Bioorganic & Medicinal Chemistry Letters xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis of new conjugated coumarin–benzimidazole hybrids and their anticancer activity

Kamaldeep Paul *, Shweta Bindal, Vijay Luxami

School of Chemistry and Biochemistry, Thapar University, Patiala 147004, India

ARTICLE INFO

Article history:
Received 9 October 2012
Revised 5 December 2012
Accepted 21 December 2012
Available online xxxx

Keywords:
Coumarin
Benzimidazole
Hybrids
Anticancer agents
Molecular docking

ABSTRACT

A series of novel coumarin–benzimidazole hybrids, 3-(1*H*-benzo[*d*]imidazol-2-yl)-7-(substituted amino)-2*H*-chromen-2-one derivatives of biological interest were synthesized. Six out of the newly synthesized compounds were screened for in vitro antitumor activity against preliminary 60 tumor cell lines panel assay. A significant inhibition for cancer cells was observed with compound **8** (more than 50% inhibition) compared with other compounds and active known drug 5-fluorouracil (in some cell lines) as positive control. Compound **8** displayed appreciable anticancer activities against leukemia, colon cancer and breast cancer cell lines.

Coumarin and its derivatives are important compounds due to their presence in naturally occurring products and their wide-range applications in agrochemicals, drugs and pharmaceuticals[1–5] such as anticancer,[6,7] anti-HIV,[8] antituberculosis,[9] anti-influenza,[10] anti-alzheimer,[11,12] anti-inflammatory,[13] antiviral[14] and antimicrobial agents.[15] Coumarin derivatives have also been shown to be novel lipid lowering agents that possess moderate triglyceride lowering activity.[16] Many coumarin derivatives have unique ability to scavenge reactive oxygen species such as hydroxyl free radicals, superoxide radicals, or hypochlorous acid to prevent free radical injury.[17] Certain coumarin derivatives have been shown to function as HIV integrase inhibitors and evaluated in the treatment of HIV infection,[18] whereas others evaluated as anti-invasive compounds due to their inhibitory activity against some serine proteases and matrix metalloproteases (MMPs).[19] 6-Nitro-7-hydroxy coumarin acts as a selective anti-proliferative agent by activating p38, stress activated protein kinase (SAPK), p21WAF1/CIP1 cyclin dependent kinase inhibitor and human renal cell carcinoma cell line, A-498.[20,21] Coumarin showed the inhibition of polymerization of tubulin and arrest cells in mitotic phase by inhibiting microtubule formation.[22] The benzimidazole moiety also exist in many biologically active natural products, synthetic compounds[23] and are well known for clinical values toward tumor cells,[24–26] and antiviral agents.[27–31] 2-Arylbenzimidazole-5-carboxylic acids were shown to inhibit the HCV NS5B RNA polymerase.[32,33] Coumarin–benzimidazole hybrids showed diverse biological activity with significant clinical potential, such as anti-angiogenesis[34] and antihepatitis.[35]

Despite numerous attempts in search for more effective antitumor agents, coumarin still remains as one of the most versatile class of compound against cancer cell lines and are an important component among the molecules in drug discovery. Most of hybrid molecules have been reported where both coumarin and benzimidazole moieties were attached via spacer[35] as shown in Figure 1(A–C). Still, rare examples are known for coumarin–benzimidazole hybrids as antitumor agents. Here, we reported the synthesis, evaluation and molecular docking of combinations of two biological active coumarin and benzimidazole moiety without any linkage or spacer and the corresponding amines at position 7 of coumarin moiety that exhibit better anticancer activity.

The synthetic strategy to obtain the targets **5**, **7**–**14** and **18** are depicted in Schemes 1 and 2. In order to synthesize compounds of amino substituted coumarin–benzimidazole hybrids, we began with the commercially available starting material 5-bromosalicylaldehyde (**1**) and diethylmalonate (**2**). 5-Bromosalicylaldehyde (**1**) was refluxed with diethylmalonate in the presence of piperidine, acetic acid and ethanol for 3 h gave 7-bromo-2-oxo-2*H*-chromene-3-carboxylic acid ethyl ester (**3**) with 86% yield followed by hydrolysis with NaOH at room temperature to obtain 7-bromo-2-oxo-2*H*-chromene-3-carboxylic acid (**4**) with 63% yield. Compound **4** was refluxed with *o*-phenylenediamine in polyphosphoric acid (PPA) for 12 h gave two types of products **5** and **6** and separated through column chromatography in 62% and 20% yields, respectively. Compound **5** was substituted with different primary amines at 7-position of coumarin ring in ethanol using triethyl-

* Corresponding author. Tel.: +91 9465670595; fax: +91 175 236 4498.
E-mail address: kpaul@thapar.edu (K. Paul).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.12.071

