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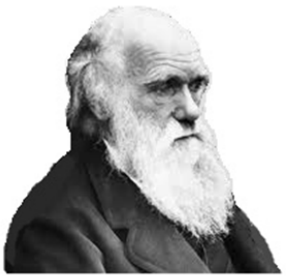

World News of Natural Sciences

An International Scientific Journal

WNOFNS 30(2) (2020) 203-212 EISSN 2543-5426

Novel Synthesis, spectral, characterization of 4,5-diphenyl-1-((tetrahydrofuran-2-yl)methyl)-2-(3,4,5-trichlorophenyl)-1H-imidazole and its applications of molecular docking, anticancer activity

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ABSTRACT

In the present study of 4,5-diphenyl-1-((tetrahydrofuran-2-yl)methyl)-2-(3,4,5-trichlorophenyl)-1H-imidazole 1 was synthesized. The synthesized imidazole compound 1 has been characterized by FT-IR, ^{1}H, ^{13}C NMR and ESI-Mass spectral studies. Molecular docking is also performed in order to explain the over-expression of estrogen receptor in 70% of liver cancer. The imidazole scaffold is a privileged scaffold for exploration of anticancer agents. The objective of the present study is to evaluate the anticancer activity of imidazole 1 in human liver cancer cell lines HepG2.

***Keywords*:** Imidazole; molecular docking; HepG2; cytotoxicity

1. INTRODUCTION

The imidazole ring represents an important class of heterocycles, which have been widely applied in natural products, biological fields and pharmaceutical industries [1]. They possess different biological properties, viz. antitumor, anti-HIV, herbicides, fungicides, antibacterial, anticonvulsant, antioxidant, antihypertensive, anti-allergy, anticancer, analgesic, anti-inflammatory, FTase, and p38 MAP kinase inhibitory activities [2-10].

(Received 10 March 2020; Accepted 01 April 2020; Date of Publication 02 April 2020)

Hence, some diseases such as gastric disease, heart disease, migrain disease are rectified by imidazole derivatives available drugs shown in **Fig 1**. It has been one of the best compound of medicinal chemistry field [11, 12]. Multicomponent reaction is biologically processed that causes a specific cell cycle block and it has been used as a stimulation for the design of new anticancer agents [13]. The excellent therapeutic properties of imidazole-related drugs have encouraged medicinal chemists to synthesize a large number of novel chemotherapeutic agents [14]. The potency and wide applicability of the imidazole pharmacophore can be attributed to its hydrogen bond donor-acceptor capability, as well as its high affinity for metals [15]. Multicomponent reaction is also used as plant growth regulators and therapeutic agents, dye super sensitized star cells (DSSCs), organic light emitting diodes (OLED) [16], and nonlinear optics (NLO) [17]. In this study, new 4,5-diphenyl-1-((tetrahydrofuran-2-yl)methyl)-2-(3,4,5-trichlorophenyl)-1H-imidazole 1 was synthesized and characterized by the spectral studies. However, this IC_{50} is categorized in moderate potential 4,5-diphenyl-1-((tetrahydrofuran-2-yl)methyl)-2-(3,4,5-trichlorophenyl)-1H-imidazole **1** and the aim of this study is to find the best-modified imidazole that binds well with ERα by replacement of the carbonyl and nitryl group of that imidazole that binds well with the Erα [18].

