Received: 8 January 2020 | Revised: 11 February 2020 | Accepted: 13 February 2020

DOI: 10.1002/ardp.202000002

FULL PAPER

DPhG ARCH PHARM Archiv der Pharmazie

Synthesis and leishmanicidal evaluation of sulfanyl- and sulfonyl-tethered functionalized benzoate derivatives featuring a nitroimidazole moiety

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Funding information
Instituto de Investigaciones Farmacéuticas (IIF), Grant/Award Number: IIF.01–2014; Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela (CDCH-UCV), Grant/Award Number: PG. 09–8819-2013/2; France–Venezuela PCP program, Grant/Award Number: 2013000438; University of Bordeaux; Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation; Centre National de la Recherche Scientifique (CNRS)

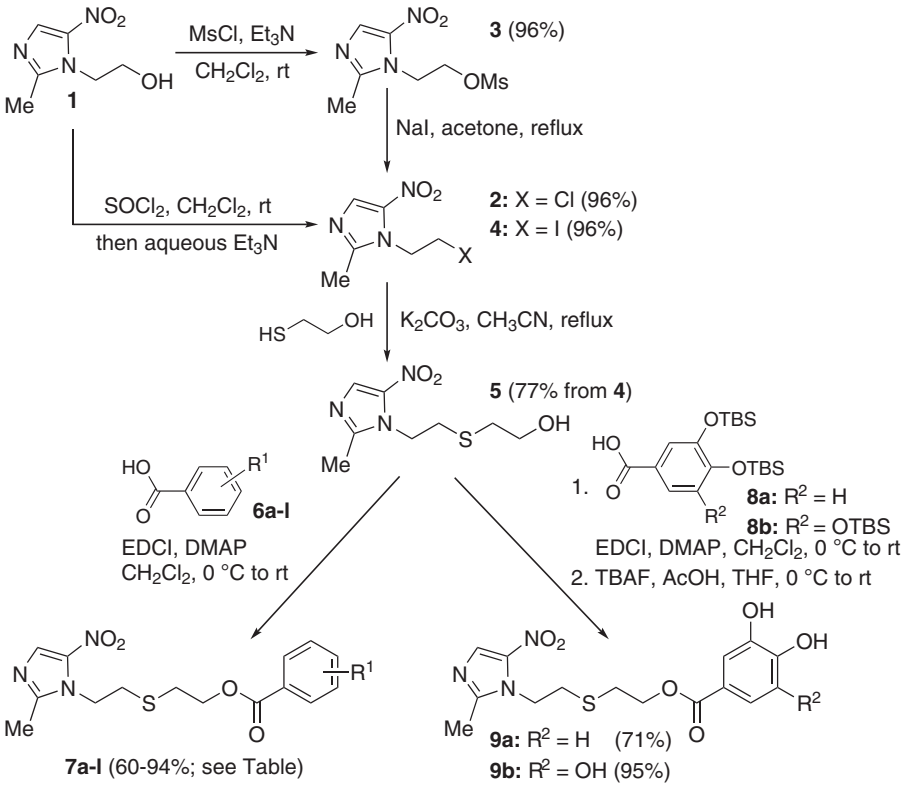
Abstract

A series of new nitroimidazole-containing derivatives was synthesized by coupling of 2-[2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethylthio]ethanol with diversely substituted benzoic acids. Upon treatment with *m*-CPBA, 12 of these sulfanyl compounds were further oxidized to their sulfonyl analogs. All the 26 synthetic compounds were examined for in vitro activity against *Leishmania (V.) braziliensis* and *Leishmania (L.) mexicana*, and some of them displayed an efficient antileishmanial activity. Among the compounds tested, the catecholic derivative 2-{[2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl]sulfanyl}ethyl 3,4-dihydroxybenzoate (**9a**, LC_{50} = 13 and 11 μM) and the pyrogallolic derivative 2-{[2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl]sulfanyl}ethyl 3,4,5-trihydroxybenzoate (**9b**, LC_{50} = 4 and 1 μM) were the most active ones against the two *Leishmania* strains.

KEYWORDS
antiproliferative agents, nitroimidazole, synthesis

1 | INTRODUCTION

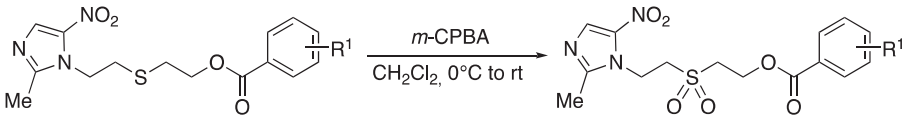
Leishmaniasis is an infectious disease caused by protozoa of the genus *Leishmania*. A recent review has shown that over 98 countries and territories are endemic for leishmaniasis transmission, with an overall prevalence of 12 million cases. Over 20 *Leishmania* species known to be infective to humans are transmitted by the bite of infected female phlebotomine sandflies, thus causing three main types of leishmaniasis: visceral (VL), cutaneous (CL), and mucocutaneous (MCL). It is estimated that approximately 0.2–0.4 million of new VL cases and 0.7–1.2 million of new CL cases occur each year. These diseases are responsible annually for approximately



SCHEME 1 Synthesis of the 2-{[2-(1*H*-imidazol-1-yl)ethyl]sulfanyl}ethyl benzoate derivatives **7a–l** and **9a,b**

20,000–40,000 deaths.[1] Leishmaniasis control relies on chemotherapy, as an effective vaccine is not available in the market, but available drugs are limited. The recommended first-line therapies include pentavalent antimony compounds, such as sodium stibogluconate and meglutamine antimoniate. However, these drugs present several disadvantages, such as toxicity, high costs, prolonged treatment, and parenteral or intralesional routes of administration. The second-line treatments include pentamidine and amphotericin B, but their use is limited due to toxicity and cost, even though lipid and liposomal formulations of amphotericin B have been developed to reduce this toxicity. Recently, the oral administration of miltefosine has been used for the treatment of VL in some countries, but despite its great efficacy, miltefosine is not free either from toxicity, as it shows teratogenic potential.[2–4] Several compounds that show leishmanicidal activity are currently in different stages of development. Among them, a few classes of compounds, such as 8-aminoquinolinic sitamaquine,[5] 7-aminoimidazoquinolinic imiquimod,[6] triazolic posaconazole,[7] 5-nitroimidazole fexinidazole,[8] as well as some natural product derivatives, such as licochalcone A,[9] have been revealed as potential new drugs for antileishmania therapy. The synthesis of several molecules showing leishmanicidal activity and that of new lead compounds, such as β-carboline alkaloids,[10] piperonylaminoacid conjugates,[11] heteroretinoid-bis(benzylidene)ketones,[12] and bispyridinium cyclophanes, have also been described.[13] In addition, the drawbacks associated with the currently available treatments have led to the development of new strategies aiming at leishmaniasis control. In this context, special attention has been given to nitroaromatic scaffolds, as such compounds are used to treat a wide variety of diseases, including Parkinson's disease, angina, and insomnia,[14–16] as well as several infections caused either by bacteria or by a range of pathogenic protozoan parasites as reported over the past 60 years.[17,18] For instance, metronidazole, tinidazole, ornidazole, benznidazole, fexinidazole, and nifurtimox are the recommended drugs for the treatment of protozoan infections.[19–23] In biological systems, nitro groups can undergo enzymatic reduction by reacting with nitroreductase enzymes. The resulting damages to the cells mainly occur in two ways, either by oxidative stress or through the formation of adducts between a protein or nonprotein thiol and some intermediate metabolites.[24] In the search for more effective alternatives to the currently used antileishmanial drugs, we synthesized a set of novel metronidazole analogs featuring an heterocyclic and basic 5-nitroimidazole head linked to a substituted benzoic acid through a dialkyl sulfur chain and further tested them in vitro against strains of *Leishmania (V.) braziliensis* (MHOM/BR/75/M2903) and *Leishmania (L.) mexicana* (MHOM/BZ/82/Bel21). The choice of this sulfur-containing spacer was related to some reported work[25] and to our previous successful experience with 7-chloroquinolin-4-ylthio derivatives, which exhibited an excellent in vitro antiplasmodial activity against chloroquine-sensitive strain of *Plasmodium berghei* and good in vivo

