Bioorganic & Medicinal Chemistry Letters 20 (2010) 4865–4869

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and anti-cancer activity of chalcone linked imidazolones

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ARTICLE INFO

Article history:
Received 24 March 2010
Revised 28 May 2010
Accepted 16 June 2010
Available online 25 June 2010

Keywords:
Chalcone linked imidazolones
Anti-cancer activity
Cell cycle arrest
G2/M
G0/G1

ABSTRACT

A series of novel chalcone linked imidazolones were prepared and evaluated for their anti-cancer activity against a panel of 53 human tumour cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. Some of these hybrids (**6**, 7 and **8**) showed good anti-cancer activity with GI_{50} values ranging from 1.26 to 13.9 μM. When breast carcinoma cells (MCF-7) were treated with 10 μM concentration of compounds TMAC, CA-4, 6 and 8 cell cycle arrest was observed in G2/M phase. Surprisingly, the increased concentration of the same compound to 30 μM caused accumulation of cells in G0/G1 phase of the cell cycle.

Petitt et al. isolated combretastatin A-4 (CA-4) in 1982 from the bark of *Combretum caffrum*, having strong antitubulin activity.[1] CA-4 binds to colchicine binding site and exhibits strong anti-cancer activity against wide variety of human cancer cell lines including MDR cancer cell lines.[2] CA-4 exhibit strong anti-cancer activity in vitro against a number of cancer cell lines whereas it shows poor antitumour activity in animal models due to low water solubility. A potent prodrug, CA-4 phosphate (CA-4P), has been developed to overcome the problem of water solubility.[3,4] It is observed that on long standing *cis* CA-4 is converted to *trans* CA-4 and further the *trans* form of these compounds exhibit reduction in antitubulin and antitumour activity. Thus many attempts have been made to retain the *cis*-olefinic bond by introducing five membered hetero cycle.[5] Structurally, in CA-4, two phenyl rings are spaced by two carbon atoms. Similarly many of the combretastatin analogues are separated by two atom bridge. However, CA-4 analogues possessing spacers of three carbon atoms are very few and the present study is focused on it. Combretastatin A-4 is thus attractive as a lead compound for the development of new anti-cancer compounds.

Chalcones (A) are an important class of anti-cancer agents that are present in edible plants. They exhibit a broad spectrum of biological activities, including anti-inflammatory,[6,7] anti-invasive[8,9] and antibacterial properties. Chalcones exhibiting potent anti-cancer activity were found to prevent tubulin polymerization by binding with colchicine binding site.[10–12]

The compounds containing imidazolone (B) chromophore are known to have a wide range of biological activities like anti-cancer, anti-inflammatory, cardioactivity and angiotensin II receptor antagonistic activity.[13] A trisubstituted imidazolone (MZ3) induced high degree of apoptosis in human leukemia cells and also have prominent cytotoxicity.[14] The compounds having imidazolone scaffold are known to inhibit hdm 2 E3 ligase activity, which ubiquitinates oncoprotein p53. It is well established in the literature that imidazolones exhibit selective blocking of hdm 2 mediated ubiquitation of p53 thus promoting apoptosis.[15] Chalcones (A),

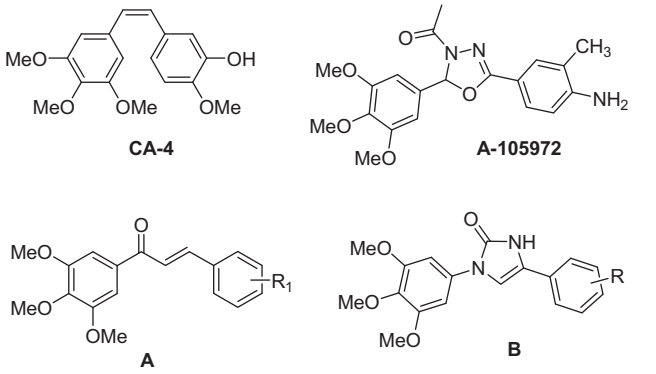

Figure 1. Chemical structures of combretastatin (CA-4), A-105972, chalcones (A) and imidazolones (B).

