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ORIGINAL PAPER

# Synthesis and antimicrobial activity of new 1,2,4-triazole-3-thiol metronidazole derivatives

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**Abstract** New 1,2,4-triazole-3-thiol metronidazole derivatives have been synthesized by treating 1,2,4-triazole-3-thiols with metronidazole tosylate (toluene-4-sulfonic acid 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl ester) in DMF and in the presence of potassium carbonate as a base. S-alkylated and N-alkylated products were obtained, with the S-alkylated being the major products. All of the newly synthesized compounds were characterized by IR, $^{1}$H NMR, $^{13}$C NMR, and high-resolution mass spectrometry. The antiparasitic activity of the compounds against *Entamoeba histolytica* and *Giardia intestinalis* was investigated. The antibacterial and antifungal activity of the compounds, assessed as minimal inhibitory concentration, was also investigated.

**Keywords** Triazoles · Metronidazole · Antiparasitic activity · Antimicrobial activity

## Introduction

The chemistry of 1,2,4-triazoles has received considerable attention because of their synthetic and biological importance. Derivatives of 1,2,4-triazole have been found to have diverse pharmacological activity, for example fungicidal, insecticidal, bactericidal, herbicidal, anti-tumor, and anti-inflammatory, among others [1]. A large number of 1,2,4-triazole-containing ring systems, for example fluconazole, intraconazole, and voriconazole have been incorporated into a wide variety of therapeutically interesting drug candidates with, for example, anti-inflammatory, CNS-stimulant, sedative, antianxiety, antimicrobial, and antimycotic activity [2]. Moreover, there are known drugs containing the 1,2,4-triazole group, including triazolam [3], alprazolam [4], etizolam [5, 6], and furacylin [7, 8]. In addition, thione-substituted 1,2,4-triazoles and their derivatives [2] have been found to have a variety of biological activity, for example antibacterial, antifungal, antitubercular, antimycobacterial, anticancer, diuretic, and hypoglycemic properties.

Because of their interesting and diverse biological activity, a series of 1,2,4-triazole derivatives has been prepared and their biological activity investigated. An extensive review that deals with the synthetic procedures employed in the preparation of mercapto and thione-substituted 1,2,4-triazoles was published in 2006. What follows is the most important work pertaining to triazoles published since 2006. Amir and co-workers [9] prepared, among others, a series of 1,2,4-triazole derivatives of (biphenyl-4-yloxy)acetic acid; these compounds were potential anti-inflammatory analgesic agents with minimum ulcerogenic potential. They synthesized a series of 5-[(biphenyl-4-yloxy)methyl]-4-alkyl/aryl-3-mercapto-4*H*-1,2,4-triazoles by cyclization of the corresponding thiosemicarbazides. Koparir and Demiradg [10] prepared a number of 4,5-substituted-4*H*-1,2,4-triazole-3-thiol derivatives, with potential antibacterial and antifungul activity, from the corresponding furan-2-carboxylic acid hydrazides and

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phenylacetic acid hydrazides. Deprez-Poulain and co-workers [11] reported the synthesis of 4*H*-1,2,4-triazole-3-thiols using di-2-pyridylthionocarbonate as the thiocarbonyl transfer agent. Very recently, Kucukguzel and his team [12] described the synthesis and wide range of antiviral activity of some novel 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones and several related thiourea derivatives; the anti-tuberculosis activity of the prepared compounds was also evaluated. Karthikeyan and Holla [13] have synthesized a series of 2,4-dichloro-5-fluorophenyl-substituted arylidenetriazolothiazolidinones from the corresponding 1,2,4-triazole-5-thiols and investigated their anti-inflammatory and antimicrobial activity. Interestingly, Supuran and his team [14] have very recently synthesized novel mercapto-1,3,4-oxadiazole and 1,2,4-triazole derivatives starting from 4-(4-halogeno-phenylsulfonyl) benzoic acid hydrazides; the prepared compounds were assayed as inhibitors of the cytosolic and tumor-associated carbonic anhydrase isoenzymes I, II, and IX. More recently, Rawat et al. [15] reported on the antibacterial activity of a series of metronidazole–triazole conjugates.

Other important drugs known for their wide range of biological activity are metronidazole, 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (**1**), and its derivatives [16–19]. They are highly effective against trichomoniasis, various forms of amoebiasis, and infections with anaerobic bacteria and protozoa [20]; metronidazole can kill or inhibit most anaerobic bacteria when the concentration in serum is in the range 2 to 8 μg/cm$^3$ [21]. In view of the wide interest in the activity and profile of triazoles and metronidazole, and as an extension of our ongoing research on the synthesis of new compounds of pharmacological interest [22–25], we describe herein the synthesis and characterization of a number of new compounds that combine metronidazole and 1,2,4-triazole-3-thiols, which, to the best of our knowledge, have not previously been described in the literature. In addition, the antiparasitic, antibacterial, and antifungal activity of the newly prepared compounds was investigated.

