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ARTICLE

Synthesis and anti-proliferative activity studies of 2-(2-(trifluoromethyl)-6-(substituted)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(substituted)acetamide derivatives

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Abstract

A series of novel imidazo[1,2-*b*]pyridazin-3-yl acetamide derivatives **(9a-9j)** were synthesized from a 3,6-dichloropyridazine. We have developed a simple strategy for the synthesis of functionally diverse imidazole, and pyridiazine derivatives were reported via a series of steps. The work involves bicyclic imidazo-pyridazine ring formation, halogenation, cynation, hydrolysis, peptide coupling, and Buchwald reaction. The structure of the synthesized compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR,^{19}F NMR, mass spectra, and elemental analysis, and purity is checked by HPLC. All synthesized compounds were screened for anticancer activity against A-549 and Du-145 cancer cell lines by MTT assay. The preliminary bioassay suggests that most of the compounds show anti-proliferation with different degrees; doxorubicin was used as positive control. The synthesized compound shows IC_{50} values in the range of 1.74μM to 16.17μM in both cell lines. The compounds **9e**, **9g**, and **9h** were active compared with doxorubicin in both the cell lines. The compounds having cyclopentyl ring are active compared with higher and lower carbon analogues.

1 | INTRODUCTION

Cancer is considered as one of the major causes of human health concerns with increasing number of patients with the time all over the world. Although many types of chemotherapeutic drugs were used for the treatment, although still, there is a challenge to identify safe and effective drug for the cancers. Drug resistance occurred during treatment is a major concern of present time. The design and development of new anti-proliferative agents with increased efficiency, less side effective, cost effective, and time concern for the treatment were the major challenges for present researchers. Considering these facts, the development of new chemotherapeutic targets with selective action has to be identified, as many classes of hetterocycle scaffolds were used for the different types of cancers. For normal functioning of cells in the human kinome, there are 518 kinases that are involved in different phases of life and all are associated with each other.[1] Different kinases were responsible for different functioning of cells; some kinases are TOR signaling, which are responsible for cell growth,[2,3] and some are protein tyrosine kinase inhibitors.[4] The imbalance in the kinases occurs in several diseases like cancer, neurodegenerative disorders, and inflammation. By considering the importance of kinases, we need to develop new kinase inhibitors with diversified activity. In present

work, we have chosen substituted imidazole and pyridazine nuclei and its derivatives for cell line and kinases study. Several heterocycles were intensively studied to discover new anticancer agents. Imidazole scaffolds were identified as one of the important classes of heterocyclic compounds because of their significant and diversified pharmacological and biological properties. Imidazole derivatives acts as anti-proliferative,[5] TGR5 receptor agonists,[6] calcitonin gene-related peptide (CGRP) antagonists,[7] and antimicrobial.[8] The imidazole scaffold plays an important role in drug discovery. The imidazole scaffold shows varied biological activities such as antifungal,[9] antibacterial,[10] anti-inflammatory, analgestic,[11] antitubercular,[12] antidepressant, antiviral,[13] anticancer,[14] anti-lishmanial.[15] The substituted imidazole shows multiple biological activities.[16–18] Recent literature review reveals that reactions involving hero atoms such as pyridazine were very advantageous in design of anti-proliferative agents as they resemble many biomolecules. Piridazine and derivatives mainly act as potent and selective factor Xia inhibitors,[19] inhibitors of TNF-α production,[20] potent PDE10a inhibitors,[21] CNS penetrant pan-muscarinic antagonists,[22] Janus kinase (JAK) inhibitors.[23] Piridazine in combination of other groups shows diversified activity like FXIa inhibitors.[24–26]

The imidazo[1,2-*b*]pyridazine was identified as one of the promising building blocks in drug discovery as it shows diversified biological activities like TNF-α inhibitors,[27] treatment of rheumatoid arthritis, treatment of dermal allergic inflammation,[28] and treatment of inflammatory diseases caused by dysfunctions of kinases. Several derivatives of imidazo-pyridazine series act as inhibitors of *Plasmodium falciparum* calcium-dependent protein kinase inhibitors,[29] some as protein kinase inhibitors.[30] By considering the diversified biological activities of imidazole and pipridazine, we have tried to incorporate both scaffolds in one framework as imidazo[1,2-*b*]pyridazin, and in continuation of our research,[31–35] for the search of anti-proliferateve target and kinase inhibitors, we have synthesized a series of compounds having imidazo[1,2-*b*]pyridazin nuclei.

2 | RESULTS AND DISCUSSION

In present work, we have done synthesis of 2-(2-(trifluoromethyl)-6-(substituted)imidazo[1,2-*b*] pyridazin-3-yl)-*N*-(substituted)acetamide (**9a-9j**) by using 3,6-dichloropyridazine (**1**) in below Scheme 1. We have optimized the synthesis of compound **9a-9j** in stepwise manner to get good yield, neat reaction profile, and

