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Synthesis of 1,2,3-triazole-linked pyrrolobenzodiazepine conjugates employing 'click' chemistry: DNA-binding affinity and anticancer activity

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Abstract—1,2,3-Triazole based molecules are useful pharmacophores for several DNA-alkylating and cross-linking agents. A series of *A*/C8, *C*/C2 and *A*/C8-*C*/C2-linked 1,2,3-triazole–PBD conjugates have been synthesized by employing 'click' chemistry. These molecules have exhibited promising DNA-binding affinity and anticancer activity in selected human cancer cell lines.

Triazoles are an important class of heterocycles, which display an ample spectrum of biological activities and are widely employed as pharmaceuticals and agrochemicals. These molecules have been reported to possess significant antibacterial, antifungal and antihelmintic activities.[1] They have been regarded as an interesting unit in terms of biological activity[2] and some of them have also shown significant anticancer activity in many of the human cell lines. In recent years, alkylating agents have been extensively studied with regard to cancer chemotherapy, this has led to the development of many new and more selective alkylating agents like some molecules based on triazole moiety as anticancer drugs.[3] The conventional route to triazoles is the Huisgen dipolar cycloaddition of alkynes with organic azides. This procedure involved the 1,3-dipolar cycloaddition of azides with organic alkynes possessing an electron-withdrawing substituent[4] to provide 1,2,3-triazoles[5].

The naturally occurring pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) usually interact covalently with DNA in a sequence-selective manner and have generated immense interest as potential anticancer and gene-targeting agents.[6] These include anthramycin (**1**), tomaymycin (**2**) and DC-81 (**3**), that are known to monoalkylate by covalent binding to the N_2 of a guanine base in the DNA minor groove through their electrophilic imine functionality with preference for Pu-G-Pu sequence (Fig. 1).[7] The development of new practical synthetic strategies has allowed the exploration of several analogues based on PBD ring system including the joining of two PBD units through various spacers and PBD unit linked to certain known antitumour agents. Further extensive studies have been carried out in both the solution[8] and solid-phase[9] synthesis of PBDs, and a sound understanding of structure–activity relationships within the family has been developed.[10] In continuation of these efforts we have designed and synthesized some conjugates of 1,2,3-triazoles tethered to the PBD moiety employing 'click' chemistry.[11] The DNA binding ability and in vitro anticancer activity for these novel conjugates have also been investigated.

In view of the biological importance of 1,2,3-triazoles and PBDs, it was of considerable interest to develop novel conjugates incorporating both the ring systems, such a combination could provide two types of DNA-alkylating moieties in a single molecule. In this context 1,2,3-triazole has been linked to *A*/C8, *C*/C2 and *A*/C8-*C*/C2-positions of the PBD moiety. Interestingly, it has been observed that solubility of 1,2,3-triazole–PBD