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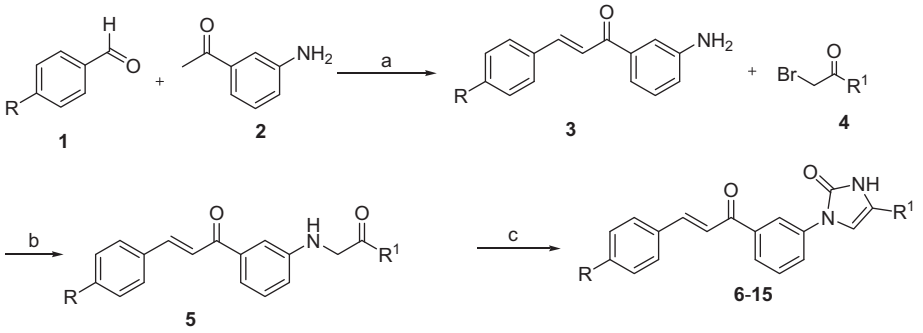
doi:10.1016/j.bmcl.2010.06.097



6: R = 4-OH-3-OMe; R^1 = Phenyl
7: R = 4-OH-3-OMe; R^1 = 4 -methoxy phenyl
8: R = 4-OH-3-OMe; R^1 = 4 -chloro phenyl
9: R = 3-OH; R^1 = phenyl
10: R = 3-OH; R^1 = 4 -chloro phenyl
11: R = 3,4,5-(MeO)3; R^1 = phenyl
12: R = 3,4,5-(MeO)3; R^1 = 4 -methoxy phenyl
13: R = 3,4,5-(MeO)3; R^1 = 3,4,5 -trimethoxy phenyl
14: R = 3,4,5-(MeO)3; R^1 = 4 chloro phenyl
15: R = 3-OH; R^1 = naphthyl

Scheme 1. Reagents and conditions: (a) 40% KOH, ethanol, 0 °C to rt, 10 h; (b) $NaHCO_3$, ethanol, 25 °C, 16 h; (c) KOCN, AcOH, 60–70 °C, 2 h.

Table 1
Anti-cancer activities of chalcono linked imidazolones against the NCI human tumours cell lines[a]

