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

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


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 $\mathrm{H}_{3}$ 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-checkpoint ${ }^{7}$ 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. ${ }^{14} \mathrm{~A}$ few representatives Aurora B inhibitors are shown in Figure 1. To date no smallmolecule 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 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 $\mathrm{C}^{21,22,23}$ 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).



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

In addition to the micromolar inhibition of the CK1 kinase by the natural product benzosceptrin $B\left(\mathrm{IC}_{50}=0.68 \mu \mathrm{M}\right)$, the pyrrole-2aminoimidazole 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) ( $\mathrm{IC}_{50}=5$ $\mu \mathrm{M}$ ), 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
(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 $\mathbf{3}$ 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 $\mathbf{9 a}$ and $\mathbf{9 b}$ 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 9 c , a Sonogashira cross-coupling with propargylamine and 10 c gave the compound 11 c .

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) Boc 2 O, DMAP, ACN, rt, 16 h, $97 \%$; (d) $\operatorname{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2} 10 \%$, CuI 10\%, $\mathrm{NEt}_{3}, 81 \%$; (e) TFA, DCM, $80 \%$; (f), EDC.HCl, HOBT, DIEA, rt, DCM, 17-56\%; (g) Propargylamine, $\mathrm{T}_{3} \mathrm{P}$, DIEA, EtOAc; (h) 3, $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2}, \mathrm{CuI}, \mathrm{Et}_{3} \mathrm{~N}, \mathrm{DMF}, 5{ }^{\circ} \mathrm{C}, 40-48 \%$; (i) $\mathrm{NaNO}_{2}, \mathrm{AcOH}, \mathrm{H}_{2} \mathrm{O}, 81 \%$; (j) NaOMe 5.4 M in MeOH, THF dry, $60 \%$; (k) $\mathrm{Zn}, \mathrm{AcOH}$, dioxane $/ \mathrm{H}_{2} \mathrm{O}, 80^{\circ} \mathrm{C}, 66 \%$; ( 1 ) LiOH. $\mathrm{H}_{2} \mathrm{O}, \mathrm{MeOH} / \mathrm{H}_{2} \mathrm{O}, 70^{\circ} \mathrm{C}, 76 \%$; (m) Propargylamine, T $\mathrm{T}_{3} \mathrm{P}$, DIEA, EtOAc, 69\%; (n) 3, $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2}, \mathrm{CuI}, \mathrm{Et}_{3} \mathrm{~N}, \mathrm{DMF}, 50^{\circ} \mathrm{C}, 52 \%$.

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 iodoimidazopyrimidine $\mathbf{3}$ provided compounds $\mathbf{1}$ 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) $\mathrm{Br}_{2}, \mathrm{AcOH}$; (b) $\mathrm{I}_{2}, ~ \mathrm{AgTFA}, \mathrm{CHCl}_{3}$; (c) $\mathrm{SO}_{2} \mathrm{Cl}_{2}, \mathrm{CHCl}_{3}$; (d) $\mathrm{Br}_{2} 1$ equiv., AcOH ; (e) propargylamine, $60^{\circ} \mathrm{C}$; (f) $3, \mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2}, \mathrm{CuI}, \mathrm{Et}_{3} \mathrm{~N}, \mathrm{DMF}, 60^{\circ} \mathrm{C}$.

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 $19 \mathrm{~d}-\mathrm{j}$ 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 $19 a$ with the alkynes $15 b$ and 15 c gave the dichloro and diiodo derivatives $20 \mathrm{k}-1$ respectively. The same reaction between the bicycle $\mathbf{1 9 c}$ and the acetylenic $\mathbf{1 5 b}$ and $\mathbf{1 5 c}$ gave the dichloro and diiodo derivatives $\mathbf{2 0 m} \mathbf{- n}$ respectively (see Supporting Information).

Scheme 3. Variation of the east bicycle


Reagents and conditions: (a) Chloroacetaldehyde, EtOH or ACN $70^{\circ} \mathrm{C}, 100 \%$; (b) NIS, ACN $64-100 \%$ ( 19 b and 19 c ) or $\mathrm{I}_{2}$, pyridine, $42 \%$ (19a) ; (c) $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2} 5 \%, \mathrm{CuI} 10 \%, \mathrm{Et}_{3} \mathrm{~N}, \mathrm{THF}, 50^{\circ} \mathrm{C}, 10-$ 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 $23 \mathrm{a}-\mathrm{h}^{43,44,45,46,47,48,49}$ 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) Chloracetaldehyde, EtOH , reflux; 79-100 \%; (b) NIS, ACN, 65-97\%; (c) R-I, $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2}, \mathrm{CuI}$, $E t_{3} \mathrm{~N}, \mathrm{THF}, 5-63 \%$.

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) $\mathrm{I}_{2}, \mathrm{KI}, \mathrm{NaOH} 1 \mathrm{~N}, \mathrm{EtOH}, 83$ \% (b) $\mathrm{PhSO}_{2} \mathrm{Cl}, \mathrm{Bu}_{4} \mathrm{NBr}, \mathrm{NaOH}, \mathrm{DCM}$ dry, $98 \%$; (c) 15a, $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2}, \mathrm{CuI}, \mathrm{Et}_{3} \mathrm{~N}, \mathrm{THF}, 60 \%$; (d) TBAF. $\mathrm{H}_{2} \mathrm{O}$, 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, ${ }^{51,52} \mathbf{3 1 b}$ and 31c, ${ }^{53,54} \mathrm{~N}$-protection of the amino-imidazopyridine with acetyl chloride or $\mathrm{Boc}_{2} \mathrm{O}$ 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 intermediaites was achieved with TFA in DCM producing into 37,38 and 43 (Scheme 6). ${ }^{55}$ In addition, Sonogashira coupling of $\mathbf{3 3 b}$ with $\mathbf{1 5 b}$ (dichloro) and $\mathbf{1 5 c}$ (diiodo) gave after deprotection the dichloro and diiodo derivatives 44 and 45 (see SI).

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


Reagents and conditions: (a) Chloroacetaldehyde, EtOH reflux, $65-93 \%$; (b) $\mathrm{Ac}_{2} \mathrm{O}, \mathrm{DCM}, 95 \%$; (c) Boc 2 O , DCM, DMAP, 39-67\%; (d)
 (h) $\mathrm{Boc}_{2} \mathrm{O}, \mathrm{Et}_{3} \mathrm{~N}, \mathrm{DMF}$ and NIS, $\mathrm{ACN}, 38 \%$; (i) $15 \mathrm{a} \mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2} 5 \%, \mathrm{CuI} 10 \%, \mathrm{Et}_{3} \mathrm{~N}$, DMF; then TFA, DCM (yields over two steps : $11 \%$ for 38 and $59 \%$ for 43 ).

The preparation of derivatives $\mathbf{4 8}$ and $\mathbf{5 0}$ having additional salifiable groups is shown in Scheme 7. The halogenated intermediates $47 a$ and $47 b$ were prepared from 46a and $46 b$ using Buchwald coupling with $N$-Boc-piperazine or morpholine in the presence of XPhos as ligand followed sequentially by iodination, Sonogashira
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, $\mathrm{Pd}_{2}(\mathrm{dba})_{3}$, XPhos, NaOtBu , toluene, $100^{\circ} \mathrm{C}$, $62 \%$; (b) Boc-piperazine, $\mathrm{Pd}_{2}(\mathrm{dba})_{3}$, XPhos, NaOtBu , toluene, $100^{\circ} \mathrm{C}$, quant.; (c) NIS, ACN 55-100\%; (d) $15 \mathrm{a}, \mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2} 5 \mathrm{~mol} \%$, $\mathrm{CuI} 10 \mathrm{~mol} \%, \mathrm{Et}_{3} \mathrm{~N}, \mathrm{THF}, 22-61 \%$ ; (e) HCl 4 N dioxane, $\mathrm{DCM}, 72 \%$

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. $N$-Bocprotected propargylamine $51 a^{57}$ was subjected to a hydrozircona-tion-hydroboration sequence with pinacol borane and Schwartz's reagent to produce the key building block $\mathbf{5 1 b}$. ${ }^{58,59}$ Suzuki coupling with the corresponding iodo compounds 19a-c gave compounds 52-54. After $N$-Boc cleavage of the latter with aqueous 4 N HCl , the resulting amines were acylated using the trichloroacetyl dibromopyrrole 14a.

Scheme 8. Synthesis of derivatives with an alkene linker


Reagents and conditions: (a) $\mathrm{Boc}_{2} \mathrm{O}, \mathrm{DMAP}, \mathrm{DCM}, 97 \%$; (b) Pinacolborane, $\mathrm{Cp}_{2} \mathrm{ZrHCl} 25 \mathrm{~mol} \%, \mathrm{Et}_{3} \mathrm{~N} 25 \mathrm{~mol} \%, 40^{\circ} \mathrm{C}, 73 \%$; (c) R-I, $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{4} 5 \mathrm{~mol} \%, \mathrm{~K}_{2} \mathrm{CO}_{3}(2 \mathrm{M})$, Dioxane, $80^{\circ} \mathrm{C}, 47-100 \%$; (d) HCl 4 N 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 2aminopyrimidine and 3 -aminopyridazin with the nitrophenyl- $\alpha$ 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 $\mathrm{Et}_{3} \mathrm{~N}$ 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) $\mathrm{SnCl}_{2} .2 \mathrm{H}_{2} \mathrm{O}, \mathrm{HCl}, \mathrm{EtOH}, 62-78 \%$; (c) Trichloroacetyl dibromopyrrole 14a $\mathrm{Et}_{3} \mathrm{~N}, 60^{\circ} \mathrm{C}, 49 \%$; (d) Dibromopyrrole carboxylic acid, $(\mathrm{COCl})_{2}, \mathrm{DCM} / \mathrm{DMF}, 72 \%$.

The preparation of the new C3 regioisomers and benzene metalinked 67a-d or benzene para-linked 68a-d was achieved from 19a$\mathbf{c}$ 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 $\mathrm{T}_{3} \mathrm{P}$ (Scheme 10).

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


Reagents and conditions: (a) i. Pd-118, $\mathrm{K}_{2} \mathrm{PO}_{4}$, dioxane $/ \mathrm{H}_{2} \mathrm{O}$, $80^{\circ} \mathrm{C}$, ii. $\mathrm{SnCl}_{2} .2 \mathrm{H}_{2} \mathrm{O}$, EtOH/HCl, reflux; (b) Dibromopyrrole carboxylic acid, $\mathrm{T}_{3} \mathrm{P}$, 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 $\mathrm{NaN}_{3}$ under acidic conditions. ${ }^{66}$ The azidomethylimidazoles were then coupled with dibromopyrrole alkynes via the $\mathrm{CuSO}_{4}$ 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, $90^{\circ} \mathrm{C}$, $48 \%$; (b) formaldehyde $37 \%$ solution, $\mathrm{NaOAc}, \mathrm{AcOH}, 100^{\circ} \mathrm{C}, 50-$ $73 \%$; (c) TFA, $\mathrm{NaN}_{3}, 70^{\circ} \mathrm{C}, 56-77 \%$; (d) $15 \mathrm{a}, \mathrm{CuSO}_{4} .5 \mathrm{H}_{2} \mathrm{O} 1$ $\mathrm{mol} \%$, Sodium ascorbate $10 \mathrm{~mol} \%$, DMF/ $\mathrm{H}_{2} \mathrm{O}, 63-89 \%$.

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 ADPGlo methodology (ADP-Glo ${ }^{\text {TM }}$ 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 ( $\mathbf{8 a}, \mathbf{8 b}$ ) and indoles ( $\mathbf{8 c}, \mathbf{8 d}$ ) 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 $\mathbf{1 6 b}$ 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 11 b 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 ( $\mathrm{IC}_{50}$ on Aurora B kinase activity were calculated from dose-response curves performed in duplicate).
8b

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 $\left(\mathrm{IC}_{50}=46.8 \mathrm{nM}\right)$ was the simplest imidazopyridine 20c while 20a and 20b showed lower activities with $\mathrm{IC}_{50}$ '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 imidazopyridine 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 $24 \mathrm{a}-24 \mathrm{~h}$ resulted in compounds less active or, at most, equipotent with the unsubstituted imidazopyridine 20c. Even the smaller fluoro substituent in $\mathbf{2 4 f}$ 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 imidazopyridine derivatives 35,37 and 43 having an H -donor at C 8 . Our assays showed that the activity of 37 was only slightly superior to that of $\mathbf{2 0}$ c but 150 times greater than that of the initial hit EL-228 (1), with an $\mathrm{IC}_{50}$ of 34.3 nM . The importance of a hydrogen donor at this position was confirmed by the compound $\mathbf{2 4 c}$ bearing a bromine C8 atom, which did not show any activity. Moreover, the imidazopyridine 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 $\mathrm{IC}_{50}$ '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 ( $\mathrm{IC}_{50}$ on Aurora B kinase activity were calculated from dose-response curves performed in duplicate).
$\mathbf{2 0 0}$

At this stage and in line with the above finding regarding the equipotency of the dichloropyrrole derivative $\mathbf{1 6 b}$ (Table 1) with EL-228 (1), we chose to combine the activity-improving 8-aminoimidazopyridine unit of $\mathrm{CJ} 2-150$ (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 ( $\mathrm{IC}_{50}$ on Aurora B kinase activity were calculated from dose-response curves performed in duplicate).
(,

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 $\mathrm{IC}_{50}$ '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 ( $\mathrm{IC}_{50}$ on Aurora B kinase activity were calculated from dose-response curves performed in duplicate)
20c
(

$>1000$


* $\overline{\text { Dr. Sylvain Picon, Ph.D. thesis, Université Paris XI, Orsay, December 9 }}{ }^{\text {th }}, 2008$.


