RESEARCH ARTICLE

Evaluation of Anticancer Activity of Curcumin Analogues Bearing a Heterocyclic Nucleus

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Abstract

We report herein an in vitro anticancer evaluation of a series of seven curcumin analogues (3a-g). The National Cancer Institute (NCI US) Protocol was followed and all the compounds were evaluated for their anticancer activity on nine different panels (leukemia, non small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer) represented by 60 NCI human cancer cell lines. All the compounds showed significant anticancer activity in one dose assay (drug concentration 10 μM) and hence were evaluated further in five dose assays (0.01, 0.1, 1, 10 and 100 μM) and three dose related parameters GI_{50}, TGI and LC_{50} were calculated for each (3a-g) in micro molar drug concentrations (μM). The compound 3d (NSC 757927) showed maximum mean percent growth inhibition (PGI) of 112.2%, while compound 3g (NSC 763374) showed less mean PGI of 40.1% in the one dose assay. The maximum anticancer activity was observed with the SR (leukemia) cell line with a GI_{50} of 0.03 μM. The calculated average sensitivity of all cell lines of a particular subpanel toward the test agent showed that all the curcumin analogues showed maximum activity on leukemia cell lines with GI_{50} values between 0.23 and 2.67 μM.

Keywords: Anticancer activity - curcumin analogues - cancer cell lines - five dose assay - pyrazole - pyrimidine

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Introduction

Today cancer is one among the major health problems worldwide. It is a genetic disease that is caused by changes to gene that control the way our cells functioning. There are more than 100 types of cancer (http://www.cancer.gov/). The statistics showed that cancer causes almost 13 percent total deaths globally surpassing cardiovascular disease. In India alone the total number of cancer cases are likely to go up from 979,786 cases in the year 2010 to 1,148,757 cases in the year 2020 (Takiar et al., 2010). A total of 1,658,370 new cases of cancer and 589,430 cancer deaths are projected to occur in the United States in 2015 (Siegel et al., 2015). It is expected that the new cases of cancer will jump to 19.3 million worldwide by 2025 (WHO World Cancer Report 2014). The types of cancer treatment include surgery, chemotherapy, radiation therapy, immunotherapy, targeted therapy and hormonal therapy. The therapeutic applications of antiproliferative drugs are often restricted to their toxic potentials, resistance, and genotoxicity (Aydemir and Bilaloglu, 2003). Thus the demand for relatively more effective and safer anticancer agents is today's need to combat against cancer.

The natural products from plants and other biological sources are expected to play a vital role in creating new and better chemotherapeutic agents. Curcumin, a β-diketone is one of the active ingredients obtained from the powdered root of Curcuma longa Linn., which showed a wide spectrum biological activity. The four different sites (aryl side chain, diketo group, double bonds, and active methylene group) of curcumin were exploited and a large no of semi-synthetic analogues as well as synthetic analogues of curcumin was prepared by medicinal chemists with significantly improved biological activity (Vyas et al., 2013; Balaji et al., 2015).

Curcumin itself is more toxic towards cancerous cells as compared to the normal human cell, (Kunwar et al., 2008). It can be concluded that curcumin analogues would have more specificity towards the cancer cells. In another study, curcumin showed autophagic and apoptotic death of K562 cell line (leukemia) (Jia et al., 2009). Several curcumin analogues were reported as anticancer agent (Sharma et al., 2015; Ahsan et al., 2013; Liang et al., 2009). Apart from anticancer activity various other activity were also reported that includes antibacterial (Lal el al., 2012; Sahu et al., 2012; Zhichang et al., 2012), anti-HIV (Singh et al., 2010), anti-inflammatory (Saja et al., 2007), antimalarial (Mishra et al., 2008; Balaji et al., 2015), anti-EGFR activity (Ahsan et al., 2013; Yadav et al., 2014).and many more have been reported for curcumin analogues. These recent development, made curcumin as an ideal

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lead compound for future drug discovery. The promising anticancer activity of curcumin inspired us to explore curcumin analogues further. The present investigation is the continuation of the previous work in which the anticancer activity was reported on 60 NCI cancer cell lines at 10 μM drug concentration (one dose assay) (Ahsan et al., 2013). And we reported herein the anticancer activity of curcumin analogues in five dose assay (0.01, 0.1, 1, 10 and 100 μM) and three dose related parameters GI_{50}, TGI and LC_{50} were calculated for each tested compounds (3a-g) in micro molar drug concentrations (μM).

