Indian Journal of Experimental Biology
Vol. 55, December 2017, pp. 831-837

Hydroxyacetamide derivatives: Cytotoxicity, genotoxicity, antioxidative and metal chelating studies

Supriyo Saha[1], Dilipkumar Pal[2*] & Sushil Kumar[3]

[1]Department of Pharmaceutical Sciences, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research. Balawala, Dehradun-248 001, Uttarakhand, India

[2]Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (A Central University), Koni, Bilaspur-495 009, Chhattisgarh, India

[3] Faculty of Pharmacy, IFTM University, Moradabad, Uttar Pradesh, India

Received 28 April 2015; *revised 15 September 2016*

Cancer is one of the most life threatening noncommunicable diseases of mankind. In India, it has been estimated to have have caused 8.8 lakh deaths in 2016 alone, and 1.74 million more people are expected to be affected by 2020. Considerable research has gone into curing cancer including drugs based hydroxyacetamide derivatives. In the present study, we studied the cytotoxic, genotoxic, antioxidative and metal chelating activity of the synthesized hydroxyacetamide derivatives. We did brine shrimp lethality assay to examine cell toxicity effect, *Allium cepa* root tip genotoxicity assay to identify *in vitro* mutagenicity on mitotic cells, DPPH scavenging activity to measure its antioxidative property and metal chelating activity was used to synchronize the receptor enactment of the hydroxyacetamide subordinates.and histone deacetylase was a metalloenzyme, so Brine shrimp lethality assay of hydroxyacetamide derivatives (FP1-FP12) demonstrated that that best activity was exhibited by FP2 with 7.7 µg/mL in comparison to standard $K_2Cr_2O_7$ with 13.83 µg/mL. As per Meyer theory, all the synthesized molecules were cytotoxic in nature. Genotoxicity study by *Alium cepa* assay demonstrated that chromosomal aberrations were seen in all phases of mitosis and FP1, FP4, FP5, FP11, FP12 data showed statistically significant as compared to the standard cyclophosphamide. Amongst them, FP10 showed the best % mitotic index with various chromosomal disorders such as C-mitosis, chromosomal bridge and sticky chromosome. Antioxidant studies were performed by DPPH free radical scavenging, reducing power assay and metal chelating ability. All the synthesized molecules indicated better reducing property and FP4 was the best molecule with free radical scavenging property in comparison to the standard ascorbic acid. FP4 showed better metal chelating activity as compared to standard EDTA. As per the outcomes, the synthesized molecules can be utilized as a better anticancer molecule to treat cancer.

Keywords: Anticancer, Antioxidative property, HDAC, Metalloenzyme

Cancer is the most lethal noncommunicable disease affecting humans. The recent report by NCDIR-NCRP, Indian Council of Medical Research (ICMR) based on Population Based Cancer Registries while estimated the fatalities due to various types of cancer to be 0.88 million in 2016 alone, has projected its burdon to reach 1.74 million by 2020[1]. Among various approaches, hydroxyacetamide derivatives have also been shown their adequate potential in fighting against different etiological variables connected with tumor[2]. HDAC enzyme, one of the main focus in anticancer medication advancement, has been isolated into three different auxiliary classes as: a class (I/II) zinc dependent; and class (III) as NAD (nicotinamide adenine dinucleotide) subordinate. These catalysts are a piece of multiprotein structures, catalyzing the removal of acetyl gathering from a lysine store on protein, including histone[3]. HDACIs (histone deacetylase inhibitors) instigate tumor development hindrance, cell signification and customized cell passing. HDACI incites cell cycle capture and develops hindrance which is typically related to the transcriptional enactment of p21WAF1/CIP1 (cycling-subordinate kinase inhibitor 1 or CDK-cooperating protein), p27KIP1 (a cell cycle administrative protein that connects with cycling-CDK2 and CDK4, repressing the cell cycle movement at G1), GADD45 (development capture and DNA harm), restraint of cyclin A, cyclin D, and thymidylate synthetase[2]. Previously, hydroxyacetamide subordinates were assessed and found active against mutagenicity and bacterial infection[4].

