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Aminoimidazo[1,2-*a*]pyridines as a new structural class of cyclin-dependent kinase inhibitors. Part 1: Design, synthesis, and biological evaluation

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Abstract—We have identified a novel structural class of protein serine/threonine kinase inhibitors comprised of an aminoimidazo[1,2-*a*]pyridine nucleus. Compounds from this family are shown to potently inhibit cyclin-dependent kinases by competing with ATP for binding to a catalytic subunit of the protein. Structure-based design approach was used to direct this chemical scaffold toward generating potent and selective CDK2 inhibitors. The discovery of this new class of ATP-site directed protein kinase inhibitors, aminoimidazo[1,2-*a*]pyridines, provides the basis of new medicinal chemistry tool in search for an effective treatment of cancer and other diseases that involve protein kinase signaling pathways.

The primary function of the cell cycle is to manage the process of replication of DNA.[1–4] Alterations in the key regulatory elements that control the phases of the cell cycle often result in uncontrolled proliferation, the basis of neoplastic disease.[5] The basic cell cycle is divided into four phases, known as G_1, S, G_2, and M. When cells cease proliferation, they exit the cycle and enter a nondividing, quiescent state known as G_0.

Cyclin-dependent kinases (CDKs) are the key players in the control of this complex process. Family members of this class of serine/threonine kinases include at least nine CDKs and over 12 different cyclin families. The most relevant enzymes for cell cycle control are CDK4 and CDK6 (involved in early G_1), CDK2 (required to complete G1 and initiate S phase), and CDK1 (controls the G_2 checkpoint and regulates entry into mitosis).

Because of the CDKs critical role in the regulation of cell cycle and the observed expression/activity pattern in most human cancers, considerable effort has been focused on the development of small molecule inhibitors[6,7] that block CDK activity.[8–17] However, the number of structural classes that act as CDKs inhibitors is limited and most of them derive from relatively nonspecific protein kinase inhibitor scaffolds that also inhibit CDKs such as staurosporins,[18] flavonoids,[19] indigoids,[20] paullones,[21] and purines.[22] Flavopiridol,[23] a flavonoid derived from an indigenous plant from India, was the first CDK modulator tested in clinical trials. Early-phase trials have shown activity in some patients with non-Hodgkin's lymphoma, renal, prostate, colon, and gastric carcinomas.[24]

We have recently showed[25] that 2-aminoimidazo[1,2-*a*]-pyridine scaffold **1** represents a new structural class of protein serine/threonine kinase inhibitors. These kind of compounds potently inhibit cyclin-dependent kinases by competing with ATP for binding to a catalytic subunit of the protein. The initial lead identification,

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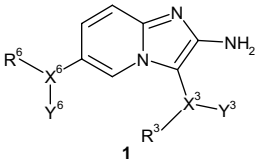
structure-based analog design, kinase inhibition data, and X-ray crystallographic structures of CDK2/inhibitor complexes were reported. We now disclose a detailed SAR study of 2-aminoimidazo[1,2-*a*]pyridines around substituents 3 and 6 of the aromatic bicyclic nucleus.



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Our initial research effort was directed to create a diversity of substituents at positions 3 and 6 of the imidazo[1,2-*a*]pyridine nucleus. The approaches for the synthesis of molecules described in this letter are based on two different routes: one makes use[26] of the reaction of 2-chloropyridines **2** with cyanamide **3** and α-bromoacetophenones **4** (route A, Scheme 1), while the other focuses on the selective and sequential functionalization[27] of 2-trifluoroamido-6-iodoimidazo[1,2-*a*]pyridine **5**[28] (route B, Scheme 2).

