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SYNTHESIS, CYTOTOXICITY, PDGFR INHIBITORY ACTIVITY AND DOCKING STUDY OF NOVEL 2-AMINOQUINOLINE-3-CARBOXAMIDE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS

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Abstract

The goal of this work was to synthesize new substituted 2-aminoquinoline-3-carboxamide derivatives from substituted anilines utilizing the Vilsmeier-Haack reaction, and then to test these compounds for in vitro anticancer activity and molecular docking in order to identify prospective lead molecules. Substituted aniline, acetanilide, 2-chloro-3-carbaldehyde to carboxylic acid as well as coupling provide the lead compounds and were characterized by physical and spectral methods. In vitro cytotoxicity testing was done by using MTT assay method. Research on the binding interaction of the most effective drugs was conducted using AutoDock molecular docking tool. Novel Series of substituted 2-aminoquinoline-3-carboxamide derivatives have been synthesized as well as verified utilizing different spectral methods for example mass spectrometry, carbon-13 nuclear magnetic resonance, nuclear magnetic resonance, and infra-red. In a cytotoxicity testing vs a breast cancer cell line, synthesized compounds showed some potential (MCF-7). Four derivatives 6b, 6c, 6j, and 6o were shown to have more efficacy than Sunitinib in an in vitro cytotoxicity assessment research. Moreover compounds 6b, 6c, 6j, and 6o exhibited higher binding score at platelet-derived growth factor receptor active sites (PDB: 5GRN) compared with standard sunitinib. This article described the synthesis of sixteen novel substituted aniline results in substituted 2-aminoquinoline-3-carboxamide derivatives. The results showed that compounds 6b, 6c, 6j, and 6o exhibited promising anticancer activity. Sunitinib is currently the only approved inhibitor of PDGFR; however the 2-aminoquinoline-3-carboxamides showed promise as a more selective alternative. The above findings were also supported by molecular docking studies.These findings may serve as models for future research and derivatization, opening the door to the development of effective and precise PDGFR inhibitors.

Key words: Docking study, Quinoline-3-carboxamides, Vilsmeier–Haack reaction Synthesis, Cytotoxicity.

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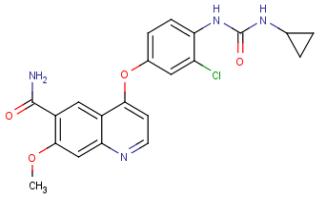
INTRODUCTION

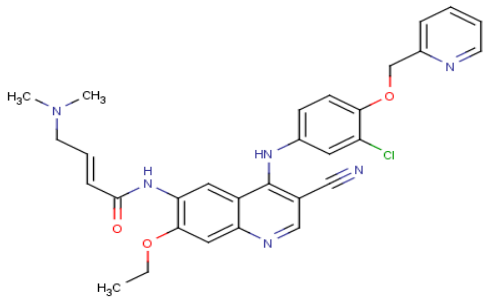
In 2020, cancer will kill approximately 10 million people throughout the globe. This is roughly one in six fatalities. Among all the numerous kinds of cancer, breast cancer is perhaps the most deadly. The anticipated 47.8% increase in female breast cancer incidence by 2020 poses a global threat to the development of effective therapies. Breast cancer has emerged as the top cause of cancer death among women. Anticancer medications either intravenously or orally can be used to treat and prevent breast cancer metastases. FDA (Food and Drug Administration) has accepted a variety of chemotherapeutics during the past 20 years; nonetheless, there are still many challenges to be overcome before an effective and typically non-toxic cancer treatment can be developed.[2] Therefore, the development of potent anticancer agents in medicinal chemistry has received considerable focus in the worldwide quest for new treatments.

Tyrosine kinases at the receptor end are transmembrane proteins called receptor tyrosine kinases (RTKs). The control of cell migration, proliferation, and angiogenesis is mediated by PDGF (platelet-derived growth factor).[3,4]PDGFRβ along with PDGFRα are two of five members of PDGF RTK (receptor tyrosine kinase) family (PDGF-AA, -BB, -AB, -CC, and -DD). A receptor's affinity for a given ligand might vary depending on the kind of receptor being studied. Cell proliferation, survival, and migration are all improved by activating PI3K (phosphatidylinositol 3-kinase)/AKT and ERK1/2 signalling transduction pathways upon stimulation of PDGFR and PDGFR, respectively. Several unfavourable outcomes, including metastasis to lymph nodes and HER2 positive, have been linked to high PDGFR expression in breast tumours. As a result, PDGFRs are being studied as potential new target for creation of tyrosine kinase inhibitors to prevent the uncontrolled cancer cell proliferation and restore a healthy physiological balance.[4]

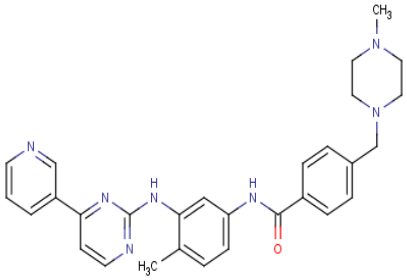
Natural quinolines may be found in numerous alkaloids with powerful tumour effects as camptothecin. When it comes to drug development, specifically for novel anticancer medicines, quinoline scaffolds and related derivatives show a broad variety of pharmacological activities. Figure1 depicts many studies that have resulted in a line of cancer medications that has been approved by the FDA.



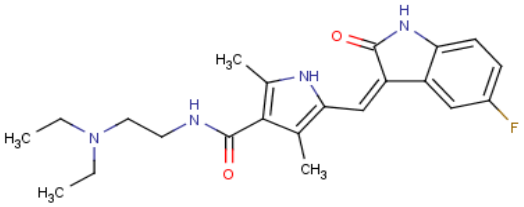
1. Lenvatinib(1)



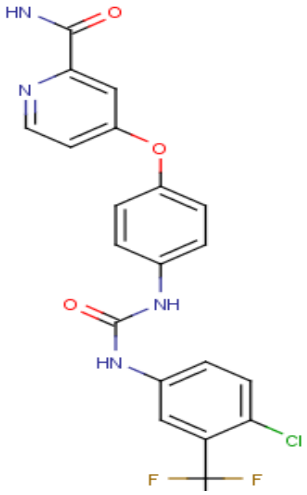
2. Neratinib



3. Imatinib



4. Sunitinib



5. Sorafenib

Figure 1. Quinoline-based multi-kinase inhibitors approved by FDA.

