SYNTHESIS, ANTICANCER AND ANTIMICROBIAL EVALUATION OF NOVEL ETHER-LINKED DERIVATIVES OF ORNIDAZOLE

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Short Title: Synthesis and Biological Activity of Ornidazole Derivatives

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INTRODUCTION: In this study, some novel 1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(substituted phenoxy)propan-2-ol derivatives (3a-g) were designed and synthesized.
METHODS: Compounds 3a-g were obtained by refluxing ornidazole (1) with the corresponding phenolic compounds (2a-g) in the presence of anhydrous K2CO3 in acetonitrile.
RESULTS: Following the structure elucidation, 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 to the drug-likeness properties were evaluated. The physico-chemical parameters (log P, TPSA, nrotb, number of hydrogen bond donors and acceptors, logS) were calculated using OSIRIS software
DISCUSSION AND CONCLUSION: All the synthesized compounds except 3a, showed significant activity (MIC=4-16 μgmL-1) against the bacterial strain B.subtilis as compared to the standard drug whereas antileukemic activities were rather limited. Furthermore, all the compounds are found to be nontoxic, selectivity index outcome indicated that antileukemic and also antimicrobial effects of the compounds were selective with good estimated oral bioavailability and drug-likeness scores

Keywords: Imidazole, ether linked, ornidazole, antimicrobial activity, cytotoxicity

INTRODUCTION

Cancer that is defined as a group of related diseases, not a single disease, is a major health problem worldwide. According to the report of World Health Organization, cancer is the second leading cause of death globally and concluded for

8.8 million death in 2015. Leukemia is a group of cancers that generally start in the bone marrow and end up high numbers of abnormal white blood cells and forms 3.7% of all new cancer cases[1]. Bloodstream infections resulting deaths in leukemia patients constitute major challenges for public health and is a situation that needs attention for cancer patients[2]. Intrinsic immune defense mechanisms keep us against invading pathogens and improving of malignancies thence 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 have reduced the disease severity and improved survival rate[3].

Over the years, there has been increased interest in the synthesis and biological research of nitrogen-based heterocycles. Among these, imidazole ring is a major pharmacophoric substructure in a number of antimicrobial and anticancer agents [4–7] like temozolomide, zoledronic acid, mercaptopurine, azomycin and ornidazole. Therefore, the search for new therapeutic agents bearing imidazole ring continues to be an attractive area of investigation in medicinal chemistry. Also, 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 covalent bond and activates the lethal effect[8]. As shown in Figure 1, analogs of metronidazole and ornidazole have been reported as antimicrobial and anticancer agents[9–11]. Additionally, five membered heterocyclic compounds containing ether-linked structure showed significant anticancer activity[12,13].

Figure 1. The design of new ornidazole derivatives on the basis of the literature.

In view of the aforementioned premises, we aimed at reporting herein the synthesis and *in vitro* anticancer and antimicrobial activity of the title compounds. Ornidazole is an antibiotic which is effective only anaerobic bacterial strains. In this paper, we planned the design and synthesis of a series of ornidazole derivatives and investigation of antimicrobial properties and anticancer activity on K562 leukemia cell line besides cytotoxic effect on 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 TLC. Melting points were determined using Schmelzpunktbestimmer SMP II apparatus and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 300 MHz Ultrashield TM spectrometer. Elemental analyses were determined by CHNS-932 (LECO). FT-IR spectra were recorded on a Schimadzu FT-IR-8400S spectrometer. ESI-MS mass spectra were acquired using a Perkin Elmer AxION 2 TOF spectrometer. The liquid chromatographic system consists 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 Agilent Chemstation Plus software. Chromatographic separation was performed at ambient temperature using a reverse phase ACE C18 (4.0×100 mm) column. All experiments were performed using ACN-H_2O (v/v, 40/60) mobile phase with UV 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 K_2CO_3 (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 1N hydrochloric acid. The resulting precipitate consisting of 1-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-3-(substitutedphenoxy)propan-2-ol **(3a-g)** derivatives were collected by filtration and finally purified by recrystallization from methanol (Scheme 1).

