ANTIBACTERIAL EFFECTS OF 1,2,4-TRIAZOLES

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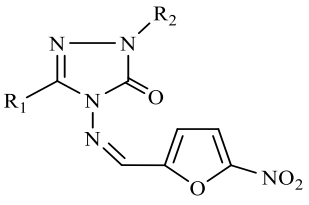
Biological activity studies have increased significantly, especially in the last 20 years. While these studies are being tried on different organic compound groups, it is noticed that these studies are concentrated on heterocyclic compounds (Aktaş Yokuş et al., 2017; Beytur et al., 2019; Çiftçi et al., 2018; Yüksek et al., 2022).

Considering the academic studies, the number of studies on the antibacterial effect of 1,2,4-triazole derivatives has increased significantly since the beginning of the 2000s. In some of these studies, activities against Gram-negative and some Gram-positive bacteria were investigated. In some studies, activities against fungi have also been tried.

In a study conducted in the 1980s examining 1,2,4-triazol-3-one derivatives, 10 new compounds were synthesized (Figure 1).



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	R_1	R_2
1a	CH_3	CH_2CO_2Et
1b	CH_3	$(CH_2)_2OH$
1c	C_2H_5	CH_2CO_2Et
1d	C_2H_5	CH_2CO_2-*n*-propyl
1e	C_2H_5	$(CH_2)_2OH$
1f	C_6H_5	CH_2CO_2-*n*-propyl
1g	C_6H_5	$(CH_2)_2OH$
1h	$p(CH_3O)C_6H_4$	CH_2CO_2Et
1i	$p(CH_3O)C_6H_4$	$(CH_2)_2OH$
1j	$p(Cl)C_6H_4$	CH_2CO_2-*n*-propyl

Figure 1. Formulas of synthesized compounds

A Gram-positive bacterial strain (*Staphylococcus aureus*) and two Gram-negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) were selected for the antibacterial activity of the 10 newly synthesized compounds. It is an important result that the activity of all synthesized compounds against Gram-positive bacteria was determined. The most interesting antibacterial activity against the selected *Staphylococcus aureus* was obtained especially in the case where the 2-hydroxy ethyl group (**1g** and **1i**) is located at the N-2 position in aromatic structures (Malbec et al., 1984).

In the early 2000s, in a study to examine the antibacterial properties of compounds containing 1,2,4-triazole ring, 1,2-bis(1,3,4-oxadiazol-2-yl)ethanes and 1,2- bis(4-amino-1,2,4-triazol-3-yl)ethanes compounds were synthesized (Figure 2).

