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PAPER

Groebke multicomponent reaction and subsequent nucleophilic aromatic substitution for a convenient synthesis of 3,8-diaminoimidazo[1,2-*a*]pyrazines as potential kinase inhibitors†

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In a program aimed at discovering novel protein kinase inhibitors, a convenient synthesis of 3,8-diaminoimidazo[1,2-*a*]pyrazines has been developed exploiting the isocyanide-based multicomponent Blackburn reaction, followed by a nucleophilic aromatic substitution with ammonia or primary and secondary amines. The potential of the reported scaffold is strengthened by the inhibition of STAT5-dependent transcription displayed by four of the synthesized compounds.

Introduction

Protein kinases represent one of the widest protein families in humans and play a pivotal role in cell growth, differentiation, apoptosis, and metabolism.[1] Inhibition of kinases has become an attractive therapeutic strategy for several diseases and a vast array of molecules that target the hinge region of the ATP-binding pocket in the catalytic domain have been identified.[2] In this context, the synthesis of chemicals with a nitrogen heterocyclic core, resembling the adenine portion of ATP, is still the most followed synthetic approach in the search for kinase inhibitors that compete with ATP in the catalytic domain.[3] Consequently, the design of novel molecular scaffolds able to mimic the purine nucleus is a primary target for medicinal chemists involved in the identification of novel kinase inhibitors. The possibility to create such a library using a single reaction sequence should furthermore facilitate the search for inhibitors, reducing the time and the costs required.

In our view, multicomponent reactions (MCRs)[4] can address this problem. Indeed, over the last decades, MCRs have attained an increasing importance in heterocyclic chemistry, allowing for the assembly of molecular arrays with high molecular diversity.[5] A survey on the most exploited structures in the kinase field showed us that the imidazo[1,2-*a*]pyrazine portion has been highly used as a general support, able to interact with the hinge region of the enzyme with one or two hydrogen bonds, while the presence of substituents at different positions was fundamental to issue the desired isoform-selectivity.[6]

In connection with our ongoing work on the application of MCRs to the synthesis of biologically active compounds,[7] in this communication we describe the development of a facile route to 3,8-diaminoimidazo[1,2-*a*]pyrazines *via* MCR with the intention to use this core structure as a general support in the discovery of novel kinase inhibitors. Surprisingly, a survey on Sci-Finder Scholar revealed that this scaffold and the use of a multicomponent reaction strategy to prepare such analogues were missed by both academic and industrial researchers involved in kinase projects, despite the strong resemblance between the adenine ring and the 3,8-diaminoimidazo[1,2-*a*]pyrazines.[8]

As the purine skeleton can have a different binding mode depending on the presence of substituents on the amino group,[9] we compared our structure with adenine and olomoucine, a well-known CDK2 kinase inhibitor (Fig. 1).[10]

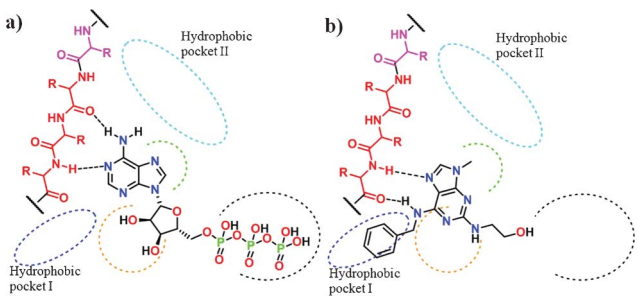

Fig. 1 Schematic representation of ATP (a) and olomoucine (b) binding the catalytic domain of protein kinases. The kinase hinge region (red), the gatekeeper (purple) and the binding pockets are indicated.

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Preliminary computational studies performed with VegaZZ[11] revealed similarity between the 3,8-diaminoimidazol[1,2-*a*]pyrazine scaffold and both ATP and olomoucine: these good superimposition (RMSD < 1 Å) could maintain the key hydrogen bonds with the hinge region (Fig. 2).

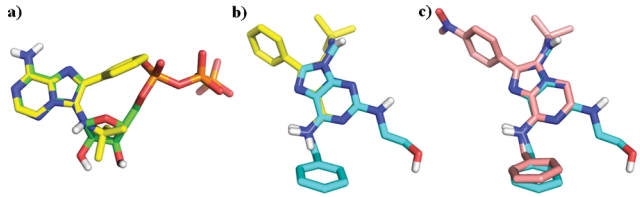
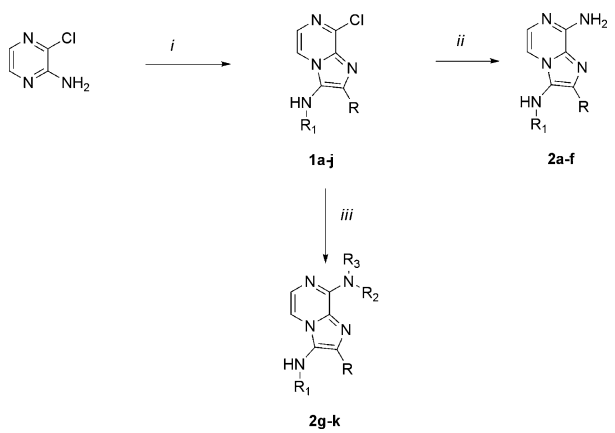


Fig. 2 Superimposition between: (a) ATP (green) and **2c** (yellow), (b) olomoucine (cyan) and **2c**, (c) olomoucine and **2g** (pink). Structures of ATP and olomoucine were obtained from Protein Data Bank, respectively from crystal structure 1E8X and 1W0X.

