



Cite this: *RSC Adv.*, 2025, 15, 15497

# Synthesis of imidazopyridines *via* NaIO<sub>4</sub>/TBHP-promoted (3 + 2) cycloaddition and biological evaluation as anticancer agents†

Huiping Luo,<sup>‡a</sup> Zhengyu Hu,<sup>‡b</sup> Jihai Shi,<sup>a</sup> Yongxin Lou,<sup>c</sup> Zhonghua Shi,<sup>a</sup> Xin Jin,<sup>a</sup> Jia Chen,<sup>b</sup> Xing Liu<sup>\*d</sup> and Qiang Huang<sup>ib\*</sup>

A novel and simple NaIO<sub>4</sub>/TBHP-promoted (3 + 2) cycloaddition reaction of propargyl alcohols and 2-aminopyridines was discovered for the synthesis of imidazo[1,2-*a*]pyridines. This protocol exhibits a broad substrate scope for both propargyl alcohols and 2-aminopyridines, with high functional group tolerance, leading to the formation of various C3-carbonylated imidazopyridines in moderate yields. More importantly, these synthesized compounds were evaluated for their antiproliferation activity against MOLM-13 and MV4-11 cells, indicating that **3n**, **5a** and **5d** possessed good bioactivity. Molecular docking analysis showed the strong interaction between **5a**, **5d** and FLT3 kinase, which have practical values in the development of kinase inhibitors.

Received 19th March 2025  
Accepted 24th April 2025

DOI: 10.1039/d5ra01949d

rsc.li/rsc-advances

## 1. Introduction

Imidazopyridines are an important class of aza-fused heterocycles, including imidazo[1,2-*a*]pyridines, imidazo[1,5-*a*]pyridines, imidazo[4,5-*c*]pyridines, imidazo[5,1-*a*]quinolones, and imidazo[2,1-*a*]isoquinolines, which has been widely used in medicinal chemistry (Fig. 1)<sup>1–7</sup> and material science.<sup>8</sup> Many reports have shown that imidazopyridines possess good antibacterial activity,<sup>9</sup> kinase inhibitory activity,<sup>10,11</sup> and anti-cancer activities.<sup>12–15</sup> Hence, new strategies to construct an imidazopyridine scaffold have received considerable interest,<sup>16–23</sup> and these scaffolds are suitable for the design and development of more effective, targeted compounds for cancer treatment.<sup>24–30</sup>

Among imidazopyridine derivatives, imidazo[1,2-*a*]pyridine is highly valuable due to its bioactivity,<sup>11</sup> and it was mainly obtained through synthetic approaches starting from 2-aminopyridines.<sup>31–34</sup> The direct annulation of acetophenone, ethylbenzene, styrene, or phenylacetylene as starting materials with 2-aminopyridine is the usual synthetic protocol,<sup>35–39</sup> which mainly achieved direct C2-arylated imidazo[1,2-*a*]pyridines. Additionally, some three-component reactions between aldehydes, 2-aminopyridines and other partners could afford

functionalization at the C3 position of the imidazo[1,2-*a*]pyridine moiety (Scheme 1a).<sup>40–44</sup> Although these excellent works provided alternative approaches for the synthesis of bioactive imidazo[1,2-*a*]pyridines, the pursuit of innovative synthetic methodologies aimed at constructing a diverse array of imidazo[1,2-*a*]pyridine derivatives is highly important.

In our previous work, we developed an I<sub>2</sub>/DTBP-promoted (3 + 2) cycloaddition reaction of 2-aminopyridines and chromones to synthesize C3-carbonylated imidazo[1,2-*a*]pyridines (Scheme 1b)<sup>45</sup> and found that the products provided key active fragments for the synthesis of cabozantinib analogues, which have the potential to be developed as an anticancer agent. Recently, we also developed a series of reactions of propargyl alcohols to synthesize various compounds, including silver-mediated aminophosphinylation of propargyl alcohols<sup>46</sup> and the DBU-promoted isomerization/addition of propargyl alcohols.<sup>47</sup> Inspired by these results, we wondered whether adoption of a (3 + 2) cyclization protocol would afford imidazo[1,2-*a*]pyridine from propargyl alcohols and 2-aminopyridines. For a proof of concept, we reported a novel and simple synthesis of imidazo[1,2-*a*]pyridine *via* NaIO<sub>4</sub>/TBHP-promoted (3 + 2) cycloaddition

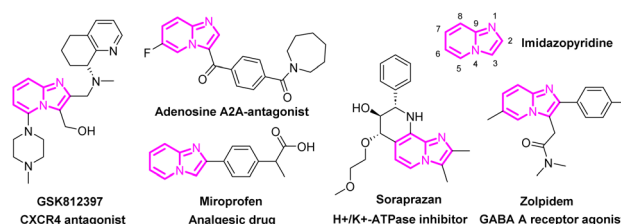


Fig. 1 Representative bioactive imidazopyridines.

<sup>a</sup>School of Pharmacy, Zunyi Medical University, Zunyi, Guizhou 563006, China. E-mail: huangqiang65@sina.com

<sup>b</sup>The First Clinical Institute, Zunyi Medical University, Zunyi, 563006, China

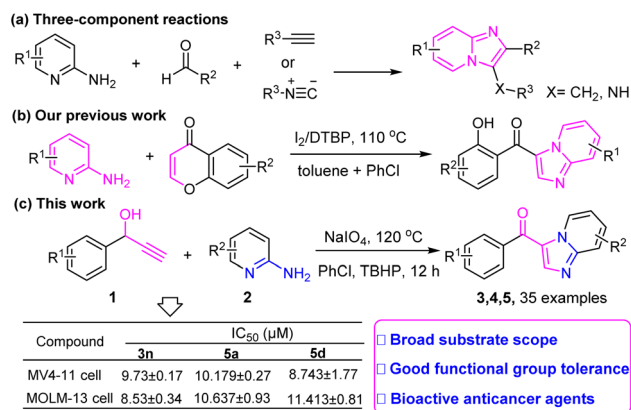
<sup>c</sup>School of Preclinical Medicine, Zunyi Medical University, Zunyi, Guizhou 563006, China

<sup>d</sup>Kweichow Moutai Hospital, Zunyi, Guizhou 563006, China. E-mail: lx-z@163.com

† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d5ra01949d>

‡ These authors contributed equally to this work.



Scheme 1 The synthesis of imidazo[1,2-*a*]pyridines.

and further evaluated the bioactivities of these products as potential anticancer agents, revealing their practical values in the development of kinase inhibitors (Scheme 1c).

