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Research Article

Romain Paoli-Lombardo, Nicolas Primas, Sébastien Hutter, Caroline Castera-Ducros, Inès Jacquet, Pierre Verhaeghe, Nadine Azas, Pascal Rathelot and Patrice Vanelle*

Structural simplification of the 3-nitroimidazo [1,2-*a*]pyridine antileishmanial pharmacophore: Design, synthesis, and antileishmanial activity of novel 2,4-disubstituted 5-nitroimidazoles

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Abstract: As part of our ongoing antileishmanial structure–activity relationship study, a structural simplification of the 3-nitroimidazo[1,2-*a*]pyridine ring to a 5-nitroimidazole moiety was conducted. A series of novel 2,4-disubstituted 5-nitroimidazole derivatives, including the 5-nitroimidazole analog of Hit A and the 4-phenylsulfonylmethyl analog of fexinidazole, were obtained by using the vicarious nucleophilic substitution of hydrogen (VNS) reaction, to substitute position 4, and by using the tetrakis(dimethylamino)ethylene methodology to modulate position 2. The molecular structures of eight novel 5-nitroimidazoles were characterized by ^1H NMR, ^{13}C NMR, LC/MS, and HRMS. The *in vitro* antileishmanial activity of these compounds was evaluated against the promastigote form of *Leishmania infantum* and their influence on cell viability was assessed on the human

hepatocyte HepG2 cell line. The 4-phenylsulfonylmethyl analog of fexinidazole showed the best selectivity index of the series, displaying good activity against both the promastigote form of *L. infantum* ($\text{EC}_{50} = 0.8 \mu\text{M}$, $\text{SI} > 78.1$) and the promastigote form of *Leishmania donovani* ($\text{EC}_{50} = 4.6 \mu\text{M}$, $\text{SI} > 13.6$), and exhibiting low cytotoxicity on the HepG2 cell line ($\text{CC}_{50} > 62.5 \mu\text{M}$).

Keywords: structural simplification, 5-nitroimidazole, vicarious nucleophilic substitution (VNS), tetrakis(dimethylamino)ethylene (TDAE), antileishmanial activity

1 Introduction

Leishmaniasis are vector-borne parasitic diseases transmitted to mammalian hosts, including humans, by the bite of infected female phlebotomine sandflies. They are caused by almost 20 species of flagellated protozoa belonging to the *Leishmania* genus [1]. These diseases are classified by the World Health Organization (WHO) as neglected tropical diseases (NTDs), a heterogeneous group of infections mainly found in tropical and subtropical developing countries and of little interest to pharmaceutical companies [2]. Present in 98 countries, leishmaniasis remain a major public health problem worldwide, potentially infecting over a billion of the world's poorest people and causing nearly a million new cases every year. These diseases are strongly associated with impoverished environments, malnutrition, lack of sanitation, and immunodeficiency (leishmania-HIV co-infection) [3]. Leishmaniasis are divided into three main clinical forms: (1) cutaneous leishmaniasis, the most common manifestation of the disease with 600,000 to 1 million new cases annually; (2) mucocutaneous leishmaniasis, predominantly found in South America, affecting the nasal and oral mucosa; and (3) life-threatening visceral leishmaniasis (VL), also known as kala-azar, with an estimated 50,000–90,000 new cases of VL

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reported annually and more than 90% of cases distributed in seven countries: Brazil, India, Ethiopia, Kenya, Somalia, South Sudan, and Sudan [4,5]. If left untreated, VL is lethal in over 95% of cases and is responsible for more than 30,000 deaths annually. In humans, VL is caused by two species of *Leishmania*: *Leishmania infantum* in Latin America and the Mediterranean Basin and *Leishmania donovani* in Africa and Asia [6]. In the absence of an effective vaccine in humans [7], treatment of VL relies on the use of a small number of drugs. Unfortunately, most of them suffer from drawbacks that limit their application [8]: severe side effects, increasing resistance and constraining administration for pentavalent antimonials [9], high cost, parenteral administration, and cold chain compliance for the liposomal form of amphotericin B [10,11], and teratogenic properties for miltefosine, the only drug available orally for the treatment of VL [12]. Moreover, the number of new antileishmanial chemical entities in clinical trials is limited with only six compounds in the DNDi portfolio, including five at early stage (phase I) [13]. Thus, the development of new effective, safe, and affordable oral drugs is urgently needed to strengthen and diversify the antileishmanial drug arsenal.

Since the discovery of their anti-infective properties in the 1940s, nitroheterocyclic compounds have been widely used to treat bacterial and parasitic infections [14,15]. In particular, 5-nitroimidazoles such as metronidazole have traditionally been used as antibacterial and anti-parasitic agents [16,17] and have been studied for decades by our team [18]. Recently, fexinidazole, a new non-genotoxic 5-nitroimidazole derivative (Figure 2), was approved as the first all-oral treatment for sleeping sickness [19], renewing interest in the development of new 5-nitroimidazoles active against other kinetoplastid infections, such as leishmaniasis. Fexinidazole and other antikinoplastid nitroaromatic compounds act as prodrugs bioactivated by the parasitic type 1 nitroreductases (NTRs) [16,17]. This enzyme, essential for *Leishmania* and *Trypanosoma* and absent in mammalian cells, can catalyze the reduction of the nitro group in the parasite cell, producing nitroso and hydroxylamine intermediates and reactive oxygen species able to form covalent adducts with DNA and leading to the death of the parasite [20]. Unfortunately, the lack of crystal structure for parasitic NTRs and their low degree of homology with bacterial NTRs rule out the molecular docking approach to design new nitroaromatic compound substrates of parasitic NTRs.

To develop novel nitroheterocyclic compounds substrate of parasitic NTRs exerting antileishmanial activity, our team has previously described a new hit compound (Hit A) in the 3-nitroimidazo[1,2-*a*]pyridine series, through pharmacomodulation work focused on positions 2 and 8 of the moiety [21]. This antileishmanial hit, substituted by a phenylsulfonylmethyl group at position 2, a chlorine atom at position 6, and a 4-chlorophenylthioether group at position 8,

showed good *in vitro* activity against the promastigote form of *L. infantum* and *L. donovani* and low cytotoxicity on the human hepatocyte (HepG2) cell line, but exhibited poor *in vitro* mouse liver microsomal stability and aqueous solubility (Table 1).

