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Condensed Emodin Derivatives and Their Applicability for the Synthesis of a Fused Heterocyclic Hypericin Derivative

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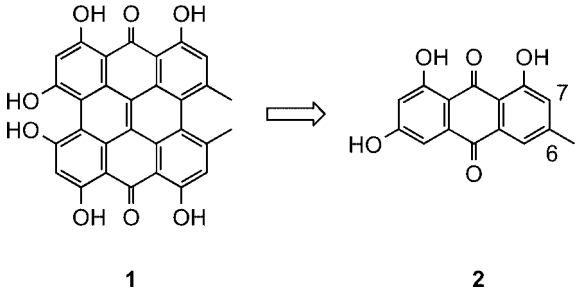
Investigations on the bifunctionalization of emodin at C(6) and C(7) with respect to the synthesis of a new class of fused heterocyclic hypericin derivatives were undertaken. The synthesis of such complex anthraquinone derivatives is possible by applying a Marschalk-type reaction with emodin-6-carbaldehyde as the key step. The newly synthesized pyridazinone-fused hypericin derivative possesses a satisfying bathochromically shifted absorption maximum making it a promising candidate for further investigations concerning a possible application in photodynamic therapy. Furthermore, the herein described strategy for the bifunctionalization of emodin provides a novel route for the synthesis of anthraquinone derivatives that are difficult to obtain by other methods.

Introduction

Hypericin (**1**) is a naturally occurring dye belonging to the family of the phenanthroperylenequinones. The most outstanding property of **1** is its ability to generate singlet oxygen and other reactive oxygen species upon irradiation with light in the presence of oxygen making it one of the most powerful photosensitizers to be found in nature.[1] Unfortunately, the applicability of **1** in photodynamic therapy (PDT) suffers from three main disadvantages: expensive isolation from natural sources (e.g. *Hypericum* species), limited solubility under physiological (aqueous) conditions, and an absorption maximum (λ_{max} = 590–600 nm) lying outside the optimum wavelength range (λ_{max} = 620–800 nm) for a photosensitizer. The first disadvantage can be overcome by semisynthetic approaches starting from the easily available anthraquinone precursor emodin (**2**)[2] (Scheme 1). Next to the cheaper availability this strategy also allows the introduction of substituents on stage of the easier to handle anthraquinone skeleton.

Accordingly, several efforts in the syntheses of novel bathochromically shifted and better soluble hypericin derivatives (second generation hypericin based photosensitizers) have been undertaken over the last years.[3,4] Because nitrogen containing heterocyclically substituted derivatives turned out to be quite promising with respect to the above mentioned aims,[4] we have now focused on a new class of nitrogen containing fused heterocyclic hypericin derivatives. These compounds should provide an even larger bathochromic shift compared to the heterocyclically substituted



1 **2**

Scheme 1. Semisynthetic approach for the synthesis of hypericin (**1**) and its derivatives starting from emodin (**2**).

derivatives reported so far[4] because the fused aromatic heterocycle is supposed to allow an efficient delocalization of the π-electrons of the phenanthroperylenequinone moiety. Therefore, the main challenge in this approach seems to be the syntheses of appropriate bifunctional emodin derivatives (carbonyl or carboxyl-substituted) allowing the introduction of nitrogen via a nucleophilic attack. Among the nitrogen-containing fused aromatics phthalazines and phthalazinones play an important role as intermediates as well as due to their biological and medicinal properties.[5]

We herein report our efforts in synthesizing bifunctionalized emodin derivatives as well as testing the applicability of these derivatives for the synthesis of a new class of phthalazinone-fused hypericin derivatives and the (photo)-chemical properties in comparison to the unsubstituted parent compound **1**.

Results and Discussion

We have recently described the regioselective functionalization of the emodin moiety at C(7)[6] via a Marschalk-

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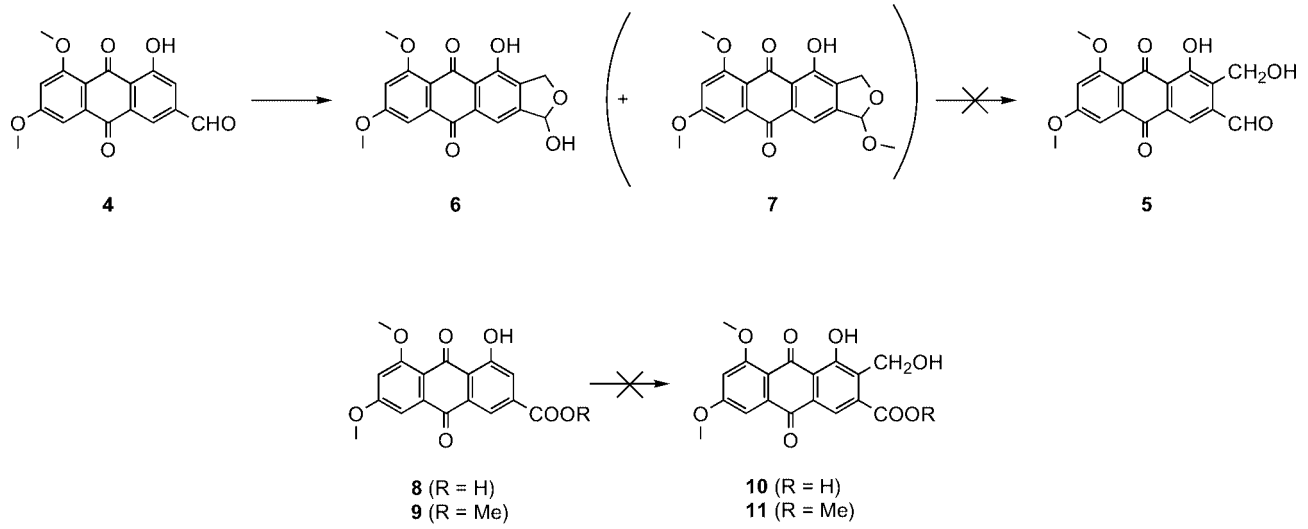
type approach.[7] This methodology provides a useful tool for the regioselective hydroxymethylenation of hydroxyanthraquinones. When carried out under reductive conditions, the deactivation of anthraquinones towards an electrophilic attack can be overcome by an intermediate reduction to the corresponding electron-rich anthrahydroquinone. Therefore this strategy plays a major role in the syntheses of anthracyclinone-type antibiotics.[8] Unfortunately, these 7-functionalized emodin derivatives turned out to be not suitable for the syntheses of 6,7-bifunctional emodin derivatives due to their unsubstituted methyl group at C(6). The activation of this methyl group towards further oxidation seems to be rather hampered as a result of the additional electron-withdrawing functional groups at C(7) as observed in our synthesis of endocrocin[6] as well as by Joshi et al. in their endocrocin synthesis starting from 6,7-dimethyl emodin, where only the 7-methyl group could be oxidized.[9]

