Bioorganic & Medicinal Chemistry 18 (2010) 6666–6677

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, anticancer activity and mitochondrial mediated apoptosis inducing ability of 2,5-diaryloxadiazole–pyrrolobenzodiazepine conjugates

Ahmed Kamal [a,*], D. Dastagiri [a], M. Janaki Ramaiah [a], E. Vijaya Bharathi [a], J. Surendranadha Reddy [a], G. Balakishan [a], Pranjal Sarma [a], S. N. C. V. L. Pushpavalli [a], Manika Pal-Bhadra [a,*], Aarti Juvekar [b], Subrata Sen [b], Surekha Zingde [b]

[a] Chemical Biology Laboratory, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500 607, India
[b] Advanced Centre for Treatment, Research & Education in Cancer, Navi Mumbai 400 611, India

ARTICLE INFO

Article history:
Received 20 May 2010
Revised 28 July 2010
Accepted 29 July 2010
Available online 1 August 2010

Keywords:
Pyrrolobenzodiazepine
2,5-Diaryloxadiazoles
Anticancer activity
Apoptosis
p53
Caspases
Cyclin dependent kinase-2 (CDK-2)

ABSTRACT

A series of 2,5-diaryloxadiazole linked pyrrolo[2,1-*c*][1,4]benzodiazepine conjugates have been prepared and evaluated for their anticancer activity. These conjugates have shown promising activity with GI_{50} values ranging from <0.1 to 0.29 μM. It is observed that some of these conjugates particularly **4a**, **4d**, **4i** and **4l** exhibit significant anticancer activity. Some detailed biological assays relating to the cell cycle aspects associated to Bax and caspases have been examined with a view to understand the mechanism of action of these conjugates.

1. Introduction

Currently cancer is one of the causes of death and it is likely to become the most common disease in the near future. It is well established that DNA is the target[1,2] for many anticancer drugs that are presently being used in the clinic. However, there are very few DNA-interactive agents that bind to DNA with high sequence selectivity. DC-81 (**1**), an antitumor antibiotic produced from *Streptomyces* species,[3] belongs to the pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) class of compounds that are potent inhibitors of nucleic acid synthesis. These compounds exhibit their antitumor activity by binding in the minor groove of double stranded DNA and forming a covalent bond to the exocyclic amino group of a central guanine within a three base pair recognition site.[4–6]

1,3,4-Oxadiazoles are an important class of heterocyclic compounds[7] with a broad range of biological activities such as antiviral,[8] antimicrobial,[9] fungicidal,[10] antineoplastic,[11] anticancer,[12] and inhibition of tyrosinase.[13] Moreover, it is considered that the presence of toxophoric –N=C–O– linkage[7] is responsible for their potent pharmacological activity. Further, 1,3,4-oxadiazole heterocyclics are very good bioisosters of amide and ester functionalities with substantial improvement in biological activity by participating in hydrogen bonding interactions with different receptors.[14,15] In recent years, some PBD conjugates have been reported as potential anticancer agents not only from this laboratory, but as well from other research groups.[16–19] Recently, Wang and co-workers have synthesized DC-81–indole and DC-81–enediyne conjugates to explore their anticancer potential. In this study, a correlation between antitumor activity and apoptosis has been well explained in such conjugates.[20,21] For the last few years, we have been involved in the development of new synthetic strategies[22,23] for the PBD ring system and also in the design of structurally modified PBDs including their conjugates.[24–28] Some representative PBD's, oxadiazoles, and their conjugates are illustrated in Figure 1.

In continuation of our efforts in search of new effective anticancer agents, we have synthesized a series of PBD conjugates by linking a diaryloxadiazole moiety to the PBD (DC-81) scaffold through the stable alkane spacers and evaluated their anticancer activity. The promising activity obtained, prompted us to investigate their role in the cell proliferation and apoptosis of human melanoma cell line (A375). Further, it is considered of interest to investigate the effect of these compounds on regulatory proteins of cell cycle progression.

* Corresponding authors. Tel.: +91 40 27193157; fax: +91 40 27193189.
E-mail addresses: ahmedkamal@iict.res.in (A. Kamal), manika@iict.res.in (M. Pal-Bhadra).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.07.067

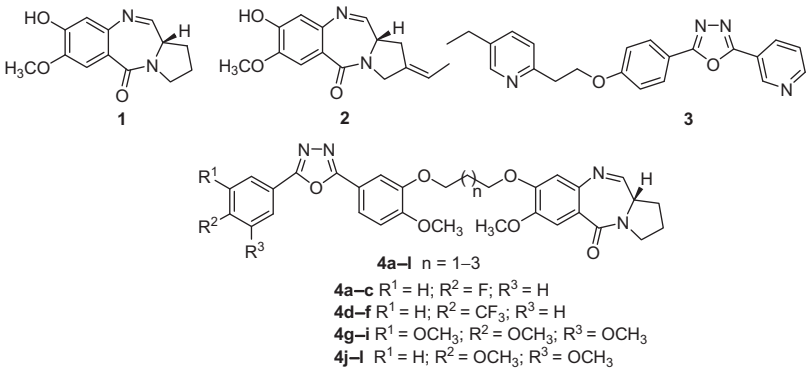


Figure 1. Structures of DC-81 (**1**), tomaymycin (**2**), 2,5-diaryloxadiazole (**3**), 2,5-diaryloxadiazole–PBD conjugates (**4a–l**).

2. Results and discussions

2.1. Chemistry

The synthesis of 2,5-diaryloxadiazoles (**9a–d**) was carried out from isovanilic ester by using the reported procedures[29,30] as shown in Scheme 1. Reaction of benzylated isovanilic ester (**5**) with hydrazine hydrate in ethanol gave isovanilic acid hydrazide (**6**) which upon treatment with substituted benzoyl chloride in dry pyridine provided the intermediates **7a–d**. Then these upon reaction with phosphorus oxychloride yielded the corresponding compounds **8a–d** which on further debenzylation provided the precursors **9a–d** in good yields.

The synthesis of C8-linked 2,5-diaryloxadiazole–PBD conjugates (**4a–l**) was carried out from the (2*S*)-*N*-{4-[*n*-bromoalkoxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**10a–c**), which were prepared by the methods reported in our earlier studies.[22–28] These upon etherification with the 2,5-diaryloxadiazole intermediates (**9a–d**) using K_2CO_3 in acetone provided the corresponding nitrothioacetal intermediates (**11a–l**) in 80–92% yield. Further, reduction of nitro compounds by using $SnCl_2.2H_2O$ in methanol followed by deprotection using $HgCl_2/CaCO_3$ afforded the desired PBD conjugates **4a–l** as shown in Scheme 2.

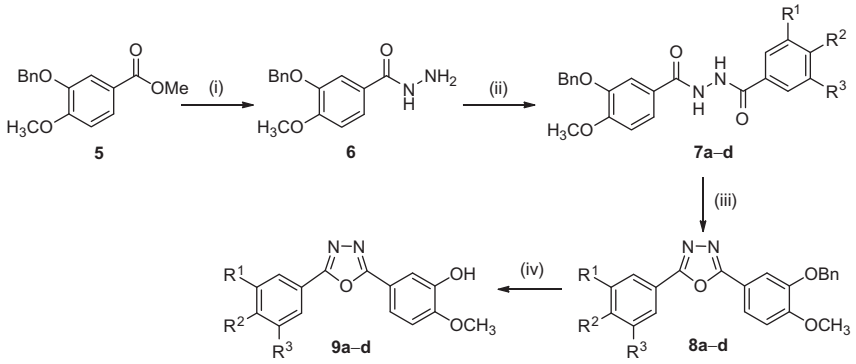
2.2. Biological results

2.2.1. Anticancer activity

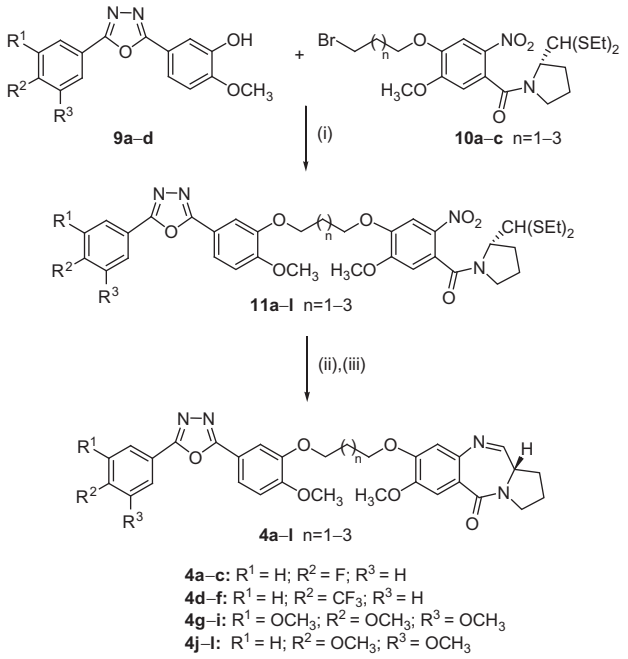
The synthesized compounds **4a–l** were evaluated for their anticancer activity in selected human cancer cell lines of lung, breast, oral, colon, prostate, ovarian and cervix by using sulforhodamine B (SRB) method. The compounds that exhibit $GI_{50} \leqslant 10^{-5}$ M are considered to be active on the respective cell lines and the results are illustrated in Table 1. All the compounds (**4a–l**) exhibited significant anticancer activity with GI_{50} values ranging from <0.1 to 0.29 μM, while the positive controls, adriamycin and DC-81 (**1**) demonstrated the GI_{50} in the range of 0.1–7.25 μM and 0.1–2.37 μM, respectively, in the cell lines employed. Interestingly, all the compounds showed promising anticancer activity, particularly against A2780, Gurav, MCF-7, Colo205, DWD cell lines. However, **4a**, **4d**, **4i** and **4l** exhibited significant activity.

Additionally, cell viability assay was carried out to investigate the cytotoxic effect mediated by the four most promising PBD conjugates (**4a**, **4d**, **4i** and **4l**) in A375 cells. These PBD conjugates exhibited cytotoxicity at 4 μM concentration, moreover these were more effective than DC-81 (**1**) and **9d** as shown in Figure 2.

Cells develop multiple DNA repair pathways such as base excision repair system (BER), nucleotide repair system (NER) and mismatch repair system (MMR) to protect themselves from different types of DNA damage.[31] TK6 are the human lymphoblastoid cells that have proficient mismatch repair system. Hence, in order to understand the potent anticancer activity for these conjugates against tumor cell lines with proficient DNA repair system, an MTT cell viability assay was carried out with these PBD conjugates (**4a**, **4d**, **4i** and **4l**) in TK6 cells. The results suggest that these compounds are functioning in those cells in which there is a DNA repair system (Fig. 3a). Further Western blot analysis showed upregulation of Chk2 protein[32] level as illustrated in Figure 3b. These stud-



Scheme 1. Reagents and conditions: (i) $NH_2{\cdot}NH_2{\cdot}H_2O$, EtOH, reflux; (ii) substituted benzoyl chlorides, pyridine, reflux, 30 min, 85–95%; (iii) $POCl_3$, reflux, 1 h, 80–87%; (iv) $EtSH–BF_3OEt_2$, CH_2Cl_2, 12 h, rt, 80–89%.



Scheme 2. Reagents and conditions: (i) K_2CO_3, acetone, reflux, 24 h, 80–92%; (ii) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 1–2 h, 70%; (iii) $HgCl_2$–$CaCO_3$, CH_3CN/H_2O (4:1), rt, 8–12 h, 55–60%.

ies indicate that these conjugates actively function in the DNA damage and repair pathway.

Prediction of lipophilicity (log *P*) and aqueous solubility (log *S*) of compounds **4a–l** was calculated using A LOGPS 2.1 software.[33,34] Compounds **4a–l** showed lipophilicity with log *P* values in the range of 3.76–5.84 (Table 2) and aqueous solubility with log *S* values in the range of 5.65–6.46 mg/L. These studies indicate that the aqueous solubility of these compounds is better than the DC-81 (**1**).

