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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DESIGN, SYNTHESIS, AND EVALUATION OF ANTIBACTERIAL POTENTIAL OF HYDRAZONE-TETHERED PYRAZOLE-THIAZOLE DERIVATIVES

HİDRAZON BAĞLI PİRAZOL-TİYAZOL TÜREVLERİNİN TASARIMI, SENTEZİ VE ANTİBAKTERİYEL POTANSİYELİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *This study includes the investigation of the antimicrobial potential of a series of compounds designed by hybridization of thiazole, hydrazone and pyrazole systems identified as antimicrobial moieties in the literature. The aim was to filter the designed compounds with drugability parameters, synthesize the selected compounds and test their antibacterial potential in silico and in vitro.*
Material and Method: *The drugability properties of synthesized compounds were determined by online scanners and the potential effects of selected compounds on E. coli and S. aureus strains were determined by disk diffusion method. Also, Autodock 4.2 software was used to determine the inhibitory potential of compounds against the dihydrofolate reductase (DHFR) enzyme.*
Result and Discussion: *In our study, among the newly designed hydrazone-linked pyrazole-thiazole compounds, the compounds determined according to their drugability parameters (17a-c) were synthesized with high efficiency. Among the compounds tested for antibacterial activity, Compound 17c formed a zone diameter of 8 mm against E. coli strain and 9 mm against S. aureus strain at a concentration of 80 µg/ml. Also, compound 17c formed a zone diameter of 7 mm against E. coli strain and 8 mm against S. aureus strain at a concentration of 40 µg/ml. Furthermore, the ADMET profiles of the presented compounds indicate that they may have suitable drugability parameters as potential antibacterial agents.*
Keywords: *Antibacterial, hybridization, in silico, synthesis*

ÖZ

Amaç: *Bu çalışma, literatürdeki antimikrobiyal kısım olarak belirlenen tiyazol, hidrazon ve pirazol sistemlerinin hibridizasyonu ile tasarlanan bir seri bileşiğin antimikrobiyal etki potansiyelinin araştırılmasını içermektedir. Tasarlanan bileşiklerin ilaçlanabilirlik parametreleri ile filtrasyonu, seçilen bileşiklerin sentezi ve antibakteriyel etki potansiyelinin in siliko ve in vitro test edilmesi hedeflenmiştir.*
Gereç ve Yöntem: *Sentezlenen bileşiklerin ilaçlanabilirlik özellikleri online tarayıcılar ile belirlenmiş ve en düşük toksisite profiline sahip bileşiklerin E. coli ve S. aureus şuşlarındaki*

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potansiyel etkisi disk difüzyon yöntemi ile belirlenmiştir. Ayrıca bileşiklerin dihidrofolat redüktaz (DHFR) enzimine karşı inhibisyon potansiyelini belirlemek için Autodock 4.2 yazılımı kullanılmıştır.
Sonuç ve Tartışma: *Çalışmamızda, yeni tasarlanan hidrazon bağlı pirazol-tiyazol bileşikleri arasında, ilaçlanabilirlik parametrelerine (17a-c) göre belirlenen bileşikler yüksek verimlilikle sentezlendi. Antibakteriyel aktivite için test edilen bileşikler arasında, Bileşik 17c, 80 µg/ml konsantrasyonunda E. coli suşuna karşı 8 mm ve S. aureus suşuna karşı 9 mm'lik bir zon çapı oluşturdu. Ayrıca, Bileşik 17c, 40 µg/ml konsantrasyonunda E. coli bakteri suşunda 7 mm'lik, S. aureus bakteri suşunda 8 mm'lik bir zon çapı oluşturdu. İlaveten, sunulan bileşiklerin ADMET profilleri, bunların potansiyel antibakteriyel ajanlar olarak uygun ilaçlanabilirlik parametrelerine sahip olabileceğini göstermektedir.*
Anahtar Kelimeler: *Antibakteriyel, hibridizasyon, in siliko, sentez*

INTRODUCTION

Nowadays, the increase in disease types and drug resistance limits the use of existing pharmaceuticals and thus reveals the need to develop new, fast, effective, and economical pharmaceuticals [1]. For this purpose, computer-aided drug design (CADD) provides a great advantage in terms of time and selectivity in discovering new drug candidates [2]. The support of structure-based drug design and ligand-based drug design strategies with *in silico* procedures and bioinformatics tools facilitates access to more effective, practical, economical, and reliable drug candidates [3,4]. In addition, it has been determined that the molecular hybridization technique, formed by combining the pharmacophoric regions of known drugs in the same molecule, is quite effective in developing new drug candidates with high selectivity and druggability potential [5].

Developing targeted drug candidates using drug design strategies is particularly important for creating new antimicrobial agents [6]. Because one of the major factors that threaten human health worldwide, especially in developing countries, is antibiotic resistance due to the abuse of antibiotics. If a solution cannot be found against this situation, it is estimated that 10 million people will lose their lives annually due to drug-resistant infections by 2050 [7]. This difficulty can be overcome by discovering new, selective, and reliable agents.

