Synthesis and Anticancer and Antimicrobial Evaluation of Novel Ether-linked Derivatives of Ornidazole

Eter Bağlı Yeni Ornidazol Türevlerinin Sentezi, Antikanser ve Antimikrobiyel Etkisinin Değerlendirilmesi

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ABSTRACT

Objectives: Some novel 1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(substituted phenoxy)propan-2-ol derivatives (3a-g) were designed and synthesized.

Materials and Methods: Compounds 3a-g were obtained by refluxing ornidazole (1) with the corresponding phenolic compounds (2a-g) in the presence of anhydrous K$_2$CO$_3$ in acetonitrile.

Results: Following the structure elucidation, the in vitro antimicrobial activity and cytotoxic effects of compounds 3a-g on K562 leukemia and NIH/3T3 mouse embryonic fibroblast cells were measured. As a part of this study, the compliance of the compounds with the drug-likeness properties was evaluated. The physico-chemical parameters (log P, TPSA, nrotb, number of hydrogen bond donors and acceptors, logS) were calculated using the software OSIRIS.

Conclusion: All the synthesized compounds except 3a showed significant activity (MIC=4-16 µg mL$^{-1}$) against the bacterial strain Bacillus subtilis as compared to the standard drug, whereas antileukemic activities were rather limited. Furthermore, all the compounds were nontoxic and the selectivity index outcome indicated that the antileukemic and antimicrobial effects of the compounds were selective with good estimated oral bioavailability and drug-likeness scores.

Key words: Imidazole, ether linked, ornidazole, antimicrobial activity, cytotoxicity

ÖZ

Amaç: Bu çalışmada bazı yeni 1-(2-metil-5-nitro-1H-imidazol-1-yl)-3-(substitüe fenoksi)propan-2-ol (3a-g) türevleri tasarlanmış ve sentezlenmiştir.

Gereç ve Yöntemler: 3a-g numaralı bileşikler Ornidazol’un (1) uygun fenolik bileşikler (2a-g) ile asetonitril içinde susuz K$_2$CO$_3$ eşliğinde isınmasıyla elde edilmiştir.

Bulgular: Yapılan in vitro antibakteriyel ve antikanser etkileri, hücrelerin: K562 lezyon hücreleri ve NIH/3T3 doku hücrelerinde değerlendirilmiştir. OSIRIS yazılımı kullanılarak fizikokimyasal parametreler (log P, TPSA, nrotb, hidrojen bağ vericileri ve alıcıları, logS) hesaplanmıştır.

Sonuç: 3a bileşigi 3a-g türevlerinden en in vitro aktivite göstermiş olup, antibakteriyel ve antikanser etkileri, sırasıyla 16 ve 8 µg mL$^{-1}$ düzeyinde olduğu tespit edilmiştir. Diğer bileşikler ise nötral veya antikanser etkisine sahiptir.

Anahtar kelimeler: Imidazol, eter bağlı, ornidazol, antimikrobiyel aktivite, sitotoksitite

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INTRODUCTION
Cancer, which is defined as a group of related diseases, not a single disease, is a major health problem worldwide. According to a report by the World Health Organization, cancer is the second leading cause of death globally and resulted in 8.8 million deaths in 2015. Leukemia is a group of cancers that generally start in the bone marrow and end up with high numbers of abnormal white blood cells and forms 3.7% of all new cancer cases. Bloodstream infections resulting in deaths in leukemia patients constitute a major challenge for public health and is a situation that needs attention for cancer patients. Intrinsic immune defense mechanisms protect us against invading pathogens and progression of malignancies; thus there is a relationship between antibacterial and antileukemic effects. Many studies for reducing the threat posed by leukemia showed that progress in antimicrobial protection and chemotherapy has reduced disease severity and improved the survival rate.

Over the years, there has been increased interest in the synthesis and biological research of nitrogen-based heterocycles. Among these, the imidazole ring is a major pharmacophoric substructure in a number of antimicrobial and anticancer agents like temozolomide, zoledronic acid, mercaptopurine, azomycin, and ornidazole. Therefore, the search for new therapeutic agents bearing the imidazole ring continues to be an attractive area of investigation in medicinal chemistry. Moreover, it has been hypothesized that a reactive intermediate formed in the microbial reduction of the nitro moiety of nitroimidazoles binds to the DNA of the microorganism with a covalent bond and activates the lethal effect. As shown in Figure 1, analogues of metronidazole and ornidazole have been reported as antimicrobial and anticancer agents. Additionally, five-membered heterocyclic compounds containing an ether-linked structure showed significant anticancer activity.