Thus, as part of our ongoing structure–activity relationship (SAR) study (Figure 1) to improve the physicochemical and pharmacokinetic properties of Hit A and to obtain an ideal candidate for further *in vivo* studies [22,23], a structural simplification of the 3-nitroimidazo[1,2-*a*]pyridine core to a 5-nitroimidazole moiety was conducted (Figure 2). In particular, the aim of this work was to synthesize the 5-nitroimidazole analog of Hit A and the 4-phenylsulfonylmethyl analog of fexinidazole to compare their biological profile. These two compounds and other 2-substituted 5-nitroimidazole derivatives were obtained, using several reactions developed by our team, such as the vicarious nucleophilic substitution of hydrogen (VNS) reaction, to substitute position 4 [24,25], and the tetrakis(dimethylamino)ethylene (TDAE) methodology, to modulate position 2 [26,27].

2 Results and discussion

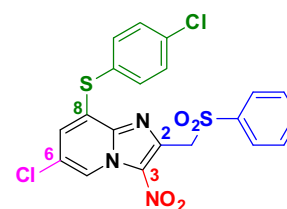
2.1 Synthesis

The 4-phenylsulfonylmethyl-5-nitroimidazole compounds **1a** and **1b** were prepared by vicarious nucleophilic substitution of hydrogen at position 4 of commercially available

Table 1: Structure and *in vitro* biological profile of Hit A

Hit A	
EC ₅₀ <i>L. donovani</i> promastigotes (μM)	1.0 ± 0.3
EC ₅₀ <i>L. infantum</i> promastigotes (μM)	0.6 ± 0.1
CC ₅₀ HepG2 (μM)	>100
Microsomal stability: half-life (min)*	3
Thermodynamic solubility at pH 7.4 (μM)	1.4

*Microsomal stability was assessed on female mouse microsomes.



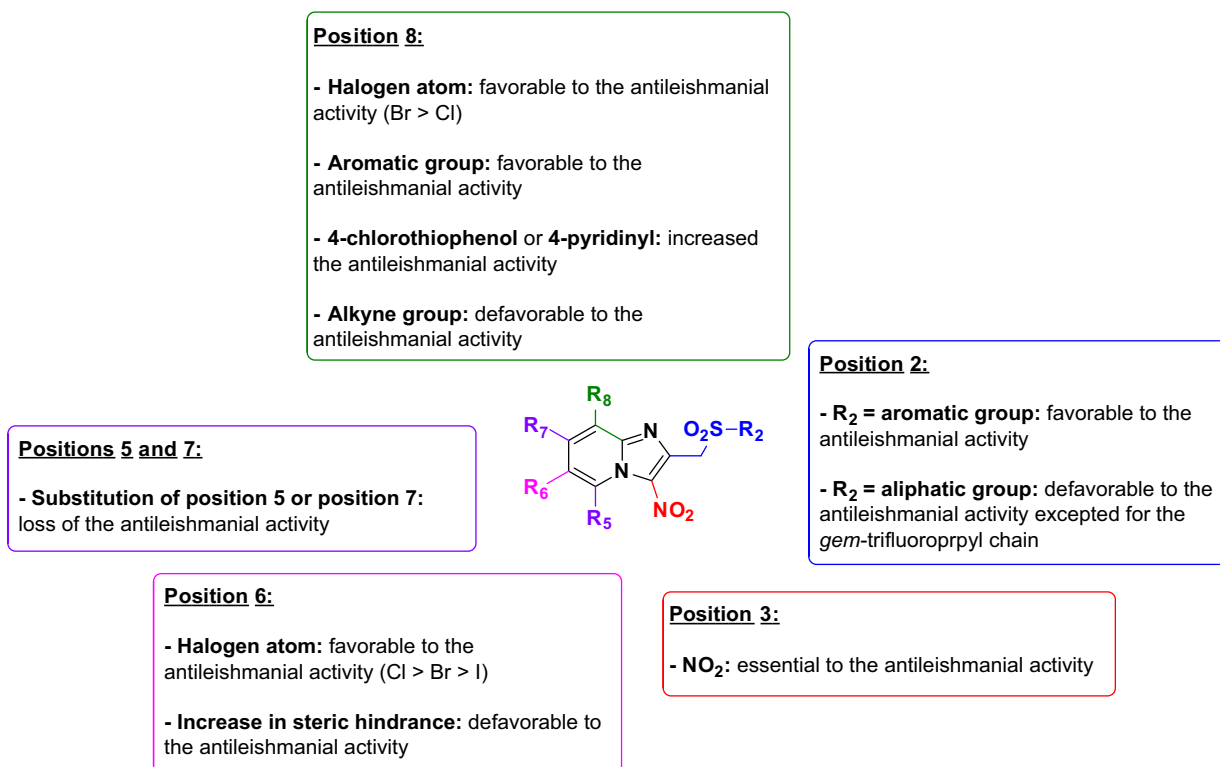


Figure 1: Antileishmanial structure–activity relationship study in 3-nitroimidazo[1,2-*a*]pyridine series.

(1-methyl-5-nitro-1*H*-imidazol-2-yl)methanol (Scheme 1), followed by a chlorination reaction at position 2 with thionyl chloride, to give the 2-chloromethyl intermediates **2a** and **2b** [28,29].

Using conditions similar to those previously reported [21], a 4-chlorophenylthioether moiety was introduced at position 2 of intermediates **2a** and **2b**, to obtain the 5-nitroimidazole analogs of Hit A **3a** and **3b** in good yields (Scheme 2).

O-alkylation reactions were performed at position 2 of intermediate **2a** in the presence of the desired phenol, to afford the 4-chlorophenol derivative **4a** and the 4-phenylsulfonylethyl analog of fexinidazole **4b** (Scheme 2).

Finally, we used a TDAE methodology previously described by our team to generate first a stable 2-methyl carbanion [27], which was reacted with *N*-tosylbenzyl-imines to give compounds **5a** and **5b** in moderate yields (Scheme 2) [30].

