



HAL
open science

From Synthetic Simplified Marine Metabolite Analogues to New Selective Allosteric Inhibitor of Aurora B Kinase

Charlotte Juillet, Ludmila Ermolenko, Dina Boyarskaya, Blandine Baratte, Beátrice Josselin, Hristo Nedev, Stéphane Bach, Bogdan Iorga, Jérôme Bignon, Sandrine Ruchaud, et al.

► To cite this version:

Charlotte Juillet, Ludmila Ermolenko, Dina Boyarskaya, Blandine Baratte, Beátrice Josselin, et al.. From Synthetic Simplified Marine Metabolite Analogues to New Selective Allosteric Inhibitor of Aurora B Kinase. *Journal of Medicinal Chemistry*, 2021, 64 (2), pp.1197-1219. 10.1021/acs.jmedchem.0c02064 . hal-03154739

HAL Id: hal-03154739

<https://hal.science/hal-03154739v1>

Submitted on 8 Mar 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

From Synthetic Simplified Marine Metabolite Analogs to New Selective Allosteric Inhibitor of Aurora B Kinase

Charlotte Juillet,[†] Ludmila Ermolenko,[†] Dina Boyarskaya,[†] Blandine Baratte,[‡] Béatrice Josselin,[‡] Hristo Nedev,[†] Stéphane Bach,^{*,‡} Bogdan I. Iorga,[†] Jérôme Bignon,[†] Sandrine Ruchaud^{*,‡} and Ali Al-Mourabit^{†*}

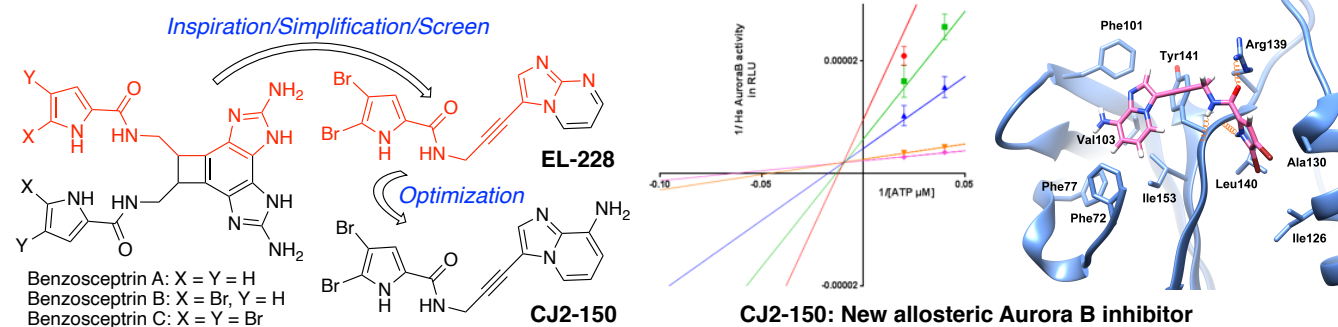
[†]Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, 91190, France

[‡]Sorbonne Université, CNRS, FR 2424, Plateforme de criblage KISSf, Station Biologique de Roscoff, Roscoff, 29680, France

^{*}Sorbonne Université, CNRS, UMR 8227, Integrative Biology of Marine Models Laboratory (LBI2M), Station Biologique de Roscoff, Roscoff, 29680, France

KEYWORDS: kinase, allosteric inhibitor, allosteric inhibitor, Aurora B, pyrrole-imidazole, mitosis, cancer

ABSTRACT:



ABSTRACT: Significant inhibition of Aurora B was achieved by the synthesis of simplified fragments of benzosceptrins and oroidin belonging to the marine pyrrole-2-aminoimidazoles metabolites isolated from sponges. Evaluation of kinase inhibition enabled the discovery of a synthetically accessible rigid acetylenic structural analog EL-228 (1), whose structure could be optimized into the potent CJ2-150 (37). Here we present the synthesis of new inhibitors of Aurora B kinase which is an important target for cancer therapy through mitosis regulation. The biologically oriented synthesis yielded several nanomolar inhibitors. The optimized compound CJ2-150 (37) showed a non-ATP competitive allosteric mode of action in a mixed-type inhibition for Aurora B kinase. Molecular docking identified a probable binding mode in the allosteric site "F" and highlighted the key interactions with the protein. We describe the improvement of the inhibitory potency and specificity of the novel scaffold as well as the characterization of the mechanism of action.

■ INTRODUCTION

Aurora kinases A, B and C are serine-threonine protein kinases, expressed in cells during mitosis that play an essential role via mitosis regulation.¹ Aurora B is a member of the chromosomal passenger complex (CPC) together with INCENP, Borealin and Survivin. It is localized at the centromere in early mitosis, and then during the anaphase, Aurora B relocates to the microtubules at the central spindle.^{2,3} Aurora B seems to be involved in chromatin compaction and condensation via the phosphorylation of histone H₃ at serine 10.^{4,5} It promotes the proper chromosomes alignment and segregation. Aurora B is a key component of the error correction machinery and is responsible for the selective destabilization of incorrect attachments between microtubules and kinetochores.^{6,7}

Aurora B inhibition results in a weakened spindle-checkpoint⁷ with chromosomes prone to mis-segregation and cytokinesis failure. As Aurora B is involved in the cleavage furrow ingression and abscission,^{8,9,10} its inhibition creates polyploidization and drags the cell into aberrant mitosis leading to its death by apoptosis. Aurora kinases are up-regulated in various tumor cells and are responsible for genomic instability associated with tumorigenesis.^{11,12,13} Their overexpression is highly correlated with poor diagnosis of cancers.

As Aurora kinases are all implicated during cell division at different stages, this family of kinases has sparked great interest over the last two decades as new targets in cancer therapy.¹⁴ A few representatives Aurora B inhibitors are shown in Figure 1. To date no small-molecule inhibitor of Aurora B has been approved by the FDA; nevertheless, numerous compounds are described in the literature^{15,16} or undergoing clinical trials at different stages.^{17,18,19} So far, all of these are ATP-competitive though this mode of binding might suffer from off-target effects due to the high degree of conservation among kinases, leading to a potential lack of specificity.

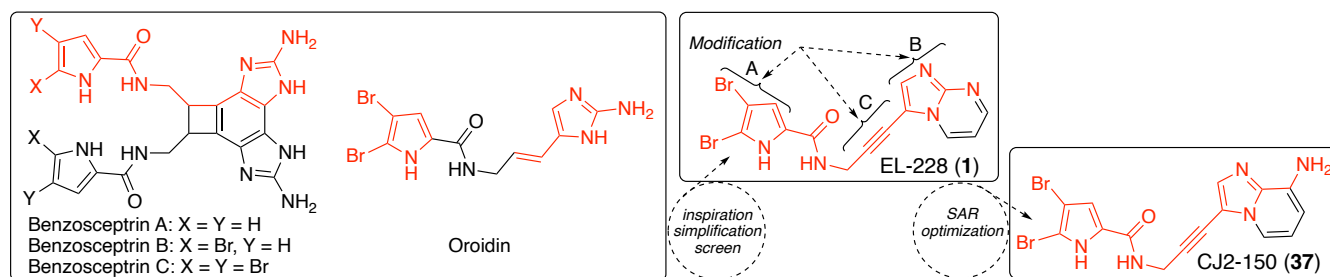


Figure 2: Fragment reasoning containing the pyrrole and the 2-aminoimidazole of the marine benzosceptrins

In addition to the micromolar inhibition of the CK1 kinase by the natural product benzosceptrin B ($IC_{50} = 0.68 \mu M$), the pyrrole-2-aminoimidazole metabolites are known to have affinity for other kinases. As part of the total synthesis of benzosceptrins A-C, various simplified intermediates were prepared and screened on a panel of kinases. From this screening, a moderately potent but relatively selective inhibitor of Aurora B kinase, EL-228 (1) ($IC_{50} = 5 \mu M$), was highlighted (Figure 2). The structure, linking a dibromopyrrole with an imidazo[1,2-a]pyrimidine heterocycle via an alkyne offered the possibility of exploring a new chemical space. These initial three structural elements A, B and C (Figure 2) were then modified consecutively in order to improve activity and explore structure-activity relationships. Among the new analogs synthesized, the most active was shown to be compound Cj2-150

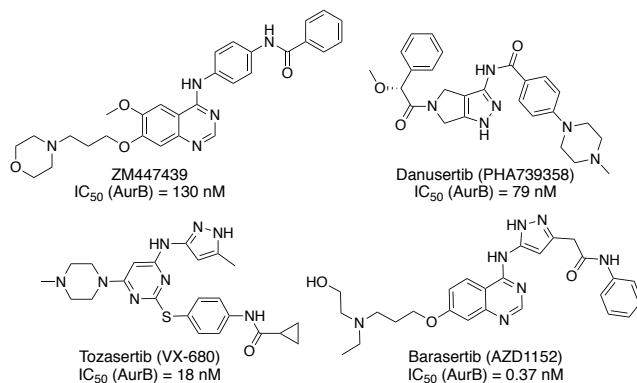


Figure 1: Examples of known ATP-competitive Aurora B inhibitors.

Consequently, instead of designing molecules targeting the ATP binding pocket for competitive inhibition, we decided to explore a different chemical space as a possible route to novel inhibitory mechanisms. For this purpose, simplification of the complex structures of bioactive natural products is often advantageous and even necessary for their valorization in drug discovery.²⁰ In this context, we considered synthetic fragments inspired by the rigid benzosceptrins A, B and C^{21,22,23} isolated by our group (Figure 2). The idea for the design of new inhibitors was to extract privileged active structural motifs of the marine benzosceptrins and oroidin and further optimize their activity.^{24,25,26}

In the present study, we describe pharmacomodulation of the hit EL-228 (1) (Figure 2), leading to the identification of a new active class of compounds similar to the oroidin scaffold, the monomer of dimeric benzosceptrin metabolite. Importantly, we have shown that these compounds are non-ATP competitive inhibitors. To the best of our knowledge, this is the first example of such a binding mode for Aurora B inhibitors (Figure 2).

(37). A study of the latter's mode of action then revealed it to be a non-ATP competitive inhibitor of Aurora B kinase, the first of its kind.

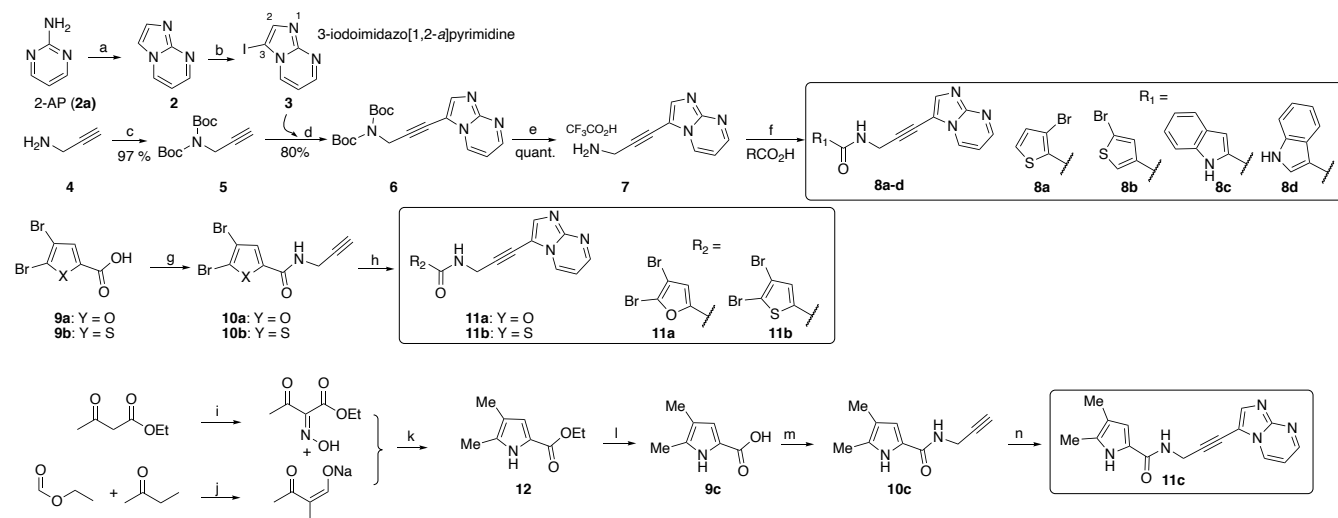
■ RESULTS AND DISCUSSION

Chemistry. *Modifications of the pyrrole moiety part A.* Our synthetic strategy began with the synthesis of the Boc propargylamine **5** from **4**²⁷ and the iodoimidazopyrimidine **3** from 2-AP **2a**.²⁸ Sonogashira coupling of the aryl halide **3** with the alkyne **5** led to the platform **6** that was deprotected to the trifluoroacetic acid salt **7**.²⁹ The last step was an EDCI-mediated acylation with various carboxylic acids to give four analogs **8a-d** (Scheme 1). The pyrrole part was replaced by the dibrominated five-membered rings furan and thiophen to produce **11a-b** from

the corresponding carboxylic acids **9a** and **9b** through the acetylenic derivatives **10a** and **10b**. Finally, another efficient synthetic route allowed us to synthesize the pyrrole bearing two methyl

groups instead of the bromines. The key pyrrole **12** was prepared according to the literature from ethylacetoacetate, ethyl formate and 2-butanone respectively (see Supporting Information).^{30, 31, 32} After hydrolysis of the ester **12** to **9c**, a Sonogashira cross-coupling with propargylamine and **10c** gave the compound **11c**.

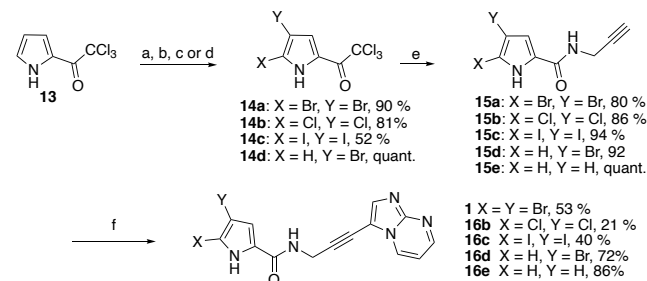
Scheme 1. General synthesis of the analogs with modified pyrrole part A



Reagents and conditions: (a) Chloroacetaldehyde, EtOH reflux, quant.; (b) NIS, ACN, 32%; (c) Boc₂O, DMAP, ACN, rt, 16 h, 97%; (d) Pd(PPh₃)₂Cl₂ 10%, CuI 10%, NEt₃, 81%; (e) TFA, DCM, 80%; (f) EDC.HCl, HOBT, DIEA, rt, DCM, 17-56%; (g) Propargylamine, T₃P, DIEA, EtOAc; (h) **3**, Pd(PPh₃)₂Cl₂, CuI, Et₃N, DMF, 50 °C, 40-48%; (i) NaNO₂, AcOH, H₂O, 81%; (j) NaOMe 5.4M in MeOH, THF dry, 60%; (k) Zn, AcOH, dioxane/H₂O, 80 °C, 66%; (l) LiOH. H₂O, MeOH/H₂O, 70°C, 76%; (m) Propargylamine, T₃P, DIEA, EtOAc, 69%; (n) **3**, Pd(PPh₃)₂Cl₂, CuI, Et₃N, DMF, 50 °C, 52%.

To explore the effect of the nature and the degree of pyrrole substitutions with halogens on the biological activity, several chlorinated, brominated or iodinated pyrrole derivatives were prepared from trichloroacetylpyrrole (**13**).^{33,34,35} Amidation of **14a-14d** with commercial propargylamine followed by Sonogashira coupling of the resulting acetylenes with the previously prepared imidoimidazopyrimidine **3** provided compounds **1** and **16b-e** in yields varying from 20% to 86% (Scheme 2).^{36, 37, 38}

Scheme 2. Variation of the nature and degree of pyrrole substitutions by halogens

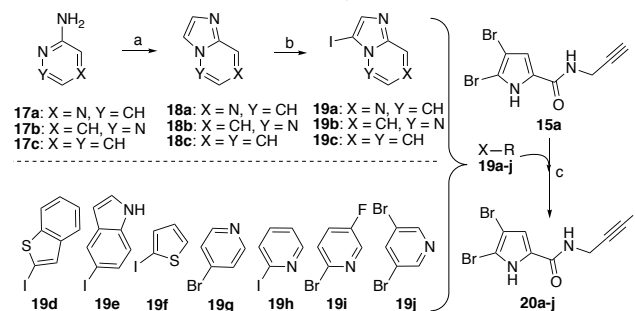


Reagents and conditions: (a) Br₂, AcOH; (b) I₂, AgTFA, CHCl₃; (c) SO₂Cl₂, CHCl₃; (d) Br₂ 1 equiv., AcOH; (e) propargylamine, 60°C; (f) **3**, Pd(PPh₃)₂Cl₂, CuI, Et₃N, DMF, 60°C.

Modifications of the bicyclic moiety part B. Nitrogen positions within the bicyclic ring and substituent effects were examined. The precursor aromatic halides **19a-c** were synthesized based on

previous literature methods. Thus, cyclization of **17a-c** followed by iodination using the two-step method as for **3** provided **19a-c** (Scheme 1),^{39, 40, 41, 42} while compounds **19d-j** were commercially available. Sonogashira coupling of **19a-j** with the alkyne **15a** then afforded compounds **20a-j** regioselectively though in rather low yields varying from 10% to 36%. In addition, Sonogashira coupling of **19a** with the alkynes **15b** and **15c** gave the dichloro and diiodo derivatives **20k-l** respectively. The same reaction between the bicyclic **19c** and the acetylenic **15b** and **15c** gave the dichloro and diiodo derivatives **20m-n** respectively (see Supporting Information).

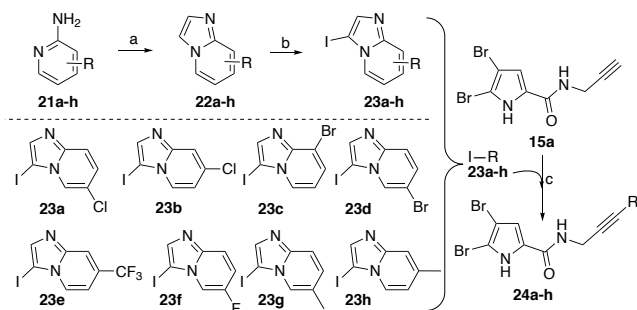
Scheme 3. Variation of the east bicycle



Reagents and conditions: (a) Chloroacetaldehyde, EtOH or ACN 70°C, 100%; (b) NIS, ACN 64-100% (**19b** and **19c**) or I₂, pyridine, 42% (**19a**); (c) Pd(PPh₃)₂Cl₂ 5%, CuI 10%, Et₃N, THF, 50°C, 10-46 %

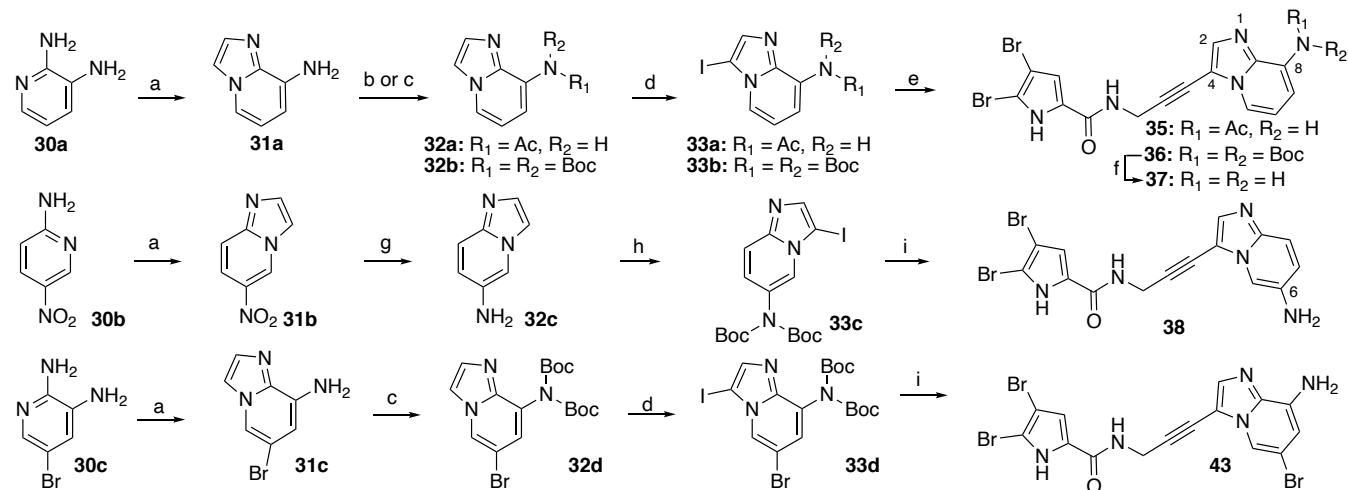
Since the most active compound was the imidazopyridine **20c**, we decided to add various substituents to the imidazopyridine moiety in order to evaluate which positions most affect activity. This was achieved from the corresponding commercially available substituted amino pyridines **21a-h** which were subjected to the previous cyclization-iodination sequence to provide **23a-h**^{43,44,45,46,47,48,49} followed by Sonogashira cross-coupling to afford compounds **24a-h** (Scheme 4, see Supplementary data for full details).

Scheme 4. Imidazopyridine substitutions



Reagents and conditions: (a) Chloroacetaldehyde, EtOH, reflux; 79-100 %; (b) NIS, ACN, 65-97%; (c) R-I, Pd(PPh₃)₂Cl₂, CuI, Et₃N, THF, 5-63 %.

Scheme 6. Introduction of an exocyclic aromatic primary amine on positions 6 and 8

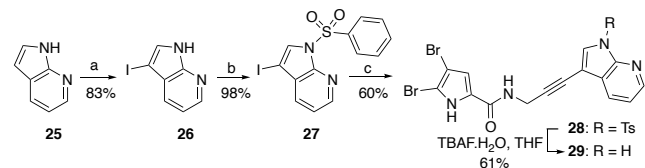


Reagents and conditions: (a) Chloroacetaldehyde, EtOH reflux, 65-93 %; (b) Ac₂O, DCM, 95%; (c) Boc₂O, DCM, DMAP, 39-67%; (d) NIS, ACN, 88 -100 %; (e) **15a**, Pd(PPh₃)₂Cl₂ 5%, CuI 10%, Et₃N, THF, 35-66%; (f) TFA, DCM, 70%; (g) SnCl₂·2H₂O, HCl, EtOH, 78%; (h) Boc₂O, Et₃N, DMF and NIS, ACN, 38%; (i) **15a** Pd(PPh₃)₂Cl₂ 5%, CuI 10%, Et₃N, DMF; then TFA, DCM (yields over two steps : 11% for **38** and 59% for **43**).

