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# Synthesis and biological activity of fluoroquinolone-pyrrolo[2,1-*c*][1,4]benzodiazepine conjugates

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**Abstract**—Fluoroquinolones have been synthesized and linked to DC-81 at C8 position through different alkyl chain spacers. These PBD conjugates have exhibited good DNA binding affinity, and a representative compound shows promising in vitro anticancer activity.


## 1. Introduction

The imine or carbinolamine-containing pyrrlo[2,1-*c*][1,4]benzodiazepines are a family of low molecular weight antitumour antibiotics originally isolated from various *Streptomyces* species[1] and examples of which include, DC-81 (**1**), anthramycin (**2**), tomaymycin and sibiromycin. These antibiotics bind selectively in the minor groove of DNA through a covalent aminal bond between the electrophilic C11 position of the PBD moiety and the nucleophilic N2-amino group of a guanine base,[2] resulting in the possible biological activity. In the literature, large number of PBD conjugates and its dimers have been synthesized and evaluated for their biological activity, particularly for their antitumour potential.[3] Thurston and co-workers have synthesized some new PBD dimers, for example, SJG-136 (**3a**) and DRG-16 (**3b**) that shows markedly superior in vitro cytotoxic potency and interstrand DNA cross-linking reactivity, and SJG-136 is presently under clinical evaluation.[4] Moreover, recently mixed imine–amide (**4**) and imine–amine PBD dimers have been designed and synthesized in this laboratory for investigating the contribution from the non-covalent interactions by one of the subunit in these dimers. It has been observed that the contribution by such a non-covalent component is very significant as DNA binding affinity is remarkable in such mixed-type of PBD dimers[5] (Fig. 1).

In literature, quinolone ring system has been extensively exploited for the development of a broad spectrum of antimicrobial drugs.[6] The compounds based on quinolones skeleton particularly fluoroquinolones are the only direct inhibitors of the DNA synthesis by binding to the enzyme DNA complex, they stabilize DNA strand breaks created by DNA gyrase and topoisomerase II and IV.[7] Further, in the literature a number of antiviral and antitumour agents have been developed in which fluorine substitution has played a key role in their biological activity. Recently, some quinolones like 7-(2,6-dimethyl-4-pyridyl) derivative WIN57294 (**5a**) have been identified as potent antineoplastic agents.[8] Similarly, 1,4-dihydro-4-oxanaphthyridine-3-carboxylic acids, for example, AT-3639 (**5b**) have displayed good in vitro as well as in vivo activity against murine tumour cells in mouse P-388 leukemia models.[9] More recently, quinobenzoxazines such as A-62176 (**5c**) demonstrated broad activity against human and murine tumour cell lines[10] (Fig. 2).

The development of conjugates or hybrid molecules between two type of cytotoxic moieties represent a new approach in the discovery of new antitumour agents, as they could posses not only high potency but also different alkylation sites, both the aspects are useful for tumour treatment. Our recent efforts in the structural modification of the PBD-ring system have led to the synthesis of a variety of PBD hybrids[11] that exhibited not only good DNA binding affinity but also promising in vitro anticancer activity. Moreover, we have also been involved in the development of new synthetic strategies[12] including solid-phase synthesis[13] for this ring system. It is observed from the literature that

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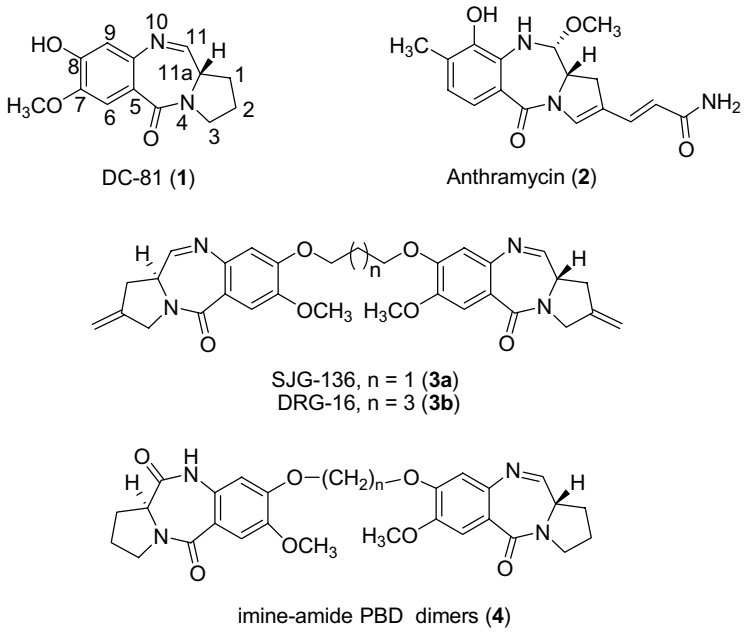