In view of the aforementioned premises, we aimed to report herein the synthesis and in vitro anticancer and antimicrobial activity of the title compounds. Ornidazole is an antibiotic that is effective only against anaerobic bacterial strains. In the present study paper, we planned the design and synthesis of a series of ornidazole derivatives and investigation of their antimicrobial properties and anticaner activity on the K562 leukemia cell line as well as their cytotoxic effect on the NIH/3T3 cell line.

EXPERIMENTAL
Chemistry
All reagents and solvents were obtained from commercial suppliers and were used without further purification. Merck silica gel 60 F254 plates were used for analytical thin-layer chromatography (TLC). Melting points were determined using a Schmelzpunktbestimmer SMP II apparatus and were uncorrected. 1H-NMR and 13C-NMR spectra were recorded on a Bruker 300 MHz Ultrashield TM spectrometer. Elemental analyses were determined by CHNS-932 (LECO). FT-IR spectra were recorded on a Shimadzu FT-IR-8400S spectrometer. ESI-MS mass spectra were acquired using a PerkinElmer AxION 2 TOF spectrometer. The liquid chromatographic system consisted of an Agilent Technologies 1100 series instrument equipped with a quaternary solvent delivery system and a model Agilent series G1315 A photodiode array detector. Chromatographic data were collected and processed using the software Agilent Chemstation Plus. Chromatographic separation was performed at ambient temperature using a reverse phase ACE C18 (4.0×100 mm) column. All experiments were performed using ACN-H2O (v/v, 40/60) mobile phase with ultraviolet detection at 254 nm.

General procedure for the synthesis of compounds 3a-g
A mixture of phenol derivatives (2a-g) (paracetamol, p-nitrophenol, thymol, methyl paraben, ethyl paraben, 4-chloro-3-methylphenol, and m-cresol) (0.005 mol) and anhydrous

Figure 1. The design of new ornidazole derivatives on the basis of the literature
K₂CO₃ (0.015 mol) in acetonitrile (30 mL) was heated for 1 h. Ornidazole (ODZ) (1) (0.005 mol) was added and the mixture was refluxed for 3 h. The reaction mixture was then poured onto ice and neutralized with 1 N hydrochloric acid. The resulting precipitate consisting of 1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(substitutedphenoxy) propan-2-ol (3a-g) derivatives was collected by filtration and finally purified by recrystallization from methanol (Scheme 1).  

1-(2-Methyl-5-nitro-1H-imidazol-1-yl)-3-[5-methyl-2-(propan-2-yl)phenoxy]propan-2-ol (3c)  
Off-white solid; Yield 92%; m.p. 163°C; Rt (min): 7.25; FT-IR ν max (cm⁻¹): 3234 (O-H); 1659 (C=O amide); 1610, 1533, 1469, 1365 (N-H bending, C=C, C=N, NO₂); 1245 (C-O). 1H-NMR (300 MHz), (DMSO-d₆/TMS) δ ppm: 1.15-1.18 [d, 6H, J=6.9 Hz, CH-(CH₃)]; 2.27 (s, 3H, Ar-CH₃); 2.486s (3H, CH₃); 3.28-3.35 [m, 1H, CH-(CH₃)]; 3.95-4.05 (m, 2H, N-CH₂-CH-CH₉-O); 4.13-4.14 (m, 1H, CH-OH); 4.27-4.70 (m, 2H, CH₂-O); 5.52 (bs, 1H, OH); 6.72-7.08 (m, 3H, Ar-H); 8.05 (s, 1H, Im-H). 13C-NMR (75 MHz, DMSO-d₆) δ ppm: 14.81(CH₃), 21.40 (CH₃), 2313 (CH₃), 26.50 (CH), 49.64 (CH₃), 68.44 (CH-OH), 70.16 (CH₉), 112.71 (CH), 121.63 (CH), 126.01 (CH), 133.40 (CH₉), 133.61 (C), 136.36 (C), 138.98 (C-N-O₂), 152.65 (C), 157.72 (C). Anal. Calcd for C₁₅H₁₇N₃O₆: C, 53.19; H, 5.11; N, 12.33. ESI-MS (m/z): 358 [M+Na]+.