In recent drug candidate development studies, the thiazole ring is used as an important building block due to its strong and electron-rich structure [8]. It has been determined that various compounds containing the thiazole nucleus have various pharmaceutical activities, including anti-inflammatory, anticancer, antiviral, antidiabetic, and anticonvulsant properties [9]. It is no coincidence that the first effective antibiotics used, penicillins, cephalosporins with thiazole units, and sulfathiazole structures from the sulfonamide group of antibiotics contain a thiazole moiety (Figure 1) [10].

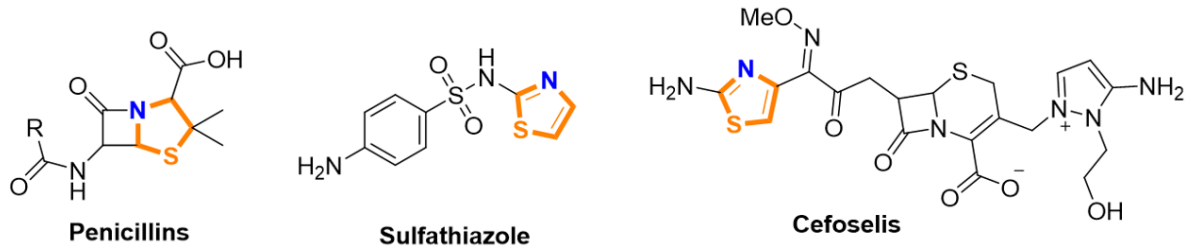

Figure 1. Antibacterial drugs containing thiazole moiety

Therefore, in this study we focused on agents containing thiazole groups, and interestingly, recent studies in the literature led us to the antimicrobial potential of thiazole-hydrazone derivatives. For example, a series of compounds (1) designed with a hydrazone bridge using molecular hybridization techniques of quinoline and thiazole groups have been reported to have antimicrobial activity via dihydrofolate reductase (DHFR) inhibition [11]. In another study, 2-pyridyl thiazole hydrazone derivatives (2) were determined to have highly effective antiprotozoal activity against *Trypanosoma cruzi* [12]. Similarly, coumarin-thiazole hydrazone derivatives (3) were determined to have

antimicrobial activity against *Mycobacterium tuberculosis* and *Candida albicans* species [13]. Additionally, several thiazole-hydrazole derivatives (4) were studied on clinically important fungi, and the compounds were found to have significant antifungal activity, particularly on *Candida* and *Cryptococcus* species and *Paracoccidioides brasiliensis* species [14]. Finally, 1,2,3-triazole-thiazole hydrazone hybrid derivatives (5) were reported as promising candidates as antibacterial, anti-candida, and anti-biofilm agents (Figure 2) [15].