SCHEME 2 Synthesis of the 2-{[2-(1*H*-imidazol-1-yl)ethyl]sulfonyl}ethyl benzoate derivatives **10a–l**



7a–l **10a–l** (61–93%; see Table)

No	R^1	Yield (%)	No	R^1	Yield (%)
10a	2-OMe	80	**10g**	3,4,5-OMe	77
10b	4-OMe	76	**10h**	4-OMe-3-NO_2	77
10c	2,3-OMe	75	**10i**	3,5-Me	80
10d	2,4-OMe	92	**10j**	4-$(CH_3)_3C$	93
10e	2,5-OMe	86	**10k**	5-Me-2-NO_2	77
10f	2,4,5-OMe	61	**10l**	4-CF_3	69

efficacy in murine models of malaria, together with an excellent in vitro and in vivo antitumoral activity against prostate cancer.[26] Our interest in metronidazole analogs as an alternative to antiprotozoan treatments also lies in the fact that side-chains attached to position 1 of the imidazole nucleus provide an interesting opportunity to quickly carry out various modifications.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

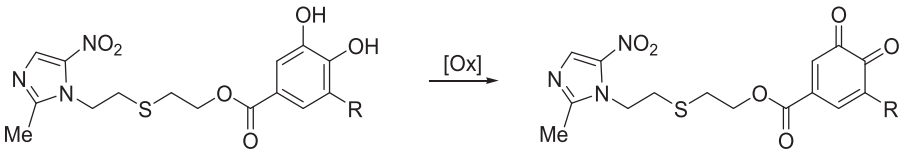
Our synthesis work began from metronidazole® **1**, whose primary alcohol was substituted by either a chlorine atom or a mesyl group. Following known procedures,[27,28] upon reaction with thionyl chloride, **1** was converted into the corresponding hydrochloride salt, which was then treated with water and Et_3N until pH 11, to obtain 1-(2-chloroethyl)-2-methyl-5-nitroimidazole (**2**) with a yield of 96% (Scheme 1). Alternatively, treatment of **1** with mesyl chloride and Et_3N in dichloromethane (CH_2Cl_2), at room temperature, afforded *O*-mesylated metronidazole (**3**) in a very good yield of 96%. Subsequent nucleophilic substitution of **3**, using sodium iodide in refluxing acetone, gave the iodinated compound **4** in 96% yield.[27,28] The nucleophilic substitution of **2** and **4** with 2-mercaptoethanol furnished the thioether-linked metronidazole analog **5**. This compound was obtained in a good yield (61%) when the reaction was carried out with **2** as the starting material, but the modified experimental protocol using **4** turned out to be more efficient and allowed us to prepare **5** in an even better yield (77%). The final compounds **7a–l** were synthesized via a coupling reaction between **5** and a series of benzoic acids, in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) and 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2. The title compounds were isolated in good-to-excellent (60–94%) yields after purification by recrystallization or by column chromatography (Scheme 1).

In addition, two polyhydroxy aromatic derivatives **9a** and **9b** were prepared, respectively, from commercially available

TABLE 1 Preliminary evaluation of the in vitro antileishmanial activity of compounds **7a–l**, **9a,b**, and **10d–l** on the *Leishmania braziliensis* and *Leishmania mexicana* promastigotes growth

		LC_{50} (mM)	
N°	R	*L. braziliensis*	*L. mexicana*
5	–	0.95 ± 0.032	2.25 ± 0.054
7a	2-OCH_3	0.417 ± 0.021	>1
7b	4-OCH_3	0.009 ± 0.002	0.215 ± 0.034
7c	2,3-OCH_3	0.432 ± .0.018	0.734 ± 0.032
7d	2,4-OCH_3	0.009 ± 0.003	0.229 ± 0.05
7e	2,5-OCH_3	0.032 ± 0.024	0.706 ± 0.021
7f	2,4,5-OCH_3	0.908 ± 0.081	0.632 ± 0.355
7g	3,4,5-OCH_3	0.464 ± 0.003	0.352 ± 0.089
7h	3-NO_2–4-OCH_3	0.253 ± 0.031	0.168 ± 0.035
7i	3,5-CH_3	0.585 ± 0.015	0.456 ± 0.017
7j	4-$C(CH_3)_3$	0.129 ± 0.017	0.188 ± 0.016
7k	2-NO_2–5-CH_3	0.442 ± 0.012	0.479 ± 0.002
7l	4-CF_3	0.356 ± 0.003	0.098 ± 0.005
9a	3,4-OH	0.013 ± 0.001	0.011 ± 0.002
9b	3,4,5-OH	0.004 ± 0.002	0.001 ± 0.001
10d	2,4-OCH_3	0.411 ± 0.042	0.420 ± 0.004
10e	2,5-OCH_3	>1	>1
10f	2,4,5-OCH_3	0.981 ± 0.049	0.037 ± 0.004
10g	3,4,5-OCH_3	0.023 ± 0.019	>1
10h	3-NO_2–4-OCH_3	0.134 ± 0.006	0.343 ± 0.075
10i	3,5-CH_3	0.120 ± 0.029	0.977 ± 0.180
10j	4-$C(CH_3)_3$	0.664 ± 0.019	0.497 ± 0.011
10k	2-NO_2–5-CH_3	0.303 ± 0.012	0.904 ± 0.045
10l	4-CF_3	0.464 ± 0.076	0.176 ± 0.025

Note: LC_{50} for compounds **5–9b**: S; **10d–l**: SO_2. Untreated control was used as control (–).



