Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 77–86
Bioorganic & Medicinal Chemistry

Synthesis of 5-deazaflavin derivatives and their activation of p53 in cells

Jennifer M. Wilson,[a] Graham Henderson,[a] Fiona Black,[a] Andrew Sutherland,[a] Robert L. Ludwig,[b] Karen H. Vousden[b] and David J. Robins[a,*]

[a]*WestCHEM, Department of Chemistry, The Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, UK*
[b]*The Beatson Institute for Cancer Research, Switchback Road, Glasgow G61 1BD, UK*

Received 12 May 2006; revised 5 October 2006; accepted 10 October 2006
Available online 12 October 2006

Abstract—A family of 5-deazaflavin derivatives has been synthesised using a two-step convergent strategy. The biological activity of these compounds was evaluated in cells, by assessing their ability to stabilize and activate p53. These compounds may act as low molecular weight inhibitors of the E3 activity of HMD2 in tumours that retain wild-type p53. Importantly, we have demonstrated that the nitro group present in all three of the original lead compounds [**1**–**3** (HL198C-E)] is not essential for observation of this biological activity.

1. Introduction

The p53 protein plays a crucial role in preventing cancer development by inducing both cell cycle arrest and apoptosis (programmed cell death) in response to oncogenic stress.[1] While both of these responses may contribute to the inhibition of tumour development, evidence suggests that activation of p53 may cause tumour specific killing, whilst inducing a potentially reversible cell cycle arrest in normal tissue.[2–6]

In unstressed cells, to allow normal proliferation, the activity and stability of the p53 protein is tightly regulated.[7] One of the key regulators of p53 stability is HDM2, a ubiquitin ligase (E3) that targets the p53 protein for degradation through the proteasome. In response to tumourigenic stress, such as DNA damage or oncogene activation, degradation of p53 by HDM2 is inhibited, resulting in a rapid increase in the concentration of the p53 protein in cells. Several pathways which inhibit the degradation of p53 by HDM2 have been reported, including the activation of DNA damage-induced kinases, which phosphorylate p53 and HDM2; and the induction of proteins involved in the response to oncogenes' activation or perturbations in ribosome assembly such as ARF and L11, which interact with HDM2 to inhibit its E3 activity.[8–14] In cancer cells that retain wild-type p53 activity these pathways are often defective and thus the p53 protein cannot be stabilized and activated in response to stress.

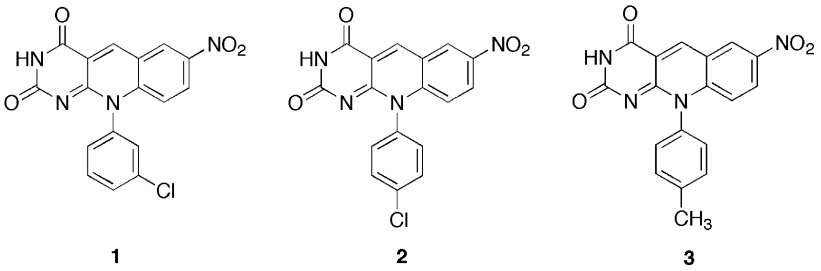
In an effort to identify small molecule inhibitors of the E3 activity of HDM2 in tumours that retain wild-type p53, we have previously carried out a high-throughput assay.[15] From a library of 10,000 compounds, forty inhibited HDM2 autoubiquitylation by more than 50%. Further testing using an in vitro gel-based assay identified a family of three compounds **1**–**3** (HL198C-E) (Fig. 1) with the 5-deazaflavin structural motif which significantly inhibited HDM2 autoubiquitylation. We now report the synthesis and biological evaluation of a small library of 5-deazaflavins to probe the structure–activity relationship of this series of compounds for their ability to stabilize and activate p53. This ability may be due to the inhibition of HDM2 autodegradation by the new compounds.

2. Chemistry

Our initial aim was to develop a short efficient synthesis of the three lead compounds **1**–**3**. The first stage of this involved the reaction of 2,4,6-trichloropyrimidine **4** with

Keywords: Deazaflavins; Heterocycle synthesis; Autoubiquitylation inhibitors; p53; Cell cycle arrest.
* Corresponding author. Tel.: +44 141 330 4378; fax: +44 141 330 4888; e-mail: D.Robins@chem.gla.ac.uk

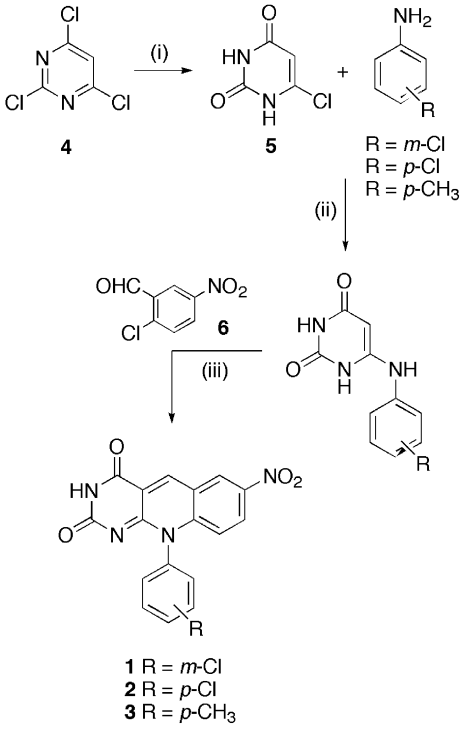
0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.10.011



1 **2** **3**

Figure 1. Structures of original lead compounds **1**–**3**.

sodium hydroxide to give 6-chlorouracil **5** in 71% yield and this was used as the key intermediate for the preparation of all the 5-deazaflavins in this study. The next stage involved a two-step convergent approach where 6-chlorouracil **5** was fused at melt temperature with the corresponding aryl amine followed by heating the resulting 6-*N*-aryl-aminouracils with 2-chloro-5-nitrobenzaldehyde **6** in DMF (Scheme 1).[16,17] This gave 10-(3-chlorophenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (**1**), 10-(4-chlorophenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (**2**) and 10-(4-methylphenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (**3**) in 26%, 22% and 79% yields, respectively, over the two steps.



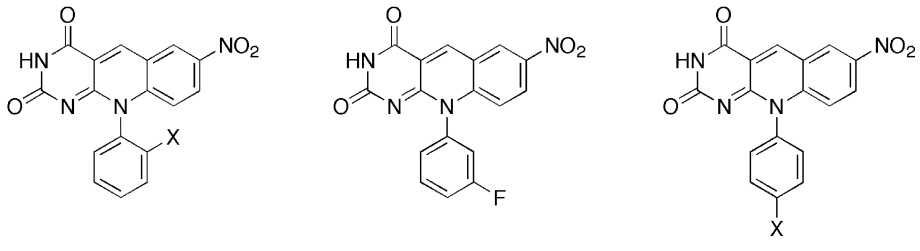
4 **5** R = *m*-Cl, R = *p*-Cl, R = *p*-CH_3 **6**

1 R = *m*-Cl
2 R = *p*-Cl
3 R = *p*-CH_3

Scheme 1. Reagents and conditions: (i) NaOH, H_2O, Δ, (71%); (ii) neat, Δ; (iii) DMF, Δ, (**1**, 26%), (**2**, 22%), (**3**, 79%).

Having developed a short route to the lead compounds that are 7-nitro-5-deazaflavins, we sought to probe the effect of size and electronic character of the substituents on the aniline portion of the molecule. A series of compounds (Fig. 2) was prepared using the two-step convergent approach described above. Here the various phenyl substituents were incorporated by reaction of 6-chlorouracil **5** with the appropriately substituted aniline. This allowed the preparation of various fluorinated compounds substituted at the *o*-, *m*- and *p*-positions (**8**, **10** and **11**), as well as the *o*-chloro compound (**7**) and an analogue with no substituent on the phenyl ring (**9**).