2.2 Biological results

The *in vitro* antileishmanial activity of intermediates **1b** and **2b** and compounds **3a** to **5b** was evaluated against the promastigote form of *L. infantum* and compared to

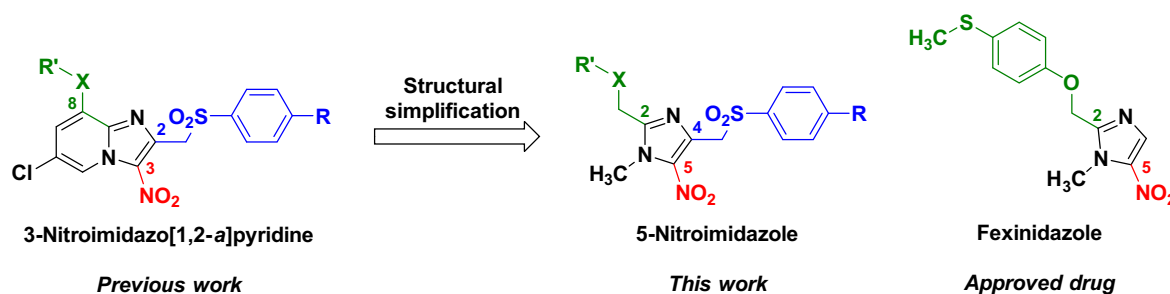
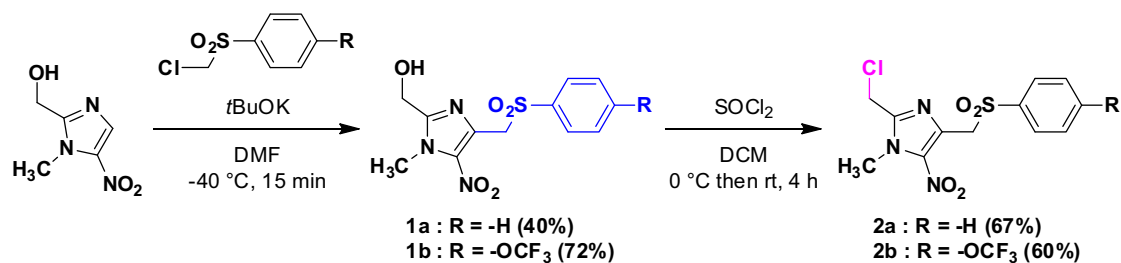
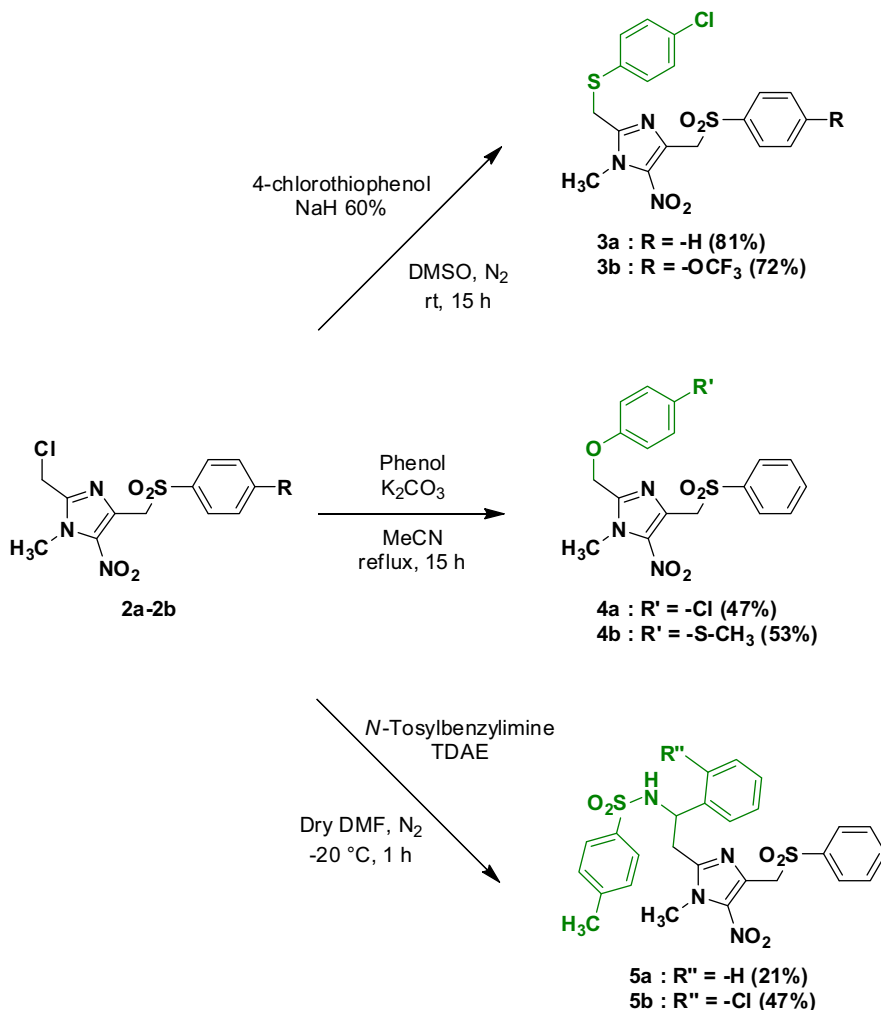


Figure 2: Structural simplification of the 3-nitroimidazo[1,2-*a*]pyridine pharmacophore into a 2,4-disubstituted 5-nitroimidazole moiety and structure of fexinidazole.

Scheme 1: Synthesis of compounds **1a–1b** and **2a–2b**.Scheme 2: Synthesis of compounds **3a–3b**, **4a–4b**, and **5a–5b**.

Hit A and to reference drugs (amphotericin B, miltefosine, and fexinidazole) (Table 2). Their influence on cell viability was assessed on the human hepatocyte HepG2, using doxorubicin as a positive control. The *in vitro* antileishmanial activity of the best compounds was also evaluated against the promastigote form of *L. donovani*.

