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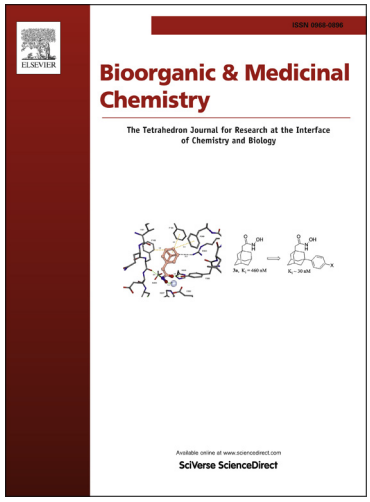

Targeting the human parasite *Leishmania donovani*: Discovery of a new promising anti-infectious pharmacophore in 3-nitroimidazo[1,2-*a*]pyridine series

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Graphical Abstract

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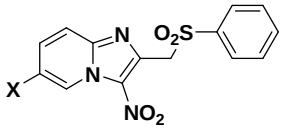

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Targeting the human parasite *Leishmania donovani*: Discovery of a new promising anti-infectious pharmacophore in 3-nitroimidazo[1,2-*a*]pyridine series

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ABSTRACT

We report herein the discovery of antileishmanial molecules based on the imidazo[1,2-*a*]pyridine ring. *In vitro* screenings of imidazopyridines belonging to our chemical library, toward the promastigotes stage of *Leishmania donovani*, J774A.1 murine and HepG2 human cells, permitted to identify 3 selective hit-compounds (**12**, **20**, **28**). New derivatives were then synthesized to allow structure-activity and -toxicity relationships analyses, enabling to characterize a lead-compound (**44**) displaying both a high potency (IC_{50} = 1,8 µM) and a good selectivity index, in comparison with 3 antileishmanial reference drug-compounds (amphotericin B, miltefosine and pentamidine). Moreover, lead-compound **44** also exhibits good *in vitro* activity against the intracellular amastigote stage of *L. donovani*. Thus, the 6-halo-3-nitro-2-(phenylsulfonylmethyl)imidazo[1,2-*a*]pyridine scaffold appears as a new promising selective antileishmanial pharmacophore, especially when substituted at position 8 by a bromine atom.

1. Introduction

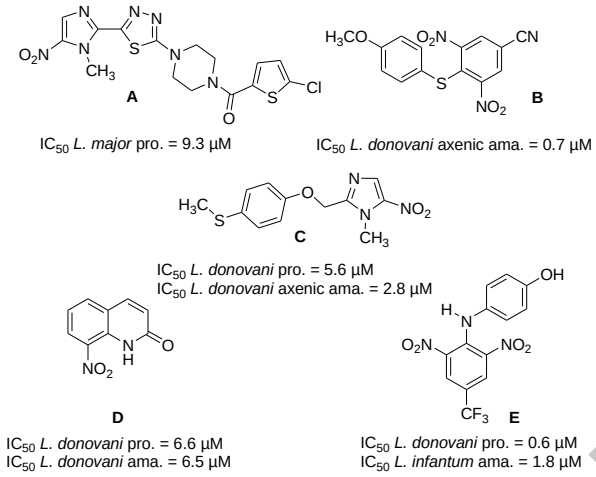
Leishmaniasis is a parasitic infection second to malaria in terms of parasite-related mortality. Leishmaniasis pathologies include cutaneous leishmaniasis (CL), muco-cutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL), depending on *Leishmania* species. Visceral leishmaniasis is the most severe clinical form of the disease, lethal in untreated patients. There are an estimated 14 million people infected by leishmaniasis in 98 countries worldwide, mainly in tropical and sub-tropical regions, but also in southern Europe, especially around the Mediterranean area. The annual incidence of leishmaniasis is 2 million with about 50.000 estimated deaths due to its visceral form. The number of cases is certainly under-evaluated as leishmaniasis are reportable diseases in only 40 countries[1]. These parasitic infections are caused by a protozoan of the *Leishmania* genus transmitted to its mammal hosts (humans, dogs, monkeys, rodents…) by the bite of an infected sandfly (*Phlebotominae*). *Leishmania* parasites exist in two major morphological stages: extracellular flagellated promastigotes in the digestive tract of their sandfly vector and non-motile intracellular amastigotes in cells of their host mononuclear phagocytic system[2,3].

There are very few drugs available for treating leishmaniasis: pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), liposomal amphotericin B, pentamidine, paromomycin and miltefosine[4,5]. Some other drugs are in clinical trials: sitamaquine, imiquimod and antifungal azoles[6,7]. Currently, the drugs used in the treatment of leishmaniasis present major limitations such as non-oral routes of administration (apart for miltefosine), high toxicity of antimonials and pentamidine, and teratogenicity for miltefosine[8]. Moreover, the expensive cost of liposomal amphotericin B, and the socio-economic problems encountered in endemic areas, lead to patients withdrawing from treatment and global emergence of resistant strains[9]. During the last decade, antimonial resistance reached epidemic dimension in Bihar, India, where about 60% of newly diagnosed visceral leishmaniasis do not respond to these molecules[10]. Evaluation of the *in vitro* susceptibility of Indian *L. donovani* patient isolates to antimonials, amphotericin B and miltefosine indicates that cross-resistance may be emerging among these three drugs. Despite the absence of reported cases of clinical resistance to miltefosine, the preliminary data of clinical trials suggest a doubling of the relapse rates, leading to predict a fast development of the resistance[11]. Thus, efficient, safe and

cheap oral antileishmanial agents are needed to safeguard against the expanding resistance problem and to overcome miltefosine's shortcomings[12].

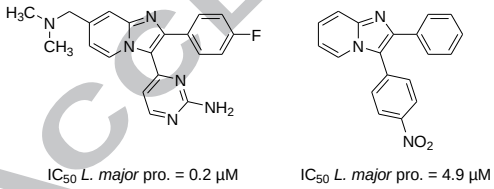
A bibliographical review about molecules exhibiting antileishmanial activity shows that nitroaromatic compounds have already been reported[13,14] as interesting agents (**Figure 1**), displaying inhibitory concentrations 50% (IC_{50}) in the micromolar range toward the promastigote and/or the amastigote stages of the parasite, in particular in 5-nitroimidazole (A and C)[15,16], dinitrodiphenylsulfane (B)[17], nitroquinolin-2(1*H*)-one (D)[18] and dinitrodiphenylamine (E)[19] series.

Figure 1. Structures of some nitroaromatic compounds displaying antileishmanial properties.



Thanks to the sequencing of the parasite genome[20], new targets were identified in order to come up with new powerful antileishmanial drugs[21]. Among them, *Leishmania* kinases appear to be of great interest. Thus, some imidazo[1,2-*a*]pyridines substituted at positions 2 and 3 have demonstrated antileishmanial activity, notably due to the inhibition of the parasite casein-kinase 1 (**Figure 2**)[22,23].

Figure 2. Structure of previously described antileishmanial imidazo[1,2-*a*]pyridines.



From these bibliographical data, and in continuation with our efforts toward the conception and preparation of original molecules bearing anti-infectious potential[24,25], focusing on the development of new antiplasmodial[26,27] and antileishmanial[18,28] agents, we recently investigated the antileishmanial potential of new imidazo[1,2-*a*]pyridine derivatives. A large series of molecules was prepared and screened *in vitro* on the promastigote stage of *Leishmania donovani*, in parallel with 3 antileishmanial drug-compounds. To assess the selectivity of action of these molecules, cytotoxicity evaluations were also conducted on the murine J774A.1 macrophage cell line and on the human HepG2 hepatocyte cell line. This medicinal chemistry study allowed us to identify a new potent antileishmanial lead-compound and also to characterize the corresponding pharmacophore whose antileishmanial spectrum of activity was more completely highlighted.

Twenty-one compounds with a 3-nitroimidazo[1,2-*a*]-pyridine backbone from our library were randomly screened against *L. donovani*. Based on the activity of these compounds, we wanted to further investigate the influence of the nitro group. Therefore, an additional 23 compounds were prepared and screened.

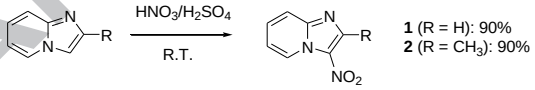
2. Results and Discussion

2.1. Chemistry

Among these 44 synthesized compounds, 21 were previously described (labeled* in the result and discussion part) and came from our internal chemical library, part of the French National Chemical Library (Chimiothèque Nationale, CNRS), while 23 were newly prepared for studying structure-activity relationships.

