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ω,ω'-Urea- and -dithioacetal-derivatives of hypericin

Joachim Zuschrader, Gerald Reiter, Heinz Falk

Institute of Organic Chemistry, Johannes Kepler University, Linz, Austria

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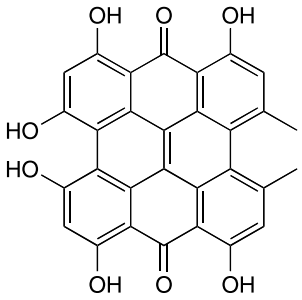
Abstract An ω,ω'-disubstituted hypericin derivative bearing two dicyclohexylurea moieties separated by propionyl chains from the chromophore and an ω,ω'-dithioacetal of hypericin were prepared. Both showed excellent production of oxidizing species comparable to hypericin when irradiated with appropriate light as shown by the photodestruction of bilirubin IXα.

Keywords Hypericin; *N*,*N*′-Dicyclohexylurea; Photodynamic agent; Dithioacetal; Oxidizing species.

Introduction

Although hypericin (**1**; 1,3,4,6,8,13-hexahydroxy-10,11-dimethyl-phenanthro[1,10,9,8-*opqra*]perylene-7,14-dione) is still ''number one'' of the photodynamic therapy sensitizer candidates, the search for second-generation photosensitizers is still proceeding [1]. This search is targeted at two main issues, namely to shift the absorption wavelength into the area of medicinal lasers on the one hand [1, 2], and to hybridize the hypericin moiety with moieties that modulate the properties of the molecule with *e.g.*, aiming at better water solubility or better interaction with cellular ingredients [3]. In this report we will address the synthesis and properties of an urea appended hypericin derivative having in mind the prominent hydrogen bonding capabilities of its carbonyl unit, and to a thioacetal derivative to explore the influence of saturated heterocyclic sulfur on the photochemical behavior of hypericin (**1**).

Correspondence: Heinz Falk, Institute of Organic Chemistry, Johannes Kepler University, Altenbergerstraße 69, 4040 Linz, Austria, Europe. E-mail: heinz.falk@jku.at



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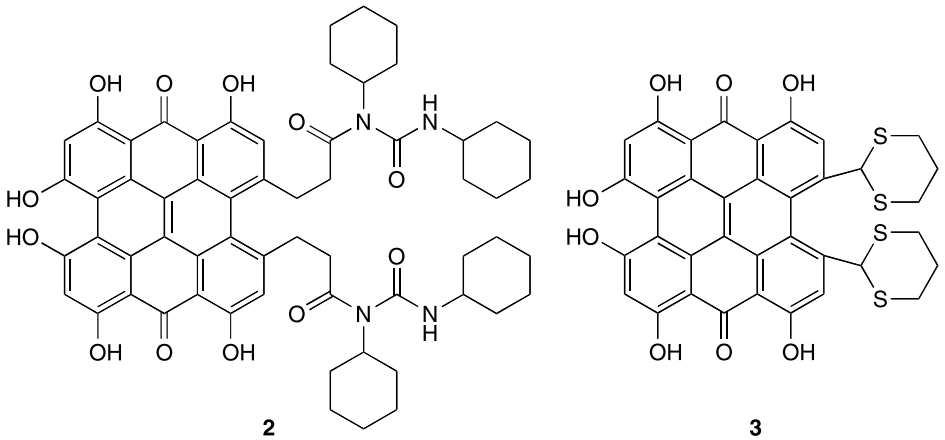
Results and discussion

The design of the target molecule was directed by leaving the hypericin moiety as untouched as possible to grant undiminished photochemical properties on the one hand, and a substituted urea unit attached some distance from the chromophore. Thus, we ended up with a hypericin-ω,ω'-dipropionic unit attached to a *N*,*N*′-dicyclohexylurea (**2**, Scheme 1). An advantage of this kind of attachment is certainly its low hydrolysis potential. On the other hand, a derivative containing un-conjugated saturated sulfur units could provide information on the influence of this atom as compared to corresponding unsaturated conjugated sulfur heterocycles. Thus, we targeted the dithioacetal **3** also (Scheme 1).

