

SYNTHESIS AND CHARACTERIZATION OF CYCLOHEXANE-1,3-DIONE DERIVATIVES AND THEIR *IN SILICO* AND *IN VITRO* STUDIES ON ANTIMICROBIAL AND BREAST CANCER ACTIVITYRAJA CHINNAMANAYAKAR¹, EZHILARASI MR^{1*}, PRABHA B¹, KULANDHAIVEL M²¹Department of Chemistry, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India. ²Department of Microbiology, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India. Email: ezhilarasi.au@gmail.com

Received: 29 October 2018, Revised and Accepted: 13 December 2018

ABSTRACT**Objective:** The objective of this study was to evaluate *in silico* and *in vitro* anticancer activity for synthesized cyclohexane-1,3-dione derivatives.**Methods:** The new series of cyclohexane-1,3-dione derivatives were synthesized based on the Michael addition reaction. Further, the structures of the synthesized compounds were confirmed by Fourier-transform infrared spectroscopy, ¹H nuclear magnetic resonance (NMR), and ¹³C NMR spectral data. Then, the *in silico* molecular docking studies were carried out using AutoDock tool version 1.5.6 and AutoDock version 4.2.5.1 docking program. The antimicrobial activity was carried out using the agar disk diffusion method, and the *in vitro* anticancer activity was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for the synthesized compound.**Results:** *In silico* docking study, compound 5c showed good binding score and binding interactions with selected bacterial proteins and breast cancer protein. Further, compound (5a-5h) was tested for their antimicrobial activity and compound 5c was only tested for anticancer activity (human breast adenocarcinoma 3,4-methylenedioxyamphetamine-MB-231 cell line). Compound 5c was found to be the most active one of all the tested compounds. In the MTT assay compound, 5c showed the LC₅₀ value of 10.31±0.003 µg/ml. In antimicrobial activity, the minimum inhibitory concentration of compound 5c is 2.5 mg/ml.**Conclusion:** An efficient synthesis of biologically active cyclohexane-1, 3-dione derivatives has been developed.**Keywords:** Dimedone, Antimicrobial activity, Antifungal activity, Molecular docking studies, Anticancer activity, 3,4-methylenedioxyamphetamine-MB-231 cell line.© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2019.v12i3.30481>**INTRODUCTION**

Cancer is one of the most stressful and life aggressive diseases which implements cruel deaths in the world [1]. It exhibits at least 100 different disease conditions which shares several common symptoms. Cancer is the second leading cause of death in developed countries in spite of its prevention, early detection, and novel therapies. The World Health Organization has warned that nearly 13.1 million people may die of cancer in 2030. Among the women community, breast cancer is the most prevalent type of cancer [2]. The breast cells spread over the body by a process called cancer metastasis. By this process, the organs such as the liver, lungs, brain, and bones get affected and it becomes a major problem affecting the survival of cancer patients. Many therapies were introduced in the recent past to deal with the recurrence of cancer even though drugs and medicines have serious side effects. Hence, the development of drugs to serve as chemoprevention agents is warranted [3].

Heterocyclic compounds play a vital role in the development of pharmacologically active molecules and organic materials [4,5]. The hydroxyl carbonyl compounds prepared by the Claisen-Schmidt reaction between aldehydes and ketone also play an important role in synthetic organic chemistry [6]. Chalcone is a captivating moiety which consists of two aromatic rings linked by enone bridge and it belongs to the flavonoid family. Chalcones show various pharmacological activities such as anticancer, antiviral, antibacterial [7], and antifungal activity [8]. The C-C bond formation has been discovered in the past decades and its applications were reviewed [9-14]. Reactions such as Knoevenagel [15], Henry [16], Aldol [17], and Michael reaction [18] are base catalyzed transformations and are mostly used in organic synthesis. Further, heterocyclic compounds exhibited specific activity and are used in the

treatment of many infectious diseases. Liu *et al.* developed successfully an efficient Michael addition between dimedone and cinnamons using unmodified chiral diphenylethylenediamine (DPEN) [19]. In the fight against the resistant bacterial strains, one of the strategies is the development of new antibacterial drugs affecting the integrity of the bacterial cell wall. According to that, many number of chalcones were evaluated for their anticancer and antibacterial activity. Studies on cyclohexane-1,3-dione remain scattered. Hence, this prompted us to undertake the present work. The antibacterial activity of cyclohexanone 1,3-dione compounds mechanism were done by *in silico* method.

Modern drug design process helps to identify and develop new ligands with a high binding affinity toward a target protein receptor. The molecular docking approaches help to reveal drug-receptor interaction to a greater detail. The study of receptor-ligand interaction is considered as one of the fundamental approaches for rational drug design and so the prediction of such interactions by molecular docking has been gaining importance [20].

The cyclohexane-1,3-dione derivatives have been synthesized by Michael addition method and characterized [21]. The new derivatives of cyclohexane-1,3-dione were synthesized and characterized by Fourier-transform infrared spectroscopy (FT-IR), ¹H nuclear magnetic resonance (NMR), and ¹³C NMR spectral data. Molecular docking studies were carried out against bacterial proteins (1UAG, 3UDI, and 2X5O) and cancer proteins (2ZOQ). Finally, the synthesized compounds were screened for antimicrobial activity by agar disk diffusion method and *in vitro* anticancer activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method by 3,4-methylenedioxyamphetamine (MDA)-MB-231 cell line.

METHODS

Dimedone (25 g) was purchased from Sigma-Aldrich. The melting points of compounds were determined to open capillary method and the values were uncorrected. The FT-IR spectrum (cm^{-1}) was recorded through KBr in FT-IR spectrometer (model Shimadzu 8400s) in the range of 400–4000 cm^{-1} . The ^1H and ^{13}C NMR spectra were recorded by Bruker 400 MHz spectrometer, and chemical shifts are recorded in value (ppm) with tetramethylsilane as an internal standard, as well as CDCl_3 used as a solvent.

General procedure for the synthesis of (E)-3-(phenyl)-1-(biphenyl-2-yl) 3-arylprop-2-en-1-one derivatives (3a-3h)

0.1 mol of various substituted aromatic aldehydes (1a-1h) and 0.1 mol of 4-acetyl biphenyl (ketone) were taken in a 100 ml beaker, and to this, approximately added 30 ml of ethanol containing 2 g of NaOH pellets. Then, the mixture was stirred well for 3 h in an ice cold bath after it was poured into the crushed ice containing 500 ml beaker and this reaction mixture was kept into overnight at room temperature. The chalcone precipitated out as solid. Then, it was filtered, dried, and recrystallized from ethanol. The purity of the compound was checked by thin-layer chromatography (TLC) using CHCl_3 as a solvent [22].

