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Synthesis and potent cytotoxicity of some novel imidazopyridine derivatives against MCF-7 human breast adenocarcinoma cell line

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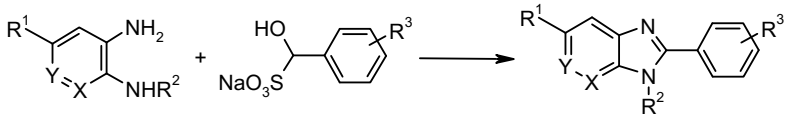


X = CH, Y = N; X = N, Y = CH

A series of novel 2-phenyl-3*H*-imidazo[4,5-*b*]pyridines and 2-phenyl-3*H*-imidazo[4,5-*c*]pyridines and their precursors were synthesized. Their *in vitro* cytotoxicity against MCF-7 human breast adenocarcinoma cell line has been investigated, and some of the tested compounds have shown high cytotoxic activity against MCF-7 cells. *N*-Hydroxy-4-(3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzenecarboximidamide was the most active compound with IC_{50} equal to 0.082 μM, which is an activity almost as high as that of a commonly used anticancer drugs docetaxel and imatinib mesylate.

Keywords: carboxamidine, 3*H*-imidazo[4,5-*b*]pyridine , 3*H*-imidazo[4,5-*c*]pyridine, cytotoxicity, MCF-7 cell line, steric hindrance.

Cancer is the biggest health issue in the world. Breast carcinoma is the second most common cancer in women and remains as the major cause of malignancy-associated deaths in humans. In the last 60 years, the development of novel chemotherapeutic agents has significantly progressed, however, the treatment of most types of solid tumors (e.g., breast and ovarian) is still a problem.[1] In addition, resistance against many existing anticancer drugs is developing, therefore, development of new potent, safe, and selective antitumor agents is strongly needed.

The imidazopyridine nucleus is considered as a bioisostere to the purine nucleus and easily interacts with large molecules such as DNA, RNA, or some proteins.[2] Hence imidazopyridines exhibit promising cytotoxic activity by inhibiting some kinase enzymes. As it is well known, kinases are involved in cellular signaling pathways that control various cellular functions, including cell division, growth, metabolism, differentiation, and survival, through reversible phosphorylation of the hydroxyl groups of tyrosine residues in proteins.[3] Cyclin-dependent kinases (CDKs) are the major regulators of the cell cycle and transcription. Inhibition of this enzyme by a series of imidazo-[4,5-*b*]pyridine and 4-heteroarylpyrimidine derivatives as anticancer agents has been reported.[4] Bavetsias et al.[5–8] reported the identification of the novel imidazo[4,5-*b*]-pyridine derivatives as potent inhibitors of some Aurora kinases. Aurora proteins A, B, and C, a small family of serine/threonine kinases, play distinct role in the regulation of mitosis. In recent years, these proteins have been actively pursued as targets for the discovery of new anticancer chemotherapeutics. As a result, several simple inhibitors of Aurora kinases identified and some of them have reached clinical evaluation.[5–8] Furthermore, very recently some imidazopyridine derivatives were published as selective potent inhibitors of TANK-binding kinase inhibitors (TBK1).[9,10] In another study, the discovery of new imidazo[4,5-*b*]pyridine derivatives, potent, highly selective inhibitors of CDK4/6 kinases, was reported,[11] in addition potent c-Met kinase inhibitory activity was also reported for new analog of 3*H*-imidazo[4,5-*b*]pyridine[12] (Fig. 1).

Prompted by the findings from the studies mentioned above, we set out to develop and synthesize novel imidazo[4,5-*b*]- or imidazo[4,5-*c*]pyridine derivatives and investigate their inhibitory activity against the MCF-7 cell line. It is well known that carboxamides, carboxamidines, and combinations of both are present in the structure of a variety of antitumor agents, such as DNA minor groove-

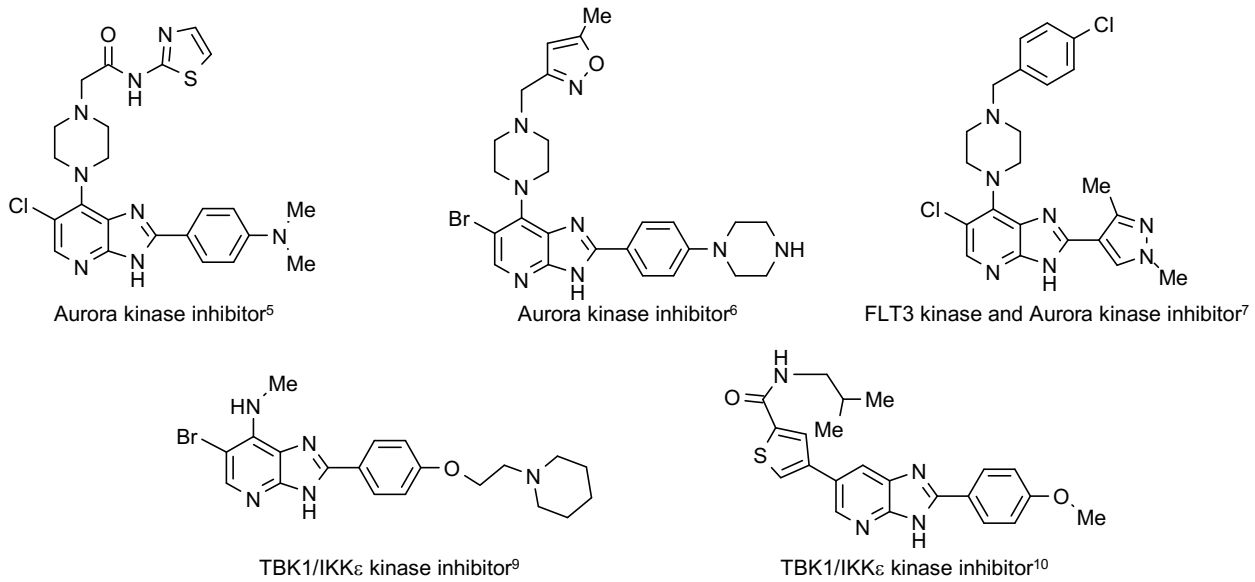


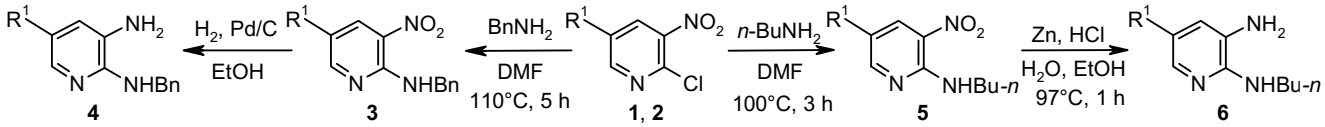
Figure 1. Previously reported imidazo[4,5-*b*]pyridines possessing anticancer activity.

binding agents netropsin, DAPI, distamycin.[13–15] In continuation[16–18] of our efforts in search of potent molecules exhibiting antimicrobial and anticancer activities, we have synthesized carboxamidine and carboxamidoxime derivatives of imidazopyridine and tested their anticancer activity, which we wish report in this paper.

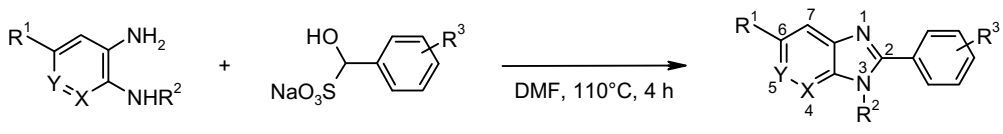
By converting chlorine atom in 2-chloro-3-nitropyridine (**1**) and 5-bromo-2-chloro-3-nitropyridine (**2**) to alkylamino group in an aromatic nucleophilic substitution reaction, and the subsequent reduction of the nitro group in compounds **3** and **5**, pyridinediamines **4** and **6** were prepared (Scheme 1) which, like the commercially available compounds **7**–**10**, served as starting compounds for the synthesis of imidazopyridines **11**–**26**. The latter were obtained by the condensation of the respective substituted pyridinediamines **4**,**6**–**10** with the corresponding $Na_2S_2O_5$-adducts of various arylaldehydes in DMF (Scheme 2).[19]

The attemt to apply the general method to the reaction of diamine **4** with sodium bisulfite adduct of 2-formylbenzenesulfonic acid failed to produce the expected imidazopyridine derivative. Using harsher conditions (130°C, 7 h) resulted in 2,3-dihydro intermediate **27** and the expected aromatic product **28** as a mixture having similar peak areas in HPLC-MS chromatogram, but neither of the two compounds could be isolated (Scheme 3). Probably, because of the strong steric congestion between SO_3H and benzyl groups, the conversion of intermediate **27** has not been complete. Hence, in order to obtain only compound **27**, the same reaction was carried out under milder conditions (in EtOH, at 75°C). This time, the mixture of three compounds

