

## Full Length Article

# Design, synthesis of new 4,5-dibenzylidene-9,10-diphenyl-1,2,7,8,9,10-hexahydroacridine-3,6-dione derivatives using extract of *Vitexnegundo*: Cytotoxic activity & molecular docking study

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## ABSTRACT

Among phytochemical and pharmaceutical investigations, the green production of silver nanoparticles has emerged as the crown jewel of this investigation. The production of acridinedione substances is greatly aided by the high catalytic activity of these nanoparticles. Structures and morphologies were determined by using a wide range of analytical techniques, including nuclear magnetic resonance (<sup>1</sup>H & <sup>13</sup>C), Fourier transform infrared spectroscopy, mass spectrometry, elemental investigation, scanning electron microscopy, also transmission electron microscopy. Acridinedione compounds' cytotoxicity was measured using the MTT assay with doxorubicin as the reference drug in a number of cancerous and healthy cell lines, comprising Human embryonic kidney cell (HEK293), liver cell (LO2), and lung cell (MRC5). The cytotoxicity compound 1h was shown to be especially active (HepG2, LC<sub>50</sub>-0.5 μM; MCF-7, LC<sub>50</sub>-0.64 μM; HeLa, LC<sub>50</sub>-0.52 μM). By eliciting IC<sub>50</sub> values larger than 100 μg/M in normal cell lines (HEK-293), (LO2), and (MRC5), the synthesised compounds proved to be safe. In addition, we present the results from an *in-silico* analysis of the Methoxsalen protein (1Z11). The binding affinity of compound 1h for the 1Z11 protein is greater than that of other compounds (-11.7 kcal/mol), but it is less than that of Doxorubicin (-10.2 kcal/mol). Hence, the chemical 1h may be used to create powerful cancer drugs.

## 1. Introduction

In the twenty-first century, cancer remains a significant health concern. Cancer is a hereditary illness that involves several different physical phenomena such as cell signalling and death, making treatment difficult [1]. Cancer is often identified as a limited sickness that evolves over time, making therapy tough. It's the leading killer in industrialised nations. Cancer symptoms include new tumour growth, irregular bleeding, prolonged coughing, unexplained weight loss, as well as changes in bowel habits [2]. Yet, the mechanism by which malnutrition increases cancer risk remains obscure [3]. Cancer is the leading cause of death in the United States and has the 2nd highest global mortality rate [4–6]. Several researchers (using radiation and chemotherapy) have spent years trying to figure out how to effectively combat cancer [7]. Chemotherapy is the sole choice for treating cancer that has progressed

to other organs, despite the fact that it has serious side effects similar to those of other treatments. Several noncytotoxic drugs inhibit cancer cell proliferation [8]. The synthesis of nanoparticles with bioactive substance is an attractive nanotechnology that is a component of green chemistry. The term "green chemistry" denotes to the evolution of chemical compounds and procedures that considerably minimise the usage of toxic components or solvents without compromising the ability to produce desirable items at an affordable price. In the wake of the effective development of green nanoparticles (NPs), numerous biological techniques were devised for the manufacture of metal NPs, including the use of enzymes [9], microorganisms [10], and plant extracts [11–13]. A profile of bioactive compounds of *Vitex negundo* has been reported, which revealed that the plant contained a high amount of total phenolic compounds and flavonoids, which are considered to be potent natural antioxidants [14–17]. Two important challenges for the

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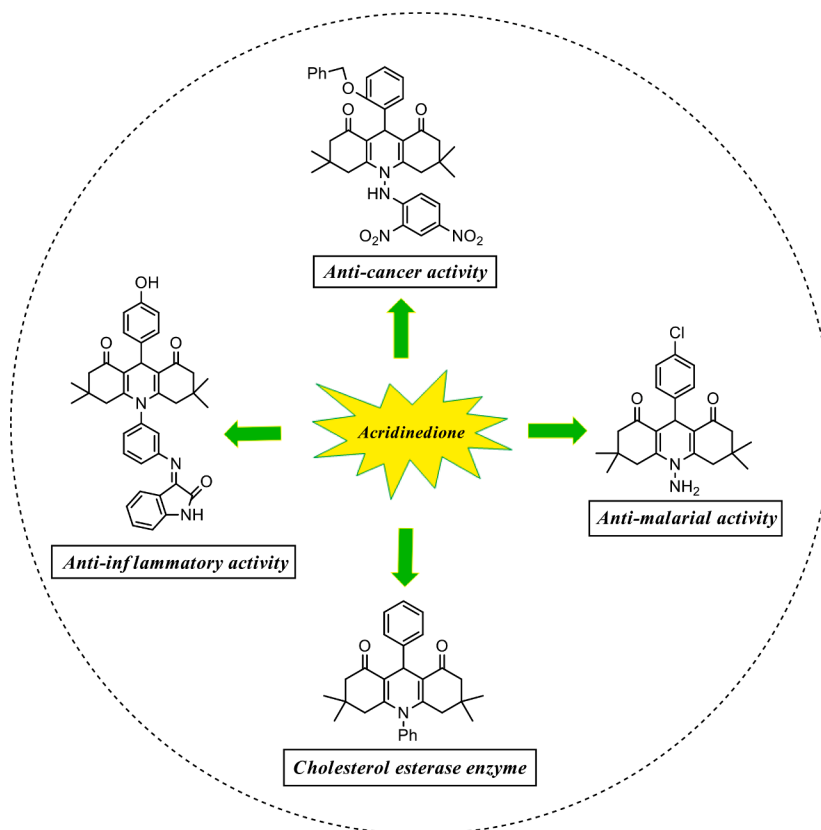


Fig. 1. Biologically active 1,8-acridinedione compounds.

use of acridones as prospective anti-cancer medications are the capacity of acceptor/donor nitrogen to intercalate among base pairs in the double-stranded DNA structure and the efficiency of tricyclic, heterocyclic, and planar arrays to intercalate between base pairs in the double-stranded DNA structure [18,19]. Furthermore, acridone analogues impede the growth of cancer cells through blocking the activity of enzymes that control DNA topology, including topoisomerase, telomerase, and cyclin-dependent kinases [20,21]. Several chemicals with important physiological effects have this basic structure. Antifungal [22], antitumor [23], anticancer [24], and antiglaucoma [25] properties have been shown to be present in acridinedione variants, as shown in Fig. 1. One approach for synthesising acridinedione is the multi component reaction (MCR) of various amines, cyclic diketones, and aldehydes utilising different catalysts [26–27]. DFT has been applied for high accuracy for quantum mechanical electronic structure calculations on the title compound with different solvents. Highest Occupied Molecular Orbital to Lowest Unoccupied Molecular Orbital is investigated in water and DMSO solvent [28–32]. Owing to the difficulty in recycling, recovering, and separating these homogenous catalysts throughout synthesis. To get over these restrictions and enhance the reaction conditions for acridinedione synthesis, a new method using reusable catalyst has been developed. To prepare substituted acridinedione derivatives on ethanol medium with milder process conditions, shortened reaction times, improved substituent variation in the components, and higher yields, we provide a simple and direct approach for the synthesis and characterization of Ag NPs, which are used as a novel and increasingly cost-effective green catalyst. Before evaluating the compounds for cytotoxicity, they were characterised using FT-IR, NMR ( $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR), mass, elemental analysis, scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

## 2. Materials and methods

### 2.1. General methods

1,3-cyclohexanedione, substituted aldehyde, and aniline were all compounds supplied by Merck. The melting points were determined using open capillary tubes that were not calibrated. All of the KBr IR spectra were captured with a Shimadzu 8201 pc ( $4000\text{--}400\text{ cm}^{-1}$ ). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded using a Bruker DRX-300 MHz instrument. The elemental analysis (of C, H, and N) was performed using a model of an elemental analyzer (Varian EL III). The GC-MS data was recorded using a Perkin Elmer GCMS clarus SQ8 (EI). The morphology of silver nanoparticles is verified by scanning electron microscopy and transmission electron microscopy. For SEM analysis, we used a VP-1450 Scanning Electron Microscope from LEO (a German company). For this work, researchers used a transmission electron microscopy (TEM) instrument with the name of LEO 912 AB. Silica gel plates and thin layer chromatography were used to analyse the chemicals for purity (TLC).