They are numbered 1 (Lenvatinib), 2 (Neratinib), 3 (Imatinib), 4 (Sunitinib), and 5 (Sorafenib).[5] Several small-molecule quinolines that inhibit protein kinases have been licenced by the FDA for use in cancer therapy.[5,6,7,8] Nitrogen atoms in the quinoline nucleus cause the p-electron density to be unevenly distributed by extracting electrons via resonance. Numerous experiments demonstrate that the quinoline nucleus has a strong propensity for binding to the active areas of proteins by forming p-p stacking and hydrogen-atom- complexes with the necessary amino acid residues.[9, 10]The initial scaffold's design plan is shown in Figure 2.In order to synthesise acetanilides from substituted anilines, we used the Vilsmeier-Haack reaction on a total of 16 different substituted 2-aminoquinoline-3-carboxamide derivatives.[11]Once the carboxylic acid has been formed and connected with different anilines, this process produced 2-chloro-3-carbaldehyde quinolines. The R group was rationally chosen to examine how electronic characteristics affect cell toxicity. This group contains alkyl, oxy-halo alkyl and halogens.

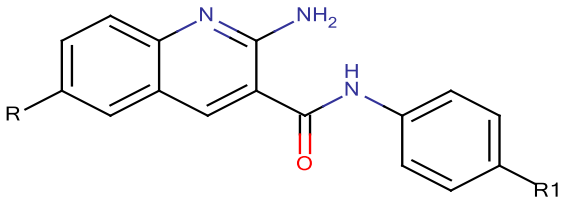


Figure 2. Designed 2-Amino Quinoline-3-Carboxamide Scaffold

We provide the first evidence that a hitherto unrecognised class of 2-aminoquinoline-3-carboxamides have promising anticancer properties. The MTT method has been utilized for assessing cytotoxic effects of several substances on MCF-7 cancer cell line. To further confirm the inhibition of PDGFR kinase, a CTG experiment has been done on MCF-7 cells.

MATERIALS AND METHODS

Chemicals, solvents, and reagents of laboratory grade quality were exclusively utilized in the synthesis. The solvents were cleaned up using tried and true techniques. Following dehydration in a vacuum desiccators and a hot air oven, each residue was recrystallized from the appropriate solvent. A % yield can be determined once the chemical has been purified.

To measure the compounds' melting points in open capillaries, we used a Thieles tube. The melting points of the following materials are listed in degrees Celsius (°C). Spots were made on a precoated silica gel-G plate (E. Merck Kieselgel 60F254) using a UV cabinet and an iodine chamber for 30 minutes prior to the thin layer chromatography technique. Here we offer the numbers that are used in the text to compare to the standard of improvement of solvent systems with higher R_f values.

Utilizing Jasco/FT/IR-4100A spectrophotometer, and KBr pellets, the infrared spectra of a number of compounds were recorded at Vineet Analytical Lab's Pune location. At the Vineet Analytical Lab in Pune, India, ^{1}H-NMR spectra have been collected utilizing a Bruker AVIII500 (500 MHz) apparatus with chloroform-d and tetramethyl silane (TMS) as an internal standard. Parts per million (ppm) chemical changes for a $CDCl_3$ solution are given in relation to TMS. Each signal's multiplicity is represented by the letter S. (singlet), m (multiple) (multiplet) bs (broad singlet), q (quartet), t (triplet), and d (doublet),.TMS (Tetramethylsilane) has been utilized as an internal standard whereasDMSO-d6 (dimethyl sulfoxide) has been utilized as solvent for ^{13}C-NMR spectrum acquisition on a Bruker AV600 (600 MHZ). The Vineet Analytical Lab's Research Instrumentation Facility used a Bruker Compass Data Analyser is 4.2 to get the mass spectra.

At the Vineet Analytical Laboratory's Research Instrumentation Facility in Pune, researchers evaluated the effectiveness of chemotherapy and radiation therapy against MCF-7 breast cancer cell line.

General Synthetic Procedure

Step-1

Para-Substituted Acetanilide Derivatives: Synthesis 0.1 mol of substituted anilines, 10ml of glacial acetic acid and 10 ml of acetic anhydride have been combined first, then heated gently for 1hour under reflux, after that poured into 200ml of freezing water to crystallize acetylated product. Crystals have been filtered and re-crystallized in an acetic acid to water solution, yielding acetanilide (1:2). In this case, the returns were anything from 94% to 100%.

Step-2

The summary of the 2-chloroquinoline-3-carbaldehyde synthesis process 3 as shown in the scheme 1.Using the approach described by Toth et al., we were able to synthesize substituted 2-chloroquinoline-3-carbaldehydes.[12]Under inert circumstances and at 0 degrees Celsius, dry N,N- DMF (dimethyl formamide) (9.6ml; 0.125mol) has been mixed to two-neck round bottom flask, after thatPOCl3 (phosphoryl chloride) (32.2ml; 0.35 mol) has been mixed slowly. After adding the specified amount of acetanilide, the solution has been heated at 82°Cin a condenser fitted with $CaCl_2$ drying tube for 24 hours under reflux. Using TLC, we were able to monitor the reaction's development and determine when it was finished. At the end of the process, the liquid was cooled to between 0 and 10 degrees Celsius, placed in 300 cc of ice water, and swirled for an hour. Yields of 2-chloroquinoline-3-carbaldehydes ranged from 43% to 74% after the light-yellow precipitate has been removed, washed with water (100ml), and after that dried.

Step-3

Turning 2-chloroquinoline-3-carbaldehydes method Chloroquinoline-3-carboxylic acids, 3 to 4 as shown in the scheme 1.

It was modified from the procedure described by A.R. Syniugin et al.[13]After dissolving the modified 2-chloroquinoline-3-carbaldehydes (4.68mmol) in n-butanol, the solution is agitated at ambient temperature for 2 hours before adding the sodium dihydrogen orthophosphate (35.61 mmol; 5.56 g) and sodium chlorite (47.47mmol; 4.29g) in 89ml of water. TLC analysis has been performed on a solution that had been vacuum-concentrated as well as diluted with 68ml of water. Next, the mixture has been separated into two layers using sodium carbonate after being acidified

with HCl to a pH of 4, at which point the 2-chloroquinoline-3-carboxylic acids 4 precipitated out as cream-colored compounds (5 g).

Step-4

An Overview of 2-Aminoquinoline-3-Carboxylic Acid Synthesis 2-chloroquinoline-3-carboxylic acids, 5's and 4's Suspensions of corresponding Acid Chloroquinolone-3-Carboxylic, or 2-CQC for short, produced the required outcome when heated to 3 (1mmol) in 26 percent aqueous NH_3 (5ml) in a stainless steel autoclave for four hours pH has been lowered to 4 with 5 percent of aqueous HCl solution after the reaction mixture had cooled. This produced a transparent solution. The IPA-DMF solution was filtered and the solid products 6a-6p was obtained.