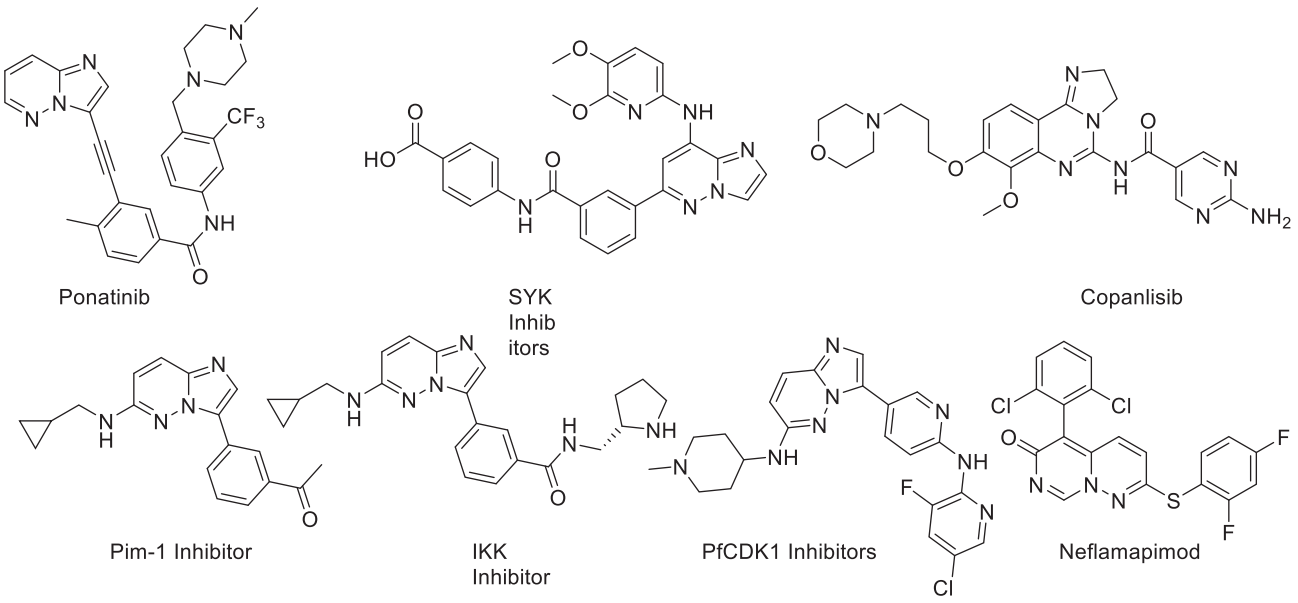
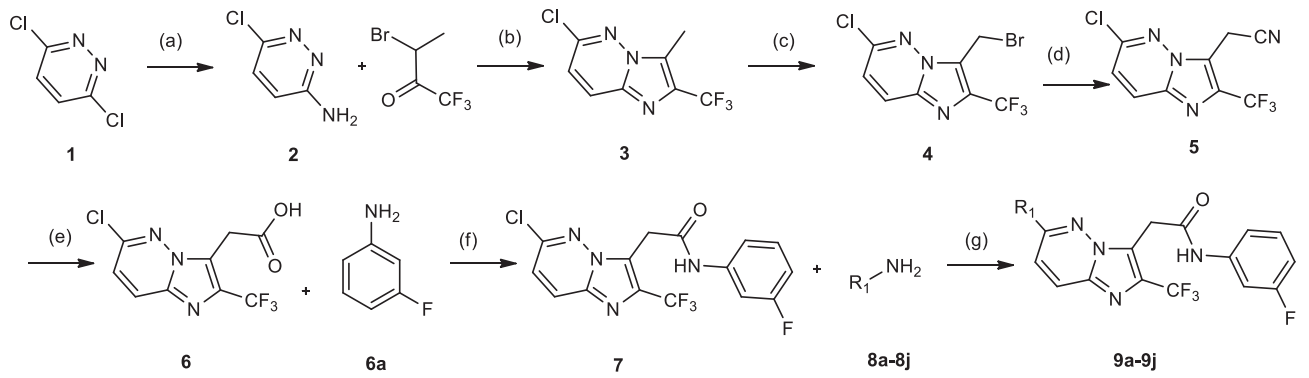


FIGURE 1 General structure of biologically active molecules having imidazo[1,2-*b*]pyridazin nuclei: Figure 1 compromising the biological active molecules reported with imidazo-pyridazine nuclei in combination. Ponatinib is an oral drug used for the treatment of chronic myeloid leukemia and Philadelphia chromosome positive acute lymphoblastic leukemia; in short, it is used as multitargeted tyrosine kinase inhibitor. Neflamapimod is used for treatment of Alzheimer and other neurodegenerative disorders. Copanlisib: drug used for the treatment of follicular lymphoma. Pim-1 inhibitor: imidazo[1,2-*b*]pyridazine 1 inhibitor; it acts as inhibitor for PIM-1 dependent cancer types. IKKβ inhibitor: It is known for inhibitor of nuclear factor kappa-B kinases. SYK inhibitors: These are known as spleen tyrosine kinase inhibitors as these are used for the treatment of autoimmune diseases as asthma and rheumatoid arthritis. PfCDK1 inhibitors: These are known as *Plasmodium falciparum* calcium-dependent protein kinase



SCHEME 1 2-(2-(Trifluoromethyl)-6-(substituted)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(substituted)acetamide (**9a-9j**): Reagents and conditions: (**a**): liq. Ammonia, −10°C to room temperature, 150°C, 8 hours; (**b**): 3-bromo-1,1,1-trifluorobutan-2-one, DME, 150°C, 4 hours; (**c**): NBS, AIBN, CAN, 100°C for 1 hour; **(d)** NaCN, DMF, H_2O, room temperature, 12 hours; **(e)** NaOH, 100°C, 2 hours; **(f)** 3-flouroaniline, EDCI, DIPEA, DCM, room temperature, 12 hours; **(g)** pyrrolidin-2-one, $Cu(OAc)_2$, K_3PO_4, 1,2-dimethylethylenediamine, DMF, 150°C, 12 hours

less harsh condition by keeping these things in mind, and the optimized steps are depicted in below Scheme 1.

Sr. No.	R_1	Sr. No.	R_1
8a = 9a	methylamine	8f = 9f	piperidine
8b = 9b	ethylamine	8g = 9g	4-methylpiperidine
8c = 9c	propylamine	8h = 9h	pyrrolidin-2-one
8d = 9d	butylamine	8i = 9i	piperidin-2-one
8e = 9e	pyrrolidine	8j = 9j	1-methylpiperazine

In step **a**, we have done the synthesis of 6-chloropyridazine-3-amine [cas no. 5469-69-2; **2**] from commercially available 3,6-dichloropyridazine (**1**). We have used liquid ammonia for the reaction; when the reaction was performed in sealed tube, the starting material was not consuming as product formation was very less as there is possibility of ammonia leakage at higher temperature. There are also strong chances of sealed tube breakage at higher temperature. We have used steel bomb vessel for performing this reaction, and it worked well getting good yields of product. The compound **2** was characterized by melting point as it matches with reported literature.

In step **b**, the compound **2** reacted with 3-bromo-1,1, 1-trifluorobutan-2-one in DME at higher temperature in sealed tube, as this cyclization required high temperature. The compound **3** was confirmed from ^{13}C NMR, as two aliphatic carbons are vanished, and only one signal was there for aromatic ring attached methyl group, also observed different spot on TLC.

The compound **3** on bromination by using crystalized NBS in acetonitrile and AIBN was added as catalyst to obtain yield >90%. We have used heating condition for this bromination as to decrease the time of reaction. At room temperature for getting good yields, we have to run the reaction for 12 hours. The new non polar spot was seen on TLC, afterisolation the ^1H NMR shows singlet of methylene peak for 2 protons confirmingformation of compound 3.