Cancer panel/cell line	GI_{50} (μM)									
	6	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**17**
Leukemia										
RPMI-8226	1.67	1.35	1.33	2.15	1.55	0.62	0.92	0.23	3.92	1.67
K-562	2.04	2.35	1.42	3.35	0.40	0.50	1.27	0.59	3.69	1.43
Non-small cell lung										
A549/ATCC	3.85	4.14	6.78	3.89	3.55	4.03	11.8	3.03	na	3.38
EKVX	3.90	13.4	9.61	15.6	2.40	2.30	4.20	9.47	na	2.64
HOP-62	1.48	5.15	2.84	6.93	2.36	4.12	5.91	3.08	na	2.26
HOP-92	2.27	2.96	1.26	5.04	1.6	10.8	14.9	nt	na	0.51
NCI-H226	2.60	4.10	2.77	24.8	2.31	14.6	35.2	14.4	na	2.43
NCI-H23	2.02	2.85	3.18	3.35	2.78	1.43	1.63	2.00	3.56	1.52
NCI-H322M	3.39	8.20	4.58	10.6	2.52	2.02	2.66	3.61	12.9	1.66
NCI-H460	1.95	3.23	3.90	2.49	2.41	1.4	1.56	1.73	3.74	1.67
NCI-H522	2.36	3.48	2.36	nt	1.65	0.95	1.85	1.05	4.10	1.29
Colon										
COLO 205	2.21	6.61	3.40	2.01	2.29	1.51	1.84	1.52	7.38	2.09
HCC-2998	1.51	2.32	1.85	4.09	13.7	1.26	1.64	1.92	2.46	2.13
HCT-116	1.40	1.93	1.46	3.02	1.23	0.33	0.37	0.74	1.39	0.40
HCT-15	2.73	3.15	1.80	3.10	2.33	1.13	1.38	1.89	3.85	1.56
HT29	3.77	3.84	2.97	3.33	1.90	0.66	1.28	1.70	2.00	1.54
KM12	1.61	1.77	1.48	1.83	1.34	1.37	1.42	1.12	5.45	1.21
SW-620	2.29	2.50	2.00	2.41	1.62	0.39	0.45	1.03	1.75	0.22
CNS										
SF-268	2.07	3.40	2.50	4.53	1.78	1.92	3.21	2.20	5.07	1.73
SF-295	3.04	13.9	10.5	18.2	1.79	1.90	4.95	4.96	24.3	1.44
SF-539	1.91	2.25	1.78	1.98	1.68	1.68	2.00	3.12	3.49	1.55
SNB-19	2.96	5.84	3.17	10.9	1.92	2.35	4.38	4.07	16.1	1.32
SNB-75	3.90	4.73	2.75	12.1	1.46	4.47	4.59	5.13	na[c]	1.88
U251	2.11	3.45	2.23	1.61	0.58	0.63	0.88	1.23	2.12	1.09
Renal										
A498	1.68	3.72	2.82	14.3	3.71	2.65	4.21	20.3	30.2	nt
786-0	1.35	4.53	2.07	2.98	1.57	1.38	1.61	1.59	2.14	1.74
ACHN	2.50	3.40	2.19	2.76	1.84	1.60	1.82	1.74	7.17	1.57
CAKI-1	2.19	4.99	2.64	13.3	1.72	1.75	3.05	2.89	na	1.45
RXF 393	2.27	5.85	6.40	1.89	1.51	1.71	1.92	1.62	3.22	1.41
SN12C	3.31	4.11	2.87	3.14	1.95	2.09	3.50	2.75	5.14	1.44
TK-10	5.29	6.08	4.67	nt[b]	2.83	3.02	3.04	3.00	6.46	3.45
UO-31	2.18	5.19	1.67	2.16	0.55	1.51	1.91	0.96	7.03	1.26
Prostate										
PC3	2.52	3.05	1.95	2.82	nt	3.26	6.22	2.63	na	1.85

Table 1 (*continued*)

Cancer panel/cell line	GI_{50} (μM)									
	6	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**17**
Ovarian										
OVCAR-3	2.29	2.70	1.56	3.08	1.56	1.23	1.78	1.48	2.40	1.33
OVCAR-4	5.19	6.08	4.64	5.30	2.21	1.88	3.57	3.77	11.4	1.68
OVCAR-5	2.04	3.38	2.00	5.44	2.19	1.88	2.64	4.38	3.11	1.89
OVCAR-8	4.11	4.97	4.18	2.80	0.99	1.62	2.53	2.32	3.85	2.33
NCI/ADR-RES	2.92	2.56	2.28	3.73	2.20	1.10	1.70	2.10	2.36	1.55
SK-OV-3	3.76	4.77	3.19	11.1	4.21	3.27	4.60	5.12	31.4	2.15
Breast										
MCF7	1.55	3.18	2.30	2.47	1.27	0.31	0.44	0.47	1.74	0.47
MDA-MB-231/ATCC	3.36	5.25	3.87	3.86	3.35	2.17	5.48	4.19	13.8	1.66
HS 578T	4.16	4.25	4.67	6.14	4.00	3.41	3.39	na	5.96	2.01
BT-549	2.78	4.57	2.52	9.32	1.45	1.93	2.59	1.52	12.3	1.89
TD-47D	2.7	3.60	3.09	4.24	1.92	2.19	3.84	3.08	6.65	1.11
Melanoma										
LOX IMVI	1.88	2.44	1.63	1.85	1.57	0.48	0.72	1.23	1.72	0.85
MALME-3M	2.67	4.42	3.06	6.21	1.83	1.31	1.79	1.80	18.1	2.08
M14	2.61	5.14	2.86	4.53	2.01	1.35	1.85	2.12	4.36	1.49
MDA-MB-435	2.14	3.35	2.25	3.12	2.06	1.20	1.86	2.11	3.63	1.71
SK-MEL-2	3.65	5.54	3.66	8.26	2.07	2.06	2.63	3.02	3.44	2.31
SK-MEL-28	1.85	2.03	2.06	5.63	3.64	1.74	2.03	1.61	10.9	3.79
SK-MEL-5	1.59	2.63	1.80	3.18	1.53	1.40	2.03	2.76	8.70	1.16
UACC-257	6.81	13.3	8.69	7.68	4.07	1.52	3.04	2.22	17.4	4.13
UACC-62	2.01	3.96	2.74	3.65	1.85	1.52	2.06	1.87	8.88	1.80

