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Full Paper

Design, Synthesis, and Evaluation of the Kinase Inhibition Potential of Pyridylpyrimidinylaminophenyl Derivatives

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In view of potent kinase inhibitors for the treatment of myriad human disorders, we synthesized some structurally variant amide/cyclic amide derivatives based on pyridylpyrimidinylaminophenyl amine, the key pharmacophore of the kinase inhibitor drug molecule, imatinib, and evaluated their kinase inhibition potency. Among the various synthesized amides, compound **20**, a cyclic amide/pyridin-2(1*H*)-one derivative, exhibited an IC_{50} value comparable to that of the drug imatinib against c-Src kinase, and another compound (**14**) containing a 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)oxy)acetamide demonstrated an IC_{50} value of 8.39 μM. Furthermore, the constitution of the cyclic amide derivative was confirmed by the single-crystal X-ray diffraction technique. These results may serve as a gateway for developing novel next-generation kinase inhibitors.

Keywords: Amide derivatives / Imatinib / Kinase inhibitors / Pyridin-2(1*H*)-one / Pyridylpyrimidinylaminophenyl

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Introduction

Protein kinases are key regulators that mediate signal transduction pathways, and regulate various cellular activities like proliferation, survival, apoptosis, metabolism, transcription, differentiation, by catalyzing the transfer of the γ-phosphate group of ATP onto a substrate [1]. Deregulation of kinase activity has emerged as a major mechanism by which cells develop pathophysiological conditions regarding cell growth and fate leading to mortal diseases such as cancer [2]. The Abl and c-Src kinases belong to the family of non-receptor tyrosine kinases that are involved in the tumorigenesis process [3, 4]. c-Src kinase is recognized to play a critical role in the genesis and progression of myriad human disorders, including cancers of the breast, colon, prostate, lung, ovary, and in myeloproliferative disorders by inducing STATs (signal transducer and activator of transcription) [5, 6]. Consequently, there are bestowed interest both in academia and industry in the development of novel and potent kinase inhibitors for therapeutic interventions in the treatment of cancer. The most popular approaches for cancer treatment since 1980's includes the use of cytotoxic agents like antimetabolic and microtubule-destabilizing drugs [7], and targeting selective signal elements for cancer cells [8]. However, the latter approach is grounded upon the development of protein kinase inhibitors that act by intruding the signaling network

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of cancer cells. Naturally occurring bioactive alkaloid like staurosporine, a prototype ATP-competitive protein kinase inhibitor with strong binding affinity, has been explored for its anticancer potency but has not made it to therapeutic usage due to its limited selectivity and lack of specificity [9]. However, it is commonly used as a positive control in kinase assays. The early success in the field of targeted therapy of cancer was achieved in 2001 by the efficacious use of Gleevec (imatinib, **1a**), the foremost FDA-approved tyrosine kinase inhibiting drug for treatment of chronic myeloid leukemia (CML) [10–12]. The discovery of imatinib (**1a**), a type II kinase inhibitor, is a pathbreaking discovery in selective cancer therapy as it works by inhibiting a constitutively active fusion protein, Bcr-Abl (present exclusively in >90% of CML cancer cells), from phosphorylating successive proteins thereby suppressing the signaling cascade essential for cancer development, thus leading to cell death by apoptosis [13]. The FDA approved imatinib has been rationally developed from a 2-phenylaminopyrimidine (PAP) scaffold found through high-throughput screening against the Bcr-Abl fusion protein, the lead structure responsible for kinase inhibition [14]. The structure was further modified for improved binding efficacy and selectivity by introduction of the pyridine moiety at C-4 position of pyrimidine leading to enhancement of protein kinase C inhibition, and the phenyl amide part for conferring high tyrosine kinase and Bcr-Abl kinase inhibition. Additional gain in selectivity toward Bcr-Abl was achieved by restricting the conformation by attachment of methyl at the C-6 position of phenyl ring [14, 15]. The crystal structure of the kinase domain of c-Abl with imatinib has shown the pyridylpyrimidinylaminophenyl (PPAP) part to interact with the ATP binding region in the Bcr-Abl fusion enzyme, thereby inhibiting its activity [16].

As a part of our enduring research program in the design and synthesis of biologically potent diversity of heterocycles we have identified few compounds that possess significant antiproliferative and kinase inhibition activity [17–23]. This study motivated us to assemble newer and more widely effective kinase inhibitors. Our synthetic approach is based on the concept of molecular hybridization, an effective tool for the cogent design of novel molecular constructs by covalently conjugating two or more active pharmacophores [24]. Such constructs are known to exhibit improved pharmacokinetic-dynamic properties, potency to act on more than one target with reduced side effects and efficacy in case of drug resistant cases [25]. Success of molecular hybridization in medicinal chemistry has been exemplified in the development of a tyrosine kinase inhibitor drug "nilotinib" (**1b**) (Fig. 1), a hybrid/variant of imatinib having the active pharmacophore PPAP (Fig. 1) [26] conserved in its structure; the drug is efficacious in imatinib resistant cases [27]. On the basis of above and considering the literature reports discussing Abl-imatinib structural interactions [16, 26–32], we decided to conjugate the scaffolds based on 2-pyridone [21, 33, 34], benzopyran-2-one [35], benzopyran-4-one [22, 36], indole acetic acid, *iso*-nicotinic acid, hippuric acid, piperic acid, 2-oxoquinolinacetic acid, 3-oxobenzooxazinacetic acid [37], and trimethoxyphenyl acrylic acid [38], with PPAP moiety to form the amide/cyclic amide derivatives.