Scheme 1



Scheme 2



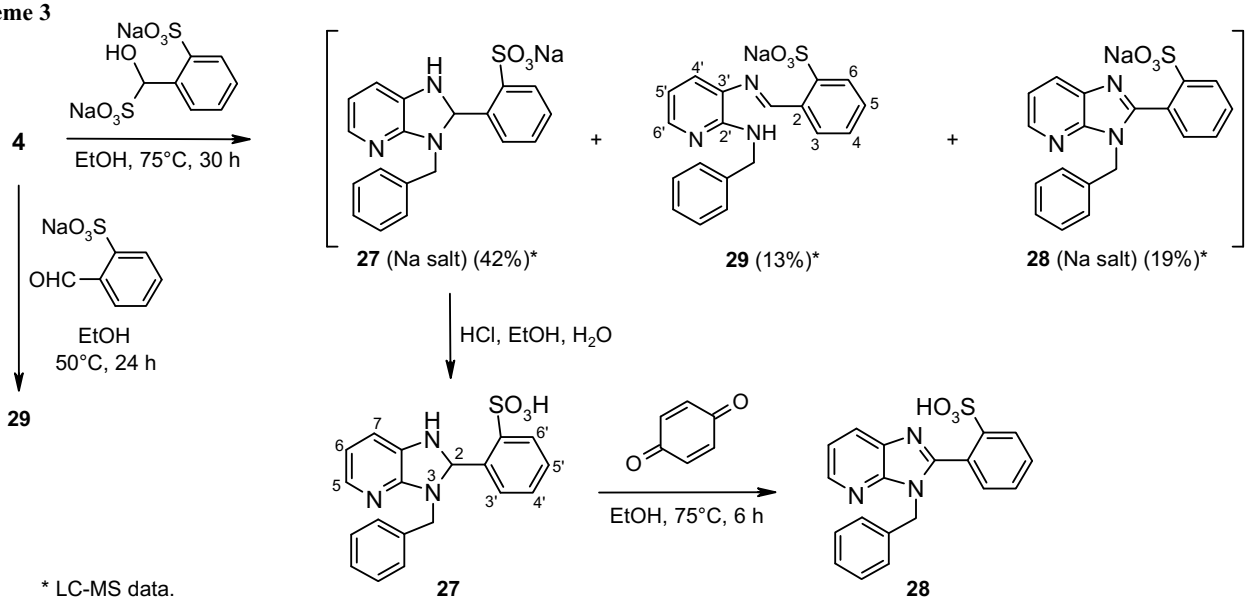
	X	Y	R^1	R^2
7	N	CH	H	H
8	CH	N	H	H
9	N	CH	Cl	H
10	N	CH	Br	H

	X	Y	R^1	R^2	R^3
11	N	CH	H	H	2-COOH
12	CH	N	H	H	2-COOH
13	N	CH	H	H	3-COOH
14	CH	N	H	H	3-COOH
15	N	CH	H	H	4-COOH
16	N	CH	Cl	H	4-COOH

	X	Y	R^1	R^2	R^3
17	N	CH	H	Bn	3-COOH
18	N	CH	H	Bn	2-COOH
19	N	CH	H	H	3-SO_3H
20	N	CH	Br	H	2-SO_3H
21	N	CH	H	Bn	3-SO_3H
22	N	CH	Br	*n*-Bu	2-SO_3H

	X	Y	R^1	R^2	R^3
23	N	CH	H	H	4-CN
24	CH	N	H	H	4-CN
25	N	CH	H	H	3-CN
26	N	CH	H	Bn	4-CN

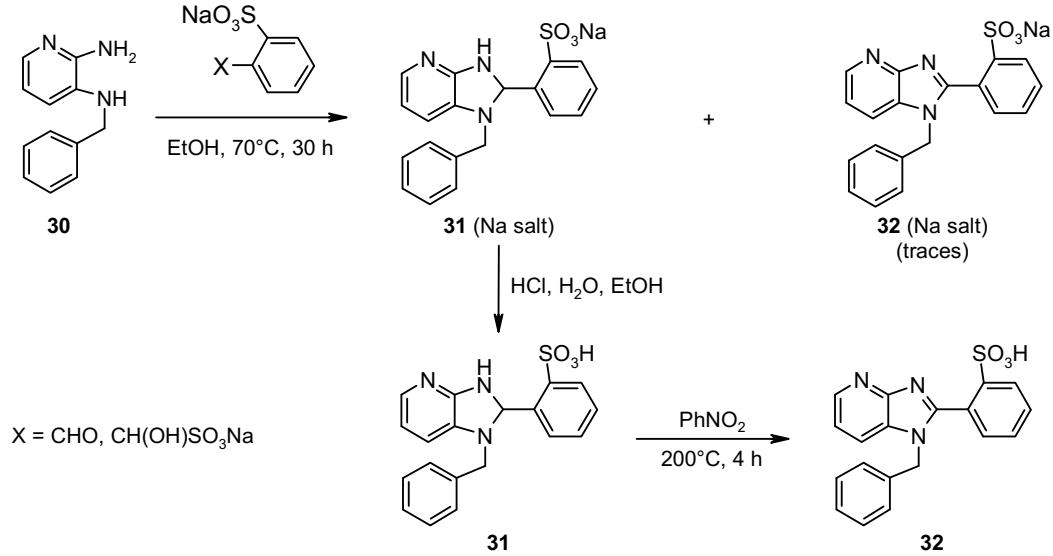
Scheme 3



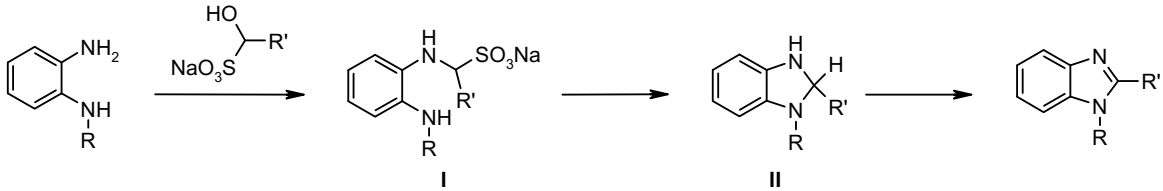
27 and **28** (both in form of sodium salt), and an open-chain Schiff base **29** has been identified chromatographically. Since compound **27** was the major product, it has been isolated by crystallization from the reaction medium; the other minor products could not be isolated. Therefore, compound **29** was prepared separately by the reaction of compound **4** and sodium 2-formylbenzenesulfonate only for the purpose of identification. Compound **27** was converted to compound **28** by using 1,4-benzoquinone as the aromatizing agent.

When we have attempted to carry out the reaction between compound **30**[20] and sodium 2-formylbenzenesulfonate or its bisulfite adduct (Scheme 4), only compound **31** and a trace amount of compound **32** have been observed, but not the corresponding Schiff base. As an mechanistic alternative to the involvement of Schiff base in the metabisulfite-mediated reaction, Ridley et al.[19] suggested that it proceeds by way of formation of an α-aminosulfonic acid derivative **I** (Scheme 5), the product normally formed in the condensation of amines and aldehyde

Scheme 4



Scheme 5



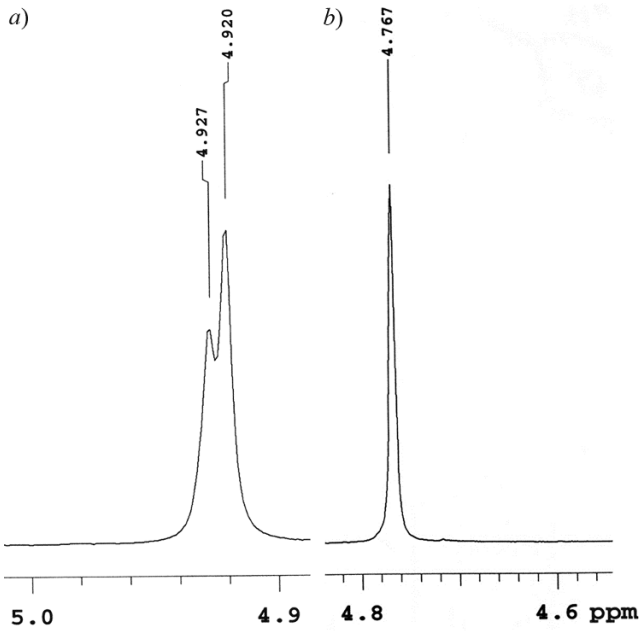


Figure 2. A fragment of the ^{1}H NMR spectrum of compound **18** at (*a*) rt and (*b*) 42°C.

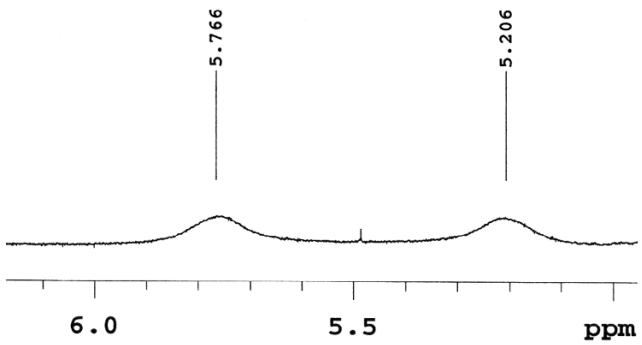


Figure 3. A fragment of the ^{1}H NMR spectrum of compound **28**.

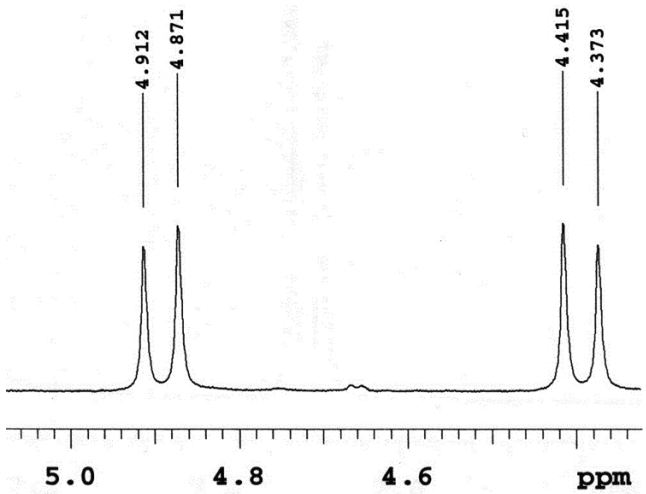


Figure 4. A fragment of the ^{1}H NMR spectrum of compound **27**.

bisulfite adducts, followed by the intramolecular nucleophilic displacement of sodium sulfite with the closure of imidazole cycle giving benzimidazoline **II**. Compound **31** was isolated in good yield, the corrresponding monoimine (Schiff base) has not detected, and a trace amount of pyridoimidazole derivative **32** (as sodium salt) has been identified in LC-MS chromatogram. In order to obtain compound **32** preparatively, nitrobenzene has been used as the aromatizing agent instead of 1,4-benzoquinone, since some unexpected impurities have been occured using 1,4-benzoquinone. Our results suggest that both mechanisms described above could be possible depending on the properties of the starting pyridinediamines.

