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Original article

Synthesis and in vitro trichomonicidal, giardicidal and amebicidal activity of *N*-acetamide(sulfonamide)-2-methyl-4-nitro-1*H*-imidazoles☆

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ABSTRACT

Two new series of imidazole derivatives (acetamides: **1**–**8** and sulfonamides: **9**–**15**) were synthesized using a short synthetic route. Compound **1** as well as the intermediate **16g** were characterized by X-ray crystallography. Imidazole derivatives **1**–**15** were tested in vitro against three unicellular parasites (*Giardia intestinalis*, *Trichomonas vaginalis* and *Entamoeba histolytica*) in comparison with benznidazole (Bzn) and metronidazole. Compound **1** [*N*-benzyl-2-(2-methyl-4-nitro-1*H*-imidazol-1-yl)acetamide] was 2 times more active than Bzn against *T. vaginalis* and *G. intestinalis* and it was as active as Bzn against *E. histolytica*. Sulfonamides showed selective toxicity against *E. histolytica* over the other parasites. Toxicity assay showed that all compounds are non-cytotoxic against MDCK cell line. The results revealed that compounds **1**–**15** have antiparasitic bioactivity in the micromolar range against the parasites tested, and could be considered as benznidazole bioisosteres.

1. Introduction

Parasitic diseases are still major problem in developing countries, affecting hundreds of millions people around the world. Since parasites are eukaryotic, they share many common features with their mammalian host, making the development of effective and selective drugs a hard task. Despite the great effort that has been done in the discovery of unique targets that afford selectivity, many of the drugs used today have serious side effects [1].

Some heterocyclic nucleus used mainly as antiparasitic drugs are: benzimidazoles-2,5(6)-substituted and 2- or 5-nitroimidazoles [2,3]. Nitroimidazoles are attractive and important reagents in organic synthesis [4–6]. The 5-nitroimidazole core, found particularly in metronidazole, the most commonly antiparasitic drug, is accepted as drug of choice for anti-infectious chemotherapy against anaerobic bacteria and parasites and also for the radiosensitization of hypoxic tumors [7].

Benznidazole [Bnz, Radanil®], a 2-nitroimidazole derivative, is an important drug for Chaga's Disease, and has been used in other parasitic diseases [8].

The Bnz mode of action is related to reductive metabolism, it functions as prodrug and must be activated by an NADH-dependent, mitochondrially localized, bacterial-like, type I nitroreductase [9].

It is well-known that the imidazole pharmacophore is an important structural core in medicinal chemistry that shows a broad spectrum of pharmacological activities. Several compounds containing the nitroimidazole scaffold have been used as antiparasitic [10,11], antimycobacterial [12], antibacterial [13,14], antitumoral [15], antioxidant, antifungal, [16] and calcium channel antagonist agents [17].

As a part of our search for basic information about the structural requirements for new antiparasitic activities of an old drug (Benznidazole), we have synthesized a series of novel *N*-acetamide (sulfonamide)-2-methyl-4-nitro-1*H*-imidazoles as analogues of this drug. The in vitro antiparasitic activity of these compounds on intestinal unicellular parasites (*Giardia intestinalis* and *Entamoeba histolytica*), and a urogenital tract parasite (*Trichomonas vaginalis*) is also reported in this paper.

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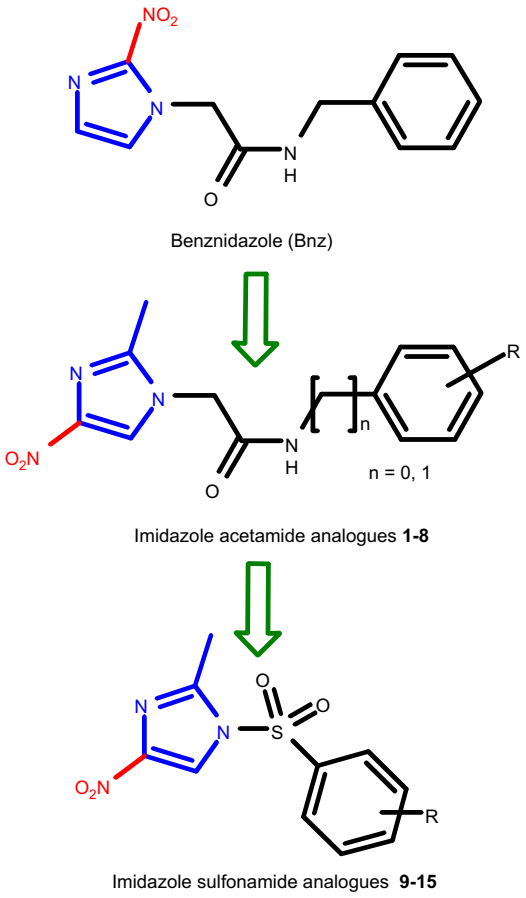
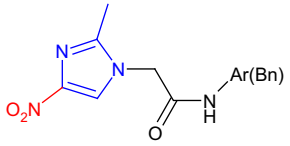
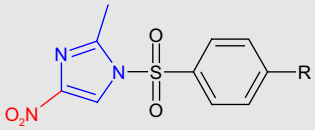


Fig. 1. Drug design of title compounds.

Table 1
Predictive values of antiparasitic effect calculated with PASS for compounds **1**–**15**.



Compound	Ar	Antiparasitic effect (formerly antiprotozoal)	
		Pa	*Pi*
1	Benzyl	0.651	0.006
2	Phenyl	0.679	0.006
3	4-Fluorophenyl	0.645	0.007
4	4-Chlorophenyl	0.661	0.006
5	4-Cyanophenyl	0.629	0.007
6	4-Nitrophenyl	0.685	0.006
7	2,6-Dichlorophenyl	0.657	0.006
8	3-(Trifluoromethyl)phenyl	0.639	0.006



Compound	Ar	Antiparasitic effect (formerly antiprotozoal)	
		Pa	*Pi*
9	$NHCOCH_3$	0.563	0.012
10	H	0.617	0.006
11	CH_3	0.591	0.008
12	Cl	0.599	0.008
13	F	0.571	0.011
14	NO_2	0.616	0.007
15	OCH_3	0.574	0.010

Pa = Probability of activity, *Pi* = Probability of inactivity.

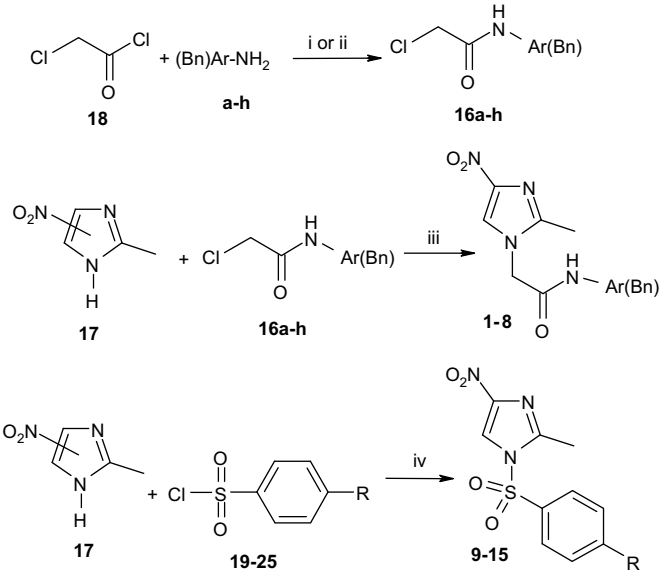
2. Results and discussion

2.1. Drug design of derivatives *1*–*15*

In our ongoing research on imidazole derivatives we have designed the compounds **1**–**8**, on the basis of the structure of antiparasitic drug benznidazole (Bzn, Fig. 1). In order to find new antiparasitic bioactivities of this old drug (besides antichagasic activity), we decided to exchange the nitro group from position 2 to position 4, and add an extra-methyl group at position 2, as in metronidazole core. The resulting 2-methyl-4-nitroimidazole scaffold was kept and it was hybridized with sulfonamide pharmacophore as shown in compounds **9**–**15**, in order to improve the antiparasitic activity. The design was also based on the biological activity predictions made by the computer software PASS® (Prediction of activity spectra for substances). This software illustrates the predicted activity spectrum of a compound as probable activity (*Pa*) and probable inactivity (*Pi*) with the accuracy of prediction reported to be as high as 85% [18–21].