SCHEME 3 Oxidation of catechol- and pyrogallol-type phenols **9a** and **9b** to the reactive *ortho*-quinone species **11a,b**

protocatechuic acid and gallic acid, whose aromatic hydroxyl functions were previously protected with *tert*-butyldimethylsilyl groups.[29] After coupling **8a** and **8b** with **5**, the protecting groups were removed efficiently upon treatment with tetra-*n*-butylammonium fluoride (TBAF), in the presence of acetic acid, to afford compounds **9a** and **9b** with very good isolated yields of 71% and 95%, respectively (Scheme 1). Further *m*-CPBA-mediated oxidation of compounds **7a–l** gave a rapid and efficient (61–93% yield) access to the corresponding sulfonyl analogs **10a–l** (Scheme 2). The chemical structures of all synthesized compounds were confirmed on the basis of their nuclear magnetic resonance (NMR) and infrared (IR) spectral data and their purity was ascertained by microanalysis. In the ^{1}H NMR spectra, the signals of the respective protons of the compounds were checked on the basis of their chemical shifts, multiplicities, and coupling constants. All compounds showed a single signal ranging from δH 7.9 to 8.5 ppm, which was assigned to H-4 of the imidazole ring. The aliphatic signals expected at upfield shifts were found from δH 2.9 to 4.5 ppm. The aromatic region of ^{1}H NMR spectra featured signal patterns ranging from δH 6.5 to 8.0 ppm and was characteristic of the substitution pattern of each aromatic ring. ^{13}C NMR spectra showed characteristic signals of the 5-nitroimidazole core, with one signal resonating at δC 140–165 ppm, which was attributed to C-5, as well as two signals observed in the δC 138–152 and 124–140 ppm regions, which were assigned to C-2 and C-4, respectively. For the carboxyl group, another characteristic signal was observed further downfield around δC 165–166 ppm.

2.2 | Biological evaluation

The synthesized compounds were evaluated for their antileishmanial activity against in vitro forms of *L. (V.) braziliensis* and *L. (L.) mexicana* (promastigotes) strains. The main results are summarized in Table 1, in which the data are reported as mean ± standard deviation after statistical analysis by one-way analysis of variance. The LC_{50} values were calculated using the sigmoid dose–response curves. In comparison with metronidazole® (**1**, LC_{50} > 1 mM), compounds **7b, 7d**, **7e**, **9a**, and **9b** showed a higher activity against promastigotes of *L. (V.) braziliensis* (LC_{50} ranging from 4 to 32 µM), but only **9a** and **9b** were active against the glucantime-resistant promastigotes of *L. (L.) mexicana* (LC_{50} of 11 and 1 µM, respectively). When compounds **7a–l** were oxidized into the corresponding sulfones **10a–l**, compounds **10g–i** were found to be the most active of this group of molecules against the *L. (V.) braziliensis* promastigotes (LC_{50} ranging from 23 to 134 µM), but these three sulfonyl analogs were revealed as being weakly active against the *L. (L.) mexicana* ones (see Table 1).

Compounds **7h**, **7j**, **7l**, **10k**, and **10l** showed a weak antileishmanial activity, whereas compounds **5**, **7a**, **7c**, **7f**, **7i**, **7k**, and **10d–f** exhibited almost no activity against *L. (V.) braziliensis* and *L. (L.) mexicana* (with the exception of **10f** on this last strain). It is evident from these results that compounds featuring aromatic hydroxyl and methoxy groups are the most active ones, and that their added hydrophilic character likely plays an essential role in producing an antileishmanial effect, with hydroxy groups being more effective than methoxy substituents. A hypothetical explanation could also be proposed on the basis of oxidative dehydrogenation of the catechol- and pyrogallol-bearing phenols **9a** and **9b** into *ortho*-quinones (i.e., **11a,b**, Scheme 3), as such electrophilic entities can be engaged in covalently trapping proteins via their nucleophilic amino acid residue side-chains, hence possibly causing inactivation of sensitive enzymes.[30,31] Introduction of electron-withdrawing or hydrophobic groups, such as NO_2, CF_3, CH_3, or $C(CH_3)_3$, leads to almost inactive compounds. It is worthy to mention that a sulfanyl group (i.e., reduced sulfur atom) was more effective than a sulfonyl group (i.e., oxidized sulfur atom).

3 | CONCLUSION

A series of novel metronidazole® derivatives has been synthesized and tested as antileishmanial agents against promastigotes of *L. (V.) braziliensis* and *L. (L.) mexicana*. Among the tested compounds, the catecholic and pyrogallolic benzoate derivatives **9a** and **9b** have shown a significant in vitro activity superior to that of the parent drug, which may result from several independent or combined causes. The improved amphiphilic character brought by the di/trihydroxylated benzoate function may lead to an increase of the concentration of the compound inside the parasite form, thus increasing the interactions with leishmanial functional proteins. Furthermore, the dehydrogenation of their catechol or pyrogallol moieties into electrophilic quinones could mediate covalent modifications of these proteins. Alternatively, these structural modifications of the parent drug may simply support a more stable physical interaction between the active compounds and their biomolecular target. Even if additional assays related to toxicity on human cells, genotoxicity, in vivo experiments, and mechanism of action will be required to estimate their real potential, these biological results revealed that these two compounds constitute promising candidates in the search for improved therapies against *L. (V.) braziliensis* and *L. (L.) mexicana*.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Melting points were determined on a Thomas microhot stage apparatus and were uncorrected. The IR spectra were recorded on a Shimadzu model 470 (KBr pellets) or a Nicolet IS5FT-IR (ID3 Zn–Se) spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker Avance 300 (300 MHz/75.5 MHz) spectrometer using $CDCl_3$ or acetone-d_6 as the solvent, and they were reported in ppm downfield from the residual $CHCl_3$ or acetone signals. Elemental analyses were obtained using a Perkin–Elmer 2400 CHN elemental analyzer, and the results were within ±0.4% of the predicted values. Chemical reagents were purchased from Aldrich Chemical Co. All solvents were distilled and dried in the usual manner. Compounds **2–4**[18,25,26] and **8a,b**[29] were prepared according to known procedures.

The original spectra of the investigated compounds are provided as Supporting Information, as are their InChI codes together with some biological activity data.

4.1.2 | Synthesis of 2-[2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethylthio]ethanol (5)

A stirred solution of **4** (5.3 mmol), 2-mercaptoethanol (10.6 mmol), and potassium carbonate (1.8 g, 12.7 mmol) in acetonitrile (50 ml) was refluxed for 12 hr, after which it was concentrated under vacuum. The resulting residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to yield crude product, which was purified by column chromatography, first eluting with EtOAc/hexane (1:1), and then with CH_2Cl_2/MeOH (9.5:0.5). Yield: 77%; Mp. 79–80°C; IR (KBr) cm^{-1}: 3,344, 1,536, 1,478, 1,465, and 1,420; ^{1}H NMR $CDCl_3$ δ ppm: 2.53 (s, 3H, CH_3), 2.70 (t, 2H, CH_2 J = 5.9 Hz), 2.90 (t, 2H, CH_2 J = 5.9 Hz), 3.75 (t, 2H, CH_2 J = 5.9 Hz), 4.47 (t, 2H, CH_2 J = 5.9 Hz), and 7.97 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.4, 31.7, 35.5, 46.3, 61.3, 133.1, 138.5, and 150.5. Anal. calcd. for $C_8H_{13}N_3O_3S$: C, 41.55; H, 5.67; N, 18.17. Found: C, 41.59; H, 5.68; N, 18.35.