Materials and Methods

Chemistry

All the chemicals were supplied by Merck (Germany), Konark Herbal (India) and S. D. Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. IR spectra were obtained on a Schimadzu 8201 PC, FT-IR spectrometer (KBr pellets). 1H NMR spectra were recorded on a Bruker AC 400 MHz spectrometer using TMS as internal standard in DMSO d6. Mass spectra were recorded on a Bruker Esquire LCMS using ESI and elemental analyses were performed on Perkin-Elmer 2400 Elemental Analyzer.

Cancer cell lines

The antiproliferative activity of the was carried out at National Cancer Institute (NCI US) on nine different panels viz. leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers of nearly 60 cancer cell lines (60 NCI cancer cell lines).

Anticancer activity

The anticancer screening was carried out on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers cell lines, nearly 60 in number according to the reported NCI US protocol (http://dtp.nci.nih.gov; Boyd et al., 1995; Monks et al., 1991; Shoemaker 2006). Using the seven absorbance measurements [time zero, (Ti), control growth, (C), and test growth in the presence of drug at the five concentration levels (Tf)], the percentage growth was calculated at each of the drug concentrations levels as: [(Tf-Ti)/(C-Ti)] x 100 for concentrations for which Tf ≥ Ti and [(Tf-Ti)/Ti] x 100 for concentrations for which Tf < Ti (http://dtp.nci.nih.gov; Ahsan et al., 2013). Three-dose response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each of the experimental agents in five dose assay. Growth inhibition of 50% (GI_{50}) was calculated from 100 × [(Tf-Ti)/(C-Ti)] = 50, which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by sulforhodamine B, SRB staining) in control cells during the drug incubation. The total growth inhibition (TGI) was calculated from Tf = Ti, which was the drug concentration resulting in total growth inhibition and signified the cytostatic effect. The LC_{50} was calculated from 100 × [(Tf-Ti)/Ti] = -50, indicating a net loss of cells following treatment which indicated the concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning (Grever et al., 1992; Alley et al., 1998; Corona et al., 2009; Ahsan et al., 2013).

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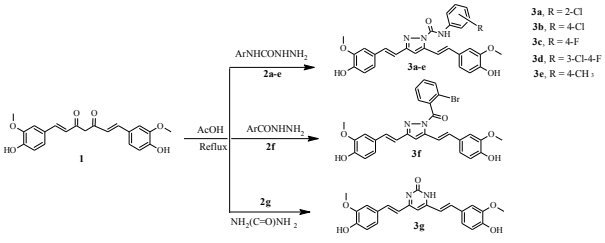
Results

Chemistry

A mixture of 1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione (curcumin) and substituted phenyl semicarbazide (2a-e)/ 2-brormophenyl hydrazide (2f)/ urea (2g) was refluxed in glacial acetic acid (AcOH) for 10-12 h to obtain the curcumin analogues (3a-g). The completion of reaction was monitored by TLC using mobile phase, hexane: ethylacetate (6:4) and purity of the compounds was checked by elemental analyses. Both the analytical and spectral data (IR, NMR and MS) of the synthesized compounds were in full harmony with the proposed structures and reported earlier. The curcumin analogues (3a-g) described herein the study are summarised in Scheme 1. The melting point and complete characterization data of the curcumin analogues (3a-g) was reported earlier (Ahsan et al., 2013).

Anticancer activity

The curcumin analogues (3a-g) showed promising



Scheme 1. Protocol for the Synthesis of Curcumin Analogues (3a-g)