In step **d**, we have done cynation of comp-**4** by using sodium cyanide in DMF: H_2O. The optimization reaction for step **d** was done by using different solvents obtaining better yields.

We have done reaction by using MeOH, toluene and DMF to get 30%, 34% and 50% yields respectively. Further we have used DMF assolvent along with combination of water for increasing the yield of compound 5. We have used 1:1 (DMF-H_2O) mixture; we got 55% yield; further, we have decreased the water content to (2:1), (3:1), (4:1), and (5:1); in all these combinations, we got yield of 80% in (4:1) combination condition. The yields in higher combinations and lower combinations of H_2O ware less as compared with 4 DMF and 1 H_2O combination. We used the same condition for scale up of compound **5**.

The compound 5 was treated with aq. NaOH solution at room temperature for 16 h there is 50% formation of compound 6. We have heated compound **5** along with NaOH at 100°C for 1 hour; there is 60% formation of product, and after 2 hours, there is >90% formation of compound **6**; we used the same condition for scale up of compound **6**.

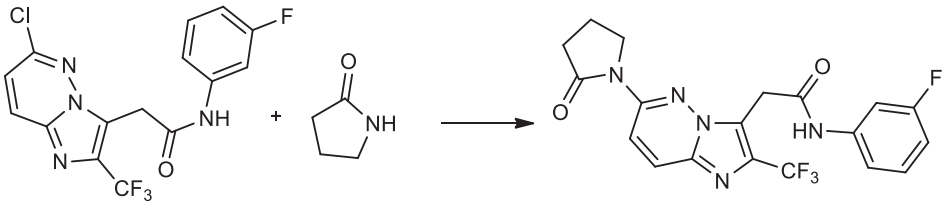
In step **f**, we have used peptide coupling condition for the reaction of compound **6** and 3-flouro aniline to obtain 88% of compound **7**. [36,37]

In step **g**, we have used Buchwald coupling reaction condition to obtain different compounds as **9a-9j** with

TABLE 1 Screening of catalyst, ligand, base, and solvent for synthesis of compound (**9h**)

Entry	Catalyst	Ligand	Base	Solvent	Temp (°C)	Yield[a] (%)
1	$Cu(OAc)_2$	i	Cs_2CO_3	Dioxane	110	20
2	$Cu(OAc)_2$	i	K_3PO_4	DMF	150	32
3	$Cu(OAc)_2$	i	KOtBu	Toluene	110	18
4	$Cu(OAc)_2$	ii	Cs_2CO_3	Dioxane	110	24
5	$Cu(OAc)_2$	ii	K_2CO_3	Toluene	110	20
6	$Cu(OAc)_2$	ii	K_3PO_4	DMF	150	75
7	$Cu(OAc)_2$	iii	Cs_2CO_3	Dioxane	110	45
8	$Cu(OAc)_2$	iii	K_3PO_4	Dioxane	110	36
9	$Cu(OAc)_2$	iii	NaOtBu	Toluene	110	30
10	$Cu(OAc)_2$	iv	Cs_2CO_3	DMF	150	40
11	$Pd(OAc)_2$	i	Cs_2CO_3	Dioxane	110	20
12	$Pd(OAc)_2$	i	K_3PO_4	Toluene	110	15
13	$Pd(OAc)_2$	i	KOtBu	Toluene	110	-
14	$Pd(OAc)_2$	ii	Cs_2CO_3	Dioxane	110	20
15	$Pd(OAc)_2$	ii	K_3PO_4	Dioxane	110	30
16	$Pd(OAc)_2$	iii	Cs_2CO_3	Dioxane	110	30
17	$Pd(OAc)_2$	iii	K_3PO_4	Dioxane	110	28
18	$Pd(OAc)_2$	iv	K_3PO_4	DMF	150	40
19	$Pd(OAc)_2$	iv	K_2CO_3	DMF	150	25

[a]Isolated yield, Comp-**7** (1 eq.), pyrrolidin-2-one (**8h**), (1.5 eq.), catalyst (10 mol%), ligand (15 mol%), base (2 eq.), solvent (10 Vol) time 12 hours.



7 8h 9h

SCHEME 2 2-(2-(Trifluoromethyl)-6-(2-oxopyrrolidin-1-yl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl) acetamide (**9h**)

>70% yields.[38] We have optimized Buchwald coupling reaction by modifying catalyst, ligand, base, solvent, and temperature to get suitable condition.

For optimization purpose, we have taken pyrrolidin-2-one as model amine reacted with comp-**7**; we have used catalyst $Cu(OAc)_2$ and $Pd(OAc)_2$, ligands as BINAP (i), 1,2-Dimethylethylenediamine (DMEDA) (ii), trans-*N*,*N*′-dimethylcyclohexane-1,2-diamine (DMCDA) (iii), and Xanthophos (iv). We have used bases like CS_2CO_3, K_2CO_3, K_3PO_4, NaOtBu, and KOtBu and solvents like dioxane, toluene, and DMF. For all the optimization reactions, we have used catalyst 10 mol%, ligands 15 mol%, base 2 equivalents, and solvents 10 vol for all the reactions. The results of the optimization reaction were given in Table 1. Synthesis of 2-(2-(trifluoromethyl)-6-(2-oxopyrrolidin-1-yl) imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (**9h**) shown in Scheme 2.

By using $Cu(OAc)_2$ as catalysts, we have used four ligands, different bases, and different solvents as in entries 1 to 10; the yields are in the range of 18% to 75%. We got 75% yield by using DMEDA as ligand K_3PO_4 as base and DMF as solvent in entry 6; we have heated the reaction at high temperature 150°C remaining reactions we have done at 110°C. By using CS_2CO_3 as base, we got yields of 30%, 24%, 45%, and 30% in dioxane and DMF as solvents in entries 1, 4, 7, and 10. By using K_3PO_4 in different solvents like toluene and dioxane, we got <40% yields in entries 2 and 8. For toluene and dioxane, we have done all the reactions at 110°C, and for DMF, we have used 150°C temperature. The reaction at higher temperature works well. In entry 10, we have used xanthophos as ligand CS_2CO_3 as base DMF as solvent, and we heated the reaction at 150 C to obtain 40% of desired product. In entries 11 to 19, we have used

$Pd(OAc)_2$ as catalyst, BINAP, DMEDA, DMCDA, xanthophos as ligands, CS_2CO_3, K_3PO_4, K_2CO_3, KOtBu, and NaOtBu as bases. We have used dioxane, toluene, and DMF as solvents; the reaction temperature and isolated yields of the product were given in Table 1. From all those optimizations, it is clear that when we use $Cu(OAc)_2$ as catalyst, DMEDA as ligand, K_3PO_4 as base, and DMF as solvent at 150°C, we obtain highest yield. The same reaction condition works well for the synthesis of remaining compounds from the series; in most of the cases, we got yields in the range of 65% to 75%. The detailed experimental procedure, workup, and yield details are given in experimental section.