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

Light yellow solid; Yield 81 %; m.p 189-190^{o}C; Rt (min.): 1.15; FT-IR υ_{max} (cm^{-1}): 3377 (O-H.); 3219 (N-H); 1659 (C=O amide); 1602, 1553, 1510, 1445, 1370 (N-H bending, C=C, C=N, NO_2); 1240 (C-O). ^{1}H-NMR (300 MHz), (DMSO-d_6/TMS) δ ppm: 2.00 (s, 3H, Ar-**CH_3**); 2.46 (s, 3H**,** CO**CH_3**); 3.95-3.97 (d, 2H, *J=5.1 Hz,* N-**CH_2**-CH-

OH); 4.05-4.13 (m, 1H, **CH**-OH); 4.24-4.62 (m, 2H, **CH_2-**O); 5.55 (bs, 1H, **OH**); 6.87-6.90 (d, 2H, *J=9.0 Hz,* Ar-**H**); 7.47-7.50 (d, 2H, *J=9.0 Hz,* Ar-**H**); 8.04 (s, 1H, **Im-H**); 9.80 (s, 1H, **NH**). ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm: 14.81 (CH_3), 24.27(CH_3), 49.37 (CH_2), 68.32 (CH-OH), 70.47 (CH_2), 114.96 (2CH), 120.92 (2CH), 133.31(C), 133.38 (CH), 139.03 (C-NO_2), 152.58 (C), 154.46 (C), 168.22 (C=O). Anal. Calcd for $C_{15}H_{18}N_4O_5$. 1/2 H_2O: C, 52.47; H, 5.58; N, 16.32 Found: C, 52.96; H, 4.91; N, 16.31. ESI-MS (m/z): 357 $[M+Na]^+$.

1-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)-3-(4-nitrophenoxy)propan-2-ol (3b)

Light brown solid; Yield 78 %; m.p 181ºC; Rt (min.): 2.71; FT-IR υ_{max} (cm^{-1}): 3375 (O-H.); 3136 (=C-H); 1593, 1531,1498, 1347 (C=C, C=N, NO_2); 1342 (C-O). ^{1}H-NMR (300 MHz), (DMSO-d_6/TMS) δ ppm: 2.47 (s, 3H, **CH_3**); 4.13-4.35 (m, 4H, 2**CH_2**); 4.57-4.63 (m, 1H, **CH-**OH); 5.62-5.64 (d, 1H, *J=4,5 Hz,* **OH**); 7.15-7.21 (d, 2H, *J=9.3 Hz,* Ar-**H**); 8.05 (s, 1H, **Im-H**); 8.21-8.26 (d, 2H, *J=9.6 Hz,* Ar-**H**). ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm: 14.82 (CH_3), 49.12 (CH_2), 68.08 (CH-OH), 71.05 (CH_2), 115.57 (2CH), 126.36 (2CH), 133.44 (CH), 139.03 (C-NO_2), 141.47 (C-NO_2), 152.61 (C), 164.09 (C). Anal. Calcd for $C_{13}H_{14}N_4O_6$: C, 48.45; H, 4.38; N, 17.38 Found: C, 49.16; H, 4.31; N, 17.36. ESI-MS (m/z): 345 $[M+Na]^+$.

