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ORIGINAL PAPER

Synthesis and biological activity of new metronidazole derivatives

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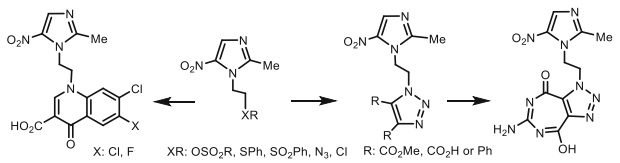
Abstract The development of new antimicrobial and antiparasitic agents offers the possibility of generating structures of increased potency. To this end, three sulphonate ester derivatives of metronidazole were synthesized. Treatment of the tosylate analogue with NaSPh and NaN_3 gave the thiophenolate and azide derivatives, respectively. Oxidation of phenylthio derivative with *m*CPBA afforded the sulfonyl analogue. Similarly, cycloaddition of azido-metronidazole with various symmetric acetylene compounds furnished the 1,2,3-triazole analogues. Treatment of dimethyl dicarboxylate metronidazole derivative with guanidine hydrochloride in the presence of base resulted in the formation of the ring-expanded (fat) derivative, triazolo-diazepam derivative of metronidazole. Treatment of chlorometronidazole with silylated quinolones gave the quinolone analogues of metronidazole. The antigardiasis and antifungal activities of the synthesized compounds were investigated. In addition, all synthesized compounds were evaluated for their in vitro anti-HIV activity in MT-4 cells as non-nucleoside reverse transcriptase inhibitors.

Graphical abstract



Keywords Anti-HIV activity · Antiparasitic/antifungal activity · Cycloadditions · Metronidazole · Triazoles

Introduction

Nitroimidazoles have important pharmaceutical applications, particularly as anaerobic antibacterials and antiprotozoal agents [1]. The antimicrobial activity of these chemotherapeutic agents inhibits the growth of both anaerobic bacteria and certain anaerobic protozoa such as *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Giardia lamblia* [2]. Some nitroimidazoles were reported as potent radiosensitizers in treatment of cancer [3–5], antitubercular therapy [6, 7], control of fertility [8], and as anti-*Helicobacter pylori* agents [9, 10]. *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. Furthermore, some 5-nitroimidazole have been tested in cell-based assays and in enzyme assays against HIV-1 recombinant reverse transcriptase [11, 12] and as anticancer agents [12]. In addition, others are under investigation for their use as hypoxic cell cytotoxines [13].

Metronidazole, 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (Flagyl) [14–16] has been regarded as the drug of choice that is commonly used for the treatment of infections caused by *Giardia lamblia* and *Trichomonas vaginalis*, and also acts as an amoebicidal in cases of amoebiasis caused by *Entamoeba histolytica*; thus several metronidazole derivatives were reported as potent antiparasitic agents [17, 18]. In 2009, Mital [19] has

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reviewed a brief account of various biological activities exhibited by synthetic nitroimidazole derivatives as well as their structure–mutagenicity relationships. Recently, Mubarak et al. [20–23] have reported the synthesis of new metronidazole derivatives with evaluation of their antibacterial and antifungal activity.

Based on these pharmacological activities, and in continuation of our work on nitroimidazoles [24–29], we report here the synthesis of new mitronidazole derivatives, with the aim to develop new antifungal, antiparasitic and anti-HIV agents.

Results and discussion

Chemistry

Prior studies on side chain modifications of metronidazole did not show remarkable structural themes that could be exploited to generate compounds with superior activity [30, 31]. However, the nitro group is critical for antimicrobial activity. We decided here to design new structural diversity of metronidazole, aiming for development of new improved antiparasitic agents. Thus, sulfonylation of metronidazole (**1**), with 4-bromobenzenesulfonyl, camphorsulfonyl, and 4-methylsulfonyl chlorides in the presence of dry pyridine afforded the sulfonate ester derivatives **2**–**4** in 76, 69, and 82 % yield, respectively. Displacement of the tosylate group at **4** [32], via SN_2 mechanism, by treatment with sodium thiophenolate at 120 °C furnished the phenylthio analogue **5** (71 %). Analogously, treatment of **4** with sodium azide in DMF resulted in SN_2 displacement of the sulfonate ester group by the azide residue **6** (89 %) (Scheme 1), which was identical in the physical properties for those prepared previously from the mesylate derivative [33]. The azide alkyne cycloaddition reaction is used widely in synthesis of various substituted 1,2,3-triazole derivatives [34], since they are suitable for generating new antimicrobial drugs. Thus, treatment of the azide **6** with three symmetrical acetylene compounds: dimethyl acetylenedicarboxylate, acetylene dicarboxylic acid, and diphenylacetylene in toluene at 100-120 °C furnished, after purification, the 1,2,3-triazole analogues **7**–**9** in 43, 37, and 63 % yield (Scheme 1).