3 | EXPERIMENTAL SECTION

All chemicals, unless otherwise specified, were purchased from commercial sources and were used without further purification. The major chemicals were purchased from Sigma Aldrich and Avra labs. The development of reactions was monitored by thin layer chromatography (TLC) analysis on Merck precoated silica gel 60 F254 aluminum sheets, visualized by UV light. Melting points were recorded on SRS Optimelt, melting point apparatus, and are uncorrected. The ^{1}H NMR spectra were recorded on a 400-MHz Varian NMR spectrometer. The ^{13}C were recorded on a 100-MHz Varian NMR spectrometer. The chemical shifts are reported as NMR spectra δppm units. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br s). LCMS mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

3.1 | Experimental procedure for synthesis of 2-(2-(trifluoromethyl)-6-(substituted)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(substituted)acetamide derivatives (9a-9j)

3.1.1 | Step a—synthesis of 6-chloropyridazin-3-amine (2)

To a stirred solution of comp-**1** (14.9 g, 1.0 mmol) in a steel bottomed flask was added Liq. ammonia (150 mL) at −10°C. Allowed reaction mixture to come to room temperature and stirred at 150°C for 8 hours. Progress of reaction was monitored by TLC and LCMS for the consumption of starting material. After completion, reaction mixture was cooled to −10°C, solid precipitates out in steel bottomed flask which is filtered. The obtained solid was washed with hexane (250 mL), cold diethyl ether (200 mL), and cold pentane (200 mL) and dried in vacuo to afford 6-chloropyridazin-3-amine (**2**, 10 g, 77.2%) [CAS - 5469-69-2] as yellow solid.

The crude obtained was used further for next reaction without purification.

3.1.2 | Step b—synthesis of 6-chloro-2-(trifluoromethyl)-3-methylimidazo[1,2-*b*]pyridazine (3)

To a stirred solution of comp-**2** (1.29 g, 1.0 mmol) and DME (13 mL) in a sealed tube was added 3-bromo-1,1,1-trifluorobutan-2-one (2.25 g, 1.1 mmol) at room temperature. Heat the reaction mixture at 150°C for 4 hours. Progress of reaction was monitored by TLC and LCMS for the consumption of starting material. After completion, reaction mixture was poured in cold H_2O (10 mL), precipitation formed in reaction mixture, which was filtered in vacuo washed with water (25 mL), brine (20 mL), cold diethyl ether (25 mL), and cold pentane (25 mL) to afford 6-chloro-2-(trifluoromethyl)-3-methylimidazo[1,2-*b*]pyridazine (**3**, 2 g, 85.3%) as brown solid.

The crude obtained was used further for next reaction without purification.

3.1.3 | Step c—synthesis of 3-(bromomethyl)-6-chloro-2-(trifluoromethyl)imidazo[1,2-*b*]pyridazine (4)

To a stirred solution of comp-**3** (2.35 g, 1.0 mmol) and ACN (25 mL) in a sealed tube was added freshly crystalized NBS (1.95 g, 1.1 mmol) and AIBN (25 mg) at room temperature. Heat the reaction mixture at 100°C for 1 hour. Progress of reaction was monitored by TLC and LCMS for the consumption of starting material. After completion, the reaction mixture was evaporated in vacuo to obtain crude, which was poured in cold H_2O (20 mL). Reaction mixture was extracted with DCM (2 × 20 mL). The organic layer was separated, washed with H_2O (15 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford 3-(bromomethyl)-6-chloro-2-(trifluoromethyl)imidazo[1,2-*b*]pyridazine (**4**, 2.9 g, 92%) as yellow solid.

The crude obtained was used further for next reaction without purification.

3.1.4 | Step d—synthesis of 2-(6-chloro-2-(trifluoromethyl)imidazo[1,2-*b*]pyridazin-3-yl)acetonitrile (5)

To a stirred solution of comp-**4** (2.35 g, 1.0 mmol) and DMF: H_2O (19 mL: 5 mL) in a round bottom flask was

carefully added NaCN (0.98 g, 2.0 mmol) at room temperature. Stirred the reaction mixture at room temperature for 12 hours. Progress of reaction was monitored by TLC and LCMS for the consumption of starting material. After completion, the reaction mixture was poured in cold H_2O (30 mL). Reaction mixture was extracted with DCM (2 × 15 mL). The organic layer was separated, washed with H_2O (3 × 15 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo to afford 2-(6-chloro-2-(trifluoromethyl)imidazo[1,2-*b*]pyridazin-3-yl)acetonitrile (**5**, 2.08 g, 80%) as off white solid.

The crude obtained was used further for next reaction without purification.

3.1.5 | Step e—synthesis of 2-(6-chloro-2-(trifluoromethyl)imidazo[1,2-*b*]pyridazin-3-yl)acetic acid (6)

To a stirred solution of comp-**5** (2.60 g, 1.0 mmol) in a round bottom flask was added aq. 20% NaOH solution (25 mL) at room temperature. Heat the reaction mixture at 100°C for 2 hours. Progress of reaction was monitored by TLC and LCMS for the consumption of starting material.

After completion, reaction mixture was poured in cold H_2O (30 mL) and extracted with DCM (25 mL). Collected the aqueous layer acidified it by using 6 N aq. HCl solution up to pH 3 solid precipitated in reaction mixture. The formed precipitation was filtered in vacuo washed with water (25 mL), brine (20 mL), and diethyl ether (25 mL) to afford 2-(6-chloro-2-(trifluoromethyl)imidazo [1,2-*b*]pyridazin-3-yl)acetic acid (**6**, 2.52 g, 90%) as a white solid.

The crude obtained was used further for next reaction without purification.

LC-MS *m*/*z* (%): 280.7 (M + H); ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 11.8 (brs, 1H, COOH), 7.70 (d, J = 8.4 Hz, 1H, ArH), 6.88 (d, J = 7.6 Hz, 1H, Ar-H), 3.388 (s, 2H, CO-CH_2).