**Figure 1.** Structures of pyrrolobenzodiazepines and their dimers.

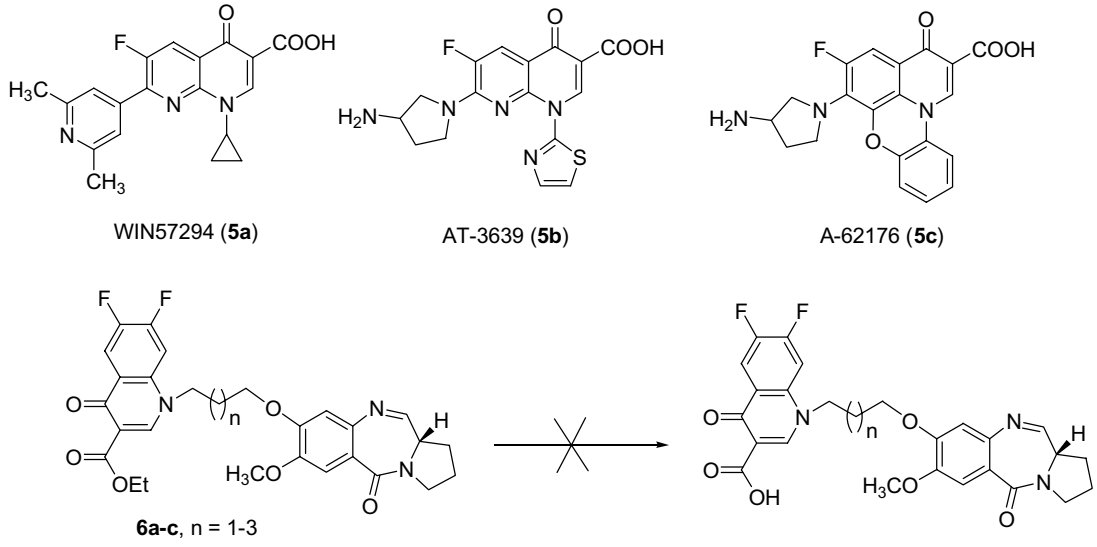



**Figure 2.** Structures of 1,8-naphthyridines, quinobenzoxazines and fluoroquinolone-PBD conjugates.

1,4-dihydro-4-oxo-3-pyridine carboxylic acids (quinolone-3-carboxylic acids) moiety is usually essential for the antitumour activity, however to prepare such a moiety linked to the PBD ring system posed synthetic problems. Numerous attempts to hydrolyze the ester functionality of the quinolone ring either before or after the imine formation has not provided the desired product. Nevertheless, attempts are in progress to prepare the free acid group containing fluoroquinolone–PBD conjugates by employing enzymatic procedures. However, it was of interest to prepare compounds, in which fluoroquinolone moiety has been linked to the PBD-ring system even without the free 3-carboxylic acid functionality at the fluoroquinolone ring particularly to understand the effect of the incorporation of fluoroquinolone moiety on the DNA binding potential. We herein report the synthesis of PBDs that are linked to the fluoroquinolone through different alkyl chain spacers at C8 position. Interestingly, these fluoroquinolone–PBDs (**6a**–**c**) have shown good DNA binding affinity and promising in vitro cytotoxicity.

## 2. Results and discussion

### 2.1. Synthesis

Synthesis of quinolone–PBD conjugates **6a**–**c** has been carried out by employing the (2*S*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-pyrrolidine-2-carboxaldehyde-diethyl thioacetal (**11**) as the starting material. This

compound has been prepared by a literature method,[14] which upon debenzylation gives (2*S*)-*N*-(4-hydroxy-5-methoxy-2-nitrobenzoyl)-pyrrolidine-2-carboxaldehyde-diethyl thioacetal (**12**). The main precursors **13a–c** have been obtained by coupling of compound **12** and *N*-alkylbromo-6,7-difluoro-4-hydroxyquinolone-3-ethyl-carboxylate (**10a–c**). These nitrothioacetals have been reduced with $SnCl_2{\cdot}2H_2O$ to give **14a–c**. The deprotection of these amino thioacetal by employing $HgCl_2$/$CaCO_3$ afford the target imine compounds **6a–c** (Scheme 2).[15] The precursors **10a–c** have been prepared by the monoalkylation of compound (**9**) with dibromoalkanes. The compound ethyl-6,7-difluoro-4-hydroxy-3-quinoline carboxylate (**9**) has been prepared by the cyclization of diethyl-2-(3,4-difluoroanilinomethylene)malonate (**8**), which has been obtained by the condensation of 3,4-difluoroaniline (**7**) with diethyl ethoxymethylenemalonate (Scheme 1).

### 2.2. Cytotoxicity

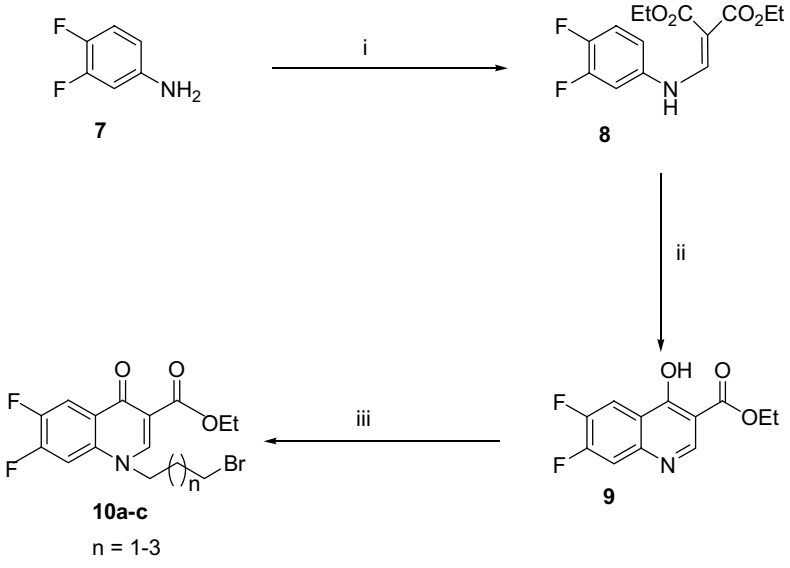
As representative members, compounds **6b–c** have been evaluated for their in vitro cytotoxicity in selected human cancer cell lines of colon (HT-29, HCT-15), lung (A-549, HOP-62), cervix (SiHa) origin by using SRB method. Usually, when the concentration of the solution is $10^{-6}$ mol/L, the inhibition of the solution is more than 50%, and then the compound is considered as an effective agent. According to this standard, it has been observed from Table 2 that both **6b** and **6c** exhibit a strong effect to HCT-15, A-549 cell lines. However, the in vitro cytotoxicity ($IC_{50}$) for naturally occurring DC-81[16] is 0.38 and 0.33 μM in L1210 and PC6 cell lines.