All of the compounds prepared so far contain a 7-nitro group. It cannot be ruled out that part of the mechanism of action of these compounds may involve oxidative stress processes.[18] Accordingly, to probe this and to elucidate further the structure–activity relationship of these 5-deazaflavins, compounds **12**–**29** (Fig. 3) without the 7-nitro group were prepared using the two step convergent approach described above. Thus, a series of compounds was prepared containing either a chloro-, fluoro- or the strongly electronegative trifluoromethyl group at the 6-, 7-, 8- or 9-positions and with either no substituent, chloro-, fluoro- or

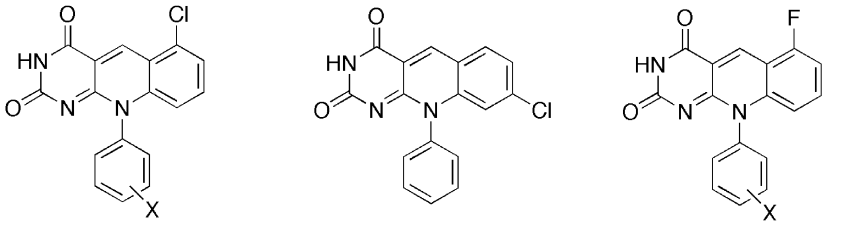


7 X = Cl (40%)
8 X = F (50%)
9 X = H (71%)

10 (47%)

11 X = F (56%)

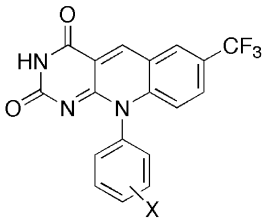
Figure 2. Structures of the 7-nitro-5-deazaflavin analogues (yields from 6-chlorouracil **5**).



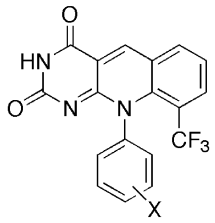
12 X = H (79%)
13 X = *o*-Cl (22%)
14 X = *p*-Cl (28%)
15 X = *p*-CH_3 (40%)
16 X = *o*-F (49%)
17 X = *m*-F (71%)
18 X = *p*-F (31%)

19 (60%)

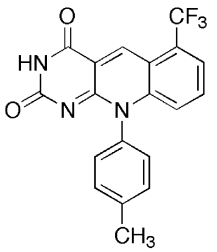
20 X = *o*-F (66%)
21 X = *m*-F (56%)
22 X = *p*-F (55%)



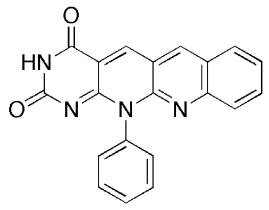
23 X = *p*-Cl (36%)
24 X = *o*-F (67%)
25 X = *m*-F (29%)
26 X = *p*-F (33%)



27 X = *o*-F (48%)
28 X = *p*-F (61%)



29 (36%)



30 (62%)

Figure 3. Structures of the 5-deazaflavin analogues (yields from 6-chlorouracil **5**).

methyl group in the aniline portion of the molecule. It is of interest to note that treatment of 6-*N*-aryl-aminouracils with 2-chloro-6-fluorobenzaldehyde produced the 6-chlorodeazaflavins **12**–**18** rather than the corresponding 6-fluoro derivatives. These reactions follow an addition–elimination process of nucleophilic aromatic substitution and fluoride is displaced rather than chloride due to the increased electronegativity of fluorine—a decrease in the electron density at the C–F bond results in faster attack there by the nucleophile compared to the C–Cl bond.[19,20] Finally, to probe further the size of the binding pocket the tetraazanaphthacene analogue **30** was also prepared.

3. Biology

Protein expression levels were assessed by Western blotting, after incubation of primary human pigment epithelial (RPE) cells with 1–20 μM of each compound for 24 h. Proteins from whole cell extracts were separated by SDS 12% polyacrylamide gel electrophoresis and analysed by Western blotting with anti-p53 DO-1 (Pharmingen), anti-HDM2 AB1/AB2 (Oncogene Science), anti-phospho-p53 (serine 15) (Cell Signaling) and anti-$21^{WAF1/CIP1}$ (Santa Cruz Biotechnology) antibodies. Blots were also probed with an anti-Cdk4 antibody (Santa Cruz Biotechnology) to monitor protein loading. The effects of some of the compounds on cell cycle progression and cell death were assessed by flow cytometry, as previously described.[21]

4. Results and discussion

Each of the 5-deazaflavin analogues was analysed for the ability to stabilize endogenous HDM2 and p53 in primary human pigment epithelial cells (RPE). After treating the cells with 1–20 μM of each compound for 24 h, HDM2 and p53 levels were assessed by Western blotting (Fig. 4 and Table 1). Several of the new compounds showed activity in the stabilization of p53 comparable to that of the previously described 5-deazaflavin **1**. Three of the analogues, **7**, **14** and **15**,

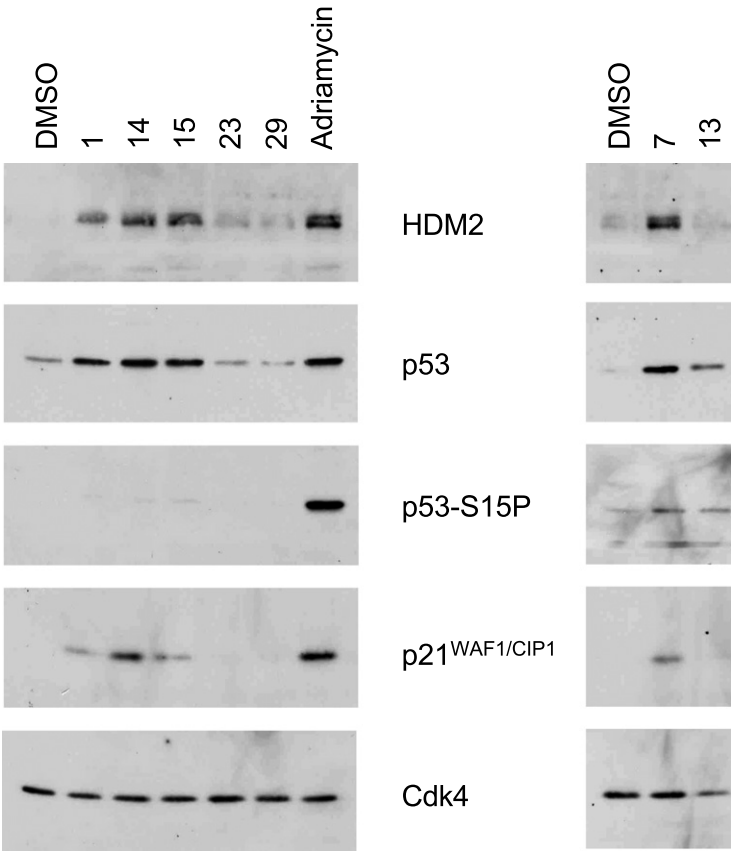


Figure 4. Western blots of protein expression levels after treatment of cells with 2.5 μM deazaflavin analogues or 0.1 μg/ml adriamycin.

Table 1. Stabilization of p53 measured by Western blotting in RPE cells treated with the analogues at the indicated concentrations for 24 h

Compound	1 μM	5–10 μM
1	–	+
7	+	++
8	ND	–
9	ND	–
10	ND	–
11	ND	–
12	ND	–
13	–	+
14	+	++
15	+	++
16	+	+
17	–	++
18	ND	–
19	ND	–
20	ND	–
21	ND	–
22	ND	–
23	–	–
24	ND	–
25	ND	–
26	–	+
27	ND	–
28	ND	–
29	–	–
30	ND	–

Results of three experiments are summarised to indicate no elevation of p53 levels (–), increase in p53 comparable with that seen with 5 μM compound 1 (+), or increase in p53 levels in excess of that seen with 5 μM compound 1 (++).
ND, not determined.

reproducibly showed stronger p53 stabilization and activity at lower concentrations than **1** (1 μM compared to 5 μM). Although stabilization of p53 with adriamycin is accompanied by activation of DNA damage-induced kinases that phosphorylate p53 at serine 15, the p53 stabilized by the 5-deazaflavin compounds was not clearly phosphorylated at this residue (Fig. 4). These results support a role for the compounds in stabilizing p53 by inhibition of HDM2, rather than the activation of a DNA damage response. The original lead compounds each contained a nitro group that could be predicted to be reduced to a nitro anion radical, which could then interact with DNA to induce DNA damage. The failure to detect p53 phosphorylation would argue against this mode of action, but more compellingly we found that some analogues without the 7-nitro group retained biological activity (Table 1). These included compounds **13**–**17** with a 6-chloro group together with compound **26** that has a 7-trifluoromethyl group.

