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PII: S0223-5234(16)30797-8

DOI: 10.1016/j.ejmech.2016.09.064

Reference: EJMECH 8930

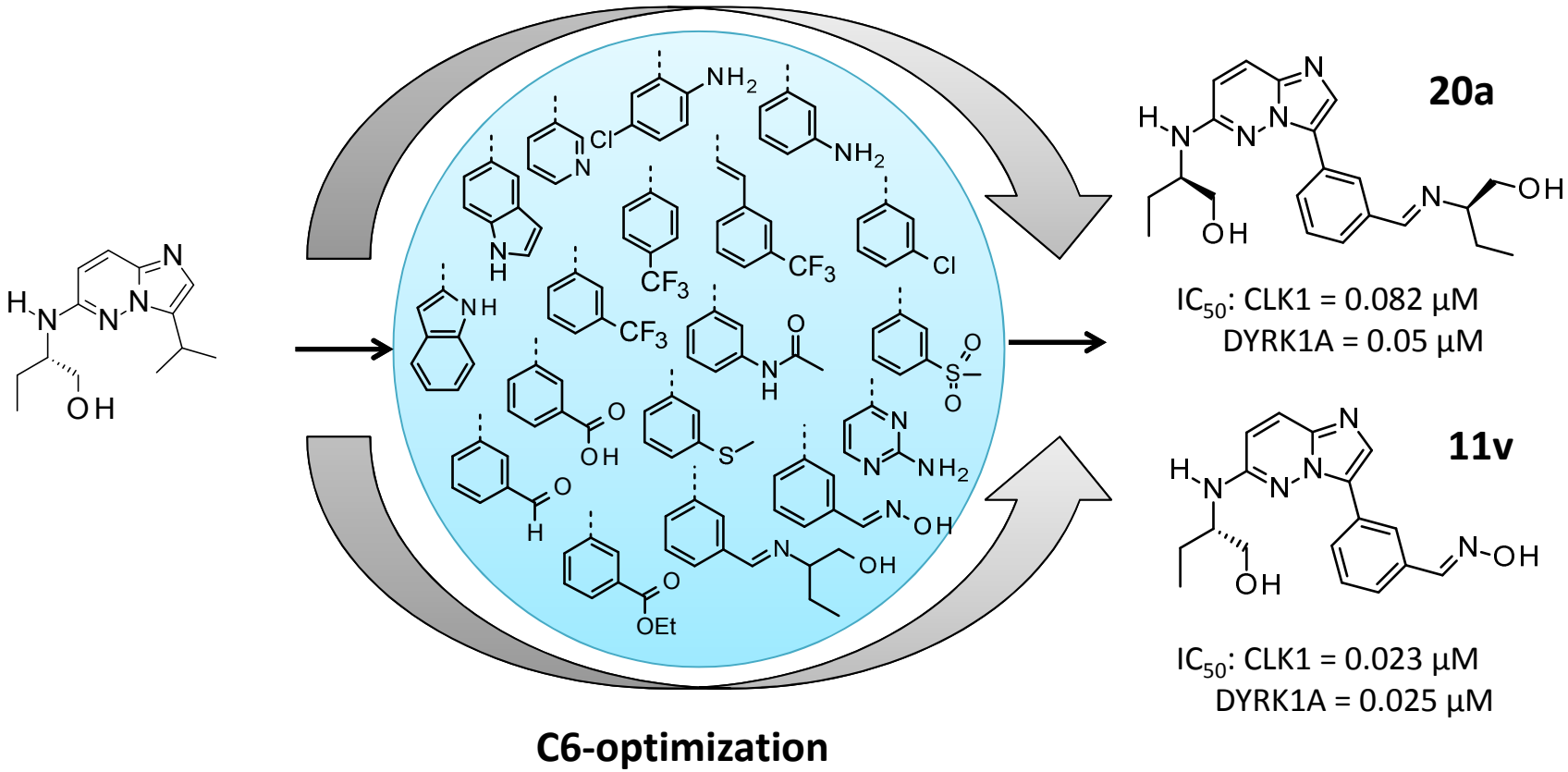
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 8 August 2015

Revised Date: 19 September 2016

Accepted Date: 20 September 2016

Please cite this article as: L.Z. Bendjeddou, N. Loaëc, B. Villiers, E. Prina, G. Späth, H. Galons, L. Meijer, N. Oumata, Exploration of the imidazo[1,2-b]pyridazine scaffold as a protein kinase inhibitor, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.09.064.


C3-optimization
20a
IC50: CLK1 = 0.082 µM
DYRK1A = 0.05 µM
11v
IC50: CLK1 = 0.023 µM
DYRK1A = 0.025 µM
C6-optimization

Exploration of the imidazo[1,2-b]pyridazine scaffold as a protein kinase inhibitor

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Running Title: imidazo[1,2-b]pyridazine kinase inhibitors

Keywords: kinase inhibitor, imidazo[1,2-b]pyridazine, unicellular parasites, *Leishmania,* DYRK1A, CLKs

Abbreviations:

AD, Alzheimer's disease; **CDKs**, cyclin-dependent kinases; **CK1**, casein kinase 1; **CLKs**, cdc2-like kinases; **CMGC** (cyclin-dependent kinase [CDK], mitogen-activated protein kinase [MAPK], glycogen synthase kinase [GSK3], CDC-like kinase [CLK]); **CML**, chronic myelocytic leukemia; **DAD**, diode array detector; **DEPC**, diethyl pyrocarbonate; **DMEM**, Dulbecco's modified Eagle's medium; **DCM**, dichloromethane; **DMSO**, dimethylsulfoxide; **DS**, Down syndrome; **DTT**, dithiothreitol; **DYRKs**, dual-specificity tyrosine phosphorylation

regulated kinases; **FBS**, fetal bovine serum; **GSH**, glutathione; **GSK-3**, glycogen synthase kinase-3; **GST**, glutathione-S-transferase; **IPTG**, isopropyl-β-D-thiogalactopyranoside; **LC**, Liquid chromatography; **MAP kinases**, mitogen-activated protein kinases; **NFTs**, neurofibrillary tangles; **MTS**, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; **PBS**, phosphate-buffered saline; **SAR**, structure activity relationship; **SRp**, serine/arginine-rich proteins; **TLC**, Thin-layer chromatography; **TMS**, tetramethylsilane.

ABSTRACT

3,6-Disubstituted imidazo[1,2-b]pyridazine derivatives were synthesized to identify new inhibitors of various eukaryotic kinases, including mammalian and protozoan kinases. Among the imidazo[1,2-b]pyridazines tested as kinase inhibitors, several derivatives were selective for DYRKs and CLKs, with IC_{50} <100 nM. The characterization of the kinome of several parasites, such as *Plasmodium* and *Leishmania*, has pointed out profound divergences between protein kinases of the parasites and those of the host. This led us to investigate the activities of the prepared compounds against 11 parasitic kinases. 3,6-Disubstituted imidazo[1,2-b]pyridazines showed potent inhibition of *Plasmodium falciparum* CLK1 (*Pf*CLK1). Compound **20a** was found to be the most selective product against CLK1 (IC_{50} = 82 nM), CLK4 (IC_{50} = 44 nM), DYRK1A (IC_{50} = 50 nM), and *Pf*CLK1 (IC_{50} = 32 nM). The compounds were also tested against *Leishmania amazonensis.* Several compounds showed anti-leishmanial activity at rather high (10 µM) concentration, but were not toxic at 1 µM or 10 µM, as judged by viability assays carried out using a neuroblastoma cell line.

1. Introduction

Reversible protein phosphorylation is the most common post-translational modification occurring in many fundamental cellular processes, such as differentiation, division, proliferation, apoptosis, and signal transduction mechanisms. Due to their strong involvement in essentially all physiological processes, dysfunctional phosphorylation can generate many human diseases, such as cancer [1], inflammation, auto-immune and neurodegenerative diseases, and parasite infections. Among the human kinome described by Manning et al. [2] in 2002, the CMGC group, which comprises nine kinase families, has been extremely well-studied as a group of therapeutic targets. Cdc2-like kinases (CLKs) and dual-specificity tyrosine-phosphorylation-regulated kinases (DYRKs) are families of conserved groups of dual-specificity kinases belonging to the CMGC group, including CDKs (cyclin-dependent kinases), MAP kinases (mitogen-activated protein kinases), GSK-3 (glycogen synthase kinase), and CLKs (Cdc2-like kinases). These two families of kinases auto-phosphorylate at tyrosine residues in their activation loop, but exclusively phosphorylate serine/threonine residues. The CLK family consists of four isoforms: CLK1, CLK2, CLK3, and CLK4. CLKs phosphorylate serine/arginine-rich splicing proteins (SRp), which are implicated in the regulation of alternative splicing [3]. CLK1 is involved in Alzheimer's disease (AD) [4] (splicing of microtubule-associated protein Tau pre-mRNA). The DYRK family comprises five isoforms, DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4. The human DYRK1A gene is located on chromosome 21 in the "Down syndrome (DS) critical region," and overexpression of DYRK1A in DS has been associated with neurodegenerative disease [5]. DYRK1A and DYRK1B are also implicated in diverse types of cancers [6,7]. Orthologs of mammalian CLKs and DYRKs are also found in unicellular parasites, such as *Leishmania*, *Trypanosoma*, *Cryptosporidium*, *Giardia*, *Toxoplasma*, and *Plasmodium*. Deregulation and dysfunction of CLKs and DYRKs have been linked to several diseases, which make these kinases attractive potential therapeutic targets.

In the present paper, we describe potent and selective CLK and DYRK inhibitors. We optimized the C-3 position of disubstituted imidazo[1,2-b]pyridazines as CLK and DYRK kinase inhibitors. Our interest in this scaffold is based on the fact that imidazo[1,2-b]pyridazines are frequently described as kinase inhibitors. Imidazo[1,2-b]pyridazines have been reported to be inhibitors of CDK2 [8], Pim [9], IKKβ [10-12], VEGFR2 [13], and Syk [14] (Figure 1). We have identified more than 20 patents issued since 2007 that describe

imidazo[1,2-b]pyridazines as kinase inhibitors, the most advanced product being Ponatinib [15], which in 2012 was launched on the market as a BCR-ABL inhibitor in the treatment of chronic myelocytic leukemia (CML). The MRC (Medical Research Council) technology team has described *Pf*PK7 [16] and *Pf*CDPK1 [17,18] inhibitors as being potential antimalarial agents. More recently, diarylimidazo[1,2-b]pyridazines have been identified and evaluated for antiplasmodial activity [19] (Figure 1).

(Figure 1)

Alzheimer's disease (AD) is a neurodegenerative disease responsible for the most common form of dementia. It is characterized by extracellular accumulation of amyloid plaque (Aβ) and intracellular deposition of neurofibrillary tangles (NFTs). NFTs mainly result from the aggregation of hyperphosphorylated microtubule-associated Tau protein. Aberrant hyperphosphorylation of Tau induces decreased microtubule binding, causing loss of function and aggregation. CLK1 [4] and DYRK1A [20] have been shown to be involved in neurodegenerative disease, particularly AD. Many CLK and DYRK inhibitors have been described over the last few years, including harmine, TG003 [21] and Indy [22], leucettines [23,24], quinazolines [25-27], KH-CB19 [3], meriolins [28], meridianins [29], imidazopyridazines [30], and most recently, furo- or thieno[3,2-d]pyrimidin-4-amines [31] derived from harmine. We describe the synthesis strategy and biological evaluation of a new series of disubstituted imidazo[1,2-b]pyridazine inhibitors of CLKs and DYRKs. The aim of this work is first to identify new mixed inhibitors of DYRK1A and CLKs, which could be further evaluated in cell and animal AD models. A second objective is to identify inhibitors of orthologous parasitic kinases which could be useful in the treatment of *Leishmania* and *Plasmodium* infections.

