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A B S T R A C T

In continuation of our pharmacomodulation work on the nitroimidazooxazole series, we report the synthesis of new 5-substituted 6-nitroimidazooxazole derivatives. Our aim was to evaluate how functionalization of the 5-position of the 6-nitroimidazooxazole scaffold affects antileishmanial and antitrypanosomal *in vitro* activities. Twenty-one original compounds were synthesized and evaluated for their *in vitro* antileishmanial (*L. donovani*) and antitrypanosomal (*T. cruzi*) properties. Pallado-catalyzed cross-coupling reactions were used to introduce an aryl or ethynyl aryl substituent in 5-position from a 5-brominated-6-nitroimidazooxazole starting product. Unfortunately, the first series of compounds bearing an aryl group in 5-position presented limited *in vitro* activities against *L. donovani* and *T. cruzi*, with $IC_{50} > 10$ μM (*vs* 0.18 μM and 2.31 μM for the reference drugs amphotericin B and benznidazole respectively). Interestingly, the second series of compounds bearing an ethynyl aryl substituent in 5-position showed more promising, particularly against *T. cruzi*. Compounds **6a**, **6b**, **6c**, **6g** and **6h** had better activity than the reference drug benznidazole ($0.92\ \mu M \leq IC_{50} \leq 2.18\ \mu M$ *vs* $IC_{50} = 2.31\ \mu M$), whereas the non-functionalized 2-methyl-6-nitro-2,3-dihydroimidazo [2,1-*b*]oxazole **2** was not active against *T. cruzi* ($IC_{50} > 10\ \mu M$).

1. Introduction

The *Kinetoplastidæ* order includes two families of flagellated eukaryotic parasites: the uniflagellate trypanosomatids and the biflagellate bodonids which of the *Trypanosomatidæ*, including *Leishmania* genus and *Trypanosoma* genus, are the cause of several human diseases classified as neglected tropical diseases [1–3]. The most widespread neglected tropical diseases with the highest death rates are visceral leishmaniasis caused by *Leishmania donovani* and *Leishmania infantum*, Chagas disease caused by *Trypanosoma cruzi* and Human African Trypanosomiasis caused by *Trypanosoma brucei* [4].

Epidemiological data from the World Health Organization (WHO) show between 700 000 and 1 million new cases of leishmaniasis worldwide every year, mainly in Africa, Asia and Latin America, with more than 20 000 deaths annually [5]. There are three clinical manifestations of leishmaniasis according to the *Leishmania* species involved: cutaneous (the most common), mucocutaneous and visceral (the most severe). The most severe visceral form leads to a fatal prognosis without treatment. Current guidelines for the management of leishmaniasis are based on various parameters, like *Leishmania* species, clinical manifestations, geographical location and tolerance profile. The main molecules used are pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), injectable amphotericin B, pentamidine isethionate and miltefosine (the only oral form available). However, there are several limitations to these conventional therapies, such side effects, long-term parenteral administration, high cost in endemic countries and resistances [6].

Concerning trypanosomiasis, it is important to distinguish between the Human African Trypanosomiasis (HAT), called sleeping

☆ This paper is dedicated to the late Professor Alain Gueiffier.

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sickness, and the American Trypanosomiasis called Chagas disease. HAT is endemic in sub-Saharan African countries, mainly in the Democratic Republic of the Congo [7]. For 50 years, the number of new cases has been decreasing. In 2018, it was less than 1000 thanks to the WHO [8]. The first oral treatment, fexinidazole, was recently approved by the European Medicines Agency (EMA) and appeared on the 2019 WHO model list of essential medicines for the management of the first and second stages of Human African Trypanosomiasis due to *Trypanosoma brucei gambiense* infection, according to the recent WHO guidelines [8–10]. On the other hand, the management of the American Trypanosomiasis is more difficult because current treatments (nifurtimox and benznidazole) are appropriate only for the first stage of the disease, usually asymptomatic and misdiagnosed. Chagas disease represents a major health problem, with a worldwide prevalence estimated at 7 million, mainly in Central and South America, and 7000 annual deaths in Latin America [11,12]. The lack of treatment for the second stage of the disease, where there is risk of cardiac and digestive complications, makes the search for new molecules active against *T. cruzi* vital [13].

Antikinetoplastid activity by 6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole derivatives was found for the first time from a screening of nitroimidazole derivatives initiated by DND*i* (the Drugs for Neglected Diseases initiative). One compound, DNDI-VL-2098, was selected for preclinical evaluation as a potential candidate for visceral leishmaniasis (Fig. 1) but its development was stopped because of testicular toxicity [14]. Further studies showed that 6-nitroimidazooxazole compounds could have antitrypanosomal activity [15,16].

Several Structure-Activity Relationships (SAR) of nitroimidazooxazoles for visceral leishmaniasis have been reported (Fig. 2) [14].

As part of our pharmacomodulation work on the nitroimidazooxazole series, we previously synthesized two new series of 5-substituted 7-nitro-2,3-dihydroimidazo[5,1-*b*]oxazole and 6-substituted 5-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole compounds that did not display antileishmanial activity [17,18].

Extending this work, we report herein the synthesis of 5-substituted 6-nitroimidazooxazole compounds aimed at determining whether 5-functionalization might yield compounds with antileishmanial activity. It has been established that 6-nitroimidazooxazole bearing a small substituent at 1-position, such as an ethyl group found in CGI-17341, leads to good antileishmanial activity (Fig. 3) [19]. Thus, we synthesized a series of 6-nitroimidazooxazole compounds substituted by a simple methyl group at 1-position and various aryl or alkyne substituents at 5-position. These new compounds were evaluated for their *in vitro* antileishmanial and antitrypanosomal properties.

2. Results and discussion

2.1. Chemistry

The most commonly described methodology for the synthesis of 6-nitroimidazooxazole uses 2,4-dinitroimidazole as starting

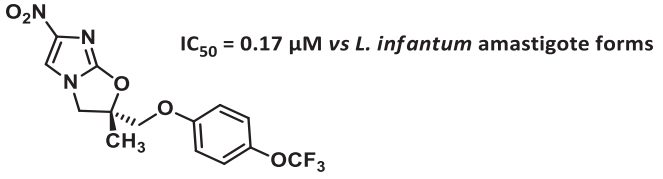


Fig. 1. Structure and *in vitro* activity of DNDI-VL-2098 [14].

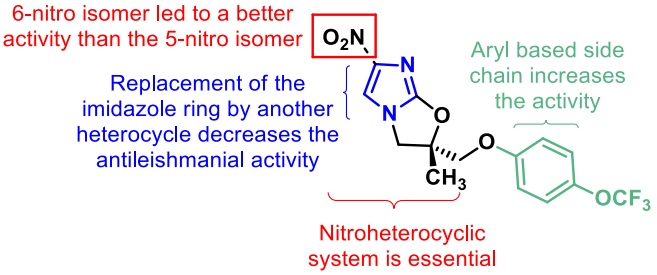


Fig. 2. Structure-Activity Relationship (SAR) of DNDI-VL-2098 for visceral leishmaniasis.

