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Cytotoxic activity of 1, 2-dihydroquinoline derivatives.

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Abstract: Earlier we reported the synthesis, characterisation antimicrobial, anti-inflammatory activity of twelve novel 1,2-Dihydroquinoline derivatives. In extension of this work, cytotoxic activity of some of the 12 novel compounds was carried out using MTT assay. From the analysis, it was observed that most of the compounds exhibited varied degree of cytotoxic activity. Among 12 synthesized compounds eight were selected, the compounds, 0402 and 0407 exhibited significant cytotoxic activity and the remaining compounds showed moderate activity. The observed activities may be due to the presence of sulphonamido, fluoro benzyl and amido moieties containing dihydroquinoline nucleus.

Keywords: 1,2-dihydroquinoline derivatives, cytotoxicity, MTT assay

Introduction

Cancer is one of the leading causes of adult deaths worldwide. In India, the International Agency for Research on Cancer estimated indirectly that about 635,000 people died from cancer in 2008, representing about 8% of all estimated global cancer deaths and about 6% of all deaths in India [1]. The absolute number of cancer deaths in India is projected to increase because of population growth, urbanization, industrialization, lifestyle changes and increasing life expectancy. World Health Organization (WHO) has estimated that the cancer deaths in India are projected to increase to 1,200,000 by 2020. Lip, Oral cavity & Pharynx Cancer in Men & Cervical Cancer in woman is the most common cancer responsible for the death in Indians.

Cytotoxicity is the quality of being toxic to cells. The cytotoxic compounds can be used to kill cancerous cells [2]. Treatment of cancer through cytotoxicity is called cytotoxic therapy. Chemotherapy and radiotherapy are forms of cytotoxic therapy. Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. They are based on various cell functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. Scientists have established different methods such as Colony Formation method, Crystal Violet method, Tritium-Labelled Thymidine Uptake method, MTT, and WST methods, which are used for counting the number of live cell. Quinoline is a heterocyclic aromatic organic compound containing nitrogen[3,4] atom as a part of the ring system[5]. Pharmaceutically, a wide variety of quinoline derivatives possess a broad range of bioactivities such as antimicrobial, anticancer, anti-malarial, antibiotic, antihypertensive, platelet-derived growth factor - receptors tyrosine kinase inhibition, DNA-intercalating carrier, anti-inflammatory, analgesic, anti-HIV, antitumor, DNA binding[6-17] capability, and many other functional material. Literature survey reveals that quinoline derivatives possess cytotoxic/anticancer activity. However, very few reports are available on the cytotoxic activity of dihydroquinolines. Hence we planned to conduct cell viability and cytotoxic activity for synthesized compounds by MTT assay. The MTT assay is based on that, the Tetrazolium **salts** form highly coloured and insoluble **Formazans** after reduction.

In the foregoing section [18-20], we preferentially wanted to shape our study in a manner to get an efficient knowledge about the target oriented development of dihydroquinoline derivatives.

Materials and Methods

Synthesis and characterization of 12 novel 1, 2-Dihydroquinoline derivatives were achieved and their antimicrobial activity was earlier reported by us [21, 22]. Briefly, total 12 derivatives were synthesised from three diverged schemes (Scheme 1, 2 & 3). The characterization of these derivatives was carried out by Melting points, IR, 1H NMR, 13C NMR and LC-MS data. FT-IR Spectra was recorded using Agilent Carry 630 FTIR with ATR instrument. 1H NMR and 13C NMR were recorded in Bruker model avance II (399.65 MHz, 1H NMR) and Bruker model avance II (100.50 MHz, 13C NMR) instruments respectively and analysis were carried out either DMSO-d6 or CD3OD depending on solubility of the compound. All the chemical Shifts

