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Design and synthesis of new anticancer pyrimidines with multiple-kinase inhibitory effect

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ABSTRACT

A new series of N-substituted-2-aminopyrimidines based on the '4-(pyridin-3-yl)pyrimidin-2-amine' scaffold of Imatinib has been designed and synthesized. A selected group from the target compounds was tested over a panel of 60 cancer cell lines at a single dose concentration of 10 μM, and the two most active compounds, **25b** and **30**, were further tested in a five-dose testing mode to determine their IC_{50} values over the 60 cell lines. Compound **30** has showed good potencies and high efficacies, and was accordingly tested at a single dose concentration of 10 μM over a panel of 54 kinases. At this concentration, the compound has showed multiple inhibitions over a number of oncogenic kinases, including ABL1, AKT1, LCK, C-SRC, PIM1, FLT3, FYN, and KDR. A molecular modeling study was made by docking of the most active compound **30** and its inactive analog **29** into the kinase domain of ABL1 to investigate their possible binding interactions.

1. Introduction

Cancer is thought to reflect a multi-step process, resulting from an accumulation of inherited and/or acquired defects in genes involved in the positive or negative regulation of cell proliferation and survival. For the development of a clinically recognizable human cancer, the activation or inactivation of as many as four or five different genes may be required.[1] The human genome encodes approximately 500 predicted protein kinases, many of them are participating in signal transduction pathways that regulate cell growth and survival, suggesting potential roles in cancer initiation and progression.[2] Indeed, several protein kinases have been directly implicated in human oncogenesis by virtue of being over-expressed or mutationally activated in cancer cells.[3] Hence, the protein kinases have been widely considered as drug targets for cancer therapy, and the targeting of such kinases either with small organic molecules[4–6] or with monoclonal antibodies[7,8] has been recently considered as a promising tool for selective treatment of such kinase dependent cancers.

Phenylaminopyrimidines (PAPs) represent a large chemical group of small molecular kinase inhibitors, and a representative example and a lead compound of such group is the Bcr-Abl tyrosine kinase inhibitor Imatinib (Gleevec, STI-571).[9] Imatinib, a PAP derivative, was the first kinase inhibitor to be approved for the treatment of cancer. After the successful approval of Imatinib as a selective anticancer drug for treatment of chronic myeloid leukemia (CML) with minimal side effects, relative to old anticancer drugs, numerous kinase inhibitors targeting oncogenic kinases have been developed. A large number of these kinase inhibitors belong to the same chemical group of Imatinib, PAPs. These compounds include inhibitors for receptor and non-receptor kinases such as Bcr-Abl,[10,11] CDK,[12] c-KIT,[13] EGFR,[11,14] Lyn,[11] and PLK1[12] kinases. Accordingly, we have designed and synthesized a new series of N-substituted-2-aminopyrimidines based on the '4-(pyridin-3-yl)pyrimidin-2-amine' scaffold of Imatinib (Fig. 1). The modifications at this scaffold were made basically at both of the amino group of the 2-aminopyrimidine moiety and the 6-position of the terminal pyridine ring.

A selected group (12 compounds) from the target compounds was tested over a panel of 60 cancer cell lines at a single dose concentration of 10 μM, and the two most active compounds, **25b** and

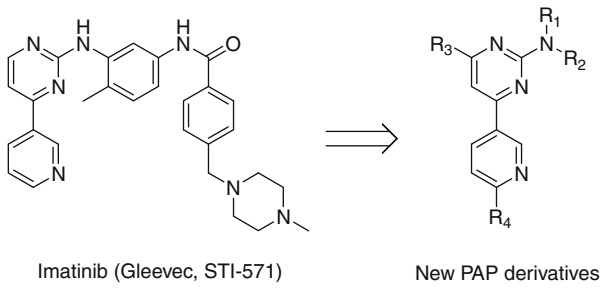

Figure 1. Structure of Imatinib and the general formula of the new PAP derivatives.

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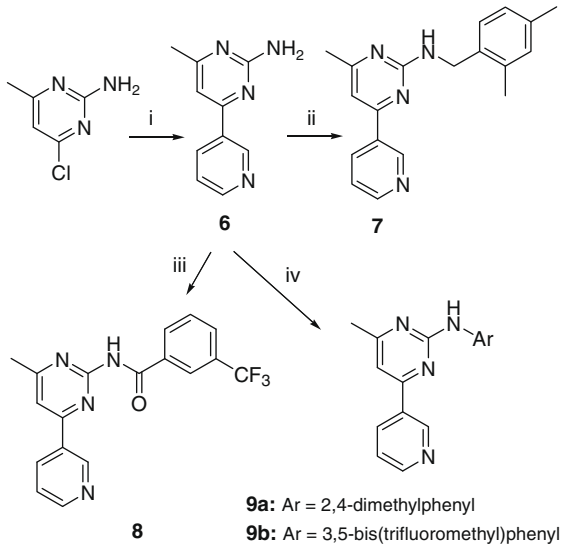
30, were further tested in a five-dose testing mode to determine their IC_{50} values over the 60 cell lines. Compound **30** has showed the highest potencies and efficacies, and was accordingly tested at a single dose concentration of 10 μM over a panel of 54 kinases, in order to determine its kinase inhibitory profile. At this concentration, the compound has showed multiple inhibitions over a number of oncogenic kinases.

2. Results and discussion

2.1. Chemistry

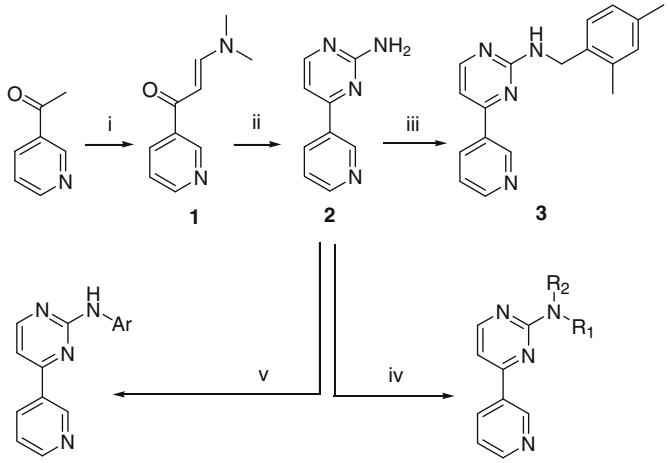
In this study, five different groups of the target compounds were prepared by following five different synthetic schemes. In Scheme 1, 3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one (**1**) was prepared in 82% yield by heating 3-acetylpyridine with excess *N,N*-dimethylformamide dimethylacetal for 4 h.[15] By refluxing compound **1** with guanidine hydrochloride in absolute ethanol and in the presence of sodium ethoxide, 4-(pyridin-3-yl)pyrimidin-2-amine (**2**) was obtained in a good yield.[16] The benzylation of the amino group of the pyrimidin-2-amine (**2**), was carried out by the dropwise addition of 2,4-dimethylbenzyl bromide to a heated solution of **2** and K_2CO_3 in DMF for the preparation of compound **3** in 39% yield.[17] A group of *N*-mono- and *N,N*-dibenzoyl derivatives (**4a–c**) was prepared by dropwise addition of the appropriate benzoyl chloride to the amine **2** in refluxing pyridine. For the preparation of the *N*-aryl derivatives (**5a–f**), a modified Buchwald–Hartwig amination protocol previously developed by our group was applied,[17] where the amine **2** was refluxed with the appropriate arylbromide in toluene and under N_2 atmosphere, in the presence of dichlorobis-(triphenylphosphine)Pd(II) as a palladium catalyst, Xantphos as a phosphine ligand, and NaO*t*-Bu as a base. By this method, the target *N*-aryl derivatives were obtained in moderate yields and in a relatively short time of only 8 h.

In Scheme 2, 4-methylpyrimidin-2-amine analogs to the benzyl, benzoyl and *N*-aryl derivatives prepared in Scheme 1 were synthesized, applying the same preparation protocols followed in Scheme 1. The only difference was the use of 4-methyl-6-(pyridin-3-yl)pyrimidin-2-amine (**6**) instead of the amine **2** used in Scheme 1. For the preparation of this amine, 2-amino-4-chloro-6-methylpyridine underwent a Suzuki coupling reaction with 3-pyridineboronic acid in a mixed solvent of acetonitrile and water in a (1:1, v/v) ratio, and in the presence of dichlorobis(triphenylphosphine)Pd(II) and Na_2CO_3.[18]



Scheme 2. Reagents and conditions: (i) 3-pyridineboronic acid, dichlorobis-(triphenylphosphine)Pd(II), Na_2CO_3, acetonitrile/water (1:1), N_2, 78 °C, 7 h, 74%; (ii) 2,4-dimethylbenzylbromidee, K_2CO_3, DMF, 130 °C, 5 h, 43%; (iii) 3-trifluoromethylbenzoylchloride, pyridine, reflux, 6 h; (iv) arylbromide, dichlorobis(triphenylphosphine) Pd(II), Xantphos, NaO*t*-Bu, toluene, N_2, reflux, 8 h, 35% (**9a**), 68% (**9b**).

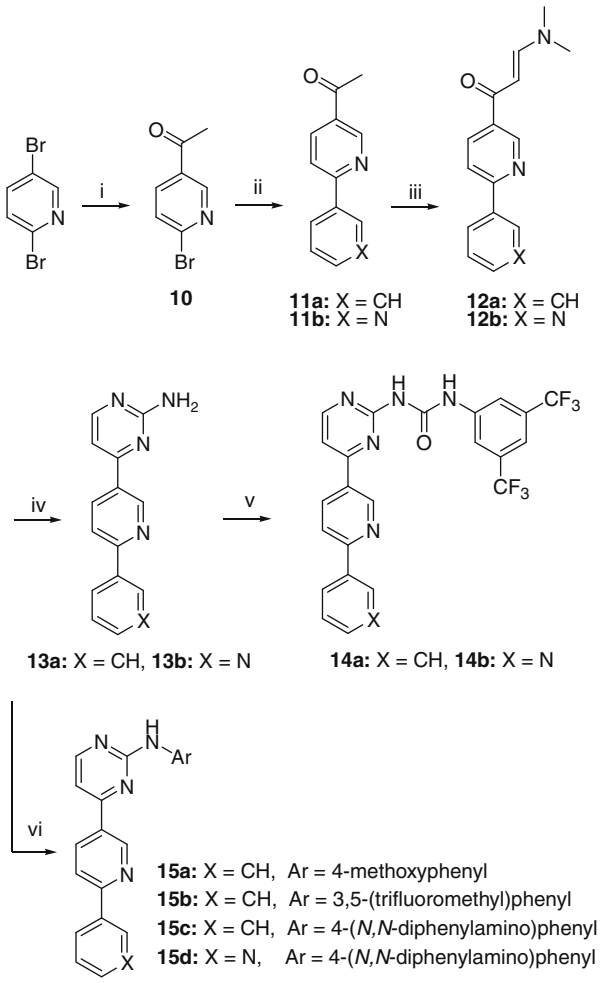
The target of Schemes 3–5 was to obtain new pyrimidine derivatives with extended structure at their pyridine tail, in order to investigate the effect of the structural modifications at that part of the compounds on activity. This structural extension was achieved by substitution with aryl or heteroaryl moieties at the 6-position of the terminal pyridine ring. In order to achieve this



5a: Ar = 5-acetamido-2-methylphenyl
5b: Ar = 3,5-bis(trifluoromethyl)phenyl
5c: Ar = 4-(*N,N*-dimethylamino)phenyl
5d: Ar = 2,4-dimethylphenyl
5e: Ar = 4-methoxyphenyl
5f: Ar = 2-naphthyl

4a: R_1 = H, R_2 = 3-trifluoromethylbenzoyl
4b: R_1 = R_2 = 3-trifluoromethylbenzoyl
4c: R_1 = R_2 = benzoyl

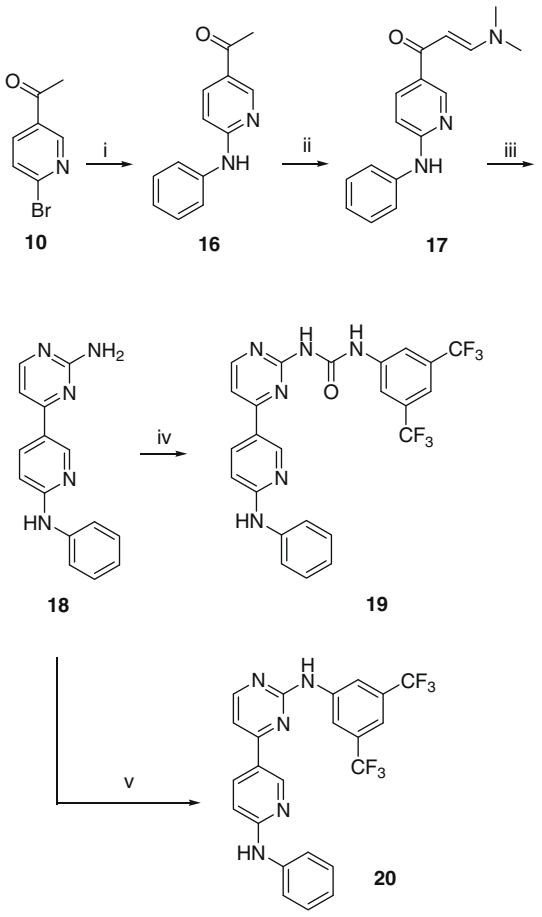
Scheme 1. Reagents and conditions: (i) *N,N*-dimethylformamide dimethylacetal, fusion, 4 h, 82%; (ii) guanidine-HCl, NaOEt, abs EtOH, reflux, 6 h, 73%; (iii) 2,4-dimethylbenzyl bromide, K_2CO_3, DMF, 130 °C, 5 h, 39%; (iv) (substituted) benzoylchloride, pyridine, reflux, 3 h, 19% (**4a**), 37% (**4b**), 52% (**4c**); (v) arylbromide, dichlorobis(triphenylphosphine)Pd(II), Xantphos, NaO*t*-Bu, toluene, N_2, reflux, 8 h, 42% (**5a**), 82% (**5b**), 39% (**5c**), 31% (**5d**), 52% (**5e**), 56% (**5f**).



