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Original Article

Synthesis and Pharmacological Characterization of Metronidazole-Oxadiazole Derivatives

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Abstract

Background: The use of antibiotics with or without prescription is increasing worldwide. With certain limitations, metronidazole (MTZ) is extensively used as an antibacterial and antiparasitic drug. Derivatives of 1,2,4-oxadiazole (ODZ) are used to modify the chemical structure of drugs. The present study aimed to synthesize new MTZ-ODZ derivatives that could potentially lead to new medications.

Methods: The reaction of MTZ with ethyl chloroacetate and potassium carbonate anhydrous was used to produce compound 7. This compound was treated with hydrazine hydrate in methanol to obtain compound 8. Carbon disulfide and potassium hydroxide were then added to obtain compound 9, which was then mixed with various α-haloketones to obtain compounds 10a to 10f. Subsequently, the structures of the new MTZ-ODZ derivatives were determined.

Results: All new compounds exhibited excellent activity against all tested organisms. The synthesized compounds showed a significant radical scavenging activity. The IC_{50} value for compounds 10a, 10b, 10c, 10d, 10e, and 10f was 70.42±0.15, 70.52±0.54, 85.21±0.85, 80.10±0.46, 82.52±0.13, and 70.45±0.12 g/mL, respectively. In terms of antigiardial activity, the IC_{50} value for compounds 10a,10b, 10c, and 10d ranged from 1.31±0.11 µM to 2.26±0.49 µM. In contrast, the IC_{50} for MTZ was 3.71±0.27 µM. Compound 10f showed the highest antigiardial activity with an IC_{50} value of 0.88±0.52 µM.

Conclusion: Most of the MTZ-ODZ derivatives showed high radical scavenging activity in the benzene ring due to the activation of certain groups, such as OCH_3, NO_2, and OH. The results suggest that the newly synthesized compounds could be used as an antiparasitic drug.

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Keywords ● Antibacterial agents ● Giardiasis ● Metronidazole ● Oxadiazoles

What's Known

- Oxadiazole is associated with azole derivatives exhibiting biological and pharmacological properties.
- Metronidazole is an antibiotic with a 5-nitroimidazole structure and is one of the most potent drugs used to treat giardiasis. However, its use is limited due to its ineffectiveness in children and the emergence of drug-resistant strains.

What's New

- Synthesis of novel metronidazole derivatives involving 1,3,4-oxadiazole moiety is presented.
- All the developed compounds are biologically active, exhibit significant radical scavenging activity, and excellent antimicrobial and antibacterial properties. It is hypothesized that they may even have additional pharmacological properties against other diseases.

Introduction

Nitrogen- and oxygen-containing heterocyclic compounds exhibit various key biological properties. Azoles are a class of five-membered heterocyclic compounds that instead of carbon atoms contain nitrogen and another heteroatom (e.g., oxygen or sulfur) as part of the ring.[1] Oxadiazole is an azole derivative in which the five-membered ring is made up of oxygen and two nitrogen atoms. Depending on the position of the heteroatoms, there are four isomers of oxadiazole, namely 1,2,4-, 1,3,4-, 1,2,3-, and

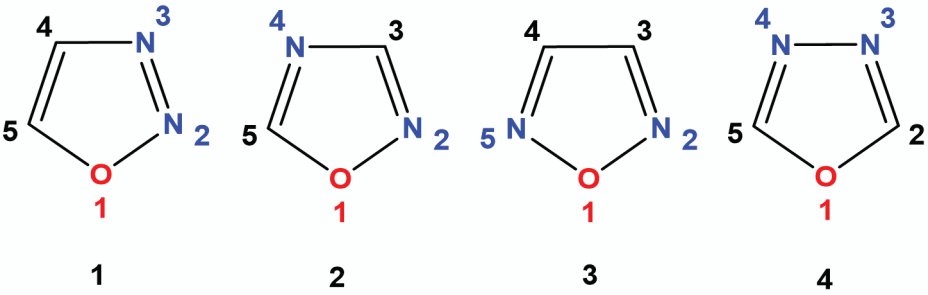

Figure 1: The structure of the four isomers of oxadiazole is illustrated.

