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Original article

Lowering of 5-nitroimidazole's mutagenicity: Towards optimal antiparasitic pharmacophore

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Abstract

To improve the antiparasitic pharmacophore, 20 5-nitroimidazoles bearing an arylsulfonylmethyl group were prepared from commercial imidazoles. The antiparasitic activity of these molecules was assessed against *Trichomonas vaginalis*, the *in vitro* cytotoxicity was evaluated on human monocytes and the mutagenicity was determined by the *Salmonella* mutagenicity assay. All IC_{50} on *T. vaginalis* were below the one of metronidazole. The determination of the specificity indexes (SIs), defined as the ratios of the cytotoxic activity and the antitrichomonas activity, indicated that 11 derivatives had a SI over the one of metronidazole. Molecules, bearing an additional methyl group on the 2-position, showed a lower mutagenicity than metronidazole. Moreover, three derivatives were characterized by a low mutagenicity and an efficient antitrichomonas activity.

Keywords: 5-Nitroimidazoles; Arylsulfonylmethyl group; Antiparasitic activity; Mutagenicity

1. Introduction

5-Nitroimidazoles are a well-established group of pharmaceutical drugs. These chemotherapeutic agents inhibit the growth of both anaerobic bacteria and some anaerobic protozoa [1]. Nowadays, among the nitroimidazole family, the leader drug is metronidazole and it uses extensively for the treatment of both infections caused by protozoa such as *Trichomonas vaginalis*, *Entamœba histolytica*, *Giardia intestinalis* and infections induced by anaerobic bacteria. However, the 5-nitroimidazoles have been found to possess a high mutagenic activity in prokaryotic microorganisms. Although a few hypotheses have been published, active research in this area has not led to a comprehensive mechanism for the mutagenic and therapeutic activities of these compounds in eukaryotic cells yet. A body of indirect evidence suggests that the nitro group reduction, directly involved in the mechanism of action of the 5-nitroimidazoles is also responsible for the expression of mutagenicity and drug residue formation in mammalian cells [2]. Effectively, the metabolic way of the 5-nitroimidazoles (inhibition of the ferredoxine reductase) has been showed to produce free radicals and cause DNA damage [3]. Furthermore, it has been assumed, that the separation of antiprotozoal and genotoxic activities is not feasible, both of theses properties being mediated through a common metabolic intermediate. A nitroimidazole possessing good pharmacological activities with no mutagenicity would, therefore, be of great interest not only from a safety point of view but would also provide a basis for further investigations of the mode of action and mechanism of expression of mutagenicity. Moreover, emergence of metronidazole-resistant *T. vaginalis* results in

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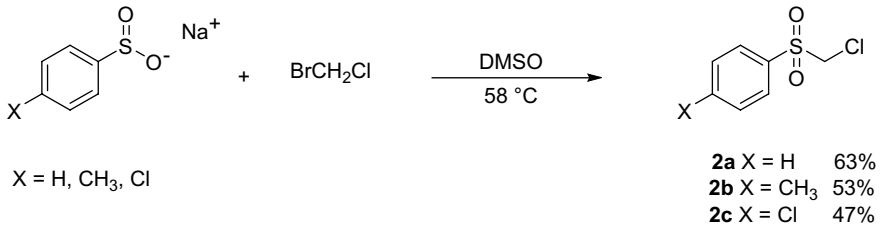


Scheme 1.

decreased success of current therapies [4]. These refractory cases are usually treated with higher doses of metronidazole, which leads to an increase in the occurrence of side effects. So, alternative curative therapies are needed.

Our previous study was based on the evaluation of mutagenic and the genotoxic activities of 48 imidazoles derived from dimetridazole and metronidazole [5]. Although substituents at the 1- and 2-positions were found to modulate these activities, all the tested compounds induced mutagenicity in the *Salmonella* mutagenicity assay and genotoxicity as assessed by the SOS chromotest. In order to enlarge the pharmacological potential of the nitroimidazole ring, we have decided, in this present study, to modulate the 4-position of easily obtainable 1-methyl-5-nitro-1*H*-imidazole [6] or commercial dimetridazole, metronidazole and secnidazole. We focused our work on the synthesis of original compounds from 1,2-dialkyl-5-nitro-1*H*-imidazoles or 1-methyl-5-nitro-1*H*-imidazole bearing an arylsulfonylmethyl group in the 4-position by vicarious nucleophilic substitution of hydrogen (VNS) of Makosza followed by the evaluations of antitrichomonas activity (TV87), THP1 cell toxicity and mutagenicity (*Salmonella* mutagenicity assay). The arylsulfonylmethyl group in the 4-position was never investigated in these areas.

2. Chemistry

Chloromethylaryl sulfones (**2a–c**) were prepared by alkylation of the corresponding sulfinate salts with bromochloromethane in DMSO at 58 °C (Scheme 1) [7].