General procedure for the synthesis of cyclohexane-1,3-dione derivatives (5a-5h)

The dimedone (0.1 mol) and chalcones 3a-3h (0.1 mol) were taken in a round bottom flask containing 30 ml ethanol. After, 1 mole of sodium acetate was added. Then, this reaction mixture was stirred and refluxed for 42 h. After the reaction mixture was poured into 500 ml beaker containing crushed ice and it was kept in overnight at room temperature. Then, it was filtered, dried, and recrystallized from ethanol. The purity of the compound was checked by TLC using CHCl_3 as a solvent.

In silico activity

Molecular docking studies

Molecular docking studies have been carried out using the AutoDock tools (ADT) version 1.5.6 and AutoDock version 4.2.5.1 docking program.

Preparation of the protein

The bacterial proteins and cancer protein were downloaded from Protein Data Bank (PDB) with id: 1UAG, 3UDI, 2X50, and 2ZOQ.

Ligand preparation

Two-dimensional (2D) structure of the cyclohexane-1,3-dione derivatives is drawn using ChemDraw Ultra 8.0 (Chemoffice2002). After that, the chem 3D ultra were used to convert the 2D structure to 3D structure of the compounds, and the energy is minimized using the semi-empirical AM1 method. All the structures are saved as PDB file format for input to ADT. Finally, all the ligand structures are saved as PDB file format to carry out docking molecular docking in AutoDock Vina.

Grid formation

A grid box with a dimension of $40 \times 40 \times 40$ Å in 0.375 Å spacing and centered on 30.473, 47.997, and 9.563 has created around the binding site of protein using ADT. The center of the box was set at ligand center and grid energy calculations have been carried out.

Docking protocol

Default parameters have been used for auto dock calculations. The energy calculation is done using genetic algorithms. The outputs are exported to Chimera 1.10 and Discovery Studio 4.5 for visual inspection of the binding modes and interaction of the compounds with amino acid residues in the active site [23].

Antimicrobial activity

The newly synthesized cyclohexane-1,3-dione compounds have been evaluated for their antimicrobial activity. The dimethyl sulfoxide was used as the solvent control of this activity. These studies were carried out by the agar disk diffusion method.

Anticancer activity

MTT assay

MDA-MB-231 (human breast adenocarcinoma) cell was initially procured from the National Center for Cell Science, Pune, India, and maintained Dulbecco's Modified Eagle's Medium (DMEM) (Sigma Aldrich, USA).

The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% fetal bovine serum, L-glutamine, and sodium bicarbonate (Merck, Germany) and an antibiotic solution containing penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), and amphotericin B (2.5 $\mu\text{g}/\text{ml}$). Cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by inverted phase contrast microscope and followed by MTT assay method.

Cell seeding in 96-well plate

Two-day-old confluent monolayer of cells trypsinized and the cells were suspended in 10% growth medium, and 100 μl cell suspension (5×10^4 cells/well) was seeded in 96-well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator.

Preparation of compound stock

Nearly 1 mg of sample was weighed and dissolved in 1 ml DMEM using a cyclomixer. The sample solution was filtered through 0.22 μm Millipore syringe filter to ensure the sterility.

Anticancer evaluation

After 24 h, the growth medium was removed, freshly prepared each compound in 5% DMEM was five times serially diluted by two-fold dilution (100 μg , 50 μg , 25 μg , 12.5 μg and, 6.25 μg) in 500 μl of 5% DMEM, and each concentration of 100 μl was added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO_2 incubator. Non-treated control cells were also maintained.

Anticancer assay by MTT method

About 15 mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 h of the incubation period, the sample content in wells was removed and 30 μl of reconstituted MTT solution was added to all test and cell control wells; the plate was gently shaken well and then incubated at 37°C in a humidified 5% CO_2 incubator for 4 h. After the incubation period, the supernatant was removed and 100 μl of MTT solubilization solution (dimethyl sulfoxide, Sigma-Aldrich, USA) was added, and the wells were mixed gently by pipetting up and down to solubilize the formazan crystals. The absorbance values were measured using microplate reader at a wavelength of 540 nm [24].

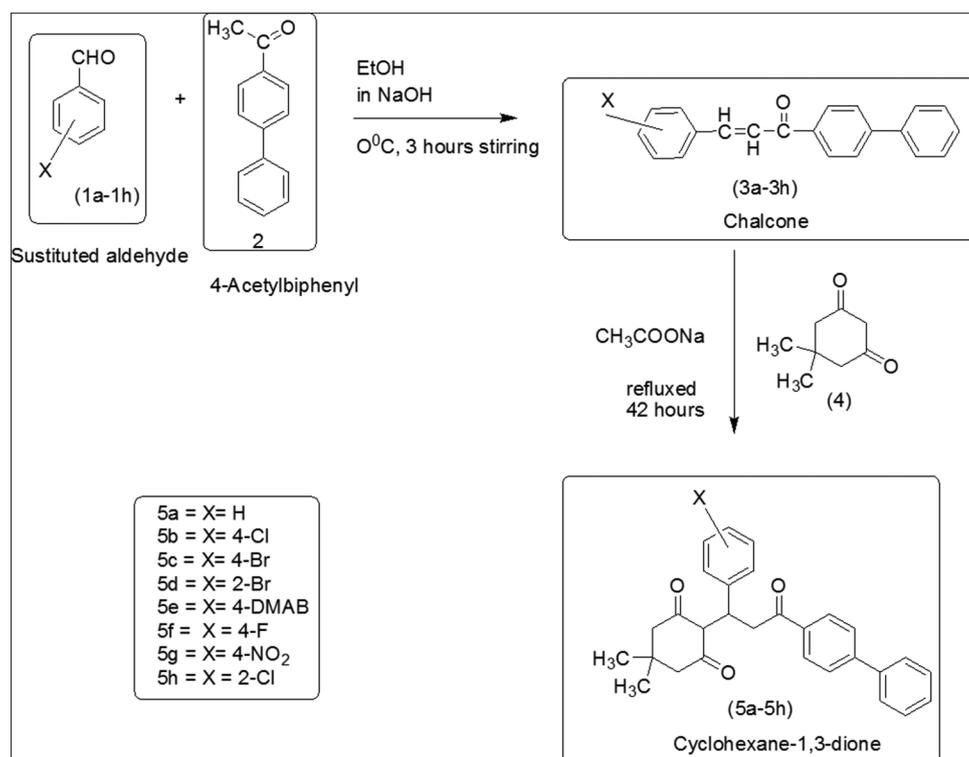
The percentage of growth inhibition was calculated using the following formula:

$$\% \text{ of viability} = \frac{\text{Mean OD} \times 100}{\text{Mean OD of control group}}$$