The preparation of derivatives **48** and **50** having additional salifiable groups is shown in Scheme 7. The halogenated intermediates **47a** and **47b** were prepared from **46a** and **46b** using Buchwald coupling with *N*-Boc-piperazine or morpholine in the presence of XPhos as ligand followed sequentially by iodination, Sonogashira

Compound **29** (Scheme 5), bearing a 7-azaindole ring system was prepared by a sequence of transformations including iodination of the commercially available 7-azaindole with *N*-iodosuccinimide, protection with benzenesulfonyl chloride, Sonogashira reaction with the alkene **15a**, and finally deprotection with TBAF in THF.⁵⁰

Scheme 5. 7-aza-indole derivative preparation

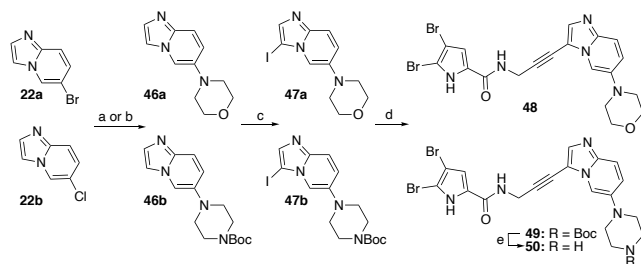


Reagents and conditions: (a) I₂, KI, NaOH 1N, EtOH, 83 % (b) PhSO₂Cl, Bu₄NBr, NaOH, DCM dry, 98 %; (c) **15a**, Pd(PPh₃)₂Cl₂, CuI, Et₃N, THF, 60% ; (d) TBAF·H₂O, THF, 61%.

To explore the effect of an exocyclic amine on the bicyclic portion of the framework, different aromatic amines were introduced at positions 6 and 8 (Scheme 6). After preparation of **31a**,^{51,52} **31b** and **31c**,^{53,54} *N*-protection of the amino-imidazopyridine with acetyl chloride or Boc₂O when necessary and subsequent iodination, followed by Sonogashira coupling allowed the synthesis of compounds **35**, **36**, **37**, **38** and **43**. The deprotection of the amines of **36** and other di-Boc intermediates was achieved with TFA in DCM producing into **37**, **38** and **43** (Scheme 6).⁵⁵ In addition, Sonogashira coupling of **33b** with **15b** (dichloro) and **15c** (di-iodo) gave after deprotection the dichloro and diiodo derivatives **44** and **45** (see SI).

coupling and then, in the case of **49**, piperazine *N*-Boc cleavage with HCl into **50**.^{34,56}

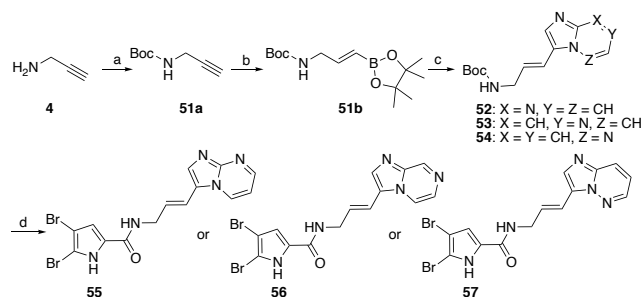
Scheme 7. Morpholine and piperazine derivatives preparation



Reagents and conditions: (a) Morpholine, Pd₂(dba)₃, XPhos, NaOtBu, toluene, 100°C, 62%; (b) Boc-piperazine, Pd₂(dba)₃, XPhos, NaOtBu, toluene, 100°C, quant.; (c) NIS, ACN 55-100%; (d) **15a**, Pd(PPh₃)₂Cl₂ 5mol%, CuI 10 mol%, Et₃N, THF, 22-61%; (e) HCl 4N dioxane, DCM, 72%

Modification of the linker moiety C. In order to vary the flexibility of the molecules, we first replaced the acetylene linker by an alkene. The alkene series was prepared as described in Scheme 8. *N*-Boc-protected propargylamine **51a**⁵⁷ was subjected to a hydrozirconation-hydroboration sequence with pinacol borane and Schwartz's reagent to produce the key building block **51b**.^{58,59} Suzuki coupling with the corresponding iodo compounds **19a-c** gave compounds **52-54**. After *N*-Boc cleavage of the latter with aqueous 4N HCl, the resulting amines were acylated using the trichloroacetyl dibromopyrrole **14a**.

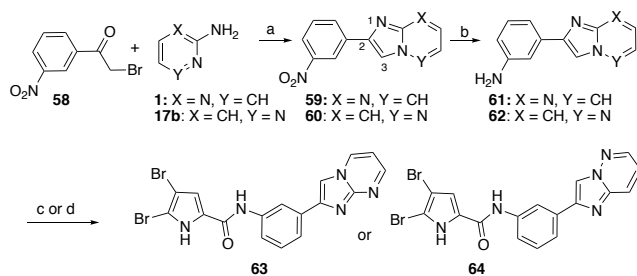
Scheme 8. Synthesis of derivatives with an alkene linker



Reagents and conditions: (a) Boc₂O, DMAP, DCM, 97%; (b) Pinacolborane, Cp₂ZrHCl 25 mol%, Et₃N 25 mol%, 40°C, 73%; (c) R-I, Pd(PPh₃)₄ 5 mol %, K₂CO₃ (2M), Dioxane, 80°C, 47-100%; (d) HCl 4N then trichloroacetyl dibromopyrrole **14a**, 37%-51%.

We next investigated aromatic linkers between our various bicyclic heterocycles and the most active dibromopyrrole structural motif. Namely, we proposed to introduce a forced orientation in a series of compounds that contain regioisomers and both meta and para benzene substitutions. Imidazopyrimidine and imidazopyridazine cores were obtained from the cyclization of the corresponding 2-aminopyrimidine and 3-aminopyridazin with the nitrophenyl- α -bromoketone **58** (Scheme 9).^{60,61,62} The nitro derivatives **59** and **60** were reduced to the corresponding aromatic amines **61** and **62** with tin (II) chloride under acidic conditions. The latter were then coupled with either trichloroacetyl dibromopyrrole **14a** in Et₃N or with the acyl chloride prepared *in situ* to afford **63** and **64**, respectively.⁶³

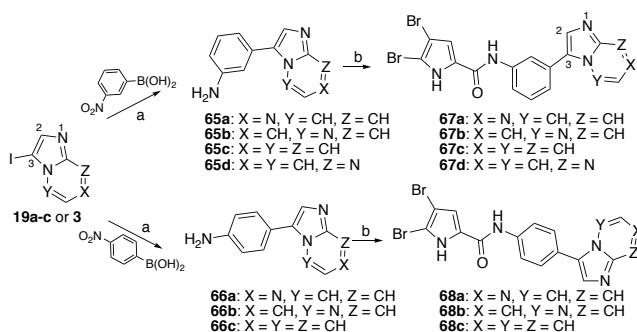
Scheme 9. Synthesis of derivatives with a meta benzene linker at C2



Reagents and conditions: (a) EtOH, reflux 41-71 %; (b) SnCl₂·2H₂O, HCl, EtOH, 62-78 %; (c) Trichloroacetyl dibromopyrrole **14a** Et₃N, 60°C, 49 %; (d) Dibromopyrrole carboxylic acid, (COCl)₂, DCM/DMF, 72 %.

The preparation of the new C3 regioisomers and benzene meta-linked **67a-d** or benzene para-linked **68a-d** was achieved from **19a-c** and **3** via a Suzuki coupling,⁶⁴ reduction of the nitro group and final acylation by dibromopyrrole carboxylic acid in presence of the coupling reagent T₃P (Scheme 10).

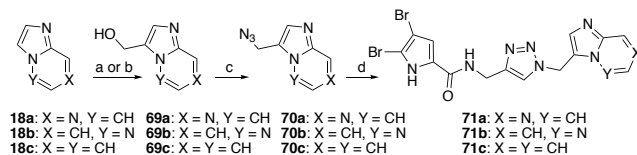
Scheme 10. Synthesis of derivatives with a meta and para benzene linker at C3.



Reagents and conditions: (a) i. Pd-118, K₂PO₄, dioxane/H₂O, 80°C, ii. SnCl₂·2H₂O, EtOH/HCl, reflux; (b) Dibromopyrrole carboxylic acid, T₃P, pyridine, EtOAc/DMF.

We also prepared derivatives comprising 1,2,3-triazole as a proton acceptor linker. Thus, bicyclic intermediates **18a-c** underwent a hydromethylation reaction in 37% formaldehyde solution.⁶⁵ The hydroxy group was replaced by an azide using NaN₃ under acidic conditions.⁶⁶ The azidomethylimidazoles were then coupled with dibromopyrrole alkynes via the CuSO₄ mediated Huisgen click reaction to afford compounds **71a-c** bearing a triazole linker with good yields (Scheme 11).⁶⁷

Scheme 11. Synthesis of derivatives with a meta and para benzene linker at C3



Reagents and conditions: (a) Formaldehyde 37% solution, 90°C, 48%; (b) formaldehyde 37% solution, NaOAc, AcOH, 100 °C, 50-73%; (c) TFA, NaN₃, 70°C, 56-77 %; (d) **15a**, CuSO₄·5H₂O 1 mol%, Sodium ascorbate 10 mol%, DMF/H₂O, 63-89 %.

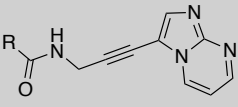
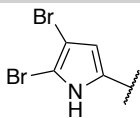
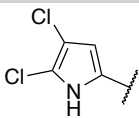
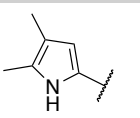
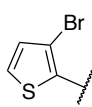
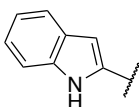
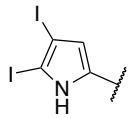
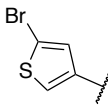
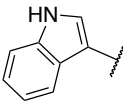
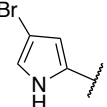
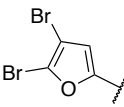
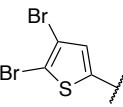

Biological Evaluation and Structure–Activity Relationship Study.

The above chemical library of new compounds was synthesized not only to create molecular diversity but also in response to results obtained from Aurora B kinase inhibition assessments during the course of our study. Kinase inhibition was measured using an ADP-Glo methodology (ADP-Glo™ Assay kit, Promega). Preliminary screening was performed on several kinases including Aurora B, allowing identification of the first hit, EL228 (**1**). Later in the project, going back and forth between synthesis and biological activity tests permitted investigation of SAR and significant improvement of Aurora B kinase inhibition. The compounds were also assessed for their effects on cell viability on human HCT116 and K562 cell lines.

Structure–Activity Relationships of part A moiety. Starting from the first hit EL228 (**1**), various heterocycles (Table 1) were considered for the modification of part A (Figure 2). Replacement of

the pyrrole moiety of EL-228 (**1**) by the regioisomeric bromothiophenes (**8a**, **8b**) and indoles (**8c**, **8d**) gave rise to analogs inactive against Aurora B (Table 1). We also explored the influence of the nature and the degree of halogen substitution on the pyrrole ring. Thus, compounds with two iodine atoms (**16c**), one bromine atom (**16d**), no halogen atom (**16e**) or having two methyl groups (**11c**) were less active than **1** or, completely inactive (**16d–e**). Only the dichlorinated pyrrole derivative **16b** was shown to be equipotent to EL-228 (**1**). This suggests that the two bromine atoms on the pyrrole moiety are essential for the proper interaction with the kinase, probably due to halogen bond interaction with the protein. Interestingly, bromine and chlorine are better tolerated than iodine, which is probably too bulky. Replacing the H-bond donating pyrrole unit of EL-228 (**1**) by H-bond acceptor units as in **11a** and **11b** led to complete loss of inhibitory activity. We therefore chose to continue our SAR study using dibromopyrrole as the optimal part A moiety.

Table 1: SAR of part A moiety (IC_{50} on Aurora B kinase activity were calculated from dose-response curves performed in duplicate).

								
	R	IC_{50} , μ M	R	IC_{50} , μ M	R	IC_{50} , μ M		
1		5	16b		1.6	11c		> 10
8a		> 10	8c		> 10	16c		> 10
8b		> 10	8d		> 10	16d		> 10
11a		> 10	11b		> 10	16e		> 10

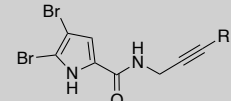
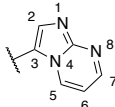

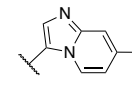
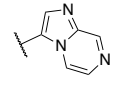

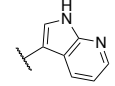
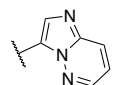
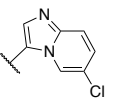
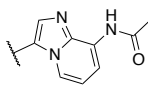
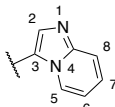
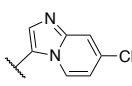
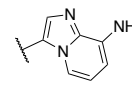
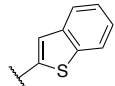
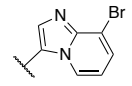
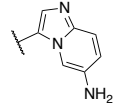
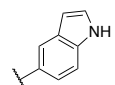
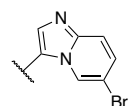
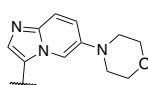
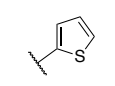
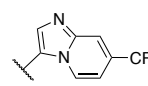
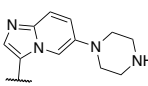
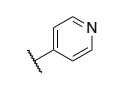
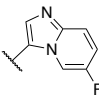
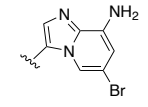
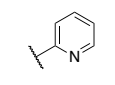

Structure–Activity Relationships of part B moiety. In our search for compounds more potent than EL-228 (**1**), we evaluated the derivatives **20a**, **20b** and **20c** built around an imidazopyrimidine fragment in which the nitrogen atom is now endocyclic or replaced by a carbon atom (Table 2). To our delight, the assays revealed that the most active Aurora B kinase inhibitor (IC_{50} = 46.8 nM) was the simplest imidazopyrimidine **20c** while **20a** and **20b** showed lower activities with IC_{50} 's of 190 and 350 nM, respectively. To further explore the influence of the nature of this heterocycle, it was replaced by other aromatic heterocycles including benzothiophene, indole, imidazole and pyridine derivatives (compounds **20d–20j**) (Table 2). Interestingly, none of these compounds demonstrated significant activity. Retaining the imidazopyrimidine moiety as optimal for Aurora B inhibition, we next examined the effect of substituents on

this ring system. Chloro-, bromo-, methyl- or trifluoromethyl substituents as in **24a–24h** resulted in compounds less active or, at most, equipotent with the unsubstituted imidazopyrimidine **20c**. Even the smaller fluoro substituent in **24f** resulted in reduced activity. At this stage, we assumed that compounds with an H-bond acceptor (mapped by the nitrogen in EL-228 (**1**)) at position 8 is not favorable for activity. Thus, we decided to prepare imidazopyrimidine derivatives **35**, **37** and **43** having an H-donor at C8. Our assays showed that the activity of **37** was only slightly superior to that of **20c** but 150 times greater than that of the initial hit EL-228 (**1**), with an IC_{50} of 34.3 nM. The importance of a hydrogen donor at this position was confirmed by the compound **24c** bearing a bromine C8 atom, which did not show any activity. Moreover, the imidazopyrimidine bearing a primary exocyclic amine at position 8 as in

37 as well as a bromine atom at position 6 as in **43**, also demonstrated good inhibitory activities with IC_{50} 's of 34.3 nM and 71 nM, respectively.

On the other hand, azaindole analog **29**, offering a reversed H-bond donor and acceptor sequence compared to CJ2-150 (**37**), did not show any inhibitory activity against Aurora B. We thus conclude that the imidazopyridine derivatives can be correlated with high activities.

Table 2: SAR of part B moiety (IC_{50} on Aurora B kinase activity were calculated from dose-response curves performed in duplicate).

								
	R	IC_{50} , nM		R	IC_{50} , nM		R	IC_{50} , nM
1		5000	20i		>1000	24h		32
20a		190	20j		>1000	29		>1000
20b		350	24a		>1000	35		87.1
20c		46.8	24b		71	37		34.3
20d		>1000	24c		>1000	38		710
20e		>1000	24d		978	48		150
20f		>1000	24e		620	50		478
20g		>1000	24f		139	43		71
20h		>1000	24g		40			

At this stage and in line with the above finding regarding the equipotency of the dichloropyrrole derivative **16b** (Table 1) with EL-228 (**1**), we chose to combine the activity-improving 8-aminoimidazopyridine unit of CJ2-150 (**37**) with the favorable dichloropyrrole and diiodopyrrole structural elements in **44** and **45**

(Table 3). Of the dihalide analogs investigated, the dibromo and exoamino derivative **37** appeared to be the most active. The dibromopyrrole unit was thus retained as part A of the molecules for the following pharmacomodulations.

Table 3: SAR with regard to various halides and bicyclic imidazoles (IC_{50} on Aurora B kinase activity were calculated from dose-response curves performed in duplicate).

N°, X	R	IC ₅₀ , nM	N°, X	R	IC ₅₀ , nM	N°, X	R	IC ₅₀ , nM
37, Br		34	20a, Br		190	20c, Br		47
44, Cl		77	20k, Cl		680	20m, Cl		160
45, I		130	20l, I		350	20n, I		140

Structure–Activity Relationships of Linker part C modification.

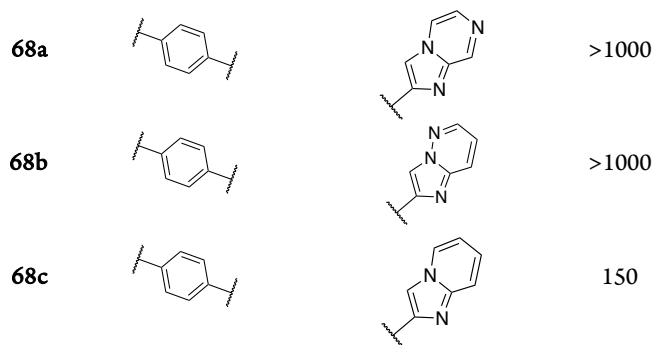
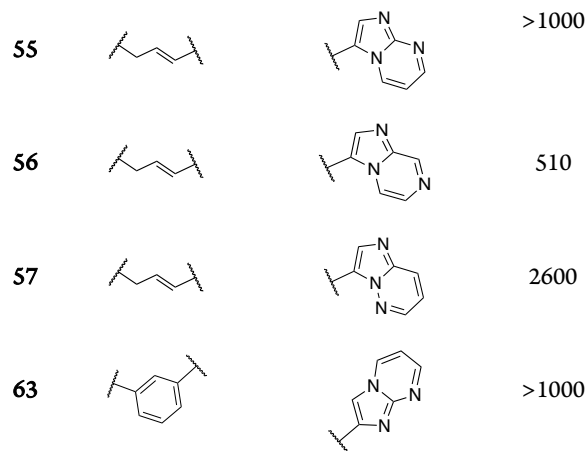
To examine whether the acetylene (part C), which serves as a linker between the pyrrole (part A) and the bicyclic platform (part B), binds these two parts in the most favorable way, we studied other linkers which allowed modification of the flexibility, the steric hindrance, the electronic distribution and the orientation angle between parts A and B of the molecule (Table 4).

Regardless of the nature of part B (pyrimidine, pyrazine, pyridazine, pyridine core), all the alternative linkers led to a dramatically decreased inhibition (Table 4). Only the alkene linker bearing the imidazopyrazine (**56**) and the para-phenyl linker combined

with the imidazopyridine (**68c**) displayed appreciable activities with IC₅₀'s of 510 nM and 150 nM, respectively. These activities were nevertheless almost three-fold less potent than their alkyne counterparts **20a** and **20c**. Moreover, the selectivity over the panel of kinases was not optimal for these compounds. At this stage, we hypothesized that the linear alkyne linker, not overly sterically demanding, favorably reduces steric clash allowing the compound to fit within a tight hydrophobic pocket of the protein. This rigidification property of an acetylene linker has previously been pointed out in the literature as a means to improve the selectivity and potency of kinase inhibitors.⁶⁸

Table 4: SAR with regard to various linkers and bicyclic imidazoles (IC₅₀ on Aurora B kinase activity were calculated from dose-response curves performed in duplicate)

	X	R	IC ₅₀ , nM		X	R	IC ₅₀ , nM
1			5000	64			>1000
20a			190	67a			>1000
20b			350	67b			>1000
20c			47	67c			4600
72*			>1000	67d			>1000



* Dr. Sylvain Picon, Ph.D. thesis, Université Paris XI, Orsay, December 9th, 2008.

Selectivity of Aurora B inhibition.

To assess their selectivity, all the synthesized derivatives were first tested on a small panel of 8 kinases involved in many human disorders notably in cancer. The assays were conducted at 10 μ M and 1 μ M concentrations and IC₅₀ values were determined for all compounds showing greater than 50% inhibition at 10 μ M. The results showed that the selected compounds on the running panel of kinases had significantly more activity on Aurora B than on all the other tested kinases. Importantly, all our products have been shown to be very active on Aurora B but inactive or significantly less active on most of the kinase panel.

At this stage, the good selectivity and potency displayed by our library of derivatives prompted us to evaluate one of these compounds on a larger panel of kinases. We thus evaluated compound CJ2-150 (**37**) for inhibitory activity on a panel of 58 protein kinases (Diversity Panel, Eurofins) covering a variety of associations with several cellular regulatory functions. The inhibition profile of CJ2-150 (**37**), evaluated at 1 μ M, is illustrated in red in a phylogenetic tree format (Figure 3). Only Aurora A (the congener of Aurora B) showed high inhibition (80%). GSK3 β and LYN kinases were 40% and 34% inhibited while all the other kinases were inhibited by less than 25%. Note that CJ2-150, used at the same concentration of 1 μ M, inhibited 88% of Aurora B kinase activity in an independent enzymatic assay (ADP-Glo, Promega) (See SI for values table). Interestingly, these results demonstrate a high selectivity for inhibition of Aurora family members by CJ2-150.

Antiproliferative profiles. The antiproliferative activity of active compounds, together with the reference Aurora B inhibitor ZM447439, was evaluated in dose-response experiments against the human cancer cell lines HCT116 (colorectal cancer) and K562 (myeloid leukemia) (Table 5). Compound **1**, **20b** and **24e** had a mild effect on K562 cell viability with EC₅₀s above 20 μ M together with little to no effect on HCT116 cells. On the other hand, three compounds, **24b**, **24f** and **24g**, showed an interesting specificity towards HCT116 cell line with EC₅₀ of 8, 3 and 5 μ M respectively in the same order of magnitude as ZM447439 (EC₅₀ of 7 μ M), and little or no effect on K562 cells. Four compounds, **37**, **48** and **68c** showed effect on cell viability on both cell lines in the range of 22 to 2 μ M, similar to ZM447439 (7 and 9 μ M on HCT116 and K562

respectively). It is to be noted that there was no obvious correlation between the cell viability effect of the compounds and their potency on Aurora B kinase. The lack of effect from certain compounds having similar or higher potency to that of ZM447439 could be explained by their low solubility or lipophilicity.