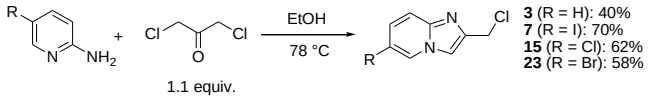
As initially reported[29,30], imidazo[1,2-*a*]pyridine and 2-methylimidazo[1,2-*a*]pyridine can be nitrated at room temperature to afford, respectively, compounds **1*** and **2*** in 90% yield (**Scheme1**).

Scheme 1. Synthesis of compounds **1*** and **2***



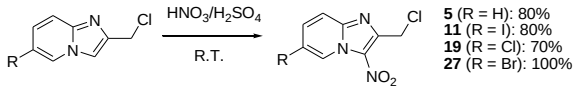
Following an operating procedure which we previously reported[31,32], 2-chloromethylimidazo[1,2-*a*]pyridines **3***, **7**, **15*** and **23*** were obtained by cyclo-condensation of 1,3-dichloroacetone with corresponding 2-aminopyridines in refluxing ethanol (**Scheme 2**).

Scheme 2. Formation of the imidazopyridine ring by a cyclo-condensation reaction



2-Chloromethyl-3-nitroimidazo[1,2-*a*]pyridines **5***, **11**, **19*** and **27*** were then obtained by nitration at room temperature[33,34] (**Scheme 3**).

Scheme 3. Nitration reactions in 2-chloromethylimidazo[1,2-*a*]series.



2-Chloromethylimidazo[1,2-*a*]pyridines bearing a hydrogen or a halogen atom at position 6 were involved into a microwave-assisted reaction with various sulfonylchlorides, according to a procedure which we previously reported,[35,36] affording corresponding sulfones in 25 to 100% yield (**Scheme 4**). The obtained molecules are presented in **Table 1**.

Scheme 4. Preparation of sulfone derivatives under microwave irradiation

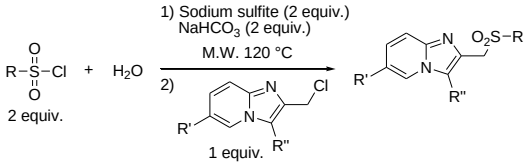
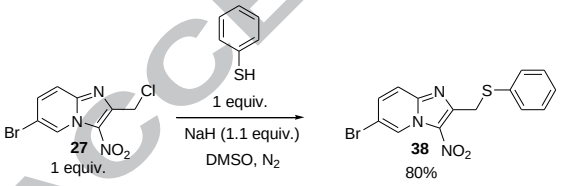


Table 1. Sulfone synthesized according to **Scheme 4**

	R-	R'-	R''-	Yield
4	C_6H_5-	-H	-H	60%
6	C_6H_5-	-H	-NO_2	60%
8	C_6H_5-	-I	-H	74%
9	4-CH_3-C_6H_4-	-I	-H	80%
10	4-Cl-C_6H_4-	-I	-H	53%
12	C_6H_5-	-I	-NO_2	90%
13	4-CH_3-C_6H_4-	-I	-NO_2	100%
14	4-Cl-C_6H_4-	-I	-NO_2	90%
16*	C_6H_5-	-Cl	-H	69%
17	4-CH_3-C_6H_4-	-Cl	-H	88%
18	4-Cl-C_6H_4-	-Cl	-H	88%
20*	C_6H_5-	-Cl	-NO_2	80%
21*	4-CH_3-C_6H_4-	-Cl	-NO_2	86%
22	4-Cl-C_6H_4-	-Cl	-NO_2	76%
24*	C_6H_5-	-Br	-H	100%
25	4-CH_3-C_6H_4-	-Br	-H	94%
26	4-Cl-C_6H_4-	-Br	-H	89%
28*	C_6H_5-	-Br	-NO_2	90%
29*	4-CH_3-C_6H_4-	-Br	-NO_2	90%
30*	4-Cl-C_6H_4-	-Br	-NO_2	60%
31	4-Br-C_6H_4-	-Br	-NO_2	80%
32	4-I-C_6H_4-	-Br	-NO_2	30%
33	4-F-C_6H_4-	-Br	-NO_2	44%
34	4-CF_3-C_6H_4-	-Br	-NO_2	60%
35	4-OCH_3-C_6H_4-	-Br	-NO_2	46%
36	CH_3-	-Br	-NO_2	25%
37	4-C_6H_5-C_6H_4-	-Br	-NO_2	40%

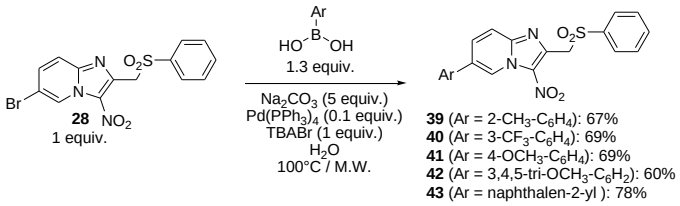
Sulfane derivative **38*** was prepared by reacting **27*** with thiophenol (**Scheme 5**) in the presence of sodium hydride in dry DMSO.[33]

Scheme 5. Preparation of sulfane derivative **38***



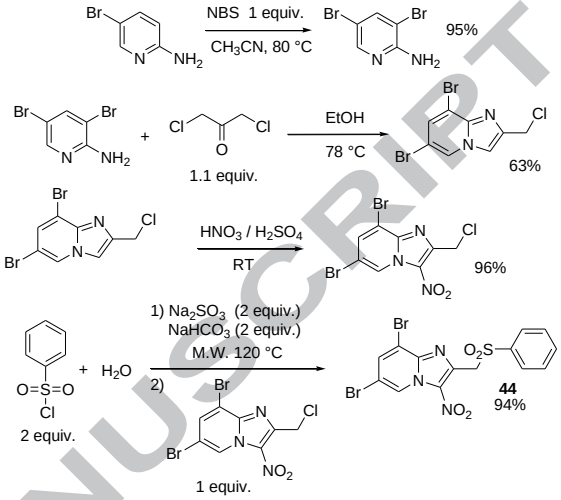
6-Bromo-3-nitro-2-phenylsulfonylmethylimidazo[1,2-*a*]pyridine **28*** was involved into microwave-assisted Suzuki-Miyaura cross-coupling reactions[37] with boronic acids (**Scheme 6**), to afford compounds **39***, **40***, **41***, **42**, **43*** in good yields[38,39].

Scheme 6. Suzuki-Miyaura coupling reactions leading to derivatives **39*-43***



Finally, molecule **44*** which synthesis was first described in our lab by Szabo *et al.*[40] was prepared in 4 steps from 2-amino-5-bromopyridine with a 54% global yield (**Scheme 7**).

Scheme 7. Four-step synthesis of lead-compound **44***



2.2. Biological activity

All molecules were first screened *in vitro* on the promastigote stage of *L. donovani* by determining their inhibitory concentrations 50% (IC_{50}) and comparing them to pentamidine, miltefosine and amphotericin B, chosen as antileishmanial reference drugs. In order to assess their selectivity of action, all molecules were also evaluated *in vitro* in regards to their cytotoxicity toward two complementary cell lines, measured by the cytotoxic concentrations 50% (CC_{50}) on the murine J774A.1 macrophage and metabolizing human hepatocyte HepG2 cell lines (positive control = doxorubicin), giving access to the corresponding selectivity indexes (SI = CC_{50} / IC_{50}). The results are presented in **Table 2**.

A global analysis of the results first shows that neither the imidazo[1,2-*a*]pyridine ring nor its nitrated counterpart **1** display any antileishmanial properties. Among all 45 tested molecules, only thirteen (**5***, **12**-**14**, **17**, **20***, **22**, **27***-**31** and **44***) presented measurable activity against the parasite, all of them including a nitro group at position 3 of the imidazo[1,2-*a*]pyridine ring, except molecule **17**. However, molecules **5***, **27*** and **29*** appeared to be toxic toward, at least, one of the cell lines used for measuring cytotoxicity. Then, out of the nine remaining nitrated molecules, only four (**12**, **20***, **28*** and **44***) displayed significant antileishmanial activities (IC_{50} < 20 µM) objectivized by significantly higher selectivity indexes (> 5 by considering the HepG2 human cell line). From a structural point of view, these four hit-molecules appear to be very closely related, sharing the same 6-halo-3-nitro-2-phenylsulfonyl-methylimidazo[1,2-*a*]pyridine scaffold.