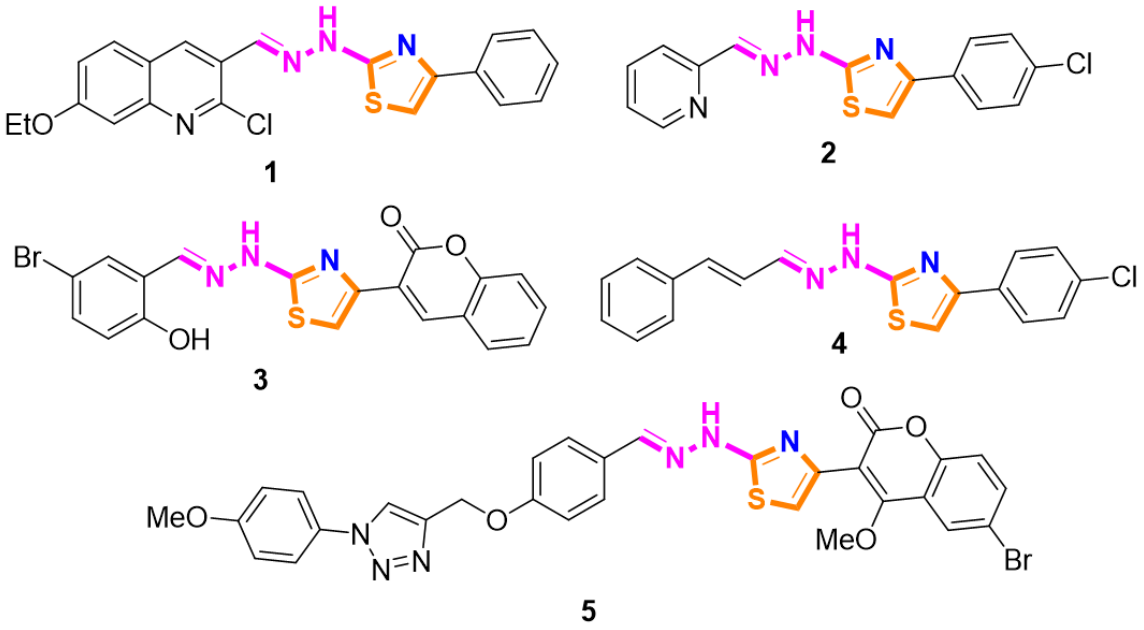

Figure 2. Thiazolyl hydrazone hybrid structures for antimicrobial effect

In addition to the unique antimicrobial effects exhibited by thiazole-hydrazone derivatives, pyrazole-hydrazone derivatives have also been reported to possess various antibacterial effects. For example, compound 6 was determined to be bactericidal for *S. aureus* and bacteriostatic for *A. baumannii* strains and was found to have low toxicity in *in vitro* and *in vivo* studies [16]. In a study in which aminoguanidine derivative 1,3-diphenyl pyrazole hydrazone derivatives were designed, compound 7 was found to be a potent antimicrobial agent against various bacterial strains with MIC values in the range of 1-8 μg/ml [17]. A more exciting group of studies in the literature involves the design of thiazole-pyrazole-hydrazone derivatives and their investigation of antimicrobial effects. For example, among the pyrazole-thiazolinone hydrazone derivatives developed for the potential treatment of drug-resistant bacterial infections, compound (8) was found to be effective against the broad-spectrum antibiotic-resistant pathogen *N. Gonorrhoeae* [18]. In another study, developed thiazole-pyrazole-hydrazone derivatives [9] were found to be effective against *S. aureus* and *Klebsiella planticola* strains. In addition, coumarin-linked pyrazole derivative thiazole hydrazones (10) were reported as broad-spectrum antibacterial agents with moderate activity against the tested strains [19]. Trisubstituted pyrazoles containing coumarin-thiazole moiety (11) were reported as moderate growth inhibitors of bacterial strains (Figure 3). Compound 11 showed the most potent activity in the series, with MIC values as low as 15.5 μg/ml [20].

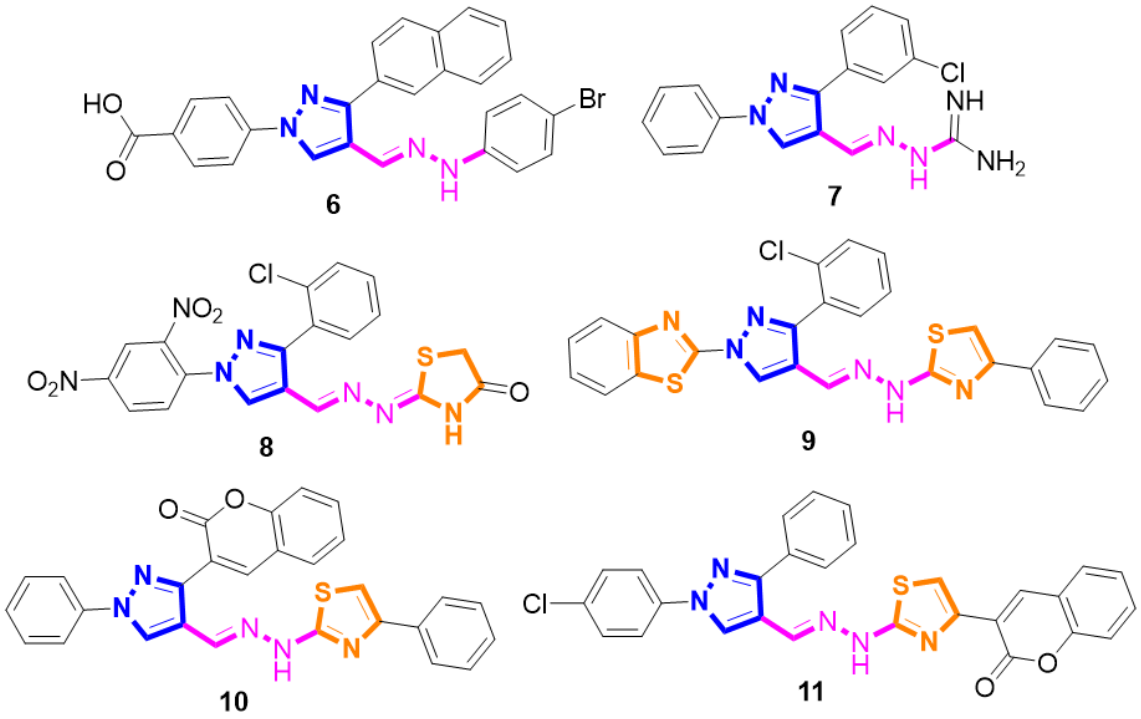

Figure 3. Thiazolyl/pyrazole-hydrazone hybrid structures for antimicrobial effect

In this study, the strong antimicrobial potential of derivatives formed by hybridization of thiazole, hydrazone, and pyrazole groups in the literature has led to the idea of molecular design in the development of new antimicrobial agents. Accordingly, a molecular design was created in which alkyl and phenyl derivatives were connected with the thiazole group, phenyl-substituted pyrazole group containing various electron-withdrawing and donating groups and finally, pyrazole and thiazole groups were connected with the hydrazone linker (Figure 4).

