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Preparation and evaluation of sulfur-containing metal chelators

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With a view to probe the structure and function of G-protein coupled receptors the synthesis of functionalized 8-mercaptoquinoline derivatives and 2-(2-pyridyl)thiophenol was achieved. A fluorescence-based method for determining the affinity of these metal chelators toward zinc ions was developed.

Introduction

Organic molecules capable of chelating metal ions are well-known and used in a variety of disciplines in science, spreading from biology and medicine, via catalysis, to photo industry. Amongst the most common chelators are 2,2′-bipyridines and 1,10-phenanthrolines, which are well characterized and widely used to chelate a variety of metal ions.[1]

G-protein coupled receptors (GPCR) or 7-transmembrane (7TM) receptors represent the most important class of drug targets.[2] The 7TM receptors are not amenable to conventional structure-based drug design, but engineered metal binding sites have proved to be a useful tool to unravel their structure and function.[2] For continued investigations we were in need of various functionalized chelators, where the metal chelator would serve as the bridging moiety between the protein structure containing a metal ion and small molecule fragments capable of interacting with neighbouring binding epitopes in the receptor protein.

Previously, we have described the preparation of a broad selection of 2,2′-bipyridines for such a purpose.[3] We were, however, interested in identifying chelators with stronger binding to zinc ions than 2,2′-bipyridine or even 1,10-phenantroline. Increasing the number of coordinating atoms in the chelator is one way to increase the complex constant, and indeed, as Fig. 1 indicates, this is the case for *e.g.* EDTA. It was in our interest, however, to keep the number of coordinating atoms to two in order to avoid crowding around the metal centre.

8-Hydroxyquinoline is another common chelator, and replacement of the oxygen atom with the softer sulfur atom should increase the strength of the complex. Indeed, 8-mercaptoquinolines have attracted interest due to their ability to chelate many metal ions, and the complexation properties of several alkyl-, aryl-, nitro- and halo-derivatives have been reported.[4] Thus, 8-mercaptoquinoline and a close analogue, 2-(2-pyridyl)-thiophenol, were two of the sulfur-containing chelators we considered. The first is commercially available, while the latter needed to be synthesized. A synthesis for this compound has been published[5] and we saw the opportunity to develop a more efficient route for introduction of the sulfur atom.

Results and discussion

Evaluating the chelating properties[6]

We wanted to investigate the chelating properties of the novel scaffolds in relation to known chelators. Several methods for measuring stability constants of metal complexes have previously been shown to be efficient.[7] Such methods include pH titrations which monitor the mass balances on hydrogen-ions as

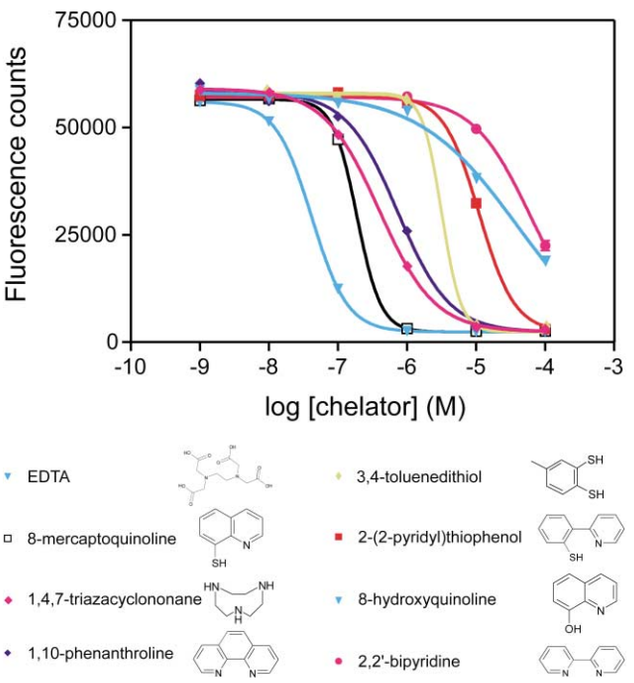

Fig. 1 Binding curves of several zinc-ion chelators.

many metal-ion chelators often are deprotonised upon metal-ion binding, including the sulfur-containing metal chelators examined here. However, as the chelators are the subject for latter biological experiments, we were interested in estimating a conditional stability constant of the chelators at physiological pH (pH = 7.4). However, the pH titration is intractable since it results in stability constants at other pH-values. Alternatively, spectrophotometric properties of the chelator can be used to estimate the stability constant, where the complex formation by metal ions may result in an altered absorption or fluorescent spectra of the ligands. We have also tested if 8-mercaptoquinoline resulted in altered absorption or fluorescent spectra as previously observed for 1,10-phenanthroline and 2,2′-bipyridine.[8] Unfortunately, we did not observe any difference in absorption or fluorescent spectra upon addition of Zn^{2+} to 8-mercaptoquinoline. To estimate if 8-mercaptoquinoline was a stronger Zn^{2+}-chelator than 2,2′-bipyridine ($\log K_1(Zn^{2+}) = 5.1$) or 1,10-phenanthroline ($\log K_1(Zn^{2+}) = 6.4$),[6] we performed a competition experiment between 8-mercaptoquinoline and 2,2′-bipyridine or 1,10-phenanthroline for Zn^{2+}. Upon addition of 8-mercaptoquinoline to 2,2′-bipyridine or 1,10-phenanthroline chelated Zn^{2+} the fluorescent signal was terminated indicating a stronger Zn^{2+}-chelation of 8-mercaptoquinoline