2.2.2. Cell cycle effects

To investigate the effect of these PBD conjugates on the cell cycle progression of human cancerous cell line A375, the DNA content of the cell nuclei was measured by flow cytometric (FACS) analysis. Drug induced hypodiploid DNA (i.e., sub-G1 phase) is the

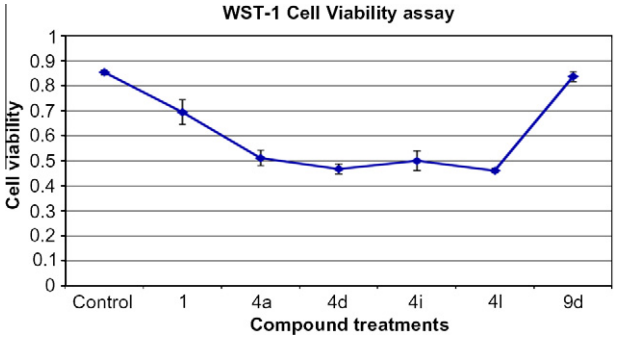


Figure 2. Effect of PBD conjugates (**4a**, **4d**, **4i** and **4l**) on cell viability (in vitro cytotoxicity). A375 cells were treated with 4 μM concentration of PBD conjugates as indicated for 24 h in 96-well plates seeded with 10,000 cells per well. OD readings were taken at 420 nm wavelength to measure the percentage of cell viability after treatment with the respective compound. DC-81 (**1**) was used as the positive control. Control: control cells (untreated cells).

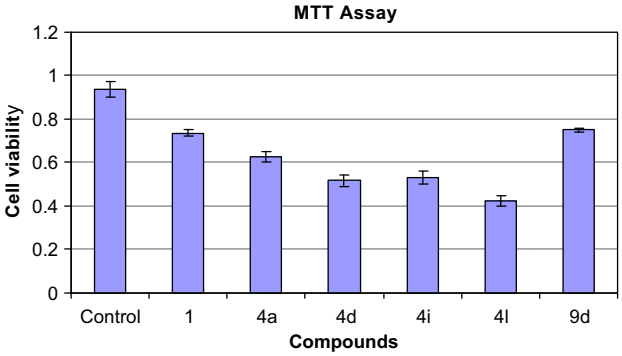


Figure 3a. Effect of PBD conjugates (**4a**, **4d**, **4i** and **4l**) on cell viability (in vitro cytotoxicity). TK6 cells were treated with 4 μM concentration of PBD conjugates as indicated for 24 h in 96-well plates seeded with 10,000 cells per well. OD readings were taken at 420 nm wavelength to measure the percentage of cell viability after treatment with the respective compound. DC-81 (**1**) was used as the positive control. Control: control cells (untreated cells).

Table 1
GI_{50} values[a] (in μM) for compounds **4a–l** in selected human cancer cell lines

Compound	A549[b]	Gurav[b]	HOP62[b]	MCF7[c]	Zr-75-1[c]	A2780[d]	DWD[e]	KB[e]	Colo205[f]	PC-3[g]	SiHa[h]
4a	0.15	0.16	0.16	<0.1	<0.1	0.14	<0.1	0.15	<0.1	<0.1	0.17
4b	0.29	0.1	0.15	0.16	0.17	<0.1	0.13	<0.1	0.15	0.17	<0.1
4c	0.14	<0.1	0.12	0.12	0.15	<0.1	<0.1	<0.1	<0.1	0.16	0.12
4d	<0.1	<0.1	<0.1	0.11	0.11	<0.1	0.11	<0.1	<0.1	0.14	<0.1
4e	0.11	0.14	0.16	0.14	0.12	0.14	0.15	0.15	0.16	0.16	0.17
4f	<0.1	<0.1	0.13	<0.1	0.11	<0.1	<0.1	0.11	0.11	0.13	0.12
4g	0.11	0.14	0.17	<0.1	0.12	0.14	<0.1	0.14	<0.1	0.14	0.18
4h	<0.1	<0.1	0.13	0.15	0.16	<0.1	0.12	<0.1	<0.1	0.16	0.13
4i	0.14	<0.1	<0.1	<0.1	0.12	<0.1	<0.1	<0.1	0.12	0.15	<0.1
4j	<0.1	0.11	0.13	<0.1	<0.1	0.11	<0.1	0.11	<0.1	<0.1	0.15
4k	<0.1	<0.1	0.13	<0.1	0.12	<0.1	<0.1	0.11	<0.1	0.13	0.13
4l	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
ADR[i]	7.25	0.17	0.14	0.17	1.79	0.16	0.10	0.17	0.14	1.81	0.17
DC-81 (**1**)	0.16	0.16	0.15	0.17	2.37	0.14	1.49	0.17	0.11	0.20	0.17

[a] 50% growth inhibition and the values are mean of three determinations.
[b] Lung cancer.
[c] Breast cancer.
[d] Ovarian cancer.
[e] Oral cancer.
[f] Colon cancer.
[g] Prostate cancer.
[h] Cervix cancer.
[i] Adriamycin.

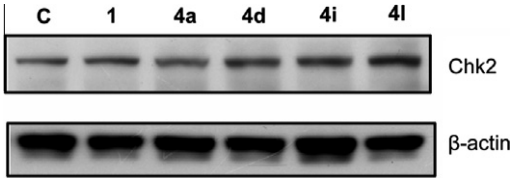


Figure 3b. Effect of PBD conjugates on the expression of chk2 protein levels. TK6 cells were treated with PBD conjugates (**4a**, **4d**, **4i**, **4l**) and DC-81 (**1**) at 4 μM concentration for 24 h. The cell lysates were collected and expression levels chk2 was determined by Western blot analysis, β-actin was used as loading control.

characteristic feature of apoptosis.[35,36] The majority of the control cells exposed to DMSO were in the G1 phase. Cells (1.53%) were in sub-G1, 53.9% in G1, 20.12% in S and 24.44% in G2/M phase. Treatment of A375 cells with the most promising compounds **4a**, **4d**, **4i** and **4l** at 4 μM concentration for 24 h induced apoptosis effects up to 6.79%, 17.93%, 13.76% and 36.66%, respectively, whereas, DC-81 (**1**) showed 15.33% of sub-G1 DNA peak. Simultaneously there was a concomitant decrease in the G2/M phase in A375 cells when treated with **4a** (5.06%), **4d** (2.26%), **4i** (2.34%) and **4d** (0.99%) compounds. Increased cells of sub-G1 phase, decrease of G2/M phase cells clearly showed that all the compounds are effective in causing apoptosis in case of A375 cells as shown in Table 3.

Table 3
Cell cycle distribution of A375 cell line at 4 μM concentration of compounds **4a**, **4d**, **4i**, **4l** and DC-81 (**1**)

Compound	Sub G1	G1	S	G2/M
Control	1.53 ± 0.05	53.9 ± 1.35	20.12 ± 0.66	24.44 ± 0.84
DC-81 (**1**)	15.33 ± 1.24	73.66 ± 2.05	8.02 ± 0.81	2.98 ± 0.02
4a	6.79 ± 0.46	74 ± 0.92	14.14 ± 0.20	5.06 ± 0.821
4d	17.93 ± 0.33	72.28 ± 0.4	7.51 ± 0.614	2.26 ± 0.13
4i	13.76 ± 0.29	73.5 ± 0.28	10.4 ± 0.43	2.34 ± 0.28
4l	36.66 ± 0.73	59.66 ± 0.47	2.68 ± 0.32	0.99 ± 0.13

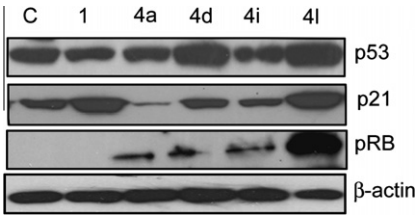


Figure 4. Effect of PBD conjugates on the expression of p53, p21 and pRb protein levels. A375 cells were treated with PBD conjugates (**4a**, **4d**, **4i**, **4l**) and DC-81 (**1**) at 4 μM concentration for 24 h. The cell lysates were collected and expression levels p53, p21 and pRb were determined by Western blot analysis, β-actin was used as loading control.

2.2.3. Effect on tumor suppressor proteins

To understand the mechanism underlying the G1 cell cycle arrest in these conjugates, we examined the G1/S check point associated tumor suppressor proteins such as p53, p21 and pRb. The tumor suppressor protein p53 regulates the transcription of p21 which in turn increases the retinoblastoma protein (pRb) leading into cell cycle arrest at G1 phase.[37,38] In order to understand the effect of PBD conjugates on p53, p21 and pRb dependent apoptotic pathway, A375 cells were treated with these conjugates at 4 μM concentration for 24 h and Western blot analysis was carried out. It was observed that, the p53, p21 and pRb levels were upregulated in the case of compounds **4d** and **4l** compared to the untreated controls. Predominantly, increase of these protein levels was more prominent in compound **4l**, as shown in Figure 4.

2.2.4. Effect on apoptotic proteins

According to previous studies apoptosis can be induced in two major pathways, including extrinsic and intrinsic apoptosis signaling.[39] Extrinsic pathway is probably mediated through the regulation of death receptors such as Fas and TNF-α located on the cell surface.[40] In order to know whether these conjugates are inducing apoptosis in an extrinsic pathway, Western blot analysis was carried out in relation to TNF-α and it was found that there was no change in the level of TNF-α (data not shown). Therefore, from this observation, it was clear that the apoptosis caused by these conjugates is independent of extrinsic pathway mediated by TNF-α.

An important consequence of intrinsic apoptotic pathway is the mitochondrial dysfunction and cytochrome *c* release.[41] The Bcl-2 family of proteins plays a crucial role in regulating the protein release from mitochondria.[42] This family is divided into two subgroups; pro-apoptotic and anti-apoptotic members. The pro-apoptotic proteins like Bax, induces cell apoptosis through mitochondrial membrane permeabilization (MMP), which leads to the release of cytochrome *c*.[43–45] cytosolic cytochrome *c* is known to cause activation of caspase-3.[46] To investigate the effect of Bax and release of cytochrome *c*, A375 cells were treated with PBD conjugates (**4a**, **4d**, **4i** and **4l**) at 4 μM concentration for 24 h and Western blot analysis was carried out. It was observed that upregulation of Bax which inturn causes an increase in cytochrome *c* protein levels as shown in Figure 5a.

One of the important characteristic features of apoptosis is activation of caspase-3 leads to the cleavage of DNA repair enzyme PARP(poly(ADP-ribose) polymerase)[47] and eventually leads to apoptosis.[48] Release of cytosolic cytochrome *c* is known to cause activation of caspase-3.[46] To assess the effect of PBD conjugates on the activation of caspase-3 and cleavage of PARP, A375 cells were treated with PBD conjugates (**4a**, **4d**, **4i** and **4l**) at a concentration of 4 μM for 24 h and the expression levels of caspase-3 was analyzed by Western blot analysis. An increase in the levels of caspase-3 was observed. An increase in the expression of cleaved PARP product specific to apoptosis was observed and the levels were more pronounced in the case of compound **4l**. This data clearly indicated that these PBD conjugates induce apoptosis through intrinsic pathway as shown in Figure 5a and b.

2.2.5. Effect of PBD conjugates on the expression of Cdk2

Further, to understand the molecular events involved in G1 phase arrest, the effects on the expression level of CDKs particularly Cdk2[49] (G1 related protein) was also investigated in A375 cells that were treated with these conjugates. Accordingly, Western blot analysis was carried out and it was observed that there was a reduction of Cdk2 levels as seen in Figure 6. The reduction of Cdk2 protein level is significantly more in case of **4l** compared to the other conjugates. Thus confirm the blockade of G1/S transition there by resulting in cell cycle arrest.