To achieve the goal, our strategy involved the MCR discovered in 1998 by the independent groups of Blackburn, Bienaymé, and Groebke, where amidines (2-aminopyridine or pyrimidine or pyrazine), aldehydes, and isocyanides react in the presence of an acidic catalyst to give 3-amino-imidazoheterocycles.[12] The 2-aminoazine behaves as a bifunctional reagent, forming a Schiff base with the aldehyde and providing a nucleophilic nitrogen to intercept the nitrilium intermediate.

Results and discussion

We started from 2-amino-3-chloropyrazine, prepared from 2,3-dichloropyrazine by nucleophilic aromatic substitution with aqueous ammonium hydroxide.[13] By reacting it with different aldehydes and isocyanides, we were able to synthesize the 3-amino-8-chloroimidazo[1,2-*a*]pyrazines. Subsequent nucleophilic aromatic substitution with aqueous ammonia or amines led to the desired 3,8-diaminoimidazo[1,2-*a*]pyrazines (Scheme 1).



Scheme 1 *Reagents and conditions*: (i) 1. RCHO (1.2 equiv.), MeCN, reflux, 2 h; 2. TMSCl (1.2 equiv.), MeCN/DCM, 30 min 3. R_1NC (1.2 equiv.), reflux, overnight; (ii) aq. NH_4OH/dioxane, 100 °C, Carius tube, overnight; (iii) R_2R_3NH (3 equiv.), EtOH, 48 h.

Most electron poor amidines, such as 2-amino-3-chloropyrazine, are known to react slowly in the Blackburn reaction and, indeed, a careful optimization of the experimental conditions was necessary. Our initial attempts to run the Blackburn reaction with the representative starting materials 2-amino-3-chloropyrazine, 3-methoxybenzaldehyde and cyclohexyl isocyanide proved to be somewhat discouraging. In the presence of a small set of catalysts such as ammonium chloride[14] or silica sulfuric acid[15] in methanol as solvent, a complex mixture was detected, and the target compound was present in less than 20%. Pre-formation of the imine intermediate was attempted using toluene as solvent,[16] but only a small conversion was observed. Finally, TMSCl was used as a promoter, following the protocol described by Krasavin *et al.*,[17] and gratifyingly after 30 min at reflux the reaction provided the desired product **1a** in 68% of yield. The imine pre-generation suppressed by-products formation and avoided the undesired formation of the regioisomeric fused imidazo-product due to the alternative reaction of the ring nitrogen with the aldehyde, as was first reported by Bradley *et al.*[18]

Different isocyanides and aldehydes were reacted with the 2-amino-3-chloropyrazine in the MCR to give 3-amino-8-chloroimidazo[1,2-*a*]pyrazines **1a–j** in good yields (Fig. 3). Cyclohexyl, pentyl, and *tert*-butyl isocyanides participated in the transformation. The reaction worked well with benzaldehyde, [1,1′-biphenyl]-4-carboxyaldehyde, electron-poor and electron-rich aromatic aldehydes. Unfortunately, aliphatic aldehydes (heptanal, isobutyraldehyde) failed to react under these conditions.

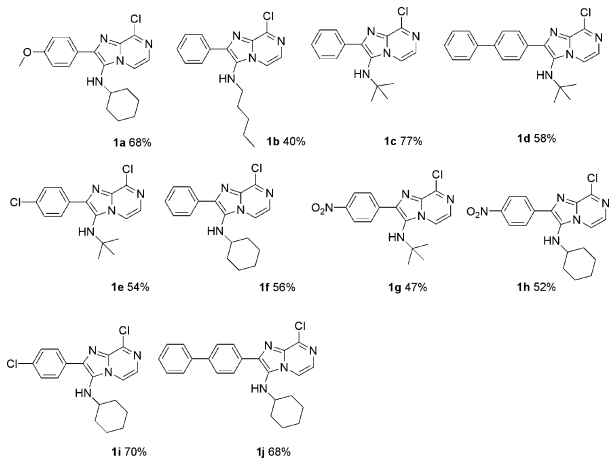


Fig. 3 Synthesized intermediates.

The 3-amino-8-chloroimidazo[1,2-*a*]pyrazines were then reacted with aqueous ammonium hydroxide in a nucleophilic aromatic substitution. The following optimized experimental conditions were followed: the starting material was dissolved in the minimum amount of dioxane, aqueous ammonium hydroxide (3 mL 100 $mmol^{-1}$) was then added, and the reaction was heated at 100 °C overnight in a Carius tube. Six 3,8-diaminoimidazo[1,2-*a*]pyrazines **2a–f** were synthesized in good to excellent yields (Fig. 4). In order to demonstrate the generality of our strategy, the reactions with primary and secondary amines were also explored. The 3-amino-8-chloroimidazo[1,2-*a*]pyrazine (1.0 equiv.) was dissolved in ethanol, the amine (benzylamine, cyclohexylamine, cyclopropylamine, and morpholine) (3.0 equiv.) was added, and the reaction was heated at reflux for 48 h. Five 3,8-diaminoimidazo[1,2-*a*]pyrazines **2g–k** were prepared in good yields (Fig. 4).

