How to Cite:
Marvaniya, V., Joshi, H. V., Shah, U. A., Patel, J. K., & Patel, J. R. (2022). Docking, synthesis and biological evaluation of pyridine ring containing Diaryl urea derivatives as anticancer agents. *International Journal of Health Sciences*, *6*(S3), 2851–2865. https://doi.org/10.53730/ijhs.v6nS3.6200

Docking, synthesis and biological evaluation of pyridine ring containing Diaryl urea derivatives as anticancer agents

Vanita Marvaniya
Research Scholar, Sankalchand Patel University, Visnagar, Gujarat, India

Hirak V. Joshi
Faculty of Pharmacy, Sankalchand Patel University, Visnagar, Gujarat, India

Ujashkumar A. Shah
Faculty of Pharmacy, Sankalchand Patel University, Visnagar, Gujarat, India

Jayvadan K. Patel
Faculty of Pharmacy, Sankalchand Patel University, Visnagar, Gujarat, India

Jimish R. Patel
Department of Pharmaceutical Chemistry, Shri B M Shah College of Pharmaceutical Education and Research, College Campus, Dhansura Road, Modasa-383315, Gujarat, India

***Abstract*---**A novel series of pyridine ring containing diaryl urea derivatives (R1-R9) were synthesized in four chemical steps using pyridine-2-carboxylic acid as starting material. The synthesized compounds were design by using Autodock vina in the crystal structure of the Kinase domain of Human B-raf (PDB ID: 4DBN) to get insights into structural requirements for anticancer activity. In vitro anticancer activity against cell line (MCF-7) showed that compounds R3, R6 and R9 were found to be the most potent (Docking score: > -12, IC50 = 17.39 µM) among the synthesized molecules.

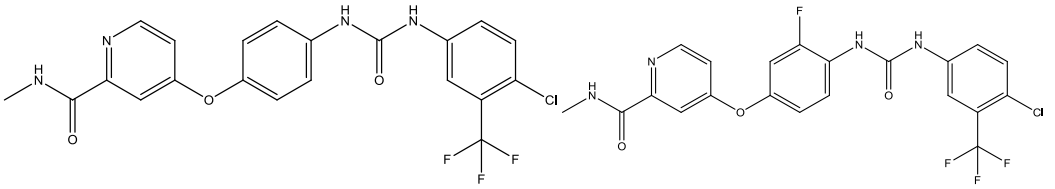
***Keywords*---**pyridine, diaryl urea, molecular docking, anticancer, MTT assay.

Introduction

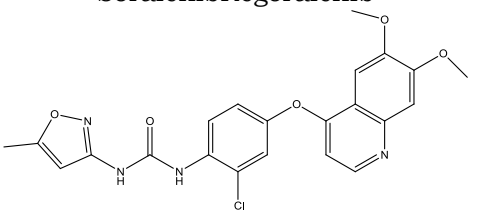
In medicinal chemistry, heterocycles are the most important pharmacophores and researchers are working to create efficient synthetic methods for obtaining

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
***Corresponding author**: Marvaniya, V.*
Manuscript submitted: 18 Dec 2021, Manuscript revised: 27 March 2022, Accepted for publication: 09 April 2022

pharmaceutically active heterocycles. Pyridines are heterocyclic compounds with six members that are frequently employed in medicinal chemistry, natural products and functional materials. Pyridine scaffolds are appealing for medication design and development. Pyridine is another major nitrogen-containing heterocycle that has gotten a lot of attention from medicinal chemists because of its wide range of uses in drug development.[1-4] A number of substituted pyridine containing diaryl urea derivatives have recently been produced and their anticancer properties described.[5-11]Several pyridine-based diaryl urea-containing small compounds, to name a few, have been approved as anticancer drugs: Sorafenib[12-15]



SorafenibRegorafenib



Tivozanib

Fig 1. Pyridine containing diaryl urea agents

As part of our work on the synthesis and biological effects of new nitrogen heterocyclic compounds. The production and in vitro anticancer activity of pyridine diaryl urea derivatives are described here. Diaryl urea functionality is the main pharmacophoric feature in several anticancer drugs, including Sorafenib and Regorafenib, Tivozanib (Figure 1), and Pyridine Diaryl Urea Derivative, which has selective inhibitory activity against RAF kinase and antiproliferative effects, particularly in human colorectal cancer cell lines MCF-7.

Experimental

Materials and Methods

The required chemicals and solvents for the synthesis were purchased from Avra Synthesis, Finar, and Spectrochem. All the chemicals were used without further purification.Precoated plates of silica gel G60 F254 (0.2 mm, Mfg. by Merck) were used for thin-layer chromatography.Melting points have been determined by using digital melting point apparatus.Visualization was made under UV light (254 and 365nm) or with iodine vapour. Spectral analysis of the synthesized compound was done with the help of FTIR-8400 (Shimadzu) using the ATR technique. The 1H NMR (400 MHz) and 13C NMR (101 MHz) spectra were recorded on the "Bruker AVANCE II Spectrometer" using DMSO-d6 as solvent and TMS as the internal reference. Mass spectra were recorded on a Jeol-JMSD 300 mass spectrometer at 70ev.

Docking study[16-19]
Ligands preparation

The nine chemical constituents of diaryl urea derivatives were collected from synthesized compounds. The ligands' two-dimensional (2D) chemical structures were sketched using ChemDraw Ultra 2008, and the energy minimizations of the prepared ligands were carried out with Chem3D Ultra and were saved in PDB format.

