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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization and biological activity of Schiff bases derived from metronidazole

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Abstract A series of novel Schiff bases; compounds **3a–j** were prepared by reacting 1-(2-aminoethyl)-2-methyl-5-nitroimidazole dihydrochloride monohydrate (1) with different aldehydes. The structures of these compounds were confirmed through different spectroscopic methods such as ^{1}H-NMR, ^{13}C-NMR and mass spectrometry and also by elemental analyses. The prepared compounds were evaluated in vitro for their antigiardial, antibacterial and antifungal activities. Compounds **3h**, **3b** and **3d** showed remarkable antigiardial activities and were found to be more active than metronidazole with IC_{50} of 0.83, 1.36 and 1.83 μM, respectively. Other compounds also exhibited antigiardial activity and were as good as or even more potent than metronidazole. Some of the newly synthesized Schiff bases exhibited more antifungal activities than the parent drug. In addition, a few of the prepared compounds exhibited modest antibacterial activity but were not as active as metronidazole.

Keywords Metronidazole · Schiff bases · Antigiardial activity

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Introduction

Schiff bases form an important class of organic compounds with a variety of uses. They have been widely used as ligands in the formation of transition metal complexes (Gulco *et al*., 2005). In addition, Schiff bases were found to exhibit biological activities including antibacterial, antifungal and anti-inflammatory (Zhou *et al*., 2007). Moreover, Schiff bases containing heterocycles have attracted much attention due to their diverse biological activity such as anticancer, antiviral, fungicidal, bactericidal and anti-HIV (Patel and Parmar, 2010). Alternatively, development of new chemotherapeutic Schiff bases is now attracting the attention of medicinal chemists (Khan *et al*., 2009).

Several research groups have been involved in the synthesis and biological screening of Schiff bases (Gulco *et al*., 2005; Zhou *et al*., 2007; Patel and Parmar, 2010; Khan *et al*., 2009; Ronad *et al*., 2008; Bertinaria *et al*., 2003). Zhou *et al*. (2007) synthesized a range of Schiff bases derived from 2-aminothiazoles and substituted benzaldehyde; the in vitro antitumor activity of the prepared compounds with three human tumour cell lines was evaluated. On the other hand, Ronad *et al*. (2008) have synthesized a series of Schiff bases from 7-amino-4-methylcoumarin and benzaldehydes and studied their anti-inflammatory and analgesic activity. They discovered that the anti-inflammatory and analgesic activities of some of the prepared Schiff bases were either comparable or more potent than the reference drug. In addition, Bertinaria and his colleagues (Bertinaria *et al*., 2003) have prepared a number of Schiff bases through the condensation of aromatic and heteroaromatic aldehydes with coumarin acetohydrazides under conventional and microwave conditions; the prepared compounds were tested for their antimicrobial activity and were found to display moderate to potent

activity against different bacterial strains. Recently, the synthesis and antiglycation activity of bis-Schiff bases of isatins have been reported by Khan and colleagues (2009); a remarkable effect on the antiglycation activity due to the presence of electron withdrawing groups at isatin was observed. Very recently, Patel and Parmar (2010) have reported on the synthesis and antimicrobial activities of novel optically active Schiff bases derived from substituted benzaldehyde with different amines and discovered that the presence of nitro, methoxy and halogen group in the phenyl ring increases the activity of the prepared compounds against bacteria.

Similarly, mertonidazole, 2-(2-methyl-5-nitro-*1H*-imidazol-1-yl)ethanol (1) and its derivatives have a wide range of biological activity (Bertinaria *et al*., 2003; Martino *et al*., 2005; Hay *et al*., 2003; Günay *et al*., 1999). They are highly effective against trichomoniasis, various forms of amoebiasis and infections with anaerobic bacteria and protozoa (Goldman and Wuest, 1981); it can kill or inhibit the majority of anaerobic bacteria when the metronidazole concentration in serum is in the range from 2 to 8 μg/ml (Salimi *et al*., 1997). Recently (Abu-Shaireh *et al*., 2009), a number of novel metronidazole derivatives have been synthesized and their anti-parasitic activity has been evaluated. In view of the wide interest in the activity and profile of Schiff bases and metronidazole, and as part of our ongoing research in the synthesis of new compounds of pharmacological interest (Abu-Shaireh *et al*., 2009; Al-Zghoul *et al*., 2005; Saadeh *et al*., 2009, 2010), we describe herein the synthesis and characterization of a number of new Schiff bases derived from metronidazole which, to the best of our knowledge, have not previously been described in the literature. The antigiardial, antibacterial and antifungal activities of the newly prepared compounds were evaluated.

