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Antimicrobial and Cytotoxic Studies of New 2-Substitue-1*H*-Benzimidazole Derivatives

Yeni 2-Sübstitüe-1*H*-Benzimidazol Türevi Bileşikler Üzerinde Antimikrobiyal ve Sitotoksik Çalışmalar

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Abstract

We synthesized a series of 2-substituted-1*H*-benzimidazole derivatives (**1-9**) of which six of them were original and elucidated their structures by spectral analyses. We also evaluated the *in-vitro* antimicrobial activities of the synthesized compounds and detected potent inhibitory action in some of the compounds. Moreover, 2-(1*H*-benzimidazole-2-yl)phenol intermediates, bearing *o*-hydroxyphenyl substituent on 1*H*-benzimidazole ring possess equal or similar results compared to the standard compound, Ceftazidime. The synthesized compounds didn't show any significant antifungal activity on *Candida albicans*. All nine 1*H*-benzimidazole derivatives were tested for their cytotoxicity through WST-1 colorimetric assay-based *in vitro* tests on the mammalian LNCaP cell line. Compounds **7** and **8** were found to have IC_{50} values of 0,09 ± 0,01 μM and 0,03 ± 0,02 μM, respectively. It is noteworthy that the values obtained with these two compounds were highly comparable to that of Doxorubicine 0,053 ± 0,003 μM, the reference drug used in our study.

Keywords: Antibacterial, antifungal, cytotoxicity, benzimidazoles, synthesis.

Introduction

Chemotherapeutic drugs have limited use in the treatment of diseases due to the resistance of the microorganisms to these drugs and/or the side effects on biological systems. The resistance acquired by microorganisms through time against chemotherapeutic agents and/or their side effects, the usage of these agents. Even though, large numbers of antimicrobial drugs avaible for medical use, there will always be a vital need to discover new agents due to antimicrobial resistance (Fidler 1998 and Özkay et al. 2011). For these reasons, there are many ongoing studies on new chemotherapeutic agents having better activity and wider spectrum (Projan 2002, Foroumadi et al. 2003).

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One of the mechanisms of chemotherapeutical activity is the inhibition of nucleic acid synthesis. It is thought that the chemotherapeutical activity is due to the benzimidazol ring system and its analogue ring systems which are structurally similar to adenine and guanine bases in the structure of nucleic acids. Studies indicate that benzimidazole derivatives and their analogues give promising chemotherapeutic activity results (Kuş et al. 2009, Ertan et al. 2009, Yıldız-Oren et al. 2004).

Benzimidazoles are very useful intermediates for the development of pharmaceutical molecules of biological interest. Some benzimidazole derivatives with different pharmacological effects, including antifungal, antihelmintic, anti-HIV, antihistaminic, anticancer, antihypertensive, antiulcer are in clinical use (Göker et al. 2002, Valdez et al. 2002, Miller et al. 2010, Lavrador et al. 2010, Tong et al. 2009, Mizuno et al. 2010 and Kuhler et al. 2002). Many reports have uncovered that the influence of the substitution at the 1-, 2- and 5- positions of the benzimidazole ring is very important for their pharmacological effects (Göker et al. 1995, Garuti et al. 2000). A number of studies confirmed that 2-(substituted-phenyl)-benzimidazoles with various types of biological activities (Estrada-Soto et al. 2006, Sharmaa et al. 2009, Penning et al. 2010).

Reports described the presence of benzimidazole or heterocyclic ring systems that are isosteric with benzimidazole is vital for antibacterial activity. Thus, substitutions at the 5-position of the heterocyclic ring have a significant role in enhancing the activity (Yalçın et al. 1992).

In this study, we prepared a number of 2-(substituted-phenyl)-benzimidazole derivatives to assess their antimicrobial and cytotoxic activities. The minimum inhibitory concentration (MIC) was determined for the test compounds as well as for the reference standards. Cytotoxicity of the compounds was also evaluated in comparison with the reference drug Doxorubicine using WST-1 colorimetric assay against human lymph node metastatic prostate cancer epithelial cells (LNCaP) cell line (Horoszewicz et al. 1983).

