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Synthesis and biological evaluation of [4-(2-phenylethenesulfonylmethyl)phenyl]-quinazolin-4-yl-amines as orally active anti-cancer agents☆

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Abstract—A new series of [4-(2-phenylethenesulfonylmethyl)phenyl]quinazolin-4-yl-amines was prepared and tested for its in vitro cytotoxic activity against a panel of 12 human cancer cell lines. Compounds **9**, **15**, **24** and **31** showed good in vitro activity and were further tested for their in vivo efficacy in the HT-29 human colon adeno carcinoma xenograft model. Compound **9** exhibited promising activity in this model. Dose–response studies for this compound against HT-29 human colon adeno carcinoma xenografts at 100, 200 and 400 mg/kg doses were performed.
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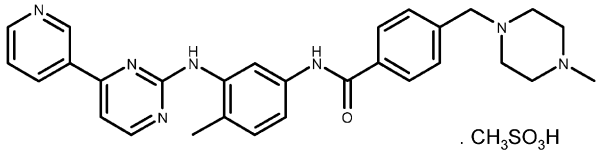
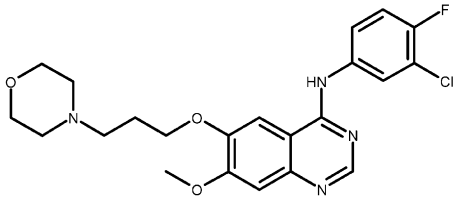
A recent trend in the discovery of anti-cancer agents is to design small molecules which interfere with signal transduction processes by blocking the cell surface receptor, by inhibiting a growth factor receptor tyrosine kinase or by inhibiting downstream signaling proteins such as mitogen-activated protein (MAP) kinase. These signal transduction inhibitors are new type of anti-cancer agents which may have several beneficial effects such as non-toxic, cytostatic and may be administered orally. Two anti-cancer drugs Imatinib (Gleevec)[1] and Gefitinib (Iressa)[2] (Fig. 1) have already received regulatory approval in cancer indications. 4-Anilinoquinazolines, a class of biologically active compounds, are known to exhibit cytotoxic activity through various mechanisms. The inhibitory properties of these compounds against epidermal growth factor receptor tyrosine kinases



Imatinib mesylate



Gefitinib

Figure 1. Structures of Imatinib mesylate (Gleevec) and Gefitinib (Iressa).

Keywords: [4-(2-Phenylethenesulfonylmethyl)phenyl]-quinazolin-4-yl-amines; Orally active anti-cancer agents; HT-29 human colon adeno carcinoma xenograft.
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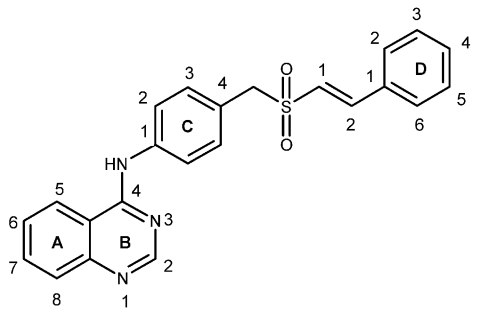
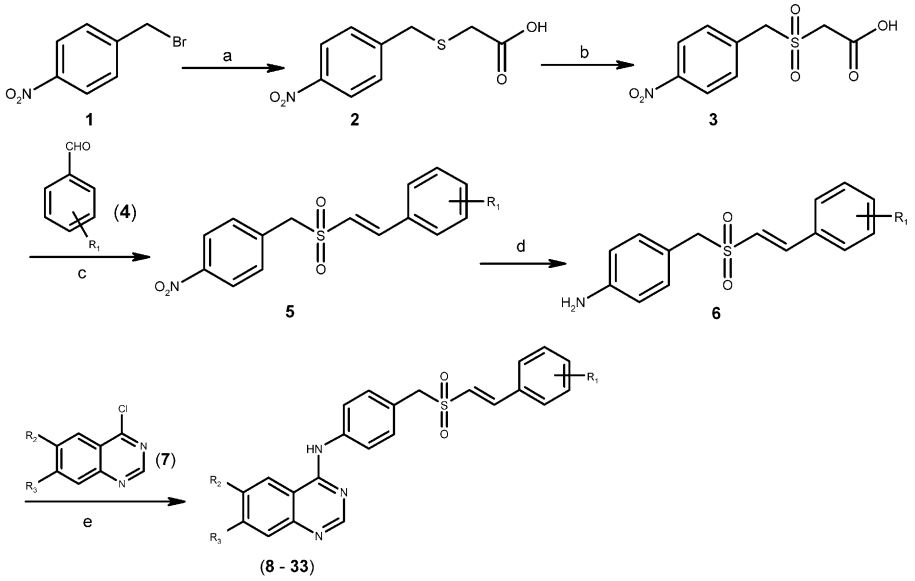


Figure 2. Structure of [4-(2-phenylethenesulfonylmethyl)phenyl]-quinazolin-4-yl-amine.

