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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new compounds derived from metronidazole and amino acids and their esters as antiparasitic agents

Ahmed T. Al-Masri · Haythem A. Saadeh · Ibrahim M. Mosleh · Mohammad S. Mubarak

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Abstract A number of new compounds derived from metronidazole and amino acids and their esters have been synthesized through a reaction between 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetic acid and a number of amino acid esters in the presence of *N*,*N*′carbonyldiimidazole (CDI). Hydrolysis of the esters derivatives with sodium hydroxide (4%) followed by acidification with hydrochloric acid (3 M) afforded the corresponding acids. The newly synthesized compounds were characterized by elemental analysis and by spectroscopic techniques such as ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. Some of the prepared compounds exhibited lethal activity against pathogenic protozoan parasites.

Keywords Metronidazole · Amino acid esters · *N*-*N*′-carbonyldiimidazole · Antiamoebic activity

Introduction

Nitroimidazole derivatives are an extremely important class of compounds; they are extensively used in the treatment of anaerobic infections and are under continuing investigation regarding their use as hypoxic cell cytotoxins, radiation sensitizers, and as anti-*Helicobacter pylori* agents (Cavalcanti *et al.*, 2004), *H. pylori*, a Gram-negative microaerophilic spiral bacterium, is the major factor in peptic ulcer diseases; highly effective treatment for *H. pylori* infections includes a combination of antisecretory and antimicrobial agents such as metronidazole (Cavalcanti *et al.*, 2004). The need for additional drugs of anti-*H. pylori* is an absolute necessity due to metronidazole-resistant *H. pylori* strains (Jenks and Edwards, 2002).

Mertonidazole, 2-(2-methyl-5-nitro-1*H*-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 infection 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 per ml (Salimi *et al.*, 1997). In view of the wide interest in the activity and profile of metronidazole, and as part of our ongoing research in the synthesis of new compounds of pharmacological interests (Al-Zghoul *et al.*, 2005; Salih *et al.*, 2006, 2007; Al-Soud *et al.*, 2008; Saadeh *et al.*, 2009; Abu-Shaireh *et al.*, 2009, Saadeh *et al.*, 2010), we report, herein, on the synthesis and characterization of a number of new compounds derived from metronidazole and amino acids and their esters, which, to the best of our knowledge, have not previously been described in the literature. In addition, the antiparasitic activities of the newly prepared compounds are presented.

A. T. Al-Masri · H. A. Saadeh · M. S. Mubarak (✉)
Department of Chemistry, University of Jordan,
Amman 11942, Jordan
e-mail: mmubarak@ju.edu.jo

I. M. Mosleh
Department of Biological Sciences, University of Jordan,
Amman 11942, Jordan

Results and discussion

Chemistry

2-(2-Methyl-5-nitro-1H-imidazol-1-yl)acetic acid (**2**), the starting material for compounds **4a–h** and **5b–g** was

synthesized through Jones oxidation of metronidazole (**1**) as shown in Scheme 1. Reaction of compound **2** with a solution of the appropriate amino acid ester (**3**) and triethyl amine in DMF, in presence of *N*,*N*′-carbonyldiimidazole, under nitrogen afforded the corresponding substituted (2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) acetamido)amino esters, **4a**–**h** as given in Scheme 1. 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 carbons. Additional support of the proposed structures comes from mass spectral data; mass spectra of the prepared compounds showed the correct molecular ions as suggested by their molecular formulas. The ^{1}H-NMR spectra of **4a**–**h** showed singlets at $\delta = 2.22$–2.47 ppm attributed to the metronidazole methyl group CH_3-C2 and singlets at $\delta \sim 7.90$ ppm ascribed to the C4-H of the metronidazole ring. Detailed ^{1}H-NMR signals are described in the experimental section. The ^{13}C-NMR spectra of the aforementioned compounds reveal clearly the presence of CH_3 signal $\delta = 14.0$–14.1 ppm for the CH_3 carbon and a carbonyl amide carbon signal at $\delta = 171.2$–173.1 ppm.

Moreover, polarimetric measurements indicated that the coupling reactions to produce the esters have proceeded with retention of configuration and racemization has been excluded. The same applies for the base promoted hydrolysis of the esters to the corresponding acids. For example, $[\alpha]^{DMF}$ for **4b** is -49.0^{o} and for **5b** -49.0^{o}.

Ethyl 2,6-bis(2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamido)hexanoate (**7**) was synthesized by reacting the diamino acid ester, lysine methyl ester (**6**) with compound **2** using the same general procedure employed for the synthesis of compounds **4b**–**h** as shown in Scheme 2. The solution was filtered off to yield the desired product in fairly good yield. The identity of the product is confirmed from ^{1}H-NMR data—which shows two signals for the two secondary amino groups—mass spectrometry, and by elemental analysis.

