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# Design, Synthesis and Biological Evaluation of 4,6-Substituted 2-Styrylquinoline Derivatives: Potential Antimicrobial and Anticancer Agents

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A series of novel 4,6-substituted 2-styrylquinoline derivatives (**3a-h**) was designed and synthesized based on the substitution in quinoline scaffolds (with Br, H, $NO_2$ at C6 position) as well as the substitution in the styryl moeity (at C4′ position). The target compounds were investigated for their antimicrobial activity against Gram-positive *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and Gram-negative bacterium *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10231) and anticancer activity against human colon adenocarcinoma HT29 cells. Compared to standard drug ciprofloxacin, compounds **3e**, **3b** and **3c** showed significant antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*. Additionally, synthesized compound **3e** had significant activity against *Candida albicans* with MIC value of 8 µg/mL. In anticancer study, the maximum cell death rate was found in compounds **3e**, **3b** and **3c** at the dose of 100 µM *i.e.*, 72.9%., 44.01% and 35.46%, respectively and the measured $IC_{50}$ were 58.84, 114.53 and 130 µM. The cytotoxic potential of compound **3e** was confirmed by AO-EtBr dual staining assay in which red fluorescence of necrotic cells lost their membrane integrity due to toxicity induced by compound **3e**. This growth inhibition and apoptotic activity of compound **3e** was dose-dependent when the colour changed from green to red. Furthermore, *in silico* studies were conducted on the synthesized compounds to elucidate the potential mechanisms of action against dihydrofolate reductase (DHFR) from *E. coli*, DNA gyrase from *P. aeruginosa*, DHFR from *S. aureus*, acetolactate synthase from *K. pneumoniae* and DHFR from *C. albicans*. The derivatives also exhibited the strongest ligand-receptor binding affinity. Additionally, ADME analysis was performed to predict the pharmacokinetic profiles of the compounds, indicating good oral drug-like properties.

**Keywords: Antimicrobial, Anticancer, *In silico* studies, 2-Styrylquinolines, Pfitzinger reaction, Molecular docking.**

## INTRODUCTION

The microbial world is the foundation of global ecology; it contains a variety of multicellular species, including bacteria, viruses and countless more [1]. Along with every surface, cavity and cellular milieu of every individual, these microscopic creatures populate every ecological niche in this world. Although penicillin was successful against many bacterial infections when it was first introduced, approximately 70 years later, more and more pathogens are developing resistance to penicillin and its derivatives as well as to all other antibiotics that are currently in use [2]. Microbial resistance is an issue that has arisen because of the increasing use of antibiotics in medical, veterinary and agricultural uses [3]. Microbes develop resistance through a multitude of mechanisms, including restricting access to the target, switching antibacterial targets through mutation and direct drug modification [4]. The upsurge of microbial resistance to currently available antimicrobial medicines, as well as its impact on global healthcare, necessitates continuing anti-infective medication research and development [5]. As a result, the ongoing quest for new antibacterial and antifungal medications is strongly encouraged [3].

Among the quinoline derivatives, styrylquinolines have recently attracted interest due to their potential as antifungal and antiviral drugs along with wide variety of actions, including anti-HIV-1 activity [6,7], antimicrobial [8,9], antimalarial [10,11] anti-Alzheimer [12,13], as well as inhibiting the proliferation of tumor cell types.

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In recent studies, 2-styryl-4-quinolinecarboxylic acids were synthesized under microwave irradiation, compounds containing 2-chloro or 4-chlorostyryl moieties showed significant antileishmanial activity [14]. Some styrylquinoline based fluorophores were also synthesized to evaluate solvatochromic behaviour. Compounds demonstrated acidochromism and molecular docking revealed key amino acid interactions identified dehydrosqualene synthase and squalene synthase as potential targets for antimicrobial activity of compounds [15]. While it is possible to swap any location in the styryl fragment, the most synthetically convenient ones are the C-2 and C-4 of quinoline. Condensing the right methyl-quinolines with aromatic aldehydes in a Perkin-like reaction makes 2- and 4-styrylquinolines easy to obtain [16,17]. 2-Styrylquinoline derivatives are active biological compounds among the several identified quinoline derivatives having antiviral, antimicrobial [18], anti-parasitic [19], antifungal [20-23], anti-inflammatory [24], analgesic [25] properties. The literature showed the earlier developed derivatives of 2-styrylquinoline exhibited strong *in vitro* antiproliferative activity against HepG2 hepatocellular carcinoma cell lines [25,26].

Based on the SAR study, the carboxyl and hydroxyl groups at C-7, C-8 in the quinoline ring is required for the activity. A bulky group attached near carboxy group at C-7 in the quinoline ring enhance the inhibitory activity of HIV integrase enzyme. Chlorine substitution at C-6 improves the anti-HIV activity of the compound, electron-withdrawing groups substitution in the styryl moiety and the chelation in the quinoline ring is important for anticancer activities. The C-4 substitution with thiadiazol found to be active in cancer cell lines of liver. The acetoxy group in the phenyl ring also positively affects the activity, but multi-substitution may decrease the activity. At the 2- and 4-position substitution of a hydroxyl group leads to Pim-1 kinase inhibition [27].

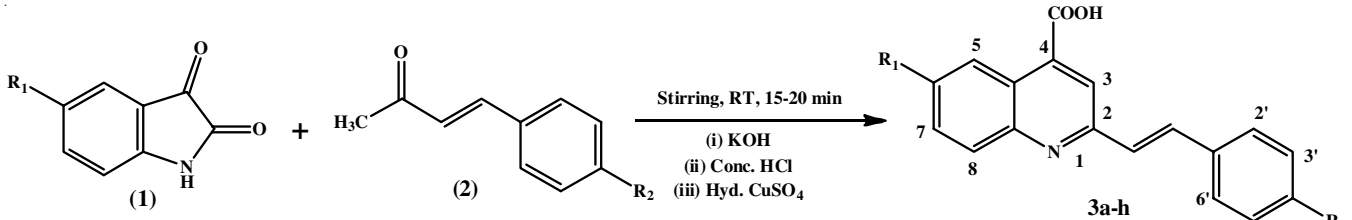
The previously reported styryl quinoline derivatives commonly feature substitutions such as halogens, alkyl groups, methoxy and hydroxyl groups at the C-2, C-4 or C-6 positions on the quinoline ring [7,27]. Additionally, nitro groups have been introduced at C-6′ on the styryl ring to enhance electronic effects; however, these modifications have not been extensively optimized for antimicrobial and anticancer activities. In this investigation, we design and synthesize a novel series of 2-styrylquinoline derivatives with substitution on C-6 of quinoline ring with Br, H, $NO_2$ and C-4′ of styryl moiety with electron-withdrawing groups from isatin and ketones using Pfitzinger reaction and evaluation of their antimicrobial and anticancer properties. Moreover, *in silico* studies were also performed to assess the ability of the tested compounds to bind to the crucial amino acids of specific targets. The molecular docking study based on the binding affinities, interaction modes and stability of the ligand-target complexes, offers the valuable insights into the compounds and their potential mechanisms of action. An *in silico* ADME analysis was also conducted to further anticipate the pharmacokinetic profile of the synthesized compounds.