Materials and Methods

Chemistry

Melting points were determined with a capillary melting point apparatus (Buchi 510, BUCHI, Flawil, Switzerland). The IR spectra of compounds were monitored as potassium bromide pellets (FT/IR-430, JASCO, Tokyo, Japan). The ^{1}H-NMR spectra were recorded at 400 MHz in the deuterated solvent indicated with chemical shifts were measured in parts per million (δ) units downfield from tetramethylsilane (TMS). Coupling constants (J) are reported in hertz (Hz) (AS 400 Mercury Plus NMR Varian, Palo Alto, USA). Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet. HRMS was recorded on a HPLC-TOF Waters Micromass LCT Premier XE (Milford, MA, USA) mass spectrometer using ESI. Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60 F_{254}) with detection by UV light (254 nm). All starting materials and reagents were high-grade commercial products.

Synthesis of 2-(1H-Benzimidazole-2-yl)phenol derivatives 1-3

The preparation of the substituted benzimidazoles is outlined in Scheme 1 (Coban et al. 2009). First of all, 0,04 mole of 2-hydroxybenzaldehyde and 0.04 mol of sodium bisulfide, dissolved in 20 mL of ethanol and water, respectively, were mixed and stirred for 60 min. at room temperature. The mixture was filtered and sodium hydroxy(2-hydroxyphenyl)methane sulfonate salt (68 %) was obtained from the crude extract. Secondly, 0,01 mol the crude salt and 0,01 mole *o*-phenylenediamine in 30 mL of dimethyl formamide (DMF) were refluxed for 2 h at 150 ^{0}C in an oil bath and the mixture was poured in an ice bath. 2-(1*H*-benzimidazol-2-yl)phenol (87,3 %) was filtered and recrystallized from methanol/water. 2-(5-Methyl-1*H*-benzimidazol-2-yl)phenol (87,5 %) was synthesized with 4-methyl-*o*-phenylenediamine as described and recrystallized from methanol/water. 4-methyl-*o*-phenylenediamine was replaced with 4-chloro-*o*-phenylenediamine for synthesis of **3**.

Synthesis of N-{2-[2-(1H-benzimidazole-2-yl)phenoxy]propyl}substitutedamine derivatives 4-9

After reacting 0,005 mole 2-(1*H*-benzimidazol-2-yl)phenol and 0,01 mole **A** (Figure 1) with 0,015 mole sodium hydroxide in 10 mL ethanol, the mixture was refluxed at 95 ^{0}C for 2 h in an oil bath. Ethanol was evaporated and the residue was extracted with ether. The compounds were obtained by preperative TLC (Ethyl acetate/n-hexane, 1:1).

Microbiology

The antimicrobial activities of compounds were tested by the microdilution method according to the *Clinical and Laboratory Standards Institute* (CLSI) (CLSI, 2008 and NCCLS, 2002). Minimum inhibitory concentrations (MICs) of compounds were determined against reference strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Candida albicans* ATCC 90028). The antibacterial and antifungal assays were performed in Mueller Hinton broth and Sabouraud dextrose broth, respectively. The broth microdilution test was performed by using sterile, disposable, multiwell microdilution plates (96 U-shaped wells). All the synthesized compounds were dissolved in DMSO and diluted with water to prepare the stock solutions (8,2 mg/mL). The twofold serial dilution with culture medium from 2048 to 1 μg/mL was made in a 96-well plate. The final inoculum size was ~10^5 CFU/mL for the antibacterial assay and ~10^4 CFU/mL for the antifungal assay. For the antibacterial assay after incubation for 24 h at 37 ± 1 ^{0}C and after incubation for 48 h at 35 ± 1 ^{0}C for the antifungal assay, in the microdilution plate the last well with no growth of microorganism and/or yeast was recorded to represent the MIC expressed in μg/mL. All experiments in the antibacterial and antifungal assays were replicated twice.

In our study the standard compounds were Ceftazidime, Amphotericin B, Fluconazole and Flucyitosine. The inhibitory activitiy results of 2-substituted-phenyl-1*H*-benzimidazoles were compared with the standard compounds.

