Improved Synthesis of Indirubin Derivatives by Sequential Build-Up of the Indoxyl Unit: First Preparation of Fluorescent Indirubins

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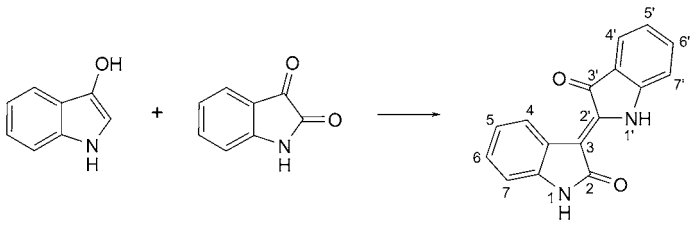
Indirubin, present in extracts of *Isatis tinctoria* and some other plant species, has promising cytotoxicity against a variety of cell lines by inhibition of cyclin-dependent kinases. Chemical synthesis of its derivatives relies on the combination of isatins and 2,3-dihydro-1*H*-indol-3-one ('indoxyl') derivatives and usually yields indigo as well as other by-products. Inspection of the hydrolysis of the long-known condensation products of 2-thioxothiazolidin-4-one with isatins gave useful hints for an improved synthesis of indirubins: this reaction does not yield quinoline derivatives but 2-(2,3-dihydro-2-oxo-1*H*-indol-3-ylidene)-2-sulfanyl acetic acids. By substitution of the sulfanyl group in this oxindoles with anilines and straightforward cyclization under *Nazarov* conditions, a broad variety of indirubins substituted in the indoxyl ring system are thus available, usually in very good purity and yield. Use of naphthylamines in this reaction sequence yields various fluorescent substances with λ_{fl} at *ca.* 630 nm.

Introduction. – Since recently, indirubin (**1a**) is under examination as an antileukemic agent. This is the result of inspecting the use of indigo dyes in the folk medicines of India, south-east Asia, and China [1]. Most prominently, this purple substance is found in the indigo dyeing material obtained from *Isatis* plant species, but also it occurs in various quantities in the aqueous extracts of several other plant species used as medicines like *Strobilanthes*- and *Polygonum* spp.

Indirubin is formed as a by-product of the oxidative dimerization of 2,3-dihydro-1*H*-indol-3-one ('indoxyl'; **2**) to yield the blue indigo dye in fermented leaves from the respective plant species, whereby **2**, in the presence of isatin (**3a**), undergoes condensation to indirubin (**1a**; see *Scheme 1*).

Scheme 1. *Indirubin* (**1a**) *Formation by Condensation of Indoxyl* **2** *with Isatin* **3a**



2 **3a** **1a**

Indirubin (**1a**) has promising cytotoxicity against a variety of cell lines where it reverses leukaemic transformation by inhibition of cyclin dependent kinases [2], and it has shown a reduced toxicity in animal experiments. It is essentially insoluble in aqueous media, a finding, which obscures the elucidation of its biological effects. For obtaining sound SAR data of the various cytotoxic effects, it will be necessary to examine a far greater number of derivatives not available at present. These derivatives should have a far better solubility. Additionally, a future aim will be to examine the inhibitory activity against protein tyrosin kinases according to an example [3], dependent on the substituents at C(4′) and C(7′) of the indol-3-one part.

Results and Discussion. – There is only one systematic access to indirubin, originating from *Baeyer*. 2-Nitrobenzaldehyde is oxidized in the presence of acetone which yield – among many side-products – indoxyl (**2**). The established way to obtain indirubins (*Scheme 1*) would then be to condense them with appropriate isatin derivatives. Additionally, **2** can be prepared *in situ* by hydrolysis of indoxyl sulfate or acetate in aqueous base solution. As a by-product, always blue indigo is formed. Access to other indoxyls apart form the unsubstituted case or the common 5-chloro or 5-bromoindoxyl is difficult but realizable [4]. These procedures cannot be applied rigorously to obtain other derivatives. To illustrate the necessity of new methods to synthesize indirubins, an example of a microbial oxidation procedure [5] can be mentioned. This, being not selective at all as a disadvantage, also fails to give reasonable amounts of substance.

We hereby describe a systematic method which allows the preparation of indirubins substituted at C(4′) to C(7′) of the indol-3-one part, avoiding thereby the preparation of indoxyls, but using the large variety of commercially available anilines. There is some older literature describing the synthesis of several differently subsituted isatins. Together with this new procedure, a large library of indirubins should be available.

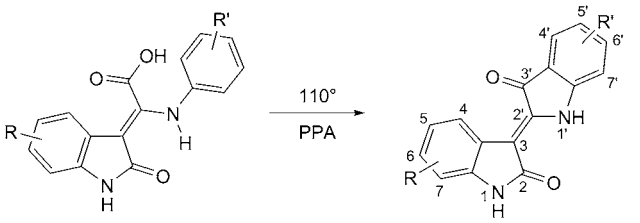
The key step of the method comprises the use of compounds **7**, substituted phenylglycines, capable to undergo a ring closure (*Scheme 2*) to indirubins **1a**–**1j**, in analogy to the old reaction of *Heumann*, who used the cyclization of *N*-phenylglycine as a source of free indoxyl. In this way, formation of free indoxyl and any coupling products resulting from it is avoided.

This concept was realized with 2-(2,3-dihydro-2-oxo-1*H*-indol-3-ylidene)-2-sulfanylacetic acids **6**, a distinct and stable class of compounds, which were substituted at their S-atom with anilines to easily yield the desired indirubin precursors **7** (*Scheme* 3).

2-Thioxothiazolidin-4-one ('rhodanin', **4**) and isatine (**3a**) reacted in boiling AcOH or by some other means to give (3*Z*)-3-(4-oxo-2-thioxo-1,3-thiazolidin-5-ylidene)-1,3-dihydro-2*H*-indol-2-one (**5a**; *Scheme* 3), which belongs to the class of 'indigoids', a generally and easily available class of dark colored compounds. Indigoid **5a** was originally reported to give during hydrolysis either **6a** or 2-hydroxy-3-sulfanylquinoline-4-carboxylic acid (**8a**) [7][8].

The formation of **6a** was questioned for several reasons [6]. After reduction, a quinoline-2-one derivative was obtained, and so the original condensation product was claimed to be **8a** in its tautomeric 2-one form. Substitution of the S-atom in the claimed quinolone by refluxing it with aniline was reported to occur and subsequently should

Scheme 2. *A New Synthesis of Indirubins from (2Z)-2-(1,2-Dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(phenylamino)ethanoic Acids* **7** *by a Ring Closure under* Nazarov *Conditions* (polyphosphoric acid (PPA), 110°)



	R	R'
7a	H	H
7b	H	4-Me
7c	H	4-Me,2-MeO
7d	H	3,4-$(MeO)_2$
7e	H	2,3-Me_2
7f	H	4-COOH
7g	7-COOH	H
7h	5-Cl	4-Cl
7i	7-Cl	2,3-Me_2
7j	5-Br	4-Me

	R	R'
1a	H	H
1b	H	5'-Me
1c	H	5'-Me,7-MeO
1d	H	5',6'-$(MeO)_2$
1e	H	6',7'-Me_2
1f	H	5'-COOH
1g	7-COOH	H
1h	5-Cl	5'-Cl
1i	7-Cl	6',7'-Me_2
1j	5-Br	5'-Me

have given a substance assumed to be 2-hydroxy-3-(phenylamino)quinoline-4-carboxylic acid (**8b**) in its tautomeric form.

We tried to use this putative **8b** from the early report (*Scheme* 3) for a desired synthesis of dibenzo[*b*,*f*][1,7]naphthyridine-6,12-dione [9]. Cyclization in polyphosphoric acid (PPA) surprisingly yielded only indirubine **1a** in excellent yield. Various promoters of the *Nazarov*-type reaction such as MsOH/phosphorous oxide suspension were less effective, but do work. Triflic acid was effective even at room temperature.

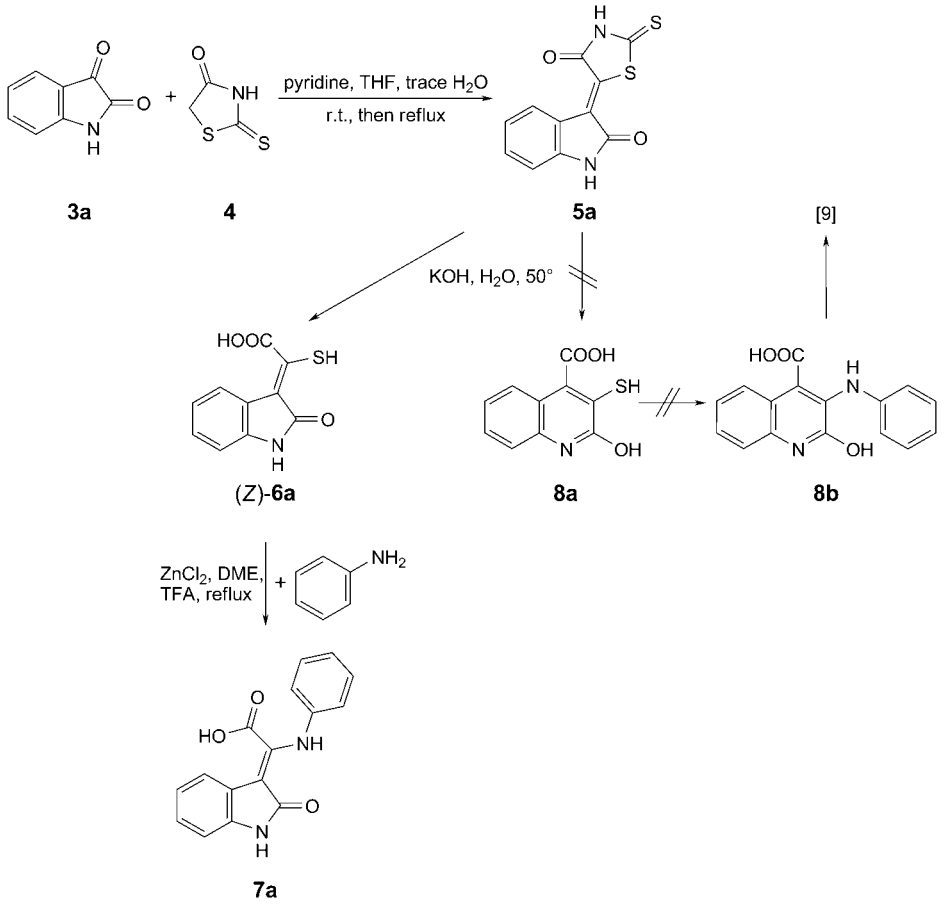
This could hardly be explained. It would be possible only if the structure **7a**, *i.e.*, ((2*Z*)-(1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)(phenylamino)ethanoic acid, is correct for this compound and not **8b**.

In contrast, real compounds **8b** could be obtained independently by an alternative route from the reaction of *N*,*N*′-diphenylhydrazines and acetylenedicarboxylates, followed by rearrangement. Refluxing the acid chloride of **8b** with *Lewis* acids gave dibenzo[*b*,*f*][1,7]naphthyridine-6,12-dione [9], and thus establishing that it is the real **8b**. Not even a trace of indirubin was detected in this reaction.

Another evidence in favor of structure **7a** is that NMR spectra of compounds of this type exhibit frequently two distinct sets of signals, often in a ratio of *ca.* 1:1, *e.g.*, compound **7h** (R = 5-Cl, R′ = 4′-Cl (1:1)) and **7i** (R = 7-Cl, R′ = 2′,3′-Me_2, (1:1.5)). These isomeric mixtures **7** did not contained any **8**. Data of the latter are known accurately from pure samples synthesized independently [9].

