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Research Article

Synthesis and antitubercular activities of acetamide-substituted benzazole derivatives

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Abstract

Multidrug-resistant *Mycobacterium tuberculosis* strains' increasing emergence and rapid spread necessitate the urgent development of innovative antimycobacterial agents. In pursuit of novel agents, a series of N-(benzazole-2-ylmethyl)-2-substituted phenylacetamide or N-(benzazole-2-ylmethyl)-2-(thiophen-2-yl)acetamide compounds (6-11) were synthesized. Their efficacy against multidrug-resistant *Mycobacterium tuberculosis* was assessed. Compounds exhibited potent antimycobacterial activity with minimum inhibitory concentrations (MIC) ranging from 1.05 to 4.10 µM and demonstrated low cytotoxicity towards fibroblast cell line (L929), as indicated by IC_{50} values ranging from 196.23 to 487.34 µM and selectivity indices (SI) ranging from 57.23 to 186.11. ADMET predictions suggested that these synthesized compounds possess drug-like properties. Our findings offer a promising starting point for designing more selective and potent antimycobacterial agents.

Keywords: Benzazole, synthesis, Mycobacterium tuberculosis, MIC, antitubercular activity

Asetamit Sübstitüte Benzazol Türevlerinin Sentezi ve Antitüberküloz Aktiviteleri

Öz

Çoklu ilaca dirençli mikobakterium tüberkülozis suşlarının artan ortaya çıkışı ve hızlı yayılımı, acil olarak yenilikçi antimikobakteriyel ajanların geliştirilmesini gerektirmektedir. Yeni ajanların geliştirilmesi amacıyla, N-(benzazol-2-ilmetil)-2-sübstitüe fenilasetamit veya N-(benzazol-2-ilmetil)-2-(tiyofen-2-il)asetamit bileşik serisi (6-11) sentezlendi. Bu bileşiklerin çoklu ilaca dirençli mikobakterium tüberkülozis 'e karşı etkinliği değerlendirildi. Bileşikler, minimum inhibitör konsantrasyonları (MIC) 1.05 ile 4.10 µM arasında değişen güçlü antimikobakteriyel aktivite sergiledi ve IC_{50} değerleri 196.23 ile 487.34 µM arasında değişen ve seçicilik indeksleri (SI) 57.23 ile 186.11 arasında olan fibroblast hücre hattına (L929) karşı düşük sitotoksisite gösterdi. ADMET çalışmaları, sentezlenen bileşiklerin ilaç benzeri özelliklere sahip olduğunu göstermektedir. Bulgularımız, daha seçici ve güçlü antimikobakteriyel ajanlar tasarlamak için umut verici bir başlangıç noktası sunmaktadır.

Anahtar Kelimeler: Benzazol, sentez, mikobakteriyum tüberkülozis, MİK, antitüberküloz aktivite

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1. Introduction

Since the early 1990s, tuberculosis (TB) has been recognized globally as a significant public health threat, accounting for the highest number of adult deaths due to infectious diseases worldwide. Mycobacterium tuberculosis is the primary species responsible for TB infections [1, 2]. Its resurgence in epidemic proportions indicates that substantial efforts are still needed to safeguard patients and healthcare professionals from its potentially fatal consequences [3]. Molecular evidence suggests that TB ranks second globally in terms of death rates caused by a single infectious agent in 2022, causing approximately twice as many deaths as HIV/AIDS. Tuberculosis remains a significant global health issue. According to the World Health Organization (WHO), there is an estimated annual increase of approximately 1.1% in TB cases worldwide, with over 10 million new TB cases reported each year. [4].

Benzazoles derivatives (benzimidazole, benzothiazole and benzoxazole) display a broad spectrum of activities, including antibacterial, antifungal, antitubercular, antimalarial, anti-inflammatory, analgesic, anti-amoebic, antiulcerative, antioxidant, antihypertensive, antiallergic, antiproliferative, antitumor, and anti-HIV-1 properties [5–7]. Due to their straightforward and rapid synthesis along with other advantageous properties, benzazoles hold promise as potential anti-TB agents. Benzazoles have consistently attracted the attention of researchers, not only from synthetic or biosynthetic perspectives but also due to its intriguing biological characteristics [8–19].

