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RESEARCH ARTICLE

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Synthesis, Antimicrobial Evaluation, and Molecular Modeling Studies of New Thiosemicarbazide-Triazole Hybrid Derivatives of (*S*)-Naproxen

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The discovery of new antimicrobial molecules is crucial for combating drug-resistant bacterial and fungal infections that pose a dangerous threat to human health. In the current research, we applied a molecular hybridization approach to synthesize original thiosemicarbazide-triazole derivatives starting from (*S*)-naproxen (**7a**–**7k**). After structural characterization using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and HR-MS, the obtained compounds were screened for their antimicrobial activities against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231 and their isolates, as well. Although all compounds were found to be moderate antimicrobial agents, in general, their antibacterial activities were better than antifungal effects. Among the tested compounds, **7j** carrying nitrophenyl group on the thiosemicarbazide functionality represented the best MIC value against *S. aureus* isolate. Finally, molecular docking studies were performed in the active pocket of *S. aureus* flavohemoglobin to rationalize the obtained biological data.

Keywords: antibacterial activity, antifungal activity, biological activity, drug design, thioether.

Introduction

Throughout history, infectious diseases caused by bacteria and fungi have constituted a danger to public health, resulting in important morbidity and mortality worldwide.[1] In spite of continuing efforts to discover and commercialize novel antimicrobial agents, there has been no decline in the prevalence or spread of infectious diseases. The primary impediment to obtaining complete control of infectious diseases is the emergence of drug-resistant microbial strains.[2,3] This circumstance highlights the critical need for the development of novel antimicrobial molecules with divergent chemical structures and mechanisms of action.

Naproxen, (*S*)-2-(6-methoxynaphthalen-2-yl)propionic acid (*Figure 1*), is one of the most frequently utilized nonsteroidal anti-inflammatory drugs providing an effective cure in inflammatory disorders and symptoms, such as pain and fever.[4] Besides these biological activities, naproxen and its derivatives have attracted great interest in recent years due to their anticancer and antimicrobial effects.[5–7]

Thiosemicarbazide (NH_2–NH–(C=S)–NH_2) is the sulfur-counterpart of semicarbazide functionality and provides chemical versatility and varying biological

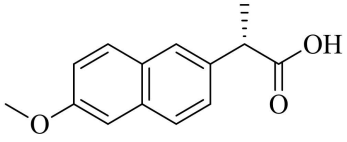

Figure 1. Chemical structure of (*S*)-naproxen.

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202100900

properties than semicarbazide due to the presence of the sulfur atom.[8] In addition to its wide range of pharmacological activities such as anticancer, antioxidant, and anti-inflammatory,[9–11] thiosemicarbazide is also a strategic functionality frequently used in the development of antimicrobial agents (*Figure 2*).[12–14]

Azoles are heterocycles with five members containing at least one nitrogen. Although they act as valuable pharmacophores for a wide range of biological activities, particularly imidazole and triazole-based compounds attract great interest due to their antifungal properties.[15] Azole antifungals, exemplified by fluconazole, voriconazole, and ketoconazole, show their mechanism by inhibiting the lanosterol 14α demethylase (CYP51) enzyme in the biosynthesis of ergosterol that is the crucial component of the fungal cell membrane.[16] In the catalytic site of CYP51, they compete with lanosterol and coordinate the iron atom of the cofactor heme via free electron pair of the nitrogen atom of the azole rings to demonstrate their antifungal activities (*Figure 3*).[17]

The popularity of azoles in antimicrobial chemotherapy is not limited to their antifungal effects. Recently, they have also attracted great interest due to the discovery of their antibacterial properties. Topically administered azole antifungals, such as ketoconazole, econazole, and miconazole, were reported to be effective particularly against Gram-positive bacteria.[19]

Molecular hybridization is a widely used strategy for drug design and development that involves the combination of two or more pharmacophores directly or with a spacer to create a unique single molecule. This method is primarily concerned with optimizing the biological profile while mitigating adverse effects.[20,21]

In light of these considerations, we purposed to apply a molecular hybridization approach to synthesize thiosemicarbazide-triazole hybrids with thioether