2.1.1. In silico PASS® screening

An approach to computer-aided prediction of the general biological activity spectra on the basis of chemical structure of a compound has been developed and marketed as computer program PASS® [18,19,21]. This software is based on a robust analysis of structure–activity relationships in a heterogeneous training set [20] including many thousands of compounds from different chemical series. Using PASS predictions the number of



Scheme 1. Reagents: (i) Et_3N, CH_2Cl_2; (ii) $NaHCO_3$, Acetone; (iii) K_2CO_3, CH_3CN; (iv) KOH, DMAP (cat.), triethylamine, CH_2Cl_2.

Table 2
Comparison of yields of synthesized 2-chloro-***N***-(aryl)acetamides **16a–h** obtained through two methodologies.

Compd	Ar	Conditions		Reaction time (h)
		Et_3N/CH_2Cl_2 % yield	$NaHCO_3$/acetone % yield	
16a	Bn	96.3	–	24
16b	Ph	92.3	89.3	24
16c	4-F-Ph	71.1	89.4	48
16d	4-Cl-Ph	93.8	86.1	24
16e	4-CN-Ph	94.1	81.8	24
16f	4-NO_2-Ph	96.5	84.8	24
16g	2,6 diCl-Ph	88.4	89.5	24
16h	3-CF_3-Ph	87.5	90.2	48

actives in the selected compounds can be increased by up to 17-fold [18]. Thus, PASS-based computer pre-screening of large databases of diverse compounds can increase the probability of finding bioactive new chemical agents, and reduce the number of compounds that have to be synthesized and tested experimentally.

Before the establishment of an in vitro antiparasitic assay, we obtained predictive values concerning biological activities by comparing the chemical structures of the designed compounds **1–15**, with structures or substructures of more than 46,000 well-known biologically active drugs included in the database of PASS® [20]. Results of prediction are presented as estimates of the probability *Pa* that the compounds are active. For *Pa* values $\geq$0.7, the corresponding compound is very likely to reveal this activity in experiments, but in that case, the chance of the compound being the analogue of a known pharmaceutical agent is also high. For *Pa* values between 0.5 and 0.7, the compound is likely to reveal this activity in experiments and the compound exhibits less similarity to the known pharmaceutical agents. For *Pa* values lower than 0.5, the compound is unlikely to reveal this activity in experiments, but if the presence of this activity is confirmed in experiments, the compound might be a new biologically active chemical entity. Predictive antiparasitic values for compounds **1–15** are summarized in Table 1. *Pa* values estimated for antiparasitic activity for compounds **1–8** (acetamides) were higher than 0.6 for all structures. These results indicated that compounds **1–8** could be likely to reveal this activity in experiments and the compounds exhibit less similarity to the known antiparasitic drugs. For sulfonamides series **9–15**, *Pa* values were also in the range of 0.5–0.6; hence, the structures are likely to reveal antiparasitic activity in experiments. For Bzn, the *Pa* value estimated for antiparasitic activity was 0.497, due to the lack of information about the other parasiticidal bioactivities rather than trypanocidal.

2.2. Chemistry

The α-chloroacetamides adequately substituted **16a** and **16b–h**, were synthesized by condensation of 2-chloroacetyl chloride (**18**) with benzylamine (**a**) and 4-substituted anilines (**b–h**) respectively, through two methodologies (Scheme 1). In the first one, we used methylene chloride as solvent and triethylamine as base. In the second one, we change the solvent and the base for acetone and sodium bicarbonate, respectively, affording the desired compounds with yields ranging from 70 to 90%. The reactions conditions, duration of reaction and yields of the products prepared are listed in Table 2.

Bimolecular nucleophilic substitution in **16a–h** with 4(5)-nitro-2-methyl-1*H*-imidazole anion (**17**) afforded compounds **1–8** (Scheme 1). Solid compounds were purified by recrystallization and the structure of the pure compounds was established by spectroscopic data. Crystals suitable for X-ray crystallography were obtained for compounds **1** and **16**g.

Compounds **9–15** were prepared in a single step (Scheme 1), starting from 4(5)-nitro-2-methyl-1*H*-imidazole anion (**17**), via a coupling reaction with arylsulfonyl chlorides **19–25**, in the presence of a catalytic amount of 4-dimethylaminopyridine and triethylamine. Title compounds were recovered with 11–89% yields. Compounds were purified by recrystallization or by column chromatography. The chemical structures of the synthesized compounds were confirmed on the basis of their spectral data.

In the nuclear magnetic resonance spectra (^{1}H NMR; δ ppm), the signals of the respective protons of the compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. In compounds **3–6**, **9**, and **11–15**, the aromatic region of the ^{1}H NMR spectrum contained an A_2B_2 pattern signals ranging from δH 7.04 to 8.24 ppm, attributable to H-2′, H-3′, H-5′ and H-6′, of the 4-substituted benzene ring. Also, we observed in all compounds a characteristic pattern for 2-methyl-4-nitroimidazole: a singlet methyl signal ranging from 2.26 to 2.33, and a singlet signal ranging 8.31–8.46 ppm, attributable to H-5 of heterocyclic ring. A singlet peak was observed for compounds **1–8**, assigned to a methylene group found in 2-substituted acetamide.

2.3. Crystallography

Single crystals of compounds **1** and **16g** were grown by slow evaporation at room temperature of DMSO and acetone, respectively. Perspective views of the molecular structures of compounds **1** and **16g** are shown in Fig. 2. The most relevant crystallographic data for **1** and **16g** have been summarized in Table 3. Selected bond lengths, bond angles, and torsion angles are outlined in Table 4. In both cases, the packing is stabilized by hydrogen bonds [22].

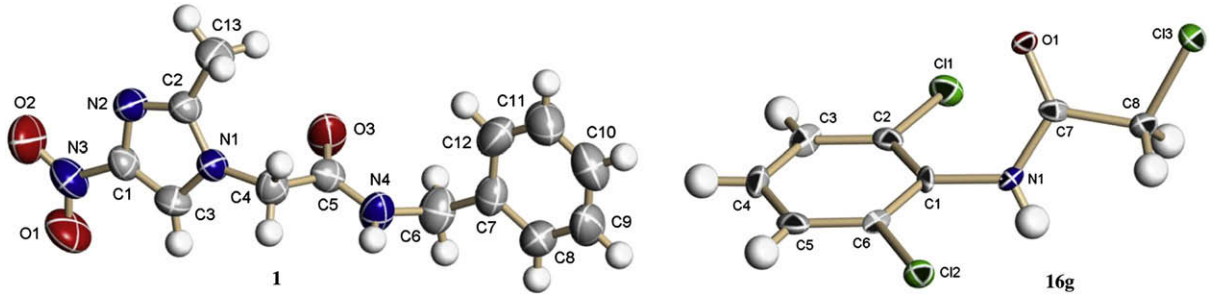


Fig. 2. The molecular structures of **1** and **16g** showing the atom labeling scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii.

Table 3
Crystallographic data for compounds **1** and **16g**.

