European Journal of Medicinal Chemistry 103 (2015) 381–395

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis, antileishmanial activity and cytotoxicity of 2,3-diaryl- and 2,3,8-trisubstituted imidazo[1,2-*a*]pyrazines

Pascal Marchand [a, *], Marc-Antoine Bazin [a], Fabrice Pagniez [b], Guillaume Rivière [a], Lizeth Bodero [a], Sophie Marhadour [a], Marie-Renée Nourrisson [a], Carine Picot [a, b], Sandrine Ruchaud [c], Stéphane Bach [c], Blandine Baratte [c], Michel Sauvain [d, e], Denis Castillo Pareja [f], Abraham J. Vaisberg [f], Patrice Le Pape [b]

[a] Université de Nantes, Nantes Atlantique Universités, Laboratoire de Chimie Thérapeutique, Cibles et Médicaments des Infections et du Cancer, IICiMed UPRES EA 1155, UFR de Sciences Pharmaceutiques et Biologiques, 1 rue Gaston Veil, 44035 Nantes, France
[b] Université de Nantes, Nantes Atlantique Universités, Laboratoire de Parasitologie et Mycologie Médicale, Cibles et Médicaments des Infections et du Cancer, IICiMed UPRES EA 1155, UFR de Sciences Pharmaceutiques et Biologiques, 1 rue Gaston Veil, 44035 Nantes, France
[c] Sorbonne Universités, UPMC Univ Paris 06, CNRS USR3151, "Protein Phosphorylation & Human Diseases" Group, Station Biologique, Place Georges Teissier, 29688 Roscoff, France
[d] Institut de Recherche pour le Développement (IRD), UMR152, Mission IRD Casilla, 18-1209 Lima, Peru
[e] Université de Toulouse, UPS, UMR152 (Laboratoire de Pharmacochimie et Pharmacologie pour le Développement, Pharma-DEV), F-31062 Toulouse Cedex 9, France
[f] Departamento de Microbiología y Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

ARTICLE INFO

Article history:
Received 22 July 2015
Received in revised form
1 September 2015
Accepted 3 September 2015
Available online 9 September 2015

Keywords:
Antileishmanial activity
Imidazo[1,2-*a*]pyrazines
Direct arylation
Casein kinase 1

ABSTRACT

A series of original 2-phenyl-3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazines and the 3-iodo precursors, bearing a polar moiety at the C-8 position, was synthesized and evaluated for their antileishmanial activities. Two derivatives exhibited very good activity against the promastigote and the amastigote forms of *Leishmania major* in the micromolar to submicromolar ranges, coupled with a low cytotoxicity against macrophages and 3T3 mouse fibroblast cells. Through *Lm*CK1 inhibition assay, investigations of the putative molecular target of these promising antileishmanial compounds will be discussed.

1. Introduction

According to a recent report from the World Health Organization (WHO) [1], leishmaniases - visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL) - collectively affect 12 million people in 98 countries, and 350 million more are at risk of infection. Moreover, there are 1.3 million new cases and 40,000 deaths attributed to leishmaniases each year and leishmaniasis is included in the neglected tropical diseases (NTDs) [2]. Clinical forms differ in immunopathologies and degree of morbidity and mortality. VL caused by *Leishmania donovani* and *Leishmania infantum* is the most severe form of leishmaniasis and is usually fatal in the absence of treatment whereas CL caused by *Leishmania major*, *Leishmania amazonensis, Leishmania mexicana*, *Leishmania braziliensis*, and *Leishmania panamensis* is significantly associated with morbidity. Cutaneous leishmaniasis is more widely distributed, with about one-third of cases occurring in each of three epidemiological regions, the Americas, the Mediterranean basin, and western Asia from the Middle East to Central Asia. The ten countries with the highest estimated case counts are: Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Iran, Peru, Sudan and Syria, and together account for 70–75% of global estimated CL incidence. More than 90% of global VL cases occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan [1–9].

These parasitic infections are caused by a protozoan of the *Leishmania* genus transmitted to its mammal hosts (humans, dogs,

* Corresponding author.
E-mail address: pascal.marchand@univ-nantes.fr (P. Marchand).

http://dx.doi.org/10.1016/j.ejmech.2015.09.002
0223-5234/

monkeys, rodents,…) by the bite of an infected sandfly (*Phlebotominae*). *Leishmania* parasites present two morphological stages: extracellular flagellated promastigotes in the digestive tract of their sandfly vector and non-motile amastigotes inside the cells of their hosts' mononuclear phagocytic system [1]. Currently, there are no effective vaccines and a number of drugs are used in the treatment of leishmaniasis. These drugs include pentavalent antimonials, amphotericin B, miltefosine, pentamidine and paromomycin. Unfortunately, many of these drugs cause side effects and high toxicities, and display a high rate of treatment failure in HIV co-infected patients. In addition, an inevitable resistance has developed in recent times in *Leishmania* parasites towards some of these drugs. Otherwise, they are costly and require long-term treatment [2–9]. Consequently, there is an urgent need to speed up the development of a new generation of more effective and safe antileishmanials.

Various classes of natural products have shown promising antileishmanial activity [10] and compounds of synthetic origin comprising a diverse group of chemical structures have been reported as antileishmanial agents [3,5,11]. These include mostly the nitrogen heterocycles for instance as quinolines [12], quinolinones [13], quinazolines [14], quinoxalines [15], acridines [16], pyrimidines [17], thienopyrimidines [18] or azoles [9].

During the last few years, our strategy to fight against leishmaniasis was the design of small heterocyclic compounds in azolylindole series [19,20] and, more recently, 2,3-diarylimidazo[1,2-*a*]pyridine series [21].

Interestingly, 2,3-diarylimidazo[1,2-*a*]pyridine derivatives exhibited good activity against the promastigote form and/or the amastigote form of *L. major*, coupled with a low cytotoxic activity against the HeLa human cell line. We studied *Lm*CK1 as a potential molecular target responsible of antiparasitic properties but, in a general approach, antileishmanial activities of the imidazo[1,2-*a*]pyridines were not clearly correlated with *Lm*CK1 inhibition. Nevertheless, the 4-pyridyl derivative **I** (Fig. 1) displayed *Lm*CK1 kinase inhibition in the submicromolar range (IC_{50} value of 0.30 μM) and IC_{50} value of 6.5 μM on *L. major* promastigote stage, constituting a good starting point for the development of new antileishmanial agents [21].

Indeed, among the different targets such as sterol biosynthetic pathway, trypanothione or methylglyoxal metabolism, parasite glycolysis, purine salvage pathway, folate biosynthesis or topoisomerases [4–6,10,22], the involvement of several parasitic protein kinases was found essential for parasite proliferation and viability [23,24]. These enzymes were validated as potential molecular targets for drug development, namely cyclin-dependent kinases (CDKs) [25–27], mitogen-activated protein kinases (MAPKs) [28–30], Aurora kinase [31], Protein kinase C (PKC) [32] or, in our field of interest, casein kinase 1 (CK1) [33–35].

In the course of our ongoing synthetic and screening programs for new biologically active imidazo[1,2-*a*]azines [21,36,37], we decided to develop bioisostere analogues of the previously described 2,3-diarylimidazo[1,2-*a*]pyridines [21]. Imidazo[1,2-*a*]pyrazines have been gaining attention in drug discovery but no biological application related to *Leishmania* diseases was found in the literature [38]. Consequently, we planned to explore such scaffold for the design of new antileishmanial agents associated with *Lm*CK1 inhibition (Fig. 1, Series A). In addition, to try to enhance *Lm*CK1 inhibition, our second strategy was to introduce a variety of polar groups, namely amines [33], at the imidazo[1,2-*a*]pyrazine C8 position, by holding constant the 4-pyridyl at the C3 position along with a phenyl or a 4-fluorophenyl substituent at the C2 position (Fig. 1, Series B).

2. Results and discussion

2.1. Chemistry

For the design of imidazo[1,2-*a*]pyrazines substituted at 2,3-ring positions, we were interested in preparing 2-phenylimidazo[1,2-*a*]pyrazine **2** as intermediate (Scheme 1).

To this end, a generally established method was the condensation of α-bromoacetophenone with 2-aminopyrazine **1** [39]. The low yield observed for the cyclization step (30%) prompted us to prepare imidazo[1,2-*a*]pyrazin-2-yl triflate **3**, as recently described by our team in imidazo[1,2-*a*]pyridine series [21,36], followed by Suzuki coupling to give the desired product **2** (Scheme 1). Thus, 2-aminopyrazine **1** reacted with ethyl bromoacetate and a subsequent treatment with *N*-phenylbis(trifluoromethanesulfonimide) led to **3** in only 33% yield. This could be due to the competitive reactivity of the N-4 nitrogen of starting 2-aminopyrazine, involved in the nucleophilic substitution mechanism, thereby preventing following cyclisation. Suzuki coupling was then performed in the presence of phenylboronic acid to produce compound **2** in satisfactory yield (67%). Considering the overall yield of the two steps of this sequence (22% *vs* 30%), this second approach was less effective. Nevertheless, the original introduction of a leaving group (OTf) at the position 2 of the imidazo[1,2-*a*]pyrazine core will allow metal-catalyzed chemistry for a broad pharmacomodulation since the corresponding halogenated derivatives are almost not described in the literature. In the aim of the obtention of 2-arylimidazo[1,2-*a*]pyrazines, a wide variety of boronic acids are commercially available for Suzuki coupling in the contrary of α-bromoacetophenones for heterocyclization reaction.

The direct arylation reaction at the 3-position was then examined using 2-phenylimidazo[1,2-*a*]pyrazine **2** and aryl halides, applying Fagnou's procedure [40,41]. In the presence of $Pd(OAc)_2$ (2 mol %), $PCy_3 \cdot HBF_4$ (4 mol %), PivOH (0.3 equiv), K_2CO_3 (1.5 equiv), the reaction proceeded smoothly (18–40 h) in DMA at 100 °C to afford 3-aryl-2-phenylimidazo[1,2-*a*]pyrazines **4a–g** in low yields due to degradation of the reaction mixture (Scheme 1, Table 1).

In the second series of compounds, the purpose of the synthetic scheme was to introduce a chlorine atom at the position 8 of the imidazo[1,2-*a*]pyrazine scaffold for further pharmacomodulation. Thus, 2,3-dichloropyrazine **5** was the starting material subjected to amination and cyclization (Scheme 2, Table 2) [42,43].

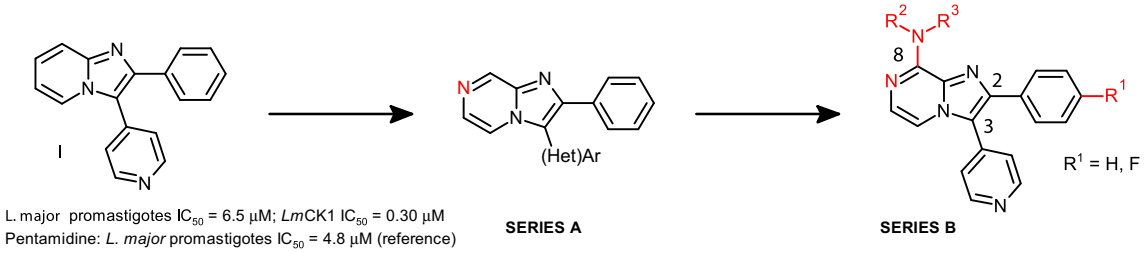
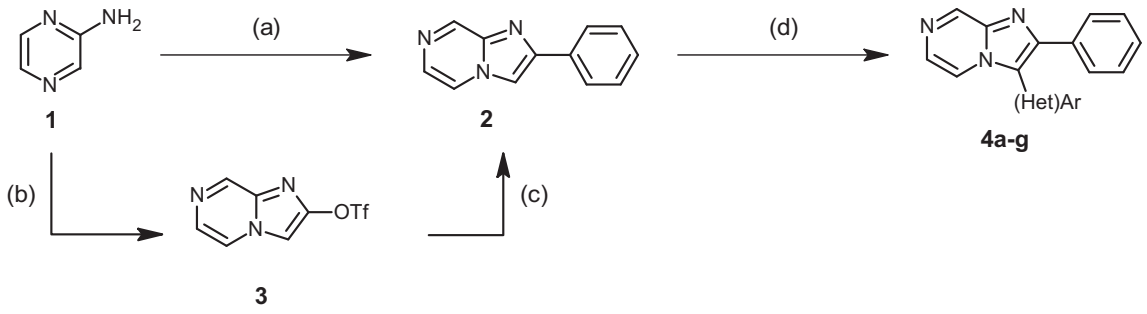


Fig. 1. Structures of target compounds.



Scheme 1. *Reagents and conditions*: (a) $BrCH_2Ph$, $NaHCO_3$, EtOH, 17 h, reflux, 30%; (b) $BrCH_2CO_2Et$, iPrOH, 90 °C, 6 h then $PhNTf_2$, Et_3N, toluene, reflux, 6 h, 33%; (c) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, EtOH, sealed tube, MW 100 °C, 10 min, 67%; (d) (Het)ArX (X = Br, I), $Pd(OAc)_2$, $PCy_3{\cdot}HBF_4$, PivOH, K_2CO_3, DMA, sealed tube, 100 °C.

Table 1
Structures of 3-aryl-2-phenylimidazo[1,2-*a*]pyrazines **4a**–**g**.

Compound	(Het)Ar	Time	Yield (%)[a]
4a	C_6H_5	40 h	15
4b	4-$MeOC_6H_4$	18 h	20
4c	4-$(NO_2)C_6H_4$	22 h	20
4d	4-$(CO_2Et)C_6H_4$	21 h	18
4e	3-ClC_6H_4	28 h	13
4f	3,5-$(Cl)_2C_6H_3$	28 h	20
4g	4-Pyridyl	30 h	22

[a] Isolated yields.

By comparison to unsubstituted aminopyrazine **1**, the heterocyclisation proceeded in better yield (55–58% *vs* 30%) in the presence of 3-chloropyrazin-2-amine **6** and suitable 2-bromoacetophenone to furnish compounds **7a**–**b**. Interestingly, placing a chlorine atom adjacent to the amino group in the ring can reduce the nucleophilicity of the N-4 nitrogen and therefore can prevent the formation of by-product. Subsequent iodination at the C-3 position of imidazo[1,2-*a*]pyrazines **7a**–**b** provided the key intermediates **8a**–**b**, which facilitated installation of different functional groups at C-3 by Suzuki coupling and at C-8 by nucleophilic substitution [38]. Thus different amines were introduced leading to heterocyclic amines **9a**–**f** in acceptable yields. In the last step, the C-3 palladium-catalyzed arylation in the presence of 4-pyridylboronic acid pinacol ester allowed preparing the target compounds **10a**–**f** in moderate to good yields.

Keeping 4-pyridyl moiety as important pharmacophore to obtain CK1 inhibition, we studied the opportunity to introduce the diversity at the C-8 position in the last step (Scheme 3).

