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 some 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]. Galcine 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 antitumor, antiviral, and antibacterial [7], and antiinflammatory activity [8]. The C-C bond formation has been discovered in the past decades and its 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 dimeredone 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 as to undertake the present work. The antibacterial activity of cyclohexane-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 the in vitro anticancer activity was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method by using unmodified chiral diphenylethylenediamine (DPEN) [19]. In the MTT assay compound 5c showed the LC50 value of 10.31±0.005 µg/ml. In antimicrobial activity, the minimum inhibitory concentration of compound 5c is 2.5 mg/ml.

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.

Conclusion: An efficient synthesis of biologically active cyclohexane-1,3-dione derivatives has been developed.

Keywords: Dimeredone, Antimicrobial activity, Antifungal activity, Molecular docking studies, Anticancer activity, 3,4-methylenedioxyamphetamine-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 \(^1\)H and \(^13\)C NMR spectra were recorded by Bruker 500 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)

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. 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 PDB Bank (PDB) with id: 1UAG, 3UDI, 2X50, and 2ZQO.

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 × 40 × 40 A\(^3\) in 0.375 A 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 µg/ml), and amphotericin B (2.5 µg/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. Non-treated control cells were also maintained.

**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...
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=0 (1705.07, 1678.07, 1598.99), 3059.10, 3034.03, 2956.87 (Ar-CH), 1487.12 (C=C), 1487.12; 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, J₂',₂=15.2Hz, J₂',₃=3.2, 1H, H-²'); 3.64 ppm
2-(1-(4-chlorophenyl)-3-oxo-3-diphenylpropyl)-5, 5-dimethylcyclohexane-1,3-dione (5b)

Yield 86%; white solid; molecular formula; C_{24}H_{24}O_2; IR (KBr): ν (cm⁻¹) at C=O (1699.17, 1703.83, 1554.55), 3087.83, 3013.90, 2986.41 (Ar-CH); 1486.20 (C=C); 694.01, 763.03, 855.69; ν (CDCl₃, 400 MHz): 1.093 (s, 3H), 2.407-2.493 (m, 4H, 6-H and 8-H), 2.59 ppm (dd, J = 3.4 Hz, 1H, H-2'a); 3.67 ppm (dd, J = 7.1 Hz, 1H, H-2'a); 3.67 ppm (dd, J = 7.1 Hz, 1H, H-2'a); 4.47 ppm (dd, J = 7.4 Hz, 1H, H-2'b); 4.91 ppm (dd, J = 3.4 Hz, 1H, H-3'); 7.194-7.938 (m, Ar-H). ν (CDCl₃, 400 MHz, δ; 30.73 (CH₃); 30.97 (CH₃), 31.738 (C-5), 26.072 (C-3'), 45.98 (C-25'). 51.654 (C-5'), 53.416 (C-3), 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).

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

Yield 86%; white solid; molecular formula; C_{24}H_{24}BrO_2; IR (KBr): ν (cm⁻¹) at C=O (1699.24, 1670.83, 1554.55), 3087.83, 3013.90, 2986.41 (Ar-CH); 1486.20 (C=C); 694.01, 763.03, 855.69; ν (CDCl₃, 400 MHz): 1.116 (s, 3H), 2.407-2.493 (m, 4H, 6-H and 8-H), 2.59 ppm (dd, J = 3.4 Hz, 1H, H-2'a); 3.67 ppm (dd, J = 7.1 Hz, 1H, H-2'a); 3.67 ppm (dd, J = 7.1 Hz, 1H, H-2'a); 4.47 ppm (dd, J = 7.4 Hz, 1H, H-2'b); 4.91 ppm (dd, J = 3.4 Hz, 1H, H-3'); 7.194-7.938 (m, Ar-H). ν (CDCl₃, 400 MHz, δ; 30.73 (CH₃); 30.97 (CH₃), 31.738 (C-5), 26.072 (C-3'), 45.98 (C-25'). 51.654 (C-5'), 53.416 (C-3), 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).