## 2. Results and discussion

### 2.1 Chemistry

Initially, various reaction conditions were conducted by mixing 1-phenylprop-2-yn-1-ol (**1a**) and 2-aminopyridine (**2a**) to evaluate different parameters. As shown in Table 1, based on our previous works,<sup>45</sup> the initial reaction was performed using TBHP as an oxidant in PhCl at 120 °C. Unfortunately, no corresponding product **3a** was observed (entry 1). Next, when 20 mol% I<sub>2</sub> was supplemented into the system, 9% yield of **3a** was obtained (entry 2). Herein, different iodine reagents were investigated, such as HI, NaI, KI, NaIO<sub>4</sub>, CuI, AgI, FeI<sub>2</sub>, and ZnI<sub>2</sub> (entries 3–10), and NaIO<sub>4</sub> showed optimal performance. Subsequently, an examination of various hydroperoxides revealed that all were inferior to TBHP (entries 11–12). We hypothesized that this phenomenon was associated with solubility and ion pairing. Given that 70% TBHP in H<sub>2</sub>O was employed, it is possible that a trace amount of H<sub>2</sub>O facilitated the ionization of NaIO<sub>4</sub>, thereby producing a synergistic oxidation effect. Similar oxidation was also observed in our recent work.<sup>48</sup> Additionally, the solvent effect was further considered. However, it seems that all of the polar solvents (like DMSO, DMF and dioxane) were unfavorable for the conversion of **3a** compared to nonpolar media, such as toluene and PhCl (entries 13–18). Here, while *tert*-butyl acetate (TBAC) served as a suitable solvent for the synthesis of compound **3a**, it gave a slightly low yield compared to PhCl (entry 15). When screening the reaction temperatures, the temperature set at 120 °C remained preferable (entries 18–20). Finally, the amount of NaIO<sub>4</sub> and **2a** was examined. The yield of **3a** was significantly increased when 40 mol% NaIO<sub>4</sub> and 3 equivalents of **2a** were used (entry 21). However, 2-aminopyridine was used in a slight excess, leading to a substantial reduction in the reaction efficiency. During the reaction process, we noticed that a highly polar substance could not be separated and remained at the origin spot on the TLC plate, potentially indicating that it could be a by-product formed from

Table 1 Optimization of the reaction condition<sup>ab</sup>

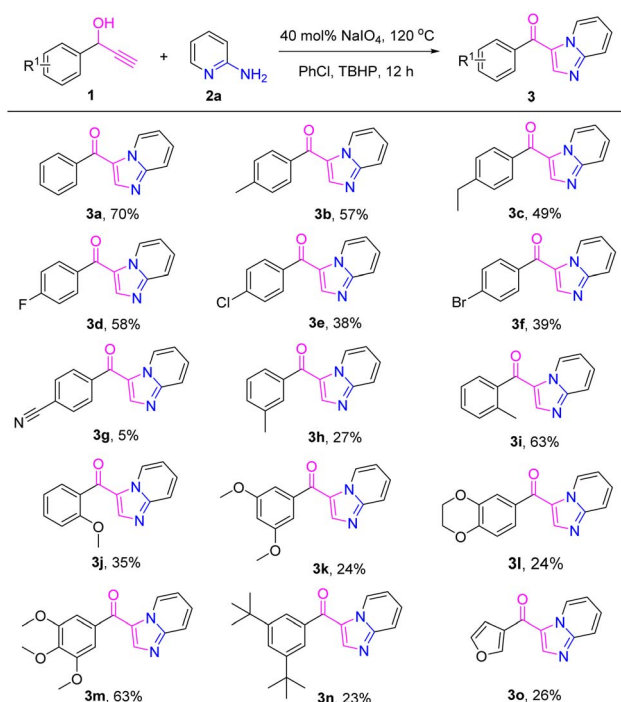
Entry	AD	Oxidant	Solvent	T (°C)	Yield (%)
1	—	TBHP	PhCl	120	0
2	I <sub>2</sub>	TBHP	PhCl	120	9
3	HI	TBHP	PhCl	120	21
4	NaI	TBHP	PhCl	120	17
5	KI	TBHP	PhCl	120	11
6	NaIO <sub>4</sub>	TBHP	PhCl	120	59
7	CuI	TBHP	PhCl	120	27
8	AgI	TBHP	PhCl	120	8
9	FeI <sub>2</sub>	TBHP	PhCl	120	33
10	ZnI <sub>2</sub>	TBHP	PhCl	120	34
11	NaIO <sub>4</sub>	DTBP	PhCl	120	7
12	NaIO <sub>4</sub>	H <sub>2</sub> O <sub>2</sub>	PhCl	120	5
13	NaIO <sub>4</sub>	TBHP	Dioxane	120	18
14	NaIO <sub>4</sub>	TBHP	Toluene	120	37
15	NaIO <sub>4</sub>	TBHP	TBAC	120	54
16	NaIO <sub>4</sub>	TBHP	DMSO	120	21
17	NaIO <sub>4</sub>	TBHP	DMF	120	25
18	NaIO <sub>4</sub>	TBHP	Toluene + PhCl	120	45
19	NaIO <sub>4</sub>	TBHP	PhCl	100	19
20	NaIO <sub>4</sub>	TBHP	PhCl	110	36
21	NaIO <sub>4</sub>	TBHP	PhCl	130	48
22	NaIO <sub>4</sub>	TBHP	PhCl	120	62 <sup>c</sup> , 70 <sup>d</sup> , 65 <sup>e</sup>
23	NaIO <sub>4</sub>	TBHP	PhCl	120	42 <sup>g</sup> , 18 <sup>h</sup>

<sup>a</sup> Reaction conditions: **1a** (0.5 mmol), **2a** (1.5 mmol), additive (20 mol%), oxidant (1.5 mmol), solvent (4 mL), 12 h, temperature 100–130 °C in sealed tube. <sup>b</sup> Yield of isolated product based on **1a**. <sup>c</sup> 30 mol% additive was used. <sup>d</sup> 40 mol% additive was used. <sup>e</sup> 50 mol% additive was used. <sup>g</sup> 1 mmol of **2a**. <sup>h</sup> 0.5 mmol of **2a** was added.

the oxidation of 2-aminopyridine in the presence of TBHP. Thus, it is necessary to utilize a significantly larger excess of 2-aminopyridine. Based on these findings, the optimal condition was determined as follows: 40 mol% NaIO<sub>4</sub> and 3 equivalents of TBHP at 120 °C in PhCl for 12 h.

