Synthesis, Characterization and in vitro Anticancer Evaluation of Novel Quinoline-3-Carboxamide Derivatives as Inhibitors of PDGFR

Ganesh S. Mhaske[1, 2],* Ashim K. Sen[1], Ashish Shah[1], Dhanya B. Sen[1], Sagar A. Jadhav[3], Rahul H. Khiste[4]

[1]Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara-391760, Gujarat, India.
[2]Department of Pharmaceutical Chemistry, Indrayani Institute of Pharmaceutical Education and Research, Talegaon Dabhade, Pune-410507, Maharashtra, India.
[3]Department of Pharmaceutical Chemistry, Dr. Shivajirao Kadam College of Pharmacy. Kasbe Digraj, Sangli-416416
[4]Department of Pharmaceutical Chemistry, Marathwada Mitra Mandal's College of Pharmacy, Pune-411033, Maharashtra, India.

Corresponding author
Ganesh S. Mhaske[1,2] *
Ph.D. Research Scholar,
Department of Pharmacy,
Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, Gujarat, India.
E-mail address:hariomganesh79@gmail.com
ORCID ID: 0000-0002-3120-6314

Abstract

Cancer cells exploit transmembrane receptor protein kinases like platelet derived growth factor (PDGF) for their survival, which leads to the development of resistance towards anticancer agents. The importance of inhibiting PDGF receptor is well established. In this article, twelve novel substituted 2-aminoquinoline-3-carboxamide derivatives were synthesized from substituted anilines using Vilsmeier–Haack reaction, producing 2-chloro-3-carbaldehyde quinolines, followed by oxidation of 2-chloro-3-carbaldehyde to the carboxylic acid and coupling this group with various anilines done by using dicyclohexylcarbodiimide (DCC) coupling reagent to form amide bonds as potential inhibitors of PDGFR is reported. The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. SAR studies suggested the importance of the electron-donating nature of the R group for the molecule to be toxic. The cytotoxicity assay of synthesized compounds was performed against breast cancer cell line (MCF-7) and found promising results. The results obtained in vitro cytotoxicity evaluation study revealed the superior activity of three derivatives (6a, 6b, and 6i) compared with that of imatinib. In conclusion, these experiments will lay the groundwork for the evolution of potent and selective PDGFR inhibitors for the treatment of cancer cells.

Keywords:PDGFR, Breast Cancer, Quinoline-3-carboxamides derivatives, Synthesis, Imatinib.
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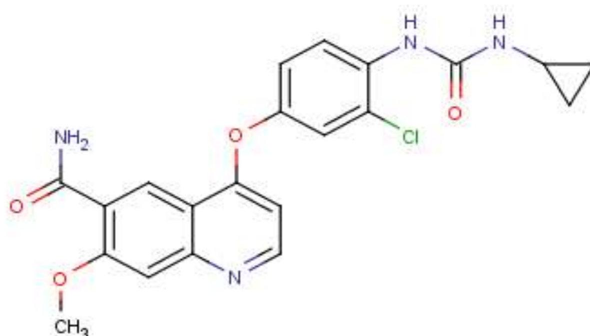
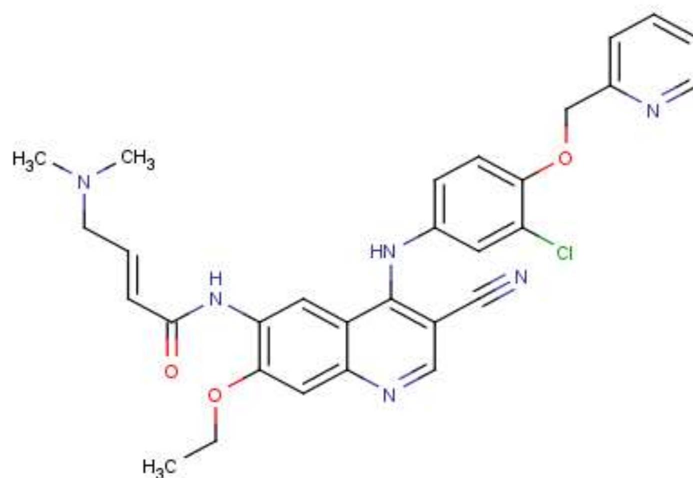
Introduction

Cancer is a first/second leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths. Among many types of cancers, breast cancer is the most common reason responsible to increase mortality rate worldwide. In 2020, there were 2.3 million women diagnosed with breast cancer and 685 000 deaths globally.[1] Breast cancer treatment can be highly effective which includes anti-cancer medicines given by mouth or intravenously to treat and/or reduce the risk of the cancer spreading metastasis. Despite the approval of a number of chemotherapeutics by the Food and Drug Administration (FDA) over the last two decades, there are still many challenges in finding an effective cancer therapy without considerable toxicity. Therefore, as part of global plan seeking novel effective discovery of anticancer agents is of great interest in the field of medicinal chemistry [1-2].