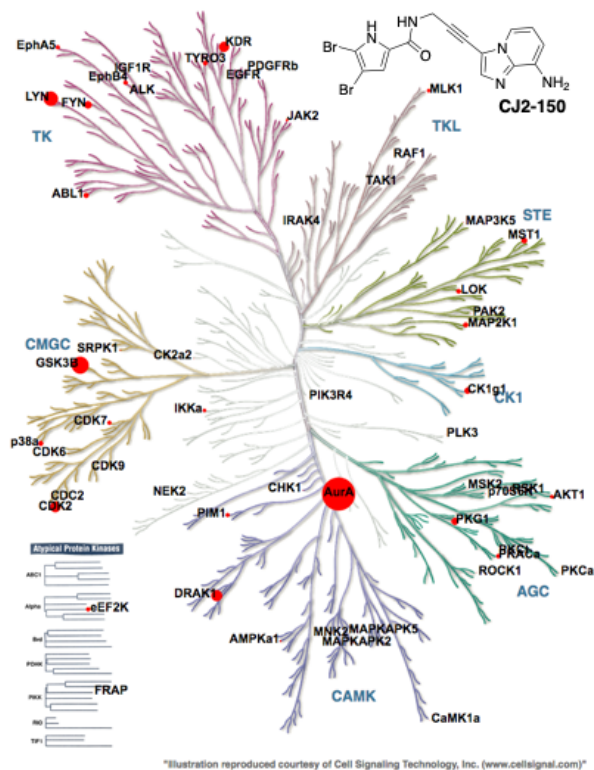


Figure 3: Kinase activity inhibition profile of CJ2-150 (**37**), screened against 58 kinases (at 1 μ M) (Eurofins), illustrated on a human kinome phylogenetic tree (dendrogram, courtesy of Cell Signalling). Each kinase tested is marked and the size of the red circle is representative of the percentage of inhibition. Precise kinase inhibition percentages are shown in a table in SI.

Table 5: Effects on cell viability of HCT116 and K562 cancer cells.

	Aurora B IC ₅₀ (nM)	HCT116 EC ₅₀ (μM)	K562 EC ₅₀ (μM)		Aurora B IC ₅₀ (nM)	HCT116 EC ₅₀ (μM)	K562 EC ₅₀ (μM)
1	5000	n.e.	51	24g	40	5	n.e.
20a	180	15	44	CJ2-150 (37)	34	11	22
20b	350	76	40	48	150	7	2
24b	71	8	32	67c	> 1000	9	22
24e	620	93	21	68c	150	12	8
24f	139	3	66	ZM447439	127	7	9

EC₅₀ (μM) on cell viability were calculated from dose-response curves made in triplicate after 72 h treatment. IC₅₀ (nM) on Aurora B activity are reported in this table. "n.e.": no effect at maximal tested concentration of 100 μM.

The most interesting compound (in terms of enzymatic inhibition activity), the exocyclic amine substituted derivatives CJ2-150 (**37**) also showed interesting activity toward cell lines viability with EC₅₀ of 11 and 22 μM for HCT116 and K562 respectively (Table 5).

Therefore, we subsequently assessed the ability of this compound, in parallel with the reference ZM447439, to affect cell viability of various human cancerous cell lines from lung carcinoma A549, ovarian cancer SKOV3, breast cancer MCF7, osteosarcoma U-2 OS, as well as immortalized retinal fibroblast RPE-1 cell line (Table 6). The results showed that CJ2-150 (**37**) as well as ZM447439 affected cell viability of all cell lines and that A549 lung carcinoma line was the most sensitive to both inhibitors with EC₅₀ of 9 and 2

μM respectively. Overall, these last results showed that, interestingly, most of our compounds affect the viability of several cell lines of cancerous origin. In addition, we conducted CJ2-150 (**37**) dose-response in viability assays on peripheral blood mononuclear cells (PBMCs) comparing non-proliferating to proliferating conditions i.e. activated with the mitogen phytohemagglutinine (PHA) (see SI Figure S1). We observed that cell viability of CJ2-150 (**37**)-treated PBMCs was much less impacted (EC₅₀>100 μM) than the proliferative condition performed with PHA (30 < EC₅₀ < 100 μM). These results showed that CJ2-150 (**37**) has very low cytotoxicity on PBMC. They further show that, as expected for an Aurora B inhibitor, proliferating cells are more affected than the non-proliferating ones.

Table 6: Effects of CJ2-150 and ZM447439 on cell viability of several human cell lines.

	A549	SKOV3	MCF7	U2OS	RPE1
CJ2-150 (37)	9	40	36	62	18
ZM447439	2	31	4	29	27

EC₅₀ (μM) on cell viability were calculated from dose-response curves made in triplicate after 72 h treatment.

Mode of action. *ATP competition assay with compound CJ2-150 (37).* The binding mode of the most potent compound CJ2-150 (**37**) was investigated by performing experiments at varied ATP and inhibitor concentrations (Figure 4). The results are represented in Michaelis-Menten and Lineweaver-Burk plots (Figure 4A,C). They showed that in the presence of an excess of ATP, Aurora B activity (represented by ADP production) decreases in response to increasing concentrations of the inhibitor. Note that in an ATP-competition situation, Aurora B activity would not be impacted by any concentration of inhibitor when in the presence of an excess of ATP (in this case the 1/ADP production versus 1/[ATP] plots at different CJ2-150 concentrations should intersect on the 1/ADP production axis). Additionally, increasing inhibitor concentration was linked with Km increase suggesting a reduced affinity of the kinase for ATP. Similar results were obtained with compound **20c** (see SI Figure S2). Furthermore, we observed that CJ2-150 IC₅₀s measured at increasing concentration of ATP showed very little variation (from 39 nM at 5 μM ATP to 94 nM at 75 μM ATP) (Figure 4B). All together, these results strongly suggest that CJ2-150 does not compete directly with ATP, implying that the compound binds outside of the ATP pocket on an

allosteric site, resulting in a mixed inhibition. They also suggest that this allosteric binding may affect the ATP pocket.

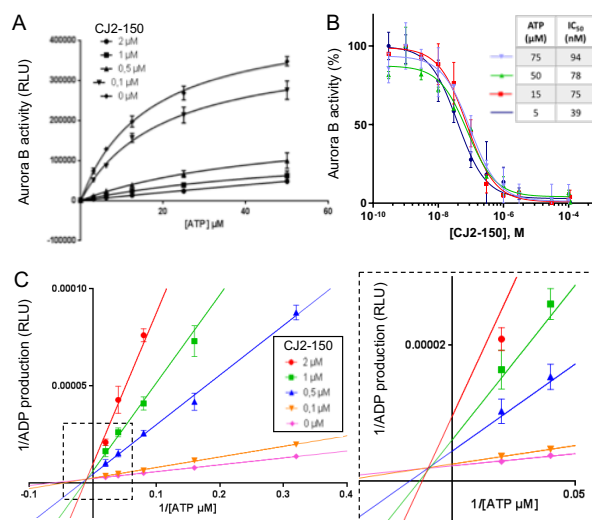


Figure 4: CJ2-150 (**37**) is a non-ATP-competitive Aurora B kinase inhibitor. A: ATP competition assay with compound CJ2-150 represented on a Michaelis-Menten graph. Aurora B kinase activity assays were performed using ADP-Glo methodology (Promega) and conducted with increasing concentrations of CJ2-150 ranging from 0.1 to 2 μM in the presence of increasing concentrations of ATP from 3.125 to 50 μM . ADP production in the reaction is directly proportional to Aurora B activity. B; Dose-response curves of CJ2-150 (from 2 nM to 100 μM) on Aurora B activity at increasing concentrations of ATP (from 5 to 75 μM), corresponding calculated IC_{50} s are showed in embedded table. C: ATP competition assay with compound CJ2-150 represented on a double reciprocal Lineweaver-Burk type plot; $1/\text{ADP}$ production expressed in RLU (Relative Light Unit) is plotted against $1/\text{ATP}$ concentration (μM). All points are mean of triplicate \pm s.e.m.

*Effect of compound CJ2-150 (**37**) on the cell cycle and mitotic progression.* We next validated that the compound CJ2-150 effectively targets the Aurora B kinase in cells and assessed its impact on mitotic progression. Several key points were examined. Firstly, the DNA cell content following treatment was analyzed to highlight the polyploidy effect that is caused by cytokinesis failure on the K562 cell line upon Aurora B inhibition. Secondly, we investigated the signal intensity of Histone H3 phosphorylated on Ser10 in U-2 OS mitotic cells; Histone H3 is a specific substrate of Aurora B. Lastly, we analyzed the distribution pattern of Aurora B during mitosis.

Cell cycle analysis. We examined the effect of our most active compound CJ2-150 at 2, 5, 10 and 20 μM on the cell cycle of K562 cells after 48 h of treatment. The DNA content of the drug-treated cells and DMSO-treated cells (control) was analyzed by flow cytometry (FACS) allowing us to determine the percentage of cells in each phase of the cell cycle. After 48 h, we observed a strong accumulation of cells with 4N DNA contents and a part of the population reaching a DNA content of 8N and above (Figure 5) in a clear dose-dependent effect. These results strongly suggested that treatment with CJ2-150 (**37**) resulted in impaired cell division and the generation of polyploid cells. This is indeed consistent with the essential role of Aurora B during the cytokinesis process.⁶⁹ In support of these results, visual examination of cells revealed an important time-dependent increase in cell size.

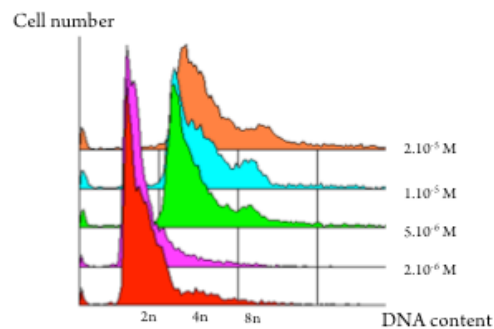


Figure 5: DNA content analysis in K562 cells treated with CJ2-150 (**37**) (for 48 h at indicated concentrations) by flow cytometry.

Phosphorylation of H3 on Ser10. In order to investigate the ability of CJ2-150 (**37**) to inhibit cellular endogenous Aurora B, we monitored the specific phosphorylation of histone H3 on serine 10 by immunofluorescence. For this purpose, we worked with U-2 OS adherent cells treated with CJ2-150 (**37**) at 10 μM or ZM447439 at 1 μM (Aurora B reference inhibitor) and a DMSO control.

After 16 h of treatment, we evaluated the levels of phospho-H3 on Ser10 (H3S10ph) by immunofluorescence and image analysis (Figure 6). Compared to the control, the H3S10ph signal was greatly reduced for both our compound and ZM447439 (Figure 6A). We noted that typical chromosome alignment and spindle defects were observed as previously reported in Aurora B depletion or inhibition experiments.⁷⁰ Quantification of the H3S10ph signal versus the DAPI signal is represented in figure 6B. The strongest reduction in the H3S10ph signal was obtained with compound CJ2-150 at 10 μM similar to that obtained with ZM447439 at 1 μM . The effect was slightly milder with CJ2-150 at 2.5 μM . These results showed that the compound, displaying an *in vitro* inhibitory IC_{50} on Aurora B 3.7 times lower than that of ZM447439 (34 nM and 127 nM respectively), required a 10 times higher concentration in cells in order to observe a level of inhibition comparable to that obtained with ZM447439 (Figure 6B). This discrepancy could be explained by different factors. It may result from the reduced solubility of CJ2-150 (**37**) in aqueous solution (cell culture media), or a lower affinity for lipid plasma membranes. Additionally, the compound can display a high metabolism rate: the amide function metabolic sensitivity and the heterocyclic probably bicyclic make the molecule prone to metabolic instability. These structural weaknesses suggest the next step which is to design analogs with improved cellular activity while improving pharmacokinetic properties. Overall, these results demonstrate that CJ2-150 is functional on cellular Aurora B kinase activity.

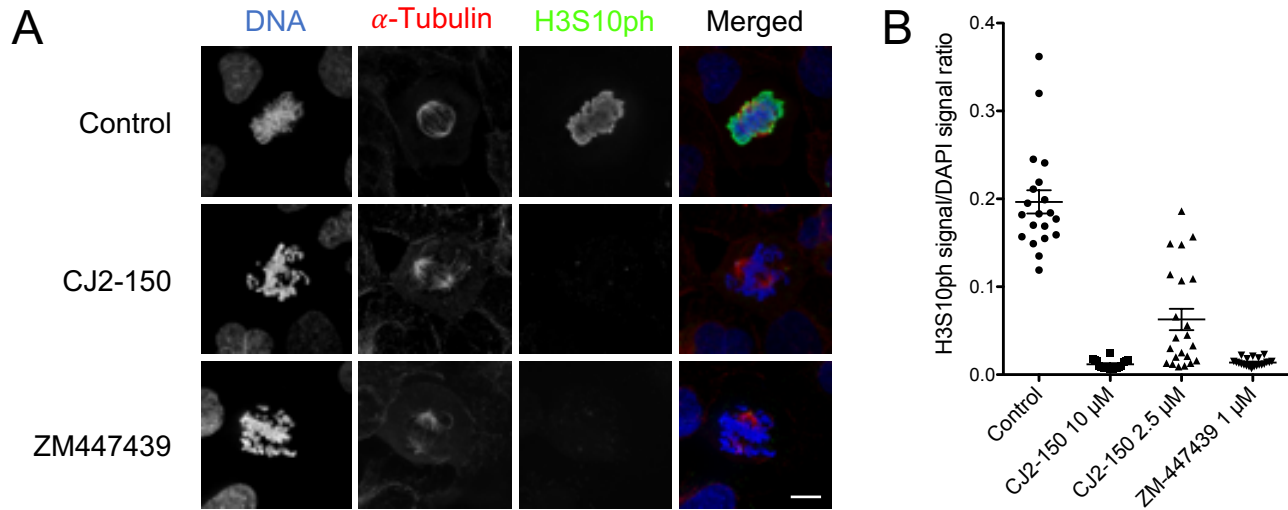


Figure 6: In-cell evaluation of Aurora B activity inhibition. **A**, immunofluorescence staining on U-2 OS cells treated with 10 μ M of CJ2-150 or 1 μ M of ZM447439 or DMSO (control) for 16 h. Aurora B activity was monitored by immuno-staining of phosphorylated Histone H3 on serine 10 (H3S10ph, green); the cytoskeleton was visualized by α -Tubulin immuno-staining (red) and DNA was visualized by DAPI staining (blue). Bar 10 μ m. **B**, quantification of H3S10ph signal over DAPI signal, representative of Aurora B activity, in prometaphase cells from the experiment described in **A**, $n \geq 20$.

We next analyzed the different mitotic phenotypes resulting from the treatment with CJ2-150 (**37**) on U-2 OS cells. We first quantified the mitotic indexes and the proportion of cells in early and late mitosis after 16 h of treatment with CJ2-150 (**37**) (10 and 2.5 μ M) or ZM447439 (1 μ M) and a DMSO control. We observed increased mitotic indexes after treatment with both CJ2-150 at 10 μ M and ZM447439 compared to the DMSO control (7.6% and 7.1% respectively compared to 4.5 %). A slight increase was observed with CJ2-150 used at 2.5 μ M (4.6%). An increased mitotic

index is the result of a mitotic delay often generated by defects in early mitosis, a phenotype encountered when Aurora B activity or protein levels are affected.^{71,72} Indeed, chromosome misalignments activate the SAC (Spindle Assembly Checkpoint) and slow down mitotic progression leading to an accumulation of cells in early mitosis. Thus, we could observe early mitosis accumulation for both CJ2-150 (**37**) at 10 μ M and ZM447439 treatments (Figure 7B)

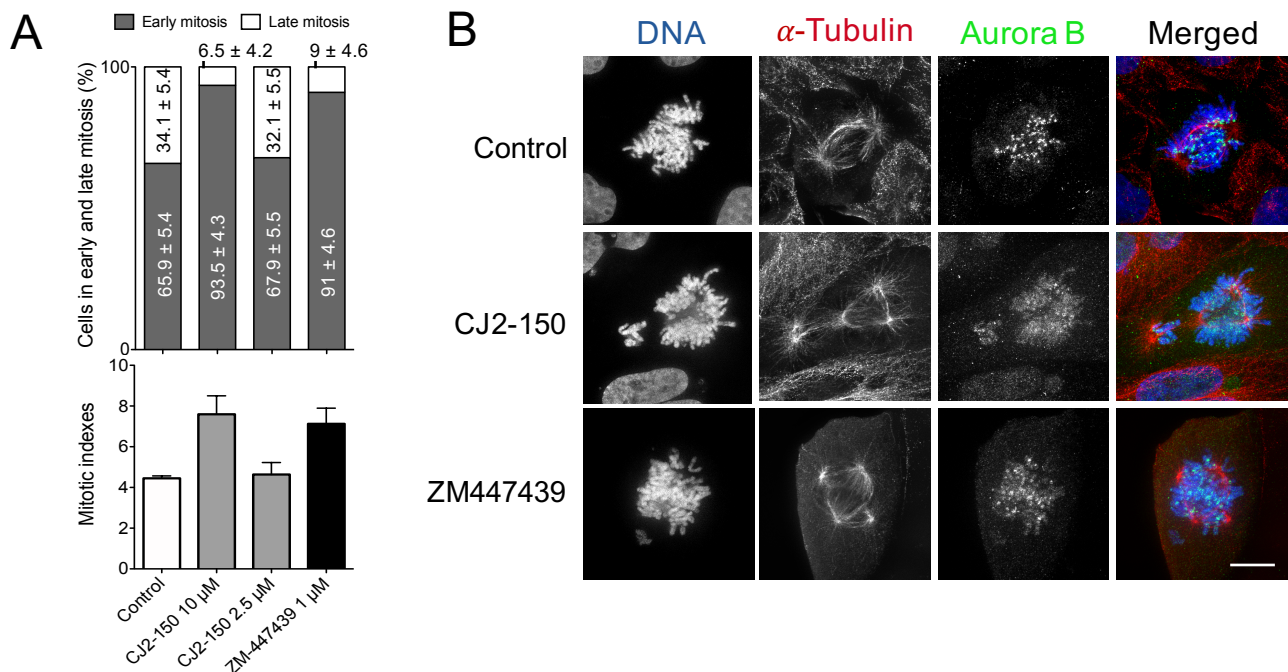


Figure 7: CJ2-150 (**37**) affects mitosis of U-2 OS cells. A, upper graph, quantification of early and late mitosis in U-2 OS cells treated with CJ2-150 (**37**) (10 and 2.5 μM) or ZM447439 (1 μM) or DMSO (control) for 16 h. $n \geq 200$; graph represent the mean of 3 independent experiments; results are mean \pm s.e.m. Lower graph, quantification of mitotic indexes in cells treated as in A. $n \geq 500$; graph represent the mean of 3 independent experiments; results are mean \pm s.e.m. B, immunofluorescence staining of prometaphase U-2 OS cells treated with CJ2-150 (10 μM) or ZM447439 (1 μM) or DMSO for 24 h. Aurora B is visualized in green, α -Tubulin in red and DNA (DAPI) in blue. Scale bar 10 μm .

We thus examined the mitotic phenotypes of U-2 OS cells treated with CJ2-150 (**37**) and ZM447439 by immunofluorescence (Figure 7B). Aurora B and α -tubulin staining allowed us to assess the kinase localization and spindle integrity respectively during mitosis. Aurora B clearly localized and concentrated at the centromere of control cells in prometaphase whereas for the cells treated with either CJ2-150 (**37**) or ZM447439, the kinase appeared delocalized from centromere to chromosome arms (Figure 7B). Indeed, Aurora B, *via* a positive phosphorylation loop with Haspin kinase, is responsible for its proper localization at the centromere in early mitosis.⁷³ The tubulin staining revealed a strong disrupting effect on spindles which often appeared multipolar. Taken together, these results showed that CJ2-150 (**37**) specifically acts on Aurora B kinase activity and therefore localization, impairing spindle structure, chromosome alignment and cytokinesis.

Molecular docking.

The mode of interaction of the most active compound, CJ2-150 (**37**) with Aurora B was investigated using molecular docking. Structural data available for Aurora B, includes only complexes with ATP-competitive ligands. However, three allosteric binding sites (“F”, “Y” and “W”) were identified for Aurora A,^{74,75,76} as well as their equivalents in Aurora B.^{77,78}

The “W” site proved to be non-specific after a detailed analysis.⁷⁴ We performed molecular docking of CJ2-150 (**37**) in the allosteric sites (type IV) “F” and “Y” of a representative Aurora B structure (PDB 4AF3),⁸¹ which provided a single cluster for the site “F” (Figure 8), whereas several clusters were obtained for the site “Y”, with the bromine atoms exposed to the solvent in all of them. Therefore, although a binding in the “Y” site cannot be formally excluded, compound CJ2-150 (**37**) most likely interacts with the site “F”, as shown in Figure 8 (see SI Figure S3 as well).

In this model, there is a very good shape complementarity between the ligand and the protein, with the imidazopyridine ring system buried in a hydrophobic subpocket formed by the residues Phe72, Phe77, Phe101, Val103, Tyr141 and Ile153 and possible π -stacking interactions with Phe72 and Tyr141, whereas the pyrrole ring interacts with a second hydrophobic subpocket with favorable hydrophobic interactions between the two bromine atoms and the residues Ile126, Ala130 and Leu140. Hydrogen bonds are established between the amide oxygen of the ligand with the side chain of Arg139, and between the N-H of amide and pyrrole moieties in the ligand with the backbone O of Tyr141 and Leu140, respectively (Figure 8). Docking of compounds **35**, **48** and **50** in the site “F” provided similar results. The docking conformation of **35** is exactly the same as for CJ2-150 (**37**), with the acetamide substituent deeply buried in the first subpocket. The same conformation is also observed for **48** and **50**, with the exception of the imidazopyridine ring system, which is flipped towards the subpocket extremity, stabilized by a π - π stacking with Phe101, but with the morpholine and piperazine substituents more solvent exposed (Figure S4). Overall, these docking results are in agreement with the SAR from Table 2

and strongly suggest a type IV allosteric inhibition.⁷⁹ Neither the competitive non-ATP mode of action nor the type of mixed inhibition was described until October 2020.⁸⁰

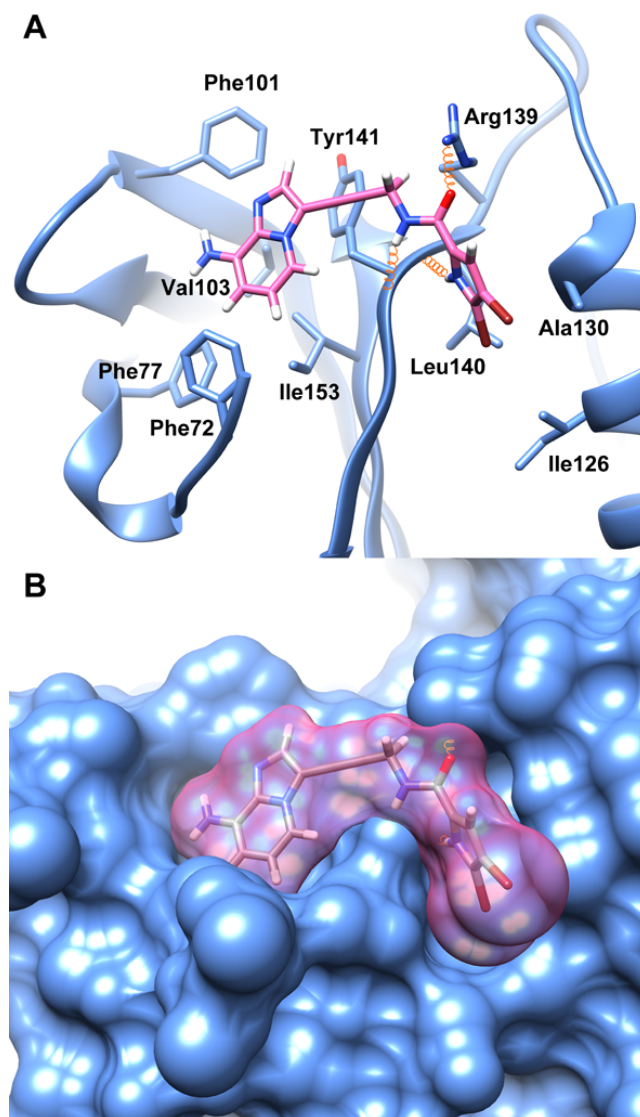


Figure 8: Docking conformation of CJ2-150, represented as pink sticks and transparent surface, into the allosteric site “F” of Aurora B (PDB 4AF3)⁸¹ colored in blue in cartoon (A) or surface (B) representation. Hydrogen bonds are represented as orange springs.