2. Results and discussion

2.1. Chemistry

A large group of disubstituted imidazo[1,2-b]pyridazines was synthesized, following routes A, B, or C, as depicted in Scheme 1. The synthesis strategy developed provides access to a broad family of disubstituted imidazo[1,2-b]pyridazines. 6-Chloroimidazo[1,2-b]pyridazine **1** was obtained by the cyclisation of 3-amino-6-chloropyridazine and chloroacetaldehyde at reflux in butanol [8], followed by bromination by N-bromosuccinimide in $CHCl_3$, which yielded **4**. A Suzuki cross-coupling reaction at the C-3 position and nucleophilic substitution [32] at C-6 yielded the desired compounds. The aromatic ring can also be introduced by direct arylation on 6-chloroimidazo[1,2-b]pyridazine [33] (route B). An alternative is to introduce the amine at C-6 and then carry out the Suzuki reaction or direct arylation. This route C synthesis allows the introduction of more sensitive groups. (Scheme 1). Indoles were introduced *via* route A at position C-3 by a Suzuki reaction, after -NH protection by Boc. Nucleophilic substitution at position C-6 was then carried out from (*S*)-2-amino-1-butanol in EtOH. Methoxy and sulfonyl groups were obtained by nucleophilic substitution, starting from **5-6**. Pyridines were then introduced at the C-3 position. 6-Chloro-3-(3-pyridyl)imidazo[1,2-b]pyridazine **6e** was prepared using route A in a 66% yield. (Scheme 1). The carbamate **14** was synthesized using route C, the ethyl carbamate being introduced on **1** by a Buchwald reaction, which afforded **7a**. Bromination of **7a** yielded **8a**, which eventually led to **14** after a Suzuki coupling reaction (Scheme 2). The acetanilide derivative **15** was prepared by reacting **6h** with 3-aminoacetanilide under Buchwald conditions (Scheme 3).

(Scheme 2 and 3)

Several pyrimidines were obtained through CH-arylation of 6-chloroimidazo[1,2-b]pyridazine **1**, using the the S-methyl derivative **23**. (Scheme 4). Oxidation of the S-methyl group provided a mixture of the corresponding sulfone and sulfoxide. Indirect amination of the sulfones **10d** and **21g** was performed by 2,4-dimethoxybenzylamine followed by acidic removal of the dimethoxybenzyl protective group. Similarly, the oxidation step was also achieved with **11o** bearing the aminobutanol group and with **21e** substituted by OCH_3. Finally, indirect amination of the pyrimidine yielded **11r** and **21i**.

(Scheme 4)

3-Formylphenyl was linked with an excellent yield (90%) on position 3 of **3**. Imine **20a** was the main product obtained from aldehyde **6m** and (*R*)-2-amino-1-butanol (Scheme 7). An alternative route to imines presented in scheme 5 gave access to acid and imine derivatives in three steps, starting from 6-chloro-3-bromoimidazo[1,2-b]pyridazine. We used the same procedure for a C-6 position for compounds **11v**, but we used route A for chlorine (**6n**) and methoxy (**21j**) groups. Amide synthesis was the last one we used to complete substituent optimization. The four possible stereoisomers *SS*(**11x**), *SR*(**11y**), *RR*(**20c**) and *RS*(**20d**) were prepared (Scheme 5). Several oximes such as **11v** were synthesized from an aldehyde in the presence of hydroxylamine and sodium acetate in methanol.

(Schemes 5)

2.2. Evaluation against kinases.

Our studies originated from testing the biological activity of product **A** (Figure 1). Among several others, this compound was prepared in the aim of identifying new DYRK and CLK inhibitors. We therefore decided to analyze the structure-activity relationship (SAR) of this class of molecules, particularly by introducing an aromatic ring at the C-3 position. Leucettine L41 was used as a reference compound in the evaluation of DYRK/CLK inhibitors. In the context of this article, IC_{50} values obtained with mammalian kinases were in accordance with those reported in a previous paper [24]. IC_{50} values of Leucettine L41 on unicellular parasite DYRK/CLK homologs are shown in table 13, which is added in the supplementary material.

The indole series revealed a slight inhibition preference for CLKs and DYRKs (Table 1). Two derivatives displaying an IC_{50} of less than 0.1 µM, were obtained, with substitutions on the indole C-5 position (0.092 µM for **22b** against CLK4 and 0.098 µM for **11a** against *Ld*DYRK1B). With the substituted indole in position C-2 (**11b**), activity is increased for CLKs/DYRKs, but a bromine atom in compound **11c** decreased the activity (Table 1). The introduction of a 3-pyridyl group at the 3-position led to rather potent inhibitors of CLKs as soon as an amino-alcohol was in position 6 (0.059 µM for **11d** against CLK4 and 0.090 µM for **12** against CLK4). (Table 2).

The optimization was pursued by introducing an arylhalide and a trifluromethylphenyl group. Comparing the various C-6 substituents, (*S*)-2-amino-1-butanol **11e** (IC_{50} CLK1 = 0.12 µM) appeared to be the most active (Table 3). 4-Trifluoromethylbenzene derivatives **10a**, **21c**, and

11f synthesized using route B displayed decreased activity and elongation of the ring, further decreasing the activity, but increasing selectivity towards CLK1 and CLK4. (Table 3).

(Table 3)

The trifluoromethyl group was replaced in the same position by chlorine, the most active compound against CLKs and DYRKs, is substituted by amino alcohol (compound **11h**, table 4). In order to increase activity and selectivity towards mammalian kinases, the C-6 position was modulated with heterocyclic aromatic and heterocyclic aliphatic amine substitutions.

(Table 4)

N-(3-Aminophenyl)acetamide derivative (**15**) and 2-piperazin-1-ylethanol derivative (**16**) displayed good inhibitory activity against CLK1 (IC_{50} values of 86 nM and 84 nM, respectively). Compound **15** also inhibited *Pf*CLK1 (IC_{50} =78 nM) (Table 4).

Converting the amine (**11i**) to an acetamide (**11k**) led to increased activity toward CLKs and DYRKs as well as toward CDK1. Compound **11k** inhibited CLK1, CLK2, CLK4, DYRK1A, DYRK1B, DYRK2, and CDK1, with IC_{50} values of less than 100 nM (Table 5).

Compound **11m** was more active than compound **11l** on CLK1, CLK2, CLK4, DYRK1A, and DYRK2 (Table 6).

In general, compared to phenyl, the pyrimidines were less active. Pyrimidine appeared not to be the most efficient aryl substitution. Compound **11m** (Table 6) was 10-fold more active than **11q** the product (Table 7) on CLK1 and DYRK1A (Table 7).

(Tables 5, 6, and 7)

The formyl-containing derivative **11u** showed good inhibitory activity against CLK1 (41 nM), CLK4 (41 nM), DYRK1A (79 nM), DYRK1B (53 nM), and DYRK2 (63 nM). Next, chlorine was replaced by fluorine to compare inhibitory activities and to observe the increased reactivity of fluorine over chlorine in nucleophilic substitution. Comparison of compounds **5b** and **6m** (Table 8) revealed decreased inhibitory activity against CDK5, DYRK1A, and DYRK2 for the fluorine derivative, but its inhibitory activity was maintained for CLKs and CK1.

Derivative **20a** selectively inhibited CLK1, CLK4, and DYRK1A with a low IC_{50}. Among all the compounds tested, **20a** seems to be the most potent; we therefore synthesized its isomer

(S)-2-amino-1-butanol via route C. Compound **11s** revealed activity, particularly against the CLK and DYRK families, but it also inhibited CDK1 and CDK2

The *S*-isomer of amino alcohol **11w** was more active than isomer R(**20a**) against CLK1 and CLK4, but less active against DYRK1A. Compound **20a** also inhibited *Pf*CLK1 (32 nM), whereas compound **11w** was 10-fold less active.

Among all the products described here, compound **11v** was the most active against CLK1 (23 nM) and CLK4 (25 nM). DYRK1A was inhibited at 33 nM by compound **21j**. It is interesting to observe the differences in activity of compounds **11w** and **20a** (Table 9) All formamides were found to be less active than the imine analogues (Table 10).

(Tables 9 and 10)

2.3. Cytotoxicity

Because these derivatives are intended to be used in the treatment of neurodegenerative diseases, it is important that they not be cytotoxic. All compounds were tested on the SH-SY5Y human neuroblastoma cell-line in a cell-survival assay. The results showed that no compound was cytotoxic at 1µM and 10µM.

Since many kinases are involved in proliferation, it may be estimated that the present compounds are not potent inhibitors of other kinases, and may therefore be considered rather selective inhibitors of the kinases under study. (See Supplementary Material, Table 12).

2.4. Antiparasitic activities

Finally, in respect to the tests on parasitic kinases, the compounds were assayed against *L. amazonensis*. Several compounds were able to reduce parasite proliferation, but at the rather high concentration of 10 µM (Table 11).

(Table 11)

3. Conclusion

In conclusion, based on the imidazo[1,2-b]pyridazine scaffold, we have identified and optimized novel CLK1 and DYRK1A inhibitors, and have established a detailed SAR. Investigation of aryl groups at the C-3 position led us to the finding that the imine group is the

most active substituent. A large number of substituents were explored, compound **20a** representing the most selective compound against CLK1 and DYRK1A, but also against *Pf*CLK1. Since parasite kinases have recently been shown to be potential targets for anti-microbial chemotherapy, we also carried out enzymatic assays with our compounds, using recombinant or purified kinases from various protozoa, including *T. brucei, T. cruzi, T. gondii, C. parvum, G. lamblia, L. donovani,* and *L. major*. Furthermore, the compounds were also assessed for anti-leishmanial activity, using a viability assay with *Leishmania amazonensis* parasites and a phenotypic HCA with intra-macrophagic parasites. Our derivatives showed much less inhibitory activity against *Leishmania* and *Trypanosoma* parasite kinases. Alternatively, the oxime derivatives **21j** and **11v** showed significant inhibitory activity against mammalian CLK1 and DYRK1A. Moreover, cell-survival evaluation showed no cytotoxicity, at least in a neuroblastoma cell line. Finally, several compounds were found to be highly potent against *L. donovani* kinases. However, no correlation was found between this enzymatic inhibitory activity and the effect on *Leishmania* survival. *In fine*, the imidazo[1,2-b]pyridazine scaffold presents a wide potential for future use *in vivo* evaluation. The co-crystal structure determination of **11v** with several kinases, as previously described in the case of leucettine L41 [24], should be undertaken. These results will guide future design in this family of imidazo[1,2-b]pyridazines**.**

4. Experimental

4.1. Buffers and chemicals

Buffer A: 10 mM $MgCl_2$, 1 mM EGTA (MW 380.4), 1 mM dithiothreitol (DTT) (MW 154.2), 25 mMTris/HCl (MW 121.1) and 50 µg/mL heparin.

Buffer C: 60 mM ß-glycerophosphate, 30 mM *p*-nitrophenylphosphate, 25 mM MOPS, 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT and 0.1 mM sodium vanadate.

All chemicals were purchased from Sigma, unless otherwise stated; the protease inhibitor cocktail was obtained from Roche.

4.2. Protein kinase assays

Kinase activities were assayed in buffer A or C at 30 °C at a final ATP concentration of 15 µmol/L. Blank values were subtracted and activities expressed in percent of maximal activity, i.e., in the absence of inhibitors. Controls were carried out with appropriate DMSO dilutions.

The GS-1, CKS, CDK7/9 tide, and RS peptide substrates were obtained from Proteogenix (Oberhausbergen, France).

CDK1/cyclin B (M phase starfish oocytes, native), CDK2/cyclin A and CDK5/p25 (human, recombinant) were prepared as previously described [33]. Their kinase activity was assayed in buffer A with 1 mg histone H1/mL, in the presence of 15 µmol/L [γ-33P] ATP (3,000 Ci/mmol; 10 mCi/mL) in a final volume of 30 µL. After 30 min incubation at 30 °C, the reaction was stopped by harvesting onto P81 phosphocellulose supernatant (Whatman) using a FilterMate harvester (Packard) and were washed in 1% phosphoric acid. Scintillation fluid was added and the radioactivity measured in a Packard counter.

CDK9/cyclin T (human, recombinant, expressed in insect cells) was assayed as described for CDK1/cyclin B, but using CDK7/9-tide (YSPTSPSYSPTSPSYSPTSPSKKKK) (8.1 µg/assay) as a substrate.

GSK-3 (porcine brain, native) and PfGSK3 (*Plasmodium falciparum*, recombinant, expressed in *E. coli* as glutathione-S-transferase (GST) fusion proteins) was assayed as described for CDK1 with 0.5 mg BSA /mL + 1 mM DTT and using a GSK-3-specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQSpEDEEE) (Sp stands for phosphorylated serine) [33].