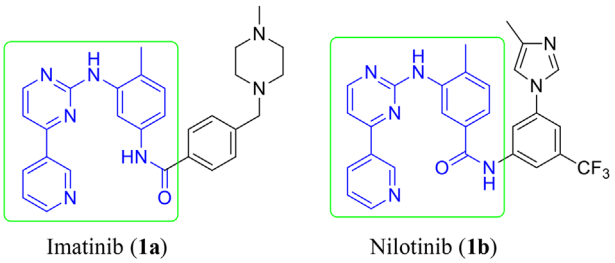


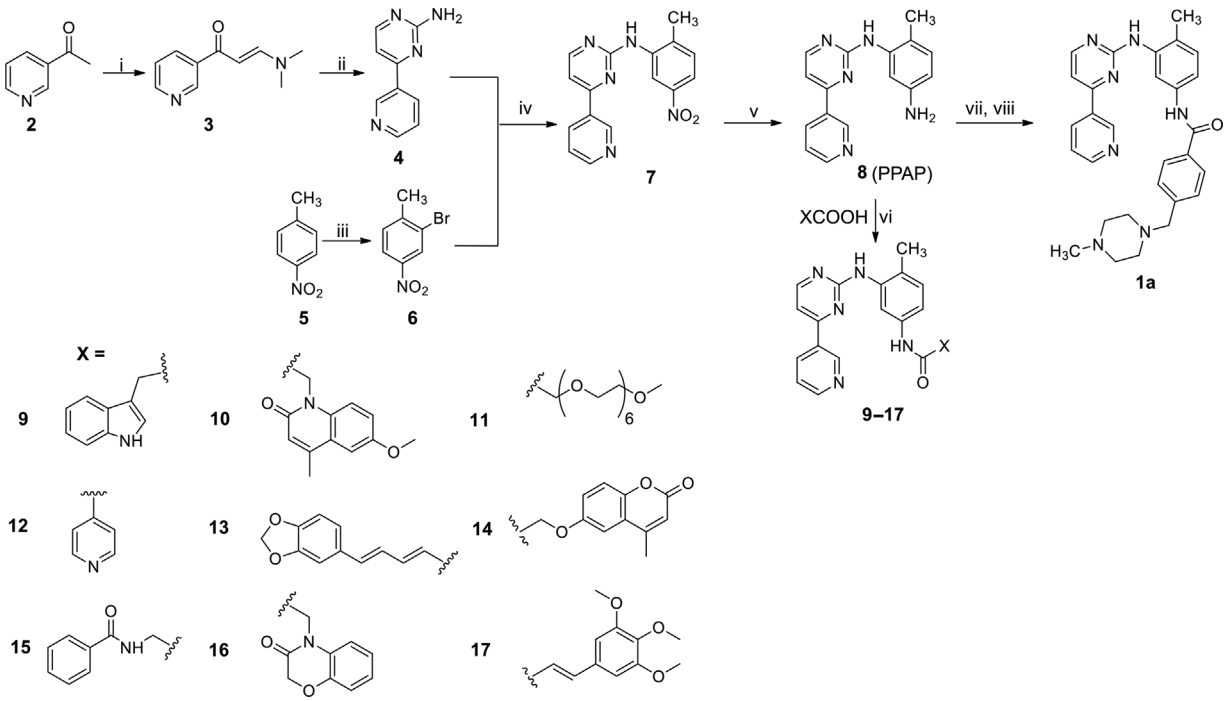
Figure 1. FDA approved tyrosine kinase inhibitors containing the boxed pyridylpyrimidinylaminophenyl (PPAP) scaffold.

Results and discussion

Chemistry

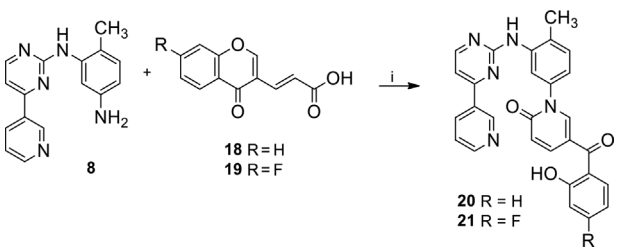
The pharmacophore 6-methyl-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (**8**), was synthesized by using a route that partially follows the reported preparation of imatinib (Scheme 1) [29]. The reaction of 3-acetylpyridine **2** with an excess of dimethylformamide dimethyl acetal (DMF DMA) in xylene gave the enaminone **3**. It was reacted further with guanidine hydrochloride and sodium hydroxide in *n*-butanol leading to the formation of the pyrimidine ring shown in compound **4**. *o*-Bromo-*p*-nitrotoluene **6** was synthesized from *p*-nitrotoluene **5** and was then coupled with **4** by Ullman type coupling using copper iodide (CuI), *N*,*N*-dimethylethylenediamine (DMEDA), potassium iodide (KI), and potassium carbonate (K_2CO_3) in dry dioxane at 100°C for 24 h leading to formation of pyrimidine nitro derivative **7**. Reduction of the nitro group of compound **7** to an amine has been previously reported by different hydrogenation strategies [39]. We adopted a very convenient methodology for this reduction reaction by employing ammonium formate with 10% Pd/C in anhydrous methanol [40]. The *in situ* generated hydrogen reduces nitro compound **7** to corresponding amine **8** in short reaction time of 3–4 h with 80% isolated yield.