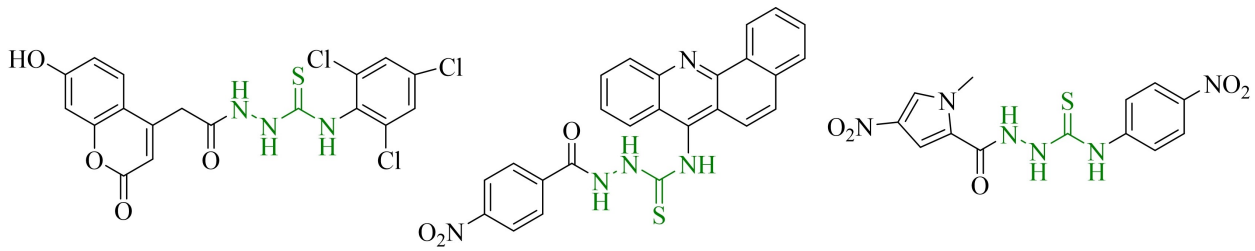

Figure 2. Chemical structures of representative antimicrobial molecules with thiosemicarbazide scaffold.

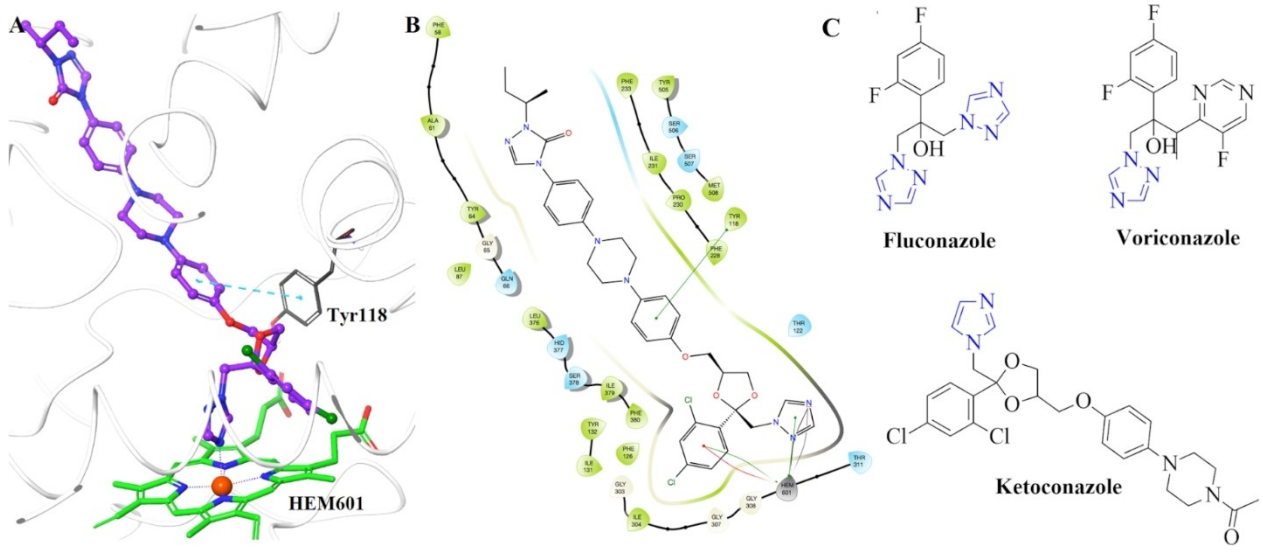

Figure 3. The binding mechanism of itraconazole in the active pocket of CYP51 from *Candida albicans* (PDB code: 5V5Z)[18] depicted in 3D (A) and 2D (B). Itraconazole (purple ball&sticks) coordinates the iron (orange sphere) of heme (green sticks) through one nitrogen atom of its triazole ring. π-π stacking with Tyr118 is represented as turquoise dashed lines. (C) Representative members of azole antifungals.

linkage (**7a**–**7k**) starting from naproxen and evaluate their antimicrobial activities (*Figure 4*). Furthermore, we carried out molecular modeling studies to provide insights into their potential antibacterial activity mechanism.

Results and Discussion

Chemistry

In this study, we synthesized eleven novel triazole and thiosemicarbazide derivatives linked by thioether functionality employing naproxen as the starting compound. The synthetic pathway for the preparation of **7a**–**7k** is outlined in *Figure 5*.