Crystal data	1	16g
Formula	$C_{13}H_{14}N_4O_3$	$C_8H_6Cl_3NO$
Crystal size (mm)	0.23 × 0.27 × 0.31	0.15 × 0.17 × 0.23
MW (g mol^{-1})	274.28	238.49
Crystal System	Orthorhombic	Orthorhombic
Space group	$Pna2_1$	$P2_12_12_1$
Cell parameters		
a (Å)	13.0874(13)	4.7166(15)
b (Å)	8.4150(8)	10.917(3)
c (Å)	11.9285(12)	18.405(5)
V (Å^3)	1313.7(2)	947.7(7)
Z	4	4
μ (mm^{-1})	0.102	0.921
ρ calcd (g cm^{-3})	1.387	1.671
Data collection		
θ limits (Å)	2.88 < θ < 27.5°	2.17 < θ < 25.0
hkl limits	−17,16; −10,10; −15,	−5,5; −12,8; −21,11
No. collected refl.	1,514,245	3387
No. ind refl. (R_{int})	1586 (0.038)	1000 (0.077)
Refinement		
R [I > 2sigma(I)]	0.074	0.053
R_w (all data)	0.170	0.116
No. of variables	186	110
GOF	1.273	1.123
$\Delta\rho_{min}$ (e Å^{-3})	−0.17	−0.52
$\Delta\rho_{max}$ (e Å^{-3})	0.27	0.50

In the crystal structure of **1** an intermolecular N4–H4···N2^{i} hydrogen bond [symmetry code: (i) ½ − *x*, ½ + *y*, ½ + *z*] links the molecules into chains running in zigzag along the diagonal of the *bc* plane (Fig. 3), with H4···N2^{i} = 2.21(5) Å, N4···N2^{i} = 3.024(6) Å and N4–H4···N2^{i} = 167(4)°.

In the crystal lattice of **16g** the molecules are linked through intermolecular N1–H1···O1^{ii} hydrogen bonds [symmetry code: (ii) −1 + *x*, *y*, *z*] into chains running along the *a* axis (Fig. 4a), with H1···O1^{ii} = 2.05(7) Å, N1···O1^{ii} = 2.827(8) Å and N1–H1···O1^{ii} = 167(7)°. The packing is further stabilized by intermolecular C(2)–Cl(1)···Cl(3)^{iii} interhalogen contacts [symmetry code: (iii) ½ + *x*, ½ − *y*, 2 + *z*] [23–28]. The C(2)–Cl(1)···Cl(3)–C(8) interaction has a Cl···Cl separation that is slightly shorter than the sum of the van der Waals radii of the chlorine atoms and exhibits an obtuse angle in C(2)–Cl(1)···Cl(3) [(C)Cl···Cl–(C) 3.437 Å, C–Cl···Cl 152.99°] (Fig. 4b).

Table 4
Selected bond lengths, bond angles and torsion angles for compound **1** and **16g**.

1		16g	
Bond lengths (Å)			
C(1)–C(3)	1.340(7)	C(2)–Cl(1)	1.735(9)
C(1)–N(2)	1.344(6)	C(6)–Cl(2)	1.759(8)
C(1)–N(3)	1.438(5)	C(1)–N(1)	1.416(10)
C(2)–N(2)	1.308(6)	N(1)–C(7)	1.346(10)
C(2)–N(1)	1.361(5)	C(7)–O(1)	1.216(9)
N(1)–C(3)	1.348(6)	C(8)–Cl(3)	1.771(8)
N(3)–O(1)	1.224(7)		
N(3)–O(2)	1.210(7)		
C(5)–O(3)	1.206(6)		
C(5)–N(4)	1.328(6)		
Bond angles (deg)			
N(2)C(2)N(1)	111.3(4)	Cl(1)C(2)C(1)	119.6(6)
N(2)C(1)N(3)	120.7(5)	Cl(2)C(6)C(1)	119.9(6)
C(1)N(3)O(1)	116.3(5)	C(1)N(1)C(7)	123.7(7)
C(1)N(3)O(2)	119.1(5)	N(1)C(7)O(1)	123.7(8)
O(3)C(5)N(4)	125.0(4)	C(7)C(8)Cl(3)	110.9(6)
N(4)C(5)C(4)	112.8(4)		
Torsion angles (deg)			
O(1)N(3)C(1)C(3)	−2.8(7)	Cl(1)C(2)C(1)N(1)	2.9(11)
O(2)N(3)C(1)N(2)	−2.5(7)	Cl(2)C(6)C(1)N(1)	0.6(11)
N(1)C(4)C(5)O(3)	23.3(7)	C(1)N(1)C(7)O(1)	−1.5(15)
O(3)C(5)N(4)C(6)	0.1(8)	O(1)C(7)C(8)Cl(3)	13.5(12)
N(4)C(6)C(7)C(8)	115.2(6)		

In conclusion, the crystal packing of compounds **1** and **16g** is determined by N–H···N and N–H···O hydrogen bonds, respectively, with consequent formation of polymeric chains. On the other hand, the Cl···Cl donor–acceptor interactions detected in the intermediate **16g** are important because halogen atoms that are covalently bond to carbon atoms are known to form cooperative short contacts to hydrogen, nitrogen, oxygen, sulfur and other halogens, this short contacts are implicated in all the fields where design and manipulation of aggregation phenomena play a key role [23–28].

2.4. In vitro antiparasitic effect

In this study fifteen new nitroimidazole derivatives (**1**–**15**), were synthesized and tested in vitro as antiparasitic agents against *G. intestinalis*, *T. vaginalis* and *E. histolytica*. The main features of these compounds are:

- The 2-nitroimidazole found in Bzn was replaced by 2-methyl-4-nitroimidazole moiety in compounds **1**–**15**.
- The substitution of the hydrogen atom at position 4 of the benzene ring by cyano, nitro, halo (–F, –Cl), or 3-trifluoromethyl and 2,6-(Cl)$_2$ groups in order to determine bioisosteric equivalence, enhancement of solubility and potential antiparasitic activity in compounds **1**–**8**.
- The replacement of sulfonamide group instead of acetamide group found in Bzn in compounds **9**–**15**.

Biological assays results against the three unicellular parasites tested are summarized in Table 5. Comparison was made among new compounds and the antiparasitic drug benznidazole. In order to compare bioactivities, 2-methyl-4(5)-nitroimidazole (Mni) and metronidazole (Met), were also tested.

In the first series (acetamide derivatives **1**–**8**), compounds **1**, **3**, **4** and **6** showed high bioactivity (<10 μM) against *T. vaginalis*. Compound **1**, with a benzylacetamide substituent (Bzn true analogue), was two-fold more active than Bzn and five-fold more potent than Mni. The same pattern was observed in compound **4**, which bears a 4-chloroacetanilide substituent. Compounds **3** and **6** (4-fluoro and 4-nitroacetanilide substituent, respectively) were three times more potent than Bzn and six-fold more actives than Mni. Compound **5** (4-CN) was as active as Bzn and three-fold more potent than Mni, whereas compounds **2** (4-H), **7** (3-CF$_3$) and **8** [2,6-(Cl)$_2$] were less potent than reference drugs.

For *G. intestinalis*, compounds **1**, **4**–**6** were more active than Bzn and Mni. The most potent compound was **5**, which was two times more active than Bzn. Compounds **3** and **7** were as active as Bzn. Nitroimidazoles **2** and **8** were the least active compounds.

Against *E. histolytica*, only compound **1** was as active as Bzn and two-fold more potent than Mni. Compounds **2**–**8** showed low bioactivity against this parasite. It is important to note that none of the compounds assayed were more active that metronidazole.