Scheme 3. Reagents and conditions: (i) *n*-BuLi (1.6 M/hexanes), *N*,*N*-dimethyl acetamide, diethyl ether, N_2, −78 °C, 1 h, 51%; (ii) arylboronic acid, dichlorobis(triphenylphosphine)Pd(II), Na_2CO_3, acetonitrile/water (1:1), N_2, 78 °C, 4 h, 67% (**11a**), 74% (**11b**); (iii) *N*,*N*-dimethylformamide dimethylacetal, fusion, 3 h, 99% (**12a**), 88% (**12b**); (iv) guanidine-HCl, NaOEt, abs EtOH, reflux, 5 h, 84% (**13a**), 90% (**13b**); (v) 3,5-bis(trifluoromethyl)phenyl isocyanate, fusion, 2 h, 68% (**14a**), 75% (**14b**); (vi) arylbromide, dichlorobis(triphenylphosphine)Pd(II), Xantphos, NaO*t*-Bu, toluene, N_2, reflux, 8 h, 82% (**15a**), 79% (**15b**), 31% (**15c**), 27% (**15d**).

target, 2,5-dibromopyridine was converted into 5-acetyl-2-bromopyridine (**10**) in 51% yield, as showed in Scheme 1, according to literature procedure,[19] by lithiation of 2,5-dibromopyridine in diethyl ether at −78 °C under nitrogen atmosphere, followed by acetylation at position 5 by *N*,*N*-dimethylacetamide. The resulted 5-acetyl-2-bromopyridine was then converted to the amines **13a** and **13b** following the procedures previously reported by our group.[17] The new pyrimidin-2-amines **13a** and **13b** were then used for the preparation of the corresponding 3,5-bis(triflorometh-yl)phenyl urea derivatives **14a** and **14b** in good yields of 68% and 75%, respectively, by fusion with 3,5-bis(trifloromethyl)phenyl isocyanate for 2 h. The synthesized amines **14a** and **14b** were also used for the preparation of a number of *N*-aryl derivatives **15a–d** using the same Buchwald–Hartwig amination protocol previously employed for the synthesis of compounds **5a–f**.[17]

In Scheme 4, the extension of structure at the pyridyl tail was carried out through a nitrogen bridge, by reacting the 5-acetyl-2-bromo-pyridine scaffold (**10**) with excess aniline under reflux for 12 h, in the presence of dichlorobis(triphenylphosphine)Pd(II) as a palladium catalyst, 1,3-bis(diphenylphosphino)propane as a phosphine ligand, and sodium *tert*-butoxide as a base. The resulted acetyl intermediate **16** was then converted to the corresponding

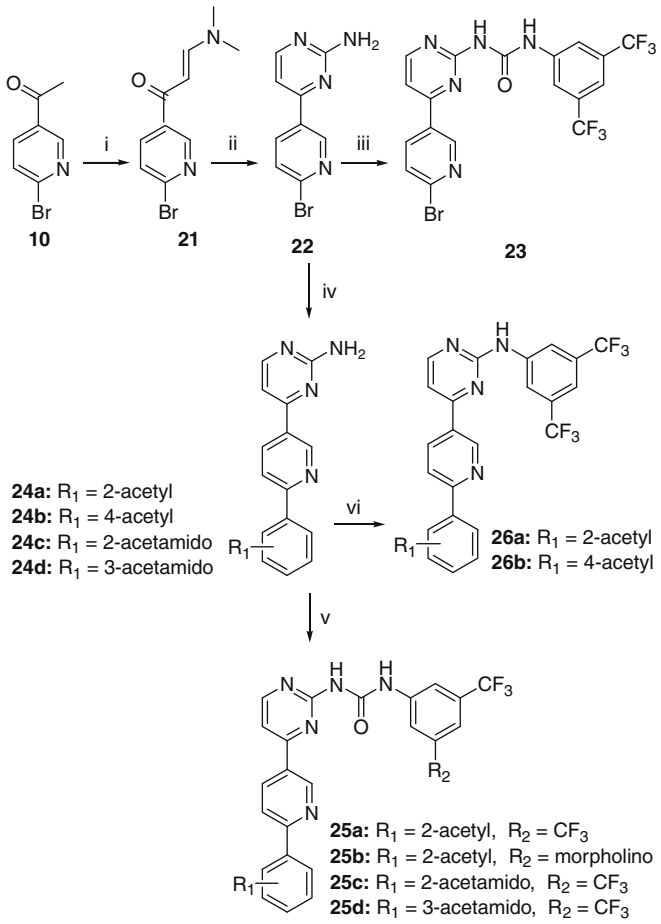


Scheme 4. Reagents and conditions: (i) aniline. dichlorobis(triphenylphosphine)Pd(II), dppp, aniline, N_2, reflux, 12 h, 38%; (ii) *N*,*N*-dimethylformamide dimethylacetal, fusion, 12 h, 94%; (iii) guanidine-HCl, NaOEt, abs EtOH, reflux, 8 h, 46%; (iv) 3,5-bis(trifluoromethyl)phenyl isocyanate, fusion, 2 h, 74%; (v) 3,5-bis(trifluoromethyl)phenyl bromide, dichlorobis(triphenylphosphine)Pd(II), Xantphos, NaO*t*-Bu, toluene, N_2, reflux, 12 h, 62%.

amine **18** in an analogous method to that used for the preparation of the amines **2**, **13a** and **13b**. Similarly to what was made in Scheme 3, the urea derivative **19** and the *N*-aryl derivative **20** were prepared following the same procedures used for the preparation of the urea and aryl derivatives **14a–b** and **15a–d**, respectively.

In Scheme 5, a number of acetyl- and acetamidophenyl analogs to the compounds prepared in Scheme 3 were synthesized. The reason for following a modified pathway here is the presence of an acetyl moiety at the newly attached phenyl ring which would interfere with the reaction of the acetyl group of 5-acetyl-2-bromopyridine (**10**) with *N*,*N*-dimethylformamide dimethylacetal if attached to the compound from the beginning. Therefore, 5-acetyl-2-bromopyridine (**10**) was first reacted with *N*,*N*-dimethylformamide dimethylacetal, followed by reaction with guanidine hydrochloride in ethanol to yield the intermediate amine **22** in a 58% yield. This amine was directly converted to the urea derivative **23** similarly to the urea derivatives **14a** and **14b**. It was also used for the preparation of the new amines **24a–d**, by Suzuki coupling with the appropriate arylboronic acids in a mixed solvent of acetonitrile and water in a (1:1, v/v) ratio, and in the presence of dichlorobis(triphenylphosphine)Pd(II) and Na_2CO_3. By following similar procedures to those used for the preparation of the urea derivatives **14a–b** and the *N*-aryl derivatives **15a–d**, compounds **25a–d** and **26a–b** were prepared, respectively.

In order to improve the solubility of compounds **15b**, **19**, **25c**, and **25d** in DMSO for the purpose of anticancer screening, the com-



Scheme 5. Reagents and conditions: (i) *N*,*N*-dimethylformamide dimethylacetal, fusion, 20 h, 88%; (ii) guanidine-HCl, NaOEt, abs EtOH, reflux, 12 h, 58%; (iii) 3,5-bis(trifluoromethyl)phenyl isocyanate, fusion, 2 h, 79%; (iv) arylboronic acid, dichlorobis(triphenylphosphine)Pd(II), Na_2CO_3, acetonitrile/water (1:1), N_2, 78 °C, 12 h, 64% (**24a**), 61% (**24b**), 46% (**24c**), 59% (**24d**); (v) 3,5-bis(trifluoromethyl)phenyl isocyanate, fusion, 2 h, 65% (**25a**), 66% (**25b**), 42% (**25c**), 45% (**25d**); (vi) 3,5-bis(trifluoromethyl)phenyl bromide, dichlorobis(triphenylphosphine)Pd(II), Xantphos, NaO*t*-Bu, toluene, N_2, reflux, 12 h, 79% (**26a**), 69% (**26b**).

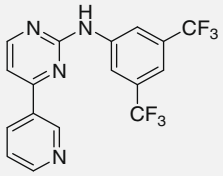
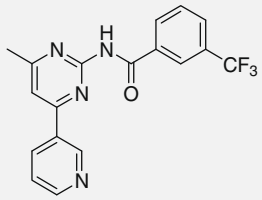
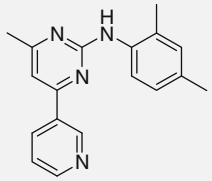
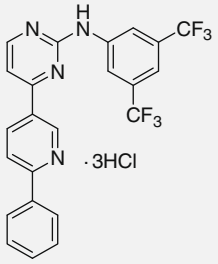
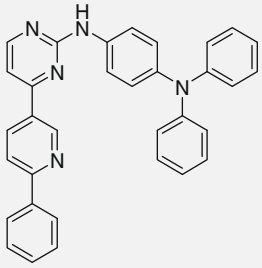
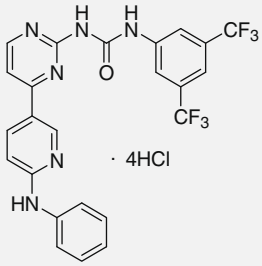
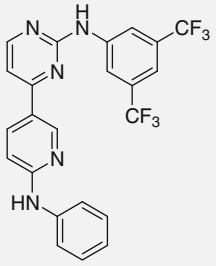
pounds were converted into their corresponding HCl-salts **27**, **28**, **29**, and **30**, respectively, by passing HCl gas into free base solutions of these compounds in toluene.

2.2. Biological screening

2.2.1. In vitro anticancer screening

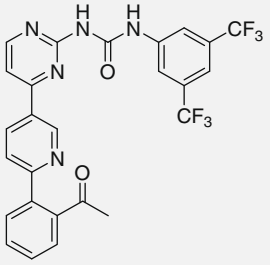
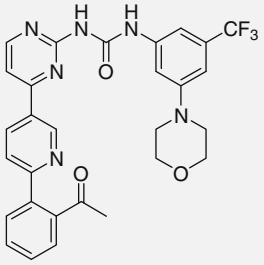
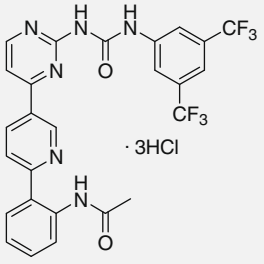
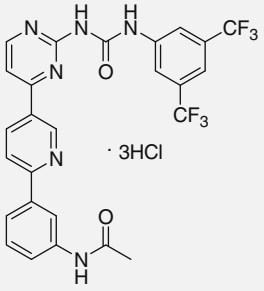
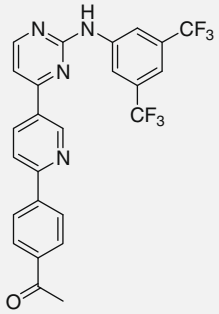
The structures of the final products were submitted to National Cancer Institute (NCI),[20] Bethesda, Maryland, USA, and the 12 compounds showed in Table 1 were selected on the basis of degree of structural variation and computer modeling techniques for evaluation of their antineoplastic activity. The selected compounds were subjected to in vitro anticancer assay against tumor cells in a full panel of 60 cell lines taken from nine different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate, and breast). The compounds were tested at a single dose concentration of 10 μM, and the percentages of growth inhibition over the sixty tested cell lines were determined. The mean inhibition percentages of all of the tested compounds over the full panel of cell lines are illustrated in Table 1 and the figures showing the inhibitions exerted by each compound over the full cell lines panel are showed in the Supplementary data. As showed in Table 1, a remarkable mean inhibition was observed with compounds **25b** (38.41%) and **30** (50.92%), while the mean inhibition was weak in the rest of the compounds.

Table 1
Compounds selected for single dose cancer cell line screening and their mean inhibitory percentages

Compd	Structure	Inhibition %[a]
5b		−0.32
8		0.62
9a		3.91
27		0.49
15c		0.97
28		−6.98
20		0.20

(*continued on next page*)

Table 1 (*continued*)

Compd	Structure	Inhibition %[a]
25a		11.31
25b		38.41
29		9.74
30		50.92
26b		−2.21

[a] Inhibition % represents the mean inhibition percentages over the 60 cell lines. The inhibition percentages are calculated by subtracting the growth percentages from 100.

As observed from the data, a great difference in activity was observed between the tested compounds in spite of close structural similarity. For example, a big difference in the mean inhibitory percentages of compounds **25a** (11.31%) and **25b** (38.41%) was observed although the only difference in between them lies in the replacement of one of the two trifluoromethyl groups of compound **25a** with a morpholino group in compound **25b**. This, however, emphasizes the positive effect of substitution with the morpholino moiety at the phenylurea part on activity. Similarly, a large difference in activity was observed between compounds **29** (9.74%) and **30** (50.92%) which are highly similar in structure. The only difference between these two compounds lies in the position of the acetamido-substituent at the distal phenyl ring, which is *ortho* in compound **29** and *meta* in compound **30**. This slight change in the relative position of this group was associated however with a very big difference in activity, which suggests a possible critical binding role for this group in compound **30** in the binding site of the molecular target of the compound. The little shift in the position of this group is thought however to distort this critical and important binding, which is reflected by this great drop in activity from compound **30** to compound **29**. From these observations and by referring again to the structures of the tested compounds we can conclude that the phenyl ring introduced to the terminal pyridyl ring has a critical effect on the anticancer activity of the compound, and that this effect is highly dependent on the type and the position of the substituent attached. Accordingly, by comparing the activities of compounds **19**, **25a**, **29**, and **30**, which all bear the same 3,5-bis(trifluoromethyl)phenylurea moiety attached to the amino group, we can conclude that the presence of a 3-acetamidophenyl moiety attached to the terminal pyridine ring in compound **30** is the best for activity. The substitution of one of the trifluoromethyl groups with a morpholine moiety was found also to have a positive impact on activity as observed in compound **25b**.

The inhibitions of the two most active compounds (**25b** and **30**) at 10 μM concentration over the full cell lines panel are presented together in Figure 2. As showed in Figure 2, compounds **25b** and **30** have showed moderate to strong inhibitions over almost all of the tested cell lines. The inhibitions approached 100% at many cell lines, and exceeded the 100% inhibition limit to exert a lethal, rather than inhibitory, effect at a number of these cell lines. The overall inhibition of compound **30** was stronger. However, compound **25b** has showed stronger mean inhibitions at melanoma and renal cancers. After the initial single dose screening of the 12 selected compounds, the compounds showing the highest activity at the single dose (compounds **25b** and **30**) were further tested in a five-dose testing mode, in order to determine their IC_{50} values over the 60 tumor cell lines. For each of these two compounds, the IC_{50} (the concentration producing 50% inhibition), TGI (the concentration producing 100% inhibition) and LC_{50} (the concentration causing 50% lethality or 50% tumor regression) were recorded. The five-dose testing results of these 2 compounds are showed in Table 2. Compound **30** has showed a great superiority over compound **25b** in the five-dose testing mode. The potency of compound **30** was much higher, with IC_{50} values in the range of 1–2 μM at the majority of cell lines, and below 1 μM in the breast cancer cells T-47D and MDA-MB-468. The compound has showed also high efficacies, being able to induce total growth inhibition (TGI) at almost all of the tested cell lines, and 50% lethality (LC_{50}) in 65% of the tested cell lines at concentrations below 100 μM.