Amides (**9–16**) were synthesized by using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbonate (EDC.HCl) as the coupling agent and hydroxybenzotriazole (HOBt) as an additive for the reaction between amine **8** and different acids in DMF in the presence of base triethylamine (TEA)/*N*,*N*-diisopropylethylamine (DIPEA) (Scheme 1). 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate (HATU) was employed as a coupling agent for the synthesis of amide **17** (Scheme 1). Amine **8** was also coupled with 4-bromomethylbenzoic acid to obtain the corresponding pyrimidine amide derivative, which was sequentially treated with 1-methylpiperazine to form imatinib (**1a**) (Scheme 1) [41].



Scheme 1. Synthesis of amide derivatives of 6-methyl-*N*-(4-(pyridin-4-yl)pyrimidin-2-yl)benzene-1,3-diamine. Reagents and conditions: i. DMF DMA, xylene, reflux; ii. guanidine hydrochloride, NaOH, *n*-butanol; iii. *N*-bromosuccinimide, H_2SO_4/H_2O (1:1); iv. CuI, KI, DMEDA, dry dioxane; v. ammonium formate, 10% Pd/C, dry methanol; vi. EDC.HCl, HOBt, DIPEA, DMF, 30°C, 24 h (for compound **17** vi. HATU, DIPEA, DMF, 30°C, 24 h); vii. 4-bromomethylbenzoic acid, EDC.HCl, HOBt, DIPEA, DMF, 30°C, 24 h; viii. 1-methylpiperazine, dioxane, 30°C, 1 h.

Cyclic amides **20**/**21** (2-pyridone derivatives) were synthesized by coupling diamine **8** with (*E*)-3-(4-oxo-4*H*-chromen-3-yl)acrylic acid and (*E*)-3-(7-fluoro-4-oxo-4*H*-chromen-3-yl)acrylic acid, respectively, in the presence of EDC.HCl and HOBt (Scheme 2). Both compounds were characterized on the basis of their spectral data. The proton noise decoupled ^{13}C NMR spectra for 5-(4-fluoro-2-hydroxybenzoyl)-1-(4-methyl-3-((4-(pyridin-3-yl)pyrimidi n-2-yl)amino)phen-yl)pyridin-2(1*H*)-one (**21**) showed a characteristic carbonyl peak at δ 193 ppm, this deshielded value ruled out the formation of simple acyclic amide and rather suggested the presence of 2-pyridone **21** formed by the Michael addition of the aromatic amine via nucleophilic nitrogen on the *O*-acylisourea intermediate of the acrylic acid followed by subsequent ring opening and closure [42]. The structure was further confirmed by single crystal X-ray diffraction (SCXRD) analysis. Compound **21** crystalizes in monoclinic *P*21 space group with two ligand units present in the asymmetric unit (Fig. 2 and Supporting Information Tables S2–S4). The characteristic carbonyl peak for the benzoyl moiety in compound **20** was also observed in a similar range, that is, at δ 191.7 ppm, thus confirming the structure of compound **20** to be similar to that of **21** as shown in Scheme 2.



Scheme 2. Synthesis of cyclic amide analogue of imatinib. Reagents and conditions: i. EDC.HCl, HOBt, DIPEA, DMF, 30°C, 24 h.

The methodology for the synthesis of (*E*)-3-(4-oxo-4*H*-chromen-3-yl)acrylic acid, (*E*)-3-(3,4,5-trimethoxyphenyl)-acrylic acid, 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)oxy)acetic acid, 2-(6-methoxy-4-methyl-2-oxoquinolin-1(2*H*)-yl)acetic acid, 2-(3-oxo-2*H*-benzo[*b*][1,4]oxazin-4(3*H*)-yl)acetic acid and methoxypolyethylene glycol acid (M_w: 350) is described in the Supporting Information (Schemes S1–S5). Other acids, such as indole acetic acid, hippuric acid, piperic acid, isonicotinic acid were procured from Sigma–Aldrich. All the amides (**9**–**17**, **20**–**21**) were obtained in moderate to good yields.

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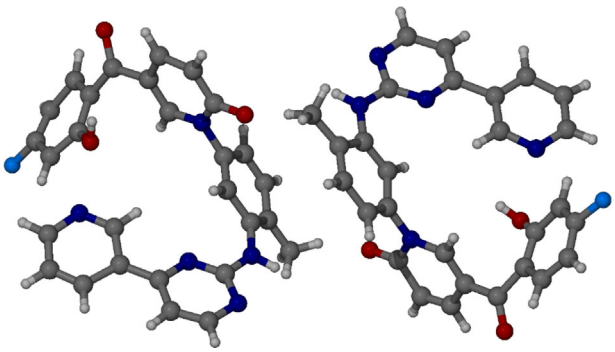

Figure 2. Molecular structure of compound **21** (CCDC Number 1483452).

Biology

Kinase inhibitory activity evaluation

The kinase inhibition potential of all the synthesized pyridylpyrimidinylaminophenyl derivatives was screened against Abl1 and c-Src kinase. Compounds were tested in single dose duplicate mode at a concentration of 50 μM (Table 1). Reactions were carried out in the presence of 10 μM ATP. Control compound, staurosporine, was tested in 10-dose IC_{50} mode with fourfold serial dilution starting at 20 μM and showed IC_{50} values of 25.2 and 1.61 nM against Abl1 and c-Src, respectively. A number of compounds showed more than 50% inhibition against Abl1 and c-Src. Among all the compounds screened, compound **20** showed maximum enzyme inhibition activity against both Abl1 and c-Src kinases. Compounds **13** and **14** did not show any inhibitory activity against c-Src kinase.

Although imatinib is marketed as a drug in the form of its mesylate salt, we have synthesized it in non-salt form for a better *in vitro* comparison with the synthesized compounds.