### 2.3. DNA interactions: thermal denaturation studies

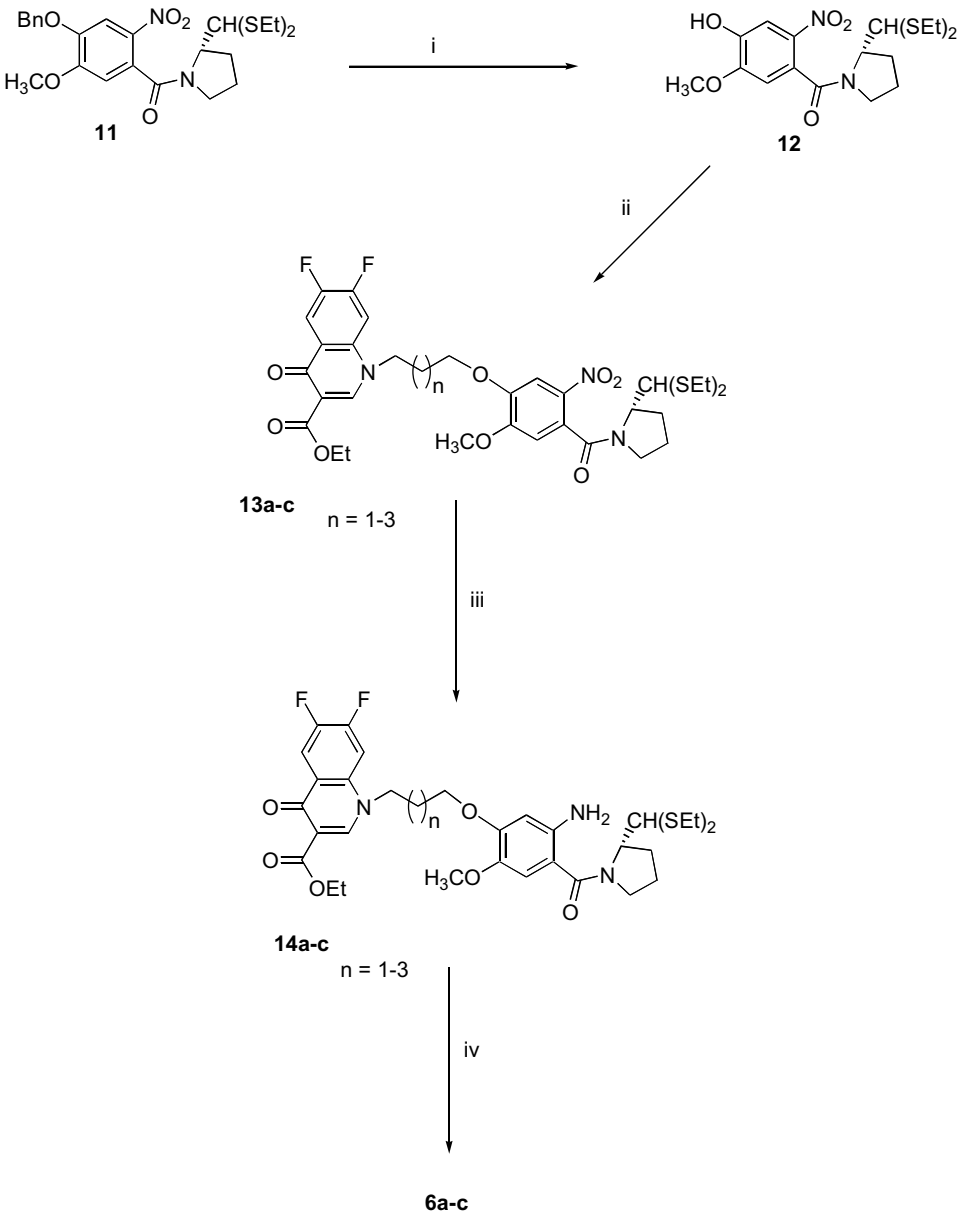
The DNA binding ability for these C8-linked PBD conjugates has been examined by thermal denaturation studies using calf thymus (CT) DNA. Melting studies show that these compounds stabilize the thermal helix → coil or melting stabilization ($\Delta T_m$) for the CT-DNA duplex at pH 7.0, incubated for 18 h at 37 °C, where PBD/DNA molar ratio is 1:5. Interestingly, in this study PBD conjugates have shown moderate melting temperature values (1.6–3.9 °C). Data for compounds **6a–c** and DC-81 are included in Table 1. It is interesting, to observe that both the PBD conjugates **6b** and **6c** the $\Delta T_m$ values are higher when the length of alkyl chain spacer is four or five. In the same experiment the naturally occurring DC-81 exhibits a $\Delta T_m$ of 0.7 °C.

### 2.4. $RED_{100}$-restriction endonuclease digestion assay

Many studies have employed restriction endonuclease inhibition to confirm the relative binding affinity of DNA-interactive small molecule ligands.[17–20] A quantitative restriction enzyme digestion ($RED_{100}$) assay has been developed in which the inhibition of DNA cleavage by *Bam*H1 is used to probe the DNA binding capability of PBD monomers.[21] We have earlier investigated this assay for preferences of base pair selectivity of the imine–amide PBD dimers.[22] Recently, this study has been carried out to determine the DNA binding ability of A-C8/C-C2-*exo* unsaturated alkoxyamido-linked PBD dimers.[23] Moreover, this technique has also been used to study the covalent DNA interaction of PBD dimers and it is capable to distinguish between the monomeric and dimeric families.[4] The *Bam*H1 cleavage sequence G↓GATCC overlaps with several favoured PBD binding sites suggesting ligand binding has the potential to inhibit the *Bam*H1 cleavage activity. The study has been carried out to determine the ability of **6a–c**, which inhibits the DNA linearization by *Bam*H1. The results of this experiment for compounds **6a**, **6b** and **6c** are shown in Figure 3 suggest that the fluoroquinolone–PBDs inhibit *Bam*H1. There are differences in the inhibitory activity exhibited by PBDs evaluated in this assay. It is observed that the ranking order is **6c > 6b > 6a** for inhibition of *Bam*H1 cleavage is in agreement with the DNA binding affinity as determined




**Scheme 1.** Reagents and conditions: (i) diethylethoxymethylenemalonate, 110 °C, 1.5 h, 98%; (ii) diphenyl ether, 250 °C, 1 h, 81%; (iii) dibromoalkanes, $K_2CO_3$, acetone, reflux, 48 h, 80–90%.




