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4-Cyano-6,7-dimethoxycarbostyrils with Solvent- and pH-Independent High Fluorescence Quantum Yields and Emission Maxima

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Highly fluorescent and stable 6,7-dimethoxy-2-oxoquinoline-4-carbonitriles (**11**) were synthesized starting from appropriate 4-hydroxyquinolones **3** via reactive 4-chloroquinolones **8** by using toluenesulfinates as catalysts. In contrast to the well-described 4-trifluoromethyl-substituted analogues **18**, *N*-substituted derivatives **11** fluoresce in water, polar, and apolar solvents in a narrow 430–440-nm window with almost constant quantum yield of 0.5. Equal excitation is possible in the broad double maximum between 385 and 410 nm yielding identical data between pH 1 and 11. These properties could lead to a broadly usable fluorescence standard. *N*-Alkylation with bromoacetate yields ester **13** in good yields. Reactive succinimidoyl (OSu) ester **15** was prepared by saponification to acid **14**. With amino acids or peptides, linking to labeled derivatives **17** was achieved under mild conditions in slightly basic aqueous media.

Introduction

Luminescence properties of most carbostyrils [quinolin-2(1*H*)-ones] have the disadvantage of shorter absorption and emission wavelengths relative to coumarins.[1] In contrast, carbostyrils have, in general, the important advantage that they are highly stable against chemicals (relative to coumarins[2] or fluorescein-type dyes), thermal and photochemical stress (e.g., relative to azodyes), and they are insensitive to oxygen quenching (e.g., relative to 1,10-phenanthroline complexes). All these properties make them very useful as, for example, fluorescence markers for biological samples. Recently, we reported systematic studies about the fluorescence properties of differently substituted carbostyrils[3] which revealed that suitable structure elements shifted the wavelengths up to 440 and 540 nm for the absorption and emission maxima, respectively.

We were able to show that 6,7-dimethoxy-4-trifluoromethylcarbostyrils with linking groups either at the N-1 or O-6 position[4] reveal very good photophysical properties, such as an absorption maximum close to the visible region ($\lambda \geq 370$ nm), large Stokes' shifts ($\lambda_F \geq 440$ nm), sufficiently high extinction coefficients (ε about 10^4), and high fluorescence quantum yields (Φ_F up to 0.3). The disadvantage of dimethoxy derivatives in terms of slightly shorter absorption and emission wavelengths compared with aminocarbostyrils is counterbalanced by advantages such as largely pH-independent photophysical properties and high chemical stability.

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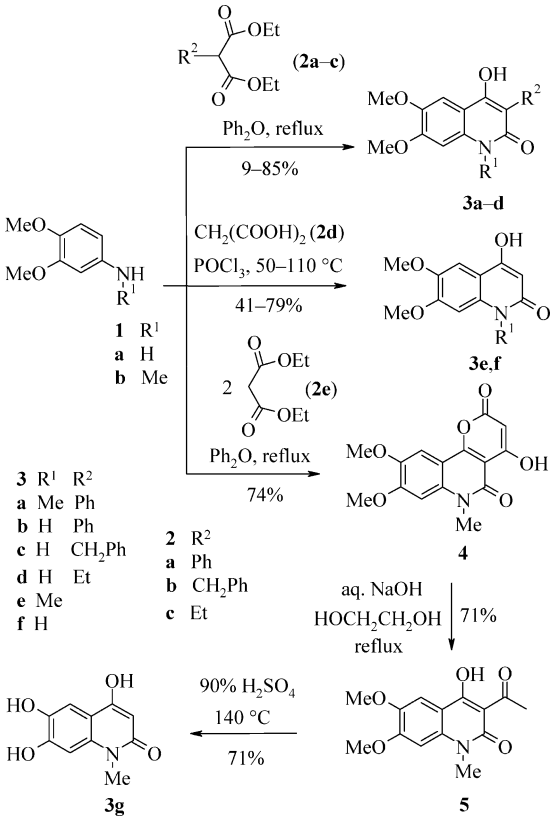
Results and Discussion

Synthesis

Searching for new carbostyril substituents with suitable electronic properties and high photophysical stability, we extended the successful push–pull concept[3] and investigated the properties of 4-cyanocarbostyrils **11**. We had synthesized some 4-cyanoquinoline-2-ones earlier in another project,[5] but they were not further synthetically used because of their low reactivity, properties that now make them in the push–pull modification very useful as fluorescent probes, as they proved to be chemically and photophysically highly stable.

In the literature there is no useful synthesis for 4-cyanocarbostyrils described. So we started the reaction sequence to desired 4-cyanocarbostyrils **11** from 4-hydroxycarbostyrils of type **3**. The synthesis of 3-substituted derivatives **3a**–**d** was easily achieved by using the general procedure described in refs.[6,7] starting from appropriately substituted anilines **1a**,**b** and alkyl- or aryl-substituted malonates **2a**–**c**. For 3-unsubstituted derivatives **3e**,**f** this reaction did not work and so we applied two known approaches: either directly with malonic acid and phosphoryl chloride as a condensation agent[7] or by the three-step pyrono route.[7–9] The direct route offers the advantage of a one-step-reaction, but phosphoryl chloride, which is used as the condensation agent, can react in a subsequent step easily with the 4-hydroxy group and the 2-oxo group of the primarily formed hydroxycarbostyril **3**. To avoid such side reactions, an excess amount of aniline **1** and malonic acid (**2d**) had to be used, which gave desired carbostyrils **3e**,**f** in moderate-to-good yields.

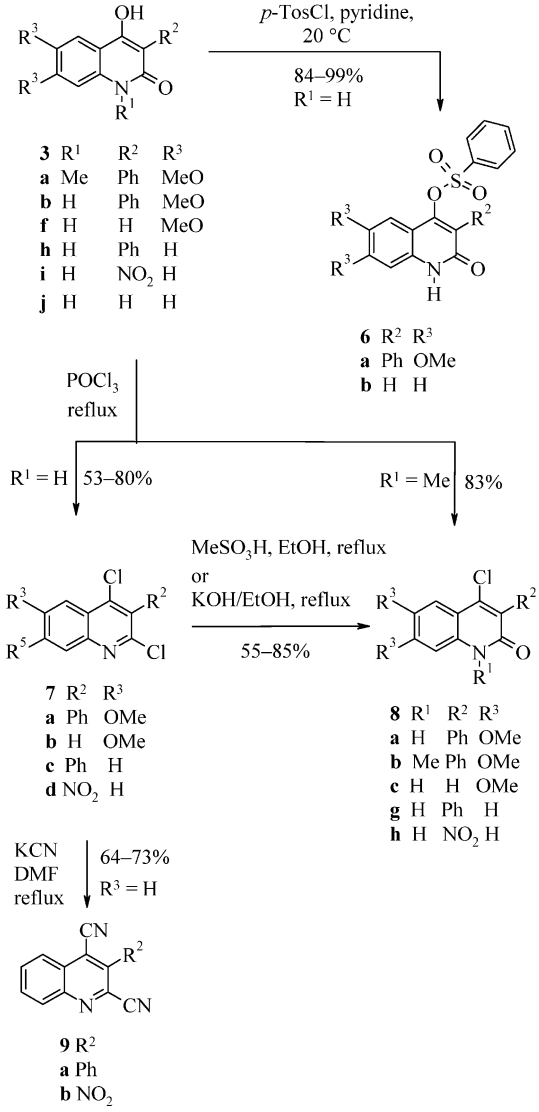
The pyrono route to 3-unsubstituted carbostyrils often gives better results and purer compounds[7–9] but needs a three-step reaction sequence. The first step involves the formation of pyranoquinolone **4** from a 2:1 reaction of diethylmalonate (**2e**) with anilines **1**, followed by a basic ring opening of the pyrone to 3-acetylcarbostyril **5** and then removal of the acetyl group by *ipso*-substitution with 90% sulfuric acid. However, in our case the reaction sequence failed in the third step: in this reaction not only the acetyl group was cleaved, but also double demethoxylation to afford 4,6,7-trihydroxycarbostyril **3g** took place (Scheme 1).



Scheme 1.

The next step in the reaction sequence to 4-cyanocarbostyrils was the introduction of a reactive leaving group in the 4-position. Experiments with 4-tosylates **6a**,**b** revealed that no reaction took place. So we directed our attention to the reactions of 2,4-dichloroquinolines **7** and 4-chlorocarbostyrils **8**, which were prepared by reaction of the corresponding hydroxycarbostyrils **3** with an excess amount of phosphoryl chloride. 1-Unsubstituted 4-hydroxycarbostyrils (such as **3b**,**f**,**h**,**i**) gave 2,4-dichloroquinolines **7a**–**d**, which could be hydrolyzed regioselectively to afford 4-chlorocarbostyrils **8a**,**c**,**g**,**h**. The best reaction conditions were found with 70% methanesulfonic acid in ethanol; alkaline hydrolysis gave significantly lower yields. The reactions of 1-substituted 4-hydroxycarbostyrils such as **3a** with phosphoryl chloride directly gave 4-chlorocarbostyrils **8b**.