In compounds **18**, **22**, **28**, and **32**, the steric hindrance between the benzylic methylene group and the COOH and SO_3H groups of the *o*-substituted 2-phenyl moiety was confirmed by their ^{1}H NMR spectra. In the spectrum of compound **18** (in CD_3OD at room temperature), benzylic protons are observed as two overlapping singlets in contrast to expected as one singlet (Fig. 2*a*). However, when the temperature was raised to 42°C the two signals coalesced into one (Fig. 2*b*). In the same solvent, benzylic protons of compound **28** have been seen separately as broad singlets at 5.21 and 5.77 ppm (Fig. 3), and these peaks did not merge even at 40°C. Finally, in the spectrum of 2,3-dihydro derivatives **27** and **31** these protons were observed as two sharp doublets (Fig. 4). (This shows us that SO_3H group causes greater steric hindrance than COOH group.)

The analogous reaction of *o*-phenylenediamine with aldehydes, yielding benzimidazoles, has been investigated using NMR spectroscopy.[21] It has been established that the reaction begins with the formation of monoimines (isolated in condensations with some aromatic aldehydes) which are cyclized to the corresponding benzimidazolines, and these compounds are converted into 2-substituted benzimidazoles. The structures of monoimines and benzimidazolines were proved unequivocally by the presence of a signal for the resonance of the azomethine and the 2-CH proton of appropriate intensity at 8.0–8.5 and 5.0–6.0 ppm, respectively.[21] The corresponding proton signals in the isomeric structures **29** and **27** have been observed at 9.60 and 7.69 ppm, respectively. However, the chemical shift value of the 2-CH in benzimidazolines is highly dependent on the NMR solvent used, e.g., the 2-CH signal of 2-phenylbenzimidazoline has been observed at 5.6 ppm in DMSO-d_6[21] and at 8.4 ppm in CCl_4.[22] In another study, monoimines and benzimidazolines have been shown exitisting in a tautomeric equilibrium[23] or they have been postulated as sequential intermediates in the synthesis of benzimidazoles from hydrazones.[24]

No added oxidizer was needed to obtain compounds without steric congestion (**11**–**26**), since the aromatic cyclization (rapid dehydrogenation) is taking place spontaneously (the oxidizer probably being O_2 from air or bisulfite anion[19]) with sodium metabisulfite adducts of aldehydes. However, under same conditions it was not possible to obtain compounds **28** and **32**. This means that, the more bulky SO_3H group, but not carboxyl group (in compound **18**), inhibits the last step of the reacion because

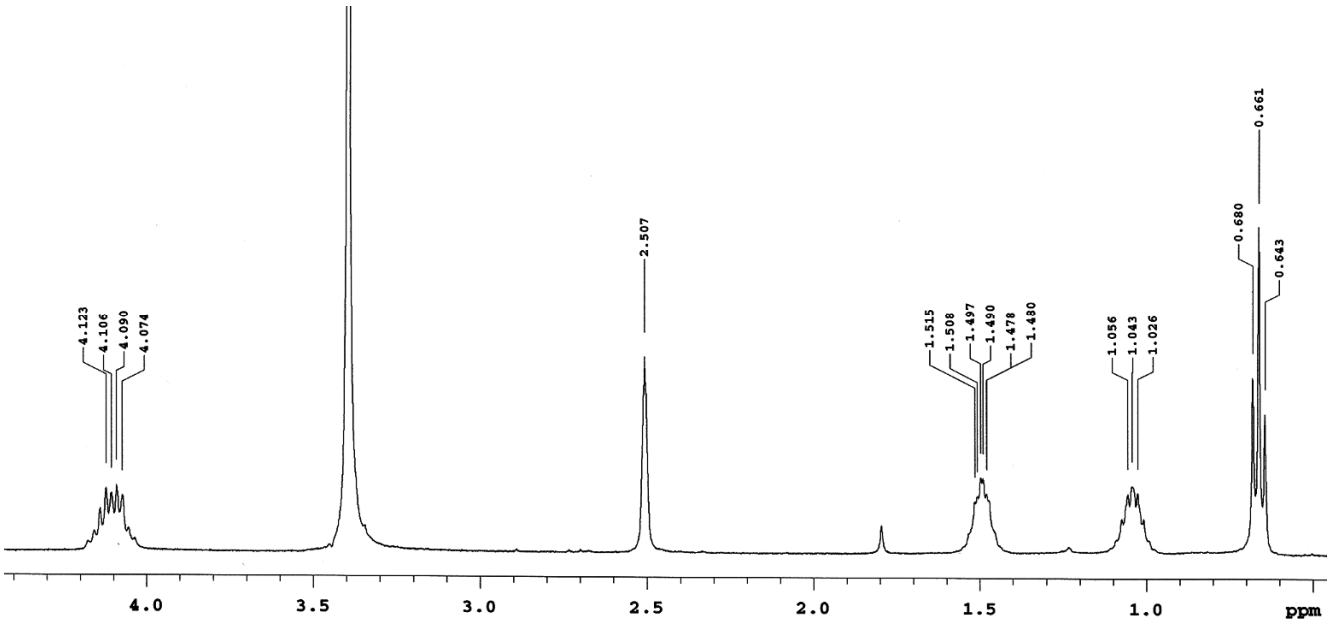


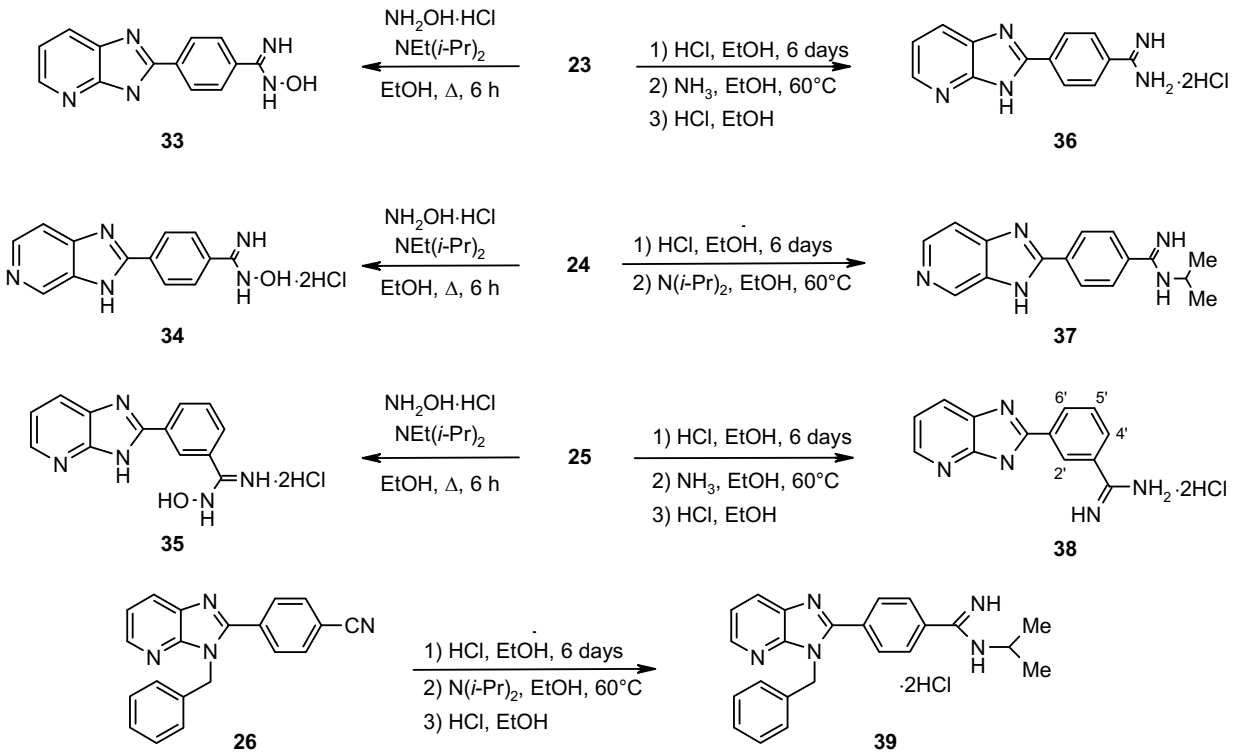
Figure 5. The aliphatic part of the ^{1}H NMR spectrum of compound **22** in DMSO-d_6.

of its steric effect. However, a similar problem has not been observed in the synthesis of compound **22**, since there is less steric congestion between SO_3H and butyl groups than between SO_3H and benzyl groups. Nevertheless, in the ^{1}H NMR spectrum of compound **22** (in DMSO-d_6), *N*-methylene protons of the *n*-butyl substituent are observed as a multiplet instead of the expected triplet (Fig. 5).

As a further step of functionalization, the cyano group of compounds **23**–**25** was converted to amidoxime group using hydroxylamine hydrochloride producing compounds **33**–**35** (Scheme 6). The cyano group of compounds **23**–**26** was also converted, using the Pinner method,[25] to the imidate esters which were not purified and characterized due to their instability, but were instead reacted with ammonia or isopropylamine to yield the corresponding carboximidamides **36**–**39**.