*Correspondence
Phone: +91 7389263761 (Mob.)
E-mail: drdilip2003@yahoo.co.in

In this communication, hydroxyacetamide subordinates (FP1-FP12) were evaluated by brine shrimp lethality assay (BSLA), *Alium cepa* root tip genotoxicity assay, DPPH free radical scavenging antioxidant studies and reducing power assaymethod. Metal chelating assay was also performed by ferrozine reagent. BSLA has been utilized as an aide to analyze cell toxicity effect of the synthesized molecule[5,6]. The minimal effort, simplicity and accessibility of modest salt water shrimp eggs made BSLA as an exceptionally helpful system for cytotoxicity. Genotoxicity assay was performed utilizing *Allium cepa* root tips as a part of *in vitro* mutagenicity on mitotic cells[7]. Free radical scavenging activity[8] and reducing power assay were also portrayed with the cancer prevention property[9-11]. As HDAC is a metalloenzyme having Zn378 as cofactor, metal chelating activity was used to synchronize the receptor enactment of the hydroxyacetamide subordinates. All the synthesized molecules were especially designed for HDAC inhibitory effect; *in vitro* HDAC inhibitory activity was mentioned in our previous research article[12].

Materials and Methods

Synthesis of hydroxyacetamide derivatives (FP1-FP12)

All the molecules (FP1-FP12) were synthesized by three steps process as follows: (i) Synthesis of 2-chloro-N-hydroxyacetamide; (ii) General method for the preparation of 2-[(4-amino-3-phenyl/3-(2-hydroxy phenyl)-4H-1,2,4-triazol-5-yl) sulfanyl]-N-hydroxyacetamide; and (iii) Synthesis of 2-[[(3-substituted phenyl-[4-{(4-(substituted phenyl) ethylidine-2-Phenyl-1,3-Imidazol-5-One}] (-4H-1,2,4-triazol-5-yl) sulfanyl]-N hydroxyacetamide. The general method of synthesis is indicated at (Fig. 1). The compounds were recrystallized from ethanol. The UV spectroscopy of synthesized molecules were done by Shimadzu UV-1700 Spectroscopy and elemental analysis was performed using a micro-analytical unit. Melting points were checked using open capillary method Veego Electronics Apparatus. The structures were chemically analyzed by FTIR, 1H NMR, Elemental analysis and Mass spectrum data. The IR spectra for synthesized compounds were recorded on a Perkin Elmer (serial no: 78625) FTIR spectrophotometer. ^{1}HNMR spectra were recorded on Bruker Avance DRX300 300MHz FTNMR spectrometer using DMSO-d6 as solvent. The chemical shifts were measured at δ units (reported as ppm) relative to TMS and signals are reported as s

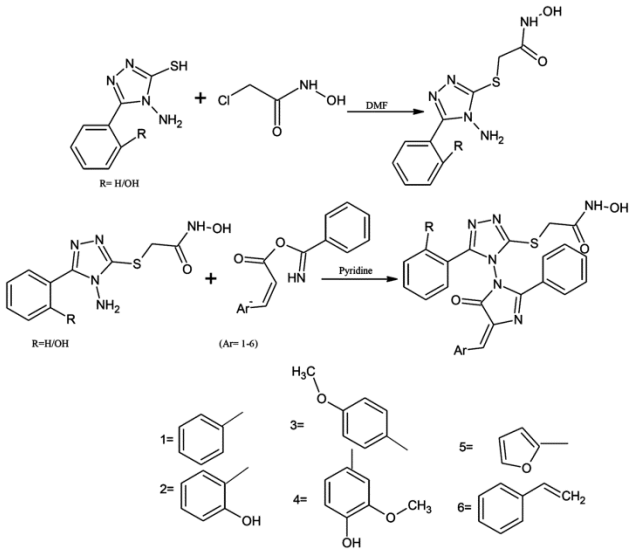

Fig. 1 — General procedure to synthesize FP1-FP12.

(singlet), d (doublet), t (triplet), q (quartet, m (multiple). Mass spectra were also recorded. All ^{1}HNMR and Mass spectra were performed in IICB, Kolkata and CDRI, Luck now. All chemicals were procured from sigma Aldrich, India, Mumbai and Merck India Ltd, Mumbai. All the reactions were routinely checked by precoated Merck thin layer chromatographic plate using toluene: methanol (9:1) as solvent system[12].

Brine shrimp lethality assay (BSLA)

Preparation of solutions for hydroxyacetamide derivatives (*FP1-FP12*)

Hydroxyacetamide derivatives solutions (FP1-FP12) were prepared by dissolving in DMSO (dimethyl sulfoxide) to get the stock solution of 1000 µg/mL concentration. The final concentration ranges of 1, 5, 10, 25, 50 and 100 µg/mL were prepared using ethyl alcohol as diluent. Pure DMSO and manufactured seawater were utilized as negative control and potassium dichromate solution was utilized as standard[13].

