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Docking, Synthesis and Anticancer Activity of 4-(4-(3-(4-Chloro-3-(trifluoromethyl)-phenyl)ureido)phenoxy)-*N*-(2-morpholinoethyl)picolinamide Derivatives

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A new series of diaryl urea derivatives bearing pyridine moiety were designed, synthesized and evaluated for their biological activity. In this study, we applied the structure-based virtual screening (SBVS) on the high similar sorafenib approved drug, selected from the DrugBank database as well as on a series of derivatives, selected from the literature. Aim was to provide new potent anticancer agents. Analysis was performed using AutoDock VINA tools. Based on the ligand binding energy. Compounds will be synthesized by chlorination of pyridine acid derivative which further coupled with amine and form amide, amide further reaction with aminophenolic moiety and form ether which is react with other aromatic amine using CDI to produce final compound and these compounds characterized by IR, NMR and mass spectroscopic techniques. The synthesized derivatives have been evaluated to their anticancer activity *in vitro* by MTT assay using MCF-7 cell line. The anticancer activity indicates that compounds **C1**, **C3**, **C6** and **C9** have better anticancer activity.

Keywords: Diaryl urea derivatives, Anticancer agents, Molecular docking, MTT assay.

INTRODUCTION

Cancer is a one of the major causes of deaths globally [1]. In developed countries, it is a major human health problem and can become the most severe life-threatening disease in the near future [2]. Cancer treatments through targeted drugs, including imatinib, gefitinib and trastuzumab, could reduce severe adverse reactions and improve the cure rates due to their high specificity. Furthermore, these drugs usually lead to severe toxicity on and/or off targets [3]. Therefore, a highly target specific therapy having the minimum toxicity must be developed for disease-free survival and improvement of the life quality of patients with cancer.

Numerous kinases involve in transduction pathway signalling inside the cancer cells. Thus, for the discovery of novel antitumor agents, these kinases are excellent therapeutic targets [4,5]. RAF proteins (Ser/Thr kinase) play a critical role in the activation of the signalling pathway of RAS-RAF-MEK-ERK and promotion of normal cell development [6,7]. RAF is mainly activated by RAS, a G protein, in response to cancer cell mutations or through the over expression of various receptor protein tyrosine kinases, including epidermal, vascular endothelial, and platelet-derived growth factors [8]. Deregulation of normal RAF signalling pathways results in their over expression in different cancers, such as hepatocellular (14%), colorectal (15%), mammary gland (10%), prostate (10%) and melanoma (60%) cancer. FDA has approved several Raf inhibitors as anticancer drugs, including dabrafenib, sorafenib and vemurafenib and some inhibitors, such as WO201106818715, remain under clinical trials [9].

A diaryl urea moiety is commonly utilized for designing anticancer drugs, including regorafenib, sorafenib, tivozanib, and linifanib. Diaryl ureas can be employed to synthesize many heterocyclic compounds having diversified biological activities, such as antimalarial [10], antithrombotic [11], anti-inflammatory [12] and antibacterial [13] properties and are fragments with considerable importance in medicinal chemistry. They can form hydrogen bonds (HBs) with biotargets [14]. Carbonyl oxygen atoms act as proton acceptors, whereas two amide nitrogen atoms serve as proton donors. This unique

structure provides urea derivatives with the ability to bind various receptors and enzymes in a biological system [15].

In past years, diarylurea moieties were considered an essential pharmacophore in medicinal chemistry [16-18] and drug design due to the nature of urea linkers, where NH moieties and their carbonyl oxygen atoms are conceived as strong HB donors and outstanding HB acceptors, respectively. Therefore, the urea linker was employed to fine-tune some drug like properties, establish certain stable pseudoheterocycles through intermolecular hydrogen bonding, and develop specific drug-target interactions [19,20].

Many design rationales of the antiproliferative agents are incorporated with urea motifs to enhance interactions and thus, affinity with target receptors, for example, several signalling pathway inhibitors and DNA-alkylating agents [21-23]. Within the kinase inhibitor domain, urea linkers play a critical role as hinge binding motifs with urea moieties embedded in heterocyclic structuresand to develop HBs with DFG motifs in the type II kinase inhibitor. Many diarylurea derivatives, including regorafenib, sorafenib and linifanib, are FDA-approved as anticancer drugs [24-31].

EXPERIMENTAL

Docking study

Ligand preparation: Nine structures of the chemical constituents of diaryl urea derivatives were obtained from the synthesized compounds. The 2D chemical structures of ligands were drawn using ChemDraw Ultra 2008. The energy of the prepared ligands was minimised using Chem3D Ultra and was saved in pdb format.

Target preparation and validation of docking method: The 3D structures of proteins were acquired from the Protein databank (PDB ID: 4DBN). The docking study was commenced by defining a binding site, which is generally a restricted region of a protein. The location and size of the binding site were visualised in PyMOL. AutoDock Vina was used to further validate the protein target.

Molecular docking analysis: The interactions and binding modes of individual synthesized compounds with 4DBN were analyzed using AutoDock Vina software. Then, docking was conducted to acquire the population of possible orientations and conformations for ligand at binding sites. In PyRx software, the protein was loaded, thereby creating a PDBQT file comprising the protein structure having hydrogen atoms in each polar residue. All the ligand bonds were rotatable. For protein-fixed ligand-flexible docking, all the calculations were performed using the lamarckian genetic algorithm. On the protein target, the docking site was defined by developing the grid box cantered on X: 35.251, Y: -27.003, Z: 5.157 and having a dimension of X: 40 Y: 40 Z: 40 Å with the exhaustiveness of 8. The optimum conformation with the lowest docked energy was selected after docking was completed. Nine AutoDock Vina runs were performed for all the scenarios for each ligand structure. For each run, the optimal pose was obtained. The average affinity of optimal poses was considered the final affinity. The complex protein-ligand interaction conformations, such as bond lengths and HBs, were analyzed using Discovery studio visualizer [32-35].