Although the principal function of HDM2 in regulating p53 activity is through ubiquitination and destabilization of the p53 protein,[22] previous studies have suggested that the interaction of HDM2 with p53 can inhibit the transcriptional activity of p53.[23] To confirm that the p53 stabilized following treatment with the deazaflavin analogues is transcriptionally active, we looked for increased expression of the p53-inducible target gene product, $p21^{WAF1/CIP1}$ (Fig. 4 and Table 2). As seen for the original compounds **1**–**3**, stabilization of p53

Table 2. Cell cycle effects of analogues in RKO and RKO E6 cells

Compound	Stabilization of p53	Induction of p21 (RPE cells)	Cell cycle/apoptosis in RKO cells	Cell cycle/apoptosis in RKO/E6 cells
1	+	+	G1/apoptosis	None
7	+	+	G2/apoptosis	Apoptosis
13	+	ND	G2/apoptosis	Apoptosis
14	+	+	G1/apoptosis	None
15	+	+	G1/apoptosis	None
23	−	−	None	None
29	−	−	None	None

p53 and $p21^{WAF1/CIP1}$ levels were assessed as increasing (+) or not increasing (−) following treatment.

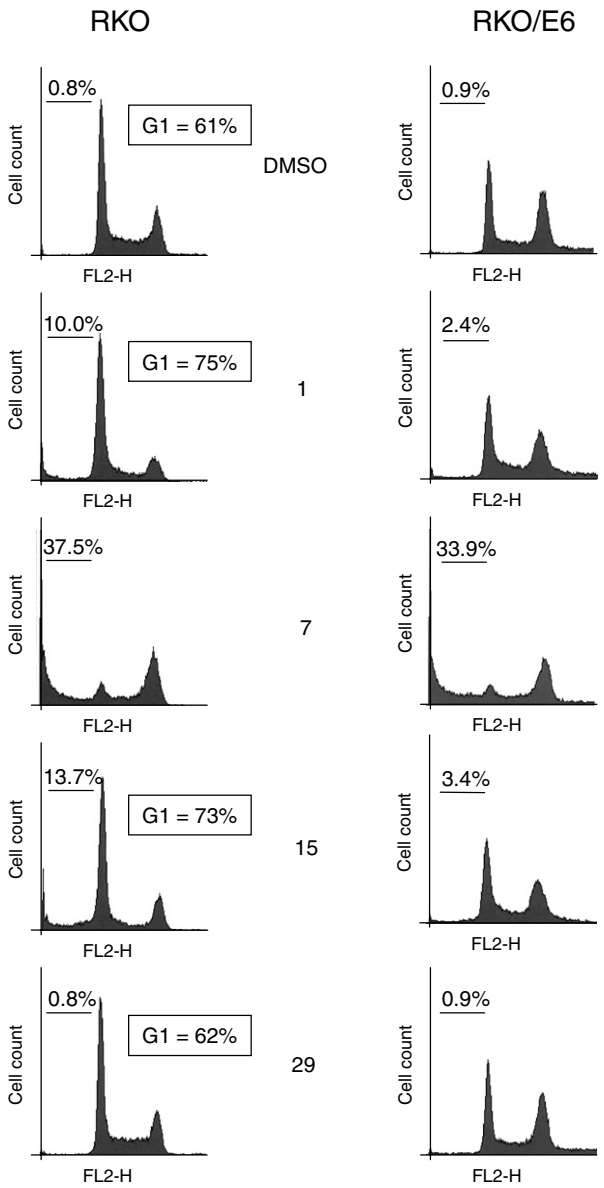


Figure 5. Effect of deazaflavin analogues on cell cycle progression and cell death.

by the active analogues identified in this study resulted in the increased expression of $p21^{WAF1/CIP1}$, indicating that the compounds both stabilize and activate p53. Some of the active compounds showed a clearly weaker ability to activate p53 than others, suggesting that they are less potent. However, in the case of compound **13** the weaker activity is likely to reflect a strong apoptotic response and the death of cells treated with this compound (see below).

The consequence of p53 activation is the induction of cell cycle arrest and apoptosis, and so we investigated the effects of some of the novel deazaflavin analogues on cell cycle progression and cell death (Fig. 5 and Table 2). In RKO cells, a colon cancer cell line that retains wild-type p53, all of the analogues that showed an ability to stabilize p53 also caused cell cycle perturbations and induced apoptosis. Control compounds that did not stabilize p53 (**23** and **29**) had no effect on cell cycle progression and did not induce significant levels of cell death. Interestingly, two clear types of responses were seen following treatment of these cells with the active analogues. Compounds **1**, **14** and **15** induced a clear G1 arrest, and some apoptosis, a pattern that was similar but less pronounced following treatment with compounds **16**, **17** and **26**. By contrast, **7** and **13** induced significant accumulation of cells in G2/M and substantial apoptosis. To determine which of these effects is dependent on p53, we examined the response of RKO/E6 cells—RKO cells expressing the HPV16 E6 protein that inactivates endogenous p53. The compounds that activated a G1 arrest and slight apoptosis in the RKO cells (**1**, **14** and **15**) had no effect in RKO/E6 cells, indicating that their activity is a reflection of the stabilization and activation of p53. However, the compounds that induced a G2/M accumulation and strong apoptosis in RKO cells (**7** and **13**) retained the strong apoptotic activity in the RKO/E6 cells, suggesting that these compounds show significant off target effects and can kill cells through p53-independent pathways. While there is a clear differential between these two groups of compounds at these concentrations, at higher concentrations all the compounds show some off target activities, as also described for the original compounds **1**–**3**.

Thus a family of 5-deazaflavin derivatives has been synthesised using a two-step convergent strategy and their ability to stabilize and activate p53 has been evaluated in cells. It is difficult to draw any conclusions about the structure–activity relationships from the biological data for these new compounds, but the most important new finding is that the nitro group present in all three of the original compounds **1**–**3** is not essential for their ability to stabilize and activate p53. Compounds **14** and **15** with the 7-nitro group replaced with a 6-chloro

group were better at stabilization of p53 and were effective at lower concentrations than the original lead compounds **1**–**3**. These new low molecular weight compounds that may inhibit the E3 activity of HMD2 in tumours that retain wild-type p53 should be useful in further biological studies.

5. Experimental

5.1. General experimental considerations

All reactions were performed under a nitrogen atmosphere unless otherwise noted. Reagents and starting materials were obtained from commercial sources and used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 spectrometer with chemical shift values in parts per million relative to residual chloroform (δ_H 7.28 and δ_C 77.2) as standard. Infrared spectra were recorded on a JASCO FTIR 410 spectrometer and mass spectra were obtained using a JEOL JMS-700 spectrometer. Melting points were determined on a Reichert platform melting point apparatus.

5.1.1. 6-Chlorouracil 5. 2,4,6-Trichloropyrimidine (15.0 g, 8.2 mmol) was added to a stirring solution of sodium hydroxide (13.2 g, 0.33 mol) and water (135 cm^3). The reaction mixture was heated under reflux for 1 h. The solution was cooled and the pH was adjusted to ~2 to 3 using concentrated hydrochloric acid (18 cm^3). The mixture was stored at 0 °C overnight. The white precipitate was filtered and recrystallised from water (9.1 g, 71%); mp 287–289 °C (lit.[24] 295–298 °C); ^{1}H NMR (DMSO-d_6, 400 MHz): 5.76 (s, 1H), 11.33 (s, 1H), 12.09 (s, 1H); *m*/*z* (FAB): 147.05 (MH$^+$, 86%).