Table 2
Lipophilicity (log *P*) and aqueous solubility (log *S*) of compounds **4a**–**l**

Compd	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	DC-81(**1**)
Log *P*	4.39	4.78	5.17	5.07	5.44	5.84	3.76	4.13	4.53	3.92	4.28	4.68	0.88
Log *S* (gm/L)	5.96	6.11	6.24	6.19	6.34	6.46	5.72	5.86	5.98	5.65	5.80	5.91	2.01

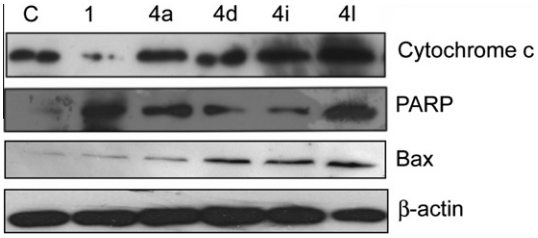
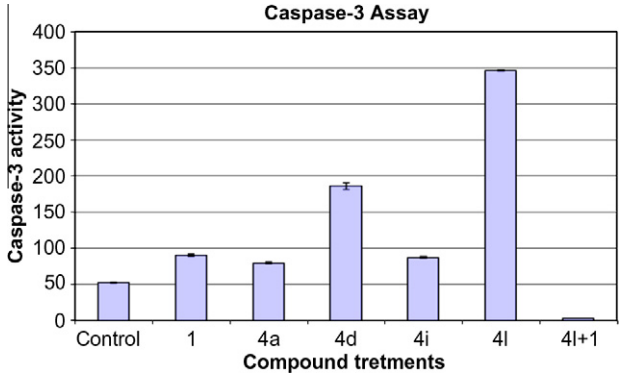


Figure 5a. Effect of PBD conjugates on cytochrome *c*, cleaved PARP and Bax levels. A375 cells were treated with compounds **4a**, **4d**, **4i**, and **4l** and DC-81 (**1**) at 4 μM concentration for 24 h. The cell lysates were collected and expression levels of cytochrome *c*, cleaved PARP and Bax were determined by Western blot analysis. β-Actin was used as loading control.



Figures 5b. Effect of PBD conjugates on caspase-3 activity in A375 cells. The increased enzymatic activity of caspase-3, in apoptosis after the treatment of PBD conjugates (**4a**, **4d**, **4i** and **4l**) at 4 μM concentration was determined by fluorimetry. The cleavage of peptide by caspase-3 releases the fluorophore AFC that was quantified at excitation wavelength of 400 nm and emission wavelength of 505 nm. 'I' represent the inhibitor used. DEVD-CHO is the inhibitor of caspase-3. DC-81 (**1**) was used as the positive control.

2.2.6. Thermal denaturation studies

Many previous reports by our group[24,17] showed strong binding of the PBD hybrids with DNA molecules that is proved both by RED assay and by computational studies. To show the DNA binding activity for these new C8-linked 2,5-diaryloxadiazole–PBD conjugates (**4a**–**l**) thermal denaturation studies were carried out using calf thymus (CT) DNA.[50] Melting studies showed that these compounds stabilize the thermal helixcoil or melting stabilization (ΔT_m) for the CT-DNA duplex at pH 7.0, incubated at 37 °C, where PBD/DNA molar ratio is 1:5. The data for compounds 4**a**–**l** is included in Table 4 and it is observed that some of these compounds have shown good DNA binding affinity compared to naturally occurring PBD-like DC-81. It is also observed that 2,5-diaryloxadiazole–PBD conjugates (**4d** and **4l**) exhibited better DNA binding affinity than other PBD conjugates and DC-81 (**1**).

2.2.7. In vivo tumor xenograft studies of compound 4l

The preliminary in vitro anticancer activities revealed that compound **4l** has shown significant anticancer activity among the series. These encouraging results provided an impetus to carry out in vivo efficacy studies using xenograft model of human prostate cancer cells (PC-3) in male NOD-SCID mice. The dose determination studies demonstrated the maximally tolerated dose (MTD) of compound **4l** as <50 mg/Kg. Hence, compound **4l** was administered at 20 mg/kg intraperitoneal (ip), on days 1, 5 and 9 (q4d) (total drug administered was 20 mg/kg). With these doses no toxicity was observed in terms of weight loss or mortality of the experimental mice (Fig. 7a and b).

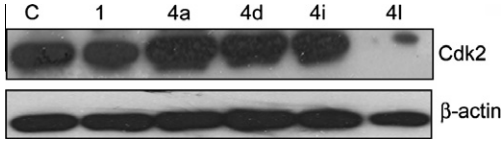


Figure 6. Effect of PBD conjugates on Cdk2 levels. A375 cells were treated with compounds **4a**, **4d**, **4i**, **4l** and DC-81 (**1**) at 4 μM concentrations for 24 h. The cell lysates were collected and expression levels of Cdk2 were determined by Western blot analysis. β-actin was used as a loading control.

Table 4
Thermal denaturation data for 2,5-diaryloxadiazoline–PBD conjugates (**4a**–**l**) with calfthyms (CT)-DNA

Compound	[PBD]: [DNA] Molar ratio[b]	ΔT_m (°C)[a] after incubation at 37 °C for	
		0 h	18 h
4a	1:5	1.9	2.0
4b	1:5	2.3	2.5
4c	1:5	1.5	1.8
4d	1:5	2.3	4.0
4e	1:5	1.7	2.1
4f	1:5	1.6	1.7
4g	1:5	1.4	1.9
4h	1:5	1.9	2.1
4i	1:5	1.1	1.6
4j	1:5	1.4	1.5
4k	1:5	2.1	2.3
4l	1:5	3.0	5.8
DC-81 (**1**)	1:5	0.3	0.7

[a] For CT-DNA alone at pH 7.00 ± 0.01, ΔT_m = 68.5 0C ± 0.01 (mean value from 10 separate determinations), all ΔT_m values are ± 0.1–0.2 °C.
[b] 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].

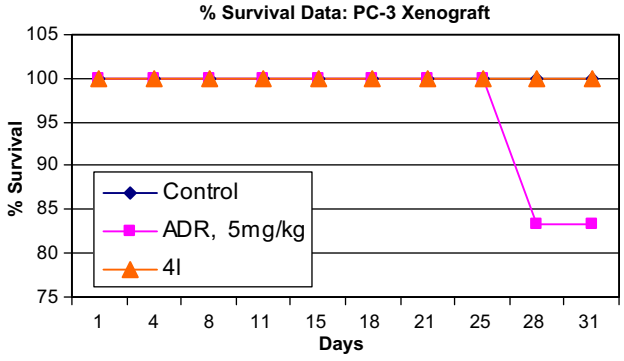


Figure 7a. NOD-SCID male mice treated with compound **4l**, Survival data of compound **4l** on PC-3 xenograft mice.

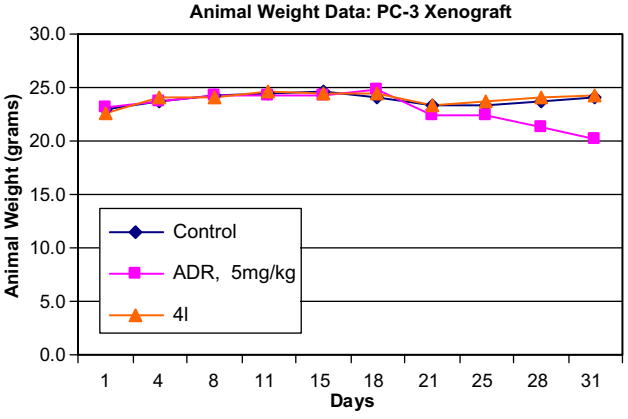


Figure 7b. Tumors weight curves in NOD-SCID male mice treated with compound **4l**.

3. Conclusion

In conclusion, a new class of 2,5-diaryloxadiazole–PBD conjugates have been synthesized and these exhibit promising anticancer activity against a number of human cancer cell lines. The cell viability assay of the two most promising compounds **4d** and **4l** showed cytotoxicity in A375 cells at 4 μM concentration. It is interesting to observe that these compounds exhibit a strong effect against cancer cells with a proficient DNA repair system. The FACS analysis also showed more population in sub-G1 phase indicating that these two PBD conjugates have apoptosis inducing ability. It is also observed from the detailed biological assays that the p53, p21 and pRb levels have been enhanced when treated with compounds **4a**, **4d**, **4i** and **4l**, interestingly it is more pronounced in case of compound **4l**. Further, release of cytochrome *c* has been observed when a Bcl-2 family protein (Bax) regulates the mitochondrial mediated pathway for these conjugates (**4a**, **4d**, **4i** and **4l**). Therefore, these conjugates have been examined for their effects on other mitochondrial mediated apoptotic proteins such as Bax, cytochrome *c*, caspase-3 and PARP. Upregulation of Bax, release of cytochrome *c*, increase in the levels of active caspase-3 and cleavage of PARP is noted, and is more pronounced in case of compound **4l**. Similarly, in case of **4l** a drastic decrease in the levels of Cdk2 has been observed. Moreover, thermal denaturation studies are also carried out, these conjugates showed better DNA binding ability. The in vivo efficacy study of compound **4l** revealed that the ability to delay the tumor growth and retention of body weight in the PC-3 human prostate cancer xenograft model suggested that 2,5-diaryloxadiazole–PBD conjugates have promising anticancer activity in the treatment of human cancer.

All these new conjugates showed significant anticancer activity with GI_{50} values in the range of <0.1–0.29 μM. Whereas, some of the PBD conjugates previously synthesized showed GI_{50} values in the range of 0.13–30.50 μM (1,2,3-triazole–PBD conjugates),[28] 0.22–30.30 μM (triazolobenzothiadiazine–PBD conjugates),[51] 0.17–30.50 μM (phosphonate linked PBD conjugates)[27] and 1.48–26.2 μM (some of the quinazolinone linked PBD conjugates).[17] In this article we highlight that by linking the 2,5-diaryloxadiazole moiety to the PBD ring system has dramatically improved the anticancer activity. These studies are likely to provide an important mechanistic insight into the action of such conjugates.

4. Experimental section

4.1. Chemistry

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H spectra were recorded on Gemini Varian-VXR-unity (200 MHz) or Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

4.2. General procedures

4.2.1. Synthesis of 3-(benzyloxy)-*N*′-(4-fluorobenzoyl)-4-methoxybenzohydrazide (7a)

A mixture of methyl 3-(benzyloxy)-4-methoxy benzoate **5** (272 mg, 1 mmol), ethanol (100 mL), and hydrazine hydrate (1.3 mL, 0.34 mol) was heated to reflux and left overnight. The solid formed was filtered and washed with cold water and ether, to obtain compound 3-(benzyloxy)-4-methoxy benzohydrazide (**6**) and as such taken for the next step.

A mixture of 3-(benzyloxy)-4-methoxy benzohydrazide **6** (272 mg, 1 mmol) and 4-fluorobenzoyl chloride (158 mg, 1 mmol), in dry pyridine (10 mL), was heated under reflux for 30 min. On cooling, the mixture was poured onto cold water (50 mL) and stirred for 10 min. The separated solid was filtered, washed thoroughly with cold water and dried to yield compound **7a** as white solid. The products were pure enough to be used in the next step without further purification (355 mg, 90%). Mp: 186–189 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.92 (s, 3H, –OCH_3), 5.18 (s, 2H, benzyl –CH_2–), 6.93 (d, 1H, J = 8.9 Hz, ArH), 7.15 (d, 1H, J = 8.9 Hz, ArH), 7.24–7.61 (m, 8H, ArH), 8.03 (dd, 2H, J = 12.8 Hz, J = 2.5 Hz, ArH), 10.24 (d, 2H, J = 8.9 Hz, –CONHNHCO–); MS (ESI): m/z 395 $[M+1]^+$.

4.2.2. 3-(Benzyloxy)-4-methoxy-*N*′-(4-(trifluoromethyl) benzoyl)benzohydrazide (7b)

Compound **7b** was prepared following the method described for the preparation of compound **7a**, employing **6** (272 mg, 1 mmol) and 4-(trifluoromethyl)benzoyl chloride (208 mg, 1 mmol) to afford compound **7b** as a white solid (413 mg, 93%). Mp: 162–165 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.88 (s, 3H, –OCH_3), 5.22 (s, 2H, benzyl –CH_2–), 7.22 (d, 1H, J = 8.7 Hz, ArH), 7.39–7.61 (m, 6H, ArH), 7.97 (d, 3H, J = 8.0 Hz, ArH), 8.17 (d, 2H, J = 8.0 Hz, ArH), 10.64 (d, 2H, J = 8.0 Hz, –CONHNHCO–); MS (ESI): m/z 445 $[M+1]^+$.