**Scheme 2.** Reagents and conditions: (i) EtSH–$BF_3OEt_2$, $CH_2Cl_2$, 12 h, rt, 76% (ii) **10a**–**c**, $K_2CO_3$, acetone, reflux, 48 h, 90–95%; (iii) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 6–8 h, 80–85%; (iv) $HgCl_2$, $CaCO_3$, $CH_3CN$–$H_2O$ (4:1), rt, 12 h, 60–70%.

**Table 1.** Thermal denaturation data for fluoroquinolone–PBD conjugates with calf thymus (CT) DNA

| PBD conjugates | [PBD]:[DNA] molar ratio[a] | $\Delta T_m$ (°C)[b] after incubation at 37 °C for | |
|---|---|---|---|
| | | 0 h | 18 h |
| **6a** | 1:5 | 1.4 | 1.6 |
| **6b** | 1:5 | 1.6 | 2.0 |
| **6c** | 1:5 | 3.4 | 3.9 |
| DC-81 | 1:5 | 0.3 | 0.7 |

[a] For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

[b] For CT-DNA alone at pH 7.00 ± 0.01, $T_m$ = 69.2 °C ± 0.01 (mean value from 20 separate determinations), all $\Delta T_m$ values are ±0.05–0.1 °C.

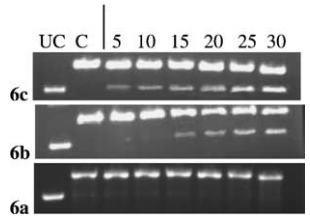



**Figure 3.** $RED_{100}$-restriction endonuclease digestion assay for fluoroquinolone–PBDs conjugates CT-DNA inhibitory activity of **6a**, **6b** and **6c** on the cleavage of plasmid pBR322 by restriction endonuclease *Bam*H1 (20 units in 2 μL) for 1 h at 37 °C. The cut (C) and uncut (UC) products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining under UV illumination. Lane 1: control pBR322; lane 2: complete digest of pBR322 by *Bam*H1.

by thermal denaturation. These results clearly demonstrate that as the linker chain increases from three to five

**Table 2.** The percentage growth inhibition data for fluoroquinolone–PBD hybrids

| | Cell lines | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Colon | | | Colon | | | Lung | | | Lung | | | Cervix | | |
| | HT-29 | | | HCT-15 | | | A-549 | | | HOP-62 | | | SiHa | | |
| Compd (mol/L) | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| **6b** | 77 | 40 | 9 | NT | 96 | 52 | 78 | 43 | 49 | 21 | 10 | 8 | 49 | 28 | 12 |
| **6c** | 79 | 65 | 25 | NT | 47 | 59 | 79 | 69 | 55 | NT | 29 | 10 | 43 | 33 | 25 |

carbon spacer as in the case of **6c** there is an enhancement in the inhibitory activity (Table 2).

## 2.5. Conclusion

In conclusion, these new quinolone–PBD conjugates exhibit DNA binding affinity and promising in vitro antitumour activity. Interestingly, upon increase of the size of the alkane linker from three to five carbons the DNA melting temperatures enhance considerably. The restriction endonuclease studies also demonstrate this aspect and suggest that these molecules selectively interact with G-sequences in DNA and low affinity with AT-rich sequences. Moreover the DNA binding potential of this quinolone–PBD conjugates is more than the naturally occurring PBD (DC-81). However, the in vitro anticancer activity for the representative compounds **6b** and **6c** is moderate.

## 3. Experimental

Reaction progress was monitored by thin-layer chromatography (TLC) using $GF_{254}$ silica gel with fluorescent indicator on glass plates. Visualization was achieved with UV light and iodine vapor unless otherwise stated. Chromatography was performed using Acme silica gel (100–200 and 60–120 mesh). The majority of reaction solvents were purified by distillation under nitrogen from the indicated drying agent and used fresh: dichloromethane (calcium hydride), tetrahydrofuran (sodium benzophenone ketyl), methanol (magnesium methoxide), acetonitrile (calcium hydride).

$^1$H NMR spectra were recorded on Varian Gemini 200 MHz spectrometer using tetramethyl silane (TMS) as an internal standard. Chemical shifts are reported in parts per million (ppm) down field from tetramethyl silane. Spin multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Coupling constants are reported in Hertz (Hz). Low-resolution mass spectra were recorded on VG-7070H Micromass mass spectrometer at 200 °C, 70 eV with trap current of 200 μA and 4 kV acceleration voltages. Optical rotations are measured on Horiba, High Sensitive Polarimeter, SEPA-300.