Inspection of the $^{1}H/^{13}C$-NMR signals of **7** indicate substantially different electronic environments in some pairs of isomers, so in **7i**, other pairs of signal are only shifted, as in **7h**. Whether these spectra really correspond to (*Z*)/(*E*) pairs or represent an admixture of structures like **13** has to be clarified (*Scheme 4*).

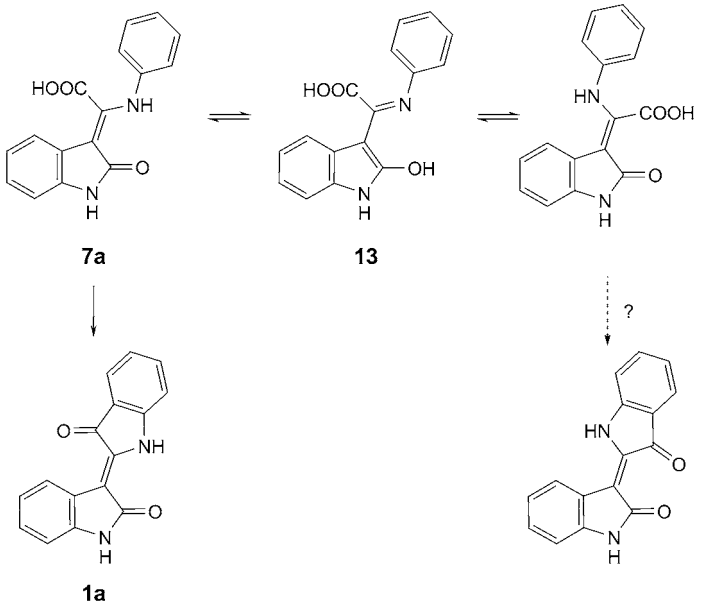
Scheme 3. *Isatin* (**3a**) *and 2-thioxothiazolidin-4-one* (rhodanine; **4**) *in Pyridine Formed an Indigoid* **5a** *that Could Hydrolyze in Aqueous Basic Solution to Yield 2-Hydroxy-3-sulfanylquinoline-4-carboxylic Acid* (**8a**) *or (2*Z*)-2-(1,2-Dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(sulfanyl)ethanoic Acid* (**6a**). It was concluded from the reaction data that **8a** was not formed. Reaction of **6a** with aniline in DME under catalysis with anh. $ZnCl_2$ and refluxing for 8 h or microwave heating for minutes yielded **7a**, which subsequently could undergo ring closure to indirubin (**1a**). If any **8b** would have been formed, cyclization to benzonaphtyridines [9] should have been observed, which was not the case.



Eventually, the question arises whether the (*E*)-form of **7**, cyclized differently from (*Z*)-**7**, or whether a disfavored isomer for indirubine exists. Close inspection of the obtained dyes revealed no hint for a large amount of such a material, but traces of so far unknown substances were detected, which would be an interesting topic for future research. The possibility of easy rotation in **13** would favor the cyclization to the ordinary indirubin, which is stabilized by the six-membered ring formed by a H-bridge between NH of one part and the CO group of the other of **1** (*Scheme 4*).

The substances **7** are also very different from compounds **8** with respect to solubility in organic media. For example, **8a** is sparingly soluble in MeOH, whereas **7a** is well

Scheme 4. *Besides the Possibility of a* (Z)/(E) *Mixture of* ***7a*** *on the Basis of* ^{1}*H- and* ^{13}*C-NMR Signal Sets Observed, Other Tautomers* **13** *May Be Responsible for an Equilibration Mechanism and the Resulting Complete Cyclization of Both Isomers to Indirubin* **1a** *with Its Favourable Configuration by Involvement of H-Brigdes*



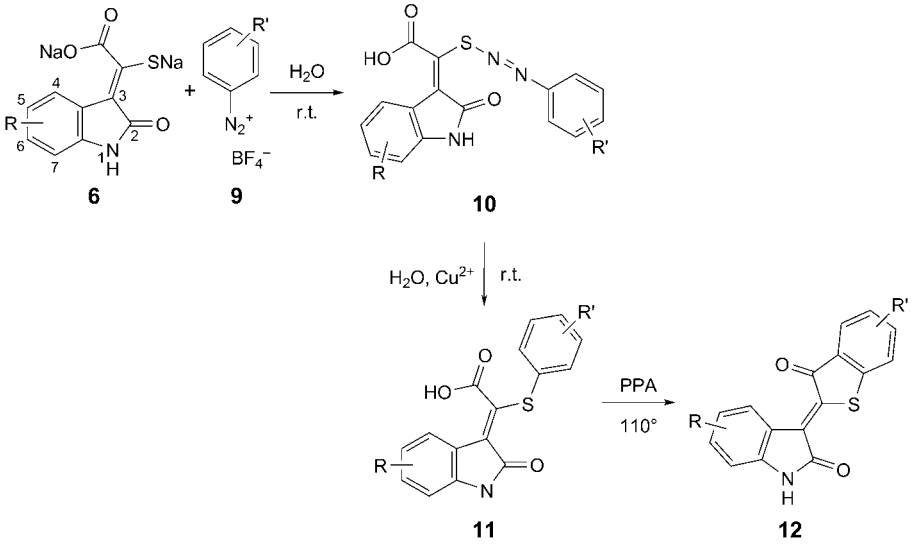
soluble. Compounds **7** display particular good H_2O solubility, rendering sometimes problems for their recovery from aqueous solutions.

Existence and good stability of compounds **7** against transformation to **8** is a strong evidence to assume also structure **6a** to be correct and not **8a**. Arylation of the SH group by using aryldiazonium tetrafluoroborates **9** and cuprous salts in MeCN according to a published procedure [10] would then yield 2-(phenylsulfanyl)acetic acids **11a** and **11b** (*Scheme 5*).

As proof of concept, cyclization of **11** to thioindirubins **12** should be achieved. Unfortunately, these arylations proved difficult. They give a multitude of products and frequently fail. But in support of the general structure **6** proposed, the acids available so far, *i.e.*, **11a** (R = 5-Me R′ = 4′-Me) and **11b** (R = 5-SO_3H, R′ = 4′-Me), do cyclize in PPA to give thioindirubines **12a** (R = 5-Me, R′ = 5′-Me) and **12b** (R = 5-SO_3H, R′ = 5′-Me), respectively, in high yield and purity. Usually, compounds **6** are obtained as single isomers by preferential crystallization. These isomers **6** are stable at room temperature and react presumably without (*E*)/(*Z*)-isomerization to **11**.

It is not clear so far under which circumstances **7a** would be transformed to **8a**, but only prolonged treatment of **6a** or **7a** at high pH values seems to cleave the N–C(2) bond of the indolone part according to isatin itself. Ring-opened isatin salts condense with (phenylsulfanyl)acetone to give indeed 3-(phenylsulfanyl)quinolin-4-carboxylic acids [11].

Scheme 5. *By Reaction of* **6** (**6g**, R = 5-Me; or **6h**, R = 5-SO_3H) *with Phenyldiazonium Salt* **9** (R′ = 4-Me) *in* H_2O *at pH 6–7, S-Arylation Could Be Observed, Catalyzed by* Cu^{2+}. Products **11** (**11a**, R = 5-Me; R′ = 4′-Me; **11b**, R = 5-SO_3H, R′ = 4′-Me) yielded thioindirubins **12** (**12a**, R = 5-Me; R′ = 5′-Me; **12b**, R = 5-SO_3H, R′ = 5′-Me) by applying *Nazarov* conditions (polyphosphoric acid (PPA), 110°).

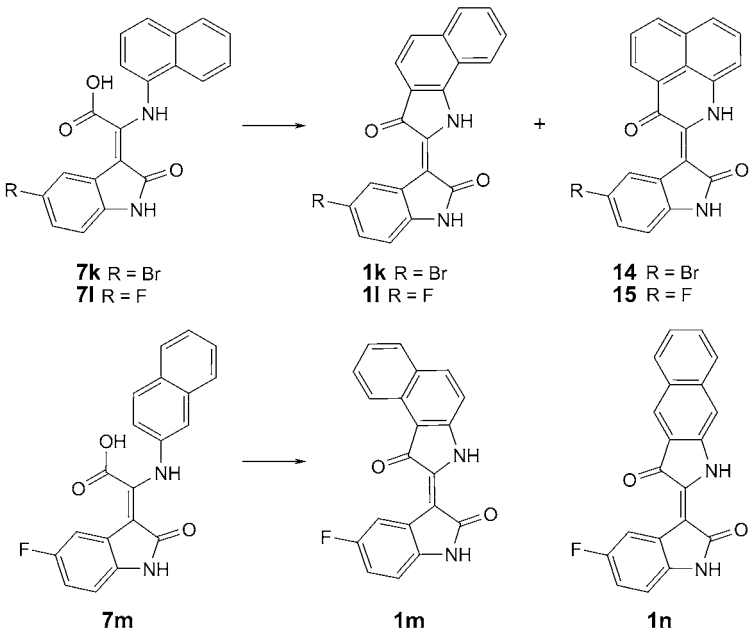


These observations and the assumption that the original structure **6a** is correct, thus opens a systematic route to indirubins. For practical use as a synthetic method, several experimental improvements in this concepts had to be found. Notably, it was found that rhodanin (**4**) can be condensed with isatines in pyridine when a small quantitiy of H_2O is added, avoiding the long-lasting procedure reported earlier. This method rarely fails. One of these cases is the reaction of 4-methyl-7-methoxyisatin. In these cases, also the synthesis of the indigoid in AcOH is difficult.

Second, the substitution of the SH group in compounds **6** to yield derivatives **7** was practicable only in the case of **7a**, because aniline itself was used as a solvent, and **7a** was sparingly soluble therein. This works sometimes for single cases even of more sensitive compound **7c**. Nevertheless, a stoichiometric variant was desirable. The yields generally being *ca.* 30–40%, assistance by using a thiophilic transition metal salt was found to be helpful. Use of basic Pb salts indeed results in lead sulfide formation, but the yield remained low. Cuprous salts resulted in complex decompositions, but use of anhydrous Zn salts gave promising results. In combination with solvents such as 1,2-dimethoxyethane (DME), the yield of compounds **7** could be improved up to 70% in a clean reaction, without additional chromatography of the products. The form of the starting material is crucial; the reaction does not proceed with sodium salts of **6**. For materials containing such salts, the yield was proportional to the content of free acid. It was found that protonation by CF_3COOH is helpful. In conclusion, a systematic combination of a variety of commercially available isatins **3** with anilines would lead to new and unusual indirubins. Examples are **1c** and **1d** of phenolic character or indirubin-carboxylic acids **1f** and **1g**. These indirubins have particularly good H_2O solubilities. Of

great help was the application of microwave heating. Often, indirubins **1** can be prepared without purification of the intermediates **7**. By treating a derivative **6** and a suitable aniline in a microwave oven, followed by simple extraction with base and reprecipitation in acid, a material is obtained of sufficient purity because of very short reaction times, *i.e.*, 8 min instead of 8 h. In this manner, also the reactions with naphthalenamines and **6d** (R = 5-Br), **6e** (R = 5-F) were carried out (*Scheme* 6), which yielded remarkable 'naphthindirubins'. It could be shown, by abriged isolation of **7k**, **7l**, and **7m**, preparation of 'napthindirubins' from **6d** (R = 5-Br) and **6e** (R = 5-F) could be accomplished in one day.