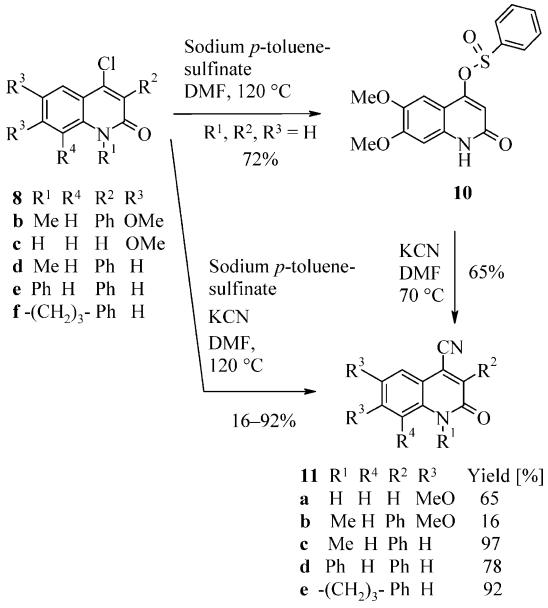
When we applied our findings from studies in the nucleophilic substitution of quinolines and quinolones,[8,10] we found that the introduction of nitrile groups by reaction with sodium or potassium cyanide in suitable organic solvents in the presence of crown ethers failed in most cases. Only with rather reactive dichloroquinolines such as **7c**,**d** did a reaction take place; however, both of the chloro atoms were substituted by cyano groups to form dinitriles **9** (Scheme 2). All attempts to find either conditions for a regioselective monosubstitution or removal of the undesired cyano group in the 2-position failed. The introduction of cyano groups into the corresponding 4-chlorocarbostyrils by these methods failed. Attempts to utilize the Rosenmund–Braun aromatic cyanation[11] with copper(I) cyanide in high-boiling solvents gave a mixture of fluorescent compounds, but separation attempts with chromatographic methods failed.



Scheme 2.

Another method, however, which was described recently,[12] allowed us to introduce the nitrile group at the 4-position of chlorocarbostyrils **8** under rather mild conditions of 120 °C by using sodium or lithium *p*-toluenesulfinate as intermediates in dimethylformamide as the solvent.

Only the reactions of 4-chlorocarbostyrils of type **8** with 3-alkyl- or 3-alkoxy-substituents in the 3-position did not proceed. In ref.[12] the intermediate sulfinates were not isolated. We decided to determine if isolation of the intermediate and a two-step reaction could produce better results; thus, as an example, we prepared sulfinate **10** (Scheme 3) from 4-chlorocarbostyril **8c**, which was then treated with potassium cyanide at 70 °C to afford 4-cyanocarbostyril **11a**. The yields and purity show that there is no advantage for the two-step reaction. So, we synthesized **11** in one step in good-to-excellent yields (65–97%), with the exception of **11b** (16%).



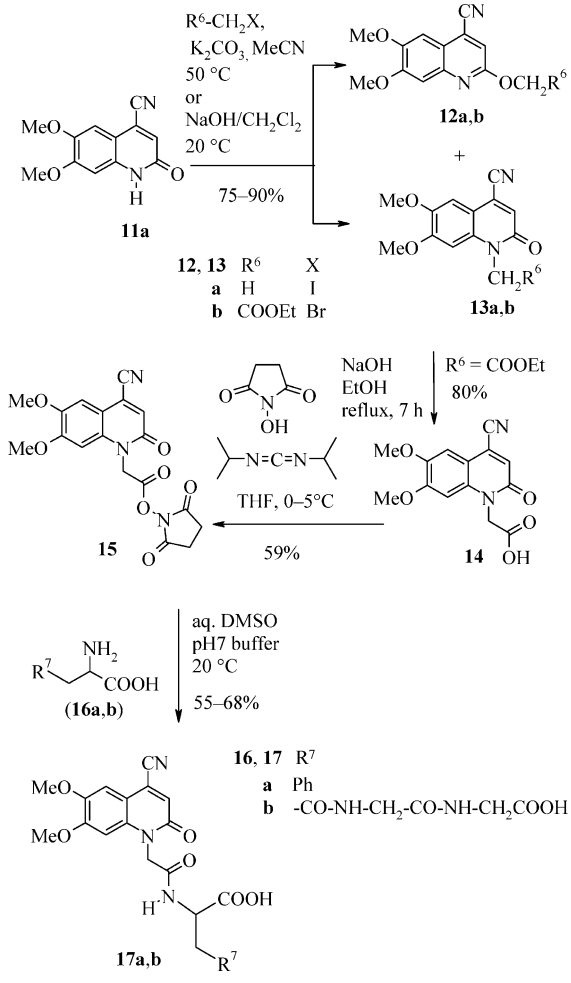
Scheme 3.

Because the yields of *N*-methyl carbostyrils (e.g., **3e**, **11b**) were rather low, we also checked the possibility of later *N*-methylation of **11a** to compare it with the use of *N*-methyl derivatives in earlier stages. Methylation with iodomethane in acetonitrile gave, as expected, in 75% yield a mixture of *O*-methylated and *N*-methylated products **12a** and **13a** (ratio 1:4), respectively, which was separated by dry flash chromatography.[18] So all routes with *N*-methyl derivatives were somewhat restricted in terms of yield; together with some disadvantages in the construction of linkers at O-6 of the carbostyrils,[4b] this route was not further completed.

Recently, we showed that the introduction of linker groups attached at N-1 of 4-trifluoromethylcarbostyrils hardly influenced the photophysical properties regarding wavelengths and Stokes' shifts; however, water as the solvent lowered the fluorescence quantum yields by about 50%[4a]

When we used bromoacetate[4a] in the *N*-alkylation reaction in a binary mixture of sodium hydroxide/water and dichloromethane in the presence of dibenzo-18-crown-6, 4-chlorocarbostyril **11a** afforded a mixture of *O*- and *N*-alkylated products, **12b** and **13b**, respectively, in 90% yield in a 1:5 ratio; the isomers were separated by dry flash chromatography. Hydrolysis of quinolinyl-1-acetate **13b** in aqueous–ethanolic sodium hydroxide gave quinolinyl-1-acetic acid **14** in excellent yield, which was subsequently transformed into reactive succinimidoyl active ester **15** (OSu ester) in dry tetrahydrofuran containing diisopropylcarbodiimide as a water scavenger. Ester **15** is stable in aqueous solution, but highly reactive towards amino groups.[13]

The reaction of OSu ester **15** with phenylalanine (**16a**) proceeded under biochemical conditions (aqueous medium, 15–20 °C) by using aqueous dimethyl sulfoxide as the solvent and an aqueous pH 7 buffer as the base; this reaction afforded amino-linked carbostyril **17a** in 70% yield. In the same manner, the reaction of tripeptide glycyl-glycyl-glycine (**16b**) afforded amino-linked carbostyril **17b** in 55% yield (Scheme 4).



Scheme 4.

Electronic Spectra

Figure 1 shows the normalized excitation and emission spectra of *N*-methyl derivative **13a** and the previously published[4b] trifluoromethyl analogue **18** in water. Both the excitation and the emission maxima of **13a** are redshifted significantly. Significantly different are the fluorescence quantum yields: whereas in DMSO they are about equal (about

0.5), **18** and similar compounds are quenched in water significantly to give a quantum yield of 0.16. A solvent-independent quantum yield as shown for cyanocarbostyrils such as **13a** is at least new in the class of carbostyrils; therefore, we measured the fluorescence spectra in two additional solvents: dichloromethane and ethanol. No quenching by oxygen was observed, as the intensities did not change after degassing with argon.

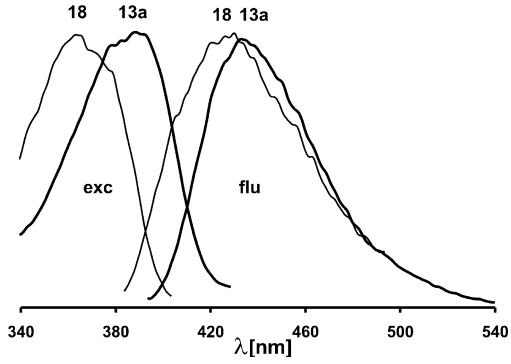
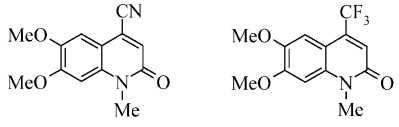


Figure 1. Excitation and emission spectra of 4-cyano- vs. 4-trifluoromethylcarbostyrils **13a** and **18** in water.