1,2,5-oxadiazoles (figure 1). Among these, 1,2,4- and 1,3,4-oxadiazoles are better known and well-studied. They are isolated from natural products and have important biological and chemical properties.[2, 3] Derivatives of 1,2,4-oxadiazole are widely used in the chemical structure of drugs due to their biological properties such as anti-inflammatory, antiviral, antifungal,[4] anticancer,[5] anticonvulsant,[6] analgesic,[7] and antidiabetic.[8, 9] For example, antibacterial drugs derived from 1,2,4-oxadiazole are used to treat *Mycobacterium tuberculosis*.[10]

The use of antibiotics with or without prescription is increasing worldwide with the consequence of more bacteria becoming resistant to antibiotics.[11, 12] It is therefore important to develop new antimicrobial agents that can combat drug resistance and prevent the less commonly diagnosed neurotoxicity.[13] Metronidazole (MTZ), an antibiotic with a chemical structure of 5-nitroimidazole, is extensively used as an antibacterial and antiparasitic drug due to its effectiveness and integrity.[14] However, polyneuropathy due to MTZ therapy has been reported.[15] MTZ is one of the most effective drugs used in the treatment of giardiasis. However, its use is limited due to its ineffectiveness in children and the emergence of drug-resistant strains.[16] MTZ has been widely used in topical and systematic applications for more than 50 years. However, there is still insufficient information regarding its use as an antioxidant. A previous study reported that MTZ does not show direct antioxidant activity in *in vitro* global systems.[17]

In general, the design of MTZ derivatives aims at reducing the common side effects of the therapeutic agent and synthesizing a new drug to prevent some microorganisms from becoming resistant. Herein, we report the synthesis of novel MTZ derivatives as a metronidazole-oxadiazole (MTZ-ODZ) compound with a 1,3,4-oxadiazole moiety. Treatment with various α-haloketones synthesized novel thio-substituted oxadiazole derivatives of MTZ as a spacer, leading to a new drug with less antimicrobial resistance.

Materials and Methods

The study was approved by the Ethics Committee of Kirkuk University, Kirkuk, Iraq (code: 169).

Instrumentation

A Stuart™ SMP3 melting point apparatus (Scientific Laboratory Supplies Ltd, Nottingham, England) was used to measure uncorrected melting points. Thin layer chromatography (Merck, Darmstadt, Germany) monitored the reactions using a pre-coated silica gel plate (kieselgel 60G/F_{254}, 0.25 mm). A Fourier transform infrared spectrophotometer (FTIR-4200, PerkinElmer, Shimadzu, Japan) was used to record the FTIR spectra at 400-4000 cm^{-1}. A Bruker Avance AV-500 spectrometer (Bruker, Bremen, Germany) recorded the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra. A Shimadzu UV-1800 UV/Vis scan spectrophotometer (Shimadzu, Kyoto, Japan) was used to record the ultraviolet, and visible light absorbed by the samples. Combustion analysis was performed on a Carlo Erba automated elemental analyzer, model 1106 (Carlo Erba, Milan, Italy). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was provided by Sigma-Aldrich, Baghdad, Iraq. The cell lines (CaCo-2 and Vero) were purchased from Thermo Fisher Scientific, Waltham, USA. All the synthesis reagents were supplied by Sigma-Aldrich, Baghdad, Iraq.

Synthesis of MTZ-ODZ Derivatives

The MTZ-ODZ was prepared according to the method described in a previous study.[18] The compound was synthesized by mixing 0.01 mol MTZ 5 with ethyl chloroacetate 6 and anhydrous potassium carbonate in 15 mL of dimethylformamide, then stirred at 25 °C for 10 hours.[19] Hydrazine hydrate (0.02 mol) was added to a solution of compound 7 (0.01 mol) dissolved in methanol (15 mL) and refluxed for 10 hours.[20] Then, 95% ethanol (15 mL), CS_2 (0.01 mol), and potassium hydroxide (KOH) (0.01 mol) were added to the reaction mixture of compound 8 (0.01 mol), and the solution

was heated for 10 hours. The solution was then concentrated and acidified with dilute HCl. The desired compound 5-[(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethoxy)methyl]-1,3,4-oxadiazole-2-thiol 9 was purified using column chromatography (figure 2).

