Anticancer Potential of Novel Amide Derivatives of Pyrido-thiazolo[5,4-b] pyridine: Synthesis and Biological Evaluation

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Abstract

A new library of amide derivatives of pyrido-thiazolo[5,4-b] pyridine (11a-j) was designed, synthesized and their chemical structures were confirmed by spectral data and analytical data. Further, all these newly synthesized compounds 11a-j were tested for their preliminary anticancer applications against a panel of human cancer cell lines such as breast cancer (MCF-7), lung cancer (A549), colon cancer (Colo-205) and ovarian cancer (A2780) by using of MTT assay and the obtained results were compared with known chemotherapeutic agents namely etoposide used as positive control.

Most of the tested compounds displayed good to moderate anticancer properties on all cell lines. Among them, five compounds, 11a, 11b, 11c, 11d and 11e, exhibited more potent activity as compared to etoposide, in which one of the compounds, 11a, displayed superior activity.

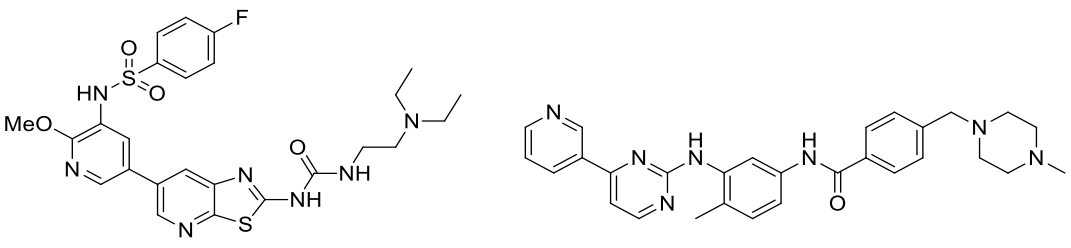
Keywords: Thiazole[5,4-b] pyridine, imatinib, pyridine, anticancer activity, breast cancer and colon cancer.

Introduction

Cancer is the most common cause of mortality globally and, despite advancements in its biology and pharmacology, it remains a critical public health concern[23]. Despite a great deal of research, the illness is still a serious health risk[29] which is the outcome of unchecked cell division and proliferation brought on by environmental and genetic variables[16]. It is predicted to overtake cardiovascular illnesses as the world's top cause of mortality in the coming years[9,27]. Even though chemotherapy is the cornerstone of cancer treatment, its application is frequently restricted because of unfavourable side effects. Furthermore, a major medical issue is the rising prevalence of drug resistance to cancer chemotherapeutic drugs[1,2]. Lately, a general ionophobic potency for divalent cations was demonstrated by the heterocyclic nitrogen derivatives[11] and a new kind of thiocyanate-selective membrane sensor was applied[12].

Nitrogen-sulfur atoms bearing hetero-aromatic molecules were the most privileged heterocycles frequently found in many bioactive molecules, materials and agrochemicals[3,19,26] They were used for novel drug discovery and development. In particular, thiazole[5,4-b] pyridine derivatives were considered the most unique class of fused. Due to their numerous biological applications, heterocyclic motifs were important intermediates in medicinal chemistry. They possessed a wide spectrum of biological activities such as Glucokinase,[25] p38 MAP kinase,[7] PPAR,[10] VEGFR-2,3 sirtuin,[5] ubiquitin ligase, [28 and] JAK3[15]. Among the various thiazole-pyridines, one of the compounds 1 (Figure 1), has a thiazole-pyridine core unit as the main backbone of the structure and displays potent anticancer activity[30].

On the other hand, pyridines were well-recognized six-membered heterocyclic molecules and were found in several synthetic, natural and biologically active scaffolds[20]. They have demonstrated a broad range of biological activities including antitumor[18], anticancer[22], antioxidant[14], antibacterial,[21] anti-inflammatory[6], antiviral[8], anticonvulsant[24], antifungal[17], antimicrobial[13]. Amongst the US Food and Drug Administration-sanctioned chemotherapeutic agents, namely imatinib (2), it contained a pyridine nucleus as part of the structure and was used for the treatment of different types of cancers[4].



a **b**

Figure 1: a) N-(5-(2-(3-(2-(diethylamino) ethyl) ureido) thiazole[5,4-b]pyridin-6-yl)-2-methoxypyridin-3-yl)-4-fluorobenzenesulfonamide, b) Imatinib

The phosphoinositide 3-kinase alpha (PI3Kα) kinase is a central component in various signalling pathways, overseeing a myriad of cellular processes that encompass cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. The structural elements of PI3Kα kinase play essential roles in its function and regulation. By understanding their structure and function, we can develop more effective strategies to target PI3Kα for therapeutic purposes.

Its structural elements include the P-loop, catalytic loop, activation loop, DFG motif, specificity pocket and hinge region. The P-loop (771–777) is a significant motif common to many nucleotide-binding proteins. In PI3Kα, the P-loop primarily serves to bind the phosphate groups of ATP, ensuring its appropriate orientation and facilitating the efficient transfer of phosphate during the catalytic reaction. Another essential component is the catalytic loop (909–920), which is vital for the kinase's enzymatic activity. It plays a role either directly or indirectly in the phosphate group's transfer to its designated substrate. The activation loop (933–958) is a part of the kinase that witnesses conformational shifts during its activation process. This loop is often pivotal in recognizing substrates or in strategically positioning the catalytic residues.

Within the activation loop, there is the DFG motif (933–935), which holds considerable importance for the kinase's function. The position of this motif often dictates if the kinase assumes an active or inactive state. Furthermore, the specificity pocket (772, 780), located in the ATP pocket, is instrumental in determining substrate specificity. It might also influence the binding of certain inhibitors. Lastly, the hinge region (849, 851) within the ATP pocket links major structural components of the kinase and is fundamental for the interactions with ATP and various kinase inhibitors (Figure 2a).