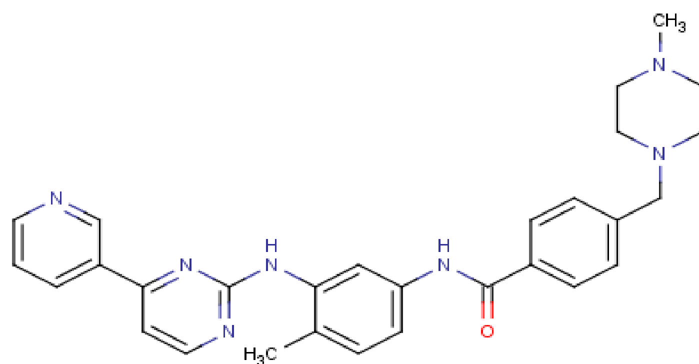
Receptor tyrosine kinases (RTKs) are transmembrane proteins composed Platelet-derived growth factor (PDGF) is a critical regulator of cell proliferation, migration and angiogenesis in various cells [3].The PDGF family consists of five isoforms (PDGF-AA, -BB, -AB, -CC, and -DD) and differentially binds to two receptor tyrosine kinases (RTKs), PDGFRα and PDGFRβ. The different receptors bind with the ligands with different affinities. Activated PDGFRα and β subsequently trigger downstream signal transduction pathways, including extracellular signal-regulated kinase 1/2 (ERK) and phosphatidylinositol 3-kinase (PI3K)/AKT for promoting cell proliferation, migration and survival. In particular, breast cancers with high PDGFRα expression are associated with lymph node metastasis and human epidermal growth factor receptor 2 (HER2) positivity. Thus, PDGFR are considered novel target to develop tyrosine kinase inhibitors that suppress the over expression of cancer cell and maintain physiological balance [4].



Lenvatinib (1)

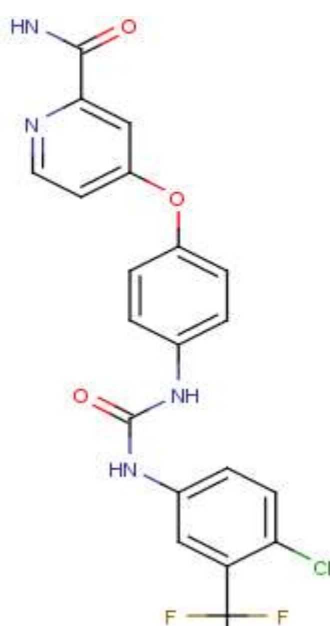


Neratinib (2)



Imatinib (3)

Sunitinib (4)



Sorafenib (5)

Quinoline is naturally present in many alkaloids having potent antitumor activity, for example, camptothecin. Quinoline scaffold and its related derivatives represent a broad spectrum of pharmacological activities, especially in drug discovery of new anticancer agents. Some of these attempts were successful in the development of a number of medications that were accepted by the FDA for the treatment of cancer [Figure **1**]. These include, but are not limited to, lenvatinib (1), neratinib (2), imatinib (3), sunitinib (4) and sorafenib (5). Food and Drug Administration (FDA) has approved various quinoline small molecules acting as protein kinases inhibitors for clinical uses in cancer disease [5-8]. The occurrence of the nitrogen atom in the quinoline nucleus withdraws electrons by resonance and interferes with the equal distribution of p electron density. It has been reported that the quinoline nucleus has a great tendency to bind to the active site of various

proteins via the formation of hydrogen bonds with its nitrogen atom and p–p stacking complexes with complementary amino acid residues [9-10].[Figure. **2]** shows the design of the novel scaffold. The R group was rationally chosen which includes halogens, alkyl, haloalkyl, and oxy-haloalkyl for studying the effect of their electronic properties on cell toxicity.

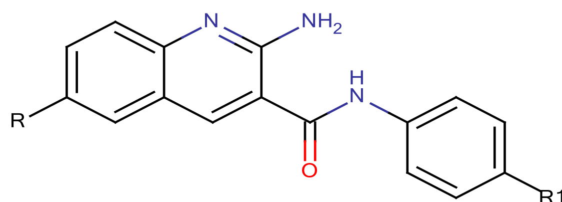


Figure 2. Designed 2-Amino Quinoline-3-Carboxamide Scaffold

Here, for the first time, a new set of 2-aminoquinoline-3-carboxamides, as potential anticancer agents, is reported. Their cytotoxicity was assessed by performing MTT assay against cancer cell line MCF-7 (breast cancer cell line). Additionally, CTG assay was performed with MCF-7 cells to confirm the inhibition of PDGFR kinase. Structure-activity relationship (SAR) studies correlated the importance of electron-donating R group with the activity of the compound.

Materials and Methods

All chemicals, solvents and reagents used for the synthetic work were of Sigma Aldrich Laboratory grade. The solvents were purified by the established methods. All the residues have been dried in vacuum desiccators as well as in hot air oven and recrystallized from appropriate solvent. The percentage yields are calculated after the purification of the compound.

The melting points of the compounds were determined in open capillaries using Thieles tube. The melting points, reported herein, are in the Celsius scale (°C) and are uncorrected. Precoated silica gel-G plate (E. Merck Kieselgel $60F_{254}$) activated at 110 °C for 30 min were used for thin layer chromatography and the spots were developed in UV cabinet as well as in iodine chamber. R_f values are reported for better comparable solvent systems, which are mentioned in text.

The IR spectra of compounds were recorded using KBr pellets on Jasco/FT/IR-4100A spectrophotometer at Vineet Analytical Lab, Pune. ^{1}H-NMR spectra were recorded on Bruker AVIII500 (500 MHz) using tetramethylsilane (TMS) as internal standard with chloroform-d as solvent at Vineet Analytical Lab, Pune, India. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS in $CDCl_3$ solution. Signal multiplicities are presented by s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet) and m (multiplet). ^{13}C-NMR spectra were recorded on Bruker AV600 (600 MH_Z) using tetramethylsilane (TMS) as internal standard with dimethyl sulfoxide (DMSO-d6) as solvent. Mass spectra were recorded using Bruker Compass Data Analys is 4.2 at Research Instrumentation Facility, Vineet Analytical Lab, Pune .

