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Synthesis, Anticancer Evaluation and Molecular Docking Studies of Isonicotinamide and Diaryl Urea Hybrid Motifs

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Asian Journal of Organic & Medicinal Chemistry

Volume: 7 Year: 2022
Issue: 2 Month: April–June
pp: 179–186
DOI: https://doi.org/10.14233/ajomc.2022.AJOMC-P382

Received: 16 March 2022
Accepted: 6 June 2022
Published: 29 June 2022

ABSTRACT

In search of new anticancer agents with improved efficacy, we designed and synthesized novel hybrid series of isonicotinamide and diaryl urea motifs (**R1-R9**). Design of series compounds carried out using docking study by Autodock vina tool. Binding energy (more than -9.7 kcal/mol) calculated using Autodock vina against Raf kinase (PDB: 4DBN). All the synthesized compounds were evaluated for them *in vitro* anticancer activity against MCF-7 cell line. The anticancer activities of the synthesized compounds were also carried. Some of the compounds (**R1**, **R8**, **R9**) showed better activities towards MCF-7 cell line by MTT assay.

KEYWORDS

Isonicotinamide, Diaryl urea, Molecular docking, Anticancer, MTT assay.

INTRODUCTION

The diaryl urea is an important fragment in medicinal chemistry due to its utility in synthesis of a variety of heterocyclic compounds with various biological activities, such as antithrombotic [1,2], antimalarial [3], antibacterial [4] and anti-inflammatory [5] characteristics. The creation and development of diaryl urea derivatives as a class of anticancer medicines has received a lot of attention in recent years [6,7]. Diaryl ureas are the backbone of various cancer treatments, including advanced, metastatic hepatocellular carcinoma [8], advanced renal cell carcinoma (RCC) [9], gastrointestinal stromal tumours (GISTs) [10] and metastatic colorectal cancer (mCRC) [11]. The ability to create H-bond interactions with pharmacological targets is its most notable property [12,13].

Sorafenib, regorafenib, linifanib, tivozanib and lenvatinib are some of the diaryl urea derivatives, which are now in clinical trials or have been utilized in clinical trials. *In vitro*, sorafenib is the first diaryl urea derivative to target the RAS-RAF-MEK-ERK signalling cascade in a variety of cancer cell lines [14].

Furthermore, diaryl urea-based derivatives have attracted attention due to their potent inhibitory activity against a variety of kinases, including Raf kinases [15], vascular endothelial growth factor receptor 2 (VEGFR-2) [16], platelet derived growth factor receptor (PDGF) [17], receptor tyrosine kinases (RTKs)

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[18] and aurora kinases [19]. In fact, the diarylurea moiety is found in several type II kinase inhibitors. These chemicals avoid kinases that are inactive, or DFG-out, by occupying a hydrophobic pocket close to the kinase [20].

EXPERIMENTAL

Avra synthesis, Finar and Spectrochem provided the synthetic chemicals and solvents for the amalgamation. The HEPES [(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), MEM-non-essential amino acid solution (100X), fetal bovine serum (FBS), antibiotic-antimycotic solution, sodium pyruvate, cell culture grade dimethyl sulfoxide (DMSO) and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (USA). Thin-layer chromatography was performed using pre-coated silica gel G60 F_{254} (0.2 mm, Merck) plates. UV light (254 and 365 nm) or iodine vapour were used to visualize the results. Using FTIR-8400 (Shimadzu) and the ATR method, an extensive examination of mixed chemicals was completed. The ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded using DMSO-d_6 as solvent and TMS as the internal reference on the Bruker AVANCE II Spectrometer. A Jeol-JMSD 300 mass spectrometer was used to record mass spectra at 70 eV.

Docking studies

Preparation of ligands: Synthesized compounds were used to collect the nine chemical components of diaryl urea derivatives. ChemDraw Ultra 2008 was used to draw the ligands' two-dimensional (2D) chemical structures and Chem3D Ultra was used to perform energy minimizations on the created ligands, which were saved in PDB format [21,22].

Docking method validation and target preparation: Three-dimensional structure of the protein was derived from the Protein Databank (PDB ID: 4DBN). The docking investigation began with the identification of a binding site, which is a limited protein area in general. PyMOL was used to visualize the size and area of this limiting site. AutoDock Vina was used to confirm the protein target [21,22].