After determining the optimized reaction conditions, we explored the substrate scope for the synthesis of imidazopyridines. Firstly, various substituted propargyl alcohols were investigated and the results are shown in Table 2. Obviously, substitution on the phenyl ring has a significant effect on the reactivity of the (3 + 2) cycloaddition. Although different substrates can react with **2a**, the corresponding products were obtained in low to moderate yields. Especially, strong electron-withdrawing groups (**3g**) such as nitrile on the phenyl ring distinctly decreased the performance of the reaction. Meta- or ortho-substituted phenyl propargyl alcohols can also be converted into the corresponding compounds **3h–3j** in appropriate yields. Importantly, multisubstituted phenyl propargyl alcohols reacted smoothly with **2a** to give products **3k–3n** in moderate yields. Finally, furan-3-yl(imidazo[1,2-*a*]pyridin-3-yl)methanone was examined in this system, affording the desired **3o** in 26% yield.



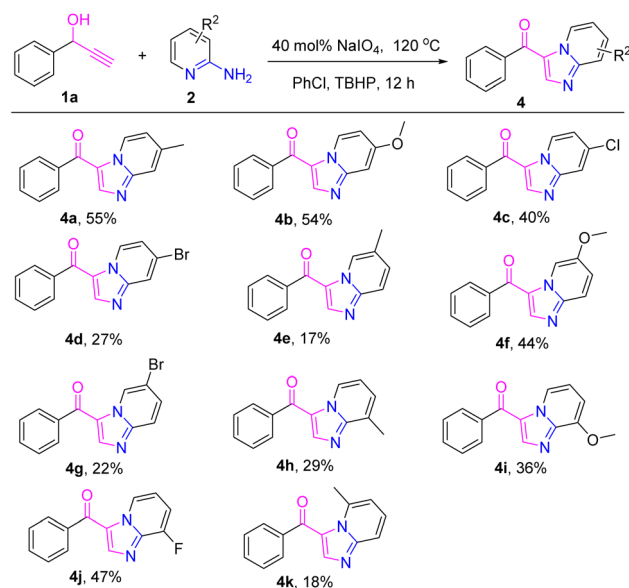
Table 2 Scope of propargyl alcohols<sup>ab</sup>

<sup>a</sup> Reaction conditions: **1** (0.5 mmol), **2a** (1.5 mmol), NaIO<sub>4</sub> (40 mol%), TBHP (1.5 mmol), PhCl (4 mL), 12 h, temperature 120 °C in sealed tube. <sup>b</sup> Yield of isolated product based on **1**.

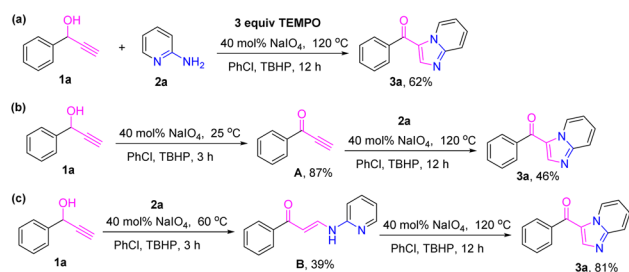
Next, the scope of various 2-aminopyridines was investigated. As shown in Table 3, aminopyridines bearing various substituents can be smoothly converted into the corresponding products **4a–4k**. Generally, the substitutions on the 3-, 4- and 5-positions of 2-aminopyridines are more compatible for the reaction compared to the 6-substituted 2-aminopyridines, such as **4k**, affording the desired products in higher yields. This outcome might be related to the steric hindrance of the substituents, which is not conducive to the final aromatization *via* (3 + 2) cycloaddition.

To gain insight into this reaction, a series of control experiments were conducted, as outlined in Scheme 1. Firstly, the radical inhibition experiments were carried out by TEMPO. The reaction could not be inhibited by TEMPO, and the desired compound **3a** was found in 62% yield (Scheme 2a), indicating that a radical mechanism might not be involved in this system. According to our previous work,<sup>47</sup> we hypothesized that the reaction involves a tandem process of oxidation, addition, and cyclization. To further investigate, we synthesized the potential intermediates **A** and **B** at low temperature, giving 87% and 39% yields, respectively. Interestingly, when using intermediates **A** and **B** under standard conditions (Scheme 2b and c), compound **3a** was obtained in yields of 46% and 81%, respectively. These results indicated that the reactive intermediates **A** and **B** might actively participate in the reaction.

Based on the results from the control experiments, the reaction is likely to proceed through the following mechanism.

Table 3 Scope of 2-aminopyridines<sup>ab</sup>

<sup>a</sup> Reaction conditions: **1a** (0.5 mmol), **2** (1.5 mmol), NaIO<sub>4</sub> (40 mol%), TBHP (1.5 mmol), PhCl (4 mL), 12 h, temperature 120 °C in sealed tube. <sup>b</sup> Yield of isolated product based on **1**.

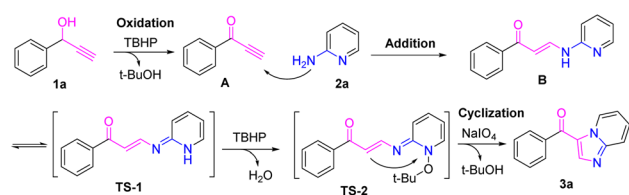


Scheme 2 Control experiments.

As shown in Scheme 3, propargyl alcohol **1a** can be oxidized into intermediate **A** under TBHP. Next, compound **A** reacted with **2a** to generate intermediate **B** through an intermolecular addition. Here, compound **B** can be reversibly converted into **TS-1**, and further oxidized into **TS-2** by TBHP. Finally, the intramolecular cyclization of **TS-2** proceeded to yield the desired **3a** product.

## 2.2 Biological evaluation

Imidazo[1,2-*a*]pyridines as potential anticancer agents have shown promising kinase inhibitory potential.<sup>11,49–51</sup> To the best



Scheme 3 Proposed reaction mechanism.



of our knowledge, considerable research studies have shown the substantial structural features of small molecule imidazopyridines and their noticeable interactions in the active sites of serine/threonine and tyrosine kinases,<sup>52–54</sup> which might be probably related to its remarkable physiochemical properties. For example, Li's group reported on the discovery of the highly ligand efficient, low molecular weight imidazopyridine analogs as FLT3 inhibitors by computer-aided virtual drug screening.<sup>43</sup> Later, they identified a novel series of imidazo[1,2-*a*]pyridine-thiophene derivatives from a NIMA-related kinase 2 (NEK2) inhibitor,<sup>42</sup> which retained inhibitory activities as type-I inhibitors of FLT3. These results indicate that the imidazo[1,2-*a*]pyridine-thiophene scaffold is promising for the development of FLT3 inhibitors. Therefore, the antiproliferation of compounds **3a–3o** and **4a–4k** to MOLM-13 and MV4-11 cells was examined.

