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ORIGINAL ARTICLE

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

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

Sevil ŞENKARDEŞ[1]*, Necla KULABAŞ[1], Özlem BİNGÖL ÖZAKPINAR[2], Sadık KALAYCI[3], Fikrettin ŞAHİN[3], İlkay KÜÇÜKGÜZEL[1], Ş. Güniz KÜÇÜKGÜZEL[1]

[1]Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Haydarpaşa, İstanbul, Turkey
[2]Marmara University, Faculty of Pharmacy, Department of Biochemistry, Haydarpaşa, İstanbul, Turkey
[3]Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering, Kayışdağı, İstanbul, Turkey

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_2CO_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-il)-3-(sübstitüe fenoksi) propan-2-ol (**3a-g**) türevleri tasarlanmış ve sentezlenmiştir

Gereç ve Yöntemler: 3a-g numaralı bileşikler Ornidazol'ün **(1)** uygun fenolik bileşikler (**2a-g**) ile asetonitril içinde susuz K_2CO_3 eşliğinde ısıtılmasıyla elde edilmiştir.

Bulgular: Yapı aydınlatılmasını takiben, *in vitro* antimikrobiyal aktivite ve K562 lösemi hücresi ve NIH/3T3 fare embriyonik fibroblast hücresi üzerinde sitotoksik etki çalışması yapılmıştır. Bu çalışmanın bir parçası olarak, bileşiklerin ilaç benzerlik özelliklerine uyumu değerlendirilmiştir. OSIRIS yazılımı kullanılarak fizikokimyasal parametreler (log P, TPSA, dönebilen bağ sayısı, hidrojen bağ vericileri ve alıcıları, logS) hesaplanmıştır.

Sonuç: Bileşik **3a** dışında sentezlenen tüm bileşikler, *Bacillus subtilis* suşuna karşı standart ilaca kıyasla anlamlı bir aktivite (MIC=4-16 μg mL^{-1}) göstermiş olmakla birlikte antilösemik aktiviteleri oldukça sınırlıdır. Ayrıca, bütün bileşiklerin hesaplanan iyi oral biyoyararlanım ve ilaç benzerlik özelliklerinin yanında non-toksik özellikte olabilecekleri öngörülmüş olup, seçicilik indeksi sonucuna göre bileşiklerin antimikrobiyal ve antilösemik etkilerinin selektif olduğu tespit edilmiştir.

Anahtar kelimeler: İmidazol, eter bağlı, ornidazol, antimikrobiyal aktivite, sitotoksisite

Presented in: The research was presented at the 12th International Symposium on Pharmaceutical Sciences on 26-29 June 2018 in Ankara, Turkey

***Correspondence: E-mail:** sevil.aydin@marmara.edu.tr, **Phone:** +90 543 474 39 17 **ORCID-ID:** orcid.org/0000-0002-0523-459X
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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.[1] 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.[2] 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.[3]

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[4-7] 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.[8] As shown in Figure 1, analogues of metronidazole and ornidazole have been reported as antimicrobial and anticancer agents.[9-11] Additionally, five-membered heterocyclic compounds containing an ether-linked structure showed significant anticancer activity.[12,13]

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 anticancer 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. ^{1}H-NMR and ^{13}C-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-H_2O (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

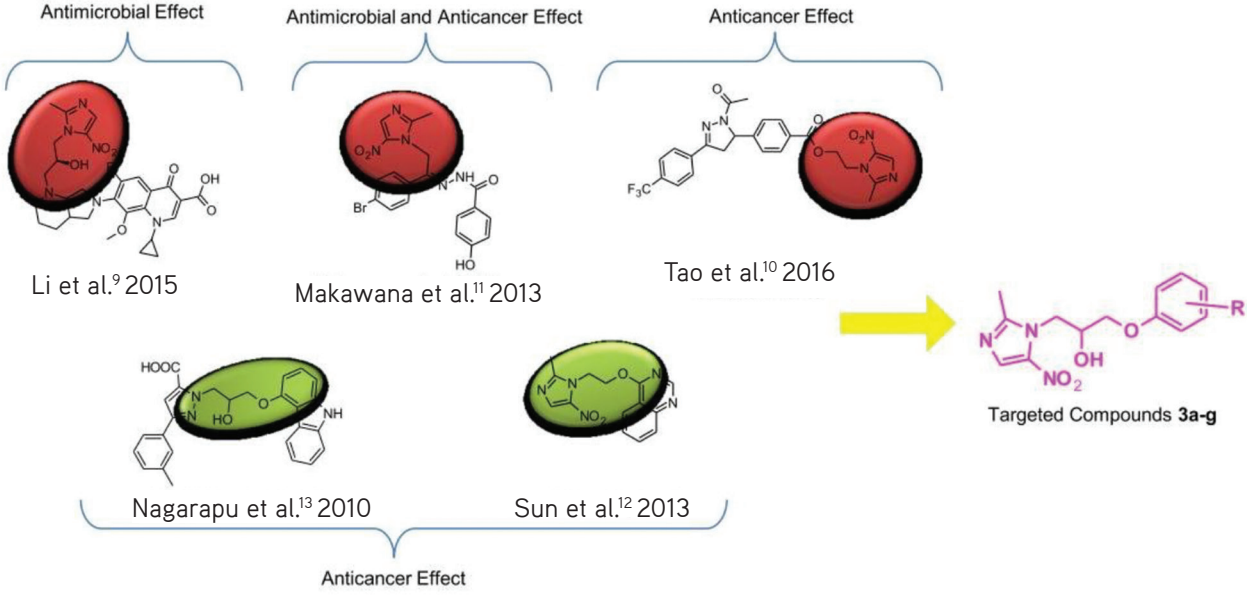
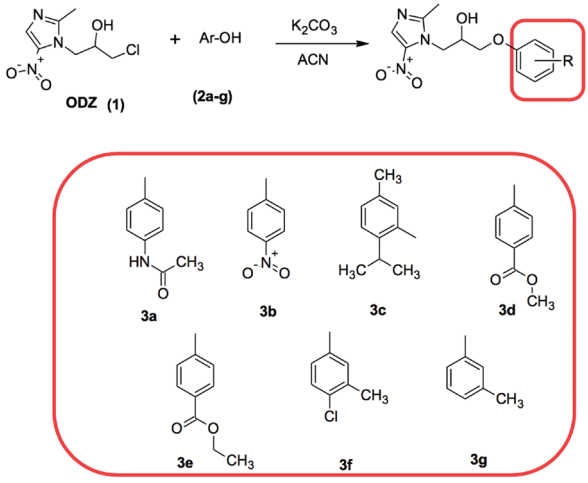