13a **18**

The data for 4-cyanocarbostyril **13a** in Table 1 show an almost complete independence of solvent influences for emission maxima and fluorescence quantum yield. All other N–CH_2–CO–R derivatives **13b**, **14**, and **17a**,**b** exhibit very similar electronic spectra; however, quantum yields of the latter are about 0.55 in water and 0.45 ± 0.05 in DMSO. The use of this type of labeling fluorophore in biological samples is therefore superior to the trifluoromethyl variants similar to **18**, which are equally linked at N-1.[4a]

Table 1. Independence of solvent influences for emission maxima and fluorescence quantum yield of *N*-methyl derivative **13a**.

Solvent	UV[a] λ_{max} [nm]	ε [$M^{-1}cm^{-1}$]	exc[a] λ_{max} [nm]	em λ_{max} [nm]	Φ
Dichloromethane	392	13000	400	430	0.48
DMSO	390	12400	400	436	0.61
Ethanol	385	13100	390	432	0.51
Water[b]	380	11000	385	432	0.50

[a] Maximum at the center of the broad signal or at the center of the sometimes visible almost equally intense double peak (see Experimental Section). [b] Identical values between pH = 1 and pH = 11.

Conclusions

The synthesis of highly fluorescent 6,7-dimethoxy-4-cyanocarbostyril **11a** can be achieved in a three-step reaction with an overall yield of about 30%. Linkers can be introduced easily at N-1, and labeled proteins such as **17b** can be obtained by an additional three-step reaction in good yields.

Absorption spectra of the new *N*-linked 6,7-dimethoxy-4-cyanocarbostyrils **13b**, **14**, and **17a**,**b** are all very similar with broad double maxima between 385 and 410 nm in polar and apolar solvents. In water, a maximum redshift of about 20 nm is observed. The extinction coefficients are high with values between 10000 and 13000. Interestingly, and in contrast to the 4-trifluoromethyl analogues, the emission values range between 430 and 440 nm in all solvents, which therefore shows varying Stokes' shifts. Most important of all, fluorescence quantum yields are almost independent of solvents and pH and high values of around 0.5 are obtained. These findings reveal that 4-cyanocarbostyrils substituted at the 6- and 7-positions with electron-donating substituents will be very useful new fluorophores that are well suited as fluorescence standards.

Experimental Section

General Remarks: Melting points were determined with a Stuart SMP3 melting point apparatus in open capillary tubes. ^1H and ^{13}C NMR spectra were recorded with a Bruker AMX 360 instrument (360 or 90 MHz) or a Bruker Avance DRX 500 instrument (500 or 125 MHz). Chemical shifts are given in ppm (δ) from the internal TMS standard. IR spectra were recorded with a Mattson Galaxy Series FTIR 7020 instrument with potassium bromide discs. Elemental analyses were performed at the Microanalytical Laboratory of the University of Vienna, Austria. Mass spectra were obtained with a HP 1100 LC/MSD mass spectral instrument (positive or negative APCI ion source, 50–200 V, nitrogen). UV/Vis spectra were recorded with a Shimadzu UV/Vis scanning spectrophotometer UV-2101 PC; concentration: 1×10^{-4} M. Excitation and emission spectra were recorded with a Shimadzu RF-5001 PC spectrofluorometer (150-W Xe lamp, 6 selectable slits: 1.5, 3, 5, 10, 15, 20 nm, R452–01 photomultiplier; monochromator: ion-blazed holographic concave grating F/2.5); concentration: 1×10^{-4} M. Determination of quantum yields: emission signals were set in relation to the known area of the emission signal of quinine sulfate at pH 1. Corrections were made for other solvents by using the factor ($n_{water}/n_{solvent}$).[2] Analytical HPLC was performed with a Shimadzu LC 20 system equipped with a diode array detector (215 and 254 nm) with a Pathfinder AS reversed phase (4.6 × 150 mm, 5 μm) column, running an acetonitrile/water gradient (30–100% acetonitrile).

All reactions were monitored by thin-layer chromatography (TLC) on 0.2-mm silica gel F-254 (Merck) plates by using UV light (254 and 366 nm) for detection. Common reagent-grade chemicals were either commercially available and were used without further purification or prepared by standard literature procedures. All optical measurements were performed by using analytical-grade solvents.

4-Hydroxy-6,7-dimethoxy-1-methyl-3-phenylquinolin-2(1*H*)-one (3a): A mixture of 3,4-dimethoxy-*N*-methylbenzenamine[4b] (**1b**; 3.72 g, 22 mmol) and diethyl phenylmalonate (**2a**; 5.24 g, 22 mmol) in diphenyl ether (25 mL) was heated under reflux for 3 h. During this time, ethanol was liberated (2.1 mL). The mixture was cooled to 20 °C, diluted with cyclohexane (50 mL), and the formed precipitate was filtered by suction. The solid was dissolved in aqueous NaOH (0.5 M, 80 mL), and the byproducts were extracted with toluene (50 mL) and decolorized with charcoal. The product was precipitated upon acidification with HCl (2 M), filtered by suction, and

dried at 40 °C under reduced pressure to afford 4.93 g (71% yield) of colorless prisms. M.p. 264–267 °C (DMF). IR (KBr): $\tilde{\nu}$ = 3180–2820 (br., m), 1625 (sh), 1610 (s) cm^{-1}. ^1H NMR (360 MHz, [D_6]-DMSO): δ = 3.61 (s, 3 H, NMe), 3.82 and 3.89 (2 s, 2×3 H, 6- and 7-OMe), 6.95 (s, 1 H, 8-H), 7.28–7.33 (m, 5 H, PhH), 7.45 (s, 1 H, 5-H), 9.80 (s, 1 H, OH) ppm. $C_{18}H_{17}NO_4$ (311.34): calcd. C 69.44, H 5.50, N 4.50; found C 69.25, H 5.78, N 4.81.

4-Hydroxy-6,7-dimethoxy-3-phenylquinolin-2(1*H*)-one (3b): A mixture of aniline **1a** (3.43 g, 22 mmol) and diethyl phenylmalonate (**2a**; 5.24 g, 22 mmol) was brought to reaction (24 h) and worked up as described for **3a** to afford 5.55 g (85% yield) of colorless prisms. M.p. 340–343 °C (DMF). IR (KBr): $\tilde{\nu}$ = 3120–2600 (br., m), 1640 (s), 1610 (s) cm^{-1}. ^1H NMR (360 MHz, [D_6]DMSO): δ = 3.82 and 3.86 (2 s, 2×3 H, 6- and 7-OMe), 6.85 (s, 1 H, 8-H), 7.30–7.35 (m, 5 H, PhH), 7.39 (s, 1 H, 5-H), 9.81 (s, 1 H, OH), 11.20 (s, 1 H, NH) ppm. $C_{17}H_{15}NO_4$ (297.31): calcd. C 68.68, H 5.09, N 4.71; found C 68.96, H 4.85, N 4.58.

3-Benzyl-4-hydroxy-6,7-dimethoxyquinolin-2(1*H*)-one (3c): A mixture of aniline **1a** (3.43 g, 22 mmol) and diethyl benzylmalonate (**2b**; 5.50 g, 22 mmol) was brought to reaction (48 h) and worked up as described for **3a** to afford 4.24 g (62% yield) of pale-brownish prisms. M.p. 207–210 °C (DMF). IR (KBr): $\tilde{\nu}$ = 3150–2850 (br., m), 1645 (s), 1615 (s) cm^{-1}. ^1H NMR (360 MHz, [D_6]DMSO): δ = 3.83 and 3.87 (2 s, 2×3 H, 6- and 7-OMe), 3.99 (s, 2 H, benzyl-CH_2), 6.83 (s, 1 H, 8-H), 7.29–7.35 (m, 5 H, PhH), 7.40 (s, 1 H, 5-H), 9.79 (s, 1 H, OH), 11.23 (s, 1 H, NH) ppm. $C_{18}H_{17}NO_4$ (311.34): calcd. C 69.44, H 5.50, N 4.50; found C 69.71, H 5.24, N 4.82.