## Results and discussion

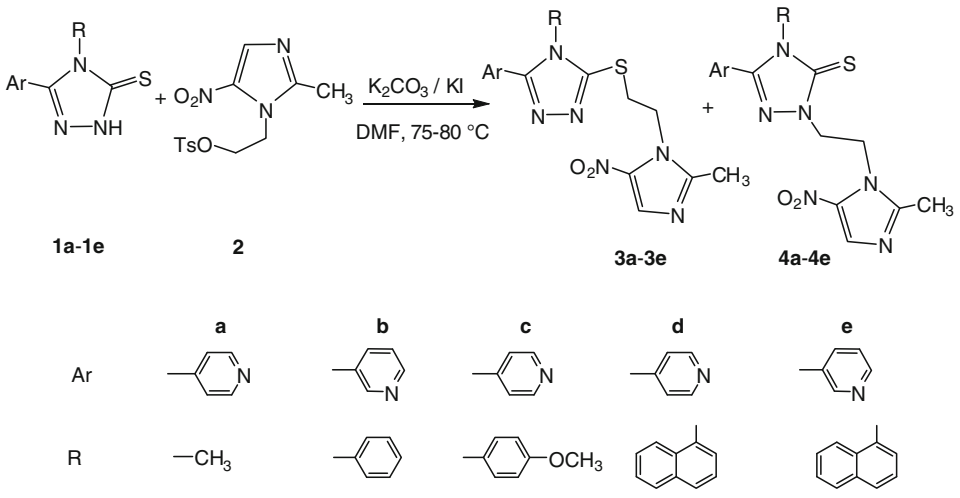
### *Chemistry*

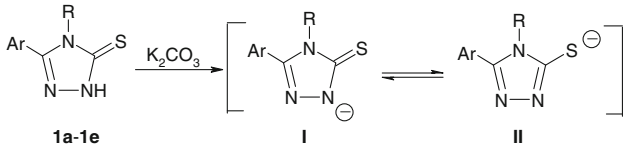
Synthesis of compounds **3a–3e** and **4a–4e** was carried out via the route shown in Scheme 1. The key starting materials, 1,2,4-triazole-3-thiones **1a–1e** [26] and metronidazole tosylate (**2**) [27] were prepared according to published procedures. Treatment of 1,2,4-triazole-3-thiones **1a–1e** with metronidazole tosylate (**2**) in warm DMF in the presence of potassium iodide and potassium carbonate afforded the desired products in moderate yields.

Under these experimental conditions, both the S-alkylated (**3a–3e**) and the N-alkylated (**4a–4e**) isomers were obtained. Flash chromatography of the crude material allowed the isolation of both isomers with an average yield of 25–32% for the S-alkylated isomers and 10–14% for the N-alkylated (ratio ~3:1). This type of regioselective alkylation reactions of thioamides is known to take place at either the sulfur or nitrogen atom. In most cases of nucleophilic substitution of an alkyl halide on a thioamide system sulfur atom attack is favored [28]; very few examples are known where attack on the nitrogen atom is preferred [29]. The outcome of these regioselective alkylations of thioamide derivatives with electrophiles depends on the character of both the thioamide derivative and the applied electrophile [30]. Under basic conditions, thioamides **1a–1e** exist in two tautomeric forms (I and II) in solution (Scheme 2); upon alkylation of I and II with **2**, compounds **4a–4e** and **3a–3e**, respectively, are obtained.

Because the major product was formed by S-alkylation, the preferred tautomeric form of the thioamides is II. Sulfur

**Scheme 1**






1a-1e I II

**Scheme 2**

atom alkylation is preferred to nitrogen atom alkylation because of sterically free access, higher partial charge, better polarizability owing to its 3*p* orbitals, and the aromaticity of the triazole ring conferred [31].

As reported in the literature [32, 33], analysis of the $^{1}$H NMR and $^{13}$C NMR spectra of the synthesized compounds provided proof of the structures of S- and N-alkylated triazoles **3a**–**3e** and **4a**–**4e**, respectively. The $CH_2$ protons adjacent to the sulfur atom in compounds **3a**–**3e** appear at about 3.60 ppm and the carbon resonates at about 31 ppm. However, $CH_2$ protons adjacent to the nitrogen atom in compounds **4a**–**4e** are more downfield-shifted to about 4.70 and the carbon resonates at about 43 ppm. Finally, the $^{13}$C NMR spectra show peaks at 169–170 ppm corresponding to the C=S group associated with the N-alkylated derivatives **4a**–**4e**, whereas the S-alkylated derivatives **3a**–**3e** gave peaks at 151.3–152 ppm for the same carbon, corresponding to C–S.