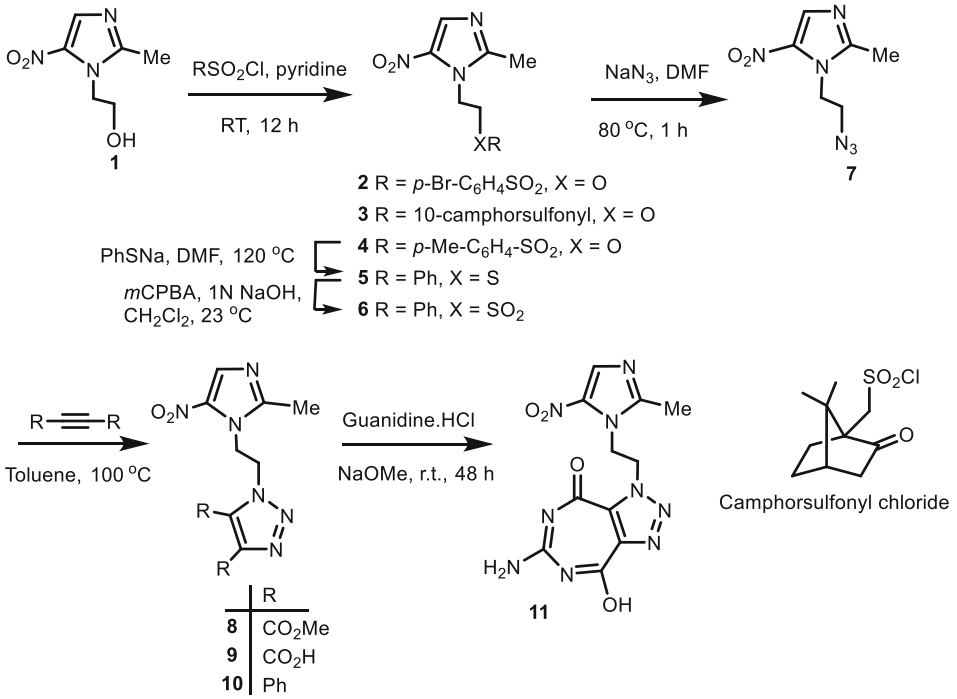
Recently, Hosmane et al. [35–37] have reported numerous papers concerning the chemical and biological activity of ring-expanded (fat) heterocycles, containing the imidazo[4,5-*e*][1,3]diazepine ring system with their potency as inhibitors of NTPases/helicases of flaviviridae including the West Nile virus (WNV), hepatitis C virus (HCV), hepatitis B virus (HBV), and Japanese encephalitis virus (JEV). Accordingly, we have undertaken synthesis of new metronidazole with triazolo-diazepine moiety as a new potential antiviral candidate. Treatment of **7** with guanidine hydrochloride in the presence of sodium methoxide at room temperature afforded **10** in 40 % yield (Scheme 1).

The structures of **2**–**10** were determined by their ^{1}H, ^{13}C, and 2D NMR spectra, since metronidazole backbone protons showed a similar pattern. H-4 of the imidazole ring appeared as singlet in the region $\delta = 8.05$–7.26 ppm, whereas methyl protons at C-2 of the same ring resonated as singlets in the region 2.52–2.41 ppm. NCH_2 protons appeared as triplets ($J \sim 5.1$ Hz), multiplets, or broad singlets at 4.65–3.68 ppm, while OCH_2 methylene protons of compounds **2**–**4** resonated at 3.67 ppm (t, $J = 5.0$ Hz), 3.46 ppm (br s.), and 4.41 ppm (t, $J = 5.1$ Hz), respectively. In addition, SCH_2 and N_3-CH_2 protons of **5** and **6** appeared as triplets at 3.79, 3.67 ppm ($J = 5.2$, 5.1 Hz), respectively. The methylene protons ($N_{triazole}$-CH_2) of **7**–**10** appeared as triplets or broad singlets at the regions 4.34–3.38 ppm. The other aliphatic and aromatic protons were fully analysed (cf. "Experimental"). The ^{13}C NMR spectra of **2**–**10** contained similar resonance signals of the metronidazole carbon atoms. Carbon atoms 2, 4, and 5 of the metronidazole scaffold resonated at the regions 152.7–149.6, 133.8–131.4, and 139.7–137.8 ppm, respectively, whereas the carbonyl carbon atom of 3 appeared at 214.0 ppm. Signals at lower field of 164.4 and 161.7 and 167.7 ppm were assigned to C=O carbon atoms of **7** and **8**, whereas the carbonyl carbon atoms at 8′ and 6′ of **10** appeared at 190.7 and 164.0 ppm, respectively. The chemical shifts between 48.9 and 41.6 ppm were assigned to the $N_{imidazole}CH_2$ carbon atom, except for those of **9**, which appeared at 59.7 ppm. The signals at 69.1, 68.1, and 68.3 ppm were assigned to CH_2O carbon atom, while the chemical shifts in the region 59.2–53.2 ppm were attributed to $N_{triazole}CH_2$ carbon atom of **7**–**10**. The signals of other carbon atoms were fully analysed (cf. "Experimental").