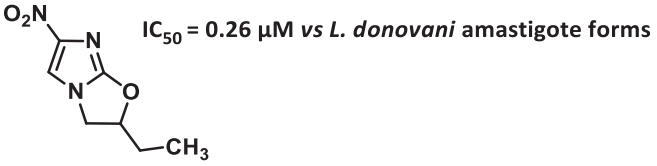
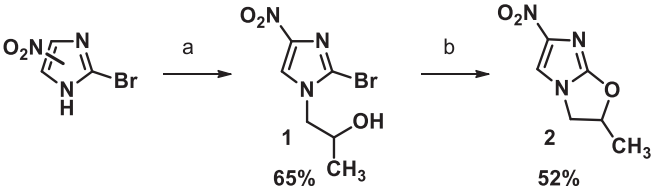


Fig. 3. Structure and *in vitro* activity of CGI-17341 [19].

product [20,21]. Recently, alternative conditions were developed for enhanced safety of the synthesis of 6-nitroimidazooxazole compounds. In accordance with Thompson *et al.* [14], we used a 2-bromo-4(5)-nitroimidazole as starting product in the presence of DIPEA as base and propylene oxide to introduce an alcohol chain at 1-position (Scheme 1). In these conditions, 4-nitro isomer was obtained in good yield and only traces of 5-nitro isomer were observed. Then, intramolecular cyclization occurred with sodium hydride, at 0 °C in DMF for 65 min, to afford the synthesis of the cyclized compound **2**.

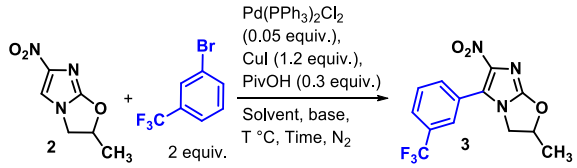
To functionalize the 5-position, we sought to optimize the CH-activation reaction between starting product **2** and arylbromide (Table 1).

The initial conditions (Table 1, entry 1) were inspired from a previously reported protocol for CH-activation reaction in 4-nitroimidazole series involving $Pd(PPh_3)_2Cl_2$, CuI and pivalic acid as catalyst, K_2CO_3 as base, in DMA as solvent at 130 °C [22]. Only traces of the desired product **3** were obtained, and unidentified degradation products were observed from LC-MS monitoring. For the optimization of the reaction conditions, the first parameters studied were the solvent and the temperature. Using DME at 110 °C, product **3** was observed by LC-MS monitoring with an extrapolated yield of 30% (Table 1, entries 3–4). Then, we changed the base and used Cs_2CO_3 (Table 1, entry 5), leading to an increased yield of 37%. Heating to more than 110 °C led to the formation of degradation products and only traces of the desired product **3** (entries 6–7). The best isolated yield obtained (Table 1, entry 5) was very moderate, so this reaction optimization was stopped. We decided to use another pallado-catalyzed cross-coupling methodology after bromination of the 5-position of compound **2** with *N*-bromosuccinimide (Scheme 2).



Scheme 1. Synthesis of 6-nitromidazooxazole **2**: a) propylene oxide (1.5 equiv.), DIPEA (5 equiv.), 110 °C, MW, 1.5 h. b) NaH (2 equiv.), DMF, N_2, 0 °C, 65 min.

Table 1
Optimization of the CH-activation reaction between substrate **2** and aryl bromide.

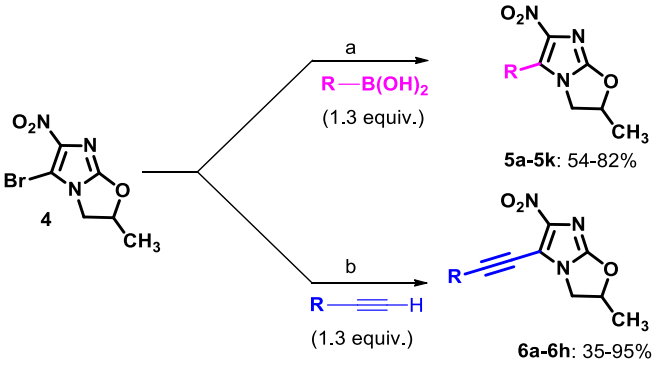


Entry	Base	Solvent	T° (°C)	Time (h)	Yield **2** (%)	Yield **3** (%)
1[a]	K_2CO_3, 1.3 equiv.	DMA	MW, 130	1.5	0	-[c]
2[a]	K_2CO_3, 1.3 equiv.	DMF	MW, 110	1.5	100	-[c]
3[a]	K_2CO_3, 1.3 equiv.	DME	MW, 110	1.5	70	30
4[a]	K_2CO_3, 3 equiv.	DME	MW, 110	1.5	70	30
5[b]	**Cs_2CO_3, 3 equiv.**	**DME**	**MW, 110**	**1.5**	**0**	**37**
6[a]	Cs_2CO_3, 3 equiv.	DME	MW, 130	1.5	0	-[c]
7[a]	Cs_2CO_3, 3 equiv.	DME	130	17	0	-[c]

[a] LC-MS monitoring.
[b] Isolated yield after purification by chromatography column.
[c] Only traces of product were observed by LC-MS monitoring.

To functionalize the 5-position of compound **4** with various aryl or alkyne substituents, we performed a Suzuki-Miyaura or a Sonogashira cross-coupling reaction using experimental conditions previously described in 5-nitroimidazole series (Scheme 3) [18].

A series of 11 new compounds was obtained by Suzuki-Miyaura reaction, with isolated yields varying from 54% {[4-(hydroxymethyl)phenyl]boronic acid} to 82% (*p*-tolylboronic acid) (Table 2). A series of 8 new compounds was synthesized by Sonogashira reaction, with isolated yields varying from 35% (3-ethynylaniline) to 95% (ethynylbenzene). To confirm the regioselectivity of the cyclization reaction, the X-ray structure crystal was determined for compound **5h** (Fig. 4).

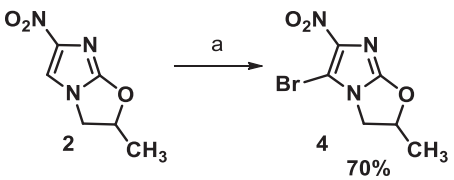


Scheme 3. General procedure for the pallado-catalyzed cross-coupling reactions on **4**: **a)** Boronic acid (1.3 equiv.), Na_2CO_3 (3 equiv.), $Pd(PPh_3)_4$ (0.05 equiv.), DME/H_2O (3/1), MW, 100 °C, 1.5 h. **b)** Alkynes (1.3 equiv.), CuI (0.1 equiv.), $Pd(PPh_3)_2Cl_2$ (0.05 equiv.), Et_3N (3 equiv.), DMF, rt, 6 h.

2.2. Biology

All 6-nitroimidazooxazole compounds synthesized were evaluated *in vitro* for their antileishmanial activity against *Leishmania donovani* promastigote and their antitrypanosomal activity against *Trypanosoma cruzi* amastigote forms. Their cytotoxicity against two different adherent cell lines was evaluated to assess their selectivity of action (Table 2). Thus, the HepG2 cell line was tested because it is a commonly used human-derived hepatocarcinome cell line expressing many of the hepatocyte-specific metabolic enzymes. The aim of this assay using HepG2 cells was to evaluate the impact of metabolic activation of this series of original synthetic compounds on cell viability [23]. Another cancer cell line was used, CHO, an epithelial cell line derived from the ovary of the Chinese hamster. These CHO cells do not display any metabolic activation, nor any immune modulation.