4.2.3. *N*′-(3-(Benzyloxy)-4-methoxybenzoyl)-3,4,5-trimethoxybenzohydrazide (7c)

Compound **7c** was prepared following the method described for the preparation of compound **7a**, employing **6** (272 mg, 1 mmol) and 3,4,5-trimethoxybenzoyl chloride (230 mg, 1 mmol) to afford compound **7c** as a white solid (396 mg, 85%). Mp: 172–174 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (s, 3H, –OCH_3), 3.84 (s, 9H, 3 × –OCH_3), 5.17 (s, 2H, benzyl –CH_2–), 7.17 (d, 1H, J = 8.3 Hz, ArH), 7.26 (s, 2H, ArH), 7.34–7.50 (m, 5H, ArH), 7.55 (d, 2H, J = 6.0 Hz, ArH), 10.40 (d, 2H, J = 9.0 Hz, –CONHNHCO–); MS (ESI): m/z 467 $[M+1]^+$.

4.2.4. 3-(Benzyloxy)-*N*′-(3,4-dimethoxybenzoyl)-4-methoxybenzohydrazide (7d)

Compound **7d** was prepared following the method described for the preparation of compound **7a**, employing **6** (272 mg, 1 mmol) and 3,4-dimethoxybenzoyl chloride (200 mg, 1 mmol) to afford compound **7d** as a white solid (414 mg, 95%). Mp: 130–133 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.94 (s, 9H, 3 × –OCH_3), 5.29 (s, 2H, benzyl –CH_2–), 7.19 (d, 1H, J = 8.0 Hz, ArH), 7.45–7.72 (m, 10H, ArH), 10.43 (d, 2H, J = 9.0 Hz, –CONHNHCO–); MS (ESI): m/z 436 $[M+1]^+$.

4.2.5. Synthesis of 2-(3-(benzyloxy)-4-methoxyphenyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole (8a)

Compound **7a** (395 mg, 1 mmol) was added to phosphorus oxychloride (6 mL) and the mixture was heated under reflux for 1 h. On cooling, crushed ice was added cautiously and the mixture was stirred for 30 min. The separated crude product was filtered, washed with water then with saturated sodium hydrogen carbon-

ate ($NaHCO_3$) solution and finally with water, dried and recrystallized from ethanol to afford compound **8a** as a white solid (327 mg, 87%). Mp: 142–144 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.00 (s, 3H, $-OCH_3$), 5.21 (s, 2H, benzyl $-CH_2-$), 6.95 (d, 1H, J = 8.8 Hz, ArH), 7.21 (t, 2H, J = 8.8 Hz, ArH), 7.30–7.47 (m, 4H, ArH), 7.55 (d, J = 1.4 Hz, 1H, ArH), 7.59 (d, J = 2.2 Hz, 1H, ArH), 7.65 (d, 1H, J = 2.2 Hz, ArH), 8.12 (dd, 2H, J = 13.9 Hz, J = 3.6 Hz, ArH); MS (ESI): m/z 377 $[M+1]^+$.

4.2.6. 2-(3-(Benzyloxy)-4-methoxyphenyl)-5-(4-(trifluoro methyl)phenyl)-1,3,4-oxadiazole (8b)

Compound **8b** was prepared following the method described for the preparation of compound **8a**, employing **7b** (445 mg, 1 mmol) to afford compound **8b** as a white solid (354 mg, 83%). Mp: 137–139 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.00 (s, 3H, $-OCH_3$), 5.20 (s, 2H, benzyl $-CH_2-$), 6.95 (d, 1H, J = 8.0 Hz, ArH), 7.29–7.47 (m, 5H, ArH), 7.56 (d, 1H, J = 2.2 Hz, ArH), 7.66 (d, 1H, J = 2.2 Hz, ArH), 7.79 (d, 2H, J = 8.0 Hz, ArH), 8.24 (d, 2H, J = 8.0 Hz, ArH); MS (ESI): m/z 427 $[M+1]^+$.

4.2.7. 2-(3-(Benzyloxy)-4-methoxyphenyl)-5-(3,4,5-trimethoxy phenyl)-1,3,4-oxadiazole (8c)

Compound **8c** was prepared following the method described for the preparation of compound **8a**, employing **7c** (467 mg, 1 mmol) to afford compound **8c** as a white solid (358 mg, 80%). Mp: 133–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 3H, $-OCH_3$), 3.97 (s, 6H, 2 × $-OCH_3$), 4.01 (s, 3H, $-OCH_3$), 5.24 (s, 2H, benzyl $-CH_2-$), 6.98 (d, 1H, J = 8.6 Hz, ArH), 7.32 (s, 2H, ArH), 7.36–7.41 (m, 3H, ArH), 7.45 (d, 2H, J = 7.0 Hz, ArH), 7.60 (d, 1H, J = 7.0 Hz, ArH), 7.68 (s, 1H, ArH); MS (ESI); m/z 449 $[M+1]^+$.

4.2.8. 2-(3-(Benzyloxy)-4-methoxyphenyl)-5-(3,4-dimethoxy phenyl)-1,3,4-oxadiazole (8d)

Compound **8d** was prepared following the method described for the preparation of compound **8a**, employing **7d** (436 mg, 1 mmol) to afford compound **8d** as a white solid (355 mg, 85%). Mp: 163–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.96 (s, 3H, $-OCH_3$), 3.99 (s, 3H, $-OCH_3$), 4.01 (s, 3H, $-OCH_3$), 5.21 (s, 2H, benzyl $-CH_2-$), 6.93 (d, 1H, J = 2.8 Hz, ArH), 6.96 (d, 1H, J = 2.2 Hz, ArH), 7.27–7.46 (m, 5H, ArH), 7.54–7.66 (m, 4H, ArH); MS (ESI); m/z 419 $[M+1]^+$.

4.2.9. Synthesis of 5-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenol (9a)

To a stirred solution of EtSH (1.18 g, 19.0 mmol) and $BF_3{\cdot}OEt_2$ (1.41 g, 10 mmol) in dichloromethane was added dropwise to the solution of compound **8a** (377 mg, 1 mmol) in dichloromethane (10 mL) at room temperature. Stirring was continued until TLC indicated completion of the reaction. The solvent was evaporated under vacuum. The residue, thus obtained was quenched with bicarbonate solution and then extracted with ethyl acetate. The combined organic phases were washed with saturated brine, dried over anhydrous Na_2SO_4 and the solvent removed under vacuum to afford the crude product. This was further purified by column chromatography using (50% EtOAc–hexane) as eluant to afford compound **9a** as a white solid (286 mg, 80%). Mp: 199–202 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.99 (s, 3H, $-OCH_3$), 7.02 (d, 1H, J = 7.8 Hz, ArH), 7.23 (t, 2H, J = 8.5 Hz, ArH), 7.37 (s, 1H, ArH), 7.59 (d, 1H, J = 7.8 Hz, ArH), 8.13 (dd, 2H, J = 14.0 Hz, J = 3.9 Hz, ArH); MS (ESI): m/z 287 $[M+1]^+$.

4.2.10. 2-Methoxy-5-(5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl) phenol (9b)

Compound **9b** was prepared following the method described for the preparation of compound **9a**, employing **8b** (427 mg, 1 mmol) and the crude product was purified by column chromatography (40% EtOAc–hexane) to afford compound **9b** as a white solid (300 mg, 89%). Mp: 183–186 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.02 (s, 3H, $-OCH_3$), 7.07 (d, 1H, J = 8.3 Hz, ArH), 7.65 (d, 1H, J = 2.2 Hz, ArH), 7.67 (s, 1H, ArH), 7.80 (d, 2H, J = 8.3 Hz, ArH), 8.26 (d, 2H, J = 8.3 Hz, ArH); MS (ESI): m/z 337 $[M+1]^+$.

4.2.11. 2-Methoxy-5-(5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl) phenol (9c)

Compound **9c** was prepared following the method described for the preparation of compound **9a**, employing **8c** (449 mg, 1 mmol) and the crude product was purified by column chromatography (70% EtOAc–hexane) to afford compound **9c** as a white solid (235 mg, 82%). Mp: 138–142 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.92 (s, 3H, $-OCH_3$), 3.97 (s, 6H, 2 × $-OCH_3$), 4.01 (s, 3H, $-OCH_3$), 7.05 (d, 1H, J = 8.0 Hz, ArH), 7.34 (s, 2H, ArH), 7.64–7.70 (m, 2H, ArH); MS (ESI): m/z 359 $[M+1]^+$.

4.2.12. 5-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxy phenol (9d)

Compound **9d** was prepared following the method described for the preparation of compound **9a**, employing **8d** (419 mg, 1 mmol) and the crude product was purified by column chromatography (60% EtOAc–hexane) to afford compound **9d** as a white solid (279 mg, 85%). Mp: 181–183 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.96 (s, 3H, $-OCH_3$), 3.99 (s, 3H, $-OCH_3$), 4.01 (s, 3H, $-OCH_3$), 6.97 (d, 1H, J = 8.4 Hz, ArH), 7.04 (d, 1H, J = 8.4 Hz, ArH), 7.58–7.70 (m, 4H, ArH); MS (ESI): m/z 329 $[M+1]^+$.

4.2.13. Synthesis of (2*S*)-*N*-{4-[3-[5-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]propyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11a)

To a solution of (2*S*)-*N*-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethylthioacetal **10a** (521 mg, 1 mmol) in acetone (10 mL) was added anhydrous K_2CO_3 (552 mg, 4 mmol) and 5-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenol **9a** (286 mg, 1 mmol). The reaction mixture was heated to reflux for 24 h. After completion of the reaction as indicated by TLC, potassium carbonate was removed by suction filtration and the solvent was removed under vacuum. The crude product thus obtained was purified by column chromatography using 50% EtOAc–hexane as eluant to afford pure compound of **11a** as a yellow solid (581 mg, 80%). Mp: 118–120 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29–1.40 (m, 6H, $-(CH_3)_2$), 1.68–2.15 (m, 4H, $-(CH_2)_2-$), 2.41–2.49 (m, 2H, $-CH_2-$), 2.66–2.84 (m, 4H, 2 × $-SCH_2-$), 3.17–3.29 (m, 2H, $-NCH_2-$), 3.93 (s, 3H, $-OCH_3$), 3.97 (s, 3H, $-OCH_3$), 4.29–4.38 (m, 4H, 2 × $-OCH_2-$), 4.65–4.72 (m, 1H, $-NCH-$), 4.85 (d, 1H, J = 3.7 Hz, $-CHS_2-$), 6.80 (s, 1H, ArH), 7.01 (d, 1H, J = 8.3 Hz, ArH), 7.21 (d, 1H, J = 8.3 Hz, ArH), 7.26 (s, 1H, ArH), 7.62 (s, 1H, ArH), 7.65 (d, 1H, J = 2.2 Hz, ArH), 7.72 (s, 1H, ArH), 8.14 (dd, 2H, J = 14.3 Hz, J = 3.7 Hz, ArH); MS (ESI): m/z 727 $[M+1]^+$.

4.2.14. (2*S*)-*N*-{4-[4-[5-(5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl) oxy]butyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11b)

This compound was prepared according to the method described for compound **11a** employing compound **10b** (535 mg, 1 mmol) and compound **9a** (286 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11b** as a yellow solid (607 mg, 82%). Mp: 97–99 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21–1.42 (m, 6H, $-(CH_3)_2$), 1.60–1.72 (m, 4H, $-(CH_2)_2-$), 2.03–2.19 (m, 4H, 2 × $-CH_2-$), 2.67–2.87 (m, 4H, 2 × $-SCH_2-$), 3.18–3.32 (m, 2H, $-NCH_2-$), 3.92 (s, 3H, $-OCH_3$), 3.96 (s, 3H, $-OCH_3$), 4.16–4.30 (m, 4H, 2 × $-OCH_2-$), 4.64–4.75 (m, 1H, $-NCH-$), 4.86 (d, 1H, J = 4.1 Hz,

$-CHS_2-$), 6.80 (s, 1H, ArH), 6.98 (d, 1H, J = 8.3 Hz, ArH), 7.25 (d, 2H, J = 8.3 Hz, ArH), 7.61–7.71 (m, 3H, ArH), 8.14 (dd, 2H, J = 14.1 Hz, J = 4.1 Hz, ArH); MS (ESI): m/z 741 $[M+1]^+$.