[a] Values are reported as GI_{50}, the concentration of the compound required to cause 50% inhibition of cell growth.
[b] nt = not tested.
[c] na = not active.

imidazolones (B) and A-105972 are the derivatives of CA-4, wherein the two aryl rings are bridged through three atoms as illustrated in Figure 1.[16]

In the present study we have linked the chalcone pharmacophore with imidazolones as outlined in Scheme 1.[17,18] Initially, amino chalcone intermediates (**3**) have been synthesized from the easily available starting materials, like aldehydes and 3-amino acetophenone. Further, these on reactions with phenacyl bromides in ethanol in the presence of sodium bicarbonate gives intermediates **5.** Finally, these intermediates are reacted with KCNO in acetic acid at 60 °C to provide the chalcone linked imidazolones.[19]

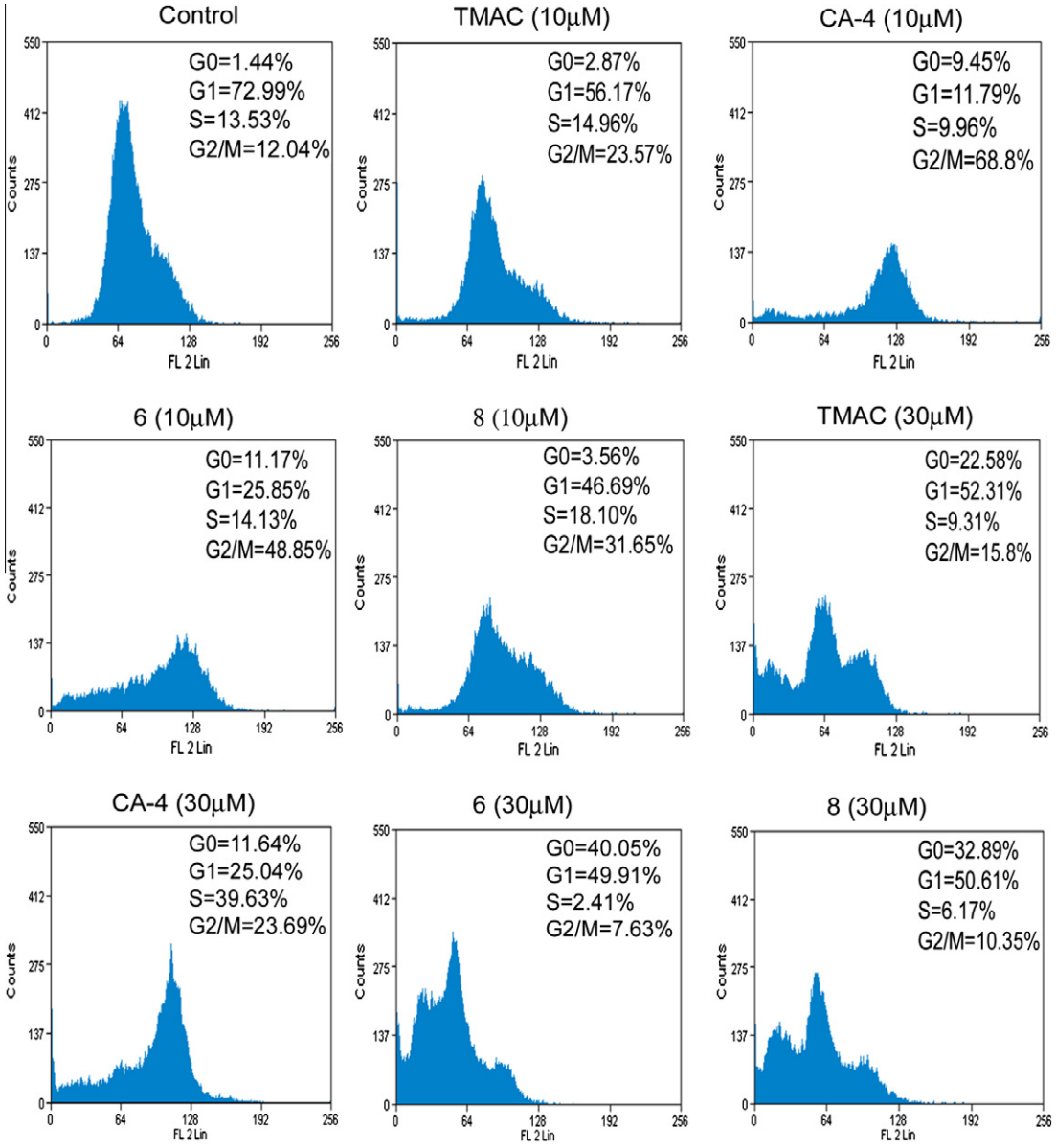


Figure 2. FACS analysis of cell cycle distribution of MCF-7 cells after treatment with chalcone imidazolones compounds TMAC, CA-4, **6** and **8** at 10 μM and at 30 μM concentration for 24 h. TMAC is the starting material. CA-4 was used as the positive control. Control is DMSO.

Compounds **6–15**, which passed the preliminary screening on the tumour cell lines were tested for five dose studies in the framework of the in vitro Anticancer Screen Program of the National Cancer Institute (Bethesda, USA) on a panel of 53 human tumour cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. The GI_{50} values for the test compounds are illustrated in Table 1.