3. ■ CONCLUSION

In this report, new type IV allosteric inhibitors of Aurora B kinase are presented. Starting from the original marine benzosceptrin and oroidin pyrrole-2-aminoimidazole natural product analog EL-228 (**1**), pharmacomodulation by the chemical synthesis of 56 analogs

and their systematic evaluation on Aurora B and others kinases was carried out. The rigid acetylenic structures were further optimized into CJ2-150 (**37**) with nanomolar activity and high selectivity for both Aurora A and B. Cell cycle and cellular effects in the presence of our compounds notably CJ2-150 resulted in impaired cell division and polyploid cells generation; consistent with the essential role of Aurora B in the cytokinesis process. Furthermore, we showed that CJ2-150 could inhibit in-cell Aurora B activity generating typical chromosome alignment, spindle and kinase

4. ■ EXPERIMENTAL SECTION

All reagents were of commercial grade and were used without further purification, unless otherwise stated. Commercially available anhydrous solvents were used for reactions conducted under inert atmosphere. Reagent grade solvents were used in all other cases. Flash column chromatography was performed on silica gel 60 (70–230 mesh, Macherey–Nagel). Thin layer chromatography was carried out using precoated silica gel F-254 plates (thickness 0.25 mm) and visualized by UV light (254 nm). The purity of the final compounds was controlled by ELSD-HPLC using Kinetex C18 2.6 x 10 mm, flow : 1 mL/min, eluent : H₂O + 0.1% formic acid/ACN + 0.1% formic acid by injection 10 μL of (1mg/mL in MeOH/DMSO : 7/3). The ELSD-HPLC profiles showed that the purity of the majority of the final products is > 95% (see SI). ¹H NMR also demonstrates the high purity of the final products (see SI). ¹H and ¹³C NMR spectra were recorded on a Bruker Advance 300 NMR spectrometer (300 and 75 MHz). Chemical shifts δ are reported in parts per million (ppm) relative to the residual solvent signals (CDCl₃, δ = 7.26 ppm for ¹H and δ = 77.16 ppm for ¹³C; DMSO-d₆, δ = 2.50 ppm for ¹H and δ = 39.52 ppm for ¹³C. DMF-d₇, δ = 2.79, 2.92, 8.03 ppm for ¹H and δ = 29.76, 34.89, 163.15 ppm for ¹³C). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants (Hz) and integration. High-resolution MS data were obtained on a Waters LCT Micromass spectrometer in positive electrospray ionization-time of flight (ESI-TOF) mode. IR spectra were recorded on a PerkinElmer Spectrum 65 spectrophotometer using an attenuated total reflectance (ATR) device (ν in cm⁻¹). Reported yields are unoptimized.

General Procedure A for Amide Synthesis.

To a solution of the appropriate carboxylic acid (1 eq.) in dry DCM were added HOBT (1.2 eq.), then EDC (1.2 eq.). DIEA was added (5 eq.) followed by the amine **7** (1 eq.). The mixture was stirred at room temperature until completion. The mixture was quenched with water and extracted with DCM. The combined organic layers were washed with water and brine, dried over MgSO₄, filtered, and concentrated under vacuum. The product was purified by flash chromatography.

General Procedure B for Sonogashira coupling.

Appropriate halide (1 eq.) was dissolved in either dry THF or dry DMF and Et₃N (2-3 eq.). The reaction mixture was degassed by argon bubbling. CuI (5-10 % mol) was introduced followed by Pd(PPh₃)₂Cl₂ (2-5 % mol). Then the alkyne (1.5-2 eq.) was added to the mixture. The reaction mixture was stirred under inert atmosphere at room temperature or 50°C overnight. The mixture was concentrated under vacuum. The crude residue was purified by

silica gel flash column chromatography and then on basic alumina gel to afford the expected product.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**1**). Compound **1** was synthesized according to general procedure B using **15a** (153 mg, 0.5 mmol) and **3** (147 mg, 0.6 mmol). Purification afforded the title compound as a pale yellow solid (110 mg, 52%). ¹H NMR (300MHz, DMSO-d₆) δ 4.44 (d, *J* = 5.3 Hz, 2H), 6.98 (s, 1H), 7.24 (m, 1H), 8.05 (s, 1H), 8.66 (m, 1H), 8.76 (t, *J* = 5.3 Hz, 1H), 8.88 (d, *J* = 6.6 Hz, 1H), 12.82 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.7, 69.4, 97.7, 98.5, 105.7, 110.5, 113.6, 128, 134.4, 152, 159.2. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₀N₅OBr₂: 423.9226. Found 423.9239. IR (cm⁻¹): 3107, 1616, 1558, 1511, 1395, 1237, 1142.

silica gel flash column chromatography and then on basic alumina gel to afford the expected product.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**1**). Compound **1** was synthesized according to general procedure B using **15a** (153 mg, 0.5 mmol) and **3** (147 mg, 0.6 mmol). Purification afforded the title compound as a pale yellow solid (110 mg, 52%). ¹H NMR (300MHz, DMSO-d₆) δ 4.44 (d, *J* = 5.3 Hz, 2H), 6.98 (s, 1H), 7.24 (m, 1H), 8.05 (s, 1H), 8.66 (m, 1H), 8.76 (t, *J* = 5.3 Hz, 1H), 8.88 (d, *J* = 6.6 Hz, 1H), 12.82 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.7, 69.4, 97.7, 98.5, 105.7, 110.5, 113.6, 128, 134.4, 152, 159.2. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₀N₅OBr₂: 423.9226. Found 423.9239. IR (cm⁻¹): 3107, 1616, 1558, 1511, 1395, 1237, 1142.

3-Bromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)thiophene-2-carboxamide (**8a**). The title compound was synthesized according to general procedure A using 3-bromothiophene-2-carboxylic acid (250 mg, 1.21 mmol) yielding **8a** as a beige amorphous solid (244 mg, 56%). ¹H NMR (300MHz, DMSO-d₆) δ 4.45 (d, *J* = 5.4 Hz, 2H), 7.19 (d, *J* = 5.4 Hz, 1H), 7.26 (dd, *J* = 4.2, 2.8 Hz, 1H), 7.83 (d, *J* = 5.5 Hz, 1H), 8.05 (s, 1H), 8.65 (dd, *J* = 2.1, 2.1 Hz, 1H), 8.77 (t, *J* = 5.1 Hz, 1H), 8.88 (dd, *J* = 1.8, 5.1 Hz, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 30, 68.8, 96.8, 106.3, 110, 100.7, 130.2, 131.8, 132.3, 133.6, 138.7, 147.8, 151.5, 160.3. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₀BrN₄OS: 362.9738. Found: 362.9742. IR (cm⁻¹): 3335, 1630, 1616, 1514, 1484, 1276.

5-Bromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)thiophene-3-carboxamide (**8b**). The title compound was synthesized according to general procedure A using 5-bromothiophene-3-carboxylic acid (250 mg, 1.21 mmol) yielding **8b** as a beige amorphous solid (216 mg, 44 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.44 (d, *J* = 5.6 Hz, 2H), 7.25 (dd, *J* = 2.2, 4 Hz, 1H), 7.62 (d, *J* = 1.8 Hz, 1H), 8.04 (s, 1H), 8.19 (d, *J* = 1.8 Hz, 1H), 8.64 (dd, *J* = 2.3, 2.3 Hz, 1H), 8.88 (dd, *J* = 2.3, 4.6 Hz, 1H), 8.96 (t, *J* = 5.3 Hz, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.4, 68.7, 96.9, 106.3, 110, 112.1, 129.3, 131.5, 133.8, 137.2, 138.7, 147.9, 151.5, 160.6. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₀BrN₄OS: 362.9738. Found: 362.9734. IR (cm⁻¹): 3249, 1658, 1555, 1512, 1486, 1424, 1262.

N-(3-(Imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)-1*H*-indole-2-carboxamide (**8c**). The title compound was synthesized according to general procedure A using 1*H*-indole-2-carboxylic acid (200 mg, 1.24 mmol) yielding **8c** as a yellow amorphous solid (220 mg,

56%). ¹H NMR (300MHz, DMSO-d₆) δ 4.53 (d, *J* = 5.5 Hz, 2H), 7.02 (t, *J* = 7.8 Hz, 1H), 7.19 (t, *J* = 8.4 Hz, 1H), 7.19 (s, 1H), 7.25 (dd, *J* = 2.2, 4.5 Hz, 1H), 7.44 (dd, *J* = 1.1, 8.4 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 8.06 (s, 1H), 8.64 (dd, *J* = 1.9, 4.3 Hz, 1H), 8.9 (dd, *J* = 1.9, 7.1 Hz, 1H), 9.1 (t, *J* = 5.2 Hz, 1H), 11.7 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.3, 68.6, 97.2, 103, 106.3, 109.9, 112.3, 119.7, 121.5, 123.5, 126.9, 131, 133.7, 136.5, 138.7, 147.9, 151.5, 160.9. HRMS (ESI+) *m/z* Calcd for (M+H)⁺ C₁₈H₁₄N₃O: 316.1198. Found: 316.1218. IR (cm⁻¹): 3241, 1631, 1616, 1553, 1309 1257.

[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)-1*H*-indole-3-carboxamide (**8d**). The title compound was synthesized according to general procedure A using 1*H*-indole-3-carboxylic acid (200 mg, 1.24 mmol) yielding **8d** as a white amorphous solid (64 mg, 17%). ¹H NMR (300MHz, DMF-d₇) δ 4.62 (d, *J* = 5.5 Hz, 2H), 7.19 (m, 2H), 7.3 (dd, *J* = 4, 6.9 Hz, 1H), 7.53 (d, *J* = 7.3 Hz, 1H), 8.05 (s, 1H), 8.26 (d, *J* = 3 Hz, 1H), 8.34 (d, *J* = 8.2 Hz, 1H), 8.6 (t, *J* = 5.2 Hz, 1H), 8.71 (dd, *J* = 2, 4.2 Hz, 1H), 9.02 (dd, *J* = 1.9, 6.8 Hz, 1H), 11.22 (s, 1H). ¹³C NMR (75 MHz, DMF-d₇) δ 28.9, 68.2, 98.1, 106.5, 110 (2C), 111.8, 120.5, 120.9, 121.9, 126.1, 128.1, 133.7, 136.1, 138.5, 147.8, 151.4, 164.4. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₈H₁₄N₃O: 316.1198. Found: 316.1209.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)furan-2-carboxamide (**11a**). Compound **11a** was synthesized according to general procedure B using **10a** (245 mg, 0.80 mmol) and **3** (150 mg, 0.61 mmol). Purification afforded the title compound as a white amorphous solid (104 mg, 40 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.44 (d, *J* = 5.6 Hz, 2H), 7.25 (dd, *J* = 4.1, 6.8 Hz, 1H), 7.44 (s, 1H), 8.05 (s, 1H), 8.65 (dd, *J* = 2.0, 4.2 Hz, 1H), 8.88 (dd, *J* = 2.0, 6.8 Hz, 1H), 9.17 (t, *J* = 5.5 Hz, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 68.8, 96.6, 103.4, 106.3, 110, 118.1, 126.3, 133.8, 138.8, 147.9, 149, 151.6, 155.8. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₉N₄O₂Br₂: 424.9072. Found: 424.9068. IR (cm⁻¹): 3214, 3010, 1681, 1513, 1485, 1284, 1192.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)thiophene-2-carboxamide (**11b**). Compound **11b** was synthesized according to general procedure B using **10b** (343 mg, 1.06 mmol) and **3** (200 mg, 0.82 mmol). Purification afforded the title compound as a beige amorphous solid (174 mg, 48%). ¹H NMR (300MHz, DMSO-d₆) δ 4.48 (d, *J* = 5.2 Hz, 2H), 7.26 (dd, *J* = 4.1, 6.7 Hz, 1H), 7.84 (s, 1H), 8.08 (s, 1H), 8.66 (dd, *J* = 1.9, 4.4 Hz, 1H), 8.90 (dd, *J* = 1.7, 7.0 Hz, 1H), 9.29 (t, *J* = 5.0 Hz, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.8, 69.1, 96.4, 106.3, 110.1, 114, 117, 130.8, 133.9, 139, 140.4, 147.9, 151.6, 159.1. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₉N₄OSBr₂: 440.8843. Found: 440.8851. IR (cm⁻¹): 3217, 2979, 1645, 1512, 1290, 1230.

N-(3-(Imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)-4,5-dimethyl-1*H*-pyrrole-2-carboxamide (**11c**). Compound **11c** was synthesized according to general procedure B using **10c** (129 mg, 0.73 mmol) and **3** (150 mg, 0.61 mmol). Purification afforded the title compound as an orange amorphous solid (94 mg, 52 %). ¹H NMR (300MHz, DMSO-d₆) δ 1.91 (s, 3H), 2.10 (s, 3H), 4.40 (d, *J* = 5.5 Hz, 2H), 6.57 (d, *J* = 2.3 Hz, 1H), 7.24 (dd, *J* = 4.2, 6.8 Hz, 1H), 8.03 (s, 1H), 8.32 (t, *J* = 5.3 Hz, 1H), 8.64 (dd, *J* = 2.0, 4.1 Hz, 1H), 8.87 (dd, *J* = 2.0, 6.9 Hz, 1H), 11.07 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 10.6, 10.7, 29, 68.2, 97.9, 106.5, 110, 112, 114.6, 122.4, 128.7, 133.7, 138.5, 147.8, 151.5, 160.5. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₆H₁₆N₃O: 294.1355. Found: 294.1342. IR (cm⁻¹): 3267, 3205, 1618, 1513, 1486, 1410, 1262, 1230, 1146.

4,5-Dichloro-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**16b**). Compound **16b** was synthesized according to general procedure B using **15b** (106 mg, 0.49 mmol) and **3** (100 mg, 0.41 mmol). Purification afforded the title compound as a pale yellow amorphous solid (28 mg, 21 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.44 (d, *J* = 5.3 Hz, 2H), 6.96 (s, 1H), 7.25 (t, *J* = 4.6 Hz, 1H), 8.05 (s, 2H), 8.66 (m, 1H), 8.78 (t, *J* = 5.2 Hz, 1H), 8.88 (d, *J* = 6.6 Hz, 1H), 12.86 (br s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 68.7, 97.1, 108.1, 108.6, 110, 110.2, 115.4, 124.3, 133.8, 138.8, 150.1, 151.5, 158.8.

N-(3-(Imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)-4,5-diiodo-1*H*-pyrrole-2-carboxamide (**16c**). Compound **16c** was synthesized according to general procedure B using **15c** (196 mg, 0.49 mmol) and **3** (120 mg, 0.49 mmol). Purification afforded the title compound as a white amorphous solid (101 mg, 40 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.43 (d, *J* = 5.2 Hz, 2H), 6.97 (d, *J* = 2.3 Hz, 1H), 7.24 (dd, *J* = 4.2, 6.8 Hz, 1H), 8.04 (s, 1H), 8.66 (m, 2H), 8.88 (d, *J* = 6.8 Hz, 1H), 12.43 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.1, 68.6, 76.2, 84.2, 97.2, 106.3, 110, 118.6, 132.2, 133.8, 138.7, 147.8, 151.5, 158.4. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₀I₂N₃O: 517.8975. Found: 517.8974. IR (cm⁻¹): 3122, 1620, 1553, 1511, 1379, 1277, 1223.

4-Bromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**16d**). Compound **16d** was synthesized according to general procedure B using **15d** and **3**. Purification afforded the title compound (72 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.44 (d, *J* = 4.3 Hz, 2H), 6.91 (m, 1H), 7.03 (m, 1H), 7.25 (dd, *J* = 4.0, 6.7 Hz, 1H), 8.07 (br s, 1H), 8.66 (m, 1H), 8.72 (t, *J* = 4.9 Hz, 1H), 8.89 (d, *J* = 5.9 Hz, 1H), 11.94 (br s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.1, 68.6, 97.3, 95.1, 110.1, 112, 121.7, 126.3, 133.8, 138.6, 147.6, 151.6, 159.4. Some quaternary carbons too broad to be seen due to very slow relaxation.

N-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**16e**). Compound **16e** was synthesized according to general procedure B using **15e** and **3**. Purification afforded the title compound (86 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.44 (d, *J* = 5.6 Hz, 2H), 6.10 (m, 1H), 6.83 (m, 1H), 6.89 (m, 1H), 7.24 (dd, *J* = 4.2, 6.7 Hz, 1H), 8.04 (s, 1H), 8.58 (t, *J* = 5.6 Hz, 1H), 8.65 (dd, *J* = 2.0, 4.1 Hz, 1H), 8.88 (d, *J* = 2.1, 6.8 Hz, 1H), 11.53 (br s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29, 68.3, 97.7, 106.4, 108.7, 110, 110.5, 121.8, 125.6, 133.7, 138.6, 147.8, 151.5, 160.4.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyrazin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20a**). Compound **20a** was synthesized according to general procedure B using **15a** (125 mg, 0.41 mmol) and **19a** (100 mg, 0.41 mmol). Purification afforded the title compound as a yellow amorphous solid (46 mg, 27%). ¹H NMR (300MHz, DMSO-d₆) δ 4.45 (d, *J* = 5.6 Hz, 2H), 6.99 (d, *J* = 2.5 Hz, 1H), 8.08 (d, *J* = 4.5 Hz, 1H), 8.10 (s, 1H), 8.51 (dd, *J* = 1.3, 4.5 Hz, 1H), 8.79 (t, *J* = 5.3 Hz, 1H), 9.16 (d, *J* = 1.3 Hz, 1H), 12.83 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 68.1, 98, 98.4, 105.3, 108.9, 113.1, 118.6, 127.5, 130.5, 138.9, 140, 143.2, 158.8. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₀N₅OBr₂: 425.9211. Found: 425.9236. IR (cm⁻¹): 3280, 2922, 1657, 1513, 1412, 1311, 1221.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*b*]pyridazin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20b**). Compound **20b** was synthesized according to general procedure B using **15a** (192 mg, 0.78 mmol) and **19b** (200 mg, 0.65 mmol). Purification afforded the

title compound as a yellow amorphous solid (24 mg, 9%). ¹H NMR (300MHz, DMSO-d₆) δ 4.43 (d, *J* = 5.5 Hz, 2H), 6.99 (d, *J* = 2.8Hz, 1H), 7.34 (dd, *J* = 4.6, 9 Hz, 1H), 8.07 (s, 1H), 8.2(dd, *J* = 1.5, 9.2Hz, 1H), 8.63 (dd, *J* = 1.5, 4.6 Hz, 1H), 8.78 (t, *J* = 5.5 Hz, 1H), 12.8 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 28.9, 69.1, 96.5, 98, 105.2, 111.6, 113.1, 118.8, 126, 127.6, 138, 139, 144.7, 158.6. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₀N₅OBr₂: 425.9211. Found: 425.9229. IR (cm⁻¹): 3141, 3119, 1659, 1562, 1523, 1293, 1240.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20c**). Compound **20c** was synthesized according to general procedure B using **15a** (282 mg, 0.92 mmol) and **19c** (150 mg, 0.62 mmol). Purification afforded the title compound as a beige amorphous solid (120mg, 46%). ¹H NMR (300MHz, DMSO-d₆) δ 4.45 (d, *J* = 5.4Hz, 2H), 6.99 (d, *J* = 1.9Hz, 1H), 7.11 (t, *J* = 6.8 Hz, 1H), 7.39 (t, *J* = 7.3Hz, 1H), 7.68 (d, *J* = 8.8Hz, 1H), 7.9 (s, 1H), 8.46 (d, *J* = 6.6Hz, 1H), 8.75 (t, *J* = 5.2 Hz, 1H), 12.8 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 69.4, 97.2, 97.9, 105.5, 113.1, 113.6, 117.4, 125.2, 126.2, 127.6, 137.7, 158.7. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₁N₄OBr₂: 422.9279. Found: 422.9325. IR (cm⁻¹): 3137, 3116, 1646, 1565, 1402, 1243.

N-(3-(benzo[*b*]thiophen-2-yl)prop-2-yn-1-yl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**20d**). Compound **20d** was synthesized according to general procedure B using **15a** (100 mg, 0.33 mmol) and **19d** (84 mg, 0.39 mmol). Purification afforded the title compound as a yellow amorphous solid (34 mg, 24 %). ¹H NMR (300MHz, DMF-d₇) δ 4.46 (d, *J* = 5.7 Hz, 2H), 7.10 (s, 1H), 7.45 (m, 2H), 7.66 (s, 1H), 7.89 (m, 1H), 7.99 (m, 1H), 8.97 (t, *J* = 5.7Hz, 1H), 13.09 (bd s, 1H). ¹³C NMR (75 MHz, DMF-d₇) δ 29, 75.2, 93, 98.4, 105.1, 113.5, 122.3, 122.4, 124.2, 125.1, 125.9, 128.4, 129.4, 139.3, 139.8, 159. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₆H₁₁Br₂N₂OS: 436.8959. Found: 436.9008.

N-(3-(1*H*-indol-5-yl)prop-2-yn-1-yl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**20e**). Compound **20e** was synthesized according to general procedure B using **15a** (200 mg, 0.65 mmol). Purification afforded the title compound as a beige amorphous solid (25 mg, 10 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.28 (d, *J* = 5.6 Hz, 2H), 6.42 (s, 1H), 7 (d, *J* = 2.8 Hz, 1H), 7.11 (dd, *J* = 1.4, 8.4 Hz, 1H), 7.37 (m, 1H), 7.39 (m, 1H), 7.64 (s, 1H), 8.66 (t, *J* = 5.4 Hz, 1H), 11.26 (s, 1H), 12.77 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 28.7, 83.7, 97.9, 101.2, 105, 111.7, 112.3, 113, 123.7, 124.3, 126.5, 127.5, 127.7, 135.5, 158.5. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₆H₁₂Br₂N₃O: 421.9327. Found: 421.9356. IR (cm⁻¹): 3415, 3154, 1639, 1556, 1504, 1236.