(EGF-RTK),[3] $p56^{lck}$,[4] and vascular endothelial growth factor receptor tyrosine kinases (VEGF-RTK),[5–7] are well documented.

In continuation of our ongoing cancer research program,[8–10] we were looking for novel quinazoline derivatives with modifications at the C(4)-aniline substituent. We report here the synthesis of a number of [4-(2-phenylethenesulfonylmethyl)phenyl]-quinazolin-4-yl-amines (Fig. 2) and the evaluation of their biological activity of in vitro growth inhibition of cancer cells, and in vivo prolongation of cancer survival in animals.



(**8 - 33**)

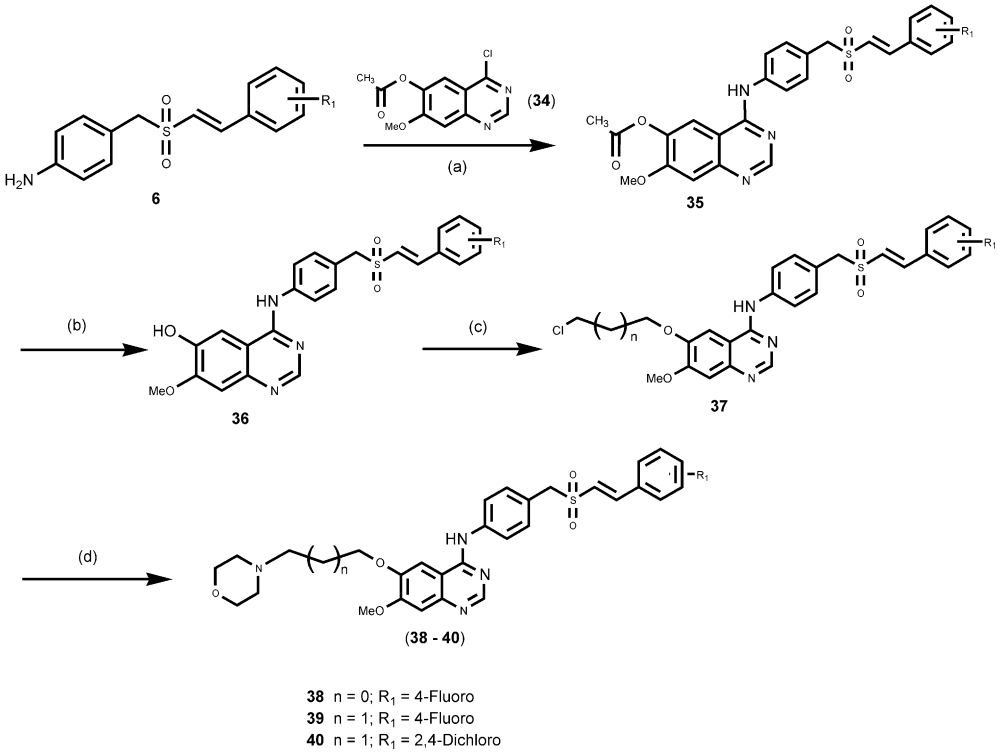
8 $R_1 = R_2 = R_3 = H$
9 R_1 = 4- fluoro; $R_2 = R_3 = H$
10 R_1 = 4- cyano; $R_2 = R_3 = H$
11 R_1 = 3,4- dichloro; $R_2 = R_3 = H$
12 R_1 = 4- bromo; $R_2 = R_3 = H$
13 R_1 = 3,5-di-t-butyl-4-hydroxy; $R_2 = R_3 = H$
14 R_1 = 4- chloro; $R_2 = R_3 = H$
15 R_1 = 2,4- dichloro; $R_2 = R_3 = H$
16 R_1 = 2,4- difluoro; $R_2 = R_3 = H$
17 R_1 = 2-chloro-4-fluoro; $R_2 = R_3 = H$
18 R_1 = 2- fluoro-4-trifluoromethyl; $R_2 = R_3 = H$
19 R_1 = 4- hydroxy; $R_2 = R_3 = H$
20 R_1 = 3,4- dichloro; R_2 = Br; $R_3 = H$
21 R_1 = 4- bromo; R_2 = Br; $R_3 = H$
22 R_1 = 4- chloro; R_2 = Br; $R_3 = H$
23 R_1 = 4- hydroxy; R_2 = Br; $R_3 = H$
24 R_1 = 2,4- dichloro; R_2 = Br; $R_3 = H$
25 R_1 = 2- fluoro-4-trifluoromethyl; R_2 = Br; $R_3 = H$
26 R_1 = 2,4- difluoro; R_2 = Br; $R_3 = H$
27 R_1 = 2,4- dichloro; $R_2 = H$; R_3 = Cl
28 R_1 = 2- fluoro-4-trifluoromethyl; $R_2 = H$; R_3 = Cl
29 R_1 = 2- chloro-4-fluoro; $R_2 = H$; R_3 = Cl
30 R_1 = 2,4- difluoro; $R_2 = H$; R_3 = Cl
31 R_1 = 2,4- dichloro; $R_2 = R_3 = OCH_3$
32 R_1 = 4- fluoro; $R_2 = R_3 = OCH_3$
33 R_1 = 4- bromo; $R_2 = R_3 = OCH_3$