4.1.3 | General procedure for the preparation of ethylsulfanylethyl benzoate derivatives 7a–l

EDCI (0.3 mmol) and DMAP (0.3 mmol) were added to an ice-cold stirred solution of substituted benzoic acid (0.3 mmol) in dry CH_2Cl_2 (10 ml). The resulting mixture was stirred at 0°C for 30 min, after which alcohol **5** (0.26 mmol) was added. The resulting mixture was stirred for 12 hr and monitored by thin-layer chromatography (TLC), eluting with cyclohexane/EtOAc (7:3). The reaction mixture was quenched with sat. aq. $NaHCO_3$ (20 ml), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 ml), and the combined organic layers were washed with water (50 ml) and brine (50 ml), dried over Na_2SO_4, filtered, and concentrated in vacuum. Purification by column chromatography furnished the benzoate derivatives **7a–l**.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 2-methoxybenzoate (**7a**)*

Mp. 132–133°C; IR (Zn–Se) cm^{-1}: 1,731, 1,458, 1,190, and 1,180; ^{1}H NMR $CDCl_3$ δ ppm: 2.50 (s, 3H, CH_3), 2.88 (t, 2H, CH_2 J = 6.7 Hz), 2.97 (t, 2H, CH_2 J = 7.1 Hz), 3.87 (s, 3H, OCH_3), 4.43 (t, 2H, CH_2 J = 6.6 Hz), 4.47 (t, 2H, CH_2 J = 7.1 Hz), 6.96 (m, 2H, Ar), 7.46 (m, 1H, Ar), 7.77 (dd, 1H J = 7.9, 1.9 Hz), and 7.92 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 31.1, 32.0, 46.2, 56.0, 63.6, 112.2, 119.6, 120.3, 131.7, 133.3, 133.9, 150.6, 159.3, and 165.8. Anal. calcd. for $C_{16}H_{19}N_3O_5S$: C, 52.59; H, 5.24; N, 11.50. Found: C, 52.63; H, 5.27; N, 11.72.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 4-methoxybenzoate (**7b**)*

Mp. 138–140°C; IR (Zn–Se) cm^{-1}: 1,740, 1,472, 1,210, and 1,187; ^{1}H NMR $CDCl_3$ δ ppm: 2.50 (s, 3H, CH_3), 2.86 (t, 2H, CH_2 J = 6.7 Hz), 2.95 (t, 2H, CH_2 J = 6.0 Hz), 3.83 (s, 3H, OCH_3), 4.41 (t, 2H, CH_2 J = 6.0 Hz), 4.46 (t, 2H, CH_2 J = 6.0 Hz), 6.89 (d, 2H, $H_{3,5}$ J = 9.0 Hz), 7.92 (s, 1H, H_4), and 7.94 (d, 2H, $H_{2,6}$ J = 9.0 Hz); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 31.1, 31.9, 46.2, 55.5, 63.2, 113.7, 122.1, 131.7, 133.2, 138.4, 150.5, 163.6, and 166.0. Anal. calcd. for $C_{16}H_{19}N_3O_5S$: C, 52.59; H, 5.24; N, 11.50. Found: C, 52.67; H, 5.25; N, 11.61.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 2,3-dimethoxybenzoate (**7c**)*

Mp. 136–138°C; IR (Zn–Se) cm^{-1}: 1,723, 1,523, 1,458, and 1,421; ^{1}H NMR $CDCl_3$ δ ppm: 2.49 (s, 3H, CH_3), 2.87 (t, 2H, CH_2 J = 6.7 Hz), 2.95 (t, 2H, CH_2 J = 7.1 Hz), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.43 (t, 2H, CH_2 J = 6.7 Hz), 4.46 (t, 2H, CH_2 J = 7.1 Hz), 7.05 (m, 2H, Ar), 7.28 (m, 1H, Ar), and 7.91 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 31.0, 31.9, 46.1, 56.1, 61.6, 63.6, 116.1, 122.2, 123.9, 125.7, 133.2, 149.2, 150.5, 153.6, and 166.0. Anal. calcd. for $C_{17}H_{21}N_3O_6S$: C, 51.64; H, 5.35; N, 10.63. Found: C, 51.69; H, 5.38; N, 10.83.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 2,4-dimethoxybenzoate (**7d**)*

Mp. 141–143°C; IR (Zn–Se) cm^{-1}: 2,941, 1,715, 1,609, 1,458, 1,417, and 1,237; ^{1}H NMR $CDCl_3$ δ ppm: 2.51 (s, 3H, CH_3), 2.87 (t, 2H, CH_2 J = 6.7 Hz), 2.97 (t, 2H, CH_2 J = 7.0 Hz), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.41 (t, 2H, CH_2 J = 6.7 Hz), 4.48 (t, 2H, CH_2 J = 7.0 Hz), 6.48 (m, 2H, $H_{3',5'}$), 7.82 (d, 1H, $H_{6'}$ J = 8.9 Hz), and 7.91 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.4, 31.1, 32.2, 45.9, 55.7, 55.9, 64.1, 99.1, 105.1, 110.1, 133.7, 134.3, 161.6, 164.8, and 165. Anal. calcd. for $C_{17}H_{21}N_3O_6S$: C, 51.64; H, 5.35; N, 10.63. Found: C, 51.72; H, 5.35; N, 10.87.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 2,5-dimethoxybenzoate (**7e**)*

Mp. 152–154°C; IR (Zn–Se) cm^{-1}: 1,745, 1,499, 1,224, and 1,202; ^1H NMR $CDCl_3$ δ ppm: 2.50 (s, 3H, CH_3), 2.88 (t, 2H, CH_2 J = 6.7 Hz), 2.97 (t, 2H, CH_2 J = 7.1 Hz), 3.77 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.43 (t, 2H, CH_2 J = 6.7 Hz), 4.47 (t, 2H, CH_2 J = 7.0 Hz), 6.90 (d, 1H, $H_{3'}$; J = 9.1 Hz), 7.01 (dd, 1H, $H_{4'}$ J = 9.1, 3.2 Hz), 7.31 (d, 1H, $H_{6'}$ J = 3.2 Hz), and 7.92 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 31.1, 32.0, 46.2, 55.9, 56.8, 63.7, 113.9, 116.3, 119.7, 120.1, 133.2, 150.6, 153.1, 153.7, and 165.7. Anal. calcd. for $C_{17}H_{21}N_3O_6S$: C, 51.64; H, 5.35; N, 10.63. Found: C, 51.66; H, 5.37; N, 10.72.

2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 2,4,5-trimethoxybenzoate (7f)

Mp. 161–163°C; IR (Zn–Se) cm^{-1}: 1,705, 1,480, 1,203, and 1,191; ^1H NMR $CDCl_3$ δ ppm: 2.50 (s, 3H, CH_3), 2.86 (t, 2H, CH_2 J = 6.7 Hz), 2.96 (t, 2H, CH_2 J = 7.1 Hz), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.40 (t, 2H, CH_2 J = 6.7 Hz), 4.46 (t, 2H, CH_2 J = 7.0 Hz), 6.49 (s, 1H, $H_{3'}$), 7.36 (s, 1H, $H_{6'}$), and 7.91 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 31.1, 32.0, 46.2, 56.1, 56.5, 57.0, 63.3, 97.7, 110.1, 114.5, 133.2, 142.6, 150.6, 153.9, 156.0, and 165.3. Anal. calcd. for $C_{18}H_{23}N_3O_7S$: C, 50.82; H, 5.45; N, 9.88. Found: C, 50.85; H, 5.47; N, 10.07.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 3,4,5-trimethoxybenzoate (**7g**)*

