Synthesis and evaluation of new pyrrolo[2,1-*c*][1,4]benzodiazepine hybrids linked to a flavone moiety

Ahmed Kamal,[a]* R. Ramu,[a] G. B. Ramesh Khanna,[a] Ajit Kumar Saxena,[b] M. Shanmugavel [b], and Renu Moti Pandita[b]

[a] Division of Organic Chemistry, Indian Institute of Chemical Technology, Hyderabad 500 007, India
[b] Division of Pharmacology, Regional Research Laboratory, Jammu 180 001, India
E-mail: ahmedkamal@iict.res.in

In honor of Dr. A.V. Rama Rao on the occasion of his 70th birthday
(received 10 Sep 04; accepted 31 Oct 04; published on the web 12 Nov 04)

Abstract
Pyrrolo[2,1-*c*][1,4]benzodiazepine hybrids linked to a flavone ring system have been designed, synthesized and evaluated for their biological activity. Flavone has been tethered to the C8 position of pyrrolobenzodiazepine through alkanedioxy spacers of varying lengths. These new type of hybrids exhibit significant DNA minor groove binding ability in comparison to the naturally occurring DC-81 and appreciable *in vitro* cytotoxicity.

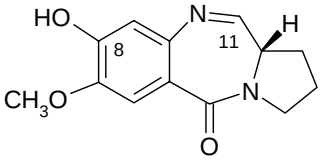
Keywords: Pyrrolobenzodiazepines, flavones, cytotoxicity, DNA-binding

Introduction

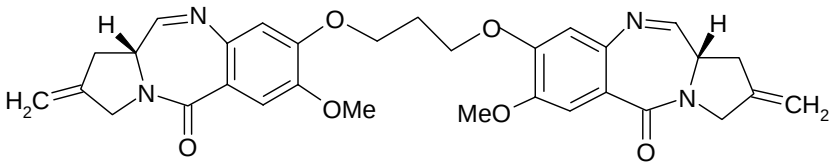
In recent years there is much interest in the design and development of DNA interactive molecules that can bind to DNA in a sequence selective manner. In spite of enormous efforts to design synthetic DNA intercalators, very few molecules are in clinical usage that exhibit sequence selectivity. The synthesis of small molecules which exhibit DNA sequence selectivity is of immense importance for targeting the rapid growth of tumour cells.

The pyrrolo[2,1-*c*][1,4]benzodazepines (PBDs) are a group of naturally occurring antitumour antibiotics produced by various *Streptomyces* species and examples of which include anthramycin, tomaymycin, sibiromycin and DC-81. The formation of a covalent bond in the minor groove of DNA by nucleophilic attack of 2-amino group of a guanine base to form an aminal linkage to C-11 is responsible for the biological activities of PBDs.[1] Studies like X-ray and DNA foot-printing on the covalent DNA-PBD adducts have demonstrated a high sequence

specificity for GC-rich DNA regions in particular, for Pu-G-Pu motifs.[2] A large number of structurally modified PBDs have been prepared and evaluated for their biological activity, particularly their antitumour and DNA binding potential.[3] A recently developed PBD dimer with C2-exomethylene substitution (SJG-136) has been selected for clinical trials (Figure 1).[4] The PBDs have been used to attach ethylenediamine tetra acetic acid (EDTA),[5] epoxide,[6] pyrrole and imidazole polyamide[7-9] and cyclopropaindole[10,11] moieties, which have exhibited sequence selective DNA-cleavating and cross linking properties. Recently, we have been involved in the development on new synthetic strategies[12] for the preparation of PBD ring systems and also in the design of structurally modified PBDs and their hybrids.[13] In this pursuit some novel PBD hybrids and conjugates have been prepared by linking the PBD moiety to other DNA interactive compounds through suitable linkers.