Anticancer activity on breast cancer cell line MCF-7, was carried out at Research Instrumentation Facility, Vineet Analytical Lab, Pune .

General Synthetic Procedure

Step-1

General procedure for synthesis of para substituted Acetanilide derivatives 2

Acetic anhydride (10ml,0.1 mol) and glacial acetic acid (10ml, 0.175 mol) were added to substituted anilines(0.1 mol), heated gently under reflux for 1 h, and poured into ice water (200 ml),where crystals of the acetylated product was formed. The crystals were filtered and recrystallized with acetic acid: water (1:2), producing the acetanilide in yields of between 94 and 100%.

Step-2

General procedure for synthesis of 2-chloroquinoline-3-carbaldehydes 3

Substituted 2-chloroquinoline-3-carbaldehydes were prepared according to the method in Toth et al. [11]. Briefly, dry N,N,-dimethyl formamide (DMF) (9.6 ml; 0.125 mol) was transferred under inert conditions to a two-neck round bottom flask, placed in an ice bath (0°C) to which phosphoryl chloride (POCl3) (32.2 ml; 0.35 mol) was added drop wise with constant stirring. The appropriate acetanilide (0.05mol) was then added and heated under reflux with a condenser fitted with a $CaCl_2$ drying tube at 82°C for 24 h. The reaction was monitored to completion using TLC. Upon completion, the mixture was cooled, poured into 300 ml ice water, and stirred for 1 h at 0– 10°C. A pale yellow precipitate formed and was filtered off, washed with 100 ml water, and dried producing 2-chloroquinoline-3-carbaldehydes in yields ranging from 43 to 74%.

Step-3

General procedure for the oxidation of 2-chloroquinoline-3-carbaldehydes 3 to 2-chloroquinoline-3-carboxylic acids 4

The procedure in George et al. [12] was carried out with modifications. The substituted 2-chloroquinoline-3- carbaldehydes (4.68 mmol) was dissolved in n-butanol (89 ml), and a solution of sodium dihydrogen orthophosphate (5.56 g; 35.61 mmol) and sodium chlorite (4.29 g; 47.47 mmol) in water (39 ml) was gradually added and stirred for 2 h at room temperature. The reaction was monitored to completion by TLC, concentrated in vacuo, and diluted with water (68 ml) and ethyl acetate (50 ml). Sodium carbonate (5 g) was added to the mixture to separate the two layers and the lower aqueous layer acidified with HCl to pH 4 where the 2-chloroquinoline-3-carboxylic acids 4 precipitated as cream-colored compounds with yields of between 54 and 72%.

Step-4

General procedure for the synthesis of 2-aminoquinoline-3-carboxylic acids 5 from 2-chloroquinoline-3-carboxylic acids 4

Suspension of corresponding 2-chloroquinoline-3-carboxylic acids 3 (1mmol) in26% aqueous NH_3 (5ml) was heated in stainless steel autoclave at 150°C during 4 hr. After reaction mixture cooling, clear solution was acidified by 5% aqueous HCl solution to pH-4.Solid products 6a–y were filtered and recrystallized from IPA–DMF mixture.

Step-5

General procedure for synthesis of 2-amino-N-phenylquinoline-3-carboxamides (6a–y)

The procedure used by Li et al. [13] for the synthesis of quinoline-3-carboxamides was followed with modifications. DCC (0.99 mmol) and 1-hydroxybenzotrizole (HOBt) (0.99 mmol) were added sequentially at room temperature to the substituted quinoline-3-carboxylic acids 4 (0.9 mmol) dissolved in 10 ml DMF. The reaction mixture was stirred for 30 min before adding the appropriate substituted aryl amines (0.99 mmol) and triethyl amine (1.895 mmol) to the mixture and stirring at room temperature for 24 h. Upon completion, the reaction mixture was poured into ice water, where it formed a whitish yellow colored precipitate. This was washed with water, dried, and purified using column chromatography on silica gel with hexane: ethyl acetate (9:1) producing 2-amino-N- phenylquinoline-3-carboxamide derivatives 6a–y in yields of between 76 and 96%.

Compound 6a Column Chromatography: 70%; hexane: ethyl acetate; Yield: 81%

IR (KBr) V_{max}/cm^{-1}: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*): δ 6.50 (s, 2H), 7.05 (*J* = 1.2, 6.9 Hz, 1H), 7.35 – 7.28 (m, 2H), 7.49 (ddd, *J* = 1.2, 7.7, 9.1 Hz, 1H), 7.73 – 7.64 (m, 3H), 7.88 – 7.82 (m, 1H), 7.95 – 7.89 (m, 1H), 8.69 (d, *J* = 2.3 Hz, 1H), 9.83 (s, 1H, NH).

^{13}C NMR (75 MHz, DMSO-d6): δ 109.0, 119.9, 124.5, 126.4, 127.7, 127.8, 128.2, 128.5, 129.4, 130.4, 137.4, 146.5, 159.1, 165.7.

MS *m/z* (%): **Exact Mass** of $C_{16}H_{13}N_3O$ 263.23, **Found** 263.11 $(M)^+$, 264.10 $(M+1)^+$, 265.10 $(M+2)^+$, 266.01 (M+3)+

Compound 6b Column Chromatography: 70%; hexane: ethyl acetate; Yield: 86%

IR (KBr) V_{max}/cm^{-1}: 3423 (N-H str., $-NH_2$), 3321 (N-H str. >NH), 3190 (C-H str. aryl), 2924, 2853 (C-H str., alkyl), 1636 (C=O str.)