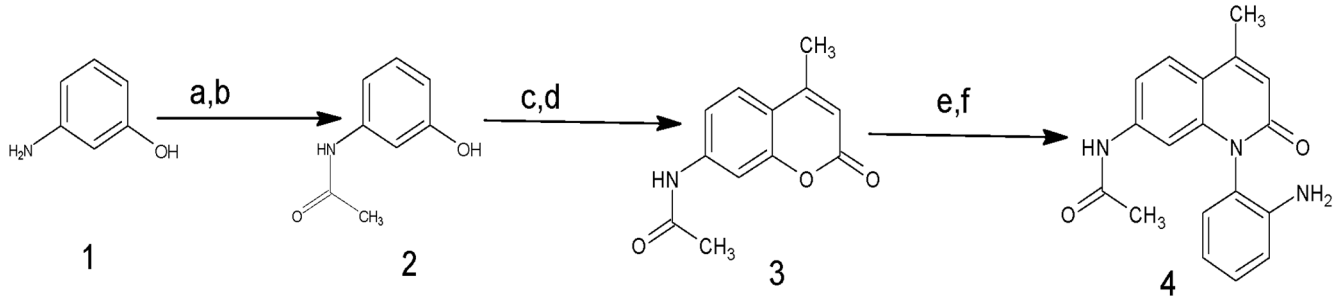
were reported in parts per million (ppm). LC-MS was recorded using Waters Alliance 2795 separations module and Waters Micromass LCT mass detector. Elemental analysis (C, H and N) was performed on an Elementar vario MICRO cube. The purity of the compound was confirmed using TLC on pre-coated silica gel plate and further purification was done using column chromatography.

Chemicals:

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS),Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Synthetic route for preparation of 1,2-dihydroquinoline derivatives is represented in **scheme 1**

Preparation of 1,2-dihydroquinoline



3-aminophenol (1) on acetylation(a,b) gives 3-hydroxyacetanilide or metacetamol (2), which on cyclisation with ethyl acetoacetate and 70% sulphuric acid gives N-(4-methyl-2-oxo-2H-chromen-7-yl)acetamide(3);further substitution with O- phenylene diamine and sodium acetate in glacial acetic acid gives of *N*-[1-(2-aminophenyl)-4-methyl-2-oxo-1,2-dihydroquinolin-7-yl]acetamide (4). Different 1,2,-dihydroquinoline derivatives were synthesised.

Cell lines and Culture medium

MCF-7 (Breast carcinoma), cell line was procured from National Centre for Cell Sciences (NCCS), une, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Experimentation Procedure for the preparation of *N*-(3-hydroxyphenyl) acetamide (metacetamol) (2):

Compound **(1)** (0.11 mol, 25g) was dissolved in acetic anhydride (a; 80 mL) and the reaction mixture was stirred at 60°C for 8 h at room temperature under nitrogen atmosphere. The excess acetic anhydride was removed under reduced pressure; the residue was dissolved in methylene dichloride (b; MDC), washed with water. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated to obtain compound **(2).** LCMS: 152 (M+1), MP: 152 °C. Yield: 82%

Procedure for the preparation of *N*-(4-methyl-2-oxo-2*H*-chromen-7-yl) acetamide (3):