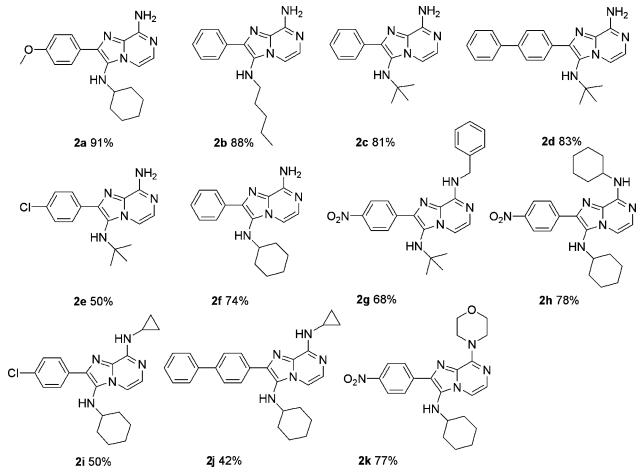


Fig. 4 Synthesized 3,8-diaminoimidazo[1,2-*a*]pyrazines.

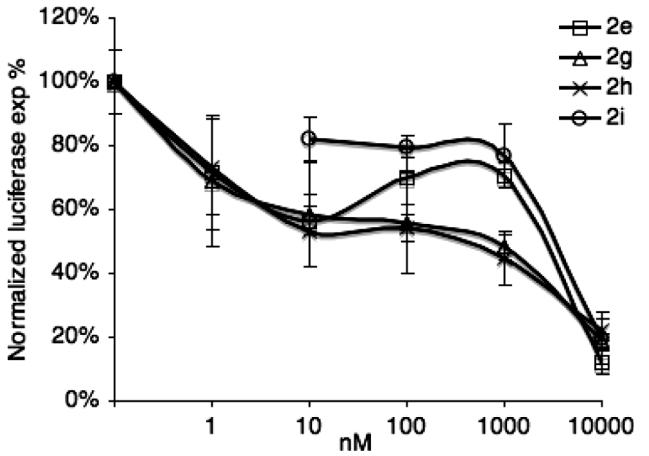


Fig. 5 HEK293 FLT3-ITD transfected with the reporter plasmid and exposed for 24 h with increasing concentration of drugs. The reported STAT5 activation is relative to the DMSO treated sample as 100%.

The synthetic strategy used and the rationale of this preliminary work were not aimed at identifying inhibitors of specific kinases. Nonetheless, we felt it necessary to investigate the putative activity of these compounds on two separate models. First, we investigated all the synthesized molecules for inhibitory activity on PI3Ks. None of the molecules displayed any significant inhibitory activity on these kinases up to a concentration of 10 μM (not shown). Second, as our aim was to demonstrate possible inhibitory activity on kinases without investigating specificity, we opted for investigating a signalling cascade dependent on multiple kinases. In brief, FLT3 is a receptor tyrosine kinase that plays a crucial role in the maturation of hematopoietic progenitors.[19] After stimulation by its specific ligand FLT3, it dimerizes, autophosphorylates and activates downstream signalling through AKT and ERK.[20] In almost 25% of acute myeloid leukemias, the FLT3 receptor presents a mutation of the juxtamembrane domain, named Internal Tandem Duplication, which leads to a conformational change and constitutive autophosphorylation.[21] The activation of this cascade leads directly or through other kinases to the phosphoryation and activation of STAT5-dependent transcription.[22] Such activation can be detected *via* a specific reporter gene that expresses luciferase (which yields light that can be read by a luminometer) when STAT5 is phosphorylated. Therefore, if upon addition of our compounds a decrease in luminescence is recorded, it can be inferred that one or more kinases are inhibited, although it does not allow to pinpoint which specific kinase is inhibited. All compounds were initially screened at a concentration of 100 nM. Of the eleven compounds tested, four were able to reduce activity by at least 60% (**2e**, **2g**, **2h**, **2i**). We therefore continued the characterization of these latter compounds by performing full dose-response curves (Fig. 5).

The four compounds were able to inhibit fully STAT5-dependent transcription at a concentration of 10 μM, suggesting that at least a fundamental kinase in this process is inhibited. FLT3 inhibitors have been described previously[23] and it is interesting to note the structural similarity between purine derivatives (*e.g.* AP23846)[24] and the structures reported by us. It would therefore not be surprising if our compounds were directly FLT3 inhibitors. This is also supported by the complete abolishment of activity observed at high concentrations.

Conclusions

In conclusion, we have developed a straightforward synthetic strategy for the synthesis of 3,8-diaminoimidazo[1,2-*a*]pyrazines, starting from readily available starting materials. This synthesis displays atom economy, efficiency and versatility, making its use feasible for a combinatorial approach. Moreover, the reported scaffold proved to be able to interfere with STAT5-dependent transcription, demonstrating its inhibitory activity on kinases.

Experimental section

Commercially available reagents and solvents were used without further purification and were purchased from Fluka–Aldrich or Lancaster. Acetonitrile was dried by distillation from CaH_2 and stored on activated molecular sieves (4 Å). Dichloromethane was dried by distillation from P_2O_5 and stored on activated molecular sieves (4 Å). When needed, the reactions were performed in oven-dried glassware under a positive pressure of dry nitrogen.