2.2.2. In vitro kinase screening

In order to investigate the mechanism of action and the kinase inhibitory profile of this new class of compounds, compound **30** with the highest potencies at the cancer cell lines was tested at a single dose concentration of 10 μM over a panel of 54 kinases at Reaction Biology Corporation using hot spot technique.[21] As showed in Figure 3, compound **30** has exerted multiple inhibitions over a number of oncogenic serine/threonine and tyrosine kinases at the test concentration. The inhibition was above 70% in ABL1 kinase, a fusion tyrosine kinase protein responsible for 90% of chronic myeloid leukemia (CML) cases,[22] AKT1 serine/threonine kinase, which is over-expressed in a number of cancers including breast, prostate, lung, pancreatic, liver, ovarian and colorectal can-

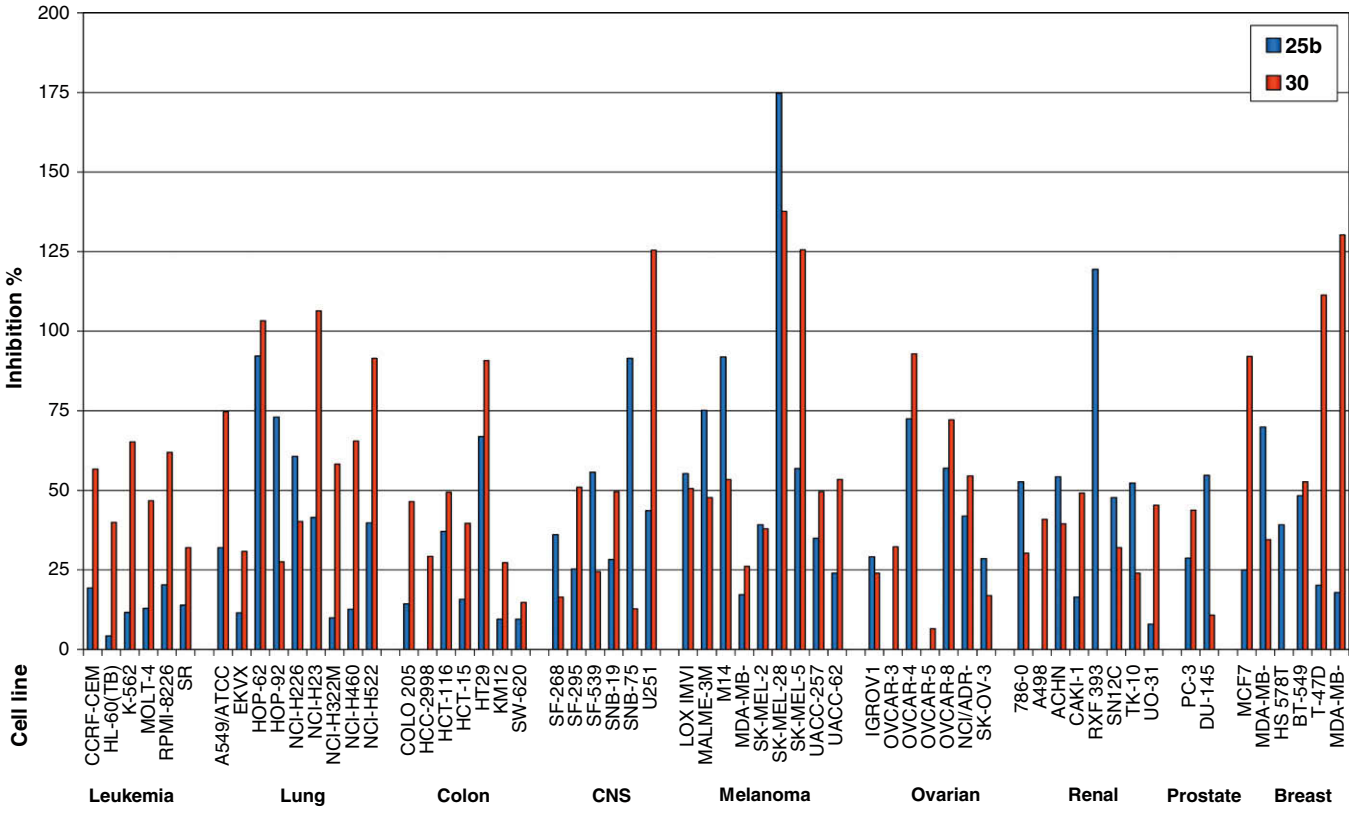


Figure 2. Multiple inhibitions of compounds **25b** and **30** at 10 μM concentration over the 60 cell lines panel.

cer,[23] and LCK kinase, which is aberrantly expressed in leukemia and colorectal cancer.[24,25] In a number of other kinases, the inhibition percentages were between 60% and 70%. These kinases include Aurora-A kinase, a mitotic serine/threonine kinase whose dysregulation results in aneuploidy and is associated with high incidence of cancers such as breast and ovarian cancers,[26] C-src (CSK) tyrosine kinase, which is over-expressed in a number of cancers, such as breast, colon, pancreas, lung, ovary and CNS cancers,[27] and PIM1 serine/threonine kinase, whose over-expression is reported in prostate cancer and hematopoietic malignancies.[8] In addition to these inhibitions, compound **30** has also inhibited three other kinases with a milder degree (with inhibition percentages ranging between 50% and 60%). These kinases are FLT3 kinase, a type III receptor tyrosine kinase whose mutations were reported in about 30% of cases of acute myeloid leukemia (AML),[28] FYN tyrosine kinase, whose over-expression aids in cellular transformation and xenograft metastasis[29] and is linked also to Alzheimer's disease pathology,[30] and KDR (VEGFR2) kinase, which plays a critical role in tumor angiogenesis in solid cancers.[31] The compound was able also to inhibit a number of other oncogenic kinases, as showed in Figure 3, but the inhibitions at these kinases were below 50%. The multiple inhibitions of compound **30** over this group of oncogenic kinases have compromised together to yield finally the strong and broad spectrum anticancer effect of the compound.

In order to get a better insight at the critical effect of the position of the acetamido-substituent in compound **30** on activity and justify for the big difference in activity between compound **30** and compound **29**, bearing the same group in the *ortho*-position, a molecular modeling study was applied using MOE.2008.10 software. In this study, both of the two compounds were docked into the binding pocket of ABL1 kinase using the crystal structure of the enzyme co-crystallized with Imatininb (PDB code 3K5V). The two compounds (**29** and **30**) were docked over the ligand atoms (Imatinib) in the binding site. The most stable docking poses of the two compounds aligned with Imatinib in the binding pocket are showed in Figure 4. Both of the two compounds occupy nearly the same space in the binding pocket, and are overlaid well over the skeleton of imatininb, with the '4-(pyridin-3-yl)pyrimidin-2-amine' scaffold in the same direction of that of Imatininb. It was found that compound **30** was fit into the kinase domain through two hydrogen bonding (HB) interactions, one of them was formed by the HB-acceptor acetamido oxygen with Met-337 in the hinge region, while the other was formed by the HB-donor distal urea NH to Glu-305 inside the binding pocket. The capability of compound **30** to occupy a similar pose to that of Imatininb suggests the possibility of compound binding to the enzyme in the inactive conformation (DGF-out conformation) similarly to Imatininb.[32]

Although compound **29** has showed almost a perfect overlay over compound **30**, it failed to establish the highly important hydrogen bonding with Met-337 in the hinge region as a result of the far positioning of its acetamido group as showed in Figures 4 and 5. This failure in initial fitting to the hinge region was associated also with a failure in hydrogen bonding to Glu-305 inside the binding pocket, which makes the overall binding of the compound in the binding site to be so weak, and consequently diminish the ability of the compound to competitively inhibit the enzyme. These results emphasize the importance of the right positioning of the acetamido group in compound **30**, and give an explanation for the lack of activity in compound **29**, since similar binding modes would be anticipated for both of the two compounds in the other kinases inhibited by compound **30**.

3. Conclusions

A new pyrimidine anticancer lead compound (compound **30**) has been developed in this study. The compound has showed good

Table 2
IC_{50}, TGI and LC_{50} values in μM of compounds **25b** and **30** over 60 cancer cell lines

	Cell line	25b			30		
		IC_{50}[a]	TGI[b]	LC_{50}[c]	IC_{50}[a]	TGI[b]	LC_{50}[c]
Leuk	HL-60(TB)	>100	>100	>100	3.78	96.1	>100
	K-562	>100	>100	>100	1.87	NA	>100
	MOLT-4	>100	>100	>100	1.98	>100	>100
Non-small cell Lung cancer	A549/ATCC	>100	>100	>100	1.48	12.6	41.9
	EKVX	>100	>100	>100	1.44	5.71	>100
	HOP-62	4.29	>100	>100	4.88	26.8	>100
	HOP-92	2.95	NA	>100	NA	NA	NA
	NCI-H23	>100	>100	>100	2.24	6.94	43.8
	NCI-H322M	>100	>100	>100	2.17	14.6	68.8
	NCI-H460	>100	>100	>100	2.06	7.07	59.7
	NCI-H522	63.2	>100	>100	1.71	5.85	35.3
Colon Cancer	COLO 205	>100	>100	>100	11.0	>100	>100
	HCT-116	>100	>100	>100	1.60	4.71	17.1
	HCT-15	>100	>100	>100	3.62	15.0	45.1
	HT29	>100	>100	>100	3.00	8.74	57.6
	KM12	>100	>100	>100	2.59	8.01	27.9
	SW-620	>100	>100	>100	2.30	5.59	>100
CNS cancer	SF-268	40.2	>100	>100	4.92	23.9	98.7
	SF-295	9.04	>100	>100	1.27	6.19	>100
	SF-539	NA	>100	>100	5.48	18.9	44.3
	SNB-19	38.7	>100	>100	4.57	17.8	47.4
	SNB-75	5.35	27.5	>100	10.9	30.5	85.8
	U251	34.9	>100	>100	2.00	5.32	18.9
Melanoma	LOX IMVI	>100	>100	>100	1.79	NA	>100
	MALME-3M	NA	>100	>100	2.01	4.48	NA
	M14	NA	>100	>100	1.83	3.76	7.74
	MDA-MB-435	>100	>100	>100	2.31	6.80	>100
	SK-MEL-2	15.9	75.5	>100	5.65	19.8	52.4
	SK-MEL-5	NA	>100	>100	1.35	2.64	5.13
	UACC-257	>100	>100	>100	1.69	4.32	13.2
	UACC-62	>100	>100	>100	2.86	12.7	35.7
Ovarian cancer	IGROV1	6.18	>100	>100	2.68	9.46	>100
	OVCAR-3	>100	>100	>100	1.85	4.23	9.69
	OVCAR-4	NA	>100	>100	1.57	11.1	39.0
	OVCAR-5	>100	>100	>100	11.8	24.5	50.8
	OVCAR-8	NA	>100	>100	2.07	5.93	26.4
	NCI/ADR-RES	NA	>100	>100	3.66	47.4	>100
	SK-OV-3	9.42	>100	>100	13.1	32.2	78.8
Renal cancer	786-0	13.0	>100	>100	3.66	15.9	39.9
	A498	>100	>100	>100	6.13	22.2	59.2
	ACHN	6.89	>100	>100	3.83	15.5	47.1
	CAKI-1	>100	>100	>100	2.37	8.34	>100
	RXF 393	1.27	3.50	NA	NA	NA	NA
	SN12C	>100	>100	>100	9.29	23.8	57.7
	TK-10	5.46	>100	>100	16.8	39.8	94.1
	UO-31	>100	>100	>100	5.51	23.2	61.7
Prost.	DU-145	NA	>100	>100	6.57	19.6	47.0
Breast cancer	MDA-MB-231	NA	>100	>100	3.72	19.7	62.0
	HS 578T	3.10	>100	>100	7.52	>100	>100
	BT-549	2.87	>100	>100	7.07	20.4	46.3
	T-47D	>100	>100	>100	0.619	8.43	>100
	MDA-MB-468	>100	>100	>100	0.837	2.35	5.85

[a] IC_{50} is the concentration producing 50% inhibition.
[b] TGI is the concentration producing 100% inhibition.
[c] LC_{50} is the concentration causing 50% lethality (50% tumor regression), NA means that the data is not available.

anticancer potencies and efficacies over a wide range of cancer cell lines. The compound was found also to exert multiple inhibitions over a number of oncogenic kinases, and it is anticipated that these contributing inhibitions are responsible for the anticancer activity of this compound. A structure–activity relationship study was also concluded from the available anticancer screening data; where it has showed the importance of the type and the position of the substituent inserted at the distal phenyl ring in determining the activity of these compounds. The good and unique activity of compound **30** within the whole series has proved that the 3-acetamido group is the best for activity. Currently, further structural modifications are subjected to the urea part of compound **30** (keeping the distal 3-acetamidophenyl ring unchanged) in order to study the effect of structural variations at that part of the compound on activity.

4. Experimental

4.1. General

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker Avance 300 spectrometer with TMS as an internal standard. The IR spectra were recorded on Perkin Elmer Spectrum GX spectrometer. Melting points were taken on a Thomas-Hoover capillary melting apparatus and are uncorrected. Chemical analyses were carried out by EA 1108 CHNS-O of Fisons Instruments. Column chromatography was performed on Merck Silica Gel 60 (230–400 mesh). TLC was carried out using glass sheets precoated with silica gel 60 F254 prepared by E. Merck. All the commercially available reagents were obtained from Aldrich and Tokyo Kasei Chemicals and generally used without further purification.

