiently than its *bis-des*-dimethylamino parent **2** [2]. Accordingly, the electron donating auxochromic group did not prevent the cycloaddition reaction, and therefore this dimerization strategy was not pursued any further.

Following this result, it was investigated if only half of the bathochromically shifting dimethylaminobenzal moieties of **8** would be sufficient to move the long-wavelength absorption band of **1** into the medicinal laser region. The synthesis of such a derivative was achieved by reacting the dimethylamino derivative **6** with emodin anthrone **10** [4]. The resulting protohypericin derivative **11** together with the corresponding symmetrical dimers, *i.e.* protohypericin and **7**, were photocyclyzed, and the desired product **12** was eventually separated in rather low yield from the symmetrical dimers **1** and **8/9**.

The constitution of **12** was immediately evident from its spectroscopic properties (see Experimental). In addition, these data together with appropriate experiments revealed detailed information on its deprotonation and photochemical behaviour, which are relevant to its potential photodynamic properties. Thus, a spectrophotometric titration showed that a three-species deprotonation system prevails within the *pH* range from 1 to 12. The absorption spectra of the three species **12**, $\mathbf{12}^{-}$, and $\mathbf{12}^{2-}$ characterized by pK_a values of 3.4 and 10.7 are displayed in Fig. 1.



No experiments were available to discriminate between **12** and its zwitterion $\mathbf{12}^{\pm}$; however, we inferred that the equilibrium would be shifted in favour of **12** because of the basicity of the nitrogen atom of **12** (vinylogous amide). This



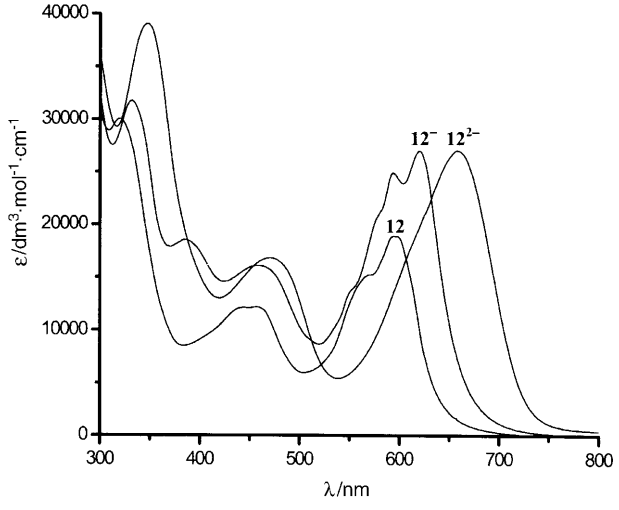


Fig. 1. Absorption spectra of the three species **12**, $\mathbf{12}^-$, and $\mathbf{12}^{2-}$ in 80% ethanol

could be substantiated by AM1 calculations, which showed **12** to be more stable by $276\,\mathrm{kJ \cdot mol^{-1}}$ than its corresponding zwitterion $\mathbf{12}^{\pm}$. These calculations also revealed that protonation of **12** to yield $\mathbf{12} \cdot \mathrm{H}^+$ would be favoured at the carbonyl group in position 14 by about $20\,\mathrm{kJ \cdot mol^{-1}}$ over the protonation at the nitrogen atom, stressing again the vinylogous amide substructure of **12**. These calculations were also in accordance with the observation that **12** could not be extracted into 1 *M* hydrochloric acid for isolation and purification purposes. The assignment of the phenolate ion $\mathbf{12}^-$ was based upon an electrospray mass spectroscopic