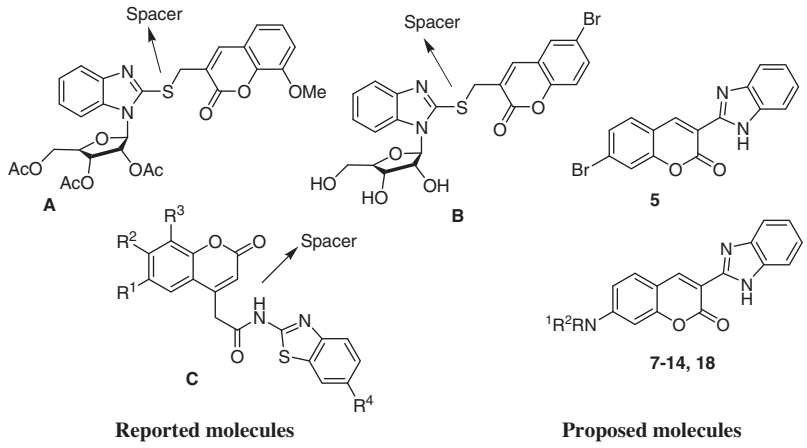
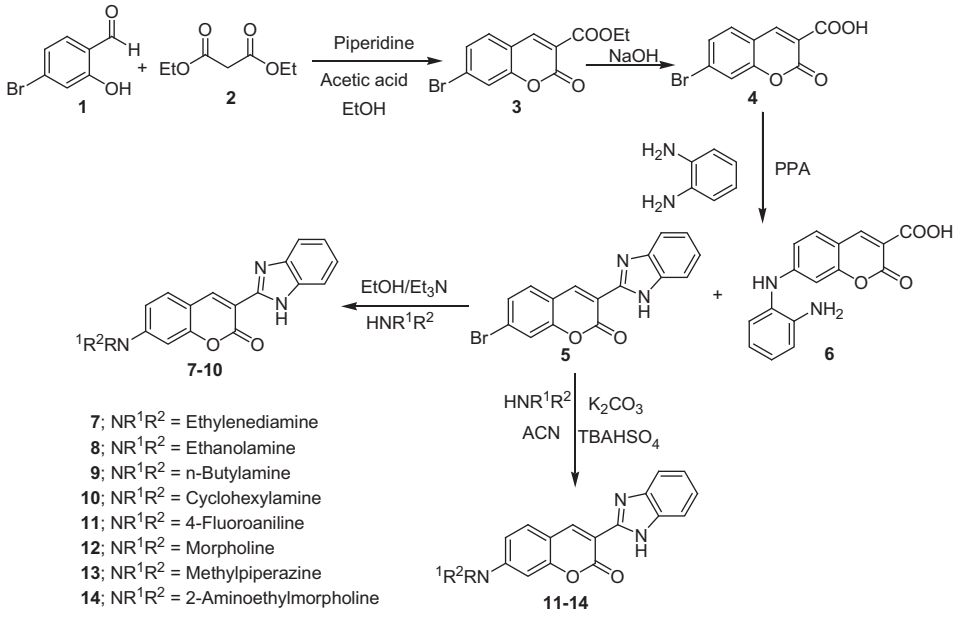
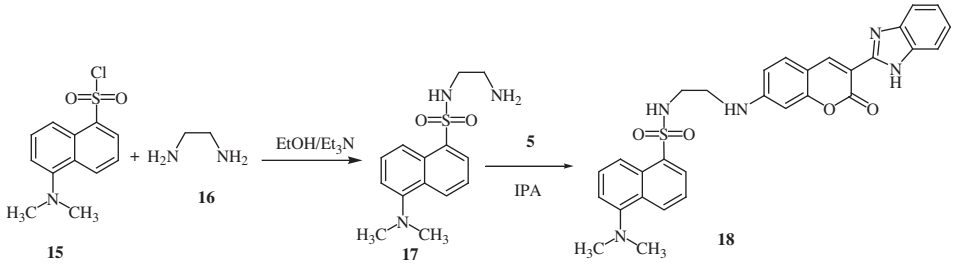


Figure 1. Reported and proposed coumarin–benzimidazole hybrids.



Scheme 1. Synthesis of 3-(1*H*-benzo[*d*]imidazol-2-yl)-2*H*-chromen-2-one analogs (**7**–**14**).



Scheme 2. Synthesis of 5-dimethylamino-naphthalene-1-sulfonic acid {2-[3-(1*H*-benzimidazol-2-yl)-2-oxo-2*H*-chromen-7-ylamino]ethyl}-amide (**18**).

amine as base to obtain compounds **7**–**10**. Compounds **11**–**14** were not synthesized via this methodology and used alternate phase transfer catalyzed approach to synthesize these compounds (Scheme 1).

Compound **5** was refluxed with primary and secondary amines using K_2CO_3 as base and $TBAHSO_4$ as catalyst in acetonitrile for 6–8 h, gave compounds **11**–**14** with moderate to good yields.

Treatment of 5-dimethylamino-naphthalene-1-sulfonyl chloride (**15**) with excess of ethylenediamine (**16**) in ethanol using triethylamine at refluxing conditions for 7 h gave 5-dimethylamino-naphthalene-1-sulfonic acid-(2-amino-ethyl)-amide (**17**) and used further without purification. Compound **17** was refluxed with 3-(1*H*-benzimidazol-2-yl)-7-bromo-chromen-2-one (**5**) in isopropyl alcohol (IPA) for 8 h and after work up gave brown crystals of

5-dimethylamino-naphthalene-1-sulfonic acid {2-[3-(1*H*-benzimidazol-2-yl)-2-oxo-2*H*-chromen-7-ylamino]ethyl}-amide (Scheme 2). All synthesized compounds were well characterized by ^{1}H and ^{13}C NMR (Supplementary data) as well as mass spectroscopy.[36]

Compounds **5**, **7**, **8**, **12**–**14** were selected by National Cancer Institute, Bethesda, Maryland, USA on the basis of degree of the structure variation and computer modeling techniques for evaluation of their anticancer activity. The selected compounds were subjected to in vitro anticancer assay against tumor cells in a full panel of 60-cell lines[37–40] at single dose concentration of 10 μM, and the percentages of growth inhibition over sixty tested cell lines were determined (Table 1).

Preliminary in vitro antitumor screening revealed that compounds **5**, **7**, **12–14** showed moderate inhibition (20.11–56.30%) for the most cancer cell lines, while compound **8** exhibited more than 50% inhibition for most of the cell lines and for some of the tested derivatives its growth inhibition was much better than 5-fluorouracil (positive control). Regarding the activity towards most active compounds **8**, Leukemia cells HL-60 (TB), CCRF-CEM, K-562, MOLT-4 and RPMI-8226 proved to be selective sensitive with GI value of 80.51%, 72.52%, 57.34%, 38.03% and 46.65%, respectively, breast cancer cells T-47D, MDA-MB-231/ATCC, MDA-MB-468, BT-549 with GI values 70.68%, 58.91%, 48.37% and 33.10%, respectively, colon cancer cells HCT-15 and HCT-116 with GI values 72.67% and 62.25%, melanoma cancer cell LOX IMVI and prostate cancer cell PC-3 GI value of 54.29% and 56.69%, respectively. Only K-562 selective to **13** with GI values of more than 50% inhibition. Other compounds also show some sensitivity towards various cell lines. Thus, Table 1 revealed that compound **8** is more active towards numerous cancer cell lines belonging to different tumor subpanels.