Analysis of molecular docking: Binding mode and interaction of 4DBN with individual synthesized compounds were performed using AutoDock Vina software. Docking was performed to acquire a populace of potential compliances and directions for the ligand at the limiting site. The protein was stacked in PyRx programming, making a PDBQT record that contains a protein structure with hydrogens in every polar buildup (Fig. 1). All obligations of ligands were set to be rotatable. All computations for protein-fixed ligand-adaptable docking were finished utilizing the Lamarckian Genetic Algorithm (LGA) technique. The docking site on the protein target was characterized by laying out a lattice box with the aspects of X: 40, Y: 40, Z: 40, Å and centred on X: 35.251, Y: -27.003, Z: 5.157 with exhaustiveness of 8. The best conformation was chosen with the lowest docked energy after the docking search was completed. Nine runs with AutoDock Vina were performed in all cases per each ligand structure and for each run, the best pose was saved. The average affinity for best poses was taken as the final affinity value. The interactions of complex protein-ligand conformations, including hydrogen bonds and the bond lengths, were analyzed using the Discovery Studio Visualizer [21,22].

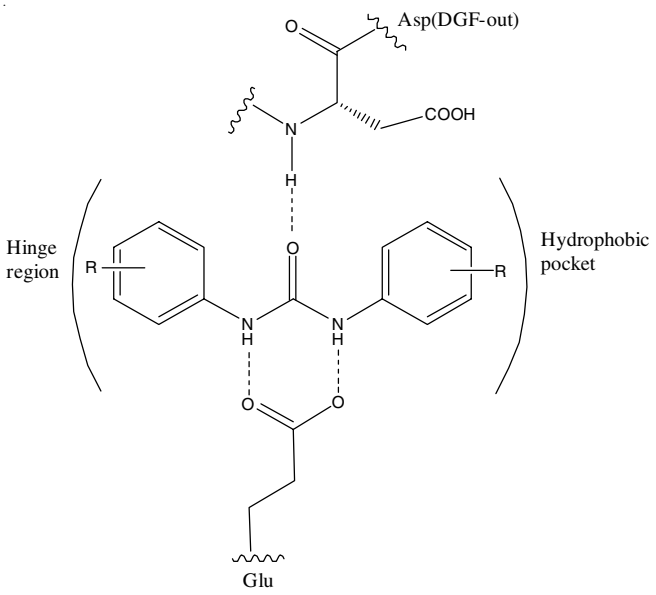


Fig. 1. Diarylureas derivatives forms hydrogen bonds with the type II kinase receptor

General procedure of 2-chloropyridine-4-carbonyl chloride (step-1): In methylene dichloride, 2-chloropyridine-4-carboxylic acid (0.08 mol) and 2-3 drops DMF are added. Thionyl chloride (0.085 mol) was added. On completion of reaction excess, thionyl chloride was removed by distillation under reduced pressure after cooling to room temperature and the resulting material was used immediately in the next stage.

2-Chloro pyridine-4-carboxamide derivatives (step-2): Triethylamine (0.0568 mol) was added to 2-chloropyridine-4-carbonyl chloride (0.0284 mol) in THF (50 mL) at 0 ºC. The amine (0.031 mol) solution in THF (25 mL) was added to the reaction mixture at a pace that kept the internal temperature below 5 ºC. The following mixture was kept at room temperature for 5 h before being concentrated under decreased pressure. To achieve step-2 in **R1-R9**, the mixture was first diluted with water, then extracted with ethyl acetate, dried over anhydrous sodium sulphate and concentrated under reduced pressure. All intermediates were confirmed by mass spectrometry and employed without purification for the next step.

2-(4-Aminophenoxy)pyridine-4-carboxamide derivatives (step-3): The reddish-brown mixture was added to a solution of 4-aminophenol (0.0183 mol) in anhydrous DMF (15 mL), which had been treated with potassium *tert*-butoxide (0.0366 mol) and agitated at room temperature for 2 h. The contents were warmed at 80 ºC for 8 h after being treated with 2-chloropyridine-4-carboxamide derivatives (0.0183 mol) and K_2CO_3 (0.009 mol). Between ethyl acetic acid derivation and water, the mixture was cooled to room temperature and separated. The natural layers were united, then washed in an immersed NaCl solution, dried over Na_2SO_4 and concentrated under reduced tension. The solids were then dried at 35 ºC for 3 h under reduced tension to get 4-(4-aminophenoxy)-pyridine-2-carboxamide derivatives (**R1-R9**).

Diaryl urea derivatives (step-4) (R1-R9): Carbonyl diimidazole (CDI) was added to 4-chloro-3-(trifluoromethyl)-aniline (0.005 mol) in anhydrous THF at 0 ºC. Reaction mixture was stirred for 1 h at room temperature and 2-(4-aminophenoxy)-