The final step of the synthetic scheme was the preparation of a series of unique thio-substituted oxadiazole derivatives of MTZ (10a-f).[21] A solution of compound 9 (0.1 mol) in ethanol (0.1 mol) of six different aromatic α-haloketones was mixed and refluxed for six hours. Next, ice-cold water was used to quench the reaction and solidify the mixture. Column chromatography was used to isolate and purify the required product. Column chromatography using petrol:ethyl acetate (1:1), as eluent was used for purification to obtain the desired derivatives of 1-(4-substituted-phenyl)-2-[(5-((2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethoxy)methyl)-1,3,4-oxadiazol-2-yl)thio]ethan-1-one (10a-f) (figure 3).

Biological Activity of MTZ-ODZ Compound
Antioxidant Evaluation

The newly developed compounds (10 a-f) and the crude products were subjected to a radical scavenging assay to evaluate their antioxidant properties using 1,1-diphenylpicryl-hydrazyl (DPPH).[22] Various concentrations of the compounds in methanol (50, 75, and 100 mg/mL) were added to 5 mL of 0.005% (w/v) methanol solution of DPPH and incubated at room temperature for 45 min. The absorbance was measured against a blank at 517 nm. The inhibition (%) of free radical production was calculated according to the formula: Inhibition (%)=[(control$_A$-sample$_A$)/blank$_A$]×100. Sample$_A$ is the absorbance of the test sample, and control$_A$ is the absorbance of all reagents except the test compound. Tests were carried out in triplicate. The IC_{50} value was determined using a fitted line to a plot. IC_{50} indicates the sample required to inhibit 50% of the DPPH free radicals.

Antimicrobial Activity

The antimicrobial evaluation of the crude and synthesized derivatives was performed using the disc diffusion method (DDM).[23-25] The experiment was carried out *in vitro* using Petri dishes. Three strains of Gram-positive and Gram-negative bacteria were used, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *viridans streptococci*, *Providencia spp.*, *Serratia marcescens*, and *Enterobacter cloacae*. Dimethyl sulfoxide (DMSO) as a solvent and a concentration of 60 µg/mL from each sample were used. Agar plates were used to culture the bacteria and incubated for 24

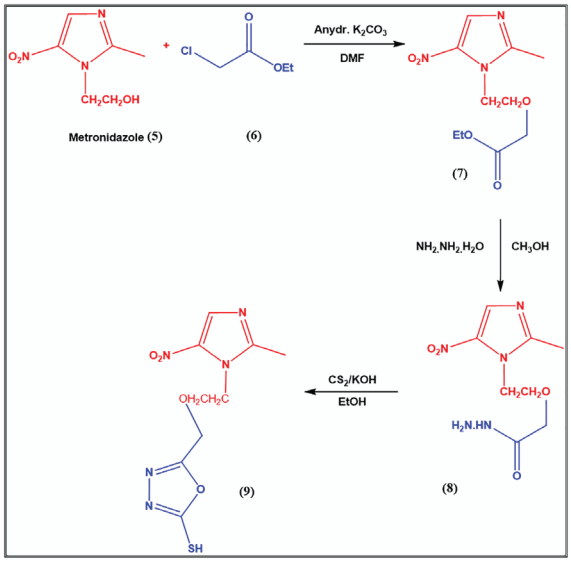

Figure 2: Illustration of the synthetic route used to synthesize metronidazole-oxadiazole derivatives.