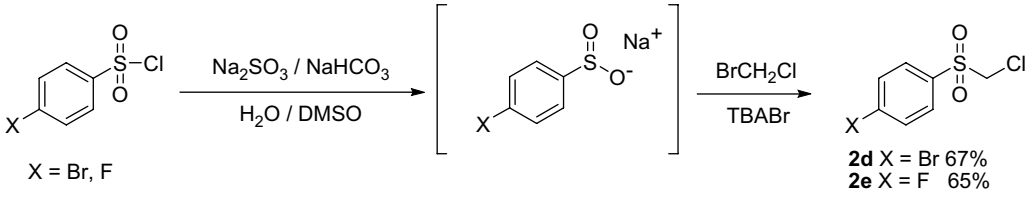
Non-commercially available sulfinate salts could be prepared from sodium sulfite-mediated reduction of the corresponding sulfonyl chlorides in water (Scheme 2). In order to optimize the procedure described by Antane et al. [8], DMSO was added in the aqueous reaction mixture to facilitate the solubilizations of the 4-bromobenzene-1-sulfonyl chloride and 4-fluorobenzene-1-sulfonyl chloride. The reduced sulfonyl chlorides were treated with a phase transfer catalyst (PTC) and an excess of bromochloromethane at 75 °C during 20 h. After work-up and recrystallization, the desired 1-bromo-4-(chloromethylsulfonyl)-benzene (**2d**) and 1-fluoro-4-(chloromethylsulfonyl)benzene (**2e**) were obtained, respectively, in 67% and 65% yield.

The 1,2-dialkyl-4-arylsulfonylmethyl-5-nitro-1*H*-imidazoles and 4-arylsulfonylmethyl-1-methyl-5-nitro-1*H*-imidazoles (Table 1) were prepared according to our published procedure by vicarious nucleophilic substitution of hydrogen from chloromethylaryl sulfones with 1-methyl-5-nitro-1*H*-imidazole (**3**) (easily obtainable) [6] or commercial dimetridazole (**4**), metronidazole (**5**), secnidazole (**6**) (Scheme 3) [9].

3. Results and discussion

The activities of 20 nitroimidazole derivatives were compared with that of precursors, 1-methyl-5-nitro-1*H*-imidazole (**3**), dimetridazole (**4**), metronidazole (**5**) and secnidazole (**6**) in four series of molecules. For the purpose of screening these new compounds, we have directly compared their IC_{50} and SI with those of precursors (Table 2). The 24-nitroimidazole derivatives showed antitrichomonas activity with IC_{50} ranging from 0.044 μM (**9c**) to 2.746 μM (**5**). The reference standards dimetridazole, metronidazole and secnidazole exhibited IC_{50} of 0.708 μM, 2.746 μM and 0.810 μM, respectively. In the 1-methyl-5-nitro-1*H*-imidazole and metronidazole series of molecules, all compounds were more effective against *T. vaginalis* than precursors.

Among the 24 tested molecules, 21 showed stronger activity against *T. vaginalis* than metronidazole and two had similar activity. The highest activity was obtained with 2-[4-(4-chlorophenylsulfonylmethyl)-2-methyl-5-nitro-1*H*-imidazol-1yl] ethanol (**9c**) with a specificity index 13136. Fifteen molecules did not have any toxicity on THP1 cells. Among remaining molecules, the cell toxicity (IC_{50}) ranged from 59 μM to 578 μM. The present study demonstrated the positive influence of the phenylsulfonylmethyl moiety in 4-position. The influence of the sulfonyl group was already observed in 2-substituted-5-nitroimidazole series [10]. The compounds



Scheme 2.

Table 1
Preparation of 4-arylsulfonylmethyl-1,2-dialkyl-5-nitro-1*H*-imidazoles and 4-arylsulfonylmethyl-1-methyl-5-nitro-1*H*-imidazoles

Entry	Sulfone derivatives			Compound number	Yield (%)
	X	R_1	R_2		
1	H	CH_3	H	**7a**	55
2	CH_3	CH_3	H	**7b**	29
3	Cl	CH_3	H	**7c**	42
4	Br	CH_3	H	**7d**	57
5	F	CH_3	H	**7e**	54
6	H	CH_3	CH_3	**8a**	28
7	CH_3	CH_3	CH_3	**8b**	20
8	Cl	CH_3	CH_3	**8c**	49
9	Br	CH_3	CH_3	**8d**	53
10	F	CH_3	CH_3	**8e**	40
11	H	$(CH_2)_2OH$	CH_3	**9a**	24
12	CH_3	$(CH_2)_2OH$	CH_3	**9b**	24
13	Cl	$(CH_2)_2OH$	CH_3	**9c**	41
14	Br	$(CH_2)_2OH$	CH_3	**9d**	51
15	F	$(CH_2)_2OH$	CH_3	**9e**	20
16	H	$CH_2CH(CH_3)OH$	CH_3	**10a**	37
17	CH_3	$CH_2CH(CH_3)OH$	CH_3	**10b**	37
18	Cl	$CH_2CH(CH_3)OH$	CH_3	**10c**	51
19	Br	$CH_2CH(CH_3)OH$	CH_3	**10d**	69
20	F	$CH_2CH(CH_3)OH$	CH_3	**10e**	17