Scheme 6. *Cyclization of* **7l** (R = F) *Derived from Naphthalen-1-amine, Interestingly Yielded Two Isomers, the Major Isomer* **15** (R = F) *Being a Benzo[*de*]quinolin-3(*2H*)-one and* **1l**, *a Regular 'Napthindirubin'* (benzo[*g*]indol-3(2*H*)-one). Whereas with **7l** (R = F) **15** is the major isomer, the Br derivative **7k** predominantly yielded the non-fluorescent **1k**. Reaction of naphthalen-2-amine derivative **7m** (R = F) did only yield the non-fluorescent 'naphthindirubin' **1m** ((*Z*)-2-(5-fluoro-2,3-dihydro-2-oxo-1*H*-indol-3-ylidene)-2,3-dihydro-1*H*-benzo[*e*]indol-1-one).



The cyclization of **7l** (R = 5-F), derived from naphthalen-1-amine, interestingly yielded two isomers. The UV-VIS and fluorescence spectra of the major isomer **15** (R = 5-F), which exhibited red-orange fluorescence, are shown in the *Figure*.

As an impurity, a non-fluorescent minor isomer **1l** (R = 5-F) was present, characterized by a better solubility. Separation was easily achieved by column chromatography. By the same treatment, **7k**, the Br derivative (R = 5-Br), gave the non-fluorescent isomer **1k** (R = 5-Br) in 22% yield and the fluorescent **14** (R = 5-Br) in a minor amount (**1k**/**14** *ca.* 3 : 1). In case of reaction of **6e** with naphthalen-2-amine, also two isomers were expected (*Scheme* 6), but only one non-flourescent product was

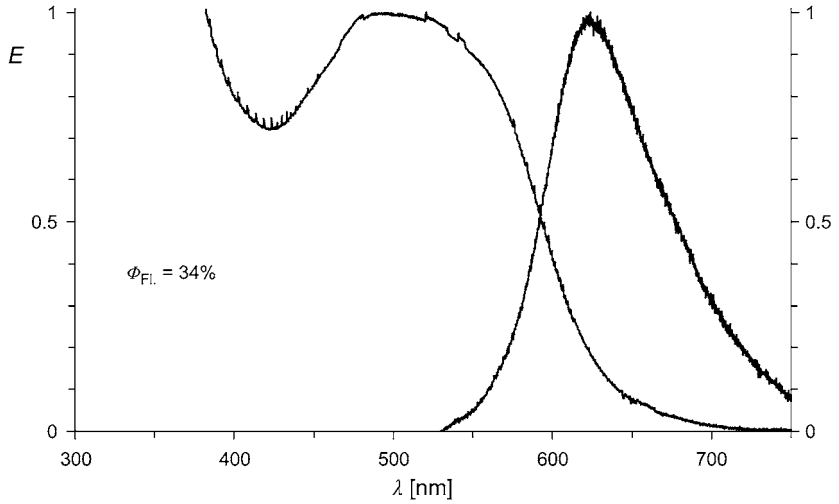


Figure. *UV/VIS and fluorescence spectrum of (2Z)-2-(5-Fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-1,2-dihydro-3*H*-benzo[*de*]quinolin-3-one* (**15**). Solvent, acetone; ambient temp. Compound shines orange-red (629 nm) with a fluorescence quantum yield of 34%.

detected, which was identified as (2*Z*)-2-(5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2,3-dihydro-1*H*-benzo[*e*]indol-1-one (**1m**), the occurrence of a naphthindirubin **1n** with the other orientation being considered as less probable due to the enhanced reactivity of the α-position. This finding raises the question if one of the isomers in case of naphthalen-1-amine, specifically the fluorescent one, is really an indirubin-like structure or should be rather formulated as 1,2-dihydro-3*H*-benzo[*de*]-quinolin-3-one **14** (R = Br) and **15** (R = F). Indeed, 1*H*-benzo[*de*]quinoline-2,3-diones show a yellow fluorescence, but not the corresponding naphthisatines (= 1*H*-benz[*g*]indole-2,3-diones). Using 2-methylnaphthalen-1-amine, only a single fluorescent compound was formed. With 2-methylnaphthalen-1-amine, no indirubin-like compound could be obtained since the 2-position was blocked.

Therefore, it was concluded that it is thus the 1*H*-1-azaphenalen-3-one element is the source of fluorescence.

Whereas the problem to obtain indirubins substituted in the indoxyl nucleus has been solved, thus allowing preparation of a wide variety of them, the method is still dependent on isatins. At present, only nitro-isatins did not lead to any indirubins, since internal reduction and disappearance of sulfur seems to take place in these cases.

Experimental Part

General. IR: *Thermo Electron Nicolet 380* (usually in KBr). ^{1}H- and ^{13}C-NMR: *Bruker AMX400*. MS: *Finnigan MAT 70*, EI: 70 eV, CI: Isobutane. Elemental analyses were performed in the Mikroanalytisches Labor, Institut für Anorganische Chemie, Technische Universität München. Microwave-asisted syntheses were carried out with *CEM* DiscoverS class[1]) single-mode synthesis system

[1]) For further information, see http://www.cem.com

(www.cem.com) interfaced with a laptop pc running CEM synergy software monitoring the reaction. The temp. was checked by an external IR sensor on the floor of the cavity. Once the target temp. was reached, the microwave system automatically started to count down the hold time[1]). For reactions, *CEM* vials (10 ml) with snap-on caps were used. The pressure was monitored by a sensor outside the snap-on caps. The upper pressure limit was set to 17 bar. Temp./pressure recording were attached to CEM synergy reaction files.

Isatins apart from commercial samples, were obtained according to literature, see, *e.g.:* isatincarboxylic acids [12–14], aminoisatins [15], methoxyisatins [16][17], benzoisatins [18][19]. Compounds **6** rarely provide clean anal. data or melting points – except for **6h**. As *S*-Me derivatives **6a′**–**6g′**, they gave good crystals with sharp melting points. Spectral data usually refer to this material if not indicated otherwise. These derivatives were usually prepared by shaking weakly alkaline solns. in H_2O/EtOH mixtures with MeI according to [7]. In several cases, the crude compounds **7** were cyclized to the indirubins directly without further purification, so in cases of **1e**, **1f**, **1h**, **1k**, **1l**, and **1m**. Certain indirubins were sparingly soluble in nearly every solvent. Good COSY spectra could be obtained only by prolonged sampling times of *ca.* 24 h.

General Procedure for the Synthesis of 2-Sulfanyl-2-(2-oxoindolin-3-ylidene)acetic Acids (**6**). A quantity of desired isatin was dissolved in pyridine in a three-necked vessel with attached frit. Slightly more than 1 equiv. of *2-thioxothiazolidin-4-one* (**4**; rhodanine, M_r 133.19) in pyridine was added, and after several min. of mixing and dissolving, H_2O (10 ml) was added. The dark red suspension became warm, while residual solid dissolved. Soon afterwards crystallization started. Some THF was added, and the mixture was heated to reflux for 1 h. When cool, after standing in the fridge, excess pyridine and solvent were sucked over the frit. Then, the vessel was gently evacuated while standing in a warm H_2O bath. Hereby, the bright red solid lost excess pyridine and decayed into a powder. From the mother liquor, by standing in the fridge, additional material could be obtained. Dil. aq. KOH soln. was prepared and added to the powder, whereby the color faded. After stirring for 1 h at *ca.* 50°, the soln. was neutralized with aq. HCl at 0° in an ice bath. Half of the acid was slowly added until the soln. became turbid, the rest was added then quickly to yield a deep orange or red preciptiate. Otherwise, a yellow material of different chemical structure was obtained. To obtain sufficiently pure material, neutralization in the cold was essential. Otherwise, red oils were obtained. In this case, stirring of the decanted oil in freshly dist. H_2O at pH *ca.* 6 for some h yielded a crystalline material. Half the way of to neutrality, often a thick yellow salt appeared. This could be isolated by sucction and used successfully to prepare the *S*-Me derivatives.

Otherwise, an aliquot of the alkaline soln. of the salt was diluted with 20% aq. EtOH, excess MeI was added, and the mixture was stirred overnight whereby yellow crystals formed. After standing overnight, they were collected and recrystallized from MeOH.

*(2Z)-2-(1,2-Dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(sulfanyl)ethanoic Acid* (**6a**). From 28 g of (0.2 mol) isatin and 26.6 g of (0.2 mol) **4**, 80 ml of pyridine, and 10 ml of H_2O. Hydrolysis with 45 g KOH in 400 ml H_2O. Acidification with 110 ml conc. HCl. Yield: 24 g (64%). Intense red powder.

*(2Z)-(1,2-Dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(methylsulfanyl)ethanoic acid* (**6a′**). Yellow needles. IR (KBr): 3552*m* (br.), 1675*s*, 1653*s*, 1574v*s*, 1473*w*, 1465*m*, 1431*m*, 1417*m*, 1370*s*, 1337*m*, 1295*m*, 1273*w*, 1229*m*, 1187*m*, 1099*w*, 1081*m*, 1011*m*, 883*w*, 789*m*, 744*m*, 734*m*, 711*w*, 670*w*, 624*m*, 594*m*. ^{1}H-NMR (CD_3OD): 7.68 (*d*, *J* = 7.5, 1 H); 7.12 (*t*-like, *J* = 8.2, 1 H); 6.97 (*t*-like, *J* = 8.2, 1 H); 6.83 (*d*, *J* = 7.5, 1 H); 2.6 (*s*, 3 H). ^{13}C-NMR ($CDCl_3$): 165.9; 163.8; 161.3; 139.9; 126.2; 124.4; 122.5; 120.5; 111.4; 108.7; 14.7.

*2-(1,2-Dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(phenylamino)ethanoic Acid* (**7a**). Compound **6a** (2.2 g, 0.01 mol) and aniline (10 ml) were mixed to homogeneity. A slightly exothermic reaction and evolution of H_2S started. Under N_2, the temp. was raised to 140°. After 30 min, the green liquid was cooled, whereby a yellow crystalline precipitate appeared. Before reaching r.t., the yellow solid was isolated by suction. The greenish material was washed with ice cold MeOH. After drying, the material was dissolved in 10% aq. NaOH (20 ml) and filtered. The filtrate was acidified by pouring it into dil. aq. HCl (20 ml), whereby a yellow powder precipitated (0.84 g). The aniline residue could be worked up as well by complete sublimation of the excess aniline *in vacuo* to yield 1 g of material. Yield: 1.84 g (64%). Yellow needles. Recrystallization from hot MeOH. IR: 3425*s* (br.), 3047*w*, 2921*w*, 2851*w*, 2606*m* (br.), 2361*w*, 1661*s*, 1620v*s*, 1595*s*, 1577*s*, 1496*m*, 1463*m*, 1385*m*, 1337*m*, 1325*m*, 1264*w*, 1226*m*, 1197*m*, 1102*w*,

1027*w*, 997*w*, 742*m*, 731*m*, 690*m*, 640*w*, 619*w*, 589*w*, 544*w*, 502*w*, 500*m*. ^{1}H-NMR ((D_8)THF, (*E*/*Z*) mixture): 11.4 (*s*, 1 H); 10.6 (*s*, 1 H); 7.4 (*t*-like, *J* = 8.0, 2 H); 7.2 (*t*-like, *J* = 8.0, 3 H); 7.15 (*t*-like, *J* = 7.0, 2 H); 7.0 (*t*-like, *J* = 7.0, 1 H); 7.0–6.9 (*m*, 4 H). ^{13}C-NMR ((D_8)THF): 171.1 (COOH); 164.75 (C(2)=O); 146.8; 139.0; 137.6; 130.0; 126.8; 125.5; 125.2; 122.3; 121.2; 118.6; 110.1; 96.4. CI-MS: 105.1 (4), 144.1 (32), 165.0 (2), 236 (100), 280.2 (12).