The 2-hydroxymethyl intermediate **1b** was not active *in vitro* against the promastigote form of *L. infantum* ($EC_{50} > 50 \mu\text{M}$)

while the 2-chloromethyl intermediate **2b** showed a good antileishmanial activity ($EC_{50} = 1.1 \mu\text{M}$) but was cytotoxic on the HepG2 cell line ($CC_{50} = 2.7 \mu\text{M}$). The substitution of the chlorine atom at position 2 of intermediate **2b** by a 4-chlorophenylthioether moiety (compound **3b**) resulted in reduced cytotoxicity on the HepG2 cell line ($CC_{50} = 11.9 \mu\text{M}$) and similar activity against the promastigote form of *L. infantum* ($EC_{50} = 2.6 \mu\text{M}$). The 5-nitroimidazole analog of Hit A **3a** showed a

Table 2: *In vitro* evaluation of molecules **1b**, **2b**, and **3a–5b** on *L. infantum* promastigotes and on the human hepatocyte HepG2 line. *In vitro* evaluation of molecules **3a** and **4b** on *L. donovani* promastigotes

	CC ₅₀ HepG2 (μM)	EC ₅₀ <i>L. inf.</i> pro. (μM)	Selectivity Index (SI) <i>L. inf. pro.</i>	EC ₅₀ <i>L. dono.</i> pro. (μM)	Selectivity Index (SI) <i>L. dono. pro.</i>
1b	>125 ^a	>50	<2.5	—	—
2b	2.7 ± 0.8	1.1 ± 0.2	2.5	—	—
3a	>50 ^a	1.2 ± 0.2	>41.7	4.1 ± 0.3	>12.2
3b	11.9 ± 1.6	2.6 ± 0.8	4.6	—	—
4a	29.5 ± 5.4	2.0 ± 0.7	14.8	—	—
4b	>62.5^a	0.8 ± 0.1	>78.1	4.6 ± 0.4	>13.6
5a	>25 ^a	9.3 ± 2.6	>2.7	—	—
5b	32.4 ± 10.6	6.2 ± 1.5	5.2	—	—
Hit A	>100	0.6 ± 0.1	>166.7	1.0 ± 0.3	>100
Doxorubicin ^b	0.20 ± 0.02	—	—	—	—
Amphotericin B ^c	8.8 ± 0.3	0.04 ± 0.01	220	0.07 ± 0.01	125.7
Miltefosine ^c	85.0 ± 8.8	18.2 ± 0.5	4.7	3.1 ± 0.2	27.4
Fexinidazole ^c	>100	1.0 ± 0.1	>100	1.2 ± 0.2	>83.3

^aThe product could not be tested at higher concentrations due to a lack of solubility in the culture medium.

^bDoxorubicin was used as a cytotoxic reference drug.

^cAmphotericin B, miltefosine, and fexinidazole were used as antileishmanial reference drugs.

Bold was used to highlight the best compound of the series.

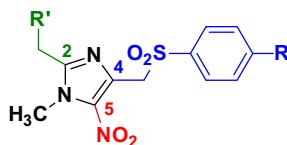
Position 2 :

R' = -Cl: increased cytotoxicity

R' = 4-chlorothiophenol: similar antileishmanial activity to Hit A

R' = 4-(methylthio)phenoxy: similar antileishmanial activity to Hit A and fexinidazole

R' = *N*-tosylbenzylimine: slight loss of antileishmanial activity



Position 4 :

- Phenylsulfonylmethyl: similar antileishmanial activity to Hit A and fexinidazole

- R = -OCF₃: increased cytotoxicity in comparison to R = -H

Position 5 :

- NO₂: essential to the antileishmanial activity

Figure 3: Antileishmanial structure–activity relationship study of novel 5-nitroimidazole derivatives.

good selectivity index (SI > 41.7) with an EC₅₀ value close to the one of fexinidazole against the promastigote form of *L. infantum* (EC₅₀ = 1.2 μM), no cytotoxicity on the HepG2 cell line at the tested concentrations and good solubility in the HepG2 culture medium (CC₅₀ > 50 μM). Compound **3a** also showed a good activity against the promastigote form of *L. donovani* (EC₅₀ = 4.1 μM) but was inferior to Hit A, fexinidazole, and miltefosine. The replacement of the sulfur atom of the 4-chlorophenylthioether moiety of compound **3a** by an oxygen atom (compound **4a**) resulted in a lower selectivity index (SI = 14.8) while remaining active against the promastigote form of *L. infantum* (EC₅₀ = 2.0 μM). The 4-phenylsulfonylmethyl analog of fexinidazole **4b** was the best compound of the

series (SI > 78.1), displaying similar activity to fexinidazole and Hit A against the promastigote form of *L. infantum* (EC₅₀ = 0.8 μM), no cytotoxicity on the HepG2 cell line at the tested concentrations and good solubility in the HepG2 culture medium (CC₅₀ > 62.5 μM). Its activity against the promastigote form of *L. donovani* (EC₅₀ = 4.6 μM) was lower than that of Hit A, fexinidazole, and miltefosine and quite similar to compound **3a**. Finally, the introduction of the two *N*-tosylbenzylimine moieties (compounds **5a** and **5b**) at position 2 of the 5-nitroimidazole scaffold resulted in a slight loss of activity against the promastigote form of *L. infantum* (EC₅₀ = 9.3 μM and EC₅₀ = 6.2 μM, respectively). The antileishmanial SAR study of the novel 5-nitroimidazole derivatives was summarized in Figure 3.

3 Conclusion

This antileishmanial SAR study focused on the structural simplification of the 3-nitroimidazo[1,2-*a*]pyridine ring to a 5-nitroimidazole moiety. Thus, 8 new 2,4-disubstituted 5-nitroimidazole derivatives were obtained, including the 5-nitroimidazole analog of Hit A (compound **3a**) and the 4-phenylsulfonylmethyl analog of fexinidazole (compound **4b**), using the VNS reaction and TDAE methodology. Compound **4b** was the best derivative of the series, showing the best selectivity index due to its good activity against the promastigote form of *L. infantum* ($EC_{50} = 0.8 \mu\text{M}$, SI > 78.1) and the promastigote form of *L. donovani* ($EC_{50} = 4.6 \mu\text{M}$, SI > 13.6), its lack of cytotoxicity on the HepG2 cell line and its good solubility in the HepG2 culture medium ($CC_{50} > 62.5 \mu\text{M}$). These encouraging results paved the way for exploring new promising fexinidazole analogs substituted at position 4 of the imidazole ring.