$^{1}$H NMR and $^{13}$C NMR spectra of all the prepared compounds were in total agreement with the suggested structures. DEPT experiments were used to differentiate secondary and quaternary carbons from primary and tertiary carbons. Additional support for the proposed structures comes from mass spectral data; mass spectra of the prepared compounds showed the correct molecular ions ($M^{+\cdot}$) as suggested by their molecular formulas. Each pair of S- and N-alkylated triazoles **3** and **4** has exactly the same molecular ion ($M^{+\cdot}$), for example the $M^{+\cdot}$ for both **3a** and **4a** is 437.1. Analyses of the molecular ions were used in the identification and characterization of these compounds.

*Antiamoebic, antigiardial, and cytotoxic activity*

The antiamoebic and antigiardial activity of the reported compounds was investigated by use of in-vitro bioassays. Their bioactivity was compared with that of the standard antiamoebic and antigiardial drug, metronidazole. The cytotoxicity of the compounds and metronidazole on two cell lines, Hep-2 and Vero cells, was also tested. The $IC_{50}$ values of the compounds against *Entamoeba histolytica*, *Giardia intestinalis*, and the two cell lines are given in Table 1.

As shown in Table 1, all the compounds tested had biological activity against *Entamoeba* and *Giardia*. Compounds **1c**, **3c**, and **3d** had the highest activity against *Entamoeba*, with $IC_{50}$ values ranging from 0.48 to 0.85 μM; other derivatives, for example **1a**, **3a**, **4a**, **4c**, **4d**, **3e**, and **4e**, also had significant activity against *Entamoeba* with $IC_{50}$ values ranging from 1.10 to 1.53 μM, which makes them more active than metronidazole itself. Additionally, compound **3b** was the most potent against *Giardia*, with an $IC_{50}$ value of 0.76 μM compared with 5.03 μM for metronidazole. When the cytotoxicity of the prepared molecules is considered (Table 1), compound **1a** seems to be the best among the

**Table 1** Antiamoebic, antigiardial, and cytotoxic activity of the tested compounds

| Compound | Mean $IC_{50}$ ± SD (μM) | | | |
|---|---|---|---|---|
| | *E. histolytica* | *G. intestinalis* | Hep-2 | Vero |
| **1a** | 1.37 ± 0.05 | 1.56 ± 0.09 | 531.46 ± 11.9 | 1,122.6 ± 9.43 |
| **3a** | 1.53 ± 0.11 | 1.21 ± 0.11 | 469.27 ± 11.4 | 614.39 ± 3.22 |
| **4a** | 1.31 ± 0.08 | 2.30 ± 0.11 | 472.29 ± 6.90 | 605.32 ± 9.89 |
| **1b** | 2.99 ± 0.16 | 3.19 ± 0.35 | 249.33 ± 11.3 | 237.52 ± 13.8 |
| **3b** | 3.27 ± 0.15 | 0.76 ± 0.10 | 297.18 ± 8.87 | 317.63 ± 9.92 |
| **4b** | 3.36 ± 0.37 | 3.19 ± 0.25 | 403.59 ± 12.6 | 287.35 ± 8.87 |
| **1c** | 0.85 ± 0.04 | 2.71 ± 0.14 | 205.38 ± 10.1 | 303.98 ± 8.87 |
| **3c** | 0.48 ± 0.02 | 1.78 ± 0.09 | 204.39 ± 9.38 | 150.25 ± 8.65 |
| **4c** | 1.10 ± 0.07 | 3.78 ± 0.11 | 142.61 ± 7.34 | 117.44 ± 12.0 |
| **1d** | 2.76 ± 0.16 | 2.53 ± 0.13 | 140.34 ± 8.29 | 141.43 ± 8.72 |
| **3d** | 0.79 ± 0.07 | 1.07 ± 0.11 | 92.61 ± 4.55 | 114.50 ± 6.69 |
| **4d** | 1.42 ± 0.22 | 1.66 ± 0.09 | 68.55 ± 3.35 | 97.72 ± 7.68 |
| **1e** | 2.27 ± 0.13 | 2.10 ± 0.16 | 548.18 ± 9.50 | 720.29 ± 11.9 |
| **3e** | 1.31 ± 0.09 | 1.77 ± 0.07 | 413.53 ± 11.2 | 565.97 ± 7.63 |
| **4e** | 1.42 ± 0.09 | 1.73 ± 0.04 | 471.86 ± 8.75 | 216.61 ± 7.90 |
| Metronidazole | 4.51 ± 0.35 | 5.03 ± 0.29 | 1,475.5 ± 15.0 | 1,515.2 ± 11.0 |

