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Synthesis and biological evaluation of 4β-benzoxazolepodophyllotoxin hybrids as DNA topoisomerase-II targeting anticancer agents

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A series of new 4β-benzoxazolepodophyllotoxin compounds (**9a-j**) were prepared and screened for cytotoxicity against four human tumour cell lines (HeLa, DU-145, A-159 and MCF-7). Among these compounds **9a, 9c**, **9f** and **9i** have shown more potent anticancer activity than etoposide with considerable IC_{50} values. Apoptosis evaluation studies were performed by using Hoechst-33258 staining method and found that specially the best active compound **9i** shows a clear nuclear damage than etoposide. Molecular docking studies were also carried out to recognize the interactions against DNA topoisomerase-II and found that the energy calculations were in good agreement with the observed IC_{50} value.

Introduction

Podophyllotoxin (**1**) (Fig. 1) is an abundant naturally occurring cyclolignan isolated from the roots and rhizomes of various species of the *Podophyllum* genus, such as *Podophyllum peltatum* and some *Juniperus* species.[1] Podophyllotoxin exhibits important antineoplastic, antiviral and antimitotic activities.[2] Its prevailing cytotoxic properties have been ascribed to its binding ability to tubulin during mitosis that inhibit microtubule assembly.[3] However, its high toxicity and low bioavailability limit its anticancer applications. In this context, semisynthetic derivatives of Podophyllotoxin have been widely used as anticancer agents.[4,5] The structural modifications and mechanism of action of podophyllotoxin have been studied since many decades, mainly at Sandoz Laboratories[6] which led to the semisynthetic etoposide (VP-16, **2**) and teniposide (VM-26, **3**).

The semisynthetic derivatives of podophyllotoxin differ significantly with the parent compound podophyllotoxin in their mechanisms of action. Although podophyllotoxin inhibits the assembly of tubulin into microtubules, in contrast etoposide and teniposide analogues exhibits inhibition of DNA topoisomerase-II, preventing the relegation of double-stranded breaks.[7] The epipodophyllotoxin derivatives **2-5** (Figure 1) are currently used in the chemotherapy.[8] However, their clinical use has encountered certain limitations, such as poor water solubility, development of drug resistance, metabolic inactivation and certain toxic effects.[9] To overcome the above limitations, herein we have shown some requisite modifications to podophyllotoxin by developing the synthesis of 4β-benzoxazolepodophyllotoxins (**9a-j**) and their efficiency in cytotoxicity studies against four human tumor cell lines (HeLa, DU-145, A-159 and MCF-7). We also report the apoptosis measurements using Hoechst-33258 staining representing nuclear shrinkage and molecular interactions with docking studies.

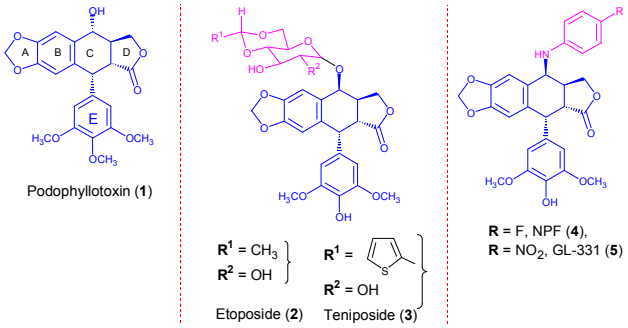

Fig. 1 Scheme represents the outline of the work.

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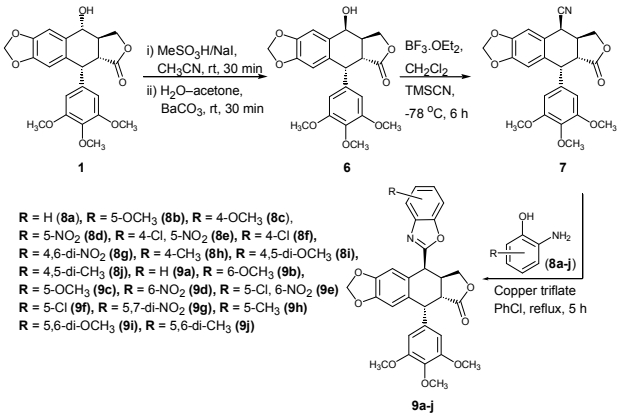
Electronic Supplementary Information (ESI) available: Docking poses for **9a**,**9c** and **9f** in DNA topoisomerase-II, Top 10 interaction poses of compound **9i** and experimental section. See DOI: 10.1039/x0xx00000x