Thiazolo rings combined with pyridines form the backbone of numerous bioactive entities, showcasing vast promise for therapeutic benefits. These heterocyclic structures are becoming pillars within medicinal chemistry due to their varied and crucial biological impacts, especially concerning oncology. For example, thiazolo pyridines exhibit a wide array of biological functions. Their known roles encompass anti-inflammatory, antibacterial, antihypertensive and muscle-relaxing effects. Moreover, these compounds are acknowledged for their antimicrobial and antifungal features. 1,3,4-thiadiazole is a nitrogen-containing heterocyclic ring with promising anticancer activity against various cancer cell lines. It exhibits inhibitory effects on diverse biological targets and is being extensively explored in the field of anticancer research.

In a recent study, a library of 1,2,4-thiadiazole-1,2,4-triazole analogs was synthesized and was evaluated for anticancer activity. Several compounds showed moderate to potent anticancer activity against breast, lung and prostate cancer cell lines. The imidazo[2,1-b] [1,3,4] thiadiazole derivatives have been investigated extensively for their biological profiles including antimicrobial, antifungal, anticancer and anti-inflammatory activities. They have also shown potential as enzyme inhibitors, indicating their significance in target-oriented drug design and discovery. Overall, the current research was conducted over the past six decades on the biological activities of derivates of thiadiazole, particularly their promising anticancer potential.

Adding to their medicinal stature is their involvement as inhibitors of beta-amyloid production, CDK2-cyclin A, uterine stimulants and coronary expanders. Remarkably, their application spans into the chemotherapy arena against conditions like leukaemia, lung tumours and skin cancers. Concerning cancer's spread, there is heightened interest in thiazolo pyridines due to their migrastatic capabilities. Tackling cancer spread remains a forefront challenge in oncology because of its intricate nature and its link to reduced patient survival. Delving into compounds like thiazolo pyridines, particularly those targeting Lysyl-tRNA synthetase (KRS), emphasizes their potential in curbing metastasis. Given the crucial role, KRS has in cancer spread, inhibiting it could offer a promising avenue to combat metastatic developments.

Additionally, nitrogen-rich compounds like pyridines and pyrimidines have garnered attention for their anticancer attributes. They have exhibited potential against several cancer types, such as breast tumors, myeloid leukemia and hepatic cancers. Aryl sulfide derivatives, often paired with thiazole structures, appear in multiple drugs with pharmaceutical potency. These structures are found in medications targeting conditions including diabetes, Alzheimer's and Parkinson's diseases. Pairing thiazole and pyridine nuclei further amplifies their therapeutic spectrum, making them effective against illnesses like infections, blood pressure anomalies and diverse cancers.

To conclude, the convergence of thiazolo rings with pyridine structures has cemented their place as dynamic and effective tools in oncology therapeutics. Spanning from antimicrobial to anticancer effects, their role in addressing metastatic challenges stands out. As research delves deeper into newer variants of these compounds, it promises groundbreaking strides in oncology and beyond, highlighting their crucial position in medicinal chemistry's future. Based on the above-mentioned biological information of thiazolo-pyridine and pyridine skeletons and continuous efforts, we have designed and synthesized amide derivatives of pyrido-thiazolo[5,4-b] pyridine (11a-j) and their chemical structures were confirmed by spectral data.

Further, all these newly synthesized compounds 11a-j were tested for their preliminary anticancer applications against a panel of human cancer cell lines, such as breast cancer (MCF-7), lung cancer (A549), colon cancer (Colo-205) and ovarian cancer (A2780).

Material and Methods

All the chemicals and reagents were used without additional purification; they were purchased from Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and Aldrich (Sigma–Aldrich, St. Louis, MO, USA). On silica gel glass plates containing 60 F-254, reactions were carried out and monitored by TLC. UV light or an iodine indicator was used to visualize the reactions on TLC. Using a Gemini Varian-VXR-unity (400 MHz, 300 MHz) device, 1H and 13C NMR spectra were acquired. Chemical shifts (d) from the internal TMS standard are reported in parts per million downfield. ESI+ software, an ESI mode positive ion trap detector and a capillary voltage of 3.98 kV were used to record ESI spectra on a mass Quattro LC. Melting points were measured without correction using an electro-thermal melting point device.

2-(4-nitrophenyl) thiazole [5,4-b] pyridine (5): ZnO (322 mg, 3.96 mmol) was added to a combination of compound 3 (10 g, 79.2 mmol) and compound 4 (11.9 g, 79.2 mmol) in anhydrous DMF (60 ml) that was agitated at room temperature under nitrogen atmosphere. Following that, the reaction mixture was agitated for six hours at reflux. Following reaction completion, TLC (10%, ethyl acetate: n-hexane) was used to monitor the reaction. The resultant mixture (3×90 mL) was added to ethyl acetate and water (2:8, v: v). In vacuum, the solvent was concentrated. Ethyl acetate/hexane (1:9) was used in column chromatography to purify the crude product, yielding pure product 5 weighing 14.8 g and having a 74% yield.

MP: 233-235^{o}C^{1}H NMR (300 MHz, DMSO-d6): δ7.45 (dd, 1H, *J* = 5.6, 5.03 Hz), 7.75 (d, 2H, *J* = 8.0 Hz), 8.12 (d, 2H, *J* = 8.0 Hz), 8.35 (d, 1H, *J* = 5.6 Hz), 8.69 (d, 1H, *J* = 5.6 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ121.3, 121.7, 122.4, 129.5, 130.3, 147.3, 150.3, 160.3, 165.3, 167.4.MS (ESI): m/z 258 $[M+H]^{+}$.