DC - 81

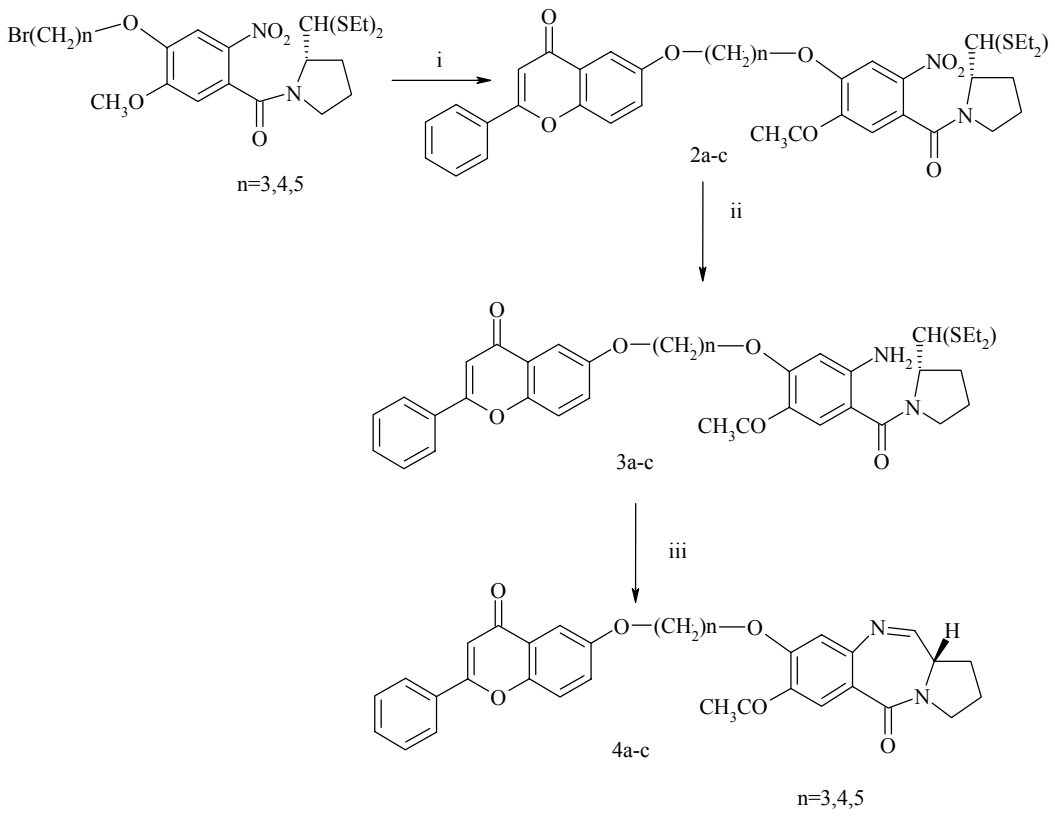


SJG - 136

Several flavonoid natural products have recently attracted attention as novel inhibitors of virus-associated reverse transcriptase.[14] These flavonoid derivatives also inhibit various cellular DNA polymerases by binding to the double stranded nucleic acid template-primer or by competing with it for a site on the enzyme. The DNA intercalation contributes to the cytotoxicity of the flavonoids.[15] In literature some flavonoids have been examined to induce enzymatic breakage by mammalian topoisomerase II.[16] Additionally the cytotoxic effects of flavonoids have demonstrated promising cytotoxicity against some selected human tumour cell lines. Taking advantage of the DNA interactive property of flavone moiety, it was considered of interest to link it to the PBD ring system through different alkane spacers for the preparation of new hybrids of PBD and evaluation of their DNA binding property as well as *in vitro* anticancer potential.

Results and Discussion

The precursor (2*S*)-*N*-[4-(n-bromoalkyloxy)-5-methoxy-2-nitrobenzoyl]-pyrrolidine-2-carbox-aldehyde diethyl thioacetal **1a-c** has been prepared following our previously described procedure.[13a] Etherification of 6-hydroxy flavone by **1a-c** gives the desired intermediate **2a-c**. This nitrothioacetal has been reduced with $SnCl_2.2H_2O$ to give **3a-c**. The deprotection of the thioacetal group afforded the desired compounds **4a-c** (Scheme).



Scheme 1. Reagents and conditions: (i) 6-hydroxyflavone, K_2CO_3, acetonitrile, reflux, 24 h; (ii) $SnSl_2$. $2H_2O$, methanol, reflux, 5 h; (iii) $HgCl_2$, $CaCO_3$, acetonitrile/H_2O, r.t., 12 h.

The DNA binding ability for these hybrids has been examined by using thermal denaturation assay with calf thymus (CT) DNA.[17] Studies have been carried out at [DNA]/[ligand] molar ratio of 5:1. The increase in helix melting temperature (ΔT_m) has been examined after 0 h and 18 h of incubation at 37 ^oC (Table 1). Interestingly, the compounds **4a** and **4c** elevates the helix melting temperature of CT-DNA by 6.2 ^oC and 2.6 ^oC after incubation for 18 h at 37 ^oC. On the other hand, the naturally occurring DC-81 exhibits a ΔT_m of 0.7 ^oC. This demonstrates that these PBD-flavone conjugates have significant DNA binding ability as illustrated in **Table 1**. The results also demonstrate that increasing chain length from three to five reduces the DNA binding affinity.

