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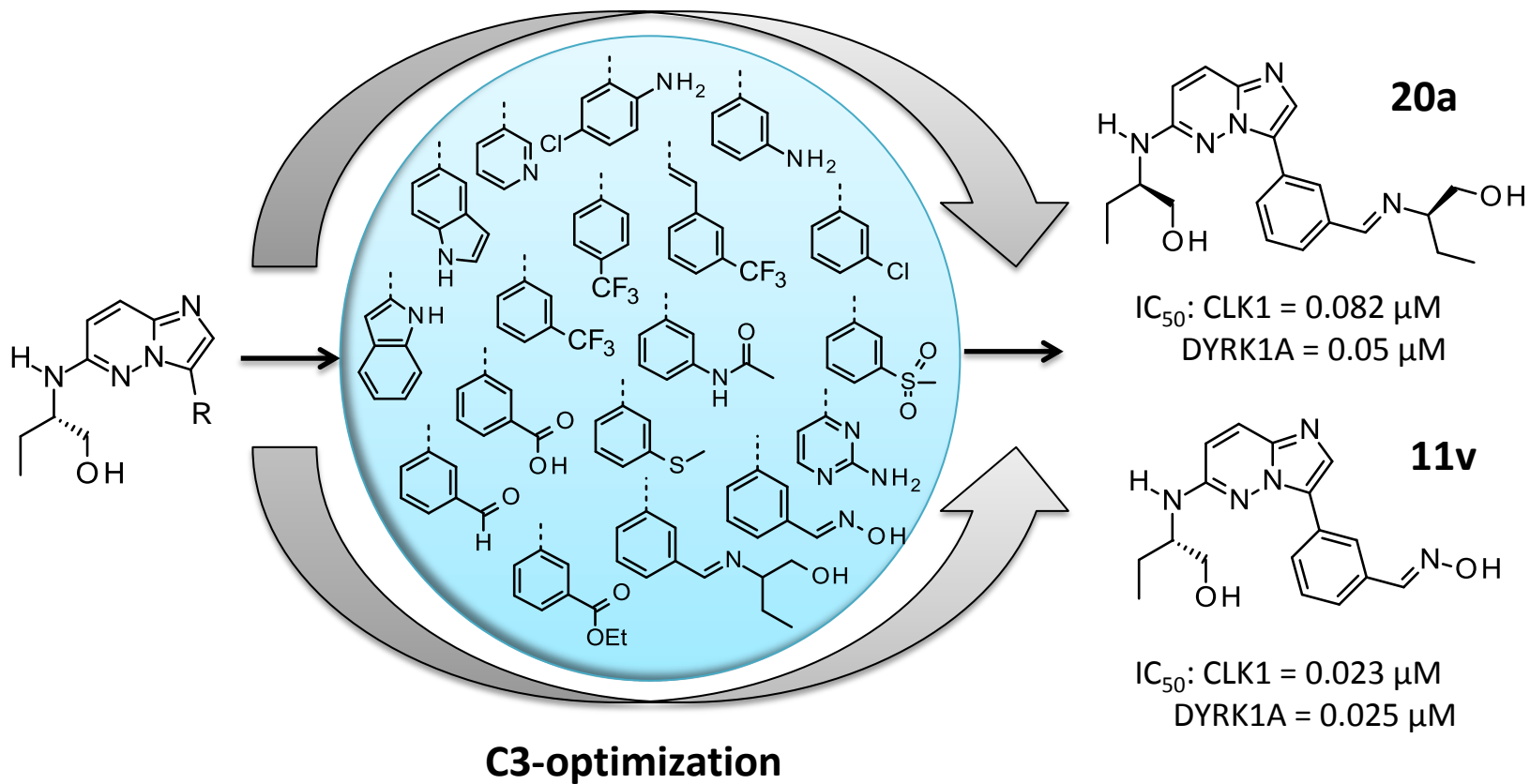
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Abstract: Cdc2-like kinases (CLKs) and dual specificity, tyrosine phosphorylation-regulated kinases (DYRKs) are involved in neurodegenerative diseases, such as Alzheimer's disease and Down syndrome. 3,6-disubstituted imidazo[1,2-b]pyridazine derivatives were synthesized to identify new inhibitors of various eukaryotic kinases, including mammalian and protozoan kinases. Among the imidazo[1,2-b]pyridazines tested for anti-kinase activity, several molecules were selective for DYRKs and CLKs, with IC₅₀ <100 nM. Some compounds showed potent inhibition of Plasmodium falciparum CLK1 (PfCLK1). Compound 20a was found to be the most selective product against CLK1 (IC₅₀ = 82 nM), CLK4 (IC₅₀ = 44 nM), DYRK1A (IC₅₀ = 50 nM), and PfCLK1 (IC₅₀ = 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.

C3-optimization



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.

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

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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

Cdc2-like kinases (CLKs) and dual specificity, tyrosine phosphorylation-regulated kinases (DYRKs) are involved in neurodegenerative diseases, such as Alzheimer's disease and Down syndrome. 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 for anti-kinase activity, several molecules were selective for DYRKs and CLKs, with $IC_{50} < 100$ nM. Some compounds 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 μ M) concentration, but were not toxic at 1 μ M or 10 μ M, 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 or 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], IKK β [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 been launched on the market as a BCR-ABL inhibitor in the treatment of chronic myelocytic leukemia (CML). The MRC 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 (A β) 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 kinase, 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 cyclization 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 to 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]pyridazines **6e** were prepared using both route A and route B. A 66% yield was obtained by the Suzuki coupling, compared to 38% with the CH-arylation reaction. Several protocols have been tested, including nucleophilic substitution for the introduction of the ethyl carbamate. Product **14** was synthesized using route C, the ethyl carbamate being introduced on **1** by a Buchwald reaction. Bromination of **7a** yielded **8a**, which 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). Among the diverse aryl groups introduced on position 3, compound **11k** resulted from the acylation of the aromatic amine **11i**.

(Schemes 2, 3, and 4)

Several pyrimidines (**11p**, **11q**, **21f** and **21g**) were obtained through CH-arylation of 6-chloroimidazo[1,2-b]pyridazine **1** (Scheme 5). The sulfones **10d** and **21g** were converted into the amines **10f**, **11r**, and **21i** (Scheme 6).