Step-5

As an illustration, the generalized synthesis of 2-amino-N-phenylquinoline-3-carboxamides is given (6a–6p)

Quinoline-3-carboxamide synthesis was altered from the Li et al. [14] technique. The substituted quinoline-3-carboxylic acid 4 (0.9mmol), 0.99mmol of DCC, and 1-hydroxybenzotrizole (HOBt) have been mixed in a total volume of 10 ml of DMF. The required substituted 0.99 mmol of aryl amines and triethyl amine were mixed, and after that for 24hours the mixture was stirred. By submerging it in frozen water, the reaction could be halted, producing a yellowish-white precipitate. After washing, drying, and purifying with water (9:1), this was separated using silica gel column chromatography with a 30% ethyl acetate solvent: 70% hexane. In general, synthesis of 2-amino-N-phenylquinoline-3-carboxamide derivatives 6a-6p resulted in yields between 76% and 96%.

Synthesis of 2-amino-N-phenylquinoline-3-carboxamide (6a)

IR (KBr) Vmax/cm^{-1} values are as follows: 3480 (N-H str.), 3357(N-H str.), 3178, 3108 (C-H str. aryl), 1570 (C=O str.) ^{1}H NMR (500 MHz, Chloroform-d): 6.50(s, 2H), 7.35-7.28(m, 2H), 7.49(ddd, J=1.2, 7.7, 9.1Hz, 1H), 7.73-7.64(m, 3H), 7.05 (J=1.2, 6.9Hz, 1H), 7.88-7.82(m, 1H), 7.95-7.98(m, 1H), 8.69 (d, *J* = 2.3 Hz, 1H), and 9.83 (s, 1H, NH). ^{13}C NMR was able to identify chemical shifts: 109.0, 119.9, 124.5, 126.4, 127.7, 127.8, 128.5, 129.2, 128.5, 129.4, 130.4, 137.4, 146.5, 159.1, and 165.7.Distribution of MS m/z by Percentage: $C_{16}H_{13}N_3O$ has been weighed and measured, and its exact mass is 263.00; the measured values are 263.11 (M)+, 264.10 (M+1)+, 265.10 (M+2)+, and 266.01 (M+3)+.

Synthesis of 2-amino-6-ethyl-N-phenylquinoline-3-carboxamide (6b)

IR (KBr) Vmax/cm^{-1} values are as follows: 3423 (N-H str., -NH_2), 3321 (N-H str. >NH), 3190 (C-H str. aryl), 2924 (C-H str. alkyl), 2853 (C-H str. alkyl), 1636 (C=O str.) ^{1}H NMR (500 MHz, Chloroform-d) 1.21 (t, J = 7.3 Hz, 3H), 2.72 - 2.64 (m, 2H), 6.50(s, 2H), 7.17-7.11(m, 2H), 7.88-7.82(m, 1H), 7.53-7.46(m, 3H), 7.69(td, J=7.9, 1.0Hz, 2H), 7.95-7.89.(s, 1H). ^{13}CNMR (75MHz, DMSO-d6): 14.6, 28.7, 109.0, 117.9, 124.5, 126.4, 127.7, 128.5, 129.4 129.9, 130.4, 137.4, 144.2, 146.5, 159.1, 165.7. MS m/z (%): Masses of $C_{18}H_{17}N_3O$ measured to be 291.35 (M+), and they were really measured to be 291.01 (M)+, 292.87 (M+1)+, 293.38 (M+2)+, and 294.02 (M+3)+.

Synthesis of 2-amino-6-methoxy-N-phenylquinoline-3-carboxamide (6c)

The following values for the IR (KBr) Vmax/cm^{-1} spectrum are: 3360, 3067(C-H str. aryl), 2851(C-H str. alkyl), 3255(N-H str., -NH_2), and 1688(C=O str.). ^{1}H NMR (500 MHz, Chloroform-d) 3.78(s, 3H), 6.50(s, 2H), 7.82–7.88(m, 1H), 6.88–6.94(m, 2H), 7.4–7.52(m, 1H), 7.92(ddd, J=1.1–2.2–8.8Hz, 1H), 7.52-7.58(m, 2H), 7.69 (td, J=1.0–7.9Hz, 1H), and 8.69(d,J(s, 1H). ^{13}C NMR(DMSO-d6, 75 MHz): 56.0, 109.0, 120.5, 124.5, 126.4, 127.7, 128.5, 129.4, 130.4, 137.4, 146.5, 159.1, 159.8, and 165.7. MS m/z (%): Exact mass of $C_{17}H_{15}N_3O_2$ was 293.32, and the values found were 293.07 (M)+, 294.09 (M+1)+, and 295.07 (M+2)+.

Synthesis of 2-amino-6-nitro-N-phenylquinoline-3-carboxamide (6d)

The values for IR (KBr) Vmax/cm^{-1} are as follows: 3360, 3067(C-H str., aryl), 2851(C-H str., alkyl), 3255(N-H str., -NH_2), and 1688 (C=O str.) are the numbers that follow:7.46–7.53m(1H), 8.69d(J=2.4Hz, 1H), 6.50s(2H), 7.66–7.74m(3H), 7.89–7.95m(1H), 7.82–7.88m(1H), 8.20–8.26m(2H), and 10.64 s(2H) were all recorded in the 500 MHz ^{1}H NMR

experiment with chloroform-d (s, 1H). The following ^{13}C NMR spectral lines were obtained in DMSO-d6 at 75 MHz: 109.0, 116.6, 124.5, 125.0, 126.4, 127.7, 128.5, 129.4, 130.4, 137.4, 146.5, 147.3, 159.1, and 165.7.MS m/z (%): Found 308.13 (M)+, 309.16 (M+1)+, and 310.18 (M+2)+ for the exact mass of $C_{16}H_{12}N_4O_3$.

Synthesis of 2-amino-6-chloro-N-phenylquinoline-3-carboxamide (6e)

The values for IR (KBr) Vmax/cm^{-1} are as follows: 3371, 3269 (N-H str. –NH_2), 1659 (C=O str.), 736 (C-Cl bend.) ^1H NMR (500 MHz, chloroform-d): 7.28-7.35 (m, 1H), 7.05(tt, J=1.2, 6.9Hz, 1H), 7.64-7.71(m, 2H), 76.45(s, 2H),.56(dd, J=2.2, 8.2Hz, 2H), 7.77(d, J=8.3Hz, 1H), and 7.91 (t, J=2.2, 8.(s, 1H). 109.0, 119.9, 124.5, 126.1, 128.0-128.3, 128.1, 129.6, 130.0, 131.9, 137.4, 146.5, 159.1, 165.7, ^{13}C NMR (75 MHz, DMSO-d6). Numbers of m/z for the MS (in percentage): 109.0, 119.9, 124.5, 126.1, 128.0-128.3, 128.1, 128.1, 128.2, 129.6, 130.0. The sum of the atomic masses of the ionised forms of $C_{16}H_{12}ClN_3O$ is 297.74, which is exactly the same as the calculated masses of 297.06 (M)+, 298.35 (M+1)+, 299.67 (M+2)+, and 300.01 (M+3)+.