4.2.15. (2*S*)-*N*-{4-[5-[5-(5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl) oxy]pentyl)oxy-5-methoxy-2-nitro benzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11c)

This compound was prepared according to the method described for compound **11a** employing compound **10c** (549 mg, 1 mmol) and compound **9a** (286 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11c** as a yellow solid (679 mg, 90%). Mp: 99–101 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.40 (m, 8H, $-(CH_3)_2$, $-CH_2-$), 1.61–1.80 (m, 4H, 2 × $-CH_2-$), 1.89–2.07 (m, 4H, 2 × $-CH_2-$), 2.66–2.84 (m, 4H, 2 × $-SCH_2-$), 3.19–3.31 (m, 2H, $-NCH_2-$), 3.94 (s, 3H, $-OCH_3$), 3.97 (s, 3H, $-OCH_3$), 4.07–4.17 (m, 4H, 2 × $-OCH_2-$), 4.66–4.74 (m, 1H, $-NCH-$), 4.86 (d, 1H, J = 3.7 Hz, $-CHS_2-$), 6.81 (s, 1H, ArH), 6.97 (d, 1H, J = 8.3 Hz, ArH), 7.24 (d, 2H, J = 8.3 Hz, ArH), 7.27 (s, 1H, ArH), 7.62–7.67 (m, 2H, ArH), 8.14 (dd, 2H, J = 13.5 Hz, J = 3.0 Hz, ArH); MS (ESI): m/z 755 $[M+1]^+$.

4.2.16. (2*S*)-*N*-{4-[3-[5-(5-(4-Trifluoromethylphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]propyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11d)

This compound was prepared according to the method described for compound **11a** employing (2*S*)-*N*-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxycarbaldehyde diethylthioacetal **10a** (521 mg, 1 mmol) and 2-methoxy-5-(5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl) phenol **9b** (336 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11d** as a yellow solid (654 mg, 85%). Mp: 101–103 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.35 (m, 6H, $-(CH_3)_2$), 1.90–2.13 (m, 2H, $-CH_2-$), 2.20–2.35 (m, 2H, $-CH_2-$), 2.40–2.49 (m, 2H, $-CH_2-$), 2.67–2.84 (m, 4H, 2 × $-SCH_2-$), 3.18–3.29 (m, 2H, $-NCH_2-$), 3.93 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 4.29–4.38 (m, 4H, 2 × $-OCH_2-$), 4.65–4.73 (m, 1H, $-NCH-$), 4.85 (d, 1H, J = 3.7 Hz, $-CHS_2-$), 6.80 (s, 1H, ArH), 7.02 (d, 1H, J = 8.3 Hz, ArH), 7.63–7.69 (m, 2H, ArH), 7.72 (s, 1H, ArH), 7.80 (d, 2H, J = 8.3 Hz, ArH), 8.26 (d, 2H, J = 8.3 Hz, ArH); MS (ESI): m/z 777 $[M+1]^+$.

4.2.17. (2*S*)-*N*-{4-[4-[5-(5-(4-Trifluoromethylphenyl)-1,3,4-oxa diazol-2-yl)-2-methoxyphenyl)oxy]butyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11e)

This compound was prepared according to the method described for compound **11a** employing compound **10b** (535 mg, 1 mmol) and compound **9b** (336 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11e** as a yellow solid (726 mg, 92%). Mp: 95–97 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23–1.35 (m, 6H, $-(CH_3)_2$), 1.89–2.17 (m, 4H, 2 × $-CH_2-$), 2.21–2.34 (m, 2H, $-CH_2-$), 2.64–2.82 (m, 4H, 2 × $-SCH_2-$), 3.17–3.30 (m, 2H, $-NCH_2-$), 3.91 (s, 3H, $-OCH_3$), 3.95 (s, 3H, $-OCH_3$), 4.16–4.30 (m, 4H, 2 × $-$OCH2$-$), 4.62–4.69 (m, 1H, $-NCH-$), 4.80 (d, 1H, J = 3.7 Hz, $-CHS_2-$), 6.73 (s, 1H, ArH), 6.94 (d, 1H, J = 8.3 Hz, ArH), 7.61 (d, 2H, J = 4.5 Hz, ArH), 7.64 (s, 1H, ArH), 7.80 (d, 2H, J = 8.3 Hz, ArH), 8.26 (d, 2H, J = 8.30 Hz, ArH); MS (ESI): m/z 791 $[M+1]^+$.

4.2.18. (2*S*)-*N*-{4-[5-[5-(5-(4-Trifluoromethylphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]pentyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11f)

This compound was prepared according to the method described for compound **11a** employing compound **10c** (549 mg, 1 mmol) and compound **9b** (336 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11f** as a yellow solid (658 mg, 82%). Mp: 91–93 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23–1.41 (m, 6H, $-(CH_3)_2$), 1.68–1.84 (m, 2H, $-CH_2-$), 1.89–2.08 (m, 6H, 3 × $-CH_2-$), 2.21–2.36 (m, 2H, $-CH_2-$), 2.66–2.86 (m, 4H, 2 × $-SCH_2-$), 3.19–3.27 (m, 2H, $-NCH_2-$), 3.94 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 4.05–4.19 (m, 4H, 2 × $-OCH_2-$), 4.62–4.74 (m, 1H, $-NCH-$), 4.84 (d, 1H, J = 3.9 Hz, $-CHS_2-$), 6.79 (s, 1H, ArH), 6.96 (d, 1H, J = 8.5 Hz, ArH), 7.62–7.69 (m, 3H, ArH), 7.80 (d, 2H, J = 8.5 Hz, ArH), 8.26 (d, 2H, J = 7.81 Hz, ArH); MS (ESI): m/z 805 $[M+1]^+$.

4.2.19. (2*S*)-*N*-{4-[3-[5-(5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]propyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11g)

This compound was prepared according to the method described for compound **11a** employing (2*S*)-*N*-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxycarbaldehyde diethylthioacetal **10a** (521 mg, 1 mmol) and 2-methoxy-5-(5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)phenol **9c** (358 mg, 1 mmol).The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11g** as a yellow solid (717 mg, 90%). Mp: 93–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24–1.38 (m, 6H, $-(CH_3)_2$), 1.48–1.84 (m, 4H, $-(CH_2)_2-$), 2.38–2.50 (m, 2H, $-CH_2-$), 2.65–2.85 (m, 4H, 2 × $-SCH_2-$), 3.14–3.31 (m, 2H, $-NCH_2-$), 3.92 (s, 6H, $-OCH_3$), 3.93 (s, 3H, $-OCH_3$), 3.98 (s, 6H, $-OCH_3$), 4.28–4.40 (m, 4H, 2 × $-OCH_2-$), 4.64–4.73 (m, 1H, $-NCH$), 4.84 (d, 1H, J = 3.7 Hz, $-CHS_2-$), 6.80 (s, 1H, ArH), 7.01 (d, 1H, J = 8.3 Hz, ArH), 7.32 (s, 2H, ArH), 7.64 (s, 2H, ArH), 7.72 (s, 1H, ArH); MS (ESI): m/z 799 $[M+1]^+$.

4.2.20. (2*S*)-*N*-{4-[4-[5-(5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]butyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11h)

This compound was prepared according to the method described for compound **11a** employing compound **10b** (535 mg, 1 mmol) and compound **9c** (358 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11h** as a yellow solid (689 mg, 85%). Mp: 88–90 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.39 (m, 6H, $-(CH_3)_2$), 1.73–1.86 (m, 4H, $-(CH_2)_2-$), 2.07–2.16 (m, 4H, 2 × $-CH_2-$), 2.64–2.82 (m, 4H, 2 × $-SCH_2-$), 3.18–3.28 (m, 2H, $-NCH_2-$), 3.90 (s, 3H, $-OCH_3$), 3.91 (s, 3H, $-OCH_3$), 3.95 (s, 3H, $-OCH_3$), 3.97 (s, 6H, 2 × $-OCH_3$), 4.16–4.27 (m, 4H, 2 × $-OCH_2-$), 4.63–4.70 (m, 1H, $-NCH-$), 4.81 (d, 1H, J = 3.7 Hz, $-CHS_2-$), 6.74 (s, 1H, ArH), 6.93 (d, 1H, J = 9.0 Hz, ArH), 7.30 (s, 2H, ArH), 7.61 (d, 2H, J = 6.7 Hz, ArH), 7.65 (s, 1H, ArH); MS (ESI): m/z 813 $[M+1]^+$.

4.2.21. (2*S*)-*N*-{4-[5-[5-(5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]pentyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11i)

This compound was prepared according to the method described for compound **11a** employing compound **10c** (549 mg, 1 mmol) and compound **9c** (358 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11i** as a yellow solid (677 mg, 82%). Mp: 78–90 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.40 (m, 8H, $-(CH_3)_2$, $-CH_2-$), 1.68–1.84 (m, 4H, 2 × $-CH_2-$), 1.90–2.08 (m, 4H, 2 × $-CH_2-$), 2.64–2.88 (m, 4H, 2 × $-SCH_2-$), 3.18–3.29 (m, 2H, $-NCH_2-$), 3.91 (s, 6H, 2 × $-OCH_3$), 3.95 (s, 3H, $-OCH_3$), 3.99 (s, 6H, 2 × $-OCH_3$), 4.07–4.18 (m, 4H, 2 × $-OCH_2-$), 4.60–4.73 (m, 1H, $-NCH-$), 4.82 (d, 1H, J = 3.9 Hz, $-CHS_2-$), 6.77 (s, 1H, ArH),

6.92 (d, 1H, J = 7.8 Hz, ArH), 7.30 (s, 2H, ArH), 7.58 (d, 1H, J = 1.5 Hz, ArH), 7.63 (s, 2H, ArH); MS (ESI): m/z 827 $[M+1]^+$.

4.2.22. (2*S*)-*N*-{4-[3-[5-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]propyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11j)

This compound was prepared according to the method described for compound **11a** employing (2*S*)-*N*-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxycarbaldehyde diethylthioacetal **10a** (521 mg, 1 mmol) and 5-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenol **9d** (328 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11j** as a yellow solid (660 mg, 86%). Mp: 106–108 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.39 (m, 6H, $-(CH_3)_2$), 1.74–1.98 (m, 4H, $-(CH_2)_2-$), 2.38–2.52 (m, 2H, $-CH_2-$), 2.70–2.87 (m, 4H, 2 × $-SCH_2-$), 3.19–3.30 (m, 2H, $-NCH_2-$), 3.94 (s, 3H, $-OCH_3$), 3.98 (s, 6H, 2 × $-OCH_3$), 4.00 (s, 3H, $-OCH_3$), 4.28–4.41 (m, 4H, 2 × $-OCH_2-$), 4.65–4.77 (m, 1H, $-NCH-$), 4.88 (d, 1H, J = 3.9 Hz, $-CHS_2-$), 6.82 (s, 1H, ArH), 6.99 (d, 1H, J = 8.5 Hz, ArH), 7.04 (s, 1H, ArH), 7.63–7.72 (m, 4H, ArH), 7.75 (s, 1H, ArH); MS (ESI): m/z 769 $[M+1]^+$.

4.2.23. (2*S*)-*N*-{4-[4-[5-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]butyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11k)

This compound was prepared according to the method described for compound **11a** employing compound **10b** (535 mg, 1 mmol) and compound **9d** (328 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11k** as a yellow solid (640 mg, 82%). Mp: 102–104 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24–1.39 (m, 6H, $-(CH_3)_2$), 1.56–1.87 (m, 4H, $-(CH_2)_2-$), 2.08–2.17 (m, 4H, 2 × $-CH_2-$), 2.68–2.86 (m, 4H, 2 × $-SCH_2-$), 3.18–3.30 (m, 2H, $-NCH_2-$), 3.92 (s, 3H, $-OCH_3$), 3.96 (s, 6H, 2 × $-OCH_3$), 4.00 (s, 3H, $-OCH_3$), 4.18–4.28 (m, 4H, 2 × $-OCH_2-$), 4.66–4.74 (m, 1H, $-NCH-$), 4.86 (d, 1H, J = 3.7 Hz, $-CHS_2-$), 6.80 (s, 1H, ArH), 6.97 (d, 2H, J = 8.3 Hz, ArH), 7.64 (d, 2H, J = 6.7 Hz, ArH) 7.68 (s, 2H, ArH); MS (ESI): m/z 783 $[M+1]^+$.

