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Synthesis and evaluation of thiol probes using the 6,7-dimethoxyquinoxalin-2(1*H*)-one fluorophore

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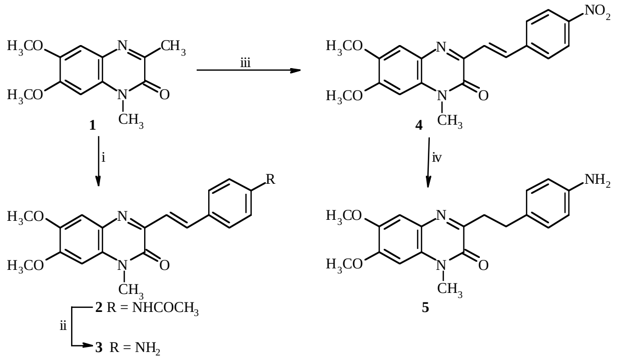
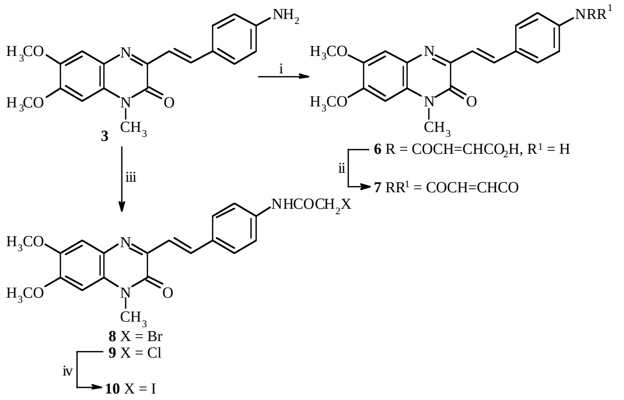
3-Substituted quinoxalin-2(1*H*)-ones, **7** and **10**, were prepared as candidate fluorescent probes for thiols and then reacted with representative thiols to give sulfides, **11**–**17**, whose fluorescence was studied.

The detection and estimation of thiols is an important procedure in environmental and biochemical investigations.[1] The usual reactive centres in fluorescent probes for thiols are maleimide or iodoacetate.[2–6] We have linked the reactive centre to the fluorophore through a chain conjugated with the quinoxalinone fluorophore so that the reactive centre is bonded to an aromatic nucleus. The molecular architecture was designed to give a degree of flexibility in the chain, an increased fluorescence emission maximum compared with that from a saturated chain, and to provide ease of formation of the maleimide and iodoacetate reactive centres.

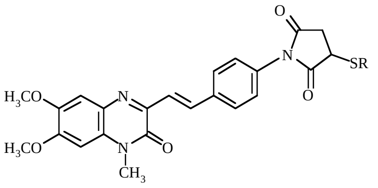
Condensation of 6,7-dimethoxy-1,3-dimethylquinoxalin-2(1*H*)-one, **1**,[13] with 4-acetamidobenzaldehyde and 4-nitrobenzaldehyde gave **2** and **4**, respectively (Scheme 1). Attempts to reduce the nitro group of **4** without reducing the olefinic function were unsuccessful and only **5** was obtained. The required amine **3** was prepared by hydrolysis of **2**. The reaction of **3** with maleimide in chloroform[20] gave the maleimic acid **6**, which was cyclised to the thiol probe, **7** (Scheme 2). The amine **3** was converted into the haloacetamides **8** and **9** by reaction of haloacetyl halides and **9** was used as a starting material for the preparation of the thiol probe **10**. Reaction of butanethiol, thiophenol, 2-hydroxyethanethiol and *N*-acetylcysteine with **7** gave the sulfides **11**–**14**, respectively, and reaction of the first three with **10** gave **15**–**17**.



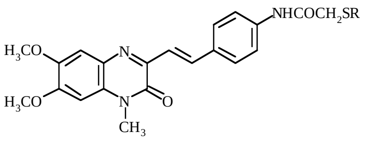
Scheme 1 Reagents and conditions: (i) 4-$CH_3CONHC_6H_4CHO$, $C_5H_{11}N$, AcOH, MeOH, N_2, reflux, 24h, 42%; (ii) aq. alc. KOH, reflux, 24h, 79%; (iii) 4-nitrobenzaldehyde, Ac_2O, reflux, 24h, 42%; (iv) PtO_2, H_2, AcOEt, r.t., 72%.



Scheme 2 Reagents and conditions: (i) maleic anhydride, $CHCl_3$, reflux, 24h, 97%; (ii) AcONa, Ac_2O, reflux, 24h, 47%; (iii) $Pr_2{}^iEtN$, THF, -40°C, $BrCH_2COCl$, 37% for **8** and $ClCH_2COCl$, 65% for **9**; (iv) NaI, acetone, N_2, reflux, 24h, 29%.



11 R = $(CH_2)_3CH_3$
12 R = Ph
13 R = CH_2CH_2OH
14 R = $CH_2CH(CO_2H)NHCOCH_3$



15 R = $(CH_2)_3CH_3$
16 R = Ph
17 R = CH_2CH_2OH

Fluorescence studies were carried out on **1**, **3**, **7**, **10** and **11**–**17**. The conjugated chain as the substituent on the quinoxalinone nucleus in **3** produced the expected bathochromic shift in the fluorescence emission maximum (λ_{em} for **1** in methanol and dichloromethane: 431 and 427 nm, respectively, but the corresponding values for **3** were 493 and 512 nm). The sulfides **15**–**17** in methanol had λ_{em} 492–493 and fluorescence quantum yield (ϕ_f) 0.24–0.29 which are little different from the values of λ_{em} 491 and ϕ_f 0.25 obtained for the probe **10**. In dichloromethane solution, there was little change in λ_{em} but an increase in ϕ_f to 0.50–0.54 for **15**–**17** compared with ϕ_f 0.25 for **10**.

Methanolic solutions of **7** showed little fluorescence (λ_{em} 495 nm, ϕ_f 0.02), presumably due to intersystem crossing between the excited π* state of the fluorophore and the n→π* state of the conjugated system of the maleimide.[7,22,23] Potentially usefully, the thiol adducts **11** to **14** showed a significant increase in both the emission maximum and the quantum yield (λ_{em} 499–504 nm, ϕ_f 0.4–0.54).

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Techniques used: ^1H NMR spectroscopy, IR and fluorescence spectroscopy, mass spectrometry, elemental analysis.

References: 23.

Schemes: 2.

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Tables: 2

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