4 Experimental

4.1 Experimental details

Melting points were determined on a Köfler melting point apparatus (Wagner & Munz GmbH, München, Germany) and were uncorrected. HRMS spectrum (ESI) was recorded on a SYNAPT G2 HDMS (Waters) at the Faculté des Sciences de Saint-Jérôme (Marseille). NMR spectra were recorded on a Bruker Avance 250 MHz or a Bruker Avance NEO 400 MHz NanoBay spectrometer at the Faculté de Pharmacie of Marseille. (^1H NMR: reference CDCl_3 $\delta = 7.26$ ppm, reference $\text{DMSO}-d_6$ $\delta = 2.50$ ppm and ^{13}C NMR: reference CDCl_3 $\delta = 76.9$ ppm, reference $\text{DMSO}-d_6$ $\delta = 39.52$ ppm). The following adsorbent was used for column chromatography: silica gel 60 (Merck KGaA, Darmstadt, Germany, particle size 0.063–0.200 mm, 70–230 mesh ASTM). TLC was performed on 5 cm × 10 cm aluminum plates coated with silica gel 60F-254 (Merck) in an appropriate eluent. Visualization was performed with ultraviolet light (234 nm). The purity determination of synthesized compounds was checked by LC/MS analyses, which were realized at the Faculté de Pharmacie of Marseille with a Thermo Scientific Accela High-Speed LC System[®] (Waltham, MA, USA) coupled using a single quadrupole mass spectrometer Thermo MSQ Plus[®]. The purity of all the synthesized compounds was >99%, except for the compound **5b** for which the purity was >90%. The RP-HPLC column is a Thermo Hypersil Gold[®] 50 mm × 2.1 mm (C_{18} bounded), with particles of a diameter of 1.9 mm. The volume of the sample injected into the column was 1 μL . Chromatographic analysis, total duration of 8 min, was on the gradient of the

following solvents: $t = 0$ min, methanol/water 50:50; $0 < t < 4$ min, linear increase in the proportion of methanol to a methanol/water ratio of 95:5; $4 < t < 6$ min, methanol/water 95:5; $6 < t < 7$ min, linear decrease in the proportion of methanol to return to a methanol/water ratio of 50:50; and $6 < t < 7$ min, methanol/water 50:50. The water used was buffered with ammonium acetate 5 mM. The flow rate of the mobile phase was 0.3 mL/min. The retention times (t_R) of the molecules analyzed were indicated in min. Reagents were purchased from Sigma-Aldrich or Fluorochem and used without further purification. Copies of ^1H NMR and ^{13}C NMR are available in the Supplementary Materials.

4.2 Synthesis of {1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1H-imidazol-2-yl}methanol (**1a**)

To a solution of *t*BuOK (1.95 g, 17.4 mmol, 4 equiv) in dry DMF (15 mL) at -40°C was added a solution of chloromethylsulfonylbenzene (1.66 g, 8.7 mmol, 2 equiv) in dry DMF (15 mL). Then, a solution of (1-methyl-5-nitro-1H-imidazol-2-yl)methanol (0.683 g, 4.35 mmol, 1 equiv) was added dropwise and the reaction mixture was stirred at -40°C for 15 min. A 2 M solution of HCl was added until pH 1 and the aqueous layer was extracted three times with ethyl acetate. Then, the organic layer was washed four times with brine, dried over anhydrous Na_2SO_4 , filtered, and evaporated. Compound **1a** was obtained after purification by chromatography on silica gel (eluent: cyclohexane-dichloromethane 2:8) as a yellow solid in 40% yield (0.541 g). mp 153°C . ^1H NMR (250 MHz, $\text{DMSO}-d_6$) δ : 7.75–7.72 (m, 3H), 7.64–7.58 (m, 2H), 5.74 (brs, 1H), 4.91 (s, 2H), 4.53 (s, 2H), 3.87 (s, 3H). ^{13}C NMR (63 MHz, $\text{DMSO}-d_6$) δ : 150.4, 138.9, 137.4, 134.2, 131.6, 129.4 (2C), 127.9 (2C), 55.9, 55.3, 34.1. LC/MS ESI+ t_R 3.17, (m/z) [$\text{M} + \text{H}$]⁺ 312.07. HRMS (+ESI): 312.0646 [$\text{M} + \text{H}$]⁺. Calcd for $\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}_5\text{S}$: 312.0649 [25].

4.3 Synthesis of {1-methyl-5-nitro-4-[(4-trifluoromethoxyphenylsulfonyl)methyl]-1H-imidazol-2-yl}methanol (**1b**)

To a solution of *t*BuOK (1.95 g, 17.4 mmol, 4 equiv) in dry DMF (15 mL) at -40°C was added a solution of 1-(chloromethyl) sulfonyl-4-(trifluoromethoxy)benzene (2.39 g, 8.7 mmol, 2 equiv) in dry DMF (15 mL). Then, a solution of (1-methyl-5-nitro-1H-imidazol-2-yl)methanol (0.683 g, 4.35 mmol, 1 equiv) was added dropwise and the reaction mixture was stirred at -40°C for 15 min. A 2 M solution of HCl was added until pH 1 and the

aqueous layer was extracted three times with ethyl acetate. Then, the organic layer was washed four times with brine, dried over anhydrous Na_2SO_4 , filtered, and evaporated. Compound **1b** was obtained after purification by chromatography on silica gel (eluent: dichloromethane-ethyl acetate 6:4) as a white solid in 72% yield (1.24 g). mp 116°C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.97–7.81 (m, 2H), 7.60 (d, $J = 8.1$ Hz, 2H), 5.71 (t, $J = 5.8$ Hz, 1H), 4.97 (s, 2H), 4.52 (d, $J = 5.8$ Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 152.1, 150.5, 137.6, 137.4, 131.3, 130.9 (2C), 121.4 (2C), 119.8 (q, $J = 258.3$ Hz), 55.9, 55.3, 34.0. LC/MS ESI+ t_R 4.80, (m/z) $[\text{M} + \text{H}]^+$ 395.97. HRMS (+ESI): 396.0472 $[\text{M} + \text{H}]^+$. Calcd for $\text{C}_{13}\text{H}_{13}\text{F}_3\text{N}_3\text{O}_6\text{S}$: 396.0472. HPLC purity >99%.