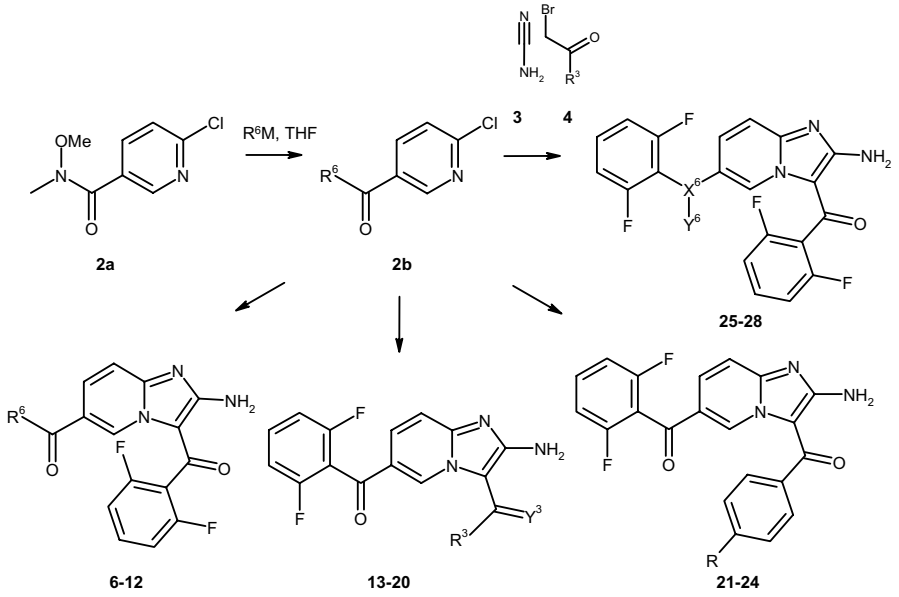
Route A started (Scheme 1) from 2-chloronicotinic acid's Weinreb amide **2a**,[28] which was provided with the adequate functionality at R^6 by treatment with the appropriate aryl lithium reagent, to give ketones **2b**. Targets **6**–**29** were directly prepared from **2b** using our one-pot procedure[26] for the formation of the 2-aminoimidazo[1,2-*a*]pyridine nucleus, by effecting chloride atom displacement with cyanamide, followed by *N*-alkylation with bromoacetophenone, and final ring-closure to target compounds.

On the other hand, route B was based (Scheme 2) on the stepwise introduction of substituents at positions 6 and 3. Halogen–metal exchange of **5**,[27] followed by reaction with the appropriate aryl aldehyde, gave alcohols **29**, bearing the desired R^6 functionality. Further protection of alcohol functional group, and then metalation at position 3, afforded, by reaction of the required electrophile, 3,6-disubstituted 2-aminoimidazo[1,2-*a*]pyridines, which were transformed into targets **30**–**41** by subsequent deprotection of the silyl ether, oxidation of the resulting alcohol, and final trifluoroacetamide removal.

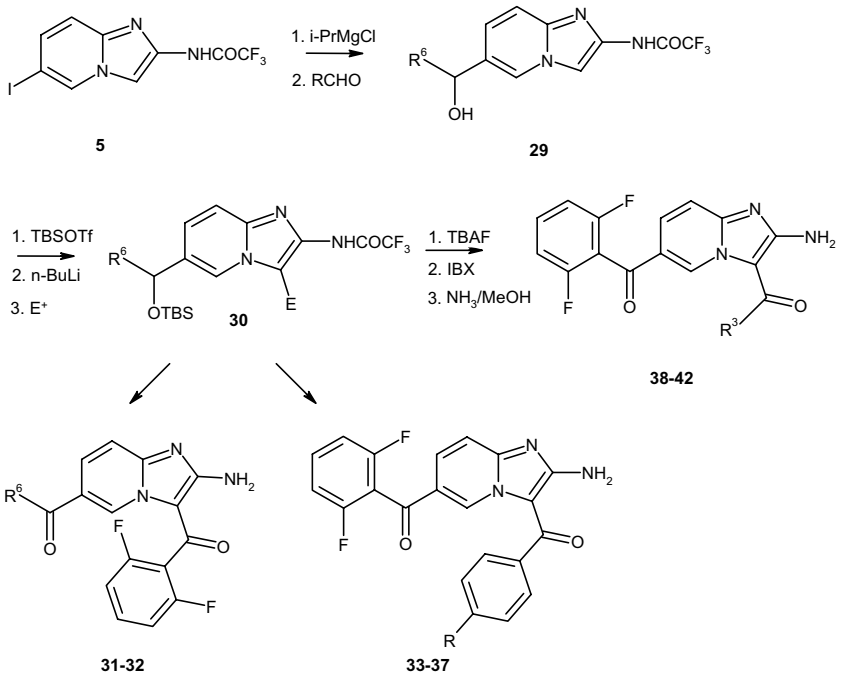
Our initial lead (**14**) proved to be quite potent as CDK2 inhibitor, and it was proved that its inhibitory activity was effected to competing with ATP for binding to catalytic subunit of the protein.[25] Initial SAR studies around R^6 (Table 1) were focused to determine the best aryl substitution pattern, and it was found that the presence of at least one substituent at position 2 was necessary to keep the potency below 1 μM. Disubstitution with electron-withdrawing functional groups at positions 2 and 6 provided the most active molecules and, among them, fluorine and chlorine (compounds **14**, **12**, and **31**) proved to be the best. The use of other functional groups, or substitution at other positions of the aryl ring, led to a significant loss in activity.