Figure 1. The design of new ornidazole derivatives on the basis of the literature

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 1 N 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 was collected by filtration and finally purified by recrystallization from methanol (Scheme 1).



Scheme 1. Synthetic route to ornidazole derivatives

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

Light yellow solid; Yield 81%; m.p. 189-190°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-1H-imidazol-1-yl)-3-(4-nitrophenoxy) propan-2-ol **(3b)***

Light brown solid; Yield 78%; m.p. 181°C; 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-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 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-1H-imidazol-1-yl) propoxy]benzoate **(3d)***

Dark brown solid; Yield 80%; m.p. 181-183°C; 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-1H-imidazol-1-yl) propoxy]benzoate **(3e)***

Yellow solid; m.p. 81-82°C, 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 and **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-1H-imidazol-1-yl)propan-2-ol **(3f)***

Dark brown solid; Yield 87%; m.p. 139-140°C; 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-1H-imidazol-1-yl)-3-(3-methylphenoxy) propan-2-ol ***(3g)***

Light brown solid; Yield 76% ; m.p. 103°C (lit. 105-106°C).[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. 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.[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.[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 leukemic 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 *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 (1×10^4 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-1*H*-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, ^{1}H NMR, ^{13}C-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 ^{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 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 IC_{50} 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 IC_{50} 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.

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
Imatinib	11.22	1104.00	98.39

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

The IC_{50} 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/

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		Fungus	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
Ampicillin	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 SI>10, MIC: Minimum inhibitory concentration

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 acceptors; [f]number of hydrogen bond donors; [g]rotatable bond; [h]number of violations; [i]drug-likeness score, ODZ: Ornidazole

datawarrior/) are given in Table 3. Drug-likeness prediction evaluates the acceptability of derivatives as drug molecules based on Lipinski[23] rule of five. ABS% was calculated by %ABS=109-[0.345 × topological polar surface area (TPSA)] according to the method described by Zhao et al.[24] TPSA,[25] 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 1.273 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.[26,27] TPSA values are closely related to the hydrogen bonding potential of a compound; those around of 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, ^{1}H-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-1*H*-imidazol-1-yl)propoxy]phenyl}acetamide (3a)

