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Research Article

Synthesis, Antimicrobial and Cytotoxic Activity Studies of Some New Benzoxazole Derivatives

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Abstract

There is an urgent need to develop new antimicrobial drugs due to the rapid development of resistance to increasing microbial infectious diseases and drugs used in their treatment. Also, the compounds synthesized for use in cancer treatment limit their use due to their cytotoxic effect on normal cells and cancerous cells, and their side effects are high. For this reason, researches continue to intensify the drugs developed for cancer treatment to be selectively effective on cancer cells and to reduce their side effects as much as possible. Within this study's scope, 7 compounds of 2-(*p*-substituted phenyl)-5-(2-substitutedacetamido) were synthesized in 3 steps, and their structures were illuminated by HRMS, ^{1}H-NMR, and ^{13}C-NMR spectroscopy methods. Their antimicrobial activities were evaluated on 10 different microorganisms. In addition, cytotoxic activities were investigated on MCF-7 and A549 cell lines by the MTT method. As a result of the study, it was determined that the synthesized compounds were both antibacterial and antifungal at MIC values ranging from 64-256 µg/mL, and compound **9** had significant cytotoxic activity on both A549 and MCF-7. The results obtained will make an important contribution to the studies conducted to develop new or alternative antimicrobial and cytotoxic agents.

Keywords: Benzoxazole, Synthesis, Antimicrobial activity, Cytotoxicity, ADME prediction.

Bazı Yeni Benzoksazol Türevlerinin Sentezi, Antimikrobiyal ve Sitotoksik Aktivite Çalışmaları

Öz

Artan mikrobiyal enfeksiyon hastalıklara ve bunların tedavisinde kullanılan ilaçlara karşı hızla direnç gelişmesi nedeniyle yeni antimikrobiyal ilaçların geliştirilmesine acil ihtiyaç duyulmaktadır. Ayrıca kanser tedavisinde kullanmak amacıyla sentezlenen bileşiklerin kanserli hücrelerde olduğu kadar normal hücrelerde de sitotoksik etki göstermesi ve yan etkilerinin fazla olması nedeniyle kullanımını sınırlı hale getirmektedir. Bu nedenle kanser tedavisi için geliştirilen ilaçların seçici olarak kanserli hücreler üzerinde etkili olması ve yan etkilerinin mümkün olduğunca azaltılması yönünde araştırmalar yoğun olarak devam etmektedir. Bu çalışma kapsamında 7 tane 2-(*p*-substitutedphenyl)-5-(2-substitutedacetamido) bileşiğinin 3 basamakta sentezi gerçekleştirildi, yapıları HRMS, ^{1}H-NMR ve ^{13}C-NMR spektrokpisi yöntemleri ile aydınlatıldı. Antimikrobiyal aktiviteleri 10 farklı mikroorganizma üzerinde değerlendirildi. Ayrıca MCF-7 ve A549 hüzre hatları üzerinde MTT metodu ile sitotoksik aktiviteleri araştırıldı. Çalışma sonucunda sentezlenen bileşiklerin 64-256 µg/mL arasında değişen MİK değerlerinde hem antibakteriyel hem de antifungal etkili oldukarı belirlenmiş ve bileşik **9**'nin hem A549 hem de MCF-7 üzerinde önemli sitotoksik aktivitesi bulunmuştur. Elde edilen sonuçlar yeni veya alternatif ilaç antimikrobiyal ve sitotoksik ajan geliştirmek için yapılan çalışmalara önemli bir katkı sağlayacaktır.

Anahtar Kelimeler: Benzoksazol, Sentez, Antimikrobiyal aktivite, Sitotoksisite, ADME tahmini.

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1. Introduction

One of the most important goals of researchers working in the field of pharmaceuticals and medicinal chemistry is to be able to design new effective chemical compounds, which may be drug substance. Antibiotics are among the most widely used drugs in the world in the first place. 35% of the health budgets of developing countries are spent on antibiotics. Unfortunately, as a result of unnecessary and inappropriate use of these commonly used and costly drugs, treatment failure, mortality, cost, resistance, the current use of antibiotics, and the need for new antimicrobials are emerging (Arandjelovic et al., 2019). However, the development of a new antibiotic has taken many years, and the costs of these studies are quite high. For this reason, the possibilities for the development of new antibiotics are gradually diminishing. Resistance to various classes of antibiotics, such as methicillin, vancomycin, rifamycin, and the like, which are effective against bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), multiresistant (MDR) *Mycobacterium tuberculosis* have been reported (Alanis, 2005; Yilmaz et al., 2017). This leads researchers to work on designing and synthesizing new antimicrobial drugs with more effective and fewer side effects. Studies have shown that structures bearing heterocyclic nuclei have a very strong microbiological effect. Since the benzoxazole ring system is the structural analog of the heterocyclic adenine and guanine bases in the nucleic acids structure, it is believed that the derivatives carrying this ring system can demonstrate their microbiological activity by inhibiting nucleic acid synthesis (Oehlers et al., 2004). For this reason, the workings of these derivatives have been increasing in recent years.