derivatives of metronidazole. This derivative was around three times more active than metronidazole against *Entamoeba* and *Giardia*. Fortunately, the boosted activity of this compound was not accompanied by any substantial increase in cytotoxicity (Table 1). In contrast, the cytotoxicity of the other derivatives with potent antiamoebic activity (**1c**, **3c**, and **3d**) was higher than that of the precursor compound metronidazole, although they remain noncytotoxic at concentrations much higher than the antiparasitic concentration of the derivatives. The other potent antigiardial derivative, **3b**, was also relatively nontoxic to the tested cell lines, with an $IC_{50}$ value of around 300 μM (Table 1). Interestingly, some of the tested compounds (e.g. **3b**, **3c**, and **1c**) had different patterns of activity against *G. intestinalis* and *E. histolytica* (Table 1). In addition, the molecular modifications on our derivatives did not render any of the compounds inactive. All other molecules had slightly higher activity than metronidazole (Table 1). Their cytotoxicity however was generally higher than that of metronidazole. The activity exhibited by the derivatives, especially compounds **1a** and **3b**, suggest that the derivatives may be used as new lead compounds in the development of new antiparasitic drugs. Although drug resistance to *Entamoeba* and *Giardia* does not, so far, appear to be a serious problem, occasional reports of failure with metronidazole [34] and the reported variations in drug sensitivities of isolates [35] may be alarming. Therefore, the importance of such biologically active, noncytotoxic metronidazole derivatives lies in their potential contribution to overcoming the problem of resistance of pathogens to the standard drugs. Additionally, because of the limited number of commercially available drugs active against anaerobic protozoan parasites and bacteria there is a serious need for new active compounds. Molecular modification of 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 **1a** and **3b**, are good drug candidates to be tested against metronidazole-resistant parasites and bacteria.

### *Antibacterial and antifungal activity*

The antimicrobial activity of the newly prepared compounds and their precursor compounds, assessed as minimal inhibitory concentration (MIC) values, are presented in Table 2. The spectrum of activity of the tested compounds covered Gram-positive, Gram-negative, and fungal cultures. With the exception of *Clostridium sporogenes* the reported antimicrobial activity was significantly lower than that of the reference tested antimicrobials. Nevertheless, it was demonstrated that compounds **3c**, **4d**, and **3e** have higher antimicrobial activity towards the Gram-positive anaerobic culture of *C. sporogenes* than the reference antimicrobial, metronidazole (Table 2). For these compounds the MIC

**Table 2** Minimum inhibitory concentrations (MIC, μM) of the tested substances and reference drugs against the tested microorganisms

| Compound | MIC (μM) | | | | |
|---|---|---|---|---|---|
| | *P. aeruginosa* | *E. coli* | *S.aureus* | *Cl. sporogenes* | *C. albicans* |
| Met-Ts | ND | ND | ND | 514.2 ± 273.6 | 2,212.2 ± 589.6 |
| **1a** | ND | ND | ND | ND | ND |
| **1b** | ND | ND | ND | ND | ND |
| **1c** | ND | ND | ND | ND | ND |
| **1d** | ND | ND | ND | ND | ND |
| **1e** | ND | ND | ND | ND | ND |
| **3a** | ND | ND | 2,136.4 ± 602.6 | 76.7 ± 42.5 | ND |
| **4a** | ND | ND | ND | 99.2 ± 35.6 | ND |
| **3b** | ND | ND | 3,832.0 ± 1,080.8 | 152.3 ± 97.8 | ND |
| **4b** | ND | ND | ND | 43.2 ± 14.4 | ND |
| **3c** | ND | 892.3 ± 251.7 | 446.2 ± 125.8 | 17.8 ± 6.3 | ND |
| **4c** | ND | ND | ND | 640.64 ± 215.1 | ND |
| **3d** | ND | ND | ND | 612.6 ± 205.7 | ND |
| **4d** | ND | ND | ND | 29.9 ± 16.0 | ND |
| **3e** | ND | ND | 555.8 ± 218.8 | 17.1 ± 6.0 | ND |
| **4e** | ND | ND | ND | 4,102.5 ± 0.0 | ND |
| Miconazole | NT | NT | NT | NT | 11.3 ± 1.2 |
| Ampicillin | ND | 21.7 ± 7.3 | 0.089 ± 0.0 | NT | NT |
| Metronidazole | NT | NT | NT | 42.9 ± 16.1 | NT |

*ND* Not detectable at concentrations as high as 0.5 mg/cm$^3$, *NT* not tested

values were 17.8, 29.9, and 17.1 μM, respectively, making them more active than metronidazole (MIC = 42.9 μM).

## Experimental

Unless otherwise indicated, all chemicals were obtained from commercial sources and were used as received. Melting points were measured with a Fischer–Johns melting-point apparatus. The IR spectra were recorded, as KBr discs, with the aid of a Thermo Nicolet Nexus 670 FT-IR instrument. We obtained $^{1}H$ and $^{13}C$ NMR with a Bruker-DPX 300 MHz spectrometer with $CDCl_3$ or dimethyl sulfoxide (DMSO)-$d_6$ as solvents; chemical shifts are reported in ppm relative to TMS as internal standard. High-resolution mass spectral data were acquired with a Bruker APEX (IV) mass spectrometer (Bremen, Germany). Elemental analyses were obtained by use of an Eurovector Euro EA3000, C, H, N, and S elemental analyzer and the results obtained agreed with the calculated percentages to within ±0.4%. Compounds were checked for purity by TLC using glass plates precoated with silica gel 60 $GF_{254}$, supplied by Fluka. 1,2,4-Triazole-3-thiones **1a**–**1e** [26] and metronidazole tosylate (**2**) [27] were synthesized and purified according to published procedures.