1-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)-3-[5-methyl-2-(propan-2-yl)phenoxy]propan-2-ol (3c)

Off-white solid; Yield 92 %; m.p 163ºC; Rt (min.): 7.25; FT-IR υ_{max} (cm^{-1}): 3234 (O-H.); 1659 (C=O amide); 1610, 1533, 1469, 1365 (N-H bending, C=C, C=N, NO_2); 1243 (C-O). ^{1}H-NMR (300 MHz), (DMSO-d_6/TMS) δ ppm: 1.15-1.18 (d, 6H, *J=6,9 Hz,* CH-(**CH_3**)$_2$); 2.27 (s, 3H, Ar-**CH_3**); 2.48(s, 3H, **CH_3**); 3.28-3.35 (m, 1H, **CH**-(CH_3)$_2$); 3.95-4.05 (m, 2H, N-**CH_2**-CH-OH); 4.13-4.14 (m, 1H, **CH**-OH); 4.27-4.70 (m, 2H, **CH_2-**O); 5.52 (bs, 1H, **OH**); 6.72-7.08 (m, 3H, Ar-**H**); 8.05 (s, 1H, **Im-H**). ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm: 14.81(CH_3), 21.40 (CH_3), 23.13 (CH_3), 23.16 (CH_3), 26.50 (CH), 49.64 (CH_2), 68.44 (CH-OH), 70.16 (CH_2), 112.71(CH), 121.63 (CH), 126.01(CH), 133.40 (CH_2), 133.61(C), 136.36 (C), 138.98 (C-NO_2), 152.65 (C), 155.72(C). Anal. Calcd for $C_{17}H_{23}N_3O_4$ .1/2 H_2O: C, 59.63; H, 7.06; N, 12.27 Found: C, 59.00; H, 6.33; N, 12.50. ESI-MS (m/z): 356 $[M+Na]^+$.

Methyl 4-[2-hydroxy-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl)propoxy]benzoate (3d)

Dark brown solid; Yield 80 %; m.p 181-183ºC; Rt (min.): 2.48; FT-IR υ_{max} (cm^{-1}): 1699 (C=O ester); 1602, 1533, 1475, 1363 (N-H bending, C=C, C=N, NO_2) 1317 (C-O). ^{1}H-NMR (300 MHz), (DMSO-d_6/TMS) δ ppm: 2.47 (s, 3H, **CH_3**); 3.82 (s, 3H, COO**CH_3**); 4.08-4.34 (m, 4H, 2**CH_2**); 4.57-4.63 (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**). ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm: 14.81(CH_3), 49.23 (CH_2), 52.29 (CH_3), 68.15 (CH-OH), 70.46 (CH_2), 115.00 (2CH), 122.57 (C), 131.71(2CH), 133.41(CH), 139.02 (C-NO_2), 152.59 (C), 162.62 (C), 166.32 (C=O). Anal. Calcd for $C_{15}H_{17}N_3O_6$: 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-1*H*-imidazol-1-yl)propoxy]benzoate (3e)

Yellow solid; m.p 81-82ºC, Rt (min.): 3.73; FT-IR υ_{max} (cm^{-1}): 3208 (O-H.); 1690 (C=O ester); 1607, 1537, 1472, 1427, 1364 (N-H bending, C=C, C=N, NO_2); 1314 (C-O). ^{1}H-NMR (300 MHz), (DMSO-d_6/TMS) δ ppm: 1.29-1.34 (t, 3H, *J=7,2 Hz*, CH_2-**CH_3**); 2.47 (s, 3H, **CH_3**); 4.11-4.62 (m, 7H, **CH_2**-CH_3 ve **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**). ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm: 14.68 (CH_3), 14.81(CH_3), 49.24 (CH_2), 60.81(CH_2), 68.15 (CH-OH), 70.46 (CH_2), 114.94 (2CH), 122.84 (C), 131.66 (2CH), 133.41(CH), 139.02 (C-NO_2), 152.59 (C), 162.57(C), 165.81(C=O). Anal. Calcd for $C_{16}H_{19}N_3O_6$: 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-(4-Chloro-3-methylphenoxy)-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl)propan-2-ol (3f)