Cancer is a group of diseases consisting of uncontrolled cells' proliferation in different organs, with additional clinical appearance, treatment, and approach (Suzuki et al., 2013; Yeliz et al., 2019). According to the World Health Organization data in 2018, the cause of death of approximately one in six people in the world is cancer. Today, the total number of deaths due to AIDS, malaria, and tuberculosis is far less than people who die from cancer. For this purpose, many new compounds have been synthesized, and successful results have been obtained in cancer cells; however, the cytotoxic effect of the compounds synthesized for use in cancer treatment on normal cells as well as on cancer cells and their excessive side effects limit their use (Fernández et al., 2010). For this reason, researches continue to intensify the drugs developed for cancer treatment to be selectively effective on cancer cells and to reduce their side effects as much as possible.

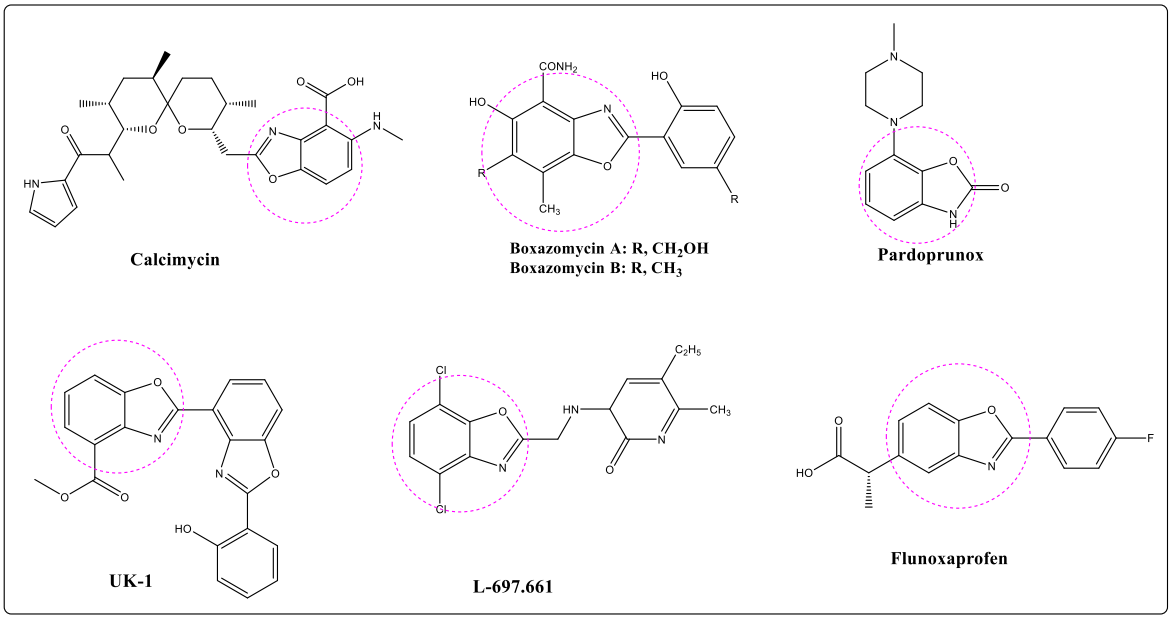


Figure 1. *Benzoxazole derivatives with various biological activities*

Benzoxazole ring is one of the important heterocyclic ring systems in drug design due to its structural similarity to adenine and guanine bases. It is thought to show its chemotherapeutic activities by inhibiting nucleic acid synthesis. This approach further increases the interest in benzoxazole derivatives (Oehlers et al., 2004). The investigations reveal that benzoxazole and its analogs are compounds that give meaningful results in terms of microbiological (Taşcı et al., 2018) and cytotoxic activity (Zilifdar et al., 2018). Benzoxazole ring system also has a broad spectrum of pharmacological effects such as anti-inflammatory (Sondhi et al., 2006), antiepileptic (Wei et al., 2009), antialzheimer (Celik et al., 2020; Temiz-Arpaci et al., 2016), anti-HIV (Sakamoto et al., 2007), topoisomerase inhibitors (Oksuzoglu et al., 2008), kinase inhibitors (Han et al., 2012), protease inhibitors (Jonckers et al., 2012). Calcimycin and boxazomycin A-B (antimicrobial), flunoxaprofen (non-steroidal anti-inflammatory), pardoprunox (antiparkinsonian), UK-1 and L-697.661 (anticancer) are examples of some drugs carrying benzoxazole ring system (Figure 1).