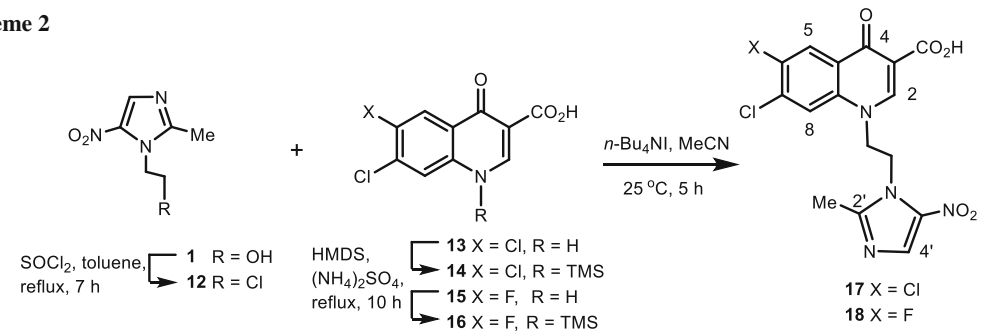
Additionally, some quinolines are a family of synthetic broad spectrum antibacterial drugs, for example ciprofloxacin is an antibiotic that can treat a number of bacterial infections [38]. This has attracted our attention towards the synthesis of new metronidazole analogues conjugated with the antibacterial quinolone bases, via silylation method [39], aiming to evaluate their antimictobial and antiviral activities. Thus, metronidazole (**1**) was converted first into the chloro analogue **12** following Aita method [40]. Treatment of the silylated quinolones **14** and **16**, prepared by refluxing bases **13** and **15** in hexamethyldisilazane (HMDS), with **12** in the presence of *n*-Bu_4NI as catalyst in dry acetonitrile as solvent at 25 °C to give **17** (67 %) and **18** (64 %), respectively (Scheme 2).

Structures of **17** and **18** were confirmed from their ^{1}H, ^{13}C NMR, and mass spectra. Compound **17** has been

Scheme 1



Scheme 2



selected for further NMR studies. From the gradient selected HMBC [41] spectrum of **17**, H-2 of the quinolone backbone at $\delta_H = 8.85$ ppm showed a $^3J_{C,H}$ heteronuclear correlation with CH_2 carbon atom at N-1′ of imidazole ring at $\delta_C = 47.0$ ppm. Two similar correlations between CH_2 protons at N-1 of quinolone moiety at $\delta_H = 3.41$ ppm and C-2 at $\delta_C = 143.7$ ppm as well as with C-8a of quinolone ring at $\delta_C = 137.7$ ppm were observed (Fig. 1).

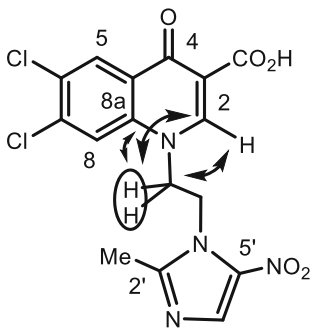


Fig. 1 $J_{C,H}$ correlations in the HMBC NMR spectrum of **17**

Antigiardiasis and antifungal activity

Compounds **2**, **3**, **5**, **6**, **8**–**11**, **17**, and **18** were screened for their antimicrobial activity by use of in vitro bioassays and

Table 1 Minimum inhibitory concentrations (MIC) of the tested substances and reference drug against the tested microorganisms

Compd.	MIC/μM		
	G. intestinals	*C. tropicalis*	*C. albicans*
2	7.90	432	>500
3	10.24	>500	>500
5	3.25	>500	>500
6	0.89	263	87
8	2.22	>500	>500
9	2.85	>500	>500
10	5.23	>500	>500
11	3.21	182	122
17	8.31	182	122
18	6.81	138	79
Metronidazole	5.03	128	64

assessed as minimal inhibitory concentration (MIC) values. Results are summarised in Table 1, in which the data for metronidazole [42] was included for comparison. The fungal strains utilised in the assays were *C. tropicalis* ATCC 13803 and *C. albicans* ATCC 40227. As shown in Table 1, compound **6** was the most potent candidate against Giardia, with an IC_{50} value of 0.89 μM compared with 5.03 μM for metronidazole. Table 1 revealed also that all synthesized compounds were inactive or moderately active against *C. tropicalis and C. albicans*, with IC_{50} values ranging from >500 μM to 87 μM compared to metronidazole.