4,5-Dibromo-*N*-(3-(thiophen-2-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20f**). Compound **20f** was synthesized according to general procedure B using **15a** (200 mg, 0.65 mmol) and **19f** (0.1 mL, 0.92 mmol). Purification afforded the title compound as a yellow amorphous solid (92 mg, 36 %). ¹H NMR (300MHz, DMF-d₇) δ 4.40 (d, *J* = 5.6 Hz, 2H), 7.09 (dd, *J* = 3.6, 5.3 Hz, 1H), 7.11 (d, *J* = 2.8 Hz, 1H), 7.31 (dd, *J* = 1.2, 3.7 Hz, 1H), 7.61 (dd, *J* = 1.2, 5.2 Hz, 1H), 8.76 (t, *J* = 5.5 Hz, 1H), 12.96 (s, 1H). ¹³C NMR (75 MHz, DMF-d₇) δ 29.2, 75.3, 90.8, 98.6, 105.3, 113.4, 122.6, 127.7, 128.3, 128.5, 132.7, 159. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₂H₉Br₂N₂OS: 390.8761. Found: 390.8794.

4,5-Dibromo-*N*-(3-(pyridin-4-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20g**). Compound **20g** was synthesized according to

general procedure B using **15a** (117 mg, 0.38 mmol) and **19g** (106 mg, 0.6 mmol). Purification afforded the title compound as a light yellow amorphous solid (43 mg, 22%). ¹H NMR (300 MHz, DMSO-d₆) δ 4.32 (d, *J* = 5.7 Hz, 2H), 6.98 (s, 1H), 7.39 (d, *J* = 6 Hz, 2H), 8.57 (m, 2H), 8.72 (t, *J* = 5.4 Hz, 1H), 12.79 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 28.6, 79.2, 92, 98, 105.2, 113.1, 125.5, 127.5, 130.1, 149.9, 158.6. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₃H₁₀Br₂N₃O: 383.9165. Found 383.9187.

4,5-Dibromo-*N*-(3-(pyridin-2-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20h**). Compound **20h** was synthesized according to general procedure B using **15a** (153 mg, 0.5 mmol) and **19h** (123 mg, 0.6 mmol). Purification afforded the title compound as a light yellow amorphous solid (50 mg, 25%). ¹H NMR (300 MHz, DMSO-d₆) δ 4.31 (d, *J* = 5.5 Hz, 2H), 6.98 (d, *J* = 2.9 Hz, 1H), 7.37 (ddd, *J* = 1.2, 4.9, 7.7 Hz, 1H), 7.49 (dt, *J* = 1.1, 7.7 Hz, 1H), 7.79 (td, *J* = 1.9, 7.7 Hz, 1H), 8.54 (d, *J* = 4.8 Hz, 1H), 8.72 (t, *J* = 5.5 Hz, 1H), 12.79 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 28.5, 81.5, 86.5, 97.9, 105.2, 113.1, 123.4, 127, 127.5, 136.8, 142.1, 149.1, 158.7. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₃H₁₀Br₂N₃O: 383.9165. Found 383.9167.

4,5-Dibromo-*N*-(3-(5-fluoropyridin-2-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20i**). Compound **20i** was synthesized according to general procedure B using **15a** (153 mg, 0.5 mmol) and **19i** (106 mg, 0.6 mmol). Purification afforded the title compound as a light yellow amorphous solid (48 mg, 25%). ¹H NMR (300 MHz, DMSO-d₆) δ 4.30 (d, *J* = 5.4 Hz, 2H), 6.98 (d, *J* = 2.4 Hz, 1H), 7.59 (dd, *J* = 4.5, 8.7 Hz, 1H), 7.74 (dt, *J* = 3.0, 9.3 Hz, 1H), 8.55 (m, 1H), 8.71 (t, *J* = 5.4 Hz, 1H), 12.78 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 28.4, 80.5, 86.4, 98, 105.2, 113, 123.9, 127.5, 128.6, 138.3, 138.5, 156.7, 158.6. Some quaternary carbons are too broad to be seen due to very slow relaxation. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₃H₉Br₂FN₃O: 401.9070. Found 401.9090.

4,5-Dibromo-*N*-(3-(5-bromopyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20j**). Compound **20j** was synthesized according to general procedure B using **15a** (142 mg, 0.46 mmol) and **19j** (110 mg, 0.46 mmol). Purification afforded the title compound as a light yellow amorphous solid (61 mg, 27%). ¹H NMR (300 MHz, DMSO-d₆) δ 4.32 (d, *J* = 5.4 Hz, 2H), 6.97 (d, *J* = 2.7 Hz, 1H), 8.15 (t, *J* = 1.8 Hz, 1H), 8.60 (d, *J* = 1.5 Hz, 1H), 8.70 (m, 2H), 12.78 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 28.6, 77.1, 91.9, 98, 105.2, 113.1, 119.9, 120.9, 127.5, 140.7, 149.7, 150.2, 158.6. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₃H₇Br₂N₃O: 459.8124. Found 459.8113.

4,5-Dichloro-*N*-(3-(imidazo[1,2-*a*]pyrazin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20k**). Compound **20k** was synthesized according to general procedure B using **15b** (133 mg, 0.61 mmol) and **19a** (100 mg, 0.41 mmol). Purification afforded the title compound as a yellow amorphous solid (90 mg, 66%, %). ¹H NMR (300MHz, DMSO-d₆) δ 4.46 (d, *J* = 5.3 Hz, 2H), 6.96 (s, 1H), 8.08 (m, 2H), 8.50 (dd, *J* = 1.4, 4.7 Hz, 1H), 8.79 (t, *J* = 5.5 Hz, 1H), 9.15 (s, 1H), 12.85 (br s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 68.1, 98.4, 108.1, 109.2, 110.2, 115.4, 118.6, 124.2, 130.5, 138.9, 140, 143.2, 158.8. HRMS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₀Cl₂N₅O: 334.0262. Found: 334.0277. IR (cm⁻¹): 3156, 3126, 1656, 1523, 1429, 1313.

N-(3-(imidazo[1,2-*a*]pyrazin-3-yl)prop-2-yn-1-yl)-4,5-diiodo-1*H*-pyrrole-2-carboxamide (**20l**). Compound **20l** was synthesized

according to general procedure B using **15c** (149 mg, 0.37 mmol) and **19a** (70mg, 0.29 mmol). Purification afforded the title compound as a beige amorphous solid (95mg, 64%). ¹H NMR (300MHz, DMSO-d₆) δ 4.44 (d, *J* = 5.5 Hz, 2H), 6.97 (d, *J* = 2.6 Hz, 1H), 8.1 (s, 2H), 8.50 (d, *J* = 4.5 Hz, 1H), 8.68 (t, *J* = 5.2 Hz, 1H), 9.16 (br s, 1H), 12.43 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.1, 68.1, 76.3, 84.2, 98.4, 109, 118.6, 130.4, 132.1, 138.9, 140.1, 143.2, 158.4. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₀I₂N₅O: 517.8975. Found: 517.8960. IR (cm⁻¹): 3154, 1641, 1553, 1346, 1310, 1226.

4,5-Dichloro-*N*-(3-(imidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**20m**). Compound **20m** was synthesized according to general procedure B using **15b** (110 mg, 0.51 mmol) and **19c** (124 mg, 0.51 mmol). Purification afforded the title compound as a white amorphous solid (63 mg, 37%). ¹H NMR (300MHz, DMSO-d₆) δ 4.45 (d, *J* = 5.3 Hz, 2H), 6.96 (s, 1H, H₄), 7.11 (t, *J* = 6.9 Hz, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 9.1 Hz, 1H), 7.89 (s, 1H), 8.45 (d, *J* = 6.7 Hz, 1H), 8.78 (t, *J* = 5.6 Hz, 1H), 12.85 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 69.5, 97.2, 108.1, 110.2, 115.4, 108.1, 113.6, 117.4, 124.3, 125.3, 126.2, 137.6, 144.9, 158.8. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₁Cl₂N₄O: 333.0310. Found: 333.0354. IR (cm⁻¹): 3151, 1650, 1524, 1431, 1306, 1255.

N-(3-(imidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-4,5-diiodo-1*H*-pyrrole-2-carboxamide (**20n**). Compound **20n** was synthesized according to general procedure B using **15c** (171 mg, 0.43 mmol) and **19c** (80 mg, 0.33 mmol). Purification afforded the title compound as a beige amorphous solid (55 mg, 33%). ¹H NMR (300MHz, DMSO-d₆) δ 4.43 (d, *J* = 5.5 Hz, 2H), 6.98 (d, *J* = 2.7 Hz, 1H), 7.11 (t, *J* = 6.7 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.89 (br s, 1H), 8.45 (d, *J* = 6.5 Hz, 1H), 8.66 (t, *J* = 5.3 Hz, 1H), 12.43 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 69.4, 76.2, 84.1, 97.3, 107.8, 113.6, 117.4, 118.6, 125.3, 126.2, 132.2, 137.6, 158.4. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₁I₂N₄O: 516.9022. Found: 516.906. IR (cm⁻¹): 3148, 1643, 1562, 1383, 1305, 1231.

4,5-Dibromo-*N*-(3-(6-chloroimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**24a**). Compound **24a** was synthesized according to general procedure B using **15a** (198 mg, 0.65 mmol) and **23a** (150 mg, 0.54 mmol). Purification afforded the title compound as a beige amorphous solid (75 mg, 31 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.46 (d, *J* = 5.5 Hz, 2H), 6.99 (d, *J* = 2.8 Hz, 1H), 7.45 (dd, *J* = 1.9, 9.4 Hz, 1H), 7.73 (d, *J* = 9.4 Hz, 1H), 7.94 (s, 1H), 8.62 (s, 1H), 8.74 (t, *J* = 5.4 Hz, 1H), 12.80 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 68.8, 98, 98, 105.2, 108.7, 113.1, 118.3, 120.8, 123.2, 127.6, 127.1, 138.2, 143.4, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₀N₄OBr₂Cl: 456.8889. Found: 456.8891. IR (cm⁻¹): 3158, 1655, 1561, 1520, 1489, 1308, 1242.

4,5-Dibromo-*N*-(3-(7-chloroimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**24b**). Compound **24b** was synthesized according to general procedure B using **15a** (169 mg, 0.55 mmol) and **23b** (62 mg, 0.22 mmol). Purification afforded the title compound as a pale yellow amorphous solid (20 mg, 20 %). ¹H NMR (300MHz, DMSO) δ 4.44 (d, *J* = 5.4 Hz, 2H), 6.99 (d, *J* = 2.9 Hz, 1H) 7.18 (dd, *J* = 2.1, 7.3 Hz, 1H), 7.88 (s, 1H), 7.91 (s, 1H) 8.46 (d, *J* = 7.3 Hz, 1H), 8.75 (t, *J* = 5.3 Hz, 1H), 12.80 (s, 1H). ¹³C NMR (75 MHz, DMSO) δ 29.2, 68.9, 97.6, 98, 105.2, 108.1, 113.1, 114.7, 116.3, 126.2, 127.5, 131.3, 138.4, 144.6, 158.7.

HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₀N₄OBr₂Cl: 456.8889. Found: 456.8879.

4,5-Dibromo-*N*-(3-(8-bromoimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**24c**). Compound **24c** was synthesized according to general procedure B using **15a** (313 mg, 1.02 mmol) and **23c** (220 mg, 0.68 mmol). Purification afforded the title compound as a beige amorphous solid (113 mg, 33 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.44 (d, *J* = 5.4 Hz, 2H), 6.99 (d, *J* = 2.9 Hz, 1H), 7.03 (t, *J* = 6.9 Hz, 1H), 7.74 (dd, *J* = 0.9, 7.2 Hz, 1H), 7.94 (s, 1H), 8.48 (dd, *J* = 0.9, 6.8 Hz, 1H), 8.75 (t, *J* = 5.3 Hz, 1H), 12.80 (s, 1H). ¹³C NMR (75 MHz, -d₆) δ 29.2, 69, 97.6, 98, 105.2, 109.6, 110.9, 113.1, 113.9, 125, 127.5, 128.7, 137.7, 142.5, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₀N₄OBr₃: 498.8405. Found: 498.8423. IR (cm⁻¹): 3135, 3116, 1642, 1560, 1521, 1398, 1240, 1212.

4,5-Dibromo-*N*-(3-(6-bromoimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**24d**). Compound **24d** was synthesized according to general procedure B using **15a** (549 mg, 1.79 mmol) and **23d** (386 mg, 1.2 mmol). Purification afforded the title compound as a pale yellow amorphous solid (32 mg, 5 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.46 (d, *J* = 5.4 Hz, 2H), 6.99 (s, 1H), 7.52 (dd, *J* = 1.9, 9.6 Hz, 1H), 7.68 (d, *J* = 9.6 Hz, 1H), 7.91 (s, 1H), 8.67 (s, 1H), 8.74 (t, *J* = 5.3 Hz, 1H), 12.81 (br s, 1H). HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₀N₄OBr₃: 500.8384. Found: 500.8393. IR (cm⁻¹): 3152, 1654, 1561, 1518, 1407, 1310, 1222.

4,5-Dibromo-*N*-(3-(7-(trifluoromethyl)imidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**24e**). Compound **24e** was synthesized according to general procedure B using **15a** (286 mg, 0.94 mmol) and **23e** (122 mg, 0.62 mmol). Purification afforded the title compound as a pale yellow amorphous solid (173 mg, 57 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.46 (d, *J* = 5.6 Hz, 2H), 6.99 (d, *J* = 1.8 Hz, 1H), 7.37 (d, *J* = 7.1 Hz, 1H), 8.11 (s, 1H), 8.20 (s, 1H), 8.65 (d, *J* = 7.1 Hz, 1H), 8.77 (t, *J* = 5.4 Hz, 1H), 12.80 (br s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 68.7, 98, 98.1, 105.2, 108.9 (d, *J* = 3.0 Hz), 109.4, 113.1, 115.6 (d, *J* = 5.0 Hz), 121.6, 125.7 (t, *J* = 36.7 Hz), 126.8, 127.5, 139.5, 143.3, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₆H₁₀N₄OBr₂F₃: 490.9147. Found: 490.9153. IR (cm⁻¹): 3154, 1638, 1564, 1314, 1168, 1131, 1052.

4,5-Dibromo-*N*-(3-(6-fluoroimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**24f**). Compound **24f** was synthesized according to general procedure B using **15a** (214 mg, 0.70 mmol) and **23f** (122 mg, 0.47 mmol). Purification afforded the title compound as a pale yellow amorphous solid (129 mg, 63 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.45 (d, *J* = 5.4 Hz, 2H), 6.99 (s, 1H), 7.49 (t, *J* = 9.2 Hz, 1H), 7.77 (dd, *J* = 5.1, 9.3 Hz, 1H), 7.94 (s, 1H), 8.63 (s, 1H), 8.74 (t, *J* = 4.9 Hz, 1H), 12.82 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.3, 69.1, 98, 98.1, 105.2, 108.9, 112.3 (d, *J* = 41.6 Hz), 113.2, 118.0 (d, *J* = 25.7 Hz), 118.4 (d, *J* = 9.3 Hz), 127.6, 138.6, 152-155.1 (d, *J* = 235.8 Hz), 158.7. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₀N₄OBr₂F: 440.9188. Found: 440.9185. IR (cm⁻¹): 3148, 1653, 1562, 1519, 1499, 1404, 1309, 1221.

4,5-Dibromo-*N*-(3-(6-methylimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**24g**). Compound **24g** was synthesized according to general procedure B using **15a** (376mg, 1.23 mmol) and **23g** (211 mg, 0.82 mmol). Purification afforded

the title compound as a pale yellow amorphous solid (105 mg, 30 %). ¹H NMR (300MHz, DMSO-d₆) δ 2.34 (s, 3H), 4.45 (d, *J* = 5.3 Hz, 2H), 7.0 (s, 1H), 7.25 (d, *J* = 8.7 Hz, 1H), 7.59 (d, *J* = 8.7 Hz, 1H), 7.82 (s, 1H), 8.25 (s, 1H), 8.74 (t, *J* = 5.2 Hz, 1H), 12.80 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 17.5, 29.2, 69.7, 97.1, 98, 105.2, 107.4, 113.1, 116.8, 122.7, 123.1, 127.6, 129.1, 137.5, 144.2, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₆H₁₃N₄OBr₂: 436.9436. Found: 436.9457. IR (cm⁻¹): 3144, 1651, 1564, 1404, 1308, 1243, 1162.

4,5-Dibromo-*N*-(3-(7-methylimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**24h**). Compound **24h** was synthesized according to general procedure B using **15a** (457mg, 1.5 mmol) and **23h** (257 mg, 1.0 mmol). Purification afforded the title compound as a beige amorphous solid (100 mg, 23 %). ¹H NMR (300MHz, DMSO-d₆) δ 2.39 (s, 3H), 4.43 (d, *J* = 5.3 Hz, 2H), 6.96 (d, *J* = 6.9 Hz, 1H), 6.99 (s, 1H), 7.47 (br s, 1H), 8.35 (br s, 1H), 8.75 (t, *J* = 5.4 Hz, 1H), 12.81 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 20.7, 29.2, 69, 97, 97.9, 105.1, 113.1, 115.8, 116, 124.5, 127.6, 136.9, 158.7. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₆H₁₃N₄OBr₂: 436.9436. Found: 436.9424. IR (cm⁻¹): 3136, 1634, 1566, 1400, 1244, 1190.

N-(3-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)prop-2-yn-1-yl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**29**). Compound **29** was synthesized according to general procedure B using **15a** (200 mg, 0.65 mmol) and **27** (301 mg, 0.78 mmol). Purification afforded the intermediate **28** as a brown amorphous solid (221 mg, 60%). **28** (125 mg, 0.22 mmol, 1.2 eq.) was dissolved in 2 mL of dry THF. A solution of TBAF (187 mg, 0.67 mmol, 3eq.) in 0.7 mL of dry THF was added to the mixture. It was stirred at room temperature for 3 h and then at 50°C for 2 h until completion of reaction. The solvent was removed under vacuum. The crude was purified by silica gel flash chromatography (elution by gradient from 100 to 90/10 DCM/MeOH) to afford the deprotected compound **29** as a beige amorphous solid (57 mg, 61%). ¹H NMR (300MHz, DMSO-d₆) δ 4.33 (d, *J* = 5.4 Hz, 2H), 7.0 (s, 1H), 7.16 (dd, *J* = 4.5, 7.9 Hz, 1H), 7.78 (s, 1H), 7.94 (d, *J* = 7.9 Hz, 1H), 8.28 (d, *J* = 4.4 Hz, 1H), 8.66 (t, *J* = 5.5 Hz, 1H), 12.0 (s, 1H), 12.76 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29, 75.4, 87.5, 94.7, 97.9, 105, 112.9, 116.3, 120.3, 127.8, 127, 130.1, 143.8, 147.6, 158.5. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₁N₄OBr₂: 422.9279. Found: 422.9273. IR (cm⁻¹): 3200, 1618, 1517, 1410, 1293, 1214.

N-(3-(8-acetamidoimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**35**). Compound **35** was synthesized according to general procedure B using **15a** (264 mg, 0.86 mmol) and **33a** (200 mg, 0.66 mmol). Purification afforded the title compound as a beige amorphous solid (110mg, 35%). ¹H NMR (300MHz, DMSO-d₆) δ 2.21 (s, 3H), 4.44 (d, *J* = 5.5 Hz, 2H), 6.99 (d, *J* = 2.7 Hz, 1H), 7.06 (t, *J* = 7Hz, 1H), 7.87 (s, 1H), 8.09 (d, *J* = 7.6Hz, 1H), 8.16 (dd, *J* = 1, 7Hz, 1H), 8.75 (t, *J* = 5.5Hz, 1H), 10.1 (s, 1H), 12.81 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 24, 29.2, 69.3, 97.2, 98, 105.2, 108.7, 112.5, 113.1, 113.9, 119.6, 127.6, 127.9, 136.1, 138.8, 158.7, 169.9. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₇H₁₄N₅O₂Br₂: 479.9494. Found: 479.9506. IR (cm⁻¹): 3115, 1636, 1552, 1522, 1412, 1276, 1222.

N-(3-(8-aminoimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**37**). Compound **37** was synthesized according to general procedure B using **15a** (466 mg, 1.52 mmol) and **33b** (350mg, 0.76 mmol). Purification afforded **36** as a brown amorphous solid (321 mg, 66%). Then **36** (320 mg,

0.5 mmol, 1eq.) was dissolved in 2.5 mL of dry DCM under argon at 0°C. TFA (0.37 mL, 5mmol, 10 eq.) was added dropwise to the reaction mixture. After 2 h, 10 eq. of TFA (0.37 mL) were again added to the mixture at -5°C. The reaction mixture was stirred at -5°C 2 h and then 2h at room temperature. The mixture was quenched with an aqueous solution of Na₂CO₃ and extracted with DCM. Collected organic layers were dried over MgSO₄, filtered and concentrated under vacuum. Flash chromatographic purification (elution by gradient from 100 to 95/5 DCM/MeOH) afforded compound **37** as a beige amorphous solid (153 mg, 70 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.42 (d, *J* = 6.6 Hz, 2H), 5.79 (s, 2H), 6.37 (dd, *J* = 1.0, 7.5 Hz, 1H), 6.83 (t, *J* = 6.9 Hz, 1H), 6.99 (s, 1H), 7.7 (m, 2H), 8.74 (t, *J* = 5.4 Hz, 1H), 12.8 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 70.1, 96.5, 98, 101.9, 105.2, 108, 112.7, 113.1, 114.9, 127.6, 135.6, 137.6, 138.9, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₂N₅OBr₂: 437.9388. Found: 437.9375. IR (cm⁻¹): 3376, 3133, 1645, 1557, 1402, 1240.

N-(3-(6-aminoimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**38**). Compound **38** was synthesized according to general procedure B using **15a** and **33c** (358 mg, 0.78 mmol). The protected intermediate was directly engaged in a TFA (1 mL) deprotection in DCM at 0°C then at room temperature. After 24h of reaction, the reaction mixture was quenched with Na₂CO₃ and concentrated under vacuum. The crude product was purified by silica gel flash chromatography (elution by gradient from 100 to 95/5 DCM/MeOH) to afford a brown amorphous solid (38 mg, 11% over two steps). ¹H NMR (500MHz, DMF-d₇) δ 4.53 (s, 2H), 5.26 (s, 2H), 7.10 (d, *J* = 9.5Hz, 1H), 7.14 (s, 1H), 7.44 (d, *J* = 9.2Hz, 1H), 7.66 (s, 1H), 7.86 (s, 1H), 8.81 (t, *J* = 4.6Hz, 1H), 12.95 (s, 1H). ¹³C NMR (75 MHz, DMF-d₇) δ 29.8, 70.8, 96.7, 98.7, 105.3, 106.6 (two carbons), 113.6, 117.4, 21.6, 128.7, 137, 138.2, 142.2, 159.2. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₂N₅OBr₂: 437.9388. Found: 437.9388. IR (cm⁻¹): 3149, 2920, 2849, 1628, 1565, 1521, 1500, 1414, 1203.