Methyl 4-(2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propoxy)benzoate (3d)  
Dark brown solid; Yield 80%; m.p. 181-183°C; Rt (min): 2.48; FT-IR ν max (cm⁻¹): 1699 (C=O ester); 1602, 1533, 1475, 1363 (N-H bending, C=C, C=N, NO₂) 1317 (C-O). 1H-NMR (300 MHz), (DMSO-d₆/TMS) δ ppm: 2.47 (s, 3H, CH₉), 3.82 (s, 3H, COOCH₃), 4.08-4.34 (m, 4H, 2CH₂), 4.47-4.63 (m, 1H, CH); 5.58-5.59 (d, 1H, J=4.8 Hz, OH); 7.05-7.09 (d, 2H, J=9.0 Hz, Ar-H); 7.90-7.95 (d, 2H, J=9.0 Hz, Ar-H); 8.05 (s, 1H, Im-H). 13C-NMR (75 MHz, DMSO-d₆) δ ppm: 14.81 (CH₉), 49.23 (CH₉), 68.15 (CH-OH), 70.46 (CH₉), 115.00 (2CH), 122.57 (C), 131.71 (2CH), 133.41 (C), 139.02 (C-N-O₂), 152.59 (C), 162.62 (C), 166.32 (C=O) Anal. Calcd for C₁₅H₁₅NO₂: C, 53.73; H, 5.11; N, 12.53 Found: C, 53.19; H, 4.77; N, 12.33. ESI-MS (m/z): 358 [M+Na]+.

Ethyl 4-(2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propoxy)benzoate (3e)  
Yellow solid; m.p. 81-82°C; Rt (min): 3.73; FT-IR ν max (cm⁻¹): 3208 (O-H); 1690 (C=O ester); 1607, 1537, 1472, 1427, 1364 (N-H bending, C=C, C=N, NO₂) 1314 (C-O). 1H-NMR (300 MHz), (DMSO-d₆/TMS) δ ppm: 1.29-1.34 (t, 3H, J=7.2 Hz, CH₃CH₂-CH₂); 2.47 (s, 3H, CH₉); 4.11-4.62 (m, 7H, CH₂-CH₂ and CH-CH-OH); 5.58-5.60 (d, 1H, J=4.8 Hz, OH); 7.05-7.08 (d, 2H, J=8.7 Hz, Ar-H); 7.91-7.94 (d, 2H, J=9.0 Hz, Ar-H); 8.05 (s, 1H, Im-H). 13C-NMR (75 MHz, DMSO-d₆) δ ppm: 14.68 (CH₉), 14.81 (CH₉), 49.24 (CH₉), 60.81 (CH-OH), 68.15 (CH-OH), 70.46 (CH₉), 114.94 (2CH), 122.84 (C), 131.66 (2CH), 133.41 (CH₉), 139.02 (C-N-O₂), 152.59 (C), 162.57 (C), 165.81 (C=O) Anal. Calcd for C₁₅H₁₅NO₂: C, 55.01; H, 5.48; N, 12.03 Found: C, 54.74; H, 5.05; N, 12.13. ESI-MS (m/z): 372 [M+Na]+.