5.1.2. General procedure A. 6-Chlorouracil (1 equiv) and aniline (3 equiv) were heated at 170 °C for 20 min. The mixture was then cooled and diethyl ether (20 cm^3) was added. The mixture was sonicated for 15 min. The white suspension was filtered and washed with water (15 cm^3), methanol (15 cm^3) and diethyl ether (15 cm^3). The white solid was placed in a desiccator and dried overnight using silica and P_2O_5.

5.1.3. 6-Phenylamino-1*H*-pyrimidine-2,4-dione. Yield 84%; mp 332–333 °C; IR (KBr): 3414 (NH), 1818 (CO), 1807 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 4.70 (s, 1H), 7.15–7.23 (m, 3H), 7.41 (t, 2H, J = 7.8 Hz), 8.28 (s, 1H), 10.20 (s, 1H), 10.50 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 75.8 (CH), 122.7 (CH), 124.7 (CH), 129.4 (CH), 137.9 (C), 150.8 (C), 152.2 (C), 164.4 (C); HRMS (FAB$^+$): *m*/*z* found 204.0771, calcd $C_{10}H_{10}N_3O_2$ (MH$^+$) 204.0773.

5.1.4. 6-(2-Chlorophenylamino)-1*H*-pyrimidine-2,4-dione. Yield 60%; mp (dec) 321–323 °C; IR (KBr): 3202 (NH), 1731 (CO), 1632 (C=C), 1540 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 4.37 (s, 1H), 7.29 (ddd, 1H, J = 8.0, 8.0, 1.6 Hz), 7.41 (ddd, 1H, J = 8.0, 8.0, 1.3 Hz), 7.46 (dd, 1H, J = 8.0, 1.6 Hz), 7.59 (dd, 1H, J = 1.3, 8.0 Hz), 8.10 (s, 1H) 10.38 (s, 1H), 10.55 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 76.1 (CH), 126.8 (CH), 127.4 (CH), 128.2 (CH), 128.4 (C), 130.1 (CH), 134.4 (C), 150.7 (C), 152.1 (C), 164.2 (C); Anal. Calcd for $C_{10}H_8ClN_3O_2$: C, 50.54; H, 3.39; N, 17.68. Found: C, 50.42; H, 3.26; N, 17.45; *m*/*z* (CI): 238.0 (MH$^+$, 91%).

5.1.5. 6-(3-Chlorophenylamino)-1*H*-pyrimidine-2,4-dione. Yield 99%; mp 328–329 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 4.76 (s, 1H), 7.18 (dt, 2H, J = 8.0, 2.0 Hz), 7.25 (d, 1H, J = 2.0 Hz), 7.39 (t, 1H, J = 8.0 Hz), 8.53 (s, 1H), 10.41 (s, 1H), 10.50 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 77.6 (CH), 121.1 (CH), 122.1 (CH), 124.4 (CH), 131.4 (CH), 133.9 (C), 140.2 (C), 151.2 (C), 152.1 (C), 164.7 (C); Anal. Calcd for $C_{10}H_8ClN_3O_2$: C, 50.54; H, 3.39; N, 17.68. Found: C, 50.39; H, 3.25; N, 17.55.

5.1.6. 6-(4-Chlorophenylamino)-1*H*-pyrimidine-2,4-dione. Yield 43%; mp (dec) 344–346 °C; IR (KBr): 3195 (NH), 1754 (CO), 1616 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 4.73 (s, 1H), 7.23 (AA′BB′ system, 2H, J = 8.6 Hz), 7.43 (AA′BB′ system, 2H, J = 8.6 Hz), 8.42 (s, 1H), 10.32 (s, 1H), 10.55 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 76.6 (CH), 124.1 (CH), 128.2 (C), 129.3 (CH), 137.1 (C), 150.9 (C), 151.9 (C), 164.4 (C); Anal. Calcd for $C_{10}H_8ClN_3O_2$: C, 50.54; H, 3.39; N, 17.68. Found: C, 50.33; H, 3.22; N, 17.44; *m*/*z* (CI): 238.04 (MH$^+$, 3%).

5.1.7. 6-(4-Methylphenylamino)-1*H*-primidine-2,4-dione. Yield 91%; mp 321–323 °C; IR (KBr): 3273 (NH), 1772 (CO), 1634 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 2.29 (s, 3H), 4.61 (s, 1H), 7.10 (AA′BB′ system, 2H, J = 8.4 Hz), 7.20 (AA′BB′ system, 2H, J = 8.4 Hz), 8.23 (s, 1H), 10.18 (s, 1H), 10.45 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 20.4 (CH_3), 75.2 (CH), 123.1 (CH), 129.8 (CH), 134.1 (C), 135.1 (C), 150.8 (C), 152.5 (C), 164.3 (C); Anal. Calcd for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34. Found: C, 60.68; H, 5.07; N, 19.10; *m*/*z* (CI): 217.04 (MH$^+$, 10%).

5.1.8. 6-(2-Fluorophenylamino)pyrimidine-2,4-dione. Yield 84%; mp > 360 °C; IR (KBr): 3247 (NH), 3092 (NH), 1736 (CO), 1626 (C=C), 1547 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 4.45 (s, 1H), 7.21–7.42 (m, 4H), 8.20 (s, 1H), 10.32 (s, 1H), 10.55 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 76.1 (CH), 116.3 (CH), 117.9 (CH), 120.4 (CH), 125.2 (CH), 127.6 (C), 150.6 (C), 152.2 (C), 158.6 (C), 164.2 (C); *m*/*z* (FAB): 222.1 (MH$^+$, 1%).

5.1.9. 6-(3-Fluorophenylamino)pyrimidine-2,4-dione. Yield 75%; mp 321–322 °C; IR (KBr): 3322 (NH), 3231 (NH), 1752 (CO), 1604 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 4.66 (s, 1H), 7.00 (dt, 2H, J = 8.0, 2.1 Hz), 7.38–7.42 (m, 2H), 8.75 (s, 1H), 10.60 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 76.8 (CH), 107.6 (CH), 109.1 (CH), 111.3 (CH), 131.0 (CH), 131.2 (CH), 139.8 (CH), 150.7 (CH), 151.7 (CH), 164.6 (C); *m*/*z* (FAB): 221.1 (MH$^+$, 4%).

5.1.10. 6-(4-Fluorophenylamino)pyrimidine-2,4-dione. Yield 76%; mp 339–340 °C; IR (KBr): 3391 (NH), 1752 (CO), 1680 (CO), 1577 (C=C) cm^{-1}; ^{1}H NMR

(DMSO-d_6, 400 MHz): 4.53 (s, 1H), 7.20–7.27 (m, 4H), 8.22 (s, 1H), 10.25 (s, 1H), 10.45 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 75.5 (CH), 115.9 (CH), 116.2 (CH), 125.3 (CH), 125.5 (CH), 134.8 (CH), 150.8 (C), 152.7 (C), 164.3 (C); *m/z* (FAB): 222.1 (MH^+, 100%).

5.1.11. 6-(4-Bromophenylamino)pyrimidine-2,4-dione. Yield 80%; mp 339–340 °C; IR (KBr): 3263 (NH), 1766 (CO), 1616 (C═C), 1554 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 4.73 (s, 1H), 7.16 (AA′BB′ system, 2H, J = 8.6 Hz), 7.55 (AA′BB′ system, 2H, J = 8.6 Hz), 8.40 (s, 1H), 10.28 (s, 1H), 10.51 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 77.1 (CH), 116.6 (C), 124.7 (CH), 132.6 (CH), 138.0 (C), 151.2 (C), 152.1 (C), 164.7 (C); Anal. Calcd for $C_{11}H_{11}BrN_3O_2$: C, 42.58; H, 2.86; N, 14.89. Found: C, 42.44; H, 2.66; N, 14.63.