N-(3-(8-amino-6-bromoimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**43**). Compound **43** was synthesized according to general procedure B using **15a** (238 mg, 0.78 mmol) and **33d** (209 mg, 0.39 mmol) to afford the protected intermediate, which was dissolved in 2 mL of DCM. The mixture was cooled with an ice bath and TFA was added (0.2 mL, 10 eq.). The mixture was stirred 8 h at room temperature. The reaction was quenched with the addition of NaOH 10 %, the resulting precipitate was filtered and rinsed with water and then DCM yielding a beige amorphous solid **43** (119 mg, 59% over two steps). ¹H NMR (300MHz, DMSO-d₆) δ 4.43 (d, *J* = 5.6 Hz, 2H), 6.18 (br s, 2H), 6.48 (d, *J* = 1.7 Hz, 1H), 6.99 (d, *J* = 2.8 Hz, 1H), 7.73 (s, 1H), 7.83 (d, *J* = 1.6 Hz, 1H), 8.72 (t, *J* = 5.5 Hz, 1H), 12.80 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 69.3, 97.3, 98, 104.6, 105.2, 108.6, 109.5, 112, 113.1, 127.6, 135.5, 137.1, 138.2, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₁N₅OBr₃: 515.8493. Found: 515.8485. IR (cm⁻¹): 3385, 3147, 1649, 1600, 1561, 1397, 1239, 1212.

N-(3-(8-aminoimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-4,5-dichloro-1*H*-pyrrole-2-carboxamide (**44**). Compound **44** was synthesized according to general procedure B using **15b** (150 mg, 0.69 mmol) and **33b** (317 mg, 0.69 mmol). Then the intermediate was dissolved in 2 mL of dry DCM under argon at 0°C. TFA (0.63 mL, 17 eq.) was added dropwise to the reaction mixture. After 2 h of reaction, 10 eq. of TFA (0.37 mL) were added to the mixture at

0°C. The reaction mixture was stirred at room temperature 7 h. The mixture was quenched with an aqueous solution of NaHCO₃ and extracted with DCM. Collected organic layers were dried over MgSO₄, filtered and concentrated under vacuum. Flash chromatographic purification (elution by gradient from 100 to 95/5 DCM/MeOH) afforded compound **44** as a beige amorphous solid (60 mg, 25 % over two steps). ¹H NMR (300MHz, DMSO-d₆) δ 4.43 (d, *J* = 5.4 Hz, 2H), 5.78 (br s, 2H), 6.38 (dd, *J* = 0.9, 7.6 Hz, 1H), 6.83 (t, *J* = 6.9 Hz, 1H), 6.96 (s, 1H, H₄), 7.70 (d, *J* = 6.9 Hz, 1H), 7.71 (s, 1H), 8.76 (t, *J* = 5.4 Hz, 1H), 12.84 (br s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 70.1, 96.5, 101.9, 108, 108.1, 110.1, 112.7, 114.9, 115.4, 124.3, 135.6, 137.5, 138.9, 158.8. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₂Cl₂N₅O: 348.0419. Found: 348.0457. IR (cm⁻¹): 3149, 1651, 1607, 1514, 1493, 1431.

N-(3-(8-aminoimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-4,5-dihydro-1*H*-pyrrole-2-carboxamide (**45**). Compound **45** was synthesized according to general procedure B using **15c** (180 mg, 0.45 mmol) and **33b** (207 mg, 0.45 mmol). Then the intermediate was dissolved in 2 mL of dry DCM under argon at 0°C. TFA (0.67 mL, 20 eq.) was added dropwise to the reaction mixture. After 2 h of reaction, 10 eq. of TFA (0.37 mL) were added to the mixture at 0°C. The reaction mixture was stirred 5 h at room temperature. The mixture was quenched with an aqueous solution of Na₂CO₃ and extracted with EtOAc. Collected organic layers were dried over MgSO₄, filtered and concentrated under vacuum. Flash chromatographic purification (elution by gradient from 100 to 90/10 DCM/MeOH) afforded compound **45** as a beige amorphous solid (97 mg, 41 % over two steps). ¹H NMR (300MHz, DMSO-d₆) δ 4.41 (d, *J* = 5.3 Hz, 2H), 5.78 (br s, 2H), 6.38 (d, *J* = 7.4 Hz, 1H), 6.82 (t, *J* = 7.4 Hz, 1H), 6.97 (d, *J* = 2.2 Hz, 1H), 7.69 (d, *J* = 5.7 Hz, 1H), 7.71 (s, 1H), 8.65 (t, *J* = 5.2 Hz, 1H), 12.42 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 70, 76.2, 84.1, 96.6, 101.9, 108.2, 112.7, 114.9, 118.6, 132.2, 135.5, 137.5, 139, 158.4. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₂I₂N₅O: 531.9131. Found: 531.9142. IR (cm⁻¹): 3126, 1628, 1552, 1494, 1375, 1288.

4,5-Dibromo-*N*-(3-(6-morpholinoimidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**48**). Compound **48** was synthesized according to general procedure B using **15a** (383mg, 1.3 mmol) and **46** (206mg, 0.63 mmol). Purification afforded the title compound as a beige amorphous solid (71mg, 22 %). ¹H NMR (300MHz, DMSO-d₆) δ 3.05 (t, *J* = 4.5 Hz, 4H), 3.75 (t, *J* = 4.5 Hz, 4H), 4.43 (d, *J* = 5.3 Hz, 2H), 6.99 (s, 1H), 7.38 (d, *J* = 9.9 Hz, 1H), 7.57 (d, *J* = 9.9 Hz, 1H), 7.64 (s, 1H), 7.76 (s, 1H), 8.79 (t, *J* = 5.3 Hz, 1H), 12.79 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 49.3, 65.8, 29.3, 69.9, 97.5, 98, 105.1, 107.8, 108.8, 113, 117.2, 121.7, 127.6, 136.9, 140.5, 141.7, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₉H₁₈N₅O₂Br₂: 507.9807. Found: 507.9807. IR (cm⁻¹): 3111, 1642, 1567, 1505, 1402, 1244, 1167, 1118.

4,5-Dibromo-*N*-(3-(6-(piperazin-1-yl)imidazo[1,2-*a*]pyridin-3-yl)prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (**50**). Compound **50** was synthesized according to general procedure B using **15a** (296 mg, 0.97 mmol) and **47** (207 mg, 0.48 mmol). Purification afforded **49** as a yellow amorphous solid. Compound **49** was further dissolved in 4 mL of DCM and cooled with an ice bath and then HCl (4N dioxane) was added (0.6 mL) with 2 mL of methanol. The reaction mixture was stirred overnight at room temperature. The solvent was removed under vacuum, the resulting solid rinsed with DCM and MeOH to afford the salt **50** a white amorphous solid (123 mg, 44% over two steps). ¹H NMR (300MHz,

DMSO-d₆) δ 3.27 (s, 4H), 3.54 (s, 4H), 4.45 (d, *J* = 5.3 Hz, 2H), 7.08 (d, *J* = 2.7 Hz, 1H), 7.92 (m, 2H), 8.03 (s, 1H), 8.41 (s, 1H), 9.28 (t, *J* = 5.2 Hz, 1H), 9.64 (br s, 2H), 12.91 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 29.2, 42.2, 45.5, 66.7, 98.1, 99.5, 105, 109.3, 113.3, 113.8, 127.2, 127.6, 127.7, 135.6, 142.1, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₉H₁₉N₆OBr₂: 506.9967. Found: 506.9964. IR (cm⁻¹): 3275, 2928, 1640, 1521, 1390, 1322, 1237.

General Procedure C for Amidation (S5-S7).

Boc-protected amine **52-54** (1 eq.) was dissolved in 2-6 mL of concentrated HCl under argon at room temperature. The reaction mixture was stirred 3-5 h until completion of reaction. The solvent was removed under vacuum. The residue was taken up with DMF and NaHCO₃ to form the free base. **14a** (1eq.) was added and the mixture was stirred overnight at room temperature. It was concentrated under vacuum and the product was purified by silica gel flash chromatography (elution by gradient from 100 to 95/5 DCM/MeOH) to yield the desired alkene.

(*E*)-4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)allyl)-1*H*-pyrrole-2-carboxamide (**55**). Compound **55** was synthesized according to general procedure C using **14a** (108mg, 0.29 mmol) and **52** (80 mg, 0.29 mmol). Purification afforded the title compound as a yellow amorphous solid (52 mg, 42 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.1 (t, *J* = 5.7 Hz, 1H), 6.38 (dt, *J* = 5.7, 16.1 Hz, 1H), 6.89 (d, *J* = 16.1 Hz, 1H), 7 (s, 1H), 7.12 (dd, *J* = 4.4, 7 Hz, 1H), 8.01 (s, 1H), 8.42 (t, *J* = 5.7 Hz, 1H), 8.52 (dd, *J* = 1.9, 4.1 Hz, 1H), 9.03 (dd, *J* = 1.9, 7.1 Hz, 1H), 12.7 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 40.8, 97.8, 104.6, 109, 112.8, 115.6, 121.5, 127.3, 128.1, 132.4, 138.8, 148, 149.4, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₂N₅O₂Br₂: 425.9388. Found: 425.9388. IR (cm⁻¹): 3102, 3048, 1619, 1562, 1511, 1243, 1151.

(*E*)-4,5-dibromo-*N*-(3-(imidazo[1,2-*a*]pyrazin-3-yl)allyl)-1*H*-pyrrole-2-carboxamide (**56**). Compound **56** was synthesized according to general procedure C using **14a** (97 mg, 0.26 mmol) and **53** (72 mg, 0.26 mmol). Purification afforded the title compound as a beige amorphous solid (57 mg, 51 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.20 (t, *J* = 5.9 Hz, 1H), 6.59 (dt, *J* = 6, 16.1 Hz, 1H), 7.06 (d, *J* = 16.1 Hz, 1H), 7.12 (s, 1H), 7.97 (d, *J* = 4.6 Hz, 1H), 8.14 (s, 1H), 8.45 (t, *J* = 5.9 Hz, 1H), 8.7 (d, *J* = 5 Hz, 1H), 9.04 (s, 1), 12.86 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 41.1, 98.6, 104.9, 113.1, 115.7, 117.9, 125.3, 129.6, 129.1, 130.1, 133.7, 141.2, 143.6, 159.3. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₂N₅O₂Br₂: 425.9388. Found: 425.9401. IR (cm⁻¹): 3377, 2647, 1624, 1511, 1487, 1410, 1313, 1161.

(*E*)-4,5-dibromo-*N*-(3-(imidazo[1,2-*b*]pyridazin-3-yl)allyl)-1*H*-pyrrole-2-carboxamide (**57**). Compound **57** was synthesized according to general procedure C using **14a** (332 mg, 0.9 mmol) and **54** (224 mg, 0.82 mmol). Purification afforded the title compound as a beige amorphous solid (125 mg, 40 %). ¹H NMR (300MHz, DMSO-d₆) δ 4.10 (t, *J* = 4.8 Hz, 1H), 6.82 (m, 2H), 7.0 (s, 1H), 7.23 (dd, *J* = 4.6, 9.2 Hz, 1H), 8.0 (s, 1H), 8.13 (dd, *J* = 1.6, 9.2 Hz, 1H), 8.49 (t, *J* = 5.6 Hz, 1H), 8.58 (dd, *J* = 1.5, 4.5 Hz, 1H), 12.73 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ 40.6, 97.9, 104.7, 112.7, 115.5, 116.9, 125.7, 125.8, 127.8, 128, 132.7, 139.3, 143.9, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₄H₁₂N₅O₂Br₂: 425.9388. Found: 425.9386. IR (cm⁻¹): 3107, 3065, 1644, 1565, 1522, 1317, 1239, 1151.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-2-yl)phenyl)-1*H*-pyrrole-2-carboxamide (**63**). **61** (50 mg, 0.24 mmol, 1 eq.) was

dissolved in 1 mL of Et₃N under argon. **14a** (114 mg, 0.31 mmol, 1.3 eq.) was added to the mixture. The reaction mixture was stirred at 60°C overnight. The solvent was removed under vacuum and the product was purified by silica gel flash chromatography (elution by gradient from 100 to 98/2 DCM/MeOH) to yield **63** as a yellow amorphous solid (54 mg, 49 %). ¹H NMR (300MHz, DMSO-d₆) δ7.07 (dd, *J* = 4.1, 6.9 Hz, 1H), 7.30 (d, *J* = 2.7 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.69 (dt, *J* = 1.1, 8.2 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 1H), 8.33 (s, 1H), 8.35 (t, *J* = 1.8 Hz, 1H), 8.54 (dd, *J* = 2.1, 4.1 Hz, 1H), 8.98 (dd, *J* = 2.1, 6.7Hz, 1H), 9.99 (s, 1H), 12.91 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ98.2, 105.9, 107.5, 108.9, 113.9, 117.3, 119.7, 120.8, 127.9, 129.2, 133.9, 135.1, 139.4, 145.1, 148, 150.4, 157.4. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₇H₁₂N₅OBr₂: 461.9388. Found: 461.9412. IR (cm⁻¹): 3149, 1610, 1558, 1400, 1223, 1198.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*b*]pyridazin-2-yl)phenyl)-1*H*-pyrrole-2-carboxamide (**64**). 4,5-Dibromo-1*H*-pyrrole-2-carboxylic acid (100 mg, 0.37 mmol, 1 eq.) was dissolved in 1 mL of anhydrous DCM and a few drops of DMF under argon atmosphere. (COCl)₂ (130 μL, 1.49 mmol, 4eq.) was added to the mixture. The mixture was stirred overnight. The solvent was removed under vacuum and 1 mL of pyridine and 2 mL of DCM were added followed by **62** (86 mg, 0.41 mmol, 1.1eq.). It was stirred at room temperature for 6 h. The reaction mixture was concentrated with toluene and purified by silica gel flash chromatography (elution by gradient from 100 to 90/10/MeOH) to afford **64** as a beige amorphous solid (77mg, 72%). ¹H NMR (300MHz, DMSO-d₆) δ7.24 (dd, *J* = 4.5, 9.2 Hz, 1H), 7.3 (d, *J* = 2.2 Hz, 1H), 7.42 (t, *J* = 7.8Hz, 1H), 7.74 (d, *J* = 7.9Hz, 1H), 7.81 (d, *J* = 7.9Hz, 1H), 8.13 (d, *J* = 9.4Hz, 1H), 8.40 (s, 1H), 8.50 (dd, *J* = 1.5, 4.4 Hz, 1H), 8.78 (s, 1H), 9.98 (br s, 1H), 12.9 (br s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ98.2, 105.9, 113.1, 113.9, 117.2, 117.8, 119.7, 121, 125, 127.9, 129.1, 133.8, 138.9, 139.3, 143.9, 144.4, 157.3. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₇H₁₂N₅OBr₂: 461.9388. Found: 461.9420. IR (cm⁻¹): 3142, 1646, 1559, 1426, 1338, 1236.

General Procedure D for Amidation with T₃P.

4,5-dibromo-1*H*-pyrrole-2-carboxylic acid (1eq.) was dissolved in EtOAc and pyridine (3eq.). T₃P (1.5eq.) was added to the mixture. After 10 min, the corresponding aniline (1.1eq.) was added with a few drops of DMF to have a homogeneous mixture. The reaction mixture was stirred at room temperature overnight.

The solvent was removed under vacuum and the product and purified by silica gel flash chromatography (elution by gradient from 100 to 90/10/MeOH) to afford the desired product.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyrazin-3-yl)phenyl)-1*H*-pyrrole-2-carboxamide (**67a**). The title compound was synthesized according to general procedure D using **65a** (43 mg, 0.20 mmol) yielding **67a** as a beige amorphous solid (55 mg, 64%). ¹H NMR (300MHz, DMSO-d₆) δ6.92 (br s, 1H), 7.36 (d, *J* = 7.6Hz, 1H), 7.5 (t, *J* = 7.6Hz, 1H), 7.88 (d, *J* = 7.6Hz, 1H), 7.97 (d, *J* = 4.7Hz, 1H), 8.06 (s, 1H), 8.12 (t, *J* = 1.9Hz, 1H), 8.67 (dd, *J* = 1.4, 4.8Hz, 1H), 9.15 (s, 1H), 9.77 (s, 1H), 13.05 (br s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ113.7, 117.5, 117.8, 119.3, 121.6, 126.4, 127.8, 129.7, 129.8, 134.4, 140.4, 140.9, 143.7, 159.8. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₇H₁₂N₅OBr₂: 461.9388. Found: 461.9385. IR (cm⁻¹): 2974, 1671, 1595, 1522, 1418, 1305.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*b*]pyridazin-3-yl)phenyl)-1*H*-pyrrole-2-carboxamide (**67b**). The title compound was synthesized according to general procedure D using **65b** (86 mg, 0.41 mmol) yielding **67b** as a beige amorphous solid (45mg, 26%). ¹H NMR (300MHz, DMSO-d₆) δ7.31 (m, 1H), 7.34 (s, 1H), 7.48 (t, *J* = 8.0Hz, 1H), 7.80 (br s, 1H), 7.83 (br s, 1H), 8.21 (m, 1H), 8.23 (m, 1H), 8.55 (s, 1H), 8.65 (d, *J* = 4.1Hz, 1H), 10.25 (s, 1H), 13.07 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ98.2, 105.8, 114.4, 127.9, 117.2, 117.9, 119.6, 121.7, 126.1, 127.3, 128.8, 129, 132.8, 139.2, 140.2, 143.9, 157.4. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₇H₁₂N₅OBr₂: 461.9388. Found: 461.9383. IR (cm⁻¹): 3157, 1665, 1562, 1433, 1291, 1233.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyridin-3-yl)phenyl)-1*H*-pyrrole-2-carboxamide (**67c**). The title compound was synthesized according to general procedure D using **65c** (182 mg, 0.87 mmol) yielding **67c** as a beige amorphous solid (225mg, 56%). ¹H NMR (300MHz, DMSO-d₆) δ7.0 (t, *J* = 6.6Hz, 1H), 7.29 (s, 1H), 7.30 (m, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 9.1 Hz, 1H), 7.78 (s, 1H), 7.80 (m, 1H), 8.08 (s, 1H), 8.62 (d, *J* = 6.7 Hz, 1H), 10.12 (s, 1H), 13.02 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ98.2, 106.1, 113, 114.2, 117.7, 118.3, 119.2, 122.5, 124, 124.6, 125, 127.8, 129.2, 129.8, 132.5, 139.6, 145.6, 157.5. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₈H₁₃N₄OBr₂: 460.9436. Found: 460.9459. IR (cm⁻¹): 3158, 1661, 1609, 1523, 1422, 1233.

4,5-Dibromo-*N*-(3-(imidazo[1,2-*a*]pyrimidin-3-yl)phenyl)-1*H*-pyrrole-2-carboxamide (**67d**). The title compound was synthesized according to general procedure D using **65d** (100 mg, 0.48 mmol) yielding **67d** as a beige amorphous solid (80 mg, 36%). ¹H NMR (300MHz, DMSO-d₆) δ7.06 (dd, *J* = 4.3, 6.8 Hz, 1H), 7.31 (s, 1H), 7.43 (t, *J* = 7.7Hz, 1H), 7.69 (d, *J* = 7.7Hz, 1H), 7.83 (d, *J* = 7.7Hz, 1H), 8.33 (s, 1H), 8.36 (s, 1H), 8.54 (dd, *J* = 2.0, 4.2 Hz, 1H), 8.98 (dd, *J* = 2.0, 6.7Hz, 1H), 10.01 (s, 1H), 12.92 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ98.2, 105.9, 107.5, 108.9, 114, 117.3, 119.7, 120.8, 127.9, 129.1, 133.8, 135.1, 139.4, 145.1, 148, 150.4, 157.3. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₇H₁₂N₅OBr₂: 461.9388. Found: 461.9412. IR (cm⁻¹): 3188, 1667, 1612, 1560, 1416, 1222.

4,5-Dibromo-*N*-(4-(imidazo[1,2-*a*]pyrazin-3-yl)phenyl)-1*H*-pyrrole-2-carboxamide (**68a**). The title compound was synthesized according to general procedure D using **66a** (43 mg, 0.20 mmol) yielding **68a** as a beige amorphous solid (44 mg, 51%). ¹H NMR (300MHz, DMSO-d₆) δ7.17 (s, 1H), 7.70 (d, *J* = 8.5Hz, 2H), 7.94 (m, 3H), 8.02 (s, 1H), 8.61 (d, *J* = 4.7Hz, 1H), 9.12 (br s, 1H), 12.86 (br s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ97.6, 107.4, 114, 117.5, 120.1, 122, 126.2, 128.1, 128.6, 129.7, 134.1, 139.5, 140.7, 143.5, 158.2. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₇H₁₂N₅OBr₂: 461.9388. Found: 461.9369. IR (cm⁻¹): 3177, 1639, 1524, 1412, 1332.

4,5-Dibromo-*N*-(4-(imidazo[1,2-*b*]pyridazin-3-yl)phenyl)-1*H*-pyrrole-2-carboxamide (**68b**). The title compound was synthesized according to general procedure D using **66b** (123 mg, 0.59 mmol) yielding **68b** as a beige amorphous solid (98 mg, 40 %). ¹H NMR (300MHz, DMSO-d₆) δ7.28 (m, 2H), 7.87 (d, *J* = 8.9Hz, 2H), 8.15 (d, *J* = 8.9Hz, 2H), 8.20 (dd, *J* = 1.6, 8.9 Hz, 1H), 8.24 (s, 1H), 8.64 (dd, *J* = 1.6, 4.5Hz, 1H), 10.0 (s, 1H), 12.95 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆) δ98.2, 106.1, 114, 116.8, 119.9, 123.5, 126, 126.7, 127.2, 127.9, 132.3, 138.4, 139.6, 143.8, 157.3. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₇H₁₂N₅OBr₂: 461.9388. Found: 461.9390. IR (cm⁻¹): 3179, 1637, 1598, 1525, 1409, 1333, 1159, 1022.

4,5-Dibromo-*N*-(4-(imidazo[1,2-*a*]pyridin-3-yl)phenyl)-1*H*-pyrrole-2-carboxamide (**68c**). The title compound was synthesized according to general procedure D using **66c** (161 mg, 0.77 mmol), yielding **68c** as a beige amorphous solid (140 mg, 40%). ¹H NMR (300MHz, DMSO-*d*₆) δ 6.97 (t, *J* = 6.7Hz, 1H), 7.29 (m, 2H), 7.65 (d, *J* = 8.0 Hz, 3H), 7.75 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 2H), 8.57 (d, *J* = 7.0Hz, 1H), 10.05 (s, 1H), 12.99 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 98.2, 106.1, 112.8, 114.1, 117.4, 120.4, 123.7, 124.1, 124.5, 124.9, 127.8, 128, 132, 138.6, 145.2, 157.4. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₈H₁₃N₄OBr₂: 460.9436. Found: 460.9435. IR (cm⁻¹): 3169, 1634, 1595, 1410, 1310, 1030.

