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Synthesis and Characterization of a Multi Ring-Fused 2-Pyridone-Based Fluorescent Scaffold

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A series of compounds based on a novel fluorescent scaffold have been synthesized. Most of the compounds displayed high quantum yields of fluorescence and unusually long fluorescence lifetimes. HeLa cells were treated with one of the compounds and its use as a fluorescent dye was demonstrated with fluorescence confocal microscopy.

Introduction

Small organic compounds with fluorescent properties have found wide application in molecular biology, e.g. as DNA-staining dyes or as intracellular probes for calcium ion concentrations. Fluorescent reporter molecules can also be covalently linked to proteins, antibodies, or known inhibitors of a specific target, to give information about cellular distribution and mode of action.[1] However, linking a fluorescent probe to a small inhibitor can significantly alter its biological properties. Here, a novel fluorescent 2-pyridone-based scaffold is presented. In addition to function as a fluorescent probe, the scaffold can easily be substituted in various ways, potentially allowing the fluorophore itself to interact with different biological targets.

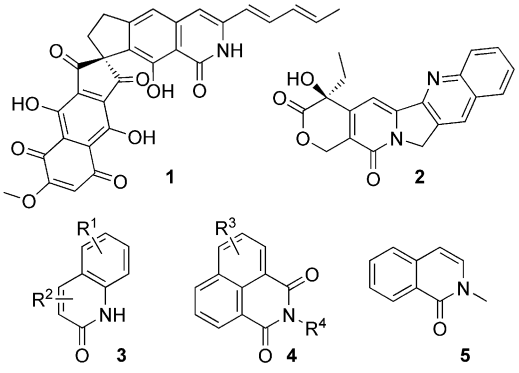

Figure 1. Examples of naturally occurring cytotoxic compounds (**1**, **2**), and fluorescent probes (**3**–**5**), containing 2-pyridone-like motifs.

There exists many naturally occurring ring fused 2-pyridones with interesting biological activity, e.g. fredericamycin A,[2] **1**, and camptotecin,[3] **2**, which both are antitumor agents (Figure 1). Carbostyrils,[4] **3**, and naphthalimides,[5] **4**, are well known fluorescent probes with versatile biological applications. Even the simple isoquinolone **5** has fluorescent properties,[6] although not as prominent.

Results and Discussion

The synthesis of bicyclic 2-pyridones like **6a** (Figure 2) has previously been described.[7] By changing the substitution pattern, these compounds become active either as antibacterial agents,[8] or as inhibitors of the formation of Alzheimer's amyloids.[9] The same compounds can also inhibit formation of a bacterial amyloid, curli, which is a virulence factor in Gram-negative bacteria.[10] Now, given the fluorescent structures **3**–**5**, we sought to ring-close the formylated derivative **7a**,[11] to form an extended aromatic scaffold with potential fluorescent properties. A related ring-closure of 4-formyldihydropyridones have been described using $TiCl_4$ in

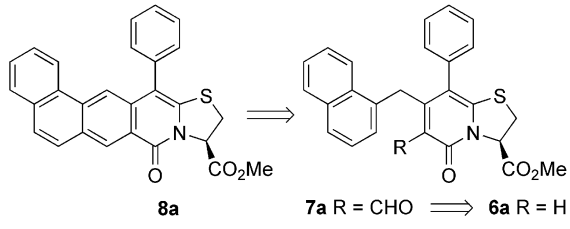

Figure 2. A route to prepare polyaromatic 2-pyridones.

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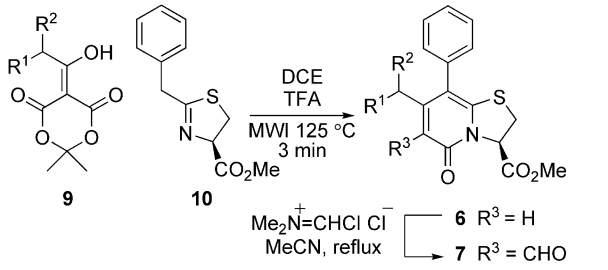
dichloromethane.[12] Initial attempts to transform **7a** under similar conditions were promising, and the generated compound **8a** was indeed fluorescent.

A series of formylated 2-pyridones were synthesized in analogy with **7a** to examine the applicability of the synthetic route for preparation of differently substituted compounds (Table 1). This offers an opportunity to fine-tune both their fluorescent properties and their potential interaction with a biological target.

Table 1. Synthesis of formylated bicyclic 2-pyridones.

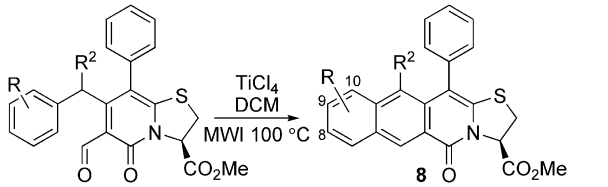


Entry	R^1	R^2	Product	% Yield	Product	% Yield
1	Ph	H	**6b**	88	**7b**	76
2	Ph	Me	**6c**[a]	63	**7c**[a]	41
3	*o*-tolyl	H	**6d**	92	**7d**	62
4	*o*-MeOPh	H	**6e**	96	**7e**	87
5	*p*-FPh	H	**6f**	93	**7f**	69

[a] Obtained as a mixture of diastereomers.

The formylated species **7** were heated with microwave irradiation in the presence of excess $TiCl_4$ to obtain the ring-closed products **8** (Table 2). The reaction worked well for most compounds by using microwave heating at 100 °C for 10–70 min. However, the electron poor fluorobenzyl-substituted compound **7f** proved more difficult to react and required heating at 160 °C for 120 min. These conditions also hydrolyzed the methyl ester, and prior to purification, the crude reaction mixture was re-methylated using TMS chloride in methanol.

Table 2. Ring closure to prepare the fluorescent compounds **8a**–**f**.



Entry	R	R^2	Reaction time [min]	Product	% Yield
1	9,10 ring-fused Ph	H	10	**8a**	79
2	H	H	50	**8b**	89
3	H	Me	20	**8c**	85
4	10-Me	H	20	**8d**	79
5	10-MeO	H	70	**8e**	64
6[a]	8-F	H	120	**8f**	51

[a] Reaction performed at 160 °C. The product was obtained after re-methylation (TMSCl, MeOH) of the initially obtained carboxylic acid.

The fluorescent properties of the polyaromatic compounds **8** were then examined. The absorption and fluorescence spectra of the compounds exhibit similar shapes over an identical wavelength region. The absorption spectra are quite broad, which enables a wide range of excitation wavelengths for measuring fluorescence (Figure 3). The fluorescence spectra cover a substantial part of the visible region. In addition, the small overlap between the lowest absorption transition and the fluorescence spectrum enables the use of relatively high dye concentrations with a negligible probability of reabsorption.