Cytotoxicity

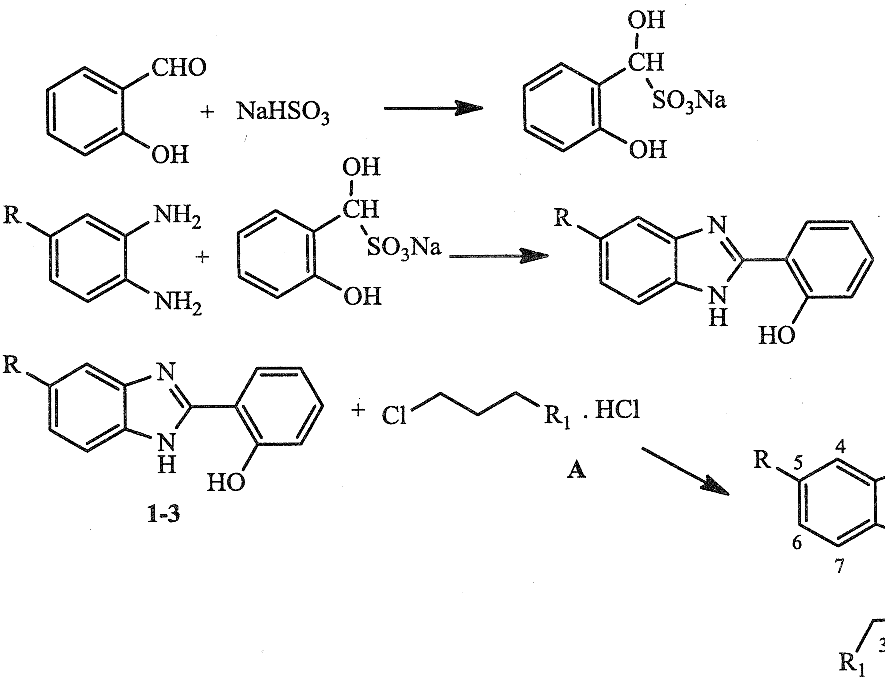
Cytotoxicity of compounds **1-9** were assessed in comparison to the reference drug Doxorubicin using WST-1 assay (Roche, Germany) (Berridge et al. 1996) against mammalian LNCaP cell line. The cells were obtained from the ATCC (ATCC CRL-1740™) and maintained in RPMI 1640 medium (Gibco,USA) containing 10% (v/v) FCS (fetal calf serum) (Gibco,USA), 1% (v/v) penicillin streptomycin and 1% (v/v) L-glutamine (Gibco,USA) and seeded in 96-well plates containing 200 μL microtitre wells at a density of 5x10^3 cells/ well in 200 μL of medium, followed by incubation at 37 ^{0}C for 24 h to allow for exponential growth. The compounds used for the testing were dissolved in 0,1 % DMSO, diluted with medium from stock solutions of 5x10^{-4} M concentration, subsequently incubated at 37 ^{0}C for 24 h. The solutions were, then, removed from wells and the cells were washed with PBS (phosphate buffer solution), and fresh medium was added. After a resting period of 24 h at 37 ^{0}C, individual wells were treated with a 20 μL of a solution of WST-1. Following the incubation at 37 ^{0}C for 1 h, absorbance readings were performed at 540 nm using VICTOR V-Beckman Plate Reader (PelkinElmer, Germany). Cell viability was calculated as a percentage of the absorbance reading of compounds comparison to control reading.

Results and Discussion

Chemistry

For the synthesis of the target compounds, the reaction sequences outlined in Figure 1 was followed. In the initial step of the synthetic process, the reaction of 2-hydroxybenzaldehyde with sodium bisulfide solution afforded sodium hydroxy(2-hydroxyphenyl)metan sulfonate salt, while the condensation reaction of this salt with unsubstituted or 5-substituted *o*-

phenylenediamine analogs were achieved in the second step (Coban et al. 2009). Following the reaction the 2-(1*H*-benzimidazol-2-yl)phenol (**1**) substituted alkyl derivatives with sodium hydroxide the reaction mixture were refluxed for two hours at 95 ^{0}C by adding aminoalkylhalogenates.



Comp.	R	R$_1$
1	H	-
2	CH$_3$	-
3	Cl	-
4	H	-N(CH$_3$)$_2$
5	H	-Piperidine
6	CH$_3$	-N(CH$_3$)$_2$
7	CH$_3$	-Piperidine
8	Cl	-N(CH$_3$)$_2$
9	Cl	-Piperidine

