Structural Optimization of a Pyridinylimidazole Scaffold: Shifting the Selectivity from p38α Mitogen-Activated Protein Kinase to c-Jun N-Terminal Kinase 3

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ABSTRACT: Starting from known p38α mitogen-activated protein kinase (MAPK) inhibitors, a series of inhibitors of the c-Jun N-terminal kinase (JNK) 3 was obtained. Altering the substitution pattern of the pyridinylimidazole scaffold proved to be effective in shifting the inhibitory activity from the original target p38α MAPK to the closely related JNK3. In particular, a significant improvement for JNK3 selectivity could be achieved by addressing the hydrophobic region I with a small methyl group. Furthermore, additional structural modifications permitted to explore structure−activity relationships. The most potent inhibitor 4-(4-methyl-2-(methylthio)-1H-imidazol-5-yl)-N-(4-morpholinophenyl)pyridin-2-amine showed an IC50 value for the JNK3 in the low triple digit nanomolar range and its binding mode was confirmed by X-ray crystallography.

INTRODUCTION

The mitogen-activated protein kinases (MAPKs) represent a family of enzymes involved in several signal transduction pathways, whose activation is part of a phosphorylation cascade triggered by diverse extracellular stimuli. Among the members of this family, the c-Jun N-terminal kinases (JNKs) mostly respond to a variety of stress stimuli such as radiation, osmotic or heat shock, oxidative insult, and proinflammatory cytokines, modulating responses such as cell survival and apoptosis.1 The JNK subfamily is encoded by the three genes jnk1, jnk2, and jnk3, which in turn give rise to 10 different isoforms through alternative splicing.2 Despite their structural homology and the partially functional redundancy, these isoforms follow a different tissue distribution pattern, JNK3 being restricted to the central nervous system, heart, and testis oppositely to the ubiquitous expression of JNK1 and 2.2,3 In addition to this, a different substrate specificity of the JNK1, 2, and 3 suggests the existence of isoform-specific roles of these enzymes, which were partially disclosed through gene knockout studies.4 There is well-documented evidence for the critical role of the JNK subfamily members in several neurodegenerative diseases such as Parkinson’s and Alzheimer’s disease, as well as in neuronal death derived by stroke and ischemia/reperfusion injury.3,6,8 Furthermore, some members of the JNKs are also involved in metabolic and inflammatory diseases, and several studies suggest that these kinases might contribute to the development and diffusion of some forms of cancer,7,9 thus emerging as particularly attractive drug targets.

Despite the intense endeavor in the research of JNK inhibitors, only a scarce number of candidates have reached clinical trial phases and to date, none of them have been approved.10−12 Until early 2010s, a major challenge in the development of JNK inhibitors has been the achievement of selectivity over the closely related p38α MAPK,11 a member of the same family which, analogously to the JNKs, participates in regulating the cellular response to stress stimuli. This protein kinase was also shown to assume a key function in diabetic complications of type II diabetes,13 and the simultaneous inhibition of JNK and p38α MAPK is assumed to obtain a synergistic effect in the treatment of some pathological conditions.14 Nevertheless, obtaining a JNK-selective inhibitor would be beneficial to fully elucidate the effective role of this protein kinase in the aforementioned pathological conditions and thereby assess its therapeutic potential. Furthermore, most of the reported clinical trials on selective p38α MAPK inhibitors have been discontinued because of the insurgence of adverse effects mostly related to liver toxicity,15 leading to...
the assumption the activity on the \( p38^{\alpha} \) MAPK to be undesired for an improved safety profile of JNK inhibitors.

Regarding the selectivity within the JNK subfamily, the achievement of JNK isoform-selective inhibitors would be desirable to dissect the contribution of the different isoforms in various pathological conditions. However, the JNK1, 2, and 3 share more than 80% sequence identity, making the development of isoform-specific inhibitors extremely challenging.

In the last decades, pyridinylimidazoles have encountered a remarkable success in the field of \( p38^{\alpha} \) MAPK inhibition. This class of inhibitors counts a large number of examples starting from the precursor \( \text{SB203580} \) to the optimized compound \( \text{LN950} \) (Figure 1), until reaching derivatives with low single digit nanomolar IC \(_{50}\) values (a review on this class of compounds has recently been published).\(^{18}\) As can be seen from Figure 1, the reported \( p38^{\alpha} \) MAPK inhibitors are also able to inhibit the JNK3 with IC \(_{50}\) values in the submicromolar range, thus offering a suitable starting point for optimization when aiming to target this enzyme. In 2016, we published compound \( 1a \) as a balanced dual JNK3/\( p38^{\alpha} \) MAPK inhibitor, which served as a precursor for the synthesis of a fluorescent probe used in fluorescence polarization-based binding assays.\(^{19,20}\) As it is evident from the biological activity of \( 1a \) in comparison to the activity of previous inhibitors, modifying the substitution pattern around the pyridinylimidazole scaffold can contribute to a shift in selectivity toward the JNK3.

Some of us have recently reported the optimization of compound \( 1a \) following a covalent inhibition approach (compound \( 1b \)), which was based on the introduction of an electrophilic moiety able to target a noncatalytic cysteine of the JNK3 that is not conserved in the closely related \( p38^{\alpha} \) MAPK.\(^{21}\) The aim of the herein presented work consists instead in the achievement of a potent and selective JNK inhibitor by structural modification of the pyridinylimidazole scaffold following the canonical concept of reversible inhibition.

### RESULTS AND DISCUSSION

**Chemistry.** Despite the overall similarity of their structures, the herein reported compounds were synthesized following considerably diverse routes, especially with regard to the construction of the five-membered heterocyclic central core. The synthesis of compounds \( 5 \) and \( 8 \) was achieved as displayed in Scheme 1. The route leading to the common intermediate \( 3 \), starting from 2-fluoro-4-methylpyridine (2), is based on the Marckwald imidazole synthesis\(^{22}\) and was previously reported by Laufer et al.\(^{23}\) The substitution on the imidazole-C2-S position was obtained by reacting imidazole-2-thione \( 3 \) with the appropriate alkyl halide. Finally, the introduction of the 4-morpholinoaniline moiety was carried out through nucleophilic aromatic substitution in acidic conditions, this representing the final step for most of the herein presented compounds. Applying these conditions to the hydroxyethyl derivative \( 6 \) unexpectedly yielded imidazol-2-one \( 8 \), instead of imidazole \( 7 \), as a result of a previously described rearrangement.\(^{24}\)

The preparation of 2,4,5-trisubstituted imidazole \( 13 \) and of 4,5-disubstituted imidazoles \( 14 \) and \( 18a-1 \) is outlined in Scheme 2. The route providing \( \alpha \)-diketone \( 10 \) starting from 2-fluoro-4-methylpyridine (2) was recently described by Ansideri et al.,\(^{19} \) whereas the synthesis of intermediates \( 16a-1 \) was achieved following a similar approach. Ethanones \( 15a-1 \) were obtained by condensation of the appropriate ethyl ester with 2-chloro-4-methylpyridine (9) and were subsequently oxidized.
Scheme 2. Synthesis of 4,5-Disubstituted Imidazoles 13, 14, and 18a–l

Reagents and conditions: (a) route reported by Ansideri et al.; (b) HCHO$_{aq}$, NH$_4$OAc, AcOH, 180 °C microwave irradiation, 2–5 min; (c) propionaldehyde, 7 M NH$_3$ in MeOH, 80 °C, 4 h; (d) 4-morpholinoaniline, 1.25 M HCl in EtOH, n-BuOH, 180 °C, 16 h; (e) ethyl arylcarboxylate or ethyl alkylcarboxylate, NaHMDS, dry THF, 0 °C, 2–3 h; and (f) SeO$_2$, AcOH, 70 °C, 2–3 h; (R$_2$ = see Table 2).

Scheme 3. Synthesis of Imidazoles 38, 39, 43–45, and 47

Reagents and conditions: (a) SOCl$_2$, reflux temperature, 5 h; (b) N,O-dimethylhydroxylamine hydrochloride, Et$_3$N, dry DCM, 16 h; (c) EtMgBr or n-PrMgBr, dry THF, –10 °C, 1–3 h; (d) NH$_2$OH·HCl, 20% NaOH$_{aq}$, MeOH, H$_2$O, 0 °C, 1–2 h; (e) TsCl, pyridine, rt, 24–72 h; (f) EtOH$_{ref}$, K$_{aq}$, 0 °C, 2–16 h; (g) concd HCl, 50 °C, 1–4 h; (h) KSCN, MeOH, reflux temperature, 4 h; (i) H$_2$O$_2$, AcOH, rt, 15 min; (j) MeI, t-BuONa, MeOH, 50 °C, 0.5–3 h; (k) 4-morpholinoaniline, 1.25 M HCl in EtOH, n-BuOH, 180 °C, 16 h; and (l) cyanamide, EtOH, reflux temperature, 2 h.
and 37 by oxidative desulfurization as well as the 2-methylsulfonylimidazoles 40–42 via monomethylation. Alternatively, compound 46 displaying a 2-aminimidazole core could be prepared by cyclization of the \( \alpha \)-aminoketone 31 with cyanamide. Intermediates 36, 37, 40–42, and 47 were then reacted with 4-morpholinoaniline, as previously mentioned, to afford the final compounds 38, 39, 43–45, and 47, respectively.

Several analogues of compound 44 featuring a different substituent at the pyridine-C2 position (compounds 48m and 48n, Scheme 4) could be prepared by nucleophilic aromatic substitution of synthone 41 with p-phenylethylamine, 1-phenylethylamine, or with diverse branched or cycloalkyl amines. In addition, compound 48h and the previously reported 48m were coupled with different acid chlorides or anhydrides to obtain the corresponding amides 48i–q (Scheme 4).

The introduction of a methyl substituent on the imidazole-N atom, providing 1,2,4,5-tetrasubstituted imidazoles 50 and 57, required a distinct approach depending on the desired N-methylated regioisomer. In fact, double nucleophilic substitution of imidazole-2-thione 34 using excess of methyl iodide almost exclusively afforded the regioisomer bearing the methyl substituent on the N atom away from the pyridine ring (49, Scheme 5). The regioselectivity of the methylation reaction was confirmed by crystal structure analysis of intermediate 49 (see Figure S1 in the Supporting Information) and was attributed to the lower steric hindrance offered by the methyl group compared to the pyridine ring. The regioisomer 54, having the methyl group on the N atom adjacent to the pyridine ring, was instead achieved by cyclizing the \( \alpha \)-aminoketone 31 with methyl isothiocyanate, followed by methylation of the sulfur of the resulting N1-methylimidazole-2-thione 51.

This approach, adapting a procedure published by Xi et al., represents an unusual route to tetrasubstituted pyridinylimidazoles and was recently reported by some of us for the preparation of tetrasubstituted imidazoles bearing two aromatic moieties at the 4 and 5 positions. The same method could also be employed, using the appropriate alkyl isothiocyanate, to achieve the N-ethyl- and the N-cyclopropylimidazole derivatives 55 and 56, respectively. Unlike the majority of the reported compounds, the introduction of the 4-morpholinoaniline moiety, yielding compounds 50 and 57–59, was carried out by palladium-catalyzed Buchwald–Hartwig aroylation.

The synthesis of the 1,5-disubstituted imidazole 66, bearing an aromatic substituent on the imidazole-N1 atom, was performed starting from 2-bromoisonicotinaldehyde (60) via a two-step procedure as depicted in Scheme 6. Such a route entails the formation of the imine derivative 61 and its direct cyclization through the Van Leusen reaction using toluene sulfonylmethylisocyanide (TOSMIC) and K₂CO₃. The analogous route was unfortunately not accessible for the synthesis of the N1-methyl substituted derivative 67 because of the instability of the corresponding imine intermediate. As an alternative, the preformed N1-methyl imidazole group was introduced through Suzuki cross-coupling reaction between 5-bromo-1-methyl-1H-imidazole (62) and pyridinyl-boronic acid chlorides or anhydride, dry pyridine, rt, 16 h;

\[ \text{Reagents and conditions: (a) KSCN, MeOH, reflux temperature, 4 h; (b) Me$_2$SiCl, CH$_2$Cl$_2$, rt, 16 h; (c) 4-morpholinoaniline, Pd$_2$(dba)$_3$, Xantphos, Cs$_2$CO$_3$, dry 1,4-dioxane, 100 °C, 18 h; (d) alkyl isothiocyanate, Et$_3$N, 60 °C, 16 h; (e) AcOH, 80 °C, 1 h; (f) Me$_3$SiCN, CH$_2$Cl$_2$, rt, 16 h; (g) 4-morpholinoaniline, Pd$_2$(dba)$_3$, XPhos, Cs$_2$CO$_3$, dry 1,4-dioxane, 100 °C, 16 h.} \]
acid 63 (Scheme 6). The last step of both routes consisted of the introduction of the 4-morpholinoaniline moiety. In the case of the 4-fluorophenyl derivative 64, this was performed by Buchwald–Hartwig amination giving compound 66, whereas the acid-catalyzed nucleophilic aromatic substitution was employed for the synthesis of compound 67.

The 1,2-disubstituted imidazole derivative 71 was obtained starting from 2-chloroisonicotinonitrile (68), which was initially reacted in a one-pot procedure described by Voss et al.31 (Scheme 7). This reaction involves the formation of an imidate, followed by substitution with acetal-protected aminoacetaldehyde and final ring closure by deprotection, affording 2-(pyridine-4-yl)imidazole 69 in good yield. At last, N-methylimidazole 70 was obtained by nucleophilic substitution with methyl iodide and subsequently reacted with 4-morpholinoaniline as previously discussed, yielding compound 71.