^{1}H NMR (500 MHz, Chloroform-*d*): δ 1.21 (t, *J* = 7.3 Hz, 3H), 2.72 – 2.64 (m, 2H), 6.50 (s, 2H), 7.17 – 7.11 (m, 2H), 7.53 – 7.46 (m, 3H), 7.69 (td, *J* = 7.9, 1.0 Hz, 2H), 7.88 – 7.82 (m, 1H), 7.95 – 7.89 (m, 1H), 8.69 (d, *J* = 2.3 Hz, 1H), 9.85 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 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* (%): **Exact Mass** of $C_{18}H_{17}N_3O$ 291.35, **Found** 291.01 $(M)^+$, 292.87 $(M+1)^+$, 293.38 $(M+2)^+$, 294.02 $(M+3)^+$

Compound 6c Column Chromatography: 70%; hexane: ethyl acetate; Yield: 88%

IR (KBr) V_{max}/cm^{-1}: 3360, 3255 (N-H str., $-NH_2$), 3067 (C-H str. aryl), 2851 (C-H str., alkyl), 1688 (C=O str.)

^{1}H NMR (500 MHz, Chloroform-*d*): δ 3.78 (s, 3H), 6.50 (s, 2H), 6.88 – 6.94 (m, 2H), 7.46 – 7.52 (m, 1H), 7.52 – 7.58 (m, 2H), 7.69 (td, *J* = 1.0, 7.9 Hz, 1H), 7.82 – 7.88 (m, 1H), 7.92 (ddd, *J* = 1.1, 2.2, 8.8 Hz, 1H), 8.69 (d, *J* = 2.4 Hz, 1H), 9.93 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 56.0, 109.0, 114.5, 120.5, 124.5, 126.4, 127.7, 128.5, 129.4, 130.4, 137.4, 146.5, 159.1, 159.8, 165.7.

MS *m/z* (%): **Exact Mass** of $C_{17}H_{15}N_3O_2$ 293.32 **Found** 293.07 $(M)^+$, 294.09 $(M+1)^+$, 295.07 $(M+2)^+$

Compound 6d Column Chromatography: 70%; hexane: ethyl acetate; Yield: 85%

IR (KBr) V_{max}/cm^{-1}: 3360, 3255 (N-H str., $-NH_2$), 3067 (C-H str. aryl), 2851 (C-H str., alkyl), 1688 (C=O str.)

^{1}H NMR (500 MHz, Chloroform-*d*): δ 6.50 (s, 2H), 7.46 – 7.53 (m, 1H), 7.66 – 7.74 (m, 3H), 7.82 – 7.88 (m, 1H), 7.89 – 7.95 (m, 1H), 8.20 – 8.26 (m, 2H), 8.69 (d, *J* = 2.4 Hz, 1H), 10.64 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 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, 165.7.

MS *m/z* (%): Exact Mass of $C_{16}H_{12}N_4O_3$ 308.29 **Found** 308.13 $(M)^+$, 309.16 $(M+1)^+$, 310.18 $(M+2)^+$

Compound 6e Column Chromatography: 70%; hexane: ethyl acetate; Yield: 86%

IR (KBr) V_{max}/cm^{-1}: 3371, 3269 (N-H str. $-NH_2$), 1659 (C=O str.), 736 (C-Cl bend.)

^{1}H NMR (500 MHz, Chloroform-*d*): δ 6.45 (s, 2H), 7.05 (tt, *J* = 1.2, 6.9 Hz, 1H), 7.28 – 7.35 (m, 1H), 7.56 (dd, *J* = 2.2, 8.2 Hz, 2H), 7.64 – 7.71 (m, 2H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.91 (t, *J* = 2.2 Hz, 1H), 8.63 (d, *J* = 2.2 Hz, 1H), 9.70 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 109.0, 119.9, 124.5, 126.1, 127.8, 128.0-128.3, 128.1, 128.2, 129.6, 130.0, 131.9, 137.4, 146.5, 159.1, 165.7.

MS *m/z* (%): Exact Mass of $C_{16}H_{12}ClN_3O$ 297.74 **Found** 297.06 $(M)^+$, 298.35 $(M+1)^+$, 299.67 $(M+2)^+$, 300.01 $(M+3)^+$

Compound 6f Column Chromatography: 70%; hexane: ethyl acetate; Yield: 89%

IR (KBr) V_{max}/cm^{-1}: 3323 (N-H str., >N-H str.), 3046 (C-H str. aryl), 2925 (C-H str. aryl), 2847 (C-H str., alkyl), 1655 (C=O str.), 784 (C-Cl bend.)

^{1}H NMR (500 MHz, Chloroform-*d*): δ 1.21 (t, *J* = 7.3 Hz, 3H), 2.64 – 2.72 (m, 2H), 6.45 (s, 2H), 7.11 – 7.17 (m, 2H), 7.46 – 7.52 (m, 2H), 7.56 (dd, *J* = 2.2, 8.2 Hz, 1H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.91 (t, *J* = 2.2 Hz, 1H), 8.63 (d, *J* = 2.2 Hz, 1H), 9.70 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 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* (%): Exact Mass of $C_{18}H_{16}ClN_3O$ 325.79 **Found** 325.10 $(M)^+$, 326.08 $(M+1)^+$, 327.56 $(M+2)^+$,328.04 $(M+4)^+$

Compound 6g Column Chromatography: 70%; hexane: ethyl acetate; Yield: 91%

IR (KBr) V_{max}/cm^{-1}: 3308 (N-H str. >NH), 2979, 2927 (C-H str., alkyl), 1698 (C=O str.), 725 (C-Cl bend.)