Similar to podophyllotoxin, benzoxazoles have also occupied unique place in the field of medicinal chemistry, due to their wide range of biological activities such as anticancer activity,[10] DNA topoisomerase-I, II annihilates,[11] antibacterial and antifungal activity.[12] It has been reported previously that the replacement of the C-4 sugar moiety of podophyllotoxin derivatives with a non-sugar substituent has improved the therapeutic value.[13] Accordingly, we have developed the following synthetic methodology to afford 4β-benzoxazolepodophyllotoxin (**9a-j**).



Scheme 1 Synthesis of 4β-benzoxazolepodophyllotoxin congeners **(9a-j).**

As shown in Scheme 1, the reaction of podophyllotoxin (**1**) with CH_3SO_3H/NaI followed by base hydrolysis using $BaCO_3$ has given epipodophyllotoxin (**6**). Compound **6** was then converted into 4β-cyanopodophyllotoxin (**7**) intermediate by the simple treatment with trimethylsilyl cyanide (TMSCN) and $BF_3{\cdot}OEt_2$/in dry CH_2Cl_2 at -15 °C for 6 hours. Thereafter, the intermediate (**7**) was reacted with substituted amino phenols in the presence of copper triflate in chlorobenzene under refluxing conditions to obtain 4β-benzoxazolepodophyllotoxin derivatives (**9a-j**) as shown in good yields. All the synthesized compounds were characterized by ^1H, ^{13}C NMR and mass spectral data.

In vitro cytotoxicity assay

The synthesized 4β-benzoxazolepodophyllotoxins (**9a-j)** were evaluated for *in vitro* cytotoxic ability against a panel of human cancer cell lines, including HeLa (cervical cancer), DU145 (prostate cancer), A549 (non-small cell lung cancer) and MCF-7 (breast carcinoma) selected using a MTT assay. The results were summarized in Table 1 and well-known standard etoposide was used as a reference standard to deduce the structure-activity relationship (SAR) for podophyllotoxin derivatives with various substitutions on benzoxazole appendage. As shown Figure 2, it is clear that the basic structural unit of appendage-1 is unchanged and efforts were made to deduce SAR by modifying the appendage-2. These newly synthesized 4β-benzoxazolepodophyllotoxins have shown moderate to good antiproliferative potential against most of the cell lines in this investigation. Among them, compounds **9a, 9c, 9f** and **9i** were exhibited superior activity with IC_{50} values ranging from 1.2-5.3 μM compared to that of etoposide IC_{50} 2.03-5.74 μM (Table 1). The best active compound **9i** with an electron donating dimethoxy substitution on appendage **2** (Fig.2) inhibits the growth of HeLa and DU145 cells with IC_{50} values of 1.3 μM and 1.2 μM respectively. In addition, this compound (**9i**) also displayed significant growth inhibition effect in A549 (IC_{50} = 1.8 μM) and MCF-7 (IC_{50} = 2.0 μM) cells.

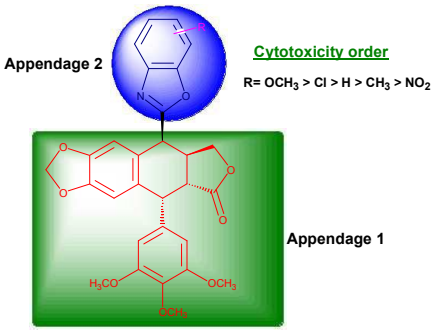


Fig. 2 Structural Activity Relationship (SAR).