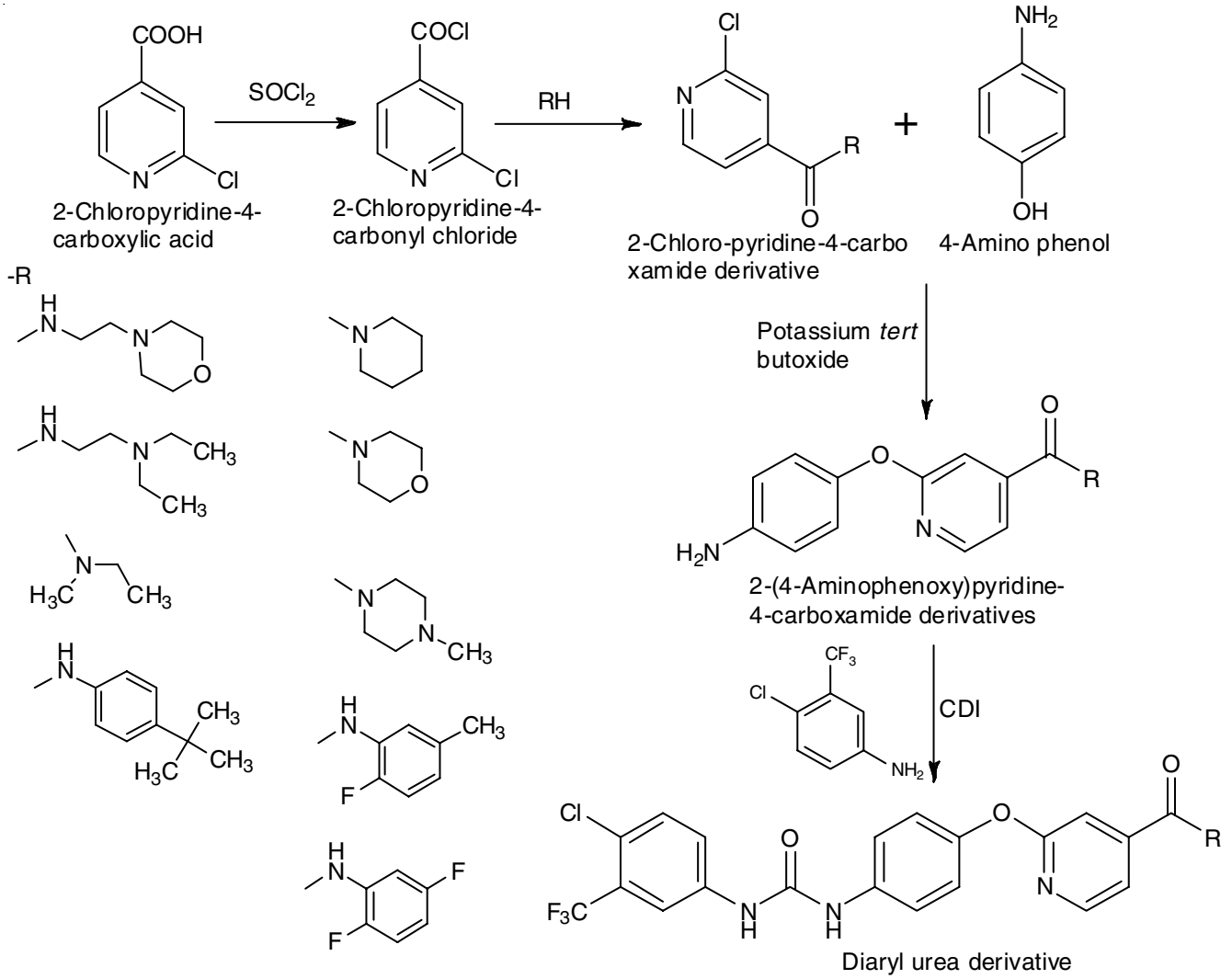
pyridine-2-carboxamide derivative (0.005 mol) (step-3) was added and further stirred for 16 h at room temperature. After completion of reaction, reaction mass was concentrated under vacuum then added water and ethyl acetate. Ethyl acetate layer was separated and dried over sodium sulphate. Concentrated the organic layer under vacuum and resulting crude material was purified by column chromatography using ethyl acetate and hexane as mobile phase. Product eluted in 30-50% ethyl acetate in hexane (**Scheme-I**).

2-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-*N*-(2-morpholinoethyl)isonicotinamide (R1): Yield: 53.50%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.35-2.37 (t, 4H, CH_2), 2.51-2.54 (t, 2H, CH_2), 3.34-3.36 (t, 2H, CH_2), 3.64-3.67 (t, 4H, CH_2), 7.35-7.36 (d, 2H, ArH), 7.63-7.64 (d, 1H, ArH), 7.74-7.76 (d, 1H, ArH), 7.89-7.91 (m, 3H, ArH), 8.05 (s, 2H, ArH and amide), 8.27 (s, 1H, ArH), 8.74-8.76 (d, 1H, ArH), 9.12 (s, 1H, CONH), 9.25 (s, 1H, CONH), ^{13}C NMR (DMSO-d_6) δ ppm: 37.7, 54.0, 55.6, 66.6, 103.2, 116.0, 118.5, 119.4(X2), 122.6(X2), 123.3, 128.4, 129.1, 129.3, 129.6, 134.3, 135.5, 147.2, 148.3, 150.6, 152.9 (C=O), 164.2, 167.0 (C=O), Mass (LC-MS): *m/z*: 564.2[M+H]$^+$, 566.2[M+2]$^+$.