We next studied position 6 (Table 2) to map the optimal spacer X^6–Y^6 between phenyl and imidazopyridine rings. The presence of an sp^2 was shown to be critical to maintain activity. Carbonyl, vinyl, or *Z*-configurated cyanovinyl substituents, were equally efficient, but activity was partially lost when either *E*-configurated olefins (**27**) or bulkier Y^6 such as tetrazole (**28**) substituents were introduced.

SAR around R^3–Y^3 (Table 3) was performed taking into consideration that the presence of a Y^3 substituent bearing a lone pair might lead to a conformational change in the molecule, by the formation of a hydrogen acceptor

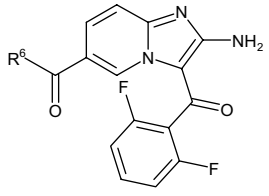


Scheme 1. Synthesis of 2-aminoimidazo[1,2-*a*]pyridines (route A).



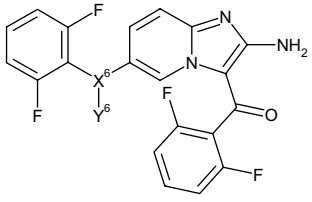
Scheme 2. Synthesis of 2-aminoimidazo[1,2-*a*]pyridines (route B).

Table 1. Enz. assay (IC_{50}) CDK2[29]



Compound	R^6	IC_{50} (nM)
14	2,6-Di-F–Ph	122
6	Ph	1927
7	2-Cl–Ph	428
8	2-CF_3–Ph	1068
9	2-Me–Ph	1294
10	2-MeO–Ph	603
11	2-CF_3O–Ph	2171
12	2,6-Di-Cl–Ph	53
31	2-Cl–6-F–Ph	33

Table 2. Enz. assay (IC_{50}) CDK2[29]

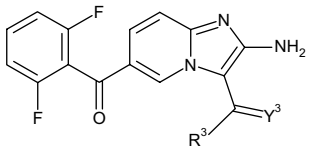


Compound	X^6–Y^6	IC_{50} (nM)
14	C=O	122
25	$C=CH_2$	63
26	(*Z*)-C=CHCN	160
27	(*E*)-C=CHCN	896
28	(*E*,*Z*)-C=CH(1*H*-tetrazol-5-yl)	1691

with one of the hydrogen atoms at *N*-2.[30] On the other hand, according to our modeling and X-ray studies,[25] this part of the molecule would need to fit in a narrow, hydrophobic pocket, and, therefore, a profound effect on activity should be expected in this region. It was found that substitution at positions 2 with electron-donating methoxy (**38**), or at positions 2 and 6 electron-withdrawing fluorine and chlorine (**14**, **42**, and **15**) functional groups, provided the most active molecules. In this regard, we also found that, when X was a sulfur atom, a fivefold improvement in activity was obtained with respect to the oxygenated analog, but only when a 2,6-disubstitution pattern containing electron-withdrawing groups (**18** and **20**) was present. On the other hand, it was also discovered that subtle changes in R^3 had a profound effect in the activity of the molecule. In this case, the presence of an electron-donating group at position 4 was well tolerated (**16** and **20**). We took advantage of this to introduce potentially solubilizing groups in the molecule.