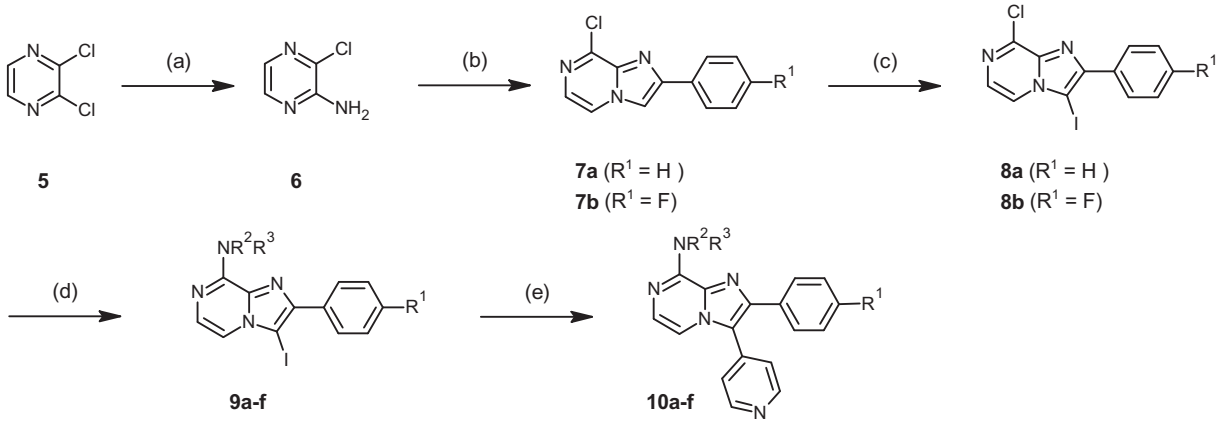
Selective arylation of the 3,8-dihalogenated precursor **8a** was achieved by the displacement of the iodine atom to furnish 2,3-diaryl derivative **11** in 51% yield. Only traces (<5%) of the 2,3,8-triaryl counterpart were detected by UPLC-MS analysis. To avoid the iodination step, direct arylation procedure was applied to starting compound **7a** but this alternative strategy provided compound **11** in low yield (27%) in comparison to the first route (43% for 2 steps). Indeed, the mobility of chlorine atom at the 8-position in metal-catalyzed reaction conditions gave dechlorinated by-products. Finally, compound **11** reacted with 4-(morpholin-4-yl) ethanamine to synthesize product **10c** in 49% yield. From compound **7a**, the overall yield for the obtention of **10c** was divided into two using the second route (21% *vs* 43%) but, in a general approach, this pathway offers the possibility of a wide diversity for further structure–activity relationship study at the 8-position considering the more convergent synthetic scheme.

2.2. Biological evaluation

2.2.1. In vitro antileishmanial activity against promastigotes

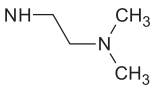
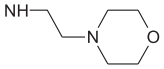
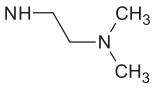
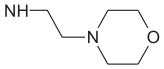
All compounds **4a**–**g**, **9a**–**f** and **10a**–**f** were evaluated for their *in vitro* antileishmanial activity upon the *L. major* strain. Pentamidine was used as the reference drug. The screening data are presented in Table 3.

When compared to the previously reported 2,3-diarylimidazo[1,2-*a*]pyridine analogues displaying IC_{50} values ranging from 4.9 to 22.5 μM on promastigote stage of the same *Leishmania* strain [21], it could be noticed that the new 2,3-diarylimidazo[1,2-*a*]pyrazines **4a**–**g**, with IC_{50} values from 10.3 to 27.6 μM, were globally found less active (Table 3). In this sub-series the SAR study was not

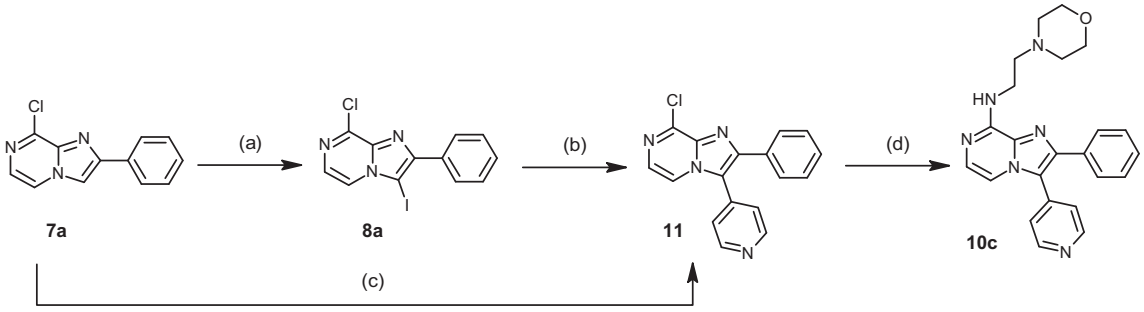


Scheme 2. *Reagents and conditions*: (a) NH_4OH, sealed tube, 100 °C, 17 h, 70%; (b) $BrCH_2(4\text{-}R^1C_6H_4)$, CH_3CN, 80 °C, 11 h, 58% (**7a**) and 18 h, 55% (**7b**); (c) NIS, CH_3CN, 80 °C, 40 h, 85% (**8a**) and 73% (**8b**); (d) R^2R^3NH, K_2CO_3, DMF, 100 °C, 5–12 h, 55–77%; (e) (4-Pyridyl)B(pin), $PdCl_2(dppf)$, K_3PO_4, dioxane/H_2O (9/1), sealed tube, 100 °C, 8 h, 44–84%.

Table 2
Structures of 2-aryl-3-iodoimidazo[1,2-*a*]pyrazines **9a**–**f** and 2-aryl-3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazines **10a**–**f**.

R^1	NR^2R^3	Compound	Time	Yield (%)[a]	Compound	Yield (%)[a]
H		**9a**	8 h	55	**10a**	64
H	$N(CH_3)_2$	**9b**	5 h	73	**10b**	63
H		**9c**	12 h	77	**10c**	66
F		**9d**	8 h	52	**10d**	44
F	$N(CH_3)_2$	**9e**	5 h	70	**10e**	84
F		**9f**	12 h	60	**10f**	52

[a] Isolated yields.



Scheme 3. *Reagents and conditions*: (a) NIS, CH_3CN, 80 °C, 40 h, 85%; (b) (4-Pyridyl)B(pin), $PdCl_2(dppf)$, K_3PO_4, dioxane/H_2O (9/1), sealed tube, 100 °C, 8 h, 51%; (c) 4-Iodopyridine, $Pd(OAc)_2$, PPh_3, Cs_2CO_3, DMF, sealed tube, 90 °C, 16 h, 27%; (d) 4-(Morpholin-4-yl)ethanamine, K_2CO_3, DMF, 100 °C, 10 h, 49%.

easy to establish since, from the 3-phenyl derivative **4a** displaying IC_{50} value of 26.2 μM, the introduction at the para position of an electron donating group (OMe, IC_{50} = 17.4 μM, compound **4b**) or electron withdrawing groups (NO_2, IC_{50} = 27.6 μM, compound **4c** and CO_2Et, IC_{50} = 17.4 μM compound **4d**) provided analogues with the same level of activity but also slightly more active. Nevertheless, the presence of a chlorine atom on the phenyl ring for compound **4e** (3-Cl, IC_{50} = 14.5 μM) and compound **4f** (3,5-diCl, IC_{50} = 10.3 μM) enhanced the antileishmanial potency. Finally, the replacement of the phenyl ring at the C-3 position of 2,3-diphenylimidazo[1,2-*a*]pyrazine **4a** by a 4-pyridyl moiety (IC_{50} = 20.1 μM, compound **4g**) allowed retaining the low antiparasitic activity.

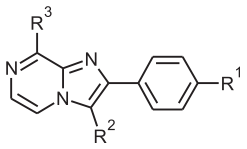
The introduction of a polar substituent in C-8 position of 2-phenyl-3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazine **4g** did not enhance the antileishmanial activity of compounds **10a**, **10b** and the (morpholin-4-yl)ethylamino counterpart **10c**. Interestingly, a slight modification of these three compounds by the design of the corresponding 4-fluorophenyl derivatives **10d**–**f** led to moderate increase in activity for **10d** and **10 f** (IC_{50} values of 35.3, 16.5 μM *vs* 68.8, 23.5 μM, respectively) but significant increase for the *N*,*N*-dimethylamino derivative **10e** (IC_{50} values of 6.4 *vs* 31.3 μM) that showed the same level of activity as pentamidine, chosen as the antileishmanial reference drug.

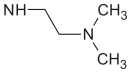
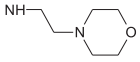
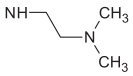
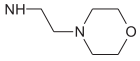
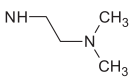
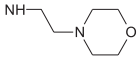
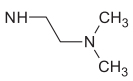
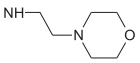
We also investigated the antileishmanial potential of precursors **9a**–**f**, bearing a iodine atom at the C-3 position of the imidazo[1,2-*a*]pyrazine heterocycle instead of a 4-pyridyl moiety. Compounds **9b**, **9c** and **9f** exhibited very promising activities with IC_{50} values ranging from 6.8, 10.6 and 2.8 μM, respectively. Especially, derivative **9f** was the most active compound of the series displaying better IC_{50} value than the reference. In a general trend for each sub-series, *N*,*N*-dimethylamino and (morpholin-4-yl)ethylamino appendages afforded more potent compound than their *N*,*N*-dimethylethane-1,2-diamine analogues with the exception of compound **9e** that remained inactive in the *L. major* promastigote assay without clear explanation. In the iodine sub-series, comparing IC_{50s} of compounds **9c** (10.6 μM) and **9f** (2.8 μM), the introduction of a fluorine atom enhanced the activity. As previously discussed, this feature was found in the 4-pyridyl sub-series. Finally, the introduction of a polar moiety at the C-8 position of the scaffold provided the best active compounds **9b**, **9c**, **9f** and **10e**, in comparison to unsubstituted molecules **4a**–**g**. Such substitution allowed recovering the level of activity previously obtained in 2,3-diarylimidazo[1,2-*a*]pyridine series [21], validating the interest of this new series of compounds as antileishmanial agents.

2.2.2. Cytotoxicity and selectivity index

Toxicity study was first performed on murine peritoneal macrophages in order to identify drugs which were less toxic and as a prelude to select drugs for *in vitro* assay on the more relevant clinical *Leishmania* amastigote stage [44]. The results concerning the cytotoxicity and selectivity index (SI) data are presented in Table 3. IC_{50} values ranged from 29.2 to 153.7 μM and most of drugs had IC_{50} more than 50 μM, indicating low level of cytotoxicity against macrophages. The SI was calculated as the ratio of cytotoxicity (IC_{50} value on macrophages) to activity (IC_{50} value on promastigotes). This *in vitro* therapeutic index showed that compounds **4g** and **10e** possessed a moderate safety profile (SI of 7.7

Table 3
In vitro antileishmanial activities against *L. major* (MHOM/IL/81/BNI) promastigotes and cytotoxicity evaluation of 3-aryl-2-phenylimidazo[1,2-*a*]pyrazines **4a**–**g**, 2-aryl-3-iodoimidazo[1,2-*a*]pyrazines **9a**–**f** and 2-aryl-3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazines **10a**–**f**.



Compd	R^1	R^2	R^3	*L. major* promastigotes IC_{50} ± SEM (μM)[a]	Cytotoxicity on macrophages IC_{50} ± SEM (μM)[a]	Selectivity index[c]	Cytotoxicity on 3T3 cells IC_{50} (μM)[a]	Selectivity index[d]
Pentamidine				4.6 ± 1.1	152.7 ± 1.7	33.2	n.d.	n.d.
4a	H	C_6H_5	H	26.2 ± 11.1 (22.5 ± 0.6)[b]	41.4 ± 6.1	1.6.	n.d.	n.d.
4b	H	4-$MeOC_6H_4$	H	17.4 ± 3.1 (7.2 ± 0.3)[b]	60.2 ± 8.8	3.5	n.d.	n.d.
4c	H	4-(NO_2)C_6H_4	H	27.6 ± 8.3 (4.9 ± 0.2)[b]	113.6 ± 4.0	4.2	n.d.	n.d.
4d	H	4-(CO_2Et)C_6H_4	H	17.5 ± 11.2 (5.2 ± 1.0)[b]	49.8 ± 7.2	2.9	n.d.	n.d.
4e	H	3-ClC_6H_4	H	14.5 ± 8.2 (n.d.)[b]	51.5 ± 7.1	3.5	n.d.	n.d.
4f	H	3,5-$(Cl)_2C_6H_3$	H	10.3 ± 4.2 (7.7 ± 0.2)[b]	35.6 ± 4.8	3.4	n.d.	n.d.
4g	H	4-Pyridyl	H	20.1 ± 12.2 (6.5 ± 1.6)[b]	153.7 ± 12.3	7.7	n.d.	n.d.
9a	H	I		24.2 ± 5.9	68.6 ± 6.4	2.8	31.9	1.3
9b	H	I	$N(CH_3)_2$	6.8 ± 0.6	29.2 ± 2.4	4.3	35.7	5.3
9c	H	I		10.6 ± 3.3	31.4 ± 4.5	3.0	235.9	22.3
9d	F	I		24.7 ± 13.2	33.8 ± 2.2	1.4	18.8	0.8
9e	F	I	$N(CH_3)_2$	>100	n.d.	n.d.	99.4	n.d.
9f	F	I		2.8 ± 0.4	93.2 ± 9.5	33.3	>267.5	>95.6
10a	H	4-Pyridyl		68.8 ± 6.2	64.9 ± 11.4	0.9	86.5	1.3
10b	H	4-Pyridyl	$N(CH_3)_2$	31.3 ± 14.3	143.9 ± 5.6	4.6	155.4	5.0
10c	H	4-Pyridyl		23.5 ± 2.9	51.7 ± 4.1	2.2	n.d.	n.d.
10d	F	4-Pyridyl		35.3 ± 13.2	57.8 ± 4.3	1.6	100.9	2.9
10e	F	4-Pyridyl	$N(CH_3)_2$	6.4 ± 0.2	42.0 ± 4.5	6.6	>375.0	>58.6
10f	F	4-Pyridyl		16.5 ± 1.3	40.3 ± 3.7	2.4	47.8	2.9

n.d.: not determined.
SEM: Standard Error of the Mean.
[a] Mean from at least two or three determinations.
[b] IC_{50} values of the 2,3-diarylimidazo[1,2-*a*]pyridine analogues [21].
[c] SI = Cytotoxicity on murine peritoneal macrophages (IC_{50})/Promastigotes (IC_{50}).
[d] SI = Cytotoxicity on BALB/3T3 clone A31 embryonic mouse fibroblast cells (IC_{50})/Promastigotes (IC_{50}).

and 6.6, respectively) and **9f** a high safety profile (SI of 33.3), in all sub-series. It is worth mentioning that the SI of **9f** was equivalent to pentamidine (SI of 33.2). Considering the SI of **9f** (>95.6) and **10e** (>58.6) on non-tumorogenic mouse fibroblast cell line 3T3, the selective activity of the compounds towards *L. major* strain was confirmed. In addition, molecules **9f** and **10e** were found among the most active antileishmanial compounds on promastigote stage of *L. major* with IC_{50} values of 2.8 and 6.4 μM, respectively. Consequently, they were suitable for further investigation on amastigote stage. Furthermore, the selectivity indexes varied between 2.4 and 4.6 for the majority of compounds justifying the interest of the series for the design antiparasitic agents.