Conditions: KOH (5 eq.), 1,2-dialkyl-5-nitro-1*H*-imidazole (1 eq.) or 1-methyl-5-nitro-1*H*-imidazole (1 eq.), chloromethylaryl sulfone (1 eq.), DMSO.

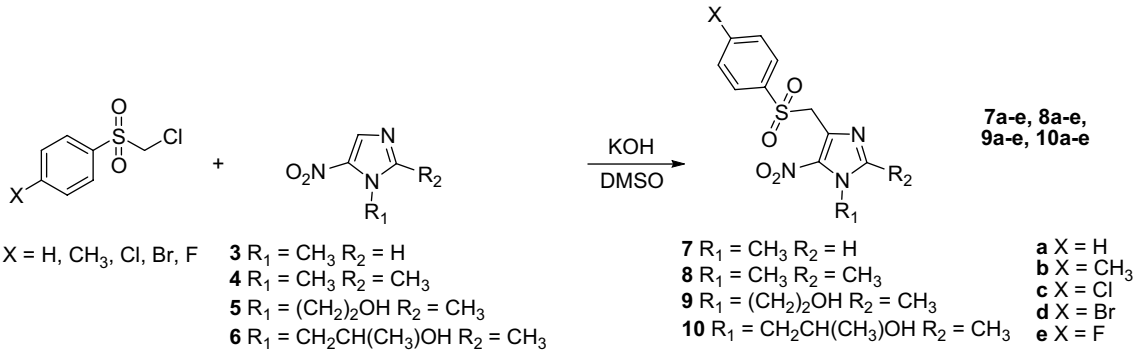
have side chains that are more hydrophobic than those of metronidazole, and this may reflect the increased activities because the site of metronidazole activation in *Trichomonas* is the membrane-localized electron transport pathway. The mechanism of action of metronidazole in Mz-susceptible parasites requires reduction of the critical nitro group to toxic radicals by ferrodoxin, which is reduced by the membrane-localized enzyme pyruvate: ferrodoxin oxido-reductase (PFOR) [11]. However, compounds **8e** and **10e** were less effective than their precursors dimetridazole and secnidazole, respectively, and **9e** exhibited the lowest antitrichomonas activity for the metronidazole series of molecules, showing that the substitution on the phenylsulfonyl group by a fluorine atom resulted in a decrease of $IC_{50\ T.\ vaginalis}$. These encouraging results had to be completed with genotoxicity evaluation by *Salmonella* mutagenicity assays.

4. The *Salmonella* mutagenicity assay

The 24-nitroimidazole derivatives were mutagenic on TA100 and YG1042. The calculated mutagenic powers (MP) are included in Table 3. The MP ranged from 0.56 rev/nmol (**9a**) to 44.03 rev/nmol (**7c**) for TA100 and from 1.29 rev/nmol (**10d**) to 222.71 rev/nmol (**4**) for YG1042. The mutagenicity could be linked to the NO_2 reduction by nitroreductase activity for 19/24 molecules as assessed by comparison of the MP_{TA100} and the MP_{YG1042}. Addition of the phenylsulfonylmethyl, (4-methylphenylsulfonyl)methyl, (4-chlorophenyl sulfonyl)methyl and (4-bromophenylsulfonyl)methyl on the 4-position resulted in significant decreases of MP_{TA100} for the **4**–**6** series of molecules. These additions either did not modify or increased the MP_{TA100} for the **3** series. These results underlined the critical role of substituents in the 2-position as the only difference between the four series was the methyl group in the 2-position of the nitroimidazole ring.

Different correlations between MP and IC_{50} were attempted. The only statistical significant correlation was found between MP_{TA100} and $IC_{50\ T.\ vaginalis}$ (Fig. 1). However, this correlation was limited to 21 molecules. The molecules **9b**, **9c** and **10b**, characterized by a low MP_{TA100} and an efficient $IC_{50\ T.\ vaginalis}$, were excluded from the non-linear regression.