Researches on the benzoxazole ring system focus on derivatives of the ring-substituted from the 2nd and 5th positions. In previous studies, some derivatives containing *p*-(substituted)/phenyl) at position 2 and 6-membered rings attached to the amide side chain at position 5 were synthesized, and promising results were obtained by examining their antimicrobial and cytotoxic activities (Temiz-Arpacı et al., 2005; Arisoy et al., 2008; Temeltas et al., 2020). Among the derivatives, compounds as effective as gentamicin against MRSA were observed; in addition, it was determined that the presence of piperazine group in the structure positively affected the activity by the quantitative

structure-effect relationships study conducted against MRSA. Based on these data, in this study, the synthesis of some compounds with the general structure of 2-(*p*-substitutedphenyl)-5-(2-substitutedacetamido)benzoxazole was carried out in three steps. The structure of the compounds was elucidated by HRMS, ^{1}H-NMR, and ^{13}C-NMR spectroscopy methods, and also melting points were determined. Antimicrobial effects of *S. aureus*, *E. faecalis, E. coli, P. aeruginosa, C. albicans* microorganisms and their drug-resistant strains in the form of minimum inhibitory concentration (MIC) were investigated. Also, cytotoxic activities were determined on A549 and MCF-7 cell lines. The estimated ADME profiles of all compounds were calculated.

2. Material and Method

Chemicals and solvents were purchased from Sigma Aldrich, Merck, Riedel de Haen, and Fluka and used without further purification. For the TLC, 60 F254 coated aluminum plates (Merck) were used, and the UV light at 254 and 366 nm wavelength was used to detect stains. The TLC mobile phase composition used in each step is indicated in the relevant method. Silica gel 60 (Merck) with a particle size of 230-400 mesh was used in column chromatography. The used column diameter is 2.4 cm, and the amount of silica gel is 30 g. Soluble compounds in the small mobile phase (3-5 mL) were applied by dissolving in the column's mobile phase. The resulting compounds, which were insoluble in the mobile phase, were used solidly by adsorption of silica gel. Melting points were determined by Electrothermal 9100 (Varian, Palo Alto, CA) instrument, and the results are given without correction. NMR spectra were analyzed on a Varian Mercury 400 MHz High-Performance Digital FT-NMR spectrometer (Palo Alto, CA, USA) dimethylsulfoxide-d_6 (DMSO-d_6) was used as a solvent. The mass spectra (high-resolution mass spectrometry [HRMS]) of all of the synthesized compounds showed molecular ion $[M+H]^+$ peaks were determined by the Shimadzu LC/MS ITTOF system.

2.1. General Procedure for Synthesis of 2-(*p*-substitutedphenyl)benzoxazole (1, 2)

20 mmol of 2,4-diaminophenol dihydrochloride and 20 mmol of *p*-substituted benzoic acid was stirred at 160-190°C for 3-4 h at 25 g of polyphosphoric acid (PPA) catalyst. At the end of the reaction, the reaction contents were poured over ice, and the solution was neutralized with 10% NaOH. The resulting precipitate was filtered, then crystallized with ethanol-water. During the synthesis studies, ethyl acetate: *n*-hexane (1:1) TLC mobile phase was used to monitor the reaction end and to check the purity of the compound (Alper-Hayta et al., 2008; Arisoy et al., 2012; Arisoy et al., 2013; Arisoy et al., 2008).

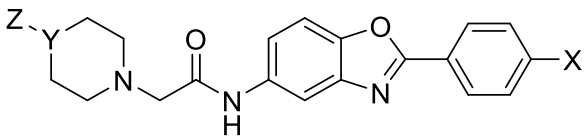
2.2. General Procedure for Synthesis 5-(2-chloroacetamido)-2-(*p*-substitutedphenyl) benzoxazole (3, 4)

6 mmol of 2-(*p*-substitutedphenyl)-5-aminobenzoxazole were dissolved in 120 ml of diethyl ether and 12 mmol of $NaHCO_3$ in 60 ml distilled water. 6 mmol of 2-chloroacetyl chloride is slowly added while the ether and water phase were stirred in the ice bath in the magnetic stirrer. Stirring is continued overnight. At the end of the reaction, the reaction medium was filtered, and crystallized from ethanol-water. During the synthesis studies, ethyl acetate: *n*-hexane (1:1) TLC mobile phase was used to monitor the reaction end and to check the purity of the compound (Alper-Hayta et al., 2008; Arisoy et al., 2012; Arisoy et al., 2013; Arisoy et al., 2008).

2.3. General Procedure for 2-(*p*-substitutedphenyl)-5-(2-substitutedacetamido)benzoxazole (5-11)

1 mmol 2-(*p*-substituted phenyl)-5-(2-chloroacetamido) benzoxazole derivative compounds were reacted with 5 mmol piperazine/piperidine derivatives at room temperature for 24 h in the presence of 1 ml TEA and 5 ml DMF. At the end of the reaction time, the mixture was poured over ice, the same volume of 10% NaOH solution is added. Extraction was carried out 2 times with chloroform. The chloroformed phases were collected, and the solvent was evaporated, and the residue was purified by column chromatography. The list of synthesized compounds was given in Table 1.During the synthesis studies, the TLC/column mobile phase of ethyl acetate: *n*-hexane (1:3) was used to monitor the end of the reaction, purify the resulting compound and purify the resulting compound by column chromatography (Alper-Hayta et al., 2008; Arisoy et al., 2012; Arisoy et al., 2013; Arisoy et al., 2008).Compounds **11** are not original. The ^{1}H-NMR, ^{13}C-NMR spectra, and HRMS results agree with those of the proposed structures.