To obtain the target compounds, initially (*S*)-naproxen ethyl ester (**1**) was prepared by the reaction of naproxen and ethanol in the presence of a catalytic amount of concentrated sulfuric acid. Then, compound **1** was heated with hydrazine hydrate (80%) in an ethanolic medium to obtain (*S*)-naproxen hydrazide (**2**). Compound **2** was reacted with 4-fluoroisothiocyanate for the formation of thiosemicarbazide functionality in compound **3**. The ring closure reaction of **3** in

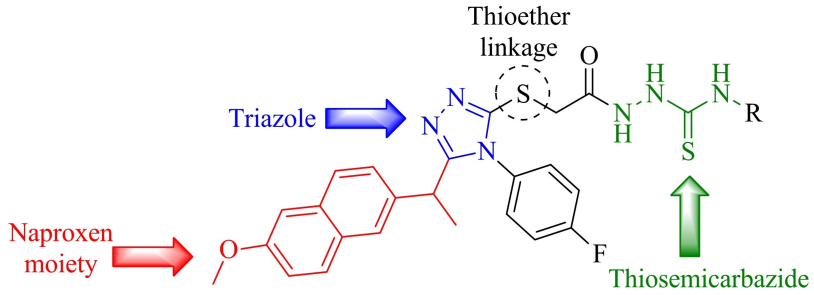

Figure 4. Design strategy of the title compounds (**7a**–**7k**).