### *Synthesis of compounds **3a**–**3e** and **4a**–**4e***

Compounds **3a**–**3e** and **4a**–**4e** were prepared according to the following procedure. A mixture of 1,2,4-triazole-3-thione **1a**–**1e** (2 mmol), metronidazole tosylate (**2**, 2 mmol), and $K_2CO_3$ (3 mmol) in 8 $cm^3$ DMF was stirred at 75–80 °C overnight. Then, 30 $cm^3$ water was added and the resulting mixture was extracted with $CHCl_3$. The organic layer was separated, washed with water (4 × 30 $cm^3$), and dried over $Na_2SO_4$. After solvent evaporation, the residue was purified by flash chromatography on silica gel using $CHCl_3$–$CH_3OH$ (95:5) to afford **3a**–**3e** and **4a**–**4e**.

*4-[4-Methyl-5-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)-ethylsulfanyl]-4H-[1,2,4]triazol-3-yl]pyridine*
(**3a**, $C_{14}H_{15}N_7O_2S$)
Yield 0.25 g (29%); m.p.: 134–135 °C; $^{1}H$ NMR ($CDCl_3$): $\delta = 2.60$ (s, 3H), 3.65 (m, 5H), 4.80 (t, $J = 6.9$ Hz, 2H), 7.58 (d, $J = 5.8$ Hz, 2H), 7.95 (s, 1H), 8.80 (d, $J = 5.8$ Hz, 2H) ppm; $^{13}C$ NMR ($CDCl_3$): $\delta = 14.6$ ($CH_3$), 31.5 (S–$CH_2$), 31.9 (N–$CH_3$), 45.3 (N–$CH_2$), 122.2 (CH), 133.5 (CH), 134.1 (C), 137.5 (C), 150.7 (CH), 151.6 (C), 152.3 (C), 153.9 (C) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{14}H_{15}N_7NaO_2S$ $[M+Na]^+$ 368.09056, found 368.09512.

*2,4-Dihydro-4-methyl-2-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-5-(4-pyridinyl)-3H-[1,2,4]triazole-3-thione*
(**4a**, $C_{14}H_{15}N_7O_2S$)
Yield 0.11 g (13%); m.p.: 138–139 °C; IR (KBr): $\bar{\nu} = 1{,}528$ $cm^{-1}$ (C=S); $^{1}H$ NMR ($CDCl_3$): $\delta = 2.30$ (s, 3H), 3.65 (s, 3H), 4.65 (t, $J = 5.7$ Hz, 2H), 4.80 (t, $J = 5.8$ Hz, 2H), 7.45 (d, $J = 6.0$ Hz, 2H), 7.95 (s, 1H), 8.80 (d, $J = 6.0$ Hz, 2H) ppm; $^{13}C$ NMR ($CDCl_3$): $\delta = 14.1$ (Ar-$CH_3$), 33.4 (N–$CH_3$), 43.9 (S-$CH_2$), 48.5 (N–$CH_2$), 122.1 (CH), 132.7 (C), 133.4 (CH), 138.9 (C), 148.6 (C), 150.6 (C), 150.9 (CH), 169.1 (C) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{14}H_{15}N_7$ $NaO_2S$ $[M+Na]^+$ 368.09056, found 368.09217; *m/z*: calcd. for $C_{14}H_{16}N_7O_2S$ $[M+H]^+$ 346.10862, found 346.11036.

*3-[5-[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethylsulfanyl]-4-phenyl-4H-[1,2,4]triazol-3-yl]pyridine*
(**3b**, $C_{19}H_{17}N_7O_2S$)
Yield 0.22 g (27%); m.p.: 179–180 °C; $^{1}H$ NMR ($CDCl_3$): $\delta = 2.40$ (s, 3H), 3.60 (t, $J = 7.0$ Hz, 2H), 4.65 (t, $J = 7.0$ Hz, 2H), 7.35 (m, 3H), 7.55 (m, 3H), 7.65 (d, $J = 2.8$ Hz, 1H), 7.95 (s, 1H), 8.48–8.55 (m, 2H) ppm; $^{13}C$ NMR ($CDCl_3$): $\delta = 14.6$ ($CH_3$), 31.5 (S–$CH_2$), 45.4 (N–$CH_2$), 123.3 (C), 124.1 (CH), 126.7 (C), 128.1 (CH), 130.6 (CH), 130.9 (CH), 133.5 (CH), 135.7 (CH), 138.9 (C), 148.7 (CH), 151.1 (CH), 151.9 (C), 152.3 (C), 152.8 (C) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{19}H_{17}NaN_7O_2S$ $[M+Na]^+$ 430.10621, found 430.10566.