Results and discussion

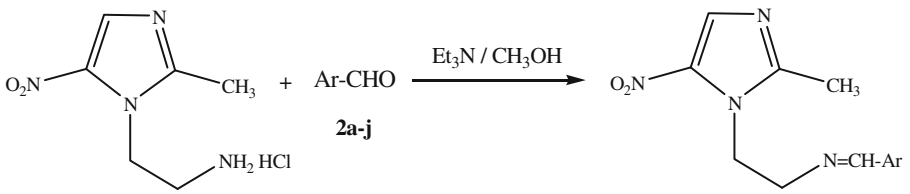
Chemistry

The Schiff bases **3a–j** were prepared by reacting 1-(2-aminoethyl)-2-methyl-5-nitroimidazole dihydrochloride monohydrate (1) with different aldehydes in methanol as shown in Scheme 1. The prepared compounds were checked for purity by TLC using glass plates precoated with silica gel 60 GF254, supplied by Fluka as stationary phase and suitable solvent system as mobile phase and was also confirmed by melting point determination. The structures of the prepared compounds were confirmed by NMR, mass spectrometry and elemental analysis. The ^{1}H and ^{13}C-NMR spectra of all prepared compounds are in total agreement with the suggested structures. DEPT experiments were employed to differentiate secondary and quaternary carbons from primary and tertiary ones. Additional supports of the proposed structures come from mass spectral data; mass spectra of the prepared compounds showed the correct molecular ions as suggested by their molecular formulas.

Biological activities

Antigiardial activity The antigiardial activity of the reported compounds was investigated using in vitro bioassays. Their bioactivity was compared with the standard antigiardial drug, metronidazole. The IC_{50} values of the compounds against *Giardia intestinalis* are given in Table 1. As shown in the table, all the tested compounds exhibited biological activity against Giardia. Compounds **3a–e**, **3i** and **3h** have IC_{50} values ranging from 0.83 to 2.8 μM compared to 3.74 μM for metronidazole. Compound 3h showed the highest antigiardial activity with IC_{50}

Scheme 1 Synthesis of compounds **3a–j**, **6**



1 **3a-j**

	a	b	c	d	e	f	g	h	i	j
Ar	F	CH_3	OCH_3	NO_2	OH	OH, Cl	Cl	S	O	N

Table 1 Antigiardial activities of the tested compounds

Mean IC_{50} ± SD (*n*) (μM)	
Compound	*G. intestinalis*
3a	2.39 ± 0.18
3b	1.36 ± 0.11
3c	2.26 ± 0.17
3d	1.83 ± 0.30
3e	2.15 ± 0.07
3f	4.32 ± 0.49
3g	7.29 ± 0.51
3h	0.83 ± 0.08
3i	2.78 ± 0.12
3j	11.20 ± 0.77
Metronidazole	3.74 ± 0.29