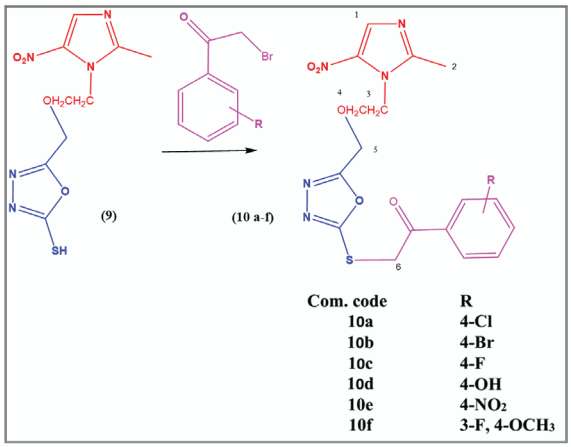

Figure 3: Illustration of the synthetic route of thio-substituted metronidazole-oxadiazole derivatives.

hours at 37 °C, pH 7.4±0.2. The bacteria strains were obtained from Kirkuk Hospital (Kirkuk, Iraq). Table 1 shows the antibacterial activity of both the crude and synthesized MTZ-ODZ derivatives.

Antigiardial Activity

Using an *in vitro* bioassay, the antigiardial activity of the derivatives was evaluated, and their biological activity was compared to that of standard MTZ.

Cytotoxicity Assay

MTT assay was used to assess cytotoxicity.[26, 27] Cells were seeded in a 96-well plate (Sigma-Aldrich) with a density of 35,000 in 250 µL volume per well. The coated plate was stored at 37 °C with 6% CO_2 for 24 hours. After 48 hours, different concentrations (0.5 to 50 µM, in RPMI containing 0.6% DMSO) of the samples were

Table 1: Evaluation of the antibacterial activity of crude metronidazole and synthesized metronidazole-oxadiazole derivatives (10a-f) against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *viridans streptococci* bacteria by calculating the diameter of the inhibition zone

Gram-positive bacteria			
Compound	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Viridans streptococci***
Metronidazole	-	-	-
10a	++	++	+++
10b	++	++	++
10c	++	-	++
10d	+	+	++
10e	++	+	+++
10f	+++	+++	+++
DMSO	-	-	-
Gram-negative bacteria			
Compound	***Providencia spp.***	***Serratia marcescens***	***Enterobacter cloacae***
Metronidazole	-	-	-
10a	++	++	+++
10b	++	++	+++
10c	+	+	+
10d	++	++	+
10e	++	+	++
10f	+++	+++	+++
DMSO	-	-	-

-No activity; +10 mm<inhibition diameter<15 mm; ++15 mm<inhibition diameter<20 mm; +++Inhibition diameter≥20 mm); DMSO: Dimethyl sulfoxide

added to the coated plate. A culture medium containing 0.6% DMSO was used as the control group. The coated plate was treated with 26 µL of MTT solution (2.6 mg/mL) and stored at 37 °C for four hours. The dish was washed with phosphate-buffered saline (pH 7.4), followed by the addition of DMSO (150 µL) per well. The absorbance was measured at a wavelength of 575-695 nm using an iMARK™ microplate absorbance reader (Bio-Rad Laboratories S.r.l, Segrate, Italy).

Statistical Analysis

Data were analyzed using SigmaPlot software, version 12.5 (Regional office in India). The results of cytotoxicity were expressed as mean±SD.

Results

Chemistry

A number of MTZ-ODZ derivatives were selected, and the reaction of MTZ with chloroacetic acid ethyl ester in the presence of K_2CO_3 was used to develop compound 7. The structure of compound **7** was determined using FTIR, which showed the characteristics of the carbonyl group, ^{1}H NMR, and ^{13}C NMR. All the charts are shown in the supplementary data file.

Compound 7: Yield: 80%, m.p. 238-240 °C. I.R (KBr, cm^{-1}) 1710 (C=O), 1600 (C=N), 1455 (C=C Ar), 1148 (C-O-C), 743, 695. δ_H (500 MHz, DMSO-d_6), 6.74 (1H, s), 4.59 (2H, m), 4.38 (2H, q, J6.0), 4.32 (2H, m), 4.29 (1H, m), 4.02 (2H, t, J4.4), 2.39 (2H, s), 1.57 (3H, t, J 6.0). δ_C (125 MHz, DMSO-d_6) 171.10, 149.16, 139.22, 133.15, 69.02, 67.49, 61.14, 31.37, 14.66, 14.10. Anal.% calc./found for $C_{10}H_{15}N_3O_5$ (m.w. 257.10) Elemental Analysis: C, 46.69/45.52; H, 5.88/5.75; N, 16.33/15.98; O, 31.10/32.54.