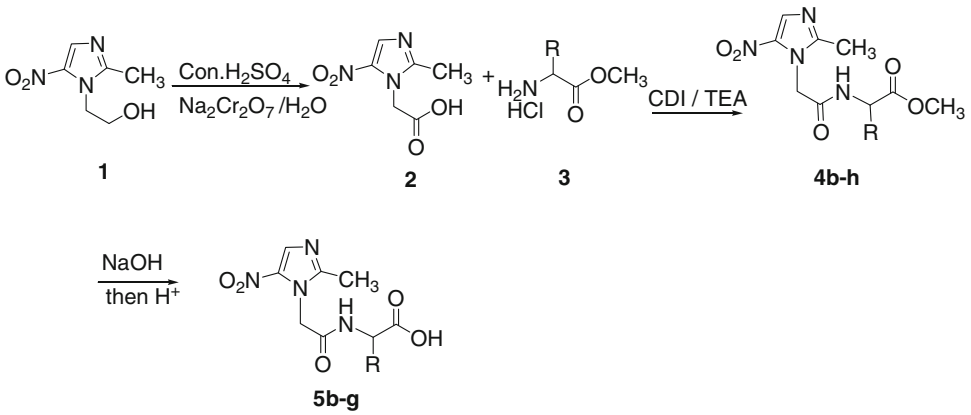
Base-pormoted hyrolysis of compounds **4a**–**g**, and **7** with 4% NaOH solution followd by acidification with HCl (3 M) yielded the corresponding amino acid derivatives of metronidazole **5b**–**g** and **8**. The identities of these compounds were confirmed by their ^{1}H NMR, ^{13}C NMR, mass spectra, and elemental analyses. The ^{1}H and ^{13}C-NMR spectra of all prepared compounds are in total agreement with their proposed structures.

Biological activity

In vitro antiamoebic and antigiardial activity and cytotoxicity

The newly synthesized metronidazole-derivatives were tested for their antiamoebic and antigiardial activity using in vitro bioassays. The cytotoxicity of these compounds on the two cell lines, Hep-2 and Vero cells, was also investigated and compared with that of the standard drug, metronidazole. The IC_{50} of the tested compounds against *Entamoeba histolytica*, *Giardia intestinalis*, and the two cell lines is given in Table 1. As indicated in the table, compounds **4a**, **4c**, **4h**, and **7** showed more potent activities against *Entamoeba* and *Giardia* than the standard drug with IC_{50} values ranging from 2.10 to 3.95 μM.

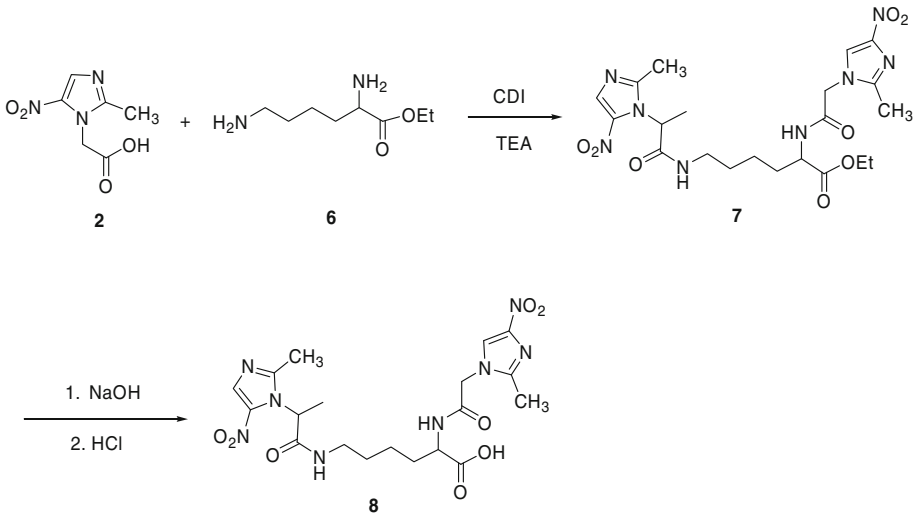
Scheme 1 Synthesis of (2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamido)esters (**4a**–**h**) and the corresponding acids **5b**–**g**



	a	b	c	d	e	f	g	h
R (aminoacid residue)	Ala	Pha	Asp	Tyr	Try	His	Lys	Pro

* **4a** is the ethyl ester analogue of **4**

Scheme 2 Synthesis of Ethyl 2,6-bis(2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamido) hexanoate (**7**) and 2,6-bis (2-(2-methyl-5-nitro-1H-imidazol-1-yl)acet-amido)hexanoic acid (**8**)



Compound **4a** displayed the highest activity against the two parasites. However, when the cytotoxicity of the prepared molecules is considered, compound **4c** appears to be better; its cytotoxicity was around four times less than that of **4a** (Table 1). The amino acid part apparently seems to influence the antiparasitic activity of the compound; introduction of some amino acid moieties such as alanine, aspartic, and proline increased the antiparasitic activity of the prepared compounds. In addition, data in Table 1 also reveal that the introduction of some groups on metronidazole can decrease its biological activities against the two tested parasites to variable degrees. Interestingly, the majority of the tested compounds exhibited almost similar activities against both *G. intestinalis and E. histolytica* (Table 1), indicating that each compound affects both parasites by a similar mechanism of action. The activities exhibited by the newly prepared compounds suggest that these new derivatives may be used as starting points in the development of new antiparasitic drugs. Moreover, the importance of such biologically active, noncytotoxic molecules, 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). Because of their low solubility in DMSO, the rest of the prepared compounds were not tested against the aforementioned parasites.