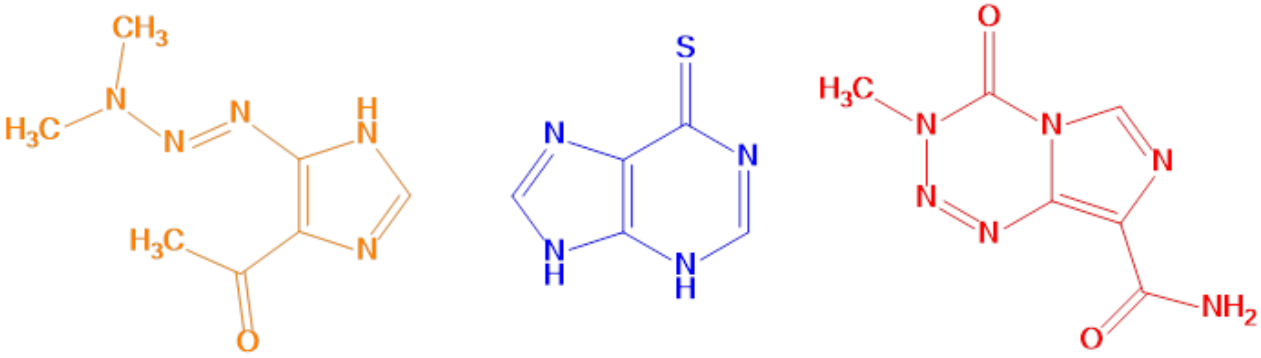


Fig. 1. Chemical structures of some imidazole-based anticancer drugs

2. EXPERIMENTAL

2. 1. Materials and Methods

All synthesis grade chemicals and reagents are purchased from Sigma-Aldrich (India). Elemental analysis has been conducted using a Thermo Scientific (FLASH 2000) Elemental Analyser. FT-IR (Fourier transform infrared) spectra are recorded between 400-4000 cm^{-1} wave number range on a Nicolet Avatar 330 FT-IR Spectrometer by the KBr pellet method. The ^{1}H and ^{13}C NMR spectrum was recorded on a Bruker Spectrometer. Mass spectrum was measured on a SCIEX-API 2000 ESI-MS spectrometer using electron spray soft ionization technique.

2. 2. Synthesis of 4,5-diphenyl-1-((tetrahydrofuran-2-yl)methyl)-2-(3,4,5-trichlorophenyl)-1H-imidazole 1

A mixture of benzil (1 mol), 3,4,5-Trichlorobenzaldehyde (1 mol), tetrahydro-furfurylamine (1 mol) and ammoniumacetate (1 mol) are taken in 50 mL round bottom flask and this mixture is dissolved in 25 mL ethanol. The reaction mixture is refluxed at 90 ºC for 8

h. The product is poured in ice water to get brown colour resin which is separated and dried. The final product is purified by column chromatography using silica gel (100-120 mesh) and the solvent petroleum ether and ethylacetate (8:2) as the eluent (**Figure 2**). Yield: 92%; M.P: 139 ºC, dark yellow solid, molecular formula: $C_{26}H_{21}Cl_3NO_2O$. FT-IR (KBr, cm^{-1}): 3056 (CH stretching); 1599 (C=N); 1319 (C-O ring stretching); 1498 (C=C); ^{1}H NMR (400MHZ, $CDCl_3$, ppm): δ = 3.86 (q, CH_2, 6H); 7.09-7.62 (m, aromatic protons), 1.51 (m, 1-H, 3-H). ^{13}C NMR (100 MHZ, $CDCl_3$ ppm); δ = 48.79 (C-6); 141.14 (C-8); 126.38-138.04 (aromatic Carbons) 152.60 (C=N) ipso carbon (C-23). ESI-MS (m/z): calcd. 482.07, found: 482.26 [M^{-1}].

2. 3. Molecular docking Procedure

2. 3. 1 Protein preparation

The imidazole 1 structure was subjected to molecular docking simulation and 3D structure-based pharmacophore models are employed to identify the molecular interactions of α-mangostin and its derivatives against estrogen receptor α (ERα) (PDB ID: 3ERT) are obtained from Brook-haven Protein Data Bank (RCSB) (http://www.rcsb.org/pdb). The protein was pre-processed and prepared using the protein preparation wizard. The unwanted protein chains and water molecules are deleted using Review and modify panel. The OPLS-2005 force field is used to minimize the complex. The grid is created using Receptor grid generation panel and imported in GLIDE Docking.

2. 3. 2 Ligand Preparation

The receptor 1 structure was drawn using Chem Office 8.0 and saved in SDF file format. The imidazole 1 structure file is imported to project table Maestro and minimized using OPLS 2005 (Optimized Potential for Liquid Stimulation) force field. The EPIK program is used to neutralize the charge and ionization of the compound.

2. 3. 3 Molecular Docking

The in-silico prepared protein and ligand are used for Molecular docking using Glide. XP docking (Extra Precession) is used to identify the interaction between protein and ligand.

2. 4. Cell culture

The HepG2 (human liver cancer cell lines) cell line (NCCS, Pune, India), are grown in DMEM supplemented with 10% FBS and antibiotics (penicillin-100 µg/mL; streptomycin-50 µg/mL). Cells are cultured at 37 ºC in 95% air, 5% CO_2 incubator.