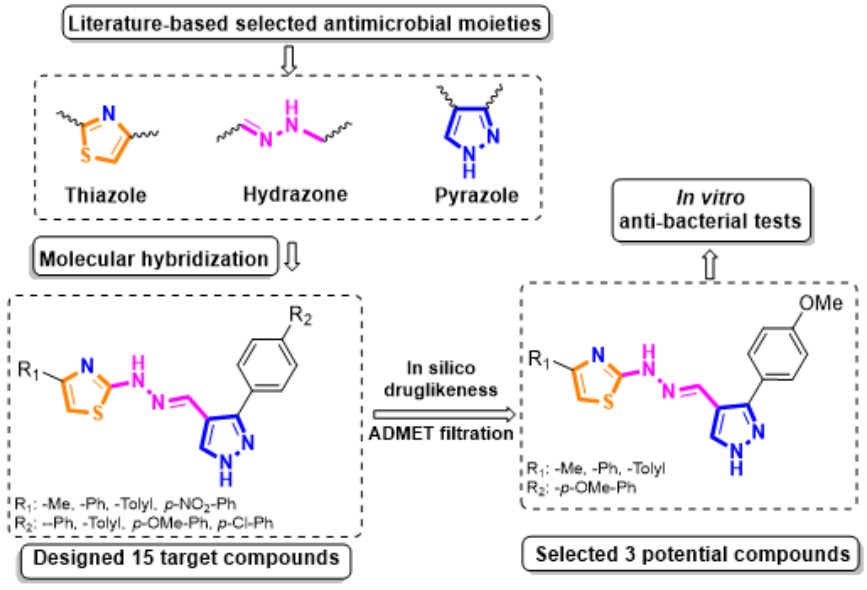

Figure 4. Rational design, structures of compounds designed from thiazole, hydrazone and pyrazole as antimicrobial moieties

As a result of various combinations with the mentioned strategy, 15 target compounds were designed (Figure 4). However, the main reasons for the failure of the designed drug candidate compounds in the clinical stages are that they do not have acceptable drug similarity, pharmacokinetic properties, and toxicity parameters. Therefore, the drug-similarity of the designed compounds and the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the candidate drugs were evaluated *in silico*. After this filtering process, the 3 candidate compounds with the lowest toxicity profiles were determined and the synthesis, characterization, and *in vitro* antibacterial effect potentials of the selected 3 compounds were tested by the disk diffusion method. In addition, the potential inhibitory effect of the compounds on dihydrofolate reductase (DHFR), one of the antimicrobial effect pathways, was investigated by molecular docking method.

MATERIAL AND METHOD

In the design of the compounds, various methyl, phenyl and electron donor (e.g. -Me), electron-withdrawing (e.g. -NO_2) phenyl groups were used for R1 groups; electron donor (e.g. -Me, -OMe), halogen (-Cl) or electron-withdrawing (e.g. -NO_2) substituted phenyl groups were used for R2 groups for antimicrobial activity. Then, drug similarity and theoretical pharmacokinetic properties of the designed compounds were investigated.

In Silico Study

The toxicity prediction of the compounds was determined using ProTox-3.0 [21]. ProTox-3.0 utilizes molecular similarity, fragment propensities, frequent features, and a machine-learning approach (fragment similarity-based CLUSTER cross-validation) across 61 models to predict various toxicity endpoints. These endpoints include acute toxicity, organ toxicity, toxicological effects, molecular initiating events, metabolism, adverse outcome pathways (Tox21), and toxicity targets. Toxic doses are typically expressed as LD_{50} mg/kg body weight values. The LD_{50} represents the median lethal dose, meaning the dose at which 50% of test subjects die upon exposure to a compound. Toxicity classes are defined according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) with LD_{50} values as follows:

- **Class I:** Fatal if swallowed ($LD_{50} \leq 5$ mg/kg)
- **Class II:** Fatal if swallowed ($5 < LD_{50} \leq 50$ mg/kg)
- **Class III:** Toxic if swallowed ($50 < LD_{50} \leq 300$ mg/kg)
- **Class IV:** Harmful if swallowed ($300 < LD_{50} \leq 2000$ mg/kg)
- **Class V:** May be harmful if swallowed ($2000 < LD_{50} \leq 5000$ mg/kg)
- **Class VI:** Non-toxic ($LD_{50} > 5000$ mg/kg)

Synthesis of the Target Compounds

General Information

^{1}H and ^{13}C NMR spectra were recorded on a Varian-Agilent Inova instrument (400 and 100 MHz, respectively) using Me_4Si (TMS) as the internal standard. Melting points were determined on a Stuart Melting Point (SMP30) analyzer using open glass capillaries. Column chromatography was performed on silica gel (60 mesh, Silycycle). Commercially available materials were used without further purification. The analyses of the C, H, and N elements of the compounds were made with the LECO 932 CHNS (St. Joseph, MI, USA) elemental analysis device. Analysis results have a maximum deviation of ±0.4 from the calculated theoretical values.