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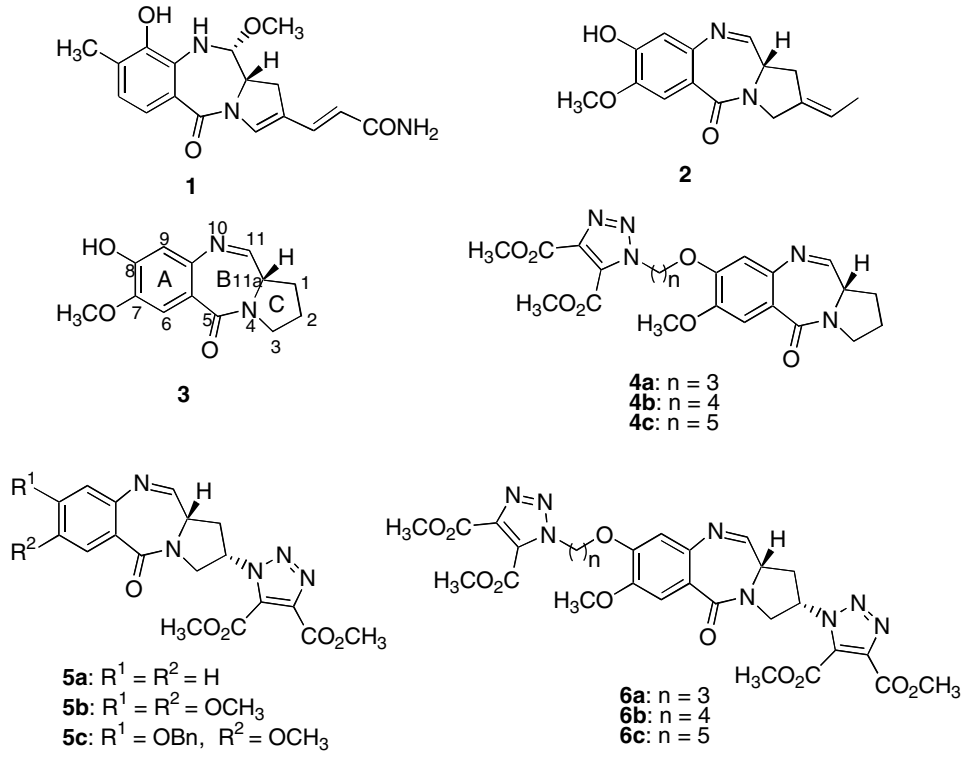
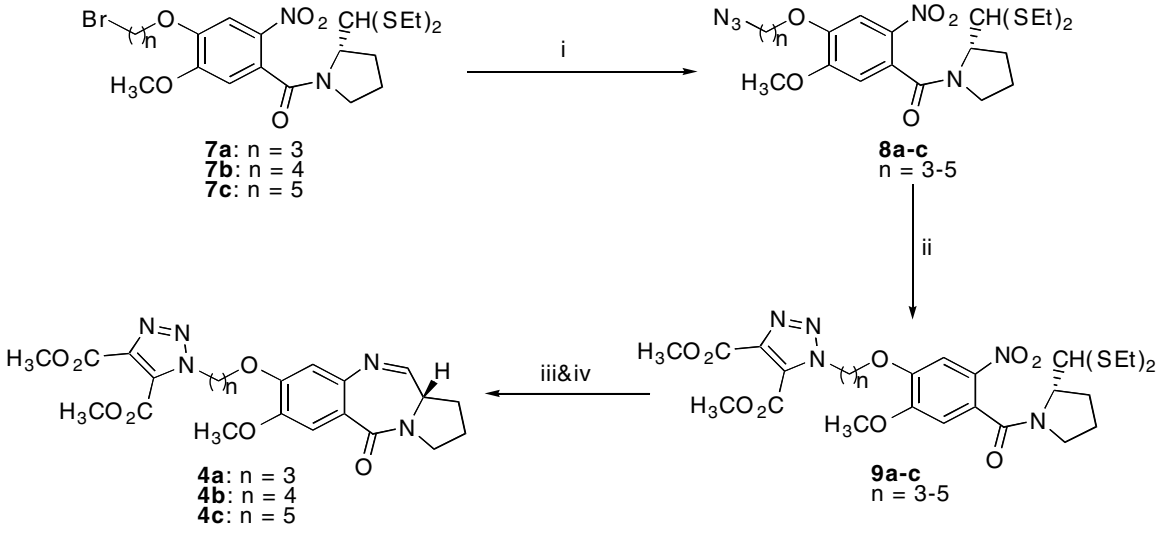


Figure 1. Chemical structures of anthramycin (**1**), tomaymycin (**2**), DC-81 (**3**) and 1,2,3-triazole–PBD conjugates (**4a**–**c**, **5a**–**c** and **6a**–**c**).