## EXPERIMENTAL

The DbkProg digital melting point device was utilized to determined the melting points and are uncorrected. The progress of the reactions were monitored using TLC on the gel plates (60 $F_{254}$). The infrared spectra were recorded from Bruker alpha-II FT/IR spectrophotometer. $^1$H & $^{13}$C NMR spectra were captured on a Bruker AVANCE 500 NMR spectrometer at 500 MHz in DMSO-$d_6$ solvent using TMS as an internal standard. Mass spectra were determined using an MSD VL ESI1 spectrometer.

**General procedure for the synthesis of 2-styryl quinoline-4-carboxylic acid derivatives (3a-h):** Novel styryl quinoline compounds were synthesized using a one-pot process that involved an enhanced Pfitzinger reaction of isatins with various ketones and catalysts in an aqueous solution. The substituted isatins (**1**, 1 mmol) and substituted ketones (**2**, 2.5 mmol) in 50 mL of 10% KOH was stirred at room temperature until completion of the reaction (**Scheme-I**). Added $CuSO_4{\cdot}5H_2O$ (0.1 mmol) and acidify the mixture to pH of 2-3 using 0.38 mL conc. HCl. A precipitate was generated by stirring the resulting mixture and the reaction was monitored for completion by TLC ($CHCl_3$/MeOH 9:1). To obtain compound **3a-h**, the precipitate was filtered, rinsed with water and recrystallized.

**(*E*)-6-Bromo-2-styryl quinoline-4-carboxylic acid (3a):** Solid crystalline light yellow, yield 70%, m.p.: 224-226 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3429 (-OH), 3071 (=C-H) *str.*, 1727 (C=O), 1605 (Ar-C=C *str.*), 1012 (C-Br); $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): 11.01 (1H, s, -OH), 9.48 (1H, s, ArH), 8.58 (1H, s, ArH), 8.19-8.18 (1H, d, $J$ = 3.6 Hz, ArH), 7.78-7.74 (2H, m, ArH), 7.62-7.54 (2H, m, ArH), 7.51 (1H, s, -CH), 7.49-7.41 (3H, m, ArH), 7.24 (1H, s, -CH); $^{13}$C NMR (500 MHz, DMSO-$d_6$, δ ppm): 169.23, 155.49, 144.57, 138.49, 133.07, 131.36, 129.29, 128.49, 127.88, 125.28, 122.23, 119.34, 112.44; MS (ESI): *m/z* 355.08 (M+); $R_f$ value: 0.52 (chloroform:methanol 9:1).




| Compd. | 3a | 3b | 3c | 3d | 3e | 3f | 3g | 3h |
|---|---|---|---|---|---|---|---|---|
| $R_1$ | -Br | $-NO_2$ | H | -Br | $-NO_2$ | H | -Br | $-NO_2$ |
| $R_2$ | H | H | -Cl | -Cl | -Cl | -OH | -OH | -OH |

**Scheme-I:** Synthesis of novel 2-styrylquinoline derivatives (**3a-h**)

**(*E*)-6-Nitro-2-styryl quinoline-4-carboxylic acid (3b):** Solid crystalline white, yield 75%, m.p.: 242-244 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3431 (-OH), 3085 (=C-H *str.*); 1746 (C=O); 1617 (Ar-C=C *str.*), 1527, 1341 (-$NO_2$); $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): 11.41 (1H, s, -OH), 10.04 (1H, s, ArH), 8.72 (1H, s, ArH), 8.55-8.50 (1H, d, *J* = 10.95 Hz, ArH), 7.75-7.74 (1H, d, *J* = 2.85 Hz, ArH), 7.62-7.54 (3H, m, ArH, -CH), 7.50-7.39 (3H, m, ArH),) 7.51 (1H, s, -CH), 7.49-7.41 (3H, m, ArH), 7.23 (1H, s, -CH); $^{13}$C NMR (500 MHz, DMSO-$d_6$, δ ppm): 167.73, 157.07, 147.87, 145.07, 137.07, 136.34, 131.96, 129.11, 128.04, 127.78, 125.03, 122.73, 121.34, 111.84; MS (ESI): *m/z* 321.08 (M+); $R_f$ value: 0.58 (chloroform:methanol 9:1).

**(*E*)-2-(4-Chlorostyryl)quinoline-4-carboxylic acid (3c):** Solid white, yield 70%, m.p.: 259-262 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3485 (-OH), 3050 (=C-H *str.*); 1732 (C=O); 1610 (Ar-C=C *str.*); 953 (C-Cl); $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): 11.33 (1H, s, -OH), 8.50 (1H, s, ArH), 8.01-7.99 (1H, d, ArH), 7.78-7.75 (2H, t, *J* = 10.95 Hz, ArH), 7.62-7.60 (1H, d, *J* = 2.85 Hz, ArH), 7.59-7.55 (4H, m, ArH), 7.51 (1H, s, -CH), 7.25 (1H, s, -CH); $^{13}$C NMR (500 MHz, DMSO-$d_6$, δ ppm): 168.28, 154.45, 146.77, 137.47, 134.19, 131.27, 129.19, 128.34, 127.83, 125.22, 122.23, 113.47; MS (ESI) *m/z* = 310.8 (M+); $R_f$ value: 0.61 (chloroform:methanol 9:1).

**(*E*)-6-Bromo-2-(4-chlorostyryl)quinoline-4-carboxylic acid (3d):** Solid light yellow, yield 85%, m.p.: 265-268 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3455 (-OH); 3072 (=C-H *str.*); 1710 (C=O); 1605 (Ar-C=C *str.*), 1012 (C-Br); 809 (C-Cl); $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): 11.71 (1H, s, -OH), 9.48 (1H, s, ArH), 8.58 (1H, s, ArH), 8.19-8.17 (1H, d, *J* = 11.85 Hz, ArH), 7.62-7.41 (3H, m, ArH, -CH), 7.25 (1H, s, -CH); $^{13}$C NMR (500 MHz, DMSO-$d_6$, δ ppm): 163.48, 154.19, 146.23, 136.27, 133.34, 131.22, 129.23, 128.02, 127.87, 124.82, 123.43, 120.32, 113.47; MS (ESI) *m/z* = 389.34 (M+); $R_f$ value: 0.72 (chloroform:methanol 9:1).

**(*E*)-2-(4-Chlorostyryl)-6-nitroquinoline-4-carboxylic acid (3e):** Solid brownish yellow, yield 75%, m.p.: 272-275 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3435 (-OH); 3023 (=C-H *str.*); 1720 (C=O); 1615 (Ar-C=C *str.*); 1531, 1347 (-$NO_2$); 836 (C-Cl); $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): 11.75 (1H, s, -OH), 10.74 (1H, s, ArH), 8.71 (1H, s, ArH), 8.56-8.52 (1H, d, *J* = 12.95 Hz, ArH), 7.75-7.74 (1H, d, *J* = 6.15 Hz, ArH), 7.62-7.55 (3H, m, ArH, -CH), 7.51-7.39 (2H, m, ArH), 7.24 (1H, s, -CH); $^{13}$C NMR (500 MHz, DMSO-$d_6$, δ ppm): 167.07, 158.04, 149.03, 145.27, 137.01, 136.44, 133.06, 129.78, 128.03, 127.58, 125.10, 122.77, 121.01, 113.64; MS (ESI) *m/z* = 355.54 (M+); $R_f$ value: 0.63 (chloroform:methanol 9:1).