Target preparation and validation of docking method

The protein's three-dimensional structure was obtained from the Protein databank (PDB ID: 4DBN). The docking study started with the definition of a binding site, in general, a restricted protein region. The size and area of this limiting site were visualized in PyMOL. The protein target was further validated with AutoDock Vina.

Molecular docking analysis

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 build-up. 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 centredon 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 analysed using the Discovery studio visualizer.

Synthetic methodology[20-24]
General procedure for 4-Chloropyridine-2-carbonyl chloride (Step-I)

Pyridine 2-carboxylic acid (0.081mol) and sodium bromide (0.013mol) are suspended inchlorobenzene. After heating to 50℃, thionyl chloride (0.40mol) was added to such a degree. Thereaction mixture was subsequently heated to 85℃ and stirred for 20 h. After cooling at roomtemperature, the majority of the chlorobenzene and excess thionyl chloride was removed bydistillation under reduced pressure and obtain material was used directly in the next stage.

General procedure for 4-Chloro-pyridine-2-carboxamide derivatives (Step-II)

A solution of 4-Chloropyridine-2-carbonyl chloride(0.0284mol) was mixed in THF (50 mL) at 0° C and triethylamine (0.0568mol). Resulting reaction mixture was

treated with amine (0.031mol) solution in THF (25 ml) at a rate which kept the internal temperature below 5° C. 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 sulphate and concentrated under reduced pressure to obtain step-II product. All intermediated confirmed by mass spectroscopy and used in next step without purification.

4-(4-Aminothiophenoxy)-pyridine-2-carboxamide derivatives (Step-III)

A solution of 4-Aminothiophenol (0.0183 mol) in anhydrous DMF (15 mL) was treated with potassium tert-butoxide (0.0366 mol), and the reddish-brown mixture was added, stirred at room temperature for 2 h. The contents were treated with 4-Chloro-pyridine-2-carboxamide derivatives (0.0183 mol) and K_2CO_3 (0.009 mol) and then heated at 80° C. for 8 h. The mixture was cooled to room temp. and separated between ethyl acetate and water. The combine 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° C. for 3 h to obtain 4-(4-Aminothiophenoxy)-pyridine-2-carboxamide derivatives as solid. All intermediated confirmed by mass spectroscopy and used in next step without purification.

General procedure for Diaryl urea derivatives (R1-R9) (Step-IV)

To the solution of 4-Chloro-3-(trifluoromethyl) aniline (0.005 mol) in anhydrous a Dichloromethane at 0° C. was added CDI (0.0052mol). The resulting solution was allowed to warm to room temp. over 1 h, was stirred at room temp. for 16 h, then was treated with 4-(4-Aminothiophenoxy)-pyridine-2-carboxamide derivatives (Step-III, 0.005mol). The resulting yellow solution was stirred at room temp. for 72 h, then was treated with water. The resulting aqueous mixture was extracted with ethyl acetate. The combined organics were dried over sodium sulphate and concentrate under reduced pressure. The residual oil purified by column chromatography using ethyl acetate and n-hexane as mobile phase to obtained diaryl urea derivatives as solid.

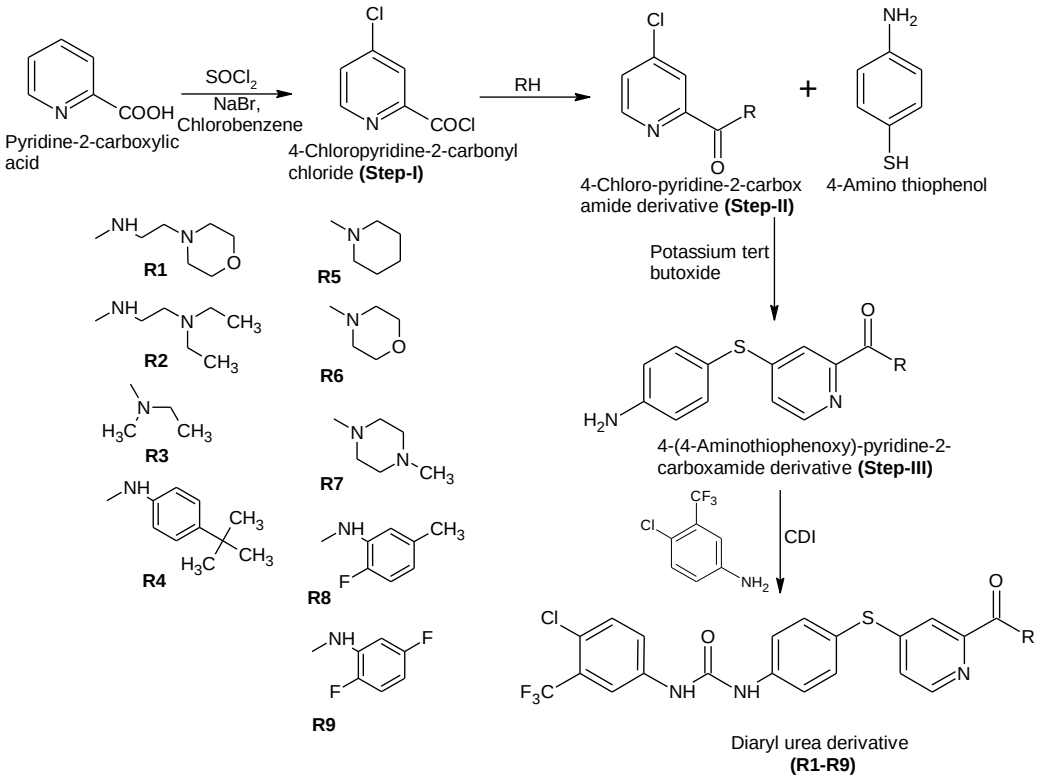


Fig 2. Reaction scheme

4-((4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl)thio)-N-(2-morpholinoethyl)picolinamide (R1)