Mp. 160–162°C; IR (Zn–Se) cm^{-1}: 1,704, 1,217, and 1,120; ^1H NMR $CDCl_3$ δ ppm: 2.50 (s, 3H, CH_3), 2.86 (t, 2H, CH_2 J = 6.8 Hz), 2.95 (t, 2H, CH_2 J = 7.2 Hz), 3.87 (s, 9H, OCH_3), 4.42 (t, 2H, CH_2 J = 6.8 Hz), 4.46 (t, 2H, CH_2 J = 7.1 Hz), 7.24 (s, 2H, $H_{2',6'}$), and 7.91 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 31.0, 31.9, 46.2, 56.3, 60.9, 63.5, 106.9, 124.7, 133.2, 142.5, 150.5, 153.0, and 165.9. Anal. calcd. for $C_{18}H_{23}N_3O_7S$: C, 50.82; H, 5.45; N, 9.88. Found: C, 50.83; H, 5.45; N, 10.12.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 4-methoxy-3-nitrobenzoate (**7h**)*

Mp. 118–120°C; IR (Zn–Se) cm^{-1}: 2,923, 2,360, 2,325, 1,711, and 1,514; ^1H NMR $CDCl_3$ δ ppm: 2.54 (s, 3H, CH_3), 2.89 (t, 2H, CH_2 J = 6.6 Hz), 2.97 (t, 2H, CH_2 J = 7.2 Hz), 4.03 (s, 3H, OCH_3), 4.47 (t, 2H, CH_2 J = 6.6 Hz), 4.50 (t, 2H, CH_2 J = 7.1 Hz), 7.15 (d, 1H, $H_{5'}$ J = 8.9 Hz), 7.94 (s, 1H, H_4), 8.19 (dd, 1H, $H_{6'}$ J = 8.8, 2.2 Hz), and 8.47 (d, 1H, $H_{2'}$ J = 2.2 Hz); ^{13}C NMR $CDCl_3$ δ ppm: 14.6, 31.1, 31.9, 46.2, 57.0, 63.8, 113.4, 122.3, 127.4, 133.4, 135.5, 139.4, 150.6, 156.4, and 164.3. Anal. calcd. for $C_{16}H_{18}N_4O_7S$: C, 46.83; H, 4.42; N, 13.65. Found: C, 46.89; H, 4.45; N, 13.81.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 3,5-dimethylbenzoate (**7i**)*

Mp. 85–87°C; IR (Zn–Se) cm^{-1}: 2,921, 1,703, 1,519, and 1,515; ^1H NMR $CDCl_3$ δ ppm: 2.34 (s, 6H, CH_3), 2.54 (s, 3H, CH_3), 2.89 (t, 2H, CH_2 J = 6.7 Hz), 2.98 (t, 2H, CH_2 J = 7.1 Hz), 4.45 (t, 2H, CH_2 J = 6.7 Hz), 4.49 (t, 2H, CH_2 J = 7.1 Hz), 7.18 (s, 1H, $H_{4'}$), 7.62 (s, 2H, $H_{2',6'}$), and 7.96 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.6, 21.2, 31.1, 32.0, 46.3, 63.5, 127.4, 129.7, 133.2, 135.0, 138.2, 150.6, and 166.8. Anal. calcd. for $C_{17}H_{21}N_3O_4S$: C, 56.18; H, 5.82; N, 11.56. Found: C, 56.23; H, 5.82; N, 11.87.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 4-tert-butylbenzoate (**7j**)*

Mp. 83–85°C; IR (Zn–Se) cm^{-1}: 2,962, 1,711, 1,605, 1,527, 1,454, and 1,352; ^1H NMR $CDCl_3$ δ ppm: 1.36 (s, 9H, CH_3), 2.56 (s, 3H, CH_3), 2.92 (t, 2H, CH_2 J = 6.0 Hz), 3.01 (t, 2H, CH_2 J = 7.3 Hz), 4.49 (t, 2H, CH_2 J = 6.6 Hz), 4.53 (t, 2H, CH_2 J = 7.1 Hz), 7.49 (d, 2H, $H_{3',5'}$ J = 8.9 Hz), 7.97 (d, 2H, $H_{2',6'}$ J = 8.7 Hz), and 7.99 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.6, 31.2, 32.0, 35.2, 46.3, 63.4, 125.5, 127.0, 129.6, 133.2, 150.6, 157.0, and 166.4. Anal. calcd. for $C_{19}H_{25}N_3O_4S$: C, 58.29; H, 6.44; N, 10.73. Found: C, 58.35; H, 6.46; N, 10.97.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 5-methyl-2-nitrobenzoate (**7k**)*

Mp. 88–90°C; IR (Zn–Se) cm^{-1}: 2,970, 1,728, 1,589, 1,523, and 1,458; ^1H NMR $CDCl_3$ δ ppm: 2.47 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 2.86 (t, 2H, CH_2 J = 6.0 Hz), 2.94 (t, 2H, CH_2 J = 7.2 Hz), 4.46 (m, 4H, CH_2), 7.40 (dd, 1H, $H_{4'}$ J = 8.3, 2.6 Hz), 7.46 (s, 1H, $H_{6'}$), 7.86 (d, 1H, $H_{3'}$ J = 8.3 Hz), and 7.92 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm:14.5, 21.5, 30.4, 31.9, 46.1, 64.8, 124.3, 128.0, 130.1, 132.1, 133.3, 145.0, 150.6, and 165.8. Anal. calcd. for $C_{16}H_{18}N_4O_6S$: C, 48.73; H, 4.60; N, 14.21. Found: C, 48.72; H, 4.61; N, 14.45.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 4-trifluoromethylbenzoate (**7l**)*

Mp. 92–94°C; IR (Zn–Se) cm^{-1}: 3,039, 1,711, 1,519, 1,450, and 1,360; ^1H NMR $CDCl_3$ δ ppm: 2.52 (s, 3H, CH_3), 2.89 (t, 2H, CH_2 J = 6.7 Hz), 2.96 (t, 2H, CH_2 J = 7.1 Hz), 4.48 (m, 4H, CH_2), 7.69 (d, 2H, $H_{3',5'}$ J = 8.1 Hz), 7.93 (s, 1H, H_4), and 8.12 (d, 2H, $H_{2',6'}$ J = 8.1 Hz); ^{19}F NMR ($CDCl_3$, 282 MHz) δ 63.14; ^{13}C NMR $CDCl_3$ δ ppm: 14.6, 31.0, 31.9, 46.2, 63.9, 125.55, 125.60, 130.1, 133.3, 150.6, and 165.2. Anal. calcd. for $C_{16}H_{16}F_3N_3O_4S$: C, 47.64; H, 4.00; N, 10.42. Found: C, 47.67; H, 4.03; N, 10.67.