Syntheses

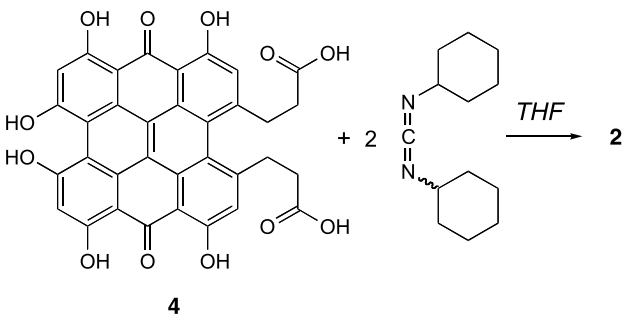
Although the preparation of ω,ω'-disubstituted hypericin derivatives usually has to start with the synthesis of the two ''halves'', which then are dimerized [1], the synthesis of **2** fortunately could be started



Scheme 1

with the already known hypericin-ω,ω'-dipropionic acid **4** [4]. The latter was reacted with *N*,*N*′-dicyclohexylcarbodiimide (*DCCD*) in *THF* solution in presence of triethylamine. This reaction provided the hypericin-ω,ω'-bisacylbis(dicyclohexylurea) **2** in 70% yield. Interestingly enough, we accidentally discovered this reaction when we tried to esterify or amidate the acid **4** by means of *DCCD* (Scheme 2).

For the preparation of the hypericin-ω,ω'-dithioacetal **3** we started from the tri-*O*-methyl protected emodinaldehyde **5** prepared recently [5] (Scheme 3). By means of the silica-gel/thionyl chloride catalyst compound **5** was efficiently acetalized [6] with 1,3-propanedithiol to yield **6** in 84% yield. This dithioacetal **6** was then reduced at the quinone part in the conventional way using the system $SnCl_2$/HBr/acetic acid providing the anthrone derivative **7** with 86% yield. Dimerization in analogy to Ref. [7] afforded the ω,ω'-disubstituted protohypericin derivative **8**. Because this product was not stable enough to be purified by chromatography, it was immediately photocyclized and thus gave the targeted hypericin-ω,ω'-dialdehyde dithioacetal **3** in about 50% overall yield for the last two steps. It might be mentioned that under some of the various conditions designed for recovering the aldehyde function from thioacetals ($AgClO_4/I_2$ [8], $Tl(NO_3)_3$ [9], and *NBS*/acetone [10]) none could be successfully applied to provide the hypericin-ω,ω'-dialdehyde.