On the contrary, structural modification of the hydroxyl group of metronidazole into sulphonyl group positively influenced the antigiardial activity, in addition to moderate activity in antifungal potency against *C. albicans.*

In vitro anti-HIV activity

Compounds **2**–**11**, **17**, and **18** were tested for their anti-HIV-1 activity in vitro, using III_B strain in human MT-4 cells, based on a Microculture Tetrazolium (MTT) assay [43, 44]. Cytotoxicity was also measured on MT-4 cells, where results are summarized in Table 2, nevirapine (BOE/BIRG587) [45] was used as a reference drug. All compounds were found to be inactive except compounds **6** and **11**, which showed remarkable anti-HIV-1 activity with EC_{50} values of >2.23 and >7.28 μM, and CC_{50} values of 24.75 and 29.12 μM, resulting in SI values of 11 and 4, respectively.

With respect to SAR, derivatization of the hydroxyl group of metronidazole with sulphonyl group (e.g. **6**) or triazolo-diazepam (e.g. **11**) moieties has considerably increased the anti-HIV activity compared for those with other functional groups. Based on the above data, compounds **6** and **11** might be considered new promising anti-HIV-1 agents to act as NNRTIs for further structural modifications and pharmacological evaluation.

Table 2 In vitro anti-HIV-1 and HIV-2 activity of some new metronidazole analogues

Compd.	Virus strain	EC_{50}/μM[a]	CC_{50}/μM[b]	SI[c]
2	III_B	>19.92	19.92	<1
	ROD	>19.92	19.92	<1
3	III_B	>105.00	>105.00	X1
	ROD	>105.00	>105.00	X1
4	III_B	>95.70	95.70	<1
	ROD	>95.70	95.70	<1
5	III_B	>18.32	>18.32	X1
	ROD	>18.32	>18.32	X1
6	III_B	2.23	24.75	11
	ROD	>24.75	24.75	<1
7	III_B	>87.34	>87.34	X1
	ROD	>87.34	>87.34	X1
8	III_B	>102.30	>102.30	X1
	ROD	>102.30	>102.30	X1
9	III_B	>97.80	>97.80	X1
	ROD	>97.80	>97.80	X1
10	III_B	>68.9	68.9	<1
	ROD	>68.9	68.9	<1
11	III_B	>7.28	29.12	4
	ROD	>29.12	29.12	<1
17	III_B	>32.23	32.23	<1
	ROD	>32.23	23.23	<1
18	III_B	>19.22	19.22	<1
	ROD	>19.22	19.22	<1
Nevirapine	III_B	0.05	>4	>80
	ROD	>4	>4	<1

Anti-HIV-1 activity measured on strain III_B

Anti-HIV-2 activity measured using strain ROD

[a] Compound concentration required to achieve 50 % protection of MT-4 cells from HIV-1 and HIV-2 induced cytopathogenic effects

[b] Compound concentration that reduces the viability of mock-infected MT-4 cells by 50 %

[c] Selectivity index (CC_{50}/EC_{50})

Conclusion

In conclusion, we have synthesized new metronidazole analogues, including the derivatization of the hydroxyl group with various potential groups. Some of these analogues exhibited activity against *Giardia* and HIV-1 which are considered as promising antiparasitic and anti-HIV

candidates for further structural development and pharmacological study.

Experimental

Melting points were measured on a Büchi melting point apparatus B-545 (BÜCHI Labortechnik AG, Switzerland). Elemental analyses (C, H, N, S) were conducted using a Vario Elementar apparatus (Shimadzu, Japan), their results were found to be in good agreement (±0.3 %) with the calculated values. NMR spectra were recorded on 400 and 600 MHz (^{1}H) and at 100 and 150.91 MHz (^{13}C) spectrometers (Bruker, Germany) with TMS as internal standard and on δ scale in ppm. Heteronuclear assignments were verified by ^{1}H-^{13}C HMBC experiment. Metronidazole tosylate (**4**) (m.p.: 152 °C; Ref. [32] 153 °C) and azide analogue **7** (m.p.: 55–58 °C; Ref. [33] 56–58 °C) were synthesized and purified according to published procedures.

General procedure for the synthesis of the imidazole arylsulfonate esters 2–4

A solution of arylsulphonyl chloride (2.00 mmol) in 10 cm^3 dichloromethane was added dropwise onto a stirred, cooled (~10 °C) solution of 342 mg metronidazole **1** (2.00 mmol) in 10 cm^3 dry pyridine. The mixture was stirred at room temperature for 6–10 h and the progress of reaction was followed by TLC. After completion of the reaction, few drops of water were added and the mixture was stirred for 1 h. Chloroform (20 cm^3) was added and the solution was portioned successively with 10 % sulphuric acid (3 × 20 cm^3), 20 cm^3 10 % $NaHCO_3$, and finally with water. The combined organic extracts was dried (Na_2SO_4), filtered, and evaporated to dryness. The crude product was recrystallized from EtOH to give the desired sulfonate ester derivative.