For the synthesis of compounds 75 and 78, presenting a methylaminothiazole central core, an approach related to Hantzsch thiazole synthesis32 was employed (Schemes 8 and 9).

Thiazole 75 was obtained starting from 1-(4-fluorophenyl)-2-(2-fluoropyridin-4-yl)ethan-1-one (72), whereas compound 78 was synthesized starting from 1-pyridyl-propan-1-one (22). Both ketones 72 and 22 were monohalogenated at the α-position under acidic conditions and then cyclized via N-methylthiourea, affording intermediates 74 and 77, respectively. Conclusively, substitution with 4-morpholinoaniline yielded the desired compounds 75 and 78.

**Biological Evaluation.** All synthesized inhibitors were evaluated by enzyme-linked immunosorbent assays15,34 to determine their ability to inhibit JNK3 and p38α MAPK, and the results are presented in Tables 1–4 and 6.

### Table 1. Core Modifications on 4-F-Phenyl-Substituted Derivatives

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Core</th>
<th>IC50 ± SD [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>38 ± 2</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>142 ± 28</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>34 ± 0.2</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>31 ± 2</td>
</tr>
<tr>
<td>66</td>
<td></td>
<td>276 ± 10</td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>15 ± 3</td>
</tr>
<tr>
<td>SB203580</td>
<td></td>
<td>58 ± 16f</td>
</tr>
<tr>
<td>SP600125</td>
<td></td>
<td>171 ± 36f</td>
</tr>
</tbody>
</table>

Data of standard inhibitors SB203580 (p38α MAPK) and SP600125 (JNK3) in our in-house activity assay are included.

IC50 values are the mean of three experiments.

The free terminal aniline moiety of compound 1a is considered to be potentially responsible for aggregation and therefore might result in assay interference, as also pointed out by analysis through the ZINC 15 pattern tool.35 For this reason, the p-phenylendiamine moiety at the pyridine-C2 position of compound 1a was modified in a 4-morpholinoaniline group, which has already been reported as a beneficial substituent in this position.36 Resulting compound 5 (Table 1) displayed extremely close inhibition values to its analogoue 1a (IC50[JNK3] = 24 nM; IC50[p38α MAPK] = 17 nM) and this moiety was, therefore, maintained constant during the investigation of other positions of the scaffold. The first attempt, which was carried out to shift the preference of compound 5 toward the JNK3, consisted of modifying the central imidazole core together with acting on the substitution at the imidazole-C2 position (Table 1). Transformation of the methylsulfanyl group at the imidazole-C2 position into an ethyl group or removal of the same group,
resulting in compounds 13 and 14, respectively, did not seem to affect the inhibitory activity on the two enzymes. Replacement of the imidazole core with an imidazol-2-one ring instead caused a decrease in the JNK3 inhibitory activity while leaving the inhibition of p38α MAPK unchanged (8: IC₅₀(JNK3) = 142 nM; IC₅₀(p38α MAPK) = 34 nM). The position of the two nitrogen atoms at the central imidazole core seems to be essential for the inhibition of both enzymes, as the different arrangements of substituents around the five-membered ring of 1,5-disubstituted imidazole 66 resulted in a drop in activity on both target kinases. On the other hand, exchange of 2-sulfanylimidazole with 2-methylaminothiazole (75) yielded an increase in inhibitory activity of 2.5- and 8-fold for JNK3 and p38α MAPK, respectively.

To assess the effect of the substituent located in the hydrophobic region (HR) I, the 4-fluorophenyl group was replaced by different aromatic, alkyl, and cycloalkyl moieties (Table 2). In terms of both ligand efficiency (LE) as well as

Table 2. Effect of Different Aryl and Alkyl Substituents at the Imidazole C4(5) Position

<table>
<thead>
<tr>
<th>Cpd</th>
<th>R</th>
<th>IC₅₀ ± SD [nM]</th>
<th>JNK3</th>
<th>p38α MAPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td></td>
<td>31 ± 2</td>
<td>21 ± 1</td>
<td></td>
</tr>
<tr>
<td>18a</td>
<td></td>
<td>37 ± 3</td>
<td>24 ± 3</td>
<td></td>
</tr>
<tr>
<td>18b</td>
<td>Br</td>
<td>60 ± 8</td>
<td>38 ± 2</td>
<td></td>
</tr>
<tr>
<td>18c</td>
<td>Br</td>
<td>131 ± 21</td>
<td>61 ± 2</td>
<td></td>
</tr>
<tr>
<td>18d</td>
<td>F</td>
<td>143 ± 12</td>
<td>22 ± 3</td>
<td></td>
</tr>
<tr>
<td>18e</td>
<td></td>
<td>31 ± 3</td>
<td>16 ± 3</td>
<td></td>
</tr>
<tr>
<td>18f</td>
<td>H₂C=N-</td>
<td>758 ± 49</td>
<td>3,259 ± 181</td>
<td></td>
</tr>
<tr>
<td>18g</td>
<td></td>
<td>1,724 ± 179</td>
<td>726 ± 21</td>
<td></td>
</tr>
<tr>
<td>18h</td>
<td></td>
<td>2,189 ± 0.136</td>
<td>1,716 ± 81</td>
<td></td>
</tr>
<tr>
<td>18i</td>
<td></td>
<td>1,757 ± 133</td>
<td>2,265 ± 177</td>
<td></td>
</tr>
<tr>
<td>18j</td>
<td></td>
<td>1,080 ± 165</td>
<td>4,023 ± 193</td>
<td></td>
</tr>
<tr>
<td>18k</td>
<td>H₂C=N-O₃</td>
<td>&gt;10,000 (32%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;10,000 (41%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>18l</td>
<td>H₂C=N-O₃</td>
<td>2,833 ± 46</td>
<td>10,000 (50%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td></td>
<td>833 ± 139</td>
<td>&gt;10,000 (41%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td></td>
<td>1,198 ± 193</td>
<td>&gt;10,000 (34%)&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup>IC₅₀ values are the mean of three experiments. <sup>b</sup>Percent inhibition at indicated concentration.

Table 3. Modification of the Core on Methyl-Substituted Derivatives

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Core</th>
<th>IC₅₀ ± SD [nM]&lt;sup&gt;a&lt;/sup&gt;</th>
<th>JNK3</th>
<th>p38α MAPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td></td>
<td>833 ± 139</td>
<td>&gt;10,000 (41%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td></td>
<td>363 ± 34</td>
<td>&gt;10,000 (48%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td></td>
<td>1,395 ± 230</td>
<td>&gt;10,000 (43%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>1,279 ± 179</td>
<td>&gt;10,000 (37%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td></td>
<td>2,514 ± 312</td>
<td>&gt;10,000 (10%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td></td>
<td>2,091 ± 108</td>
<td>&gt;10,000 (21%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td></td>
<td>6,509 ± 1,326</td>
<td>&gt;10,000 (40%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>67</td>
<td></td>
<td>714 ± 21</td>
<td>&gt;10,000 (32%)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>71</td>
<td></td>
<td>&gt;10,000 (42%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;10,000 (15%)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>78</td>
<td></td>
<td>2,500 ± 92</td>
<td>&gt;10,000 (9%)&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup>IC₅₀ values are the mean of three experiments. <sup>b</sup>Percent inhibition at indicated concentration.

Table 4. Effect of Small Alkyl Substituents in the HR I

<table>
<thead>
<tr>
<th>Cpd</th>
<th>R</th>
<th>IC₅₀ ± SD [nM]&lt;sup&gt;a&lt;/sup&gt;</th>
<th>JNK3</th>
<th>p38α MAPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>H₃C</td>
<td>562 ± 21</td>
<td>&gt;10,000 (43%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>H₃C,N-</td>
<td>363 ± 34</td>
<td>&gt;10,000 (48%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>H₃C,N-</td>
<td>1,095 ± 64</td>
<td>&gt;10,000 (38%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>IC₅₀ values are the mean of three experiments. <sup>b</sup>Percent inhibition at indicated concentration.
lipophilic LE (LLE), the 4,5-disubstituted derivative 14 is the most efficient one out of the series of Table 1 and serves, therefore, as the optimal starting point for these modifications. Moreover, this scaffold presents a substantially equal activity compared to its S-methylated analogue 5, along with a convenient synthetic strategy, facilitating the preparation of a broad range of derivatives.

Most of the 4,5-disubstituted pyridinylimidazoles having an aromatic moiety at the imidazole-C4 position (compounds 18a–f) revealed to be potent inhibitors for both enzymes, displaying IC_{50} values down to the low double digit nanomolar range. In general, addressing the HR I with a phenyl or monosubstituted phenyl ring resulted in dual inhibitors displaying a slight preference toward p38α MAPK over JNK3. This trend is most distinct in the case of compound 18d having a 3-(trifluoromethyl)phenyl moiety, which presents a 6-fold higher activity for p38α MAPK than for JNK3. The only aromatic substituent stepping out of this trend was the heteroaromatic N-methylpyrazole of compound 18f producing an overall decrease in activity on both kinases while conserving a moderate preference toward JNK3 (18f: IC_{50}(JNK3) = 758 nM; IC_{50}(p38α MAPK) = 3259 nM). These findings indicate that substitution on the phenyl ring is not beneficial when pursuing selectivity on JNK3 and instead seems to be counterproductive, increasing the activity on p38α MAPK. The reason behind this lack of selectivity can be intuitively explained by considering the dimensions of the hydrophobic pocket known as HR I in the two target kinases. This cleft is wider in the p38α MAPK than in JNK3 mostly because of a difference in the "gatekeeper" residue (Thr106 in p38α MAPK vs Met146 in JNK3). However, as already mentioned in some co-crystallization studies, aromatic moieties can induce a shift of the flexible side chain of Met146 (JNK3), thus essentially abolishing the size differences between the two pockets. As a proof of that, even the bulky 2-naphthyl group of compound 18e seems to be accommodated in the "reshaped" hydrophobic pocket of JNK3, therefore resulting in a high inhibitory potency. Moreover, attempts of substituting the ortho and meta positions of the phenyl ring, seeking for additional interactions, did not succeed and produced negative outcomes instead (compounds 18b–d).

The replacement of the aromatic ring at the imidazole-C4 position by cycloalkyl moieties resulted in a dramatic decrease in activity for both enzymes, with IC_{50} values in the low micromolar range. The only exception was the cyclohexyl derivative 18g that was able to interact with p38α MAPK with an IC_{50} value of 726 nM, 2-fold more potent than on JNK3. The inhibitory effect of compounds 18g–j on p38α MAPK, decreasing alongside the reduction of the ring size, is symptomatic of a gradually diminished capability of the cyclic group to occupy the spacious cavity of the enzyme. On the other side, JNK3 activity of derivatives 18g–i, bearing a four- to six-membered ring at the imidazole-C4 position, remained substantially constant, although significantly decreased compared to the parent compound 14. An analogous scenario occurred in the case of compounds featuring branched aliphatic groups at the same position. The isopropyl derivative 18l, analogously to the closely related 18j, showed a significant drop in activity on p38α MAPK, while conserving an IC_{50} value on JNK3 in the low micromolar range. On the other hand, introduction of a tert-butyl moiety (18k) resulted in a complete loss of activity on both JNK3 and p38α MAPK. Because of their flexibility and low electron density, cyclic and branched aliphatic groups are presumably unable to promote the Met146 shift and therefore cannot fit in the narrow hydrophobic back pocket of JNK3. A reasonable consequence of this would therefore consist of the flip of the imidazole ring, directing the branched or cyclic alkyl moieties away from the hydrophobic back pocket of the JNK3, thus explaining the similarity of the inhibitory activity regardless of the substituent size.

In agreement with the trend of the series, methyl- and ethyl-substituted imidazoles 38 and 39, respectively, displayed no inhibition of the p38α MAPK (IC_{50} > 10 μM), however, preserving activity on the JNK3. In particular, the methyl derivative 38 represented the sole compound of this series reaching a submicromolar activity on JNK3 without any remarkable effect on the p38α MAPK. Moreover, this inhibitor also represents the most efficient selective inhibitor of this series in terms of LE and LLE and was therefore chosen as the starting point for further investigations.

Once the methyl substituent at the imidazole-C4 position was selected, our attention was refocused on the central core (Table 3). Because of the presence of the methyl substituent, all derivatives presented in this series lost their potency on the p38α MAPK, with each one displaying an IC_{50} value higher or equal to 10 μM. Altering the arrangement of the substituents around the imidazole ring proved beneficial in the case of the 1,5-disubstituted imidazole 67, slightly increasing its potency compared to the precursor 38, whereas it was deleterious for the 1,2-disubstituted derivative 71. Replacement of the imidazole core with a 2-aminomethyl thiazole (78) also revealed to be detrimental for the inhibitory activity. A different approach consisted of the introduction of an additional substituent on the imidazole-N atom, together with a reintroduction of the S-methyl group at the C2 position, yielding the tetrasubstituted imidazole scaffold already reported in potent dual JNK3/p38α MAPK as well as JNK3 selective inhibitors. In the case of p38α MAPK, the effect of an additional alkyl substituent on the imidazole ring has been reported to be strictly dependent on the position of the substituted N atom. Several examples have demonstrated alkylation of the imidazole-N atom away from the pyridine ring to cause a severe reduction of the activity because of the impossibility to establish a hydrogen bond with the Lys53 of the p38α MAPK. Because the same interaction has shown to also occur in the binding to JNK3 (Lys93), an analogous effect was expected on this enzyme as well and was confirmed by the remarkably reduced JNK3 inhibition by compound 50, carrying a methyl group on the distal imidazole N atom. On the other hand, because several tetrasubstituted JNK3/p38α MAPK inhibitors have been reported with an alkyl substituent on the imidazole N adjacent to the pyridine ring, we assumed this modification to be suitable with our 4-methyl substituted scaffold as well. However, derivatives 57 and 58, featuring a methyl and an ethyl substituent on the N atom proximal to pyridine, respectively, unexpectedly presented an even lower potency on JNK3 than the supposedly "wrong" regiosomer 50. The drop in activity appeared to increase with the size of the alkyl substituent, as N-cyclopropyl substituted 59 was almost 3-fold less active compared to its N-methyl analogue 57. This outcome suggests that despite not hampering the formation of a H bond with the Lys93, alkyl substituents at the imidazole N atom proximal to pyridine reduce the tightness of the binding to the JNK3 active site.
To complete this series, starting from 4,5-disubstituted imidazole 38, the original S-methyl group or a free amino substituent was introduced at the imidazole-C2 position, affording compounds 44 and 47, respectively. Although the 2-amino imidazole derivative showed a drop in activity compared to the parent compound 38, reintroduction of the S-methyl group at the imidazole-C2 position surprisingly produced a 2-fold increase in the inhibitory potency on JNK3 (44: IC<sub>50</sub>(JNK3) = 363 nM; IC<sub>50</sub>(p38α MAPK) > 10 μM). This outcome prompted us to reconsider our previous assumption regarding the role of the 2-methylsulfanyl moiety. Although the S-methyl group exerts no influence on the inhibitory activity when the 4-fluorophenyl moiety is installed at the imidazole-C4 position, it has a significant impact in the case of 4-methyl imidazole derivatives.