3.1.6 | Step f—synthesis of 2-(6-chloro-2-(trifluoromethyl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (7)

To a stirred solution of Comp-**6** (0.28 g, 0.01 mmol) in DCM (5 mL) was added EDCI (0.28 g, 0.015 mmol) and DIPEA (0.52 mL, 0.03 mmol) at 0°C. Added 3-flouroaniline (0.13 g, 0.012 mmol) at 0°C and stirred reaction mixture at room temperature for 12 hours. Progress of the reaction was monitored by TLC and LCMS. After completion, the reaction mixture was diluted with cold water (10 mL). The reaction mixture was extracted with DCM (2 × 10 mL). The organic layer was separated, washed with 1 N aq. cold HCl (5 mL), brine (5 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude obtained was purified by washing with 15:85% of DCM: hexane (20 mL), cold diethyl ether (20 mL), and cold pentane (20 mL) to afford2-(6-chloro-2-(trifluoromethyl)imidazo[1,2-*b*]pyridazin-3-yl)-N-(3-fluorophenyl)acetamide (**7**, 0.33 g, 88%) as a white solid.

IR (KBr) (ν_{max}, cm^{-1}): 1625 (C═O), 1588 and 1520 (Ar). LC-MS *m*/*z* (%): 372.7 (M + H); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (s, 1H, NH), 7.42 (d, J = 7.2 Hz, 1H, ArH), 7.36-7.25 (m, 2H, ArH), 7.22 (d, J = 7.2 Hz, 1H, ArH), 6.80 (d, J = 7.6 Hz, 1H, ArH), 6.70 (d, J = 6.8 Hz, 1H, ArH), 3.40 (s, 2H, CO-CH_2).

3.1.7 | Step g—synthesis of 2-(2-(trifluoromethyl)-6-(2-oxopyrrolidin-1-yl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (9h)

To a stirred solution of Comp-**7** (0.19 g, 0.5 mmol) in DMF (5 mL) was added pyrrolidin-2-one (0.065 g, 0.75 mmol), $Cu(OAc)_2$ (0.019 g, 10 mol%), K_3PO_4 (0.216 g, 1.0 mmol), and *N*,*N*-dimethylethylenediamine (0.028 g, 15 mol%). Degas reaction mixture using argon and heat reaction mixture to 150°C for 12 hours. Progress of reaction was monitored by TLC and LCMS for the consumption of starting material. After completion, reaction mixture was poured in cold H_2O (10 mL) and stirred for 10 minutes. Reaction mixture was extracted with EtOAc (2 × 10 mL), collected organic layer washed with H_2O (5 mL) and Brine (5 mL). Separated organic layer dried over anhydrous Na_2CO_3 and evaporated in vacuo to obtain crude material. Crude was purified by silica gel (100-200 mesh) column chromatography by using (ethyl acetate-hexane; 30:60%) to obtain 2-(2-(trifluoromethyl)-6-(2-oxopyrrolidin-1-yl)imidazo[1,2-*b*] pyridazin-3-yl)-N-(3-fluorophenyl)acetamide (**9h**, 160 mg, 75%) as a white solid.

3.1.8 | 2-(2-(Trifluoromethyl)-6-(methylamino)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (9a)

Yellow Solid; m.p. 163-164°C; Yield - 68%; IR (KBr) (ν_{max}, cm^{-1}): 1627 (C═O), 1582 and 1520 (Ar). Anal. calc. for $C_{16}H_{13}F_4N_5O$: C, 52.32; H, 3.57; N, 19.07; Found: C, 52.36; H, 3.61; N, 19.04. LC-MS *m*/*z* (%): 367.3 (M + H); HPLC-98.9% RT-8.22 minutes; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H, NH), 7.43 (d, J = 7.2 Hz, 1H, ArH), 7.38-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.88 (d, J = 7.6 Hz, 1H, ArH), 6.72 (d, J = 6.8 Hz,

1H, ArH), 5.16 (q, 1H, CH_3-NH), 3.42 (s, 2H, CO-CH_2), 2.46 (d, J = 6.6 Hz, 3H, NH-CH_3). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ 24.3, 28.7, 110.1, 112.2, 117.2, 118.2, 120.2, 120.4, 124.6, 130.4, 135.6, 136.3, 136.8, 162.3, 163.4, 168.6.

3.1.9 | 2-(6-(Ethylamino)-2-(trifluoromethyl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (9b)

Gray Solid; m.p. 167-168°C; Yield - 70%; IR (KBr) (ν_{max}, cm^{-1}): 1624 (C═O), 1580 and 1524 (Ar). Anal. calc. for $C_{17}H_{15}F_4N_5O$: C, 53.55; H, 3.96; N, 18.37; Found: C, 53.51; H, 3.99; N, 18.34; Mol. Wt.: 381.33; HPLC-97.2% RT-8.16 minutes; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H, NH), 7.43 (d, J = 7.2 Hz, 1H, ArH), 7.38-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.88 (d, J = 7.6 Hz, 1H, ArH), 6.72 (d, J = 6.8 Hz, 1H, ArH), 5.16 (q, 1H, CH_3-NH), 3.42 (s, 2H, CO-CH_2), 3.16-3.08 (q, J = 14.4, 7.2 Hz, 2H, CH_3-CH_2), 1.16 (s, 3H, CH_2-CH_3).^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ14.8, 28.7, 40.4, 110.2, 112.2, 117.4, 118.2, 120.2, 120.4, 124.6, 130.4, 135.6, 136.3, 136.8, 162.2, 163.3, 168.6.

3.1.10 | 2-(2-(Trifluoromethyl)-6-(propylamino)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (9c)

Gray Solid; m.p. 181-182°C; Yield - 69%; IR (KBr) (ν_{max}, cm^{-1}): 1620 (C═O), 1576 and 1524 (Ar). Anal. calc. for $C_{18}H_{17}F_4N_5O$: C, 54.68; H, 4.33; N, 17.71; Found: C, 54.69; H, 4.38; N, 17.75; Mol. Wt.: 395.35; HPLC-98.3% RT-7.89 minutes; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H, NH), 7.43 (d, J = 7.2 Hz, 1H, ArH), 7.38-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.88 (d, J = 7.6 Hz, 1H, ArH), 6.72 (d, J = 6.8 Hz, 1H, ArH), 5.16 (q, 1H, CH_3-NH), 3.42 (s, 2H, CO-CH_2), 3.16-3.14 (d, J = 12.6, 6.4 Hz, 2H, CH_2-CH_2), 1.54 (q, J = 14.8, 7.4 Hz, 2H, CH_3-CH_2), 0.94 (t, J = 7.4 Hz, 3H, CH_2-CH_3). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ 11.2, 24.3, 28.7, 47.4, 110.4, 112.2, 117.1, 118.5, 120.2, 120.4, 124.6, 130.2, 135.6, 136.3, 136.6, 162.3, 163.6, 168.8.