2. 4. 1. Anticancer activity, Cell viability assay

The cytotoxicity of imidazole compound 1 was tested against HepG2 cell lines using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells are seeded into a 96-well plate at a density of 1.5×10^4 cells per well and incubated in medium containing imidazole **1** at concentration ranging from 0.9 to 500 µM for 48 h. Triplicate wells are maintained for each treatment, 100 µL of MTT added to each well. It is incubated at 37 ºC for 4 h to allow MTT to the formation of formazan crystals by reacting with MTT and metabolically active cells. The medium with MTT is discarded from the wells carefully. Each well is added with 100 µL of DMSO to dissolve intracellular formazan crystals, and the plates

are shaken for 10 min. Using enzyme-linked immunosorbent assay (ELISA) reader, absorbance is read out at 405 nm. The cell images are examined using a fluorescence microscope. The percentage of survival is calculated using the formula: % survival = [live cell number (test)/live cell number (control)] × 100.

3. RESULTS AND DISCUSSION

3. 1. Chemistry

In the present study, we prepared the compound 1 by mixing of benzil, Trichlorobenzaldehyde, tetrahydro- furfurylamine and ammoniumacetate in ethanol. The synthesis routine of compound 1 is given in Figure 2. The obtained data, such as FT-IR, NMR, Mass data, confirmed the formation imidazole 1.

3. 2. Molecular docking studies

Molecular docking is performed in order to evaluate how small molecule and the target macromolecule fit together. This can be useful for developing better drug candidates and also for understanding the nature and extent of binding of molecules with the receptor. Therefore, molecular docking studies are carried out in order to explain *in silico* cytoxicity studies, a specific protein tyrosine kinase 3ERT, (**Fig. 3a**) is identified as the target for cytotoxicity compounds. Their PDB file is obtained from the protein data bank and used after removal of all bound water, ligands and co-factors. The molecular docking studies have been carried out to evaluate the binding affinity of imidazole molecule 1 with these enzymes (Fig. 3**b**). Several important interactions from the docking pose are observed notably LEU 525, ILE 424, MET 421, GLY 420, GLU 419, VAL 418ASP 351, GLU 353, ARG 394, LEU 391, MET 388, LEU 387, LEU 384, TRP 383, LEU 428, LYS 520, GLY 521, MET 522, MET343, LEU346, THR 347, LEU 349, ALA 359, and HIF 524 between the receptor and imidazole molecule 1 (Fig. 3**c**) in proper binding orientation.