4.4 Synthesis of 2-(chloromethyl)-1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1H-imidazole (2a)

To a solution of {1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1H-imidazol-2-yl}methanol (**1a**) (0.5 g, 1.61 mmol, 1 equiv) in dichloromethane (15 mL) at 0°C was added thionyl chloride (234 μL , 3.22 mmol, 2 equiv) and the reaction mixture was stirred at room temperature for 4 h. A 1 M K_2CO_3 solution was added to neutralize and the aqueous layer was extracted three times with dichloromethane. Then, the organic layer was washed four times with brine, dried over anhydrous Na_2SO_4 , filtered, and evaporated. Compound **2a** was obtained after purification by chromatography on silica gel (eluent: petroleum ether-ethyl acetate 5:5) as a brown solid in 67% yield (0.354 g). mp 154°C. ^1H NMR (250 MHz, CDCl_3) δ : 7.87–7.75 (m, 2H), 7.70–7.59 (m, 1H), 7.55–7.49 (m, 2H), 4.82 (s, 2H), 4.63 (s, 2H), 3.97 (s, 3H). ^{13}C NMR (63 MHz, CDCl_3) δ : 145.9, 138.8, 137.9, 134.3, 132.4, 129.3 (2C), 128.4 (2C), 55.9, 40.0, 34.7. LC/MS ESI+ t_R 4.28, (m/z) $[\text{M} + \text{H}]^+$ 330.05/332.04. HRMS (+ESI): 330.0311 $[\text{M} + \text{H}]^+$. Calcd for $\text{C}_{12}\text{H}_{13}\text{ClN}_3\text{O}_4\text{S}$: 330.0310 [25].

4.5 Synthesis of 2-{chloromethyl}-1-methyl-5-nitro-4-[(4-trifluoromethoxyphenylsulfonyl)methyl]-1H-imidazole (2b)

To a solution of {1-methyl-5-nitro-4-[(4-trifluoromethoxyphenylsulfonyl)methyl]-1H-imidazol-2-yl}methanol (**1b**) (0.5 g, 1.26 mmol, 1 equiv) in dichloromethane (15 mL) at 0°C was added thionyl chloride (183 μL , 2.52 mmol, 2 equiv) and the reaction mixture was stirred at room temperature for 4 h. A 1 M K_2CO_3 solution was added to neutralize and the aqueous layer was extracted three times with dichloromethane. Then, the organic layer was washed four times with brine, dried

over anhydrous Na_2SO_4 , filtered, and evaporated. Compound **2b** was obtained after purification by chromatography on silica gel (eluent: dichloromethane) as a white solid in 60% yield (0.314 g). mp 84°C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.91–7.81 (m, 2H), 7.59 (d, $J = 8.1$ Hz, 2H), 5.00 (s, 2H), 4.90 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 152.1, 146.1, 137.6, 137.3, 131.5, 130.9 (2C), 121.4 (2C), 119.8 (q, $J = 258.8$ Hz), 55.1, 36.0, 34.3. LC/MS ESI+ t_R 6.31, (m/z) $[\text{M} + \text{H}]^+$ 412.21/414.18. HRMS (+ESI): 414.0132 $[\text{M} + \text{H}]^+$. Calcd for $\text{C}_{13}\text{H}_{12}\text{ClF}_3\text{N}_3\text{O}_5\text{S}$: 414.0133. HPLC purity >99%.

4.6 Synthesis of 2-[[4-(chlorophenyl)thio]methyl]-1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1H-imidazole (3a)

To a sealed 20 mL flask containing sodium hydride (60% dispersion in mineral oil) (0.049 g, 1.21 mmol, 2 equiv) in dimethylsulfoxide (3 mL), 4-chlorothiophenol (0.175 g, 1.21 mmol, 2 equiv) was added under N_2 atmosphere. The reaction mixture was stirred at room temperature for 30 min. Then, a solution of 2-(chloromethyl)-1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1H-imidazole (**2a**) (0.2 g, 0.61 mmol, 1 equiv) in dimethylsulfoxide (6 mL) was injected. The reaction mixture was stirred for 15 h at room temperature. The mixture was slowly poured into an ice-water mixture and precipitated. The solid was collected by filtration and dried under reduced pressure. Compound **3a** was obtained after purification by chromatography on silica gel (eluent: dichloromethane) as a white solid in 81% yield (0.214 g). mp 105°C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.71 (t, $J = 7.2$ Hz, 1H), 7.62 (d, $J = 7.4$ Hz, 2H), 7.55 (t, $J = 7.6$ Hz, 2H), 7.44 (d, $J = 8.5$ Hz, 2H), 7.37 (d, $J = 8.5$ Hz, 2H), 4.88 (s, 2H), 4.44 (s, 2H), 3.81 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 147.5, 138.7, 137.4, 134.0, 133.1, 131.9, 131.6, 131.3 (2C), 129.2 (2C), 129.0 (2C), 127.8 (2C), 55.2, 34.2, 29.1. LC/MS ESI+ t_R 5.67, (m/z) $[\text{M} + \text{H}]^+$ 437.92/439.98. HRMS (+ESI): 438.0342 $[\text{M} + \text{H}]^+$. Calcd for $\text{C}_{18}\text{H}_{17}\text{ClN}_3\text{O}_4\text{S}_2$: 438.0344. HPLC purity >99%.