5-iodo-2-(4-nitrophenyl) thiazolo[5,4-b] pyridine (6): A solution of compound 5 (14 g, 54.4 mmol) in 100 mL of acetonitrile was agitated at room temperature in the presence of nitrogen. NIS (12.2g, 54.4 mmol) was added to this gradually and it was agitated for 12 hours. Following the TLC reaction's conclusion, the mixture was cooled, filtered to remove succinimide and then cleaned with ethyl acetate.

After separating the organic layer and drying it with Na_2SO_4, the filtrate was rinsed with water and brine. The targeted product 5, weighing 11.9 g and having a 57% yield with white solid, was obtained by purifying the crude compound using column chromatography and ethyl acetate/hexane (1:9).

MP: 239-241^{0}C^{1}H NMR (300 MHz, DMSO-d6): δ7.42 (dd, 1H, *J* = 5.6, 5.03 Hz), 7.73 (d, 2H, *J* = 8.0 Hz), 8.11 (d, 2H, *J* = 8.0 Hz), 8.32 (d, 1H, *J* = 5.6 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ118.4, 120.5, 121.6, 122.5, 129.5, 136.4, 147.4, 151.5, 165.3, 167.4.MS (ESI): m/z 384 $[M+H]^{+}$.

2-(4-nitrophenyl)-5-(pyridine-4-yl) thiazolo[5,4-b] pyridine (8): Reaction mixture was added and agitated at 100^{o}C for 12 hours. The mixture contained intermediate 5 (11 g, 28.7 mmol), Pd (PPh3)4 (3.3 g, 2.87 mmol) in 1,4-dioxane (100 mL), pyridin-4-ylboronic acid (6) (3.5 g, 28.7 mmol), Cs_2CO_3 (28 g, 86.1 mmol) and 5 ml of water. After bringing the reaction mixture down to room temperature, the solvent was drawn out using a vacuum. Water (100 mL) was added after the residue had been dissolved in 150 mL of ethyl acetate. After separation, the organic phase was dried over sodium sulfate that had been dehydrated. By employing ethyl acetate/hexane (3:7) in column chromatography, the crude compound was refined to yield compound 8, which was 7.2 g of light brown solid with a 75% yield.

MP: 247-249^{0}C^{1}H NMR (300 MHz, DMSO-d6): δ7.47 (dd, 1H, *J* = 5.7, 5.03 Hz), 7.74 (d, 2H, *J* = 8.1 Hz), 7.96 (d, 2H, *J* = 5.4 Hz), 8.13 (d, 2H, *J* = 8.1 Hz), 8.34 (d, 1H, *J* = 5.6 Hz), 8.72 (d, 2H, J = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ118.4, 120.5, 121.6, 122.5, 122.9, 129.4, 146.3, 147.3, 150.5, 151.3, 158.3, 165.4, 167.5.MS (ESI): m/z 335 $[M+H]^{+}$.

4-(5-(pyridin-4-yl) thiazolo[5,4-b] pyridin-2-yl) aniline (9): To a compound 8 combination (7 g, 20.9 mmol) in 100 mL of THF, add Raney-Ni (3.6 g, 62.7 mmol) to this. It was shaken for three hours at room temperature while exposed to a hydrogen environment. The reaction was filtered and then given an ethyl acetate wash after it was finished. The ethyl acetate was vacuum-sealed and evaporated, the pure compound of 9—5.3 g of off-white solid with an 83% yield, was obtained.

MP: 250-252^{0}C^{1}H NMR (300 MHz, DMSO-d6): δ5.67 (brs, 2H), 7.47 (dd, 1H, *J* = 5.7, 5.03 Hz), 7.68 (d, 2H, *J* = 8.1 Hz), 7.96 (d, 2H, *J* = 5.4 Hz), 8.09 (d, 2H, *J* = 8.1 Hz), 8.35 (d, 1H, *J* = 5.6 Hz), 8.71 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ115.4, 118.5, 120.3, 122.5, 122.9, 129.4, 146.4, 147.4, 149.4, 150.4, 151.3, 158.5, 167.5.MS (ESI): m/z 305 $[M+H]^{+}$.

3,4,5-Trimethoxy-N-(4-(5-(pyridin-4-yl)thiazolo[5,4-b]pyridin-2-yl)phenyl)benzamide (11a):30 millilitre's of dry THF were used to dissolve compound 9 (200 mg, 0.65 mmol). Next, 3,4,5-trimethoxybenzoic acid (10a) (139 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol) were added. Twelve hours were spent stirring the reaction mixture at room temperature. Following the completion of the reaction, the solvent was vacuum-evaporated. The compound was extracted using ethyl acetate and then dried over anhydrous Na_2SO_4 before being cleaned with a saturated solution of $NaHCO_3$. By using ethyl acetate/hexane (1:1) in column chromatography, the crude product was refined to produce pure chemical 11a, 210.8 mg in 64(yield as an off-white solid).

MP: 285-287^{0}C^{1}H NMR (300 MHz, DMSO-d6): δ3.78 (s, 3H), 3.89 (s, 6H), 7.25 (s, 2H), 7.47 (dd, 1H, *J* = 5.7, 5.03

Hz), 7.70 (d, 2H, *J* = 8.2 Hz), 7.96 (d, 2H, *J* = 5.4 Hz), 8.10 (d, 2H, *J* = 8.2 Hz), 8.36 (d, 1H, *J* = 5.6 Hz), 8.45 (s, 1H), 8.72 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): *δ*57.3, 57.7, 105.3, 118.5, 118.9, 120.4, 122.5, 122.9, 129.4, 130.4, 138.4, 140.2, 146.4, 147.3, 150.4, 151.3, 154.4, 158.5, 165.6, 167.5.MS (ESI): m/z 499 $[M+H]^+$.