We calculated the compliance of compounds to the Lipinski's rule of five.[41] Briefly, this simple rule is based upon the observation

Table 1
Percentages growth inhibition of compounds **5**, **7**, **8**, **12–14** over the full panel of 60 tumor cell lines at 10 μM

Cell line type	Cell line name	**5**	**7**	**8**	**12**	**13**	**14**	5-Fluorouracil
Leukemia	CCRF-CEM	–	–	72.52	–	26.47	–	57.1
	HL-60(TB)	–	–	80.51	–	27.15	–	47.9
	K-562	–	–	57.34	–	56.39	–	42.3
	MOLT-4	–	–	38.03	–	–	–	43.1
	RPMI-8226	–		46.65	–	35.44	–	41.4
	SR	21.50	–	–	–	–	nt	24.8
Non-small cell lung cancer	A549/ATCC	–	–	–	–	–	–	34.2
	HOP-62	–	–	24.75	–	–	–	47.8
	HOP-92	20.11	–	–	20.70	41.12	23.26	50.6
	NCI-H226	–	–	29.98	–	–	–	69.5
	NCI-H23	–	–	32.85	–	25.50	–	39.0
	NCI-H322M	–	-	29.69	–	–	–	59.5
	NCI-H522	20.59	–	23.66	–	–	–	58.0
Colon Cancer	HCC-2998	–	–	–	–	–	–	L
	HCT-116	–	–	62.25	–	–	–	17.8
	HCT-15	–	20.58	72.67	–	–	–	26.5
	KM12	–	–	–	–	–	–	40.7
	SW-620	–	–	21.38	–	–	–	–
CNS cancer	SF-268	–	–	26.15	–	–	–	59.0
	SF-295	–	–	24.51	–	–	–	69.0
	SF-539	–	–	25.85	–	–	–	L
	SNB-75	22.87	–	–	–	–	22.91	65.9
	U251	–	–	20.74	–	–	–	50.3
Melanoma	LOX IMVI	–	–	54.29	–	–	–	30.4
	MALME-3M	–	–	29.78	–	–	–	58.2
	M14	–	–	25.43	–	–	–	nt
	SK-MEL-2	–	–	–	–	–	–	95.5
	SK-MEL-28	–	–	–	–	–	–	19.5
	SK-MEL-5	–	–	35.75	–	–	–	nt
	UACC-257	–	–	27.96	–	25.30	–	33.7
	UACC-62	–	–	27.16	–		–	39.7
Ovarian cancer	IGROV1	–	–	24.51	–	20.11	–	51.2
	OVCAR-3	–	–	32.83	–	–	–	47.4
	OVCAR-4	–	–	44.75	–	29.75	–	59.4
	OVCAR-5	–	–	–	–	–	–	44.3
	OVCAR-8	–	–	31.45	–	–	–	nt
	NCI/ADR-RES	–	–	23.70	–	–	–	47.6
	SK-OV-3	–	–	–	–	–	–	77.5
Renal cancer	A498	–	–	–	–	–	–	L
	ACHN	–	–	–	–	–	–	39.3
	CAKI-1	–	–	22.41	–	–	–	39.4
	SN 12C	–	–	22.41	–	–	–	54.0
	UO-31	22.67	–	41.85	24.87	34.60	34.31	41.3
Prostate cancer	PC-3	–	20.28	56.69	–	–	–	58.2
	DU-145	23.65	–	–	–	–	–	35.5
Breast cancer	MCF7	29.96	–	62.12	62.12	26.33	–	11.5
	MDA-MB-231/ATCC	26.00	33.98	58.91	–	–	–	78.1
	HS 578T	25.08	–	–	–	–	–	L
	BT-549	–	–	33.10	–	–	–	37.8
	T-47D	28.42	–	70.68	–	23.12	–	56.7
	MDA-MB-468	–	–	48.37	–	31.72	–	nt

–GI% less than 20%, nt: not tested, L: lethal.

that most biological active drugs have a molecular weight (MW) of 500 or less, a log*P* no higher than 5, five or fewer hydrogen bond donor sites and ten or fewer hydrogen bond acceptor sites (N or O atoms). These results display in Table 2, show that all the synthesized compounds comply with this rule.

Introduction of primary and secondary substituted amines to coumarin moiety resulted decrease in log*P* (lipophilic factor) but increased TPSA (total polar surface area) and molar refractometry (steric factors) values determined by SciGress program[42] for each compound. Within the series of these compounds, compound **8** (most active in this series) has higher TPSA and lower log*P* and molar refractometry which showed not lipophilicity but hydrophilicity is the key role for antitumor activity and less steric interaction (MR = 90.7) favors the higher activity. From Table 2 it is clear that lipophilicity and molar refractometry of the molecules are not a crucial factor for the activity and differences in their bioactivity cannot be attributed to these parameters.

Although the cellular target were not defined in the experimental anticancer investigations of these molecules, to look into the possible interactions at the enzymatic level, we carried out the docking studies[43] of compound **8** in the active site of topoisomerase II (Topo II, pdb ID 1BJT, enzymes responsible for DNA replication), ribonucleotide reductase (RNR, pdbID 4R1R) and dihydrofolate reductase (DHFR) containing NADPH and folate (pdb ID 3FRD). Docking of compound **8** in the active site of Topo II showed H-bond interaction between N atom of benzimidazole moiety with L557 (d = 2.75 Å) amino acid residue of active site, carbonyl group and oxygen atom of coumarin moiety with M522 (d = 1.88 Å) and I523 (d = 2.93 Å) amino acid residues respectively, NH group of ethanolamine with V446 (d = 2.93 Å) and L447 (d = 1.73 Å) and OH group of ethanolamine with NH (d = 2.60 Å) and CO (d = 2.05 Å) groups of A453 amino acid residues (Fig. 2A).

Similarly compound **8** was also docked with active site of ribonucleotide reductase (another enzyme involved in the propagation of cancer, synthesis of raw material for replication of DNA). It showed H-bond interaction between NH group and N atom of benzimidazole with L1725 (d = 2.66 Å and d = 2.69 Å) and T1944 (d = 2.99 Å) amino acid residues respectively, carbonyl oxygen and O atom of coumarin with Q1933 (d = 2.71 Å) and N1755 (d = 2.27 Å) amino acid residues respectively and NH group of ethanolamine with I523 (d = 2.44 Å) amino acid residue (Fig. 2B).