2.2.3. *In vitro antileishmanial activity against amastigotes*

In view to study antileishmanial activity against amastigotes, compounds were selected according to their high level of activity against *L. major* promastigotes and/or their selectivity index. Considering these criteria, compounds **4g**, **9f** and **10e** were selected

for testing against intracellular *L. major* amastigotes. The data are indicated in Table 4. Molecule **10b** was added to the set of compounds in order to check the influence of the presence of a fluorine atom on the scaffold towards the antileishmanial activity on amastigotes, as previously discussed in the context of promastigote assay.

Compounds **4g**, **9f** and **10e** provided very high activities against the intracellular amastigote stage of *L. major*. Compound **4g** displayed IC_{50} value at the level of the reference (IC_{50} of 2.7 μM for **4g** *vs* IC_{50} of 3.0 μM for pentamidine) and compounds **10e** (IC_{50} of 0.8 μM) and **9f** (IC_{50} of 0.2 μM) were found 4–15-fold more active than the pentamidine, respectively. In addition, very low toxicity was measured for the molecules affording significant SI ranging from 52.5 to 466 and better than pentamidine (SI of 50.9). Moreover, the values were enhanced in comparison with the promastigote stage (Table 4). It is noteworthy to highlight that the variation of IC_{50} values is conserved from promastigotes to amastigotes. Considering structure-activity relationships, it was difficult to conclude about the importance of the presence of the polar moiety at the C-8 position of imidazo[1,2-*a*]pyrazine ring towards amastigote testing. Indeed, compound **10b** (IC_{50} value of 9.4 μM), bearing a *N*,*N*-dimethylamino group, was less active than its unsubstituted analogue **4g** (IC_{50} value of 2.7 μM), and the most active compound **9f** (IC_{50} value of 0.2 μM) was substituted by a iodine atom at the C-3 position and a (morpholin-4-yl)ethylamino appendage at the C-8 position. An interesting structural feature for enhancing antileishmanial activity of this series is clearly the presence of a fluorine atom on the molecule since, once again, compound **10b** (IC_{50} value of 9.4 μM) was 12-fold less active than its fluoro-substituted counterpart **10e** (IC_{50} value of 0.8 μM). During the process of invasion, the parasite initiates the formation of a membrane, the socalled parasitophorous vacuole membrane, which surrounds the intracellular parasite. Penetration of the two membranes (i.e. plasmatic and vacuole) is therefore an important criterion for activity and can differ from a compound to the other, depending on its hydrophobic character. To discuss this point of view, Log *P* values close to 2.9 (**10b**) and 3.0 (**10e**) were calculated using Log *P* Plugin of MarvinSketch software from ChemAxon. Taking into account these data, the difference of Log *P* was not enough to explain the variation of activity by lipophilic consideration.

For comparison with another strain, all the compounds were also evaluated against axenic amastigotes of a cloned line of *L. amazonensis* strain (MHOM/BR/76/LTB-012) but unfortunately no promising activity was denoted since compounds displayed IC_{50} values from 31.4 to 153.7 μM and low SI ranging from 1 to 5 [18].

The lack of activity on axenic amastigotes of *L. amazonensis* does not bode inefficiency of the molecule on intracellular amastigotes of this species given the metabolic and environmental differences of clinical stage [45,46].

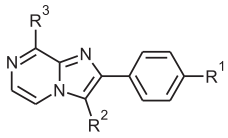
2.2.4. Kinase inhibitory activities

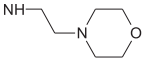
To investigate mechanism of action, compounds were tested for *L. major* CK1 inhibition [33–35]. The results presented in Table 5 showed that 4-pyridyl moiety at the C-3 position of the imidazo[1,2-*a*]pyrazine ring is a structural requirement to obtain *Lm*CK1 inhibition.

Indeed, 4-pyridyl derivatives **4g** and **10a**–**f** displayed IC_{50} values ranging from 0.48 to 1.3 μM whereas the corresponding arylated or iodinated analogues remained inactive. Comparing the level of activities between unsubstituted compound **4g** (IC_{50} value of 1.3 μM) and the C-8 substituted counterparts **10a**–**f** (IC_{50} values from 0.48 to 2.4 μM), an increase of activity was observed for molecules **10a**, **10b**, **10d** and **10f** exhibiting 2-fold more potent *Lm*CK1 inhibition. The two other analogues **10c** (IC_{50} value of 2.4 μM) and **10e** (IC_{50} value of 0.99 μM) were less active than **4g** or as active as this reference compound, respectively. These results prompted us to conclude that *Lm*CK1 inhibition was not related to the nature of the amine side chain in C-8 position of the imidazo[1,2-*a*]pyrazine based scaffold.

Taking into account the first results, antileishmanial activities against promastigotes and amastigotes were not correlated with the inhibition of *Lm*CK1, except for compounds **10e** and **4g**. In addition, no selectivity was observed against the corresponding protozoa model since the selected compounds were also able to inhibit porcine brain *Sus scrofa* CK1 (*Ss*CK1) in the micromolar range. Finally, through biological data of the most active compound **9f** against both promastigotes and amastigotes (Table 5), it clearly appeared that a different target is to be investigated to explain

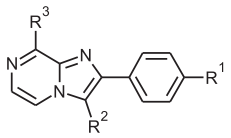
Table 4
In vitro antileishmanial activities of 2-arylimidazo[1,2-*a*]pyrazines **4g**, **9f**, **10b** and **10e** against promastigotes and intracellular amastigotes of a strain of *L. major*, and cytotoxicity evaluation.

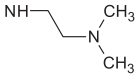
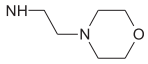
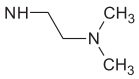
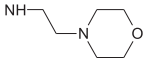
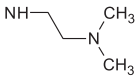
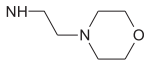
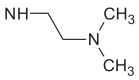
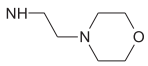


Compd	R^1	R^2	R^3	*L. major 1* promastigotes IC_{50} ± SEM (μM)[a]	*L. major 1* amastigotes IC_{50} ± SEM (μM)[a]	Cytotoxicity on macrophages IC_{50} ± SEM (μM)[a]	Selectivity index amastigotes[b]
Pentamidine				4.6 ± 1.1	3 ± 1	152.7 ± 1.7	50.9 (33.2)[d]
4g	H	4-Pyridyl	H	20.1 ± 12.2	2.7[c]	153.7 ± 12.3	57.0 (7.7)[d]
9f	F	I		2.8 ± 0.4	0.2 ± 0.1	93.2 ± 9.5	466 (33.3)[d]
10b	H	4-Pyridyl	$N(CH_3)_2$	31.3 ± 14.3	9.4 ± 7.9	143.9 ± 5.6	15.3 (4.6)[d]
10e	F	4-Pyridyl	$N(CH_3)_2$	6.4 ± 0.2	0.8[c]	42.0 ± 4.5	52.5 (6.6)[d]

SEM: Standard Error of the Mean.
[a] Mean from two determinations.
[b] SI = Cytoxicity (IC_{50})/Amastigotes (IC_{50}).
[c] In one of the two experiments, IC_{50} was below the lower tested concentration, so that SEM calculation was not possible.
[d] SI = Cytoxicity (IC_{50})/Promastigotes (IC_{50}).

Table 5
In vitro antileishmanial activities against promastigotes and intracellular amastigotes of a *L. major* strain and inhibition of *Leishmania major* CK1 and porcine brain *Sus scrofa* CK1 activities of 3-aryl-2-phenylimidazo[1,2-*a*]pyrazines **4a**–**g**, 2-aryl-3-iodoimidazo[1,2-*a*]pyrazines **9a**–**f** and 2-aryl-3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazines **10a**–**f**.



Compd	R^1	R^2	R^3	*L. major* promastigotes IC_{50} ± SEM (μM)[a]	*L. major* amastigotes IC_{50} ± SEM (μM)[a]	*L. major* CK1 IC_{50} (μM)[a]	Native porcine brain *Ss*CK1δ/ε IC_{50} (μM)[a]
Pentamidine				4.6 ± 1.1	3 ± 1		
4a	H	C_6H_5	H	26.2 ± 11.1		>10	n.d.
4b	H	4-$MeOC_6H_4$	H	17.4 ± 3.1		>10	n.d.
4c	H	4-(NO_2)C_6H_4	H	27.6 ± 8.3		>10	n.d.
4d	H	4-(CO_2Et)C_6H_4	H	17.5 ± 11.2		>10	n.d.
4e	H	3-ClC_6H_4	H	14.5 ± 8.2		n.d.	n.d.
4f	H	3,5-$(Cl)_2C_6H_3$	H	10.3 ± 4.2		>10	n.d.
4g	H	4-Pyridyl	H	20.1 ± 12.2	2.7[b]	1.3	3.3
9a	H	I		24.2 ± 5.9		>10	>10
9b	H	I	$N(CH_3)_2$	6.8 ± 0.6		>10	>10
9c	H	I		10.6 ± 3.3		>10	>10
9d	F	I		24.7 ± 13.2		>10	>10
9e	F	I	$N(CH_3)_2$	>100		>10	>10
9f	F	I		2.8 ± 0.4	0.2 ± 0.1	>10	>10
10a	H	4-Pyridyl		68.8 ± 6.2		0.51	1.6
10b	H	4-Pyridyl	$N(CH_3)_2$	31.3 ± 14.3	9.4 ± 7.9	0.48	2.4
10c	H	4-Pyridyl		23.5 ± 2.9		2.4	3.3
10d	F	4-Pyridyl		35.3 ± 13.2		0.52	1.6
10e	F	4-Pyridyl	$N(CH_3)_2$	6.4 ± 0.2	0.8[b]	0.99	2.1
10f	F	4-Pyridyl		16.5 ± 1.3		0.62	2.7

n.d.: not determined.
SEM: Standard Error of the Mean.
[a] Mean from at least two or three determinations.
[b] In one of the two experiments, IC_{50} was below the lower tested concentration, so that SEM calculation was not possible.

promising activities of these new antileishmanial agents.

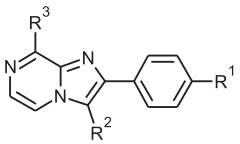
2.2.5. Antiproliferative activity

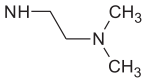
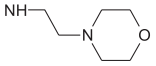
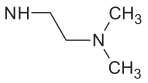
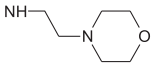
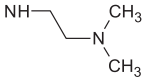
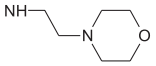
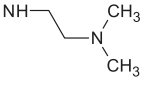
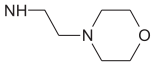
In order to explore more in depth the biological profile of the new imidazo[1,2-*a*]pyrazines **9a**–**f** and **10a**–**f**, the antiproliferative activity of these compounds was also studied against a panel of seven human cancer cell lines, as depicted in Table 6.

Considering the GI_{50} value of 5-fluorouracil (5FU), used as reference in the assay, all the compounds displayed very low cytotoxicity towards the studied cell lines, with the exception of derivatives **9a** and **9d**, and MCF-7 (human breast adenocarcinoma) tumour cell line. Indeed, MCF-7 cell line was sensitive to imidazo [1,2-*a*]pyrazines **9a**, **9b**, **9d**, **9e**, **10a** and **10d**, bearing *N*,*N*-dimethylethane-1,2-diamino or *N*,*N*-dimethylamino groups at the C-8 position of the scaffold and independently of the substitution at the position C-3 position (iodine atom or 4-pyridyl). Nevertheless, with GI_{50} values ranging from 3 to 9 μg/mL, they remained 10-fold–30-fold less active than the reference (GI_{50} values of 0.3 μg/mL). In addition, it was highlighted that *N*'-(3-iodo-2-phenylimidazo[1,2-*a*]pyrazin-8-yl)-*N*,*N*-dimethylethane-1,2-diamine **9a** and especially its fluorine analogue **9d** showed the best activity against all the panel of cell lines, exhibiting GI_{50} values from 4 to 13 μg/mL but except DU145 for **9a** and K562 for **9d**.

Finally, as very interesting result, no antiproliferative activity

Table 6
Antiproliferative activity of 2-aryl-3-iodoimidazo[1,2-*a*]pyrazines **9a**–**f** and 2-aryl-3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazines **10a**–**f** against various cancer cell lines.



Compd	R^1	R^2	R^3	Human cancer cell lines[a] GI_{50} (μg/mL)[b]						
				H460	HuTu80	DU145	MCF-7	M-14	HT-29	K562
5FU[c]				0.3	0.4	1.0	0.3	0.7	0.4	0.9
9a	H	I		13	12	20	9	4	4	5
9b	H	I	$N(CH_3)_2$	47	44	39	3	>125	38	16
9c	H	I		121	33	47	29	179	22	18
9d	F	I		9	6	13	5	4	4	43
9e	F	I	$N(CH_3)_2$	25	22	40	3	>125	>125	>125
9f	F	I		>125	73	>125	>125	>125	>125	>125
10a	H	4-Pyridyl		40	32	47	6	27	6	15
10b	H	4-Pyridyl	$N(CH_3)_2$	70	50	90	32	84	56	48
10c	H	4-Pyridyl		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10d	F	4-Pyridyl		34	24	52	8	19	8	14
10e	F	4-Pyridyl	$N(CH_3)_2$	>125	77	>125	52	>125	>125	>125
10f	F	4-Pyridyl		20	28	39	19	36	27	21

n.d.: not determined.
[a] Mean from three determinations.
[b] H460, human large cell lung cancer; HuTu 80, human duodenal carcinoma; DU145, human prostate carcinoma; MCF-7, human breast adenocarcinoma; M−14, human melanoma; HT-29, human colon adenocarcinoma; K562, human chronic myelogenous leukaemia cells.
[c] 5FU: 5-Fluorouracil.

was detected towards all tumour cell lines and non-tumour cells 3T3 when treating by the compounds **10e** and especially **9f**, highlighting the interest of such molecules as candidates for the development of safe and effective antileishmanial drugs.

3. Conclusion

To the best of our knowledge, we have developed for the first time very promising imidazo[1,2-*a*]pyrazine derivatives, acting as potent antileishmanial agents on both promastigote and amastigote forms of *L. major* and displaying high SI. From previous results, the design of 2-phenyl-3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazine **4g** provided interesting starting point to obtain antiamastigotes activity. The modulation at the C-3 position of the scaffold was envisaged by Suzuki coupling from iodinated precursors or by direct arylation. Following these pathways and by introducing polar moiety at the C-8 position, two sub-series of interesting antileishmanial compounds were highlighted with 3-iodoimidazo[1,2-*a*]pyrazine **9f** and 3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazine **10e** as hit compounds exhibiting very good activity and high therapeutic index, especially on amastigote stage of the parasite. Moreover, the presence of an additional fluorine atom in the molecules, in terms of physicochemical behaviour, was denoted as a relevant criterion to enhance antiparasitic activity. In the aim to identify putative mechanisms of action involved in antileishmanial properties, 4-pyridyl moiety at the C-3 position of the heterocyclic core was found as the pharmacophore to target *Lm*CK1 but unfortunately no selective inhibition towards mammalian *Ss*CK1 was obtained. Further pharmacomodulation will be dedicated to selectivity approach and will be developed to reach nanomolar inhibition that could be necessary to link clearly *Lm*CK1 inhibition to antileishmanial properties since CK1 was established as one of the major protein kinase families expressed and active in *Leishmania* at both stages.

