Imidazo[1,2-*a*]pyridin-3-amines as potential HIV-1 non-nucleoside reverse transcriptase inhibitors.

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Abstract

During random screening of a small in-house library of compounds, certain substituted imidazo[1,2-*a*]pyridines were found to be weak allosteric inhibitors of HIV-1 reverse transcriptase (RT). A library of these compounds was prepared using the Groebke reaction and a subset of compounds prepared from 2-chlorobenzaldehyde, cyclohexyl isocyanide and a 6-substituted 2-aminopyridine showed good inhibitory activity in enzymatic (RT) and HIV anti-infectivity MAGI whole cell assays. The compound showing the best anti-HIV-1 IIIB whole cell activity (MAGI IC_{50} = 0.18 μM, IC_{90} = 1.06 μM), along with a good selectivity index (>800), was 2-(2-chlorophenyl)-3-(cyclohexylamino)imidazo[1,2-*a*]pyridine-5-carbonitrile **38**.

Keywords

Groebke reaction; imidazo[1,2-*a*]pyridine; NNRTI; reverse transcriptase; antiretroviral; HIV

1. Introduction

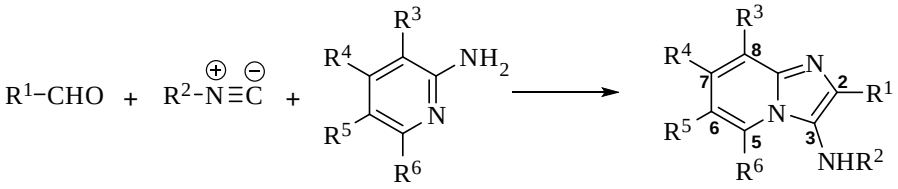
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are indispensable first-line drugs in the fight against HIV-AIDS. One advantage of this class is the excellent therapeutic window,[1] making the approved NNRTIs among the least toxic of the clinically approved antiretrovirals.[2] These drugs act by binding to a lipophilic, non-substrate binding pocket located about 10Å from the substrate binding site. Binding induces conformational changes in the catalytic site, slowing catalytic activity markedly.[3] About fifty structurally diverse classes of NNRTIs are known.[4] The first generation of NNRTIs (exemplified by nevirapine and delavirdine) are sensitive to the development of drug resistance and even a single amino acid mutation in the NNRTI binding region of RT results in a loss of compound efficacy. The second generation NNRTI efavirenz, however, maintains activity against a number of different NNRTI mutants. The most recently approved NNRTI etravirine remains active against a wide range of NNRTI mutants, and this is attributed to the molecule's ability to adopt multiple conformations in the RT binding pocket.[5] Recent literature indicates the ongoing interest in the design and development of novel NNRTI scaffolds capable of similar activity.[6-12]

Imidazo[1,2-*a*]pyridines have been shown to possess a broad range of biological activities and have been investigated for treatment of conditions such as gastric disease,[13,14] heart disease,[15] migraines[16] and viral diseases,[17-21] amongst others. The pharmacology of these compounds has also been extensively investigated.[22] As part of our ongoing interest in the biological activity of these compounds, a small in-house library of compounds was screened against HIV-1 RT, leading to identification of *N*-cyclohexyl-2-isopropylimidazo[1,2-*a*]pyridin-3-amine **1** (Table 1) as a possible NNRTI. This compound was reasonably active against wild-type RT in an enzymatic assay, but poorly active in a whole cell anti-HIV infectivity assay. Based on this structure, a larger library of imidazo[1,2-*a*]pyridines was prepared and screened in this preliminary investigation for improved activity against wt RT,[23] selected examples of which are presented below.

2. Results and Discussion

2.1 Chemistry

Compounds were prepared using the multi-component Groebke coupling reaction[24, 25] between an aldehyde, isocyanide and 2-aminopyridine (Scheme 1). In this study, the reactions were catalysed by Montmorillonite K-10 clay using either conventional heating or microwave conditions.[26]



Scheme 1. Groebke reaction

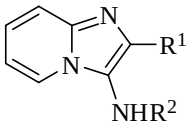
Using Groebke methodology, three distinct zones can be varied in the imidazo[1,2-*a*]pyridine scaffold: the "aldehydic" region that generates R^1 at C-2, the R^2 "isocyanide" region at C-3 and positions R^3-R^6, around the 2-aminopyridine ring (Scheme 1). Commercially available 2-aminopyridines, aldehydes and isocyanides with suitable substituents were used where possible, while certain 2-aminopyridines and isocyanides were prepared by standard methods. Yields from the Groebke reaction varied widely (13-83%).

2.2. Biological Results

The inhibitory activity of compounds was assessed using a colorimetric HIV-1 RT assay. Percentage residual RT enzyme activity after incubation with 50 μM test compound was determined relative to untreated controls. During this investigation, percentage residual activity was used as an initial guide to compound activity (Table 1). This approach was found to be reasonable for the set of compounds under evaluation (*vide infra*).

Isopropyl-substituted product **1** was identified as a potential NNRTI (41% residual RT activity) in the original screen. Simple variation of the isocyanide used in the Groebke reaction to vary the identity of the pendant amino group, exemplified by compounds **2** - **5**, afforded only a marginal improvement in the case of cyclopentylamine **4**. Other variations tried, including various simple aryl, linear- or branched aliphatic groups, showed no significant RT activity (data not shown). The cyclohexyl derivatives at R^2 were then chosen for further investigation based both on the availability of the starting isocyanide and the lack of a definitive difference between the activities of the cyclohexyl- and cyclopentyl derivatives screened thus far.

Table 1. Residual RT activities of simple 2,3-disubstituted imidazo[1,2-*a*]pyridines at 50μM



No.	R^1	R^2	% Res RT Act[a]	No.	R^1	R^2	% Res RT Act[a]
1	Propyl, i-	Hexyl, c-	40.6	**18**	Phenyl 2-trifluoromethyl-	Hexyl, c-	73.2
2	Propyl, i-	Pentyl, n-	83.7	**19**	4-trifluoromethyl-	Hexyl, c-	88.3
3	Propyl, i-	Pentyl, 2-	54.9	**20**	3-cyano-	Hexyl, c-	100.2
4	Propyl, i-	Pentyl, c-	33.7	**21**	4-cyano-	Hexyl, c-	90.8

5	Propyl, i-		Butyl, n-	54.0	**22**		4-nitro-	Hexyl, c-	86.1
6	Propyl, c-		Hexyl, c-	72.9	**23**		2-hydroxy-	Hexyl, c-	87.0
7	Butyl, 2-		Hexyl, c-	54.8	**24**		4-hydroxy-	Hexyl, c-	89.4
8	Octyl, n-		Hexyl, c-	92.4	**25**		4-methoxy-	Hexyl, c-	84.5
9	Phenyl		Hexyl, c-	74.6	**26**		4-dimethylamino-	Hexyl, c-	91.6
10	Phenyl	2-chloro-	Hexyl, c-	7.1	**27**		2,4-dichloro-	Hexyl, c-	39.9
11		3-chloro-	Hexyl, c-	76.0	**28**		2,5-dichloro-	Hexyl, c-	36.8
12		4-chloro-	Hexyl, c-	77.6	**29**		2,6-dichloro-	Hexyl, c-	55.5
13		2-bromo-	Hexyl, c-	29.7	**30**		2,6-difluoro-	Hexyl, c-	17.8
14		3-bromo-	Hexyl, c-	92.3	**31**		2,4,5-trifluoro-	Hexyl, c-	18.6
15		4-bromo-	Hexyl, c-	102.7	**32**		2,3,6-trichloro-	Hexyl, c-	83.5
16		2-fluoro-	Hexyl, c-	13.8		Nevirapine			1.0
17		4-fluoro-	Hexyl, c-	45.4					

[a]Percentage residual RT enzyme activity after incubation with 50 μM test compound determined relative to untreated controls.

Keeping the isocyanide component constant while changing the identity of the starting aldehyde afforded no improvement in activity for the aliphatic groups (**6** – **8)**. Simple aryl groups at C-2, however, yielded more interesting results. An unsubstituted phenyl moiety afforded very poor inhibition (**9**). However, a dramatic improvement in activity was observed when using 2-chlorobenzaldehyde as the aldehyde partner in the reaction (**10**). Changing this component to the 3- or 4-chlorobenzaldehyde produced much poorer inhibitors (**11** and **12**). Similar trends occurred with the fluoro- and bromobenzaldehydes (**13** - **17**), producing reasonable inhibitors with the corresponding 2-halophenyl derivatives (**13** and **16**), although both were still poorer inhibitors than the initial 2-chlorophenyl product (**10**). Introducing a sterically more demanding electron-withdrawing group at the 2- or 4-position of the phenyl group (**18** and **19**) failed to improve matters, as did other such substitutions with cyano- or nitro groups (**20** - **22**). Electron donating groups similarly abrogated activity (**23** - **26**). This was expected, taking into account the known lipophilicity of the NNRTI binding pocket.

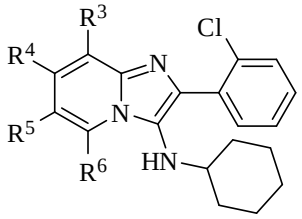
Introducing additional 4-, 5- or 6-chloro substituents to the 2-chlorophenyl ring (**27** - **29**) weakened the inhibition relative to **10**, while trichloro product **32** showed effectively no activity. Interestingly, though, products **30** and **31** with two and three fluoro substituents, respectively, retained activity relative to the parent 2-fluorophenyl compound (**16**). This suggests that a steric constraint governs the activity at this site, in addition to the need for lipophilicity suggested by the preference for halo- substituents.

Attempts to improve the activity of compound **10** to near or better than that of nevirapine focussed on variations around the six-membered ring of the imidazo[1,2-*a*]pyridine system (Table 2). Initially continuing to use residual RT activity to measure inhibition, monomethylation at position R^3-R^6 (**33** - **36**) showed decreasing activity in the order $R^6 > R^5 > R^3 > R^4$, with the compound substituted at R^6 (**36**) having improved

activity relative to the unsubstituted parent compound (**10**). This also proved to be the case in terms of RT IC_{50} values, with compound **36** having a value of 1.55 μM relative to the 4.4 μM of compound **10**.