CK1δ/ε (porcine brain, native) and LmCK1 (*Leishmania major*, recombinant, expressed in *E. coli* as HIS fusion proteins) was assayed as described for CDK1, but in buffer C, and using 25 µM CKS peptide (RRKHAAIGpSAYSITA), a CK1-specific substrate [33].

DYRK1A, 1B, 2, and 3 (human, recombinant, expressed in *E. coli* as GST fusion proteins) were purified by affinity chromatography on glutathione-agarose and assayed as described for CDK1/cyclin B with 0.5 mg BSA /mL + 1 mM DTT and using Woodtide (KKISGRLSPIMTEQ) (1.5µg/assay) as a substrate, a residue of transcription factor FKHR.

CLK1, 2, 3, and 4 (mouse, recombinant, expressed in *E. coli* as GST fusion proteins) were assayed as described for CDK1/cyclin B with 0.5 mg BSA /mL + 1 mM DTT and RS peptide (GRSRSRSRSRSR) (1µg/assay) as a substrate.

Protozoan DYRKs and CLKs (recombinant, expressed in *E. coli)* were assayed as described for CDK1/cyclin B with 0.5 mg BSA /mL + 1 mM DTT and Woodtide or RS peptide for DYRKs or CLKs isoforms.

4.3. Production and purification of the parasitic kinases

The parasitic kinase genes (Table 13) were optimized for expression in *E. coli*, synthesized and cloned in pGEX-6P-1 (GE Healthcare) with the type II restriction enzymes *BamHI* and *XhoI* for glutathione S-transferase (GST) fusion at the N-terminus (GenScript).

The plasmids were transformed into chemically competent *E. coli* BL21(DE3), which were grown overnight at 37°C in 2xYT medium containing 100 µg/mL ampicillin. These cultures were used to inoculate 1 L volumes of 2xYT medium (containing 100 µg/mL ampicillin) in 5L flasks. The cultures were allowed to grow at 37 °C before the temperature was decreased to 18 °C. At an optical density (OD600) of about 1.0, protein expression was induced overnight at 18°C with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The bacteria were harvested by centrifugation and frozen at -20 °C.

Cells were resuspended in lysis buffer (PBS pH 7.4, 1% NP40, 1 mM DTT, 1 mM EDTA, 1 mM PMSF) in the presence of protease inhibitor cocktail (Roche), and lysozyme was added to 1 mg/mL and incubated for 1h at 4 °C with gentle agitation. Cells were then sonicated on ice before $MgCl_2$ (6 mM) and benzonase (Novagen, 25 U/mL) were added to degrade nucleic acids and decrease sample viscosity. After centrifugation at 4 °C, the soluble fraction was collected and proteins were bound to glutathione (GSH) sepharose 4B beads (GE Healthcare). Beads were washed three times with lysis buffer and once with buffer C (25 mM MOPS pH 7.0, 60 mMβ-glycerophosphate, 15 mM p-nitrophenylphosphate, 15 mM EGTA, 15 mM $MgCl_2$, 2 mM DTT, 1 mM sodium vanadate). Proteins were eluted with elution buffer (buffer C containing 30 mM reduced GSH, 15% glycerol, and pH adjusted to 8.5).

4.4. Cell culture conditions; cell-survival quantification

SH-SY5Y cells were grown in DMEM medium from Invitrogen (Cergy-Pontoise, France). All media were supplemented with antibiotics (penicillin-streptomycin) from Lonza and 10% volume of fetal calf serum from In vitrogen. Cells were cultured at 37 °C with 5% CO_2. Drug treatments were carried out on exponentially growing cultures at the indicated concentrations. Control experiments were also carried out, using appropriate dilutions of DMSO (maximum 1% DMSO). Cell Titer 96® containing the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) reagent was purchased from Promega (Madison, WI, USA). Cell viability was determined by measuring the reduction of MTS as described [34].

4.5. Antileishmanial activity

4.5.1. Parasites

L. amazonensis strain LV79 (MPRO/BR/1972/M1841) expressing the mCherry fluorescent reporter was propagated in Swiss nu/nu mice. Amastigotes were isolated from mouse lesions and used for HCA. Promastigotes were differentiated from lesion-derived amastigotes and grown in M199 medium at 26 °C for use in dye reduction assay to assess compound toxicity on host cell-free parasites [35].

4.5.2. Viability assay on host cell-free *Leishmania*

An enzymatic assay which incorporates a REDOX indicator (resazurin), which fluoresces in response to cellular metabolic reduction (resofurin), was used to quantify the toxic effect of selected compounds on *L. amazonensis* promastigotes in axenic conditions. Compounds were tested at three final concentrations (20, 4, and 0.8 µM) in quadruplicate.

Supporting information,

1. Supplementary material 1. Experimental procedures for the synthesis of all compounds. Table 12: cell survival after treatment by prepared compounds. Table 13: Inhibition of parasitic kinases by Leucettine L41. Table 14: Parasitic kinase genes used for the expression of tested kinases.
2. Supplementary material 2 and 3. ^{1}H NMR, ^{13}C NMR, and MS spectra.

Acknowledgements

This research was supported by grants from the 'Fonds Unique Interministériel" (FUI) PHARMASEA, and TRIAD projects (HG, LM), the "Association France-Alzheimer (Finistère)" (LM), the "Fondation Jérôme Lejeune" (LM), ANR grant TRANSLEISH and EEC FP7 European Union 7th Framework Program Knowledge-based Bioeconomy (FP7-KBBE) grant BLUEGENICS (LM).

References

(1) Zhang, J.; Yang, P. L.; Gray, N. S. Targeting Cancer with Small Molecule Kinase Inhibitors. Nat. Rev. Cancer 2009, 9, 28–39.

(2) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The Protein Kinase Complement of the Human Genome. Science 2002, 298, 1912–1934.

(3) Fedorov, O.; Huber, K.; Eisenreich, A.; Filippakopoulos, P.; King, O.; Bullock, A. N.; Szklarczyk, D.; Jensen, L. J.; Fabbro, D.; Trappe, J.; Rauch, U.; Bracher, F.; Knapp, S. Specific CLK Inhibitors from a Novel Chemotype for Regulation of Alternative Splicing. Chem. Biol. 2011, 18, 67–76.

(4) Jain, P.; Karthikeyan, C.; Moorthy; N.S.; Waiker, D. K.; Jain, A. K.; Trivedi, P. Human CDC2-Like Kinase 1 (CLK1): A Novel Target for Alzheimer's Disease. Curr. Drug Targets 2014, 15, 539–550.

(5) Becker, W.; Sippl, W. Activation, Regulation, and Inhibition of DYRK1A. FEBS J. 2011, 278, 246–256.

(6) Ionescu, A.; Dufrasne, F.; Gelbcke, M.; Jabin, I.; Kiss, R. DYRK1A Kinase Inhibitors with Emphasis on Cancer. Mini Rev. Med. Chem. 2012, 1315–1329.

(7) Friedman, E. Mirk/dyrk1B Kinase in Ovarian Cancer. Int. J. Mol. Sci. 2013, 14, 5560–5575.

(8) Byth, K. F.; Cooper, N.; Culshaw, J. D.; Heaton, D. W.; Oakes, S. E.; Minshull, C. a; Norman, R. a; Pauptit, R. a; Tucker, J. a; Breed, J.; Pannifer, A.; Rowsell, S.; Stanway, J. J.; Valentine, A. L.; Thomas, A. P. Imidazo[1,2-B]pyridazines: A Potent and Selective Class of Cyclin-Dependent Kinase Inhibitors. Bioorg. Med. Chem. Lett. 2004, 14, 2249–2252.

(9) Bullock, A. N.; Debreczeni, J. E.; Fedorov, O. Y.; Nelson, A.; Marsden, B. D.; Knapp, S. Structural Basis of Inhibitor Specificity of the Human Protooncogene Proviral Insertion Site in Moloney Murine Leukemia Virus (PIM-1) Kinase. J. Med. Chem. 2005, 48, 7604–7614.

(10) Shimizu, H.; Tanaka, S.; Toki, T.; Yasumatsu, I.; Akimoto, T.; Morishita, K.; Yamasaki, T.; Yasukochi, T.; Iimura, S. Discovery of imidazo[1,2-B]pyridazine Derivatives as IKKbeta Inhibitors. Part 1: Hit-to-Lead Study and Structure-Activity Relationship. Bioorg. Med. Chem. Lett.2010, 20, 5113–5118.

(11) Shimizu, H.; Yasumatsu, I.; Hamada, T.; Yoneda, Y.; Yamasaki, T.; Tanaka, S.; Toki, T.; Yokoyama, M.; Morishita, K.; Iimura, S. Discovery of imidazo[1,2-B]pyridazines as IKKβ Inhibitors. Part 2: Improvement of Potency in Vitro and in Vivo. Bioorg. Med. Chem. Lett. 2011, 21, 904–908.

(12) Shimizu, H.; Yamasaki, T.; Yoneda, Y.; Muro, F.; Hamada, T.; Yasukochi, T.; Tanaka, S.; Toki, T.; Yokoyama, M.; Morishita, K.; Iimura, S. Discovery of imidazo[1,2-B]pyridazines as IKKβ Inhibitors. Part 3: Exploration of Effective Compounds in Arthritis Models. Bioorg. Med. Chem. Lett. 2011, 21, 4550–4555.

(13) Matsumoto, S.; Miyamoto, N.; Hirayama, T.; Oki, H.; Okada, K.; Tawada, M.; Iwata, H.; Nakamura, K.; Yamasaki, S.; Miki, H.; Hori, A.; Imamura, S. Structure-Based Design, Synthesis, and Evaluation of imidazo[1,2-b]pyridazine and imidazo[1,2-a]pyridine Derivatives as Novel Dual c-Met and VEGFR2 Kinase Inhibitors. Bioorg. Med. Chem. 2013, 21, 7686–7698.

(14) Lucas, M. C.; Goldstein, D. M., Kuglstatter H. C.; Andreas, Liu, W.; Luk, K.C.; Fernando Padilla, F.; Slade, M.; Villaseñor, A.G.; Wanner, J.; Xie, W.; Zhang, X.; Liao, C. Rational Design of Highly Selective Spleen Tyrosine Kinase Inhibitors. J. Med. Chem. 2012, 55, 10414–10423.

(15) Huang, W.-S.; Metcalf, C.; Sundaramoorthi, R.; Wang, Y.; Zou, D.; Thomas, R. M.; Zhu, X.; Cai, L.; Wen, D.; Liu, S.; Romero, J.; Qi, J.; Chen, I.; Banda, G.; Lentini, S. P.; Das, S.; Xu, Q.; Keats, J.; Wang, F.; Wardwell, S.; Ning, Y.; Snodgrass, J. T.; Broudy, M. I.; Russian, K.; Zhou, T.; Commodore, L.; Narasimhan, N. I.; Mohemmad, Q. K.; Iuliucci, J.; Rivera, V. M.; Dalgarno, D. C.; Sawyer, T. K.; Clackson, T.; Shakespeare, W. C. Discovery of 3-[2-(imidazo[1,2-B]pyridazin-3-Yl)ethynyl]-4-Methyl-N-{4-[(4-Methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide (AP24534), a Potent, Orally Active Pan-Inhibitor of Breakpoint Cluster Region-Abelson (BCR-ABL) Kinase Including Th. J. Med. Chem. 2010, 53, 4701–4719.

(16) Bouloc, N.; Large, J. M.; Smiljanic, E.; Whalley, D.; Ansell, K. H.; Edlin, C. D.; Bryans, J. S. Synthesis and in Vitro Evaluation of Imidazopyridazines as Novel Inhibitors of the Malarial Kinase PfPK7. Bioorg. Med. Chem. Lett. 2008, 18, 5294–5298.

(17) Chapman, T. M.; Osborne, S. a; Bouloc, N.; Large, J. M.; Wallace, C.; Birchall, K.; Ansell, K. H.; Jones, H. M.; Taylor, D.; Clough, B.; Green, J. L.; Holder, A.Substituted

Imidazopyridazines Are Potent and Selective Inhibitors of Plasmodium Falciparum Calcium-Dependent Protein Kinase 1 (PfCDPK1). Bioorg. Med. Chem. Lett. 2013, 23, 3064–3069.

(18) Timothy M. Chapman, Simon A. Osborne, Claire Wallace, Kristian Birchall, Nathalie Bouloc, Hayley M. Jones, Keith H. Ansell, Debra L. Taylor, Barbara Clough, Judith L. Green, and A. A. H. Optimization of an Imidazopyridazine Series of Inhibitors of Plasmodium Falciparum Calcium-Dependent Protein Kinase 1 (PfCDPK1). J. Med. Chem. 2014, 57, 3570–3587.