Figure 1. The synthesis pathway and the structures of the compounds

Structure elucidations of the final compounds were performed with IR, ^{1}H-NMR, and HRMS spectroscopic methods. The IR spectra, obtained in solid phase at 2400-3200 cm^{-1}, are based on N-H--N type hydrogen bonds, which are characteristic for benzimidazole derivatives. Moreover, the N–H stretching at 3390-3460 cm^{-1}, C=C and C=N stretching at 1650-1500 cm^{-1} and 1500-1400 cm^{-1} corresponding to benzenoid ring at 1000 cm^{-1} and 960 cm^{-1} (Preston, 1981), asymmetric and symmetric C-O-C stretching at 1200-1275 cm^{-1} and 1020-1075 cm^{-1} (Silverstein et al. 1981) (Table 1). ^{1}H-NMR results were interpreted in experimental section. The aromatic proton signals of *o*-hydroxyphenyl substituent at position 2- were observed within prospective chemical shift values and divisions while the hydrogen atoms of 1*H*-benzimidazole ring at positions 4- and 7- were not detected at the prospective divisions (Table 2). These results were noteworthy in that it may suggest a proton exchange due to 1,3-tautomerization (Sridharan et al. 2005).

Microbiology

All described benzimidazoles, **1-9**, were tested for their *in vitro* growth inhibitory activity by broth microdilution method (CLSI, 2008 and NCCLS, 2002) against *Escherichia coli* ATCC (American Tissue Cell Culture Collection) 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 as gram-positive and gram-negative bacterias, and

Candida albicans ATCC 90028 as yeast. All final compounds were dissolved in dimethylsulfoxide (DMSO) and diluted with water to prepare the stock solutions. It was determined that the DMSO had no anti-microbial activity against any of the test microorganisms (data not shown).

The MIC value results were compared with the reference antibiotics. All of the compounds were more effective to gram-positive bacteria (*Staphylococcus aureus*) than gram-negative bacterias (*Esherichia coli, Pseudomonas aeruginosa).* MIC values of intermediates on *Staphylococcus aureus* organisms were exceptionally effective compared with the other compounds. In regard to their antibacterial activity against *S. aureus* ATCC 29213 the phenol derivatives could be arranged in the following decreasing order of activity, compound **1**>**2**>**3** (Table 3). Unsubtitiuted benzimidazole ring derivative compound **1** found two fold more active than the reference compound Ceftazidime while compound **2** having methyl substitution on benzimidazol ring displayed an equal activity with the reference drug. However, phenol derivative with chloro atom on 5- position (**3**) didn't show a significant activity result. On the other hand, the biological activity results of compound **4-9** established that etherification of phenol group with the aminopropyl chain had an unfavorable effect on the activity. Among these series of compounds, compounds **5** and **6** were found to be more active than the others.

MIC values of compounds examined and there was no significant antifungal activity on *Candida albicans.*

Table 1. Chemical analyses of compounds.

Compound	**m. p. (^{0}C)**	**Yield %**	**IR ν_{max} (cm^{-1})**	**HRMS (ESI +) [M+H$^+$]**
1 ($C_{13}H_{10}ON_2$)	240 (Grey powder) 234-236 (Coburn et al.1987)	87.3	3285 (O-H st), 3032 (C-H), 1602 (C=C ring), 1252 (C-O)	calcd. 211.0871, found 211.0871
2 ($C_{14}H_{12}ON_2$)	238.7 (Dark brown powder) 240-242 (Han et al. 2008)	87.5	3235 (O-H), 3024 (C-H), 1598 (C=C ring), 1260 (C-O)	calcd. 225.1028, found 225.1025
3 ($C_{13}H_9ON_2Cl$)	288.3(Dark purple powder) 279-281 (Han et al. 2008)	87	3327 (O-H), 3007 (C-H), 1585 (C=C ring), 1261 (C-O)	calcd. 245.0482, found 245.0479
4 ($C_{18}H_{21}ON_3$)	a tawny oil	27	3433 (N-H), 2920 (C-H), 1603 (C=C ring), 1458 (C-N ring), 1241 (assym. C-O-C), 1051 (sym. C-O-C ring)	calcd. 296.1763, found 296.1771
5 ($C_{21}H_{24}ON_3$)	a light brown oil	22	3325 (N-H), 2849 (C-H), 1717 (C=C ring), 1497 (C-N ring), 1262 (assym. C-O-C), 1025 (sym. C-O-C ring)	calcd. 336.2076, found 336.2076
6 ($C_{19}H_{23}ON_3$)	a dark brown oil	21	3071 (N-H), 2918 (C-H), 1603 (C=C ring), 1461 (C-N ring), 1239 (assym. C-O-C), 1054 (sym. C-O-C ring)	calcd. 310.1919, found: 310.1911
7 ($C_{22}H_{26}ON_3$)	a dark brown oil	18	3419 (N-H), 2852 (C-H), 1605 (C=C ring), 1447 (C-N ring), 1260 (assym. C-O-C), 1018 (sym. C-O-C ring)	calcd. 350.2232, found 350.2230
8 ($C_{18}H_{20}ON_3Cl$)	a pink oil	18	3413 (N-H), 2921 (C-H), 1583 (C=C ring), 1466 (C-N ring), 1242 (assym. C-O-C), 1087 (sym. C-O-C ring)	calcd. 330.1373, found 330.1375
9 ($C_{21}H_{24}ON_3Cl$)	a red oil	15	3434 (N-H), 2851 (C-H), 1602 (C=C ring), 1458 (C-N ring), 1261 (assym. C-O-C), 1037 (sym. C-O-C ring)	calcd. 370.1686, found 370.1686