## 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 $10 \mu \mathrm{M}$ and 1 $\mu \mathrm{M}$ concentrations and $\mathrm{IC}_{50}$ values were determined for all compounds showing greater than $50 \%$ inhibition at $10 \mu \mathrm{M}$. 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 $1 \mu \mathrm{M}$, is illustrated in red in a phylogenetic tree format (Figure 3). Only Aurora A (the congener of Aurora B) showed high inhibition (80\%). GSK3 $\beta$ 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 $1 \mu \mathrm{M}$, 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 K 562 cell viability with $\mathrm{EC}_{50 \mathrm{~s}}$ above $20 \mu \mathrm{M}$ together with little to no effect on HCT116 cells. On the other hand, three compounds, $\mathbf{2 4 b}, \mathbf{2 4 f}$ and $\mathbf{2 4 g}$, showed an interesting specificity towards HCT116 cell line with $\mathrm{EC}_{50}$ of 8,3 and $5 \mu \mathrm{M}$ respectively in the same order of magnitude as $\mathrm{ZM} 447439\left(\mathrm{EC}_{50}\right.$ of $\left.7 \mu \mathrm{M}\right)$, 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 $2 \mu \mathrm{M}$, similar to ZM447439 ( 7 and $9 \mu \mathrm{M}$ 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 $1 \mu \mathrm{M}$ ) (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.

|  | $\begin{gathered} \text { Aurora B } \\ \mathrm{IC}_{50}(\mathrm{nM}) \end{gathered}$ | $\begin{gathered} \operatorname{HCT}^{\mathrm{EC}_{50}(\mu \mathrm{M})} \end{gathered}$ | $\begin{gathered} \mathrm{K} 562 \\ \mathrm{EC}_{50}(\mu \mathrm{M}) \end{gathered}$ |  | $\begin{gathered} \text { Aurora B } \\ \mathrm{IC}_{50}(\mathrm{nM}) \end{gathered}$ | $\begin{gathered} \text { HCT116 } \\ \text { EC }_{50}(\mu \mathrm{M}) \end{gathered}$ | $\begin{gathered} \mathrm{K} 562^{\mathrm{EC}_{50}(\mu \mathrm{M})} \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 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 |

$\mathrm{EC}_{50}(\mu \mathrm{M})$ on cell viability were calculated from dose-response curves made in triplicate after 72 h treatment. IC ${ }_{50}(\mathrm{nM})$ on Aurora B activity are reported in this table. "n.e.": no effect at maximal tested concentration of $100 \mu \mathrm{M}$.

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 $\mathrm{EC}_{50}$ of 11 and $22 \mu \mathrm{M}$ 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 $\mathrm{EC}_{50}$ of 9 and 2
$\mu \mathrm{M}$ 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) doseresponse 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 ( $\mathrm{EC}_{50}>100 \mu \mathrm{M}$ ) than the proliferative condition performed with PHA ( $30<\mathrm{EC}_{50}<100 \mu \mathrm{M}$ ). 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 |

$\mathrm{EC}_{s}(\mu \mathrm{M})$ 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 Mickaelis-Menten and Lineweaver-Burk plots (Figure $4 \mathrm{~A}, \mathrm{C})$. 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 IC sos measured at increasing concentration of ATP showed very little variation (from 39 nM at $5 \mu \mathrm{M}$ ATP to 94 nM at $75 \mu \mathrm{M}$ 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 Mickaelis-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 $2 \mu \mathrm{M}$ in the presence of increasing concentrations of ATP from 3.125 to $50 \mu \mathrm{M}$. ADP production in the reaction is directly proportional to Aurora B activity. B; Dose-response curves of CJ2-150 (from 2 nM to $100 \mu \mathrm{M}$ ) on Aurora B activity at increasing concentrations of ATP (from 5 to $75 \mu \mathrm{M}$ ), corresponding calculated IC $\mathrm{S}_{50}$ are showed in embedded table. C: ATP competition assay with compound CJ2-150 represented on a double reciprocal Lineweaver-Burk type plot; 1/ADP production expressed in RLU (Relative Light Unit) is plotted against 1/ATP concentration $(\mu \mathrm{M})$. 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 U2 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 $20 \mu \mathrm{M}$ 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 4 N DNA contents and a part of the population reaching a DNA content of 8 N 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 H 3 on serine 10 by immunofluorescence. For this purpose, we worked with U-2 OS adherent cells treated with CJ2-150 (37) at $10 \mu \mathrm{M}$ or ZM447439 at $1 \mu \mathrm{M}$ (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 $10 \mu \mathrm{M}$ similar to that obtained with ZM447439 at $1 \mu \mathrm{M}$. The effect was slightly milder with CJ2-150 at $2.5 \mu \mathrm{M}$. These results showed that the compound, displaying an in vitro inhibitory $\mathrm{IC}_{50}$ 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 metabolization rate: the amide function metabolic sensitivity and the heterocyclic probably bicycle 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 $10 \mu \mathrm{M}$ of CJ2-150 or $1 \mu \mathrm{M}$ 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 $\alpha$-Tubulin immuno-staining (red) and DNA was visualized by DAPI staining (blue). Bar $10 \mu \mathrm{~m}$. B, quantification of H3S10ph signal over DAPI signal, representative of Aurora B activity, in prometaphase cells from the experiment described in $\mathrm{A}, \mathrm{n} \geq 20$.

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 $2.5 \mu \mathrm{M}$ ) or ZM447439 ( $1 \mu \mathrm{M}$ ) and a DMSO control. We observed increased mitotic indexes after treatment with both CJ2-150 at 10 $\mu \mathrm{M}$ 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 $2.5 \mu \mathrm{M}(4.6 \%)$. 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 $10 \mu \mathrm{M}$ 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 $2.5 \mu \mathrm{M}$ ) or ZM447439 ( $1 \mu \mathrm{M}$ ) or DMSO (control) for $16 \mathrm{~h} . \mathrm{n} \geq 200$; 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 ( $10 \mu \mathrm{M}$ ) or ZM447439 ( $1 \mu \mathrm{M}$ ) or DMSO for 24 h . Aurora B is visualized in green, $\alpha$-Tubulin in red and DNA (DAPI) in blue. Scale bar $10 \mu \mathrm{~m}$.

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 $\alpha$-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 $\pi$-stacking interactions with Phe 72 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 $\mathrm{N}-\mathrm{H}$ of amide and pyrrole moieties in the ligand with the backbone O of Tyr 141 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 $\mathbf{5 0}$, with the exception of the imidazopyridine ring system, which is flipped towards the subpocket extremity, stabilized by a $\pi$ - $\pi$ 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.so


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
localization defects thus, validating its cellular functionality on Au rora B kinase. ATP-competition assay complemented with molecular docking showed that CJ2-150 (37) and compound 20c do not compete directly with ATP, but binds outside of the ATP pocket on an allosteric site (type IV) in a mixed-type inhibition. Our novel family of acetylenic compounds provides new entry to selective inhibitors of Aurora B since the selectivity of kinase inhibitors is often an inherent challenge for the types of inhibitors that target the highly conserved ATP binding pocket.

## 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 (70230 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 : $1 \mathrm{~mL} / \mathrm{min}$, eluent : $\mathrm{H}_{2} \mathrm{O}+0.1 \%$ formic acid $/ \mathrm{ACN}$ $+0.1 \%$ formic acid by injection $10 \mu \mathrm{~L}$ of $(1 \mathrm{mg} / \mathrm{mL}$ in $\mathrm{MeOH} / \mathrm{DMSO}: 7 / 3$. The ELSD-HPLC profiles showed that the purity of the majority of the final products is $>95 \%$ (see SI). 1 H NMR also demonstrates the high purity of the final products (see SI). ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR spectra were recorded on a Bruker Advance 300 NMR spectrometer ( 300 and 75 MHz ). Chemical shifts $\delta$ are reported in parts per million ( ppm ) relative to the residual solvent signals $\left(\mathrm{CDCl}_{3}, \delta=7.26 \mathrm{ppm}\right.$ for ${ }^{1} \mathrm{H}$ and $\delta=77.16 \mathrm{ppm}$ for ${ }^{13} \mathrm{C}$; DMSO-d6, $\delta=2.50 \mathrm{ppm}$ for ${ }^{1} \mathrm{H}$ and $\delta=39.52 \mathrm{ppm}$ for ${ }^{13} \mathrm{C}$. DMF$\mathrm{d} 7, \delta=2.79,2.92,8.03 \mathrm{ppm}$ for ${ }^{1} \mathrm{H}$ and $\delta=29.76,34.89,163.15$ ppm for $\left.{ }^{13} \mathrm{C}\right)$. Data are reported as follows: chemical shift, multiplicity ( $\mathrm{s}=$ singlet, $\mathrm{d}=$ doublet, $\mathrm{t}=$ triplet, $\mathrm{q}=$ quartet, $\mathrm{m}=$ multiplet, $\mathrm{br}=$ broad $)$, coupling constants $(\mathrm{Hz})$ and integration. Highresolution 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 ( $v$ in $\mathrm{cm}-1$ ). 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 $\mathrm{MgSO}_{4}$, filtered, and concentrated under vacuum. The product was purified by flash chromatography.
General Procedure B for Sonogashira coupling.
Appropriate halide (1eq.) was dissolved in either dry THF or dry DMF and $\mathrm{Et}_{3} \mathrm{~N}$ (2-3 eq.). The reaction mixture was degased by argon bubbling. $\mathrm{CuI}(5-10 \% \mathrm{~mol})$ was introduced followed by $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{2} \mathrm{Cl}_{2}(2-5 \% \mathrm{~mol})$. Then the alkyne (1.5-2 eq.) was added to the mixture. The reaction mixture was stirred under inert atmosphere at room temperature or $50^{\circ} \mathrm{C}$ 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 15 a ( $153 \mathrm{mg}, 0.5 \mathrm{mmol}$ ) and $3(147 \mathrm{mg}, 0.6 \mathrm{mmol})$. Purification afforded the title compound as a pale yellow solid ( $110 \mathrm{mg}, 52 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\delta 4.44(\mathrm{~d}, J=5.3 \mathrm{~Hz}, 2 \mathrm{H}), 6.98(\mathrm{~s}, 1 \mathrm{H}), 7.24(\mathrm{~m}, 1 \mathrm{H})$, $8.05(\mathrm{~s}, 1 \mathrm{H}), 8.66(\mathrm{~m}, 1 \mathrm{H}), 8.76(\mathrm{t}, J=5.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.88(\mathrm{~d}, J=6.6$ $\mathrm{Hz}, 1 \mathrm{H}), 12.82(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.7$, 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) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+}$ $\mathrm{C}_{14} \mathrm{H}_{10} \mathrm{~N}_{5} \mathrm{OBr}_{2}: 423.9226$. Found 423.9239. IR $\left(\mathrm{cm}^{-1}\right): 3107,1616$, 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-bromothio-phene-2-carboxylic acid ( $250 \mathrm{mg}, 1.21 \mathrm{mmol}$ ) yielding 8a as a beige amorphous solid ( $244 \mathrm{mg}, 56 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.45(\mathrm{~d}, J=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 7.19(\mathrm{~d}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.26$ (dd, $J=4.2,2.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.83(\mathrm{~d}, J=5.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.05(\mathrm{~s}, 1 \mathrm{H})$, $8.65(\mathrm{dd}, J=2.1,2.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.77(\mathrm{t}, J=5.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.88(\mathrm{dd}, J$ $=1.8,5.1 \mathrm{~Hz}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 30,68.8$, 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) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+}$ $\mathrm{C}_{14} \mathrm{H}_{10} \mathrm{BrN}_{4} \mathrm{OS}: 362.9738$. Found: 362.9742 . IR $\left(\mathrm{cm}^{-1}\right): 3335$, 1630, 1616, 1514, 1484, 1276.
5-Bromo- $N$-(3-(imidazo[1,2-a] pyrimidin-3-yl)prop-2-yn-1-
yl )thiophene-3-carboxamide ( $\mathbf{8 b}$ ). The title compound was synthesized according to general procedure A using 5-bromothio-phene-3-carboxylic acid ( $250 \mathrm{mg}, 1.21 \mathrm{mmol}$ ) yielding $\mathbf{8 b}$ as a beige amorphous solid ( $216 \mathrm{mg}, 44 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.44(\mathrm{~d}, J=5.6 \mathrm{~Hz}, 2 \mathrm{H}), 7.25(\mathrm{dd}, J=2.2,4 \mathrm{~Hz}, 1 \mathrm{H})$, $7.62(\mathrm{~d}, J=1.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.04(\mathrm{~s}, 1 \mathrm{H}), 8.19(\mathrm{~d}, J=1.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.64$ (dd, $J=2.3,2.3 \mathrm{~Hz}, 1 \mathrm{H}$ ), 8.88 (dd, $J=2.3,4.6 \mathrm{~Hz}, 1 \mathrm{H}$ ), $8.96(\mathrm{t}, J$ $=5.3 \mathrm{~Hz}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.4,68.7,96.9$, 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 ( $\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{14} \mathrm{H}_{10} \mathrm{BrN}_{4} \mathrm{OS}$ : 362.9738. Found: 362.9734 . IR $\left(\mathrm{cm}^{-1}\right): 3249,1658,1555,1512$, 1486, 1424, 1262.
$N$-(3-(Imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-yl)-1 $H$-indole2 -carboxamide ( $\mathbf{8 c}$ ). The title compound was synthesized according to general procedure A using 1 H -indole-2-carboxylic acid (200 $\mathrm{mg}, 1.24 \mathrm{mmol}$ ) yielding 8 c as a yellow amorphous solid ( 220 mg ,
$56 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.53$ ( $\mathrm{d}, J=5.5 \mathrm{~Hz}, 2 \mathrm{H}$ ), $7.02(\mathrm{t}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.19(\mathrm{t}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.19(\mathrm{~s}, 1 \mathrm{H}), 7.25$ (dd, $J=2.2,4.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.44(\mathrm{dd}, J=1.1,8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.62(\mathrm{~d}, J$ $=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.06(\mathrm{~s}, 1 \mathrm{H}), 8.64(\mathrm{dd}, J=1.9,4.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.9(\mathrm{dd}$, $J=1.9,7.1 \mathrm{~Hz}, 1 \mathrm{H}), 9.1(\mathrm{t}, J=5.2 \mathrm{~Hz}, 1 \mathrm{H}), 11.7(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.3,68.6,97.2,103,106.3,109.9,112.3$, 119.7, 121.5, 123.5, 126.9, 131, 133.7, 136.5, 138.7, 147.9, 151.5, 160.9. HRMS (ESI+) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{18} \mathrm{H}_{14} \mathrm{~N}_{5} \mathrm{O}$ : 316.1198. Found: 316.1218 . IR $\left(\mathrm{cm}^{-1}\right): 3241,1631,1616,1553$, 13091257.
[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 ( $200 \mathrm{mg}, 1.24$ mmol ) yielding 8 d as a white amorphous solid ( $64 \mathrm{mg}, 17 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( $300 \mathrm{MHz}, \mathrm{DMF}-\mathrm{d} 7$ ) $\delta 4.62$ (d, $J=5.5 \mathrm{~Hz}, 2 \mathrm{H}$ ), 7.19 (m, $2 \mathrm{H}), 7.3(\mathrm{dd}, J=4,6.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.53(\mathrm{~d}, J=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.05(\mathrm{~s}$, $1 \mathrm{H}), 8.26(\mathrm{~d}, J=3 \mathrm{~Hz}, 1 \mathrm{H}), 8.34(\mathrm{~d}, J=8.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.6(\mathrm{t}, J=$ $5.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.71(\mathrm{dd}, J=2,4.2 \mathrm{~Hz}, 1 \mathrm{H}), 9.02(\mathrm{dd}, J=1.9,6.8 \mathrm{~Hz}$, $1 \mathrm{H}), 11.22(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMF-d7) $\delta 28.9,68.2$, 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) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{18} \mathrm{H}_{14} \mathrm{~N}_{5} \mathrm{O}: 316.1198$. 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 $\mathbf{1 0 a}(245 \mathrm{mg}, 0.80 \mathrm{mmol})$ and 3 ( $150 \mathrm{mg}, 0.61 \mathrm{mmol}$ ). Purification afforded the title compound as a white amorphous solid ( $104 \mathrm{mg}, 40 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.44$ (d, $J=5.6 \mathrm{~Hz}, 2 \mathrm{H}$ ), 7.25 (dd, $J=4.1$, $6.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.44(\mathrm{~s}, 1 \mathrm{H}), 8,05(\mathrm{~s}, 1 \mathrm{H}), 8.65(\mathrm{dd}, J=2.0,4.2 \mathrm{~Hz}$, $1 \mathrm{H}), 8.88(\mathrm{dd}, J=2.0,6.8 \mathrm{~Hz}, 1 \mathrm{H}), 9.17(\mathrm{t}, J=5.5 \mathrm{~Hz}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,68.8,96.6,103.4,106.3,110$, 118.1, 126.3, 133.8, 138.8, 147.9, 149, 151.6, 155.8. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{14} \mathrm{H}_{9} \mathrm{~N}_{4} \mathrm{O}_{2} \mathrm{Br}_{2}$ : 424.9072. Found: 424.9068. IR ( $\mathrm{cm}^{-1}$ ): 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 11 b was synthesized according to general procedure B using $\mathbf{1 0 b}(343 \mathrm{mg}, 1.06$ mmol ) and $3(200 \mathrm{mg}, 0.82 \mathrm{mmol})$. Purification afforded the title compound as a beige amorphous solid ( $174 \mathrm{mg}, 48 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 4.48$ (d, $J=5.2 \mathrm{~Hz}, 2 \mathrm{H}$ ), 7.26 (dd, $J=4.1$, $6.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.84(\mathrm{~s}, 1 \mathrm{H}), 8.08(\mathrm{~s}, 1 \mathrm{H}), 8.66(\mathrm{dd}, J=1.9,4.4 \mathrm{~Hz}$, $1 \mathrm{H}), 8.90(\mathrm{dd}, J=1.7,7.0 \mathrm{~Hz}, 1 \mathrm{H}), 9.29(\mathrm{t}, J=5.0 \mathrm{~Hz}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.8,69.1,96.4,106.3,110.1,114$, 117, 130.8, 133.9, 139, 140.4, 147.9, 151.6, 159.1. HMRS (ESI) $m / z$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{14} \mathrm{H}_{9} \mathrm{~N}_{4} \mathrm{OSBr}_{2}$ : 440.8843. Found: 440.8851. IR ( $\mathrm{cm}^{-1}$ ): 3217, 2979, 1645, 1512, 1290, 1230.
$N$-(3-(Imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-yl)-4,5-dime-thyl-1 $H$-pyrrole-2-carboxamide (11c). Compound 11c was synthesized according to general procedure B using $\mathbf{1 0 c}(129 \mathrm{mg}, 0.73$ $\mathrm{mmol})$ and $3(150 \mathrm{mg}, 0.61 \mathrm{mmol})$. Purification afforded the title compound as an orange amorphous solid ( $94 \mathrm{mg}, 52 \%$ ). ${ }^{1} \mathrm{H}$ NMR (300MHz, DMSO-d6) $\delta 1.91$ ( s, 3H), 2.10 ( $\mathrm{s}, 3 \mathrm{H}), 4.40(\mathrm{~d}, J=5.5$ $\mathrm{Hz}, 2 \mathrm{H}), 6.57(\mathrm{~d}, J=2.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.24(\mathrm{dd}, J=4.2,6.8 \mathrm{~Hz}, 1 \mathrm{H})$, $8.03(\mathrm{~s}, 1 \mathrm{H}), 8,32(\mathrm{t}, J=5.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.64(\mathrm{dd}, J=2.0,4.1 \mathrm{~Hz}, 1 \mathrm{H})$, $8.87(\mathrm{dd}, J=2.0,6.9 \mathrm{~Hz}, 1 \mathrm{H}), 11.07(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 10.6,10.7,29,68.2,97.9,106.5,110,112,114.6$, 122.4, 128.7, 133.7, 138.5, 147.8, 151.5, 160.5. HMRS (ESI) m/z Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{16} \mathrm{H}_{16} \mathrm{~N}_{5} \mathrm{O}$ : 294.1355. Found: 294.1342. IR $\left(\mathrm{cm}^{-1}\right): 3267,3205,1618,1513,1486,1410,1262,1230,1146$.