Accordingly, it seemed necessary to introduce the functional group at C(7) on an already 6-functionalized emodin derivative. As no Marschalk type reactions on additionally deactivated anthraquinones (eg. *ortho* to a carbaldehyde or carboxylic acid) have been reported so far, this step turned out to be the main challenge in this approach.

Syntheses

The tri-*O*-methyl-protected emodin-6-carbaldehyde (**3**)[10] was chosen as starting material. BBr_3-mediated regioselective cleavage of the phenol ether at C(8) gave the potential synthon **4** in 81% yield. Carrying out the Marschalk reaction on **4** with CH_2O in alkaline MeOH at 0 °C ($Na_2S_2O_4$ as the reducing agent and subsequent H_2O_2 reoxidation) a significant conversion of the starting material was observed. The ^1H NMR spectrum proved the successful substitution at C(7) as only three aromatic signals were obtained, but no aldehyde peak and also no typical methylol signals were observed. Thus, formation of the targeted 7-hydroxymethylemodinaldehyde **5** could be excluded. Because the mass spectrum of the isolated product showed the exact molar mass as expected for the target compound **5** the corresponding lactol **6** (the structure of which would be in accordance with both, mass and ^1H NMR spectra and was later on proved by means of 2D NMR experiments) turned out to be the direct product of this Marschalk-type reaction. Interestingly enough, formation of the lactol **6** seems to be favored over the corresponding *ortho*-hydroxyalkylated aldehyde **5** due to the high stability of the entropically favored five-membered ring system. Unfortunately, it was not possible to cleave the hemiacetal **6** to **5** under a variety of conditions (HCl, HBr, montmorillonite), leaving either the starting material untouched or resulting in decomposition. The high stability of this compound towards ether cleavage is in agreement with literature statements,[11] where an intramolecularly cyclyzed hemiacetal is reported to be the more stable and therefore predominant species.

Due to the high reactivity of the lactol **6** towards further methylation of the secondary alcohol in the alkaline methanol-containing reaction medium giving the mixed acetal **7** upon prolonged reaction time, this reaction turned out to be rather tricky. Reaction time plays an important role in the synthesis of **6**. Thus, directing the reaction towards a total conversion of the starting material (> 60 min) always gave the *O*-methylated **7** as the main product. In contrast, a limited reaction time (ca. 30 min) resulted in a conversion rate of only 60–65% with the target **6** as the main product (53% yield). It is noteworthy that the total yield of this step could not be improved by a total conversion of the aldehyde **4** into the mixed acetal **7** followed by acid-catalyzed cleavage to **6**. Therefore, neither in the case of total conversion followed by subsequent ether cleavage nor with reduced reaction time the yield of isolated **6** was higher than 50–55%. Accordingly, it turned out to be more reasonable to limit