Molecular docking study was also carried out for compounds **8** with active site of DHFR. Docking of compound **8** in the active group of benzimidazole moiety (N-atom) with L5 (d = 2.63 Å) amino acid residue, OH group of ethanolamine with H23 amino acid residues of carbonyl group (d = 2.74 Å) and NH group (d = 2.93 Å) (Fig. 2C).

Therefore, docking of compound **8** in the active site of these enzymes indicated the probable mode of action for anticancer activities. Remarkable, such correlations between the experimental data and docking studies are useful for refining the structure of the molecules and improving their efficiencies.

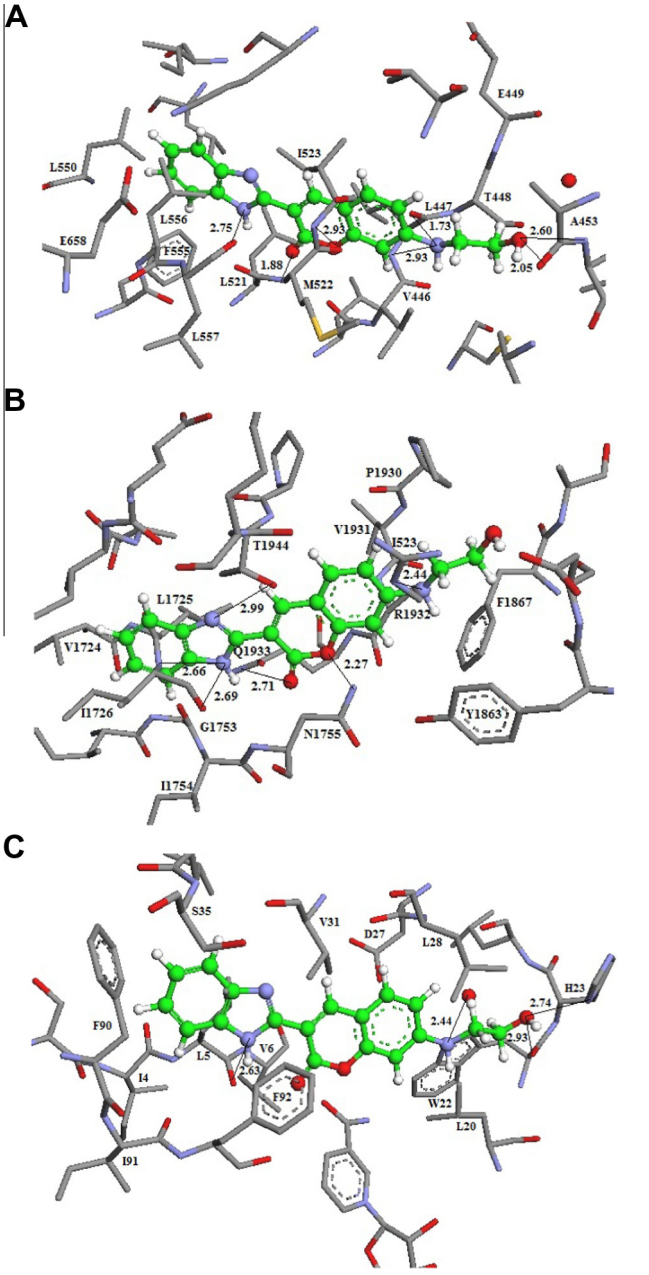


Figure 2. Compound **8** docked in active site of (A) Topo II, (B) RNR and (C) DHFR; H-bonds between compound **8** and different amino acids residues are visible. Carbon atoms are given in different colour.

Table 2
TPSA, molar refractometry and calculated Lipinski's rule of five for compounds under biological investigation

Comp No	Log*P*[a]	MR[b]	TPSA[c]	MW[d]	nON[e]	nOHNH[f]	Nviolations[g]
5	4.3	81.9	58.8	341	4	1	0
7	2.1	92.4	96.9	320	6	4	0
8	2.7	90.7	91.1	321	6	3	0
12	3.5	97.7	71.4	347	6	1	0
13	3.5	104.5	65.4	360	6	1	0
14	3.2	111.5	83.3	390	7	2	0

[a] Calculated lipophilicity.
[b] Molar refractometry (Å^3).
[c] Total polar surface area (hydrophilicity).
[d] Molecular weight.
[e] No of hydrogen bond acceptor.
[f] No of hydrogen bond donors.
[g] No of violations from Lipinski's rule of five.

In conclusion, the present work led to the development of novel antitumor molecules **5**, **7**, **8**, **12**, **13** and **14**. From the preliminary biological activity of coumarin–benzimidazole hybrids, compound **8** exhibits much better growth inhibition for most of the cell lines. We found that the introduction ethanolamine at position-7 of coumarin–benzimidazole hybrid (**8**) shows higher selectivity against leukemia cancer cells (CCRF-CEM, HL-60(TB), K-562, RPMI-8226), colon cancer cells (HCT-116, HCT-15), melanoma cancer cells (LOX IMVI, UACC-257) and breast cancer cells (MCF7, T-47D). Molecular docking also showed H-bonding interaction in the active site of Topo II, RNR and DHFR that also supports its activity. These compounds will be useful as templates for the synthesis of more potent antitumor agents.

Please cite this article in press as: Paul, K.; et al. *Bioorg. Med. Chem. Lett.* (2013), http://dx.doi.org/10.1016/j.bmcl.2012.12.071

Acknowledgements

We thank to Council of Scientific and Industrial Research (02(0034)/11/EMR-II) and Department of Science and Technology, New Delhi (SR/FT/CS-40/2010) for the research grant. We also thank Punjab University, Chandigarh for recording NMR and mass spectra. NCI, USA is gratefully acknowledged for investigating anti-tumor activities.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.071.