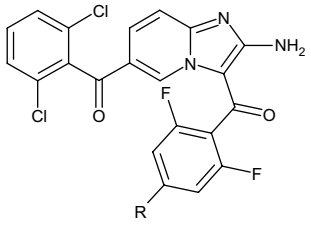
For the introduction of potentially solubilizing side chains at 4-position of R^3 (Table 4), 2,6-dichlorophenyl substitution was used as R^3. It was found, as we had expected, that only small substituents (**21**, **36**, and **37**) would fit in the molecule without losing activity; bulkier functional groups showed reduced activity (**22** and **23**). On the other hand, the nature of the substituent seemed to have little effect on the activity.

Table 3. Enz. assay (IC_{50}) CDK2[29]



Compound	R^3	Y^3	IC_{50} (nM)
13	Ph	O	324
14	2,6-Di-F–Ph	O	122
38	2-MeO–Ph	O	68
39	2-Thienyl	O	547
40	4-Cl–Ph	O	1711
41	4-Piridyl	O	2538
42	2,6-Di-Cl–Ph	O	121
15	2-Cl–6-F–Ph	O	102
16	2,6-Di-F–4-MeO–Ph	O	91
17	Ph	S	214
18	2,6-Di-F–Ph	S	26
19	2,6-Di-MeO–Ph	S	462
20	2,6-Di-F–4-MeO–Ph	S	29

Table 4. Enz. assay (IC_{50}) CDK2[29]



Compound	R	IC_{50} (nM)
21	OH	52
22	$OCH_2CH_2NEt_2$	984
23	OCH_2CH_2(4-methylpiperazine)	812
36	CO_2H	95
37	$CONH_2$	46

Table 5. Selectivity enz. assay (IC_{50}) with different kinases[29]

Compound	CDK2 IC_{50} (nM)	CDK4 IC_{50} (nM)	CDK1 IC_{50} (nM)
14	122	1081	57
31	33	810	104
32	53	693	106
25	63	1033	93
26	160	1553	53
28	1691	1703	96
13	324	5053	326
38	68	1973	475
42	121	359	209
15	102	827	239
16	91	838	132
17	214	1894	165
18	26	786	105
20	29	464	143
33	52	455	81
36	95	2589	328
37	46	1144	154

We finally studied the selectivity profile of selected compounds against a variety of kinases. The results are summarized in Table 5. Most compounds proved to be selective for CDKs versus PKA, CAMKII, and GSK-3β, with selectivity ranging from 4 to >400-fold. Among the CDKs, all compounds showed less activity against CDK4 than against either CDK1 or CDK2; the best selectivity for CDK1 versus CDK2 was observed for **14**, **26**, and **28** (2–18-fold), while CDK2 versus CDK1 selectivity was achieved with **38**, **31**, **18**, **20**, and **37** (3–7-fold). In this sense, **20** was >5-fold selective for CDK2 versus CDK1 and CDK4, and >100-fold selective for CDK2 versus PKA, CAMKII, and GSK-3β. Overall, these results provided us with useful tools for the preparation of either selective or dual CDK inhibitors.

In summary, we have found a new structural class of protein serine/threonine kinase inhibitors based on the novel 2-aminoimidazo[1,2-*a*]pyridine core. Compounds from this family are shown to potently inhibit either CDK1 or CDK2 by competing with ATP for binding to catalytic subunit of the protein. The discovery of this new class of ATP-site directed protein kinase inhibitors, aminoimidazo[1,2-*a*]pyridines, provides a new medicinal chemistry tool in the search for an effective treatment of cancer and other diseases that involve protein kinase signaling pathways.

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29. See Ref. 25 for details.
30. Full results of this study will be reported elsewhere.