^{1}H NMR (500 MHz, Chloroform-*d*): δ 3.78 (s, 3H), 6.45 (s, 2H), 6.88 – 6.94 (m, 2H), 7.52 – 7.58 (m, 3H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.91 (t, *J* = 2.2 Hz, 1H), 8.63 (d, *J* = 2.2 Hz, 1H), 9.79 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 56.0, 109.0, 114.5, 120.5, 124.5, 126.1, 128.1, 129.6, 130.0, 131.9, 137.4, 146.5, 159.1, 159.8, 165.7.

MS *m/z* (%): Exact Mass of $C_{17}H_{14}ClN_3O_2$ 327.76 **Found** 327.08 $(M)^+$, 328.09 $(M+1)^+$, 329.16 $(M+2)^+$,330.08 $(M+3)^+$

Compound 6h Column Chromatography: 70%; hexane: ethyl acetate; Yield: 94%

IR (KBr) V_{max}/cm^{-1}: 3343 (N-H str. >NH), 3089 (C-H str. aryl), 1638 (C=O str.), 780 (C-Cl str.)

^{1}H NMR (500 MHz, Chloroform-*d*): δ 6.98 (s, 2H), 7.56 (dd, *J* = 2.2, 8.2 Hz, 1H), 7.68 – 7.74 (m, 3H), 7.91 (t, *J* = 2.2 Hz, 1H), 8.20 – 8.26 (m, 2H), 8.63 (d, *J* = 2.2 Hz, 1H), 10.54 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 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* (%): Exact Mass of $C_{16}H_{11}ClN_4O_3$ 342.74 **Found** 342.56 $(M)^+$, 343.14 $(M+1)^+$, 344.68 $(M+2)^+$,345.07 $(M+3)^+$

Compound 6i Column Chromatography: 70%; hexane: ethyl acetate; Yield: 96%

IR (KBr) V_{max}/cm^{-1}: 3370, 3296 (N-H str., -NH_2), 2781, 2637 (C-H str., alkyl), 1658 (C=O str.), 1331 (C-F str.)

^{1}H NMR (500 MHz, Chloroform-*d*): δ 6.98 (s, 2H), 7.05 (tt, *J* = 1.2, 6.9 Hz, 1H), 7.26 – 7.35 (m, 2H), 7.64 – 7.71 (m, 3H), 7.91 (dd, *J* = 4.7, 8.0 Hz, 1H), 8.58 (d, *J* = 2.2 Hz, 1H), 9.76 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 109.0, 110.5, 119.4, 119.9, 124.5, 127.8, 128.0, 128.3, 128.1, 128.2, 131.0, 137.4, 146.5, 159.1, 159.7, 165.7.

MS *m/z* (%): Exact Mass of $C_{16}H_{12}FN_3O$ 281.28 **Found** 281.02 $(M)^+$, 282.03 $(M+1)^+$, 283.89 $(M+2)^+$, 284.01 $(M+3)^+$

Compound 6j Column Chromatography: 70%; hexane: ethyl acetate; Yield: 83%

IR (KBr) V_{max}/cm^{-1}: 3404 (N-H str., -NH_2), 3050 (C-H str. aryl), 2988 (C-H str., alkyl), 1620 (C=O str.), 1271 (C-F str.)

^{1}H NMR (500 MHz, Chloroform-*d*): δ 1.21 (t, *J* = 7.3 Hz, 3H), 2.64 – 2.72 (m, 2H), 6.98 (s, 2H), 7.14 (dt, *J* = 0.9, 8.0 Hz, 2H), 7.26 – 7.34 (m, 1H), 7.46 – 7.52 (m, 2H), 7.68 (dt, *J* = 2.5, 12.0 Hz, 1H), 7.91 (dd, *J* = 4.8, 8.0 Hz, 1H), 8.58 (d, *J* = 2.2 Hz, 1H), 9.70 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 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* (%): Exact Mass of $C_{18}H_{16}FN_3O$ 309. 34 **Found** 309.38 $(M)^+$, 310.01 $(M+1)^+$, 311.03 $(M+2)^+$

Compound 6k Column Chromatography: 70%; hexane: ethyl acetate; Yield: 83%

IR (KBr) V_{max}/cm^{-1}: 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*): δ 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, 12.0 Hz, 1H), 7.91 (dd, *J* = 4.7, 8.0 Hz, 1H), 8.58 (d, *J* = 2.2 Hz, 1H), 9.84 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 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.

MS *m/z* (%): Exact Mass of $C_{17}H_{14}FN_3O_2$ 311.31 **Found** 311.06 $(M)^+$, 312.25 $(M+1)^+$, 313.08 $(M+2)^+$, 314.03 $(M+3)^+$

Compound 6l Column Chromatography: 70%; hexane: ethyl acetate; Yield: 84%

IR (KBr) V_{max}/cm^{-1}: 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*): δ 6.98 (s, 2H), 7.26 – 7.34 (m, 1H), 7.64 – 7.74 (m, 3H), 7.91 (dd, *J* = 4.8, 8.0 Hz, 1H), 8.20 – 8.26 (m, 2H), 8.58 (d, *J* = 2.2 Hz, 1H), 10.54 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d6): δ 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* (%): Exact Mass of $C_{16}H_{11}FN_4O_3$326.28 **Found** 326.08 $(M)^+$, 327.02 $(M+1)^+$, 328.59 $(M+2)^+$