All the tested molecules were found to be mutagenic in the *Salmonella* mutagenicity assay using the most sensitive strain TA100 [12,13]. It has been shown that mono- or bicyclic nitroaromatics revert the base substitution strains (such as TA100) more efficiently and nitro compounds with three or more aromatic rings induce mainly mutations in frameshift tester strains (such as TA98). Comparison of MP between TA100 and YG1042 showed that the nitro group reduction played a key role in the mutagenic activity for 19 molecules (Table 3). For the five remaining molecules, the nitroreduction could not be statistically demonstrated although the MP_{YG1042} were always above the MP_{TA100}. The nitroaromatics are generally reduced to hydroxylamines that are subsequently converted to electrophilic nitrogen species. The nitronium ions react with the nucleophilic sites of cellular macromolecules (such as DNA). Furthermore, the methyl group at the 2-position and the arylsulfonylmethyl group at the 4-position modulated the nitroreduction at the 5-position. We have shown that the



Scheme 3.

Table 2
Antitrichomonas activity and cell toxicity

Mol.	IC_{50} *Trichomonas* (μM)	$IC_{50\ THP\ 1}$ (μM)	$SI_{Trichomonas}$
3	0.629	>1966[a]	>3125[a]
7a	0.199	>888[a]	>4462[a]
7b	0.155	>846[a]	>5458[a]
7c	0.158	174	1101
7d	0.394	>694[a]	>1761[a]
7e	0.334	>835[a]	>2500[a]
4	0.708	>1771[a]	>2501[a]
8a	0.240	>846[a]	>3525[a]
8b	0.355	242	684
8c	0.918	68	74
8d	0.660	326	493
8e	2.553	>797[a]	>312[a]
5	2.746	>1460[a]	>531[a]
9a	1.536	>768[a]	>500[a]
9b	0.067	59	880
9c	0.044	578	13136
9d	1.635	124	75
9e	1.747	>728[a]	>416[a]
6	0.810	>1350[a]	>1666[a]
10a	0.615	418	679
10b	0.124	>707[a]	>5701[a]
10c	0.732	>668[a]	>912[a]
10d	0.418	126	301
10e	2.378	>699[a]	>293[a]

Mol: molecules and IC_{50}: mean of three independent experiments.
[a] No toxicity at the highest tested concentration related to the solubility of the molecule.

replacement of the methyl group at the 2-position by a lactam group could double the mutagenicity of metronidazole [5]. In the present study, the methyl group at the 2-position combined with the arylsulfonylmethyl group at the 4-position lowered the MP_{TA100} of dimetridazole, metronidazole and secnidazole series. The arylsulfonylmethyl group alone did not modify the MP_{TA100} of the 1-methyl-5-nitro-1*H*-imidazole series.

In eukaryotic cells, 5-nitroimidazoles are primarily metabolized by the oxidative P450 enzymes (IA, IIB, IIC and IIIA) to generate hydroxyl and acetic acid metabolites. The hydroxyl metabolite has been shown to be more mutagenic than the original compound [14]. Another hypothesis is the production of radical oxygen species through the futile cycle of incomplete nitroreduction in mammalian cells [15].

In conclusion, a good correlation was demonstrated between the antiprotozoal activity and the mutagenicity for 21 molecules which reflects their ability to damage DNA through covalent binding and induction of DNA breaks [16]. However, three molecules were excluded from this correlation. They were characterized by a low mutagenicity and high antiparasitic activity. Although the mechanism of action explaining their biological activity remains to be elucidated, these newly synthesized compounds allow the disconnection between mutagenicity and biological activity for the first time. This approach offers the possibility of synthesizing new and potentially safer 5-nitroimidazoles.

T. vaginalis tests are not standardized [17], but under anaerobic conditions, they are well documented [10]. Nevertheless, in regard to other studies [4,18] which explain that metronidazole therapeutic use is done in microaerophilic conditions, it could appear interesting to continue our work by testing aerobic conditions. Furthermore, it could be valuable to use not only a metronidazole sensitive reference strain, but also a clinical strain with elevated minimum lethal concentration (MLC). These complementary studies could allow the selection of new interesting molecules.

5. Experimental section

5.1. Synthetic methodology

Yields refer to purified products and are not optimized. Melting points were determined on Büchi melting point B-540 apparatus and are uncorrected. NMR experiments were recorded on a Bruker Avance 200 spectrometer (operating at 200 MHz for ^1H and 50 MHz for ^{13}C). ^1H and ^{13}C NMR shifts (δ) were reported in parts per millions (ppm) with respect to $CDCl_3$ 7.26 ppm for ^1H and 77.00 ppm for ^{13}C and DMSO-d_6 2.50 for ^1H and 39.70 ppm for ^{13}C. Multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Coupling constant (J) are in Hertz (Hz). Elemental analyses were performed on a Thermo Finnigan EA 1112 at the Spectropole of the University of Aix-Marseille III. Thin-layer chromatography (TLC) was performed on 5 cm × 10 cm aluminium plates coated with silica gel 60F-254 (Merck) in an appropriate solvent. Column chromatography was performed over silica gel 60 (70–230 mesh). Procedures for the synthesis of compounds **2a–d**, **7a–d**, **8a–d**, **9a–d** and **10a–d** have been previously described [8]. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.