(Schemes 5 and 6)

3-formylphenyl)boronic acid was linked with 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 8 gave access to acid and imine derivatives in three steps, starting from 6-chloro-3-bromoimidazo[1,2-b]pyridazine. Several oximes were synthesized from an aldehyde in the presence of hydroxylamine and sodium acetate in methanol. We used the same procedure (Scheme 8) 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 9).

Schemes 7, 8, and 9

2.2. Evaluation against kinases.

Our studies originated from testing the biological activity of product **A** (Figure 2). 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 product in the evaluation of nov DYRK/CLK inhibitors. In the context of this article, IC₅₀ values obtained with mammalian kinases were in accordance with those reported in a previous paper [24]. IC₅₀ values of leucettine L41 on unicellular parasite DYRK/CLK homologs are shown in table 13, which is added in Supplementary Material.

The indole series revealed a slight inhibition preference for CLKs and DYRKs (Table 1). Two products displaying an IC₅₀ 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 for compound **11c** decreased the activity (Table 1). These results encouraged us to continue optimization by introducing an arylhalide and a trifluoromethylphenyl group. Comparing the various C-6 substituents, (*S*)-2-amino-1-butanol **11e** (IC₅₀ 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-3 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 (IC₅₀ values of 86 nM and 84 nM, respectively). Compound **15** also inhibited *Pf*CLK1 (IC₅₀ = 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 IC₅₀ 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)

Compound **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.

Compound **20a** selectively inhibited CLK1, CLK4, and DYRK1A with a low IC₅₀. Among all the compounds tested, **20a** seems to be the most potent product; 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 products are intended to be used in the treatment of neurodegenerative diseases, it is important that they should 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 1 μ M and 10 μ M.

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, compounds were assayed against *L. amazonensis*. Several compounds were able to reduce parasite proliferation, but at the rather high concentration of 10 μ M (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 was 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 products 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 on 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] is to 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 MgCl₂, 1 mM EGTA (MW 380.4), 1 mM dithiothreitol (DTT) (MW 154.2), 25 mM Tris/HCl (MW 121.1) and 50 µg/mL heparin.

Buffer C: 60 mM β-glycerophosphate, 30 mM *p*-nitrophenylphosphate, 25 mM MOPS, 5 mM EGTA, 15 mM MgCl₂, 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) (pS 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 MgCl₂ (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 mM β-glycerophosphate, 15 mM p-nitrophenylphosphate, 15 mM EGTA, 15 mM MgCl₂, 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% CO₂. 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)-2H-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) that 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. ¹H NMR, ¹³C NMR, IR, and MS spectra.

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List of tables figure and schemes:

Table 1. Kinase inhibition values (IC_{50} in μM) for indole derivatives. IC_{50} values were estimated from dose-response curves, and are shown in μM . >10 , less than 50% inhibition at 10 μM .

Table 2. Kinase inhibition values (IC_{50} in μM) for pyridine derivatives. 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} in μM) for halogens derivatives. IC_{50} values were estimated from dose-response curves, and are shown in μM . >10 , less than 50% inhibition at 10 μM .

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Table 5. Kinase inhibition values (IC_{50} in μM) for amino and acetamido derivatives. IC_{50} values were estimated from dose-response curves, and are shown in μM . >10, less than 50% inhibition at 10 μM .

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Table 7. Kinase inhibition values (IC_{50} in μM) for pyrimidine derivatives. IC_{50} values were estimated from dose-response curves and are shown in μM >10, less than 50% inhibition at 10 μM .

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Table 9. Kinase inhibition values (IC_{50} in μM) for imine and oxime derivatives.

Table 10. Kinase inhibition values (IC_{50} in μM) for amide derivatives. 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. Three compound concentrations (20, 4, and 0.8 μM) were tested in quadruplicate for three 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 \pm standard deviation is shown for every tested compound. PI above 30% and 75% are highlighted in bold and underlined, respectively.

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

Scheme 1. General synthetic strategy, C3, C6-disubstituted imidazo[1,2-b]pyridazines. Reagent and conditions: (a) i *n*BuOH, 18h, reflux, ii H₂O, NaOH; (b) NBS, CHCl₃, 8 h, 20 °C; (c) boronic acid or ester, Pd[P(C₆H₅)₃]₄, Na₂CO₃ (2 M), 1,4-dioxane, 100 °C; (d) 2-amino-1-butanol, 160-180 °C; (e) Pd(OAc)₂, xantphos, tBuOK, H₂O, 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(C₆H₅)₃, K₂CO₃, toluene, 110 °C; (i) KF, DMSO/toluene, 170 °C, 6 h, 30%.

Scheme 2. Procedure for the synthesis of compound **14**. Reagent and conditions: (a) Pd(OAc)₂, xantphos, *t*-BuOK, H₂O, 1,4-dioxane, 100 °C, 24 h, 27%; (b) NBS, CHCl₃, 20 °C, 4 h, 100%; (c) 3-chlorophenylboronic pinacol ester, Pd[P(Ph)₃]₄, Na₂CO₃ (2 M), 1,4-dioxane, 100 °C, 6 h, 5 %.

Scheme 3. Procedure for the synthesis of compound **15**. Reagents and conditions: (a) Pd(OAc)₂, xantphos, *t*-BuOK, H₂O, 1,4-dioxane, 100 °C, 24 h, 30%.

Scheme 4. Procedure for the synthesis of acetamides **111** and **18**. Reagents and conditions: (a) acetic anhydride, DMAP, Et₃N, DCM, 20 °C, 2 h, 79%; (b) (S)-2-amino-1-butanol, 160 °C, 7 h, 7%; (b') (S)-2-amino-1-butanol, 180 °C, 7 h, 26%; (a') acetic anhydride, DMAP, Et₃N, DCM, r.t., 2 h, 57%.

Scheme 5. Procedure for the synthesis of pyrimidine derivatives. Reagents and conditions: (a) TMSBr, CH₃CN, 40 °C, 27 h, 98%; (b) 6-chloro-imidazo[1,2-b]pyridazine, Pd(OAc)₂, PPh₃, K₂CO₃, toluene, 2 days, 110 °C, 38%; (c) mCPBA, DCM, r.t.; (d) MeONa, MeOH, 6 h, 80 °C; (e) (S)-2-amino-1-butanol, 170 °C, 4 h.

Scheme 6. Procedure for the synthesis of pyrimidine derivatives. Reagents and conditions: (g) 1,4-dioxane, 8 h, reflux; (h) TFA, DCM, 20 °C; (i) (S)-2-amino-1-butanol, 6 h, 160 °C.