2-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-*N*-(2-(diethylamino)ethyl)isonicotinamide (R2): Yield: 56.30%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.02-1.04 (t, 6H, CH_3), 2.45-2.53 (m, 6H, CH_2), 3.34-3.36 (t, 2H, CH_2), 6.63-6.65 (d, 1H, ArH), 6.80-6.82 (d, 2H, ArH), 7.26 (s, 1H, ArH), 7.44-7.46 (d, 2H, ArH), 7.74-7.76 (d, 1H, ArH), 7.90-7.92 (d, 1H, ArH), 7.96-7.78 (d, 1H, ArH), 8.06 (s, 2H, ArH and amide) 9.11 (s, 1H, CONH), 9.25 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 13.3, 37.7, 49.6, 53.7, 103.1, 116.1, 118.7, 119.5(X2), 122.6(X2), 123.3, 128.5, 129.1, 129.4, 129.5, 134.3, 135.5, 147.2, 148.2, 150.8, 153.0 (C=O), 164.1, 167.3 (C=O), Mass (LC-MS): *m/z*: 550.5[M+H]$^+$, 552.4[M+2]$^+$.

2-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-*N*-ethyl-*N*-methyl isonicotinamide (R3): Yield: 60.0%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.34-1.36 (t, 3H, CH_3), 3.46 (s, 3H, CH_3), 3.74-3.76 (t, 2H, CH_2), 6.62-6.64 (d, 1H, ArH), 6.80-6.82 (d, 2H, ArH), 7.27 (s, 1H, ArH), 7.44-7.46 (d, 2H, ArH), 7.74-7.76 (d, 1H, ArH), 7.90-7.92 (d, 1H, ArH), 7.96-7.98 (d, 1H, ArH), 8.05 (s, 1H, ArH), 9.11 (s, 1H, CONH), 9.24 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 12.5, 36.2, 46.5, 103.3, 116.2, 118.9, 119.5(X2), 122.6(X2), 123.4, 128.6, 129.2, 129.4, 129.5, 134.3, 135.5, 147.2, 148.2, 150.8, 153.0 (C=O), 164.1, 172.0 (C=O), Mass (LC-MS): *m/z*: 493.2[M+H]$^+$, 495.1[M+2]$^+$.

***N*-(4-(*tert*-Butyl)phenyl)-2-(4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenoxy)isonicotinamide (R4):** Yield: 53.5%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.36 (s,



Scheme-I: Synthesis of diaryl urea derivatives

9H, CH_3), 6.64-6.66 (d, 1H, ArH), 6.81-6.83 (d, 2H, ArH), 7.24-7.26 (d, 2H, ArH), 7.28 (s, 1H, ArH), 7.45-7.47 (d, 2H, ArH), 7.61-7.63 (d, 2H, ArH), 7.74-7.76 (d, 1H, ArH), 7.91-7.93 (d, 1H, ArH), 7.96-7.78 (d, 1H, ArH), 8.05 (s, 1H, ArH) 9.11 (s, 1H, CONH), 9.15 (s, 1H, CONH), 9.24 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 31.3, 34.3, 103.3, 116.2, 118.9, 119.5(X2), 121.2 (X2), 122.6(X2), 123.4, 127.9(X2), 128.6, 129.2, 129.4, 129.5, 134.3, 134.8, 135.5, 146.9, 147.2, 148.2, 150.8, 153.0 (C=O), 164.1, 164.8 (C=O). Mass (LC-MS): *m/z*: 583.3 $[M+H]^+$, 585.2 $[M+2]^+$.

1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-((4-(piperidine-1-carbonyl)pyridin-2-yl)oxy)phenyl)urea (R5): Yield: 62.20%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.50-1.53 (m, 4H, piperidine ring), 1.57-1.60 (t, 2H, piperdine ring), 3.76-3.78 (t, 4H, piperidine ring), 6.63-6.65 (d, 1H, ArH), 6.80-6.82 (d, 2H, ArH), 7.28 (s, 1H, ArH), 7.45-7.47 (d, 2H, ArH), 7.74-7.76 (d, 1H, ArH), 7.90-7.92 (d, 1H, ArH), 7.96-7.98 (d, 1H, ArH), 8.06 (s, 1H, ArH) 9.12 (s, 1H, CONH), 9.25 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 24.2, 25.4, 47.7, 103.3, 116.2, 118.9, 119.5(X2), 122.7(X2), 123.3, 128.5, 129.2, 129.4, 129.5, 134.4, 135.6, 147.3, 148.4, 150.8, 153.1 (C=O), 164.1, 172.4 (C=O) Mass (LC-MS): *m/z*: 519.1 $[M+H]^+$, 521.1 $[M+2]^+$.