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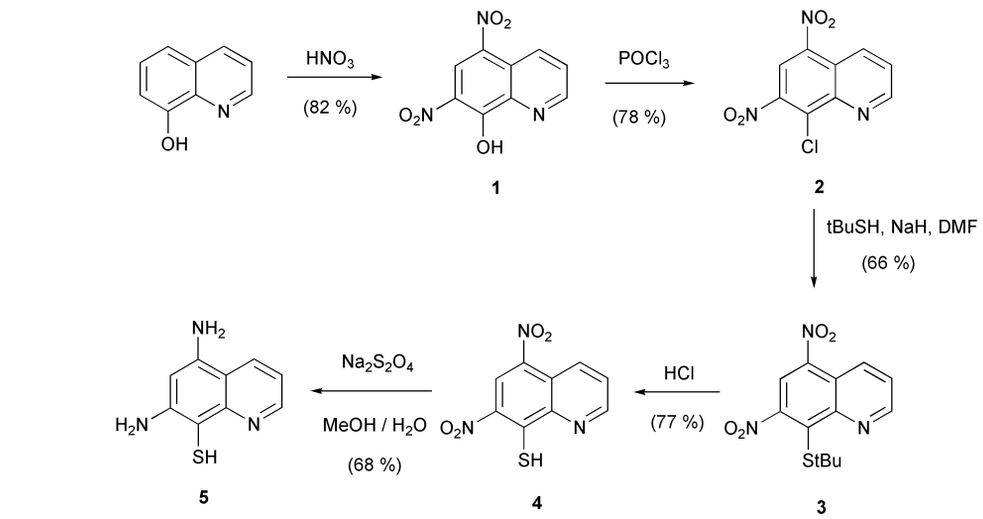
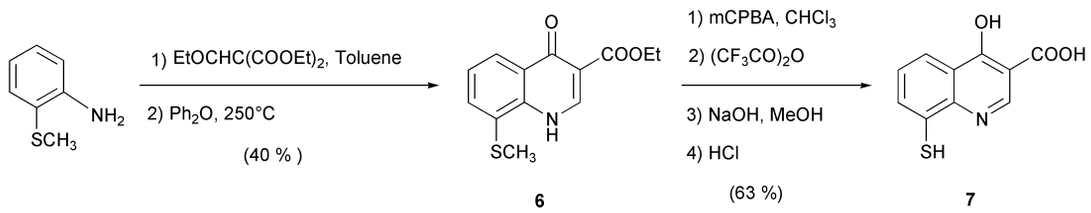


Scheme 1



Scheme 2

compared to 2,2′-bipyridine or 1,10-phenanthroline (data not shown). To further develop this competition assay for Zn^{2+} we used FluoZin-3 as a Zn^{2+}-probe which upon chelation of Zn^{2+} becomes a strong fluorophore. The assay was performed as a competition experiment between a fixed concentration of FluoZin-3 and the different chelators for Zn^{2+} at physiological pH, enabling us to rank the affinity of different chelators according to their Zn^{2+}-affinity.

Results of binding assay

As shown in Fig. 1, the order by which the tested chelators displaced Zn^{2+} from FluoZin-3 was: EDTA > 8-mercaptoquinoline>1,4,7-triazacyclononane>1,10-phenanthroline>3,4-toluenedithiol > 2-(2-pyridyl)thiophenol > 8-hydroxyquinoline = 2,2′-bipyridine. These results support the previous data which indicated that 8-mercaptoquinoline is a stronger Zn^{2+}-chelator than both 2,2′-bipyridine and 1,10-phenanthroline.

Our expectations regarding the suitability of 8-mercaptoquinoline were confirmed, since the fluorescence data indicated that it possessed the highest affinity for Zn^{2+} of the tested bidentate chelators and these results are in agreement with preliminary studies.[4] We know from the literature that 1,10-phenanthroline has a $\log K_1 = 6.4$ for Zn^{2+} (at pH 7.4).[6] Thus the current data indicates that 8-mercaptoquinoline is likely to have a higher $\log K_1$ value than 6.4 as it showed stronger Zn^{2+} binding than 1,10-phenanthroline in the fluorescent assay, yet a lower affinity than EDTA. Fig. 1 also shows that replacing both coordinating groups in a bidentate chelator with sulfur, as in 3,4-toluenedithiolate, did not result in a more potent chelator. The FluoZin-3 competition assay we have established here is a simple method as it only requires a conventional fluorescent plate reader which within few minutes can read a microtiter plate with 96 or 384 data points. A similar approach can be applied to other metal ions, simply by identifying other chelators with fluorescent properties for the applied metal ion. FluoZin-3 is likely to also respond by fluorescence upon chelation of metal ions similar to Zn^{2+}, such as Cu^{2+} and Ni^{2+}.[9]

Based on these results, we concluded that 8-mercaptoquinoline fulfilled our requirements from a chelation point of view. The next step was to develop efficient routes to suitably functionalized 8-mercaptoquinolines which could be used for further derivatisations.