4.2.24. (2*S*)-*N*-{4-[5-[5-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]pentyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (11l)

This compound was prepared according to the method described for compound **11a** employing compound **10c** (549 mg, 1 mmol) and compound **9d** (328 mg, 1 mmol). The crude product was purified by column chromatography (50% EtOAc–hexane) to afford compound **11l** as a yellow solid (700 mg, 88%). Mp: 101–103 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24–1.39 (m, 6H, $-(CH_3)_2$), 1.68–1.86 (m, 2H, $-CH_2-$), 1.92–2.11 (m, 6H, $-CH_2)_3$), 2.22–2.35 (m, 2H, $-CH_2-$), 2.68–2.85 (m, 4H, 2 × $-SCH_2-$), 3.19–3.33 (m, 2H, $-NCH_2-$), 3.94 (s, 3H, $-OCH_3$), 3.97 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 4.00 (s, 3H, $-OCH_3$), 4.10–4.18 (m, 4H, 2 × $-OCH_2-$), 4.68–4.76 (m, 1H, $-NCH-$), 4.88 (d, 1H, J = 3.7 Hz, $-CHS_2-$), 6.83 (s, 1H, ArH), 6.99 (d, 2H, J = 8.3 Hz, ArH), 7.64–7.69 (m, 5H, ArH); MS (ESI): m/z 796 $[M+1]^+$.

4.2.25. Synthesis of 7-methoxy-8-{3-[5-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]propyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepine-5-one (4a)

To a solution of compound **11a** (726 mg, 1.0 mmol) in methanol (20 mL), $SnCl_2.2H_2O$ (5 mmol) was added and refluxed for 1–2 h. The methanol was evaporated in vacuum and the aqueous layer was then carefully adjusted to pH 8 with 10% $NaHCO_3$ solution and then extracted with ethyl acetate. The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford the amino diethylthioacetal, which due to potential stability problems preceded for the next step. A solution of amino diethylthioacetal (1 mmol), $HgCl_2$ (2.26 mmol) and $CaCO_3$ (2.46 mmol) in CH_3CN–water (4:1) was stirred slowly at room temperature until TLC indicated complete loss of starting material (12 h). 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 5% $NaHCO_3$ (20 mL) and brine (20 mL), and the combined organic phase was dried over anhydrous Na_2SO_4. The organic layer was evaporated in vacuum and purified by column chromatography (MeOH/$CHCl_3$, 3%) to give the final product **4a** as a white solid (332 mg, 58% yield). Mp 102–104 °C; $[\alpha]_D^{27} = +118.5$ (c = 1 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.60–1.82 (m, 2H, $-CH_2-$), 2.01–2.14 (m, 2H, $-CH_2-$), 2.37–2.51 (m, 2H, $-CH_2-$), 3.48–3.88 (m, 3H, $-NCH_2-$, $-NCH-$), 3.94 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 4.26–4.38 (m, 4H, 2 × $-OCH_2-$), 6.88 (s, 1H, ArH), 7.03 (d, 2H, J = 9.1 Hz, ArH), 7.25 (s, 2H, ArH), 7.52 (s, 1H, ArH), 7.64 (t, 1H, J = 1.6 Hz, ArH), 7.69 (d, 1H, J = 4.1 Hz, imine–H), 8.15 (dd, 2H, J = 14.1 Hz, J = 3.3 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 164.5, 163.8, 162.9, 162.3, 159.1, 151.4, 150.6, 149.5, 147.6, 140.5, 129.0, 128.9, 120.1, 116.4, 116.1, 112.2, 111.4, 110.3, 109.6, 68.4, 56.0, 53.6, 46.5, 29.5, 25.7, 24.1, 22.5 ppm; IR (KBr) (U_{max}/cm^{-1}): ν 3359 (br), 2930, 1606, 1499, 1463, 1432, 1271, 1226, 1025, 846, 743, 656, 611; MS (ESI): m/z 573 $[M+1]^+$; HRMS (ESI m/z) for $C_{31}H_{30}N_4O_6F$ calcd 573.2149, found 573.2152 $[M+1]^+$.

4.2.26. 7-Methoxy-8-{4-[5-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]butyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4b)

Compound **4b** was prepared according to the method described for compound **4a**, employing compound **11b** (740 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 3%) to afford compound **4b** as a white solid (329 mg, 56% yield). Mp: 114–116 °C; $[\alpha]_D^{27} = +159.5$ (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56–1.68 (m, 4H, 2 × $-CH_2-$), 2.03–2.15 (m, 4H, 2 × $-CH_2-$), 3.71–3.86 (m, 3H, $-NCH_2-$, $-NCH-$), 3.93 (s, 3H, $-OCH_3$), 3.96 (s, 3H, $-OCH_3$), 4.18–4.27 (m, 4H, 2 × $-OCH_2-$), 6.82 (s, 1H, ArH), 7.01 (d, 1H, J = 8.0 Hz, ArH), 7.24 (d, 2H, J = 8.0 Hz, ArH), 7.26 (s, 1H, ArH), 7.51 (s, 1H, ArH), 7.61 (s, 1H, ArH), 7.68 (d, 1H, J = 4.3 Hz, imine–H), 8.14 (dd, 2H, J = 13.9 Hz, J = 3.6 Hz, ArH); IR (KBr) (U_{max}/cm^{-1}): ν 3342 (br), 2936, 1606, 1498, 1464, 1430, 1272, 1225, 1025, 846, 743, 655, 610, 520 cm^{-1}; MS (ESI): m/z 587 $[M+1]^+$; HRMS (ESI m/z) for $C_{32}H_{32}N_4O_6F$ calcd 587.2305, found 587.2276 $[M+1]^+$.

4.2.27. 7-Methoxy-8-{5-[5-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]pentyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4c)

Compound **4c** was prepared according to the method described for compound **4a**, employing compound **11c** (754 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 3%) to afford compound **4c** as a white solid (402 mg, 55% yield). Mp: 95–97 °C; $[\alpha]_D^{27} = +147.5$ (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58–1.77 (m, 4H, 2 × $-CH_2-$), 1.91–2.06 (m, 6H, 3 × $-CH_2-$), 3.69–3.86 (m, 3H, $-NCH_2-$, $-NCH-$), 3.94 (s, 3H, $-OCH_3$), 3.97 (s, 3H, $-OCH_3$), 4.10–4.16 (m, 4H, 2 × $-OCH_2-$), 6.80 (s, 1H, ArH), 6.98 (d, 1H, J = 8.0 Hz, ArH), 7.24 (d, 2H, J = 8.0 Hz, ArH), 7.26 (s, 1H, ArH), 7.51 (s, 1H, ArH), 7.59 (s, 1H, ArH), 7.67 (d, 1H, J = 3.6 Hz, imine–H), 8.14 (dd, 2H, J = 13.5 Hz, J = 3.0 Hz, ArH); IR (KBr) (U_{max}/cm^{-1}): ν 3380 (br), 2933, 1607, 1498, 1464, 1273, 1225, 1024, 847, 741, 654, 610, 520 cm^{-1}; MS (ESI): m/z 601 $[M+1]^+$; HRMS (ESI m/z) for $C_{33}H_{34}N_4O_6F$ calcd 601.2462, found 601.2476 $[M+1]^+$.

4.2.28. 7-Methoxy-8-{3-[5-(5-(4-trifluoromethylphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]propyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepine-5-one (4d)

Compound **4d** was prepared according to the method described for compound **4a**, employing compound **11d** (776 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 3%) to afford compound **4d** as a white solid (361 mg, 58% yield). Mp: 98−100 °C; $[\alpha]_D^{27} = +158.5$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.53–2.19 (m, 4H, 2 × $-CH_2-$), 2.29–2.52 (m, 2H, $-CH_2-$), 3.79–3.95 (m, 3H, $-NCH_2-$, –NCH–), 3.97 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 4.22–4.42 (m, 4H, 2 × $-OCH_2-$), 6.87 (s, 1H, ArH), 7.04 (d, 2H, $J = 8.5$ Hz, ArH), 7.51 (s, 1H, ArH), 7.66 (d, 1H, $J = 3.8$ Hz, imine–H), 7.69 (d, 1H, $J = 6.2$ Hz, ArH), 7.81 (d, 2H, $J = 7.7$ Hz, ArH), 8.26 (d, 2H, $J = 7.7$ Hz, ArH); IR (KBr) (U_{max}/cm^{-1}): ν 3415 (br), 2932, 1609,1502,1465,1427, 1325, 1275, 1172, 1129, 1065, 1020, 853, 752, 597 cm^{-1}; MS (ESI): m/z 623 [M+1]$^+$; HRMS (ESI m/z) for $C_{32}H_{30}N_4O_6F_3$ calcd 623.2117, found 623.2098 [M+1]$^+$.

4.2.29. 7-Methoxy-8-{4-[5-(5-(4-trifluoromethylphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]butyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepine-5-one (4e)

Compound **4e** was prepared according to the method described for compound **4a**, employing compound **11e** (790 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 3%) to afford compound **4e** as a white solid (357 mg, 56% yield). Mp: 103−105 °C; $[\alpha]_D^{27} = +171.5$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72–1.98 (m, 4H, 2 × $-CH_2-$), 2.05–2.18 (m, 4H, 2 × $-CH_2-$), 3.53–3.88 (m, 3H, $-NCH_2-$, $-NCH-$), 3.94 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 4.09–4.29 (m, 4H, 2 × $-CH_2-$), 6.83 (s, 1H, ArH), 7.01 (d, 2H, $J = 8.7$ Hz, ArH), 7.51 (s, 1H, ArH), 7.67 (d, 1H, $J = 4.1$ Hz, imine-H), 7.69 (d, 1H, $J = 5.8$ Hz, ArH), 7.81 (d, 2H, $J = 8.0$ Hz, ArH), 8.27 (d, 2H, $J = 8.0$ Hz, ArH); IR (KBr) (U_{max}/cm^{-1}): ν 3417 (br), 2930, 1608, 1502, 1466, 1429, 1324, 1275, 1225, 1172, 1129, 1068, 1017, 853, 786, 751, 727, 650, 598, 471 cm^{-1}; MS (ESI): m/z 637 [M+1]$^+$; HRMS (ESI m/z) for $C_{33}H_{32}N_4O_6F_3$ calcd 637.2273, found 637.2283 [M+1]$^+$.

4.2.30. 7-Methoxy-8-{5-[5-(5-(4-trifluoromethylphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]pentyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepine-5-one (4f)

Compound **4f** was prepared according to the method described for compound **4a**, employing compound **11f** (804 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 3%) to afford compound **4f** as a white solid (384 mg, 59% yield). Mp: 100−102 °C; $[\alpha]_D^{27} = +162.5$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.62–1.70 (m, 4H, 2 × $-CH_2-$), 2.01–2.12 (m, 6H, 3 × $-CH_2-$), 3.79–3.95 (m, 3H, $-NCH_2-$, –NCH–), 3.93 (s, 3H, $-OCH_3$), 3.96 (s, 3H, $-OCH_3$), 4.15–4.22 (m, 4H, 2 × $-OCH_2-$), 6.87 (s, 1H, ArH), 7.04 (d, 1H, $J = 8.5$ Hz, ArH), 7.14 (d, 2H, $J = 8.0$ Hz, ArH), 7.24 (s, 1H, ArH), 7.26 (s, 1H, ArH), 7.69 (s, 1H, ArH), 7.41 (d, 1H, $J = 3.9$ Hz, imine−H), 8.16 (d, 2H, $J = 7.7$ Hz, ArH); IR (KBr) (U_{max}/cm^{-1}): ν 3414 (br), 2928, 2875, 1691, 1603, 1507, 1460, 1431, 1380, 1244, 1176, 1090, 1055, 1023, 870, 833, 756 cm^{-1}; MS (ESI): m/z 651[M+1]$^+$; HRMS (ESI m/z) for $C_{34}H_{34}N_4O_6F_3$ calcd 651.2430, found 651.2411 [M+1]$^+$.