Yield (%): 52.8, IR (KBr, Vmax, cm^{-1}): 3327 (NH str), 1724 (C=O str), 1648 (C=O str), 1338 (CF3), 1116 (Aliphatic ether), 804(C-Cl) 699 (C-S), ^{1}H NMR (400MHz, DMSO) δ ppm: 2.35-2.37 (t, 4H, CH2), 2.51-2.54 (t, 2H, CH2), 3.34-3.36 (t, 2H, CH2), 3.64-3.67 (t, 4H, CH2), 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), 9.10 (s, 1H, CONH), 9.24 (s, 1H, CONH), ^{13}C NMR (DMSO) δ ppm: 54, 55.8 (x2), 57.6, 66.7 (x2), 118.8, 120.5 (x2), 123.3, 124.9, 126.4 (x2), 128.5, 128.9, 129.1, 129.3, 129.5, 132.0, 134.3, 136.2, 140.5, 148.2, 151.9, 152.9, 160.7. Mass (LC-MS): m/z: 581.1$[M+H]^{+}$,583.2$[M+2]^{+}$

4-((4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl)thio)-N-(2-(diethylamino)ethyl)picolinamide (R2)

Yield (%): 48.6, IR (KBr, Vmax, cm^{-1}):3383 (NH str), 1703 (C=O str), 1656 (C=O str), 1312 (CF3), 1175 (C-N), 799 (C-Cl), 721 (C-S), ^{1}H NMR (400MHz, DMSO) δ ppm: 1.05-1.07 (t, 6H, CH3), 2.46-2.53 (m, 6H, CH2), 3.35-3.37 (t, 2H, CH2), 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), 8.26 (s, 1H, ArH), 8.73-8.76 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.24 (s, 1H, CONH),^{13}C NMR (DMSO) δ ppm: 13.3 (x2), 49.6 (x2), 57.6, 120.5 (x2), 123.3, 124.9, 126.4 (x2), 128.5, 128.9, 129.1, 129.3,

129.5, 132, 134.3, 136.2, 140.5, 148.2, 151.9, 152.9, 160.7.Mass (LC-MS): m/z: 567.2[M+H]$^+$, 569.1[M+2]$^+$

4-((4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl)thio)-N-ethyl-N-methylpicolinamide (R3)

Yield (%):50.8,IR (KBr, Vmax, cm^{-1}):3422 (NH str), 1719 (C=O str), 1593 (C=O str), 1311 (CF3), 1174 (C-N), 823, (C-Cl), 662 (C-S), ^{1}H NMR (400MHz, DMSO) δ ppm: 1.34-1.37 (t, 3H, methyl), 3.47 (s, 3H, methyl), 3.75-3.85 (m, 2H, 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, 1H, ArH), 8.26 (s, 1H, ArH), 8.74-8.77 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.25 (s, 1H, CONH),^{13}C NMR (DMSO) δ ppm: 12.5, 35.8, 46.1, 118.8, 120.5 (x2), 123.3, 124.9, 126.4(x2), 128.5, 128.9, 129.1, 129.3, 129.5, 132, 134.3, 136.2, 140.5, 148.2, 151.9, 152.9, 162.0.Mass (LC-MS): m/z: 509.9[M+H]$^+$, 510.8[M+2]$^+$

N-(4-(tert-butyl)phenyl)-4-((4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl)thio)picolinamide (R4)

Yield (%):55.0IR (KBr, Vmax, cm^{-1}):3424 (NH str), 1687 (C=O str), 1639 (C=O str), 1307 (CF3), 801 (C-Cl), 696 (C-S),^{1}H NMR (400MHz, DMSO) δ ppm: 1.29 (s, 9H), 7.28-7.30 (d, 2H, ArH), 7.35-7.36 (d, 2H, ArH), 7.58-7.60 (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), 9.10 (s, 1H, CONH), 9.24 (s, 1H, CONH),^{13}C NMR (DMSO) δ ppm: 31.3 (x3), 34.2, 118.8, 120.5 (x2), 121.2(x2), 123.3, 124.9, 126.4 (x2), 127.9 (x2), 128.5, 128.9, 129.1, 129.3, 129.5, 132.0, 134.3, 134.8, 136.2, 140.5, 146.9, 148.2, 151.9, 152.9, 162.6. Mass (LC-MS): m/z: 600.2[M+H]$^+$, 601.2[M+2]$^+$

1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(4-((2-(piperidine-1-carbonyl)pyridin-4-yl)thio)phenyl)urea (R5)