With these results, we could conclude that compounds **1**–**8** displayed a selective toxicity against *T. vaginalis* over the other unicellular parasite tested. However, compound **1** (which is a benznidazole true congener), was more active than this drug

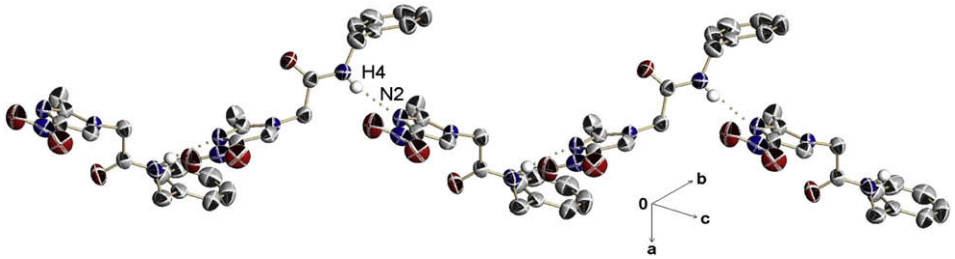


Fig. 3. A view of the crystal packing compound **1** showing the formation of zigzag chains. Hydrogen bonds are represented by dotted lines and H atoms not involved in hydrogen-bonding interactions have been omitted for clarity.

against the three parasites tested. The re-positioning of the nitro group and the addition of methyl substituent in **1**, make the molecule more active than the parent drug (Bzn).

In the last series (sulfonamide derivatives **9**–**15**), biological assay results against *T. vaginalis* showed that all compounds were more active than Bzn and Mni. In particular, compounds **9**, **11**, **12**, **14** and **15**, substituted at position 4 of the benzenesulfonamide scaffold with acetamido, methyl, chloro, nitro and methoxy groups respectively, showed high bioactivity ($<10\ \mu M$). Compound **14** was the most active showing an $IC_{50} = 2.93\ \mu M$ (six-fold more potent than Bzn and fourteen-fold more potent than Mni). Against *G. intestinalis*, compounds **10**–**15** were more potent than Bzn and Mni, whereas compound **9** was as active as the two reference drugs. Compounds **14** and **15** were the most actives against this parasite; showing two times more potency than reference drugs.

In vitro antiamoebic activity exhibited by compounds **9**–**15** was acceptable. All of them showed high bioactivity in the range of 3.55–8.56 μM. Compounds **9** and **11** were as active as Bzn and two times more active than Mni. With these biological results, we could conclude that the introduction of benzenesulfonamide core instead of acetamide substituent enhances the antiparasitic activity of 2-methyl-4-nitroimidazole derivatives. However, none of the compounds assayed were more active that metronidazole.

2.5. In vitro cytotoxic effect

Compounds **1**–**15** were evaluated for their toxicity against MDCK cell line. Compounds **2**–**15** were non-cytotoxic, with IC_{50}s ranging from 321.30 to 1270.30 μM. The selectivity index (SI) of the compounds defined as the ratio of cytotoxicity to biological activity ($SI = CC_{50}$ MDCK cells/IC_{50} parasites) was calculated. It is generally considered that biological efficacy is not due to in vitro cytotoxicity when $SI \geq 10$. Most of the compounds possessing good in vitro antiparasitic activity have shown decent selectivity index (Table 5). Compound **1** showed a CC_{50} of 77.55 μM, whereas metronidazole displayed a $CC_{50} = 68\ \mu M$. All compounds were less cytotoxic than metronidazole.

Since nitroimidazole mode of action is related to reductive metabolism, they must be activated by an NADH-dependent, mitochondrially localized, bacterial-like, type I nitroreductase. According to the position of the nitro substituent in the imidazole ring (2-, 4- and 5-), the reduction potentials are different. In relation to their biological activity, 2-nitroimidazole derivatives are preferably used as radiosensitizers, while 5-nitroimidazole derivatives are mainly used as antiparasitic or antibacterial drugs, and 4-nitroimidazoles are relatively more inert [29]. However, the descriptions of the mutagenic and carcinogenic properties of some 2- and 5-nitroimidazole derivatives have also increased the

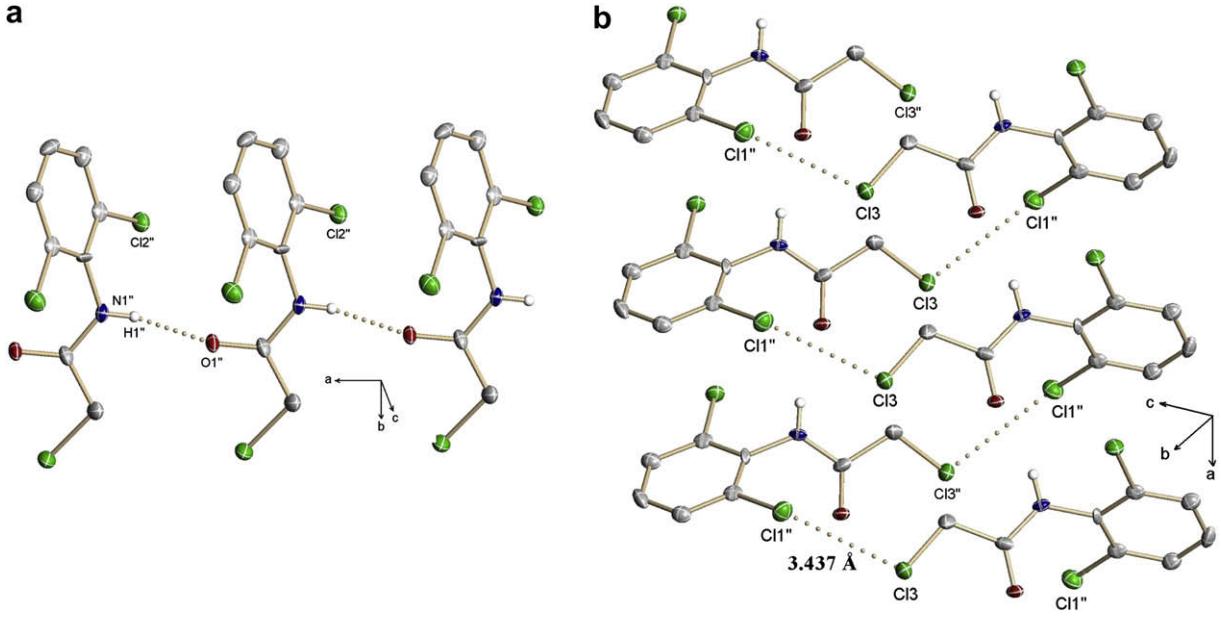
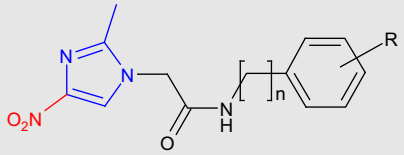
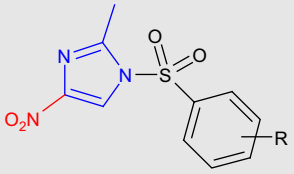


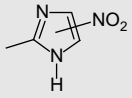
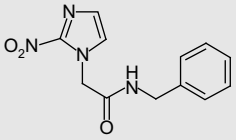
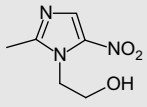
Fig. 4. Packing diagrams of compound **16g** showing the formation of chains along *a* axis. Hydrogen bonds and interhalogen contacts are represented by dotted lines and H atoms not involved in hydrogen bonding have been omitted for clarity.

Table 5
Physicochemical properties and in vitro antiparasitic bioactivity of imidazoleacetamides **1**–**8** and imidazolesulfonamides **9**–**15**.