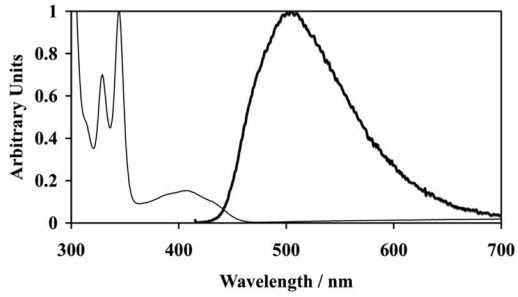


Figure 3. The shapes of the absorption (thin line) and corrected fluorescence spectrum (thick line) of **8b**, when dissolved in glycerol, at 277 K.

With the exception of compound **8a**, the fluorescence quantum yields of are quite high, and the fluorescence lifetimes are unusually long (ca. 8–20 ns) (Table 3). Compound **8a** has a more typical lifetime (ca. 1 ns).[1] The influence of solvent viscosity is not of great importance for the fluorescence lifetimes of these compounds. The fluorescence quenching caused by dissolved O_2 is not dramatically increased, despite the fact that the viscosity is lower by three orders of magnitude in ethanol, as compared to glycerol. Assuming that the O_2 solubility is known (0.56 mmol/L), the quenching constant can be estimated from the measured fluorescence lifetimes of the substances in the presence and absence of dissolved O_2. The obtained values are reasonable,[1] typically in the range of 10^{10}–10^{11} $mol^{-1}\,L\,s^{-1}$. Similar results are observed for these compounds, when dissolved in dichloromethane (data not shown).

Table 3. The quantum yield of fluorescence (Φ_f) and fluorescence lifetime (τ_f) of different polyaromatic 2-pyridones when dissolved in glycerol (g) at 277 K. 1,2-propanediol (p) was chosen as solvent for compound **8a**, due to its poor solubility in glycerol. Ethanol (e) was used to exemplify a solvent of low viscosity at 293 K.

Entry		Φ_f[a]	τ_f [ns][b]	Φ_f[a]	τ_f [ns][b]	τ_f [ns][b,c]
1	**8a**	0.06(p)	1.6(p)	0.11(e)	1.1(e)	1.0(e)
2	**8b**	0.72(g)	17.0(g)	0.44(e)	14.0(e)	19.0(e)
3	**8c**	0.46(g)	11.3(g)	0.29(e)	9.0(e)	11.0(e)
4	**8d**	0.66(g)	17.1(g)	0.38(e)	14.4(e)	19.5(e)
5	**8e**	0.56(g)	14.0(g)	0.34(e)	8.1(e)	10.1(e)
6	**8f**	0.57(g)	16.2(g)	0.27(e)	14.3(e)	17.9(e)

[a] The fluorescence reference used is described elsewhere[13] and λ_{ex} = 410 nm. [b] λ_{ex} = 404 nm. [c] Degassed with argon.

The photo stability of compounds **8a** and **8b** were also investigated. Samples dissolved in chloroform were exposed to high-energy white light (approximately 770 mW/cm^2). It

was found that after 44 min, the absorption spectra of the first two bands of substance **8a** had decreased by 50%. The same effect could be seen after 54 min for sample **8b**. This value can be compared to a half-life of 12 min for a defined and used BODIPY probe dissolved in chloroform, upon exposure to approximately eight times higher light intensity (6 W/cm^2), in the same wavelength range.[14] The BODIPY probe used in the reference has a quantum yield > 95%. For both **8a** and **8b**, the absorbance increased at wavelengths shorter than 300 nm following the photon-bleaching, which is compatible with the generation of smaller aromatic systems produced by photo-degradation. The intensities of the used high-energy light sources are at least one order of magnitude greater than those used in conventional spectroscopic experiments. A similar experiment was performed where two samples containing **8a** and **8b** in chloroform were exposed to white light from a desktop lamp, which exposed the samples to an intensity of approximately 2 mW/cm^2. Then, the absorption decrease due to sample degradation was less than 5% after an exposure time of 24 hours.

The high quantum yields of fluorescence of these compounds facilitate their use as fluorescent markers in biological systems. This was demonstrated by the use of one of the most fluorescent compounds (**8b**) for staining HeLa cells. Fluorescence microscopy shows that the compound was taken up and distributed in almost the entire cell (Figure 4, A–B). The fluorophore appeared to be concentrated in areas rich in microtubule. However, cross staining with antibodies against α-tubulin revealed only partial overlap, with accumulation of the compound around the cellular nucleus (Figure 4, C–E). Neither could any clear effect on microtubule polymerization be detected (see Supporting Information). Nonetheless, this exemplifies the use of this compound as a cell staining dye.

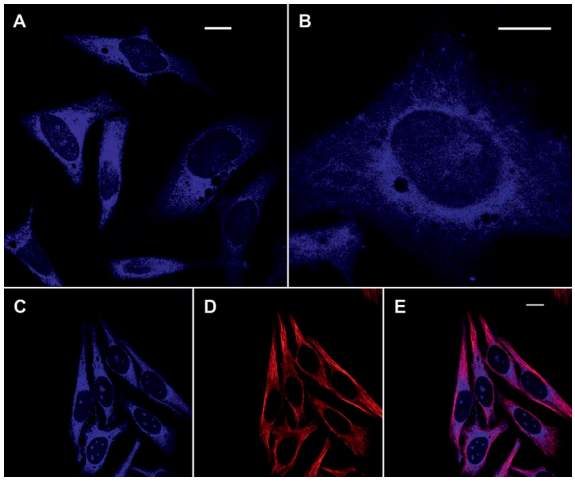


Figure 4. Fluorescence confocal microscopy of HeLa cells. Scale bars are 10 μm. A and B) Cells incubated presence of 100 μM **8b** before fixation with methanol. C) Cells treated with 100 μM **8b** after fixation with methanol. The wavelength used for excitation was 408 nm (violet diode laser). D) α-tubulin stained cells. The excitation wavelength used was 543 nm. E) Picture C and D merged.

In addition to being used as a dye, the scaffold can potentially be used as a fluorescent tag, using the methyl ester as a linking point. However, then the scaffold must show a limited cytotoxicity profile. Hence, the cytotoxic effects of **8b** were evaluated for three different human cell types commonly used in cytotoxicity tests, T-cell leukemia cell line CCRF-CEM, U937 lymphoma cells, and normal peripheral blood mononuclear cells (PBMC). The LD_{50} values (i.e. concentrations leading to 50% survival) after 72 h exposure, was determined to 37 and 23 μM for U937 and PBMC cells, respectively. The LD_{50} for the CCRF-CEM cells was estimated to be > 100 μM (Figure 5). Similar survival data were obtained for cells treated for 24 h. In these rather sensitive cell systems this is considered to be moderate cytotoxic potential, considerably lower than that of, for example, the lipophilic fluorescent probe calcein/AM with a LD_{50} of 0.25 μM in U937.[15] These data indicate that **8b** might be useful as a fluorescent probe in biological systems.