References and notes

1. Egan, D. A.; Cox, D.; Kennedy, O. R.; Moran, E.; Prosser, E.; Thornes, R. D. *Drugs Metab. Rev.* **1990**, *22*, 503.
2. Modranka, J. N.; Nawrot, E.; Graczyk, J. *Eur. J. Med. Chem.* **2006**, *41*, 1301.
3. Karali, N.; Kocabalkanli, A.; Gursoy, A.; Ates, O. *Farmaco* **2002**, *57*, 589.
4. Cacic, M.; Trkovnik, M.; Cacic, F.; Schon, E. H. *Molecules* **2006**, *11*, 134.
5. Hoult, J. R. S.; Paya, M. *Gen. Pharmacol.* **1996**, *27*, 713.
6. Devji, T.; Reddy, C.; Woo, C.; Awale, S.; Kadota, S.; Corrio-Moniz, D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5770.
7. Reddy, N. S.; Mallireddigari, M. R.; Cosenza, S.; Gumireddy, K.; Bell, S. C.; Reddy, E. P.; Reddy, M. V. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4093.
8. Xue, H.; Lu, X.; Zheng, P.; Liu, L.; Han, C.; Hu, J.; Liu, Z.; Ma, T.; Li, Y.; Wang, L.; Chen, Z.; Liu, G. *J. Med. Chem.* **2010**, *53*, 1397.
9. Manvar, A.; Bavishi, A.; Radadiya, A.; Patel, J.; Vora, V.; Dodia, N.; Rawal, K.; Shah, A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4728.
10. Yeh, J.-Y.; Coumar, M. S.; Horng, J.-T.; Shiao, H.-Y.; Lee, H.-L. *J. Med. Chem.* **2010**, *53*, 1519.
11. Anand, P.; Singh, B.; Singh, N. *Bioorg. Med. Chem. Lett.* **2012**, *20*, 1175.
12. Piazzi, L.; Cavalli, A.; Colizzi, F.; Belluti, F.; Bartolini, M.; Mancinni, F.; Recanatini, M.; Andrisana, V.; Rampa, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 423.
13. Lin, C.-M.; Huang, S.-T.; Lee, F.-W.; Kuo, H.-S.; Lin, M.-H. *Bioorg. Med. Chem. Lett.* **2006**, *14*, 4402.
14. Curini, M.; Epifano, F.; Maltese, F.; Marcotullio, M. C.; Gonzales, S. P.; Rodriguez, J. C. *Aust. J. Chem.* **2003**, *56*, 59.
15. Shi, Y.; Zhou, C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 956.
16. Madhavan, G. R.; Balraju, V.; Mallesham, B.; Chakrabarti, R.; Lohray, V. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2547.
17. Paya, M.; Halliwell, B.; Hoult, J. R. S. *Biochem. Pharmacol.* **1992**, *44*, 205.
18. Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K. H. *Med. Res. Rev.* **2003**, *23*, 322.
19. Kempen, I.; Papapostolou, D.; Thierry, N.; Pochet, L.; Counerotte, S.; Masereel, B.; Foidart, J. M.; Reboud-Ravaux, M.; Noel, A.; Pirotte, B. *Br. J. Cancer.* **2003**, *88*, 1111.
20. Finn, G.; Creaven, B.; Egan, D. *Eur. J. Pharmacol.* **2003**, *481*, 159.
21. Finn, G. J.; Creaven, B. S.; Egan, D. A. *Cancer Lett.* **2004**, *205*, 69.
22. Morita, H.; Dota, T.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3665.
23. (a) Boiani, M.; González, M. *Mini-Rev. Med. Chem.* **2005**, *5*, 409; (b) El-On, J. *Acta Trop.* **2003**, *85*, 243.
24. (a) Andrzejewska, M.; Yépez-Mulia, L.; Cedillo-Rivera, R.; Tapia, A.; Vilpo, L.; Vilpo, J.; Kazimierczuk, Z. *Eur. J. Med. Chem.* **2002**, *37*, 973; (b) Gumus, F.; Algul, O.; Eren, G.; Eroglu, H.; Diril, N.; Gur, S.; Ozkul, A. *Eur. J. Med. Chem.* **2003**, *38*, 473; (c) Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G.; Kumar, P. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4791; (d) Chauhan, P. M. S.; Martins, C. J. A.; Horwell, D. C. *Bioorg. Med. Chem. Lett.* **2005**, *13*, 3513.
25. (a) Garuti, L.; Roberti, M.; Malagoli, M.; Rossi, T.; Castelli, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2193; (b) Demirayak, S.; Mohsen, U. A.; Karaburun, A. C. *Eur. J. Med. Chem.* **2002**, *37*, 255.
26. (a) Lukevics, E.; Arsenyan, P.; Shestakova, I.; Domracheva, I.; Nesterova, A.; Pudova, O. *Eur. J. Med. Chem.* **2001**, *36*, 507; (b) Handratta, V. D.; Vasaitis, T. S.; Njar, V. C. O.; Gediya, L. K.; Kataria, R.; Chopra, P., Jr.; Farquhar, R.; Guo, Z.; Qiu, Y.; Brodie, A. M. H. *J. Med. Chem.* **2005**, *48*, 2972.
27. (a) Komazin, G.; Ptak, R. G.; Emmer, B. T.; Townsend, L. B.; Drach, J. C. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1725; (b) Bretner, M.; Baier, A.; Kopanska, K.; Najda, A.; Schoof, A.; Reinholz, M.; Lipniacki, A.; Piasek, A.; Kulikowsi, T.; Borowski, P. *Antiviral Chem. Chemother.* **2005**, *16*, 315.
28. Devivar, R. V.; Kawashima, E.; Revankar, G. R.; Breitenbach, J. M.; Kreske, E. D.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1994**, *37*, 2942.
29. Beaulieu, P. L.; Bös, M.; Bousquet, Y.; DeRoy, P.; Fazal, G.; Gauthier, J.; Gillard, J.; Goulet, S.; McKercher, G.; Poupart, M.-A.; Valois, S.; Kukolj, G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 967.
30. Beaulieu, P. L.; Bousquet, Y.; Gauthier, J.; Gillard, J.; Marquis, M.; McKercher, G.; Pellerein, C.; Valois, S.; Kukolj, G. *J. Med. Chem.* **2004**, *47*, 6884.
31. McKercher, G.; Beaulieu, P. L.; Lamarre, D.; LaPlante, S.; Lefebvre, S.; Pellerin, C.; Thauvette, L.; Kukolj, G. *Nucleic Acids Res.* **2004**, *32*, 422.
32. Hirashima, S.; Suzuki, T.; Ishida, T.; Noji, S.; Yata, S.; Ando, I.; Komatsu, M.; Ikeda, S.; Hashimoto, H. *J. Med. Chem.* **2006**, *49*, 4721.
33. Ishida, T.; Suzuki, T.; Hirashima, S.; Mizutani, K.; Yoshida, A.; Ando, I.; Ikeda, S.; Adachi, T.; Hashimoto, H. *Bioorg. Med. Chem. Lett.* **1859**, *2006*, 16.
34. Lee, S.; Sivakumar, K.; Shin, W.-S.; Xie, F.; Wang, Q. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4596.
35. (a) Neyts, J.; Clercq, E. D.; Singha, R.; Chang, Y. H.; Das, A. R.; Chakraborty, S. K.; Hong, S. C.; Tray, S.-C.; Hsu, M.-H.; Hwu, J. R. *J. Med. Chem.* **2009**, *52*, 1486; (b) Hwu, J. R.; Singha, R.; Hong, S. C.; Chang, Y. H.; Das, A. R.; Vliegen, I.; Clercq, E. D.; Neyts, J. *Antiviral Res.* **2008**, *77*, 157.