As seen in Fig. 2, monosubstituted imidazo[1,2-*a*]pyridin-3-yl(phenyl)methanone derivatives **3** on the phenyl ring failed to clearly improve their antiproliferation for MOLM-13 and MV4-11 cells, and compounds **3a–3i** showed moderate to low inhibition at 50  $\mu$ M and  $IC_{50}$  value. Fortunately, multiple substituents on the phenyl ring (such as 3,4-dioxane, 3,5-*di-t*-Bu) could significantly enhance their antiproliferative effect, and compounds **3l**, **3m** and **3n** gave more than 70% of inhibition rate for the MOLM-13 and MV4-11 cells. Additionally, we found that imidazo[1,2-*a*]pyridines bearing various substituents at different positions have a key effect on the antiproliferation for MOLM-13 and MV4-11 cells. Notably, the 6-site substituted imidazo[1,2-*a*]pyridin-3-yl(phenyl)methanone derivatives **4e–4g** exhibited better antiproliferative activity compared to others.

Furthermore, the  $IC_{50}$  values of compounds **3a–3o** and **4a–4k** to MOLM-13 and MV4-11 cells were further explored using the reported FLT3 inhibitor Cabozantinib as a positive control drug. As presented in Table 4, compounds **3l–3n**, **4e–4g** demonstrated superior bioactivity against MOLM-13 and MV4-11 cells, with compound **3n** notably exhibiting  $IC_{50}$  values of  $8.53 \pm 0.34$  nM and  $9.73 \pm 0.17$  nM against MOLM-13 and MV4-11 cells, respectively. Notably, the 6-position substituted imidazo[1,2-*a*]pyridines (**4e–4g**), bearing methyl, methoxy, and bromo groups, emerged as particularly promising candidates. These structural modifications at the C6 position appear to significantly enhance the inhibitory potency against FLT3-driven leukemic cells.

Based on the above analysis of the structure–activity relationship, we considered that 3,4-dioxane, 3,5-*di-t*-Bu on the benzene ring and 6-site substituted methyl, methoxy, bromide substituents might be responsible for the improvement in antiproliferation. Herein, a series of imidazo[1,2-*a*]pyridine derivatives **5** were synthesized using the NaIO<sub>4</sub>/TBHP-promoted (3 + 2) cycloaddition method. Their antiproliferative activity was then further evaluated, as summarized in Table 5. Compound **5a–5e** exhibited good antiproliferative activity for MOLM-13 and MV4-11 cells compared to compounds **3** and **4**. Among all compounds, **5a** and **5d** showed similar bioactivity, which have the potential for the development of FLT3 inhibitors.

To the best of our knowledge, MOLM-13 and MV4-11 cells are FLT3-ITD-expressing human leukemia cell lines. Here, compounds **5a**, **5d** and **3n** showed good anti-proliferative activity against MV4-11 and MOLM-13. To gain insight into the binding mode of **5a**, **5d** and **3n** in the kinase domain of FLT3, a molecular docking study was carried out using the X-ray structure of quizartinib in complex with FLT3-ITD kinase (PDB ID: 4XUF). Fig. 3 shows the overlying docking results of quizartinib, **5a**, **5d** and **3n**. Obviously, quizartinib perfectly occupies the binding pocket of ATP adenosine and the region of

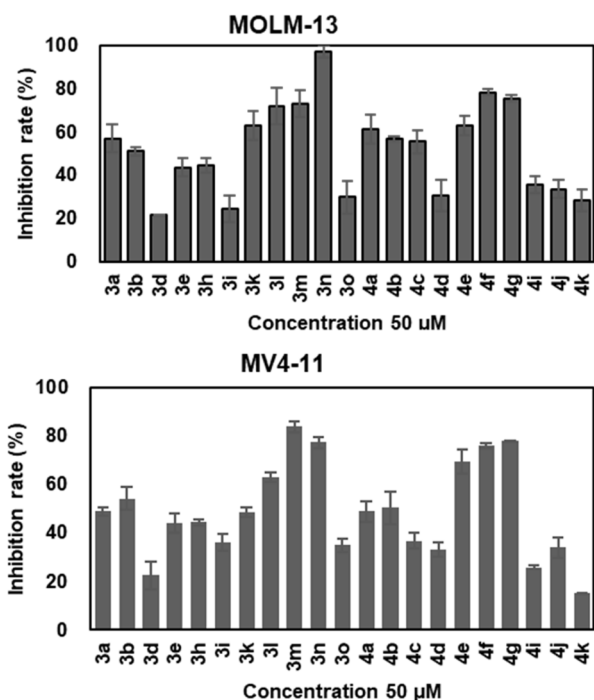


Fig. 2 Inhibition rate of compounds **3a–3o** and **4a–4k** for MOLM-13 and MV4-11 cells.

Table 4 Bio-evaluation of compounds **3a–3o** and **4a–4k**

Compound	$IC_{50}$ ( $\mu$ M)	
	MOLM-13	MV4-11
<b>3a</b>	$28.99 \pm 7.07$	$44.17 \pm 1.34$
<b>3b</b>	$34.80 \pm 3.47$	$32.05 \pm 3.97$
<b>3d</b>	$84.35 \pm 8.19$	$92.52 \pm 6.20$
<b>3e</b>	$24.44 \pm 9.96$	$32.28 \pm 5.21$
<b>3h</b>	$30.29 \pm 1.98$	$87.34 \pm 1.81$
<b>3i</b>	$31.74 \pm 3.13$	$87.75 \pm 0.62$
<b>3l</b>	$13.24 \pm 0.41$	$10.63 \pm 0.44$
<b>3m</b>	$19.79 \pm 1.57$	$19.91 \pm 1.68$
<b>3n</b>	$8.53 \pm 0.34$	$9.73 \pm 0.17$
<b>4a</b>	$86.57 \pm 5.43$	$43.82 \pm 1.48$
<b>4b</b>	$42.41 \pm 4.38$	$30.80 \pm 1.15$
<b>4c</b>	$10.64 \pm 0.40$	$41.79 \pm 8.01$
<b>4e</b>	$17.95 \pm 0.86$	$8.19 \pm 1.87$
<b>4f</b>	$19.94 \pm 1.06$	$18.69 \pm 2.38$
<b>4g</b>	$10.43 \pm 0.37$	$13.54 \pm 3.65$
<b>4i</b>	$44.19 \pm 3.97$	$72.58 \pm 9.05$
<b>4j</b>	$47.07 \pm 5.95$	$84.39 \pm 6.86$
Cabozantinib	0.045	0.003