Synthesis of Semicarbazone Derivative

Initially, 5 mmol p-methoxys-acetophenone (12) was dissolved/suspended in ethanol (10 ml) and magnetically stirred with 6 mmol semicarbazide (13) and 7.5 mmol NaOAc. The reaction mixture was refluxed at 70°C for 4 hrs., and the completion of the reaction was checked by TLC. Afterward, the experiment was terminated and cooled to room temperature, and the formed particles were filtered out. Solid particles washed several times with distilled water were allowed to dry at room temperature. The obtained semicarbazone derivative (14) were used for the next step without further purification [22].

Synthesis of Pyrazole-Carboxaldehyde Derivative

A chilled solution of 3 mmol *N*,*N*-dimethyl formamide (DMF), and 3 mmol $POCl_3$ was added dropwise on each other and stirred for 15 min at 0°C. A solution of 1 mmol semicarbazone derivative (14) in DMF (3ml) was added dropwise to the reaction mixture and heated at 80°C for 3h. The reaction mixture was cooled to room temperature, transferred to 20 ml of an ice-water mixture, and stirred for 30 min. Then, 10% aqueous sodium hydroxide (NaOH) solution was added dropwise to the reaction mixture until pH 8. The resulting precipitate was filtered, washed with water (15ml), and dried in the open air. The crude product (15) was purified by column chromatography on silica gel eluting with n-hexane/EtOAc (5/1) [23].

Synthesis of Thiosemicarbazone Derivative

5 mmol pyrazole-carboxaldehyde derivative derivative (15) was dissolved in ethanol (10 ml) and magnetically stirred with 6 mmol thiosemicarbazide. The reaction mixture was refluxed at 70°C for 8-16 h., and the completion of the reaction was checked by TLC. Afterward, the experiment was terminated and cooled to room temperature, and the formed particles were filtered out. Solid particles washed several times with distilled water were allowed to dry at room temperature. The furnished thiosemicarbazone derivative (16) was used for the next step without further purification [24].

Synthesis of Target Compounds

4 mmol thiosemicarbazone derivative (16) was dissolved in 10 ml of ethanol by heating. 5 mmol of chloroacetone or alpha bromo acetophenone derivatives were added to the reaction medium and reflux was performed for 1 hour. As the reaction progressed, solid particles began to form, and the reaction was terminated. The reaction mixture, which was cooled to room temperature, was transferred to 50 ml of ice-water mixture and magnetically stirred for 30 min. The solid particles formed were separated by filtration, washed in 15 ml of an ethanol-water mixture (1:1), and dried in an oven at 40°C [25]. Title compounds (17a-c) were purified by chromatography on silica gel eluting with hexane/EtOAc (1:1). The structure of newly synthesized compounds was elucidated based on elemental analysis and NMR spectral data. The physical properties and spectral data of the compounds are presented below.

(E)-2-(2-((3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)-4-methyl thiazole (17a)

Gray powder, M.p. 206-208°C, Yield: 85%, Rf: 0.72 (ethyl acetate/n-hexane (1/1)). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.36 (s, 1H, -CH=N), 8.03 (bs, 1H, Ar-H), 7.57-7.55 (m, AA'BB' system, 2H, Ar-H), 7.07-7.04 (m, AA'BB' system, 2H, Ar-H), 6.55 (d, *J*=0.9 Hz, 1H, Ar-H), 3.80 (s, 3H, -OCH_3), 2.20 (quasi q, *J*= 0.9 Hz, 3H, -CH_3). ^{13}C NMR (100 MHz, d_6-DMSO) δ 167.8, 160.3, 141.9, 141.3, 130.5, 130.2, 123.1, 115.0, 113.4, 130.6, 56.0, 15.5. FT-IR (ATR cm^{-1}): 3116, 2917, 2837, 1615, 1559, 1423, 1286, 1088, 945, 814, 716, 659. Anal. calcd. for $C_{15}H_{15}N_5OS$: C: 57.49; H: 4.82; N: 22.35; Found: C: 57.46; H: 4.85; N: 22.36.