Dark brown solid; Yield 87 %; m.p 139-140 ºC; Rt (min.): 7.40; FT-IR υ_{max} (cm^{-1}): 3144 (O-H.); 1593, 1530, 1483, 1362 (N-H bending, C=C, C=N, NO_2); 1292 (C-O). ^{1}H-NMR (300 MHz), (DMSO-d_6/TMS) δ ppm: 2.30 (s, 3H, Ar-**CH_3**); 2.49 (s, 3H**,** Im-**CH_3**); 3.99-4.00 (m, 2H, N-**CH_2**-CH-OH); 4.10 (m, 1H, **CH**-OH); 4.24-4.61 (m, 2H, **CH_2**-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**). ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm: 14.81(CH_3), 20.25 (CH_3), 49.30 (CH_2), 68.22 (CH-OH), 70.52 (CH_2), 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 $C_{14}H_{16}ClN_3O_4$: C, 51.62; H, 4.95; N, 12.90 Found: C, 51.68; H, 4.55; N, 12.75. ESI-MS (m/z): 348 $[M+Na]^+$.

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

Light brown solid; Yield 76 % ; m.p 103 ºC (lit. 105-106 ºC)[14]. Rt (min.): 3.44; FT-IR υ_{max} (cm^{-1}): 3140 (O-H.); 1586, 1530, 1497, 1470, 1370 (N-H bending, C=C, C=N, NO_2); ^{1}H-NMR (300 MHz), (DMSO-*d_6*/TMS) δ ppm: 2.29 (s, 3H, Ar-**CH_3**); 2.47 (s, 3H**,** Im-**CH_3**); 3.97-3.99 (m, 2H, N-**CH_2**-CH-OH); 4.10 (m, 1H, **CH**-OH); 4.57-4.63 (m, 2H, **CH_2-**O); 5.51-5.53 (d, 1H, *J=5,4 Hz*, **OH**); 6.73-7.20 (m, 4H, Ar-**H**); 8.04 (s, 1H, **Im-H**). ^{13}C-NMR (75 MHz, DMSO-*d_6*) δ ppm: 14.80 (CH_3), 21.56 (CH_3), 49.38 (CH_2), 68.32 (CH-OH), 70.11(CH_2), 111.93 (CH), 115.62 (CH), 122.02 (CH), 129.71 (CH), 133.38 (CH), 139.03 (C-NO_2), 139.47 (C), 152.58 (C), 158.76 (C). Anal. Calcd for $C_{14}H_{17}N_3O_4$: C, 57.72; H, 5.88; N, 14.42 Found: C, 58.25; H, 5.45; N, 14.24. ESI-MS (m/z): 314 $[M+Na]^+$.

Biological Part

Antimicrobial activity

All synthesized compounds were evaluated for antimicrobial activity. Activity experiments were carried out in Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering. Gram positive and Gram negative bacteria, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 10231 were used in activity studies. Antimicrobial activities of the compounds tested against that bacteria species based on disc diffution and microwell dilution assay.

Disc-diffusion assay

Antimicrobial properties of compounds were investigated by using disc-diffusion assay as described in the literature[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 negative control. The inoculated plates were incubated at 36±1 °C for 24 h for bacterial strains and 27±1 °C for 72 h for fungal isolate. Antimicrobial activity was determined by measuring zone of inhibition around the discs.

Micro-well dilution assay

The sensitivity of the bacterial strains towards the compounds was quantitatively evaluated from the MIC values obtained by the micro-well dilution method [16].

The inocula of the bacterial strains were prepared from 12-h broth cultures and 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 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 negative control. Contents of each well were mixed on 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 the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, VT, U.S.A.) 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. Ampicilin and fluconazole were used as positive control for the bacteria and fungi, respectively.

Anticancer activity

Cell culture

Human leukemic cell line, K562 (ATCC, CCL-243) and mouse embryonicnic fibroblast cell line NIH/3T3 (ATCC, CRL-1658) were maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal bovine serum), 1% L-Glutamine and penicillin/streptomycin (Gibco) at 37°C in a humidified incubator with 5% CO_2.