**(*E*)-2-(4-Hydroxystyryl)quinoline-4-carboxylic acid (3f):** Solid yellow, yield 72%, m.p.: 243-245 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3375 (-OH); 3174 (=C-H *str.*); 1763 (C=O); 1614 (Ar-C=C *str.*); $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): 11.01 (1H, s, -COOH), 9.49 (1H, s, -OH), 8.58 (1H, s, ArH), 8.01 (1H, d, *J* = 3.6 Hz, ArH), 7.99-7.75 (3H, m, ArH), 7.62-7.55 (2H, m, ArH), 7.51 (1H, s, -CH), 7.25 (1H, s, -CH) 6.78-6.77 (2H, d, *J* = 3.5 Hz, ArH); $^{13}$C NMR (500 MHz, DMSO-$d_6$, δ ppm): 169.43, 159.46, 155.26, 144.57, 138.49, 132.57, 130.26, 128.58, 128.49, 127.98, 125.25, 122.23, 117.30, 111.27; MS-ESI: 292.12 (M+); $R_f$ value: 0.67 (chloroform:methanol 9:1).

**(*E*)-6-Bromo-2-(4-hydroxystyryl)quinoline-4-carboxylic acid (3g):** Solid yellowish brown, yield 67%, m.p.: 217-220 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3429 (-OH); 3071 (=C-H *str.*); 1727 (C=O); 1605 (Ar-C=C *str.*); 809 (C-Br); $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): 11.90 (1H, s, -COOH), 10.19 (1H, s, -OH), 8.79 (1H, s, ArH), 8.01-7.99 (1H, m, Ar-H), 7.78-7.57 (1H, m, -ArH), 7.62-7.59 (1H, m, ArH), 7.55 (1H, s, -CH), 7.51-7.40 (2H, m, ArH), 7.25 (1H, s, -CH), 6.99-6.98 (2H, s, -ArH); $^{13}$C NMR (500 MHz, DMSO-$d_6$, δ ppm): 160.58, 158.13, 144.62, 144.57, 138.49, 132.57, 130.26, 128.57, 127.98, 120.12, 118.94; MS-ESI: 371.07 (M+); $R_f$ value: 0.69 (chloroform: methanol 9:1).

**(*E*)-2-(4-Hydroxystyryl)-6-nitroquinoline-4-carboxylic acid (3h):** Solid yellowish red, yield 69%, m.p.: 255-257 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3431 (-OH); 3085 (=C-H *str.*); 1746 (C=O); 1618 (Ar-C=C *str.*); 1527, 1341 (N=O); $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): 11.35 (1H, s, -COOH), 10.11 (1H, s, -ArH), 9.88 (1H, s, -OH), 8.60 (1H, s, ArH), 8.13-8.14 (1H, d, *J* = 8.5 Hz ArH), 7.85-7.81 (3H, m, -ArH, -CH), 7.41 (1H, s, -CH), 6.67-6.66 (2H, d, *J* = 5.5 Hz, -ArH); $^{13}$C NMR (500 MHz, DMSO-$d_6$, δ ppm): 163.46, 156.23, 150.60, 147.68, 133.89, 131.63, 126.31, 125.70, 123.197, 117.71, 116.11; MS-ESI: 337.19 (M+); $R_f$ value: 0.71 (chloroform:methanol 9:1).

**Antimicrobial studies:** The antimicrobial efficacy of 2-styrylquinoline derivatives (**3a-h**) against *Staphylococcus aureus* (ATCC-25923), *Enterococcus faecalis* (ATCC-29212), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853) and *Candida albicans* (ATCC 10231) was assessed using the broth microdilution method with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as the indicator. Bacterial strains were cultured in nutrient broth and incubated at 37 ºC for 24 h before the assay. Subsequently, the bacterial suspension was adjusted to 0.5 McFarland standard (corresponding to $10^5$ CFU/mL) using a Densi-Check-Meter. Minimum inhibitory concentrations (MICs) of various styryl quinoline derivatives were determined in sterile 96-well microtiter plates with flat bottoms (Himedia). Initially, 200 µL of 0.5 McFarland standard bacterial suspension was added to each well. Then, twelve two-fold serially diluted concentrations of the styryl quinoline derivatives were prepared, starting from 64 µg/mL. Positive controls consisted of wells containing bacterial strains treated with ciprofloxacin, while wells with only bacterial suspension served as negative controls. For the antifungal activity, the MTT assay was assessed using *Candida albicans* (ATCC 10231), with fluconazole used as the reference positive control. The sealed 96-well microtiter plates, covered with a tight-fitting lid and wrapped in paraffin tape, were incubated at 37 ºC for 24 h. Following the initial incubation period, 5 mg/mL of MTT was added to each well and maintained at 37 ºC for another 4 h. The formation of blue colour (formazan) following MTT treatment indicated the presence of viable cells, whereas no colour change (remaining yellow) indicated the antimicrobial efficacy of the tested compound [28].

The minimum inhibitory concentration (MIC) values, calculated as the concentrations at which bacterial growth was inhibited relative to the compound-free control, were expressed in micrograms per milliliter. The studies were repeated three

times in separate trials and the mean MIC values were used to report the results.

**Molecular docking studies:** The structural model of the dihydrofolate reductase (DHFR) enzyme of *E. coli* (pdb id: 6cqa), DNA gyrase of *P. aeruginosa* (pdb id: 6m1j), DHFR of *S. aureus* (pdb id: 2w9s), acetolactate synthase of *K. pneumoniae* (pdb id: 5d6r), DHFR of *C. albicans* (pdb id: 1ai9), human p53 receptor (pdb id: 1tup), topoisomerase enzyme (pdb id: 3qx3) and tyrosine kinase receptor (pdb id: 2hyy), from RCSB Protein Data Bank, was used in the current docking study [29]. The macromolecular receptors that were prepared were saved in the Autodock default format (*.pdbqt). To begin docking analysis, the extended conformations of the ligands and the macromolecular binding residues of the target proteins were covered by the imaginary grid-box that was generated for each of the receptor targets.

***In silico* pharmacokinetic properties prediction:** The Swiss ADME, a free Webware for studying a molecule's pharmacokinetic profile, was used to estimate the kinetic profile of ADME-designated 2-styrylquinoline derivatives [30]. When evaluating a drug's similarity, several factors are taken into account, including physico-chemical qualities, lipophilicity and solubility patterns. These factors are derived from Lipinski's rule of five, consensus logP and ESOL LogS, as well as molecular weight and H-bond modulator [31]. The target compounds were also evaluated for their gastrointestinal absorption and brain permeability.

