Research Article

Synthesis of Isatin Derivatives Using Silver Nanoparticles as Green Catalyst: Study of Molecular Docking Interactions in SARS-CoV-2 3c-Like Protease and Determination of Cytotoxic Activities of the Compounds

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The coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). As isatin-containing compounds exhibit several remarkable biological activities, isatin derivatives were prepared to combat the global pandemic caused by SARS-CoV-2 that is gripping the world. Herein, the synthesis of novel isatin derivatives has been reported. The cytotoxic activities of the compounds were determined using cancer cell (MCF-7) and normal cell (MCF-10A and MRC-5) lines. *In silico* molecular docking experiments were conducted using AutoDock Vina. We have successfully predicted the binding energies and the number of hydrogen bonds present. We have also identified the residues involved in hydrogen bond formation. The target compounds were synthesized using Schiff base following cyclization and Knoevenagel condensation reactions. We have focused on the recyclable synthesis of silver nanoparticles (AgNPs) using the extracts obtained from *Dipteryx odorata*. The extracts were used to reduce silver ions for the production of AgNPs. The synthesized nanoparticles exhibited excellent catalytic activities during the synthesis of isatin derivatives in ethanol. The formation of the target isatin derivatives has been confirmed using the Fourier transform-infrared (FT-IR), proton nuclear magnetic resonance (¹H NMR) spectroscopy, ¹³C NMR spectroscopy, mass spectrometry, and elemental analysis techniques. Compound 3e was found to be the most active compound when tested against the MCF-7 cancer cell line (IC₅₀ = 20.5 μM). The activity was comparable to the activities of standard doxorubicin and other compounds. *In silico* molecular docking experiments were conducted to study the spike protein in SARS-CoV-2 (PDB ID: 6LU7). Compound 3c exhibited high binding ability (−9.4 kcal/mol). The inhibition ability was studied using hydroxychloroquine as a standard. Results from docking studies revealed that the inhibition ability of compound 3c was higher than the inhibition abilities exhibited by other compounds. The synthesized compound 3e is a potential antiviral drug that can be used for treating the COVID-19 disease.

1. Introduction

The coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, a novel coronavirus). SARS-CoV-2 attracted the attention of the World Health Organization (WHO) in the early part of 2020, though the presence of the virus was first detected in China in November 2019. SARS-CoV-2 is a virus that causes pneumonia. The WHO declared on the 11th of December (2019) that an infectious disease caused by SARS-CoV-2 was spreading across Wuhan (the cultural and economic hub of central China). The disease rapidly spread across 25 countries [1] and human-to-human transmission of COVID-19 was observed [2]. The disease was declared a pandemic by WHO. When a human being infected with the virus coughs, sneezes, or talks, tiny liquid droplets are expelled from the nose and/or mouth. The disease is transmitted when a person is exposed to these droplets. The Gamaleya National Center of Epidemiology and Microbiology (the Gambia, Moscow, Russia) developed the Sputnik V vaccine [3]. The SARS-CoV-2 virus is a single-stranded positive-sense RNA virus with a spike protein on the surface that imparts the crown-like appearance to the virus [4, 5]. The COVID-19 epidemic is not the first human coronavirus outbreak in the twenty-first century [6, 7]. The genome of HCoV consists of single-stranded positive-sense RNA [8–11]. It is important to synthesize compounds that can be used as drugs to treat COVID-19. Green chemistry involves the use of environmentally friendly or mildly hazardous reagents for the production of chemicals. Processes that reduce or eliminate the use of dangerous reagents or solvents and provide a cost-effective synthesis method are followed in the field of green chemistry for the synthesis of target compounds [12]. Microorganisms [13] and plant extracts [14–16] play an important role in the synthesis of nanoparticles (NPs). The isatin derivatives can be used to develop drugs to combat COVID-19, and the derivatives also exhibit anticancer properties. Cancer is the second leading cause of death in the world [17–20]. Uncontrolled proliferation and growth of cells cause cancer [21–23]. A mass of cancerous cells grows to form a tumor which in some cases invade other parts of the body and metastasize [24]. Most types of tumors can be surgically removed. Chemotherapy or radiation-based treatment methods can also be used to treat cancer. Chemotherapy or other methods cannot be used to treat most types of metastatic cancers. In these cases, the cancer cells are inherently resistant to chemotherapeutics or become resistant to the medication used during treatment [25]. Several chemotherapeutic anticancer drugs directly inhibit DNA synthesis, resulting in cell death. Unfortunately, in most cases, they do not specifically target tumor cells and thus exhibit high toxicity [26–30]. Analysis of the reports published by the American Cancer Society revealed that breast cancer is the second leading cause and lung cancer is the leading cause of cancer death in women. Four different phase I trials were conducted with the compound sunitinib, but the use of the compound had to be discontinued as it exhibited high activity [31–34]. The biological activities of the isatin derivatives are demonstrated in

Figure 1 [35–37]. Methisazone (isatin-thiosemicarbazone) functions as a prophylactic agent and is used to treat various viral diseases [38]. Isatin and 2-thioxo-imidazolidine-4-one derivatives exhibit excellent antiviral, anticancer, and other pharmacological activities. Herein, the synthesis and characterization of silver NPs (AgNPs) have been reported. The NPs were used as effective and highly efficient green catalysts during the preparation of isatin-based thiosemicarbazone conjugates.

The 2-thioxo-imidazolidine-4-one moiety is also present in our newly synthesized compound and the sulfur analog of hydantoin, in which one carbonyl group is substituted by a thiocarbonyl group [39]. Thioxoimidazolidine derivatives show anticancer, anti-inflammatory, and antiviral activities in Figure 2 [40–44].

Based on the evidence presented above, new medicines for the treatment of killer diseases such as antiviral and anticancer activities are urgently needed. The cytotoxic activities were determined, and the *in silico* molecular docking technique was used to study the effect of the compounds on the spike proteins of SARS-CoV-2.

2. Materials and Methods

2.1. General Methods. The chemicals, such as isatin, thiosemicarbazide, and various aldehydes, were purchased from Sigma-Aldrich with 99% purity. Melting points are recorded in open capillary tubes and were uncorrected. On a Shimadzu 8201 pc, the FT-IR spectra were reported using the KBr pellet process ($4000\text{--}400\text{ cm}^{-1}$). An Avance Bruker DRX-300 MHz spectrometer was used for NMR (^1H -NMR and ^{13}C -NMR) analysis using CDCl_3 as a solvent. The mass spectrum was recorded by the PerkinElmer GCMS model Clarus SQ 8 (EI). The morphology of silver nanoparticles is confirmed by using SEM and TEM. For SEM research, a Scanning Electron Microscope (SEM) model VP-1450 (LEO, Co., Germany) was used. For transmission electron microscopy (TEM) analysis, an LEO 912 AB instrument was used. Elemental analyses (C, H, and N) were performed using an elemental analyzer (Version EL III). The purity of the compound was determined by using thin-layer chromatography (TLC) with a silica gel plate.

2.2. Preparation of *Dipteryx odorata* Extract. A 7 g of dried fresh powder of *Dipteryx odorata* was mixed with 200 ml of water and stirred for 45 minutes on a magnetic stirrer to make an aqueous extract. After boiling for 30 minutes, the mixture was strained, cooled, and filtered. The boiled extract was kept refrigerated and used in subsequent experiments.

2.2.1. Synthesis of AgNPs. The 25 ml of 2 mM silver nitrate solution was stored in an RB flask, and *Dipteryx odorata* extract was added for reduction into Ag^+ ions. The mixture, which included silver nitrate solution and plant extracts, was stirred and heated for 52 hours at 80°C with 600 rpm. The color of the solution changed from yellow to dark brown. The nanoparticle solution was purified by repeated centrifugation at 12,000 rpm for 30 minutes and then decanted and

FIGURE 1: Biologically active compounds of isatin derivatives.