General Procedure E for Click Reaction.

Corresponding azide (1 eq.) was dissolved in DMF/H₂O (10:2). The mixture was degassed with bubbling argon 5 min. Then sodium ascorbate (10 mol%) was added followed by the alkyne **15a** (1 eq.). CuSO₄·5H₂O (1mol %) was finally added to the reaction mixture. It was stirred at room temperature overnight under argon. The mixture was filtered, rinsed with DCM then with water. The resulting solid was dried to afford the desired triazole.

4,5-Dibromo-*N*-((1-(imidazo[1,2-*a*]pyridin-3-ylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-pyrrole-2-carboxamide (**71a**). The title compound was synthesized according to general procedure E using **70a** (146 mg, 0.84 mmol) and **15a** (256 mg, 0.84 mmol), yielding **71a** as a white amorphous solid (310 mg, 77%). ¹H NMR (300MHz, DMSO-*d*₆) δ 4.42 (d, *J* = 6.0 Hz, 2H), 6.09 (s, 2H), 6.92 (s, 1H), 8.01 (m, 2H), 8.06 (s, 1H), 8.59 (t, *J* = 6.0 Hz, 1H), 8.64 (m, 1H), 9.12 (s, 1H), 12.69 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 34.1, 41.6, 97.8, 104.8, 112.9, 118.2, 123, 123.7, 127.9, 129.3, 136, 143.3, 145.3, 158.8. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation.

Protein kinase assays. Kinase enzymatic activities were assayed in 384-well plates using the ADP-Glo™ assay kit (Promega, Madison, WI). This assay provides an homogeneous and high-throughput screening method to measure kinase activity by quantifying the amount of ADP produced during a kinase reaction.⁸² Briefly, the reactions were carried out in a final volume of 6 µl for 30 min at 30°C in appropriate kinase buffer, with either protein or peptide as substrate in the presence of 10 µM ATP (40mM Tris pH 7.5, 20mM MgCl₂ and 0.1mg/ml of BSA). Afterwards, 6 µl of ADP-Glo™ Kinase Reagent was added to stop the kinase reaction. After an incubation time of 50 min at room temperature (RT), 12 µl of Kinase Detection Reagent was added for one hour at RT. The transmitted signal was measured using the Envision (PerkinElmer, Waltham, MA) microplate luminometer and expressed in Relative Light Unit (RLU). In order to determine the half maximal inhibitory concentration (IC₅₀), the assays were performed in duplicate in the absence or presence of increasing doses of the tested

compounds. Kinase activities are expressed in % of maximal activity, i.e. measured in the absence of inhibitor. Peptide substrates were obtained from Proteogenix (Schiltigheim, France). Aurora B kinase assay was validated using a model inhibitor employed under the same conditions as those for the tested compounds: ZM-447439 (#S1103, Selleckchem).

HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₃Br₂N₈O: 480.9559. Found: 480.9598. IR (cm⁻¹): 3443, 3277, 1630, 1564, 1525, 1405, 1229.

4,5-Dibromo-*N*-((1-(imidazo[1,2-*b*]pyridazin-3-ylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-pyrrole-2-carboxamide (**71b**). The title compound was synthesized according to general procedure E using **70b** (127 mg, 0.73 mmol) and **15a** (223 mg, 0.73 mmol), yielding **71b** as a white amorphous solid (310 mg, 89%). ¹H NMR (300MHz, DMSO-*d*₆) δ 4.41 (d, *J* = 5.8 Hz, 2H), 5.99 (s, 2H), 6.92 (s, 1H), 7.30 (dd, *J* = 4.6, 9.2 Hz, 1H), 7.95 (s, 2H), 8.18 (dd, *J* = 1.5, 9.2 Hz, 1H), 8.58 (m, 2H), 12.69 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 34.1, 41.9, 97.8, 104.7, 112.8, 118, 122.3, 122.9, 125.9, 127.9, 134.2, 144, 145, 146.2, 158.7. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₅H₁₃Br₂N₈O: 480.9559. Found: 480.9564. IR (cm⁻¹): 3146, 3128, 1635, 1566, 1523, 1243.

4,5-Dibromo-*N*-((1-(imidazo[1,2-*a*]pyridin-3-ylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-pyrrole-2-carboxamide (**71c**).

The title compound was synthesized according to general procedure E using **70c** (202 mg, 1.2 mmol) and **15a** (356 mg, 1.2 mmol), yielding **71c** as a white amorphous solid (349 mg, 63%). ¹H NMR (300MHz, DMSO-*d*₆) δ 4.41 (d, *J* = 5.7 Hz, 2H), 6.03 (s, 2H), 6.91 (s, 1H), 7.0 (t, *J* = 6.8 Hz, 1H), 7.31 (t, *J* = 6.8 Hz, 1H), 7.62 (m, 1H), 7.78 (m, 1H), 7.99 (s, 1H), 8.55 (m, 1H), 8.6 (t, *J* = 5.6 Hz, 1H), 12.69 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 34.1, 42.2, 97.8, 104.7, 112.5, 112.9, 117.2, 122.8, 124.8, 125, 127.9, 134.3, 145.2, 147, 158.7. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HMRS (ESI) *m/z* Calcd for (M+H)⁺ C₁₆H₁₄Br₂N₇O: 479.9606. Found: 479.9621. IR (cm⁻¹): 3263, 1629, 1519, 1501, 1407, 1317, 1227.

Buffers: (A) 10 mM MgCl₂, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 µg/ml heparin; (B) 25 mM MOPS, pH7.2, 12.5 mM β-glycerophosphate, 25 mM MgCl₂, 5 mM EGTA, 2 mM EDTA, 0.25 mM DTT.

In this study the following protein kinases were analyzed: AuroraB (human, recombinant, expressed by baculovirus in Sf9 insect cells, SignalChem, product #A31-10G) was assayed in buffer B with 0.2 µg/µl of myelin basic protein (MBP) as substrate; CDK2/CyclinA (cyclin-dependent kinase-2, human kindly provided by Dr. A. Echaliier-Glazer, Leicester, UK) was assayed in buffer A with 0.8 µg/µl of histone H1 as substrate; CDK5/p25 (human, recombinant, expressed in bacteria) was assayed in buffer A with 0.8 µg/µl of histone H1 as substrate; CDK9/CyclinT (human, recombinant, expressed by baculovirus in Sf9 insect cells) were assayed in buffer A with 0.27 µg/µl of the following peptide: YSPTSPSYSPSYSPSYSPSKKKK, as substrate; GSK3β (human, recombinant, expressed by baculovirus in Sf9 insect cells) were assayed in buffer A with 0.010 µg/µl of GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSpEDEEE); CK1ε (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer A with 0.022 µg/µl of the following peptide: RRKHAAGSpAYSITA ("Sp" stands for phosphorylated serine) as CK1-specific substrate; Haspin-kd (human, kinase domain, amino acids 470 to 798, recombinant, expressed in bacteria) was assayed in buffer A with 0.007 µg/µl of Histone H3 (1-21) peptide (ARTKQTARKSTGGKAPRKQLA) as substrate; Pim-1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed

in buffer A with 0.8 $\mu\text{g}/\mu\text{l}$ of histone H1 (Sigma #H5505) as substrate; *Mm*CLK1 (from *Mus musculus*, recombinant, expressed in bacteria) was assayed in buffer A with 0.027 $\mu\text{g}/\mu\text{l}$ of the following peptide: GRSRSRSRSRSR as substrate; *Rn*DYRK1A-kd (*Rattus norvegicus*, amino acids 1 to 499 including the kinase domain, recombinant, expressed in bacteria, DNA vector kindly provided by Dr. W. Becker, Aachen, Germany) was assayed in buffer A with 0.033 $\mu\text{g}/\mu\text{l}$ of the following peptide: KKISGRLSPIMTEQ as substrate.

ATP competition assay. Human recombinant Aurora B activity was assayed *in vitro*, according to the ADP-Glo method (Promega), in the absence or presence of CJ2-150 (2; 1; 0.5 or 0.1 μM) and increasing concentrations of ATP (3,125 to 50 μM). The kinase assay was performed, in triplicate, in buffer B with 0.2 $\mu\text{g}/\mu\text{l}$ of MBP (0.2 $\mu\text{g}/\mu\text{l}$) as substrate in buffer B and carried out in a final volume of 6 μl for 30 min at 30°C. The reaction was then continued as described above (cf. part Protein kinase assays). The signals obtained, expressed in RLU, allow drawing of a double-reciprocal plot of the Michaelis-Menten equation (also known as the Lineweaver-Burk plot).

Immunofluorescence. For immunofluorescence experiments, cells were grown on glass coverslips, fixed with 4% paraformaldehyde in PBS, permeabilized by 0.15% Triton-X100 for 2 minutes and processed using standard protocols. Images were acquired with a Coolsnap HQ2 CCD camera (Photometrics) on a Zeiss Axio microscope (Carl Zeiss) using a 100x NA 1.40 objective. Image acquisition and processing were performed using Metamorph (Molecular Device). Deconvolution was performed using the AutoQuant module in Metamorph. Quantification of signal intensity was performed using ImageJ software (NIH). Primary antibodies used in this study included rabbit polyclonal anti-phospho-Ser10 Histone H3 (1/1000 dilution, Millipore), rabbit polyclonal anti-Aurora B (1/100, Life Technologies) and mouse monoclonal anti- α -Tubulin (Clone B512, Sigma).

Cell Culture and Proliferation Assay. Cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA) or from the European collection of cell culture (ECACC, England) and were cultured according to the supplier's instructions. K562R (doxorubicin-resistant) leukemia cells were a generous gift from Pr. J.P. Marie (France). Human HCT-116 colorectal carcinoma, MCF7 breast adenocarcinoma, A549 lung carcinoma, U2OS osteosarcoma and SK-OV-3 ovary carcinoma were grown in Gibco McCoy's 5A supplemented with 10% fetal calf serum and 1% glutamine. K562 and K562R leukemia cells were grown in RPMI 1640 supplemented with 10% fetal calf serum and 1% glutamine. hTERT-RPE1 cells were cultured in DMEM/F12 medium containing 10% fetal calf serum and 1% glutamine. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

Cell viability was determined by a luminescent assay according to the manufacturer's instructions (Promega, Madison, WI, USA). For IC₅₀ determination, the cells were seeded in 96-well plates (3×10^3 cells/well) containing 90 μL of growth medium. After 24 h of culture, the cells were treated with the tested compounds at 10 different final concentrations. Each concentration was obtained from serial dilutions in culture medium starting from the stock solution. Control cells were treated with the vehicle. Experiments were performed in triplicate.

After 72 h of incubation, 100 μL of CellTiter Glo Reagent was added for 15 min before recording luminescence with a spectrophotometric plate reader PolarStar Omega (BMG LabTech). The dose-response curves were plotted with Graph Prism software and the IC₅₀ values were calculated using the Graph Prism software from polynomial curves (four or five-parameter logistic equations).

PBMC Viability Assay. Isolation of peripheral blood mononuclear cells (PBMC) from human donor blood samples was performed by Ficoll density gradient centrifugation. The separated PBMC were resuspended at a final concentration of 5.56×10^5 cells/mL in RPMI media supplemented with 10% fetal bovine serum. Cell viability assays were performed on both phytohemagglutinin (PHA)-activated and non-activated PBMC using the luminescent CellTiter-Glo protocol from Promega.

Cell cycle analysis. After 24h of treatment with CJ2-151, K562 cells were fixed in 70% ethanol and stained with propidium iodide solution containing RNase A. DNA content was further determined by flow cytometry using FC500 (Beckman Coulter).

Molecular docking. Three-dimensional structures of the ligands were generated using CORINA 4.2.0 (Molecular Networks GmbH, Germany and Altamira, LLC, USA). Molecular docking calculations were performed using the GOLD software⁸³ from the CCDC_2020 suite, with the Aurora B structure 4AF3⁷¹ as receptor and the GoldScore scoring function. The interaction between Aurora B and the INCENP protein structure 4AF3 cover both "F" and "Y" sites, therefore ensuring that these two sites are accessible for docking. The site "F" was defined as a sphere with 10 Å diameter around a virtual point situated at mid-distance between the atoms CE2 of Phe72 and CZ of Phe101. The site "Y" was defined as a sphere with 10 Å diameter around the atom CD1 of Ile150. All other parameters had default values and 50 conformers were generated for each site. The analysis of docking poses was performed with UCSF Chimera,⁸⁴ as well as the generation of molecular modeling images.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Additional experimental procedures, NMR spectra, specificity inhibition table, Dose-effect PBMC viability, Michaelis-Menten graph for ATP competition, docking conformation of

compound CJ2-150 in the "F" allosteric site, ELSD-HPLC Profiles and the CSV file with formula strings with the associated biochemical and biological data.

AUTHOR INFORMATION

Corresponding Authors

*ali.almourabit@cnrs.fr (A. Al-Mourabit)

*sandrineruchaud@sb-roscoff.fr (Sandrine Ruchaud)

Author Contributions

Charlotte Juillet did most of the synthetic work as well as most of the biological analysis and the mode of action studies, and co-authored the manuscript.

Ludmila Ermolenko and Dina Boyarskaya prepared some analogs Blandine Baratte performed biochemical experiments and cowrote the experimental part.

Béatrice Josselin performed biological evaluations.

Sandrine Ruchaud and Stéphane Bach co-supervised the screening against protein kinases. Sandrine Ruchaud, supervised the biological and biochemical evaluation and co-authored the manuscript.

Jerome Bignon, supervised the cell cycle analysis and co-authored the corresponding experimental part.

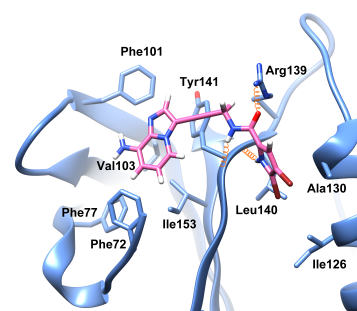
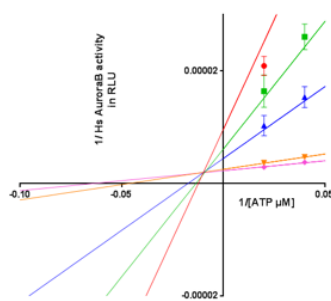
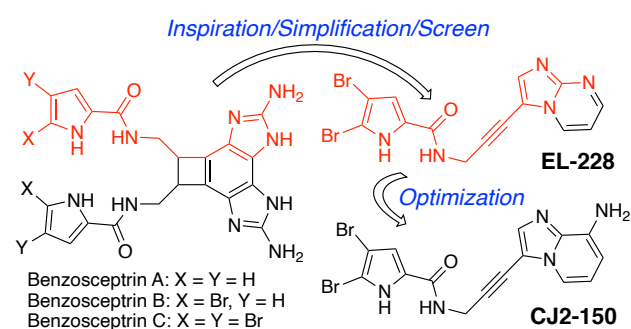
Hristo Nedev carried out the molecular modeling experiments and analyzed the results.

Bogdan I. Iorga, analyzed the docking results and co-authored the manuscript.

Ali Al-Mourabit conceived and directed the project, supervised the findings of this work and co-authored the manuscript.

Funding Sources

Graphical Abstract



CNRS, Cancéropôle Grand-Ouest and La Ligue Contre le Cancer

Conflict of interest

Stéphane Bach is a founder and member of the scientific advisory board of SeaBeLife Biotech, which is developing novel therapies for treating liver and kidney acute disorders. This work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ABBREVIATIONS

CK1, Casein kinase 1; CPC, chromosomal passenger complex; DAPI, 4',6-diamidino-2-phenylindole; DTT, dithiothreitol; EDCl, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; ELSD, Evaporative light scattering detector; ESI-TOF, Electrospray Ionization Time-of-Flight; FACS, Fluorescence-activated Cell Sorting; HPBT, 1-Hydroxybenzotriazole; INCENP, Inner centromere protein; IR, infrared; MBP, myelin basic protein; MOPS, 3-(N-morpholino)propanesulfonic acid; PBMCs, peripheral blood mononuclear cells, PHA, phytohemagglutinine; RLU; Relative Light Unit; SAC, spindle assembly checkpoint.

ACKNOWLEDGMENT

The authors thank Dr. Robert H. Dodd for careful reading and improvements of the manuscript with regard to English usage. We thank Céline Moriou for the HPLC profiles, Marine Peyneau and Luc Dechaisemartin (Paris-Saclay, Inserm, Châtenay-Malabry) for PBMC viability assay. We thank Paris-Saclay University for doctoral fellowships.

Financial support from CNRS is gratefully acknowledged. The authors wish to thank: Cancéropôle Grand-Ouest, French IBiSA and Biogenouest for supporting the KISSf screening facility, FR2424 (Roscoff, France). The authors also wish to thank "La Ligue Contre le Cancer" committee (Districts 29, 22, 56, 45 and 79) for financial support.