5.2. 1-Fluoro-4-(chloromethylsulfonyl)benzene (**2e**) [19]

A stirred mixture of 4-fluorobenzene-1-sulfonyl chloride (3.00 g, 15.4 mmol), sodium sulfite (3.30 g, 26.2 mmol), and sodium bicarbonate (2.20 g, 26.3 mmol) in water (20 mL) was heated at 80 °C for 6 h. The crude sodium sulfinate solution was allowed to cool for 30 min, and then treated with bromochloromethane (15 mL, 230 mmol) and tetra-*N*-butylammonium bromide (1.00 g, 3.10 mmol). The resultant mixture was heated at 75 °C for 10 h and poured into an ice–water mixture. The residue was extracted with chloroform (3 × 50 mL). The combined organic layers were washed with water (3 × 30 mL), brine, dried over $MgSO_4$ and removed under reduced pressure. The crude solid residue was purified by column chromatography eluting with chloroform/petroleum ether (7:3) and recrystallized from propan-2-ol giving a white solid (2.07 g, 65%): mp 88 °C (propan-2-ol) (Ref. [19] mp 88–89 °C); ^1H NMR ($CDCl_3$, 200 MHz) δ 4.53 (s, 2H, CH_2), 7.25–7.34 (m, 2H, CH × 2), 7.97–8.04 (m, 2H, CH × 2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 58.5 (CH_2), 116.8 (d, $J = 23$ Hz, CH × 2), 131.6 (C), 132.4 (d, $J = 10$ Hz, CH × 2), 166.6 (d, $J = 258$ Hz, C). Anal. $C_7H_6ClFO_2S$: (C, H, S).

Table 3
Mutagenic activities (MA) and mutagenic powers (MP) of the 24-nitroimidazole derivatives

Mol.	Mutagenic activities												
	TA100						YG1042						
	r^2	P	P_E	MA	MP	Ratio	r^2	P	P_E	MA	MP	Ratio	Comment
3	0.99	$<10^{-5}$	0.08	121.79	**15.48**	*1.85*	0.98	$<10^{-5}$	0.05	1551.06	**197.14**	2.34	NO_2 (12.7)
7a	0.99	$<10^{-5}$	0.05	50.91	**14.32**	*1.71*	0.99	$<10^{-5}$	0.08	176.64	**49.69**	0.59	NO_2 (3.5)
7b	0.99	$<10^{-5}$	0.52	69.71	**20.59**	*2.46*	0.99	$<10^{-5}$	0.99	185.37	**54.74**	0.65	NO_2 (2.7)
7c	0.99	$<10^{-5}$	0.13	139.44	**44.03**	*5.27*	0.96	$<10^{-5}$	0.23	167.38	**52.85**	0.63	No NO_2 (1.2)
7d	0.99	$<10^{-5}$	0.94	41.41	**14.92**	*1.78*	0.98	$<10^{-5}$	0.32	164.42	**59.22**	0.70	NO_2 (4.0)
7e	0.99	$<10^{-5}$	0.53	35.17	**10.53**	*1.26*	0.99	$<10^{-5}$	0.85	157.34	**47.09**	0.56	NO_2 (4.5)
4	0.98	$<10^{-5}$	0.54	153.80	**21.71**	*2.60*	0.99	$<10^{-5}$	0.53	1578.04	**222.71**	2.64	NO_2 (10.3)
8a	0.99	$<10^{-5}$	0.05	27.91	**8.24**	*0.99*	0.98	$<10^{-5}$	0.62	74.18	**21.91**	0.26	NO_2 (2.7)
8b	0.98	$<10^{-5}$	0.98	15.03	**4.65**	*0.56*	0.99	$<10^{-5}$	0.09	54.97	**17.00**	0.20	NO_2 (3.7)
8c	0.96	$<10^{-5}$	0.16	7.15	**2.36**	*0.28*	0.98	$<10^{-5}$	0.74	25.21	**8.31**	0.10	NO_2 (3.5)
8d	0.98	$<10^{-5}$	0.06	17.61	**6.59**	*0.79*	0.98	$<10^{-5}$	0.06	32.91	**12.32**	0.15	NO_2 (1.9)
8e	0.97	$<10^{-5}$	0.57	4.72	**1.48**	*0.18*	0.97	$<10^{-5}$	0.07	24.26	**7.60**	0.09	NO_2 (5.1)
5	0.96	$<10^{-5}$	0.09	48.85	**8.36**	***1.00***	0.95	$<10^{-5}$	0.46	469.79	**84.34**	**1.00**	NO_2 (10.1)
9a	0.74	$2E^{-3}$	0.82	1.72	**0.56**	*0.07*	0.95	$<10^{-5}$	0.92	8.36	**2.72**	**0.03**	NO_2 (4.9)
9b	0.77	$7E^{-5}$	0.20	4.62	**1.57**	*0.19*	0.89	$<10^{-5}$	0.29	6.14	**2.08**	**0.02**	No NO_2 (1.3)
9c	0.94	$<10^{-5}$	0.14	6.22	**2.24**	*0.27*	0.96	$<10^{-5}$	0.54	14.73	**5.30**	**0.06**	NO_2 (2.4)
9d	0.84	$1E^{-5}$	0.74	2.73	**1.10**	*0.13*	0.95	$<10^{-5}$	0.06	9.123	**3.73**	**0.04**	NO_2 (3.4)
9e	0.80	$1E^{-5}$	0.47	1.91	**0.66**	*0.08*	0.90	$<10^{-5}$	0.67	10.09	**3.46**	**0.04**	NO_2 (5.3)
6	0.97	$<10^{-5}$	0.53	25.74	**4.77**	*0.57*	0.81	$2E^{-5}$	0.24	128.44	**23.78**	**0.28**	NO_2 (5.0)
10a	0.69	$5E^{-4}$	0.15	2.58	**0.88**	*0.10*	0.68	$6E^{-4}$	0.50	10.76	**3.65**	**0.04**	NO_2 (4.2)
10b	0.87	$<10^{-5}$	0.55	2.07	**0.73**	*0.09*	0.81	$2E^{-5}$	0.06	6.76	**2.39**	**0.03**	NO_2 (3.3)
10c	0.93	$<10^{-5}$	0.28	2.18	**0.81**	*0.10*	0.58	$4E^{-3}$	0.13	3.72	**1.39**	**0.02**	No NO_2 (1.7)
10d	0.97	$<10^{-5}$	0.75	3.36	**1.41**	*0.17*	0.67	$7E^{-6}$	0.66	3.08	**1.29**	**0.02**	No NO_2 (0.9)