1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-((4-(morpholine-4-carbonyl)pyridin-2-yl)oxy)phenyl)urea (R6): Yield: 52.80%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.50-3.52 (t, 2H, morpholine ring), 3.63-3.65 (t, 2H, morpholine ring), 6.64-6.66 (d, 1H, ArH), 6.80-6.82 (d, 2H, ArH), 7.28 (s, 1H, ArH), 7.46-7.48 (d, 2H, ArH), 7.75-7.77 (d, 1H, ArH), 7.90-7.92 (d, 1H, ArH), 7.96-7.98 (d, 1H, ArH), 8.06 (s, 1H, ArH) 9.12 (s, 1H, CONH), 9.24 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 46.5, 66.2, 103.1, 116.1, 118.8, 119.4(X2), 122.6(X2), 123.3, 128.5, 129.1, 129.3, 129.5, 134.3, 135.3, 147.2, 148.2, 150.8, 152.9 (C=O), 164.1, 168.9 (C=O) Mass (LC-MS): *m/z*: 521.1 $[M+H]^+$, 523.0$[M+2]^+$.

1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-((4-(4-methylpiperazine-1-carbonyl)pyridin-2-yl)oxy)phenyl)-urea (R7): Yield: 57.50%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.23 (s, 3H, CH_3), 2.27 (t, 4H, piperazine ring), 3.19-3.21 (t, 4H, piperazine ring), 6.63-6.65 (d, 1H, ArH), 6.80-6.82 (d, 2H, ArH), 7.28 (s, 1H, ArH), 7.46-7.49 (d, 2H, ArH), 7.75-7.78 (d, 1H, ArH), 7.90-7.92 (d, 1H, ArH), 7.95-7.98 (d, 1H, ArH), 8.06 (s, 1H, ArH) 9.10 (s, 1H, CONH), 9.23 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 46.6, 50.1, 51.5, 103.1, 116.1, 118.8, 119.4 (X2), 122.6 (X2), 123.3, 126.6, 128.5, 129.1, 129.3, 129.5, 134.3, 135.3, 147.2, 148.2, 150.8, 152.9 (C=O), 164.1, 168.9 (C=O) Mass (LC-MS): *m/z*: 534.3 $[M+H]^+$, 536.3 $[M+2]^+$.

2-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-*N*-(2-fluoro-5-methyl phenyl)isonicotinamide (R8): Yield: 49.25%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.34 (s, 3H, CH_3), 6.64-6.66 (d, 1H, ArH), 6.81-6.83 (d, 2H, ArH), 6.94-6.95 (d, 1H, ArH), 7.09-7.11 (d, 1H, ArH), 7.28 (s, 1H, ArH), 7.45-7.47 (d, 2H, ArH), 7.70 (s, 1H, ArH), 7.74-7.76 (d, 1H, ArH), 7.91-7.93 (d, 1H, ArH), 7.96-7.78 (d, 1H, ArH), 8.05 (s, 1H, ArH) 9.11 (s, 1H, CONH), 9.15 (s, 1H, CONH), 9.24 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 21.3, 103.1, 113.2, 116.1, 118.8, 119.0, 119.4 (X2), 121.6, 122.6 (X2), 122.9, 123.3, 128.5, 129.1, 129.3, 129.5, 134.2, 134.3, 135.3, 147.2, 148.2, 150.8, 152.9 (C=O), 155.3, 164.1, 164.7 (C=O). Mass (LC-MS): *m/z*: 559.2 $[M+H]^+$, 561.2 $[M+2]^+$.

2-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenoxy)-N-(2,5-difluorophenyl)isonicotinamide (R9): Yield: 48.50%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 6.64-6.66 (d, 1H, ArH), 6.81-6.83 (d, 2H, ArH), 6.95-6.97 (d, 1H, ArH), 7.28 (s, 1H, ArH), 7.35-7.37 (d, 1H, ArH), 7.45-7.47 (d, 2H, ArH), 7.73 (s, 1H, ArH), 7.74-7.76 (d, 1H, ArH), 7.91-7.93 (d, 1H, ArH), 7.96-7.78 (d, 1H, ArH), 8.05 (s, 1H, ArH) 9.11 (s, 1H, CONH), 9.15(s, 1H, CONH), 9.24 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ ppm: 103.1, 111.7, 112.7, 113.3, 116.1, 118.8, 119.4 (X2), 120.7, 122.6 (X2), 123.3, 128.5, 129.1, 129.3, 129.5, 134.3, 135.3, 147.1, 148.2, 150.8, 152.9 (C=O), 153.9, 158.7, 164.1, 164.7 (C=O). Mass (LC-MS): *m/z*: 563.1 $[M+H]^+$, 564.1 $[M+2]^+$.