Table 1. Thermal denaturation data for flavone-PBD hybrids with CT-DNA

Compound	Induced ΔT_m °C after incubation at 37 °C	
	0 h	18 h
4a	3.8	6.2
4b	1.6	1.8
4c	2.1	2.6
DC-81	0.3	0.7

For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.8 °C ± 0.01 (mean value from 10 separate determinations), all ΔT_m values are ± 0.1 - 0.2 °C. 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].

Primarily, compounds **4a-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 sulforhodamine B (SRB) method.[18] The results are expressed as percent of cell growth inhibition determined relative to that of untreated control cells (Table 2). Usually, when the concentration of the compound solution is 10^{-6} mol/L, the inhibition of the solution is more than 50%, and then compound is considered as an effective agent. According to this standard, it has been observed from Table 2 that **4a** exhibit a strong effect to HT-29, HCT-15, HOP-62 cell lines and compound **4c** suppressing HCT-15 and A-549 cell growth by 69% and 63%. The comparison of the data in Table 2 reveals the importance of an alkane spacer. As the alkane spacer increases from three to four the growth inhibition activity has decreased. This observation has been supported by thermal denaturation studies.

Table 2. The percentage growth inhibition data for flavone-PBD hybrids

Compd	Cell lines				
	HT-29	HCT-15	A-549	HOP-62	SiHa
(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}
4a	91 87 76	a 4 66	92 a a	a 93 73	a 52 49
4b	88 68 31	a 64 49	90 a a	a 79 12	a 60 18
4c	91 78 50	a 68 69	91 52 63	a 85 46	a 42 13

a: not tested

In conclusion, we report the synthesis, DNA binding ability and *in vitro* cytotoxicity of the new PBD-flavone hybrids. These results show that an increase in linker chain length reduces the DNA binding affinity and *in vitro* growth inhibition of certain cancer cell lines. One of the hybrids with three carbon chain spacer (**4a**) exhibited significant DNA binding affinity as well as *in vitro* cytotoxicicty.

Experimental Section

General Procedures. 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 vapour 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) and acetonitrile (calcium hydride).

^{1}H NMR spectra were recorded on Varian Gemini 200 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Spin multiplicities are described as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Coupling constants are reported in Hertz (Hz). Mass spectra obtained using a FAB-MS spectrophotometer.

(2*S*)-*N*-4-[3-(2-Phenyl-4-oxo-4*H*-[1]benzopyran-6-yloxy)propoxy]-5-methoxy-2-nitrobenzoyl pyrrolidine-2-carboxaldehyde diethyl thioacetal (2a). To a solution of (2*S*)-*N*-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl) pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1a**) (521 mg, 1 mmol) in dry acetonitrile (30 mL) was added anhydrous K_2CO_3 (552 mg, 4 mmol) and 6-hydroxy flavone (238 mg, 1 mmol). The reaction mixture was refluxed for 24 h. The potassium carbonate was removed by filtration and the solvent was evaporated under vacuum, the crude product was purified by column chromatography (ethyl acetate-hexane 6:4) to afford the compound **2a** as a yellow oil. (584 mg, 86%). ^{1}H NMR ($CDCl_3$): δ 1.28-1.40 (m, 6H), 1.70-2.45 (m, 6H), 2.62-2.85 (m, 4H), 3.12-3.30 (m, 2H), 3.95 (s, 3H), 4.32 (t, 4H), 4.60-4.70 (m, 1H), 4.82 (d, 1H), 6.78 (s, 2H), 7.24-7.30 (m, 1H), 7.46-7.60 (m, 5H), 7.70 (s, 1H), 7.85-7.95 (m, 2H). FABMS 679 (M^+), Calcd for $C_{35}H_{38}N_2O_8S_2$: C, 61.93; H, 5.64; N, 4.13, S, 9.45; found: C, 61.85; H, 5.72; N, 4.07; S, 9.48.