Compound 7 was treated with hydrazine hydrate in methanol to obtain compound 8 after heating for 10 hours. Solid 8 was purified by recrystallization from ethanol and the addition of dilute HCl. Then, 0.01 mol from KOH and carbon disulfide was added to the solution of compound **8**, and the reaction mixture refluxed for 12 hours to produce compound 9 with a 70% yield.[28] FTIR, ^{1}H NMR, and ^{13}C NMR for the structure of compound 9 are presented below.

Compound 9: Yield: 70%, m.p. 221-223 °C. I.R (KBr, cm^{-1}) 1600 (C=N), 1458 (C=C Ar), 1150 (C-O-C), 1253, 1190 (C-S-C), 743, 695. δ_H (500 MHz, DMSO-d_6) 7.08 (1H, s), 5.55 (1H, s), 3.52 (1H, t, J5.5), 3.41 (1H, t, J5.5), 3.00 (2H, t, J5.5), 2.77 (1H, s), 1.47 (3H, s). δ_C (125 MHz, DMSO-d_6) 178.59, 153.02, 149.16, 139.22, 133.15, 69.02, 59.01, 31.37, 14.10. Anal. % calc./found for $C_9H_{11}N_5O_4S$ (m.w. 285.28) Elemental Analysis: C, 37.89/36.85; H, 3.89/3.57; N, 24.55/25.78; O, 22.43/23.41; S, 11.24/12.5.

In an oil bath, compound 9 and six different aromatic α-haloketones were mixed in ethanol and heated for six hours at 105-110 ˚C. The resulting solid product was separated and purified by column chromatography using

petrol:ethyl acetate (1:1) as eluent to obtain the desired compounds 10a-f. FTIR, ^{1}H NMR, and ^{13}C NMR for the structure of synthesized derivatives are presented below.

Compound 10a: Yield: 65%, m.p. 115-117 ˚C. I.R (KBr, cm^{-1}) 1711 (C=O), 1601 (C=N), 1456 (C=C Ar), 1147 (C-O-C), 1253, 1190 (C-S-C), 743, 695. δ_H (500 MHz, DMSO-d_6) 6.41 (1H, s), 6.35-6.26 (3H, m), 6.17 (1H, d, J7.3), 3.28 (2H, s), 3.02 (2H, s), 2.97 (2H, t, J3.7), 2.60 (2H, t, J3.7), 1.10 (3H, s). δ_C (125 MHz, DMSO-d_6) 193.97, 165.44, 155.70, 154.19, 149.25, 139.31, 138.61, 133.24, 129.88, 128.49, 123.11, 110.41, 71.00, 59.10, 56.84, 42.50, 39.52, 14.19. Anal. % calc./found for $C_{17}H_{16}ClN_5O_5S$ (m.w. 437.86). Elemental Analysis: C, 46.63/45.34; H, 3.68/3.12; Cl, 8.10/7.94; N, 16.00/15.5; O, 18.27/17.45; S, 7.32/6.45.