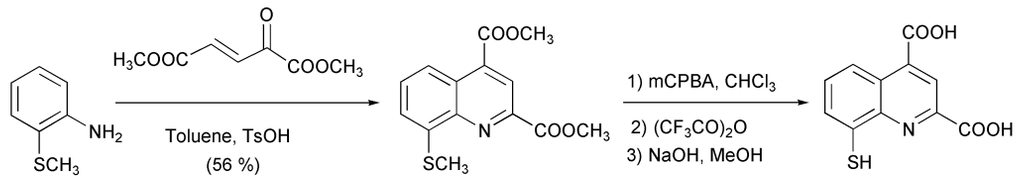
Preparation of sulfur-containing chelators

Synthesis of substituted 8-mercaptoquinolines

The synthesis of quinolines and their derivatives has been of considerable interest for many years,[10] and methods still continue to be developed[11] as a large number of natural products and drugs contain this heterocyclic nucleus. Also substituted 8-mercaptoquinolines are known in the literature, *e.g.* 5-carboxy-8-mercaptoquinolines are described as intermediates for the synthesis of H^+,K^+–ATPase inhibitors.[12] We now wish to report several procedures for the preparation of functionalized 8-mercaptoquinolines.

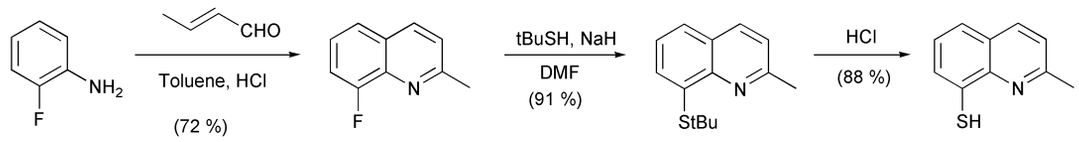
First we focused our interest on the synthesis of 5,7-diamino-8-mercaptoquinoline **5** which was prepared in five steps starting from 8-hydroxyquinoline (Scheme 1). Nitration of 8-hydroxyquinoline afforded the dinitro derivative **1** which was reacted with phosphorus oxychloride to give the known chloroquinoline **2**. Displacement of the chlorine with 2-methyl-2-propanethiol under basic conditions yielded the quinoline **3**, *cf.* the synthesis of compound **14**. The *tert*-butylthio group was then hydrolyzed under acidic conditions and the nitro groups were reduced with sodium dithionite to gain access to the expected product **5** in good yield.

The synthesis of 4-hydroxy-8-mercaptoquinoline-3-carboxylic acid **7** was performed by the Gould–Jacobs reaction sequence[13] (Scheme 2). Thus condensation of 2-(methylthio)aniline with diethylethoxymethylenemalonate followed by intramolecular thermal cyclisation in refluxing diphenyl ether gave the quinolone **6** in 40% yield. The thiomethyl group was then deprotected by expanding upon the one-pot procedure reported by Young *et al.*,[14] involving a Pummerer rearrangement of the corresponding sulfoxide. After hydrolysis of the



8 9

Scheme 3



10 11 12

Scheme 4

ethyl ester, the 8-mercaptoquinoline **7** was obtained in 63% overall yield.

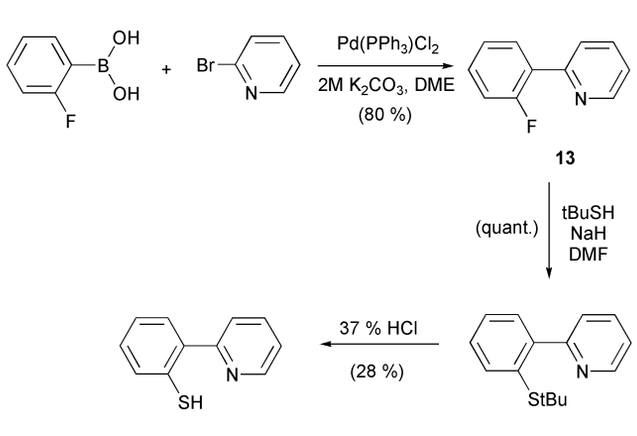
A modified Doebner–Miller sequence[15] between 2-(methylthio)aniline and dimethyl 2-ketoglutaconate[16] allowed us to prepare the diester **8** in 56% yield (Scheme 3). The methylthio group was then cleaved under conditions previously described and the esters saponified to afford 8-mercaptoquinoline-2,4-dicarboxylic acid **9**.

2-Methylquinoline-8-thiol **12** was synthesized in three steps starting from 2-fluoroaniline (Scheme 4). The Doebner–Miller cyclisation of 2-fluoroaniline with crotonaldehyde was carried out in a two-phase solvent system without oxidant, according to the recent procedure of Matsugi *et al.*,[17] and led to the fluoroquinoline **10** in 72% yield. Nucleophilic substitution with 2-methyl-2-propanethiol followed by acidic hydrolysis of the *tert*-butylthio group gave the desired 8-mercaptoquinoline **12**.

These functionalized 8-mercaptoquinolines are now ready for attaching side-chains by the chemistry disclosed in our previous publications.[3]

Synthesis of 2-(2-pyridyl)thiophenol

2-(2-Pyridyl)thiophenol **15** was synthesized in a three-step procedure, starting from 2-fluorophenyl boronic acid (Scheme 5). This acid was coupled to the pyridyl moiety by a Suzuki cross-coupling protocol[18] to yield the fluorobenzene **13** in high yield. The fluorine was then displaced by a sulfur-nucleophile in a nucleophilic aromatic substitution. Various candidates were tried out, and 2-methyl-2-propanethiol turned out to be the most reactive, although the reaction needed to be left for three days for completion to the *tert*-butyl protected compound **14**. Finally, deprotection under strong acidic conditions resulted in the free thiophenolic compound **15**, although in a modest yield.