Benzazole rings are recognized by living organisms due to their isosteric nature with DNA bases (purine and pyrimidine cores) and their presence in natural structures like the amino acid tryptophan. Despite numerous studies on the antimicrobial activities of benzazole and bioisostere structures and their substituted derivatives, literature on compounds carrying amid linkages in benzazole derivatives is scarce. Particularly, structure-activity studies conducted on the compounds registered in the literature and those designed in this study suggest that the compounds designed in this study may possess antitubercular activity [20–27].

Previous research revealed that benzothiazole and benzoxazole groups demonstrate superior antimicrobial activities compared to other benzazole groups. This led to prioritizing these groups in our current work, with plans to compare them with other benzazole groups in future studies [8, 28–30].

The amide functional group is crucial in the structure of many biologically active molecules. Amide structures are not only stable but also possess highly positive and negative electric charge densities, making them polar structures. This polarity allows drugs containing amide structures to easily interact with biological receptors and enzymes. In addition to facilitating interactions with biological targets, amide-containing drugs are also resistant to rapid metabolic degradation in the body's complex environment. Consequently, due to its significant bond polarity, stability, and conformational versatility, the amide bond stands out as one of the most effective functional groups. The amide functional group is crucial in the structure of biomolecules, including numerous clinically approved drugs. Consequently, the use of the amide bond was planned in the designed bis structures [31–34].

Recent studies on benzazoles and the above literature survey encouraged us to synthesize some new compounds containing benzazole moiety hoping to obtain new compounds with potential antitubercular activity [27]. The inclusion of the amide functional group in the benzoxazole structure has the potential to contribute to the development of various activities. Current studies

have shown that such modifications can enhance biological activity. In this context, our presented study can inspire the development of more effective antituberculosis agents. In our previous research, we investigated the antitubercular effects of compounds with a benzimidazole structure, an isosteric analog of benzoxazole and benzothiazole. In this study, some benzazole derivatives containing an amide linker were designed and synthesized (Figure 1). The results demonstrated significant antitubercular activity [35, 36, 37].

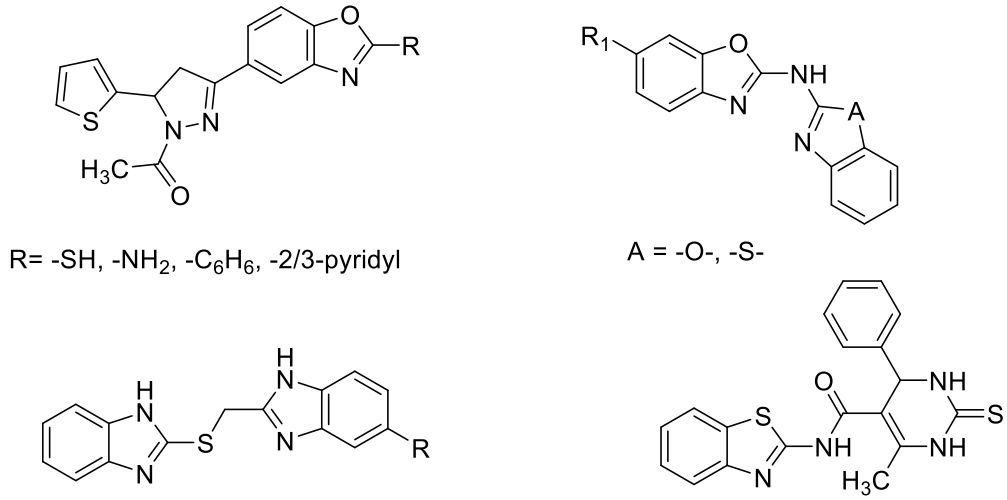


Figure 1. Structures of antitubercular active benzazole compounds

Here, we describe the synthesis of a series of N-(benzazole-2-ylmethyl)-2-substituted phenylacetamide or N-(benzazole-2-ylmethyl)-2-(thiophen-2-yl)acetamide compounds. The structures of the synthesized derivatives were elucidated by the spectroscopic data. Then, we determined antitubercular effects of the synthesized compounds by *in vitro* activity.