4.2.31. 7-Methoxy-8-{3-[5-(5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]propyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepine-5-one (4g)

Compound **4g** was prepared according to the method described for compound **4a**, employing compound **11g** (798 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 3%) to afford compound **4g** as a white solid (368 mg, 57% yield). Mp: 86–88 °C; $[\alpha]_D^{27} = +112.5$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.51–1.78 (m, 4H, 2 × $-CH_2-$), 1.92–2.14 (m, 2H, $-CH_2-$), 3.73–3.88 (m, 3H, $-NCH_2-$, –NCH–), 3.96 (s, 9H, 3 × $-OCH_3$), 4.00 (s, 6H, 2 × $-OCH_3$), 4.20–4.39 (m, 4H, 2 × $-OCH_2-$), 6.88 (s, 1H, ArH), 7.03 (d, 1H, $J = 8.7$ Hz, ArH), 7.24 (d, 1H, $J = 8.0$ Hz, ArH), 7.34 (s, 1H, ArH), 7.63 (s, 1H, ArH), 7.66 (d, 1H, $J = 4.2$ Hz, imine–H), 8.14 (dd, 2H, $J = 5.1$ Hz, ArH; IR (KBr) (U_{max}/cm^{-1}): ν 3404 (br), 2928, 1603, 1497, 1460, 1464, 1421, 1337, 1245, 1179,1127, 1024, 868, 813, 738, 652 cm^{-1}; MS (ESI): m/z 645 [M+1]$^+$; HRMS (ESI m/z) for $C_{34}H_{37}N_4O_9$ calcd 645.2560, found 645.2553 [M+1]$^+$.

4.2.32. 7-Methoxy-8-{4-[5-(5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]butyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepine-5-one (4h)

Compound **4h** was prepared according to the method described for compound **4a**, employing compound **11h** (812 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 3%) to afford compound **4h** as a white solid (362 mg, 55% yield). Mp: 76−78 °C; $[\alpha]_D^{27} = +123.5$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56–1.71 (m, 4H, 2 × $-CH_2-$), 2.02–2.16 (m, 4H, 2 × $-CH_2-$), 3.70–3.89 (m, 3H, $-NCH_2$, $-NCH-$), 3.93 (s, 6H, 2 × $-OCH_3$), 3.97 (s, 9H, 3 × $-OCH_3$), 4.13–4.26 (m, 4H, 2 × $-OCH_2-$), 6.82 (s, 1H, ArH), 7.00 (d, 2H, $J = 8.7$ Hz, ArH), 7.34 (s, 2H, ArH), 7.52 (s, 1H, ArH), 7.65 (d, 1H, $J = 1.4$ Hz, ArH), 7.68 (d, 1H, $J = 4.3$ Hz, imine−H); IR (KBr) (U_{max}/cm^{-1}): ν 3364 (br), 2933, 1600, 1497, 1463, 1422, 1338, 1313, 1245, 1179, 1127, 1009, 865, 737, 653 cm^{-1}; MS (ESI): m/z 659 [M+1]$^+$; HRMS (ESI m/z) for $C_{34}H_{39}N_4O_9$ calcd 659.2717, found 659.2695 [M+1]$^+$.

4.2.33. 7-Methoxy-8-{5-[5-(5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]pentyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepine-5-one (4i)

Compound **4i** was prepared according to the method described for compound **4a**, employing compound **11i** (826 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 3%) to afford compound **4i** as a white solid (390 mg, 58% yield). Mp: 79−80 °C; $[\alpha]_D^{27} = +152.5$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.55–1.78 (m, 4H, 2 × $-CH_2-$), 1.87–2.12 (m, 6H, 3 × $-CH_2-$), 3.55–3.86 (m, 3H, $-NCH_2-$, $-NCH-$), 3.93 (s, 6H, 2 × $-OCH_3$), 3.98 (s, 9H, 3 × $-OCH_3$), 4.07–4.18 (m, 4H, 2 × $-OCH_2-$), 6.80 (s, 1H, ArH), 6.98 (d, 2H, $J = 9.1$ Hz, ArH), 7.35 (s, 2H, ArH), 7.51 (s, 1H, ArH), 7.65 (d, 1H, $J = 2.4$ Hz, ArH), 7.67 (d, 1H, $J = 4.1$ Hz, imine−H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.1, 163.7, 161.9, 153.2, 151.1, 150.3, 149.2, 145.1, 141.8, 126.9, 119.9, 118.6, 115.8, 111.8, 111.1, 109.9, 109.5, 103.7, 68.2, 60.5, 56.0, 55.7, 53.2, 46.2, 29.1, 28.1, 23.7, 22.0 ppm; IR (KBr) (U_{max}/cm^{-1}): ν 3415 (br), 2937, 2677, 1602, 1497, 1463, 1421,1337, 1245, 1178, 1127, 1006, 868, 767, 737, 616 cm^{-1}; MS (ESI): m/z 673 [M+1]$^+$; HRMS (ESI m/z) for $C_{34}H_{41}N_4O_9$ calcd 673.2873, found 673.2861 [M+1]$^+$.

4.2.34. 7-Methoxy-8-{3-[5-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]propyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzo- diazepine-5-one (4j)

Compound **4j** was prepared according to the method described for compound **4a**, employing compound **11j** (768 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 3%) to afford compound **4j** as a white solid (351 mg, 57% yield). Mp: 74−76 °C; $[\alpha]_D^{27} = +135.5$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.57–1.81 (m, 4H, 2 × $-CH_2-$), 1.99–

2.18 (m, 2H, $-CH_2-$), 3.74–3.85 (m, 3H, $-NCH_2-$, –NCH–), 3.96 (s, 6H, $2 \times -OCH_3$), 4.00 (s, 3H, $2 \times -OCH_3$), 4.24–4.37 (m, 4H, $2 \times -OCH_2-$), 6.87 (s, 1H, ArH), 6.99 (d, 2H, $J = 8.8$ Hz, ArH), 7.02 (d, 2H, $J = 8.0$ Hz, ArH), 7.52 (s, 1H, ArH), 7.65 (d, 1H, $J = 4.0$ Hz, imine–H), 7.68 (d, 2H, $J = 8.8$ Hz, ArH); IR (KBr) (U_{max}/cm^{-1}): ν 3424 (br), 2926, 1609, 1500, 1462, 1330, 1273, 1143, 1025, 868, 739, 603 cm^{-1}; MS (ESI): m/z 615 $[M+1]^+$; HRMS (ESI m/z) for $C_{33}H_{35}N_4O_8$ calcd 615.2454, found 615.2432 $[M+1]^+$.

4.2.35. 7-Methoxy-8-{4-[5-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]butyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4k)

Compound **4k** was prepared according to the method described for compound **4a**, employing compound **11k** (782 mg, 1.0 mmol). The crude product was purified by column chromatography ($MeOH/CHCl_3$, 3%) to afford compound **4k** as a white solid (352 mg, 56% yield). Mp: 76–78 °C; $[\alpha]_D^{27} = +139.5$ ($c = 1$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.59–1.77 (m, 4H, $2 \times -CH_2-$), 2.03–2.17 (m, 4H, $2 \times -CH_2-$), 3.56–3.63 (m, 1H, –NCH–), 3.69–3.88 (m, 2H, $-NCH_2-$), 3.93 (s, 3H, $-OCH_3$), 3.97 (s, 6H, $2 \times -OCH_3$), 4.00 (s, 3H, $-OCH_3$), 4.15–4.26 (m, 4H, $2 \times -OCH_2-$), 6.83 (s, 1H, ArH), 6.99 (d, 3H, $J = 8.3$ Hz, ArH), 7.51 (s, 2H, ArH), 7.65 (d, 1H, $J = 4.0$ Hz, imine-H), 7.69 (d, 2H, $J = 8.4$ Hz, ArH); IR (KBr) (U_{max}/cm^{-1}): ν 3416 (br), 2935, 1605, 1500, 1464, 1336, 1273, 1178, 1140, 1104, 1023, 867, 814, 739, 656 cm^{-1}; MS (ESI): m/z 629 $[M+1]^+$; HRMS (ESI m/z) for $C_{34}H_{37}N_4O_8$ calcd 629.2611, found 629.2568 $[M+1]^+$.

4.2.36. 7-Methoxy-8-{5-[5-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl)oxy]pentyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4l)

Compound **4l** was prepared according to the method described for compound **4a**, employing compound **11l** (795 mg, 1.0 mmol). The crude product was purified by column chromatography ($MeOH/CHCl_3$, 3%) to afford compound **4l** as a white solid (386 mg, 60% yield). Mp: 78-80 °C; $[\alpha]_D^{27} = +142.5$ ($c = 1$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.65–1.76 (m, 4H, $2 \times -CH_2-$), 1.94–2.10 (m, 6H, $3 \times -CH_2-$), 3.55–3.63 (m, 1H, –NCH–), 3.70–3.86 (m, 2H, $-NCH_2-$), 3.94 (s, 3H, $-OCH_3$), 3.97 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 4.00 (s, 3H, $-OCH_3$), 4.10–4.16 (m, 4H, $2 \times -CH_2-$), 6.80 (s, 1H, ArH), 6.96–7.01 (m, 3H, ArH), 7.52 (s, 1H, ArH), 7.65 (d, 1H, $J = 3.6$ Hz, imine–H), 7.67 (d, 2H, $J = 4.6$ Hz, ArH), 7.70 (d, 1H, $J = 1.8$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.5, 164.1, 162.3, 153.7, 153.0, 151.8, 151.4, 149.2, 147.6, 146.2, 145.2, 140.4, 138.3, 120.2, 116.5, 116.3, 112.2, 111.4, 110.9, 110.3, 109.7, 109.3, 68.6, 56.1, 55.9, 53.6, 46.5, 29.5, 28.6, 24.1, 22.4 ppm; IR (KBr) (U_{max}/cm^{-1}): ν 3418 (br), 2936, 1690, 1598, 1511, 1461, 1427, 1369, 1337, 1259, 1126, 1014, 895, 820, 757, 636 cm^{-1}; MS (ESI): m/z 643 $[M+1]^+$; HRMS (ESI m/z) for $C_{34}H_{39}N_4O_8$ calcd 643.2767, found 643.2760 $[M+1]^+$.

4.3. In vitro evaluation of cytotoxicity

Cell viability was assessed by the MTT based assay using WST-1 (premix WST-1 cell proliferation Assay system, Takara), is more sensitive than MTT. Briefly, A375 and TK6 cells were seeded in a 96-well plate (TPP) at a cell density of 10,000 cells/well. After overnight incubation, the cells were treated with compounds **4a**, **4d**, **4i**, **4l** and DC-81 (**1**) at 4 μM concentration and incubated for 24 h. The medium was then discarded and replaced with fresh 100 μL media followed by addition of 10 μL of WST-1 dye. Plates were incubated at 37 °C for 30 min. Optical density (OD) was read at 420 nm using Multimode Varioskan FLASH (Thermo-Fischer Scientifics).

4.4. Cell culture

A375 (Human melanoma cells) was obtained from ATCC, USA. A375 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen), supplemented with 10% fetal calf serum and 100 U/mL penicillin and 100 mg/mL streptomycin sulfate (Sigma). The cells were passaged and maintained at 37 °C in a humidified atmosphere containing 5% CO2. TK6 cells were cultured in RPMI 1640 medium supplemented with L-glutamine (2 mM) 10% fetal bovine serum (FBS). Cell lines were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

4.5. Cell cycle analysis

5×10^5 A375 cells were seeded in 60 mm dish and were allowed to grow for 24 h. Compounds **4a**, **4d**, **4i**, **4l** and DC-81(**1**) at 4 μM concentration were added to the culture media and the cells were incubated for an additional 24 h. Harvesting of cells was done with Trypsin–EDTA, fixed with ice-cold 70% ethanol at 4 °C for 30 min, washed with PBS and incubated with 1 mg/mL RNaseA solution (Sigma) at 37 °C for 30 min. Cells were collected by centrifugation at 2000 rpm for 5 min and further stained with 250 μL of DNA staining solution [10 mg of propidium iodide (PI), 0.1 mg of trisodium citrate, and 0.03 mL of Triton X-100 were dissolved in 100 mL of sterile MilliQ water at room temperature for 30 min in the dark]. The DNA contents of 20,000 events were measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed using Summit Software.