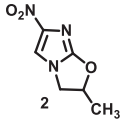
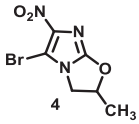
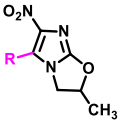
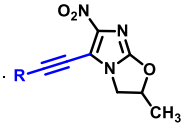
Intermediate product **2** without a substituent in 5-position showed better activity against *L. donovani* (IC_{50} = 0.10 μM) than the antileishmanial reference drug amphotericin B (IC_{50} = 0.18 μM), with low cytotoxicity (CC_{50} > 500 μM) and a very high selectivity index (SI > 5000). Some non-functionalized 6-nitroimidazooxazoles in 5-position are known to exhibit antikinetoplastid activity [16]. However, this compound **2** did not show antitrypanosomal activity, with an IC_{50} > 10 μM. The addition of a bromine atom (**4**) led to limited activity against *L. donovani* and *T. cruzi* (IC_{50} = 6.71 μM). The addition of an aryl group directly linked to the imidazooxazole bicycle (**5a-5k**) led to a loss of activity against *L. donovani* and *T. cruzi* (IC_{50} > 10 μM), while these compounds displayed no cytotoxicity whatever the used cell line (51 μM < CC_{50} ≤ 500 μM). An acetylenic function between the imidazooxazole bicycle and the aryl group (compounds **6a-6h**) enhanced the activity against *L. donovani* and *T. cruzi* but at the expense of their cytotoxicity (3.54 μM ≤ CC_{50} ≤ 18.14 μM), leading to low selectivity indexes. Nevertheless, the difference between the two cell lines is not sufficient to conclude on the higher toxicity of metabolite compounds.



Scheme 2. Synthesis of 5-bromo-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole **4**: a) NBS (1 equiv.), CH_3CN, rt, 1 h.

Regarding antileishmanial activity, only compound **6b** with an ethynyl-3-methoxyphenyl substituent and compound **6f** with an ethynyl-4-(*tert*-butyl)phenyl substituent have a selectivity index > 8. Moreover, compound **6f** displays activity close to the activity of the antileishmanial reference drug amphotericin B (IC_{50} = 0.61 μM *vs* 0.18 μM). The functionalization of the acetylenic group with a saturated cycle (**6d**, **6g**) or an alcohol function (**6c**, **6g**) seems to reduce the activity on *L. donovani*. According to the clogP values, the two most active compounds (**6b** and **6f**) of the acetylenic series are the most lipophilic (clogP ≥ 3). Contrastingly, the three least lipophilic compounds **6c**, **6d** and **6g** (clogP < 2.1) are the least active. Hydrophilic substituents seem to lead to decreased

Table 2
In vitro antileishmanial (*L. donovani*) and antitrypanosomal (*T. cruzi*) activity and cytotoxicity of synthesized imidazooxazoles.

	Compounds	R	Yield (%)	clogP[a]	CC_{50} HepG2 μM[b]	CC_{50} CHO μM[b]	*L. donovani* promastigote		*T.cruzi* amastigote	
							IC_{50}[c] (μM)	IS[d]	IC_{50}[e] (μM)	IS[f]
Intermediate products		–	55	0.66	>500	>500[j]	0.10 ± 0.01	>5000	>10	–
		–	70	1.24	20.05 ± 2.96	9.19	6.71 ± 2.8	1.4	>10	–
	**3[k] or 5a[l]**	3-CF_3-C_6H_4	79	2.82	>250	>250[j]	>10	–	>10	–
	5b	4-CH_2OH–C_6H_4	54	2.05	>500	>500[j]	>10	–	>10	–
	5c	3,4,5-CH_3O–C_6H_2	80	2.51	>250	>125[j]	>10	–	>10	–
	5d	4-Cl-C_6H_4	60	2.91	>140	>250[j]	>10	–	>10	–
	5e	4-CH_3-C_6H_4	82	2.67	>125	>250[j]	>10	–	>10	–
	5f	3-CN-C_6H_4	59	2.28	>500	>500[j]	>10	–	>10	–
	5g	C_6H_5	63	2.51	>500	>500[j]	>10	–	>10	–
	5h	5-methylthiophen-2-yl	80	2.86	>250	>125[j]	>10	–	>10	–
	5i	4-$CH_3(CH_2)_2O$–C_6H_4	76	3.63	>62.5	>125[j]	>10	–	>10	–
	5j	4-CHO-C_6H_4	58	2.07	>51	255.19 ± 2.42	>10	–	>10	–
	5k	4-CF_3O–C_6H_4	80	3.56	>184	>125[j]	>10	–	>10	–
	**6a**	4-CH_3-C_6H_4	83	2.94	4.44 ± 0.7	5.47 ± 0.6	1.66 ± 0.7	3.3	2.18 ± 0.6	2.5
	6b	**3-CH_3O–C_6H_4**	**85**	**3.00**	**6.41 ± 1.9**	**12.86 ± 0.3**	**1.58 ± 0.9**	**8.1**	**0.92 ± 0.6**	**14**
	6c	4-CH_2OH–C_6H_4	64	2.08	9 ± 0.8	8.77 ± 0.2	4.11 ± 1.7	2.1	1.61 ± 0.4	5.4
	6d	Cyclopropyl	45	2.03	14.77 ± 4.2	6.45 ± 0.1	4.59 ± 1.8	1.4	4.1 ± 1.8	1.6
	6e	3-NH_2-C_6H_4	35	2.23	8.30 ± 2.1	18.14 ± 1.2	3.49 ± 0.8	5.2	3.63 ± 2.0	5
	6f	**4-(*tert*-butyl)-C_6H_4**	**76**	**4.48**	**4.22 ± 0.9**	**6.62 ± 0.6**	**0.61 ± 0.4**	**10.8**	2.86 ± 1.2	2.3
	6g	Cyclopentanol	75	1.50	8.35 ± 3.1	6.65 ± 0.6	4.67 ± 2.9	1.4	2.11 ± 0.4	3.1
	6h	C_6H_5	95	2.65	5.40 ± 2.0	3.54 ± 0.3	2.08 ± 0.7	1.7	1.46 ± 0.3	2.4
Control drugs	**Doxorubicin**[g]	–	–	–	0.06 ± 0.01	4.65 ± 0.08	–	–	–	–
	Amphotericin B[h]	–	–	–	–	–	0.18 ± 0.03	–	–	–
	Benznidazole[i]	–	–	–		–	–	–	2.31 ± 0.1	–

[a] Calculated by Alogps version 2.1 software.
[b] Results are the mean ± SD of three independent biological replicates.
[c] Results are the mean ± SD of at least three independent biological replicates.
[d] SI = CC_{50} CHO cells/IC_{50} *L. donovani*.
[e] Results are the mean ± SD of four independent biological replicates.
[f] SI = CC_{50} CHO cells/IC_{50} *T. cruzi*.
[g] Doxorubicin was used as a cytotoxic reference drug.
[h] Amphotericin B was used as antileishmanial reference drug.
[i] Benznidazole was used as antitrypanosomal reference drug.
[j] The product could not be tested at higher concentrations in aqueous medium.
[k] The product was synthesized by direct arylation.
[l] The product was synthesized by Suzuki-Miyaura reaction.

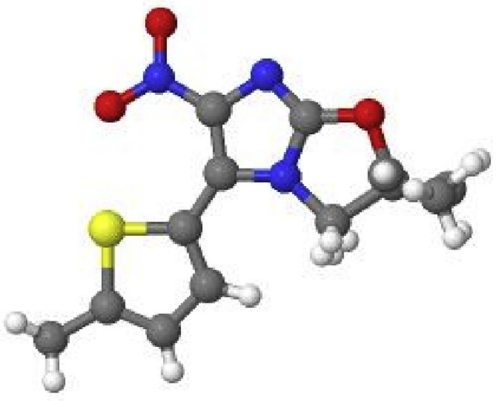


Fig. 4. X-ray structure of compound **5h**.

antileishmanial activity.