Yield (%):48.5,IR (KBr, Vmax, cm^{-1}):3420 (NH str), 1700 (C=O str), 1609 (C=O str), 1311 (CF3), 1124 (C-N), 797 (C-S), 698 (C-S)**,**^{1}H NMR (400MHz, DMSO) δ ppm: 1.54-1.64 (m, 4H, piperidine), 1.67-1.71 (t, 2H, piperidine), 3.68-3.77(t, 4H, piperidine), 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, 1H, ArH), 8.27 (s, 1H, ArH), 8.74-8.76 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.24 (s, 1H, CONH),^{13}C NMR (DMSO) δ ppm: 24.2, 25.4 (x2), 47.3(x2), 118.8, 120.5 (x2), 123.3, 124.9, 126.4 (x2), 128.5, 128.9, 129.1, 129.3, 129.5, 132, 134.3, 136.2, 140.5, 148.2, 151.9, 152.9, 172.2. Mass (LC-MS): m/z: 536.0[M+H]$^+$, 537.9[M+2]$^+$

1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(4-((2-(morpholine-4-carbonyl)pyridin-4-yl)thio)phenyl)urea (R6)

Yield (%): 53.78,IR (KBr, Vmax, cm^{-1}):3422 (NH str), 1712 (C=O str), 1594 (C=O str), 1316 (CF3), 1002 (Aliphatic ether), 828 (C-Cl), 798 (C-S)**,**^{1}H NMR (400MHz, DMSO) δ ppm:3.47-3.57 (t, 4H, morpholine), 3.60-3.68 (t, 4H, morpholine), 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, 1H, ArH), 8.27 (s, 1H, ArH), 8.74-8.76 (d, 1H, ArH), 9.10 (s, 1H,

CONH), 9.24 (s, 1H, CONH),^{13}C NMR (DMSO) δ ppm: 46.1(x2), 66.2 (x2), 118.8, 120.5(x2), 123.3, 124.9, 126.4 (x2), 128.5, 128.9, 129.1, 129.3, 129.5, 132, 134.3, 136.2, 140.5, 148.2, 151.9, 152.9, 168.6. Mass (LC-MS): m/z: 538.1[M+H]$^+$,540.0[M+2] $^+$

1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(4-((2-(4-methylpiperazine-1-carbonyl)pyridin-4-yl)thio)phenyl)urea (R7)

Yield (%): 50.90,IR (KBr, Vmax, cm^{-1}):3422 (NH str), 1700 (C=O str), 1587 (C=O str), 1309 (CF3), 819, 699 (C-Cl)**,**^{1}H NMR (400MHz, DMSO) δ ppm:2.19 (s, 3H, n-methyl), 2.25-2.29 (t, 4H, piperazine), 3.21-3.27 (t, 4H, piperazine), 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, 1H, ArH), 8.27 (s, 1H, ArH), 8.74-8.76 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.24 (s, 1H, CONH),^{13}C NMR (DMSO) δ ppm:46.6, 49.7, 49.7, 51.5 (x2), 118.8, 120.5 (x2), 123.3, 124.9, 126.4 (x2), 128.5, 128.9, 129.1, 129.3, 129.5, 132, 134.3, 136.2, 140.5, 148.2, 151.9, 152.9, 168.6.Mass (LC-MS): m/z:556.2[M+H]$^+$, 558.1[M+2] $^+$

4-((4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl)thio)-N-(2-fluoro-5-methylphenyl)picolinamide (R8)

Yield (%): 62.30,IR (KBr, Vmax, cm^{-1}):3345 (NH str), 1711 (C=O str), 1631 (C=O str), 1300 (CF3), 1284(C-F), 828 (C-Cl), 703 (C-S),^{1}H NMR (400MHz, DMSO) δ ppm:2.35 (s, 3H, CH3), 7.35-7.36 (d, 2H, ArH), 7.10-7.20 (m, 2H, ArH), 7.63-7.65 (d, 2H, ArH), 7.74-7.76 (d, 1H, ArH), 7.89-7.91 (m, 3H, ArH), 8.05 (s, 1H, ArH), 8.27 (s, 1H, ArH), 8.74-8.76 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.16 (S, 1H, CONH), 9.24 (s, 1H, CONH),^{13}C NMR (DMSO) δ ppm: 21.3, 113.2, 118.8, 119, 120.5 (x2), 121.6, 122.9, 123.3, 124.9, 126.4 (x2), 128.5, 128.9, 129.1, 129.3, 129.5, 132, 134.2, 134.5, 136.2, 140.5, 148.5, 151.9, 152.9, 155.3, 162.6. Mass (LC-MS): m/z: 576.9[M+H]$^+$, 578.9[M+2] $^+$

4-((4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl)thio)-N-(2,5-difluorophenyl)picolinamide (R9)

Yield (%): 58.20,IR (KBr, Vmax, cm^{-1}):3421 (NH str), 1704 (C=O str), 1614 (C=O str), 1313 (CF3), 1288 (C-F), 798 (C-Cl), 700 (C-S)**,**^{1}H NMR (400MHz, DMSO) δ ppm:7.00-7.10 (m, 2H, ArH), 7.35-7.36 (d, 2H, ArH), 7.63-7.65 (d, 1H, ArH), 7.74-7.76 (d, 2H, ArH), 7.89-7.91 (m, 3H, ArH), 8.05 (s, 1H, ArH), 8.27 (s, 1H, ArH), 8.74-8.76 (d, 1H, ArH), 9.10 (s, 1H, CONH), 9.16 (S, 1H, CONH), 9.24 (s, 1H, CONH),^{13}C NMR (DMSO) δ ppm: 111.7, 112.7, 113.3, 118.8, 120.5 (x2), 120.7, 123.3, 124.9, 126.4 (x2), 128.5, 128.9, 129.1, 129.3, 129.5, 132.0, 134.3, 136.2, 140.5, 148.2, 151.9, 152.9, 153.9, 158.7, 162.6. Mass (LC-MS): m/z: 579.9[M+H]$^+$, 581.9[M+2]