Cell viability assay

Cell viability effects of compounds **3a-g** were evaluated *in vitro* using the MTT colorimetric method against the K-562 and NIH/3T3 cell lines at different doses [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 into each well to a final concentration of 0.5 mg/mL and incubated for 4 h. The culture medium was removed and 100 µL of the SDS buffer was added to solubilize the purple formazan product. Absorbances at wavelengths of 570 and 630 nm were 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 analysed by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison tests 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-1*H*-imidazol-1-yl)-3-(substituted phenoxy)propan-2-ol (**3a-g**) derivatives. TLC and HPLC data confirmed new products. The purity of compounds was established from sharp melting points and elemental analysis data. Compound **3g** was reported to be synthesized previously but no theoretical and biological information about this compound has been presented in the available literature.[14]

The FT-IR, ^{1}H NMR, ^{13}C-NMR and ESI-MS data were in agreement with the proposed structures of compounds **3a-g**. In particular, the FT-IR spectrum of **3a** showed absorption band at 1659 cm^{-1} and 3129 cm^{-1} due to C=O and NH functions, respectively. In the NMR spectra NH proton was resonated at expected regions. FT-IR spectra of compounds, **3d** and **3e** revealed the presence of bands for C=O ester groups. While ^{1}H-NMR spectra of compounds **3d** and **3e** revealed the presence of methyl and ethyl protons attributed to the ester group. The aryl methyl group 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 aliphatic region. LC-MS/MS analysis data displayed $[M+Na]^{+}$ corresponding to new compounds.

Scheme 1. Synthetic route to ornidazole derivatives.

Pharmacological screening

Cytotoxicity of compounds towards K562 and NIH/3T3 cells

In MTT test, K562 cell line and NIH/3T3 cell line were incubated wtih the compounds **3a-g** at various concentration. After the completetion of incubation period, cytotoxic effects of the compounds were examined and IC_{50} values with selectivity index were calculated. Results are presented in Table 1.

Table 1. IC_{50} values of the compounds against K562 and NIH/3T3 cells for 24 h.

MTT assay was performed to determine the antiproliferative effects of compounds **3a-g** on 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 MTT test. The selectivity index values of all of the compounds also determined to compare their selectivity (Table 1). Compounds **3a-g** showed weaker cytotoxicity on K562 cell line also 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 IC_{50} value 116.55 μM. Besides, these compounds exhibited selectivity due to their low cytotoxicity on healthy cells with selectivity indices between 1.94 and 4.02.

The IC_{50} values of these compounds for NIH/3T3 cell line were higher than their IC_{50} values for K562 cell line. These results suggested this series of compounds possessed selectivity for K562 cancer cell line. As a result, compounds **3b** and **3g** showed more selective antileukemic activity than other compounds.

Antimicrobial activity

The synthesized derivatives were screened for their antimicrobial activity against two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains and *Candida albicans* fungi strain. The zone of inhibitons and minimum inhibitory concentrations 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 *S.aureus* only (MIC; 64 μg/ml). On the other hand, all the tested compounds exhibited significiantly active against *B.subtilis* (MIC; 4-16 μg/ml, with a range of inhibition zones 12-20 mm) comparable with that of 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 the least MIC value as 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. species and gram-positive, aerobic and spore-forming microorganism, belongs to Bacillus genus[19]. *B.subtilis* ATCC 6633 is

used in EN 13704 as an obligatory test organism in the form of endospores, and can also be used in EN 13797 mod. as a standard test organism in testing for sporicidial efficacy.[20]

Table 2. *In vitro* antimicrobial activity of the synthesized compounds **3a-g**, MIC in μg mL^{-1} (zones of inhibition, mm)

SI is commonly used to estimate the therapeutic window of a drug and to identify drug candidates for further studies. According to literature [21,22] candidates for new drugs must have a SI>10, with MIC values lower than 6.25 μg/mL and a 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).