The antiproliferative activity of compounds **11**–**22**, **28**, **33**–**39** was assessed against MCF-7 human breast cancer

Scheme 6



cells using the so-called 48h-MTT cytotoxicity assay,[26,27] and the results are summarized in Table 1. Among the first series of compounds **11**–**22**, **28**, only compound **28** showed a limited activity with the IC_{50} value of 0.907 μM towards the MCF-7 cell line. To investigate the effect of carboximidamide and *N*-hydroxycarboximidamide groups, we tested a series of imidazopyridines **33**–**39**. It is noteworthy that almost all of them exhibited potent cytotoxic activity against the MCF-7 cell line. Among them, compound **33** showed the highest activity with the IC_{50} value of 0.082 μM (Table 1). This means that *N*-hydroxycarboximidamide group presents the best inhibitory profile. Similarly, compounds **34** (IC_{50} 0.097 μM) and **35** (IC_{50} 0.100 μM) containing *N*-hydroxycarboximidamide group displayed potent cytotoxic activity. *N*-Alkyl substitution of carboxamidine group and imidazole moiety, caused a dramatic reduction of the cytotoxic effect (compounds **37** and **39**). In the light of the observed promising antiproliferative activity, the mechanism of action of compounds **33**–**35** will be evaluated later with some biochemical assays.

In summary, a new series of 2-phenylimidazopyridines have been successfully synthesized starting from 2,3- or 3,4-pyridinediamines, and sodium bisulphite and aromatic aldehydes. The structure of all new compounds has been confirmed by ^{1}H and ^{13}C NMR, mass spectroscopy, and elemental analysis. The steric interaction between the *o*-sulfo or *o*-carboxyl group with *N*-alkyl groups was discussed.

Table 1. Cytotoxicity expressed as IC_{50} of compounds **11**–**22, 28, 33**–**39** in the 48h-MTT assay

Compound	X	Y	R^1	R^2	R^3	IC_{50},* μM
11	N	CH	H	H	2-COOH	3.395
12	CH	N	H	H	2-COOH	3.492
13	N	CH	H	H	3-COOH	2.998
14	CH	N	H	H	3-COOH	0.212
15	N	CH	H	H	4-COOH	3.011
16	N	CH	Cl	H	4-COOH	1.416
17	N	CH	H	Bn	3-COOH	3.996
18	N	CH	H	Bn	2-COOH	2.604
19	N	CH	H	H	3-SO_3H	0.277
20	N	CH	Br	H	2-SO_3H	0.401
21	N	CH	H	Bn	3-SO_3H	6.221
22	N	CH	Br	*n*-Bu	2-SO_3H	3.498
28	N	CH	H	Bn	2-SO_3H	0.907
33	N	CH	H	H	4-C(=NH)NHOH	0.082
34	CH	N	H	H	4-C(=NH)NHOH	0.097
35	N	CH	H	H	3-C(=NH)NHOH	0.100
36	N	CH	H	H	4-C(=NH)NH_2	1.103
37	CH	N	H	H	4-C(=NH)NHCH$(CH_3)_2$	2.611
38	N	CH	H	H	3-C(=NH)NH_2	0.119
39	N	CH	H	Bn	4-C(=NH)NHCH$(CH_3)_2$	0.430
Docetaxel						0.013
Imatinib mesylate						0.049

* IC_{50} values were calculated from the cell growth inhibition percentages obtained with ten different concentrations.

Compounds containing *N*-hydroxycarboximidamide group at the phenyl ring showed the highest *in vitro* cytotoxic activity against human breast adenocarcinoma MCF-7 cell line. This result makes these compounds worthy of a further investigation.

Experimental

^{1}H and ^{13}C NMR spectra (400 and 100 MHz, respectively) were recorded on a Varian Mercury 400 FT spectrometer, internal standard – TMS. The NMR signals were assigned with COSY (compound **32**), HSQC (compounds **17**, **22**, **26**, **28**, **32**–**36**, **38**–**39**) and HMBC (compounds **34**, **36**) methods. To eliminate the tautomeric effect of imidazole ring in the ^{1}H NMR spectra, some of the compounds were dissolved in DMSO-d_6, a tiny amount of dry NaH and 2–3 drops of D_2O were added into the NMR tube, stirred well, and observed visually for a while. If there was any turbidity remaining, the solution was centrifuged. Mass spectra were recorded on a Waters Micromass ZQ spectrometer connected with a Waters Alliance HPLC instrument, using a C-18 column and a mixture MeCN–MeOH–0.1 % HCOOH in MeCN–H_2O, 65:10:10:15, as eluent; ionization method – ESI(+) or ESI(–). The calculated element contents were adjusted for the solvents visible in ^{1}H NMR and water, as the compounds were hygroscopic. Elemental analysis was performed on a Leco CHNS-932 instrument. Melting points were measured on an Büchi B-540 capillary melting point apparatus and were not corrected. Commercially available starting materials, reagents, and dry solvents were purchased from Aldrich-Sigma and Merck. Compound **30** was prepared according to a published procedure.[20]

***N*-Benzyl-3-nitropyridin-2-amine (3)**. A mixture of compound **1** (1.58 g, 10 mmol) and benzylamine (1.07 g, 10 mmol) in DMF (1 ml) was heated for 5 h at 110°C. The mixture was allowed to cool, and water was added. The resultant yellow precipitate was filtered off, washed with water, and crystallized from ethanol. Yield 1.35 g (59 %). Mp 80–81°C (mp 78°C (EtOH)[28]).

***N*^{2}-Benzyl-2,3-pyridinediamine (4)**. Compound **3** (0.65 g, 3.26 mmol) in ethanol (30 ml) was subjected to hydrogenation using 40 psi of H_2 and 10 % Pd/C until the uptake of H_2 had ceased. The catalyst was filtered off on a bed of Celite and washed with ethanol. The filtrate was concentrated in vacuum. The powder-like residue was used for the subsequent steps without crystallization. Yield 0.58 g (90 %). Mp 86–88°C (mp 88°C[28]).

5-Bromo-*N*-butyl-3-nitropyridin-2-amine (5). A mixture of compound **2** (1.19 g, 5.02 mmol) and *n*-butylamine (0.74 g, 10 mmol) in DMF (1 ml) was heated for 3 h at 100°C. The mixture was allowed to cool, and water was added. The resultant yellow precipitate was filtered off, washed with water, and crystallized from ethanol. Yield 0.76 g (55 %). Mp 63–64°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.96 (3H, t, *J* = 7.2, CH_3); 1.40–1.46 (2H, m, $C\underline{H}_2CH_3$); 1.62–1.67 (2H, m, $C\underline{H}_2CH_2CH_3$); 3.57–3.62 (2H, m, $NHC\underline{H}_2$); 8.20 (1H, br. s, NH); 8.41 (1H, d, *J* = 2.4, H-6); 8.51 (1H, d, *J* = 2.4, H-4). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 156.7; 151.5; 136.9; 128.1; 104.3; 41.4; 31.6; 20.4;

14.0. Mass spectrum, *m/z* (I_{rel}, %): 276 [M(^{81}Br)+H]$^+$ (98), 276 [M(^{79}Br)+H]$^+$ (100). Found, %: C 39.17; H 4.37; N 15.32. $C_9H_{12}BrN_3O_2$. Calculated, %: C 39.43; H 4.41; N 15.33.

5-Bromo-N^2-(*n*-butyl)pyridine-2,3-diamine (6). The mixture of compound **5** (0.6 g, 2.19 mmol) in 25% HCl (15 ml) and EtOH (10 ml) was stirred vigorously. Zinc dust (1.5 g, 23 mmol) was added to this mixture in several portions at room temperature. After the addition of zinc dust was completed, the mixture was heated on a water bath at 97°C for 1 h. Reaction mixture was cooled and made alkaline with 10% NaOH solution, then extracted with EtOAc. The extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Residue was purified by column chromatography (eluent *n*-hexane–AcOEt, 1:3). Yield 0.26 g (49%). Dark-purple powder. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.87 (3H, t, *J* = 7.2, CH_3); 1.31–1.39 (2H, m, C$\underline{H}_2$CH$_3$); 1.50–1.58 (2H, m, C$\underline{H}_2$CH$_2$CH$_3$); 3.20 (2H, br. s, NH_2); 3.30 (2H, t, *J* = 7.6, NHC$\underline{H}_2$); 4.04 (1H, br. s, NH); 6.86 (1H, d, *J* = 2.4, H-6); 7.68 (1H, d, *J* = 2.4, H-4). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 149.4; 139.2; 129.7; 124.0; 107.4; 41.9; 32.0; 20.5; 14.2. Mass spectrum, *m/z* (I_{rel}, %): 246 [M(^{81}Br)+H]$^+$ (98), 244 [M(^{79}Br)+H]$^+$ (100).

Synthesis of compounds 11–26 (General method). An aromatic aldehyde (6.0 mmol) was dissolved in EtOH (20 ml), and sodium metabisulfite (0.64 g, 3.37 mmol) in H_2O (3 ml) was added in portions. The reaction mixture was stirred vigorously and more EtOH was added. The mixture was kept in a refrigerator for several hours. The precipitate was filtered and dried. The mixture of the obtained sodium aryl(hydroxy)methanesulfonate (1.0 mmol) and pyridine-diamine **4**, **6**–**10** (1.0 mmol) in DMF (1–2 ml) was heated at 110°C for 4 h. The reaction mixture was cooled, poured into water, and the crude product was filtered off if it was solid, or, alternatively, it was extracted with chloroform, dried over anhydrous Na_2SO_4, evaporated, then crystallized from appropriate solvent, unless stated otherwise.

2-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)benzoic acid (11). Yield 0.065 g (27%). Mp 269–270°C (DMF–EtOH, 5:95). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 7.23 (1H, dd, *J* = 8.0, *J* = 4.8, H-6); 7.62 (1H, td, *J* = 7.6, *J* = 1.6, H-5'); 7.68 (1H, td, *J* = 7.6, *J* = 1.2, H-4'); 7.77 (1H, dd, *J* = 7.6, *J* = 1.2, H-6'); 7.83 (1H, dd, *J* = 7.6, *J* = 1.2, H-3'); 7.97 (1H, dd, *J* = 8.0, *J* = 1.6, H-7); 8.32 (1H, dd, *J* = 4.8, *J* = 1.6, H-5). Mass spectrum, *m/z* (I_{rel}, %): 240 [M+H]$^+$ (100). Found, %: C 63.34; H 4.22; N 16.56. $C_{13}H_9N_3O_2$. Calculated (with 3.6% H_2O), %: C 62.89; H 4.06; N 16.92.