### 3.1. Synthesis of diethyl 2-(3,4-difluoroanilinomethylene)malonate (8)

The 3,4-difluoroaniline (2.54 g, 20 mmol) reacted with diethyl ethoxymethylenemalonate (4.32 g, 21.6 mmol) at 100 °C for 80 min. During this time, a light nitrogen flow was introduced to the reaction mixture to remove the ethanol formed. Then, hexane was added to dissolve the product. The reaction mixture was warmed (100 °C) and allowed to cool to room temperature to give corresponding malonate as a white crystalline compound **8** (5.04 g, 85%): mp 76–77 °C; IR (KBr) 1739 $cm^{-1}$ (ester), 1688 $cm^{-1}$; $^1$H NMR $CDCl_3$: $\delta$ (ppm) 1.20 (t, 3H, $J$ = 7.12 Hz), 1.24 (t, 3H, $J$ = 7.12 Hz), 4.18 (q, 2H, $J$ = 7.12 Hz), 4.24 (q, 2H, $J$ = 7.12 Hz), 6.79–6.88 (m, 1H), 6.92–6.88 (m, 1H), 7.07–7.25 (m, 1H), 8.27 (d, 1H, $J$ = 12.83 Hz), 10.97 (d, NH, $J$ = 12.83 Hz); MS (EI) $m/z$; 299. Anal. Calcd for $C_{14}H_{15}F_2NO_4$: C, 56.19; H, 5.05; N, 4.68. Found: C, 56.09; H, 4.95; N, 4.59.

### 3.2. Synthesis of ethyl 6,7-difluoro-4-hydroxy-3-quinolinecarboxylate (9)

Diphenylether (14 mL) was heated in an oil bath at 250 °C and the malonate **8** (3.56 g, 12.0 mmol) was slowly added. The mixture was kept under reflux for 1 h. During this time vapours evolved and a white solid was formed. The solid was filtered and washed with hexane to remove the excess of diphenylether. The corresponding ester **9** was obtained as a white solid.

### 3.3. Synthesis of ethyl-1-(4-bromopropyl)-6,7-difluoro-4-oxo-1,4-dihydro-3-quinolinecarboxylate (10a)

To a solution of **9** (1.00 g, 3.9 mmol) in dry acetone (15 mL) was added anhydrous potassium carbonate (2.72 g, 19.7 mmol) and the 1,3-dibromopropane (2.40 g, 11.8 mmol). The reaction mixture was refluxed in an oil bath for 48 h and the reaction was monitored by TLC using ethyl acetate–hexane (8:2) as a solvent system. The potassium carbonate was removed by suction filtration and the solvent removed under vacuum to afford the crude product. This was further purified by column chromatography using ethyl acetate–hexane (6:4) as a solvent system to give pure product **10a** (1.17 g, 80%): IR (KBr) 1718 $cm^{-1}$ (ester), 1608 $cm^{-1}$ (keto); $^1$H NMR $CDCl_3$: $\delta$ (ppm) 1.42 (t, 3H, $J$ = 6.90 Hz), 1.85–2.06 (m, 2H), 3.40 (t, 2H, $J$ = 6.13 Hz), 4.19 (t, 2H, $J$ = 6.90 Hz), 4.37 (q, 2H, $J$ = 7.66 Hz), 7.44 (q, 1H, $J_{H-F}$ = 6.04 Hz), 8.15 (t, 1H, $J_{H-F}$ = 9.06 Hz), 8.36 (s, 1H); MS (EI) $m/z$ 374 $(M+1)^+$. Anal. Calcd for $C_{15}H_{14}BrF_2NO_3$: C, 48.15; H, 3.77; N, 3.74. Found: C, 48.02; H, 3.44; N, 3.64.

### 3.4. Synthesis of ethyl 1-(4-bromobutyl)-6,7-difluoro-4-oxo-1,4-dihydro-3-quinoline-carboxylate (10b)

Compound **10b** was prepared according to the method described for compound **10a** employing compound **9**

(1.00 g, 3.9 mmol) 1,4-dibromobutane (2.57 g, 11.8 mmol) and $K_2CO_3$ (2.72 g, 19.7 mmol) to afford the crude product, which was purified by column chromatography using ethyl acetate–hexane (8:2) to afford **10b** (1.36 g, 90%): IR (KBr) 1718 $cm^{-1}$ (ester), 1608 $cm^{-1}$ (keto); $^1H$ NMR $CDCl_3$: $\delta$ (ppm) 1.42 (t, 3H, $J$ = 6.90 Hz), 1.85–2.06 (m, 2H), 3.40 (t, 2H, $J$ = 6.13 Hz), 4.19 (t, 2H, $J$ = 6.90 Hz), 4.37 (q, 2H, $J$ = 7.66 Hz), 7.50 (q, 1H, $J_{H-F}$ = 6.04 Hz), 8.18, (t, 1H, $J_{H-F}$ = 9.06 Hz), 8.36 (s, 1H); MS (EI) $m/z$ 389 $(M+1)^+$. Anal. Calcd for $C_{16}H_{16}BrF_2NO_3$: C, 49.50; H, 4.15; N, 3.61. Found: C, 49.53; H, 3.89; N, 3.45.

### 3.5. Synthesis of ethyl 1-(4-bromopentyl)-6,7-difluoro-4-oxo-1,4-dihydro-3-quinoline-carboxylate (10c)

Compound **10c** was prepared according to the method described for compound **10a** employing compound **9** (1.00 g, 3.9 mmol) 1,5-dibromopentane (2.73 g, 11.8 mmol) and $K_2CO_3$ (2.72 g, 19.7 mmol) to afford the crude product, which was purified by column chromatography using ethyl acetate–hexane (8:2) to afford **10c** (1.41 g, 90%): IR (KBr) 1718 $cm^{-1}$ (ester), 1608 $cm^{-1}$ (keto); $^1H$ NMR $CDCl_3$: $\delta$ (ppm) 1.41 (t, 3H, $J$ = 7.55 Hz), 1.55–1.65 (m, 2H), 1.87–1.98 (m, 4H), 3.39 (t, 2H, $J$ = 6.04 Hz), 4.13 (t, 2H, $J$ = 7.55 Hz), 4.35 (q, 2H, $J$ = 6.75 Hz), 7.17 (q, 1H, $J_{H-F}$ = 6.04 Hz), 8.24 (t, 1H, $J_{H-F}$ = 9.06 Hz), 8.36 (s, 1H); MS (EI 70 eV) $m/z$ 402 $(M+1)^+$. Anal. Calcd for $C_{17}H_{18}BrF_2NO_3$: C, 50.76; H, 4.51; N, 3.48. Found: C, 50.82; H, 4.34; N, 3.23.