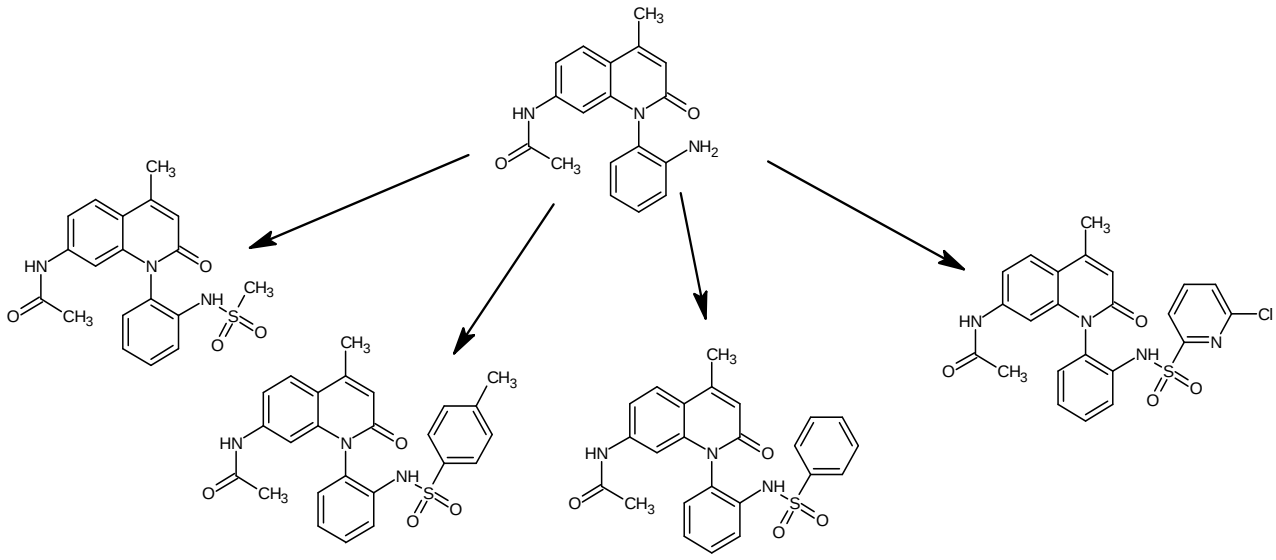
A mixture of 3-hydroxy acetanilide (metacetamol) (0.1 mole, 15.1g) and ethylacetoacetate (c, 0.1mole) with 70% sulphuric acid (d, 50 ml) was heated carefully for 5 h. The resulting solution was cooled and poured over crushed ice (250 g). The crude product was filtered off and washed repeatedly with water, dried and recrystallized from hot water to result in title compound (**3).** LCMS: 205 (M+1), MP: 243 °C. Yield: 62%.

Procedure for the preparation of *N*-[1-(2-aminophenyl)-4-methyl-2-oxo-1,2-dihydroquinolin-7-yl]acetamide (4):

A mixture of *N*-(4-methyl-2-oxo-2*H*-chromen-7-yl) acetamide (0.01 mole, 2.17g), o-phenylenediamine (e; 0.01 mole, 1.08g) and sodium acetate (f; 5 g) in glacial acetic acid (15 ml) was refluxed for 8 h and cooled. The separated solid was filtered and recrystallized from methanol: water (1:2) to give title compound (**4).** LCMS: 237.8 (M+1), MP: 285 °C. Yield: 70%.

General Procedure for the preparation of sulphonamides containing dihydroquinoline nucleus (0401 to 0404):

Equimolar quantities of compound (**4)** (0.001 mol, 0.5 g), and different substituted sulphonyl chlorides (0.001 mol) such as methyl-, p-tolyl-**,** phenyl- and 2-chloro pyridyl-sulfonyl chloride, and tetra ethyl amine (TEA, 0.003 moles, 0.57 g) were stirred in dry MDC (10 mL) under nitrogen condition at room temperature for 12 h. The reaction was monitored by TLC; mixture was washed with water and brine. The organic phase was dried over Na_2SO_4 and evaporated on vacuum. Residue was purified by column chromatography using petroleum ether:ethyl acetate as eluent (7:3) to get sulphonamides dihydroquinoline nucleus **(0401 to 0404)** in good yield **(scheme 2)**.