Finally, the possibility is obvious that inhibition of another target contributes to the antileishmanial effect of the most active compound **9f**, bearing a iodine atom at the C-3 position of the imidazo[1,2-*a*]pyrazine ring instead of a 4-pyridyl group. Taking into account the level of activity on *Leishmania* coupled with a putative original mechanism of action and high protozoa selectivity of the compound **9f**, we plan to evaluate its activity in an experimental murine model of leishmaniasis, and to extend the assessment to other species of protozoa, i.e., trypanosomatidae and not trypanosomatidae.

4. Experimental protocols

4.1. Chemistry

All reactions were carried out under argon. All reactions were monitored by TLC analysis using Merck silicagel 60F-254 thin-layer plates. Column chromatography was carried out on silicagel Merck 60 (70–230 mesh ASTM). Melting points were determined on an Electrothermal IA 9000 melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were performed in DMSO-d_6 using a Bruker AVANCE 400 MHz spectrometer. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane as internal standard and coupling constants (J) are given in hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet). Mass spectra were recorded using an Electrospray Ionization Method with Waters ZQ 2000 spectrometer. Microwave reactions were carried out in a CEM Discover microwave reactor in sealed vessels (monowave, maximum power 300 W, temperature control by IR sensor, fixed temperature). Elemental analyses were performed on a Thermo Scientific Elemental Analyser Flash EA 1112 and were found within ±0.4% of the theoretical values.

4.1.1. 2-phenylimidazo[1,2-a]pyrazine (**2**)

4.1.1.1. Method A. To a solution of 2-aminopyrazine **1** (2.38 g, 25.0 mmol) in ethanol (500 mL) were added 2-bromoacetophenone (4.98 g, 25.0 mmol) and sodium hydrogenocarbonate (6.30 g, 75.0 mmol). The mixture was refluxed overnight, cooled to room temperature and the solvent was evaporated *in vacuo*. The residue was diluted with ethyl acetate, then water was added and the aqueous layer was extracted with ethyl acetate (2 x 100 mL). The combined organic extracts were dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by neutral alumina column chromatography using dichloromethane/methanol (98/2) as eluent to give the compound **2** (1.46 g, 30%) as a yellow solid.

4.1.1.2. Method B. Imidazo[1,2-*a*]pyrazin-2-yl trifluoromethane sulfonate **3** (100 mg, 0.4 mmol) was introduced into a 10 mL sealed tube with phenylboronic acid (98 mg, 0.8 mmol), sodium carbonate (169 mg, 1.6 mmol) and tetrakis(triphenylphosphine)palladium(0) (23 mg, 5 mol%) in ethanol (3 mL). The mixture purged with argon and heated at 100 °C for 10 min under microwave irradiation (70 W). After cooling, the resulting solution was poured into water and the organic layer was extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were washed with brine and water, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by neutral alumina column chromatography using dichloromethane/methanol (99/1) as eluent to give the compound **2** (49 mg, 67%) as a yellow powder. R_f 0.40 (CH_2Cl_2/MeOH 98:2); Mp: 102 °C (lit. [39]: 100–102 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.40–7.55 (m, 3H, H_{ar}), 7.93 (d, 1H, J = 4.5 Hz, H_5), 8.07 (dd, 2H, J = 7.2 and 1.2 Hz, H_{ar}), 8.62 (dd, 2H, J = 4.5 and 1.3 Hz, H_6), 8.64 (s, 1H, H_3), 9.12 (d, 1H, J = 1.3 Hz, H_8); ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.5 (CH), 120.0 (CH), 125.9 (2 CH), 128.5 (CH), 128.8 (2 CH), 129.1 (CH), 132.9 (C), 140.3 (C), 142.6 (CH), 146.2 (C); MS (ESI) *m/z* (%): 196.1 (100) $[M+H]^+$. Anal. calcd for $C_{12}H_9N_3$: C 73.83, H 4.65, N 21.52. Found: C 74.02, H 4.66, N 21.59.

4.1.2. Imidazo[1,2-a]pyrazin-2-yl trifluoromethanesulfonate (**3**)

To a stirred solution of 2-aminopyrazine **1** (300 mg, 3.0 mmol) in isopropanol (6 mL) was added ethyl bromoacetate (0.40 mL, 3.6 mmol). The mixture was heated at 90 °C for 6 h, then it was cooled to room temperature and the solvent was evaporated *in vacuo*. The residue was diluted with toluene (40 mL), *N*-phenylbis(trifluoromethanesulfonimide) (2.14 g, 6.0 mmol) and triethylamine (0.75 mL) were added. The solution was refluxed for 6 h, cooled to room temperature and poured into water. The aqueous layer was extracted with ethyl acetate (2 x 50 mL), the combined organic extracts were dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using dichloromethane/methanol (95/5) as eluent to afford the compound **3** (262 mg, 33%) as a yellow oil. R_f 0.48 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, 1H, J = 4.6 Hz, H_5), 8.42 (s, 1H, H_3), 8.67 (dd, 1H, J = 4.6 and 1.5 Hz, H_6), 9.16 (d, 1H, J = 1.5 Hz, H_8); ^{13}C NMR (100 MHz, DMSO-d_6): δ 103.7 (C), 118.5 (q, $J_{C\text{-}F}$ = 319 Hz, CF_3), 120.7 (CH), 130.6 (CH), 135.2 (C), 142.9 (CH), 147.2 (C); MS (ESI) *m/z* (%): 268.0 (100) $[M+H]^+$. Anal. calcd for $C_7H_4F_3N_3O_3S$: C 31.47, H 1.51, N 15.73. Found: C 31.59, H 1.52, N 15.77.

4.1.3. Representative direct arylation procedure for the synthesis of 3-aryl-2-phenylimidazo[1,2-a]pyrazines **4a–g**

2-Phenylimidazo[1,2-*a*]pyrazine **2** (1.0 mmol) was introduced into a 10 mL vial with (hetero)arylhalide (2.0 mmol), palladium acetate (0.04 mmol), tricyclohexylphosphine tetrafluoroborate (0.08 mmol), pivalic acid (0.6 mmol) and potassium carbonate (3.0 mmol) in *N*,*N*-dimethylacetamide (4 mL). The vial was purged with argon for 10 min and then sealed. The mixture was heated at 100 °C for 18–40 h and then it was cooled to room temperature. Water was added and the aqueous layer was extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were washed with brine and water, dried over sodium sulfate, filtered, concentrated *in vacuo* and purified by silica gel column chromatography. Trituration with diisopropylic ether afforded the compounds **4a–g**.

*4.1.3.1. 2,3-diphenylimidazo[1,2-a]pyrazine (**4a**).* Compound **4a** was obtained following the representative procedure, using 2-phenylimidazo[1,2-*a*]pyrazine **2** (300 mg, 1.5 mmol), bromobenzene (0.15 mL, 3.0 mmol) and heating for 40 h. The crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1/1) as eluent to give **4a** (61 mg, 15%) as an orange solid. R_f 0.28 (PET/EtOAc 1:1); Mp: 138 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.31–7.37 (m, 2H, H_{ar}), 7.52–7.63 (m, 8H, H_{ar}), 7.87 (d, 1H, J = 4.6 Hz, H_5), 8.09 (dd, 1H, J = 4.6 and 1.2 Hz, H_6), 9.16 (d, 1H, J = 1.2 Hz, H_8); ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.0 (CH), 122.2 (C), 127.7 (2CH), 128.0 (C), 128.2 (CH), 128.5 (2CH), 129.6 (CH), 129.7 (3CH), 130.4 (2CH), 133.2 (C), 139.4 (C), 142.9 (CH), 143.3 (C); MS (ESI) *m/z* (%): 272.1 (100) $[M+H]^+$. Anal. calcd for $C_{18}H_{13}N_3$: C 79.68, H 4.83, N 15.49. Found: C 79.42, H 4.84, N 15.45.

*4.1.3.2. 3-(4-methoxyphenyl)-2-phenylimidazo[1,2-a]pyrazine (**4b**).* Compound **4b** was obtained following the representative procedure, using 2-phenylimidazo[1,2-*a*]pyrazine **2** (100 mg, 0.5 mmol), 4-bromoanisole (0.12 mL, 1.0 mmol) and heating for 18 h. The crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1/1) as eluent to give **4b** (31 mg,

20%) as a brown powder. R_f 0.30 (PET/EtOAc 1:1); Mp: 109 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (s, 3H, CH_3), 7.16 (d, 2H, J = 8.6 Hz, H_{ar}), 7.30–7.37 (m, 3H, H_{ar}), 7.46 (d, 2H, J = 8.6 Hz, H_{ar}), 7.65 (dd, 2H, J = 8.3 and 1.6 Hz, H_{ar}), 7.85 (d, 1H, J = 4.6 Hz, H_5), 8.05 (dd, 1H, J = 4.6 and 1.2 Hz, H_6), 9.13 (d, 1H, J = 1.2 Hz, H_8); ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.3 (CH_3), 115.2 (2CH), 117.0 (CH), 119.8 (C), 122.1 (C), 127.6 (2CH), 128.0 (CH), 128.5 (2CH), 129.6 (CH), 131.8 (2CH), 133.4 (C), 139.3 (C), 142.8 (CH), 143.0 (C), 160.0 (C); MS (ESI) m/z (%): 302.1 (100) $[M+H]^+$. Anal. calcd for $C_{19}H_{15}N_3O$: C 75.73, H 5.02, N 13.94. Found: C 75.98, H 5.04, N 13.97.

*4.1.3.3. 3-(4-nitrophenyl)-2-phenylimidazo[1,2-a]pyrazine (**4c**).* Compound **4c** was obtained following the representative procedure, using 2-phenylimidazo[1,2-*a*]pyrazine **2** (100 mg, 0.5 mmol), 1-bromo-4-nitrobenzene (202 mg, 1.0 mmol) and heating for 22 h. The crude product was purified by silica gel column chromatography using dichloromethane/ethanol (95/5) as eluent to afford **4c** (31 mg, 20%) as a brown powder. R_f 0.37 (CH_2Cl_2/EtOH 95:5); Mp: 140 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.37–7.39 (m, 3H, H_{ar}), 7.57–7.60 (m, 2H, H_{ar}), 7.85 (d, 2H, J = 8.7 Hz, H_{ar}), 7.94 (d, 1H, J = 4.6 Hz, H_5), 8.28 (dd, 1H, J = 4.6 and 1.1 Hz, H_6), 8.42 (d, 2H, J = 8.7 Hz, H_{ar}), 9.21 (d, 1H, J = 1.4 Hz, H_8); ^{13}C NMR (100 MHz, DMSO-d_6): δ 112.7 (C), 115.3 (C), 117.29 (CH), 124.6 (2CH), 128.1 (2CH), 128.5 (CH), 128.7 (2CH), 130.0 (CH), 131.7 (2CH), 132.6 (C), 135.0 (C), 140.3 (C), 143.1 (CH), 144.7 (C); MS (ESI) m/z (%): 317.2 (100) $[M+H]^+$. Anal. calcd for $C_{18}H_{12}N_4O_2$: C 68.35, H 3.82, N 17.71. Found: C 68.52, H 3.79, N 17.61.

*4.1.3.4. Ethyl 4-(2-phenylimidazo[1,2-a]pyrazin-3-yl)benzoate (**4d**).* Compound **4d** was obtained following the representative procedure, using 2-phenylimidazo[1,2-*a*]pyrazine **2** (100 mg, 0.5 mmol), ethyl 4-bromobenzoate (0.16 mL, 1.0 mmol) and heating for 21 h. The crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1/1) as eluent to give **4d** (30 mg, 18%) as a brown powder. R_f 0.35 (PET/EtOAc 1:1); Mp: 123 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.35 (t, 3H, J = 8.0 Hz, CH_3), 4.32 (q, 2H, J = 8.0 Hz, CH_2), 7.34–7.37 (m, 3H, H_{ar}), 7.59 (dd, 2H, J = 6.9 and 1.8 Hz, H_{ar}), 7.70 (d, 2H, J = 8.1 Hz, H_{ar}), 7.90 (d, 1H, J = 4.6 Hz, H_5), 8.14 (d, 2H, J = 8.1 Hz, H_{ar}), 8.20 (d, 1H, J = 4.6 Hz, H_6), 9.18 (s, 1H, H_8); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.1 (CH_3), 61.0 (CH_2), 117.1 (CH), 121.1 (C), 127.9 (2CH), 128.3 (CH), 128.6 (2CH), 129.9 (CH), 130.2 (2CH), 130.4 (C), 130.6 (2CH), 132.7 (C), 132.9 (C), 139.7 (C), 143.0 (CH), 143.9 (C), 165.2 (C=O); MS (ESI) m/z (%): 344.2 (100) $[M+H]^+$. Anal. calcd for $C_{21}H_{17}N_3O_2$: C 73.45, H 4.99, N 12.24. Found: C 73.64, H 5.01, N 13.28.

*4.1.3.5. 3-(3-chlorophenyl)-2-phenylimidazo[1,2-a]pyrazine (**4e**).* Compound **4e** was obtained following the representative procedure, using 2-phenylimidazo[1,2-*a*]pyrazine **2** (100 mg, 0.5 mmol), 3-chlorobromobenzene (0.12 mL, 1.0 mmol) and heating for 28 h. The crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1/1) as eluent to give **4e** (20 mg, 13%) as a brown solid. R_f 0.36 (PET/EtOAc 1:1); Mp: 221 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.33–7.40 (m, 3H, H_{ar}), 7.49–7.52 (m, 1H, H_{ar}), 7.59–7.68 (m, 5H, H_{ar}), 7.89 (d, 1H, J = 4.6 Hz, H_5), 8.16 (dd, 1H, J = 4.6 and 1.4 Hz, H_6), 9.17 (d, 1H, J = 1.4 Hz, H_8); ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.3 (CH), 120.7 (C), 127.8 (2CH), 128.3 (CH), 128.6 (2CH), 129.3 (CH), 129.6 (CH), 129.8 (CH), 130.1 (CH), 130.2 (C), 131.5 (CH), 133.0 (C), 134.1 (C), 139.5 (C), 142.9 (CH), 143.7 (C); MS (ESI) m/z (%): 306.1 (100) $[M+H]^+$, 308.2 (35) $[M+H+2]^+$. Anal. calcd for $C_{18}H_{12}N_3Cl$: C 70.71, H 3.96, N 13.74. Found: C 70.95, H 3.98, N 13.66.