4.2. Synthesis

4.2.1. 3-(Dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one (1)[15]

A mixture of 3-acetylpyridine (10 g, 0.083 mol) and *N,N*-dimethylformamide dimethylacetal (13.8 g, 0.116 mol) was heated under reflux in an oil bath for 4 h. The excess unreacted *N,N*-dimethylformamide dimethylacetal was removed by distillation, followed by removal of the residual part under vacuum. The solid residue was triturated with water (100 mL) and then extracted with methylene chloride (200 mL × 3). The organic layer was separated, dried over anhydrous $MgSO_4$, and then evaporated under vacuum to yield the crude product. Crystallization from the mixed solvent ethanol/chloroform (1:1, v/v) yielded pure **1** as reddish orange crystals. Yield (12.0 g, 82%); mp 70–71 °C (62–70 °C);[15] ^{1}H NMR ($CDCl_3$): δ 2.90 (s, 3H), 3.12 (s, 3H), 5.62 (d, J = 12.2 Hz, 1H), 7.30 (dd, J = 3.6, 5.4 Hz, 1H), 7.79 (d, J = 12.2 Hz, 1H), 8.14 (d, J = 7.8 Hz, 1H), 8.60 (d, J = 4.8 Hz, 1H), 9.03 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 37.35, 45.18, 91.79, 123.25, 135.03, 135.62, 148.85, 151.38, 154.68, 186.30.

4.2.2. 4-(Pyridin-3-yl)pyrimidin-2-amine (2)[16]

To a solution of NaOEt in absolute ethanol, made by dissolving sodium metal (0.69 g, 30.0 mmol) in absolute ethanol (100 mL), guanidine hydrochloride (2.86 g, 30.0 mmol) was added in one portion and stirred at room temperature for 1 h. 3-(Dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one (**1**) (5.28 g, 30 mmol) was dissolved in absolute ethanol (20 mL) and added to the reaction mixture. The mixture was heated under reflux for 6 h, and was then left to cool at room temperature, followed by cooling in an ice bath. The formed product was separated by filtration, washed with cold ethanol (20 mL), then with water (50 mL), and left to dry. Crystallization from ethanol yielded pure **2** as pale yellow needle crystals. Yield (3.8 g, 73.3%); mp 192 °C (186–188 °C);[16] ^{1}H NMR (DMSO-d_6): δ 6.78 (s, 2H), 7.20 (d, J = 6.0 Hz, 1H), 7.52 (dd, J = 3.0, 4.8 Hz, 1H), 8.34–8.40 (m, 2H), 8.67 (d, J = 3.6 Hz, 1H), 9.23 (s, 1H).

4.2.3. *N*-(2,4-Dimethylbenzyl)-4-(pyridin-3-yl)pyrimidin-2-amine (3)[17]

A solution of 2,4-dimethylbenzylbromide (0.17 g, 0.87 mmol) in DMF (5 mL) was added dropwise over a period of 4 h to a mixture of 4-(pyridin-3-yl)pyrimidin-2-amine (**2**) (0.15 g, 0.87 mmol) and K_2CO_3 (0.12 g, 0.87 mmol) in DMF (5 mL) while heating (130 °C) and stirring. After complete addition of 2,4-dimethylbenzylbromide, heating and stirring were maintained for 1 h. The reaction

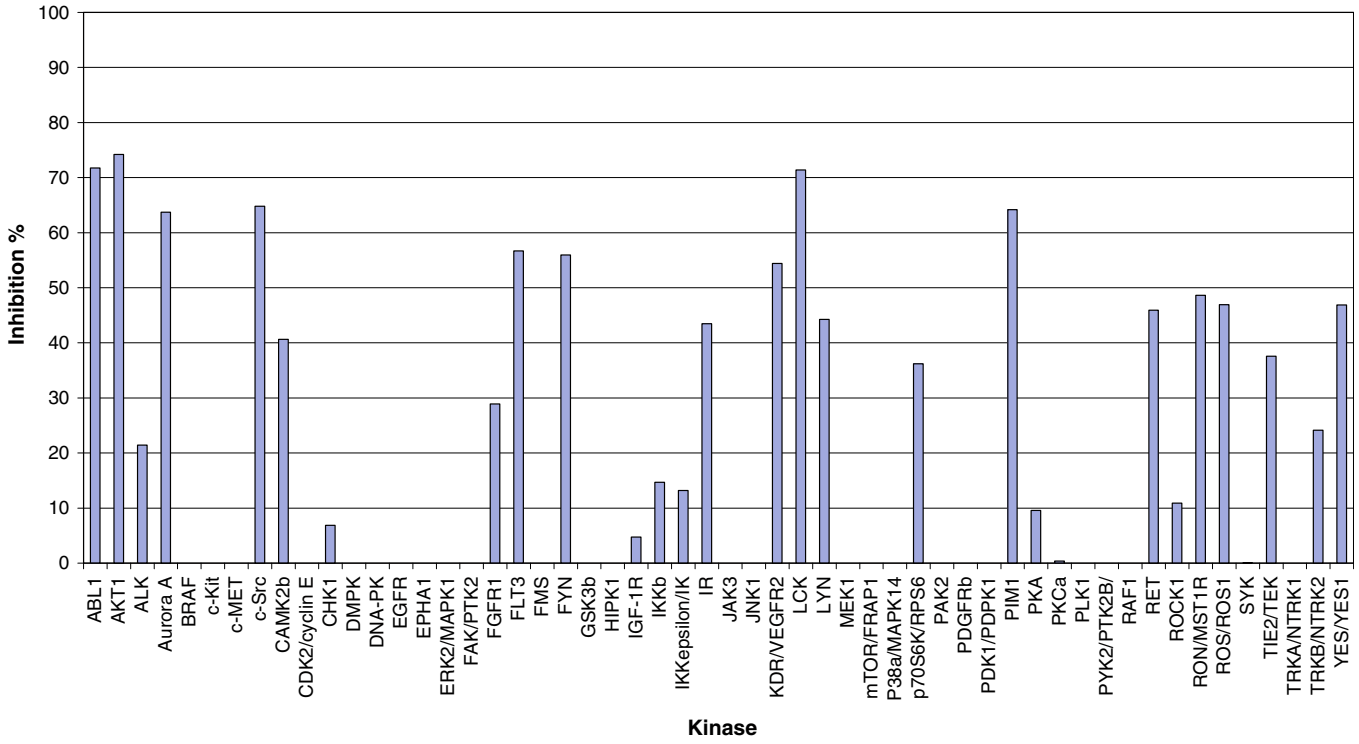


Figure 3. Inhibition percentages of compound **30** at a single dose concentration of 10 μM over 54 kinases.

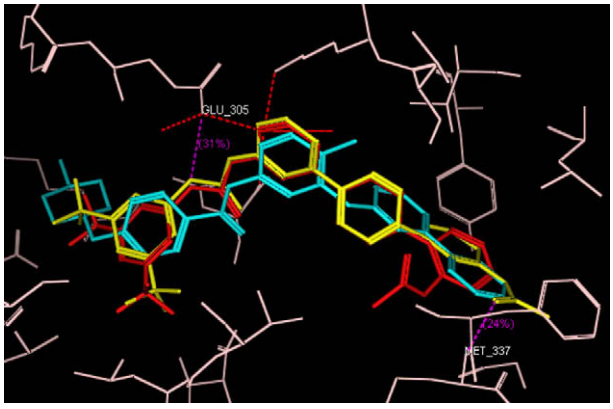


Figure 4. Docking poses for compounds **29** (red) and **30** (yellow) overlaid to Imatinib (cyan) in the kinase domain of ABL1 kinase. The docking structures show the acetamido group of **29** shifted away from Met-337 and incapable of HB formation.

mixture was left to cool to room temperature, and then poured over ice water (50 mL). The resulted precipitate was filtered, dried, and then purified by column chromatography (silica gel, ethyl acetate/hexane, 1:1, v/v) to give **3** as brownish needles. Yield (0.10 g, 39%); mp 82.5–83.5 °C; IR ν/cm^{-1}: 3231, 1597, 1566, 1345, 794; ^{1}H NMR ($CDCl_3$): δ 2.15 (s, 3H), 2.32 (s, 3H), 4.66 (d, J = 4.4 Hz, 2H), 5.86 (s, 1H), 6.98–7.11 (m, 3H), 7.26 (d, J = 7.2 Hz, 1H), 7.40 (dd, J = 2.7, 5.1 Hz, 1H), 8.30–8.33 (m, 2H), 8.69 (d, J = 3.3 Hz, 1H), 9.23 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 19.06, 21.03, 43.53, 106.42, 123.53, 126.70, 128.46, 131.30, 133.05, 133.67, 134.42, 136.31, 137.14, 148.55, 151.24, 158.99, 162.48; Anal. Calcd for $C_{18}H_{18}N_4$: C, 74.46; H, 6.25; N, 19.30. Found: C, 74.06; H, 6.25; N, 19.20.

4.2.4. General procedure for the synthesis of compounds 4a–c

To a solution of the amine **2** (100 mg, 0.58 mmol) in pyridine (5 mL) under reflux was added the appropriate benzoyl chloride (1.31 mmol) in one portion. Reflux was maintained for 2 h, and then the reaction mixture was cooled and poured over crushed ice to induce precipitation of the crude product. The resulted precipitate was then filtered, dried, and purified by the suitable method.

4.2.4.1. 3,5-Bis(trifluoromethyl)-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzamide (4a). It was separated by column chromatography (silica gel, ethyl acetate). Yield (38 mg, 19%); mp 238–239 °C; IR ν/cm^{-1}: 3436, 2920, 1661, 1591, 1410, 1357, 1318, 1166, 1114; ^{1}H NMR ($CDCl_3$): δ 7.36 (dd, J = 2.1, 4.9 Hz, 1H), 7.45 (d, J = 5.1 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.8 (d, J = 7.5 Hz, 1H), 8.16 (d, J = 7.5 Hz, 1H), 8.23 (s, 1H), 8.29 (d, J = 7.5 Hz, 1H), 8.59 (d, J = 3.9 Hz, 1H), 8.71 (d, J = 5.1 Hz, 1H), 9.23 (s, 1H), 9.96 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 112.54, 121.79, 123.80, 124.85, 125.40, 128.81, 129.39, 130.49, 131.78, 134.82, 135.45, 148.40, 151.82, 158.17, 159.40, 163.10, 164.74.

4.2.4.2. *N,N*-Bis(3,5-bis(trifluoromethyl)benzoyl))-4-(pyridin-3-yl)pyrimidin-2-amine (4b). It was separated by column chromatography (silica gel, ethyl acetate). Yield (111 mg, 37%); mp 134–135 °C; IR ν/cm^{-1}: 3436, 1717, 1691, 1616, 1579, 1416, 1380, 1335, 1278, 1234, 1168, 1130, 1097, 1072, 818, 697; ^{1}H NMR ($CDCl_3$): δ 7.36 (dd, J = 3.0, 4.8 Hz, 1H), 7.53–7.60 (m, 3H), 7.77 (d, J = 7.5 Hz, 2H), 7.99–8.05 (m, 3H), 8.12 (s, 2H), 8.70–8.74 (m, 2H), 9.01 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 114.42, 121.53, 123.80, 125.14, 126.10, 126.15, 129.41, 129.50, 130.82, 131.31, 131.75, 131.98, 132.20, 134.57, 135.01, 148.34, 152.46, 159.86, 160.30, 163.77, 171.05.

4.2.4.3. *N,N*-Bis(benzoyl))-4-(pyridin-3-yl)pyrimidin-2-amine (4c). The product was purified by crystallization from a mixed solvent of ethanol and water (1:2, v/v). Yield (115 mg, 52%); mp 199–200 °C; IR ν/cm^{-1}: 3436, 1710, 1687, 1577, 1373, 1287, 1255, 704; ^{1}H NMR ($CDCl_3$): δ 7.42 (d, J = 7.8 Hz, 4H), 7.47–7.60

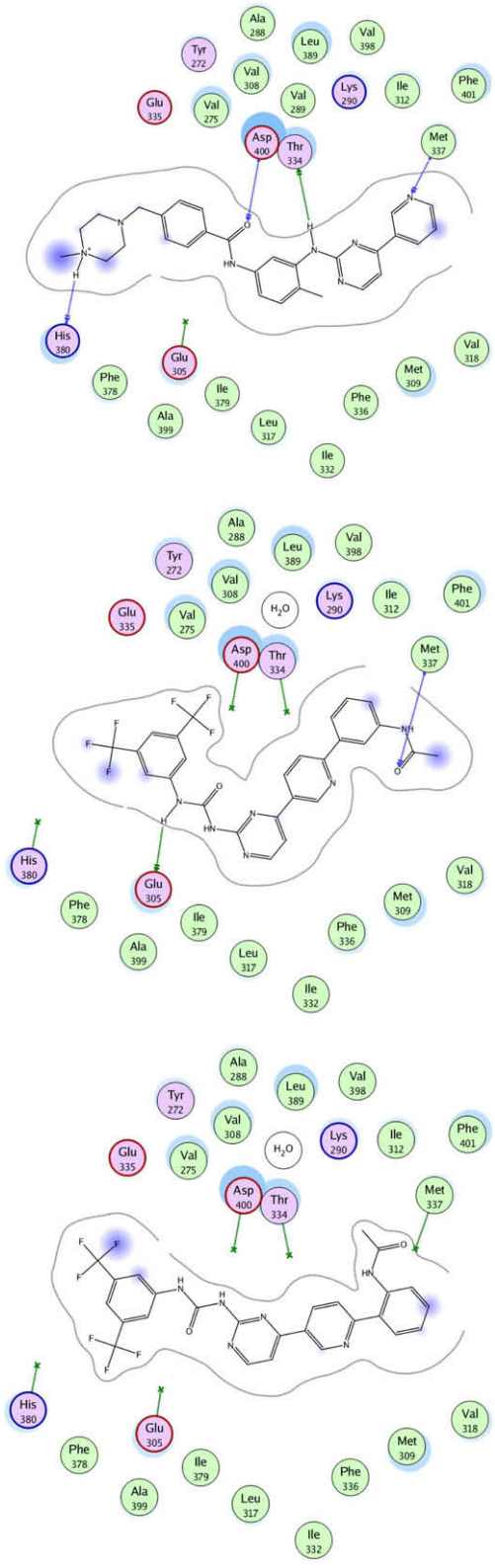


Figure 5. 2D-presentation for the binding interactions in Imatinib (upper panel), compound **30** (middle panel) and compound **29** (lower panel).

(m, 4H), 7.87 (d, J = 7.2 Hz, 4H), 8.06 (d, J = 7.8 Hz, 1H), 8.69 (d, J = 4.2 Hz, 1H), 8.77 (d, J = 5.1, 1H), 8.97 (s, 1H); ^{13}C NMR ($CDCl_3$): 113.89, 123.70, 128.73, 129.27, 131.26, 132.83, 134.50, 134.66, 148.44, 152.10, 159.64, 161.02, 163.37, 172.61.