5.1.12. 6-(4-Nitrophenylamino)pyrimidine-2,4-dione. Yield 48%; mp 328–329 °C; IR (KBr): 3270 (NH), 3103 (NH), 1734 (CO), 1624 (C═C), 1570 (NO_2), 1344 (NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 5.12 (s, 1H), 7.34 (AA′BB′ system, 2H, J = 9.1 Hz), 8.21 (AA′BB′ system, 2H, J = 9.1 Hz), 9.20 (s, 1H), 10.60 (s, 1H), 10.70 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 81.8 (CH), 119.7 (CH), 125.8 (CH), 142.0 (C), 146.0 (C), 150.3 (C), 151.1 (C), 165.5 (C). HRMS (EI): *m/z* found 248.0543, calcd $C_{10}H_8N_4O_4$ (MH^+) 248.0546. *m/z* (EI): 248.0 (MH^+, 6%).

5.1.13. General procedure B. A suspension of substituted uracil (1 equiv) and DMF (15 cm^3) was prepared. The chlorobenzaldehyde (1.2 equiv) was added and the reaction mixture was heated at 110 °C for 90 min. The yellow transparent solution was cooled and water (25 cm^3) was added. The resulting precipitate was filtered and washed with water (15 cm^3). The yellow solid was placed in a desiccator and dried overnight using silica and P_2O_5.

5.1.14. 10-(3-Chlorophenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (1). Yield 26%; mp 368–369 °C; IR (KBr): 3400 (NH), 1714 (CO), 1614 (C═C), 1571, 1510 (NO_2), 1340 (NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.95 (d, 1H, J = 9.5 Hz), 7.49 (t, 1H, J = 4.6 Hz), 7.69 (s, 1H), 7.76 (dd, 2H, J = 4.7, 1.9 Hz), 8.45 (dd, 1H, J = 9.5, 2.7 Hz), 9.24 (d, 1H, J = 2.7 Hz), 9.32 (s, 1H), 11.46 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 117.8 (CH), 118.9 (CH), 120.8 (C), 127.6 (CH), 127.7 (CH), 128.9 (CH), 130.3 (CH), 132.5 (CH), 134.8 (C), 138.8 (C), 142.7 (CH), 143.3 (C), 145.2 (C), 156.6 (C), 159.6 (C), 161.5 (C), 162.7 (C); *m/z* (EI): 368.4 (M^+, 1%).

5.1.15. 10-(4-Chlorophenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (2). Yield 52%; mp > 380 °C; IR (KBr): 3072 (NH), 1716 (CO) 1570 (C═C), 1484 (NO_2), 1344 (NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.96 (d, 1H, J = 9.5 Hz), 7.53 (d, 2 H, J = 6.7 Hz), 7.80 (d, 2H, J = 6.7 Hz), 8.43 (dd, 1H, J = 2.7 Hz), 9.22 (d, 1H, J = 2.7 Hz), 9.31 (s, 1H), 11.31 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 117.6 (C), 118.9 (CH), 121.2 (C), 127.5 (CH), 128.7 (CH), 130.7 (CH), 130.9 (CH), 134.8 (C), 136.5 (C), 142.6 (CH), 143.3 (C), 145.1 (C), 156.6 (C), 159.7 (C), 161.7 (C); *m/z* (FAB): 369.2 (MH^+, 5%).

5.1.16. 10-(4-Methylphenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (3). Yield 87%; mp > 380 °C; IR (KBr): 3408 (NH), 1716 (CO), 1614 (C═C), 1485 (NO_2), 1340 (NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 2.47 (s, 3H), 6.91 (d, 1H, J = 9.5 Hz), 7.42 (m, 4H), 8.44 (dd, 1H, J = 9.5, 2.6 Hz), 9.20 (d, 1H, J = 2.6 Hz), 9.38 (s, 1H), 11.26 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 21.2 (CH_3), 117.9 (C), 119.0 (CH), 120.8 (C), 127.3 (CH), 128.3 (2 CH), 128.6 (CH), 131.2 (2 CH), 135.0 (C), 139.7 (C), 142.4 (CH), 143.2 (C), 145.6 (C), 156.7 (C), 159.9 (C), 161.8 (C); *m/z* (FAB): 349.3 (MH^+, 56%).

5.1.17. 10-(2-Chlorophenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (7). Yield 66%; mp 326–328 °C; IR (KBr): 3144 (NH), 2826 (CH), 1683 (CO), 1617 (C═C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.88 (d, 1H, J = 9.6 Hz), 7.56–7.70 (m, 3H), 7.81–7.89 (m, 1H), 8.48 (dd, 1H, J = 9.6, 2.8 Hz), 9.27 (s, 1H), 9.32 (d, 1H, J = 2.8 Hz), 11.43 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 117.2 (C), 117.7 (CH), 120.4 (C), 127.5 (CH), 129.0 (CH), 129.5 (CH), 130.4 (CH), 130.9 (CH), 131.6 (C), 131.9 (CH), 134.2 (C), 142.7 (CH), 143.3 (C). 143.8 (C), 156.2 (C), 158.8 (C), 161.1 (C); HRMS (FAB): *m/z* found 369.0391, calcd $C_{17}H_9N_4O_4Cl$ (MH^+) 369.0388.

5.1.18. 10-(2-Fluorophenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (8). Yield 60%; mp > 360 °C; IR (KBr): 2558 (NH), 1685 (CO), 1630 (C═C), 1571 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.94 (d, 1H, J = 9.6 Hz), 7.52–7.61 (m, 4H), 8.45 (dd, 1H, J = 9.6, 2.8 Hz), 9.21 (d, 1H, J = 2.8 Hz), 9.31 (s, 1H), 11.35 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 117.5 (C), 118.5 (CH), 120.4 (CH), 127.2 (CH), 128.3 (CH), 129.7 (CH), 130.4 (CH), 137.2 (C), 145.1 (C), 156.3 (C), 161.4 (C); Anal. Calcd for $C_{17}H_9FN_4O_4$: C, 57.96; H, 2.58; N, 15.90. Found: C, 57.49; H, 2.65, N, 15.43; *m/z* (FAB): 353.2 (MH^+, 5%).

5.1.19. 7-Nitro-10-phenyl-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (9). Yield 84%; mp > 360 °C; IR (KBr): 3220 (NH), 1722 (CO), 1610 (C═C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.87 (d, 1 H, J = 9.2 Hz), 7.47–7.75 (m, 2H), 7.65–7.75 (m, 3H), 8.45 (dd, 1H, J = 9.2, 2.4 Hz), 9.23 (d, 1H, J = 2.4 Hz), 9.31 (s, 1H), 11.32 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 117.5 (C), 118.5 (CH), 120.4 (C), 127.2 (CH), 128.3 (CH), 128.4 (CH), 129.7 (CH), 130.4 (CH), 137.2 (C), 142.1 (C), 142.9 (C), 145.1 (C), 156.3 (C), 159.5 (C), 161.4 (C); Anal. Calcd for $C_{17}H_{10}N_4O_4$: C, 61.08; H, 3.02; N, 16.76. Found: C, 60.69; H, 2.75; N, 16.39; *m/z* (FAB): 335.2 (MH^+, 13%).

5.1.20. 10-(3-Fluorophenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (10). Yield 63%; mp 371–372 °C; IR (KBr): 3467 (NH), 1707 (CO), 1612 (C═C), 1522 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.93 (d, 1H, J = 9.4 Hz), 7.50 (t, 1H, J = 4.5 Hz), 7.65 (dd, 1H,

J = 4.5, 2.0 Hz), 7.9 (br s, 1H), 8.40 (dd, 1H, J = 9.4, 2.6 Hz), 9.11 (d, 1H, J = 2.6 Hz), 9.3 (s, 1H), 11.3 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 115.9 (CH), 118.5 (CH), 120.3 (CH), 127.1 (CH), 128.4 (CH), 132.2 (CH), 132.5 (CH), 138.3 (CH), 142.3 (CH), 142.9 (C), 156.2 (C), 159.3 (CH), 161.3 (C), 161.6 (C), 161.6 (C), 162.5 (C), 164.1 (C); Anal. Calcd for $C_{17}H_9FN_4O_4$: C, 57.96; H, 2.58; N, 15.90. Found: C, 57.53; H, 2.22, N, 15.43; *m*/*z* (FAB): 353.2 (MH^+, 2%).