3-Ethyl-4-hydroxy-6,7-dimethoxyquinolin-2(1*H*)-one (3d): A mixture of aniline **1a** (3.43 g, 22 mmol) and diethyl ethylmalonate (**2c**; 4.03 g, 22 mmol) was brought to reaction (48 h) and worked up as described for **3a** to afford 0.49 g (9%) of pale green-brownish prisms. M.p. 229–234 °C (DMF). IR (KBr): $\tilde{\nu}$ = 3160–2890 (br., m), 1650 (s), 1610 (s) cm^{-1}. ^1H NMR (360 MHz, [D_6]DMSO): δ = 1.28 (t, J = 7.1 Hz, 3 H, ethyl-CH_3), 2.36 (q, J = 7.1 Hz, 2 H, ethyl-CH_2), 3.85 and 3.88 (2 s, 2×3 H, 6- and 7-OMe), 6.79 (s, 1 H, 8-H), 7.40 (s, 1 H, 5-H), 9.78 (s, 1 H, OH) ppm. $C_{13}H_{15}NO_4$ (249.27): calcd. C 62.64, H 6.07, N 5.62; found C 62.38, H 5.87, N 5.91.

4-Hydroxy-6,7-dimethoxy-1-methylquinolin-2(1*H*)-one (3e): A mixture of 3,4-dimethoxy-*N*-methylbenzenamine[4b] (**1b**; 4.22 g, 25 mmol) and dry malonic acid (**2d**; 2.57 g, 25 mmol) in phosphoryl chloride (2.65 mL, 17 mmol) was heated to 110 °C until strong foaming decreased and the mixture solidified (about 30 min). The mixture was suspended in water and stirred for 24 h until complete crystallization had taken place. The solid was dissolved in aqueous NaOH (0.5 M, 100 mL), decolorized with charcoal, filtered, and acidified with HCl (6 M). The precipitate was filtered by suction, washed with water (250 mL), and dried at 40 °C under reduced pressure to afford 1.63 g (41% yield) of pale-brown microprisms. M.p. 262.4 °C (DMF). IR (KBr): $\tilde{\nu}$ = 3480–3120 (br., m), 2950–2820 (m), 1665 (s) cm^{-1}. ^1H NMR (360 MHz, [D_6]DMSO): δ = 3.61 (s, NMe), 3.76 and 3.78 (2 s, 2×3 H, 6- and 7-OMe), 5.60 (s, 1 H, 3-H), 6.80 (s, 1 H, 8-H), 7.15 (s, 1 H, 5-H), 11.05 (s, 1 H, NH) ppm. $C_{12}H_{13}NO_4$ (235.24): calcd. C 61.27, H 5.57, N 5.95; found C 61.62, H 5.88, N 5.57.

4-Hydroxy-6,7-dimethoxyquinolin-2(1*H*)-one (3f): A mixture of aniline **1a** (6.12 g, 40 mmol) and dry malonic acid (4.38 g, 42 mmol) in phosphoryl chloride (6.2 g, 40 mmol) was heated at 50 °C for 2 h, then the temperature was raised slowly up to 90 °C and kept there for 30 min. The obtained residue was dissolved in NaOH solution (0.5 M, 100 mL) and acidified with concentrated HCl to pH 2–3. A light-green precipitate was formed, which was filtered, washed with water, and dried at 40 °C under reduced pressure to afford 7.10 g (79% yield) of pale-green prisms. M.p. >320 °C (DMF/ethanol); ref.[14] m.p. >320 °C. IR (KBr): $\tilde{\nu}$ = 3570–3250 (br., s), 2920 (s), 1685 (s), 1641 (sh), 1617 (m), 1605 (sh), 1518 (s) cm^{-1}. ^1H NMR (360 MHz, [D_6]DMSO): δ = 3.76 and 3.78 (2 s, 2×3 H, 6- and 7-OMe), 5.61 (s, 1 H, 3-H), 6.81 (s, 1 H, 8-H), 7.15 (s, 1 H, 5-H), 11.00 (s, 1 H, NH) ppm. MS: *m*/*z* (%) = 222 (8) [M + 1], 221 (100) [M]. $C_{11}H_{11}NO_4$ (221.21): calcd. C 59.73, H 5.01, N 6.33; found C 59.55, H 4.87, N 6.68.

4,6,7-Trihydroxy-1-methylquinolin-2(1*H*)-one (3g): 3-Acetylquinolone **5** (1.39 g, 5 mmol) was dissolved in 90% H_2SO_4 (4 mL) and heated for 15 min at 140 °C. Then, the solution was poured into ice/water (100 mL). The obtained suspension was kept for 24 h at 20 °C, and it was then filtered, washed with water (250 mL), and dried at 40 °C under reduced pressure to afford 0.74 g (71% yield) of light-brownish prisms. M.p. 310–311 °C (ethanol/water). IR (KBr): $\tilde{\nu}$ = 3400–2800 (br., s), 1621 (s), 1590 (m), 1561 (s), 1526 (m) cm^{-1}. ^1H NMR (360 MHz, [D_6]DMSO): δ = 3.40 (s, 3 H, NMe), 5.65 (s, 1 H, 3-H), 6.77 (s, 1 H, 8-H), 7.19 (s, 1 H, 5-H), 9.21 (s, 1 H, OH), 9.79 (s, 1 H, OH), 10.90 (s, 1 H, NH) ppm. MS: *m*/*z* (%) = 208 (10) [M + 1], 207 (100) [M], 192 (8). $C_{10}H_9NO_4$ (207.19): calcd. C 57.97, H 4.38, N 6.76; found C 58.18, H 4.13, N 6.44.

4-Hydroxy-8,9-dimethoxy-6-methyl-2*H*-pyrano[3,2-*c*]quinoline-2,5-(6*H*)-dione (4): A mixture of 3,4-dimethoxy-*N*-methylbenzenamine[4b] (**1b**; 3.34 g, 20 mmol) and diethyl malonate (**2e**; 6.41 g, 40 mmol) in diphenyl ether (20 mL) was heated under reflux in a distillation apparatus equipped with a 20-cm Vigreux column. During 3–4 h, the liberated ethanol was distilled until no more ethanol was formed (about 3.6 mL). The reaction mixture was cooled to 100 °C and treated with dioxane (20 mL). The precipitate was filtered by suction, washed with dioxane (20 mL) to remove diphenyl ether, and dried at 40 °C under reduced pressure to afford 4.48 g (74% yield) of yellow prisms. M.p. 315–316 °C (DMF). IR (KBr): $\tilde{\nu}$ = 3550–3450 (br., s), 1739 (s), 1677 (m), 1622 (m), 1576 (m), 1520 (s) cm^{-1}. ^1H NMR (360 MHz, CF_3COOD): δ = 4.25 (s, 3 H, NMe), 4.43 and 4.45 (2 s, 2×3 H, 8- and 9-OMe), 6.25 (s, 1 H, 3-H), 7.51 (s, 1 H, 10-H), 8.16 (s, 1 H, 7-H) ppm. MS: *m*/*z* (%) = 304 (24) [M + 1], 303 (100) [M], 289 (55), 259 (100). $C_{15}H_{13}NO_6$ (303.27): calcd. C 59.41, H 4.32, N 4.62; found C 59.11, H 4.50, N 4.79.

3-Acetyl-4-hydroxy-6,7-dimethoxy-1-methylquinolin-2(1*H*)-one (5): A mixture of pyranoquinoline **4** (3.03 g, 10 mmol) and aqueous NaOH (1 M, 3 mL) in 1,2-dihydroxyethane (30 mL) was heated to gentle boiling for 1 h and then poured into ice/water (100 mL). The obtained solution was slowly acidified with concentrated HCl (*attention: strong foaming!*). The precipitate was filtered by suction, washed with water (2×250 mL), and dried at 40 °C under reduced pressure to afford 1.97 g (71% yield) of yellow prisms. M.p. 238–239 °C (toluene). IR (KBr): $\tilde{\nu}$ = 2939 (w), 1653 (s), 1594 (s), 1563 (s), 1520 (s) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 2.82 (s, 3 H, acetyl-Me), 3.66 (s, 3 H, NMe), 3.98 and 4.05 (2 s, 2×3 H, 8- and 9-OMe), 6.69 (s, 1 H, 8-H), 7.55 (s, 1 H, 5-H) ppm. MS: *m*/*z* (%) = 278 (14) [M + 1], 277 (100) [M], 262 (43). $C_{14}H_{15}NO_5$ (277.28): calcd. C 60.65, H 5.45, N 5.05; found C 60.83, H 5.48, N 4.95.

6,7-Dimethoxy-2-oxo-3-phenyl-1,2-dihydroquinolin-4-yl 4-Methylbenzenesulfonate (6a): A mixture of hydroxyquinolone **3b** (9.78 g, 33 mmol), *p*-tosyl chloride (9.44 g, 49.5 mmol), and 4-(*N*,*N*-dimethyl)aminopyridine (200 mg) in dry pyridine (200 mL) was stirred at 20 °C for 24 h. Then the mixture was poured into ice/water (500 mL), and the solid was filtered by suction, washed subsequently with water (200 mL) and ethanol (50 mL), and then crystallized from DMF to afford 14.7 g (99% yield) of colorless prisms.