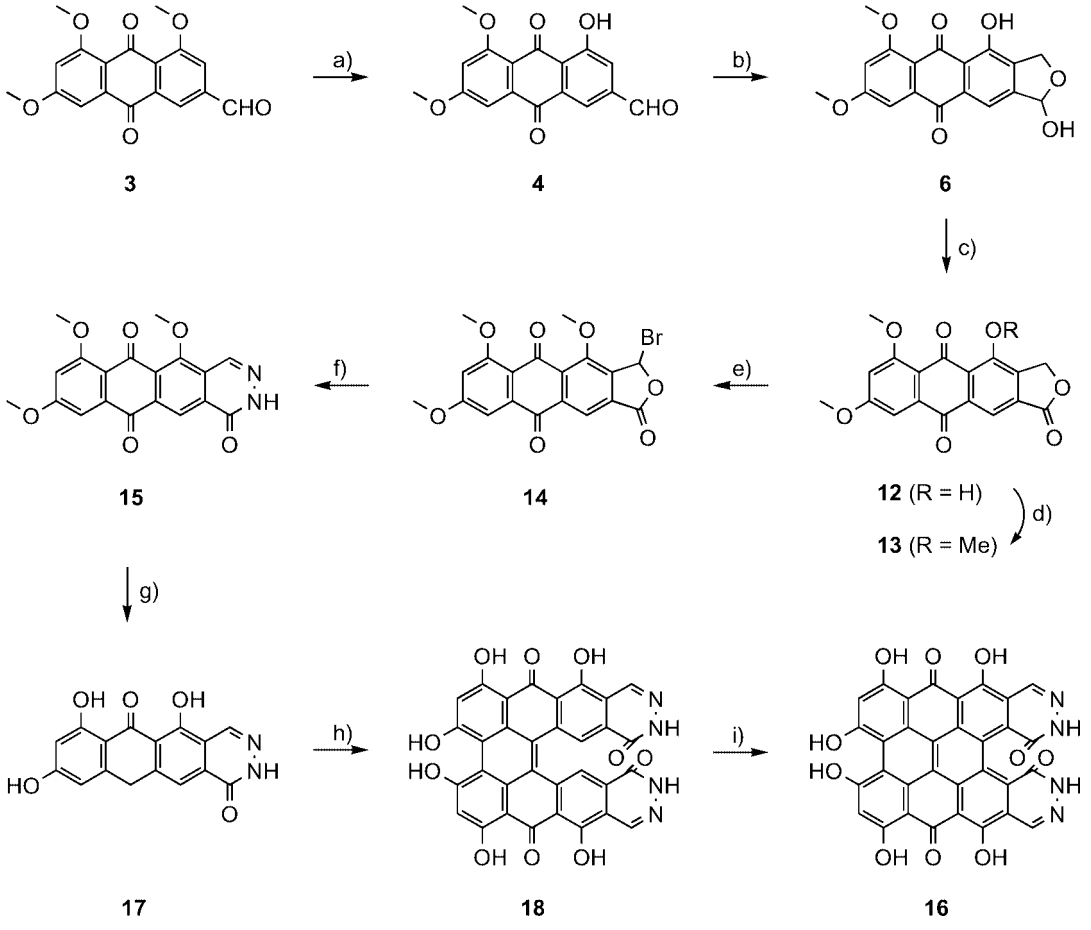


Scheme 2. Marschalk reaction of the aldehyde **4** with HCHO (alkaline MeOH, $Na_2S_2O_4$, 0 °C, and subsequent H_2O_2 reoxidation) gave the lactol **6** whereas the acid **8** and its methyl ester **9** remain unconverted under these conditions.

the reaction time to 30–35 min and to recycle unconverted **4**.

In contrast to the aldehyde **4**, the carboxylic acid **8**[12] as well as the corresponding methyl ester **9** turned out to be completely unreactive towards Marschalk conditions (Scheme 2). Because the Hammett σ-parameters (describing the electronic properties of the system) are nearly the same for ester, carboxyl, and carbonyl functionality,[13] the higher reactivity of the carbaldehyde **4** may be rationalized by the formation of a less electron-withdrawing C(6)-formyl-sulfite/bisulfite addition product as a result of the oxidation of the dithionite ion to sulfite/bisulfite ions during the reaction. This addition product was removed during the oxidative workup to provide lactol **6**. Thus, carboxyl derivatives are not suitable for this kind of reaction. Accordingly, the only way found to introduce an additional functional group at C(7) of an already 6-functionalized emodin derivative was with the aldehyde **3** as the starting material yielding the lactol **6** as a useful synthon with respect to further modifications. Five-membered hemiacetals are known to be very useful intermediates in various fields.[11,14] Rodrigo et al.[14c] reported the use of anthraquinone-lactols in the syntheses of anthracyclinones. Rutledge et al.[14a] as well as Rodrigo et al.[14c] used similar lactols as starting materials for the syntheses of anthra[*c*]furans. However, these syntheses required rather harsh reaction conditions and are reported to proceed in low yields only. Furthermore, isobenzofurans are known to be rather unstable and reactive compounds[15] making them less promising for the intended approach than the initially targeted phthalazinone fused system.

Therefore, it was necessary to find a way to convert the lactol to the corresponding pyridazinone. Oxidation of **6** with pyridinium chlorochromate (PCC) afforded the corresponding lactone **12** (77% yield), which is in accordance to the results obtained by Krohn et al. in a similar case.[14d] This strategy provides a way to overcome the limited reactivity of the emodic acid **8** and its ester **9** towards an electrophilic attack. A possible strategy for the conversion of lactones to the corresponding phthalazinones involves the radical side chain bromination of the lactone followed by subsequent treatment with hydrazine hydrate.[5] To avoid any interference in the bromination, protection of the remaining free phenolic group of **12** was necessary. Treatment with dimethyl sulfate (DMS) gave the tri-*O*-methylated lactone **13** in 82% yield. Subsequent side-chain bromination with NBS/benzoyl peroxide yielded the key intermediate **14** in



Scheme 3. a) BBr_3/CH_2Cl_2, 0 °C, 1 h, 81%; b) $Na_2S_2O_4/CH_2O$ (37%)/MeOH/NaOH (1 N), 0 °C, subsequent H_2O_2 oxidation, 53%; c) PCC/CH_2Cl_2, 18 h, 77%; d) DMS/K_2CO_3/acetone, reflux, 17 h, 82%; e) NBS/benzoyl peroxide/CCl_4, reflux, 16 h, 84%; f) $N_2H_4{\cdot}H_2O$/ethanol, 24 h, 77%;g) $SnCl_2{\cdot}2H_2O$/HBr(47%)/AcOH, reflux, 60 min, 85%; h) $FeSO_4{\cdot}7H_2O$/pyridine *N*-oxide/pyridine/piperidine, 115 °C, 60 min; i) *hν*, acetone, 30 min, 43% (based on **17**).

84%. Finally, nucleophilic addition of hydrazine hydrate ($N_2H_4 \cdot H_2O$, 2.5 equiv.) in refluxing ethanol gave the desired pyridazinone-fused emodin derivative **15** in 77% yield.