Mol.: molecule; Mod.: mathematical model selected for the regression (Mar-1, Mar-2, Mar-3 and Mar-4 take into account the mutagenic and toxic effects); r^2: square of the correlation coefficient; P: model probability (should be <0.05); P_E: error probability of the model (should be >0.05); MA: mutagenic activities (number of revertants/μg); MP: mutagenic powers (number of revertants/nmol); Ratio: ratio of the MP of the molecule and the MP of metronidazole for each strain; Comment NO_2: mutagenicity that is linked to the reduction of the NO_2 substituent by comparison of the MP of TA100 and YG1042; and Comment no NO_2: mutagenicity that is not linked to the reduction of the NO_2 substituent by comparison of the MP of TA100 and YG1042. In brackets the value of the ratio MPYG1042/MPTA100 is indicated. Ratios ≥ 2 reflected MP linked to NO_2 group reduction.

*5.3. General procedure for the synthesis of compounds **7e–10e***

To a stirred suspension of powdered KOH (21.90 mmol) in DMSO (20 mL) were added a solution of corresponding 5-nitroimidazole (4.40 mmol) and 1-fluoro-4-(chloromethylsulfonyl)benzene (**2e**) (4.40 mmol) in DMSO (15 mL) and the reaction was carried out at room temperature for 15 min. The mixture was poured into an ice–water mixture, acidified with 1 N HCl until pH = 1–2 and extracted with chloroform (3 × 50 mL). The combined organic layers were washed with water (3 × 30 mL), dried over $MgSO_4$ and removed under reduced pressure. After purification by chromatography over silica gel, washing with acetone and recrystallization from appropriate solvent, corresponding 4-arylsulfonylmethyl-1-alkyl-5-nitro-1*H*-imidazole (**7e–10e**) was obtained.

*5.4. 4-(4-Fluorobenzenesulfonylmethyl)-1-methyl-5-nitro-1H-imidazole (**7e**)*

Following general procedure, compound **7e** was purified by column chromatography eluting with ethyl acetate and recrystallized in butan-1-ol giving a white solid (54%): mp 206 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.97 (s, 3H, CH_3), 4.87 (s, 2H, CH_2), 7.16–7.25 (m, 2H, CH × 2), 7.51 (s, 1H, CH), 7.82–7.89 (m, 2H, CH × 2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 36.1 (CH_3), 56.1 (CH_2), 116.5 (d, $J = 23$ Hz, CH × 2), 131.3 (d,