Table 1. *List of synthesized compounds*



Compound	X	Y	Z
5	-C_2H_5	C	Ph
6	-C_2H_5	C	Br
7	-Cl	N	(*p*-NO_2)-Ph
8	-Cl	N	Ph-C=O
9	-Cl	N	-C_2H_5
10	-Cl	N	(*p*-F)-Ph
11	-Cl	N	(*p*-Cl)-Ph

2-(*p*-ethylphenyl)-5-(2-(4-phenylpiperidine-1-yl)acetamido) benzoxazole (5)

Yield 50%, Mp: 164-166°C. ^{1}H-NMR δ ppm (400 MHz, DMSO-d_6): 9.92 (s, 1H, -NH), 8.19 (d, J = 2.1 Hz, 1H, Ar-H), 8.10 (d, J = 8.2 Hz, 2H, Ar-H), 7.72 (d, J = 8.8 Hz, 1H, Ar-H), 7.63 (dd, J = 8.8, 2.1 Hz, 1H, Ar-H), 7.53 (s, 1H, Ar-H), 7.45 (d, J = 8.1 Hz, 2H, Ar-H), 7.29 (d, J = 6.6 Hz, 3H, Ar-H), 7.19 (td, J = 6.1, 2.7 Hz, 1H, Ar-H), 3.19 (s, 2H, -CH_2), 3.00 (d, J = 11.1 Hz, 2H, -CH_2), 2.71 (q, J = 7.6 Hz, 2H, -CH_2), 2.29 (td, J = 11.5, 2.7 Hz, 2H, -CH_2), 1.90 – 1.77 (m, 2H, -CH_2), 1.76 (s, 2H, -CH_2), 1.74 (d, J = 10.1 Hz, 1H, -CH_2), 1.22 (t, J = 7.6 Hz, 3H, -CH_3). ^{13}C-NMR δ ppm (100 MHz, DMSO-d6): 169.08, 163.56, 148.69, 146.84, 146.71, 142.16, 136.28, 129.20, 128.79, 127.78, 127.22, 126.48, 124.39, 118.24, 110.99, 110.72, 62.68, 54.36, 41.84, 33.39, 28.63, 15.65. HRMS (m/z): $[M+H]^+$ calcd for $C_{28}H_{29}N_3O_2$: 440.22598; found: 440.23534.

2-(*p*-ethylphenyl)-5-(2-(4-bromopiperidine-1-yl)acetamido) benzoxazole (6)

Yield 55%, Mp: 197-199°C. ^{1}H-NMR δ ppm (400 MHz, DMSO-d_6): 9.89 (s, 1H, -NH), 8.16 (d, J = 2.0 Hz, 1H, Ar-H), 8.11 – 8.08 (m, 2H, Ar-H), 7.70 (d, J = 8.8 Hz, 1H, Ar-H), 7.60 (dd, J

= 8.9, 2.1 Hz, 1H, Ar-H), 7.46 – 7.43 (m, 2H, Ar-H), 4.42 (s, 1H, -CH), 3.16 (s, 2H, -CH_2), 2.72 (dq, J = 15.2, 7.6, 6.8 Hz, 4H, -CH_2), 2.43 (d, J = 10.7 Hz, 2H, -CH_2), 2.16 (q, J = 3.4 Hz, 2H, -CH_2), 2.02 (dt, J = 8.4, 3.9 Hz, 2H, -CH_2), 1.22 (t, J = 7.6 Hz, 3H, -CH_3). ^{13}C-NMR δ ppm (100 MHz, DMSO-d_6): 168.84, 163.55, 148.68, 146.85, 142.13, 136.23, 129.19, 127.78, 124.38, 118.30, 110.95, 110.81, 62.00, 52.25, 51.84, 36.29, 28.63, 15.64. HRMS (m/z): $[M+H]^+$ calcd for $C_{22}H_{24}BrN_3O_2$: 442.10519; found: 442.11380.

2-(*p*-chlorophenyl)-5-(2-(4-(p-nitrophenylpiperazine-1-yl)acetamido)benzoxazole (7)

Yield 70%, Mp: 208-210°C. 1H-NMR δ ppm (400 MHz, DMSO-d_6): 10.01 (s, 1H, -NH), 8.22 – 8.17 (m, 3H, Ar-H), 8.06 (d, J = 9.2 Hz, 2H, Ar-H), 7.68 (td, J = 21.9, 20.9, 8.8 Hz, 4H, Ar-H), 7.05 (d, J = 9.3 Hz, 2H, Ar-H), 3.56 (d, J = 5.0 Hz, 4H, (2)-CH_2), 3.25 (s, 2H), 2.69 (d, J = 5.1 Hz, 4H, (2)-CH_2). ^{13}C-NMR δ ppm (100 MHz, DMSO-d_6): 168.66, 162.48, 155.19, 146.96, 142.01, 137.30, 137.17, 136.5, 129.98, 129.46, 126.21, 125.73, 118.71, 113.12, 111.21, 110.88, 61.83, 52.67, 46.73. HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{22}ClN_5O_4$: 492.13603; found: 492.14437.