Culture, harvesting and experimental procedure for brine shrimp lethality assay

Brine shrimp eggs were procured from Sahaymatha Salterns Pvt Ltd, 13A/1, Pillaiyar Koil road Meenakshipuram, Tulicorin-628002 and stored at −20°C. Simulated seawater was prepared by dissolving 38 g of sea salt in 1000 mL of distilled water for bringing forth the shrimp eggs. The sea water was placed in a little plastic compartment (as bring forth chamber) with a part for dull (secured) and light regions. Shrimp eggs were included into the dim side of the chamber while the light over the other side

(light) will pull in the brought forth shrimp. Two days were allowed as incubation period to developed nauplii. Following two days, when the shrimp hatchings were prepared, 4 mL of the artificial seawater was added to every test tube and 10 brine shrimps were brought into every tube. In this way, there was a sum of 30 shrimps at every test tube. At this point the volume was made up with fake seawater up to 5 mL for each test tube. Then the test tubes were left revealed under the light. The quantity of surviving shrimps was checked and recorded over 24 h. Probit analysis was used to calculate the lethality focus (LC_{50}), which was surveyed at 95% certainty interims. LC_{50} of less than 100 ppm was considered as strong (dynamic). The mortality rate (% M) was computed by separating the quantity of dead nauplii by the aggregate number and afterward duplicated by 100%. This was to guarantee that the mortality of the nauplii was credited to the bioactive mixes among the combined atoms.

Allium cepa assay

Pretreatment

Alium cepa knobs were developed in faucet water at room temperature (20ºC) for 2-3 days. When the roots were 2-4 cm long, the globules were treated with the hydroxyacetamide derivatives solutions (FP1-FP12) and standard cyclophosphamide (both @ 1000 µg/mL), and (1:99) DMSO:water as a control. The arrangements were changed day by day and after 48 h, root tips from every globule was gathered, altered in Carnoy's fixative (1:3 acetic acid: liquor) for 24 h. It was then continued with slides planning in 70% alcohol[14].

Slides preparation

After pretreatment, the root tips were washed for a couple of minutes with distilled water. They were hydrolyzed with 1 N HCl at 60-70°C for 5 min. After hydrolysis, the roots were washed. At this point, around 1-2 mm of the root tip was cut and set on the slide. A little drop of aceto-orcein stain was dropped on the root tip and left for 2 min. After that, the root tips were squashed with metal poles and another little drop of aceto-orcein stain was included and left for another 2 min. The cover slip was deliberately brought down to maintain a strategic distance from air pockets and the sides of the slides were fixed with liquid paraffin[15].

Observation of specimens

The slides were seen under the light magnifying lens at 400×630 amplification. An Olympus light magnifying instrument with a computerized camera was used as a piece of solicitation to get the unmistakable picture of the chromosome distortions. Photomicrographs were made and 1000 cells every slide were investigated. The mitotic index, micronucleus formation in interphase and chromosomal abberations in mitotic stages was identified by the examination. The total experimental procedure was repeated in a triplicate manner to calculate mitotic index[16]. The mitotic index was obtained as follows:

Mitotic index = Number of cells in mitosis/Total number of cells.

Antioxidant studies

Free radical scavenging activity by DPPH method

One mL of DPPH methanolic solution (concentration 0.16 mmol/L) was added to each hydroxyacetamide derivative solutions (FP1-FP12) with concentration range 10-100 µg/mL[10]. The mixtures were vortexed for 1 min and after that they were left to stay at room temperature in dark. After 30 min, absorbance was measured at 517 nm for each sample[15].

Radical scavenging activity was calculated by DPPH free radical method by utilizing $A_{control}$ (absorbance of the control, no antioxidant was included) and A_{sample} (absorbance of the hydroxyacetamide solution). Dyed DPPH solution, arranged by including 1.0 mL of DPPH arrangement (c = 0.16 mmol/L) to 1.0 mL ascorbic acid solution (g = 1 mg/mL), was utilized as a blank. DPPH radical scavenging was calculated considering the concentration that scavenges 50% of DPPH free radical and along these lines having RSA = 50%. The antioxidant activity of ascorbic acid as standard reference was also assayed[17].