Scheme 1. (a) $HSCH_2COOH$ (2 equiv), KOH (4 equiv), water, DMSO, rt, 0.5 h, 93%; (b) potassium peroxymonosulfate (Oxone™; 2 equiv), *t*-BuOH/water/CH_2Cl_2 (1:1:1), rt, 12 h, 98%; (c) benzylamine (0.2 equiv), glacial acetic acid, reflux, 3–5 h, 85–90%; (d) $SnCl_2{\cdot}2H_2O$ (4 equiv), EtOH, reflux, 2–4 h, 75–85%; (e) 2-propanol, reflux, 3–5 h, 88–92%.

The general procedure for the synthesis of compounds **8**–**33** is outlined in Scheme 1. (4-Nitrobenzylsulfanyl)-acetic acid (**2**) was prepared from 4-nitrobenzylbromide and mercaptoaceticacid. The sulfide of **2** was oxidized to sulfone using potassium peroxymonosulfate (Oxone™) to give (4-nitrophenylmethanesulfonyl)acetic acid (**3**). Compound **3** on Knoevenagel reaction with various substituted benzaldehydes (**4**) in glacial aceticacid in the presence of benzylamine, yielded respective 1-nitro-4-(2-phenylethenesulfonylmethyl)-benzenes (**5**). The nitro groups of compounds **5** were reduced with stannous chloride in refluxing ethanol to give corresponding 4-(2-phenylethenesulfonylmethyl)-phenylamines (**6**) in good yields. The amino compounds (**6**) were finally reacted with different substituted chloroquinazolines (**7**)[11,12] in 2-propanol under reflux conditions to give [4-(2-phenylethenesulfonylmethyl)phenyl] - quinazolin - 4 - yl - amines (**8**–**33**).

Quinazoline derivatives (**38**–**40**) having a side chain at the C-6 position were prepared as shown in Scheme 2. 6-Acetyloxy-7-methoxy-4-chloroquinazoline (**34**)[11,12] was reacted with 4-(2-phenylethenesulfonylmethyl)-phenylamines (**6**) in refluxing 2-propanol to give compounds **35**. Deprotection of acetyl group of compounds **35** with ammonium hydroxide resulted in compound **36**. The hydroxy compounds (**36**) were reacted with 1-bromo-2-chloroethane/1-bromo-3-chloropropane to give compounds **37** having a terminal halogen. Compounds **38**–**40** with morpholinoethoxy/propoxy substituents at the C-6 position were obtained from corresponding compounds **37** by reaction with morpholine.