Cmpd	*n*	R	Mp (°C)	Yield (%)	MS FAB (+)	IC_{50} (μM)				Selectivity index CC_{50}/IC_{50}		
						T. vaginalis	*G. intestinalis*	*E. histolytica*	CC_{50} (μM) MDCK cell	*T.v*	*G. i*	*E. h*
1	1	H	198.0–200.9	90.1	275	7.98	17.70	3.96	77.55	10	4	20
2	0	H	292.0–293.8	80.1	261	24.64	49.11	26.60	1136.60	46	23	43
3	0	4-F	287.0–289.9	72.0	279	5.61	21.04	11.19	828.06	148	39	74
4	0	4-Cl	297.0–300.2	92.1	295	9.18	16.02	18.11	642.35	70	40	35
5	0	4-CN	280.7–282.0	81.5	286	14.20	11.25	41.23	672.37	47	60	16
6	0	4-NO_2	317.0–319.1	79.8	306	6.63	15.44	15.34	798.03	120	52	52
7	0	2,6-diCl	220.1–223.9	61.4	329	37.02	22.02	25.28	396.49	11	18	16
8	0	3-CF_3	250.0–254.0	60.7	329	67.43	37.59	29.08	435.03	6	12	15



Cmpd	R	Mp (°C)	EIMS	IC_{50} (μM)				Selectivity index CC_{50}/IC_{50}		
				T. vaginalis	*G. intestinalis*	*E. histolytica*	CC_{50} (μM) MDCK cell	*T.v*	*G. i*	*E. h*
9	–$NHCOCH_3$	152.0–154.0	324	8.73	20.18	3.89	1031.39	118	51	265
10	–H	129.0–131.3	267	12.52	11.99	6.74	1270.30	101	106	188
11	–CH_3	147.1–150.2	281	9.96	13.97	3.55	987.59	99	71	278
12	–Cl	174.3–175.8	301	7.62	15.34	4.03	578.37	76	38	144
13	–F	152.5–155.1	285	11.48	10.62	4.44	813.32	71	77	183
14	–NO_2	160.1–161.1	312	2.93	7.50	7.28	611.35	209	82	84
15	–OCH_3	160.0–161.5	297	8.41	6.55	8.56	321.30	38	49	38
Mni				40.74	20.40	8.38				
Bnz				18.62	22.58	4.27				
Met				0.29	5.36	0.77	68.0			

interest in minor mutagenic 4-nitroimidazoles [14,30]. Although the nitro reduction is crucial for any biological activity, there are still no conclusive results about the incidence of the nitro position in their biological activity [31].

3. Conclusion

We have synthesized new *N*-acetamide(sulfonamide)-2-methyl-4-nitro-1*H*-imidazoles **1**–**15**, and screened them for their in vitro antiparasitic activity. The obtained results are very promising since many of the compounds showed activity comparable with benznidazole, and even better potency. Bzn is an anti-chagasic drug, but this study also shows other antiparasitic bioactivities. The bioactivity observed against these three unicellular parasites suggests that compounds **1**–**8** (acetamide derivatives) showed mainly trichomonicidal and giardicidal properties, whereas compounds **9**–**15** (sulfonamide derivatives) shown anti-amoebic activity. None of the compounds assayed were more active than metronidazole. Toxicity assay showed that all compounds are non-cytotoxic against MDCK cell line.

This study demonstrated that the bioisosteric replacement of 2-nitroimidazole ring by 2-methyl-4-nitroimidazole scaffold, results in retention and an enhancement of antiparasitic bioactivity. Further optimization and pharmacokinetic characterization of this series are in progress in our laboratory.

4. Experimental

4.1. Instruments

Melting points were determined on an EZ-Melt MPA120 automated melting point apparatus from Stanford Research Systems and are uncorrected. Reactions were monitored by TLC on 0.2 mm precoated silica gel 60 F254 plates (E. Merck). ^{1}H and 13C NMR were recorded on a Varian INOVA 400 and Varian Mercury 200 instruments, respectively. Chemical shifts are given in ppm relative to tetramethylsilane (Me_4Si, $\delta = 0$) in DMSO-d_6; J values are given in Hz. The following abbreviations are used: s, singlet; d, doublet; q, quartet; dd, doublet of doublet; t, triplet; m, multiplet; bs, broad signal. MS were recorded on a JEOL JMS-700 spectrometer by Electron Impact or Fast Atom Bombarded [(FAB^+)]. Predictive values of antiparasitic activities were also investigated using the chemistry software server PASS (http://195.178.207.233/PASS/) [19]. Starting materials benzylamine (**a**), aniline (**b**), 4-fluoroaniline (**c**), 4-chloroaniline (**d**), 4-aminobenzonitrile (**e**), 4-nitroaniline (**f**), 2,6-dichloroaniline (**g**), 3-(trifluoromethyl)aniline (**h**), 2-methyl-4(5)-nitro-1*H*-imidazole (**17**), 2-chloroacetyl chloride (**18**), and 4-substituted benzenesulfonyl chlorides (**19**–**25**) were commercially available from Aldrich and used without purification.

4.2. General method of synthesis of N-aryl-2-(2-methyl-4-nitro-1H-imidazol-1-yl)acetamides (**1**–**8**)

A solution of 2-methyl-4(5)-nitro-1*H*-imidazole (3.93 mmol, 1.0 equiv) and potassium hydroxide (4.72 mmol, 1.2 equiv) in acetonitrile (10 mL) was stirred for 30 min until the anion was formed (yellow solution). This mixture was added to a solution of substituted α-chloroacetamides **16a**–**h** (4.33 mmol, 1.1 equiv) in acetonitrile (5 mL) and stirred under reflux for 8 h. Solvent was removed under vacuum, and the residue obtained was washed with water. The crude solid product was then recrystallized from appropriate solvent.

4.2.1. N-Benzyl-2-(2-methyl-4-nitro-1H-imidazol-1-yl) acetamide (**1**)

Yield 0.91 g (83.9%) of white solid. Mp 198.0–200.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3*H*, CH_3), 4.33 (d, 2*H*, CH_2Ph, $J = 6.0$ Hz), 4.85 (s, 2*H*, CH_2CO), 7.24–7.36 (m, 5*H*, Ph), 8.31 (s, 1*H*, H-5), 8.82 (dd, 1*H*, N–H, $J = 6$ Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.6 (CH_3), 42.5 (CH_2Ph), 48.8 (CH_2CO), 123.3 (C-5), 126.9 (C-4′), 127.3 (C-2′, C-6′), 128.3 (C-3′, C-5′), 138.6 (C-1′), 145.0 (C-4), 145.7 (C-2), 165.4 (C=O). MS (FAB^+): m/z 275 $(M+H)^+$. HRMS (FAB^+) Calcd. for $C_{13}H_{15}N_4O_3$ $[M+H]^+$ 275.2826, found: 275.1259. Anal. Calcd. for $C_{13}H_{14}N_4O_3$: C, 56.93; H, 5.14; N, 20.43. Found: C, 57.05; H, 5.23; N, 19.77.

4.2.2. 2-(2-Methyl-4-nitro-1H-imidazol-1-yl)-N-phenylacetamide (**2**)

Yield 0.46 g (75.8%) of white solid. Mp 292.0–293.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 5.00 (s, 2H, CH_2CO), 7.08 (dd, 1H, H-4′, $J = 7.6$ Hz,), 7.33 (dd, 2H, H-3′, $J = 7.8$ Hz, $J = 7.8$ Hz), 7.58 (d, 2H, H-2′, $J = 7.6$ Hz), 8.46 (s, 1H, H-5), 8.82 (s, 1H, N–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.7 (CH_3), 49.3 (CH_2CO), 119.1 (2C, C-2′, C-6′), 123.5 (C-5), 123.7 (C-4′), 128.9 (2C, C-3′, C5′), 138.3 (C-1′), 145.0 (C-4), 146.0 (C-2), 164.2 (C=O). MS (FAB^+): m/z 261 $(M+H)^+$. HRMS (FAB^+) Calcd. for $C_{12}H_{15}N_4O_3$ $[M+H]^+$ 261.2560 found: 261.1085. Anal. Calcd. for $C_{12}H_{12}N_4O_3$: C, 55.38; H, 4.65; N, 21.53. Found: C, 55.14; H, 4.61; N, 21.34.