FIGURE 2: Anticancer and anti-inflammatory or biological activities of 2-thiohydantoin derivatives.

dried in an oven at 70°C overnight. The synthesis of AgNPs is presented in Scheme 1.

2.3. General Process for the Synthesis of Compound 1. Isatin (2.94 g, 0.02 mol), thiosemicarbazide (3.64 g, 0.04 mol), and green catalyst AgNPs were refluxed for 2 h to give compound 1. TLC was used to track the reaction, which used the eluting solvents hexane:ethyl acetate (1:2), until the sample was purified and washed with water before being dried to yield the crude sample. The crude sample dissolved in DMF and centrifuged to separate the catalyst and later the catalyst was washed several times with EtOH, then dried and reutilized three times for the same reaction. Then, the product was recrystallized with ethanol. Yellow solid; yield: 98%; mp: 244°C; IR (KBr) 3475 (NH₂), 3421 (NHstr), 3143 (Ar-CHstr), 1682 (C=Nstr), 1496 (Ar-C=C) cm⁻¹; ¹H NMR

(300 MHz, CDCl₃), δ 12.85 (s, 2H, thiosemicarbazide=NH), 11.24 (s, 1H, isatin-NH), 7.53 (d, 1H, isatin, *J* = 7.5 Hz), 7.32 (t, 1H, isatin, *J* = 7.7 Hz), 7.06 (t, 1H, isatin, *J* = 7.2 Hz), 6.92 (d, 1H, isatin, *J* = 7.8 Hz), 6.89 (s, 4H, thiosemicarbazide-NH₂); ¹³C NMR (300 MHz, CDCl₃) δ 176.84 (2C, C-2,3, C=S), 142.62 (2C, C-1,4, C=N), 142.50 (1C, C-10, isatin), 132.72 (1C, C-8, isatin), 131.30 (1C, C-6, isatin), 122.45 (1C, C-9, isatin), 119.73 (1C, C-7, isatin), 111.22 (1C, C-5, isatin). EI-MS (relative intensity %): *m/z* 293.05 (M⁺, 10.9%). Elemental analysis (C₁₀H₁₁N₇S₂): calculated C, 40.94, H, 3.78, N, 33.42; found C, 40.96, H, 3.80, N, 33.45.

2.3.1. General Process for the Synthesis of Compound 2. The compound 1 (2.9 g, 0.01 mol) underwent cyclization with chloroethyl acetate (2.14 ml, 0.02 mol), fused sodium acetate (0.82 g, 0.01 mol), and AgNPs used as a catalyst and refluxed

SCHEME 1: Synthesis of silver nanoparticles from extract of *Dipteryx odorata*.

for 3 h to give compound 2. The reaction was checked by TLC with eluting solvents hexane:ethyl acetate (1:2). The obtained product was recrystallized with ethanol. Brown color solid; Yield: 95%; mp; 236°C; IR (KBr) 3421 (NHstr), 3143 (Ar-CHstr), 1699 (C=O), 1682 (C=Nstr), 1496 (Ar-C=C), 1135 (C=S) (cm^{-1}); ^1H NMR (300 MHz, CDCl_3) δ 12.89 (s, 2H, imidazolidine-NH), 10.68 (s, 1H, isatin-NH), 7.53 (d, 1H, isatin, $J = 7.5$ Hz), 7.30 (t, 1H, isatin, $J = 7.7$ Hz), 7.06 (t, 1H, isatin, $J = 7.4$ Hz), 6.92 (d, 1H, isatin, $J = 7.8$ Hz), 2.75 (s, 4H, imidazolidine- CH_2); ^{13}C NMR (300 MHz, CDCl_3) δ 179.61 (2C, C-4,7, C=S), 162.93 (2C, C-2,5, C=O), 145.75 (1C, C-14, isatin), 142.53 (2C, C-1,8, C=N), 132.79 (1C, C-12, isatin), 131.33 (1C, C-6, isatin), 122.48 (1C, C-13, isatin), 119.79 (1C, C-11, isatin), 111.22 (1C, C-9, isatin), 39.13 (2C, C-3,6); EI-MS (relative intensity %): m/z 373.04 (M^+ , 19.3%). Elemental analysis ($\text{C}_{14}\text{H}_{11}\text{N}_7\text{O}_2\text{S}_2$): calculated C, 45.03, H, 2.97, N, 26.26; found C, 40.05, H, 2.99, N, 26.28.

2.3.2. General Process for the Synthesis of Compound 3a. Compound 2 (0.74 g, 0.002 mol) was mixed with ethanol and aromatic aldehyde, and AgNPs were used as a catalyst. The mixture was refluxed for 3 h and recrystallized by ethanol. The reaction was confirmed by TLC with eluting solvents hexane:ethyl acetate (1:2) to give 3a. The remaining compounds (3b–3n) were synthesized by the above-mentioned procedure. Light yellowish brown color solid; yield: 90%; mp: 230°C; IR (KBr); 3422 (NHstr), 3170 (Ar-CHstr), 1673 (C=O), 1697 (C=Nstr), 1493 (Ar-C=C), 1130 (C=S) (cm^{-1}); ^1H NMR (300 MHz, CDCl_3) δ 12.84 (s, 2H, imidazolidine-NH), 10.65 (s, 1H, isatin-NH), 7.73 (d, 4H, $J = 10.20$, Ar-H), 7.56 (d, 1H, isatin, $J = 7.5$ Hz), 7.51 (t, 4H, Ar-H), 7.39 (t, 2H, Ar-H), 7.32 (t, 1H, isatin, $J = 7.7$ Hz), 7.01 (t, 1H, isatin, $J = 7.4$ Hz), 6.90 (d, 1H, isatin, $J = 7.8$ Hz), 6.89 (s, 2H, imidazolidine=CH); ^{13}C NMR (300 MHz, CDCl_3) δ (ppm): 179.05 (2C, C-4,7, C=S), 162.43 (2C, C-2,5, C=O), 154.53 (2C, C-1,8, C=N), 145.70 (1C, C-14, isatin), 142.03 (2C, C-3,6, imidazolidine-CH), 132.76 (1C, C-12, isatin), 132.25, 130.81, 128.26, 121.98, 119.31 (14C, C-3',6', Ar), 131.30 (1C, C-6, isatin), 122.45 (1C, C-13, isatin),

119.89 (1C, C-11, isatin), 110.71 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 549.10 (M^+ , 30.6%). Elemental analysis ($\text{C}_{28}\text{H}_{19}\text{N}_7\text{O}_2\text{S}_2$): calculated for C, 61.19, H, 3.48, N, 17.84; found C, 61.21, H, 3.50, N, 17.86.