2. Material and Methods

2.1. Chemistry

All reagents utilized were of analytical grade, sourced from Sigma-Aldrich, and employed without further purification. Thin-layer chromatography (TLC) was conducted on silica gel-coated F254 Merck plates to monitor reaction progress and product mixtures. Melting points were measured using an Electrothermal-9200 digital apparatus, with recorded values remaining uncorrected. Nuclear magnetic resonance (NMR) spectra, encompassing both ^{1}H NMR and ^{13}C NMR, were acquired employing a Bruker 400 NMR spectrometer for ^{1}H NMR and a Bruker 100 NMR spectrometer for ^{13}C NMR. The NMR spectra were acquired in deuterated solvents such as dimethyl sulfoxide (DMSO-d6) or chloroform (CDCl3), with chemical shifts (δH) reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Fourier transform infrared attenuated total reflection (FT-IR ATR) spectra were recorded using a Varian FTS1000 FT-IR spectrometer equipped with a Diamond/ZnSe prism (4000–525 cm^{-1}; number of scans: 250; resolution: 1 cm^{-1}) in the solid state.

2.1.1. General procedure for the synthesis of target compounds (6-11):

Aminomethylbenzazoles (2-aminomethylbenzoxazole/2-aminomethylbenzothiazole) (1.1 mmol) were dissolved in dichloromethane (DCM) (15 mL). 2-Phenylacetyl chloride derivatives or -(thiophen-2-yl)acetyl chloride (1.1 mmol) were added dropwise to the initial solution at 0

°C over a period of 10-30 minutes. The reaction mixture was stirred at 0 °C for two hours. Subsequently, 5 drops of diisopropylethylamine (DIPEA) were added and the reaction mixture was refluxed under a reversing cooler for 24 hours. After confirming the completion of the reaction with TLC, the reaction was terminated, and the solvent was evaporated. DCM (25 mL) was added, and the organic phase was washed with 1N HCl (3 x 20 mL), followed by saturated $NaHCO_3$ solution (2 x 15 mL) and 15 mL of distilled water. The organic phase was separated, treated with $MgSO_4$, filtered, and the solvent was evaporated under reduced pressure. The resulting product was purified by column chromatography using an appropriate n-hexane/ethyl acetate mobile phase. The obtained products were recrystallized using an appropriate solvent [38].

2.2. Antitubercular activity

2.2.1. Agar proportion method

Following the guidelines set by the Clinical Laboratory Standards Institute (CLSI), the minimum inhibitory concentration (MIC) values for each synthesized compound were determined using duplicate agar dilution techniques [39]. Each assay included both positive and negative growth controls. Isoniazid (INH; Sigma I3377) and Rifampisin (RIF; Sigma-Aldrich R7382) served as control agents. The MTB H37Rv strain, provided by the National Tuberculosis Reference Laboratory at Refik Saydam National Public Health Agency in Ankara, Turkey, was used as the standard strain.

2.2.2. Cytotoxic activity

Cytotoxic impact of the investigated compounds were assessed using fibroblast L929 and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay [40, 41].

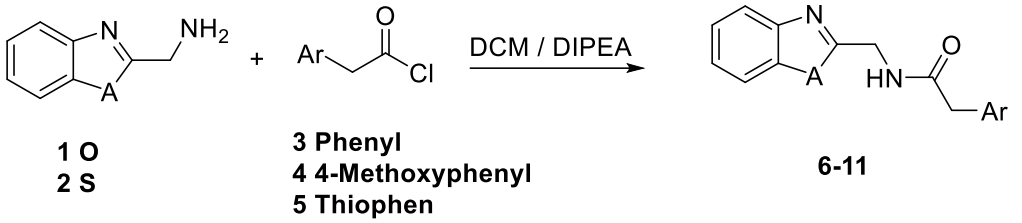
2.3. In silico ADMET prediction

In ADMET studies, the Maestro 11.8 (Schrodinger) program was used. The compounds were minimized after being prepared with LigPrep, and their ADME properties were investigated using the Qikprop module [42]. Predicted toxic properties of compounds were calculated using DataWarrior 5.5.0.