Melting points were determined in open glass capillary with a Stuart scientific SMP3 apparatus and are uncorrected. All the compounds were checked by IR (FT-IR THERMO-NICOLET AVATAR), ^{1}H and ^{13}C APT (JEOL ECP 300 MHz), and mass spectrometry (Thermo Finningan LCQ-deca XP-*plus*) equipped with an ESI source and an ion trap detector. Chemical shifts are reported in part per million (ppm). Column chromatography was performed on silica gel (Merck Kieselgel 70-230 mesh ASTM) using the indicated eluants. Thin layer chromatography (TLC) was carried out on 5 × 20 cm plates with a layer thickness of 0.25 mm (Merck Silica gel 60 F_{254}). When necessary they were developed with $KMnO_4$. Purity of tested compounds was established by elemental analysis. Elemental analysis (C, H, N) of the target compounds are within ± 0.4% of the calculated values.

General procedure for Groebke–Blackburn reaction (1a–j)

2-Amino-3-chloropyrazine (1.0 equiv.) is dissolved in acetonitrile and aldehyde (1.2 equiv.) is added under nitrogen atmosphere. The reaction is refluxed for 2 h. The solvent is removed under vacuum

and the resulting solid is taken up with toluene and evaporated (× 3). A solution of TMSCl (1.2 equiv.) in dichloromethane is added and the reaction is stirred at room temperature for 30 min under nitrogen atmosphere. Then, a solution of the isocyanide (1.2 equiv.) in acetonitrile is added and the reaction is stirred at reflux overnight. The solvent is evaporated and the crude material is purified by column chromatography to afford the desired product.

***N*-[8-chloro-2-(4-methoxyphenyl)imidazo[1,2-a]pyrazin-3-yl]-*N*-cyclohexylamine, 1a.** Eluent: PE/EtOAc 7 : 3, 68%, amorphous yellow solid, (found: C, 63.99; H, 5.98; N 15.81; $C_{19}H_{21}ClN_4O$ requires C, 63.95; H, 5.93; N 15.70%); ν_{max}(KBr)/cm^{-1} 3278, 2938, 2852, 1506, 1250, 1033, 836, 793; δ_H(300 MHz, $CDCl_3$) 7.90 (3 H, m), 7.57 (1 H, d, *J* 4.4), 6.93 (2 H, d, *J* 8.8), 3.82 (3 H, s), 3.20 (1 H, br s), 2.94 (1 H, m), 1.78–1.56 (5 H, m), 1.25–1.10 (5 H, m); δ_C(75 MHz, $CDCl_3$) 159.0, 142.1, 139.0, 132.6, 128.1, 126.9, 126.5, 124.9, 114.8, 113.3, 56.1, 54.6, 33.5, 24.0 (2C); *m*/*z* 357 (M+H)$^+$.

8-Chloro-*N*-pentyl-2-phenylimidazo[1,2-*a*]pyrazin-3-amine, 1b. Column chromatography: PE/EtOAc 5 : 5; yield = 40%; orange solid; mp = 122–123 °C (dec) (found: C, 64.80; H, 5.99; N 17.71; $C_{17}H_{19}ClN_4$ requires C, 64.86; H, 6.08; N 17.80%); ν_{max}(KBr)/cm^{-1} 2921, 2856, 1464, 1350, 1198, 1076, 951, 702; δ_H(300 MHz, $CDCl_3$) 7.86 (3 H, m), 7.57 (1 H, d, *J* 4.7), 7.39 (2 H, m), 7.30 (1 H, m), 3.35 (1 H, br s), 3.01 (2 H, t, *J* 7.1), 1.52 (2 H, quint, *J* 7.1), 1.28 (4 H, m), 0.84 (3 H, t, *J* 7.1); δ_C(75 MHz, $CDCl_3$) 142.8, 138.1, 132.9, 132.5, 129.3, 128.3, 127.9, 127.0, 126.9, 114.9, 47.8, 30.0, 28.7, 22.1, 13.6; MS (ESI) *m*/*z* 315 (M+H)$^+$.

***N*-(*tert*-butyl)-8-chloro-2-phenylimidazo[1,2-a]pyrazin-3-amine, 1c.** Column chromatography: PE/EtOAc 8 : 2; yield = 77%; brown solid; mp = 122–123 °C (found: C, 63.90; H, 6.05; N 18.60; $C_{16}H_{17}ClN_4$ requires C, 63.89; H, 5.70; N 18.63%); ν_{max}(KBr)/cm^{-1} 3287, 2967, 1604, 1459, 1348, 1197, 954, 780, 702; δ_H(300 MHz, $CDCl_3$) 7.88 (2 H, m), 7.39 (5 H, m), 1.05 (9 H, s); δ_C(75 MHz, $CDCl_3$) 143.0, 142.6, 133.7, 133.1, 128.9, 128.5 (2C), 128.3 (2C), 126.5, 56.6, 30.5; MS (ESI) *m*/*z* 301 (M+H)$^+$.

***N*-(2-[1,1′-biphenyl]-4-yl-8-chloroimidazo[1,2-a]pyrazin-3-yl)-*N*-(*tert*-butyl)amine, 1d.** Column chromatography: PE/EtOAc 8 : 2; yield = 58%; orange solid; mp = 177–178 °C (found: C, 70.20; H, 5.70; N 14.90; $C_{22}H_{21}ClN_4$ requires C, 70.11; H, 5.62; N 14.87%); ν_{max}(KBr)/cm^{-1} 2969, 2933, 1545, 1466, 1352, 1217, 951, 776; δ_H(300 MHz, $CDCl_3$) 7.97 (3 H, m), 7.58 (4 H, m), 7.50 (1 H, d, *J* 4.6), 7.38 (2 H, m), 7.30 (1 H, m), 3.25 (1 H, br s), 0.99 (9 H, s); δ_C(75 MHz, $CDCl_3$) δ 142.6, 142.0, 140.4 (2C), 139.9, 133.6, 132.2, 128.4, 128.3, 127.1, 126.7, 126.6 (2C), 116.0, 56.6, 30.0; MS (ESI) *m*/*z* 377 (M+H)$^+$.