4,5-Dichloro-N-(3-(imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-$\mathrm{yl})-1 \mathrm{H}$-pyrrole-2-carboxamide ( $\mathbf{1 6 b}$ ). Compound 16 b was synthesized according to general procedure B using $\mathbf{1 5 b}(106 \mathrm{mg}, 0.49$ $\mathrm{mmol})$ and $3(100 \mathrm{mg}, 0.41 \mathrm{mmol})$. Purification afforded the title compound as a pale yellow amorphous solid ( $28 \mathrm{mg}, 21 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.44$ (d, $J=5.3 \mathrm{~Hz}, 2 \mathrm{H}$ ), 6.96 (s, $1 \mathrm{H}), 7.25(\mathrm{t}, J=4.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.05(\mathrm{~s}, 21 \mathrm{H}), 8.66(\mathrm{~m}, 1 \mathrm{H}), 8.78(\mathrm{t}$, $J=5.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.88(\mathrm{~d}, J=6.6 \mathrm{~Hz}, 1 \mathrm{H}), 12.86(\mathrm{br} \mathrm{s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( $75 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d} 6$ ) $\delta 29.2,68.7,97.1,108.1,108.6,110$, 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-diiodo1 H -pyrrole-2-carboxamide (16c). Compound 16 c was synthesized according to general procedure B using $15 c$ ( $196 \mathrm{mg}, 0.49$ $\mathrm{mmol})$ and $3(120 \mathrm{mg}, 0.49 \mathrm{mmol})$. Purification afforded the title compound as a white amorphous solid ( $101 \mathrm{mg}, 40 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.43(\mathrm{~d}, J=5.2 \mathrm{~Hz}, 2 \mathrm{H}), 6.97(\mathrm{~d}, J=2.3$ $\mathrm{Hz}, 1 \mathrm{H}), 7.24(\mathrm{dd}, J=4.2,6.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.04(\mathrm{~s}, 1 \mathrm{H}), 8.66(\mathrm{~m}, 2 \mathrm{H})$, $8.88(\mathrm{~d}, J=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 12.43(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.1,68.6,76.2,84.2,97.2,106.3,110,118.6,132.2$, 133.8, 138.7, 147,8, 151.5, 158.4. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{14} \mathrm{H}_{10} \mathrm{I}_{2} \mathrm{~N}_{5} \mathrm{O}: 517.8975$. Found: 517.8974. IR $\left(\mathrm{cm}^{-1}\right)$ : 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 16 d was synthesized according to general procedure B using 15d and 3. Purification afforded the title compound ( $72 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\delta 4.44(\mathrm{~d}, J=4.3 \mathrm{~Hz}, 2 \mathrm{H}), 6.91(\mathrm{~m}, 1 \mathrm{H}), 7.03(\mathrm{~m}, 1 \mathrm{H})$, $7.25(\mathrm{dd}, J=4.0,6.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.07(\mathrm{br} \mathrm{s}, 1 \mathrm{H}), 8.66(\mathrm{~m}, 1 \mathrm{H}), 8.72$ $(\mathrm{t}, J=4.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.89(\mathrm{~d}, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 11.94(\mathrm{br} \mathrm{s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.1,68.6,97.3,95.1,110.1,112$, 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 $H$-pyr-role-2-carboxamide (16e). Compound $16 e$ was synthesized according to general procedure $B$ using $15 e$ and 3 . Purification afforded the title compound ( $86 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSOd6) $\mathcal{\delta} 4.44(\mathrm{~d}, J=5.6 \mathrm{~Hz}, 2 \mathrm{H}), 6.10(\mathrm{~m}, 1 \mathrm{H}), 6.83(\mathrm{~m}, 1 \mathrm{H}), 6.89$ $(\mathrm{m}, 1 \mathrm{H}), 7.24(\mathrm{dd}, J=4.2,6.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.04(\mathrm{~s}, 1 \mathrm{H}), 8.58(\mathrm{t}, J=$ $5.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.65(\mathrm{dd}, J=2.0,4.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.88(\mathrm{~d}, J=2.1,6.8 \mathrm{~Hz}$, $1 \mathrm{H}), 11.53$ (br s, 1H). ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29,68.3$, 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 ( $125 \mathrm{mg}, 0.41$ mmol ) and 19a ( $100 \mathrm{mg}, 0.41 \mathrm{mmol}$ ). Purification afforded the title compound as a yellow amorphous solid ( $46 \mathrm{mg}, 27 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d} 6) \delta 4.45(\mathrm{~d}, J=5.6 \mathrm{~Hz}, 2 \mathrm{H}), 6.99(\mathrm{~d}, J=2.5$ $\mathrm{Hz}, 1 \mathrm{H}), 8.08(\mathrm{~d}, J=4.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.10(\mathrm{~s}, 1 \mathrm{H}), 8.51(\mathrm{dd}, J=1.3$, $4.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.79(\mathrm{t}, J=5,3 \mathrm{~Hz}, 1 \mathrm{H}), 9.16(\mathrm{~d}, J=1.3 \mathrm{~Hz}, 1 \mathrm{H}), 12.83$ ( $\mathrm{s}, 1 \mathrm{H}$ ). ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,68.1,98,98.4$, 105.3, 108.9, 113.1, 118.6, 127.5, 130.5, 138.9, 140, 143.2, 158.8. HRMS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{14} \mathrm{H}_{10} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 425.9211. Found: 425.9236. IR ( $\mathrm{cm}^{-1}$ ): 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 15 a ( $192 \mathrm{mg}, 0.78$ mmol ) and 19b ( $200 \mathrm{mg}, 0.65 \mathrm{mmol}$ ). Purification afforded the
title compound as a yellow amorphous solid ( $24 \mathrm{mg}, 9 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.43$ (d, $J=5.5 \mathrm{~Hz}, 2 \mathrm{H}$ ), 6.99 (d, $J=$ $2.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.34(\mathrm{dd}, J=4.6,9 \mathrm{~Hz}, 1 \mathrm{H}), 8.07(\mathrm{~s}, 1 \mathrm{H}), 8.2(\mathrm{dd}, J=$ $1.5,9.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.63(\mathrm{dd}, J=1.5,4.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.78(\mathrm{t}, J=5.5 \mathrm{~Hz}$, 1H), $12.8(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 28.9,69.1$, 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 $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{14} \mathrm{H}_{10} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 425.9211. Found: 425.9229. IR $\left(\mathrm{cm}^{-1}\right): 3141,3119,1659,1562$, 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 ( $282 \mathrm{mg}, 0.92$ $\mathrm{mmol})$ and $19 \mathrm{c}(150 \mathrm{mg}, 0.62 \mathrm{mmol})$. Purification afforded the title compound as a beige amorphous solid ( $120 \mathrm{mg}, 46 \%$ ). ${ }^{1} \mathrm{H}$ NMR (300MHz, DMSO-d6) $\delta 4.45$ (d, $J=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 6.99$ (d, $J=$ $1.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.11(\mathrm{t}, J=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.39(\mathrm{t}, J=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.68$ $(\mathrm{d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.9(\mathrm{~s}, 1 \mathrm{H}), 8.46(\mathrm{~d}, J=6.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.75(\mathrm{t}, J$ $=5.2 \mathrm{~Hz}, 1 \mathrm{H}), 12.8(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta$ 69.4, 97.2, 97.9, 105.5, 113.1, 113.6, 117.4, 125.2, 126.2, 127.6, 137.7, 158.7. HMRS (ESI) $m / z$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{11} \mathrm{~N}_{4} \mathrm{OBr}_{2}$ : 422.9279. Found: 422.9325. IR $\left(\mathrm{cm}^{-1}\right): 3137,3116,1646,1565$, 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 15 a ( $100 \mathrm{mg}, 0.33 \mathrm{mmol}$ ) and $19 \mathrm{~d}(84 \mathrm{mg}, 0.39 \mathrm{mmol})$. Purification afforded the title compound as a yellow amorphous solid ( $34 \mathrm{mg}, 24 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMF-d7) $\mathcal{\delta} 4.46$ (d, $J=5.7 \mathrm{~Hz}, 2 \mathrm{H}$ ), $7.10(\mathrm{~s}, 1 \mathrm{H}), 7.45$ $(\mathrm{m}, 2 \mathrm{H}), 7.66(\mathrm{~s}, 1 \mathrm{H}), 7.89(\mathrm{~m}, 1 \mathrm{H}), 7.99(\mathrm{~m}, 1 \mathrm{H}), 8.97(\mathrm{t}, J=$ $5.7 \mathrm{~Hz}, 1 \mathrm{H}), 13.09(\mathrm{bd} \mathrm{s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMF-d7) $\delta 29$, 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) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{16} \mathrm{H}_{11} \mathrm{Br}_{2} \mathrm{~N}_{2} \mathrm{OS}: 436.8959$. Found: 436.9008.
$N$-(3-(1 $H$-indol-5-yl)prop-2-yn-1-yl)-4,5-dibromo-1 $H$-pyrrole-2carboxamide (20e). Compound 20e was synthesized according to general procedure B using $15 \mathrm{a}(200 \mathrm{mg}, 0.65 \mathrm{mmol})$. Purification afforded the title compound as a beige amorphous solid ( $25 \mathrm{mg}, 10$ \%). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d} 6) \delta 4.28(\mathrm{~d}, J=5.6 \mathrm{~Hz}, 2 \mathrm{H})$, $6.42(\mathrm{~s}, 1 \mathrm{H}), 7(\mathrm{~d}, J=2.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.11(\mathrm{dd}, J=1.4,8.4 \mathrm{~Hz}, 1 \mathrm{H})$, $7.37(\mathrm{~m}, 1 \mathrm{H}), 7.39(\mathrm{~m}, 1 \mathrm{H}), 7.64(\mathrm{~s}, 1 \mathrm{H}), 8.66(\mathrm{t}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H})$, $11.26(\mathrm{~s}, 1 \mathrm{H}), 12.77(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\mathcal{\delta}$ 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 $(\mathrm{M}+\mathrm{H})^{+} \quad \mathrm{C}_{16} \mathrm{H}_{12} \mathrm{Br}_{2} \mathrm{~N}_{3} \mathrm{O}:$ 421.9327. Found: 421.9356. IR ( $\mathrm{cm}^{-1}$ ): 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 20 f was synthesized according to general procedure B using 15 a ( $200 \mathrm{mg}, 0.65 \mathrm{mmol}$ ) and 19 f ( $0.1 \mathrm{~mL}, 0.92 \mathrm{mmol}$ ). Purification afforded the title compound as a yellow amorphous solid ( $92 \mathrm{mg}, 36 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMF-d7) $\mathcal{\delta} 4.40(\mathrm{~d}, J=5.6 \mathrm{~Hz}, 2 \mathrm{H}), 7.09(\mathrm{dd}, J=3.6,5.3 \mathrm{~Hz}, 1 \mathrm{H})$, $7.11(\mathrm{~d}, J=2.8 \mathrm{HZ}, 1 \mathrm{H}), 7.31(\mathrm{dd}, J=1.2,3.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.61(\mathrm{dd}$, $J=1.2,5.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.76(\mathrm{t}, J=5.5 \mathrm{~Hz}, 1 \mathrm{H}), 12.96(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( $75 \mathrm{MHz}, \mathrm{DMF}-\mathrm{d} 7$ ) $\delta 29.2,75.3,90.8,98.6,105.3,113.4$, 122.6, 127.7, 128.3, 128.5, 132.7, 159. HRMS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{12} \mathrm{H}_{9} \mathrm{Br}_{2} \mathrm{~N}_{2} \mathrm{OS}: 390.8761$. Found: 390.8794.
4,5-Dibromo- $N$-(3-(pyridin-4-yl)prop-2-yn-1-yl)-1 H -pyrrole-2carboxamide $\mathbf{( \mathbf { 2 0 }} \mathbf{~}$ ). Compound $\mathbf{2 0 g}$ was synthesized according to
general procedure B using $\mathbf{1 5 a}(117 \mathrm{mg}, 0.38 \mathrm{mmol})$ and $\mathbf{1 9 g}$ (106 $\mathrm{mg}, 0.6 \mathrm{mmol}$ ). Purification afforded the title compound as a light yellow amorphous solid ( $43 \mathrm{mg}, 22 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\mathcal{\delta} 4.32(\mathrm{~d}, \mathrm{~J}=5.7 \mathrm{~Hz}, 2 \mathrm{H}), 6.98(\mathrm{~s}, 1 \mathrm{H}), 7.39(\mathrm{~d}, J=6$ $\mathrm{Hz}, 2 \mathrm{H}), 8.57(\mathrm{~m}, 2 \mathrm{H}), 8.72(\mathrm{t}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H}), 12.79(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 28.6,79.2,92,98,105.2,113.1$, $125.5,127.5,130.1,149.9,158.6$. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HRMS (ESI) $m / z$ Calcd for (M+H) ${ }^{+} \mathrm{C}_{13} \mathrm{H}_{10} \mathrm{Br}_{2} \mathrm{~N}_{3} \mathrm{O}: 383.9165$. Found 383.9187.
4,5-Dibromo-N-(3-(pyridin-2-yl)prop-2-yn-1-yl)-1H-pyrrole-2carboxamide (20h). Compound $\mathbf{2 0 h}$ was synthesized according to general procedure B using 15 a ( $153 \mathrm{mg}, 0.5 \mathrm{mmol}$ ) and 19 h (123 $\mathrm{mg}, 0.6 \mathrm{mmol}$ ). Purification afforded the title compound as a light yellow amorphous solid ( $50 \mathrm{mg}, 25 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\mathcal{\delta} 4.31(\mathrm{~d}, \mathrm{~J}=5.5 \mathrm{~Hz}, 2 \mathrm{H}), 6.98(\mathrm{~d}, J=2.9 \mathrm{~Hz}, 1 \mathrm{H})$, 7.37 (ddd, $J=1.2,4.9,7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.49(\mathrm{dt}, J=1.1,7.7 \mathrm{~Hz}, 1 \mathrm{H})$, $7.79(\mathrm{td}, J=1.9,7.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.54(\mathrm{~d}, J=4.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.72(\mathrm{t}, J=$ $5.5 \mathrm{~Hz}, 1 \mathrm{H}), 12.79(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 28.5$, 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 $(M+H)^{+} \mathrm{C}_{13} \mathrm{H}_{10} \mathrm{Br}_{2} \mathrm{~N}_{3} \mathrm{O}$ : 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 $\mathbf{2 0 i}$ was synthesized according to general procedure B using 15 a ( $153 \mathrm{mg}, 0.5 \mathrm{mmol}$ ) and $19 i(106 \mathrm{mg}, 0.6 \mathrm{mmol})$. Purification afforded the title compound as a light yellow amorphous solid ( $48 \mathrm{mg}, 25 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 4.30(\mathrm{~d}, J=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 6.98(\mathrm{~d}, J=2.4 \mathrm{~Hz}$, $1 \mathrm{H}), 7.59(\mathrm{dd}, J=4.5,8.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.74(\mathrm{dt}, J=3.0,9.3 \mathrm{~Hz}, 1 \mathrm{H})$, $8.55(\mathrm{~m}, 1 \mathrm{H}), 8.71(\mathrm{t}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H}), 12.78(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR $(75$ MHz , DMSO-d6) $\delta 28.4,80.5,86.4,98,105.2,113,123.9,127.5$, 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) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{13} \mathrm{H}_{9} \mathrm{Br}_{2} \mathrm{FN}_{3} \mathrm{O}: 401.9070$. Found 401.9090.
4,5-Dibromo- N -(3-(5-bromopyridin-3-yl)prop-2-yn-1-yl)-1 H -pyrrole-2-carboxamide (20j). Compound $\mathbf{2 0} \mathbf{j}$ was synthesized according to general procedure $B$ using 15 a ( $142 \mathrm{mg}, 0.46 \mathrm{mmol}$ ) and $19 \mathrm{j}(110 \mathrm{mg}, 0.46 \mathrm{mmol})$. Purification afforded the title compound as a light yellow amorphous solid ( $61 \mathrm{mg}, 27 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 4.32(\mathrm{~d}, J=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 6.97(\mathrm{~d}, J=2.7$ $\mathrm{Hz}, 1 \mathrm{H}), 8.15(\mathrm{t}, J=1.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.60(\mathrm{~d}, J=1.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.70$ $(\mathrm{m}, 2 \mathrm{H}), 12.78(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 28.6$, 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) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{13} \mathrm{H}_{7} \mathrm{Br}_{3} \mathrm{~N}_{3} \mathrm{O}$ : 459.8124. Found 459.8113.