Scheme 7. Procedure for the synthesis of imines. Reagents and conditions: (a) KF, DMSO/toluene, 8 h, 180°C, 46%; (b) NBS, CHCl₃, 6 h, r.t., 93%; (c) 3-formylphenylboronic acid, Pd[P(C₆H₅)₃]₄, Na₂CO₃ (2 M), 1,4-dioxane, 100 °C, 6 h, 63%; (d) (R)-2-amino-1-butanol, 135 °C, 2 h.

Scheme 8. Procedure for the synthesis of acid and ethyl ester derivative. Reagents and conditions : (a) (S)-2-amino-1-butanol, 8 h, 180 °C, 69%; (c) ethyl 3-bromobenzoate, Pd(OAc)₂, P(C₆H₅)₃, K₂CO₃, toluene, 8h, 110°C, 10 %; (c) i: NaOH (1N), THF/EtOH, ii: HCl (1N), H₂O, 28%; (d) 3-formylphenylboronic acid, Pd[P(C₆H₅)₃]₄, Na₂CO₃ (2 M), 1,4-dioxane, 100 °C, 12 h. (e) (S)-2-amino-1-butanol, 3 days, 20 to 60 °C.

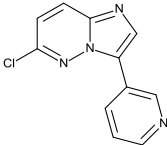
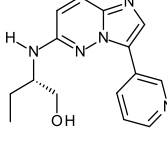
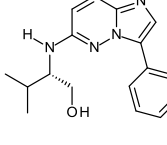
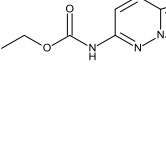
Scheme 9: Procedure of the synthesis of amide. Reagents and conditions: (a) (S or R)-2-amino-1-butanol, DCC/HOBt, Et₃N, THF/DCM, 20 °C, 24h; (b) (S or R)-2-amino-1-butanol, 180 °C, 12 h.

Table 1. Kinase inhibition values : IC₅₀^a in μM for indole derivatives

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
LdDYRK1B	1.8	1.2	3.1	0.098	0.15	0.13
LdDYRK3	>10	9.2	>10	>10	10	10
LdDYRK4	>10	>10	>10	>10	10	10
TbCLK1	>10	>10	>10	>10	10	10
TcCLK1	>10	>10	>10	>10	10	10
CpCLK1	1.2	2.7	4.2	3.1	10	10
GiCLK	5.9	5.2	8.1	1.1	2.1	2.9
TgCLK	2.8	4.1	4.1	4.1	10	10
LmDYRK2	4	5	>10	3.9	4.1	10
LmCLK	>10	>10	>10	10	10	10
PfCLK1	1.6	1.3	0.9	2.2	2.2	10

^a IC₅₀ values were estimated from dose-response curves and are shown in μM. >10, less than 50% inhibition at 10 μM.

Table 2. Kinase inhibition values: IC₅₀^a in μM for pyridine derivatives.

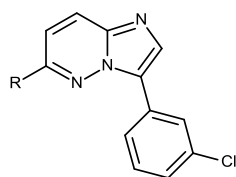
				
	6e	11d	12	13
Compd				
CDK1	>10	3	2.3	>10
CDK2	71	3.4	3.2	>10
CDK5	4.7	1.7	1.4	>10
CDK9	>10	2.1	1.2	>10
CK1	4.1	5.1	1.2	>10
CLK1	0.89	0.19	0.18	>10
CLK2	4.1	0.71	0.59	>10
CLK3	42	3.9	3.3	>10
CLK4	0.4	0.059	0.09	>10
DYRK1A	13	0.87	0.39	>10
DYRK1B	20	0.51	0.13	>10
DYRK2	11	0.6	0.19	>10
DYRK3	16	0.95	0.32	>10
GSK-3	51	3	5.1	>10
LdDYRK1B	>10	0.69	0.39	>10
LdDYRK3	>10	>10	>10	>10
LdDYRK4	>10	>10	>10	>10
TbCLK1	>10	>10	>10	>10
TcCLK1	>10	>10	>10	>10
CpCLK1	>10	2.2	3.8	>10

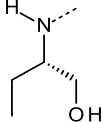
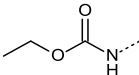
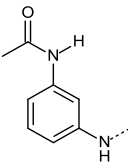
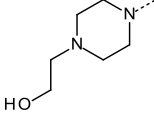
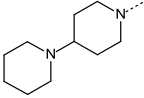
<i>GlCLK</i>	>10	0.71	4.2	>10
<i>TgCLK</i>	>10	4.9	5.2	>10
<i>LmDYRK2</i>	>10	5.7	6.5	>10
<i>LmCLK</i>	>10	>10	>10	>10
<i>PfCLK1</i>	>10	3	1.3	>10

^a IC₅₀ values were estimated from dose-response curves and are shown in μM >10, less than 50% inhibition at 10 μM .

<i>TcCLK1</i>	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
<i>CpCLK1</i>	>10	>10	>10	3	2.3	>10	>10	>10	>10	>10	>10
<i>GlCLK</i>	>10	>10	>10	>10	>10	>10	>10	3.9	>10	>10	>10
<i>TgCLK</i>	>10	>10	>10	2	>10	>10	>10	>10	>10	>10	>10
<i>LmDYRK2</i>	>10	>10	>10	7.1	>10	>10	7.8	>10	>10	>10	>10
<i>LmCLK</i>	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
<i>PfCLK1</i>	8	>10	>10	2.9	8.6	4.5	4.5	3.9	7	>10	>10

^a IC₅₀ values were estimated from dose-response curves and are shown in μM . >10, less than 50% inhibition at 10 μM .

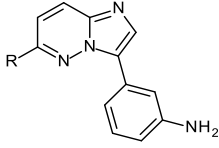
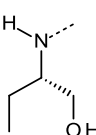
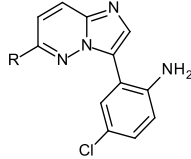
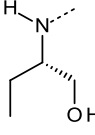
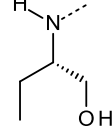
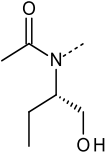
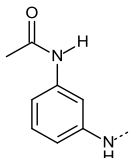
Table 4. Kinase inhibition values: IC₅₀^a in μM for chlorine derivatives.