*2-(1,2-Dihydro-2-oxo-3*H*-indol-3-ylidene)-2-[(4-methylphenyl)amino]ethanoic Acid* (**7b**). Compound **6a** (2.2 g, 0.01 mol) was mixed with *p*-toluidine (10 g). The yellow mass was heated for 20 min at 140° under Ar at normal pressure in a *Kugelrohr* distillation apparatus or in some glassware which allows distillation of the easily crystallizing anilines. Then, pressure was lowered, and the excess *p*-toluidine was sublimed off. The dry green material was dissolved in aq. 10% NaOH soln. (20 ml) and filtered. The filtrate was acidified by pouring it into 20 ml of dil. aq. HCl whereby a yellow powder precipitates that was collected and air-dried. Yield: 1.98 g (68%). Recrystallization from hot MeOH. IR: 3281*s*, 3229*s*, 3065*w*, 3026*w*, 2927*w*, 2862*w*, 1710*s*, 1612v*s*, 1516*s*, 1475*w*, 1458*w*, 1403*m*, 1322*s*, 1248*s*, 1222*s*, 1197*s*, 1103*s*, 1020*m*, 1000*m*, 969*w*, 874*m*, 847*m*, 833*m*, 802*m*, 781*m*, 745*m*, 728*m*, 685*m*, 641*m*, 600*m*, 567*m*, 518*s*, 486*m*, 473*w*, 440*w*. ^{1}H-NMR ((D_8)THF, (*E*/*Z*) mixture): 11.8 (*s*, 1 H); 9.9 (*s*, 1 H); 7.44 (*d*, *J* = 7.5, 1 H); 7.2–7.14 (*m*, 4 H); 7.0 (*t*-like, *J* = 7.0, 1 H); 6.9–6.8 (*m*, 2 H); 2.35 (*s*, 3 H). ^{13}C-NMR ((D_8)THF): 171.6 (COOH); 164.5 (NCO); 148.0 (Ar–NR); 137.4 (ArNCO); 137.1 (NC); 135.8; 130.6 (2 arom. C); 130.5; 127.4; 125.6; 122.15 (2 arom. C); 121.8; 121.75; 114.0; 111.25; 96.0; 20.75 (Me). CI-MS: 294.3 (14), 250.3 (100), 150.2 (18), 133.1 (23), 118.1 (16).

General Procedure for the Synthesis of Indirubin Derivatives. Compound **7** (*ca.* 300–500 mg) was suspended in polyphosphoric acid (PPA; 10 g) and heated in an oil bath to *ca.* 80° by stirring. The temp. was then raised to 120° (internal) and kept for 1 h. A dark color appears. The hot melt was poured into 200 ml of dist. H_2O and stirred for 2 h. A purple solid was collected by filtration and washed with dist. H_2O to neutral pH. After drying in air, the filter paper keeping the dark solid was extracted in a *Soxhlet* apparatus with 200 ml acetone for some h. The solvent was stripped off (*ca.* 80%), then the liquid was left for evaporation in air. Intense purple needles.

5′-Methylindirubin (*=(3Z)-1,3-Dihydro-3-(1,3-dihydro-5-methyl-3-oxo-2*H*-indol-2-ylidene)-2*H*-indol-2-one*; **1b**). From **7b** (300 mg). Yield: 260 mg (92%). IR: 3444*s* (br.), 3303*s*, 3186*s* (br.), 3053*m* (sh), 2918*m*, 2875*m* (sh), 1710*s*, 1663v*s*, 1619v*s*, 1684v*s*, 1492v*s*, 1464*m*, 1385*w*, 1334*m*, 1292*m*, 1197v*s*, 1193v*s*, 1128*s*, 1043*w*, 1022*m*, 961*w*, 813*w*, 777*w*, 744*w*, 711*w*, 618*w*, 583*w*, 565*w*. ^{1}H-NMR ((D_6)DMSO): 11.15 (*s*, 1 H); 11.1 (*s*, 1 H); 8.63 (*d*, *J* = 7.5, 1 H); 7.32 (*s*, 1 H); 7.27 (*d*, *J* = 8.2, 1 H); 7.18 (*d*, *J* = 8.2, 1 H); 7.12 (*t*-like, *J* = 7.5, 1 H); 6.89 (*t*-like, *J* = 7.5, 1 H); 6.78 (*d*, *J* = 7.5, 1 H); 2.32 (*s*, 3 H). ^{13}C-NMR ((D_6)DMSO): 189.4; 171.7; 151.3; 141.4; 139.4; 138.7; 131.2; 129.8; 125.3; 124.9; 124.8; 122.2; 121.9; 119.8; 113.9; 110.2; 106.7; 20.8.

*(2Z)-2-(1,2-Dihydro-2-oxo-3*H*-indol-3-ylidene)-2-[(2-methoxy-4-methylphenyl)amino]ethanoic Acid* (**7c**). **6a** (1 g, 4.5 mmol) was mixed with 5 g of 2-methoxy-4-methylanilin and treated as described for **7b**. After complete stripping of excess 2-methyl-4-methoxyaniline, the residue was dissolved in MeOH and submitted to column chromatography CC *Kieselgel G60* (60 g), column diameter, 3 cm; height, 20 cm; eluent, MeOH/acetone gradient). Yield: 300 mg (21%). IR: 3443*s* (br.), 2997*m*, 2828*m*, 1645v*s*, 1616*s*, 1587*s*, 1504*m*, 1455*w*, 1386*w*, 1347*w*, 1229*w*, 1267*w*, 1216*m*, 1163*w*, 1102*w*, 1047*m*, 1000*w*, 973*w*, 875*w*, 856*w*, 793*m*, 745*w*, 679*w*, 610*m*, 562*w*, 518*w*. ^{1}H-NMR ((D_6)acetone): 7.3 (*d*, *J* = 7.6, 1 H); 7.23 (*d*, *J* = 8.8, 1 H); 7.0 (*dt*-like, *J* = 7.5, 1.7, 1 H); 6.95 (*d*, *J* = 7.4, 1 H); 6.85 (*dt*-like, *J* = 7.4, 1.5, 1 H); 6.82 (*d*, *J* = 3.2, 1 H); 6.75 (*dd*, *J* = 8.5, 3.2, 1 H); 3.76 (*s*, 3 H); 2.73 (*s*, 3 H). ^{13}C-NMR ((D_6)acetone): 173.3 (COOH); 158.6 (CONH); 137.4 (ArN); 136.0 (ArO); 131.0 (ArN); 125.4; 124.9; 124.7; 123.4; 121.25; 118.8; 116.7; 116.2; 112.2; 112.0; 109.8; 55.5 (MeO); 55.29 (Me). CI-MS: 324.4 (16), 280.4 (100), 265.3 (18), 247.4 (5.8), 239 (3.0), 219.3 (2.5).

5′-Methoxy-7′-methylindirubin (*=(3Z)-1,3-Dihydro-3-(1,3-dihydro-7-methoxy-5-methyl-3-oxo-2*H*-indol-2-ylidene)-2*H*-indol-2-one*; **1c**). Cyclization of 0.147 g **7c** in 10 g of PPA. Reaction seemed to be complete after 30 min at 110°. The melt was dissolved in 200 ml of dist. H_2O. Compound **1c** was soluble in H_2O at r.t., so KCl (15 g) was added. After standing overnight, a dark blue solid was collected by filtration. Yield: 69 mg (50%). The blue filtrate was treated with 10 g *XAD 1600 N* resin (*Rohm & Haas*) by stirring for 2 h to extract a second crop of **1c**. After elution of the dry resin with MeOH, 30 mg of **1c** was obtained from this solid phase. IR: 3222*s* (br.), 3055*m*, 2923*s*, 2848*s*, 1664v*s*, 1615*s*, 1595*m*, 1496v*s*,

1461*s*, 1421*m*, 1382*w*, 1331*s*, 1288*s*, 1265*s*, 1246*m*, 1207*m*, 1195*m*, 1173*s*, 1146*m*, 1100*w*, 1064*w*, 1041*s*, 996*m*, 959*m*, 932*m*, 875*w*, 816*m*, 775*m*, 760*w*, 731*w*, 699*w*, 676*w*, 608*m*, 599*m*, 542*w*. ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, 1 H); 10.3 (*s*, 1 H); 8.68 (*d*, *J* = 7.4, 1 H); 7.25 (*t*-like, *J* = 7.4, 1 H); 7.11 (*s*, 1 H); 7.04–7.33 (*m*, 2 H); 6.92 (*d*, *J* = 7.6, 1 H); 2.26 (*s*, 3 H); 1.35 (*s*, 3 H). ^{13}C-NMR ((D$_6$)DMSO): 188.3; 171.5; 156.0; 154.7; 145.4; 140.7; 139.3; 129.4; 125.9; 122.8; 121.5; 121.2; 119.1; 109.8; 106.5; 104.5; 26.3; 14.9.

(2Z)-(1,2-Dihydro-2-oxo-3 H-indol-3-ylidene)[(3,4-dimethoxyphenyl)amino]ethanoic Acid (**7d**). Compound **6a** (0.63 g, 2.8 mmol) and 3,4-dimethoxyaniline (0.9 g, 5.8 mmol) was dissolved in 1,2-dimethoxyethane (DME; 5 ml). After addition of a few drops CF_3COOH and dry $ZnCl_2$ (0.8 g, 5.8 mmol), the red soln. was refluxed 6 to 8 h (8 min microwave). The soln. was worked up by pouring it into 15% dil. aq. HCl (50 ml), isolation of the solids, dissolving it in dil. aq. NaOH (5 ml), and repreciptiation with 15% dil. aq. HCl. Losses because of good H_2O solubility. Yield: 0.5 g (50%) (with microwave: 62%). Yellow powder ^{1}H-NMR ((D$_6$)DMSO): 11.3 (*s*, 1 H); 10.7 (*s*, 1 H); 7.18 (*d*, *J* = 7.4, 1 H); 7.02 (*t*-like, *J* = 7.4, 1 H); 6.95 (*d*, *J* = 8.6, 1 H); 6.93–6.85 (*m*, 3 H); 6.78 (*dd*, *J* = 2.5, 8.6, 1 H); 3.75 (*s*, 3 H); 3.73 (*s*, 3 H). ^{13}C-NMR ((D$_6$)DMSO): 170.5; 164.2; 149.2; 147.1; 146.7; 136.9; 131.7; 124.3; 122.0; 120.5; 117.7; 113.4; 112.4; 109.4; 106.7; 94.8; 55.8; 55.5. CI-MS: 340 (2), 339 (5), 298 (39), 296 (36), 246 (7), 207 (10), 206 (100), 154 (61), 153 (38).

5′,6′-Dimethoxyindirubin (=*(3Z)-1,3-Dihydro-3-(1,3-dihydro-5,6-dimethoxy-3-oxo-2*H*-indol-2-ylidene)-2*H*-indol-2-one*; **1d**). Cylization of 0.26 g of **7d** in 5 g of PPA. Workup in 200 ml of dist. H_2O. Dark powder, separated from a red soln. Yield: 108 mg (43%). An additional crop of 43 mg could be extracted from the soln. by application of 10 g of *XAD 1600N* resin (*Rohm & Haas*). Compound seems to be sensitive to air. Extraction of the dark powder with acetone in a *Soxhlet* extractor left considerable dark residue insoluble in acetone. TLC of the extract (toluol/acetone 5 :2) showed also presence of a little amount of the other possible regioisomer. Purification by CC *Kieselgel G 60* (60 g), cyclohexane/acetone 3 :1; column diameter, 3 cm; height, 20 cm). Yellow material (minor isomer eluating first) was discarded. The main fraction was collected, and the solvent was stripped off *in vacuo* until precipitation occured. The dark flakes were collected by centrifugation, and the clear supernatant was discarded. Yield: 20 mg. IR: 2961 (sh), 2924*m*, 2856*w*, 1663*s*, 1620*s*, 1596*m*, 1490*s*, 1464*m*, 1352*w*, 1311*m*, 1290*m*, 1269*w*, 1244*w*, 1208*s*, 1156*m*, 1133*m*, 1099*w*, 1022*s*, 956*w*, 856*m*, 773*w*, 743*w*, 669*w*. ^{1}H-NMR ((D$_6$)DMSO): 10.8 (*s*, 1 H); 10.6 (*s*, 1 H); 8.8 (*d*, *J* = 7.4, 1 H); 7.21 (*t*-like, *J* = 7.4, 1 H); 7.08 (*s*, 1 H); 7.07 (*s*, 1 H); 6.99 (*t*-like, *J* = 7.4, 1 H); 6.88 (*d*, *J* = 7.4, 1 H); 3.85 (*s*, 3 H); 3.75 (*s*, 3 H). ^{13}C-NMR ((D$_6$)DMSO): 187.7; 172.3; 159.0; 151.5; 146.0; 142.0; 141.2; 130.3; 126.0; 122.7; 122.6; 111.4; 110.9; 107.5; 107.0; 98.1; 57.4; 57.2. CI-MS: 322 (3), 321 (2), 306 (3), 305 (2), 292 (2), 291 (2), 278 (17), 248 (20), 247 (14), 166 (42), 165 (58), 164 (14), 151 (13), 135 (13), 134 (100), 133 (51).