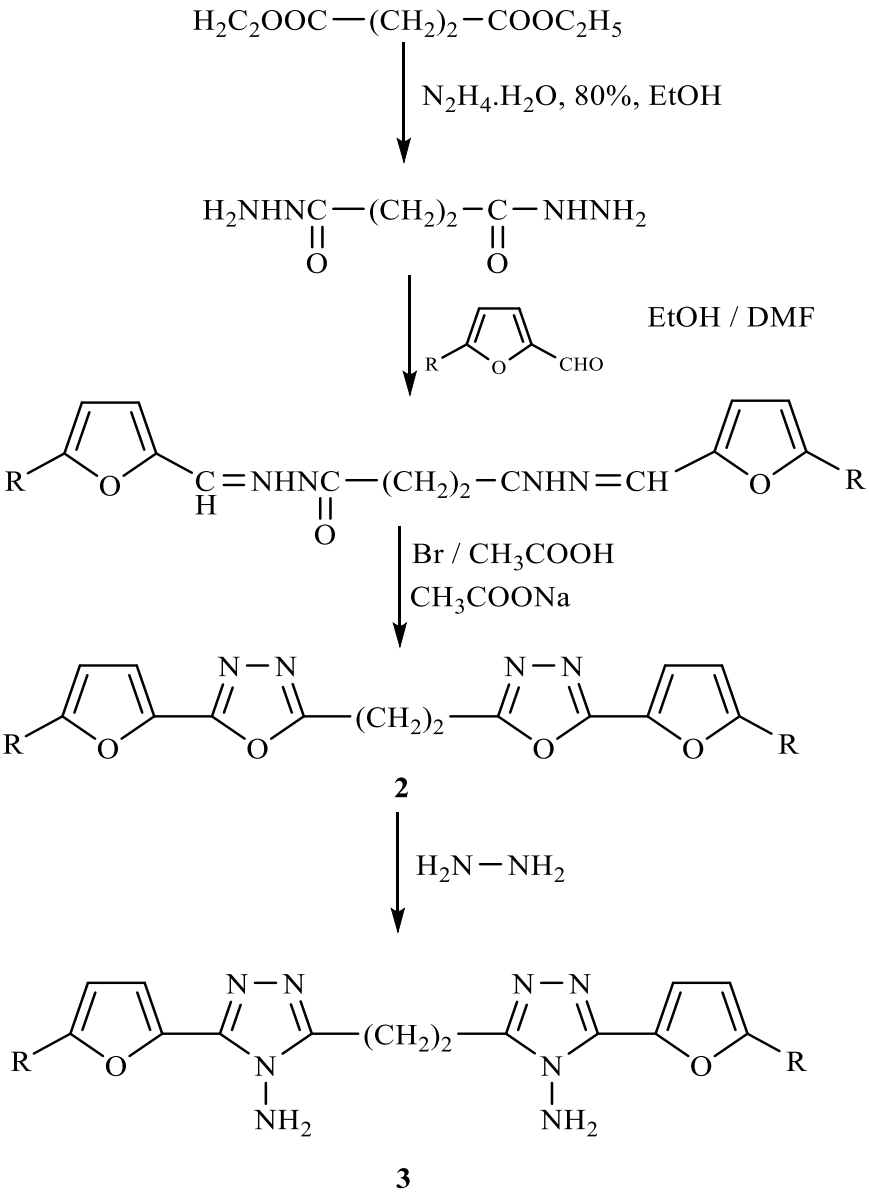

Figure 2. R= a=H, b=NO_2, c=p-nitrophenyl, d=p-chlorophenyl, e=p-bromophenyl, f=1,2,4-dichlorophenyl

When the antibacterial activity of the synthesized bis-oxadiazolylethanes (**2**) and bis-triazolylethanes (**3**) was examined, the presence of activity against selected Gram positive and Gram negative bacteria was determined. Obtaining results that compete with Furacin, which was chosen as the standard drug, was noted as an important result. The antibacterial activity results of the synthesized compounds are given in Table 1 (Holla et al., 2000).

Table 1. Antibacterial activity of bis-oxadiazolylethanes (**2** type) and bis-triazolylethanes (**3** type)

	MIC (μg/mL)			
Compound	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*
2c	6	6	6	3
2d	6	6	6	6
2e	6	6	6	12,5
3c	6	6	6	6
3e	6	6	6	6
3f	6	6	6	6
Furacin	12,5	12,5	12,5	6

In a study conducted in 2005, 1,2,4 triazole derivative compounds were synthesized and significant activities were determined especially against *S. aureus*, *S. epidermidis* and *B. subtilis* strains. The synthesis steps of the synthesized compounds are given in Figure 3.