Conclusions

We have successfully synthesized and characterized a number of new compounds derived from metronidazole and amino acids and their esters. The newly synthesized compounds were screened for their antiparasitic activity and results showed that these compounds exhibited lethal activity against pathogenic protozoan parasites.

Experimental section

Chemicals

The following chemicals and materials were used without further purification:

Metronidazole, *N,N′*-carbonyldiimidazole, L-phenylalanine methyl ester hydrochloride, L-phenylalanine ethyl ester hydrochloride, and L-alanine methyl ester hydrochloride were obtained from Acros. L-Lysine ethyl ester dihydrochloride, L-lysine methyl ester dihydrochloride, and L-proline methyl ester dihydrochloride were purchased from Fluka. L-aspartic acid dimethyl ester hydrochloride, L-tyrosine methyl ester, L-tryptophan methyl ester hydrochloride, and L-histidine methyl ester dihydrochloride were obtained from Aldrich. Thin-layer chromatography was carried out using glass plates, precoated with silica gel 60 GF_{254}, supplied by Fluka.

Instrumentation

Melting points were measured with a Fischer-Johns melting point apparatus and were uncorrected. The ^{1}H and ^{13}C NMR spectra were acquired with the aid of a Bruker-DPX 300 MHz spectrometer (Germany) and are reported in ppm (δ) relative to TMS as an internal standard and in DMSO-d_6 as solvent. Coupling constants are in Hz; abbreviations: s = singlet, d = doublet, t = triplet, m = complex multiplets; bs = broad singlet. EIMS spectra were measured using a Finnegan MAT TSQ-70 spectrometers (Finnegan MAT, USA); ion source temperature = 200°C. High resolution mass spectral data were obtained with a Brucker APEX (IV) mass spectrometer (Bremen, Germany). 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

Table 1 The biological activity of the derivative compounds and metronidazole against the parasites, *Entamoeba histolytica* and *Giardia intestinalis*, and cancer (Hep-2) and nonmalignant (Vero) cells

Compound	IC_{50} ± SD (half the growth in negative control) μM		IC_0± SD (growth as in negative control) μM	
	Entamoeba histolytica	*Giardia intestinalis*	Hep-2 cells	Vero cells
4a	2.10 ± 0.20	2.18 ± 0.30	69.74 ± 2.70	72.17 ± 2.02
4b	22.13 ± 2.90	44.43 ± 4.83		
4c	2.21 ± 0.20	2.26 ± 0.34	304.16 ± 8.58	314.00 ± 12.77
4d	18.29 ± 2.60	17.79 ± 1.25		
4e	8.29 ± 1.10	16.57 ± 0.98		
4g	8.91 ± 0.4	8.93 ± 1.08		
4h	2.54 ± 0.55	2.70 ± 0.75	168.85 ± 5.01	203.62 ± 15.32
7	3.95 ± 0.25	3.84 ± 0.55		
5b	23.45 ± 1.55	22.22 ± 1.05		
5e	17.25 ± 1.15	18.00 ± 0.65		
5f	16.17 ± 1.21	17.21 ± 0.70		
Metronidazole	4.40 ± 0.60	5.17 ± 0.75	869.33 ± 25.32	903 ± 16.09

within ±0.4%. Compounds were checked for their purity by TLC using glass plates, precoated with silica gel 60 GF_{254}, supplied by Fluka.

Synthesis of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetic acid (**2**)

The title compound was synthesized according to the following general procedure: metronidazole (**1**) (5 g, 29.0 mmol) was added to a solution of sulfuric acid (5 ml) and ice-water (20 ml); the solution was stirred in an ice-bath until all metronidazole was dissolved. A solution of sodium dichromate (5 g, 20 mmol) in water (5 ml) was added drop-wise and the mixture was stirred overnight at room temperature, where the solution color changed to green. The solution was then extracted several times with ether; evaporation of the ether under reduced pressure gave 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetic acid (**2**) (3 g, 55%, Mp 172–174°C, Lit. Mp 176–178°C) (Khabnadideh *et al.*, 2007).

Compounds **4a–h** and **7** were synthesized and purified according to the following general procedure: A solution of *N,N′*-carbonyldiimidazole (CDI) (0.9 g, 5.50 mmol) in DMF (10 cm^3) was added to a solution of (2-methyl-5-nitro-1*H*-imidazol-1-yl)acetic acid (**2**) (1.0 g, 5.40 mmol) in DMF (3 ml) under N_2. After stirring for 15 min, a solution of the appropriate amino acid ester hydrochloride (**3**) (5.40 mmol) and triethylamine (1.0 ml) in DMF (4 ml) was added. The solution was then stirred overnight under N_2 and monitored by TLC. After consumption of the starting materials, most of the DMF was evaporated under reduced pressure and the residue was treated with water and kept in the freezer for 1 h. The desired products were collected by filtration. Using the aforementioned general procedure, the following compounds were synthesized.