4.2.3. N-(4-Fluorophenyl)-2-(2-methyl-4-nitro-1H-imidazol-1-yl) acetamide (**3**)

Yield 0.80 g (91.3%) of white solid. Mp 287.0–289.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 5.03 (s, 2H, CH_2CO), 7.39 (dd, 2H, H-2′, $J = 8.8$ Hz), 7.63 (dd, 2H, H-3′, $J = 5.0$ Hz, $J_{H3'-P} = 9.0$ Hz), 8.35 (s, 1H, H-5), 10.79 (s, 1H, N–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.7 (CH_3), 49.3 (CH_2CO), 115.4 (d, 2C, C-3′, C-5′, $J_{C3'-F} = 21.2$ Hz), 120.9 (d, 2C, C-2′, C-6′, $J_{C2'-F'} = 21.2$ Hz), 123.4 (C-5), 134.8 (C-1′), 145.0 (C-4), 146.0 (C-2), 158.1 (d, C-4′, $J_{C4'-F} = 238.3$ Hz), 164.2 (C=O). MS (FAB^+): m/z 279 $(M+H)^+$. HRMS (FAB^+) Calcd. for $C_{12}H_{12}N_4O_3F$ $[M+H]^+$ 279.2465, found: 279.1194.

4.2.4. N-(4-Chlorophenyl)-2-(2-methyl-4-nitro-1H-imidazol-1-yl) acetamide (**4**)

Yield 0.52 g (74.1%) of white solid. Mp 297.0–300.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 5.01 (s, 2H, CH_2CO), 7.39 (d, 2H, H-3′, $J = 8.8$ Hz), 7.62 (d, 2H, H-2′, $J = 8.8$ Hz), 8.33 (s, 1H, H-5), 10.60 (s, 1H, N–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.6 (CH_3), 49.3 (CH_2CO), 120.7 (2C, C-2′, C-6′), 123.4 (C-5), 127.3 (C-4′), 128.7 (2C, C-3′, C-5′), 137.2 (C-1′), 145.0 (C-4), 146.0 (C-2), 164.4 (C=O). MS (FAB^+): m/z 295 $(M+H)^+$. HRMS (FAB^+) Calcd. for $C_{12}H_{12}N_4O_3Cl$ $[M+H]^+$ 295.7011, found: 295.0586. Anal. Calcd. for $C_{12}H_{11}N_4O_3Cl$: C, 48.91; H, 3.76; N, 19.01. Found: C, 48.60 H, 3.55; N, 18.90.

4.2.5. N-(4-Cyanophenyl)-2-(2-methyl-4-nitro-1H-imidazol-1-yl) acetamide (**5**)

Yield 0.85 g (94.6%) of white solid. Mp 280.7–282.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 5.10 (s, 2H, CH_2CO), 7.87 (m, 4H, H-2′, H-3′), 8.35 (s, 1H, H-5), 11.23 (s, 1H, N–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.6 (CH_3), 49.5 (CH_2CO), 105.4 (C-4′), 118.7 (CN), 119.2 (2C, C-2, C-6′), 123.4 (C-5), 133.3 (2C, C-3′, C-5′), 142.6 (C-1′), 145.0 (C-4), 146.0 (C-2), 165.2 (C=O). MS (FAB^+): m/z 286 $(M+H)^+$. HRMS (FAB^+) Calcd. for $C_{13}H_{12}N_5O_3$ $[M+H]^+$ 286.2655, found: 286.1410.

4.2.6. 2-(2-Methyl-4-nitro-1H-imidazol-1-yl)-N-(4-nitrophenyl)acetamide (**6**)

Yield 0.85 g (88.2%) of white solid. Mp 317.0–319.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H, CH_3), 5.13 (s, 2H, CH_2CO), 7.86 (d, 2H, H-3′, $J = 8.8$ Hz), 8.17 (d, 2H, H-2′, $J = 9.6$ Hz), 8.33 (s, 1H, H-5), 11.73 (s, 1H, N–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.2 (CH_3), 50.2 (CH_2CO), 119.7 (2C, C-2, C-6′), 124.1 (C-5), 125.7 (2C, C-3′, C-5′), 143.1 (C-4), 145.4 (C-4′), 145.8 (C-1′), 146.8 (C-2), 166.3 (C=O). MS (FAB^+): m/z 306 $(M+H)^+$.

4.2.7. N-(2,6-Dichlorophenyl)-2-(2-methyl-4-nitro-1H-imidazol-1-yl)acetamide (**7**)

Yield 0.30 g (30.0%) of white solid. Mp 220.1–223.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.33 (s, 3H, CH_3), 5.11 (s, 2H, CH_2CO), 7.38 (dd, 1H, H-4′, $J = 8.0$ Hz), 7.57 (d, 2H, H-3′, $J = 8.0$ Hz), 8.38 (s, 1H, H-5), 10.43 (b, 1H, N–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.6 (CH_3), 48.7 (CH_2CO), 123.5 (C-5), 128.6 (2C, C-3′, C-5′), 129.6 (C-4′), 132.0 (C-1′), 133.3 (2C, C-2′, C-6′), 145.0 (C-4), 145.8 (C-2), 164.7 (C=O). MS (FAB^+): m/z 329 $(M+H)^+$. HRMS (FAB^+) Calcd. for $C_{12}H_{10}N_4O_3Cl_2$ $[M+H]^+$ 329.1388, found: 329.0239. Anal. Calcd. for $C_{12}H_{10}N_4O_3Cl_2$: C, 43.79; H, 3.06; N, 17.02. Found: C, 42.58; H, 3.26; N, 16.98.

4.2.8. 2-(2-Methyl-4-nitro-1H-imidazol-1-yl)-N-[3-(trifluoromethyl)phenyl]acetamide (**8**)

Yield 0.92 g (71.5%) of white solid. Mp 250.0–254.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.32 (s, 3H, CH_3), 5.08 (s, 2H, CH_2CO), 7.44 (d, 1H, H-4′, $J = 7.6$ Hz), 7.58 (d, 1H, H-5′, $J = 8.0$ Hz), 7.81 (d, 1H, H-6′, $J = 8.0$ Hz), 8.12 (s, 1H, H-2′), 8.36 (s, 1H, H-5), 11.17 (s, 1H,

N–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.7 (CH_3), 49.4 (CH_2CO), 115.2 (C-2′), 120.0 (C-4′), 122.7 (q, CF_3, $J_{C3'-F} = 258.0$ Hz), 129.6 (C-3′), 130.1 (C-6′), 139.2 (C-5′), 146.0 (C-2), 145.0 (C-4), 165.0 (C=O). MS (FAB$^+$): m/z 329 $(M+H)^+$. HRMS (FAB$^+$) Calcd. for $C_{13}H_{12}N_4O_3F_3$ $[M+H]^+$ 329.2540, found: 329.0950.

4.3. General method of synthesis of 1-(arylsulfonyl)-2-methyl-4-nitro-1H-imidazoles (**9**–**15**)

To a solution of 2-methyl-4(5)-nitro-1*H*-imidazole (3.00 mmol) in dichloromethane (10 mL) were added triethylamine (3.30 mmol, 1.1 equiv) and a catalytic amount of 4-dimethylaminopyridine (DMAP). After stirring at room temperature for 15 min, a solution of 4-substituted benzenesulfonyl chloride (3.30 mmol, 1.1 equiv) in 5 mL of dichloromethane was added droop by droop. The reaction mixture was stirred at 40 °C under nitrogen atmosphere for 6–10 h. After complete conversion as indicated by TLC, the solvent was removed in vacuo, the residue was neutralized with saturated $NaHCO_3$ solution, and the aqueous layer was extracted with ethyl acetate (3 × 15 mL), washed with water (3 × 20 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the precipitated solids were recrystallized from a mixture of ethanol–water.