2-(*p*-chlorophenyl)-5-(2-(4-benzoylpiperazine-1-yl) acetamido)benzoxazole (8)

Yield 45%, Mp: 198-200°C. ^{1}H-NMR δ ppm (400 MHz, DMSO-d_6): δ 9.94 (s, 1H, -NH), 8.19 (s, 3H, Ar-H), 7.67 (dd, J = 21.5, 13.4 Hz, 5H, Ar-H), 7.45 –7.39 (m, 4H, Ar-H), 3.69 (s, 3H, -CH_3), 3.23 (s, 2H, -CH_2), 2.64 – 2.56 (m, 5H, (2)-CH_2 and -CH). ^{13}C-NMR δ ppm (100 MHz, DMSO-d_6): 169.42, 168.64, 162.46, 146.94, 141.99, 137.15, 136.43, 136.36, 129.96, 129.84, 129.44, 129.08, 128.90, 127.36, 125.72, 118.70, 111.18, 111.05, 110.88, 61.83. 51.48, 47.81. HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{23}ClN_4O_3$: 475.14587; found: 475.15574.

2-(*p*-chlorophenyl)-5-(2-(4-ethylpiperazine-1-yl)acetamido) benzoxazole (9)

Yield 40%, Mp: 162-164°C. ^{1}H-NMR δ ppm (400 MHz, DMSO-d_6): 9.90 (s, 1H, -NH), 8.24 – 8.09 (m, 3H, Ar-H), 7.73 (d, J = 8.8 Hz, 1H, Ar-H), 7.67 (dd, J = 8.7, 6.7 Hz, 2H, Ar-H), 7.66 – 7.56 (m, 1H, Ar-H), 3.34 (s, 2H, -CH_2), 2.53 (s, 4H, (2)-CH_2), 2.44 (s, 4H, (2)-CH_2), 2.32 (q, J = 7.2 Hz, 2H, -CH_2), 0.99 (t, J = 7.1 Hz, 3H, -CH_3). ^{13}C-NMR δ ppm (100 MHz, DMSO-d_6): ^{13}C-NMR δ ppm (DMSO-d_6): 168.81, 165.92, 163.44, 162.58, 146.93, 142.05, 136.37, 130.39, 130.30, 123.55, 123.52, 118.37, 117.13, 116.91, 111.11, 110.73, 62.37, 53.35, 52.66, 52.06, 12.51. HRMS (m/z): [M+H]+ calcd for $C_{21}H_{23}ClN_4O_2$: 399.15095; found: 399.15948.

2-(*p*-chlorophenyl)-5-(2-(4-(*p*-fluorophenyl)piperazine-1-yl)acetamido)benzoxazole (10)

Yield 55%, Mp: 190-192°C. ^{1}H-NMR δ ppm (400 MHz, DMSO-d_6): 9.95 (s, 1H, -NH), 8.17 (s, 1H, Ar-H), 7.65 (dd, J = 19.3, 8.8 Hz, 4H, Ar-H), 7.04 (t, J = 8.5 Hz, 3H, Ar-H), 6.95 (d, J = 9.4 Hz, 3H, Ar-H), 3.32 (s, 2H, -CH_2), 2.70 (d, J = 5.3 Hz, 8H, (4)-CH_2). ^{13}C-NMR δ ppm (100 MHz, DMSO-d_6): ^{13}C-NMR δ ppm (DMSO-d_6): 168.74, 162.46, 157.61, 155.27, 148.40, 146.94, 142.01, 137.15, 136.47, 129.96, 129.44, 125.72, 118.65, 117.63, 117.55, 115.82, 115.60, 111.19, 110.82, 62.16, 53.18, 49.31, 40.59, 40.38, 40.17, 39.96, 39.75, 39.54, 39.33. HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{22}ClFN_4O_2$: 465.14153.15095; found: 465.14125.