Synthesis of 2-amino-6-chloro-N-(4-ethylphenyl) quinoline-3-carboxamide (6f)

Maximum IR(KBr) Vmax/cm^{-1}:3323 (N-H str.,>N-H str.), 3046(C-H str. aryl), 1655(C=O str.), 2847(C-H str., alkyl), 2925(C-H str. aryl), 784(C-Cl bend.) 500MHz ^1H NMR in chloroform-d:1.21(t, J=7.3Hz, 3H), 2.64-2.72m, 7.77d, J=8.3Hz, 6.45s, 7.11-7.17m, 7.46-5.52m, 7.56dd, J=2.2, 8.2Hz, 1H), 1H, 7.91t, J=2.2 Hz, 1H, 8.63 d(s, 1H). ^{13}C NMR (75MHz, DMSO-d6): 14.6, 28.7, 109.0, 117.9, 124.5, 126.1, 128.1, 129.6, 129.8, 130.1, 129.9, 130.0, 131.9, 137.4, 144.2, 146.5, 159.1, 165.7. MS m/z (%): Found 325.10 (M)+, 326.08 (M+1)+, 327.56 (M+2)+, and 328.04 (M+4)+ for an exact mass of $C_{18}H_{16}ClN_3O$.

Synthesis of 2-amino-6-chloro-N-(4-methoxyphenyl) quinoline-3-carboxamide (6g)

Maximum IR (KBr) Vmax/cm^{-1} values are 3308 for N-H str. >NH, 2979 for C-H str., alkyl, 2927 for C-H str., 1698 for C=O str., and 725 for C-H str (C-Cl bend.) 500 MHz, Chloroform-d; ^1H NMR: 6.45(s, 2H); 3.78(s, 3H); 7.52–7.58(m, 3H); 6.88–6.94(m, 2H); 7.77(d, J=8.3Hz, 1H); 7.91(t, J = 2.2 Hz, 1H); 8.63(d, J=2.2Hz, 1H); 9.79(s, 1H). ^{13}C NMR measurements included 56.0, 109.0, 114.5, 120.5, 124.5, 126.1, 128.1, 1296, 130.0, 131.9, 137.4, 146.5, 159.1, 159.8, and 165.7. (at 75 MHz in DMSO-d6). MS m/z (per cent) According to the calculations, the masses of 327.76 g of $C_{17}H_{14}ClN_3O_2$ are 327.08 g (M)+, 328.09 g (M+1), 329.16 g (M+2)+, and 330.08 g (M+3)+, respectively.

Synthesis of 2-amino-6-chloro-N-(4-nitrophenyl) quinoline-3-carboxamide (6h)

Maximum IR (KBr) Vmax/cm^{-1} values are 3343 (N-H str. >NH), 3089 (C-H str. aryl), 1638 (C=O str.), 780 (C-Cl str.), ^1H NMR(500 MHz, Chloroform-d): 6.98(s, 2H), 8.20-8.26(m, 2H), 7.56(dd, J=2.2, 8.2Hz, 1H), 7.68-7.74(m, 3H), 7.91(t, J=2.2Hz, 1H), 8.63(d, J=2.2Hz, 1H), 10.54(d, J=2.2Hz, 1(s, 1H). Using a 75 MHz DMSO-d6 ^{13}C NMR spectrometer, we get the following spectral lines: 109.0, 116.6, 124.5, 125.0, 126.1, 128.1, 129.6, 130.0, 131.9, 137.4, 146.5, 147.3, 159.1, 165.7. MS m/z (%): $C_{16}H_{11}ClN_4O_3$ found to have an exact mass of 342.74, with component ions of 342.56 (M)+, 343.99 (M+1), 344.68 (M+2), and 345.07 (M+3).

Synthesis of 2-amino-6-fluoro-N-phenylquinoline-3-carboxamide (6i)

The following values for Vmax/cm^{-1} in the infrared (KBr): 3370, 3296 (N-H str., -NH_2), 1658(C=O str.), 2781 (C-H str., alkyl), and 1331 (C-F str.) 500 MHz ^1H NMR (Chloroform-d) 7.26-7.35m, 3H7.64-7.71m, 7.91dd, J=4.7, 8.0Hz, 1H), 8.58 d, J=2.2Hz, 1H, 9.76 s, 6.98 s, 7.05tt, J=1.2, 6.9Hz, 1H. (s, 1H). Values for the ^{13}C NMR at 75 MHz in DMSO-d6 are as follows: 109.0, 110.5, 119.4, 119.9, 124.5, 127.8, 128.0, 128.3, 128.1, 128.5, 131.0, 137.4, 146.5, 159.1, 159.7, and 165.7. (%) MS m/z $C_{16}H_{12}FN_3O$'s precise mass To get to 281.28, we first found 281.02 (M)+, then 282.03 (M+1)+, then 283.89 (M+2)+, and finally 284.01 (M+3)+.

Synthesis of 2-amino-N-(4-ethylphenyl)-6-fluoroquinoline-3-carboxamide (6j)

IR (KBr) Vmax/cm^{-1} values include, 1271 (C-H str., alkyl) (C-F str.), 1620(C=O str.), 2988(C-H str., alkyl), 3050(C-H str. aryl), and 3404(N-H str., -NH_2): 500 MHz ^1H NMR (Chloroform-d) 9.70(s, 1H), 8.58(d, J=2.2Hz,

1H), 7.91(dd, J=4.8, 8.0Hz, 1H), 7.68 dt(J=2.5, 12.0 Hz 1H), 7.46-7.52 MHz(m, 2H), 7.26-7.34MHz(m, 1H), 7.14dt (J=0.9, 8.0Hz, 2H), 6.98s(H), 2.64-2.72MHz(m, 2H), 1.21(t, J=7.3Hz, 3H), ^{13}C NMR(75 MHz, DMSO-d6): 14.6, 28.7, 109.0, 110.5, 117.9, 119.4, 124.5, 128.1, 129.9, 131.0, 137.4, 144.2, 146.5, 159.1, 159.7, 165.7. MS m/z (%): $C_{18}H_{16}FN_3O$ 309.34 g, found to have an exact mass of 309.38 (M)+, 310.01 (M+1)+, and 311.03 (M+2)+.