### Anticancer activity

**Cell cultures:** The National Center for Cell Sciences (NCCS) in Pune, India, supplied the human colon adenocarcinoma HT29 cells. The cells were cultivated in DMEM media and let to grow in a humidified incubator with 5% $CO_2$ at 37 ºC. Antibiotics (100 units/mL penicillin, 100 µg/mL streptomycin), 2 millimolars of L-glutamine and 10% heat-inactivated fetal bovine serum (FBS) were added to the DMEM.

**Cytotoxicity assay:** A cytotoxicity experiment was conducted using human colon adenocarcinoma HT29 cells to determine the effects of 2-styrylquinoline derivatives. After being cultivated in 96-well plates for 24 h with $3 \times 10^3$ cells per well, the test compounds were incubated with varying doses (10, 25, 50 and 100 µM) for an additional 24 h. After the incubation period ended, 10 µL of MTT solution, with a stock concentration of 5 mg/mL, was applied to each well and left to incubate for 4 h in the dark. A microtiter plate reader (Fluostar OPTIMA, BMG LABTECH, Offenburg, Germany) was used to record the absorbance at 570 nm after 4 h of removing the medium and dissolving the formazan crystals in 100% DMSO. The suppression of cell growth was determined by measuring the relative absorbance of MTT. There was no reduction in cell viability in the dimethyl sulfoxide vehicle control. The standard drug used in this study was cisplatin.

**Fluorescence staining for detection of apoptotic cells:** The HT29 cell suspensions ($1 \times 10^5$ cells $mL^{-1}$ per well) were cultured in six-well plates for 24 h. For additional 24 h, cells were subjected to the potent test compound **3e** at 25 and 50 µM concentrations. All cells used as controls were cultured in DMEM. The AOEB dye mixture was made with 100 µg $mL^{-1}$ of acridine orange (AO) and ethidium bromide (EtBr), the amounts of which were identical. The cells, both treated and controlled, were rinsed with PBS after 24 h and then fixed with 4% paraformaldehyde for 20 min. Following a wash with 1× PBS, cells were stained in each well with 1 mL of AO-EtBr. Two washes with PBS were performed at 5 min intervals following 2 min of incubation and the cells were examined using a fluorescence microscope (EVOS, Thermo Fisher Scientific, MA, USA).

## RESULTS AND DISCUSSION

An enhanced Pfitzinger reaction of isatins with different ketones in the presence of KOH was used to synthesize the novel 2-styrylquinoline-4-carboxylic acid derivatives (**3a-h**) using one-pot synthesis (**Scheme-I**). The end product was attained after acidifying the mixture with conc. HCl and copper sulfate. The progess of the reaction was monitored with TLC plates and the compounds were purified by column chromatography using $CHCl_3$:$CH_3OH$ (9:1) solvent system. The structures and purities of the target compounds were confirmed by IR, $^1$H NMR, $^{13}$C NMR and mass spectroscopies.

The synthesized compounds **3a-h** were characterized by $^1$H NMR, $^{13}$C NMR, FTIR and mass spectroscopic analysis. The FT-IR spectra of compounds **3a-h** displayed the peak at 1760-1650 $cm^{-1}$, which confirmed the presence of C=O group of carboxyl in the styryl-quinoline skeleton and reveals a strong absorption bands in the range, which arise due to the extended conjugation effect. The -OH of carboxyl also showed broad peaks at around 3300 $cm^{-1}$ in all the compounds. The presence of N=O group in compounds **3b**, **3e** and **3h** are appeared in the range of 1531-1527 $cm^{-1}$. The aromatic ring showed the characteristic absorption bands in the range of 3179-3023 $cm^{-1}$. The peaks for halogens (-Cl & -Br) appeared in the range from 1000-800 $cm^{-1}$ in compounds **3a**, **3c**, **3d**, **3e** and **3g**. In $^1$H NMR spectrum of all synthesized compounds **3a-h** showed the one singlet proton typical for the -CH group with chemical shift value δ 7.51-7.25 ppm range, confirming the presence of styryl ring in all final compounds. The appearance of one proton of -COOH at the highly downfield side with chemical shift value ranges from δ 11.90-11.01 ppm confirmed the synthesis of 2-styrylquinoline-4-carboxylic acid scaffold in all the synthesized compounds. Apart from these, the peaks for varied aromatic protons appeared as doublet, triplet and multiplets in the range of δ 8.40-7.20 ppm chemical shift value. The slight change in the chemical shift value due to different electron densities revealed the substitution of varied substituents like –Br, -$NO_2$, -Cl and –OH groups in compounds **3a**, **3b**, **3c** and **3f** respectively, wehreas compound **3g** showed one broad peak signal for the -OH group at δ 9.49 ppm. In the $^{13}$C NMR spectra, the respective peaks were observed against specific carbon and provided information about carbon nuclei's position and nature (primary, secondary, tertiary or quaternary) from their chemical shift values. In the $^{13}$C NMR spectra of compounds **3a-h**, the carbonyl group (-CO) of carboxyl appeared at around δ 169.8-167.7 ppm. The aromatic carbons appeared with the chemical shift value ranges from δ 133-123 ppm. The mole-

cular ion peaks for the compounds **3a-h** in the mass spectra of these newly synthesized compounds confirmed the formation of the 4,6-substituted 2-styrylquinoline derivatives.

**Antimicrobial studies:** The minimum inhibitory concentration (MIC) were evaluated against *S. aureus* (ATCC-25923) revealed varying levels of inhibitory activity for 2-styrylquinoline derivatives. Among these derivatives, **3d** and **3f** exhibited a MIC of 8 µg/mL, indicating weak inhibitory potency, whereas, derivatives **3a**, **3b**, **3c**, **3e**, **3g** and **3h** displayed an MIC of 4 µg/mL, suggesting a moderate inhibitory effect (Table-1).

Against *E. faecalis* (ATCC-29212), compounds **3b**, **3c** and **3e** had significant MICs of 2 µg/mL, while compound **3a** showed moderate inhibition at 4 µg/mL. However, compounds **3d**, **3f**, **3g** and **3h** exhibited mild antibacterial activity at 8 µg/mL compared to the positive control. In case of *E. coli* (ATCC-25922) tested derivatives showed variation in their inhibitory potential. Compound **3e** showed the significant MIC of 2 µg/mL, although **3b** and **3d** demonstrated moderate inhibition at 4 µg/mL whereas **3a**, **3c**, **3f**, **3g**, **3h** derivatives showed their weak antibacterial activity at 8 µg/mL.

The minimum inhibitory concentration (MIC) results for 2-styrylquinoline derivatives against *P. aeruginosa* (ATCC-27853) revealed that derivatives **3a**, **3b**, **3c**, **3d** and **3h** demonstrated weak bioactivity at a MIC of 8 µg/mL. Derivative **3e** displayed a moderate MIC of 4 µg/mL, whereas derivative **3f** and **3g** showed lower bioefficacy at MIC of 16 µg/mL. Similarly, all derivatives showed anticandida activity at MIC of 16 µg/mL besides derivative **3e**, which shows anticandida activity at 8 µg/mL.