Table 2. ^{1}H NMR results for the compounds

1	**^{1}H-NMR:** 7.02 (td, 1H, *J*= 1.2, 7.8 Hz, H-5′), 7.04 (d, 1H, *J*= 8.2 Hz, H-3′), 7.29 (m, 2H, H-5, H-6), 7.39 (td, 1H, *J*= 1.6, 8.2 Hz, H-4′), 7.66 (m, 2H, H-4, H-7) 8.06 (dd, 1H, *J*= 1.2, 7.8 Hz, H-6′), 13.15 (bs, 1H, Ar-NH)
2	**^{1}H-NMR:** 2.48 (s, 3H, Ar-CH$_3$), 6.99 (t, 1H, *J*= 7.6 Hz, H-5′), 7.01 (d, 1H, *J*= 8.0 Hz, H-3′), 7.08 (bs, 1H, H-6), 7.35 (dd, 1H, *J*= 7.2, 8.4 Hz, H-4′), 7.48 (m, 1H, H-4), 7.57 (d, 1H, *J*= 7.2 Hz, H-7), 8.00 (d, 1H, *J*= 8.0 Hz, H-6′), 13.08 (s, 1H, Ar-NH)
3	**^{1}H-NMR:** 5.75 (s, 1H, Ar-OH), 7.03 (td, 1H, *J*= 1.2, 7.8 Hz, H-5′), 7.05 (dd, 1H, *J*= 0.8, 8.4 Hz, H-3′), 7.30 (dd, 1H, *J*= 2.0, 8.8 Hz, H-6), 7.40 (td, 1H, *J*= 1.6, 7.8 Hz, H-4′), 7.67 (d, 1H, *J*= 8.0 Hz, H-7), 7.73 (bs, 1H, H-4), 8.06 (dd, 1H, *J*= 1.6, 7.8 Hz, H-6′)
4	**^{1}H-NMR:** 2.11 (quin, 2H, *J*= 5.85 Hz, H-2″), 2.39 (s, 6H, -N(CH$_3$)$_2$), 2.61 (t, 2H, *J*= 5.85 Hz, H-3″), 4.29 (t, 2H, *J*= 5.85 Hz, H-1″), 7.03 (d, 1H, *J*= 8.00 Hz, H-3′), 7.12 (td, 1H, *J*= 1.2, 7.8 Hz, H-5′), 7.25 (dd, 2H, *J*= 3.12, 5.66 Hz, H-5, H-6), 7.39 (td, 1H, *J*= 2, 7.8 Hz, H-4′), 7.65 (bs, 2H, H-4, H-7), 8.61 (dd, 1H, *J*= 1.6(2.0), 8.2 Hz, H-6′)
5	**^{1}H-NMR:** 1.48 (quin, 2H, *J*= 5.85 Hz, H-4 of piperidine ring), 1.62 (quin, 4H, *J*= 5.85 Hz, H-3, H-5 of piperidine ring), 2.17 (quin, 2H, *J*= 6.63 Hz, H-2″), 2.44 (m, 4H, H-2, H-6 of piperidine ring), 2.55 (t, 2H, *J*= 7.02 Hz, H-3″), 4.31 (t, 2H, *J*= 6.24 Hz, H-1″), 7.07 (d, 1H, *J*= 8.58 Hz, H-3′), 7.13 (td, 1H, *J*= 1.17, 7.60 Hz, H-5′), 7.26 (m, 2H, H-5, H-6), 7.40 (td, 1H, *J*= 1.56, 7.8 Hz, H-4′), 7.52 (dd, 1H, *J*= 3.12, 5.60 Hz, H-4 or H-7), 7.70 (dd, 1H, *J*= 3.12, 5.60 Hz, H-4 or H-7), 8.57 (dd, 1H, *J*= 1.95, 7.8 Hz, H-6′)