of 0.83 μM and was around five times more potent than metronidazole. The molecular modifications on our derivatives did not render any of the compounds inactive, but **3f**, **3g** and **3j** exhibited less antigiardial activity than metronidazole. The giardicidal activity exhibited by the derivatives, especially compounds **3h**, **3b** and **3d** suggest that the derivatives may be used as new lead compounds in the development of new antiparasitic drugs. This cidal activity of the prepared Schiff bases could extend to cover other related anaerobic protozaol parasites such as *Entamoeba histolytica* and *Trichomonas vaginalis*; this, however, needs further investigations. Although, drug resistance of *Giardia* and other target pathogens such as *E. histolytica* does not, so far, appear to be a serious problem, occasional reports of failure with metronidazole (Knight, 1980) and the reported variations in drug sensitivities of isolates may be alarming (Majewska *et al*., 1991; Aley *et al*., 1994). Therefore, the importance of such biologically active metronidazole derivatives lies in their potential contribution to overcome the problem of resistance of pathogens to the standard drugs (Elizondo *et al*., 1996; Hager and Rapp, 1992). In addition, because of the limited number of drugs available in the market against anaerobic protozoan parasites and bacteria there is a serious need for new active compounds. The molecular modification on the original drugs, therefore, offers alternatives that may bypass the already developed mechanisms adopted by the anaerobic pathogens against the standard drugs. Our new compounds, especially **3h**, **3b** and **3d** are good drug candidates to be tested against metronidazole-resistant parasites and bacteria.

Antimicrobial activity The antibacterial and antifungal activities of the prepared compounds are presented in Table 2. Results revealed that the Schiff bases derived from metronidazole were significantly less active in their antimicrobial activity towards anaerobic culture of *Clostridium sporogenes* relative to the parent drug, metronidazole. In addition, as shown in Table 2, the relatively weak activity displayed by metronidazole against the tested gram positive and gram negative bacteria is weak, however, Schiff base formation led to the increased activity of **3f** towards both gram positive and gram negative species tested. Interestingly, Schiff base formation of metronidazole increased the antifungal activity, where the parent drug was devoid of any anti-candida effect under the experimental conditions. The tested compounds exhibited MIC values ranging from 0.17 to 2.0 mM.

Table 2 Minimum inhibitory concentrations (MIC) (mM) of the prepared compounds and reference substances against tested microorganisms

Compound	MIC (mM)			
	C. sporogenes	*E. coli*	*S. aureus*	*C. albicans*
Metronidazole	0.0456 ± 0	5.48	2.05	NA
Ampicillin (mg/ml)	–	0.0023	0.000036	–
Miconazole (mg/ml)	–	–		0.0029
3a	1.133 ± 0.493	4.30 ± 0	5.09 ± 2.93	1.99 ± 1.30
3b	1.438 ± 0.500	4.37 ± 0	6.89 ± 0	1.00 ± 0.66
3c	0.203 ± 0	4.34 ± 1.88	4.34 ± 1.88	0.34 ± 0.12
3d	0.257 ± 0.112	NA	NA	0.45 ± 0.29
3e	0.285 ± 0.124	2.24 ± 1.02	2.00 ± 1.31	0.36 ± 0.12
3f	1.013 ± 0.438	0.76 ± 0	0.76 ± 0	0.44 ± 0.29
3g	0.267 ± 0.116	2.14 ± 0.92	2.67 ± 0.92	0.17 ± 0.06
3h	0.369 ± 0.129	7.10 ± 0	7.10 ± 0	2.07 ± 1.36
3i	0.236 ± 0	8.85 ± 1.09	8.85 ± 1.09	NA
3j	0.376 ± 0.131	8.46 ± 1.03	8.46 ± 1.03	NA

NA not achieved at concentration ≤1.25 mg/ml

Conclusions

A series of new metronidazole-derived Schiff bases were prepared by reacting 1-(2-aminoethyl)-2-methyl-5-nitroimidazole dihydrochloride monohydrate with different aldehydes. Structures of the newly synthesized compounds were confirmed by means of ^{1}H-NMR, ^{13}C-NMR, mass spectrometry and elemental analyses. The prepared compounds were tested in vitro for their antibacterial, antigiardial and antifungal activity. Some of them displayed remarkable antigiardial activity and were more potent than metronidazole itself. Similarly, the newly synthesized Schiff bases exhibited more antifungal activities than the parent drug, metronidazole. In contrast, a modest antibacterial activity was exhibited by some of the prepared compounds.