When comparing the activities of the hit-molecules to the one of compound **6**, it can be concluded that a halogen atom at position 6 of the scaffold is necessary for providing antileishmanial activity to this series. Comparison of the hit-molecules to non-nitrated analogs **8**, **16*** and **24*** shows that the nitro group is mandatory for conferring activity in the studied series. Moreover, sulfane analog **38*** of sulfone hit-molecule **28*** appears very toxic, indicating that the sulfone group is also required for designing selective antileishmanial molecules. Finally, when studying the influence of the nature of the sulfone moiety (compounds **28*** to **37**), it can be concluded that the

unsubstituted phenylsulfonyl moiety is the only one which confers satisfying antileishmanial profile.

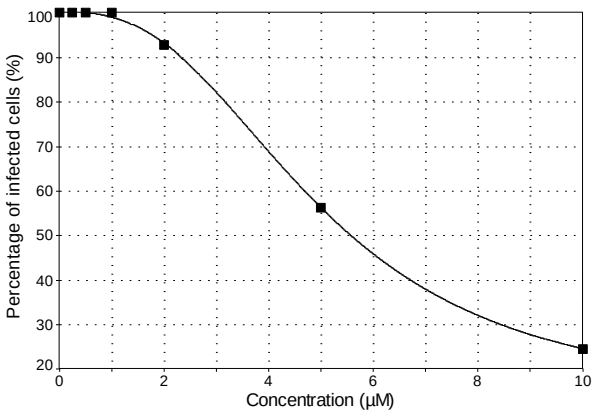
Thus, from all these structure-activity relationships data, the 6-halo-3-nitro-2-phenylsulfonylmethylimidazo[1,2-*a*]pyridine scaffold corresponds to the minimal structural skeleton required for displaying *in vitro* antileishmanial activity against the promastigotes stage of *Leishmania donovani* and then, appears as a new antileishmanial pharmacophore. Molecule **28***, chosen as a good representative of this pharmacophore, has an IC_{50} value of 14 µM, 2.3 times higher than the one of pentamidine and 4.5 times higher than the one of miltefosine. Its CC_{50} values make it less toxic than pentamidine and amphotericin B and globally as slightly toxic as miltefosine, reaching a HepG2 selectivity index >7, much better than the one of pentamidine, and about 2 times inferior to the one of miltefosine.

Indeed, 6,8-dibrominated imidazopyridine **44***, corresponding to the previously described pharmacophore bearing an additional bromine atom at position 8, appeared to be the lead-compound in the tested series. Such molecule is about eight times more active than hit-molecule **28***, presents a very good activity (IC_{50} = 1.8 µM), better than the ones of both pentamidine and miltefosine and a selectivity index (SI >17.2, considering the HepG2 human cell line) standing higher than the ones of pentamidine and miltefosine.

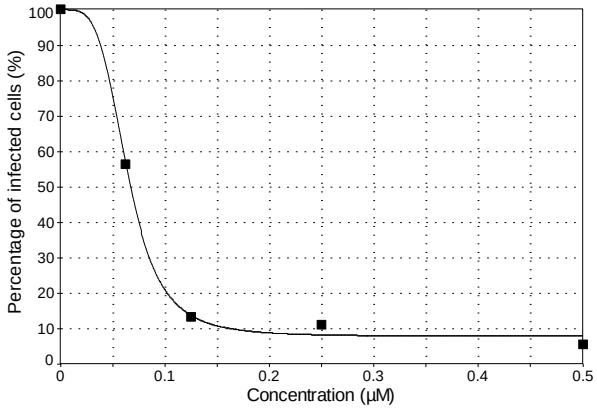
In order to investigate and define more precisely the antileishmanial potential of hit-molecule **28*** and lead-molecule **44***, these two molecules were tested on the promastigote stage of *Leishmania infantum* (second causing agent of visceral leishmaniasis worldwide and species responsible for visceral leishmaniasis in the endemic Mediterranean area) and on the amastigote stage of *Leishmania donovani*. Remembering that only amastigotes develop in the mammalian hosts of the parasite, this last evaluation is fundamental so as to identify molecules which could be used for treating human visceral leishmaniasis. The intracellular amastigotes assay was done according to the method of da Luz *et al.*[41] which uses a brief exposure of promastigotes to an acidic pH to insure optimal metacyclogenesis[42,43] just prior to macrophages infection, leading to a higher final cell density and more reproducible intracellular infection[41,44]. Results were compared to the IC_{50} values obtained with three antileishmanial reference-drugs: pentamidine, miltefosine and amphotericin B (**Table 3**, **Figure 3**). Finally, to complete the evaluation of these two molecules, their selectivity of action among protozoa was assessed by determining their *in vitro* antiplasmodial IC_{50} on the K1 multi-resistant strain of *Plasmodium falciparum*, in comparison with atovaquone, chosen as an antimalarial reference drug.

Figure 3. Dose-response curves of compound **44*** (A) and amphotericine B (B) toward the amastigote stage of *L. donovani*.

(A)



(B)

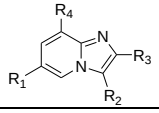


Hit-compound **28*** does not show any interesting activity toward either *L. infantum* promastigotes or *L. donovani* amastigotes whereas lead-compound **44*** exhibits quite good *in vitro* activity against both of them. Like all antileishmanial reference drugs, **44*** is slightly less active toward the amastigote stage of the parasite (IC_{50} = 5.5 µM) than the promastigote one (IC_{50} = 1.8 µM). Nevertheless, concerning the amastigote stage of *L. donovani*, amphotericin B remains the most active drug (IC_{50} = 0.07 µM), but the activity of 44* is interesting (Figure 3). Indeed, **44*** is much more active than pentamidine (IC_{50} > 20 µM) and approximately as active as miltefosine (IC_{50} = 6.8 µM) (Table 3). Moreover, concerning antiprotozoal profile, **44*** appears rather selective, in comparison with reference drug-compounds, being 3 to 9 times less active toward the *Plasmodium* genus (IC_{50} = 16 µM) than the *Leishmania* one.

3. Conclusions

In vitro screening of a series of imidazo[1,2-*a*]pyridines permitted us to identify selective molecules targeting the promastigote stage of *Leishmania donovani* and displaying low cytotoxicity. From numerous derivatives, structure-activity relationships could be studied and allowed the characterization of the corresponding pharmacophore: 6-halo-3-nitro-2-phenylsulfonyl-methylimidazo[1,2-*a*]pyridine. Substitution of this pharmacophore at position 8 by a bromine atom generates lead-compound **44*** which appears as a potent and selective antileishmanial molecule targeting both the pro- and intracellular amastigote stages of the human parasite. Molecule **44*** will next be the starting point for additional pharmaceutical chemistry work exploring both position 6 and key-position 8, trying to improve, among others, solubility in biological media. Enzymatic assays on several *Leishmania* kinases will also be carried out.

Table 2. *In vitro* antileishmanial activity and cytotoxicity of imidazo[1,2-*a*]pyridine derivatives.