36. *Experimental*: All chemicals and solvents of commercial grade used without further purification and were supplied by loba, spectrochemicals and Aldrich chemicals. Melting points were determined in capillaries and are uncorrected. ^{1}H and ^{13}C NMR spectra were run on Bruker 400 and 100 MHz NMR spectrometer respectively, using $CDCl_3$ and DMSO-d_6 as solvents. The chemical shifts were expressed in parts per million with TMS as internal reference. *J* Values are given in hertz. Chromatography was performed with silica gel 60–120 mesh and reactions were monitored by thin layer chromatography (TLC) with silica plate coated with silica gel HF-254. Petroleum ether/ethyl acetate and chloroform/methanol were the adopted solvent system.
General procedure for the synthesis of 3-(1*H*-benzo[*d*]imidazol-2-yl)-7-bromo-2*H*-chromen-2-one (**5**). A mixture of bromosalicylaldehyde (**1**) (2 g, 9.94 mmol), diethylmalonate (**2**) (3.18 g, 19.8 mmol), piperidine (0.5 ml) and acetic acid (0.5 ml) in ethanol (70 ml) were heated under reflux for 3 h. Water was added to the reaction mixture at 0 °C to get crystalline solid and washed with 50% cold ethanol. White fine powdered ester of ethyl 7-bromo-2-oxo-2*H*-chromene-3-carboxylate (**3**) (yield = 86%); mp 148–150 °C was obtained. Ester (**3**) (2 g, 6.7 mmol) was refluxed with EtOH and 0.5% NaOH for 2 h and then acidified to pH 2.0 using concentrated hydrochloric acid at 0 °C to give 7-bromo-2-oxo-2*H*-chromene-3-carboxylic acid (**4**) (yield = 63%); mp 178–180 °C. Acid (**4**) (1 g, 3.7 mmol) was then refluxed with 1 equiv of *o*-phenylenediamine (0.4 g, 3.7 mmol) in polyphosphoric acid (2–3 ml) for 12 h. After the completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature, treated with 50 ml water and ammonia until the reaction mixture becomes neutral, filtered and washed with water to obtain two products. The residues were separated by column chromatography on silica gel using chloroform/methanol (80:20) as eluents to get yellow powdered 3-(1*H*-benzo[*d*]imidazol-2-yl)-7-bromo-2*H*-chromen-2-one (**5**) and 7-(2-aminophenylamino)-2-oxo-2*H*-chromene-3-carboxylic acid (**6**).
3-(1*H*-Benzo[*d*]imidazol-2-yl)-7-bromo-2*H*-chromen-2-one (**5**). Yellow powder; yield 62%; mp >250 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.52 (br s, 1H, NH), 9.11 (s, 1H, CH), 8.20 (t, 1H, *J* = 2.32 Hz, ArH), 7.80 (dd, 1H, ^2J = 6.44 Hz, ^3J = 2.36 Hz, ArH), 7.69 (t, 2H, *J* = 9.12 Hz, ArH), 7.45 (d, 1H, *J* = 8.84 Hz, ArH), 7.21 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.69, 152.16, 145.18, 142.81, 140.75, 134.95, 134.79, 131.27, 122.78, 122.11, 120.79, 118.47, 118.17, 117.67, 116.73, 112.75; EIMS, *m*/*z*: 342 (M^++1). 7-(2-Aminophenylamino)-2-oxo-2*H*-chromene-3-carboxylic acid (**6**). Yellow powder; yield 20%; mp >250 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.19 (s, 1H, NH), 9.16 (s, 1H, CH), 8.34 (d, 1H, *J* = 3.72 Hz, ArH), 7.86 (t, 2H, *J* = 9.96 Hz, ArH), 7.16 (t, 1H, *J* = 3.12 Hz, ArH), 6.98 (t, 1H, *J* = 1.44 Hz, ArH), 6.85 (dd, 1H, ^2J = 3.00 Hz, ^3J = 4.12 Hz, ArH), 6.71 (dd, 1H, ^2J = 1.60 ^3J = 7.36 Hz, ArH), 4.73 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.67, 152.05, 146.57, 145.01, 140.50, 134.89, 131.91, 130.99, 122.80, 122.11, 118.41, 117.98, 117.60, 117.21, 116.92, 112.60; EIMS, *m*/*z*: 296 (M^++1)
General procedure for preparation of 7-(substituted amino)-3-(1*H*-benzo[*d*]imidazol-2-yl)-2*H*-chromen-2-one (**7**–**10**). A mixture of 3-(1*H*-benzo[*d*]imidazol-2-yl)-7-bromo-2*H*-chromen-2-one (**5**) (100 mg, 0.029 mmol), amines (0.058 mmol) (excess in case of ethylenediamine), triethylamine (2 drops) in ethanol (10 ml) were heated under reflux for 6–8 h. The reaction mixture was cooled and extracted with chloroform and water. The organic layer was separated, dried over anhydrous sodium sulphate, filtered and concentrated. The obtained residue was column chromatographed on silica gel using chloroform/methanol (80:20) as eluents to afford compounds **7–10**. 3-(1*H*-Benzo[*d*]imidazol-2-yl)-7-(2-ethylenediamino)-2*H*-chromen-2-one (**7**). Brown crystals; yield 77.41%; mp 138–140 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.22 (s, 1H, NH), 8.46 (s, 1H, CH), 7.93 (s, 1H, ArH), 7.49 (s, 2H, ArH), 7.39 (m, 2H, ArH), 7.14 (t, 1H, *J* = 2.24 Hz, ArH), 6.82 (d, 1H, *J* = 9.08 Hz, ArH), 3.76 (s, 2H, NH_2), 3.69 (t, 2H, *J* = 3.28 Hz, CH_2), 3.57 (t, 2H, *J* = 5.72 Hz, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 165.46, 159.75, 151.74, 151.64, 142.01, 133.83, 131.99, 131.34, 131.14, 124.42, 121.96, 119.96, 117.04, 116.68, 111.57, 110.23, 108.20, 60.81, 60.33; EIMS, *m*/*z*: 322 (M^++1). 3-(1*H*-Benzo[*d*]imidazol-2-yl)-7-(2-hydroxyethyl amino)-2H-chromen-2-one (**8**). Yellow liquid; yield 79%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.21 (s, 1H, NH), 9.12 (s, 1H, CH), 8.20 (t, 1H, *J* = 2.96 Hz, ArH), 7.80 (dd, 1H, ^2J = 2.76 Hz, ^3J = 9.20 Hz, ArH), 7.67 (t, 2H, *J* = 9.12 Hz, ArH), 7.45 (d, 1H, *J* = 12.84 Hz, ArH), 7.23 (d, 2H, *J* = 3.08 Hz, ArH), 3.74 (t, 2H, *J* = 6.44 Hz, CH_2), 3.68 (t, 2H, *J* = 3.96 Hz, CH_2), 2.76 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.81, 160.27, 158.12, 157.38, 147.83, 140.67, 134.13, 133.03, 131.22, 122.91, 121.12, 119.80, 118.61, 108.69, 48.73, 46.48; EIMS, *m*/*z*: 321 (M^++1). 3-(1*H*-Benzo[*d*]imidazol-2-yl)-7-(butylamino)-2*H*-chromen-2-one (**9**). Yellow liquid; yield 50%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.22 (s, 1H, NH), 9.11 (s, 1H, CH), 8.19 (t, 1H, *J* = 2.32 Hz, ArH), 7.78 (dd, 1H, ^2J = 2.36 Hz, ^3J = 9.20 Hz, ArH), 7.67 (t, 2H, *J* = 5.28 Hz, ArH), 7.43 (d, 1H, *J* = 12.84 Hz, ArH), 7.21 (m, 2H, ArH), 3.30 (q, 2H, *J* = 5.96 Hz, CH_2), 1.58 (m, 2H, CH_2), 1.41 (m, 2H, CH_2), 0.92 (t, 3H, *J* = 7.28 Hz,

CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.93, 157.79, 152.98, 152.34, 142.76, 133.70, 132.57, 131.98, 131.66, 126.03, 120.81, 120.74, 117.52, 117.33, 112.13, 110.38, 108.85, 46.10, 26.02, 26.07, 13.79; EIMS, *m/z*: 334 (M^++1). 3-(1*H*-Benzo[*d*]imidazol-2-yl)-7-(cyclohexylamino)-2*H*-chromen-2-one (**10**). Brown liquid; yield 52%; ^1H NMR (DMSO-d_6, 400 MHz): δ 12.20 (br s, 1H, NH), 9.00 (s, 1H, CH), 8.14 (t, 2H, *J* = 9.96 Hz, CH), 7.46 (s, 2H, ArH), 7.10 m, 3H, ArH), 3.67 (m, 1H, CH), 1.78 (t, 2H, *J* = 5.08 Hz, CH_2), 1.67 (m, 2H, CH_2), 1.56 (m, 2H, CH_2), 1.31 (m, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 157.33, 156.97, 151.74, 151.64, 142.01, 133.83, 131.99, 131.34, 131.14, 124.42, 121.96, 119.96, 117.04, 116.68, 111.57, 110.23, 46.31, 45.33, 25.08, 13.28; EIMS, m/z: 360 (M^++1). General procedure for preparation of 3-(1*H*-benzo[*d*]imidazol-2-yl)-7-(substituted amino)-2*H*-chromen-2-one (**11**–**14**). A mixture of 3-(1*H*-benzo[*d*]imidazol-2-yl)-7-bromo-2*H*-chromen-2-one (**5**) (100 mg, 0.029 mmol), amines (0.058 mmol), potassium carbonate (0.035 mmol) and catalytic amount of tetrabutylammonium hydrogen sulphate ($TBAHSO_4$) in acetonitrile (10 ml) were heated under reflux for 6–8 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled and extracted with chloroform and water. The organic layer was separated, dried over anhydrous sodium sulphate, filtered and concentrated. The obtained residue was column chromatographed on silica gel using chloroform:methanol as eluents to afford pure compounds **11**–**14**. (1*H*-benzo[*d*]imidazol-2-yl)-7-(4-fluorophenylamino)-2*H*-chromen-2-one (**11**). Brown liquid; yield 74%; ^1H NMR ($CDCl_3$, 400 MHz): δ 9.06 (s, 1H, CH), 8.53 (d, 1H, *J* = 7.8 Hz, ArH), 8.06 (s, 1H, ArH), 7.96 (d, 1H, *J* = 11.08 Hz, ArH), 7.93 (t, 1H, *J* = 3.00 Hz, ArH), 7.79 (d, 1H, *J* = 5.44 Hz, ArH), 7.56 (m, 3H, ArH), 7.36 (d, 1H, *J* = 4.64 Hz, ArH), 7.16 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.04, 155.82, 150.63, 150.44, 150.03, 140.61, 137.08, 134.28, 133.81, 129.73, 129.37, 127.74, 126.73, 126.50, 126.21, 123.66, 121.89, 121.21, 119.01, 118.20, 114.23, 113.72, 111.59; EIMS, *m/z*: 372 (M^++1). 3-(1*H*-Benzo[*d*]imidazol-2-yl)-7-morpholino-2*H*-chromen-2-one (**12**). Brown liquid; yield 74%; ^1H NMR ($CDCl_3$, 400 MHz): δ 11.78 (br s, 1H, NH), 9.01 (s, 1H, CH), 7.93 (dd, 1H, ^2J = 1.88 Hz, ^3J = 7.76 Hz, ArH), 7.67 (m, 3H, ArH), 7.46 (m, 1H, ArH), 7.40 (m, 1H, ArH), 7.21 (dd, 1H, ^2J = 3.16 Hz, ^3J = 6.08 Hz, ArH), 3.81 (t, 4H, *J* = 4.88 Hz, CH_2), 3.70 (t, 4H, *J* = 5.6 Hz, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.84, 148.49, 144.66, 136.78, 130.70, 130.10, 129.11, 128.87, 128.40, 127.80, 126.23, 123.47, 122.38, 120.88, 119.00, 116.38, 115.77, 68.78, 63.04; EIMS, *m/z*: 348 (M^++1).