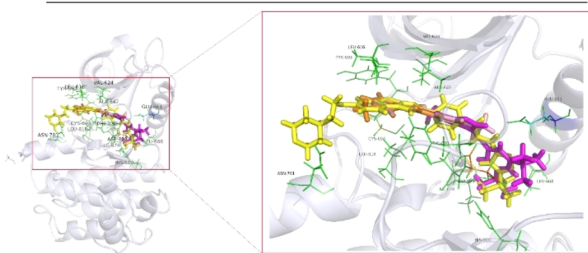




**Table 5** Structure and bio-evaluation scope of imidazo[1,2-*a*]pyridines 5

<b>5a</b> , yield: 30% MV4-11: IC <sub>50</sub> = 10.637±0.93 μM MOLM-13: IC <sub>50</sub> = 10.179±0.27 μM	<b>5b</b> , yield: 30% MV4-11: IC <sub>50</sub> = 11.39±1.75 μM MOLM-13: IC <sub>50</sub> = 41.685±7.62 μM	<b>5c</b> , yield: 22% MV4-11: IC <sub>50</sub> = 12.63±0.42 μM MOLM-13: IC <sub>50</sub> = 14.29±2.05 μM
<b>5d</b> , yield: 28% MV4-11: IC <sub>50</sub> = 11.413±0.81 μM MOLM-13: IC <sub>50</sub> = 8.743±1.77 μM	<b>5e</b> , yield: 31% MV4-11: IC <sub>50</sub> = 38.970±0.36 μM MOLM-13: IC <sub>50</sub> = 37.643±2.61 μM	<b>Cabozantinib</b> MV4-11: IC <sub>50</sub> = 0.051 μM MOLM-13: IC <sub>50</sub> = 0.006 μM

Compounds	Quizartinib	5a	5d	3n
E <sub>score</sub> (kcal/mol)	-10.86	-5.62	-6.59	-6.32

**Fig. 3** Overlying docking results of quizartinib, 5a, 5d and 3n.

phosphate in the catalytic loop, giving  $-10.86$  kcal mol<sup>-1</sup> of the binding energy, which is consistent with the type II FLT3 inhibitor. However, when **5a**, **5d** and **3n** were docked with the FLT3 crystal, the hydrophobic pocket was just partly occupied, giving low binding energies of  $-5.62$ ,  $-6.59$ , and  $-6.32$  kcal mol<sup>-1</sup>, respectively.

Moreover, the independent binding modes of quizartinib, **5a**, **5d** and **3n** were shown in Fig. S1 (ESI<sup>†</sup>). The benzo[*d*]imidazo[2,1-*b*]thiazole scaffold and urea of quizartinib formed three key hydrogen bonds with Cys694, Glu661 and Asp829 in the hinge region of FLT3. The 2-morpholinoethoxy moiety of quizartinib extended out of the binding site, and was thus exposed to the solvent. This binding mode manifests the stable interaction between quizartinib and FLT3 kinase (Fig. S1a<sup>†</sup>). However, for compound **5a**, only the back pocket composed of the Glu661, Leu668, Cys828, and Asp829 residues was occupied. Although the imidazo[1,2-*a*]pyridine scaffold of **5a** formed one hydrogen bond with Glu661 and two pi-H interactions with Cys828 (Fig. S1b<sup>†</sup>), the binding pocket of ATP adenosine in FLT3 was still exposed, which might weaken the binding interaction. In contrast, compounds **5d** and **3n** could better occupy the binding pocket of ATP adenosine in FLT3, forming one key hydrogen bond with Cys694 through the N atom receptor of the imidazo

[1,2-*a*]pyridine scaffold (Fig. S1c and d<sup>†</sup>), which improved the binding stability. Together, these docking analysis results may further help us in conducting the structural modification, and in developing novel, potent and selective FLT3 inhibitors.

## 3. Experimental section

### 3.1 General information

All commercially available reagent grade chemicals were purchased from Acros and Alfa Aesar Chemical Company, and used as received without further purification unless otherwise stated. All of the <sup>1</sup>H, and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> on a Bruker advance 400 MHz NMR. The high-resolution mass spectra (HRMS) were obtained on a Waters UPLC-Xevo TQMS (PDA Detector)/Quattro Premier XE triquadrupole mass spectrometer by ESI<sup>†</sup> method. Flash column chromatography was conducted on silica gel (200–300 mesh).

MOLM13 cells were purchased from Wuhan Pricella Biotechnology Co., Ltd, and MV4-11 cells were provided by Suzhou Haixing Biosciences Co., Ltd. The cell lines were maintained following ATCC recommendation in Roswell Park Memorial Institute (RPMI) 1640, Dulbecco's Modified Eagle's Medium (DMEM) or Minimum Essential Medium (MEM) with 10% fetal bovine serum (FBS) (Sigma-Aldrich, Castle Hill, NSW, Australia). All cell lines were cultured at 37 °C in a humidified incubator in the presence of 5% CO<sub>2</sub> atmosphere.

### 3.2 General procedure for the synthesis of compounds 3, 4 and 5

A solution of propargyl alcohols (0.5 mmol), 2-aminopyridine (1.5 mmol), TBHP (1.5 mmol) and NaIO<sub>4</sub> (40 mol%) in chlorobenzene (4.0 mL) was stirred at 120 °C for 12 h. After the reaction was finished, the mixture was concentrated under vacuum to remove any residual solvent and the residue was purified by flash column chromatography using petroleum ether/ethyl acetate (3/1, V/V) as an eluent to afford the desired compound.

### 3.3 CCK8 assay

The cytotoxic effect of the imidazo[1,2-*a*]pyridine derivatives was determined by CCK8 assay. Cell lines (MV4-11, MOLM-13) were inoculated in 96-well plates at a density of  $3 \times 10^4$  cells per well. After incubation for 24 h, cells were incubated with different concentrations of the compounds for 24 h. Then, 10 μL CCK8 was added to each well, the wells were further incubated for 4 h, and the luminescence was measured in a multilabel reader (Envision2014, PerkinElmer, USA). Data were normalized to control groups (DMSO) and represented by the mean of three independent measurements with a standard error of <20%. IC<sub>50</sub> values were calculated using Prism 5.0 (GraphPad Software, San Diego, CA).

### 3.4 Molecular docking

The crystal structure of FLT3 (PDB ID: 4XUF) was retrieved from the Protein Data Bank (PDB). Initially, the PDB file was prepared by removing crystallographic water molecules and the bound ligand, followed by assigning partial charges. The 3D structure



of the ligand was generated and saved as a pdbqt file using AutoDock software. Once the protein and ligand structures were prepared, molecular docking was conducted with AutoDock Vina. Docking simulations were carried out by placing the ligands into the active site of FLT3, with the grid box set to  $20 \times 20 \times 20 \text{ \AA}^3$ , centered at the original ligand's binding site. Binding affinity was evaluated based on the docking scores, where a more negative binding energy suggested stronger interactions between the ligand and the FLT3 receptor. For detailed interaction analysis, Discovery Studio Visualizer was employed, allowing visualization of the molecular interactions between the ligands and protein.