Amongst all the compounds tested for five dose studies, three compounds that is, **6**, **7**, **8** have showed reproducible results. The antitumour activity (GI_{50}) of these compounds **6**, **7** and **8** are in the range of 1.35–6.81, 1.35–13.9 and 1.26–10.5 μM, respectively.

From the cell cycle analysis in FACS studies,[20] it is observed that compounds **6** and **8** exhibited significant cell cycle arrest. At 10 μM concentration we have tested these compounds for the possible regulatory role on cell cycle. The control cells have shown 1.44%, 72.99%, 13.53% and 12.04% of G0, G1, S and G2/M phase of the cell cycle, respectively. (*E*)-1-(3-Aminophenyl)-3-(3,4,5-trimethoxyphenyl)-2-propen-1-ne(TMAC), the starting material has shown 23.57% of G2/M cell cycle arrest. The positive control CA-4, compounds **6** and **8** have shown 68.8% 48.85% and 31.65% of G2/M arrest, respectively as seen in (Figs. 2 and 3a).

This data clearly showed that these compounds arrest the cell cycle at G2/M phase. Moreover this data further revealed that compound **6** is the most potent one amongst the two investigated. Surprisingly, the increased concentrations of these compounds to 30 μM have shown enhanced accumulation of cells at G0 phase and drastic reduction of cells at G2/M phase. The increased cells in G0 phase indicated the extent of apoptosis, as it is obvious that increased concentration after certain threshold leads to apoptosis. At 30 μM concentration the control cells showed 1.44% and TMAC, CA-4, **6** and **8** showed 22.58%, 11.64%, 40.05% and 32.89% of G0 phase cells (Fig. 3b). The increase of G0 cells concomitantly decreased the G2/M phase cells that is, 15.8%, 23.69%, 7.63% and 10.35% of TMAC, CA-4, compounds **6** and **8**. This data obtained