4.2.5. General procedure for the synthesis of compounds 5a–f

A mixture of the amine **2** (150 mg, 0.87 mmol), the appropriate arylbromide (0.96 mmol), dichlorobis(triphenylphosphine)Pd(II) (0.061 g, 0.087 mmol), Xantphos (0.05 g, 0.087 mmol) and sodium *tert*-butoxide (0.25 g, 2.61 mmol) was refluxed in toluene (10 mL) under nitrogen atmosphere for 8 h.

4.2.5.1. *N*-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)acetamide (5a)[33]. Toluene was removed under vacuum, and the residue was triturated with water (100 mL) and extracted with methylene chloride (100 mL × 2). The undissolved solid was then filtered, and washed with cold water to yield the pure product. Yield (117 mg, 42%); mp 220–221 °C; IR ν/cm^{-1}: 3416, 3210, 3008, 1660, 1590, 1539, 1455, 1419, 800; ^{1}H NMR (CD_3OD): δ 2.13 (s, 3H), 2.29 (s, 3H), 7.2 (d, J = 8.2 Hz, 1H), 7.25(d, J = 8.4 Hz, 1H), 7.35 (d, J = 5.2 Hz, 1H), 7.56 (dd, J = 3.0, 4.9 Hz, 1H), 8.04 (s, 1H), 8.45 (d, J = 5.2 Hz, 1H), 8.56 (d, J = 8.0 Hz, 1H), 8.65 (d, J = 4.0 Hz, 1H), 9.27 (s, 1H).

4.2.5.2. *N*-(3,5-Bis(trifluoromethyl)phenyl)-4-(pyridin-3-yl)pyrimidin-2-amine (5b). Toluene was filtered while hot to remove insoluble metal impurities. After cooling of toluene filtrate, the pure product **5b** was crystallized out as off-white crystals. The crystals were filtered, washed with toluene (10 mL), then with water (50 mL), and dried. Yield (274 mg, 82%); mp 233–234 °C; IR ν/cm^{-1}: 3239, 3082, 2992, 1559, 1383, 1277, 1194, 1124, 995, 880, 799, 680; ^{1}H NMR (DMSO-d_6): δ 7.57–7.70 (m, 3H), 8.49 (d, J = 6.3 Hz, 1H), 8.59 (s, 2H), 8.72–8.75 (m, 2H), 9.36 (s, 1H), 10.50 (s, 1H); ^{13}C NMR (DMSO-d_6): 109.79, 118.08, 123.90, 130.34, 130.77, 131.77, 134.31, 142.37, 148.13, 151.80, 159.48, 159.75, 161.72.

4.2.5.3. *N1,N1*-Dimethyl-*N4*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,4-diamine (5c)[34]. After cooling of toluene, the pure product **5c** was crystallized out as yellow crystals. The crystals were filtered, washed with toluene (10 mL), then with water (50 mL), and dried. Yield (98 mg, 39%); mp 165–167 °C (171–174 °C);[34] ^{1}H NMR ($CDCl_3$): δ 6.81 (d, J = 8.9 Hz, 2H), 7.06 (s, 1H), 7.10 (d, J = 5.1 Hz, 1H), 7.43 (dd, J = 3.0, 4.9 Hz, 1H), 7.51 (d, J = 8.9 Hz, 2H), 8.35 (d, J = 8.0 Hz, 1H), 8.46 (d, J = 5.1 Hz, 1H), 8.72 (d, J = 4.7 Hz, 1H), 9.28 (d, J = 1.4 Hz, 1H).

4.2.5.4. *N*-(2,4-Dimethylphenyl)-4-(pyridin-3-yl)pyrimidin-2-amine (5d)[34]. Toluene was removed under vacuum, and the residue was triturated with water (50 mL) and extracted with ethyl acetate (100 mL × 2). The organic layer was separated, dried over anhydrous $MgSO_4$, and then evaporated under vacuum. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexane, 1:1, v/v) to give **5d** as a yellow powder. Yield (74 mg, 31%); mp 122–123 °C (113–115 °C);[34] IR ν/cm^{-1}: 3210, 2922, 1595, 1571, 1451, 1290, 797; ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 3H), 2.34 (s, 3H), 6.94 (s, 1H), 7.08–7.14 (m, 3H), 7.42 (dd, J = 3.0, 4.8 Hz, 1H), 7.82 (d, J = 8.1 Hz, 1H), 8.34 (d, J = 8.1 Hz, 1H), 8.47 (d, J = 5.1 Hz, 1H), 8.71 (d, J = 3.3 Hz, 1H), 9.25 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 18.12, 20.89, 107.77, 122.91, 123.62, 127.14, 129.84, 131.30, 132.79, 133.94, 134.50, 148.56, 151.40, 159.16, 162.56.

4.2.5.5. *N*-(4-Methoxyphenyl)-4-(pyridin-3-yl)pyrimidin-2-amine (5e)[34]. Toluene was removed under vacuum, and the residue was triturated with water (50 mL). The formed precipitate was filtered, washed with cold water (100 mL), dried, and then crystallized from ethanol to give **5e** as yellow crystals. Yield (126 mg, 52%); mp 182–183 °C (121–122 °C);[34] IR ν/cm^{-1}: 3435, 1575, 1509, 1423, 1242, 801; ^{1}H NMR (DMSO-d_6): δ 3.74 (s, 3H), 6.92 (d, J = 9.0 Hz, 2H), 7.42 (d, J = 5.2 Hz, 1H), 7.59 (dd, J = 4.0, 4.5 Hz, 1H), 7.68 (d,

J = 8.7 Hz, 2H), 8.49 (d, J = 7.8 Hz, 1H), 8.55 (d, J = 4.7 Hz, 1H), 8.73 (d, J = 4.0 Hz, 1H), 9.34 (s, 1H), 9.58 (s, 1H).

4.2.5.6. *N*-(Naphthalen-2-yl)-4-(pyridin-3-yl)pyrimidin-2-amine (5f)[17]. Toluene was removed under vacuum, and the residue was triturated with water (50 mL). The formed precipitate was filtered, washed with cold water (100 mL), dried, and then crystallized from ethanol to give **5f** as yellow plates. Yield (144 mg, 56%); mp 199–200 °C; IR ν/cm^{-1}: 3247, 1571, 1548, 1447, 1434, 798; ^{1}H NMR (DMSO-d_6): δ 7.36 (t, J = 7.8 Hz, 1H), 7.46 (t, J = 7.3 Hz, 1H), 7.56 (d, J = 5.1 Hz, 1H), 7.63 (dd, J = 2.9, 4.8 Hz, 1H), 7.78–7.88 (m, 4 H), 8.54–8.57 (m, 2H),8.67 (d, J = 5.1 Hz, 1H), 8.76 (d, J = 4.5 Hz, 1H), 9.40 (s, 1H), 10.03 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 108.98, 114.43, 121.19, 124.49, 126.77, 127.46, 127.89, 128.50, 129.46, 134.18, 134.93, 138.55, 148.68, 152.03, 160.69; Anal. Calcd for $C_{19}H_{14}N_4$: C, 76.49; H, 4.73; N, 18.78. Found: C, 76.60; H, 4.70; N, 18.38.

4.2.6. 4-Methyl-6-(pyridin-3-yl)pyrimidin-2-amine (6)[18]

A mixture of 2-amino-4-chloro-6-methylpyridine (2.70 g, 18.76 mmol), 3-pyridineboronic acid (2.54 g, 20.66 mmol), dichlorobis(triphenylphosphine)Pd(II) (0.36 g, 0.512 mmol) and Na_2CO_3 (1.40 g, 13.2 mmol) was placed in a mixed solvent of acetonitrile and water (1:1, v/v, 150 mL). N_2 gas was bubbled into this mixture for 10 min, and then the mixture was heated at 78 °C while stirring under N_2 atmosphere for 7 h. The reaction mixture was left to cool at room temperature, poured into ice water (100 mL), and then extracted with ethyl acetate (100 mL × 3). The organic layer was separated, washed with water (100 mL × 3), dried over anhydrous $MgSO_4$, and then evaporated under vacuum to yield the crude product which was then crystallized from ethanol to yield pure yellow crystals of **6**. Yield (2.58 g, 74%); mp 191–192 °C (192–194 °C);[18] ^{1}H NMR (CD_3OD): δ 2.41 (s, 3H), 7.13 (s, 1H), 7.56 (dd, J = 2.9, 4.9 Hz, 1H), 8.49 (d, J = 8.0 Hz, 1H), 8.64 (d, J = 5.0 Hz, 1H), 9.21 (s, 1H).

4.2.7. *N*-(2,4-Dimethylbenzyl)-4-methyl-6-(pyridin-3-yl)pyrimidin-2-amine (7)[17]

The procedure used for the synthesis of compound **3** was adapted for the synthesis of this compound. After pouring the reaction mixture over ice water, the aqueous solution was extracted with ethyl acetate (100 mL × 2). The organic layer was separated, dried over anhydrous $MgSO_4$, and then evaporated under vacuum to yield the crude product, which was then purified by column chromatography (silica gel, ethyl acetate/hexane, 1:1, v/v) to give **7** as brown needle crystals. Yield (0.113 g, 43%); mp 125–126 °C; IR ν/cm^{-1}: 3254, 2920, 1604, 1557, 1341, 808; ^{1}H NMR ($CDCl_3$): 2.32 (s, 3H), 2.37 (s, 3H), 2.43 (s, 3H), 4.68 (d, J = 7.5 Hz, 2H), 5.41 (s, 1H), 6.90 (s, 1H), 6.98–7.02 (m, 2H), 7.25 (d, J = 7.8 Hz, 1H), 7.33 (dd, J = 3.5, 4.8 Hz, 1H), 8.31 (d, J = 7.8 Hz, 1H), 8.67 (d, J = 4.8 Hz, 1H), δ 9.22 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 19.08, 21.01, 24.38, 43.44, 106.23, 123.48, 126.66, 128.34, 131.24, 133.34, 133.94, 134.45, 136.21, 137.03, 148.55, 151.01, 162.13, 162.48, 168.95; Anal. Calcd for $C_{19}H_{20}N_4$: C, 74.97; H, 6.62; N, 18.41. Found: C, 74.99; H, 6.65; N, 18.90.

4.2.8. 3-(Trifluoromethyl)-*N*-(4-methyl-6-(pyridin-3-yl)pyrimidin-2-yl)benzamide (8)

To a stirred and heated solution of 4-methyl-6-(pyridin-3-yl)pyrimidin-2-amine (0.1 g, 0.54 mmol) in pyridine (5 mL), 3-(trifluoromethyl)benzoyl chloride (0.134 g, 0.65 mmol) was added dropwise over a period of 1 h while heating and stirring were maintained. Reflux was maintained for 30 min, and then the reaction mixture was cooled and poured over crushed ice to induce precipitation of the crude product. The resulted precipitate was then filtered, dried, and purified by silica column using ethyl acetate as the mobile phase. Yield (56 mg, 29%), mp 148–149 °C; IR ν/cm^{-1}: 3437, 1689, 1630, 1607, 1540, 1439, 1336, 1261, 1172, 1113; ^{1}H NMR ($CDCl_3$): δ 2.65 (s, 3H), 7.43 (s, 1H), 7.46 (dd, J = 3.0, 4.8 Hz, 1H), 7.69 (dd, J = 7.7 Hz, 1H), 7.87 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 8.1 Hz, 1H), 8.22 (s, 1H), 8.45 (d, J = 7.9 Hz, 1H), 8.66 (s, 1H), 8.75 (d, J = 3.7 Hz, 1H), 9.25 (s, 1H); ^{13}C NMR (CDCl3): δ 27.08, 115.03, 126.46, 127.68, 131.66, 133.82, 134.81, 137.86, 151.02, 154.02, 160.79, 165.03, 167.52, 172.69.

4.2.9. General procedure for synthesis of compounds 9a, 9b

A mixture of 4-methyl-6-(pyridin-3-yl)pyrimidin-2-amine (0.162 g, 0.87 mmol), the appropriate arylbromide (0.96 mmol), dichlorobis(triphenylphosphine)Pd(II) (0.061 g, 0.087 mmol), Xantphos (0.05 g, 0.087 mmol) and sodium *tert*-butoxide (0.25 g, 2.61 mmol) was refluxed in toluene (10 mL) under nitrogen atmosphere for 8 h.

4.2.9.1. 4-Methyl-*N*-(2,4-dimethylphenyl)-6-(pyridin-3-yl)pyrimidin-2-amine (9a)[17]. Toluene was removed under vacuum, and the residue was triturated with water (50 mL), and extracted with ethyl acetate (100 mL × 2). The organic layer was separated, dried over $MgSO_4$, and then evaporated till dryness. The crude product was further purified by silica column using the mixed solvent of ethyl acetate and hexane (1:1, v/v) as mobile phase to yield **9a** as an off-white powder. Yield (88 mg, 34.8%); mp 95–96 °C; IR ν/cm^{-1}: 3215, 2921, 1597, 1581, 1552, 1448, 1343, 804; ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 6H), 2.49 (s, 3H), 6.90 (s, 1H), 7.04–7.10 (m, 3H), 7.41 (dd, J = 3.0, 5.0 Hz, 1H) 7.97 (d, J = 8.1 Hz, 1H), 8.32 (d, J = 8.1 Hz, 1H), 8.69 (d, J = 3.9 Hz, 1H), 9.24 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 18.16, 20.83, 24.42, 107.49, 121.97, 123.55, 127.02, 128.68, 131.16, 133.04, 133.08, 134.49, 134.90, 148.58, 151.19, 160.79, 162.18, 169.15; Anal. Calcd for $C_{18}H_{18}N_4$: C, 74.46; H, 6.25; N, 19.30. Found: C, 74.46; H, 6.50; N, 19.00.