Ethyl 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)-3-phenylpropanoate (**4a**)

Yield 1.20 g (73%), Mp 69–71°C. ^{1}H-NMR (DMSO-d_6) δ (ppm) = 1.26 (t, 3H, J = 7.14 Hz), 2.39 (s, 3H), 2.98–3.15 (m, 2H), 4.18 (q, 2H, J = 7.14 Hz), 4.82–4.98 (m, 3H) 6.50 (d, 1H, J = 7.81 Hz), 7.04–7.06 (m, 2H),7.21–7.28 (m, 3H), 7.91 (s, 1H). ^{13}C-NMR (DMSO-d_6): δ (ppm) = 14.1 (CH_3), 14.1 (CH_3), 37.7 (CH_2), 48.4 (CH_2), 53.4 (CH), 62.1 (CH_2); 127.4 (CH), 128.7 (CH), 129.3 (CH); 132.9 (CH); 135.4 (C); 138.4 (C); 151.4 (C); 164.7 (C); 171.2 (C). MS (70 eV) ($C_{18}H_{22}N_4O_4$), *m/z* (% rel. Int.): 358 (8) $[M]^+$; 343 (45); 315 (32); 288 (10); 270 (6); 242 (20); 176 (96); 150 (87); 132 (90); 92 (100); 53 (96). Anal. Calcd. for $C_{18}H_{22}N_4O_4$: C, 60.32; H, 6.19; N, 15.63. Found: C, 60.11; H, 6.08; N, 15.81.

Methyl 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido) propanoate (**4b**)

Yield 0.75 g (80%), Mp 142–144°C. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.40 (d, 3H, J = 7.18 Hz), 2.46 (s, 3H), 3.73 (s, 3H), 4.95 (d, 2H, J = 2.52 Hz), 4.52–4.62 (m, 1H), 6.72 (d, 1H, J = 6.85 Hz),7.93 (s, 1H). ^{13}C-NMR (DMSO-d_6): δ (ppm) = 14.1 (CH_3), 18.2 (CH_3), 48.4 (CH_2), 48.5 (CH_3), 52.8 (CH), 132.7 (CH), 138.5 (C), 151.5 (C), 164.7 (C), 173.1 (C). MS (70 eV) $C_{10}H_{14}N_4O_5$, *m/z* (% rel. Int.): 270 (18) $[M]^+$; 253 (27); 165 (86); 140 (59); 136 (45); 111

(88); 102 (67); 80 (92); 74 (82); 53 (100). HRMS (EIMS) *m/z*: calcd. for $C_{10}H_{14}N_4O_5$ $[M]^+$ 270.09642 found 270.09622. Anal. Calcd. for $C_{10}H_{14}N_4O_5$: C, 44.44; H, 5.22; N, 20.73. Found: C, 44.51; H, 5.16; N, 20.42.

Methyl 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)-3-phenylpropanoate (**4c**)

Yield 1.20 g (75%), Mp 88–90°C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.35$ (s, 3H), 2.86–3.06 (m, 2H), 3.70 (s, 3H), 4.50–4.97 (m, 1H), 4.82–4.97 (m, 2H), 7.04–7.06 (m, 2H), 7.19–7.24 (m,3H); 7.88 (s, 1H); 8.89 (d, 1H, $J = 7.76$ Hz). ^{13}C-NMR (DMSO-d_6): $\delta = 14.0$ (CH_3), 37.6 (CH_2), 48.2 (CH_2), 52.6 (CH_3), 53.4 (CH), 127.3 (CH), 128.7 (CH), 129.2 (CH), 132.7 (CH), 135.5 (C), 138.6 (C), 151.5 (C), 165.0 (C); 171.8 (C). MS (70 eV) ($C_{16}H_{18}N_4O_5$), *m/z* (% rel. Int.): 346 (2) $[M]^+$; 328 (56); 300 (24); 241 (6); 202 (3); 178 (4); 162 (96); 150 (60); 131 (71); 80 (84); 53 (100); 28 (73). Anal. Calcd. for $C_{16}H_{18}N_4O_5$: C, 55.49; H, 5.24; N, 16.18. Found: C, 55.57; H, 5.29; N, 16.13.

Dimethyl 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)succinate *(**4d**)*

Yield 1.07 g (64%); Mp 109–110°C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.47$ (s, 3H), 2.81–3.07 (m, 2H), 3.67 (s, 3H), 3.74 (s, 3H), 4.80–4.86 (m, 1H), 4.98 (d, 2H, $J = 3.5$ Hz), 7.06 (d, 1H, $J = 7.85$), 7.94 (s, 1H), 7.06 (d, 1H, $J = 7.85$ Hz). ^{13}C-NMR (DMSO-d_6): $\delta = 14.1$ (CH_3), 35.7 (CH_2), 48.4 (CH_2), 48.8 (CH), 52.8 (CH_3), 53.1 (CH_3), 132.7 (CH), 138.5 (C), 151.4 (C), 165.1 (C), 170.7 (C), 171.6 (C). MS (70 eV) $C_{12}H_{16}N_4O_7$, *m/z* (% rel. Int.): 328 (8) $[M]^+$; 311 (12); 283 (100); 269 (23); 222 (30); 180 (6); 168 (21); 140 (22); 111 (49); 80 (6); 28 (84); 15 (86). Anal. Calcd. for $C_{12}H_{16}N_4O_7$: C, 43.90; H, 4.91; N, 17.07. Found: C, 44.01; H, 4.84; N, 17.15.