***N*-(*tert*-butyl)-8-chloro-2-(4-chlorophenyl)imidazo[1,2-a]pyrazin-3-amine, 1e.** Column chromatography: PE/EtOAc 8 : 2; yield = 54%; yellow solid; mp = 115–117 °C (found: C, 57.40; H, 4.90; N 16.85; $C_{16}H_{16}Cl_2N_4$ requires C, 57.33; H, 4.81; N 16.71%); ν_{max}(KBr)/cm^{-1} 3317, 3284, 2962, 2924, 1603, 1462, 1342, 1205, 950, 832; δ_H(300 MHz, $CDCl_3$) 8.00 (1 H, d, *J* 4.7), 7.84 (2 H, d, *J* 9.6), 7.55 (1 H, d, *J* 4.7), 7.32 (2 H, d, *J* 9.6), 0.98 (9 H, s); δ_C(75 MHz, $CDCl_3$) 143.0, 141.5, 134.1, 133.8, 132.0, 129.5, 128.5, 127.1, 126.6, 116.1, 56.9, 30.2; MS (ESI) *m*/*z* 335 (M+H)$^+$.

8-Chloro-*N*-cyclohexyl-2-phenylimidazo[1,2-*a*]pyrazin-3-amine, 1f. Column chromatography: PE/EtOAc 7 : 3; yield = 56%; brown solid; mp = 187–188 °C (found: C, 66.30; H, 45.95; N 17.00; $C_{18}H_{19}ClN_4$ requires C, 66.15; H, 5.86; N 17.14%); ν_{max}(KBr)/cm^{-1} 3303, 2931, 2850, 1536, 1455, 1355, 945, 775, 609; δ_H(300 MHz, $CDCl_3$) 7.99 (3 H, m), 7.66 (1 H, d, *J* 4.4), 7.47 (2 H, m), 7.37 (1 H, m), 2.97 (1 H, m), 1.81–1.56 (5 H, m), 1.25–1.12 (5 H, m); δ_C(75 MHz, $CDCl_3$) 142.7, 139.0, 132.9, 132.6 (2C), 128.2, 127.8, 127.0, 126.8, 115.1, 56.4, 33.8, 25.0, 24.3; MS (ESI) *m*/*z* 327 (M+H)$^+$.

***N*-(*tert*-butyl)-8-chloro-2-(4-nitrophenyl)imidazo[1,2-a]pyrazin-3-amine, 1g.** Column chromatography: PE/EtOAc 8 : 2; yield = 47%; yellow solid; mp = 167–168 °C (found: C, 55.71; H, 4.75; N 20.30; $C_{16}H_{16}ClN_2O_2$ requires C, 55.58; H, 4.66; N 20.25%); ν_{max}(KBr)/cm^{-1} 2924, 1598, 1518, 1346, 1201, 949, 866; δ_H(300 MHz, $CDCl_3$) 8.23 (4 H, m), 8.06, (1 H, d, *J* 4.4), 7.61 (1 H, d, *J* 4.4), 3.27 (1 H, br s), 1.04 (9 H, s); δ_C(75 MHz, $CDCl_3$) 147.0, 143.4, 139.9, 139.8, 134.1, 128.6, 127.5, 127.3, 123.3, 116.0, 57.0, 30.1; MS (ESI) *m*/*z* 346 (M+H)$^+$.

***N*-[8-chloro-2-(4-nitrophenyl)imidazo[1,2-a]pyrazin-3-yl]-*N*-cyclohexylamine, 1h.** Column chromatography: PE/EtOAc 5 : 5; yield = 52%; orange solid; mp = 157–158 °C (found: C, 58.23; H, 4.98; N 18.73; $C_{16}H_{16}Cl_2N_4$ requires C, 58.14; H, 4.88; N 18.84%); ν_{max}(KBr)/cm^{-1} 3340, 3092, 2925, 2852, 1514, 1342, 1108, 953, 862, 719; δ_H(300 MHz, $CDCl_3$) 8.30 (4 H, s), 7.95 (1 H, d, *J* 4.7), 7.67 (1 H, d, *J* 4.7), 2.99 (1 H, m), 1.84–1.57 (5 H, m), 1.27–1.15 (5 H, m); δ_C(75 MHz, $CDCl_3$) 147.2, 143.9, 139.5, 138.0, 136.8, 133.8, 127.8, 127.7, 123.8, 115.4, 57.2, 34.3, 25.3, 24.7; MS (ESI) *m*/*z* 372 (M+H)$^+$.

***N*-[8-chloro-2-(4-chlorophenyl)imidazo[1,2-a]pyrazin-3-yl]-*N*-cyclohexylamine, 1i.** Column chromatography: PE/EtOAc 5 : 5; yield = 70%; yellow solid; mp = 137–139 °C (found: C, 59.90; H, 5.10; N 15.48; $C_{18}H_{18}Cl_2N_4$ requires C, 59.84; H, 5.02; N 15.51%); ν_{max}(KBr)/cm^{-1} 3295, 3065, 2925, 2852, 1466, 1349, 1088, 944, 793; δ_H(300 MHz, $CDCl_3$) 7.89 (3 H, m), 7.56 (1 H, d, *J* 4.4), 7.35 (2 H, d, *J* 8.5), 3.23 (1 H, br s), 2.91 (1 H, m), 1.76–1.55 (5 H, m), 1.22–1.08 (5 H, m); δ_C(75 MHz, $CDCl_3$) 140.7, 138.2, 134.1, 132.9, 131.4, 128.7, 128.6, 127.3, 128.1, 115.4, 56.8, 34.1, 25.4, 24.6; MS (ESI) *m*/*z* 361 (M+H)$^+$.