(19) Le Manach, C.; Gonzàlez Cabrera, D.; Douelle, F.; Nchinda, A. T.; Younis, Y.; Taylor, D.; Wiesner, L.; White, K. L.; Ryan, E.; March, C.; Duffy, S.; Avery, V. M.; Waterson, D.; Witty, M. J.; Wittlin, S.; Charman, S. a; Street, L. J.; Chibale, K. Medicinal Chemistry Optimization of Antiplasmodial Imidazopyridazine Hits from High Throughput Screening of a SoftFocus Kinase Library: Part 1..J. Med. Chem., 2014, 57, 2789–2798.

(20) Wegiel, J.; Gong, C.-X.; Hwang, Y.-W. The Role of DYRK1A in Neurodegenerative Diseases. FEBS J. 2011, 278, 236–245.

(21) Muraki, M.; Ohkawara, B.; Hosoya, T.; Onogi, H.; Koizumi, J.; Koizumi, T.; Sumi, K.; Yomoda, J.; Murray, M. V; Kimura, H.; Furuichi, K.; Shibuya, H.; Krainer, A. R.; Suzuki, M.; Hagiwara, M. Manipulation of Alternative Splicing by a Newly Developed Inhibitor of Clks. J. Biol. Chem. 2004, 279, 24246–24254.

(22) Ogawa, Y.; Nonaka, Y.; Goto, T.; Ohnishi, E.; Hiramatsu, T.; Kii, I.; Yoshida, M.; Ikura, T.; Onogi, H.; Shibuya, H.; Hosoya, T.; Ito, N.; Hagiwara, M. Development of a Novel Selective Inhibitor of the Down Syndrome-Related Kinase Dyrk1A. Nat. Commun. 2010, 1, 86.

(23) Debdab, M.; Carreaux, F.; Renault, S.; Soundararajan, M.; Fedorov, O.; Filippakopoulos, P.; Lozach, O.; Babault, L.; Tahtouh, T.; Baratte, B.; Ogawa, Y.; Hagiwara, M.; Eisenreich, A.; Rauch, U.; Knapp, S.; Meijer, L.; Bazureau, J.-P. Leucettines, a Class of Potent Inhibitors of cdc2-like Kinases and Dual Specificity, Tyrosine Phosphorylation Regulated Kinases Derived from the Marine Sponge Leucettamine B: Modulation of Alternative Pre-RNA Splicing. J. Med. Chem. 2011, 54, 4172–4186.

(24) Tahtouh, T.; Elkins, J. M.; Filippakopoulos, P.; Soundararajan, M.; Burgy, G.; Durieu, E.; Cochet, C.; Schmid, R. S.; Lo, D. C.; Delhommel, F.; Oberholzer, A. E.; Pearl, L. H.; Carreaux, F.; Bazureau, J.-P.; Knapp, S.; Meijer, L. Selectivity, Cocrystal Structures, and Neuroprotective Properties of Leucettines, a Family of Protein Kinase Inhibitors Derived from the Marine Sponge Alkaloid Leucettamine B. J. Med. Chem. 2012, 55, 9312–9330.

(25) Rosenthal, A. S.; Tanega, C.; Shen, M.; Mott, B. T.; Bougie, J. M.; Nguyen, D.-T.; Misteli, T.; Auld, D. S.; Maloney, D. J.; Thomas, C. J. Potent and Selective Small Molecule Inhibitors of Specific Isoforms of Cdc2-like Kinases (Clk) and Dual Specificity Tyrosine-Phosphorylation-Regulated Kinases (Dyrk). Bioorg. Med. Chem. Lett. 2011, 21, 3152–3158.

(26) Coombs, T. C.; Tanega, C.; Shen, M.; Wang, J. L.; Auld, D. S.; Gerritz, S. W.; Schoenen, F. J.; Thomas, C. J.; Aubé, J. Small-Molecule Pyrimidine Inhibitors of the cdc2-like (Clk) and Dual Specificity Tyrosine Phosphorylation-Regulated (Dyrk) Kinases: Development of Chemical Probe ML315. Bioorg. Med. Chem. Lett. 2013, 23, 3654–3661.

(27) Pan, Y.; Wang, Y.; Bryant, S. 3D-QSAR Characterization of 6-Arylquinazolin-4-Amines as Cdc2-like Kinase 4 (Clk4) and Dual Specificity Tyrosine-Phosphorylation-Regulated Kinase 1A. J. Chem. Inf. 2013, 4, 938–947.

(28) Echalier, A.; Bettayeb, K.; Ferandin, Y.; Lozach, O.; Clément, M.; Valette, A.; Liger, F.; Marquet, B.; Morris, J. C.; Endicott, J. a; Joseph, B.; Meijer, L. Meriolins (3-(pyrimidin-4-Yl)-7-Azaindoles): Synthesis, Kinase Inhibitory Activity, Cellular Effects, and Structure of a CDK2/cyclin A/meriolin Complex. J. Med. Chem.2008, 51, 737–751.

(29) Giraud, F.; Alves, G.; Debiton, E.; Nauton, L.; Théry, V.; Durieu, E.; Ferandin, Y.; Lozach, O.; Meijer, L.; Anizon, F.; Pereira, E.; Moreau, P. Synthesis, Protein Kinase Inhibitory Potencies, and in Vitro Antiproliferative Activities of Meridianin Derivatives. J. Med. Chem. 2011, 54, 4474–4489.

(30) Bullock, A. N.; Das, S.; Debreczeni, J. E.; Rellos, P.; Fedorov, O.; Niesen, F. H.; Guo, K.; Papagrigoriou, E.; Amos, A. L.; Cho, S.; Turk, B. E.; Ghosh, G.; Knapp, S. Kinase Domain Insertions Define Distinct Roles of CLK Kinases in SR Protein Phosphorylation. Structure 2009, 17, 352–362.

(31) Loidreau, Y.; Marchand, P.; Dubouilh-Benard, C.; Nourrisson, M.-R.; Duflos, M.; Loaëc, N.; Meijer, L.; Besson, T. Synthesis and Biological Evaluation of N-Aryl-7-methoxybenzo[b]furo[3,2-D]pyrimidin-4-Amines and Their N-arylbenzo[b]thieno[3,2-D]pyrimidin-4-Amine Analogues as Dual Inhibitors of CLK1 and DYRK1A Kinases. Eur. J. Med. Chem. 2013, 59, 283–295.

(32) Gray. N. S.; Wodicka, L.; Thunnissen, A. M., Norman, T.C.; Kwon, S.; Espinoza, F.H; Morgan, D.O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S.H; Lockhart, D.J; Schulz, P.G. Exploiting Chemical Libraries, Structure, and Genomics in the Search for Kinase Inhibitors. Science 1998, 281, 533–538.

(33) Oumata, N.; Bettayeb, K.; Ferandin, Y.; Demange, L.; Lopez-Giral, A.; Goddard, M.-L.; Myrianthopoulos, V.; Mikros, E.; Flajolet, M.; Greengard, P.; Meijer, L.; Galons, H. Roscovitine-Derived, Dual-Specificity Inhibitors of Cyclin-Dependent Kinases and Casein Kinases 1. J. Med. Chem. 2008, 51, 5229–5242.

(34) Ribas, J.; Boix J. Cell differentiation caspase inhibition and macromolecular synthesis blockage but not BCL-2 or BCL-XL proteins protect SH-SY5Y cells from apoptosis triggered by two CDK inhibitory drugs. Exp. Cell Res. 2004, 295, 9-24.

(35). Aulner, N.; Danckaert A.; Rouault-Hardoin E.; Desrivot J.; Helynck O.; Commere PH.; Munier-Lehmann H.; Späth GF.; Shorte SL.; Milon G.; Prina E. High content analysis of primary macrophages hosting proliferating Leishmania amastigotes: application to anti-leishmanial drug discovery. PLoS Negl Trop Dis. 2013;7: e2154.

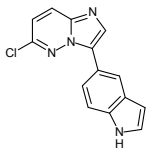
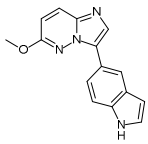
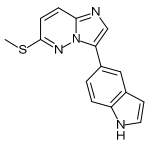
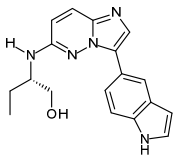
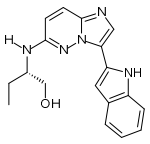
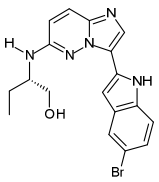
List of tables figure and schemes:

Scheme 3. Reagents and conditions: (a) Acetic anhydride, DMAP, Et_3N, DCM, 20 °C, 2 h; (b) (*S*)-2-Amino-1-butanol, 160 °C, 7 h; (b') (*S*)-2-Amino-1-butanol,180 °C, 7 h; (a') Acetic anhydride, DMAP, Et_3N, DCM, r.t, 2 h.

Scheme 4. Reagents and conditions**:** (a) TMSBr, CH_3CN, 40 °C, 27 h; (b) 6-Chloro-imidazo[1,2-b]pyridazine, $Pd(OAc)_2$, PPh_3, K_2CO_3, toluene, 2 days, 110 °C, 38%; (c) *m*CPBA, DCM, r.t; (d) MeONa, MeOH, 6 h, 80 °C; (e) (*S*)-2-Amino-1-butanol, 170 °C, 4 h. (f) 2,4-Dimethoxybenzylamine 3 Eq. 1,4-dioxane, 8 h, reflux; (g) TFA, DCM, 20 °C.

Scheme 5. Reagents and conditions : (a) (*R*) or (*S*)-2-Amino-1-butanol, 8 h, 180 °C; (c) Ethyl 3-bromobenzoate, $Pd(OAc)_2$, $P(C_6H_5)_3$, K_2CO_3, toluene, 8 h, 110 °C, 10 %; (c) i: NaOH (1N), THF/EtOH, ii: HCl (1N), H_2O, 28%; (d) 3-Formylphenylboronic acid, $Pd[P(C_6H_5)_3]_4$, Na_2CO_3 (2 M), 1,4-dioxane, 12 h, 100 °C. (e) (*S*)-2-Amino-1-butanol 2.5 Eq., 3 days 20 to 60 °C. (f) (*S* or *R*)-2-Amino-1-butanol, DCC/HOBt, Et_3N, THF/DCM, 20 °C, 24 h. (g) (*S* or *R*)-2-Amino-1-butanol, DCC/HOBt, Et_3N, THF/DCM, 20 °C, 24 h; (b) (*S* or *R*)-2-Amino-1-butanol, 180 °C, 12 h; (h) KF, DMSO/Toluene, 8 h, 180 °C; (i) $NH_2OH.HCl$, NaOAc 1.1 Eq, MeOH-H_2O 10%, 20 °C, 16 h.

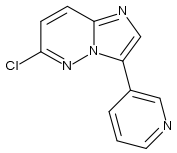
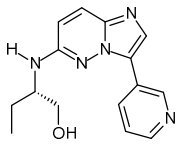
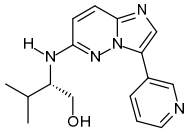
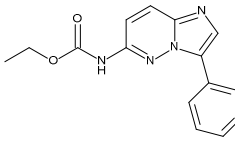
Table 1. Kinase inhibition values : IC_{50} [a] in µM for indole derivatives. Leucettine L41 was used as reference compound.[b]

						
Compd	**6a**	**21a**	**22b**	**11a**	**11b**	**11c**
CDK1	4.1	4.3	2.7	2.7	1.8	1.7
CDK2	5.9	>10	9.1	41	4.1	9.1
CDK5	6.7	5.1	4.2	3.1	1.2	8.3
CDK9	5	3.1	2	2.8	1.1	0.22
CK1	2.8	1.2	1.2	5	2.1	10
CLK1	0.13	0.22	0.13	0.27	0.11	1.2
CLK2	1	0.71	0.22	2.2	0.38	1.9
CLK3	5.2	3.1	5.8	21	2.4	10
CLK4	0.12	0.18	0.092	0.21	0.12	1
DYRK1A	2.4	0.47	0.58	0.35	0.34	0.71
DYRK1B	1.2	1.2	1.4	0.96	0.22	2.1
DYRK2	0.61	0.32	0.44	0.38	0.21	1.2
DYRK3	4.7	0.91	1.2	1.8	0.12	0.61
GSK-3	5.6	4.3	>10	2.7	2.3	10
***Ld*DYRK1B**	1.8	1.2	3.1	0.098	0.15	0.13
***Ld*DYRK3**	>10	9.2	>10	>10	10	10
***Ld*DYRK4**	>10	>10	>10	>10	10	10
***Tb*CLK1**	>10	>10	>10	>10	10	10
***Tc*CLK1**	>10	>10	>10	>10	10	10
***Cp*CLK1**	1.2	2.7	4.2	3.1	10	10
***Gl*CLK**	5.9	5.2	8.1	1.1	2.1	2.9
***Tg*CLK**	2.8	4.1	4.1	4.1	10	10
***Lm*DYRK2**	4	5	>10	3.9	4.1	10
***Lm*CLK**	>10	>10	>10	10	10	10
***Pf*CLK1**	1.6	1.3	0.9	2.2	2.2	10

[a] IC_{50} values were estimated from dose-response curves and are shown in µM. >10, less than 50% inhibition at 10 µM.