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

Docking studies of cyclohexane-1,3-dione derivatives (5a-5h) were individual, docked in silico molecular docking studies of cyclohexane-1,3-dione derivatives (5a-5h) show better binding affinity 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, 1UAG, 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 side chains. 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 interactions were obtained. Cyclohexane-1,3-dione derivatives has been explained.
### 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|
| 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 |
| 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 |
| 3UDI               | -9.6    |Nil                                |LYS A:137, ILE A:148, PHE A:72, ILE A:140 |
| 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 |
| 3UDI               | -9.6    |Nil                                |LYS A:185, PRO A:184, ALA A:66, LYS A:147, 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, LYS A:147, TYR A:144, PHE A:72 |
| 3UDI               | -8.8    |LYS A:137                          |LYS A:147, TYR A:144, PHE A:72        | LEU A:416, SE A:112, LYS A:319  |
| 2X50               | -8.3    |Nil                                |PHE A:303                             | LYS A:137, ILE A:148, TYR A:144 |
|                   |         |                                   |                                        | 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               | −8.1    | Nil                               | VAL A:232, VAL A:328                   | NIL                           | ASN A:331, ASP A:214, HIS A:267 |
| 3UDI               | −8.9    | Nil                               | ILE A:148                              | LYS A:137, ILE A:140, GLU A:67 |
| 2X5O               | −8.6    | ASN A:178, ASN A:211, ASN A:271   | ALA A:320                              | ASP A:214, HIS A:267          |
| 1UAG               | −8.1    | PHE A:422, SER A:415, LYS A:115, ASN A:138 | LEU A:416, ALA A:414, PHE A:161, LYS A:348, LEU A:15 | NIL                           | HIS A:183 |
| 3UDI               | −9.1    | PHE A:149                          | LEU A:141, LYS A:137, ILE A:148, LYS A:147, PRO A:187, PHE A:149 | PHE A:72, ILE A:140, TYR A:144 |
| 2X5O               | −8.3    | ASN A:211                          | ARG A:221, ALA A:414                    | PHE A:422, PHE A:303          |
| 1UAG               | −8.6    | ASN A:138                          | LYS A:319, LYS A:348, ALA A:414, PHE A:422 | LEU A:416, HIS A:183          |
| 3UDI               | −9.5    | Nil                               | LYS A:185, PRO A:184, ALA A:66, LYS A:147, PHE A:72, TYR A:144 | TYR A:144, ILE A:48          |
| 2X5O               | −8.1    | ASN A:138, LYS A:319               | ALA A:414, LEU A:416                    | PHE A:161, PHE A:422          |
| 1UAG               | −7.8    | LYS A:319, LYS A:319, SER A:112, SER A:415, ASN A:138 | LEU A:263, LEU A:333               | NIL                           |
| 3UDI               | −7.6    | SER A:470, TYR A:485, SER A:487, LYS A:669, THR A:670, THR A:672, TYR A:707 | NIL                           | NIL                           |
| 2X5O               | −8.3    | Nil                               | PHE A:303, GLU A:266                    | PHE A:303, GLU A:266          |
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 (2X5O). 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 2X5O 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 2ZOQ. 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 2ZOQ protein

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

![Graph showing percentage viability vs. concentration (µg/ml)]

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

| 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.     | *Staphylococcus aureus*   | 9  8 10 9 10 7 9 8                           |
| 2.     | *Streptococcus pyogenes*  | 12 10 10 10 12 11 12 11                       |
| 3.     | *Escherichia coli*        | 12 8 9 11 11 11 10 10                       |
| 4.     | *Pseudomonas sp.*         | 12 7 10 11 11 10 10 9                       |

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

| S. No. | 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     |

Antimicrobial susceptibility testing against *Candida albicans*

| S. No. | 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 dinedone moiety. This compound has only one alkyl and pi-alkyl interaction with PRO A: 373 formed at the benzene ring of biphenyl moiety.
The synthesized compounds (5a-5h) were subjected to insilico analysis against different bacterial proteins (1UAG, 3UDI, and 2X5O). Compound 5c showed binding scores as −9.4, −9.6, and −9.1 kcal/mol against 1UAG, 3UDI, and 2X5O proteins indicating a very good affinity. In a similar way when these compounds were docked against the human breast cancer protein Z2QG, 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 LC50 value of this compound is 10.31±0.03 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 showed 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 Michaid addition reaction. The structures of the cyclohexane-1,3-dione derivatives were confirmed by FT-IR, ^1H, and ^13C NMR spectral data. The docking study was carried out for cyclohexane-1,3-dione derivatives (5a-5h) using bacterial proteins (1UAG, 3UDI, and 2X5O) and cancer protein (Z2QG). From this result, the receptor and the compound have greater potency of interactions in a binding site.

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