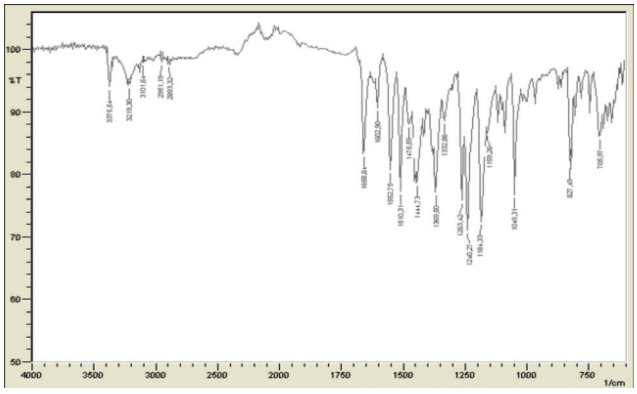

Figure S1. IR spectrum for compound 3a

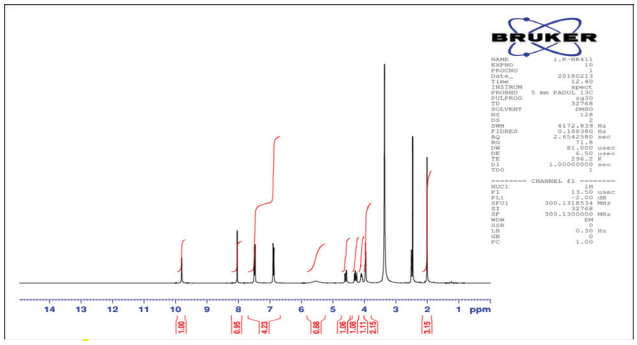

Figure S2. ^{1}H-NMR spectrum for compound 3a

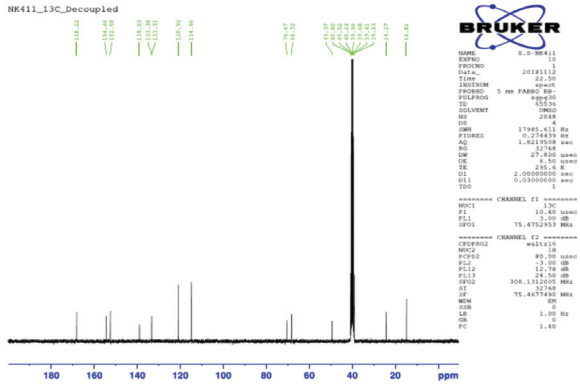

Figure S3. ^{13}C-NMR spectrum for compound 3a

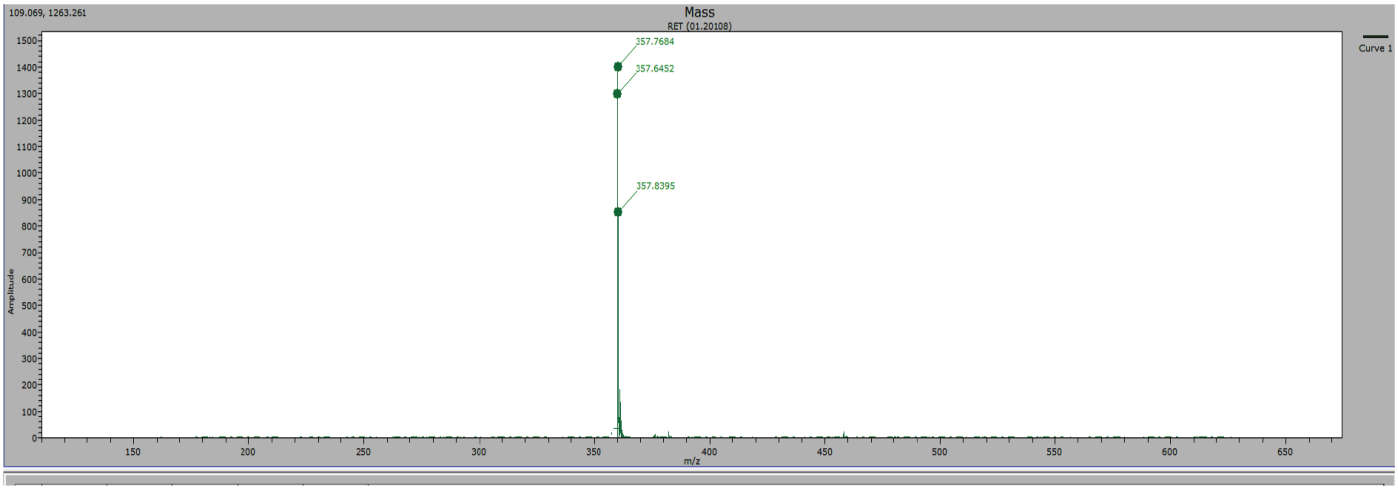

Figure S4. MS spectrum for compound 3a

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

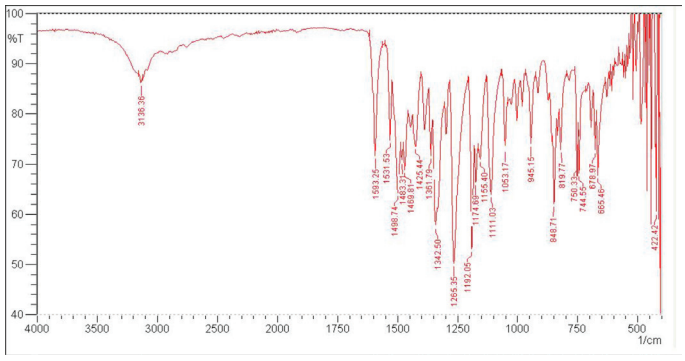