4,5-Dichloro-N-(3-(imidazo[1,2-a]pyrazin-3-yl)prop-2-yn-1-yl)-1H-pyrrole-2-carboxamide (20k). Compound 20k was synthesized according to general procedure B using 15 b ( $133 \mathrm{mg}, 0.61$ mmol ) and 19 a ( $100 \mathrm{mg}, 0.41 \mathrm{mmol}$ ). Purification afforded the title compound as a yellow amorphous solid ( $90 \mathrm{mg}, 66 \%, \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 4.46$ (d, $J=5.3 \mathrm{~Hz}, 2 \mathrm{H}$ ), 6.96 (s, $1 \mathrm{H}), 8.08(\mathrm{~m}, 2 \mathrm{H}), 8.50(\mathrm{dd}, J=1.4,4.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.79(\mathrm{t}, J=5.5$ $\mathrm{Hz}, 1 \mathrm{H}), 9.15(\mathrm{~s}, 1 \mathrm{H}), 12.85(\mathrm{br} \mathrm{s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,68.1,98.4,108.1,109.2,110.2,115.4,118.6$, 124.2, 130.5, 138.9, 140, 143.2, 158.8. HMRS (ESI) m/z Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C} 14 \mathrm{H} 10 \mathrm{Cl} 2 \mathrm{~N} 5 \mathrm{O}: 334.0262$. Found: 334.0277. IR ( $\mathrm{cm}-1$ ): 3156, 3126, 1656, 1523, 1429, 1313.
$N$-(3-(imidazo[1,2-a]pyrazin-3-yl)prop-2-yn-1-yl)-4,5-diiodo-1H-pyrrole-2-carboxamide (201). Compound 201 was synthesized
according to general proce-dure $B$ using 15 c ( $149 \mathrm{mg}, 0.37 \mathrm{mmol}$ ) and 19a ( $70 \mathrm{mg}, 0.29 \mathrm{mmol}$ ). Purification afforded the title compound as a beige amorphous solid ( $95 \mathrm{mg}, 64 \%$ ). ${ }^{1} \mathrm{H}$ NMR (300MHz, DMSO-d6) $\delta 4.44(\mathrm{~d}, \mathrm{~J}=5.5 \mathrm{~Hz}, 2 \mathrm{H}), 6.97(\mathrm{~d}, J=2.6$ $\mathrm{Hz}, 1 \mathrm{H}), 8.1(\mathrm{~s}, 2 \mathrm{H}), 8.50(\mathrm{~d}, J=4.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.68(\mathrm{t}, J=5.2 \mathrm{~Hz}$, $1 \mathrm{H}), 9.16(\mathrm{br} \mathrm{s}, 1 \mathrm{H}), 12.43(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSOd6) $\delta 29.1,68.1,76.3,84.2,98.4,109,118.6,130.4,132.1,138.9$, 140.1, 143.2, 158.4. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+}$ C14H10I2N5O: 517.8975. Found: 517.8960. IR ( $\mathrm{cm}-1$ ): 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 ( 20 m ). Compound 20 m was synthesized according to general pro-cedure B using 15 b ( $110 \mathrm{mg}, 0.51$ $\mathrm{mmol})$ and $19 \mathrm{c}(124 \mathrm{mg}, 0.51 \mathrm{mmol})$. Purification afforded the title compound as a white amorphous solid ( $63 \mathrm{mg}, 37 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 4.45(\mathrm{~d}, J=5.3 \mathrm{~Hz}, 2 \mathrm{H}), 6.96(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H} 4)$, $7.11(\mathrm{t}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.40(\mathrm{t}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.68(\mathrm{~d}, J=9.1$ $\mathrm{Hz}, 1 \mathrm{H}), 7.89(\mathrm{~s}, 1 \mathrm{H}), 8.45(\mathrm{~d}, J=6.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.78(\mathrm{t}, J=5.6 \mathrm{~Hz}$, $1 \mathrm{H}), 12.85(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,69.5$, 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 $(\mathrm{M}+\mathrm{H})^{+}$ 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-
1H-pyrrole-2-carboxamide (20n). Compound 20n was synthesized according to general pro-cedure B using $15 \mathrm{c}(171 \mathrm{mg}, 0.43$ mmol ) and 19 c ( $80 \mathrm{mg}, 0.33 \mathrm{mmol}$ ). Purification afforded the title compound as a beige amorphous solid ( $55 \mathrm{mg}, 33 \%$ ). ${ }^{1} \mathrm{H}$ NMR (300MHz, DMSO-d6) $\delta 4.43$ (d, $J=5.5 \mathrm{~Hz}, 2 \mathrm{H}$ ), 6.98 (d, $J=2.7$ $\mathrm{Hz}, 1 \mathrm{H}), 7.11(\mathrm{t}, J=6.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.39(\mathrm{t}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.68(\mathrm{~d}$, $J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.89(\mathrm{br} \mathrm{s}, 1 \mathrm{H}), 8.45(\mathrm{~d}, J=6.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.66(\mathrm{t}, J$ $=5.3 \mathrm{~Hz}, 1 \mathrm{H}), 12.43(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta J$ 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 $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{11} \mathrm{I}_{2} \mathrm{~N}_{4} \mathrm{O}: 516.9022$. Found: 516.906. IR ( $\mathrm{cm}-1$ ): 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 15 a ( 198 mg , 0.65 mmol ) and 23a ( $150 \mathrm{mg}, 0.54 \mathrm{mmol}$ ). Purification afforded the title compound as a beige amorphous solid ( $75 \mathrm{mg}, 31 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 4.46(\mathrm{~d}, J=5.5 \mathrm{~Hz}, 2 \mathrm{H}), 6.99(\mathrm{~d}, J$ $=2.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.45(\mathrm{dd}, J=1.9,9.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.73(\mathrm{~d}, J=9.4 \mathrm{~Hz}$, $1 \mathrm{H}), 7.94(\mathrm{~s}, 1 \mathrm{H}), 8.62(\mathrm{~s}, 1 \mathrm{H}), 8.74(\mathrm{t}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H}), 12.80(\mathrm{~s}$, 1H). ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,68.8,98,98,105.2$, 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 $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{10} \mathrm{~N}_{4} \mathrm{OBr}_{2} \mathrm{Cl}: 456.8889$. Found: 456.8891. IR ( $\mathrm{cm}^{-1}$ ): 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 15 a ( 169 mg , 0.55 mmol ) and 23 b ( $62 \mathrm{mg}, 0.22 \mathrm{mmol}$ ). Purification afforded the title compound as a pale yellow amorphous solid ( $20 \mathrm{mg}, 20 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( $300 \mathrm{MHz}, \mathrm{DMSO}$ ) $\mathcal{\delta} 4.44$ (d, $J=5.4 \mathrm{~Hz}, 2 \mathrm{H}$ ), 6.99 (d, $J$ $=2.9 \mathrm{~Hz}, 1 \mathrm{H}) 7.18(\mathrm{dd}, J=2.1,7.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.88(\mathrm{~s}, 1 \mathrm{H}), 7.91(\mathrm{~s}$, $1 \mathrm{H}) 8.46(\mathrm{~d}, J=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.75(\mathrm{t}, J=5.3 \mathrm{~Hz}, 1 \mathrm{H}), 12.80(\mathrm{~s}$, $1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( $75 \mathrm{MHz}, \mathrm{DMSO}$ ) $\delta 29.2,68.9,97.6,98,105.2$, 108.1, 113.1, 114.7, 116.3, 126.2, 127.5, 131.3, 138.4, 144.6, 158.7.

HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{10} \mathrm{~N}_{4} \mathrm{OBr}_{2} \mathrm{Cl}$ : 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 $23 \mathrm{c}(220 \mathrm{mg}, 0.68 \mathrm{mmol})$. Purification afforded the title compound as a beige amorphous solid ( $113 \mathrm{mg}, 33 \%$ ). ${ }^{1} \mathrm{H}$ NMR (300MHz, DMSO-d6) $\delta 4.44(\mathrm{~d}, J=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 6.99(\mathrm{~d}, J$ $=2.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.03(\mathrm{t}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.74(\mathrm{dd}, J=0.9,7.2 \mathrm{~Hz}$, $1 \mathrm{H}), 7.94(\mathrm{~s}, 1 \mathrm{H}), 8.48(\mathrm{dd}, J=0.9,6.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.75(\mathrm{t}, J=5.3$ $\mathrm{Hz}, 1 \mathrm{H}), 12.80(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( $75 \mathrm{MHz},-\mathrm{d} 6$ ) $\delta 29.2,69,97.6$, 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 $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{10} \mathrm{~N}_{4} \mathrm{OBr}_{3}$ : 498.8405. Found: 498.8423. IR ( $\mathrm{cm}^{-1}$ ): 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 15 a ( 549 mg , 1.79 mmol ) and $23 \mathrm{~d}(386 \mathrm{mg}, 1.2 \mathrm{mmol})$. Purification afforded the title compound as a pale yellow amorphous solid ( $32 \mathrm{mg}, 5 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 4.46$ (d, $J=5.4 \mathrm{~Hz}, 2 \mathrm{H}$ ), 6.99 (s, $1 \mathrm{H}), 7.52(\mathrm{dd}, J=1.9,9.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.68(\mathrm{~d}, \mathrm{~J}=9.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.91$ $(\mathrm{s}, 1 \mathrm{H}), 8.67(\mathrm{~s}, 1 \mathrm{H}), 8.74(\mathrm{t}, J=5.3 \mathrm{~Hz}, 1 \mathrm{H}), 12.81(\mathrm{br} \mathrm{s}, 1 \mathrm{H})$. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{10} \mathrm{~N}_{4} \mathrm{OBr}_{3}$ : 500.8384. Found: 500.8393. IR $\left(\mathrm{cm}^{-1}\right): 3152,1654,1561,1518,1407,1310$, 1222.