The solvents and chemicals required for the synthesis were obtained from the reputed commercial sources like Finar, Avra Synthesis and Spectrochem. All the chemicals were used as received without further purification. The precoated plates of silica gel G60 F_{254} (0.2 mm, Merck) were utilized for TLC. Visualization was achieved with iodine vapour or under UV light (254 and 365 nm). The spectra of the fabricated compound were analysed through FTIR-8400 (Shimadzu) by using the ATR technique. The ^{13}C NMR (101 MHz) and ^{1}H NMR (400 MHz) spectra were measured on the Bruker AVANCE II spectrometer by employing DMSO-d_6 and TMS as the solvent and internal reference, respectively. The mass spectra were recorded at 70 eV on a Jeol-JMSD 300 mass spectrometer.

The MEM-nonessential amino acid solution (100X), HEPES [(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)], antibiotic-antimycotic solution, sodium pyruvate, foetal bovine serum (FBS), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and cell culture grade dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich (USA). All other chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany).

Cell lines and cell culture: Human breast adenocarcinoma (MCF-7) cell lines were obtained from National Center for Cell Science (NCCS), Pune, India. The MCF-7 cell was cultured in MEM media supplemented with 1% nonessential amino acids, 10% FBS and 0.5 mL of antibiotic-antimycotic solution (100X) (10,000 units/mL of penicillin, 10,000 µg/mL of streptomycin, and 25 µg/mL of Gibco amphotericin B. The cell lines were stored under sterile conditions with 95% air and 5% CO_2 at 37 ºC and weekly subcultured with 0.05% trypsin and 0.02% EDTA. The media were changed after every 2 to 3 days.

The solutuions were freshly prepared in DMSO with cell culture grade at 100 mM stock concentration. The exponentially growing MCF-7 cells were treated with various compounds (100 µM) for 24 h. The cells treated with DMSO (0.1%) were regarded as vehicle control.

Anticancer activity: The MTT assay was used to examine cell proliferation. For 24 h, cells of 2×10^4 MCF-7 were treated with several fabricated compounds. Then, these cells were washed using DPBS and incubated with MTT (0.5 mg/mL) in the dark for 4 h at 37 ºC. After incubation, MTT was removed. Subsequently, DMSO was added to all the wells. Absorbance was measured at 570 nm (the reference wavelength was 650 nm) by employing the Multimode microplate reader (SpectraMax M2e, Molecular Devices, USA). The results were reported as the percentage of cell proliferation.

Synthesis

Step-I: 4-Chloropicolinoyl chloride (Int1): Sodium bromide (0.013 mol) and 2-picolinic acid (0.081 mol) were suspended in chlorobenzene. After heating to 50 ºC, 0.40 mol of $SOCl_2$ was added. Subsequently, the reaction mixture was heated to 85 ºC, and then stirred for 20 h. After the mixture cooled down to room temperature, under reduced pressure, excess thionyl chloride and most of chlorobenzene were

removed through distillation. The material obtained was directly used in the subsequent stages [36-40].

Step-II: 4-Chloropyridine-2-carboxamide derivatives (IntS1-9): A solution of 4-chloropicolinoyl chloride (0.0284 mol) was mixed in THF (50 mL) at 0 ºC and triethylamine (0.0568 mol) was added. Resulting reaction mixture was treated with amine (0.031 mol) solution in THF (25 mL), which kept the internal temperature below 5 ºC. The resulting mixture was stored at room temperature for 5 h, then concentrated under reduced pressure. Finally, the mixture was diluted with water, extracted with ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain **IntS1-9**. All the intermediated products were confirmed by mass spectroscopy and used in next step without purification.

Step-III: 4-(4-Aminophenoxy)pyridine-2-carboxamide derivatives (IntS1a-9a): A solution of 4-aminophenol (0.0183 mol) in anhydrous DMF (15 mL) was treated with potassium *tert*-butoxide (0.0366 mol), a reddish-brown mixture was stirred at room temperature for 2 h. The contents were treated with 4-chloropyridine-2-carboxamide derivatives (**IntS1-9**) (0.0183 mol) and K_2CO_3 (0.009 mol) and then heated at 80 ºC for 8 h. The mixture was cooled to room temperature and separated between ethyl acetate and water. The combined organic layers were washed with a saturated NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting solids were dried under reduced pressure at 35 ºC for 3 h to obtain 4-(4-aminophenoxy)pyridine-2-carboxamide derivatives (**IntS1a-9a**) as solid. All the intermediated compounds confirmed by mass spectroscopy and used in next step without purification.

Step-IV: Diaryl urea derivatives (C1-C9): To a solution of 4-chloro-3-(trifluoromethyl)aniline (0.005 mol) in anhydrous dichloromethane at 0 ºC was added CDI (0.0052 mol). The resulting solution was allowed to warm to room temperature over 1 h, stirred for 16 h, then treated with 4-(4-aminophenoxy)pyridine-2-carboxamide derivatives (**IntS1a-9a**) (0.005 mol). The resulting yellow solution was stirred at room temperature for 72 h, then treated with water. The resulting aqueous mixture was extracted with ethyl acetate, while the combined organics were dried over sodium sulphate and concen-trated under reduced pressure. The residual oil purified by column chromatography using ethyl acetate and *n*-hexane as mobile phase to obtain diaryl urea derivatives as solid.