(E)-2-(2-((3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)-4-phenyl thiazole (17b) [25]

Light orange powder, M.p. 234-235°C, Yield: 91%. Rf: 0.63 (ethyl acetate/n-hexane (1/1)). ^{1}H NMR (400 MHz, d_6-DMSO) δ 13.26 (bs, 1H, -NH), 11.77 (bs, 1H, -NH), 8.09 (s, 1H, -CH=N), 7.85-7.83 (m, AA'BB' system, 2H, Ar-H), 7.64-7.53 (m, 2H, Ar-H), 7.46-7.33 (m, 3H, Ar-H), 7.31-7.27 (m, 2H, Ar-H), 7.15-7.04 (m, 2H, Ar-H), 3.83 (s, 3H, -OCH_3). ^{13}C NMR (100 MHz, d_6-DMSO) δ 168.6, 160.0, 150.9, 135.3, 130.0, 129.0, 127.9, 126.0, 114.8, 114.7, 113.9, 103.6, 55.8. FT-IR (ATR cm^{-1}): 3085, 2923, 2824, 1702, 1565, 1509, 1435, 1339, 1237, 1168, 1106, 1044, 914, 827, 752, 690. Anal. calcd. for $C_{20}H_{17}N_5OS$: C: 63.98; H: 4.56; N: 18.65; Found: C: 64.01; H: 4.55; N: 18.68.

(E)-2-(2-((3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)-4-(*p*-tolyl) thiazole (17c)

Light brown powder, M.p. 229-230°C, Yield: 93%. Rf: 0.66 (ethyl acetate/n-hexane (1/1)). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.16 (s, -CH=N), 7.97 (s, 1H, Ar-H), 7.72-7.66 (m, AA'BB' system, 2H, Ar-H), 7.60-7.55 (m, AA'BB' system, 2H, Ar-H), 7.23-7.17 (m, 3H, Ar-H), 7.09-7.04 (m, AA'BB'

system, 2H, Ar-H), 3.81 (s, 3H, -OCH_3), 2.30 (s 3H, -CH_3). ^{13}C NMR (100 MHz, d_6-DMSO) δ 168.8, 160.2, 149.1, 145.0, 138.1, 137.9, 135.4, 131.5, 130.2, 129.9, 126.3, 123.3, 114.9, 113.9, 103.2, 56.0, 21.5. FT-IR (ATR cm^{-1}): 3153, 2936, 2849, 1664, 1584, 1503, 1423, 1249, 1168, 1100, 1020, 938, 814, 728, 685. Anal. calcd. for $C_{20}H_{17}N_5OS$: C: 64.46; H: 4.92; N: 17.98; Found: C: 64.50; H: 4.89; N: 17.99.

Antimicrobial Tests

The inhibition zone diameter, an indicator of the reaction that prevents the growth of bacteria by the synthesized compounds (17a-c), was measured by the disk diffusion method [26]. Bacterial concentrations adjusted according to 10^8 CFU/ml 0.5 McFarland were used in this study. *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive) bacterial strains were used in the research. 3 antibiotics [Gentamicin (10 µg), Amoxicillin (25 µg), Tetracycline (30 µg)] and 6 mm blank disks (BIOANALYSE Blank Disc in Cartridge ASD10011) were used for test strains. The compound concentrations were adjusted to 80 µg/ml and 40 µg/ml. After the bacteria were cultured in the media by the spreading technique, the extract-impregnated disks were placed in petri dishes. It was incubated for 16-24 hours at 37°C. At the end of incubation, the zones formed around the discs were measured in mm.

Molecular Docking Studies

Molecular docking studies were conducted using AutoDock 4.2 software to determine the interactions of selected and control compounds with the crystal structure of DHFR enzyme (PDB ID: 1DLS) obtained from RCSB Protein Data Bank (www.rcsb.org). Ligand compounds were drawn in 3D using Gaussview 5.0 and optimized using DFT method with the help of Gaussian 03 package based on theoretical level of B3LYP method and 6–31G basis set. The position and location of natural ligand methotrexate were taken as reference for target binding sites in crystal structure. In docking studies, x: 31.815; y: 19.694; z: -1.861 coordinate centers were determined. After re-docking, cluster RMSD value (Root Mean Square Deviation: 0-2 A° range) was determined to be within acceptable range for both targets in 10 different conformations of methotrexate. Then, a grid box with 50×50×50 points centered on the predicted positions and a grid point spacing of 0.375 Å was created for docking studies. The docking operations started with the removal of unwanted solvents, water, and ligands from the protein structures. Then, polar hydrogen atoms, Gasteiger partial charges, and Kollman charges were added to the targets. Rotatable bonds of the compounds were also checked. Target sites of the selected compounds similar to known compounds were docked under validated conditions using Autodock 4.2 software. Lamarckian genetic algorithm (10 runs) approach was applied and the population size was set to 300 for each simulation. The conformation with the highest receptor affinity was selected to determine the binding energy and the ligand-receptor complex of the corresponding conformation was formed.