1-(2-Chloro-3-methylphenoxy)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (3f)  
Dark brown solid; Yield 87%; m.p. 139-140°C; Rt (min): 7.40; FT-IR ν max (cm⁻¹): 3144 (O-H); 1593, 1530, 1483, 1362 (N-H bending, C=C, C=N, NO₂); 1292 (C-O). 1H-NMR (300 MHz), (DMSO-d₆/TMS) δ ppm: 2.30 (s, 3H, Ar-CH₃); 2.49 (s, 3H, Im-CH₃); 3.99-4.00 (m, 2H, N-CH₂-CH-CH₉-O); 4.10 (m, 1H, CH-CH-OH); 4.24-4.61 (m, 2H, CH₉-O); 5.54-5.55 (d, 1H, J=3.9 Hz, OH); 6.80-7.32 (m, 3H, Ar-H); 8.04 (s, 1H, Im-H). 13C-NMR (75 MHz, DMSO-d₆) δ ppm: 2.47 (s, 3H, Ar-CH₃); 2.486s (3H, CH₃); 3.28-3.35 [m, 1H, CH-(CH₃)]; 3.95-4.05 (m, 2H, N-CH₂-CH-CH₉-O); 4.13-4.14 (m, 1H, CH-OH); 4.27-4.70 (m, 2H, CH₂-O); 5.52 (bs, 1H, OH); 6.72-7.08 (m, 3H, Ar-H); 8.05 (s, 1H, Im-H). 13C-NMR (75 MHz, DMSO-d₆) δ ppm: 14.81 (CH₉), 21.40 (CH₃), 2313 (CH₃), 26.50 (CH), 49.64 (CH₃), 68.44 (CH-OH), 70.16 (CH₉), 112.71 (CH), 121.63 (CH), 126.01 (CH), 133.40 (CH₉), 133.61 (C), 136.36 (C), 138.98 (C-N-O₂), 152.65 (C), 157.72 (C). Anal. Calcd for C₁₅H₁₅NO₂: C, 53.73; H, 5.11; N, 12.53 Found: C, 53.19; H, 4.77; N, 12.33. ESI-MS (m/z): 358 [M+Na]+.
ppm: 14.81 (CH$_3$), 20.25 (CH$_3$), 49.30 (CH$_3$), 68.22 (CH-CH$_2$), 70.52 (CH$_3$), 114.15 (CH), 117.70 (CH), 125.28 (C), 129.97 (CH), 133.40 (CH), 136.98 (C), 139.02 (C-NO$_2$), 152.58 (C), 157.53 (C). Anal. Calcd for C$_{14}$H$_{17}$N$_3$O$_4$: C, 57.72; H, 5.88; N, 14.42. Found: C, 58.25; H, 5.45; N, 14.24. ESI-MS (m/z): 314 [M+Na]$^+$. 

1-(2-Methyl-5-nitro-1H-imidazol-1-yl)-3-(3-methylphenoxy) propan-2-ol (3g)

Light brown solid; Yield 76%; m.p. 103°C (lit. 105-106°C).\textsuperscript{14} $^\text{13}$C-NMR (75 MHz, DMSO-$d_6$) δ ppm: 27.13 (CH$_3$), 31.68 (CH$_2$), 49.38 (CH$_2$), 68.32 (CH-CH$_2$), 70.11 (CH$_3$), 111.93 (CH), 115.62 (CH$_2$), 122.02 (CH), 129.71 (CH), 133.38 (CH), 139.02 (C-NO$_2$), 139.47 (C), 152.58 (C), 157.86 (C). Anal. Calcd for C$_{14}$H$_{16}$ClN$_3$O$_4$: C, 51.62; H, 4.95; N, 12.90. Found: C, 51.68; H, 4.55; N, 12.75. ESI-MS (m/z): 348 [M+Na]$^+$. 

**Biological part**

**Antimicrobial activity**

All synthesized compounds were evaluated for antimicrobial activity. The activity experiments were carried out in Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering. The gram-positive and gram-negative bacteria Escherichia coli ATCC 10536, Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 15442, and Bacillus subtilis (B. subtilis) ATCC 6633 and Candida albicans ATCC 10231 were used in the activity studies. Antimicrobial activities of the compounds tested against these species were based on disc-diffusion and micro-well dilution assays. 

**Disc-diffusion assay**

The antimicrobial properties of the compounds were investigated by disc-diffusion assay as described in the literature.\textsuperscript{15} For this aim, 100 µL of freshly prepared microbial suspensions containing $10^8$ CFU/mL of bacteria and $10^4$ spore/mL of fungi were spread on nutrient agar, Sabouraud dextrose agar, and potato dextrose agar, respectively. Black discs (6 mm) impregnated with imidazole derivatives (20 µL) of the specified concentrations were placed on the inoculated plates. Distilled water (20 µL) was used as a negative control. The inoculated plates were incubated at 36±1°C for 24 h for the bacterial strains and 27±1°C for 72 h for the fungal isolate. Antimicrobial activity was determined by measuring the zone of inhibition around the discs.