*2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-bromobenzenesulfonate (**2**, $C_{12}H_{12}BrN_3O_5S$)*

From 511 mg 4-bromobenzenesulfonyl chloride. Yield: 580 mg (76 %) as colourless crystals; m.p.: 144–145 °C; $R_f = 0.48$; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.94$ (s, 1H, H-4), 7.51 (d, 2H, $J_{2',3'} = 8.2$ Hz, $H_{arom.}$-3′+$H_{arom.}$-5′), 7.21 (d, 2H, $J_{5',6'} = 8.2$ Hz, $H_{arom.}$-2′+$H_{arom.}$-6′), 4.35 (t, 2H, $J = 5.0$ Hz, NCH_2), 3.67 (t, 2H, $J = 5.0$ Hz, OCH_2), 2.50 (s, 3H, C_2-Me) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): $\delta = 151.6$ (C-2), 145.2 ($C_{arom.}$-1′), 138.2 (C-5), 132.9 (C-4), 132.9 ($C_{arom.}$-3′ + $C_{arom.}$-5′), 129.4 ($C_{arom.}$-2′ + $C_{arom.}$-6′), 129.2 (C–Br), 69.1 (OCH_2), 44.9 (NCH_2), 13.9 (C_2-*Me*) ppm; MS (FAB): *m/z* = 312/314 ($[M+Na]^+$).

*2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl (7,7-dimethyl-2-oxobicylo[2.2.1]heptan-1-yl)methanesulfonate (**3**, $C_{16}H_{23}N_3O_5S$)*

From 501 mg camphorsulphonyl chloride. Yield: 530 mg (69 %) as colouirless crystals; m.p.: 135–137 °C; $R_f = 0.62$; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.94$ (s, 1H, H-4), 4.65 (br s, 4H, NCH_2+OCH_2), 3.46 (d, 2H, $J = 6.5$ Hz, CH_2SO_2), 2.93 (m, 2H, CH_2-2″), 2.52 (s, 3H, C_2-Me), 1.91 (m, 1H, H-4′), 1.45 (m, CH_2-5′+CH_2-6′), 0.81 (s, 6H, $C_{7''}$-Me_2) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): $\delta = 214.5$ (C=O), 151.4 (C-2), 138.2 (C-5), 133.0 (C-4), 68.5 (OCH_2), 57.8 (C-4′), 48.2 (CH_2SO_2), 45.7 (CH_2-2′), 42.6 (C-7′), 42.4 (NCH_2), 35.2 (C-6′), 31.9 (C-5′), 29.7 (C-7), 19.4 (C_7-Me_2), 14.1 (C_2-*Me*) ppm; MS (FAB): *m/z* = 408 ($[M+Na]^+$).

*2-Methyl-5-nitro-1-[2-(phenylthio)ethyl]-1H-imidazole (**5**, $C_{12}H_{13}N_3O_2S$)*

To a solution of 650 mg **4** (2.00 mmol) in 10 cm^3 DMF was added 330 mg sodium thiophenolate (2.00 mmol) and the mixture was stirred at 120 °C for 6 h. After cooling, the mixture was evaporated to dryness and the residue was partitioned between $CHCl_3$ (3 × 15 cm^3) and 15 cm^3 water. The combined organic extracts was dried (Na_2SO_4), filtered, and evaporated to dryness. The crude product was purified on a short SiO_2 column (5 g), by elution in gradient with MeOH (0–5 %) and chloroform as eluent to give **5** (400 mg, 71 %) as colourless crystals. M.p.: 154–156 °C; $R_f = 0.80$; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 8.00$ (s, 1H, H-4), 7.63–7.11 (m, 5H, H_{arom}), 4.34 (t, 2H, $J = 5.2$ Hz, NCH_2), 3.40 (t, 2H, $J = 5.2$ Hz, OCH_2), 2.45 (s, 3H, C_2-Me), 7.92 (s, 1H, H-4), 7.60 (d, 2H, $J_{2',3'} = 8.3$ Hz, $H_{arom.}$-3′+$H_{arom.}$-5′), 7.40 (d, 2H, $J_{5',6'} = 8.3$ Hz, $H_{arom.}$-2′+$H_{arom.}$-6′), 4.57 (t, 2H, $J = 5.0$ Hz, NCH_2), 4.41 (t, 2H, $J = 5.0$ Hz, OCH_2), 2.41 (s, 3H, C_2-Me), 2.40 (s, 3H, Me-Ar) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): $\delta = 151.9$ (C-2), 137.8 (C-5+$C_{arom.}$-1′), 132.9 (C-4), 132.8 ($C_{arom.}$-2′+$C_{arom.}$-6′), 128.4 ($C_{arom.}$-3+$C_{arom.}$-6′), 125.5 ($C_{arom.}$-4′), 59.7 (NCH_2), 34.0 (SCH_2), 14.1 (C_2-*Me*) ppm; MS (FAB): *m/z* = 264 ($[M+H]^+$).