### 2.2. Preparation of *Vitex negundo* extract

*Vitex negundo* green leaves were washed and dried in an oven dryer at  $40^\circ\text{C}$  for 48 h. The dried leaves were ground into a powder and stored at  $20^\circ\text{C}$  for further analysis. Overnight at  $40^\circ\text{C}$ , 20 grammes of coarsely chopped leaves were extracted with methanol (ratio 1:10 w/v) in a water bath with shaking. A vacuum pump was used to collect the residue again after it had been filtered using Whatman filter paper No. 1. Using a rotational vacuum evaporator maintained at  $40^\circ\text{C}$ , all of the solvent was removed. After the extract was concentrated, it was stored in dark vials at  $4^\circ\text{C}$ .

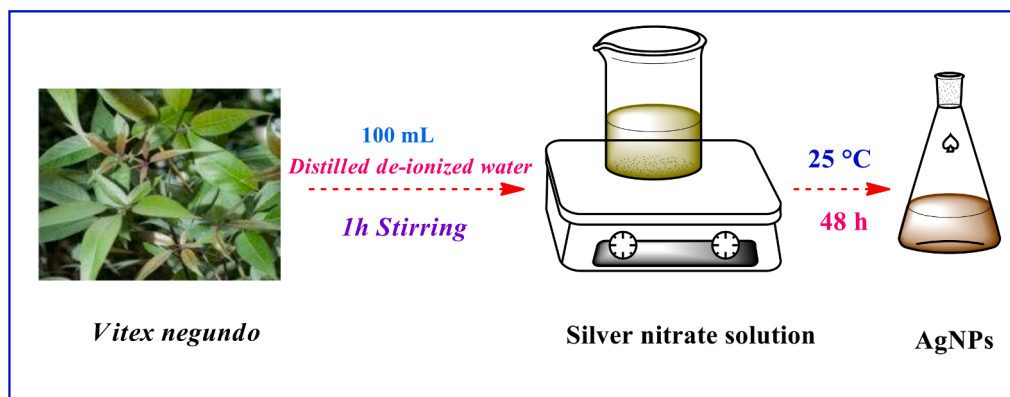
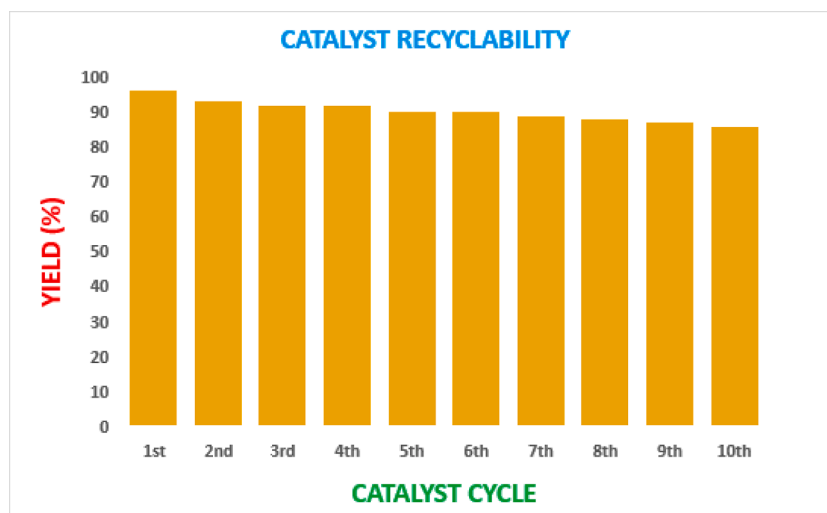
Fig. 2. Synthesis of Ag nanoparticles from *Vitex negundo*.

Fig. 3. Recyclability of Ag nanoparticle.

Table 1

Catalyst recyclability.

| Entry | Catalyst use     | Yield (%) |
|-------|------------------|-----------|
| 1     | 1 <sup>st</sup>  | 96        |
| 2     | 2 <sup>nd</sup>  | 93        |
| 3     | 3 <sup>rd</sup>  | 92        |
| 4     | 4 <sup>th</sup>  | 92        |
| 5     | 5 <sup>th</sup>  | 90        |
| 6     | 6 <sup>th</sup>  | 90        |
| 7     | 7 <sup>th</sup>  | 89        |
| 8     | 8 <sup>th</sup>  | 88        |
| 9     | 9 <sup>th</sup>  | 87        |
| 10    | 10 <sup>th</sup> | 86        |

### 2.3. Synthesis of AgNPs

The crude extract (0.5 g) was vigorously mixed into 100 mL of distilled de-ionized water for 1 hour. Following that, a hundred millilitres ( $1 \times 10^{-1}$  M) of  $\text{AgNO}_3$  was weighed and agitated for 48 h at room temperature (25 °C). Throughout the incubation period, silver nanoparticles were gradually obtained. The synthesis of Ag-NPs is shown in Fig. 2.

### 2.4. Recovery of catalyst

Fig. 3 shows the recovery of the catalyst salvaged from at least 10 run times, with a slight loss in catalytic activity. The lessening of activity

could be detected with the regenerated catalyst on salvaging due to the surface area of the catalyst during the reaction, or partial loss of the basic sites/regeneration. The application of the catalyst was inspected by optimizing the reaction conditions. A number of aldehydes were selected for the condensation reaction with the Ag-NPs (1 mole %) catalyst at room temperature in a solvent-free setting, and the yield is reported in Table 1.

### 2.5. General procedure for the synthesis of 4-benzylidene-5-ethylidene-9,10-diphenyl-1,2,7,8,9,10-hexahydroacridine-3,6(4H,5H)-dione (1a)

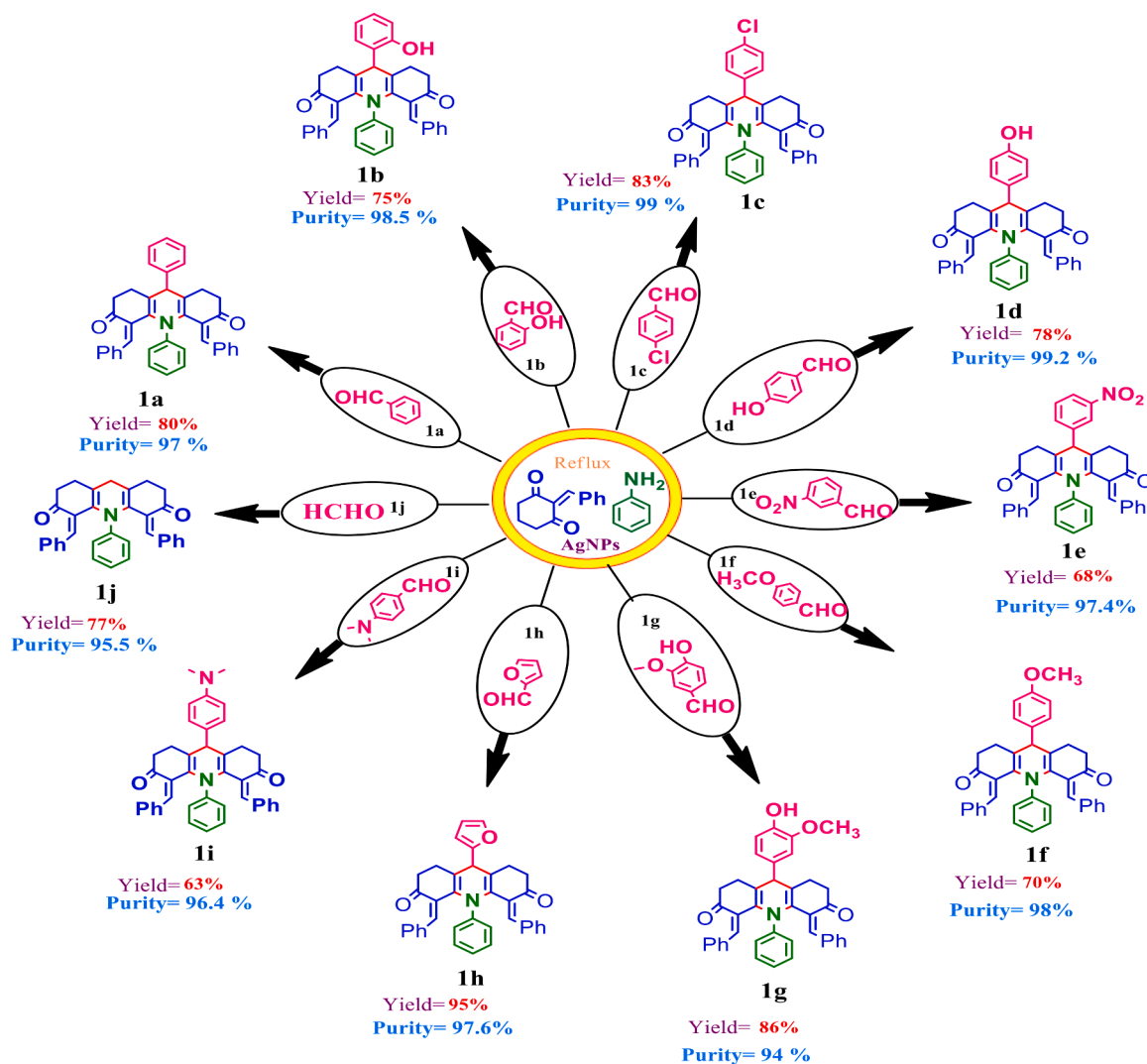
We compounded substituted 1,3-cyclohexanedione derivative (0.02 mol), benzaldehyde (0.01 mol), and aniline (0.01 mol) as well as AgNPs as a catalyst in ethanol solution to give a yellow precipitate. The product was filtered and dried after being rinsed with ice cold water. TLC was used to validate the product. Ethanol was used to recrystallize the precipitate. Compounds (1b–1j) were synthesized using the same procedure (Scheme 1).