Experimental section

Chemistry

All reagents were used as received from commercial sources without further purification. Progress of reactions was monitored by thin layer chromatography (TLC) using glass plates pre-coated with silica gel (E. Merck Kiesegel 60 F254 layer thickness 0.25 mm). Melting points were measured with a Fischer-Johns melting point apparatus and were uncorrected. ^{1}H- and ^{13}C-NMR spectra were acquired with the aid of a Bruker DPX 300 MHz spectrometer (Germany) with TMS as the internal standard. Chemical shifts are expressed in δ units; J values for 1H–1H, 1H–F and ^{13}C–F coupling constants are given in Hertz. High resolution mass spectra (HRMS) were obtained (in positive/or negative mode) using electrospray ion trap (ESI) technique by collision-induced dissociation on a Bruker APEX-4 (7-Tesla) instrument (Bremen, Germany). The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1% formic acid) and infused using a syringe pump with a flow rate of 2 µl/min. External calibration was conducted using arginine cluster in a mass range *m/z* 175–871. Elemental analyses were obtained using a Eurovector Euro EA3000, C, H, N and S elemental analyzer and the obtained results agreed with the calculated percentages to within ±0.4%. Compounds were checked for their purity by TLC using glass plates, pre-coated with silica gel 60 GF254, supplied by Fluka.

*Synthesis of imines **3a–j***

Compounds **3a–j** were synthesized and purified according to the following general procedure: A mixture of 1-(2-aminoethyl)-2-methyl-5-nitroimidazole dihydrochloride monohydrate 1 (2.50 mmol), triethylamine (5.50 mmol) and the corresponding aldehyde (2.60 mmol) in methanol (15 ml) was stirred at room temperature for 24 h. The desired products were collected by filtration, washed with water and then recrystallized form methanol. Using the aforementioned general procedure, the following compounds were synthesized.

*(4-Fluoro-benzylidene)-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl]-amine (**3a**)* Yield 77%; Mp 134–135°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.45$ (s, 3H), 3.95 (t, $J = 5.5$ Hz, 2H), 4.65 (t, $J = 5.5$ Hz, 2H), 7.05 (m, $J = 7.9$ Hz, 2H), 7.60 (m, $J = 7.9$ Hz, 2H), 7.92 (s, 1H), 8.05 (s, 1H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.9$ (CH_3), 47.0 (CH_2), 60.6 (CH_2), 116.0 (d, $^{2}J_{C-F} = 22$ Hz, C), 130.1 (d, $^{4}J_{C-F} = 3.6$ Hz, CH), 133.4 (CH), 138.5 (C), 151.6 (C), 162.2 (CH), 163.7 (d, $^{1}J_{C-F} = 250$ Hz, C). HRMS (EIMS) *m/z*: $[M + Na]^+$ calcd. for $C_{13}H_{13}FN_4\ NaO_2$ 299.09202; found 299.09257. Anal. Calcd. for $C_{13}H_{13}FN_4O_2$: C, 56.52; H, 4.74; N, 20.28. Found: C, 56.41; H, 4.69; N, 20.13.

*(4-Methyl-benzylidene)-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl]-amine (**3b**)* Yield 86%; Mp 163–164°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.35$ (s, 3H), 2.45 (s, 3H), 3.95 (t, $J = 5.5$ Hz, 2H), 4.65 (t, $J = 5.5$ Hz, 2H), 7.15 (d, $J = 7.9$ Hz, 2H), 7.50 (d, $J = 7.9$ Hz, 2H), 7.90 (s, 1H), 8.00 (s, 1H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.6$ (CH_3), 21.6 (CH_3), 47.1 (CH_2), 60.8 (CH_2), 128.2 (CH), 129.5 (CH), 132.8 (C), 133.4 (CH), 141.8 (C), 151.6 (C), 163.5 (CH). HRMS (EIMS) *m/z*: $[M + Na]^+$ calcd. for $C_{14}H_{16}N_4NaO_2$ 295.11709; found 295.12575. Anal. Calcd. for $C_{14}H_{16}N_4O_2$: C, 61.75; H, 5.92; N, 20.58. Found: C, 61.68; H, 5.87; N, 20.44.