Methyl 3-(4-hydroxyphenyl)-2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)propanoate (**4e**)

Yield 1.05 g (78%); Mp 106–108°C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.24$ (s, 3H), 2.74–2.93 (m, 2H), 3.56 (s, 3H), 4.37–4.44 (m, 1H), 4.96 (d, 2H, $J = 8.07$ Hz), 6.63 (d, 2H, $J = 8.40$ Hz), 6.96 (d, 2H, $J = 8.42$ Hz), 7.99 (s, 1H), 8.83 (d, 1H, $J = 7.82$ Hz), 9.26 (s, 1H, O*H*). ^{13}C-NMR (DMSO-d_6): $\delta = 14.1$ (CH_3), 36.6 (CH_2), 48.1 (CH_2), 52.4 (CH), 54.5 (CH_3), 115.5 (CH), 127.3 (C), 130.5 (CH), 132.8 (CH), 138.1 (C), 152.2 (C), 156.6 (C), 166.1 (C), 172.1 (C). MS (70 eV) $C_{16}H_{18}N_4O_6$, *m/z* (% rel. Int.): 362 (1) $[M]^+$; 303 (10); 256 (2); 227 (9); 197 (98); 150 (41); 147 (100); 107 (60); 77 (48); 68 (47). Anal. Calcd. for $C_{16}H_{18}N_4O_6$: C, 53.04; H, 5.01; N, 15.46. Found: C, 52.91; H, 5.12; N, 15.59.

Methyl 3-(1H-indol-3-yl)-2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)propanoate (**4f**)

Yield 1.38 g (69%); Mp 179–181°C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.22$ (s, 3H), 3.06–3.14 (m, 2H), 3.55 (s, 3H), 4.56–4.64 (m, 1H), 4.97 (d, 2H, $J = 10.8$ Hz), 6.96–7.04 (m, 2H), 7.14 (s, 1H), 7.31 (d, 1H, $J = 7.9$ Hz), 7.45 (d, 1H, $J = 7.71$ Hz); 7.99 (s, 1H), 8.87 (d, 1H, $J = 7.52$ Hz), 10.90 (s, 1H, N*H*-indole). ^{13}C-NMR (DMSO-d_6): $= 14.1$ (CH_3), 27.6 (CH_2), 48.2 (CH_2), 52.4 (CH), 53.9 (CH_3), 109.6 (C), 111.9 (CH), 118.4 (CH); 118.9 (CH), 121.5 (CH), 124.3 (C), 127.6 (C), 132.8 (CH), 136.6 (C), 139.0 (C), 152.2 (C), 166.2 (C), 172.3 (C). MS (70 eV) ($C_{18}H_{19}N_5O_5$), *m/z* (% rel. Int.): 385 (36) $[M]^+$; 326 (5); 280 (1); 201 (93); 197 (21); 170 (23); 131 (100); 103 (43); 80 (48); 53 (98); 28 (46). Anal. Calcd. for $C_{18}H_{19}N_5O_5$: C, 56.10; H, 4.97; N, 20.76. Found: C, 56.24; H, 4.86; N, 20.64.

Methyl 3-(1H-imidazol-4(5)-yl)-2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)propanoate (**4g**)

Yield 1.31 g, (63%), Mp 220–222°C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.29$ (s, 3H), 2.82–2.97 (m, 2H), 3.57 (s, 3H), 4.47–4.54 (m, 1H), 5.00 (s, 2H), 6.8 (s, 1H), 7.49 (s, 1H), 7.99 (s, 1H), 8.79 (d, 2H, $J = 6.98$ Hz), 11.84 (bs, 1H). ^{13}C-NMR (DMSO-d_6): $\delta = 14.1$ (CH_3), 29.7 (CH_2), 48.2 (CH_2), 52.4 (CH_3), 53.1 (CH), 132.8 (CH), 132.8 (CH), 135.4 (CH), 135.5 (C), 139.0 (C), 152.2 (C), 166.2 (C), 172.2 (C). MS (70 eV) ($C_{13}H_{16}N_6O_5$), *m/z* (% rel. Int.): 336 (4) $[M]^+$; 290 (37); 277 (29); 240 (8); 185 (27); 152 (31); 136 (30); 110 (26); 82 (66); 53 (75); 28 (100). Anal. Calcd. for $C_{13}H_{16}N_6O_5$: C, 46.43; H, 4.80; N, 24.99. Found: C, 46.52; H, 4.87; N, 25.07.

1-[2-(Methyl-5-notroimidazol-1-yl)acetyl]pyrolidine-2-carboxylic acid methyl ester (**4h**)

Yield 1.60 g (68%); Mp 115–118°C). ^{1}H-NMR (DMSO-d_6): $\delta = 1.83$–1.90 (m, 2H), 2.12–2.24 (m, 2H), 2.33 (s, 3H), 3.56 (s, 3H), 3.63–3.72 (m, 2H), 4.28–4.32 (m, 1H), 5.25 (s, 2H), 8.01 (s, 1H). ^{13}C-NMR (DMSO-d_6): $\delta = 14.0$ (CH_3), 24.9 (CH_2), 29.1 (CH_2), 46.4 (CH_2), 48.1 (CH_2), 52.3 (CH_3), 59.2 (CH), 132.7 (CH); 139.1 (C), 152.1 (C),164.8 (C), 172.4 (C). HRMS (ESI) *m/z*: calcd for $C_{12}H_{17}N_4O_5$ $[M + H]^+$ 297.111990, found 297.12008. Anal. Calcd. for $C_{12}H_{16}N_4O_5$: C, 48.65; H, 5.44; N, 18.91. Found: C, 48.53; H, 5.39; N, 18.74.