4-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-*N*-(2-morpholinoethyl)picolinamide (C1): Yield: 50.6%. IR (KBr, ν_{max}, cm^{-1}): 3338 (NH *str.*), 1708 (C=O *str.*), 1645 (C=O *str.*), 1302 (CF_3), 1229 (arom. ether), 1025 (aliph. ether), 681 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.34-2.36 (t, 4H, CH_2), 2.50-2.53 (t, 2H, CH_2), 3.34-3.36 (t, 4H, CH_2), 3.63-3.66 (t, 4H, CH_2), 3.75-3.85 (m, 2H, CH_2), 7.12-7.16 (d, 2H, ArH), 7.47-7.49 (d, 2H, ArH), 7.70-7.76 (d, 2H, ArH), 7.89-7.91 (d, 1H, ArH), 8.07 (s, 1H, ArH), 8.13 (s, 1H, ArH), 8.58-8.59 (d, 1H, ArH), 9.11 (s, 1H, CONH), 9.25 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 54.1, 55.8, 57.6, 66.7, 109.1, 114.3, 117.2, 120.9, 121.8, 122.8, 123.4, 124.6, 126.7, 132.3, 137.5, 139.7, 148.2, 150.6, 152.8, 164.2 (C=O), 166.4 (C=O). Mass (LC-MS): *m/z* 564.5[M+H]$^+$, 566.4 [M+2]$^+$.

4-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-*N*-(2-(diethylamino)ethyl)picolinamide (C2): Yield: 49.25%. IR (KBr, ν_{max}, cm^{-1}): 3376 (NH *str.*), 1708 (C=O *str.*), 1645 (C=O *str.*), 1301 (CF_3), 1200 (arom. ether), 1134 (C-N), 684 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.04-1.06 (t, 6H, CH_3), 2.45-2.53 (m, 6H, CH_2), 3.34-3.36 (t, 4H, CH_2), 3.63-3.66 (t, 4H, CH_2), 3.75-3.85 (m, 2H, CH_2), 7.12-7.16 (d, 2H, ArH), 7.47-7.49 (d, 2H, ArH), 7.70-7.76 (d, 2H, ArH), 7.89-7.91 (d, 1H, ArH), 8.07 (s, 1H, ArH), 8.13 (s, 1H, ArH), 8.58-8.59 (d, 1H, ArH), 9.11 (s, 1H, CONH), 9.25 (s, 1H, CONH). Mass (LC-MS): *m/z* 550.5 [M+H]$^+$, 552.4 [M+2]$^+$.

4-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-*N*-ethyl-*N*-methyl picolinamide (C3): Yield: 45.8%. IR (KBr, ν_{max}, cm^{-1}): 3338 (NH *str.*), 1708 (C=O *str.*), 1646 (C=O *str.*), 1329 (CF_3), 1230 (arom. ether), 1168 (C-N), 683 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.34-1.37 (t, 3H, methyl), 3.47 (s, 3H, methyl), 3.75-3.85 (m, 2H, CH_2), 7.13-7.15 (d, 2H, ArH), 7.48-7.49 (d, 2H, ArH), 7.71-7.76 (d, 2H, ArH), 7.89-7.91 (d, 1H, ArH), 8.07 (s, 1H, ArH), 8.13 (s, 1H, ArH), 8.58-8.59 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.25 (s, 1H, CONH). Mass (LC-MS): *m/z* 493.3 [M+H]$^+$, 495.4 [M+2]$^+$.

***N*-(4-(*tert*-Butyl)phenyl)-4-(4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenoxy)picolinamide (C4):** Yield: 52.10%. IR (KBr, ν_{max}, cm^{-1}): 3340 (NH *str.*), 3078 (C-H *str.*), 1710 (C=O *str.*), 1650 (C=O *str.*), 1333 (CF_3), 1233 (arom. ether), 683 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.29 (s, 9H), 7.33-7.49 (m, 5H, ArH), 7.52-7.53 (d, 2H, ArH), 7.72-7.76 (d, 2H, ArH), 7.88-7.89 (d, 2H, ArH), 7.97-8.17 (s, 4H, ArH), 8.53 (s, 1H, ArH), 9.10 (s, 1H, CONH), 9.30 (s, 1H, CONH), 9.45 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 31.4, 34.3, 109.3, 114.4, 117.1, 120.8, 121.2, 121.8, 122.8, 123.4, 124.5, 126.7, 127.9, 132.2, 134.8, 137.5, 139.6, 146.9, 148.2, 150.6, 152.8, 164.4. Mass (LC-MS): *m/z* 583.9 [M+H]$^+$, 585.9 [M+2]$^+$.

1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-((2-(piperidine-1-carbonyl)pyridin-4-yl)oxy)phenyl)urea (C5): Yield: 61.58%. IR (KBr, ν_{max}, cm^{-1}): 3339 (NH *str.*), 1707 (C=O *str.*), 1647 (C=O *str.*), 1327 (CF_3), 1225 (arom. ether), 1134 (C-N), 682 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.54-1.64 (m, 4H, piperidine), 1.67-1.71 (t, 2H, piperidine), 3.68-3.77 (t, 2H, piperidine), 7.13-7.16 (d, 2H, ArH), 7.47-7.49 (d, 2H, ArH), 7.71-7.75 d, 2H, ArH), 7.89-7.90 (d, 1H, ArH), 8.07 (s, 1H, ArH), 8.12 (s, 1H, ArH), 8.58-8.59 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.25 (s, 1H, CONH). Mass (LC-MS): *m/z* 519.2 [M+H]$^+$, 521.2 [M+2]$^+$.