The quinazoline derivatives prepared in this study were tested for their cytotoxic activity against breast (MCF7/ADR, MCF7), CNS (U251), colon (SW620, HT29), lung (H522), melanoma (UACC62), ovarian (SKOV3, OVCAR8), prostate (DU145, PC3) and renal (ACHN) human cancer cell lines. The selected active analogues were then further evaluated for their in vivo anti-cancer activity with HT-29 human colon adeno carcinoma xenografts. The *E*,*Z* isomers of the compounds were tested for their activity without separation. The concentration that caused 50% inhibition of cancer cell growth against various cancer cell lines were expressed as GI_{50} values and were given in Table 1. We started our SAR studies with an unsubstituted [4-(2-phenylethenesulfonylmethyl)phenyl]-quinazolin-4-ylamine (**8**). This particular compound showed activity only against SKOV3 and DU145 cell lines with GI_{50} values of 5.0 and 2.0 μM, respectively. Then various analogues were prepared with different substitutions on ring D (Fig. 2). Among 4-substituted derivatives **9**, **10**, **12**, **14** and **19** on ring D, 4-fluoro (**9**)[14] compound exhibited very good activity against various cell lines. Particularly, renal, CNS and prostate cell lines were highly sensitive towards this compound with GI_{50} values in the range of 0.01–1.0 μM. This compound was also active against HT29 of colon and SKOV3, OVCAR8 of ovarian cell lines in the range of 1.0–2.0 μM. The 4-hydroxy compound (**19**) showed activity at submicromolar concentrations in CNS and renal cell lines and showed moderate activity in HT-29 and OVCAR8 cell lines. However 4-cyano (**10**), 4-bromo (**12**) and 4-chloro (**14**) compounds were found to be inactive. Among the dis-



Scheme 2. (a) 2-Propanol, reflux, 3–4 h, 75–80%; (b) aq NH_3, MeOH, rt, 2–4 h, 65–72%; (c) 1-bromo-2-chloroethane/1-bromo-3-chloropropane (2 equiv), K_2CO_3 (3 equiv), DMF, rt, 6–8 h, 58–65%; (d) morpholine (2 equiv), K_2CO_3 (2 equiv), KI (1 equiv), DMF, 80 °C, 2–4 h, 50–65%.

Table 1. In vitro cytotoxic activities of [4-(2-phenylethenesulfonylmethyl)phenyl]-quinazolin-4-yl-amines

Compd	GI50 values in μM											
	Breast		CNS	Colon		Lung	Melanoma	Ovarian		Prostate		Renal
	MCF7/ADR	MCF7	U251	SW620	HT29	H522	UACC62	SKOV3	OVCAR8	DU145	PC3	ACHN
8	>100	74.0	>100	>100	>100	>100	>100	5.0	>100	2.0	>100	58.0
9	nt	16.0	0.01	100.0	1.0	13.0	5.0	2.0	2.0	0.3	1.0	0.01
10	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11	22.0	28.0	11.0	41.0	11.0	32.0	21.0	22.0	14.0	nt	35.0	28.0
12	>100	91.0	>100	>100	>100	>100	>100	1.0	38.0	55.0	42.0	0.2
13	>100	13.0	38.0	9.0	17.0	45.0	6.0	3.0	5.0	1.0	7.0	>100
14	>100	52.0	49.0	77.0	73.0	46.0	13.0	54.0	>100	>100	43.0	>100
15	5.0	4.0	4.0	14.0	24.0	12.0	2.0	2.0	4.0	3.0	19.0	0.1
16	>100	>100	>100	86.0	>100	nt	>100	69.0	>100	nt	>100	31.0
17	66.0	21.0	100.0	100.0	100.0	100.0	100.0	44.0	100.0	nt	100.0	73.0
18	100.0	100.0	100.0	100.0	100.0	100.0	100.0	39.0	100.0	nt	100.0	100.0
19	nt	100.0	0.3	100.0	2.0	100.0	10.0	14.0	1.0	19.0	14.0	0.02
20	nt	14.0	>100	5.0	27.0	>100	30.0	nt	0.1	17.0	11.0	nt
21	nt	5.0	11.0	>100	10.0	20.0	>100	nt	2.0	3.0	34.0	nt
22	nt	46.0	30.0	45.0	>100	13.0	15.0	nt	3.0	1.0	2.0	nt
23	nt	32.0	>100	94.0	9.0	27.0	28.0	nt	>100	3.0	42.0	nt
24	>100	4.0	4.0	>100	28.0	8.0	2.0	1.0	3.0	2.0	4.0	0.1
25	85.0	32.0	30.0	55.0	100.0	40.0	22.0	5.0	15.0	nt	58.0	16.0
26	100.0	100.0	32.0	100.0	100.0	100.0	3.0	2.0	100.0	nt	100.0	8.0
27	>100	25.0	53.0	>100	59.0	76.0	32.0	48.0	>100	nt	38.0	8.0
28	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
29	>100	15.0	2.0	>100	>100	nt	72.0	>100	>100	37.0	73.0	17.0
30	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
31	24.0	11.0	13.0	2.0	6.0	6.0	21.0	2.0	2.0	1.4	5.0	15.0
32	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
33	7.0	32.0	11.0	5.0	24.0	8.0	63.0	50.0	6.0	nt	9.5	4.0
38	nt	100.0	100.0	100.0	100.0	100.0	100.0	36.0	48.0	nt	100.0	66.0
39	3.0	3.0	18.0	>100	>100	>100	55.0	>100	>100	4.0	28.0	0.2
40	>100	54.0	4.0	7.0	4.0	nt	23.0	2.4	12.0	1.3	5.0	3.4