4.3.1. N-{4-[(2-Methyl-4-nitro-1H-imidazol-1-yl) sulfonyl]phenyl}acetamide (**9**)

Yield 0.55 g (43.4%) of white solid. Mp 152.0–154.0 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.07 (s, 3H, 4″-$COCH_3$), 2.36 (s, 3H, 2′-CH_3), 7.57 (s, 4H, H-2″, H-3″, H-5″, H-6″), 8.27 (s, 1H, H-4) 10.08 (s, 1H, *N*–H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 3.8 (C-2́), 24.0 ($COCH_3$), 118.0 (C-3″, C-5″), 119.5 (C-4), 126.7 (C2″, C6″), 139.8 (C-5), 142.0 (C-1″), 145.1 (C-2), 146.2 (C-4″), 168.4 (CO). MS (EI): m/z 324. Anal. Calcd. for $C_{12}H_{12}N_4O_5S$: C, 44.44; H, 3.73; N, 17.28. Found: C, 44.05; H, 3.28; N, 17.80.

4.3.2. 2-Methyl-4-nitro-1-(phenylsulfonyl)-1H-imidazole (**10**)

Yield 0.15 g (14%) of white solid. Mp 129.0–131.3 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.47 (s, 3H, 2′-CH_3), 7.37 (m, 3H, H-4″, H-5″, H-6″, $J = 8.0$, $J = 2.0$, Hz), 7.65 (m, 2H, H-2″ H-6″, $J = 8.0$, $J = 2.0$ Hz), 8.25 (s, 1H, H-4). ^{13}C NMR (50 MHz, DMSO-d_6) δ 14.8 (C-2′), 119.0 (C-4), 125.7 (C-2″, C-6″), 127.9 (C-3″, C-5″), 128.5 (C-4″), 144.0 (C-1″), 128.5 (C-5), 144.5 (C-1″), 149.9 (C-2). MS (EI) m/z (% int. rel.) 267 (20), 141 (70), 77 (100). Anal. Calcd. for $C_{10}H_9N_3O_4S$: C, 44.94; H, 3.39; N, 15.72. Found: C, 44.02; H, 4.07; N, 15.52.

4.3.3. 2-Methyl-1-[(4-methylphenyl)sulfonyl]-4-nitro-1H-imidazole (**11**)

Yield 0.53 g (48.4%) of white solid. Mp 147.1–150.2 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.5 (s, 3H, 4″-CH_3), 2.57 (s, 3H, 2′-CH_3), 7.46 (s, 2H, H-2″, H-6″, $J = 8.4$, $J = 2.4$ Hz), 7.67 (s, 2H, H-3″, H-5″ $J = 8.4$, $J = 2.4$ Hz), 8.21 (s, 1H, H-4). ^{13}C NMR (50 MHz $CDCl_3$) δ 15.3 (CH_3-C-2′), 22.2 (CH_3-C-4″), 118.7 (C-4), 128.1 (C-2″, C-6″), 131.0 (C-3″, C-5″) 133.1 (C-5) 145.1 (C-1″) 148.0 (C-2). MS (EI) m/z (% int. rel.) 281 (10), 155 (70), 91 (100), 65 (23). Anal. Calcd. for $C_{11}H_{11}N_3O_4S$: C, 46.97; H, 3.94; N, 14.94. Found: C, 46.63; H, 3.91; N, 14.74.

4.3.4. 1-[(4-Chlorophenyl)sulfonyl]-2-methyl-4-nitro-1H-imidazole (**12**)

Yield 0.51 g (45.1%) of white solid. Mp 174.3–175.8 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.59 (s, 3H, 2′-CH_3), 7.65 (m, 2H, H-3″, H-5″, $J = 8.7$, $J = 2.7$ Hz), 7.94 (m, 2H, H-2″, H-6″, $J = 8.7$, $J = 2.7$ Hz), 8.21 (s, 1H, H-4). ^{13}C NMR (50 MHz $CDCl_3$) δ: 15.4 (C-2′), 118.7 (C-4), 129.0 (C-2″, C-6″), 129.5 (C-5), 130.7 (C-3″, C-5″), 134.5 (C-1″), 143.4 (C-4″), 145.4 (C-2). MS (EI) m/z (% int. rel.) 301 (10), 175 (93), 111 (100), 75 (27). Anal. Calcd. for $C_{10}H_8ClN_3O_4S$: C, 39.81; H, 2.67; N, 13.93. Found: C, 39.56; H, 2.54; N, 13.85.

4.3.5. 1-[(4-Fluorophenyl)sulfonyl]-2-methyl-4-nitro-1H-imidazole (**13**)

Yield 0.15 g (14.3%) of white solid. Mp 152.5–155.1 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.59 (s, 3H, H-2′), 7.36 (dd, 2H, H-3″, H-5″, $J = 9.0$, $J = 3.3$ Hz), 8.05 (dd, 2H, H-2″, H-6″, $J = 8.0$, $J = 3.0$ Hz), 8.21 (s, 1H H-4). ^{13}C NMR (50 MHz $CDCl_3$) δ 15.4 (C-2′), 117.9–118.2 (C-3″, C-5″, $J = 23.3$ Hz), 118.7 (C-4), 131.2–131.3 (C-2″, C-6″, $J = 9.8$ Hz), 132.1 (C-5, C-1″), 145.3 (C-2), 167.0 (C-4″, $J_{C-F} = 259.5$ Hz). MS (EI) m/z (% int. rel.) 285 (19), 159 (100), 95 (96), 75 (16). Anal. Calcd. for $C_{10}H_8FN_3O_4S$: C, 42.11; H, 2.83; N, 14.73. Found: C, 41.70; H, 2.86; N, 15.17.

4.3.6. 2-Methyl-4-nitro-1-[(4-nitrophenyl)sulfonyl]-1H-imidazole (**14**)

Yield 0.13 g (10.8%) of white solid. Mp 160.1–161.1 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.18 (s, 3H, 2″-CH_3), 7.87 (d, 2H, H-3″, H-5″, $J = 8.0$ Hz), 8.24 (d, 3H, H-2″, H-6″, $J = 8,0$ Hz). ^{13}C NMR (50 MHz DMSO-d_6) δ 14.2 (C-2′), 120 (C-4), 123.8 (C-5″, C-3″), 127.5 (C-2″, C-4″, C-6″), 146.9 (C-1″), 148.0 (C-2), 155.0 (C-5). MS (FAB$^+$) m/z 312 (M^+). Anal. Calcd. for $C_{10}H_8N_4O_6S$: C, 38.46; H, 2.58; N, 17.94. Found: C, 37.84; H, 2. 63; N, 17.50.

4.3.7. 1-[(4-Methoxyphenyl)sulfonyl]-2-methyl-4-nitro-1H-imidazole (**15**)

Yield 1.03 g (89%) of white solid. Mp 160–161.5 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.52 (s, 3H, H-2′), 3.87 (s, 3H, OCH_3), 7.04 (dd, 2H, H-3″, H-5″, $J = 8.8$ Hz, $J = 1.6$ Hz), 7.68 (dd, 2H, H-2″, H-6″, $J = 8.8$ Hz, $J = 1.6$ Hz), 8.14 (s, 1H, H-4). ^{13}C NMR (50 MHz $CDCl_3$) δ 17.5 (C-2), 56.6 (O–CH_3), 11.8 (C-4), 115.6 (C-5″, C-3″), 128. 7 (C-1″), 131.2 (C-5), 131.2 (C-5, C-2″, C-6″), 142.5 (C-2), 166.3 (C-4″). MS (EI) m/z (% int. rel.) 297 (7), 171 (100), 107 (48), 77 (34).

4.4. General method of synthesis of 2-chloro-N-arylacetamides (**16a**–**h**)

4.4.1. Method A

A solution of substituted amine **a**–**h** (6.20 mmol, 1 equiv) and triethylamine (6.83 mmol, 1.1 equiv) in dichloromethane (10 mL) was added over a period of 30 min to a solution of 2-chloroacetyl chloride (6.83 mmol, 1.1 equiv) in dichloromethane (3 mL). The reaction mixture was kept under stirring until the product was formed as a solid (24 h). Dichloromethane was removed under vacuum, and the residue obtained washer with water.