2.4. Antibacterial and Antifungal Activity

Standard strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231, and clinical isolates of the bacteria obtained from Trakya University Faculty of Medicine Microbiology Laboratory were included in the study. Vancomycin, ampicillin, gentamicin, ciprofloxacin, cefotaxime, amphotericin B, and fluconazole were used as standard drugs and dissolved in the solvents recommended by CLSI guidelines. Stock solutions of the tested compounds were dissolved in DMSO (Merck). Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Sabouraud Dextrose Agar (SDA), Sabouraud Liquid Medium (SLM), and RPMI-1640 medium with L-glutamine buffered with MOPS (pH:7) were used in the study. MHA, MHB, SDA, and SLM were sterilized with an autoclave at 121ºC for 15-20 minutes, and RPMI-1640 was sterilized by filtration. Susceptibility testing was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M100-S25 and M27-A3 (CLSI, 2018; Edition, 2007). 100 µL of MHB and RPMI-1640 medium with L-glutamine (Sigma) buffered with MOPS (pH:7) were added to each well of the microplates for bacteria and fungi, respectively. The inoculation's bacterial suspensions were prepared at 10^5 CFU/mL by diluting fresh cultures at McFarland 0.5 density. Suspensions of the yeast at McFarland density were diluted 1:100 and 1:20, respectively and $2{,}5x10^3$ CFU/mL were inoculated to the twofold-diluted solution of the compounds. Stock solutions of the tested compounds and standard drugs were diluted two-fold in the wells of the microplates, so the solution of the synthesized compounds and standard drugs were prepared at 512, 256, 128, 64, 32, 16, 8, 4 µg/mL and standard drugs were prepared at 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 µg/mL concentrations. All solvents and diluents, pure microorganisms, and pure media were used in control wells. A 10 µl microorganism inoculum was added to each well of the microplates. Microplates, including bacteria were incubated at 37°C for 16–20 h and microplates, including fungi were incubated at 35 °C for 24-48 h. After incubation, the lowest concentration of the compounds that completely inhibits macroscopic growth was determined and reported as minimum inhibitory concentrations (MICs).

2.5. Cell Culture and Cell Proliferation Assay

The effects of **5-11** on the proliferation of A549 (lung cancer cell line) and MCF-7 (breast cancer cell line) cells were determined by MTT assay. The MCF-7 and A549 were cultured in the incubator, and the proliferating cells were passaged into new 25 and 75 cm² flasks, and cell stocks were prepared for use in experiments. Cells were placed on DMEM medium; glutamine, 10% Fetal Bovine Serum (FBS), and 1% penicillin-streptomycin antibiotic mixture was added and grown under suitable conditions. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved in phosphate buffer (PBS). Before each run, the solution was prepared fresh from MTT powder. MCF-7 and A549 cells in DMEM medium, 5% CO_2 at 37°C was cultured in an incubator. In the evaluation of whether the cells multiply enough and cell viability, sowing was performed by counting on the cell counting device. Subsequently, the substances prepared in the wells on the plates were applied and left for 24 h incubation. At the end of the incubation periods, the medium in the plate was discarded, and 100 µL of MTT solution was added to the cells in each 96-well, and the cells were

incubated in the incubator for 2 h. At the end of the incubation, 100 µL of DMSO was placed in each well, and the absorbance values were read in the ELISA device with a wavelength of 570 nm. Results were calculated according to the formula of viability and in % determined.

2.6. *In silico* ADME prediction

In addition to having a high pharmacological activity and low toxicity profile, a compound candidate to be a drug should have ideal pharmacokinetics. Within the scope of computer-aided drug development studies in recent years, estimates of absorption, distribution, metabolism, and absorption (ADME) profiles of drug candidates can be made. In this study, various pharmacokinetic parameter values such as log P, TPSA, nrotb, molecular weight, and hydrogen bond donor-receptor number of the compounds synthesized using the Molinspiration software program were calculated (Cheminformatics, 2018). Drug-likeness scores were computed using the Molsoft program (Molsoft, 2004).

3. Results and Discussion

3.1. Chemistry

In this study, 7 pieces 2-(*p*-substituted phenyl)-5-(2-substitutedacetamido)benzoxazole derivatives, 1 of which are not original (compound **11**), were synthesized in three steps and the synthesis steps for the preparation of the target compounds are shown in Figure 2.