Reducing power assay

One mL of significant concentration of hydroxyacetamide solutions (FP1-FP12) were blended with 2.5 mL phosphate buffer and 2.5 mL of potassium ferricyanide. The mixtures were incubated at 50°C for 20 min. 2.5 mL of trichloroacetic acid was added to the mixture, then it was centrifuged at 3000 RPM for 10 min[18]. About 2.5 mL of upper layer was brought and blended with 2.5 mL distilled water and 0.5 mL of ferric chloride solution and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture was indicative of increased reducing power[19].

Metal chelating ability

One mL of significant concentration of hydroxyl-acetamide solutions (FP1-FP12) with concentration

range (10-100) µg/mL were added to 0.1 mL of $FeCl_2$ solution with concentration of 2 mmol/L. The response was initiated by the addition of 0.2 mL ferrozine solution (c=5 mmol/L). The mixtures were shaken overwhelmingly and left at room temperature for 10 min[4]. When the response was reached to harmony, absorbance was set at 562 nm. Metal chelating activity was figured by absorbance of the negative control $A_{control}$ (no chelating specialists was included) and A_{sample} (absorbance of the hydroxyl-acetamide solution). Metal chelating activity of hydroxyacetamide derivatives (FP1-FP12) was calculated as EC_{50}, *i.e.* the concentration that chelates 50% of Fe2+ particles. Ethylenediamine tetraacetic acid (EDTA) was used as a standard for this test[20].

Statistical analysis

All outcomes from brine shrimp lethality and genotoxicity assay were validated using one way ANOVA followed by Dunnett's t test with $*P < 0.05$, 95% level of centrality. Factual examination was performed by Graphpad Prism 6.0 statistical tool.

Results

Characterization of the hydroxyacetamide derivatives (FP1-FP12)

All the characterization of synthesized molecules (FP1-FP12) was chemically characterized by FTIR, ^{1}H NMR, Elemental analysis and Mass spectrometry information (EIMS) which anticipated the formation of the structure. As per the spectral data of compound FP1-FP6, FTIR and ^{1}H NMR showed presence of characteristic peak at 1600, 3100 and 3300 cm^{-1} (for C=O, -OH and -NH stretching), 3000 cm^{-1} (phenyl group), 1380 cm^{-1} (for etheric linkage) and δ around 2.500, 7.200, 7.800 and 3.38 ppm due to hydroxamic OH, Aromatic -H, -NH and $-CH_2$ group, respectively but the presence of para amino group of the triazole moiety δ 5.793 ppm was missing in the final set of compound. In case of compounds FP7-FP12, as per the ^{1}H NMR spectroscopy, spectral data at δ5.622 ppm indicated the absence of para amino group in the triazole moiety. Further, the structures were established by mass spectra data in accordance to their molecular formula.

Brine shrimp lethality assay

From the resuls of brine shrimp lethality assay (Fig 2), it is found that with the exception of FP7, all other molecules have noteworthy r^2 value of 0.9323 and F value of 74.55 in comparison to standard $K_2Cr_2O_7$ as standard. The activity diagram follows the order as: FP2>FP6>FP9>FP11=FP12. The best activity was demonstrated by FP2 with 7.7 µg/mL.

Allium cepa assay

The aggregate results are reported Figs. 3 and 4. The outcomes from *Alium cepa* gentoxicity assay demonstrated that with the exception of FP1, FP4, FP5, FP11, FP12; all other molecules were measurably critical with r^2 value of 0.9630 and F value of 140.2 in examination to standard. The activity order as % mitotic index was as follows: FP8>FP3=FP6>FP2>FP1>FP5 and the % Mitotic Index of standard cyclophosphamide and FP10 were 4.2 and 2.4, respectively. However, % mitotic index of the control was 7.9.

Antioxidant studies

Free radical scavenging activity by DPPH method

Antioxidant property of all the synthesized molecules (FP1-FP12) were performed by DPPH free radical scavenging activity using ascorbic acid as standard substance. The activity order was as follows: FP4>FP3>FP5>FP11>FP6 (Fig. 5). Among them FP4

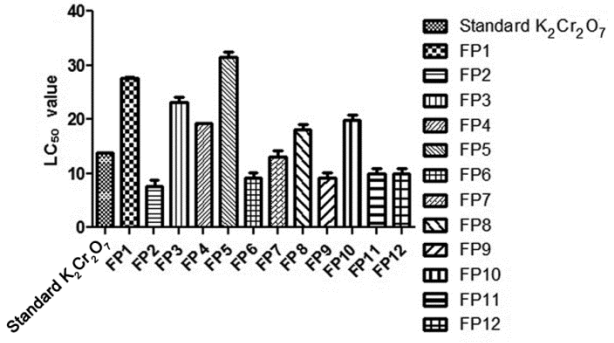

Fig. 2 — Brine shrimp lethality assay data of FP1-FP12 with standard potassium dichromate.