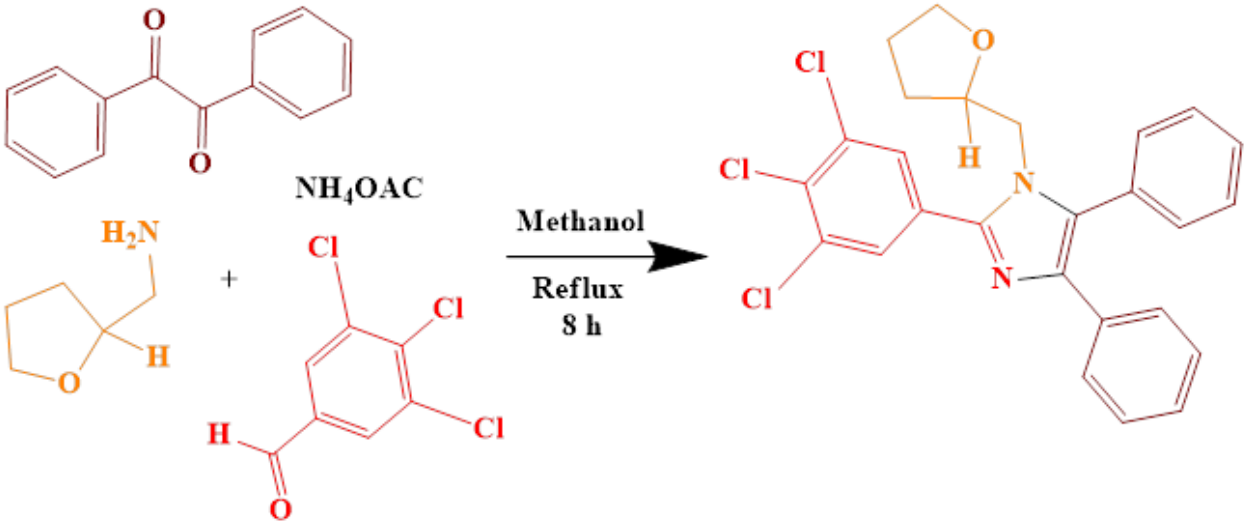
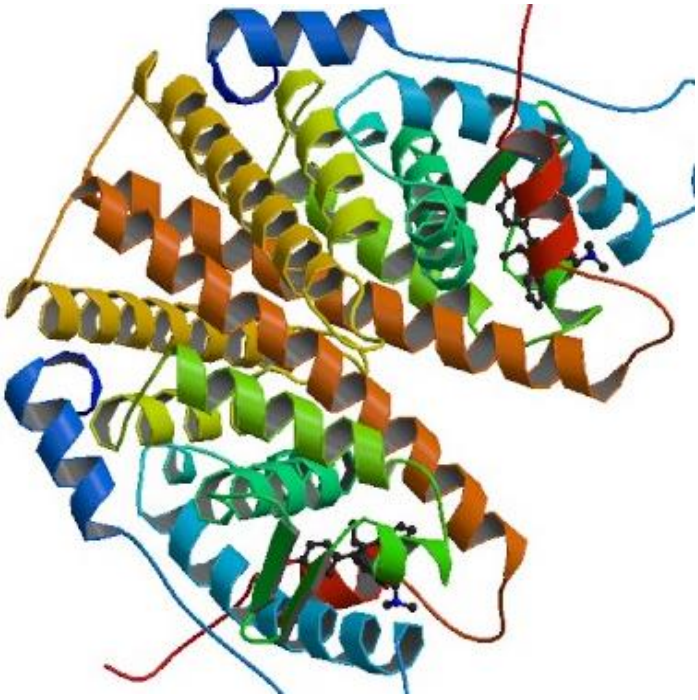
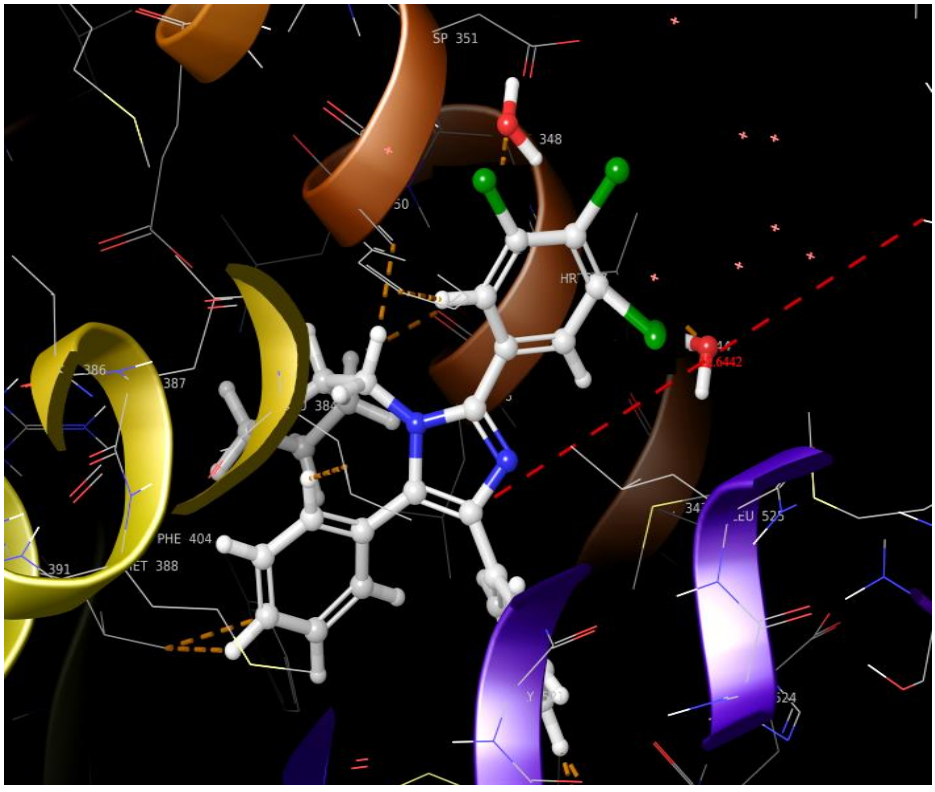