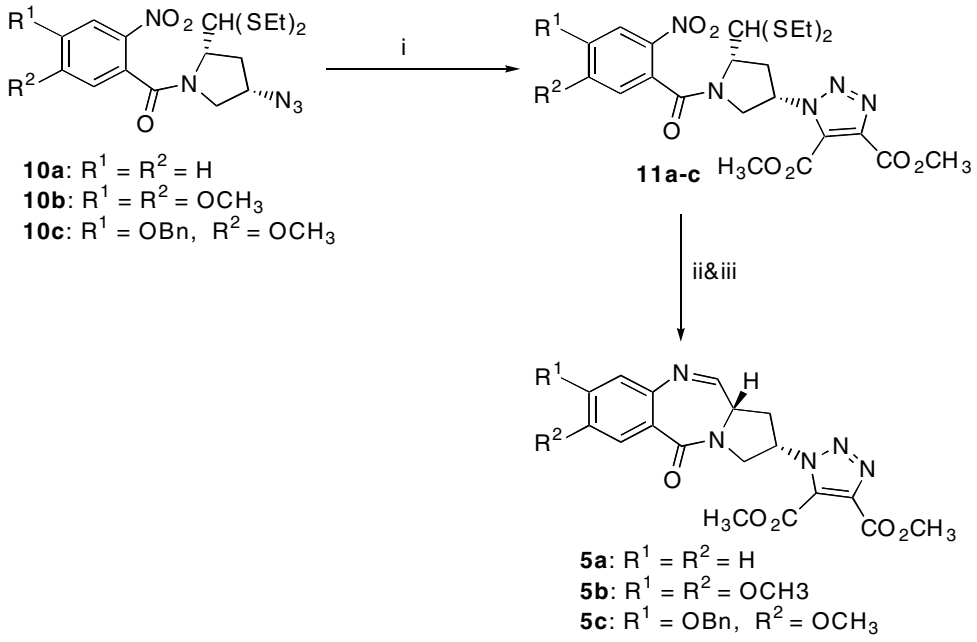
conjugates is enhanced in most of the organic solvents; this may be attributed to the ester functionalities that are present in the 1,2,3-triazole moiety.

Synthesis of (2*S*)-*N*-[4-(bromoalkoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetals (**7a**–**c**) has been accomplished by employing the reported method.[12] These upon azidation with NaN_3 followed by cycloaddition with dimethyl acetylenedicarboxylate produces the corresponding **9a**–**c**[13] through a 'click' reaction. This process provides the biologically diverse molecules in a single step. These triazole linked nitrothioacetal intermediates **9a**–**c** upon reduction with $SnCl_2.2H_2O$ and deprotection followed by cyclization with $HgCl_2/CaCO_3$ affords the desired *A*/C8-linked triazole–PBD conjugates (**4a**–**c**) [14] as shown in Scheme 1.