nt, not tested.

Table 2. Results of Xenograft studies for compounds **9**, **15**, **24**, **31**

Compd	Dose (mg/kg)	% Inhibition	Mortality	Max. mean% body weight loss
9	300	84	0/6	5
15	300	22	0/6	7
24	300	22	1/6	4
31	300	68	0/6	12

ubstituted derivatives, 3,4-dichloro (**11**), 2,4-dichloro (**15**), 2,4-difluoro (**16**), 2-chloro-4-fluoro (**17**) and 2-fluoro-4-trifluoromethyl (**18**), compound **15** displayed potent activity across most of the tested cell lines with GI_{50} values ranging from 0.1 to 5.0 μM. The trisubstituted compound 3,5-di-*t*-butyl-4-hydroxy (**13**) was less active.

To study further SAR of compounds **9** and **15** by introducing substitutions at the 6 and/or 7 positions of quinazoline moiety, compounds **20**–**33** have been prepared. In this group, even though compounds did not exhibit expected enhancement in activity, compounds **24** and **31** showed moderate activity in various cell lines. Aiming to improve the solubility of compound **31**, compound **40** with a 6-(3′-morpholino)propyloxy substituent was prepared. The compound retained activity in various cell lines though no improvement was observed. The corresponding 4-fluoro (**39**) compound did not show activity.

Compounds **9**, **15**, **24** and **31** were selected for assessing their in vivo anti-cancer activity in HT-29 human colon adeno carcinoma xenografts. HT-29 tumors were initiated in athymic nude mice (females, 20–25 g) by implantation of tumor fragments (~60 mm^3) from established tumors. Tumor fragments were implanted subcutaneous (sc) into the axillary region of the animal. When the tumors reached a size of ~100 mm^3, 300 mg/kg of experimental compounds were administered per oral once daily for 20 days (QD×20 schedule). The sc tumors were measured with vernier calipers, mice were weighed every alternate day and the volumes were calculated using the equation $V = (D \times d^2)/2$ where '*V*' is the tumor volume in mm^3, '*D*' is the longest diameter in mm and '*d*' is the shortest diameter in mm.[13]

Among these, compounds **9** and **31** showed encouraging in vivo activity (Table 2). Compound **9** was then evaluated for its in vivo dose–response relationship in HT-29 human adeno carcinoma xenografts at 100, 200 and 400 mg/kg doses. The results illustrated in Figure 3. Each bar in the figure represents the mean percentage inhibition ± standard deviation of six replicates where control mean was set to 0%. Compound **9** at an oral dose of 100 mg/kg exhibited 50% tumor volume reduction at the end of the treatment.

In conclusion, recent efforts in the discovery of anti-cancer drugs have been focused on searching for orally active anti-cancer agents. Our efforts in this area lead to

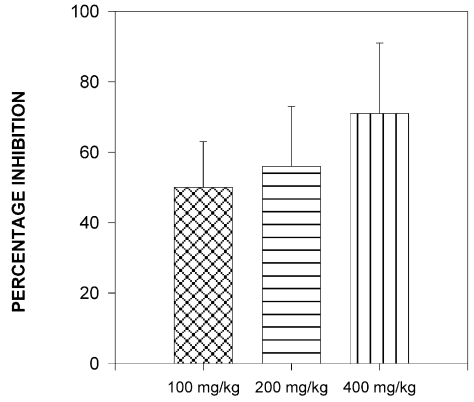


Figure 3. Dose–response relationship of compound **9** in HT-29 human adeno carcinoma xenografts.

the discovery of [4-(2-phenylethenesulfonylmethyl)phenyl]-quinazolin-4-yl-amines and we demonstrated oral activity for these compounds against HT-29 human adeno carcinoma xenografts. This class of compounds may be further explored as anti-cancer agents by replacing the quinazoline moiety with various heterocycles to improve their pharmacological activity.