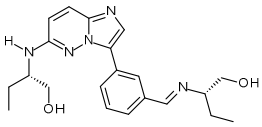
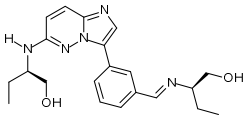
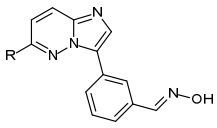
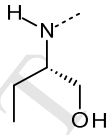
[b] Leucettine L41 [24] DYRK1A: IC_{50} = 0.040 µM; CLK1: IC_{50} = 0.090 µM (See also table the table in supplementary material.

Table 2. Kinase inhibition values: IC_{50} [a] in µM for pyridine derivatives.

Compd				
	6e	**11d**	**12**	**13**
CDK1	>10	3	2.3	>10
CDK2	71	3.4	3.2	>10
CDK5	4.7	1.7	1.4	>10
CDK9	>10	2.1	1.2	>10
CK1	4.1	5.1	1.2	>10
CLK1	0.89	0.19	0.18	>10
CLK2	4.1	0.71	0.59	>10
CLK3	42	3.9	3.3	>10
CLK4	0.4	0.059	0.09	>10
DYRK1A	13	0.87	0.39	>10
DYRK1B	20	0.51	0.13	>10
DYRK2	11	0.6	0.19	>10
DYRK3	16	0.95	0.32	>10
GSK-3	51	3	5.1	>10
***Ld*DYRK1B**	>10	0.69	0.39	>10
***Ld*DYRK3**	>10	>10	>10	>10
***Ld*DYRK4**	>10	>10	>10	>10
***Tb*CLK1**	>10	>10	>10	>10
***Tc*CLK1**	>10	>10	>10	>10
***Cp*CLK1**	>10	2.2	3.8	>10
***Gl*CLK**	>10	0.71	4.2	>10
***Tg*CLK**	>10	4.9	5.2	>10
***Lm*DYRK2**	>10	5.7	6.5	>10
***Lm*CLK**	>10	>10	>10	>10
***Pf*CLK1**	>10	3	1.3	>10

[a] IC_{50} values were estimated from dose-response curves and are shown in µM >10, less than 50% inhibition at 10 µM.

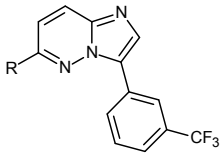
Table 9. Kinase inhibition values: [a]IC_{50} in µM for imine and oxime derivatives.

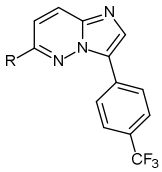
					
R			Cl	OMe	

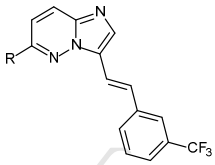
Compd	**11w**	**20a**	**6n**	**21j**	**11v**
CDK1	0.59	5.3	>10	1.1	0.71
CDK2	1.2	5.9	>10	2.8	1.4
CDK5	0.59	>10	>10	1.8	1
CDK9	0.9	1.3	>10	2.2	2
CK1	2.7	0.19	0.12	0.25	2.1
CLK1	0.066	0.082	0.12	0.25	0.023
CLK2	0.16	0.16	0.13	0.059	0.052
CLK3	1.9	2.3	0.62	0.31	1.4
CLK4	0.042	0.044	>10	2.2	0.025
DYRK1A	0.12	0.05	0.078	0.033	0.11
DYRK1B	0.11	0.11	0.68	0.17	0.08
DYRK2	0.5	0.38	0.73	0.17	0.39
DYRK3	1.4	3.1	0.51	0.14	0.92
GSK-3	>10	3.2	>10	11	>10
***Ld*DYRK1B**	0.48	1	4.1	0.98	0.41
***Ld*DYRK3**	3.1	3.8	>10	6	3.3
***Ld*DYRK4**	>10	>10	>10	>10	>10
***Tb*CLK1**	>10	>10	>10	>10	>10
***Tc*CLK1**	>10	>10	>10	>10	>10
***Cp*CLK1**	1.8	0.46	2.2	1.5	1.2
***Gl*CLK**	2.3	3	2.4	2.9	2
***Tg*CLK**	1.4	1.7	>10	6.3	0.73
***Lm*DYRK2**	1.7	0.81	7.8	4.2	0.98
***Lm*CLK**	>10	>10	>10	>10	>10
***Pf*CLK1**	0.37	0.032	0.83	1.1	0.31

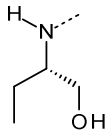
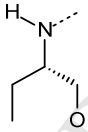
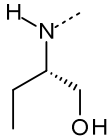
[a] IC_{50} values were estimated from dose-response curves and are shown in µM. >10, less than 50% inhibition at 10 µM.

Table 3. Kinase inhibition values: IC_{50} [a] in µM for halogens and CF_3 derivatives





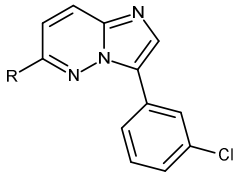


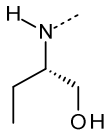
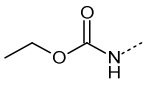
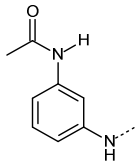
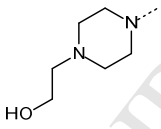
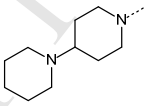
R	Cl	F	OMe	SMe		Cl	OMe		Cl	OMe	

Compd	**6f**	**5a**	**21b**	**22c**	**11e**	**10a**	**21c**	**11f**	**6g**	**21d**	**11g**
CDK1	7.5	>10	3.3	1.4	1.3	>10	5.1	1.7	>10	>10	>10
CDK2	45	>10	12	7.8	82	>10	5.1	5.3	>10	>10	>10
CDK5	4.7	>10	18	0.75	50	>10	>10	7.1	>10	>10	>10
CDK9	>10	>10	8.1	4	1.7	>10	>10	7.5	>10	>10	3.7
CK1	8.1	>10	10	5.9	>10	3.6	2.4	3.8	5.1	>10	>10
CLK1	0.58	2.8	0.79	0.33	0.12	2.7	1.1	0.18	0.59	0.51	0.79
CLK2	2.8	6.3	1.5	0.54	0.38	5.9	1.8	1.4	1.3	0.89	9.1
CLK3	>100	>10	12	12	39	8.8	>10	>10	>10	>10	>10
CLK4	0.57	1.5	0.61	0.23	0.17	2.1	0.98	0.18	0.32	0.22	0.41
DYRK1A	7.8	4.2	1.3	1	0.22	7.3	2.8	1.1	8.9	>10	>10
DYRK1B	0.82	>10	3.4	1.7	0.19	>10	3.6	1.1	7.1	>10	>10
DYRK2	0.48	6.3	1.2	1.8	0.18	8.6	2.5	0.78	7.1	>10	>10
DYRK3	>10	>10	5.3	3	>100	>10	4.9	2.1	>10	>10	>10
GSK-3	>100	>10	22	12	91	>10	>10	>10	>10	>10	>10
***Ld*DYRK1B**	2.1	>10	2.2	4.1	1.2	>10	3.1	0.42	>10	>10	>10
***Ld*DYRK3**	>10	>10	>10	>10	>10	>10	>10	>10	>10	6.4	2.2
***Ld*DYRK4**	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
***Tb*CLK1**	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
***Tc*CLK1**	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
***Cp*CLK1**	>10	>10	>10	3	2.3	>10	>10	>10	>10	>10	>10
***Gl*CLK**	>10	>10	>10	>10	>10	>10	>10	3.9	>10	>10	>10
***Tg*CLK**	>10	>10	>10	2	>10	>10	>10	>10	>10	>10	>10
***Lm*DYRK2**	>10	>10	>10	7.1	>10	>10	7.8	>10	>10	>10	>10
***Lm*CLK**	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
***Pf*CLK1**	8	>10	>10	2.9	8.6	4.5	4.5	3.9	7	>10	>10

[a] IC_{50} values were estimated from dose-response curves and are shown in µM. >10, less than 50% inhibition at 10 µM.

Table 4. Kinase inhibition values: IC_{50}[a] in µM for chlorine derivatives.



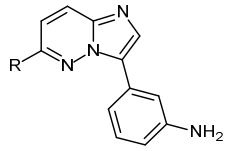
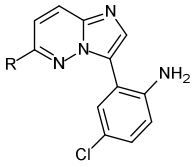
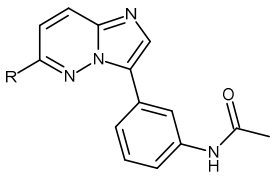
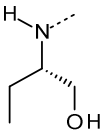
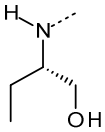
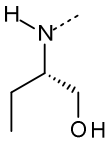
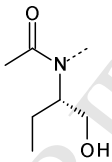
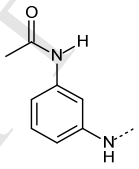
R	Cl					

Compd	**6h**	**11h**	**14**	**15**	**16**	**17**
CDK1	6.7	1.1	>10	0.89	8.2	>10
CDK2	10	>10	>10	4	>10	>10
CDK5	6.8	0.61	>10	0.39	>10	>10
CDK9	4.8	0.22	>10	2.1	1	0.62
CK1	6.7	1.1	>10	>10	0.9	0.4
CLK1	0.23	0.048	0.63	0.086	0.084	1.8
CLK2	2.1	0.22	1.2	0.13	1.2	1.9
CLK3	>10	>10	>10	1.8	>10	>10
CLK4	0.15	0.043	0.52	0.1	0.51	0.8
DYRK1A	1.4	0.075	0.42	0.11	0.51	2.8
DYRK1B	1.7	0.051	1.1	0.18	0.52	3.6
DYRK2	1.3	0.05	1.6	0.11	0.44	3.2
DYRK3	3	0.081	7.1	5.3	0.43	1.1
GSK-3	>10	>10	>10	>10	>10	>10
***Ld*DYRK1B**	6.2	0.19	>10	0.59	1.8	>10
***Ld*DYRK3**	>10	>10	>10	>10	>10	>10
***Ld*DYRK4**	>10	>10	>10	>10	>10	>10
***Tb*CLK1**	>10	>10	>10	>10	>10	>10
***Tc*CLK1**	>10	>10	>10	>10	>10	>10
***Cp*CLK1**	5.1	6.1	>10	>10	>10	>10

***Gl*CLK**	>10	>10	>10	>10	>10	>10
***Tg*CLK**	7.1	>10	>10	>10	>10	>10
***Lm*DYRK2**	8.3	>10	>10	0.5	8.9	>10
***Lm*CLK**	>10	>10	>10	>10	>10	>10
***Pf*CLK1**	3.4	1.2	>10	0.078	1.2	4.1

[a]IC_{50} values were estimated from dose-response curves and are shown in µM. >10, less than 50% inhibition at 10 µM.

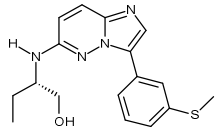
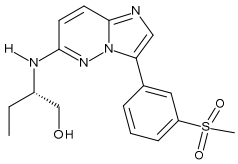
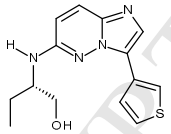
Table 5. Kinase inhibition values IC_{50}[a] in μM) for amino and acetamido derivatives.