*(4-Methoxy-benzylidene)-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl]-amine (**3c**)* Yield 80%; Mp 69–71°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.38$ (s, 3H), 3.85 (s, 3H), 3.95 (t, $J = 5.5$ Hz, 2H), 4.65 (t, $J = 5.5$ Hz, 2H), 6.90 (d, $J = 8.0$ Hz, 2H), 7.55 (d, $J = 8.0$ Hz, 2H), 7.93 (s, 1H), 8.00 (s, 1H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.8$ (CH_3), 47.2 (CH_2), 60.7 (CH_3), 60.7 (CH_2), 114.2 (CH), 128.3 (C), 129.8 (CH), 133.3 (CH), 138.4 (C), 151.5 (C), 162.1 (C), 162.9 (CH). HRMS (EIMS) *m/z*: $[M - H]^+$ calcd. for $C_{14}H_{15}N_4O_3$ 287.11442, found 287.11496. Anal. Calcd. for $C_{14}H_{16}N_4O_3$: C, 58.32; H, 5.59; N, 19.43 found C, 58.25; H, 5.56; N, 19.33.

*[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethyl]-(4-nitro-benzylidene)-amine (**3d**)* Yield 81%; Mp 190–191°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.45$ (s, 3H), 4.00 (t, $J = 5.6$ Hz, 2H), 4.65 (t, $J = 5.6$ Hz, 2H), 7.80 (d, $J = 8.0$ Hz, 2H), 7.93 (s, 1H), 8.15 (s, 1H), 8.20 (d, $J = 8.0$ Hz, 2H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.8$ (CH_3), 46.8 (CH_2), 60.7 (CH_2), 124.1 (CH), 128.9 (CH), 133.4 (CH), 138.4 (C), 140.6 (C), 151.3 (C), 161.4 (CH). HRMS (EIMS) *m/z*: $[M]^+$ calcd. for

$C_{13}H_{13}N_5O_4$ 303.09676; found 303.09730. Anal. Calcd. for $C_{13}H_{13}N_5O_4$: C, 51.48; H, 4.32; N, 23.09 found C, 51.39; H, 4.29; N, 23.98.

*2-{[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethylimino]-methyl}-phenol (**3e**)* Yield 91%; Mp 105–106°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.45$ (s, 3H), 4.00 (t, $J = 5.6$ Hz, 2H), 4.70 (t, $J = 5.6$ Hz, 2H), 6.85 (t, $J = 7.1$ Hz, 1H), 6.95 (d, $J = 7.7$ Hz, 1H), 7.15 (dd, $J = 7.6$, 1.5 Hz, 1H), 7.35 (dt, $J = 7.7$, 1.7 Hz, 1H), 7.95 (s, 1H), 8.20 (s, 1H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.7$ (CH_3), 46.8 (CH_2), 59.4 (CH_2), 117.1 (CH), 118.1(C), 119.2 (CH), 131.9 (CH), 133.2 (CH), 133.6 (CH), 138.5 (C), 151.2 (C), 160.7 (C), 167.9 (CH). HRMS (EIMS) *m/z*: $[M + H]^+$ calcd. for $C_{13}H_{15}N_4O_3$ 275.11442; found 275.11387. Anal. Calcd. for $C_{13}H_{14}N_4O_3$: C, 56.93; H, 5.14; N, 20.43. Found: C, 56.85; H, 5.11; N, 20.34.