References and notes

1. Joensuu, H.; Dimitrijevic, S. *Ann. Med.* **2001**, *33*, 451.
2. Muhsin, M.; Graham, J.; Kirkpatrick, P. *Nat. Rev. Drug Discov.* **2003**, *2*, 515.
3. Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D. H.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 267.
4. Myers, M. R.; Setzer, N. N.; Spada, A. P.; Zulli, A. L.; Hsu, C. Y. J.; Zilberstein, A.; Johnson, S. E.; Hook, L. E.; Jacoski, M. V. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 417.
5. Wedge, S. R.; Ogilvie, D. J.; Dukes, M.; Kendrew, J.; Chester, R.; Jackson, J. A.; Boffey, S. J.; Valentine, P. J.; Curwen, J. O.; Musgrove, H. L.; Graham, G. A.; Hughes, G. D.; Thomas, A. P.; Stokes, E. S. E.; Curry, B.; Richmond, G. H. P.; Wadsworth, P. F.; Bigley, A. L.; Hennequin, L. F. *Cancer Res.* **2002**, *62*, 4645.
6. Hennequin, L. F.; Stokes, E. S. E.; Thomas, A. P.; Johnstone, C.; Ple, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.; Curwen, J. O. *J. Med. Chem.* **2002**, *45*, 1300.
7. Hennequin, L. F.; Thomas, A. P.; Johnstone, C.; Stokes, E. S. E.; Ple, P. A.; Lohmann, J. J. M.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Curwen, J. O.; Kendrew, J.; Brempt, C. L. V. *J. Med. Chem.* **1999**, *42*, 5369.
8. Sharma, V. M.; Adi Seshu, K. V.; Vamsee Krishna, C.; Prasanna, P.; Chandra Sekhar, V.; Venkateswarlu, A.; Rajagopal, S.; Ajaykumar, R.; Deevi, S. D.; Mamidi, N. V. S. R.; Rajagopalan, R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1679.
9. Sharma, V. M.; Prasanna, P.; Adi Seshu, K. V.; Renuka, B.; Rao, C. V. L.; Kumar, G. S.; Narasimhulu, C. P.; Babu, P. A.; Puranik, R. C.; Subramanyam, D.; Venkateswarlu, A.; Rajagopal, S.; Kumar, K. B. S.; Rao, C. S.; Mamidi, N. V. S. R.; Deevi, S. D.; Ajaykumar, R.; Rajagopalan, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2303.
10. Subrahmanyam, D.; Sharma, V. M.; Venkateswarlu, A.; Sastry, T. V. S. R.; Srinivas, A. S. S. V.; Vamsi Krishna, C.; Deevi, D. S.; Babu, M. J.; Damodaran, K. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 369.
11. Gibson, K. H.; Grundy, W.; Godfrey, A. A.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Scarlett, L.; Barker, A. J.; Brown, D. S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2723.
12. Barker, A. J.; Gibson, K. H.; Grundy, W.; Godfrey, A. A.; Barlow, J. J.; Healy, M. P.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Scarlett, L.; Henthorn, L.; Richards, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1911.
13. Rusnak, D. W.; Lackey, K.; Affleck, K. *Mol. Can. Ther.* **2001**, *1*, 85.
14. Compound **9**: mp 192–193 °C; IR: 3432.1, 1621.8, 1572.7, 1535.4 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.6 (s, 2H), 7.30 (t, 2H, $J = 8.8$ Hz), 7.38 (m, 2H), 7.49 (d, 2H, $J = 8.4$ Hz), 7.70 (m, 4H), 7.83 (t, 1H, $J = 8.0$ Hz), 7.88 (d, 1H, $J = 8.8$ Hz), 8.08 (t, 1H, $J = 8.4$ Hz), 8.67 (d, 1H, $J = 8.4$ Hz), 8.80 (s, 1H), 11.30 (bs, 1H, –NH); CIMS *m*/*z* 420 (MH^+, 100%); CHN: calcd C 65.86%; H 4.33%; N 10.02%. Found C 65.80%; H 4.27%; N 10.30%.