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
<i>Ld</i> DYRK1B	6.2	0.19	>10	0.59	1.8	>10
<i>Ld</i> DYRK3	>10	>10	>10	>10	>10	>10
<i>Ld</i> DYRK4	>10	>10	>10	>10	>10	>10
<i>Tb</i> CLK1	>10	>10	>10	>10	>10	>10
<i>Tc</i> CLK1	>10	>10	>10	>10	>10	>10
<i>Cp</i> CLK1	5.1	6.1	>10	>10	>10	>10

<i>GI</i>CLK	>10	>10	>10	>10	>10	>10
<i>Tg</i>CLK	7.1	>10	>10	>10	>10	>10
<i>Lm</i>DYRK2	8.3	>10	>10	0.5	8.9	>10
<i>Lm</i>CLK	>10	>10	>10	>10	>10	>10
<i>Pf</i>CLK1	3.4	1.2	>10	0.078	1.2	4.1

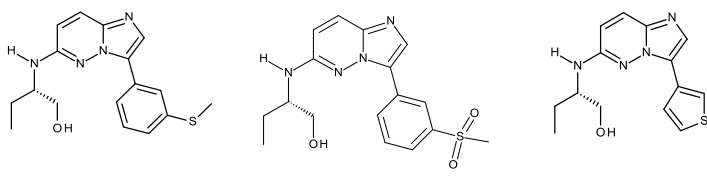
^aIC₅₀ values were estimated from dose-response curves and are shown in μM. >10, less than 50% inhibition at 10 μM.

Table 5. Kinase inhibition values IC₅₀^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
LdDYRK1B	8.9	2.8	>10	>10	0.11	2.2	0.69
LdDYRK3	>10	>10	>10	>10	1.1	>10	7
LdDYRK4	>10	>10	>10	>10	>10	>10	>10
TbCLK1	>10	>10	>10	>10	>10	>10	>10
TcCLK1	>10	>10	>10	>10	>10	>10	3.9
CpCLK1	>10	>10	>10	>10	3	>10	8.2
GlCLK	>10	>10	>10	>10	1.1	>10	4.4
TgCLK	>10	>10	>10	>10	>10	>10	6.1
LmDYRK2	>10	>10	>10	>10	3.3	3	3.2
LmCLK	>10	>10	>10	>10	>10	>10	>10
PfCLK1	>10	>10	>10	>10	3.2	3.1	2.1

^a IC₅₀ 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
LdDYRK1B	0.42	1.6	0.88
LdDYRK3	0.42	3.3	>10
LdDYRK4	>10	>10	>10
TbCLK1	>10	>10	>10
TcCLK1	>10	>10	>10
CpCLK1	>10	4.1	3.3

<i>GlCLK</i>	>10	2.1	2.8
<i>TgCLK</i>	>10	3.2	5.1
<i>LmDYRK2</i>	2.2	2.2	5.2
<i>LmCLK</i>	>10	>10	>10
<i>PfCLK1</i>	0.22	0.21	1.4

^aIC₅₀ values were estimated from dose-response curves and are shown in μM . >10, less than 50% inhibition at 10 μM .

<i>Tb</i>CLK1	>10	>10	>10	>10	>10	>10	>10
<i>Tc</i>CLK1	>10	>10	>10	>10	>10	>10	>10
<i>Cp</i>CLK1	>10	>10	>10	>10	>10	>10	2.3
<i>Gl</i>CLK	>10	>10	>10	>10	>10	>10	>10
<i>Tg</i>CLK	>10	>10	>10	>10	>10	>10	>10
<i>Lm</i>DYRK2	>10	>10	>10	>10	>10	>10	3.7
<i>Lm</i>CLK	>10	>10	>10	>10	>10	>10	>10
<i>Pf</i>CLK1	>10	>10	>10	>10	>10	>10	3.1

^a IC₅₀ 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: IC₅₀^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
<i>Ld</i> DYRK1B	7.3	>10	0.43	2.1	0.13
<i>Ld</i> DYRK3	>10	>10	4.8	>10	1.3
<i>Ld</i> DYRK4	>10	>10	>10	>10	>10
<i>Tb</i> CLK1	>10	>10	>10	>10	>10

<i>Tc</i>CLK1	>10	>10	>10	>10	>10
<i>Cp</i>CLK1	>10	>10	1	8.8	0.88
<i>Gl</i>CLK	>10	>10	1.6	>10	0.75
<i>Tg</i>CLK	>10	>10	2.8	1.9	0.98
<i>Lm</i>DYRK2	>10	>10	1.3	5.3	0.52
<i>Lm</i>CLK	>10	>10	>10	>10	>10
<i>Pf</i>CLK1	2.4	>10	0.32	2.1	0.19

^a IC₅₀ 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: ^aIC₅₀ 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
<i>Ld</i> DYRK1B	0.48	1	4.1	0.98	0.41
<i>Ld</i> DYRK3	3.1	3.8	>10	6	3.3
<i>Ld</i> DYRK4	>10	>10	>10	>10	>10
<i>Tb</i> CLK1	>10	>10	>10	>10	>10
<i>Tc</i> CLK1	>10	>10	>10	>10	>10
<i>Cp</i> CLK1	1.8	0.46	2.2	1.5	1.2
<i>Gl</i> CLK	2.3	3	2.4	2.9	2

<i>TgCLK</i>	1.4	1.7	>10	6.3	0.73
<i>LmDYRK2</i>	1.7	0.81	7.8	4.2	0.98
<i>LmCLK</i>	>10	>10	>10	>10	>10
<i>PfCLK1</i>	0.37	0.032	0.83	1.1	0.31

^a IC₅₀ values were estimated from dose-response curves and are shown in μM . >10, less than 50% inhibition at 10 μM .

<i>CpCLK1</i>	>10	8.7	9.3	>10	>10	>10
<i>GlCLK</i>	>10	>10	>10	>10	>10	>10
<i>TgCLK</i>	>10	>10	>10	>10	>10	>10
<i>LmDYRK2</i>	>10	>10	>10	>10	>10	>10
<i>LmCLK</i>	>10	>10	>10	>10	>10	>10
<i>PfCLK1</i>	3.9	4.9	0.59	6.9	4.2	0.81