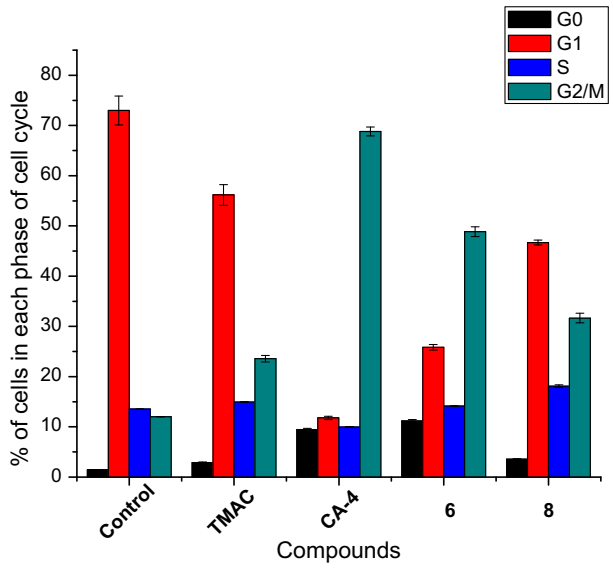


Figure 3a. Histogram depicting the different phases of cell cycle in MCF-7 cells exposed to chalcone imidazolone hybrid compounds (**6** and **8**) at 10 μM (values indicated are mean of three experiments).

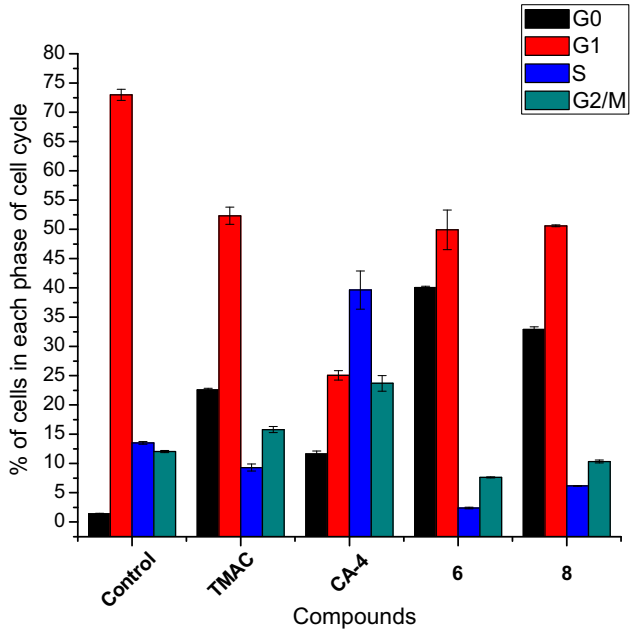


Figure 3b. Histogram depicting the different phases of cell cycle in MCF-7 cells exposed to chalcone imidazolone hybrid compounds (**6** and **8**) at 30 μM concentration. CA-4 was used as a positive control. TMAC was the starting material for the chalcone imidazolone compounds (values indicated are mean of three experiments).

from these studies clearly revealed the G2/M arrest caused by these compounds. The concentration of 10 μM concentration was found to be optimum by our in vitro cytotoxic studies.

Previous studies by Twiddy et al. have discovered that caspase-7 and PARP play an important role in causing apoptosis in human breast cancer cells.[21] Mooney and coworkers have also reported that activation of the caspase cascade leads to cleavage of DNA repair enzyme poly-ADP-ribose polymerase (PARP) in Staurosporine induced apoptosis in breast cancer cells.[22] Therefore, in the present investigation human breast cancer cells (MCF-7) were treated with 30 μM concentration of compounds CA-4, **6, 8** and TMAC. After