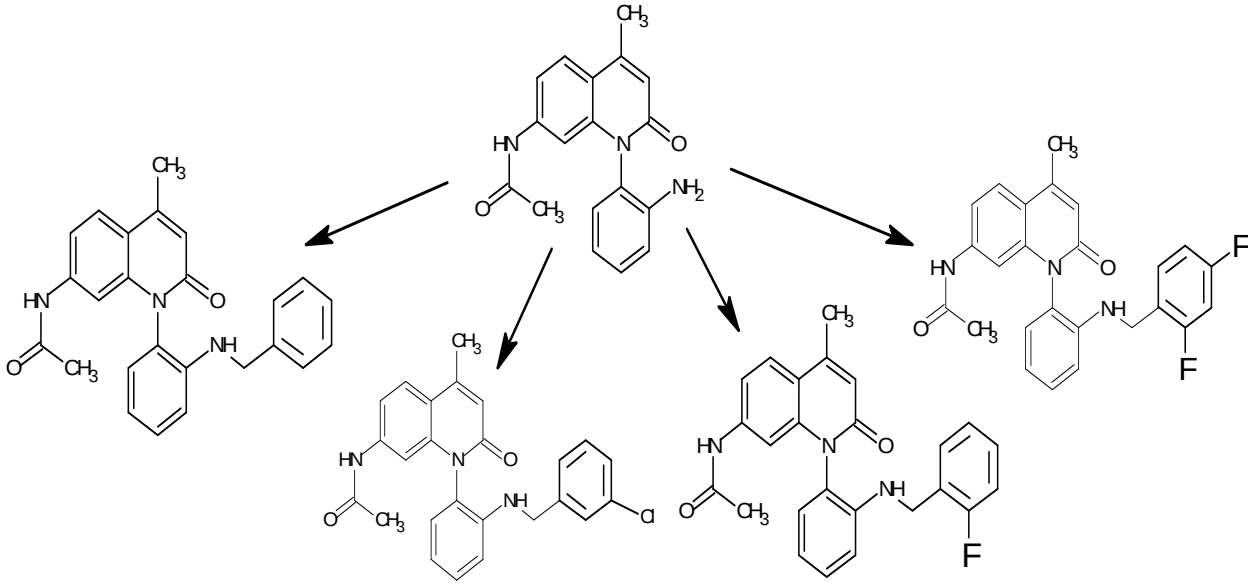


04 01 **04 02** **0403** **0404**

Scheme 2

General Procedure for the preparation of benzylated dihydroquinoline nucleus (0405 to 0408):

Equimolar quantities of compound **(4)** (0.5 g, 0.001 mol) and different substituted benzyl bromides (0.001 mol) such as benzyl-, 2-chloro benzyl-, 2-fluoro benzyl- and 2,4-difluoro benzyl bromides**,** K_2CO_3 (0.003 moles, 0.57 g), were stirred in dry ACN (10 mL) under nitrogen at room temperature for 10 h. The reaction was monitored by TLC and reaction mixture was filtered. The organic phase was dried over anhydrous Na2SO4 and evaporated on (under) vacuum. Residue was purified by column chromatography using petroleum ether: ethyl acetate as eluent (8:2) to get benzylated dihydroquinolinenucleus **(0405 to 0408)** in good yield **(scheme 3).**

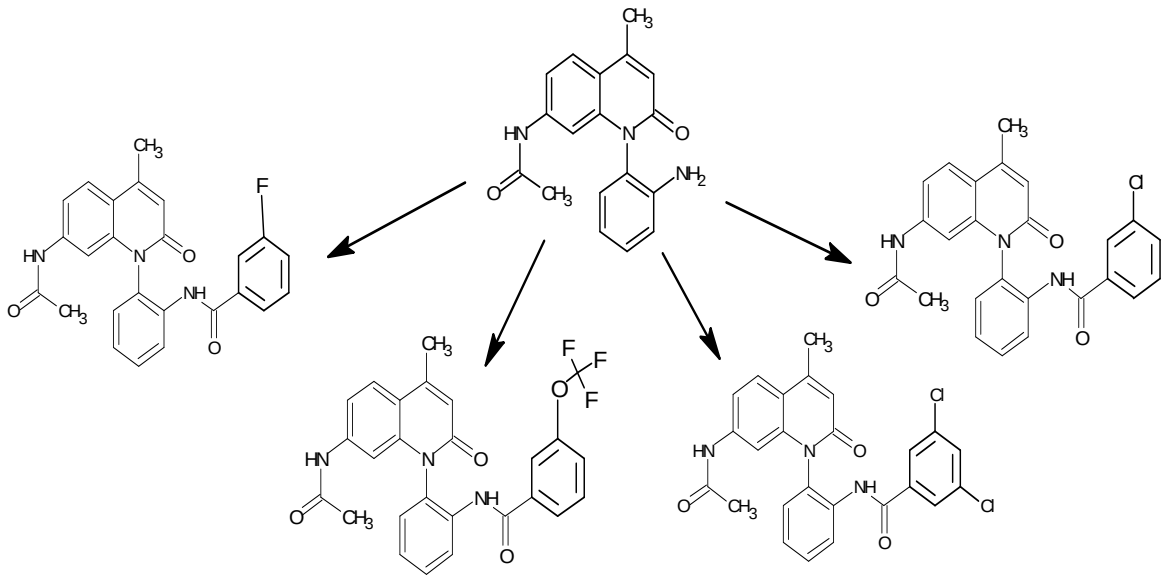


0405 **0406** **0407** **0408**

Scheme 3

General Procedure for the preparation of amides containing dihydroquinoline nucleus (0409 to 0412):