3,5-Dimethoxy-N-(4-(5-(pyridin-4-yl)thiazolo[5,4-b] pyridin-2-yl)phenyl)benzamide (11b): Using the same procedure as for compound 11a, compound 11b was made by combining 9 (200 mg, 0.65 mmol) with 3,5-dimethoxy benzoic acid (10b) (118 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol). The crude product was then purified using column chromatography with ethyl acetate/hexane (1:1) to yield pure compound 11b, which was obtained as 231.6 mg with 75(yield as a white solid.

Mp: 282-284^{0}C^{1}H NMR (300 MHz, DMSO-d6): *δ*3.77 (s, 6H), 6.63 (s, 1H), 7.12 (s, 2H), 7.47 (dd, 1H, *J* = 5.7, 5.03 Hz), 7.70 (d, 2H, *J* = 8.2 Hz), 7.96 (d, 2H, *J* = 5.4 Hz), 8.10 (d, 2H, *J* = 8.2 Hz), 8.36 (d, 1H, *J* = 5.6 Hz), 8.46 (s, 1H), 8.72 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): *δ*57.3, 102.4, 113.3, 118.5, 118.9, 120.4, 122.5, 122.9, 129.4, 130.4, 138.3, 146.5, 147.3, 150.4, 151.6, 158.4, 161.4, 165.4, 167.4; MS (ESI): m/z 469 $[M+H]^+$.

4-Methoxy-N-(4-(5-(pyridin-4-yl)thiazolo[5,4-b]pyridin-2-yl)phenyl)benzamide (11c): Utilizing 9 (200 mg, 0.65 mmol) in conjunction with 4-methoxybenzoic acid (10c) (99 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol), compound 11c was prepared using the same procedure as compound 11a. The crude product was then purified by column chromatography using ethyl acetate/hexane (1:1) to yield pure compound 11c, 244.5 mg, with an 85 (yield as a white solid.

MP: 274-276^{0}C^{1}H NMR (300 MHz, DMSO-d6): *δ*3.88 (s, 3H), 7.10 (d, 2H, *J* = 7.6 Hz), 7.47 (dd, 1H, *J* = 5.7, 5.03 Hz), 7.51 (d, 2H, *J* = 7.6 Hz), 7.69 (d, 2H, *J* = 8.2 Hz), 7.96 (d, 2H, *J* = 5.4 Hz), 8.11 (d, 2H, *J* = 8.2 Hz), 8.35 (d, 1H, *J* = 5.6 Hz), 8.47 (s, 1H), 8.71 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): *δ*57.2, 115.3, 118.4, 118.9, 120.4, 122.4, 122.4, 129.5, 130.3, 134.4, 138.4, 146.5, 147.4, 150.5, 151.6, 158.5, 160.5, 165.6, 167.5.MS (ESI): m/z 439 $[M+H]^+$.

4-Methyl-N-(4-(5-(pyridin-4-yl)thiazolo[5,4-b]pyridin-2-yl)phenyl)benzamide (11d): Using 9 (200 mg, 0.65 mmol) with 4-methylbenzoic acid (10d) (85 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol), compound 11d was prepared by the procedure outlined for compound 11a. The crude product was then purified by column chromatography using ethyl acetate/hexane (1:1) to yield pure compound 11d as 199.6 mg with 72(yield as white solid).

MP: 260-262^{0}C^{1}H NMR (300 MHz, DMSO-d6): *δ*2.32 (s, 3H), 7.16 (d, 2H, *J* = 7.3 Hz), 7.47 (dd, 1H, *J* = 5.7, 5.03 Hz), 7.50 (d, 2H, *J* = 7.3 Hz), 7.69 (d, 2H, *J* = 8.2 Hz), 7.96 (d, 2H, *J* = 5.4 Hz), 8.11 (d, 2H, *J* = 8.2 Hz), 8.35 (d, 1H, *J* = 5.6 Hz), 8.44 (s, 1H), 8.71 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): *δ*24.4, 118.6, 118.9, 120.4, 122.4, 122.8, 129.3, 129.6, 130.2, 134.6, 138.5, 142.5, 146.4, 147.3, 150.5, 151.4, 158.6, 165.6, 167.4.MS (ESI): m/z 423 $[M+H]^+$.

4-(Dimethylamino)-N-(4-(5-(pyridin-4-yl)thiazolo[5,4-b] pyridin-2-yl)phenyl) benzamide (11e): Using the same procedure as for compound 11a, compound 11e was made using 9 (200 mg, 0.65 mmol), 4-(dimethylamino)benzoic acid (10e) (107 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol). The crude product was then purified using column chromatography with ethyl acetate/hexane (1:1) to yield pure compound 11e, 212.7 mg with 72(yield as a white solid.

MP: 266-268^{0}C^{1}H NMR (300 MHz, DMSO-d6): *δ*2.86 (s, 6H), 7.15 (d, 2H, *J*= 7.4 Hz), 7.48-7.55 (m, 3H), 7.69 (d, 2H, *J* = 8.2 Hz), 7.95 (d, 2H, *J* = 5.4 Hz), 8.12 (d, 2H, *J* = 8.2 Hz), 8.36 (d, 1H, *J* = 5.6 Hz), 8.46 (s, 1H), 8.71 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): *δ*41.3, 113.4, 118.4, 118.9, 120.5, 122.4, 122.8, 129.4, 130.3, 134.4, 138.4, 146.3, 147.4, 150.3, 151.6, 151.9, 158.5, 165.4, 167.5.MS (ESI): m/z 452 $[M+H]^+$.