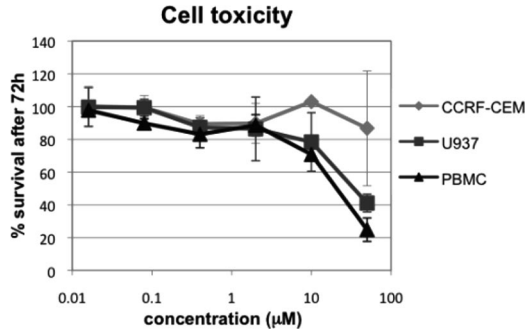


Figure 5. Cell survival after 72 h in presence of compound **8b** at different concentrations. Three different human cell lines were tested: T-cell leukemia cell line CCRF-CEM, lymphoma cell line U-937, and normal peripheral blood mononuclear cells (PBMC). Error bars indicate the standard deviation of duplicates.

Conclusions

Here we present a new scaffold with useful fluorescent properties. The scaffold can hold independently variable substituents, which allows for tuning of its biological properties. A high quantum yield of fluorescence was obtained for most compounds, regardless of the electronic nature of their substituents. Unusually long fluorescence lifetimes were also observed. HeLa cells were stained with one of the synthesized compounds and fluorescence microscopy suggests that the compound is located primarily in the vicinity of microtubules and surrounding the cell nucleus. In addition to functioning as a dye in itself, the limited cytotoxicity of the compound enables its potential use as a fluorescent tag, utilizing the methyl ester as a linking point.

Experimental Section

General: All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. Microwave heated reactions were conducted in sealed Emrys™ process vials with an Initiator™ 2.0 microwave instrument

from Biotage. Temperatures were monitored with an IR probe. TLC was performed on Silica Gel 60 F_{254} using UV light detection. Silica gel chromatography employed normal phase silica gel (60 Å, 230–400 mesh, Merck, grade 9385). Optical rotations were measured with a Perkin–Elmer 343 polarimeter at 20 °C and 589 nm. IR spectra were recorded on a Bruker Equinox 55 spectrometer equipped with an ATR-device. The ^1H and ^{13}C NMR spectra were recorded at 298 K with a Bruker DRX-400 or DRX-360 spectrometer and calibrated using the residual peak of $CHCl_3$ as internal standard ($CHCl_3$ δ_H = 7.26 ppm, $CDCl_3$ δ_C = 77.16 ppm). HRMS was conducted using a Micromass Q-Tof Ultima™ mass spectrometer with electrospray (ES^+) ionization. Polyethylenglycol PEG-400 was used as calibration chemical. LRMS was conducted on a Micromass ZQ mass spectrometer with ES^+ ionization. The absorption spectra were recorded by a Varian Cary, 5000 UV/Vis spectrometer. The fluorescence spectra were transcribed by means of a Fluorolog®-3 (Jobin Yvon) spectrometer equipped with Glan–Thompson polarisers. The bandwidth of the excitation and emission light was 2 nm. All fluorescence spectra were corrected. The time-resolved fluorescence decays were measured by means of the time-correlated single photon-counting technique using a Fluorolog® TCSPC (Horiba) spectrometer. For the pulsed excitation, a NanoLED (Horiba) was used in combination with an interference filter, centred at 398.8 nm (HBW = 11.4 nm). The fluorescence lifetime was calculated by using a deconvolution method[1] based on the Levenberg–Maquardt algorithm.[16] Cell images were aquired using a Nikon e-C1plus fluorescence confocal microscope. The pictures were analysed with Nikon E2-C1 software and processed in Adobe® Photoshop CS4.

General Synthesis of Acyl Meldrum's Acids 9: The carboxylic acid (**I**) to be reacted (1.0 equiv.), Meldrum's acid (1.1 equiv.), and 4-(dimethylamino)pyridine (1.6 equiv.) were dissolved in CH_2Cl_2 (6.0 mL/mmol **I**) and cooled to 0 °C. Dicyclohexylcarbodiimide (1.15 equiv.) in CH_2Cl_2 (1.5 mL/mmol **I**) was added dropwise over 60 min. The reaction was allowed to attain room temp. while stirring overnight. The resulting mixture was cooled to 0 °C, filtered and washed three times with 6% $KHSO_4$(aq.). The combined aqueous phases were extracted once with CH_2Cl_2. The combined organic phases were washed once with brine, dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was taken up in ethyl acetate, which made **9f** precipitate, this precipitate was collected and used without further purification. In all other cases undissolved substance was filtered off and the solvent was concentrated under reduced pressure. The crude products were used without further purification.

Compound **9a** and **9b** was synthesized as described previously.[7,17]

5-(1-Hydroxy-2-phenylpropylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (9c): By following the general procedure for synthesis of acyl Meldrum's acids, 1502 mg (10.00 mmol) racemic 2-phenylpropionic acid was converted to 2732 mg (99%) crude product **9c**. IR: $\tilde{\nu}_{max}$ = 1736, 1662 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 15.70 (br. s, 1 H, OH), 7.46–7.41 (m, 2 H, H_{Ar}), 7.36–7.23 (m, 3 H, H_{Ar}), 5.46 (q, J = 7.1 Hz, 1 H, C*H*CH_3), 1.73 (s, 3 H, CH_3), 1.62 (s, 3 H, CH_3), 1.59 (d, J = 7.1 Hz, 3 H, CHC*H*$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 198.5, 171.0, 160.2, 140.1, 128.7 (2 C), 128.2 (2 C), 127.6, 104.9, 90.8, 42.7, 26.9, 26.6, 18.1 ppm.

5-(1-Hydroxy-2-*o*-tolylethylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (9d): By following the general procedure for synthesis of acyl Meldrum's acids, 1502 mg (10.00 mmol) *o*-tolylacetic acid was converted to 1426 mg (52%) crude product **9d**. IR: $\tilde{\nu}_{max}$ = 1726, 1656 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 15.49 (br. s, 1 H, OH), 7.23–7.11 (m, 4 H, H_{Ar}), 4.49 (s, 2 H, CH_2), 2.33 (s, 3 H, $ArCH_3$), 1.74 [s, 6 H, $C(CH_3)_2$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.3, 170.5, 160.3, 137.0, 132.7, 130.4, 129.9, 127.6, 126.1, 104.9, 91.6, 38.9, 26.7 (2 C), 19.7 ppm.

5-[1-Hydroxy-2-(2-methoxyphenyl)ethylidene]-2,2-dimethyl-1,3-dioxane-4,6-dione (9e): By first following the general procedure for synthesis of acyl Meldrum's acids, 1663 mg (10.01 mmol) *o*-methoxyphenylacetic acid was converted to a crude product that was further purified by dissolving it in CH_2Cl_2 and extract it with saturated $NaHCO_3$(aq.) three times. The aqueous phase was washed once with a small amount of CH_2Cl_2, acidified with aqueous HCl (to pH ≈ 2) and then extracted with CH_2Cl_2. The organic phase was dried with anhydrous Na_2SO_4, filtered and concentrated to 2189 mg (75%) product **9e**. IR: $\tilde{\nu}_{max}$ = 1737, 1655 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 15.37 (br. s, 1 H, OH), 7.29–7.23 (m, 1 H, H_{Ar}), 7.17 (d, J = 7.3 Hz, 1 H, H_{Ar}), 6.94–6.84 (m, 2 H, H_{Ar}), 4.45 (s, 2 H, CH_2), 3.77 (s, 3 H, OCH_3), 1.73 [s, 6 H, $C(CH_3)_2$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.8, 170.5, 160.3, 157.4, 131.0, 128.8, 122.6, 120.5, 110.4, 104.8, 91.4, 55.4, 36.6, 26.6 (2 C) ppm.