Figure S5. IR spectrum for compound 3b

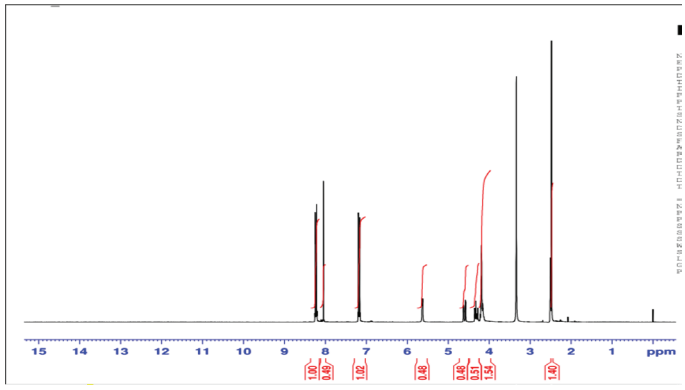

Figure S6. [1]H-NMR spectrum for compound 3b

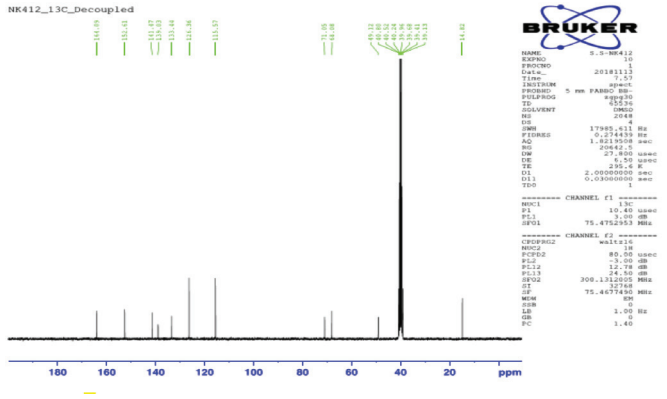

Figure S7. [13]C-NMR spectrum for compound 3b

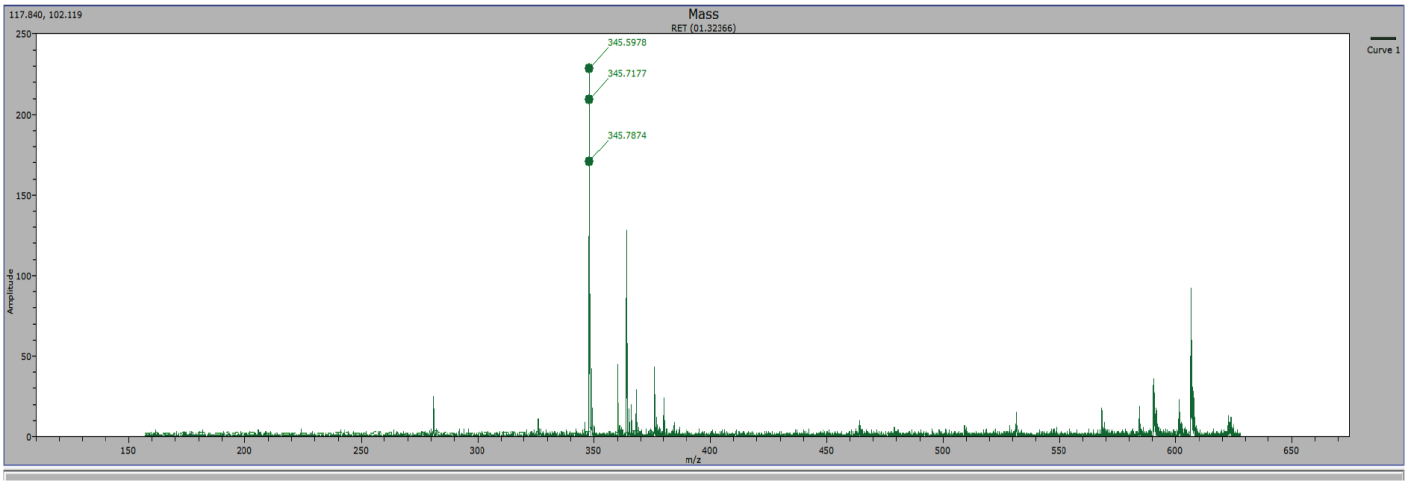

Figure S8. MS spectrum for compound 3b

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

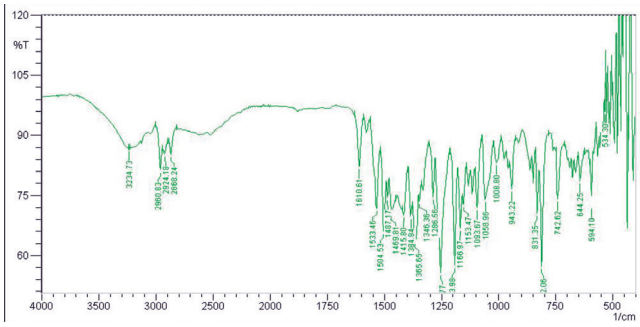

Figure S9. IR spectrum for compound 3c

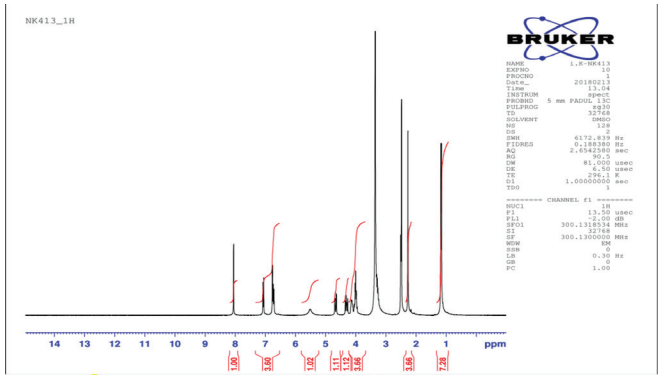