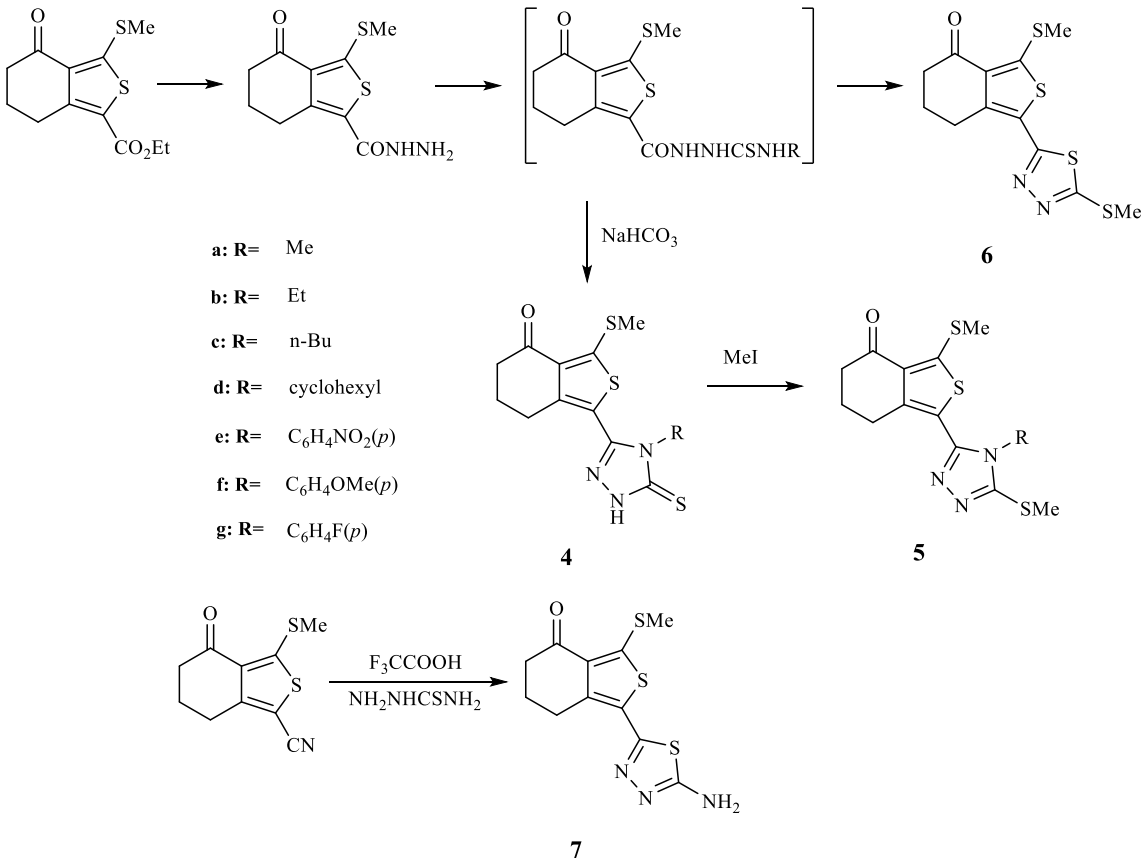

Figure 3. Reaction steps of synthesized compounds

The inhibition zone diameter values obtained as a result of the antibacterial effect examinations of the synthesized compounds are given in the table below.

Table 2. Antimicrobial effect examination data of compounds of type **4-7** (inhibition zone-mm)

Compound	*S. aureus*	*S. epidermidis*	*B. cereus*	*B. subtilis*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
4a	-	-	-	-	-	-	-	-
4b	-	-	-	-	-	-	-	-
4c	17	26	-	14	-	-	-	-
4d	-	-	-	-	-	-	-	-
4e	18	25	-	23	14	-	-	-
4f	-	-	-	11	-	-	-	-
4g	-	-	-	-	-	-	-	-
5a	-	-	-	-	-	-	-	-
5b	-	-	-	-	-	-	-	-
5c	17	26	16	16	-	-	-	-
5d	-	-	-	-	-	-	-	-
5e	18	24	12	21	14	-	-	-
5f	-	-	-	-	-	-	-	-
5g	-	-	-	-	-	-	-	-

6a	-	-	-	28	-	-	12	11
6b	-	-	-	20	-	-	13	8
6c	-	-	-	22	-	-	11	9
6d	-	-	-	-	-	-	-	12
6e	-	-	-	33	-	-	14	10
7	-	-	-	-	-	-	-	-
Genta.	23	20	28	30	27	23	NT	NT
Nitrof.	21	-	20	18	21	20	NT	NT
Amph.	NT	NT	NT	NT	NT	NT	22	22

Genta: *Gentamycin*; Nitrof: *Nitrofurantoin*; Amph: *Amphotricin*; NT: Not tested

When the antimicrobial examination results were examined, it was observed that some compounds showed moderate activity and some compounds showed high activity. Activity against Gram-positive bacteria was observed when n-butyl or p-nitrophenyl groups were present at the 4 position of 1,2,4-triazole in compounds of type **4** and **5**. No significant change was observed in the antibacterial effects of compounds **5c** and **5e** obtained from S-methylation of compounds **4**c and **4e**. No antimicrobial effect was found in **7** compounds.