5.1.21. 10-(4-Fluorophenyl)-7-nitro-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (11). Yield 74%; mp > 360 °C; IR (KBr): 3051 (NH), 1709 (CO), 1613 (C═C), 1569 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.94 (d, 1H, J = 9.6 Hz), 7.52–7.61 (m, 4H), 8.45 (dd, 1H, J = 9.6, 2.8 Hz), 9.21 (d, 1H, J = 2.8 Hz), 9.31 (s, 1H), 11.35 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 117.8 (CH, d, J = 23 Hz), 118.9 (CH), 120.8 (C), 127.6 (CH), 128.7 (CH), 131.3 (CH, d, J = 9.2 Hz), 133.8 (C), 138.0 (C), 142.6 (CH), 143.3 (C), 145.6 (C), 156.6 (C), 160.0 (C), 162.7 (C, d, J = 246 Hz), 161.74 (C); *mz* (FAB): 353.2 (MH^+, 8%).

5.1.22. 6-Chloro-10-phenyl-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (12). Yield 94%; mp (dec) 338–340 °C; IR (KBr): 3381 (NH), 3234 (NH), 1702 (CO), 1669 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.70 (dd, 1H, J = 8.0, 1.2 Hz), 7.43–7.45 (m, 2H, 7-H), 7.63–7.71 (m, 5H), 9.04 (s, 1H), 11.27 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 116.7 (C), 116.8 (CH), 118.4 (C), 124.9 (CH), 128.3 (CH), 129.5 (CH), 130.3 (CH), 133.7 (C), 135.2 (CH), 136.5 (CH), 137.6 (C), 143.1 (C), 156.3 (C), 158.5 (C), 161.6 (C); HRMS (FAB): *m*/*z* found 324.0536, calcd $C_{17}H_{10}N_3O_2Cl$ (MH^+) 324.0540.

5.1.23. 6-Chloro-10-(2-chlorophenyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (13). Yield 36%; mp 305–307 °C; IR (KBr): 3142 (NH), 2801 (NH), 1709 (CO), 1685 (CO), 1607 (C═C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.72 (d, 1 H, J = 10.8 Hz), 7.62–7.66 (m, 1H), 7.68–7.79 (m, 4H), 7.85–7.96 (m, 1H), 9.89 (s, 1H), 10.33 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 115.9 (CH), 116.5 (C), 118.4 (C), 125.4 (CH), 129.5 (CH), 130.4 (CH), 130.8 (CH), 131.0 (C), 131.7 (CH), 134.1 (C), 134.5 (C), 135.9 (CH), 137.2 (C), 141.9 (C), 156.2 (C), 157.9 (C), 161.3 (C); HRMS (FAB): *m*/*z* found 358.0151, calcd $C_{17}H_9N_3O_2Cl_2$ (MH^+) 358.0150.

5.1.24. 6-Chloro-10-(4-chlorophenyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (14). Yield 64%; mp (dec) 220–222 °C; IR (KBr): 3254 (NH), 1667 (CO), 1602 (C═C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.55 (d, 1 H, J = 8.8 Hz), 7.50 (AA′BB′ system, 2H, J = 8.4 Hz), 7.59–7.65 (m, 2H), 7.79 (AA′BB′ system, 2H, J = 8.4 Hz), 9.07 (s, 1H), 11.29 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 116.7 (C), 116.8 (CH), 118.5 (C), 124.9 (CH), 130.3 (CH), 130.4 (CH), 133.8 (C), 134.2 (C), 135.3 (CH), 136.3 (C), 136.7 (CH), 142.9 (C). 156.2 (C), 158.6 (C), 161.5 (C); HRMS (FAB): *m*/*z* found 358.0152, calcd $C_{17}H_9N_3O_2Cl_2$ (MH^+) 358.0150.

5.1.25. 6-Chloro-10-(4-methylphenyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (15). Yield 44%; mp > 360 °C; IR (KBr): 3131 (NH), 3000 (NH), 1707 (CO), 1656 (C═C); ^{1}H NMR (DMSO-d_6, 400 MHz): 2.49 (s, 3H), 6.75 (d, 1H, J = 9.2 Hz), 7.30 (AA′BB′ system, 2H, J = 8.2 Hz), 7.50 (AA′BB′ system, 2H, J = 8.2 Hz), 7.66–7.73 (m, 2H, 7-H), 9.02 (s, 1H), 11.24 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 20.8 (CH_3), 116.9 (CH), 118.4 (C), 124.8 (CH), 125.3 (C), 127.9 (CH), 130.7 (CH), 133.7 (C), 134.9 (C), 135.1 (CH), 136.4 (CH), 139.1 (C) 143.3 (C), 156.3 (C), 158.6 (C), 161.6 (C); HRMS (FAB): *m*/*z* found 338.0700, calcd $C_{18}H_{12}N_3O_2Cl$ (MH^+) 338.0696.

5.1.26. 6-Chloro-10-(2-fluorophenyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (16). Yield 58%; mp 323–324 °C; IR (KBr): 3523 (NH), 1694 (CO), 1629 (CO), 1504, 1229, 754 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.81 (s, 1H), 7.55–7.71 (m, 4H), 7.72–7.81 (m, 3H), 11.38 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 116.3 (CH), 117.2 (CH), 118.3 (CH), 119.8 (CH), 120.5 (CH), 124.6 (CH), 124.7 (C), 128.3 (CH), 129.6 (C), 130.1 (CH), 132.6 (C), 140.6 (C), 148.9 (C), 159.8 (C), 160.3 (C), 162.2 (C), 164.8 (C); Anal. Calcd for $C_{17}H_9FClN_3O_2$: C, 59.75; H, 2.65; N, 12.30; Found: C, 59.39; H, 2.42; N, 12.45; *m*/*z* (FAB): 342.1 (MH^+, 3%).

5.1.27. 6-Chloro-10-(3-fluorophenyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (17). Yield 95%; mp 326–328 °C; IR (KBr): 3475 (NH), 1707 (CO), 1671 (CO), 1522 (C═C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.81 (s, 1H), 7.55–7.71 (m, 4H), 7.72–7.81 (m, 3H), 11.38 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 104.3 (CH), 107.6 (CH), 114.3 (CH), 116.8 (CH), 117.5 (CH), 125.1 (C), 129.1 (CH), 130.2 (CH), 131.3 (CH), 132.3 (C), 136.4 (C), 138.5 (CH), 141.5 (C), 158.6 (C), 161.1 (C), 162.4 (C), 164.7 (C); Anal. Calcd for $C_{17}H_9FClN_3O_2$: C, 59.75; H, 2.65; N, 12.30. Found; C, 59.51; H, 2.43; N, 12.67; *m*/*z* (FAB): 342.1 (MH^+, 3%).

5.1.28. 6-Chloro-10-(4-fluorophenyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (18). Yield 41%; mp > 360 °C; IR (KBr): 3273 (NH), 1717 (CO), 1650 (C═C), 1578 cm^{-1};^{1}H NMR (DMSO-d_6, 400 MHz): 6.93 (d, 1H, J = 9.4 Hz), 7.50 (t, 1H, J = 4.3 Hz), 7.65 (dd, 1H, J = 4.3, 2.0 Hz), 7.9 (s, 1H), 8.40 (dd, 1H, J = 9.4, 2.6 Hz), 9.11 (1H, d, J = 7.2 Hz), 9.3 (1H, s), 11.3 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 116.3 (CH), 117.8 (CH), 119.5 (CH), 121.6 (CH), 123.6 (C), 127.9 (CH), 131.2 (CH), 133.5 (C), 135.2 (CH), 136.2 (C), 139.5 (C), 143.2 (C), 158.3 (C), 162.2 (C), 164.2 (C); *mz* (FAB): 342.1 (MH^+, 2%).