2-(3*H*-Imidazo[4,5-*c*]pyridin-2-yl)benzoic acid (12). Yield 0.057 g (24%). Mp 271–272°C (DMF–EtOH, 5:95). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 7.67–7.69 (2H, m, H-4',5'); 7.80 (1H, d, *J* = 5.6, H-7); 7.88–7.91 (1H, m, H-6'); 8.04–8.06 (1H, m, H-3'); 8.38 (1H, d, *J* = 6.0, H-6); 9.01 (1H, s, H-4). Mass spectrum, *m/z* (I_{rel}, %): 240 [M+H]$^+$ (100). Found, %: C 59.87; H 4.70; N 16.44. $C_{13}H_9N_3O_2$. Calculated (with 7.7% H_2O), %: C 60.27; H 4.36; N 16.22.

3-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)benzoic acid (13). Yield 0.187 g (78%). Mp >370°C (EtOH). ^{1}H NMR spectrum (DMSO-d_6 + NaH + D_2O), δ, ppm (*J*, Hz): 7.01–7.04 (1H, m, H-6); 7.42 (1H, t, *J* = 7.6, H-5'); 7.87 (1H, d, *J* = 8.0, H-5); 7.94 (1H, d, *J* = 7.6, H-6'); 8.14 (1H, d, *J* = 4.8, H-7); 8.26 (1H, d, *J* = 7.6, H-4'); 8.77 (1H, s, H-2'). ^{13}C NMR spectrum (DMSO-d_6 + NaH + D_2O), δ, ppm: 171.1; 159.5; 157.8; 141.6; 140.5; 136.5; 133.5; 130.4; 128.4; 128.3; 128.2; 123.2; 116.1. Mass spectrum, *m/z* (I_{rel}, %): 240 [M+H]$^+$ (100). Found, %: C 64.40; H 3.78; N 17.28. $C_{13}H_9N_3O_2$. Calculated (with 1.9% H_2O), %: C 64.06; H 3.92; N 17.24.

3-(3*H*-Imidazo[4,5-*c*]pyridin-2-yl)benzoic acid (14). Yield 0.172 g (72%). Mp >370°C (EtOH). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 7.60 (1H, br. d, *J* = 4.0, H-7); 7.71 (1H, t, *J* = 7.2, H-5'); 8.09 (1H, d, *J* = 7.6, H-6'); 8.31 (1H, d, *J* = 6.0, H-6); 8.46 (1H, d, *J* = 8.0, H-4'); 8.82 (1H, s, H-2'); 8.96 (1H, s, H-4). Mass spectrum, *m/z* (I_{rel}, %): 240 [M+H]$^+$ (100). Found, %: C 61.65; H 4.14; N 16.58. $C_{13}H_9N_3O_2$. Calculated (with 5.3% H_2O), %: C 61.77; H 4.19; N 16.63.

4-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)benzoic acid (15). Yield 0.158 g (66%). Mp >370°C (EtOH). ^{1}H NMR spectrum (DMSO-d_6 + D_2O), δ, ppm (*J*, Hz): 7.28 (1H, dd, *J* = 8.4, *J* = 4.8, H-6); 8.02 (1H, br. d, *J* = 7.6, H-7); 8.08 (2H, dd, *J* = 6.8, *J* = 1.6, H-2',6'); 8.26 (2H, dd, *J* = 6.8, *J* = 1.6, H-3',5'); 8.34 (1H, br. dd, *J* = 4.4, *J* = 0.8, H-5). Mass spectrum, *m/z* (I_{rel}, %): 240 [M+H]$^+$ (100). Found, %: C 60.14; H 4.48; N 16.01. $C_{13}H_9N_3O_2$. Calculated (with 7.7% H_2O), %: C 60.27; H 4.36; N 16.22.

4-(6-Chloro-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzoic acid (16). Yield 0.126 g (46%). Mp >370°C (DMF–EtOH, 5:95). ^{1}H NMR spectrum (DMSO-d_6 + NaH + D_2O), δ, ppm (*J*, Hz): 7.68 (1H, d, *J* = 2.8, H-7); 7.87 (2H, d, *J* = 8.0, H-2',6'); 7.90 (1H, d, *J* = 2.8, H-5); 8.16 (2H, d, *J* = 8.4, H-3',5'). ^{13}C NMR spectrum (DMSO-d_6 + NaH + D_2O), δ, ppm: 171.2; 164.5; 159.7; 140.1; 139.7; 137.7; 137.3; 129.6; 126.4; 121.6; 120.7. Mass spectrum, *m/z* (I_{rel}, %): 272 [M–H]$^+$ (100), 274 [M–H+2]$^+$ (33). Found, %: C 56.63; H 2.82; N 15.44. $C_{13}H_8ClN_3O_2$. Calculated (with 0.7% H_2O), %: C 56.67; H 3.00; N 15.25.

3-(3-Benzyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzoic acid (17). Yield 0.151 g (46%). Mp 218–220°C (2-PrOH). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 5.62 (2H, s, PhC$\underline{H}_2$); 6.97–6.99 (2H, m, H-3,5 Ph); 7.18–7.25 (3H, m, H-2,4,6 Ph); 7.36 (1H, dd, *J* = 8.4, *J* = 5.2, H-6); 7.63 (1H, t, *J* = 8.0, H-5'); 7.98 (1H, d, *J* = 8.0, H-6'); 8.07 (1H, d, *J* = 8.0, H-4'); 8.17 (1H, dd, *J* = 8.0, *J* = 1.6, H-7); 8.30 (1H, s, H-2'); 8.39 (1H, dd, *J* = 5.2, *J* = 1.2, H-5). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 167.3 (C=O); 153.7; 149.3; 144.9 (C-5); 137.5; 135.2; 133.5 (C-6'); 132.1; 131.5 (C-4'); 130.8; 130.4 (C-2'); 130.0 (C-5'); 129.4 (C-2,6 Ph); 128.1 (C-4 Ph); 127.9 (C-7); 126.9 (C-3,5 Ph); 119.7 (C-6); 46.7(CH_2). Mass spectrum, *m/z* (I_{rel}, %): 330 [M+H]$^+$ (100). Found, %: C 70.30; H 4.92; N 11.90. $C_{20}H_{15}N_3O_2$. Calculated (with 2.7% H_2O and 4.4% 2-PrOH), %: C 70.52; H 5.13; N 11.89.

2-(3-Benzyl-1*H*-imidazo[4,5-*b*]pyridin-2-yl)benzoic acid (18). Purified by column chromatography, eluent CH_2Cl_2–MeOH–AcOH, 50:10:0.2. Yield 0.072 g (22%). Mp 226–228°C. ^{1}H NMR spectrum (CD_3OD), δ, ppm (*J*, Hz): 4.92 and 4.93 (2H total, 2s, PhC$\underline{H}_2$); 6.89–6.91 (2H, m, H-3,5 Ph); 7.11–7.16 (3H, m, H-2,4,6 Ph); 7.22 (1H, d, *J* = 7.2, H-6'); 7.37 (1H, dd, *J* = 8.0, *J* = 4.8, H-6); 7.56 (1H, td, *J* = 7.2, *J* = 0.8, H-5'); 7.66 (1H, td, *J* = 7.6, *J* = 0.8, H-4');

8.07 (1H, dd, *J* = 8.0, *J* = 1.6, H-7); 8.18 (1H, d, *J* = 8.0, H-3'); 8.41 (1H, dd, *J* = 4.8, *J* = 1.2, H-5). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 167.3; 115.6; 147.6; 144.0; 136.3; 134.3; 132.1; 131.5 (2C); 130.8 (2C); 130.6; 128.3; 127.6; 127.3; 126.8; 118.8; 46.3. Mass spectrum, *m*/*z* (I_{rel}, %): 330 $[M+H]^+$ (100). Found, %: C 71.47; H 4.44; N 12.09. $C_{20}H_{15}N_3O_2$. Calculated (with 2.1% H_2O), %: C 71.37; H 4.73; N 12.48.

3-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)benzenesulfonic acid (19). Yield 0.58 g (56%). Mp >340°C (2-PrOH). ^{1}H NMR spectrum (CD_3OD + NaH + D_2O), δ, ppm (*J*, Hz): 6.98 (1H, dd, *J* = 7.6, *J* = 5.2, H-6); 7.50 (1H, t, *J* = 8, H-5'); 7.83 (1H, dt, *J* = 8, *J* = 1.6, H-6'); 7.86 (1H, dd, *J* = 8.0, *J* = 1.6, H-7); 8.07 (1H, dd, *J* = 4.8, *J* = 1.6, H-5); 8.35 (1H, dt, *J* = 8.4, *J* = 1.2, H-4'); 8.77 (1H, t, *J* = 1.6, H-2'). ^{13}C NMR spectrum (CD_3OD + NaH + D_2O), δ, ppm: 162.7; 159.5; 145.1; 140.0; 139.1; 136.2; 129.0; 128.2; 125.6; 124.7; 123.5; 114.8. Mass spectrum, *m*/*z* (I_{rel}, %): 274 $[M–H]^+$ (100). Found, %: C 42.06; H 4.15; N 12.53; S 9.29. $C_{12}H_9N_3O_3S$. Calculated (with 19.1% H_2O), %: C 42.37; H 4.80; N 12.36; S 9.42.

2-(6-Bromo-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzenesulfonic acid (20). Yield 0.127 g (36%). Mp 257–261°C (2-PrOH). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 7.31–7.39 (2H, m, H-4',5'); 7.61 (1H, dd, *J* = 7.6, *J* = 1.6, H-6'); 7.85 (1H, d, *J* = 2.4, H-7); 7.93–7.96 (2H, m, H-3',5). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 166.7; 159.6; 145.2; 140.6; 138.6; 136.2; 133.0; 129.3; 128.6; 127.5; 124.8; 108.6. Mass spectrum, *m*/*z* (I_{rel}, %): 352 $[M–H]^+$ (98), 354 $[M–H+2]^+$ (100). Found, %: C 36.01; H 3.17; N 10.67; S 8.12. $C_{12}H_8BrN_3O_3S$. Calculated (with 11.3% H_2O), %: C 36.10; H 3.28; N 10.52; S 8.03.