experiment in analogy to $\mathbf{1}^-$ [5], which showed that at *pH* 6.1 the $[M–H]^-$ ion is the only one that can be detected. Moreover, in *DMSO*-d_6 only one ^{1}H NMR OH signal at 18.3 ppm was observed, which is characteristic [6] of a hydroxyl proton in position 3/4 strongly bonded intramolecularly to the 4/3 phenolate ion. Accordingly, at physiological *pH* values $^{(3-)}\mathbf{12}$ will be the predominating species (AM1 calculations revealed that 3- and 4-deprotonation is of virtually identical energy). Further deprotonation yields $\mathbf{12}^{2-}$ – based on AM1 calculations the second deprotonation site 6 (yielding $^{(3-,6-)}\mathbf{12}$) was found to be more stable than the most stable of the other possible species by about 30 kJ · mol^{-1}. Comparison of the absorption spectra of **12** in several solvents pointed also to a prevalence of the $^{(3-)}\mathbf{12}$ ion in such solutions. Thus, the bathochromic shift of more than 30 nm to about 630 nm compared to hypericin (**1**) together with a moderately high extinction coefficient would make **12** a proper candidate to match the medicinal laser wavelength. It should also be mentioned that the fluorescence spectra of the three species were quite different from each other. Thus, in 80% ethanol and at $pH = 0.6$ it displayed an emission band at 621 nm ($\Phi_F = 0.07$), at $pH = 6.9$ at 644 nm ($\Phi_F = 0.03$), and at $pH > 12$ at 718 nm ($\Phi_F = 0.02$).

The tautomeric situation of **12** was investigated in some detail by means of AM1 calculations. Accordingly, the 7,14-dioxo tautomer proved to be much more stable than all other conceivable tautomers, with a difference in their enthalpies of formation of 79 kJ · mol^{-1} between the 7,14-dioxo tautomer and the next one in the stability series, the 1,6-dioxo tautomer. In addition, in analogy to the results obtained for **1** and $^{(3-)}\mathbf{1}$ [8] it could also be confirmed that for $^{(3-)}\mathbf{12}$ the 7,14-dioxo tautomer shown in the formulae predominates.

With respect to the photophysical and photochemical behaviour of $^{(3-)}\mathbf{12}$, which is of outmost importance for its intended use, its fluorescence spectrum displayed an emission which was *Stokes*-shifted by nearly 20 nm. This is quite large compared with the only 5 nm of $^{(3-)}\mathbf{1}$ [9]. Together with the rather small fluorescence quantum yield Φ_F of only about one tenth of that of hypericin ($^{(3-)}\mathbf{1}$) [9] this pointed to excited state processes different from those of $^{(3-)}\mathbf{1}$. At this point the use of **12** as a photodynamic agent seemed even more promising since a low fluorescence quantum yield could point to an enhanced intersystem crossing, which would be necessary to sensitize active oxygen species. However, hypericin sensitized photooxidation of bilirubin, which has been shown to provide a sensitive measure of the generation of sensitized active oxygen species – in peculiar of singlet oxygen and superoxide radicals [10] – revealed that $^{(3-)}\mathbf{12}$ did not produce the sensitized singlet oxygen and superoxide species thought to be the most active agents in hypericin photodynamic therapy [1] (Fig. 2). Accordingly, a non-radiative channel other than intersystem crossing (or a suppressed capacity of the triplet state to sensitize singlet oxygen) is operating in **12**. Perhaps an intramolecular quenching process similar to the intermolecular quenching of the triplet state of **1** by amines [11] might be operative.

In conclusion, **12** as a monomethyl conjugated and amine functionalized molecule showed a promising bathochromic long-wavelength shift together with a reduced fluorescence yield. However, the auxochromic dimethylamino substituent of the benzal residue led to a dramatic change in the photochemical behaviour of **12** and, in particular, its phenolate ion as compared to **1**, making it virtually

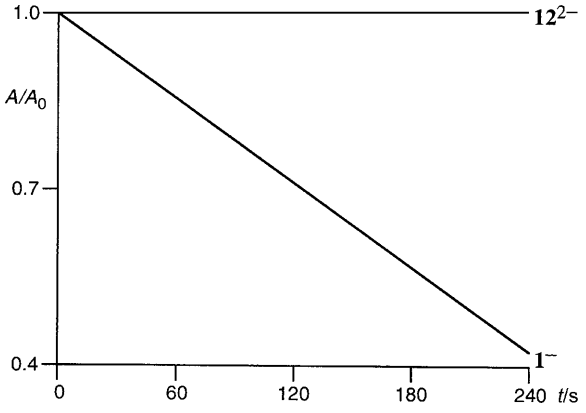


Fig. 2. Hypericin derivative sensitized photooxidation of bilirubin: normalized absorption changes (A/A_0) with time of solutions of disodium bilirubinate together with $^{(3-)}$**1** or $^{(3-)}$**12** in aereated 80% ethanol upon irradiation at $\lambda > 570$ nm

non-reacting with respect to the generation of active oxygen species and thus unsuited for its use as a photodynamic therapy agent. Nevertheless, the behaviour of this compound added a valuable facet to the chemistry and photochemistry of hypericin.