anticancer activity in single dose assay with mean percent growth inhibition (PGI) ranging between 112.2 and 40.1 percent (Ahsan et al., 2013). The compound 3d showed maximum anticancer activity with PGI of 112.2%, while compound 3g showed less anticancer activity with PGI of 40.1% (Figure 1). All the compounds (3a-g) was found to be active and met the pre-determine criterion of growth inhibition and thus further chosen for the NCI full panel of five dose assay at five different drug concentrations (0.01, 0.1, 1, 10 and 100 μM). Three-dose response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each of the experimental agents in five dose assay is given in Table 1. The compound 3a showed highest sensitivity to SR (leukemia) with GI_{50} of 0.03 μM and least sensitivity to OVCAR-5 (ovarian cancer) with GI_{50} of 3.30 μM. The best value of TGI was being noted on SR (leukemia) with 0.09 μM. Only in 15 cell lines the compound 3a registered LC_{50} value of >100 μM. The compound 3b showed highest sensitivity to SR (leukemia) with GI_{50} of 0.06 μM and least sensitivity to HT29 (colon cancer) with GI_{50} of 2.52 μM. The best value of TGI was being noted on MDA-MB 435 (melanoma) with 0.37 μM. Only in 24 cell lines the compound 3b registered LC_{50} value of >100 μM. The compound 3c showed highest sensitivity to MDA-MB 435 (melanoma) with GI_{50} of 0.23 μM and least sensitivity to SW620 (colon cancer) with GI_{50} of 54.90 μM. The best value of TGI was being noted on MDA-MB 435 (melanoma) with 0.57 μM. Only in 42 cell lines the compound 3c registered LC_{50} value of >100 μM. The compound 3d showed highest sensitivity to SR (leukemia) with GI_{50} of 0.04 μM and least sensitivity to COLO205 (colon cancer) with GI_{50} of 1.73 μM. The best value of TGI was being noted on SR (leukemia) with 0.29 μM. Only in 24 cell lines the compound 3d registered LC_{50} value of >100 μM. The compound 3e showed highest sensitivity to SR (leukemia) with GI_{50} of 0.03 μM and least sensitivity to OVCAR-5 (ovarian cancer) with GI_{50} of 2.67 μM. The best value of TGI was being noted on SR (leukemia) with 0.15 μM. Only in 24 cell lines compound 3e registered LC_{50} value of >100 μM. The compound 3f showed highest sensitivity to SR (leukemia) with GI_{50} of 0.34 μM and least sensitivity to EKVX (non small cell lung cancer) with GI_{50} of 4.72 μM. The best value of TGI was being noted on RXF (renal cancer) with 3.96 μM. Nearly on 57 cell lines compound 3f registered LC_{50} value of >100 μM. The compound 3g showed highest sensitivity to HT29 (colon cancer) with GI_{50} of 1.30 μM and least sensitivity to NCI ADR-RES (ovarian cancer) with GI_{50} of 16.7 μM. The best value of TGI was being noted on HCT-116 (colon cancer) with 1.24 μM. Nearly on 50 cell lines compound 3g registered LC_{50} value of >100 μM. All the curcumin analogues showed promising anticancer activity with GI_{50} between 0.03 μM (SR; leukemia) and 54.9 μM (SW620; colon cancer). Further average sensitivity on a particular panel of cell lines was calculated for each compounds (3a-g) showed a relatively higher sensitivity towards leukemia

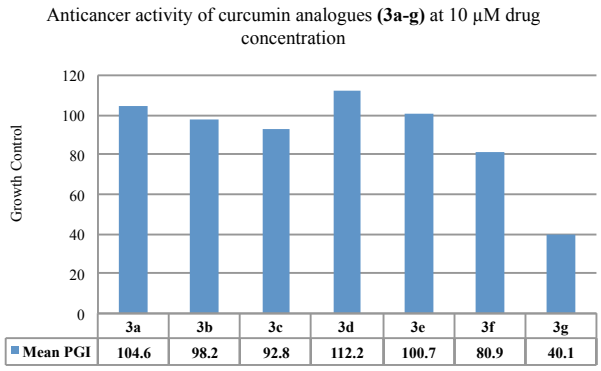
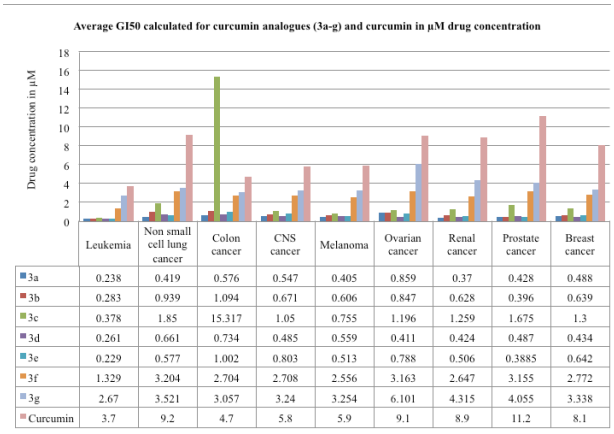