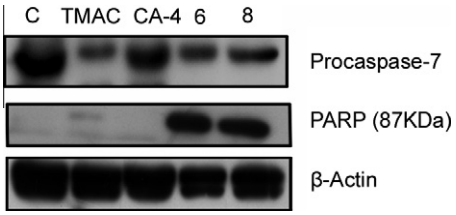


Figure 4. Effect of chalcone linked imidazolones (**TMAC,** CA-4, **6 and 8**) on the expression of apoptotic proteins (ProCaspase-7) and cleavage PARP. Here MCF7 cells treated with 30 μM concentration of compounds. CA-4 was used as positive control. Cell lysates were collected and western blot analysis was carried out with the above mentioned antibodies and β-actin was used as loading control.

24 h cell lysates were extracted and subjected to Western blot analysis using antibodies procaspase-7 and cleavage specific PARP. It was observed that there is a decrease in the level of procaspase-7 and cleaved PARP (87 kDa) in compounds **6** and **8**, and there is no significant difference in case of CA-4 and TMAC. Thus indicating that in compounds **6** and **8** apoptotic cell death takes place in a caspase dependent pathway as shown in Figure 4.

In conclusion, in the present study, a series of novel chalcone linked imidazolones were prepared and evaluated for their anti-cancer activity against a panel of 53 human tumour cell lines derived from nine different cancer types. Some of these hybrids like **6** and **8** showed good anti-cancer activity with GI_{50} values ranging from 1.26 to 10.5 μM. The FACS analysis data clearly showed that these compounds (**6** and **8)** arrest the cell cycle at G2/M phase. Some of the compounds like **6** and **8** from this series have shown promising activity and useful in the design and development of new molecules based on these leads.

Acknowledgements

We thank the National Cancer Institute, Bethesda, for anti-cancer assay in human cell lines. The authors (G.R.K., P.R. and A.V.) thank to CSIR and UGC, New Delhi for financial assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.097.

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19. The spectral data of final compounds **6** and **8** are given below 1-3-[(*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoyl]phenyl-4-phenyl-2,3-dihydro-1*H*-2-imidazolone (**6**): mp 141–151 °C; IR 3283, 1689, 1659, 1580 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.67 (1H, br s), 8.01 (1H, s), 7.81 (1H, d, J = 7.30 Hz), 7.64 (1H, d, J = 7.28 Hz), 7.52 (1H, s), 7.36 (1H, d, J = 16.02 Hz), 7.32 (2H, m), 7.16 (2H, m), 7.03 (1H, d, J = 16.02 Hz), 6.95 (3H, m), 6.73 (2H, m), 3.73 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 188.6, 152.3, 149.8, 147.8, 145.3, 138.9, 137.5, 129.4, 128.9, 128.7, 127, 126.1, 125, 124.6, 124, 123.3, 122.5, 119.9, 118.6, 115.5, 111.9, 106.7, 55.76; MS (ESI): m/z 413 $[M+H]^+$; purity 98%, C18 column, acetonitrile–water (mobile phase), wavelength 289 nm; yellow solid. 4-(4-Chlorophenyl)-1-3-[(*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoyl]phenyl-2,3-dihydro-1*H*-2-imidazolone (**8**): mp 217–221 °C; IR: 3356, 1710, 1657, 1580 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.23 (1H, br s), 9.74 (1H, s), 8.4 (1H, s), 8.05 (2H, m), 7.84 (1H, br d), 7.71 (4H, m), 7.5 (3H, m), 7.31 (1H, d, J = 15.92 Hz), 6.82 (1H, d, J = 15.92 Hz), 3.86 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 188.6, 152.2, 149.7, 147.8, 145.3, 138.9, 137.3, 131.3, 129.3, 128.6, 127.8, 126.1, 125, 124.9, 124.6, 123.9, 121.4., 119.9, 118.6, 115.5, 111.9, 107.5, 55.70; MS (ESI): m/z 447 $[M+H]^+$; purity 95%, C18 column, acetonitrile–water (mobile phase), wavelength 289 nm; yellow solid.
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