4.7 Synthesis of 2-[[4-(chlorophenyl)thio]methyl]-1-methyl-5-nitro-4-[(4-trifluoromethoxyphenylsulfonyl)methyl]-1H-imidazole (3b)

To a sealed 20 mL flask containing sodium hydride (60% dispersion in mineral oil) (0.038 g, 0.96 mmol, 2 equiv) in dimethylsulfoxide (3 mL), 4-chlorothiophenol (0.139 g, 0.96 mmol, 2 equiv) was added under N_2 atmosphere. The reaction mixture was stirred at room temperature for 30 min. Then, a solution of 2-(chloromethyl)-1-methyl-5-nitro-4-[(4-trifluoromethoxyphenylsulfonyl)methyl]-

1*H*-imidazole (**2b**) (0.2 g, 0.48 mmol, 1 equiv) in dimethylsulfoxide (6 mL) was injected. The reaction mixture was stirred for 15 h at room temperature. The mixture was slowly poured into an ice–water mixture, extracted three times with ethyl acetate. Then, the organic layer was washed four times with brine, dried over anhydrous Na₂SO₄, filtered, and evaporated. Compound **3b** was obtained after purification by chromatography on silica gel (eluent: dichloromethane) as a white solid in 72% yield (0.18 g). mp 93°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.81–7.73 (m, 2H), 7.58–7.50 (m, 2H), 7.46–7.33 (m, 4H), 4.95 (s, 2H), 4.42 (s, 2H), 3.82 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 152.0, 147.6, 137.5, 137.4, 133.2, 131.7, 131.6, 131.3 (2C), 130.8 (2C), 129.0 (2C), 121.3 (2C), 119.8 (d, *J* = 258.5 Hz), 55.1, 34.2, 29.1. LC/MS ESI+ *t*_R 6.32, (*m/z*) [M + H]⁺ 521.87/523.80. HRMS (+ESI): 522.0166 [M + H]⁺. Calcd for C₁₉H₁₆ClF₃N₃O₅S₂: 522.0167. HPLC purity >99%.

4.8 Synthesis of 2-[(4-chlorophenoxy)methyl]-1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1*H*-imidazole (**4a**)

To a solution of 2-chloromethyl-1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1*H*-imidazole (**2a**) (0.2 g, 0.61 mmol, 1 equiv) in acetonitrile (10 mL), 4-chlorophenol (124 μL, 1.22 mmol, 2 equiv) and potassium carbonate (0.211 g, 1.53 mmol, 2.5 equiv) were added. The reaction mixture was stirred and heated under reflux for 15 h. Then, the mixture was slowly poured into an ice–water mixture, extracted three times with dichloromethane, dried over anhydrous Na₂SO₄, filtered, and evaporated. Compound **4a** was obtained after purification by chromatography on silica gel (eluent: dichloromethane) as a white solid in 47% yield (0.12 g). mp 122°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.78–7.66 (m, 3H), 7.58 (t, *J* = 7.6 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 5.26 (s, 2H), 4.94 (s, 2H), 3.89 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 156.3, 145.8, 138.8, 137.8, 134.1, 131.6, 129.3 (2C), 129.3 (2C), 127.8 (2C), 125.4, 116.9 (2C), 62.2, 55.1, 34.3. LC/MS ESI+ *t*_R 5.57, (*m/z*) [M + H]⁺ 421.87/423.92. HRMS (+ESI): 422.0573 [M + H]⁺. Calcd for C₁₈H₁₇ClN₃O₅S₂: 422.0572. HPLC purity >99%.

4.9 Synthesis of 1-methyl-2-[[4-(methylthio)phenoxy]methyl]-5-nitro-4-[(phenylsulfonyl)methyl]-1*H*-imidazole (**4b**)

To a solution of 2-chloromethyl-1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1*H*-imidazole (**2a**) (0.2 g, 0.61 mmol, 1 equiv) in acetonitrile (10 mL), 4-methylsulfonylphenol (0.171 g, 1.22 mmol, 2 equiv) and potassium carbonate (0.211 g, 1.53 mmol, 2.5 equiv)

were added. The reaction mixture was stirred and heated under reflux for 15 h. Then, the mixture was slowly poured into an ice–water mixture, extracted three times with dichloromethane, dried over anhydrous Na₂SO₄, filtered, and evaporated. Compound **4b** was obtained after purification by chromatography on silica gel (eluent: dichloromethane) as a yellow solid in 53% yield (0.14 g). mp 122°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.75–7.69 (m, 3H), 7.59–7.55 (m, 2H), 7.26 (d, *J* = 8.5 Hz, 2H), 7.04 (d, *J* = 8.6 Hz, 2H), 5.23 (s, 2H), 4.94 (s, 2H), 3.89 (s, 3H), 2.43 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 155.6, 146.1, 138.8, 137.8, 134.1, 131.6, 129.9, 129.3 (2C), 128.7 (2C), 127.8 (2C), 115.9 (2C), 62.1, 55.1, 34.3, 16.3. LC/MS ESI+ *t*_R 5.53, (*m/z*) [M + H]⁺ 433.91. HRMS (+ESI): 434.0840 [M + H]⁺. Calcd for C₁₉H₂₀N₃O₅S₂: 434.0839. HPLC purity >99%.

4.10 Synthesis of 4-methyl-*N*-(2-{1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1*H*-imidazol-2-yl}-1-phenylethyl)benzenesulfonamide (**5a**)

To a solution of 2-chloromethyl-1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1*H*-imidazole (**2a**) (0.2 g, 0.61 mmol, 1 equiv) in dry DMF (7 mL) at –20°C was added (*E*)-*N*-benzylidene-4-methylbenzenesulfonamide (0.239 g, 0.92 mmol, 1.5 equiv). Then, tetrakis(dimethylamino)ethylene (142 μL, 0.61 mmol, 1 equiv) was added under N₂ atmosphere and the reaction mixture was stirred at –20°C for 1 h. The mixture was slowly poured into an ice–water mixture and precipitated. The solid was collected by filtration and dried under reduced pressure. Compound **5a** was obtained after purification by chromatography on silica gel (eluent: dichloromethane–ethyl acetate 9:1) as a white solid in 21% yield (0.07 g). mp 113°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.47 (s, 1H), 7.79–7.67 (m, 3H), 7.59 (t, *J* = 7.7 Hz, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.31–7.19 (m, 5H), 7.16 (d, *J* = 8.0 Hz, 2H), 4.82 (s, 2H), 4.59 (t, *J* = 7.1 Hz, 1H), 3.52 (s, 3H), 3.08 (dd, *J* = 15.0, 9.1 Hz, 1H), 2.98 (dd, *J* = 15.0, 5.8 Hz, 1H), 2.27 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 148.5, 142.2, 141.1, 138.7, 138.5, 136.3, 134.1, 132.7, 129.2 (2C), 129.1 (2C), 128.3 (2C), 128.0 (2C), 127.4, 126.4 (2C), 126.0 (2C), 56.3, 55.2, 34.7, 33.8, 20.8. LC/MS ESI+ *t*_R 6.37, (*m/z*) [M + H]⁺ 555.15. HRMS (+ESI): 555.1368 [M + H]⁺. Calcd for C₂₆H₂₇N₄O₆S₂: 555.1367. HPLC purity > 99%.