15 14

Scheme 5

Conclusion

In summary, we have devised synthetic routes to introduce the handles carboxy, amino and hydroxy groups in 8-mercaptoquinolines, which can be used for further elaborations. We have also shown the utility of 2-methyl-2-propanethiol for the introduction of sulfur by nucleophilc displacement. Furthermore, we have developed a method to identify relative binding affinities toward zinc ions for a variety of chelators.

Experimental

General

Melting points (mp) were determined with a Büchi B-545 apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 1600 instrument or Perkin-Elmer FT-IR Spectrum One instrument. ^{1}H and ^{13}C NMR were performed on a Bruker Avance DPX300 spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C). All chemical shifts (δ) are given in ppm relative to the residual deuterated solvent signals. Coupling constants (J) are reported in Hertz. Mass spectra were recorded on a Perkin-Elmer Sciex API 150 spectrometer in the electro-spray ionisation mode. LC-MS was recorded on a Agilent 1100 Series, using a C8 (150 × 4.6 mm) analytical column. Thin-layer chromatography (TLC) was carried out on Merck silica gel $60F_{254}$ precoated plates and flash column chromatography was performed on silica gel Merck 60 (230–400 mesh). All solvents were HPLC grade or purified by distillation. All starting material were commercially available (purchased from Aldrich) and were used without further purification.

Fluorescence based chelator strength assay

For evaluation of the relative chelator strength for Zn^{2+}, a fluorescence based assay was performed. The tested chelator was dissolved in 100 µl buffer (20 mM sodiumphosphate buffer pH 7.4, 100 mM NaCl, 200 µM 2-mercaptoethanol, 20 nM $ZnCl_2$ and 1 µM FluoZin-3 (Molecular Probes, Eugene, OR) and incubated for 2 h at room temperature. The 2-mercaptoethanol was added to reduce dimerised 8-mercaptoquinoline. After incubation the fluorescence of fluozin-3 was measured in a NovoStar reader (BMG, Germany). Each sample in the microtiter plate was measured for 0.2 s with a 485 nm excitation filter (slit = 12 nm) and a 520 nm emission filter (slit = 12 nm). Binding curves was analysed by nonlinear regression using Prism 3.0 (GraphPad Software, San Diego, CA).

5,7-Dinitroquinolin-8-ol (1)[19]

To a solution of 65% HNO_3 (50 mL) cooled at 0 °C was added portion-wise 8-hydroxyquinoline (5 g, 34.4 mmol). The solution was stirred at 40 °C for 1 h and then poured onto ice with

vigorous stirring. The precipitate was filtered off, washed with water and dried over P_2O_5 to provide **1** (6.61 g, 82%) as a greenish solid; mp 316 °C (lit.[19] 318 °C); ν_{max} (KBr)/cm^{-1}: 2956, 1644, 1582, 1547, 1515, 1340, 1252, 1202, 1011, 830, 743; ^{1}H NMR (DMSO-d$_6$): δ_H 4.99 (1H, br s, OH), 8.25 (1H, dd, J = 8.7, 5.0, H-3), 8.92 (1H, dd, J = 5.0, 1.4, H-4), 9.21 (1H, s, H-6), 9.77 (1H, dd, J = 8.7, 1.4, H-2); ^{13}C NMR (DMSO-d$_6$): δ_C 123.1(C), 127.5 (C), 128.1 (CH), 128.2 (CH), 130.4 (CH), 131.1 (C), 138.2 (C), 142.2 (CH), 142.6 (CH). *m*/*z* 236 (M + 1).

8-Chloro-5,7-dinitroquinoline (2)[20]

A solution of **1** (1 g, 4.25 mmol) in excess phosphorus oxychloride (50 mL) was refluxed for 2 h. After removing of the excess phosphorous oxychloride under reduced pressure, the residue was poured onto ice and the obtained precipitate was filtered off, washed with water and dried over P_2O_5 to provide **2** (840 mg, 78%) as a yellow solid; mp 152 °C (lit.[20] 152–153 °C); ν_{max} (KBr)/cm^{-1}: 3064, 1561, 1531, 1401, 1332, 1009, 964, 820, 809, 786; ^{1}H NMR ($CDCl_3$): δ_H 7.90 (1H, dd, J = 8.9, 4.1, H-3), 8.80 (1H, s, H-6), 9.19 (1H, dd, J = 8.9, 1.5, H-4), 9.31 (1H, dd, J = 4.1, 1.5, H-2); ^{13}C NMR ($CDCl_3$): δ_C 119.1 (CH), 122.6 (C), 122.8 (C), 125.4 (C), 126.0 (CH), 132.3 (CH), 134.3 (C), 144.3 (C), 153.2 (CH). *m*/*z* 254 (M + 1).

8-(*tert*-Butylthio)-5,7-dinitroquinoline (3)

To a solution of **2** (200 mg, 0.79 mmol) in THF (20 mL) was added 2-methyl-2-propanethiol (0.18 mL, 1.58 mmol) and triethylamine (0.22 mL, 1.58 mmol). The resulting mixture was refluxed for 12 h. After evaporation of the solvent, flash chromatography (hexane–EtOAc (98 : 2)) provided **5** (159 mg, 66%) as an orange solid; mp 138 °C; Found: C, 50.96; H, 4.38; N, 13.81; S, 10,66. $C_{13}H_{13}N_3O_4S$ requires C, 50.81; H, 4.26; N, 13.67; S, 10.43%; ν_{max} (KBr)/cm^{-1}: 2963, 1526, 1401, 1367, 1333, 1162, 821, 792; ^{1}H NMR ($CDCl_3$): δ_H 1.37 (9H, s, $SC(CH_3)_3$), 7.81 (1H, dd, J = 8.8, 4.1, H-3), 8.47 (1H, s, H-6), 9.05 (1H, m, H-4), 9.30 (1H, dd, J = 3.8, 1.1, H-2); ^{13}C NMR ($CDCl_3$): δ_C 31.6 (CH_3); 52.8 (C); 117.3 (CH), 121.4 (C), 121.6 (C), 124.8 (CH), 132.1 (CH), 145.4 (C), 149.6 (C), 149.8 (C), 152.8 (CH). *m*/*z* 308 (M + 1).