4.1.4 | General procedure for the preparation of ethylsulfanylethyl di- and trihydroxybenzoates 9a and 9b

EDCI (0.3 mmol) and DMAP (0.3 mmol) were added to an ice-cold stirred solution of 3,4-bis(*tert*-butyldimethylsilyloxy)benzoic acid (**8a**)[29] or 3,4,5-tris(*tert*-butyldimethylsilyloxy)benzoic acid (**8b**)[29] (0.3 mmol) in dry CH_2Cl_2 (10 ml). The resulting mixture was stirred at 0°C for 30 min, after which alcohol **5** (0.26 mmol) was added. The resulting mixture was stirred for 12 hr and monitored by TLC, eluting with cyclohexane/EtOAc (7:3). The reaction mixture was quenched

with sat. aq. $NaHCO_3$ (20 ml), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 ml) and the combined organic layers were washed with water (50 ml) and brine (50 ml), dried over Na_2SO_4, filtered and concentrated in vacuum. The subsequent desilylation step was adapted from our previously described procedure.[29] Dropwise acetic acid (1.5 mmol) and tetrabutylammonium fluoride (1.5 mmol, TBAF 0.1 M in THF) were added to a stirred ice-cold solution of this crude material in THF (25 ml). The resulting mixture was stirred for 3 hr and monitored by TLC, eluting with CH_2Cl_2/MeOH (9.5:0.5). After evaporation of the solvent, CH_2Cl_2 (50 ml) was added. The organic layer was washed with sat. aq. $NaHCO_3$ (50 ml), water (50 ml), and brine (50 ml), dried over Na_2SO_4, filtered, and concentrated in vacuum. The resulting white powder was purified by column chromatography, eluting with CH_2Cl_2/MeOH (from 9.5:0.5 to 9:1).

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 3,4-dihydroxybenzoate (**9a**)*

Mp. 185–187°C; IR (Zn–Se) cm^{-1}: 2,966, 1,699, 1,589, 1,274, and 1,176; ^1H NMR $CDCl_3$ δ ppm: 2.56 (s, 3H, CH_3), 2.95 (t, 2H, CH_2 J = 6.7 Hz), 3.08 (t, 2H, CH_2 J = 7.0 Hz), 4.40 (t, 2H, CH_2 J = 6.6 Hz), 4.61 (t, 2H, CH_2 J = 7.2 Hz), 6.91 (d, 1H, $H_{5'}$ J = 8.3 Hz), 7.44 (dd, 1H, $H_{6'}$ J = 8.3, 2.0 Hz), 7.51 (d, 1H, $H_{2'}$ J = 2.0 Hz), and 7.93 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 31.6, 31.9, 46.2, 64.3, 116.1, 116.4, 121.7, 133.4, 139.0, 146.2, 152.1, and 166.7. Anal. calcd. for $C_{15}H_{17}N_3O_6S$: C, 49.04; H, 4.66; N, 11.44. Found: C, 49.10; H, 4.70; N, 11.69.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfanyl}ethyl 3,4,5-trihydroxybenzoate (**9b**)*

Mp. 198–200°C; IR (Zn–Se) cm^{-1}: 3,367, 1,696, 1,684, 1,225, 1,171, and 1,040; ^1H NMR acetone-d_6 δ ppm: 2.57 (s, 3H, CH_3), 2.95 (t, 2H, CH_2 J = 6.6 Hz), 3.09 (t, 2H, CH_2 J = 7.0 Hz), 4.38 (t, 2H, CH_2 J = 6.6 Hz), 4.62 (t, 2H, CH_2 J = 7.2 Hz), 7.12 (s, 2H, $H_{2',6'}$), 7.93 (s, 1H, H_4), and 8.29 (bs, 3H, OH); ^{13}C NMR acetone-d_6 δ ppm: 14.5, 31.4, 32.2, 46.8, 64.0, 110.0, 121.7, 133.4, 139.0, 146.2, 152.1, and 166.6. Anal. calcd. for $C_{15}H_{17}N_3O_7S$: C, 46.99; H, 4.47; N, 10.96. Found: C, 47.05; H, 4.61; N, 11.17.

4.1.5 | General procedure for the preparation of ethylsulfonylethyl benzoate derivatives 10a–l

Meta-chloroperoxybenzoic acid (0.6 mmol, purity of 70%) was added to an ice-cold stirred solution of substituted ester **7a–l** (0.2 mmol) in dry CH_2Cl_2 (10 ml) under an N_2 atmosphere. The resulting mixture was allowed to warm to room temperature for 6 hr. The reaction mixture was quenched with sat. aq. $NaHCO_3$ (10 ml) and $Na_2S_2O_3$ (5 ml). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 25 ml). The combined organic layers were washed with water (50 ml) and brine (50 ml), dried over Na_2SO_4, filtered, and concentrated in vacuum. Purification by column chromatography, eluting with EtOAc/cyclohexane (9:1), furnished the sulfonyl benzoate derivatives **10a–l**.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 2-methoxybenzoate (**10a**)*

Mp. 101–103°C; IR (Zn–Se) cm^{-1}: 2,933, 1,715, 1,601, 1,523, and 1,470; ^1H NMR $CDCl_3$ δ ppm: 2.53 (s, 3H, CH_3), 3.45 (t, 2H, CH_2 J = 5.6 Hz), 3.62 (t, 2H, CH_2 J = 6.5 Hz), 3.82 (s, 3H, OCH_3), 4.71 (t, 2H, CH_2 J = 5.7 Hz), 4.76 (t, 2H, CH_2 J = 6.7 Hz), 6.97 (m, 2H, $H_{4',5'}$), 7.49 (m, 1H, $H_{3'}$), 7.76 (dd, 1H, $H_{6'}$ J = 7.7, 1.8 Hz), and 7.93 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.4, 39.1, 53.2, 53.8, 56.0, 58.0, 112.3, 118.5, 120.6, 132.0, 134.6, 159.3, and 165.4. Anal. calcd. for $C_{16}H_{19}N_3O_7S$: C, 48.36; H, 4.82; N, 10.57. Found: C, 48.39; H, 4.86; N, 10.79.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 4-methoxybenzoate (**10b**)*

Mp. 103–105°C; IR (Zn–Se) cm^{-1}: 3,141, 1,719, 1,519, and 1,450; ^1H NMR $CDCl_3$ δ ppm: 2.58 (s, 3H, CH_3), 3.46 (t, 2H, CH_2 J = 5.6 Hz), 3.58 (t, 2H, CH_2 J = 6.6 Hz), 3.87 (s, 3H, OCH_3), 4.73 (t, 2H, CH_2 J = 5.8 Hz), 4.79 (t, 2H, CH_2 J = 6.6 Hz), 6.93 (d, 2H, $H_{3',5'}$ J = 9.0 Hz), 7.92 (d, 2H, $H_{2',6'}$ J = 9.0 Hz), and 7.95 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 39.0, 53.5, 53.9, 55.7, 57.6, 114.1, 121.2, 131.9, 133.8, 151.3, 164.1, and 165.6. Anal. calcd. for $C_{16}H_{19}N_3O_7S$: C, 48.36; H, 4.82; N, 10.57. Found: C, 48.41; H, 4.83; N, 10.81.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 2,3-dimethoxybenzoate (**10c**)*

Mp. 114–116°C; IR (Zn–Se) cm^{-1}: 2,361, 1,711, 1,503, and 1,450; ^1H NMR $CDCl_3$ δ ppm: 2.58 (s, 3H, CH_3), 3.49 (t, 2H, CH_2 J = 5.7 Hz), 3.68 (t, 2H, CH_2 J = 6.7 Hz), 3.86 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.77 (t, 2H, CH_2 J = 5.7 Hz), 4.80 (t, 2H, CH_2 J = 6.7 Hz), 7.12 (m, 2H, $H_{4',6'}$), 7.30 (m, 1H, $H_{5'}$), and 7.96 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.4, 39.0, 53.3, 53.7, 56.1, 58.2, 61.7, 116.6, 122.1, 124.3, 124.7, 133.6, 149.2, 151.2, 153.7, and 165.5. Anal. calcd. for $C_{17}H_{21}N_3O_8S$: C, 47.77; H, 4.95; N, 9.83. Found: C, 47.78; H, 4.97; N, 10.05.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 2,4-dimethoxybenzoate (**10d**)*