Figure 1. Mean Percent Growth Inhibition (PGI) of Curcumin Analogues (3a-g) at 10 μM drug Concentration on NCI 60 Cancer Cell Lines



	Leukemia	Non small cell lung cancer	Colon cancer	CNS cancer	Melanoma	Ovarian cancer	Renal cancer	Prostate cancer	Breast cancer
3a	0.238	0.419	0.576	0.547	0.405	0.859	0.37	0.428	0.488
3b	0.283	0.939	1.094	0.671	0.606	0.847	0.628	0.396	0.639
3c	0.378	1.85	15.317	1.05	0.755	1.196	1.259	1.675	1.3
3d	0.261	0.661	0.734	0.485	0.559	0.411	0.424	0.487	0.434
3e	0.229	0.577	1.002	0.803	0.513	0.788	0.506	0.3885	0.642
3f	1.329	3.204	2.704	2.708	2.556	3.163	2.647	3.155	2.772
3g	2.67	3.521	3.057	3.24	3.254	6.101	4.315	4.055	3.338
Curcumin	3.7	9.2	4.7	5.8	5.9	9.1	8.9	11.2	8.1

Figure 2. The Calculated Average GI_{50} of Curcumin Analogues (3a-g) in μM Drug Concentration

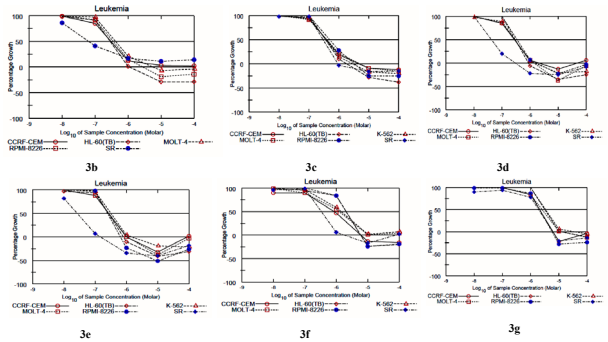


Figure 3. Dose response curve curcumin analogues (3b-g) against the most sensitive panel (leukemia cell lines)

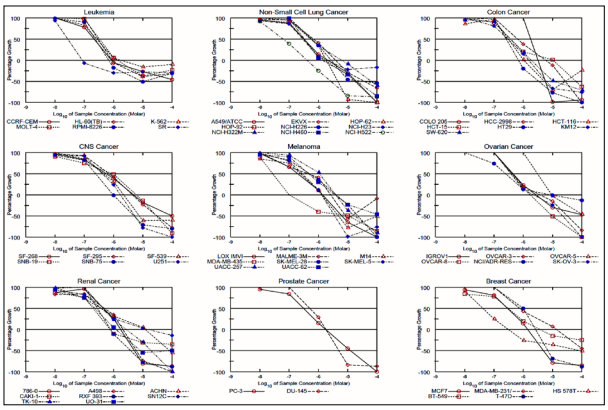


Figure 4. Dose Response Curve Curcumin Analogues (3a) Against the Nine Different Panels of Cancer Cell Lines

Table 1. NCI *In Vitro* Testing Results of Curcumin Analogues (3a-g) at Five Dose Level in μM