4.6. Caspase-3 assay

We have used Apo alert caspase-3 fluorescent assay kit (Clonetech, CA) according to manufacturer's recommendations. A375 cells were treated with compounds **4a**, **4d**, **4i**, **4l** and DC-81(**1**) at 4 μM concentrations as obtained from FACS analysis. Here the substrate and inhibitor (I) used are DEVD-AFC and DEVD-CHO, respectively. The DEVD-AFC substrate, DEVD-AFC+ DEVD-CHO is added to the cell lysate and incubation was carried out at 37 °C for 1 h. Readings were taken at excitation wavelength 400 nm and emission wavelength 505 nm.

4.7. Protein extraction and Western blot analysis

Total cell lysates were isolated from cultured A375 cells after compound treatments as mentioned earlier were obtained by lysing the cells in ice-cold RIPA buffer (1× PBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS containing protease inhibitors). After centrifugation at 12,000 rpm for 10 min, the protein in supernatant was quantified by Bradford method (BIO-RAD) using Multimode varioscan instrument (Thermo-Fischer Scientifics). Thirty micrograms of protein per lane was applied in 12% SDS–polyacrylamide gel. After electrophoresis, the protein was transferred to polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences). The membrane was blocked at room temperature for 2 h in TBS + 0.1% Tween20 (TBST) containing 5% blocking powder (Santa Cruz). The membrane was washed with TBST for 5 min, and primary antibody was added and incubated at 4 °C overnight (O/N). Rabbit polyclonal β-actin, mouse monoclonal cytochrome *c* and cleaved PARP were obtained from Imgenex. Rabbit polyclonal Bax (p-19), TNFR1 (H271), Cdk2 (M2) and mouse monoclonal p53 (pab1801) were from Santa Cruz, p21 antibody was obtained from upstate and Chk2 antibody was purchased from Imgenex. After three TBST washes, the membrane was incubated with corresponding horseradish peroxidase-labeled secondary antibody (1:2000) (Santa Cruz) at room temperature for 1 h. Membranes were washed with TBST three times for 15 min and the protein

blots were visualized with chemiluminescence reagent (Thermo-Fischer Scientifics Ltd). The X-ray films were developed with developer and fixed with fixer solution.

4.8. Thermal denaturation studies

Compounds **4a**–**l** subjected to DNA thermal melting (denaturation) studies using duplex form calf thymus DNA (CT-DNA) using modification reported procedure.[48] Working solutions were produced by appropriate dilution in aqueous buffer (10 mM NaH_2PO_4/NaH_2PO_4, 1 mM Na_2EDTA, pH 7.00+0.01) containing CT-DNA, (100 lM in phosphate) and the PBD (20 μM) were prepared by addition of concentrated PBD solutions in methanol to obtain a fixed [PBD]/[DNA] molar ratio of 1:5 The DNA–PBD solutions were incubated at 37 °C for 0 h prior to analysis sample were monitored a 260 nm using a Beckman DU-7400 spectrophotometer fitted with high performance temperature controller. Heating was applied at a rate of 1 °C min^{-1} in the 40–90 °C range. DNA helixcoil transition temperatures (Tm) were determined from the maxima in the $d(A_{260})/dT$ derivative plots. Results for each compound are shown as mean ± standard derivation from the least three determinations and are corrected for the effects of methanol cosolvent using a linear correction term. Ligand-induced alteration in DNA melting behavior are given by ΔT_m = Tm (DNA + PBD) − Tm (DNA alone), where the Tm value for the PBD free CT-DNA is 68.5 ± 0.001 the fixed [PBD]/[DNA] ratio used did not result in binding saturation of the host DNA duplex for any compound examined.

Acknowledgments

The authors D.D.G., E.V.B., J.S.N.R. are thankful to CSIR, New Delhi, for the award of research fellowships. We are also thankful to the Department of Biotechnology (BT/PR/7037/Med/14/933/2006), New Delhi; for financial assistance.

References and notes

1. Martinez, R.; Garcia, L. C. *Curr. Med. Chem.* **2005**, *12*, 127.
2. Cargill, C.; Bachmann, E.; Zbinden, G. J. *Natl. Cancer Inst.* **1974**, *53*, 481.
3. Thurston, D. E. *Molecular Aspects of Anticancer Drug-DNA Interactions*; The Macmillan Press: London, UK, 1993. pp 54–88.
4. Petrusek, R. L.; Uhlenhopp, E. L.; Duteau, N.; Hurley, L. H. *J. Biol. Chem.* **1982**, *257*, 6207.
5. Hurley, L. H.; Reck, T.; Thurston, D. E.; Langley, D. R.; Holden, K. G.; Hertzberg, R. P.; Hoover, J. R. E.; Gallagher, G. J.; Faucette, L. F.; Mong, S. M.; Johnson, R. K. *Chem. Res. Toxicol.* **1988**, *1*, 258.
6. Wang, Z.; Shi, H. *J. Heterocycl. Chem.* **2001**, *38*, 355.
7. Rigo, B.; Couturier, D. *J. Heterocycl. Chem.* **1985**, *22*, 287.
8. Tan, T. M.; Chen, Y.; Kong, K. H.; Bai, J.; Li, Y.; Lim, S. G.; Ang, T. H.; Lam, Y. *Antivir. Res.* **2006**, *71*, 7.
9. Gaonkar, S. L.; Rai, K. M. L.; Prabhuswamy, B. *Eur. J. Med. Chem.* **2006**, *41*, 841.
10. Li, Y.; Liu, J.; Zhang, H.; Yang, X.; Liu, Z. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2278.
11. Aboraia, A. S.; Rahman, H. M. A.; Mahfouz, N. M.; Gendy, M. A. *Bioorg. Med. Chem. Lett.* **2006**, *14*, 1236.
12. Yadav, M. R.; Shirude, S. T.; Puntambekar, D. S.; Patel, P. J.; Prajapati, H. B.; Parmar, A.; Balaraman, R.; Giridhar, R. *Acta Pharm.* **2007**, *57*, 13.
13. Khan, M. T.; Choudhary, M. I.; Khan, K. M.; Rani, M.; Rahman, A. U. *Bioorg. Med. Chem.* **2005**, *13*, 3385.
14. Guimaraes, C. R. W.; Boger, D. L.; Jorgensen, W. L. *J. Am. Chem. Soc.* **2005**, *127*, 17377.
15. Rahman, V. P. M.; Mukhtar, S.; Ansari, W. H.; Lemiere, G. *Eur. J. Med. Chem.* **2005**, *40*, 173.
16. Thurston, D. E.; Morris, S. J.; Hartley, J. A. *Chem. Commun.* **1996**, *3*, 563.
17. Kamal, A.; Bharathi, E. V.; Ramaiah, M. J.; Dastagiri, D.; Reddy, J. S.; Viswanath, A.; Sultana, F.; Pushpavalli, S. N. C. V. L.; Bhadra, M. P.; Srivastava, H. K.; Sastry, G. N.; Juvekar, A.; Sen, S.; Zingde, S. *Bioorg. Med. Chem.* **2010**, *18*, 526.
18. 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.
19. Damayanthi, Y.; Reddy, B. S. P.; Lown, J. W. *J. Org. Chem.* **1999**, *64*, 290.
20. Wang, J. J.; Shen, Y. K.; Hu, W. P.; Hsieh, M. C.; Lin, F. L.; Hsu, M. K.; Hsu, M. H. *J. Med. Chem.* **2006**, *49*, 1442.
21. Hu, W. P.; Liang, J. J.; Kao, C. L.; Chen, Y. C.; Chen, C. Y.; Tsai, F. Y.; Wu, M. J.; Chang, L. S.; Chen, Y. L.; Wang, J. J. *Bioorg. Med. Chem.* **2009**, *17*, 1172.
22. Kamal, A.; Reddy, G. S. K.; Raghavan, S. *Bioorg. Med. Chem. Lett.* **2001**, *13*, 387.
23. Kamal, A.; Shankaraiah, N.; Devaiah, V.; Reddy, K. L. *Tetrahedron Lett.* **2006**, *47*, 6553.
24. 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.
25. Kamal, A.; Ramesh, G.; Ramulu, P.; Srinivas, O.; Rehana, T.; Sheelu, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3451.
26. Kamal, A.; Devaiah, V.; Reddy, K. L.; Kumar, M. S. *Bioorg. Med. Chem.* **2005**, *13*, 2021.
27. Kamal, A.; Kumar, P. P.; Seshadri, B. N.; Srinivas, O.; Kumar, M. S.; Sen, S.; Kurian, N.; Juvekar, A.; Zingde, S. *Bioorg. Med. Chem.* **2008**, *16*, 3895.
28. Kamal, A.; Shankaraiah, N.; Devaiah, V.; Reddy, K. L.; Juvekar, A.; Kurian, N.; Zingde, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1468.
29. Kadi, A. A.; Brollosy, N. R.; Omar, A.; Deeb, A.; Habib, E.; Tarek, M.; Ibrahim, A.; Emam, A. E. *Eur. J. Med. Chem.* **2007**, *42*, 235.
30. Rai, N. P.; Narayanaswamy, V. K.; Shashikanth, S.; Arunachalam, P. N. *Eur. J. Med. Chem.* **2009**, *44*, 4522.
31. Watanabe, M.; Yatagai, F. *Radiat. res.* **2002**, *158*, 678.
32. Aliouat Denis, C. M.; Dendouga, N.; Wyngaert, V. I.; Goehlmann, H.; Steller, U.; Vandeweyer, I.; Slycken, N. V.; Andries, L.; Kass, S.; Luyten, W.; Janicot, M.; Vialard, J. E. *Mol. Cancer. Res.* **2005**, *3*, 627.
33. ALOGPS 2.1 software available at http://www.vcclab.org/lab/alogps.
34. Tetko, I. V.; Tanchuk, V. Y. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 1136.
35. Telford, W. G.; King, L. E.; Fraker, P. J. *Cytometry* **1992**, *13*, 137.
36. Fraker, P. J.; King, L. E.; Lill-Elghanian, D.; Telford, W. G. *Methods Cell. Biol.* **1995**, *46*, 57.
37. Sampath, D.; Plunkett, W. *Curr. Opin. Oncol.* **2001**, *13*, 484.
38. Kaelin, W. G. *Mol. Cell.* **2005**, *18*, 623.
39. Nicholson, D. W.; Thornberry, N. A. *Trends Biochem. Sci.* **1997**, *22*, 299.
40. Green, D. R.; Reed, J. C. *Science* **1998**, *287*, 1309.
41. Wang, X.; Jiang, X. *Annu. Rev. Biochem.* **2004**, *3*, 87.
42. Shimizu, S.; Narita, M.; Tsujimoto, Y. *Nature* **1999**, *399*, 483.
43. Debatin, K. M.; Krammer, P. H. *Oncogene* **2004**, *23*, 2950.
44. Adams, J. M.; Cory, S. *Science* **1998**, *281*, 1322.
45. Cory, S.; Adams, J. M. *Nat. Rev. Cancer.* **2002**, *2*, 245.
46. Kluck, R. M.; Wetzel, E. B.; Green, D. R.; New Meyer, D. D. *Science* **1997**, *275*, 1132.
47. Hu, W. P.; Yu, H. S.; Sung, P. J.; Tsai, F. Y.; Shen, Y. K.; Chang, L. S.; Wang, J. J. *Chem. Res. Toxicol.* **2007**, *20*, 905.
48. Salvesen, G. S.; Dixit, V. M. *Cell* **1997**, *91*, 443.
49. Morgan, D. O. *Nature* **1995**, *374*, 131.
50. Jones, G. B.; Davey, C. L.; Jenkins, T. C.; Kamal, A.; Kneale, G. G.; Neidle, S.; Webster, G. D.; Thurston, D. E. *Anti-Cancer. Drug. Des.* **1990**, *5*, 249.
51. Kamal, A.; Khan, M. N. A.; Srikanth, Y. V. V.; Reddy, K. S.; Juvekar, A.; Sen, S.; Kurian, N.; Zingde, S. *Bioorg. Med. Chem.* **2008**, *16*, 7804.