## 4. Conclusions

In summary, a novel and simple  $\text{NaIO}_4$ /TBHP-promoted (3 + 2) cycloaddition from propargyl alcohols and 2-aminopyridines was developed for the synthesis of imidazo[1,2-*a*]pyridines. This practical reaction has a wide range of substrate scope for both propargyl alcohols and 2-aminopyridines to give various C3-carbonylated imidazopyridines in moderate yields, and showed high functional group tolerance. More importantly, these synthesized compounds exhibited antiproliferation for MOLM-13 and MV4-11 cells, and **3n**, **5a** and **5d** possessed similar bioactivity. Molecular docking analysis indicated the strong interaction between **5a**, **5d** and FLT3 kinase, which have potential for the development of FLT3 inhibitors.

## Data availability

The data supporting the characterization of this study are included in the ESI file.†

## Author contributions

Huiping Luo and Zhengyu Hu drafted the experimental section of the manuscript and conducted the synthesis of compounds **5** and CCK8 assay in this research. Jihai Shi performed the investigation of the substrates. Yongxin Lou and Zhonghua Shi contributed to the bioanalysis. Xin Jin and Jia Chen provided the methodology and formal analysis. Xing Liu and Qiang Huang designed the study and revised the manuscript. Qiang Huang contributed to the supervision, project administration, funding acquisition and review & editing. All authors agreed to the final version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

All authors are deeply grateful for financial support from the Guizhou Provincial Natural Science Foundation (QKHJC-ZK [2022]592), National Natural Science Foundation of China (82360679), China National University Student Innovation & Entrepreneurship Development Program (2024106610959), and

the Health and Wellness Special Program of the 2024 Scientific Research and Talent Development Fund Project at Kweichow Moutai Hospital.

## Notes and references

- 1 D. K. Soumyashree, R. S. Keri, D. S. Reddy, M. S. Kumari, L. Naik, A. Kumar, N. Kadam, P. N. Patil, H. Shanavaz and B. Padmashali, Design, synthesis, single-crystal X-ray and docking studies of imidazopyridine analogues as potent anti-TB agents, *J. Mol. Struct.*, 2024, **1295**, 136540.
- 2 R. Hussain, H. Ullah, S. Khan, Y. Khan, T. Iqbal, R. Iqbal, H. S. Almoallim and M. J. Ansari, A promising acetylcholinesterase and butyrylcholinesterase inhibitors: *In vitro* enzymatic and *in silico* molecular docking studies of benzothiazole-based oxadiazole containing imidazopyridine hybrid derivatives, *Results Chem.*, 2024, **7**, 101503.
- 3 R. Hussain, W. Rehman, F. Rahim, S. Khan, M. Taha, Y. Khan, A. Sardar, I. Khan and S. A. A. Shah, Discovery of imidazopyridine derived oxadiazole-based thiourea derivatives as potential anti-diabetic agents: Synthesis, *in vitro* antioxidant screening and *in silico* molecular modeling approaches, *J. Mol. Struct.*, 2023, **1293**, 136185.
- 4 N. P. Mishra, S. Mohapatra, T. Das and S. Nayak, Imidazo [1,2-*a*]pyridine as a promising scaffold for the development of antibacterial agents, *J. Heterocycl. Chem.*, 2022, **59**, 2051–2075.
- 5 D. Vanda, P. Zajdel and M. Soural, Imidazopyridine-based selective and multifunctional ligands of biological targets associated with psychiatric and neurodegenerative diseases, *Eur. J. Med. Chem.*, 2019, **181**, 111569.
- 6 L. Dymińska, Imidazopyridines as a source of biological activity and their pharmacological potentials-infrared and Raman spectroscopic evidence of their content in pharmaceuticals and plant materials, *Bioorg. Med. Chem.*, 2015, **23**, 6087–6099.
- 7 G. Volpi, E. Laurenti and R. Rabezzana, Imidazopyridine Family: Versatile and Promising Heterocyclic Skeletons for Different Applications, *Molecules*, 2024, **29**, 2668.
- 8 P. A. Chaudhran and A. Sharma, Progress in the Development of Imidazopyridine-Based Fluorescent Probes for Diverse Applications, *Crit. Rev. Anal. Chem.*, 2022, **54**, 2148–2165.
- 9 B. K. R. Sanapalli, A. Ashames, D. K. Sigalapalli, A. B. Shaik, R. R. Bhandare and V. Yele, Synthetic Imidazopyridine-Based Derivatives as Potential Inhibitors against Multi-Drug Resistant Bacterial Infections: A Review, *Antibiotics*, 2022, **11**, 1680.
- 10 R. Esfandiari, P. Moghimi-Rad, M. H. Bule, E. Sour, H. Nadri, M. Mahdavi, R. Ghobadian and M. Amini, New Imidazo[1,2-*a*]Pyridin-2-yl Derivatives as AChE, BChE, and LOX Inhibitors; Design, Synthesis, and Biological Evaluation, *Lett. Drug Des. Discov.*, 2023, **20**, 1784–1798.
- 11 F. Peytam, Z. Emamgholipour, A. Mousavi, M. Moradi, R. Foroumadi, L. Firoozpour, F. Divsalar, M. Safavi and A. Foroumadi, Imidazopyridine-based kinase inhibitors as