In a closer evaluation concerning the influence of the alkyl chain in position 4 of the imidazole core combined with the 2-methylsulfanyl moiety in C2 position, the methyl group (44) emerged once more as the substituent presenting the optimal length to target the JNK3 HR I, when compared to the 4-unsubstituted and to the 4-ethyl derivatives 43 and 45, respectively (Table 4). Comparison of imidazoles 5 (Table 1) and 44 (Table 4) reveals the replacement of the 4-fluorophenyl ring at the imidazole-C4(S) position by a smaller methyl group to result in a 1 order of magnitude loss in JNK3 inhibition and in a complete loss of p38α MAPK inhibitory activity.

To elucidate the binding mode of the 4-methyl-substituted-5-(pyridine-4-yl)imidazole derivatives, as well as to gain insight into the role of the 2-methylsulfanyl group, crystal structures of JNK3 in complex with compounds 38 and 44 were determined (Figure 2).

**Figure 2.** Crystal structures of JNK3 in complex with inhibitors 38 (A) and 44 (B) featuring a pyridinylimidazole scaffold. Only the JNK3 active site is shown. The protein backbone is displayed in gray. The compounds, the side chain of gatekeeper Met146, and a part of the Gly-rich loop are highlighted in stick display. Active site residues with common orientations and interactions are shown in light blue, whereas residues that differ between both complexes are highlighted in the same color as the respective inhibitor. Side chains for which multiple orientations are observed (Asn194 in complex with 38 and Asn152 in complex with 44) are shown in both orientations. Water molecules are represented as red spheres and hydrogen bonds are shown as black dashed lines.

The structures revealed a similar binding mode of the inhibitors within the adenosine 5′-triphosphate (ATP) pocket of JNK3 (Figure 2). As expected, both scaffolds interacted with the hinge region of the kinase via two hydrogen bonds involving the main chain carbonyl and backbone amine groups of Met149 and mimicking the interactions of the enzyme with ATP as well as with its nonhydrolyzable analogue β,γ-methyleneadenosine-5′-triphosphate (AMP–PCP, Figure S3, Supporting Information). In both structures, the imidazole-N atom distal from the pyridine ring is part of a network of water-mediated hydrogen bonds, involving the side chain of Lys93 and the main chain of Leu206. Further water-mediated hydrogen bonds in the JNK3-38 crystal structure (Figure 2A) include the side chain of Asn194, whereas in the JNK3-44 structure (Figure 2B), the backbone of Gly76 and the side chain of Asp207 are involved. The structure of JNK3 in complex with inhibitor 38 also showed that the imidazole-N atom proximal to the pyridine ring participates in a water-mediated hydrogen bond with the Asn152 side chain and the same interaction seems to be present in the JNK3-44, thus explaining the detrimental effect produced by the substitution of this position (compounds 57–59). Multiple hydrophobic interactions comprising the gatekeeper Met146 and the side chains of Ile70, Val78, Val196, and Leu206 were also observed. These interactions have been previously described by Scapin et al. and confer JNK3 selectivity as they cannot be formed in the larger binding pocket of p38α MAPK. The methyl group present in both inhibitors was oriented toward the HR I, which resulted in an identical orientation of the side chain of the gatekeeper residue Met146. The 4-morpholinoaniline moiety, which occupied the solvent-exposed HR II, exhibited higher flexibility and no direct interactions with JNK3, that is, this moiety likely contributes barely or not at all to the binding.

A major structural difference between the two complex structures was observed for the Gly-rich loop. In the JNK3-38 complex structure, no electron density for residues Gly71–Gly76 was visible because of high local flexibility, a phenomenon also encountered in other JNK3 crystal structures. In the JNK3-44 complex, however, the electron density for this loop was clearly defined, hinting to a structural stabilization of this region upon interaction with the 2-methylsulfanyl moiety in compound 44.

A superposition of our inhibitor complex structures with crystal structures of JNK3 bound to AMP–PCP and the dual JNK3/p38α MAPK inhibitor by Scapin et al. (PDB code: 1PMN) yielded insights into the structural basis for the observed selectivity of compounds 38 and 44 (Figure 3).

As can be seen from this structural comparison, no movement of the gatekeeper Met146 side chain is induced by compounds 38 and 44 when compared to the AMP–PCP complex, contrary to the dual kinase inhibitor studied by Scapin et al. In the latter crystal structure, an induced fit of side chain 146 occurred to accommodate the dichlorophenyl moiety of the dual kinase inhibitor. Conversely, it appears that the methyl substituent of compounds 38 and 44 was unable to occupy the wider HR I of the p38α MAPK, while possessing the optimal length to target the respective region of JNK3. Therefore, this moiety determined the selectivity achieved over p38α MAPK, demonstrated by the activities of compounds listed in Tables 2–4. In the case of AMP–PCP and compound 44, another result of the interaction is a downward positioning of the flexible Gly-rich loop. A similar compression of the binding pocket caused by a repositioning of the Gly-rich loop was reported for a JNK3 complex crystal structure by Kamenecka et al. and might be a result of hydrophobic interactions and water-mediated hydrogen bonds provided by inhibitor 44, which stabilized this otherwise flexible section. Overall, as a result of inhibitor binding, the JNK3 ATP binding pocket in our crystal structures appears somewhat narrower in comparison to the p38α MAPK binding.
A further approach in the pursuit of a tighter binding with the JNK3 consisted of modifying the amino moiety at the pyridine-C2 position (Table 6). An initial attempt was carried out by introducing α-methyl(phenyl)alkylamino moieties (compounds 48a−b) as well as cycloalkylamino groups (compounds 48c−e). The former moieties have been reported in potent p38α MAPK inhibitors, for example, LN950 (Figure 1) and ML3403,46 and were thus introduced to evaluate their effect on JNK3 inhibitory potency. In detail, these substituents were hypothesized to yield an increase in the JNK3 inhibitory activity while conserving selectivity over the p38α MAPK because of the combination with the 4-methyl substituent on the imidazole ring. However, the 3-methyl-2-butyramino group (48a) resulted in a loss of activity compared to the 4-morpholinoaniline precursor 44, although maintaining some selectivity over the p38α MAPK. Substitution with the α-methylbenzylamine, giving rise to compound 48b, was instead counterproductive as it not only caused a tremendous drop in JNK3 inhibition but also a recovery of the activity on the p38α MAPK (48b: IC_{50}[JNK3] = 7610 nM; IC_{50}[p38α MAPK] = 3460 nM). On the other hand, although not reaching the potency of the parent compound 44, the JNK3 inhibitory activity of compounds bearing cycloalkylamino moieties at the pyridine-C2 position (48c−e) increased alongside the size of the aliphatic ring, a trend suggesting the importance of hydrophobic interactions in this area of the molecule. Nevertheless, replacement of the cyclohexyl ring of 48e with the similar tetrahydropyranly group (48f) yielded, unexpectedly, a remarkable loss of activity on JNK3.

A possible strategy to gain activity and selectivity on JNK3 would consist of targeting the side chain of Gln155 as this residue is replaced by a shorter Asn in the p38α MAPK.46 As suggested by the structure of the JNK3−44 complex (Figure 2), this amino acidic residue is located about 4 Å away from the 4-morpholinoaniline-N atom but cannot be reached because of the rigidity of this substituent. Moreover, the 4-morpholinoaniline moiety is only able to accept a hydrogen bond, whereas the Gln residue has the potential to act as both acceptor and donor of hydrogen bonds. For this reason, trans-4-aminocyclohexanol and trans-1,4-diaminocyclohexyl moieties were selected for compounds 48g and 48h, respectively, because of a higher flexibility and their additional capability to donate hydrogen bond interactions. In particular, the former moiety is also present in the structure of clinical candidate CC-930, wherein it is reported to interact with the aforementioned Gln155,47 and included in potent p38α MAPK inhibitors.48 Unfortunately, despite preserving the selectivity over the p38α MAPK, none of the two inhibitors 48g and 48h succeeded in overcoming the activity of the parent compound 44 on the JNK3, with the latter displaying a 3-fold drop in potency. This observation suggests an inability of the introduced moiety to form the desired interaction with the Gln155 side chain or this interaction being compensated by other factors. Additionally, it underlines the necessity of the aromatic moiety at the pyridine-C2 amino function for the binding to the JNK3. The significantly lower activity of compound 48h could also derive from the not tolerated protonation of its terminal amino functionality. With the aim to reach the Gln155 side chain by the introduction of an additional hydrogen bond acceptor, a series of amides of compound 48h and of its aromatic counterpart 48m was synthesized. This approach also permits to seek additional interactions with the enzyme HR II.

Table 5. Determined Melting Temperatures (T_m) for JNK3 Alone and in Complex with Inhibitors 38 and 44

<table>
<thead>
<tr>
<th>sample</th>
<th>T_m (°C) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNK3</td>
<td>46.28 ± 0.58</td>
</tr>
<tr>
<td>JNK3-38</td>
<td>53.87 ± 0.04</td>
</tr>
<tr>
<td>JNK3-44</td>
<td>54.83 ± 0.04</td>
</tr>
</tbody>
</table>

“Data represent mean value ± SD of a single experiment performed in triplicate. nanoDSF measurements (Figure S2, Supporting Information) were conducted using Prometheus NT.48 (NanoTemper Technologies, Munich).
Unfortunately, in neither of the two series, the introduction of amide moieties permitted to gain an inhibitory activity comparable with the precursor 44. In the series featuring a cycloaliphatic amine (48i–l), only the small acetamide derivative 48i exhibited an almost similar activity to the precursor, whereas bulkier alkyl and aromatic residues displayed a 2- to 3-fold decrease in potency. In an analogous fashion, when considering the series derived from the aromatic intermediate 48m, compounds bearing a tert-butyl or a cyclohexyl amide (48p and 48q, respectively) showed a significant drop in inhibitory activity, with IC_{50} values in the micromolar range. On the other hand, both inhibitors carrying an acetamido or benzamido moiety (48n and 48o, respectively) were still able to inhibit the JNK3 with a potency akin to the free amine derivative 48m. The comparison of the two amide series also supports the theory of a higher suitability of aromatic substituents at the pyridine-C2 amino position when targeting the JNK3.

Compound 44 resulted as the best inhibitor of the synthesized series and was, therefore, further investigated to achieve a comprehensive characterization. At a first instance, to evaluate the intra-JNK selectivity, compound 44 was tested on the three JNK isoforms (Table 7). As expected, compound 44 inhibited the three isoforms with a similar potency but showed a moderate preference for JNK1 and JNK3 over JNK2.

Moreover, inhibitor 44 was further screened against a panel of 45 diverse kinases to achieve a preliminary evaluation of its selectivity within the kinome. Out of the kinase panel, 10 kinases (including JNK1) were inhibited more than 50% at a testing concentration of 10 μM (Table S3, Supporting Information).

Additional studies were aimed at evaluating the inhibition of the human-ether-à-go-go related gene (hERG) potassium channels as well as liver cytochromes P450 (CYP450) to highlight potential liabilities of the synthesized scaffold. As displayed in Table 8, compound 44 showed a reduced interference with the hERG channels (IC_{50} > 10 μM).