4.2.9.2. *N*-(3,5-Bis(trifluoromethyl)phenyl)-4-methyl-6-(pyridin-3-yl)pyrimidin-2-amine (9b). Toluene was filtered while hot to remove insoluble metal impurities. After cooling of toluene filtrate, the pure product **9b** was crystallized out as pale yellow crystals. The crystals were filtered, washed with toluene (10 mL), then with water (50 mL), and dried. Yield (235 mg, 68%); mp 254–255 °C; IR ν/cm^{-1}: 3265, 3085, 2964, 1543, 1385, 1277, 1267, 1186, 1124, 874, 809, 701, 680; ^{1}H NMR (DMSO-d_6): δ 2.08 (s, 3H), 7.56–7.60 (m, 3H), 8.47 (d, J = 8.0 Hz, 1H), 8.61 (s, 2H), 8.73 (d, J = 4.6 Hz, 1H), 9.33 (d, J = 1.3 Hz, 1H), 10.42 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 24.26, 109.66, 113.77, 118.39, 122.14, 124.27, 125.76, 130.79, 131.21, 132.43, 134.60, 143.03, 148.53, 152.03, 159.78, 161.80, 169.87.

4.2.10. 5-Acetyl-2-bromopyridine (10)[19]

Under N_2 atmosphere, *n*-BuLi (8.1 mL, 13 mmol, 1.6 M in hexane) was added to a mixture of 2,5-dibromopyridine (3.08 g, 13 mmol) and anhydrous Et_2O (50 mL) at −78 °C. The reaction mixture was stirred for 30 min and then *N*,*N*-dimethylacetamide (1.4 mL, 15 mmol) was added and stirred at −78 °C to ambient temperature within 1 h. The whole mixture was poured into saturated NH_4Cl solution and then extracted with Et_2O (150 mL × 3). The combined organic layer was washed with brine, dried, and concentrated. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexane, 1:7.5, v/v) to give **8** as a yellowish white solid. Yield (1.3 g, 50.5%), mp 127–129 °C (124–128 °C);[19] ^{1}H NMR ($CDCl_3$): δ 2.62 (s, 3H), 7.61 (d, J = 8.2 Hz, 1H), 8.07 (d, J = 7.6 Hz, 1H), 8.89 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 26.79, 128.44, 131.41, 137.70, 146.96, 150.42, 195.65.

4.2.11. General procedure for the synthesis of compounds 11a, 11b

A mixture of 5-acetyl-2-bromopyridine (**10**) (2.0 g, 10 mmol), appropriate arylboronic acid (11 mmol), dichlorobis(triphenyl-

phosphine)Pd(II) (192 mg, 0.273 mmol) and Na_2CO_3 (0.75 g, 7 mmol) was placed in mixed solvent of acetonitrile and water (1:1, 80 mL). N_2 gas was bubbled into this mixture for 10 min, and then the mixture was heated at 78 °C while stirring under N_2 atmosphere for 4 h. The reaction mixture was left to cool at room temperature, poured into ice water (100 mL), and then extracted with methylene chloride (150 mL × 3). The organic layer was separated, dried over anhydrous $MgSO_4$, and then evaporated under vacuum to yield the crude product which was then crystallized from ethanol to yield the pure compounds **11a** and **11b**.

4.2.11.1. 1-(6-Phenylpyridin-3-yl)ethanone (11a)[17]. It was obtained as silvery needle crystals. Yield (1.32 g, 67%); mp 119–120 °C (118 °C [22]); IR ν/cm^{-1}: 1677, 1588, 1262, 740; ^{1}H NMR ($CDCl_3$): δ 2.68 (s, 3H), 7.49–7.55 (m, 3H), 7.86 (d, J = 8.4 Hz, 1H), 8.08 (d, J = 7.2 Hz, 2H), 8.31 (dd, J = 2.2, 6.2 Hz, 1H), 9.25 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 26.76, 120.18, 127.39, 128.96, 130.11, 136.43, 138.13, 150.12, 160.60.

4.2.11.2. 1-(6-(Pyridin-3-yl)pyridin-3-yl)ethanone (11b)[17]. It was obtained as buff plates. Yield (1.46 g, 73.6%); mp 103–104 °C; IR ν/cm^{-1}: 1680, 1586, 1269, 818; ^{1}H NMR (CD_3OD): δ 2.68 (s, 3H), 7.63 (dd, J = 3.0, 4.8 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 8.44 (dd, J = 2.1, 6.0 Hz, 1H), 8.58 (d, J = 8.1 Hz, 1H), 8.66 (d, J = 4.5 Hz, 1H), 9.23 (s, 1H), 9.28 (s, 1H); ^{13}C NMR (CD_3OD): δ 25.49, 120.51, 124.25, 131.48, 134.36, 135.80, 137.00, 147.29, 149.29, 149.78, 157.27, 197.02; Anal. Calcd for $C_{12}H_{10}N_2O$: C, 72.71; H, 5.08; N, 14.13. Found: C, 72.96; H, 5.25; N, 14.30.

4.2.12. General procedure for the synthesis of compounds 12a, 12b

The appropriate 1-(6-(substituted)pyridin-3-yl)ethanone (**11a**) or (**11b**) (2.5 mmol) and *N,N*-dimethylformamide dimethylacetal (0.6 g, 5 mmol) were refluxed together in an oil bath for 3 h. The excess unreacted *N,N*-dimethylformamide dimethylacetal was removed under vacuum. The solid residue was triturated with water (30 mL) and then extracted with methylene chloride (100 mL × 2). The organic layer was separated, dried over anhydrous $MgSO_4$, and then evaporated under vacuum to yield the crude product which was used for the next step without further purification.

4.2.12.1. 3-(Dimethylamino)-1-(6-phenylpyridin-3-yl)prop-2-en-1-one (12a)[17]. It was obtained as yellow powder. Yield (0.62 g, 99%); mp 151–152 °C; IR ν/cm^{-1}: 1644, 1589, 1567, 1537, 1285, 1242, 769, 745; ^{1}H NMR ($CDCl_3$): δ 2.97 (s, 3H), 3.19 (s, 3H), 5.74 (d, J = 12.3 Hz, 1H), 7.42–7.53 (m, 3H), 7.81 (d, J = 8.3 Hz, 1H), 7.87 (d, J = 12.2 Hz, 1H), 8.05 (d, J = 7.4 Hz, 2H), 8.28 (dd, J = 1.6, 6.6 Hz, 1H), 9.18 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 37.46, 45.30, 91.71, 120.46, 127.32, 128.95, 129.84, 134.14, 136.96, 137.79, 148.10, 154.74, 158.37, 185.40; Anal. Calcd for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.86; H, 6.25; N, 11.50.

4.2.12.2. 3-(Dimethylamino)-1-(6-(pyridin-3-yl)pyridin-3-yl)prop-2-en-1-one (12b)[17]. It was obtained as brown powder. Yield (0.56 g, 88.4%); mp 163–164 °C; IR ν/cm^{-1}: 1639, 1590, 1565, 1538, 1274, 1260, 780; ^{1}H NMR ($CDCl_3$): δ 2.95 (s, 3H), 3.17 (s, 3H), 5.70 (d, J = 12.2 Hz, 1H), 7.40 (dd, J = 2.8, 5.0 Hz, 1H), 7.79 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 12.2 Hz, 1H), 8.27 (d, J = 8.2 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 8.64 (d, J = 4.5 Hz, 1H), 9.17 (s, 1H), 9.22 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 37.42, 45.29, 91.74, 120.02, 123.63, 134.30, 134.52, 136.20, 148.43, 149.29, 150.25, 154.71, 156.26, 185.70; Anal. Calcd for $C_{15}H_{15}N_3O$: C, 71.13; H, 5.97; N, 16.59. Found: C, 71.16; H, 5.99; N, 16.70.

4.2.13. General procedure for the synthesis of compounds 13a, 13b

To a solution of NaOEt in absolute ethanol, made by dissolving sodium metal (27 mg, 1.185 mmol) in absolute ethanol (20 mL), guanidine hydrochloride (114 mg, 1.185 mmol) was added in one portion and stirred at room temperature for 1 h. The appropriate 3-(dimethylamino)-1-(6-(substituted)pyridin-3-yl)prop-2-en-1-one (**12a**) or (**12b**) (1.185 mmol) was dissolved in absolute ethanol (10 mL) and added to the reaction mixture. The mixture was heated under reflux for 5 h, and was then left to cool at room temperature, followed by cooling in an ice bath. The formed product was separated by filtration, washed with cold ethanol (20 mL), then with water (50 mL), and left to dry.

4.2.13.1. 4-(6-Phenylpyridin-3-yl)pyrimidin-2-amine (13a)[17]. It was obtained as silver crystals. Yield (246 mg, 83.5%); mp 211–212 °C; IR ν/cm^{-1}: 3317, 3156, 1648, 1590, 1571, 1542, 1478, 743; ^{1}H NMR (DMSO-d_6): δ 6.80 (s, 2H), 7.26 (d, J = 3.0 Hz, 1H), 7.48–7.55 (m, 3H), 8.11 (d, J = 8.7 Hz, 1H), 8.17 (d, J = 7.1 Hz, 2H), 8.36 (d, J = 2.7 Hz, 1H), 8.50 (dd, J = 2.1, 6.3 Hz, 1H), 9.32 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 106.38, 120.48, 127.21, 129.34, 130.05, 131.54, 135.69, 138.40, 148.49, 157.84, 159.83, 161.73, 164.28; Anal. Calcd for $C_{15}H_{12}N_4$: C, 72.56; H, 4.87; N, 22.57. Found: C, 72.99; H, 4.90; N, 22.80.

4.2.13.2. 4-(6-(Pyridin-3-yl)pyridin-3-yl)pyrimidin-2-amine (13b)[17]. It was obtained as yellow plates. Yield (267 mg, 90.3%); mp >300 °C; IR ν/cm^{-1}: 3438, 3324, 1621, 1586, 1566, 1545, 1477, 803; ^{1}H NMR (DMSO-d_6): δ 6.82 (s, 2H), 7.29 (d, J = 5.4 Hz, 1H), 7.55 (dd, J = 3.2 , 4.8 Hz, 1H), 8.21 (d, J = 8.1 Hz, 1H), 8.37 (d, J = 5.1 Hz, 1H), 8.50–8.55 (m, 2H), 8.67 (d, J = 3.6 Hz, 1H), 9.34 (d, J = 1.5 Hz, 1H), 9.36 (d, J = 1.5 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 106.52, 121.01, 124.41, 132.14, 133.87, 134.67, 135.91, 148.35, 148.70, 150.74, 155.63, 159.92, 161.55, 164.23; Anal. Calcd for $C_{14}H_{11}N_5$: C, 67.46; H, 4.45; N, 28.10. Found: C, 67.15; H, 4.55; N, 28.30.

4.2.14. General procedure for the synthesis of compounds 14a, 14b

A mixture of the amine **13a** or **13b** (0.34 mmol) and 3,5-bis(trifluoromethyl)phenyl isocyanate (173 mg, 0.68 mmol) was heated without solvent on an oil bath at 120 °C for 2 h. The crude product was then crystallized from DMSO to yield the pure urea derivatives **14a** and **14b**.

4.2.14.1. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-(6-phenylpyridin-3-yl)pyrimidin-2-yl)urea (14a). Yield (116 mg, 68%); mp >300 °C; IR ν/cm^{-1}: 3094, 2971, 2911, 1722, 1585, 1411, 1388, 1277, 1177, 1127, 743; ^{1}H NMR (DMSO-d_6): δ 7.51–7.58 (m, 3H), 7.76 (s, 1H), 7.90 (d, J = 5.3 Hz, 1H), 8.15–8.22 (m, 3H), 8.35 (s, 2H), 8.66 (dd, J = 2.1, 6.3 Hz, 1H), 8.85 (d, J = 4.5 Hz, 1H), 9.47 (d, J = 1.8 Hz, 1H), 10.64 (s, 1H), 12.04 (s, 1H).

4.2.14.2. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-(6-(pyridin-3-yl)pyridin-3-yl)pyrimidin-2-yl)urea (14b). Yield (128 mg, 75%); mp 200–201 °C; IR ν/cm^{-1}: 3436, 1714, 1625, 1587, 1447, 1385, 1284, 1252, 1166, 1134; ^{1}H NMR (DMSO-d_6): δ 7.57–7.59 (m, 1H), 7.77 (s, 1H), 7.92 (d, J = 5.1 Hz, 1H), 8.29–8.36 (m, 3H), 8.55 (d, J = 6.3 Hz, 1H), 8.67–8.69 (m, 2H), 8.85 (d, J = 4.8 Hz, 1H), 9.38 (s, 1H), 9.51 (s, 1H), 10.66 (s, 1H), 12.04 (s, 1H).

4.2.15. General procedure for the synthesis of compounds 15a–d

A mixture of the appropriate amine **13a** or **13b** (0.29 mmol), the appropriate arylbromide (0.32 mmol), dichlorobis(triphenylphosphine)Pd(II) (20 mg, 0.029 mmol), Xantphos (17 mg, 0.029 mmol) and sodium *tert*-butoxide (42 mg, 0.44 mmol) was refluxed in toluene (7 mL) under nitrogen atmosphere for 8 h. The reaction mixture was left to cool at room temperature, and then cooled in an ice bath. The formed solid was filtered, washed with cold toluene (10 mL), then with water (50 mL).

4.2.15.1. *N*-(4-Methoxyphenyl)-4-(6-phenylpyridin-3-yl)pyrimidin-2-amine (15a)[17]. It was obtained as yellow crystalline powder. Yield (84 mg, 82%); mp >300 °C; IR ν/cm^{-1}: 3462, 1579, 1511, 1456, 1430, 1262, 1240, 1097, 1039, 804; ^{1}H NMR (DMSO-d_6): δ 3.74 (s, 3H), 6.92 (d, J = 9.0 Hz, 2H), 7.48–7.56 (m, 4H), 7.71 (d, J = 8.7 Hz, 2H), (m, 3H), 8.55–8.58 (m, 2H), 9.41 (s, 1H), 9.57 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 55.65, 107.96, 114.24, 120.66, 121.38, 127.25, 129.39, 131.32, 133.90, 135.90, 138.31, 148.59, 154.83, 158.12, 159.79, 160.78; Anal. Calcd for $C_{22}H_{18}N_4O$: C, 74.56; H, 5.12; N, 15.81. Found: C, 74.30; H, 5.60; N, 15.90.

4.2.15.2. *N*-(3,5-Bis(trifluoromethyl)phenyl)-4-(6-phenylpyridin-3-yl)pyrimidin-2-amine (15b). It was obtained as white powder. Yield (105 mg, 79%); mp 113–115 °C; IR ν/cm^{-1}: 3276, 3098, 1578, 1558, 1383, 1275, 1184, 1129, 882, 818, 785, 739, 680; ^{1}H NMR (DMSO-d_6): δ 7.49–7.57 (m, 3H), 7.63 (s, 1H), 7.72 (d, J = 5.2 Hz, 1H), 8.16–8.22 (m, 3H), 8.57–8.61 (m, 3H), 8.73 (d, J = 5.1 Hz, 1H), 9.43 (d, J = 1.7 Hz, 1H), 10.50 (s, 1H).