2-[1,1′-Biphenyl]-4-yl-8-chloro-*N*-cyclohexylimidazo[1,2-*a*]pyrazin-3-amine, 1j. Column chromatography: PE/EtOAc 5 : 5; yield = 68%; yellow solid; mp = 139–140 °C (found: C, 71.63; H, 5.79; N 14.01; $C_{16}H_{16}Cl_2N_4$ requires C, 71.54; H, 5.75; N 13.91%); ν_{max}(KBr)/cm^{-1} 3307, 3277, 2925, 2852, 1484, 1357, 1201, 945, 769, 734; δ_H(300 MHz, $CDCl_3$) 8.04 (2 H, d, *J* 8.2), 7.89 (1 H, d, *J* 4.4), 7.64 (4 H, m), 7.54 (1 H, d, *J* 4.4), 7.43 (2 H, t, *J* 7.7), 7.33 (1 H, t, *J* 7.7), 3.32 (1 H, br d), 2.97 (1 H, m), 1.80–1.48 (5 H, m), 1.27–1.13 (5 H, m); δ_C(75 MHz, $CDCl_3$) 142.8, 140.6, 140.5, 140.1, 138.9, 133.2, 131.7, 128.6, 128.0, 127.4, 127.2, 126.9, 126.7, 115.3, 56.5, 34.0, 25.3, 24.4; MS (ESI) *m*/*z* 403 (M+H)$^+$.

General procedure with ammonia (2a–f)

The 3-amino-8-chloroimidazo[1,2-*a*]pyrazine (0.200 mmol) is dissolved in dioxane in a Carius tube and 33% ammonium hydroxide in water (5 mL) is added. The reaction is stirred at 100 °C for three days. EtOAc is added and the organic phase is washed with water (× 1), brine (× 1) and dried over sodium sulfate. The crude material is purified by column chromatography.

***N*-[8-amino-2-(4-methoxyphenyl)imidazo[1,2-a]pyrazin-3-yl]-*N*-cyclohexylamine, 2a.** Column chromatography: PE/EtOAc 4 : 6; yield = 91%; brown solid; mp = 107–109 °C (found: C, 67.71; H, 6.93; N 20.72; $C_{19}H_{23}N_5O$ requires C, 67.63; H, 6.87; N 20.76%); ν_{max}(KBr)/cm^{-1} 2926, 2852, 1611, 1507, 1250, 1174, 1031, 838; δ_H(300 MHz, $CDCl_3$) 7.87 (2 H, d, *J* 8.8), 7.43 (1 H, d, *J* 4.9), 7.22 (1 H, d, *J* 4.9), 6.99 (2 H, d, *J* 8.8), 3.84 (3 H, s), 3.13 (1 H, d, *J* 5.8), 2.93 (1 H, m), 1.81–1.56 (5 H, m), 1.25–1.15 (5 H, m); MS (ESI) *m*/*z* 337 (M+H)$^+$.

***N*-(8-amino-2-phenylimidazo[1,2-a]pyrazin-3-yl)-*N*-pentylamine, 2b.** Column chromatography: PE/EtOAc 5 : 5; yield = 88%; yellow solid; mp = 132–133 °C (dec) (found: C, 69.21; H, 7.23; N 23.59; $C_{17}H_{21}N_5$ requires C, 69.12; H, 7.17; N 23.71%); ν_{max}(KBr)/cm^{-1} 2956, 2924, 2853, 1516, 1028; δ_H(300 MHz, $CDCl_3$) 7.87 (2 H, m), 7.50–7.26 (5 H, m), 3.20 (1 H, br t), 3.04 (2 H, q, *J* 6.9), 1.57 (2 H, quint, *J* 6.9), 1.35–1.18 (4 H, m), 0.85 (3 H, t, *J* 7.1); δ_C(75 MHz, $CDCl_3$) 149.9, 135.7, 134.1, 129.6, 129.2, 128.6, 128.0, 127.4, 127.1, 108.3, 48.9, 30.7, 30.0, 29.5, 14.3; MS (ESI) *m*/*z* 296 (M+H)$^+$.

***N*-(8-amino-2-phenylimidazo[1,2-a]pyrazin-3-yl)-*N*-(*tert*-butyl)-amine, 2c.** Column chromatography: PE/EtOAc 4 : 6; yield = 81%; yellow solid; mp = 133–134 °C (dec) (found: C, 68.35; H, 6.73; N 24.92; $C_{16}H_{19}N_5$ requires C, 68.30; H, 6.81; N 24.89%); ν_{max}(KBr)/cm^{-1} 3467, 3292, 2923, 1742, 1637, 1517, 1203, 755, 699; δ_H(300 MHz, $CDCl_3$) 7.84 (2 H, m), 7.54 (1 H, d, *J* 4.7), 7.40 (2 H, m), 7.29 (2 H, m), 1.02 (9 H, s); δ_C(75 MHz, $CDCl_3$) 149.1, 139.1, 134.2, 128.8, 128.0, 127.6, 127.0, 126.8, 125.9, 108.5, 56.1, 29.8; MS (ESI) *m*/*z* 282 (M+H)$^+$.