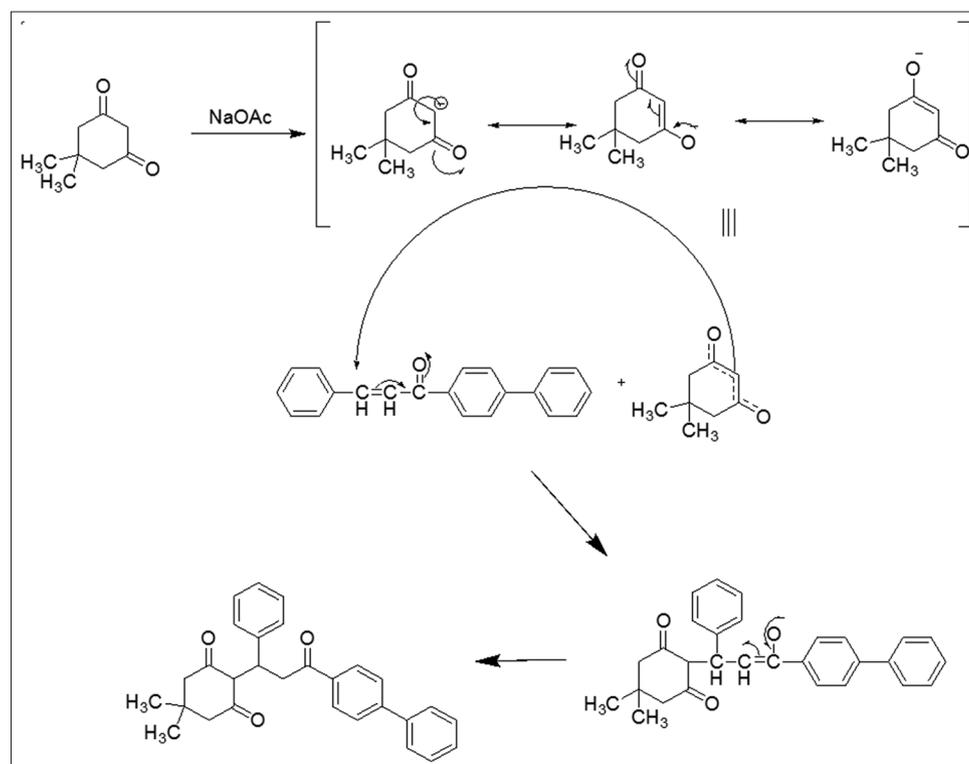
RESULTS AND DISCUSSION

Chemistry

In the present research work, the new series of cyclohexane-1,3-dione derivatives (5a-5h) were synthesized, which is shown in Scheme 1. The first step in Scheme 1 is the condensation reaction between substituted aldehydes and 4-acetyl biphenyl in the presence of a base to give compound (3a-3h). Finally, the compounds were recrystallized



Scheme 1: Synthetic pathway for cyclohexane-1,3-dione derivatives (5a-5h)



Scheme 2: Possible mechanism for the formation of cyclohexane-1,3-dione derivatives (5a-5h)

by using ethanol solvent. The chalcones (3a-3h) react with dimedone in the presence of sodium acetate by Michael addition reaction to give cyclohexane-1,3-dione derivatives. The cyclohexane-1,3-dione derivatives were purified by column chromatography using CHCl₃ as the solvent. The structure of the synthesized compounds (5a-5h) was confirmed by spectral techniques such as FT-IR, ¹H NMR, and ¹³C NMR. The possible mechanism for this reaction is shown in Scheme 2.

5, 5-dimethyl-2-(3-oxo-1-diphenyl-3-phenylpropyl) cyclohexane-1,3-dione (5a)

Yield 69%; white solid; molecular formula; C₂₉H₂₈O₃; IR (KBr): C=O (1705.07, 1678.07, 1598.99), 3059.10, 3034.03, 2956.87 (Ar-CH), 1487.12 (C=C), 694.37, 729.09, 759.95, 833.25; ¹H NMR (CDCl₃, 400 MHz): 1.032 (s, 3H), 1.096 (s, 3H), 2.405-2.448 (m, 4H, H-6 and H-8); 2.57 ppm (dd, J_{2a,2b}=15.2Hz, J_{2a,3}=3.2, 1H, H-2'a); 3.64 ppm

(dd, $J_{2b,2a}=15.8$ Hz, $J_{2b,3}=3.8$ Hz, 1H, H-2'b); 4.41 ppm (dd, $J_{3,2a}=7$ Hz, $J_{3,2b}=4.6$ Hz, H-3'); 7.259–7.958 (m, Ar-H). 13 C NMR, (CDCl₃, 100 MHz): 30.539 (CH₃) at C-5, 30.639 (CH₃) at C-5, 38.952 (C-5), 26.076 (C-3'), 45.724 (C-2'), 52.518 (C-4), 53.387 (C-6), 100.361 (C-2), 196.638 (C-1), 202.815 (C-3), 203.895 (C-1), Ar-C (127.247–129.199), IPSO carbon (146.232, 139.742, 136.985, 134.892).

2-(1-(4-chlorophenyl)-3-oxo-3-diphenylpropyl)-5, 5-dimethylcyclohexane-1,3-dione (5b)

Yield 86%; white solid; molecular formula; C₂₉H₂₇ClO₅; IR (KBr): C=O (1701.22, 1600.92, 1556.55), 3057.17, 3034.03, 2958.80 (Ar-CH), 1489.05 (C=C), 698.23, 758.02, 833.25; 1 H NMR (CDCl₃, 400 MHz): 0.978 (s, 3H), 1.016 (s, 3H), 2.419–2.462 (m, 4H, H-6 and H-8); 2.58 ppm (dd, $J_{2a,2b}=14.8$ Hz, $J_{2a,3}=3.6$ Hz, 1H, H-2'a); 3.64 ppm (dd, $J_{2b,2a}=16.6$ Hz, $J_{2b,3}=2.6$ Hz, 1H, H-2'b); 4.38 ppm (dd, $J_{3,2a}=7.6$ Hz, $J_{3,2b}=4.4$ Hz, H-3'); 7.259–7.958 (m, Ar-H). 13 C NMR, CDCl₃, 400 MHz, δ : 30.497 (CH₃), 30.669 (CH₃), 31.162 (C-5), 26.049 (C-3'), 44.998 (C-2'), 52.461 (C-4), 53.334 (C-6), 116.542 (C-2), 196.358 (C-1'), 201.749 (C-1), 202.522 (C-3), Ar-C (127.157–135.475), IPSO carbon (146.851, 146.401, 140.533, 139.683).