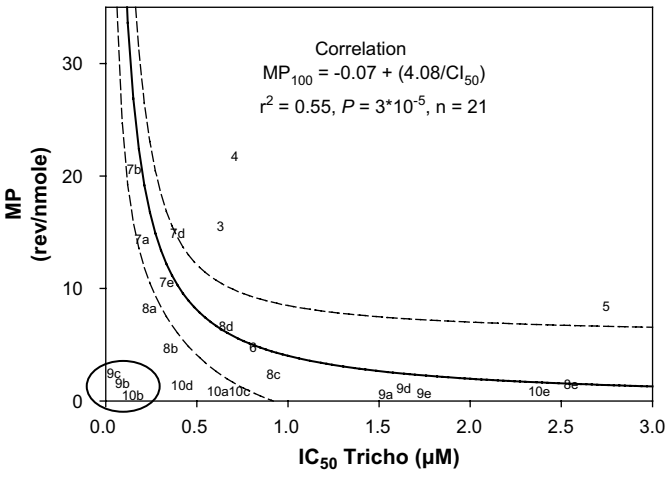


Fig. 1. Correlation between the mutagenic powers determined with TA100 and the IC_{50} on *Trichomonas vaginalis*. Circle: the molecules **9b**, **9c** and **10b** were excluded from the non-linear regression analysis. The dashed lines indicate the 95% confidence levels.

$J = 10$ Hz, CH × 2), 133.5 (C), 135.1 (C), 139.9 (CH), 142.8 (C), 166.1 (d, $J = 257$ Hz, C). Anal. $C_{11}H_{10}FN_3O_4S$: (C, H, N, S).

5.5. 4-(4-Fluorobenzenesulfonylmethyl)-1,2-dimethyl-5-nitro-1H-imidazole (8e)

Following general procedure, compound **8e** was purified by column chromatography eluting with ethyl acetate and recrystallized in propan-1-ol giving a white solid (40%): mp 183 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.48 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 4.84 (s, 2H, CH_2), 7.17–7.26 (m, 2H, CH × 2), 7.85–7.92 (m, 2H, CH × 2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.1 (CH_3), 34.1 (CH_3), 56.1 (CH_2), 116.5 (d, $J = 23$ Hz, CH × 2), 131.3 (d, $J = 10$ Hz, CH × 2), 132.5 (C), 135.2 (C), 137.1 (C), 148.7 (C), 166.0 (d, $J = 257$ Hz, C). Anal. $C_{12}H_{12}FN_3O_4S$: (C, H, N, S).

5.6. 2-[4-(4-Fluorobenzenesulfonylmethyl)-2-methyl-5-nitroimidazol-1-yl]ethanol (**9e**)

Following general procedure, compound **9e** was purified by column chromatography eluting with chloroform/acetone (6:4) and recrystallized in propan-1-ol giving a beige solid (20%): mp 189 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.41 (s, 3H, CH_3), 3.57–3.65 (m, 2H, CH_2), 4.31 (t, $J = 5.2$ Hz, 2H, CH_2), 4.91 (s, 2H, CH_2), 5.04 (t, $J = 5.3$ Hz, 1H, OH), 7.39–7.48 (m, 2H, CH × 2); 7.72–7.79 (m, 2H, CH × 2); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 14.1 (CH_3), 48.9 (CH_2), 55.7 (CH_2), 59.8 (CH_2), 116.6 (d, $J = 23$ Hz, CH × 2), 131.4 (d, $J = 10$ Hz, CH × 2), 133.0 (C), 135.1 (C), 136.6 (C), 150.1 (C), 166.4 (d, $J = 253$ Hz, C). Anal. $C_{13}H_{14}FN_3O_4S$: (C, H, N, S).

5.7. 1-[4-(4-Fluorobenzenesulfonylmethyl)-2-methyl-5-nitroimidazol-1-yl]propan-2-ol (**10e**)

Following general procedure, compound **10e** was purified by column chromatography eluting with chloroform/acetone (7:3), washed with petroleum ether and recrystallized in propan-2-ol giving a yellow solid (17%): mp 128 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30 (d, $J = 5.6$ Hz, 3H, CH_3), 1.87 (broad s, 1H, OH), 2.52 (s, 3H, CH_3), 3.96–4.46 (m, 3H, CH_2 and CH), 4.83 (s, 2H, CH_2), 715–7.88 (m, 4H, CH × 4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.7 (CH_3), 21.3 (CH_3), 53.4 (CH_2), 56.3 (CH_2), 67.3 (CH), 116.4 (d, $J = 23$ Hz, CH × 2), 131.4 (d, $J = 10$ Hz, CH × 2), 133.1 (C), 135.1 (C), 136.6 (C), 150.2 (C), 166.0 (d, $J = 257$ Hz, C). Anal. $C_{14}H_{16}FN_3O_5S$: (C, H, N, S).