1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-((2-(morpholine-4-carbonyl)pyridin-4-yl)oxy)phenyl)urea (C6): Yield: 52.60%. IR (KBr, ν_{max}, cm^{-1}): 3326 (NH *str.*), 1708 (C=O *str.*), 1624 (C=O *str.*), 1331 (CF_3), 1229 (arom. ether), 1025 (aliph. ether), 691 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.47-3.57 (t, 4H, morpholine), 3.60-3.68 (t, 4H, morpholine), 7.13-7.16 (d, 2H, ArH), 7.48-7.50 (d, 2H, ArH), 7.71-7.76 d, 2H, ArH), 7.89-7.90 (d, 1H, ArH), 8.07 (s, 1H, ArH), 8.12 (s, 1H, ArH), 8.58-8.59 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.25 (s, 1H, CONH). Mass (LC-MS): *m/z* 521.8 [M+H]$^+$, 523.6 [M+2].

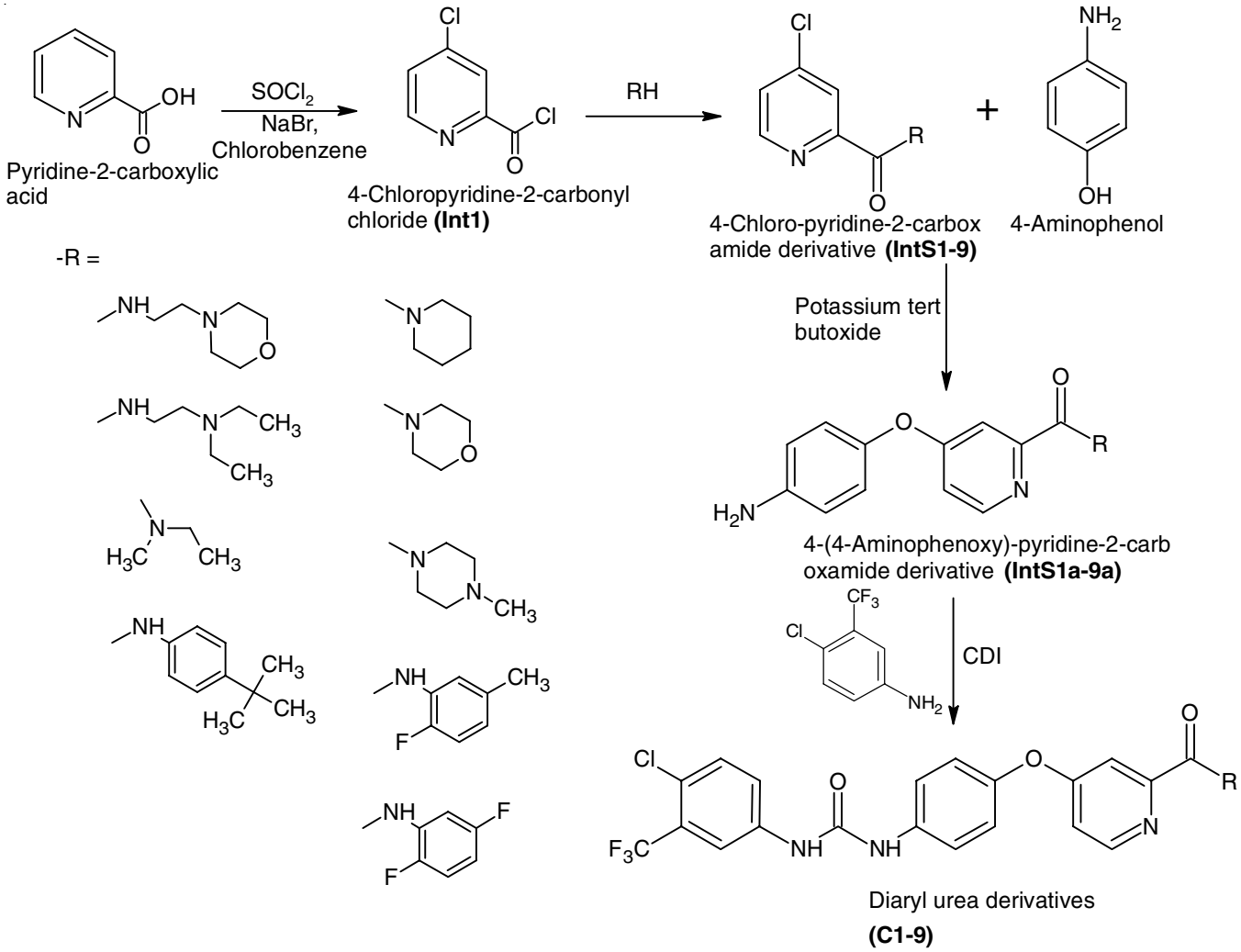
1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-((2-(4-methylpiperazine-1-carbonyl)pyridin-4-yl)oxy)phenyl)-urea (C7): Yield: 56.75%. IR (KBr, ν_{max}, cm^{-1}): 3375 (NH *str.*), 1710 (C=O *str.*), 1650 (C=O *str.*), 1328 (CF_3), 1231 (arom. ether), 686 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.19 (s, 3H, N-methyl), 2.25-2.29 (t, 4H, piperazine), 3.21-3.27 (t, 4H, piperazine), 7.14-7.15 (d, 2H, ArH), 7.47-7.49 (d, 2H, ArH), 7.71-7.75 d, 2H, ArH), 7.88-7.90 (d, 1H, ArH), 8.07 (s, 1H, ArH), 8.12 (s, 1H, ArH), 8.55-8.59 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.25 (s, 1H, CONH). Mass (LC-MS): *m/z* 534.7 $[M+H]^+$, 536.6 $[M+2]^+$.