Synthesis of 2-amino-6-fluoro -N-(4-methoxyphenyl) quinoline-3-carboxamide (6k)

IR (KBr) Vmax/cm^{-1} values are 3419 (N-H str., -NH_2), 3064, 3028 (C-H str. aryl), 1677 (C=O str.), 1211 (C-F str.)^{1}H NMR (500 MHz, Chloroform-d): 3419 (N-H str., -NH_2), 3064, 3028 (C-H str. aryl), 1677 (C=O str.), 1211 (C-F str.) 3.78 (s, 3H), 6.88-6.94 (m, 2H), 6.98 (s, 2H), 7.26-7.34(m, 1H), 7.52-7.58(m, 2H), 7.68(dt, J=2.5, 12.0Hz, 1H), 7.91(dd, J=4.7, 8.0Hz, 1H), 8.58(d, J=2.2Hz, 1H), 9.84(s, 1H).56.0, 109.0, 110.5, 114.5, 119.4, 120.5, 124.5, 128.1, 131.0, 137.4, 146.5, 159.1, 159.6, 159.9, 159.7, 159.8, 165.7 were all observed by ^{13}C NMR (75MHz,DMSO-d6). MS m/z (%): 313.08(M+2)+, 312.25(M+1)+, 311.06(M)+, and 314.03 (M+3)+ were all found to be within 0.01% of the value obtained of 311.31 for $C_{17}H_{14}FN_3O_2$.

Synthesis of 2-amino-6-fluoro -N-(4-nitrophenyl) quinoline-3-carboxamide (6l)

IR (KBr) Vmax/cm^{-1} values includes 3370 (N-H str. >NH), 3075 (C-H str. aryl), 2984 (C-H str., alkyl), 1656 (C=O str.), 1317 (C-F str.) ^{1}H NMR (500 MHz, Chloroform-d) at 8.58 (d, J=2.2Hz, 1H), 8.20-8.26(m, 2H), 7.91(dd, J=4.8, 8.0Hz, 1H), 7.64-7.74(m, 3H), 7.26-7.34(m, 1H), 6.98(s, 2H), and 10.54 at 3370(N-H (s, 1H).The includes the application shifts were observed by ^{13}C NMR(75MHz, DMSO-d6): 109.0, 110.5, 116.6, 119.4, 124.5, 125.0, 128.1, 131.0, 137.4, 146.5, 147.3, 159.1, 159.7, 165.7. MS m/z (%): $C_{16}H_{11}FN_4O3$ had an exact mass of 326.28, and it was determined to be 326.08 (M)+, 327.02 (M+1)+, and 328.59 (M+2)+.

Synthesis of 2-amino-6-methoxy -N-phenyquinoline-3-carboxamide (6m)

Vmax/cm^{-1} at 3373 "(N-H str.>NH), 2922 (C-H str. aryl), 2851 (C-H str., alkyl), and 1677 (C=O str.) in the infrared (KBr). 500 MHz ^{1}H NMR (Chloroform-d): 3.80 (s, 3H), 6.98 (s, 2H), 7.22-7.30(m, 2H), 7.37-7.47 (m, 3H), 7.57(s, 5H), 7.54-7.61(m, 2H), 7.80(d, J=8.4Hz, 1H), 8.59(d, J=2.2Hz, 1H), 10.26.(s, 1H). ^{13}C NMR (75MHz, DMSO-d6): 56.0(1C,s), 106.4(1C,s), 109.0(1C,s), 119.9(2C,s), 121.2(1C, s), 124.5(1C, s), 127.6(1C,s), 127.8(1C, s), 128.2(2C,s), 130.9(1C,s), 137.4(1C, s), 146.5(1C, s), 158.1(1C,s), 159.1 (1C, s), 165.7(1C, s). MS m/z (%): 293.07 (M)+, 294.09 (M+1)+, and 295.07 (M+2)+ were all found to be the correct values for the formula mass of $C_{17}H_{15}N_3O_2$.

Synthesis of 2-amino-N-(4-ethylphenyl)-6-methoxyquinoline-3-carboxamide (6n)

These molecules have the following IR (KBr) Vmax/cm^{-1} values: 3361(N-H str., -NH_2), 3265(N-H str.>NH), 2759(C-H str. alkyl), 2840(C-H str.aryl), and 1603(C=O str.). 500MHz ^{1}H NMR(Chloroform-d): 1.21(t, J=7.3Hz, 3H), 2.64-2.72(m,2H), 3.80s, 2H, 6.98s, 2H, 7.14dt, J=0.9, 8.0Hz, 2H), 7.22-27.17m, 1H, 7.46-52m, 2H.(s, 1H), 7.29 d, J=2.3Hz, 1H,. The following signals were detected by ^{13}C NMR (75 MHz, DMSO-d6): 14.6 (1C, s), 28.7(1C, s), 56.0(1C, s), 106.4(1C, s), 109.0(1C, s), 117.9(2C, s), 121.2(1C, s), 124.5(1C, s), 127.6(1C, s), 129.9(2C, s)(1C, s).MS m/z (%): $C_{19}H_{19}N_3O_2$ had a precise mass of 321.37 and was found to have the following isotope abundances: 321.83 (M)+, 322.15 (M+1)+, 323.56 (M+2)+, 324.69 (M+3)+.

Synthesis of 2-amino-6-methoxy-N-(4-methoxyphenyl) quinoline-3-carboxamide (6o)

The following are the IR (KBr) Vmax/cm^{-1} values: N-H str., -NH_2: 3367; N-H str.,>NH: 3315; C-H str., aryl: 3119; C-H str., alkyl: 2931; C=O str., aryl: 1707; C-H str., aryl: 2931; C-H str., >NH: 3315; N-H These ^{1}H NMR spectra were produced using chloroform-d as the solvent and a spectrometer running at 500 MHz: 6.88-6.94 (m, 2H), 7.22-7.30(m,2H), 6.98(s,2H), 7.52-7.58(m,2H), 8.59(d, J=11.3Hz, 2H), 3.79 (d, J=11.3Hz, 4H) (s,1H). The following signals were detected by ^{13}C NMR (75 MHz, DMSO-d6): 56.0-56.0 (2C, 56.0(s), 56.0(s), 106.4(s), 109.0(s), 114.5(s), 120.5(s), 121.2(s), 124.5(s), 127.6(s), 130.9(s), and 137.4(s) (1C, s). $C_{18}H_{17}N_3O_3$ (precise mass) Values for the several

sequences include: 296.06 (M)+, 298.67 (M+1)+, 297.35 (M+2)+, and 299.81 (M+3)+.