### 3.6. 2-Di(ethylsulfanyl)methyl-(2*S*)-tetrahydro-1*H*-1-pyrrolyl-4-hydroxy-5-methoxy-2-nitrophenylmethanone (12)

To a stirred solution of EtSH (1.91 g, 19 mmol) and $BF_3{\cdot}OEt_2$ (1.41 g, 10 mmol) in DCM was added dropwise compound **11** (0.49 g, 1 mmol) in DCM (10 mL) at rt. Stirring was continued until TLC indicated completion of the reaction. The solvent was evaporated in vacuum. The residue was quenched with water (25 mL) and then extracted with ethyl acetate ($3 \times 25$ mL). The combined organic phase washed with brine ($1 \times 25$ mL) dried over $Na_2SO_4$ and the solvent removed in vacuum to afford the crude product. This was further purified by column chromatography using ethyl acetate–hexane (7:3) to afford the compound **12** (0.30 g, 75%); $^1H$ NMR $CDCl_3$: $\delta$ (ppm) 1.20–1.40 (m, 6H), 1.75–2.35 (m, 4H), 2.70–2.88 (m, 4H), 3.20–3.32 (m, 2H), 3.95 (s, 1H), 4.60–4.70 (m, 1H), 4.85 (d, 1H, $J$ = 6.30 Hz), 6.20–6.32 (br s, 1H), 6.75 (s, 1H), 7.75 (s, 1H); MS (EI) $m/z$ $400M^+$. Anal. Calcd for $C_{17}H_{24}N_2O_5S_2$: C, 50.98; H, 6.04; N, 6.99. Found: C, 50.63; H, 5.98; N, 6.58.

### 3.7. Ethyl-1-(3-4-[2-di(ethylsulfanyl)methyl-(2*S*)-tetrahydro-1*H*-1-pyrrolylcarbonyl]-2-methoxy-5-nitrophenoxy-propyl)-6,7-difluoro-4-oxo-1,4-dihydro-3-quinolinecarboxylate (13a)

To a stirred solution of **12** (0.40 g, 1 mmol) in acetone (15 mL) was added anhydrous potassium carbonate (0.69 g, 5.0 mmol) and compound **10a** (0.39 g, 1.1 mmol). The reaction mixture was refluxed in an oil bath for 48 h and the reaction was monitored by TLC using ethyl acetate–hexane (8:2) as a solvent system. The potassium carbonate was removed by suction filtration and solvent was removed under vacuum to afford the crude product. This was further purified by column chromatography using ethyl acetate–hexane (8:2) as a solvent system to give product **13a** (0.61 g, 90%): $^1H$ NMR $CDCl_3$: $\delta$ (ppm) 1.32–1.40 (m, 10H), 1.98–2.02 (m, 3H), 2.07–2.17 (m, 3H), 2.29–2.31 (m, 1H), 2.65–2.87 (m, 4H), 3.20–3.27 (m, 2H), 3.94 (s, 3H), 4.17–4.23 (m, 2H), 4.29–4.40 (m, 4H), 4.62–4.68 (m, 1H), 4.80 (d, 1H, $J$ = 3.77 Hz), 6.80 (s, 1H), 7.36 (q, 1H, $J_{H-F}$ = 6.79 Hz), 7.59 (s, 1H), 8.19 (t, 1H, $J_{H-F}$ = 9.06 Hz), 8.53 (s, 1H); MS (FAB) 693 $(M+1)^+$. Anal. Calcd for $C_{32}H_{37}F_2N_3O_8S_2$: C, 55.40; H, 5.38; N, 6.06. Found: C, 55.44; H, 5.43; F, 5.34; N, 5.98.

### 3.8. Ethyl-1-(4-4-[2-di(ethylsulfanyl)methyl-(2*S*)-tetrahydro-1*H*-1-pyrrolylcarbonyl]-2-methoxy-5-nitrophenoxybutyl)-6,7-difluoro-4-oxo-1,4-dihydro-3-quinoline-carboxylate (13b)

Compound **13b** was prepared according to the method described for compound **13a** employing compound **12** (0.400 g, 1 mmol), **10b** (0.41 g, 1.1 mmol) and $K_2CO_3$ (0.69 g, 5 mmol) to afford the crude product, which was purified by column chromatography with ethyl acetate–hexane (8:2) to afford compound **13b** (0.65 g, 95%): $^1H$ NMR $CDCl_3$: $\delta$ (ppm) 1.32–1.38 (m, 10H), 1.92–2.00 (m, 3H), 2.09–2.19 (m, 3H), 2.32–2.35 (m, 1H), 2.58–2.78 (m, 6H), 3.20–3.29 (m, 2H), 3.98 (s, 3H), 4.21–4.25 (m, 2H), 4.31–4.38 (m, 4H), 4.63–4.69 (m, 1H), 4.85 (d, 1H, $J$ = 3.78 Hz), 6.82 (s, 1H), 7.38 (q, 1H, $J_{H-F}$ = 6.83 Hz), 7.61 (s, 1H), 8.19 (t, 1H, $J_{H-F}$ = 9.09 Hz), 8.55 (s, 1H); MS (FAB) 707 $(M+1)^+$. Anal Calcd for: $C_{33}H_{39}F_2N_3O_8S_2$: C, 56.00; H, 5.55; N, 5.94. Found: C, 55.86; H, 5.33; F, 5.39; N, 5.78.