6. Antitrichomonas activity

T. vaginalis parasites (TVR87) were grown in *Trichomonas* medium TM 161 medium (Oxoid) supplemented with 10% heat-inactivated horse serum (Eurobio, Paris, France). Parasites to be used in drug susceptibility assays were grown for one day following regular subculturing and were in the log phase of growth.

In vitro drug susceptibility assays were carried out using standard procedure [20]. Test compounds were dissolved in DMSO (analytical grade, Sigma). The compounds were tested in the concentration range of 0.01–5 μg/mL.

Working concentrations of 5×10^3 trophozoites/mL were selected for the assay. Triplicate culture of the *T. vaginalis* was incubated anaerobically at 37 °C in the presence of a range of concentration of nitroimidazole derivatives. A second culture containing DMSO (final concentration of 0.1%) served as the negative control. After a 48 h-incubation period, antitrichomonas activity was assessed by trypan blue staining to determine the number of viable cells. The IC values were defined as the concentration of drug inducing a mortality of 50% of parasites compared to the control ($IC_{50\ Trichomonas}$). They were calculated by non-linear regression analysis processed on dose–response curves, using the Table curve program (Jandel scientific).

7. Cells toxicity assays

In vitro toxicity of nitroimidazoles was assessed on human monocytes (THP1 cells) maintained at 37 °C in 6% CO_2, in a medium of RPMI (Eurobio, Paris, France) supplemented with 10% foetal calf serum (Eurobio, Paris, France), 25 mM of HEPES, 25 mM of $NaHCO_3$ (Gibco-BRL, Paisley, Scotland), and 1% of L-glutamine/penicillin–streptomycin mix. Cells were replicated every 7 days [21].

THP1 cell (10^5 cells/mL) were incubated with different concentrations of tested products dissolved in DMSO. A viability control free of drug and a control containing DMSO (final concentration of 0.5%) were performed in parallel. After a 72 h-incubation at 37 °C and 6% CO_2 in complete RPMI medium, cell growth and viability were measured by flow cytometry after staining the monocytes by 5 μL of propidium iodide (1 mg/mL) (Sigma) [22].

Antiproliferative activity was evaluated by counting the number of living cells on a 100 μL-sample. Inhibitory concentration 50 ($IC_{50\ human}$) was defined as the concentration of drug inducing a reduction of 50% in the THP1 cell proliferation compared to the control [23].

A specificity index (SI) corresponding to the ratio of toxicities on human cells and on parasite was calculated according to the following formula: $SI_{\ T.\ vaginalis} = IC_{50\ human}/IC_{50\ T.\ vaginalis}$.

8. The *Salmonella* mutagenicity assay

The *Salmonella* mutagenicity [24] test was carried out according to De Méo et al. [25]. *Salmonella typhimurium* strain TA100 was a generous gift of B.N. Ames (Berkeley, CA, USA). This strain has been shown to be the most sensitive to the mutagenic effect of nitroimidazole derivatives [5,26]. *S. typhimurium* YG1042 strain was obtained from T. Nohmi (National Institute of Hygienic Sciences, Tokyo, Japan). This strain is a derivative of TA100 and includes an additional

pYG233 plasmid [27]. It overproduces nitroreductase and *O*-acetyltransferase enzymes and is more sensitive than TA100 to nitroaromatics and aromatic amines. The tester strains were grown in Oxoid Nutrient Broth No. 2 with ampicillin (25 μg/mL) for TA100 and ampicillin (25 μg/mL) and kanamycine (25 μg/mL) for YG1042. After an overnight incubation, the following ingredients were added to 12×75 mm polystyrene tubes: (i) various volumes of tested molecules not exceeding 10 μL to avoid the potential bactericidal activity of the solvent control (DMSO); (ii) 0.1 mL of phosphate buffered saline (PBS, 0.15 M pH 7.4) and (iii) 0.1 mL of the strain culture. The mixtures were incubated at 37 °C for 60 min with rapid shaking. At the end of the contact period, 2-mL volumes of melted top agar containing 50 nM histidine and biotin were added to the tubes and the mixtures were immediately poured on Vogel–Bonner (VB) agar plates. After a 48-h incubation period, the revertant colonies were counted with a bacterial enumeration program (Spiral System Instrument Inc., Bethesda, MD, USA). For each molecule, triplicate plates of four consecutive doses and quadricate plates of the solvent control were assayed. Throughout this study, the number of spontaneous revertants averaged 144 ± 12/plate and 140 ± 19/plate for TA100 and YG1042, respectively. The dose–response relationships were calculated by non-linear regression analyses using four models [28] that integrated mutagenic and toxic responses. The maximal slopes, defined as the first derivative at the origin, were named mutagenic activities (MA) and were expressed as the numbers of revertants per μg. The mutagenic powers (MP) were expressed as the numbers of revertants per nmol (rev/nmol).

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