5-[2-(4-Fluorophenyl)-1-hydroxyethylidene]-2,2-dimethyl-1,3-dioxane-4,6-dione (9f): By following the general procedure for synthesis of acyl Meldrum's acids, 1546 mg (10.03 mmol) *p*-fluorophenylacetic acid was converted to 1840 mg (65%) crude product **9f**. IR: $\tilde{\nu}_{max}$ = 1738, 1646 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 15.33 (br. s, 1 H, OH), 7.39–7.33 (m, 2 H, H_{Ar}), 7.04–6.96 (m, 2 H, H_{Ar}), 4.38 (s, 2 H, CH_2), 1.71 [s, 6 H, $C(CH_3)_2$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 194.4, 170.6, 162.4 (d, J = 246.0 Hz), 131.4 (d, J = 8.0 Hz, 2 C), 129.8 (d, J = 3.5 Hz), 115.7 (d, J = 21.4 Hz, 2 C), 105.2, 91.5, 40.1, 27.0 (2 C) ppm.

General Procedure for Synthesis of Bicyclic Pyridones 6: Thiazoline **10**[7] (1.0 equiv.) and acyl Meldrum's acid **9** (2.5 equiv.) were dissolved in 1,2-dichloroetane (1.6 mL/mmol **10**). Trifluoroacetic acid (2.0 equiv.) was added and the solution was heated with microwave irradiation for 3 min at 125 °C. The reaction was diluted with CH_2Cl_2, quenched with saturated $NaHCO_3$(aq.), and extracted three times with CH_2Cl_2. The combined organic phases were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified with silica gel chromatography using heptane/ethyl acetate 2:1→1:4 as mobile phase to afford **6** as white foam.

Compound **6a** was prepared as described previously.[7]

(*R*)-Methyl 7-Benzyl-2,3-dihydro-8-phenylthiazolo[3,2-*a*]pyridine-5-one-3-carboxylate (6b): By following the general synthesis of bicyclic pyridones, 708 mg (3.01 mmol) thiazoline **10** and 1974 mg (7.53 mmol) **9b** was converted to 995 mg (88%) **6b**. $[\alpha]_D$ = –166 (c = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1750, 1650 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.31 (m, 3 H, H_{Ar}), 7.21–7.08 (m, 5 H, H_{Ar}), 6.92–6.86 (m, 2 H, H_{Ar}), 6.13 (s, 1 H, 6-H), 5.62 (dd, J = 8.5, 2.5 Hz, 1 H, H_α), 3.81 (s, 3 H, CO_2CH_3), 3.61 (dd, J = 11.7, 8.5 Hz, 1 H, H_β), 3.57 (s, 2 H, $ArCH_2$), 3.42 (dd, J = 11.7, 2.5 Hz, 1 H, H_β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.6, 161.4, 154.6, 146.8, 137.7, 136.3, 130.3, 129.9, 129.1 (2 C), 128.8, 128.7, 128.4 (2 C), 128.3, 126.5, 116.3, 115.5, 63.6, 53.3, 39.6, 31.7 ppm. HRMS: m/z = 378.1167 [M + H], $C_{22}H_{20}NO_3S$ requires 378.1164.

(3*R*)-Methyl 2,3-Dihydro-8-phenyl-7-(1-phenylethyl)thiazolo[3,2-*a*]-pyridine-5-one-3-carboxylate (6c): By following the general synthesis of bicyclic pyridones, 353 mg (1.50 mmol) thiazoline **10** and 1038 mg (3.76 mmol) **9c** was converted to 371 mg (63%) **6c**. $[\alpha]_D$ = –143 (c = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1752, 1649 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.36 (m, 1 H, H_{Ar}), 7.34–7.28 (m, 1 H, H_{Ar}), 7.27–7.22 (m, 1 H, H_{Ar}), 7.20–7.04 (m, 4 H, H_{Ar}), 6.36 and 6.35 (s, 1 H, 6-H), 5.64–5.58 (m, 1 H, H_α), 3.88–3.78 (m, 1 H,

C*H*CH_3), 3.81 and 3.80 (s, 3 H, CO_2CH_3), 3.59 (dd, J = 11.7, 8.6 Hz, 1 H, H_β), 3.42–3.36 (m, 1 H, H_β), 1.40 and 1.39 (d, J = 7.1 Hz, 3 H, CHCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.5, 161.2, 159.1, 146.8 and 146.7 (1 C), 143.8 and 143.7 (1 C), 136.4 and 136.2 (1 C), 130.8, 130.5 and 130.2 (1 C), 129.6, 128.6, 128.6 and 128.5 (1 C), 128.3 (2C, split), 128.2 and 128.1 (1 C), 127.5 (split), 126.3 (split), 116.4 and 116.3 (1 C), 113.2 and 113.1 (1 C), 63.6, 53.2, 41.4 (split), 31.5, 21.6 (split) ppm. HRMS: m/z = 392.1323 [M + H], $C_{23}H_{22}NO_3S$ requires 392.1320.

(*R*)-Methyl 2,3-Dihydro-8-phenyl-7-(*o*-tolylmethyl)thiazolo[3,2-*a*]-pyridine-5-one-3-carboxylate (6d): By following the general synthesis of bicyclic pyridones, 361 mg (1.53 mmol) thiazoline **10** and 1028 mg (3.72 mmol) **9d** was converted to 552 mg (92%) **6b**. $[a]_D$ = –130 (c = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1752, 1650 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43–7.33 (m, 3 H, H_{Ar}), 7.25–7.17 (m, 2 H, H_{Ar}), 7.13–7.04 (m, 3 H, H_{Ar}), 7.02–6.95 (m, 1 H, H_{Ar}), 5.87 (s, 1 H, 6-H), 6.62 (dd, J = 8.7, 2.6 Hz, 1 H, H_α), 3.80 (s, 3 H, CO_2CH_3), 3.63 (dd, J = 11.6, 8.7 Hz, 1 H, H_β), 3.57–3.44 (m, 2 H, $ArCH_2$), 3.42 (dd, J = 11.6, 2.6 Hz, 1 H, H_β), 2.02 (s, 3 H, $ArCH_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.5, 161.3, 154.3, 146.5, 136.4, 136.3, 136.0, 130.3, 130.2, 130.0, 129.7, 128.9 (2C, split), 128.3, 126.9, 126.1, 116.2, 114.7, 63.5, 53.3, 37.3, 31.7, 19.4 ppm. HRMS: m/z = 392.1327 [M + H], $C_{23}H_{22}NO_3S$ requires 392.1320.