Regarding the activity of the compounds **9b** and **9c** that are structural isomers possessing monomethoxy substitutions at C6 and C5 of phenyl ring without any change at appendage **1** have exhibited varied cytotoxicity (Entry 2&3, Table 1). When compared to compound **9b**, the structural isomer **9c** with a C5-OCH_3 exhibited a profound activity against all the four cancer cell lines used in this study with considerable IC_{50} values. Compound **9f** with a chloro (Cl) group at C5 position of phenyl ring has also shown a considerable effect on the cell growth with a highest inhibition particularly in MCF-7 (IC_{50} = 1.2 μM). On the other hand, compound **9e** with a chloro (Cl) group at C5 and an electron withdrawing nitro (–NO_2) substitution at C6 position has shown very minimum effect on the growth. The results presented in Table 1 also provide a finding that while the presence of weak electron donating substitution like –CH_3 (**9h, 9j**) and a neutral -H (**9a)** at appendage-2 can show moderate potency, those contains electron withdrawing –NO_2substituents (**9d and 9g**) have shown diminished cytotoxic effect. Based on structural diversity, the optimal order of substitutions on the phenyl ring of appendage-2 is methoxy > chloro > unsubstituted > methyl > nitro (Fig. 2). Overall, these results suggest that the best active compounds like **9a, 9c, 9f** and **9i** had shown excellent antiproliferative activity compared to that of positive control etoposide.

Chromatin condensation by Hoechst-33258 staining

DNA fragmentation is the most seen marker of apoptosis and one of the major pathways of cell death. A classic characteristic of apoptosis is chromatin condensation and

nuclear fragmentation/shrinkage.[14] Hoechst-33258 staining is a commonly employed technique to distinguish the compact chromatin of apoptotic nuclei to identify replicating cells and to sort cells based on their DNA content. To elucidate, whether 4β-benzoxazolepodophyllotoxin congeners (**9c** and **9i**) induced cytotoxicity by cellular apoptosis, HeLa cells were exposed with 3 μM concentration of representative compounds for 24 hours. The results demonstrated that the active compounds condensed the nuclear content considerably. Specifically the best active compound **9i** exhibited a clear nuclear damage in comparison to etoposide (**Eto**). (Fig. 3).

Cells were treated with **9c, 9i** andetoposide (**Eto**) at 3 μM concentration and control (DMSO) for 24 hours, washed with PBS, and incubated with Hoechst-33258 stain (4 mg mL^{-1}) for 20 minutes to measure chromatin condensation. Micro photographed images were captured using fluorescence microscopy equipped with DAPI filter.

Table 1 *In vitro* cytotoxicity ($^{a}IC_{50}$ μM) of 4β-benzoxazolepodophyllotoxin compounds (**9a–j**).

Entry	Compound	[b]HeLa	[c]DU145	[d]A549	[e]MCF-7
1	**9a**	2.8±014	3.2±02	4.3±1.3	2.6±01
2	**9b**	5±0.3	6.8±2.4	11.7±1.7	4.8±2.4
3	**9c**	1.5±0.08	1.9±0.14	4.0±0.2	2.5±0.13
4	**9d**	5.6±0.6	7.9±0.7	8.6±0.3	6.0±0.4
5	**9e**	14.3±0.9	16.7±1.0	8.4±0.7	10.8±0.8
6	**9f**	2.2±0.13	5.3±0.7	2.6±0.12	1.2±0.05
7	**9g**	11.7±0.8	14.1±1.7	11.3±0.8	10.3±1.1
8	**9h**	22.8±1.1	12.0±1.0	23.0±1.1	14.5±1.5
9	**9i**	1.3±0.03	1.2±0.04	1.8±0.05	2.0±0.06
10	**9j**	14.8±0.8	28.7±2.2	21.8±1.1	5.8±0.23
11	Etoposide	5.74±0.37	2.58±0.25	2.03±0.12	2.61±0.32

[a] Each data represents as mean ± S.D values. from three different experiments performed in triplicates. [b] HeLa: human cervical cancer cell line. [c]DU145: human prostate cancer cell line. [d]A549: human lung cancer epithelial cell line. [e]MCF-7: human breast carcinoma cell line.

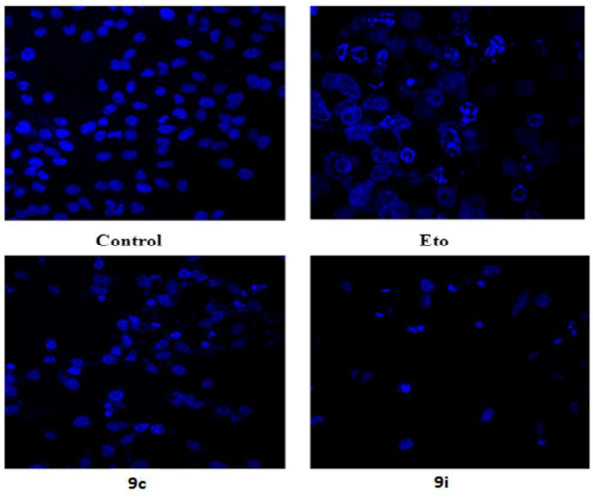


Fig. 3 Benzoxazolepodophyllotoxin congeners cause apoptosis in HeLa cells.