4.2.15.3. *N1*,*N1*-Diphenyl-*N4*-(4-(6-phenylpyridin-3-yl)pyrimidin-2-yl)benzene-1,4-diamine (15c)[17]. It was obtained as yellow powder. Yield (44 mg, 31%); mp 210–211 °C; IR ν/cm^{-1}: 3460, 1575, 1527, 1507, 1493, 1422, 1277, 744, 695; ^{1}H NMR ($CDCl_3$): δ 6.98–7.72 (m, 18H), 7.89 (d, J = 8.1 Hz, 1H), 8.10 (d, J = 6.9 Hz, 2H), 8.45 (dd, J = 2.5, 6.1 Hz, 1H), 8.51 (d, J = 5.1 Hz, 1H), 9.40 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 107.92, 108.12, 119.44, 120.31, 120.46, 122.30, 122.74, 123.60, 125.62, 127.13, 128.90, 129.00, 129.10, 129.60, 130.93, 134.90, 135.26, 138.58, 142.75, 147.98, 148.53, 158.92, 159.15, 160.33, 162.48; Anal. Calcd for $C_{33}H_{25}N_5$: C, 80.63; H, 5.13; N, 14.25. Found: C, 80.46; H, 5.55; N, 14.20.

4.2.15.4. *N1*,*N1*-Diphenyl-*N4*-(4-(6-(pyridin-3-yl)pyridin-3-yl)-pyrimidin-2-yl)benzene-1,4-diamine (15d)[17]. It was obtained as yellow powder. Yield (39 mg, 27.3%); mp 230–231 °C; IR ν/cm^{-1}: 3429, 1582, 1530, 1507, 1493, 1422, 1281, 751, 695; ^{1}H NMR (DMSO-d_6): δ 6.96 (d, J = 6.9 Hz, 6H), 7.05 (d, J = 8.8 Hz, 2H), 7.24–7.30 (m, 4H), 7.56 (d, J = 5.1 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H), 8.25 (d, J = 8.1 Hz, 1H), 8.52 (d, J = 8.1 Hz, 1H), 8.60–8.68 (m, 3H), 9.35 (s, 1H), 9.45 (s, 1H), 9.82 (s, 1H); ^{13}C NMR ($CDCl_3$): 100.37, 108.54, 120.84, 121.17, 122.59, 123.10, 124.43, 126.18, 129.86, 131.87, 133.82, 134.72, 136.15, 136.96, 141.37, 148.00, 148.40, 148.88, 159.92, 161.57; Anal. Calcd for $C_{32}H_{24}N_6$: C, 78.03; H, 4.91; N, 17.06. Found: C, 78.06; H, 4.99; N, 17.20.

4.2.16. 1-(6-(Phenylamino)pyridin-3-yl)ethanone (16)

A mixture of 5-acetyl-2-bromopyridine (**10**; 1.0 g, 5.0 mmol), dichlorobis(triphenylphosphine)Pd(II) (140 mg, 0.2 mmol), 1,3-bis(diphenylphosphino)propane (210 mg, 0.5 mmol) and sodium *tert*-butoxide (1.2 g, 12.5 mmol) in aniline (5 mL) was refluxed under N_2 atmosphere for 12 h. After reaction completion, excess aniline was removed under vacuum, and the residue was purified with column chromatography (silica gel, ethyl acetate/hexane 1:3, v/v). Yield (0.4 g, 38%); mp 128–129 °C; IR ν/cm^{-1}: 3240, 1675, 1609, 1590, 1572, 1391, 1278, 1142, 754; ^{1}H NMR ($CDCl_3$): δ 2.53 (s, 3H), 6.85 (d, J = 8.9 Hz, 1H), 7.14–7.20 (m, 1H), 7.39–7.40 (m, 4H), 7.97 (br s, 1H), 8.05 (dd, J = 2.1, 8.9 Hz, 1H), 8.78–8.79 (m, 1H); ^{13}C NMR ($CDCl_3$): 26.11, 107.16, 121.93, 124.55, 124.77, 129.52, 137.62, 138.89, 151.04, 158.92, 195.33.

4.2.17. (*E*)-3-(Dimethylamino)-1-(6-(phenylamino)pyridin-3-yl)prop-2-en-1-one (17)

A mixture of 1-(6-(phenylamino)pyridin-3-yl)ethanone (**16**; 0.37 g, 1.72 mmol) and *N*,*N*-dimethylformamide dimethylacetal (0.41 g, 3.44 mmol) was heated under reflux in an oil bath for 12 h. The excess unreacted *N*,*N*-dimethylformamide dimethylacetal was removed under vacuum. The solid residue was triturated with water (50 mL) and then extracted with methylene chloride (100 mL × 3). The organic layer was separated, dried over anhydrous $MgSO_4$, and then evaporated under vacuum to yield the product (**17**). Yield (0.43 g, 93.6%); mp 187–189 °C; IR ν/cm^{-1}: 3283, 3038, 1635, 1583, 1547, 1496, 1422, 1387, 1318, 1253, 1115, 1061, 896, 751; ^{1}H NMR ($CDCl_3$): δ 2.94 (s, 6H), 5.65 (d, J = 9.0 Hz, 1H), 7.05–7.10 (m, 1H), 7.27–7.45 (m, 4H), 7.56 (s, 1H), 7.79 (d, J = 12.3 Hz, 1H), 8.07 (dd, J = 2.1, 8.7 Hz, 1H), 8.78 (s, 1H); ^{13}C NMR ($CDCl_3$): 38.67, 44.91, 91.38, 107.81, 120.83, 123.21, 126.69, 127.01, 129.22, 129.37, 137.20, 148.94, 153.82, 185.7.

4.2.18. 4-(6-(Phenylamino)pyridin-3-yl)pyrimidin-2-amine (18)

To an ethanolic solution of sodium ethoxide prepared by dissolving sodium metal (40 mg, 1.74 mmol) in absolute ethanol (10 mL) was added guanidine hydrochloride (170 mg, 1.74 mmol). The mixture was stirred at room temperature for 1 h, then 3-(dimethylamino)-1-(6-(phenylamino)pyridin-3-yl)prop-2-en-1-one (**17**; 0.4 g, 1.5 mmol) in 10 mL of absolute ethanol was added. The temperature was raised to reflux, and the mixture was heated for 8 h. The reaction mixture was left to cool to room temperature then cooled in ice water. The crystallized product (**18**) was collected by filtration, washed first with cold ethanol, then with water. Yield (0.21 g, 46%); mp >300 °C; IR ν/cm^{-1}: 3342, 3175, 1653, 1594, 1574, 1540, 1497, 1471, 1443, 1393, 1344, 1292, 806; ^{1}H NMR (DMSO-d_6): δ 6.56 (s, 2H); 6.89–6.97 (m, 2H), 7.06 (d, J = 5.2 Hz, 1H), 7.29 (t, J = 7.7 Hz, 2H), 7.70 (d, J = 8.1 Hz, 2H), 8.18–8.24 (m, 2H), 8.86 (d, J = 1.9 Hz, 1H), 9.38 (s, 1H); ^{13}C NMR (DMSO-d_6): 105.01, 110.60, 119.20, 121.70, 123.42, 129.15, 135.72, 141.45, 147.34, 157.59, 159.06, 162.36, 164.11.

4.2.19. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-(6-(phenylamino)-pyridin-3-yl)pyrimidin-2-yl)urea (19)

A mixture of 4-(6-(phenylamino)pyridin-3-yl)pyrimidin-2-amine (**18**; 100 mg, 0.38 mmol) and 3,5-bis(trifluoromethyl)phenyl isocyanate (193 mg, 0.76 mmol) was heated without solvent on an oil bath at 120 °C for 1 h. The mixture was then crystallized from DMSO to yield the pure product (**19**). Yield (145 mg, 74%); mp 278–279 °C; IR ν/cm^{-1}: 3134, 3011, 2974, 1708, 1587, 1388, 1282, 1181, 1135, 739; ^{1}H NMR (DMSO-d_6): δ 7.05 (d, J = 8.8 Hz, 2H), 7.35 (t, J = 7.8 Hz, 2H), 7.70–7.77 (m, 4H), 8.33 (s, 2H), 8.38 (d, J = 8.6 Hz, 1H), 8.68 (d, J = 5.4 Hz, 1H), 9.01 (s, 1H), 9.97 (s, 1H), 10.59 (s, 1H), 12.09 (s, 1H).

4.2.20. *N*-(3,5-Bis(trifluoromethyl)phenyl)-4-(6-(phenylamino)-pyridin-3-yl)pyrimidin-2-amine (20)

A mixture of 4-(6-(phenylamino)pyridin-3-yl)pyrimidin-2-amine-(**18**; 100 mg, 0.38 mmol), 3,5-bis(trifluoromethyl)phenylbromide (123 mg, 0.42 mmol), dichlorobis(triphenylphosphine)Pd(II) (26 mg, 0.038 mmol), Xantphos (22 mg, 0.038 mmol) and sodium *tert*-butoxide (55 mg, 0.57 mmol) was refluxed in toluene (7 mL) under nitrogen atmosphere for 12 h. The reaction mixture was left to cool at room temperature, and then cooled in an ice bath. The formed solid was filtered, washed with cold toluene (10 mL), then with water (50 mL) to yield pure **20** as yellow crystals. Yield (112 mg, 62%); mp 273–274 °C; IR ν/cm^{-1}: 3288, 3221, 3133, 2994, 1591, 1552, 1524, 1372, 1281, 1172, 1123, 877, 813, 699, 681; ^{1}H NMR (DMSO-d_6): δ 6.95–7.00 (m, 2H), 7.32 (t, J = 7.6 Hz, 2H), 7.51 (d, J = 5.1 Hz, 1H), 7.60 (s, 1H), 7.71 (d, J = 8.0 Hz, 2H), 8.30 (d, J = 8.9 Hz, 1H), 8.60 (s, 3H), 9.01 (s, 1H), 9.52 (s, 1H), 10.35 (s, 1H).

4.2.21. (*E*)-1-(6-Bromopyridin-3-yl)-3-(dimethylamino)prop-2-en-1-one (21)

A mixture of 5-acetyl-2-bromopyridine (**10**; 2.5 g, 12.5 mmol) and *N*,*N*-dimethylformamide dimethylacetal (3.0 g, 25 mmol) was heated under reflux in an oil bath for 20 h. The excess unreacted *N*,*N*-dimethylformamide dimethylacetal was removed under vac-

uum. The solid residue was triturated with water (100 mL) and then extracted with methylene chloride (200 mL × 3). The organic layer was separated, dried over anhydrous $MgSO_4$, and then evaporated under vacuum to yield the crude product (**21**) which was purified by crystallization from the mixed solvent of ethyl acetate and hexane. Yield (2.8 g, 88%); mp 122–123 °C; IR ν/cm^{-1}: 1645, 1578, 1571, 1534, 1437, 1418, 1087, 900, 790; ^{1}H NMR ($CDCl_3$): δ 2.86 (s, 3H); 3.10 (s, 3H), 5.52 (d, J = 12.1 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.77 (d, J = 12.1 Hz, 1H), 7.94–7.96 (m, 1H), 8.73 (s, 1H); ^{13}C NMR ($CDCl_3$): 37.46, 45.32, 91.44, 127.80, 134.88, 137.62, 144.22, 149.32, 154.94, 184.89.

4.2.22. 4-(6-Bromopyridin-3-yl)pyrimidin-2-amine (22)

To an ethanolic solution of sodium ethoxide prepared by dissolving sodium metal (0.27 g, 11.37 mmol) in absolute ethanol (30 mL) was added guanidine hydrochloride (1.12 g, 11.73 mmol). The mixture was stirred at room temperature for 1 h, then 1-(6-bromopyridin-3-yl)-3-(dimethylamino)prop-2-en-1-one (**21**; 2.7 g, 10.6 mmol) in 30 mL of absolute ethanol was added. The temperature was raised to reflux, and the mixture was heated for 12 h. The reaction mixture was left to cool to room temperature then cooled in ice water. The crystallized product (**22**) was collected by filtration, washed first with cold ethanol, then with water. Yield (1.54 g, 58%); mp 192–193 °C; IR ν/cm^{-1}: 3482, 3301, 3170, 1634, 1582, 1568, 1479, 1463, 1436, 1217, 1087, 815; ^{1}H NMR (DMSO-d_6): δ 6.82 (s, 2H); 7.21 (d, J = 5.1 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 8.32–8.37 (m, 3H), 9.02 (d, J = 2.3 Hz, 1H); ^{13}C NMR (DMSO-d_6): 128.65, 132.76, 137.83, 143.61, 149.23, 160.08, 160.83, 164.19, 164.24.

4.2.23. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-(6-bromopyridin-3-yl)pyrimidin-2-yl)urea (23)

A mixture of the amine **22** (100 mg, 0.4 mmol) and 3,5-bis(trifluoromethyl)phenyl isocyanate (203 mg, 0.8 mmol) was heated without solvent on an oil bath at 120 °C for 2 h. The mixture was then crystallized from DMSO to yield the pure product (**23**). Yield (160 mg, 79%); mp 139–141 °C; IR ν/cm^{-1}: 3281, 3128, 3024, 1722, 1580, 1472, 1382, 1280, 1171, 1146, 1031, 879, 683; ^{1}H NMR (DMSO-d_6): δ 7.18 (d, J = 8.4 Hz, 1H), 7.74–7.76 (m, 2H), 8.34 (s, 2H), 8.51 (d, J = 9.6 Hz, 1H), 8.72 (d, J = 5.4 Hz, 1H), 8.80 (s, 1H), 10.54 (s, 1H), 11.98 (s, 1H).

4.2.24. General procedure for the synthesis of compounds 24a–d

A mixture of the amine **22** (0.4 g, 1.6 mmol), the appropriate aryl boronic acid (1.76 mmol), dichlorobis(triphenylphosphine)-Pd(II) (30 mg, 0.044 mmol) and Na_2CO_3 (120 mg, 1.12 mmol) was placed in mixed solvent of acetonitrile and water (1:1, v/v, 20 mL). N_2 gas was bubbled into this mixture for 10 min, and then the mixture was heated at 78 °C while stirring under N_2 atmosphere for 12 h. The reaction mixture was left to cool at room temperature, and then poured into ice water (100 mL). The resulted solid was collected by filtration, washed with water, and crystallized from ethanol to yield the pure products **24a–d**.