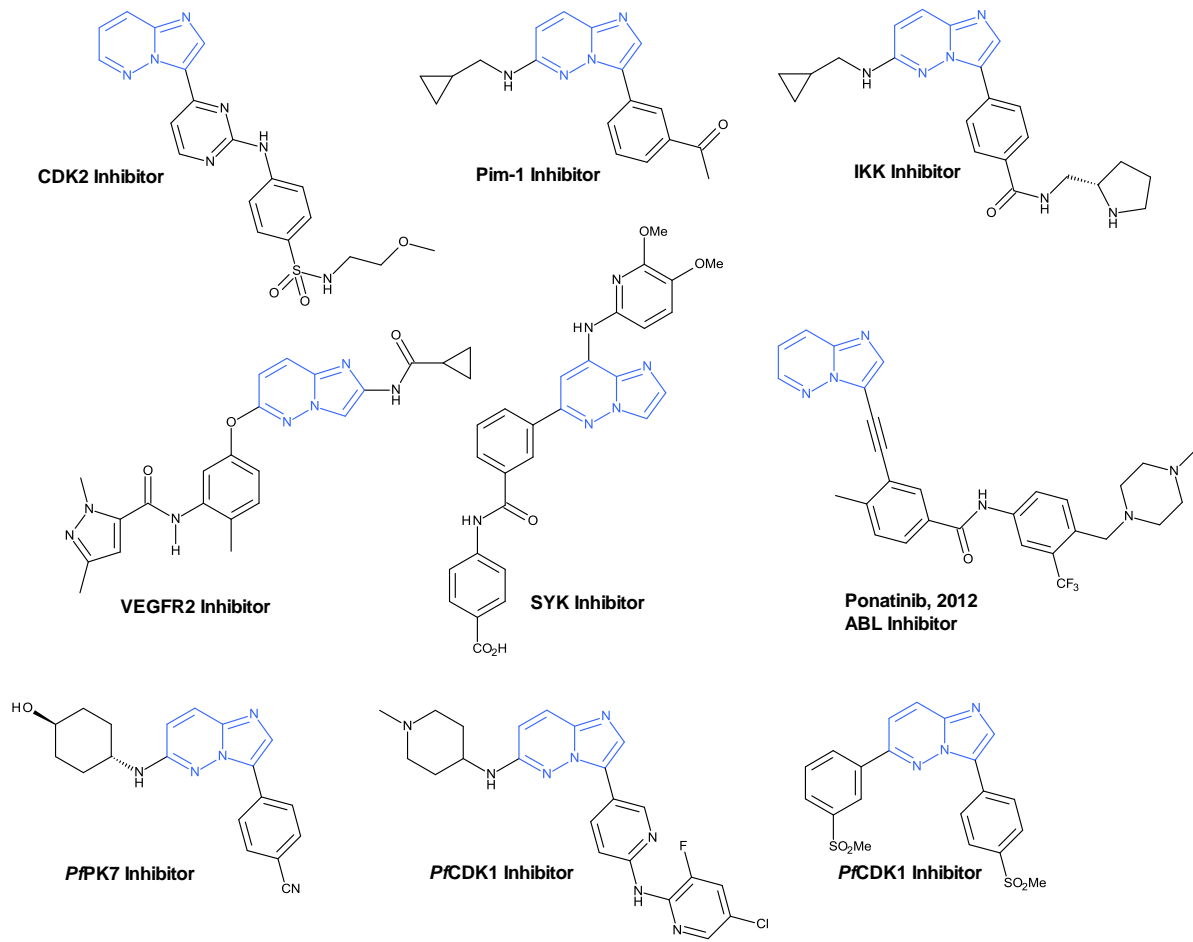
^a IC₅₀ values were estimated from dose-response curves and are shown in μM . >10, less than 50% inhibition at 10 μM .

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 \pm standard deviation is shown for every tested compound.

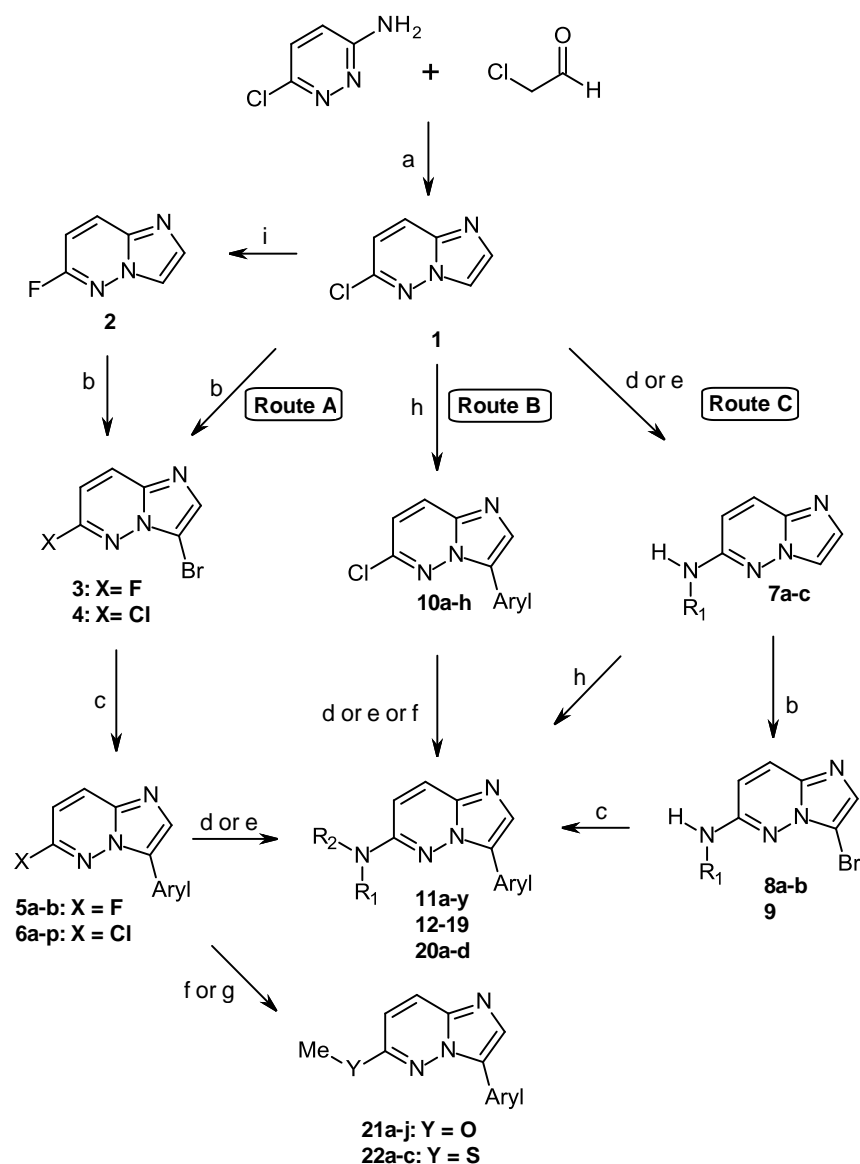
Cmpd	20 μM		4 μM		0.8 μM	
	Mean	StDev	Mean	StDev	Mean	StDev
10a	22,4	2,8	-1,6	2,3	-0,7	2,0
11a	-13,1	7,8	3,1	2,7	-5,9	2,9
11b	74,3	3,1	28,3	2,2	-1,8	2,3
11c	100,0	0,1	-4,7	0,9	5,4	11,2
11d	1,8	2,8	3,7	3,5	-7,0	3,6
11e	59,5	13,6	-4,8	8,0	-3,7	2,8
11f	-16,3	5,8	-12,1	1,1	-3,5	1,5
11g	93,3	0,9	-8,3	5,9	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