N°	-R_1	-R_2	-R_3	-R_4	*L. donovani* promastigotes IC_{50} (µM)[a]	J774 Cytotoxicity CC_{50} (µM)[a]	Selectivity Index[b]	HepG2 Cytotoxicity CC_{50} (µM)[a]	Selectivity Index[c]
-	-H	-H	-H	-H	>50	>100	ND[g]	>100	ND[g]
1*	-H	-NO_2	-H	-H	>50	>100	ND[g]	>100	ND[g]
2*	-H	-NO_2	-CH_3	-H	>50	>100	ND[g]	>100	ND[g]
3*	-H	-H	Cl-CH_2-	-H	>50	>100	ND[g]	>100	ND[g]
4	-H	-H	C_6H_5-SO_2-CH_2-	-H	>50	>100	ND[g]	>100	ND[g]
5*	-H	-NO_2	Cl-CH_2-	-H	15.1 (± 1.2)	4.8 (± 0.7)	0.3	8.7 (± 1.2)	0.6
6	-H	-NO_2	C_6H_5-SO_2-CH_2-	-H	>50	>100	ND[g]	>62.5[d]	ND[g]
8	-I	-H	C_6H_5-SO_2-CH_2-	-H	>50	5.2 (± 0.6)	<0.1	5.0 (± 0.9)	<0.1
9	-I	-H	4-CH_3-C_6H_4-SO_2-CH_2-	-H	>50	25 (± 0.8)	<0.5	>12.5[d]	ND[g]
10	-I	-H	4-Cl-C_6H_4-SO_2-CH_2-	-H	>50	44.2 (± 5.4)	<0.8	39.1 (± 4.0)	<0.8
12	**-I**	**-NO_2**	**C_6H_5-SO_2-CH_2-**	-H	**12.6** (± 0.8)	**27** (± 0.6)	**2.1**	**>100**	**>7.9**
13	-I	-NO_2	4-CH_3-C_6H_4-SO_2-CH_2-	-H	39.2 (± 0.4)	34.8 (± 1.3)	0.9	>50	>1.3
14	-I	-NO_2	4-Cl-C_6H_4-SO_2-CH_2-	-H	29.1 (± 1.0)	43.6 (± 1.7)	1.5	>50	>1.7
16*	-Cl	-H	C_6H_5-SO_2-CH_2-	-H	>50	13.3 (± 0.7)	<0.3	16.5 (± 1.1)	<0.3
17	-Cl	-H	4-CH_3-C_6H_4-SO_2-CH_2-	-H	33.5 (± 0.9)	33.3 (± 1.2)	0.9	68.3 (± 1.3)	2.0
18	-Cl	-H	4-Cl-C_6H_4-SO_2-CH_2-	-H	>50	68.7	<1.3	71.5	<1.4
20*	**-Cl**	**-NO_2**	**C_6H_5-SO_2-CH_2-**	-H	**18.2** (± 0.6)	**>100**	**>5.5**	**>100**	**>5.5**
21*	-Cl	-NO_2	4-CH_3-C_6H_4-SO_2-CH_2-	-H	>50	>94.0[d]	ND[g]	>100	ND[g]
22	-Cl	-NO_2	4-Cl-C_6H_4-SO_2-CH_2-	-H	23.0 (± 0.8)	42.4 (± 1.6)	1.8	>25[d]	1.1
23*	-Br	-H	Cl-CH_2-	-H	>50	68.4 (± 2.3)	<1.4	>100	ND[g]
24*	-Br	-H	C_6H_5-SO_2-CH_2-	-H	>50	6.4 (± 0.3)	<0.1	7.3 (± 0.9)	0.15
25	-Br	-H	4-CH_3-C_6H_4-SO_2-CH_2-	-H	>50	20.9 (± 1.8)	<0.4	28.9 (± 1.5)	<0.6
26	-Br	-H	4-Cl-C_6H_4-SO_2-CH_2-	-H	>50	43.9 (± 2.1)	<0.9	23.0 (± 0.4)	<0.5
27*	-Br	-NO_2	Cl-CH_2-	-H	9.6 (± 0.8)	4.9 (± 0.9)	0.5	15.6 (± 1.1)	1.6
28*	**-Br**	**-NO_2**	**C_6H_5-SO_2-CH_2-**	-H	**14.0** (± 0.8)	**34.3** (± 2.3)	**2.5**	**>100**	**>7.1**
29*	-Br	-NO_2	4-CH_3-C_6H_4-SO_2-CH_2-	-H	44.1 (± 2.8)	44.3 (± 4.6)	1.0	5.9 (± 0.8)	0.13
30*	-Br	-NO_2	4-Cl-C_6H_4-SO_2-CH_2-	-H	45.4 (± 3.6)	51.1 (± 6.1)	1.1	5.5 (± 0.3)	0.12
31	-Br	-NO_2	4-Br-C_6H_4-SO_2-CH_2-	-H	24.3 (± 1.1)	28.2 (± 1.4)	1.1	39 (± 2.1)	1.6
32	-Br	-NO_2	4-I-C_6H_4-SO_2-CH_2-	-H	>50	16.2 (± 0.7)	<0.3	20.3 (± 0.2)	<0.4
33	-Br	-NO_2	4-F-C_6H_4-SO_2-CH_2-	-H	>50	>92.8[d]	ND[g]	>100	ND[g]
34	-Br	-NO_2	4-CF_3-C_6H_4-SO_2-CH_2-	-H	>50	>25[d]	ND[g]	31.7 (± 1.1)	<0.6
35	-Br	-NO_2	4-OCH_3-C_6H_4-SO_2-CH_2-	-H	>50	>92.5[d]	ND[g]	>100	ND[g]
36	-Br	-NO_2	CH_3-SO_2-CH_2-	-H	>50	>100	ND[g]	>100	ND[g]
37	-Br	-NO_2	4-C_6H_5-C_6H_4-SO_2-CH_2-	-H	>50	54.4 (± 3.4)	<1.1	>100	ND[g]
38*	-Br	-NO_2	C_6H_5-S-CH_2-	-H	>10[d]	>6[d]	ND[g]	0.53 (± 0.06)	<0.05
39*	2-CH_3-C_6H_4-	-NO_2	C_6H_5-SO_2-CH_2-	-H	>10[d]	5.0 (± 0.6)	<0.5	>9.4[d]	ND[g]
40*	3-CF_3-C_6H_4-	-NO_2	C_6H_5-SO_2-CH_2-	-H	NS[h]	NS[h]	ND[g]	NS[h]	ND[g]
41*	4-OCH_3-C_6H_4-	-NO_2	C_6H_5-SO_2-CH_2-	-H	NS[h]	NS[h]	ND[g]	NS[h]	ND[g]
42	3,4,5-tri-OCH_3-C_6H_2-	-NO_2	C_6H_5-SO_2-CH_2-	-H	>50	5.0 (± 0.8)	<0.1	34.7 (± 1.7)	<0.7
43*	2-Naphthyl-	-NO_2	C_6H_5-SO_2-CH_2-	-H	NS[h]	NS[h]	ND[g]	NS[h]	ND[g]
44*	**-Br**	**-NO_2**	**C_6H_5-SO_2-CH_2-**	**-Br**	**1.8** (± 0.8)	**>25**[d]	**>13.4**	**>31**[d]	**>17.2**

Ref.	Amphotericin B[e]	0.06 (± 0.02)	3.1 (± 0.3)	51.6	8.8 (± 0.6)	147
Ref.	Miltefosine[e]	3.1 (± 0.06)	94.6 (± 2.1)	30.5	50.3 (± 1.5)	16.2
Ref.	Pentamidine[e]	6.0 (± 0.8)	1.0 (± 0.7)	0.1	2.3 (± 0.5)	0.4
Ref.	Doxorubicin[f]	-	0.02 (± 0.03)	-	0.2 (± 0.05)	-

[a]The values are means ± SD of three independent experiments.

[b]Selectivity Index was calculated according to the formula : SI = (J774 CC_{50}) / (*L. donovani* IC_{50})

[C]Selectivity Index was calculated according to the formula : SI = (HepG2 CC_{50}) / (*L. donovani* IC_{50})

[d]Determination of the IC_{50} or CC_{50} value was limited by lack of solubility in the culture medium

[e]Amphotericin B, pentamidine and miltefosine were used as antileishmanial drug compounds of reference.

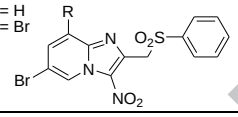
[f]Doxorubicin was used as a cytotoxic drug compound of reference.

[g]ND = not determinable

[h]NS = non-soluble

Table 3. *In vitro* antileishmanial profile and antiplasmodial activity of hit compounds **28*** and **44***.

28: R = H
44: R = Br



Compound	*L. infantum* promastigotes	*L. donovani* promastigotes	*L. donovani* amastigotes	*P. falciparum* (K1)
	IC_{50} (µM)[a]	**IC_{50} (µM)[a]**	**IC_{50} (µM)[a]**	**IC_{50} (µM)[a]**
28*	>25[d]	14.0 (± 0.8)	>25[d]	41.2 (± 1.3)
44*	3.3 (± 0.7)	1.8 (± 0.8)	5.5 (± 0.2)	16.0 (± 0.7)
Pentamidine[b]	8.2 (± 0.1)	6.0 (± 0.8)	>20	-
Miltefosine[b]	11.6 (± 0.4)	3.1 (± 0.06)	6.8 (± 0.3)	-
Amphotericin B[b]	0.04 (± 0.01)	0.06 (± 0.02)	0.07 (± 0.02)	-
Atovaquone[c]	-	-	-	0.0015 (± 0.0007)

[a] The values are means ± SD of three independent experiments.