### 2.6. Cytotoxic activity

The newly synthesized compounds (1a–1j) were screened for their cytotoxic activity according to a procedure described in previous literature [33]. Three cancer cell lines were treated with these compounds at one primary cytotoxic assay dose of 100  $\mu\text{M}$  for 48 h (MTT anticancer assay). Doxorubicin was used as a standard. The three cell lines used in the present investigation were HepG2 (liver) and Hela (cervical), MCF-7

**Table 2**Cytotoxic activity of synthesized compounds (1a-1j)<sup>a</sup>.

| Cpds                      | MCF7                           |                   |                                | HeLa                           |                   |                                | HepG2                       |                   |                                |
|---------------------------|--------------------------------|-------------------|--------------------------------|--------------------------------|-------------------|--------------------------------|-----------------------------|-------------------|--------------------------------|
|                           | GI <sub>50</sub><br>( $\mu$ M) | TGI<br>( $\mu$ M) | LC <sub>50</sub><br>( $\mu$ M) | GI <sub>50</sub><br>( $\mu$ M) | TGI<br>( $\mu$ M) | LC <sub>50</sub><br>( $\mu$ M) | GI <sub>50</sub> ( $\mu$ M) | TGI<br>( $\mu$ M) | LC <sub>50</sub><br>( $\mu$ M) |
| 1a                        | -                              | -                 | >100                           | -                              | -                 | 100 $\pm$ 0.02                 | cb53.2 $\pm$ 0.07           | 68.3 $\pm$ 0.12   | >100                           |
| 1b                        | 56.1 $\pm$ 0.04                | 75.1 $\pm$ 0.04   | >100                           | 61.0 $\pm$ 0.06                | 79.2 $\pm$ 0.05   | >100                           | 42.2 $\pm$ 0.96             | 13.1 $\pm$ 0.03   | 56.8 $\pm$ 0.03                |
| 1c                        | 64.9 $\pm$ 0.05                | 33.7 $\pm$ 0.08   | 39.4 $\pm$ 0.28                | 51.3 $\pm$ 0.15                | 77.2 $\pm$ 0.08   | 66.7 $\pm$ 0.10                | 46.3 $\pm$ 0.56             | 55.3 $\pm$ 0.06   | 59.9 $\pm$ 0.06                |
| 1d                        | 0.89 $\pm$ 0.12                | 09.3 $\pm$ 0.05   | 65.0 $\pm$ 0.38                | 08.9 $\pm$ 0.04                | 16.8 $\pm$ 0.07   | 55.9 $\pm$ 0.08                | 01.0 $\pm$ 0.67             | 0.25 $\pm$ 0.26   | 54.0 $\pm$ 0.08                |
| 1e                        | 34.4 $\pm$ 0.22                | 49.3 $\pm$ 0.02   | 66.3 $\pm$ 0.09                | 44.2 $\pm$ 0.08                | 62.1 $\pm$ 0.04   | >100                           | 25.9 $\pm$ 1.56             | 28.2 $\pm$ 0.14   | 44.8 $\pm$ 0.01                |
| 1f                        | 28.6 $\pm$ 0.08                | 31.8 $\pm$ 0.04   | 42.3 $\pm$ 0.11                | 31.6 $\pm$ 0.11                | 44.7 $\pm$ 0.01   | 67.4 $\pm$ 0.07                | 39.1 $\pm$ 1.47             | 36.8 $\pm$ 0.02   | 57.5 $\pm$ 0.26                |
| 1g                        | 12.9 $\pm$ 0.16                | 45.3 $\pm$ 0.07   | 10.3 $\pm$ 0.03                | 52.9 $\pm$ 0.01                | 81.3 $\pm$ 0.06   | 45.3 $\pm$ 0.03                | 21.3 $\pm$ 1.63             | 41.0 $\pm$ 0.16   | 72.1 $\pm$ 0.16                |
| 1h                        | 44.0 $\pm$ 0.04                | 07.4 $\pm$ 0.01   | 0.64 $\pm$ 0.15                | 07.9 $\pm$ 0.03                | 11.9 $\pm$ 0.12   | 0.52 $\pm$ 0.06                | 2.00 $\pm$ 0.86             | 1.00 $\pm$ 0.08   | 0.50 $\pm$ 0.08                |
| 1i                        | 55.6 $\pm$ 0.10                | 66.0 $\pm$ 0.09   | 59.3 $\pm$ 0.09                | 59.0 $\pm$ 0.08                | 59.3 $\pm$ 0.01   | 65.0 $\pm$ 0.01                | 29.3 $\pm$ 1.26             | 18.3 $\pm$ 0.09   | >100                           |
| 1j                        | 66.0 $\pm$ 0.09                | 39.3 $\pm$ 0.08   | 78.6 $\pm$ 0.14                | 3.3 $\pm$ 0.02                 | 48.3 $\pm$ 0.09   | 58.3 $\pm$ 0.08                | 50.3 $\pm$ 1.74             | 35.3 $\pm$ 0.02   | 66.8 $\pm$ 0.02                |
| Doxorubicin<br>(standard) | 0.02 $\pm$ 0.05                | 0.21 $\pm$ 0.18   | 0.74 $\pm$ 0.02                | 0.05 $\pm$ 0.10                | 0.41 $\pm$ 0.03   | 0.88 $\pm$ 0.12                | 0.01 $\pm$ 0.58             | 0.13 $\pm$ 0.04   | 0.58 $\pm$ 0.01                |

<sup>a</sup> Each compound was tested in triplicate. All error bars represent mean $\pm$ SD from three independent experiments.**Fig. 4.** Optimization of reaction conditions (1a-1j).

(breast). In the current protocol, all cell lines were pre-incubated on a microtiter plate. The results of each test were reported as the growth percentage of treated cells compared to untreated control cells (Table 2). The screening results were expressed in terms of the growth inhibitor concentration (GI<sub>50</sub>), total growth of inhibition (TGI), and lethal concentration (LC<sub>50</sub>). To each well of the 96-well plates, 10,000 cells were

added and incubated in a CO<sub>2</sub> incubator for 24 h at 37° C. Then, the medium was flicked off, and 95  $\mu$ L of fresh medium and 5  $\mu$ L of drug were added. The plate was incubated for 48 h at 37° C. The 20  $\mu$ L medium was removed, 20  $\mu$ L of MTT was added, and the assay was done. The plate was again incubated in CO<sub>2</sub> incubator for 4–6 h at 37° C. The entire medium was carefully removed, and the formazan crystal solution

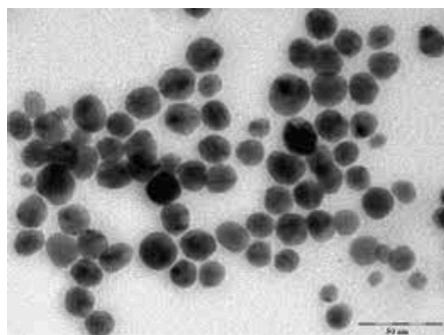


Fig. 5. TEM image of silver nanoparticle.