4-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-*N*-(2-fluoro-5-methylphenyl)picolinamide (C8): Yield: 48.37%. IR (KBr, ν_{max}, cm^{-1}): 3332 (NH *str.*), 1709 (C=O *str.*), 1650 (C=O *str.*), 1322 (CF_3), 1271 (C-F), 1226 (arom. ether), 681 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.35 (s, 3H, CH_3), 7.10-7.20 (m, 4H, ArH), 7.47-7.49 (d, 2H, ArH), 7.70-7.76 (m, 3H, ArH), 7.89-7.91 (d, 1H, ArH), 8.07 (s, 1H, ArH), 8.13 (s, 1H, ArH), 8.58-8.59 (d, 1H, ArH), 9.11 (s, 1H, CONH), 9.25 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 21.2, 109.0, 113.2, 114.4, 117.2, 119.0, 120.9, 121.6, 121.8, 122.9, 123.4, 124.4, 126.6, 132.2, 134.2, 137.5, 139.7, 148.2, 150.6, 152.8, 155.1, 164.4. Mass (LC-MS): *m/z* 559.9 $[M+H]^+$, 561.8 $[M+2]^+$.

4-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-N-(2,5-difluorophenyl)picolinamide (C9): Yield: 47.60%. IR (KBr, ν_{max}, cm^{-1}): 3312 (NH *str.*), 1706 (C=O *str.*), 1643 (C=O *str.*), 1342 (CF_3), 1280 (C-F), 1224 (arom. ether), 676 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.10-7.16 (m, 3H, ArH), 7.30-7.31 (d, 1H, ArH), 7.47-7.49 (d, 2H, ArH), 7.70-7.76 (m, 3H, ArH), 7.89-7.91 (d, 1H, ArH), 8.07 (s, 1H, ArH), 8.13 (s, 1H, ArH), 8.58-8.59 (d, 1H, ArH), 9.11 (s, 1H, CONH), 9.25 (s, 1H, CONH). Mass (LC-MS): *m/z* 563.5 $[M+H]^+$, 565.4 [M+2].

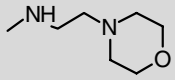
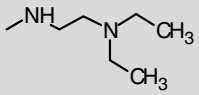
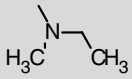
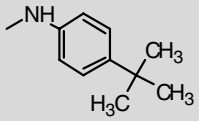
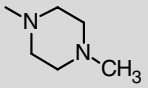
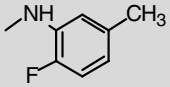
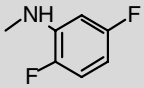
RESULTS AND DISCUSSION

The synthetic methods adapted for the synthesis of the diaryl urea derivatives are represented in **Scheme-I**. Picolinic acid was reacted with thionyl chloride in presence of sodium bromide and chlorobenzene solvent at 85 ºC, after chlorination produce 4-chloropicolinoyl chloride (**Int1**). **Int1** was reacted with nine different amines using triethylamine base and THF solvent, by acid chloride reaction with amine produce **IntS1-9**. **IntS1-9** was reacted with 4-amino phenol in the presence of



Scheme-I: Synthesis of diaryl urea derivatives

TABLE-1
BINDING ENERGY AND AMINO ACID INTERACTIONS OF DOCKING STUDY

Compound	RH (amine)	Autodock Vina binding energy (kcal/mol)	Amino acid interactions
C1		-11.2	LYS:A482, GLU:A500, VAL:A508, LEU:A504, ILE:A591, ASP:A593, GLY:A592, ILE:A512, PHE:A594, CYS:A581, LEU:A566, ALA:A480, VAL:A470, TRP:A580, ASN:A579.
C2		-9.8	VAL:B470, ALA:B480, ASP:B593, LEU:B513, LYS:B482, GLU:B500, TRP:B530, ALA:B597, CYS:B531, ILE:B462.
C3		-11.0	HIS:B538, THR:B593, ALA:B480, ASP:B593, GLU:B500, LYS:B482, VAL:B470, TRP:B530, CYS:B531, ALA:B597, ILE:B462, LEU:B513.
C4		-11.9	LEU:B504, GLY:B533, GLU:B532, TRP:B530, CYS:B531, ALA:B597, ALA:B480, VAL:B470, LYS:B482.
C5		-10.3	ALA:A480, VAL:A470, ASP:A593, LYS:A482, GLU:A500, ILE:A462, LEU:A513, TRP:A530, GLY:A533, GLU:A532.
C6		-10.9	SER:A535, ILE:A462, CYS:A531, TRP:A530, VAL:A470, THR:A528, ALA:A480, LYS:A482, LEU:A513, ASP:A593, GLU:A500.
C7		-10.3	ALA:A480, VAL:A470, ASP:A593, GLU:A500, LYS:A482, LEU:A513, ILE:A462, TRP:A530, GLY:A533, GLU:A532.
C8		-11.6	SER:B534, VAL:B599, THR:B598, ILE:B591, LEU:B513, LYS:B482, GLY:B592, ASP:B593, LEU:B504, ALA:B480, ALA:B597, THR:B528, VAL:B470, CYS:B531, TRP:B530, PHE:B594.
C9		-12.3	ILE:A591, GLY:A592, LEU:A566, HIS:A573, ASP:A593, ILE:A512, PHE:A594, ALA:A480, VAL:A470, PHE:A582, ILE:A462, TRP:A530, LEU:A504, VAL:A503, GLU:A500, LYS:A482, CYS:A531.

potassium tertiary butoxide to produce **IntS1a-9a**. 4-Chloro-3-(trifluoromethyl)aniline reacted with CDI then **IntS1a-S9a** added to form diaryl urea derivative (**C1-C9**). All the intermediates were confirmed by mass spectroscopy and used in next step without purification. All final compounds were purified by column chromatography with 47 to 62% yield. The structure of synthesized compounds **C1-C9** was confirmed through IR, Mass, ^{1}H NMR and ^{13}C NMR data.