Synthesis of 2-amino-6-methoxy-N-(4-nitrophenyl) quinoline-3-carboxamide (6p)

The following are the greatest infrared radiation reflection intensities (KBr): Other examples are C=O str, C-H alkyl 2936, C-H aryl2979, and N-H>NH 3308. 1698. 8.20-8.26(m,2H)(s,1H), 7.80(d,J=8.3Hz,1H), 7.68-7.74(m,2H), 7.22-7.30(m,2H), 6.98(s,2H), 3.80(s,3H), 8.20-8.26(m,2H), 10.54(^{1}H NMR, 500MHz, Chloroform-d), 8.59(d, J=2.1Hz,1H).The following signals were found using ^{13}C NMR (75 MHz, DMSO-d6): 146.5 (1C(1C,s), 137.4(1C,s), 130.9(1C,s), 127.6(1C,s), 125.0(2C,s), 124.5(1C,s), 121.2(1C,s), 116.6(2C,s), 109.0(1C,s), 106.4(1C,s), 56.0 (1C,s)". MS m/z (%): With an exact mass of 338.32, $C_{17}H_{14}N_4O_4$ has been found to have the isotopes 338.08 (M)+, 339.82 (M+1), 340.01 (M+2)+, and 341.06 (M+3)+.

CTG ASSAY

The ability of the potential synthesised drugs to stop the spread of MCF-7 cancer cells was evaluated using an MTT luminous cell viability assay.[15] To prepare the cells for the treatment, after seeding at 1 1×105 cells per well density in 96-well plates (the cell count has been obtained utilising Neubauer's chamber), and they were cultured at 370°C for 24hours. After 24 hours of development in DMEM with 10% foetal bovine serum (FBS), the cells had reached confluence (FBS), the cells have been subjected to successive dilutions of drug molecules with concentrations ranging from 10 µL to 80 µLEach well received 10µ l of the 5 mg/mL MTT reagent after the first 24 hours, and the plates underwent a further 4 hours of incubation. By swiping the plate, we were able to fully remove the medium. Next, we loaded each well with 200 µl of acidic isopropanol". After one hour, the absorbance at 492nm has been calculated using a 96 well plate reader. Here, the active drug was sunitinib, whereas the harmful control was DMSO.

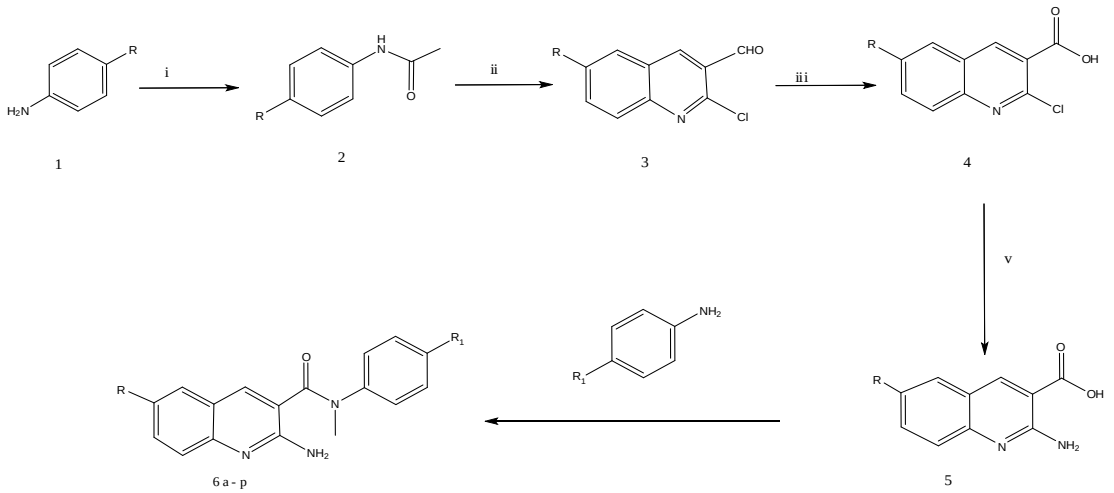
MOLECULAR DOCKING STUDY

Several programmes, including Discovery Studio Visualizer v20.1.0.19295, MGL Tools 1.5.6, and AutoDock 1.1.2, were used to study the molecular docking interactions of 2-aminoquinoline-3-carboxamide derivatives with the target receptor (PDB:5GRN).It was discovered that the RCSB-PDB database contains crystal structures of PDGFRA coupled to WQ-C-159 with the PDB Id 5GRN. Expressed at a resolution of 1.70 nanometres in human cells, 5GRN belongs to the family of transferase inhibitor.[21]

RESULTS AND DISCUSSION

Synthesis of 2-aminoquinoline-3-carboxamide derivatives

The method for making 2-aminoquinoline-3-carboxamide derivatives is set out in Scheme 1.Compound (2) was made by treating anilines containing substituent's (1) with acetic anhydride and acetic acid (2). The 2-chloroquinoline-3-carbaldehydes (3) were synthesised by cyclising compound 2 with DMF and $POCl_3$ at 150°C.[12]$NaH_2PO_4.2H_2O$ was used to hydrolyze compound 3 to yield 2-chloroquinoline-3-carboxylic acid (4)[13] which, after being heated in 26% aqueous NH3 at 150 °C, yielded 2-aminoquinoline-3-carboxylic acid (5). Two different types of 2-aminoquinoline-3-carboxamides (6) 14 were synthesised by reacting compound 5 with a number of substituted aryl amines in the presence of coupling agents including Et_3N, HOBt, along with DCC. Table 1 displays the results of the physical characterisation of the produced compounds 6a-6p.



Scheme 1. Synthetic route of 2-aminoquinoline-3-carboxamide derivatives 6a-p

Reaction conditions: A substituted arylamine, DCC, Et_3N, HOBt, DMF, 24 hours; acetic anhydride, CH_3COOH, 1 hour; DMF, $POCl_3$, 24 hours; butan-1-ol, $NaClO_2$, $NaH_2PO_4.2H_2O$, 2 hours; NH_3, 4 hours; and a substituted arylamine, NH_3, 4 hours.