**Micro-well dilution assay**

The sensitivity of the bacterial strains towards the compounds was quantitatively evaluated from the minimum inhibitory concentration (MIC) values obtained by the micro-well dilution method.\textsuperscript{16} The inocula of the bacterial strains were prepared from 12-h broth cultures and the suspensions were adjusted to 0.5 McFarland standard turbidity. Compounds dissolved in DMSO were first prepared at the highest concentration to be tested (1024 µg/mL), and then serial two-fold dilutions were made in order to obtain a concentration range from 2 to 1024 µg/mL in 15-ml sterile test tubes containing nutrient broth. The 96-well plates were prepared by dispensing into each well 95 µL of nutrient broth and 5 µL of the inoculum. Two hundred microliters of nutrient broth without inoculum was transferred into the first wells as a positive control. Aliquots (100 µL) taken from the 200 µg/mL stock solution were added to the second well. One hundred microliters from the respective serial dilutions was transferred into 5 consecutive wells. The last well containing 195 µL of nutrient broth without compound and 5 µL of the inoculum on each strip was used as a negative control. 

The contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth in each medium was determined by reading the absorbance (Abs) at 630 nm using an ELx800 universal microplate reader (BioTek Instruments Inc., Highland Park, VT, USA) and confirmed by plating 5-µL samples from clear wells on nutrient agar medium. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. Ampicillin and fluconazole were used as positive controls for the bacteria and fungi, respectively.

**Anticancer activity**

**Cell culture**

The human leukaemic cell line K562 (ATCC, CCL-243) and mouse embryonic fibroblast cell line NIH/3T3 (ATCC, CRL-1658) were maintained in Dulbecco’s Modified Eagle Medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and penicillin/streptomycin (Gibco) at 37°C in a humidified incubator with 5% CO$_2$. 

**Cell viability assay**

The cell viability effects of compounds 3a-g were evaluated \textit{in vitro} using the MTT colorimetric method against the K-562 and NIH/3T3 cell lines at different doses\textsuperscript{17,18} at the Marmara University, Faculty of Pharmacy, Department of Biochemistry. Briefly, the cells (1x10$^4$ cells/well) were seeded onto 96-well plates and incubated overnight. Then the cells were treated with different concentrations of compounds for 48 h. After the incubation period, MTT was added to each well to a final concentration of 0.5 mg/mL followed by incubation for 4 h. The culture medium was removed and 100 µL of the SDS buffer was added to solubilize the purple formazan product. Abs at wavelengths of 570 and 630 nm was measured by a microplate reader (BioTek, Winooski, VT, USA). Cell viability was expressed as a percentage of the untreated control cells.

**Statistical analysis**

The data were reported as means±standard deviations and analyzed by one-way analysis of variance followed by Tukey’s multipl comparison test using GraphPad Prism 5. Differences between means at p<0.05 level were considered significant.
RESULTS AND DISCUSSION

Chemistry

Scheme 1 outlines the synthetic pathway used to obtain the ether-linked derivatives 3a-g. The reaction of ornidazole (1) with phenol compounds 2a-g yielded the 1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(substituted phenoxy)propan-2-ol (3a-g) derivatives. The TLC and high performance liquid chromatography data confirmed the new products. The purity of the compounds was established from sharp melting points and elemental analysis data. Compound 3g was reported to be synthesized previously but no theoretical or biological information about this compound has been presented in the available literature.14

The FT-IR, 1H NMR, 13C-NMR, and ESI-MS data were in agreement with the proposed structures of compounds 3a-g (Figures S1-S28, in the Supplementary File). In particular, the FT-IR spectrum of 3a showed absorption bands at 1659 cm⁻¹ and 3129 cm⁻¹ due to C=O and NH functions, respectively. In the NMR spectra the NH proton resonated at the expected regions. The FT-IR spectra of compounds 3d and 3e revealed the presence of bands for C=O ester groups, while the 1H-NMR spectra of the compounds. The aryl methyl groups in the 3f and 3g molecules were clearly demonstrated by their typical signals at δ 2.29-2.30 ppm along with a singlet in the aliphatic region. The LC-MS/MS analysis data displayed [M+Na]⁺ corresponding to new compounds.