Equimolar quantities of compound (**4)** (0.001 mole, 0.5 g) and different substituted acid chlorides (0.001 mol) such as 3-fluoro benzoyl-**,** 3-trifluoromethoxyl benzoyl-, 3,5-dichloro benzoyl- and 3-chloro benzoyl-chlorides,TEA (0.003 moles, 0.57 g), were stirred in dry MDC (10 mL) under nitrogen at room temperature for 10 h. The reaction was monitored by TLC, reaction mixture was washed with water and brine. The organic phase was dried over anhydrous Na_2SO_4 and evaporated on (under) vacuum. Residue was purified by column chromatography using petroleum ether:ethyl acetate as eluent (7:3) to get amides containing dihydroquinoline nucleus**(0409 to 0412)** in good yield. These derivative are represented in **scheme 4.**



0409 0410 0411 0412

Scheme 4

Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

Principle: The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

MTT ASSAY:

Measurement of cell viability and proliferation by MTT assay where the tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means.

Procedure of MTT assay:

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 micro titre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, wash with medium. 100 µl of different concentrations of test drugs and were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} * 100$$

Results and Discussion:

The cytotoxic effects of some newly synthesized compounds of dihydro quinolones derivatives against the MCF-7 (Breast carcinoma) cells were determined using the standard MTT-dye reduction assay for cell viability. The cytotoxic potential of the free ligands was

evaluated as well. The retrieved MTT-formazan absorption values are summarized in Table 1. The 72 h exposure of both cell lines with the tested compounds resulted in a concentration dependent reduction of cell viability as assessed by the MTT-dye reduction assay, which enabled the construction of concentration–response curves. In addition the corresponding CTC_{50} values were derived in order to allow a quantitative merit for assessment of the relative potencies of the agents under investigation (**Table 1**).

Table 1: Cytotoxic properties of test drugs against MCF-7 cell line

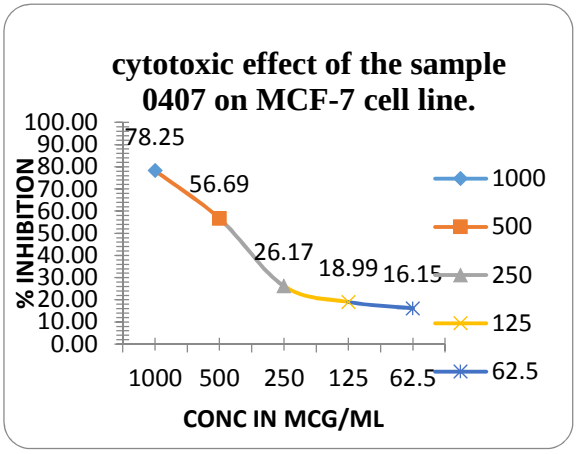
Sl. No	Name of Test sample	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	**0407**	1000 500 250 125 62.5	78.25±0.6 56.69±0.5 26.17±0.7 18.99±0.1 16.15±0.1	446.70±0.4
2	**0408**	1000 500 250 125 62.5	17.21±0.2 15.00±0.3 12.40±0.2 7.90±0.2 5.39±0.2	>1000±0.00
3	**0410**	1000 500 250 125 62.5	20.31±0.2 17.43±0.2 14.40±0.2 10.48±0.1 8.17±0.4	>1000±0.00
4	**0401**	1000 500 250 125 62.5	18.47±0.3 15.15±0.3 10.95±0.4 6.84±0.1 3.80±0.3	>1000±0.00
5	**0404**	1000 500 250 125 62.5	26.79±0.5 22.59±0.2 18.15±0.2 13.97±0.2 11.63±0.3	>1000±0.00
6	**0402**	1000 500 250 125 62.5	58.50±0.3 21.52±0.2 17.32±0.1 13.13±1.8 8.46±0.2	893.33±0.5