Regarding antitrypanosomal activity, five compounds (**6a, 6b, 6c**, **6g** and **6h**) have IC_{50} values lower than that of the antitrypanosomal reference drug benznidazole. One compound (**6b**) seems particularly interesting, with an $IC_{50} < 1$ μM on *T. cruzi* and a selectivity index >10. The other compounds show homogeneous antitrypanosomal activity, and there are no significant differences according to the substituents of the acetylenic group. We do not observe a correlation between antitrypanosomal activity and the lipophilicity of the compounds.

We observed a difference in the activity of our compounds between *L. donovani* and *T. cruzi*. Indeed, *L. donovani* activity decreased with the 5-functionalization by an acetylenic function from IC_{50} = 0.10 μM (SI > 5000) for compound **2** to 0.61 μM ≤ IC_{50} ≤ 4.67 μM (1.4 ≤ SI ≤ 10.8) for compounds **6a-6h** whereas *T. cruzi* activity increased from $IC_{50} \geq 10$ μM for compound **2** to 0.92 μM ≤ IC_{50} ≤ 4.1 μM for compounds **6a-6h**. It has been described in the literature that the dissociation of the imidazole derivatives activity between *Leishmania* and

Trypanosoma could come from a difference in the affinity for an enzyme involved in the synthesis of sterols, the 14α-demethylase (CYP51) [24–26]. Indeed, the CYP51 active site volume and surface area are smaller in *Leishmania* than in *T. cruzi* [24]. This hypothesis could explain that the functionalization of the 5-position of our compounds leads to a steric hindrance which reduced binding to the *Leishmania* CYP51 active site with for consequence a limited activity on *Leishmania*. These results suggest that the anti *T. cruzi* activity could come from the inhibition of CYP51 and not only from the reduction of the nitro group frequently involved in the antikinetoplastid activity of nitro derivatives [27,28]. This work opens up new perspectives for pharmacomodulation to reduce the toxicity of our compounds, which is currently the factor limiting their therapeutic interest. The synthesis of non-nitrate analogues by deletion or replacement of the nitro group and replacement of the acetylenic function are the first modifications to be carried out in the attempt to reduce toxicity and increase the selectivity index of our compounds on *T. cruzi*.

3. Conclusion

Twenty-one new 5-substituted 6-nitroimidazooxazole compounds were synthesized and evaluated for their antileishmanial and antitrypanosomal activities. On the one hand, 5-functionalization led to lower antileishmanial activity and higher cytotoxicity compared to compound **2** bearing a hydrogen atom at 5-position. On the other hand, some 5-substituted compounds led to better activity against *T. cruzi* than compound **2** and the reference drug benznidazole. Biological results showed that the first series of eleven compounds with an aryl group directly linked to the 6-nitroimidazooxazole bicycle (**5a-5k**) was not active. The introduction of an acetylenic function between the 6-nitroimidazooxazole bicycle and the aryl group enhanced activity but also cytotoxicity. However, one compound (**6b**) bearing an ethynyl-3-methoxyphenyl substituent showed good activity against *T. cruzi*, with a selectivity index >10. In further extensions of this work, the acetylenic group will be replaced by an ethylenic or saturated chain and the nitro group will be replaced or deleted.

4. Experimental section

4.1. Chemistry

4.1.1. General procedure

All commercially available solvents and reagents were purchased from commercial suppliers (Fluorochem or Sigma Aldrich) and used without further purification. Melting points were determined in open capillary tubes with a Büchi apparatus B-540 and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker Avance 250 or 400 MHz instrument at the Faculté de Pharmacie de Marseille and Faculté des Sciences de St Jérôme (Marseille) at 24 °C in $CDCl_3$ and DMSO-d_6 solvents. Chemical shifts are given in δ values referenced to the solvent and using tetramethylsilane (TMS) as an internal standard. High resolution mass spectra were recorded on a QStar Elite mass spectrometer at the Spectropôle department of the Faculté des Sciences de St Jérôme. PEG was the matrix for HRMS. Silica Gel 60 (Merck, particle size 0.063–0.200 mm, 70–230 mesh ASTM) was used for column chromatography. TLC was performed on aluminium plates coated with silica gel 60F-254 (Merk) in an appropriate eluent, with visualization undo ultraviolet light (234 nm). Progress of the reactions and purity of the synthesized compounds were monitored by LC-MS analyses realized at the Faculté de Pharmacie de Marseille on a Thermo Scientific Accela High Speed LC System® coupled with a single quadrupole mass spectrometer Thermo MSQ Plus®. The RP-HPLC column used is a Thermo Hypersil Gold® 50 _ 2.1 mm (C13 bounded), with particles of 1.9 mM diameter. The volume of sample injected on the column was 1 mL. The chromatographic analysis, total duration of 8 min, was performed with the following gradient of solvents: t = 0 min, water/methanol 50/50; 0 < t < 4 min, linear increase in the proportion of water to a water/methanol ratio of 95/5; 4 < t < 6 min, water/methanol 95/5; 6 < t < 7 min, linear decrease in the proportion of water to return to a ratio water/methanol of 50/50; 6 < t < 7 min, water/methanol 50/50. The water used was buffered with 5 mM ammonium acetate. Microwave reactions were performed with a Biotage® Initiator Microwave oven using 2–5 mL sealed vials; temperatures were measured with an IR-sensor and reaction times are given as hold times.

4.1.2. Synthesis of intermediate products

*4.1.2.1. 1-(2-Bromo-4-nitro-1H-imidazole-1-yl)propan-2-ol **(1)**.* To a solution of 2-bromo-4(5)-nitro-1*H*-imidazole [29] (500 mg, 2.61 mmol, 1 equiv.) in DIPEA (1.69 g, 13 mmol, 2.3 mL, 5 equiv.), propylene oxyde (227 mg, 3.91 mmol, 0.27 mL, 1.5 equiv.) was added. The reaction mixture was stirred for 1.5 h at 110 °C under microwave irradiation. After cooling, the reaction mixture was poured into an ice-$NaHCO_3$ aqueous solution (60 mL) and extracted four times with CH_2Cl_2 (4 × 60 mL). The organic layer was washed three times with brine (3 × 100 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 99:1 and recrystallized from *i*-PrOH).

Yield: 391 mg (65%); yellow solid; mp 116 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.12 (d, $^{3}J_{H\text{-}H}$ = 5.6 Hz, 3H, CH_3); 3.88–4.08 (m, 3H, CH, CH_2); 5.17 (d, $^{3}J_{H\text{-}H}$ = 5.2 Hz, 1H, OH); 8.51 (s, 1H, CH_{Ar}).

^{13}C NMR (62.5 MHz, DMSO-d_6): δ = 20.9 (CH_3); 55.5 (CH_2); 65.1 (CH); 121.4 (C); 125.4 (CH); 146.4 (C).

HRMS: *m*/*z* $[M + H]^+$ calcd for $[C_6H_8BrN_3O_3]^+$: 249.9822; found: 249.9824.

*4.1.2.2. 2-Methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazole **(2)**.* A solution of 1-(2-bromo-4-nitro-1*H*-imidazole-1-yl)propan-2-ol **1** (1 g, 40 mmol, 1 equiv.) in *N,N*-dimethylformamide anhydrous (20 mL) under N_2 at 0 °C was treated with 60% NaH (321 mg, 80 mmol, 2 equiv.). The resulting mixture was stirred at 0 °C for 65 min, quenched with ice/aqueous $NaHCO_3$ (50 mL) and extracted with ethyl acetate (5 × 50 mL). The organic layer was washed five times with brine (5 × 100 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 99:1 and recrystallized from *i*-PrOH).