CTG Assay

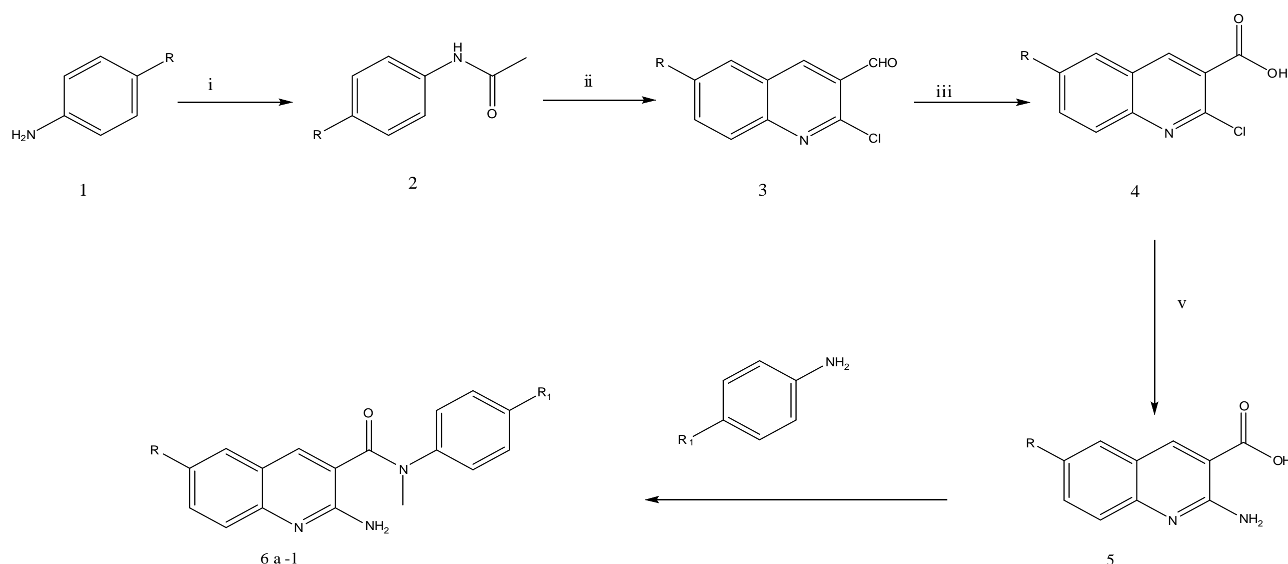
Growth inhibition of MCF-7 cancer cells by the synthesized drug candidates were evaluated by performing MTT luminescent cell viability assay. [14] Cells were seeded at a density of $1{\times}10^5$ cells/ml per well in 96-well plates (cell count was taken on Neubauer's chamber) incubated at 37^0C for 24 hours before treatment. Cells were treated with various concentrations of drug molecules (from 10 µL to 80 µL) by doing serial dilution in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal bovine serum (FBS) and incubated for 24 hours. After 24 hours, 10 µL of 5 mg/ml MTT reagent was added in each well and incubated for 4 hours. The entire medium was removed by flicking the plate and 200 µl of acidic isopropanol was added in each well. After 1hr the absorbance was measured after 1 hour at 492 nm on 96 well plate reader. Imatinib was used as a positive control, and DMSO was used as a negative control.

Results and Discussion:

Synthesis of 2-aminoquinoline-3-carboxamide derivatives

2-aminoquinoline-3-carboxamide derivatives were synthesized by following Scheme **1**.

Substituted anilines (1) were treated with acetic anhydride and acetic acid to yield compound (2). Compound 2 was cyclized in the presence of DMF and $POCl_3$ at 150°C to yield 2-chloroquinoline-3-carbaldehydes (3) [11]. Compound 3 was subjected to hydrolysis by treating it with $NaH_2PO_4.2H_2O$ to obtain 2-chloroquinoline-3-carboxylic acid (4) [12], which was converted to 2-aminoquinoline-3-carboxylic acid (5) when heated at 150°C in 26% aqueous NH_3. Compound 5 was converted to the various 2-aminoquinoline-3-carboxamides (6) by treating it with various substituted aryl amines in the presence of coupling reagent DCC, HOBt and Et_3N [13].



Scheme 1. Synthesis of 2-aminoquinoline-3-carboxamide derivatives

Reaction conditions: (i) Acetic anhydride, CH_3COOH, 1 hr; (ii) DMF, $POCl_3$, 24 hrs;(iii) $NaClO_2$,

$NaH_2PO_4.2H_2O$, butan-1-ol, 2hrs; (iv) NH_3, 4hrs; (v) Substituted arylamine, DCC, Et_3N, HOBt, DMF, 24hrs.

Compound ID	R	R_1
6a	H	H
6b	H	C_2H_5
6c	H	OCH_3
6d	H	NO_2
6e	Cl	H
6f	Cl	C_2H_5
6g	Cl	OCH_3
6h	Cl	NO_2
6i	F	H
6j	F	C_2H_5
6k	F	OCH_3
6l	F	NO_2

Table 1. R and R_1 substituent's of compound 6

Cytotoxicity Assessment of Compounds 6a-l

MCF-7, breast cancer cell line, was chosen to study the cytotoxic properties of the synthesized compounds. This particular cell line was chosen because of the ability to generate an unlimited amount of RNA/DNA to enable validation and downstream functional studies. It has been seen that PDGFR kinase can regulate the cell cycle through alpha expression. Therefore, the cytotoxicity of the synthesized quinoline-3-carboxamides against MCF-7 was assessed using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay kit. [15-17] The cells were treated with a varying range of inhibitor concentrations (10 µg/ml - 80 µg/ml) for 24 hrs. From the set of twelve novel quinoline-3-carboxamides, three compounds 6a, 6b, and 6h exhibited levels of toxicity that were comparable with Imatinib, MCF-7 in cell line. Fig. 3 shows the IC_{50} curves of compounds 6a to 6l and Imatinib, and Table 2 gives the IC_{50} (µg/ml) for 6a - l, and imatinib against MCF-7 cell line. From Table 2, it is evident that the compounds 6a, 6b, and 6i inhibited the MCF-7 cell growth with IC_{50} values that were moderate compared with that of the existing PDGFR inhibitor, Imatinib.