Concentrating on substitution at R^5 and R^6, similar trends were seen with the compounds having cyano-, bromo- and chloro- substituents. Compounds substituted at R^6 were more active than the corresponding compounds substituted at R^5 (compare **37**, **40** and **42** with **38**, **41** and **43**). Compound **39**, with fluoro substitution at R^5 had similar activity to compounds **40** and **42**. A preference for lipophilic groups at R^6 is evident. On the other hand, electron donating groups, exemplified by methoxy compound **44**, weren't tolerated. Similar or poorer activities were observed with other electron donating groups (data not shown). Combining the effects of substituents at R^5 and R^6 didn't result in a recovery of activity, with compound **45** showing poorer activity than either the methyl- (**36**) or bromo- (**42**) monosubstituted compounds. An interesting exception is compound **46** derived from quinoline, which had reasonable activity despite effective disubstitution.

Table 2. RT and whole-cell inhibitory activity of 2-(2-chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amines



No.	R^3	R^4	R^5	R^6	% Res RT Act[a]	RT IC_{50}[b] (μM)	MAGI IC_{50}[c] (μM)	MAGI CC_{50}[d] (μM)	SI[e]
10					7.1	4.4	0.41	60.4	0.16[a]
33	methyl				18.8	7.18	3.44	> 294	> 86
34		methyl			56.8	NT	NT	NT	NT
35			methyl		10.8	5.39	0.64	57.4	90
36				methyl	3.4	1.55	0.19	20.5	108
37			cyano		10.1	6.60	< 0.63	140	> 222
38				cyano	4.7	3.47	0.18	156	868
39			fluoro		17.0	11.28	1.88	160	85
40			chloro		20.0	14.47	0.84	>200	>238
41				chloro	3.9	3.74	0.24	152	633
42			bromo		20.2	12.20	2.19	> 200	> 91
43				bromo	6.1	4.62	3.15	> 200	64
44				methoxy	43.2	NT	NT	NT	NT
45			bromo	methyl	33.5	18.89	73.00	> 200	> 3

46	CH=CHCH=CH-	12.5	7.52	0.60	146	243
Nevirapine		1.0	0.67	0.09	>10	>111

[a]Percentage residual RT enzyme activity after incubation with 50 μM test compound determined relative to untreated controls.
[b]Compound concentration (μM) required to inhibit the RT enzyme by 50%.
[c] Compound concentration (μM) required to inhibit virus replication in MAGI-R5 cells by 50%.
[d] Compound concentration (μM) required to reduce the viability of MAGI-R5 cells by 50%.
[e] Selectivity index (CC_{50}/IC_{50}).
NT: not tested

In terms of effective enzymatic inhibition, compounds substituted at R^5 (**35**, **37**, **39**-**40**, **42**) all showed low micromolar enzymatic inhibition; although these were still several fold weaker than nevirapine. Interestingly, the compounds substituted at R^6 with methyl, cyano and chloro moieties (**36**, **38**, **41**) showed favourable whole cell IC_{50} values of around 0.2 μM, and IC_{90} values of 1.04, 1.06 and 1.10 μM, respectively; about double that of nevirapine (IC_{50} = 0.09 μM and IC_{90} = 0.60 μM). The relationship of the IC_{90} to the IC_{50} values for these compounds is similar to that of nevirapine, due to the fact that the dose-response curves have similar Hill slope factors (1.16 for nevirapine; 1.15 to 1.39 for our compounds). Compound **38** had the best antiviral activity (MAGI IC_{50} = 0.18 μM) and a good selectivity index of 867. In addition, of the compounds tested, only compound **36** had a CC_{50} value of < 50 μM, indicating a lack of significant toxicity of this type of compound compared to their IC_{50} values. This favourable profile in itself makes these inhibitors worthy of further investigation.

To assess the confidence with which percentage residual enzymatic activity could be used as a means of predicting anti-HIV activity for this series of compounds, the relationship between percentage residual enzyme activity and antiviral activity obtained for that compound from the MAGI whole cell assay was examined (Figure 1). A good correlation between the two was found; compounds that yielded enzyme activity under 40% at 50 μM produced MAGI IC_{50} values less than 4.5 μM (Figure 1A, points within dashed rectangular box). Nineteen compounds adhered to this trend. There were only two exceptions that had less than 40% enzyme activity, but higher MAGI IC_{50} values (18 - 73 μM) (Figure 1A, circled data points). The rectangular box region of Figure 1A is presented on a more convenient scale in Figure 1B, which demonstrates a close correlation between the HIV RT activity of the test compounds and the MAGI cell antiviral activities. Three compounds (circled) deviated somewhat from the trend and produced whole cell activities slightly higher than expected from the RT enzyme activities. If these three outliers are excluded, the correlation between the enzyme and whole cell results, as determined by linear regression, is r^2 = 0.86. The results thus reveal an empirical correlation between HIV RT enzyme inhibition and MAGI whole cell anti-HIV activity for compounds in the present series.

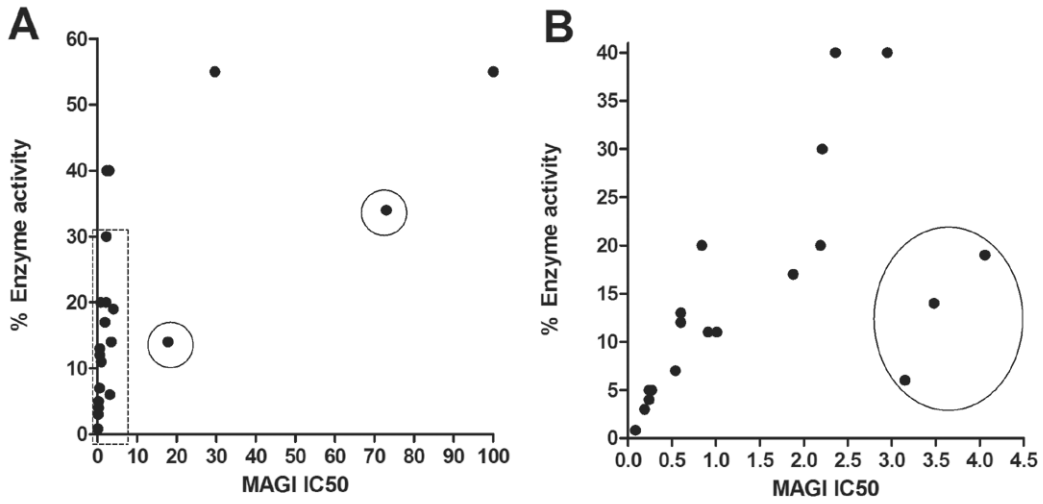


Figure 1: Correlation between the RT activity and corresponding MAGI antiviral activity of test compounds. A total of 23 compounds were subjected to the analysis (A). The 19 compounds with % enzyme activity ≤40% and MAGI IC_{50} <4.5% are shown in B.

2.3. Molecular modelling and analysis

In order to attempt to rationalise the results, compound **38** was docked into the HIV-1 NNRTI binding site using CDOCKER (Accelrys® Discovery Studio 2.5.5). The crystal structures of both the wild-type and K103N mutant forms of HIV-1 RT containing the diarylpyrimidine inhibitor rilpivirine (TMC-278) were used (pdb codes MEE and 3MEG, respectively).[27] Etravirine (TMC-125) and rilpivirine (TMC-278) were simultaneously docked to ensure docking robustness (see Experimental section).

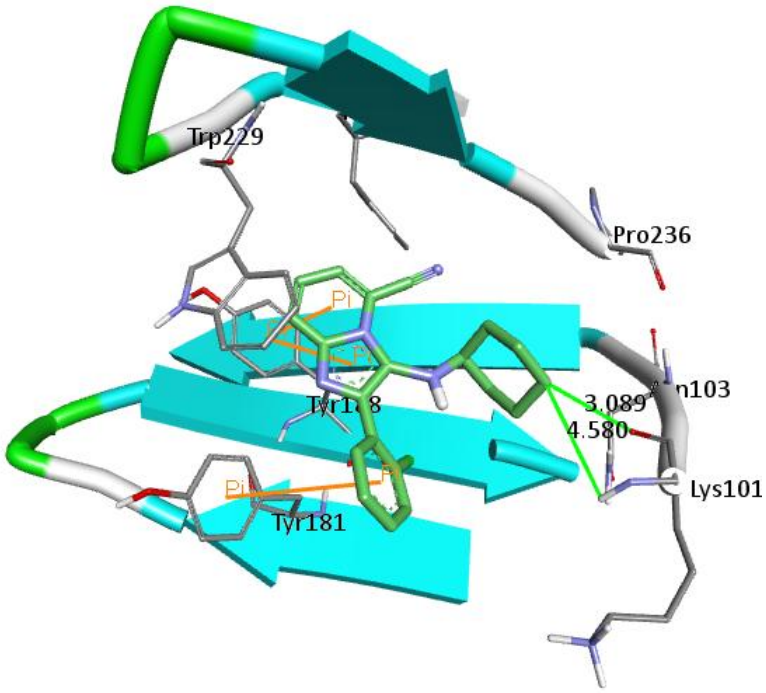


Figure 2. Docked pose of compound **38** in the NNRTI binding site. π-π Stacking interactions between the compound and the aromatic rings of Tyr181 and Tyr188 are indicated in orange. Interatomic distances between C-4 of the cyclohexyl ring and the carbonyl oxygen as well as the amino nitrogen of Lys101 are indicated in green.