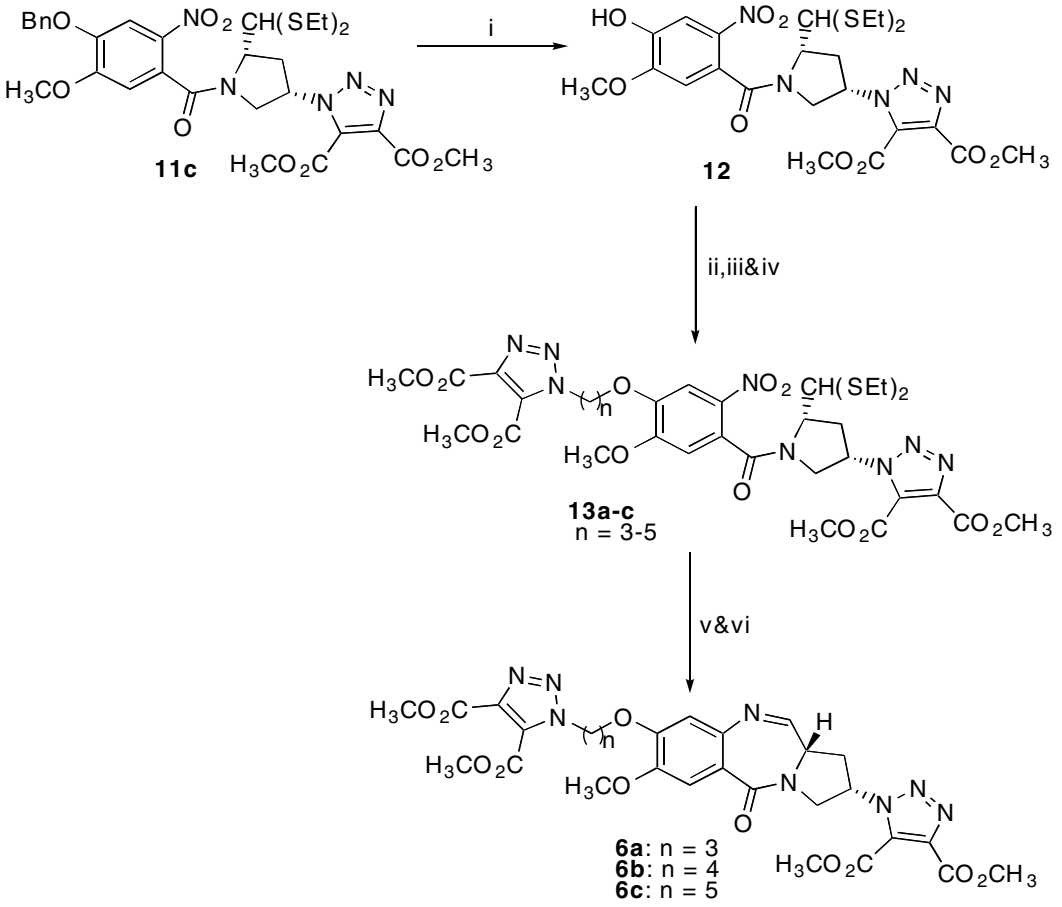
Synthesis of (2*S*,4*S*)-*N*-[2-nitrobenzoyl]-4-azidopyrrolidine-2-carboxaldehyde diethylthioacetals (**10a**–**c**) has been carried out by the reported method.[15] These com-



Scheme 1. Reagents and conditions: (i) NaN_3 (0.5 M) in DMSO, 80 °C, 6 h, 85–88%; (ii) dimethyl acetylenedicarboxylate, dry benzene, reflux, 6 h, 87–92%; (iii) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 5 h, 75–83%; (iv) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O (4:1), rt, 12 h, 58–62%.



Scheme 2. Reagents and conditions: (i) dimethylacetylene dicarboxylate, dry benzene, reflux, 6 h, 88–90%; (ii) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 5 h, 80–85%; (iii) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O (4:1), rt, 12 h, 58–61%.



Scheme 3. Reagents and conditions: (i) $BF_3{\cdot}OEt_2$, EtSH, CH_2Cl_2, rt, 12 h, 75%; (ii) dibromoalkane spacers, K_2CO_3, dry DMF, 12 h, rt, 78–80%; (iii) NaN_3 (0.5 M) in DMSO, 80 °C, 6 h, 89–92%; (iv) dimethyl acetylene dicarboxylate, dry benzene, reflux, 6 h, 88–90%; (v) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 5 h, 78–85%; (vi) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O (4:1), rt, 12 h, 58–62%.

Table 1. Thermal denaturation data for 1,2,3-triazole-linked PBD conjugates (**4a–c**, **5a–c** and **6a–c**) with calf thymus CT-DNA

Compound	[PBD]/[DNA] Molar ratio[a]	ΔTm[b] (°C)	After incubation at 37 °C
		0 h	18 h
4a	1:5	1.5	3.1
4b	1:5	0.0	0.0
4c	1:5	2.2	3.6
5a	1:5	1.0	2.3
5b	1:5	1.1	2.8
5c	1:5	0.9	2.2
6a	1:5	2.6	4.5
6b	1:5	0.0	0.0
6c	1:5	3.1	5.9
DC-81	1:5	0.3	0.7

[a] For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.6 °C ± 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].

pounds **10a–c** upon cycloaddition with dimethyl acetylenedicarboxylate afford the corresponding **11a–c**. Further steps are similar to that which have been described in the previous method to afford the desired *C*/C2-linked triazole–PBD conjugates **5a–c**[14] as shown in Scheme 2.