3. Results and Discussion

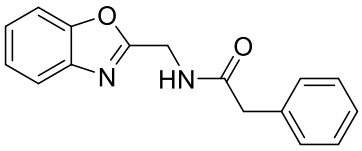
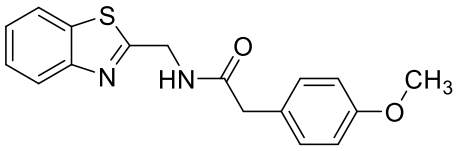
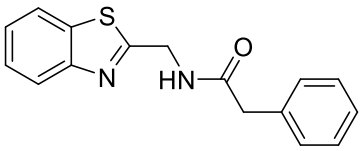
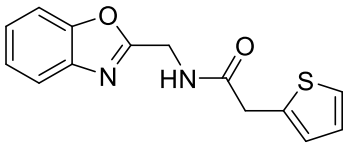
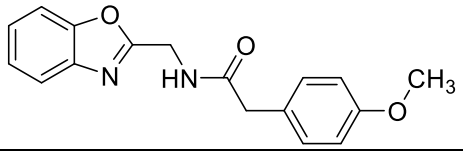
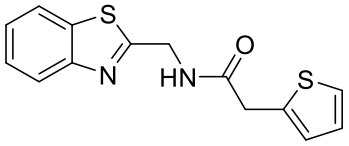
3.1. Chemistry

The target molecules were synthesized in single steps as depicted in Scheme 1. 2-Aminomethylbenzazoles (**1, 2**) and non/4-methoy-2-phenylacetyl chloride (**3, 4**) or 2-(thiophen-2-yl)acetyl chloride (**5**) under reflux, target compounds N-(benzo[d]oxazol or benzo[d]thiazol-2-ylmethyl)-2-phenylacetamide and N-(benzo[d]thiazol or benzo[d]thiazol -2-ylmethyl)-2-(thiophen-2-yl)acetamide (**6-11**) were obtained. Table 1 provides the chemical structures of all compounds.



Scheme 1. General synthetic procedure for compounds **6-11**

Table 1. Structure of all synthesized compounds.

Comp. No	Structure	Comp. No	Structure
6		9	
7		10	
8		11	

N-(benzo[d]oxazol-2-ylmethyl)-2-phenylacetamide (6)
White solid, m.p. 215-216 ºC, R_f, 0.53 in chloroform/methanol (9.5:0.5), Yield; 54%.
^{1}H NMR (ppm): δ= 7.49–7.47 (dd, J = 7.0, 2.7 Hz, 2H, aromatic-H), 7.25–7.22 (dd, J = 7.0, 2.7 Hz, 1H, aromatic-H), 7.21 (m, 1H, aromatic-H), 7.20–7.16 (m, 1H, aromatic-H), 7.14–7.12 (m, 1H, aromatic-H), 5.29 (s, 2H, aliphatic-CH_2N-), 3.56 (d, J = 2.1 Hz, 2H, aliphatic-CH_2CO-).
^{13}C NMR (ppm): δ= 172.2, 148.5, 138.2, 133.2, 129.3, 128.7, 127.4, 123.1, 115.5, 60.3, 40.8.
FT-IR (cm^{-1}): 3028, 2969, 2946, 1739, 1444, 1356, 1216.
N-(benzo[d]thiazol-2-ylmethyl)-2-phenylacetamide (**7**)
White solid, m.p. 192 ºC, R_f, 0.55 in chloroform/methanol (9.5:0.5), Yield; 50%.
^{1}H NMR (ppm): *δ*= 7.85 – 7.81 (m, 2H, aromatic -H), 7.42- 7. 40 (ddd, *J* = 8.2, 7.3, 1.3 Hz, 1H, aromatic -H), 7.38 – 7.29 (m, 1H, aromatic -H), 7.23 – 7.17 (m, 4H, aromatic -H), 7.14 – 7.10 (m, 1H, aromatic -H), 4.69 (s, 2H, aliphatic -CH_2-N), 3.85ppm (s, 2H, aliphatic -CH_2CO-).
^{13}C NMR (ppm) *δ*= 174.6, 172.2, 172.1, 154.0, 136.6, 136.2, 130.3, 129.6, 127.9, 127.4, 126.4, 123.4, 123.0, 43.75, 42.4.
FT-IR (cm^{-1}) 3253, 3015, 2969, 2945, 1738, 1365, 1228.
N-(benzo[d]oxazol-2-ylmethyl)-2-(4-methoxyphenyl)acetamide (8)
White yellow solid, m.p. 220 ºC, R_f, 0.50 in chloroform/methanol (9.5:0.5), Yield; 65%.
^{1}H NMR (ppm) *δ*= 7.51 – 7.47 (m, 2H, aromatic -H), 7.21 – 7.17 (m, 2H, aromatic -H), 7.08 – 7.05 (m, 2H, aromatic -H), 6.77 – 6.75 (m, 2H, aromatic -H), 5.30 (s, 2H, aliphatic -CH_2N-), 3.70 (s, 3H, aliphatic -OCH_3), 3.53 ppm (s, 2H, aliphatic -CH_2CO-).
^{13}C NMR (ppm) *δ*= 172.7, 159.0, 148.4, 137.9, 137.9, 130.4, 130.3, 125.2, 123.2, 115.5, 115.4, 114.2, 60.2, 55.2, 40.03 ppm.
FT-IR (cm^{-1}) 3065, 2969, 1737, 1365, 1228, 1216.