*4.1.3.6. 3-(3,5-dichlorophenyl)-2-phenylimidazo[1,2-a]pyrazine (**4f**).* Compound **4f** was obtained following the representative procedure, using 2-phenylimidazo[1,2-*a*]pyrazine **2** (200 mg, 1.0 mmol), 1,3-dichloro-5-iodobenzene (680 mg, 2.0 mmol) and heating for 28 h. The crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1/1) as eluent to afford **4f** (68 mg, 20%) as a brown powder. R_f 0.34 (PET/EtOAc 1:1); Mp: 199 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.35–7.42 (m, 3H, H_{ar}), 7.59–7.61 (m, 2H, H_{ar}), 7.66 (d, 2H, J = 1.9 Hz, H_{ar}), 7.84 (t, 1H, J = 1.9 Hz, H_{ar}), 7.92 (d, 1H, J = 4.6 Hz, H_5), 8.24 (dd, 1H, J = 4.6 and 1.3 Hz, H_6), 9.18 (d, 1H, J = 1.3 Hz, H_8); ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.6 (CH), 119.4 (C), 127.9 (2CH), 128.4 (CH), 128.6 (2CH), 129.2 (2CH), 129.3 (CH), 129.8 (CH), 131.6 (C), 132.8 (C), 135.1 (2C), 139.6 (C), 142.9 (CH), 143.9 (C); MS (ESI) m/z (%): 340.1 (100) $[M+H]^+$, 342.1 (65) $[M+H+2]^+$, 344.1 (11) $[M+H+4]^+$. Anal. calcd for $C_{18}H_{11}Cl_2N_3$: C 63.55, H 3.26, N 12.35. Found: C 63.41, H 3.24, N 13.31.

*4.1.3.7. 2-phenyl-3-(pyridin-4-yl)imidazo[1,2-a]pyrazine (**4g**).* Compound **4g** was obtained following the representative procedure, using 2-phenylimidazo[1,2-*a*]pyrazine **2** (200 mg, 1.0 mmol), 4-bromopyridine hydrochloride (389 mg, 2.0 mmol) and heating for 30 h. The crude product was purified by silica gel column chromatography using dichloromethane/ethanol (99/1) as eluent to afford **4g** (60 mg, 22%) as a beige powder. R_f 0.10 (CH_2Cl_2/EtOH 99:1); Mp: 126 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.37–7.40 (m, 3H, H_{ar}), 7.56–7.60 (m, 4H, H_{ar} and H_{pyr}), 7.93 (d, 1H, J = 4.6 Hz, H_5), 8.31 (dd, 1H, J = 4.6 and 1.2 Hz, H_6), 8.78 (d, 2H, J = 5.2 Hz, H_{pyr}), 9.21 (d, 1H, J = 1.2 Hz, H_8); ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.3 (CH), 119.6 (C), 124.7 (2CH), 128.1 (2CH), 128.5 (CH), 128.6 (2CH), 130.0 (CH), 132.8 (C), 136.0 (C), 139.9 (C), 143.1 (CH), 144.4 (C), 150.8 (2CH); MS (ESI) m/z (%): 273.2 (100) $[M+H]^+$. Anal. calcd for $C_{17}H_{12}N_4$: C 74.98, H 4.44, N 20.58. Found: C 75.21, H 4.46, N 20.65.

*4.1.4. 3-chloropyrazin-2-amine (**6**)*

2,3-Dichloropyrazine **5** (5.0 g, 33.6 mmol) in 28% aqueous ammonia solution (20 mL) in a reactor Parr was stirred at 100 °C for 17 h. After cooling, the resulting mixture was filtered, the solid was washed with water and dried *in vacuo* to afford compound **6** (3.0 g, 70%) as a white powder. R_f 0.37 (cyHex/EtOAc 1:1); Mp: 170 °C (lit. [43]: 169.4–170.1 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.83 (bs, 2H, NH_2), 7.60 (d, 1H, J = 2.4 Hz, H_5), 7.98 (d, 1H, J = 2.4 Hz, H_6); ^{13}C NMR (100 MHz, DMSO-d_6): δ 130.6 (CH), 132.6 (C), 141.4 (CH), 152.7 (C); MS (ESI) m/z (%): 129.8 (100) $[M+H]^+$, 131.8 (40). $[M+H+2]^+$.

*4.1.5. Cyclization step for the synthesis of 8-chloro-2-phenylimidazo [1,2-a]pyrazine derivatives **7a** and **7b***

*4.1.5.1. 8-chloro-2-phenylimidazo[1,2-a]pyrazine (**7a**).* To 3-chloropyrazin-2-amine **6** (500 mg, 3.9 mmol) in acetonitrile (7 mL) was added 2-bromoacetophenone (920 mg, 4.6 mmol). The reaction mixture was stirred at 80 °C for 11 h. After cooling, the resulting mixture was diluted with dichloromethane and neutralized by a saturated sodium hydrogenocarbonate aqueous solution. The organic layer was dried over sodium sulfate, filtered and concentrated under *vacuum*. The crude product was purified by silica gel column chromatography using cyclohexane, then cyclohexane/ethyl acetate (1/1) as eluent to give **7a** (521 mg, 58%) as a white powder. R_f 0.42 (cyHex/EtOAc 1:1); Mp: 206 °C (lit. [42]: 206–208 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (t, 1H, J = 7.6 Hz, H_{ar}), 7.54 (t, 2H, J = 7.6 Hz, H_{ar}), 7.77 (d, 1H, J = 4.4 Hz, H_6), 8.08 (d, 2H, J = 7.2 Hz, H_{ar}), 8.66 (d, 1H, J = 4.4 Hz, H_5), 8.77 (s, 1H, H_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.2 (CH), 120.6 (CH), 126.1 (2CH), 127.7 (CH), 128.9 (CH), 129.03 (2CH), 132.5 (C), 137.3 (C), 141.3 (C), 146.5 (C); MS (ESI) m/z (%): 230.0 (100) $[M+H]^+$, 232.0 (35) $[M+H+2]^+$. Anal. calcd for $C_{12}H_8ClN_3$: C 62.76, H 3.51, N 18.30. Found: C 62.99, H 3.52, N 18.35.

*4.1.5.2. 8-chloro-2-(4-fluorophenyl)imidazo[1,2-a]pyrazine (**7b**).* Compound **7b** was obtained using the same procedure as **7a** by reaction of 3-chloropyrazin-2-amine **6** (500 mg, 3.9 mmol) with 2-bromo-4'-fluoroacetophenone (1.00 g, 4.6 mmol) and heating for 18 h to afford **7b** (529 mg, 55%) as white powder. R_f 0.46 (cyHex/EtOAc 1:1); Mp: 191 °C (lit. [42]: 188–190 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.38 (t, 2H, J = 8.8 Hz, H_{ar}), 7.78 (d, 1H, J = 4.6 Hz, H_6), 8.13 (dd, 2H, J = 8.8 and 5.2 Hz, H_{ar}), 8.67 (d, 1H, J = 4.6 Hz, H_5), 8.77 (s, 1H, H_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.2 (CH), 116.1 (d, $^2J_{C-F}$ = 22 Hz, 2CH), 120.7 (CH), 127.8 (CH), 128.4 (d, $^3J_{C-F}$ = 9 Hz, 2CH), 129.2 (d, $^4J_{C-F}$ = 3 Hz, C), 137.4 (C), 141.3 (C), 145.6 (C), 162.6 (d, $^1J_{C-F}$ = 245 Hz, C); MS (ESI) *m/z* (%): 248.0 (100) $[M+H]^+$, 250.0 (35) $[M+H+2]^+$. Anal. calcd for $C_{12}H_7ClFN_3$: C 58.20, H 2.85, N 16.97. Found: C 58.40, H 2.87, N 16.92.

*4.1.6. Iodination step for the synthesis of 8-chloro-3-iodo-2-phenylimidazo[1,2-a]pyrazine derivatives **8a** and **8b***

*4.1.6.1. 8-chloro-3-iodo-2-phenylimidazo[1,2-a]pyrazine (**8a**).* To 8-chloro-2-phenylimidazo[1,2-*a*]pyrazine **7a** (500 mg, 2.2 mmol) in acetonitrile (10 mL) was added *N*-iodosuccinimide (489 mg, 2.2 mmol). The reaction mixture was stirred at 80 °C for 40 h. After cooling, the resulting mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over sodium sulfate, filtered and concentrated under *vacuum*. The crude product was purified by silica gel column chromatography using cyclohexane, then cyclohexane/ethyl acetate (1/1) as eluent to afford **8a** (655 mg, 85%) as a yellow powder. R_f 0.69 (cyHex/EtOAc 1:1); Mp: 155 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.52 (t, 1H, J = 7.2 Hz, H_{ar}), 7.59 (t, 2H, J = 7.2 Hz, H_{ar}), 7.89 (d, 1H, J = 4.6 Hz, H_6), 8.11 (d, 2H, J = 7.2 Hz, H_{ar}), 8.55 (d, 1H, J = 4.6 Hz, H_5); ^{13}C NMR (100 MHz, DMSO-d_6): δ 70.2 (C), 121.0 (CH), 128.4 (2CH), 128.7 (CH), 128.8 (2CH), 129.1 (CH), 132.70 (C), 140.1 (C), 141.5 (C), 148.6 (C); MS (ESI) *m/z* (%): 355.9 (100) $[M+H]^+$, 357.9 (35) $[M+H+2]^+$. Anal. calcd for $C_{12}H_7ClIN_3$: C 40.54, H 1.98, N 11.82. Found: C 40.67, H 1.99, N 11.85.

*4.1.6.2. 8-chloro-3-iodo-2-(4-fluorophenyl)imidazo[1,2-a]pyrazine (**8b**).* Compound **8b** was obtained using the same procedure as **8a** by reaction of 8-chloro-2-(4-fluorophenyl)imidazo[1,2-*a*]pyrazine **7b** (1.00 g, 4.0 mmol) with *N*-iodosuccinimide (908 mg, 4.0 mmol) to give **8b** (1.10, 73%) as beige powder. R_f 0.76 (cyHex/EtOAc 1:1); Mp: 194 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.41 (t, 2H, J = 8.8 Hz, H_{ar}), 7.86 (d, 1H, J = 4.6 Hz, H_6), 8.12 (dd, 2H, J = 8.8 and 5.5 Hz, H_{ar}), 8.52 (d, 1H, J = 4.6 Hz, H_5); ^{13}C NMR (100 MHz, DMSO-d_6): δ 70.1 (C), 115.5 (d, $^2J_{C-F}$ = 26 Hz, 2CH), 120.8 (CH), 128.4 (CH), 129.0 (d, $^4J_{C-F}$ = 3 Hz, C), 130.4 (d, $^3J_{C-F}$ = 9 Hz, 2CH), 139.9 (C), 141.2 (C), 147.5 (C), 162.4 (d, $^1J_{C-F}$ = 245 Hz, C); MS (ESI) *m/z* (%): 373.9 (100) $[M+H]^+$, 375.9 (35) $[M+H+2]^+$. Anal. calcd for $C_{12}H_6ClFIN_3$: C 38.58, H 1.62, N 11.25. Found: C 38.70, H 1.63, N 11.22.

*4.1.7. Representative amination procedure for the synthesis of 2-aryl-3-iodoimidazo[1,2-a]pyrazines **9a**–**f***

To 8-chloro-3-iodo-2-phenylimidazo[1,2-*a*]pyrazine derivatives **8a** or **8b** (1.0 mmol) in *N*,*N*-dimethylformamide (12 mL) was added amine (2.0 mmol) and potassium carbonate (1.0 mmol). The reaction mixture was purged with argon through the septum and then stirred at 100 °C for 5–12h. The resulting mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over sodium sulfate, filtered and concentrated under *vacuum*. The crude product was purified by silica gel column chromatography.

*4.1.7.1. N′-(3-iodo-2-phenylimidazo[1,2-a]pyrazin-8-yl)-N,N-dimethylethane-1,2-diamine (**9a**).* Compound **9a** was obtained following the representative procedure for 8 h, using 8-chloro-3-iodo-2-phenylimidazo[1,2-*a*]pyrazine **8a** (300 mg, 0.85 mmol) and *N*,*N*-dimethylethylenediamine (184 μL, 1.7 mmol). The crude product was purified by silica gel column chromatography using dichloromethane then dichloromethane/ethanol (95/5) as eluent to afford **9a** (190 mg, 55%) as a beige powder. R_f 0.12 (CH_2Cl_2/EtOH 9:1); Mp: 126 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.23 (s, 6H, $2CH_3$), 2.56 (t, 2H, J = 6.4 Hz, CH_2), 3.62 (q, 2H, J = 6.3 Hz, CH_2), 7.33 (t, 1H, J = 5.5 Hz, NH), 7.39–7.44 (m, 2H, H_{ar} and H_5), 7.51 (t, 2H, J = 7.5 Hz, H_{ar}), 7.60 (d, 1H, J = 4.7 Hz, H_6), 8.02 (d, 2H, J = 7.5 Hz, H_{ar}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.8 (CH_2), 45.0 ($2CH_3$), 57.5 (CH_2), 67.0 (C), 109.7 (CH), 127.7 (2CH), 128.0 (CH), 128.2 (2CH), 129.2 (CH), 133.3 (C), 135.4 (C), 145.0 (C), 148.6 (C); MS (ESI) *m/z* (%): 408.0 (100) $[M+H]^+$. Anal. calcd for $C_{16}H_{18}IN_5$: C 47.19, H 4.45, N 17.20. Found: C 47.35, H 4.47, N 17.28.

*4.1.7.2. N′-(3-iodo-2-phenylimidazo[1,2-a]pyrazin-8-yl)-N,N-dimethylamine (**9b**).* Compound **9b** was obtained following the representative procedure for 5 h, using 8-chloro-3-iodo-2-phenylimidazo[1,2-*a*]pyrazine **8a** (200 mg, 0.56 mmol) and *N*,*N*-dimethylamine (57 μL, 1.1 mmol). The crude product was purified by silica gel column chromatography using cyclohexane/ethyl acetate (95/5) as eluent to afford **9b** (150 mg, 73%) as a white powder. R_f 0.48 (cyHex/EtOAc 6:4); Mp: 142 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.52 (s, 6H, $2CH_3$), 7.42 (t, 1H, J = 7.5 Hz, H_{ar}), 7.46 (d, 1H, J = 4.5 Hz, H_5), 7.52 (t, 2H, J = 7.5 Hz, H_{ar}), 7.69 (d, 1H, J = 4.5 Hz, H_6), 8.04 (d, 2H, J = 7.5 Hz, H_{ar}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 39.4 ($2CH_3$), 67.1 (C), 110.4 (CH), 127.7 (2CH), 128.2 (CH), 128.5 (2CH), 129.1 (CH), 133.3 (C), 136.3 (C), 144.2 (C), 149.5 (C); MS (ESI) *m/z* (%): 365.1 (100) $[M+H]^+$. Anal. calcd for $C_{14}H_{13}IN_4$: C 46.17, H 3.60, N 15.38. Found: C 46.02, H 3.58, N 15.33.