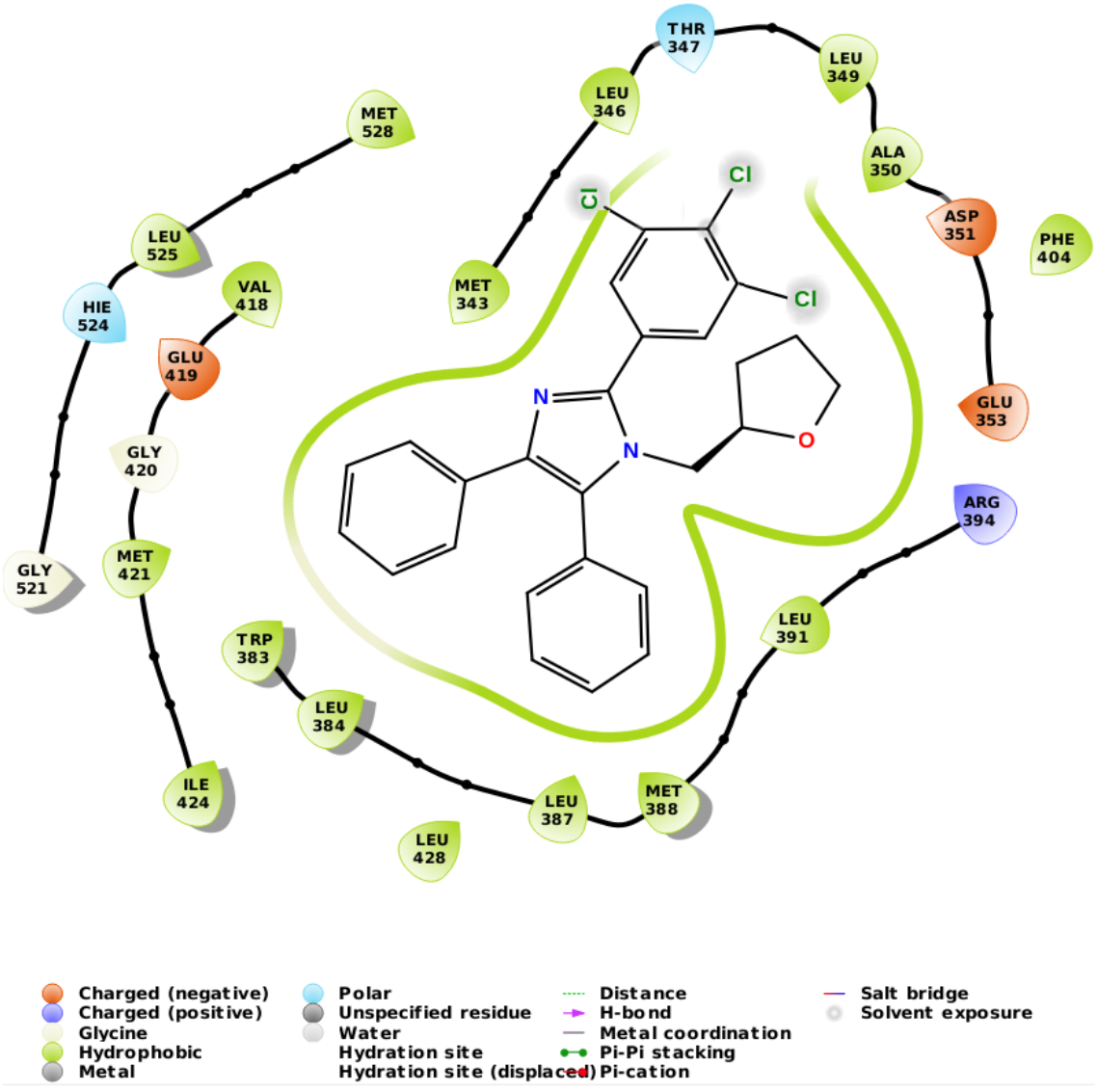
Figure 2. Synthetic procedure of imidazole compound 1.



(a)



(b)



(c)

Fig. 3. (a) Molecular docking study of PDB ID: 3ERT with compound 1, and **(b, c)** the various non-covalent interactions between the compound 1/3d and 2d images.

The binding score from iGEMDOCK is found to be –7.22 kcal/mol, due to the cumulative vander Wall contribution and H-bonding interactions. Ligand map (Fig. 3b) is used to visualize comprehensively the basic receptor-imidazole interactions of the docked compound and binding site region of the receptor. Number of secondary interactions, such as hydrophobic, hydrogen bonds and vander Waals forces and their pattern is assigned with the help of diagram generated by Molegro Molecular Viewer software.