(*R*)-Methyl 7-(2-Methoxybenzyl)-2,3-dihydro-8-phenylthiazolo[3,2-*a*]pyridine-5-one-3-carboxylate (6e): By following the general synthesis of bicyclic pyridones, 353 mg (1.50 mmol) thiazoline **10** and 1096 mg (3.75 mmol) **9e** was converted to 589 mg (96%) **6e**. $[a]_D$ = –145 (c = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1752, 1650 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.30 (m, 3 H, H_{Ar}), 7.25–7.19 (m, 2 H, H_{Ar}), 7.17–7.10 (m, 1 H, H_{Ar}), 6.91–6.86 (m, 1 H, H_{Ar}), 6.82–6.77 (m, 1 H, H_{Ar}), 6.74 (d, J = 8.3 Hz, 1 H, H_{Ar}), 5.98 (s, 1 H, 6-H), 5.58 (dd, J = 8.5, 2.5 Hz, 1 H, H_α), 3.77 (s, 3 H, CO_2CH_3), 3.65 (s, 3 H, $ArOCH_3$), 3.63–3.44 (m, 3 H, H_β and $ArCH_2$), 3.38 (dd, J = 11.7, 2.5 Hz, 1 H, H_β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.5, 161.3, 157.1, 154.8, 146.0, 136.4, 130.7, 130.2, 129.8, 128.6 (2C, split), 128.0 (2C, split), 126.2, 120.3, 116.3, 114.6, 110.3, 63.4, 55.1, 53.1, 33.6, 31.5 ppm. HRMS: m/z = 408.1281 [M + H], $C_{23}H_{22}NO_4S$ requires 408.1270.

(*R*)-Methyl 7-(4-Fluorobenzyl)-2,3-dihydro-8-phenylthiazolo[3,2-*a*]-pyridine-5-one-3-carboxylate (6f): By following the general synthesis of bicyclic pyridones, 118 mg (0.50 mmol) thiazoline **10** and 350 mg (1.25 mmol) **9f** was converted to 185 mg (93%) **6f**. $[a]_D$ = –142 (c = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1752, 1651 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.27 (m, 3 H, H_{Ar}), 7.12–7.02 (m, 2 H, H_{Ar}), 6.84–6.72 (m, 4 H, H_{Ar}), 6.06 (s, 1 H, 6-H), 5.58 (dd, J = 8.7, 2.6 Hz, 1 H, H_α), 3.75 (s, 3 H, CO_2CH_3), 3.57 (dd, J = 11.8, 8.7 Hz, 1 H, H_β), 3.50 (s, 2 H, $ArCH_2$), 3.37 (dd, J = 11.8, 2.6 Hz, 1 H, H_β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.3, 161.4 (d, J = 245.0 Hz), 161.1, 154.1, 146.9, 136.0, 133.3 (split), 130.4 (d, J = 8.0 Hz, 2 C), 130.1, 129.7, 128.6 (2C, split), 128.1, 115.8, 115.1, 115.0 (d, J = 21.3 Hz, 2 C), 63.4, 53.1, 38.6, 31.5 ppm. HRMS: m/z = 396.1070 [M + H], $C_{22}H_{19}FNO_3S$ requires 396.1070.

General Procedure for Formylation of 6: Pyridone **6** (1.0 equiv.) was added to a mixture of Arnold's reagent (4.0 equiv.) in acetonitrile (25 mL/mmol **6**). (The Arnold's reagent were either freshly prepared or used as the commercially available salt.) The mixture was then refluxed for 3 h. If necessary, (judged by LC-MS), additional Arnold's reagent was added and the = mixture was further refluxed until **6** was completely consumed. The solvent was removed under reduced pressure and the residue was taken up in CH_2Cl_2 and washed with saturated $NaHCO_3$(aq.). The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified with silica gel chromatography using heptane/ethyl acetate 4:1→1:4 as mobile phase to afford **7** as yellow foam.

Preparation of Arnold's Reagent in Acetonitrile: Oxalyl chloride (1.0 equiv.) was added dropwise to DMF (1.0 equiv.) in acetonitrile (6.25 mL/mmol dmF). The solution was stirred 15 min at room temp. and then used directly.

Compound **7a** was prepared as described previously.[11]

(*R*)-Methyl 7-Benzyl-6-formyl-2,3-dihydro-8-phenylthiazolo[3,2-*a*]-pyridine-5-one-3-carboxylate (7b): By following the general procedure for formylation, 283 mg (0.75 mmol) **6b** was converted to 232 mg (76%) **7b**. Four equivalents of freshly prepared Arnold's reagent was used. $[a]_D$ = –284 (c = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1751, 1674, 1639 cm^{-1}. ^{1}H NMR (360 MHz, $CDCl_3$): δ = 10.44 (s, 1 H, CHO), 7.35–7.22 (m, 3 H, H_{Ar}), 7.12–7.04 (m, 3 H, H_{Ar}), 7.03–6.91 (m, 2 H, H_{Ar}), 6.77–6.70 (m, 2 H, H_{Ar}), 5.70 (dd, J = 9.0, 2.6 Hz, 1 H, H_α), 4.35–4.15 (m, 2 H, $ArCH_2$), 3.82 (s, 3 H, CO_2CH_3), 3.64 (dd, J = 12.1, 9.0 Hz, 1 H, H_β), 3.42 (dd, J = 12.1, 2.6 Hz, 1 H, H_β) ppm. ^{13}C NMR (90 MHz, $CDCl_3$): δ = 191.0, 167.9, 162.4, 157.9, 156.5, 137.8, 135.0, 130.3, 130.0, 128.7 (3 C), 128.3 (2 C), 128.0 (2 C), 125.9, 117.9 (2C, split), 64.1, 53.4, 34.9, 31.4 ppm. LRMS: m/z = 406 [M + H], $C_{23}H_{20}NO_4S$ requires 406.

(3*R*)-Methyl 6-Formyl-2,3-dihydro-8-phenyl-7-(1-phenylethyl)-thiazolo[3,2-*a*]pyridine-5-one-3-carboxylate (7c): By following the general procedure for formylation, 230 mg (0.59 mmol) **6c** was converted to 101 mg (41%) **7c**. In total 12 equiv. of commercially available Arnold's reagent was used, **6c** was however never fully consumed. $[a]_D$ = –159 (c = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1752, 1673, 1633 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.37 and 10.33 (s, 1 H, CHO), 7.36–7.29 (m, 1 H, H_{Ar}), 7.28–7.18 (m, 2 H, H_{Ar}), 7.13–6.97 (m, 4 H, H_{Ar}), 6.90–6.82 (m, 2 H, H_{Ar}), 6.56–6.33 (m, 1 H, H_{Ar}), 5.73–5.68 (m, 1 H, H_α), 5.34–5.07 (m, 1 H, C*H*CH_3), 3.87 (s, 3 H, CO_2CH_3), 3.69–3.60 (m, 1 H, H_β), 3.45–3.39 (m, 1 H, H_β), 1.55 and 1.51 (d, J = 7.2 Hz, 3 H, CHCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 191.5 and 191.2 (1 C), 168.2, 164.6 and 164.4 (1 C), 162.3 and 162.2 (1 C), 157.0 and 156.8 (1 C), 142.6 and 142.5 (1 C), 136.1 (split), 130.9 (split), 130.6 and 130.2 (1 C), 128.8 and 128.6 (1 C), 128.5 (split), 128.4, 128.0 (2C, split), 127.0 (2C, split), 125.8 (split), 118.3 and 118.2 (1 C), 117.7 (split), 64.5 (split), 53.7, 38.4 and 37.8 (1 C), 31.4 (split), 17.7 and 17.6 (1 C) ppm. LRMS: m/z = 420 [M + H] $C_{24}H_{22}NO_4S$ requires 420.