In continuation of our later efforts to explore the diversity in both *A*/C8 and *C*/C2-positions of the PBD ring system, synthesis of such 1,2,3-triazole–PBD conjugates (**6a–c**) has been carried out accordingly. Compound **11c** has been prepared by employing the similar procedure as described in the previous protocol of Scheme 2. Compound **11c** upon debenzylation with $BF_3 \cdot OEt_2$/EtSH affords the corresponding debenzylated product **12**. This upon etherification with different dibromoalkanes, azidation followed by cycloaddition with dimethyl acetylenedicarboxylate, provides the precursors **13a–c**. Further steps are also similar to that which have been described in the above procedure to yield the desired *A*/C8 and *C*/C2-linked 1,2,3-triazole–PBD conjugates **6a–c** as illustrated in Scheme 3.

The DNA-binding ability for these 1,2,3-triazole linked PBD conjugates has been determined by thermal denaturation studies using calf thymus (CT)-DNA. These studies have been carried out at PBD/DNA molar ratio of 1:5. Interestingly, most of the compounds (**4a**, **4c**; **5a–c** and **6a**, **6c**) elevate the helix melting temperature of CT-DNA in the range of 2.2–5.9 °C after 18 h incubation at 37 °C. It is observed that in compounds **4a–c** to **6a–c**, when the linker spacer comprises of odd number of carbons the DNA-binding ability is good. However, the DNA-binding ability is completely absent in the compounds when there are even number of carbons in the linker spacer (**4b** and **6b**). Interestingly, further in compounds **6a** and **6c** the DNA-binding affinity is significant, when the triazole moiety is present at both C8 as well as C2-positions. In case of compounds **5a–c** there is not much difference in their ΔT_m values, wherein the triazole moiety is linked at C2-position of the PBD ring without an alkane spacer. Compound **6c** has shown the highest ΔT_m value of 5.9 °C upon 18 h of incubation at 37 °C. Whereas, the naturally occurring DC-81 (**3**) exhibits a ΔT_m of 0.7 °C after 18 h of incubation under similar conditions (Table 1).

Compounds **4a–c**, **5b**, **5c** and **6a–c** have been evaluated for their in vitro anticancer activity in selected human cancer cell lines of breast, oral, ovary, colon, lung, prostate and cervix by using sulforhodamine B (SRB) method.[16,17] The compounds exhibiting $GI_{50} \leqslant 10^{-5}$ M (10 μM) are considered to be active on the respective cell lines. Table 2 reveals that compound **6c** exhibits broad-spectrum activity in the cell lines used. However, compound **6b** is found to be inactive and **5a** has not been evaluated. The activity of the compounds ranged from GI_{50} 0.12 to 30.50 μM. The positive control compound adriamycin demonstrated highly significant activity with the GI_{50} in the range from 0.10 to 7.25 μM and for DC-81 the GI_{50} ranged from 0.10 to 2.37 μM. The efficacy of

Table 2. GI_{50} values (μM) for compounds **4a–c**, **5b**, **5c** and **6a–c** in selected human cancer cell lines

Compound	Zr-75-1[a]	MCF7[a]	KB[b]	Gurav[b]	DWD[b]	A2780[c]	Colo205[d]	A549[e]	PC3[f]	SiHa[g]
4a	2.53	—[h]	26.60	25.10	25.50	2.48	2.09	24.70	28.70	2.12
4b	2.12	30.50	2.36	2.39	2.24	0.13	0.21	28.71	25.40	2.04
4c	1.93	—[h]	7.19	26.83	26.01	26.01	2.17	6.12	29.14	2.15
5b	2.47	2.17	26.40	2.39	2.26	0.914	1.09	—[h]	2.23	0.21
5c	—[h]	27.55	—[h]	—[h]	29.71	25.81	27.90	—[h]	—[h]	2.04
6a	—[h]	2.12	1.05	2.42	—[h]	0.19	2.01	7.12	—[h]	2.17
6b	—[h]	—[h]	—[h]	—[h]	—[h]	—[h]	—[h]	—[h]	—[h]	—[h]
6c	5.51	2.05	1.92	1.82	0.15	0.16	1.92	2.41	0.17	0.12
ADR	1.79	0.18	0.17	0.17	0.10	0.17	0.15	7.25	1.81	0.18
DC-81	2.37	0.17	0.17	0.16	1.49	0.14	0.11	0.16	0.20	0.17