***N*-(8-amino-2-[1,1′-biphenyl]-4-ylimidazo[1,2-a]pyrazin-3-yl)-*N*-(*tert*-butyl)amine, 2d.** Purification: filtration and water washes; yield = 83%; pale yellow solid; mp = 150–152 °C (found: C, 73.99; H, 6.38; N 19.61; $C_{22}H_{23}N_5$ requires C, 73.92; H, 6.49; N 19.59%); ν_{max}(KBr)/cm^{-1} 3475, 3286, 3096, 2971, 1635, 1519, 1199, 844, 759; δ_H(300 MHz, $CDCl_3$) 7.96 (3 H, m), 7.67 (4 H, m), 7.56 (1 H, d, *J* 4.6), 7.46 (2 H, m), 7.29 (1 H, m), 5.72 (1 H, br s), 2.02 (2 H, br s), 1.07 (9 H, s); δ_C(75 MHz, $CDCl_3$) 153.6, 144.6, 144.5, 143.0, 137.2 (2C), 132.8, 132.4, 131.5, 131.1, 130.9 (2C), 130.7, 112.8, 60.5, 34.1; MS (ESI) *m*/*z* 358 (M+H)$^+$.

***N*-[8-amino-2-(4-chlorophenyl)imidazo[1,2-a]pyrazin-3-yl]-*N*-(*tert*-butyl)amine, 2e.** Column chromatography: PE/EtOAc 5 : 5; yield = 50%; yellow solid; mp = 154–155 °C (dec) (found: C, 60.89; H, 5.81; N 22.20; $C_{16}H_{18}ClN_5$ requires C, 60.85; H, 5.75; N 22.18%); ν_{max}(KBr)/cm^{-1} 2961, 2884, 2806, 1635, 1522, 1482, 1439, 1202, 1012, 832; δ_H(300 MHz, $CDCl_3$) 7.88 (2 H, d, *J* 8.5), 7.51 (1 H, d, *J* 4.7), 7.39 (2 H, d, *J* 8.5), 7.28 (1 H, d, *J* 4.7), 5.76 (2 H, br s), 2.98 (1 H, br s), 0.99 (9 H, s); δ_C(75 MHz, $CDCl_3$) 149.8, 138.4, 133.5, 133.4, 129.5, 129.3, 128.6, 127.7, 108.8, 126.1, 56.5, 30.5; MS (ESI) *m*/*z* 316 (M+H)$^+$.

***N*-(8-amino-2-phenylimidazo[1,2-a]pyrazin-3-yl)-*N*-cyclohexylamine, 2f.** Column chromatography: PE/EtOAc 5 : 5; yield = 74%; brown solid; mp = 134–135 °C (found: C, 70.39; H, 6.90; N 22.89; $C_{18}H_{21}N_5$ requires C, 70.33; H, 6.89; N 22.78%); ν_{max}(KBr)/cm^{-1} 2360, 2341, 764, 749; δ_H(300 MHz, $CDCl_3$) 7.94 (2 H, m), 7.45 (3 H, m), 7.31 (1 H, m), 7.26 (1 H, m), 3.16 (1 H, br d), 2.98 (1 H, m), 1.80–1.58 (5 H, m), 1.27–1.13 (5 H, m); δ_C(75 MHz, $CDCl_3$) 149.8, 136.0, 133.8, 128.9, 128.6, 128.0 (2C), 127.5, 127.3, 108.1, 57.1, 34.3, 25.8, 25.0; MS (ESI) *m*/*z* 308 (M+H)$^+$.

General procedure with amine (2g–k)

The 3-amino-8-chloroimidazo[1,2-*a*]pyrazine (1.0 equiv.) is dissolved in ethanol and the amine (3.0 equiv.) is added. The reaction is stirred at reflux for 24 h. The solvent is evaporated under vacuum and the crude material is purified by column chromatography.

***N*-benzyl-*N*-[3-(*tert*-butylamino)-2-(4-nitrophenyl)imidazo[1,2-a]pyrazin-8-yl]amine, 2g.** Column chromatography: PE/EtOAc 8 : 2; yield = 68%; orange oil; (found: C, 66.40; H, 5.95; N 20.17; $C_{23}H_{24}N_6O_2$ requires C, 66.33; H, 5.81; N 20.18%); ν_{max}(KBr)/cm^{-1} 3399, 2973, 1599, 1547, 1341, 1203, 856, 739 cm^{-1}; δ_H(300 MHz, $CDCl_3$) 8.24 (4 H, s), 7.43–7.27 (7 H, m), 6.41 (1 H, br t), 4.78 (2 H, d, *J* 5.8), 2.95 (1 H, br s), 1.08 (9 H, s); δ_C(75 MHz, $CDCl_3$) 148.9, 146.5, 141.3, 138.3, 136.1, 130.0, 128.6, 128.3, 128.0, 127.8, 127.4, 127.0, 123.5, 107.3, 56.8, 44.6, 30.4; MS (ESI) *m*/*z* 417 (M+H)$^+$.