RESULT AND DISCUSSION

Identification of Candidate Compounds

Potential toxicity of 15 compounds was assessed based on their LD_{50} values. Three of the 15 compounds (17a, 17b, and 17c) had higher LD_{50} values (Table 1) compared to the other 12 compounds (Table S1). In other words, these three compounds must be used in higher doses to cause toxicity in healthy animals compared to the other 12 compounds. Therefore, these three compounds were identified as candidates for further studies.

Table 1. Predictive acute toxicities of candidate compounds

Compounds	SMILES	LD_{50} (mg/kg) (ProTox-3.0)
17a	COC1=CC=C(C=C1)C1=NNC=C1\C=N\NC1=NC(C)=CS1	1000
17b	COC1=CC=C(C=C1)C1=NNC=C1\C=N\NC1=NC(=CS1)C1=CC=CC=C1	1000
17c	COC1=CC=C(C=C1)C1=NNC=C1\C=N\NC1=NC(=CS1)C1=CC=C(C)C=C1	1000

All three compounds have an LD_{50} of 1000 mg/kg, which means they fall under 'Harmful if swallowed ($300 < LD_{50} \leq 2000$ mg/kg)'. The other 13 compounds fall under Class III

Synthesis of the Target Compounds

The synthetic approach adopted to obtain the target compounds is shown in Figure 4. This method consists of four steps; initially, the conversion of *p*-methoxy-substituted acetophenone (12) with semicarbazide (13) to semicarbazone derivative (14) and their next step reactions with Vilsmeier-Haack reagents ($POCl_3$, DMF) to pyrazole-carboxaldehyde derivative (15) were prepared. Then, by reaction of the carbaldehyde group with thiosemicarbazone (16) and then with chloroacetone or phenacyl derivatives, the targeted thiazolyl/pyrazole-hydrazone hybrid derivatives (17a-c) were furnished in high yields (Figure 5).

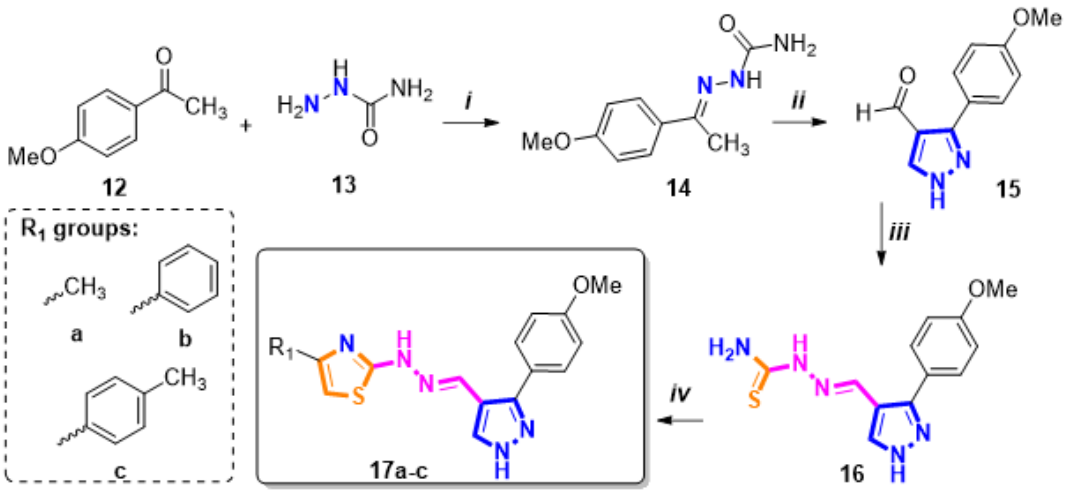

Figure 5. Total synthesis scheme of target compounds, *i*: NaOAc, EtOH, Reflux, 4 h. *ii*: DMF, $POCl_3$, 80°C, 3 h. *iii*: thiosemicarbazide, EtOH, Reflux, 2h. *iv:* Chloroacetone or phenacyl/*p*-methyl-phenacyl bromide, Reflux, 2h

The characterization of the synthesized compounds was completed by ^{1}H-NMR, ^{13}C-NMR, elemental analysis, and FT-IR. Particularly, in the ^{1}H-NMR spectrum, only the -NH group attached to pyrazole and hydrazone -NH groups of molecule 17b showed resonance as broad singlets at 13.26 ppm and 11.77 ppm, respectively. The proton attached to the hydrazone carbon in compounds 17a, 17b, and 17c appeared as singlets at 8.36 ppm, 8.09 ppm and 8.16 ppm, respectively. The -CH protons of the pyrazole ring were observed between 8.03-7.97 and the -CH protons of the thiazole ring were observed among other aromatics except 17a. The -CH_3 group attached to thiazole in compound 17a was found to interact with thiazole -CH (J=0.9 Hz). The aromatic protons of the p-methoxy phenyl group in all structures resonated as the AA'BB' system and all aliphatic proton peaks were consistent with the structures. The carbon number and resonance region in the ^{13}C-NMR spectrum of the compounds matched the structures characteristically.