[b]Amphotericin B, pentamidine and miltefosine were used as antileishmanial drug compounds of reference.

[c]Atovaquone was used as a reference antiplasmodial drug compound.

[d]Molecule **28** could not be tested at higher concentrations due to lack of solubility in the culture mediu

4. Experimental

4.1. Biology

4.1.1. Antileishmanial evaluation

Leishmania species used in this study were *L. donovani* MHOM/IN/00/DEVI and *L. infantum* MCAN/ES/98/LLM-877, all provided by the CNR *Leishmania* (Montpellier, France).

Antileishmanial activity on promastigotes

The effects of the tested compounds on the growth of *Leishmania donovani* and *Leishmania infantum* promastigotes were assessed by MTT assay[45]. Briefly, promastigotes in log-phase in Schneider's medium supplemented with 20% fetal calf serum (FCS), 2 mM L-glutamine and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin), were incubated at an average density of 10^6 parasites/mL in sterile 96-well plates with various concentrations of compounds dissolved in DMSO (final concentration less than 0.5% v/v), in duplicate. Appropriate controls treated by DMSO, pentamidine, miltefosine or amphotericin B (reference drugs purchased from Sigma Aldrich) were added to each set of experiments. After a 72 h incubation period at 27 °C, parasite metabolic activity was determined. Each plate-well was then microscope-examined for detecting possible precipitate formation. Plates were centrifuged at 900 g for 10 min and the supernatant removed. After the addition of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), 0.5 mg/mL in Schneider's medium, 100 µL/well, plates were incubated for 6 h at 27 °C. The plates were subsequently centrifuged at 900 g for 10 min and the supernatant removed. The pellet was dissolved in 100 µl of DMSO and the absorbance measured in a plate reader at 570 nm. Inhibitory concentration 50% (IC_{50}) was defined as the concentration of drug required to inhibit by 50% the metabolic activity of *Leishmania donovani and Leishmania infantum* promastigotes compared to the control. IC_{50} were calculated by non-linear regression analysis processed on dose–response curves, using TableCurve 2D V5 software. IC_{50} values represent the mean value calculated from three independent experiments.

Antileishmanial activity on intracellular amastigotes

The effects of the tested compounds on the growth of *Leishmania* amastigotes were assessed according to the method of da Luz *et al*[41]. in the following way. 500 µL of J774A.1 cells were seeded in sterile chamber-slides at an average density of $5x10^4$ cells/mL and incubated for 24 h at 37 °C and 6% CO_2. *Leishmania donovani* promastigotes were centrifuged at 900 g for 10 min and the supernatant replaced by the same volume of Schneider 20% FCS pH 5.4 and incubated for 24 h at 27 °C. J774A.1 cells were then infected by acidified promastigotes at an average density of $5x10^5$ cells/mL (10:1 ratio) and chamber-slides incubated for 24 h at 37 °C. Then, in duplicate, the medium containing various concentrations of tested-compounds was added (final DMSO concentration being inferior to 0.5% v/v). Appropriate controls treated with or without solvent (DMSO), and various concentrations of pentamidine, miltefosine and amphotericin B were added to each set of experiments. After 120 h incubation at 37 °C and 6% CO_2, well supernatant was removed. Cells were fixed with analytical grade methanol and stained with 10% Giemsa. The percentage of infected macrophages in each assay was determined microscopically by counting at least 200 cells in each sample (31% ± 0.5%). IC_{50} was defined as the concentration of drug necessary to produce a 50% decrease of infected macrophages compared to the control. IC_{50} were calculated by non-linear regression analysis processed on dose–response curves, using TableCurve 2D V5 software. IC_{50} values represent the mean value calculated from three independent experiments.

4.1.2. Antiplasmodial evaluation

In this study, a K1 culture-adapted *P. falciparum* strain resistant to chloroquine, pyrimethamine and proguanil was used in an *in vitro* culture. Maintenance in continuous culture was done as described previously by Trager and Jensen[46]. Cultures were maintained in fresh A+ human erythrocytes at 2.5% hematocrit in complete medium (RPMI 1640 with 25 mM HEPES, 25 mM $NaHCO_3$, 10% of A+ human serum) at 37 C under reduced O_2 atmosphere (gas mixture 5% O_2, 5% CO_2, and 90% N_2). Parasitaemia was maintained daily between 1% and 6%. The *P. falciparum* drug susceptibility test was carried out by comparing quantities of DNA in treated and control cultures of parasite in human erythrocytes according to a SYBR Green I fluorescence-based method[47] using a 96-well fluorescence plate reader. Parasite culture was synchronized at ring stage with 5% sorbitol. Compounds were incubated in a total assay volume of 200 µL (RPMI, 2% hematocrit and 1% parasitaemia) for 72 h in a humidified atmosphere (5% O_2 and 5% CO_2) at 37 °C, in 96-well flat bottom plates. Triplicate assays were performed for each sample. After incubation, 170 µL supernatant was discarded and cells were washed with 150 µL 1X PBS. 15 µL re-suspended cells were transferred to 96-well flat bottom non-sterile black plates (Greiner Bio-one) already containing 15 µL of the SYBR Green I lysis buffer (2XSYBR Green, 20 mM Tris base pH 7.5, 20 mM EDTA, 0.008% w/v saponin, 0.08% w/v Triton X-100). Negative control, treated by solvents (DMSO or H_2O) and positive control (atovaquone) were added to each set of experiments. Plates were incubated for 15 min at 37 °C and then read on a TECAN Infinite F-200 spectrophotometer with excitation and emission wavelengths at 497 and 520 nm, respectively. The concentrations of compounds required to induce a 50% decrease of parasite growth (IC_{50} K1) were calculated from three independent experiments.

4.1.3. Cytotoxicity evaluation

The evaluation of the tested molecules cytotoxicity on the HepG2 (hepatocarcinoma cell line purchased from ATCC, ref HB-8065) J774A.1 (mouse macrophage cell line ECACC, Salisbury UK) and HFF (human foreskin fibroblast) cell lines was done according to the method of Mosmann[45] with slight modifications. Briefly, cells in 100 µL of complete medium, [RPMI supplemented with 10% FCS, 1% L-glutamine (200 mM) and penicillin (100 U/mL) / streptomycin (100 µg/mL)] were inoculated into each well of 96-well plates and incubated at 37 °C in a humidified 6% CO_2 with 95% air atmosphere. After a 24 h incubation, 100 µL of medium with various product concentrations were added and the plates were incubated for 72 h at 37 °C. Each plate-well was then microscope-examined for detecting possible precipitate formation before the medium was aspirated from the wells. 100 µL of MTT solution (0.5 mg / mL in PBS) were then added to each well with 100 µl of medium without FCS. Cells were incubated for 2 h at 37 °C. After this time, the MTT solution was removed and DMSO (100 µL) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (300 rpm) for 5 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength with a microplate spectrophotometer. DMSO was used as blank and doxorubicin (purchased from Sigma Aldrich) as positive control. Cell viability was calculated as percentage of control (cells incubated without compound). The 50% cytotoxic concentration was determined from the dose–response curve by using the TableCurve 2D V5 software.

4.2. Chemistry

4.2.1. General

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were determined on a Bruker Avance 200 MHz or 400 MHz instrument, at the Faculté de Pharmacie de Marseille and Faculté des Sciences de St Jérôme. Chemical shifts are given in δ values referenced to the solvent. High resolution mass spectra were recorded on a QStar Elite at the Spectropôle department of the Faculté des Sciences de St Jérôme. Elemental analyses were carried out with a Thermo Finnigan EA 1112 apparatus at the Spectropole department of the Faculté des Sciences de St Jérôme. Silica Gel 60 (Merck 70-230) was used for column chromatography. The progress of the reactions was monitored by thin layer chromatography using Kieselgel 60 F254 (Merck) plates.

Imidazo[1,2-*a*]pyridine was purchased from Sigma Aldrich.

Compounds **1**[29], **2**[30], **3**[32], **5**[33], **15**[33], **16**[33], **19**[33], **20**[33], **21**[48], **23**[33], **24**[33], **27**[33], **28**[33], **29**[48], **30**[49], **38**[33], **39**[34], **40**[34], **41**[34], **43**[34], **44**[40] were prepared as previously describe and are presented with a * in the result and discussion part.