4.4.2. Method B

A solution of substituted amine **a**–**h** (17.99 mmol, 1 equiv) and sodium bicarbonate (21.60 mmol, 1.2 equiv) in acetone (15 mL) was added over a period of 30 min to a solution of 2-chloroacetyl chloride (19.80 mmol, 1.1 equiv) in acetone (5 mL) The reaction mixture was kept under stirring until the product was formed as a solid (24 h). Acetone was removed under vacuum, and the residue obtained was washed with water.

4.4.3. N-Benzyl-2-chloroacetamide (**16a**)

White solid, Mp 93.4–94.8 °C (Lit. 92–93 °C [32]).

4.4.4. 2-Chloro-N-phenylacetamide (**16b**)

White solid, Mp 136.0–139.0 °C (Lit 134–135 °C [33]).

4.4.5. 2-Chloro-N-(4-fluorophenyl)acetamide (**16c**)

Grey solid, Mp 112.0–115.0 °C (Lit. 109–112 °C [34]).

*4.4.6. 2-Chloro-N-(4-chlorophenyl)acetamide (**16d**)*

White solid, Mp 169.8–172.0 °C (Lit 168–170 °C [33]). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.88 (s, 2H, $COCH_2Cl$); 7.00 (d, 2H, H-3. H-5′, $J = 8.8$ Hz); 7.33 (d, 2H, H-2, H-6, $J = 8.8$ Hz); 9.56 (bs, 1H, N–H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 42.9 ($\underline{C}H_2Cl$); 120.8 (2C, C-2, C-6); 128.3 (2C, C-3, C-5); 128.5 (C-4); 136.3 (C-1); 164.4 (C═O) ppm.

*4.4.7. 2-Chloro-N-(4-cyanophenyl)acetamide (**16e**)*

Beige solid, Mp 184.0–186.6.0 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.90 (d, 2H, $COCH_2Cl$, $J = 2.0$ Hz); 7.33 (dd, 2H, H-2, H-6, $J = 2.0$, 8.8 Hz); 7.53 (dd, 2H, H-3, H-5, $J = 2.0$, 8.8 Hz); 9.92 (s, 1H, N–H) ppm, ^{13}C NMR (50 MHz, $CDCl_3$) δ 43.0 ($\underline{C}H_2Cl$), 106.4 (C-4), 118.5 (CN), 119.5 (2C, C-2, C-6), 132.6 (2C, C-3, C-5), 142.0 (C-1), 165.0 (C═O) ppm.

*4.4.8. 2-Chloro-N-(4-nitrophenyl)acetamide (**16f**)*

Green solid, Mp 184.0–185.6 °C (Lit 181–183 °C [33]).

*4.4.9. 2-Chloro-N-(2,6-dichlorophenyl)acetamide (**16g**)*

White solid, Mp 178.0 °C (Lit. light Brown 172 °C [35]). ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.34 (s, 2H, $COCH_2Cl$), 7.56 (d, 2H, H-3, H-5, $J = 8.0$ Hz), 7.37 (d, 1H, H-4, $J = 7.4$, Hz), 10.26 (s, 1H, N–H) ppm, ^{13}C NMR (50 MHz, DMSO-d_6) δ 42.3 ($\underline{C}H_2Cl$), 128.5 (2C, C-3, C-5), 129.5 (C-4), 132.1 (C-1), 133.5 (2C, C-2, C-6), 164.8 (C═O) ppm.

*4.4.10. 2-Chloro-N-[3-(trifluoromethyl)phenyl] acetamide (**16h**)*

White solid, Mp 73.3–75.5 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 4.22 (s, 2H, $COCH_2Cl$); 7.47 (d, 1H, H-4, $J = 8.0$ Hz); 7.47 (dd, 1H, H-5, $J = 8.0$ Hz); 7.77 (d, 1H, H-6, $J = 8.0$ Hz); 7.85 (s, 1H, H-2); 8.40 (bs, 1H, N–H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 43.0 ($\underline{C}H_2Cl$); 117.0 (q, C-2, $J_{C-F} = 3.8$ Hz); 122.0 (q, C-4′, $J_{C-F} = 6.0$ Hz); 122.7 (q, C-3, $J_{C-F} = 65.15$ Hz); 123.3 (C-6); 129.0 (C-5); 127.2 (q, CF_3, $J_{C-F} = 320.0$ Hz); 137.3 (C-1); 164.2 (C═O).

4.5. X-ray crystallography

X-ray diffraction studies were performed on a Bruker-APEX diffractometer with a CCD area detector ($\lambda_{MOK\alpha} = 0.71073$ Å, monochromator: graphite). Frames were collected at 100 K for compound **1** and 293 K for compound **16g** via ω/φ-rotation at 10 s per frame (SMART) [36]. The measured intensities were reduced to F^2 and corrected for absorption with SADABS (SAINT-NT) [37]. Corrections were made for Lorentz and polarization effects. Structure solution, refinement and data output were carried out with the SHELXTL-NT program package [38,39]. Non-hydrogen atoms were refined anisotropically. All hydrogen atoms (except for H4 in compound **1** and H1 in compound **16g**) were placed in geometrically calculated positions using a riding model. Atoms H4 and H1 were located in a difference Fourier map and were freely isotropically refined. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications nos. CCDC-692184, 692185. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk, www: http://www.ccdc.cam.ac.uk).

4.6. Biological assays

4.6.1. In vitro antiparasitic assay

G. intestinalis strain IMSS:0696:1, *T. vaginalis* strain GT3 and *E. histolytica* HM1-IMSS were cultured in axenic conditions in TYI-S-33 modified medium, supplemented with 10% calf serum and bovine bile. In vitro susceptibility assays were performed using a method previously described [40,41]. Briefly: 4×10^4 trophozoites of *G. lamblia* or *T. vaginalis* or *E. histolytica* were incubated for 48 h at 37 °C with increasing concentrations of synthesized compounds, Benznidazole, metronidazole and 2-methyl-4(5)-nitro-1*H*-imidazole. As the negative control, trophozoites were incubated with DMSO used in the experiments. After the incubation, the trophozoites were washed and subcultured for another 48 h in fresh medium alone. At the end of this period, trophozoites were counted and the 50% inhibitory concentration (IC_{50}) was calculated by Probit analysis. Experiments were carried out in triplicate and repeated at least twice.

4.6.2. Cytotoxicity test

Cytotoxicity on host cells is a very important criterion for assessing the selectivity of the observed antiparasitic activity. The cytotoxicity assay was performed according to Rahman et al. [42], where, 1.5×10^4 viable cells from the cell line were seeded in a 96-well plate and incubated for 24–48 h. Dog kidney cells (MDCK) were grown in DMEM media supplemented with 10% (v/v) Fetal Bovine Serum with 100 U/mL penicillin and 100 mg/mL streptomycin and maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity. When cells reached >80% confluence, the medium was replaced and the cells were treated with the compounds at 6.25, 12.5, 25, 50 and 100 μg/mL dissolved in DMSO at a maximum concentration of 0.05%. After 72 h of incubation, 10 μL of a 0.005% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5 mg/mL) was added to each well and incubated at 37 °C for 4 h. The medium was removed and the formazan, a product generated by the activity of dehydrogenases in cells, was dissolved in acidified isopropanol (0.4 N HCl). The amount of MTT–formazan is directly proportional to the number of living cells and was determined by measuring the optical density (OD) at 540 nm using a Bio-assay reader. Metronidazole was used as a positive control, whereas untreated cells were used as negative controls. The concentration of the crude extract that killed 50% of the cells (CC_{50}) was calculated by GraphPad Prim 4 software. All determinations were performed in triplicate [42].

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