- potential anticancer agents: A review, *Bioorg. Chem.*, 2023, **140**, 106831.
- 12 M. Pan, V. Solozobova, N. C. Kuznik, N. Jung, S. Gräßle, V. Gourain, Y. M. Heneka, C. A. Cramervon Clausbruch, O. Fuhr, R. S. N. Munuganti, *et al.*, Identification of an Imidazopyridine-Based Compound as an Oral Selective Estrogen Receptor Degradar for Breast Cancer Therapy, *Cancer Res. Commun.*, 2023, **3**, 1378–1396.
  - 13 B. O. Sucu, Biological Evaluation of Imidazopyridine Derivatives as Potential Anticancer Agents against Breast Cancer Cells, *Med. Chem. Res.*, 2022, **31**, 2231–2242.
  - 14 J. Wang, H. Wu, G. Song, D. Yang, J. Huang, X. Yao, H. Qin, Z. Chen, Z. Xu and C. Xu, A Novel Imidazopyridine Derivative Exerts Anticancer Activity by Inducing Mitochondrial Pathway-Mediated Apoptosis, *BioMed Res. Int.*, 2020, **2020**, e4929053.
  - 15 D. K. Soumyashree, D. S. Reddy, H. Nagarajaiah, L. Naik, H. M. Savanur, C. D. Shilpa, M. Sunitha Kumari, H. Shanavaz and B. Padmashali, Imidazopyridine Chalcones as Potent Anticancer Agents: Synthesis, Single-Crystal X-ray, Docking, DFT and SAR Studies, *Arch. Pharm.*, 2023, **356**, 2300106.
  - 16 F. A. Sofi, K. Gogde, D. Mukherjee and M. H. Masoodi, Sustainable approaches towards the synthesis of functionalized imidazo[1,2-*a*]pyridines: Recent advancements, *J. Mol. Struct.*, 2024, **1297**, 137012.
  - 17 S. Kitaoka, Y. Kitagawa, R. Nozoe and K. Nobuoka, Syntheses and properties of imidazopyridine-based ionic liquids, *J. Ionic Liq.*, 2024, **4**, 100100.
  - 18 A. B. Gadekar, Sonam, V. N. Shinde, Bhawani, K. Rangan and A. Kumar, Visible light-driven difluoroalkoxylation of imidazopyridines using N-fluorobenzenesulfonimide as fluorinating agent, *Adv. Synth. Catal.*, 2024, **366**, 4794–4800.
  - 19 Z. Y. Feng, Y. B. Fan, C. C. Qiang, P. Liu and P. P. Sun, Electrochemical C3-methylthiolation of imidazopyridines with dimethyl sulfoxide, *Green Chem.*, 2024, **26**, 3517–3521.
  - 20 Z. C. Tang, G. Hong, J. Chen, T. Huang, Z. C. Zhou and L. M. Wang, Electrochemical-induced solvent-tuned selective transfer hydrogenation of imidazopyridines with carbazates as hydrogen donors, *Green Chem.*, 2023, **25**, 9705–9710.
  - 21 H. K. Indurthi, S. Das, P. Saha and D. K. Sharma, K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>-Mediated C-3 Formylation of imidazopyridines using glyoxylic acid, *Eur. J. Org. Chem.*, 2023, **26**, e202300829.
  - 22 M. E. Firuz, S. Rajai-Daryasarei, F. Rominger, A. Biglari and S. Balalaie, Mn-mediated direct regioselective C-H trifluoromethylation of imidazopyridines and quinoxalines, *J. Org. Chem.*, 2023, **88**, 10599–10608.
  - 23 L. L. Shi, T. T. Li and G. J. Mei, Recent advances in transition-metal-free C-H functionalization of imidazo[1,2-*a*]pyridines, *Green Synth. Catal.*, 2022, **3**, 227–242.
  - 24 N. R. Vanam and J. S. Anireddy, Synthesis and biological evaluation of tetrazole fused imidazopyridine derivatives as anticancer agents, *Chem. Data Collect.*, 2023, **48**, 101092.
  - 25 P. Pragyaandipta, R. K. Pedapati, P. K. Reddy, A. Nayek, R. K. Meher, S. K. Guru, S. Kantevari and P. K. Naik, Rational design of novel microtubule targeting anticancer drugs N-imidazopyridine noscapinoids: chemical synthesis and experimental evaluation based on *in vitro* using breast cancer cells and *in vivo* using xenograft mice model, *Chem.-Biol. Interact.*, 2023, **382**, 110606.
  - 26 R. Boddiboyena, G. N. Reddy, N. V. Seelam, M. Sarma, D. Kolli, M. Rajeswari and M. R. Gudisela, Synthesis and biological evaluation of novel amide derivatives of 1,2,4-oxadiazole-imidazopyridines as anticancer agents, *Chem. Data Collect.*, 2023, **46**, 101036.
  - 27 Z. Q. Liu, Q. Zhang, Y. L. Liu, X. Q. Yu, R. H. Chui, L. L. Zhang, B. Zhao and L. Y. Ma, Recent contributions of pyridazine as a privileged scaffold of anticancer agents in medicinal chemistry: an updated review, *Bioorg. Med. Chem.*, 2024, **111**, 117847.
  - 28 K. Armendariz-Barrientos, L. A. Pérez, S. Lagunas-Rivera, Y. Alcaraz-Contreras, M. A. García-Revilla, H. Prado-Garcia, R. García-Becerra and M. A. Vazquez, Novel N-quaternary coumarin-3-yl-imidazo[1,2-*a*]pyridines as fluorescent hybrids: Their synthesis and biological evaluation in cancer cells, *Results Chem.*, 2025, **13**, 101959.
  - 29 M. H. Yang, B. Basappa, S. N. Deveshegowda, A. Ravish, A. Mohan, O. Nagaraja, M. Madegowda, K. S. Rangappa, A. Deivasigamani, V. Pandey, P. E. Lobie, K. M. Hui, G. Sethi and K. S. Ahn, A novel drug prejudice scaffold-imidazopyridine-conjugate can promote cell death in a colorectal cancer model by binding to  $\beta$ -catenin and suppressing the Wnt signaling pathway, *J. Adv. Res.*, 2024, DOI: [10.1016/j.jare.2024.07.022](https://doi.org/10.1016/j.jare.2024.07.022).
  - 30 W. Daoudi, M. Azzouzi, M. Aaddouz, N. D. A. O. Hajedris, M. Abdalla, A. J. Obaidullah, K. K. Yadav and A. E. Aatiaoui, Synthesis, characterization, DFT, ADMET, MD analysis and molecular docking of C-3 functionalized imidazo[1,2-*a*]pyridine motifs, *J. Mol. Struct.*, 2024, **1312**, 138658.
  - 31 S. A. Babu, P. V. Varsha, S. Poulse, S. Varughese and J. John, Copper-catalyzed annulation of electrophilic benzannulated heterocycles with 2-aminopyridine and 2-aminoquinoline: direct access toward polyring-fused imidazo[1,2-*a*]pyridines, *J. Org. Chem.*, 2023, **88**, 10027–10039.
  - 32 V. H. Luu, H. V. M. Trinh, D. H. Tran, T. Q. Le and T. T. Nguyen, Cyclization of sulfoxonium ylides with 2-aminopyridines towards imidazo[1,2-*a*]pyridines, *Tetrahedron Lett.*, 2025, **156**, 155438.
  - 33 P. Kushwaha, Rashi, A. Bhardwaj and D. Khan, Synthetic approaches toward imidazo-fused heterocycles: A comprehensive review, *J. Heterocycl. Chem.*, 2024, **61**, 1807–1869.
  - 34 A. Kumar Bagdi, S. Santra, K. Monir and A. Hajra, Synthesis of Imidazo[1,2-*a*]Pyridines: A Decade Update, *Chem. Commun.*, 2015, **51**, 1555–1575.
  - 35 S. K. Samanta and M. K. Bera, Iodine mediated oxidative cross coupling of 2-aminopyridine and aromatic terminal alkyne: a practical route to imidazo[1,2-*a*]pyridine derivatives, *Org. Biomol. Chem.*, 2019, **17**, 6441–6449.
  - 36 Y. Liu, Y. X. Zhang and J. W. Sun, Copper-promoted annulation of terminal alkynes with 2-aminopyridines to