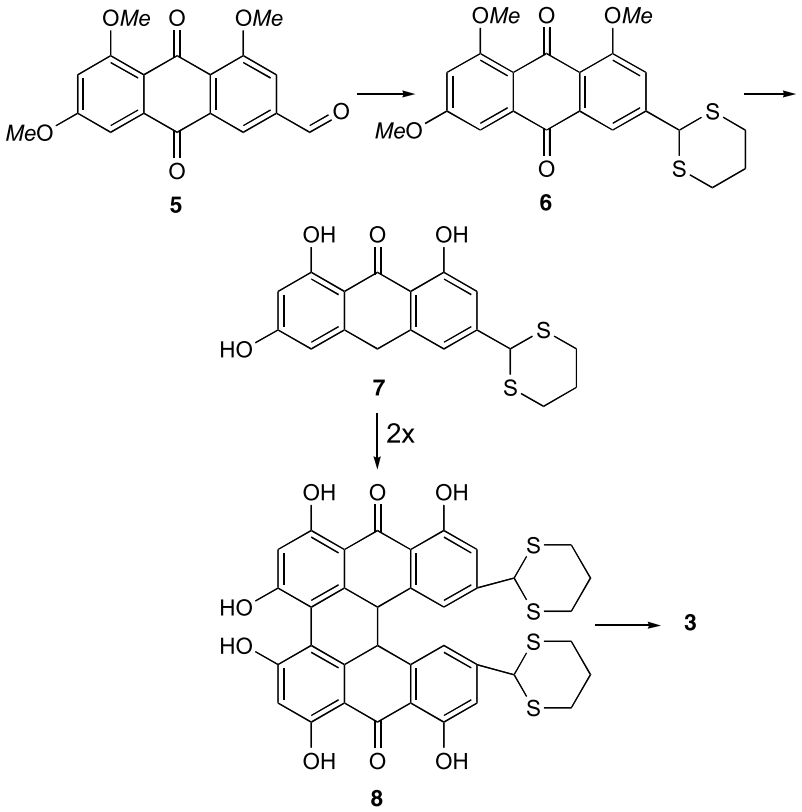


Scheme 2

Properties

As could be expected for non-conjugated hypericin derivatives the long-wavelength absorption band of the two derivatives **2** and **3** are only marginally shifted as compared with hypericin (**1**), with the first one a few nm hypsochromically shifted, whereas the latter is more or less absorbing at the same wavelength – both with about the same pattern of the absorption spectra as **1**. The fluorescence spectra are also very similar. The most interesting property with respect to photodynamic therapy is the ability to produce oxidizing species and/or singlet oxygen. We applied the recently developed method of photosensitized destruction of bilirubin IXα to test for this ability [11]. As shown in Fig. 1 both derivatives display similar but slightly diminished activities as hypericin (**1**) itself. In particular, in the case of the saturated sulfur-containing derivative, the photosensitization of oxidizing species seems to be significantly reduced.

In conclusion, we prepared two derivatives **2** and **3** of hypericin (**1**) with two urea and two thioheterocyclic moieties non-conjugatedly attached. Both show comparable photodynamic activity and might be candidates for second-generation photodynamic agents aimed at certain interactions with cell components.



Scheme 3

Experimental

The characterizations of the products were established using mp (*Kofler* microscope, Reichert), ^{1}H NMR (Bruker Avance DPX 200 MHz, *DMSO*-d_6), IR (Bruker Tensor 27, KBr), MS (Thermofinnigan LCQ Deca XP Plus), fluorescence (Varian Carey Eclipse fluorescence), and UV-Vis data (Varian Cary 100 Bio). The production of singlet oxygen/oxidizing species by **1**, **2**, and **3** was monitored by bilirubin-IXα-degradation according to Ref. [11]. The long-wavelength absorption band intensities of the three compounds were made equal by adjusting concentrations to provide comparable light absorptions for the three compounds. *N*,*N*′-Dicyclohexyl-carbodiimide was obtained from Merck and used as obtained. Compounds **4** and **5** were prepared according to Refs. [4] and [5]. Silica-gel loaded with thionyl chloride was prepared according to Ref. [6].

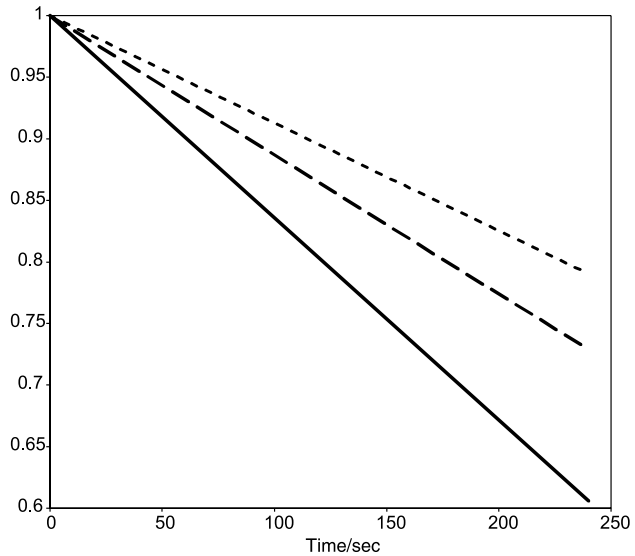


Fig. 1 Hypericin derivative sensitized photooxidation of bilirubin IXα: normalized absorption (A/A_0) *vs.* time curves of solutions of disodium bilirubinate IXα together with the sodium salts of either hypericin (**1**) (—), derivative **2** (– – –), and derivative **3** (- - -)