The docked pose of compound **38** (Figure 2) and the accompanying 2D interaction map (Figure 3) show that the core imidazo[1,2-*a*]pyridine ring system fits into the aromatic-rich pocket bracketed by Tyr188, Phe227 and Trp229. The imidazo[1,2-*a*]pyridine ring is parallel to the aromatic ring of Tyr188, allowing for π-π stacking to stabilise this interaction. No CH-π interaction is possible, as the imidazo[1,2-*a*]pyridine scaffold projects between the indole ring of Trp229 and Tyr188, possibly due to the need to accommodate the projecting cyano group. A second π-π stacking interaction between Tyr181 and the chlorophenyl moiety contributes to the stabilisation of the complex. Furthermore, the cyclohexyl ring lies in a hydrophobic pocket lined by the mutable Lys/Arg103 residue, probably associating with it through van der Waals interactions. Interestingly, C-4 of the cyclohexyl group lies about 3Å from the carbonyl oxygen and 4.5Å away from the hydrogen on the NH group of Lys101. Hydrogen bonds to this and the adjacent Lys/Arg103 residue confer the remarkable increase in activity associated with newer NNRTIs.[28]

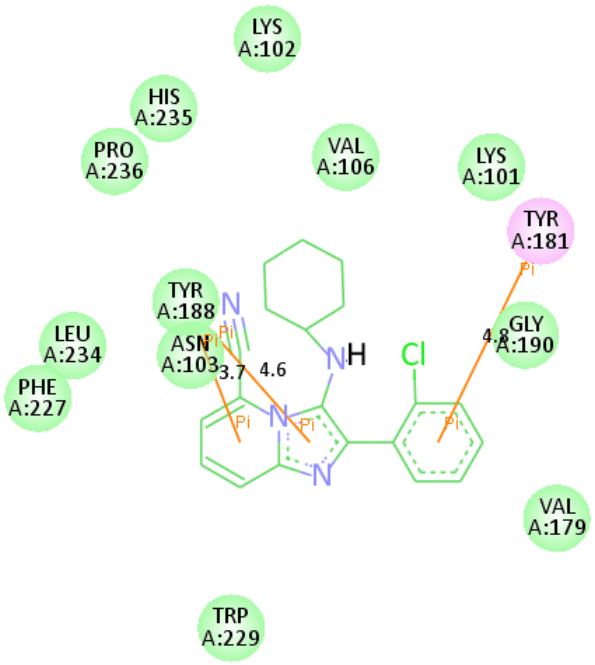
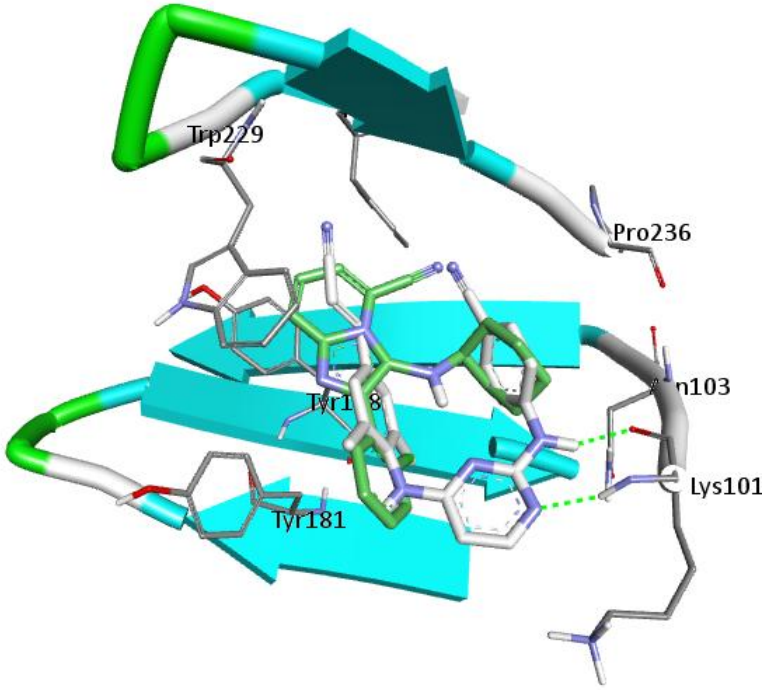
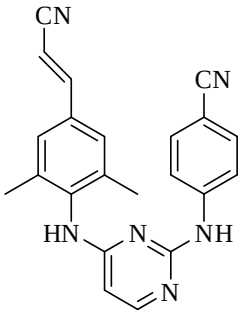


Figure 3. 2D interaction map of docked compound **38**, with π-π stacking interactions and distances indicated in orange.

An overlay of compound **38** with TMC-278 sheds further light on the observed activity (Figure 4) The 2-chloro moiety occupies a small hydrophobic pocket similar to that of the one methyl group of the dimethylanilino moiety of TMC-278, while groups at the 3-position of the phenyl ring would not be accommodated as readily. Similarly, the cyano group at R^6 occupies a cavity between Pro236 and Phe227, similar to the cyano group on TMC-278. The cyano group at R^5 could be accommodated with a small repositioning, while groups at R^3 or R^4 would clash with Phe227 and Trp229. The relationship between the cyclohexyl group and the hydrogen bonding groups on TMC-278 is clearly evident in the figure. This suggests sites of further investigation to improve the activities of the new series of compounds presented here, by introducing hydrogen bonding.





TMC-278

Figure 4. Overlay of the docked poses of TMC-278 (white) and compound **38** (green). Hydrogen bonds between TMC-278 and Lys101 are indicated as dashed green lines.

2.4. PAMPA permeability

The membrane permeabilities of a selection of compounds from Table 2 were assessed in a parallel artificial membrane permeability assay (PAMPA) as a preliminary guide to the likelihood of their passive absorption. The compounds were tested along with standard markers for low, intermediate and high permeability (atenolol, pindolol and metoprolol, respectively; Figure 5). All the compounds demonstrated similar intermediate to high permeabilities. These data could support a potentially favourable absorption profile for thes imidazo[1,2-*a*]pyridin-3-amines in this study.

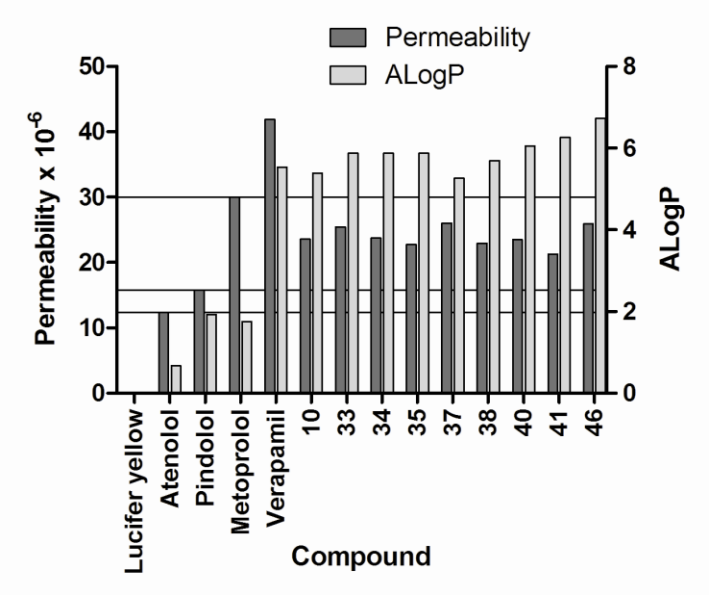


Figure 5. PAMPA permeabilities and AlogP values for HIV RT inhibitors. Compounds were incubated in PAMPA plates for 5 h at 37 ºC at a starting concentration of 100 μM in the donor wells. Atenolol, pindolol and metoprolol were included as permeability markers, while lucifer yellow was used to control for intactness of the permeability barrier. ALogP is the atom-based method published by Ghose and Crippen[29] for determining the water-octanol partition coefficient for a 3D compound and is a reliable measure of hydrophobicity.

3. Conclusion

A number of imidazo[1,2-*a*]pyridin-3-amines were synthesised in a one-pot procedure from appropriate aldehydes, isocyanides and 2-aminopyridines using the Groebke reaction. They were tested for their anti-HIV activity. Compounds possessing alkyl groups at R^1 (derived from the aldehydic component) were poor inhibitors of or inactive against RT. Compounds substituted at R^1 with phenyl or substituted phenyl were also poorly active, with the striking exception of those bearing a 2-halophenyl group. In particular compounds substituted at R^1 with 2-chlorophenyl; and with cyano, methyl or chloro at R^6 (derived from 2-amino-6-substituted pyridine) showed promising activity. Novel compound 2-(2-chlorophenyl)-3-(cyclohexylamino)imidazo[1,2-*a*]pyridine-5-carbonitrile (**38**) exhibited the best activity profile, with a whole-cell anti-HIV IC_{50} of 0.18 μM in the MAGI assay and a good selectivity index of 868 (cf. nevirapine, MAGI IC_{50} of 0.09 μM). In addition, a selection of these compounds showed good membrane permeability in a parallel artificial membrane permeability (PAMPA) assay. Future investigations will expand on improving the promising profile of these compounds, with particular emphasis on introducing the structural changes suggested by the molecular modelling results.

4. Experimental

4.1 Chemistry

NMR spectra were run on either a Varian 200 MHz Gemini 2000 instrument, or on a 400 MHz Varian INOVA instrument. Samples were referenced against chloroform at 77.00 ppm for ^{13}C and against tetramethylsilane at 0.00 ppm for ^{1}H. Spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), quin (quintet), ddd (double doublet of doublets), td (triplet of doublets), dt (doublet of triplets) and m (multiplet). High resolution mass spectra were recorded on a Waters SYNAPT G1 HDMS mass spectrometer operated in electrospray mode. Leucine enkephalin (50 pg/ml) was used as reference calibrant to obtain typical mass accuracies between 1 and 3 mDa. Melting points were determined using a Mettler FP62 capillary melting point apparatus and are uncorrected. All reagents were of reagent grade purchased from Sigma-Aldrich (Schnelldorf, Germany) and were used without any further purification. Solvents used for chromatography or extractions were distilled prior to use. Thin-layer chromatography was carried out using precoated aluminium-backed plates (Merck silicagel 60 F_{254}) visualised under UV light (λ=254 nm). Column chromatography was performed on Fluka silica gel 60 (70-230 mesh).

4.2 General procedure for the Groebke reaction

Cyclopentyl isocyanide was prepared as previously described.[30] The appropriate 2-aminoheterocycle [pyridine or quinoline] (1.33 mmol), aldehyde (1.33 mmol) and isocyanide (1.36 mmol) in dioxane (2.5 ml) were heated in the presence of montmorillonite K-10 clay (250 mg) for 5-8 h at 95–100 ºC, or irradiated with microwave energy at 150 W and 100 °C for 15-30 min in a sealed pressure tube. After reaction completion, the liquid was filtered from the clay and the clay was rinsed with dioxane or ethyl acetate (2 x 2.5 ml). The solvent was removed *in vacuo* and the residue purified by column chromatography (elution hexane: ethyl acetate) to afford the required product.

4.2.1 *N*-Cyclohexyl-2-isopropylimidazo[1,2-*a*]pyridin-3-amine (1)

Yield 25%, as white needles; mp 116-118 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.02 (d, *J* = 6.8 Hz, 1H), 7.49 (d, *J* = 8.9 Hz, 1H), 7.07– 6.95 (m, 1H), 6.72 (dd, *J* = 3.7, 9.7 Hz, 1H), 3.14 (quin, *J* = 6.8 Hz, 1H), 2.85 (br m, 2H), 2.00–1.51 (m, 5H), 1.38 and 1.34 (2 x s, 6H), 1.32-1.09 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 144.7, 141.4, 123.1, 122.8, 122.4, 117.0, 110.9, 57.2, 34.3, 26.2, 25.9, 25.0, 22.8. HRMS (ESI): *m/z* 258.1963 $(M+H)^+$; calc. for $C_{16}H_{24}N_3$: 258.1970.