3-(1*H*-Benzo[*d*]imidazol-2-yl)-7-(4-methylpiperazin-1-yl)-2*H*-chromen-2-one (**13**). Brown liquid; yield 70%; ^1H NMR (DMSO-d_6, 400 MHz): δ 12.49 (br s, 1H, NH), 9.12 (s, 1H, CH), 7.94 (d, 1H, *J* = 6.28 Hz, ArH), 7.69 (m, 3H, ArH), 7.48 (m, 2H, ArH), 7.19 (m, 1H, ArH), 3.88 (s, 4H, CH_2), 2.60 (t, 4H, *J* = 4.28 Hz, CH_2), 2.39 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.76, 162,88, 152.17, 148.19, 131.97, 131.30, 130.22, 129.44, 128.47, 122.84, 122.13, 121.27, 115.80, 115.50, 110.48, 108.14, 52.81, 44.38, 25.59; EIMS, *m/z*: 361 (M^++1).

3-(1*H*-Benzo[*d*]imidazol-2-yl)-7-(2-morpholinoethyl amino)-2*H*-chromen-2-one (**14**). Brown liquid; yield 65%; ^1H NMR ($CDCl_3$, 400 MHz): δ 10.93 (s, 1H, NH), 8.94 (s, 1H, CH), 8.14 (d, 1H, *J* = 1.62 Hz, ArH), 7.46 (d, 1H, *J* = 8.64 Hz, ArH), 7.31 (m, 5H, ArH), 3.73 (m, 4H, CH_2), 3.61 (s, 4H, CH_2), 2.45 (t, 2H, *J* = 4.52 Hz, CH_2), 2.27 (t, 2H, *J* = 4.36 Hz, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.12, 151.87, 141.99, 133.33, 132.30, 132.20, 131.68, 122.91, 120.87, 117.85, 116.51, 112.42, 110.78, 108.55, 54.05, 45.37, 45.02, 42.93; EIMS, *m/z*: 391 (M^++1).

5-Dimethylamino-naphthalene-1-sulfonic acid {2-[3-(1*H*-benzimidazol-2-yl)-2-oxo-2*H*-chromen-7-ylamino]ethyl}-amide (**18**). 5-Dimethylamino-naphthalene-1-sulfonyl chloride (**15**) (100 mg, 0.37 mmol) in EtOH (10 ml) was refluxed with ethylenediamine (0.23 g, 3.7 mmol) using triethylamine (2 drops) as base for 7 h. After completion of reaction (monitored by TLC), the reaction mixture was cooled, extracted with chloroform, dried over anhydrous sodium sulphate, filtered and concentrated to get orange crystals of 5-dimethylamino-naphthalene-1-sulfonic acid-(2-amino-ethyl)-amide (**17**). Compound **17** (80 mg, 0.27 mmol) was refluxed with 3-(1*H*-benzo[*d*]imidazol-2-yl)-7-bromo-2*H*-chromen-2-one (**5**) (90 mg, 0.27 mmol) in IPA for 8 h. The obtained residue was column chromatographed on silica gel using chloroform/methanol as eluents to afford compound **18**. Dark brown crystals; yield 60%; mp 170–175 °C; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.89 (s, 1H, CH), 8.53 (d, 2H, *J* = 8.40 Hz, ArH), 8.41 (d, 2H, J = 7.40 Hz, ArH), 8.17 (s, 1H, ArH), 8.04 (d, 1H, *J* = 7.72 Hz, ArH), 7.98 (d, 1H, *J* = 8.08 Hz, ArH), 7.86 (d, 1H, *J* = 10.36 Hz, ArH), 7.76 (d, 2H, *J* = 8.40 Hz, ArH), 7.63 (t, 1H, *J* = 7.72 Hz, ArH), 7.50 (t, 2H, *J* = 7.88 Hz, ArH), 4.06 (s, 2H, NH), 3.97 (t, 2H, *J* = 13.64 Hz, CH_2), 2.92 (t, 2H, *J* = 9.28 Hz, CH_2), 2.83 (s, 6H, N–CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.08, 162.62, 162.18, 161.75, 157.35, 155.92, 152.17, 146.86, 142.55, 142.29, 141.42, 139.40, 134.32, 131.96, 131.77, 118.18, 117.27, 117.04, 116.83, 116.70, 114.33, 109.81, 106.53, 52.81, 45.68, 25.59; EIMS, *m/z*: 554 (M^++1).

37. Grever, M. R.; Sehepartz, S. A.; Chabners, B. A. *Semin. Oncol.* **1992**, *19*, 622.
38. Monks, A.; Schudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
39. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.
40. *Antitumor methodology*: The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2.0 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 μl at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Aliquot of 100 μl of this drug dilution was added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing 5 times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA).
41. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **2001**, *46*, 3.
42. SciGress Ultra: Molecular Modeling System 7.7.0.47 (1981, FujitsuKyushu Systems, Fukuoka-Shi, Japan), http://jp.fujitsu.com/group/kyushu/en.
43. Compounds were constructed and docked with builder toolkit of the software package ArgusLab 4.0.1 (www.arguslab.com).