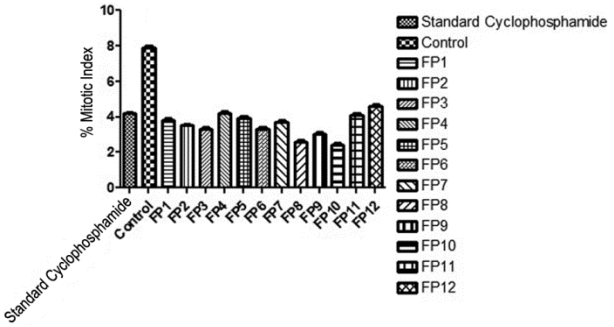

Fig.3 — Genotoxicity by *Alium cepa* data of FP1-FP12 with standard cyclophosphamide.

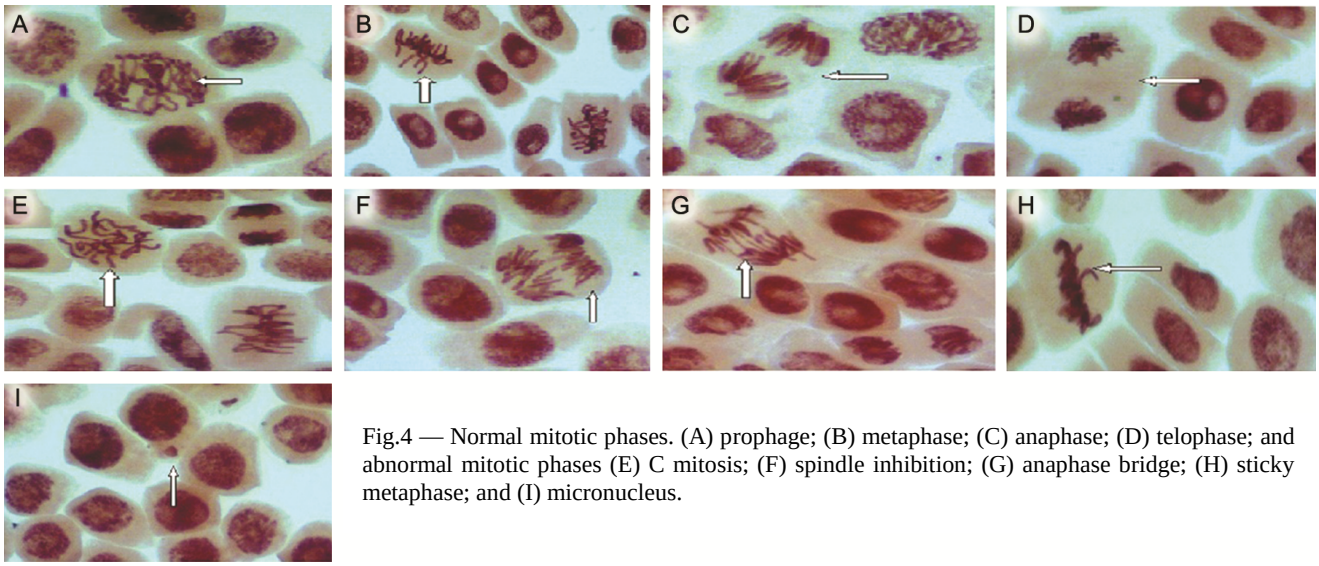

Fig.4 — Normal mitotic phases. (A) prophage; (B) metaphase; (C) anaphase; (D) telophase; and abnormal mitotic phases (E) C mitosis; (F) spindle inhibition; (G) anaphase bridge; (H) sticky metaphase; and (I) micronucleus.

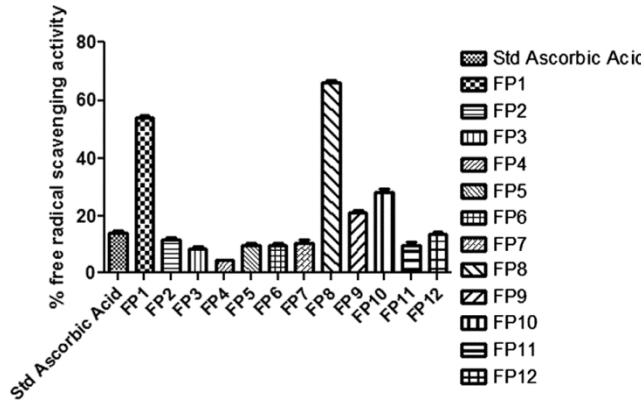

Fig. 5 — DPPH scavenging data of FP1-FP12 with standard ascorbic acid.