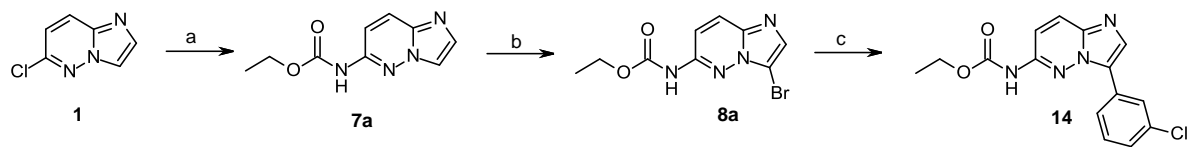
Figure 1. The imidazo[1,2-b]pyridazine scaffold: selected kinase inhibitors.



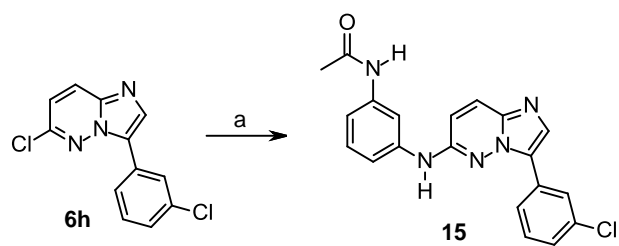
Scheme 1. General synthetic strategy, C3, C6-disubstituted imidazo[1,2-b]pyridazines. Reagent and conditions: (a) i *n*BuOH, 18h, reflux, ii H₂O, NaOH; (b) NBS, CHCl₃, 8 h, 20 °C; (c) boronic acid or ester, Pd[P(C₆H₅)₃]₄, Na₂CO₃ (2 M), 1,4-dioxane, 100 °C; (d) 2-amino-1-butanol, 160-180 °C; (e) Pd(OAc)₂, xantphos, *t*-BuOK, H₂O, 1,4-dioxane, 100 °C, 48h; (f) NaOMe, MeOH, 48 h, reflux; (g) NaSMc, DMSO, r.t or 40 °C, 2 h ; (h) aryl/heteroaryl halide, Pd(OAc)₂, P(C₆H₅)₃, K₂CO₃, toluene, 110 °C; (i) KF, DMSO/toluene, 170 °C, 6 h.



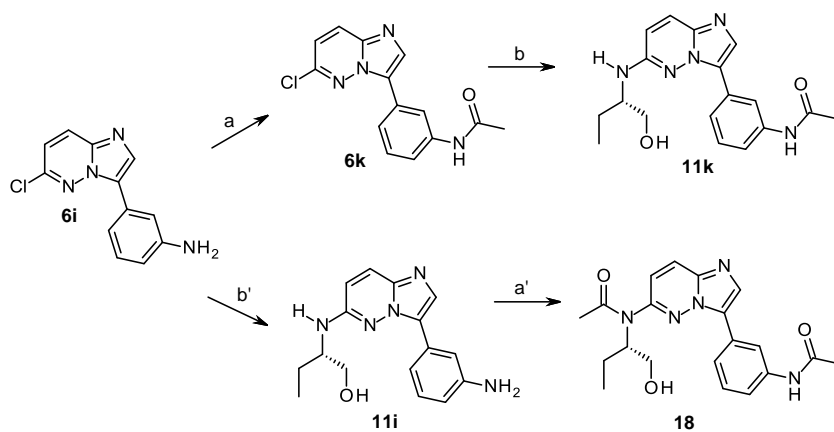
Scheme 2. Procedure for the Synthesis of compound **14**. Reagent and conditions : (a) Pd(OAc)₂, xantphos, *t*-BuOK, H₂O, 1,4-dioxane, 100 °C, 24 h, 27%; (b) NBS, CHCl₃, 20 °C, 4 h, 100%; (c) 3-chlorophenylboronic pinacol ester, Pd[P(Ph)₃]₄, Na₂CO₃ (2 M), 1,4-dioxane, 100 °C, 6 h , 5 %