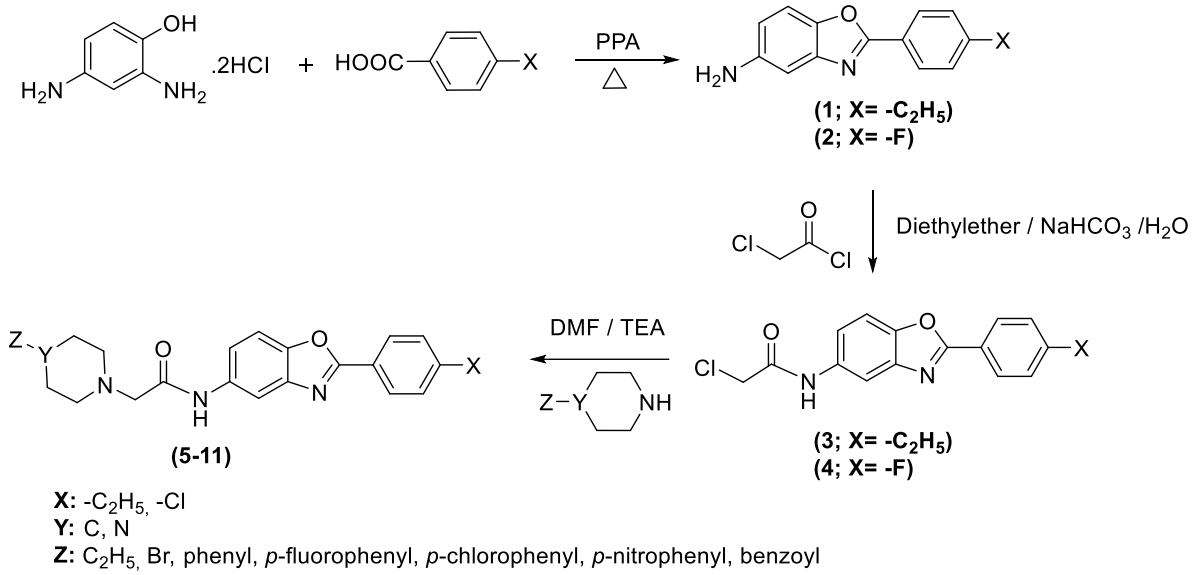


Figure 2. *General procedure for the preparation of* ***5-11***

During the synthesis process, the reaction medium was checked with TLC, and the compounds were purified by column chromatography as described in synthesis methods. The compounds' structure was confirmed by HRMS, ^{1}H-NMR and ^{13}C-NMR spectroscopic methods, and melting points were determined. In the ^{1}H NMR spectra of compounds, the NH proton's signal was observed at 10.01–10.12 ppm as a broad singlet band. -CH_2 protons in the acetamido group were observed as singlets between 3.16-3.34 ppm. CH_2 protons of piperazine and piperidine ring were recorded at 2.28-3.75 ppm as a triplet band or multiplet. In general, aliphatic protons were observed at 1.13-3.92 ppm and aromatic protons at 6.70-8.12 ppm. Also, the ^{13}C-NMR spectra and HRMS of the compounds were compatible with their formulas. The results were presented in the Material and method.

3.2. Cytotoxic Activity

MTT assay is a quantitative colorimetric assay for measuring cellular growth, cell survival, and cell proliferation based on live cells' ability. This method is based on the reduction of tetrazolium salts catalyzed by mitochondrial enzyme systems. Tetrazolium salts are generally colorless as substrates, substances that produce colored products as a result of the mitochondrial activity of living cells. MTT is a tetrazolium salt used for this purpose. Although it is yellow as a substrate, it forms water-insoluble blue-purple formazan salts when bound explicitly to the succinate-dehydrogenase (SDH) enzyme in mitochondria of living cells. Meanwhile, the color created is measured colorimetrically, and the amount of formazan formed gives the number of viable cells.

All synthesized compounds were tested at 100 µM concentration for cytotoxic activity against MCF-7 and A549 cell lines by MTT analysis, and 5-Fluorouracil was used as a standard drug at 64 µM concentration. Measurements were taken as a result of a 24-h incubation period. The percentage of live cell presence values against these two cell lines were given in Table 2. The results showed that the compounds had moderate activity against two cell lines. While compound **9** showed the best activity, it reduced viability in A549 cells to 71.29% and viability in MCF-7 cells to 70%. The compounds generally showed higher activity against the MCF-7 cell line. In both cell lines, a compound having an ethyl group at the 4th position of the piperazine ring showed better activity.

Table 2. *Percent live-cell presence values from 100 µM concentration*

Compound	MCF-7 % Vitality	A549 % Vitality
5	95.8	102.21
6	92.0	101.26
7	100.0	100.32
8	94.4	89.91
9	**70.0**	**71.29**
10	86.6	102.21
11	99.4	85.49
Contol	100.0	100.00
DMSO Control	85.2	93.38
5-Fluorourasil (64 µM)	73.13	73.99

3.3. Antimicrobial Activity

All the newly synthesized benzoxazoles were tested *in vitro* for antibacterial activity against *Staphylococcus aureus* ATCC 29213, *S. aureus* isolate [meticilline-resistant (MRSA)], *Enterococcus faecalis* ATCC 29212, *E. faecalis* isolate (resistant to vancomycin) as Gram-positive bacteria; *Escherichia coli* ATCC 25922, *E. coli* isolate [has extended spectrum beta-lactamase (ESBL) enzyme], *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* isolate (resistant to gentamicin) as Gram-negative bacteria, and antifungal activities of the compounds were evaluated against *Candida albicans* ATCC 10231, and *C. albicans* isolate. The standard drugs employed while assessing antimicrobial activities were vancomycin, ampicillin, gentamycin, ciprofloxacin, cefotaxime, fluconazole, and amphotericin B. The MIC values were determined by microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The antimicrobial activity MIC (µg / mL) of the compounds and reference drugs are given in Table 3. Compound 11 has been synthesized before, its antimicrobial effects have been evaluated, and these data are given in the relevant literature. Generally, synthesized benzoxazole derivatives showed weak activity against *S. aureus* and MRSA with MIC: 256 µg/mL. When activity against *E. faecalis* is examined; compound **5** showed a moderate activity compared to reference drugs with MIC: 64 µg/mL. Compounds in general; it has been observed to have a weak antimicrobial effect against *E. coli, P. aeruginosa*, and its isolates. While the antifungal activities of the compounds against *C. albicans* and *C. albicans* isolate vary between 64-128 µg/mL.