(2*S*)-*N*-4-[4-(2-Phenyl-4-oxo-4*H*-[1]benzopyran-6-yloxy)butoxy]-5-methoxy-2-nitrobenzoyl pyrrolidine-2-carboxaldehyde diethyl thioacetal (2b). The compound **2b** was prepared following the method described for the compound **2a,** employing **1b** (535 mg, 1 mmol) and the crude product was purified by column chromatography to afford the compound **2b** as a yellow oil. (582 mg, 84%). ^{1}H NMR ($CDCl_3$): δ 1.28-1.40 (m, 6H), 1.75-2.34 (m, 8H), 2.65-2.84 (m, 4H), 3.18-3.30 (m, 2H), 3.95 (s, 3H), 4.15-4.26 (m, 4H), 4.62-4.70 (m, 1H), 4.82 (d, 1H), 6.77 (s, 2H), 7.22-7.28 (m, 1H), 7.45-7.58 (m, 5H), 7.62 (s, 1H), 7.88-7.94 (m, 2H). FABMS 693 (M^+), Calcd for $C_{36}H_{40}N_2O_8S_2$: C, 62.41; H, 5.82; N, 4.04, S, 9.25; found: C, 62.52; H, 5.88; N, 3.97; S, 9.28.

(2*S*)-*N*-4-[5-(2-Phenyl-4-oxo-4*H*-[1]benzopyran-6-yloxy)pentyloxy]-5-methoxy-2-nitrobenzoyl pyrrolidine-2-carboxaldehyde diethyl thioacetal (2c). The compound **2c** was prepared following the method described for the compound **2a,** employing **1c** (549 mg, 1 mmol) and the crude product was purified by column chromatography to afford the compound **2c** as a yellow oil.

(593 mg, 84%). ^{1}H NMR ($CDCl_3$): δ 1.30-1.40 (m, 6H), 1.70-2.32 (m, 10H), 2.65-2.85 (m, 4H), 3.18-3.30 (m, 2H), 3.96 (s, 3H), 4.08-4.16 (m, 4H), 4.62-4.70 (m, 1H), 4.82 (d, 1H), 6.78 (s, 2H), 7.22-7.30 (m, 1H), 7.45-7.55 (m, 5H), 7.62 (s, 1H), 7.88-7.96 (m, 2H). FABMS 707 (M^+), Calcd for $C_{37}H_{42}N_2O_8S_2$: C, 62.87; H, 5.99; N, 3.96, S, 9.07; found: C, 62.82; H, 6.05; N, 3.92; S, 9.11.

(2*S*)-*N*-4-[3-(2-Phenyl-4-oxo-4*H*-[1]benzopyran-6-yloxy)propoxy]-5-methoxy-2-aminobenzoyl pyrrolidine-2-carboxaldehyde diethyl thioacetal (3a). The compound **3a** (679 mg, 1 mmol) dissolved in methanol (20 mL) and added $SnCl_2.2H_2O$ (1.13 gr, 5 mmol) was refluxed for 5 h. 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 (2x30 mL). The combined organic phase was washed with brine (15 mL), dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford the amino diethyl thioacetal **3a** as yellow oil (506 mg, 78%) and this was directly used in the next step.

(2*S*)-*N*-4-[4-(2-Phenyl-4-oxo-4*H*-[1]benzopyran-6-yloxy)butoxy]-5-methoxy-2-aminobenzoyl pyrrolidine-2-carboxaldehyde diethyl thioacetal (3b). The compound **3b** was prepared following the method described for the compound **3a**, employing the compound **2b** (693 mg, 1 mmol) to afford the amino diethyl thioacetal **3b** as a yellow oil (510 mg, 77%).

(2*S*)-*N*-4-[5-(2-Phenyl-4-oxo-4*H*-[1]benzopyran-6-yloxy)pentyloxy]-5-methoxy-2-aminobenzoyl pyrrolidine-2-carboxaldehyde diethyl thioacetal (3c). The compound **3c** was prepared following the method described for the compound **3a**, employing the compound **2c** (707 mg, 1 mmol) to afford the amino diethyl thioacetal **3c** as a yellow oil (528 mg, 78%).