Molecular modeling studies

The docking studies were carried out by employing AutoDock software and **9i**, **9i+Eto** (Etoposide) structures have been docked against the crystal structure of human DNA topoisomerase-II receptor that was retrieved from protein data bank with PDB: 3QX3. The best active compound **9i** occupied the exact site, where the positive control etoposide binds with the protein. The yellow coloured stick (**9i**) was sandwiched between and surrounded by most important amino acid residues like Asp-479, Arg-503, Met-782, Gln-778, Ala-779, Gly-478, Gly-1023, Leu-852, Glu-477 and Phe-720. A strong hydrogen bonding was observed between -O of trimethoxy phenyl ring and -NH of Asp-479 (O---HN, distance: 3.0 Å). In addition, the -O of methoxy phenyl ring has shown Vander wall interactions with -NH of Arg-503. Another hydrogen bond was noticed between O of lactone ring and NH of the amino acid residue Gln-778 (O---HN, distance: 3.0 Å). Interestingly, the modified structural unit benzoxazole also exhibited considerable interactions with topoisomerase along with nucleotide of DNA. In comparison, methoxy -O of benzoxazole unit forms weak interactions with NH of Met-782. Furthermore, some hydrophobic interactions were observed between the compound **9i** and amino acid residues like Ala-779, Gly-478, Gly-1023, Leu-852, Glu-477 and Phe-720. Overall, the docking results reveal that the compound **9i** interacts with topoisomerase-II in a similar manner with respect to that of etoposide. A superimposition pose also demonstrates that the

newly synthesized compounds induce the antiproliferative potential by inhibiting the DNA topoisomerase-II (Fig. 4). Additionally, the docking interactions for the succeeding active compounds **9a**, **9c** and **9f** of the same series is also evaluated (Fig. S1 see ESI†). The calculated docking parameters were summarized in Table 2.

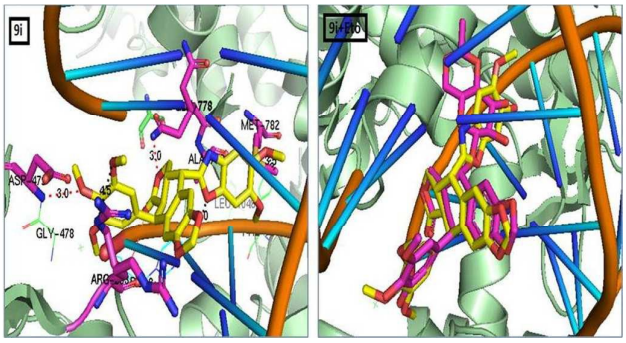


Fig. 4 Molecular docking poses for **9i** and **9i**+etoposide (**Eto**) in DNA topoisomerase-II.

Table 2 Estimated free energy of binding and inhibition constant of the representative molecules **9a**, **9c**, **9f**, **9i** and Etoposide

Entry	Compound	Free Energy of Binding	Inhibition Constant, Ki
1	**9a**	-13.24 kcal/mol	195.61 pM
2	**9c**	-13.18 kcal/mol	219.98 pM
3	**9f**	-13.42 kcal/mol	144.96 pM
4	**9i**	-12.91 kcal/mol	342.40 pM
5	**Etoposide**	-8.54 kcal/mol	551.55 nM

Molecular docking poses for 9i and 9i+etoposide (Eto) in DNA topoisomerase-II: The most potent compound **9i** was shown as yellow colour sticks, the interacted amino acid residues were represented as magenta sticks and the surrounded amino acids are shown as lines. The hydrogen bonding interactions with ASP-479, GLN-778 and ARG-503 were denoted as red dots. The protein DNA topoisomerase-II was shown as a pale green ribbon. The hydrophobic interactions were also shown as black dots. A pose of superimposition of potent compound and etoposide (**9i+Eto**) demonstrated the similar interactions with respect to positive control. Besides, different docking poses of compound 9i were represented in Fig S2, ESI†. The representation of yellow (**9i**) and magenta (**Eto**) sticks were proposed in the image. PyMOL was used to visualize the docking poses.