*4.1.7.3. 3-iodo-N-[2-(morpholin-4-yl)ethyl]-2-phenylimidazo[1,2-a]pyrazin-8-amine (**9c**).* Compound **9c** was obtained following the representative procedure for 12 h, using 8-chloro-3-iodo-2-phenylimidazo[1,2-*a*]pyrazine **8a** (100 mg, 0.28 mmol) and 4-(morpholin-4-yl)ethanamine (36 μL, 0.56 mmol). The crude product was purified by silica gel column chromatography using cyclohexane/ethyl acetate (95/5) then cyclohexane/ethyl acetate (1/1) as eluent to afford **9c** (100 mg, 77%) as a beige powder. R_f 0.10 (cyHex/EtOAc 1:1); Mp: 146 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.42–2.46 (m, 4H, $4H_{morph}$), 2.57 (t, 2H, J = 6.4 Hz, CH_2), 3.55–3.62 (m, 6H, CH_2 and $4H_{morph}$), 7.39–7.44 (m, 3H, H_{ar}, H_5 and NH), 7.51 (t, 2H, J = 7.5 Hz, H_{ar}), 7.59 (d, 1H, J = 4.7 Hz, H_6), 8.01 (d, 2H, J = 7.5 Hz, H_{ar}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.3 (CH_2), 53.4 ($2CH_2$), 57.1 (CH_2), 66.3 ($2CH_2$), 66.9 (C), 109.8 (CH), 128.0 (2CH), 128.3 (CH), 128.6 (2CH), 129.6 (CH), 133.3 (C), 135.6 (C), 145.1 (C), 148.7 (C); MS (ESI) *m/z* (%): 450.0 (100) $[M+H]^+$. Anal. calcd for $C_{18}H_{20}IN_5O$: C 48.12, H 4.49, N 15.59. Found: C 48.30, H 4.51, N 15.63.

*4.1.7.4. N′-[2-(4-fluorophenyl)-3-iodoimidazo[1,2-a]pyrazin-8-yl]-N,N-dimethylethane-1,2-diamine (**9d**).* Compound **9d** was obtained following the representative procedure for 8 h, using 8-chloro-3-iodo-2-(4-fluorophenyl)imidazo[1,2-*a*]pyrazine **8b** (300 mg, 0.80 mmol) and *N*,*N*-dimethylethylenediamine (175 μL, 1.6 mmol). The crude product was purified by silica gel column chromatography using dichloromethane then dichloromethane/ethanol (95/5) as eluent to afford **9d** (177 mg, 52%) as a beige powder. R_f 0.11 (CH_2Cl_2/EtOH 9:1); Mp: 127 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 6H, $2CH_3$), 2.60 (t, 2H, J = 6.3 Hz, CH_2), 3.59 (q, 2H, J = 6.3 Hz, CH_2), 7.33–7.40 (m, 3H, H_{ar} and NH), 7.43 (d, 1H, J = 4.7 Hz, H_5), 7.59 (d, 1H, J = 4.7 Hz, H_6), 8.04 (dd, 2H, J = 8.7 and 5.6 Hz, H_{ar}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.5 (CH_2), 44.7 ($2CH_3$), 57.2 (CH_2), 67.3 (C), 109.6 (CH), 115.2 (d, $^2J_{C-F}$ = 22 Hz, 2CH), 129.3 (CH), 129.6 (d, $^4J_{C-F}$ = 3 Hz, C), 129.7 (d, $^3J_{C-F}$ = 8 Hz, 2CH), 135.6 (C), 144.4 (C), 148.8 (C), 162.1 (d, $^1J_{C-F}$ = 243 Hz, C); MS (ESI) *m/*

z (%): 426.1 (100) $[M+H]^+$. Anal. calcd for $C_{16}H_{17}FIN_5$: C 45.19, H 4.03, N 16.47. Found: C 45.31, H 4.05, N 16.52.

*4.1.7.5. 2-(4-fluorophenyl)-3-iodo-N,N-dimethylimidazo[1,2-a]pyrazin-8-amine (**9e**).* Compound **9e** was obtained following the representative procedure for 5 h, using 8-chloro-3-iodo-2-(4-fluorophenyl)imidazo[1,2-*a*]pyrazine **8b** (300 mg, 0.80 mmol) and *N*,*N*-dimethylamine (81 μL, 1.6 mmol). The crude product was purified by silica gel column chromatography using cyclohexane/ethyl acetate (95/5) as eluent to afford **9e** (215 mg, 70%) as a white powder. R_f 0.48 (cyHex/EtOAc 6:4); Mp: 129 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.48 (s, 6H, 2xCH_3), 7.33 (t, 2H, J = 8.9 Hz, H_{ar}), 7.43 (d, 1H, J = 4.6 Hz, H_5), 7.64 (d, 1H, J = 4.6 Hz, H_6), 8.05 (dd, 2H, J = 8.7 and 5.7 Hz, H_{ar}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 39.5 ($2CH_3$), 67.0 (C), 110.4 (CH), 115.4 (d, $^2J_{C\text{-}F}$ = 21 Hz, 2CH), 129.1 (CH), 129.7 (d, $^3J_{C\text{-}F}$ = 8 Hz, 2CH), 129.8 (d, $^4J_{C\text{-}F}$ = 3 Hz, C), 136.2 (C), 143.3 (C), 149.3 (C), 162.0 (d, $^1J_{C\text{-}F}$ = 244 Hz, C); MS (ESI) *m/z* (%): 383.0 (100) $[M+H]^+$. Anal. calcd for $C_{14}H_{12}FIN_4$: C 44.00, H 3.16, N 14.66. Found: C 43.85, H 3.17, N 14.70.

*4.1.7.6. 2-(4-fluorophenyl)-3-iodo-N-[2-(morpholin-4-yl)ethyl]imidazo[1,2-a]pyrazin-8-amine (**9f**).* Compound **9f** was obtained following the representative procedure for 12 h, using 8-chloro-3-iodo-2-(4-fluorophenyl)imidazo[1,2-*a*]pyrazine **8b** (300 mg, 0.80 mmol) and 4-(morpholin-4-yl)ethanamine (211 μL, 1.6 mmol). The crude product was purified by silica gel column chromatography using cyclohexane/ethyl acetate (95/5) then cyclohexane/ethyl acetate (1/1) as eluent to afford **9f** (225 mg, 60%) as a white powder. R_f 0.10 (cyHex/EtOAc 1:1); Mp: 167 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.43–2.59 (m, 6H, CH_2 and $4H_{morph}$), 3.55–3.62 (m, 6H, CH_2 and $4H_{morph}$), 7.36 (t, 2H, J = 8.8 Hz, H_{ar}), 7.43 (d, 1H, J = 4.7 Hz, H_5), 7.44–7.48 (m, 1H, NH), 7.59 (d, 1H, J = 4.7 Hz, H_6), 8.04 (dd, 2H, J = 8.8 and 5.6 Hz, H_{ar}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.4 (CH_2), 53.3 ($2CH_2$), 56.9 (CH_2), 66.2 ($2CH_2$), 66.8 (C), 109.8 (CH), 115.3 (d, $^2J_{C\text{-}F}$ = 25 Hz, 2CH), 129.5 (CH), 129.7 (d, $^4J_{C\text{-}F}$ = 3 Hz, C), 129.9 (d, $^3J_{C\text{-}F}$ = 8 Hz, 2CH), 135.4 (C), 144.3 (C), 148.6 (C), 162.0 (d, $^1J_{C\text{-}F}$ = 243 Hz, C); MS (ESI) *m/z* (%): 468.0 (100) $[M+H]^+$. Anal. calcd for $C_{18}H_{19}FIN_5O$: C 46.27, H 4.10, N 14.99. Found: C 46.43, H 4.13, N 15.03.

*4.1.8. Representative Suzuki coupling procedure for the synthesis of 2-aryl-3-(pyridin-4-yl)imidazo[1,2-a]pyrazines **10a–f***

To *N*-substituted 2-aryl-3-iodoimidazo[1,2-*a*]pyrazin-8-amines ***9a–f*** (1.0 mmol) in dioxane/water (9:1) (10 mL) was added 4-pyridylboronic acid pinacol ester (1.1 mmol), potassium phosphate tribasic (2.5 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (0.05 mmol). The vial was purged with argon for 10 min and then sealed. The mixture was heated at 100 °C for 8 h and then it was cooled to room temperature. Water was added and the aqueous layer was extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were washed with brine and water, dried over sodium sulfate, filtered, concentrated *in vacuo*. The crude product was purified by silica gel column chromatography to afford the compounds **10 a–f**.

*4.1.8.1. N,N-Dimethyl-N′-[2-phenyl-3-(pyridin-4-yl)imidazo[1,2-a]pyrazin-8-yl]ethane-1,2-diamine **10a**.* Compound **10a** was obtained following the representative procedure, using *N*′-(3-iodo-2-phenylimidazo[1,2-*a*]pyrazin-8-yl)-*N*,*N*-dimethylethane-1,2-diamine **9a** (90 mg, 0.22 mmol). The crude product was purified by silica gel column chromatography using dichloromethane then dichloromethane/ethanol (95/5) as eluent to afford **10a** (51 mg, 64%) as a beige powder. R_f 0.10 (CH_2Cl_2/EtOH 9:1); Mp: 126 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 6H, $2CH_3$), 2.58 (t, 2H, J = 6.3 Hz, CH_2), 3.61 (q, 2H, J = 6.3 Hz, CH_2), 7.31–7.39 (m, 5H, $3H_{ar}$, H_5 and NH), 7.44 (d, 1H, J = 4.7 Hz, H_6), 7.49 (d, 2H, J = 5.7 Hz, H_{pyr}), 7.54 (d, 2H, J = 7.5 Hz, H_{ar}), 8.74 (d, 2H, J = 5.7 Hz, H_{pyr}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.4 (CH_2), 44.7 ($2CH_3$), 57.3 (CH_2), 106.8 (CH), 120.6 (C), 124.4 (2CH), 127.7 (2CH), 127.8 (CH), 128.3 (2CH), 129.0 (CH), 132.4 (C), 133.2 (C), 136.7 (C), 141.4 (C), 148.9 (C), 150.5 (2CH); MS (ESI) *m/z* (%): 359.2 (100) $[M+H]^+$. Anal. calcd for $C_{21}H_{22}N_6$: C 70.37, H 6.19, N 23.45. Found: C 70.60, H 6.21, N 23.52.

*4.1.8.2. N,N-dimethyl-2-phenyl-3-(pyridin-4-yl)imidazo[1,2-a]pyrazin-8-amine **10b**.* Compound **10b** was obtained following the representative procedure, using *N*′-(3-iodo-2-phenylimidazo[1,2-*a*]pyrazin-8-yl)-*N*,*N*-dimethylamine **9b** (200 mg, 0.55 mmol). The crude product was purified by silica gel column chromatography using cyclohexane/ethyl acetate (95/5) then cyclohexane/ethyl acetate (1/1) as eluent to afford **10b** (110 mg, 63%) as a beige powder. R_f 0.22 (AcOEt); Mp: 185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.35 (s, 6H, $2CH_3$), 7.27–7.40 (m, 4H, $3H_{ar}$ and H_5), 7.43 (d, 1H, J = 4.6 Hz, H_6), 7.48–7.56 (m, 4H, $2H_{pyr}$ and $2H_{ar}$), 8.76 (d, 2H, J = 5.7 Hz, H_{pyr}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 39.5 ($2CH_3$), 107.5 (CH), 119.9 (C), 124.9 (2CH), 127.6 (2CH), 127.9 (CH), 128.5 (2CH), 128.9 (CH), 133.2 (C), 133.3 (C), 136.9 (C), 140.4 (C), 149.7 (C), 150.8 (2CH); MS (ESI) *m/z* (%): 316.2 (100) $[M+H]^+$. Anal. calcd for $C_{19}H_{17}N_5$: C 72.36, H 5.43, N 22.21. Found: C 72.51, H 5.45, N 22.28.

*4.1.8.3. N-[2-(morpholin-4-yl)ethyl]-2-phenyl-3-(pyridin-4-yl)imidazo[1,2-a]pyrazin-8-amine **10c***

4.1.8.3.1. Method A. Compound **10c** was obtained following the representative procedure, using 3-iodo-*N*-[2-(morpholin-4-yl) ethyl]-2-phenylimidazo[1,2-*a*]pyrazin-8-amine **9c** (70 mg, 0.15 mmol). The crude product was purified by silica gel column chromatography using dichloromethane then dichloromethane/ethanol (95/5) as eluent to afford **10 c** (40 mg, 66%) as a beige powder.

4.1.8.3.2. Method B. Compound **10c** was obtained following the representative procedure used for the synthesis of compounds **9a–f** with 8-chloro-2-phenyl-3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazine **11** (80 mg, 0.26 mmol) as starting material for 10 h. The crude product was purified by silica gel column chromatography using dichloromethane then dichloromethane/ethanol (95/5) as eluent to afford **10 c** (51 mg, 49%) as a beige powder. R_f 0.68 (CH_2Cl_2/EtOH 95:5); Mp: 159 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.42–2.46 (m, 4H, $4H_{morph}$), 2.60 (t, 2H, J = 6.4 Hz, CH_2), 3.56–3.64 (m, 6H, CH_2 and $4H_{morph}$), 7.32–7.39 (m, 4H, $3H_{ar}$ and NH), 7.43 (d, 1H, J = 4.6 Hz, H_5), 7.49–7.55 (m, 5H, $2H_{pyr}$, $2H_{ar}$ and H_6), 8.74 (d, 2H, J = 5.6 Hz, H_{pyr}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.2 (CH_2), 53.5 ($2CH_2$), 57.2 (CH_2), 66.4 ($2CH_2$), 107.2 (CH), 120.8 (C), 124.8 (2CH), 128.1 (2CH), 128.2 (CH), 128.7 (2CH), 129.5 (CH), 132.7 (C), 133.4 (C), 136.9 (C), 141.6 (C), 149.1 (C), 150.9 (2CH); MS (ESI) *m/z* (%): 401.2 (100) $[M+H]^+$. Anal. calcd for $C_{23}H_{24}N_6O$: C 68.98, H 6.04, N 20.99. Found: C 68.75, H 6.02, N 20.92.

*4.1.8.4. N′-[2-(4-Fluorophenyl)-3-(pyridin-4-yl)imidazo[1,2-a]pyrazin-8-yl]-N,N-dimethylethane-1,2-diamine **10d**.* Compound **10d** was obtained following the representative procedure, using *N*'-[2-(4-fluorophenyl)-3-iodoimidazo[1,2-*a*]pyrazin-8-yl]-*N*,*N*-dimethylethane-1,2-diamine **9d** (100 mg, 0.24 mmol). The crude product was purified by silica gel column chromatography using dichloromethane then dichloromethane/ethanol (95/5) as eluent to afford **10d** (40 mg, 44%) as a beige powder. R_f 0.11 (CH_2Cl_2/EtOH 9:1); Mp: 137 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 6H, $2CH_3$), 2.59 (t, 2H, J = 6.2 Hz, CH_2), 3.61 (q, 2H, J = 6.2 Hz, CH_2), 7.22 (t, 2H, J = 8.8 Hz, H_{ar}), 7.34 (d, 1H, J = 4.7 Hz, H_5), 7.40 (t, 1H, J = 5.5 Hz, NH), 7.44 (d, 1H, J = 4.7 Hz, H_6), 7.49 (d, 2H, J = 5.9 Hz, H_{pyr}), 7.55 (dd, 2H, J = 8.6 and 5.6 Hz, H_{ar}); 8.74 (d, 2H, J = 5.9 Hz, H_{pyr}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.4 (CH_2), 44.7 ($2CH_3$), 57.3 (CH_2),

106.8 (CH), 115.2 (d, $^{2}J_{C-F}$ = 21 Hz, 2CH), 120.5 (C), 124.5 (2CH), 129.1 (CH), 129.5 (d, $^{4}J_{C-F}$ = 3 Hz, C), 129.7 (d, $^{3}J_{C-F}$ = 8 Hz, 2CH), 132.4 (C), 136.5 (C), 140.5 (C), 148.9 (C), 150.7 (2CH), 161.9 (d, $^{1}J_{C-F}$ = 243 Hz, C); MS (ESI) *m/z* (%): 377.2 (100) $[M+H]^+$. Anal. calcd for $C_{21}H_{21}FN_6$: C 67.00, H 5.62, N 22.33. Found: C 67.20, H 5.63, N 22.40.