Figure S10. [1]H-NMR spectrum for compound 3c

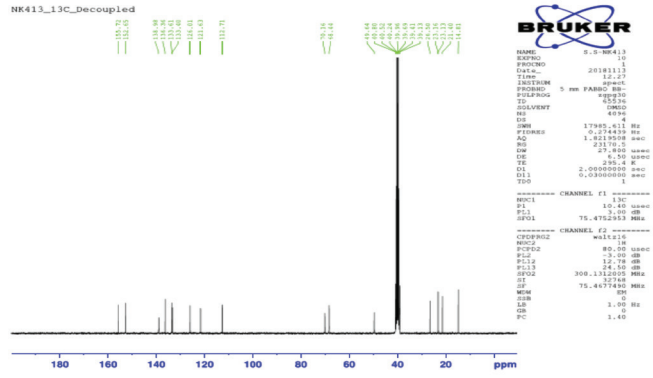

Figure S11. [13]C-NMR spectrum for compound 3c

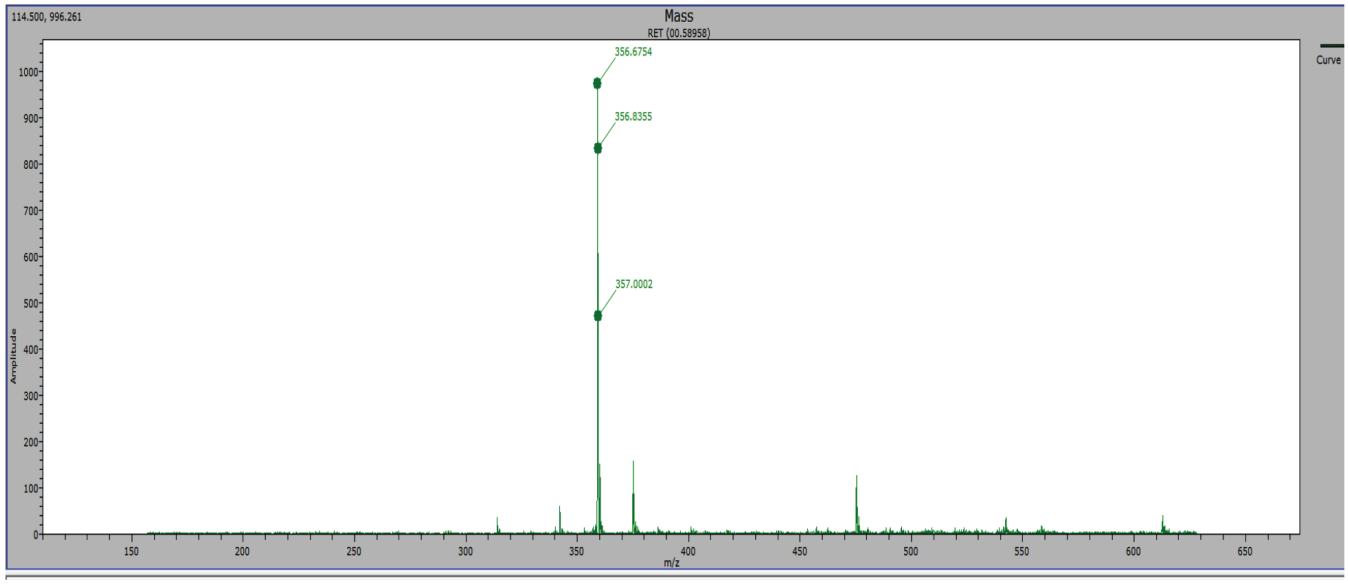

Figure S12. MS spectrum for compound 3c

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

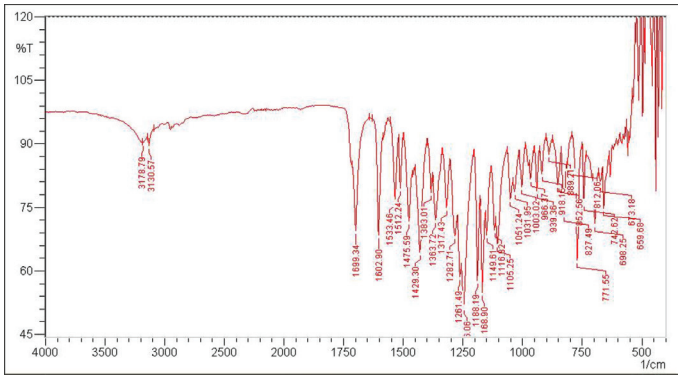

Figure S13. IR spectrum for compound 3d

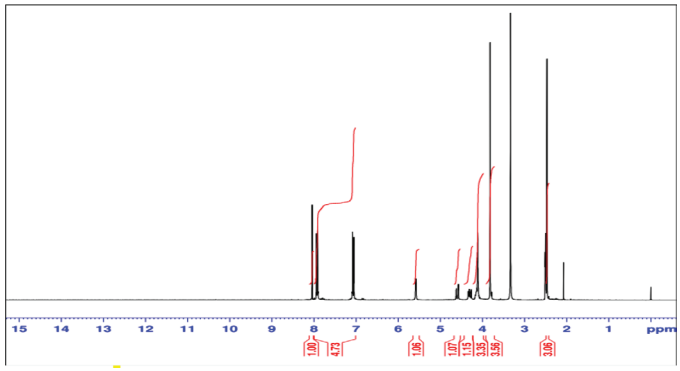

Figure S14. ^{1}H-NMR spectrum for compound 3d