Compound ID	Concentrations of compounds (IC_{50} µg/ml), n=3			
	10	**20**	**40**	**80**
6a	**35.38**	**47.29**	**60.29**	**86.29**
6b	**33.87**	**47.67**	**63.38**	**81.24**
6c	22.25	37.12	5.12	73.34
6d	28.95	41.83	54.83	77.83
6e	24.25	38.98	54.57	72.37
6f	27.78	29.64	42.95	65.26
6g	29.69	42.47	55.32	78.29
6h	26.45	43.94	56.93	79.68
6i	**37.43**	**51.96**	**67.43**	**84.26**
6j	29.61	43.39	59.61	77.67
6k	30.45	44.85	60.45	78.32
6l	30.65	44.24	60.51	78.83
Imatinib	**50.58**	**64.13**	**80.26**	**97.84**

Table 2: % Control growth against Breast Cancer lines MCF-7

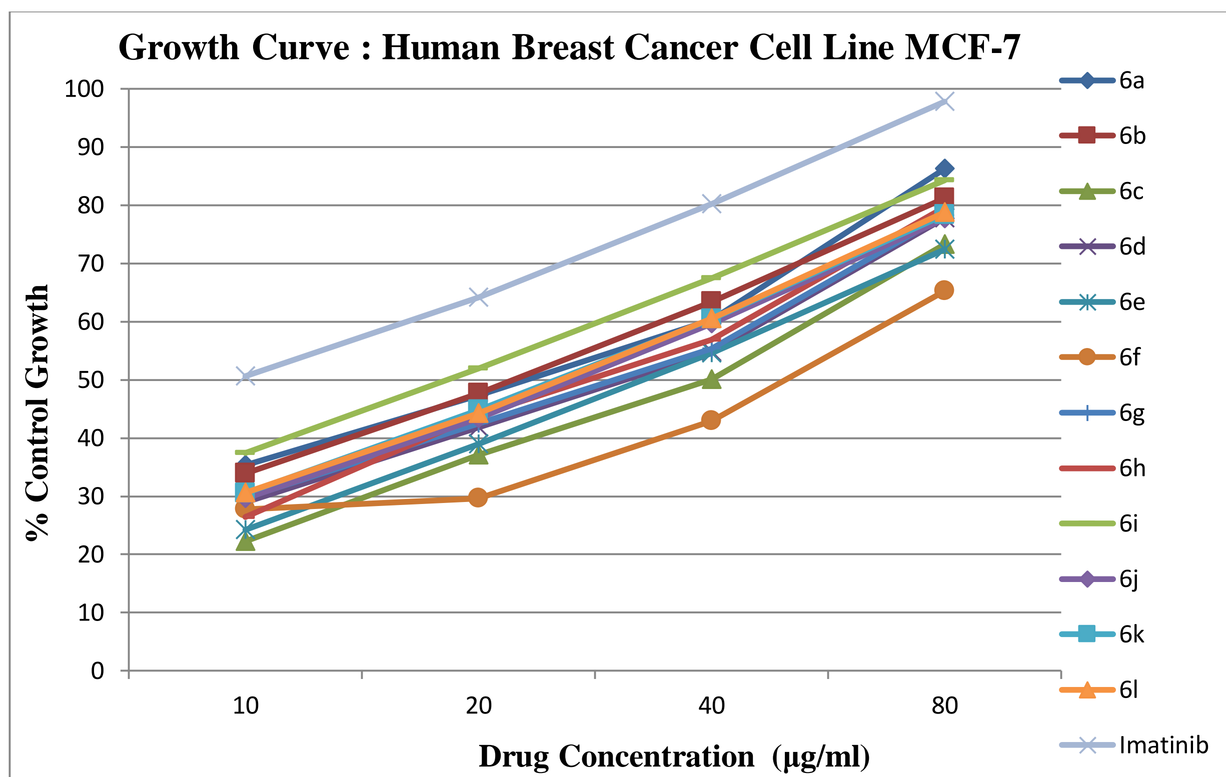


Figure 3: IC_{50} curves of compounds 6a to 6l and Imatinib

Structure-Activity Relationship (SAR)

Structure-Activity relationship helps in understanding why a certain molecule is toxic and relating it to its structure forms the basis of medicinal chemistry and drug discovery upon analyzing the structure-activity relationship (SAR), it was understood that the substituent R_1 in 6a, 6b, and 6i were electron-donating in nature (-H in 6a, $-C_2H_5$ in 6b, and –F in 6i). Though fluorine is an electron-withdrawing group owing to its high electronegativity, it is known that it can act as a π-donor.[18]

Since compound 6a showed relatively higher cytotoxicity in comparison with 6b and 6i, the ortho (6a') and meta (6a") variants of the same were synthesized. On performing the cytotoxicity assay of the variants, the following trend in the IC_{50} (µg/ml)values was observed.

meta >>> ortho > para

This trend in the **IC_{50} (µg/ml)** values is in accordance with the electron-donating power of R_1group based on its position, i.e., the donating nature of an electron-donating group (EDG) is the highest at the ortho and para positions and lowest lowest at the meta position rendering the meta variant to be least cytotoxic. Ortho (6a') variant is less toxic in comparison with that of compound 6b, may be because of the steric effect.