Ethyl 2,6-bis(2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamido)hexanoate (**7**)

Yield 0.95 g (69%), Mp 116–118°C. ^{1}H-NMR (DMSO-d_6): $\boldsymbol{\delta} = 1.13$ (t, 3H, $J = 7.0$ Hz), 1.24–1.39 (m, 4H),

1.53–1.76 (m, 2H), 2.33 (s, 6H), 3.01–3.08 (m, 2H), 4.04 (q, 2H, $J = 7.0$ Hz), 4.14–4.22 (m, 1H); 4.90 (s, 2H), 5.01 (s, 2H),7.99 (s, 2H), 8.31 (t, 1H), 8.78 (d, 1H, $J = 7.78$ Hz). ^{13}C-NMR (DMSO-d_6): $\boldsymbol{\delta} = 14.1$ (CH_3), 14.2 (CH_3), 14.4 (CH_3), 22.9 (CH_2), 28.9 (CH_2), 31.0 (CH_2), 38.9 (CH_2), 48.2 (CH_2), 48.5 (CH_2), 52.6 (CH); 61.1 (CH_2), 132.8 (CH), 139.0 (C), 152.3 (C), 165.9 (C), 166.3 (C), 172.1 (C). MS (70 eV) ($C_{20}H_{28}N_8O_8$), *m/z* (% rel. Int.): 508 (31) $[M]^+$; 462 (24); 446 (26); 249 (20); 205 (100); 138 (20); 111 (37). Anal. Calcd. for $C_{20}H_{28}N_8O_8$: C, 47.24; H, 5.55; N, 22.04. Found: C, 47.33; H, 5.42; N, 21.89.

Syntehsis 2- or 3-substituted 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)substituted propanoic acid (**5b–g**)

These compounds were synthesized via base-pormoted hyrolysis of their corresponding esters followd by acidification with HCl (6 M). The following general procedure was employed in the synthesis: each of compounds **4b–g** (5.50 mmol) was dissolved in 4% NaOH (40 ml) and stirred overnight. After the completion of the reaction, the solution was acidified with HCl (3 M) ($4.5 < pH < 6.5$). The precipitate was collected by filtration, washed with distilled water, dried in vacuum and purified using silica gel plates ($CHCl_3$:MeOH 95: 5) to obtain yellowish crystals. The structures of compounds were confirmed by NMR, mass spectrometry, and elemental analysis. The following compounds were synthesized using the aforementioned general procedure.

2-(2-(2-Methyl-5-nitro-1H-imidazol-1-yl)acetamido) propanoic acid (**5b**)

Yield 1.50 g (90%); Mp 183–185°C. ^{1}H-NMR (DMSO-d_6): = 1.25 (d, 3H, $J = 6.85$ Hz), 2.47 (s, 3H), 4.15–4.20 (m, 1H), 4.97 (d, 2H, $J = 4.80$ Hz), 7.97 (s, 1H); 8.70 (d, 1H, $J = 6.60$ Hz). ^{13}C-NMR (DMSO-d_6): $\delta = 14.0$ (CH_3), 17.7 (CH_3), 48.2 (CH_2), 48.4 (CH), 132.6 (CH), 139.0 (C), 152.3 (C), 166.0 (C); 174.2 (C). MS (70 eV) $C_9H_{12}N_4O_5$, *m/z* (% rel. Int.): 239 (24); 224 (2); 210 (100); 164 (29); 136 (35); 111 (65); 94 (28); 80 (74); 51 (70); 28 (85); 18 (60). HRMS (EIMS) *m/z*: calcd. for $C_9H_{11}N_4O_5$ $[M\text{-}H]^+$ 255.07295 found 255.07349. Anal. Calcd. for $C_9H_{12}N_4O_5$: C, 42.19; H, 4.72; N, 21.87. Found: C, 42.05; H, 4.86; N, 21.99.

2-(2-(2-Methyl-5-nitro-1H-imidazol-1-yl)acetamido)-3-phenylpropanoic acid (**5c**)

Yield 1.90 g (81%); Mp 230–232°C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.21$ (s, 3H), 2.86–3.06 (m, 2H), 4.43–4.47 (m, 1H), 4.94 (d, 2H, $J = 11.9$ Hz), 7.17–7.27 (m, 5H, aromatic hydrogens), 7.98 (s, 1H), 8.73 (d, 1H, $J = 8.08$ Hz, N*H*), 12.87 (bs, 1H). ^{13}C-NMR (DMSO-d_6): $\delta = 14.1$ (CH_3), 37.3 (CH_2), 48.1 (CH_2), 54.1 (CH), 127.0 (CH), 128.7 (CH); 129.6 (CH); 132.8 (CH), 137.7 (C), 139.0 (C), 152.2 (C), 165.9 (C), 172.9 (C). HRMS (EIMS) *m/z*: calcd. for $C_{15}H_{15}N_4O_6$ $[M\text{-}H]^+$ 331.10425, found 331.10414. Anal. Calcd. for $C_{15}H_{16}N_4O_5$: C, 54.21; H, 4.85; N, 16.86. Found: C, 54.32; H, 4.76; N, 16.81.