These results stated the variability of the antimicrobial potencies among 2-styrylquinoline derivatives against each tested microbial pathogen demonstrating promising efficacy. According to structural activity relationship (SAR) studies, the presence of electron-withdrawing group at 4$^{th}$ position of the styrylquinoline core structure is considered effective for antimicrobial activity (Table-1). For positive control, ciprofloxacin was used against bacterial strains while ketoconazole was used against *C. albicans*. In comparison to derivatives, ciprofloxacin showed antibacterial efficacy at MIC of 0.5 µg/mL while ketoconazole exhibited anticandida activity at MIC of 2 µg/mL.

## Anticancer studies

**MTT assay:** In comparison to compounds **3b** and **3c**, the MTT assay showed that compound **3e** had strong cytotoxic (anticancer) effects at doses ranging from 10 to 100 µM against Human colon adenocarcinoma HT29 cell lines. The MTT colorimetric assay was used to detect the decrease in cell viability because of the anticancer impact, using mitochondrial dehydrogenase enzymes from viable cells. The results demonstrated that compound **3e** action was dose-dependent and there was substantial cell death after 24 h (Fig. 1). At low doses (10 µM)

TABLE-1
ANTIMICROBIAL ACTIVITY DATA OF SYNTHESIZED NOVEL 2-STYRYLQUINOLINE DERIVATIVES (**3a-h**)

| Compound | Minimum inhibition concentration* (MIC) in µg/mL | | | | |
|---|---|---|---|---|---|
| | *S. aureus* (ATCC-25923) | *E. faecalis* (ATCC-29212) | *E. coli* (ATCC-25922) | *P. aeruginosa* (ATCC-27853) | *C. albicans* (ATCC 10231) |
| **3a** | 4 | 4 | 8 | 8 | 16 |
| **3b** | 4 | 2 | 4 | 8 | 16 |
| **3c** | 4 | 2 | 4 | 8 | 16 |
| **3d** | 8 | 8 | 4 | 8 | 16 |
| **3e** | 4 | 2 | 2 | 4 | 8 |
| **3f** | 8 | 8 | 8 | 16 | 16 |
| **3g** | 4 | 8 | 8 | 16 | 16 |
| **3h** | 4 | 8 | 8 | 8 | 16 |
| Positive control (Ciprofloxacin) | 0.5 | 0.5 | 0.5 | 0.5 | – |
| Positive control (Fluconazole) | – | – | – | – | 2 |

*Performed in triplicate (n = 3)

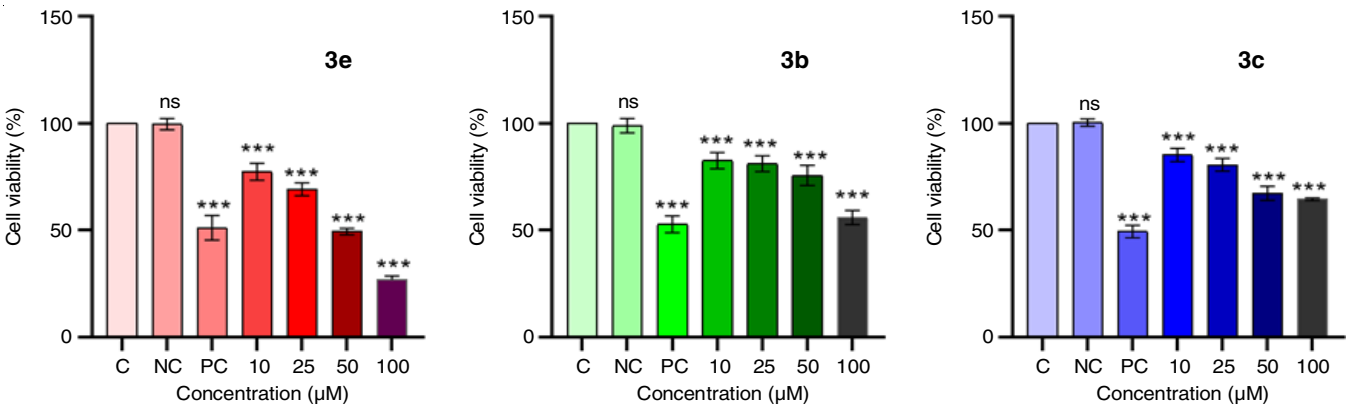



Fig. 1. *In vitro* cytotoxicity of compounds **3e**, **3b** and **3c**. Cell viability assay of **3e**, **3b** and **3c** on HT29 cell lines by 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide assay

of compounds **3e**, **3b** and **3c**, cell death rates were less than 22.67%, 17.45% and 14.81%, respectively. However, at high dosages (100 µM) increased significantly higher to 72.9%, 44.01% and 35.46%. Compared with these results, the inhibitory concentrations ($IC_{50}$) measured were 58.84 µM, 114.53 µM and 130 µM for compounds **3e**, **3b** and **3c** (Table-2). This study revealed that compound **3e** caused significant cytotoxicity in HT29 cell lines.

TABLE-2
$IC_{50}$ VALUES OF THE SYNTHESIZED NOVEL 2-STYRYL-QUINOLINE DERIVATIVES (**3a-h**) ON HT29 CELL LINES

| Compounds | $IC_{50}$ | Compounds | $IC_{50}$ |
|---|---|---|---|
| **3a** | 160.32 | **3e** | 58.84 |
| **3b** | 114.53 | **3f** | 146.21 |
| **3c** | 130.00 | **3g** | 139.22 |
| **3d** | 143.24 | **3h** | 151.65 |

**AO-EtBr dual staining assay:** The majority of anticancer medications trigger the death of cancer cells. Consequently, studying apoptosis is far more crucial than using the standard MTT cell viability test, which does not differentiate between live and dead cells [32]. One of the best ways to identify cell death is with the Dual AO/EtBr test, which is also easy to use and reliable [33]. Fig. 2 shows fluorescent microscopy pictures of untreated HT29 cells and cells treated with test chemical **3e** at 25 µM and 50 µM doses. Normal cell morphology, apoptotic cell death and necrosis of HT29 were identified by fluorescence microscopy, which was based on membrane integrity and DNA staining with AO-EtBr. Apoptotic cells, characterized by nuclear shrinkage and blebbing, appear orange in fluorescent microscopy, in contrast to the green fluorescence of normally functioning cells. The red fluorescence was caused by necrotic cells losing their membrane integrity as a result of the toxicity induced by compound **3e**. The growth inhibition and apoptotic activity of compound **3e** in the AO-EtBr experiment were shown to be dose-dependent when the colour changed from green to red. As Das *et al*. [34] found when the two strains are combined, injured cells labeled with EtBr illuminate orange or red, but healthy cells with intact membranes shine green when stained with AO. The results demonstrated that the addition of compound **3e** enhances the apoptosis of HT29 cells through the membrane blebbing and nuclear fragmentation.