Yield: 373 mg (55%); white solid; mp 62 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.67 (d, $^{3}J_{H\text{-}H}$ = 6.3 Hz, 3H, CH_3); 3.88 (dd, $^{3}J_{H\text{-}H}$ = 7.5 Hz, $^{2}J_{H\text{-}H}$ = 10.2 Hz, 1H, CH_2); 4.40 (dd, $^{3}J_{H\text{-}H}$ = 8.1 Hz, $^{2}J_{H\text{-}H}$ = 10.2, 1H, CH_2); 5.40–5.48 (m, 1H, CH); 7.54 (s, 1H, CH_{Ar}).

^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 20.3 (CH_3); 50.2 (CH_2); 83.8 (CH); 112.5 (CH); 156.5 (C) (C–NO_2 was not observed in these conditions).

HRMS: *m*/*z* $[M + H]^+$ calcd for $[C_6H_7N_3O_3]^+$: 170.0560; found: 170.0561.

*4.1.2.3. 5-Bromo-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazole **(4)**.* To a solution of 2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole **2** (300 mg, 1.77 mmol, 1 equiv.) in acetonitrile (7 mL), *N*-bromosuccinimide (1.77 mmol, 315 mg, 1 equiv.) was added and the reaction mixture was stirred 1 h at room temperature. After evaporation under reduced pressure, the residue was purified by

column chromatography (silica gel, CH_2Cl_2/MeOH, 99:1 and recrystallized from *i*-PrOH).

Yield: 307 mg (70%); yellow solid; mp 135 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.68 (d, $^3J_{H\text{-}H}$ = 6.4, 3H, CH_3); 3.85 (dd, $^3J_{H\text{-}H}$ = 7.6 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 4.38 (dd, $^3J_{H\text{-}H}$ = 7.9 Hz, $^2J_{H\text{-}H}$ = 10.2, 1H, CH_2); 5.45–5.54 (m, 1H, CH).

^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 20.5 (CH_3); 49.9 (CH_2); 80.3 (C); 83.9 (CH); 96.9 (C); 155.3 (C).

HRMS: *m/z* $[M + H]^+$ calcd for $[C_6H_6BrN_3O_3]^+$: 247.9665; found: 247.9662.

*4.1.3. General procedure for the Suzuki-Miyaura reaction of **4** with various boronic acids*

To a sealed glass vial were added the brominated derivative **4** (100 mg, 0.4 mmol, 1 equiv.), boronic acid derivative (1.3 equiv.), $Pd(PPh_3)_4$ (5 mol%), and Na_2CO_3 (3 equiv.) in DME/H_2O mixture (1.5 mL/0.5 mL). The vessel was capped, and the solution was heated for 1.5 h at 100 °C. After cooling, the reaction mixture was filtered on Celite. The solution was poured in water (40 mL) and extracted three times with CH_2Cl_2 (40 mL). The organic layer was washed three times with brine (3 × 100 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 99:1) and recrystallized from *i*-PrOH.

*4.1.3.1. 2-Methyl-6-nitro-5-[3-(trifluoromethyl)phenyl]-2,3-dihydroimidazo[2,1-b]oxazole (**5a** = **3**).* Yield: 100 mg (79%); brown oil solidified by trituration in cyclohexane.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.68 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 3.80 (dd, $^3J_{H\text{-}H}$ = 7.5 Hz, $^2J_{H\text{-}H}$ = 10.2 Hz, 1H, CH_2); 4.35 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^2J_{H\text{-}H}$ = 10.2 Hz, 1H, CH_2); 5.45–5.54 (m, 1H, CH); 7.61–7.65 (m, 1H, CH_{Ar}); 7.72–7.77 (m, 3H, $3CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.5 (CH_3); 50.1 (CH_2); 84.2 (CH); 122.5 (C); 125.2 (CH); 126.2 (q, $^3J_{C\text{-}F}$ = 4.4 Hz, CH); 127.0 (q, $^3J_{C\text{-}F}$ = 3.7 Hz, CH); 127.7 (CH); 129.5 (C); 131.6 (q, $^1J_{C\text{-}F}$ = 281.0 Hz, C); 133.1 (C); 142.7 (C); 155.5 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{13}H_{10}F_3N_3O_3]^+$: 314.0747; found: 314.0746.

*4.1.3.2. [4-(2-Methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazol-5-yl)phenyl]methanol (**5b**).* Yield: 60 mg (54%); yellow solid; mp 80 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.67 (d, $^3J_{H\text{-}H}$ = 6.2 Hz, 3H, CH_3); 3.78 (dd, $^3J_{H\text{-}H}$ = 7.6 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 4.31 (dd, $^3J_{H\text{-}H}$ = 7.9 Hz, $^2J_{H\text{-}H}$ = 10.1, 1H, CH_2); 4.78 (s, 2H, CH_2); 5.43–5.48 (m, 1H, CH); 7.50 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 2H, $2CH_{Ar}$); 7.53 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 2H, $2CH_{Ar}$). (OH was not observed in these conditions).

^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 20.4 (CH_3); 49.9 (CH_2); 64.6 (CH_2); 83.8 (CH); 125.7 (C); 126.1 (C); 127.0 (2CH); 128.2 (C); 129.5 (2CH); 143.1 (C); 155.1 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{13}H_{13}N_3O_4]^+$: 276.0979; found: 276.0980.

*4.1.3.3. 2-Methyl-6-nitro-5-(3,4,5-trimethoxyphenyl)2,3-dihydroimidazo[2,1-b]oxazole (**5c**).* Yield: 108 mg (80%); yellow solid; mp 173 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.67 (d, $^3J_{H\text{-}H}$ = 6.2 Hz, 3H, CH_3); 3.79 (dd, $^3J_{H\text{-}H}$ = 7.7 Hz, $^2J_{H\text{-}H}$ = 10.2 Hz, 1H, CH_2); 3.87 (s, 6H, $2OCH_3$); 3.91 (s, 3H, OCH_3); 4.35 (dd, $^3J_{H\text{-}H}$ = 7.9 Hz, $^2J_{H\text{-}H}$ = 10.2 Hz, 1H, CH_2); 5.44–5.50 (m, 1H, CH); 6.76 (s, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.5 (CH_3); 50.2 (CH_2); 56.6 ($2OCH_3$); 61.1 (OCH_3); 83.9 (CH); 107.2 (2CH); 121.8 (C); 127.2 (C); 139.9 (C); 142.2 (C); 153.5 (2C); 155.0 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{15}H_{17}N_3O_6]^+$: 336.1190; found: 336.1191.

*4.1.3.4. 5-(4-Chlorophenyl)-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazole (**5d**).* Yield: 67 mg (60%); yellow solid (solidification after trituration in cyclohexane); mp 71 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.66 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 3.76 (dd, $^3J_{H\text{-}H}$ = 7.7 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 4.32 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 5.44–5.49 (m, 1H, CH); 7.45–7.51 (m, 4H, $4CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4 (CH_3); 49.9 (CH_2); 83.8 (CH); 125.1 (C); 127.5 (C); 129.1 (2CH); 130.6 (2CH); 131.6 (C); 136.3 (C); 155.2 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{12}H_{10}ClN_3O_3]^+$: 280.0483; found: 280.0486.