It has been determined that the **6a-e** compounds obtained by changing the amino group of the **7**-type compounds have antifungal activity and a significant effect against *B. subtilis*. In addition, it was observed that compound **4e** was the most active compound against *S. aureus* and was close to the standard drug nitrofurantoin. Compounds **4c** and **5c** were found to be effective against *S. epidermidis* and compete with the standard drug Gentamicin. **6a**, **6b** and **6e** showed a significant effect against *B. subtilis*. This effect is greater for compound 6e than for Nitrofurantoin (Tehranchian et al., 2005).

In the study, in which a wide group of compounds were synthesized, 1,2,4-triazole derivatives were synthesized and Schiff Base and Mannich Bases were obtained from these compounds. This study is extremely important for comparing the antimicrobial activity of 1,2,4-triazole derivatives and Schiff Bases and Mannich Bases synthesized from them. The synthesis mechanism of the compounds synthesized in the study is given in the diagram.

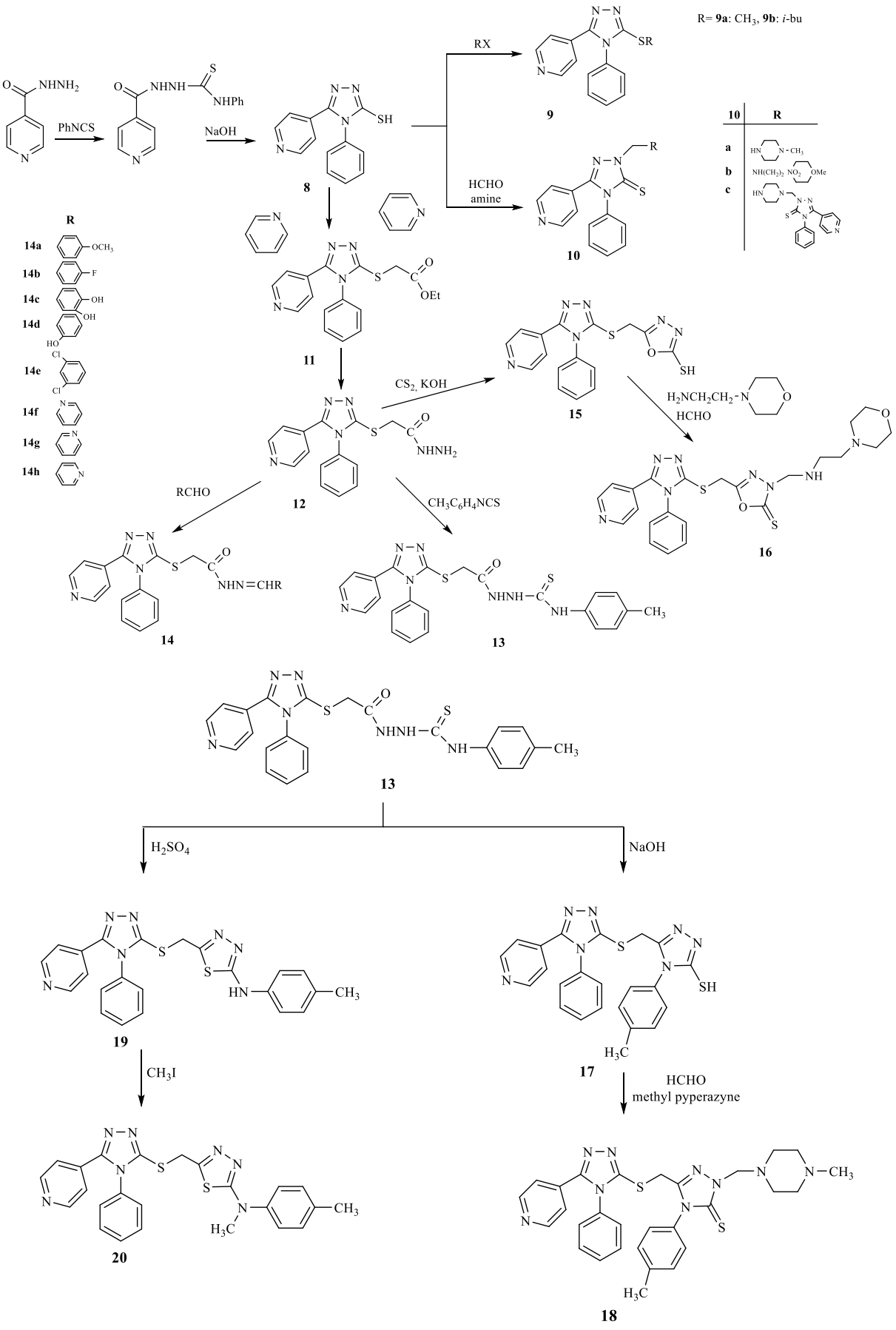

Figure 4. Synthesis mechanism of compounds **8-20**

In the study, Agar Well Diffusion Method was used for antimicrobial activity analysis. Inhibition zone diameters of synthesized compounds are given in Table 3.

Table 3. Inhibition zones of synthesized compounds

Compound	Microorganisms and effective inhibition zone diameters (mm)							
	Ec.	*Yp.*	*Pa.*	*Ef.*	*Sa.*	*Bc.*	*Ct.*	*Ca.*
8*	-	-	-	-	-	-	-	-
9a*	30	30	30	30	25	30	9	9
9b*	25	30	30	30	25	30	8	8
10a*	24	24	30	28	24	23	-	-
10b*	20	22	14	16	21	15	-	-
10c*	-	-	-	-	-	-	-	-
11*	-	-	-	-	-	-	13	13
12*	-	-	-	-	-	-	-	-
13*	-	-	-	-	-	-	7	7
14a*	24	30	30	28	28	25	-	-
14b*	30	25	38	30	30	23	-	-
14c*	-	-	-	-	-	-	-	-
14d*	30	30	30	30	25	25	-	-
14e*	-	-	-	-	-	-	-	-
14f*	42	30	40	20	24	20	-	-
14g*	8	7	-	-	-	-	-	-
14h*	-	-	-	-	-	-	-	-
15**	-	-	-	-	8	6	6	6
16**	6	-	-	-	-	-	-	-
17**	-	-	-	15	-	6	-	-
18**	-	-	-	-	-	-	-	-
19**	31	22	35	20	25	20	-	-
20**	28	16	15	7	22	17	-	-
Etanol	-	-	-	-	-	-	11	11