REFERENCES

- (1) Vader, G.; Lens, S.M.A. The Aurora Kinase Family in Cell Division and Cancer. *Biochim. Biophys. Acta*. **2008**, *1786*, 60–72.
- (2) Carmena, M.; Wheelock, M.; Funabiki, H.; Earnshaw, W. C. The Chromosomal Passenger Complex (CPC): From Easy Rider to the Godfather of Mitosis. *Nature Rev. Mol. Cell Biol.* **2012**, *13*, 789–803.
- (3) Van der Waal, M. S.; Hengeveld, R. C. C.; van der Horst, A.; Lens, S. M. A. Cell Division Control by the Chromosomal Passenger Complex. *Exp. Cell Res.* **2012**, *318*, 1407–1420.
- (4) Gurley, L. R.; D'anna, J. A.; Barham, S. S.; Deaven, L. L.; Tobey, R. A. Histone Phosphorylation and Chromatin Structure during Mitosis in Chinese Hamster Cells. *Eur. J. Biochem.* **1978**, *84*, 1–15.
- (5) Willems, E.; Dedobbeleer, M.; Digregorio, M.; Lombard, A.; Lumapat, P. N.; Rogister, B. The Functional Diversity of Aurora Kinases: A Comprehensive Review. *Cell Div.* **2018**, *13*, 1–17.
- (6) Sarangapani, K. K.; Asbury, C. L. Catch and Release: How Do Kinetochores Hook the Right Microtubules during Mitosis? *Trends Genet.* **2014**, *30*, 150–159.
- (7) Krenn, V.; Musacchio, A. The Aurora B Kinase in Chromosome Bi-Orientation and Spindle Checkpoint Signaling. *Front. Oncol.* **2015**, *5*, 225. doi: 10.3389/fonc.2015.00225.
- (8) Lens, S. M. A.; Medema, R. H. Cytokinesis Defects and Cancer, *Nat. Rev. Cancer*, **2019**, *19*, 32–45.
- (9) Steigemann, P.; Wurzenberger, C.; Schmitz, M.H.A.; Held, M.; Guizetti, J.; Maar, S.; Gerlich, D.W. Aurora B-Mediated Abscission Checkpoint Protects against Tetraploidization, *Cell*, **2009**, *136*, 473–484.
- (10) Ruchaud, S.; Carmena, M.; Earnshaw, W. C. Chromosomal Passengers: Conducting Cell Division. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 798–812.
- (11) Keen, N.; Taylor, S. Aurora-Kinase Inhibitors as Anticancer Agents. *Nat. Rev. Cancer*, **2004**, *4*, 927–936.
- (12) Sen, S.; Zhou, H.; White, R. A. A Putative Serine/Threonine Kinase Encoding Gene BTAK on Chromosome 20q13 is Amplified and Overexpressed in Human Breast Cancer Cell Lines. *Oncogene*. **1997**, *14*, 2195–2200.
- (13) Bischoff, J. R. A Homologue of Drosophila Aurora Kinase Is Oncogenic and Amplified in Human Colorectal Cancers. *EMBO J.* **1998**, *17*, 3052–3065.
- (14) Pollard, J. R.; Mortimore, M. Discovery and Development of Aurora Kinase Inhibitors as Anticancer Agents, *J. Med. Chem.* **2009**, *52*, 2629–2651.
- (15) Bavetsias, V.; Large, J. M.; Sun, C.; Boulou, N.; Kosmopoulou, M.; Matteucci, M.; Wilsher, N. E.; Martins, V.; Reynolds, J.; Atrash, B.; Urban, F.; Valenti, M.; De Haven Brandon, A.; Box, G.; Raynaud, F. I.; Workman, P.; Eccles, S. A.; Bayliss, R.; Blagg, J.; Linardopoulos, S.; McDonald, E. Imidazo[4,5-b]pyridine Derivatives As Inhibitors of Aurora Kinases: Lead Optimization Studies toward the Identification of an Orally Bioavailable Preclinical Development Candidate, *J. Med. Chem.* **2010**, *53*, 5213–5228.
- (16) Sharma, A.; Luxami, V.; Paul, K. Purine-benzimidazole Hybrids: Synthesis, Single Crystal Determination and in vitro Evaluation of Antitumor Activities, *Eur. J. Med. Chem.* **2015**, *93*, 414–422.
- (17) Borisa, A. C.; Bhatt, H. G. A Comprehensive Review on Aurora Kinase: Small Molecule Inhibitors and Clinical Trial Studies. *Eur. J. Med. Chem.* **2017**, *140*, 1–19.
- (18) Dar, A. A.; Goff, L. W.; Majid, S.; Berlin, J.; El-Rifai, W. Aurora Kinase Inhibitors - Rising Stars in Cancer Therapeutics? *Mol. Cancer Ther.* **2010**, *9*, 268–278.
- (19) Carles, F.; Bourg, S.; Meyer, C.; Bonnet, P. PKIDB: A Curated, Annotated and Updated Database of Protein Kinase Inhibitors in Clinical Trials. *Molecules*. **2018**, *23*, 908.
- (20) Wang, S.; Dong, G.; Sheng, C. Structural Simplification of Natural Products. *Chem. Rev.* **2019**, *119*, 4180–4220.
- (21) Al-Mourabit, A.; Zancanella, M. A.; Tilvi, S.; Romo, D. Biosynthesis, Asymmetric Synthesis, and Pharmacology Including Cellular Targets of the Pyrrole-2-Aminoimidazole Marine Alkaloids. *Nat. Prod. Rep.* **2011**, *28*, 1229–1260.
- (22) Appenzeller, J.; Tilvi, S.; Martin, M.-T.; Gallard, J.-F.; El-bitar, H.; Dau, E. T. H.; Debitus, C.; Laurent, D.; Moriou, C.; Al-Mourabit, A. Benzosceptrins A and B with a Unique Benzocyclobutane Skeleton and Nagelamide S and T from Pacific Sponges. *Org. Lett.* **2009**, *11*, 4874–4877.
- (23) Kubota, T.; Araki, A.; Yasuda, T.; Fromont, J.; Aoyama, K.; Mikami, Y.; Walchli, M.R.; Kobayashi, J. Benzosceptrin C, a New Dimeric Bromopyrrole Alkaloid from Sponge *Agelas* sp. *Tetrahedron Lett.* **2009**, *50*, 7268–7270.
- (24) Tran, M. Q.; Nguyen, T. B.; Sawadogo, W. R.; Ermolenko, L.; Song, S.; Retailleau, P.; Diederich, M.; Al-Mourabit, A. Unaromatized Tetrahydrobenzimidazole Synthesis from p-Benzoquinone and N-Arylamidines and their Cytotoxic Potential, *Eur. J. Org. Chem.* **2018**, 5878–5884.
- (25) Song, S.; Lee, J.-Y.; Ermolenko, L.; Mazumder, A.; Ji, S.; Ryu, H.; Kim, H.; Kim, D.-W.; Lee, J.-W.; MDicato, M.; Christov, C.; Schneckeburger, M.; Cerella, C.; Gérard, D.; Boyer, B.-O.; Al-Mourabit, A.; Diederich, M. Tetrahydrobenzimidazole TMQ0153 triggers Apoptosis, Autophagy and Necroptosis Crosstalk in Chronic Myeloid Leukemia. *Cell Death & Disease*, **2020**, *11*:109.
- (26) Benchekrout, M.; Ermolenko, L.; Tran M. Q.; A. Vagneux, A.; Nedev, H.; Delehouz, C.; Souab, M.; Baratte, B.; Josselin, B.; Iorga, B.-I.; Ruchaud, S.; Bach, S.; Al-Mourabit, A. Discovery of Simplified Benzazole Fragments Derived from the Marine Benzosceptrin B as Necroptosis Inhibitors Involving the Receptor Interacting Protein Kinase-1, *Eur. J. Org. Chem.* **2020**, 201:112337. doi.org/10.1016/j.ejmech.2020.112337.
- (27) Sasmal, P.K.; Chandrasekhar, A.; Sridhar, A.; Iqbal, J. Novel one-step Method for the Conversion of Isothiocyanates to 2-Alkyl(aryl)aminothiazoles, *Tetrahedron*, **2008**, *64*, 11074–11080.
- (28) Rivall, Y.; Grassyl, G.; Taudou, A.; Ecale, R. Antifungal Activity in vitro of some Imidazo[1,2-a]pyrimidine Derivatives, *Eur. J. Med. Chem.*, **1991**, *26*, 13–18.
- (29) Lindel, T.; Hochgurtel, M. Synthesis of the Marine Natural Product Oroidin and Its Z-Isomer, *J. Org. Chem.* **2000**, *65*, 2806–2809.
- (30) Dasgupta, R.; Rajalu, G.G.; Dickson, J.K. Novel Oxazole Compounds as β -Catenin Modulators and Uses thereof, WO2016/81451, 2016.
- (31) Yasui, K.; Morioka, Y.; Hanasaki, K.; Antipruritics, EP1477186, 2004.
- (32) Kuwano, R.; Kashiwabara, M.; Ohsumi, M.; Kusano, H. Catalytic Asymmetric Hydrogenation of 2,3,5-Trisubstituted Pyrroles, *J. Am. Chem. Soc.* **2008**, *130*, 3, 808–809.
- (33) Essa, A.H.; Lerrick, R.I.; Tuna, F.; Harrington, R.W.; Clegg, W.; Hall, M.J. Reduction of 2,2,2-Trichloro-1-Aryl-Ethanones by RMgX: Mechanistic Investigations and the Synthesis of Substituted α,α -Dichloro-Ketones, *Chem. Commun.* **2013**, *49*, 2756–2758.
- (34) Ponasik, J.A.; Kassab, D.J.; Ganem, B. Synthesis of the Antifouling Poly-Amine Pseudoceratidine and its Analogs: Factors Influencing Biocidal Activity, *Tetrahedron Lett.* **1996**, *37*, 6041–6044.
- (35) Clark, B.R.; O'Connor, S.; Fox, D.; Leroy, J.; Murphy, C.D. Org. Production of Anticancer Polyenes through Precursor-directed Biosynthesis, *Biomol. Chem.* **2011**, *9*, 6306–6311.
- (36) Allmann, T.M.; Moldovan, R.P.; Jones, P.G.; Lindel, T. Synthesis of Hydroxypyrrolone Carboxamides Employing Selectfluor, *Chem. Eur. J.* **2016**, *22*, 111–115.
- (37) Huigens, R.B.; Rogers, S.A.; Steinhauer, A.T.; Melander, A. 2-Aminobenzimidazole That Inhibits and Disperses Gram-Positive Biofilms through a Zinc-Dependent Mechanism, *C. Org. Biomol. Chem.* **2009**, *7*, 794–802.
- (38) Poverlein, C.; Jacobi, N.; Mayer, P.; Lindel, T. Synthesis of 4(S)-Acyl-2-aminoimidazoles and Vinyllogues, *Synthesis*, **2007**, *23*, 3620–3626.
- (39) Loeber, S.; Huebner, H.; Gmeiner, P. Azaindole Derivatives with High Affinity for the Dopamine D4 Receptor: Synthesis, Ligand Binding Studies and Comparison of Molecular Electrostatic Potential Maps, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 97–102.
- (40) Enguehard, C.; Renou, J.L.; Collot, V.; Hervet, M.; Rault, S.; Gueffier, A. Reactivity of 3-Iodoimidazo[1,2-a]pyridines Using a Suzuki-Type Cross-Coupling Reaction, *J. Org. Chem.* **2000**, *65*, 6572–6575.
- (41) Jingtao, C.; Xuanping, G.; Meng, H.; Xinsheng, L.; Method for Synthesizing 3-Iodoimidazo[1,2-b]pyrazine, CN105218549A, **2016**.
- (42) Sablayrolles, C.; Cros, G.H.; Milhavet, J.C.; Rechenq, E.; Chapat, J.P.; Boucard, M.; Serrano, J.J.; McNeill, J.H. Synthesis of Imidazo[1,2-a]pyrazine Derivatives with Uterine-Relaxing, Antibronchospastic, and Cardiac-Stimulating Properties *J. Med. Chem.* **1984**, *27*, 206–212.
- (43) Reddy, K.; Martinez Botella, G.; Griffin, A.M.; Marron, B.E. Compounds and their Methods of Use, WO2018098500A1, **2018**.

- (44) Bischoff, F.P.; Berthelot, D.; De Cleyn, M.; Macdonald, G.; Minne, G.; Oehlich, D.; Pieters, S.; Surkyn, M.; Trabanco, A.A.; Tresadern, G.; Van Brandt, S.; Velter, I.; Zaja, M.; Borghys, H.; Masungi, C.; Mercken, G.; Gijzen, H.J.M. Design and Synthesis of a Novel Series of Bicyclic Heterocycles as Potent γ -Secretase Modulators, *J. Med. Chem.* **2012**, *55*, 9089–9106.
- (45) El Akkaoui, A.; Bassoude, I.; Koubachi, J.; Berteina-Raboin, S.; Mouaddib, A.; Guillaume, G. Pd-catalyzed Regiocontrolled Sonogashira and Suzuki Cross-coupling Reaction of 3,6-Dihaloimidazo[1,2-a]pyridines: One-pot Double-coupling Approach, *Tetrahedron*, **2011**, *67*, 7128–7138.
- (46) Mu, B.; Li, J.; Zou, D.; Wu, Y.; Chang, J.; Wu, Y. Pd-Catalyzed Tandem Cyclization via C–H Arylation and Acylation for the Construction of Polycyclic Scaffolds, *Org. Lett.* **2016**, *18*, 5260–5263.
- (47) Gardan, S.; Mayer, S.; Stephan, S. New adenosine Receptor Ligands and Uses Thereof, EP2210891, **2019**.
- (48) Barda, D.A.; Burkholder, T.P.; Clayton, J.R.; Hao, Y.; Heath, P.C.; Henry, J.R.; Knobloch, J.M.; Mendel, D.; McLean, J.A.; Remick, D.M.; Rempala, M.E.; Wang, Z.Q.; Yip, Y.Y.M.; Zhong, B. Imidazo (1,2-a)pyridine Compounds as Vegf-R2 inhibitors, WO 2006/91671, **2006**.
- (49) Berdini, V.; Besong, G.E.; Callaghan, O.; Carr, M. G.; Congreve, M.S.; Gill, A.L.; Griffiths-Jones, C.M.; Madin, A.; Murray, C.W.; Nijjar, R.K.; O'Brien, M.A.; Pike, A.; Saxty, G.; Taylor, R.D.; Vickerstaffe, E. New Compounds, WO2008/78100, **2008**.
- (50) Baltus, C. B.; Jorda, R.; Marot, C.; Berka, K.; Bazgier, V.; Krystof, V.; Prié, G.; Viaud-Massuard, M.C. Synthesis, Biological Evaluation and Molecular Modeling of a Novel Series of 7-Azaindole Based Tri-heterocyclic Compounds as Potent CDK2/Cyclin E Inhibitors, *Eur. J. Med. Chem.* **2016**, *108*, 701–719.
- (51) Tichenor, M.S.; Keith, J.M.; Jones, W.M.; Pierce, J.M.; Merit, J.; Hawryluk, N.; Seierstad, M.; Palmer, J.A.; Webb, M.; Karbarz, M.J.; Wilson, S.J.; Wennerholm, M.L.; Woestenborghs, F.; Beerens, D.; Luo, L.; Brown, S.M.; De Boeck, M.; Chaplan, S.R.; Breitenbucher, J.G. Heteroaryl Urea Inhibitors of Fatty Acid Amide Hydrolase: Structure–Mutagenicity Relationships for Arylamine Metabolites, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7357–7362.
- (52) Garamvolgyi, R.; Dobos, J.; Sipos, A.; Boros, S.; Illyes, E.; Baska, F.; Kekesi, L.; Szabadkai, I.; Szantai-Kis, C.; Keri, G.; Orfi, L. Design and Synthesis of New Imidazo[1,2-a]pyridine and Imidazo [1,2-a]pyrazine Derivatives with Antiproliferative Activity Against Melanoma Cells, *Eur. J. Med. Chem.* **2016**, *108*, 623–643.
- (53) Chao, H.; Li, L.; Chunshen, Z. Preparation Process of 8-Iodo-6-Nitroimidazo[1,2-a]pyridine, CN107129496, **2017**.
- (54) Dwyer, M.P.; Paruch, K.; Alvarez, C.; Doll, R.J.; Keertikar, K.; Duca, J.; Fischmann, T.O.; Hruza, A.; Madison, V.; Lees, E.; Parry, D.; Seghezzi, W.; Sgambellone, N.; Shanahan, F.; Wiswell, D.; Guzi, T.J. Versatile Templates for the Development of Novel Kinase Inhibitors: Discovery of Novel CDK Inhibitors, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6216–6219.
- (55) Huang, W.S.; Metcalf, C.A.; Sundaramoorthi, R.; Wang, Y.; Zou, D.; Thomas, R.M.; Zhu, X.; Cai, L.; Wen, D.; Liu, S.; Romero, J.; Qi, J.; Chen, L.; Banda, G.; Lentini, S.P.; Das, S.; Xu, Q.; Keats, J.; Wang, F.; Wardwell, S.; Ning, Y.; Snodgrass, J.T.; Broudy, M.L.; Russian, K.; Zhou, T.; Commodore, L.; Narasimhan, N.I.; Mohemmad, Q.K.; Iuliucci, J.; Rivera, V.M.; Dalgarno, D.C.; Sawyer, T.K.; Clackson, T.; Shakespeare, W.C. Discovery of 3-[2-(Imidazo[1,2-b]pyridazin-3-yl)ethyl-N-(4-methyl-N-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide (AP24534), a Potent, Orally Active Pan-inhibitor of Breakpoint Cluster Region-abelson (BCR-ABL) Kinase Including the T315I Gatekeeper Mutant, *J. Med. Chem.* **2010**, *53*, 4701–4719.
- (56) Desroy, N.; Housseman, C.; Bock, X.; Joncour, A.; Bienvenu, N.; Cherel, L.; Labeguery, V.; Rondet, E.; Peixot, C.; Grassot, J.M.; Picolet, O.; Annot, D.; Triballeau, N.; Monjardet, A.; Wakselman, E.; Roncoroni, V.; Le Tallec, S.; Blaque, R.; Cottreaux, C.; Vandervoort, N.; Christophe, T.; Mollat, P.; lamers, M.; Auberval, M.; Hrvacic, B.; Ralic, J.; Oste, L.; Van Der Aar, E.; Brys, R.; Heckmann, B. Discovery of 2-[[2-Ethyl-6-[4-[2-(3-hydroxyazetidin-1-yl)-2-oxoethyl]piperazin-1-yl]-8-methylimidazo[1,2-a]pyridin-3-yl]methyl-mino]-4-(4-fluorophenyl)thiazole-5-carbonitrile (GLPG1690), a First-in-Class Autotaxin Inhibitor Undergoing Clinical Evaluation for the Treatment of Idiopathic Pulmonary Fibrosis, *J. Med. Chem.* **2017**, *60*, 3580–3590
- (57) Molander, G. A.; Cadoret F. Synthesis of the Stereogenic Triad of the Halicyclamine A Core, *Tetrahedron Lett.* **2011**, *52*, 2199–2202.
- (58) Casimiro-Garcia, A.; Bigge, C.F.; Davis, J.A.; Padalino, T.; Pulaski, J.; Ohren, J.F.; McConnell, P.; Kane, C.D.; Royer, L.J.; Stevens, K.A.; Auerbach, B.J.; Collard, W.T.; McGregor, C.; Fakhoury, S.A.; Schaum, R.P.; Zhou, H. Effects of Modifications of the Linker in a Series of Phenylpropanoic Acid Derivatives: Synthesis, Evaluation as PPAR α /c Dual Agonists, and X-ray Crystallographic Studies, *Bioorg. Med. Chem.* **2008**, *16*, 4883–4907.
- (59) Wang, Y.D.; Kimball, G.; Prashad, A.M.; Wang, Y. Zr-Mediated Hydroboration: Stereoselective Synthesis of Vinyl Boronic Esters, *Tetrahedron Lett.* **2005**, *46*, 8777–8780.
- (60) Loubidi, M.; Jouha, J.; Tber, Z.; Khouli, M.; Suzenet, F.; Akssira, M.; Erdogan, M.A.; Kose, F.A.; Dagci, T.; Armagan, G.; Saso, L.; Guillaume, G. Efficient Synthesis and First Regioselective C-6 Direct Arylation of Imidazo[2,1-c][1,2,4]triazine Scaffold and their Evaluation in H₂O₂-Induced Oxidative Stress, *Eur. J. Med. Chem.* **2018**, *145*, 113–123.
- (61) Marchand, P.; Bazin, M.A.; Pagniez, F.; Riviere, G.; Bodero, L.; Marchadour, S.; Nourrisson, M.R.; Picot, C.; Ruchaud, S.; Bach, S.; Barratte, B.; Sauvain, M.; Castillo Pareja, D.; Vaisberg, A.J.; Le Pape, P. Synthesis, Antileishmanial Activity and Cytotoxicity of 2,3-Diaryl- and 2,3,8-Trisubstituted Imidazo[1,2-a]pyrazines, *Eur. J. Med. Chem.* **2015**, *103*, 381–395.
- (62) Zidar, N.; Jakopin, Z.; Madge, D.J.; Chan, F.; Tytgat, J.; Peigneur, S.; Sollner Dolenc, M.; Tomasi, T.; Ilas, J.; Peterlin Masic, L.; Kikelj, D. Substituted 4-Phenyl-2-aminoimidazoles and 4-Phenyl-4,5-dihydro-2-aminoimidazoles as Voltage-gated Sodium Channel Modulators *Eur. J. Med. Chem.* **2014**, *74*, 23–30.
- (63) Bandini, M.; Bottoni, A.; Eichholzer, A.; Miscione, G.P.; Stenta, M. Asymmetric Phase-Transfer-Catalyzed Intramolecular N-Alkylation of Indoles and Pyrroles: A Combined Experimental and Theoretical Investigation *Chem. Eur. J.* **2010**, *16*, 12462–12473.
- (64) Lee, H.J.; Cho, C.W. Chemoselective Suzuki Cross-Coupling Reactions of Chiral Pyrrolizines, *Bull. Korean Chem. Soc.* **2011**, *32*, 359–362.
- (65) Mavel, S.; Renou, J.L.; Galtier, C.; Allouchi, H.; Snoeck, R.; Andrei, G.; De Clercq, E.; Balzarini, J.; Gueffier, A. Influence of 2-Substituent on the Activity of Imidazo[1,2-a] Pyridine Derivatives Against Human Cytomegalovirus, *Bioorg. Med. Chem.* **2002**, *10*, 941–946.
- (66) Giovanni, R.; Cui, Y.; Doods, H.; Ferrara, M.; Just, S.; Kuelzer, R.; Lingard, I.; Mazzaferro, R.; Rudolf, K. New Somatostatin Receptor Subtype 4 (SSTR4) Agonists, WO2014/184275, **2014**.
- (67) Bevilacqua, V.; King, M.; Chaumontet, M.; Nothisen, M.; Gabillet, S.; Buisson, D.; Puente, C.; Wagner, A.; Taran, F. Copper-Chelating Azides for Efficient Click Conjugation Reactions in Complex Media, *Angew. Chem. Int. Ed.* **2014**, *53*, 5872–5876.
- (68) Assadieskandar, A.; Yu, C.; Maisonneuve, P.; Kurinov, I.; Sichi, F.; Zhang, C.; Rigidification Dramatically Improves Inhibitor Selectivity for RAF Kinases, *Med. Chem. Lett.* **2019**, *10*, 1074–1080.
- (69) Hindriksen, S.; Meppelink, A.; Lens, S. Functionality of the chromosomal passenger complex in cancer. *Biochem. Soc. Trans.* **2015**, *43*, 23–32
- (70) Ting, T.-C.; Chang, M.-Y.; Hsu, T.-Y.; Wang, W.-P.; Hsieh, Y.-J.; Chang, C.-J. Vanadocene Dichloride Inhibits Cell Proliferation by Targeting Aurora B. *Metallomics* **2018**, *10*, 1099–1106.
- (71) Suzuki, T.; Miyazaki, K.; Kita, K.; Ochi, T. Trivalent Dimethylarsenic Compound Induces Histone H3 Phosphorylation and Abnormal Localization of Aurora B Kinase in HepG2 Cells. *Toxicol. Appl. Pharmacol.* **2009**, *241*, 275–282.
- (72) Kaestner, P.; Stolz, A.; Bastians, H. Determinants for the Efficiency of Anticancer Drugs Targeting Either Aurora-A or Aurora-B Kinases in Human Colon Carcinoma Cells. *Mol. Cancer Ther.* **2009**, *8*, 2046–2056.
- (73) Wang, F.; Ulyanova, N. P.; vann peu plus haut.; Patnaik, D.; Lens, S. M. A.; Higgins, J. M. G. A Positive Feedback Loop Involving Haspin and Aurora B Promotes CPC Accumulation at Centromeres in Mitosis. *Curr. Biol.* **2011**, *21*, 1061–1069.
- (74) McIntyre, P.J.; Collins, P.M.; Vrzal, L.; Birchall, K.; Arnold, L.H.; Mpamhanga, C.; Coombs, P.J.; Burgess, S.G.; Richards, M.W.; Winter, A.; Veverka, V.; Delft, F.V.; Merritt, A.; Bayliss, R.; Characterization of Three Druggable Hot-Spots in the Aurora-A/TPX2 Interaction Using Biochemical, Biophysical, and Fragment-Based Approaches. *ACS Chem Biol.* **2017**, *12*, 2906–2914.
- (75) Zhang, R.J.; Collins, P.M.; Foley, D.J.; Arter, C.; von Delft, F.; Bayliss, R.; Warriner, S.; Nelson, A.; Construction of a Shape-Diverse Fragment Set: Design, Synthesis and Screen against Aurora-A Kinase. *Chemistry*, **2019**, *25*, 6831–6839.
- (76) Janeček, M.; Rossmann, M.; Sharma, P.; Emery, A.; Huggins, D.J.; Stockwell, S.R.; Stokes, J.E.; Tan, Y.S.; Almeida, E.G.; Hardwick, B.; Narvaez, A.J.; Hyvönen, M.; Spring, D.R.; McKenzie, G.J.; Venkitaraman, A.R.; Allosteric Modulation of AURKA Kinase Activity by a Small-molecule Inhibitor of its Protein-protein Interaction with TPX2. *Sci Rep.* **2016**, *6*, 28528. DOI: 10.1038/srep28528.
- (77) Sessa, F.; Mapelli, M.; Ciferri, C.; Tarricone, C.; Areces, L.B.; Schneider, T.R.; Stukenberg, P.T.; Musacchio, A. Mechanism of Aurora B Activation by INCENP and Inhibition by Hesperadin. *Mol Cell.* **2005**, *18*, 379–391.

(78) Xu, Z, Ogawa, H, Vagnarelli, P, Bergmann, JH, Hudson, DF, Ruchaud, S, Fukagawa, T, Earnshaw, WC, Samejima, K. INCENP-aurora B Interactions Modulate Kinase Activity and Chromosome Passenger Complex localization. *J. Cell. Biol.* **2009**, *187*, 637-653. Erratum in: *J. Cell. Biol.* **2010**, *188*, 611.

(79) Martinez R.; Defnet A.; Shapiro P. Avoiding or Co-Opting ATP Inhibition: Overview of Type III, IV, V, and VI Kinase Inhibitors. In: Shapiro P. (ed.) *Next Generation Kinase Inhibitors*, Springer, Cham, Switzerland, **2020**, 29–59. doi: 10.1007/978-3-030-48283-1_3.

(80) Lakkaniga N. R.; Zhang L.; Belachav B.; Gunaganti N.; Frett B.; Li H. Discovery of SP-96, The First non-ATP-competitive Aurora Kinase B Inhibitor, for Reduced Myelosuppression. *Eur. J. Med. Chem.* **2020**, *203*, 1–11.

(81) Elkins, J.M.; Santaguida, S.; Musacchio, A.; Knapp, S. Crystal Structure of Human Aurora B in Complex with INCENP and VX-680. *J. Med. Chem.* **2012**, *55*, 7841–7848.

(82) « Zegzouti, H.; Zdanovskaia, M.; Hsiao, K.; Goueli, S.A.; ADP-Glo: A Bioluminescent and Homogeneous ADP Monitoring Assay for Kinases, *Assay Drug Dev Technol.* **2009**, *7*, 560–572.

(83) Verdonk, M.L.; Cole, J.C.; Hartshorn, M.J.; Murray, C.W.; Taylor, R.D. Improved protein-ligand docking using GOLD. *Proteins* **2003**, *52*, 609-623.

(84) Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera-a Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.* **2004**, *25*, 1605–1612.