*4.1.3.5. 2-Methyl-6-nitro-5-(p-tolyl)-2,3-dihydroimidazo[2,1-b]oxazole (**5e**).* Yield: 86 mg (82%); yellow solid; mp 174 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.66 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 2.41 (s, 3H, CH_3); 3.75 (dd, $^3J_{H\text{-}H}$ = 7.6 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 4.30 (dd, $^3J_{H\text{-}H}$ = 8.0 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 5.41–5.46 (m, 1H, CH); 7.27 (d, $^3J_{H\text{-}H}$ = 3.7 Hz, 2H, $2CH_{Ar}$); 7.43 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4 (CH_3); 21.4 (CH_3); 49.8 (CH_2); 83.7 (CH); 123.7 (C); 127.3 (C); 129.1 (2CH); 129.4 (2CH); 140.5 (C); 142.0 (C); 154.9 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{13}H_{13}N_3O_3]^+$: 260.1030; found: 260.1029.

*4.1.3.6. 3-(2-Methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazol-5-yl)benzonitrile (**5f**).* Yield: 64 mg (59%); yellow solid; mp 159 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.68 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 3.84 (dd, $^3J_{H\text{-}H}$ = 7.7 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 4.38 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 5.49–5.54 (m, 1H, CH); 7.62 (t, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H, CH_{Ar}); 7.74 (d, $^3J_{H\text{-}H}$ = 7.8 Hz, 1H, CH_{Ar}); 7.83 (d, $^3J_{H\text{-}H}$ = 6.0 Hz, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.3 (CH_3); 50.0 (CH_2); 84.0 (CH); 113.3 (C); 115.8 (C); 117.7 (C); 128.3 (C); 129.7 (CH); 132.5 (CH); 133.3 (CH); 133.9 (CH); 142.9 (C); 155.5 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{13}H_{10}N_4O_3]^+$: 271.0826; found: 271.0826.

*4.1.3.7. 2-Methyl-6-nitro-5-phenyl-2,3-dihydroimidazo[2,1-b]oxazole (**5g**).* Yield: 62 mg (63%); yellow solid; mp 153 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.67 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 3.78 (dd, $^3J_{H\text{-}H}$ = 7.7 Hz, $^2J_{H\text{-}H}$ = 10.0 Hz, 1H, CH_2); 4.30 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^2J_{H\text{-}H}$ = 10.0 Hz, 1H, CH_2); 5.44–5.50 (m, 1H, CH); 7.47–7.54 (m, 5H, $5CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4 (CH_3); 49.9 (CH_2); 83.7 (CH); 126.7 (C); 126.9 (C); 128.7 (2CH); 129.3 (2CH); 130.1 (CH); 142.2 (C); 155.0 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{12}H_{11}N_3O_3]^+$: 246.0873; found: 246.0873.

*4.1.3.8. 2-Methyl-5-(5-methylthiophen-2-yl)-6-nitro-2,3-dihydroimidazo[2,1-b]oxazole (**5h**).* Yield: 86 mg (80%); yellow solid; mp 155 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.69 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 2.55 (s, 3H, CH_3); 3.97 (dd, $^3J_{H\text{-}H}$ = 7.5 Hz, $^2J_{H\text{-}H}$ = 9.8 Hz, 1H, CH_2); 4.53 (dd, $^3J_{H\text{-}H}$ = 8.3 Hz, $^2J_{H\text{-}H}$ = 9.7 Hz, 1H, CH_2); 5.45–5.50 (m, 1H, CH); 6.83 (d, $^3J_{H\text{-}H}$ = 3.5 Hz, 1H, CH_{Ar}); 7.50 (d, $^3J_{H\text{-}H}$ = 3.7 Hz, 1H, CH_{Ar}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 15.5 (CH_3); 20.7 (CH_3); 51.5 (CH_2); 83.9 (CH); 123.0 (C); 125.1 (C); 126.4 (CH); 131.1 (CH); 141.4 (C); 144.8 (C); 155.1 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{11}H_{11}N_3O_3S]^+$: 266.0594; found: 266.0593.

*4.1.3.9. 2-Methyl-6-nitro-5-(4-propoxyphenyl)-2,3-dihydroimidazo [2,1-b]oxazole **(5i)**.* Yield: 93 mg (76%); yellow solid; mp 116 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.06 (t, $^3J_{H\text{-}H}$ = 4.4 Hz, 3H, CH_3); 1.67 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 1.82−1.87 (m, 2H, CH_2); 3.78 (dd, $^3J_{H\text{-}H}$ = 7.6 Hz, $^2J_{H\text{-}H}$ = 10.2 Hz, 1H, CH_2); 3.98 (t, $^3J_{H\text{-}H}$ = 6.5 Hz, 2H, CH_2); 4.30 (dd, $^3J_{H\text{-}H}$ = 7.9 Hz, $^2J_{H\text{-}H}$ = 10.0 Hz, 1H, CH_2); 5.42−5.47 (m, 1H, CH); 6.98 (d, $^3J_{H\text{-}H}$ = 8.8 Hz, 2H, $2CH_{Ar}$); 7.48 (d, $^3J_{H\text{-}H}$ = 8.7 Hz, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.5 (CH_3); 20.4 (CH_3); 22.5 (CH_2); 49.9 (CH_2); 69.7 (CH_2); 83.6 (CH); 114.7 (2CH); 118.4 (C); 130.8 (2CH); 141.9 (C); 154.8 (C); 160.5 (C). (C−NO_2 was not observed in these conditions).

HRMS: *m*/*z* $[M+H]^+$ calcd for $[C_{15}H_{17}N_3O_4]^+$: 304.1292; found: 304.1288.

*4.1.3.10. 4-(2-Methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazol-5-yl) benzaldehyde **(5j)**.* Yield: 64 mg (58%); yellow solid; mp 74 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.70 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 3.84 (dd, $^3J_{H\text{-}H}$ = 7.8 Hz, $^2J_{H\text{-}H}$ = 9.8 Hz, 1H, CH_2); 4.36 (dd, $^3J_{H\text{-}H}$ = 8.2 Hz, $^2J_{H\text{-}H}$ = 9.8 Hz, 1H, CH_2); 5.48−5.55 (m, 1H, CH); 7.72 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 2H, $2CH_{Ar}$); 8.0 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 2H, $2CH_{Ar}$); 10.08 (s, 1H, CH_{Ar}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.5 (CH_3); 50.2 (CH_2); 84.1 (CH); 125.3 (C); 129.9 ($2CH_{Ar}$); 130.1 ($2CH_{Ar}$); 132.5 (C); 137.0 (C); 143.0 (C); 155.7 (C); 191.3 (C).

HRMS: *m*/*z* $[M+H]^+$ calcd for $[C_{13}H_{11}N_3O_4]^+$: 274.0822; found: 274.0822.

*4.1.3.11. 2-Methyl-6-nitro-5-[4-(trifluoromethoxy)phenyl]-2,3-dihydroimidazo[2,1-b]oxazole **(5k)**.* Yield: 105 mg (80%); yellow solid (solidification after trituration in cyclohexane); mp 73 °C.