2-(1-(4-bromo phenyl)-3-oxo-3-diphenylpropyl)-5, 5-dimethyl cyclohexane-1,3-dione (5c)

Yield 84%; white solid; molecular formula; C₂₉H₂₇BrO₅; IR (KBr): C=O (1703.14, 1598.99, 1550.77), 3024.38, 2956.87 (Ar-CH), 1485.19 (C=C), 696.30, 725.23, 761.88, 835.18; 1 H NMR (CDCl₃, 400 MHz): 0.879 (s, 3H), 1.255 (s, 3H), 2.422–2.465 (m, 4H, H-6 and H-8), 2.58 ppm (dd, $J_{2a,2b}=15.2$ Hz, $J_{2a,3}=3.2$ Hz, 1H, H-2'a); 3.63 ppm (dd, $J_{2b,2a}=18.9$ Hz, $J_{2b,3}=3.9$ Hz, 1H, H-2'b); 4.37 ppm (dd, $J_{3,2a}=7.4$ Hz, $J_{3,2b}=4.2$ Hz, H-3'); 7.234–7.950 (m, ph-H). 13 C NMR, (CDCl₃, 400 MHz), δ : 30.686 (CH₃) at C-5, 30.503 (CH₃) at C-5, 38.884 (C-5), 26.059 (C-3'), 45.040 (C-2'), 52.444 (C-4), 53.323 (C-6), 100.093 (C-2), 196.322 (C-1'), 202.531 (C-1), 203.522 (C-3), Ar-C (122.331–131.889), IPSO carbon (146.412, 139.677, 135.980, 134.696).

2-(1-(2-bromo phenyl)-3-oxo-3-diphenylpropyl)-5, 5-dimethyl cyclohexane-1,3-dione (5d)

Yield 78%; white solid; molecular formula; C₂₉H₂₇BrO₅; IR (KBr): C=O (1705.07, 1691.57, 1598.99), 2954.95, 2922.16 (Ar-CH), 1481.33 (C=C), 694.37, 731.02, 765.74, 839.03; 1 H NMR (CDCl₃, 400 MHz): 1.116 (s, 3H), 1.159 (s, 3H), 2.354–2.476 (m, 4H, H-6 and H-8), 2.58 ppm (dd, $J_{2a,2b}=19.4$ Hz, $J_{2a,3}=3.8$ Hz, 1H, H-2'a), 3.61 ppm (dd, $J_{2b,2a}=18.4$ Hz, $J_{2b,3}=3.6$ Hz, 1H, H-2'b); 4.92 ppm (dd, $J_{3,2a}=8.4$ Hz, $J_{3,2b}=3.2$ Hz, H-3'); 6.863–7.892 (m, ph-H). 13 C NMR, (CDCl₃, 400 MHz); 28.608 (CH₃) at C-5, 30.745 (CH₃) at C-5, 38.884 (C-5), 27.869 (C-3'), 42.347 (C-2'), 43.420 (C-4), 50.780 (C-6), 116.134 (C-2), 197.565 (C-1'), 200.473 (C-1), 203.589 (C-3), Ar-C (123.745–131.512), IPSO carbon (146.120, 134.814, 132.107, 131.910).

2-(1-(4-dimethylamino phenyl)-3-oxo-3-diphenylpropyl)-5, 5-dimethylcyclohexane-1,3-dione (5e)

Yield 70%; white solid; molecular formula; C₃₁H₃₃NO₅; IR (KBr): C=O (1676.14, 1662.85, 1516.05), 3026.31, 2953.02 (Ar-CH), 1458.18 (C=C), 692.44, 738.74, 759.95, 819.75; 1 H NMR (CDCl₃, 400 MHz): 1.012 (s, 3H), 1.049 (s, 3H), 2.283–2.435 (m, 4H, H-6 and H-8), 2.89 ppm (dd, $J_{2a,2b}=11.8$ Hz, $J_{2a,3}=7.4$ Hz, 1H, H-2'a), 3.60 ppm (dd, $J_{2b,2a}=6.8$ Hz, $J_{2b,3}=3.6$ Hz, 1H, H-2'b); 4.65 ppm (dd, $J_{3,2a}=7$ Hz, $J_{3,2b}=3.8$ Hz, H-3'); 2.525 (s, 3H), 2.5497 (s, 3H); 7.197–7.703 (m, Ar-H). 13 C NMR, (CDCl₃, 400 MHz); 27.857 (CH₃) at C-5, 28.250 (CH₃) at C-5, 33.455 (C-5), 22.145 (C-3'), 46.143 (C-2'), 54.070 (C-4), 56.823 (C-6), 108.423 (C-2), 195.565 (C-1'), 201.763 (C-1), 202.489 (C-3), 40.717 (CH₃) at N(CH₃)₂, 40.860 (CH₃) at N(CH₃)₂, Ar-C (112.295–129.512), IPSO carbon (149.005, 146.421, 139.766, 135.115).

2-(1-(4-fluorophenyl)-3-oxo-3-diphenylpropyl)-5, 5-dimethyl cyclohexane-1,3-dione derivative (5f)

Yield 74%; white solid; molecular formula; C₂₉H₂₇FO₅; IR (KBr): C=O (1681.43, 1698.39, 1703.69), 3058.41 (Ar-CH), 1463.72 (C=C), 687.90, 712.40, 740.38, 803.17; 1 H NMR (CDCl₃, 400 MHz): 1.093

(s, 3H), 1.068 (s, 3H), 2.273–2.439 (m, 4H, H-6 and H-8), 2.75 ppm (dd, $J_{2a,2b}=11.6$ Hz, $J_{2a,3}=7.1$ Hz, 1H, H-2'a); 3.67 ppm (dd, $J_{2b,2a}=7.3$ Hz, $J_{2b,3}=3.8$ Hz, 1H, H-2'b); 4.61 ppm (dd, $J_{3,2a}=7.5$ Hz, $J_{3,2b}=3.6$ Hz, H-3'); 6.893–7.719 (m, Ar-H). 13 C NMR, (CDCl₃, 400 MHz); 27.38 (CH₃) at C-5, 29.71 (CH₃) at C-5, 32.13 (C-5), 23.739 (C-3'), 41.016 (C-2'), 55.910 (C-4), 58.711 (C-6), 114.810 (C-2), 198.513 (C-1'), 203.901 (C-1), 204.171 (C-3), Ar-C (118.873–128.501), IPSO carbon (147.59, 146.30, 144.81, 139.91).

2-(1-(4-nitrophenyl)-3-oxo-3-diphenylpropyl)-5, 5-dimethyl cyclohexane-1,3-dione derivative (5g)

Yield 77%; white solid; molecular formula; C₂₉H₂₇NO₅; IR (KBr): C=O (1699.17, 1703.82, 1598.41), 3089.51, 2086.61 (Ar-CH), 1489.07 (C=C), 683.81, 724.23, 759.32, 855.69; 1 H NMR (CDCl₃, 400 MHz): 0.936 (s, 3H), 1.170 (s, 3H), 2.419–2.460 (m, 4H, H-6 and H-8), 2.59 ppm (dd, $J_{2a,2b}=15.4$ Hz, $J_{2a,3}=3.7$ Hz, 1H, H-2'a); 3.67 ppm (dd, $J_{2b,2a}=18.3$ Hz, $J_{2b,3}=3.7$ Hz, 1H, H-2'b); 4.51 ppm (dd, $J_{3,2a}=7.1$ Hz, $J_{3,2b}=4.3$ Hz, H-3'); 7.137–7.943 (m, Ar-H). 13 C NMR, (CDCl₃, 400 MHz), δ : 31.684 (CH₃) at C-5, 31.592 (CH₃) at C-5, 39.730 (C-5), 27.941 (C-3'), 44.142 (C-2'), 54.041 (C-4), 55.767 (C-6), 112.42 (C-2), 195.672 (C-1'), 201.337 (C-1), 203.741 (C-3), Ar-C (120.08–129.31), IPSO carbon (144.83, 141.09, 139.03, 139.17).