Conclusion:

Selective inhibition of the PDGFR kinases has been a huge challenge since they share a high percentage of sequence similarity. Here, a new set of 2-aminoquinoline-3-carboxamide based small molecules, as potent cytotoxic agents, is designed and reported. Three molecules from the synthesized set of compounds showed good cytotoxicity against MCF-7. SAR studies indicate the importance of the substituent being electron donating in nature and the position to be para in order to obtain maximum effect against cancer cells. Additionally, the 2-aminoquinoline-3-carboxamides were reasonably more selective when compared to that of the existing PDGFR inhibitor, imatinib. In conclusion, these experiments will lay the groundwork for the evolution of potent and selective PDGFR

inhibitors for the treatment of breast cancer cells.

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Abbreviations:
PDGFR = Platelet derived growth factor receptor
DCC = Dicyclohexylcarbodiimide
RTK = Receptor tyrosine kinase
ERK = Extracellular signal-regulated kinase
PI3K = Phosphatidylinositol 3-kinase
HER = Human epidermal growth factor receptor
MTT = (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)
CTG = Cell-Titer Glo
SAR = Structure-activity relationship
TMS = Tetramethylsilane
Hrs = Hours
EDG = Electron Donating Group
DMSO = Dimethyl sulphoxide
DMF = Dimethyl formamide
TLC = Thin layer chromatography
HOBt = 1-hydroxybenzotrizole
DMEM = Dulbecco's Modified Eagle Medium
FBS = Fetal bovine serum
Et_3N = Triethyl amine
$CDCl_3$ = Deuterated chloroform

References:

1. The World Health Organization, Cancer 2021 https://www.who.int/news-room/fact-sheets/detail/cancer (accessed: December 2021)
2. H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, *CA Cancer J. Clin.*2021, *71*, 209.
3. Mohamed Mokhtar, Khadijah S. Alghamdi, Nesreen S. Ahmed, DinaBakhotmah & Tamer S. Saleh. Design and green synthesis of novel quinolinonederivatives of potential anti-breast cancer activity against MCF-7 cell line targeting multi-receptor tyrosine kinases, Journal of Enzyme Inhibition and Medicinal Chemistry, 2021, 36 (1), 1453-1470.
4. Sangmin Kim, Daeun You, Yisun Jeong, Sun Young Yoon, Sung A Kim, Jeong Eon Lee. Inhibition of platelet-derived growth factor receptor synergistically increases thepharmacological effect of tamoxifen in estrogen receptor α positive breast cancer. Oncology Letters, 2021, 21 (4), 1-8.
5. Cortes JE, Gambacorti-Passerini C, Deininger MW, et al Bosutinib versus imatinib for newly diagnosed chronic myeloid leukemia: results from the randomized BFORE trial. J Clin Oncol 2018;36:231–7.
6. Zsch€abitz S, Gr€ullich C. Lenvantinib: a tyrosine kinase inhibitor of VEGFR 1-3, FGFR 1-4, PDGFRa, KIT and RET. Recent Result Cancer Res 2018; 211:187–98.
7. Markowitz JN, Fancher KM. Cabozantinib: a multitargeted oral tyrosine kinase inhibitor. Pharmacotherapy 2018; 38: 357–69.
8. Deeks ED. Neratinib: first global approval. Drugs 2017;77: 1695–704.
9. Martorana A, Monica GL, Lauria A. Quinoline-based molecules targeting c-met, EGF, and VEGF receptors and the proteins involved in related carcinogenic pathways. Molecules 2020; 25: 4279.
10. Marella A, Tanwar OP, Saha R, et al. Quinoline: a versatile heterocyclic. Saudi Pharm J 2013; 21:1–12.
11. Toth, J.; Blasko, G.; Dancso, A.; Toke, L.; Nyerges, M. Synth Commun 2006, 36, 3581.
12. George, I. R.; Lewis, W.; Moody, C. J. Tetrahedron 2013, 69, 8209.
13. Li, W. Y.; Xiong, X. O.; Zhao, D. M.; Shi, Y. F.; Yang, Z. H.; Yu, C.; Fan, P. W.; Cheng, M. S.; Shen, J. K. Molecules 2012, 17, 5497.
14. Hosamani KM, Reddy DS, Devarajegowda HC. Microwave-assisted synthesis of new fluorinated coumarin–

pyrimidine hybrids as potent anticancer agents, their DNA cleavage and X-ray crystal studies. RSC Adv 2015;5: 11261–71.

15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays.J Immunol Methods 1983;65:55–63.
16. Vistica DT, Skehan P, Scudiero D, et al. Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. Cancer Res 1991;51:2515–20.
17. Edmondson JM, Armstrong LS, Martinez AO. A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures. J Tissue Cult Meth 1988;11:15–7.
18. Carroll, T.X.; Thomas, T.D.; Bergersen, H.; Børve, K.J.; Saethre L.J. Fluorine as a π donor. Carbon 1s photoelectron spectroscopy and proton affinities of fluorobenzenes. J. Org. Chem., **2006**, 71(5), 1961-1968.

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