4.2.2 2-Isopropyl-*N*-pentylimidazo[1,2-*a*]pyridin-3-amine (2)

Yield 63%, as an orange crystalline solid; mp 66-68 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.98 (d, *J* = 6.8 Hz, 1H), 7.48 (d, *J* = 9.0 Hz, 1H), 7.04 (dd, *J* = 6.5, 8.5 Hz, 1H), 6.71 (t, *J* = 6.9 Hz, 1H), 3.12 (quin, *J* = 7.1 Hz, 1H), 2.96 (t, *J* = 7.0 Hz, 2H), 1.73–1.42 (m, 2H), 1.42–1.10 (m, 4H), 1.38 and 1.34 (2 x s, 6H), 0.90 (t, *J* = 7.0

Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 143.9, 141.2, 124.5, 122.7, 122.1, 117.1, 111.0, 49.3, 30.5, 29.2, 26.4, 22.8, 22.6, 14.0. HRMS (ESI): *m/z* 246.1950 $(M+H)^+$; calc. for $C_{15}H_{24}N_3$: 246.1970.

4.2.3 2-Isopropyl-*N*-(pentan-2-yl)imidazo[1,2-*a*]pyridin-3-amine (3)

Yield 49%, as a yellow solid; mp 83-84 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.12–7.92 (m, 1H), 7.59–7.40 (m, 1H), 7.05 (ddd, *J* = 1.2, 6.7, 8.9, 1H), 6.71 (dd, *J* = 0.8, 13.6, 1H), 3.25–2.98 (m, 2H), 2.95–2.56 (m, 1H), 1.63–1.40 (m, 4H), 1.37 (dd, *J* = 2.9, 6.9, 6H), 1.06 (d, *J* = 6.3, 3H), 0.93 (dd, *J* = 5.5, 8.4, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 144.9, 141.5, 123.2, 122.7, 122.4, 117.1, 110.9, 53.7, 40.0, 26.2, 22.7, 21.0, 19.5, 14.2; HRMS (ESI): *m/z* 246.1967 $(M+H)^+$; calc. for $C_{15}H_{24}N_3$: 246.1970.

4.2.4 *N*-Cyclopentyl-2-isopropylimidazo[1,2-*a*]pyridin-3-amine (4)

Yield 20%, as a beige solid; mp 112-114 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.18–7.92 (m, 1H), 7.49–7.52 (m, 1H), 7.14–6.94 (m, 1H), 6.82–6.61 (m, 1H), 3.80–3.47 (m, 1H), 3.09 (q, *J* = 6.8 Hz, 1H), 2.90–2.66 (m, 1H), 1.93–1.43 (m, 8H), 1.38 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 160.2, 155.8, 138.1, 126.2, 116.3, 105.8, 105.2, 58.2, 37.3, 37.0, 34.2, 25.1, 18.2, 18.0. HRMS (ESI): *m/z* 244.1812 $(M+H)^+$; calc. for $C_{15}H_{22}N_3$: 244.1814.

4.2.5 *N*-Butyl-2-isopropylimidazo[1,2-*a*]pyridin-3-amine (5)

Yield 67%, as a yellow crystalline solid; mp 71-73 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.92 (1H), 7.47 (d, *J* = 9.0 Hz, 1H), 6.97 (ddd, *J* = 0.9, 6.7, 8.6 Hz, 1H), 6.64 (td, *J* = 0.6 , 6.7 Hz, 1H), 3.14 (quin, *J* = 7.0 Hz, 1H), 2.97 (t, *J* = 6.8 Hz, 2H), 2.91 (br s, 1H), 1.65–1.34 (m, 4H), 1.38 and 1.35 (2 x s, 6H), 0.87 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 144.0, 141.2, 124.5, 122.6, 122.1, 117.1, 111.0, 49.0, 32.9, 26.5, 22.8, 20.2, 13.9. HRMS (ESI): *m/z* 232.1808 $(M+H)^+$; calc. for $C_{14}H_{22}N_3$: 232.1814.

4.2.6 *N*-Cyclohexyl-2-cyclopropylimidazo[1,2-*a*]pyridin-3-amine (6)

Yield 32%, as a white solid; mp 85-87 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.05–7.94 (m, 1H), 7.46–7.33 (m, 1H), 7.01 (ddd, *J* = 1.3, 6.7, 9.0 Hz, 1H), 6.69 (td, *J* = 1.0, 6.7 Hz, 1H), 3.07–2.67 (m, 2H), 2.11–1.82 (m, 3H), 1.82–1.67 (m, 2H), 1.67–1.48 (m, 1H), 1.41–1.10 (m, 6H), 1.05 (dt, *J* = 4.1, 5.2 Hz, 2H), 1.01–0.85 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.2, 140.5, 124.8, 122.7, 121.9, 116.6, 110.8, 57.2, 34.4, 25.9, 24.9, 8.2, 7.7. HRMS (ESI): *m/z* 256.1810 $(M+H)^+$; calc. for $C_{16}H_{22}N_3$: 256.1814.

4.2.7 2-*sec*-Butyl-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (7)

Yield 20%, as a yellow solid; mp 82-84 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.14–7.93 (m, 1H), 7.48 (dt, *J* = 1.3, 9.5 Hz, 1H), 7.04 (ddd, *J* = 1.2, 6.7, 8.8 Hz, 1H), 6.71 (td, *J* = 1.1, 6.9 Hz, 1H), 3.10–2.63 (m, 3H), 2.12–1.48 (m, 6H), 1.38 (d, *J* = 6.8 Hz, 3H), 1.48–1.06 (m, 5H), 0.85 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (50 MHz,

$CDCl_3$) δ 143.8, 141.8, 124.4, 123.0, 122.7, 117.1, 111.1, 57.6, 34.6, 33.6, 30.2, 26.1, 25.2, 21.1, 12.9. HRMS (ESI): *m/z* 272.2127 $(M+H)^+$; calc. for $C_{17}H_{26}N_3$: 272.2127.

4.2.8 *N*-Cyclohexyl-2-octylimidazo[1,2-*a*]pyridin-3-amine (8)

Yield 71%, as a yellow solid; mp 56-58 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.01 (d, *J* = 6.8 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.12–6.92 (m, 1H), 6.70 (t, *J* = 6.7 Hz, 1H), 2.98–2.77 (m, 2H), 2.77–2.58 (m, 2H), 1.95–1.48 (m, 7H), 1.48–1.00 (m, 15H), 0.98–0.72 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.4, 139.7, 124.4, 122.7, 122.4, 116.9, 110.9, 57.2, 34.3, 31.9, 29.9, 29.7, 29.5, 29.3, 27.5, 25.9, 24.9, 22.7, 14.1. HRMS (ESI): *m/z* 328.2747 $(M+H)^+$; calc. for $C_{21}H_{34}N_3$: 328.2753.

4.2.9 *N*-Cyclohexyl-2-phenylimidazo[1,2-*a*]pyridin-3-amine (9)

Yield 78%, as a white solid; mp 176-178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17–7.94 (m, 2H), 7.58–7.39 (m, 2H), 7.36–7.22 (m, 2H), 7.17–6.88 (m, 2H), 6.77 (t, *J* = 6.6 Hz, 1H), 3.13 (br s, 1H), 2.97 (br s, 1H), 1.90–1.38 (m, 5H), 1.32–0.81 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.7, 136.7, 134.6, 129.6, 128.7, 127.5, 127.3, 124.1, 122.9, 117.6, 111.8, 57.2, 34.4, 26.0, 25.1. HRMS (ESI): *m/z* 292.1782 $(M+H)^+$; calc. for $C_{19}H_{22}N_3$: 292.1814.

4.2.10 2-(2-Chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (10)

Yield 54%, as a viscous yellow oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.17 (dt, *J* = 1.0, 6.9 Hz, 1H), 7.70 (dd, *J* = 3.0, 6.4 Hz, 1H), 7.62–7.52 (m, 1H), 7.52–7.29 (m, 3H), 7.16 (ddd, *J* = 1.1, 6.6, 9.0 Hz, 1H), 6.82 (td, *J* = 0.9, 6.7 Hz, 1H), 3.29 (d, *J* = 6.7 Hz, 1H), 2.85–2.53 (m, 1H), 1.88–1.31 (m, 5H), 1.30–0.77 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.6, 135.0, 134.0, 132.6, 129.4, 129.1, 126.9, 126.3, 123.7, 122.8, 117.5, 111.6, 56.4, 33.9, 25.7, 24.6. HRMS (ESI): *m/z* 326.1440 $(M+H)^+$; calc. for $C_{19}H_{21}ClN_3$: 326.1424.

4.2.11 2-(3-Chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (11)

Yield 35%, as a cream solid; mp 167-169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (dd, *J* = 1.6, 3.2 Hz, 1H), 7.97 (d, *J* = 6.8 Hz, 1H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.45 (d, *J* = 9.2 Hz, 1H), 7.29 (t, d, *J* = 8.0 Hz, 1H), 7.23 (ddd, *J* = 1.2, 2.0, 8.0 Hz, 1H), 7.05 (ddd, *J* = 1.2, 6.4, 8.8 Hz, 1H), 6.69 (td, *J* = 0.8, 6.8 Hz, 1H), 3.07 (br d, *J* = 4.4 Hz, 1H), 2.98-2.82 (m, 1H), 1.94–1.42 (m, 5H), 1.42–0.92 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.3, 136.1, 134.9, 134.1, 129.4, 126.8, 126.7, 125.1, 124.6, 123.9, 122.4, 117.1, 111.4, 56.6, 33.9, 25.4, 24.5. HRMS (ESI): *m/z* 326.1423 $(M+H)^+$; calc. for $C_{19}H_{21}ClN_3$: 326.1424.

4.2.12 2-(4-Chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (12)

Yield 32%, as an off-white crystalline solid; mp 198-199 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.13–7.98 (m, 3H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.46–7.37 (m, 2H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.79 (t, *J* = 6.8 Hz, 1H), 3.13–2.84 (m, 2H), 1.95–1.46 (m, 6H), 1.30–1.09 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.1, 141.7, 133.1, 132.9,

128.6, 128.3, 125.1, 124.0, 122.6, 117.5, 111.6, 56.9, 34.2, 25.7, 24.8. HRMS (ESI): *m/z* 326.1422 $(M+H)^+$; calc. for $C_{19}H_{21}Cl\ N_3$: 326.1424.