With the potential hypericin synthon **15** available in reasonable yield, the synthesis of the corresponding hypericin derivative **16** was carried out in three steps. First, a combined deprotection/reduction of the tri-*O*-methylated **15** with 47% HBr in AcOH and $SnCl_2 \cdot H_2O$ (as carried out earlier[16]) provided the corresponding anthron **17** in 85% yield. Oxidative dimerization of **17** with $FeSO_4 \cdot 7H_2O$, pyridine *N*-oxide, pyridine, and piperidine (according to ref.[2]) gave the light-sensitive protohypericin **18**. Due to its instability, crude **18** was directly used without purification for the photocyclization to the targeted pyridazinone fused hypericin **16**. The product **16** was obtained in 43% yield (based on **17**) after purification by means of gel filtration (Scheme 3).

Purification of **16** turned out to be difficult as it showed a high affinity to silica gel, which made elution and therefore purification by silica gel based column chromatography not desirable, although a qualitative analysis by means of TLC was possible in principle. However, the highest purity (at least 90% as judged by ^{1}H NMR) could be obtained by Sephadex LH-20 based gel chromatography with acetone as the mobile phase.

In conclusion, synthesis of the first representative of a novel class of hypericin derivatives could be achieved in a nine-step synthesis starting from the carbaldehyde **3** with an overall yield of 6%. All derivatives could be characterized by their NMR, UV/Vis, and mass spectra (except for the unstable protohypericin **18**) as detailed in the Exp. Sect. Complete NMR peak assignments were achieved by means of HMBC, HSQC, and NOESY 2D NMR methods.

Chemical and Photochemical Properties

Following the demands for second-generation hypericin-based photosensitizers, solubility, UV/Vis absorption characteristic, and photosensitizing ability of **16** were investigated. It was found that this compound is stable in principle, but temperatures above 80–90 °C, especially in the presence of air, might lead to some decomposition. Heating of solutions in low-boiling solvents like acetone, THF, or MeOH is allowed. The phthalazinone **16** was found to be rather soluble in a variety of common organic solvents such as acetone, THF, and DMSO, which are known to be good solvents for hypericin-like compounds.[1] In addition, **16** showed a satisfying (but lower) solubility in apolar organic solvents such as $CHCl_3$ and EtOAc allowing an extractive work-up with H_2O if necessary. Furthermore, **16** is also sparingly soluble in 50% aqueous acetone and even better in 80% aqueous EtOH, which allows the use under physiological conditions.

As might be inferred from the planar formula of **16** (Scheme 3), the two carbonyl groups of the pyridazinone groups might sterically hinder each other. To elucidate this aspect investigations concerning the conformation and the orientation of these two carbonyl groups within the hypericin **16** by means of force field and semiempirical calculations were undertaken. First, force field calculations were carried out by means of the MM2+ model[17] on the propeller and the butterfly conformations. Similar as in the case of several hypericin derivatives,[18] the propeller conformation turned out to be the more stable one ($\Delta H \approx 4.5$ kJ/mol). Subsequent semiempirical calculation by means of MOPAC[19] also proved the propeller conformation to be the more stable one ($\Delta H \approx 4.0$ kJ/mol) (Figure 1).

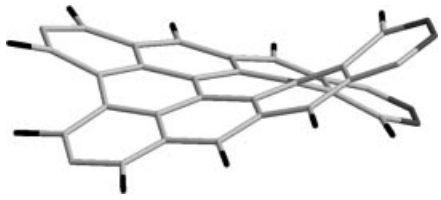


Figure 1. MOPAC-derived propeller conformation of the heterocyclically fused hypericin derivative **16** (H atoms are not shown for clarity).

In addition, these calculations showed that the carbonyl oxygen at C(1) and C(18) do not hinder each other due to the propeller conformation of the skeleton. MOPAC-based calculations of the dihedral angels of **16** gave a value of 26.4° on the *bay*-OH side (27.1° for the unsubstituted **1**) and 34.8° on the pyridazinone side (34.2° for the methyl side of **1**). Thus, introduction of the heterocycle does not heavily affect the conformation of the phenanthroperylenequinone moiety itself. Furthermore, the pyridazinone carbonyl groups were found to be in plane with the aromatic system. Thus, any interruption of the chromophore due to a steric hindrance of these carbonyl groups can be neglected, which is of main interest with respect to a bathochromically shifted absorption maximum.

As intended, the extension of the chromophoric system by the phthalazinone heterocycles leads to a significant shift of the long-wavelength absorption maximum (λ_{max} = 615–625 nm depending on the solvent) of about 25 nm in comparison to the unsubstituted **1**. The molar extinction coefficient of **16** was found to be in the order of 7500–9000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$, thus lying in a similar range as for several recently synthesized hypericin derivatives but significantly lower than in the parent compound **1** (ε = 40000–50000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$[1,4]), which might be due i.a. to association phenomena, protonation, and/or other equilibria.

The nature of this compound seems to be strongly solvent-depending as **16** shows no typical hypericin-like red fluorescence in $CHCl_3$ and DMSO, which is also in accordance with its UV/Vis spectra in these two solvents showing a broad maximum in the range of 650–660 nm but with a very low extinction. These results mainly differ from the spectra obtained in acetone, EtOAc, or THF where "normal" hypericin-like UV/Vis and fluorescence spectra and satisfying extinctions could be obtained. Furthermore, also no NMR spectrum in DMSO could be obtained as no defined peaks could be observed. In contrast, acetone as the solvent gave the expected ^{1}H NMR spectrum, showing that

only one certain species is predominant in this solvent. These results may be due to aggregation or staggering phenomena leading to a non-monomolecular appearance in DMSO and $CHCl_3$, whereas the spectra observed in acetone, THF, or 80% aqueous EtOH indicate the predominance of the not-aggregated and monomolecular form of **16**. As this tendency is in contrast to the parent compound **1**,[1] the fused pyridazinone substituent is supposed to heavily influence the tendency of the phenanthroperylenequinone skeleton towards aggregation.