3-(3-Benzyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzenesulfonic acid (21). Yield 0.120 g (33%). Mp 304–306°C (MeOH). ^{1}H NMR spectrum (CD_3OD), δ, ppm (*J*, Hz): 5.69 (2H, s, $PhCH_2$); 6.99–7.02 (2H, m, H-3,5 Ph); 7.19–7.22 (3H, m, H-2,4,6 Ph); 7.41 (1H, dd, *J* = 8.0, *J* = 4.8, H-6); 7.56 (1H, t, *J* = 8.0, H-5'); 7.68 (1H, d, *J* = 8.0, H-6'); 8.02 (1H, dt, *J* = 8.0, *J* = 1.2, H-4'); 8.13 (1H, dd, *J* = 7.6, *J* = 1.2, H-7); 8.24–8.25 (1H, m, H-2'); 8.44 (1H, dd, *J* = 4.8, *J* = 1.6, H-5). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 154.5; 148.4; 146.3; 144.5; 136.6; 134.6; 130.8; 129.8; 128.9; 128.6; 127.9; 127.6; 127.2; 126.7 (2C); 119.3; 46.5. Mass spectrum, *m*/*z* (I_{rel}, %): 364 $[M–H]^+$ (98). Found, %: C 54.27; H 4.87; N 10.38; S 7.72. $C_{19}H_{15}N_3O_3S$. Calculated (with 12.9 % H_2O), % : C 54.40; H 5.04 ; N 10.02 ; S 7.64.

2-(6-Bromo-3-butyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)-benzenesulfonic acid (22). Purified by column chromatography, eluent CH_2Cl_2–MeOH–AcOH, 50:10:0.2, and afterwards recrystallized from MeOH. Yield 0.121 g (30%). Mp 260–270°C (decomp.). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 0.66 (3H, t, *J* = 7.6, CH_3); 1.03–1.06 (2H, m, $C\underline{H}_2CH_3$) ; 1.48–1.52 (2H m, $C\underline{H}_2CH_2CH_3$); 4.07–4.12 (2H, m, NCH_2); 7.35 (1H, d, *J* = 7.2, H-6'); 7.51 (1H, t, *J* = 7.2, H-5'); 7.60 (1H, t, *J* = 7.6, H-4'); 7.92 (1H, d, *J* = 8.0, H-3'); 8.29 (1H, d, *J* = 2.0, H-7); 8.43 (1H, d, *J* = 2.0, H-5). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 157.5; 148.1; 147.3; 143.4 (C-5); 136.5; 131.6 (C-6'); 130.6 (C-4'); 129.2 (C-7); 129.1 (C-5'); 128.5 (C-3'); 127.4; 112.9; 43.3 (NCH_2); 31.2 ($C\underline{H}_2CH_2CH_3$); 19.9 ($C\underline{H}_2CH_3$); 13.9 (CH_3). Mass spectrum, *m*/*z* (I_{rel}, %): 410 $[M(^{81}Br)–H]^+$ (99), 408 $[M(^{79}Br)–H]^+$ (100). Found, %: C 42.55; H 3.99; N 9.50; S 6.76. $C_{16}H_{16}BrN_3O_3S$. Calculated (with 7.8% H_2O and 3.2% AcOH), %: C 42.9; H 4.58; N 9.11; S 6.95.

4-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)benzonitrile (23). Yield 0.105 g (48%). Mp >370°C. (MeOH). ^{1}H NMR spectrum (DMSO-d_6 + NaH + D_2O), δ, ppm (*J*, Hz): 6.90 (1H, dd, *J* = 7.6, *J* = 4.8, H-6); 7.76 (1H, dd, *J* = 8.0, *J* = 0.8, H-7); 7.80 (2H, d, *J* = 8.0, H-2',6'); 8.05 (1H, d, *J* = 4.8, H-5); 8.39 (2H, d, *J* = 8.0, H-3',5'). Mass spectrum, *m*/*z* (I_{rel}, %): 221 $[M+H]^+$ (100). Found, %: C 69.93; H 3.72; N 25.24. $C_{13}H_8N_4$. Calculated (with 1.6% H_2O), %: C 69.75; H 3.70; N 25.03.

4-(3*H*-Imidazo[4,5-*c*]pyridin-2-yl)benzonitrile (24). Yield 0.095 g (43%). Mp >370°C. (MeOH). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 7.69 (1H, d, *J* = 5.2, H-7); 8.07 (2H, d, *J* = 8.0, H-2',6'); 8.35 (1H, d, *J* = 5.6, H-5); 8.40 (2H, d, *J* = 8.0, H-3',5'); 9.02 (1H, s, H-4). Mass spectrum, *m*/*z* (I_{rel}, %): 221 $[M+H]^+$ (100). Found, %: C 66.37; H 3.80; N 23.31. $C_{13}H_8N_4$. Calculated (with 6.9% H_2O), %: C 66.04; H 4.18; N 23.69.

3-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)benzonitrile (25). Yield 0.113 g (51%). Mp 342–344°C. (MeOH). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 7.29 (1H, dd, *J* = 8.4, *J* =4.8, H-6); 7.81 (1H, t, *J* = 8.0, H-5'); 8.01 (1H, dt, *J* = 6.4, *J* = 1.2, H-6'); 8.08 (1H, br. d, *J* = 7.6, H-7); 8.4 (1H, br. d, *J* = 4.0, H-5); 8.54 (1H, dt, *J* = 6.4, *J* = 1.2, H-4'); 8.61 (1H, t, *J* = 1.2, H-2'); 13.5 (1H, br. s, NH). Mass spectrum, *m*/*z* (I_{rel}, %): 221 $[M+H]^+$ (100). Found, %: C 70.60; H 3.65; N 25.25. $C_{13}H_8N_4$. Calculated, %: C 70.90; H 3.66; N 25.44.

4-(3-Benzyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzonitrile (26). Yield 0.197 g (64%). Mp 182–183°C. (MeOH). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 5.72 (2H, s, $PhCH_2$); 6.85–7.05 (2H, m, H-3,5 Ph); 7.20–7.28 (3H, m, H-2,4,6 Ph); 7.41 (1H, dd, *J* = 8.4, *J* = 4.8, H-6); 7.96 (2H, d, *J* = 8.4, H-2',6'); 7.99 (2H, d, *J* = 8.4, H-3',5'); 8.23 (1H, dd, *J* = 8.0, *J* = 1.2, H-7); 8.45 (1H, dd, *J* = 4.8, *J* = 1.6, H-5). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 152.8; 149.2; 145.4 (C-5); 137.4; 135.2; 134.8; 133.4 (C-3',5'); 130.3 (C-2',6'); 129.4 (C-2,6 Ph); 128.3 (C-7); 128.2 (C-4 Ph); 126.9 (C-3,5 Ph); 119.9 (C-6); 118.9; 113.4; 46.8 (CH_2). Mass spectrum, *m*/*z* (I_{rel}, %): 311 $[M+H]^+$ (100). Found, %: C 75.33; H 4.30; N 17.44. $C_{20}H_{14}N_4$. Calculated (with 2.8% H_2O), %: C 75.22; H 4.73; N 17.54.

2-(3-Benzyl-2,3-dihydro-1*H*-imidazo[4,5-*b*]pyridin-2-yl)-benzenesulfonic acid (27). A mixture of compound **4** (0.20 g, 1.0 mmol) and sodium metabisulfite adduct of sodium 2-formylbenzenesulfonate (0.312 g, 1.00 mmol), prepared as in the general method above, in EtOH (10 ml) was stirred at 75°C for 30 h. The reaction mixture was then cooled to room temperature. The solid was filtered off, suspended in MeOH–H_2O, a few drops of 1 N HCl was added, and precipitate was filtered off and recrystallized from MeOH. Yield 0.120 g (33%). Mp 269–270°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 4.37 (1H, d, *J* =16.4) and 4.87 (1H, d, *J* = 16.4, $PhC\underline{H}_2$); 6.43 (1H, d,

J = 7.2, H-5); 6.58 (1H, t, *J* = 7.6, H-6); 7.02 (1H, d, *J* = 6.4, H-7); 7.20–7.33 (7H, m, H Ar); 7.47–7.49 (1H, m, H Ar); 7.69 (1H, s, 2-CH); 7.74–7.76 (1H, m, H-3'); 7.8 (1H, br. s, NH, D_2O-exchangeable). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 149.11; 146.8; 138.8; 135.4; 135.3; 130.3; 129.3; 129.2; 128.3 (2C); 128.2; 127.1; 120.1; 114.9; 107.4; 78.03; 46.8. Mass spectrum, *m*/*z* (I_{rel}, %): 366 $[M–H]^+$ (100). Found, %: C 61.07; H 4.88; N 11.25; S 8.44. $C_{19}H_{17}N_3O_3S$. Calculated (with 1.2% H_2O), %: C 61.36; H 4.74; N 11.29; S 8.62.