Panel	Cell line	3a (NSC 763372)			3b (NSC 757924)			3c (NSC 757925)			3d (NSC 757927)			3e (NSC 763373)			3f (NSC 757928)			3g (NSC 763374)		
		GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Leukemia	CCRF-CEM	0.22	0.84	>100	0.29	>100	>100	0.39	4.91	>100	0.28	NT	>100	0.27	NT	>100	0.84	6.14	>100	2.1	6.22	>100
	HL-60(TB)	0.31	0.89	>100	0.29	1.05	>100	0.37	1.82	>100	0.31	0.88	>100	0.26	0.796	>100	2.07	6	>100	3.74	12.1	>100
	K-562	0.27	1.78	>100	NT	NT	NT	0.37	2.91	>100	0.29	1.45	>100	0.32	1.53	>100	1.45	>100	>100	3.66	35.8	>100
	MOLT-4	0.33	1.38	>100	0.42	5.82	>100	0.39	5.21	>100	0.28	1.4	>100	0.27	1.07	>100	1.23	>100	>100	2.55	11.7	>100
	RPMI-8226	0.27	0.68	NT	0.35	2.76	>100	0.49	3.34	>100	0.36	1.73	>100	0.25	0.647	NT	2.05	6	>100	2.1	5.68	>100
	SR	0.03	0.09	>100	0.06	>100	>100	0.25	0.933	>100	0.044	0.29	>100	0.03	0.15	>100	0.34	NT	>100	1.91	6.18	>100
Non-Small Cell Lung Cancer	A549/ATCC	0.32	2.07	19.1	0.71	10.6	40.9	2.39	23.9	>100	0.38	3.39	>100	0.42	3.73	44.5	3.05	45.3	>100	5.2	27.6	>100
	EKVX	0.67	3.84	37.3	1.51	11.7	76.5	3.52	24.9	>100	1.43	8.76	>100	0.99	5.02	>100	4.72	>100	>100	3.89	72.8	>100
	HOP-62	0.54	1.84	4.56	0.54	1.92	5.55	1.11	8.13	54.4	0.42	1.53	4.01	0.75	2.18	5.45	3.41	16.3	>100	4.11	17.6	81.2
	HOP-92	0.31	1.64	17.3	2.18	7.28	>100	2.76	8.08	>100	0.92	6.59	>100	0.62	3.24	62.8	3.82	9.65	>100	2.28	9.24	>100
	NCI-H226	0.62	2.74	33.4	1.85	7.11	30.3	2.73	9.83	>100	1.3	3.5	NT	1.03	4.48	>100	3.29	56.5	>100	3.48	22.5	>100
	NCI-H23	0.29	1.9	>100	0.34	1.38	NT	1.18	6.37	>100	0.32	NT	>100	0.39	NT	>100	2.68	16.9	>100	2.32	-	>100
	NCI-H322M	0.61	6.32	64.8	0.78	9.35	48.6	2.16	11.4	>100	0.57	5.65	>100	0.5	4.7	86	3.5	40.1	>100	4.72	80.5	>100
	NCI-H460	0.33	1.37	20.9	0.32	2.14	>100	0.62	10.7	>100	0.35	1.1	NT	0.33	1.44	40.4	2.99	>100	>100	3.12	18.5	>100
	NCI-H522	0.06	0.41	2.65	0.21	0.8	50.9	0.26	1.38	>100	0.22	1.01	NT	0.17	5.99	48	1.38	6.32	>100	2.57	7.95	>100
Colon Cancer	COLO 205	1.79	3.19	5.68	2.03	3.52	6.13	1.9	3.4	6.11	1.73	3.2	5.94	1.84	3.28	5.85	2.31	4.63	9.26	2.03	4.03	7.99
	HCC-2998	0.65	5.83	27.1	1.51	7.62	32.7	2.25	5.63	35.7	0.588	2.89	14.7	2.23	9.43	38.3	2.48	6.37	>100	3.55	1.43	93.8
	HCT-116	0.31	1.05	NT	0.36	1.88	36.1	0.41	10.5	45.3	0.35	NT	NT	0.27	1.13	>100	3.06	>100	>100	2.48	1.24	>100
	HCT-15	0.37	10.4	62.3	0.45	11.8	>100	0.57	17.6	>100	0.38	2.74	>100	0.39	10.9	>100	2.79	>100	>100	5.43	>100	>100