Anticancer activity

Cell lines and cell cultures: The National Center for Cell Science (NCCS), Pune, India, provided a human bosom adenocarcinoma (MCF-7) cell line. MCF-7 cells were grown in MEM media with 10% FBS (foetal bovine serum), 1% non-essential amino acids and 0.5 mL antibiotic-antimycotic solution (100X) (10,000 units/mL penicillin, 10,000 g/mL streptomycin and 25 g/mL Gibco Amphotericin B). The cell lines were kept sterile at 37 ºC with 5% CO_2 and 95% air, with weekly sub-cultures using 0.02% EDTA and 0.05% trypsin. After ingesting a high rate of nutritious agents in the cell culture medium or utilizing the complete surface on which they can reproduce, cells multiplying in cultures gradually lose their proliferation rate and cell growths slow down.

Culture growth: The synthesized compounds were dissolved in cell culture grade DMSO at a stock concentration of 100 mM. For 24 h, exponentially developing MCF-7 cells were treated with several compounds (100 M). As a vehicle control, cells were treated with 0.1% DMSO.

Assay for cell proliferation: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to measure cell growth [23-25]. The MCF-7 cells (2×10^4 M) were treated for 24 h with a series of synthesized compounds. After that, the cells were rinsed in DPBS and incubated in the dark at 37 ºC for 4 h with MTT (0.5 mg/mL). MTT was removed after the incubation period and DMSO was applied to each well. Using a Multimode micro-plate reader, the absorbance was measured at 570 nm with a reference wavelength of 650 nm (SpectraMax M2e, Molecular Devices, USA).

RESULTS AND DISCUSSION

Molecular docking study: The AutoDock Vina programme was used to conduct the docking investigation. This tool was created to anticipate how tiny compounds will bind to a known 3D structure's receptor. Docking of nine compounds was done in attempt to uncover new potential cancer-treating molecules. For each ligand, AutoDoc Vina, which is used in the PyRx tool, created nine alternatives conformations, which were categorized by binding affinity (kcal/mol). Table-1 shows synthesized compounds with binding free energies ranging from -9.7 to -13.5 kcal/mol. Table-1 shows that the binding free

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

Compound code	Autodock Vina binding energy (kcal/mol)	Amino acid interactions
R1	-11.6	Phe594A, Lys482A, Leu513A, Asp593A, Glu500A, Ala480A, Asn599A
R2	-9.7	Ser534A, lle462A, Phe582A, Phe594A, Trp530A
R3	-11.7	Phe594A, Lys482A, Leu513A, Ala480A, Asp593A
R4	-13.5	Ala597B, Phe582B, Ala480B, Phe594B, Leu513B, Asp593B, Lys482B, Glu500B
R5	-10.6	Trp530A, Phe582A, lle462A
R6	-10.4	Trp530A, Phe582A, lle462A
R7	-11.6	Trp530B, Phe582B, Val599B, lle462B, Lys482B
R8	-13.0	Phe582A, Leu513A, Phe594A, Ala480A, Lya482A, Glu500A, Asp593A
R9	-12.7	Phe582A, Lys482A, Leu513A, Phe594A, Ala480A, Asp593A, Glu500A
Sorafenib	-8.5	Asp554B, Asp478A, Val510B

energy of all the synthesized compounds is greater than **R2** (-9.7 kcal/mol). Similarly, molecular docking investigations revealed that synthesized compounds **R4** (-13.5 kcal/mol) and **R8** (-13.0 kcal/mol) have a high binding affinity score for the spike protein. Figs. 2 and 3 depict 2D and 3D molecular interactions of all the synthesized compounds, including the traditional hydrogen link, carbon hydrogen bond, halogen interaction, pi-cation, pi-sulfur, pi-pi stacked, pi-pi T-shaped, alkyl and pi-alkyl interactions.

The synthetic scheme for the diaryl urea derivatives is shown in **Scheme-I**. 2-Chloro pyridine-4-carboxylic acid was reacted with thionyl chloride and to get acid chloride (step-1), which was then reacted with different amines in presence of triethylamine base. In step-2, derivatives were reacted with 4-amino phenol in the presence of potassium tertiary butoxide to get carboxamide derivatives. Then, in step-4, 4-chloro-3-(trifluoromethyl)aniline interacted with CDI to get the diaryl urea derivatives (**R1-R9**). All intermediates were confirmed by mass spectrometry and employed without purification in the next step. With a yield of 48% to 60% obtained after all of the final compounds were purified using column chromatography. The synthesized compounds **R1-R9** was confirmed through Mass, ^{1}H NMR and ^{13}C NMR data.