Regarding interaction with hepatic enzymes, compound 44 displayed low to moderate inhibition of four of the five tested

### Table 6. Influence of Substituents at the Pyridine-C2 Position

<table>
<thead>
<tr>
<th>Cpd</th>
<th>R</th>
<th>IC_{50} ± SD [nM]</th>
<th>JNK3</th>
<th>p38a MAPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>48a</td>
<td>-</td>
<td>2,590 ± 173</td>
<td>&gt;10,000 (34%)</td>
<td></td>
</tr>
<tr>
<td>48b</td>
<td>-</td>
<td>7,610 ± 145</td>
<td>3,460 ± 1,680</td>
<td></td>
</tr>
<tr>
<td>48c</td>
<td>-</td>
<td>3,040 ± 194</td>
<td>&gt;10,000 (36%)</td>
<td></td>
</tr>
<tr>
<td>48d</td>
<td>-</td>
<td>1,566 ± 124</td>
<td>&gt;10,000 (47%)</td>
<td></td>
</tr>
<tr>
<td>48e</td>
<td>-</td>
<td>671 ± 74</td>
<td>&gt;10,000 (31%)</td>
<td></td>
</tr>
<tr>
<td>48f</td>
<td>-</td>
<td>2,632 ± 806</td>
<td>&gt;10,000 (27%)</td>
<td></td>
</tr>
<tr>
<td>48g</td>
<td>-</td>
<td>457 ± 72</td>
<td>&gt;10,000 (35%)</td>
<td></td>
</tr>
<tr>
<td>48h</td>
<td>-</td>
<td>941 ± 75</td>
<td>&gt;10,000 (46%)</td>
<td></td>
</tr>
<tr>
<td>48i</td>
<td>-</td>
<td>819 ± 141</td>
<td>&gt;10,000 (37%)</td>
<td></td>
</tr>
<tr>
<td>48j</td>
<td>-</td>
<td>1,216 ± 723</td>
<td>&gt;10,000 (12%)</td>
<td></td>
</tr>
<tr>
<td>48k</td>
<td>-</td>
<td>2,231 ± 80</td>
<td>&gt;10,000 (10%)</td>
<td></td>
</tr>
<tr>
<td>48l</td>
<td>-</td>
<td>2,538 ± 261</td>
<td>&gt;10,000 (13%)</td>
<td></td>
</tr>
<tr>
<td>48m</td>
<td>-</td>
<td>353 ± 58</td>
<td>&gt;10,000 (38%)</td>
<td></td>
</tr>
<tr>
<td>48n</td>
<td>-</td>
<td>415 ± 40</td>
<td>&gt;10,000 (32%)</td>
<td></td>
</tr>
<tr>
<td>48o</td>
<td>-</td>
<td>432 ± 37</td>
<td>&gt;10,000 (33%)</td>
<td></td>
</tr>
<tr>
<td>48p</td>
<td>-</td>
<td>1,539 ± 340</td>
<td>&gt;10,000 (27%)</td>
<td></td>
</tr>
<tr>
<td>48q</td>
<td>-</td>
<td>2,373 ± 504</td>
<td>&gt;10,000 (15%)</td>
<td></td>
</tr>
</tbody>
</table>

"IC_{50} values are the mean of three experiments. Percent inhibition at indicated concentration. According to the ZINC patterns tool, compound 48m represents a potential pan-assay interference compound. However, this compound was synthesized as the intermediate for the preparation of inhibitors 48n–q. To estimate the impact of the amide moiety present in compounds 48n–q on the inhibition of the two kinases, the activities of 48m are listed in this table.

### Table 7. Inhibition Data of Compound 44 on the Three JNK Isoforms

<table>
<thead>
<tr>
<th>IC_{50} [nM] (^{a})</th>
<th>JNK1</th>
<th>JNK2</th>
<th>JNK3</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td>119</td>
<td>468</td>
<td>184</td>
</tr>
</tbody>
</table>

\(^{a}\)Compound 44 was tested by Reaction Biology corporation (Malvern, PA, USA) using a radiometric assay.

### Table 8. Inhibitory Activity of Compound 44 on hERG Channels and on the Most Relevant CYP Isoforms

<table>
<thead>
<tr>
<th>CYP450 inhibition [% inhibition at 10 μM]</th>
<th>hERG inhibition [% inhibition at 10 μM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A9</td>
<td>38.8</td>
</tr>
<tr>
<td>2C9</td>
<td>51.5</td>
</tr>
<tr>
<td>2C19</td>
<td>53.9</td>
</tr>
<tr>
<td>2D6</td>
<td>35.6</td>
</tr>
<tr>
<td>3A4</td>
<td>19.0</td>
</tr>
<tr>
<td>19.0</td>
<td>75.1</td>
</tr>
</tbody>
</table>
isoenzymes, this representing a significantly cleaner profile in comparison with previously reported inhibitors of this class.48 However, the elevated blockage of the most abundant CYP450 isoform 3A4 still constitutes a serious limit, which needs to be solved by subsequent optimization strategies.

Finally, additional tests were performed to assess the metabolic stability of methyl-substituted pyridylimidazole 44 upon incubation with human liver microsomes. One of the most serious limitations of previously reported 2-alkylsulfinyl-imidazoles is their severe metabolization consisting of oxidation of the thioether function to the corresponding sulfoxide.48 Nevertheless, in vitro assays performed on compound 44 demonstrated a substantial metabolic stability, as approximately 80% of the unmodified compound was still present after 4 h incubation (Figure S6, Supporting Information). The major metabolite formed still appears to be represented by the sulfoxide derivative (8.49%), although modifications at the 4-morpholinoaniline substituent might also be present.

■ CONCLUSIONS

Optimization of 4-(4-fluorophenyl)-5-(pyridin-4-yl)imidazole-based p38α MAPK inhibitors by modification of the five-membered heterocyclic core, the aryl moiety at the imidazole-C4 position, and the pyridine-C2 amino function resulted in a novel series of JNK3 inhibitors exhibiting high selectivity over the closely related p38α MAPK. Biological evaluation of the different pyridinyl-substituted five-membered rings provided valuable insights into the structure activity relationship of this scaffold with respect to JNK3 and p38α MAPK inhibitory potencies. By addressing the HR 1 with a small methyl group, a significant selectivity toward JNK3 was achieved. This feature is not yet reported for this class of compounds, which have been generally described as p38α MAPK inhibitors. The binding mode at the ATP binding site of the enzyme for this class of compounds was confirmed by X-ray structures of JNK3 crystals incubated with imidazoles 38 and 44. The most potent inhibitor 4-(4-(4-methyl-2-(methylthio)-1H-imidazol-5-yl)-N-(4-morpholino phenyl)pyridin-2-amine (44) inhibits the JNK3 in the low triple digit nanomolar range, is metabolically stable, and the mixture was stirred at 0 °C for 3 h. After cooling to room temperature (rt), the formed solid residue of Se was removed by filtration and the filtrate was washed with saturated NaHCO3 solution four times. Finally, the organic phase was washed with saturated NaCl solution, dried over anhydrous Na2SO4, and concentrated at reduced pressure. The residue was purified by flash column chromatography.

General Procedure for the Synthesis of Compounds 15a—1 (General Procedure B). In a three-neck round-bottom flask under anhydrous conditions, 2-chloro-4-methylpyridine (9) (1 equiv) and the appropriate ethyl ester (1 equiv) were dissolved in dry tetrahydrofuran (THF) (2 mL). After cooling the reaction mixture to 0 °C, 2 M sodium bis(trimethylsilyl)amide (NaHDSM) in dry THF (2.2 equiv) was added dropwise and the mixture was stirred at 0 °C for 1.5–5 h. After adding H2O, the aqueous phase was extracted three times with dichloromethane (DCM) or EtOAc and washed with NaCl saturated solution. The combined organic layers were dried over anhydrous Na2SO4 and the solvent was evaporated at reduced pressure. The residue was purified by flash column chromatography.
derivatives 16a–l (1 equiv) and NH₄OAc (10 equiv) were suspended in 3 mL of glacial AcOH and after that a 37% aqueous solution of formaldehyde (1 equiv) was added. The reaction vessel was heated in a CEM microwave reactor at 180 °C, with an initial power of 200 W, for 2–5 min. The mixture was added dropwise to NH₄OH concentrated solution at 0 °C. The suspension obtained was extracted three times with EtOAc and the combined organic layers were dried over anhydrous Na₂SO₄ and concentrated at reduced pressure. The residue was purified by flash column chromatography.

**General Procedure for the Synthesis of Compounds 48a–h (General Procedure E).** In a pressure vial, 2-chloro-4-(4-methyl-2-(methylthio)-1H-imidazol-5-yl)pyridin-2-yl)pyridin-2-yl)ethane-1,2-dione (41) was suspended in ≈2 mL of cycloalkylamine (in the case of solid amine, 20 equiv of amine was added and the mixture was suspended in ≈2 mL of n-butanol). The closed vial was then heated at 180 °C and stirred for 48–120 h. The reaction mixture was poured in H₂O and the aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated at reduced pressure. The residue was finally purified by flash column chromatography.

**General Procedure for the Synthesis of Compounds 48i–l and 48n–q (General Procedure F).** Under an argon atmosphere, trans-N1-(4-(4-methyl-2-(methylthio)-1H-imidazol-5-yl)pyridin-2-yl)cyclohexane-1,4-diamine (48h) or N1-(4-(4-methyl-2-(methylthio)-1H-imidazol-5-yl)pyridin-2-yl)-benzene-1,4-diamine (48m) was dissolved in 1.5 mL of dry pyridine and after that the appropriate acid chloride or anhydride was added and the reaction mixture was stirred for rt for 16 h. The reaction mixture was poured in H₂O and the aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated at reduced pressure. The residue was finally purified by flash column chromatography.

**2-Fluoro-4-(4-(fluorophenyl)-2-(methylthio)-1H-imidazol-5-yl)pyridine (4).** The title compound was synthesized as described in the literature and analytical data were in agreement with the reported ones.

**4-(4-Fluorophenyl)-2-(methylthio)-1H-imidazol-5-yl)-N-(4-morpholinophenyl)pyridin-2-amine (5).** The title compound was synthesized according to general procedure A starting from 4-(2-ethyl-4-(4-morpholinophenyl)pyridin-2-yl)-1H-imidazol-5-yl)-N-(4-morpholinophenyl)pyridin-2-yl)ethane-1,2-dione (10). The title compound was synthesized according to the literature and the analytical data were in agreement with the reported ones.

**4-(2-Ethyl-4-(fluorophenyl)-1H-imidazol-5-yl)-2-fluoropyridine (11).** To a solution of 10 (250 mg, 1.01 mmol) in MeOH (5 mL), 7 M ammonia in MeOH (2.89 mL, 20.23 mmol) and propionaldehyde (88.11 mg, 1.52 mmol) were added and the reaction mixture was heated to reflux temperature and stirred for 4 h. After cooling down, the solvent was evaporated at reduced pressure and the residue was purified by flash column chromatography (SiO₂, DCM/ EtOH 97:03 to 94:06), obtaining 125 mg of the desired product (43% yield); ¹H NMR (300 MHz, DMSO-d₆): δ 1.28 (t, J = 7.6 Hz, 3H), 2.64–2.77 (m, 2H), 7.09 (s, 1H), 17.1–7.40 (m, 3H), 7.47–7.56 (m, 2H), 8.06 (d, J = 5.4 Hz, 1H), 12.41 ppm (br s, 1H); MS-ESI m/z: [M + H]⁺ calcld for C₂₈H₂₄F₂N₂O₂ 481; found, 483; HPLC (method 2): tᵣ = 3.680 min.

**4-(2-Ethyl-4-(fluorophenyl)-1H-imidazol-5-yl)-N-(4-morpholinopyridin-2-yl)pyridin-2-amine (13).** The title compound was synthesized according to general procedure A starting from 4-(2-ethyl-4-(fluorophenyl)-1H-imidazol-5-yl)-2-fluoropyridine (11) (85 mg, 0.30 mmol) and 4-morpholinonoline (80.2 mg, 0.45 mmol). The crude residue was purified twice by flash column chromatography (SiO₂, DCM/ EtOH 96:04 to 94:06) and (RP-C18, iso-propanol/H₂O 1:1), obtaining 32 mg of the desired compound (24% yield); ¹H NMR (300 MHz, DMSO-d₆): δ 1.27 (t, J = 7.6 Hz, 3H), 2.69 (q, J = 7.6 Hz, 2H) 2.96–3.06 (m, 4H), 3.70–3.79 (m, 4H), 6.63–6.92 (m, 3H), 6.97 (br s, 1H), 7.12–7.57 (m, 4H), 7.86–8.08 (m, 1H), 8.60–8.79 (m, 1H), 12.20 ppm (br s, 1H); ¹³C NMR (101 MHz, DMSO-d₆): δ 12.7, 21.2, 49.4, 66.2, 106.1, 106.6, 111.4, 111.7, 111.9, 112.0, 123.3, 126.2, 126.9, 129.5 (δ, J = 8.1 Hz), 150.5 (δ, J = 8.0 Hz), 133.7, 134.2, 134.8, 137.9, 138.7, 141.9, 142.7, 145.6, 145.9, 147.3, 148.1, 156.7, 161.9 ppm (δ, J = 244.4 Hz); MS–FAB m/z: [M⁺] for C₂₉H₂₄F₂N₂O⁺ 442; found, 444.2; m/z: [M – H]⁻ calcld for C₂₉H₂₄F₂N₂O⁻ 442; found, 442.2; HPLC (method 2): tᵣ = 4.960 min (98.6%).

**4-(4-Fluorophenyl)-1H-imidazol-5-yl)-N-(4-morpholinopyridin-2-yl)pyridin-2-amine (14).** The title compound was synthesized according to general procedure A starting from 2-fluoro-4-(4-(fluorophenyl)-1H-imidazol-5-yl)pyridine (12) (70 mg, 0.27 mmol) and 4-morpholinonoline (71.3 mg, 0.40 mmol). Purification by flash column chromatography (SiO₂, DCM/EtOH 95:05 to 90:10) afforded 70 mg of the desired compound (62% yield); ¹H NMR (250 MHz, DMSO-d₆): δ 2.93–3.07 (m, 4H), 3.66–3.79 (m, 4H),
The title compound was synthesized according to general procedure A starting from compound 17a (100 mg, 0.39 mmol) (for the synthesis of 17a see Supporting Information) and 4-morpholinomethylphenyl (90.9 mg, 0.51 mmol). Purification by flash column chromatography (SiO₂, DCM/EtOH 97:03 to 90:10) afforded 138 mg of the desired compound (89% yield); ¹H NMR (300 MHz, DMSO-d₆): δ 2.92−3.08 (m, 4H), 3.66−3.80 (m, 4H), 6.68 (dd, J = 5.3, 0.9 Hz, 1H), 6.82 (d, J = 9.0 Hz, 2H), 6.93 (br s, 1H), 7.30−7.53 (m, 7H), 7.82 (s, 1H), 7.97 (d, J = 5.3 Hz, 1H), 8.70 (s, 1H), 12.66 ppm (br s, 1H); ¹³C NMR (101 MHz, DMSO-d₆): δ 49.4, 66.1, 106.5, 111.8, 115.9, 120.1, 127.6, 128.1, 128.6, 132.0, 134.0, 136.0, 142.4, 145.7, 147.2, 156.6 ppm; MS-ESI m/z: [M + H]⁺ calcld for C₂₅H₂₀ClN₅O, 432.1; found, 432.2; m/z: [M − H]⁻ calcld for C₂₅H₂₀ClN₅O, 430.2; found, 429.8; HPLC (method 2): tᵣ = 5.434 min (99.1%).