Experimental

Solvents were of *p.a.* quality. ^{1}H and ^{13}C NMR, UV/Vis, fluorescence, and mass spectra were recorded using Bruker DRX 500 and DPX 200, Hewlett Packard 8453 UV/Vis, Hitachi 4010F, and Hewlett Packard 59987 quadrupole instruments. HSQC NMR experiments for signal assignments were carried out using standard parameters. The fluorescence quantum yield was determined as described previously [9]. The emodin phosphonium salt **4** was prepared according to Ref. [3]. Emodin anthrone **10** was synthesized in the usual way [6]. Hypericin sensitized photooxidation of bilirubinate was executed as described in Ref. [10]. AM1 calculations [12] were performed at the SGi Origin 2000 of the LIZENS using the MOPAC package [13, 14].

(E)-6-(2-(4-Dimethylaminophenyl)-ethenyl)-1,3,8-trimethoxy-anthraquinone (**5**; $C_{27}H_{25}NO_5$)

A mixture of 5 g 60% **4** (4.6 mmol), 2.17 g dry and pulverized K_2CO_3 (15.73 mmol), 1.45 g 18-crown-6 (5.5 mmol), and 293 cm^3 dry CH_2Cl_2 was refluxed for 15 min. To this dark blue ylide solution, a solution of 11.47 g commercial (Fluka) 4-dimethylamino-benzaldehyde (76.9 mmol) in 293 cm^3 dry CH_2Cl_2 was added in three portions, refluxing the mixture between the additions for 40 min. After refluxing for additional 30 min the cooled reaction mixture was diluted with 400 cm^3 CH_2Cl_2, filtered, and extracted with brine. The brine phase was extracted with 3×100 cm^3 CH_2Cl_2, and the organic phase was dried over Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica using $CHCl_3$:EtOAc = 1:1 as the eluent.

Yield: 0.86 g (42%); m.p.: 237–240°C; ^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 7.80–7.30 (m, $2H_{Ph} + 4H_{ar} + HC{\equiv}CH$), 7.15 (m, H_{ar}-4), 6.19 (m, H_{ar}-2), 6.74 (d, $J = 8.4$ Hz, XX′ part of an AA′XX′ system, H_{Ph}-3,5), 3.95 (s, OCH_3), 3.89 (s, OCH_3), 2.96 (s, $N(CH_3)_2$) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 50 MHz): 183.4 (C=O), 179.6 (C=O), 163.3 (C_{ar}–OCH_3), 161.0 (C_{ar}–OCH_3), 159.4 (C_{ar}–OCH_3), 143.5, 135.6, 133.7, 131.7, 131.4, 128.9, 128.6, 128.3, 121.9, 121.2, 117.7, 115.7, 114.9, 105.2 ($13C_{ar} + C{=}C$), 56.32 (OCH_3), 56.26 (OCH_3), 55.9 (OCH_3) ppm (the $N(CH_3)_2$ signal was masked

by the solvent peak); UV/Vis (*DMSO*; $c = 1.10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 479$ (12630), 368 (24570), 275 (27070) nm.

(E)-9,10-Dihydro-6-(2-(4-dimethylaminophenyl)-ethenyl)-1,3,8-trihydroxy-anthracene-9-one (**6**; $C_{24}H_{21}NO_4$)

To 1.57 g **5** (3.57 mmol) dissolved in 100 cm^3 glacial acetic acid, 7.0 g $SnCl_2 \cdot 2H_2O$ (31 mmol) and 20 cm^3 47% HBr were added, and the reaction mixture was refluxed for 2 h. Upon cooling to 15°C for 2 h the product was filtered, washed with a few drops of glacial acetic acid, and dried in high vacuum.