Assay for cell proliferation: Table-2 compares the *in vitro* cytotoxicity of the synthesized compounds to that of the reference standard Sorafenib. Among the series of compounds **R1**, **R8** and **R9** exhibited better anticancer activity compared to that of the reference standard. Most of these pyridine based diaryl urea derivatives have shown good to excellent anticancer activity.

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

Compound	IC_{50} μM (MCF-7)	Compound	IC_{50} μM (MCF-7)
R1	12.1	**R6**	18.1
R2	23.6	**R7**	21.7
R3	14.6	**R8**	13.3
R4	13.9	**R9**	12.4
R5	25.9	Sorafenib	21.8

Conclusion

In this study, a structure-based virtual screening (SBVS) was applied on the high similar sorafenib approved drug and selected from literature. SBVS was performed by using AutoDock Vina tools. Nine compounds show strong and stable interactions in AutoDock Vina tools. **R4**, **R8** and **R9** compounds shown better binding energy among all derivatives. 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 physical and spectral data. Further, all the synthesized compounds **R1-R9** have been subjected to *in vitro* assay anticancer, applications. Among the series of compounds **R1**, **R8** and **R9** exhibited better anticancer activity compared to that of the reference standard. Most of these pyridine based diaryl urea derivatives have shown good to excellent anticancer activity and also shows significant binding energy using AutoDock Vina tools. Further, appropriate modifications of the compounds may show significant biological activities.

ACKNOWLEDGEMENTS

This research was supported by Sankalchand Patel University, Visnagar, India.

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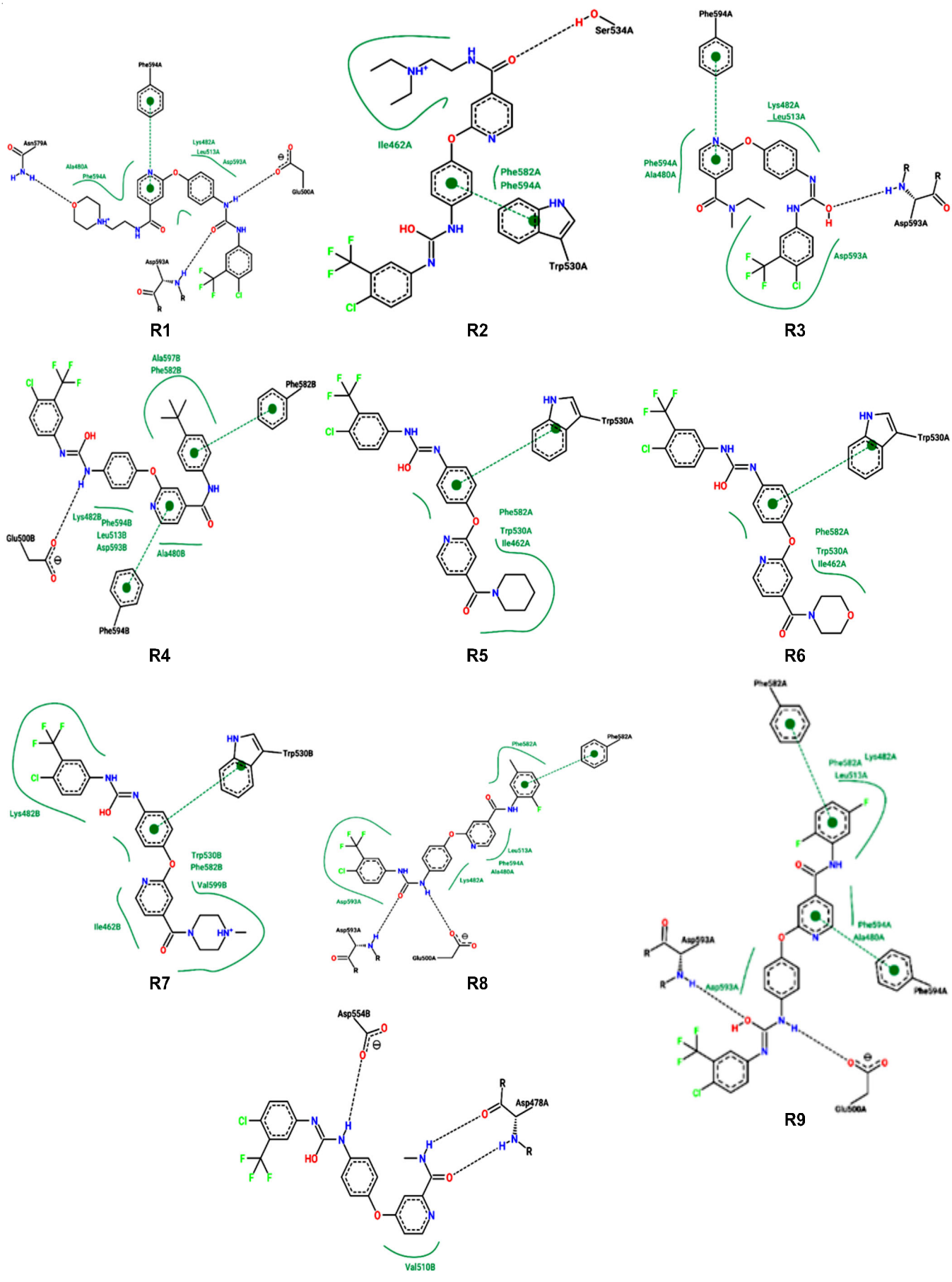