Experimental

Chemicals and Reagents

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization of TLC was achieved by UV light or iodine indicator. ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) spectra were recorded on Gemini Varian-VXR-unity (200 and 400 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 in Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an electro thermal melting point apparatus, and are uncorrected.

Materials and methods

Cell culture and reagents

The cell lines used in this study were obtained from the American Type Culture Collection (ATCC). DU145 (human prostate carcinoma epithelial) cells have cultured in Eagle's minimal essential medium (MEM) containing nonessential amino acids, 1mM sodium pyruvate, and 10% FBS. HeLa (human epithelial cervical cancer), MCF-7 (human breast cancer) and A549 (human lung carcinoma epithelial) were co-cultured in Dulbecco's modified Eagle's medium (DMEM) containing nonessential amino acids and 10% FBS. All the cells maintained under humidified atmosphere of 5% CO_2 at 37 °C. Cells were trypsinized when sub confluent from T75 flasks/90mm dishes and seeded on to 96-well test plates at a density of 1×10^4 cells/well in complete medium, treated with compounds at a desired concentrations and harvested as required.[15]

In vitro cytotoxicity assay

Cell viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The pale yellow coloured tetrazolium salt (MTT) reduced to a dark blue water-insoluble formazan by metabolically active cells and that is measured quantitatively after dissolving in DMSO (dimethyl sulfoxide). The absorbance of the soluble form of formazan is directly proportional to the number of viable cells. Cells were seeded at a density of 1×10^4 cells in 200 µL of medium per well of 96-well plate. Further, the plates were incubated prior to the addition of the experimental compounds for 24 hours. Later, cells were treated with vehicle alone (0.4% DMSO in medium) or compounds (drugs were dissolved in DMSO previously) at a different concentrations (1, 10 and 25 µM) for 48 hours. The assay was completed with the addition of MTT (5%, 10 µL) and incubated for 60 min at 37 °C. The supernatant was aspirated and plates were air dried and the MTT-formazan crystals were dissolved in 100 µL of DMSO. The optical density (O.D) was measured at 560 nm using TECAN Multimode reader. The growth percentage of each treated well of 96-well plate has been calculated based on the test wells relative to control wells. The cell growth inhibition was calculated by

generating dose response curves as a plot of the percentage of surviving cells versus drug concentration. The antiproliferative activity of the cancer cells to the test compounds was expressed in terms of IC_{50} values, which is defined as a concentration of compound that produced 50% absorbance reduction relative to control.[16]

Hoechst-33258 staining

HeLa cells were incubated for a period of 24 hours in the presence or absence of test compounds **9c, 9i** and etoposide (**Eto**) (3 μM). At the end of treatment, the medium was removed, cells were washed with medium without FBS, and Hoechst-33258 stain (Invitrogen cat. no. H3570) was added to the cells for 20 minutes at 37 °C under humidified atmosphere. The HeLa cells were washed twice with medium. The cells were covered with medium and observed under a fluorescence microscope equipped with DAPI filter.[17]

Molecular modeling

AutoDock was used to dock **9i** derivatives in etoposide (**Eto**) binding site of human DNA topoisomerase-II.[18] Initial Cartesian coordinates for the protein-ligand complex structure were derived from the crystal structure of DNA topoisomerase-II (PDB ID: 3QX3). The protein targets were prepared for molecular docking simulation by removing water molecules, bound ligands. Hydrogen atoms and Kollman charges were added to each protein atom. AutoDock Tools (ADT) was used to prepare and analyze the docking simulations for the AutoDock program. The coordinates of each compound were generated using Chemdraw11 followed by MM2 energy minimization. Grid map in AutoDock that defines the interaction of protein and ligands in binding pocket was defined. The grid map was used with 60 points equally in each x, y, and z direction. AutoGrid 4 was used to produce grid maps for AutoDock calculations where the search space size utilized grid points of 0.375 Å. The Lamarckian genetic algorithm was chosen to search for the best conformers. Each docking experiment was performed 100 times, yielding 100 docked conformations. The parameters used for the docking were as follows: population size of 150; random starting position and conformation; maximal mutation of 2 Å in translation and 50 degrees in rotations; elitism of 1; a mutation rate of 0.02 and crossover rate of 0.8; and local search rate of 0.06. Simulations were performed with a maximum of 1.5 million energy evaluations and a maximum of 50000 generations. Final docked conformations were clustered using a tolerance of 1.0 Å root mean square deviation. The best model was picked based on the best stabilization energy. Final figures for molecular modelling were generated by using PyMol.[19]