Compound 10b: Yield: 75%, m.p. 119-121 ˚C. I.R (KBr, cm^{-1}) 1721 (C=O), 1600 (C=N), 1459 (C=C Ar), 1150 (C-O-C), 1263, 1192 (C-S-C), 744, 697. δ_H (500 MHz, DMSO-d_6), 7.98 (2H, d, J7.5), 7.70 (2H, d, J7.5), 6.80 (1H, s), 4.98 (2H, s), 4.53 (2H, s), 4.48 (1H, t, J3.7), 4.38 (1H, t, J3.7), 4.12 (2H, t, J3.7), 2.52 (3H, s). δ_C (125 MHz, DMSO-d_6) 193.94, 164.45, 154.29, 154.96, 140.19, 138.81, 142.40, 142.40, 135.09, 131.09, 130.13, 129.70, 72.00, 60.10, 45.29, 23.61, 13.42. Anal. % calc./found for $C_{17}H_{16}BrN_5O_5S$ (m.w. 482.31) Elemental Analysis: C, 42.34/42.15; H, 3.34/3.84; Br, 16.57/15.64; N, 14.52/15.24; O, 16.59/17.45; S, 6.65/6.31.

Compound 10c: Yield: 67%, m.p. 121-123 °C. I.R (KBr, cm^{-1}) 1701 (C=O), 1621 (C=N), 1449 (C=C Ar), 1150 (C-O-C), 1263, 1195 (C-S-C), 741, 694. δ_H (500 MHz, DMSO-d_6) 7.81-7.68 (2H, m), 7.07 (2H, t, J7.8), 6.60 (1H, s), 4.53 (1H, s), 4.37 (2H, t, J7.1), 4.19 (2H, s), 4.09 (1H, t, J7.1), 3.81 (2H, t, J7.1), 1.24 (3H, t, J6.7). δ_C (125 MHz, DMSO-d_6) 193.94, 168.26, 166.17, 165.44, 154.19, 153.96, 139.19, 133.20, 133.17, 131.13, 131.08, 129.12, 116.27, 116.06, 71.00, 59.10, 43.28, 22.60, 12.42. Anal. % calc./found for $C_{17}H_{16}FN_5O_5S$ (m.w. 421.40) Elemental Analysis: C, 48.45/47.54; H, 3.83/3.12; F, 4.51/3.87; N, 16.62/15.47; O, 18.98/17.87; S, 7.61/7.12.

Compound 10d: Yield: 55%, m.p. 134-136 °C. I.R (KBr, cm^{-1}) 3200-3600 (OH), 1712 (C=O), 1631 (C=N), 1436 (C=C Ar), 1137 (C-O-C), 1233, 1150 (C-S-C), 743, 698. δ_H (500 MHz, DMSO-d_6) 8.35 (1H, s), 7.73 (1H, s), 7.61 (2H, d, J7.5), 6.83 (2H, d, J7.5), 4.44 (2H, s), 4.25-4.11 (3H, m), 4.05 (1H, t, J4.0), 3.74 (2H, t, J4.0), 1.24 (3H, t, J6.7). δ_C (125 MHz, DMSO-d_6) 193.94, 165.44, 163.01, 154.19, 153.96, 139.19, 131.83, 129.12, 128.62, 116.31, 71.00, 59.10, 43.28, 22.60, 12.42. Anal. % calc./found for $C_{17}H_{17}N_5O_6S$ (m.w. 419.41). Elemental Analysis: C, 48.68/47.45; H, 4.09/5.10; N, 16.70/15.54; O, 22.89/21.65; S, 7.64/6.95.

Compound 10e: Yield: 57%, m.p. 127-129 °C. I.R (KBr, cm^{-1}) 1700 (C=O), 1600 (C=N), 1450 (C=C Ar), 1140 (C-O-C), 1250, 1191 (C-S-C), 742, 695. δ_H (500 MHz, DMSO-d_6) 6.87 (1H, d, J7.4), 6.48 (1H, s), 6.45-6.36 (2H, m), 3.24 (2H, s), 3.17 (1H, t, J7.2), 2.93 (2H, s), 2.73 (1H, t, J7.2), 2.57-2.49 (3H, m), 1.08 (3H, s). δ_C (125 MHz, DMSO-d_6) 193.97, 165.44, 154.41, 154.19, 149.25, 142.95, 142.18, 139.31, 133.24, 124.78, 124.15, 114.80, 71.00, 59.10, 56.84, 42.50, 39.52, 14.19. Anal. % calc./found for $C_{17}H_{16}N_6O_7S$ (m.w. 448.08) Elemental Analysis: C, 45.54/44.54; H, 3.60/2.94; N, 18.74/17.54; O, 24.98/25.14; S, 7.15/6.87.