Table 1. Percentage of inhibition by compounds against Abl1 and c-Src.

Compound	Normalized % inhibition	
	Abl1	c-Src
1a	100.29	70.68
9	85.29	74.54
10	43.14	11.44
11	46.56	15.05
12	86.06	66.34
13	63.17	NA
14	78.36	NA
15	43.41	9.35
16	82.11	1.09
17	88.09	42.31
20	90.98	92.81

All the experiments were performed in triplicate (± SD).

Imatinib (**1a**) showed IC_{50} values of 0.27, >50, 35.1, and 0.26 μM against Abl1, EGFR, c-Src, and Lck, respectively. Staurosporine exhibited IC_{50} values of 31.3, 79.5, 3.0, and 1.4 nM against Abl1, EGFR, c-Src, and Lck, respectively. The cyclic amide derivative **20** exhibited higher c-Src inhibition as compared to imatinib. It showed Abl1, c-Src, and Lck kinase inhibition with IC_{50} values of 2.7, 2.8, and 9.9 μM, respectively, while it did not show high inhibitory activity against EGFR. Compound **14** containing a 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)oxy)acetamide moiety exhibited less kinase inhibition. It demonstrated IC_{50} values of 8.4, 99.7, and 14.4 μM against Abl1, EGFR, and Lck, respectively, while no significant inhibitory activity was observed against c-Src. Compound **14** showed higher selectivity against Lck when compared with imatinib. The data suggest it is possible to generate selectivity with appropriate substitution on PPAP scaffold (Table 2).

Cell viability assay

The activity of the compounds was examined on the cell viability of human leukemia cell line CCRF-CEM for 72 h at a concentration of 50 μM (Fig. 3). Compounds **1a**, **10**, **12**, and **20** exhibited modest antiproliferative activity.

Among all the compounds, compound **20** reduced the cell viability of human leukemia cell line (CCRF-CEM) by 56.2%. Compounds **13** and **14** were not active against c-Src; also these compounds did not show any significant anticancer activity. There was no clear correlation between kinase inhibitory activity and anticancer assay possibly due to differences in cellular uptake and solubility of the compounds. Optimized compounds such as compound **20** that demonstrated both tyrosine kinase inhibition and anticancer activity could generate analogs with higher efficacy profile at the site of action.

Conclusions

A series of structurally varied novel PPAP amide derivatives were designed, synthesized, and evaluated for their enzyme inhibition activity against tyrosine kinases. The structures of

Table 2. IC_{50} values for compounds 14 and 20 against Abl1, EGFR, c-Src, and Lck.

Compound	IC_{50} (μM)[a]			
	Abl1	EGFR	c-Src	Lck
14	8.39	99.70	NA[b]	14.40
20	2.67	NA	2.79	9.96
Imatinib (**1a**)	0.27	>50	35.10	0.26
Staurosporine	0.031	0.079	0.0029	0.0014

[a] All data were calculated based on triplicate assays; [b] No significant inhibitory activity was observed at tested concentration (data could not be fit to an IC_{50} curve).

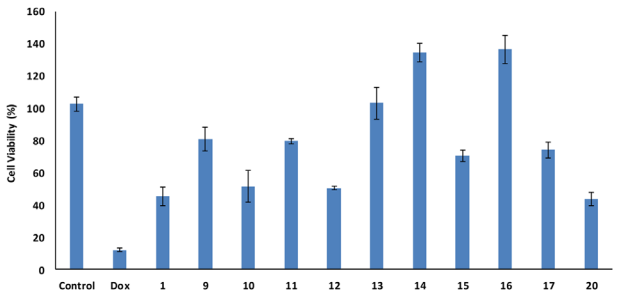

Figure 3. Normalized viability of CCRF-CEM cells determined after 72 h treatment of compounds at 50 μM concentration. The untreated DMSO control was set to 100% viability. Doxorubicin (Dox) was applied at a concentration of (10 μM) and served as a positive toxic control. All the experiments were performed in triplicate (±SD).

all the compounds were well established by FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS. In addition, the structure of a cyclic amide was established on the basis of SCXRD data. Among the 10 novel amides synthesized, compound **20**, a cyclic amide/pyridin-2(1*H*)-one derivative exhibited an IC_{50} value better than the drug imatinib against c-Src kinase, and compound **14** containing a 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)oxy)acetamide demonstrated an IC_{50} value of 8.39 μM against Abl1 kinase. To the best of our knowledge this is the first report of synthesis of cyclic amide (2-pyridone) derivative of PPAP and its screening of kinase inhibition. The kinase inhibitory study reflected the potency and selectivity of synthesized molecular hybrids, which can serve as leads for further exploration and development of kinase inhibitors.

Experimental

Chemistry

General

All commercially available compounds were used as received without further purification. All the solvents were distilled and some even dried prior to their use. Reactions were monitored by pre-coated TLC plates (Merck silica gel $60F_{254}$); the spots were visualized either by UV light, or by spraying with Ninhydrin stain solution. Silica gel (100–200 mesh) was used for column chromatography. All of the chemicals and reagents were procured from Spectrochem Pvt. Ltd., India and Sigma–Aldrich, USA. Melting points (m.p.) were measured on a Büchi M-560 equipment and are uncorrected. Infrared spectra were recorded on a PerkinElmer SPECTRUM BX FTIR and SPECTRUM XIFT IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Jeol-400 (400 MHz, 100.5 MHz) NMR spectrometer using tetramethylsilane (TMS) as an internal standard. The chemical shift values are on a δ scale and the coupling constant values (*J*) are in Hertz. The HRMS data were recorded on Agilent 6210 ESI-TOF, Agilent Technologies, Santa Clara, CA, USA.