Mp. 120–122°C; IR (Zn–Se) cm^{-1}: 2,941, 1,711, 1,601, 1,458, and 1,360; ^1H NMR $CDCl_3$ δ ppm: 2.53 (s, 3H, CH_3), 3.43 (t, 2H, CH_2 J = 5.5 Hz), 3.61 (t, 2H, CH_2 J = 6.6 Hz), 3.80 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.66 (t, 2H, CH_2 J = 5.6 Hz), 4.75 (t, 2H, CH_2 J = 6.6 Hz), 6.44 (d, 1H, $H_{3'}$ J = 2.3 Hz), 6.49 (dd, 1H, $H_{5'}$ J = 8.8, 2.3 Hz), 7.79 (d, 1H, $H_{6'}$ J = 8.7 Hz), and 7.91 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.4, 39.0, 53.2, 53.9, 55.7, 55.9, 57.6, 99.1, 105.1, 110.6, 133.7, 134.3, 151.3, 161.6, 164.8, and 165.1. Anal. calcd. for $C_{17}H_{21}N_3O_8S$: C, 47.77; H, 4.95; N, 9.83. Found: C, 47.83; H, 4.95; N, 9.97.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 2,5-dimethoxybenzoate (**10e**)*

Mp. 137–139°C; IR (Zn–Se) cm^{-1}: 2,933, 1,723, 1,533, 1,470, and 1,364; ^1H NMR $CDCl_3$ δ ppm: 2.49 (s, 3H, CH_3), 2.87 (t, 2H, CH_2 J = 6.7 Hz), 2.95 (t, 2H, CH_2 J = 7.1 Hz), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.43 (t, 2H, CH_2 J = 6.7 Hz), 4.46 (t, 2H, CH_2 J = 7.1 Hz), 7.05 (m, 2H, $H_{4',6'}$), 7.28 (m, 1H, $H_{5'}$), and 7.91 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 31.0, 31.9, 46.1, 56.1, 61.6, 63.6, 116.1, 122.2,

123.9, 125.7, 133.2, 149.2, 150.5, 153.61, and 166.0. Anal. calcd. for $C_{17}H_{21}N_3O_8S$: C, 47.77; H, 4.95; N, 9.83. Found: C, 47.79; H, 4.96; N, 10.01.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 2,4,5-trimethoxybenzoate (**10f**)*

Mp. 140–142°C; IR (Zn–Se) cm^{-1}: 2,929, 1,715, 1,613, 1,511, 1,462, and 1356; ^1H NMR $CDCl_3$ δ ppm: 2.56 (s, 3H, CH_3), 3.46 (t, 2H, CH_2 J = 5.4 Hz), 3.65 (t, 2H, CH_2 J = 6.5 Hz), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.71 (t, 2H, CH_2 J = 5.6 Hz), 4.78 (t, 2H, CH_2 J = 6.5 Hz), 6.49 (s, 1H, $H_{3'}$), 7.39 (s, 1H, $H_{6'}$), and 7.93 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 39.1, 53.2, 53.9, 56.3, 56.6, 56.9, 57.9, 97.6, 109.0, 114.6, 133.6, 143.0, 151.3, 154.6, 155.9, and 165.2. Anal. calcd. for $C_{18}H_{23}N_3O_9S$: C, 47.26; H, 5.07; N, 9.19. Found: C, 47.30; H, 5.12; N, 9.41.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 3,4,5-trimethoxybenzoate (**10g**)*

Mp. 147–149°C; IR (Zn–Se) cm^{-1}: 2,923, 1,730, 1,711, 1,503, and 1,449; ^1H NMR $CDCl_3$ δ ppm: 2.58 (s, 3H, CH_3), 3.49 (t, 2H, CH_2 J = 5.9 Hz), 3.58 (t, 2H, CH_2 J = 6.6 Hz), 3.89 (s, 6H, OCH_3), 3.91 (s, 3H, OCH_3), 4.76 (t, 2H, CH_2 J = 6.0 Hz), 4.78 (t, 2H, CH_2 J = 6.6 Hz), 7.25 (s, 2H, $H_{2',6'}$), and 7.95 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 39.2, 53.1, 53.6, 56.4, 57.7, 61.0, 107.1, 123.8, 133.8, 143.0, 151.3, 153.2, and 165.7. Anal. calcd. for $C_{18}H_{23}N_3O_9S$: C, 47.26; H, 5.07; N, 9.19. Found: C, 47.27; H, 5.09; N, 9.33.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 4-methoxy-3-nitrobenzoate (**10h**)*

Mp. 135–137°C; IR (Zn–Se) cm^{-1}: 2,929, 2,365, 1,744, 1,711, 1,523, and 1,458; ^1H NMR $CDCl_3$ δ ppm: 2.60 (s, 3H, CH_3), 3.49 (t, 2H, CH_2 J = 5.7 Hz), 3.59 (t, 2H, CH_2 J = 6.7 Hz), 4.05 (s, 3H, OCH_3), 4.81 (m, 4H, CH_2), 7.17 (d, 1H, $H_{5'}$ J = 8.9 Hz), 7.97 (s, 1H, H_4), 8.17 (dd, 1H, $H_{6'}$ J = 8.8, 2.2 Hz), and 8.47 (d, 1H, $H_{2'}$ J = 2.2 Hz); ^{13}C NMR $CDCl_3$ δ ppm: 14.6, 39.2, 53.4, 57.1, 58.1, 113.7, 121.3, 127.6, 134.0, 135.5, 151.4, and 156.8. Anal. calcd. for $C_{16}H_{18}N_4O_9S$: C, 43.44; H, 4.10; N, 12.66. Found: C, 43.48; H, 4.17; N, 12.81.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 3,5-dimethylbenzoate (**10i**)*

Mp. 105–107°C; IR (Zn–Se) cm^{-1}: 2,929, 1,703, 1,597, 1,458, and 1,258; ^1H NMR $CDCl_3$ δ ppm: 2.35 (s, 6H, CH_3), 2.58 (s, 3H, CH_3), 3.47 (t, 2H, CH_2 J = 5.8 Hz), 3.59 (t, 2H, CH_2 J = 6.6 Hz), 4.75 (t, 2H, CH_2 J = 5.8 Hz), 4.79 (t, 2H, CH_2 J = 6.6 Hz), 7.22 (s, 1H, $H_{4'}$), 7.58 (s, 2H, $H_{2',6'}$), and 7.96 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 21.3, 39.1, 53.4, 53.9, 57.8, 127.5, 128.8, 133.8, 135.6, and 138.6. Anal. calcd. for $C_{17}H_{21}N_3O_6S$: C, 51.64; H, 5.35; N, 10.63. Found: C, 51.67; H, 5.40; N, 10.89.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 4-tert-butylbenzoate (**10j**)*