***N*-cyclohexyl-*N*-[3-(cyclohexylamino)-2-(4-nitrophenyl)imidazo[1,2-a]pyrazin-8-yl]amine, 2h.** Column chromatography: PE/EtOAc 8 : 2; yield = 78%; orange solid; mp = 149–150 °C (dec) (found: C, 66.40; H, 6.97; N 19.30; $C_{24}H_{30}N_6O_2$ requires C, 66.34; H, 6.96; N 19.34%); ν_{max}(KBr)/cm^{-1} 2929, 2850, 1508, 1340, 1108, 854; δ_H(300 MHz, $CDCl_3$) 8.28 (4 H, s), 7.34 (1 H, d, *J* 4.9), 7.25 (1 H, d, *J* 4.9), 5.93 (1 H, br d), 3.02 (1 H, br d), 2.94 (1 H, m), 2.12 (1 H, m), 1.82–1.15 (20 H, m); δ_C(75 MHz, $CDCl_3$) 148.6, 146.5, 140.9, 132.9, 129.9, 128.9, 128.8, 127.0, 124.0, 106.0, 57.4, 49.3, 34.4, 33.3, 25.7, 25.6, 25.1, 24.9; MS (ESI) *m*/*z* 435 (M+H)$^+$.

***N*-[2-(4-chlorophenyl)-3-(cyclohexylamino)imidazo[1,2-a]pyrazin-8-yl]-*N*-cyclopropylamine, 2i.** Column chromatography: PE/EtOAc 8 : 2; yield = 50%; brown solid; mp = 79–80 °C (found: C, 66.08; H, 6.40; N 18.40; $C_{21}H_{24}ClN_5$ requires C, 66.04; H, 6.33; N 18.34%); ν_{max}(KBr)/cm^{-1} 2929, 2852, 1541, 1490, 1091, 1013, 836; δ_H(300 MHz, $CDCl_3$) 7.92 (2 H, d, *J* 8.2), 7.40–7.28 (4 H, m), 2.95 (3 H, m), 1.84–1.55 (5 H, m), 1.22–1.05 (5 H, m), 0.90 (4 H, m); δ_C(75 MHz, $CDCl_3$) 150.9, 135.9, 135.0, 134.6, 133.7, 130.1 (2C), 129.5, 129.0, 108.6, 58.4, 35.6, 27.0, 26.1, 25.0, 8.60; MS (ESI) *m*/*z* 382 (M+H)$^+$.

***N*-[2-[1,1′-biphenyl]-4-yl-3-(cyclohexylamino)imidazo[1,2-a]pyrazin-8-yl]-*N*-cyclopropylamine, 2j.** Column chromatography: PE/EtOAc 5 : 5; yield = 42%; brown oil (found: C, 76.66; H, 6.98; N 16.65; $C_{27}H_{29}N_5$ requires C, 76.56; H, 6.90; N 16.53%); ν_{max}(KBr)/cm^{-1} 2950, 2852, 1538, 1512, 1091, 1013, 836, 720; δ_H(300 MHz, $CDCl_3$) 8.03 (2 H, d, *J* 8.2), 7.70–7.35 (9 H, m), 2.95 (2 H, m), 1.86–1.56 (5 H, m), 1.22–1.05 (5 H, m), 0.96 (2 H, m), 0.83 (2 H, m); δ_C(75 MHz, $CDCl_3$) 149.4, 146.3, 140.9 (2C), 140.6 (2C), 132.4, 129.2, 127.9, 127.7, 127.6, 127.5, 127.3, 107.6, 57.4 (2C), 34.6, 25.9, 25.1, 7.67; MS (ESI) *m*/*z* 424 (M+H)$^+$.

***N*-cyclohexyl-8-morpholino-2-(4-nitrophenyl)imidazo[1,2-a]pyrazin-3-amine, 2k.** The product precipitates during the reaction and is filtered under vacuum; yield = 77%; yellow amorphous solid (found: C, 62.60; H, 6.23; N 19.91; $C_{22}H_{26}N_6O_3$ requires C, 62.54; H, 6.20; N 19.89%); ν_{max}(KBr)/cm^{-1} 2936, 2856, 1598, 1510, 1338, 1115; δ_H(300 MHz, $CDCl_3$) 8.27 (4 H, m), 7.38 (2 H, m), 4.32 (4 H, t, *J* 4.1), 3.89 (4 H, t, *J* 4.1), 2.98 (1 H, m), 1.80–1.58 (5 H, m), 1.27–1.13 (5 H, m); δ_C(75 MHz, $CDCl_3$) 151.7, 148.6, 142.8, 134.5,

132.8, 130.2, 130.0, 129.1, 126.0, 109.7, 69.2, 59.3, 48.9, 36.5, 27.7, 27.0; MS (ESI) m/z 423 $(M+H)^+$.

Cell culture and detection of STAT5 phosphorylation and activation

HEK293 stable expressing FLT3-ITD (kind gift of Prof. Thomas Fisher) where cultured in RPMI1640 10% FCS, 1% Pen/Strep and Glutamine. 10 000 cells where plated in a 96 well plate in 50 µL of medium 6 h before transfection. For transfection, 0.2 µL of Lipofectamine 2000 (Invitrogen) was mixed in 20 µL of Opti-MEM (Invitrogen) with 50 ng of LHRE-FF plasmid (Lactogene Hormone Responsive Element-Firefly luciferase) and 50 ng of pGL4.73 renilla luciferase plasmid (Promega) that served, respectively, as reporter of STAT5 activation and normalization of transfection and viability. After 18 h, the cells were exposed for 24 h to the compounds dissolved in DMSO, which served as a negative control. Luciferase activity was then measured by a commercial luciferase assay (Promega). All experiments were conducted at least in triplicate.

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