4.2.1.1. 2-Chloromethyl-6-iodoimidazo[1,2-*a*]pyridine 7

To a solution of 2.95 mmol of 1,3-dichloroacetone in absolute ethanol (120 mL) were added 2.27 mmol of 2-amino-5-iodopyridine. The mixture was stirred and heated under reflux for 4 h. The solvent was evaporated *in vacuo*. The residue was poured into water and basified with a saturated aqueous solution of Na_2CO_3. The solution was extracted three times with chloroform. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by chromatography on a silica gel, eluting with dichloromethane-ethyl acetate (8-2) and recrystallized from isopropanol to give, the expected product as a white solid in 70% yield (0.46 g).
White solid, mp 150-151 °C (Isopropanol). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 4.74 (2H, s), 7.35-7.36 (2H, m), 7.56 (1H, s), 8.35-8.36 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 39.3 (CH_2), 75.4 (C), 110.5 (CH), 118.8 (CH), 130.6 (CH), 133.1 (CH), 143.7 (C), 143.9 (C). Anal. Calcd. For $C_8H_6ClIN_2$: C, 32.85; H, 2.07; N, 9.58%. Found: C, 32.88; H, 2.07; N, 9.63%.

4.2.1.2. 2-Chloromethyl-6-iodo-3-nitroimidazo[1,2-*a*]pyridine 11

To a solution of 1.71 mmol of 2-chloromethyl-6-iodoimidazo[1,2-*a*]pyridine in concentrated sulfuric acid (30 mL) cooled by an ice-water bath, nitric acid 65% (5.1 mmol.) was added. The reaction mixture was stirred for 3 h at room temperature. The reaction mixture was then slowly poured into a water-ice-mixture making the desired product precipitate. The yellow solid was collected by filtration, dried under reduced pressure and recrystallized from isopropanol to give, the expected product in 80% yield (0.46 g).
Yellow solid, mp 211-212 °C (Isopropanol). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.10 (2H, s), 7.62 (1H, dd, J = 0.8 and 9.3 Hz), 7.87 (1H, dd, J = 1.5 and 9.3 Hz), 9.69 (1H, dd, J = 0.8 and 1.5 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 38.6 (CH_2), 80.8 (C), 119.4 (CH), 132.3 (CH), 138.7 (C), 139.2 (CH), 143.4 (C), 147.4 (C). Anal. Calcd. For $C_8H_5ClIN_3O_2$: C, 28.47; H, 1.49; N, 12.45%. Found: C, 28.53; H, 1.47; N, 12.60%.

4.2.1.3. General procedure for the preparation of sulfone derivatives 4, 6, 8, 9, 10, 12-14, 16, 17, 18, 22, 25, 26, 31-37 from 2-chloromethylated imidazo[1,2-*a*]pyridines

A aqueous solution (15 mL) containing 4 mmol of corresponding sulfonyl chloride, 4 mmol of sodium sulfite, 4 mmol of sodium hydrogenocarbonate and 2 mmol corresponding 2-chloromethylated imidazo[1,2-*a*]pyridines was heated under microwave irradiation (120 °C, 5 bars) in a sealed vial. After disappearance of the starting materials (about 1 hour), monitored by TLC, the solid was collected by filtration and dried under reduced pressure to give, after purification by column chromatography on silica gel and/or recrystallization, the expected product in 25 to 100% yield.

4.2.1.4. 2-Phenylsulfonylmethylimidazo[1,2-*a*]pyridine 4

Compound **4** was obtained, after recrystallization from ethanol, as a white solid in 60% yield (0.33 g), mp 170-171 °C (Ethanol). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 4.59 (2H, s), 6.75-6.82 (1H, m), 7.11-7.19 (1H, m), 7.42-7.50 (3H, m), 7.56-7.64 (1H, m), 7.70-7.80 (3H, m), 8.06-8.09 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 57.0 (CH_2), 112.8 (CH), 112.9 (C), 117.6 (CH), 125.0 (CH), 125.7 (CH), 128.3 (CH*2), 129.0 (CH*2), 133.7 (CH), 133.8 (C), 138.7 (CH), 144.9 (C). Anal. Calcd. For $C_{14}H_{12}N_2O_2S$: C, 61.75; H, 4.44; N, 10.29%. Found: C, 61.82; H, 4.49; N, 10.12%.

4.2.1.5. 3-Nitro-2-phenylsulfonylmethylimidazo[1,2-*a*]pyridine 6

Compound **6** was obtained, after recrystallization from isopropanol, as a beige solid in 60% yield (0.38 g), mp 208-209 °C (Isopropanol). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.16 (2H, s), 7.27-7.35 (1H, m), 7.49-7.56 (2H, m), 7.63-7.71 (2H, m), 7.83-7.90 (3H, m), 9.36-9.40 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 56.8 (CH_2), 117.2 (CH), 118.7 (CH), 127.7 (CH), 128.3 (CH*2), 129.2 (CH*2), 131.1 (CH), 134.2 (CH), 138.7 (C), 139.1 (C), 139.5 (C), 145.0 (C). Anal. Calcd. For $C_{14}H_{11}N_3O_4S$: C, 52.99; H, 3.49; N, 13.24%. Found: C, 52.92; H, 3.47; N, 13.14%.

4.2.1.6. 6-Iodo-2-phenylsulfonylmethylimidazo[1,2-*a*]pyridine 8

Compound **8** was obtained, after recrystallization from isopropanol, as a beige solid in 74% yield (0.59 g), mp 242-243 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.81 (2H, s), 7.30 (1H, d, J = 9.4 Hz), 7.39 (1H, dd, J = 1.5 and 9.4 Hz), 7.54-7.78 (6H, m), 8.94-8.95 (1H, m). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 55.7 (CH_2), 76.4 (C), 113.5 (CH), 118.0 (CH), 128.1 (CH*2), 129.3 (CH*2), 131.6 (CH), 132.5 (CH), 133.9 (CH), 134.5 (C), 139.0 (C), 142.7 (C). Anal. Calcd. For $C_{14}H_{11}IN_2O_2S$: C, 42.23; H, 2.78; N, 7.03%. Found: C, 42.23; H, 2.79; N, 6.90%.

4.2.1.7. 6-Iodo-2-tosylmethylimidazo[1,2-*a*]pyridine 9

Compound **9** was obtained, after recrystallization from isopropanol, as a beige solid in 80% yield (0.66 g), mp 241-242 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 2.38 (3H, s), 4.75 (2H, s), 7.29-7.42 (4H, m), 7.64 (2H, d, J = 8.3 Hz), 7.78 (1H, s), 8.94-8.95 (1H, m). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 21.2 (CH_3), 55.8 (CH_2), 76.4 (C), 113.4 (CH), 118.0 (CH), 128.1 (CH*2), 129.7 (CH*2), 131.6 (CH), 132.5 (CH), 134.7 (C), 136.2 (C), 142.7 (C), 144.4 (C). Anal. Calcd. For $C_{15}H_{13}IN_2O_2S$: C, 43.70; H, 3.18; N, 6.80. Found: C, 43.84; H, 3.20; N, 6.80%.

4.2.1. 8. 2-[(4-Chlorophenylsulfonyl)methyl]-6-iodoimidazo-[1,2-*a*]pyridine 10

Compound **10** was obtained, after recrystallization from isopropanol, as a grey solid in 53% yield (0.46 g), mp 179-180 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.86 (2H, s), 7.30 (1H, d, J = 9.5 Hz), 7.40 (1H, dd, J = 1.6 and 9.5 Hz), 7.63-7.79 (5H, m), 8.94-8.95 (1H, m). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 55.6 (CH_2), 76.4 (C), 113.6 (CH), 118.0 (CH), 129.4 (CH*2), 130.2 (CH*2), 131.7 (CH), 132.6 (CH), 134.4 (C), 137.8 (C), 139.0 (C), 142.8 (C). Anal. Calcd. For $C_{14}H_{10}ClIN_2O_2S$: C, 38.86; H, 2.33; N, 6.47%. Found: C, 38.90; H, 2.43; N, 6.79%.

4.2.1.9. 6-Iodo-3-nitro-2-phenylsulfonylmethylimidazo[1,2-*a*]-pyridine 12

Compound **12** was obtained, after recrystallization from isopropanol, as a beige solid in 90% yield (0.79 g), mp 237-238 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.85 (2H, s), 7.31 (1H, d, J = 9.4 Hz), 7.40 (1H, dd, J = 1.6 and 9.4 Hz), 7.63-7.79 (5H, m), 8.95 (1H, dd, J = 0.9 and 1.6 Hz). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 55.6 (CH_2), 76.5 (C), 113.6 (CH), 118.1 (CH), 129.4 (CH*2), 130.2 (CH*2), 131.7 (CH), 132.6 (CH), 134.4 (C), 137.8 (C), 139.0 (C), 142.8 (C). Anal. Calcd. For $C_{14}H_{10}IN_3O_4S$: C, 37.94; H, 2.27; N, 9.48%. Found: C, 37.99; H, 2.21; N, 9.46%.