Mp. 111–113°C; IR (Zn–Se) cm^{-1}: 2,978, 1,711, 1,601, 1,523, and 1,458; ^1H NMR $CDCl_3$ δ ppm: 1.31 (s, 9 H, *t*-Bu), 2.53 (s, 3H, CH_3), 3.46 (t, 2H, CH_2 J = 5.8 Hz), 3.59 (t, 2H, CH_2 J = 7.3 Hz), 4.71 (t, 2H, CH_2 J = 5.9 Hz), 4.76 (t, 2H, CH_2 J = 6.7 Hz), 7.43 (d, 2H, $H_{3',5'}$ J = 8.7 Hz), 7.86 (d, 2H, $H_{2',6'}$ J = 8.7 Hz), and 7.89 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.4, 31.1, 35.2, 38.9, 53.2, 53.6, 57.6, 125.7, 126.0, 129.5, 133.7, 138.3, 151.3, 157.6, and 165.8. Anal. calcd. for $C_{19}H_{25}N_3O_6S$: C, 53.89; H, 5.95; N, 9.92. Found: C, 53.92; H, 5.98; N, 10.19.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 5-methyl-2-nitrobenzoate (**10k**)*

Mp. 115–117°C; IR (Zn–Se) cm^{-1}: 2,917, 2,316, 1,736, 1,711, and 1,519; ^1H NMR $CDCl_3$ δ ppm: 2.48 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 3.44 (t, 2H, CH_2 J = 5.5 Hz), 3.49 (t, 2H, CH_2 J = 6.7 Hz), 4.75 (m, 4H, CH_2), 7.42 (m, 1H, $H_{4'}$), 7.44 (s, 1H, $H_{6'}$), 7.89 (d, 1H, $H_{3'}$ J = 9.1 Hz), and 7.92 (s, 1H, H_4); ^{13}C NMR $CDCl_3$ δ ppm: 14.4, 21.5, 39.0, 53.0, 59.0, 124.5, 127.3, 130.1, 132.5, 133.7, 145.1, 145.7, 151.3, and 165.5. Anal. calcd. for $C_{16}H_{18}N_4O_8S$: C, 45.07; H, 4.26; N, 13.14. Found: C, 45.12; H, 4.32; N, 13.40.

*2-{[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]sulfonyl}ethyl 4-trifluoromethylbenzoate (**10l**)*

Mp. 128–130°C; IR (Zn–Se) cm^{-1}: 2,982, 2,361, 1,711, 1,531, 1,503, and 1,454; ^1H NMR $CDCl_3$ δ ppm: 2.58 (s, 3H, CH_3), 3.49 (t, 2H, CH_2 J = 5.7 Hz), 3.59 (t, 2H, CH_2 J = 6.6 Hz), 4.81 (m, 2H, CH_2), 7.73 (d, 2H, $H_{3',5'}$ J = 8.8 Hz), 7.96 (s, 1H, H_4), and 8.11 (d, 2H, $H_{2',6'}$ J = 8.8 Hz); ^{19}F NMR ($CDCl_3$, 282 MHz) δ 63.20; ^{13}C NMR $CDCl_3$ δ ppm: 14.5, 39.2, 53.3, 58.1, 125.8, 125.9, 130.2, 134.0, 151.4, and 164.9. Anal. calcd. for $C_{16}H_{16}F_3N_3O_6S$: C, 44.14; H, 3.70; N, 9.65. Found: C, 44.16; H, 3.75; N, 9.89.

4.2 | Antileishmania assays

4.2.1 | Culture and maintenance of the parasite

International reference strains of *L. (V.) braziliensis* (MHOM/BR/75/M2903) and *L. (L.) mexicana* (MHOM/BZ/82/Bel21) were thawed and cultured in RPMI 1640 medium (Gibco-BRL) at room temperature with 10% fetal bovine serum inactivated by heating at 56°C for 30 min. Finally, antibiotics (penicillin/streptomycin), at concentrations of 100 and 1,000 units, respectively, were added. For the experiments, parasites were collected in the logarithmic phase of growth (5th day of culture) by centrifugation at 3,000 rpm and washed three times with saline phosphate buffer, pH 8.0. The pellet was resuspended in fresh medium and the parasites were adjusted to a concentration of 1×10^6 cells/ml.[32]

4.2.2 | Antileishmania activity on promastigote proliferation

Each compound was diluted to a concentration of 50 mg/ml in appropriate solvent (dimethyl sulfoxide) and dilutions between 10 and

500 μg/ml were prepared subsequently for the experiments. Different concentrations of each compound were used for the different species of *Leishmania*, that is, *L. (V.) braziliensis* or *L. (L.) mexicana*, to investigate the response of the parasite to each compound. A daily sample of 5 μl was taken for cell counting. The count was performed in triplicate for 7 days until the culture reached the stationary phase of growth. The effect of each compound over the different *Leishmania* species was evaluated.[32]

4.2.3 | Calculation of cell viability and LC_{50}

To evaluate the effect of the compounds on cell viability and to calculate LC_{50}, two methods were used.

Indirect method

Parasites were incubated with various concentrations of the respective compound for 18–24 hr; thereafter, 10 μl (10 mg/ml) of methyl-thiazole tetrazolium (MTT; Sigma-Aldrich) was added and incubated for 4 hr. After incubation, the reaction was stopped with lysis buffer (50% isopropyl alcohol, 10% sodium dodecyl sulfate), and then the optical density (OD) was measured at 570 nm in a spectrophotometer (Bio-Rad). Cell viability is directly proportional to OD. A higher number of living cells have a greater color intensity because they have a high capacity to metabolize the MTT. For each experiment, different controls were used, including cells treated with solvent only and controls without and with meglumine antimoniate (i.e., drug of choice in the treatment of leishmaniasis). The effect of each compound on the growth of the parasites in relation to controls was used to estimate the concentration that causes the death of 50% of the cells in a given time (LC_{50}).

Direct method

This method is based on the comparison between two doses, X_1 and X_2, such that the density of parasites (Y_1) to the X dose 1 is greater than half of the density found in the control (I), and the density of parasites Y_2 found to the dose X_2 is less than half of the control. Then, we can calculate the lethal concentration 50 (LC_{50}) using the algorithm previously described by Huber and Koella.[33] For promastigote, 1×10^6 parasites were added in 2 ml of medium SDM 79 at pH 7.2, supplemented with 10% fetal serum and 100 μl of penicillin–streptomycin in sterile six-well plates, treated with different concentrations of the compound, and incubated at 26°C. Also, 5 μl of each sample was taken daily, by triplicate and the respective controls. Viability was determined by counting the cells stained with Trypan blue.

ACKNOWLEDGMENTS

We thank the Instituto de Investigaciones Farmacéuticas (IIF) and Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela (CDCH-UCV) (grants IIF.01–2014, PG. 09–8819-2013/2), and the France–Venezuela PCP program No. 2013000438, as well as the University of Bordeaux, the Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation and the Centre National de la Recherche Scientifique (CNRS) for financial support. This study has benefited from the analytical facilities and expertise of the CESAMO platform at the University of Bordeaux and of the CNRS-UMS 3033 at the European Institute of Chemistry and Biology.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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How to cite this article: Rodríguez M, Gutiérrez J, Domínguez J, et al. Synthesis and leishmanicidal evaluation of sulfanyl- and sulfonyl-tethered functionalized benzoate derivatives featuring a nitroimidazole moiety. *Arch Pharm.* 2020;e2000002. https://doi.org/10.1002/ardp.202000002