Supplementary information

Synthesis, biological evaluation and X-ray crystallographic studies of imidazo[1,2-*a*]pyridine-based *Mycobacterium tuberculosis* glutamine synthetase inhibitors

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Experimental

General information

NMR spectra were recorded on a Varian Mercury plus spectrometer at 25 °C for ^{1}H NMR at 399.9 MHz, ^{13}C NMR at 100.5 MHz and ^{19}F NMR at 376.16 MHz. The chemical shifts (δ) are reported in ppm and referenced indirectly to TMS via the solvent signals (1H, $CDCl_3$ at 7.26 ppm, CD_3OD at 3.31 ppm; ^{13}C, $CDCl_3$ at 77.16 ppm, CD_3OD at 49.0 ppm) All microwave-assisted synthesis was carried out in a Smith/Emrys^{TM} or Initiator synthesizers single mode cavity instruments producing controlled irradiation at 2450 MHz (Biotage AB, Uppsala, Sweden). HPLC-MS was performed on a Gilson HPLC system with a Finnigan AQA quadrupole mass spectrometer with detection by UV (DAD) and MS (ESI^+), or a Gilson HPLC system with a Finnigan Thermoquest MSQ quadrupole mass spectrometer with detection by a SEDERE ELSD (Sedex 55) and MS (ESI^+), using an Onyx Monolithic C18 column (50×4.6 mm). Both systems employed a flow rate of 4 mL/min and an H_2O/CH_3CN/0.05% HCOOH gradient. Preparative RP-HPLC was performed by UV-triggered fraction collection with a similar Gilson-Finnigan AQA system and a Zorbax SB C8 column (150×21.2 mm) at a flow rate of 10 mL/min and an H_2O/CH_3CN/0.05% HCOOH gradient. Molecular mass (HR-ESI-MS) was determined on a mass spectrometer equipped with an electrospray ion source. Analytische Laboratorien GmbH, Lindlar, Germany performed the elemental analysis. Column chromatography was performed using silica gel 60 (particle size 0.040–0.063 mm, Sigma-Aldrich). Thin-layer chromatography was performed with aluminum sheets coated with silica gel 60 F_{254} (0.2 mm E. Merck), using UV-light for visualization. All products were >95% pure according to LC-UV and ^{1}H NMR or passed elemental analysis.

All reactants and reagents were commercially available and used without further purification unless otherwise stated. Compounds **3n**[1] and **3p**[1] are known compounds and **3h** is commercially available, but there are no NMR data reported in the literature. Compounds **3a**–**3m**, **3o**, **4a**–**4e** and **5a**–**5l** are all new.

Protein expression and purification

Escherichia coli strain GJ4745, lacking adenylyltransferase, was the kind gift of Dr. Gowrishankar (Center for Cellular and Molecular Biology, Hyderabad, India). Protein was over-expressed and purified in this strain, using methods described previously,[2] to provide unadenylylated *Mt*GS.

Enzyme inhibition studies

Compounds were dissolved in DMSO to prepare stock solutions (1 and 10 mM) that were stored frozen at 4 °C. Inhibition screening assays and IC_{50} determination were performed essentially as described earlier.[3] A typical 100-µL reaction contained 50 mM HEPES-HCl (pH 7.5), 25 mM $MgCl_2$, 1 mM ATP, 30 mM NH_4Cl, 30 mM L-glutamate, and 7 nM of *Mt*GS (subunit concentration). For inhibition screening and IC_{50} determinations, DMSO solvent concentration was 2.5% (v/v) and 2% (v/v), respectively. Compound concentration during inhibition screening was 25 µM; for IC_{50} determination, compound was prepared in an appropriate concentration range by 2-fold serial dilutions in DMSO. The incubation was carried out for 1 h at room temperature, after which released inorganic phosphate was detected using a P_iColorlock Gold reagent kit purchased from Innova Biosciences, UK. IC_{50} values were determined by nonlinear regression of $Y = Lo+[(Hi-Lo)/(1+X/IC_{50})]$ as described earlier.[3] Regression analysis was performed using the SOLVER function in the Microsoft Excel spreadsheet software[4-6] with the goal of maximizing the square of the correlation coefficient (R^2), and including the constraint that IC_{50} was greater than or equal to 0. The reported IC_{50} values are an average from three separate experiments, and reported together with the standard deviation.

Test of inhibition of Pi-detection assay

The effect of each compound on the Pi-detection assay was investigated by incubating 25 µM sodium phosphate with 25 µM compound under the same conditions as for the enzymatic assay but excluding *Mt*GS and ATP. The mixture was incubated at room temperature for 60 min, then the color was developed and quantified as described for the enzymatic assay. The signal obtained was then compared with that from a compound-free phosphate control; compounds deviating by more than 5% from the control were flagged as interfering with the color development.

Characterization of building blocks

Isocyanides and *ortho*, *meta* or *para* monosubstituted benzaldehydes that were available from ABCR, Acros, Aldrich, Alfa, Apollo, Fluka, Frontier, Matrix, Maybridge, Sigma, Strem and TCI at the time were extracted from ACD[7] and saved as four separate SD files. These SD files were translated into mol2 format using the Concord standalone module of SYBYL.[8] A set of chemical descriptors was calculated for each data set (Table S1).

Table S1. Descriptors used to characterize building blocks.

Calculated octanol/water partition coefficient (CLOGP)	Hydrophobe (Hy)	Rotatable bonds (RB)
Calculated molecular refractivity (CMR)	Bond count (BC)	Ring count (RC)
Hydrogen bond acceptors (HBA)	Polar surface area (PSA)	Area (A)
Hydrogenbond donors (HBD)	Polar volume (PV)	Volume (V)
22 charged partial surface area (CPSA) descriptors (C_N1, C_N2, C_P1, C_P2)	Molecular weight (MW)	Atom count (AC)

[a]As implemented in SYBYL v.7.3

The descriptors were chosen for their ability to represent the size and polar nature of compounds, and their ease of interpretability. Each descriptor set was later compressed using principal component analysis (PCA) in SIMCA-P+[9] (Table S2). Since 22 charged polar surface area (CPSA) descriptors were present in the original descriptor set they were first compressed using PCA in order to reduced their combined influence. These were instead represented by four latent variables for the aldehydes, and three variables for the isocyanides in the final descriptor table.

Table S2. PCA diagnostics for the 4 datasets.

Dataset	# cmpd	A[a]	R^2X[b]	Q^2 [c]	SEU[d]	LENU[e]
R_i	52	2	0.70	0.56	3.81	2.12
R_o	75	2	0.64	0.45	3.42	2.43
R_m	85	2	0.67	0.50	3.24	2.37
R_p	160	2	0.66	0.52	3.19	2.16

[a]A is the number of latent variables for the indicated position used in the design
[b]R^2X is the goodness of fit
[c]Q^2 is the goodness of prediction
[d]SEU is the eigenvalue of the last component used in the design.
[e]LENU is the eigenvalue of the first component not used in the design.

The loading plots for the different positions were very similar for the four data sets. The first latent variable is characterized by large loadings of e.g. molecular weight (MW), calculated molar refractivity (CMR) and volume (V), reflecting the size of the building block whereas the second component mainly reflects the polarity as judged by the descriptors calculated octanol-water partition coefficient (CLOGP) and polar surface area (PSA) (Figure S1).

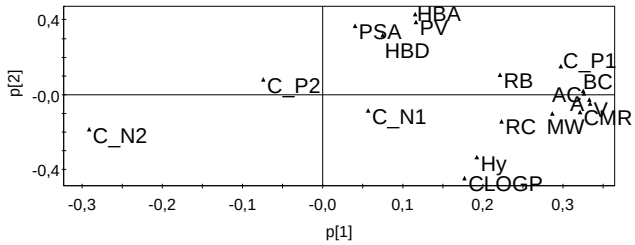

Figure S1. Representative (showing R^m) loading plot for the 4 datasets. First component mainly describes size whereas the second component reflects polarity. Abbreviations for the descriptors are as stated in Table S1.