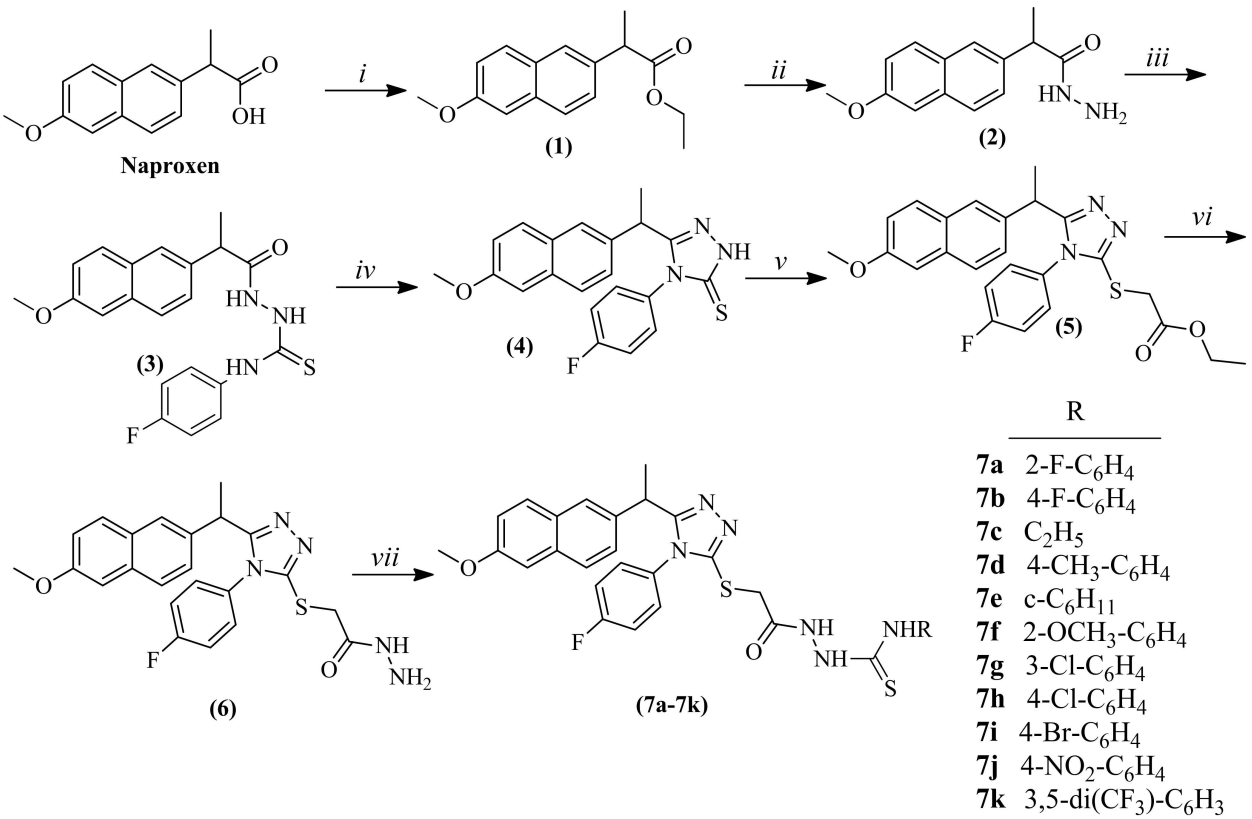

Figure 5. Synthesis of the compounds derived from naproxen. (*i*) $CH_3OH/d.H_2SO_4$, (*ii*) $NH_2NH_2.H_2O/C_2H_5OH$, (*iii*) 4-F-C_6H_4-NCS/butanol, (*iv*) 4 N NaOH, (*v*) $BrCH_2COOC_2H_5$, K_2CO_3, acetone, (*vi*) $NH_2NH_2{\cdot}H_2O$/EtOH, (*vii*) butanol/R-NCS.

basic medium yielded compound **4** carrying triazolethione moiety. The reaction of compound **4** with equimolar ethyl α-bromoacetate in the presence of potassium carbonate resulted in the formation of compound **5**. The hydrazide functionality in compound **6** was obtained by the reaction of compound **5** with hydrazine hydrate in ethanol.[22,23] Our final thiosemicarbazide derivatives, **7a**–**7k**, were synthesized by the reaction of compound **6** with various isothiocyanates. All obtained compounds were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and HR-MS spectroscopic data. The FT-IR data demonstrated that hydrazide C=O stretching bands varied from 1735 cm^{-1} to 1666 cm^{-1} confirming the formation of the amide functionality on thiosemicarbazides (**7a**–**7k**). N–H and C=S thiocarbonyl stretching bands were observed at 3232–3186 cm^{-1} and 1273–1265 cm^{-1}, respectively. The absorption bands at 1604 cm^{-1} were due to the presence of the C=N stretch of the triazole ring system. ^{1}H-NMR spectra data of N^{1}, N^{2}, and N^{3} protons (**7a**–**7k**) confirmed the formation of thiosemicarbazide functionality. The thiosemicarbazide protons were detected between 7.83–9.70, 9.31–10.70, and 10.20–11.63 ppm, respectively. Alkyl –S–CH_2-protons were seen at 3.80-4.02 ppm. The formation of thiosemicarbazides was also confirmed by ^{13}C-NMR studies. The C=O, C=S, and –CH_2- peaks of compounds **7a**–**7k** were detected between 182.96-175.68, 167.47-161.46, and 34.68-33.96 ppm, respectively. HRMS studies were performed on all thiosemicarbazide compounds and approved the molecular weights within <5 mmu.

Biological Evaluation

The antimicrobial activities of **7a**–**7k** were tested *in vitro* against Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli*), fungi (*Candida albicans*), and clinical isolates of these microorganisms. *Table 1* contains the minimum inhibitory concentrations (MICs) of the synthesized compounds, as well as ampicillin, gentamicin, vancomycin (antibacterial), and fluconazole (antifungal) as positive controls.

According to the results of the biological experiments, it was determined that all tested compounds had moderate antimicrobial activity in the range of 64–512 μg/mL. **7a**–**7k** represented better antibacterial effects than their antifungal activities. MIC value of the compounds on *E. coli* ATCC 25922 was found as 128 μg/mL. The studied compounds showed their antibacterial activities against *S. aureus* as well as its clinical isolate with the MIC value of 256 μg/mL, except for **7j**. This compound stepped forward as the most active molecule in this series with its MIC value of 64 μg/mL on *S. aureus* isolate. It is critical to note that compound **7j** carries nitro substituent in on the *para* position of its phenyl ring.

Molecular Modeling Studies

According to the obtained MIC values against bacteria and fungi, the title compounds were moderate antimicrobial agents. This situation prompted us to rationalize the biological activity results by molecular

Table 1. Minimum inhibitory concentrations (MIC, μg/mL) of the compounds against tested microorganisms.