2-(1-(2-chlorophenyl)-3-oxo-3-diphenylpropyl)-5, 5-dimethyl cyclohexane-1,3-dione derivative (5h)

Yield 82%; white solid; molecular formula; C₂₉H₂₇ClO₅; IR (KBr): C=O (1699.24, 1607.83, 1554.55), 3087.83, 3013.90, 2986.41 (Ar-CH), 1486.20 (C=C), 694.01, 763.03, 854.09; 1 H NMR (CDCl₃, 400 MHz): 0.971 (s, 3H), 1.013 (s, 3H), 2.407–2.493 (m, 4H, H-6 and H-8), 2.57 ppm (dd, $J_{2a,2b}=14.3$ Hz, $J_{2a,3}=3.1$ Hz, 1H, H-2'a); 3.61 ppm (dd, $J_{2b,2a}=16.7$ Hz, $J_{2b,3}=3.1$ Hz, 1H, H-2'b); 4.47 ppm (dd, $J_{3,2a}=7.4$ Hz, $J_{3,2b}=4.9$ Hz, H-3'); 7.194–7.938 (m, Ar-H). 13 C NMR, CDCl₃, 400 MHz, δ : 30.731 (CH₃), 30.971 (CH₃), 31.738 (C-5), 26.072 (C-3'), 45.988 (C-2'), 51.653 (C-4), 53.413 (C-6), 117.40 (C-2), 196.19 (C-1'), 201.028 (C-1), 203.627 (C-3), Ar-C (123.740–134.891), IPSO carbon (145.937, 143.970, 140.71, 139.99).

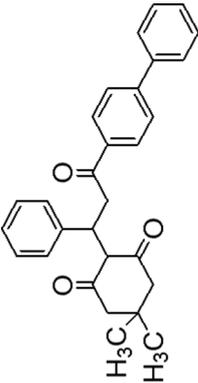
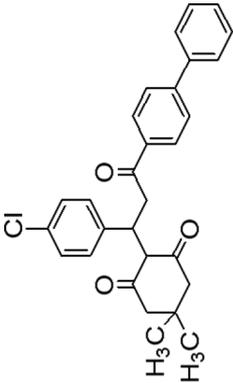
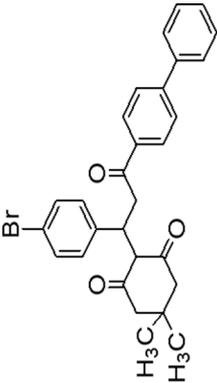
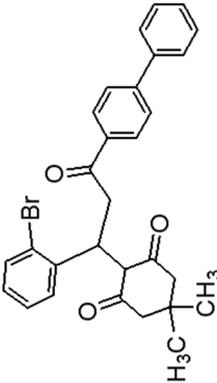
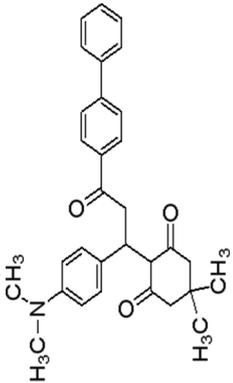
Docking studies of cyclohexane-1, 3-dione derivatives (5a-5h)

In the present study, *in silico* molecular docking studies of cyclohexane-1,3-dione derivatives (5a-5h) against bacterial proteins (PDB id: 1UAG, 3UDI, and 2X50) and breast cancer protein (PDB id: 2ZOQ) have been carried out using ADT version 1.5.6 and AutoDock version 4.2.5.1 docking program. The molecular docking studies are reported in terms of binding affinity score and the compound which have a better interactions had a lower affinity score. In the ligand, cyclohexane-1,3-dione derivatives (5a-5h) were individual, docked with bacterial proteins. Docking results are shown in Table 1. From the table, cyclohexane-1,3-dione derivatives (5a-5h) exhibit good docking scores compared to standard drug (amoxicillin). Based on the docking score, hydrophobic and hydrophilic interaction, cyclohexane-1,3-dione derivatives (5a-5h) show better binding affinity compared to the standard drug. The proteins IUAG, 3UDI, and 2X50 were involved in the cell wall synthesis mechanism. Molecular docking studies show that this cyclohexane-1,3-dione derivatives bind well in the active site pocket of bacterial proteins and interact with the active site of amino acid residues. The best compound of this cyclohexane-1,3-dione derivatives has been explained.

Binding affinity value

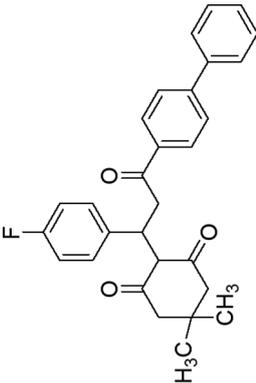
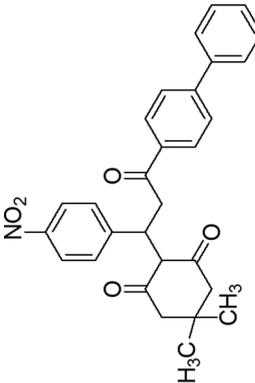
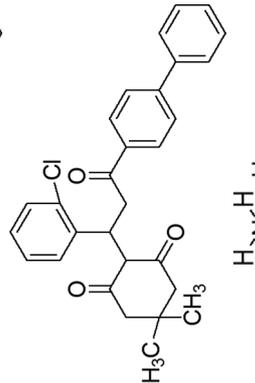
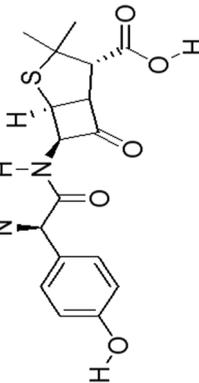
Finally, the binding affinity value, conventional hydrogen bond, hydrophobic interaction such as alkyl and pi-alkyl interactions, and other interactions were obtained. Cyclohexane-1, 3-dione derivatives showed good interaction with the studied proteins. Compound 5c showed good binding score with 1UAG (–9.3 kcal/mol), 3UDI (–9.6 kcal/mol), and 2X50 (–9.4 kcal/mol) compared with other synthesized compound of this series (5a-5h) and standard drug. Other synthesized compounds (5a-5h) binding affinity score are shown in Table 1.