(5,5')-3,3'-((1-Indolin-2,3-diylidenebis(azanylylide-ne))bis(5-nitrobenzylidene)-2-thioxoimidazolidin-4-one (3b): light brown solid; yield: 96%; mp: 192°C; IR (KBr); 3427 (NHstr), 3175 (Ar-CHstr), Ar), 1676 (C=O), 1693 (C=Nstr), 1497 (Ar-C=C), 1473 (NO_2 str), 1134 (C=S) (cm^{-1}); ^1H NMR (300 MHz, CDCl_3) δ 12.85 (s, 2H, imidazolidine-NH), 10.70 (s, 1H, isatin-NH), 8.28 (s, 2H, Ar-H), 8.10 (d, 2H, $J = 7.0$ Hz, Ar-H), 7.73 (d, 2H, $J = 7.3$ Hz, Ar-H), 7.56 (t, 2H, Ar-H), 7.50 (d, 1H, isatin, $J = 7.5$ Hz), 7.32 (t, 1H, isatin, $J = 7.7$ Hz), 7.01 (t, 1H, isatin, $J = 7.4$ Hz), 6.92 (d, 1H, isatin, $J = 7.8$ Hz), 6.87 (s, 2H, imidazolidine=CH); ^{13}C NMR (300 MHz, CDCl_3) δ 179.08 (2C, C-4,7, C=S), 162.45 (2C, C-2,5, C=O), 154.48 (2C, C-1,8, C=N), 148.10, 137.04, 135.96, 128.22, 121.94, 119.34, 117.92 (14C, C-3',6', Ar), 145.72 (1C, C-14, isatin), 142.05 (2C, C-3,6, imidazolidine-CH), 132.78 (1C, C-12, isatin), 131.30 (1C, C-6, isatin), 121.98 (1C, C-13, isatin, Ar), 119.85 (1C, C-11, isatin), 110.73 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 640.08 (M^+ , 31%). Elemental analysis ($\text{C}_{28}\text{H}_{17}\text{N}_9\text{O}_6\text{S}_2$): calculated C, 52.58, H, 2.68, N, 19.71; found C, 52.60, H, 2.71, N, 19.73.

(5,5')-3,3'-((1,1')-Indoline-2,3-diylidenebis(azanylylide-ne))bis(5-(2-hydroxybenzylidene)-2-thioxoimidazolidin-4-one (3c): brown solid; yield: 97%; mp: 226°C; IR (KBr) 3420 (NHstr), 3264 (OHstr), 3177 (Ar-CHstr), 1674 (C=O), 1695 (C=Nstr), 1497 (Ar-C=C), 1136 (C=S) (cm^{-1}); ^1H NMR (300 MHz, CDCl_3) δ 12.53 (s, 2H, imidazolidine-NH), 10.63 (s, 1H, isatin-NH), 9.85 (s, 2H, OH), 8.22 (d, 2H, $J = 7.1$ Hz, Ar-H), 7.53 (d, 1H, isatin, $J = 7.5$ Hz), 7.30 (t, 1H, isatin, $J = 7.7$ Hz), 7.25 (t, 2H, Ar-H), 7.04 (t, 1H, isatin, $J = 7.4$ Hz), 6.94 (d, 1H, isatin, $J = 7.8$ Hz), 6.83 (s, 2H, imidazolidine=CH), 6.80 (t, 2H, Ar-H), 6.56 (d, 2H, $J = 7.4$ Hz, Ar-H); ^{13}C NMR (300 MHz, CDCl_3) δ 179.04 (2C, C-4,7, C=S), 162.45 (2C, C-2,5, C=O), 154.48 (2C, C-1,8, C=N), 145.72 (1C, C-14, isatin), 142.05 (2C, C-3,6, imidazolidine-CH), 142.00, 130.83, 126.70, 120.29, 119.30, 118.85,

FIGURE 3: TEM image of silver nanoparticle.

116.05 (14C, C-3',6', Ar), 132.78 (1C, C-12, isatin), 131.30 (1C, C-6, isatin), 121.89 (1C, C-13, isatin), 119.61 (1C, C-11, isatin), 110.73 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 582.10 (M^+ , 31%). Elemental analysis ($C_{28}H_{19}N_7O_4S_2$): calculated C, 57.82, H, 3.29, N, 16.86; found C, 57.80, H, 3.30, N, 16.83.

(5,5')-3,3'-((1)-Indoline-2,3-diylidenebis(azanylylidene)bis(5-(4-hydroxybenzylidene)-2-thioxoimidazolidin-4-one (3d): yellow orange solid; yield: 95% mp: 228°C; IR (KBr) 3426 (NHstr), 3262 (OHstr), 3173 (Ar-CHstr), 1670 (C=O), 1693 (C=Nstr), 1494 (Ar-C=C), 1133 (C=S) (cm^{-1}); 1H NMR (300 MHz, $CDCl_3$) δ 12.80 (s, 2H, imidazolidine-NH), 10.76 (s, 1H, isatin-NH), 9.41 (s, 2H, OH), 7.73 (d, 4H, $J = 7.6$ Hz, Ar-H), 7.53 (d, 1H, isatin, $J = 7.5$ Hz), 7.30 (t, 1H, isatin, $J = 7.7$ Hz), 7.02 (t, 1H, isatin, $J = 7.4$ Hz), 7.01 (d, 4H, $J = 7.3$ Hz, Ar-H), 6.92 (d, 1H, isatin, $J = 7.8$ Hz), 6.89 (s, 2H, imidazolidine=CH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 179.05 (2C, C-4,7, C=S), 162.43 (2C, C-2,5, C=O), 156.48 (2C, C-1,8, C=N), 145.72 (1C, C-14, isatin), 142.05 (2C, C-3,6, imidazolidine-CH), 142.03, 130.81, 121.98, 119.31, 110.71 (14C, C-3',6', Ar), 132.25 (1C, C-12, isatin), 131.36 (1C, C-6, isatin), 121.89 (1C, C-13, isatin), 119.61 (1C, C-11, isatin), 110.73 (1C, C-9, isatin); EI-MS (relative intensity%): m/z 582.10 (M^+ , 31%). Elemental analysis ($C_{28}H_{19}N_7O_4S_2$): calculated C, 57.82, H, 3.29, N, 16.86; found C, 57.84, H, 3.32, N, 16.88.

(5,5')-3,3'-((1,1')-Indoline-2,3-diylidenebis(azanylylidene)bis(5-(4-chlorobenzylidene)-2-thioxoimidazolidin-4-one (3e): dark yellow solid; yield: 98% mp: 230°C; IR (KBr)

FIGURE 4: SEM image of biosynthesized AgNPs.

3420 (NHstr), 3173 (Ar-CHstr), 1670 (C=O), 1693 (C=Nstr), 1494 (Ar-C=C), 1133 (C=S), 759 (C-Cl) (cm^{-1}); 1H NMR (300 MHz, $CDCl_3$) δ 12.78 (s, 2H, imidazolidine-NH), 10.73 (s, 1H, isatin-NH), 7.54 (d, 1H, isatin, $J = 7.5$ Hz), 7.50 (d, 4H, $J = 7.2$ Hz, Ar-H), 7.34 (t, 1H, isatin, $J = 7.7$ Hz), 7.32 (d, 4H, $J = 7.1$ Hz, Ar-H), 7.06 (t, 1H, isatin, $J = 7.4$ Hz), 6.96 (d, 1H, isatin, $J = 7.8$ Hz), 6.83 (s, 2H, imidazolidine=CH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 179.08 (2C, C-4,7, C=S), 162.45 (2C, C-2,5, C=O), 156.43 (2C, C-1,8, C=N), 145.70 (1C, C-14, isatin), 142.40, 130.81, 128.45, 128.12, 119.25 (14C, C3',6', Ar), 142.03 (2C, C-3,6, imidazolidine-CH), 132.23 (1C, C-12, isatin), 131.34 (1C, C-6,

SCHEME 2: Synthesis of isatin derivatives. Reagents and conditions: (1) $\text{NH}_2\text{NHC}=\text{S}-\text{NH}_2$, EtOH, and AgNPs; (2) $\text{CH}_2\text{ClCOOEt}$, CH_3COONa , EtOH, and AgNPs; (3a–3n) RCHO , EtOH, and AgNPs.

isatin), 121.85 (1C, C-13, isatin), 119.60 (1C, C-11, isatin), 110.73 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 618.03 (M^+ , 32.2%). Elemental analysis ($\text{C}_{28}\text{H}_{17}\text{Cl}_2\text{N}_7\text{O}_2\text{S}_2$): calculated C, 54.37, H, 2.77, N, 15.85; found C, 54.35, H, 2.75, N, 15.88.