7	**0409**	1000 500 250 125 62.5	24.37±0.4 20.02±0.3 17.32±0.3 10.53±0.2 6.98±0.4	>1000±0.00
8	**0411**	1000 500 250 125 62.5	27.62±1.1 21.70±0.3 18.55±0.4 11.77±0.6 5.72±0.4	>1000±0.00

The human breast cancer cell line MCF-7 was treated with some synthesised dihydroquinoline derivatives. Among the eight (0407, 0408, 0410, 0401, 0404, 0402, 0409 and 0411) tested compounds, 0407and 0402 showed significant cytotoxic effect in comparison with the control. The rest of the compound has varied cytotoxic effect. The inhibitory concentration per cent (CTC 50%) was estimated and the varied results among the samples are shown in **Table (1)**.

Figure 1.Graphical representation of cytotoxic effect of drugs on MCF-7 cell lines.

1.



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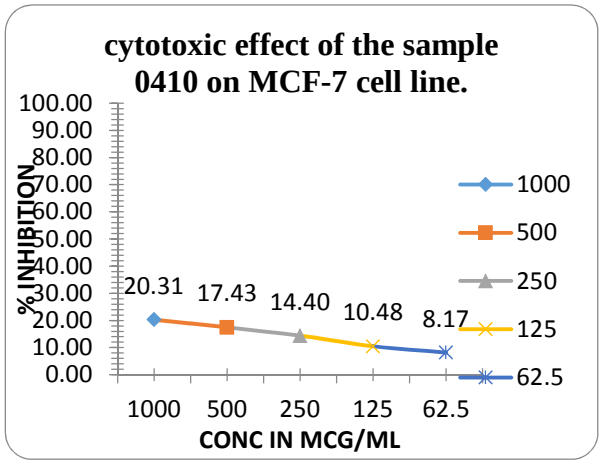

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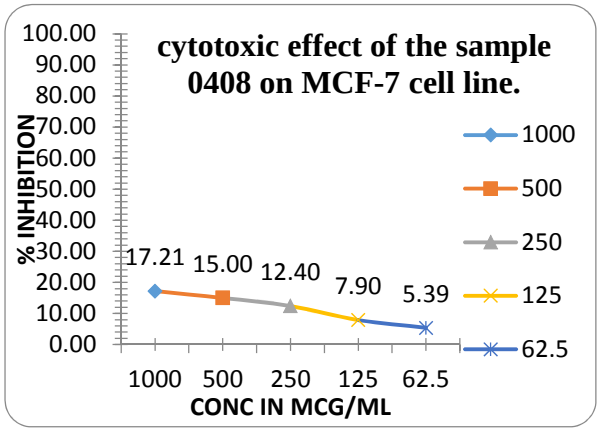

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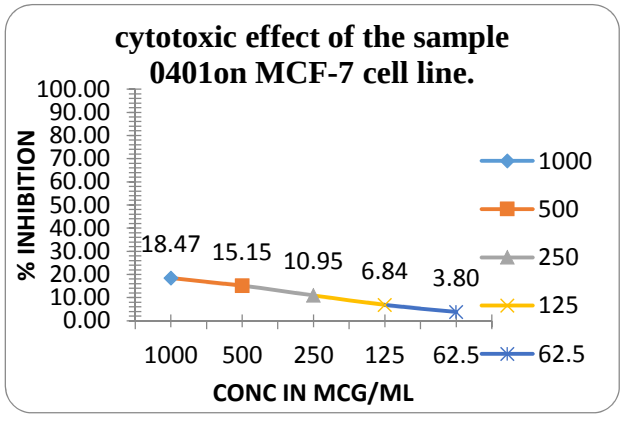