**Molecular docking studies:** Molecular docking analyses have revealed that 2-styrylquinoline derivatives (**3a-h**) were found to be responsible for executing antibacterial, antifungal and anticancer activities. These derivatives were found to be interacting with the DHFR enzyme of *E. coli*, *S. aureus* and DNA gyrase of *P. aeruginosa* and *E. faecalis* for antimicrobial

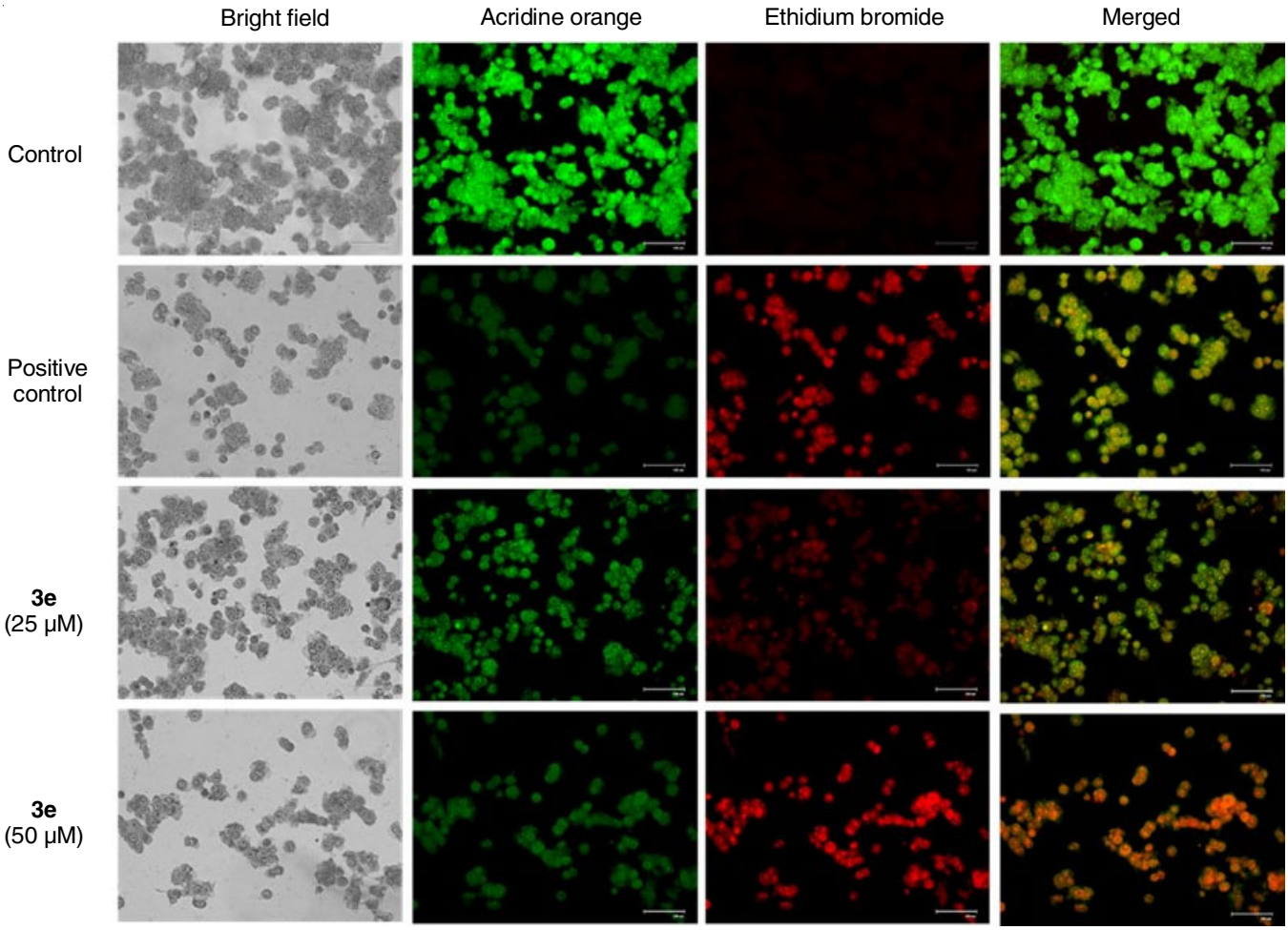



Fig. 2. Acridine orange-ethidium bromide staining of HT29 cells. Images captured by fluorescence microscopy of HT29 cells stained with acridine orange-ethidium bromide after being treated with compound **3e** for 24 h

and DHFR enzyme of *C. albicans* for antifungal effects and with the various target proteins of cancer such as p53, topoisomerase and tyrosine Kinase.

Among all the compounds, **3e** found to be most potent as per the best binding scores against all targets -8.87, -8.95, -9.33, -6.85 & -7.85 Kcal $mol^{-1}$ against DHFR of *E. coli* (6CQA), DNA gyrase of *P. aeruginosa* (6M1J), DHFR of *S. aureus* (2W9S), acetolactate synthase of *E. faecanilis* (1YZF) and DHFR of *C. albicans* (4HOE), respectively (Table-3).

These designed molecules displayed varied interactions with the desired amino acids residues such as Ala6, Ala7, Met16, Asn18 Leu28, Phe31, Lys32, Thr46, Ser49, Ile50, Arg52, Leu54, Arg57, Ile94, Tyr100 of DHFR enzyme of *E. coli*; Val6, Leu20, Leu28, Ile31, Ile50, Lys52, Leu54, Pro55, Arg57, Phe92, Tyr98 of DHFR enzyme of *S. aureus* and Met25, Ile33, Phe36, Lys37, Thr58, Ser61, Ile62, Leu69, Pro70, Arg72 of DHFR enzyme of *C. albicans via* hydrogen bonding, π-π interactions, alkyl-π, carbon-hydrogen bonding and van der Waals forces. Among all, compound **3e** exhibited the highest binding energy against all the target proteins (Fig. 3). The docking studies revealed the structure-activity relationship of designed novel 2-styrylquinoline derivatives that styryl ring is required for the activity as it interacted with crucial amino acids of all the target proteins *e.g.* Leu28, Ile50 (*E. coli*), Leu28, Ile31, Leu54, Ile50, (*S. aureus*) and Ile31, Lys37 (*C. albicans*) *via* π-π interactions, alkyl-π bondings. The styryl group itself is important for the activity as interacted with active sites using pi-bond *e.g.* Ala6, Met16, Phe31 (*E. coli*), Leu20, Phe92 (*S. aureus*) and Ile62 (*C. albicans*). Substitution of -Cl at position 4 of the styryl also interacted with all the target proteins *e.g.* Ala6 of *E. coli*, Val6, Tyr98 of *S. aureus via* hydrophobic interactions. Carboxyl group of quinoline scaffold also interacted with Leu28, Arg57 of S. aureus and Arg72 of *C. albicans*. The nitro group at position-6 of quinoline interacted with Arg52 of *E.*