4.1.8.5. 2-(4-fluorophenyl)-N,N-dimethyl-3-(pyridin-4-yl)imidazo[1,2-a]pyrazin-8-amine ***10e****.* Compound **10e** was obtained following the representative procedure, using 2-(4-fluorophenyl)-3-iodo-*N*,*N*-dimethylimidazo[1,2-*a*]pyrazin-8-amine **9e** (150 mg, 0.39 mmol). The crude product was purified by silica gel column chromatography using cyclohexane/ethyl acetate (9/1) then cyclohexane/ethyl acetate (1/1) as eluent to afford **10e** (110 mg, 84%) as a white powder. R_f 0.22 (AcOEt); Mp: 141 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.53 (s, 6H, $2CH_3$), 7.20 (t, 2H, *J* = 8.8 Hz, H_{ar}), 7.35 (d, 1H, *J* = 4.5 Hz, H_5), 7.41 (d, 1H, *J* = 4.7 Hz, H_6), 7.50 (d, 2H, *J* = 5.7 Hz, H_{pyr}), 7.55 (dd, 2H, *J* = 8.6 and 5.6 Hz, H_{ar}); 8.76 (d, 2H, *J* = 5.7 Hz, H_{pyr}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 39.1 ($2CH_3$), 107.2 (CH), 115.2 (d, $^{2}J_{C-F}$ = 21 Hz, 2CH), 119.7 (C), 124.6 (2CH), 128.7 (CH), 129.3 (d, $^{3}J_{C-F}$ = 8 Hz, 2CH), 129.4 (d, $^{4}J_{C-F}$ = 3 Hz, C), 133.2 (C), 136.7 (C), 139.4 (C), 149.6 (C), 150.6 (2CH), 1618 (d, $^{1}J_{C-F}$ = 244 Hz, C); MS (ESI) *m/z* (%): 334.2 (100) $[M+H]^+$. Anal. calcd for $C_{19}H_{16}FN_5$: C 68.46, H 4.84, N 21.01. Found: C 68.69, H 4.86, N 21.07.

4.1.8.6. 2-(4-fluorophenyl)-N-[2-(morpholin-4-yl)ethyl]-3-(pyridin-4-yl)imidazo[1,2-a]pyrazin-8-amine ***10f****.* Compound **10f** was obtained following the representative procedure, using 2-(4-fluorophenyl)-3-iodo-*N*-[2-(morpholin-4-yl)ethyl]imidazo[1,2-*a*] pyrazin-8-amine **9f** (150 mg, 0.32 mmol). The crude product was purified by silica gel column chromatography using dichloromethane then dichloromethane/ethanol (95/5) as eluent to afford **10f** (70 mg, 52%) as a beige powder. R_f 0.17 (CH_2Cl_2/EtOH 9:1); Mp: 156 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.42–2.46 (m, 4H, $4H_{morph}$), 2.59 (t, 2H, *J* = 6.1 Hz, CH_2), 3.53–3.67 (m, 6H, CH_2 and $4H_{morph}$), 7.22 (t, 2H, *J* = 8.8 Hz, H_{ar}), 7.33 (d, 1H, *J* = 4.7 Hz, H_5), 7.43 (d, 1H, *J* = 4.7 Hz, H_6), 7.47–7.50 (m, 1H, NH), 7.49 (d, 2H, *J* = 5.8 Hz, H_{pyr}), 7.55 (dd, 2H, *J* = 8.6 and 5.6 Hz, H_{ar}); 8.74 (d, 2H, *J* = 5.8 Hz, H_{pyr}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.0 (CH_2), 53.3 ($2CH_2$), 56.9 (CH_2), 66.2 ($2CH_2$), 107.0 (CH), 115.5 (d, $^{2}J_{C-F}$ = 22 Hz, 2CH), 120.5 (C), 124.6 (2CH), 129.4 (CH), 129.7 (d, $^{4}J_{C-F}$ = 3 Hz, C), 129.9 (d, $^{3}J_{C-F}$ = 8 Hz, 2CH), 132.4 (C), 136.5 (C), 140.5 (C), 148.9 (C), 150.8 (2CH), 161.9 (d, $^{1}J_{C-F}$ = 244 Hz, C); MS (ESI) *m/z* (%): 419.2 (100) $[M+H]^+$. Anal. calcd for $C_{23}H_{23}FN_6O$: C 66.01, H 5.54, N 20.08. Found: C 66.24, H 5.56, N 20.14.

4.1.9. 8-chloro-2-phenyl-3-(pyridin-4-yl)imidazo[1,2-a]pyrazine ***11***

4.1.9.1. Method A. Compound **11** was obtained following the representative procedure used for the synthesis of compounds **10a**–**f** with 8-chloro-3-iodo-2-phenylimidazo[1,2-*a*]pyrazine **8a** (200 mg, 0.56 mmol) as starting material. The crude product was purified by silica gel column chromatography using dichloromethane then dichloromethane/ethanol (95/5) as eluent to afford **11** (88 mg, 51%) as a beige powder.

4.1.9.2. Method B. To a stirred solution of 8-chloro-2-phenylimidazo[1,2-*a*]pyrazine **7a** (100 mg, 0.43 mmol) in DMF (2 mL) was added 4-iodopyridine (179 mg, 0.86 mmol), cesium carbonate (213 mg, 0.65 mmol), triphenylphosphine (46 mg, 0.17 mmol) and palladium acetate (20 mg, 0.09 mmol). The vial was purged with argon for 10 min and then sealed. The mixture was heated at 90 °C for 16 h and then it was cooled to room temperature. Water was added and the aqueous layer was extracted with ethyl acetate (2 x 20 mL). The combined organic extracts were washed with brine and water, dried over sodium sulfate, filtered, concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using dichloromethane then dichloromethane/ethanol (95/5) as eluent to afford **11** (36 mg, 27%) as a beige powder.

R_f 0.42 (CH_2Cl_2/EtOH 9:1); Mp: 212 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.49–7.53 (m, 3H, H_{ar}), 7.61–7.63 (m, 4H, H_{ar} and H_{pyr}), 7.79 (d, 1H, *J* = 4.4 Hz, H_6), 8.35 (d, 1H, *J* = 4.4 Hz, H_5), 8.84 (d, 2H, *J* = 5.6 Hz, H_{pyr}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.3 (CH), 122.2 (C), 124.9 (2CH), 128.3 (2CH), 128.5 (CH), 128.9 (2CH), 129.0 (CH), 132.5 (C), 136.0 (C), 137.1 (C), 141.9 (C), 144.6 (C), 151.1 (2CH); MS (ESI) *m/z* (%): 307.1 (100) $[M+H]^+$, 309.1 (35) $[M+H+2]^+$. Anal. calcd for $C_{17}H_{11}ClN_4$: C 66.56, H 3.61, N 18.26. Found: C 66.76, H 3.59, N 18.31.

4.2. Biological evaluation

4.2.1. In vitro antileishmanial activity

Compounds were diluted in dimethylsulfoxide (DMSO) in view to obtain stock solutions (10 mM). Dilutions of each compound were realized in medium in accordance with cell line culture and at a maximum final concentration of 1% DMSO.

4.2.1.1. Strains. L. major (MHOM/IL/81/BNI) promastigotes were maintained by weekly subpassages in Schneider's insect medium (Sigma chemical Co. St Louis, Mo) supplemented with 13% heat-inactivated foetal bovine serum (FBS, Sigma–Aldrich) at 26 °C [19]. Axenically grown amastigote forms of *L. amazonensis* (MHOM/BR/76/LTB-012) were maintained by weekly subpassages in MAA/20 medium at 32 ± 1 °C [47].

4.2.1.2. Antileishmanial activity against promastigote stage of Leishmania major. Promastigote susceptibility testing was performed with a spectrofluorimetric micromethod previously described [48]. Briefly, 100 μL of a 10^6 promastigotes/mL suspension were placed into wells of a 96-well microplate (Nunc®). The cultures were exposed for 96 h at 26 °C to the drugs (100, 10 and 1 μM). Four hours before measurement, 10 μL of resazurin solution (700 μM) were added. Then fluorescence was measured at 590 nm with an excitation at 550 nm. Appropriate controls treated by DMSO, pentamidine (reference drugs purchased from Sigma–Aldrich) were added to each two sets of experiments.

4.2.1.3. Antileishmanial activity against amastigote stage of L. major. Activity against the intracellular amastigote stage of the parasite was determined after infection of Balb/c mice peritoneal macrophages (CE Janvier, Le Genest, France). 100 μL of a peritoneal macrophage suspension were placed into a 24-well plate (Nunc®) on glass slides (10 mm diameter) at 1.5 10^5 cell/mL in RPMI 1640 with 15% FBS at 37 °C and 5% CO_2. Following a 24 h-incubation to allow attachment, macrophages were infected with 100 μL of a stationary phase promastigotes (1.5 × 10^6 promastigotes/mL in RPMI 1640 medium plus 15% FBS) and then incubated for a 24 h-period at 37 °C and 5% CO_2 for infection. Macrophage culture was washed and exposed to drugs at concentrations of 100, 10, 1 and 0.1 μM. After 4 days, cultures were fixed with methanol, stained with May-Grunwald-Giemsa and microscopically examined. The average number of amastigotes per macrophage was determined by counting the number of amastigotes in 100 randomly chosen macrophages in each duplicate well. IC_{50} values were calculated by using the values of the number of amastigotes per macrophage [19]. Pentamidine was used as a reference at the concentrations of 100, 10 and 1 μM. Experiments were done twice.

4.2.1.4. Antileishmanial activity against axenic amastigote stage of Leishmania amazonensis. The *in vitro* leishmanicidal activity of compounds was determined in axenic cultures of amastigotes of

L. amazonensis. To estimate the 50% inhibitory concentration (IC_{50}) of the compounds, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) micromethod was used as previously described [49].

4.2.2. SsCK1 and LmCK1 (lmjF35.1010) kinases inhibitory activities
4.2.2.1. Buffer. Buffer A: 60 mM β-glycerophosphate, 30 mM *p*-nitrophenylphosphate, 25 mM Mops (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 0.1 mM sodium vanadate, 1 mM phenylphosphate.

4.2.2.2. Kinase preparations and assays. Kinase activities were assayed in Buffer A, at 30 °C, at a final ATP concentration of 15 μM. Blank values were subtracted and activities expressed in % of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO. *Ss*CK1 and *Lm*CK1 peptide substrates were obtained from Proteogenix (Oberhausbergen, France).

*Ss*CK1δ/ε (*S. scrofa* porcine brain, native) was assayed in three-fold diluted buffer A, as previously described [50,51] but using 25 μM CKS peptide (RRKHAAIGpSAYSITA), a *Ss*CK1-specific substrate [52]. Its kinase activity was assayed in buffer A, with 1 mg histone H1/mL, in the presence of 15 μM [γ-^{33}P] ATP (3000 Ci/mmol; 10 mCi/mL) in a final volume of 30 μL. After incubation for 30 min at 30 °C, the reaction was stopped by deposing 25 mL onto P81 phosphocellulose papers (Whatman) using a FilterMate harvester (Perkin–Elmer) and were washed in 1% phosphoric acid. Scintillation fluid was added and the radioactivity measured in a Perkin–Elmer counter. *Lm*CK1.2 (LmjF35.1010, *L. major*, recombinant, His-fusion protein in *Escherichia coli*) [34] was purified by affinity chromatography on cobalt beads and its kinase activity was assayed as described for *Ss*CK1.

4.2.3. Cell cytotoxicity bioassays
4.2.3.1. Cells. Cytotoxicity of the compounds was evaluated in various tumour cell lines and in a normal cell lines using the sulforhodamine B (SRB) assay method [18]. Cell lines tested include BALB/3T3 (non-tumorogenic, BALB/c mouse embryo cells), HuTu 80 (human duodenal carcinoma), H460 (human lung large cell carcinoma), M–14 (human amelanotic melanoma), DU145 (human prostate carcinoma), MCF-7 (human breast adenocarcinoma), HT-29 (human colon adenocarcinoma), and K562 (human chronic myelogenous leukaemia).

4.2.3.2. Macrophages. Murine macrophages were harvested from peritoneal cavities of 6–8 week-old female BALB/c mice in ice-cold M199 medium supplemented with 10% FBS. Extracted cells were immediately deposited on sterile 4 × 4 mm cover glasses and placed in each well of a 96-well plate. Plates were incubated for 24 h at 37 °C, 5% CO_2 to allow cell adhesion. Pre-warmed complete M199 medium was used twice to remove non-adherent cells. A neutral red method was employed to determine cell concentration. Approximately 7×10^4 viable cells were deposited in each well for adhesion [18].

4.2.3.3. Determination of toxicity to macrophages. Murine peritoneal macrophages were treated with appropriate dilutions of tested compounds and the trypan blue dye exclusion method was used [44]. Dilutions of 10, 1 and 0.1 μM in complete medium were then added to achieve a final volume of 100 μL. The culture was continued for another 48 h at 37 °C in 5% CO_2. After this incubation, the number of viable cells was scored by hematocytometer using 0.4% trypan blue solution in PBS. The half-maximal cytotoxic dose 50 for each cell type was determined. All experiments were repeated 3 times.

4.2.3.4. Cytotoxicity assays. To determine the cytotoxicity of the compounds, cells were plated into 96-well tissue culture plates and in their corresponding growth medium, and incubated at 37 °C in a 5% CO_2 and 95% air humidified atmosphere for 24 h to allow cells to attach. A plate containing each of these cells was fixed *in situ* with trichloroacetic acid (TCA) in order to obtain the cell values at cero time before adding the compounds. The rest of the plates containing the different cell lines received serial 4-fold dilutions of the compound to be tested. The plates were then incubated at 37 °C in a 5% CO_2 and 95% air humidified atmosphere for 48 h. The assay was terminated by the addition of cold TCA. TCA treated plates were incubated at 4 °C for 1 h and then washed 5 times with tap water to remove TCA and air dried. Background optical densities were measured in wells incubated with growth medium without cells. TCA-fixed cells were stained for 30 min with 0.2% (w/v) SRB dissolved in 1% acetic acid. At the end of the staining period unbound dye was removed by washing 4 times with 1% acetic acid. After air drying the plates, bound dye was solubilized with 10 mM Tris base (pH 10.5) and the absorbance read on an automated plate reader at a wavelength of 510 nm. The GI_{50} value was defined as the concentration of test sample resulting in a 50% reduction of absorbance as compared with untreated controls that received a serial dilution of the solvent in which the test samples were dissolved, and was determined by linear regression analysis. For K562 cells, which grow in suspension, instead of fixing and staining with SRB, cells were counted using a Coulter counter. 5-Fluorouracil (5-FU) was used as a reference compound for the testing.