*4-Chloro-2-{[2-(2-methyl-5-nitro-imidazol-1-yl)-ethylimino]-methyl}-phenol (**3f**)* Yield 86%; Mp 160–161°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.45$ (s, 3H), 4.00 (t, $J = 5.6$ Hz, 2H), 4.70 (t, $J = 5.6$ Hz, 2H), 6.90 (t, $J = 8.6$ Hz, 1H), 7.20 (s, 1H), 7.30 (d, $J = 6.2$, 1H), 7.95 (s, 1H), 8.10 (s, 1H), 12.6 (s, 1H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.6$ (CH_3), 46.7 (CH_2), 59.3 (CH_2), 118.8 (CH), 118.9 (C), 123.8 (C), 130.9 (CH), 133.1 (CH), 133.6 (CH), 138.5 (C), 151.0 (C), 159.3 (C), 166.8 (CH). HRMS (EIMS) *m/z*: $[M - H]^+$calcd. for $C_{13}H_{12}ClN_4O_3$ 307.05979; found 307.06034. Anal. Calcd. for $C_{13}H_{13}ClN_4O_3$: C, 50.58; H, 4.24; N, 18.15. Found: C, 50.52; H, 4.19; N, 18.06.

*(2-Chloro-benzylidene)-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl]-amine (**3g**)* Yield 80%; Mp 142–143°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.50$ (s, 3H), 3.85 (t, $J = 5.5$ Hz, 2H), 4.65 (t, $J = 5.5$ Hz, 2H), 7.38 (m, 3H), 7.58 (d, $J = 7.7$ Hz, 1H), 7.15 (dd, $J = 7.6$, 1.5 Hz, 1H), 7.55 (t, $J = 7.7$, 1H), 7.90 (s, 1H), 8.00 (s, 1H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.8$ (CH_3), 46.9 (CH_2), 60.6 (CH_2), 126.5 (CH), 127.8 (CH), 130.1 (CH), 131.3 (CH), 133.4 (CH), 135.0 (C), 137.0 (C), 138.6 (C), 151.5 (C), 162.2 (C). HRMS (EIMS) *m/z*: $[M + H]^+$ calcd. for $C_{13}H_{14}ClN_4O_2$ 293.08053; found 293.07998. Anal. Calcd. for $C_{13}H_{13}ClN_4O_2$: C, 53.34; H, 4.48; Cl, 12.11; N, 19.14. Found: C, 53.25; H, 4.46; N, 19.07.

*[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethyl]-thiophen-2-ylmethylene-amine (**3h**)* Yield 89%; Mp 88–90°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.40$ (s, 3H), 3.85 (t, $J = 5.4$ Hz, 2H), 4.55 (t, $J = 5.4$ Hz, 2H), 6.95 (t, 1H), 7.15 (d, $J = 5.0$ Hz, 1H), 7.30 (d, $J = 5.0$ Hz, 1H), 7.80 (s, 1H), 8.05 (s, 1H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.8$ (CH_3), 47.0 (CH_2), 60.3 (CH_2), 127.7 (CH), 129.7 (CH), 131.6 (CH), 133.3 (CH), 138.5 (C), 141.4 (C), 151.8 (C), 156.7 (C). HRMS (EIMS) *m/z*: $[M + Na]^+$ calcd. for $C_{11}H_{12}N_4NaO_2S$ 287.05786; found 287.05732. Anal. Calcd. for $C_{11}H_{12}N_4O_2S$: C, 49.99; H, 4.58; N, 21.20; S, 12.13 Found: C, 49.91; H, 4.55; N, 21.13; S, 12.04.

*Furan-2-ylmethylene-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl]-amine (**3i**)* Yield 67%; Mp 74–76°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.50$ (s, 3H), 3.90 (t, $J = 5.6$ Hz, 2H), 4.65 (t, $J = 5.6$ Hz, 2H), 6.45 (dd, $J = 3.5$, 1.8 Hz 1H), 6.70 (d, $J = 3.5$ Hz, 1H), 7.50 (d, $J = 3.5$ Hz, 1H), 7.85 (s, 1H), 7.95 (s, 1H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.9$ (CH_3), 47.0 (CH_2), 61.0 (CH_2), 111.9 (CH), 115.4 (CH), 133.4 (CH), 138.5 (C), 145.5 (CH), 150.9 (C), 151.6 (C), 152.0 (CH). HRMS (EIMS) *m/z*: $[M - H]^+$ calcd. for $C_{11}H_{11}N_4O_3$ 247.08312; found 247.08366. Anal. Calcd. for $C_{11}H_{12}N_4O_3$: C, 53.22; H, 4.87; N, 22.57. Found: C, 53.17; H, 4.83; N, 22.46.