6′,7′-Dimethylindirubin (=*(3Z)-1,3-Dihydro-3-(1,3-dihydro-6,7-dimethyl-3-oxo-2*H*-indol-2-ylidene)-2*H*-indol-2-one*; **1e**). Compound **6a** (2.0 g, 10 mmol) was mixed with 10 g 2,3-dimethylaniline and treated as described for **7b**. After evaporation to dryness in a high vacuum, the material was directly subjected to 20 g of PPA and cyclized for 1 h at 110°. By workup in 500 ml of dist. H_2O, dark violet material separated. After filtration, drying in air, and extraction of the solid with acetone, a dark powder was obtained. Yield: 1.9 g (72%). IR: 3432*s* (br.), 1698*w*, 1664vs, 1615v*s*, 1592*s*, 1492*w*, 1462*m*, 1434*m*, 1382*w*, 1316*w*, 1293*s*, 1270*w*, 1209*s*, 1180*m*, 1150*w*, 1082*m*, 1030*m*, 994*m*, 964*w*, 801*w*, 771*m*, 746*m*, 698*w*, 673*w*. ^{1}H-NMR ((D$_6$)DMSO): 10.95 (*s*, 1 H); 10.5 (*s*, 1 H); 8.68 (*d*, *J* = 8.6, 1 H); 7.43 (*d*, *J* = 7.4, 1 H); 7.25 (*t*-like, *J* = 7.4, 1 H); 7.0 (*t*, *J* = 7.4, 1 H); 6.9–6.88 (*m*, 2 H); 2.29 (*s*, 3 H); 2.16 (*s*, 3 H). ^{13}C-NMR ((D$_6$)DMSO): 187.9; 171.6; 150.5; 147.0; 140.7; 139.5; 129.3; 124.5; 123.6; 121.9; 121.4; 121.2; 119.5; 117.3; 109.8; 106.3; 20.1; 11.6.

Indirubin-5-carboxylic Acid (=*(2Z)-2,3-Dihydro-2-(1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-3-oxo-1*H*-indole-5-carboxylic Acid*; **1f**). Compound **6a** (0.663 g, 3 mmol) was dissolved in 5 ml of DME and 1 g of ethyl 4-aminobenzoate was added. After addition of three drops of CF_3COOH and 408 mg of $ZnCl_2$, the mixture was refluxed for 24 h and worked up with 50 ml of 5% aq. NaOH. Precipitation occurred by acidification. Yield: 0.23 g. Another crop of 100 mg could be obtained by repeated extraction of the solid residue from the initial workup with 5% aq. NaOH.

A part of the dried material **1f** (70 mg) was cyclized in 15 g of PPA at a prolonged reaction time of 1.5 h. Workup in 150 ml of H_2O. The product had considerable solubility in H_2O. The purple acid was

isolated by collection of the solid phase (5 g of *Amberlite XAD 1600 N*, *Rohm & Haas*) after 1 d stirring. The acid was isolated by elution with acetone. Yield: 60 mg. IR: 3428*s* (br.), 3200 (sh), 2918*m*, 2849*w*, 1680*s*, 1667*s*, 1617*s*, 1538*w*, 1494*m*, 1463*m*, 1384*w*, 1331*w*, 1284*w*, 1262*m*, 1209*m*, 1176*m*, 1117*w*, 961*w*. ^{1}H-NMR ((D_6)DMSO): 11.27 (*s*, 1 H); 10.9 (*s*, 1 H); 8.77 (*d*, *J* = 7.4, 1 H); 8.2–8.1 (*m*, 2 H); 7.47 (*d*, *J* = 8.6, 1 H); 7.27 (*t*-like, *J* = 7.4, 1 H); 7.02 (*t*-like, *J* = 6.4, 1 H); 6.9 (*d*, *J* = 7.3, 1 H).

*(3Z)-3-[Carboxy(sulfanyl)methylidene]-2,3-dihydro-2-oxo-1*H-*indole-7-carboxylic Acid* (**6b**). From isatin-7-carboxylic acid (= 2,3-dihydro-2,3-dioxo-1*H*-indole-7-carboxylic acid; 5.8 g, 0.03 mol) and 3.99 g rhodanine in 40 ml pyridine and 5 ml of dist. H_2O. When few crystals appeared, 50 ml of THF was added. Reflux for 1 h. Isolation of the red compound after standing in the fridge. Hydrolyis in 6 g NaOH/100 ml H_2O. After 1 h/50° stirring, neutralization with 45 ml 17% aq. HCl. Red powder, yield 6.7 g (83%).

*(3Z)-3-[Carboxy(methylsulfanyl)methylidene]-2,3-dihydro-2-oxo-1*H-*indole-7-carboxylic Acid* (**6b'**). Yellow needles. IR: 3421*s* (br.), 1669*s*, 1601*s*, 1558*s*, 1507*w*, 1476*m*, 1436*m*, 1375*s*, 1334*m*, 1293*m*, 1241*m*, 1188*m*, 1158*w*, 1084*w*, 860*w*, 782*m*, 750*w*, 733*m*, 712*m*, 668*w*, 628*w*. ^{1}H-NMR ((D_6)DMSO): 6.9 (*d*, *J* = 7.6, 1 H); 6.8 (*d*, *J* = 7.6, 1 H); 6.17 (*t*-like, *J* = 7.6, 1 H); 1.58 (*s*, 3 H). ^{13}C-NMR ((D_6)DMSO): 173.6; 169.0; 166.7; 157.3; 139.7; 128.0; 125.8; 123.7; 121.2; 117.4; 113.7; 14.2.

Indirubin-7-carboxylic Acid (= *(3Z)-2,3-Dihydro-2-oxo-3-(1,3-dihydro-3-oxo-2*H-*indol-2-ylidene)-1*H-*indole-7-carboxylic Acid*; **1g**). Compound **6b** (0.5 g) was suspended in 5 ml of DME together with 3–4 of drops of CF_3COOH, and 0.35 g (2 equiv.) freshly dist. aniline was added. A yellow precipitate formed, and 0.5 g of $ZnCl_2$ was added. The mixture was properly closed with plastic septum and placed in a microwave oven (heating 2 min). The temp. reached 170° and 7 bar. After cooling, the green liquid and black solid were dissolved in 25 ml of H_2O with 0.8 g of NaOH. After standing for 10 min and filtering, the dark soln. was acidified with 17% aq. HCl. A beige precipitate formed, which was isolated by suction and dried. Yield 0.28 g. This material was cyclized with 5 g of PPA at 110° for 45 min. The hot melt was poured into 100 ml of dist. H_2O. A red precipitate was isolated, which was collected by filtration and purified by dissolving in dil. aq. NaOH, and reprecipitated with 17% aq. HCl. Yield: 210 mg. IR: 3397*s*, 3322*s*, 1686*s*, 1654*m*, 1637*m*, 1607v*s*, 1585*s*, 1480*m*, 1466*s*, 1442*m*, 1393*w*, 1348*w*, 1311*s*, 1267*m*, 1219*m*, 1178v*s*, 1156v*s*, 1093*w*, 992*s*, 876*w*, 797*w*, 775*w*, 749*m*, 709*w*, 686*w*, 637*w*, 602*w*, 576*w*. ^{1}H-NMR ((D_6)DMSO): 11.06 (*s*, 1 H); 10.27 (*s*, 1 H); 8.94 (*d*, *J* = 8.2, H–C(4′)); 7.74 (*d*, *J* = 8.9, H–C(6′)); 7.65 (*d*, *J* = 7.5, H–C(4)); 7.57 (*t*-like, *J* = 8.2, H–C(6)); 7.36 (*d*, *J* = 7.5, H–C(7)); 7.11 (*t*-like, *J* = 7.5, H–C(5′)); 7.02 (*t*-like, *J* = 7.5, H–C(5)). ^{13}C-NMR ((D_6)DMSO): 189.5; 171.3; 167.8; 153.0; 141.9; 140.0; 138.2 (C(5)); 130.3 (C(6′)); 128.9 (C(4′)); 125.3 (C(7)); 123.3 (C(6)); 122.5 (C(5′)); 121.8; 119.6; 114.2 (C(4)); 112.1; 105.16. CI-MS: 307 (8), 306 (39), 288 (27), 263 (11), 262 (62), 261 (6), 260 (23), 248 (3), 234 (34), 206 (13), 205 (25), 204 (19), 192 (10), 191 (78), 164 (7), 163 (61), 162 (12), 145 (50), 144 (67), 135 (16), 119 (100), 118 (12), 117 (50), 116 (17).

The residue of this acid/base treatment, recrystallized from acetone, was *methyl (3Z)-2-oxo-3-(3-oxo-1,3-dihydro-2*H-*indol-2-ylidene)-2,3-dihydro-1*H-*indole-7-carboxylate* (**1g'**). ^{1}H-NMR ((D_6)DMSO): 11.1 (*s*, 1 H); 10.52 (*s*, 1 H); 8.96 (*d*, *J* = 7.35, 1 H); 7.73 (*d*, *J* = 8.6, 1 H); 7.62 (*d*, *J* = 7.4, 1 H); 7.55 (*t*-like, *J* = 8.6, 1 H); 7.39 (*d*, *J* = 8.6, 1 H); 7.11 (*t*-like, *J* = 7.4, 1 H); 7.01 (*d*, *J* = 7.4, 1 H); 3.85 (*s*, 3 H). CI-MS: 321 (19), 320 (100), 289 (11), 288 (38), 263 (12), 262 (59), 261 (10), 260 (26), 234 (21), 205 (13.4).

