Syntheses of Fluorescent Analogs of NSC73306, a Dual-Mode Agent against p-Glycoprotein-Expressing Cells, for Mechanistic Studies of its Actions

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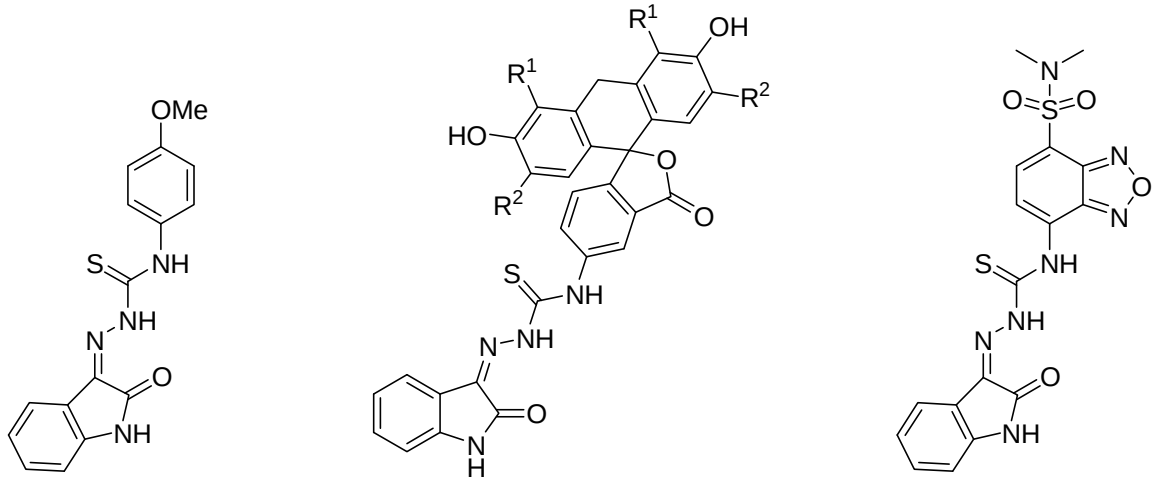
Background

Multi-drug resistance (MDR) presents a significant challenge for the development of new drugs and for current chemotherapy of cancer.[1] Although some agents have shown promise *in vitro* for circumventing MDR by inhibition of efflux transports (p-glycoprotein), subsequent clinical trials against leukemias and lymphomas have not been fully successful, perhaps in some part due to interference with endogenous function of p-glycoprotein in normal tissues.[2] An alternative strategy for resolving MDR is to develop novel agents that exploit the expression of p-glycoprotein and this has led to discovery of NSC73306, a lead compound that selectively kills high levels of p-glycoprotein expressing cells.[2] NSC73306 has been shown to act effectively in two ways, by eliminating p-glycoprotein over-expressing cells and by acting as a modulator that is capable of resensitizing ABCG2-expressing cancer cells to chemotherapy agents.[3] In order to further investigate mechanisms of NSC73306 action, fluorescent derivatives **1-4** were made by replacing the phenol methyl ether segment of the original agent with fluorescein or nitrobenzoxadiazole fluorophores (Figure 1). The following describes the syntheses of these fluorescent NSC73306 analogs.



NSC73306 **1** R^1 = H, R^2 = H
2 R^1 = Br, R^2 = Br
3 R^1 = H, R^2 = F **4**

Figure 1. Structures of NSC73306 and its fluorescent analogs **1-4**.

Chemistry

Parallel synthesis of the compounds **1-4** was performed by one-step conjugation of commercially available isatin 3-hydrazone with the respective isothiocyanate in the presence of diisopropylethylamine (DIPEA). The products were purified by preparative HPLC.

Experimental

General. APCI mass spectrometry (APCI-MS) was performed on LC/MSD TrapXCl Agilent Technologies instrument. Analytical and preparative HPLC analysis was performed on Agilent 1200 series instrument equipped with multi-wavelength detector using an Agilent Eclipse XDB C18, 5.0 μm, 4.6 x 150 mm column and 21.2x50 mm column. Solvent A was 0.1% TFA in water, Solvent B was 0.1% TFA in acetonitrile, and a linear gradient of 5%B to 95%B over 12 min was used. ^{1}H-NMR spectra was recorded with a Varian spectrometer at 400MHz. Chemical shifts are reported in parts per million (δ) and are referenced to tetramethylsilane (TMS). All chemicals are commercially available from Aldrich or Invitrogen.