M.p. >310 °C (dec.) (DMF). IR (KBr): $\tilde{\nu}$ = 3100–2850 (br., m), 1660 (sh), 1650 (s), 1635 (s), 1615 (m), 1595 (w), 1575 (w) cm^{-1}. ^1H NMR (360 MHz, CF_3COOH): δ = 2.23 (s, Me), 3.91 and 3.96 (2 s, 2×3 H, 6- and 7-OMe), 6.99 (s, 1 H, 8-H), 7.11–7.17 (m, 9 H, 5 PhH, 4 TosH), 7.59 (s, 1 H, 5-H) ppm. $C_{24}H_{21}NO_6S$ (451,50): calcd. C 63.85, H 4.69, N 3.10; found C 63.67, H 4.58, N 3.24.

2-Oxo-1,2-dihydroquinolin-4-yl 4-Methylbenzenesulfonate (6b): Hydroxyquinolone **3j** (3.67 g, 23 mmol), *p*-tosyl chloride (5.69 g, 30 mmol), and 4-(*N*,*N*-dimethyl)aminopyridine (200 mg) in dry pyridine (200 mL) were brought to reaction and worked up as described for **6a** to afford 6.05 g (84% yield) of colorless prisms. M.p. 237–238 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 1650 (w), 1610 (w), 1510 (w) cm^{-1}. ^1H NMR (360 MHz, [D$_6$]DMSO): δ = 2.40 (s, 3 H, Me), 7.21 (d, *J* = 7.0 Hz, 1 H, 8-H), 7.46 (t, *J* = 7.0 Hz, 1 H, Aryl-H), 7.65 (t, *J* = 7.0 Hz, 1 H, Aryl-H), 7.85 (dd, *J* = 7.0, 1.5 Hz, 1 H, 5-H), 12.30 (s, 1 H, NH) ppm. $C_{16}H_{13}NO_4S$ (315.35): calcd. C 60.94, H 4.16, N 4.44; found C 61.33, H 4.28, N 4.46.

2,4-Dichloro-6,7-dimethoxy-3-phenylquinoline (7a): A mixture of hydroxyquinolone **3b** (8.72 g, 29 mmol) and phosphoryl chloride (50 mL) was heated for 1 h under reflux. The excess amount of phosphoryl chloride was removed in vacuo, and the residue was poured into ice/water (300 mL) and brought to pH = 4–6 with aqueous NaOH (5 M). The precipitate was washed with water (200 mL) and filtered by suction to afford 5.13 g (53% yield) of colorless prisms. M.p. 285–290 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3050–2850 (br., w), 1640 (m), 1615 (m), 1555 (w), 1510 (sh), 1500 (s) cm^{-1}. ^1H NMR (360 MHz, [D$_6$]DMSO): δ = 3.80 and 3.96 (2 s, 2×3 H, 6- and 7-OMe), 7.37 (m, 1 H, ArH), 7.39 (m, 1 H, ArH), 7.47 (m, 1 H, ArH), 7.49 (m, 1 H, ArH), 7.51 (m, 1 H, ArH), 7.53 (m, 1 H, ArH), 7.55 (s, 1 H, ArH) ppm. $C_{17}H_{13}Cl_2NO_2$ (334.20): calcd. C 61.10, H 3.92, N 4.19; found C 60.88, H 4.12, N 4.37.

2,4-Dichloro-6,7-dimethoxyquinoline (7b): Hydroxyquinolone **3f** (6.60 g, 30 mmol) and phosphoryl chloride (50 mL) were brought to reaction (8 h) and worked up as described for **7a** to afford 6.20 g (80% yield) of colorless prisms. M.p. 160–161 °C (ethanol); ref.[15] m.p. 159 °C. IR (KBr): $\tilde{\nu}$ = 3550–3350 (br., m), 3007 (m), 2983 (w), 2945 (w), 1617 (m), 1570 (s), 1504 (s) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 4.03 and 4.06 (2 s, 2×3 H, 6- and 7-OMe), 7.37 (s, 1 H, 3-H), 7.38 (s, 1 H, 8-H), 7.39 (s, 1 H, 5-H) ppm. MS: *m*/*z* (%) = 260 (75) [M + 2], 259 (17) [M + 1], 258 (100) [M], 223 (11). $C_{11}H_9Cl_2NO_2$ (258.11): calcd. C 51.19, H 3.51, N 5.43; found C 51.45, H 3.20, N 5.67.

4-Chloro-6,7-dimethoxy-3-phenylquinolin-2(1*H*)-one (8a): A mixture of dichloroquinoline **7a** (200 mg, 0.6 mmol), aqueous KOH (5 M, 10 mL) and 18-crown-6 (10 mg) in ethanol (30 mL) was heated for 4 d. Insoluble parts were removed by filtration, and the solution was acidified with HCl (6 M) to pH = 1–2. The solid was separated by suction filtration to afford 130 mg (69% yield) of colorless prisms. M.p. >300 °C (dec) (ethanol/water). IR (KBr): $\tilde{\nu}$ = 3560–3350 (br., m), 3007 (w), 2927 (m), 2848 (m), 2792 (sh), 1658 (s), 1625 (w), 1596 (m), 1510 (s) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 3.92 and 4.07 (2 s, 2×3 H, 6- and 7-OMe), 6.78 (s, 1 H, 8-H), 7.38–7.53 (m, 6 H, PhH and 5-H), 11.83 (br. s, NH) ppm. $C_{17}H_{14}ClNO_3$ (315.76): calcd. C 64.67, H 4.47, N 4.44; found C 64.39, H 4.66, N 4.76.

4-Chloro-6,7-dimethoxy-1-methyl-3-phenylquinolin-2(1*H*)-one (8b): A mixture of hydroxyquinolone **3a** (5.42 g, 17 mmol) and phosphoryl chloride (30 mL) was heated under reflux for 1 h. The excess amount of phosphoryl chloride was removed in vacuo, and the residue was poured into ice/water (200 mL) and neutralized to pH = 4–6 with aqueous NaOH (5 M). The precipitate was filtered by suction and washed with water (100 mL) to afford 4.64 g (83% yield) of yellowish prisms. M.p. 171–176 °C (ethanol/water). IR (KBr): $\tilde{\nu}$ = 3450–3250 (br., w), 2960 (w), 1635 (m), 1610 (s) cm^{-1}. ^1H NMR (360 MHz, [D$_6$]DMSO): δ = 4.09 and 4.22 (2 s, 2×3 H, 6- and 7-OMe), 4.66 (s, 3 H, NMe), 7.29–7.31 (m, 1 H, 8-H), 7.37–7.44 (m, 2 H, PhH), 7.58–7.64 (m, 2 H, PhH), 7.72 (s, 1 H, PhH), 7.84 (s, 1 H, 5-H) ppm. $C_{18}H_{16}ClNO_3$ (329.79): calcd. C 65.56, H 4.89, N 4.25; found C 65.22, H 4.97, N 4.01.

4-Chloro-6,7-dimethoxyquinolin-2(1*H*)-one (8c): A solution of dichloroquinoline **7b** (5.16 g, 20 mmol), 70% CH_3SO_3H (9.60 g, 100 mmol) and ethanol (80 mL) was heated under reflux for 28 h. After cooling, the mixture was poured into ice/water (150 mL) and brought to pH = 4–6 with aqueous NaOH (2 M). The obtained solid was filtered by suction, washed with water (150 mL), dried at 40 °C under reduced pressure, and crystallized from ethanol to afford 2.63 g (55% yield) of dark-brownish prisms. M.p. 258–259 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3403 (m), 2957 (w), 2929 (m), 2909 (sh), 2853 (w), 1657 (s), 1624 (m), 1606 (w), 1545 (w), 1513 (s) cm^{-1}. ^1H NMR (360 MHz, [D$_6$]DMSO): δ = 3.82 and 3.83 (2 s, 2×3 H, 6- and 7-OMe), 6.60 (s, 1 H, 3-H), 6.90 (s, 1 H, 8-H), 7.17 (s, 1 H, 5-H), 11.82 (s, 1 H, NH) ppm. MS: *m*/*z* (%) = 241 (23) [M + 2], 240 (12) [M + 1], 239 (100) [M], 204 (100) [M – 35]. $C_{11}H_{10}ClNO_3$ (239.66): calcd. C 55.13, H 4.21, N 5.84; found C 55.49, H 4.53, N 5.45.