*2-Methyl-5-nitro-1-[2-(phenylsulfonyl)ethyl]-1H-imidazole (**6**, $C_{12}H_{13}N_3O_4S$)*

To a stirred cold solution of 74 mg **5** (0.28 mmol) in 10 cm^3 CH_2Cl_2 was added 100 mg *m*-chloroperbenzoic acid (*m*CPBA, 0.58 mmol). After stirring for 6 h at 25 °C, the solution was evaporated to dryness and the residue was partitioned between $CHCl_3$ (2 × 10 cm^3) and 10 cm^3 water. The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was poured onto a SiO_2 column (10 g), with MeOH, in gradient (0–10 %), and $CHCl_3$ as eluent to give **6** (60 mg, 73 %), as white crystals. M.p.: 149–151 °C; $R_f = 0.8$; ^{1}H NMR

(400 MHz, DMSO-d_6): $\delta = 8.11$ (m, 2H, H_{arom}), 7.91 (s, 1H, H-4), 7.65–7.48 (m, 3H, H_{arom}), 4.32 (t, 2H, $J = 5.1$ Hz, NCH_2), 3.48 (t, 2H, $J = 5.1$ Hz, OCH_2), 2.42 (s, 3H, C_2-Me) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): δ 151.3 (C-2), 138.0 (C-5+$C_{arom.}$-1′), 132.3 (C-4+$C_{arom.}$-4), 131.3 ($C_{arom.}$-2′+$C_{arom.}$-6′), 128.6 ($C_{arom.}$-3+$C_{arom.}$-6′), 125.5 ($C_{arom.}$-4′), 59.4 (SCH_2), 35.1 (NCH_2), 14.0 (C_2-*Me*) ppm; MS (FAB): $m/z = 296$ ($[M+H]^+$).

General procedure of cycloaddition reaction of metronidazole azide 7 with substituted acetylene compounds

To a stirred solution of 392 mg **7** (2.00 mmol) in 10 cm^3 dry toluene was added acetylene derivative (2.10 mmol) and the mixture was heated, with stirring, at 100–120 °C for 5–6 h. After cooling, the mixture was evaporated to dryness and the residue was partitioned between $CHCl_3$ (3x15 cm^3) and 15 cm^3 water. The combined organic extracts was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified on a short column of silica gel (5 g) and elution, in gradient, with MeOH (0–20 %) and $CHCl_3$ as eluent to afford the desired 1,2,3-triazole analogue.

*Dimethyl 1-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-1,2,3-triazole-4,5-dicarboxylate (**8**, $C_{12}H_{14}N_6O_6$)*
From 296 mg dimethyl acetylenedicarboxylate. Yield: 291 mg (43 %) as yellow powder. M.p.: 260–263 °C (dec.); $R_f = 0.89$; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 8.01$ (s, 1H, H-4), 4.35 (m, 4H, $N_{imidazole}CH_2$+$N_{triazole}CH_2$), 3.67, 3.36 (2 s, 6H, 2xCO_2Me), 2.45 (C_2-Me) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): $\delta = 164.4$, 161.7 (*CO_2*Me), 152.7 (C-2), 139.5 (C-5), 133.2 (C-4), 121.5 ($C_{triazole}$-4), 117.7 ($C_{triazole}$-5), 53.9 (CO_2*Me*), 53.2 ($N_{triazole}$-CH_2), 41.6 ($N_{imidazole}$-CH_2), 11.4 (C_2-*Me*) ppm; MS (FAB): $m/z = 339$ ($[M+H]^+$).

*1-[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]-1,2,3-triazole-4,5-dicarboxylic acid (**9**, $C_{10}H_{10}N_6O_6$)*
From 240 mg acetylene dicarboxylic acid. Yield: 230 mg (37 %) as yellow powder. M.p.: 290–294 °C (dec.); $R_f = 0.39$; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 8.02$ (s, 1H, H-4), 4.35 (m, 4H, $N_{imidazole}CH_2$+$N_{triazole}CH_2$), 2.46 (C_2-Me) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): $\delta = 167.7$ (CO_2H), 151.7 (C-2), 139.7 ($C_{triazole}$-5), 138.2 (C-5), 132.8 (C-4+$C_{triazole}$-4), 59.6 ($N_{triazole}$-CH_2), 48.2 ($N_{imidazole}$-CH_2), 14.1 (C_2-*Me*) ppm; MS (FAB): $m/z = 333$ ($[M+Na]^+$).