[a] Breast cancer.
[b] Oral cancer.
[c] Ovary cancer.
[d] Colon cancer.
[e] Lung cancer.
[f] Prostate cancer.
[g] Cervix cancer.
[h] GI_{50} value not attained at the concentrations used in the assay; ADR (adriamycin); DC-81.

the compounds is as shown here in the descending order **6c > 4b > 6a > 5b > 4c** and **4a > 5c**. In terms of sensitivity for the cell lines it is observed that SiHa cell line is the most sensitive followed by A2780 > DWD > MCF7 > Colo205 > PC3 > A549 > Gurav and KB > Zr-75-1 (GI_{50} 0.12–30.50 μM). Overall, the in vitro anticancer activity exhibited by these new 1,2,3-triazole-linked PBD conjugates is promising.

In conclusion, some new 1,2,3-triazole-linked pyrrolobenzodiazepine conjugates have been synthesized by utilizing the 'click' process (1,3-dipolar cycloaddition). Some of the synthesized compounds have shown noticeable DNA-binding affinity and potential anticancer activity in selected human cancer cell lines with increased solubility. Further, detailed mechanistic and molecular modelling studies for these compounds are in progress.

Acknowledgments

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References and notes

1. (a) Hardman, J.; Limbird, L.; Gilman, A. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 9th ed.; McGraw-Hill: New York, 1996; p. 988; (b) Gennaro, A. R.; Remington. *The Science and Practice of Pharmacy*, Mack Easton, PA, 1995; Vol. II, pp 1327; (c) Richardson, K.; Whittle, P. J. *Eur. Pat. Appl. EP* **1984**, *115*, 416; Richardson, K.; Whittle, P. J. *Chem. Abstr.* **1984**, *101*, 230544; (d) Ammermann, E.; Loecher, F.; Lorenz, G.; Janseen, B.; Karbach, S.; Meyer, N. *Brighton Crop Prot. Conf. Pests. Dis.* **1990**, *2*, 407; Ammermann, E.; Loecher, F.; Lorenz, G.; Janseen, B.; Karbach, S.; Meyer, N. *Chem. Abstr.* **1991**, *114*, 223404h; (e) Heindel, N. D.; Reid, J. R. *J. Heterocycl. Chem.* **1980**, *17*, 1087.
2. (a) Dehne, H. In *Methoden der Organischen Chemie (Houben-Weyl)*; Schumann, E., Ed.; Thieme: Stuttgart, 1994; Vol. E8d, p 305; (b) Wamhoff, H.. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; 5, p 669.
3. De las Heras, F. G.; Alonso, R.; Alonso, G. *J. Med. Chem.* **1979**, *22*, 496.
4. (a) Journet, M.; Cai, D.; Kowal, J. J.; Larsen, R. D. *Tetrahedron Lett.* **2001**, *42*, 9117; (b) Katritzky, A. R.; Zhang, Y.; Singh, S. *Heterocycles* **2003**, *60*, 1225.
5. (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem.* **2002**, *114*, 2708; (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
6. Thurston, D. E. *Br. J. Cancer* **1990**, *80*, 65.
7. Thurston, D. E. In *Molecular Aspects of Anticancer Drug-DNA Interactions*; Neidle, S., Waring, M. J., Eds.; Macmillan: London, 1993; p 54.
8. (a) Kamal, A.; Ramesh, G.; Srinivas, O.; Ramulu, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 471; (b) Kamal, A.; Reddy, K. L.; Reddy, G. S. K.; Reddy, B. S. N. *Tetrahedron Lett.* **2004**, *45*, 3499; (c) Kamal, A.; Ramesh, G.; Laxman, N.; Ramulu, P.; Srinivas, O.; Neelima, K.; Kondapi, A. K.; Srinu, V. B.; Nagarajaram, H. A. *J. Med. Chem.* **2002**, *45*, 4679; (d) Kamal, A.; Devaiah, V.; Reddy, K. L.; Kumar, M. S. *Bioorg. Med. Chem.* **2005**, *13*, 2021; (e) Kamal, A.; Reddy, K. L.; Devaiah, V.; Shankaraiah, N.; Kumar, M. S.; Reddy, G. S. K. *Lett. Drug Design Discov.* **2005**, *1*, 55.