Design of a 16-compound library

A library was designed using the FHDoE approach,[10] with compounds **1a–1d** as templates, which were based on an HTS hit.[11] This method generates a library biased towards the hit structure by combining two design layers with a focused building block selection. Substitutions were allowed in the benzaldehyde (R^o, R^m and R^p) and isocyanide (R^1) building blocks as indicated in structure **3** (Figure 1). To explore the effect that different substitutions on the benzaldehyde have on activity, the unsubstituted benzaldehyde was chosen as center point in this region, even though it was inactive. The cyclopentylamine from **1a–1d** was chosen as center point for R^1. A first 2^4 full factorial design was performed to decide the substitution pattern. Here a (-) setting corresponds to the case that no substitution should be made, i.e. keeping a hydrogen at the R^o, R^m and R^p position, respectively, in the aldehyde building block and/or keeping the R^1 substituent as cyclopentylamine (indicated by shaded cells in Table S3). A second 2^{8-4} fractional factorial design was then made to decide the final design matrix (Table S3). Each building block was characterized by two latent variables as described above, and the columns were reordered to receive better design properties. The quality of the resulting screening design matrix was inspected in Modde version 8.0. Since chemical space is a discontinuous space filled with holes, not all combinations decided by the design could be found. In those cases (compounds **3b** and **3j**) a compound resembling the setting sought was selected.

Table S3. Final design matrix, showing how the principal properties in each position should be combined to yield the 16 compounds. A "0-setting" corresponds to making no change, i.e. keep the R^1 substituent as cyclopentyl or have the position unsubstituted, leaving a hydrogen at the position (R^o, R^m, R^p) respectively.

Str.	t1 R^1	t2 R^1	t1 R^o	t2 R^o	t1 R^m	t2 R^m	t1 R^p	t2 R^p
1	-	-	-	-	-	-	-	-
2	0	0	+	+	+	-	-	+
3	+	+	-	-	0	0	-	+
4	0	0	+	+	0	0	-	-
5	+	-	0	0	+	-	+	-
6	0	0	0	0	-	-	+	+
7	-	+	0	0	0	0	+	+
8	0	0	0	0	0	0	+	-
9	+	-	-	+	-	+	0	0
10	0	0	+	-	+	+	0	0
11	-	+	-	+	0	0	0	0
12	0	0	+	-	0	0	0	0
13	-	-	0	0	+	+	0	0
14	0	0	0	0	-	+	0	0
15	+		0	0	0	0	0	0
16	0	0	0	0	0	0	0	0

Molecular docking

The structure of *Mt*Gs bound to **5b** was prepared for docking using the protein preparation tool from Schrödinger, as implemented within Maestro.[12] All waters were deleted together with the phosphate coordinating with the metal. Hydrogens were added and a 2^+ formal charge was added to the metal. The H-bonding pattern was optimized and the structure was minimized with the OPLS2001 force field, allowing a maximum RMSD deviation of 0.30 Å, with the Impact module (version 5.0207). Compound **5b** was identified as the ligand, and the grid box for docking was centered upon it. The grid was calculated with default settings. GlideXP[13-16] docking was performed employing a vdW scaling of 0.80, collecting at most 10 poses per compound. The suggested setup was validated by successful retrospective docking of **5b** (Figure S2), ADP[2] and the previously identified diketopurine analogue (**2**).[11] To simplify the analysis of the docking results of the synthesized compounds (Table 1, 2 and 3) a positional constraint for the bromine atom to the carbonyl oxygen of His278 was introduced.

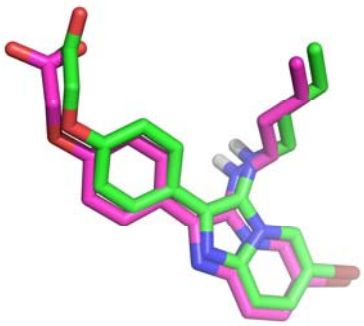

Figure S2. Best ranked docking pose by Glide score of **5b** (green carbon atoms) compared to the X-ray pose (magenta).

Chemistry

General procedure for the synthesis of substituted 6-bromo-imidazo[1,2-*a*]pyridines.
Aldehyde (1.0 equiv), isocyanide. (0.95 equiv), 2-amino-5-bromopyridine (1.0 equiv), $MgCl_2$ (0.11 equiv) and ethanol (2.5 mL) were added to a microwave vial (2-5 mL) and sealed under air with a Teflon-coated septum. After microwave irradiation for 20 min at 160ºC using a fixed hold time, the solvent was removed. The crude product was then purified by filtration, recrystallization or flash chromatography unless otherwise stated.

[6-Bromo-2-(2,3,4-trifluorophenyl)imidazo[1,2-*a*]pyridin-3-yl]*iso*-propylamine (3a)
According to the general procedure, in a 2 mmol scale, using 2,3,4-trifluoro-benzaldehyde and *iso*-propyl isocyanide. Purification was done by recrystallization from EtOH. Compound **3a** was obtained as yellow crystals in a 33% yield (183 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (dd, *J*=1.9, 0.9 Hz, 1H), 7.61 (m, 1H), 7.42 (dd, *J*=9.4, 0.9 Hz, 1H), 7.22 (dd, *J*=9.4, 1.9 Hz, 1H), 7.10 (m, 1H), 3.20 (t, *J*=6.2 Hz, 1H), 3.12 (m, 1H), 1.00 (d, *J*=6.2 Hz, 6H). ^{19}F NMR (400 MHz, $CDCl_3$): δ -134.8- -134.9 (m, 1F), -136.0- -136,2 (m, 1F), -160.4- -160.6 (m, 1F). Elemental analysis, found: C 49.76; H 3.20; N 10.74. Calcd for $C_{16}H_{13}BrF_3N_3$: C 50.02; H 3.41; N 10.94.

4-(6-Bromo-3-cyclopentylaminoimidazo[1,2-*a*]pyridin-2-yl)-2-trifluoromethylphenol (3b)
According to the general procedure, in a 2 mmol scale, using 4-hydroxy-3-trifluoromethylbenzaldehyde and cyclopentyl isocyanide. Purification was done by column chromatography on silica gel (EtOAc:iso-hexane 2:3). Compound **3b** was obtained as light yellow crystals in a 20% yield (179 mg). ^{1}H NMR (400 MHz, $(CD_3)_2SO$: δ 8.51 (dd, *J*=1.9, 0.8 Hz, 1H), 8.44 (d, *J*=2.1 Hz, 1H), 8.27 (dd, *J*=8.7, 2.1 Hz, 1H), 7.47 (dd, *J*=9.5, 0.8 Hz, 1H), 7.28 (dd, *J*=9.5, 1.9 Hz, 1H), 7.08 (d, *J*=8.7 Hz, 1H), 4.81 (d, *J*=5.0 Hz, 1H), 3.52 (m, 1H), 1.53-1.75 (m, 4H), 1.38-1.53 (m, 4H). ^{13}C NMR (100.5 MHz, $(CD_3)_2SO$: δ 155.0, 139.0, 134.9, 131.7, 126.7, 126.0, 125.0, 124.7 (q, *J*=5.1 Hz), 124.2 (q, *J*=272.0 Hz), 123.1, 117.9, 117.0, 115.3 (q, *J*=29.5 Hz), 105.6, 58.5, 32.7, 23.2. Elemental analysis, found: C 51.58; H 4.02; N 9.38. Calcd for $C_{19}H_{17}BrF_3N_3O$: C 51.83; H 3.89; N 9.54.