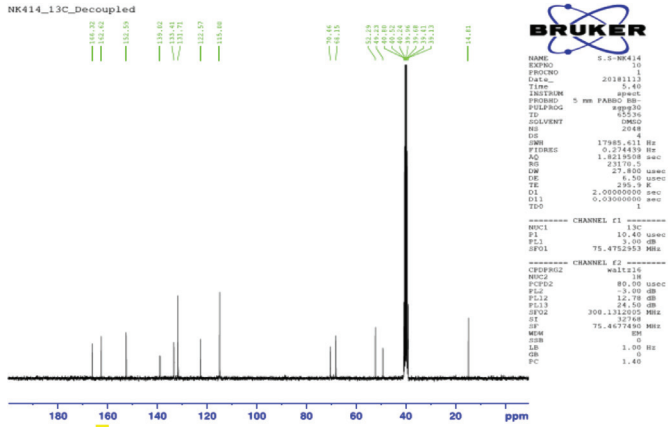

Figure S15. ^{13}C-NMR spectrum for compound 3d

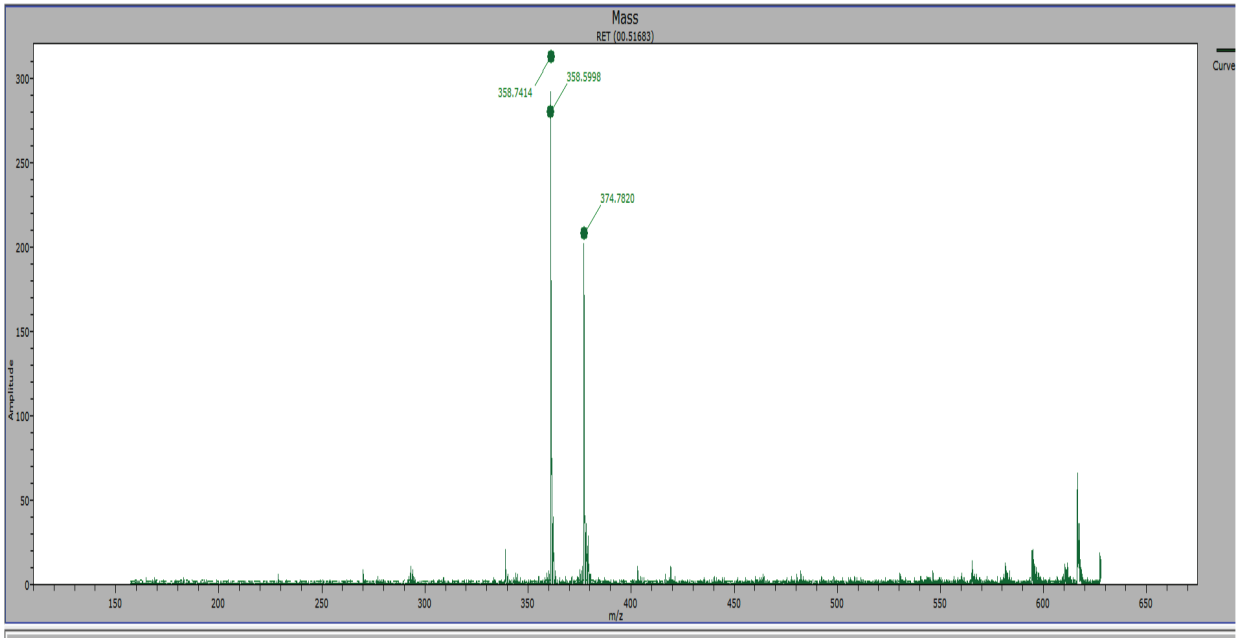

Figure S16. MS spectrum for compound 3d

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

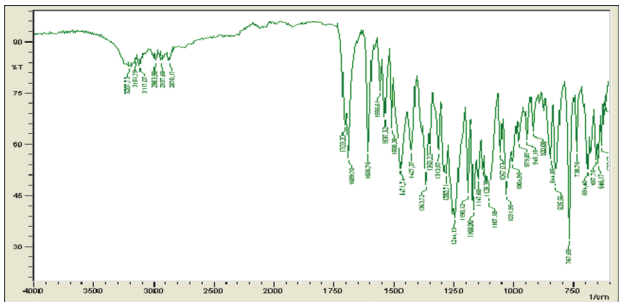

Figure S17. IR spectrum for compound 3e

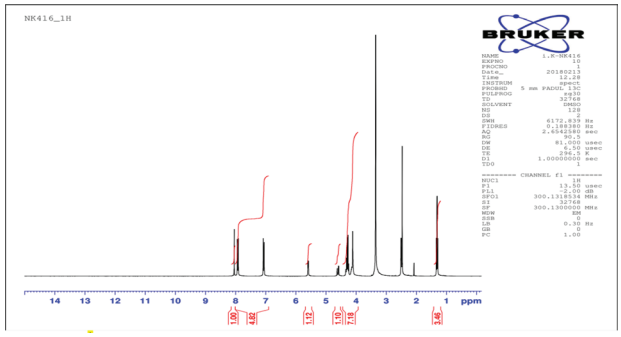

Figure S18. ^{1}H-NMR spectrum for compound 3e

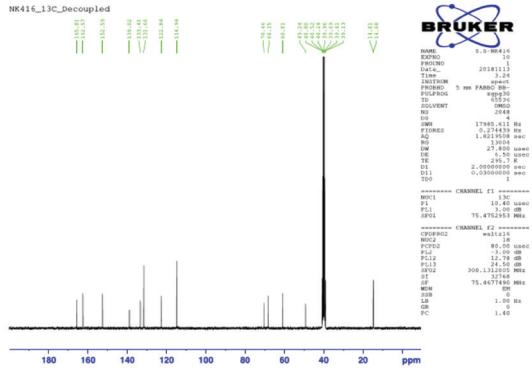