9. (a) Kamal, A.; Reddy, K. L.; Devaiah, V.; Shankaraiah, N.; Reddy, D. R. S. *Mini-Rev. Med. Chem.* **2006**, *6*, 53; (b) Kamal, A.; Reddy, G. S. K.; Raghavan, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 387; (c) Kamal, A.; Reddy, G. S. K.; Reddy, K. L. *Tetrahedron Lett.* **2001**, *42*, 6969; (d) Kamal, A.; Reddy, G. S. K.; Reddy, K. L.; Raghavan, S. *Tetrahedron Lett.* **2002**, *43*, 2103; (e) Kamal, A.; Shankaraiah, N.; Devaiah, V.; Reddy, K. L. *Tetrahedron Lett.* **2006**, *47*, 6553; (f) Kamal, A.; Shankaraiah, N.; Reddy, K. L.; Devaiah, V. *Tetrahedron Lett.* **2006**, *47*, 4253; (g) Kamal, A.; Reddy, K. L.; Devaiah, V.; Shankaraiah, N. *Synlett* **2004**, 1841.
10. (a) Gregson, S. J.; Howard, P. W.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2277; (b) Kamal, A.; Reddy, K. L.; Devaiah, V.; Shankaraiah, N. *Synlett* **2004**, 2533; (c) Kamal, A.; Devaiah, V.; Reddy, K. L.; Shankaraiah, N. *Adv. Synth. Catal.* **2006**, *348*, 249.
11. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem.* **2001**, *113*, 2056; (b) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
12. Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G.; Kumar, P. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4791.
13. Biagi, G.; Giorgi, I.; Livi, O.; Manera, C.; Scartoni, V.; Betti, L.; Giannaccini, G.; Lucacchini, A. *Farmaco II* **1999**, *54*, 615.
14. **Selected data**: **compound 4a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, 1H, J = 4.39 Hz); 7.42 (s, 1H); 6.67 (s, 1H); 4.80–4.89 (m, 2H); 4.08–4.13 (m, 1H); 3.98–4.03 (m, 1H); 3.94 (s, 3H); 3.89 (s, 3H); 3.88 (s, 3H); 3.75–3.82 (m, 1H); 3.64–3.69 (m, 1H); 3.51–3.57 (m, 1H); 2.45–2.51 (m, 2H); 2.28–2.34 (m, 2H); 2.02–2.08 (m, 2H); FABMS: *m/z* 472 [M^++H]; **compound 4b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, 1H, J = 4.91 Hz); 7.43 (s, 1H); 6.70 (s, 1H); 4.61–4.65 (t, 2H, J = 7.37 Hz); 3.97–4.08 (m, 2H); 3.91 (s, 3H); 3.89 (s, 3H); 3.85 (s, 3H); 3.71–3.77 (m, 1H); 3.63–3.67 (m, 1H); 3.46–3.53 (m, 1H); 2.22–2.26 (m, 2H); 2.04–2.11 (m, 2H); 1.95–2.01 (m, 2H); 1.80–1.87 (m, 2H); FABMS: *m/z* 486 [M^++H]; **compound 4c**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, 1H, J = 3.90 Hz); 7.50 (s, 1H); 6.78 (s, 1H); 4.63 (t, 2H, J = 7.01, 7.79 Hz); 4.03–4.12 (m, 2H); 3.99 (s, 3H); 3.97 (s, 3H); 3.93 (s, 3H); 3.78–3.84 (m, 1H); 3.70–3.74 (m, 1H); 3.54–3.60 (m, 1H); 2.30–2.34 (m, 2H); 1.97–2.07 (m, 4H); 1.88–1.93 (m, 2H); 1.50–1.58 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.7; 22.3; 23.8; 27.9; 29.2; 31.2; 46.3; 50.1; 52.3; 53.1; 55.8; 68.1; 76.5; 77.4; 110.2; 111.3; 119.8; 129.4; 139.5; 140.3; 147.4; 150.4; 158.6; 160.2; 162.2; 164.3; FABMS: *m/z* 500 [M^++H]; **compound 5a**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.50–7.57 (m, 4H); 7.61 (d, 1H); 5.80–5.88 (m, 1H); 4.44–4.53 (m, 1H); 4.04 (s, 3H); 4.00 (s, 3H); 3.26–3.31 (m, 1H); 2.99–3.07 (m, 1H); 2.18–2.37 (m, 2H); EIMS: *m/z* 383 [M^+]; **compound 5b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97–7.99 (d, 1H, J = 4.39 Hz); 7.51 (s, 1H); 6.85 (s, 1H); 5.63–5.69 (m, 1H); 4.35–4.41 (m, 1H); 4.13–4.17 (m, 1H); 4.03 (s, 3H); 3.99 (s, 3H); 3.96 (s, 3H); 3.94 (s, 3H); 3.84–3.89 (m, 1H); 3.26–3.32 (m, 1H); 3.03–3.11 (m, 1H); FABMS: *m/z* 444 [M^++H]; **compound 5c**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.43 (d, 1H, J = 7.01 Hz); 7.24–7.34 (m, 6H); 7.10 (s, 1H); 5.12 (s, 2H); 4.88–4.99 (m, 1H); 4.38–4.42 (m, 1H); 3.95 (s, 3H); 3.89 (s, 3H); 3.85 (s, 3H); 3.12–3.22 (m, 1H); 2.93–2.97 (m, 1H); 2.21–2.31 (m, 1H); 2.12–2.19 (m, 1H); FABMS: *m/z* 520 [M^++H]; **compound 6a**: ^{1}H NMR (400 MHz, $CDCl_3$):