4-Chloro-N-(4-(5-(pyridin-4-yl)thiazolo[5,4-b]pyridin-2-yl)phenyl)benzamide (11f): The preparation of compound 11f was carried out by the protocol outlined for the preparation of compound 11a. This involved the use of 9 (200 mg, 0.65 mmol) in conjunction with 4-chlorobenzoic acid (10f) (102 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol). The crude product was then purified by column chromatography using ethyl acetate/hexane (1:1) to yield pure compound 11f, with a yield of 79(mg as a white solid.

MP: 270-272^{0}C ^{1}H NMR (300 MHz, DMSO-d6): *δ*7.47 (dd, 1H, *J* = 5.6, 5.02 Hz py-5), 7.67-7.76 (m, 4H), 7.80 (d, 2H, *J* = 7.7 Hz), 7.95 (d, 2H, *J* = 5.4 Hz), 8.12 (d, 2H, *J* = 8.2 Hz), 8.36 (d, 1H, *J* = 5.6 Hz), 8.48 (s, 1H), 8.71 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): *δ*118.5, 118.9, 120.3, 122.4, 122.8, 129.4, 129.8, 130.5, 134.4, 134.7, 138.3, 146.4, 147.3, 150.5, 151.5, 158.6, 165.6, 167.5.MS (ESI): m/z 443 $[M+H]^+$.

14-Bromo-N-(4-(5-(pyridin-4-yl)thiazolo[5,4-b]pyridin-2-yl)phenyl)benzamide(11g): By using 9 (200 mg, 0.65 mmol) with 4-bromobenzoic acid (10g, 131 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol), compound 11g was prepared by the procedure outlined for compound 11a. The crude product was then purified by column chromatography using ethyl acetate/hexane (1:1) to yield pure compound 11g as 234.2 mg with 73(yield as white solid).

MP: 276-278^{0}C^{1}H NMR (300 MHz, DMSO-d6): *δ*7.47 (dd, 1H, *J* = 5.6, 5.02 Hz), 7.69-7.78 (m, 4H), 7.82 (d, 2H, *J* = 7.7 Hz), 7.95 (d, 2H, *J* = 5.4 Hz), 8.12 (d, 2H, *J* = 8.2 Hz),

8.36 (d, 1H, *J* = 5.6 Hz), 8.48 (s, 1H), 8.71 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): *δ*118.4, 118.8, 120.4, 122.3, 122.7, 123.3, 129.4, 130.5, 132.5, 134.6, 138.4, 146.5, 147.4, 150.4, 151.5, 158.5, 165.6, 167.5.MS (ESI): m/z 488 $[M+2]^+$.

4-Nitro-N-(4-(5-(pyridine-4-yl)thiazolo[5,4-b]pyridin-2-yl)phenyl)benzamide(11h): This compound 11h was prepared following the method described for the preparation of the compound 11a, employing 9(200 mg, 0.65mmol) with 4-nitrobenzoic acid (10h) (109 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (1:1) to afford pure compound 11 239.6 mg with 85% yield as a white solid.

MP: 287-289^{0}C^{1}H NMR (300 MHz, DMSO-d6): *δ*7.47 (dd, 1H, *J* = 5.6, 5.02 Hz), 7.68 (d, 2H, J = 8.2 Hz), 7.95 (d, 2H, *J* = 5.4 Hz), 8.06-8.13 (m, 3H), 8.35-8.41 (m, 3H), 8.52 (s, 1H), 8.71 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): *δ*118.5, 118.9, 120.5, 121.6, 122.3, 122.7, 129.4, 130.2, 134.4, 138.4, 146.5, 147.4, 150.5, 151.4, 158.6, 165.4, 165.7, 167.4.MS (ESI): m/z 454 $[M+H]^+$.

4-Cyano-N-(4-(5-(pyridin-4-yl)thiazolo[5,4-b]pyridin-2-yl)phenyl)benzamide (11i): This compound 11i was prepared following the method described for the preparation of compound 11a, employing 9 (200 mg, 0.65mmol) with 4-cyano benzoic acid (10i) (96 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (1:1) to afford pure compound 11i as 216.7 mg with 76% yield as white solid.