TABLE-3
BINDING ENERGY DATA of SYNTHESIZED NOVEL 2-STYRYLQUINOLINE DERIVATIVES (**3a-h**) AGAINST VARIOUS BACTERIAL AND FUNGAL STRAINS

| Compound | Binding energy (kcal/mol) | | | | |
|---|---|---|---|---|---|
| | *E. coli* DHFR (6cqa) | *P. aeruginosa* DNA Gyrase (6m1j) | *S. aureus* DHFR (2w9s) | *E. faecanilis* Acetolactate synthase (1yzf) | *C. albicans* DHFR (4hoe) |
| **3a** | -7.81 | -7.24 | -7.83 | -6.33 | -7.10 |
| **3b** | -8.42 | -8.30 | -8.85 | -6.27 | -7.57 |
| **3c** | -7.80 | -7.62 | -7.60 | -6.50 | -7.63 |
| **3d** | -7.41 | -7.44 | -7.52 | -6.64 | -7.23 |
| **3e** | -8.87 | -8.95 | -9.33 | -6.85 | -7.85 |
| **3f** | -7.49 | -7.13 | -7.00 | -6.34 | -7.44 |
| **3g** | -7.50 | -7.37 | -7.10 | -5.84 | -7.40 |
| **3h** | -8.06 | -7.29 | -8.65 | -5.50 | -7.02 |
| Ciprofloxacin | -7.64 | -7.86 | -7.21 | -5.62 | – |

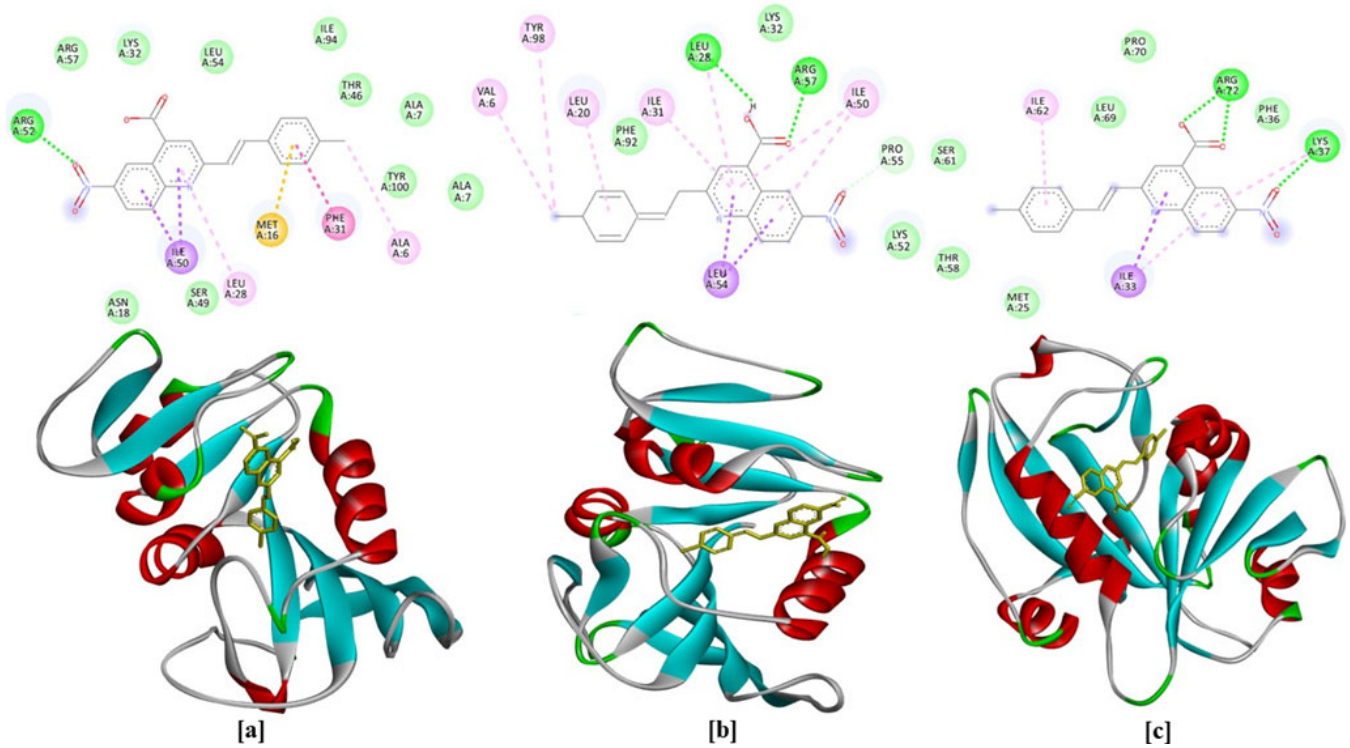



Fig. 3. 2D and 3D Binding conformations of compound **3e** against DHFR of *E. coli* (a), *S. aureus* (b) and *C. albicans* (c), respectively

*coli*, Pro55 of *S. aureus* and Lys37 of *C. albicans*. All the synthesized compounds were also docked with important targets of cancer such as p53, topoisomerase and tyrosine kinase and the binding energy was calculated for all compounds are tabulated in Table-4.

TABLE-4
DOCKING SCORES OF SYNTHESIZED 2-STYRYLQUINOLINE DERIVATIVES (**3a-h**) AGAINST CANCER TARGET PROTEINS p53, TOPOISOMERASE AND TYROSINE KINASE

| Compound | Anticancer activity | | |
|---|---|---|---|
| | P53 (1tup) | Topoisomerase (3qx3) | Tyrosine kinase (2hyy) |
| **3a** | -4.97 | -5.39 | -5.44 |
| **3b** | -6.15 | -7.78 | -7.39 |
| **3c** | -6.23 | -7.91 | -8.81 |
| **3d** | -5.13 | -5.81 | -5.71 |
| **3e** | -6.90 | -8.68 | -8.92 |
| **3f** | -6.82 | -8.39 | -5.35 |
| **3g** | -6.64 | -8.41 | -8.82 |
| **3h** | -6.40 | -7.95 | -8.25 |
| Cisplatin | -3.41 | -3.41 | -3.70 |

Though, all the compounds showed good binding scores against cancer target proteins, compound **3e** was found to be best based on the highest binding score against all targets. The 3D binding mode of compound **3e** against anticancer target topoisomerase is shown in Fig. 4. Compound **3e** showed varied interactions in the active pocket of one of target protein topoisomerases such as Arg503, Gly504, Gln778, Met782, Met781 and Pro819. SAR revealed that all the structural features like the basic quinoline ring system, styryl group with carbon spacer of 2, the electron-withdrawing group at position 4 and nitro at 6 positions of styryl moiety involved in interactions *via* hydrogen bonding, π-π interactions, alkyl-π, carbon-hydrogen bonding, hydrophobic interactions and van der Waals forces. Thus, all these structural features could be the reasons for the *in vitro* activity data, which makes these synthesized compounds therapeutic active anticancer agents.

**ADMET prediction:** The Swiss ADME program was used to conduct the ADME experiments of novel 2-styrylquinolines (**3a-h**). The drug-like characteristics of the derivatives led to their identification. According to Lipinski's rule of five, which states that HB donor ≤ 5, HB acceptor ≤ 10, logPo/w < 5 and MW < 500, all the screened compounds were drug-like. These compounds showed important ADME profile with improved pharmacokinetic and physico-chemical characteristics (Table-5). Parameters such as TPSA, Log P consensus and Log S ESOL revealed the analogues' polarity, lipophilicity and solubility. Additionally, the high gastrointestinal absorption of all compounds guaranteed their high bioavailability. All the top-ranked compounds have drug-like properties, according to the expected ADME (absorption, distribution, metabolism and excretion) profile.