Figure S19. ^{13}C-NMR spectrum for compound 3e

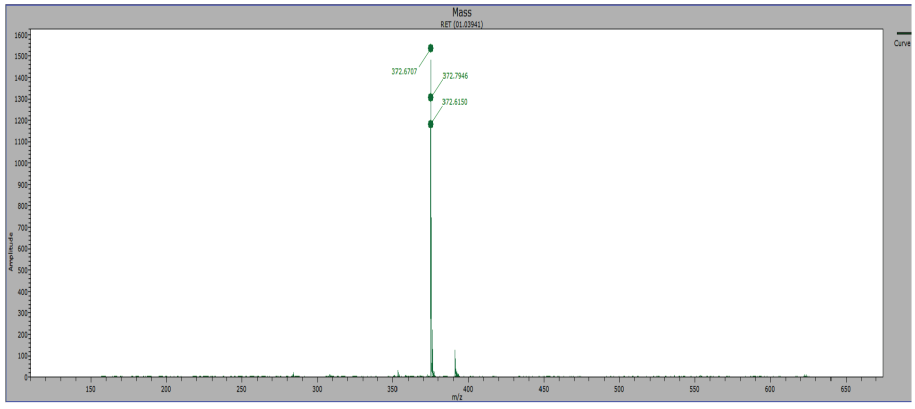

Figure S20. MS spectrum for compound 3e

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

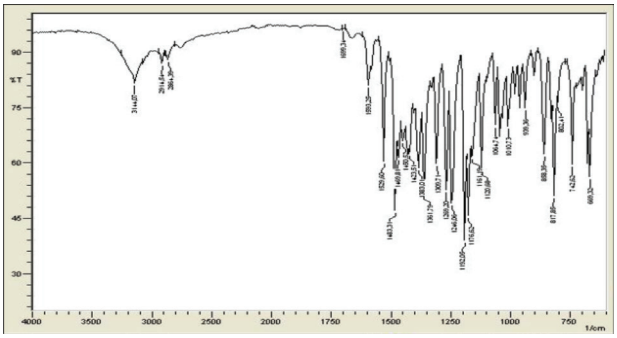

Figure S21. IR spectrum for compound 3f

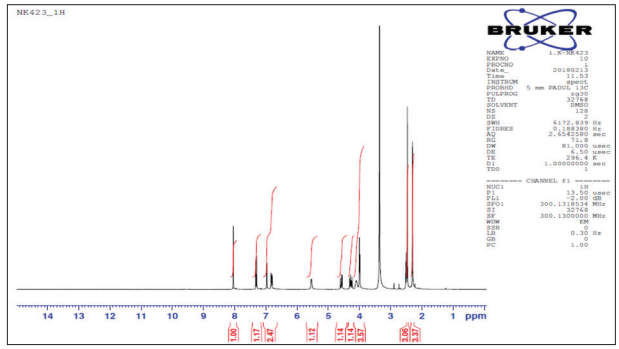

Figure S22. ^{1}H-NMR spectrum for compound 3f

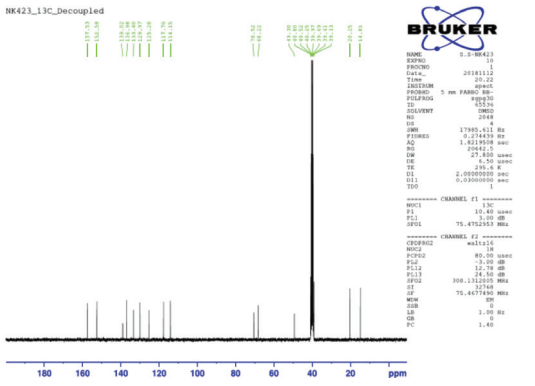

Figure S23. ^{13}C-NMR spectrum for compound 3f