N-(benzo[d]thiazol-2-ylmethyl)-2-(4-methoxyphenyl)acetamide (9)
White solid, m.p. 202 °C, R_f, 0.44 in chloroform/methanol (9.5:0.5), Yield; 40%.
^{1}H NMR ppm δ= 7.85-7.42 (ddd, J = 8.1, 1.2, 0.5 Hz, 2H, aromatic -H), 7.40- 7.38 (ddd, J = 8.1, 7.3, 1.2 Hz, 1H, aromatic -H), 7.33 – 7.29 (m, 1H, aromatic -H), 7.14 – 7.10 (m, 2H, aromatic -H), 6.76 – 6.73 (m, 2H, aromatic -H), 4.69 (s, 2H, aliphatic -CH_2N-), 3.84 (s, 2H, aliphatic -CH_2CO-) 3.64 ppm (s, 3H, aliphatic -OCH_3).
^{13}C NMR ppm δ= 175.0, 172.1, 160.2, 154.0, 136.2, 131.3, 128.5, 127.4, 126.4, 123.4, 123.0, 115.1, 55.7, 43.7, 42.4.
FT-IR (cm^{-1}) 3255, 3001, 2969, 2945, 1739, 1365, 1216.

N-(benzo[d]oxazol-2-ylmethyl)-2-(thiophen-2-yl)acetamide (10)
White yellow solid, m.p. 195 °C, R_f, 0.50 in chloroform/methanol (9.5:0.5), Yield; 30%.
^{1}H NMR (ppm) δ= 7.52- 7.50 (dd, J = 5.5, 2.9 Hz, 2H, aromatic -H), 7.22 – 7.16 (m, 2H, aromatic -H), 7.15 – 7.14 (dd, J = 5.5, 1.0 Hz, 1H, aromatic -H), 6.88 - 6.86 (dt, J = 5.5, 2.9 Hz, 1H, aromatic -H), 6.84 – 6.81 (m, 1H, aromatic -H), 5.33 (s, 2H, aliphatic -NCH_2-), 3.81ppm (d, J = 1.0 Hz, 2H, aliphatic-$COCH_2$-).
^{13}C NMR (ppm) δ= 171.3, 148.2, 134.0, 127.3, 127.0, 125.4, 123.2, 115.5,115.5, 115.2, 60.7, 35.0.
FT-IR (cm^{-1}) 3015, 2945, 1738, 1579, 1375, 1217, 1078.

N-(benzo[d]thiazol-2-ylmethyl)-2-(thiophen-2-yl)acetamide (11)
White solid, m.p. 240 °C, R_f, 0.52 in chloroform/methanol (9.5:0.5), Yield; 30%.
^{1}H NMR (ppm) δ= 7.82 - 7.81 (dd, J = 2.8, 1.0 Hz, 1H, aromatic -H), 7.80 – 7.78 (m, 1H, aromatic -H), 7.40 – 7.36 (m, 1H aromatic -H), 7.31 – 7.27 (m, 1H, aromatic -H), 7.19- 7.17 (dd, J = 5.1, 1.0 Hz, 1H, aromatic-H), 6.90- 6.87 (d, J = 3.4 Hz, 1H, aromatic -H), 6.87- 6.86 (dd, J = 5.1, 3.4 Hz, 1H, Ar-H), 4.67 (s, 2H, aliphatic -NCH_2-), 3.74 ppm (s, 2H, aliphatic -$COCH_2$-).
^{13}C NMR (ppm) δ= 173.2, 172.2, 154.0, 137.5, 136.2, 127.9, 127.9, 127.4, 126.4, 126.0, 123.4, 123.0, 42.7, 37.7.
FT-IR (cm^{-1}) 3291, 3015, 2969, 1739, 1655, 1433, 1253, 1229.