			
R	Cl 	Cl  	 

Compd	**6i**	**11i**	**6j**	**11j**	**11k**	**18**	**19**
CDK1	>10	6.1	5.1	3.2	0.071	0.52	0.17
CDK2	41	7.1	61	22	0.41	1.3	0.58
CDK5	29	3.8	71	17	0.19	1.1	0.22
CDK9	7.3	3.8	>10	3.4	0.8	3.5	2.1
CK1	>10	>10	>10	>10	1.8	>10	>10
CLK1	0.38	0.57	1	1.2	0.028	0.038	0.061
CLK2	1.9	1.4	3.9	3	0.028	0.18	0.03
CLK3	19	6.1	43	2.8	0.28	8.2	1.8
CLK4	0.12	0.44	0.54	1.3	0.027	0.04	0.065
DYRK1A	9.4	2.5	14	11	0.039	0.19	0.063
DYRK1B	9.1	1.8	28	13	0.038	0.13	0.032
DYRK2	7	1.9	19	21	0.043	0.12	0.04
DYRK3	15	5.1	41	17	0.11	0.93	0.52
GSK-3	61	41	69	65	5.2	>10	5.2
***Ld*DYRK1B**	8.9	2.8	>10	>10	0.11	2.2	0.69
***Ld*DYRK3**	>10	>10	>10	>10	1.1	>10	7
***Ld*DYRK4**	>10	>10	>10	>10	>10	>10	>10
***Tb*CLK1**	>10	>10	>10	>10	>10	>10	>10
***Tc*CLK1**	>10	>10	>10	>10	>10	>10	3.9
***Cp*CLK1**	>10	>10	>10	>10	3	>10	8.2
***Gl*CLK**	>10	>10	>10	>10	1.1	>10	4.4
***Tg*CLK**	>10	>10	>10	>10	>10	>10	6.1
***Lm*DYRK2**	>10	>10	>10	>10	3.3	3	3.2
***Lm*CLK**	>10	>10	>10	>10	>10	>10	>10
***Pf*CLK1**	>10	>10	>10	>10	3.2	3.1	2.1

[a] IC_{50} values were estimated from dose-response curves and are shown in μM. >10, less than 50% inhibition at 10 μM.

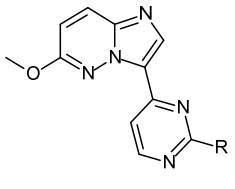
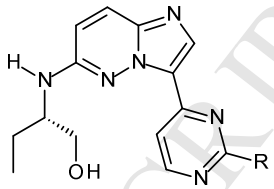
Table 6. Kinase inhibition values IC_{50}[a] in µM for compound **11m**, **11n** and **11o**.

			
Compd	**11l**	**11m**	**11n**
CDK1	0.51	0.27	0.8
CDK2	1.1	0.39	2.2
CDK5	0.82	0.89	0.59
CDK9	0.32	1.1	0.6
CK1	0.61	>10	0.088
CLK1	0.081	0.041	0.15
CLK2	0.18	0.047	0.52
CLK3	2.5	0.37	4.7
CLK4	0.1	0.048	0.042
DYRK1A	0.12	0.093	0.47
DYRK1B	0.09	0.14	0.41
DYRK2	0.12	0.082	0.6
DYRK3	1.3	2.1	1.7
GSK-3	>10	>10	>10
***Ld*DYRK1B**	0.42	1.6	0.88
***Ld*DYRK3**	0.42	3.3	>10
***Ld*DYRK4**	>10	>10	>10
***Tb*CLK1**	>10	>10	>10
***Tc*CLK1**	>10	>10	>10
***Cp*CLK1**	>10	4.1	3.3

***Gl*CLK**	>10	2.1	2.8
***Tg*CLK**	>10	3.2	5.1
***Lm*DYRK2**	2.2	2.2	5.2
***Lm*CLK**	>10	>10	>10
***Pf*CLK1**	0.22	0.21	1.4

[a]IC_{50} values were estimated from dose-response curves and are shown in μM. >10, less than 50% inhibition at 10 μM.

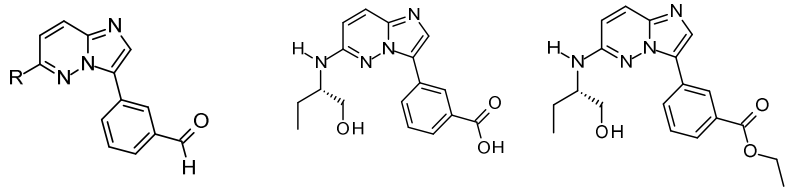
Table 7. Kinase inhibition values IC_{50}[a] in µM for pyrimidine derivatives.

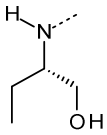
							
R	SMe	SOMe	NH_2	SMe	SOMe	SO_2Me	NH_2
Compd	**21e**	**21f**	**21i**	**11o**	**11p**	**11q**	**11r**
CDK1	>10	>10	7.9	2.8	>10	>10	4.1
CDK2	>10	>10	>10	4	>10	>10	5.1
CDK5	>10	>10	>10	2.2	>10	>10	5.1
CDK9	>10	>10	5.6	>10	>10	>10	2.2
CK1	>10	>10	1.6	>10	>10	>10	1.2
CLK1	1.2	5.5	1.2	0.81	0.91	0.51	0.25
CLK2	1.8	8.2	1.3	0.69	1.6	0.81	1.1
CLK3	>10	>10	>10	>10	>10	6.9	7.8
CLK4	0.38	1.8	0.31	0.31	0.29	0.2	0.13
DYRK1A	0.93	5.1	1.5	1.7	4.4	1.8	3.2
DYRK1B	>10	>10	3.8	1.1	3.5	1.9	2.2
DYRK2	1.2	>10	1.4	>10	>10	10	0.4
DYRK3	7.9	>10	2.3	>10	>10	>10	0.32
GSK-3	>10	>10	>10	>10	>10	>10	4.1
***Ld*DYRK1B**	>10	>10	>10	>10	>10	>10	6
***Ld*DYRK3**	>10	>10	>10	>10	>10	>10	>10
***Ld*DYRK4**	>10	>10	>10	>10	>10	>10	>10

***Tb*CLK1**	>10	>10	>10	>10	>10	>10	>10
***Tc*CLK1**	>10	>10	>10	>10	>10	>10	>10
***Cp*CLK1**	>10	>10	>10	>10	>10	>10	2.3
***Gl*CLK**	>10	>10	>10	>10	>10	>10	>10
***Tg*CLK**	>10	>10	>10	>10	>10	>10	>10
***Lm*DYRK2**	>10	>10	>10	>10	>10	>10	3.7
***Lm*CLK**	>10	>10	>10	>10	>10	>10	>10
***Pf*CLK1**	>10	>10	>10	>10	>10	>10	3.1

[a] IC_{50} values were estimated from dose-response curves and are shown in µM >10, less than 50% inhibition at 10 µM.

Table 8. Kinase inhibition values: IC_{50}[a] in µM for aldehyde, acid and ester derivatives.

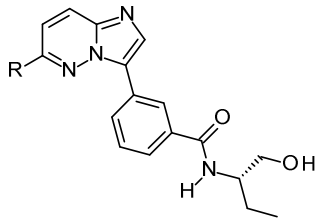
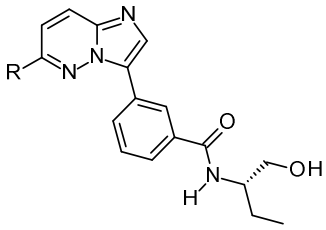
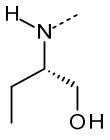
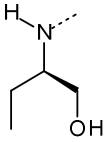
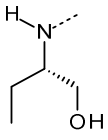
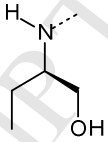


R	Cl	F			

Compd	**6m**	**5b**	**11u**	**11t**	**11s**
CDK1	7.9	>10	0.76	>10	0.36
CDK2	>10	>10	>10	>10	0.68
CDK5	0.82	>10	0.48	>10	1.1
CDK9	7.6	>10	0.58	>10	1.1
CK1	0.61	0.7	1.2	>10	0.82
CLK1	0.31	1.8	0.041	0.69	0.057
CLK2	1.6	3.9	0.22	1.2	0.032
CLK3	>10	>10	2.9	>10	0.82
CLK4	0.19	0.58	0.041	0.32	0.037
DYRK1A	0.65	3.1	0.079	1.6	0.057
DYRK1B	2.1	4.1	0.053	1.2	0.037
DYRK2	0.82	3	0.063	1.6	0.048
DYRK3	1.7	>10	0.46	6.6	0.11
GSK-3	>10	>10	5.9	>10	>10
***Ld*DYRK1B**	7.3	>10	0.43	2.1	0.13
***Ld*DYRK3**	>10	>10	4.8	>10	1.3
***Ld*DYRK4**	>10	>10	>10	>10	>10
***Tb*CLK1**	>10	>10	>10	>10	>10
***Tc*CLK1**	>10	>10	>10	>10	>10
***Cp*CLK1**	>10	>10	1	8.8	0.88
***Gl*CLK**	>10	>10	1.6	>10	0.75
***Tg*CLK**	>10	>10	2.8	1.9	0.98
***Lm*DYRK2**	>10	>10	1.3	5.3	0.52
***Lm*CLK**	>10	>10	>10	>10	>10
***Pf*CLK1**	2.4	>10	0.32	2.1	0.19

[a] IC_{50} values were estimated from dose-response curves and are shown in μM. >10, less than 50% inhibition at 10 μM.

Table 10. Kinase inhibition values: IC_{50}[a] in µM for amide derivatives.

						
R	Cl			Cl		
	6o	**11x**	**20d**	**6p**	**11y**	**20c**
CDK1	6.1	5.4	>10	>10	>10	>10
CDK2	>10	4.7	>10	>10	>10	>10
CDK5	>10	6.1	>10	>10	>10	>10
CDK9	>10	>10	>10	>10	>10	>10
CK1	6.1	>10	>10	>10	>10	>10
CLK1	0.23	0.21	1.2	0.62	0.9	2.1
CLK2	1.4	0.61	2.2	1.9	1.1	1.9
CLK3	>10	4.1	4.1	>10	6.8	6.1
CLK4	0.22	0.17	1.2	0.61	0.68	1.8
DYRK1A	2.1	1.4	2.1	9	7.9	2.2
DYRK1B	1.2	1.2	3.6	7.2	1.8	4.6
DYRK2	3.1	2.5	3.9	8.2	4.5	7.2
DYRK3	1.9	8.1	3.9	10	>10	10
GSK-3	>10	>10	>10	>10	>10	>10
***Ld*DYRK1B**	8	4.1	6.9	6.1	7.9	8.1
***Ld*DYRK3**	8.5	>10	>10	>10	>10	>10
***Ld*DYRK4**	>10	>10	>10	>10	>10	>10
***Tb*CLK1**	>10	>10	>10	>10	>10	>10
***Tc*CLK1**	>10	>10	>10	>10	>10	>10
***Cp*CLK1**	>10	8.7	9.3	>10	>10	>10
***Gl*CLK**	>10	>10	>10	>10	>10	>10
***Tg*CLK**	>10	>10	>10	>10	>10	>10
***Lm*DYRK2**	>10	>10	>10	>10	>10	>10
***Lm*CLK**	>10	>10	>10	>10	>10	>10
***Pf*CLK1**	3.9	4.9	0.59	6.9	4.2	0.81