5-(4-Methoxybenzylidene)-3-(-4-(4-methoxybenzylidene)-5-oxo-2-thioxoimidazolidin-1-yl)imino)indolin-2-ylidene)amino)-2-thioxoimidazolidin-4-one (3f): dark yellow solid; yield: 94% mp: 234°C; IR (KBr) 3420 (NHstr), 3173 (Ar-CHstr), 1670 (C=O), 1693 (C=Nstr), 1494 (Ar-C=C), 1133 (C=S) (cm^{-1}); ^1H NMR (300 MHz, CDCl_3) δ 12.73 (s, 2H, imidazolidine-NH), 10.75 (s, 1H, isatin-NH), 7.88 (d, 4H, $J = 7.3$ Hz, Ar-H), 7.56 (d, 1H, isatin, $J = 7.5$ Hz), 7.37 (t, 1H, isatin, $J = 7.7$ Hz), 7.08 (t, 1H, isatin, $J = 7.4$ Hz), 7.03 (d, 4H, $J = 7.1$ Hz, Ar-H), 6.94 (d, 1H, isatin, $J = 7.8$ Hz), 6.85 (s, 2H, imidazolidine=CH), 3.80 (s, 6H, OCH_3); ^{13}C NMR (300 MHz, CDCl_3) δ 179.04 (2C, C-4,7, C=S), 162.43 (2C, C-2,5, C=O), 157.90, 132.22, 127.80, 119.25, 114.42, 55.81 (16C- C-3',6', Ar), 156.46 (2C, C-1,8, C=N), 145.73 (1C, C-14, isatin), 142.06 (2C, C-3,6, imidazolidine-CH), 132.25 (1C, C-12, isatin), 131.32 (1C, C-6, isatin), 121.83 (1C, C-13, isatin), 119.60 (1C, C-11, isatin), 110.71 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 610.13 (M^+ , 33%). Elemental analysis ($\text{C}_{30}\text{H}_{23}\text{N}_7\text{O}_4\text{S}_2$): calculated C, 59.10, H, 3.80, N, 16.08; found C, 59.12, H, 3.82, N, 16.10.

(5,5')-3,3'-((1,1')-Indoline-2,3-diylidenebis(azanylylidene))bis(5-(3-phenylallylidene)-2-thioxoimidazolidin-4-one (3g): light brown solid; yield: 90% mp: 226°C; IR (KBr) 3423 (NHstr), 3170 (Ar-CHstr), 1672 (C=O), 1690 (C=Nstr), 1492 (Ar-C=C), 1130 (C=S) (cm^{-1}); ^1H NMR (300 MHz, CDCl_3) δ 12.75 (s, 2H, imidazolidine-NH), 10.74 (s, 1H, isatin-NH), 7.60 (d, 4H, $J = 7.3$ Hz, Ar-H), 7.56 (d, 1H, isatin, $J = 7.5$ Hz), 7.37 (t, 1H, isatin, $J = 7.7$ Hz), 7.35 (m, 4H, Ar-H), 7.30 (m, 2H, Ar), 7.05 (d, 4H, Ar), 7.04 (t, 1H, isatin, $J = 7.4$ Hz), 6.93 (d, 1H, isatin, $J = 7.8$ Hz), 6.86 (s, 2H, imidazolidine=CH); ^{13}C NMR (300 MHz, CDCl_3) δ 179.06 (2C, C-4,7, C=S), 162.40 (2C, C-2,5, C=O), 156.42 (2C, C-1,8, C=N), 145.76 (1C, C-14,

TABLE 1: Optimization of substituent for compounds 3a–3n.

Compounds	R	Time (hrs)	Yield (%)
3a	PhCHO	3	90
3b	3- NO_2 PhCHO	3	96
3c	2-OHPhCHO	3	97
3d	4-OHPhCHO	3	95
3e	4-ClPhCHO	3	98
3f	4- OCH_3 PhCHO	3	94
3g	CH=CH-PhCHO	3	90
3h	Citral	3	93
3i	4- $\text{N}(\text{CH}_3)_2$ PhCHO	3	91
3j	Vanillin	3	98
3k	Furfural	3	91
3l	Crotanal	3	89
3m	Glutral	3	92
3n	HCHO	3	87

isatin), 142.03 (2C, C-3,6, imidazolidine-CH), 141.02, 137.62, 130.32, 130.25, 126.43, 125.26, 119.26 (18C, C-3',6', Ar), 132.25 (1C, C-12, isatin), 131.35 (1C, C-6, isatin), 121.81 (1C, C-13, isatin), 119.60 (1C, C-11, isatin), 110.72 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 602.14 (M^+ , 35%). Elemental analysis ($\text{C}_{32}\text{H}_{23}\text{N}_7\text{O}_2\text{S}_2$): calculated C, 63.88, H, 3.85, N, 16.29; found C, 63.86, H, 3.83, N, 16.31.

(5,5')-3,3'-((1,1')-Indoline-2,3-diylidenebis(azanylylidene))bis(5-(3,7-dimethylocta-2,6-dien-1-ylidene)-2-thioxoimidazolidin-4-one (3h): light yellowish brown solid; yield: 93% mp: 230°C; IR (KBr) 3426 (NHstr), 3174 (Ar-CHstr), 1674 (C=O), 1692 (C=Nstr), 1494 (Ar-C=C), 1135 (C=S) (cm^{-1}); ^1H NMR (300 MHz, CDCl_3) δ 12.73 (s, 2H, imidazolidine-NH), 10.72 (s, 1H, isatin-NH), 7.52 (d, 1H, isatin, $J = 7.5$ Hz), 7.37 (t, 1H, isatin, $J = 7.7$ Hz), 7.06 (t, 1H, isatin, $J = 7.4$ Hz), 6.99 (d, 1H, isatin, $J = 7.8$ Hz), 6.90 (s, 2H, imidazolidine=CH), 6.45, 5.15, 2.01, 1.85 (30H, m, citral); ^{13}C NMR (300 MHz, CDCl_3) δ 177.04 (2C, C-4,7, C=S), 162.33

FIGURE 5: Structure of newly synthesized compounds of isatin-connected thioxoimidazolidine derivatives 1, 2, and 3a–3n.

TABLE 2: Cytotoxic activity screening of compounds (1, 2, and 3a–3n) against breast cancer cell line (MCF-7) compound in various concentrations (μM).