Compound	*S. aureus*	*S. aureus**	*E. coli*	*E. coli**	*C. albicans*	*C. albicans**
	ATCC 29213		ATCC 25922		ATCC 10231	
7a	256	256	128	256	512	512
7b	256	256	128	256	512	512
7c	256	256	128	256	512	512
7d	256	256	128	256	512	512
7e	256	256	128	256	512	512
7f	256	256	128	256	512	512
7g	256	256	128	256	512	512
7h	256	256	128	256	512	512
7i	256	256	128	256	512	512
7j	256	**64**	128	256	512	512
7k	256	256	128	256	512	512
Ampicilin	2	32	16	16	–	–
Gentamycin	1	8	1	16	–	–
Vancomycin	1	4	–	–	–	–
Fluconazole	–	–	–	–	1	1

*S. aureus**: *S. aureus* isolate (MRSA); *E. coli**: *E. coli* isolate (contains broad spectrum β-lactamase enzyme); *C. albicans**: *Candida albicans* isolate.

docking. Antibacterial properties of azoles are attributed to the inhibition of a bacterial heme-containing enzyme called flavohemoglobin (flavoHb). FlavoHbs protect bacteria from the lipid membrane oxidative damage produced during nitrosative stress.[24] Some azole antifungals such as ketoconazole and miconazole show their antibacterial activities through coordinating the heme iron (*Figure 6*) in a similar mechanism detected for CYP51 structures co-crystallized with azoles.[25]

As **7j** stands forward among the tested molecules with a MIC value of 64 μg/mL against *S. aureus* isolate, we chose it as the representative compound in this series. To establish the potential biological target and rationalize the obtained bioactivity results, we docked **7j** into the catalytic site of a homology model of *S. aureus* flavohemoglobin (*Figure 7*).

The binding energy of **7j** was estimated as −8.89 kcal/mol whereas it was calculated as −17.56 for the original ligand econazole in the active pocket of *S. aureus* flavohemoglobin. This situation showed that **7j** had an affinity to the active site of the enzyme but cannot bind to the same pocket as strongly as econazole. When the putative binding pose of **7j** in the active site of *S. aureus* flavohemoglobin was analyzed, it was observed that the compound interacted with the enzyme through π-π stacking with Phe28 in addition to further hydrophobic contacts. Although **7j** was also oriented towards the heme to mediate π-π interaction with its core triazole moiety, the nitrogen atom could not form the required coordination with the iron. Additionally, we monitored the distance between the heme iron and the closest nitrogen atom of the triazole ring of **7j** as 4.11 Å. The same distance was measured as 2.03 Å and 2.11 Å for the co-crystallized ligand econazole in the active pocket of 4G1B and miconazole in 3OZU, respectively.

Consequently, our hybrid compounds were moderate antimicrobial agents, although they carry 1,2,4-triazole rings as an important pharmacophore to block the heme cofactor. According to the obtained molecular docking results, we surmise that the title molecules could target heme-containing enzymes but the lack of coordination between the triazole nitrogen and heme iron is a significant factor for the moderate biological efficiency observed for **7a**–**7k**.

Conclusions

In the present study, we reported the synthesis of eleven novel thiosemicarbazide-triazole hybrids (**7a**–**7k**) derived from (*S*)-naproxen and linked via thioether functionality. All molecules were *in vitro* tested for their antibacterial and antifungal activities. Compound **7j**, bearing nitrophenyl moiety as the modified substituent on the backbone, demonstrated the best MIC value of 64 μg/mL against *S. aureus* isolates in this series. The rest of the molecules were identified with moderate antimicrobial activities with the MIC values of 128–512 μg/mL. Molecular docking studies in the binding pocket of *S. aureus* flavohemoglobin sug-