Fulfilling the demand for a bathochromically shifted absorption maximum as well as showing a sufficient solubility in a variety of organic solvents, it was of main interest to investigate the photosensitizing ability of this compound. The ability for the generation of singlet oxygen and/or reactive oxygen species was studied by means of the light sensitized destruction of bilirubin[20] of the pyridazinone fused **16** in comparison to the unsubstituted parent compound **1** (Figure 2).

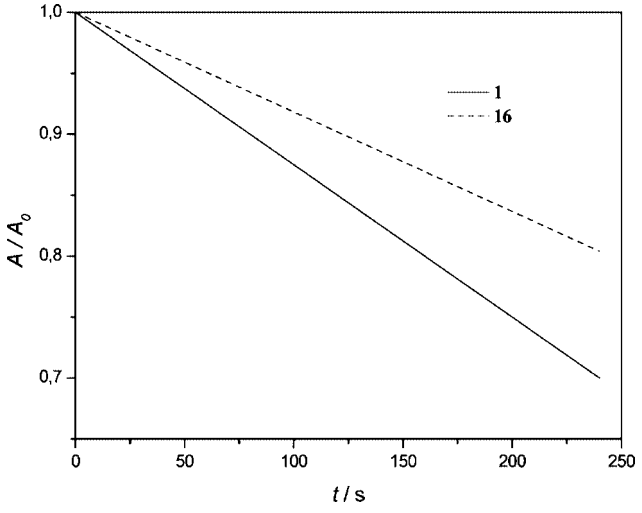


Figure 2. Photooxidation of bilirubin IXα sensitized by a hypericin derivative in aerated 80% aqueous ethanol (irradiation at $\lambda >$ 570 nm). Normalized absorption (A/A_0) vs. time of solutions of disodium bilirubinate IXα with **1** and **16**.

It was found, that the light-sensitized bilirubin destruction by means of the novel heterocyclically fused **16** was slightly less efficient than in the case of the unsubstituted **1**. Although not ideal for a second-generation hypericin based photosensitizer, this result proves that the fused nitrogen-containing heterocycle does not seriously limit the photosensitizing potential of this new class of hypericin-based photosensitizers. Surprisingly, the fluorescence quantum yields observed for solutions of **16** in acetone (Φ_f = 0.03) and 80% aqueous EtOH (Φ_f = 0.02) are lower than expected from the slightly limited ability for the generation of singlet oxygen and/or reactive oxygen species. Thus, a strong influence of the fused heterocycle on the intersystem crossing as well as on the fluorescence quantum yield might be supposed. On the other hand, an enhanced internal conversion may be assumed.

Conclusion

We have shown that the synthesis of fused heterocyclic emodin derivatives gives access to a new class of interesting bathochromically shifted hypericin derivatives with satisfying solubility and a significant ability for the generation of singlet oxygen/reactive oxygen species upon irradiation by light. Furthermore, the strategy described for the bifunctionalization of emodin via a Marschalk-type approach on the carbaldehyde **4** provides a novel route for the synthesis of useful anthraquinoid intermediates. It can be assumed that the formyl group of the synthon plays an important intermediate part within this approach, as compounds with similar electron-withdrawing compounds do not undergo this reaction.

Experimental Section

All chemicals were obtained from commercial suppliers and used without purification unless otherwise stated. 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 NOESY mixing time was set to 400 ms. UV/Vis, fluorescence, and mass spectra were recorded with a Varian Cary 100 Bio UV/Vis, Varian Cary Eclipse fluorescence, and a Thermo Finnigan LCQ Deca XP-Plus. The hypericin sensitized photooxidation of bilirubinate IXα was determined according to ref.[20]. The fluorescence quantum yields were determined according to ref.[21]. The aldehyde **3** was prepared according to ref.[10]. The purity of all compounds was judged by their ^{1}H NMR spectra to be at least 97% unless stated otherwise.

6-Formyl-8-hydroxy-1,3-dimethoxyanthraquinone (4): An Ar-flushed stirred solution of **3** (114 mg, 0.349 mmol) in absolute CH_2Cl_2 (45 mL) was cooled to –5 °C. After addition of BBr_3 (140 μL, 1.48 mmol), the dark red solution was stirred for one hour at ca. 0 °C. The solution was then poured on ice/H_2O and extracted with $CHCl_3$/H_2O. The combined organic layers were evaporated to dryness and the resulting residue purified by column chromatography ($CHCl_3$/EtOAc, 10:1) to give 90 mg of **4** (0.288 mmol, 83% yield). M.p. 200–202 °C. TLC: R_f = 0.59 ($CHCl_3$/EtOAc, 10:1). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C): δ = 3.99 (s, 3 H, 1-OCH$_3$), 4.00 (s, 3 H, 3-OCH$_3$), 7.06 (s, 1 H, 1-H), 7.33 (s, 1 H, 4-H), 7.77 (s, 1 H, 7-H), 8.06 (s, 1 H, 5-H), 10.10 (s, 1 H, 6-CHO), 13.13 (s, 1 H, 8-OH) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 30 °C): δ = 56.4 (1-OCH$_3$ or 3-OCH$_3$), 56.8 (3-OCH$_3$ or 1-OCH$_3$), 104.6 (C-2), 105.0 (C-4), 114.2 (C-9a), 117.5 (C-5), 120.1 (C-8a), 124.4 (C-7), 133.3 (C-10a), 136.8 (C-4a), 140.7 (C-6), 161.8 (C-8), 163.5 (C-1), 165.6 (C-3), 181.6 (C-10), 186.4 (C-9), 192.4 (CHO) ppm. HMBC, HSQC, and NOESY data were according to structure. ESI-MS (MeOH + 1 vol.-% HCOOH, positive ion mode): *m*/*z* = 313 ([M + H]$^+$). UV/Vis ($CHCl_3$): λ_{max} = 303 (66), 427 (100) nm (rel. int.).