4.2.1.10. 6-Iodo-3-nitro-2-tosylmethylimidazo[1,2-*a*]pyridine 13

Compound **13** was obtained, after recrystallization from isopropanol, as a yellow solid in 100% yield (0.91 g), mp 210-211 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 2.40 (3H, s), 5.17 (2H, s), 7.39 (2H, d, J = 8.0 Hz), 7.62 (2H, d, J = 8.0 Hz), 7.75 (1H, dd, J = 0.8 and 9.2 Hz), 8.05 (1H, dd, J = 1.6 and 9.2 Hz), 9.44 (1H, dd, J = 0.8 and 1.6 Hz). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 21.3 (CH_3), 56.2 (CH_2), 83.0 (C), 119.1 (CH), 128.2 (CH*2), 129.9 (CH*2), 132.3 (CH), 136.2 (C), 139.0 (C), 139.3 (C), 139.6 (CH), 143.2 (C), 144.9 (C). Anal. Calcd. For $C_{15}H_{12}IN_3O_4S$: C, 39.40; H, 2.65; N, 9.19%. Found: C, 39.55; H, 2.66; N, 9.21%.

4.2.1.11. 2-[(4-Chlorophenylsulfonyl)methyl]-6-iodo-3-nitro-imidazo[1,2-*a*]pyridine 14

Compound **14** was obtained, after recrystallization from isopropanol, as a yellow solid in 90% yield (0.86 g), mp 208-209 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 5.25 (2H, s), 7.64-7.77 (5H, m), 8.05 (1H, dd, J = 1.6 and 9.3 Hz), 9.44-9.45 (1H, m). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 56.1 (CH_2), 83.1 (C), 119.1 (CH), 129.6 (CH*2), 130.3 (CH*2), 132.4 (CH), 137.7 (C), 138.7 (C), 139.0 (C), 139.5 (C), 139.7 (CH), 143.2 (C). Anal. Calcd. For $C_{14}H_9ClIN_3O_4S$: C, 35.20; H, 1.90; N, 8.80%. Found: C, 35.27; H, 1.97; N, 9.20%.

4.2.1.12. 6-Chloro-2-(tosylmethyl)imidazo[1,2-*a*]pyridine 17

Compound **17** was obtained, after recrystallization from isopropanol, as a pale blue solid in 88% yield (0.56 g), mp 179-180 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 2.38 (3H, s), 4.78 (2H, s), 7.26 (1H, dd, J = 1.8 and 9.6 Hz), 7.36-7.67 (4H, m), 7.51 (1H, d, J = 9.6 Hz), 7.84 (1H, s), 8.84 (1H, dd, J = 0.7 and 1.8 Hz). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 21.2 (CH_3), 55.8 (CH_2), 114.2 (CH), 117.6 (CH), 119.3 (C), 124.9 (CH), 125.9 (CH), 128.1 (CH*2), 129.7 (CH*2), 135.4 (C), 136.2 (C), 142.6 (C), 144.4 (C). Anal. Calcd. For $C_{15}H_{13}ClN_2O_2S$: C, 56.16; H, 4.08; N, 8.73%. Found: C, 55.91; H, 4.07; N, 8.79%.

4.2.1.13. 6-Chloro-2-[(4-chlorophenylsulfonyl)methyl]-imidazo[1,2-*a*]pyridine 18

Compound **18** was obtained, after recrystallization from isopropanol, as a pale grey solid in 88% yield (0.60 g), mp 185-186 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.87 (2H, s), 7.27 (1H, dd, J = 1.9 and 9.6 Hz), 7.52 (1H, d, J = 9.6 Hz), 7.64-7.78 (4H, m), 7.85 (1H, s), 8.92 (1H, dd, J = 0.5 and 1.9 Hz). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 55.6 (CH_2), 114.4 (CH), 117.6 (CH), 119.3 (CH), 125.0 (CH), 126.0 (CH), 129.4 (CH*2), 130.2 (CH*2), 135.1 (C), 137.8 (C), 139.0 (C), 142.7 (C). Anal. Calcd. For $C_{14}H_{10}Cl_2N_2O_2S$: C, 49.28; H, 2.95; N, 8.21%. Found: C, 49.33; H, 2.92; N, 8.21%.

4.2.1.14. 6-Chloro-2-[(4-chlorophenylsulfonyl)methyl]-3-nitro-imidazo[1,2-*a*]pyridine 22

Compound **22** was obtained, after recrystallization from benzene, as a white solid in 76% yield (0.58 g), mp 199-200 °C (benzene). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.12 (2H, s), 7.49-7.82 (6H, m), 9.44-9.45 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 56.7 (CH_2), 118.9 (CH), 125.7 (CH), 126.0 (C), 129.6 (CH*2), 129.8 (CH*2), 132.5 (CH), 137.6 (C), 139.0 (C), 139.4 (C), 141.1 (C), 143.1 (C). Anal. Calcd. For $C_{14}H_9Cl_2N_3O_4S$: C, 43.54; H, 2.35; N, 10.88%. Found: C, 43.62; H, 2.34; N, 10.90%.

4.2.1.15. 6-Bromo-2-(tosylmethyl)imidazo[1,2-*a*]pyridine 25

Compound **25** was obtained, after recrystallization from isopropanol, as a grey solid in 94% yield (0.68 g), mp 197-198 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 2.38 (3H, s), 4.77 (2H, s), 7.32 (1H, dd, J = 1.8 and 9.6 Hz), 7.35-7.67 (4H, m), 7.45 (1H, d, J = 9.6 Hz), 7.83 (1H, s), 8.92 (1H, dd, J = 0.7 and 1.8 Hz). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 21.2 (CH_3), 55.8 (CH_2), 106.3 (C), 114.0 (CH), 117.8 (CH), 127.0 (CH), 127.9 (CH), 128.1 (CH*2), 129.7 (CH*2), 135.2 (C), 136.2 (C), 142.6 (C), 144.4 (C). Anal. Calcd. For $C_{15}H_{13}BrN_2O_2S$: C, 49.33; H, 3.59; N, 7.67%. Found: C, 49.33; H, 3.65; N, 7.96%.

4.2.1.16. 6-Bromo-2-(4-chlorophenylsulfonylmethyl)imidazo-[1,2-*a*]pyridine 26

Compound 2**6** was obtained, after recrystallization from isopropanol, as a grey solid in 89% yield (0,68 g), mp 187-188 °C (Isopropanol). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.87 (2H, s), 7.33 (1H, dd, J = 1.9 and 9.6 Hz), 7.45 (1H, d, J = 9.6 Hz), 7.64-7.78 (4H, m), 7.83 (1H, s), 8.92 (1H, dd, J = 0.7 and 1.9 Hz). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 55.6 (CH_2), 106.3 (C), 114.2 (CH), 117.9 (CH), 127.1 (CH), 128.1 (CH), 129.4 (CH*2), 130.2 (CH*2), 134.9 (C), 137.8 (C), 139.0 (C), 142.7 (C). Anal. Calcd. For $C_{14}H_{10}BrClN_2O_2S$: C, 43.60; H, 2.61; N, 7.26%. Found: C, 43.62; H, 2.62; N, 7.55%.

4.2.1.17. 6-Bromo-2-[(4-bromophenylsulfonyl)methyl]-3-nitro-imidazo[1,2-*a*]pyridine 31

Compound **31** was obtained, after purification by column chromatography on silica gel (eluent: CH_2Cl_2-ACOEt 8/2) and recrystallization from isopropanol, as a beige solid in 80% yield (0.76 g), mp 228-229 °C (Isopropanol). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.12 (2H, s), 7.65-7.79 (6H, m), 9.54-9.55 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 56.6 (CH_2), 112.6 (C), 119.1 (CH), 127.7 (CH), 129.8 (C), 129.9 (CH*2), 132.6 (CH*2),

134.7 (CH), 138.1 (C), 138.7 (C), 139.2 (C), 143.3 (C). Anal. Calcd. For $C_{14}H_9Br_2N_3O_4S$: C, 35.39; H, 1.91; N, 8.84%. Found: C, 35.28; H, 1.87; N, 8.95%.