Fig. 2. 2D views of the binding site interactions of sorafenib all synthetic compounds **R1-R9** and sorafenib

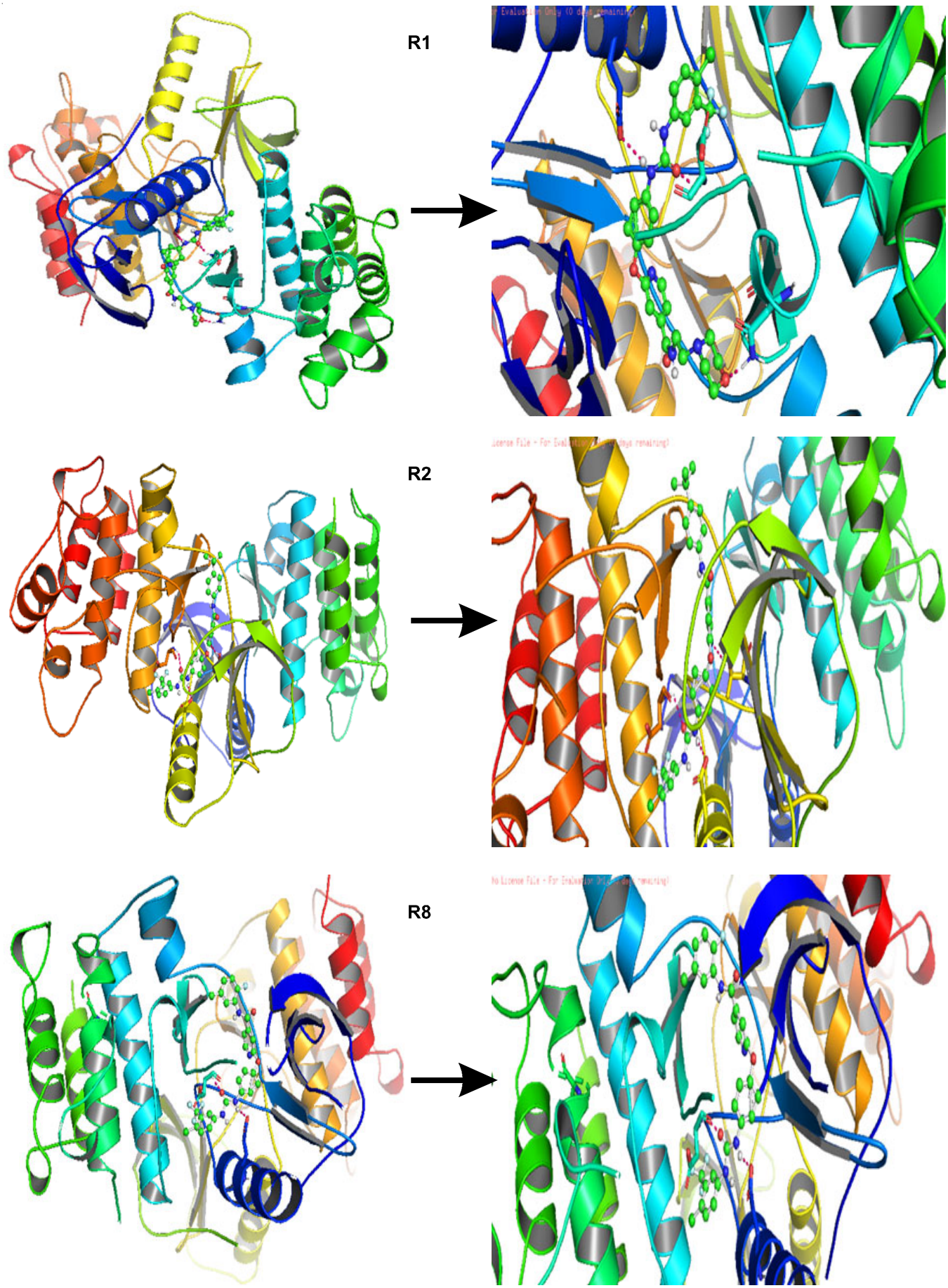

Fig. 3. 3D views of the binding site interactions of all synthetic compounds **R1**, **R4**, **R8** (dotted line indicate hydrogen bonding)

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