The NMR and HRMS spectra (Figs. S1–S11) and InChI codes along with biological activity details (Table S1) of the investigated compounds are provided as Supporting Information.

*N-(2-Methyl-5-nitrophenyl)-4-(pyridin-3-yl)pyrimidin-2-amine (**7**)*

An oven dried round bottom flask was charged with compound **4** (1 mmol), CuI (0.25 mmol), KI (2.0 mmol), K_2CO_3 (2.0 mmol), and anhydrous dioxane under N_2 atmosphere. *o*-Bromo-*p*-nitrotoluene **6** (1.0 mmol) synthesized from *p*-nitrotoluene **5** following the literature report [43], and DMEDA (0.25 mmol) were added to the reaction flask, the reaction mixture was refluxed at 120°C for 15 h, then cooled to room temperature. Concentrated ammonia (4 mL) and brine (20 mL) were added to the reaction mixture and the solution was filtered over Celite, extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was purified by column chromatography on silica gel to give the desired product as a pale-yellow solid in 55% yield, m.p.: 198–201°C (lit.: 194–199°C) [29].

*6-Methyl-N-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (**8**)*

An oven dried round bottom flask was charged with *N*-(2-methyl-5-nitrophenyl)-4-(pyridin-3-yl)pyrimidin-2-amine (**7**) (3.2 mmol, 1 g) under N_2 atmosphere, anhydrous methanol (50 mL) was then added and the reaction mixture was stirred for 10 min. Ammonium formate (55.3 mmol) and Pd/C (100 mg) were added sequentially to the reaction mixture under inert atmosphere. The reaction mixture was allowed to stir for 4 h at 30°C. Completion of the reaction was confirmed by the disappearance of the starting material using TLC. The reaction mixture was filtered, the filtrate was concentrated, and the residue was taken up in chloroform and the organic layer was washed with water. The organic layer was collected and dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was then subjected to column chromatography over silica gel. The pure diamine (**8**) was eluted in 1.5–2.5% methanol/chloroform as a yellow solid in 80% isolated yield. m.p.: 142–144°C (lit.: 140–143°C) [29].

*General procedure for the synthesis of amide derivatives (**9**–**17**, **20**, **21**)*

To the solution of an acid (2.7 mmol) in 10 mL DMF, EDC.HCl (2.1 mmol) and HOBt (2.1 mmol) were added sequentially at 0°C. After 30 min, the amine **8** (1.8 mmol) was added, followed by the addition of TEA (5.4 mmol) at 25–30°C. The reaction mixture was allowed to stir for 24 h. The completion of reaction was monitored by the disappearance of the amine on the TLC. The reaction mixture was concentrated under reduced pressure to remove DMF. The residue was taken up in chloroform and washed with cold water. The organic layer was washed with saturated sodium bicarbonate solution, subsequently with 3%

HCl solution and then with brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified with column chromatography using a gradient of methanol/chloroform over silica gel.

*2-(1H-Indol-3-yl)-N-(4-methyl-3-((4-(pyridin-3-yl)-pyrimidin-2-yl)amino)phenyl)acetamide (**9**)*
Compound **9** was obtained as a brown solid in 89% yield; m.p.: 201–203°C; IR (KBr, cm^{-1}): 3404, 3105, 1637, 1596, 1581, 1521, 1480, 1437, 1401; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.90 (s, 1H), 10.04 (s, 1H), 9.25 (d, 1H, $J = 1.53$ Hz), 8.91 (s, 1H), 8.66 (d, 1H, $J = 4.58$ Hz), 8.48 (d, 1H, $J = 5.3$ Hz), 8.44–8.41 (m, 1H), 7.93 (s, 1H), 7.61 (d, 1H, $J = 7.63$ Hz), 7.42–7.38 (m, 2H), 7.35 (d, 1H, $J = 8.39$ Hz), 7.32–7.29 (m, 1H), 7.26 (s, 1H), 7.13 (d, 1H, $J = 8.39$ Hz), 7.08–7.04 (m, 1H), 6.98–6.95 (m, 1H), 3.72 (s, 2H), 2.18 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 169.5, 161.5, 161.1, 159.5, 151.3, 148.1, 137.8, 137.4, 136.1, 134.4, 132.2, 130.1, 127.2, 126.8, 123.9, 123.8, 121.0, 118.7, 118.4, 115.8, 115.4, 111.4, 108.7, 107.5, 39.0, 17.6; HRMS, *m/z*: calculated for $C_{26}H_{22}N_6O$ $[M+H]^+$ 435.1928, found 435.1933.

*2-(6-Methoxy-4-methyl-2-oxoquinolin-1(2H)-yl)-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-acetamide (**10**)*
Compound **10** was obtained as an off-white solid in 78% yield; m.p.: 209–211°C; IR (KBr, cm^{-1}): 3435, 3268, 3052, 2959, 1677, 1663, 1582, 1528, 1423; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.30 (s, 1H), 9.17 (d, 1H, $J = 1.53$ Hz), 8.86 (s, 1H), 8.61 (d, 1H, $J = 4.58$ Hz), 8.44 (d, 1H, $J = 4.88$ Hz), 8.40–8.38 (m, 1H), 7.91 (s, 1H), 7.37–7.31 (m, 3H), 7.21–7.16 (m, 3H), 7.11 (d, 1H, $J = 8.54$ Hz), 6.52 (s, 1H), 5.05 (s, 2H), 3.77 (s, 3H), 2.40 (s, 3H), 2.15 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 165.6, 161.5, 161.0, 160.5, 159.4, 154.1, 151.2, 148.0, 146.7, 137.9, 136.8, 134.5, 133.9, 132.1, 130.2, 126.8, 123.7, 121.4, 120.4, 118.5, 116.2, 115.5, 115.1, 107.9, 107.5, 55.5, 44.9, 18.6, 17.6; HRMS, *m/z*: calculated for $C_{29}H_{26}N_6O_3$ $[M+H]^+$ 507.2139, found 507.2138.