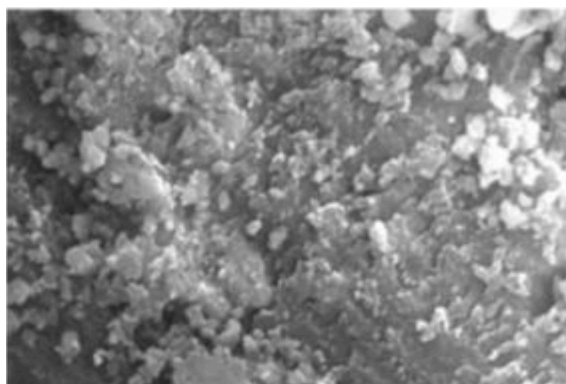


Fig. 6. SEM micrograph of the synthesized AgNPs using *Vitex negundo* leaves extract.

Table 3

In vitro cytotoxicity of synthesised derivatives (1a-1j) on normal cells<sup>a</sup>.

| Compounds | MRC5<br>IC <sub>50</sub> (μM) | HEK-293<br>IC <sub>50</sub> (μM) | LO2<br>IC <sub>50</sub> (μM) |
|-----------|-------------------------------|----------------------------------|------------------------------|
| 1a        | 56.36 ± 0.08                  | 69.06 ± 0.12                     | 67.48 ± 0.12                 |
| 1b        | 77.21 ± 0.02                  | 62.12 ± 0.07                     | 70.17 ± 0.18                 |
| 1c        | 76.66 ± 0.04                  | 51.14 ± 0.01                     | 79.10 ± 0.07                 |
| 1d        | 58.25 ± 0.09                  | 76.14 ± 0.13                     | 66.24 ± 0.04                 |
| 1e        | 70.76 ± 0.18                  | 57.09 ± 0.24                     | 56.01 ± 0.10                 |
| 1f        | 59.24 ± 0.34                  | 66.17 ± 0.08                     | 68.24 ± 0.24                 |
| 1g        | 66.32 ± 0.47                  | 67.01 ± 0.16                     | 58.22 ± 0.28                 |
| 1h        | 89.61 ± 0.28                  | 88.24 ± 0.34                     | 80.54 ± 0.15                 |
| 1i        | 74.41 ± 0.11                  | 75.44 ± 0.01                     | 65.70 ± 0.31                 |
| 1j        | 71.14 ± 0.13                  | 52.71 ± 0.09                     | 70.12 ± 0.29                 |

<sup>a</sup> Each compound was tested in triplicate. All error bars represent mean ± SD from three independent experiments.

was made up with DMSO solvent. The molecular stain absorbance was read at 570 nm in a plate reader (Bio-Rad Laboratories Inc., CA, and USA). Cell viability was calculated as (Absorbance 570 nm of Test/Absorbance 570 nm of Control) X 100.

## 2.7. Molecular docking

Compounds **1a-1j**, Doxorubicin, and the Human microsomal cytochrome P450 2A6 protein in association with Methoxsalen (PDB ID: 1Z11) were investigated for their binding mechanism and interactions via Autodock vina 1.1.2. [34–36]. The inhibitor is effectively fills the active site cavity without sustainability perturbing the chemical structure. Compounds 1a-1j and Doxorubicin were developed using Chem3D Pro 12.0 and Chem Draw Ultra 12.0. AutoDock Tools 1.5.6 was used to generate the input files used by AutodockVina. The grid coordinates for

the protein of interest are as follows: centre x,y,z: 56.316, 77.155, 60.326; size x,y,z: 24, 20, 20; and spacing 1.0. Exhaustiveness is seen to be represented by the number 8. The default settings for the other Vina docking parameters are not specified. Discovery Studio 2019 was used to visually evaluate the data, with the chemical with the lowest binding affinity value being awarded the highest score.

## 3. Results and discussion

### 3.1. Chemistry

The use of silver nanoparticles as a catalyst is shown in Scheme 2 as a potential technique for the manufacture of acridinedione derivative products. It is hypothesised that the silver nano particle induces the polarisation of carbonyl groups, aldehyde, and 1,3-cyclohexanedione. We also think silver nanoparticles set the stage for the synthesis of 1,3-cyclohexanedione analogues and facilitate the creation of the proper imine through a condensation reaction with aniline. Acquired imine undergoes tautomerization to generate enamine. Thus, silver nanoparticles play a role in the formation of two reactive intermediates, I and II, that react via Michael addition to form an adduct and then undergo tautomerization. A water molecule is lost during the tautomerization of the Michael adduct, resulting in a simple intramolecular ring closure and the desired product, **1a-1j**. Fig. 4 shows the optimization of reaction conditions (**1a-1j**).

#### Mechanism

**Synthesis of 4,5-dibenzylidene-9,10-diphenyl-1,2,7,8,9,10-hexahydroacridine-3,6-dione 1a** IR (KBr) (cm<sup>-1</sup>): 3234 (Ar-H), 2850 (-CH<sub>2</sub>), 1710 (C=O), 1110(C-N); <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): 7.64-6.25 (m, 5H, Phenyl), 7.40-7.33 (m, 10H<sub>2</sub>), 7.30-7.23 (m, 5H, Ph), 6.20 (s, 2H, =CH-Phenyl), 4.43 (s, 1H, CH-Phenyl), 2.98-2.07 (m, 8H, Cyclohexanedione); <sup>13</sup>C NMR (300 MHz, DMSO-d<sub>6</sub>): 194.41, 142.22, 141.23, 140.24, 137.75, 134.56, 132.07, 129.58, 128.69, 127.70, 122.81, 122.41, 109.72, 40.33, 35.04, 20.35; EI-MS: 546 (M<sup>+</sup>, 20%); Elemental analysis (C<sub>39</sub>H<sub>31</sub>N<sub>2</sub>O<sub>2</sub>): Calculated: C, 83.84; H, 5.73; N, 2.57 %; Found: C, 83.82; H, 5.71; N, 2.55 %.

**Synthesis of 4,5-dibenzylidene-9-(2-hydroxyphenyl)-10-phenyl-1,2,7,8,9,10-hexahydro acridine-3,6-dione 1b** IR (KBr) (cm<sup>-1</sup>): 3400 (OH), 3095 (Ar-H), 2855 (-CH<sub>2</sub>), 1723 (C=O), 1113 (C-N); <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): 7.64-6.25 (m, 5H, Phenyl), 7.40-7.33 (m, 10H, Phenyl- Cyclohexanedione), 7.09-6.83 (m, 4H, Ph-OH), 6.16 (s, 2H, =CH-Phenyl), 5.35 (s, 1H, OH), 4.43 (s, 1H, CH-Phenyl), 2.98-2.07 (m, 8H, Cyclohexanedione); <sup>13</sup>C NMR (300 MHz, DMSO-d<sub>6</sub>): 194.45, 156.16, 141.27, 140.28, 137.79, 134.50, 132.01, 130.42, 129.53, 128.54, 127.95, 127.16, 122.87, 122.68, 122.49, 115.80, 109.71, 35.02, 34.13, 20.34; EI-MS: 591 (M<sup>+</sup>, 20%); Elemental analysis (C<sub>39</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub>): Calculated: C, 79.30; H, 5.12; N, 4.74 %; Found: C, 79.28; H, 5.10; N, 4.72 %.

**Synthesis of 4,5-dibenzylidene-9-(4-chlorophenyl)-10-phenyl-1,2,7,8,9,10-hexahydro acridine-3,6-dione 1c** IR (KBr) (cm<sup>-1</sup>): 3095 (Ar-H), 2860 (-CH<sub>2</sub>), 1745 (C=O), 1191 (C-N), 748 (C-Cl); <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): 7.64-6.25 (m, 5H, Phenyl), 7.40-7.33 (m, 10H, Phenyl Cyclohexanedione), 7.37-7.17 (m, 4H, Ph-Cl), 6.21 (s, 2H, =CH-Phenyl), 4.43 (s, 1H, CH-Phenyl), 2.98-2.07 (m, 8H, Cyclohexanedione); <sup>13</sup>C NMR (300 MHz, DMSO-d<sub>6</sub>): 194.42, 141.23, 140.34, 140.25, 137.76, 134.57, 132.08, 131.39, 130.40, 129.51, 128.72, 128.53, 127.94, 122.85, 122.46, 109.77, 40.38, 35.09, 20.30; EI-MS: 561 (M<sup>+</sup>, 20%); Elemental analysis (C<sub>39</sub>H<sub>31</sub>N<sub>2</sub>O<sub>3</sub>): Calculated: C, 83.40; H, 5.56; N, 2.49 %; Found: C, 83.38; H, 5.54; N, 2.47 %.