3.1.11 | 2-(6-(Butylamino)-2-(trifluoromethyl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (9d)

Brown Solid; m.p. 162-163°C; Yield- 75%; IR (KBr) (ν_{max}, cm^{-1}): 1628 (C═O), 1586 and 1516 (Ar). Anal. calc. for $C_{19}H_{19}F_4N_5O$: C, 55.74; H, 4.68; N, 17.11; Found: C, 55.70; H, 4.62; N, 17.08; Mol. Wt.: 409.38; HPLC-99.3% RT-8.44 minutes; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H, NH), 7.43 (d, J = 7.2 Hz, 1H, ArH), 7.38-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.88 (d, J = 7.6 Hz, 1H, ArH), 6.72 (d, J = 6.8 Hz, 1H, ArH), 5.16 (q, 1H, CH_3-NH), 3.42 (s, 2H, CO-CH_2), 3.16-3.09 (d, J = 12.6, 6.4 Hz, 2H, CH_2-CH_2), 1.58-142 (q, J = 14.8, 7.4 Hz, 4H, CH_2-CH_2), 0.96 (t, J = 7.4 Hz, 3H, CH_2-CH_3). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ 12.8, 20.3, 28.7, 33.5, 47.2, 110.2, 112.2, 117.2, 118.2, 120.2, 120.4, 124.8, 130.4, 135.6, 136.3, 137.4, 162.4, 163.4, 168.6.

3.1.12 | 2-(2-(Trifluoromethyl)-6-(pyrrolidin-1-yl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (9e)

Off white Solid; m.p. 136-137°C; Yield - 68%; IR (KBr) (ν_{max}, cm^{-1}): 1628 (C═O), 1580 and 1520 (Ar). Anal. calc. for $C_{19}H_{17}F_4N_5O$: C, 56.02; H, 4.21; N, 17.19; Found: C, 55.95; H, 4.18; N, 17.22; Mol. Wt.: 407.36; HPLC-99.1% RT-7.87 minutes; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H, NH), 7.43 (d, J = 7.2 Hz, 1H, ArH), 7.38-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.88 (d, J = 7.6 Hz, 1H, ArH), 6.72 (d, J = 6.8 Hz, 1H, ArH), 3.42 (s, 2H, CO-CH_2), 2.78 (t, J = 8.4, 4.4 Hz, 4H, N-$(CH_2)_2$), 1.58 (q, J = 7.6, 3.8 Hz, 4H, CH_2-$(CH_2)_2$). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ 25.6, 28.7, 52.8, 110.1, 112.1, 117.7, 118.7, 120.2, 120.4, 124.6, 130.4, 135.6, 136.3, 136.8, 156.3, 163.4, 168.4.

3.1.13 | 2-(2-(Trifluoromethyl)-6-(piperidin-1-yl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (9f)

Off white Solid; m.p. 139-140°C; Yield - 64%; IR (KBr) (ν_{max}, cm^{-1}): 1632 (C═O), 1584 and 1528 (Ar). Anal. calc. for $C_{20}H_{19}F_4N_5O$: C, 57.00; H, 4.54; N, 16.62; Found: C, 57.06; H, 4.59; N, 16.67; Mol. Wt.: 421.39; HPLC-98.2% RT-7.44 minutes; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H, NH), 7.43 (d, J = 7.2 Hz, 1H, ArH), 7.38-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.88 (d, J = 7.6 Hz, 1H, ArH), 6.72 (d, J = 6.8 Hz, 1H, ArH), 3.42 (s, 2H, CO-CH_2), 2.82 (t, J = 8.4, 4.4 Hz, 4H, N-$(CH_2)_2$), 1.48 (q, J = 7.6, 3.8 Hz, 4H, CH_2-$(CH_2)_3$). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ 24.6, 26.1, 28.7, 52.4, 110.1, 112.2, 117.2, 118.2, 120.2, 120.4, 124.6, 130.4, 135.6, 136.3, 136.8, 157.3, 163.8, 168.2.

3.1.14 | 2-(2-(Trifluoromethyl)-6-(4-methylpiperidin-1-yl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl)acetamide (9g)

Yellow Solid; m.p. 149-150°C; Yield - 52%; IR (KBr) (ν_{max}, cm^{-1}): 1634 (C═O), 1588 and 1528 (Ar). Anal.

calc. for $C_{21}H_{21}F_4N_5O$: C, 57.93; H, 4.86; N, 16.08; Found: C, 57.90; H, 4.89; N, 16.02; Mol. Wt.: 435.42; HPLC-99.8% RT-7.38 minutes; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H, NH), 7.43 (d, J = 7.2 Hz, 1H, ArH), 7.38-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.88 (d, J = 7.6 Hz, 1H, ArH), 6.72 (d, J = 6.8 Hz, 1H, ArH), 3.42 (s, 2H, CO-CH_2), 2.82 (t, J = 8.4, 4.4 Hz, 4H, N-$(CH_2)_2$), 1.54 (q, J = 8.0, 4.0 Hz, 1H, CH_3-CH), 1.48 (q, J = 7.6, 3.8 Hz, 4H, CH_2-$(CH_2)_2$), 1.02 (d, J = 6.2 Hz, 3H, CH-CH_3). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ 20.3, 28.7, 31.9, 32.2, 48.6, 110.4, 112.6, 117.2, 118.2, 120.2, 120.4, 124.6, 130.4, 135.6, 136.3, 136.4, 157.3, 163.1, 168.3.