Antimicrobial Tests

In this study, it was observed that among the tested compounds, 17b did not show any antibacterial activity against both *E. coli* and *S. aureus* bacterial strains. Compound 17a at 80 µg/ml concentration induced a 7 mm and 8 mm zone diameter against *E. coli* and *S. aureus* strains respectively while at 40 mg/ml concentration, it induced a 7 mm zone diameter only against *S. aureus* strain. Compound 17c at 80 µg/ml concentration induced a 8 mm zone diameter against *E. coli* strain and a 9 mm zone diameter against *S. aureus* strain. At 40 µg/ml concentration of compound 17c, 7 mm and zone diameters were observed for *E. coli* and *S. aureus* strains, respectively. In the antibiotic disks used in the study, Gentamicin 17 mm, Amoxicillin 20 mm, and Tetracycline 22 mm zone diameters were determined for *E. coli* strain, while Gentamicin 21 mm, Amoxicillin 15 mm and Tetracycline 24 mm zone diameters

were determined for *S. aureus* strain. It was determined that the highest antibacterial effect was at 80 µg/ml of compound 17c in *S. aureus* strain (Table 2).

The results of the study show that the antibacterial activity of the compounds depends on their concentration. In particular, compound 17c is seen to have a stronger antibacterial effect at higher concentrations. In addition, it was found that the antibacterial activity of the compounds was lower compared to antibiotics, but they could show significant effects at certain concentrations. This result indicates that the compounds can be used as potential antibacterial agents, but their activities need to be optimized. In further studies, the effectiveness of the compounds against different bacterial strains and their mechanisms of action need to be investigated in more detail.

Table 2. Antibacterial activity results of compounds tested against *E. coli* and *S. aureus* strains

Entry	*Escherichia coli*		*Staphylococcus aureus*	
	80 µg/ml	**40 µg/ml**	**80 µg/ml**	**40 µg/ml**
17a	7 mm	-	8 mm	7 mm
17b	-	-	-	-
17c	8 mm	7 mm	9 mm	8 mm
	Escherichia coli		***Staphylococcus aureus***	
Gentamicin (CN)	17		21	
Amoxicillin (AX)	20		15	
Tetracycline (TE)	22		24	

-: None, Gentamicin: 10 µg, Amoxicillin: 25 µg, Tetracycline:30 µg

Molecular Docking Studies

Target-oriented designs in the development of new antimicrobial agents allow the development of more rational drug candidates. Our literature review found that the inhibitory potential of thiazolyl hydrazone derivatives against DHFR enzyme, one of the antimicrobial targets, was as effective as methotrexate control. Based on this, the DHFR inhibition potential of the compounds developed in this study was compared with methotrexate control by molecular docking method and the results are presented in Table 3.

Table 3. Docking scores of compounds (17a-c) and methotrexate against DHFR enzyme

Compounds	DHFR (PDB ID: 1DLS)	
	Binding affinity (kcal/mol)	**Interacted amino acid residues**
Methotrexate	-11.02	Ile7, Val8, Ala9, Glu30, Phe31, Ser59, Ile60, Asn64, Arg70, Val115
17a	-7.06	Ala9, Phe31, Ser59, Ile60, Asn64, Leu67, Val115
17b	-8.68	Ala9, Trp24, Leu27, Phe31, Phe34, Ile60, Leu67, Val115
17c	-9.47	Tyr22, Phe31, Pro61, Leu67, Pro66, Lys68, Arg70

According to the molecular docking results, the compounds were able to form complexes with the DHFR enzyme by establishing Methotrexate-like interactions. In addition, although the binding affinity of the compounds is lower than Methotrexate, it can be said that they have a very high binding affinity with DHFR binding energies between -7.06 and -9.47 kcal/mol. In addition, it is seen in the supp info file that the compounds are positioned in the DHFR active site similar to Methotrexate. All these data can be interpreted as compounds with antimicrobial potential may act through inhibition of the DHFR enzyme.

As a result, a series of hybrid compound derivatives were designed in which thiazole and pyrazole systems were connected with hydrazone. Among the designed compounds, compounds 17a-c were determined to have more suitable drugability parameters and were synthesized. The antibacterial effect potential of the synthesized compounds on E. coli and S. aureus strains was tested and especially

compound 17c was found to be a potential antibacterial agent. In addition, molecular docking studies showed that the synthesized compounds (especially compound 17c) have the potential to inhibit the DHFR enzyme, which is an important antibacterial pathway, and therefore the antibacterial effect may be due to the inhibition of this enzyme. In addition, this study will contribute to the development of effective new antibacterial agents by optimizing the structural properties of the compounds and investigating their activities and mechanisms of action against different bacterial strains in more detail.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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