5,7-Dinitroquinoline-8-thiol (4)

A solution of **3** (300 mg, 0.98 mmol) in 35% HCl was heated at 100 °C for 15 h. The mixture was then cooled to 0 °C and the pH adjusted to 8 by addition of aqueous 6 M NaOH. After extraction with EtOAc, the organic layer was dried on $MgSO_4$, filtered and evaporated. Purification by flash chromatography (dichloromethane–EtOAc (9 : 1)) provided **4** (188 mg, 77%) as an orange solid; mp 148 °C; Found: C, 43.25; H, 2.18; N, 16.81; S, 12.98. $C_9H_5N_3O_4S$ requires C, 43.03; H, 2.01; N, 16.73; S, 12.76%; ν_{max} (KBr)/cm^{-1}: 3183, 2504, 1639, 1589, 1526, 1446, 1330, 890, 854, 801, 756; ^{1}H NMR (DMSO-d$_6$): δ_H 3.10 (1H, br s, SH), 7.37 (1H, m, H-3), 7.94 (1H, s, H-6), 8.58 (1H, m, H-4), 8.78 (1H, m, H-2); ^{13}C NMR (DMSO-d$_6$): δ_C 112.3 (C), 112.7 (CH), 116.0 (CH), 119.2 (CH), 145.6 (C), 148.0 (C), 148.2 (C), 149.4 (C), 151.5 (CH). *m*/*z* 252 (M + 1).

5,7-Diaminoquinoline-8-thiol (5)

Sodium dithionite (1.11 g, 6.40 mmol) was added in small portions over 30 min to a solution of **4** (160 mg, 0.64 mmol) in methanol–water (30 mL, 1 : 1). The resulting red precipitate was filtered off and washed with water. After recrystallisation from ethanol, **5** was obtained as a dark red solid (188 mg, 77%); mp 260 °C (decomp.); Found: C, 56.70; H, 4.80; N, 22.12; S, 16,91. $C_9H_9N_3S$ requires C, 56.52; H, 4.74; N, 21.97; S, 16.76%; ν_{max} (KBr)/cm^{-1}: 3470, 3330, 2904, 1630, 1585, 1536, 1443, 1233, 1154, 824, 776; ^{1}H NMR (DMSO-d$_6$): δ_H 3.29 (1H, br s, SH), 6.16 (1H, s, H-6), 7.10 (1H, m, H-3), 8.32 (1H, m, H-4), 8.70 (1H, m, H-2); ^{13}C NMR (DMSO-d$_6$): δ_C 98.0 (CH), 111.8 (CH), 114.9 (C), 115.2 (C), 139.0 (CH), 140.6 (CH), 141.3 (C), 147.1 (C), 156.1 (C). *m*/*z* 192 (M + 1).

Ethyl 8-(methylthio)-4-oxo-1,4-dihydroquinoline-3-carboxylate (6)[21]

To a solution of 2-(methylthio)aniline (1 g, 7.18 mmol) in toluene (80 mL) was added diethyl ethoxymethylenemalonate (2.33 g, 10.78 mmol) and the mixture was refluxed for 2 h. The solvent was evaporated and the residue was added over 30 min to a solution of diphenyl ether (40 mL) heated at 250 °C. The mixture was kept under reflux for 1 h, quickly cooled to 50 °C and diluted with hexane. The resulting brown precipitate was filtered off, washed with hexane and dried under reduced pressure to provide **6** as a pale brown solid (765 mg, 40%); mp 202 °C (lit.[21] 206–207 °C); ν_{max} (KBr)/cm^{-1}: 3149, 2980, 1711, 1604, 1529, 1437, 1380, 1283, 1190, 1110, 1025, 930, 773, 742; ^{1}H NMR (DMSO-d$_6$): δ_H 1.27 (3H, t, CH_3, J = 7.0), 2.53 (3H, s, SCH_3), 4.21 (2H, q, CH_2, J = 7), 7.39 (1H, m), 7.79 (1H, m), 8.06 (1H, m), 8.45 (1H, m, H-2), 11.6 (1H, br s, NH); ^{13}C NMR (DMSO-d$_6$): δ_C 14.2 (CH_3), 17.3 (SCH_3), 59.6 (CH_2), 110.0 (C), 118.5 (C), 124.6 (CH), 126.5 (C), 127.7 (C), 129.9 (CH), 133.9 (CH). 137.6 (C), 145.1 (CH), 164.4 (CO), 173.3 (CO). *m*/*z* 264 (M + 1).