(*R*)-Methyl 6-Formyl-2,3-dihydro-8-phenyl-7-(*o*-tolylmethyl)-thiazolo[3,2-*a*]pyridine-5-one-3-carboxylate (7d): By following the general procedure for formylation, 256 mg (0.65 mmol) **6d** was converted to 171 mg (62%) **7d**. In total 9 equiv. of commercially available Arnold's reagent was used. $[a]_D$ = –232 (c = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1755, 1675, 1644 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.43 (s, 1 H, CHO), 7.34–7.18 (m, 3 H, H_{Ar}), 7.06–6.96 (m, 4 H, H_{Ar}), 6.90–6.85 (m, 1 H, H_{Ar}), 6.77–6.72 (m, 1 H, H_{Ar}), 5.77 (dd, J = 9.1, 2.7 Hz, 1 H, H_α), 4.23–3.97 (m, 2 H, $ArCH_2$), 3.89 (s, 3 H, CO_2CH_3), 3.72 (dd, J = 11.8, 9.1 Hz, 1 H, H_β), 3.49 (dd, J = 11.8, 2.7 Hz, 1 H, H_β), 1.86 (s, 1 H, $ArCH_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 191.0, 168.1, 162.5, 158.8, 156.2, 136.7, 136.4, 135.2, 130.2, 129.8 (2 C, split), 128.9, 128.8 (2 C), 126.6, 126.1, 125.3, 118.7, 118.3, 64.2, 53.7, 32.7, 31.6, 19.5 ppm. LRMS: m/z = 420 [M + H], $C_{24}H_{22}NO_4S$ requires 420.

(*R*)-Methyl 7-(2-Methoxybenzyl)-6-formyl-2,3-dihydro-8-phenyl-thiazolo[3,2-*a*]pyridine-5-one-3-carboxylate (7e): By following the general procedure for formylation, 244 mg (0.60 mmol) **6e** was converted to 227 mg (87%) **7e**. In total 18 equiv. of commercially avail-

able Arnold's reagent was used. $[\alpha]_D$ = –276 (*c* = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1752, 1680, 1641 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 10.43 (s, 1 H, CHO), 7.31–7.18 (m, 3 H, H_{Ar}), 7.11–7.04 (m, 1 H, H_{Ar}), 7.01–6.89 (m, 2 H, H_{Ar}), 6.78–6.69 (m, 2 H, H_{Ar}), 6.65 (d, *J* = 8.2 Hz, 1 H, H_{Ar}), 5.73 (dd, *J* = 8.9, 2.9 Hz, 1 H, H_α), 4.26–4.15 (m, 2 H, $ArCH_2$), 3.87 (s, 3 H, CO_2CH_3), 3.67 (dd, *J* = 11.8, 8.9 Hz, 1 H, H_β), 3.55 (s, 3 H, $ArOCH_3$), 3.44 (dd, *J* = 11.8, 2.9 Hz, 1 H, H_β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 190.7, 168.0, 162.1, 158.9, 156.7, 156.0, 135.1, 130.0, 129.7, 128.5 (2C, split), 128.4, 127.9, 127.0, 126.7, 120.1, 118.5, 118.2, 109.8, 64.0, 55.0, 53.4, 31.3, 29.1 ppm. LRMS: *m*/*z* = 436 [M + H], $C_{24}H_{22}NO_5S$ requires 436.

(*R*)-Methyl 7-(4-Fluorobenzyl)-6-formyl-2,3-dihydro-8-phenylthiazolo[3,2-*a*]pyridine-5-one-3-carboxylate (7f): By following the general procedure for formylation, 178 mg (0.45 mmol) **6f** was converted to 131 mg (69%) **7f**. In total 16 equiv. of commercially available Arnold's reagent was used. $[\alpha]_D$ = –225 (*c* = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1752, 1673, 1640 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 10.45 (s, 1 H, CHO), 7.39–7.27 (m, 3 H, H_{Ar}), 7.03–6.98 (m, 1 H, H_{Ar}), 6.96–6.91 (m, 1 H, H_{Ar}), 6.82–6.74 (m, 2 H, H_{Ar}), 6.71–6.64 (m, 2 H, H_{Ar}), 5.73 (dd, *J* = 9.1, 2.9 Hz, 1 H, H_α), 4.31–4.13 (m, 2 H, $ArCH_2$), 3.86 (s, 3 H, CO_2CH_3), 3.70 (dd, *J* = 12.0, 9.1 Hz, 1 H, H_β), 3.47 (dd, *J* = 12.0, 2.9 Hz, 1 H, H_β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 191.4, 168.0, 162.5, 161.3 (d, *J* = 244.5 Hz), 157.9, 156.6, 135.1, 133.5 (split), 130.5, 130.2, 129.9 (2C, split), 129.0 (2C, split), 128.9, 118.1, 117.9, 114.9 (d, *J* = 21.3 Hz, 2 C), 64.2, 53.6, 34.2, 31.5 ppm. LRMS: *m*/*z* = 424 [M + H], $C_{23}H_{19}FNO_4S$ requires 424.

General Procedure for Synthesis of Multicyclic Pyridones 8a–e: A pyridone of general structure **7** (1.0 equiv.) was dissolved in CH_2Cl_2 (10 mL/mmol **7**) 1.0 M $TiCl_4$ in CH_2Cl_2 (2.0 mL/mmol **7**, 2.0 equiv.) was added and the solution was heated with microwave irradiation for 10 (**8a**) or 20 (**8b**–**e**) minutes at 100 °C. Unless full conversion of **7** was indicated by TLC, more 1.0 M $TiCl_4$ in CH_2Cl_2 was added and the mixture was heated with microwave irradiation at 100 °C. The mixture was then quenched with 1 M HCl (aq.) and extracted three times with CH_2Cl_2. The combined organic phases were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified with silica gel chromatography using heptane/ethyl acetate 2:1→1:2 as mobile phase. The products were obtained as yellow foams.