### 3.9. Ethyl-l-1-(5-4-[2-di(ethylsulfanyl)methyl-(2*S*)-tetrahydro-1*H*-1-pyrrolylcarbonyl]-2-methoxy-5-nitrophenoxypentyl)-6,7-difluoro-4-oxo-1,4-dihydro-3-quinoline-carboxylate (13c)

Compound **13c** was prepared according to the method described for compound **13a** employing compound **12** (0.40 g, 1 mmol), **10c** (0.42 g, 1.1 mmol) and $K_2CO_3$ (0.69 g, 5 mmol) to afford the crude product, which was purified by column chromatography with ethyl acetate–hexane (8:2) to afford compound **13c** (0.66 g, 94%); $^1H$ NMR $CDCl_3$: $\delta$ (ppm) 1.32–1.36 (m, 10H), 1.93–2.02 (m, 3H), 2.10–2.19 (m, 3H), 2.31–2.34 (m, 1H), 2.56–2.79 (m, 8H), 3.20–3.28 (m, 2H), 3.96 (s, 3H), 4.22–4.26 (m, 2H), 4.32–4.39 (m, 4H), 4.64–4.70 (m, 1H), 4.86 (d, 1H, $J$ = 3.76 Hz), 6.85 (s, 1H), 7.40 (q, 1H, $J_{H-F}$ = 6.87 Hz), 7.63 (s, 1H), 8.21 (t, 1H, $J_{H-F}$ = 9.12 Hz), 8.58 (s, 1H), MS (FAB) 721 $(M+1)^+$. Anal. Calcd for $C_{34}H_{41}F_2N_3O_8S_2$: C, 56.58; H, 5.72; N, 5.82; Found: C, 56.33; H, 5.43; N, 5.88.

### 3.10. Ethyl-1-(3-5-amino-4-[2-di(ethylsulfanyl)methyl-(2*S*)-tetrahydro-1*H*-1-pyrrolylcarbonyl]-2-methoxyphenoxypropyl)-6,7-difluoro-4-oxo-1,4-dihydro-3-quinolinecarboxylate (14a)

Compound **13a** (0.50 g, 0.74 mmol) dissolved in methanol (10 mL) and added $SnCl_2{\cdot}2H_2O$ (0.83 g, 3.70 mmol) was refluxed for 1.5 h. The progress of the reaction was monitored by TLC. The reaction mixture was cooled and the methanol was evaporated under vacuum and the residue was carefully adjusted to pH 8 with saturated $NaHCO_3$ solution and then extracted with ethyl acetate ($3 \times 30$ mL). The combined organic phase was dried over $Na_2SO_4$ and evaporated under vacuum to afford the crude product **14a**.

### 3.11. Ethyl-1-(4-5-amino-4-[2-di(ethylsulfanyl)methyl-(2*S*)-tetrahydro-1*H*-1-pyrrolylcarbonyl]-2-methoxyphenoxybutyl)-6,7-difluoro-4-oxo-1,4-dihydro-3-quinolinecarboxylate (14b)

Compound **14b** was prepared according to the method described for compound **14a** employing compound **13b** (0.50 g, 0.72 mmol) and $SnCl_2{\cdot}2H_2O$ (0.81 g, 3.62 mmol) to afford the amino diethyl thioacetal **14b**.

### 3.12. Ethyl-1-(5-5-amino-4-[2-di(ethylsulfanyl)methyl-(2*S*)-tetrahydro-1*H*-1-pyrrolylcarbonyl]-2-methoxyphenoxypentyl)-6,7-difluoro-4-oxo-1,4-dihydro-3-quinolinecarboxylate (14c)

Compound **14c** was prepared according to the method described for compound **14a** employing compound **13c** (0.50 g, 0.71 mmol) and $SnCl_2{\cdot}2H_2O$ (0.80 g, 3.54 mmol) to afford the amino diethyl thioacetal **14c**.

### 3.13. Ethyl-6,7-difluoro-1-3-[7-methoxy-5-oxo-(11a*S*)-2,3,5,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-8-yloxy]propyl-4-oxo-1,4-dihydro-3-quinolinecarboxylate (6a)

A solution of **14a** (0.15 g, 0.232 mmol), $HgCl_2$ (0.15 g, 0.534 mmol) and $CaCO_3$ (0.05 g, 0.488 mmol) in acetonitrile–water (4:1, 12 mL) was stirred at room temperature for 12 h until TLC (ethyl acetate), indicates complete loss of starting material. The reaction mixture was diluted with ethyl acetate (30 mL) and filtered through a Celite bed. The clear yellow organic supernatant was washed with saturated $NaHCO_3$ (20 mL) and the combined organic phase is dried over $Na_2SO_4$. The organic solvent was evaporated in vacuum and purified by column chromatography using ethyl acetate–methanol (9:1) to afford **6a** (0.09 g, 60%): $^1$H NMR ($CDCl_3$): $\delta$ (ppm) 1.35–1.45 (m, 3H), 1.96–2.20 (m, 4H), 2.22–2.38 (m, 2H), 3.40–3.80 (m, 4H), 3.82–3.86 (m, 2H), 3.95 (s, 3H), 4.05–4.23 (m, 2H), 4.32–4.45 (m, 1H), 6.70 (s, 1H), 7.40 (t, 1H, $J_{H-F} = 12.03$ Hz), 7.50 (s, 1H), 7.60 (d, 1H, $J = 4.4$ Hz), 8.30 (q, 1H, $J_{H-F} = 11.2$ Hz), 8.6 (s, 1H); MS (FAB) 540 $[M+1]^+$. Anal. Calcd for $C_{28}H_{27}F_2N_3O_6$: C, 62.33; H, 5.04; N, 7.79. Found: C, 62.11; H, 4.99; N, 7.69. $[\alpha]_D^{26}$ +310 (*c* 0.5, $CHCl_3$).