4-(6-Bromo-3-cyclohexylaminoimidazo[1,2-*a*]pyridin-2-yl)-3-chlorophenol (3c)
According to the general procedure, in a 2 mmol scale, using 2-chloro-4-hydroxybenzaldehyde and cyclohexyl isocyanide. Purification was done by recrystallization

from EtOH. Compound **3c** was obtained as brown crystals in 60% (459 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (dd, *J*=1.9, 0.8 Hz, 1H), 7.49 (dd, *J*=9.4, 0.8 Hz, 1H), 7.22-7.28 (m, 2H), 6.84 (d, *J*=2.4 Hz, 1H), 6.60 (dd, *J*=8.5, 2.4 Hz, 1H), 3.23 (d, *J*=6.8 Hz, 1H), 2.67 (m, 1H), 1.45-1.70 (m, 5H), 0.95-1.17 (m, 5H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 159.3, 139.5, 135.6, 133.2, 132.7, 128.1, 126.7, 123.2, 122.3, 117.3, 117.1, 115.3, 107.3, 56.5, 33.9, 25.7, 24.7. Elemental analysis, found: C 54.12; H 4.86; N 10.12. Calcd for $C_{19}H_{19}BrClN_3O$: C 54.24; H 4.55; N 9.99.

[6-Bromo-2-(4-chloro-2-methanesulfonylphenyl)imidazo[1,2-*a*]pyridin-3-yl]cyclopentylamine (3d)

According to the general procedure, in a 1 mmol scale using 4-chloro-2-methylsulphonylbenzaldehyde and cyclopentyl isocyanide. Purification was done by recrystallization from EtOH. Compound **3d** was obtained as yellow crystals in a 62% yield (281 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (dd, *J*=1.9, 0.9 Hz, 1H), 8.22 (d, *J*=2.2 Hz, 1H), 7.68 (dd, *J*=8.2, 2.2 Hz, 1H), 7.52 (d, *J*=8.2 Hz, 1H), 7.47 (d, *J*=9.4 Hz, 1H), 7.32 (dd, *J*=9.4, 1.9 Hz, 1H), 3.57 (brs, 1H), 3.29 (m, 1H), 2.91 (s, 3H), 1.51-1.65 (m, 4H), 1.38-1.51 (m, 2H), 1.18-1.31 (m, 2H). ^{13}C NMR (100.5 MHz, $(CD_3)_2SO$): δ 141.3, 137.8, 135.0, 133.3, 133.0, 132.9, 132.0, 129.3, 128.4, 126.3, 123.0, 118.2, 106.0, 57.8, 45.5, 32.6, 23.0. Elemental analysis, found: C 48.57; H 4.06; N 8.90. Calcd for $C_{19}H_{19}BrClN_3O_2S$: C 48.68; H 4.09; N 8.96.

[2-(3,4-Bis-benzyloxyphenyl)-6-bromoimidazo[1,2-*a*]pyridin-3-yl]-(4-methoxyphenyl)amine (3e)

According to the general procedure, in a 2 mmol scale, using 3,4-bis-benzyloxybenzaldehyde and 4-methoxyphenyl isocyanide. The crude product was washed with EtOH and **3e** was obtained as white crystals in a 55% yield (570 mg). ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 8.14 (dd, *J*=1.9, 0.8 Hz, 1H), 7.90 (s, 1H), 7.73 (d, *J*=2.0 Hz, 1H), 7.56-7.60 (m, 2H), 7.27-7.45 (m, 10H), 7.08 (d, *J*=8.6 Hz, 1H), 6.78 (m, 2H), 6.47 (m, 2H), 5.14 (s, 2H), 4.96 (s, 2H), 3.63 (s, 3H). ^{13}C NMR (100.5 MHz, $(CD_3)_2SO$): δ 152.6, 148.0, 142.0, 139.9, 138.9, 137.9, 137.3, 137.1, 128.4, 128.3, 127.8, 127.7, 127.5, 127.4, 126.4, 124.4, 122.6, 119.7, 118.2, 115.1, 114.4, 114.1, 112.7, 106.1, 104.3, 70.0, 69.9, 55.3. Elemental analysis, found: C 67.13; H 4.89; N 6.78. Calcd for $C_{34}H_{28}BrN_3O_3$: C 67.33; H 4.65; N 6.93.

[6-Bromo-2-(3-fluoro-4-morpholin-4-yl-phenyl)imidazo[1,2-*a*]pyridin-3-yl]cyclopentylamine (3f)

According to the general procedure, in a 1 mmol scale, using 3-fluoro-4-(*N*-

morpholino)benzaldehyde and cyclopentyl isocyanide. Purification was done by recrystallization from EtOH. Compound **3f** was obtained as light yellow crystals in a 33% yield (150 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (dd, *J*=1.9, 0.8 Hz, 1H), 7.74-7.82 (m, 2H), 7.45 (dd, *J*=9.4, 0.8 Hz, 1H), 7.20 (dd, *J*=9.4, 1.9 Hz, 1H), 6.98 (m, 1H), 3.87-3.92 (m, 4H), 3.64 (m, 1H), 3.14-3.18 (m, 5H), 1.65-1.83 (m, 4H), 1.52-1.65 (m, 2H), 1.40-1.52 (m, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 155.4 (d, *J*=244.4 Hz), 139.7, 139.1 (d, *J*=8.6 Hz), 136.5, 128.4 (d, *J*=8.3 Hz), 127.4, 125.2, 123.0 (d, *J*=2.9 Hz), 122.6, 118.3 (d, *J*=3.6 Hz), 117.8, 114.8 (d, *J*=22.7 Hz), 106.6, 66.9, 58.9, 50.7 (d, *J*=3.2 Hz), 33.4, 23.6. Elemental analysis, found: C 57.35; H 5.32; N 12.19. Calcd for $C_{22}H_{24}BrFN_4O$: C 57.52; H 5.27; N 12.20.

[6-Bromo-2-(4-dimethylaminophenyl)imidazo[1,2-*a*]pyridin-3-yl]cyclopentylamine (3h)

According to the general procedure, in a 2 mmol scale, using 4-(*N*,*N*-dimethylamino)benzaldehyde and cyclopentyl isocyanide. Purification was done by recrystallization from EtOH. Compound **3h** was obtained as yellow crystals in a 72% yield (577 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (dd, *J*=2.0, 0.8 Hz, 1H), 7.85 (m, 2H), 7.42 (dd, *J*=9.4, 0.8 Hz, 1H), 7.14 (dd, *J*=9.4, 2.0 Hz, 1H), 6.78 (m, 2H), 3.65 (m, 1H), 3.19 (brs, 1H), 3.01 (s, 6H), 1.64-1.84 (m, 4H), 1.51-1.64 (m, 2H), 1.39-1.51 (m, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 150.1, 139.3, 137.7, 128.1, 127.1, 124.6, 122.8, 121.2, 117.4, 112.3, 106.4, 59.1, 40.5, 33.6, 23.8. Elemental analysis, found: C 59.98; H 5.70; N 13.97. Calcd for $C_{20}H_{23}BrN_4$: C 60.16; H 5.81; N 14.03.