4-Chloro-3-phenylquinolin-2(1*H*)-one (8g): A solution of 2,4-dichloro-3-phenylquinoline[10a] (**7c**; 2.74 g, 10 mmol), 70% CH_3SO_3H (4.80 g, 50 mmol), and ethanol (30 mL) was brought to reaction (40 h, 60 °C) and worked up as described for **8c** to afford 2.42 g (89% yield) of colorless prisms. M.p. 252–254 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3250–2600 (br., m), 1650 (s), 1620 (sh), 1595 (m), 1560 (w) cm^{-1}. ^1H NMR (360 MHz, [D$_6$]DMSO): δ = 7.25–7.55 (m, 7 H, ArH), 7.55–7.75 (t, *J* = 7 Hz, 1 H, ArH), 7.90 (dd, *J* = 7, 1.5 Hz, 1 H, 5-H), 12.20 (s, 1 H, NH) ppm. $C_{15}H_{10}ClNO$ (255.70): calcd. C 70.46, H 3.94, Cl 13.86, N 5.48; found C 70.45, H 3.82, Cl 13.62, N 5.46.

4-Chloro-3-nitroquinolin-2(1*H*)-one (8h): A solution of 2,4-dichloro-3-nitroquinoline[16] (**7d**; 2.43 g, 10 mmol), 70% CH_3SO_3H (4.80 g, 50 mmol), and ethanol (30 mL) was brought to reaction (24 h, 60 °C) and worked up as described for **8c** to afford 1.80 g (85% yield) of light-brownish prisms. M.p. 278 °C (ethanol); ref.[17] m.p. 275 °C. IR (KBr): $\tilde{\nu}$ = 3250–2650 (br., m), 1690 (sh), 1670 (s), 1655 (sh), 1620 (sh), 1610 (m), 1545 (m), 1520 (m) cm^{-1}. $C_9H_5ClN_2O_3$ (224.60): calcd. C 48.13, H 2.24, N 12.47; found C 48.34, H 2.36, N 12.33.

3-Phenylquinoline-2,4-dicarbonitrile (9a): A mixture of 2,4-dichloro-3-phenylquinoline[10a] (**7c**; 2.70 g, 10 mmol), KCN (1.32 g, 20 mmol), and 18-crown-6 (0.10 g) in dry DMF (30 mL) was heated under reflux for 24 h. The mixture was cooled to 20 °C, poured into ice/water (100 mL), allowed to stand for 12 h at 20 °C, and then the solid was filtered by suction (*attention: evolution of HCN is possible!*) and dried at 40 °C under reduced pressure to afford 1.70 g (69% yield) of yellow microprisms. M.p. 254 °C (ligroin). IR (KBr): $\tilde{\nu}$ = 2225 (m), 1650 (m), 1605 (m), 1560 (s) cm^{-1}. $C_{17}H_9N_3$ (255.28): calcd. C 79.98, H 3.55, N 16.46; found C 79.63. H 3.87, N 16.07.

3-Nitroquinoline-2,4-dicarbonitrile (9b): 2,4-Dichloro-3-nitroquinoline[16] (**7d**; 2.40 g, 10 mmol) in dry acetonitrile (30 mL) was brought to reaction (48 h) and worked up as described for **9a** to afford 1.60 g (73% yield) of yellowish microprisms. M.p. 194 °C (ligroin). IR (KBr): $\tilde{\nu}$ = 2225 (m), 1605 (m), 1550 (s) cm^{-1}. $C_{11}H_4N_4O_2$ (224.18): calcd. C 58.94, H 1.80, N 24.99; found C 59.77, H 1.63, N 24.88.

6,7-Dimethoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-Methylbenzenesulfinate (10): A mixture of chloroquinolone **8c** (0.239 g, 1 mmol) and sodium *p*-toluenesulfinate (267 mg, 1.5 mmol) in DMF (60 mL) was heated at 120 °C for 20 h and then poured into water (150 mL). The obtained solid was filtered by suction, washed with water (100 mL), and dried at 40 °C under reduced pressure to afford 251 mg (72% yield) of yellow prisms. M.p. 295–296 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3650–3350 (br., m), 2969 (w), 2942 (m), 2904 (w), 2844 (m), 1674 (s), 1625 (w), 1595 (w), 1516 (s) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 2.43 (s, 3 H, Me), 3.93 and 4.02 (2 s, 2×3 H, 6- and 7-OMe), 6.87 (s, 1 H, 3-H), 7.34–7.37 (m, 3 H, ArH), 7.73 (s, 1 H, ArH), 7.89 (d, J = 7.0 Hz, 2 H, ArH), 12.97 (s, 1 H, NH) ppm. MS: *m*/*z* (%) = 360 (15) [M + 1], 359 (100) [M], 344 (24), 221 (9). $C_{18}H_{17}NO_5S$ (359.40): calcd. C 60.16, H 4.77, N 3.90; found C 60.47, H 4.35, N 4.21.

6,7-Dimethoxy-2-oxo-1,2-dihydroquinoline-4-carbonitrile (11a)

Method A: A mixture of chloroquinolone **8c** (2.39 g, 10 mmol), sodium *p*-toluenesulfinate (3.80 g, 21 mmol), and KCN (1.65 g, 25 mmol) in dry DMF (60 mL) was heated at 120 °C for 20 h with vigorous stirring. After cooling to room temperature, the mixture was poured into ice/water (200 mL) and acidified with concentrated HCl to pH = 1–2. (*attention: evolution of HCN is possible!*). The obtained solid was filtered by suction, washed with water, dried, and crystallized from acetonitrile to afford 1.50 g (65% yield) of yellow prisms.

Method B: A mixture of quinolinyl tolyl sulfinate **10** (359 mg, 1.0 mmol) and potassium cyanide (100 mg, 1.5 mmol) in dry DMF (6 mL) was heated at 70 °C for 6 h. Then, the mixture was poured into water (50 mL) and acidified with concentrated HCl to pH = 1–2 (*attention: evolution of HCN is possible!*). The obtained solid was filtered by suction, washed with water, and dried at 40 °C under reduced pressure to afford 152 mg (65% yield) of yellow prisms. M.p. 295–296 °C (acetonitrile). IR (KBr): $\tilde{\nu}$ = 3550–3400 (br., m), 2921 (m), 2832 (w), 2218 (m), 1664 (s), 1625 (w), 1511 (s) cm^{-1}. ^1H NMR (360 MHz, [D_6]DMSO): δ = 3.79 and 3.86 (2 s, 2×3 H, 6- and 7-OMe), 6.85 (s, 1 H, 3-H), 7.24 (s, 1 H, 8-H), 8.54 (s, 1 H, 5-H), 12.28 (s, 1 H, NH) ppm. UV (DMSO): λ = 398, 385 nm. UV (water): λ = 380 nm. MS: *m*/*z* (%) = 231 (15) [M + 1], 230 (100) [M]. $C_{12}H_{10}N_2O_3$ (230.23): calcd. C 62.61, H 4.38, N 12.17; found C 62.69, H 4.65, N 11.65.

6,7-Dimethoxy-1-methyl-2-oxo-3-phenyl-1,2-dihydroquinoline-4-carbonitrile (11b): A mixture of chloroquinolone **8b** (1.98 g, 6 mmol), sodium *p*-toluenesulfinate (2.67 g, 15 mmol), and potassium cyanide (1.19 g, 18 mmol) was brought to reaction (65 h) and worked up as described for **11a** (Method A). Further purification was performed by dry flash chromatography (Merck Silica gel 60 H, 5–40 μm)[18] with hexane/ethyl acetate (6:4) as eluent. The samples with the product (checked by TLC) were combined, and the solvent was removed in vacuo. The crude product was crystallized from ethanol to afford 310 mg (16% yield) of yellow prisms. M.p. 179–185 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 2960 (sh), 2920 (m), 2840 (sh), 2205 (w), 1638 (s), 1620 (sh), 1583 (m) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 3.67 and 3.79 (2 s, 2×3 H, 6- and 7-OMe), 4.02 (s, 3 H, NMe), 6.82 (s, 1 H, 8-H), 7.36–7.55 (m, 6 H, ArH) ppm. UV (DMSO): λ = 398, 385 nm. UV (water): λ = 380 nm. MS: *m*/*z* (%) = 307 (21), 306 (100) [M – 14], 257 (100). $C_{19}H_{16}N_2O_3$ (320.35): calcd. C 71.24, H 5.03, N 8.74; found C 70.85, H 5.22, N 8.37.