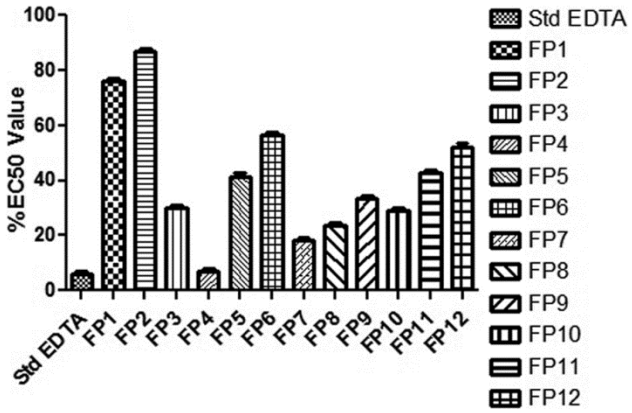

Fig. 6 — Metal chelating activity data of FP1-FP12 with standard EDTA.

demonstrated the best scavenging action having IC_{50} value of 4.25 µg/mL as FP4 molecule contain 4-hydroxy-3-methoxy phenyl group bound with phenyl linked triazolated hydroxyacetamide, whereas IC_{50} value of standard ascorbic acid was 14.33 µg/mL. The outcomes from free radical scavenging action demonstrated that with the exception of FP2, FP7, FP12 and all other molecules were statistically significant with r^2 value of 0.9879 and F value of 441.7 in comparison with the standard.

Reducing power assay

All the synthesized molecules were tested according to the potassium ferricyanide procedure and their absorbance were measured by UV-Vis spectrophotometer using ascorbic acid as standard. Amongst the test substances, FP1, FP2, FP3, FP4 which contain phenyl,2-hydroxy phenyl,4-methoxy phenyl,4-hydroxy-3-methoxy phenyl group bound with phenyl linked triazolated hydroxyacetamide showed maximum reducing power (Table 1). It was also found that absorbance level was increased with increasing concentration level.

Metal chelating ability

The results (Fig. 6) from the metal chelating activity shows that with the exception of FP4, all other molecules were measurably significant in examination to standard EDTA with r^2 value of 0.9903 and F value of 550.6. Among the test samples, EC_{50} value of FP4 was found as 6.90 µg/mL, whereas the value is 5.92 µg/mL for standard EDTA with. In spite of the fact that HDAC receptor is a metal initiated enzyme with Zn378 as cofactor, the metal chelating activity mirrored that FP4 ties effortlessly with the receptor dynamic site.

Table 1 — Reducing power assay of compound FP1-FP12 with the reference standard ascorbic acid

Synthesized molecule	Concentration (µg/mL)	Absorbance
FP1	10	0.5652
	20	0.6934
	30	0.8097
	40	0.9448
	50	1.1844
FP2	10	0.6355
	20	0.7559
	30	0.9310
	40	1.0592
	50	1.1274
FP3	10	0.7588
	20	0.8903
	30	1.0572
	40	1.1526
	50	1.2461
FP4	10	0.8678
	20	0.9533
	30	1.1752
	40	1.2846
	50	1.4146
FP5	10	0.7728
	20	0.8525
	30	0.9255
	40	1.0812
	50	1.2102
FP6	10	0.7908
	20	0.8352
	30	0.9265
	40	1.0132
	50	1.1802
FP7	10	0.6836
	20	0.7721
	30	0.9289
	40	1.0122
	50	1.1145
FP8	10	0.3373
	20	0.5423
	30	0.6873
	40	0.8467
	50	0.9199
FP9	10	0.3862
	20	0.4924
	30	0.6321
	40	0.7718
	50	0.8523
FP10	10	0.3376
	20	0.4678
	30	0.6265
	40	0.7256
	50	0.8580
FP11	10	0.3727
	20	0.4590
	30	0.6039
	40	0.7348
	50	0.8344
FP12	10	0.3910
	20	0.4478
	30	0.6136
	40	0.7293
	50	0.8421
Standard Ascorbic Acid	10	0.4216
	20	0.5623
	30	0.6789
	40	0.7914
	50	0.8835