DMSO	-	-	-	-	-	-	-	-
Amp.	10	18	18	10	35	15	-	-
Flu.	-	-	-	-	-	-	25	25

Ec: E. coli-ATCC 25922; Yp: Y. pseudotuberculosis-ATCC 911; Pa: P. aeruginosa-ATCC 27853; Ef: E. Faecalis-ATCC 29212; Sa: S. aureus-ATCC 25923; Bc: B. cereus-ATCC 60193; Ct: C. tropicalis-ATCC13803; Ca: C. albicans-ATCC 60193; Amp: Ampicillin; Flu: Fluconazole; (-) no activity; * in DMSO; ** in ethanol.

When the compounds synthesized in the study were examined, no activity of type **8** compounds was found. Except for the **10c** compound, other Mannich Bases had a low effect on *candida* species, but gave effective results against other tested microorganisms. While a moderate effect of **11**-type compounds was observed only on *candida* species, no biological activity was found in **12**-type compounds. Low activity of **13**-type triazole derivatives was observed against *candida* species.

Low activities of the compounds obtained from the conversion of the **12**-type hydrazide structure to the **15**-type 1,3,4-oxadiazole ring were determined against *S. aureus, B. cereus, C. tropicalis* and *C. albicans*. Compounds of type **16**, which are derivatives of Mannich Base of type **15** compounds, have been found to have low activity against *E. coli*. On the other hand, the conversion of compound **13** to compound **17** did not cause a significant change in activity. Similarly, the biological activity of compound **18** was not observed. However, the effect of **19** types of compounds obtained from the reaction of **13** compounds with H2SO4 was high on bacteria except fungi. Compound **20** obtained from the methylation of compound **19** also showed a significant effect against selected bacteria.

In addition, **14a**, **14b**, **14d** and **14f** compounds of **14**-type Schiff Bases obtained from the reaction of **7**-type compounds with aldehydes had a significant effect on other bacteria except *candida* species. The effect of some of the synthesized compounds on the observed standard drugs is perhaps the most important result of the study.