Biological Evaluation[25-30]
Cell lines and cell culture

A human bosom adenocarcinoma (MCF-7) cell line was obtained from National Center for Cell Science (NCCS), Pune, Maharashtra, India. MCF-7 cells were cultured MEM media supplemented with 10% FBS (fetal bovine serum), 1%

nonessential amino acids, Add 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 kept under sterile conditions at 37°C with 5% CO_2 and 95% air, also subculture weekly using 0.02% EDTA and 0.05% trypsin. Cells proliferating in cultures gradually lose their proliferation rate after consuming a high rate of nutrient agents in the cell culture medium or using the entire surface in which they can reproduce, and cell growths and deaths occur over time. In this period, the division of culture is the most accurate process to be done, and this process is called passaging. The media were changed every 2–3 days.

Treatments of compounds

Compounds were freshly prepared in cell culture grade DMSO at the stock concentration of 100mM. Exponentially growing MCF-7 cells were treated with different Compounds (10 to 60µM) for 24 h. Cells treated with DMSO (0.1%) were considered as vehicle control.

Results and Discussion

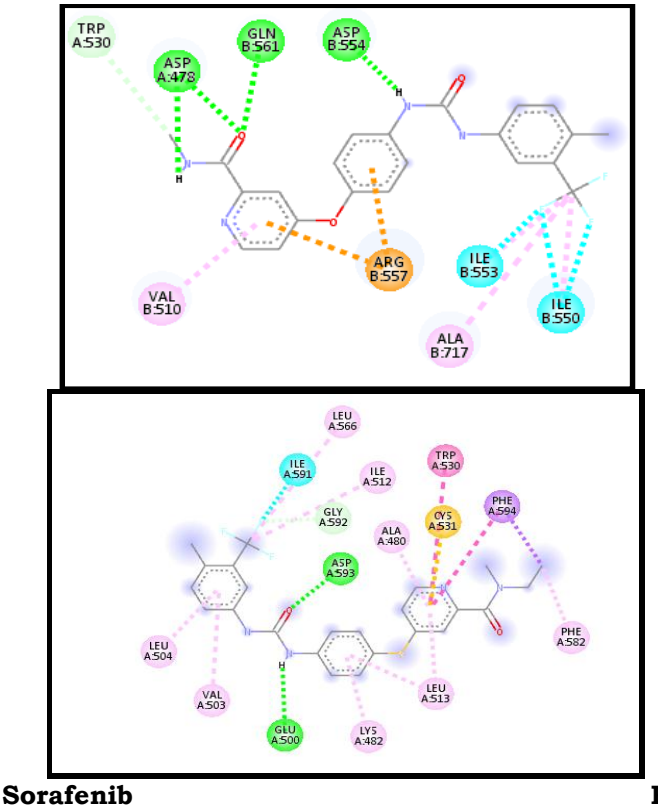
Molecular docking studies

The docking study was performed using the AutoDock Vina program. This program was designed to predict how small molecules were bound to a receptor of a known 3D structure. In order to find newpotential compounds for treating cancer, docking of nine compounds was performed. AutoDoc Vina implicated in the PyRx tool generated nine differentconformations for each ligand which are classified bybinding affinity (kcal/mol). Synthesized compounds have free energy of binding in the range −8.9 to −12.2 kcal/mol are presented in Table 1
Table 1, synthesized compounds have a binding free energy greaterthan sorafenib Similarly, it was observed that synthesized compounds have a high binding affinity score for the spike protein through molecular docking experiments. All compounds molecular interactions like conventional hydrogen bond, carbon hydrogen bond, halogen interaction, Pi-cation, Pi-sulfur, Pi-Pi stacked, Pi-Pi T-shaped, alkyl and Pi-alkyl interactions shown in figures 3 and 4.

Table 1
Molecular Docking of Compounds R1-R9

Compound code	H-Bonds	AutoDock Vina Binding energy (kcal/mol)	Amino acid interactions
R1	4	-8.9	HIS:B539, ASP:B586, CVS:B531, THR:B528
R2	2	-9.7	ASP:B593, LYS:B482
R3	2	-10.9	ASP:A593, GLU:A500
R4	0	-11.4	--
R5	3	-10.9	ASP:B593, GLU:B500, CYS:B531
R6	2	-10.0	ASP:A539, GLY:A533
R7	2	-11.2	ASP:B593, LYS:B482
R8	4	-11.6	ASP:B593, LYS:B482, ALA:B597, VAL:B599