*[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethyl]-pyridin-4-ylmethylene-amine (**3j**)* Yield 90%; Mp 109–110°C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.45$ (s, 3H), 4.00 (t, $J = 5.5$ Hz, 2H), 4.70 (t, $J = 5.5$ Hz, 2H), 7.50 (d, $J = 5.8$ Hz, 2H), 7.90 (s, 1H), 8.10 (s, 1H), 8.65 (d, $J = 5.8$ Hz, 2H). ^{13}C-NMR ($CDCl_3$): $\delta = 14.8$ (CH_3), 46.7 (CH_2), 60.7 (CH_2), 121.2 (CH), 133.4 (CH), 138.4 (C), 141.9 (CH), 150.7 (C), 151.3 (C), 161.9 (CH). HRMS (EIMS) *m/z*: $[M - H]^+$ calcd. for $C_{12}H_{12}N_5O_2$ 258.09910; found 258.09972. Anal. Calcd. for $C_{12}H_{13}N_5O_2$: C, 55.59; H, 5.05; N, 27.01. Found: C, 55.35; H, 5.01; N, 26.74.

Antigiardial activity

Test organism

Giardia intestinalis WB strain (ATCC number 30957) was grown in a modified YI-S medium with antibiotics. The parasite was cultivated in 15 ml screw-capped borosilicate glass tubes containing 13 ml medium. The tubes were incubated on a 15° horizontal slant at 36–37°C. Culture maintenance and sub-culturing was performed as described in a previous publication (Saadeh *et al*., 2009). *Giardia* was harvested from confluent cultures by chilling of the tubes on ice for 5–10 min to detach cells, followed by centrifugation at $800\times g$ for 5 min.

Antigiardial assay

The antigiardial activity of the prepared molecules and metronidazole as the standard antigiardial drug were tested as described (Saadeh *et al*., 2009). In brief, the tested compounds and metronidazole were dissolved in dimethyl sulfoxide (DMSO) then in medium and filter-sterilized. Twofold dilutions starting at 15 μg/ml were prepared in a final volume of 15 ml to exclude air from the tube. Each tube was inoculated with 20,000 cells of Giardia. Each

compound was assayed in duplicate in each of three independent experiments. In each assay, the appropriate controls were performed, including the one without any compound and another with metronidazole as the positive control. The biological activity of the compounds was evaluated by counting the parasites in each tube using the standard hemacytometer. For counting, the parasites were harvested as described in the above section. In each count, trypan blue was employed to distinguish live from dead parasites (Aley *et al*., 1994).

Antibacterial and antifungal activity

Broth microdilution method in 96 microtitre plates (Cellstar®, Greinerbio-one, Germany) was employed to assess the antimicrobial activity of the prepared Schiff bases. In brief, stock solution (10 mg/ml) of each substrate was prepared in DMSO under aseptic conditions. The first experimental well was filled with the sabauroud dextrose broth (190 µl) and the other wells were filled with 100 µl of the sabauroud dextrose broth. A volume of 10 µl of each substance stoke solutions was added to the first well and a twofold serial dilution was then carried out across the plate. The antimicrobial activity of the reference substances of ampicillin and miconazole (both obtained as a gift from Dar Al Dawa Pharmaceutical Company, Naour-Jordan) and metronidazole was determined in the same manner and their results were used as quality control. Overnight batch cultures (10 µl) of *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538P, *C. sporogenes* ATCC 19404 and *Candida albicans* ATCC 10231 were used to inoculate the wells to achieve a final inoculum size of 1 × 106 cfu/ml and the plate was incubated for 24 h at 37°C. Growth media, conditions and positive and negative controls were performed according to published procedures (Saadeh *et al*., 2010). MIC was expressed as the mean concentration between the well showing growth and the well showing no growth. Growth was detected as turbidity (630 nm), relative to an un-inoculated well using a microtitre plate reader (Biotek, USA). Each MIC determination was carried out in triplicate.

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