4,5-Dibromo- $N$-(3-(7-(trifluoromethyl)imidazo[1,2-a] pyridin-3-yl)prop-2-yn-1-yl)-1H-pyrrole-2-carboxamide (24e). Compound 24e was synthesized according to general procedure B using 15a $(286 \mathrm{mg}, 0.94 \mathrm{mmol})$ and $23 \mathrm{e}(122 \mathrm{mg}, 0.62 \mathrm{mmol})$. Purification afforded the title compound as a pale yellow amorphous solid (173 $\mathrm{mg}, 57 \%) .{ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 4.46$ (d, $J=5.6 \mathrm{~Hz}$, $2 \mathrm{H}), 6.99(\mathrm{~d}, J=1.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.37(\mathrm{~d}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.11$ (s, $1 \mathrm{H}), 8.20(\mathrm{~s}, 1 \mathrm{H}), 8.65(\mathrm{~d}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.77(\mathrm{t}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H})$, 12.80 (br s, 1H). ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,68.7,98$, 98.1, 105.2, 108.9 (d, $J=3.0 \mathrm{~Hz}$ ), 109.4, 113.1, 115.6 (d, $J=5.0$ Hz ), 121.6, 125.7 ( $\mathrm{t}, J=36.7 \mathrm{~Hz}$ ), 126.8, 127.5, 139.5, 143.3, 158.7. HMRS (ESI) $m / z$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{16} \mathrm{H}_{10} \mathrm{~N}_{4} \mathrm{OBr}_{2} \mathrm{~F}_{3}$ : 490.9147. Found: 490.9153. IR $\left(\mathrm{cm}^{-1}\right): 3154,1638,1564,1314$, 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 24 f was synthesized according to general procedure $B$ using 15 a ( 214 mg , $0.70 \mathrm{mmol})$ and $23 \mathrm{f}(122 \mathrm{mg}, 0.47 \mathrm{mmol})$. Purification afforded the title compound as a pale yellow amorphous solid ( $129 \mathrm{mg}, 63$ \%). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.45(\mathrm{~d}, J=5.4 \mathrm{~Hz}, 2 \mathrm{H}$ ), $6.99(\mathrm{~s}, 1 \mathrm{H}), 7.49(\mathrm{t}, J=9.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.77(\mathrm{dd}, J=5.1,9.3 \mathrm{~Hz}, 1 \mathrm{H})$, $7.94(\mathrm{~s}, 1 \mathrm{H}), 8.63(\mathrm{~s}, 1 \mathrm{H}), 8.74(\mathrm{t}, J=4.9 \mathrm{~Hz}, 1 \mathrm{H}), 12.82(\mathrm{~s}, 1 \mathrm{H})$. ${ }^{13} \mathrm{C}$ NMR ( 75 MHz, DMSO-d6) $\delta 29.3,69.1,98,98.1,105.2$, $108.9,112.3(\mathrm{~d}, J=41.6 \mathrm{~Hz}$ ), 113.2, $118.0(\mathrm{~d}, J=25.7 \mathrm{~Hz}), 118.4$ $(\mathrm{d}, J=9.3 \mathrm{~Hz}), 127.6,138.6,152-155.1(\mathrm{~d}, J=235.8 \mathrm{~Hz}),$,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) ${ }^{+}$ $\mathrm{C}_{15} \mathrm{H}_{10} \mathrm{~N}_{4} \mathrm{OBr}_{2} \mathrm{~F}: 440.9188$. Found: 440.9185 . IR $\left(\mathrm{cm}^{-1}\right): 3148$, 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 ( $\mathbf{2 4 g}$ ). Compound $\mathbf{2 4 g}$ was synthesized according to general procedure $B$ using 15 a ( 376 mg , 1.23 mmol ) and $\mathbf{2 3 g}$ ( $211 \mathrm{mg}, 0.82 \mathrm{mmol}$ ). Purification afforded
the title compound as a pale yellow amorphous solid ( $105 \mathrm{mg}, 30$ \%). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 2.34$ ( $\mathrm{s}, 3 \mathrm{H}$ ), 4.45 (d, $J=$ $5.3 \mathrm{~Hz}, 2 \mathrm{H}), 7.0(\mathrm{~s}, 1 \mathrm{H}), 7.25(\mathrm{~d}, J=8.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.59(\mathrm{~d}, J=8.7$ $\mathrm{Hz}, 1 \mathrm{H}), 7.82(\mathrm{~s}, 1 \mathrm{H}), 8.25(\mathrm{~s}, 1 \mathrm{H}), 8.74(\mathrm{t}, J=5.2 \mathrm{~Hz}, 1 \mathrm{H}), 12.80$ $(\mathrm{s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 17.5,29.2,69.7,97.1$, 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 $(M+H)^{+} \mathrm{C}_{16} \mathrm{H}_{13} \mathrm{~N}_{4} \mathrm{OBr}_{2}$ : 436.9436. Found: 436.9457. IR ( $\mathrm{cm}^{-1}$ ): 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 15 a ( 457 mg , 1.5 mmol ) and $23 \mathrm{~h}(257 \mathrm{mg}, 1.0 \mathrm{mmol})$. Purification afforded the title compound as a beige amorphous solid ( $100 \mathrm{mg}, 23 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 2.39$ ( $\mathrm{s}, 3 \mathrm{H}$ ), $4.43(\mathrm{~d}, J=5.3 \mathrm{~Hz}$, 2H), 6.96 (d, $J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 6.99(\mathrm{~s}, 1 \mathrm{H}), 7.47(\mathrm{br} \mathrm{s}, 1 \mathrm{H}), 8.35$ (br s, 1H), $8.75(\mathrm{t}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H}), 12.81(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR (75 MHz, DMSO-d6) $\delta 20.7,29.2,69,97,97.9,105.1,113.1,115.8$, $116,124.5,127.6,136.9,158.7$. Some quaternary carbons and aromatic CH too broad to be seen due to very slow relaxation. HMRS (ESI) $m / z$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{16} \mathrm{H}_{13} \mathrm{~N}_{4} \mathrm{OBr}_{2}$ : 436.9436. Found: 436.9424. IR ( $\mathrm{cm}^{-1}$ ): 3136, 1634, 1566, 1400, 1244, 1190.
$N$-(3-( $1 H$-pyrrolo[2,3-b]pyridin-3-yl)prop-2-yn-1-yl)-4,5-di-bromo- $1 H$-pyrrole-2-carboxamide (29). Compound 29 was synthesized according to general procedure B using $15 \mathrm{a}(200 \mathrm{mg}, 0.65$ $\mathrm{mmol})$ and 27 ( $301 \mathrm{mg}, 0.78 \mathrm{mmol}$ ). Purification afforded the intermediate 28 as a brown amorphous solid ( $221 \mathrm{mg}, 60 \%$ ). 28 ( 125 $\mathrm{mg}, 0.22 \mathrm{mmol}, 1.2 \mathrm{eq}$.) was dissolved in 2 mL of dry THF. A solution of TBAF ( $187 \mathrm{mg}, 0.67 \mathrm{mmol}, 3 \mathrm{eq}$. ) in 0.7 mL of dry THF was added to the mixture. It was stirred at room temperature for 3 h and then at $50^{\circ} \mathrm{C}$ 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 $90 / 10$ $\mathrm{DCM} / \mathrm{MeOH}$ ) to afford the deprotected compound 29 as a beige amorphous solid ( $57 \mathrm{mg}, 61 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.33(\mathrm{~d}, J=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 7.0(\mathrm{~s}, 1 \mathrm{H}), 7.16(\mathrm{dd}, J=4.5,7.9 \mathrm{~Hz}$, 1 H ), $7.78(\mathrm{~s}, 1 \mathrm{H}),, 7.94(\mathrm{~d}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.28(\mathrm{~d}, J=4.4 \mathrm{~Hz}$, $1 \mathrm{H}), 8.66(\mathrm{t}, J=5.5 \mathrm{~Hz}, 1 \mathrm{H}), 12.0(\mathrm{~s}, 1 \mathrm{H}), 12.76(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29,75.4,87.5,94.7,97.9,105,112.9$, 116.3, 120.3, 127.8, 127, 130.1, 143.8, 147.6, 158.5. HMRS (ESI) $m / z$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{11} \mathrm{~N}_{4} \mathrm{OBr}_{2}$ : 422.9279. Found: 422.9273. IR ( $\mathrm{cm}^{-1}$ ): 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 15 a ( 264 mg , 0.86 mmol ) and 33a ( $200 \mathrm{mg}, 0.66 \mathrm{mmol}$ ). Purification afforded the title compound as a beige amorphous solid $110 \mathrm{mg}, 35 \%) .{ }^{1} \mathrm{H}$ NMR (300MHz, DMSO-d6) $\delta 2.21(\mathrm{~s}, 3 \mathrm{H}),, 4.44(\mathrm{~d}, J=5.5 \mathrm{~Hz}$, $2 \mathrm{H}), 6.99(\mathrm{~d}, J=2.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.06(\mathrm{t}, J=7 \mathrm{~Hz}, 1 \mathrm{H}), 7.87(\mathrm{~s}, 1 \mathrm{H})$, $8.09(\mathrm{~d}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.16(\mathrm{dd}, J=1,7 \mathrm{~Hz}, 1 \mathrm{H}), 8.75(\mathrm{t}, J=$ $5.5 \mathrm{~Hz}, 1 \mathrm{H}), 10.1(\mathrm{~s}, 1 \mathrm{H}), 12.81(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR $(75 \mathrm{MHz}$, DMSO-d6) $\delta 24,29.2,69.3,97.2,98,105.2,108.7,112.5,113.1$, 113.9, 119.6, 127.6, 127.9, 136.1, 138.8, 158.7, 169.9. HMRS (ESI) $m / z$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{17} \mathrm{H}_{14} \mathrm{~N}_{5} \mathrm{O}_{2} \mathrm{Br}_{2}$ : 479.9494. Found: 479.9506. IR ( $\mathrm{cm}^{-1}$ ): 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 15 a ( 466 mg , 1.52 mmol ) and 33b ( $350 \mathrm{mg}, 0.76 \mathrm{mmol}$ ). Purification afforded 36 as a brown amorphous solid ( $321 \mathrm{mg}, 66 \%$ ). Then 36 ( 320 mg ,
0.5 mmol , leq.) was dissolved in 2.5 mL of dry DCM under argon at $0^{\circ} \mathrm{C}$. TFA ( $0.37 \mathrm{~mL}, 5 \mathrm{mmol}, 10 \mathrm{eq}$.) was added dropwise to the reaction mixture. After $2 \mathrm{~h}, 10$ eq. of TFA $(0.37 \mathrm{~mL})$ were again added to the mixture at $-5^{\circ} \mathrm{C}$. The reaction mixture was stirred at $5^{\circ} \mathrm{C} 2 \mathrm{~h}$ and then 2 h at room temperature. The mixture was quenched with an aqueous solution of $\mathrm{Na}_{2} \mathrm{CO}_{3}$ and extracted with DCM. Collected organic layers were dried over MgSO 4 , filtered and concentrated under vacuum. Flash chromatographic purification (elution by gradient from 100 to $95 / 5 \mathrm{DCM} / \mathrm{MeOH}$ ) afforded compound 37 as a beige amorphous solid ( $153 \mathrm{mg}, 70 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\delta 4.42(\mathrm{~d}, J=6.6 \mathrm{~Hz}, 2 \mathrm{H}), 5.79$ $(\mathrm{s}, 2 \mathrm{H}), 6.37(\mathrm{dd}, J=1.0,7.5 \mathrm{~Hz}, 1 \mathrm{H}), 6.83(\mathrm{t}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H})$, $6.99(\mathrm{~s}, 1 \mathrm{H}), 7.7(\mathrm{~m}, 2 \mathrm{H}), 8.74(\mathrm{t}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H}), 12.8(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,70.1,96.5,98,101.9,105.2$, 108, 112.7, 113.1, 114.9, 127.6, 135.6, 137.6, 138.9, 158.7. HMRS (ESI) $m / z$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 437.9388. Found: 437.9375. IR ( $\mathrm{cm}^{-1}$ ): 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 ( $358 \mathrm{mg}, 0.78 \mathrm{mmol}$ ). The protected intermediate was directly engaged in a TFA ( 1 mL ) deprotection in DCM at $0^{\circ} \mathrm{C}$ then at room temperature. After 24 h of reaction, the reaction mixture was quenched with $\mathrm{Na}_{2} \mathrm{CO}_{3}$ and concentrated under vacuum. The crude product was purified by silica gel flash chromatography (elution by gradient from 100 to $95 / 5 \mathrm{DCM} / \mathrm{MeOH}$ ) to afford a brown amorphous solid ( $38 \mathrm{mg}, 11 \%$ over two steps). ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMF}-\mathrm{d} 7$ ) $\delta 4.53(\mathrm{~s}, 2 \mathrm{H}), 5,26(\mathrm{~s}, 2 \mathrm{H}), 7.10(\mathrm{~d}, J=$ $9.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.14(\mathrm{~s}, 1 \mathrm{H}), 7.44(\mathrm{~d}, J=9.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.66(\mathrm{~s}, 1 \mathrm{H})$, $7.86(\mathrm{~s}, 1 \mathrm{H}), 8.81(\mathrm{t}, J=4.6 \mathrm{~Hz}, 1 \mathrm{H}), 12.95(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 $\mathrm{MHz}, \mathrm{DMF}-\mathrm{d} 7$ ) $\delta 29.8,70.8,96.7,98.7,105.3,106.6$ (two carbons), 113.6, 117.4, 21.6, 128.7, 137, 138.2, 142.2, 159.2. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 437.9388 . Found: 437.9388. IR ( $\mathrm{cm}^{-1}$ ): 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-1H-pyrrole-2-carboxamide (43). Compound 43 was synthesized according to general procedure B using 15a (238 $\mathrm{mg}, 0.78 \mathrm{mmol})$ and $33 \mathrm{~d}(209 \mathrm{mg}, 0.39 \mathrm{mmol})$ 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 $\mathrm{NaOH} 10 \%$, the resulting precipitate was filtered and rinsed with water and then DCM yielding a beige amorphous solid 43 ( $119 \mathrm{mg}, 59 \%$ over two steps). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\mathcal{\delta} 4.43(\mathrm{~d}, J=5.6 \mathrm{~Hz}, 2 \mathrm{H}), 6.18$ (br s, 2H), $6.48(\mathrm{~d}, J=1.7 \mathrm{~Hz}, 1 \mathrm{H}), 6.99(\mathrm{~d}, J=2.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.73$ $(\mathrm{s}, 1 \mathrm{H}), 7.83(\mathrm{~d}, J=1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.72(\mathrm{t}, J=5.5 \mathrm{~Hz}, 1 \mathrm{H}), 12.80(\mathrm{~s}$, 1H). ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,69.3,97.3,98,104.6$, 105.2, 108.6, 109.5, 112, 113.1, 127.6, 135.5, 137.1, 138.2, 158.7. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{11} \mathrm{~N}_{5} \mathrm{OBr}_{3}: 515.8493$. Found: 515.8485. IR ( $\mathrm{cm}^{-1}$ ): 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 $\mathbf{1 5 b}$ ( $150 \mathrm{mg}, 0.69$ mmol ) and 33b ( $317 \mathrm{mg}, 0.69 \mathrm{mmol}$ ). Then the intermediate was dissolved in 2 mL of dry DCM under argon at $0^{\circ} \mathrm{C}$. TFA $(0.63 \mathrm{~mL}$, 17 eq.) was added dropwise to the reaction mixture. After 2 h of reaction, 10 eq. of TFA $(0.37 \mathrm{~mL})$ were added to the mixture at
$0^{\circ} \mathrm{C}$. The reaction mixture was stirred at room temperature 7 h . The mixture was quenched with an aqueous solution of $\mathrm{NaHCO}_{3}$ and extracted with DCM. Collected organic layers were dried over $\mathrm{MgSO}_{4}$, filtered and concentrated under vacuum. Flash chromatographic purification (elution by gradient from 100 to $95 / 5$ $\mathrm{DCM} / \mathrm{MeOH}$ ) afforded compound 44 as a beige amorphous solid ( $60 \mathrm{mg}, 25 \%$ over two steps). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\mathcal{\delta}$ $4.43(\mathrm{~d}, J=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 5.78(\mathrm{br} \mathrm{s}, 2 \mathrm{H}), 6.38(\mathrm{dd}, J=0.9,7.6 \mathrm{~Hz}$, $1 \mathrm{H}), 6.83(\mathrm{t}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 6.96(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H} 4), 7.70(\mathrm{~d}, J=6.9 \mathrm{~Hz}$, $1 \mathrm{H}), 7.71(\mathrm{~s}, 1 \mathrm{H}), 8.76(\mathrm{t}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H}), 12.84(\mathrm{br} \mathrm{s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,70.1,96.5,101.9,108,108.1$, 110.1, 112.7, 114.9, 115.4, 124.3, 135.6, 137.5, 138.9, 158.8. HMRS (ESI) m/z Calcd for ( $\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{12} \mathrm{Cl}_{2} \mathrm{~N}_{5} \mathrm{O}: 348.0419$. Found: 348.0457. IR $\left(\mathrm{cm}^{-1}\right): 3149,1651,1607,1514,1493,1431$.
N -(3-(8-aminoimidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-yl)-4,5-diiodo- $1 H$-pyrrole-2-carboxamide (45). Compound 45 was synthesized according to general procedure B using $15 c(180 \mathrm{mg}, 0.45$ $\mathrm{mmol})$ and $\mathbf{3 3 b}(207 \mathrm{mg}, 0.45 \mathrm{mmol})$. Then the intermediate was dissolved in 2 mL of dry DCM under argon at $0^{\circ} \mathrm{C}$. TFA $(0.67 \mathrm{~mL}$, 20 eq.) was added dropwise to the reaction mixture. After 2 h of reaction, 10 eq. of TFA $(0.37 \mathrm{~mL})$ were added to the mixture at $0^{\circ} \mathrm{C}$. The reaction mixture was stirred 5 h at room temperature. The mixture was quenched with an aqueous solution of $\mathrm{Na}_{2} \mathrm{CO}_{3}$ and extracted with EtOAc. Collected organic layers were dried over $\mathrm{MgSO}_{4}$, filtered and concentrated under vacuum. Flash chromatographic purification (elution by gradient from 100 to $90 / 10$ $\mathrm{DCM} / \mathrm{MeOH}$ ) afforded compound 45 as a beige amorphous solid ( $97 \mathrm{mg}, 41 \%$ over two steps). ${ }^{1} \mathrm{H}$ NMR (300MHz, DMSO-d6) $\delta$ $4.41(\mathrm{~d}, J=5.3 \mathrm{~Hz}, 2 \mathrm{H}), 5.78(\mathrm{br} \mathrm{s}, 2 \mathrm{H}), 6.38(\mathrm{~d}, J=7.4 \mathrm{~Hz}, 1 \mathrm{H})$, $6.82(\mathrm{t}, J=7.4 \mathrm{~Hz}, 1 \mathrm{H}), 6.97(\mathrm{~d}, J=2.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.69(\mathrm{~d}, J=5.7 \mathrm{~Hz}$, $1 \mathrm{H}), 7.71(\mathrm{~s}, 1 \mathrm{H}), 8.65(\mathrm{t}, J=5.2 \mathrm{~Hz}, 1 \mathrm{H}), 12.42(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta$ 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 $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{12} \mathrm{I}_{2} \mathrm{~N}_{5} \mathrm{O}: 531.9131$. Found: 531.9142. IR $\left(\mathrm{cm}^{-1}\right): 3126,1628,1552,1494,1375,1288$.
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 ( $383 \mathrm{mg}, 1.3 \mathrm{mmol}$ ) and 46 ( $206 \mathrm{mg}, 0.63 \mathrm{mmol}$ ). Purification afforded the title compound as a beige amorphous solid ( $71 \mathrm{mg}, 22$ \%). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\delta 3.05(\mathrm{t}, J=4.5 \mathrm{~Hz}, 4 \mathrm{H})$, $3.75(\mathrm{t}, J=4.5 \mathrm{~Hz}, 4 \mathrm{H}), 4.43(\mathrm{~d}, J=5.3 \mathrm{~Hz}, 2 \mathrm{H}), 6.99(\mathrm{~s}, 1 \mathrm{H}), 7.38$ (d, $J=9.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.57(\mathrm{~d}, J=9.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.64(\mathrm{~s}, 1 \mathrm{H}),, 7.76(\mathrm{~s}$, $1 \mathrm{H}), 8.79(\mathrm{t}, J=5.3 \mathrm{~Hz}, 1 \mathrm{H}), 12.79(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR $(75 \mathrm{MHz}$, DMSO-d6) $\delta 49.3,65.8,29.3,69.9,97.5,98,105.1,107.8,108.8$, 113, 117.2, 121.7, 127.6, 136.9, 140.5, 141.7, 158.7. HMRS (ESI) $m / z$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \quad \mathrm{C}_{19} \mathrm{H}_{18} \mathrm{~N}_{5} \mathrm{O}_{2} \mathrm{Br}_{2}$ : 507.9807. Found: 507.9807. IR ( $\mathrm{cm}^{-1}$ ): 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 $(296 \mathrm{mg}, 0.97 \mathrm{mmol})$ and $47(207 \mathrm{mg}, 0.48 \mathrm{mmol})$. 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 ( 4 N dioxane) was added $(0.6 \mathrm{~mL})$ with 2 mL of methanol. The reaction mixture was stirred overnight at room temprature. The solvent was removed under vacuum, the resulting solid rinsed with DCM and MeOH to afford the salt 50 a white amorphous solid ( $123 \mathrm{mg}, 44 \%$ over two steps). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$,

DMSO-d6) $\mathcal{S} 3.27(\mathrm{~s}, 4 \mathrm{H}), 3.54(\mathrm{~s}, 4 \mathrm{H}), 4.45(\mathrm{~d}, J=5.3 \mathrm{~Hz}, 2 \mathrm{H})$, $7.08(\mathrm{~d}, J=2.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.92(\mathrm{~m}, 2 \mathrm{H}), 8.03(\mathrm{~s}, 1 \mathrm{H}), 8.41(\mathrm{~s}, 1 \mathrm{H})$, $9.28(\mathrm{t}, J=5.2 \mathrm{~Hz}, 1 \mathrm{H}), 9.64(\mathrm{br} \mathrm{s}, 2 \mathrm{H}), 12.91(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 29.2,42.2,45.5,66.7,98.1,99.5,105$, 109.3, 113.3, 113.8, 127.2, 127.6, 127.7, 135.6, 142.1, 158.7. HMRS (ESI) $m / z$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{19} \mathrm{H}_{19} \mathrm{~N}_{6} \mathrm{OBr}_{2}$ : 506.9967. Found: 506.9964. IR $\left(\mathrm{cm}^{-1}\right): 3275,2928,1640,1521,1390,1322$, 1237.