Table 1: Molecular docking data for cyclohexane-1,3-dione derivatives (5a-5h) with bacterial proteins

Compound structure	Protein	Binding affinity values (kcal/mol)	Conventional hydrogen bond interaction	Alkyl and pi-alkyl interaction	Other bond interaction
	1UAG	-8.7	Nil	ARG A:186, ALA A:414, LYS A:319	PHE A:422, PHE A:161
	3UDI	-9.3	Nil	ALA:66, PRO A:184, PHE A:72, LYS A:147, TYR A:144	TYR A:144, ILE A:148, LYS A:137
	2X50	-8.7	ASN A:178	ALA A:328, VAL A:232	ASP A:214, HIS A:267
	1UAG	-9.2	ASN A:268, LEU A :299	LEU A:333	PHE A:333
	3UDI	-9.0	LYS A:137	ILEA:140, TYR A:144, PRO A:184, ILE A:148	LYS A:147, ILE A:148, CLU A:67
	2X50	-8.4	ASN A:268	LEU A:333	PHE A:303
	1UAG	-9.4	LEU A:416, SER A:415	LEU A:416, PRO A:41, ALA A:414, ARG A:186, PHE A:161	Nil
	3UDI	-9.6	Nil	LYS A:137, ILE A:148, PHE A:72, ILE A:140	TYR A:144
	2X50	-9.4	ASN A:211	ALA A:328, VAL A:232, HIS A:267	HIS A:267, ASP A:213
	1UAG	-8.0	SER A:415, LEU A:416, LYS A:348	LEU A:15, PRO A: 41, ALA A:414, PHE A:422	Nil
	3UDI	-9.6	Nil	LYS A:185, PRO A:184, ALA A:66, LYS A:147, TYR A:144, PHE A:72	LYS A:137, ILE A:148, TYR A:144
	2X50	-8.3	LYS A:348	LYS A:348, HIS A:183	PHE A:422, GLU A:423
	1UAG	-9.2	ASN A:138, LYS A:348	LEU A:416	LEU A:416, SE A:112, LYS A:319
	3UDI	-8.8	LYS A:137	LYS A:147, TYR A:144, PHE A:72	LYS A:137, ILE A:148, TYR A:144
	2X50	-8.3	Nil	PHE A:303	GLN A:266

(Contd...)

Table 1: (Continued)

Compound structure	Protein	Binding affinity values (kcal/mol)	Conventional hydrogen bond interaction	Alkyl and pi-alkyl interaction	Other bond interaction
	1UAG 3UDI 2X50	-8.1 -8.9 -8.6	Nil Nil ASN A:178, ASN A:211, ASN A:271	VAL A:232, ALA A:328 ILE A:148 ALA A:328	ASN A:331, ASP A:214, HIS A:267 LYS A:137, ILE A:140, GLU A:67 ASP A:214, HIS A:267
	1UAG 3UDI 2X50	-8.1 -9.1 -8.3	PHE A:422, SER A:415, LYS A:115, ASN A:138 PHE A:149 ASN A:211	LEU A:416, ALA A:414, PHE A:161, LYS A:348, LEU A:15 LEU A:141, LYS A:137, ILE A:148, LYS A:147, PRO A:187, PHE A:149 ARG A:221, ALA A:414	HIS A:183 PHE A:422, PHE A:303
	1UAG 3UDI 2X50	-8.6 -9.5 -8.1	ASN A:138 Nil ASN A:138, LYS A:319	LYS A:319, LYS A:348, ALA A:414, PHE A:422 LYS A:185, PRO A:184, ALA A:66, LYS A:147, PHE A:72, TYR A:144 ALA A:414, LEU A:416	LEU A:416, HIS A:183 TYR A:144, ILE A:48 PHE A:161, PHE A:422
	1UAG 3UDI 2X50	-7.8 -7.6 -8.3	LYS A:319, LYS A:319, SER A:112, SER A:415, ASN A:138, SER A:470, TYR A:485, SER A:487, LYS A:669, THR A:670, THR A:672, Nil	LEU A:263, LEU A:333 TYR A:707 Nil	Nil Nil PHE A:303, GLN A:266