*2,4-Dihydro-2-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)-ethyl]-4-phenyl-5-(3-pyridinyl)-3H-[1,2,4]-triazole-3-thione* (**4b**, $C_{19}H_{17}N_7O_2S$)
Yield 0.08 g (10%); m.p.: 203–204 °C; IR (KBr): $\bar{\nu} = 1{,}529$ $cm^{-1}$ (C=S); $^{1}H$ NMR ($CDCl_3$): $\delta = 2.40$ (s, 3H), 4.75 (t, $J = 5.8$ Hz, 2H), 4.85 (t, $J = 5.6$ Hz, 2H), 7.20 (m, 3H), 7.50 (m, 4H), 8.00 (s, 1H), 8.45 (d, $J = 2.1$ Hz, 1H), 8.60 (dd, $J_1 = 2.1$, $J_2 = 5.8$ Hz, 1H) ppm; $^{13}C$ NMR ($CDCl_3$): $\delta = 14.2$ ($CH_3$), 43.9 (S–$CH_2$), 48.6 (N–$CH_2$), 121.2 (C), 123.4 (CH), 126.5 (C), 128.0 (CH), 130.2 (CH), 130.5 (CH), 133.4 (CH), 134.3 (C), 135.4 (CH), 138.9 (C), 148.8 (CH), 150.7 (C), 151.7 (CH) 169.5 (C) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{19}H_{17}N_7NaO_2S$ $[M+Na]^+$ 430.10621, found 430.11119.

*4-[4-(4-Methoxyphenyl)-5-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethylsulfanyl]-4H-[1,2,4]triazol-3-yl]pyridine*
(**3c**, $C_{20}H_{19}N_7O_3S$)
Yield: 0.28 g (32%); m.p.: 164–165 °C; $^{1}H$ NMR ($CDCl_3$): $\delta = 2.60$ (s, 3H), 3.55 (t, $J = 7.0$ Hz, 2H), 3.85 (s, 3H), 4.80 (t, $J = 7.0$ Hz, 2H), 6.95 (d, $J = 8.5$ Hz, 2H), 7.13 (d, $J = 8.5$ Hz, 2H), 7.32 (d, $J = 5.2$ Hz, 2H), 7.92 (s, 1H),

8.55 (d, $J = 5.2$ Hz, 2H) ppm; $^{13}$C NMR ($CDCl_3$): $\delta = 14.6$ ($CH_3$), 31.0 (S–$CH_2$), 45.2 (N–$CH_2$), 55.7 (O–$CH_3$), 115.7 (CH), 121.3 (CH), 125.4 (C), 128.3 (CH), 133.4 (CH), 133.8 (C), 138.4 (C), 150.3 (CH), 151.4 (C), 153.1 (C), 153.9 (C), 161.2 (C) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{20}H_{19}N_7NaO_3S$ $[M+Na]^+$ 460.11678, found 460.11623.

*2,4-Dihydro-4-(4-methoxyphenyl)-2-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-5-(4-pyridinyl)-3H-[1,2,4]triazole-3-thione* (**4c**, $C_{20}H_{19}N_7O_3S$)
Yield 0.12 g (14%); m.p.: 175–176 °C; IR (KBr): $\bar{\nu} = 1{,}531$ cm$^{-1}$ (C=S); $^1$H NMR ($CDCl_3$): $\delta = 2.40$ (s, 3H), 3.85 (s, 3H), 4.75 (t, $J = 5.4$ Hz, 2H), 4.85 (t, $J = 5.4$ Hz, 2H), 7.01 (d, $J = 8.9$ Hz, 2H), 7.08 (d, $J = 6.1$ Hz, 2H), 7.13 (d, $J = 8.9$ Hz, 2H), 7.97 (s, 1H), 8.55 (d, $J = 6.1$ Hz, 2H) ppm; $^{13}$C NMR ($CDCl_3$): $\delta = 14.2$ ($CH_3$), 43.8 (S–$CH_2$), 48.7 (N–$CH_2$), 55.7 (O–$CH_3$), 115.4 (CH), 121.5 (CH), 126.6 (C), 129.1 (CH), 132.3 (C), 133.4 (CH), 139.2 (C), 147.7 (C), 150.5 (CH), 150.7 (C), 160.8 (C), 170.2 (C) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{20}H_{19}N_7NaO_3S$ $[M+Na]^+$ 460.11678, found 460.12292.