R9	1	-12.2	LYS:B482
Reference Drug Sorafenib	3	-8.5	ASP:A478, GLN:B561, ASP:B554



Sorafenib **R3**

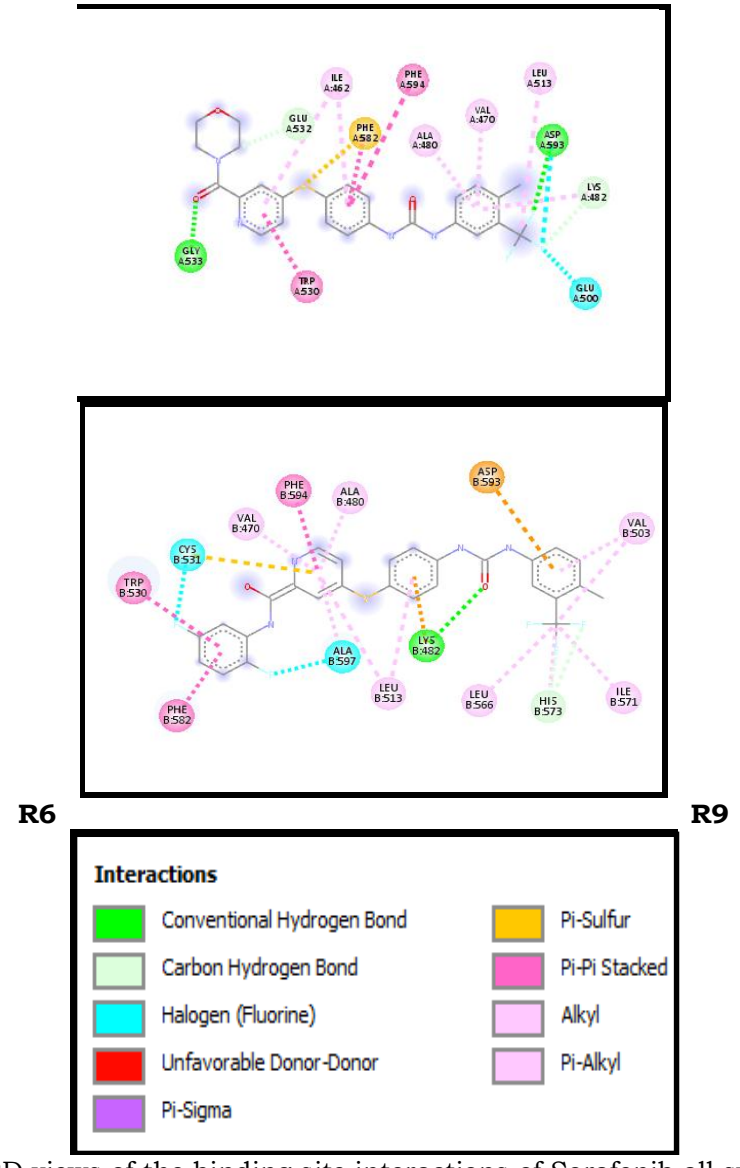


Fig 3. 2D views of the binding site interactions of Sorafenib all synthetic compounds R3, R6, R9

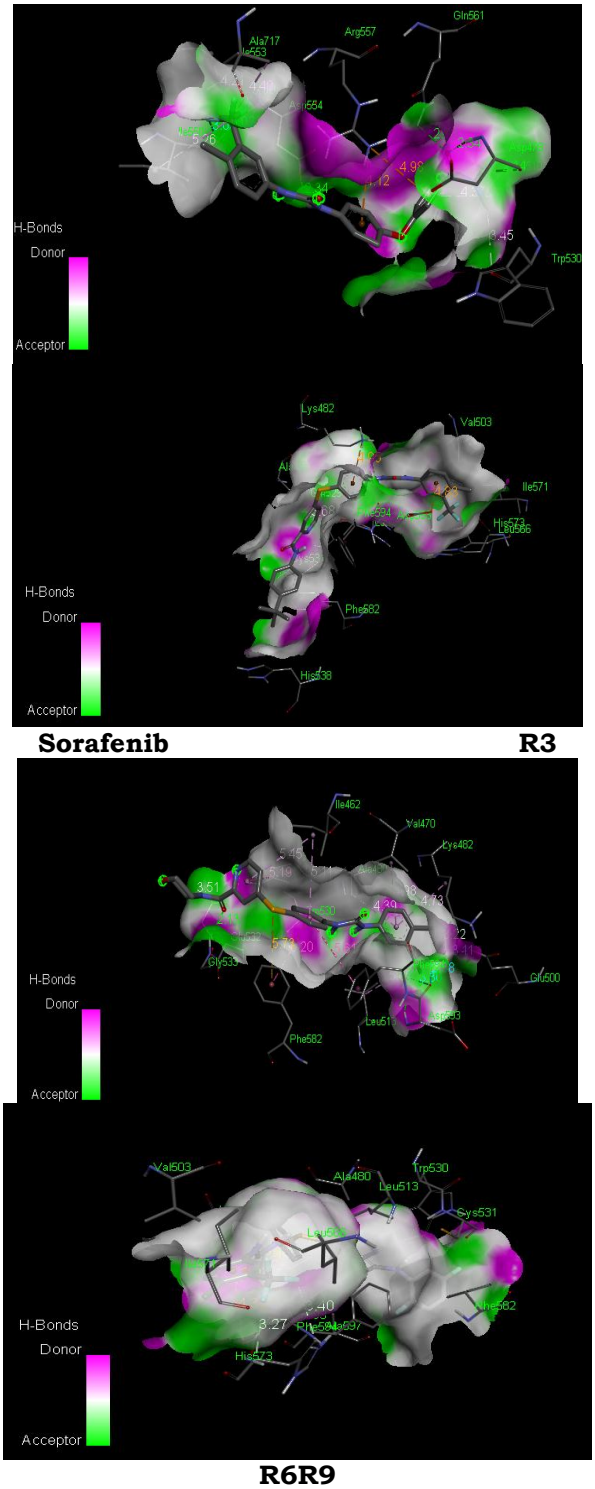


Fig 4. 3D view of Sorafenib the all the novel synthetic compounds (R3, R6, R9) conformation