1,4-Dihydroxy-6,8-dimethoxyanthra[2,3-*c*]furan-5,10(1*H*,3*H*)-dione (6): A stirred suspension of **4** (66 mg, 0.211 mmol) in MeOH (100 mL) was cooled to 0 °C. After addition of aqueous NaOH

(10 mL, 1 N) the resulting dark red solution was flushed with Ar. A solution of $Na_2S_2O_4$ (160 mg, 0.92 mmol) in H_2O (2 mL) was added through a septum, followed by the addition of aqueous HCHO (0.5 mL, 37%, stabilized with MeOH). The cooled solution was stirred for 35 min, re-oxidized with H_2O_2 (20 mL, 3%) and acidified with aqueous HCl (3%). The resulting yellow suspension was extracted with brine and $CHCl_3$ several times. The combined organic layers were washed with H_2O and the solvents evaporated to dryness. The crude product was purified by column chromatography ($CHCl_3$/EtOAc, 3:1) to yield **6** as an orange solid (38 mg, 0.111 mmol, 53%). M.p. 208–212 °C. TLC: R_f = 0.26 ($CHCl_3$/EtOAc, 3:1). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C): δ = 3.98 (s, 3 H, 6-OCH_3), 3.99 (s, 3 H, 8-OCH_3), 4.99 (d, 1 H, $^2J_{3,3}$ = 14.34 Hz, 3-H), 5.13 (dd, 1 H, $^2J_{3,3}$ = 14.34 Hz, $^3J_{1,3}$ = 2.14 Hz, 3-H), 6.41 (dd, 1 H, $^3J_{1,1\text{-OH}}$ = 7.93 Hz, $^3J_{1,3}$ = 2.14 Hz, 1-H), 7.03 (d, 1 H, $^3J_{7,9}$ = 2.44 Hz, 7-H), 7.05 (d, 1 H, $^3J_{1,1\text{-OH}}$ = 14.34 Hz, 1-OH), 7.32 (d, 1 H, $^3J_{7,9}$ = 2.44 Hz, 9-H), 7.59 (s, 1 H, 11-H), 13.34 (s, 1 H, 4-OH) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 30 °C): δ = 56.1 (6-OCH_3), 56.6 (8-OCH_3), 68.8 (-CH_2-), 100.8 (-CHOH-), 104.4 (C-7), 104.8 (C-9), 112.6 (C-11), 113.9 (C-5a), 116.5 (C-4a), 133.0 (C-10a), 134.1 (C-3a), 136.8 (C-9a), 147.4 (C-11a), 156.0 (C-4), 163.2 (C-6), 165.3 (C-8), 181.6 (C-10), 186.8 (C-5) ppm. HMBC, HSQC, and NOESY data were according to structure. APCI-MS (MeOH, negative ion mode): m/z = 341 ([M – H]$^-$). UV/Vis ($CHCl_3$): λ_{max} = 420 (100) nm (rel. int.).

4-Hydroxy-6,8-dimethoxyanthra[2,3-*c*]furan-1,5,10(3*H*)-trione (12): A solution of **6** (85 mg, 0.248 mmol) and PCC (400 mg, 1.85 mmol) in CH_2Cl_2 (400 mL) was stirred at room temperature for 18 h. After extraction with H_2O the combined organic layers were evaporated to dryness. The crude product was purified by column chromatography ($CHCl_3$/EtOAc, 3:1) to yield **12** as an orange solid (65 mg, 0.191 mmol, 77%). M.p. 219–222 °C. TLC: R_f = 0.74 ($CHCl_3$/EtOAc, 3:1). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C): δ = 4.02 (s, 6 H, 6-OCH_3 and 8-OCH_3), 5.55 (s, 2 H, -CH_2-), 7.10 (s, 1 H, 7-H), 7.38 (s, 1 H, 9-H), 7.91 (s, 1 H, 11-H), 13.45 (s, 1 H, 4-OH) ppm. ^{13}C NMR not possible to record due to the limited solubility of compound **12**. 2D NMR spectra also gave only a limited number of correlations due to the low solubility. ESI-MS (MeOH, positive ion mode): m/z = 341 ([M + H]$^+$). UV/Vis ($CHCl_3$): λ_{max} = 419 (100) nm (rel. int.).