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

Prediction of Drug-Likeness Properties of 3a-g

The *in silico* drug-likeness properties obtained by OSIRIS Property Explorer (http://www.openmolecules.org/datawarrior/) are given in Table 3. Drug-likeness prediction evaluates the acceptability of derivatives as drug molecules based on Lipinski's rule of five[23]. Absorption (%ABS) was calculated by: %ABS = 109 – [0.345×topological polar surface area (TPSA)] according to the method of Zhao et al. [24] TPSA [25], cLogP, number of rotatable bonds, and violations of Lipinski's "Rule of Five" were calculated using OSIRIS online property calculation toolkit.

The rule of five is a set of defined parameters, to predict if a chemical compound has a promising or viable pharmacological or biological activity as drug in oral administration. These parameters are: 1) The molecule should not contain more than 5 hydrogen bond donors, 2) No more than 10 hydrogen bond acceptors, 3) The

molecular weight should be lower than 500, 4) The value for cLog P 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 1,273 for all designed molecules, while the values of log S were between -1,674 and -2,748. There are more than 80% of the drugs on the market having an estimated S value greater than 4.[26,27] TPSA values is closely related to the hydrogen bonding potential of a compound, around of 160 or more are expected to exhibit 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 as tools to minimize attrition in the process of drug discovery and refers to the similarity of compound properties to existing oral drugs. Generally, the drug- likeness score values of compounds were greater than that of ornidazole using a starting compound except **3d** and **3e**.

Table 3. Drug-likeness calculations and Lipinski parameters of the compounds

CONCLUSION

A series of ether linked imidazole compounds, which 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 by IR, ^{1}H-NMR and ESI-MS.

As a result, all compounds showed minor anticancer effect on K562 leukemia cell line. In addition, were found to be the most promising antimicrobial agent in the series due to their selective antimicrobial activity against *B.subtilis* with MIC value of 4-8 μg/ml when compared with reference agent except **3a** and **3g**. The compounds with nitro and chloro substituents were observed as the most active in the series against

B.subtilis. We can say that, the inhibition of spores of *B. subtilis* is related to their sporicidal activity.

In particular, all the compounds are found to be nontoxic. This outcome indicated that, antileukemic and also antimicrobial effects of the compounds were selective and good oral bioavailability and druglikeness properties. So we can say that, these ether linked imidazole derivatives have given an excellent starting point to design noncytotoxic anticancer and antibacterial agents.