2-(2-(2-Methyl-5-nitro-1H-imidazol-1-yl)acetamido)succinic acid (**5d**)

Yield 1.80 g (35%); Mp 215–217°C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.32$ (s, 3H), 3.19 (d, 2H, $J = 6.9$ Hz), 4.51 (t, 1H), 5.01 (s, 2H), 7.98 (s, 1H), 8.69 (d, 1H, $J = 7.7$ Hz), 12.68 (bs, 2H). ^{13}C-NMR (DMSO-d_6): $\delta = 14.2$ (CH_3), 36.6 (CH_2), 48.3 (CH_2), 48.8 (CH), 132.8 (CH), 138.0 (CH), 153.1 (CH), 166.5 (CH), 172.5 (CH), 172.1 (CH). HRMS (EIMS) *m/z*: calcd. for $C_{10}H_{11}N_4O_6$ $[M\text{-}H]^+$ 283.06786, found 283.06821. Anal. calcd. for $C_{10}H_{12}N_4O_6$: C, 42.26; H, 4.26; N, 19.71. Found: C, 42.19; H, 4.19, N, 19.63.

3-(4-Hydroxyphenyl)-2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamido)propanoic acid (**5e**)

Yield 2.00 g (46%); Mp 254–256°C. $[\alpha]^{DMF} = +25.2^o$. ^{1}H-NMR (DMSO-d_6): $\delta = 2.23$ (s, 3H), 2.70–2.95 (m, 2H), 4.31–4.37 (m, 1H,), 4.95 (d, 2H, $J = 9.68$ Hz), 6.63 (d, 2H, $J = 8.24$ Hz), 6.97 (d, 2H, $J = 8.22$ Hz), 7.98 (s, 1H), 8.66 (d, 1H, $J = 7.96$ Hz); 9.20 (s, 1H), 12.81 (bs, 1H, O*H*). ^{13}C-NMR (DMSO-d_6): $\delta = 14.0$ (CH_3), 36.6 (CH_2), 48.1 (CH_2), 54.4 (CH), 115.4 (CH), 127.7 (C), 130.6 (CH), 132.8 (CH), 139.2 (C), 152.2 (C), 156.5 (C), 165.9 (C), 173.0 (C). MS (70 eV) ($C_{15}H_{16}N_4O_6$), *m/z* (% rel. Int.): 348 (6) $[M]^+$; 330 (2); 242 (11); 185 (75); 164 (77); 128 (35); 108 (49); 77 (73); 53 (100); 28 (88). HRMS (EIMS) *m/z*: calcd. for $C_{15}H_{15}N_4O_6$ $[M\text{-}H]^+$ 347.09971 found 347.09971. Anal. Calcd. for $C_{15}H_{16}N_4O_6$: C, 51.72; H, 4.63; N, 16.09. Found: C, 51.83; H, 4.68; N, 16.21.

3-(1H-indol-3-yl)-2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)propanoic acid (**5f**)

Yield 2.10 g (94%); Mp 174–176°C. ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.19 (s, 3H), 3.03–3.13 (m, 2H), 4.51–4.57 (m, 1H), 4.98 (d, 2H, $J = 10.81$ Hz), 6.92–7.05 (m, 2H), 7.13 (s, 1H), 7.29 (d, 1H, $J = 7.87$ Hz); 7.49 (d, 1H, $J = 7.43$ Hz), 7.98 (s, 1H), 8.68 (s, 1H), 10.91 (s, 1H). ^{13}C-NMR (DMSO-d_6): $\delta = 14.0$ (CH_3), 27.9 (CH_2), 48.3 (CH_2), 54.0 (CH), 110.2 (C), 111.8 (CH), 118.7 (CH), 118.9 (CH), 121.4 (CH), 124.1 (CH), 127.8 (C), 132.7

(CH), 136.5 (C), 139.0 (C), 152.2 (C), 165.8 (C), 172.3 (C). HRMS (EIMS) *m/z*: calcd. for $C_{17}H_{16}N_5O_5$ $[M\text{-}H]^+$ 370.11515, found 370.11553. Anal. Calcd. for $C_{17}H_{17}N_5O_5$: C, 54.98; H, 4.61; N, 18.86. Found: C, 54.85; H, 4.66; N, 18.76.