Chemistry

The synthetic methods adapted for the synthesis of the diaryl urea derivatives are represented in Scheme I. Pyridine-2-carboxylic acid was reacted with thionyl chloride in presence of sodium bromide and chlorobenzene solvent at 85°C temperature after chlorination produce 4-Chloropyridine-2-carbonyl chloride (Step-I). Step-I product was reacted withnine different amines using triethylamine base and THF solvent, by acid chloride reaction with amine produce step-II. Step-II product was reacted with 4-Amino thiophenol in presence of Potassium tertiary butoxide and produce step-III. 4-Chloro-3-(trifluoromethyl)aniline reacted with CDI then with step-III product added to form pyridine containing diaryl urea derivatives(R1-R9). All intermediates confirmed by mass spectroscopy and used in next step without purification. All final compounds purified by column chromatography with 45 to 65% yield. The structure of synthesized compounds R1-R9was confirmed through IR, Mass, ^{1}H NMR and ^{13}C NMR data.

Biological Activity
In-vitro Anticancer Activity

The molecules R1-R9 were assessed against MCF-7 cell lineby MTT assay. In this experiment the compound R6 (IC50 = 16.84 µM) and compound R9 (IC50 = 17.39 µM) displayed potent activity compared with standard drug Sorafenib (IC50 =29.30µM) (Table-2, Fig.-4).

Table 2
In vitro Anticancer Activity of R1-R9 Derivatives

Compound	IC50 µM (MCF-7)
R1	22.44
R2	28.79
R3	21.64
R4	24.48
R5	31.54
R6	16.84
R7	29.04
R8	22.48
R9	17.39
Sorafenib	29.30

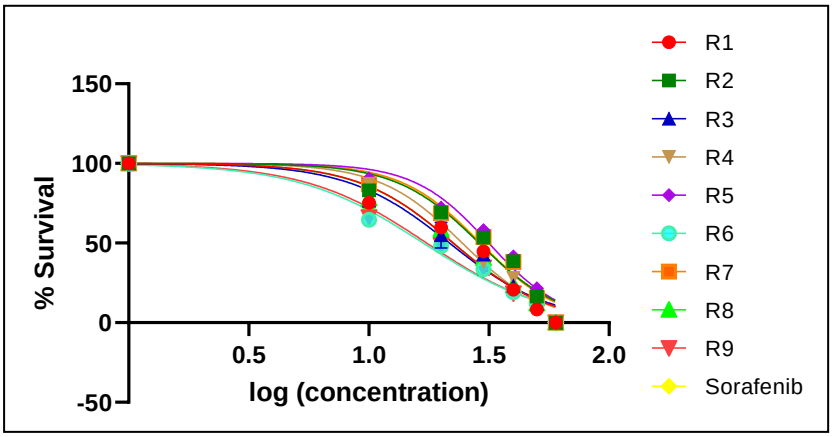


Fig 4. %Cell survival vs log concentration graph of Sorafenib and all synthesize Compounds

Conclusion

In this research, a structure-based virtual screening (SBVS) was applied on the high similar sorafenibapproved drug and selected from literature. SBVS was performed byusing AutoDock Vinatools.Nine compounds show strong and stable interactions in AutoDock Vinatools. 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-R9have been subjected to in vitro assay anticancer, applicationsAmong the series of compoundsR3, R6, and R9exhibited 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 show significant binding energy using AutoDock Vina tools. Further, appropriate modifications of the compounds may show significant biological activities.

Acknowledgement

This research was supported by Sankalchand Patel University, Visnagar, Gujarat, India, which provides research work facilities. We thank our colleagues from the research guide and co-guide who provided insight and expertise that greatly assisted the research.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Anjaneyulu V, Krishnaiah V, RajeshKumar K and Leelavathi P, An Efficient One Pot Multicomponent Synthesis of Dihydro Pyrazolyl Bithiazole