6	**^{1}H-NMR:** 2.10 (t, 2H, *J*= 5.85 Hz, H-2″), 2.37 (s, 6H, -N(CH$_3$)$_2$), 2.50 (s, 3H, Ar-CH$_3$), 2.59 (t, 2H, *J*= 5.85 Hz, H-3″), 4.28 (t, 2H, *J*= 5.85 Hz, H-1″), 7.04 (dd, 1H, *J*= 8.19 Hz, H-3′), 7.11 (t, 1H, *J*= 7.41 Hz, H-5′), 7.37 (td, 1H, *J*= 1.56, 7.8 Hz, H-4′), 7.52 (s, 1H, H-4 or H-7), 8.59 (dd, 1H, *J*= 1.95, 7.8 Hz, H-6′)
7	**^{1}H-NMR**: 1.47 (quin, 2H, *J*= 5.85 Hz, H-4 of piperidine ring), 1.62 (quin, 4H, *J*= 5.85 Hz, H-3, H-5 of piperidine ring), 2.16 (quin, 2H, H-2″), 2.45 (m, 4H, H-2, H 6 of piperidine ring), 2.50 (s, 3H, Ar-CH$_3$), 2.55 (t, 2H, *J*= 7.02 Hz, H-3″), 4.30 (t, 2H, *J*= 6.63 Hz, H-1″), 7.06 (m, 2H, H-3′, H-5′), 7.12 (td, 1H, *J*=1.17, 7.8 Hz, H-6), 7.32 (m, 1H, H-4), 7.38 (td, 1H, *J*= 1.56, 7.41 Hz, H-4′), 7.61 (m, 1H, H-7), 8.56 (dd, 1H, *J*= 1.56, 7.8 Hz, H-6′), 10.65 (bs, 1H, Ar-NH)
8	**^{1}H-NMR:** 2.11 (quin, 2H, *J*= 5.46 Hz, H-2″), 2.38 (s, 6H, -N(CH$_3$)$_2$), 2.49 (t, 2H, *J*= 5.66 Hz, H-3″), 4.29 (t, 2H, *J*= 5.66 Hz, H-1″), 7.03 (d, 1H, *J*= 8.58 Hz, H-3′), 7.13 (td, 1H, *J*= 0.78, 7.61 Hz, H-5′), 7.21 (d, 1H, *J*= 8.97 Hz, H-6), 7.26 (bs, 2H, H-4, H-7), 7.41 (td, 1H, *J*= 1.76, 7.8 Hz, H-4′), 8.57 (dd, 1H, *J*= 1.56, 7.8 Hz, H-6′)
9	**^{1}H-NMR:** 1.49 (m, 2H, H-4 of piperidine ring), 1.63 (quin, 4H, *J*= 5.46 Hz, H-3, H-5 of piperidine ring), 2.18 (t, 2H, *J*= 6.63 Hz, H-2″), 2.46 (m, 4H, H-2, H-6 of piperidine ring), 2.57 (t, 2H, *J*= 7.02 Hz, H-3″), 4.33 (t, 2H, *J*= 6.24 Hz, H-1″), 7.09 (d, 1H, *J*= 8.58 Hz, H-3′), 7.15 (td, 1H, *J*= 7.60 Hz, H-5′), 7.23 (dd, 1H, *J*= 1.95, 8.36 Hz, H-6), 7.42 (td, 1H, *J*= 1.56, 8.58 Hz, H-4′), 8.53 (dd, 1H, *J*= 1.56, 7.8 Hz, H-6′)