Pharmacological screening

Cytotoxicity of compounds towards K562 and NIH/3T3 cells

In the MTT test, the K562 cell line and NIH/3T3 cell line were incubated with compounds 3a-g at various concentrations. After the completion of the incubation period, the cytotoxic effects of the compounds were examined and the IC₅₀ values with the selectivity index were calculated. The results are presented in Table 1.

The MTT assay was performed to determine the antiproliferative effects of compounds 3a-g on the K562 leukemia cell line. In order to check for toxicity on healthy cells, the effects of compounds 3a-g on NIH/3T3 mouse embryonic fibroblast cells were investigated using the MTT test. The selectivity index values of all of the compounds were also determined to compare their selectivity (Table 1). Compounds 3a-g showed weaker cytotoxicity on the K562 cell line, while all the synthesized compounds were found to be nontoxic on healthy cells and safe for human consumption. Among them, compound 3a was the most effective, with an IC₅₀ value of 116.55 µM. Moreover, these compounds exhibited selectivity due to their low cytotoxicity on healthy cells with selectivity indices between 1.94 and 4.02. The IC₅₀ values of these compounds for the NIH/3T3 cell line were higher than those for the K562 cell line. These results suggested that this series of compounds possessed selectivity for the K562 cancer cell line. As a result, compounds 3b and 3g showed more selective antileukemic activity than the other compounds.

Antimicrobial activity

The synthesized derivatives were screened for their antimicrobial activity against two gram-positive (B. subtilis and Staphylococcus aureus) and two gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacterial strains and Candida albicans fungus strain. The zone of inhibition and minimum inhibitory concentration results are presented in Table 2.

The results of the antimicrobial screening of the tested compounds revealed that 3f, bearing chlorine, showed moderate antibacterial activity against Staphylococcus aureus only (MIC: 64 µg/mL). On the other hand, all the tested compounds exhibited significant activity against B. subtilis (MIC: 4-16 µg/mL, with a range of inhibition zones 12-20 mm) comparable with that of the reference compound ampicillin except 3a. Compounds 3b and 3f, bearing nitro and chloro groups at the para position, were the most effective synthetic compounds, having lower MIC values compared to the other compounds. B. subtilis was used as a model organism as it belongs to the same group as pathogenic Bacillus anthracis etc. and is a gram-positive, aerobic, and spore-forming microorganism belonging to the genus Bacillus.19 B. subtilis ATCC 6633 is used in EN 13704 as an obligatory test organism in the form of endospores.20

SI is commonly used to estimate the therapeutic window of a drug and to identify drug candidates for further studies. According to the literature,21,22 candidates for new drugs must have a SI>10, with MIC values lower than 6.25 µg/mL and low cytotoxicity, as is indeed the case for compounds 3b-3f. The synthesized compounds were evaluated for cytotoxicity in NIH/3T3 cells at concentrations ten times the MIC (Table 2).

The rest of the compounds showed no significant activity against the other microorganisms tested when compared to that of standard drugs at the same concentration as that of the test compounds.

Prediction of the drug-likeness properties of 3a-g

The in silico drug-likeness properties obtained by OSIRIS Property Explorer (http://www.openmolecules.org/
Drug-likeness prediction evaluates the acceptability of derivatives as drug molecules based on Lipinski’s rule of five. ABS% was calculated by $\%\text{ABS} = 109 - [0.345 \times \text{topological polar surface area (TPSA)}]$ according to the method described by Zhao et al. TPSA, cLogP, number of rotatable bonds, and violations of Lipinski’s rule of five were calculated using the online property calculation toolkit OSIRIS.

The rule of five is a set of defined parameters to predict if a chemical compound has promising or viable pharmacological or biological activity as a drug in oral administration. These parameters are: 1) The molecule should not contain more than 5 hydrogen bond donors, 2) And no more than 10 hydrogen bond acceptors, 3) The molecular weight should be lower than 500, 4) The value for cLogP should not be higher than 5. The parameters in the rule of five were fully covered for the set of our synthesized compounds. The values of log P ranged from -0.59 to 1.27 for all designed molecules, while the values of log S were between -1.674 and -2.748. More than 80% of the drugs on the market have an estimated S value greater than 4. TPSA values are closely related to the hydrogen bonding potential of a compound; those around 160 or more indicate poor intestinal absorption. Hence, these parameters suggest that the compounds are expected to exhibit good oral bioavailability and intestinal absorption.