Conclusions

In summary, a series of 4β-benzoxazolepodophyllotoxin (**9a-j**) were prepared and screened for their cytotoxicity against four human tumour cell lines (HeLa, DU-145, A-549 and MCF-7) and found to be more potent than etoposide. Some of these 4β-benzoxazolepodophyllotoxins (**9a-j**) have shown promising activity with considerable IC_{50} values. Among these compounds specifically **9a, 9c**, **9f** and **9i** showed more potent anticancer activity than etoposide. Further, the compounds **9c** and **9i** were evaluated for Hoechst-33258 staining and noticed a clear nuclear damage in comparison to etoposide. Finally the most active compound **9i** was also investigated for molecular docking interactions against DNA topoisomerase-II and compared with etoposide. The energy calculations were in good agreement with the observed IC_{50} values.

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Notes and references

1 (a) S. Renouard, T. Lopez, O. Hendrawati, P. Dupre, J. Doussot, A. Falguieres, C. Ferroud, D. Hagege, F. Lamblin, E.Laine and C.Hano, *J. Agric. Food Chem*., 2011, **59**, 8101;(b) A. L. Eyberger, R. Dondapati and J. R. Porter, *J. Nat. Prod.*, 2006, **69**, 1121.

2 (a) T. Imbert, *Biochimie*1998, **80**, 207; (b) G. R. Pettit, M. F. Baumann and K. N. Rangammal, *J. Med. Chem.*, 1962, **5**, 800; (c) M. Gordaliza, M. A. Castro, M. D. García-Grávalos, P. Ruiz, J. M. M. del Corral and A. San Feliciano,*Arch Pharm*., 1994, **327**, 175; (d) M. Gordaliza, P. A. Garcia, J. M. del Corral, M. A. Castro andM. A. Gomez-Zurita, *Toxicon*2004, **44**, 441.

3 (a) K. H. Lee, *J. Nat. Prod*., 2004, **67**, 273; (b) M. J. Schilstra, S. R. Martin and P. M. Bayley, *The Journal of Biological Chemistry,* 1989, **264**,8827;(c)M. A. Jordan, D. Thrower, L. Wilson, *J Cell Sci*., 1992, **102**, 401; (d) S. K. U. Hossain, D. Dixit and E. Sen, *Eur. J. Med. Chem*., 2013, 68, 47; (e) Y. Liu, L. H. Rakotondraibe, P. J. Brodie, J. D. Wiley, M. B. Cassera, J. S. Miller, F. Ratovoson, E. Rakotobe, V. E. Rasamison, and D. G. I. Kingston, *J. Nat. Prod.*, 2015, **78**, 1330; (f) Y. Q. Liu, L. Yang, X.Tian, *Curr. Bioact. Compds*.,2007, **3**, 37.

4 (a) T. Utsugi, J. Shibata, Y. Sugimoto, K. Aoyagi, K. Wierzba, T. Kobunani, T. Terada, T. Oh-hara, T. Tsuruo and Y.Yamada, *Cancer Res*., 1996, **56**, 2809;(b) H. -Y. Lin, Z. -K. Li, H. -W. Han, H. -Y. Qiu, H. -W. Gu, Y. -H. Yang and X. -M. Wang, *RSC Adv.*, 2015, **5**, 47511; (c) H.-Y. Lin, L. -F. Bai, F. Wang, X. Wu, L. –J. Han, S. K. Baloch, Y. -H. Yang and X. -M. Wang, *RSC Adv.*, 2015, **5**, 27775; (d) X. Yang, C. Zhang and L. Wu, *RSC Adv.*, 2015, **5**, 18945; (e) A. H. Banday, V. V. Kulkarni and V. J. Hruby, *Med. Chem. Commun.*, 2015, **6**, 94; (f) X. Zhang, J. Zhang, M. Su, Y. Zhou, Y. Chen, J. Li and W. Lu, *RSC Adv.*, 2014, **4**, 40444; (g) D. Subrahmanyam, B. Renuka, C. Rao, P. Sagar, D. Deevi, J. Babu and K.Vyas, *Bioorg. Med. Chem. Lett.,* 1998, **8**, 1391.