Table 3. In vitro antimicrobial activity of benzimidazole compounds (µg/mL)

Compound	***E. coli* (25922)**[a]	***P. aeruginosa* (27853)**	***S. aureus* (29213)**	***C. albicans* (90028)**
1	1024	1024	4	NA
2	1024	1024	8	NA
3	1024	1024	64	NA
4	1024	1024	256	512
5	1024	1024	128	512
6	1024	1024	128	512
7	1024	1024	256	512
8	1024	1024	256	512
9	1024	1024	256	512
Ceftazidime	0,25	1	8	-
	(0,06-0,5)[b]	(1-4)	(4-16)	-
Amphotericin B	-	-	-	0,5-2,0[c]
Fluconazole	-	-	-	0,25-1,0[c]
Flucytosine (5FC)	-	-	-	0,5-2,0[c]

a) ATCC number; b) MIC limits for reference bacterial strains; c) MIC limits for reference *C. albicans*

Cytotoxic activity

The newly synthesized compounds were evaluated for their ability to display cytotoxicity against mammalian LNCaP cell line and the results are expressed as IC_{50} values. Doxorubicine a well known anticancer drug was used as the positive control for comparison (Theyer et al. 1993). The resulting IC_{50} values were determined by WST-1 colorimetric assay and are reported in Table 4. As listed in Table 4, nine target compounds showed IC_{50} values ranging from 91 to 0,030 µM. The cytotoxic activity results for 5-substituted 1*H*-benzimidazol ring displayed an order of $Cl>CH_3>H$ for phenol derivatives (**1-3**), $Cl>H>CH_3$ for the derivatives dimethylamine substituted propyl chain (**4, 6, 8**) and $CH_3>Cl>H$ for the derivatives piperidine substituted propyl chain (**5, 7, 9**). Among all the compounds, compound **7** (0,09 ± 0,01 µM) showed closer activity results compared to the reference drug Doxorubicin (0,053 ± 0,003 µM) while compound **8** (0,03 ± 0,02 µM) found to be more active than the reference drug. These results suggest that introduction of a chlorine atom to the 5 position of benzimidazole ring may be enhanced the cytotoxic activity.

Table 4. The cytotoxicity data of the tested compounds against mammalian LNCaP (human prostatic epithelial cells) cell line

Compound	**IC_{50} ± SEM (µM)**
1	72 ± 38,85
2	34 ± 3,61
3	27 ± 6,99
4	35 ± 9,30
5	91 ± 1,77
6	60 ± 7,20
7	0,09 ± 0,01
8	0,03 ± 0,02
9	14 ± 3,49
Doxorubicine	0,053 ± 0,003

Conclusion

In conclusion, a series of novel 1*H*-benzimidazole derivatives were designed, synthesized and evaluated for their ability to display antimicrobial activity against a panel of selected microorganisms. According to the MIC value results, all of the compounds were more effective to gram-positive bacteria than gram-negative bacterias. Moreover, compound **2** exhibited the same MIC value and compound **1** displayed the half of the MIC value (8 and 4 µg/mL, respectively) against *S. aureus* compared to the reference drug Ceftazidime (8 µg/mL). On the other hand, MIC value results indicated that introduction of propylamine chain to the *o*-hydoxyphenol structure had no significant effect on the activity.

Moreover, all the compounds were tested for their cytotoxicity through WST-1 based *in vitro* tests on the mammalian LNCaP (human prostatic epithelial cells) cell line in order to determine their IC_{50} values. Among the tested compounds, compound **7** showed similar cytotoxic activity results compare to the reference drug Doxorubicine, while compound **8** found considerably more active than the reference drug.

Özet

Bir seri 2-sübstitüe-1*H*-benzimidazol türevi (1-9) bileşik sentez edilmiş ve spektral analizlerle yapıları aydınlatılmıştır. Bu bileşiklerin altı tanesi orijinal maddedir. Ayrıca, sentezi yapılmış bileşiklerin *in-vitro* antimikrobiyal aktiviteleri de çalışılmış ve bazı bileşiklerin güçlü inhibitör etkileri tespit edilmiştir. Üstelik 1*H*-benzimidazol halkası üzerinde *o*-hidroksifenil sübstitüenti taşıyan 2-(1*H*-benzimidazol-2-il)fenol ara ürünleri standart bileşik olan Seftazidim ile karşılaştırıldıklarında onunla eşit ya da ondan daha güçlü bulunmuşlardır. Sentezlenen bileşikler *Candida albicans* üzerinde önemli herhangi bir antifungal aktivite göstermemişlerdir. Dokuz bileşiğin hepsi sitotoksisiteleri bakımından, memeli LNCaP hücre hattı üzerinde *in vitro* kolorimetrik çalışma esasına dayalı WST-1 testi ile denenmişlerdir. **7** ve **8** nolu bileşiklerin IC_{50} değerleri sırasıyla 0,09 ± 0,01 µM ve 0,03 ± 0,02 µM olarak bulunmuştur. Bu iki bileşik ile elde edilen değerlerin, çalışmada referans bileşik olarak kullanılan Doksorubisin (0,053 ± 0,003 µM) ile çok benzer olduğu dikkati çekmektedir.

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