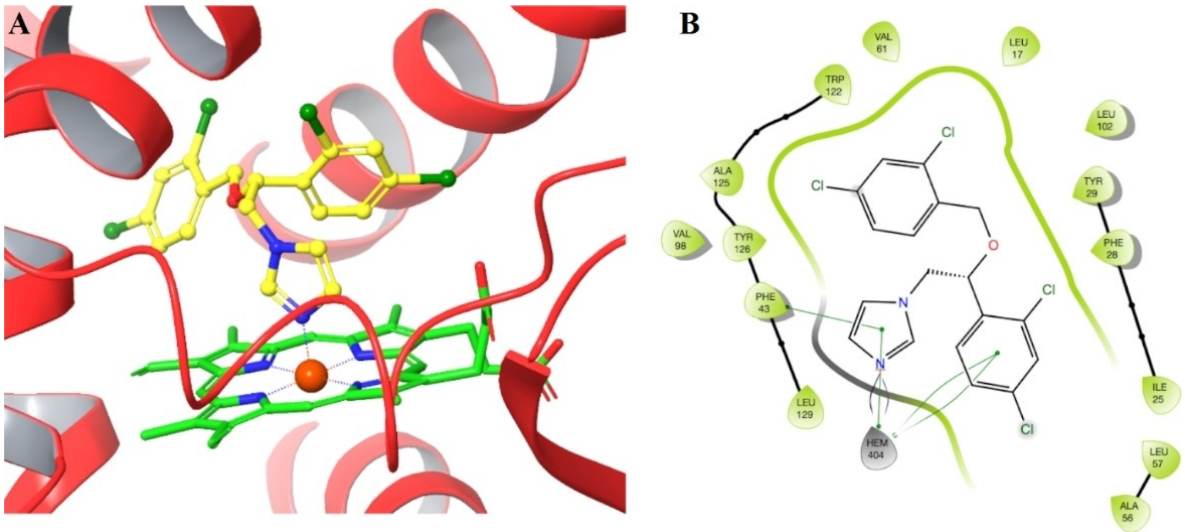

Figure 6. The binding mode of miconazole in the X-ray crystal structure of flavohemoglobin from *Ralstonia eutropha* (PDB code: 3OZU)[25] in 3D (a) and 2D (b) representations. Miconazole and protein backbone are shown as yellow ball&sticks and red cartoons, respectively.

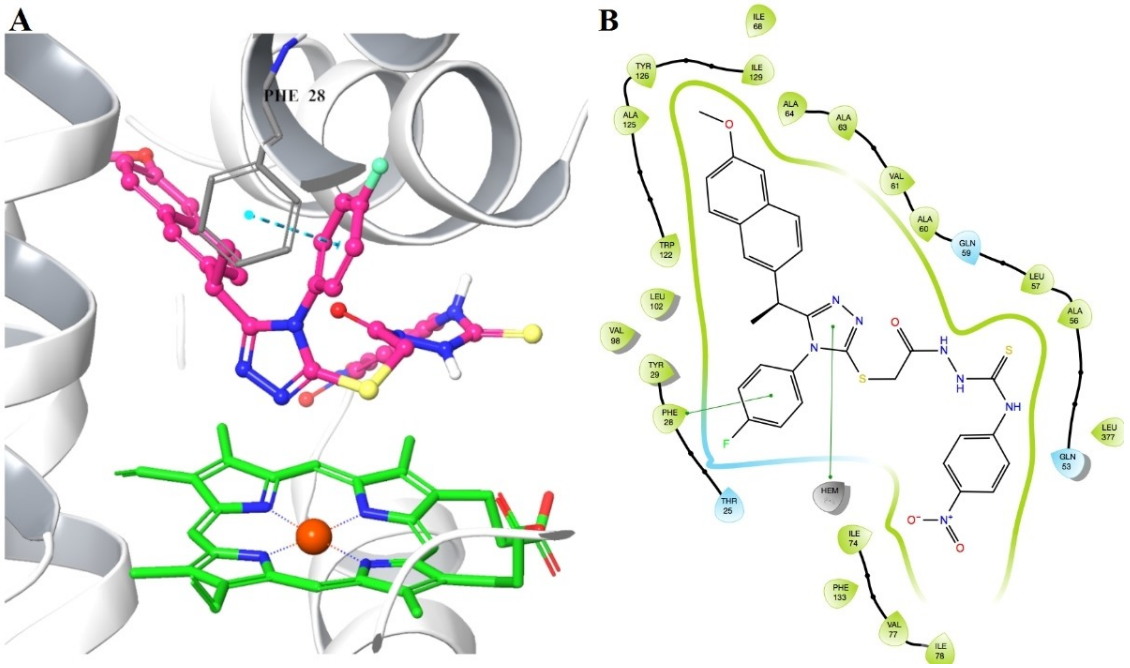

Figure 7. 3D (A) and 2D (B) representations of proposed binding mode of **7j** in the catalytic site of *S. aureus* flavohemoglobin. **7j** is shown as pink ball&sticks while heme and heme iron are represented as green stick and orange CPK, respectively. π-π stacking is represented as turquoise dashed lines.

gested that the studied compounds could target heme-containing enzymes. The chemical modifications that allow the triazole nitrogen's free-electron pair to interact with the heme-iron may be beneficial to this class of molecules by providing enhanced antimicrobial activity.