4-(4-(2-Chlorophenyl)-1H-imidazole-5-yl)-N-(4-morpholinophenyl)pyridin-2-amine (18b). The title compound was synthesized according to general procedure A starting from compound 17b (100 mg, 0.34 mmol) (for the synthesis of 17b see Supporting Information) and 4-morpholinomethylphenyl (90.9 mg, 0.51 mmol). Purification by flash column chromatography (SiO₂, DCM/EtOH 97:03 to 90:10) afforded 127 mg of the desired compound (87% yield); ¹H NMR (300 MHz, DMSO-d₆): δ 2.85−3.17 (m, 4H, 3.39−3.89 (m, 4H), 6.51 (d, J = 4.8 Hz, 1H), 6.81 (d, J = 8.5 Hz, 2H), 6.92 (br s, 1H), 7.28 (d, J = 7.5 Hz, 2H), 7.37−7.67 (m, 4H), 7.79−8.01 (m, 2H), 8.60 (s, 1H), 12.63 ppm (br s, 1H); ¹³C NMR (101 MHz, DMSO-d₆): δ 49.4, 66.2, 104.8, 110.4, 110.5, 115.9, 120.1, 127.4, 129.8, 130.5, 130.6, 132.5, 133.3, 134.0, 136.0, 136.0, 142.8, 145.7, 146.1, 147.4, 156.7 ppm; MS-ESI m/z: [M + H]⁺ calcld for C₂₅H₂₁ClN₅O, 432.1; found, 432.1; m/z: [M − H]⁻ calcld for C₂₅H₂₁ClN₅O, 430.15; found, 429.8; HPLC (method 2): tᵣ = 5.671 min (99.4%).

4-(4-(2-Bromophenyl)-1H-imidazol-5-yl)-N-(4-morpholinophenyl)pyridin-2-amine (18c). The title compound was synthesized according to general procedure A starting from compound 17c (100 mg, 0.30 mmol) (for the synthesis of 17c see Supporting Information) and 4-morpholinomethylphenyl (80.2 mg, 0.45 mmol). Purification by flash column chromatography (SiO₂, DCM/EtOH 97:03 to 90:10) afforded 100 mg of the desired compound (71% yield); ¹H NMR (300 MHz, DMSO-d₆): δ 3.00−3.13 (m, 4H, 3.62−3.95 (m, 4H), 6.51 (d, J = 4.2 Hz, 1H), 6.82 (m, J = 8.0 Hz, 3H), 7.09−7.60 (m, 5H), 7.67−8.01 (m, 3H), 8.63−8.97 (m, 1H), 12.66 ppm (br s, 1H); ¹³C NMR (101 MHz, DMSO-d₆): δ 49.3, 66.1, 104.7, 110.3, 115.9, 120.4, 124.0, 127.9, 130.6, 130.8, 132.5, 132.9, 133.6, 135.8, 145.9, 145.9, 146.9, 146.6, 156.4 ppm; MS-ESI m/z: [M + H]⁺ calcld for C₂₆H₂₂BrN₅O, 476.1; found, 476.0; m/z: [M − H]⁻ calcld for C₂₆H₂₂BrN₅O, 474.1; found, 473.9; HPLC (method 2): tᵣ = 3.669 min (99.3%).

N-(4-Morpholinophenyl)-4-(4-(3-(trifluoromethyl)phenyl)-1H-imidazol-5-yl)pyridin-2-amine (18d). The title compound was synthesized according to general procedure A starting from compound 17d (100 mg, 0.30 mmol) (for the synthesis of 17d see Supporting Information). Purification by flash column chromatography (SiO₂, DCM/EtOH 97:03 to 90:10) afforded 120 mg of the desired compound (86% yield); ¹H NMR (300 MHz, DMSO-d₆): δ 2.96−3.10 (m, 4H), 3.68−3.80 (m, 4H), 6.73 (d, J = 5.4 Hz, 1H), 6.80−6.92 (m, 3H), 7.34 (d, J = 8.8 Hz, 1H), 7.61−7.81 (m, 3H), 7.84 (br s, 1H), 7.96 (s, 1H), 7.99−8.09 (m, 1H), 8.94 (br s, 1H), 13.01 ppm (br s, 1H); MS-ESI m/z: [M + H]⁺ calcld for C₂₅H₂₃F₂N₅O, 466.2; found, 465.9; m/z: [M − H]⁻ calcld for C₂₅H₂₃F₂N₅O, 464.18; found, 463.8; HPLC (method 2): tᵣ = 5.413 min (100%).

N-(4-Morpholinophenyl)-4-(4-(napthalen-2-yl)-1H-imidazol-5-yl)pyridin-2-amine (18e). The title compound was synthesized according to general procedure A starting from compound 17e (100 mg, 0.327 mmol) (for the synthesis of 17e see Supporting Information) and 4-morpholinomethylphenyl (87.5 mg, 0.49 mmol). Purification by flash column chromatography (SiO₂, DCM/EtOH 100:00 to 90:10) afforded 88 mg of the desired compound (60% yield); ¹H NMR (250 MHz, DMSO-d₆): δ 2.79−2.96 (m, 4H), 3.61−3.80 (m, 4H), 6.53 (d, J = 9.0 Hz, 2H), 6.80 (d, J = 5.1 Hz, 1H), 6.87 (br s, 1H), 7.18 (d, J = 8.8 Hz, 2H), 7.47−7.65 (m, 3H), 7.88 (s, 1H), 7.90−8.12 (m, 5H), 8.60 (s, 1H), 12.72 ppm (br s, 1H); ¹³C NMR (101 MHz, DMSO-d₆): δ 49.3, 66.1, 106.0, 111.8, 115.7, 120.1, 126.2, 126.5, 127.6, 128.0, 128.1, 132.3, 133.1, 133.7, 136.4, 145.6, 147.7, 156.6 ppm; MS-ESI m/z: [M + H]⁺ calcld for C₂₃H₂₂N₅O, 448.2; found, 448.3; m/z: [M − H]⁻ calcld for C₂₃H₂₂N₅O, 446.2; found, 446.3; HPLC (method 2): tᵣ = 5.541 min (98.5%).
The title compound was synthesized according to general procedure A starting from compound 17h (100 mg, 0.40 mmol) (for the synthesis of 17h see Supporting Information) and 4-morpholinooxazoline (107.0 mg, 0.60 mmol). Purification by flash column chromatography (SiO2, DCM/MeOH 97:03 to 9:1) afforded 107 mg of the desired compound (69% yield); 1H NMR (300 MHz, DMSO-d6): δ 1.57-1.86 (m, 6H), 1.86-2.05 (m, 2H), 2.96-3.06 (m, 4H), 3.30-3.50 (m, 1H), 3.69-3.77 (m, 4H), 6.85-6.93 (m, 3H), 6.96 (s, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.70 (s, 1H), 8.05 (d, J = 5.4 Hz, 1H), 8.78 ppm (s, 1H); 13C NMR (101 MHz, DMSO-d6): δ 25.1, 32.9, 36.3, 49.5, 66.2, 106.1, 111.6, 116.0, 120.3, 130.9, 131.5, 134.3, 134.8, 142.7, 145.7, 147.2, 156.8 ppm; MS-ESI m/z: [M + H]+ calcld for C24H29N5O, 404.2; found, 404.4; 124 mg of the desired compound (76% yield); 1H NMR (300 MHz, methanol-d4): δ 1.31 (d, J = 7.0 Hz, 6H), 3.07-3.16 (m, 4H), 3.25-3.45 (m, 1H), 3.80-3.91 (m, 4H), 6.86-6.94 (m, 2H), 6.96-7.03 (m, 2H), 7.30-7.39 (m, 2H), 7.65 (s, 1H), 8.03 ppm (d, J = 6.2 Hz, 1H); 13C NMR (101 MHz DMSO-d6): δ 22.4, 24.6, 49.5, 66.1, 106.2, 111.6, 115.9, 119.8, 131.9, 134.4, 134.5, 134.6, 143.8, 145.5, 150.8 ppm; MS-ESI m/z: [M + H]+ calcld for C21H21N5O, 362.2; found, 362.4; M+H (method 2): tR = 2.860 min (99.6%).

4-(2-Chloro-4-(4-methyl-1H-imidazol-5-yl)-pyridin-2-amine (36)). Compound 34 (1.0 g, 4.43 mmol) was suspended in glacial AcOH (10 mL) and subsequently 30% H2O2 (602.7 mg, 17.72 mmol) was added dropwise and the reaction mixture was stirred at rt for 15 min. After adding H2O, the pH was adjusted to 8 using K2CO3 saturated solution and the aqueous phase was extracted five times with EtOAc. The combined organic layers were dried over anhydrous Na2SO4 and concentrated at reduced pressure, affording 230 mg of the product which was used in the following step without further purification (25% yield); 1H NMR (300 MHz, DMSO-d6): δ 2.47 (s, 3H), 7.62 (dd, J = 5.3, 1.3 Hz, 1H), 7.65 (br s, 1H), 7.69 (s, 1H), 8.33 ppm (d, J = 5.2 Hz, 1H); 13C NMR (101 MHz, DMSO-d6): δ 11.7, 118.8, 119.1, 127.9, 130.5, 134.9, 145.9, 149.8, 150.8 ppm; MS-ESI m/z: [M + H]+ calcld for C15H15ClN2O, 294.0; found, 294.0; m/z: [M − H]+ calcld for C14H13ClN2O, 292.0; found, 291.8; HPLC (method 2): tR = 1.375 min.

4-(2-Chloro-4-(4-ethyl-1H-imidazol-5-yl)pyridin-2-amine (37)). Compound 35 (400 mg, 1.67 mmol) (for the synthesis of compound 35 see Supporting Information) was suspended in glacial AcOH (10 mL) and subsequently 30% H2O2 (227.2 mg, 6.68 mmol) was added dropwise and the reaction mixture was stirred at rt for 40 min. The reaction mixture was concentrated at reduced pressure and after that 20 mL of K2CO3 saturated solution was added. The aqueous layer was extracted five times with EtOAc and the combined organic layers were dried over anhydrous Na2SO4 and concentrated at reduced pressure, affording 230 mg of the product which was used in the following step without further purification (71% yield); 1H NMR (300 MHz, DMSO-d6): δ 1.22 (t, J = 7.4 Hz, 3H), 2.85 (q, J = 7.4 Hz, 2H), 7.58 (d, J = 5.0 Hz, 1H), 7.62 (s, 1H), 7.70 (s, 1H), 8.33 ppm (d, J = 5.1 Hz, 1H); 13C NMR (101 MHz, DMSO-d6): δ 13.4, 18.8, 119.1, 119.4, 129.7, 147.0, 156.1, 158.3, 158.7 ppm; ESI-MS m/z: [M + H]+ calcld for C15H16N2O, 278.2; found, 278.3; ESI-MS m/z: [M − H]+ calcld for C15H14N2O, 276.2; found, 276.1; HPLC (method 2): tR = 2.860 min (100%).
4-(4-Methyl-1H-imidazol-5-yl)-N-(4-morpholinophenyl)-pyridin-2-amine (38). The title compound was synthesized according to general procedure A starting from compound 36 (100 mg, 0.47 mmol). The crude product was purified twice by flash column chromatography (SiO2, DCM/MeOH 90:10 to 80:20, (SiO2, EtOAc), obtaining 38 mg of the desired compound (74% yield); 1H NMR (400 MHz, DMSO-d6): δ 2.42 (s, 3H), 2.94–3.09 (m, 4H), 3.63–3.81 (m, 4H), 6.88 (d, J = 9.0 Hz, 2H), 6.94 (d, J = 5.1 Hz, 1H), 7.07 (s, 1H), 7.51 (d, J = 9.1 Hz, 2H), 7.60 (s, 1H), 8.04 (d, J = 5.4 Hz, 1H), 8.69 (s, 1H), 12.15 ppm (br s, 1H); 13C NMR (101 MHz, DMSO-d6): δ 11.4, 49.5, 66.2, 105.3, 110.8, 116.0, 119.7, 124.7, 133.0, 133.9, 134.6, 145.7, 147.2, 156.8 ppm; MS-ESI m/z: [M + H]+ calcd for C20H23N5O, 336.2; found, 336.2; m/z: [M – H]– calcd for C19H21N5OS, 324.2; found, 324.2; HPLC (method 2): tR = 1.653 min.