4.11 *N*-[1-(2-Chlorophenyl)-2-{1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1*H*-imidazol-2-yl}ethyl]-4-methylbenzenesulfonamide (**5b**)

To a solution of 2-chloromethyl-1-methyl-5-nitro-4-[(phenylsulfonyl)methyl]-1*H*-imidazole (**2a**) (0.2 g, 0.61 mmol, 1

equiv) in dry DMF (7 mL) at -20°C was added (*E*)-*N*-(2-chlorobenzylidene)-4-methylbenzenesulfonamide (0.27 g, 0.92 mmol, 1.5 equiv). Then, tetrakis(dimethylamino)ethylene (142 μL , 0.61 mmol, 1 equiv) was added under N_2 atmosphere and the reaction mixture was stirred at -20°C for 1 h. The mixture was slowly poured into an ice–water mixture, extracted three times with ethyl acetate. Then, the organic layer was washed four times with brine, dried over anhydrous Na_2SO_4 , filtered, and evaporated. Compound **5b** was obtained after purification by chromatography on silica gel (eluent: dichloromethane) as a beige solid in 47% yield (0.17 g). mp 136°C . ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 8.67 (d, $J = 9.4$ Hz, 1H), 7.79–7.66 (m, 3H), 7.66–7.55 (m, 3H), 7.39–7.33 (m, 3H), 7.33–7.21 (m, 2H), 7.14 (d, $J = 8.1$ Hz, 2H), 5.03 (ddd, $J = 9.9, 9.4, 5.0$ Hz, 1H), 4.80 (s, 2H), 3.64 (s, 3H), 2.99 (dd, $J = 15.1, 9.9$ Hz, 1H), 2.93 (dd, $J = 15.1, 5.0$ Hz, 1H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 147.9, 142.3, 138.8, 138.0, 136.5, 134.0, 132.7, 130.9, 129.2 (3C), 129.1 (2C), 129.0 (2C), 128.2, 128.0 (2C), 127.6, 126.0 (2C), 55.3, 52.7, 34.0, 33.9, 20.8. LC/MS ESI + t_{R} 5.77, (m/z) $[\text{M} + \text{H}]^+$ 588.82/590.85. HRMS (+ESI): 589.0978 $[\text{M} + \text{H}]^+$. Calcd for $\text{C}_{26}\text{H}_{26}\text{ClN}_4\text{O}_6\text{S}_2$: 589.0977. HPLC purity = 91%.

4.12 Antileishmanial activity against *L. infantum* promastigotes

L. infantum promastigotes (expressing luciferase activity, kindly provided by Professor Thierry Lang of the Institut Pasteur) were used in the following tests [31]. *L. infantum* promastigotes were routinely grown at 27°C in Schneider's Drosophila Medium (Dominique DUTSCHER SAS, France) supplemented with 10% fetal calf serum, 2% human urine, sodium pyruvate 0.04 mM, hemin 0.1 $\mu\text{g}/\text{mL}$, HEPES 5 mM, streptomycin 100 $\mu\text{g}/\text{mL}$, penicillin 100 U/mL, L-glutamine 2 mM (culture medium). The effects of the tested compounds on the growth of *L. infantum* promastigotes were assessed according to the Mosmann protocol [32]. Briefly, promastigotes in log-phase were incubated at an average density of 10^6 parasites/mL (100 $\mu\text{L}/\text{well}$) 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 (100 $\mu\text{L}/\text{well}$) treated by DMSO and reference drugs (purchased for the majority from Sigma Aldrich) were added to each set of experiments. After a 72 h incubation period at 27°C , each plate well was then microscope-examined to detect possible precipitate formation. 20 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich, Saint-Louis, Missouri, USA) solution (5 mg/mL in PBS) were added to each well and incubated at 27°C for 4 h. The reaction was stopped by the addition of 100 μL of 50% isopropanol – 10% sodium

dodecyl sulfate. Plates were shaken at 400 rpm for 10 min. The absorbance was measured at 570 nm with a TECAN Infinite F-200 spectrophotometer (Tecan Trading AG, Switzerland). Efficient concentration 50% (EC_{50}) was defined as the concentration of drug required to inhibit by 50% the metabolic activity of *L. infantum* promastigotes compared to the control. EC_{50} values were calculated by non-linear regression analysis processed on dose–response curves, using TableCurve 2D V5 software.

4.13 Cytotoxicity evaluation on HepG2 cell line

HepG2 cell line (hepatocarcinoma cell line purchased from ATCC, ref HB-8065) was maintained at 37°C , 5% CO_2 with 90% humidity in MEM supplemented with 10% foetal bovine serum, 1% L-glutamine (200 mM) and penicillin (100 U/mL)/streptomycin (100 mg/mL) (complete MEM medium). The tested molecules' cytotoxicity was assessed according to the method of Mosmann with slight modifications [32]. Briefly, 5×10^3 cells in 100 μL of complete medium were inoculated into each well of 96-well plates and incubated at 37°C in a humidified 5% CO_2 . After 24 h incubation, 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 72 h at 37°C . Triplicate assays were performed for each sample. Each plate well was microscope-examined to detect possible precipitate formation before the medium was aspirated from the wells. 100 μL of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) solution (0.5 mg/mL in medium without FCS) were then added to each well. Cells were incubated for 2 h at 37°C , following which the MTT solution was removed and DMSO (100 μL) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (700 rpm) for 10 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength using a TECAN INFINITE F200 Microplate Reader. DMSO was used as blank and doxorubicin (purchased from Sigma Aldrich) as positive control. Cell viability was calculated as the percentage of control (cells incubated without compound). The 50% cytotoxic concentration (CC_{50}) was determined from the dose–response curve using the TableCurve 2D v5.0 software (Jandel Scientific). CC_{50} values represent the mean value calculated from three separate experiments.

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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