Yield: 1.33 g (97%); m.p.: > 320°C; ^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 12.49 (s, OH-1), 12.37 (s, OH-8), 10.79 (s, OH-3), 7.52 (d, $J = 8.4$ Hz, AA′ part of an AA′XX′ system, H_{Ph}-2,6), 7.39 (d, $J = 16.3$ Hz, HC=C), 7.23 (d, $J = 16.3$ Hz, C=CH), 7.14 (s, H_{ar}-5), 7.00 (s, H_{ar}-7), 6.83 (d, $J = 8.4$ Hz, XX′ part of an AA′XX′ system, H_{Ph}-3,5), 6.44 (s, H_{ar}-4), 6.24 (m, H_{ar}-2), 4.36 (s, CH_2), 2.99 (s, $N(CH_3)_2$) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 50 MHz): 190.5 (C=O), 164.9 (C_{ar}–OH), 164.5 (C_{ar}–OH), 162.0 (C_{ar}–OH), 145.4, 144.9, 142.3, 133.1, 128.4, 125.4, 122.6, 122.5, 116.6, 113.5, 113.0, 11.3, 108.5, 107.4, 101.0 (13C_{ar} + C=C), 32.4 (CH_2) ppm (the $N(CH_3)_2$ signal was masked by the solvent peak); UV/Vis (*DMSO*, $c = 1.10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 443$ (21500), 298 (8660), 258 (11670) nm.

Dimerization of **6**

This reaction sequence was executed 0.1 mmolar in analogy to Ref. [2], but in a qualitative way using UV/Vis spectroscopy as the monitor and without isolation and further characterization of intermediates and product. Thus, the protohypericin derivative was characterized by absorption bands at $\lambda_{max}(DMSO) = 589$ (33), 386 (65), and 269 (100) nm (in parentheses: relative intensities). The desired product **8** exhibited a long-wavelength band at 655 nm, whereas the final product **9** displayed a hypericin-like UV/Vis spectrum similar to its parent compound **3** [2] with a long-wavelength band at 589 nm.

(E)-10-(2-(4-Dimethylaminophenyl)-ethenyl)-1,3,4,6,8,13-hexahydroxy-11-methyl-phenanthro [1,10,9,8-opqra]perylene-7,14-dione (**12**; $C_{39}H_{25}NO_8$)

To a solution of 113 mg **6** (0.29 mmol) and 450 mg **10** (1.76 mmol) in 10.5 cm^3 absolute pyridine and 1.5 cm^3 absolute piperidine, 1.05 g pyridine-N-oxide (11.04 mmol) and 28.5 mg $FeSO_4 \cdot 7H_2O$ (*p.a.*; 0.10 mmol) were added, and the reaction mixture was stirred for 1 h at 100–110°C under Ar and exclusion of light. After cooling to room temperature, the violet coloured reaction mixture was poured into 73 cm^3 of 2 *M* HCl. After standing for 30 min it was centrifuged, and the residue was washed twice with 3% HCl, three times with H_2O, and dried over silica. The residue (500 mg) consisting of a mixture of the protohypericines including **11** was dissolved in 4 dm^3 acetone and irradiated with a 700 W Hg high pressure lamp for 1.5 h under admission of air. The resulting wine red solution was evaporated, washed with 20 cm^3 MeOH, and dried over silica. The residue containing hypericin (**1**) and its derivatives **8/9** and **12** was chromatographed on a silica plate (20 × 20 × 0.2 cm) using *THF*:glacial acetic acid = 10:1. The band containing **12** was triturated three times with 50 cm^3 *THF*:methanol = 1:1 and filtered. After evaporation, the residue was chromatographed twice with MeOH on a Sephadex LH 20 column.