5 (*a*) A.Kamal andN. L. Gayatri, *Tetrahedron Lett.,*1996, **37**, 3359; (*b*) A.Kamal, B.Laxminarayana andN. L. Gayatri, *Tetrahedron Lett*.,1997, **38**, 6871; (*c*) A.Kamal andY.Damayanthi, *Bioorg. Med. Chem. Lett*.,1997, **7**, 657; (*d*) A.Kamal, N. L.Gayatri, N. V. Rao, *Bioorg. Med. Chem. Lett*.,1998, **8**, 3097; (*e*) A.Kamal, N.Laxman andG.Ramesh, *Bioorg. Med. Chem. Lett*.,2000, **10**, 2059; (*f*) A.Kamal, N. Laxman andG.Ramesh, Indian Patent Nos. 191366 and

192429; (*g*) A.Kamal, B. A.Kumar andM.Arifuddin, *Tetrahedron Lett*.,2004, **44**, 8457; (*h*) A.Kamal, B. A.Kumar, M.Arifuddin andG. D. Sunanda, *Bioorg. Med. Chem*.,2003, **11**, 5135; (*i*) A. Kamal, E. Laxman, G. B. R. Khanna, P. S. M. M. Reddy, T. Rehana, M. Arifuddin, K. Neelima, A. K. Kondapi andS. G. Dastidar, *Bioorg. Med. Chem*., 2004, **12**, 4197; (*j*) A.Kamal, B. A.Kumar, P.Suresh, S. K.Agrawal, G.Chashoo, S. K.Singh andA. K. Saxena, *Bioorg. Med. Chem*.,2010, **18**, 8493; (*k*) A.Kamal, B. A. Kumar, P.Suresh, N.Shankaraiah andM. S. Kumar, *Bioorg. Med. Chem. Lett*.,2011, **21**, 350; (*l*) A.Kamal, P.Suresh, A. Mallareddy, B. A.Kumar, P. V.Reddy, P.Raju, J. R.Tamboli, T. B.Shaik, N.Jain andS. V. Kalivendi, *Bioorg. Med. Chem*.,2011, **19**, 2349; (*m*) A. Kamal, T. S. Reddy, S. Polepalli, S. Paidakula, V.Srinivasulu, V. G.Reddy, N.Jain andN.Shankaraiah, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 3356.

6 (a) H. Stähelin and A.von Wartburg, *Cancer Res*.,1991, **51**, 5; (b) H. F. Stähelin and A von Wartburg, *Cancer Research* 1991, 51, 5; (c) H. Stähelin, A. von Wartburg, *Progress in Drug Research* 1989, 33, 169; (d) H. Connor, *J. R. Soc. Med*., 2005, **98**, 517.

7 (a) T. L. Macdonald, E. K. Lehnert, J. T. Loper, K. C. Chow and W. E. Ross, In *DNA Topoisomerasesin Cancer*; Potmesil, M., Kohn, K. W., Eds.; Oxford University: New York, 1991, 199; (b) Y. J. You, *Curr. Pharm. Des*.,2005, **11**, 1695.

8 (a) H.Stahelin, *Eur. J. Cancer*1973, **9**, 215; (b) Z. Zhang, Z. Liu, L. Ma, S. Jiang, Y. Wang, H. Yu, Q. Yin, J. Cui, and Y. Li, *Mol. Pharmaceutics*, 2013, **10**, 2426; (c) A. Mariani, A. Bartoli, M. Atwal, K. C. Lee, C. A. Austin and R. Rodriguez, *J. Med. Chem.*, 2015, **58**, 4851; (d) H.Stahelin, *Eur. J. Cancer*1970, **6***,* 303; (e) B.Issell, *Cancer Chemother. Pharmacol*.,1982, **7**, 73.