### 3.14. Ethyl-6,7-difluoro-1-4-[7-methoxy-5-oxo-(11a*S*)-2,3,5,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-8-yloxy]butyl-4-oxo-1,4-dihydro-3-quinolinecarboxylate (6b)

Compound **6b** was prepared according to the method described for compound **6a** employing **14b** (0.20 g, 0.303 mmol), $HgCl_2$ (0.19 g, 0.689 mmol) and $CaCO_3$ (0.063 g, 0.637 mmol) to afford compound **6b** (0.14 g, 68%): $^1$HNMR ($CDCl_3$): $\delta$ (ppm) 1.33–1.42 (m, 3H), 1.99–2.18 (m, 6H), 2.24–2.39 (m, 2H), 3.43–3.85 (m, 4H), 3.85–3.89 (m, 2H), 3.97 (s, 3H), 4.09–4.25 (m, 2H), 4.35–4.45 (m, 1H), 6.78 (s, 1H), 7.44 (t, 1H, $J_{H-F} = 12.03$ Hz), 7.54 (s, 1H), 7.63 (d, 1H, $J = 4.4$ Hz), 8.36 (q, 1H, $J_{H-F} = 11.2$ Hz), 8.65 (s, 1H); MS (FAB) 554 $[M+1]^+$. Anal. Calcd for $C_{29}H_{29}F_2N_3O_6$: C, 62.92; H, 5.28; N,7.59. Found: C, 62.71; H, 5.09; N, 7.44. $[\alpha]_D^{26}$ +368 (*c* 0.5, $CHCl_3$).

### 3.15. Ethyl-6,7-difluoro-1-5-[7-methoxy-5-oxo-(11a*S*)-2,3,5,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-8-yloxy]pentyl-4-oxo-1,4-dihydro-3-quinolinecarboxylate (6c)

Compound **6c** was prepared according to the method described for compound **6a** employing **14c** (0.20 g, 0.297 mmol), $HgCl_2$ (0.19 g, 0.683 mmol) and $CaCO_3$ (0.06 g, 0.65 mmol) to afford compound **6c** (0.14 g, 70%): $^1$HNMR ($CDCl_3$): $\delta$ 1.35–1.45 (m, 3H), 1.96–2.20 (m, 8H), 2.22–2.38 (m, 2H), 3.40–3.80 (m, 4H), 3.82–3.86 (m, 2H), 3.95 (s, 3H), 4.05–4.23 (m, 2H), 4.32–4.45 (m, 1H), 6.70 (s, 1H), 7.40 (t, 1H, $J_{H-F} = 12.03$ Hz), 7.50 (s, 1H), 7.60 (d, 1H, $J = 4.4$ Hz), 8.28 (q, 1H, $J_{H-F} = 11.2$ Hz), 8.6 (s, 1H); MS (FAB) 568 $[M+1]^+$. Anal. Calcd for $C_{30}H_{31}F_2N_3O_6$: C, 63.48; H, 5.50; N, 7.40. Found: C, 63.23; H, 5.39; N, 7.29. $[\alpha]_D^{26}$ +392 (*c* 0.5, $CHCl_3$).

### 3.16. Thermal denaturation studies

Compounds were subjected to thermal denaturation studies with duplex-form calf thymus DNA (CT-DNA) using an adaptation of a reported procedure.[24] Working solutions in aqueous buffer (10 mM $NaH_2PO_4/Na_2HPO_4$, 1 mM $Na_2EDTA$, pH 7.00 + 0.01) containing CT-DNA (100 μm in phosphate) and the PBD (20 μm) were prepared by addition of concentrated PBD solutions in MeOH to obtain a fixed [PBD]/[DNA] molar ratio of 1:5. The DNA–PBD solutions were incubated at 37 °C for 0, and 18 h prior to analysis. Samples were monitored at 260 nm using a Beckman DU-7400 spectrophotometer fitted with high performance temperature controller, and heating was applied at 1 °C min$^{-1}$ in the 40–90 °C range. DNA helix coil transition temperatures ($T_m$) were obtained from the maxima in the $(dA_{260})/dT$ derivative plots. Results are given as the mean ± standard deviation from three determinations and are corrected for the effects of MeOH co-solvent using a linear correction term.[25] Drug-induced alterations in DNA melting behaviour are given by $\Delta T_m = T_m(\text{DNA + PBD}) - T_m$ (DNA alone), where the $T_m$ value for the PBD-free CT-DNA is 69.2 ± 0.01. The fixed [PBD]/[DNA] ratio used did

not result in binding saturation of the host DNA duplex for any compound examined.

### 3.17. In vitro evaluation of cytotoxic activity

In routine compounds **6b–c** have been evaluated for their in vitro cytotoxicity in selected human cancer cell lines of colon (HT-29, HCT-15), lung (A-549, HOP-62) and cervix (SiHa) origin. A protocol of 48 h continuous drug exposure has been used and a sulforhodamine B (SRB) protein assay has been used to estimate cell viability or growth.[26] The results are expressed as percent of cell growth inhibition determined relative to that of untreated control cells.

### 3.18. Restriction endonuclease inhibition

Stock solutions of each PBD (100 μM) were prepared by dissolving each compound in DMSO (Sigma). These were stored at −20 °C. Plasmid (pBR 322) containing single *Bam*H1 site was used in this assay. Restriction endonuclease and the relevant buffer were obtained from NEB. The DNA fragment (500 ng) was incubated with each PBD (see Fig. 3 for PBD concentrations) in a final volume of 16 μL for 16 h at 37 °C. Next 10 × *Bam*H1 buffer (2 μL) was added, and the reaction mixture was made up to 20 μL with *Bam*H1 (20 units) and then incubated for 1 h at 37 °C. Then loaded on to a 1% agarose gel electrophoresis in Tris–acetate EDTA buffer at 80 V for 2 h. The gels were stained with ethidium bromide and photographed.

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