Experimental Section

Chemistry

Materials and Methods

All chemicals and solvents were purchased from Sigma–Aldrich and Merck. Reaction progress was controlled by thin-layer chromatography (TLC) on precoated 60 F_{254} plates (petroleum ether/ethyl acetate (40:60. v/v. t: 25 °C) mixture as mobile phase). Melting points were determined on a digital melting-point Schmelzpunktbestimmer 9300 SMP II apparatus and were uncorrected. FT-IR spectra were recorded on Shimadzu FT-IR-8400S spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra of the compounds, dissolved in (D_6)DMSO, were detected on a BRUKER AM 400 spectrometer at Erciyes University Technology Research and Application Center (TAUM). MS spectra were obtained on an LC/MS High-Resolution Time of Flight (TOF) Agilent 1200/6530 instrument at Atatürk University-East Anatolian High Technology Research and Application Center (DAYTAM).

Preparation of Compounds **1**–**6**

Compounds **1**–**6** were prepared according to the previously reported methods.[22,23] Briefly, (*S*)-naproxen ethyl ester (**1**) was prepared by the reaction of naproxen and ethanol in the presence of a few drops of concentrated sulfuric acid. Then, heating compound **1** with hydrazine hydrate (80 %) in ethanolic medium yielded (*S*)-naproxen hydrazide (**2**). The obtained molecule was reacted with 4-fluoroisothiocyanate in butanol to give compound **3**. After that, compound **4** carrying triazole-thione moiety was obtained by the ring closure reaction of **3** in a basic medium. Compound **5** was prepared in a solution of compound **4** and acetone with an equimolar amount of ethyl α-bromoacetate in the existence of potassium carbonate. The reaction of **5** with hydrazine hydrate in ethanol gave compound **6**.

*General procedure for the synthesis of N-substituted 2-({[5-[1-(6-methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulfanyl}acetyl)hydrazine-1-carbothioamide* (**7a**–**7k**). Compound **6**, 2-((4-(4-fluoro-

phenyl)-5-(1-(6-methoxynaphthalen-2-yl)ethyl)-4*H*-1,2,4-triazol-3-yl)thio)acetohydrazide, (0.01 mol) and equimolar amount of substituted isothiocyanate were heated on magnetic stirrer for 8 h at 100 °C in 25 ml of butanol. Butanol was evaporated after cooling the reaction mixture to room temperature. The obtained product was dried and recrystallized from ethanol to give the final compounds.

Antimicrobial Assay

The antimicrobial activity of compounds **7a**–**7k** against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 (Gram-positive and Gram-negative bacteria, respectively), *Candida albicans* ATCC 10231 (fungus), and the clinical isolates of these microorganisms were evaluated by using the Microbroth dilution method.[26–28]

Molecular Modeling Studies

The homology model of *S. aureus* flavohemoglobin was built using Swiss-Model[29] based on the X-ray structure of yeast flavohemoglobin with a ligand (econazole) and heme cofactor in the catalytic site with PDB code: 4G1B[30] as the template and the structural geometry of the generated model was analyzed using PROCHECK.[31] **7j** was docked into the binding site of the built homology model of *S. aureus* flavohemoglobin using AutoDock 4.2,[32] implemented in LigandScout 4.2.[33]

Supporting Information

Structural characterization data and FT-IR, ^{1}H-NMR, ^{13}C-NMR and HR-MS spectra of the synthesized compounds as well as the detailed methods for biological activity and computational studies are provided as *Supporting Information*.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

Author Contribution Statement

M. İ. H. designed the study, synthesized the compounds, elucidated their structures, and wrote the manuscript. *U. İ.* performed biological assays. *M. G. G.* carried out computational studies and wrote the manuscript. *Ş. G. K.* supervised the work and edited the manuscript.

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