Derivatives as Potential Anticancer Agents and Their Molecular Docking Studies." Research Square., 20, 2022.
2. Supaluk P, Ratchanok P , Apilak W, Nujarin S, Veda P , Somsak R and Virapong P ,Roles of Pyridine and Pyrimidine Derivatives as Privileged Scaffolds in Anticancer Agents. Mini Rev Med Chem,17, 2007, 869-901.
3. Sarah, P.; David, M., et al."Mechanisms of anticancer drugs",ScottBrown's Otorhinolaryngology: Head and Neck Surgery 7 Ed; CRC Press, 2008; Vol. pp. 34-46.
4. Sinthupoom N, Prachayasittikul V, Prachayasittikul S, Ruchirawat, S, Prachayasittikul V,Nicotinic acid and derivatives as multifunctional pharmacophores for medical applications.Eur. Food Res. Technol., 240, 20015, 1-17.
5. Barot, K, Jain, S., Kremer, L., Singh, S., Ghate, M. , Recent advances and therapeutic journey of coumarins: current status and perspectives. Med. Chem. Res.,24, 2015, 2771-2798.
6. Meropi S., Nikolaos L., et al.Synthesis, Docking Study and Kinase Inhibitory Activity of a Number of New Substituted Pyrazolo[3,4-c]pyridines. Chem. Pharm. Bull., 65, 2017, 66–81.
7. Espinosa, E., Zamora, P., Feliu, J., Gonzalez B., et al.Classification of anticancer drugs—a new system based on therapeutic targets. Cancer Treat. Rev.,29, 2013, 515-523.
8. Rajeev S. Samant and Lalita A. Shevde, Recent Advances in Anti-Angiogenic Therapy of Cancer. Oncotarget, 2, 2011, 122-134.
9. Dobbelstein, M., Moll, U. Targeting tumour-supportive cellular machineries in anticancer drug development. Nat. Rev. Drug Discov.,13, 2014, 179-196.
10. Pommier, Y., Leo, E., Zhang, H., Marchand, C., Topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem. Biol., **17**, 2010, 421-433.
11. Chiablaem, K., Lirdprapamongkol K., Keeratichamroen S., Surarit R.,Curcumin Suppresses Vasculogenic Mimicry Capacity of Hepatocellular Carcinoma Cells through STAT3 and PI3K/AKT Inhibition. J. Anticancer Res., 34, 2014, 1857-1864.
12. Mohamed N, Hadia A., Hany S., Ibrahim D., Wagdy M., and Hatem A. Pyridine-Ureas as Potential Anticancer Agents: Synthesis and In Vitro Biological Evaluation.Molecules,23,20087, 1459. doi:10.3390/molecules23061459.
13. Wilhelm S., Carter C., LynchM., LowingerT.,Dumas J., Smith, R., Schwartz, B., Simantov, R., Kelley, S., Hepatocellular Carcinoma: Basic and Transitional Research.Nat. Rev. Drug Discov., 5, 2006,835–844 .
14. DiGiulio, S. FDA Approves Stivarga for Advanced GIST. Oncol. Times., 35, 2013,12.
15. Cui J.J., Tran-Dube M., Shen H., Nambu M., Kung P.P., Pairish M., Jia L., Meng J., Funk L., Botrous I., Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK).J. Med. Chem. 54, 2011, 6342–6363.
16. Patel, J. R., Shah, U. A., et al. Exploring novel endothelin receptor blocker as anti-hypertensive agents identified from a natural drugs library using induced fit docking and biological assay. Med. Plants - Int. J. Phytomed. Relat. Ind. (3), 2020, 405-413.

17. Rina Herowati et al. Molecular Docking Studies of Chemical Constituents of Tinospora cordifolia on Glycogen Phosphorylase. Procedia Chem.13,2014, 63 – 68.
18. Samar Salam Qawoogha et al, Identification of potential anticancer phytochemicals against colorectal cancer by structure-based docking studies.J. Recept. Signal Transduct. Res.2020 1-9. DOI: 10.1080/10799893.2020.1715431.
19. Joshi, H. V., Patel, J. K., et al, In silico screening of natural compounds to identify lead as interleukin 17A receptor blockers as antihypertensive agents. Thai J. Pharm. Sci.,44(3), 2020, 168-173.
20. Wei Li1., Xin Zhai1., Zheng Zhong.,et al. Design, Synthesis and Evaluation of Novel Rhodanine containing Sorafenib Analogs as Potential Antitumor Agents.Pharm. Chem. Life Sci. 11.2011, 349–357. DOI 10.1002/ardp.201000326.
21. LU Chen S., Tang K., LI Yan., et al.Synthesis and in vitro antitumor activities of novel benzyl urea analogues of sorafenib.Acta Pharmaceutica Sinica. 48 (5), 20132, 709 −717. DOI 0513-4870 (2013) 05-0709-09.
22. Xiangkai Kong,Zeyu Yao, et al. Design, synthesis and biological evaluation of thiourea and nicotinamide-containing sorafenib analogs as antitumor agents.Med Che Comm. 2015; 1-5. DOI: 10.1039/C4MD00536H.
23. Chunjiang Wu.; Shan Xu.;et al. Design, synthesis and biological evaluation of phenylpicolinamide sorafenib derivatives as antitumor agents.Med Chem Res. 2017; DOI 10.1007/s00044-017-2045-0.
24. Balakrishna M., Lekkala Ravinda., et al. The significance of N-methylpicolinamides in the development of anticancer therapeutics: Synthesis and structure-activity relationship (SAR) studies. Bioorganic Chemistry. 86, 2019, 513–537.
25. Dimov S., Mavrova, A., et al. Anti- cancer in Medicinal Chemistry (Formerly current Medicinal Chemnisrt- Anti Cancer Agents).21(11), 2021.1441-1450.
26. Sanjeev Kumar., Ekta Lathwal.,et al. Synthesis of pyrazole based novel aurone analogs and their cytotoxic activity against MCF-7 Cell line. Chemical Data Collection. 2020; doi: https://doi.org/10.1016/j.cdc.2020.100.
27. Amani G. A., Kareem A. M., et al. Biosynthesis, Characterization, and Evaluation of the Cytotoxic Effects of Biologically Synthesized Silver Nanoparticles from Cyperus conglomeratus Root Extracts on Breast Cancer Cell Line MCF-7. Biological Trace Element Research. 2019; https://doi.org/10.1007/s12011-019-01791-7.
28. Wenbao W., Di W.,et al. Synthesis of new sarsasapogenin derivatives with cytotoxicity and apoptosis-inducing activities in human breast cancer MCF-7 cells. Eur. J. Med. Chem.127. 2017,62-71.