*N-(4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)-phenyl)methoxypolyethyleneglycol amide (**11**)*
Compound **11** was obtained as a colorless semi solid in 77% yield; IR (KBr, cm^{-1}): 3311, 2874, 1677, 1577, 1528, 1447; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 9.26 (s, 1H), 8.86 (s, 1H), 8.70 (d, 1H, $J = 3.81$ Hz), 8.51–8.45 (m, 3H), 7.44–7.41 (dd, 1H, $J = 7.63$ Hz and 5.34 Hz), 7.25–7.23 (m, 1H), 7.18–7.16 (m, 1H), 4.13 (s, 2H), 3,79–3.50 (m, 26H), 3.38–3.33 (m, 3H), 2.32 (s, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$, δ): 167.7, 162.2, 160.3, 158.8, 151.0, 148.2, 137.3, 135.7, 134.6, 132.3, 130.3, 124.6, 123.4, 115.2, 113.4, 107.8, 71.5, 70.8, 70.2, 70.1, 70.0, 69.8, 58.7, 17.4.

*N-(4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)-phenyl)isonicotinamide (**12**)*
Compound **12** was obtained as an off-white solid in 90% yield; m.p.: 199–201°C; IR (KBr, cm^{-1}): 3231, 3103, 3043, 2371, 2345, 1675, 1654, 1647, 1589, 1560, 1533, 1499, 1483, 1446, 1424, 1408; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.46 (s, 1H), 9.27 (d, 1H, $J = 1.53$ Hz), 8.99 (s, 1H), 8.78 (d, 2H, $J = 5.34$ Hz), 8.68 (d, 1H, $J = 4.58$ Hz), 8.51 (d, 1H, $J = 5.34$ Hz), 8.49–8.45 (m, 1H), 8.10 (s, 1H), 7.86 (d, 2H, $J = 4.58$ Hz), 7.53–7.47 (m, 2H), 7.43 (d, 1H, $J = 4.58$ Hz), 7.23 (d, 1H, $J = 8.39$ Hz), 2.23 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 163.8, 161.6, 161.1, 159.5, 151.4, 150.2, 148.2, 142.0, 137.9, 136.6, 134.4, 132.2, 130.2, 128.1, 123.8, 121.6, 117.1, 116.7, 107.6, 17.7; HRMS, *m/z*: calculated for $C_{22}H_{18}N_6O$ $[M+H]^+$ 383.1615, found 383.1619.

*(2E,4E)-5-(Benzo[d][1,3]dioxol-5-yl)-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)penta-2,4-dienamide (**13**)*
Compound **13** was obtained as an off-white solid in 82% yield; m.p.: 245–247°C; IR (KBr, cm^{-1}): 3448, 3256, 1648, 1615, 1582, 1533, 1451; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.06 (s, 1H), 9.25 (d, 1H, $J = 1.53$ Hz), 8.95 (s, 1H), 8.67 (d, 1H, $J = 4.58$ Hz), 8.49 (d, 1H, $J = 5.34$ Hz), 8.44–8.46 (m, 1H), 7.95 (s, 1H), 7.53–7.50 (dd, 1H, $J = 8.39$ Hz and 4.58 Hz), 7.42–7.37 (m, 2H), 7.32–7.25 (m, 2H), 7.15 (d, 1H, $J = 8.39$ Hz), 7.01–6.90 (m, 4H), 6.28 (d, 1H, $J = 15.26$ Hz), 6.04 (s, 2H), 2.10 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 163.6, 161.6, 161.1, 159.4, 151.4, 148.2, 147.9, 147.8, 140.7, 138.8, 137.8, 137.4, 134.4, 132.2, 130.8, 130.2, 127.1, 125.1, 124.5, 123.8, 122.9, 115.9, 115.5, 108.4, 107.5, 105.7, 101.3, 17.6; HRMS, *m/z*: calculated for $C_{28}H_{23}N_5O_3$ $[M+H]^+$ 478.1874, found 478.1883.

*2-((4-Methyl-2-oxo-2H-chromen-6-yl)oxy)-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)acetamide (**14**)*
Compound **14** was obtained as light yellow solid in 56% yield; m.p.: 237–239°C; IR (KBr, cm^{-1}): 3347, 2362, 1707, 1683, 1576, 1533, 1415, 1247, 1171, 1058, 933, 872; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.07 (s, 1H), 9.24 (d,1H, $J = 0.76$ Hz), 8.96 (s, 1H), 8.67 (d, 1H, $J = 4.58$ Hz), 8.50 (d, 1H, $J = 3.81$ Hz), 8.45–8.43 (m, 1H), 7.94 (s, 1H), 7.49–7.46 (m, 1H), 7.42 (d, 1H, $J = 5.34$ Hz), 7.39–7.31 (m, 4H), 7.18 (d, 1H, $J = 7.63$ Hz), 6.40 (s, 1H), 4.80 (s, 2H), 2.40 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 166.0, 161.5, 161.0, 159.8, 159.4, 154.1, 152.8, 151.3, 148.1, 147.5, 137.9, 136.2, 134.4, 132.1, 130.2, 127.7, 123.7, 120.1, 119.6, 117.5, 116.5, 116.1, 114.8, 109.6, 107.5, 67.7, 18.1, 17.6; HRMS, *m/z*: calculated for $C_{28}H_{23}N_5O_4$ $[M+H]^+$ 494.1823, found 494.1828.