**Synthesis of 4,5-dibenzylidene-9-(4-hydroxyphenyl)-10-phenyl-1,2,7,8,9,10-hexahydro acridine-3,6-dione 1d** IR (KBr) (cm<sup>-1</sup>): 3400 (OH), 3029 (Ar-H), 2865 (-CH<sub>2</sub>), 1701 (C=O), 1256 (C-N); <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): 7.64-6.25 (m, 5H, Phenyl), 7.40-7.33 (m, 10H, Phenyl- Cyclohexanedione), 7.06-6.63 (m, 4H, Ph-OH), 6.19 (s, 2H, =CH-Phenyl), 5.35 (s, 1H, OH), 4.43 (s, 1H, CH-Ph), 2.98-2.07 (m, 8H, Cyclohexanedione); <sup>13</sup>C NMR (300 MHz, DMSO-d<sub>6</sub>): 194.43, 155.54,



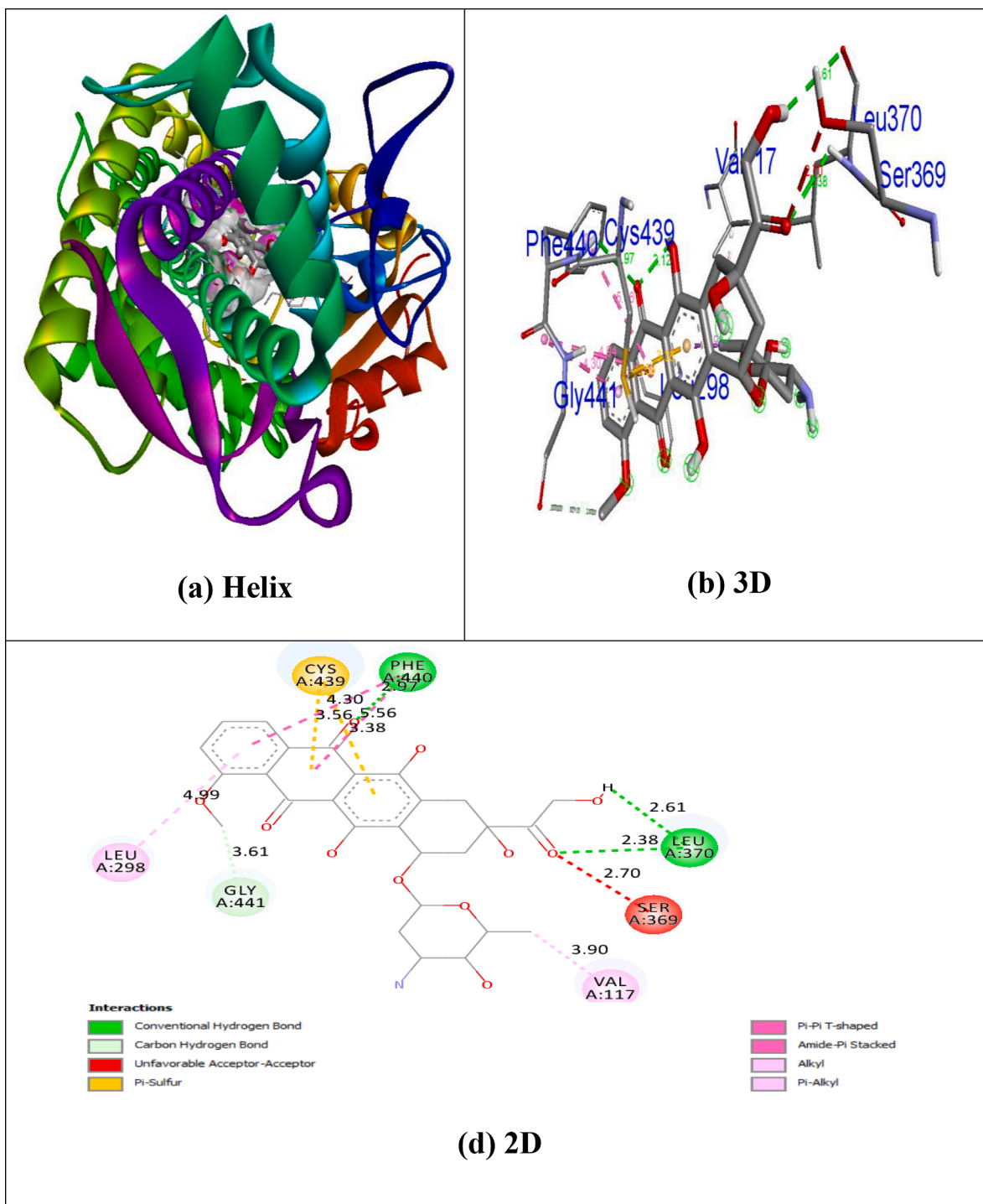


Fig. 7. Interaction kinds of compound 1h in the binding pocket of 1Z11 receptor.

141.25, 140.26, 137.77, 134.88, 134.59, 132.00, 130.41, 129.52, 128.53, 127.94, 122.85, 122.46, 115.87, 109.78, 40.39, 35.00, 20.31; EI-MS: 580 (M<sup>+</sup>, 20%); Elemental analysis (C<sub>39</sub>H<sub>30</sub>ClNO<sub>2</sub>): Calculated: C, 80.75; H, 5.21; N, 2.41 %; Found: C, 80.73; H, 5.19; N, 2.39 %.

**Synthesis of 4,5-dibenzylidene-9-(3-nitrophenyl)-10-phenyl-1,2,7,8,9,10-hexahydroacridine-3,6-dione 1e** IR (KBr) (cm<sup>-1</sup>): 3027 (Ar-H), 2872 (-CH<sub>2</sub>), 1717 (C=O), 1673 (NO<sub>2</sub>), 1349 (C-N); <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): 8.12-7.62 (m, 4H, Ph-NO<sub>2</sub>), 7.64-6.25 (m, 5H, Phenyl), 7.40-7.33 (m, 10H, Phenyl- Cyclohexanedione), 6.18 (s, 2H, =CH-Phenyl), 5.35 (s, 1H, OH), 4.43 (s, 1H, CH-Phenyl), 2.98-2.07 (m, 8H, Cyclohexanedione); <sup>13</sup>C NMR (300 MHz, DMSO-d<sub>6</sub>): 194.44,

145.25, 143.16, 141.27, 140.28, 137.79, 134.50, 133.81, 132.02, 129.53, 128.54, 127.95, 122.86, 122.47, 121.88, 120.99, 109.701, 40.3, 35.02, 20.33; EI-MS: 562 (M<sup>+</sup>, 20%); Elemental analysis (C<sub>39</sub>H<sub>31</sub>NO<sub>3</sub>): Calculated: C, 83.40; H, 5.56; N, 2.49 %; Found: C, 83.38; H, 5.54; N, 2.47 %.

**Synthesis of 4,5-dibenzylidene-9-(4-methoxyphenyl)-10-phenyl-1,2,7,8,9,10-hexahydroacridine-3,6-dione 1f** IR (KBr) (cm<sup>-1</sup>): 3027 (Ar-H), 2900 (-CH<sub>2</sub>), 1752 (C=O), 1285 (C-N); <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): 7.64-6.25 (m, 5H, Phenyl), 7.40-7.33 (m, 10H, Phenyl- Cyclohexanedione), 7.12-6.87 (m, 4H, Ph-OCH<sub>3</sub>), 6.15 (s, 2H, =CH-Phenyl), 4.43 (s, 1H, CH-Phenyl), 3.83 (s, 3H, OCH<sub>3</sub>), 2.98-2.07