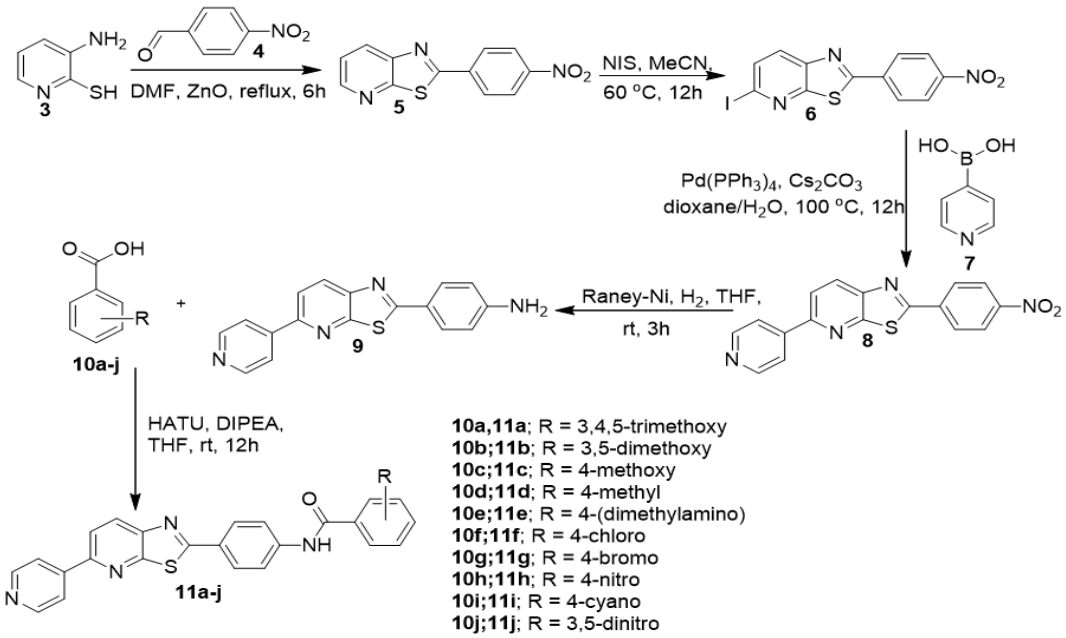
MP: 298-300^{0}C^{1}H NMR (300 MHz, DMSO-d6): *δ*7.47 (dd, 1H, *J* = 5.6, 5.02 Hz), 7.71 (d, 2H, *J* = 8.2 Hz), 7.95 (d, 2H, *J* = 5.4 Hz), 8.08-8.14 (m, 3H), 8.36-8.42 (m, 3H), 8.51 (s, 1H), 8.70 (d, 2H, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d6): *δ*111.6, 118.4, 118.8, 119.3, 120.4, 122.4, 122.8, 129.2, 129.5, 133.3, 134.5, 138.5, 146.5, 147.3, 150.6, 151.5, 158.6, 165.4, 167.5.MS (ESI): m/z 434 $[M+H]^+$.

3,5-Dinitro-N-(4-(5-(pyridin-4-yl)thiazolo[5,4-b]pyridin-2-yl)phenyl)benzamide (11j): This compound 11j was prepared following the method described for the preparation of compound 11a, employing 9 (200 mg, 0.65mmol) with 3,5-dinitrobenzoic acid (10j) (139 mg, 0.65 mmol), HATU (494 mg, 1.3 mmol) and DIPEA (0.11 ml, 1.95 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (1:1) to afford pure compound 11j as 256.4 mg with 78% yield as white solid.

MP: 325-327^{0}C^{1}H NMR (300 MHz, DMSO-d6): *δ*7.47 (dd, 1H, *J* = 5.6, 5.02 Hz), 7.72 (d, 2H, *J* = 8.3 Hz), 7.95 (d, 2H, *J* = 5.4 Hz), 8.12 (d, 2H, *J* = 8.3 Hz ar), 8.36 (d, 1H, *J* = 5.6, 5.02 Hz), 8.53 (s, 1H), 8.70 (d, 2H, *J* = 5.4 Hz), 8.75 (s, 1H), 8.84 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d6): *δ*116.3, 118.5, 118.9, 120.4, 120.8, 122.5, 122.9, 129.5, 137.3, 138.4, 146.5, 147.4, 150.5, 151.4, 158.6, 165.4, 165.6, 167.5. MS (ESI): m/z 499 $[M+H]^+$.

Results and Discussion

Chemistry: The amide derivatives of pyridine-thiazolo[5,4-b] pyridine (11a-j) were developed according to scheme 1. Condensation was the initial phase and then there was a cyclization reaction between 3-aminopyridine-2-thiol (**3**) and 4-nitrobenzaldehyde (4) in presence of ZnO nano particle in dry DMF stirred for 6 hours to give pure 2-(4-nitrophenyl)thiazolo[5,4-b]pyridine (5). This intermediate 5 was reacted with N-iodo succinamide in acetonitrile at 60^{0}C for 12 hours to give5-iodo-2-(4-nitrophenyl)thiazolo[5,4-b]pyridine (6).



Scheme 1: Preparation of amide derivatives of pyridine-thiazolo[5,4-b] pyridine (11a-j)

Table 1
Anticancer activity data of target compounds11a-j with IC_{50} in µM.

Compound	[c]MCF-7	[d]A549	[e]Colo-205	[f]A2780
11a	0.14±0.096	0.10±0.091	0.22±0.072	0.17±0.085
11b	1.56±0.66	1.21±0.43	1.30±0.57	1.43±0.74
11c	1.97±0.71	1.68±0.82	1.77±0.68	1.64±0.87
11d	2.01±1.78	2.24±1.45	2.72±1.84	2.55±1.33
11e	2.85±1.93	2.14±1.62	2.83±1.98	2.48±1.78
11f	10.4±5.43	ND	ND	ND
11g	ND	4.21±3.08	5.28±3.51	6.84±2.77
11h	ND	8.31±4.76	8.92±4.87	ND
11i	7.32±5.62	5.66±3.45	ND	ND
11j	ND	3.46±2.11	5.47±3.21	ND
Etoposide	2.11 ± 0.024	3.08 ± 0.135	0.13 ± 0.017	1.31 ± 0.27

ND = Not determined.
[a] Each data represents as mean ±S. D values. From three different experiments performed in triplicates. [bc]MCF-7: human breast cancer cell line. [d]A549: human lung cancer cell line. [e]Colo-205: human colon cancer cell line. [f]A2780: human ovarian cancer cell line.