*(2Z)-2-(5-Chloro-1,2-dihydro-2-oxo-3*H-*indol-3-ylidene)-2-(sulfanyl)ethanoic Acid* (**6c**). From 5-chloroisatine (4.5 g, 0.024 mol), 3.6 g of rhodanine, in 40 ml of pyridine, as described for **6a**. Reflux with 50 ml of THF, hydrolysis with 5.66 g of KOH in 75 ml of dist H_2O, and then neutralization by dropwise addition of 12–15% aq. HCl (*ca.* 45–50 ml) afforded the product. Sometimes sticky material was isolated from the mother liquid. This was stirred for some h in neutral dist. H_2O, whereby crystalization took place. Yield: 5.12 g (81%). Red powder. *S*-Me derivative (**6c'**): IR: 3418 (br.), 1684*s*, 1568v*s*, 1469*s*, 1429*w*, 1364*s*, 1300*s*, 1257*w*, 1228*m*, 1192*m*, 1174*m*, 1109*w*, 1085*s*, 957*w*, 923*w*, 853*w*, 804*m*, 778*m*, 724*w*, 685*w*, 659*m*, 617*s*, 592*w*, 559*w*. ^{1}H-NMR ((D_6)DMSO): 10.23 (*s*, 1 H); 7.49 (*d*, *J* = 1.9, 1 H); 7.1 (*dd*, *J* = 1.9, 8.3, 1 H); 6.75 (*d*, *J* = 8.3, 1 H); 2.46 (*s*, 3 H). ^{13}C-NMR ((D_6)DMSO): 165.6; 163.8; 163.2; 138.2; 126.2; 125.7; 124.3; 121.9; 110.9; 109.9; 14.9. FAB-MS: 270 (0.83), 271 (0.17), 272 (0.36), 273 (0.18). CI-MS: 241 (9), 240 (9), 239 (22), 228 (18), 227 (40), 226 (54), 225 (100), 224 (3), 210 (12), 206 (6), 196 (3), 194 (11), 192 (27), 180 (20), 179 (4), 178 (34), 164 (18), 163 (2).

*(2Z)-(5-Chloro-1,2-dihydro-2-oxo-3*H-*indol-3-ylidene)[(4-chlorophenyl)amino]ethanoic Acid* (**7h**). From **6c** (1.2 g) in 10 ml of DME, adding 2–3 drops CF_3COOH, 1.27 g of *p*-chloraniline and 0.68 g of

anh. $ZnCl_2$. Reflux for 8 h and workup yielded 0.68 g (40%) of **7h** (1.5:1 (*Z*)/(*E*)-mixture). ^{1}H-NMR ((D_6)DMSO; major isomer): 11.5 (*s*, 1 H); 10.8 (*s*, 1 H); 7.45 (*d*, *J* = 7.4, 2 H); 7.27 (*d*, *J* = 7.4, 2 H); 7.19 (*s*, 1 H); 7.08 (*d*, *J* = 7.3, 1 H); 6.9 (*d*, *J* = 7.3, 1 H). ^{13}C-NMR ((D_6)DMSO): 170.7 (C=O, major isomer); 164.2 (C=O, minor isomer); 149.2 (NC=O, major isomer); 145.2 (NC=O, minor isomer); 137.7 (major isomer); 135.8 (minor isomer); from DEPT-90: 129.7 (2 arom. CH, major isomer); 129.0 (2 arom. CH, minor isomer); 125.1; 124.2; 123.0 (2 arom. CH, major isomer); 121.3; 117.9; 117.0 (2 arom. CH, minor isomer); 111.0 (olef. C, major isomer); 105.0 (olef. C, minor isomer); 95.2 (olef. C, major isomer); 94.8 (olef. C, minor isomer).

5,5′-Dichloroindirubin (*=(3Z)-5-Chloro-3-(5-Chloro-1,3-dihydro-3-oxo-2*H*-indol-2-ylidene)-1,3-dihydro-2*H*-indol-2-one*; **1h**). From **7h** (150 mg) in 5 g of PPA, heating for 30 min at 130°. Workup in 100 ml of dist. H_2O. Precipitate was collected, dried, and extracted with acetone. Yield: 146 mg (97%). Pure material, intense purple powder. IR: 3432*s* (br.), 3336*s*, 3300*s*, 2921*s*, 2851*m*, 1674v*s*, 1663v*s*, 1616*s*, 1592*m*, 1539*w*, 1468*s*, 1382*w*, 1293*m*, 1278*m*, 1215*m*, 1172*m*, 1125*w*, 1112*w*, 1018*w*, 983*w*, 889*w*, 823*m*, 816*m*, 775*w*, 714*w*, 668*w*, 653*m*, 592*m*. ^{1}H-NMR ((D_6)DMSO): 11.1 (*s*, 1 H); 11.0 (*s*, 1 H); 8.8 (*s*, 1 H); 7.7 (*s*, 1 H); 7.6 (*dd*, *J* = 2.5, 8.6, 1 H); 7.45 (*d*, *J* = 8.6, 1 H); 7.3 (*dd*, *J* = 2.5, 8.6, 1 H); 6.9 (*d*, *J* = 8.6, 1 H). ^{13}C-NMR ((D_6)DMSO): 187.7; 170.4; 151.1; 139.7; 139.0; 136.6; 128.7; 125.7; 125.1; 123.9; 123.7; 122.8; 120.2; 115.3; 110.8; 106.0. CI-MS: 333 (9), 332 (8), 331 (12), 330 (8), 308 (6), 307 (20), 306 (26), 305 (31), 304 (34), 229 (5), 228 (37), 227 (31), 226 (100), 225 (57), 224 (4).

*(2Z)-2-(7-Chloro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(sulfanyl)ethanoic Acid* (**6d**). 7-Chloroisatine (9 g, 0.05 mol), rhodanin (7.2 g, 0.054 mol), 60 ml of pyridine, 10 ml of dist. H_2O. 180 ml of THF: crystalline precipitate (9 g). From the mother liquor another 4.5 g; total yield: 13.5 g (91%). Hydrolysis: 5.7 g of NaOH in 25 ml of dist. H_2O with 9 g precipitate. At 50–60° after 1 h, a greenish solid appeared. Addition of 5 ml of dist. H_2O, the solid dissolved leaving a dark yellow liquid. After standing in the fridge, a mass of needles crystallized: the sodium salt of **6d**. It was directly used to prepare *(2Z)-(7-chloro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(methylsulfanyl)ethanoic acid* (**6d′**). Yellow needles. IR (KBr): 3437 (br.), 1690*w*, 1568*m*, 1445*w*, 1368*w*, 1330*m*, 1317*m*, 1294*w*, 1240*w*, 1170*m*, 1144*m*, 1084*w*, 1053*w*, 955*w*, 788*w*, 749*w*, 722*m*, 637*w*, 600*w*, 534*m*. ^{1}H-NMR ((D_6)DMSO): 10.5 (*s*, 1 H); 7.5 (*d*, *J* = 7.5, 1 H); 7.11 (*d*, *J* = 8.2, 1 H); 6.93 (*t*-like, *J* = 8.2, 1 H); 2.47 (*s*, 3 H). ^{13}C-NMR ((D_6)DMSO): 165.6; 164.3; 163.0; 137.1; 126.3; 125.8; 121.7; 120.9; 113.1; 111.2; 14.9. CI-MS: 268 (3), 270 (1), 241 (14), 240 (19), 239 (32), 228 (20), 227 (40), 226 (64), 225 (100), 210 (11), 192 (33), 180 (18), 164 (13).

*(2Z)-2-(7-Chloro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2-[(2,3-dimethylphenyl)amino]ethanoic Acid* (**7i**). *(2Z)-(7-Chloro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(methylsulfanyl)ethanoic acid* (**6d′**; 1 g, 3.7 mmol) was suspended with 2,3-dimethylanilin (0.94 g, 7.8 mmol) in 8 ml of AcOH and heated to reflux. After complete dissolution of all solid matter, a yellow precipitate appeared after 2 h. When cold, the liquid was subjected to suction, and the yellow solid was washed with EtOH, and dried. Yield: 760 mg (57%). Yellow powder, ^{1}H-NMR ((D_6)DMSO; 2:1 mixture of isomers): 11.71 (*s*, 1 H, major isomer); 10.6 (*s*, 1 H, minor isomer); 7.6 (*d*, *J* = 8.2, 1 H, major isomer); 7.54 (*d*, *J* = 7.5, 1 H, minor isomer); 7.38 (*d*, *J* = 7.5, 1 H, major isomer); 7.24 (*d*, *J* = 8.2, 1 H, minor isomer); 7.05–6.98 (*m*, *ca.* 3 H); 6.94 (*d*, *J* = 7.5, 1 H, minor isomer); 6.91 (*d*, *J* = 8.2, 1 H, major isomer); 6.81 (*dd*, *J* = 7.5, 7.5, 1 H, major isomer); 2.49–2.51 (*m*, *ca.* 6 H); 2.25 (*s*, 3 H, major isomer); 2.22 (*s*, 3 H, minor isomer). ^{13}C-NMR ((D_6)DMSO; 2:1 mixture of isomers): 171.2 (C=O, major isomer); 165.0 (C=O, minor isomer); 163.8 (C=O, major isomer); 158.9 (C=O, minor isomer); 171.2; 163.8; 137.85; 137.0; 132.3; 127.1; 126.7; 126.1; 125.9; 122.4; 121.8; 121.3; 120.9; 118.8; 116.8; 112.85; 20.4; 13.5. CI-MS: 342 (38), 343 (12), 344 (15), 345 (3), 346 (1), 300 (24), 299 (23), 298 (100), 281 (6), 192 (12), 178 (36), 167 (20).

7-Chloro-6′7′-dimethylindirubin (*=(2Z)-2-(7-Chloro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2-[(2,3-dimethylphenyl)amino]ethanoic Acid*; **1i**). Compound **7i** (342 mg, 1 mmol) cyclized in 27 g of PPA at 120° for 1 h. After workup in 100 ml of dist. H_2O, the precipitate was collected, dried, and extracted with acetone. Yield: 270 mg (83%). Dark red powder. ^{1}H-NMR ((D_6)DMSO): 11.32 (*s*, 1 H); 10.58 (*s*, 1 H); 8.62 (*d*, *J* = 7.8, 1 H); 7.42 (*d*, *J* = 7.8, 1 H); 7.28 (*d*, *J* = 7.8, 1 H); 7.0 (*d*, *J* = 7.8, 1 H); 6.92 (*t*-like, *J* = 8.2, 1 H); 2.3 (*s*, 3 H); 2.17 (*s*, 3 H).

*(2Z)-2-(5-Bromo-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(sulfanyl)ethanoic Acid* (**6e**). *a*) Bromoisatin (13.5 g, 0.06 mol), 7.98 g of rhodanin (0.061 mol) in 70 ml pyridine, induced by adding 3.5 ml of dist. H_2O. When the liquid separated solids (after 30 min), 50 ml of THF was added, and the mixture was

refluxed 2 h. From time to time, 10 ml of THF were added (total 100 ml). After cooling overnight, the red solid was isolated by suction and dried in air. After hydrolysis in 100 ml of dist. H_2O and 9.6 g of NaOH, the dark soln. was kept at 60° for 1 h, whereby a yellow soln. appeared, which was neutralized and acidified with 17% HCl at 0° to pH 1. A bright red powder apperars. Yield: 15.5g (86%).

b) Pyridinium Salt of **6e**. Hydrolysis of 70 g of indigoid (obtained from refluxing equimolar amounts of 5-bromoisatin and rhodanin in AcOH for 8 h) in 120 ml of H_2O and 16 g NaOH at 60° gave a yellow precipitate, which was isolated by suction. Yield: 19 g. ^{1}H-NMR (D_2O): 8.77 (*d*, *J* = 2.0, 1 H); 8.38 (*d*, *J* = 4.1, 2 H); 7.73 (*t*-like, *J* = 4.1, 1 H); 7.4 (*s*, 1 H); 7.31 (*t*-like, *J* = 4.1, 2 H); 7.15 (*dd*, *J* = 2.0, 8.1, 1 H); 6.7 (*d*, *J* = 8.0, 1 H). ^{13}C-NMR (D_2O): 184.8; 179.6; 167.9; 148.9; 138.0; 136.9; 128.9; 127.4; 124.8; 124.1; 114.0; 113.6; 110.7.