2-Chloro-4-(4-(ethyl-2-(methylthio)-1H-imidazol-5-yl)-1H-imidazol-5-yl)-pyridine (42). In a pressure vial, compound 35 (400 mg, 1.67 mmol) (for the synthesis of compound 35 see Supporting Information) and t-BuONa (160.5 mg, 1.67 mmol) were dissolved in dry MeOH (15 mL) and after cooling the reaction mixture to 0 °C, methyl iodide (203 μL, 3.26 mmol) was added. The vial was tightly closed and the mixture was stirred at 50 °C for 1 h. The solvent was evaporated at reduced pressure and the residue was purified by flash column chromatography (SiO2, DCM/MeOH 99:01 to 95:05, affording 378 mg of the product (89% yield); 1H NMR (300 MHz, CDCl3): δ 1.32 (t, J = 7.6 Hz, 3H), 2.63 (s, 3H), 2.89 (q, J = 7.6 Hz, 2H), 7.50 (dd, J = 5.3, 1.5 Hz, 1H), 7.64 (br s, 1H), 8.35 ppm (dd, J = 5.3, 0.4 Hz, 1H); 13C NMR (101 MHz, CDCl3): δ 13.5, 16.6, 19.4, 119.3, 120.7, 134.5, 135.1, 141.7, 145.0, 149.4, 151.9 ppm; MS-ESI m/z: [M + H]+ calcd for C13H12ClN5S, 254.0; found, 254.0; m/z: [M – H]– calcd for C12H11ClN5S, 252.0; found, 252.0; HPLC (method 2): tR = 3.575 min.

4-(2-(methylthio)-1H-imidazol-5-yl)-N-(4-morpholinophenyl)pyridin-2-amine (43). The title compound was synthesized according to general procedure A starting from 40 (100 mg, 0.44 mmol) and 4-morpholinooaniline (117.6 mg, 0.66 mmol). Purification by flash column chromatography (SiO2, DCM/MeOH 100:0 to 90:10) afforded 92 mg of the desired compound (57% yield); 1H NMR (400 MHz, DMSO-d6): δ 2.54–2.62 (m, 3H), 2.92–3.09 (m, 4H), 3.63–3.78 (m, 4H), 6.87 (d, J = 7.8 Hz, 2H), 6.96 (d, J = 4.5 Hz, 1H); 13C NMR (101 MHz, DMSO-d6): δ 15.3, 49.5, 66.2, 104.1, 109.5, 116.0, 116.8, 119.7, 120.3, 134.6, 139.2, 142.0, 144.5, 147.3, 156.9 ppm; MS–FAB m/z: [M] calcd for C19H21N5O, 367.1; found, 367.2; HPLC (method 1): tR = 2.934 min (96.4%).

2-Chloro-4-(2-(methylthio)-1H-imidazol-5-yl)-pyridine (44). Under an argon atmosphere, compound 33 (500 mg, 2.36 mmol) (for the synthesis of compound 33 see Supporting Information) and t-BuONa (454 mg, 4.72 mmol) were dissolved in dry MeOH (20 mL) and after cooling the reaction mixture to 0 °C, methyl iodide (147.5 μL, 2.36 mmol) was added and the reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was then heated to 55 °C and stirred for 3 h. After cooling to rt, the solvent was evaporated at reduced pressure and H2O was added. The aqueous phase was then extracted twice with EtOAc and the combined organic layers were dried over anhydrous Na2SO4 and concentrated at reduced pressure. The residue was finally purified by flash column chromatography (SiO2, DCM/MeOH 100:0 to 90:10) giving 396 mg of the desired compound (74% yield); 1H NMR (400 MHz, DMSO-d6): δ 2.59 (s, 3H), 7.64–7.72 (m, 1H), 7.73–7.79 (m, 1H), 8.03 (s, 1H), 8.31 (dd, J = 5.3, 1.8 Hz, 1H), 12.70 ppm (br s, 1H); MS-ESI m/z: [M + H]+ calcd for C19H21N5O, 324.2; found, 324.2; m/z: [M – H]– calcd for C19H20N5O, 302.2; found, 302.2; HPLC (method 2): tR = 1.774 min (99.4%).

4-(4-(Ethyl-2-(methylthio)-1H-imidazol-5-yl)-1H-imidazol-5-yl)-pyridin-2-amine (45). The title compound was synthesized according to general procedure A starting from compound 42 (100 mg, 0.39 mmol) and 4-morpholinooaniline (103.4 mg, 0.58 mmol). Purification by flash column chromatography (SiO2, DCM/MeOH 99:01 to 90:10) afforded 51 mg of the desired compound (33% yield); 1H NMR (300 MHz, DMSO-d6): δ 1.21 (t, J = 7.5 Hz, 3H), 2.55 (s, 3H), 2.78 (q, J = 7.4 Hz, 2H), 2.96–3.08 (m, 4H), 3.66–3.81 (m, 4H), 6.69–6.96 (m, 3H), 7.03 (br s, 1H), 7.52 (d, J = 8.8 Hz, 2H), 8.03 (d, J = 5.3 Hz, 1H), 8.67–8.81 (m, 1H), 12.07–12.37 ppm (m, 1H); 13C NMR (101 MHz, DMSO-d6): δ 13.9, 15.3, 18.7, 49.5, 66.2, 105.5, 110.8, 116.0, 129.3, 134.6, 147.3, 156.9 ppm; MS–FAB m/z: [M] calcd for C19H21N5O, 367.1; found, 367.2; HPLC (method 1): tR = 3.024 min (96.4%).
119.8, 133.2, 134.5, 139.7, 143.0, 145.5, 147.3, 151.6, 156.8 ppm; MS-ESI m/z: [M + H]^+ calcd for C_{16}H_{22}N_4S, 301.2; found, 301.2; HPLC (method 2): t_R = 3.45 min (95%).

4-(4-Methyl-2-(methylthio)-1H-imidazol-5-yl)-N-(tetrahydro-2H-pyran-4-yl)pyridin-2-amine (48f). The title compound was synthesized according to general procedure E starting from 41 (80 mg, 0.33 mmol) and 4-aminothiophen-2-carboxylic acid (120 h). The crude residue was purified by flash column chromatography (SiO_2, DCM/MeOH 97:03 to 90:10), affording 35 mg of pure product (34% yield); 1^H NMR (400 MHz, DMSO-d_6): δ 1.74 (s, 3H), 2.36 (br s, 3H), 2.54 (br s, 3H), 2.72–3.38 (m, 1H), 3.63 (d, J = 7.1 Hz, 1H), 6.53–6.92 (m, 2H), 7.86 (d, J = 5.3 Hz, 1H), 12.23 ppm (br s, 1H); 13C NMR (101 MHz, DMSO-d_6): δ 15.4, 16.7, 17.9, 19.2, 32.1, 50.5, 104.1, 108.4, 127.0, 134.5, 139.0, 142.9, 146.5, 158.6 ppm; HPLC (method 1): t_R = 3.355 min (95%).

4-(4-Methyl-2-(methylthio)-1H-imidazol-5-yl)-N-(1-phenylethyl)pyridin-2-amine (48b). The title compound was synthesized according to general procedure E starting from 41 (100 mg, 417 mmol) and 1-phenylethylamine. The crude residue was purified by flash column chromatography (SiO_2, DCM/MeOH 97:03 to 90:10), affording 83 mg of pure product (49% yield); 1^H NMR (300 MHz, DMSO-d_6): δ 1.55–1.73 (m, 2H), 1.77–1.95 (m, 2H), 2.19–2.41 (m, 3H), 2.53 (s, 3H), 4.16–4.40 (m, 1H), 6.41–6.77 (m, 3H), 7.84–7.97 (m, 1H), 12.08–12.34 ppm (m, 1H); 13C NMR (101 MHz, DMSO-d_6): δ 11.3, 14.7, 15.5, 30.7, 46.1, 103.3, 109.0, 126.6, 134.5, 138.7, 142.7, 147.5, 158.3 ppm; MS-ESI m/z: [M + H]^+ calcd for C_{17}H_{17}N_5S, 329.1; found, 329.0; HPLC (method 2): t_R = 2.748 min (100%).
pound was synthesized according to general procedure E starting from 41 (100 mg, 0.42 mmol) and trans-4-amino-cyclohexanol (484 mg, 4.20 mmol) and adding 2 mL of n-butanol (120 h). The crude residue was purified by flash column chromatography (SiO2, DCM/ETOH 92:08 to 80:20), affording 37 mg of pure product (27% yield); 1H NMR (400 MHz, DMSO-d6): δ 1.10–1.35 (m, 4H), 1.76–2.00 (m, 4H), 2.22–2.42 (m, 3H), 2.52 (s, 3H), 3.41 (br s, 1H), 3.61 (m, 1H), 4.47–4.70 (m, 1H), 6.14–6.85 (m, 3H), 7.78–8.02 (m, 1H), 12.06–12.36 ppm (m, 1H); 13C NMR (101 MHz, DMSO-d6): δ 11.3, 15.5, 30.6, 34.1, 48.5, 68.5, 104.1, 108.7, 126.5, 134.5, 138.6, 142.5, 147.3, 158.7 ppm; MS-ESI m/z: [M + H]+ calcd for C18H25N5OS, 358.2; found, 358.1; HPLC (method 2): tR = 4.076 min (97%).

N-(trans-4-((4-Methyl-2-(methylthio)-1H-imidazol-5-yl)pyridin-2-yl)(trans-N1-(4-(4-Methyl-2-(methylthio)-1H-imidazol-5-yl)pyridin-2-yl)amino)cyclohexyl)cyclohexanecarboxamide (48h). The title compound was synthesized according to general procedure F starting from 48h (120 mg, 0.38 mmol) and cyclohexane carbonyl chloride (83 mg, 0.57 mmol). Purification by flash column chromatography (SiO2, DCM/ETOH 95:05 to 80:20) afforded 29 mg of the desired product (18% yield); 1H NMR (300 MHz, DMSO-d6): δ 1.05–1.43 (m, 9H), 1.54–1.87 (m, 7H), 1.90–2.10 (m, 3H), 2.23–2.42 (m, 3H), 2.53 (s, 3H), 3.45–3.72 (m, 2H), 6.15–6.84 (m, 3H), 7.56 (d, J = 7.6 Hz, 1H), 7.76–8.03 (m, 1H), 12.20 ppm (br s, 1H); MS-ESI m/z: [M + H]+ calcd for C23H27N5OS, 428.2; found, 428.0; m/z: [M − H]− calcd for C23H27N5OS, 426.2; found, 426.1; HPLC (method 2): tR = 5.391 min (98%).

N-(trans-4-((4-Methyl-2-(methylthio)-1H-imidazol-5-yl)pyridin-2-yl)(trans-N1-(4-(4-Methyl-2-(methylthio)-1H-imidazol-5-yl)pyridin-2-yl)amino)cyclohexyl)cyclohexanecarboxamide (48i). The title compound was synthesized according to general procedure F starting from 48i (120 mg, 0.38 mmol) and pyridine (m, 3H), 7.56 (d, J = 7.6 Hz, 1H), 7.76–8.03 (m, 1H), 12.20 ppm (br s, 1H); MS-ESI m/z: [M + H]+ calcd for C23H27N5OS, 428.2; found, 428.0; m/z: [M − H]− calcd for C23H27N5OS, 426.2; found, 426.1; HPLC (method 2): tR = 4.114 min (99%).

N-(trans-4-((4-Methyl-2-(methylthio)-1H-imidazol-5-yl)pyridin-2-yl)(trans-N1-(4-(4-Methyl-2-(methylthio)-1H-imidazol-5-yl)pyridin-2-yl)amino)cyclohexyl)cyclohexanecarboxamide (48j). The title compound was synthesized according to general procedure F starting from 48j (120 mg, 0.38 mmol) and pivaloyl chloride (69 mg, 0.57 mmol). Purification by flash column chromatography (SiO2, DCM/ETOH 90:10 to 80:20) afforded 29 mg of the desired product (19% yield); 1H NMR (400 MHz, DMSO-d6): δ 1.08 (s, 9H), 1.15–1.40 (m, 4H), 1.64–1.77 (m, 2H), 1.92–2.05 (m, 2H), 2.25–2.41 (m, 3H), 2.53 (s, 3H), 3.46–3.70 (m, 2H), 6.23–6.77 (m, 3H), 7.13 (d, J = 8.1 Hz, 1H), 7.89 (d, J = 4.5 Hz, 1H), 12.04–12.35 ppm (m, 1H); 13C NMR (101 MHz, DMSO-d6): δ 11.3, 15.5, 27.4, 31.0, 31.6, 37.8, 47.5, 48.6, 104.1, 108.6, 126.6, 134.4, 138.7, 142.6, 147.1, 158.5, 176.5 ppm; MS-ESI m/z: [M + H]+ calcd for C23H27N5OS, 428.2; found, 428.0; m/z: [M − H]− calcd for C23H27N5OS, 426.2; found, 426.1; HPLC (method 2): tR = 5.391 min (98%).
N-(4-((4-Methyl-2-(methylthio)-1H-imidazol-5-yl)-pyridin-2-yl)amino)phenyl)cyclohexanecarboxamide (48p).

The title compound was synthesized according to general procedure F starting from 48m (100 mg, 0.32 mmol) and cyclohexane carbonyl chloride (70 mg, 0.48 mmol). Purification by flash column chromatography (SiO2, DCM/ EtOH 97:03 to 90:10) afforded 40 mg of the desired product (30% yield); 1H NMR (300 MHz, DMSO-d6); δ 1.11–1.50 (m, 5H), 1.56–1.85 (m, 5H), 2.22–2.36 (m, 1H), 2.74 (s, 3H), 2.55 (s, 3H), 6.94 (d, J = 5.0 Hz, 1H), 7.09 (br s, 1H), 7.47 (d, J = 9.0 Hz, 2H), 7.58 (d, J = 9.0 Hz, 2H), 8.06 (d, J = 5.4 Hz, 1H), 8.91 (s, 1H), 9.62 (s, 1H), 12.28 ppm (br s, 1H); 13C NMR (101 MHz, DMSO-d6); δ 11.5, 15.4, 25.2, 25.4, 29.2, 44.7, 105.7, 110.9, 118.3, 119.7, 127.1, 132.5, 134.2, 137.3, 139.4, 142.8, 147.2, 156.4, 173.6 ppm; MS-ESI m/z: [M + H]+ calcd for C23H21N5OS, 416.1; found, 415.7; m/z: [M − H]− calcd for C22H23N5O5S, 414.1; found, 413.7; HPLC (method 2); tR = 4.357 min (97%).