This map helps us to analyze the way molecule interacts with the ligand. The Figures showed that the molecule strongly interacts with the amino acids LEU 525, ILE 424, MET 421, GLY 420, GLU 419, VAL 418ASP 351, GLU 353, ARG 394, LEU 391, MET 388, LEU 387,

LEU 384, TRP 383, LEU 428, LYS 520, GLY 521, MET 522, MET343, LEU346, THR 347, LEU 349, ALA 359, and HIF 524 shown in **Table 1**.

These strong interactions help the compound to bury well inside the cavity of the target protein and acts as a potent cytotoxicity agent. Moreover, the geometrical descriptor of the compound is also carried out to show the extent of the surface interaction of the imidazole molecule 1 (Fig. 3c). The green circle represents the minimal projection area, while the yellow circle to the maximal projection area.

These area is associated with the van der Waals radius around the molecule. Interactions between drugs and amino-acids residue of targeted enzymes/proteins are relied in general on the geometrical descriptors, i.e. the molecular surface area, molecular volume, principal moments of inertia of molecule, solvent accessible, molecular surface area, etc. of the exploited drugs that associated with the binding there by boosting its activity as these are related to the three-dimensional structure.

Table 1. Molecular docking studies of compound 1

Comp.	glide gscore	glide evdw	glide ecoul	glide energy	Interacting Residues
1	–7.221	–28.531	–0.619	–26.351	LEU 525, ILE 424, MET 421, GLY 420, GLU 419, VAL 418ASP 351, GLU 353, ARG 394, LEU 391, MET 388, LEU 387, LEU 384, TRP 383, LEU 428, LYS 520, GLY 521, MET 522, MET343, LEU346, THR 347, LEU 349, ALA 359, HIF 524,
glide evdw = van der Waals interaction energies, glide ecoul = Coulomb interaction energies					

3. 3. Cytotoxicity

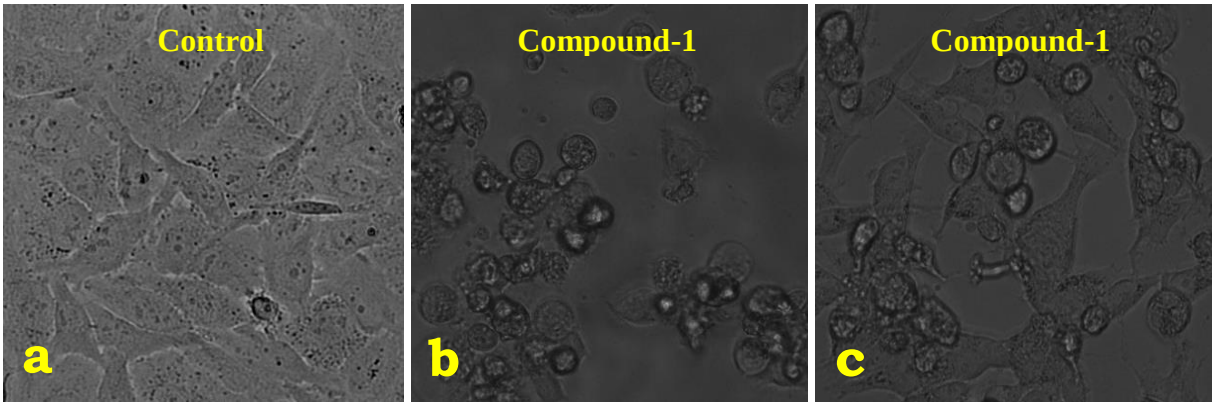


Fig. 4. Live cell images of imidazole compound **1**: (a) before and (b-h) after HepG2 cell lines treatment with imidazole compound **1** examined by fluorescence

The cytotoxicity responses of compound 1 with various concentrations are added and it is clearly evident from the cellular imaging. It is efficient candidate for monitoring changes in the intracellular concentration under certain biological conditions, and it has justified its cytotoxicity, MTT assay in HepG2 cancer cells treated with various concentrations of compound 1 for up to 5 h. The compound 1 shows significant cytotoxicity effects on HepG2 cancer cells for at least up to 4 hours at 20 μM (**Fig. 4**).