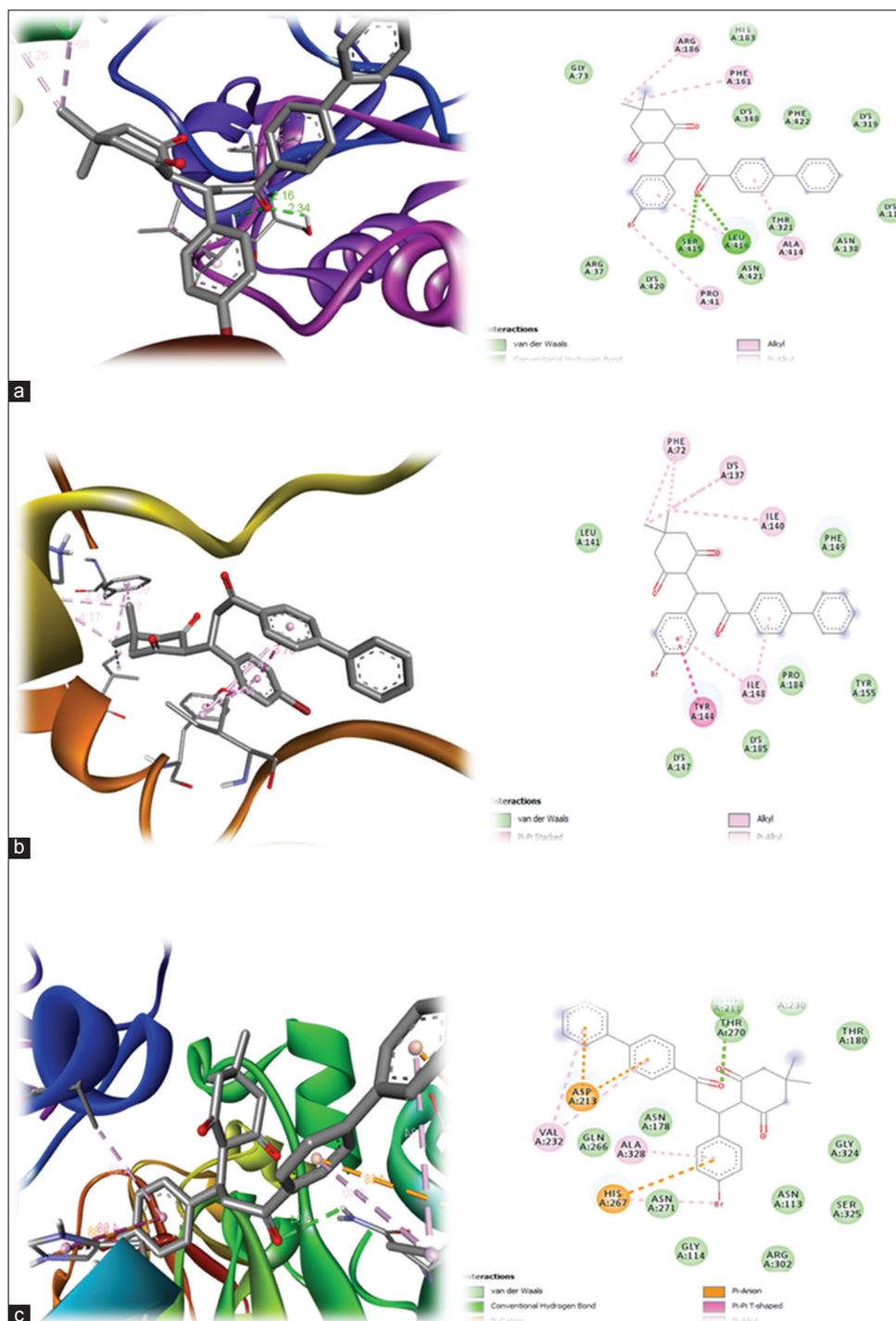


Fig. 1: (a) Two-dimensional (2D) and three-dimensional (3D) images of compound 5c docked with 1UAG protein. (b) 2D and 3D images of compound 5c docked with 3UDI protein. (c) 2D and 3D images of compound 5c docked with 2X50 protein

Conventional hydrogen bond interaction

Based on high binding affinity value, compound 5c has two conventional hydrogen bond interactions (LEU A: 416 and SER A: 415) with carbonyl moiety of the biphenyl ring (1UAG); compound 5c has no hydrogen bond interaction with 3UDI protein; compound 5c has only one hydrogen bond interaction (ASN A: 211) with carbonyl moiety of the biphenyl ring (2X50). Other synthesized compounds (5a-5h) conventional hydrogen bond interactions are shown in Table 1.

Hydrophobic interaction

Based on high binding affinity value, compound 5c has five hydrophobic interactions LEU A: 416, PRO A: 41, ALA A: 414, ARG A: 186, and

PHE A: 161 with 1UAG protein; compound 5c has four conventional hydrogen bond interactions LYS A: 137, ILE A: 148, PHE A: 72, and ILE A: 140 with 3UDI protein; compound 5c has three hydrophobic interactions ALA A: 328, VAL A: 232, and HIS A: 267 with 2X50 protein. Other synthesized compounds (5a-5h) hydrophobic interactions are shown in Table 1. 2D and 3D images of compound 5c are shown in Fig. 1.

The new series of cyclohexane-1,3-dione derivatives (5a-5h) were subjected to molecular docking studies against breast cancer protein 2Z0Q. Compound 5c has high binding affinity score than other cyclohexane-1,3-dione derivatives of this series. For that reason, that compound was performed *in vitro* anticancer activity against

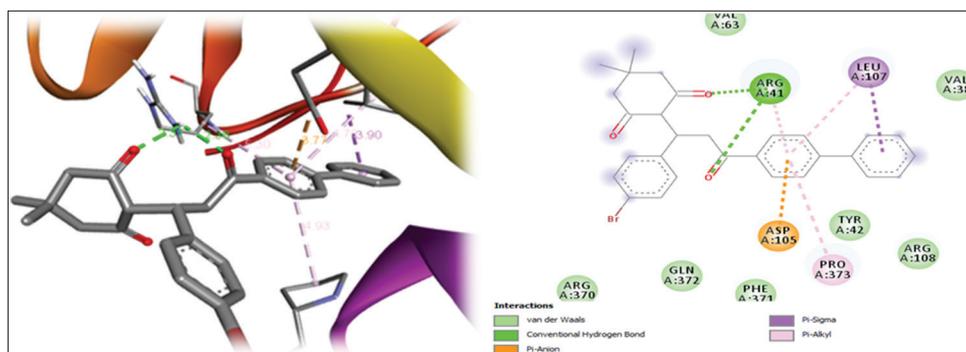


Fig. 2: Two-dimensional and three-dimensional images of compound 2-(1-(4-bromo phenyl)-3-oxo-3-diphenylpropyl)-5,5-dimethylcyclohexane-1,3-dione (5c) docked with 2Z0Q protein

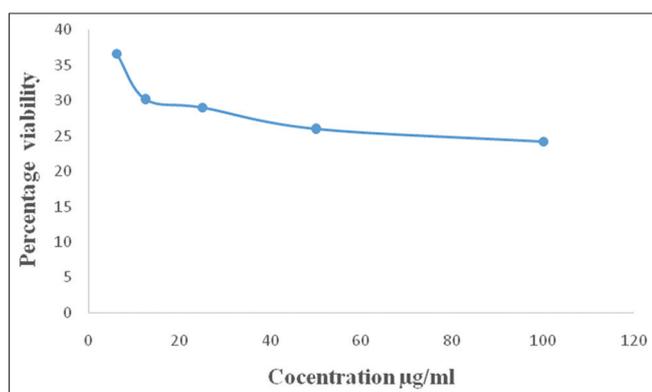


Fig. 3: *In vitro* anticancer activity screening for compound (5c) at low concentration

Table 2: Binding affinity value and the conventional hydrogen bond of cyclohexane-1, 3-dione derivatives (5a-5h) against human breast cancer protein 2Z0Q

Compound	Binding affinity (kcal/mol)	Conventional hydrogen bond
5a	-9.1	1
5b	-8.8	1
5c	-9.6	1
5d	-9.5	1
5e	-9.1	-
5f	-8.9	1
5g	-8.7	1
5h	-9.4	1

Table 3: Antibacterial activity with different strains for cyclohexane-1,3-dione derivatives (5a-5h)

S. No.	Bacteria	Zone of inhibition at 2.5 mg/ml (diameter in mm)							
		5a	5b	5c	5d	5e	5f	5g	5h
1.	<i>Staphylococcus aureus</i>	9	8	10	9	10	7	9	8
2.	<i>Streptococcus pyogenes</i>	12	10	10	10	12	11	12	11
3.	<i>Escherichia coli</i>	12	8	9	11	11	11	10	10
4.	<i>Pseudomonas sp.</i>	12	7	10	11	11	10	10	9

Table 4: Antifungal activity test for cyclohexane-1,3-dione derivatives (5a-5h)

S. No.	Compound	Zone of inhibition (diameter in mm)		
		10 mg/ml	5 mg/ml	2.5 mg/ml
1.	5a	16	13 mm	12 mm
2.	5b	14 mm	13 mm	11 mm
3.	5c	13 mm	11 mm	9 mm
4.	5d	12 mm	10 mm	9 mm
5.	5e	13 mm	11 mm	10 mm
6.	5f	12 mm	12 mm	10 mm
7.	5g	11 mm	10 mm	11 mm
8.	5h	14 mm	12 mm	10 mm

MDA-MB-231 cell line. In the ligand, cyclohexane-1,3-dione derivative (5a-5h) values are shown in Table 2. 2D and 3D images of compound 5c are shown in Fig. 2. Compound 5c showed one conventional hydrogen bond interaction with the same amino acid (ARG A: 41) formed at carbonyl group of the biphenyl moiety and dimedone moiety. This compound has only one alkyl and pi-alkyl interaction with PRO A: 373 formed at the benzene ring of biphenyl moiety.