3.2. Biological Assay

3.2.1. In Vitro Antitubercular Activity Studies

The agar proportion method was used to screen all target compounds against MTB H37Rv [35, 43, 44]. Isoniazid (INH) and rifampicin (RIF) served as positive control drugs in the assay. Table 2 displays the in vitro antimycobacterial activity, represented by the MIC values, for both the target compounds and the standard drugs. The three benzothiazole derivatives (7, 9, 11) show significant activities and other compounds exhibit moderate activities. The results of the antimycobacterial activity tests are presented in Table 2.

Table 2. The Anti-TB activity results of all compounds

Compounds	*M. tuberculosis* H37Rv (μM)	cLogP
6	4.10	1.991
7	2.82	2.656
8	3.30	2.575
9	1.38	1.910
10	2.95	1.637
11	1.05	2.302
INH	0.12	-0.668
RIF	1.00	3.710

3.2.2 Cytotoxicity Assay

In this study, a selectivity index (SI) was also calculated to assess the cytotoxicity of the synthesized compounds (**6-11**) and determine their suitability as therapeutic candidates. The cytotoxicity of compounds **6-11** was evaluated on normal human cells using the MTT cytotoxicity assay performed on fibroblast L929 cells [31,32]. The results indicated that IC_{50} values for the selected compounds ranged from 196.23 to 487.34 μM, suggesting a low risk of toxicity with favorable selectivity indices (SI) ranging from 57.23 to 186.11 (Table 3).

Table 3. *In vitro* cytotoxic effect for compounds on fibroblast L929 cells with their selectivity indices.

Compound	IC_{50} ± SEM L929 cells (μM)	MIC Mtb H37Rv (μM)	SI[a]
6	234.65±3.25	4.10	57.23
7	225.62±2.87	2.82	80.00
8	389.99±2.74	3.30	118.18
9	196.23±4.12	1.38	142.20
10	487.34±5.58	2.95	165.20
11	195.42±2.38	1.05	186.11

[a]Selectivity index (SI) calculated by dividing L929 cells IC_{50} (mean ± standard error of the mean [SEM]) by MIC against Mtb H37Rv.

3.3. In silico ADMET prediction

In this study, various ADMET properties of the compounds were assessed using in silico methods. The fulfillment of physicochemical parameters is essential for compounds to qualify as potential drugs, as many compounds fail to reach their target sites due to inadequate physicochemical properties.

All compounds adhere to Lipinski's rule of five and Jorgensen's rule of 3%, indicating their potential to possess favorable pharmacokinetic properties critical for drug development. Lipinski's rule of five suggests that compounds with optimal membrane permeability and oral bioavailability typically exhibit no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, a molecular weight below 500 Da, and a LogP value less than 5. Similarly, Jorgensen's rule of 3% predicts good oral absorption based on additional parameters. The adherence to these rules suggests that the compounds are likely to be well-absorbed and distributed in the body, undergo manageable metabolism, and exhibit low toxicity.

The high human oral absorption (> 95%) of all compounds is indicative of their efficient oral absorption, a crucial factor in drug development. This high oral absorption suggests that the compounds can effectively reach systemic circulation, thereby potentially enhancing their therapeutic efficacy. Moreover, good oral absorption is often associated with improved patient compliance, as oral administration is typically more convenient compared to other routes.

The LogP values of all compounds fall within the recommended range (2.365–3.258), indicating suitable lipophilicity levels. Lipophilicity, reflecting a compound's ability to dissolve in fats, significantly influences its penetration of cell membranes, thereby affecting its pharmacokinetic behavior. Thus, compounds with LogP values within this range may exhibit favorable pharmacokinetic properties related to ADME, suggesting their potential effectiveness and safety as therapeutic agents.

Furthermore, the compounds do not block the predicted HERG K+ channels, exhibit high Caco-2 cell permeability, and have no estimated toxic effects. These findings collectively suggest that the compounds possess favorable drug-like properties.