(*R*)-Methyl 10,11-Dihydro-13-phenylnaphtho[1,2-*g*]thiazolo[3,2-*b*]-isoquinoline-8-one-10-carboxylate (8a): By following the general procedure for synthesis of **8**, 124 mg (0.27 mmol) **7a** was converted to 95 mg (79%) **8a**. In total, 2 equiv. of $TiCl_4$ and 10 min of heating were used. $[\alpha]_D$ = –88 (*c* = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1745, 1652 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 9.95–8.90 (m, 1 H, 7-H), 8.44 (s, 1 H, 14-H), 8.29 (d, *J* = 8.3 Hz, 1 H, H_{Ar}), 7.83–7.76 (m, 2 H, H_{Ar}), 7.64 (d, *J* = 8.9 Hz, 1 H, H_{Ar}), 7.61–7.46 (m, 7 H, H_{Ar}), 5.84–5.78 (m, 1 H, H_α), 3.85 (s, 3 H, CO_2CH_3), 3.72–3.64 (m, 1 H, H_β), 3.53–3.47 (m, 1 H, H_β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.0, 161.3, 138.3, 136.6, 135.0 (split), 133.6, 133.1, 130.8, 130.4, 129.8, 129.7, 129.3, 129.1, 129.0, 128.7, 128.4, 127.9, 127.3, 127.2, 126.8, 123.4, 122.6, 117.1, 113.1, 63.1, 53.3, 31.6 ppm. HRMS: *m*/*z* = 438.1170 [M + H], $C_{27}H_{20}NO_3S$ requires 438.1164.

(*R*)-Methyl 2,3-Dihydro-12-phenylbenzo[*g*]thiazolo[3,2-*b*]isoquinoline-5-one-3-carboxylate (8b): By following the general procedure for synthesis of **8**, 61 mg (0.15 mmol) **7b** was converted to 52 mg (89%) **8b**. In total, 5 equiv. of $TiCl_4$ and 50 min of heating were used. $[\alpha]_D$ = –291 (*c* = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1749, 1654 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 9.02 (s, 1 H, 6-H), 8.01 (d, *J* = 8.1 Hz, 1 H, H_{Ar}), 7.73 (d, *J* = 8.2 Hz, 1 H, H_{Ar}), 7.62 (s, 1 H, 11-H), 7.57–7.39 (m, 7 H, H_{Ar}), 5.80 (dd, *J* = 8.0, 2.0 Hz, 1 H, H_α), 3.84 (s, 3 H, CO_2CH_3), 3.68 (dd, *J* = 11.5, 8.0 Hz, 1 H, H_β), 3.49 (dd, *J* = 11.5, 2.0 Hz, 1 H, H_β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.1, 161.6, 137.5, 136.7, 135.7, 134.1, 131.0, 130.7, 130.4, 129.7, 129.4, 129.2, 129.0, 128.4, 128.3, 127.9, 125.9, 122.8, 121.8, 112.8, 62.9, 53.3, 31.6 ppm. HRMS: *m*/*z* = 388.1013 [M + H], $C_{23}H_{18}NO_3S$ requires 388.1007.

(*R*)-Methyl 2,3-Dihydro-11-methyl-12-phenylbenzo[*g*]thiazolo[3,2-*b*]isoquinoline-5-one-3-carboxylate (8c): By following the general procedure for synthesis of **8**, 63 mg (0.15 mmol) **7c** was converted to 51 mg (85%) **8c**. In total, 2 equiv. of $TiCl_4$ and 20 min of heating were used. $[\alpha]_D$ = –215 (*c* = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1749, 1648 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.99 (s, 1 H, 6-H), 8.06–7.96 (m, 2 H, H_{Ar}), 7.61–7.54 (m, 1 H, H_{Ar}), 7.53–7.36 (m, 5 H, H_{Ar}), 7.22–7.15 (m, 1 H, H_{Ar}), 5.85 (dd, *J* = 8.0, 2.1 Hz, 1 H, H_α), 3.84 (s, 3 H, CO_2CH_3), 3.59 (dd, *J* = 11.5, 8.0 Hz, 1 H, H_β), 3.45 (dd, *J* = 11.5, 2.1 Hz, 1 H, H_β), 2.18 (s, 3 H, $ArCH_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.2, 161.7, 141.9, 139.7, 136.3, 131.8, 131.0, 130.2, 130.0 (2C, split), 129.1, 128.9 (2C, split), 128.6, 128.4, 127.7, 125.6, 124.4, 123.6, 112.7, 62.9, 53.4, 31.3, 18.8 ppm. HRMS: *m*/*z* = 402.1170 [M + H], $C_{24}H_{20}NO_3S$ requires 402.1164.

(*R*)-Methyl 2,3-Dihydro-10-methyl-12-phenyl-benzo[*g*]thiazolo[3,2-*b*]isoquinoline-5-one-3-carboxylate (8d): By following the general procedure for synthesis of **8**, 105 mg (0.25 mmol) **7d** was converted to 79 mg (79%) **8d**. In total, 2 equiv. of $TiCl_4$ and 20 min of heating were used. $[\alpha]_D$ = –255 (*c* = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1751, 1655 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.99 (s, 1 H, 6-H), 7.91–7.84 (m, 1 H, H_{Ar}), 7.76 (s, 1 H, 11-H), 7.58–7.43 (m, 5 H, H_{Ar}), 7.39–7.30 (m, 2 H, H_{Ar}), 5.80 (dd, *J* = 7.8, 2.1 Hz, 1 H, H_α), 3.84 (s, 3 H, CO_2CH_3), 3.67 (dd, *J* = 11.6, 7.8 Hz, 1 H, H_β), 3.49 (dd, *J* = 11.6, 2.1 Hz, 1 H, H_β), 2.47 (s, 3 H, $ArCH_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.1, 161.6, 137.4, 136.8, 135.1, 134.3, 134.0, 131.2, 130.7, 130.3, 130.1, 129.1, 128.9, 128.8, 128.3, 127.8, 125.7, 122.4, 118.4, 113.3, 62.9, 53.3, 31.6, 19.4 ppm. HRMS: *m*/*z* = 402.1170 [M + H], $C_{24}H_{20}NO_3S$ requires 402.1164.

(*R*)-Methyl 2,3-Dihydro-10-methoxy-12-phenylbenzo[*g*]thiazolo[3,2-*b*]isoquinoline-5-one-3-carboxylate (8e): By following the general procedure for synthesis of **8**, 109 mg (0.25 mmol) **7e** was converted to 67 mg (64%) **8e**. In total, 8 equiv. of $TiCl_4$ and 70 min of heating were used. $[\alpha]_D$ = –269 (*c* = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1744, 1658 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.96 (s, 1 H, 6-H), 8.01 (s, 1 H, 11-H), 7.59 (d, *J* = 8.3 Hz, 1 H, H_{Ar}), 7.57–7.43 (m, 5 H, H_{Ar}), 7.39–7.32 (m, 1 H, H_{Ar}), 6.79 (d, *J* = 7.6 Hz, 1 H, H_{Ar}), 5.78 (dd, *J* = 8.0, 2.0 Hz, 1 H, H_α), 3.88 (s, 3 H, CH_3), 3.83 (s, 3 H, CH_3), 3.65 (dd, *J* = 11.5, 8.0 Hz, 1 H, H_β), 3.47 (dd, *J* = 11.5, 2.0 Hz, 1 H, H_β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.1, 161.6, 155.3, 137.2, 136.9, 133.6, 132.0, 130.7, 130.4, 129.1 (2C, split), 129.0, 128.5, 128.2, 126.0, 123.1, 121.5, 116.6, 113.4, 105.3, 63.0, 55.6, 53.3, 31.6 ppm. HRMS: *m*/*z* = 418.1119 [M + H], $C_{24}H_{20}NO_4S$ requires 418.1113.