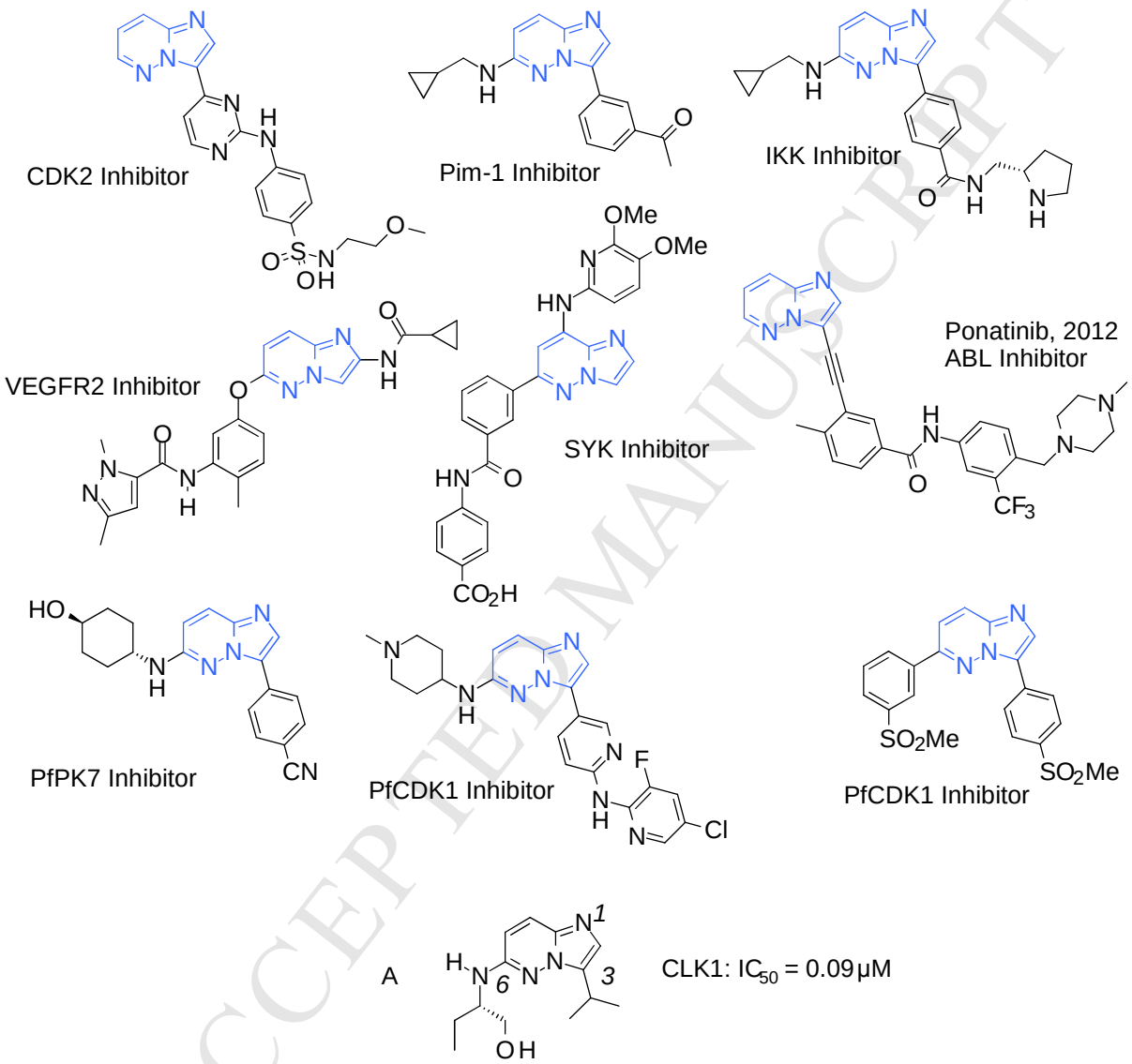
[a] IC_{50} values were estimated from dose-response curves and are shown in µM. >10, less than 50% inhibition at 10 µM.

Table 11. Anti-leishmanial activity on promastigotes. A miniaturized dye reduction assay (resazurin-based assay) was used to estimate the leishmanicidal activity of each compound (PMID:11227767). Three compound concentrations (20, 4 and 0.8 µM) were tested in quadruplicates for 3 days on promastigotes. Following subtraction of the mean background fluorescence value from the medium controls data were expressed as Percent of Inhibition (PI) as compared to untreated parasite culture wells. The mean+/- standard deviation is shown for every tested compound.

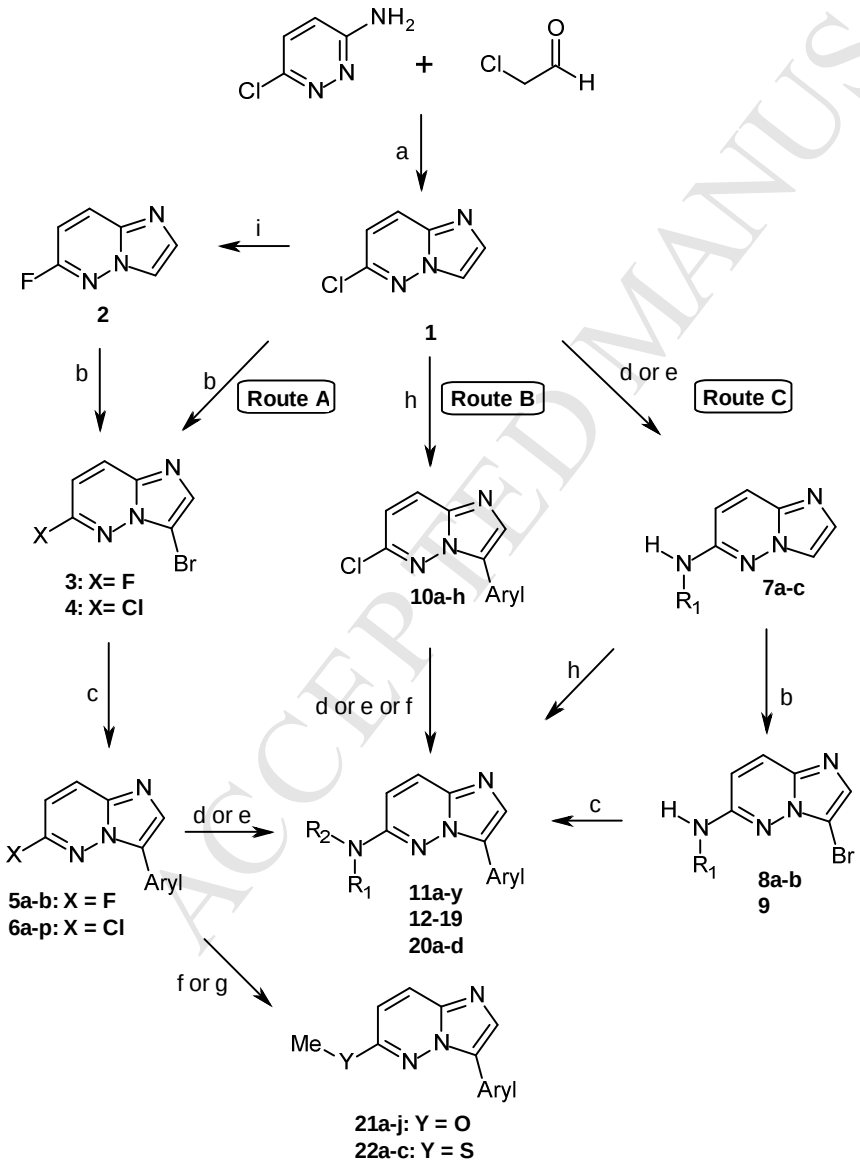
Cmpd	**20 µM**		**4 µM**		**0.8 µM**	
	Mean	StDev	Mean	StDev	Mean	StDev
10a	22,4	2,8	-1,6	2,3	-0,7	2,0
11a	-13,1	7,8	3,1	2,7	-5,9	2,9
11b	74,3	3,1	28,3	2,2	-1,8	2,3
11c	100,0	0,1	-4,7	0,9	5,4	11,2
11d	1,8	2,8	3,7	3,5	-7,0	3,6
11e	59,5	13,6	-4,8	8,0	-3,7	2,8
11f	-16,3	5,8	-12,1	1,1	-3,5	1,5
11g	93,3	0,9	-8,3	5,9	4,6	4,7
11h	-2,5	12,9	-23,5	2,0	-2,3	3,7
11i	12,0	6,4	6,8	2,0	-3,3	1,9
11j	-37,0	3,4	5,0	2,4	-0,8	7,3
11k	2,9	7,3	20,8	1,4	-8,5	1,2
11l	86,5	3,7	-25,6	5,3	-7,6	1,1
11m	-9,6	18,4	18,3	1,3	0,2	9,6
11n	21,5	4,7	35,1	2,6	3,5	1,8
11o	39,6	7,5	24,9	1,5	-2,8	1,6
11p	15,2	5,2	1,4	3,8	-5,5	1,1
11q	11,5	6,6	1,2	0,6	-4,9	1,3
11r	20,8	7,9	34,5	2,1	2,1	2,7
11s	84,7	3,8	-13,1	3,7	-9,5	2,3
11t	13,5	9,1	16,1	3,0	-8,1	2,5
11u	-33,2	4,1	-4,3	4,0	0,0	7,3
11v	12,4	18,1	31,9	5,2	-5,1	1,8
11w	-17,0	23,0	26,6	3,8	-7,7	4,0
11x	9,4	6,5	3,2	3,1	-1,8	2,9
11y	98,8	0,1	-36,2	2,4	-10,7	1,3
12	-23,6	8,8	16,5	4,2	-2,5	2,6
13	3,4	2,9	-0,4	5,2	0,7	1,2
14	16,8	5,3	21,3	2,4	3,1	4,2
15	-11,9	0,9	-1,9	3,9	0,0	4,9
16	40,7	6,9	12,9	5,2	-5,6	5,3
17	99,8	0,1	16,5	16,4	-4,3	5,3
18	1,6	1,7	26,2	3,8	-1,3	8,1
19	17,8	3,5	32,5	5,1	-5,2	5,2
20a	-23,2	4,3	12,3	9,8	-1,3	2,8
20c	25,7	2,8	10,8	3,2	-9,5	5,1

20d	91,9	0,6	19,8	5,2	-12,3	5,3
21a	-11,6	5,5	26,5	1,1	4,7	4,4
21b	-9,0	2,8	12,8	1,9	2,9	5,1
21c	22,4	6,3	3,7	3,2	0,0	2,4
21d	37,2	6,8	16,5	6,1	0,6	4,6
21e	0,7	3,4	34,6	3,1	-5,8	2,2
21f	13,7	5,8	16,7	0,9	-3,2	2,3
21i	4,2	8,7	6,2	8,8	-3,5	2,5
21j	8,6	6,2	9,6	8,0	6,0	4,5
22b	-24,2	4,2	26,9	3,9	2,7	7,8
22c	-15,0	5,7	-2,1	1,5	0,7	4,0
Roscovitine	-20,3	2,5	-3,8	4,3	-8,8	3,9
MRT2-08	19,2	4,3	0,7	6,3	-8,7	1,0

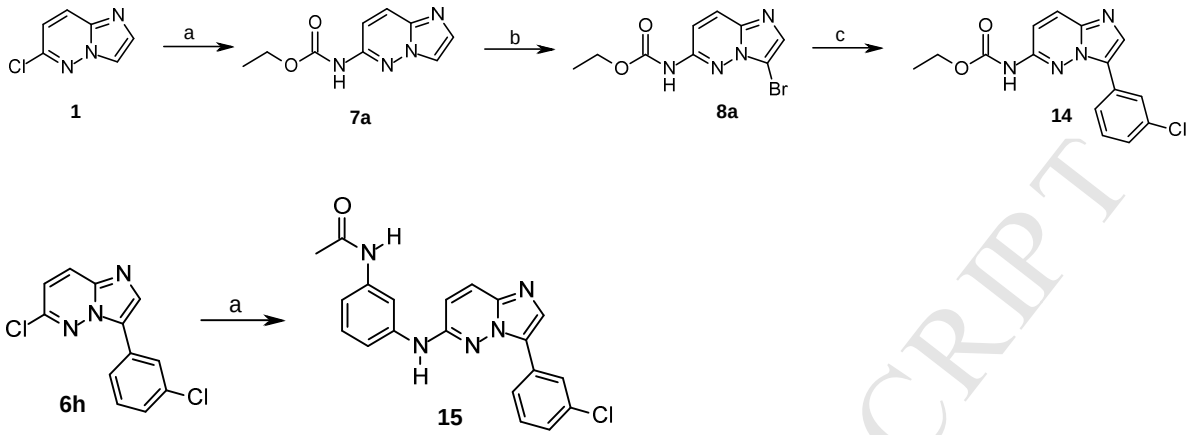
Figure 1. The imidazo[1,2-b]pyridazine scaffold: selected kinase inhibitors and initial hit A.