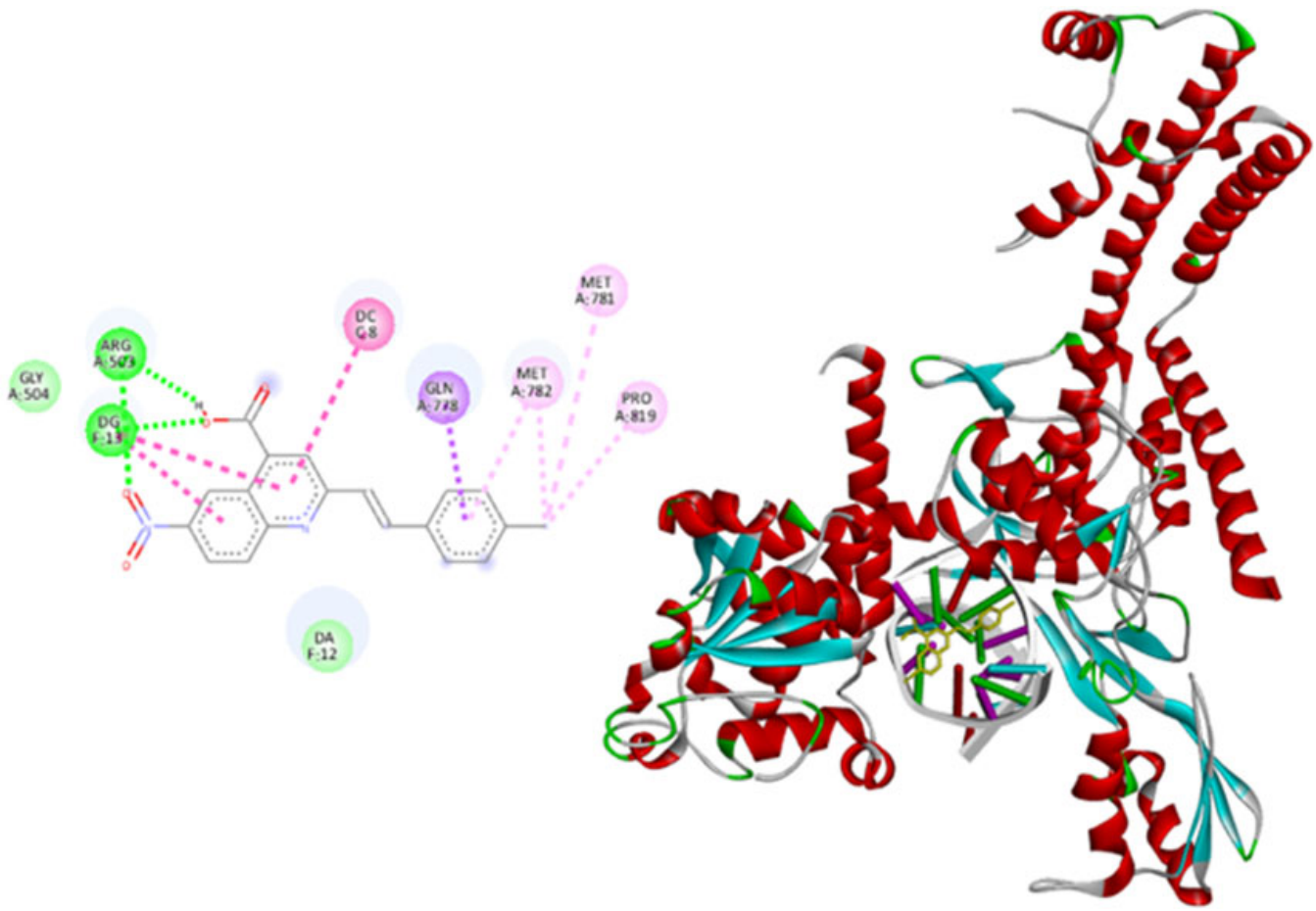



Fig. 4. 2D and 3D Binding conformations of compound **3e** against human topoisomerase II β-enzyme

TABLE-5
ADME PROFILE AND THE PHYSICO-CHEMICAL PARAMETERS OF
THE NOVEL SYNTHESIZED 2-STYRYLQUINOLINE DERIVATIVES (**3a-h**)

| Compd. | m.w. | HB Acc | HB Don | TPSA | Consensus Log P | Ali Log S | Rule of five | Brain permeant | GI absorption |
|---|---|---|---|---|---|---|---|---|---|
| **3a** | 354.20 | 3 | 1 | 50.19 | 3.89 | -5.49 | 0 | Yes | High |
| **3b** | 320.30 | 5 | 1 | 96.01 | 2.51 | -5.56 | 0 | No | High |
| **3c** | 309.75 | 3 | 1 | 50.19 | 3.81 | -5.43 | 0 | Yes | High |
| **3d** | 388.64 | 3 | 1 | 50.19 | 4.43 | -6.15 | 0 | Yes | High |
| **3e** | 354.74 | 5 | 1 | 96.01 | 3.03 | -6.22 | 0 | No | High |
| **3f** | 291.36 | 3 | 2 | 126.55 | 3.80 | -4.84 | 0 | Yes | High |
| **3g** | 370.20 | 3 | 2 | 140.43 | 4.57 | -5.55 | 0 | Yes | High |
| **3h** | 338.31 | 5 | 2 | 142.33 | 2.58 | -4.27 | 0 | No | High |

## Conclusion

Bacterial resistance is one of the major frightening situation for the world although scientists are continuously working to counter this ongoing situation of resistance and to develop new chemical entities. These include the development of novel compounds that target specific resistance determinants. Thus, in the current study, the novel 2-styrylquinolines derivatives were designed, synthesized and biologically evaluated as potential antimicrobial agents with additional anticancer activities. The novel 2-styrylquinoline-4-carboxylic acid derivatives (**3a-h**) were synthesized using an efficient one-pot method. The antibacterial activities of the synthesized compounds were screened against one Gram-positive and two Gram-negative bacteria. Among all, compound **3e** exhibited maximum inhibitory response to all bacterial stains, comparison to ciprofloxacin taken as standard. In the structural activity relationship studies, 2-styrylquinoline was greatly impacted by the presence of 6-nitro phenyl functionality through ethene linker at the C-2 position. SAR analysis also confirmed that the presence of an electron-withdrawing group at the 4th position of styrylquinoline core structure is considered to be effective for antibacterial activity Also, compound **3e** was confirmed to be significant against *C. albicans* strain with MIC range of 8 µg/mL and remaining derivatives were found to be weakly active with MIC value of 16 µg/mL. Furthermore, synthesized derivatives were also evaluated for anticancer agents using MTT assay against HT29 cell lines. The results showed the dose-dependent activity of compound **3e** and extensive cell death after 24 h and the measured $IC_{50}$ was 58.84 µM. The *in vitro* results were validated using *in silico* molecular docking studies. The results showed the optimal docking and binding potential of the synthesized compounds against varied target proteins. The ADME prediction has also shown a promising result, which indicates the drug-likeliness of all the synthesized compounds. The novel designed compounds can be taken as lead potential antimicrobial agents with additional anticancer potential.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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