5.1.29. 8-Chloro-10-phenyl-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (19). Yield 71%; mp > 360 °C; IR (KBr): 3335 (NH), 1700 (CO), 1659 (CO); ^{1}H NMR (DMSO-d_6, 400 MHz): 6.50 (d, 1 H, J = 1.6 Hz), 7.38–7.40 (m, 2H), 7.53 (dd, 1H, J = 8.8, 1.6 Hz), 7.57–7.65 (m, 3H), 8.21 (d, 1H, J = 8.8 Hz), 9.08 (s, 1H), 11.10 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 115.9 (C), 116.1 (CH), 119.8 (C), 124.7 (CH), 128.3 (CH), 129.6 (C), 130.4 (CH), 133.2 (CH), 137.1 (C), 139.3 (C), 141.7 (CH), 142.5 (C), 156.3 (C), 158.5 (C), 161.7 (C); Anal.

Calcd for $C_{17}H_{20}ClN_3O_2$: C, 63.07; H, 3.11; N, 12.98. Found: C, 62.85; H, 3.11; N, 12.75; *m/z* (FAB): 324.2 (MH^+, 100%).

5.1.30. 6-Fluoro-10-(2-fluorophenyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (20). Yield 79%; mp 333–335 °C; IR (KBr): 3434 (NH), 1715 (CO), 1668 (CO), 1565 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 6.81 (s, 1H), 7.55–7.71 (m, 4H), 7.72–7.81 (m, 3H), 11.38 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 102.1 (CH), 109.5 (C), 112.2 (CH), 115.9 (CH), 118.5 (CH), 121.7 (CH), 124.3 (CH), 127.1 (CH), 128.3 (C), 129.1 (CH), 135.6 (C), 143.2 (C), 157.8 (C), 159.7 (C), 160.3 (C), 161.2 (C), 169.4 (C); Anal. Calcd for $C_{17}H_9F_2N_3O_2$: C, 62.77; H, 2.79; N, 12.92. Found; C, 62.51; H, 2.42; N, 13.28; *m/z* (FAB): 326.3 (MH^+, 2%).

5.1.31. 6-Fluoro-10-(3-fluorophenyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (21). Yield 75%; mp 337–339 °C; IR (KBr): 3439 (NH), 1710 (CO), 1677 (CO), 1531 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 6.81 (s, 1H), 7.55–7.71 (m, 4H), 7.72–7.81 (m, 3H), 11.38 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 101.3 (CH), 104.3 (CH), 107.2 (CH), 109.6 (C), 112.5 (CH), 113.4 (CH), 125.6 (CH), 128.6 (CH), 130.3 (CH), 137.6 (C), 139.5 (C), 142.7 (C), 157.2 (C), 158.6 (C), 161.3 (C), 164.2 (C), 169.8 (C); Anal. Calcd for $C_{17}H_9F_2N_3O_2$: C, 62.77; H, 2.79; N, 12.92. Found: C, 63.19; H, 3.01, N, 12.58; *m/z* (FAB): 326.3 (MH^+, 1%).

5.1.32. 6-Fluoro-10-(4-fluorophenyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (22). Yield 72%; mp 334–336 °C; IR (KBr): 3475 (NH), 1707 (CO), 1671 (CO), 1522 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 6.60 (d, 1 H, J = 8.8 Hz), 7.49–7.55 (m, 4H), 7.72 (m, 1H), 8.90 (s, 1H), 9.11 (d, 1H, J = 8.4 Hz), 11.25 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 103.2 (CH), 109.5 (C), 113.7 (CH), 115.9 (2 CH), 118.9 (2 CH), 125.6 (CH), 129.7 (CH), 139.6 (C), 134.8 (C), 142.9 (C), 151.2 (C), 156.9 (C), 157.9 (C), 163.5 (C), 169.1 (C); Anal. Calcd for $C_{17}H_9F_2N_3O_2$: C, 62.77; H, 2.79; N, 12.92. Found; C, 62.49; H, 2.91; N, 12.63; *m/z* (FAB): 326.2 (MH^+, 3%).

5.1.33. 10-(4-Chlorophenyl)-7-(trifluoromethyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (23). Yield 83%; mp 342–343 °C; IR (KBr): 3155 (NH), 1690 (CO), 1618 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 6.97 (d, 1H, J = 9.2 Hz), 7.54 (AA′BB′ system, 2H, J = 8.6 Hz), 7.79 (AA′BB′ system, 2H, J = 8.6 Hz), 8.01 (dd, 1H, J = 9.2, 2.0 Hz), 8.54 (d, 1H, J = 2.0 Hz), 9.22 (s, 1H), 11.27 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 117.0 (C), 118.4 (CH), 120.6 (C), 123.3 (C), 124.9 (CF_3, J = 33 Hz), 128.9 (CH), 130.5 (CH), 131.2 (CH), 132.8 (CH), 134.3 (C), 136.1 (C), 142.1 (CH), 143.6 (C). 156.3 (C), 159.8 (C), 161.5 (C); Anal. Calcd for C, 55.19; H, 2.31; N, 10.73. Found: C, 54.99; H, 2.25; N, 10.59; *m/z* (FAB): 392.00 (MH^+, 100%).

5.1.34. 10-(2-Fluorophenyl)-7-(trifluoromethyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (24). Yield 49%; mp > 360 °C; IR (KBr): 3523 (NH), 1694 (CO), 1651 (CO), 1504 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 6.81 (s, 1H), 7.55–7.71 (m, 4H), 7.73–7.81 (m, 3H), 11.38 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 115.9 (CH), 118.9 (CH), 119.2 (CH), 120.1 (CH), 120.9 (C), 124.2 (CH), 124.5 (CH), 124.9 (C), 125.1 (CH), 129.3 (CH), 133.7 (C), 135.4 (C), 140.2 (C), 143.8 (C), 159.1 (C), 160.3 (C), 163.5 (C), 169.3 (C); Anal. Calcd for $C_{18}H_9F_4N_3O_2$: C, 57.61; H, 2.42; N, 11.20. Found; C, 57.91; H, 2.80; N, 11.54; *m/z* (FAB): 376.1 (MH^+, 1%).

5.1.35. 10-(3-Fluorophenyl)-7-(trifluoromethyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (25). Yield 39%; mp 326–328 °C; IR (KBr): 3263 (NH), 1707 (CO), 1671 (CO), 1522 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 6.81 (s, 1H), 7.55–7.71 (m, 4H), 7.73–7.81 (m, 3H), 11.38 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 105.9 (CH), 106.3 (CH), 110.7 (CH), 115.6 (CH), 119.2 (C), 121.3 (CH), 123.2 (C), 124.2 (CH), 126.3 (CH), 127.1 (C), 129.8 (CH), 135.2 (C), 136.9 (C), 143.8 (C), 159.9 (C), 163.2 (C), 164.2 (C), 169.7 (C); Anal. Calcd for $C_{18}H_9F_4N_3O_2$: C, 57.61; H, 2.42; N, 11.20. Found: C, 57.34; H, 2.79; N, 11.67; *m/z* (FAB): 376.1 (MH^+, 1%).

5.1.36. 10-(4-Fluorophenyl)-7-(trifluoromethyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (26). Yield 43%; mp 361–363 °C; IR (KBr): 3545 (NH), 1707 (CO), 1661 (CO), 1525 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 6.81 (s, 1H), 7.55–7.71 (m, 4H), 7.73–7.81 (m, 3H), 11.38 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 116.4 (CH), 118.3 (C), 120.1 (C), 120.6 (CH), 123.8 (CH), 124.4 (CH), 124.7 (C), 133.7 (C), 135.3 (CH), 136.6 (CH), 138.8 (C), 142.8 (C), 156.2 (C), 158.4 (C), 161.5 (C), 164.0 (C); Anal. Calcd for $C_{18}H_9F_4N_3O_2$: C, 57.61; H, 2.42; N, 11.20. Found: C, 57.37; H, 2.71; N, 10.94; *m/z* (FAB): 376.1 (MH^+, 1%).