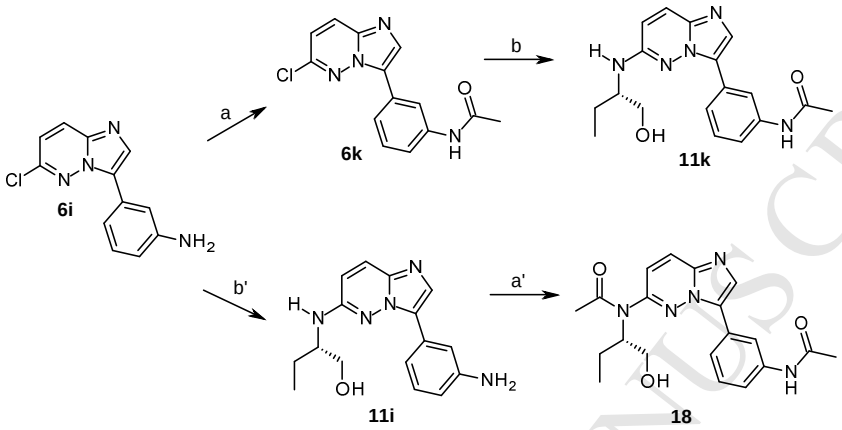
Scheme 1. General synthetic strategy, C3, C6-disubstituted imidazo[1,2-b]pyridazines. Reagents and conditions: (a) i *n*BuOH, 18h, reflux, ii H_2O, NaOH; (b) NBS, $CHCl_3$, 8 h, 20 °C; (c) boronic acid or ester, $Pd[P(C_6H_5)_3]_4$, Na_2CO_3 (2 M), 1,4-dioxane, 100 °C; (d) 2-amino-1-butanol, 160-180 °C; (e) $Pd(OAc)_2$, xantphos, *t*-BuOK, H_2O, 1,4-dioxane, 100 °C, 48 h; (f) NaOMe, MeOH, 48 h, reflux; (g) NaSMe, DMSO, r.t or 40 °C, 2 h; (h) aryl/heteroaryl halide, $Pd(OAc)_2$, $P(C_6H_5)_3$, K_2CO_3, toluene, 110 °C; (i) KF, DMSO/toluene, 170 °C, 6 h.



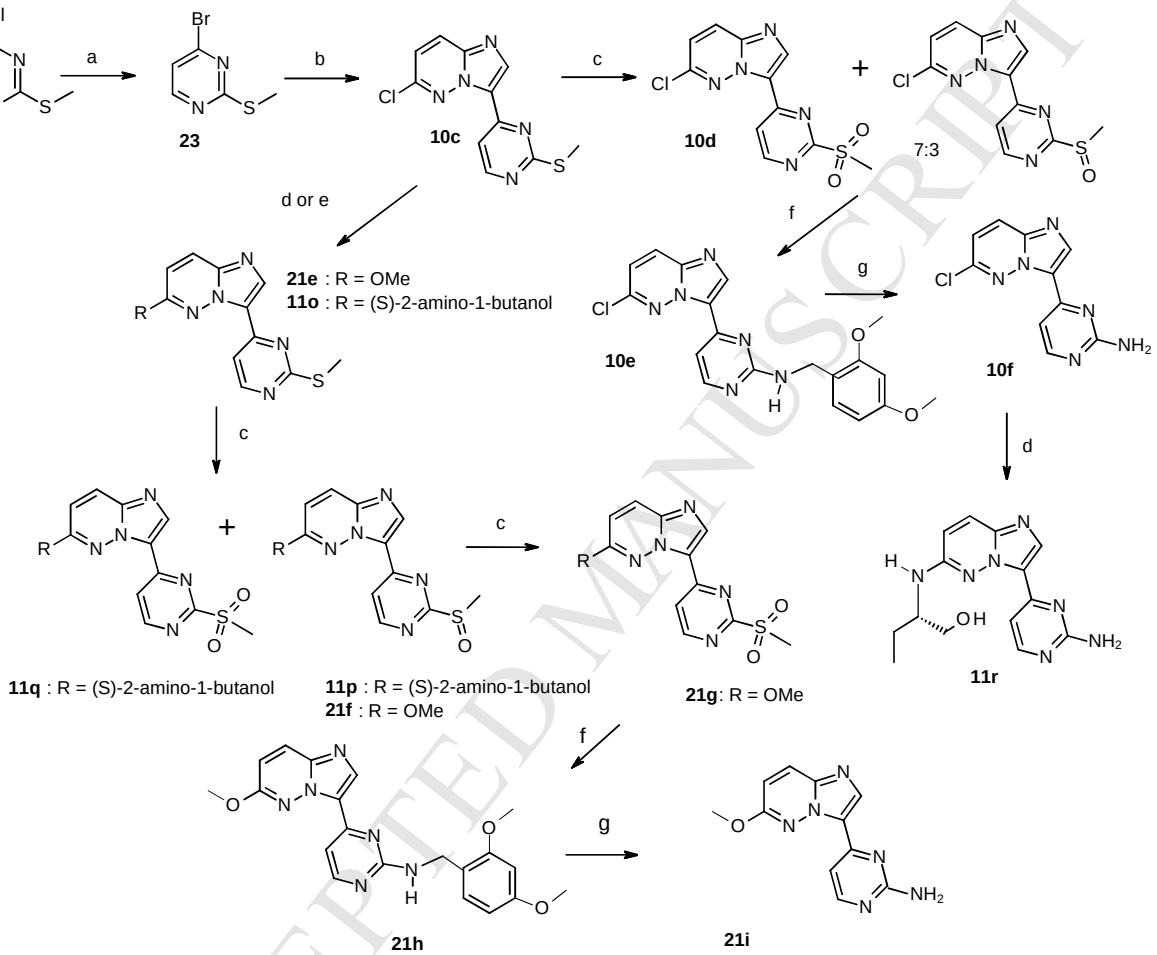
Scheme 2. Reagents and conditions: (a) Ethyloxycarbamate or 3-aminoacetanilide, $Pd(OAc)_2$, xantphos, *t*-BuOK, H_2O, 1,4-dioxane, 100 °C, 24 h, 27%; (b) NBS, $CHCl_3$, 20 °C, 4 h, 100%; (c) 3-Chlorophenylboronic pinacol ester, $Pd[P(Ph)_3]_4$, Na_2CO_3 (2 M), 1,4-dioxane, 100 °C.



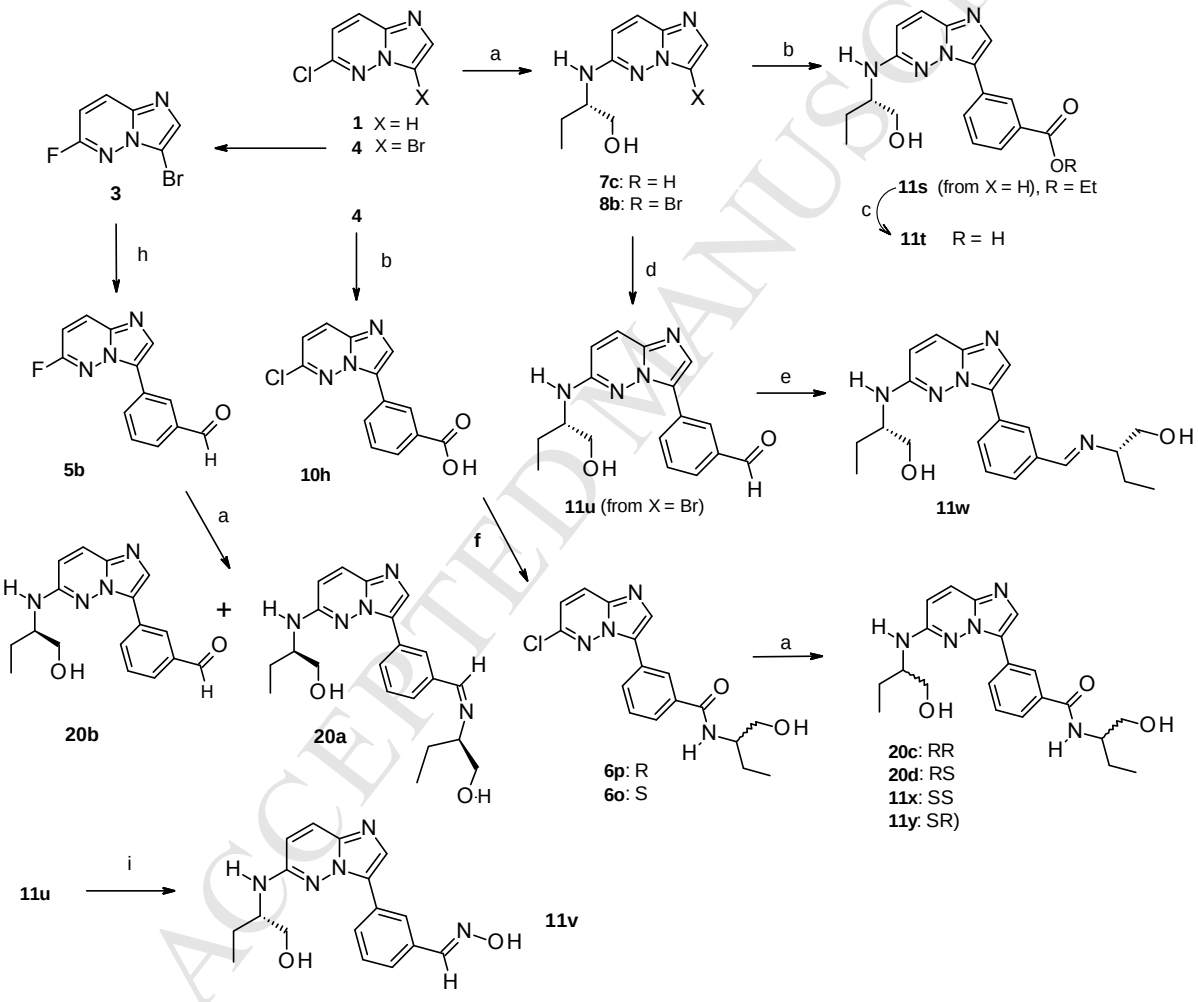
Scheme 3. Reagents and conditions: (a) Acetic anhydride, DMAP, Et_3N, DCM, 20 °C, 2 h; (b) (*S*)-2-Amino-1-butanol, 160 °C, 7 h; (b') (*S*)-2-Amino-1-butanol,180 °C, 7 h; (a') Acetic anhydride, DMAP, Et_3N, DCM, r.t, 2 h.



Scheme 4. Reagents and conditions**:** (a) TMSBr, CH_3CN, 40 °C, 27 h; (b) 6-Chloro-imidazo[1,2-b]pyridazine, $Pd(OAc)_2$, PPh_3, K_2CO_3, toluene, 2 days, 110 °C, 38%; (c) *m*CPBA, DCM, r.t; (d) MeONa, MeOH, 6 h, 80 °C; (e) (*S*)-2-Amino-1-butanol, 170 °C, 4 h. (f) 2,4-Dimethoxybenzylamine, 1,4-dioxane, 8 h, reflux; (g) TFA, DCM, 20 °C.



Scheme 5. Reagents and conditions : (a) (*R*) or (*S*)-2-Amino-1-butanol, 8 h, 180 °C; (c) Ethyl 3-bromobenzoate, $Pd(OAc)_2$, $P(C_6H_5)_3$, K_2CO_3, toluene, 8 h, 110 °C, 10 %; (c) i: NaOH (1N), THF/EtOH, ii: HCl (1N), H_2O, 28%; (d) 3-Formylphenylboronic acid, $Pd[P(C_6H_5)_3]_4$, Na_2CO_3 (2 M), 1,4-dioxane, 12 h, 100 °C. (e) (*S*)-2-Amino-1-butanol 2.5 Eq., 3 days 20 to 60 °C. (f) (*S* or *R*)-2-Amino-1-butanol, DCC/HOBt, Et_3N, THF/DCM, 20 °C, 24 h. (g) (*S* or *R*)-2-Amino-1-butanol, DCC/HOBt, Et_3N, THF/DCM, 20 °C, 24 h; (b) (*S* or *R*)-2-Amino-1-butanol, 180 °C, 12 h; (h) KF, DMSO/Toluene, 8 h, 180 °C; (i) $NH_2OH.HCl$, NaOAc 1.1 Eq, MeOH-H_2O 10%, 20 °C, 16 h.



Highlights

- A large variety of imidazo[1,2-b]pyridazines were prepared.
- The prepared compounds were assayed against a panel of mammalian and protozoan kinases.
- Several molecules were found to be selective DYRK and CLK inhibitors.
- Despite their potencies against the kinases studied, the compounds were not cytotoxic.