7-Methoxy-8-{3-[2-phenyl-4-oxo-4*H*-[1]benzopyran-6-yloxy]propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (4a). A solution of amino thioacetal **3a** (649 mg, 1 mmol), $HgCl_2$ (597 mg, 2.2 mmol) and $CaCO_3$ (240 mg, 2.4 mmol) in acetonitrile-water (4:1) was stirred slowly at room temperature for 12 h, until TLC indicate the complete disappearance of starting material. The reaction mixture was diluted with ethyl acetate (30 mL) and filtered through a celite. The clear yellow organic supernatant was extracted with ethyl acetate (2x20 mL). The organic layer was washed with saturated $NaHCO_3$ (20 mL), brine (20 mL) and the combined organic phase was dried over anhydrous Na_2SO_4. The organic layer was evaporated under vacuum and the crude product was purified by column chromatography (5% $CHCl_3$-MeOH) to afford the compound **4a** as a pale yellow solid (273 mg, 52%). This material was repeatedly evaporated from $CHCl_3$ in vacuum to generate the imine form, m.p.: 73-75 ^oC, ^{1}H NMR ($CDCl_3$): δ 1.98-2.12 (m, 3H), 2.22-2.45 (m, 3H), 3.50-3.88 (m, 3H), 3.95 (s, 3H), 4.24-4.37 (m, 4H), 6.77 (s, 1H), 6.81 (s, 1H), 7.20-7.30 (m, 1H), 7.42-7.65 (m, 7H), 7.85-7.95 (m, 2H). IR 2931, 2851, 1625, 1458, 1365, 1256, 1194, 1025 cm^{-1}, FABMS 525 (M+1), Calcd for $C_{31}H_{28}N_2O_6$: C, 70.98; H, 5.38; N, 5.34; found: C, 70.82; H, 5.46; N, 5.22.

7-Methoxy-8-{4-[2-phenyl-4-oxo-4*H*-[1]benzopyran-6-yloxy]butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (4b). The compound **4b** was prepared following the method described for the compound **4a** employing **3b** (663 mg, 1 mmol), to afford the compound **4b** as a pale yellow solid (291 mg, 54%), m.p.: 72-74 ^oC, ^{1}H NMR ($CDCl_3$): δ 1.95-2.40 (m, 8H), 3.56-3.85 (m, 3H), 3.92 (s, 3H), 4.05-4.25 (m, 4H), 6.75 (s, 2H), 7.20-7.30 (m,

1H), 7.40-7.66 (m, 7H), 7.85-7.95 (m, 2H). IR 2930, 2849, 1626, 1458, 1363, 1256, 1194, 1024 cm^{-1}, FABMS 539 (M+1), Calcd for $C_{32}H_{30}N_2O_6$: C, 71.36; H, 5.61; N, 5.20; found: C, 71.58; H, 5.38; N, 5.32.

7-Methoxy-8-{5-[2-phenyl-4-oxo-4*H*-[1]benzopyran-6-yloxy]pentyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (4c). The compound **4c** was prepared following the method described for the compound **4a** employing **3c** (677 mg, 1 mmol), to afford the compound **4c** as a pale yellow solid (298 mg, 54%), m.p.: 68-70 °C, ^{1}H NMR ($CDCl_3$): δ 1.92-2.45 (m, 10H), 3.54-3.85 (m, 3H), 3.95 (s, 3H), 4.10-4.32 (m, 4H), 6.75 (s, 2H), 7.20-7.32 (m, 1H), 7.42-7.66 (m, 7H), 7.85-7.95 (m, 2H). IR 2933, 2851, 1626, 1458, 1364, 1256, 1197, 1023 cm^{-1}, FABMS 553 (M+1), Calcd for $C_{33}H_{32}N_2O_6$: C, 71.72; H, 5.84; N, 5.07; found: C, 71.85; H, 5.65; N, 5.25.

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. 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, 18, 36 and 48 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. Drug-induced alterations in DNA melting behaviour are given by: $\Delta T_m = T_m$(DNA+PBD)$-T_m$ (DNA alone), where the T_m value for the PBD-free CT-DNA is 69.0 ± 0.01. The fixed [PBD]/[DNA] ratio used did not result in binding saturation of the host DNA duplex for any compound examined.

In vitro evaluation of cytotoxic activity. In vitro cytotoxicity was evaluated using human cancer cell lines HT-29, HCT-15, A-549,HOP-62 and SiHa. Viable cells were seeded in growth medium into 96-well microtiter plates and allowed to attach overnight. A protocol of 48 h continuous drug exposure was used,[18a] and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth.[18b]

Acknowledgements

Two of the authors R.R, G.B.R.K are grateful to CSIR, New Delhi for the award of research fellowship.