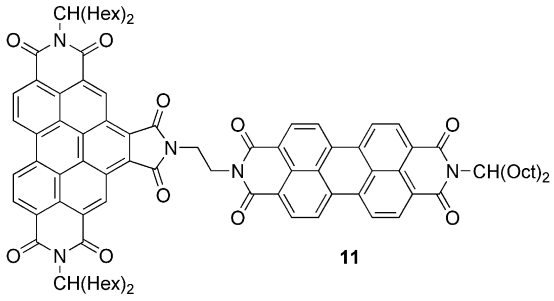
(*R*)-Methyl 8-Fluoro-2,3-dihydro-12-phenylbenzo[*g*]thiazolo[3,2-*b*]-isoquinoline-5-one-3-carboxylate (8f): 64 mg (0.15 mmol) **7f** was dissolved in 1.5 mL of CH_2Cl_2, 1.5 mL of 1.0 M $TiCl_4$ in CH_2Cl_2 (1.5 mmol) was added and the mixture was heated with microwave irradiation for 2 h at 160 °C. The reaction was quenched with 1 M HCl (aq.) and extracted three times with CH_2Cl_2. The combined organic phases were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. As LC-MS indicated significant hydrolysis of the methyl ester of **8f**, the residue was dissolved in 3.2 mL of methanol and 0.56 mL (4.4 mmol) chlorotrimethylsilane was added. The mixture was stirred overnight at room temp. and then concentrated under reduced pressure. The residue was purified with silica gel chromatography using heptane/ethyl acetate

2:1→1:2 as mobile phase. 31 mg (51%) **8f** was obtained as yellow foam. $[\alpha]_D$ = –110 (*c* = 1.0, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1749, 1655 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.94 (s, 1 H, 6-H), 7.76–7.70 (m, 1 H, H_{Ar}), 7.65–7.40 (m, 6 H, H_{Ar}), 7.62 (s, 1 H, 11-H), 7.33–7.26 (m, 1 H, H_{Ar}), 5.79 (dd, *J* = 7.9, 2.2 Hz, 1 H, H_α), 3.84 (s, 3 H, CO_2CH_3), 3.72–3.64 (m, 1 H, H_β), 3.50 (dd, *J* = 11.6, 2.2 Hz, 1 H, H_β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.0, 161.3, 160.5 (d, *J* = 247.8 Hz), 137.5, 136.6, 133.7 (split), 132.9, 131.5 (d, *J* = 9.4 Hz), 130.8, 130.6 (d, *J* = 8.8 Hz), 130.4, 129.3, 129.1, 128.8 (d, *J* = 6.3 Hz), 128.5, 123.6, 123.6, 122.1, 119.5 (d, *J* = 26.3 Hz), 112.7, 111.8 (d, *J* = 20.5 Hz), 63.0, 53.4, 31.6 ppm. HRMS: *m*/*z* = 406.0921 [M + H], $C_{23}H_{17}FNO_3S$ requires 406.0913.

Fluorescence Quantum Yield Determination: The fluorescence quantum yields (Φ) were calculated by comparison with a reference (ref) substance,[1] by means of the equation given below.

$$\Phi = \Phi_{ref} n^2 F\{1 - \exp[-A_{ref}(\lambda_{ex})\ln 10]\}/n_{ref}{}^2 F_{ref}\{1 - \exp[-A(\lambda_{ex})\ln 10]\}$$

F and F_{ref} are the integrated fluorescence spectra of the unknown and the reference sample, respectively. *A* and A_{ref} denote the corresponding absorbance at the excitation wavelength (λ_{ex}), and *n* is the refractive index for the media in which the substances are dissolved. The used refractive indices were 1.47 (glycerol), 1.43 (1,2-propanediol) and 1.42 (dichloromethane). Compound **11** (Figure 6) dissolved in chloroform was used as fluorescence reference sample.[13]



11

Figure 6. Structure of the fluorescence reference compound **11**.

Photo Stability Measurement: The photo stability of compounds **8a** and **8b** were investigated when dissolved in chloroform. The samples were exposed to approximately 770 mW/cm^2, by means of a high intensity Xe-light source. A water-cooled long pass filter was used to selectively remove all wavelengths below 330 nm. In order to prevent evaporation, a water-cooled cuvette holder was used and quartz lenses were implemented to focus the light into the cuvette.

Fluorescence Confocal Microscopy of HeLa-cells Treated with Compound 8b: HeLa cells (DSMZ) were grown in RPMI supplemented with 10% fetal calf serum (PromoCell), 20 mM HEPES and 2 mM L-glutamine. The incubations were performed at 37 °C in the presence of 5% CO_2. HeLa cells were seeded on glass cover slips (Ø = 12 mm) in 24-wells tissue culture plate and incubated for 24 h prior to addition of compound.

HeLa-cells Incubated in Presence of 8b: Medium supplemented with compound **8b** (100 μM compound in 0.5% DMSO), or only 0.5% DMSO, were added to the cells. After 20 h at 37 °C, the cells were washed twice with PBS and fixed in methanol for 5 min. For fluorescence confocal microscopy, DMSO treated cells were used to set the background.

Cross-Staining of α-Tubulin: HeLa cells were grown in the absence of compound **8b** and fixed as described above. α-Tubulin staining was performed overnight (4 °C) with a monoclonal antibody (1:1000 in BSA-PBS). To visualize α-tubulin, a secondary anti-mouse Rhodamine (TRITC)-conjugated antibody (diluted 1:500 in BSA-PBS) was used (incubated at 37 °C for 1 h). Thereafter, cells were stained with compound **8b** (100 μM compound and 0.5% DMSO in PBS) at 37 °C for 1 h and subsequently washed twice in PBS.

Toxicity Evaluation: The fluorometric microculture cytotoxicity assay (FMCA) is a total cell kill assay, based on the ability of cells with intact cell membranes to convert non-fluorescent diacetate (FDA) to fluorescent fluorescein. The details of the method has been presented previously,[18] and briefly, cell suspensions are exposed to varying concentrations of the test substance (in duplicates) in 96-well microtiter plates for 72 h whereafter the cells are washed and FDA is added. Fluorescence is measured after 30 min incubation. The fluorescence is proportional to the number of living cells, and results presented as survival index (fraction of surviving cells vs. untreated control wells). Two different human cell lines were used, the lymphoma cell line U-937 GTB and the T-cell leukemia cell line CCRF-CEM, both of which are considered generally "sensitive" to xenobiotics. In addition normal peripheral blood mononuclear cells (PBMC) from two healthy donors were included in the analysis.

Supporting Information (see also the footnote on the first page of this article): NMR spectra for all new compounds and data from the evaluation of compound **8b** in a microtubule polymerization assay.

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