The lack of blockage of the predicted HERG K+ channels implies a reduced risk of cardiac side effects, a common concern in drug development. Additionally, the compounds' high Caco-2 cell permeability indicates efficient gastrointestinal absorption, essential for oral drug administration. Moreover, the absence of estimated toxic effects implies a favorable safety profile, enhancing the compounds' potential as promising candidates for further development as therapeutic agents.

Table 4. Some predicted pharmacokinetic properties of compounds 6-11.

Property or Descriptor	6	7	8	9	10	11	Range or recommended values
MW	266,299	282,359	296,325	312,386	272,321	288,382	(130.0 - 725.0)
SASA	530,086	544,13	578,797	601,329	524,522	536,111	(300.0 - 1000.0)
PSA	61,451	49,617	69,912	58,937	61,662	50,831	(7.0 - 200.0)
donorHB	1	1	1	1	1	1	(0.0 – 6.0)
accptHB	4,5	4	5,25	4,75	4,5	4	(2.0 – 20.0)
QPlogPo/w	2,419	2,838	2,612	3,258	2,365	2,927	(-2.0 / 6.5)
QPlogHERG	-4,289	-4,417	-4,566	-4,689	-4,103	-4,106	(concern below -5)
QPPCaco	1373,382	1331,351	1672,682	1982,716	1200,483	1708,186	(<25 poor, >500 great)
QPPMDCK	1188,467	1539,599	1435,989	2945,398	1813,418	3516,471	(<25 poor, >500 great)
Lipinski Rule of 5 Violations	0	0	0	0	0	0	maximum is 4
Jorgensen Rule of 3 Violations	0	0	0	0	0	0	maximum is 3
% Human Oral Absorption	100	100	100	100	95,906	100	>80% is high <25% is poor
Mutagenic	none	none	none	none	none	none	none
Tumorogenic	none	none	none	none	none	none	none
Reproductive Effective	none	none	none	none	none	none	none
Irritant	none	none	none	none	none	none	none

MW: Molecular weight of the molecule. **SASA:** Total solvent accessible surface area in square angstroms using a probe with a 1.4 Å radius. **PSA:** Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms. **accptHB:** Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution. **donorHB:** Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution. **QPlogPo/w:** Predicted octanol/water partition coefficient. **QPlogHERG:** Predicted IC50 value for blockage of HERG K + channels. **QPPCaco:** Predicted apparent Caco-2 cell permeability in nm/sec. **QPPMDCK:** Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier.

4. Conclusion

The objective of the current study was to synthesize and examine the antitubercular activities of some new benzazole derivatives with the hope of determining new structures that could be used as potent antitubercular agents.

A novel series of benzazole derivatives (6-11) was synthesized and characterized using various spectroscopic methods. The target compounds (6-11) were assessed for their antitubercular activities against MTB H37Rv. All compounds demonstrated significant activity, with compound 11 exhibiting the most notable efficacy in the series, displaying a MIC value of 1.05 μM and a selectivity index (SI) of 186.11. This meticulous examination results in the identification of a notably consistent structure-activity relationship, suggesting that the presence of a methoxy group as a substituent enhances both the activity and selectivity of the compounds compared to those without substituents. Moreover, the benzazole structure substituted with a thiophene ring exhibits notably stronger antitubercular activity than the other compounds. Interestingly, the activity of the thiophene group against MTB H37Rv is notable.

To further investigate the effectiveness of linker groups, it is imperative to conduct studies on numerous compounds. These findings from the contribution of antitubercular activity underscore the necessity of comparing benzazole derivatives with linker groups consisting of at least four atoms to those with different atoms. Furthermore, the contributions of aromatic ring substituents to the activity require thorough evaluation.

Future research endeavors aim to enhance the antitubercular efficacy and elucidate the precise mechanism of action.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Gursoy S., Ozturk E. Ş. and Zoatier B. written manuscript; Gursoy S., Ozturk E. Ş., Ulger M. and O. Algul designed the study, prepared protocols, analyzed the data; Gursoy S., Ozturk E. S. and Ulger M. and Zoatier B.; performed the experiments and participated in discussions. All the authors were responsible for the data acquisition, review and editing of the paper.

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