4.2.1.18. 6-Bromo-2-[(4-iodophenylsulfonyl)methyl]-3-nitro-imidazo[1,2-*a*]pyridine 32

Compound **32** was obtained, after purification by column chromatography on silica gel (eluent: CH_2Cl_2) and recrystallization from toluene, as a white solid in 30% yield (0.31 g), mp 260-261 °C (Toluene). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.12 (2H, s), 7.55-7.60 (2H, m), 7.73-7.75 (2H, m), 7.88-7.93 (2H, m), 9.55-9.56 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 56.1 (CH_2), 103.4 (C), 111.7 (C), 119.1 (CH), 128.0 (CH), 129.9 (CH*2), 130.7 (C), 135.0 (CH), 138.4 (CH*2), 138.5 (C), 139.4 (C), 143.1 (C). Anal. Calcd. For $C_{14}H_9BrIN_3O_4S$: C, 33.21; H, 1.74; N, 8.05%. Found: C, 32.56; H, 1.72; N, 8.13%.

4.2.1.19. 6-Bromo-2-[(4-fluorophenylsulfonyl)methyl]-3-nitro-imidazo[1,2-*a*]pyridine 33

Compound **33** was obtained, after purification by column chromatography on silica gel (eluent: CH_2Cl_2-ACOEt 9/1) and recrystallization from toluene, as a beige solid in 44% yield (0.36 g), mp 243-244 °C (Toluene). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 5.22 (2H, s), 7.37-7.45 (2H, m), 7.76-8.01 (4H, m), 9.36-9.37 (1H, m). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 56.2 (CH_2), 111.7 (C), 116.8 (CH*2, d, J = 23 Hz), 119.1 (CH), 128.1 (CH), 131.7 (CH*2, d, J = 10 Hz), 133.0 (C, d, J = 225 Hz), 135.0 (CH), 135.1 (C, d, J = 9 Hz), 139.0 (C), 139.5 (C), 143.1 (C). Anal. Calcd. For $C_{14}H_9BrFN_3O_4S$: C, 40.60; H, 2.19; N, 10.14%. Found: C, 40.47; H, 2.25; N, 10.18%.

4.2.1.20. 6-Bromo-3-nitro-2-(4-trifluoromethyl)phenyl-sulfonylmethylimidazo[1,2-*a*]pyridine 34

Compound **34** was obtained, after purification by column chromatography on silica gel (eluent: CH_2Cl_2-ACOEt 9/1) and recrystallization from toluene, as a pale yellow solid in 60% yield (0.55 g), mp 239-240 °C (Toluene). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.16 (2H, s), 7.68-7.85 (4H, m), 8.01-8.06 (2H, m), 9.56-9.57 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 56.6 (CH_2), 112.7 (C), 119.1 (CH), 123.0 (C, q, J = 272.0 Hz), 126.4 (CH*2, q, J = 3.7 Hz), 127.7 (CH), 129.1 (CH*2), 134.8 (CH), 135.9 (C, q, J = 33.3 Hz), 138.7 (C), 138.9 (C), 142.7 (C), 143.3 (C). Anal. Calcd. For $C_{15}H_9BrF_3N_3O_4S$: C, 38.81; H, 1.95; N, 9.05%. Found: C, 38.78; H, 1.83; N, 9.37%.

4.2.1.21. 6-Bromo-2-[(4-methoxyphenylsulfonyl)methyl]-3-nitroimidazo[1,2-*a*]pyridine 35

Compound **35** was obtained, after purification by column chromatography on silica gel (eluent: CH_2Cl_2-ACOEt 9/1) and recrystallization from toluene, as a pale yellow solid in 46% yield (0.39 g), mp 228-229 °C (Toluene). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.83 (3H, s), 5.13 (2H, s), 7.04-7.09 (2H, m), 7.61-7.66 (2H, m), 7.85-7.99 (2H, m), 9.36-9.37 (1H, m). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 56.0 (CH_3), 56.5 (CH_2), 111.6 (C), 114.7 (CH*2), 119.1 (CH), 128.0 (CH), 130.5 (CH*2), 130.7 (C), 134.9 (CH), 139.0 (C), 139.8 (C), 143.0 (C), 163.7 (C). Anal. Calcd. For $C_{15}H_{12}BrN_3O_5S$: C, 42.27; H, 2.84; N, 9.86%. Found: C, 42.53; H, 2.84; N, 9.81%.

4.2.1.22. 6-Bromo-2-(methylsulfonylmethyl)-3-nitroimidazo-[1,2-*a*]pyridine 36

Compound 3**6** was obtained, after purification by column chromatography on silica gel (eluent: CH_2Cl_2-ACOEt 9/1) and recrystallization from isopropanol, as a beige solid in 25% yield (0.17 g), mp 233-234 °C (Isopropanol). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 3.12 (3H, s), 5.03-5.04 (2H, m), 7.74-7.76 (2H, m), 9.59-9.60 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 41.3 (CH_3), 54.5 (CH_2), 111.6 (C), 119.1 (CH), 128.1 (CH), 130.9 (C), 135.0 (CH), 140.4 (C), 143.2 (C). Anal. Calcd. For $C_9H_8BrN_3O_4S$: C, 32.25; H, 2.41; N, 12.58%. Found: C, 32.64; H, 2.43; N, 12.54%.

4.2.1.23. 2-(Biphenyl-4-ylsulfonyl)methyl-6-bromo-3-nitro-imidazo[1,2-*a*]pyridine 37

Compound 3**7** was obtained, after purification by column chromatography on silica gel (eluent: CH_2Cl_2-ACOEt 9/1) and recrystallization from toluene, as a yellow solid in 40% yield (0.38 g), mp 199-200 °C (Toluene). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.17 (2H, s), 7.43-7.53 (3H, m), 7.59-7.63 (2H, m), 7.74-7.76 (4H, m), 7.91-7.95 (2H, m) 9.54-9.55 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 56.8 (CH_2), 112.5 (C), 119.1 (C), 127.4 (CH*2), 127.7 (CH), 127.9 (CH*2), 128.8 (CH), 128.9 (CH*2), 129.1 (CH*2), 134.6 (CH), 137.5 (CH), 138.7 (C), 139.0 (C), 139.5 (C), 143.3 (C), 147.2 (C). Anal. Calcd. For $C_{20}H_{14}BrN_3O_4S$: C, 50.86; H, 2.99; N, 8.90%. Found: C, 51.14; H, 3.03; N, 8.77%.

4.2.1.24. 3-Nitro-2-(phenylsulfonylmethyl)-6-(3,4,5-tri-methoxyphenyl)imidazo[1,2-*a*]pyridine 42

A solution of 1.26 mmol of 6-bromo-3-nitro-2-phenylsulfonylmethylimidazo[1,2-*a*]pyridine, 1.63 mmol of 3,4,5-trimethoxyphenylboronic acid, 6.3 mmol of Na_2CO_3, 0.12 mmol of $Pd(PPh_3)_4$, 1.26 mmol of TBAB and water (15 mL) were heated under microwave irradiation (105 °C, 300 W) for 1 h. After disappearance of the starting materials (monitored by TLC), the solid was collected by filtration and dried under reduced pressure to give, after purification by column chromatography on silica gel (eluent: CH_2Cl_2-ethyl acetate 8/2) and recrystallization from isopropanol, the expected product in 60% yield (0.37 g).
Beige solid, mp 216-217 °C (Isopropanol). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 3.92 (3H, s), 3.95 (6H, s), 5.17 (2H, s), 6.77 (2H, s), 7.45-7.71 (5H, m), 7.88-7.92 (2H, m) 9.51-9.52 (1H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 56.4 (CH_3*2), 56.9 (CH_2), 61.0 (CH_3), 104.9 (CH), 118.3 (CH), 124.8 (CH), 128.3 (CH*2), 129.2 (CH*2), 131.4 (C), 131.6 (CH), 131.9 (CH), 132.1 (C), 134.1 (CH), 138.7 (C), 139.2 (C), 139.3 (C), 139.7 (C), 144.1 (C), 154.0 (C*2). Anal. Calcd. For $C_{23}H_{21}N_3O_7S$: C, 57.14; H, 4.38; N, 8.69%. Found: C, 57.08; H, 4.39; N, 8.43%.

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