2-(3-Benzyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzenesulfonic acid (28). A mixture of compound **27** (0.184 g, 0.50 mmol) and 1,4-benzoquinone (0.054 g, 0.50 mmol) in EtOH (20 ml) was heated at 75°C for 6 h. The reaction mixture was cooled to room temperature, concentrated, acidified with dilute acetic acid (5%), and diluted with some water. The solid was filtered off and recrystallized from *n*-hexane–2-PrOH, 1:1. Yield 0.148 g (81%). Mp 317–318°C. ^1H NMR spectrum (CD_3OD), δ, ppm (*J*, Hz): 5.21 (1H, br. s) and 5.76 (1H, br. s, $PhCH_2$); 6.89–6.91 (2H, m, H-3,5 Ph); 7.015 (1H, d, *J* = 7.2, H-6'); 7.13–7.18 (3H, m, H-2,4,6 Ph); 7.35 (1H, td, *J* = 7.2, *J* = 0.8, H-5'); 7.39 (1H, dd, *J* = 8.4, *J* = 5.2, H-6); 7.63 (1H, td, *J* = 7.6, *J* = 0.8, H-4'); 8.09–8.14 (2H, m, H-7,3'); 8.41 (1H, dd, *J* = 4.8, *J* = 1.2, H-5). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 155.9; 148.7; 145.9; 145.5 (H-5); 137.9; 134.9; 132.9 (H-6'); 131.9 (H-4'); 131.1 (C-6); 129.6 (C-2,6 Ph); 129.1 (C-7); 128.7 (C-4 Ph); 128.6 (C-3,5 Ph); 127.9 (C-3'); 127.7; 120.2 (C-5'); 47.8 (CH_2). Mass spectrum, *m*/*z* (I_{rel}, %): 364 $[M–H]^+$ (100). Found, %: C 62.55; H 4.11; N 11.6; S 8.66. $C_{19}H_{15}N_3O_3S$. Calculated, %: C 62.5; H 4.14; N 11.5; S 8.77.

Sodium 2-({[2-(benzylamino)pyridin-3-yl]imino}methyl)-benzenesulfonate (29). A mixture of compound **4** (0.20 g, 1.0 mmol) and sodium 2-formylbenzenesulfonate (0.208 g, 1.00 mmol) in EtOH (10 ml) was stirred at 50°C for 24 h. The reaction mixture was cooled to room temperature, EtOH was evaporated, and the solid was recrystallized from MeOH–EtOAc, 1:1. Yield 0.31 g (84 %). Yellow solid. Mp 280–285°C. ^1H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 4.64 (2H, d, *J* = 6.4, $PhCH_2$); 6.56 (1H, dd, *J* = 7.2, *J* = 5.2, H-5'); 6.77 (1H, t, *J* = 6.4, CH_2NH, D_2O-exchangeable); 7.14 (1H, dd, *J* = 7.2, *J* = 1.6, H-4'); 7.19–7.83 (8H, m, H Ar); 7.87 (1H, dd, *J* = 5.2, *J* = 1.6, H-6'); 8.34 (1H, m, H-6); 9.60 (1H, s, CH=N). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 160.1; 153.6; 148.3; 145.5; 141.2; 132.4; 132.2; 130.1; 128.7; 127.9; 127.2; 126.9; 126.6; 126.2; 122.6; 111.8; 43.6. Mass spectrum, *m*/*z* (I_{rel}, %): 366 $[M–H]^+$ (100). Found, %: C 49.15; H 5.03; N 9.05; S 6.48. $C_{19}H_{16}N_3O_3SNa$. Calculated (with 15.6% H_2O), %: C 49.34; H 5.42; N 9.09; S 6.93.

2-(1-Benzyl-2,3-dihydro-1*H*-imidazo[4,5-*b*]pyridin-2-yl)-benzenesulfonic acid (31). A mixture of compound **30**[20] (0.20 g, 1.0 mmol) and sodium metabisulfite adduct of sodium 2-formylbenzenesulfonate (0.312 g, 1.00 mmol) (or sodium 2-formylbenzenesulfonate (0.208 g, 1.00 mmol)) in EtOH (10 ml) were stirred at 70°C for 30 h. The reaction mixture was cooled to room temperature. The solid was filtered off, suspended in MeOH–H_2O, 1:1, and a few drops of 1 N HCl was added, and precipitate was filtered and recrystallized from MeOH. Yield 0.265 g (72%). Mp 285–290°C. ^1H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 4.20 (1H, d, *J* = 16.4) and 4.61 (1H, d, *J* = 16.4, $PhCH_2$); 6.39 (1H, d, *J* = 7.2, H-5); 6.51 (1H, t, *J* = 6.8, H-6); 6.90 (1H, d, *J* = 6.8, H-7); 7.20–7.30 (5H, m, H Ar); 7.37–7.47 (2H, m, H Ar); 7.55 (1H, dd, *J* = 7.6, *J* = 1.6, H-6'); 7.69 (1H, s, 2-CH); 7.82 (1H, dd, *J* = 7.6, *J* = 1.6, H-3'); 9.76 (1H, br. s, NH, D_2O-exchangeable); 12.8 (1H, br. s). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 149.1; 146.8; 138.8; 135.4; 135.3; 130.3; 129.3; 129.2; 128.3 (2C); 128.2; 127.1; 120.1; 114.9; 107.4; 78.03; 46.8. Mass spectrum, *m*/*z* (I_{rel}, %): 366 $[M–H]^+$ (100). Found, %: C 61.07; H 4.88; N 11.25; S 8.44. $C_{19}H_{17}N_3O_3S$. Calculated (with 1.2% H_2O), %: C 61.35; H 4.74; N 11.29; S 8.62.

2-(1-Benzyl-1*H*-imidazo[4,5-*b*]pyridin-2-yl)benzenesulfonic acid (32). A solution of compound **31** (0.092 g, 0.25 mmol) in nitrobenzene (2 ml) was heated at 200°C for 4 h. The precipitate was filtered off and recrystallized from *n*-hexane–2-PrOH, 1:1, twice. Yield 0.019 g (21%). Mp >300°C. ^1H NMR spectrum (CD_3OD), δ, ppm (*J*, Hz): 5.36 (2H, br. s, $PhCH_2$); 7.01–7.04 (2H, m, H-3,5 Ph); 7.22–7.24 (3H, m, H-2,4,6 Ph); 7.29 (1H, dd, *J* = 8.4, *J* = 4.8, H-6); 7.33 (1H, dd, *J* = 7.2, *J* = 0.8, H-3'); 7.49 (1H, td, *J* = 7.2, *J* = 0.8, H-4'); 7.65 (1H, td, *J* = 7.2, *J* = 0.8, H-5'); 7.82 (1H, dd, *J* = 8.4, *J* = 1.6, H-7); 8.13 (1H, dd, *J* = 7.6, *J* = 1.2, H-6'); 8.41 (1H, dd, *J* = 4.8, *J* = 1.6, H-5). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 157.6; 155.6; 146.2; 145.1 (C-5); 137.3; 132.8; 131.9 (C-5'); 131.2 (C-4'); 129.8 (C-2,6 Ph); 129.2 (C-7,6'); 129.0 (C-4 Ph); 128.3 (C-3,5 Ph); 127.8 (C-3'); 121.6 (C-7); 119.6 (C-6); 50.1 (CH_2). Mass spectrum, *m*/*z* (I_{rel}, %): 366 $[M+H]^+$ (100). Found, %: C 60.33; H 4.96; N 11.40; S 8.41. $C_{19}H_{15}N_3O_3S$. Calculated (with 3.4% H_2O and 1.6% 2-PrOH), %: C 60.36; H 4.51; N 10.94; S 8.35.

Synthesis of compounds 33–35 (General method). Hydroxylamine hydrochloride (0.10 g, 1.43 mmol) followed by *N*,*N*-diisopropylethylamine (0.184 g, 1.43 mmol) was added to a stirred solution of compound **23**–**25** (1.0 mmol) in ethanol (35 ml). The solution was heated to reflux for 6 h and then concentrated. Water was added, and the precipitate was washed with water and crystallized from ethanol. In order to obtain the HCl salts of compounds **34** and **35**, the free bases were dissolved in ethanol, and dry HCl gas was passed through the solution, and the precipitate was filtered off and dried in vacuum.

***N*-Hydroxy-4-(3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzenecarboximidamide (33)**. This compound was obtained in the base form. Yield 0.142 g (56%). Mp 293–294°C (EtOH). ^1H NMR spectrum (DMSO-d_6 + NaH + D_2O), δ, ppm (*J*, Hz): 6.87 (1H, dd, *J* = 7.6, *J* = 4.8, H-6); 7.68 (2H, d, *J* = 8.0, H-2',6'); 7.75 (1H, dd, *J* = 8.0, *J* = 0.8, H-7); 7.98 (1H, dd, *J* = 4.8, *J* = 1.6, H-5); 8.23 (2H, d, *J* = 8.0, H-3',5'). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 162.6; 160.5; 151.2; 139.8 (C-5); 139.6; 136.1; 134.1; 127.1 (C-3',5'); 124.8 (C-2',6'); 123.1 (C-7); 114.5 (H-6). Mass spectrum, *m*/*z* (I_{rel}, %): 254 $[M+H]^+$ (100). Found, %: C 61.11; H 4.29; N 27.29. $C_{13}H_{11}N_5O$. Calculated (with 0.7% H_2O), %: C 61.21; H 4.42; N 27.45.

***N*-Hydroxy-4-(3*H*-imidazo[4,5-*c*]pyridin-2-yl)benzene-**

carboximidamide dihydrochloride (34). Yield 0.121 g (37%). Mp 325–330°C (decomp.). ^{1}H NMR spectrum (D_2O), δ, ppm (*J*, Hz): 7.85 (2H, d, *J* = 8.0, H-2',6'); 8.07 (1H, d, *J* = 6.8, H-7); 8.21 (2H, d, *J* = 8.0, H-3',5'); 8.45 (1H, d, *J* = 6.8, H-6); 9.14 (1H, s, H-4). ^{13}C NMR spectrum (D_2O), δ, ppm: 160.2 (C-2); 158.4 (C-1'); 146.6 (C-7a); 138.3 (C-3a); 133.6 (C-6); 132.6 (C-4); 131.5 (C-4'); 128.7 (C-2',6'); 128.6 (C-3',5'); 128.5 (CNO); 111.3 (C-7). Mass spectrum, *m*/*z* (I_{rel}, %): 254 $[M+H]^+$ (100). Found, %: C 47.17; H 3.88; N 21.32. $C_{13}H_{11}N_5O\cdot 2HCl$. Calculated (with 1.4% H_2O), %: C 47.21; H 4.11; N 21.18.