Drug-likeness estimations have been used to minimize attrition in the process of drug discovery and refer to the similarity of compound properties to those of existing oral drugs. Generally, the drug-likeness scores of compounds were greater than that of ornidazole using a starting compound, except 3d and 3e.

### CONCLUSION
A series of ether-linked imidazole compounds that are derivatives of ornidazole were synthesized, structurally identified, and tested for antimicrobial and antileukemic activity. All the synthesized molecules were achieved in good yields by following a simple method. The projected structures of synthesized compounds 3a-g were well supported by the spectral characterization data using IR, 1H-NMR, and ESI-MS.

As a result, all compounds showed a minor anticancer effect on the K562 leukemia cell line. In addition, they were found to be the most promising antimicrobial agents in the series due to their selective antimicrobial activity against *B. subtilis* with...
MIC values of 4-8 µg/mL when compared with the reference agent, except 3a and 3g. The compounds with nitro and chloro substituents were the most active in the series against B. subtilis. We can surmise that the inhibition of spores of B. subtilis is related to their sporicidal activity.

In particular, all the compounds were nontoxic. This outcome indicated that the antileukemic and antimicrobial effects of the compounds were selective and they had good oral bioavailability and drug-likeness properties. Thus, we can conclude that these ether-linked imidazole derivatives could be an excellent starting point to design new anticancer and antibacterial agents.

Conflict of Interest: No conflict of interest was declared by the authors.

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N-{4-[2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propoxy]phenyl}acetamide (3a)

**Figure S1.** IR spectrum for compound 3a

**Figure S2.** $^1$H-NMR spectrum for compound 3a

**Figure S3.** $^{13}$C-NMR spectrum for compound 3a

**Figure S4.** MS spectrum for compound 3a
1-(2-Methyl-5-nitro-1H-imidazol-1-yl)-3-(4-nitrophenoxy)propan-2-ol (3b)

**Figure S5.** IR spectrum for compound 3b

**Figure S6.** $^1$H-NMR spectrum for compound 3b

**Figure S7.** $^{13}$C-NMR spectrum for compound 3b

**Figure S8.** MS spectrum for compound 3b
1-(2-Methyl-5-nitro-1H-imidazol-1-yl)-3-[5-methyl-2-(propan-2-yl)phenoxy]propan-2-ol (3c)

**Figure S9.** IR spectrum for compound 3c

**Figure S10.** $^1$H-NMR spectrum for compound 3c

**Figure S11.** $^{13}$C-NMR spectrum for compound 3c

**Figure S12.** MS spectrum for compound 3c
Methyl 4-[2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propoxy]benzoate (3d)

Figure S13. IR spectrum for compound 3d

Figure S14. ¹H-NMR spectrum for compound 3d

Figure S15. ¹³C-NMR spectrum for compound 3d

Figure S16. MS spectrum for compound 3d
Ethyl 4-[2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propoxy]benzoate (3e)

Figure S17. IR spectrum for compound 3e

Figure S18. $^1$H-NMR spectrum for compound 3e

Figure S19. $^{13}$C-NMR spectrum for compound 3e

Figure S20. MS spectrum for compound 3e

1-(4-Chloro-3-methylphenoxy)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (3f)

Figure S21. IR spectrum for compound 3f

Figure S22. $^1$H-NMR spectrum for compound 3f
1-(2-Methyl-5-nitro-1H-imidazol-1-yl)-3-(3-methylphenoxy)propan-2-ol (3g)

**Figure S23.** $^{13}$C-NMR spectrum for compound 3f

**Figure S24.** MS spectrum for compound 3f

**Figure S25.** IR spectrum for compound 3g

**Figure S26.** 1H-NMR spectrum for compound 3g

**Figure S27.** 13C-NMR spectrum for compound 3g

**Figure S28.** MS spectrum for compound 3g