4.2.24.1. 1-(2-(5-(2-Aminopyrimidin-4-yl)pyridin-2-yl)phenyl)-ethanone (24a). Yield (296 mg, 64%); mp 155–156 °C; IR ν/cm^{-1}: 3372, 3314, 3178, 1698, 1646, 1567, 1472, 1438, 1288, 1268, 1249, 1237, 1216, 817, 601; ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H); 5.16 (s, 2H), 7.03 (s, 1H), 7.39–7.72 (m, 5H), 8.41 (d, J = 2.3 Hz, 2H), 9.24 (s, 1H); ^{13}C NMR ($CDCl_3$): 30.64, 107.59, 122.30, 127.67, 129.10, 129.25, 130.37, 131.29, 135.28, 138.03, 141.76, 147.92, 159.27, 162.55, 163.33, 204.13.

4.2.24.2. 1-(4-(5-(2-Aminopyrimidin-4-yl)pyridin-2-yl)phenyl)-ethanone (24b). Yield (283 mg, 61%); mp 268–269 °C; IR ν/cm^{-1}: 3374, 3297, 3158, 1682, 1636, 1589, 1574, 1471, 1365, 1266, 815, 604; ^{1}H NMR (DMSO-d_6): δ 2.64 (s, 3H), 6.83 (s, 2H), 7.29 (d, J = 5.2 Hz, 1H), 8.09 (d, J = 8.4 Hz, 2H), 8.22 (d, J = 8.4 Hz, 1H), 8.32 (d, J = 8.4 Hz, 2H), 8.38 (d, J = 5.1 Hz, 1H), 8.53 (dd, J = 2.2, 6.2 Hz, 1H), 9.36 (d, J = 1.9 Hz, 1H).

4.2.24.3. *N*-(2-(5-(2-Aminopyrimidin-4-yl)pyridin-2-yl)phenyl)-acetamide (24c). Yield (225 mg, 46%); mp 198–199 °C; IR ν/cm^{-1}: 3893, 3456, 3324, 3176, 1674, 1587, 1534, 1457, 1322, 1241, 815, 776, 757; ^{1}H NMR (DMSO-d_6): δ 6.85 (s, 2H), 7.22–7.30 (m, 2H), 7.44 (t, J = 7.7 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.99 (d, J = 8.5 Hz, 1H), 8.21 (d, J = 8.3 Hz, 1H), 8.39 (d, J = 5.1 Hz, 1H), 8.56 (d, J = 8.1 Hz, 1H), 9.39 (s, 1H), 11.60 (s, 1H); ^{13}C NMR (DMSO-d_6): 24.95, 106.40, 122.91, 123.49, 124.39, 130.08, 130.39, 131.13, 136.07, 137.56, 147.13, 158.92, 159.98, 161.39, 164.29, 168.67.

4.2.24.4. *N*-(3-(5-(2-Aminopyrimidin-4-yl)pyridin-2-yl)phenyl)-acetamide (24d). Yield (288 mg, 59%); mp 246–247 °C; IR ν/cm^{-1}: 3310, 3149, 1670, 1652, 1613, 1592, 1572, 1475, 1371, 821, 801; ^{1}H NMR (DMSO-d_6): δ 2.21 (s, 3H), 5.19 (s, 2H), 7.12 (d, J = 3.9 Hz, 1H), 7.19 (t, J = 5.6 Hz, 1H), 7.45 (t, J = 5.5 Hz, 1H), 7.71 (d, J = 5.3 Hz, 1H), 7.85 (d, J = 6.3 Hz, 1H), 8.43 - 8.46 (m, 2H), 8.56 (d, J = 6.0 Hz, 1H), 9.28 (s, 1H); ^{13}C NMR (DMSO-d_6): 24.52, 106.40, 117.75, 120.43, 121.84, 129.72, 131.64, 135.75, 138.86, 140.39, 148.44, 157.67, 159.84, 161.71, 164.28, 168.96.

4.2.25. General procedure for the synthesis of compounds 25a–d

A mixture of the appropriate amine **24a–d** (0.34 mmol) and 3,5-bis(trifluoromethyl)phenyl isocyanate (173 mg, 0.68 mmol) was heated without solvent on an oil bath at 120 °C for 2 h. The crude product was then purified by the suitable method to yield pure **25a–d**.

4.2.25.1. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-(6-(2-acetylphenyl)pyridin-3-yl)pyrimidin-2-yl)urea (25a). The residue was dissolved in methylene chloride and purified by column chromatography (silica gel, ethyl acetate/hexane, 1:1, v/v). Yield (120 mg, 65%); mp 226–227 °C; IR ν/cm^{-1}: 3092, 2963, 2925, 2853, 1724, 1695, 1584, 1410, 1387, 1286, 1185, 1126, 877, 833, 742; ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 3H), 7.58–7.64 (m, 3H), 7.77 (s, 1H), 7.81 (d, J = 7.2 Hz, 1H), 7.90 (d, J = 5.4 Hz, 1H), 7.98 (t, J = 8.4 Hz, 1H), 8.33–8.36 (m, 2H), 8.67 (d, J = 8.4 Hz, 1H), 8.84 (d, J = 5.1 Hz, 1H), 9.38 (s, 1H), 10.62 (s, 1H), 11.97 (s, 1H).

4.2.25.2. 1-(4-(6-(2-Acetylphenyl)pyridin-3-yl)pyrimidin-2-yl)-3-(3-(trifluoromethyl)-5-morpholinophenyl)urea (25b). The residue was stirred in ethyl acetate (10 mL) for 20 min, and the undissolved solid was filtered and washed with ethyl acetate (10 mL) to yield pure **25b**. Yield (126 mg, 66%); mp 238–239 °C; IR ν/cm^{-1}: 3092, 2968, 2917, 2856, 1713, 1687, 1619, 1586, 1568, 1447, 1416, 1239, 1118, 838, 741; ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 3H), 3.17 (s, 4H), 3.67 (s, 4H), 6.91 (s, 1H), 7.24 (s, 1H), 7.60–7.86 (m, 6H), 7.97 (d, J = 8.2 Hz, 1H), 8.62 (d, J = 7.8 Hz, 1H), 8.81 (d, J = 5.0 Hz, 1H), 9.36 (s, 1H), 10.41 (s, 1H), 11.68 (s, 1H); ^{13}C NMR (DMSO-d_6): 30.82, 48.21, 66.31, 106.29, 108.72, 111.60, 123.03, 127.95, 129.35, 129.70, 129.88, 130.45, 130.65, 130.84, 136.26, 137.88, 140.70, 142.08, 148.21, 151.99, 152, 42, 158.44, 159.63, 159.80, 162.26, 203.27.

4.2.25.3. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-(6-(2-acetamidophenyl)pyridin-3-yl)pyrimidin-2-yl)urea (25c). The residue was crystallized from DMSO to yield pure **25c**. Yield (80 mg, 42%); mp 284–285 °C; IR ν/cm^{-1}: 3440, 3231, 3088, 2971, 1726, 1682, 1585, 1413, 2388, 1287, 1181, 1124, 879, 757, 553; ^{1}H NMR (DMSO-d_6): δ 2.05 (s, 3H), 7.28 (t, J = 7.3 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.73 (s, 1H), 7.83 (d, J = 7.6 Hz, 1H), 7.91 (d,

J = 5.3 Hz, 1H), 8.05–8.13 (m, 2H), 8.34 (s, 2H), 8.74 (d, J = 7.0 Hz, 1H), 8.85 (d, J = 5.2 Hz, 1H), 9.51 (s, 1H), 10.56 (s, 1H), 11.32 (s, 1H), 11.89 (s, 1H).

4.2.25.4. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-(6-(3-acetamidophenyl)pyridin-3-yl)pyrimidin-2-yl)urea (25d). The residue was crystallized from DMSO to yield pure **25d**. Yield (86 mg, 45%); mp >300 °C; IR ν/cm^{-1}: 3094, 2971, 2923, 1719, 1534, 1473, 1412, 1387, 1280, 1175, 1134, 880, 795, 683, 554; ^{1}H NMR (DMSO-d_6): δ 2.09 (s, 3H), 7.46 (t, J = 8.0 Hz, 1H), 7.73–7.76 (m, 2H), 7.82 (d, J = 7.3 Hz, 1H), 7.90 (d, J = 5.3 Hz, 1H), 8.10 (d, J = 8.4 Hz, 1H), 8.35 (s, 2H), 8.45 (s, 1H), 8.66 (d, J = 6.8 Hz, 1H), 8.84 (d, J = 5.3 Hz, 1H), 9.46 (s, 1H), 10.19 (s, 1H), 10.63 (s, 1H), 12.02 (s, 1H).

4.2.26. General procedure for the synthesis of compounds 26a and 26b

A mixture of the appropriate amine **24a** and **24b** (0.34 mmol), 3,5-bis(trifluoromethyl)phenylbromide (111 mg, 0.38 mmol), dichlorobis(triphenylphosphine)Pd(II) (24 mg, 0.034 mmol), Xantphos (20 mg, 0.034 mmol) and sodium *tert*-butoxide (50 mg, 0.52 mmol) was refluxed in toluene (7 mL) under nitrogen atmosphere for 12 h. The reaction was filtered while hot to remove the insoluble metal impurities. The toluene filtrate was left to cool at room temperature, and then cooled in an ice bath. The formed solid was filtered, washed with cold toluene (10 mL), then with water (50 mL).

4.2.26.1. 1-(2-(5-(2-(3,5-Bis(trifluoromethyl)phenylamino)pyrimidin-4-yl)pyridin-2-yl)phenyl)ethanone (26a). Yield (135 mg, 79%); mp 197–199 °C; IR ν/cm^{-1}: 3464, 3269, 2075, 3002, 1694, 1571, 1554, 1439, 1384, 1276, 1189, 1131, 786, 680, 594; ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 3H), 7.48–7.62 (m, 4H), 7.73 (d, J = 5.2 Hz, 1H), 7.84 (d, J = 7.3 Hz, 1H), 7.99 (d, J = 8.3 Hz, 1H), 8.61–8.63 (m, 3H), 8.74 (d, J = 5.2 Hz, 1H), 9.35 (s, 1H), 10.52 (s, 1H).

4.2.26.2. 1-(4-(5-(2-(3,5-Bis(trifluoromethyl)phenylamino)pyrimidin-4-yl)pyridin-2-yl)phenyl)ethanone (26b). Yield (118 mg, 69%); mp >300 °C; IR ν/cm^{-1}: 3473, 3356, 1684, 1586, 1555, 1477, 1460, 1437, 1385, 1171, 1124, 815; ^{1}H NMR (DMSO-d_6): δ 2.63 (s, 3H), 7.61 (s, 1H), 7.73 (d, J = 5.2 Hz, 1H), 8.07 (d, J = 8.3 Hz, 2H), 8.25 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 8.3 Hz, 2H), 8.59–8.62 (m, 3H), 8.73 (d, J = 5.1 Hz, 1H), 9.46 (s, 1H), 10.51 (s, 1H).

4.3. HCl-salt formation

The free bases **15b**, **19**, **25c**, and **25d** were changed to the corresponding HCl-salts **27**, **28**, **29**, and **30**, respectively, according to the following procedure: The appropriate starting free base **15b**, **19**, **25c**, and **25d** (0.15 mmol) was dissolved in dry toluene (10 mL) by heating and stirring under reflux. After complete dissolution of the compound, refluxing was stopped, and the solution was saturated with hydrochloride gas while stirring is maintained for 30 min. The resulted precipitate (HCl-salt) was filtered, washed throughly with toluene (50 mL) and dried.

4.4. Cell line screening

Cell line screening was applied at the National Cancer Institute (NCI), Bethesda, Maryland, USA.[20] applying the following procedure. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μl at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-fold or ½log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

- $[(Ti-Tz)/(C-Tz)] \times 100$ for concentrations for which $Ti \geqslant Tz$.
- $[(Ti-Tz)/Tz] \times 100$ for concentrations for which $Ti<Tz$.

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (IC_{50}) is calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti-Tz)/Tz] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

4.5. Enzyme screening

Kinase assays were performed at Reaction Biology Corporation using the 'HotSpot' assay platform.[21] Kinase Assay Protocol. Reaction Buffer: base Reaction buffer; 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij35, 0.02 mg/mL BSA, 0.1 mM Na_3VO_4, 2 mM DTT, 1% DMSO. Reaction procedure: To a freshly prepared buffer solution was added any required cofactor for the enzymatic reaction, followed by the addition of the selected kinase at a concentration of 20 μM. The contents were mixed gently, and then

the compound under test (compound **30**) dissolved in DMSO was added to the reaction mixture in the 10 μM concentration. 339-ATP (specific activity 500 μCi/μl) was added to the mixture in order to initiate the reaction, and the mixture was incubated at room temperature for 2 h. Staurosporine was used as a control compound in a five-dose IC_{50} mode with 10-fold serial dilutions starting at 20 μM, and the reaction was carried out at 10 μM ATP concentration.

4.6. Molecular modeling

Docking studies were performed using 'Molecular Operating Environment (MOE) version 2008.10', Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, H3A 2R7, Canada. Compounds **29** and **30** were built using the builder interface of the MOE program and subjected to energy minimization using the included Forcefield MMFF94x calculations. The X-ray crystallographic structure of ABL1 kinase complexed with Imatinib (PDB ID: 3K5V) was obtained from Protein Data Bank.[35] The enzyme was prepared for docking studies as follows: (i) Hydrogen atoms were added to the structure with their standard geometry. (iii) The whole protein structure containing the ligand inside was subjected to energy minimization to relax any abnormally strained parts of the structure. (iii) The two ligands **29** and **30** were docked individually into the binding site containing the Imatininb ligand, setting the receptor to 'receptor + solvent', the site to 'ligand atoms', and the pharmacophore to 'none'. Placement method was set to default 'Triangle Matcher', the first scoring function, 'Rescoring 1' was set to the default 'London dG' and the Retain dropdown was set to 10. Refinement inside the binding site was allowed using forcefield calculations so as to allow energy minimization of the docking poses inside the binding pocket. The refinement scoring function 'Rescoring 2' was set to none and the retain dropdown was set to 10. The final refined poses were ranked by the MM/GBVI binding free energy estimation, and the poses showing the lowest free energy (the most stable poses) were selected.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.037.

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