3.1.15 | 2-(2-(Trifluoromethyl)-6-(2-oxopyrrolidin-1-yl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl) acetamide (9h)

White Solid; m.p. 183-184°C; Yield - 75%; IR (KBr) (ν_{max}, cm^{-1}): 1636 (C═O), 1576 and 1524 (Ar). Anal. calc. for $C_{19}H_{15}F_4N_5O_2$: C, 54.16; H, 3.59; N, 16.62; Found: C, 54.10; H, 3.63; N, 16.64; Mol. Wt.: 421.7; HPLC-98.2% RT-7.67 minutes; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H, NH), 7.97 (d, J = 7.6 Hz, 1H, ArH), 7.43 (d, J = 7.2 Hz, 1H, ArH), 7.38-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.72 (d, J = 6.8 Hz, 1H, ArH), 3.42 (s, 2H, CO-CH_2), 3.02 (t, J = 6.4 Hz, 2H, N-CH_2), 2.56 (d, J = 6.2 Hz, 2H, CO-CH_2), 2.16 (q, J = 6.8, 2.2 Hz, 2H, CH_2-CH_2). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ 18.6, 28.7, 33.2, 49.2, 110.8, 112.8, 117.2, 118.2, 120.2, 120.4, 124.7, 130.4, 135.4, 136.3, 136.8, 154.3, 163.6, 168.8, 174.2.

3.1.16 | 2-(2-(Trifluoromethyl)-6-(2-oxopiperidin-1-yl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl) acetamide (9i)

Off white Solid; m.p. 180-190°C; Yield - 75%; IR (KBr) (ν_{max}, cm^{-1}): 1628 (C═O), 1580 and 1530 (Ar). Anal. calc. for $C_{20}H_{17}F_4N_5O_2$: C, 55.17; H, 3.94; N, 16.09; Found: C, 55.16; H, 3.97; N, 16.04; Mol. Wt.: 435.37; HPLC-99,1% RT-8.38 minutes; ^{1}H NMR (400 MHz,DMSO-d6)δ 8.21 (s, 1H, NH), 7.97 (d, J = 7.6 Hz, 1H, ArH), 7.44 (d, J = 7.2 Hz, 1H, ArH), 7.36-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.74 (d, J = 6.8 Hz, 1H, ArH), 3.44 (s, 2H, CO-CH_2), 3.03 (t, J = 6.4 Hz, 2H, N-CH_2), 2.57 (d, J = 6.2 Hz, 2H, CO-CH_2), 1.98 (q, J = 6.8, 2.2 Hz, 2H, CH_2-CH_2), 1.81 (q, J = 6.8, 2.2 Hz, 2H, CH_2-CH_2). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ 22.3, 26.6, 28.7, 32.4, 48.8, 110.8, 113.2, 117.6, 118.2, 120.2, 121.4, 124.6, 130.4, 135.6, 136.3, 136.8, 153.3, 162.4, 167.3, 169.8.

3.1.17 | 2-(2-(Trifluoromethyl)-6-(4-methylpiperazin-1-yl)imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(3-fluorophenyl) acetamide (9j)

Yellow Solid; m.p. 188-189°C; Yield - 80%; IR (KBr) (ν_{max}, cm^{-1}): 1622 (C═O), 1578 and 1518 (Ar). Anal. calc. for $C_{20}H_{20}F_4N_6O$: C, 55.04; H, 4.62; N, 19.26; Found: C, 55.01; H, 4.67; N, 19.29; Mol. Wt.: 436.41; HPLC-98.9% RT-8.22 minutes; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H, NH), 7.43 (d, J = 7.2 Hz, 1H, ArH), 7.38-7.28 (m, 2H, ArH), 7.24 (d, J = 7.2 Hz, 1H, ArH), 6.88 (d, J = 7.6 Hz, 1H, ArH), 6.72 (d, J = 6.8 Hz, 1H, ArH), 3.42 (s, 2H, CO-CH_2), 3.16 (t, J = 8.4, 4.4 Hz, 4H, N-$(CH_2)_2$), 2.48 (t, J = 7.6, 3.8 Hz, 4H, N-$(CH_2)_2$), 2.27 (s, 3H, N-CH_3). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm) = δ 26.7, 42.1, 46.7, 56.6, 110.2, 114.2, 116.2, 118.2, 120.2, 122.4, 124.6, 130.4, 135.6, 136.3, 136.8, 153.3, 164.4, 167.6.

3.2 | Biological evaluation

All the synthesized compounds were tested for their in vitro anticancer activity against human lung cancer cell line and human prostate cancer cell lines. The anticancer activity test is performed according to the procedure developed by the National Cancer Institute (NCI, USA) in the "In vitro Anticancer Drug Discovery Screen" that uses the 3-(4,5-dimethythiazol-2yl)-2,5-diphenyl tetrazolium bromide by mitochondrial succinate dehydrogenase.[39,40] All the newly synthesized compounds **9a-9j** are evaluated for their antiproliferative activities against two human cancer cell lines. The results are summarized in Table 2. These values represent the concentration required to inhibit 50% cell population compared with the control cells treated with DMSO and positive control Doxorubicin under similar conditions. The synthesized compounds were evaluated for their in vitro anticancer activity against human lung cancer cell line (A549) and prostate cell line (DU-145).

3.3 | Cell culture

Human cancer cell lines A549 (lungs) and DU-145 (prostate) were grown in DMEM + GlutaMax (Invitrogen, Carlsbad, CA, USA), supplemented with 10% heat-inactivated

TABLE 2 In vitro anticancer screening of the synthesized compounds against two cell lines; data are expressed as IC_{50} (μM)± SD (n = 3)

Sr. No.	A-549[a]	Si[b]	DU-145[c]	Si[b]	HUVEC[d]
9a	12.27 ± 0.10	6.85	17.26 ± 0.08	4.87	84.06 ± 0.03
9b	8.12 ± 0.06	10.10	7.11 ± 0.11	11.53	82.03 ± 0.05
9c	11.10 ± 0.07	7.84	9.17 ± 0.16	9.49	87.11 ± 0.08
9d	18.08 ± 0.14	4.76	13.48 ± 0.10	6.39	86.16 ± 0.05
9e	2.12 ± 0.04	38.26	2.06 ± 0.07	33.79	81.11 ± 0.07
9f	4.18 ± 0.11	21.31	5.10 ± 0.04	17.47	89.11 ± 0.08
9g	3.10 ± 0.24	28.08	4.10 ± 0.24	21.23	87.06 ± 0.05
9h	1.73 ± 0.18	46.85	1.76 ± 0.06	46.05	81.06 ± 0.06
9i	9.77 ± 0.03	9.11	16.42 ± 0.11	5.42	89.04 ± 0.08
9j	14.13 ± 0.12	6.16	8.36 ± 0.04	10.42	87.11 ± 0.04
Doxorubicin	1.82 ± 0.06	48.02	1.72 ± 0.12	50.81	87.04 ± 0.06

[a]A-549: Human lung cancer cell line.
[b]Selectivity Index (SI): IC50 of pure compound in normal cell line/IC50 of same compound. in cancer cell line. IC50, the concentration required to inhibit 50% of cell population.
[c]DU-145: Human prostate cancer cell line.
[d]HUVEC: Human Umbilical vein endothelial cell line.

bovine serum (Gibco) and penicillin-streptomycin (Gibco, Gaithersburg, MD, USA) at 37°C in a humified chamber with 5% CO_2 supply.