4.2.13 2-(2-Bromophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (13)

Yield 22%, as a brown solid; mp 43-45 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.43–7.97 (m, 1H), 7.64 (td, *J* = 1.5, 7.7 Hz, 1H), 7.58–7.48 (m, 1H), 7.39 (td, *J* = 1.3, 7.5 Hz, 1H), 7.31–7.21 (m, 1H), 7.21–7.13 (m, 1H), 7.11 (dd, *J* = 1.3, 6.7 Hz, 1H), 6.80 (td, *J* = 1.1, 6.7 Hz, 1H), 3.42–3.09 (m, 1H), 2.82–2.48 (m, 1H), 1.80–1.33 (m, 5H), 1.21–0.81 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.4, 136.1, 132.7, 132.5, 129.3, 127.3, 125.8, 123.5, 122.9, 122.8, 117.5, 111.5, 56.3, 33.8, 25.6, 24.5. HRMS (ESI): *m/z* 370.0892 $(M+H)^+$; calc. for $C_{19}H_{21}BrN_3$: 370.0919.

4.2.14 2-(3-Bromophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (14)

Yield 10%, as a brown solid; mp 79-81 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.35–8.21 (m, 1H), 8.15–8.04 (m, 1H), 8.04–7.90 (m, 1H), 7.61–7.47 (m, 1H), 7.43 (ddd, *J* = 1.1, 1.9, 7.9 Hz, 1H), 7.29 (dd, *J* = 5.4, 10.3 Hz, 1H), 7.13 (ddd, *J* = 1.3, 6.7, 9.0 Hz, 1H), 6.79 (td, *J* = 1.1, 6.8 Hz, 1H), 3.29–2.66 (m, 2H), 1.69 (m, 5H), 1.41–0.74 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.6, 136.6, 131.0, 130.0, 130.0, 129.9, 125.3, 124.1, 122.7, 122.6, 117.5, 111.7, 57.0, 34.3, 25.7, 24.8. HRMS (ESI): *m/z* 370.0911 $(M+H)^+$; calc. for $C_{19}H_{21}BrN_3$: 370.0919.

4.2.15 2-(4-Bromophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (15)

Yield 30%, as a pale yellow solid; mp 166-168 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.09–8.00 (m, 1H), 7.94 (d, *J* = 8.6 Hz, 2H), 7.58–7.43 (m, 3H), 7.10 (ddd, *J* = 1.3, 6.7, 9.0 Hz, 1H), 6.75 (td, *J* = 1.1, 6.8 Hz, 1H), 3.01 (s, 2H), 1.93–1.39 (m, 5H), 1.16 (s, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.6, 135.6, 133.5, 131.4, 128.5, 124.8, 124.0, 122.6, 121.1, 117.3, 111.6, 56.8, 34.3, 25.7, 24.8. HRMS (ESI): *m/z* 370.0926 $(M+H)^+$; calc. for $C_{19}H_{21}Br\ N_3$: 370.0919.

4.2.16 *N*-Cyclohexyl-2-(2-fluorophenyl)imidazo[1,2-*a*]pyridin-3-amine (16)

Yield 45%, as a viscous brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 6.9 Hz, 1H), 7.92 (td, *J* = 2.3, 7.6 Hz, 1H), 7.54 (d, *J* = 9.1 Hz, 1H), 7.38–7.21 (m, 2H), 7.18–7.05 (m, 2H), 6.79 (t, *J* = 6.7 Hz, 1H), 3.45 (t, *J* = 7.8 Hz, 1H), 2.91–2.46 (m, 1H), 1.88–1.34 (m, 5H), 1.21–0.83 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.4, 157.9, 141.7, 131.3 (d, *J* = 2.3 Hz), 131.2 (d, *J* = 4.6 Hz), 128.8 (d, *J* = 8.4 Hz), 126.6, 124.3 (d, *J* = 3.1 Hz), 123.5, 122.6, 122.3 (d, *J* = 14.5 Hz), 117.0, 115.3 (d, *J* = 23.6 Hz), 111.2, 56.3, 33.6, 25.3, 24.4. HRMS (ESI): *m/z* 310.1724 $(M+H)^+$; calc. for $C_{19}H_{21}FN_3$: 310.1720.

4.2.17 *N*-Cyclohexyl-2-(4-fluorophenyl)imidazo[1,2-*a*]pyridin-3-amine (17)

Yield 41%, as a white solid; mp 167-169 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.13–7.92 (m, 3H), 7.52 (dt, *J* = 1.0, 9.1 Hz, 1H), 7.22–6.97 (m, 3H), 6.77 (td, *J* = 1.1, 6.8 Hz, 1H), 3.31–2.47 (m, 2H), 1.93–1.40 (m, 5H), 1.40–0.86 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 164.7, 160.0, 142.1, 131.3, 129.6, 129.4, 124.9, 124.0,

123.4, 117.9, 116.4, 115.7, 112.2, 57.3, 35.3, 26.1, 25.0. HRMS (ESI): *m/z* 310.1721 $(M+H)^+$; calc. for $C_{19}H_{21}FN_3$: 310.1720.

4.2.18 *N*-Cyclohexyl-2-(2-(trifluoromethyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (18)

Yield 21%, as an off-white solid; mp 109-111 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.28–7.99 (m, 1H), 7.85–7.71 (m, 1H), 7.66–7.40 (m, 4H), 7.13 (td, *J* = 1.3, 6.7 Hz, 1H), 6.80 (td, *J* = 1.1, 6.8 Hz, 1H), 3.28–2.83 (m, 1H), 2.66 (m, 1H), 1.90–1.35 (m, 5H), 1.23–0.80 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.1, 138.2, 135.3, 132.8, 131.2, 128.1, 126.9, 126.1 (q, *J* = 5.1, 1H), 123.5, 122.8, 121.4, 117.6, 111.5, 56.3, 33.8, 25.6, 24.5. HRMS (ESI): *m/z* 360.1688 $(M+H)^+$; calc. for $C_{20}H_{21}F_3N_3$: 360.1688.

4.2.19 *N*-Cyclohexyl-2-(4-(trifluoromethyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (19)

Yield 26%, as a beige solid; mp 138-140 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.22 (d, *J* = 8.2, 2H), 8.08 (dt, *J* = 1.0, 6.0, 1H), 7.68 (d, *J* = 8.3, 2H), 7.60–7.45 (m, 1H), 7.15 (ddd, *J* = 1.2, 6.7, 9.0, 1H), 6.80 (td, *J* = 1.0, 6.7, 1H), 3.29–2.67 (m, 2H), 1.96–1.42 (m, 5H), 1.42–0.91 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.8, 138.0, 135.1, 128.5, 126.9, 125.6, 125.3 (dd, *J* = 3.8, 7.6), 124.5, 124.3, 122.6, 117.6, 111.8, 56.9, 34.2, 25.7, 24.8; HRMS (ESI): *m/z* 360.1683 $(M+H)^+$; calc. for $C_{20}H_{21}N_3F_3$: 360.1688.

4.2.20 3-(3-(Cyclohexylamino)imidazo[1,2-*a*]pyridin-2-yl)benzonitrile (20)

Yield 76%, as an off-white solid; mp 139-141 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (t, *J* = 1.6 Hz, 1H), 8.37 (dt, *J* = 1.5, 7.6 Hz, 1H), 8.14–7.94 (m, 1H), 7.62–7.45 (m, 3H), 7.16 (ddd, *J* = 1.1, 6.7, 9.0 Hz, 1H), 6.81 (td, *J* = 1.0, 6.8 Hz, 1H), 3.04 (d, *J* = 4.7 Hz, 1H), 2.94 (ddd, *J* = 4.4, 10.0, 14.3 Hz, 1H), 1.88–1.75 (m, 2H), 1.75–1.63 (m, 2H), 1.63–1.49 (m, 1H), 1.42–0.92 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.9, 135.9, 134.6, 131.0, 130.4, 130.3, 129.1, 125.3, 124.5, 122.6, 119.0, 117.6, 112.5, 112.0, 56.9, 34.2, 25.6, 24.8. HRMS (ESI): *m/z* 317.1775 $(M+H)^+$; calc. for $C_{20}H_{21}N_4$: 317.1766.

4.2.21 4-(3-(Cyclohexylamino)imidazo[1,2-*a*]pyridin-2-yl)benzonitrile (21)

Yield 62%, as a yellow solid; mp 169-171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (dd, *J* = 1.7, 8.5 Hz, 2H), 8.05 (dt, *J* = 1.0, 6.9 Hz, 1H), 7.69 (dd, *J* = 1.7, 8.5 Hz, 2H), 7.53 (dt, *J* = 1.0, 9.1 Hz, 1H), 7.17 (ddd, *J* = 1.2, 6.7, 9.1 Hz, 1H), 6.81 (td, *J* = 1.0, 6.8 Hz, 1H), 3.06 (d, *J* = 4.9 Hz, 1H), 2.95 (ddd, *J* = 4.6, 10.0, 14.4 Hz, 1H), 1.87–1.76 (m, 2H), 1.76–1.64 (m, 2H), 1.64–1.51 (m, 1H), 1.33–1.03 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.9, 139.2, 134.7, 132.2, 127.2, 126.1, 124.6, 122.6, 119.2, 117.8, 112.0, 110.2, 57.0, 34.2, 25.6, 24.8. HRMS (ESI): *m/z* 317.1765 $(M+H)^+$; calc. for $C_{20}H_{21}N_4$: 317.1766.

4.2.22 *N*-Cyclohexyl-2-(4-nitrophenyl)imidazo[1,2-*a*]pyridin-3-amine (22)

Yield 28%, as a rust-coloured solid; mp 203-205 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.49–8.21 (m, 4H), 8.07 (dt, *J* = 1.0, 6.9 Hz, 1H), 7.55 (d, *J* = 8.3 Hz, 1H), 7.18 (ddd, *J* = 1.3, 6.7, 9.1 Hz, 1H), 6.83 (td, *J* = 1.0, 6.8 Hz, 1H), 3.28–2.79 (m, 2H), 2.04–1.47 (m, 5H), 1.47–0.97 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 146.6,

142.1, 141.1, 134.4, 127.3, 126.5, 124.8, 123.8, 122.7, 117.9, 112.2, 57.1, 34.4, 25.7, 24.9. HRMS (ESI): *m/z* 337.1670 $(M+H)^+$; calc. for $C_{19}H_{21}N_4O_2$: 337.1665.