Cpd. no.	10 (μM)	25 (μM)	50 (μM)	100 (μM)	IC ₅₀ (μM)
1	0.892 $\pm$ 0.013	0.724 $\pm$ 0.032	0.534 $\pm$ 0.042	0.621 $\pm$ 0.024	64.2
2	0.996 $\pm$ 0.002	0.526 $\pm$ 0.008	0.523 $\pm$ 0.050	0.542 $\pm$ 0.030	50.0
3a	0.954 $\pm$ 0.089	0.654 $\pm$ 0.042	0.472 $\pm$ 0.024	0.441 $\pm$ 0.010	55.1
3b	0.910 $\pm$ 0.048	0.698 $\pm$ 0.072	0.460 $\pm$ 0.020	0.602 $\pm$ 0.051	43.4
3c	0.810 $\pm$ 0.056	0.435 $\pm$ 0.005	0.452 $\pm$ 0.007	0.482 $\pm$ 0.015	30.1
3d	0.657 $\pm$ 0.065	0.458 $\pm$ 0.001	0.533 $\pm$ 0.052	0.523 $\pm$ 0.022	25.3
3e	0.560 $\pm$ 0.073	0.394 $\pm$ 0.007	0.421 $\pm$ 0.031	0.450 $\pm$ 0.001	20.5
3f	0.974 $\pm$ 0.031	0.679 $\pm$ 0.114	0.563 $\pm$ 0.037	0.854 $\pm$ 0.061	40.7
3g	1.786 $\pm$ 0.021	0.690 $\pm$ 0.062	0.551 $\pm$ 0.042	0.412 $\pm$ 0.006	42.8
3h	0.925 $\pm$ 0.034	0.874 $\pm$ 0.345	0.526 $\pm$ 0.003	0.535 $\pm$ 0.019	44.6
3i	1.870 $\pm$ 0.025	0.956 $\pm$ 0.321	0.751 $\pm$ 0.070	0.610 $\pm$ 0.007	38.7
3j	0.923 $\pm$ 0.003	0.887 $\pm$ 0.025	0.819 $\pm$ 0.301	0.646 $\pm$ 0.036	46.0
3k	0.957 $\pm$ 0.348	0.609 $\pm$ 0.013	0.954 $\pm$ 0.004	0.480 $\pm$ 0.022	52.3
3l	0.920 $\pm$ 0.049	0.730 $\pm$ 0.125	0.835 $\pm$ 0.060	0.672 $\pm$ 0.035	45.9
3m	0.963 $\pm$ 0.002	0.593 $\pm$ 0.070	0.856 $\pm$ 0.025	0.468 $\pm$ 0.032	43.7
3n	0.895 $\pm$ 0.008	0.882 $\pm$ 0.054	0.760 $\pm$ 0.046	0.473 $\pm$ 0.045	39.0
DOX	0.801 $\pm$ 0.054	0.586 $\pm$ 0.034	0.670 $\pm$ 0.023	0.768 $\pm$ 0.043	30.10

NA: compound having IC₅₀ > μM ; each value is the mean of three experiments $\pm$ standard error.

(2C, C-2,5, C=O), 156.23 (2C, C-1,8, C=N), 145.68 (1C, C-14, isatin), 142.10 (2C, C-3,6, imidazolidine-CH), 140.01, 132.05, 125.15, 117.20, 40.05, 26.50, 24.50, 19.30, 18.45 (20C, C-3',6', citral), 132.15 (1C, C-12, isatin), 131.20 (1C, C-6, isatin), 121.80 (1C, C-13, isatin), 119.62 (1C, C-11, isatin), 110.73 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 642.26 (M^+ , 41%). Elemental analysis ($\text{C}_{34}\text{H}_{39}\text{N}_7\text{O}_2\text{S}_2$): calculated C, 63.62, H, 6.12, N, 15.28; found C, 63.64, H, 6.15, N, 15.30.

(5,5')-3,3'-((1,1')-Indoline-2,3-diylidenebis(azanylylidene))bis(5-(4-dimethylamino) benzyldene)-2-thioxoimidazolidin-4-one (3i): dark brown solid; yield: 91% mp: 226°C; IR (KBr) 3425 (NHstr), 3171 (Ar-CHstr), 1673 (C=O), 1694 (C=Nstr), 1490 (Ar-C=C), 1137 (C=S) (cm^{-1}); ^1H NMR (300 MHz, CDCl_3) δ 12.71 (s, 2H, imidazolidine-NH), 10.73 (s, 1H, isatin-NH), 7.63 (d, 4H, $J=9.1$ Hz, p-aminophenyl), 7.52 (d, 1H, isatin, $J=7.5$ Hz), 7.33 (t, 1H, isatin, $J=7.7$ Hz), 7.04 (t, 1H, isatin, $J=7.4$ Hz), 6.97 (d, 1H, isatin, $J=7.8$ Hz), 6.93 (s, 2H, imidazolidine=CH), 6.75 (d, 4H, $J=9.2$ Hz, p-aminophenyl), 4.03 (s, 12H, $\text{N}(\text{CH}_3)_2$); ^{13}C NMR (300 MHz, CDCl_3) δ 177.04 (2C, C-4,7, C=S), 162.28 (2C, C-2,5, C=O), 156.30 (2C, C-1,8, C=N), 151.70, 128.90, 120.82, 117.23, 111.63, 20.45 (18C, C-3',6', p-aminophenyl), 145.70 (1C, C-14, isatin), 142.11 (2C, C-3,6, imidazolidine-CH), 132.13 (1C, C-12, isatin), 131.23 (1C, C-6, isatin), 121.84 (1C, C-13, isatin), 119.64 (1C, C-11, isatin), 110.72 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 636.19 (M^+ , 40%). Elemental analysis ($\text{C}_{32}\text{H}_{29}\text{N}_9\text{O}_2\text{S}_2$): calculated C, 60.45, H, 4.60, N, 19.83; found C, 60.47, H, 4.62, N, 19.80.

(5,5')-3,3'-((1,1')-Indoline-2,3-diylidenebis(azanylylidene))bis(5-(4-hydroxy-3-methoxy benzyldene)-2-thioxoi-

TABLE 3: *In vitro* cytotoxicity of isatin derivatives (1, 2, and 3a–3n) on normal cells.

Copd. no.	MCF-10A IC ₅₀ (μM)	MRC5 IC ₅₀ (μM)
1	75.26	70.25
2	63.10	65.87
3a	70.15	73.30
3b	58.31	54.63
3c	73.40	75.45
3d	77.38	80.31
3e	80.06	86.12
3f	56.34	50.08
3g	59.50	54.29
3h	67.14	61.42
3i	52.18	52.84
3j	65.03	67.13
3k	83.26	56.27
3l	78.07	60.52
3m	71.39	58.76
3n	63.02	79.07

Each compound was tested in triplicate. All error bars represent mean $\pm$ SD from three independent experiments.

midazolidin-4-one (3j): brown solid; yield: 98% mp: 228°C; IR (KBr) 3428 (NHstr), 3262 (OHstr), 3175 (Ar-CHstr), 1676 (C=O), 1695 (C=Nstr), 1492 (Ar-C=C), 1133 (C=S) (cm^{-1}); ^1H NMR (300 MHz, CDCl_3) δ 12.70 (s, 2H, imidazolidine-NH), 10.75 (s, 1H, isatin-NH), 7.50 (d, 1H, isatin, J

FIGURE 6: The cytotoxic sequence of the synthetic compounds.

= 7.5 Hz), 7.35 (t, 1H, isatin, $J = 7.7$ Hz), 7.30 (s, 2H, vanillin), 7.15 (s, 2H, vanillin), 7.06 (t, 1H, isatin, $J = 7.4$ Hz), 7.01 (d, 2H, vanillin), 6.99 (d, 1H, isatin, $J = 7.8$ Hz), 6.90 (s, 2H, imidazolidine=CH), (5.38 (s, 2H, OH), 3.83 (s, 6H, (OCH₃)₂); ¹³C NMR (300 MHz, CDCl₃) δ 177.02 (2C, C-4,7, C=S), 162.30 (2C, C-2,5, C=O), 156.25 (2C, C-1,8, C=N), 147.60, 146.90, 130.50, 127.73, 123.50, 117.20, 115.60, 56.20 (16C, C-3',6', vanillin), 145.68 (1C, C-14, isatin), 142.10 (2C, C-3,6, imidazolidine-CH), 132.15 (1C, C-12, isatin), 131.20 (1C, C-6, isatin), 121.80 (1C, C-13, isatin), 119.62 (1C, C-11, isatin), 110.73 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 642.12 (M^+ , 33%). Elemental analysis (C₃₀H₂₃N₇O₆S₂): calculated C, 56.15, H, 3.61, N, 15.28; found C, 56.18, H, 3.63, N, 15.30.