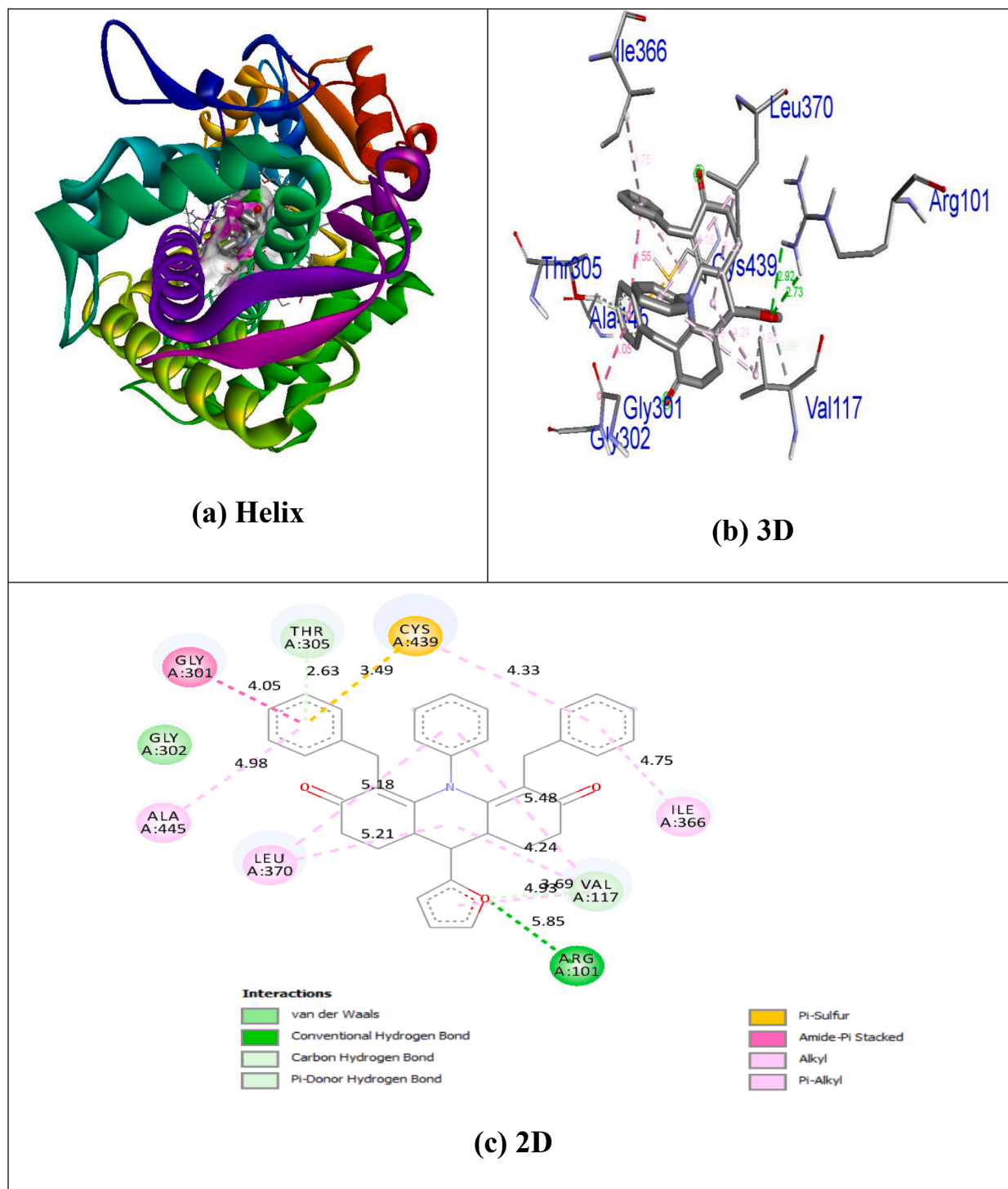


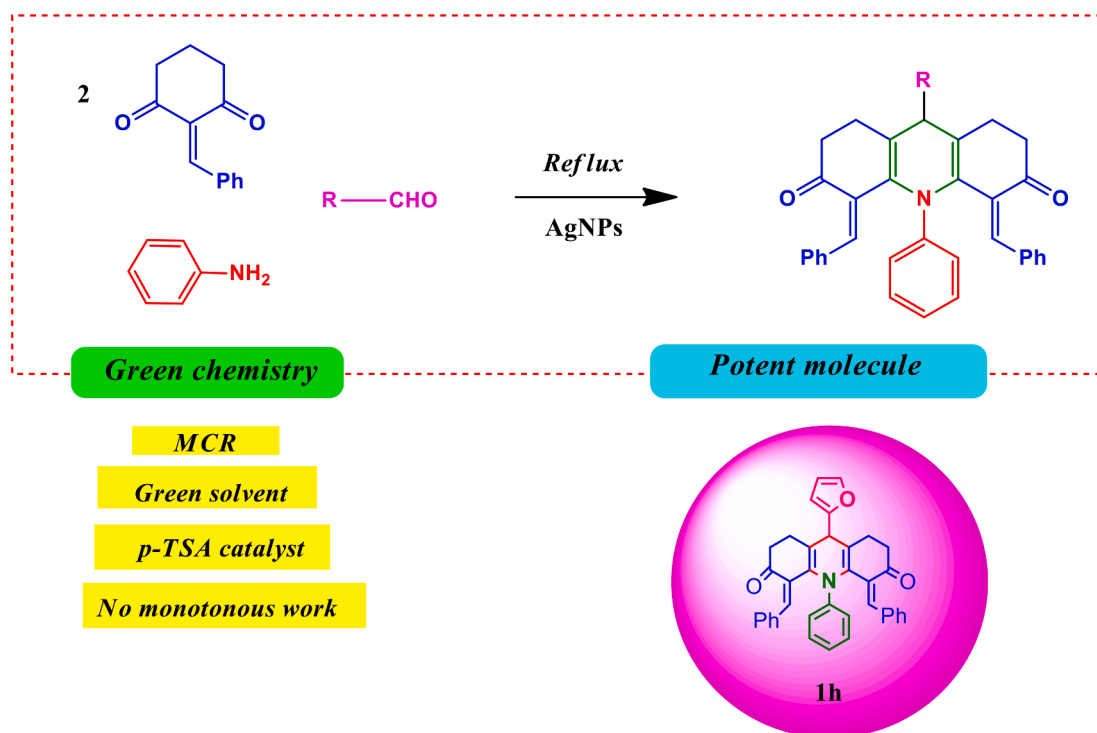
Fig. 8. Interaction kinds of compound Doxorubicin in the binding pocket of 1Z11 receptor.

(m, 8H, Cyclohexanedione);  $^{13}\text{C}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ): 194.47, 157.68, 141.29, 140.20, 137.71, 134.52, 132.03, 130.04, 129.55, 128.56, 127.97, 122.88, 122.49, 114.20, 109.71, 55.82, 40.33, 35.04, 20.35; EI-MS: 592 ( $\text{M}^+$ , 20%); Elemental analysis ( $\text{C}_{40}\text{H}_{33}\text{NO}_4$ ): Calculated: C, 81.20; H, 5.62; N, 2.37 %; Found: C, 81.18; H, 5.60; N, 2.35 %.

**Synthesis of 4,5-dibenzylidene-9-(4-hydroxy-3-methoxyphenyl)-10-phenyl-1,2,7,8,9,10-hexahydroacridine-3,6-dione (1g)** IR (KBr) ( $\text{cm}^{-1}$ ): 3300 (OH), 3027 (Ar-H), 2960 ( $-\text{CH}_2$ ), 1787 ( $\text{C}=\text{O}$ ), 1118 (C-N);  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ): 7.64-6.25 (m, 5H, Phenyl), 7.40-7.33 (m, 10H, Phenyl- Cyclohexanedione), 6.84-6.62 (m, 3H, Ph-

van), 6.17 (s, 2H,  $=\text{CH}$ -Phenyl), 5.35 (s, 1H, OH), 4.43 (s, 1H, CH-Phenyl), 3.83 (s, 3H,  $-\text{OCH}_3$ ), 2.98-2.07 (m, 8H, Cyclohexanedione);  $^{13}\text{C}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ): 194.46, 147.47, 145.78, 141.29, 140.20, 137.71, 135.82, 134.53, 132.04, 129.55, 128.56, 127.97, 122.88, 122.79, 122.40, 115.51, 114.22, 109.73, 56.14, 40.65, 35.06, 20.37; EI-MS: 576 ( $\text{M}^+$ , 20%); Elemental analysis ( $\text{C}_{40}\text{H}_{33}\text{NO}_3$ ): Calculated: C, 83.45; H, 5.78; N, 2.43 %; Found: C, 83.43; H, 5.76; N, 2.41 %.

**Synthesis of 4,5-dibenzylidene-9-(4-(furan-2-yl)phenyl)-10-phenyl-1,2,7,8,9,10-hexahydroacridine-3,6-dione (1h)** IR (KBr) ( $\text{cm}^{-1}$ ): 3029 (Ar-H), 2925 ( $-\text{CH}_2$ ), 1787 ( $\text{C}=\text{O}$ ), 1300 (C-O), 1110 (C-



**Scheme 1.** Synthesis of acridinedione derivatives by using AgNPs.

N);  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ): 7.64–6.25 (m, 5H, Ph), 7.58–6.11 (m, 3H, Phenyl-furfural), 7.40–7.33 (m, 10H, Phenyl- Cyclohexanedione), 6.14 (s, 2H, =CH-Phenyl), 4.43 (s, 1H, CH-Phenyl), 2.98–2.07 (m, 8H, Cyclohexanedione);  $^{13}\text{C}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ): 194.49, 152.50, 142.11, 141.22, 140.23, 137.74, 132.05, 129.56, 128.57, 127.98, 122.89, 122.40, 110.61, 106.72, 109.73, 41.54, 35.05, 20.36; EI-MS: 589 ( $\text{M}^+$ , 20%); Elemental analysis ( $\text{C}_{41}\text{H}_{36}\text{N}_2\text{O}_2$ ): Calculated: C, 83.64; H, 6.16; N, 4.76 %; Found: C, 83.62; H, 6.14; N, 4.74 %.