[6-Bromo-2-(2,3-dimethoxyphenyl)imidazo[1,2-*a*]pyridin-3-yl]-(2-morpholin-4-yl-ethyl)amine (3i)

According to the general procedure, in a 2 mmol scale, using 2,3-dimethoxybenzaldehyde and 4-(2-isocyanoethyl)morpholine. Purification was done by column chromatography on silica gel (EtOAc:iso-hexane 2:3). Compound **3i** was obtained as a yellow solid 50% yield (461 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (dd, *J*=1.9, 0.8 Hz, 1H), 7.42 (dd, *J*=9.4, 0.8 Hz, 1H), 7.36 (dd, *J*=7.9, 1.5 Hz, 1H), 7.14 (dd, *J*=9.4, 1.9 Hz, 1H), 7.14 (m, 1H), 6.90 (dd, *J*=8.2, 1.5 Hz, 1H), 4.90 (t, *J*=6.5 Hz, 1H), 3.90 (s, 3H), 3.57 (s, 3H), 3.49-3.54 (m, 4H), 2.88 (m, 2H), 2.22 (m, 2H), 2.10-2.16 (m, 4H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 152.8, 146.1, 140.1, 132.9, 128.9, 128.7, 126.5, 124.8, 123.0, 122.9, 118.3, 111.4, 106.5, 66.9, 61.4, 57.9, 55.7, 53.6, 44.2. Elemental analysis, found: C 54.91; H 5.52; N 11.89. Calcd for $C_{21}H_{25}BrN_4O_3$: C 54.67; H 5.46; N 12.14.

[2-(2-Benzyloxy-3-methoxyphenyl)-6-bromoimidazo[1,2-*a*]pyridin-3-yl]cyclopentylamine (3j)

According to the general procedure, in a 2 mmol scale, using 2-benzyloxy-3-methoxybenzaldehyde and cyclopentyl isocyanide. Purification was done by column chromatography on silica gel (EtOAc:iso-hexane 2:3). Compound **3j** was obtained as a yellow solid in a 68% yield (670 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (dd, *J*=1.9, 0.9 Hz, 1H), 7.38-7.42 (m, 2H), 7.02-7.22 (m, 7H), 6.95 (dd, *J*=8.1, 1.5 Hz, 1H), 4.70 (s, 2H), 4.14 (d, *J*=8.8 Hz, 1H), 3.90 (s, 3H), 3.26 (m, 1H), 1.42-1.59 (m, 4H), 1.30-1.42 (m, 2H), 1.11-1.24 (m, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 153.0, 144.8, 140.0, 136.4, 134.4, 129.6, 128.9, 128.6, 128.1, 128.0, 126.5, 125.1, 123.1, 122.9, 118.2, 111.8, 106.3, 76.4, 59.4, 56.0, 33.1, 23.3. Elemental analysis, found: C 63.40; H 5.45; N 8.51. Calcd for $C_{26}H_{26}BrN_3O_2$: C 63.42; H 5.32; N 8.53.

2-(6-Bromo-3-*tert*-butylaminoimidazo[1,2-*a*]pyridin-2-yl)phenol (3k)

According to the general procedure, in a 2 mmol scale, using 2-hydroxybenzaldehyde and *tert*-butyl isocyanide. Purification was done by column chromatography on silica gel (EtOAc:iso-hexane 2:3). Compound **3k** was obtained as a yellow solid in a 19% yield (123 mg). ^{1}H NMR (400 MHz, CD_3OD, $CDCl_3$): δ 8.45-8.43 (m, 1H), 7.87 (dd, *J*= 7.77, 1.75 Hz, 1H), 7.40 (dt, *J*=9.39, 0.90, 1H), 7.30-7.29 (m, 2H), 6.98-6.90 (m, 2H), 1.05 (s, 9H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 155.2, 141.3, 138.9, 130.4, 130.2, 128.7, 124.1, 123.8, 121.2, 120.5, 117.7, 117.7, 107.3, 57.7, 29.8. Elemental analysis, found: C 55.66; H 5.14; N 11,44. Calcd for $C_{17}H_{18}BrN_3O$: C 56.68; H 5.04; N 11.66.

(2-Biphenyl-2-yl-6-bromoimidazo[1,2-*a*]pyridin-3-yl)cyclopentylamine (3l)

According to the general procedure, in a 2 mmol scale, using biphenyl-2-carbaldehyde and cyclopentyl isocyanide. Purification was done by recrystallization from EtOH. Compound **3l** was obtained as a yellow solid in a 66% yield (571 mg).^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04-8.00 (m, 1H), 7.84-7.77 (m, 1H), 7.50-7.40 (m, 4H), 7.30-7.22 (m, 5H), 7.15 (dd, *J*=1.97, 9.28 Hz, 1H), 3.10-3.02 (m, 1H), 1.52-1.22 (m, 8H), 1.02-0.90 (m, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 141.8, 140.5, 140.3, 138.6, 133.0, 131.9, 130.1, 128. 9, 128.9, 128.6, 128.0, 127.5, 127.0, 126.6, 123.0, 118.2, 106.6, 59.1, 33.1, 23.5. HRMS (ESI^+) Calcd for $C_{24}H_{23}BrN_3$ ($M+H^+$), 432.1075; Found, 432.1070.

[6-Bromo-2-(3-morpholin-4-yl-phenyl)imidazo[1,2-*a*]pyridin-3-yl]isopropylamine (3m)

According to the general procedure, in a 2 mmol scale, using 3-morpholin-4-yl-benzaldehyde and *iso*-propyl isocyanide. Purification was done by column chromatography on silica gel (EtOAc:iso-hexane 1:1). Compound **3m** was obtained as brown oil in 81% yield (319 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13-8.20 (m, 1H), 7.64-7.58 (m, 1H), 7.45-7.39 (m, 1H), 7.31-7.23 (m, 1H), 7.14-7.07 (m, 1H), 6.87-6.80 (m, 1H), 3.90-3.75 (m, 4H), 3.30-3.25 (m, 1H) 3.25-3.15 (m, 4H), 3.10 (s, 1H), 1.03 (d, *J*=6.30 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 151.6, 139.8, 138.0, 134.8, 129.2, 127.2, 125.4, 122.8, 118.5, 117.9, 115.0, 114.5, 106.5, 66.9, 49.3, 49.0, 23.4. HRMS (ESI^+) Calcd for $C_{20}H_{24}BrN_4O$ ($M+H^+$), 415.1133; Found, 415.1139.

[6-Bromo-2-(3-methoxyphenyl)imidazo[1,2-*a*]pyridin-3-yl]cyclopentylamine (3n)[1]

According to the general procedure, in a 2 mmol scale, using 3-methoxybenzaldehyde and cyclopentyl isocyanide. Purification was done by recrystallization from EtOH. Compound **3n** was obtained as yellow crystals in a 62% yield (489 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (dd, *J*=2.0, 0.8 Hz, 1H), 7.53-7.59 (m, 2H), 7.42 (dd, *J*=9.4, 0.8 Hz, 1H), 7.34 (m, 1H), 7.17 (dd, *J*=9.4, 2.0 Hz, 1H), 6.68 (ddd, *J*=8.2, 2.6, 1.0 Hz, 1H), 3.88 (s, 3H), 3.63 (m, 1H), 3.14 (d, *J*=4.2 Hz, 1H), 1.63-1.80 (m, 4H), 1.50-1.63 (m, 2H), 1.37-1.50 (m, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 160.0, 139.8, 137.5, 135.4, 129.6, 127.5, 126.1, 122.9, 119.7, 118.2, 113.9, 112.5, 106.8, 59.3, 55.5, 33.6, 23.7. Elemental analysis, found: C 58.91; H 5.08; N 10.86. Calcd for $C_{19}H_{20}BrN_3O$: C 59.08; H 5.22; N 10.88.

(6-Bromo-2-phenyl-imidazo[1,2-*a*]pyridin-3-yl)phenethylamine (3o)

According to the general procedure, in a 2 mmol scale, using benzaldehyde and 2-phenylethyl isocyanide. Purification was done by recrystallization from EtOH. Compound **3o** was obtained as light yellow crystals in a 58% yield (312 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (dd, *J*=1.9, 0.9 Hz, 1H), 7.74 (m, 2H), 7.37 (d, *J*=9.4 Hz, 1H), 7.15-7.34 (m, 8H), 7.10 (dd, *J*=9.4, 1.9 Hz, 1H), 3.25 (m, 2H), 3.12 (m, 1H), 2.86 (t, *J*=6.5 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 139.9, 139.0, 136.7, 133.7, 129.1, 129.0, 128.9, 127.9. 127.4, 127.1, 127.0, 126.2, 122.7, 118.2, 106.8, 49.1, 36.8. Elemental analysis, found: C 64.18; H 4.51; N 10.56. Calcd for $C_{21}H_{18}BrN_3$: C 64.30; H 4.62; N 10.71.