3,3′-(1,6,8,10,11,13-Hexahydroxy-7,14-dioxo-7,14-dihydrophenanthro[1,10,9,8-opqra]perylene-3,4-diyl)bis(N-cyclohexyl-N-(cyclohexylcarbamoyl)propanamide) (**2**, $C_{60}H_{64}N_4O_{12}$) A solution of 20 mm^3 molten *N*,*N*′-dicyclohexylcarbodiimide in 2 cm^3 dry *THF* was heated to 40°C under Ar atmosphere. After addition of 20 mg **4** (0.03 mmol) dissolved in 10 cm^3 dry *THF* and 20 mm^3 triethylamine, the reaction mixture was kept at this temperature for 5 h. By evaporation of the solvent under reduced pressure and subsequent extraction with ethyl acetate/water a black solid was obtained. Purification by means of column chromatography with chlorofom:methanol = 10:1 on silica yielded 23 mg **2** (70%). Mp >350°C; $R_f = 0.55$ ($CHCl_3$:*Me*OH = 5:1); ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): $\delta = 18.48$ (s, 2ar–O*H*), 14.72 (s, 2ar–O*H*), 14.08 (s, 2ar–O*H*), 7.77 and 7.79 (s, 2NH, D-exchangeable), 7.45 (s, 2ar–H) ppm, –CH_2– not observable due to solvent overlap; ESI-MS (negative ion mode): $m/z = 1031$ ([M − H]$^-$); IR (KBr): $\bar{\nu} = 3328$, 2928, 1628, 1576, 1539, 1449, 1437, 1312, 1271,

1244, 1088, 1045, 892, 641 cm^{-1}; UV-Vis (80% *Et*OH, $c = 9.5.10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 546 (589), 584 (1421) nm (dm^3 mol^{-1} cm^{-1}); UV-Vis (*DMSO*, $c = 9.5.10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 555 (737), 588 (1284) nm (dm^3 mol^{-1} cm^{-1}); fluorescence (80% *Et*OH, $c = 2.4.10^{-6}$ mol dm^{-3}, λ_{ex} = 550 nm): λ_{em} (rel. int.) = 596 (100), 641 (9) nm; fluorescence (*DMSO*, $c = 1.0.10^{-7}$ mol dm^{-3}, λ_{ex} = 550 nm): λ_{em} (rel. int.) = 604 (100), 653 (30) nm.

3,4-Di(1,3-dithian-2-yl)-1,3,4,6,8,13-hexahydroxy-phenanthro[1,10,9,8-opqra]perylene-7,14-dione (**3**, $C_{36}H_{24}O_8S_4$)
In 2500 cm^3 acetone the raw-product **8** was dissolved and irradiated for 25 min by means of a 700 W high pressure lamp with fluorescence screen (Philips, HPL-N) under stirring and air admission. After evaporation of the solvent the resulting black residue was purified by column chromatography over silica using $CHCl_3$:*Me*OH (3:1) as solvent to yield 73 mg **3** (*ca.* 50% from **7**). Mp >350°C. TLC: $R_f = 0.65$ ($CHCl_3$:CH_3OH = 3:1), $R_f = 0.44$ ($CH_3COOC_2H_5$:$CHCl_3$ = 2:1). ^{1}H NMR (200 MHz, *DMSO*-d_6, 30°C; for comparison reasons the numbering scheme of **1** is retained and is not in accord with the IUPAC numbering scheme of the name above): δ = 14.26 (s, ar–OH), 14.63 (s, ar–OH), 7.70 (s, ar–H5, ar–H2), 6.64 (s, ar–H12, ar–H9), 6.14 (s, 10-CHR_2, 11-CHR_2) ppm; ESI-MS (CH_3OH + 1% NH_3, negative ion mode): m/z = 712.8 ($[M-H]^-$); UV-Vis (acetone): λ_{max} = 326 (95), 451 (48), 552 (42), 596 (67) nm (rel. int.).