4,6,8-Trimethoxyanthra[2,3-*c*]furan-1,5,10(3*H*)-trione (13): A stirred solution of **12** (41 mg, 0.120 mmol), K_2CO_3 (160 mg, 1.15 mmol), and DMS (0.5 mL, 5.26 mmol) in acetone (130 mL) was refluxed for 17 h. After extraction with $CHCl_3/H_2O$ the combined organic layers were evaporated to dryness. The crude product was purified by column chromatography ($CHCl_3$/EtOAc, 3:1) to yield **13** as a yellow solid (35 mg, 0.098 mmol, 82%). M.p. 233–236 °C. TLC: R_f = 0.43 ($CHCl_3$/EtOAc, 3:1). ^{1}H NMR (500 MHz, $CDCl_3$, 30 °C): δ = 3.98 (s, 3 H, 8-OCH_3), 4.00 (s, 3 H, 6-OCH_3), 4.08 (s, 3 H, 4-OCH_3), 5.48 (s, 2 H, -CH_2-), 6.82 (d, 1 H, $^3J_{7,9}$ = 2.14 Hz, 7-H), 7.39 (d, 1 H, $^3J_{7,9}$ = 2.14 Hz, 9-H), 8.52 (s, 1 H, 11-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 30 °C): δ = 56.2 (8-OCH_3), 56.8 (6-OCH_3), 62.1 (4-OCH_3), 68.6 (-CH_2-), 102.8 (C-9), 105.7 (C-7), 118.2 (C-5a), 119.8 (C-11), 130.6 (C-4a or C-3a), 131.2 (C-3a or C-4a), 136.6 (C-9a), 136.8 (C-10a), 144.5 (C-11a), 155.6 (C-4), 162.1 (C-6), 164.9 (C-8), 169.3 (C-1), 181.2 (C-5), 182.5 (C-10) ppm. HMBC, HSQC, and NOESY data were in agreement with the assumed structure. ESI-MS (MeOH, positive ion mode): m/z = 355 ([M + H]$^+$). UV/Vis ($CHCl_3$): λ_{max} = 347 (77), 399 (100) nm (rel. int.).

3-Bromo-4,6,8-trimethoxyanthra[2,3-*c*]furan-1,5,10(3*H*)-trione (14): A stirred solution of **13** (38 mg, 0.107 mmol), NBS (94 mg, 0.528 mmol), and benzoyl peroxide (9 mg, 0.037 mmol) in CCl_4 (125 mL) was refluxed for 16 h. After extraction with H_2O the combined organic layers were evaporated to dryness. The crude product was purified by column chromatography ($CHCl_3$/EtOAc, 3:1) to yield **14** as a yellow-brown solid (39 mg, 0.090 mmol, 84%). M.p. 145–149 °C. TLC: R_f = 0.67 ($CHCl_3$/EtOAc, 3:1). ^{1}H NMR (500 MHz, $CDCl_3$, 30 °C): δ = 3.99 (s, 3 H, 8-OCH_3), 4.01 (s, 3 H, 6-OCH_3), 4.16 (s, 3 H, 4-OCH_3), 6.83 (d, 1 H, $^3J_{7,9}$ = 1.83 Hz, 7-H), 7.38 (d, 1 H, = 1.83 Hz, 9-H), 7.45 (s, 1 H, -CHBr-), 8.52 (s, 1 H, 11-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 30 °C): δ = 56.3 (8-OCH_3), 56.8 (6-OCH_3), 63.1 (4-OCH_3), 72.5 (-CHBr-), 103.0 (C-9), 105.8 (C-7), 118.0 (C-5a), 119.7 (C-11), 128.4 (C-4a), 132.3 (C-3a), 136.4 (C-9a), 138.3 (C-10a), 146.2 (C-11a), 156.0 (C-4), 162.2 (C-6), 165.0 (C-8), 165.8 (C-1), 180.7 (C-5), 182.1 (C-10) ppm. HMBC, HSQC, and NOESY data were in agreement with the assumed structure. ESI-MS (MeOH, positive ion mode): m/z = 385 ([M – Br + MeOH]$^+$). UV/Vis ($CHCl_3$): λ_{max} = 348 (91), 405 (100) nm (rel. int.).

5,7,9-Trimethoxynaphtho[2,3-*g*]phthalazine-1,6,11(2*H*)-trione (15): An Ar-flushed solution of **14** (31 mg, 0.071 mmol) and $N_2H_4 \cdot H_2O$ (8 μL, 0.16 mmol) in EtOH (95 mL) was refluxed for 24 h. After extraction with $CHCl_3/H_2O$ and evaporation of the organic layers, the crude product was purified by column chromatography ($CHCl_3$/EtOAc, 1:1) to yield **15** as a yellow solid (20 mg, 0.054 mmol, 77%). M.p. 203–206 °C. TLC: R_f = 0.42 ($CHCl_3$/EtOAc, 1:1). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C): δ = 3.96 (s, 3 H, 7-OCH_3 or 9-OCH_3), 3.98 (s, 3 H, 9-OCH_3 or 7-OCH_3), 4.04 (s, 3 H, 5-OCH_3), 7.07 (d, 1 H, $^3J_{8,10}$ = 2.44 Hz, 8-H), 7.28 (d, 1 H, $^3J_{8,10}$ = 2.44 Hz, 10-H), 8.58 (s, 1 H, -CH=N), 8.60 (s, 1 H, 12-H), 13.07 (s, 1 H, -NH-) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 30 °C): no signals due to the limited solubility of compound **15**. ^{13}C signals assigned by 2D experiments: δ = 55.9 (7-OCH_3 and 9-OCH_3), 63.6 (5-OCH_3), 102.7 (C-10), 105.0 (C-8), 117.5 (C-6a), 118.7 (C-12), 129.7 (C-4a), 132.3 (C-4), 157.1 (C-6), 158.6 (C-1), 161.7 (C-7), 164.2 (C-9), 181.9 (C-11) ppm. APCI-MS (MeOH, positive ion mode): m/z = 367 ([M + H]$^+$). UV/Vis ($CHCl_3$): λ_{max} = 381 (100) nm (rel. int.).