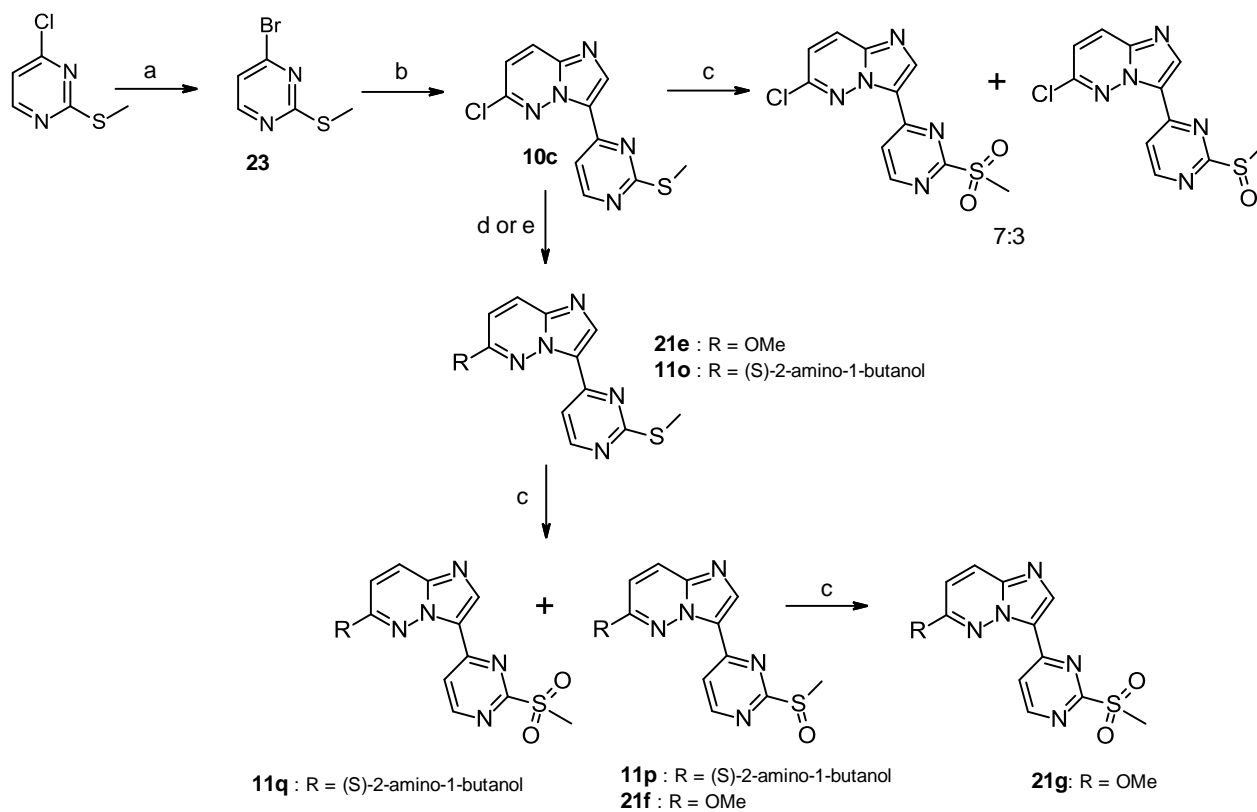
Scheme 3. Procedure for the synthesis of compound **15**. Reagents and conditions: (a) Pd(OAc)₂, xantphos, *t*-BuOK, H₂O, 1,4-dioxane, 100 °C, 24 h, 30%.



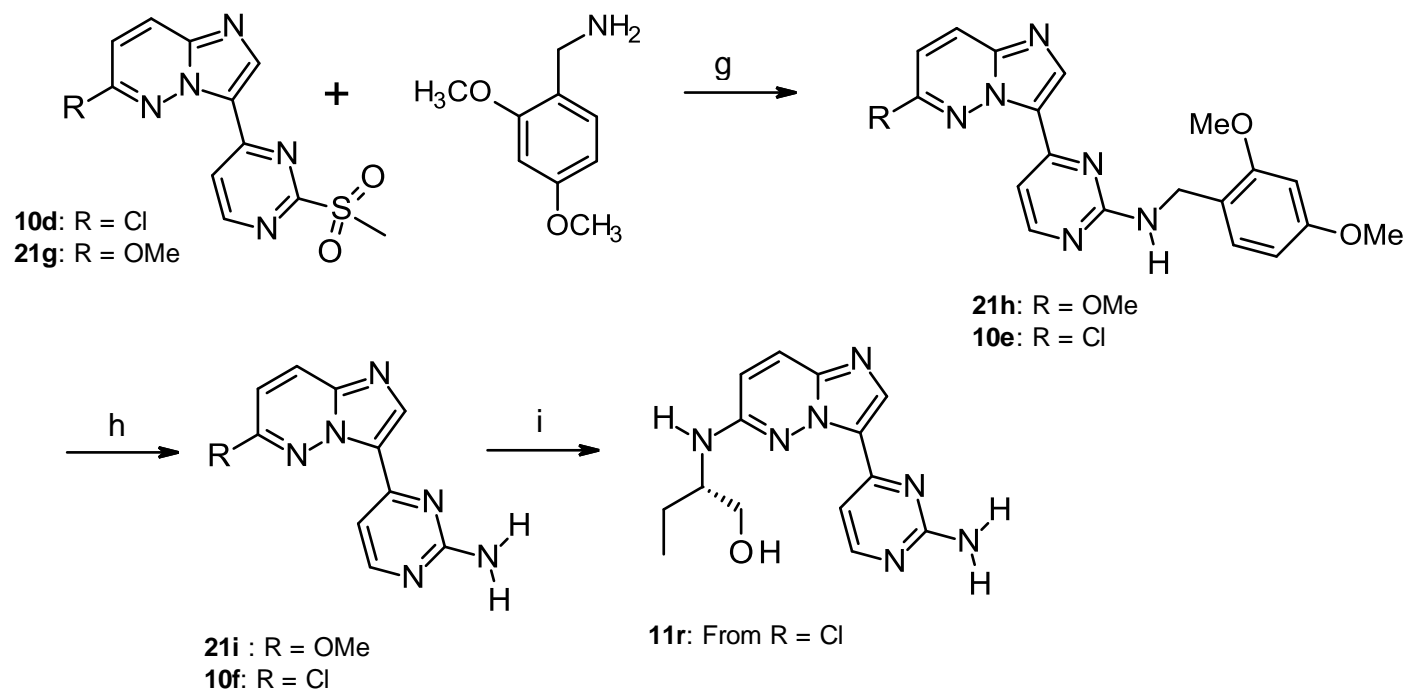
Scheme 4. Procedure for the synthesis of acetamides **11i** and **18**. Reagents and conditions: (a) acetic anhydride, DMAP, Et₃N, DCM, 20 °C, 2 h, 79%; (b) (S)-2-amino-1-butanol, 160 °C, 7 h, 7%; (b') (S)-2-amino-1-butanol, 180 °C, 7 h, 26%; (a') acetic anhydride, DMAP, Et₃N, DCM, r.t, 2 h, 57%.