Compound **C1** was characterized by FTIR with peak at 1708 cm^{-1} and 1645 cm^{-1} due to C=O, at C=O stretching, also produce characteristic peak at 1302 cm^{-1} due to CF_3 group, peak at 1229 cm^{-1} due to aromatic ether and at 681 cm^{-1} due to C-C. The ^{1}H NMR spectra characterized with all aliphatic proton, aromatic protons peak and produce peak at δ 9.11 and δ 9.25 ppm due to diaryl urea protons. ^{13}C NMR spectra show the peak at δ ppm 164.2 and 166.4 ppm due to carbonyl carbons. Mass spectra gives Molecular ion peak at 564.5 *m/z* and 566.4 *m/z* isotopic mass peak due to chloro group.

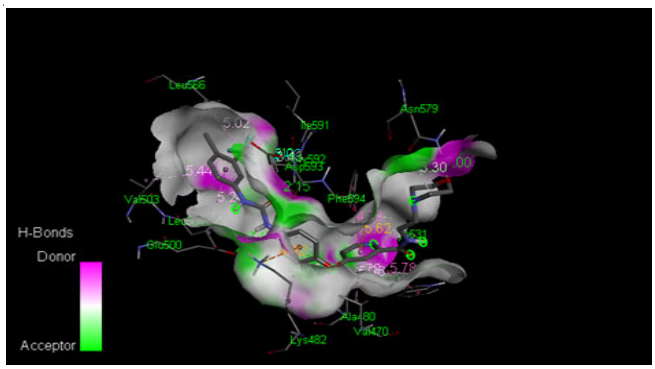
Docking study: To search novel potential compounds for cancer treatment, nine compounds were docked. For each ligand, AutoDoc vina incorporated with the PyRx tool provided nine conformations, which were classified on the basis of binding affinity (kcal/mol). The prepared compounds exhibited the free binding energy of -9.8 to -12.3 kcal/mol (Table-1).

All the synthesized compounds had a free binding energy of higher than that of compound **C2** (-9.8 kcal/mol). Similarly, for the spike protein, the fabricated compounds **C9** (-12.3 kcal/mol) and **C4** (-11.9 kcal/mol) exhibited a high binding affinity score, according to the molecular docking results. The molecular interactions, such as conventional HBs, halogen interaction, carbon HBs, pi-sulphur, pi-cation pi-pi stacked, alkyl and pi-alkyl and pi-pi T-shaped interactions, of all the compounds are shown in Fig. 1.

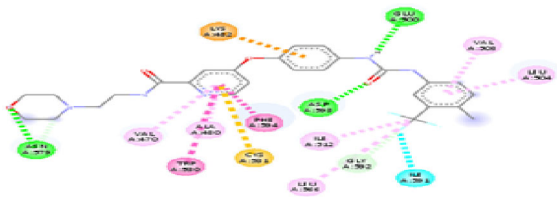
Anticancer activity: The molecules C1-C9 were assessed against MCF-7 cell line by MTT assay. In this experiment, the compounds **C1**, **C3**, **C6**, **C9** displayed potent activity compared with standard doxorubicin (IC_{50} = 29.30 µg/mL) (Table-2).

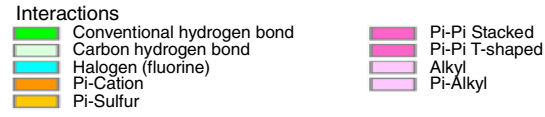
Conclusion

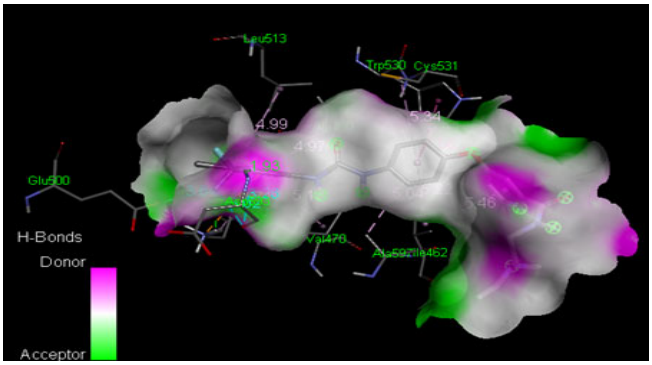
In this study, a structure-based virtual screening (SBVS) was applied on the high similar sorafenib approved drug and selected from literature. A new series of diaryl urea derivatives bearing pyridine acid compounds show strong and stable inter-