General Procedure C for Amidation (55-57).
Boc-protected amine 52-54 (1 eq.) was dissolved in $2-6 \mathrm{~mL}$ 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 $\mathrm{NaHCO}_{3}$ to form the free base. 14a (leq.) 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 $95 / 5$ $\mathrm{DCM} / \mathrm{MeOH})$ 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 $14 \mathrm{a}(108 \mathrm{mg}, 0.29 \mathrm{mmol})$ and 52 ( $80 \mathrm{mg}, 0.29 \mathrm{mmol}$ ). Purification afforded the title compound as a yellow amorphous solid ( $52 \mathrm{mg}, 42 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d} 6) \delta 4.1(\mathrm{t}, J=5.7 \mathrm{~Hz}, 1 \mathrm{H}), 6.38(\mathrm{dt}, J=5.7$, $16.1 \mathrm{~Hz}, 1 \mathrm{H}), 6.89(\mathrm{~d}, J=16.1 \mathrm{~Hz}, 1 \mathrm{H}), 7(\mathrm{~s}, 1 \mathrm{H}), 7.12(\mathrm{dd}, J=$ $4.4,7 \mathrm{~Hz}, 1 \mathrm{H}), 8.01(\mathrm{~s}, 1 \mathrm{H}), 8.42(\mathrm{t}, J=5.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.52(\mathrm{dd}, J=$ $1.9,4.1 \mathrm{~Hz}, 1 \mathrm{H}), 9.03(\mathrm{dd}, J=1.9,7.1 \mathrm{~Hz}, 1 \mathrm{H}), 12.7(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 40.8,97.8,104.6,109,112.8,115.6$, 121.5, 127.3, 128.1, 132.4, 138.8, 148, 149.4, 158.7. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \quad \mathrm{C}_{14} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{O}_{2} \mathrm{Br}_{2}$ : 425.9388. Found: 425.9388. IR ( $\mathrm{cm}^{-1}$ ): 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 $14 \mathrm{a}(97 \mathrm{mg}, 0.26 \mathrm{mmol})$ and 53 ( $72 \mathrm{mg}, 0.26 \mathrm{mmol}$ ). Purification afforded the title compound as a beige amorphous solid ( $57 \mathrm{mg}, 51 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\delta 4.20(\mathrm{t}, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 6.59(\mathrm{dt}, J=6,16.1 \mathrm{~Hz}, 1 \mathrm{H})$, $7.06(\mathrm{~d}, J=16.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.12(\mathrm{~s}, 1 \mathrm{H}), 7.97(\mathrm{~d}, J=4.6 \mathrm{~Hz}, 1 \mathrm{H})$, $8.14(\mathrm{~s}, 1 \mathrm{H}), 8.45(\mathrm{t}, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.7(\mathrm{~d}, J=5 \mathrm{~Hz}, 1 \mathrm{H}), 9.04$ ( $\mathrm{s}, 1$ ), $12.86(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 41.1,98.6$, 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 $(\mathrm{M}+\mathrm{H})^{+}$ $\mathrm{C}_{14} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{O}_{2} \mathrm{Br}_{2}: 425.9388$. Found: 425.9401 . IR $\left(\mathrm{cm}^{-1}\right): 3377$, 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 $14 \mathrm{a}(332 \mathrm{mg}, 0.9 \mathrm{mmol})$ and $54(224 \mathrm{mg}, 0.82 \mathrm{mmol})$. Purification afforded the title compound as a beige amorphous solid ( $125 \mathrm{mg}, 40 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\mathcal{\delta} 4.10(\mathrm{t}, J=4.8 \mathrm{~Hz}, 1 \mathrm{H}), 6.82(\mathrm{~m}, 2 \mathrm{H}), 7.0(\mathrm{~s}, 1 \mathrm{H})$, 7.23 (dd, $J=4.6,9.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.0(\mathrm{~s}, 1 \mathrm{H}), 8.13(\mathrm{dd}, J=1.6,9.2 \mathrm{~Hz}$, 1 H ), $8.49(\mathrm{t}, J=5.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.58$ (dd, $J=1.5,4.5 \mathrm{~Hz}, 1 \mathrm{H})$, 12.73(s, 1H). ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 40.6,97.9,104.7$, 112.7, 115.5, 116.9, 125.7, 125.8, 127.8, 128, 132.7, 139.3, 143.9, 158.7. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{14} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{O}_{2} \mathrm{Br}_{2}$ : 425.9388. Found: 425.9386 . IR $\left(\mathrm{cm}^{-1}\right): 3107,3065,1644,1565$, 1522, 1317, 1239, 1151.
4,5-Dibromo- N -(3-(imidazo[1,2-a]pyrimidin-2-yl)phenyl)-1 H -pyrrole-2-carboxamide ( 63 ). $61(50 \mathrm{mg}, 0.24 \mathrm{mmol}, 1 \mathrm{eq}$.$) was$
dissolved in 1 mL of $\mathrm{Et}_{3} \mathrm{~N}$ under argon. 14 a ( $114 \mathrm{mg}, 0.31 \mathrm{mmol}$, 1.3 eq.) was added to the mixture. The reaction mixture was stirred at $60^{\circ} \mathrm{C}$ overnight. The solvent was removed under vacuum and the product was purified by silica gel flash chromatography (elution by gradient from 100 to $98 / 2 \mathrm{DCM} / \mathrm{MeOH}$ ) to yield 63 as a yellow amorphous solid ( $54 \mathrm{mg}, 49 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 7.07(\mathrm{dd}, J=4.1,6.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.30(\mathrm{~d}, J=2.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.43(\mathrm{t}, J$ $=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.69(\mathrm{dt}, J=1.1,8.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.83(\mathrm{~d}, J=8.1 \mathrm{~Hz}$, $1 \mathrm{H}), 8.33(\mathrm{~s}, 1 \mathrm{H}), 8.35(\mathrm{t}, J=1.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.54(\mathrm{dd}, J=2.1,4.1$ $\mathrm{Hz}, 1 \mathrm{H}), 8.98(\mathrm{dd}, J=2.1,6.7 \mathrm{~Hz}, 1 \mathrm{H}), 9.99(\mathrm{~s}, 1 \mathrm{H}), 12.91(\mathrm{~s}, 1 \mathrm{H})$. ${ }^{13}$ C NMR ( 75 MHz , DMSO-d6) $\delta 98.2,105.9,107.5,108.9,113.9$, 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 $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 461.9388. Found: 461.9412. IR $\left(\mathrm{cm}^{-1}\right): 3149,1610,1558,1400$, 1223, 1198.
4,5-Dibromo- $N$-(3-(imidazo[1,2-b]pyridazin-2-yl)phenyl)-1 $H$ -pyrrole-2-carboxamide (64). 4,5-Dibromo-1H-pyrrole-2-carboxylic acid ( $100 \mathrm{mg}, 0.37 \mathrm{mmol}, 1 \mathrm{eq}$.) was dissolved in 1 mL of anhydrous DCM and a few drops of DMF under argon atmosphere. $(\mathrm{COCl})_{2}(130 \mu \mathrm{~L}, 1.49 \mathrm{mmol}, 4 \mathrm{eq}$. $)$ 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 $62(86 \mathrm{mg}, 0.41 \mathrm{mmol}, 1.1 \mathrm{eq}$.). 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 $90 / 10 / \mathrm{MeOH}$ ) to afford 64 as a beige amorphous solid ( $77 \mathrm{mg}, 72 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\delta 7.24$ (dd, $J$ $=4.5,9.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.3(\mathrm{~d}, J=2.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.42(\mathrm{t}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H})$, $7.74(\mathrm{~d}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.81(\mathrm{~d}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.13(\mathrm{~d}, J=9.4 \mathrm{~Hz}$, $1 \mathrm{H}), 8.40(\mathrm{~s}, 1 \mathrm{H}), 8.50(\mathrm{dd}, J=1.5,4.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.78(\mathrm{~s}, 1 \mathrm{H}), 9.98$ (br s, 1H), 12.9 (br s, 1H). ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 98.2$, 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 $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 461.9388. Found: 461.9420. IR $\left(\mathrm{cm}^{-1}\right): 3142,1646,1559,1426,1338,1236$.

## General Procedure D for Amidation with $\mathrm{T}_{3} \mathrm{P}$.

4,5-dibromo-1H-pyrrole-2-carboxylic acid (1eq.) was dissolved in EtOAc and pyidine (3eq.). $\mathrm{T}_{3} \mathrm{P}$ (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 productand purified by silica gel flash chromatography (elution by gradient from 100 to $90 / 10 / \mathrm{MeOH}$ ) to afford the desired product.
4,5-Dibromo- $N$-(3-(imidazo[1,2-a]pyrazin-3-yl)phenyl)-1 $H$-pyr-role-2-carboxamide (67a). The title compound was synthesized according to general procedure D using 65 a ( $43 \mathrm{mg}, 0.20 \mathrm{mmol}$ ) yielding 67a as a beige amorphous solid ( $55 \mathrm{mg}, 64 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 6.92$ (br s, 1 H ), 7.36 (d, $J=7.6 \mathrm{~Hz}, 1 \mathrm{H}$ ), $7.5(\mathrm{t}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.88(\mathrm{~d}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.97(\mathrm{~d}, J=4.7 \mathrm{~Hz}$, $1 \mathrm{H}), 8.06(\mathrm{~s}, 1 \mathrm{H}), 8.12(\mathrm{t}, J=1.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.67(\mathrm{dd}, J=1.4,4.8 \mathrm{~Hz}$, $1 \mathrm{H}), 9.15(\mathrm{~s}, 1 \mathrm{H}), 9.77(\mathrm{~s}, 1 \mathrm{H}), 13.05(\mathrm{br} \mathrm{s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz, DMSO-d6) $\delta 113.7,117.5,117.8,119.3,121.6,126.4,127.8$, 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 $(M+H)^{+} \mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 461.9388. Found: 461.9385. IR ( $\mathrm{cm}^{-1}$ ): 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 $\mathbf{6 5 b}(86 \mathrm{mg}, 0.41$ mmol ) yielding 67 b as a beige amorphous solid ( $45 \mathrm{mg}, 26 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\mathcal{\delta 7 . 3 1}(\mathrm{m}, 1 \mathrm{H}), 7.34(\mathrm{~s}, 1 \mathrm{H}), 7.48(\mathrm{t}$, $J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.80(\mathrm{br} \mathrm{s}, 1 \mathrm{H}), 7.83(\mathrm{brs}, 1 \mathrm{H}), 8.21(\mathrm{~m}, 1 \mathrm{H}), 8.23$ $(\mathrm{m}, 1 \mathrm{H}), 8.55(\mathrm{~s}, 1 \mathrm{H}), 8.65(\mathrm{~d}, J=4.1 \mathrm{~Hz}, 1 \mathrm{H}), 10.25(\mathrm{~s}, 1 \mathrm{H}), 13.07$ $(\mathrm{s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta$ 98.2, 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 $(M+H)^{+}$ $\mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 461.9388. Found: 461.9383. IR $\left(\mathrm{cm}^{-1}\right): 3157$, 1665, 1562, 1433, 1291, 1233.
4,5-Dibromo- $N$-(3-(imidazo[1,2-a]pyridin-3-yl)phenyl)-1 $H$-pyr-role-2-carboxamide (67c). The title compound was synthesized according to general procedure D using $\mathbf{6 5 c}(182 \mathrm{mg}, 0.87 \mathrm{mmol})$ yielding 67 c as a beige amorphous solid ( $225 \mathrm{mg}, 56 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\delta 7.0(\mathrm{t}, J=6.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.29(\mathrm{~s}, 1 \mathrm{H}), 7.30$ $(\mathrm{m}, 1 \mathrm{H}), 7.37(\mathrm{~d}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.52(\mathrm{t}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.68(\mathrm{~d}$, $J=9.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.78(\mathrm{~s}, 1 \mathrm{H}), 7.80(\mathrm{~m}, 1 \mathrm{H}), 8.08(\mathrm{~s}, 1 \mathrm{H}), 8.62(\mathrm{~d}$, $J=6.7 \mathrm{~Hz}, 1 \mathrm{H}), 10.12(\mathrm{~s}, 1 \mathrm{H}), 13.02(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 98.2,106.1,113,114.2,117.7,118.3,119.2,122.5$, 124, 124.6, 125, 127.8, 129.2, 129.8, 132.5, 139.6, 145.6, 157.5. HMRS (ESI) $m / z$ Calcd for $(M+H)^{+} \mathrm{C}_{18} \mathrm{H}_{13} \mathrm{~N}_{4} \mathrm{OBr}_{2}$ : 460.9436 . Found: 460.9459. IR ( $\left.\mathrm{cm}^{-1}\right): 3158,1661,1609,1523,1422,1233$. 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 $65 \mathrm{~d}(100 \mathrm{mg}, 0.48$ mmol ) yielding 67 d as a beige amorphous solid ( $80 \mathrm{mg}, 36 \%$ ). ${ }^{1} \mathrm{H}$ NMR (300MHz, DMSO-d6) $\mathcal{\delta} 7.06(\mathrm{dd}, J=4.3,6.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.31$ $(\mathrm{s}, 1 \mathrm{H}), 7.43(\mathrm{t}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.69(\mathrm{~d}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.83(\mathrm{~d}, J$ $=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~s}, 1 \mathrm{H}), 8.36(\mathrm{~s}, 1 \mathrm{H}), 8.54(\mathrm{dd}, J=2.0,4.2 \mathrm{~Hz}$, $1 \mathrm{H}), 8.98(\mathrm{dd}, J=2.0,6.7 \mathrm{~Hz}, 1 \mathrm{H}), 10.01(\mathrm{~s}, 1 \mathrm{H}), 12.92(\mathrm{~s}, 1 \mathrm{H})$. ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 98.2,105.9,107.5,108.9,114$, 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 $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 461.9388. Found: 461.9412. IR $\left(\mathrm{cm}^{-1}\right): 3188,1667,1612,1560$, 1416, 1222.
4,5-Dibromo- $N$-(4-(imidazo[1,2-a]pyrazin-3-yl)phenyl)-1 $H$-pyr-role-2-carboxamide (68a). The title compound was synthesized according to general procedure D using $66 \mathrm{a}(43 \mathrm{mg}, 0.20 \mathrm{mmol}$ ) yielding 68a as a beige amorphous solid ( $44 \mathrm{mg}, 51 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz, DMSO-d6) $\delta 7.17$ ( $\mathrm{s}, 1 \mathrm{H}$ ), $7.70(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 2 \mathrm{H}), 7.94$ $(\mathrm{m}, 3 \mathrm{H}), 8.02(\mathrm{~s}, 1 \mathrm{H}), 8.61(\mathrm{~d}, J=4.7 \mathrm{~Hz}, 1 \mathrm{H}), 9.12(\mathrm{br} \mathrm{s}, 1 \mathrm{H})$, 12.86 (br s, 1H). ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 97.6,107.4$, 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) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+}$ $\mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{OBr}_{2}$ : 461.9388. Found: 461.9369. IR $\left(\mathrm{cm}^{-1}\right): 3177$, 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 66 b ( $123 \mathrm{mg}, 0.59$ mmol ) yielding 68 b as a beige amorphous solid ( $98 \mathrm{mg}, 40 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 7.28(\mathrm{~m}, 2 \mathrm{H}), 7.87(\mathrm{~d}, J=8.9 \mathrm{~Hz}$, 2 H ), 8.15 (d, $J=8.9 \mathrm{~Hz}, 2 \mathrm{H}$ ), 8.20 (dd, $J=1.6,8.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.24$ $(\mathrm{s}, 1 \mathrm{H}), 8.64(\mathrm{dd}, J=1.6,4.5 \mathrm{~Hz}, 1 \mathrm{H}), 10.0(\mathrm{~s}, 1 \mathrm{H}), 12.95(\mathrm{~s}, 1 \mathrm{H})$. ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 98.2,106.1,114,116.8,119.9$, 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 ( $\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{5} \mathrm{OBr}_{2}: 461.9388$. Found: 461.9390. IR ( $\mathrm{cm}^{-1}$ ): 3179, 1637, 1598, 1525, 1409, 1333, 1159, 1022.