(6-Bromo-2-phenylimidazo[1,2-*a*]pyridin-3-yl)cyclopentylamine (3p)[1]

According to the general procedure, in a 0.5 mmol scale, using benzaldehyde and cyclopropyl isocyanide. The crude product was washed with cold EtOH and **3p** was obtained as a white solid in a 47% yield (84 mg). ^{1}H NMR ($CDCl_3$) δ 8.24 (dd, *J*=1.9 Hz, 0.9 Hz, 1H), 7.99 (m,

2H), 7.46 (m, 2H), 7.43 (dd, *J*=9.4 Hz, 0.9 Hz, 1H), 7.43 (m, 1H), 7.18 (dd, *J*=9.4 Hz, 1.9 Hz, 1H), 3.46 (m, 1H), 3.11 (d, *J*=4.6 Hz, 1H), 1.81-1.66 (m, 4H), 1.60-1.52 (m, 2H), 1.48-1.40 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 140.1, 137.9, 134.1, 128.7, 127.8, 127.4, 127.3, 126.0, 122.9, 118.3, 106.7, 59.3, 33.6, 32.7. Elemental analysis, found: C 60.55; H 5.02; N 11.76. Calcd for $C_{18}H_{18}BrN_3$: C 60.68; H 5.09; N 11.79.

2-[4-(6-Bromo-3-isopropylaminoimidazo[1,2-*a*]pyridin-2-yl)phenoxy]ethanol (4a)

According to the general procedure, in a 1 mmol scale, using 4-(2-hydroxyethoxy)benzaldehyde and *iso*-propyl isocyanide. Purification was done by column chromatography on silica gel (EtOAc:iso-hexane 2:3). Compound **4a** was obtained as a yellow solid in 46% yield (155 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (dd, *J*=1.9, 0.8 Hz, 1H), 7.92 (m, 2H), 7.45 (dd, *J*=9.4, 0.8 Hz, 1H), 7.19 (dd, *J*=9.4, 1.9 Hz, 1H), 6.96 (m, 2H), 4.12 (m, 2H), 3.98 (m, 2H), 3.36 (m, 1H), 3.18 (brs, 1H), 2.45 (brs, 1H), 1.08 (d, *J*=6.3 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 158.3, 139.8, 137.6, 128.4, 127.3, 126.7, 124.5, 122.8, 117.7, 114.6, 106.5, 69.2, 61.4, 49.0, 23.3. HRMS (ESI^+) Calcd for $C_{18}H_{21}BrN_3O_2$ ($M+H^+$), 390.0817; Found, 390.0814.

2-{4-[6-Bromo-3-(4-fluorophenylamino)imidazo[1,2-*a*]pyridin-2-yl]phenoxy}ethanol (4b)

According to the general procedure, in a 1 mmol scale, using 4-(2-hydroxyethoxy)benzaldehyde and 1-fluoro-4-isocyanobenzene. Purification was done by column chromatography on silica gel (EtOAc:iso-hexane 4:1). Purification was done by recrystallization from EtOH. Compound **4b** was obtained as yellow crystals in a 39% yield (128 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 8.12 (dd, *J*=1.94, 0.90 Hz, 1H), 7.90-795 (m, 2H), 7.57 (dd, *J*=9.4, 0.90 Hz, 1H), 7.38 (dd, *J*=9.4, 1.95 Hz, 1H), 7.00-6.90 (m, 4H), 6.53-6.45 (m, 2H), 4.83 (t, *J*=5.47 Hz, 1H), 4.00-3.95 (m, 2H), 3.70-3.65 (m, 2H. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 159.2, 156.5 (d, *J*=233.5 Hz), 142.3 (d, *J*=1.81 Hz), 140.8, 139.1, 128.5, 128.4, 126.2, 123.3, 119.3, 118.9, 116.7 (d, *J*=22,3 Hz), 115.2, 114.8 (d, *J*=7.50 Hz), 106.8, 70.2, 60.2. ^{19}F NMR (400 MHz, $CDCl_3$): δ -125.9- -125.6 (m, 1F). HRMS (ESI^+) Calcd for $C_{21}H_{18}BrFN_3O_2$ ($M+H^+$), 442.0566; Found, 442.0572.

2-[4-(6-Bromo-3-cyclopentylaminoimidazo[1,2-*a*]pyridin-2-yl)phenoxy]ethanol (4c)

According to the general procedure, in a 1 mmol scale, using 4-(2-hydroxyethoxy)benzaldehyde and cyclopentyl isocyanide. Purification was done by recrystallization from EtOH. Compound **4c** was obtained as a yellow solid in 45% yield (353 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (dd, *J*=1.9, 0.9 Hz, 1H), 7.93 (m, 2H), 7.43 (dd,

J=9.4, 0.9 Hz, 1H), 7.18 (dd, *J*=9.4, 1.9 Hz, 1H), 6.97 (m, 2H), 4.12 (m, 2H), 3.95 (m, 2H), 3.62 (m, 1H), 3.11 (brs, 1H), 2.49 (brs, 1H), 1.62-1.81 (m, 4H), 1.49-1.61 (m, 2H), 1.35-1.49 (m, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 158.4, 139.4, 137.0, 128.5, 127.6, 126.2, 125.1, 122.8, 117.5, 114.6, 106.8, 69.2, 61.4, 59.0, 33.4, 23.6. Elemental analysis, found: C 57.43; H 5.53; N 9.94. Calcd for $C_{20}H_{22}BrN_3O_2$: C 57.70; H 5.33; N 10.09.

2-[4-(6-Bromo-3-propylaminoimidazo[1,2-*a*]pyridin-2-yl)phenoxy]ethanol (4d)

Propylamine (1.00 mL, 12.2 mmol) and ethyl formiate (3.00 mL, 36.5 mmol) was loaded in a 5 ml smith vial. The reaction mixture was irridated with microwaves to 130°C for 30 min. The volatile starting materials were evaporated giving the crude *N*-propylformamide as slightly yellow oil. 0.213 g (2.45 mmol) of the crude *N*-propylformamide was dissolved in dry THF (5 mL) and triethyl amine (1.70 mL, 12.2 mmol) was added. The mixture was cooled to –78°C followed by addition of a solution of phosphoryl chloride (0.270 mL, 2.96 mmol) in 2 ml THF. The reaction was stirred at –78°C for 15 min and thereafter 30 min at rt. Water (15 mL) was added and the mixture was extracted with diethyl ether (2×15 mL). The combined organic layers were dried with $MgSO_4$ and evaporated to orange oil. Three component reaction in 2.5 mmol scale according to the general procedure with 4-(2-hydroxyethoxy)benzaldehyde and crude *n*-propyl isocyanide. Purification was done by column chromatography on silica gel (EtOAc:iso-hexane 1:1 to 9:1). Compound **4d** was obtained as a yellow solid in 29% yield (164 mg). ^{1}H NMR (400 MHz, $CDCl_3$:D_2O, 1:0.01): δ 8.17 (dd, *J*=1.9, 0.9 Hz, 1H), 7.88 (m, 2H), 7.42 (dd, *J*=9.4, 0.9 Hz, 1H), 7.17 (dd, *J*=9.4, 1.9 Hz, 1H), 7.00 (m, 2H), 4.13 (m, 2H), 3.98 (m, 2H), 2.99 (t, *J*=7.1 Hz, 2H), 1.62 (m, 2H), 0.99 (t, *J*=7.4 Hz, 3H). ^{13}C NMR (100.5 MHz, $CDCl_3$:D_2O, 1:1): δ 158.4, 139.9, 136.8, 128.5, 127.2, 127.1, 125.9, 122.6, 118.1, 114.9, 106.6, 69.4, 61.6, 50.3, 24.1, 11.7. Elemental analysis, found: C 55.81; H 5.48; N 10.51. Calcd for $C_{18}H_{20}BrN_3O_2$: C 55.40; H 5.17; N 10.77.