4-Hydroxy-8-mercaptoquinoline-3-carboxylic acid (7)

m-Chloroperbenzoic acid (180 mg, 1.04 mmol) was added, at 0 °C, to a stirred solution of **6** (250 mg, 0.95 mmol) in chloroform (50 mL) over 30 min. The mixture was then allowed to warm to room temperature and stirred for 1 h. A saturated $NaHCO_3$ solution was added, the organic layer was separated, dried over $MgSO_4$, filtered and evaporated. The crude sulfoxide was dissolved in an excess of trifluoroacetic anhydride (10 mL) and the solution refluxed for 30 min. After evaporation of the anhydride, the residue was dissolved in methanol (30 mL) and a solution of NaOH 1M (3 mL) was added. The mixture was refluxed for 1 h, cooled to room temperature and the methanol evaporated. A 33% HCl solution was added dropwise to the aqueous layer at 0 °C. The white precipitate which was collected was filtered off, washed with water and dried over P_20_5 to provide **7** (132 mg, 63%) as a white solid; mp 278–280 °C; Found : C, 54.40; H, 3.22; N, 6.39; S, 14.62. $C_{10}H_7NO_3S$ requires C, 54.29; H, 3.19; N, 6.33; S, 14.49%; ν_{max} (KBr)/cm^{-1}: 3060, 2516, 1709, 1606, 1553, 1470, 1376, 1332, 1271, 1202, 1066, 937, 781, 685; ^{1}H NMR (DMSO-d$_6$): δ_H 3.24 (1H, br s, SH), 7.41 (1H, m, H-6), 7.54 (1H, m, H-7), 8.36 (1H, m, H-5), 8.56 (1H, s, H-2), 12.6 (1H, br s, OH); ^{13}C NMR (DMSO-d$_6$): δ_C 108.2 (C), 124.5 (C), 125.7 (C), 125.9 (CH), 128.2 (CH), 139.7 (C), 141.5 (CH). 145.2 (CH), 165.7 (C), 178.3 (CO). *m*/*z* 222 (M + 1).

Dimethyl 8-(methylthio)quinoline-2,4-dicarboxylate (8)

To a stirred solution of 2-(methylthio)aniline (200 mg, 1.44 mmol) and dimethyl 2-ketoglutaconate[16] (297 mg, 1.72 mmol) in toluene (30 mL), *p*-toluenesulfonic acid (55 mg, 0,29 mmol) was added and the mixture was refluxed for 4 h. After cooling to room temperature and evaporation of the solvent, the residue was dissolved in EtOAc, washed with saturated $NaHCO_3$ followed by 0.1 M HCl. The organic layer was separated, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography (dichloromethane–EtOAc (8 : 2)) provided the diester **8** as a white solid (235 mg, 56%); mp 128 °C; Found: C, 57.84; H, 4.62; N, 4.93; S, 11.22. $C_{14}H_{13}NO_4S$ requires C, 57.72; H, 4.50; N, 4.81; S, 11.01%; ν_{max} (KBr)/cm^{-1}: 2944, 1719, 1434, 1239, 1154, 1137, 818, 761, 712; ^{1}H NMR ($CDCl_3$): δ_H 2.60 (3H, s, SCH_3), 4.07 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 7.35 (1H, m, H-6), 7.70 (1H, m, H-7), 8.55 (1H, m, H-5), 8.72 (1H, s, H-3); ^{13}C NMR ($CDCl_3$): δ_C 13.8 (SCH_3), 52.4 (OCH_3), 52.7 (OCH_3), 61.6 (C),

120.1 (CH), 122.2 (CH), 123.1 (CH), 126.1 (C), 129.7 (CH), 135.6 (C), 142.4 (C), 145.1 (C), 145.3 (C), 164.7 (CO), 165.4 (CO). *m*/*z* 292 (M + 1).

8-Mercaptoquinoline-2,4-dicarboxylic acid (9)

To a stirred solution of **8** (200 mg, 0.69 mmol) in chloroform (80 ml) at 0 °C was added *m*-chloroperbenzoic acid (130 mg, 0.76 mmmol) over 30 min. The mixture was then allowed to warm to room temperature and stirred for 1 h. A saturated $NaHCO_3$ solution was added, the organic layer was separated, dried on $MgSO_4$ and evaporated. The crude sulfoxide was dissolved in excess of trifluoroacetic anhydride (5 mL) and the solution refluxed for 30 min. After evaporation of the excess anhydride, the residue was dissolved in methanol (30 mL) and a solution of 1 M NaOH (3 ml) was added. The mixture was refluxed for 1 h, cooled to room temperature and the methanol evaporated. A 33% HCl solution was added dropwise to the aqueous phase at 0 °C. The white precipitate obtained was collected by filtration, washed with water and dried over P_2O_5 to provide **9** (112 mg, 65%) as a white solid; mp 310 °C (decomp.); Found : C, 53.14; H, 2.90; N, 5.78; S, 13,02; $C_{11}H_7NO_4S$ requires C, 53.01; H, 2.83; N, 5.62; S, 12.86%; ν_{max} (KBr)/cm^{-1}: 2923, 2580, 1704, 1597, 1445, 1261, 1203, 1154, 821, 764; ^{1}H NMR (DMSO-d$_6$): δ_H 3.19 (1H, br s, SH), 7.62 (1H, m, H-6), 7.78 (1H, m, H-7), 8.53 (1H, m, H-5), 8.85 (1H, s, H-3), 12.7 (1H, br s, OH), 12.9 (1H, br s, OH); ^{13}C NMR (DMSO-d$_6$): δ_C 122.0 (CH), 123.2 (CH), 126.1 (C), 126.5 (CH), 130.0 (CH), 136.3 (C), 137.7 (C), 145.0 (C), 147.7 (C), 165.4 (CO), 166.7 (CO); *m*/*z* 250 (M + 1).