4,5-Dibromo- N -(4-(imidazo[1,2-a]pyridin-3-yl)phenyl)-1 H -pyr-role-2-carboxamide (68c). The title compound was synthesized according to general procedure $D$ using 66 c ( $161 \mathrm{mg}, 0.77 \mathrm{mmol}$ ), yielding 68 c as a beige amorphous solid ( $140 \mathrm{mg}, 40 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\delta 6.97(\mathrm{t}, J=6.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.29(\mathrm{~m}, 2 \mathrm{H})$, $7.65(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 3 \mathrm{H}), 7.75(\mathrm{~s}, 1 \mathrm{H}), 7.93(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 2 \mathrm{H}), 8.57$ (d, $J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 10.05(\mathrm{~s}, 1 \mathrm{H}), 12.99(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz, DMSO-d6) $\delta 98.2,106.1,112.8,114.1,117.4,120.4,123.7$, 124.1, 124.5, 124.9, 127.8, 128, 132, 138.6, 145.2, 157.4. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{18} \mathrm{H}_{13} \mathrm{~N}_{4} \mathrm{OBr}_{2}$ : 460.9436. Found: 460.9435. IR ( $\mathrm{cm}^{-1}$ ): 3169, 1634, 1595, 1410, 1310, 1030.

## General Procedure E for Click Reaction.

Corresponding azide ( 1 eq .) was dissolved in DMF/ $\mathrm{H}_{2} \mathrm{O}$ (10:2). The mixture was degased with bubbling argon 5 min . Then sodium ascorbate ( $10 \mathrm{~mol} \%$ ) was added followed by the alkyne 15 a ( $1 \mathrm{eq}$. .). $\mathrm{CuSO}_{4} .5 \mathrm{H}_{2} \mathrm{O}(1 \mathrm{~mol} \%)$ 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] pyrazin-3-ylmethyl)-1 $\mathrm{H}^{-}$
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 ( $146 \mathrm{mg}, 0.84 \mathrm{mmol}$ ) and 15a ( $256 \mathrm{mg}, 0.84 \mathrm{mmol}$ ), yielding 71a as a white amorphous solid ( $310 \mathrm{mg}, 77 \%$ ). ${ }^{1} \mathrm{H}$ NMR (300MHz, DMSO-d6) $\delta 4.42(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 6.09(\mathrm{~s}, 2 \mathrm{H})$, $6.92(\mathrm{~s}, 1 \mathrm{H}), 8.01(\mathrm{~m}, 2 \mathrm{H}), 8.06(\mathrm{~s}, 1 \mathrm{H}), 8.59(\mathrm{t}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H})$, $8.64(\mathrm{~m}, 1 \mathrm{H}), 9.12(\mathrm{~s}, 1 \mathrm{H}), 12.69(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\mathcal{\delta} 34.1,41.6,97.8,104.8,112.9,118.2,123,123.7$, $127.9,129.3,136,143.3,145.3,158.8$. 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-GloTM 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 $6 \mu \mathrm{l}$ for 30 min at $30^{\circ} \mathrm{C}$ in appropriate kinase buffer, with either protein or peptide as substrate in the presence of $10 \mu \mathrm{M}$ ATP ( 40 mM Tris pH 7.5 , 20 mM MgCl 2 and $0.1 \mathrm{mg} / \mathrm{ml}$ of BSA). Afterwards, $6 \mu \mathrm{l}$ of ADPGloTM Kinase Reagent was added to stop the kinase reaction. After an incubation time of 50 min at room temperature (RT), $12 \mu \mathrm{l}$ 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 $\left(\mathrm{IC}_{50}\right)$, 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: ZM447439 (\#S1103, Selleckchem).

HMRS (ESI) $m / z$ Calcd for ( $\mathrm{M}+\mathrm{H})^{+} \mathrm{C}_{15} \mathrm{H}_{13} \mathrm{Br}_{2} \mathrm{~N}_{8} \mathrm{O}: 480.9559$. Found: 480.9598. IR ( $\mathrm{cm}^{-1}$ ): 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 ( $127 \mathrm{mg}, 0.73 \mathrm{mmol}$ ) and $15 \mathrm{a}(223 \mathrm{mg}, 0.73 \mathrm{mmol})$, yielding 71 b as a white amorphous solid ( $310 \mathrm{mg}, 89 \%$ ). ${ }^{1} \mathrm{H}$ NMR ( 300 MHz , DMSO-d6) $\delta 4.41$ (d, $J=5.8 \mathrm{~Hz}, 2 \mathrm{H}$ ), 5.99 ( s, 2 H ), $6.92(\mathrm{~s}, 1 \mathrm{H}), 7.30(\mathrm{dd}, J=4.6,9.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.95(\mathrm{~s}, 2 \mathrm{H}), 8.18$ (dd, $J=1.5,9.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.58(\mathrm{~m}, 2 \mathrm{H}), 12.69(\mathrm{~s}, 1 \mathrm{H}) .{ }^{13} \mathrm{C} \operatorname{NMR}(75$ MHz, DMSO-d6) $\delta 34.1,41.9,97.8,104.7,112.8,118,122.3$, 122.9, 125.9, 127.9, 134.2, 144, 145, 146.2, 158.7. HMRS (ESI) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \quad \mathrm{C}_{15} \mathrm{H}_{13} \mathrm{Br}_{2} \mathrm{~N}_{8} \mathrm{O}: 480.9559$. Found: 480.9564. IR $\left(\mathrm{cm}^{-1}\right): 3146,3128,1635,1566,1523,1243$.

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 ( $202 \mathrm{mg}, 1.2 \mathrm{mmol}$ ) and 15 a ( $356 \mathrm{mg}, 1.2 \mathrm{mmol}$ ), yielding 71c as a white amorphous solid ( $349 \mathrm{mg}, 63 \%$ ). ${ }^{1} \mathrm{H}$ NMR $(300 \mathrm{MHz}$, DMSO-d6) $\delta 4.41(\mathrm{~d}, J=5.7 \mathrm{~Hz}, 2 \mathrm{H}), 6.03(\mathrm{~s}, 2 \mathrm{H})$, $6.91(\mathrm{~s}, 1 \mathrm{H}), 7.0(\mathrm{t}, J=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.31(\mathrm{t}, J=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.62$ $(\mathrm{m}, 1 \mathrm{H}), 7.78(\mathrm{~m}, 1 \mathrm{H}), 7.99(\mathrm{~s}, 1 \mathrm{H}), 8.55(\mathrm{~m}, 1 \mathrm{H}), 8.6(\mathrm{t}, J=5.6$ $\mathrm{Hz}, 1 \mathrm{H}), 12.69$ ( $\mathrm{s}, 1 \mathrm{H}$ ). ${ }^{13} \mathrm{C}$ NMR ( 75 MHz , DMSO-d6) $\delta 34.1$, 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) $\mathrm{m} / \mathrm{z}$ Calcd for $(\mathrm{M}+\mathrm{H})^{+} \quad \mathrm{C}_{16} \mathrm{H}_{14} \mathrm{Br}_{2} \mathrm{~N}_{7} \mathrm{O}: ~ 479.9606$. Found: 479.9621. IR ( $\mathrm{cm}^{-1}$ ): 3263, 1629, 1519, 1501, 1407, 1317, 1227.

Buffers: (A) $10 \mathrm{mM} \mathrm{MgCl} 2,1 \mathrm{mM}$ EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, $50 \mu \mathrm{~g} / \mathrm{ml}$ heparin; (B) 25 mM MOPS, pH 7.2 , $12.5 \mathrm{mM} \beta$-glycerophosphate, $25 \mathrm{mM} \mathrm{MgCl} 2,5 \mathrm{mM}$ 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 $\mu \mathrm{g} / \mu \mathrm{l}$ of myelin basic protein (MBP) as substrate; CDK2/CyclinA (cyclin-dependent kinase-2, human kindly provided by Dr. A. Echalier-Glazer, Leicester, UK) was assayed in buffer A with 0.8 $\mu \mathrm{g} / \mu \mathrm{l}$ of histone H 1 as substrate; CDK5/p25 (human, recombinant, expressed in bacteria) was assayed in buffer A with $0.8 \mu \mathrm{~g} / \mu \mathrm{l}$ of histone H 1 as substrate; CDK9/CyclinT (human, recombinant, expressed by baculovirus in $\mathrm{Sf9}$ insect cells) were assayed in buffer A with $0.27 \mu \mathrm{~g} / \mu \mathrm{l}$ of the following peptide: YSPTSPSYSPTSPSYSPTSPSKKKK, as substrate; GSK3 $\beta$ (human, recombinant, expressed by baculovirus in Sf9 insect cells) were assayed in buffer A with $0.010 \mu \mathrm{~g} / \mu \mathrm{l}$ of GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSpEDEEE); $\mathrm{CK} 1 \varepsilon$ (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer A with $0.022 \mu \mathrm{~g} / \mu \mathrm{l}$ of the following peptide: RRKHAAIGSpAYSITA ("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 $0.007 \mu \mathrm{~g} / \mu \mathrm{l}$ of Histone H3 (1-21) peptide (ARTKQTARKSTGGKAPRKQLA) as substrate; Pim-1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed
in buffer A with $0.8 \mu \mathrm{~g} / \mu \mathrm{l}$ of histone H 1 (Sigma \#H5505) as substrate; MmCLK1 (from Mus musculus, recombinant, expressed in bacteria) was assayed in buffer A with $0.027 \mu \mathrm{~g} / \mu \mathrm{l}$ of the following peptide: GRSRSRSRSRSR as substrate; RnDYRK1A-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 $0.033 \mu \mathrm{~g} / \mu \mathrm{l}$ 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 $\mu \mathrm{M})$ and increasing concentrations of ATP $(3,125$ to $50 \mu \mathrm{M})$. The kinase assay was performed, in triplicate, in buffer B with $0.2 \mu \mathrm{~g} / \mu \mathrm{l}$ of MBP $(0.2 \mu \mathrm{~g} / \mu \mathrm{l})$ as substrate in buffer $B$ and carried out in a final volume of $6 \mu \mathrm{l}$ for 30 min at $30^{\circ} \mathrm{C}$. 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 Lin-eweaver-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) us-ing 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 antiAurora B (1/100, Life Technologies) and mouse monoclonal anti-$\alpha$-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 $37^{\circ} \mathrm{C}$ in a humidified atmosphere containing $5 \% \mathrm{CO}_{2}$.

Cell viability was determined by a luminescent assay according to the manufacturer's instructions (Promega, Madison, WI, USA). For $\mathrm{IC}_{50}$ determination, the cells were seeded in 96 -well plates (3 $\times 10^{3}$ cells/well) containing $90 \mu \mathrm{~L}$ 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, $100 \mu \mathrm{~L}$ 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 $\mathrm{IC}_{50}$ values were calculated using the Graph Prism software from polynomial curves (four or five-parameter logistic equations).
PBMC Viability Assay. Isolation of periferal 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 $5.56 \times 105$ cells $/ \mathrm{mL}$ in RPMI media supplemented with $10 \%$ fetal bovine serum. Cell viability assays were performed on both phytohemagglutinin (PHA)activated and non-activated PMBC 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 software ${ }^{83}$ from the CCDC_2020 suite, with the Aurora B structure 4AF3 ${ }^{71}$ 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 $10 \AA$ 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 $10 \AA$ 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, MickaelisMenten 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.

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## 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.

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## 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; EDCI, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; EGTA, ethylene glycol-$\operatorname{bis}(\beta$-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.

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## Graphical Abstract


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