Acknowledgements

We thank ChemAxon Ltd for providing the MarvinSketch software. For *Lm*CK1 and *Ss*CK1 evaluation, this work was supported by Cancéropôle Grand Ouest – Line “Valorisation des produits de la mer”.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.09.002.

References

[1] World Health Organization, Leishmaniasis, Fact sheet N°375 updated February 2015. Information site: http://www.who.int/mediacentre/factsheets/fs375/en/.
[2] S. Antinori, L. Schifanella, M. Corbellino, Leishmaniasis: new insights from an old and neglected disease, Eur. J. Clin. Microbiol. Infect. Dis. 31 (2012) 109–118.
[3] J.N. Sangshetti, F.A. Kalam Khan, A.A. Kulkarni, R. Arote, R.H. Patil, Antileishmanial drug discovery: comprehensive review of the last 10 years, RSC Adv. 5 (2015) 32376–32415.
[4] A.S. Nagle, S. Khare, A.B. Kumar, F. Supek, A. Buchynskyy, C.J.N. Mathison, N.K. Chennamaneni, N. Pendem, F.S. Buckner, M.H. Gelb, V. Molteni, Recent developments in drug discovery for leishmaniasis and human african trypanosomiasis, Chem. Rev. 114 (2014) 11305–11347, 4.2.1.
[5] H. Hussain, A. Al-Harrasi, A. Al-Rawahi, I.R. Green, S. Gibbons, Fruitful decade for antileishmanial compounds from 2002 to Late 2011, Chem. Rev. 114 (2014) 10369–10428.
[6] N. Singh, M. Kumar, R.K. Singh, Leishmaniasis: current status of available drugs and new potential drug targets, Asian Pac. J. Trop. Med. (2012) 485–497.
[7] T. Kobets, I. Grekov, M. Lipoldová, Leishmaniasis: prevention, parasite detection and treatment, Curr. Med. Chem. 19 (2012) 1443–1474.
[8] T.S. Tiuman, A.O. Santos, T. Ueda-Nakamura, B.P. Dias Filho, C.V. Nakamura, Recent advances in leishmaniasis treatment, Int. J. Infect. Dis. 15 (2011) e525–e532.
[9] S. Malik, S. Kumar, A. Choudhary, A. Kumar, A. Singh, G. Avasthi, Leishmaniasis: current treatment strategies and future opportunities, J. Chem. Pharm. Res. 2 (2010) 70–91.
[10] N. Singh, B.B. Mishra, S. Bajpai, R.K. Singh, V.K. Tiwari, Natural product based leads to fight against leishmaniasis, Bioorg. Med. Chem. 22 (2014) 18–45.
[11] D. Pathak, M. Yadav, N. Siddiqui, S. Kushawah, Antileishmanial agents: an

updated review, Der Pharma Chem. 3 (2011) 239–249.
[12] M. Jain, S.I. Khan, B.L. Tekwani, M.R. Jacob, S. Singh, P.P. Singh, R. Jain, Synthesis, antimalarial, antileishmanial, and antimicrobial activities of some 8-quinolinamine analogues, Bioorg. Med. Chem. 13 (2005) 4458–4466.
[13] C. Kieffer, A. Cohen, P. Verhaeghe, L. Paloque, S. Hutter, C. Castera-Ducros, M. Laget, S. Rault, A. Valentin, P. Rathelot, N. Azas, P. Vanelle, Antileishmanial pharmacomodulation in 8-nitroquinolin-2(1*H*)-one Series, Bioorg. Med. Chem. 23 (2015) 2377–2386.
[14] K.C. Agarwal, V. Sharma, N. Shakya, S. Gupta, Design and synthesis of novel substituted quinazoline derivatives as antileishmanial agents, Bioorg. Med. Chem. Lett. 19 (2009) 5474–5477.
[15] J. Cogo, V. Kaplum, D.P. Sangi, T. Ueda-Nakamura, A.G. Corrêa, C.V. Nakamura, Synthesis and biological evaluation of novel 2,3-disubstituted quinoxaline derivatives as antileishmanial and antitrypanosomal agents, Eur. J. Med. Chem. 90 (2015) 107–123.
[16] C.D. Giorgio, K. Shimi, G. Boyer, F. Delmas, J.P. Galy, Synthesis and antileishmanial activity of 6-mono-substituted and 3,6-di-substituted acridines obtained by acylation of proflavine, Eur. J. Med. Chem. 42 (2007) 1277–1284.
[17] C.C. Musonda, G.A. Whitlock, M.J. Witty, R. Brun, M. Kaiser, Synthesis and evaluation of 2-pyridyl pyrimidines with in vitro antiplasmodial and antileishmanial activity, Bioorg. Med. Chem. Lett. 19 (2009) 401–405.
[18] J.C. Aponte, A.J. Vaisberg, D. Castillo, G. Gonzalez, Y. Estevez, J. Arevalo, M. Quiliano, M. Zimic, M. Verástegui, E. Málaga, R.H. Gilman, J.M. Bustamante, R.L. Tarleton, Y. Wang, S.G. Franzblau, G.F. Pauli, M. Sauvain, G.B. Hammonda, Trypanoside, anti-tuberculosis, leishmanicidal, and cytotoxic activities of tetrahydrobenzothienopyrimidines, Bioorg. Med. Chem. 18 (2010) 2880–2886.
[19] F. Pagniez, H. Abdala, P. Marchand, M. Le Borgne, S. Robert-Piessard, P. Le Pape, 3-(α-Azolylbenzyl)indoles: in vitro and in vivo anti-Leishmania activity and mechanism of action, J. Enzym. Inhib. Med. Chem. 21 (2006) 277–283.
[20] P. Marchand, M. Le Borgne, Y.M. Na, F. Pagniez, N. Alvarez, G. Le Baut, P. Le Pape, Synthesis and antileishmanial activity of 3-(α-azolylbenzyl)indoles, J. Enzym. Inhib. Med. Chem. 17 (2002) 353–358.
[21] S. Marhadour, P. Marchand, F. Pagniez, M.-A. Bazin, C. Picot, O. Lozach, S. Ruchaud, M. Antoine, L. Meijer, N. Rachidi, P. Le Pape, Synthesis and biological evaluation of 2,3-diarylimidazo[1,2-*a*]pyridines as antileishmanial agents, Eur. J. Med. Chem. 58 (2012) 543–556.
[22] A.K. Shukla, B.K. Singh, S. Patra, V.K. Dubey, Rational approaches for drug designing against leishmaniasis, Appl. Biochem. Biotechnol. 160 (2010) 2208–2218.
[23] C. Merritt, L.E. Silva, A.L. Tanner, K. Stuart, M.P. Pollastri, Kinases as druggable targets in trypanosomatid protozoan parasites, Chem. Rev. 114 (2014) 11280–11304.
[24] D.P. Rotella, Recent results in protein kinase inhibition for tropical diseases, Bioorg. Med. Chem. Lett. 22 (2012) 6788–6793.
[25] E. Øezníèková, A. Popa, T. Gucký, M. Zatloukal, L. Havlíèek, V. Bazgier, K. Berka, R. Jorda, I. Popa, A. Nasereddin, C.L. Jaffe, V. Kryštof, M. Strnad, 2,6,9-trisubstituted purines as CRK3 kinase inhibitors with antileishmanial activity in vitro, Bioorg. Med. Chem. Lett. 25 (2015) 2298–2301.
[26] R. Jorda, N. Sacerdoti-Sierra, J. Voller, L. Havlíček, K. Kráčalíková, M.W. Nowicki, A. Nasereddin, V. Kryštof, M. Strnad, M.D. Walkinshaw, C.L. Jaffe, Anti-leishmanial activity of disubstituted purines and related pyrazolo[4,3-d]pyrimidines, Bioorg. Med. Chem. Lett. 21 (2011) 4233–4237.
[27] L.A.T. Cleghorn, A. Woodland, I.T. Collie, L.S. Torrie, N. Norcross, T. Luksch, C. Mpamhanga, R.G. Walker, J.C. Mottram, R. Brenk, J.A. Frearson, I.H. Gilbert, P.G. Wyatt, Identification of inhibitors of the *Leishmania* cdc2-related protein kinase CRK3, ChemMedChem 6 (2011) 2214–2224.
[28] M.A. Morales, P. Pescher, G.F. Späth, *Leishmania major* MPK7 protein kinase activity inhibits intracellular growth of the pathogenic amastigote stage, Eukaryot. Cell 9 (2010) 22–30.
[29] P. Saravanan, S.K. Venkatesan, C.G. Mohan, S. Patra, V.K. Dubey, Mitogen-activated protein kinase 4 of *Leishmania* parasite as a therapeutic target, Eur. J. Med. Chem. 45 (2010) 5662–5670.
[30] M. Wiese, *Leishmania* MAP kinases – familiar proteins in an unusual context, Int. J. Parasitol. 37 (2007) 1053–1062.
[31] K.M. Grant, Targeting the cell cycle in the pursuit of novel chemotherapies against parasitic protozoa, Curr. Pharm. Des. 14 (2008) 917–924.
[32] N. Alvarez-Rueda, M. Biron, P. Le Pape, Infectivity of *Leishmania mexicana* is associated with differential expression of protein kinase C-like triggered during a cell-cell contact, PLoS One 4 (2009) e7581, 1–9.
[33] J.J. Allocco, R. Donald, T. Zhong, A. Lee, Y.S. Tang, R.C. Hendrickson, P. Liberator, B. Nare, Inhibitors of casein kinase 1 block the growth of *Leishmania major* promastigotes in vitro, Int. J. Parasitol. 36 (2006) 1249–1259.
[34] N. Rachidi, J.-F. Taly, E. Durieu, O. Leclercq, N. Aulner, E. Prina, P. Pescher, C. Notredame, L. Meijer, G.F. Späth, Pharmacological assessment defines *Leishmania donovani* casein kinase 1 as a drug target and reveals important functions in parasite viability and intracellular infection, Antimicrob. Agents Chemother. 58 (2014) 1501–1515.
[35] M. Dan-Goor, A. Nasereddin, H. Jaber, C.L. Jaffe, Identification of a secreted casein kinase 1 in *Leishmania donovani*: effect of protein over expression on parasite growth and virulence, PLoS ONE 8 (2013) e79287, 1–10.
[36] S. Marhadour, M.-A. Bazin, P. Marchand, An efficient access to 2,3-diarylimidazo[1,2-*a*]pyridines *via* imidazo[1,2-*a*]pyridine-2-yltriflate through a Suzuki cross-coupling reaction-direct arylation sequence, Tetrahedron Lett. 53 (2012) 297–300.
[37] M.-A. Bazin, S. Marhadour, A. Tonnerre, P. Marchand, Exploration of versatile reactions on 2-chloro-3-nitroimidazo[1,2-*a*]pyridine: expanding structural diversity of C2- and C3-functionalized imidazo[1,2-*a*]pyridines, Tetrahedron Lett. 54 (2013) 5378–5382.
[38] R. Goel, V. Luxami, K. Paul, Recent advances in development of imidazo[1,2-*a*] pyrazines: synthesis, reactivity and their biological applications, Org. Biomol. Chem. 13 (2015) 3525–3555.
[39] S. Myadaraboina, M. Alla, V. Saddanapu, V.R. Bommenaa, A. Addlagatta, Structure activity relationship studies of imidazo[1,2-*a*]pyrazine derivatives against cancer cell lines, Eur. J. Med. Chem. 45 (2010) 5208–5216.
[40] B. Liégeault, D. Lapointe, L. Caron, A. Vlassova, K. Fagnou, Establishment of broadly applicable reaction conditions for the palladium-catalyzed direct arylation of heteroatom-containing aromatic compounds, J. Org. Chem. 74 (2009) 1826–1834.
[41] B.M. Johnson, M.P. Huestis, Sequential C3 and C5 direct C–H arylation of imidazo[1,2-*a*]pyrazines with (hetero)aryl bromides, Eur. J. Org. Chem. (2014) 1589–1593.
[42] X. Cao, Z. Sun, Y. Cao, R. Wang, T. Cai, W. Chu, W. Hu, Y. Yang, Design, synthesis, and structure–activity relationship studies of novel fused heterocycles-linked triazoles with good activity and water solubility, J. Med. Chem. 57 (2014) 3687–3706.
[43] J.M. Bartolome-Nebreda, F. Delgado, M.L. Martín-Martín, C.M. Martínez-Viturro, J. Pastor, H.M. Tong, L. Iturrino, G.J. Macdonald, W. Sanderson, A. Megens, X. Langlois, M. Somers, G. Vanhoof, S. Conde-Ceide, Discovery of a potent, selective, and orally active phosphodiesterase 10A inhibitor for the potential treatment of schizophrenia, J. Med. Chem. 57 (2014) 4196–4212.
[44] D. Castillo, J. Arevalo, F. Herrera, C. Ruiz, R. Rojas, E. Rengifo, A. Vaisberg, O. Lock, J.-L. Lemesre, H. Gornitzka, M. Sauvain, Spirolactone iridoids might be responsible for the antileishmanial activity of a Peruvian traditional remedy made with *Himatanthus sucuuba* (Apocynaceae), J. Ethnopharmacol. 112 (2007) 410–414.
[45] N. Gupta, N. Goyal, A.K. Rastogi, *In vitro* cultivation and characterization of axenic amastigotes of *Leishmania*, Trends Parasitol. 17 (2001) 150–153.
[46] P. Pescher, T. Blisnick, P. Bastin, G.F. Späth, Quantitative proteome profiling informs on phenotypic traits that adapt Leishmania donovani for axenic and intracellular proliferation, Cell. Microbiol. 13 (2011) 978–991.
[47] D. Sereno, J.-L. Lemesre, Axenically cultured amastigote forms as an *in vitro* model for investigation of antileishmanial agents, Antimicrob. Agents Chemother. 41 (1997) 972–976.
[48] P. Le Pape, F. Pagniez, H. Abdala-Valencia, A new automatized fluorometric assay for anti-*Leishmania* drug screening, Acta Parasitol. 47 (2002) 79–81.
[49] D. Sereno, J.-L. Lemesre, Use of an enzymatic micromethod to quantify amastigote stage *of Leishmania amazonensis in vitro*, Parasitol. Res. 83 (1997) 401–403.
[50] S. Leclerc, M. Garnier, R. Hoessel, D. Marko, J.A. Bibb, G.L. Snyder, P. Greengard, J. Biernat, E.-M. Mandelkow, G. Eisenbrand, L. Meijer, Indirubins inhibit glycogen synthase kinase-3β and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease, J. Biol. Chem. 276 (2001) 251–260.
[51] S. Bach, M. Knockaert, J. Reinhardt, O. Lozach, S. Schmitt, B. Baratte, M. Koken, S.P. Coburn, L. Tang, T. Jiang, D.C. Liang, H. Galons, J.F. Dierick, L.A. Pina, F. Meggio, F. Totzke, C. Schächtele, A.S. Lerman, A. Carnero, Y. Wan, N. Gray, L. Meijer, Roscovitine targets, protein kinases and pyridoxal kinase, J. Biol. Chem. 280 (2005) 31208–31219.
[52] J. Reinhardt, Y. Ferandin, L. Meijer, Purification of CK1 by affinity chromatography on immobilised axin, Protein Expr. Purif. 54 (2007) 101–109.