3-(1H-imidazol-4-yl)-2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)propanoic acid (**5g**)

Yield 1.85 g (32%); Mp 250–253°C). ^{1}H-NMR (DMSO-d_6): $\delta = 2.25$ (s, 3H), 2.78–2.99 (m, 2H), 4.18–4.22 (m, 1H), 5.00 (d, 2H, $J = 7.98$ Hz), 6.72 (s, 1H), 7.47 (s, 1H), 7.96 (s, 1H), 8.41 (m, 1H), 11.84 (bs, 1H). ^{13}C-NMR (DMSO-d_6): $\delta = 14.1$ (CH_3), 29.7 (CH_2); 48.3 (CH_2); 54.6 (CH), 119.2 (CH), 132.7 (CH), 134.8 (CH), 139.1 (C), 152.2 (C), 165.2 (C), 174.3 (C), 182.3 (C). HRMS (EIMS) *m/z*: calcd. for $C_{12}H_{13}N_6O_5$ $[M\text{-}H]^+$ 322.09474, found 322.09451. Anal. Calcd. for ($C_{12}H_{14}N_6O_5$): C, 44.72; H, 4.38; N, 26.82. Found: C, 44.67; H, 4.34; N, 26.73.

Synthesis of 2,6-bis(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamido)hexanoic acid (**8**)

The title compound was prepared, as given in Scheme 2, by dissolving compound **7** (2.80 g, 5.50 mmol) in 4% NaOH (40 ml) and the solution was stirred overnight at room temperature. The solution was then acidified with HCl (3 M) at pH 4.5, and the precipitate was collected by filtration, washed with distilled water, dried in vacuum, and purified using silica gel plates ($CHCl_3$:MeOH 95:5) to obtain yellowish crystals. (1.40 g, 54%, m.p. 220–221°C).

Yield 1.40 g, (54%); Mp 220–221°C. ^{1}H-NMR (DMSO-d_6): $\delta = 1.23$–1.46 (m, 4H), 1.51–1.82 (m, 2H), 2.33 (s, 6H), 2.93–3.14 (m, 2H), 4.14-4.19 (m, 1H), 4.91 (s, 2H), 5.01 (d, 2H, $J = 7.95$ Hz), 7.99 (s, 2H), 8.36 (t, 1H), 8.70 (d, 1H, $J = 7.78$ Hz). ^{13}C-NMR (DMSO-d_6): $\delta = 14.2$ (CH_3), 14.2 (CH_3), 23.0 (CH_2), 28.9 (CH_2), 31.1 (CH_2), 39.0 (CH_2), 48.2 (CH_2), 48.2 (CH_2), 52.4 (CH), 132.7 (CH), 139.0 (C), 152.3 (C), 165.8 (C); 166.2 (C), 173.7 (C). HRMS (EIMS) *m/z*: calcd. for $C_{18}H_{23}N_8O_8$ $[M\text{-}H]^+$ 479.16389, found 479.16353. Anal. Calcd. for $C_{18}H_{24}N_8O_8$: C, 45.00; H, 5.04; N, 23.32. Found: C, 45.18; H, 4.95; N, 23.43.

Biological activity

Test organisms

Entamoeba histolytica HK-9 strain (ATCC number 30015) was cultured in LYI-S-2 medium supplemented with antibiotics. *Giardia intestinalis* WB strain (ATCC number 30957) was grown in a modified YI-S medium with antibiotics. Both parasites were 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). *Entamoeba* and *Giardia* were 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.

Antiamoebic and antigiardial activity assay

The antiamoebic and antigiardial activities of the prepared compounds and metronidazole (the reference drug) were tested as previously described (Saadeh *et al.*, 2009). Briefly, the prepared compounds and metronidazole were separately dissolved in dimethyl sulfoxide (DMSO) then in medium and filter sterilized. Two-fold dilutions ranging from 0.1 to 60 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 the parasite under investigation. Each compound was assayed in duplicates in each of three independent experiments. In each assay, the appropriate controls were performed, including the one without any of the prepared compounds or metronidazole 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. In each count, trypan blue was employed to distinguish live from dead parasites (Aley *et al.*, 1994). The 50% inhibitory concentration (IC_{50}) was employed as a parameter for the antiamoebic and antigiardial activity. The IC_{50} is the concentration of tested compound or metronidazole which cuts the number of parasites to half that in the negative control (growth medium + DMSO + parasites).

Cytotoxicity assay

The cytotoxicity of the prepared compounds and metronidazole was investigated on Hep-2 and Vero cells using the standard cytotoxicity assay and the trypan blue exclusion method as described before (Saadeh *et al.*, 2009). Briefly, 100 μl portions of each cell suspension containing 10^5 cells/ml RPMI medium-10% heat inactivated fetal calf serum (FCS) were added to the wells of 96-well plates, incubated for 24 h, and the RPMI-FCS medium in each well was then replaced with 150 μl fresh medium. Solutions of the compounds and metronidazole were dissolved in DMSO, prepared in the RPMI-FCS medium, and filter sterilized. Then, 150 μl two-fold serial dilutions of each of the compounds and the reference drug starting at a concentration of 500 μg/ml in RPMI-FCS medium were prepared in the plates. After 48 h incubation, the number of cells in each well was determined using a hemacytometer. Each tested compound or metronidazole was assayed in duplicate in each of the three independent experiments. In

each assay, the negative controls (without any compound or reference drug) were included in duplicates. IC_0 was the concentration at which the number of cells in the tube was more than or equal to that of the negative control (IC_0 = no inhibition of cell growth).

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