1-Methyl-2-oxo-3-phenyl-1,2-dihydroquinoline-4-carbonitrile (11c): A mixture of 4-chloro-1-methyl-3-phenylquinolin-2(1*H*)-one[8] (**8d**; 2.70 g, 10 mmol), sodium *p*-toluenesulfinate (3.80 g, 21 mmol), and KCN (1.65 g, 25 mmol) was brought to reaction and worked up as described for **11a** (Method A) to afford 3.56 g (97% yield) of orange needles. M.p. 202–203 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3050 (w), 2240 (m), 1640 (s), 1610 (m), 1600 (m), 1570 (w) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 3.81 (s, 3 H, NMe), 7.42–7.50 (m, 5 H, ArH), 7.55–7.65 (m, 2 H, ArH), 7.70 (t, J = 7 Hz, 1 H, ArH), 8.05 (dd, J = 7, 1.5 Hz, 1 H, 5-H) ppm. $C_{17}H_{12}N_2O$ (260.30): calcd. C 78.44, H 4.65, N 10.76; found C 78.18, H 4.64, N 10.38.

2-Oxo-1,3-diphenyl-1,2-dihydroquinoline-4-carbonitrile (11d)

Method A: A mixture of 4-chloro-1,3-diphenylquinolin-2(1*H*)-one[8] (**8e**; 3.31 g, 10 mmol), lithium *p*-toluenesulfinate (4.05 g, 25 mmol), and KCN (1.65 g, 25 mmol) were brought to reaction and worked up as described for **11a** (Method A) to afford 2.51 g (78% yield).

Method B: A mixture of chloroquinolone **8e** (3.31 g, 10 mmol), sodium *p*-toluenesulfinate (4.45 g, 25 mmol), and KCN (1.65 g, 25 mmol) were brought to reaction and worked up as described for **11a** (Method A) to afford 2.48 g (77% yield) of yellow microprisms. M.p. 224 °C (dioxane). IR (KBr): $\tilde{\nu}$ = 2215 (m), 1650 (s), 1605 (w), 1590 (w) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 6.75 (d, J = 7.0 Hz, 1 H, ArH), 7.20–7.75 (m, 12 H, ArH), 8.05 (d, J = 7.0 Hz, 1 H, 5-H) ppm. $C_{22}H_{14}N_2O$ (322.37): calcd. C 81.97, H 4.38, N 8.69; found C 81.70, H 4.40, N 8.63.

3-Oxo-2-phenyl-6,7-dihydro-3*H*,5*H*-benzo[*ij*]quinolizin-1-carbonitrile (11e): A mixture of 1-chloro-2-phenyl-6,7-dihydro-3*H*,5*H*-benzo[*ij*]quinolizin-3-one[8] (**8f**; 4.44 g, 15 mmol), potassium cyanide (1.98 g, 30 mmol), and sodium *p*-toluenesulfinate (4.05 g, 23 mmol) was brought to reaction and worked up as described for **11a** (Method A) to afford 4.32 g (92%) of yellow microprisms. M.p. 197 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 2900 (w), 2220 (m), 1640 (s), 1590 (s) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 2.00–2.21 (m, 2 H, CH_2), 3.00–3.10 (m, 2 H, Ar-CH_2), 4.10–4.20 (m, 2 H, N-CH_2), 7.30–7.60 (m, 7 H, ArH), 7.74 (d, J = 8.0 Hz, 1 H, ArH), 7.95 (d, J = 7.5 Hz, 1 H, 10-H) ppm. $C_{19}H_{14}N_2O$ (286.34): calcd. C 79.70, H 4.93, N 9.78; found C 79.31, H 4.87, N 9.43.

2,6,7-Trimethoxyquinoline-4-carbonitrile (12a): A mixture of 4-cyanoquinolone **11a** (2.30 g, 10 mmol), iodomethane (2.82 g, 20 mmol), and potassium carbonate (1.38 g, 5 mmol) in dry acetonitrile (90 mL) was stirred at 50 °C for 5 h. Then, the reaction mixture was poured into water (100 mL), and the obtained solid was filtered, washed with water, and dried at 40 °C under reduced pressure. TLC analysis showed two isomeric products: *N*-methylated quinolone **13** and *O*-methylated quinoline **12**; the yield was 1.8 g (75%). The mixture was separated by dry flash column chromatography (Merck Silica gel 60 H, 5–40 μm).[18] Toluene as the eluent afforded *O*-methylated quinoline **12a** (360 mg, 15% yield) as pale-yellow prisms. M.p. 184–185 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3550–3360 (w), 2949 (m), 2222 (s), 1622 (w), 1605 (s), 1576 (w) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 4.00 and 4.06 (2 s, 2×3 H, 6- and 7-OMe), 4.14 (s, 3 H, 2-OMe), 7.01 (s, 1 H, 3-H), 7.23 (s, 1 H, 8-H), 8.23 (s, 1 H, 5-H) ppm. $C_{13}H_{12}N_2O_3$ (244.25): calcd. C 63.93, H 4.95, N 11.47; found C 63.61, H 4.58, N 11.19.

Ethyl [(4-Cyano-6,7-dimethoxyquinolin-2-yl)oxy]acetate (12b): To a stirred mixture of 4-cyanoquinolone **11a** (2.30 g, 10 mmol) was added aqueous NaOH (8 M, 5 mL), dibenzo-18-crown-6 (150 mg, 0.25 mmol) in dichloromethane (200 mL), and ethyl bromoactate (0.55 mL, 50 mmol), and the mixture was stirred 12–14 h until starting material **11a** had disappeared (TLC check). Then, the mixture was filtered, and the solvent was removed under reduced pressure. The combined solids were rinsed with hexane (40 mL), filtered, washed with hexane, and dried at 40 °C under reduced pressure. TLC analysis showed two isomeric products: *N*-alkylated quinolone **13b** and *O*-alkylated quinoline **12b**; the yield was 2.84 g

(90%). The mixture was separated by dry flash column chromatography (Merck Silica gel 60 H, 5–40 μm).[18] Toluene as eluent afforded *O*-alkylated quinoline **12b** (470 mg, 15% yield) as light-green prisms. M.p. 158–159 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3480–3420 (br., w), 3000–2930 (br., w), 2227 (m), 1753 (s), 1655 (w), 1621 (m), 1607 (m), 1511 (s) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 1.27 (t, J = 7.1 Hz, 3 H, ethyl-CH_3), 4.02 and 4.04 (2 s, 2 × 3 H, 6- and 7-OMe), 4.27 (q, J = 7.1 Hz, 2 H, ethyl-CH_2), 5.07 (s, 2 H, acetate-CH_2), 7.02 (s, 1 H, 3-H), 7.14 (s, 1 H, 8-H), 8.26 (s, 1 H, 5-H) ppm. MS: *m*/*z* (%) = 317 (10) [M + 1], 316 (100) [M]. $C_{16}H_{16}N_2O_5$ (316.32): calcd. C 60.76, H 5.10, N 8.86; found C 61.11, H 5.24, N 8.51.

6,7-Dimethoxy-1-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile (13a): The reaction was performed as described for *O*-methylated quinoline **12a**: dry flash column chromatography[18] of the mixture of quinolines **12a/13a** with toluene/acetone (9:1) as the eluent gave *N*-methylated quinolone **13a** (1.44 g, 60% yield) as pale-yellow prisms. M.p. 260–261 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3500–3350 (br., w), 3045 (m), 2963 (m), 2919 (w), 2226 (m), 1632 (s), 1592 (w), 1567 (w), 1524 (m) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 3.77 and 3.96 (2 s, 2 × 3 H, 6- and 7-OMe), 4.06 (s, 3 H, NMe), 6.77 (s, 1 H, 3-H), 6.97 (s, 1 H, 8-H), 8.09 (s, 1 H, 5-H) ppm. UV (DMSO): λ (ε, $M^{-1}cm^{-1}$) = 390 (12400) nm. Fluorescence data: see Table 1. MS: *m*/*z* (%) = 245 (23) [M + 1], 244 (100) [M]. $C_{13}H_{12}N_2O_3$ (244.25): calcd. C 63.93, H 4.95, N 11.47; found C 63.68, H 5.23, N 11.71.