(5,5')-3,3'-((1,1')-Indoline-2,3-diylidenebis(azanylylidene))bis(5-(furan-2-ylmethylene)-2-thioxoimidazolidin-4-one) (3k): yellowish brown solid; yield: 91% mp: 240°C; IR (KBr) 3428 (NHstr), 3173 (Ar-CHstr), 1675 (C=O), 1692 (C=Nstr), 1490 (Ar-C=C), 1139 (C=S) (cm⁻¹); ¹H NMR (300 MHz, CDCl₃) δ 12.73 (s, 2H, imidazolidine-NH), 10.72 (s, 1H, isatin-NH), 7.54 (d, 1H, isatin, $J = 7.5$ Hz), 7.53 (d, 2H, furyl ring, $J = 3.6$ Hz), 7.35 (t, 1H, isatin, $J = 7.7$ Hz), 7.02 (t, 1H, isatin, $J = 7.4$ Hz), 6.95 (d, 1H, isatin, $J = 7.8$ Hz), 6.93 (s, 2H, imidazolidine=CH), 6.35 (t, 2H, furyl ring), 6.10 (d, 2H, furyl ring, $J = 3.3$ Hz); ¹³C NMR (300 MHz, CDCl₃) δ 179.04 (2C, C-4,7, C=S), 162.38 (2C, C-2,5, C=O), 156.40 (2C, C-1,8, C=N), 152.10, 142.60, 117.26, 110.85, 105.70 (10C, C-3',6', furyl ring), 145.74 (1C, C-14, isatin), 142.01 (2C, C-3,6, imidazolidine-CH), 132.23 (1C, C-12, isatin), 131.33 (1C, C-6, isatin), 121.84 (1C, C-13, isatin), 119.62 (1C, C-11, isatin), 110.70 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 530.07 (M^+ , 26%). Elemental analysis (C₂₄H₁₅N₇O₄S₂): calculated C, 54.43, H, 2.86, N, 18.52; found C, 54.46, H, 2.83, N, 18.54.

(5,5')-3,3'-((1,1')-Indoline-2,3-diylidenebis(azanylylidene))bis(5-(but-2-en-1-ylidene)-2-thioxoimidazolidin-4-one) (3l): light brown solid; yield: 89% mp: 250°C; IR (KBr) 3428 (NHstr), 3172 (Ar-CHstr), 1676 (C=O), 1696 (C=Nstr), 1492 (Ar-C=C), 1137 (C=S) (cm⁻¹); ¹H NMR (300 MHz, CDCl₃) δ 12.73 (s, 2H, imidazolidine-NH), 10.72 (s, 1H, isatin-NH), 7.52 (d, 1H, isatin, $J = 7.5$ Hz), 7.45 (d, 2H, $J = 7.3$ Hz, crotonal), 7.37 (t, 1H, isatin, $J = 7.7$ Hz), 7.06 (t, 1H, isatin, $J = 7.4$ Hz), 6.99 (d, 1H, isatin, $J =$

7.8 Hz), 6.90 (s, 2H, imidazolidine=CH), 6.30 (d, 2H, $J = 7.5$ Hz, crotonal), 5.75 (s, 4H, crotonal), 2.10 (s, 6H, CH₃); ¹³C NMR (300 MHz, CDCl₃) δ 177.02 (2C, C-4,7, C=S), 162.30 (2C, C-2,5, C=O), 156.28 (2C, C-1,8, C=N), 145.65 (1C, C-14, isatin), 142.12 (2C, C-3,6, imidazolidine-CH), 134.45, 129.50, 117.24, 26.63, 14.45 (10C, C-3',6', crotonal), 132.12 (1C, C-12, isatin), 131.23 (1C, C-6, isatin), 121.78 (1C, C-13, isatin), 119.60 (1C, C-11, isatin), 110.70 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 478.11 (M^+ , 24%). Elemental analysis (C₂₄H₂₃N₇O₂S₂): calculated C, 57.01, H, 4.59, N, 19.39; found C, 57.03, H, 4.61, N, 19.41.

(5,5)-5,5'-((1,1')-Indoline-2,3-diylidenebis(azanylylidene))bis(5-oxo-2-thioxoimidazolidin-1-yl-4-ylidene)dipentanal (3m): light brown solid; yield: 92% mp: 244°C; IR (KBr) 3425 (NHstr), 3170 (Ar-CHstr), 1673 (C=O), 1694 (C=Nstr), 1494 (Ar-C=C), 1133 (C=S) (cm⁻¹); ¹H NMR (300 MHz, CDCl₃) δ 12.76 (s, 2H, imidazolidine-NH), 10.73 (s, 1H, isatin-NH), 9.75 (s, 2H, glutral), 7.50 (d, 1H, isatin, $J = 7.5$ Hz), 7.37 (t, 1H, isatin, $J = 7.7$ Hz), 7.04 (t, 1H, isatin, $J = 7.4$ Hz), 6.98 (d, 1H, isatin, $J = 7.8$ Hz), 6.93 (s, 2H, imidazolidine=CH), 2.40 (d, 2H, $J = 1.5$ Hz, glutral), 2.20 (d, 2H, $J = 1.5$ Hz, glutral), 1.86 (t, 2H, glutral); ¹³C NMR (300 MHz, CDCl₃) δ 202.05, 117.24, 43.65, 24.03, 21.75 (10C, C-3',6', glutral), 177.02 (2C, C-4,7, C=S), 162.30 (2C, C-2,5, C=O), 156.28 (2C, C-1,8, C=N), 145.65 (1C, C-14, isatin), 142.12 (2C, C-3,6, imidazolidine-CH), 132.12 (1C, C-12, isatin), 131.23 (1C, C-6, isatin), 121.78 (1C, C-13, isatin), 119.60 (1C, C-11, isatin), 110.70 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 538.11 (M^+ , 26%). Elemental analysis (C₂₄H₂₃N₇O₂S₂): calculated C, 53.62, H, 4.31, N, 18.24; found C, 53.65, H, 4.33, N, 18.26.

3,3'-((1,1')-Indoline-2,3-diylidenebis(azanylylidene))-bis(5-methylene-2-thioxoimidazolidin-4-one) (3n): light brown solid; yield: 87% mp: 242°C; IR (KBr) 3423 (NHstr), 3174 (Ar-CHstr), 1676 (C=O), 1694 (C=Nstr), 1492 (Ar-C=C), 1130 (C=S) (cm⁻¹); ¹H NMR (300 MHz, CDCl₃) δ 12.72 (s, 2H, imidazolidine-NH), 10.70 (s, 1H, isatin-NH), 7.50 (d, 1H, isatin, $J = 7.5$ Hz), 7.37 (t, 1H, isatin, $J = 7.7$ Hz), 7.04 (t, 1H, isatin, $J = 7.4$ Hz), 6.98 (d, 1H, isatin, $J = 7.8$ Hz), 6.93 (s, 2H, imidazolidine=CH), 5.80 (s, 2H, formyl); ¹³C NMR (300 MHz, CDCl₃) δ 177.05 (2C, C-4,7, C=S), 162.35 (2C, C-2,5, C=O), 156.26 (2C, C-1,8, C=N), 145.62 (1C, C-14, isatin), 142.10 (2C, C-3,6, imidazolidine-

FIGURE 7: (a) Helix structure of compound 3c; (b) molecular surface of compound 3c; (c) 3D structure of compound 3c; (d) 2D interaction modes of compound 3c within the binding site of 6LU7 protein.

CH), 132.13 (1C, C-12, isatin), 131.20 (1C, C-6, isatin), 121.76 (1C, C-13, isatin), 119.64 (1C, C-11, isatin), 117.20, 102.40 (4C, C-3',6', formyl), 110.68 (1C, C-9, isatin); EI-MS (relative intensity %): m/z 398.04 (M^+ , 21%). Elemental analysis ($C_{16}H_{11}N_7O_2S_2$): calculated C, 48.35, H, 2.79, N, 24.67; found C, 48.33, H, 2.82, N, 24.65.