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

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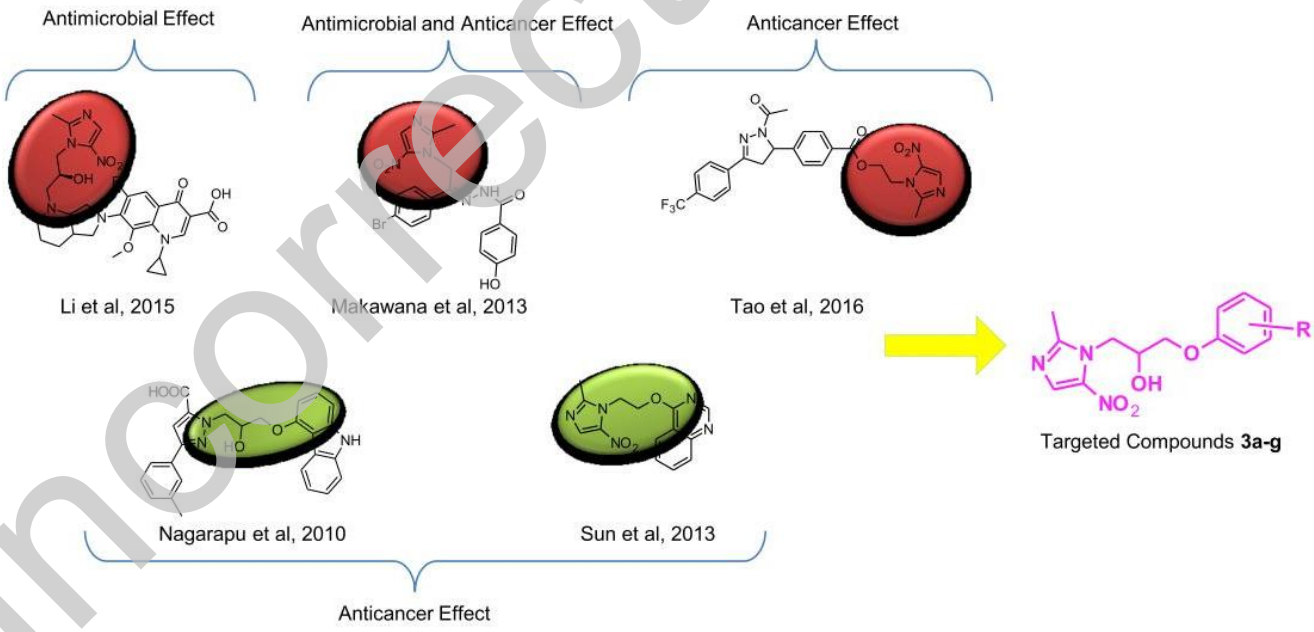
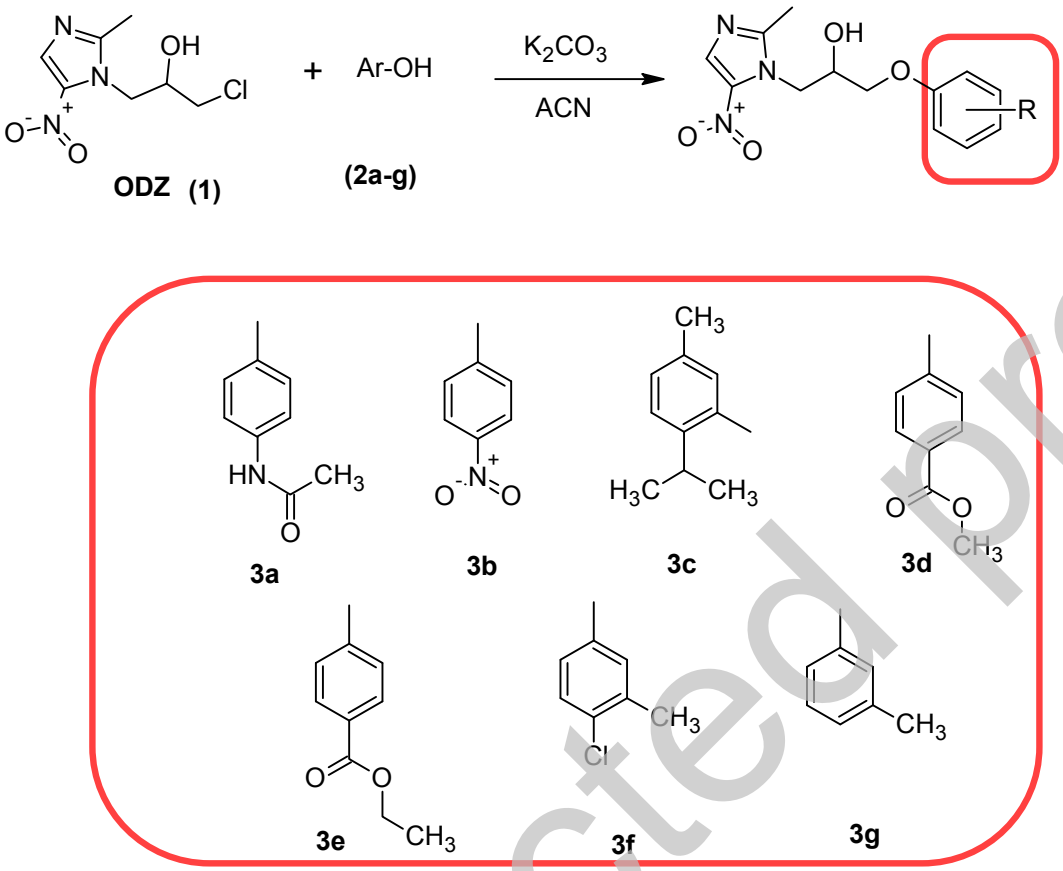