N-(4-((4-Methyl-2-(methylthio)-1H-imidazol-5-yl)-pyridin-2-yl)amino)pivalalamide (48q). Under an argon atmosphere, tris(diphenylidenaceton)dipalladium(0) (Pd2(dba)3) (17.5 mg, 0.02 mmol) and 9,9-dimethyl-4,5-bis(diphenylphosphino)xantren (XantPhos) (22.1 mg, 0.04 mmol) were dissolved in dry 1,4-dioxane (5 mL) and stirred for 10 min. After that compound 49 (50 mg, 0.21 mmol), Cs2CO3 (138.1 mg, 0.42 mmol), and 4-morpholinonoline (56.7 mg, 0.32 mmol) were added and the reaction mixture was heated to 100 °C and stirred for 15 h. After cooling to rt, the reaction mixture was diluted with DCM and the solid residue was removed by filtration. The filtrate was then concentrated at reduced pressure and the residue was purified by flash column chromatography (DCM/EtOH 100:0 to 90:10) giving 61 mg of the desired product (73% yield); 1H NMR (400 MHz, DMSO-d6); δ 2.36–2.44 (m, 3H), 2.54 (m, 3H), 2.93–3.05 (m, 4H), 3.45–3.55 (m, 3H), 3.67–3.76 (m, 4H), 6.79–6.96 (m, 3H), 7.07 (s, 1H), 7.54 (d, J = 7.3 Hz, 2H), 8.00–8.09 (m, 9H), 8.76 ppm (br s, 1H); 13C NMR (101 MHz, DMSO-d6); δ 10.5, 15.8, 30.5, 49.5, 66.2, 106.0, 111.0, 115.9, 119.5, 128.4, 134.4, 136.4, 140.9, 142.9, 145.4, 147.2, 156.7 ppm; MS–FAB m/z: [M] calcd for C22H24N5O5S, 395.2; found, 395.3; HPLC (method 1): tR = 3.178 min (98.9%).

5-(2-Chloropyridin-4-yl)-1,4-dimethyl-1,3-dihydro-2H-imidazole-2-thione (51). In a pressure vial, compound 31 (200 mg, 0.77 mmol) (for the synthesis of compound 31 see Supporting Information) and methyl isothiocyanate (284 mg, 3.88 mmol) were suspended in triethylamine (2 mL), and after closing the vial tightly, the reaction mixture was stirred at 60 °C for 16 h. The excess of triethylamine was evaporated at reduced pressure and the residue was suspended in glacial AcOH and stirred at 80 °C for 1.5 h. The reaction mixture was concentrated at reduced pressure and after that NaHCO3 saturated solution (20 mL) was added and the aqueous phase was extracted four times with EtOAc. The combined organic layers were washed with H2O and NaCl saturated solution, dried over anhydrous Na2SO4, and concentrated at reduced pressure. Finally, the residue was purified by flash column chromatography (SiO2, DCM/EtOH 100:0 to 95:05) affording 110 mg of the desired product (60% yield); 1H NMR (300 MHz, DMSO-d6); δ 2.11 (s, 3H), 3.45 (s, 3H), 7.47 (dd, J = 5.2, 1.4 Hz, 1H), 7.55–7.63 (m, 1H), 8.47 (d, J = 5.1 Hz, 1H), 12.51 ppm (br s, 1H); 13C NMR (75 MHz, DMSO-d6); δ 9.6, 32.4, 122.7, 122.9, 123.3, 124.4, 139.5, 150.2, 150.9, 161.8 ppm; MS-ESI m/z: [M − H]− calcd for C14H13ClIN,S, 238.0; found, 238.0; HPLC (method 2): tR = 2.533 min.

5-(2-Chloropyridin-4-yl)-1-ethyl-4-methyl-1,3-dihydro-2H-imidazole-2-thione (52). The title compound was prepared following the same procedure of compound 51 starting from 31 (200 mg, 0.77 mmol) (for the synthesis of compound 31 see Supporting Information) and ethyl isothiocyanate (335.5 mg, 3.85 mmol). Purification by flash column chromatography (SiO2, DCM/EtOH 99:01 to 95:05) afforded 128 mg of the desired compound (65% yield); 1H NMR (300 MHz, CDCl3); δ 1.15 (t, J = 6.9 Hz, 3H), 2.14 (s, 3H), 4.04 (q, J = 6.8 Hz, 2H), 7.11 (d, J = 4.5 Hz, 1H), 7.21 (s, 1H), 8.46 (d, J = 4.8 Hz, 1H), 12.32 ppm (br s, 1H); 13C NMR (101 MHz, CDCl3); δ 9.6, 14.1, 40.3, 122.5, 123.2, 124.2, 128.9, 139.5, 150.4, 152.4, 160.1 ppm; MS-ESI m/z: [M − H]− calcd for C14H12ClIN,S, 252.0; found, 252.0; HPLC (method 2): tR = 3.168 min.

5-(2-Chloropyridin-4-yl)-1-cyclopropyl-4-methyl-1,3-dihydro-2H-imidazole-2-thione (53). The title compound was prepared following the same procedure of compound 51 starting from 31 (500 mg, 1.94 mmol) (for the synthesis of 1H), 7.46–7.73 (m, 7H), 7.90–8.00 (m, 2H), 8.10 (d, J = 5.3 Hz, 1H), 9.01 (s, 1H), 10.12 (s, 1H), 12.29 ppm (br s, 1H); 13C NMR (101 MHz, DMSO-d6); δ 14.2, 15.9, 106.4, 111.5, 118.6, 121.6, 128.0, 128.8, 129.2, 131.4, 131.7, 132.5, 135.6, 138.6, 139.9, 143.7, 147.7, 156.9, 165.5 ppm; MS-ESI m/z: [M + H]+ calcd for C23H21N5OS, 416.1; found, 415.7; m/z: [M − H]− calcd for C22H23N5O5S, 414.1; found, 413.7; HPLC (method 2); tR = 4.357 min (97%).
compound 31 see Supporting Information) and cyclopropyl isothiocyanate (962.4 mg, 9.70 mmol). Purification by flash column chromatography (SiO2, DCM/THF 100:0 to 10:0) afforded 335 mg of the desired compound (60% yield);1 H NMR (300 MHz, DMSO-d6): δ 0.43 – 0.53 (m, 2H), 0.79 – 0.96 (m, 2H), 2.09 (s, 3H), 3.17 – 3.29 (m, 1H), 7.49 (dd, J = 5.2, 1.4 Hz, 1H), 7.55 – 7.64 (m, 1H), 8.45 (d, J = 5.2 Hz, 1H), 12.42 ppm (br s, 1H);13C NMR (101 MHz, DMSO-d6): δ 6.5, 9.6, 27.0, 122.5, 122.9, 123.3, 124.4, 139.6, 149.6, 149.2, 150.4, 163.9 ppm; MS-ESI m/z: [M + H]+ calcd for C13H14ClN3S, 280.1; found, 279.6; HPLC (method 2): tR = 3.02 min.

2-Chloro-4-(1,4-dimethyl-2-(methylthio)-1H-imidazol-5-yl)pyridine (54). In a pressure vial, compound 51 (285 mg, 1.19 mmol) and t-BuONa (114.3 mg, 1.19 mmol) were dissolved in dry MeOH (15 mL), and after cooling the reaction mixture to 0 ºC, methyl iodide (217 μL, 3.48 mmol) was added. The vial was tightly closed and the mixture was stirred at 50 ºC for 30 min. After evaporating the solvent at reduced pressure, H2O was added and the aqueous phase was extracted four times with EtOAc. The combined organic layers were washed with H2O and NaCl saturated solution, dried over anhydrous Na2SO4 and concentrated at reduced pressure, giving 290 mg of the product which was used in the following step without further purification (95% yield);1 H NMR (300 MHz, CDCl3): δ 2.28 (s, 3H), 2.66 (s, 3H), 3.52 (s, 3H), 7.12 (dd, J = 5.2, 1.5 Hz, 1H), 7.22 – 7.24 (m, 1H), 8.44 ppm (d, J = 5.1 Hz, 1H);13C NMR (101 MHz, CDCl3): δ 13.7, 15.8, 32.3, 122.0, 123.5, 126.8, 138.5, 141.1, 145.5, 149.9, 152.0 ppm; MS-ESI m/z: [M + H]+ calcd for C12H12ClN3S, 264.0; found, 264.0; 1H NMR (300 MHz, CDCl3): δ 6.81 (br s, 1H), 6.91 (d, J = 5.1 Hz, 1H);13C NMR (101 MHz, CDCl3): δ 13.7, 15.9, 32.2, 49.6, 66.8, 107.2, 114.1, 116.7, 124.2, 128.4, 131.6, 137.5, 140.4, 144.3, 148.5, 157.1 ppm; MS-ESI m/z: [M + H]+ calcd for C12H12ClN3S, 264.0; found, 263.6; HPLC (method 2): tR = 1.72 min (99.3%).

2-Chloro-4-(1-ethy1-4-methyl-2-(methylthio)-1H-imidazol-5-yl)pyridine (55). The title compound was synthesized following the same procedure of compound 54 starting from S1 (150 mg, 0.49 mmol), t-BuOna (47 mg, 0.49 mmol), and methyl iodide (90 μL, 1.44 mmol) giving 120 mg of the product, which was used in the following step without further purification (95% yield);1 H NMR (300 MHz, CDCl3): δ 1.13 (t, J = 7.2 Hz, 3H), 2.15 (s, 3H), 2.57 (s, 3H), 3.85 (q, J = 7.2 Hz, 2H), 7.06 (dd, J = 5.1, 1.5 Hz, 1H), 7.13 – 7.18 (m, 1H), 8.35 ppm (dd, J = 5.1, 0.5 Hz, 1H);13C NMR (75 MHz, CDCl3): δ 13.3, 15.5, 15.6, 39.7, 122.0, 123.5, 126.8, 138.5; MS-ESI m/z: [M + H]+ calcd for C12H14ClN3S, 246.2; found, 246.0; HPLC (method 2): tR = 2.25 min.

2-Chloro-4-(1-cyclopropyl-4-methyl-2-(methylthio)-1H-imidazol-5-yl)pyridine (56). The title compound was synthesized following the same procedure of compound 54 starting from S3 (210 mg, 0.74 mmol), t-BuOna (71.4 mg, 0.74 mmol) and methyl iodide (135 μL, 2.16 mmol) giving 200 mg of the product, which was used in the following step without further purification (95% yield);1 H NMR (300 MHz, CDCl3): δ 0.59 – 0.71 (m, 2H), 0.93 – 1.03 (m, 2H), 2.27 (s, 3H), 2.67 (s, 3H), 3.03 – 3.14 (m, 1H), 7.19 (dd, J = 5.2, 1.5 Hz, 1H), 7.27 – 7.31 (m, 1H), 8.39 ppm (d, J = 5.1 Hz, 1H);13C NMR (75 MHz, CDCl3): δ 13.9, 13.9, 14.6, 26.1, 121.7, 123.1, 126.9, 138.1, 141.3, 148.3, 149.3, 151.8 ppm; MS-ESI m/z: [M + H]+ calcd for C12H14ClN3S, 280.1; found, 280.0; HPLC (method 2): tR = 2.76 min.

4-(1,4-Dimethyl-2-(methylthio)-1H-imidazol-5-yl)-N-(4-morpholinophenyl)pyridin-2-amine (57). In an argon-flushed pressure tube, compound 54 (100 mg, 0.39 mmol), 4-morpholinoline (105.3 mg, 0.59 mmol), Pd2(dba)3 (36.1 mg, 0.04 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) (37.18 mg, 0.08 mmol), and Cs2CO3 (770.2 mg, 2.36 mmol) were suspended in dry 1,4-dioxane, and after the vial was tightly closed, the mixture was stirred at 100 ºC for 3 h. The solvent was evaporated at reduced pressure and after that NH4Cl saturated solution was added to the residue and the aqueous phase was extracted five times with EtOAc. The combined organic layers were washed twice with H2O and NaCl saturated solution, dried over anhydrous Na2SO4 and concentrated at reduced pressure. Finally, the residue was purified twice by flash column chromatography (SiO2, DCM/THF 95:05 to 90:10) and (SiO2, DCM/MeOH 97:03) giving 30 mg of the desired product (20% yield);1 H NMR (300 MHz, CDCl3): δ 2.23 (s, 3H), 2.62 (s, 3H), 3.07 – 3.23 (m, 4H), 3.46 (s, 3H), 3.83 – 3.94 (m, 4H), 6.52 – 6.62 (m, 2H), 6.72 (br s, 1H), 6.92 (d, J = 8.3 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 8.19 ppm (d, J = 5.1 Hz, 1H);13C NMR (101 MHz, CDCl3): δ 13.7, 15.9, 32.2, 49.6, 66.8, 107.2, 114.1, 116.7, 124.2, 128.4, 131.6, 137.5, 140.4, 144.3, 148.5, 157.1 ppm; MS-ESI m/z: [M + H]+ calcd for C12H14ClN3S, 346.2; found, 346.5; m/z: [M + H]+ calcd for C12H14ClN3S, 346.2; found, 349.4; HPLC (method 2): tR = 2.116 min (99.3%).
147.1, 148.3, 156.9 ppm; MS-ESI m/z: [M + H]+ calcd for C24H22FN5O, 422.2; found, 422.0; m/z: [M − H]− calcd for C24H22FN5O, 422.2; found, 421.0; HPLC (method 2): fR = 2.989 min (98.0%).