Discussion

According to Meyer LC_{50}[21] estimation brine shrimp lethality assay, LC_{50} value of less than 1000 µg/mL is lethal while LC_{50} value of more than 1000 µg/mL is nonpoisonous. Hence, all the synthesized molecules are confirmed to be cytotoxic in nature. Amongst the synthesized molecules, FP2 showed the best cytotoxic activity. In case of genotoxicity study, chromosomal aberrations were seen in all phases of mitosis. At high fixation 1000 µg/mL, sticky chromosomes and chromosomal scaffolds were the most well-known chromosome variations watched. *Alium cepa* assay empowered the evaluation of critical hereditary endpoints, which are indication of mitotic file and chromosome deviation. Mitotic list is portrayed by the aggregate number of separating cells in cell cycle[22]. Mitotic record is utilized as a pointer of cell expansion biomarkers which measures the extent of cells in the mitotic period of the cell cycle. Subsequently, the decline in the mitotic record of *A. cepa* in meristematic cells can be deciphered as cell passing. A few sorts of chromosome abnormalities are considered in the four periods of cell division (prophase, metaphase, anaphase and telophase) to assess chromosomal anomalies[23]. Chromosome deviations investigations permit estimation of genotoxic impacts, as well as empower assessment of their clastogenic and aneugenic activities[24]. The low mitotic record reflects direct genotoxic impact. The cells of *A cepa* root tip after treatment with integrated particles arrangement indicated diminished in mitotic record. FP1, FP4, FP5, FP11, FP12 were statistically significant data as compared to cyclophosphamide but FP10 showed the best % mitotic index[25]. Various chromosomal disorders such as C-mitosis, chromosomal bridge and

sticky chromosome were suggested that the drug molecule may became a better anticancer molecule. FP4 showed better scavenging property which confirm that molecule with ortho hydroxyl group or para hydroxyl-3-methoxy group bound with phenyl linked hydroxyacetamide scaffold has the capability to serve as an anticancer agent. FP4 showed better metal chelating ability[26] in comparison to the standard EDTA molecule. So all molecules may deliver as a boon for our mankind to conquer cancer manifestation and other related diseases and add some new light in this research area[27,28].

References

1 NCDIR-NCRP report, *Incidence, Distribution, Trends in Incidence Rates and Projections of Burden of Cancer*, Three-year report of Population Based Cancer Registries (2012-2014) [NCDIR-NCRP (ICMR), Bengaluru], 2016.

2 Huong TTL, Dung DTM, Dung PTP, Huong PT, Vu TK, Hahn H, Han BW, Kim J, Pyo M, Han SB, Nama NH, Novel 2-oxoindoline-based hydroxamic acids: synthesis, cytotoxicity, and inhibition of histone deacetylation. *Tetrahed Lett*, 56 (2015) 6425.

3 Peng FW, Wua TT, Zi-Wei Ren ZW, Xue JY, Shi L, Hybrids from 4-anilinoquinazoline and hydroxamic acid as dual inhibitors of vascular endothelial growth factor receptor-2 and histone deacetylase. *Bioorg Med Chem Lett*, 25 (2015) 5137.

4 Pal DK & Saha S, Hydroxyacetamide — A novel molecule for anticancer therapy. *J Adv Pharm Tech Res*, 3 (2012) 92.

5 Wang CY & Lee LH, Mutagenicity and antibacterial activity of hydroxamic acids. *American Soc Microbiol*, 11 (1977) 753.

6 Pal D, Banerjee S & Ghosh A, Dietary Induced Cancer Prevention: An Expanding Research Arena of Emerging Diet, related to health care system. *J Adv Pharm Tech Res*, 3 (2012) 16.

7 Mondal A, Maity TK, Pal D & Sannigrahi S, HPTLC Determination of Phenolic Compounds and antioxidant activity of the aerial parts of Melothria heterophylla Lour Cogn. *Int J Drug Develop & Res*, 2 (2010) 750.

8 Peter F & Tomaz A, Allium Chromosome Aberration Test for Evaluation Effect of Cleaning Municipal Water with Constructed Wetland (CW) in Sveti Tomaz, Slovenia. *J Bioremed Biodeg*, 4 (2013) 1.

9 Nayak AK, Pal D, Pany DP & Mohanty B, Evaluation of *Spinacia oleracea* L. leaves mucilage as an innovative suspending agent. *J Adv Pharm Tech Res*, 1 (2010) 338.