9 (a) K. Kobayashi and M.J. Ratain, *Cancer Chemother. Pharmacol*.,1994, **34**, S64; (b) G. Fleischhack, S. Reif, C. Hasan, U. Jaehde, S. Hettmer, and U. Bode, *Br J Cancer*., 2001, 84, 1453; (c) D. Starks, D. Prinz, A. Armstrong, L. Means, S. Waggoner and R. DeBernardo, *Case Reports in Obstetrics and Gynecology*, 2011, 2011, Article ID 837160, (d) L. Bello, G. Carrabba, C. Giussani, V. Lucini, F. Cerutti, F. Scaglione, J. Landré, M. Pluderi, G. Tomei, R. Villani, R. S. Carroll, P. McL Black and A. Bikfalvi, *Cancer Res.,* 2001*,* **61**, 7501; (e) M. C. Chamberlain, D. D. Tsao-Wei and S. Groshen, *CANCER* 2006, **106**, 2021.

10 (a) D. Kumar, M. R. Jacob, M. B. Reynolds and S. M. Kerwin, *Bioorg. Med. Chem.,*2002*,* **10***,* 3997.(b) S. Aiello, G. Wells, E. L. Stone, H. Kadri, R. Bazzi, D. R. Bell, M. F. G. Stevens, C. S. Matthews, T. D. Bradshaw and A. D. Westwell, *J. Med. Chem.*, 2008, **51**, 5135; (c) S. -T. Huang, I-J. Hsei, C. Chen, *Bioorg. Med. Chem.,* 2006, **14**, 6106.

11 E. Oksuzoglu, B. Tekiner-Gulbas, S. Alper, O. Temiz-Arpaci, T. Ertan, I. Yildiz, N. Diril, E. Sener-Aki and I.Yalcin, *J. Enzyme Inhibition Med. Chem.,* 2008, **23**, 37.

12 (a) J.Vinsova, V.Horak, V.Buchta, J.Kaustova, *Molecules***,** 2005,**10**, 783; (b) R. N.Brown, R.Cameron, D. K.Chalmers, S.Hamilton, A. Luttick, G. Y.Krippner, D. B.McConnell, R.Nearn, P. C. Stanislawski, S. P.Tucker andK. G. Watson, *Bioorg. Med. Chem. Lett.*,2005,**15**, 2051.

13 (a) S. Y.Liu, B. D.Hwang, M.Haruna, Y.Imakura, K. H.Lee andY. C. Cheng, *Mol. Pharmacol*.,1989, **36**, 78; (b) C. -T. Zi, G. -T. Li, Y. Li, J. Zhou, Z. -T. Ding, Z. -H. Jiang and J. -M. Hu, *Nat. Prod. Bioprospect*. 2015, **5**, 83; (c) C. Hu, D. Xu, W. Du, S. Qian, L. Wang, J. Lou, Q. He, B. Yang and Y. Hu, *Mol. BioSyst.*, 2010, **6**, 410.

14 (a) E. Pasquier andM. Kavallaris, *IUBMB Life*. 2008, **60**, 165; (b) Z. Lu, C. Zhang and Z. Zhai, PNAS, 2005, 10, 2778; (c) Y.-Q. Liu, J. Tian, K. Qian, X.-B. Zhao, S. L. Morris-Natschke, L. Yang, X. Nan, X. Tian and K. -H. Lee, *Med. Res. Rev*., 2015, **35**, 1.

15 M. A. Reddy, N. Jain, D. Yada, C. Kishore, J. R. Vangala, P. R. Surendra, A. Addlagatta, S. V. Kalivendi andB. Sreedhar, *J. Med. Chem*.,2011, **54**, 6751.

16 A. S. Kumar, M. A. Reddy, N. Jain, C. Kishor, T. R. Murthy, D. Ramesh, B. Supriya, A. Addlagatta, S. V. Kalivendi andB. Sreedhar, *Eur .J .Med. Chem.,*2013, **60**, 305.

17 Y. Zhang, X. Wang, W. Fang, X. Cai, F. Chu, X. Liao andJ. Lu, *Bioinorg. Chem. Appl.,* 2013, 437134.

18 AutoDock, version4.0; http://www.scripps.edu/mb/olson/doc/autodock/.

19 W. L. DeLano, Scientific, San Carlos, CA, USA, http://www.pymol.org., 2002.