*1-[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]-4,5-diphenyl-1H-1,2,3-triazole (**10**, $C_{20}H_{18}N_6O_2$)*
From 374 mg diphenylacetylene. Yield: 472 mg (63 %) as yellow powder. M.p.: 153–155 °C; $R_f = 0.64$; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 8.01$ (s, 1H, H-4), 7.48-7.10 (m, 10H, H_{arom}), 4.35 (t, 2H, $J = 5.0$ Hz, $N_{imidazole}CH_2$), 3.67 (t, 2H, $J = 5.0$ Hz, $N_{triazole}CH_2$), 2.45 (s, 3H, C_2-Me) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): $\delta = 151.9$ (C-2), 142.4 ($C_{triazole}$-4), 139.7 ($C_{triazole}$-5), 137.0 ($C_{imidazole}$-5), 133.8 ($C_{triazole}$-4), 132.8, 127.9, 127.4, 125.4 ($C_{arom.}$), 59.6 ($N_{triazole}$-CH_2), 48.1 ($N_{imidazole}$-CH_2), 14.2 (C_2-*Me*) ppm; MS (FAB): $m/z = 375$ ($[M+H]^+$).

*6-Amino-8-hydroxy-3-[2(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-[1,2,3]triazolo[4,5-e][1,3]diazepine-4-one (**11**, $C_{11}H_{11}N_9O_4$)*
To a cooled stirred solution of sodium methoxide (from 750 mg sodium and 15 cm^3 MeOH) was added 380 mg guanidine hydrochloride (4.00 mmol) and the solution was stirred at ice-bath for 30 min then filtered from sodium chloride. The filtrate was added to a stirred cooled solution of 332 mg **8** (1.00 mmol) in 20 cm^3 MeOH and the stirring was continued at room temperature for 48 h. The mixture was filtered and the filtrate was neutralised with 2 M hydrochloric acid and the precipitate was filtered and dried, followed by suspension with 20 cm^3 methanol. The suspension was heated under reflux for 30 min and filtered as a hot mixture. The filtrate was evaporated to dryness to give **11** (133 mg, 40 %) as a yellow crystals. M.p.: 195–198 °C; $R_f = 0.31$; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 8.95$ (br s, 2H, NH_2), 7.26 (s, 1H, H-4), 5.50 (br s, 1H, OH), 3.68 (s, 2H, $N_{imidazole}CH_2$), 3.38 (s, 2H, $N_{triazole}CH_2$), 2.43 (s, 3H, C_2-Me) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): $\delta = 190.7$ ($C_{8'}$=O), 164.0 ($C_{6'}$=O), 158.2 (C-4′), 149.6 (C-2), 139.5 ($C_{imidazole}$-5+C-8a′), 139.3 (C-3a′), 131.4 ($C_{imidazole}$-4), 59.2 ($N_{triazole}$-CH_2), 48.9 ($N_{imidazole}$-CH_2), 11.3 (C_2-*Me*) ppm; MS (FAB): $m/z = 356$ ($[M+Na]^+$).

*1-(2-Chloroethyl)-5-methyl-2-nitro-1H-imidazole (**12**)*
This compound was prepared according to the procedure of Atia [40] from 300 mg metronidazole (**1**, 1.75 mmol) and 240 mg thionyl chloride (2.00 mmol). Yield: 272 mg (82 %); m.p.: 86–88 °C (Ref. [39] 85–87 °C).

6,7-Dihalo-1-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acids
General procedure. A suspension of the quinolone bases **13** and **15** (0.76 mmol) in 20 cm^3 hexamethyldisilazane and a few crystals of $(NH_4)_2SO_4$ were heated under reflux for 10 h. After cooling, the solution was evaporated to dryness and the residue was dissolved in 20 cm^3 dry acetonitrile to give the silylated quinolones **14** and **16**. Chloride **12** (100 mg, 0.53 mmol) in 10 cm^3 dry acetonitrile was added dropwise followed by addition of 150 mg *n*-Bu_4NI. After stirring at 25 °C for 5 h, the solution was evaporated to dryness and the residue was partitioned between $CHCl_3$ (3 × 15 cm^3) and 15 cm^3 water. The combined organic extracts were dried ($MgSO_4$) and evaporated to dryness.

Crystallization from EtOH/hexane afforded the pure desired product.

*6,7-Dichloro-1-[2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**17**, $C_{16}H_{12}Cl_2N_4O_5$)*

From 196 mg **13**. Yield: 209 mg (67 %); m.p.: 188–200 °C (dec.); $R_f = 0.59$; ^{1}H (600 MHz, DMSO-d_6) $\delta = 8.85$ (s, 1H, H-2), 8.58 (s, 1H, H-5), 8.01 (s, 1H, H-8), 7.24 (s, 1H, H-4′), 3.89 (s, 2H, $N_{imidazole}CH_2$), 3.41 (s, 2H, $N_{quinol.}CH_2$), 2.41 (s, 3H, C_2-Me) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): $\delta = 177.2$ (C-9), 165.0 (C-4), 151.9 ($C_{imidazol}$-2′), 143.7 (C-2), 139.0 (C-7), 137.7 (C-8a), 137.0 ($C_{imidazole}$-5′), 131.9 (C-6), 128.5 (C-5), 125.6 (C-4a), 118.0 (C-8), 110.1 (C-3), 56.9 ($N_{quinol.}$-CH_2), 47.0 ($N_{imidazole}$-CH_2), 14.1 (C_2-*Me*) ppm; MS (FAB): $m/z = 411/413$ ($[M+H]^+$).