	HT29	0.29	0.7	3.4	2.52	6.37	>100	2.49	NT	>100	1.31	4.26	>100	1.5	3.73	9.29	2.41	NT	>100	1.3	2.67	5.48
	KM12	0.29	1.51	6.15	0.34	1.45	12	44.7	7.18	>100	0.31	1.15	4.05	0.3	1.52	13.9	2.22	>100	>100	3.44	14.2	>100
	SW-620	0.36	1.88	10.9	0.45	2.5	>100	54.9	10.2	74.1	0.47	1.52	NT	0.48	3.51	64.2	3.66	>100	>100	3.17	16.6	>100
CNS Cancer	SF-268	0.56	4.77	>100	0.51	3.61	28.7	0.63	7.39	>100	0.45	2.02	NT	0.5	5.08	76.5	2.44	>100	>100	4.58	>100	>100
	SF-295	0.69	4.44	31	1.6	11.6	>100	2.63	9.71	>100	0.54	2.6	>100	1.99	7.93	>100	3.12	35.1	>100	2.75	9.87	>100
	SF-539	0.46	2.23	7.69	0.48	2.22	8.41	0.4	3.22	88.3	0.46	1.89	NT	0.55	3.63	>100	2.58	NT	>100	3.87	>100	>100
	SNB-19	0.91	5.92	29	0.64	10.5	45.1	1.19	29.9	>100	0.68	2.14	NT	1.03	8.73	36.7	2.7	>100	>100	3.37	>100	>100
	SNB-75	0.24	0.97	5.04	0.42	2.58	>100	0.79	5.14	66.9	0.38	1.35	NT	0.31	1.03	5.45	2.79	15.4	>100	1.72	12	>100
	U251	0.41	1.71	5.3	0.55	5.46	45.7	0.66	10.9	>100	0.39	1.52	NT	0.44	3.61	72.7	2.62	>100	>100	3.15	13.5	66.1
	LOX IMVI	0.19	1.39	6.32	0.33	2.4	>100	0.49	4.7	>100	0.35	NT	NT	0.45	1.89	NT	2.48	NT	>100	3.16	>100	>100
	MALME-3M	0.44	2.64	NT	0.6	3.15	20	0.75	8.62	>100	0.61	2.24	NT	1.06	3.64	>100	3.27	43.6	>100	3.91	>100	>100
Melanoma	M14	0.24	1.3	4.77	0.38	1.52	17.7	0.43	7.87	>100	0.41	1.41	NT	0.26	1.11	5.7	3.07	>100	>100	3.12	15.4	>100
	MDA-MB-435	0.03	0.1	10.8	0.13	0.37	1.07	0.228	0.57	>100	0.08	0.388	NT	0.07	0.35	>100	0.69	8.06	>100	2.72	18.2	>100
	SK-MEL-2	NT	NT	NT	0.38	1.95	>100	0.56	3.38	>100	0.53	2.19	NT	NT	NT	NT	2.37	5.92	>100	2.88	6.7	>100
	SK-MEL-28	0.5	4.06	22.2	1.16	4.6	25.7	0.97	5.81	87.9	0.78	3.51	33.5	0.69	5.87	32	2.57	8.71	>100	3.05	13.1	91.5
	SK-MEL-5	0.27	1.29	3.64	0.35	1.34	4.07	0.39	2.2	23.6	0.35	1.28	NT	0.29	1.24	3.87	2.56	13.3	>100	3.7	36.1	>100
	UACC-257	1.09	3.86	17.3	1.43	6.5	29.3	1.92	9.29	>100	1.44	6.14	>100	0.81	4.48	47.8	3.36	40.1	>100	3.36	17.9	>100
	UACC-62	0.47	3.7	>100	0.58	2.38	7.79	1.04	4.02	>100	0.48	2.39	>100	0.46	2.25	NT	2.64	8.77	>100	3.39	43.2	>100
Ovarian Cancer	IGROV1	0.5	2.72	>100	0.59	4.88	>100	2.56	22	>100	0.52	NT	>100	0.56	3.2	>100	4.11	>100	>100	4.41	>100	>100
	OVCAR-3	0.47	3.69	32.3	0.35	1.06	59.6	0.45	2.74	>100	0.28	0.645	30.5	0.35	1.45	9.43	2.52	NT	>100	3.8	33.5	>100
	OVCAR-4	NT	NT	NT	0.98	7.15	>100	1.7	7.28	>100	0.59	5.65	84	0.69	5.95	53.2	3.64	22.8	>100	2.46	16.8	>100