***N*-Hydroxy-3-(3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzenecarboximidamide dihydrochloride (35)**. Yield 0.120 g (37%). Mp 282–285°C (decomp.). ^{1}H NMR spectrum (D_2O), δ, ppm (*J*, Hz): 7.72 (1H, dd, *J* = 8.0, *J* = 5.6, H-6); 7.83 (1H, t, *J* = 7.6, H-5'); 7.96 (1H, d, *J* = 7.6, H-6'); 8.34 (1H, d, *J* = 7.6, H-4'); 8.41 (1H, br. s, H-2'); 8.52–8.55 (2H, m, H-5,7). ^{13}C NMR spectrum (D_2O), δ, ppm: 163.4; 159.5; 150.7; 139.9 (C-7); 135.4 (C-4'); 134.4 (C-6'); 134.3; 133.7 (C-5'); 131.8 (C-5); 130.9; 129.9 (C-2'); 129.4; 122.4 (C-6). Mass spectrum, *m*/*z* (I_{rel}, %): 254 $[M+H]^+$ (100). Found, %: C 47.90; H 3.99; N 21.50. $C_{13}H_{11}N_5O\cdot 2HCl$. Calculated, %: C 47.86; H 4.01; N 21.47.

Synthesis of compounds 36–39 (General method). Compound **23**–**26** (1.0 mmol) was suspended in absolute EtOH, cooled in an ice–salt bath, and dry HCl gas was passed through the solution for 40 min. The solution was stirred in a stoppered flask at room temperature for 6 days and then diluted with dry ether. The precipitated imidate ester hydrochloride was washed with ether, dried under vacuum at room temperature, and used immediately without characterization. A suspension of imidate ester hydrochloride in absolute EtOH (10 ml) was stirred with NH_3 (NH_3 gas let into the reaction mixture) or isopropylamine (1.5–2-fold excess) overnight at 60°C. The reaction mixture was evaporated and treated with dilute Na_2CO_3 solution. In order to obtain the HCl salts of compounds **38** and **39**, the free bases were dissolved in ethanol and dry HCl gas was passed through the solution, and the precipitate was filtered off and dried in vacuum.

4-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)benzenecarboximidamide dihydrochloride (36). Yield 0.128 g (41%). Mp 337–339°C. ^{1}H NMR spectrum (D_2O), δ, ppm (*J*, Hz): 7.59 (1H, dd, *J* = 8.4, *J* = 5.6, H-6); 7.86 (2H, d, *J* = 8.0, H-2',6'); 8.12 (2H, d, *J* = 8.0, H-3',5'); 8.35 (1H, dd, *J* = 8.0, *J* = 0.8, H-7); 8.44 (1H, dd, *J* = 5.6, *J* = 1.2, H-5). ^{13}C NMR spectrum (D_2O), δ, ppm: 168.4 (C-2); 158.1 (C-1'); 151.4 (C-3a); 141.9 (C-5); 134.7 (C-4'); 134.0 (C-7a); 133.5 (CNO-carboxamidine); 131.5 (CH-2',6'); 131.1 (CH-3',5'); 130.7 (CH-7); 122.5 (CH-6). Mass spectrum, *m*/*z* (I_{rel}, %): 238 $[M+H]^+$ (100). Found, %: C 46.89; H 4.38; N 20.94. $C_{13}H_{11}N_5\cdot 2HCl$. Calculated (with 7.5% H_2O), %: C 46.55; H 4.74; N 20.88.

***N*-Isopropyl-4-(3*H*-imidazo[4,5-*c*]pyridin-2-yl)benzenecarboximidamide (37)**. It was crystallized from EtOAc–MeOH, 3:1. Yield 0.177 g (53.5%). Mp 265–270°C (bubbling). ^{1}H NMR spectrum (CD_3OD + NaH + D_2O), δ, ppm (*J*, Hz): 1.26 (6H, d, *J* = 6, $CH(C\underline{H}_3)_2$); 3.92–3.95 (1H, m, $C\underline{H}(CH_3)_2$); 7.51 (1H, d, *J* = 5.4, H-7); 7.67 (2H, d, *J* = 8.0, H-2',6'); 7.97 (1H, d, *J* = 5.8, H-6); 8.30 (2H, d, *J* = 8.0, H-3',5'); 8.73 (1H, s, H-4). ^{13}C NMR spectrum (CD_3OD + NaH + D_2O), δ, ppm: 163.6; 163.4; 151.5; 144.4; 137.7; 137.4; 137.2; 137.1; 127.4; 126.7; 111.5; 43.5; 21.6. Mass spectrum, *m*/*z* (I_{rel}, %): 280 $[M+H]^+$ (100). Found, %: C 55.69; H 6.97; N 19.21. $C_{16}H_{17}N_5$. Calculated (with 16.2% H_2O and 9.6% MeOH), %: C 55.87; H 7.45; N 19.16.

3-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)benzenecarboximidamide dihydrochloride (38). Yield 0.142 g (45 %). Mp 327–330°C. ^{1}H NMR spectrum (D_2O), δ, ppm (*J*, Hz): 7.68 (1H, dd, *J* = 8.0, *J* = 6.0, H-6); 7.79 (1H, t, *J* = 7.6, H-5'); 7.97–7.99 (1H, m, H-6'); 8.35–8.38 (1H, m, H-4'); 8.47 (1H, t, *J* = 2.0, H-2'); 8.48 (1H, dd, *J* = 6.0, *J* = 1.2, H-7); 8.53 (1H, dd, *J* = 8.0, *J* = 1.2, H-5). ^{13}C NMR spectrum (D_2O), δ, ppm: 169.1; 160.0; 150.8; 139.7 (C-7); 135.9 (C-4'); 134.7 (C-6'); 134.6; 133.7 (C-5'); 132.5; 132.0 (C-5); 130.9; 130.3 (C-2'); 122.4 (C-6). Mass spectrum, *m*/*z* (I_{rel}, %): 238 $[M+H]^+$ (100). Found, %: C 50.06; H 4.29; N 22.20. $C_{13}H_{11}N_5\cdot 2HCl$. Calculated, %: C 50.34; H 4.23; N 22.58.

***N*-Isopropyl-4-(3-benzyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzenecarboximidamide dihydrochloride (39)**. Yield 0.126 g (28 %). Mp 95–100°C (bubbling); 290–295°C. ^{1}H NMR spectrum (CD_3OD), δ, ppm (*J*, Hz): 1.39 (6H, d, *J* = 6.8, $CH(C\underline{H}_3)_2$); 4.03–4.06 (1H, m $C\underline{H}(CH_3)_2$); 5.73 (2H, s, $PhCH_2$); 6.99 (2H, d, *J* = 8.0, H-3,5 Ph); 7.20–7.25 (3H, m, H-2,6,4 Ph); 7.45 (1H, dd, *J* = 8.4, *J* = 4.8, H-6); 7.86 (2H, d, *J* = 8.4, H-2',6'); 7.93 (2H, d, *J* = 8.4, H-3',5'); 8.18 (1H, dd, *J* = 8.0, *J* = 1.2, H-7); 8.46 (1H, dd, *J* = 4.8, *J* = 1.2, H-5). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 162.9; 153.6; 148.5; 145.1 (C-5); 136.7; 134.7; 134.3; 131.6; 129.8 (C-3',5'); 128.7 (C-2,6 Ph or C-4 Ph); 128.5 (C-2',6'); 127.7 (C-2,6 Ph or C-4 Ph); 127.5 (H-7); 126.3 (H-3",5"); 119.6 (H-6); 46.5 (CH_2); 46.0 ($\underline{C}(CH_3)_2$); 20.3 ($(CH(\underline{C}H_3)_2$). Mass spectrum, *m*/*z* (I_{rel}, %): 370 $[M+H]^+$ (100). Found, %: C 61.25; H 6.04; N 15.87. $C_{23}H_{23}N_5\cdot 2HCl$. Calculated (with 1.6% H_2O), %: C 61.20; H 5.81; N 15.51.

Cytotoxicity Assay. The synthesized compounds were dissolved in ethanol (70%) to provide 1×10^{-2} mol/l stock solutions, these solutions were diluted within the range between 1×10^{-3} to 1×10^{-10} mol/l. The human breast cancer cell line MCF-7 (HTB 22) was purchased from the American Type Culture Collection (LGC Promochem). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, PAA Laboratories GmbH) with 10% fetal bovine serum (FBS, Lonza), 1% antibiotics (peniciline and streptomycin, PAA) and 1% L-glutamine (PAA) at humidified atmosphere (37°C and 5% CO_2).

The effects of test samples on cell viability were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay by the method of Mossman[26] in modification by Kuzma et al.[27] A 180 µl volume of the cell suspension containing 5×10^4 cells was seeded to each well of a 96-well microtiter plate, and the cells were grown in a humidified atmosphere of 5% CO_2 in air at 37°C. The test samples were added to a final concentration ranged between 100 µM to 10 nM, and the plates were incubated for 48 h. Control experiments were performed under the same conditions with addition of solvent (70% EtOH). Following incubation, the culture medium

was removed and exchanged for a fresh one. MTT solution (20 µl, 5 mg/ml in phosphate buffer solution, Sigma) was added per well and incubated at 37°C for 2 h. The metabolically active cells reduced MTT dye to formazan crystals. The medium was then removed, and the blue MTT-formazan was dissolved in DMSO (Merck). The extent of the reduction of MTT within the cells was quantified by measuring the absorbance at 540 nm on a Thermo MultiSkan GO microplate reader and compared with controlled, untreated cells. The experiments were performed within triplicates, and the concentration of test compounds required to reduce survival of cells by 50% (IC_{50}) was determined from the graph of the amount of visible cells plotted against the test compound concentration.

Supporting material to this article containing ^{1}H NMR spectra of compounds **5**, **6**, **11**–**29**, **31**–**39** and ^{13}C NMR spectra of compounds **5**, **6**, **13**, **16**–**22**, **25**–**29**, **31**–**39** is available for the authorized users.

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