Further, compound 6 underwent Suzuki coupling with pyridin-4-ylboronic acid (7) by using $Pd(PPh_3)_4$ catalyst and Cs_2CO_3 in 1,4-dioxane/water and was stirred at 100^0C for 12hours to give compound 2-(4-nitrophenyl)-5-(pyridin-4-yl)thiazolo[5,4-b]pyridine (8) with good yield. Then compound 8 underwent reduction with 10%Pd/C in dry THF under hydrogen atmosphere at room temperature for 3 hours to give compound 4-(5-(pyridin-4-yl) thiazolo[5,4-b] pyridin-2-yl) aniline (9). Finally, compound 9 was coupled with different types of aromatic carboxylic acids (10a-j) in the presence of HATU and DIPEA in dry THF at room temperature for 12 hours to give final target compounds 11a-j.

Biological Evaluation

***In vitro* cytotoxicity:** Using the MTT assay, a panel of human cancer cell lines including breast cancer (MCF-7), lung cancer (A549), colon cancer (Colo-205) and ovarian cancer (A2780), were used to test the preliminary anticancer applications of all these newly synthesized compounds, 11a-j. The results were compiled in table 1. Etoposide, a well-known chemotherapy drug, was employed as a positive control. On every cell line tested, the majority of the drugs showed good to moderate anticancer activities. Five of the compounds, 11a, 11b, 11c, 11d and 11e, showed action that was more potent than etoposide. whereby compound 11a showed better activity than the others.

After these compounds' structure-activity relationship was further investigated, compound 11a with the 3,4,5-trimethoxy group on the aryl moiety connected to the amide functionality showed good activity, according to the results. (MCF-7=0.14±0.096 µM; A549=0.10±0.091 µM; Colo-205= 0.22±0.072 µM and A2780=0.17±0.085 µM). Compound 11b bearing 3,5-dimethoxy group on the aryl ring showed slightly lower activity (MCF-7=1.56±0.66 µM; A549=1.21±0.43 µM; Colo-205=1.30±0.57 µM and A2780=1.43±0.74 µM) than with 11a. Similarly, 4-methoxy substituent having compound 11c displayed dramatic decreased activity (MCF-7=1.97±0.71µM; A549=1.68±0.82 µM; Colo-205=1.77±0.68 µM and A2780=1.64±0.87 µM) than with 11a and 11b. The compounds with weak electron-rich groups contained compounds 11d (4-methyl) and 11e (4-dimethylamino) exhibited acceptable activities on all cell lines (MCF-7=2.01±1.78 µM; A549=2.24±1.45 µM; Colo-205=2.72±1.84 µM and A2780=2.55±1.33 µM) (MCF-7=2.85±1.93 µM; A549=2.14±1.62 µM; Colo-205=2.83±1.98 µM and A2780=2.48±1.78 µM) respectively. Replacement of the 4-(dimethylamino) group with strong electron-withdrawing groups resulted in compounds 11f (4-chloro), 11g (4-bromo), 11h (4-nitro), 11i (4-cyano) and 11j (3,5-dinitro) displaying moderate activities.

Docking Studies: The crystal structure of PI3Kα, identified by the PDB code: 4JPS, was taken from the Protein Information Bank. Docking processes were executed using the PyRX (0.8) software, which incorporates the Vina module. Both the receptor and molecular ligands underwent processing via the PyRX utilities. The compound's geometry was refined by energy reduction with the Universal Force Field (UFF) in PyRX v0.8. The grid box was centralized on the binding site of pyrido-thiazolo[5,4-b] pyridine and the ATP active site of PI3Kα, as identified by PyRX utilities. The PyMOL system facilitated the visualization of the docked entities. Interactions between the ligands and target proteins were examined with the LigPlot+2D software (version 2.2). The outcomes of the docking were showcased as the binding energy scores (kcal/mol) for the protein-ligand pairing.

MTT assay: A 96-well tissue culture micro titer plate was divided into individual wells and 100 µL of the entire medium containing 1×104 cells was added to each well. Prior to the experiment, the plates were incubated for eighteen hours at 370C in a humidified 5% CO_2 incubator. Following the removal of the media, each well received 100 µL of fresh medium containing the test chemicals and

etoposide (Eto) at various concentrations such as 0.5, 1 and 2 µM. The wells were then incubated for 24 hours at 37^0C. Next, the medium was thrown away and 10 µL of MTT dye was added. For two hours, plates were incubated at 37^0C. The formazan crystals that were obtained were dissolved in 100 µL of extraction buffer. Using a multimode Varioskan instrument, Themo Scientific microplate reader, the optical density (O.D.) was measured at 570 nm. The medium's DMSO content never went above 0.25%.

Molecular docking studies of phosphatidyl no- sitol 3-kinases (PI3Kα) with pyrido-thiazolo[5,4-b] pyridine: The PI3Kα structure (PDB code: 4JPS) served as the framework for docking pyrido-thiazolo[5,4-b] pyridine derivatives into its active site. The binding patterns of all compounds are depicted in fig. 2b. Interaction strengths between PI3Kα and its ligands were determined by assessing binding energy values (kcal/mol) and discerning bonding types such as hydrogen, hydrophobic and electrostatic bonds. Interestingly, these compounds formed robust hydrogen bonds and/ or hydrophobic interactions with amino acid residues of key structural elements, predominantly via the thiazole and pyridine rings. Out of the entire set, all compounds showcased compelling interactions with PI3Kα, selected based on their binding energies.