3.4 | Cytotoxicity assay

Cells were seeded (105 cells/well) in 96-well flat-bottom plates (Becton-Dickinson Labware, Franklin Lakes, NJ, USA) a day before treatment and grown overnight. Compounds were dissolved in dimethyl sulfoxide (DMSO; Sigma) and finally prepared as 1.0 mg/mL stocks, respectively, in the culture media. The final concentration of DMSO never exceeded 0.1% in the treatment doses. Six different doses of compounds (400μM, 200μM, 100μM, 50μM, 25μM, and 10μM) were further prepared by diluting the stocks in culture media, and cells were treated (in triplicate/dose). Doxorubicin was included as standard reference drug (positive control), and untreated culture was considered as negative control. The cultures were further incubated for 48 hours. At 48-hour post-treatment, cell viability test was performed using TACS MTT Cell Proliferation and Viability Assay Kit (TACS) as per manufacturer's instructions. The optical density (OD) was recorded at 570 nm in a microplate reader (ELx800, BioTek, Winooski, VT, USA), and cell survival fraction was determined.[39,40] The cell survival fraction was calculated as [(A-B)/A], where A and B are the OD of untreated and of treated cells, respectively. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared with the reference drug Doxorubicin, and the results are given in Table 2.

For lung cancer cell line (A549), the compounds **9e**, **9g**, and **9h** are most active with 38.26%, 28.08%, and 46.85% inhibitions with IC_{50} value of 2.12μM, 3.10μM, and 1.73μM, respectively. The compounds **9f** is moderately active with 21.31% inhibition and with IC_{50} value of 4.18μM; the remaining compounds were less active with IC_{50} values in the range of 8.12μM to 18.08μM. For Du-145 cell line, the compounds **9e** and **9h** are most active with IC_{50} value of 2.06μM and 1.76μM and inhibitions of 33.79% and 46.05%, respectively. For compounds **9f** and **9g** are moderately active with IC_{50} value of 5.10μM to 4.10μM and inhibitions of 17.47% and 21.23%, respectively; remaining compounds are very less active or inactive with $IC5_0$ value in the range of 7.11 to 17.26. Compound **9h** is most active for A-549 and Du-145 cell line with IC_{50} value of 1.73μM and 1.76μM, respectively. Similar results were obtained for compound **9e** for cell lines A-549 and Du-145 with IC_{50} value of 2.12μM and 2.06μM, respectively. The remaining compounds were mostly inactive for both the cancer cell lines.

The SAR can be drawn like compounds having methyl acetamide, ethyl acetamide, propyl acetamide and butyl acetamide wareless active for both the cancer cell lines. These derivatives don't involve anyring structure and electron donating or electron with drawing substituent. These compounds showed very less activity among the synthesized compounds. Instead of straight chain, compounds were replaced by chain compounds like pyrrolidine, piperidine, 4-methylpiperidine, and pyrrolidin-2-one; the activity of final targets is increasing for both the cell lines. In those targets, the ring strain on the molecule plays a key role in the activity. We

TABLE 3 Inhibitory activity of selected compounds against panel of five human kinases

Kinase	9e (% Inh.)	9g (% Inh.)	9h (% Inh.)
CDK2/cyclinA	16	42	51
CDK5/P25	36	28	44
EGFR	72	66	63
mTOR	54	48	66
PDK1	23	28	39

tried to introduce a hetero atom in the ring system like pyrrolidin-2-one, piperidin-2-one, and 1-methylpiperazine; the activity was decreased to greater extent. The compound with pyrrolidine-2-one is more active than standard for both the cell lines. As the ring size increases to piperidin-2-one, there is decrease in activity because of larger ring size. The less activity was seen in the case of 1-methylpiperazine. The rings having oxygen as a heteroatom and larger rings are less active. The compounds having electron donating substituent's ware less active than compounds having ring structures attached with them. The compounds having electron deficient hetero atom shows decrease in activity due to ring delocalization.

The active compounds from cell line studies were further tested for their kinase inhibitors as these targets show good percentage of inhibitions for some kinases, which is key aspect in target identification in cancer, by following the earlier protocols.[41–45]

The compounds **9e**, **9g**, and **9h** are most active in cell line studies, so further, we have tested them for their activity against a panel of five human kinase at 10μM concentration. The inhibition results are summarized in Table 3. Protein kinase plays a key role in cell proliferation, differentiation of cell, migration of cell, survival of cell, and angiogenesis of cells.

Among CMGC kinase family for CDK2/cyclinA and CDK5/P25 kinases, the inhibition is in the range of 16% to 44% for all the compounds. The compounds **9e**, **9g**, and **9h** show 72%, 66%, and 63% inhibitions, respectively, (Table 3) for EGFR kinase. For mTOR kinase, the inhibitions are in the range of 48% to 66% for all the compounds. For PDK1 kinase, the inhibitor for all the compounds has very less inhibitions in the range of 23% to 39%. The active compounds in cell line data were most active for protein kinase inhibitors; all the compounds **9e**, **9g**, and **9h** show proposing inhibitions for EGFR and m-TOR kinases.

4 | CONCLUSION

We have synthesized 2-(2-(trifluoromethyl)-6-(substituted) imidazo[1,2-*b*]pyridazin-3-yl)-*N*-(substituted)acetamide derivatives (**9a-9j**) from 3,6-dichloropyridazine through a series of reactions including imidazo-pyridazine ring formation, halogenation, cynation, peptide coupling, and Buchwald reaction. We have tried to report simple reaction condition, easy workup, short reaction time, and good to high yields. The synthesized compounds were screened for anticancer activity against A-549 and Du-145 cancer cell lines. The compounds **9e**, **9g**, and **9h** are most active with IC_{50} values in the range of 1.73μM to 3.10μM. The compounds **9e**, **9g**, and **9h** show promising inhibitions (>54%) for EGFR and m-TOR human kinases. The compounds having electron-donating substituents along with aliphatic ring and without electron deficient atom show promising activity and greater inhibitions for protein kinases.

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