(Z)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)-5-(2-(2-oxoindolin-3ylidene)hydrazinecarbothio-amido)benzoic acid (1). Isatin 3-hydrazone (31 mg, 0.19 mol), fluorescein isothiocyanate (82

mg, 0.21 mmol) and DIPEA (42 μL, 0.24 mmol) were dissolved in dimethylformamide (4 mL). After stirring for 48 hours a yellow solid had precipitated. Dimethylsulfoxide (3 mL) was added to dissolve the precipitate. The resulting solution was purified by HPLC to provide a yellow powder (62 mg, yield 59%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.00 (s, 1H), 11.36 (s, 1H), 11.14 (s, 1H), 10.21 (br, 2H), 8.43 (d, 1H, J = 1.8 Hz), 8.17 (dd, 1H, J = 1.8 Hz and 8.3 Hz), 7.84 (d, 1H, J = 7.4 Hz), 7.46 (dt, 1H, J = 1.2 Hz and 7.8 Hz), 7.39 (d, 1H, J = 8.2 Hz), 7.20 (t, 1H, J = 7.5 Hz), 7.02 (d, 1H, J = 7.8 Hz), 6.75 (d, 2H, J = 2.2 Hz), 6.69-6.63 (m, 4H). MS (*m/z*): 551.3 $(M+H)^+$.

(Z)-5-(2-(2-oxoindolin-3-ylidene)hydrazinecarbothioamido)-2-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (2). Isatin 3-hydrazone (25 mg, 0.16 mmol) and eosin isothiocyanate (99 mg, 0.14 mmol) were treated in the same manner as in the preparative procedure for **1** to provide **2 (**56 mg, 46% yield) as an orange powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.95 (s, 1H), 11.30 (s, 1H), 11.11 (s, 1H), 10.85 (br, 2H), 8.43 (s, 1H), 8.16 (dd, 1H, J = 1.5 Hz and 8.3 Hz), 7.79 (d, 1H, J = 7.6 Hz), 7.54 (d, 1H, J = 8.2 Hz), 7.40 (t, 1H, J = 7.7 Hz), 7.14 (t, 1H, J = 7.5 Hz), 7.11 (d, 1H, J = 10.0 Hz), 6.98 (s, 2H). MS (*m/z*): 866.9 $(M+H)^+$.

(Z)-2-(4,5-difluoro-6-hydroxy-3-oxo-3H-xanthen-9-yl)-5-(2-(2-oxoindolin-3-ylidene) hydrazinecarbothioamido)benzoic acid (3). Isatin 3-hydrazone (40 mg, 0.25 mmol) and eosin isothiocyanate (86 mg, 0.20 mmol) were treated in the same manner as the preparative procedure for **1** to provide **3 (**67 mg, 57% yield) as an orange powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.98 (s, 1H), 11.33 (s, 1H), 11.05 (s, 1H), 10.82 (br, 2H), 8.37 (dd, 1H, J = 1.8 Hz and 8.4 Hz), 8.10 (d, 1H, J = 1.5 Hz), 7.78 (d, 1H, J = 7.4 Hz), 7.67 (d, 1H, J = 1.2 Hz), 7.44 (t, 1H, J = 7.6 Hz), 7.14 (t, 1H, J = 7.6 Hz), 7.01-6.95 (m, 3H), 6.87 (d, 2H, J = 9.3 Hz). MS (*m/z*): 587.3 $(M+H)^+$.

(Z)-N-(7-(N,N-dimethylsulfamoyl)benzo[c][1,2,5]oxadiazol-4-yl)-2-(2-oxoindolin-3-ylidene)hydrazinecarbothioamide (4). Isatin 3-hydrazone (110 mg, 0.68 mmol) and NBD isothiocyanate (184 mg, 0.65 mmol) were treated in the same manner as the preparative procedure for **1** to provide **4 (**58 mg, 20% yield) as a yellow powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.17 (s, 1H), 11.31 (s, 1H), 11.21 (br, 1H), 8.42 (d, 1H, J = 6.9 Hz), 8.14 (d, 1H, J =

7.6 Hz), 7.62 (d, 1H, *J* = 7.4 Hz), 7.38 (dt, 1H, *J* = 0.9 Hz and 7.7 Hz), 7.11 (t, 1H, *J* = 7.5 Hz), 6.93 (d, 1H, *J* = 7.8 Hz), 2.79 (s, 6H). MS (*m/z*): 446.8 $(M+H)^+$.

References

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