^{1}H NMR (400 MHz, $CDCl_3$): 1.66 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 3.79 (dd, $^3J_{H\text{-}H}$ = 7.8 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 4.34 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^2J_{H\text{-}H}$ = 10.0 Hz, 1H, CH_2); 5.45−5.50 (m, 1H, CH); 7.32 (d, $^3J_{H\text{-}H}$ = 8.3 Hz, 2H, $2CH_{Ar}$); 7.60 (d, $^3J_{H\text{-}H}$ = 8.7 Hz, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.6 (CH_3); 50.2 (CH_2); 84.2 (CH); 120.6 (q, $^1J_{C\text{-}F}$ = 259.0 Hz, C); 121.2 (2CH); 125.5 (C); 125.8 (C); 131.4 (2CH); 142.7 (C); 150.5 (C); 155.5 (C).

HRMS: *m*/*z* $[M+H]^+$ calcd for $[C_{13}H_{10}F_3N_3O_4]^+$: 330.0696; found: 330.0698.

*4.1.4. General procedure for the Sonogashira reaction of **4** with various alkynes*

To a sealed glass vial were added the brominated derivative **4** (100 mg, 0.4 mmol, 1 equiv.), alkyne derivative (0.6 mmol, 1.5 equiv.), CuI (7.7 mg, 0.04 mmol, 0.1 equiv.), $Pd(PPh_3)_2Cl_2$ (14.2 mg, 0.02 mmol, 5 mol%), and Et_3N (82 mg, 0.8 mmol, 0.11 mL, 3 equiv.) in DMF (3 mL). The vessel was capped and then evacuated and backfilled with N_2 (process repeated 3 ×). The solution was stirred at room temperature for 6 h. The reaction mixture was then filtered on Celite, poured in water (40 mL) and extracted three times with ethyl acetate (40 mL). The organic layer was washed five times with brine (5 × 100 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 99:1) and recrystallized from *i*-PrOH.

*4.1.4.1. 2-Methyl-6-nitro-5-(p-tolylethynyl)-2,3-dihydroimidazo[2,1-b]oxazole **(6a)**.* Yield: 94 mg (83%); yellow solid; mp 128 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.69 (d, $^3J_{H\text{-}H}$ = 6.2 Hz, 3H, CH_3); 2.38 (s, 3H, CH_3); 3.88−3.93 (m, 1H, CH_2); 4.44 (m, 1H, CH_2); 5.50 (m, 1H, CH); 7.19 (d, $^3J_{H\text{-}H}$ = 7.7 Hz, 2H, $2CH_{Ar}$); 7.46 (d, $^3J_{H\text{-}H}$ = 7.8 Hz, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.6 (CH_3); 21.8 (CH_3); 49.4 (CH_2); 75.3 (C); 84.4 (CH); 101.5 (C); 109.7 (C); 118.1 (C); 129.5 (2CH); 132.0 (2CH); 140.7 (C); 155.1 (C). (C−NO2 was not observed in these conditions).

HRMS: *m*/*z* $[M+H]^+$ calcd for $[C_{15}H_{13}N_3O_3]^+$: 284.1030; found: 284.1031.

*4.1.4.2. 5-[(3-Methoxyphenyl)ethynyl]-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazole **(6b)**.* Yield: 103 mg (85%); yellow solid; mp 148 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.69 (d, $^3J_{H\text{-}H}$ = 6.3 Hz, 3H, CH_3); 3.82 (s, 3H, CH_3); 3.90−3.97 (m, 1H, CH_2); 4.42−4.49 (m, 1H, CH_2); 5.46−5.53 (m, 1H, CH); 6.97 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H, CH); 7.06 (s, 1H, CH_{Ar}); 7.15 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 1H, CH); 7.28−7.30 (m, 1H, CH_{Ar}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.8 (CH_3); 49.6 (CH_2); 55.7 (CH_3); 75.7 (C); 84.6 (CH); 101.0 (C); 109.5 (C); 116.9 (CH); 116.9 (CH); 122.3 (C); 124.8 (CH); 130.0 (CH); 148.1 (C); 155.4 (C); 159.7 (C).

HRMS: *m*/*z* $[M+H]^+$ calcd for $[C_{15}H_{13}N_3O_4]^+$: 300.0979; found: 300.0985.

*4.1.4.3. {4-[(2-Methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazol5-yl) ethynyl]phenyl}methanol **(6c)**.* Yield: 78 mg (64%); yellow solid; mp 192 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.57 (d, $^3J_{H\text{-}H}$ = 6.3 Hz, 3H, CH_3); 4.05 (dd, $^3J_{H\text{-}H}$ = 7.4 Hz, $^2J_{H\text{-}H}$ = 9.9 Hz, 1H, CH_2); 4.51 (dd, $^3J_{H\text{-}H}$ = 8.2 Hz, $^2J_{H\text{-}H}$ = 9.9 Hz, 1H, CH_2); 4.56 (d, $^3J_{H\text{-}H}$ = 5.5 Hz, 2H, CH_2); 5.35 (t, $^3J_{H\text{-}H}$ = 5.7 Hz, 1H, OH); 5.54−5.63 (m, 1H, CH); 7.43 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 2H, $2CH_{Ar}$); 7.58 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 19.5 (CH_3); 48.5 (CH_2); 62.2 (CH_2); 76.0 (C); 85.4 (CH); 99.8 (C); 109.4 (C); 118.4 (C); 126.5 (2CH); 131.3 (2CH); 145.0 (C); 146.3 (C); 155.0 (C).

HRMS: *m*/*z* $[M+H]^+$ calcd for $[C_{15}H_{13}N_3O_4]^+$: 300.0979; found: 300.0981.

*4.1.4.4. 5-(Cyclopropylethynyl)-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazole **(6d)**.* Yield: 50 mg (45%); yellow solid; mp 137 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.91−1.04 (m, 4H, $2CH_2$); 1.51−1.59 (m, 1H, CH); 1.65 (d, $^3J_{H\text{-}H}$ = 6.5 Hz, 3H, CH_3); 3.80 (dd, $^3J_{H\text{-}H}$ = 7.4 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 4.33 (dd, $^3J_{H\text{-}H}$ = 8.2 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 5.41−5.49 (m, 1H, CH).

^{13}C NMR (100 MHz, $CDCl_3$): 1.0 (CH); 9.9 ($2CH_2$); 20.7 (CH_3); 49.4 (CH_2); 62.7 (C); 84.4 (CH); 107.2 (C); 110.3 (C); 147.7 (C); 154.7 (C).

HRMS: *m*/*z* $[M+H]^+$ calcd for $[C_{11}H_{11}N_3O_3]^+$: 234.0873; found: 234.0874.

*4.1.4.5. 3-[(2-Methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazol-5-yl) ethynyl]aniline **(6e)**.* Yield: 42 mg (35%); yellow solid; mp 176 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.57 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 4.02 (dd, $^3J_{H\text{-}H}$ = 7.3 Hz, $^2J_{H\text{-}H}$ = 10.0 Hz, 1H, CH_2); 4.49 (dd, $^3J_{H\text{-}H}$ = 8.3 Hz, $^2J_{H\text{-}H}$ = 10.0 Hz, 1H, CH_2); 5.38 (s, 2H, NH_2); 5.56 (m, 1H, CH); 6.68−6.78 (m, 2H, $2CH_{Ar}$); 7.09−7.13 (m, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 19.7 (CH_3); 48.7 (CH_2); 75.3 (C); 85.5 (CH); 101.0 (C); 109.8 (C); 116.0 (CH); 116.1 (CH); 119.0 (CH); 120.6 (C); 129.5 (CH); 146.4 (C); 149.0 (C); 155.1 (C).

HRMS: *m*/*z* $[M+H]^+$ calcd for $[C_{14}H_{12}N_4O_3]^+$: 285.0982; found: 285.0983.