*4-[5-[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethylsulfanyl]-4-(1-naphthalenyl)-4H-[1,2,4]triazol-3-yl]pyridine* (**3d**, $C_{23}H_{19}N_7O_2S$)
Yield 0.25 g (28%); m.p.: 197–198 °C; $^1$H NMR ($CDCl_3$): $\delta = 2.60$ (s, 3H), 3.60 (t, $J = 6.8$ Hz, 2H), 4.80 (t, $J = 6.8$ Hz, 2H), 6.95–7.22 (m, 3H), 7.45 (d, $J = 7.4$ Hz, 1H), 7.52 (t, $J = 7.0$ Hz, 1H), 7.60 (m, 2H), 7.90 (s, 1H), 8.01 (d, $J = 8.2$ Hz, 1H), 8.10 (d, $J = 8.4$ Hz, 1H) 8.42 (d, $J = 6.2$ Hz, 2H) ppm; $^{13}$C NMR ($CDCl_3$): $\delta = 14.7$ ($CH_3$), 31.1 (S–$CH_2$), 45.3 (N–$CH_2$), 120.7 (CH), 121.2 (CH), 125.6 (CH), 126.2 (CH), 127.8 (CH), 128.9 (CH), 129.0 (CH), 129.1 (C), 129.4 (C), 131.7 (CH), 133.4 (CH), 133.6 (C), 134.5 (C), 138.4 (C), 150.4 (CH), 151.4 (C), 153.7 (C), 154.5 (C) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{23}H_{19}N_7NaO_2S$ $[M+Na]^+$ 480.12186, found 480.12305.

*2,4-Dihydro-2-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)-ethyl]-4-(1-naphthalenyl)-5-(4-pyridinyl)-3H-[1,2,4]triazole-3-thione* (**4d**, $C_{23}H_{19}N_7O_2S$)
Yield 0.13 g (14%); m.p.: 209–210 °C; IR (KBr): $\bar{\nu} = 1{,}530$ cm$^{-1}$ (C=S); $^1$H NMR ($CDCl_3$): $\delta = 2.50$ (s, 3H), 4.85 (t, $J = 5.3$ Hz, 2H), 4.95 (t, $J = 5.3$ Hz, 2H), 6.95 (d, $J = 6.2$ Hz, 2H), 7.30 (d, $J = 6.8$ Hz, 1H), 7.35 (d, $J = 7.3$ Hz, 1H), 7.55 (m, 3H), 7.95 (d, $J = 8.2$ Hz, 1H), 8.00 (s, 1H), 8.05 (d, $J = 8.42$ Hz, 1H), 8.40 (d, $J = 7.2$ Hz, 2H) ppm; $^{13}$C NMR ($CDCl_3$): $\delta = 14.5$ ($CH_3$), 43.9 (S–$CH_2$), 48.6 (N–$CH_2$), 120.9 (CH), 121.6 (CH), 125.7 (CH), 127.2 (CH), 127.5 (CH), 128.5 (CH), 129.1 (CH), 129.3 (C), 130.7(C), 131.5 (CH), 132.1 (C), 133.4 (CH), 134.4 (C), 139.1 (C), 148.3 (C), 150.4 (CH), 150.7 (C), 170.5 (C) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{23}H_{19}N_7NaO_2S$ $[M+Na]^+$ 480.12186, found 480.12708.

*3-[5-[2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethylsulfanyl]-4-(1-naphthalenyl)-4H-[1,2,4]triazol-3-yl]pyridine* (**3e**, $C_{23}H_{19}N_7O_2S$)
Yield 0.24 g (26%); m.p.: 160–161 °C; $^1$H NMR ($CDCl_3$): $\delta = 2.60$ (s, 3H), 3.60 (t, $J = 6.4$ Hz, 2H), 4.80 (t, $J = 6.4$ Hz, 2H), 7.13 (m, 1H), 7.20 (d, $J = 5.4$ Hz, 1H), 7.42 (d, $J = 7.9$ Hz, 1H), 7.50-7.58 (m, 3H), 7.72 (d, $J = 7.9$ Hz, 1H), 7.95 (s, 1H), 7.95 (d, $J = 7.9$ Hz, 1H), 8.05 (d, $J = 8.1$ Hz, 1H), 8.45-8.55 (m, 2H) ppm; $^{13}$C NMR ($CDCl_3$): $\delta = 14.7$ ($CH_3$), 31.1 ($CH_2$), 45.3 ($CH_2$), 121.2 (CH), 121.4 (C), 122.8 (C), 123.5 (CH), 125.4 (CH), 125.6 (CH), 126.4 (CH), 127.8 (CH), 128.8 (CH), 129.1 (CH), 129.3 (C), 131.1 (C), 131.7 (CH), 133.5 (CH), 134.6 (CH), 146.3 (C), 147.8 (C), 150.9 (CH), 151.5 (CH) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{23}H_{19}N_7NaO_2S$ $[M+Na]^+$ 480.12186, found 480.12503.