2-[4-(6-Bromo-3-pentylaminoimidazo[1,2-*a*]pyridin-2-yl)phenoxy]ethanol (4e)

According to the general procedure, in a 2 mmol scale, using 4-(2-hydroxyethoxy)benzaldehyde and *n*-pentyl isocyanide. Purification was done by recrystallization from EtOH. Compound **4e** was obtained as yellow crystals in 68% yield (540 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (dd, *J*=1.9, 0.7 Hz, 1H), 7.88 (m, 2H), 7.46 (dd, *J*=9.4, 0.7 Hz, 1H), 7.19 (dd, *J*=9.4, 1.9 Hz, 1H), 6.97 (m, 2H), 4.02 (m, 2H), 3.98 (m, 2H), 3.25 (brs, 1H), 3.00 (m, 2H), 1.57 (m, 2H), 1.23-1.45 (m, 4H), 0.90 (t, *J*=7.1 Hz). ^{13}C NMR

(100.5 MHz, $CDCl_3$): δ 158.4, 139.4, 136.1, 128.3, 127.5, 126.3, 125.8, 122.6, 117.6, 114.8, 106.8, 69.3, 61.4, 48.2, 30.4, 29.2, 22.5, 14.0. Elemental analysis, found: C 57.39; H 5.79; N 9.94. Calcd for $C_{20}H_{24}BrN_3O_2$: C 57.42; H 5.78; N 10.04.

2-{[4-(6-Bromo-3-butylaminoimidazo[1,2-*a*]pyridin-2-yl)phenyl]methylamino}ethanol (5a)

According to the general procedure, in a 1 mmol scale, using 4-[N-(2-hydroxy-ethyl)-N-methylamino]benzaldehyde and *n*-butyl isocyanide. Purification was done by recrystallization from EtOH. Compound **5a** was obtained as a yellow solid after purification by preparative HPLC and freeze drying in 58% yield (193 mg). ^{1}H NMR (400 MHz, CD_3OD:$CDCl_3$, 2:1): δ 8.28 (dd, *J*=1.9, 0.8 Hz, 1H), 7.77 (m, 2H), 7.34 (dd, *J*=9.4, 0.8 Hz, 1H), 7.23 (dd, *J*=9.4, 1.9 Hz, 1H), 6.81 (m, 2H), 3.75 (m, 2H), 3.51 (m, 2H), 3.04 (s, 3H), 2.97 (t, *J*=7.1 Hz, 2H), 1.52 (m, 2H), 1.39 (m, 2H), 0.89 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, CD_3OD:$CDCl_3$, 2:1): δ 150.0, 140.0, 137.0, 128.8, 128.3, 126.6, 123.4, 121.3, 117.0, 112.7, 107.4, 59.8, 55.2, 48.3, 39.2, 33.4, 20.8, 14.2. Elemental analysis, found: C 57.34; H 6.07; N 13.28. Calcd for $C_{20}H_{25}BrN_4O$: C 57.56; H 6.04; N 13.42.

[4-(6-Bromo-3-butylaminoimidazo[1,2-*a*]pyridin-2-yl)phenoxy]acetic acid (5b)

According to the general procedure, in a 1 mmol scale, using (4-formylphenoxy)acetic acid and *n*-butyl isocyanide. Purification was done by column chromatography on silica gel (EtOAc:MeOH:HCOOH 1:1:0.02). Compound **5b** was obtained as yellow crystals in 36% yield (151 mg). ^{1}H NMR (400 MHz, $(CD_3)_2SO$:$CDCl_3$, 1:1): δ 8.38 (dd, *J*=1.9, 0.8 Hz, 1H), 8.03 (m, 2H), 7.35 (dd, *J*=9.4, 0.8 Hz, 1H), 7.13 (dd, *J*=9.4, 1.9 Hz, 1H), 6.89-6.94 (m, 2H), 4.62 (s, 2H), 4.58 (m 1H), 2.85-2.95 (m, 2H), 1.44-1.53 (m, 2H), 1.29-1.39 (m, 2H), 0.85 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, $SO(CD_3)_2$:$CDCl_3$, 1:1): δ 168.6, 155.5, 137.2, 133.4, 126.3, 125.8, 125.2, 124.6, 121.2, 116.0, 112.7, 104.0, 63.1, 45.8, 30.9, 18.3, 12.3. Elemental analysis, found: C 54.85; H 5.01; N 9.89. Calcd for $C_{19}H_{20}BrN_3O_3$: C 54.56; H 4.82; N 10.05.

2-[[4-(6-Bromo-3-butylaminoimidazo[1,2-*a*]pyridin-2-yl)phenyl]-(2-hydroxyethyl)amino]ethanol (5c)

According to the general procedure, in a 1 mmol scale, using 4-[*N*,*N*-bis-(2-hydroxyethyl)amino]benzaldehyde and *n*-butyl isocyanide. Purification was done by recrystallization from EtOH. Compound **5c** was obtained as a yellow solid after purification by preparative HPLC and freeze drying in 32% yield (114 mg). ^{1}H NMR (400 MHz, CD_3OD): δ 8.35 (dd, *J*=1.9, 0.9 Hz, 1H), 7.79-7.83 (m, 2H), 7.35 (dd, *J*=9.4, 0.8 Hz, 1H), 7.27 (dd, *J*=9.4, 1.9 Hz, 1H), 6.81-6.85 (m, 2H), 3.76 (t, *J*=6.1 Hz, 4H), 3.59 (t, *J*=6.1 Hz,

4H), 2.95 (t, *J*=7.0 Hz, 2H), 1.54-1.44 (m, 2H), 1.33-1.44 (m, 2H), 0.89 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, CD_3OD): δ 149.0, 140.7, 137.9, 129.4, 128.4, 127.3, 123.9, 122.3, 117.6, 113.0, 107.5, 60.4, 55.0, 48.5, 33.8, 21.3, 14.3. Elemental analysis, found: C 56.25; H 6.10; N 12.38. Calcd for $C_{21}H_{27}BrN_4O_2$: C 56.38; H 6.08; N 12.52.

[6-Bromo-2-(4-propoxyphenyl)imidazo[1,2-*a*]pyridin-3-yl]butylamine (5d)

According to the general procedure, in a 1 mmol scale, using 4-propoxybenzaldehyde and *n*-butyl isocyanide. Purification was done by column chromatography on silica gel (EtOAc:Isohexane 2:3). Compound **5d** was obtained as a yellow solid 82% yield (330 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (dd, *J*=1.9, 0.8 Hz, 1H), 7.89-7.90 (m, 2H), 7.41 (dd, *J*=9.4, 0.8 Hz, 1H), 7.15 (dd, *J*=9.4, 1.9 Hz, 1H), 6.96-7.01 (m, 2H), 3.97 (t, *J*=6.6 Hz, 2H), 2.99-3.09 (m, 3H), 1.78-1.89 (m, 2H), 1.54-1.63 (m, 2H), 1.38-1.48 (m, 2H), 1.06 (t, *J*=7.4 Hz, 3H), 0.94 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 159.0, 139.9, 137.0, 128.4, 127.0, 126.5, 125.9, 122.65, 118.1, 114.9, 106.5, 69.7, 48.2, 32.9, 22.8, 20.3, 14.1, 10.7. Elemental analysis, found: C 59.88; H 6.16; N 10.48. Calcd for $C_{20}H_{24}BrN_3O$: C 59.71; H 6.01; N 10.44.