5.1.37. 10-(2-Fluorophenyl)-9-(trifluoromethyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (27). Yield 57%; mp > 360 °C; IR (KBr): 3297 (NH), 1695 (CO), 1647 (CO), 1532 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 5.50 (s, 1H), 7.00–7.40 (m, 4H), 7.65–7.90 (m, 3H), 11.01 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 115.9 (C), 116.1 (CH), 117.3 (CH), 118.4 (C), 119.3 (CH), 120.4 (CH), 122.2 (CH), 125.2 (CH), 127.6 (CH), 127.9 (C), 129.2 (C), 129.6 (CH), 135.2 (C), 143.9 (C), 159.6 (C), 159.9 (C), 163.7 (C), 169.3 (C); Anal. Calcd for $C_{18}H_9F_4N_3O_2$: C, 57.61; H, 2.42; N, 11.20. Found; C, 57.34; H, 2.79; N, 11.37; *m/z* (FAB): 376.1 (MH^+, 3%).

5.1.38. 10-(4-Fluorophenyl)-9-(trifluoromethyl)-10*H*-pyrimido[4,5-*b*]quinoline-2,4-dione (28). Yield 80%; mp 327–329 °C; IR (KBr): 3296 (NH), 1699 (CO), 1597 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 5.50 (s, 1H), 7.00–7.40 (m, 4H), 7.65–7.90 (m, 3H), 11.01 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 115.8 (C), 116.2 (CH), 117.9 (C), 118.9 (CH), 120.5 (CH), 124.6 (CH), 127.9 (C), 128.1 (CH), 129.4 (CH), 134.5 (C), 137.4 (C), 142.2 (C), 149.3 (C), 157.2 (C), 158.1 (C), 161.0 (C); Anal. Calcd for $C_{18}H_9F_4N_3O_2$: C, 57.61; H, 2.42; N, 11.20. Found; C, 57.34; H, 2.79; N, 11.37; *m/z* (FAB): 376.1 (MH^+, 2%).

5.1.39. 10-(4-Methylphenyl)-6-(trifluoromethyl)-10*H*-pyrimido-[4,5-*b*]quinoline-2,4-dione (29). Yield 40%; mp > 360 °C; IR (KBr): 3145 (NH), 3006 (CH), 1714 (CO), 1670 (CO) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 2.47 (s, 3H), 6.91 (d, 1H, $J = 9.2$ Hz), 7.32 (AA′BB′ system, 2H, $J = 8.2$ Hz), 7.50 (AA′BB′ system, 2H, $J = 8.2$ Hz), 8.00 (dd, 1H, $J = 9.2$, 2.0 Hz), 9.22 (br s, 1H), 10.33 (s, 1H), 11.23 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 20.8 (CH), 117.0 (CH), 118.5 (CH), 120.5 (C), 122.4 (C), 124.4 (CF_3, q, $J = 33$ Hz), 128.0 (CH), 128.8 (CH), 130.3 (CH), 130.8 (CH), 134.7 (C), 139.1 (C), 141.9 (CH), 143.9 (C). 156.4 (C), 159.3 (C), 161.6 (C); Anal. Calcd for C, 61.46; H, 3.26; N, 11.32. Found: C, 61.34; H, 3.09; N, 11.24; *mz* (FAB): 372.10 (MH^+, 93%).

5.1.40. 12-Phenyl-12*H*-1,3,11,12-tetraazanaphthacene-2,4-dione 30. Yield 74%; mp (dec) 353–357 °C; IR (KBr): 3230 (NH), 1713 (CO), 1628 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): 7.40–7.42 (m, 2H), 7.56–7.58 (m, 1H), 7.62–7.68 (m, 4H), 7.85–7.87 (m, 1H), 8.32 (d, 1H, $J = 7.6$ Hz), 9.21 (s, 1H), 9.34 (s, 1H), 11.3 (s, 1H); Anal. Calcd for $C_{20}H_{12}N_4O_2$: C, 70.58; H, 3.55; N, 16.46. Found: C, 70.47; H, 3.46; N, 16.34; *m*/*z* (FAB): 341.2 (MH^+, 1%).

Acknowledgments

We thank the University of Glasgow for a Scholarship (J.M.W.) and CRUK for supporting R.L.L., K.H.V. and J.M.W.

References and notes

1. Vousden, K. H.; Lu, X. *Nat. Rev. Cancer* **2002**, *2*, 594.
2. Schmitt, C. A.; Fridman, S. J.; Yang, M.; Baranov, E.; Hoffman, R. M.; Lowe, S. W. *Cancer Cell* **2002**, *1*, 289.
3. Symonds, H.; Krall, L.; Remington, L.; Saenzrobles, M.; Lowe, S.; Jacks, T.; Van Dyke, T. *Cell* **1994**, *78*, 703.
4. Fazeli, A.; Steen, R. G.; Dickinson, S. L.; Bautista, D.; Dietrich, W. F.; Bronson, R. T.; Bresalier, R. S.; Lander, E. S.; Costa, J.; Weinberg, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10199.
5. Liu, G.; Parant, J. M.; Lang, G.; Chau, P.; Chavez-Reyes, A.; El-Naggar, A. K.; Multani, A.; Chang, S.; Lozano, G. *Nat. Genet.* **2004**, *36*, 63.
6. Ryan, K. M.; O'Prey, J.; Vousden, K. H. *Cancer Res.* **2004**, *64*, 4415.
7. Michael, D.; Oren, M. *Semin. Cancer Biol.* **2003**, *13*, 49.
8. Ljungman, M. *Neoplasia* **2000**, *2*, 208.
9. Meek, D. W.; Knippschild, U. *Cancer Res.* **2003**, *1*, 1017.
10. Dai, M. S.; Zeng, S. X.; Jin, Y.; Sun, X. X.; David, L.; Lu, H. *Mol. Biol. Cell* **2004**, *24*, 7654.
11. Jin, A.; Itahana, K.; O'Keefe, K.; Zhang, Y. *Mol. Cell. Biol.* **2004**, *24*, 7669.
12. Lohrum, M. A.; Ludwig, R. L.; Kubbutat, M. H. G.; Hanlon, M.; Vousden, K. H. *Cancer Cell* **2003**, *3*, 577.
13. Sherr, C. J.; Weber, J. D. *Curr. Opin. Genet. Dev.* **2000**, *10*, 94.
14. Zhang, Y.; Wolf, G. W.; Bhat, K.; Jin, A.; Allio, T.; Burkhart, W. A.; Xiong, Y. *Mol. Cell. Biol.* **2003**, *23*, 8902.
15. Yang, Y.; Ludwig, R. L.; Jensen, J. P.; Pierre, S. A.; Medaglia, M. V.; Davydov, I. V.; Safiran, Y. J.; Oberoi, P.; Kenten, J. H.; Phillips, A. C.; Weissman, A. M.; Vousden, K. H. *Cancer Cell* **2005**, *7*, 547.
16. Nagamatsu, T.; Hashiguchi, Y.; Yoneda, F. *J. Chem. Soc., Perkin Trans. 1* **1984**, 561.
17. Yoneda, F.; Shinozuka, K.; Tsukuda, K. *J. Heterocycl. Chem.* **1979**, *16*, 1365.
18. Martindale, J. L.; Holbrook, N. J. *J. Cell. Physiol.* **2002**, *192*, 119.
19. Loudon, J. D.; Shulman, N. J. *J. Chem. Soc.* **1941**, 772.
20. Suhr, H. *Chem. Ber.* **1963**, *97*, 3268.
21. Rowan, S.; Ludwig, R. L.; Haupt, Y.; Bates, S.; Lu, X.; Oren, M.; Vousden, K. H. *EMBO J.* **1996**, *15*, 827.
22. Marine, J. C.; Francoz, S.; Maetens, M.; Wahl, G.; Toledo, F.; Lozano, G. *Cell Death Diff.* **2006**, *13*, 927.
23. Lin, J.; Chen, J.; Eleubaas, B.; Lenne, A. J. *Genes Dev.* **1994**, *8*, 1235.
24. Iwata, M.; Bruice, T. C.; Carrell, H. L.; Glusker, J. P. *J. Am. Chem. Soc.* **1980**, *102*, 5036.