*N-(2-((4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)-phenyl)amino)-2-oxoethyl)benzamide (**15**)*
Compound **15** was obtained as light yellow solid in 72% yield; m.p.: 150–152°C; IR (KBr, cm^{-1}): 3434, 3270, 2927, 2362, 1674, 1647, 1584, 1557, 1531, 1457; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.99 (s, 1H), 9.23 (s, 1H), 8.90 (s, 1H), 8.81 (t, 1H, $J = 6.10$ Hz), 8.64 (d, 1H, $J = 3.66$ Hz), 8.49 (d, 1H, $J = 4.88$ Hz), 8.46–8.44 (m,1H), 7.92–7.88 (m, 3H), 7.53–7.45 (m, 4H), 7.40 (d, 1H), 7.30–7.28 (dd, 1H, $J = 7.93$ Hz and 1.83 Hz), 7.15 (d, 1H, $J = 7.93$ Hz), 4.05 (d, 2H, $J = 5.49$ Hz), 2.18 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 167.5, 166.6, 161.5, 161.0, 159.4, 151.2, 148.0, 137.8, 136.9, 134.4, 133.9, 132.1, 131.3, 130.1,

128.2, 127.3, 126.8, 123.8, 115.8, 115.4, 107.5, 43.2, 17.5; HRMS, *m/z*: calculated for $C_{25}H_{22}N_6O_2$ $[M+H]^+$ 439.1877, found 439.1878.

*N-(4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)-phenyl)-2-(3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)-acetamide (**16**)*
Compound **16** was obtained as white solid in 74% yield; m.p.: 261–263°C, IR (KBr, cm^{-1}): 3449, 3274, 2360, 1683, 1670, 1552, 1532, 1402; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.26 (s, 1H), 9.22 (d, 1H, $J = 1.83$ Hz), 8.91 (s, 1H), 8.66 (d, 1H, $J = 3.05$ Hz), 8.48 (d, 1H, $J = 4.88$ Hz), 8.45–8.43 (m, 1H), 7.94 (s, 1H), 7.43–7.40 (m, 2H), 7.25–7.20 (dd, 1H, $J = 7.93$ Hz and 1.83 Hz), 7.15 (d, 1H, $J = 8.54$ Hz), 7.05–6.99 (m, 4H), 4.72 (s, 2H), 4.69 (s, 2H), 2.18 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 165.0, 164.5, 161.5, 161.0, 159.4, 151.3, 148.1, 144.5, 137.9, 136.6, 134.5, 132.2, 130.2, 129.1, 127.0, 123.7, 123.5, 122.7, 116.5, 115.6, 115.4, 115.3, 107.6, 66.9, 44.1, 17.6; HRMS, *m/z*: calculated for $C_{26}H_{22}N_6O_3$ $[M+H]^+$ 467.1826, found 467.1839.

*(E)-N-(4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)-phenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide (**17**)*
Compound **17** was obtained as off-white solid in 80% yield; m.p.: 246–248°C. IR (KBr, cm^{-1}): 3347, 3270, 2926, 1659; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.16 (s, 1H), 9.27 (d, 1H, $J = 1.53$ Hz), 9.00 (s, 1H), 8.68 (d, 1H, $J = 4.58$ Hz), 8.51 (d, 1H, $J = 5.34$ Hz), 8.46 (d, 1H, $J = 8.39$ Hz), 7.98 (s, 1H), 7.54–7.50 (m, 2H), 7.42 (d, 2H, $J = 5.34$ Hz), 7.18 (d, 1H, $J = 8.39$ Hz), 6.96 (s, 2H), 6.78 (d, 1H, $J = 15.26$ Hz), 3.83 (s, 6H), 3.69 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 163.4, 161.7, 160.9, 159.3, 153.1, 151.3, 148.1, 140.0, 138.8, 137.9, 137.3, 134.4, 132.1, 130.3, 130.2, 127.3, 123.8, 121.7, 115.9, 115.5, 107.5, 105.0, 60.1, 55.8, 17.7; HRMS; *m/z:* calculated for $C_{28}H_{27}N_5O_4$ $[M+H]^+$ 498.2136 found 498.2130.

*5-(2-Hydroxybenzoyl)-1-(4-methyl-3-((4-(pyridin-3-yl)-pyrimidin-2-yl)amino)phenyl)pyridin-2(1H)-one (**20**)*
Compound **20** was obtained as yellow solid in 60% yield; m.p.: 124–126°C; IR (KBr, cm^{-1}): 3270, 3053, 2925, 1671, 1582, 1445, 1289; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.32 (s, 1H), 9.24 (s, 1H), 9.08 (s, 1H), 8.69 (d, 1H, $J = 4.58$ Hz), 8.50 (d, 1H, $J = 5.50$ Hz), 8.47–8.43 (m, 1H), 7.92–7.88 (m, 2H), 7.78–7.77 (m, 1H), 7.52–7.47 (m, 2H), 7.37–7.33 (m, 3H), 7.12–7.10 (dd, 1H, $J = 9.16$ Hz and 1.53 Hz), 6.93–6.87 (m, 2H), 6.61 (d, 1H, $J = 9.16$ Hz), 2.31 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 191.3, 161.4, 161.1, 160.6, 159.5, 155.5, 151.4, 148.0, 145.7, 138.7, 138.4, 138.0, 134.5, 132.7, 132.2, 132.0, 130.8, 129.9, 124.8, 123.9, 122.2, 121.8, 119.7, 119.3, 116.9, 116.5, 108.0, 17.8; HRMS; *m/z*: calculated for $C_{28}H_{21}N_5O_3$ $[M+H]^+$ 476.1717, found 476.1719.