*(2Z)-2-(5-Bromo-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2-[(4-methylphenyl)amino]ethanoic Acid* (**7j**). Compound **6e** (5.22 g, 0.017 mol) was mixed with 10 g of molten 4-methylaniline and treated as described for **7a**: 3.6 g (55%) of **7j**. Dull yellow powder. IR: 3376*s* (br.), 3246*s* (br.), 2919*m*, 2857*m*, 1710*s*, 1620v*s*, 1577*m*, 1517*m*, 1474*m*, 1384*w*, 1308*m*, 1240*m*, 1225*s*, 1192*m*, 1116*w*, 1064*w*, 1019*w*, 876*w*, 809*m*, 747*w*, 676*w*, 594*w*, 551*w*. ^{1}H-NMR ((D_8)THF, *ca.* 2 : 1 mixture of isomers): 11.7 (*s*, 1 H, major isomer); 11.6 (minor isomer); 9.8 (*s*, 1 H, major isomer); 9.7 (minor isomer); 7.4 (*s*, 1 H, major isomer); 7.3 (major isomer); 7.15 (*m*, 5 H); 7.12 (minor isomer); 7.0 (minor isomer); 6.85, 6.8 (*d*, 1 H, major isomer); 6.7 (minor isomer); 2.3 (*s*, 3 H, major isomer); 2.2 (minor isomer). ^{13}C-NMR (D_2O):172.0 (minor isomer); 171.6; 164.8 (minor isomer); 164.5; 148.1; 146.8 (minor isomer); 137.3 (minor isomer); 137.1 (minor isomer); 135.7; 130.6; 130.5; 130.2; 127.3 (minor isomer); 125.6; 125.0; 122.1; 121.8 (minor isomer); 121.7; 121.2; 119.3; 114.0; 111.2; 109.8; 97.5 (minor isomer); 96.0; 30.5 (minor isomer); 24.1. CI-MS: 374 (1), 330 (17), 294 (3), 284 (10), 213 (25), 211 (23), 183 (6), 147 (14), 107 (19).

*(2Z)-2-(5-Bromo-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-1,2-dihydro-3*H*-benzo[g]indol-3-one* (**1k**). Obtained from **6e** (852 mg) and naphthalen-1-amine (858 mg) in 10 ml of DME/402 mg of $ZnCl_2$, after 18 h reflux. After workup with 50 ml of dist. H_2O/0.5 g NaOH and acidification, yellow powder (662 mg) was obtained. Alternatively, a mixture of above mentioned compounds was properly shaken or stirred in an appropriate reagent tube closed with a plastic septum and subjected to microwave conditions for 2 min. Temp. reached 170° at 7 bar. Yield was identical compared to thermal treatment. This material without further workup was cyclized in 20 g of PPA at 120°. The product contained two isomers in a ratio of *ca.* 3 : 1. TLC showed a non-fluorescent bluish major isomer at R_f 0.3, another compound **14** at R_f 0.1, which was fluorescent in an orange tone. The precipitate resulting from the workup in H_2O after drying in air was extracted with acetone for 1 h, only to exploit bad solubility of the minor isomer. Then, extraction was terminated, and the solvent was stripped off: pure **1k**. Yield: 132 mg. Dark powder. IR: 3433*m*, 2922*m*, 2851*m*, 1692*sh*, 1665v*s*, 1631*s*, 1618*s*, 1577*s*, 1529*s*, 1464*s*, 1445*s*, 1421*m*, 1389*m*, 1324*m*, 1284*m*, 1201*s*, 1175*s*, 1140*w*, 1116*w*, 1007*w*, 979*w*, 878*w*, 805*w*, 761*m*, 711*w*, 672*w*, 647*w*, 540*w*. ^{1}H-NMR ((D_6)DMSO): 11.12 (*s*, 1 H); 11.08 (*s*, 1 H); 9.0 (*s*, 1 H); 8.25 (*d*, *J* = 8.6, 1 H); 8.0 (*d*, *J* = 8.6, 1 H); 7.74 (*t*-like, *J* = 7.4, 1 H); 7.66 (*t*-like, *J* = 7.4, 1 H); 7.62 (*d*, *J* = 7.4, 1 H); 7. 52 (*d*, *J* = 7.4, 1 H); 7.45 (*d*, *J* = 7.4, 1 H); 6.89 (*d*, *J* = 7.4, 1 H). ^{13}C-NMR ((D_6)DMSO): 187.2; 170.8; 152.3; 140.4; 139.3; 137.8; 132.1; 130.6; 128.6; 127.1; 126.9; 122.6; 121.6; 119.6; 119.4; 113.7; 113.4; 111.6; 110.8; 107.6.

*(2Z)-2-(5-Fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(sulfanyl)ethanoic Acid* (**6f**). Obtained from 5-fluorisatin (2.57 g, 0.015 mol) with rhodanin (2.0 g) in 10 ml of pyridine, and 10 drops dist. H_2O. After solidification, addition of 15 ml of THF, and reflux for 1 h, all solvents were evaporated *in vacuo*. The solid was hydrolyzed in 55 ml of H_2O/2.4 g of NaOH. After stirring for 1 h, the soln. was neutralized with 50 ml of dil. HCl at 0°. The red solid was hygroscopic and oily, and needed to be dried *in vacuo*. Otherwise, stirring the sticky solid overnight in 20 ml dist. H_2O resulted in a red powder. Yield: 3.28 g (91%).

*(2Z)-(5-Fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2-(methylsulfanyl)ethanoic Acid* (**6f′**). IR: 3418 (br.), 1684*s*, 1568v*s*, 1469*s*, 1429*w*, 1364*s*, 1300*s*, 1257*w*, 1228*m*, 1192*m*, 1174*m*, 1109*w*, 1085*s*, 957*w*, 923*w*, 853*w*, 804*m*, 778*m*, 724*w*, 685*w*, 659*m*, 617*s*, 592*w*, 559*w*. ^{1}H-NMR ((D_6)DMSO): 10.23 (*s*, 1 H); 7.49 (*dd*, ^3J(H,F) = 9.8, ^5J(H,H) = 2.5, 1 H); 7.1 (*dt*, ^3J(H,F) = 8.7, ^3J(H,H) = 8.7, ^4J(H,H) = 2.5, 1 H); 6.75 (*dd*, ^4J(H,F) = 4.9, ^3J(H,H) = 8.6, 1 H); 2.46 (*s*, 3 H).

*(2Z)-2-(5-Fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-1,2-dihydro-3*H*-benzo[de]quinolin-3-one* (**15**). Obtained from **6f** (930 mg), naphthalen-1-amine (1.1 g), and $ZnCl_2$ (0.52 g) in 5 ml of DME. The

mixture of the above mentioned compounds was properly shaken or stirred in an appropriate reagent tube closed with a plastic septum and subjected to microwave conditions for 2 min. The temp. reached 170° at 7 bar. Workup in 50 ml of H_2O/0.5 g NaOH and neutralization with dil. HCl at 0° yielded the crude material: 1.2 g. This material (738 mg) was dissolved in 15 ml of H_2O/1 g of NaOH, filtered, and reprecipitated by addition of dil. HCl. Yield: 440 mg. This material was cyclized in 10 g of PPA. After workup in 250 ml of H_2O, the dark insoluble residual material was collected by filtration. After drying, the filter was extracted in a *Soxhlet* with acetone. Yield: 390 mg. TLC showed presence of a minor isomer. Chromatography of 50 mg of this material with 80 g of *Kieselgel G60* with hexane/acetone 3:1 as eluent provided a deep red eluate, which was concentrated and subjected to prep. HPLC (*Kieselgel G60*, column diameter, 2.5 cm; length, 25 cm; eluent: heptane acetone 1:1; at 12 ml/min; $\lambda_{monitor}$ at 400 and 500 nm). An impurity was eluted at 6.5 min and discarded. The minor non-fluorescent isomer **1l** eluted at 8.9 min and the major product at 14 min. Fractions were collected and evaporated *in vacuo*. Yield of fluorescent **15**: 38 mg. IR: 3477*s* (br.), 3366*s*, 3250*m*, 2926*w*, 1645*s*, 1626*s*, 1573v*s*, 1555*w*, 1528*m*, 1502*w*, 1479*w*, 1467*w*, 1399*m*, 1365*w*, 1349*w*, 1310*m*, 1279*m*, 1260*s*, 1184*w*, 1133*w*, 969*w*, 893*w*, 802*w*, 787*w*, 749*w*, 683*w*, 624*w*, 585*w*. ^{1}H-NMR ((D$_6$)DMSO): 12.14 (*s*, 1 H); 11.36 (*s*, 1 H); 10.25 (*d*, J = 8.6, 1 H); 9.39 (*dd*, ^3J(H,F) = 13.5, ^5J(H,H) = 2.5, 1 H); 8.85 (*d*, J = 8.6, 1 H); 8.76 (*d*, J = 7.4, 1 H); 7.8 (*t*-like, J = 8.6, 1 H); 7.62 (*d*, J = 7.4, 1 H); 7.39 (*dd*, ^4J(H,F) = 4.9, ^3J(H,H) = 8.6, 1 H); 7.33 (*ddd*, ^3J(H,F) = 8.6, ^3J(H,H) = 8.6, ^4J(H,H) = 2.45, 1 H); 6.85 (*d*, J = 8.6, 1 H). CI-MS: 302 (97), 301 (3).

*(2Z)-2-(5-Fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)-2,3-dihydro-1*H*-benz[*e*]indol-1-one* (**1m**). Obtained from **6f** (418 mg, 1.75 mmol) and naphthalen-2-amine (500 mg, 3.5 mmol) in 5 ml of DME, traces of CF_3COOH, and 470 mg of $ZnCl_2$. After heating under microwave condition (160°, 2 min) or 8 h conventional reflux the soln. was added to 50 ml of dist. H_2O/600 mg of NaOH. The solid residue was extracted once more with fresh base. After filtration from the residue, the filtrate was acidified: Yellow amorphous solid. Yield: *ca.* 220 mg. This solid was cyclized in 15 g of PPA at 110°/1h. The melt was dissolved in 150 ml of H_2O, whereby brown solid remained. After filtration, the dry filter paper was washed with acetone to give a pink soln. Yield of the amorphous product: 200 mg. According to TLC, there was a certain amount of naphthalen-2-amine in the solid. Chromatography with 90 g of *Kieselgel G60* (column, 4 cm diameter; eluted with hexane/acetone 1:1). Up to 140 ml of eluate, traces of unknown substances, and naphthalen-2-amine, and then **1m** were eluated. This material was chromatographed once more to remove traces of naphthalen-2-amine. Yield: 30 mg. IR: 3440*m*, 3325*m*, 3223*m*, 3050*m*, 2919*m*, 2851*m*, 1697*m*, 1665*s*, 1680v*s*, 1630*m*, 1580v*s*, 1535*s*, 1465*s*, 1452*s*, 1391*w*, 1331*w*, 1285*w*, 1256*m*, 1236*s*, 1188*m*, 1139*m*, 1085*m*, 1059*w*, 993*w*, 950*m*, 807*m*, 778*w*. ^{1}H-NMR ((D$_6$)DMSO): 11.1 (*s*, 1 H); 10.85 (*s*, 1 H); 8.76 (*dd*, ^3J(H,F) = 10.9, ^5J(H,H) = 3.4, 1 H); 8.66 (*d*, J = 8.0, 1 H); 8.16 (*d*, J = 9.6, 1 H); 7.91 (*d*, J = 8.0, 1 H); 7.69–7.65 (*m*, 2 H); 7.4 (*t*, J = 9.6, 1 H); 7.13 (*ddd*, ^3J(H,F) = 2.9, ^3J(H,H) = 9.04, ^4J(H,H) = 3.9, 1 H); 6.88 (*dd*, ^4J(H,F) = 4.0, ^3J(H,H) = 9.0, 1 H).