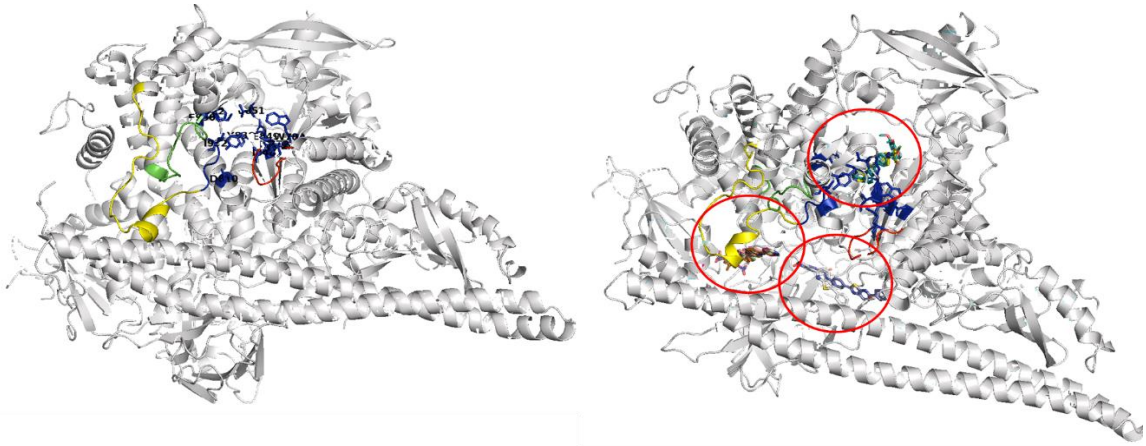


Figure 2: a) Cartoon model of PI3Kα kinase with key features color-coded: P-loop (771–777) binding ATP's phosphate groups in red, enzymatic catalytic loop (909–920), activation loop (933–958) in green featuring the essential DFG motif (933–935), specificity pocket (772, 780) within the ATP pocket and the hinge region (849, 851) depicted in blue, emphasizing ATP interactions and inhibitor binding.
b) Illustration of the ligand's docking poses as stick models and enclosed in circles, located in close spatial proximity to both the ATP binding site and the p-loop, as well as near the oncogenic mutation residues of PI3Kα.

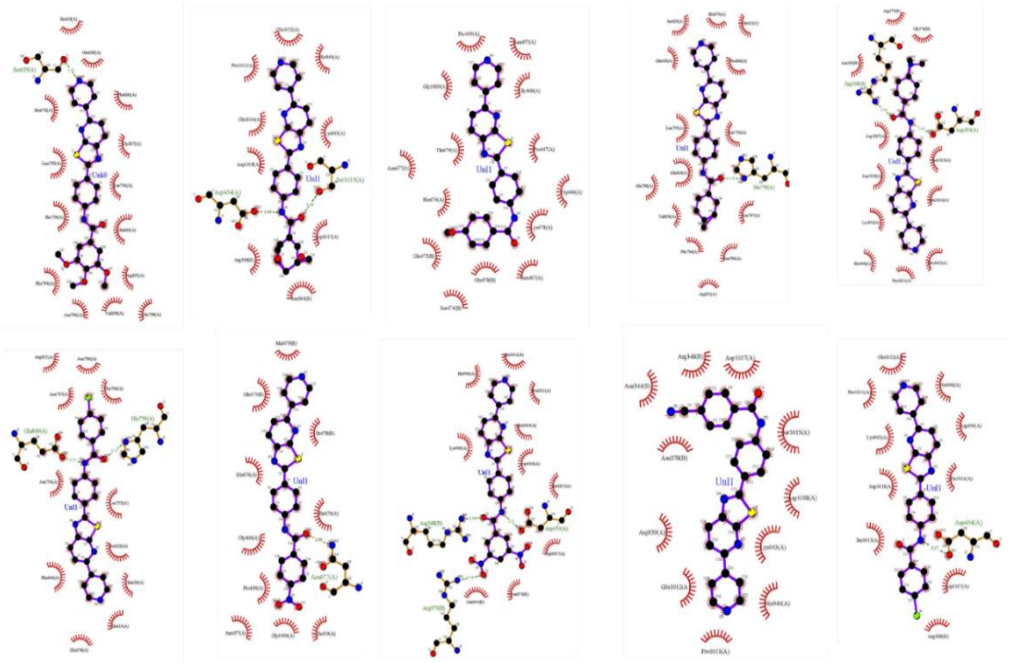


Figure 3: At the catalytic active site, PI3Kα engages with all ligand configurations (see top left). Using LigPlot, 2-D interaction visuals between PI3Kα and the listed compounds are displayed. The sequences of these interaction plots are presented in the order of compounds (11a-j)

Fig. 2 highlights the potent docking interactions of these five compounds with PI3Kα, presenting binding energies ranging from –9.5 to –10.6 kcal/mol. Further analyses indicated that these selected compounds bonded with PI3Kα primarily through hydrogen and hydrophobic interactions, notably with the tetrazole and Imidazopyridine rings, signifying a robust connection with the active site residues. The observed hydrogen bonds contributed to the unique configuration of the compound-enzyme complex. Given their significant interactions at PI3Kα's catalytic active site, it is inferred that tetrazole-integrated Imidazopyridine derivatives might hold potential anticancer properties.

Conclusion

In conclusion, spectral data was used to corroborate the chemical structures of a new library of amide derivatives of pyrido-thiazolo[5,4-b] pyridine (11a-j) that we designed and synthesized. Additionally, using the MTT assay, all of these recently synthesized compounds 11a-j were evaluated for their potential anticancer effects against a panel of human cancer cell lines, including breast cancer (MCF-7), lung cancer (A549), colon cancer (Colo-205) and ovarian cancer (A2780).

The results of these tests were compiled in table 1. Etoposide, a well-known chemotherapy drug, was employed as a positive control. On every cell line tested, the majority of the drugs showed good to moderate anticancer activities. Five of the compounds 11a, 11b, 11c, 11d and 11e showed greater potency when compared to etoposide. In this instance, compound 11a demonstrated better activity.

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