10 Asif M & Acharya M, Bioactivity of 6-Aryl-4,5-dihydropyridazin-3(2H)-ones using Brine Shrimp (Artemia salina) lethality assay. *Int J Phar Chem*, 3 (2013) 40.

11 Pal D & Mitra S, A preliminary study on the *in vitro* antioxidant activity of the stems of *Opunta vulgaris*. *J Adv Pharm Tech Res*, 1 (2010) 268.

12 Ding C, Chen S, Zhang C, Hud G, Zhang W, Li L, Chen YZ, Tan C & Jiang Y, Synthesis and investigation of novel 6-(1,2,3-triazol-4-yl)-4-aminoquinazolin derivatives possessing hydroxamic acid moiety for cancer therapy, *Bioorg & Med Chem*, 25 (2017) 27.

13 Saha S, Pal DK & Kumar S, Design, synthesis and antiproliferative activity of hydroxyacetamide derivatives against HeLa cervical carcinoma cell and breast cancer cell line. *Trop J Pharm Res*, 15 (2016) 1401.

14 Geethaa S, Surash R, Sreenivasan S, Nizam MM, Sabariah I & Sharif MM, Brine shrimp lethality and acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. seed methanolic extract. *Pharmacogn Res*, 2 (2010) 215.

15 Rank J & Nielsen MH, *Allium cepa* anaphase–telophase root tip chromosome aberration assay on *N*-methyl-*N*-nitrosourea, maleic hydrazide, sodium azide, and ethyl methanesulfonate. *Mutat Res Genet Toxicol Environ Mutagen*, 39 (1997) 121.

16 Lacine A, Erdi K, Yasin A, Zeyneb A & Mustafa K, Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic Thermopsis turcica. *Saudi J Bio Sci*, 20 (2013) 235.

17 Shah AJ, Lakkad BC & Rao MV, Genotoxicity in lead human lymphocytes evaluated by micronucleus and comet assays. *Indian J Exp Biol*, 54 (2016) 502.

18 Jayanthi P & Lalitha P, Reducing power of the solvent extracts of *Eichhornia Crassipes* (Mart.) SOLMS. *Int J Pharm Pharm Sci*, 3 (2011) 126.

19 Mandal A, Maity T & Pal D, Oxygen dependent regulation of Ehrlich Ascites carcinoma cell respiration by garlic acid and rutin isolated from *Melothria heterophylla* (lour) Cogn. *Asian J Chem*, 24 (2012) 2648.

20 Nimse SB & Pal D, Free Radicals, Natural antioxidants, and their reaction mechanisms. *RSC Advances*, 5 (2015) 27986.

21 Yadav AS & Bhatnagar D, Free radical scavenging activity, metal chelation and antioxidant power of some of the Indian spices. *Biofactors*, 31 (2007) 219.

22 Meyer BN, Ferrigini NR, Putnam JE, Jacobson LB, Nichols DE & Mclaughlin JL, Brine Shrimp: a convenient general bioassay for active plant constituents. *J Med Plant Res*, 45(1982) 31.

23 Feretti D, Zerbini I, Zani C, Ceretti E, Moretti M & Monarca S, Allium cepa chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. *Food Addit Contam*, 24 (2007) 561.

24 Kwan YP, Ibrahim D, Umi KY, Chen Y & Sreenivasan S, Genotoxicity of *Euphorbia hirta*: an *Allium cepa* assay. *Molecules*, 17 (2012) 7782.

25 Aparadh VT, Naik VV & Karadge BA, Antioxidative properties (TPC, DPPH, FRAP, metal chelating ability, reducing power and TAC) within some *Cleome* Species. *Ann. Bot (Roma)*, 2 (2012) 49.

26 Pal D, Sannigrahi S & Mazumder UK, Analgesic and anticonvulsant Effects of isolated saponin from the leaves of *Clerodendrum infortunatum* Linn. in Mice, *Indian J Exp Biol*, 47 (2009) 743.

27 Pal D & Mazumder UK, Isolation of compound and CNS depressant activities of *Micania scandens* Willd with special emphasis to brain biogenic amines in mice. *Indian J Exp Biol*, 52 (2014) 1186.

28 Rani P, Pal DK, Hegde RR & Hashim SR, Leuckart synthesis and pharmacological assessment of novel acetamide derivatives. *Anticancer Agent Medicinal Chem*, 16 (2016) 898.