*(2Z)-2-(1,2-Dihydro-5-methyl-2-oxo-3*H*-indol-3-ylidene)-2-(sulfanyl)ethanoic Acid* (**6g**). To 5-methylsatin (7.36 g, 0.045 mol) and rhodanin (7.2 g, 0.05 mol), dissolved in 60 ml of pyridine at 50°, 20 ml of dist. H_2O and 100 ml of THF were added, and the soln. was heated at 65° for 2 h. Crystallization overnight at 4°: yield: 13 g. Often hydrolysis with 9.5 g of NaOH in 40 ml of warm H_2O (*ca.* 50°, 1 h), the soln. was stared for a couple of h in a fridge at 0°. From time to time crystals were isolated. Yield: 8 g (67%).

*(2Z)-2-(1,2-Dihydro-5-methyl-2-oxo-3*H*-indol-3-ylidene)-2-(methylsulfanyl)ethanoic acid* (**6g′**). Yellow needles. IR: 3415*s* (br.), 1684*s*, 1591*s*, 1575v*s*, 1484*m*, 1420*w*, 1365*m*, 1311*m*, 1274*w*, 1236*w*, 1210*m*, 1150*m*, 1121*m*, 1080*m*, 803*m*, 752*w*, 731*w*, 670*w*, 618*m*, 588*m*. ^{1}H-NMR ((D$_6$)DMSO): 10.02 (*s*, 1 H); 7.37 (*s*, 1 H); 6.86 (*d*, J = 7.5, 1 H); 6.65 (*d*, J = 7.5, 1 H); 2.44 (*s*, 3 H); 2.25 (*s*, 3 H). ^{13}C-NMR ((D$_6$)DMSO): 166.02; 163.73; 161.0; 137.7; 128.9; 126.5; 124.6; 123.3; 111.25; 108.35; 21.2; 14.7. CI-MS: 221 (3), 220 (7), 219 (34), 206 (10), 205 (100), 204 (15), 190 (30), 186 (33), 172 (57), 162 (13), 160 (11).

*(3Z)-1,3-Dihydro-5-methyl-3-(5-methyl-3-oxo-1-benzothiophen-2(3*H*)-ylidene)-2*H*-indol-2-one* (**12a**). Compound (**6g**, 1.07 g) was dissolved together with 3 g of AcONa in 25 ml of dist. H_2O. At *ca.* 10°, a soln. of 4-methylphenyldiazonium acetate, prepared from 0.7 g of *p*-toluidine in 5 ml of AcOH, and 0.55 g of butyl nitrite, at 0° was added. The pH of the soln. dropped from 8 to *ca.* 4–5. After stirring the precipitate, some grains of $CuCl_2 \cdot 2\,H_2O$, dissolved in 2 ml of dist. H_2O were added. The ice bath was removed, and from time to time K_2CO_3 soln. (total 1 g in 10 ml of H_2O) was added, followed by 5 ml of

MeOH. Gradually N_2 evolved. Then, stirring was continued overnight. After manually removing a layer of brown resin, the soln. was filtered, acidified and the yellow precipitate of *(2Z)-2-(5-methyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-2-[(4-methylphenyl)sulfanyl]ethanoic acid* (**11a**; 800 mg) was isolated by suction. Yellow powder. This material **11a** was cyclized in 10 g of PPA at 95°/30 min. Workup in 250 ml of H_2O and isolation by suction afforded **12a**. Yield: 85%. Dark red powder. IR: 3432 (br.), 3179 (br.), 2916*w*, 2855*w*, 1697*s*, 1670v*s*, 1658*sh*, 1620*m*, 1604*m*, 1578*w*, 1472*s*, 1323*m*, 1306*m*, 1279*m*, 1237*w*, 1216*w*, 1165*w*, 1083*w*, 1058*s*, 1019*w*, 932*m*, 906*w*, 816*w*, 772*m*, 671*w*, 592*w*. ^{1}H-NMR ((D$_6$)DMSO): 10.98 (*s*, 1 H); 8.85 (*s*, 1 H); 7.66 (*s*, 1 H); 7.62 (*d*, *J* = 8.2, 1 H); 7.56 (*d*, *J* = 7.8, 1 H); 7.21 (*d*, *J* = 8.2, 1 H); 6.82 (*d*, *J* = 7.8), 2.36 (*s*, 3 H); 2.32 (*s*, 3 H). CI-MS: 311 (8), 310 (39), 309 (25), 308 (100), 307 (16), 148 (15).

*Sodium (3Z)-3-[Carboxy(sulfanyl)methylidene]-2,3-dihydro-2-oxo-1*H*-indole-5-sulfonate* (**6h**). Obtained from rhodanin (37.5 g, 0.28 mol), sodium isatinsulfonate (43.5 g, 0.17 mol), 400 ml of pyridine, 15 ml of dist. H_2O. Hydrolysis with 200 ml of dist. H_2O and 34 g of NaOH gave a soln. from which only a minor quantitiy of crystals was separated. The mother liquor was diluted with the same volume MeOH and kept for a week at –25°. Crystals were isolated by suction and dried. Yield: 51 g (65%) of **6h**. IR: 3455*s*, 1674*s*, 1627*s*, 1587*w*, 1547v*s*, 1467*m*, 1385*m*, 1302*w*, 1230*m*, 1212*m*, 1191*m*, 1128*w*, 1099*m*, 1061*m*, 1029*m*, 820*w*, 780*w*, 720*w*, 679*w*, 622*m*. ^{1}H-NMR (D$_2$O): 9.5 (*s*, 1 H); 7.44 (*d*, *J* = 8.0, 1 H); 6.9 (*d*, *J* = 8.0, 1 H). ^{13}C-NMR (D$_2$O):179.8 (COOH); 168.4(CONH); 140.3; 135.4; 127.1; 121.9; 118.9; 116.3; 114.1; 109.3.

*(2Z)-2-(1,2-Dihydro-2-oxo-5-sulfo-3*H*-indol-3-ylidene)-2-[(4-methylphenyl)sulfanyl]ethanoic Acid* (**11b**). Sodium salt **6h** (352 mg, 0.9 mmol) was dissolved in 20 ml of dist. H_2O, one or two tiny crystals of $CuCl_2 \cdot 2\ H_2O$ were added, and the soln. was stirred until it turned greenish and clear again; pH was *ca.* 8–9. Separately, 0.6 g of *p*-toluidine was dissolved in 5 ml of dist. H_2O, mixed with 2.5 ml of 50% HBF_4 aq. soln., and cooled to 0°. To this soln., a chilled soln. of $NaNO_2$ (450 mg) was added. After dissolution of all solids, a new mass of solid platelets appears. This *p*-methylphenyldiazonium tetrafluoroborate was isolated by suction and dried in a stream of N_2 some h (yield: 740 mg). A soln. of 267 mg of this diazonium salt (1.2 equiv.) in 5 ml of dist. H_2O was added to the chilled soln. of **6h** prepared above. The soln. became black, but the color faded immediately, and gas evolution started. Stirring was continued at r.t. After stirring overnight, some resin developed, which was filtered off. The soln. was made acidic with 3 ml of dil. aq. HCl, and 10 g of a collector resin *XAD 1600 N* (*Rohm & Haas*) were added. The resin was collected, rinsed with dist. H_2O to pH 7, and dried. The resin was packed in a small column and eluted with MeOH. The yellow soln. contained a major fraction of **11b** as an oily material which was subjected to CC. The total amount of formed **11b** could not be collected from the mixture by this method, even by increasing ionic strength by addition of KCl or some more *XAD*. The pure material was a yellow powder. Yield: 200 mg (50%). IR: 3402v*s*, 1664*s*, 1622v*s*, 1618v*s*, 1577*s*, 1523*m*, 1498*w*, 1463*w*, 1397*m*, 1351*w*, 1300*w*, 1262*m*, 1215*s*, 1174*m*, 1124*w*, 1097*m*, 1036*m*, 1014*m*, 806*w*, 722*w*, 625*m*, 573*w*, 545*m*, 497*w*. ^{1}H-NMR ((D$_6$)DMSO): 11.8 (*s*, 1 H); 10.34 (*s*, 1 H); 7.75 (*s*, 1 H); 7.28 (*d*, *J* = 8.6, 2 H); 7.22 (*d*, *J* = 7.4, 1 H); 7.09 (*d*, *J* = 8.6, 2 H); 6.7 (*d*, *J* = 7.4, 1 H); 2.25 (*s*, 3 H). ^{13}C-NMR ((D$_6$)DMSO): 171.5; 164.3; 157.1; 140.0; 137.15; 135.0; 132.4; 129.5 (*p*-Ar); 123.6; 120.1; 119.5 (*p*-Ar′); 116.15; 106.9; 91.0; 20.4(Me). CI-MS: 392.9 (3), 391.9 (23), 390.9 (100), 278.9 (20), 246.9 (23).

*(3Z)-2,3-Dihydro-3-(5-methyl-3-oxo-1-benzothiophen-2(3*H*)-ylidene)-2-oxo-1*H*-indole-5-sulfonic Acid* (**12b**). Obtained from **11b** (250 mg) in PPA (15 g) at 110°. After hydrolysis in 500 ml of dist. H_2O, 25 g of NaCl were added, the liquid was then extracted with butan-2-one in a liquid–liquid extraction apparatus. Solvent was evaporated and the residual red dye was crystallized from BuOH by evaporation in a glass dish. Alternatively, the diluted soln. was subjected to solid-phase extraction with 10 g of *XAD 1600 N* resin (*Rohm & Haas*) and stirring for 2 h. The resin was collected by filtration, washed to pH 7, whereby the red dye leaked out again, so it was stopped. The dried resin was packed in a small column and extracted with MeOH. The red soln. was evaporated *in vacuo*. Purification by CC over *Kieselgel G 60* (40 g), in a column of 4-cm internal diameter; solvent: MeOH/acetone 1:4. IR: 3371v*s* (br.), 2923*w*, 2852*w*, 1693*m*, 1658v*s*, 1613*s*, 1470*m*, 1306*m*, 1198*s*, 1099*s*, 1059*m*, 1030*s*, 912*m*, 821*w*, 770*w*, 725*w*, 619*m*, 539*m*, 401*w*. ^{1}H-NMR ((D$_6$)DMSO): 11.12 (*s*, 1 H); 9.38 (*s*, 1 H); 7.70–7.67 (*m*, 2 H); 7.6 (*d*, *J* = 8.6, 1 H); 7.55 (*d*, *J* = 7.4, 1 H); 6.86 (*d*, *J* = 8.6, 1 H); 2.38 (*s*, 3 H).

*(2Z)-(1,2-Dihydro-7-methoxy-4-methyl-2-oxo-3*H*-indol-3-ylidene)(sulfanyl)ethanoic Acid* (**6i**). 4-Methyl-7-methoxyisatin (6.36 g, 0.33 mol) was dissolved together with rhodanin (4.5 g, 0.34 mol) in

50 ml of AcOH. AcONa (4 g) was melted to dryness and added. The mixture was refluxed for 8 h. A dark solid formed, which was isolated by suction and washed with dist. H_2O. Yield 6.42 g (64%). Hydrolysis with 12 g of NaOH in 75 ml of dist. H_2O at 60° for 4 h, stirring overnight at r.t., and neutralizing with conc. HCl to pH 1 yielded a solid, which was stirred for some hours, whereby a red cristalline powder formed. Yield 5.6 g (68%).

*(2*Z*)-2-(1,2-Dihydro-7-methoxy-4-methyl-2-oxo-3*H*-indol-3-ylidene)-2-(methylsulfanyl)ethanoic Acid.* Yellow to green needles. ^{1}H-NMR ((D$_6$)DMSO): 6.72 (*d*, *J* = 8.3, 1 H); 6.58 (*d*, *J* = 8.3, 1 H); 3.73 (*s*, 3 H); 2.4 (*s*, 3 H).

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