Compound 10f: Yield: 65%, m.p. 132-134 °C. I.R (KBr, cm^{-1}) 1710 (C=O), 1603 (C=N), 1459 (C=C Ar), 1150 (C-O-C), 1251, 1191 (C-S-C), 742, 693. δ_H (500 MHz, DMSO-d_6) 7.74 (1H, s), 7.51 (2H, ddd, J16.0, 7.7, 1.4), 6.96 (1H, dd, J7.5, 5.0), 4.33 (2H, s), 4.29 (1H, t, J7.2), 4.20 (2H, s), 4.04 (1H, t, J7.2), 3.75 (2H, t, J7.2), 3.72 (3H, s), 1.25 (3H, t, J6.7). δ_C (125 MHz, DMSO-d_6) 193.99, 193.96, 165.44, 154.22, 154.19, 153.96, 153.03, 152.82, 152.13, 139.19, 129.72, 129.66, 129.12, 126.47, 126.44, 115.76, 115.55, 114.42, 114.37, 71.00, 59.10, 56.86, 56.82, 43.28, 22.60, 12.42. Anal. % calc./found for $C_{18}H_{18}FN_5O_6S$ (m.w. 451.43) Elemental Analysis: C, 47.89/46.54; H, 4.02/5.47; F, 4.21/5.14; N, 15.51/16.47; O, 21.26/22.14; S, 7.10/6.98.

Antimicrobial Activity

The crude MTZ and MTZ-ODZ were evaluated for antimicrobial activity using the DDM method. Three strains from both Gram-positive and Gram-negative bacteria were used. The antibacterial activity was measured by calculating the diameter of the zone of inhibition around the disc (table 1).

Antigiardial Activity

In vitro bioassay was used to evaluate the efficacy of antigiardial compounds (table 2). The IC_{50} value of the compounds 10a, 10b, 10c, and 10d ranged from 1.31±0.11 to 2.26±0.49 µM, while it was 3.71±0.27 µM for MTZ. Compound 10e showed the best antigiardial activity with an IC_{50} value of 0.88±0.52 µM.

Antioxidant Activity

The crude and synthesized derivatives were further assessed for their antioxidant properties using DPPH (table 3). Most of the synthesized compounds showed a significant radical scavenging activity. The IC_{50} values for compounds 10a, 10b, 10c, 10d, 10e, and

Table 2: Antigiardial activity of metronidazole and synthesized metronidazole-oxadiazole

Compound	*Giardia intestinalis*
Metronidazole	3.71±0.27
10a	2.15±0.07
10b	1.81±0.30
10c	1.31±0.11
10d	2.26±0.49
10e	11.20±0.78
10f	0.88±0.52

Data are drived from triplicate tests and expressed as mean±SD μM of IC_{50} values

10f were 70.42±0.15, 70.52±0.54, 85.21±0.85, 80.10±0.46, 82.52±0.13, and 70.45±0.12 μg/mL, respectively. The standard rate was 60.10 μg/mL at 100 μg/mL. The results show that radical scavenging activity in DPPH increased with an increase in concentration.

Cytotoxicity Assessment

MTT assay was used to measure cytotoxicity. *In vitro* activity of compounds 10a-10f was evaluated using Caco-2 and Vero cells. The results of cell viability (IC_{50} values) are presented in table 4.

Discussion

A number of MTZ-ODZ derivatives were developed and ^{1}H NMR, ^{13}C NMR, FTIR, and elemental analysis were used to confirm the structure of the new compounds. Evaluation of antimicrobial activity showed that the crude MTZ had no antibacterial activity against the selected bacteria strains. This was expected, since MTZ is active against anaerobic bacteria, whereas the selected bacteria were aerobic. Since the 1,3,4-oxadiazole group was biologically added to MTZ,[5] all MTZ-ODZ derivatives had good antibacterial activity against the selected bacteria given the extent of the inhibition zones.