4.2.23 2-(3-(Cyclohexylamino)imidazo[1,2-*a*]pyridin-2-yl)phenol (23)

Yield 34%, as a pale yellow solid; mp 149-151 °C; ^{1}H NMR (200 MHz, DMSO-d_6 + CD_3OD) δ 8.36 (d, *J* = 6.8 Hz, 1H), 8.17 (dd, *J* = 1.7, 8.1 Hz, 1H), 7.54 (d, *J* = 9.0 Hz, 1H), 7.40–7.15 (m, 2H), 7.10–6.85 (m, 3H), 3.05–2.81 (m, 1H), 1.99–1.44 (m, 5H), 1.44–1.01 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6 + CD_3OD) δ 156.8, 141.6, 135.9, 129.8, 129.1, 125.8, 125.1, 123.9, 119.9, 117.9, 116.9, 113.0, 57.7, 34.2, 26.4, 25.5. HRMS (ESI): *m/z* 308.1767 $(M+H)^+$; calc. for $C_{19}H_{22}N_3O$: 308.1763.

4.2.24 4-(3-(Cyclohexylamino)imidazo[1,2-*a*]pyridin-2-yl)phenol (24)

Yield 60%, as a fine white solid; decomp. >240 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.26 (d, *J* = 6.9 Hz, 1H), 8.03 (d, *J* = 8.6 Hz, 2H), 7.41 (d, *J* = 8.9 Hz, 1H), 7.28–7.01 (m, 1H), 6.83 (m + d, *J* = 7.3 Hz, 3H), 4.60 (d, *J* = 5.5 Hz, 1H), 3.45–3.38 (m, 1H), 3.00–2.65 (m, 1H), 2.01–1.40 (m, 5H), 1.40–0.85 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 154.7, 138.5, 133.8, 126.1, 123.8, 122.4, 121.3, 121.2, 114.4, 113.2, 109.0, 54.5, 31.8, 23.8, 22.8. HRMS (ESI): *m/z* 308.1754 $(M+H)^+$; calc. for $C_{19}H_{22}N_3O$: 308.1763.

4.2.25 *N*-Cyclohexyl-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridin-3-amine (25)

Yield 27%, as a yellow solid; mp 149-151 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.11 (d, *J* = 6.8 Hz, 2H), 8.02 (d, *J* = 8.6 Hz, 2H), 7.16–7.08 (m, 1H), 7.02–6.98 (d, *J* = 8.6 Hz, 2H), 6.80–6.73 (m, 1H), 3.87 (s, 3H), 3.07 (br s, 1H), 3.00-2.91 (m, 1H), 1.80–1.59 (br m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 158.3, 141.6, 138.3, 137.1, 128.6, 127.3, 124.3, 123.9, 118.5, 117.3, 116.2, 57.1, 55.5, 34.4, 26.0, 25.1. HRMS (ESI): *m/z* 322.1888 $(M+H)^+$; calc. for $C_{20}H_{24}N_3O$: 322.1919.

4.2.26 *N*-Cyclohexyl-2-(4-(dimethylamino)phenyl)imidazo[1,2-*a*]pyridin-3-amine (26)

Yield 45%, as golden brown needles; mp 166-168 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.14–8.04 (m, 1H), 7.94 (d, *J* = 8.7, 2H), 7.54–7.47 (m, 1H), 7.07 (ddd, *J* = 1.3, 6.6, 8.9, 1H), 6.81 (d, *J* = 8.9, 2H), 6.73 (td, *J* = 0.9, 6.9, 1H), 3.01 (s, 6H), 1.93–1.43 (m, 5H), 1.43–0.93 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 149.7, 141.4, 137.3, 131.8, 127.8, 123.1, 122.8, 122.4, 116.9, 112.3, 111.0, 56.8, 40.4, 34.2, 25.8, 24.8; HRMS (ESI): *m/z* 335.2220 $(M+H)^+$; calc. for $C_{21}H_{27}N_4$: 335.2236.

4.2.27 *N*-Cyclohexyl-2-(2,4-dichlorophenyl)imidazo[1,2-*a*]pyridin-3-amine (27)

Yield 41%, as a viscous brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (ddd, *J* = 0.9, 1.6, 6.9 Hz, 1H), 7.64–7.60 (m, 1H), 7.56–7.52 (m, 1H), 7.51–7.48 (m, 1H), 7.38–7.33 (m, 1H), 7.19–7.12 (m, 1H), 6.81 (tt, *J* = 1.8, 9.2 Hz, 1H), 3.19 (d, *J* = 6.0 Hz, 1H), 2.67 (dd, *J* = 3.8, 9.0 Hz, 1H), 1.65 (t, *J* = 11.2 Hz, 2H), 1.59 (dd, *J* = 4.6, 8.9 Hz, 2H), 1.17–0.92 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.8, 134.3, 134.1, 133.3, 133.1, 132.8,

129.3, 127.3, 126.4, 123.9, 122.9, 117.6, 111.7, 56.4, 33.9, 25.6, 24.6. HRMS (ESI): *m/z* 360.1025 $(M+H)^+$; calc. for $C_{19}H_{20}Cl_2N_3$: 360.1034.

4.2.28 *N*-Cyclohexyl-2-(2,5-dichlorophenyl)imidazo[1,2-*a*]pyridin-3-amine (28)

Yield 15%, as a yellow solid; mp 110-112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (dd, *J* = 0.8, 6.8, 1H), 7.68 (d, *J* = 2.4, 1H), 7.50 (dd, *J* = 1.2, 9.2, 1H), 7.39 (d, *J* = 8.4, 1H), 7.31–7.24 (m, 1H), 7.22-7.06 (m, 1H), 6.81 (td, *J* = 1.2, 6.8, 1H), 3.24 (br s, *J* = 7.6, 1H), 2.70–2.58 (m, 1H), 1.79–1.42 (m, 5H), 1.24–0.89 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.6, 135.4, 133.6, 132.7, 132.1, 130.5, 129.0, 128.5, 126.5, 124.1, 122.9, 117.3, 111.8, 56.3, 33.8, 25.5, 24.5; HRMS (ESI): *m/z* 360.1037 $(M+H)^+$; calc. for $C_{19}H_{20}N_3Cl_2$: 360.1034.

4.2.29 *N*-Cyclohexyl-2-(2,6-dichlorophenyl)imidazo[1,2-*a*]pyridin-3-amine (29)

Yield 32%, as a yellow solid; mp 55-57 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (dt, *J* = 1.2, 6.8 Hz, 1H), 7.54 (dt, *J* = 1.2, 9.2 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.26 (dd, *J* = 7.2, 8.4 Hz, 1H), 7.11 (ddd, *J* = 1.2, 6.8, 9.2 Hz, 1H), 6.78 (td, *J* = 1.2, 6.8 Hz, 1H), 2.94 (d, *J* = 6.4 Hz, 1H), 2.82–2.68 (m, 1H), 1.81–1.68 (m, 2H), 1.62–1.55 (m, 2H), 1.55–1.40 (m, 1H), 1.21–0.95 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.2, 136.0, 132.7, 132.6129.7, 127.7, 126.5, 123.3, 122.6, 117.5, 111.3, 56.2, 33.5, 25.3, 24.3. HRMS (ESI): *m/z* 360.1042 $(M+H)^+$; calc. for $C_{19}H_{20}Cl_2N_3$: 360.1034.

4.2.30 *N*-Cyclohexyl-2-(2,6-difluorophenyl)imidazo[1,2-*a*]pyridin-3-amine (30)

Yield 13%, as a beige solid; mp 87-89 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (dt, *J* = 1.2, 6.9 Hz, 1H), 7.57 (dt, *J* = 1.1, 9.1 Hz, 1H), 7.34 (tt, *J* = 6.4, 8.3 Hz, 1H), 7.14 (ddd, *J* = 1.3, 6.6, 9.1 Hz, 1H), 7.03 (m, 2H), 6.80 (td, *J* = 1.1, 6.8 Hz, 1H), 3.12 (br d, *J* = 7.6 Hz, 1H), 2.79–2.60 (m, 1H), 1.84–1.66 (m, 2H), 1.66–1.54 (m, 2H), 1.54–1.42 (m, 1H), 1.21–0.92 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9 (d, *J* = 30.4 Hz), 159.4 (d, *J* = 27.6 Hz), 142.0, 129.8, 129.7, 127.8, 124.0, 122.8, 117.8, 111.8 (d, *J* = 15.2 Hz), 111.6 (d, *J* = 27.6 Hz), 56.5, 34.0, 25.6, 24.7. HRMS (ESI): *m/z* 328.1630 $(M+H)^+$; calc. for $C_{19}H_{20}F_2N_3$: 328.1625.

4.2.31 *N*-Cyclohexyl-2-(2,4,5-trifluorophenyl)imidazo[1,2-*a*]pyridin-3-amine (31)

Yield 17%, as a viscous brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (dt, *J* = 1.1, 6.9 Hz, 1H), 7.57 (dt, *J* = 1.1, 9.1 Hz, 1H), 7.35 (tt, *J* = 6.4, 8.4 Hz, 1H), 7.15 (ddd, *J* = 1.3, 6.6, 9.1 Hz, 1H), 7.18-6.97 (m, 1H), 6.81 (td, *J* = 1.1, 6.8 Hz, 1H), 3.11 (br d, *J* = 6.4 Hz, 1H), 2.79-2.61 (m, 1H), 1.82-1.63 (m, 2H), 1.69–1.56 (m, 2H), 1.56–1.38 (m, 1H), 1.07 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9 (d, *J* = 30.8 Hz), 159.4 (d, *J* = 30.4 Hz), 129.8, 129.6, 129.5, 142.0, 129.7, 127.7, 123.8, 122.7, 117.7 (d, *J* = 24.4 Hz), 111.5 (d, *J* = 30.4 Hz), 56.5, 34.0, 25.6, 24.7. HRMS (ESI): *m/z* 346.1534 $(M+H)^+$; calc. for $C_{19}H_{19}F_3N_3$: 346.1531.

4.2.32 *N*-Cyclohexyl-2-(2,3,6-trichlorophenyl)imidazo[1,2-*a*]pyridin-3-amine (32)

Yield 18%, as a brown oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.23–8.07 (m, 1H), 7.62–7.50 (m, 1H), 7.47 (d, *J* = 8.7 Hz, 1H), 7.36 (d, *J* = 8.7 Hz, 1H), 7.18 (td, *J* = 1.3, 6.7 Hz, 1H), 6.82 (td, *J* = 0.9, 6.7 Hz, 1H), 2.94–2.62

(m, 2H), 1.85–1.35 (m, 5H), 1.35–0.71 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.8, 141.6, 134.8, 134.5, 133.0, 131.9, 130.5, 128.3, 126.6, 123.8, 122.9, 117.8, 111.7, 56.6, 33.8, 25.6, 24.6. HRMS (ESI): *m/z* 394.0645 $(M+H)^+$; calc. for $C_{19}H_{19}Cl_3N_3$: 394.0645.