2.4. Cytotoxic Activity. Sulforhodamine B stain (SRB) assays were tested for cytotoxic activity of compounds 1, 2, and 3a–3n using the previous procedure [45]. Cells were plated for

24 hours in a 96-multiwell plate (104 cells/well). To allow cells to adhere to the plate's wall before being treated with the compound(s). Compounds were soluble in DMSO. Different concentrations of the compound under investigation (10, 25, 50, and 100 M) were injected into the cell monolayer. For each concentration, triplicate wells were prepared. Monolayer cells were incubated for 48 h at 37°C with the compound(s) and 5% CO_2 in the atmosphere. After 48 h, with 0.4% (W/V) SRB dissolved in 1 percent acetic acid, the cells were fixed, washed, and stained for 30 min. Four washes with 1% acetic acid

FIGURE 8: (a) Helix structure of hydroxychloroquine; (b) molecular surface of hydroxychloroquine; (c) 3D structure of hydroxychloroquine; (d) 2D interaction modes of compound hydroxychloroquine within the binding site of 6LU7 protein.

eliminated the residual unbound dye and the attached stain was recovered with a Tris-EDTA buffer. The strength of the color was calculated by an ELISA reader. Color intensity was measured in an ELISA reader. The molar concentration needed for cell viability inhibition (IC_{50}) of 50% was calculated and compared with the control doxorubicin.

2.5. Molecular Docking Studies. The binding interactions and association of synthetic isatin analogs 1, 2, 3a–3n, and

hydroxychloroquine against target protein (PDB ID: 6LU7) with AutoDock Vina 1.1.2 [46] were investigated using molecular docking studies. The Protein Data Bank (<http://www.rcsb.org>) was used to obtain the crystal structure of the SARS-CoV-2 coronavirus core protease (PDB ID: 6LU7). ChemDraw Ultra 12.0 and Chem3D Pro 12.0 software suites were used to sketch the 3D structures of the ligand molecules and to energy minimization. AutoDock Tools 1.5.6 was used to build the AutoDock Vina input

TABLE 4: Molecular docking interaction of compounds 1, 2, and 3a–3n against protease of SARS-CoV-2 coronavirus in complex with isatin derivatives (PDB ID: 6LU7).

Compounds	Main protease of SARS-CoV-2 coronavirus (PDB ID: 6LU7)		
	Binding affinity (kcal/mol)	No. of H-bonds	H-bonding residues
1	-6.1	5	His41, Leu141, Cys145, His164
2	-7.3	3	Met165, Glu166, Thr190
3a	-7.8	2	Asn142, Glu166
3b	-8.9	4	Leu141, Gly143, His164, Glu166
3c	-9.4	5	Phe140, Asn142, His164, Arg188, Thr190
3d	-8.7	2	Thr190
3e	-8.2	2	Asn142, His164
3f	-8.1	1	His164
3g	-7.8	0	—
3h	-6.2	1	Gly143
3i	-7.2	3	Asn142, Glu166
3j	-7.8	1	Thr26
3k	-8.3	2	Cys145, Glu166
3l	-8.1	1	His164
3m	-7.2	0	—
3n	-7.9	3	Met165, Glu166, Thr190
Hydroxychloroquine	-6.2	3	Ser144, Cys145, His164

scripts. In build, a cocrystallized inhibitor molecule was used to identify the binding pocket with the discovery studio program. The amino acid residues Thr24, Thr26, Phe140, Asn142, Gly143, Cys145, His163, His164, Glu166, and His172 were present in the binding pocket. SARS-CoV-2 coronavirus main protease quest grid has been recognized as center x : -10.656, center y : 17.223, and center z : 67.024 in dimensions x : 20, y : 20, and z : 20 in 1.0 Å spacing. The importance of completeness was set to eight. The additional restrictions were fixed and not specified by default for Auto-Dock Vina. The compound which consumes the smallest binding affinity value is the best score compound, and the implications have been visually analyzed with Discovery Studio 2019.

3. Results and Discussion

3.1. Characterization of Silver Nanoparticles

3.1.1. Transmission Electron Microscopy (TEM). TEM analysis was used to describe the morphology and particle size of AgNPs. Figure 3 depicts an outline of green synthesized AgNPs, which demonstrates the NPs' spherical morphology and the average size of 20 nm. The nanoparticles are well distributed, and sizes are in the range of 0–25 nm. Therefore, the TEM analysis clearly indicates high quality and smaller size AgNps.

3.1.2. Scanning Electron Microscopy (SEM). The SEM examination revealed well-defined uniformly spherical AgNPs with no agglomeration and a small scale. *Dipteryx odorata* has a lot of charged surfaces, which makes it suitable for

binding metal ions from their aqueous precursor solution. Figure 4 shows an SEM representation of AgNOs.

3.1.3. Recovery of Catalyst. In the biosynthetic phase, catalyst recovery is critical. We have studied the recyclability of the AgNPs three times with almost the same catalytic activity, by using the reaction of isatins 1, 2, and 3a–3n. After adding DMF, the AgNPs were separated by centrifugation and washed with EtOH to eliminate the residual substance. The above procedure was repeated for three cycles.

3.2. Chemistry. The synthetic method involves the use of thiosemicarbazide for the hydrolysis of compounds 1, 2, and 3a–3n. Previously reported protocols were followed to synthesize the compounds [47, 48]. AgNPs were used as the green catalysts to synthesize the target compounds. The AgNPs are cost-effective and easily available. They do not get consumed during the chemical reactions and increase the rate of the reactions. The AgNPs were more active than other noble metal-based catalysts [49, 50]. The method followed for the synthesis of the target compounds involves a green pathway. The products are obtained within a short time. The compounds were obtained in excellent yields. The pathway followed for the synthesis of the isatin derivatives 1, 2, and 3a–n are presented in Scheme 2. The optimizations of the substituent are given in Table 1. The chemical structures of the synthesized isatin derivatives are presented in Figure 5.

Fourier transform-infrared, proton nuclear magnetic resonance (^1H NMR) spectroscopy, ^{13}C NMR spectroscopy, and mass spectrometry techniques were used to characterize

SCHEME 3: Pathway mechanism of compound 3c interacts with spike protein.

FIGURE 9: Interaction of compound 3c with SARS-CoV-2 spike protein.

FIGURE 10: Interaction of hydroxychloroquine with SARS-CoV-2 spike protein.

the compounds. A molecular ion peak was observed at $m/z = 292.06$ in the high-resolution mass spectral profile recorded for compound 1. The FT-IR spectral profiles recorded for compounds 1, 2, and 3a–3n were analyzed. Absorption bands at 3475, 3421, 3170, 1699, 1682, and 1135 cm^{-1} , corresponding to the $-\text{NH}_2$, $-\text{NH}$, $-\text{Ar-CH}$, $-\text{C=O}$, $-\text{C=N}$, and $-\text{C=S}$ groups were observed. Signals at 12.85 (corresponding to the $-\text{N-NH}$ unit in isatin), 11.24 (corresponding to the $-\text{NH}$ unit in isatin), 6.89 (corresponding to the NH_2 units in the imidazolidine ring), and 2.75 (corresponding to the CH_2 units in the imidazolidine ring) ppm were observed in the $^1\text{H-NMR}$ spectral profile. The

the compounds. A molecular ion peak was observed at $m/z = 292.06$ in the high-resolution mass spectral profile recorded for compound 1. The FT-IR spectral profiles recorded for compounds 1, 2, and 3a–3n were analyzed. Absorption bands at 3475, 3421, 3170, 1699, 1682, and 1135 cm^{-1} , corresponding to the $-\text{NH}_2$, $-\text{NH}$, $-\text{Ar-CH}$, $-\text{C=O}$, $-\text{C=N}$, and $-\text{C=S}$ groups were observed. Signals at 12.85 (corresponding to the $-\text{N-NH}$ unit in isatin), 11.24 (corresponding to the $-\text{NH}$ unit in isatin), 6.89 (corresponding to the NH_2 units in the imidazolidine ring), and 2.75 (corresponding to the CH_2 units in the imidazolidine ring) ppm were observed in the $^1\text{H-NMR}$ spectral profile. The

FIGURE 11: SARS-CoV-2 analysis of highly active compound 3e.

signals in the range of 7.53–6.92 were attributed to the Ar-CH units present in the imidazolidine ring. Signals at 179.61, 162.93, 142.53, and 39.13 ppm were observed in the ¹³C-NMR spectral profile. The signals were attributed to the –C=S, –C=O, –C=N, and –CH₂ carbon atoms present in the imidazole ring.