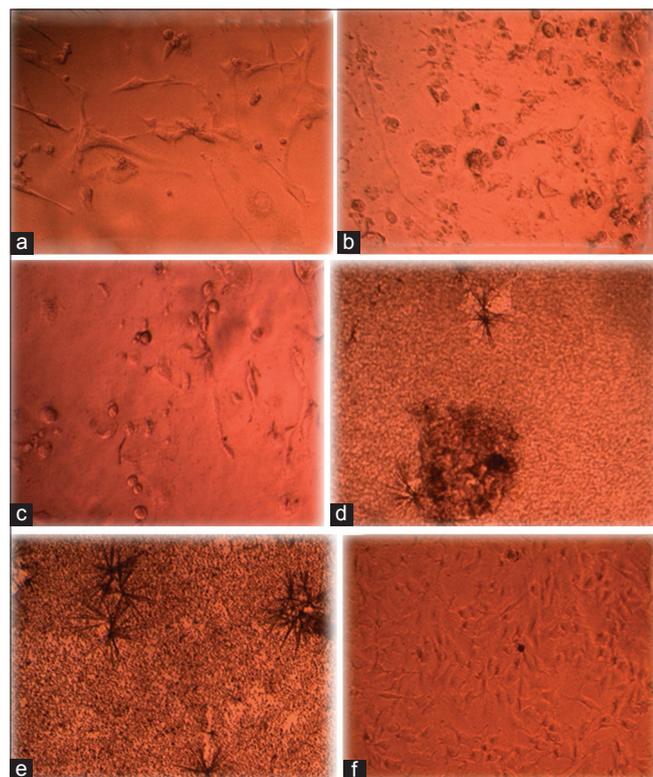


Fig. 4: An *in vitro* anticancer activity of compound 5c. 3,4-methylenedioxyamphetamine-MB-231 cells were incubated for 48 h in the presence of (a) 5c at 6.25 µg/ml, (b) 5c at 12.5 µg/ml, (c) 5c at 25 µg/ml, (d) 5c at 50 µg/ml, (e) 5c at 100 µg/ml, (f) untreated control

The synthesized compounds (5a-5h) were subjected to insilico analysis against different bacterial proteins (1UAG, 3UDI, 2X50). Compound 5c showed binding scores as -9.4, -9.6, and -9.1 kcal/mol against 1UAG, 3UDI, and 2X50 proteins indicating a very good affinity. In a similar way when these compounds were docked against the human breast cancer protein Z2OQ, compound 5c showed a binding score of -9.6 kcal/mol and thereby indicated that it is a good candidate for further studies. Hence, *in vitro* studies (MTT assay of antimicrobial studies) were carried out to confirm its activities.

MTT assay study for compound 2-(1-(4-bromophenyl)-3-oxo-3-diphenylpropyl)-5,5-dimethyl cyclohexane-1,3-dione derivative

From this result, the *in vitro* anticancer activity was performed by MTT assay method for compound 2-(1-(4-bromophenyl)-3-oxo-3-diphenylpropyl)-5,5-dimethyl cyclohexane-1,3-dione (5c). Based on binding affinity score, the *in vitro* anticancer activity was done using various concentrations (100, 50, 25, 12.5, and 6.25 µg/ml) of the sample 5c against the MDA-MB-231 cell line. From this result, synthesized compound 5c exhibited a good activity at low concentration (6.25 µg/ml). The LC_{50} value of this compound is 10.31 ± 0.003 µg/ml. Experiments were conducted in triplicate and the mean value was calculated.

Antimicrobial activity

The new series of cyclohexane-1,3-dione derivatives (5a-5h) were screened for antimicrobial activity. The results are shown in Table 3. From Table 3, compound 5c showed a good zone of inhibition against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas* sp. Compound 5a showed a good zone of inhibition against *S. pyogenes*, *Escherichia coli*, and *Pseudomonas* sp. Compound 5b showed a good zone of inhibition against *S. pyogenes*. Compound 5d showed a good zone of inhibition against *E. coli* and *Pseudomonas* sp. Compound 5e showed a good zone of inhibition against *S. pyogenes*. Compound 5f showed a good zone of inhibition against *S. pyogenes* and *E. coli*. Compound 5g showed a good zone of inhibition against *S. pyogenes*. Compound 5h showed a good zone of inhibition against *S. pyogenes*. These compound values are shown in Table 3.

The cyclohexane-1,3-dione derivatives were screened for antifungal activity at different concentrations such as 10, 5, and 2.5 mg/ml. The results are shown in Table 4. From Table 4, compound 5a shown the good zone of inhibition against *Candida albicans* (12 mm at 2.5 mg/ml).

CONCLUSION

The new series of cyclohexane-1,3-dione derivatives were synthesized by Michal addition reaction. The structures of the cyclohexane-1,3-dione derivatives were confirmed by FT-IR, 1H , and ^{13}C NMR spectral data. The docking study was carried out for cyclohexane-1,3-dione derivatives (5a-5h) using bacterial proteins (1UAG, 3UDI, and 2X50) and cancer protein (Z2OQ). From this result, the receptor and the compound have greater potency of interactions in a binding site. Especially, the compound 5c showed good binding score with cancer protein. For that reason, anticancer (human breast adenocarcinoma) activity was studied only compound 5c. The LC_{50} value of compound 5c is 10.3134 µg/ml. Furthermore, antimicrobial studies were carried out for cyclohexane 1,3-dione derivatives (5a-5h). From the antimicrobial and antifungal screening, it was observed that all compounds exhibited good activity against Gram-positive and Gram-negative strains. The present research work could be useful to develop the *in vivo* studies of the synthesized compounds.

ACKNOWLEDGMENT

We thank the Biogenix Research Center for conducting the MDA-MB-231 cell line anticancer assay for our compound 2-(1-(4-bromophenyl)-3-oxo-3-diphenylpropyl)-5,5-dimethyl cyclohexane-1,3-dione.

AUTHORS' CONTRIBUTIONS

Designed the experiments: Raja Chinnamanayakar and Ezhilarasi M. R. Performed the reactions: Raja Chinnamanayakar.

Performed the antimicrobial activity: Kulandhaivel. Performed the molecular docking study: Raja Chinnamanayakar. Performed the anticancer activity: Biogenix Research Center, Trivandrum. Wrote the paperwork: Raja Chinnamanayakar, Ezhilarasi M.R, and Prabha B. Revised the article: Ezhilarasi M.R and Prabha B.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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