4.2.33 2-(2-Chlorophenyl)-*N*-cyclohexyl-8-methylimidazo[1,2-*a*]pyridin-3-amine (33)

Yield 83%, as a brown oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.73–7.67 (m, 1H), 7.58–7.22 (m, 4H), 7.03–6.92 (m, 1H), 3.37–3.19 (m, 1H), 2.81–2.62 (m, 1H), 2.38 (s, 3H),1.85–1.42 (m, 5H), 1.22–1.08 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 146.1, 142.8, 141.3, 139.5, 135.9, 133.3, 130.2, 129.6, 127.2, 122.7, 121.3, 118.6, 57.0, 34.2, 26.5, 25.9, 19.5. HRMS (ESI): *m/z* 340.1581 $(M+H)^+$; calc. for $C_{20}H_{23}ClN_3$: 340.1581.

4.2.34 2-(2-Chlorophenyl)-*N*-cyclohexyl-7-methylimidazo[1,2-*a*]pyridin-3-amine (34)

Yield 48%, as a viscous brown oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.11 (d, *J* = 6.8 Hz, 1H), 7.72–7.68 (m, 1H), 7.53–7.26 (m, 4H), 6.72 (dd, *J* = 1.2, 6.8 Hz, 1H), 3.34–3.17 (m, 1H), 2.81–2.58 (m, 1H), 2.45 (s, 3H), 1.80–1.42 (m, 5H), 1.23–0.97 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 142.4, 135.2, 134.4, 134.7, 133.9, 130.2, 129.3, 127.7, 126.2 123.3, 116.6, 115.3, 57.4, 34.7, 26.4, 25.2, 21.8. HRMS (ESI): *m/z* 340.1581 $(M+H)^+$; calc. for $C_{20}H_{23}ClN_3$: 340.1581.

4.2.35 2-(2-Chlorophenyl)-*N*-cyclohexyl-6-methylimidazo[1,2-*a*]pyridin-3-amine (35)

Yield 81%, as a yellow solid; mp 107-109 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.74–7.54 (m, 1H), 7.52–7.40 (m, 2H), 7.40–7.18 (m, 2H), 6.98 (dd, *J* = 1.7, 9.2 Hz, 1H), 3.40–3.07 (m, 1H), 2.82–2.51 (m, 1H), 2.35 (s, 3H), 1.91–1.31 (m, 5H), 1.23–0.80 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 140.7, 135.0, 134.2, 132.5, 129.3, 128.9, 126.7, 125.9, 121.0, 120.3, 116.8, 56.3, 33.8, 25.6, 24.6, 18.4. HRMS (ESI): *m/z* 340.1582 $(M+H)^+$; calc. for $C_{20}H_{23}ClN_3$: 340.1581.

4.2.36 2-(2-Chlorophenyl)-*N*-cyclohexyl-5-methylimidazo[1,2-*a*]pyridin-3-amine (36)

Yield 58%, as a yellow solid; mp 136-138 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.71–7.68 (m, 1H), 7.52–7.26 (m, 4H), 7.12–7.08 (m, 1H), 6.49 (d, *J* = 6.6 Hz, 1H), 3.25–3.18 (m, 1H), 3.03 (s, 3H), 2.71–2.51 (m, 1H), 1.80–1.45 (m, 5H), 1.26–0.81 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 143.2, 137.3, 136.6, 134.6, 132.7, 132.5, 129.2, 128.9, 128.1, 126.7, 123.8, 115.7, 113.2, 58.7, 33.4, 25.7, 25.0, 19.8. HRMS (ESI): *m/z* 340.1581 $(M+H)^+$; calc. for $C_{20}H_{23}ClN_3$: 340.1581.

4.2.37 2-(2-Chlorophenyl)-3-(cyclohexylamino)imidazo[1,2-*a*]pyridine-6-carbonitrile (37)

Yield 49%, as a yellow solid; mp 133-135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65–8.55 (m, 1H), 7.68–7.63 (m, 1H), 7.61 (ddd, *J* = 0.9, 3.4, 9.3 Hz, 1H), 7.53–7.46 (m, 1H), 7.43–7.32 (m, 2H), 7.27–7.19 (m, 1H), 3.53–3.13 (m, 1H), 2.86–2.55 (m, 1H), 1.60 (m, 5H), 1.20–0.95 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ

140.5, 137.5, 132.8, 132.6, 132.3, 129.8, 129.6, 129.4, 127.4, 127.1, 123.2, 118.5, 117.1, 97.8, 56.6, 33.8, 25.4, 24.5. HRMS (ESI): *m/z* 351.1361 $(M+H)^+$; calc. for $C_{20}H_{20}ClN_4$: 351.1376.

4.2.38 2-(2-Chlorophenyl)-3-(cyclohexylamino)imidazo[1,2-*a*]pyridine-5-carbonitrile (38)

4.2.38.1 2-Amino-6-cyanopyridine: A mixture of 2-amino-6-bromopyridine (0.51 g, 29.6 mmol), cupric cyanide monohydrate (1.76 g, 89.3 mmol) and dry DMF (30 ml) was boiled at 100 °C for 18 h. The brown gum that resulted was diluted with 50 ml 25% ammonia solution and stirred until all the copper salts had dissolved. Extraction with ethyl acetate and concentration, followed by column chromatography (elution 30-50% ethyl acetate: hexane), afforded a brown oil (17.1 mg, 5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 7.3, 8.4 Hz, 1H), 7.04 (dd, *J* = 0.7, 7.3 Hz, 1H), 6.73–6.66 (m, 1H), 4.92 (br s, 2H). This compound was used immediately in the three component reaction.

4.2.38.2 2-(2-Chlorophenyl)-3-(cyclohexylamino)imidazo[1,2-*a*]pyridine-5-carbonitrile.

Yield 72%, as a thick yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, *J* = 0.9, 9.0 Hz, 1H), 7.62 (dt, *J* = 3.1, 5.4 Hz, 1H), 7.54–7.47 (m, 1H), 7.45–7.34 (m, 3H), 7.17 (dd, *J* = 7.1, 8.9 Hz, 1H), 3.33 (d, *J* = 7.1 Hz, 1H), 2.79 (d, *J* = 3.3 Hz, 1H), 1.76–1.72 (m, 2H), 1.65–1.62 (m, 2H), 1.48–1.41 (m, 1H), 1.28–0.95 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.1, 138.4, 133.1, 132.4, 129.8, 129.7, 128.1, 127.1, 124.3, 124.3, 122.9, 121.9, 114.0, 108.0, 57.2, 32.7, 25.6, 24.5. HRMS (ESI): *m/z* 351.1351 $(M+H)^+$; calc. for $C_{20}H_{20}ClN_4$: 351.1376.

4.2.39 2-(2-Chlorophenyl)-*N*-cyclohexyl-6-fluoroimidazo[1,2-*a*]pyridin-3-amine (39)

Yield 83%, as a dark brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 1.8 Hz, 1H), 7.71–7.67 (m, 1H), 7.48–7.44 (m, 2H), 7.38–7.33 (m, 2H), 7.24 (d, *J* = 1.8 Hz, 1H), 3.33 (d, *J* = 4.0 Hz, 1H), 2.66 (s, 1H), 1.77–1.60 (m, 3H), 1.51 (m, 2H), 1.12–0.97 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.4, 152.1, 139.2, 136.7, 133.6, 132.4, 129.5, 129.3, 127.6, 127.6, 127.0, 118.1, 118.0, 116.0, 115.7, 109.5, 109.1, 56.3, 33.8, 25.6, 24.6. HRMS (ESI): *m/z* 344.1299 $(M+H)^+$; calc. for $C_{19}H_{20}ClFN_3$: 344.1330.

4.2.40 6-Chloro-2-(2-chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (40)

Yield 72%, as an off-white solid; mp 130-132 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.25–8.11 (m, 1H), 7.75–7.56 (m, 1H), 7.55–7.39 (m, 2H), 7.39–7.25 (m, 2H), 7.10 (ddd, *J* = 1.2, 1.9, 9.5 Hz, 1H), 3.27 (d, *J* = 6.8 Hz, 1H), 2.80–2.46 (m, 1H), 1.82–1.34 (m, 5H), 1.23–0.76 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 139.8, 136.4, 133.6, 132.6, 132.4, 129.4, 129.3, 126.9, 126.7, 124.9, 120.7, 120.1, 118.0, 56.4, 33.8, 25.6, 24.5. HRMS (ESI): *m/z* 360.1019 $(M+H)^+$; calc. for $C_{19}H_{20}Cl_2N_3$: 360.1034.

4.2.41 5-Chloro-2-(2-chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (41)

Yield 28%, as an orange solid; mp 91-93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63–7.56 (m, 1H), 7.49 (dddd, *J* = 1.3, 1.8, 4.5, 5.0 Hz, 2H), 7.39–7.30 (m, 2H), 7.08–6.99 (m, 1H), 6.80–6.73 (m, 1H), 3.31 (d, *J* = 6.8 Hz, 1H), 2.81 (s, 1H), 1.67 (d, *J* = 12.5 Hz, 2H), 1.56–1.40 (m, 3H); 1.12–0.96 (m, 3H), 0.95–0.80 (m, 2H); ^{13}C

NMR (101 MHz, $CDCl_3$) δ 143.6, 137.5, 133.7, 133.2, 132.6, 129.5, 129.3, 128.2, 126.7, 126.0, 123.6, 116.8, 114.1, 58.6, 32.8, 25.7, 24.5. HRMS (ESI): *m/z* 360.1014 $(M+H)^+$; calc. for $C_{19}H_{20}Cl_2N_3$: 360.1034.

4.2.42 6-Bromo-2-(2-chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (42)

Yield 66%, as a cream solid; mp 144-146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33–8.21 (m, 1H), 7.70–7.56 (m, 1H), 7.52–7.45 (m, 1H), 7.45–7.40 (m, 1H), 7.40–7.29 (m, 2H), 7.20 (dd, *J* = 1.9, 9.5 Hz, 1H), 3.25 (d, *J* = 6.9 Hz, 1H), 2.66 (dd, *J* = 3.4, 6.5 Hz, 1H), 1.75–1.62 (m, 2H), 1.61–1.52 (m, 2H), 1.52–1.39 (m, 1H), 1.18–0.86 (m, 5H); ^{13}C NMR (101 MHz, CDCl3) δ 140.0, 136.2, 133.6, 132.6, 132.5, 129.5, 129.4, 127.1, 127.0, 126.6, 123.0, 118.3, 106.7, 56.4, 33.8, 25.6, 24.5. HRMS (ESI): *m/z* 404.0512 $(M+H)^+$; calc. for $C_{19}H_{20}BrClN_3$: 404.0529.