5,7,9-Trihydroxynaphtho[2,3-*g*]phthalazine-1,6(2*H*,11*H*)-dione (17): An Ar-flushed solution of **15** (35 mg, 0.095 mmol) in glacial AcOH (18 mL) was heated to reflux. After addition of $SnCl_2 \cdot 2H_2O$ (160 mg, 0.718 mmol) in HBr (3.5 mL, 47%) the solution was refluxed for further 60 min. The solution was then poured on ice/H_2O, extracted with EtOAc, and the solvents evaporated to dryness. The residue was washed with water giving **17** as a light brown solid (25 mg, 0.081 mmol, 85%). M.p. decomp. ≥ 205 °C. TLC: R_f = 0.15 ($CHCl_3$/EtOAc, 1:1). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C): δ = 4.57 (s, 2 H, -CH_2-), 6.28 (s, 1 H, 8-H), 6.49 (s, 1 H, 10-H), 7.71 (s, 1 H, 12-H), 8.48 (s, 1 H, 4-H), 11.09 (s, 1 H, 9-OH), 12.15 (s, 1 H, 7-OH), 12.80 (s, 1 H, 5-OH), 13.47 (s, 1 H, -NH) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 30 °C): no signals due to the limited solubility and instability of compound **17**. ^{13}C signals assigned by 2D experiments: δ = 32.7 (-CH_2-), 100.9 (C-8), 107.5 (C-10), 108.6 (C-6a), 114.1 (C-12), 116.7 (C-5a), 117.9 (C-4a), 131.5 (C-12a), 132.1 (C-4), 144.1 (C-10a or C-11a), 145.1 (C-11a or C-10a), 158.6 (C-5), 159.2 (C-1), 164.7 (C-9), 165.8 (C-7) ppm. ESI-MS (MeOH, negative ion mode): m/z = 309 ([M – H]$^-$). UV/Vis (EtOAc): λ_{max} (rel. int.) = 293 (100), 305 (90), 368 (98) nm.

5,7,9,10,12,14-Hexahydroxydiphthalazino[6,7-*ao*]perylene-1,6,13,18-(1*H*,18*H*)-tetraone (18): A mixture of **17** (38 mg, 0.122 mmol), $FeSO_4 \cdot 7H_2O$ (3.7 mg, 0.01 mmol), and pyridine *N*-oxide (87 mg, 0.92 mmol) in dry pyridine (1.2 mL) and dry piperidine (0.1 mL) was stirred under Ar in the dark at 115 °C for 1 h. After cooling

to room temperature, the mixture was poured into aqueous HCl (9 mL, 2 N) and stirred for further 30 min at room temperature (in the dark). After centrifugation the residue was first washed two times with HCl (3%) and then washed acid free with H_2O. Drying over P_2O_5 yielded **18** as a black solid (32 mg). M.p. > 350 °C. ESI-MS (MeOH, negative ion mode): m/z = 613 ([M – H]$^-$). UV/Vis (acetone): λ_{max} (rel. int.) = 619 (91), 572 (100) nm. Due to its light-instability, crude **18** was used without purification for the photocyclization to **16**.

5,7,9,10,12,14-Hexahydroxypyridazino[4′,5′-3,4]pyridazino[4′′,5′′-5,6]phenanthro[1,10,9,8-*opqra*]perylene-1,6,13,18-(1*H*,18*H*)-tetraone (16): A solution of crude **18** (32 mg, 0.052 mmol) in acetone (900 mL) was irradiated for 30 min by means of a 700-W mercury high-pressure lamp with fluorescence screen (Philips) whilst stirring and air admission. After evaporation of the solvent crude **16** was obtained as a black solid (30 mg, 0.049, 95%). Gel chromatography on Sephadex LH-20 with acetone gave **16** in a purity of at least 90% (judged by ^{1}H NMR) in 43% yield (based on **17**). M.p. > 350 °C. TLC: R_f = 0.88 (THF), 0.77 ($CHCl_3$:MeOH, 3:2). ^{1}H NMR (500 MHz, [D_6]acetone, 30 °C): δ = 6.68 (s, 2 H, ar-H), 8.89 (s, 2 H, -CH=N), 11.81 (s, 2 H, -NH-), 14.40 (s, 2 H, 1-OH and 6-OH or 8-OH and 13-OH), 15.95 (s, 2 H, 8-OH and 13-OH or 1-OH and 6-OH), 18.70 (s, 1 H, 3-OH) ppm. ^{13}C NMR and 2D NMR not obtainable due to the limited solubility of compound **16**. ESI-MS (acetone/H_2O, 1:1, negative ion mode): m/z = 611 ([M – H]$^-$). UV/Vis (acetone, $c = 4.4\cdot10^{-5}$ mol·dm^{-3}): λ_{max} (ε) = 509 (2300), 573 (4200), 621 (7900) nm (dm^3·mol^{-1}·cm^{-1}). UV/Vis (THF, $c = 3.3\cdot10^{-5}$ mol·dm^{-3}): λ_{max} (ε) = 513 (2500), 577 (4200), 625 (8400) nm (dm^3·mol^{-1}·cm^{-1}). UV/Vis (EtOH/H_2O, 4:1, $c = 2.9\cdot10^{-5}$ mol·dm^{-3}): λ_{max} (ε) = 503 (1600), 570 (3200), 617 (6600) nm (dm^3·mol^{-1}·cm^{-1}). Fluorescence (acetone, $c \approx 2\cdot10^{-7}$ mol·dm^{-3}, λ_{ex} = 550 nm): λ_{em} (rel. int.) = 631 (100), 679 (41) nm, Φ_f = 0.03. Fluorescence (THF, $c \approx 8\cdot10^{-7}$ mol·dm^{-3}, λ_{ex} = 550 nm): λ_{em} (rel. int.) = 635 (100), 684 (36) nm. Fluorescence (EtOH/H_2O, 4:1, $c \approx 6\cdot10^{-7}$ mol·dm^{-3}, λ_{ex} = 550 nm): λ_{em} (rel. int.) = 631 (100), 676 (56) nm, Φ_f = 0.02.

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