References

1. (a) Thurston, D. E. *In Molecular Aspects of Anticancer Drug DNA Interactions*; Neidle, S.; Waring, M. J.; Eds.; McMillan:London, 1993; p 54. (b) Tender, M. D.; Korman, S. *Nature* **1963**, *199*, 501. (c) Hurley, L. H.; Petrusek, R. L. *Nature* **1979**, *282*, 529.
2. Kopka, M. L.; Goodsell, D. S.; Baikalov, I.; Grzeskowiak, K.; Cascio, D.; Dickerson, R. E. *Biochemistry* **1994**, *33*, 13593.
3. Kamal, A.; Rao, M. V.; Laxman, N.; Ramesh, G.; Reddy, G. S. K. *Curr. Med. Chem. Anti-Cancer Agents*. **2002**, *2*, 215.
4. Gregson, S. J.; Howard, P. H.; Hartley, J. A.; Brooks, N. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* **2001**, *44*, 737.
5. Thurston, D. E.; Morris, S. J.; Hartley, J. A. *Chem. Commun.* **1996**, 563
6. Wilson, S. C.; Howard, P. W.; Forrow, S. M.; Hartley, J. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* **1999**, *42*, 4028.
7. Baraldi, P. G.; Balboni, G.; Cacciari, B.; Guiotto, A.; Manfredini, S.; Romagnoli, R.; Spalluto, G.; Thurston, D. E.; Howard, P. W.; Bianchi, N.; Rutigliano, C.; Mischiati, C.; Gambari, R. *J. Med. Chem*. **1999**, *42*, 5131.
8. Damayanthi, Y.; Reddy, B. S. P.; Lown, J. W. *J. Org. Chem*. **1999**, *64*, 290.
9. Kumar, R.; Lown, J. W. *Org. Biomol. Chem.* **2003**, *1*, 3327.
10. Zhou, Q.; Duan, W.; Simmons, D.; Shayo, Y.; Raymond, M. A.; Dorr, R. T.; Hurley, L. H. *J. Am. Chem. Soc.* **2001**, *123*, 4865.
11. Tercel, M.; Stribbling, S. M.; Sheppard, H.; Siim, B. G.; Wu, K.; Pullen, S. M.; Botting, K. J.; Wilson, W. R.; Denny, W. A. *J. Med. Chem*. **2003**, *46*, 2132.
12. (a) Kamal, A.; Laxman, E.; Reddy, P. S. M. M. *Tetrahedron Lett.* **2000**, *41*, 7743. (b) Kamal, A.; Reddy, G. S. K.; Raghavan, S. *Bioorg. Med. Chem. Lett*. **2001**, *13*, 387. (c) Kamal, A.; Reddy, P. S. M. M.; Reddy, D. R. *Tetrahedron Lett.* **2003**, *44*, 2557.
13. (a) Kamal, A.; Ramesh, G.; Laxman, N.; Ramulu, P.; Srinivas, O.; Neelima, K.; Kondapi, A. K.; Srinu, V. B.; Nagarajaram, H. M. *J. Med.Chem.* **2002**, *45*, 4679. (b) Kamal, A.; Reddy, B. S. N.; Reddy, G. S. K.; Ramesh, G. *Bioorg. Med .Chem. Lett*. **2002**, *12*, 1933. (c) Kamal, A.; Ramesh, G.; Ramulu, P.; Srinivas, O.; Rehana, T.; Sheelu, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3451. (d) Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3517. (e) Kamal, A.; Srinivas, O.; Ramulu, P.; Ramesh, G.; Kumar, P. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3577. (f) Kamal, A.; Reddy, P. S. M. M.; Reddy, D. R. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 2669.
14. (a) Ono, K.; Nakane, H.; Fukushima, M.; Chermann, J. C.; Barre-Sinoussi, F. *Biochem. Biophys. Res. Commun.* **1989**, *160*, 982. (b) Ono, K.; Nakane, H.; Fukushima, M.; Chermann, J. C.; Barre-Sinoussi, F. *Eur. J. Biochem.* **1990**, *190*, 469.
15. (a) Wang, T.C.; Zhao, Y.L.; Liou, S. S. *Helv. Chim. Acta.* **2002**, *85*, 1382. (b) Chen, Y.L.; Chen, P.H.; Chung, C. H.; Li, K. C.; Jeng, H. Y.; Tzeng, C. C. *Helv. Chim. Acta.* **2003**, *86*, 778.

16. Austin, C.A.; Patel, S.; Ono, K.; Nakane, H.; Fisher, L. M. *Biochem. J.* **1992**, *282*, 883.
17. Puvvada, M. S.; Hartley, J. A.; Jenkins, T. C.; Thurston, D. E. *Nucleic Acids Res.* **1993**, *21*, 3671.
18. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Wolff, A. V.; Goodrich, M. G.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757. (b) Shekan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.