*5-(4-Fluoro-2-hydroxybenzoyl)-1-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)pyridin-2(1H)-one (**21**)*
Compound **21** was obtained as off-white solid in 76% yield; m.p.: 169–171°C; IR ($CHCl_3$ solution, cm^{-1}): 3005, 1672, 1583, 1440, 1256; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.03 (s, 1H), 9.23 (s, 1H), 8.69–8.68 (m, 1H), 8.52 (d, 1H, $J = 5.34$ Hz), 8.47–8.46 (m, 1H), 8.38–8.36 (m, 1H), 8.05–8.04 (m, 1H), 7.87–7.84 (m, 1H), 7.65–7.61 (m, 1H), 7.44–7.41 (m, 1H), 7.36 (d, 1H, $J = 8.39$ Hz), 7.23–7.22 (m, 1H), 7.14 (s, 1H), 7.03–7.01 (m, 1H), 6.75–6.70 (m, 2H), 6.63–6.58 (m, 1H), 2.42 (s, 3H); ^{13}C NMR (100.5 MHz, DMSO-d_6, δ): 193.7, 166.9 (d, $^1J_{CF} = 256.87$ Hz), 163.9 (d, $^3J_{CF} = 14.30$ Hz), 162.3, 161.9, 160.0, 159.1, 151.2, 148.2, 143.8, 138.9, 138.3 (d, $^4J_{CF} = 5.75$ Hz), 134.6, 133.6 (d, $^3J_{CF} = 11.5$ Hz), 132.3, 131.2, 128.4, 123.7, 120.8, 120.5, 118.6, 117.2, 116.7, 108.6, 107.1 (d, $^2J_{CF} = 23.00$ Hz), 105.1 (d, $^2J_{CF} = 23.96$ Hz), 17.8; HRMS, *m/z*: calculated for $C_{28}H_{20}FN_5O_3$ $[M+H]^+$ 494.1623, found 494.1614.

*N-(4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)-phenyl)-4-((4-methylpiperazin-1-yl)methyl)benzamide (**1a**)*
Compound **1a** was obtained as off-white solid in 62% yield, m.p.: 201–204°C (lit.: 206–209°C); HRMS, m/z: calculated for $C_{29}H_{31}N_7O$ $[M+H]^+$ 494.2663, found 494.2668.

Biology

Kinase inhibitory activity assay
All the compounds were first screened in duplicate at 50 μM for Abl1 and c-Src kinase. Two potent compounds that showed enzyme inhibition more than 60% were selected to test against four kinases, in duplicate. Compounds were tested in a 10-dose IC_{50} mode with threefold serial dilution starting at 50 μM. Control compound, staurosporine, was tested in a 10-dose IC_{50} with fourfold serial dilution starting at 20 μM. All kinase reactions were performed in the presence of 10 μM ATP. The compounds were pre-incubated with the enzyme and substrate mixture about 20 min, and then ^{33}P-ATP (specific activity 10 μCi/μL) was added to start the reaction. The reaction was carried out for 2 h at room temperature. Curve fits were performed when the activities at the highest concentration of compounds were less than 65%. The detailed procedure is given in Supporting Information Table S1.

Cell culture and cell viability assay
Cell culture: Human leukemia cell line CCRF-CEM (ATCC no. CCL-119) purchased from American Type Culture Collection Organization. 75 cm^2 cell culture flasks were used to grow cells using RPMI-16 medium. The medium was supplemented with growth protein (10% fetal bovine serum), and antibiotics (1% penicillin/streptomycin mixture, 10000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl). The environment was adjusted at CO_2 (5%), air (95%) at 37°C.

Cell viability assay: In order to measure the cell viability, CellTiter 96 aqueous one solution (Promega, USA) was employed. In brief, when cells reached 75–80% degree of confluency, 50000 cells/well were seeded in each well in 96-well microplate in 100 μL of RPMI-16 medium. This process was followed by adding the compounds (50 μM) into each well in triplicate in 96-well plate. As a positive control in this assay, doxorubicin (Dox, 10 μM) was employed in terms of cell-viability. After incubation period is over (72 h), a volume of

20 μL of CellTiter 96 aqueous solution was added into each well. The plates were transferred into the incubator for 1 h under humidified atmosphere at 37°C. After the incubation time was over, microplate was employed to measure the formazan absorbance at 490 nm. The wells containing cells with no treatment were used as negative control. The results of the assay were calculated as a percentage of the negative control.

Crystallography

A single crystal structure of compound **21** was collected on the Oxford Diffraction Xcalibur CCD diffractometer with the graphite monochromatic MoKα radiation (graphite crystal monochromator, $\lambda = 0.71073$ Å) at 173 K. The structure was solved by direct methods using SHELXS-97 and refined using SHELXL-97 [44]. X-Seed [45] was used as the graphical interface for the SHELX program suite. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed in calculated positions using riding models. The remaining electron density corresponding to solvent of crystallization (chloroform) within the voids was removed using the SQUEEZE routine of PLATON [46]. The solvent-accessible void volumes were also calculated using PLATON. The data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

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