Evaluation of the antigiardial activity showed that all derivatives possessed biological activity against *Giardia* and that the developed compounds can be used to develop a new antiparasitic drug. This is significantly important due to the scarcity of available drugs that can combat anaerobic parasitic protozoa and bacteria. Modification of original drugs at the molecular level allows a better understanding of the nature of antibiotic resistance of anaerobic pathogens to regular pharmaceuticals.

The result of the antioxidant evaluation showed that, compared to the original drug, most of the synthesized compounds had a significant radical scavenging activity because of the addition of OCH_3, NO_2, and OH. The significant activity of the developed compound could be attributed to nitrogen, oxygen, and sulfur atoms.

Cytotoxicity was measured by MTT assay to determine *in vitro* activity of the new compounds using Caco-2 and Vero cells. In line with previous studies,[29, 30] our results showed that most derivatives (6 μM) were not cytotoxic to these cells.

Table 3: *In vitro* antioxidant activity of crude and synthetic derivatives using different concentrations of the compounds (50, 75, and 100 μg/mL)

Compound	Concentration (μg/mL)			
	50	75	100	IC_{50}
Metronidazole	ns	ns	ns	ns
10a	37.12±0.70	38.21±0.54	38.10±1.72	70.42±0.15
10b	39.14±0.22	40.57±0.32	42.71±0.25	70.52±0.54
10c	65.21±0.26	66.30±0.35	67.21±0.29	85.21±0.85
10d	55.10±0.42	56.24±0.37	27.31±0.35	80.10±0.46
10e	60.21±1.45	62.30±0.20	65.20±0.15	82.52±0.13
10f	61.70±0.31	67.76±0.29	71.98±0.17	70.45±0.12
Ascorbic acid	78.01±0.75	81.01±0.85	84.21±0.41	60.10±0.82
Blank	ns	ns	ns	ns

ns: No scavenging activity; Data are drived from triplicate tests and expressed as mean±SD.

Table 4: Cell viability of Caco-2 and Vero cells after two-day treatment with compounds 10a-f.

Compound	Cell viability* (%)	
	Vero cells	Caco-2 cells
10a	98.4±3.2[a]	91.8±19.2
10b	103.5±4.4	97.5±4.2
10c	102.4±7.1	112.5±5.2
10d	106.2±8.2	145.8±4.3
10e	104.2±6.2	95.7±4.4
10f	106.7±2.2	109.4±4.5

*6 μM from the derivatives were used in the assays, [a]Data from triplicate tests and expressed as mean±SD.

Some drugs consist of oxadiazole core moiety, e.g., Bredon (anti-inflammatory), Irrigor (anesthetic, vasodilator), and Libexin (antitussive).[31] In a previous study, Pattanayak, and Kaliyaperumal incorporated bioactive pharmacophores such as 1,3,4-thiadiazole and Schiff base moiety into MTZ without altering the nitro group. They used the synergy resulting from the inclusion of the imidazole ring, thiadiazole ring, and Schiff's base pharmacophore. Since each of these pharmacophores had antibacterial and anthelmintic activity, their incorporation into a single molecule was expected to improve the antibacterial and anthelmintic activity of the compound as well as its safety and efficacy.[32]

Conclusion

New MTZ-ODZ compounds were synthesized and pharmacological properties (e.g., antioxidant, antimicrobial, and antigiardial) were determined. Cell viability was evaluated by treating Caco-2 and Vero cells with the compounds. Compared to the crude MTZ, all compounds exhibited moderate to good antibacterial activity. Compounds 10d-f showed significant antimicrobial activity and 10f significant antigiardial activity. Most of the synthesized compounds showed significant radical scavenging activity and were also found to have biological properties. Further studies on the synthesized compounds are recommended to identify additional pharmacological properties against other diseases associated with free radicals.

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Authors' Contribution

M.M: Study concept and design, data analysis and interpretation, writing the manuscript. N.H: Conducting experiments, data analysis, and interpretation, preparing reagents, materials, analysis tools, and data, and writing the manuscript. Both authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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