2-[4-(6-Bromo-3-butylaminoimidazo[1,2-*a*]pyridin-2-yl)phenoxy]acetamide (5e)

According to the general procedure, in a 1 mmol scale, using 2-(4-formylphenoxy)acetamide and *n*-butyl isocyanide. Purification was done by recrystallization from MeOH. Compound **5e** was obtained as a yellow solid in 33% yield (138 mg). ^{1}H NMR (400 MHz, $(CD_3)_2SO$:$CDCl_3$, 1:1): δ 7.78 (dd, *J*=1.9, 0.8 Hz, 1H), 7.46-7.50 (m, 2H), 6.77 (dd, *J*=9.4, 0.8 Hz, 1H), 6.59 (dd, *J*=9.4, 1.9 Hz, 1H), 6.45-6.41 (m, 2H), 3.90 (s, 2H), 3.71 (t, *J*=6.0 Hz, 1H), 2.35-2.42 (m, 2H), 0.92-1.01 (m, 2H), 0.76-0.86 (m, 2H), 0.32 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, $(CD_3)_2SO$:$CDCl_3$, 1:1): δ 169.3, 155.8, 138.0, 134.2, 127.1, 126.7, 125.6, 125.3, 121.8, 116.5, 113.5, 104.7, 66.0, 46.5, 31.5, 18.9, 12.8. Elemental analysis, found: C 54.41; H 5.21; N 13.20. Calcd for $C_{19}H_{21}BrN_4O_2$: C 54.69; H 5.07; N 13.43.

{6-Bromo-2-[4-(2-dimethylaminoethoxy)phenyl]imidazo[1,2-*a*]pyridin-3-yl}butylamine (5f)

According to the general procedure, in a 1 mmol scale, using 4-(2-dimethylaminoethoxy)benzaldehyde and *n*-butyl isocyanide. Purification was done by recrystallization from MeOH:EtOAc. Compound **5f** was obtained as a yellow solid in 30% yield (129 mg). ^{1}H NMR (400 MHz, $(CD_3)_2SO$:$CDCl_3$, 1:1): δ 8.39 (dd, *J*=1.9, 0.8 Hz, 1H), 8.04 (m, 2H), 7.32 (dd, *J*=9.4, 0.8 Hz, 1H), 7.13 (dd, *J*=9.4, 1.9 Hz, 1H), 6.97 (m, 2H), 4.54

(t, *J*=6.0 Hz, 1H), 4.25-4.30 (m, 2H), 3.13-3.27 (m, 2H), 2.89 (m, 2H), 2.66 (s, 6H), 1.43-1.53 (m, 2H), 1.28-1.39 (m, 2H), 0.84 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, $(CD_3)_2SO$:$CDCl_3$, 1:1): δ 156.0, 137.3, 133.6, 126.4, 125.5, 125.2, 124.6, 121.3, 116.0, 112.8, 104.0, 63.3, 55.7, 45.9, 43.4, 30.9, 18.4, 12.4. HRMS (ESI$^+$) Calcd for $C_{21}H_{28}BrN_4O$ (M+H$^+$), 431.1446; Found, 431.1461.

[4-(6-Bromo-3-butylaminoimidazo[1,2-*a*]pyridin-2-yl)phenoxy]acetonitrile (5g)

According to the general procedure, in a 2 mmol scale, using (4-formylphenoxy)acetonitrile and *n*-butyl isocyanide. Purification was done by recrystallization from EtOH. Compound **5g** was obtained as a yellow solid after purification by preparative HPLC and freeze drying in 55% yield (396 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (dd, *J*=2.0, 0.9 Hz, 1H), 7.93-7.97 (m, 2H), 7.42 (dd, *J*=9.4, 0.9 Hz, 1H), 7.18 (dd, *J*=9.4, 2.0 Hz, 1H), 7.02-7.06 (m, 2H), 4.80 (s, 2H), 3.14 (m, 1H), 3.00 (dd, *J*=6.9, 12.8 Hz, 2H), 1.52-1.62 (m, 2H), 1.36-1.47 (m, 2H), 0.92 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 156.0, 139.5, 135.6, 128.6, 128.5, 127.5, 126.1, 122.6, 117.8, 115.1, 115.0, 106.8, 53.6, 47.9, 32.8, 20.1, 13.9. Elemental analysis, found: C 57.02; H 4.92; N 13.94. Calcd for $C_{19}H_{19}BrN_4O$: C 57.15; H 4.80; N 14.03.

2-[3-(6-Bromo-3-butylaminoimidazo[1,2-*a*]pyridin-2-yl)phenoxy]ethanol (5h)

According to the general procedure, in a 1.3 mmol scale, using 3-hydroxyethyleneoxybenzaldehyde and *n*-butyl isocyanide. Purification was done by column chromatography on silica gel (EtOAc:iso-hexane 7:3). Compound **5h** was obtained as a yellow solid after purification by preparative HPLC and freeze drying in 37% yield (187 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (dd, *J*=1.9, 0.8 Hz, 1H), 7.52 (dd, *J*=2.5, 1.6 Hz, 1H), 7.43-7.49 (m, 2H), 7.30 (m, 1H), 7.18 (dd, *J*=9.5, 1.9 Hz, 1H), 6.85 (ddd, *J*=8.2, 2.6, 0.9, 1H), 4.15 (t, *J*=4.6 Hz, 2H), 3.97 (t, *J*=4.6 Hz, 2H), 3.53 (brs, 2H), 3.00 (t, *J*=7.1 Hz, 2H), 1.50-1.60 (m, 2H), 1.35-1.46 (m, 2H), 0.91 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 159.0, 139.4, 135.8, 134.8, 129.6, 127.6, 126.8, 122.6, 119.5, 117.8, 114.4, 112.9, 106.9, 69.3, 61.3, 48.0, 32.7, 20.1, 13.9. HRMS (ESI$^+$) Calcd for $C_{19}H_{23}BrN_3O_2$ (M+H+), 404.0974; Found, 404.0977.

5-(6-Bromo-3-butylaminoimidazo[1,2-*a*]pyridin-2-yl)-2-methoxyphenol (5i)

According to the general procedure, in a 2 mmol scale, using 3-hydroxy-4-methoxybenzaldehyde and *n*-butyl isocyanide. Purification was done by recrystallization from EtOH. Compound **5i** was obtained as yellow crystals in 43% yield (353 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (dd, *J*=1.9, 0.9 Hz, 1H), 7.54 (d, *J*=2.1 Hz, 1H), 7.43-7.47 (m, 2H), 7.19

(dd, *J*=9.5, 1.9 Hz, 1H), 6.91 (d, *J*=8.5 Hz, 1H), 3.90 (s, 3H), 3.25 (m, 1H), 3.00 (q, *J*=6.7 Hz, 2H), 1.56 (m, 2H), 1.41 (m, 2H), 0.92 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 146.6, 145.9, 139.3, 136.0, 127.4, 126.6, 126.0, 122.6, 119.1, 117.6, 113.3, 111.0, 106.8, 55.9, 48.0, 32.7, 20.2, 13.9. Elemental analysis, found: C 55.53; H 5.30; N 10.74. Calcd for $C_{18}H_{20}BrN_3O_2$: C 55.40; H 5.17; N 10.77.