Table 3. *In vitro antimicrobial MIC values (µg/mL) of* ***5-10*** *and reference drugs*

Compound	Gram-positive bacteria				Gram-negative bacteria				Fungus	
	S.a	S.a.*	E.f.	E.f*	E.c.	E.c.*	P.a.	P.a.*	C.a.	C.a.*
5	256	256	64	256	256	256	128	128	128	128
6	256	256	128	128	128	256	128	128	64	128
7	256	256	128	128	256	256	128	128	64	128
8	256	256	128	128	256	256	128	128	128	128
9	256	256	128	128	256	256	128	128	64	128
10	256	256	128	128	256	256	128	128	128	128
Ampicillin	2	>16	2	>16	8	>16	-	-	-	-
Vancomycin	2	2	1	8	-	-	-	-	-	-
Gentamicin	0.25	>16	-	-	0.5	>8	0.5	>8	-	-
Ciprofloxacin	0.5	>16	2	>4	0.0156	>2	0.125	>2	-	-
Cefotaxime	1	>16	-	-	0.125	>8	8	-	-	-
Fluconazole	-	-	-	-	-	-	-	-	0.125	>4
Amphotericin B	-	-	-	-	-	-	-	-	0.5	0.5

S.a.: *Staphylococcus aureus* ATCC 29213; S.a.*: *S. aureus* isolate (MRSA); E.f: *Enterococcus faecalis* ATCC 29212; E.f *: *E. faecalis* isolate (Vancomycin resistant -VREF); E.c.: *E. coli* ATCC 25922; E.c.*: *E. coli* isolate (contains broad spectrum β-lactamase enzyme); P.a.: *Pseudomonas aeruginosa* ATCC 27853; P.a.*: *P. aeruginosa* isolate (gentamicin resistant); C.a: *Candida albicans* ATCC 10231; C. a.*: *Candida albicans* isolate.
-*: not determined

3.4. *In Silico* ADME Prediction

Lipophilic, estimated volume, topological polar surface area, %ABS, molecular weight, rotatable bond number, hydrogen-bonded receptor number, hydrogen bond donor count of all compounds were given in Table 4. According to the five rules of Lipinski (molecular log P ≤5, molecular weight ≤500, number of hydrogen bond receptors ≤10, and number of hydrogen bond donors ≤5), an active oral drug must fully comply with this rule or violate at most one of them (Lipinski, 2004). The absorption percentage was calculated using % ABS=109-(0.345xTPSA), and the compounds showed a good absorption profile in the range of 71.93-88.86% (Kilic-Kurt et al., 2019). Some of the compounds did not comply with Lipinski's log P but did not make more than one violation. Drug likeness score calculations qualitatively assess the chances of compounds becoming drugs for oral bioavailability, and compounds with zero or negative values are not considered drug-like candidates. Compounds **7**, **10**, and **11** had the lowest drug similarity score, while other compounds had a good drug-likeness score above 1.

Table 4. *Calculated ADME parameters* ***(5-11)***

Comp.	Log P ≤5	TPSA -	%ABS -	MW ≤500	nON ≤10	nOHNH ≤5	nviolations ≤1	nrotb -	Volume -	Drug-likeness score
5	6.58	58.37	88.86	439.56	5	1	1	6	415.79	1.25
6	5.24	58.37	88.86	442.36	5	1	1	5	362.27	1.00
7	5.43	107.43	71.93	491.94	9	1	1	6	415.25	0.76
8	4.33	78.68	81.85	474.95	7	1	0	5	410.90	1.35
9	4.15	61.61	87.74	398.89	6	1	0	5	353.88	1.27
10	5.63	61.61	87.74	464.93	6	1	1	5	396.85	0.79
11	6.14	61.61	87.74	481.38	6	1	1	5	405.46	0.79

MW: Molecular weight. TPSA: Topological polar surface area. %ABS: Percentage absorption. nrotb: Number of rotatable bonds. *n*ON: Number of hydrogen acceptors. *n*OHNH: Number of hydrogen donors. Log P: Log octanol/water partition coefficient.

4. Conclusions and Recommendations

In this study, a series of 2,5-disubstituted benzoxazole derivative compounds that we hope may be a new antimicrobial and cytotoxic agent was synthesized, and their antimicrobial and cytotoxic activities were determined. When the MIC values of the synthesized compounds and reference drugs were examined, it was observed that the reference drugs generally showed better antimicrobial activity. On the other hand, compound **9** showed the best cytotoxic activity on MCF-7 and A549 cell lines, with a reduction of 70% and 71.29% viability. The compounds also show a good predictive ADME profile. When all results are evaluated together; this study will make an important contribution to the studies for the development of safe, non-toxic new or alternative drugs.

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