8-Fluoro-2-methylquinoline (10)

Toluene (30 mL) and crotonaldehyde (3.71 mL, 45 mmol) were added to a solution of 2-fluoroaniline (2.5 g, 22.50 mmol) in aqueous 6 M HCl (100 mL) and heated at 100 °C. Heating was continued for 2 h and the mixture was then allowed to cool to room temperature. The aqueous layer was separated and neutralized with 6 M NaOH solution. After extraction with dichloromethane (2 × 50 mL), the organic layer was separated, dried over $MgSO_4$, filtered and evaporated. Purification by flash chromatography (hexane–EtOAc (8 : 2)) provided **10** (2.6 g, 82%) as a colourless oil; Found: C, 74.68; H, 5.18; F, 11.94; N, 8.88. $C_{10}H_8FN$ requires C, 74.52; H, 5.00; F, 11.79; N, 8.69%; ν_{max} (film)/cm^{-1}: 3056, 2970, 1607, 1567, 1504, 1476, 1430, 1327, 1235, 1083, 833, 755; ^{1}H NMR ($CDCl_3$): δ_H 2.75 (3H, s, CH_3), 7.30–7.36 (3H, m), 7.49 (1H, m), 7.99 (1H, d, *J* = 9.9, H-4); ^{13}C NMR ($CDCl_3$): δ_C 25.0 (CH_3), 112.8 (CH), 122.5 (CH), 122.6 (CH), 124.8 (CH), 127.6 (C), 135.3 (CH), 137.4 (C). 155.3 (C), 158.8 (C). *m*/*z* 162 (M + 1).

8-(*tert*-Butylthio)-2-methylquinoline (11)

2-Methyl-2-propanethiol (0.42 ml, 3.72 mmol) and sodium hydride (149 mg, 3.72 mmol) were added to a solution of **10** (300 mg, 1.86 mmol) in DMF (30 mL). The resulting mixture was refluxed for 12 h. After evaporation of the solvent, the residue was dissolved in EtOAc and washed with water (2 × 50 mL). The organic layer was separated, dried over $MgSO_4$, filtered and evaporated. Purification by flash chromatography (dichloromethane–EtOAc (95 : 5)) provided **11** (392 mg, 91%) as a yellow solid; mp 32 °C; Found: C, 72.82; H, 7.53; N, 6.11; S, 14.02. $C_{14}H_{17}NS$ requires C, 72.68; H, 7.41; N, 6.05; S, 13.86%; ν_{max} (KBr)/cm^{-1}: 2962, 1597, 1547, 1494, 1421, 1360, 1313, 1237, 1154, 1013, 976, 838, 797, 771, 662; ^{1}H NMR ($CDCl_3$): δ_H 1.38 (9H, s, $C(CH_3)_3$), 2.79 (3H, s, CH_3), 7.29 (1H, d, *J* = 8.0, H-3), 7.41 (1H, dd, *J* = 7.7, 7.8, H-6), 7.73 (1H, d, *J* = 8.0, H-4), 7.97–8.06 (2H, m); ^{13}C NMR ($CDCl_3$): δ_C 25.1 (CH_3), 30.9 ($3CH_3$), 46.4 (C), 121.6 (CH), 124.5 (CH), 126.5 (C), 128.0 (CH), 132.7 (C), 136.0 (CH), 137.6 (CH), 148.3 (C), 158.7 (C). *m*/*z* 232 (M + 1).

2-Methylquinoline-8-thiol (12)

A solution of **11** (170 mg, 0.73 mmol) in 35% HCl solution (50 mL) was heated at 90 °C for 12 h. The mixture was then allowed to cool to room temperature and the pH adjusted to 8 by addition of aqueous 6 M NaOH. After extraction with EtOAc, the organic layer was dried over $MgSO_4$, filtered and evaporated. Purification by flash chromatography (dichloromethane–EtOAc (95 : 5)) afforded **12** (113 mg, 88%) as a yellow solid; mp 260–262 °C; Found: C, 68.62; H, 5.23; N, 8.11; S, 18.52. $C_{10}H_9NS$ requires C, 68.54; H, 5.18; N, 7.99; S, 18.30%; ν_{max} (KBr)/cm^{-1}: 2919, 1597, 1497, 1418, 1370, 1315, 1242, 1196, 1134, 1013, 974, 823, 785, 760, 663; ^{1}H NMR ($CDCl_3$): δ_H 2.85 (3H, s, CH_3), 7.31–7.39 (2H, m), 7.57 (1H, m), 7.86 (1H, m, H-7), 8.06 (1H, d, *J* = 8.4, H-4); ^{13}C NMR ($CDCl_3$): δ_C 24.8 (CH_3), 122.1 (CH), 124.0 (CH), 124.3 (CH), 125.4 (CH), 125.9 (C), 134.1 (C), 135.7 (CH). 145.0 (C), 158.0 (C); *m*/*z* 176 (M + 1).