**Synthesis of 4,5-dibenzylidene-9-(4-(dimethylamino)phenyl)-10-phenyl-1,2,7,8,9,10-hexa hydroacridine-3,6-dione 1i)** IR (KBr) ( $\text{cm}^{-1}$ ): 3020 (Ar-H), 2967 ( $-\text{CH}_2$ ), 1780 ( $\text{C}=\text{O}$ ), 1125 (C-N);  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ): 7.64–6.25 (m, 5H, Phenyl), 7.40–7.33 (m, 10H, Phenyl- Cyclohexanedione), 6.95–6.64 (m, 4H, Phenyl), 6.23 (s, 2H, =CH-Phenyl), 4.43 (s, 1H, CH-Phenyl), 3.83 (s, 3H,  $\text{OCH}_3$ ), 3.06 (s, 6H,  $-(\text{CH}_3)_2$ ), 2.98–2.07 (m, 8H, Cyclohexanedione);  $^{13}\text{C}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ): 194.48, 148.19, 141.20, 140.21, 137.72, 132.03, 131.74, 129.55, 128.56, 128.17, 127.98, 122.89, 122.40, 112.01, 109.72, 41.33, 35.04, 20.35; EI-MS: 536 ( $\text{M}^+$ , 20%); Elemental analysis ( $\text{C}_{37}\text{H}_{29}\text{NO}_3$ ): Calculated: C, 82.97; H, 5.46; N, 2.61 %; Found: C, 82.95; H, 5.44; N, 2.59 %.

**Synthesis of 4,5-dibenzylidene-10-phenyl-1,2,7,8,9,10-hexahydroacridine-3,6-dione 1j)** IR (KBr) ( $\text{cm}^{-1}$ ): 3025 (Ar-H), 2955 ( $-\text{CH}_2$ ), 1745 ( $\text{C}=\text{O}$ ), 1125 (C-N);  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ): 7.64–6.25 (m, 5H, Phenyl), 7.40–7.33 (m, 10H, Phenyl- Cyclohexanedione), 6.13 (s, 2H, =CH-Phenyl), 4.43 (s, 1H, CH-Phenyl), 2.98–2.07 (m, 8H, Cyclohexanedione), 3.15 (s, 1H,  $\text{HCHO}$ );  $^{13}\text{C}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ): 194.40, 141.21, 140.22, 137.73, 132.04, 129.55, 128.56, 127.97, 122.88, 122.49, 109.70, 36.21, 35.02, 20.33; EI-MS: 470 ( $\text{M}^+$ , 20%); Elemental analysis ( $\text{C}_{33}\text{H}_{27}\text{NO}_2$ ): Calculated: C, 84.41; H, 5.80; N, 2.98 %; Found: C, 84.39; H, 5.78; N, 2.96 %.

### 3.2. Transmission electron microscopy (TEM)

TEM was used to examine AgNPs for information on their size and shape. TEM image is providing higher resolution and also measure the nanoparticle size. Greenly synthesised AgNPs are spherical and, on average, 20 nm in size, as seen in Fig. 5.

### 3.3. Scanning electron microscopy (SEM)

The SEM study showed that the AgNPs were tiny, well-defined, and spherical in shape. The huge number of charged surfaces of Vitex negundo makes it a good candidate for chelating metal ions in their aqueous precursor solution. The SEM image shows 13m rang for aggregates of molecules. SEM picture of AgNPs shown in Fig. 6.

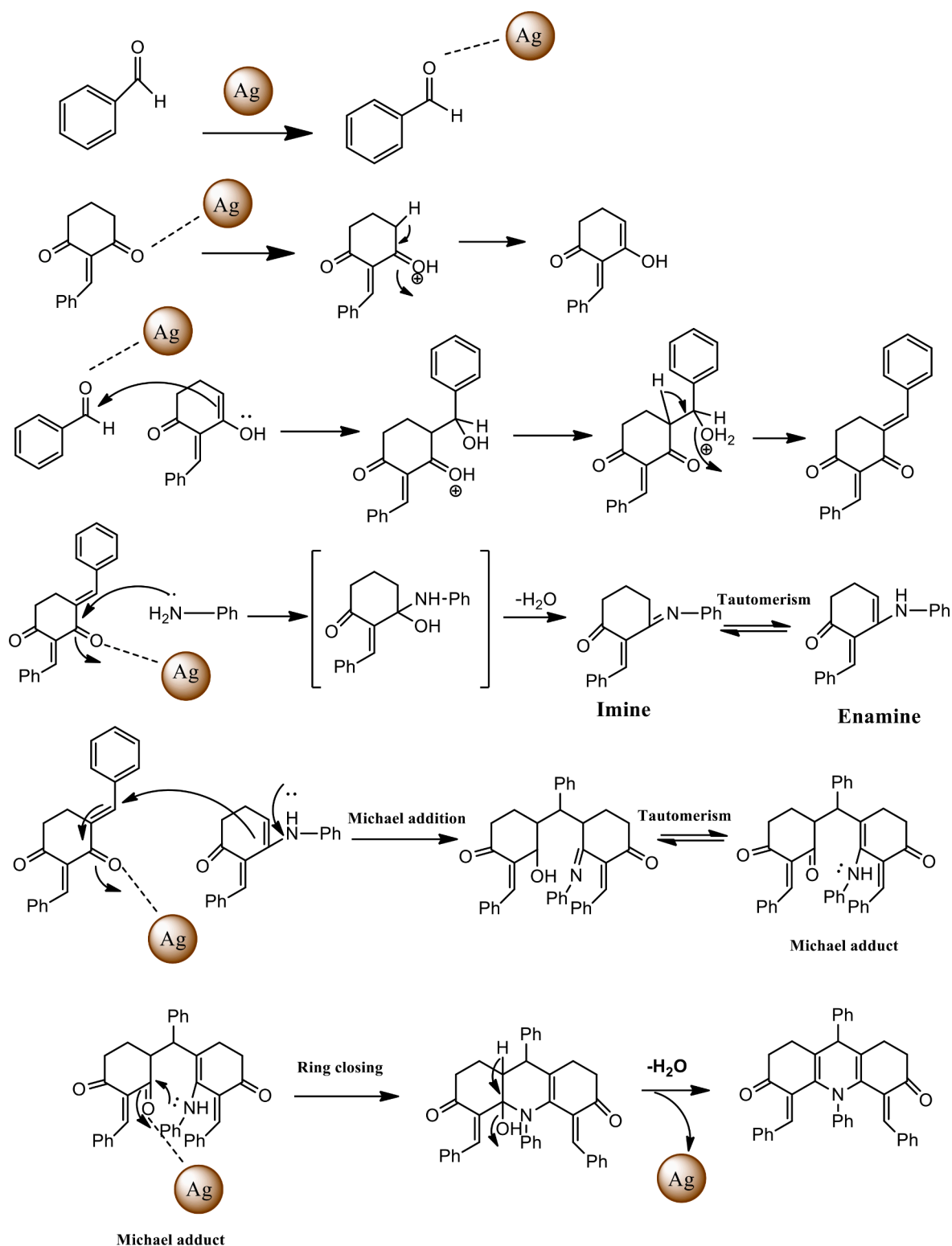
### 3.4. Cytotoxic activity

Compounds **1a–1j** are assessed for their cytotoxic potential using a procedure previously published by the US National Cancer Institute [37]. Growth inhibition at 50% ( $\text{GI}_{50}$ ), tumour growth inhibition (TGI), and median lethal concentration ( $\text{LC}_{50}$ ) were determined. After 1 hour, there was considerable action towards (HepG2,  $\text{LC}_{50}$ -0.52  $\mu\text{M}$ , MCF-7,  $\text{LC}_{50}$ -0.64  $\mu\text{M}$ , and HeLa,  $\text{LC}_{50}$ -0.52  $\mu\text{M}$ ) with these compounds. Doxorubicin was employed since it is a gold standard medication. Human embryonic kidney (HEK-293), lung (MRC-5), and liver cells were used in the MTT experiment to determine cytotoxicity (LO2). In vitro test findings revealed that these drugs had little to no influence on normal kidney cell development since their  $\text{IC}_{50}$  values were all larger than 100. Hence, these compounds have shown that they are not toxic to healthy cells, and compound **1h** may serve as a starting point for the creation of even more effective agents against the cancer cell lines HepG2 (liver), MCF-7 (breast), and HeLa (cervical). Both the in vitro cytotoxicity of acridinedione derivatives (**1a–1j**) on normal cells (Table 3) and the results of the cytotoxic screening of compounds (**1a–1j**) are shown in Table 2.