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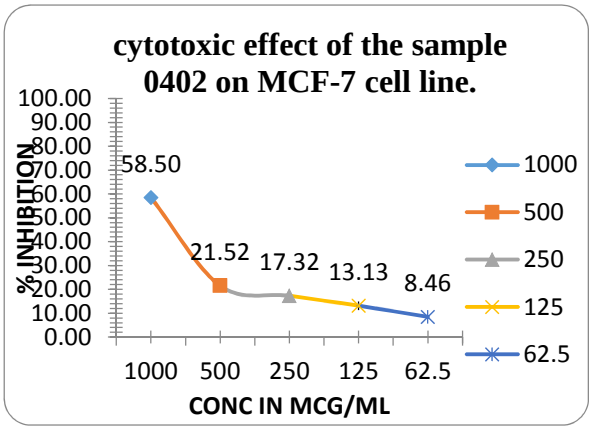

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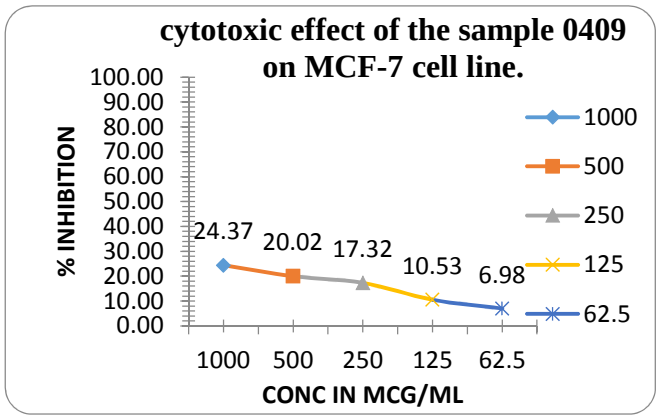

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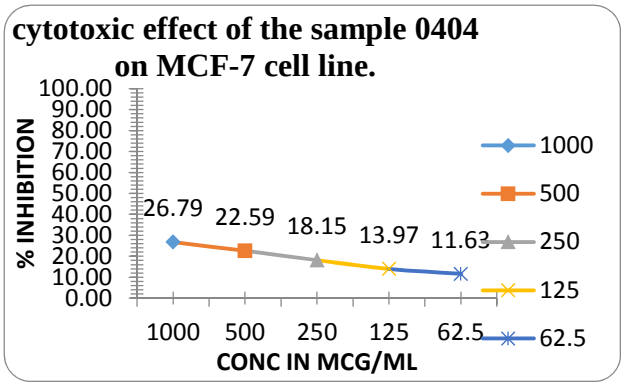

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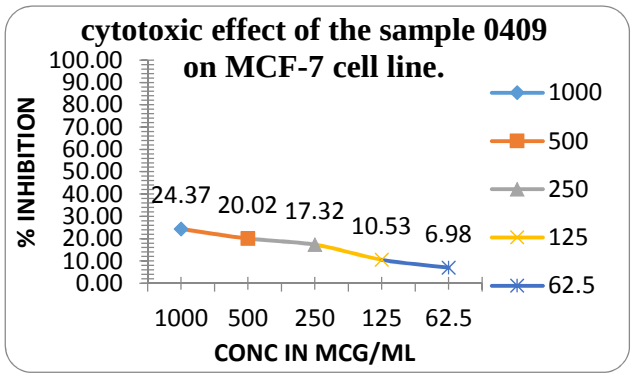

The cytotoxic effect of the tested compounds may be observed due to structure activity relationship possessing N-aniline, 2-keto, 4-methyl, 7-amido, fluoro benzyl and sulphonamide as a substituent.

Conclusions

In the present research, some 1,2-dihydroquinoline derivatives were synthesized and screened for their cytotoxic activities. The compounds 0407, 0402 possess significant cytotoxic activity and the remaining compounds showed moderate activity. The observed activities may be due to the presence of sulphonamido, fluoro benzyl and amido moieties containing dihydroquinoline nucleus. Among 12 synthesized derivatives of novel 1,2- dihydroquinoline, eight were selected for cytotoxic activity. All tested compounds showed promising activity and possess active pharmacophore for further studies.

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