*7-Chloro-6-fluoro-1-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**18**, $C_{16}H_{12}ClFN_4O_5$)*

From 196 mg **15**. Yield: 209 mg (67 %); m.p.: 188–200 °C (dec.); $R_f = 0.53$; ^{1}H (600 MHz, DMSO-d_6) $\delta = 8.83$ (s, 1H, H-2), 8.25 (d, 1H, $J_{H\text{-}5,F} = 8.5$ Hz, H-5), 8.03 (d, 1H, $J_{H\text{-}8,F} = 5.8$ Hz, H-8), 7.23 (s, 1H, H-4′), 3.87 (s, 2H, $N_{imidazole}CH_2$), 3.40 (s, 2H, $N_{quinol.}CH_2$), 2.43 (s, 3H, C_2-Me) ppm; ^{13}C NMR (150.91 MHz, DMSO-d_6): $\delta = 177.8$ (CO_2H), 166.3 (C-4), 156.7 (d, $J_{C\text{-}6,F} = 248$ Hz, C-6), 151.7 ($C_{imidazol}$-2′), 148.3 (C-2), 137.2 ($C_{imidazole}$-5′), 136.6 (C-8a), 128.0 (d, $J_{C\text{-}4a,F} = 5.6$ Hz, C-4a), 126.1 (d, $J_{C\text{-}7,F} = 21$ Hz, C-7), 120.2 (C-8), 119.5 (C-3), 113.2 (d, $J_{C\text{-}5,F} = 23$ Hz, C-5), 56.6 ($N_{quinol.}$-CH_2), 47.5 ($N_{imidazole}$-CH_2), 14.0 (C_2-*Me*) ppm; MS (FAB): $m/z = 394/396$ ($[M+H]^+$).

Antifungal and antigiadriasis activity

The antifungal activity of the synthesized compounds and metronidazole, determined as MIC, was assessed using microdilution method recommended by NCCLS [46] against *C. tropicalis* ATCC 13803 and *C. albicans* ATCC 40227. Test compounds were dissolved in 10 % DMSO, to produce a 2000 μg/cm^3 stock solution. These test tubes were serially diluted to give a concentration of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μg/cm^3. SDB (Sabouraud Dextrose Broth) was used for fungus. The cell density of each inoculum was adjusted in sterile water of a 0.5 McFarland standard. A final concentration of 106 CFU/cm^3 was obtained for fungus, Microbial inocula were added to the two fold diluted samples. The test tubes were incubated 18–24 h at 37 ± 1 °C for 2–5 days at 25 ± 1 °C for fungus. Metronidazole was used as standard drug. The highest dilution of the test compound that completely inhibited the growth of test organism was considered as the MIC value of the test compound and was expressed in μg/cm^3. Each compound was assayed in duplicate in each of three independent experiments. The antigiardial activity of the prepared compounds and metronidazole as the standard drug were tested as described elsewhere [47]. Briefly, the tested compounds and metronidazole were dissolved in DMSO then in medium and filter-sterilized. In a 15-cm^3 glass tube, two fold dilutions starting at 15 mg/cm^3 were prepared in a final volume of 15 cm^3 to exclude air from the tube. Each tube was inoculated with 20,000 cells of the parasite being tested (*Giardia*). Each compound was assayed in duplicate in each of three independent experiments. In each assay, the appropriate controls were performed, including 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 by use of a standard haemocytometer. In each count, trypan blue was used to distinguish live from dead parasite.

HIV-1 reverse transcriptase inhibition assay

Evaluation of the antiviral activity of compounds **2**–**11**, **17**, and **18** against the HIV-1 strain (III_B) and the HIV-2 strain (ROD) in MT-4 cells was performed using an MTT assay as described previously [43, 44]. In brief, stock solutions (10 times final concentration) of test compounds were added in 25-mm^3 volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments). Untreated control, HIV and mock-infected cell samples, were included for each sample. HIV-1 (III_B) [48] or HIV-2 (ROD) [49] stock (50 mm^3) at 100–300 $CCID_{50}$ (50 % cell culture infectious dose) or culture medium was added to either of the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells [50] were centrifuged for 5 min at 1000 rpm, and the supernatant was discarded. The MT-4 cells were resuspended at 6 × 105 cells per cm^3, and 50-mm^3 volumes were transferred to the microtiter tray wells. Five days after infection, the viability of the mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

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