Yield: 8 mg (5%); ^{1}H NMR (*DMSO*-d_6, δ, 500 MHz): 18.33 (s, OH-3), 14.73 (br s, OH-1,6,8,13), 7.63 (d, $J = 16.3$ Hz, HC=C), 7.43 (d, $J = 8.6$ Hz, AA′ part of an AA′XX′ system, H_{Ph}-2,6), 7.41 (s, H_{ar}-9,12), 7.03 (d, $J = 16.3$ Hz, C=CH), 6.78 (d, $J = 8.6$ Hz, XX' part of an AA′XX′ system, H_{Ph}-3,5), 6.58 (s, H_{ar}-2,5), 2.97 (s, $N(CH_3)_2$), 2.74 (s, CH_3) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 125 MHz): 183.4, 182.8 (2 C=O), 174.6, 174.3 (C_{ar}–O-3,4), 168.14, 168.13 (C_{ar}–O-1,6), 161.8, 150.6 (C_{ar}–O-8,13),

144.1, 142.9 (C_{ar}-10,11), 142.7 (C_{Ph}-4), 138.0 (C=), 132.1 (C_{Ph}-2,6), 128.2 (C_{Ph}-3,5), 127.1, 127.0 (C_{ar}-3a,3b), 126.7, 126.2 (C_{ar}-6b,14b), 124.5 (=C), 124.2 (C_{Ph}-1), 121.6, 120.3 (C_{ar}-7c,14c), 121.0, 120.0 (C_{ar}-10a,10b), 119.5, 119.4 (C_{ar}-7b,13b), 112.3, 112.2 (C_{ar}-9,12), 108.8, 108,6 (C_{ar}-6a,14a), 105.64, 105.63 (C_{ar}-2,5), 102.3, 102.1 (C_{ar}-7a,13a), 40.5 ($N(CH_3)_3$), 24.4 (CH_3) ppm (signal assignment by $^1H/^{13}C$ HSQC experiments); UV/ Vis (*DMSO*, $c = 1.10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 633$ (26800), 625 (24600), 603 (22770), 464 (16660), 392 (19570) 338 (28700), 260 (41230) nm; UV/ Vis (80% EtOH, $c = 1.10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 623$ (26700), 597 (23720), 463 (17210), 457 (17240), 384 (19570) nm; UV/ Vis (*THF*): λ_{max}(rel. int.) = 630 (76), 601 (45), 582 (45), 464 (29), 382 (40), 337 (63), 289 (68), 243 (100) nm; UV/ Vis (acetone): λ_{max}(rel. int.) = 628 (64), 581 (41), 464 (27), 383 (37), 336 (59), 210 (100) nm; UV/ Vis (CH_3CN): λ_{max}(rel. int.) = 623 (34), 581 (22), 459 (15), 383 (20), 334 (32), 273 (57), 252 (59), 200 (100) nm; UV/ Vis (MeOH): λ_{max}(rel. int.) = 618 (40), 593 (30), 581 (26), 458 (19), 383 (22), 331 (39), 284 (42), 203 (100) nm; UV/ Vis (H_2O): $\lambda_{max} = 640$ (sh), 600 nm; fluorescence (*DMSO*, $c = 1 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): λ_{em}(rel. int.) = 653 (45), 603 (100) nm, $\Phi_F \approx 0.03$; fluorescence (80% EtOH, $c = 1 \cdot 10^{-7}$ mol · dm^{-3}, $pH = 0.6$, $\lambda_{ex} = 550$ nm): λ_{em}(rel. int.) = 621 (100), 597 (70; sh) nm, $\Phi_F \approx 0.07$; fluorescence (80% EtOH, $c = 1 \cdot 10^{-7}$ mol · dm^{-3}, $pH = 6.9$, $\lambda_{ex} = 550$ nm): λ_{em}(rel. int.) = 641 (34), 596 (100) nm, $\Phi_F \approx 0.03$; fluorescence (80% EtOH, $c = 1 \cdot 10^{-7}$ mol · dm^{-3}, $pH > 12$, $\lambda_{ex} = 550$ nm): λ_{em}(rel. int.) = 718 (100) nm, $\Phi_F \approx 0.02$; electrospray MS (*i*-propanol/NH_4OAc buffer, $pH = 6.1$): $m/z = 634$ $[M–H]^-$.

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