3.3. Cytotoxic Activity. The newly synthesized isatin conjugates (1, 2, and 3a–3n) were screened for their cytotoxic activity using Sulforhodamine B stain (SRB) anticancer assay (different concentrations of 10, 25, 50, and 100 μM at 48 h) against the MCF-7 cancer cell line. Table 2 shows the different concentrations of the compounds under test (10, 25, 50, and 100 mM) that were added to the cell monolayer. The molar concentration required for 50% inhibition of cell viability (IC₅₀) was calculated and compared to the reference drug doxorubicin. The cytotoxicity of the compounds in normal breast cells (MCF-10A) and lung cells (MRC-5) was evaluated by using the MTT assay. The assay found that these compounds did not affect normal cell growth (IC₅₀ value > 100). The *in vitro* cytotoxicity of indoline-2,3-dione derivatives of normal cell lines is given in Table 3. The compound 3e is highly active (MCF-7, IC₅₀ = 20.5 μM), compared with standard doxorubicin and other compounds; compound 3c has the equipotent active (MCF-7, IC₅₀ = 30.1 μM); and compound 3d has moderately active (MCF-7, IC₅₀ = 25.3 μM). Hence, compound 3e (IC₅₀ = 20.5 μM) was relatively more effective than the other compounds. Finally, these compounds have exposed their safety to normal cells, and compound 3e can be used as a lead compound to further develop more effective drugs for the MCF-7 (breast) cancer cell line. The cytotoxic sequence of the synthetic compounds is given in Figure 6.

3.4. Docking Studies. Docking expressions were carried out in order to gain a better understanding of the biological activity mechanism. The compounds 1, 2, and 3a–3n were analyzed by the AutoDock Vina for their docking behaviour against the SARS-CoV-2 coronavirus main protease (PDB ID: 6LU7). The overall studied inhibitors indicate negative binding energy. The compound 3c demonstrated remarkable inhibition ability with a binding energy value of –9.4 kcal/mol over other compounds 1 (–6.1 kcal/mol), 2 (–7.3 kcal/mol), 3a (–7.8 kcal/mol), 3b (–8.9 kcal/mol), 3d

(–8.7 kcal/mol), 3e (–8.2 kcal/mol), 3f (–8.1 kcal/mol), 3g (–7.8 kcal/mol), 3h (–6.2 kcal/mol), 3i (–7.2 kcal/mol), 3j (–7.8 kcal/mol), 3k (–8.3 kcal/mol), 3l (–8.1 kcal/mol), 3m (–7.2 kcal/mol), and 3n (–7.9 kcal/mol) and hydroxychloroquine (–6.2 kcal/mol) in the target protein. The approvable bond distance between H-acceptor and H-donor atoms is less than 3.5 Å, which plays an important role in ligand binding stability [51]. The distance between hydrogen-bound compounds in the target protein 6LU7 was less than 3.5 Å, indicating that the protein and ligands were forming a stable hydrogen bond. The 6LU7 receptor has five hydrogen bonding interactions with the compound 3c. The amino acid residues Phe140, Asn142, His164, Arg188, and Thr190 were entangled by the hydrogen linkage, which had bond lengths of 2.65, 2.62, 3.19, 2.66, and 2.49 Å. His41, Leu141, Cys145, Met165, and Glu166 amino acid residues were in hydrophobic contact. Figure 7 shows the hydrogen bonding and hydrophobic contacts of amino acid residues in the 6LU7 protein with compound 3c. The compound hydroxychloroquine displays three hydrogen bonding associations with the 6LU7 receptor. The amino acid residues of Ser144, Cys145, and His164 were entangled through the relationship of the hydrogen linkage with bond lengths between 2.43, 2.62, and 2.66 Å. His41, Leu141, and Met165 amino acid residues were in hydrophobic contact, with the compound hydroxychloroquine; Figure 8 shows hydrogen bonding and hydrophobic contacts in 6LU7 protein amino acid residues. Compound 3c had a higher ability to inhibit SARS-CoV-2 coronavirus key protease (PDB ID: 6LU7) than other compounds, according to the results. The findings are summarized in Table 4. The pathway mechanism of compound 3c and its interactions with the spike protein are illustrated in Scheme 3. The interaction of compound 3c and hydroxychloroquine with the SARS-CoV-2 spike protein is given in Figures 9 and 10.

3.5. Structure Activity Relationship. The highly active isatin derivatives of compound 3e and its SARS-CoV-2 are shown in Figure 11. The biological data of the selected isatin derivatives 1, 2, and 3a–3n evidenced that compound 3e is the most efficacious (MCF-7, IC₅₀ (20.5 μM)) than control doxorubicin. Due to the presence of the thioxoimidazolidine ring fused with chlorobenzaldehyde, it was established to have high cytotoxic activity against the MCF-7 cancer cell

line. The reason is that chlorine is an electron withdrawing group and is present in the paraposition on the benzene ring connected to the thioxoimidazolidine backbone. The effect of 4-chloro substitution was favorable to the activities of the compounds. The substitution of both phenyl rings enhances the activity of the compound 3e. The remaining compounds exhibited weak cytotoxicity to the MCF-7 cancer cell line.

4. Conclusions

We have developed an environmentally friendly green synthetic method for the synthesis of AgNPs using *Dipteryx odorata*. A simple method was followed for the reduction of the silver ions to produce AgNPs. The NPs exhibited excellent catalytic activities during the preparation of compounds 1, 2, and 3a–3n in ethanol. The synthetic method is simple, green, cost-effective, and easy to implement. The compounds could be synthesized in high yields within a short time. The cytotoxic activities of the newly synthesized isatin conjugates (1, 2, and 3a–3n) were determined. The *in silico* molecular docking technique was also used to study the compounds. Compound 3e was found to be the most effective against the MCF-7 cancer cell line ($IC_{50} = 20.5 \mu M$). The activity was comparable to the activities of the standard doxorubicin and other compounds. Satisfactory docking scores were obtained when compounds 1, 2, and 3a–3n were studied. The interaction residues present in the spike protein of SARS-CoV-2 (PDB ID: 6LU7) were identified. A significantly high binding affinity (-9.4 kcal/mol) was recorded for compound 3c when hydroxychloroquine was used as a standard. The binding affinity recorded for compound 3c was higher than the binding affinities recorded for the other compounds. The synthesized compound 3e is a potential lead molecule that can be used to treat cancer. Compound 3c can be potentially used to develop antiviral drugs for the treatment of COVID-19.

Data Availability

The supplementary file data used to support the findings of this study are included within the supplementary information file (available here).

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Materials

The supplementary material used to support the findings of this study is included within the supplementary information files. (The supplementary file contains 1H -NMR and ^{13}C -NMR spectra.) (*Supplementary Materials*)

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