H-Bonds
Donor
Acceptor

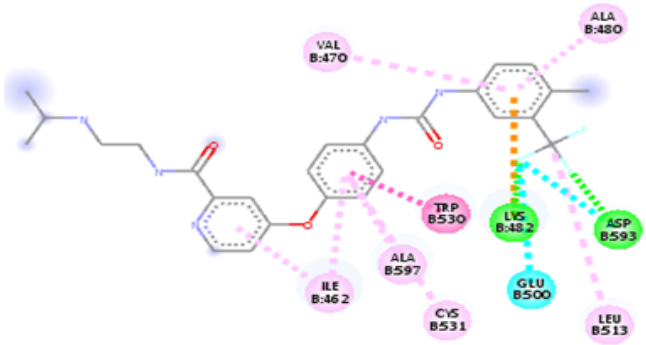
C1

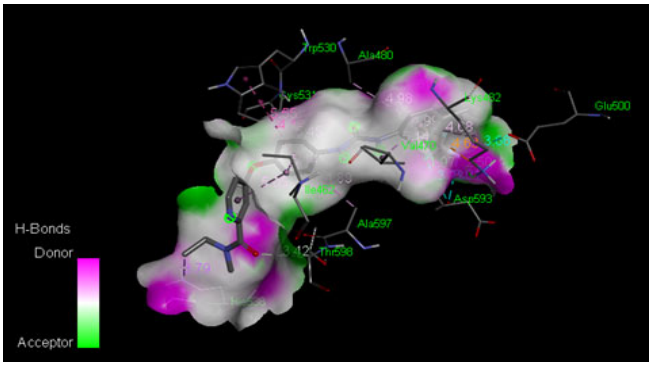



Interactions
Conventional hydrogen bond
Carbon hydrogen bond
Halogen (fluorine)
Pi-Cation
Pi-Sulfur
Pi-Pi Stacked
Pi-Pi T-shaped
Alkyl
Pi-Alkyl