2-(2-Pyridyl)fluorobenzene (13)

2-Fluorophenylboronic acid (3 g, 21.4 mmol) and 2-bromopyridine (1.64 mL, 17.2 mmol) were dissolved in aqueous 2 M K_2CO_3 (20 mL) and DME (40 mL). The mixture was degassed for approximately 30 min. $Pd(PPh_3)_2Cl_2$ was added and the mixture was heated to 80 °C overnight. The reaction mixture was allowed to cool to room temperature, filtered through Celite, and partioned between H_2O (200 mL) and EtOAc (200 mL). The organic layer was separated and dried over $MgSO_4$, filtered, and evaporated under vacuum. Purification by flash chromatography (dichloromethane–10% NH_3 in methanol (10 : 0.2)) provided **13** as a light orange oil (2.4 g, 80%); Found: C, 76.42; H, 4.78; F, 11.12; N, 8.27. $C_{11}H_8FN$ requires C, 76.29; H, 4.66; F, 10.97; N, 8.09%; ν_{max} (film)/cm^{-1}: 3054, 3011, 1615, 1588, 1494, 1464, 1456, 1427, 1303, 1248, 1220, 1208, 1154, 1111, 1095, 1061, 1025, 990, 947, 862, 827, 792, 757, 719, 611; ^{1}H NMR ($CDCl_3$): δ_H 7.13–7.23 (1H, ddd, *J* = 1.3, 8.1, 11.5), 7.24–7.33 (2H, m), 7.34–7.45 (1H, m), 7.73–7.85 (2H, m), 7.95–8.04 (1H, dt, *J* = 2.0, 7.9) and 8.71–8.78 (1H, m, H-2); ^{13}C NMR ($CDCl_3$): δ_C 116.1 (d, *J* = 22.5, CH), 122.3 (CH), 124.4 (d, *J* = 2.7, CH), 124.5 (d, *J* = 2.8, CH), 127.4 (d, *J* = 11.5, C), 130.4 (d, *J* = 8.2, CH), 131.0 (d, *J* = 2.7, CH), 136.3 (CH), 149.7 (CH), 153.3 (d, *J* = 2.2, C), 160.4 (d, *J* = 249.7, C); *m*/*z* 174 (M + 1).

2-(2-(*tert*-Butylthio)phenyl)pyridine (14)

DMF (10 mL) was degassed for approximately 1 h. Sodium hydride (231 mg, 5.77 mmol) and 2-methyl-2-propanethiol (715 μL, 5.77 mmol) were added and formation of gas was observed. The mixture was stirred for about 10 min. 2-(2-Pyridyl)fluorobenzene, **13**, (500 mg, 2.89 mmol) was added, and the reaction mixture turned orange. The mixture was heated to 120 °C for 3 days under N_2 atmosphere. The mixture was allowed to cool to room temperature and extracted with H_2O (50 mL) and EtOAc (70 mL). The organic layer was washed with H_2O (50 mL), dried over $MgSO_4$, filtered and evaporated under vacuum at 60 °C providing **14** as an orange oil (747 mg, quant.); Found: C, 74.32; H, 7.26; N, 5.82; S, 13.33. $C_{15}H_{17}NS$ requires C, 74.03; H, 7.04; N, 5.76; S, 13.17%; ν_{max} (film)/cm^{-1}: 3412 (br), 2257, 2130, 1658 (br), 1204, 1135, 1049, 1026, 1002, 827, 765; ^{1}H NMR ($CDCl_3$): δ_H 1.05 (9H, s), 7.22–7.27 (1H, ddd, *J* = 1.5, 4.9, 7.0), 7.35–7.43 (1H, dt, *J* = 1.7, 7.5), 7.46–7.53 (1H, dt, *J* = 1.3, 7.5), 7.64–7.76 (4H, m), 8.67–8.72 (1H, m, H-2); ^{13}C NMR ($CDCl_3$): δ_C 30.9 (CH_3), 47.5 ($C(CH_3)_3$), 121.7 (CH), 126.8 (CH), 128.1 (CH), 129.2 (CH), 130.7 (C), 130.8 (CH), 134.8 (CH), 139.5 (CH), 147.1 (C), 148.9 (CH), 159.3 (C); *m*/*z* 244 (M + 1).

2-(2-Pyridyl)thiophenol (15)

A solution of *tert*-butyl-2-(2-pyridyl)thiophenol **14** (200 mg, 0.82 mmol) in conc. HCl (4 mL) was heated at 110 °C overnight.

The mixture was allowed to cool to room temperature and extracted with H_2O (15 mL) and EtOAc (20 mL). The aqueous layer was separated and the pH was adjusted to approximately 7, and extracted with EtOAc (2 × 20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated under vacuum at 40 °C. The product was purified by chromatography on Silica (EtOAc–heptane (1 : 1)) providing **15** as a pale wax (43 mg, 28%); Found: C, 70.02; H, 4.21; N, 7.52; S, 16.87. $C_{11}H_9NS$ requires C, 70.55; H, 4.84; N, 7.48; S, 17.12%; ν_{max} (film)/cm^{-1}: 3053, 3007, 1583, 1568, 1478, 1461, 1432, 1421, 1296, 1257, 1154, 1131, 1095, 1076, 1036, 1021, 990, 945, 894, 870, 794, 751, 679, 621; ^{1}H NMR ($CDCl_3$): δ_H 7.25–7.35 (3H, m), 7.50–7.55 (1H, m), 7.59–7.65 (1H, m), 7.76–7.85 (2H, m) and 8.73–8.77 (1H, m, H-2); ^{13}C NMR ($CDCl_3$): δ_C 122.4 (CH), 123.5 (CH), 126.3 (CH), 127.7 (CH), 129.4 (CH), 129.4 (CH), 136.6 (CH), 136.9 (C), 139.2 (C), 148.7 (CH), 157.8 (C); *m*/*z* 186 (M-1).

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