Table 1. Physical characterization data of synthesized compounds 6a-6p

Compound ID	R	R_1	Molecular Formula	Melting Point (^{0}C)	Molecular weight	% yield
6a	H	H	$C_{16}H_{13}N_3O$	142-144	263.29	81
6b	H	C_2H_5	$C_{18}H_{17}N_3O$	164-166	291.35	86
6c	H	OCH_3	$C_{17}H_{15}N_3O_2$	182-184	293.32	88
6d	H	NO_2	$C_{16}H_{12}N_4O_3$	162-164	308.29	85
6e	Cl	H	$C_{16}H_{12}ClN_3O$	168-170	297.74	86
6f	Cl	C_2H_5	$C_{18}H_{16}ClN_3O$	170-172	325.79	89
6g	Cl	OCH_3	$C_{17}H_{14}ClN_3O_2$	122-124	327.76	91
6h	Cl	NO_2	$C_{16}H_{11}ClN_4O_3$	154-156	342.74	94
6i	F	H	$C_{16}H_{12}FN_3O$	138-140	281.28	96
6j	F	C_2H_5	$C_{18}H_{16}FN_3O$	160-162	309.34	83
6k	F	OCH_3	$C_{17}H_{14}FN_3O_2$	148-150	311.31	83
6l	F	NO_2	$C_{16}H_{11}FN_4O_3$	169-171	326.28	84
6m	OCH_3	H	$C_{17}H_{15}N_3O_2$	195-197	293.32	78
6n	OCH_3	C_2H_5	$C_{19}H_{19}N_3O_2$	166-168	321.37	79
6o	OCH_3	OCH_3	$C_{18}H_{17}N_3O_3$	169-171	323.35	78
6p	OCH_3	NO_2	$C_{17}H_{14}N_4O_4$	180-182	338.32	76

Cytotoxicity Assessment of Compounds 6a-p

In order to conduct cytotoxicity analyses, MCF-7 breast cancer cell line has been selected because to its high viability and low technical requirements. We chose this cell line due to its capacity to generate an endless supply of RNA/DNA for use in later validation and functional testing. It is known that PDGFR kinase's ability to govern the cell cycle is under the control of alpha expression. To find out whether the synthetic 2-aminoquinoline-3-carboxamides were cytotoxic to the MCF-7 cell line, we employed MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) test kit. After being given a treatment for 24 hours, the cells were subjected to inhibitor dosages ranging from 10µg/ml-80µg/ml [15-17]. Sunitinib's toxicity, a medicine used to treat the MCF-7 cell line, was compared to 16 newly discovered quinoline-3-carboxamides, four of which (6b, 6c, 6j, and 6o) showed comparable toxicity. The IC_{50} (µg/ml) values and percentages of inhibition against the MCF-7 cell line for compounds 6a-6p and Sunitinib are reported in Table 2, and Figure 3 depicts the same for the MCF-7 cell line. Table 2 demonstrates that when compared to the currently used PDGFR inhibitor, Sunitinib, the IC_{50} values of compounds 6b, 6c, 6j, and 6o for inhibiting the proliferation of MCF-7 cells were modest.

Table 2. % Control Growth against Breast Cancer Lines MCF-7

Compound ID	**Concentration of Compounds (IC_{50}µg/ml), where n=3**			
	10	**20**	**40**	**80**
6a	35.81	41.62	54.13	67.55
6b	44.67	47.67	67.13	77.26
6c	15.17	25.79	52.35	79.35
6d	19.06	22.51	41.16	65.04
6e	17.35	27.47	47.53	74.52
6f	21.84	27.66	48.51	71.42
6g	19.16	29.00	41.16	71.04
6h	19.35	26.47	49.53	72.52
6i	19.08	22.50	41.62	68.04
6j	16.84	25.66	48.51	75.42
6k	19.06	22.50	41.62	74.04
6l	22.26	31.35	53.71	68.12
6m	12.26	20.35	51.71	74.12
6n	17.26	24.47	48.35	75.57
6o	12.35	24.47	48.35	75.57
6p	19.06	22.50	42.62	71.04
Sunitinib	**29.06**	**33.02**	**64.18**	**81.98**