*4.1.4.6. 5-{[4-(Tert-butyl)phenyl]ethynyl}-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazole **(6f)**.* Yield: 100 mg (76%); yellow solid; mp 154 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.32 (s, 9H, $3CH_3$); 1.70 (d, $^3J_{H\text{-}H}$ = 6.2 Hz, 3H, CH_3); 3.90−3.95 (m, 1H, CH_2); 4.42−4.47 (m, 1H, CH_2); 5.48−5.53 (m, 1H, CH); 7.40−7.42 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 2H, $2CH_{Ar}$); 7.52 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): 20.5 (CH_3); 31.1 ($3CH_3$); 35.0 (C); 49.3 (CH_2); 75.2 (C); 84.2 (CH); 101.3 (C); 109.6 (C); 118.0 (C); 125.7 (2CH); 131.8 (2CH); 153.6 (C); 155.0 (C). (C−NO2 was not observed in these conditions).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{18}H_{19}N_3O_3]^+$: 326.1499; found: 326.1500.

*4.1.4.7. 1-[(2-Methyl-6-nitro-2,3-dihydroimidazo[2,1-b]oxazol-5-yl) ethynyl]cyclopentanol **(6g)**.* Yield: 100 mg (75%); yellow solid; mp 172 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.55 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 1.70−1.77 (m, 4H, $2CH_2$); 1.93 (m, 4H, $2CH_2$); 3.92 (dd, $^3J_{H\text{-}H}$ = 7.4 Hz, $^2J_{H\text{-}H}$ = 10.0 Hz, 1H, CH_2); 4.40 (dd, $^3J_{H\text{-}H}$ = 8.3 Hz, $^2J_{H\text{-}H}$ = 10.0 Hz, 1H, CH_2); 5.49−5.57 (m, 1H, CH); 5.60 (s, H, OH).

^{13}C NMR (100 MHz, DMSO-d_6): 19.7 (CH_3); 23.0 (2CH); 41.8 (2CH); 48.6 (CH_2); 68.9 (C); 73.1 (C); 85.4 (CH); 106.4 (C); 109.6 (C); 146.1 (C); 154.9 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{13}H_{15}N_3O_4]^+$: 278.1135; found: 278.1140.

*4.1.4.8. 2-Methyl-6-nitro-5-(phenylethynyl)-2,3-dihydroimidazo[2,1-b]oxazole **(6h)**.* Yield: 103 mg (95%); yellow solid; mp 172 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.69 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3H, CH_3); 3.92 (dd, $^3J_{H\text{-}H}$ = 7.5 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 4.44 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^2J_{H\text{-}H}$ = 10.1 Hz, 1H, CH_2); 5.46−5.54 (m, 1H, CH); 7.35−7.40 (m, 3H, $3CH_{Ar}$); 7.55−7.57 (m, 2H, $2CH_{Ar}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.8 (CH_3); 49.6 (CH_2); 75.9 (C); 84.6 (CH); 101.1 (C); 109.6 (C); 121.4 (C); 128.9 (2CH); 130.3 (CH); 132.3 (2CH); 148.1 (C); 155.4 (C).

HRMS: *m/z* $[M+H]^+$ calcd for $[C_{14}H_{11}N_3O_3]^+$: 270.0873; found: 270.0876.

*4.1.5. Crystal data for compound **5h***

$C_{11}H_{11}N_3O_3S$, M = 265.29, a = 15.1000 (3) Å, b = 8.7277(2) Å, c = 179273(5) Å, α = 90, β = 90, γ = 90, V = 2362.61(10) Å^3, T = 298 K, space group P1 Z = 8,7755 reflections measured, 2264 independent reflections (R_{int} = 2264). The final R_1 values were 0.0445 ($I > 2\sigma(I)$). The final w$R(F^2)$ values were 0.1259 ($I > 2\sigma(I)$). The final R_1 values were 0.0501 (all data). The final w$R(F^2)$ values were 0.1310 (all data). The goodness of fit on F^2 was 1.143. CCDC 1951503 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.cdcc.cam.ac.uk/data_request/cif from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: + 44 (1223) 336033; email: deposit@ccdc.cam.ac.uk.

4.2. Biology

4.2.1. Antileishmanial in vitro activity assay against L. donovani promastigote forms

L. donovani (MHOM/IN/00/DEVI) used in this study was provided by the CNR Leishmania (Montpellier, France). The effects of the tested compound on the growth of *L. donovani* (MHOM/IN/00/DEVI) promastigotes were assessed by MTT assay [30]. 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 and amphotericin B (reference drug purchased from Sigma-Aldrich) were added to each set of experiments. After 72 h incubation period at 27 °C, parasite metabolic activity was determined. Each well was microscopically examined for precipitate formation. To each well was added 20 μL of a 5 mg/mL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] solution followed by incubation for another 4 h. The enzyme reaction was stopped by addition of 100 μL of 50% isopropanol/10% sodium dodecyl sulfate [31]. Plates were shaken vigorously (300 rpm) for 10 min, and the absorbance measured in a plate reader at 570 nm in a BIO-TEK ELx808 Absorbance Microplate Reader. The IC_{50} was defined as the concentration of drug required to inhibit by 50% of the metabolic activity of *L. donovani* promastigotes compared to the control. IC_{50} values were calculated by non-linear regression analysis of data from dose-response curves, using TableCurve 2D V5.0 software (Systat Software, San Jose, CA, USA). IC_{50} values is are reported as means calculated from at least three independent experiments.

4.2.2. Antitrypanosomal activity assay on T. cruzi amastigotes

Experiments were performed using tdTomato labeled trypomastigotes with the modifications described by Recher et al. [32] IC_{50} values were determined by non-linear regression analysis using SigmaPlot.

4.2.3. Cytotoxicity in vitro assays on CHO and HepG2 cell lines

A cytotoxicity evaluation was realized according to the method of Mosmann [30] with slight modifications [33] to determine the cytotoxic concentrations 50% (CC_{50}) and using doxorubicin as a cytotoxic reference compound. These assays were performed toward two adherent cell lines: i) the human HepG2 cell line (hepatocarcinoma cell line purchased from ATCC, ref HB-8065), ii) the mammal CHO cell line (Chinese Hamster Ovarian cell line purchased from ATCC, ref CCL-61).

Briefly, an adjusted culture of cells in 100 μL of culture medium supplemented with 10% FCS, 1% L-glutamine (200 mM) and penicillin (100 U/mL)/streptomycin (100 μg/mL) (complete RPMI medium), was inoculated into each well of 96-well plates and incubated at 37 °C in a humidified 5% CO_2. After 24 h incubation to obtain adherent cells, 100 μL of medium with various product concentrations dissolved in DMSO (final concentration less than 0.5% v/v) were added and the plates were incubated for 24 h (CHO) to 72 h (HepG2) at 37 °C. Duplicate assays were performed for each sample. Each plate-well was then microscope-examined for detecting possible precipitate formation before the medium was aspirated from the cells. 100 μL of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazoliumbromide) solution (0.5 mg/mL in medium without FCS) were then added to each well. Cells were incubated for 2 h at 37 °C to allow MTT oxidation by mitochondrial dehydrogenase in the viable cells. 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 spectrophotometer using a BIO-TEK ELx808 Absorbance Microplate Reader. 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 concentrations (HepG2 CC_{50} and CHO CC_{50} respectively) were determined by non-linear regression analysis processed on dose-response curves, using the Table Curve software 2D v.5.0. CC_{50} values represent the mean value calculated from three independent experiments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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