### 3.5. Molecular Docking interaction results

To better grasp the future development of biological response, docking studies were added as docking studies can provide an understanding on biological activities of the molecule [38–43]. Docking effects of compounds **1a–1j** and Doxorubicin regulation with protein 1Z11 were evaluated using the Autodock Vina programme [38–39]. The binding affinity of compound **1h** for the 1Z11 protein is greater than that





**Scheme 2.** Plausible mechanism for the formation of the catalyzed by AgNPs.

of any other compound (-11.7 kcal/mol), whereas the binding affinity of Doxorubicin is lower (-10.2) kcal/mol. Compound 1h establishes a single hydrogen bond with the 1Z11 receptor. The hydrogen bonding interaction included the amino acid residue Arg101 (bond length: 5.85). Val117, Gly301, Gly302, Thr305, Ile366, Leu370, Cys433, and Ala445 were all involved in hydrophobic interactions via the formation of three hydrogen bonds with the 1Z11 receptor, doxorubicin controls it. The hydrogen bonding interaction included the amino acid residues Leu370

(2.38, 2.61) and Phe440 (2.97). Hydrophobic interactions were shown to be of relevance to Val117, Leu298, Ser369, Cys439, and Gly441. Figs. 7-8 show the hydrogen bonding and hydrophobic interactions of amino acid residues in the 1Z11 protein with the 1a-1j and Doxorubicin compounds, respectively. The results indicate that the target protein's compound 1h has a significant inhibitory effect on Doxorubicin. Interaction data from Molecular Docking is shown in Tables 4 and 5 [40-43].

**Table 4**

Molecular docking interaction of compounds **1a-1j** and **Doxorubicin** against protein 1Z11.

| Compound           | Human microsomal cytochrome P450 2A6 complexed with methoxsalen (PDB ID: 1Z11) |                |                                   |
|--------------------|--------------------------------------------------------------------------------|----------------|-----------------------------------|
|                    | Binding affinity (kcal/mol)                                                    | No. of H-bonds | H-bonding residues                |
| <b>1a</b>          | -10.6                                                                          | -              | -                                 |
| <b>1b</b>          | -8.3                                                                           | 2              | Arg128 and Gly301                 |
| <b>1c</b>          | -6.8                                                                           | 1              | Leu370                            |
| <b>1d</b>          | -8.1                                                                           | 4              | Val116, Val117, Gly301 and Arg437 |
| <b>1e</b>          | -8.0                                                                           | 2              | Thr309 and Gln360                 |
| <b>1f</b>          | -7.8                                                                           | 1              | Thr309                            |
| <b>1g</b>          | -8.2                                                                           | 2              | Thr309 and Gln360                 |
| <b>1h</b>          | -11.7                                                                          | 1              | Arg101                            |
| <b>1i</b>          | -5.3                                                                           | 0              | -                                 |
| <b>1j</b>          | -11.0                                                                          | 1              | Leu370                            |
| <b>Doxorubicin</b> | -10.2                                                                          | 3              | Leu370 and Phe440                 |

**Table 5**

Hydrophobic interaction of synthesized compounds (1a-1j) and Doxorubicin.

| Compounds  | Hydrophobic interaction                                  |                                      |                  |           |                  |                                      |                  |
|------------|----------------------------------------------------------|--------------------------------------|------------------|-----------|------------------|--------------------------------------|------------------|
|            | Vander Waals                                             | Conventional H- Bond                 | Pi-sigma         | Pi-Sulfur | Pi-Pi T shaped   | Alkyl                                | Pi-alkyl         |
| <b>1a</b>  | Arg369<br>Ser433 Arg437<br>Asn438 Phe480                 | -                                    | Leu370<br>Dhr305 | Cys439    | -                | Ile366                               | Val365           |
| <b>1b</b>  | Phe209<br>Phe480<br>Phe111<br>Asn297<br>Leu298<br>Phe440 | Gly301<br>Arg128                     | Thr305           | -         | Phe118<br>Phe107 | Cos439<br>Val117                     | Ile336<br>Leu370 |
| <b>1c</b>  | Gly301<br>Phe118<br>Phe209<br>Ser369                     | Leu370                               | -                | -         | Phe432           | Val365                               | Pro431<br>Cos439 |
| <b>1d</b>  | Phe209<br>Phe480<br>Phe111<br>Leu298<br>Phe440<br>Phe118 | Val116<br>Val117<br>Arg437<br>Gly301 | Thr305           | -         | Phe118<br>Phe107 | Ile336<br>Leu376                     | Ile300<br>Cys439 |
| <b>1e</b>  | Ile300<br>Ser369<br>Thr305<br>Val306                     | Gln360<br>Thr309                     | Gly301           | Cys439    | Phe440<br>Asn297 | Val365<br>Leu370                     | Leu298<br>Val117 |
| <b>1f</b>  | Phe209<br>Ile300<br>Arg101<br>Phr305<br>Pro431           | Thr309                               | Gly301           | -         | Phe480<br>Asn297 | Val365<br>Cys439<br>Leu298<br>Val117 | Leu370           |
| <b>1g</b>  | Asn297<br>Ile300<br>Phe209<br>Ser369<br>Phr307           | Gln360<br>Thr309                     | -                | Cys439    | Phe480<br>Phe440 | Val365                               | Val117<br>Leu370 |
| <b>1h</b>  | Gly302                                                   | Arg101                               | -                | Cys439    | -                | Ile366                               | Ala445 Leu370    |
| <b>1i</b>  | Gly302<br>Ile300<br>Phe209<br>Ala445<br>Thr305<br>Phe480 | -                                    | Phe432           | Cys439    | Phe440           | Leu370                               | Ile366           |
| <b>1j</b>  | Leu298<br>Gly441<br>Val306<br>Thr305                     | Leu370                               | -                | Cys439    | Phe440           | Val117                               | Ile366           |
| <b>Dox</b> | -                                                        | Leu370<br>Phe440                     | -                | Cys439    | -                | -                                    | -                |

#### 4. Conclusion

Finally, we concentrate on a green and safe method for producing silver nanoparticles (AgNPs) using *Vitex negundo* extract, which can easily convert silver ions into AgNPs. In the synthesis of acridinedione analogues in aqueous ethanol, these nanoparticles demonstrate exceptional catalytic ability employing a one-pot three-component condensation of 1,3-cyclohexanedione variations, aniline with substituted aldehyde, and AgNPs as a green catalyst. The cytotoxicity of the acridinedione compounds was assessed using the MTT test with doxorubicin as the reference drug against three cancer cell lines and three normal cell lines (human embryonic kidney cell (HEK293), liver cell (LO2), and lung cell (MRC5). When compared to other compounds examined for the same purpose (HepG2, LC<sub>50</sub>-0.5 M, MCF-7, LC<sub>50</sub>-0.64 M, and HeLa, LC<sub>50</sub>-0.52), compound **1h** shown substantial activity. The IC<sub>50</sub> values for cytotoxicity in normal cell lines (HEK-293, LO2, and MRC5) were all greater than 100 g/mL, showing the compounds' safety. In comparison to other medications, **1h** has a higher binding affinity (-11.7 kcal/mol) for the 1Z11 protein, whereas Doxorubicin has a lower affinity (-10.2 kcal/mol). As a result, compound **1h** holds significant potential as a molecule for use in the development of cancer therapeutics.

## CRediT authorship contribution statement

**Perumal Gobinath:** Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Conceptualization. **Ponnusamy Packialakshmi:** Visualization, Validation, Investigation, Formal analysis. **Govindasamy Thilagavathi:** Writing – original draft, Investigation, Formal analysis, Data curation. **Natarajan Elangovan:** Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Renjith Thomas:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Formal analysis, Data curation. **Radhakrishnan Surendrakumar:** Writing – original draft, Visualization, Validation, Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.chphi.2024.100483](https://doi.org/10.1016/j.chphi.2024.100483).

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