3-(6-Bromo-3-(butylamino)imidazo[1,2-*a*]pyridin-2-yl)phenol (5j)

According to the general procedure, in a 1 mmol scale, using 3-hydroxy benzaldehyde and *n*-butyl isocyanide. Compound **5j** was obtained as a yellow solid in 42% yield (150 mg). ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 9.33 (s, 1H), 8.47 (dd, *J*=1.9, 0.9 Hz, 1H), 7.54-7.57 (m, 2H), 7.43 (dd, *J*=9.3, 0.9 Hz, 1H), 7.22 (dd, *J*=9.5, 1.9 Hz, 1H), 7.18 (t, *J*=8.2 Hz, 1H), 6.66 (ddd, *J*=8.1, 2.3, 1.2 Hz, 1H), 4.78 (t, *J*=6.1 Hz, 1H), 2.89 (q, *J*=6.4 Hz, 2H), 1.42-1.49 (m, 2H), 1.26-1.35 (m, 2H), 0.81 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100.5 MHz, $(CD_3)_2SO$): δ 158.0, 139.1, 136.1, 135.1, 129.9, 128.4, 127.0, 123.5, 118.7, 118.1, 114.9, 114.2, 106.2, 47.9, 32.9, 20.4, 14.5. Elemental analysis, found: C 57.10; H 5.16; N 11.62. Calcd for $C_{17}H_{18}BrN_3O$: C, 56.68; H, 5.04; N, 11.66.

3-(6-Bromo-3-(butylamino)imidazo[1,2-*a*]pyridin-2-yl)benzoic acid (5k)

According to the general procedure, in a 1 mmol scale, using 3-formyl benzoic acid and *n*-butyl isocyanide. After filtration compound **5k** was obtained as an off white solid in 60% yield (233 mg). ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 12.96 (s, 1H), 8.78 (t, *J*=1.8 Hz, 1H), 8.55 (dd, *J*=2.0, 0.9 Hz, 1H), 8.35 (dt, *J*=7.8, 1.3 Hz, 1H), 7.84 (dt, *J*=7.9, 1.3 Hz, 1H), 7.54 (t, *J*=7.8 Hz, 1H), 7.47 (dd, *J*=9.4, 0.9 Hz, 1H), 7.27 (dd, *J*=9.4, 1.9 Hz, 1H), 4.90 (t, *J*=6.1 Hz, 1H), 2.90 (q, *J*=6.7 Hz, 2H), 1.42-1.52 (m, 2H), 1.27-1.37 (m, 2H), 0.81 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (100.5 MHz, $(CD_3)_2SO$): δ 168.1, 139.5, 135.2, 134.0, 131.7, 131.1, 129.3, 128.9, 128.5, 128.1, 127.5, 123.8, 118.8, 106.5, 48.0, 32.9, 20.4, 14.5. Elemental analysis, found: C 55.88; H 4.83; N 10.77. Calcd for $C_{18}H_{18}BrN_3O_2$: C, 55.68; H, 4.67; N, 10.82.

4-(6-Bromo-3-butylaminoimidazo[1,2-*a*]pyridin-2-yl)-2-methoxyphenol (5l)

According to the general procedure, in a 2 mmol scale, using 4-hydroxy-3-methoxybenzaldehyde and *n*-butyl isocyanide. Compound **5l** was obtained as a light yellow solid in 38% yield (0.282 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (dd, *J* = 0.8, 1.9 Hz, 1H), 7.58 (d, *J* = 1.9 Hz, 1H), 7.42 (dd, *J* = 0.8, 9.4 Hz, 1H), 7.39 (dd, *J* = 1.9, 8.2 Hz, 1H), 7.16 (dd, *J* = 1.9, 9.4 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 6.39 (br. s., 1H), 3.93 (s, 3H), 2.96 - 3.12 (m, 2H), 1.85 (br. s, 1H), 1.52 - 1.66 (m, 2H), 1.35 - 1.49 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100.5 MHz, $CDCl_3$): δ 147.2, 145.7, 139.8, 137.0, 127.1, 126.4, 126.0, 122.6, 119.9, 118.0, 114.6, 110.2, 106.6, 56.2, 48.1, 33.0, 20.4, 14.1. HRMS (ESI^+) Calcd for $C_{18}H_{20}BrN_3O_2$ ($M+H^+$), 390.0817; Found, 390.0810.

X-ray crystallography

Table S4. Statistics for X-ray data collection and refinement.

Data collection[a]	
Cell axial lengths (Å)	132.6, 227.4, 202.4
Space group	$C222_1$
Molecules in asymmetric unit	6
Resolution range (Å)	20–2.0 (2.1–2.0)
No. of reflections measured	802,559 (91,063)
No. of unique reflections	202,868 (26,661)
Average multiplicity	4.0 (3.4)
Completeness (%)	99.1 (96.4)
R_{meas}[b]	7.8 (39.0)
$\langle I/\sigma I \rangle$	15.5 (4.7)
Wilson B-factor (Å^2)	27.1
Refinement statistics	
Resolution range (Å)	20–2.0
No. of reflections used in working set	192,509
No. of reflections for R_{free} calculation	10,190
R-value, R_{free} (%)	16.7, 19.8
No. of non-hydrogen atoms	24,804
No. of solvent molecules	1,926
Mean B-factor, protein atoms (Å^2)	20.8
Mean B-factor, ligand (**5b**) atoms (Å^2)	44.6
Mean B-factor, MSO-P atoms (Å^2)	16.1
Mean B-factor, phosphate atoms (Å^2)	22.5
Mean B-factor, magnesium ions (Å^2)	23.0
Mean B-factor, solvent atoms (Å^2)	28.9
Mean B-factor, chloride ions (Å^2)	22.9
Ramachandran plot outliers (%)[c]	1.06
r.m.s. deviation from ideal bond length (Å)[d]	0.014
r.m.s. deviation from ideal bond angle (°)[d]	1.43

[a] Values in parentheses are for the highest resolution shell.
[b] The multiplicity-weighted value, as defined by Diederichs and Karplus.[17]
[c] Calculated using strict-boundary Ramachandran plot.[18]
[d] Using the parameters of Engh and Huber.[19]

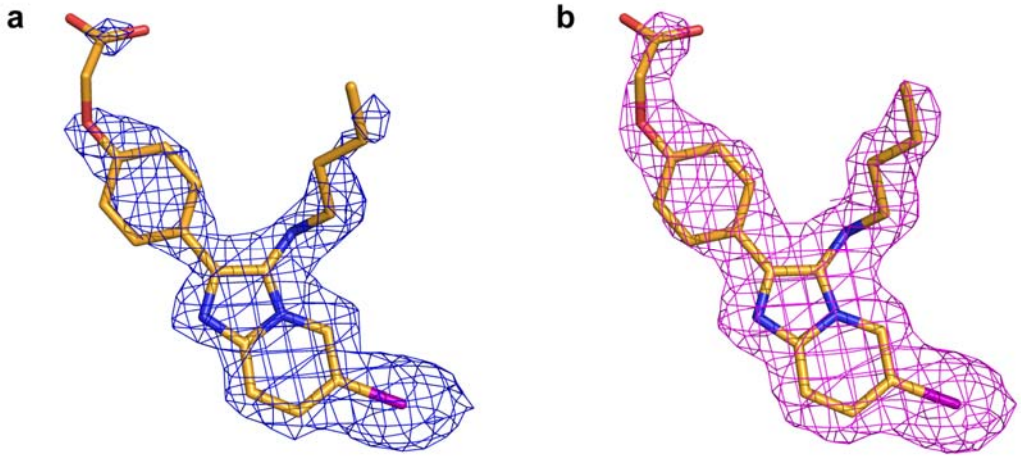

Figure S3. (a) Electron density ($2|F_o|-|F_c|$ map) for **5b** in the A molecule, contoured at 1 σ (0.35 e/Å^3). (b) 6-fold averaged electron density contoured at 1 σ (where 1 σ = 0.25 e/Å^3); map is NCS averaged in Coot using the A molecule as reference.

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