	OVCAR-5	3.3	9.35	>100	2.47	7.52	>100	NT	NT	NT	NT	NT	NT	2.67	6.73	>100	NT	NT	NT	10	28.4	80.2
	OVCAR-8	0.25	7.61	9.53	0.73	3.76	71.5	1.23	4.53	>100	0.46	2.39	>100	0.47	2.66	58.8	2.75	12.4	>100	3.01	12.7	>100
	NCI/ADR-RES	0.25	7.91	>100	0.36	3.58	>100	0.49	26.5	>100	0.29	NT	>100	0.39	NT	>100	3.72	>100	>100	16.7	>100	>100
	SK-OV-3	0.39	2.2	21.9	0.45	1.7	6.5	0.74	3.86	31.8	0.31	1.23	8.38	0.38	1.43	5.89	2.24	5.31	>100	2.33	4.89	>100
Renal Cancer	786-0	0.45	1.74	5.1	0.52	1.78	4.84	0.73	8.1	72	0.44	1.5	3.98	0.38	1.93	6.43	3.19	>100	>100	2.73	33	>100
	A498	0.49	2.08	5.99	1.86	5.22	19.2	2.46	6.09	20.5	0.61	1.95	4.9	1.21	4.38	18.3	3.04	8.71	>100	3.11	25.2	>100
	ACHN	0.37	12.2	81.3	0.52	11.4	48	NT	NT	NT	NT	NT	NT	0.41	12.1	>100	NT	NT	NT	8.84	>100	>100
	CAKI-1	0.2	0.76	>100	0.37	79.8	>100	0.89	40.3	NT	0.38	7.61	>100	0.39	4.67	>100	2.2	>100	>100	3.38	>100	>100
	RXF 393	0.33	0.82	3.83	0.25	0.75	3.14	0.4	1.73	NT	0.23	7.78	3.44	0.35	1.13	4.47	1.67	3.96	>100	2.24	4.93	27.8
	SN 12C	0.39	15.8	>100	0.45	21.6	>100	0.9	46.9	>100	0.31	NT	NT	0.44	>100	>100	2.22	>100	>100	4.38	>100	>100
	TK-10	0.51	2.73	19.2	0.66	6.19	28.9	2.9	12.3	>100	0.65	3.4	>100	0.61	4.36	34.5	3.85	33	>100	6.13	36	>100
	UO-31	0.23	1.22	NT	0.39	4.24	40.4	0.52	3.48	>100	0.35	1.46	51.8	0.26	1.44	>100	2.36	34.1	>100	3.71	58.6	>100
Prostate Cancer	PC-3	0.31	1.79	12.4	0.41	6.71	>100	1.39	8.73	>100	0.5	NT	>100	0.36	1.87	34.2	3.02	66.9	>100	3.2	18.6	>100
	DU-145	0.54	1.82	5	0.38	1.47	6.96	1.96	10.9	38.5	0.47	1.59	4.34	0.42	2.08	13	3.29	34.8	>100	4.91	35.4	>100
Breast Cancer	MCF7	0.29	1.4	4.83	0.44	3.34	40	0.4	5.67	62.2	0.4	1.65	NT	0.41	2.16	19.6	3	13	>100	2.99	11.4	66.5
	MDA-MB-231/ ATCC	0.76	13.4	>100	0.64	6.79	>100	2.56	>100	>100	0.74	NT	>100	1.21	2.71	>100	3.59	>100	>100	3.59	>100	>100
	HS 578T	0.04	0.31	81.3	0.43	-	>100	0.69	6.11	>100	0.35	2.74	>100	0.28	0.69	>100	2.75	48.6	>100	1.8	6.75	>100
	BT-549	0.3	3.82	>100	0.76	4.56	>100	0.96	3.87	>100	0.46	1.82	NT	0.31	2.34	>100	2.12	7.75	>100	2.06	8.08	>100
	T-47D	1.01	2.64	6.92	1.24	4.96	44.3	2.02	7.01	48.2	0.41	1.86	6.16	1.01	2.53	6.32	2.93	13.2	>100	6.5	3.92	>100
	MDA-MB-468	NT	NT	NT	0.32	2.36	44	1.17	4.42	>100	0.24	0.76	8.23	NT	NT	NT	2.24	5.81	>100	NT	NT	NT
Sum		27.1	-	-	42.6	-	-	166.4	-	-	29.5	-	-	36.1	-	-	155.5	-	-	220.2	-	-
Total cancer cell lines tested			57			59			58			58			58			58			59	
MID[a]			0.47			0.72			2.83			0.51			0.62			2.68			3.73	