The synthesized compound 1 was examined for cytotoxic activity on HepG2 cell line by means of MTT test that allows us to assess the effect of complexes on cellular mitochondrial metabolism. Cells were tested for two days with increasing concentrations of tested compound. Microscopic images of control cancer cells and apoptotic morphological changes in HepG2 cell line treated with compound 1 was given in Fig. 4.

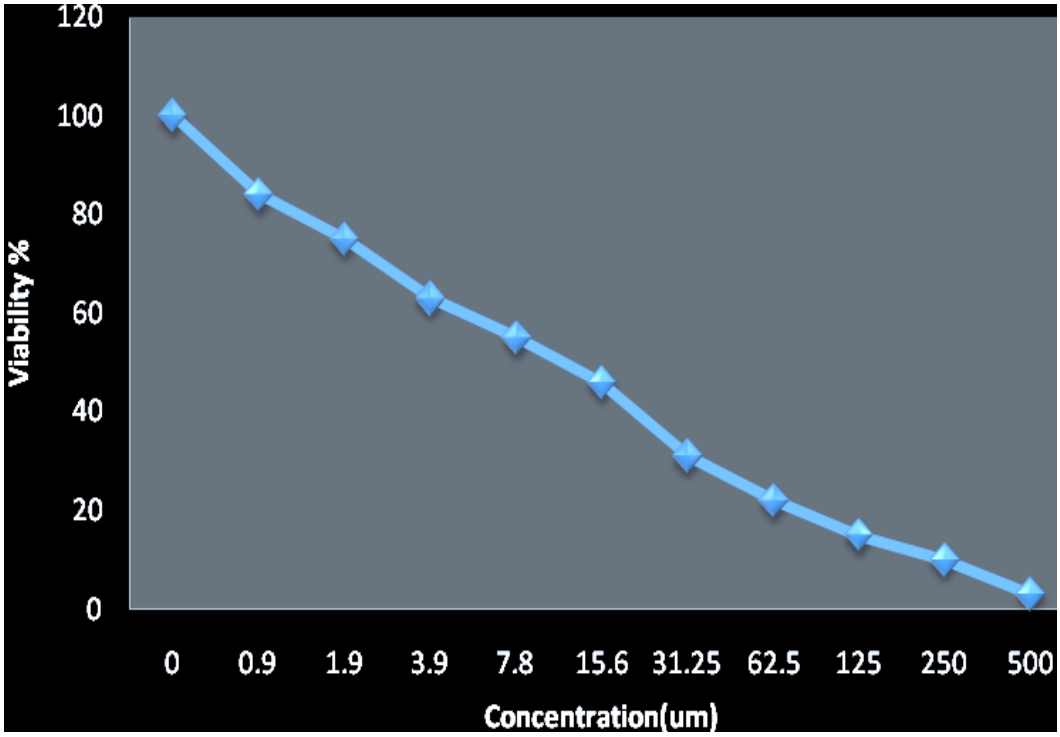


Table 2. The IC_{50} values of compound **1** against HepG2 cell lines.

Anticancer effect of compound 1 on HepG2 cell line	
Concentration (μM)	**Cell Viability %**
	1
0	100
0.9	84
1.9	75

3.9	63
7.8	55
15.6	46
31.25	31
62.5	22
125	15
250	10
500	3

The results showed that compound 1 has cytotoxicity and IC_{50} values of synthesized compound are shown in **Fig. 5**. The compound 1 exhibits broad inhibition on the HepG2 cell lines with IC_{50} values of 45.98%. The compound 1 possessed a more potent inhibitory effect against the cancer cells and it has shown the highest IC_{50} value. It is convincing us to suggest that the electronic effect may be one of the factors in determining the anticancer activities of compound 1. The IC_{50} values of compound 1 against HepG2 cell lines results is given in **Table 2**.

4. CONCLUSION

Overall, the novel imidazole derivative of compound **1** was successfully synthesized and characterized by FT-IR, ^{1}H, ^{13}C NMR and Mass spectral studies. Molecular docking has been performed in order to explain the response of the imidazole derivative against human liver cancer cell lines HepG2. The preliminary *in-vitro* anticancer investigation of novel imidazole has indicated the anticancer potency of the compound. The compound **1** indicated a significant anticancer activity against HepG2 human hepatocellular carcinoma cell line. The novel imidazole compound was proved to be the best anti-breast cancer agent.

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