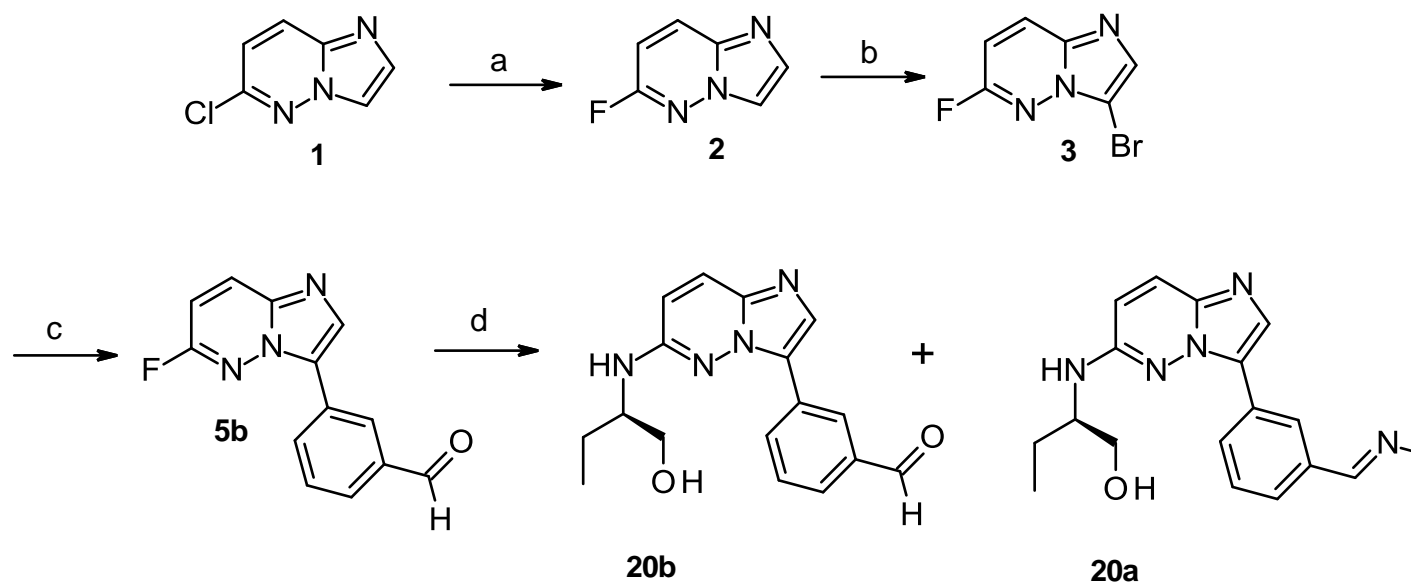
Scheme 5. Procedure for the synthesis of pyrimidine derivatives. Reagents and conditions : (a) TMSBr, CH₃CN, 40 °C, 27 h, 98% ; (b) 6-chloro-imidazo[1,2-b]pyridazine, Pd(OAc)₂, PPh₃, K₂CO₃, toluene, 2 days, 110 °C, 38% ; (c) mCPBA, DCM, r.t; (d) MeONa, MeOH, 6 h, 80 °C; (e) (S)-2-amino-1-butanol, 170 °C, 4 h.



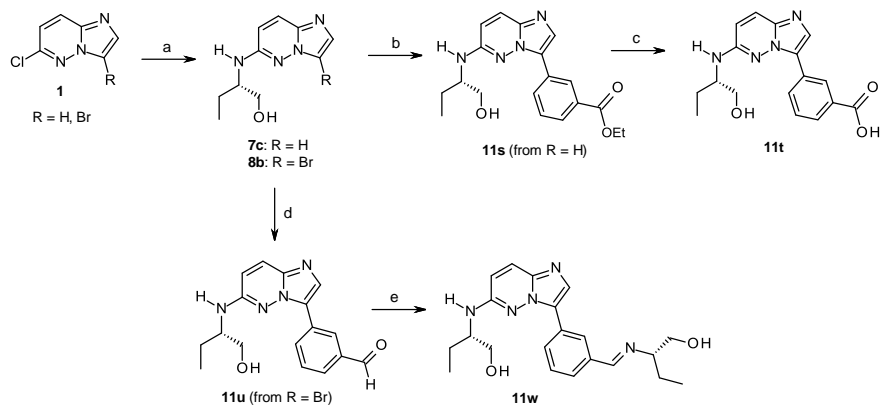
Scheme 6. Procedure for the synthesis of pyrimidine derivatives. Reagents and conditions: (g) 1,4-dioxane, 8 h, reflux; (h) TFA, DCM, 20 °C; (i) (S)-2-amino-1-butanol, 6 h, 160 °C..



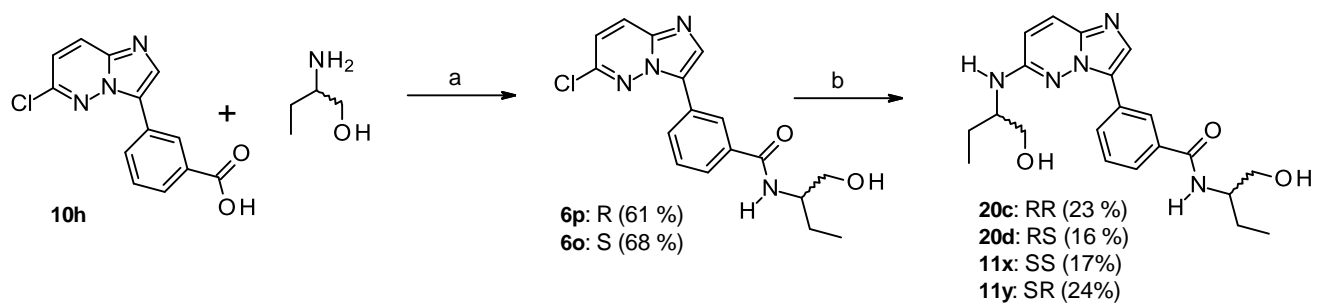
Scheme 7. Procedure for the synthesis of imines. Reagents and conditions : (a) KF, DMSO/Toluene, 8h, 180°C, 46%; (b) NBS, CHCl₃, 6 h, r.t, 93%; (c) 3-formylphenylboronic acid, Pd[P(C₆H₅)₃]₄, Na₂CO₃ (2 M), 1,4-dioxane, 6 h, 100 °C, 63%; (d) (R)-2-amino-1-butanol, 135 °C, 2 h.



Scheme 8. Procedure for the synthesis of acid and ethyl ester derivative. Reagents and conditions : (a) (S)-2-amino-1-butanol, 8 h, 180 °C, 69 %; (c) Ethyl 3-bromobenzoate, Pd(OAc)₂, P(C₆H₅)₃, K₂CO₃, toluene, 8 h, 110 °C, 10 %; (c) i: NaOH (1N), THF/EtOH, ii: HCl (1N), H₂O, 28%; (d) 3-formylphenylboronic acid, Pd[P(C₆H₅)₃]₄, Na₂CO₃ (2 M), 1,4-dioxane, 12 h, 100 °C. (e) (S)-2-amino-1-butanol, 3 days 20 to 60 °C.



Scheme 9: Procedure of the synthesis of amide. Reagents and conditions: (a) (S or R)-2-amino-1-butanol, DCC/HOBt, Et₃N, THF/DCM, 20 °C, 24 h; (b) (S or R)-2-amino-1-butanol, 180 °C, 12 h.



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