[a]The average sensitivity of all cell lines toward the test agent in μM.

Table 2. The Average GI_{50} Calculated for Curcumin Analogues (3a-g) and Curcumin in μM Drug Concentration

Panel (Cancer cell line)	3a	3b	3c	3d	3e	3f	3g	Curcumin*
Leukemia	0.24	0.28	0.38	0.26	0.23	1.33	2.67	3.7
Non small cell lung cancer	0.42	0.94	1.85	0.66	0.58	3.2	3.52	9.2
Colon cancer	0.58	1.09	15.3	0.73	1	2.7	3.06	4.7
CNS cancer	0.55	0.67	1.05	0.48	0.8	2.7	3.24	5.8
Melanoma	0.4	0.61	0.75	0.56	0.51	2.56	3.25	5.9
Ovarian cancer	0.86	0.85	1.19	0.41	0.79	3.16	6.1	9.1
Renal cancer	0.37	0.63	1.26	0.42	0.51	2.65	4.31	8.9
Prostate cancer	0.43	0.39	1.68	0.49	0.39	3.15	4.05	11.2
Breast cancer	0.49	0.64	1.3	0.43	0.64	2.77	3.34	8.1

*GI_{50} values for curcumin were obtained from online NCI database (COMPARE data vector search, compound ID NSC 32982)

cell lines with average GI_{50} value ranging between 0.24 and 2.67 μM (Table 2). The average sensitivity of each compound (3a-g) on a particular panel of cell lines is shown in Figure 2. All these curcumin analogues showed comparatively higher activity than curcumin except for the compound 3c which showed maximum GI_{50} of 15.3 μM on colon cancer. The anticancer data of curcumin in Table 2 and Figure 2 were taken from the published work (Paul et al., 2014 and NCI database compound ID NSC 32982). All the curcumin analogues showed higher sensitivity towards a panel of leukemia cell lines. A dose response curve of each compound (3b-g) against a panel of leukemia cell lines is given in Figure 3. The average sensitivity (MID) of all cancer cell lines towards the test agents (3a-g) was calculated in μM. The data showed that the MID for compound 3a was found to be 0.47 μM which was found to be comparatively less than that of the MID calculated for other curcumin analogues (Table 1). The order of sensitivity of compound 3a towards different panels of cell lines followed as leukemia, renal cancer, melanoma, non small cell lung cancer, prostate cancer, breast cancer, CNS cancer, colon cancer and ovarian cancer (Figure 2). The dose response curve of compounds 3b-g is given in Figure 3 and the dose response curve of compounds 3a on the nine different panels of cell lines is given in Figure 4.

Discussion

Curcumin gained immense attention as a medicinal drug in modern medicine now a day. Undesirable side effect of synthetic pharmaceutical compelled us to search for natural approaches to disease prevention and treatment with the hope that naturally occurring compounds may be better tolerated than their synthetic counterparts. Hence we have taken curcumin as starting material and modified them to semi-synthetic analogues of curcumin as anticancer agent. All the compounds showed promising anticancer activity in single dose assay and met the pre-determine criterion of growth inhibition and thus further chosen for the NCI full panel of five dose assay at five different drug concentrations (0.01, 0.1, 1, 10 and 100 μM). In five the dose assay all the curcumin analogues showed higher values of sensitivity towards the panel of leukemia cell lines. The average sensitivity (GI_{50}) of compound 3a to all cancer cell lines (NCI 60 cell lines) was found to be the highest among the tested compounds. The best result of TGI was observed on SR (leukemia) with 0.09 μM by compound 3a. All the curcumin analogues showed GI_{50} between 0.03 μM (SR; leukemia) and 54.9 μM (SW620; colon cancer) and showed promising result in five dose assay. Some of the curcumin analogues reported earlier showed epidermal growth factor receptor (EGFR) tyrosine kinase as a potential target for anticancer activity (Ahsan et al., 2013). Furthermore we can say that the curcumin analogues evaluated here in five dose assay may perhaps targeted EGFR tyrosine kinase and showed anticancer activity. The anticancer activity of all these curcumin analogues are promising and hence could be subjected further for quantitative structure activity relationship (QSAR) studies to acquire more information and drug discovery.

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