*2,4-Dihydro-2-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)-ethyl]-4-(1-naphthalenyl)-5-(3-pyridinyl)-3H-[1,2,4]triazole-3-thione* (**4e**, $C_{23}H_{19}N_7O_2S$)
Yield: 0.12 g (13%); m.p.: 173–174 °C; IR (KBr): $\bar{\nu} = 1{,}525$ cm$^{-1}$ (C=S); $^1$H NMR ($CDCl_3$): $\delta = 2.50$ (s, 3H), 4.80 (t, $J = 5.4$ Hz, 2H), 4.90 (t, $J = 5.4$ Hz, 2H), 7.05 (dd, $J_1 = 8.1$, $J_2 = 4.9$ Hz, 1H), 7.35 (m, 3H), 7.55 (m, 3H), 7.93 (m, 1H), 7.98 (s, 1H), 8.05 (d, $J = 8.2$ Hz, 1H), 8.34 (d, $J = 2.1$ Hz, 1H), 8.48 (dd, $J_1 = 4.8$, $J_2 = 1.4$ Hz, 1H) ppm; $^{13}$C NMR ($CDCl_3$): $\delta = 14.5$ ($CH_3$), 44.0 (S–$CH_2$), 48.6 (N–$CH_2$), 121.3 (C), 121.7 (CH), 123.4 (CH), 125.6 (CH), 127.3 (CH), 127.4 (CH), 128.5 (CH), 129.1 (CH), 129.4 (C), 130.7 (C), 131.5 (CH), 133.4 (CH), 134.5 (C), 134.8 (CH), 139.2 (C), 148.2 (CH), 148.4 (C), 150.8 (C), 151.6 (CH), 170.2 (C) ppm; HRMS (EIMS) *m/z*: calcd. for $C_{23}H_{19}N_7NaO_2S$ $[M+Na]^+$ 480.12186, found 480.12131.

### *Antiamoebic and antigiardial activity*

To test the antiamoebic and antigiardial activity of the compounds *E. histolytica* HK-9 strain (ATCC number 30015) cultured in LYI-S-2 medium and *G. intestinalis* WB strain (ATCC number 30957) grown in a modified YI-S medium were used in all assays. The antiamoebic and antigiardial activity of the prepared compounds and metronidazole as the standard antiamoebic and antigiardial drug were tested as described elsewhere [25]. Briefly, the tested compounds and metronidazole were dissolved in DMSO then in medium and filter-sterilized. In a 15-cm$^3$ glass tube, twofold dilutions starting at 15 μg/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 (*Entamoeba* or *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 hemacytometer. In each count, trypan blue was used to distinguish live from dead parasites.

The cytotoxicity of the reported compounds and the reference drug, metronidazole, was investigated on Hep-2 and Vero cells using the standard cytotoxicity assay and the trypan blue exclusion method [25]. Briefly, 100 $mm^3$ portions of each cell suspension were added to the wells of 96-well plates, incubated for 24 h, and the medium in each well was then replaced with 150 $mm^3$ fresh medium. Solutions of the compounds or the reference drug were dissolved in DMSO, prepared in medium, and filter sterilized. Then, 150 $mm^3$ twofold serial dilutions of each of the compounds and the reference drug starting at a concentration of 1,000 $\mu g/cm^3$ in culture medium were prepared on the plates. After 48 h incubation, the number of cells in each well was determined by use of a hemacytometer. Each compound was assayed in duplicate in each of three independent experiments. In each assay the negative controls (without any compound or reference drug) were included in duplicates.

*Antimicrobial activity*

The antimicrobial activity of the compounds, ampicillin, miconazole (both obtained as a gift from Dar Al Dawa Pharmaceutical Company, Naour-Jordan), and metronidazole, determined as MIC, was assessed using the broth microdilution method recommended by CLSI with some modifications [36]. Overnight cultures of *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538P, *C. sporogenes* ATCC 19404, and *Candida albicans* ATCC 10231 were used. Aerobic bacteria were grown in nutrient broth medium (Oxoid, UK). *C. sporogenes* ATCC 19404 was grown in thioglycollate medium (Oxoid, UK), and *C. albicans* was grown in Sabaroud medium (Hi Media, India). Batches of medium (20 $cm^3$) were inoculated from fresh culture slopes and incubated overnight at 37 °C. Anaerobic growth conditions for *C. sporogenes* were achieved by incubating the cultures in an anaerobic jar (Oxoid, UK) and using gas-generating sachets of $CO_2$ Gen. (Oxoid, UK).

MIC was determined by using the twofold broth dilution method in 96 well microtitre plates (Cellstar®, Greinerbio-one, Germany). Stock solution of each substrate (10 $mg/cm^3$) was prepared in DMSO (Tedia, USA) under aseptic conditions. The first experimental well was filled with 190 $mm^3$ media and the other wells were filled with 100 $mm^3$. Each substance stock solution (10 $mm^3$) was added to the first well. Twofold serial dilution was then carried out across the plate. Overnight batch culture (10 $mm^3$) was used to inoculate the wells to achieve a final inoculum size of $1 \times 10^6$ $cfu/cm^3$ and the plate was incubated for 24 h at 37 °C. 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, by use of a microtitre plate reader (Biotek, USA). MIC for *C. sporogenes* ATCC 19404 was determined in micro centrifuge tubes (Bio Basic). Negative controls were performed using only sterile broth and positive controls were performed with only overnight culture and 10 $mm^3$ DMSO. Each MIC determination was carried out in triplicate.

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