2-Bromo-4-(1-(4-fluorophenyl)-1H-imidazol-5-yl)pyridine (64). 2-Bromoisonicotinaldehyde (60, 300 mg, 1.61 mmol), 4-fluoroaniline (179 mg, 1.61 mmol), and AcOH (160 μL) were dissolved in EtOH and the reaction mixture was stirred at reflux temperature for 2 h. After cooling to rt, the solvent was evaporated at reduced pressure and the residue was suspended in a mixture of MeOH and 1,2-dimethoxyethane (8 mL) and transferred into a three-neck round-bottom flask under an argon atmosphere. TOSMIC (471.5 mg, 2.41 mmol) and K2CO3 (445 mg, 3.22 mmol) were dissolved in dry toluene (15 mL) and the reaction mixture was stirred for 3 h at 80 °C. After that both 2,2-dimethoxyethan-1-amine was added and the mixture was stirred at reflux temperature for 3 h. The mixture was cooled at rt and the solvent was evaporated at reduced pressure. The residue obtained was purified by flash chromatography (SiO2, DCM to DCM/MeOH 95:0.5) yielding 360 mg of the desired compound (70% yield); 1H NMR (250 MHz, DMSO-d6): δ 7.03 (d, J = 4.3 Hz, 1H), 7.30–7.50 (m, 5H), 7.69 (br s, 1H), 8.08 (br s, 1H), 8.24 ppm (d, J = 4.3 Hz, 1H); 13C NMR (101 MHz, DMSO-d6): δ 118.1, 118.6, 124.8, 140.7, 141.9, 150.5, 151.1 ppm; MS-ESI m/z: [M + H]+ calcld for C19H18BrN5F, 415.2; found, 415.3; HPLC (method 1): fR = 5.08 min (100%).

4-(1-Methyl-1H-imidazol-5-yl)-N-(4-morpholinophenyl)pyridin-2-amine (67). The title compound was synthesized according to general procedure A starting from 65 (140 mg, 0.72 mmol) and 4-morpholinooaniline (192.5 mg, 1.08 mmol).

Under an argon atmosphere, compound 65 (140 mg, 0.72 mmol) was dissolved in MeOH (8 mL), and 4-morpholinooaniline (192.5 mg, 1.08 mmol) was added and the reaction mixture was stirred at 0 °C for 1 h. After that both 2,2-dimethoxyethan-1-amine was added and the mixture was stirred at reflux temperature for 30 min. After cooling to rt, the mixture was diluted with MeOH (8 mL) and then 6 N HCl solution (7.2 mL, 43.2 mmol) was added and the mixture was stirred at reflux temperature for 18 h. The solvent was evaporated at reduced pressure and after that a 10% solution of K2CO3 was added to the residue until reaching pH = 10. The precipitate obtained was filtered off and washed with H2O, affording 2.01 g of the product which was used for the following step without further purification (77% yield); 1H NMR (400 MHz, DMSO-d6): δ 7.30 (d, J = 4.3 Hz, 1H), 7.37–7.42 (m, 2H), 7.99 (s, 2H), 8.70 ppm (s, 1H); 13C NMR (101 MHz, DMSO-d6): δ 118.1, 118.6, 124.8, 140.7, 141.9, 150.5, 151.1 ppm; MS-ESI m/z: [M + H]+ calcld for C19H18BrN5F, 415.2; found, 413.2; HPLC (method 1): fR = 2.048 min (100%).

2-Chloro-4-(1H-imidazol-2-yl)pyridine (69). To a solution of 2-chloroisonicotinonitrile (68) (2.0 g, 14.44 mmol) in MeOH (8 mL), a 30% solution of NaOMe in MeOH (260 μL, 1.44 mmol) was added and the reaction mixture was stirred at 40 °C for 1 h. After that both 2,2-dimethoxyethan-1-amine (1.56 mL, 14.44 mmol) and AcOH (1.56 mL, 27.27 mmol) were added dropwise and the mixture was stirred at reflux temperature for 30 min. After cooling to rt, the mixture was diluted with MeOH (8 mL) and then 6 N HCl solution (7.2 mL, 43.2 mmol) was added and the mixture was stirred at reflux temperature for 18 h. The solvent was evaporated at reduced pressure and after that a 10% solution of K2CO3 was added to the residue until reaching pH = 10. The precipitate obtained was filtered off and washed with H2O, affording 2.01 g of the product which was used for the following step without further purification (77% yield); 1H NMR (400 MHz, DMSO-d6): δ 7.30 (br s, 2H), 7.77–7.91 (m, 1H), 7.95 (br s, 1H), 8.36–8.49 (m, 1H), 13.03 ppm (br s, 1H); 13C NMR (101 MHz, DMSO-d6): δ 118.1, 118.6, 124.8, 140.7, 141.9, 150.5, 151.1 ppm; MS-ESI m/z: [M + H]+ calcld for C6H5ClN3, 180.0; found, 179.8; m/z: [M − H]− calcld for C6H5ClN3, 178.0; found, 177.8; HPLC (method 1): fR = 2.346 min.

2-Chloro-4-(1-methyl-1H-imidazol-2-yl)pyridine (70). Under an argon atmosphere, compound 69 (1.72 g, 9.61 mmol) was dissolved in dry DMF (20 mL) and after cooling the reaction mixture to 0 °C, NaH (231 mg, 9.61 mmol) was added and the mixture was stirred at 0 °C for 15 min. After that methyl iodide (1.61 mL, 25.6 mmol) was added dropwise and the reaction mixture was let to heat to rt and stirred for 90 min. The mixture was poured in H2O and the aqueous phase was extracted three times with DC. The combined organic layers were dried over anhydrous Na2SO4 and the solvent was evaporated at reduced pressure. Finally, the residue was treated with a mixture of n-hexane/EtOAc 40:1 and the solid obtained was filtered off and dried in vacuo, affording 793 mg of the product which was used for the following step without further purification (43% yield); 1H NMR (400 MHz, DMSO-d6): δ 3.82–3.93 (m, 3H), 7.09 (br s, 1H), 7.40 (br s, 1H), 7.71–
7.8 (m, 1H), 7.80 (br s, 1H), 8.40–8.53 ppm (m, 1H); 13C NMR (101 MHz, DMSO-d$_6$): δ 34.8, 121.0, 121.6, 125.8, 128.7, 140.9, 142.3, 150.1, 150.8 ppm; MS-ESI m/z: [M + H]$^+$ calcld for C$_8$H$_8$ClN$_3$O, 194.0; found, 193.8; HPLC (method 1): $t_R$ = 1.161 min.

4-(1-Methyl-1H-imidazo-2-yl)-N-(4-morpholinophenyl)pyridin-2-amine (71). The title compound was synthesized according to general procedure A starting from 70 (150 mg, 0.77 mmol) and 4-morpholinonoline (205 mg, 1.15 mmol). Purification by flash column chromatography (SiO$_2$, DCM/ EtOH 95:05) afforded 100 mg of the desired compound (39% yield); $^1$H NMR (400 MHz, DMSO-d$_6$): δ 2.93–3.09 (m, 4H), 3.66–3.77 (m, 4H), 3.81 (s, 3H), 6.90 (d, $J = 8.3$ Hz, 2H), 7.05–7.04 (m, 2H), 7.09 (s, 1H), 7.31 (s, 1H), 7.52 (d, $J = 8.1$ Hz, 2H), 8.15 (d, $J = 4.8$ Hz, 1H), 8.89 ppm (br s, 1H); $^{13}$C NMR (101 MHz, DMSO-d$_6$): δ 34.6, 49.4, 66.1, 107.8, 111.9, 115.9, 120.0, 124.5, 127.9, 133.9, 138.5, 144.3, 145.8, 147.6, 156.7 ppm; FAB−MS m/z: [M] calcld for C$_9$H$_8$F$_2$N$_3$S, 304.3; found, 304.1; HPLC (method 1): $t_R$ = 2.359 min (100%).

2-Bromo-1-(2-chloropyridin-4-yl)propan-1-one (76). The title compound was synthesized (39% yield); $^1$H NMR (250 MHz, DMSO-d$_6$): δ 2.87 (d, $J = 4.9$ Hz, 3H), 2.94–3.07 (m, 4H), 3.64–3.82 (m, 4H), 6.37 (dd, $J = 5.4$, 1.7 Hz, 1H), 6.54 (d, $J = 1.0$ Hz, 1H), 6.82 (m, $J = 9.0$ Hz, 2H), 7.12–7.25 (m, 2H), 7.27–7.38 (m, 2H), 7.42–7.55 (m, 2H), 7.85 (q, $J = 4.6$ Hz, 1H), 7.93 (d, $J = 5.4$ Hz, 1H), 8.69 ppm (s, 1H); $^{13}$C NMR (101 MHz, DMSO-d$_6$): δ 30.7, 49.3, 66.1, 107.6, 112.7, 115.2 (d, $J = 21.2$ Hz), 115.8, 116.3, 120.0, 130.8 (d, $J = 8.1$ Hz), 131.8 (d, $J = 2.9$ Hz), 133.7, 141.0, 145.8, 146.7, 147.8, 156.7, 161.7 (d, $J = 244.4$ Hz), 167.3 ppm; MS-ESI m/z: [M + H]$^+$ calcld for C$_{10}$H$_{10}$ClN$_3$, 240.2; found, 240.1; HPLC (method 2): $t_R$ = 8.518 min (98.2%).

2-Bromo-1-(2-chloropyridin-4-yl)propan-1-one (76). 1-(2-Chloropyridin-4-yl)propan-1-one (22) (3.0 g, 17.68 mmol) was dissolved in 20 mL of AcOH and cooled to 0 °C. The reaction mixture was stirred at room temperature for 1 h. After cooling to rt, the solvent was evaporated at reduced pressure, and after that, the residue was purified by flash column chromatography (SiO$_2$, hexane/EtOAc 7:3), obtaining 495 mg of the desired compound (99% yield): 1H NMR (250 MHz, DMSO-d$_6$): δ 2.87 (d, $J = 4.9$ Hz, 3H), 2.94–3.07 (m, 4H), 3.64–3.82 (m, 4H), 6.37 (dd, $J = 5.4$, 1.7 Hz, 1H), 6.54 (d, $J = 1.0$ Hz, 1H), 6.82 (m, $J = 9.0$ Hz, 2H), 7.12–7.25 (m, 2H), 7.27–7.38 (m, 2H), 7.42–7.55 (m, 2H), 7.85 (q, $J = 4.6$ Hz, 1H), 7.93 (d, $J = 5.4$ Hz, 1H), 8.69 ppm (s, 1H); $^{13}$C NMR (101 MHz, DMSO-d$_6$): δ 30.7, 49.3, 66.1, 107.6, 112.7, 115.2 (d, $J = 21.2$ Hz), 115.8, 116.3, 120.0, 130.8 (d, $J = 8.1$ Hz), 131.8 (d, $J = 2.9$ Hz), 133.7, 141.0, 145.8, 146.7, 147.8, 156.7, 161.7 (d, $J = 244.4$ Hz), 167.3 ppm; MS-ESI m/z: [M + H]$^+$ calcld for C$_{10}$H$_{10}$ClN$_3$, 240.2; found, 240.1; HPLC (method 2): $t_R$ = 8.518 min (98.2%).

2-Bromo-1-(2-chloropyridin-4-yl)propan-1-one (76). The title compound was synthesized according to general procedure A starting from 77 (150 mg, 0.62 mmol) and 4-morpholinonoline (165.8 mg, 0.93 mmol). Purification by flash column chromatography (SiO$_2$, DCM/EtOH 95:05) afforded 42 mg of the desired compound (70% yield); $^1$H NMR (250 MHz, DMSO-d$_6$): δ 2.40 (s, 3H), 2.82 (d, $J = 4.9$ Hz, 3H), 2.95–3.07 (m, 4H), 3.67–3.80 (m, 4H), 6.81–6.93 (m, 3H), 7.01 (br s, 1H), 7.34 (q, $J = 4.6$ Hz, 2H).
5.343 (99.4%).

for C_{20}H_{23}N_{5}O_{5}S, 380.2; found, 380.2; HPLC (method 2): t_{R} = 3.543 (99.4%).

■ ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00668.

Detailed procedures for the preparation of intermediates 17a–I and 33–35; crystal structure of compound 49; melting curves and method description of the nanoDSF experiments; experimental procedures for the crystallization and structure determination of JNK3 in the complex with compounds 38, 44, and AMP–PCP; selectivity screening of 44; and in vitro metabolic stability study for compound 44 (PDF)

SMILES strings of tested compounds (CSV)

Accession Codes
The atomic coordinates and structures factors of complex structures containing compounds 38 and 44 and AMP–PCP were deposited in the Protein Data Bank (PDB) with the respective accession codes 6EMH, 6EKD, and 6EQ9, respectively. The authors will release the atomic coordinates and experimental data upon article publication.

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Notes
The authors declare no competing financial interest.

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■ ABBREVIATIONS

MAPK, mitogen activated protein kinase; JNK, c-Jun N terminal kinase; TOSMIC, tolune sulfonylmethylisocyanide; HR, hydrophobic region; AMP–PCP, β,γ-methyleneadenosine-5′-triphosphate; nanoDSF, nano differential scanning fluorimetry; hERG, human-ether-a-go-go related gene; CYP450, cytochrome P450; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; DAD, diode array detector; NMR, nuclear magnetic resonance; ESI-MS, electrospray ionization mass spectrometer

■ REFERENCES