δ 7.58 (d, 1H, J = 2.34 Hz); 7.48 (s, 1H); 6.75 (s, 1H); 5.07–5.26 (m, 1H); 4.80–4.89 (m, 2H); 4.30–4.37 (m, 2H); 3.96 (s, 3H); 3.95 (s, 6H); 3.93 (s, 3H); 3.92 (s, 3H); 3.73–3.75 (m, 1H); 2.66–2.92 (m, 2H); 2.44–2.53 (m, 4H); FABMS: *m*/*z* 655 [M^++H]; **compound 6b**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.58 (d, 1H, J = 3.64 Hz); 7.49 (s, 1H); 6.78 (s, 1H); 5.31–38 (m, 1H); 5.11–5.24 (m, 1H); 4.70 (m, 2H); 4.28–4.34 (m, 2H); 3.95 (s, 15H);3.11–3.21 (m, 2H); 2.06–2.19 (m, 2H); 1.79–1.85 (m, 4H); FABMS: *m*/*z* 669 [M^++H]; **compound 6c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, 1H, J = 4.72 Hz); 7.56 (s, 1H); 6.77 (s, 1H); 5.22 (m, 1H); 5.16–5.26 (m, 1H); 4.71 (m, 2H); 4.31–4.37 (m, 2H); 3.96 (s, 15H); 3.15–3.21 (m, 2H); 2.16–2.21 (m, 2H); 2.02–2.12 (m, 4H); 1.76–1.92 (m, 4H); FABMS: *m*/*z* 683 [M^++H].

15. Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3955.
16. Vichai, V.; Kirtikara, K. *Nat. Protoc.* **2006**, *1*, 1112.
17. Skehn, P.; Storeng, R.; Scudiero, A.; Monks, J.; McMohan, D.; Vistica, D.; Jonathan, T. W.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.