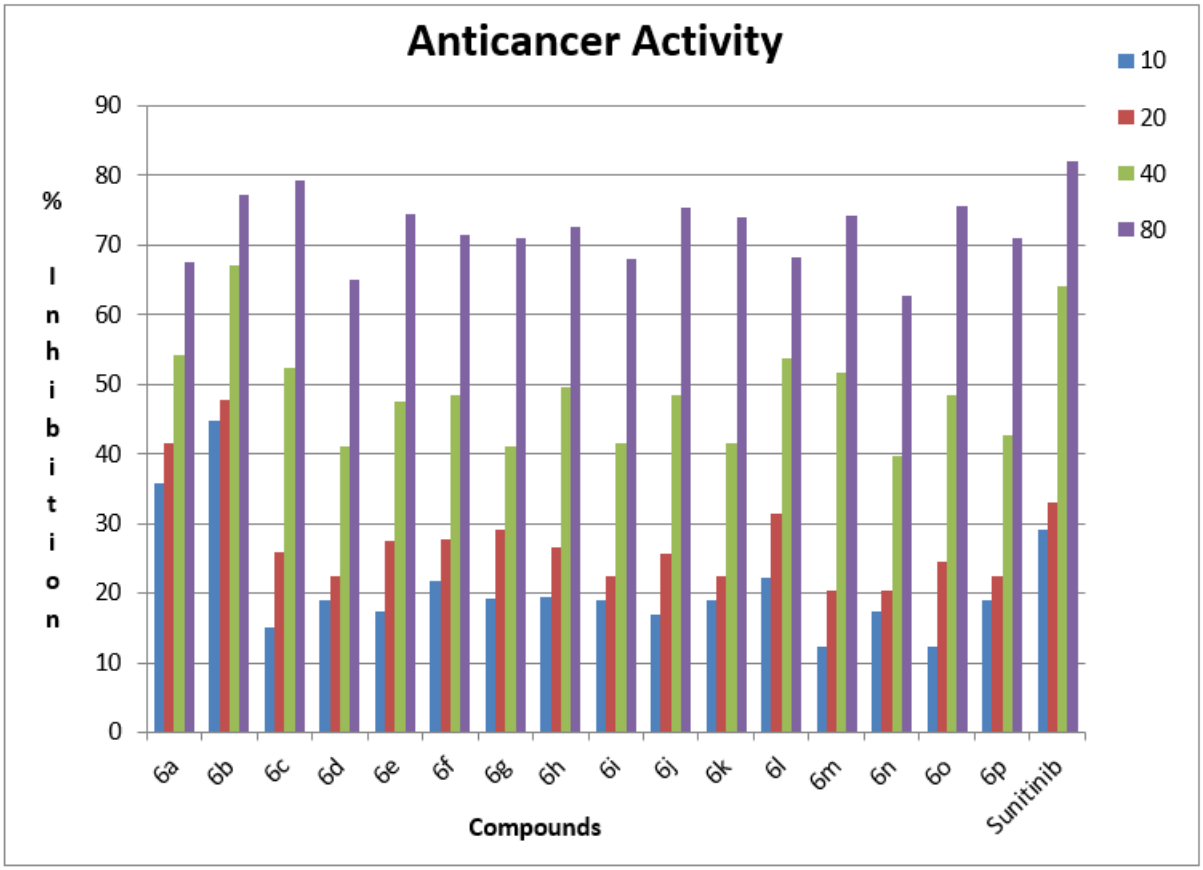


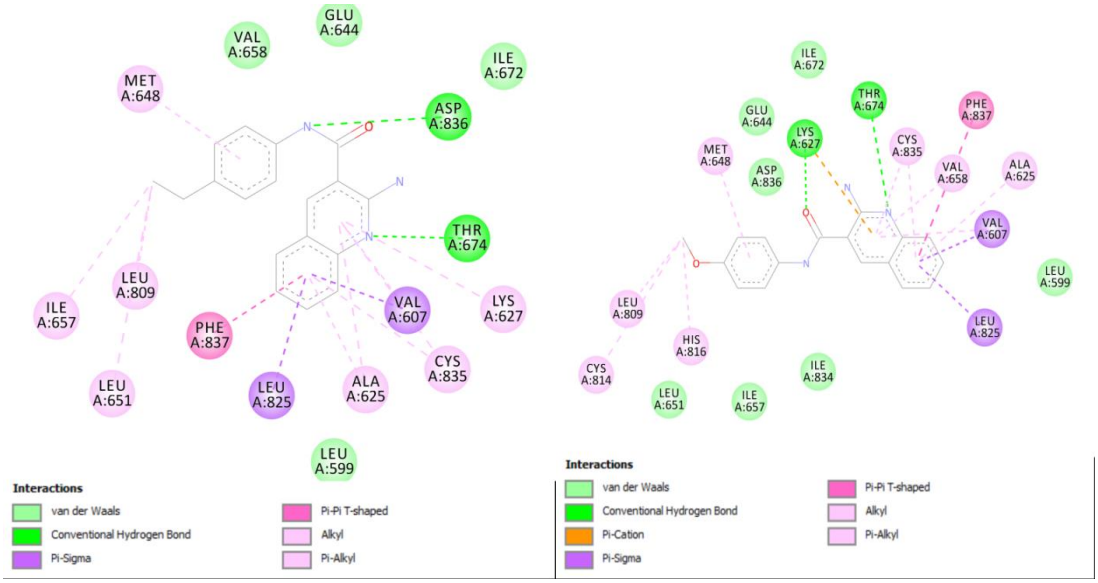
Figure 3. % inhibition of compounds 6a to 6p and Sunitinib

Docking studies

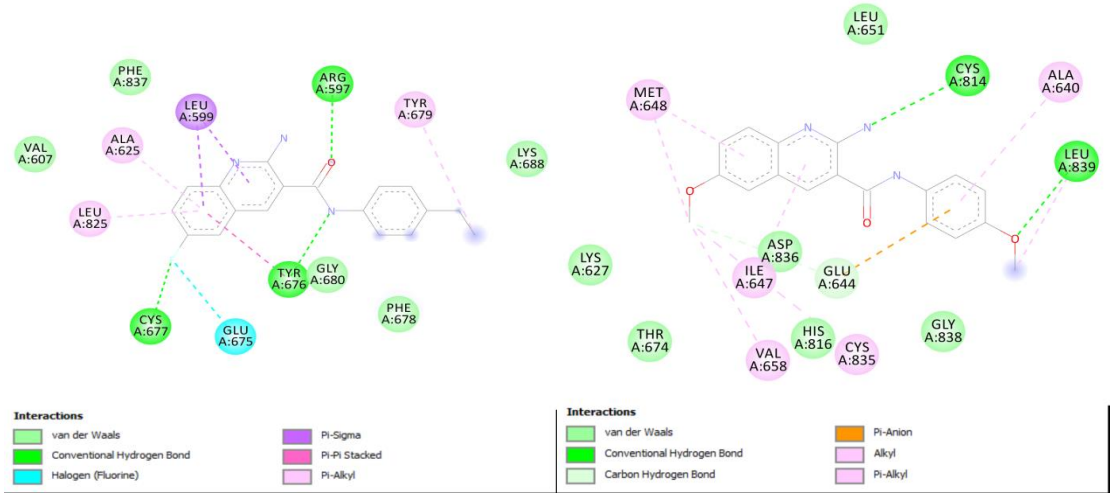
Many compounds' X-ray crystal structures, including Homo sapiens domain (5GRN) structure, were used to virtually dock a subset of synthesised 2-aminoquinoline-3-carboxamide derivatives as in Figure 4.[22] by using AutoDock vina docking software. Table 3 displays the comprehensive molecular docking data.

Table 3.Molecular binding affinities of AQCM derivatives as anti-cancer agents with PDGFR kinase inhibitor (PDB ID: 5GRN)

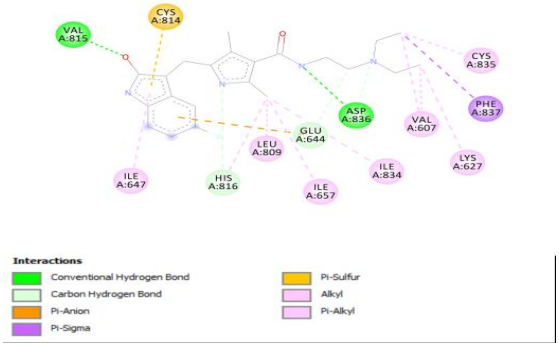
Compound ID	R	R_1	Number of H-bonds	Amino acids involved in hydrogen binding	Binding scores (kcal/mol)
6b	-H	$-C_2H_5$	2	Thr 674, Asp 836, Val 607, Leu 825	-10.8
6c	-H	$-OCH_3$	2	Thr 674, Lys 627, Val 607, Leu 825	-10.9
6j	-F	$-C_2H_5$	3	Arg 597, Tyr676, Cys 677,Glu 675, Leu 599	-11.0
6o	$-OCH_3$	$-OCH_3$	2	Cys 814, Leu 839,Ile 647, Met 648	-10.9
Sunitinib (STD)			2	Ile 834, Asp 836, Phe 837, Val 815	-7.4



6b 6c



6j 6o



Sunitinib (STD)

Figure 4. Docking of 6b, 6c, 6j, and 6o molecules with Sunitinib (STD) in 2D using Discovery Studio Visualizer.

Conclusion

Inhibiting PDGFR kinases selectively has been difficult due to their high degree of sequence similarity. In this research, we present the design along with preliminary synthesis of new small compounds with significant cytotoxic action, all of which are based on 2-aminoquinoline-3-carboxamide. The cytotoxicity of four 6b, 6c, 6j, and 6o of the produced chemicals was particularly impressive against MCF-7. Sunitinib is currently the only approved inhibitor of PDGFR, however the 2-aminoquinoline-3-carboxamides showed promise as a more selective alternative. Molecular docking analyses corroborated the findings from the experiments. In the future, PDGFR inhibitors that are more effective and selective will be used to treat breast cancer cells as a consequence of the knowledge obtained from our investigations.

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