3-(1,3-Dithian-2-yl)-1,6,8-trimethoxyanthracene-9,10-dione (**6**, $C_{21}H_{20}O_5S_2$)
A solution of 156 mg **5** (0.48 mmol) in 12 cm^3 benzene, 70 mm^3 1,3-propanedithiol (0.70 mmol) and 93 mg silica-gel loaded with thionyl chloride was stirred under *Ar* at 60°C for 12 h. The mixture was extracted five times with saturated $NaHCO_3$/ethyl acetate, washed several times with distilled water and evaporated under reduced pressure to obtain 170 mg **6** (84%). The purification was achieved by column chromatography over silica using $CHCl_3$:*Me*OH (15:1) as the solvent. Mp 228–232°C; $R_f = 0.82$ ($CHCl_3$:CH_3OH = 15:1); ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C; here and below for comparison reasons the numbering scheme of emodin is retained and is not in accord with the IUPAC numbering scheme of the name above): δ = 7.78 (s, ar–H5), 7.52 (s, ar–H7), 7.17 (s, ar–H4), 6.95 (s, ar–H2), 5.54 (s, 6-CHR_2), 3.91 (9H, s, ar–OCH_3), 3.10 (s, *R*–S–CH_2–*R*), 2.95 (s, *R*–S–CH_2–*R*), 2.75 (s, *R*–CH_2–*R*) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6, 30°C): δ = 184 (C9), 181 (C10), 164 (C3), 162 (C1), 159 (C8), 146 (C6), 117 (C7), 118 (C5), 103 (C2), 106 (C4), 56 (ar–OCH_3), 50 (6-CHR_2), 31 (*R*–S–CH_2–*R*), 29 (*R*–CH_2–*R*) ppm; HSQC (*DMSO*-d_6, 30°C): ar–*H*2 ↔ *C*2, ar–*H*4 ↔ *C*4, ar–*H*5 ↔ *C*5, ar–*H*7 ↔ *C*7, 6-CHR_2 ↔ 6-CHR_2, ar–OCH_3 ↔ ar–OCH_3, *R*–S–CH_2–*R* ↔ *R*–S–CH_2–*R*, *R*–CH_2–*R* ↔ *R*–CH_2–*R*; HMBC (*DMSO*-d_6, 30°C): *C*2 → ar–*H*4, *C*4 → ar–*H*2, C5 → ar–H7, 6–CHR_2, ar–H5, ar–H4, ar–H2, C7 → ar–H5, ar–H7, C6 → 6-CHR_2, C3 → ar–OCH_3, C8 → ar–OCH_3, C1 → ar–OCH_3.

3-(1,3-Dithian-2-yl)-1,6,8-trihydroxyanthracen-9(10H)-one (**7**, $C_{18}H_{16}O_4S_2$)
A solution of 110 mg **6** (0.93 mmol) in 16 cm^3 glacial acetic acid, 7.7 cm^3 HBr (47%), and 463 mg $SnCl_2 \cdot 2H_2O$ were stirred at 90°C under *Ar* with protection from light for 60 min. Afterwards, the mixture was extracted twice with H_2O/ethyl acetate, and after evaporation of the solvent purification was achieved by column chromatography over silica using $CHCl_3$:*Me*OH (15:1) as the solvent, and finally the residue was dried over night over P_2O_5 to yield 79.5 mg (86%) **7** as a yellow solid. Mp 175°C (dec); $R_f = 0.60$ ($CHCl_3$:CH_3OH = 15:1); ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): δ = 12.30 (d, ar–OH), 10.92 (s, ar–OH), 7.02 (s, ar–H5), 6.87 (s, ar–H7), 6.42 (s, ar–H4), 6.24 (s, ar–H2), 5.42 (s, 6-CHR_2), 4.31 (s, *R*–CH_2–*R*), 2.95 (d, *R*–S–CH_2–*R*), 2.45 (s, *R*–CH_2–*R*) ppm; ESI-MS (CH_3OH + 1% NH_3, negative ion mode): m/z = 372.8 ($[M-H]^-$).

10,13-Di(1,3-dithian-2-yl)-1,3,4,6,8,15-hexahydroxydibenzo-[a,o]perylene-7,16-dione (**8**, $C_{36}H_{26}O_8S_4$)
A mixture of 75 mg **7** (0.21 mmol), 2.8 mg $Fe_2(SO_4)_3 \cdot 7H_2O$ (0.007 mmol), and 110.4 mg pyridine-*N*-oxide (1.16 mmol) in 2 cm^3 dry pyridine and 110 mm^3 dry piperidine were stirred under Ar with protection from light at 105°C for 1 h. After cooling to room temperature the reaction mixture was poured into 5 cm^3 2 *M* HCl and stirred for 30 min at room temperature in the dark. After centrifugation the residue was washed three times with 3% HCl and four times with distilled H_2O, and dried over night in vacuum over P_2O_5 resulting in **8** as a black solid. Because **8** proved to be rather unstable (photocyclization), it was immediately photocyclized. UV-Vis (acetone): λ_{max} = 368 (86), 449 (40), 547 (45), 584 (48), 629 (37) nm (rel. int.).

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