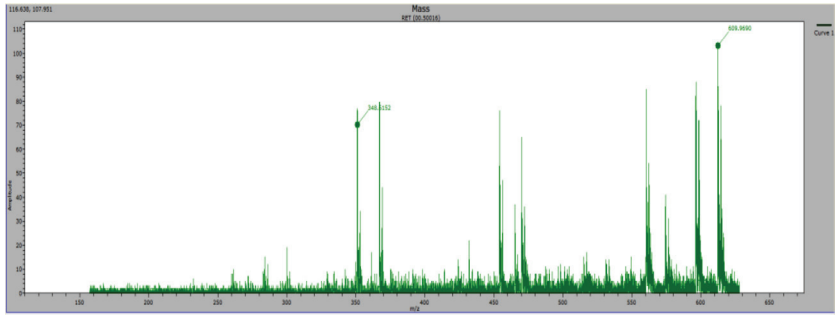

Figure S24. MS spectrum for compound 3f

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

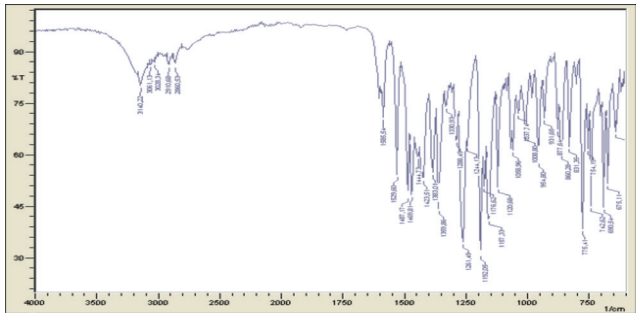

Figure S25. IR spectrum for compound 3g

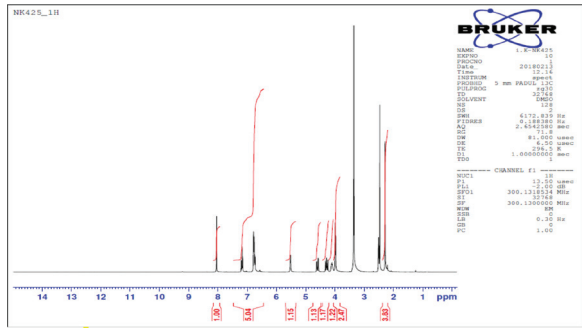

Figure S26. 1H-NMR spectrum for compound 3g

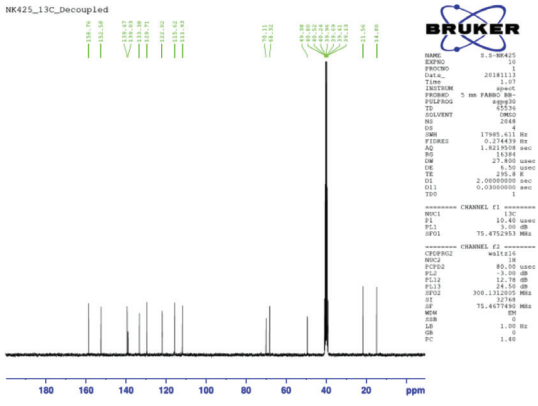

Figure S27. 13C-NMR spectrum for compound 3g

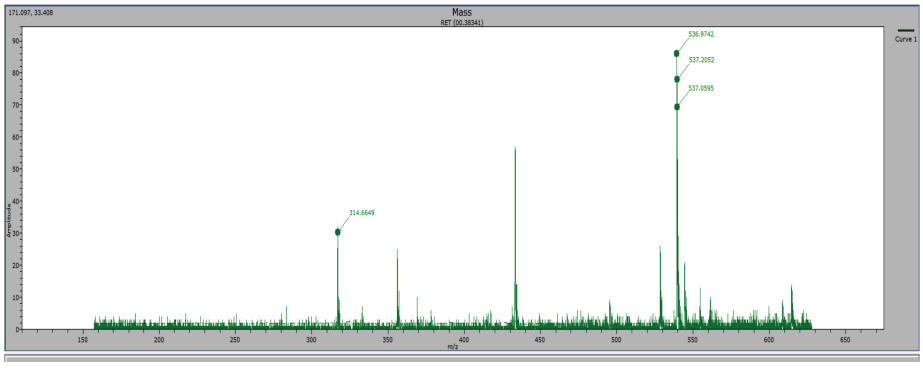

Figure S28. MS spectrum for compound 3g