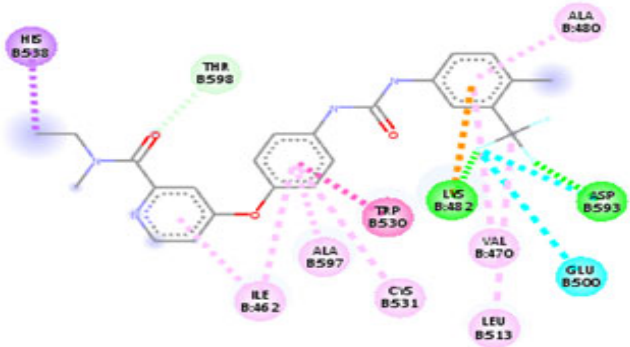

H-Bonds
Donor
Acceptor

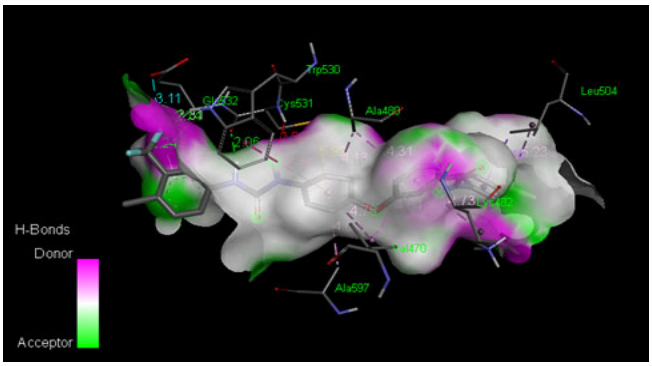
C2


ALA B:480
VAL B:470
TRP B530
LYS B:482
ASP B593
ALA B597
ILE B:462
GLU B500
CYS B531
LEU B513

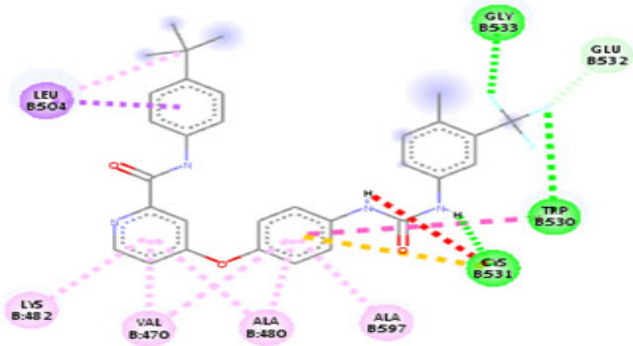

H-Bonds
Donor
Acceptor

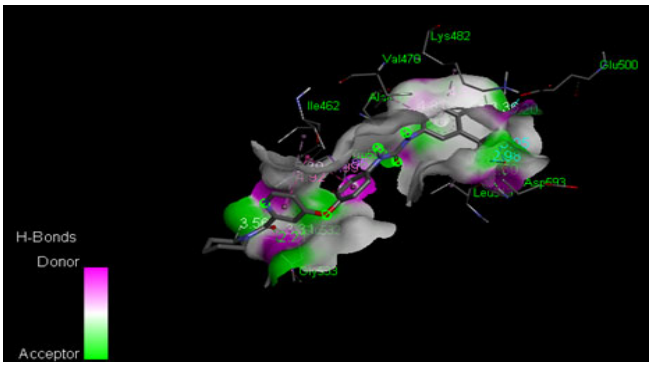
C3


HIS B538
THR B598
ALA B:480
LYS B:482
ASP B593
TRP B530
VAL B:470
ALA B597
GLU B500
ILE B:462
CYS B531
LEU B513

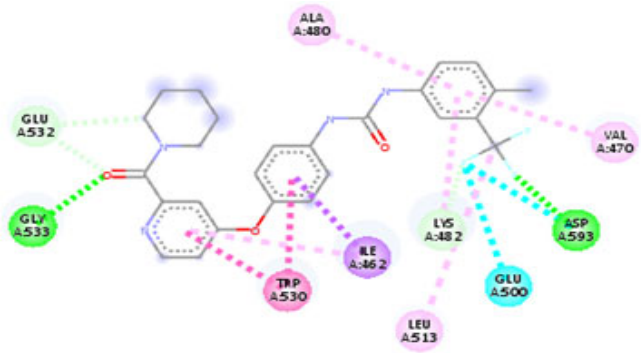

H-Bonds
Donor
Acceptor

C4


GLY B533
GLU B532
LEU B504
TRP B530
CYS B531
LYS B:482
VAL B:470
ALA B:480
ALA B597


H-Bonds
Donor
Acceptor

C5


ALA A:480
GLU A532
VAL A:470
GLY A533
LYS A:482
ASP A593
ILE A:462
TRP A530
GLU A500
LEU A513

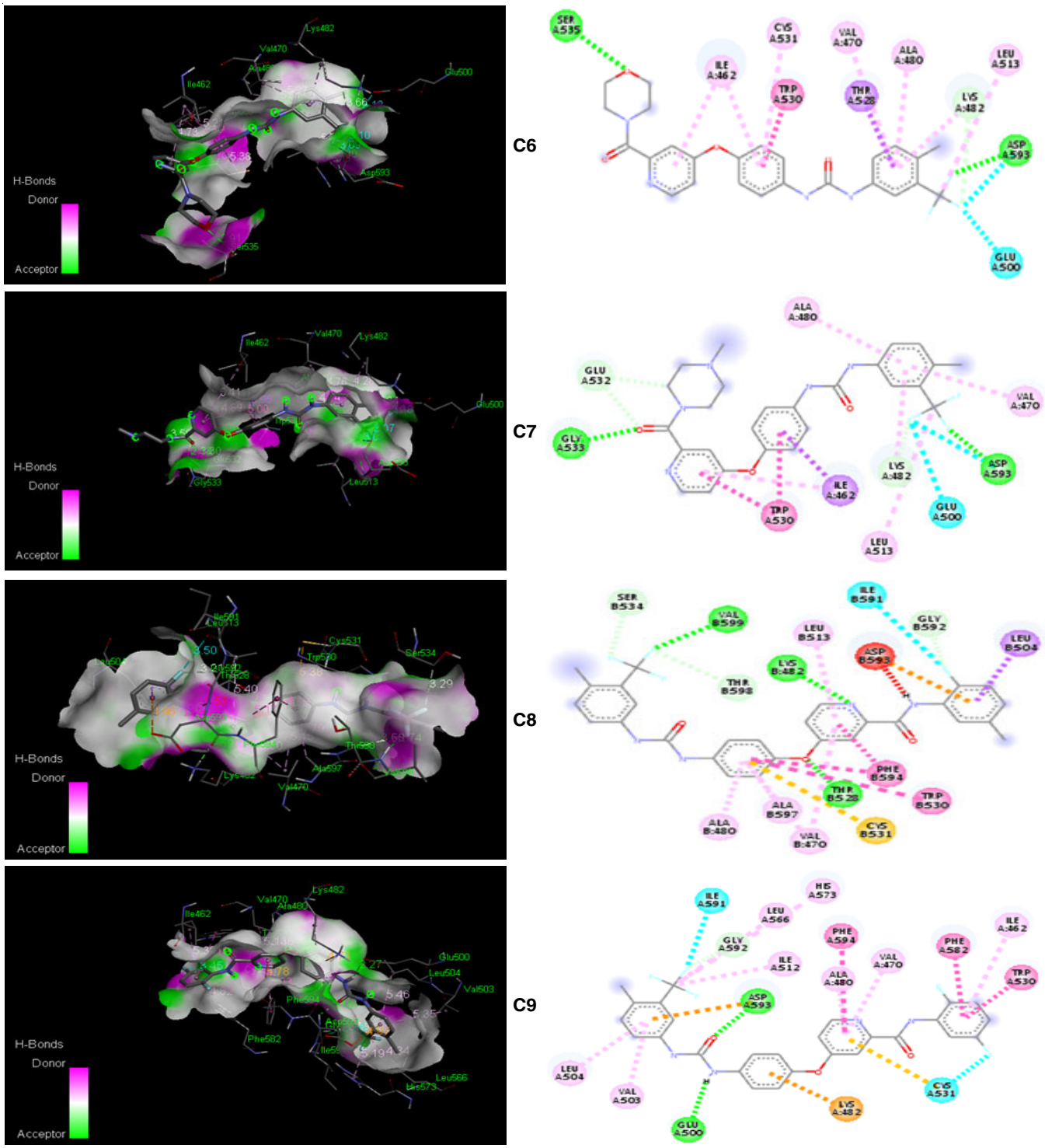


Fig. 1. 3D and 2D views of the binding site interactions of the synthesized diaryl urea derivatives (**C1-C9**)

TABLE-2
In vitro CYTOTOXICITY OF THE SYNTHESIZED COMPOUNDS AND DOXORUBICIN

Compound	IC_{50} μM (MCF-7)	Compound	IC_{50} μM (MCF-7)
C1	12.39	C6	13.15
C2	21.63	C7	21.83
C3	13.44	C8	25.12
C4	17.69	C9	11.48
C5	28.87	Doxorubicin	29.30

actions in AutoDock VINA tools. Total of nine derivatives have been synthesized by eco-friendly procedures. The chemical structures of the novel synthetic compounds were confirmed on the basis of spectral data. These have been evaluated for the anticancer activity screening tests. Some of these novel derivatives **C1**, **C3**, **C6** and **C9** exhibited better anticancer activity compared to that of the reference standard. Most of these diaryl urea derivatives have shown good to excellent anticancer activity and show significant binding energy using

autodock vina tools. Further, appropriate modifications of the compounds may show significant biological activities

CONFLICT OF INTEREST

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

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