4.2.43 5-Bromo-2-(2-chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine (43)

Yield 74%, as a viscous brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60–7.57 (m, 1H), 7.54–7.52 (m, 1H), 7.50–7.48 (m, 1H), 7.37–7.29 (m, 2H), 7.03–6.94 (m, 2H), 3.28 (d, *J* = 6.2 Hz, 1H), 2.97–2.62 (m, 1H), 1.66 (d, *J* = 12.4 Hz, 2H), 1.59–1.35 (m, 3H), 1.16–0.97 (m, 3H), 0.97–0.75 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.7, 138.2, 133.9, 133.3, 132.6, 129.5, 129.2, 128.5, 126.6, 123.8, 118.6, 117.3, 112.2, 58.3, 32.7, 25.7, 24.5; HRMS (ESI): *m/z* 404.0516 $(M+H)^+$; calc. for $C_{19}H_{20}BrClN_3$: 404.0529.

4.2.44 6-Bromo-2-(2-chlorophenyl)-*N*-cyclohexyl-5-methylimidazo[1,2-*a*]pyridin-3-amine (45)

Yield 70%, as a pale yellow solid; mp 115-117 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.71–7.55 (m, 1H), 7.55–7.42 (m, 1H), 7.42–7.29 (m, 2H), 7.25 (d, *J* = 4.3 Hz, 2H), 3.20–3.16 (m, 1H), 3.18 (s, 3H), 2.55 (s, 1H), 1.75–1.32 (m, 5H), 1.16–0.65 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 142.1, 138.2, 137.9, 135.1, 134.1, 132.7, 132.5, 129.4, 129.2, 128.3, 126.9, 116.3, 109.9, 58.7, 33.0, 25.7, 24.7, 17.9. HRMS (ESI): *m/z* 418.0676 $(M+H)^+$; calc. for $C_{20}H_{22}BrClN_3$: 418.0686.

4.2.45 2-(2-Chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]quinolin-1-amine (46)

Yield 30%, as a viscous yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.44 (dt, *J* = 5.7, 11.5 Hz, 1H), 7.74 (dt, *J* = 4.5, 9.0 Hz, 1H), 7.71–7.65 (m, 1H), 7.62–7.55 (m, 1H), 7.52–7.47 (m, 1H), 7.46–7.40 (m, 3H), 7.39–7.36 (m, 1H), 7.36–7.33 (m, 1H), 3.60–3.39 (m, 1H), 2.86–2.67 (m, 1H), 1.74 (dd, *J* = 12.6, 18.3 Hz, 2H), 1.60–1.36 (m, 3H), 1.11–0.86 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.7, 134.8, 132.7, 131.3, 129.6, 129.4, 129.3, 129.1, 128.4, 127.7, 127.4, 127.0, 125.7, 124.4, 124.3, 117.5, 117.1, 56.6, 33.1, 25.6, 24.6. HRMS (ESI): *m/z* 376.1576 $(M+H)^+$; calc. for $C_{23}H_{23}Cl\ N_3$: 376.1581.

4.3.1 2-(2-Chlorophenyl)-*N*-cyclohexyl-5-methoxyimidazo[1,2-*a*]pyridin-3-amine (44)

A solution of 5-bromo-2-(2-chlorophenyl)-*N*-cyclohexylimidazo[1,2-*a*]pyridin-3-amine **43** (81.0 mg, 0.200 mmol) in sodium methoxide (2 eq., 0.400 mmol) was refluxed for 4 hours. After being allowed to cool to room temperature the mixture was quenched with water and extracted with ethyl acetate. Organic extracts

were dried over magnesium sulphate and filtered, before excess solvent was removed on a rotary evaporator. Purified by column chromatography using ethyl acetate : hexane as eluant gave 2-(2-chlorophenyl)-*N*-cyclohexyl-5-methoxyimidazo[1,2-*a*]pyridin-3-amine (**44**). Yield 98%, as a brown solid; mp 79-81 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.64–7.57 (m, 1H), 7.51–7.39 (m, 1H), 7.38–7.19 (m, 2H), 7.21–7.11 (m, 1H), 7.04–6.88 (m, 1H), 5.92 (d, *J* = 7.3, 1H), 4.08 (s, 3H), 2.89–2.64 (m, 1H), 1.79–1.41 (m, 6H), 1.21–0.75 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 152.0, 143.7, 134.5, 134.2, 132.6, 129.4, 129.1, 126.3, 124.2, 111.7, 89.1, 57.9, 57.1, 33.8, 26.1, 24.6. HRMS (ESI): *m/z* 356.1502 $(M+H)^+$; calc. for $C_{20}H_{22}ClN_3O$: 356.1454.

4.4 Biological Screening

4.4.1 RT Enzyme assay

The inhibitory activity of compounds was assessed using an ELISA-based colorimetric HIV-1 reverse transcriptase assay (Cat. No. 11468120910, Roche Diagnostics GmbH, Germany) carried out according to the manufacturer's instructions. Percentage residual RT enzyme activity after incubation with 50 µM test compound was determined relative to untreated controls. The kit reaction involves the incorporation of biotin and DIG-labelled nucleotides into cDNA strands polymerized on an RNA template by the action of HIV-1 reverse transcriptase (RT). The cDNA products are bound to streptavidin-coated 96-well plate inserts, and their associated DIG-moieties detected by incubation with anti-DIG antibodies conjugated to horseradish peroxidase (HRP). The amount of bound antibody was measured by incubation with a colorimetric HRP substrate, followed by absorbance reading at 405 nm using a Tecan Infinite F500 multiwell spectrophotometer. In the screening assays, compounds were prepared as 10 mM stocks in DMSO and incubated with HIV-1 reverse transcriptase and substrate at a final concentration of 50 µM in duplicate wells for 1 hour, before proceeding with the rest of the kit protocol. After subtraction of background Abs_{405} values (wells without RT enzyme) from all well readings, residual enzyme activity in compound wells was calculated as a percentage of controls without inhibitor. In-house validation experiments suggest that the assay yields highly reproducible percentage enzyme activity values, both intra-experimentally (average coefficient of variation for replicates = 7%) and inter-experimentally (average coefficient of variation for enzyme activity values = 14%). Nevirapine was included as an internal standard in all assays.

To determine RT IC_{50} values for test compounds, the assay was carried out with serial 4-fold dilutions of the compounds at 8 different concentrations, with 400 µM as the highest final concentration. Duplicate wells were used for each concentration point in the dilution series. Validation experiments suggested that the IC_{50} values obtained for individual compounds are highly reproducible inter-experimentally. The average 95% confidence interval for IC_{50} values determined on three separate occasions was ±20.2%.

4.4.2. MAGI whole cell assays

Whole cell HIV assays were carried out by the Southern Research Institute (SRI, Frederick, MD, USA) according to their in-house protocols. Briefly, MAGI-R5 cells transgenically expressing CD4 and CCR5 and containing an LTR-β-galactosidase reporter construct were incubated with test compounds (triplicate samples) for 15–30 min prior to infection with HIV-1 IIIB. Compounds were added to the cells as 6 log serial dilutions with a highest concentration of 200 μM. After a 48 h incubation, levels of viral infection were quantified using a chemiluminescence β-galactosidase enzyme assay (Perkin Elmer Applied Biosystems) and the IC_{50} (concentration inhibiting virus replication by 50%) calculated from dose-response curves. In addition, compound toxicity was assessed in replicate MAGI-R5 plates incubated with test compounds for 48 h using the tetrazolium-based dye MTS cell viability assay (CellTiter 96 Reagent, Promega, WI). The latter was used to derive the CC_{50} (concentration decreasing cell viability by 50%) and SI (selectivity index: CC_{50}/IC_{50}).

4.4.3 Permeability assay

The assay employed BD Gentest pre-coated 96-well PAMPA (parallel artificial membrane permeability assay) plates (BD Biosciences, Bedford, Massachusetts, USA). Prior to use, the plates were warmed to room temperature for 60 minutes. Test compounds were diluted to 100 μM in PBS and 300 μL added to the wells of the donor plate. After adding 200 μL PBS to the receiver plate wells, the plates were fitted together and incubated with shaking at 37ºC for 5 hours. After incubation, the plates were separated, 100 μL from the receiver and donor plate wells transferred to a UV-transparent 96-well plate and compound concentration in the two samples determined with a multi-well spectrophotometer at 220 nm. The experiment was calibrated by using standards that control for low (atenolol), intermediate (pindolol) and high (metoprolol) permeability. Lucifer yellow is a non-permeable fluorescent molecule that was used to control for the integrity of the phospholipid barriers.

4.5 Molecular modeling

The crystal structures of both the wild-type (pdb code 3MEE) and K103N mutant (pdb code 3MEG) forms of HIV-1 RT complex with the diarylpyrimidine inhibitor rilpivirine (TMC-278) were used for molecular modelling studies using Accelrys® Discovery Studio 2.5.5. The structures were cleaned of any errors such as open valences, incorrect bond orders and incomplete amino acid side chains, and then typed with the charmm forcefield. The backbone was subjected to a fixed atom constraint to maintain the gross structure of the complex, after which the amino acid side-chains, waters and bound molecule were subjected to mild minimisation to optimise van der Waals clashes and X-H bond distances using a conjugate gradient protocol. The electrostatics of the system were calculated and optimised. All waters of crystallisation were then removed, and compound **38**, along with etravirine (TMC-125) and rilpivirine (TMC-278) were docked using CDOCKER as an ensemble of a maximum of 255 conformers to cover the rotamer space of the molecules efficiently. The docked poses were allowed to undergo cycles of simulated annealing to the rigid protein to optimise their docked poses. The poses were ranked according to CDOCKER_INTERACTION_ENERGY,

and the poses in each case visually inspected for chemical and physical inconsistencies. The two check compounds were overlaid on their crystal structure poses, and rms deviations for each determined. TMC-278 was docked within 0.62Å rmsd of the crystal structure pose, while TMC-125 in the 3MEG protein had a similar pose (rmsd of 1.40Å) to its crystallised form (pdb code 3MED), with the one phenyl ring turned ~50º relative to the co-crystallised ligand in 3MED.

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