- assemble 2-halogenated imidazo[1,2-*a*]pyridines, *J. Heterocycl. Chem.*, 2019, **56**, 2804–2810.
- 37 J. Tan, P. H. Ni, H. W. Huang and G. J. Deng, Metal- and base-free synthesis of imidazo[1,2-*a*]pyridines through elemental sulfur-initiated oxidative annulation of 2-aminopyridines and aldehydes, *Org. Biomol. Chem.*, 2018, **16**, 4227–4230.
  - 38 Z. T. Zhou, D. Y. Luo, G. R. Li, Z. T. Yang, L. Cui and W. G. Yang, Copper-catalyzed three-component reaction to synthesize polysubstituted imidazo[1,2-*a*]pyridines, *RSC Adv.*, 2022, **12**, 20199–20205.
  - 39 P. Szuroczi, L. B. Jenei, V. Sandor, A. Benyei and L. Kollar, Synthesis and functionalization of 2-iodoimidazo[1,2-*a*]pyridines in palladium-catalysed amino-, aryloxy- and alkoxy-carboxylations, *Tetrahedron*, 2025, **174**, 134489.
  - 40 W. Q. Zhang, L. H. Shen and J. Zhang, Iodine mediated annulation of triethylamine, aldehydes and 2-aminopyridines for the Synthesis of 3-formyl-imidazo [1,2-*a*]pyridine derivatives, *Adv. Synth. Catal.*, 2024, **366**, 3796–3801.
  - 41 M. B. Yadav and T. J. Yeon, One-pot sequential regioselective intramolecular synthesis of furo[3,2-*c*]chromen and imidazo [1,2-*a*]pyridine *via* catalyst-free and basic conditions, *J. Heterocycl. Chem.*, 2023, **60**, 2053–2062.
  - 42 L. A. Martinho and C. K. Z. Andrade, HPW-Catalyzed environmentally benign approach to imidazo[1,2-*a*]pyridines, *Beilstein J. Org. Chem.*, 2024, **20**, 628–637.
  - 43 P. Kaur, K. K. Gurjar, T. Arora, D. Bharti, M. Kaur, V. Kumar, J. Parkash and R. Kumar, Efficient synthesis and mechanistic insights for the formation of imidazo[1,2-*a*]pyridines *via* multicomponent decarboxylative coupling using chitosan-supported copper catalysts, *Mol. Catal.*, 2023, **550**, 113582.
  - 44 R. Krishnamoorthy and P. Anaikutti, Iodine catalyzed synthesis of imidazo[1,2-*a*]pyrazine and imidazo[1,2-*a*]pyridine derivatives and their anticancer activity, *RSC Adv.*, 2023, **13**, 36439–36454.
  - 45 Q. Huang, L. J. Wu, J. H. Shi, J. D. Li, W. Lu, F. S. Tang, L. Zhu, W. W. Zhong and C. K. Zhao, I<sub>2</sub>/DTBP Promoted synthesis of C3-carbonylated imidazopyridines from chromones and 2-aminopyridines *via* (3+2) cycloaddition, *Synthesis*, 2023, **55**, 2570–2580.
  - 46 Q. Huang, L. Zhu, D. Yi, X. H. Zhao and W. Wei, Silver-mediated aminophosphinylation of propargyl alcohols with aromatic amines and H-phosphine oxides leading to  $\alpha$ -aminophosphine oxides, *Chin. Chem. Lett.*, 2020, **31**, 373–376.
  - 47 J. L. Kang, Y. F. Liu, R. H. Cui, J. M. Shi, J. Zhang, H. B. Wang and Q. Huang, Silver(I)-catalyzed and DBU-promoted isomerization/addition of propargyl alcohols to amines to access  $\beta$ -aminoketones, *Tetrahedron Lett.*, 2024, **145**, 155163.
  - 48 H. Wang, L. H. Xu, X. Liu, Y. Shi, Z. Yao, Y. Zhou and Q. Huang, NaIO<sub>4</sub>/air-initiated phosphorylation of alcohols with H-phosphine oxides for the construction of P(O)–O bonds in water, *Org. Biomol. Chem.*, 2024, **22**, 7518.
  - 49 C. Hamdouchi, B. Zhong, J. Mendoza, E. Collins, C. Jaramillo, J. E. De Diego, D. Robertson, C. D. Spencer, B. D. Anderson, S. A. Watkins, F. Zhang and H. B. Brooks, Structure-based design of a new class of highly selective aminoimidazo[1,2-*a*]pyridine-based inhibitors of cyclin dependent kinases, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1943–1947.
  - 50 M. K. Elgohary, M. S. Elkotamy, S. T. Al-Rashood, F. A. Binjubair, R. S. Alarifi, H. A. Ghabbour, W. M. Eldehna and H. A. Abdel-Aziz, Exploring antitumor activity of novel imidazo[2,1-*b*]thiazole and imidazo[1,2-*a*]pyridine derivatives on MDA-MB-231 cell line: Targeting VEGFR-2 enzyme with computational insight, *J. Mol. Struct.*, 2025, **1322**, 140579.
  - 51 Q. Song, Q. R. Zhang, X. J. Fan, F. Kayaat, R. Lv, J. Li and Y. Wang, The discovery of novel imidazo[1,2-*a*]pyridine derivatives as covalent anticancer agents, *Org. Biomol. Chem.*, 2024, **22**, 5374–5384.
  - 52 L. T. Zhang, N. R. Lakkaniga, J. B. Bharate, N. McConnell, X. Q. Wang, A. Kharbanda, Y.-K. Leung, B. Frett, N. P. Shah and H.-Y. Li, Discovery of imidazo[1,2-*a*]pyridine-thiophene derivatives as FLT3 and FLT3 mutants inhibitors for acute myeloid leukaemia through structure-based optimization of an NEK2 inhibitor, *Eur. J. Med. Chem.*, 2021, **225**, 113776.
  - 53 B. Frett, N. McConnell, C. C. Smith, Y. X. Wang, N. P. Shah and H.-Y. Li, Computer aided drug discovery of highly ligand efficient, low molecular weight imidazopyridine analogues as FLT3 inhibitors, *Eur. J. Med. Chem.*, 2015, **94**, 123–131.
  - 54 M. S. Elkotamy, M. K. Elgohary, S. T. Al-Rashood, H. Almahli, W. M. Eldehna and H. A. Abdel-Aziz, Novel imidazo[2,1-*b*]thiazoles and imidazo[1,2-*a*]pyridines tethered with indolinone motif as VEGFR-2 inhibitors and apoptotic inducers: Design, synthesis and biological evaluations, *Bioorg. Chem.*, 2024, **151**, 107644.