Figure 1. The design of new ornidazole derivatives on the basis of the literature.



Scheme 1. Synthetic route to ornidazole derivatives.

Table 1. IC_{50} values of the compounds against K562 and NIH/3T3 cells for 24 h.

Compound	IC_{50} (μM)		SI*
	K562 Cell line	NIH/3T3 Cell line	
3a	116.55	341.75	2.93
3b	131.68	479.25	3.64
3c	126.33	317.00	2.51
3d	166.75	324.10	1.94
3e	276.59	773.75	2.80
3f	127.88	178.10	1.39
3g	166.46	669.40	4.02
İmatinib	11.22	1104.00	98.39

SI=IC_{50} on normal cells / IC_{50} on cancer cells

Table 2. *In vitro* antimicrobial activity of the synthesized compounds **3a-g**, MIC in μg mL^{-1} (zones of inhibition, mm)

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi	SI* IC_{50}/MIC
	S.aureus	*B.subtilis*	*E.coli*	*P.aeruginosa*	*C.albicans*	
3a	256 (8)	256 (10)	512 (0)	256 (0)	512 (0)	1.28
3b	256 (9)	4 (20)	512 (0)	256 (0)	512 (0)	119.81
3c	128 (8)	8 (12)	512 (0)	512 (0)	256 (0)	39.63
3d	128 (9)	8 (18)	512 (0)	512 (0)	512 (0)	40.51
3e	128 (9)	8 (19)	512 (0)	512 (0)	512 (0)	96.72
3f	64 (0)	4 (17)	256 (0)	512 (0)	512 (0)	44.53
3g	256 (7)	16 (18)	256 (0)	512 (0)	512 (0)	41.84
Ampicilin	10	10	25	50	-	
Fluconazole	-	-	-	-	6.25	

*Evaluation for cytotoxicity in NIH/3T3 cells at concentrations up to ten times the MIC for *B.subtilis*. The activity/cytotoxicity criterion is a SI > 10.

Table 3. Drug-likeness calculations and Lipinski parameters of the compounds

Comp	Mol. weight	cLogP[a]	cLogS[b]	TPSA[c]	%ABS[d]	HBA[e]	HBD[f]	nrotb[g]	nviol[h]	Drug-likeness[i]
3a	334.331	0.0263	-1.875	122.20	66.84	9	2	7	0	2.9626
3b	322.275	-0.5985	-1.993	138.92	61.07	10	1	7	0	2.3314
3c	333.337	1.854	-2.748	93.10	76.88	7	1	7	0	2.1639
3d	335.315	0.2361	-1.674	119.40	67.81	9	1	8	0	0.08249
3e	349.342	0.6424	-1.974	119.4	67.81	9	1	9	0	-1.3929
3f	325.751	1.273	-2.613	93.1	76.88	7	1	6	0	2.3696
3g	291.306	0.667	-1.877	93.1	76.88	7	1	6	0	2.3314
ODZ	219.627	-0.4663	-0.984	83.87	80.07	6	1	4	0	1.8208

[a] Octanol/water partition coefficient (in log), calculated lipophilicity; [b]water solubility in log (moles/L); [c]topological polar surface area; [d]absorbtion [e] number of hydrogen bond acceptor; [f]number of hydrogen bond donor; [g]rotatable bond [h]number of violation [i]drug-likeness score