Ethyl (4-Cyano-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-4-yl)acetate (13b): The reaction was performed as described for *O*-alkylated quinoline **12b**: Dry flash column chromatography[18] of the mixture of quinolines **12b/13b** with toluene/acetone (9:1) as eluent afforded *N*-alkylated quinolone **13b** (2.37 g, 75% yield) as yellow prisms. M.p. 220–221 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3480–3400 (br., w), 2922 (m), 2963 (s), 2226 (s), 1732 (s), 1657 (s), 1625 (m), 1567 (w), 1560 (m), 1536 (w) cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ = 1.28 (t, J = 7.1 Hz, 3 H, ethyl-CH_3), 3.96 and 3.99 (2 s, 2 × 3 H, 6- and 7-OMe), 4.26 (q, J = 7.1 Hz, 2 H, ethyl-CH_2), 5.11 (s, 2 H, acetate-CH_2), 6.53 (s, 1 H, 3-H), 6.99 (s, 1 H, 8-H), 8.14 (s, 1 H, 5-H) ppm. UV (DMSO): λ (ε, $M^{-1}cm^{-1}$) = 385 (10400) nm. Fluorescence (H_2O): λ (Φ_F) = 435 (0.53) nm. Fluorescence (DMSO): λ (Φ_F) = 436 (0.46) nm. MS: *m*/*z* (%) = 317 (10) [M + 1], 316 (100) [M], 270 (47) [M – 46]. $C_{16}H_{16}N_2O_5$ (316.32): calcd. C 60.76, H 5.10, N 8.86; found C 60.97, H 5.02, N 8.63.

(4-Cyano-6,7-dimethoxy-2-oxo-1,2-dihydroquinolin-1-yl)acetic Acid (14): A solution of quinolinylacetate **13b** (316 mg, 1.0 mmol) in ethanol (20 mL) and aqueous NaOH (1 M, 2 mL) was heated under reflux for 7 h. Ethanol was then removed under reduced pressure, and the residue was dissolved in water (10 mL) under cooling. The mixture was acidified with concentrated HCl to pH = 1–2, and the resulting precipitate was filtered by suction and washed with water, which afforded 232 mg (80% yield) as yellow prisms. M.p. 281–282 °C (ethyl acetate). IR (KBr): $\tilde{\nu}$ = 3522 (s), 3400 (m), 2229 (w), 1736 (m), 1641 (s), 1626 (s), 1596 (w), 1566 (s), 1514 (s) cm^{-1}. ^1H NMR (360 MHz, [D_6]DMSO): δ = 3.82 and 3.92 (2 s, 2 × 3 H, 6- and 7-OMe), 5.09 (s, 2 H, acetate-CH_2), 6.99 (s, 1 H, 3-H), 7.35 (s, 1 H, 8-H), 8.63 (s, 1 H, 5-H) ppm. UV (DMSO): λ (ε, $M^{-1}cm^{-1}$) = 398, 385 (9800) nm. UV (water): λ (ε, $M^{-1}cm^{-1}$) = 380 (8700) nm. MS: *m*/*z* (%) = 289 (16) [M + 1], 288 (80) [M], 244 (28), 243 (28), 184 (100). $C_{14}H_{12}N_2O_5$ (288.26): calcd. C 58.33, H 4.20, N 9.72; found C 57.95, H 4.34, N 9.38.

1-{2-[(2,5-Dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-4-carbonitrile (15): *N*-Hydroxysuccinimide (115 mg, 1.0 mmol) was added slowly with stirring to a solution of quinolinylacetic acid **14** (288 mg, 1.0 mmol) in dry tetrahydrofuran (20 mL) at 0 °C. Then *N*,*N*-diisopropylcarbodiimide (125 mg, 1.0 mmol) was added at 0–5 °C dropwise whilst stirring, which formed a yellowish-white precipitate; the mixture was stirred at 0–5 °C for about 15 h, and the solvent was removed under reduced pressure. The obtained solid was filtered by suction and washed with dry tetrahydrofuran. The solid was then stirred in dry ethanol (50 mL) at 20 °C for 30 min to remove *N*,*N*-diisopropylurea formed during the reaction. Suction filtration afforded 288 mg (59% yield) of pale-yellow prisms. M.p. 232–233 °C (ethanol). IR (KBr): $\tilde{\nu}$ = 3300–3550 (br., s), 2909 (m), 2223 (m), 1809 (w), 1783 (m), 1745 (s), 1660 (sh), 1646 (m), 1621 (m), 1561 (m) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 2.87 (s, 4 H, 2 succinimide-CH_2), 3.97 and 4.14 (2 s, 2 × 3 H, 6- and 7-OMe), 5.56 (s, 2 H, acetate-CH_2), 6.64 (s, 1 H, 3-H), 6.98 (s, 1 H, 8-H), 8.15 (s, 1 H, 5-H) ppm. UV (DMSO): λ = 398, 385 nm. UV (water): λ = 380 nm. MS: *m*/*z* (%) = 386 (5) [M + 1], 385 (42) [M], 288 (17), 270 (100). $C_{18}H_{15}N_3O_7$ (385.34): calcd. C 56.11, H 3.92, N 10.90; found C 56.34, H 3.89, N 11.29.

***N*-[(4-Cyano-6,7-dimethoxy-2-oxo-1,2-dihydroquinolin-1-yl)acetylamino]-3-phenylpropionic Acid (17a):** To a solution of (D, L)-phenylalanine (**16a**; 19 mg, 0.1 mmol) in 90% aqueous DMSO (1.5 mL) was added a solution of OSu ester **15** (38 mg, 0.1 mmol) in 90% aqueous DMSO (1.5 mL) dropwise at 20 °C. Then, aqueous pH 7 buffer solution (Merck 9887, 0.75 mL) was added. The mixture was stirred for 14 h at 20 °C, poured into water (25 mL), and then acidified with concentrated HCl to pH = 1–2. A solid separated, which was filtered by suction and washed with an excess amount of water to afford 29 mg (68% yield) of dark-yellow prisms. M.p. 242–243 °C (acetone). IR (KBr): $\tilde{\nu}$ = 3550–3350 (br., w), 3295 (m), 2227 (m), 1710 (m), 1654 (s), 1633 (s), 1562 (m), 1549 (w) cm^{-1}. ^1H NMR (500 MHz, [D_6]DMSO): δ = 2.90 and 3.05 (2 m, 2 H, $PhCH_2$), 3.78 and 3.82 (2 s, 2 × 3 H, 6- and 7-OMe), 4.42–4.44 (m, 1 H, N-CH), 4.85 (d, J = 16.4 Hz, 1 H, 1/2 acetate-CH_2), 5.12 (d, J = 16.4 Hz, 1 H, 1/2 acetate-CH_2), 6.60 (s, 1 H, 3-H), 7.21 (m, 5 H, PhH), 7.33 (s, 1 H, 8-H), 8.60 (s, 1 H, 5-H), 8.69 (d, J = 12.0 Hz, 1 H, NH) ppm. UV (DMSO): λ (ε, $M^{-1}cm^{-1}$) = 385 (13300) nm. Fluorescence (H_2O): λ (Φ_F) = 433 (0.73) nm. Fluorescence (DMSO): λ (Φ_F) = 436 (0.60) nm. $C_{23}H_{21}N_3O_6$ (435.44): calcd. C 63.44, H 4.86, N 9.65; found C 63.12, H 4.51, N 10.03.

(2-{2-[2-(4-Cyano-6,7-dimethoxy-2-oxo-1,2-dihydroquinolin-1-yl)-acetylamino]acetylamino}acetylamino)acetic Acid (17b): Glycyl-glycyl-glycine (**16b**; 20 mg, 0.1 mmol) was brought to reaction and worked up as described for labeled phenylalanine **17a** to afford 25 mg (55% yield) of dark-yellow prisms. M.p. 238–239 °C (acetone). IR (KBr): $\tilde{\nu}$ = 3354 (s), 3302 (s), 3071 (m), 3047 (m), 2948 (m), 2922 (m), 2224 (m), 1734 (m), 1639 (s), 1633 (s), 1566 (s), 1530 (w) cm^{-1}. ^1H NMR (500 MHz, [D_6]DMSO): δ = 3.67 (d, J = 5.04 Hz, 2 H, glycyl-CH_2), 3.72 (d, J = 5.76 Hz, 2 H, glycyl-CH_2), 3.79 (d, J = 5.76 Hz, 2 H, glycyl-CH_2), 3.81 and 3.92 (2 s, 2 × 3 H, 6- and 7-OMe), 5.06 (s, 2 H, acetate-CH_2), 6.83 (s, 1 H, 3-H), 7.34 (s, 1 H, 8-H), 8.08 (t, J = 5.79 Hz, 1 H, NH), 8.25 (t, J = 5.79 Hz, 1 H, NH), 8.60 (s, 1 H, 5-H), 8.62 (t, J = 5.79 Hz, 1 H, NH) ppm. UV (DMSO): λ (ε, $M^{-1}cm^{-1}$) = 385 (11600) nm. Fluorescence (H_2O): λ (Φ_F) = 434 (0.56) nm. Fluorescence (DMSO): λ (Φ_F) = 436 (0.46) nm. $C_{20}H_{21}N_5O_8$ (459.42): calcd. C 52.29, H 4.61, N 15.24; found C 51.90, H 4.48, N 15.52.

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