Again, in a study on biological activity investigations, 6-substituted-2-amino-benzothiazole and 5-substituted-1-(1H-1,2,4-triazol-1-yl)-ethanone reacted in the presence of concentrated HCl and formaldehyde to form Mannich base. The antibacterial and antifungal effects of nine synthesized new compounds against pathogenic bacteria were investigated in vitro. The bacterial and fungal strains used for this purpose were *S. aureus, B. subtilis, P. aeruginosa, E. coli, A. niger* and *C. albicans.*

The R groups contained in the 6-substituted-benzothiazole derivative and 5-substituted-ethanone derivative contained in the 9 synthesized Mannich bases are given in Table 4.

Table 4. Substituent groups of synthesized compounds

Compound	6-substituted	5-substituted
21a	$-CH_3$	C_2H_5
21b	$-CH_3$	C_6H_5
21c	$-CH_3$	$C_6H_4CH_3$-*p*
21d	$-NO_2$	C_2H_5
21e	$-NO_2$	C_6H_5
21f	$-NO_2$	$C_6H_4CH_3$-*p*
21g	-Cl	C_2H_5
21h	-Cl	C_6H_5
21i	-Cl	$C_6H_4CH_3$-*p*

Ciprofloxacin was used as the standard drug in the antibacterial effect studies and Fluconazole was used as the standard drug in the antifungal studies. When the results are presented in a table, it is seen that the inhibition zones obtained from Gram positive and Gram negative bacteria are as in the table.

Table 5. Activity results of synthesized compounds against gram-negative and gram-positive bacteria (zone diameter-mm)

	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*B. subtilis*	*E.coli*	*P. aeruginosa*
21a	06	06	08	12
21b	09	08	08	10
21c	12	08	12	06
21d	14	06	12	08
21e	04	09	14	11
21f	07	06	06	10
21g	07	08	10	11
21h	02	12	07	12
21i	07	14	20	19
Ciprofloxacin	23	21	23	23

Evaluation of results according to inhibition diameter: <5.5 mm negative effect (-); 5.5-10mm low effect (+); 11-16mm moderate effect (++); ≥17 mm high effect (+++) (Demirbaş et. al., 2004).

The zone diameter values obtained against the fungal strains used are presented in the table below.

Table 6. Activity of synthesized compounds against fungal strains

	A.niger Zone diameter (mm)	C. albicans Zone diameter (mm)
21a	12	12
21b	09	07
21c	12	12
21d	09	03
21e	10	06
21f	14	04
21g	10	14
21h	12	11
21i	11	06
Fluconazole	19	16

Evaluation of results according to inhibition diameter: <5.5 mm negative effect (-); 5.5-10mm low effect (+); 11-16mm moderate effect (++); ≥17 mm high effect (+++) (Demirbaş et. al., 2004).

The effect of **21d** and **21c** compounds against S.aureus strain is moderate. Other compounds have been found to be less effective. Against the B. subtilis strain, **21h** and **21i** compounds showed moderate effects, while the others had a low effect. When *E. coli* is considered, the effect of compound **21i** is high and close to the standard drug value. While the effect level of **21c**, **21d**, **21e** compounds was determined at medium level, the effect level of other synthesized compounds was found to be low. The effect of compound *1i* was also high in *P. aureginosa* strain. The effect of **21a**, **21e**, **21g** and **21h** compounds is moderate. The effect of other compounds was determined to be low.

The most important result obtained from the study is that the antibacterial effect of the synthesized Mannich bases is present in all compounds. The different effect values obtained are the effect of the substituent groups.

In fungi, the effect level of compounds **21a**, **21c**, **21f**, **21h** and **21i** was found to be moderate against *A.niger* strain. The effect value of other compounds is low. In *C. albicans* strain, the effect level of **21a**, **21c**, **21g** and **21h** compounds is moderate. In other compounds, the effect value is low. The absolute effect of Mannich Bases against fungi is a promising result as a compound group (Bele and Singhvi, 2009).

The mechanism of a different study on the antimicrobial activities of Schiff Bases and Mannich Bases, which contain 1,2,4-triazole, is presented in the diagram.

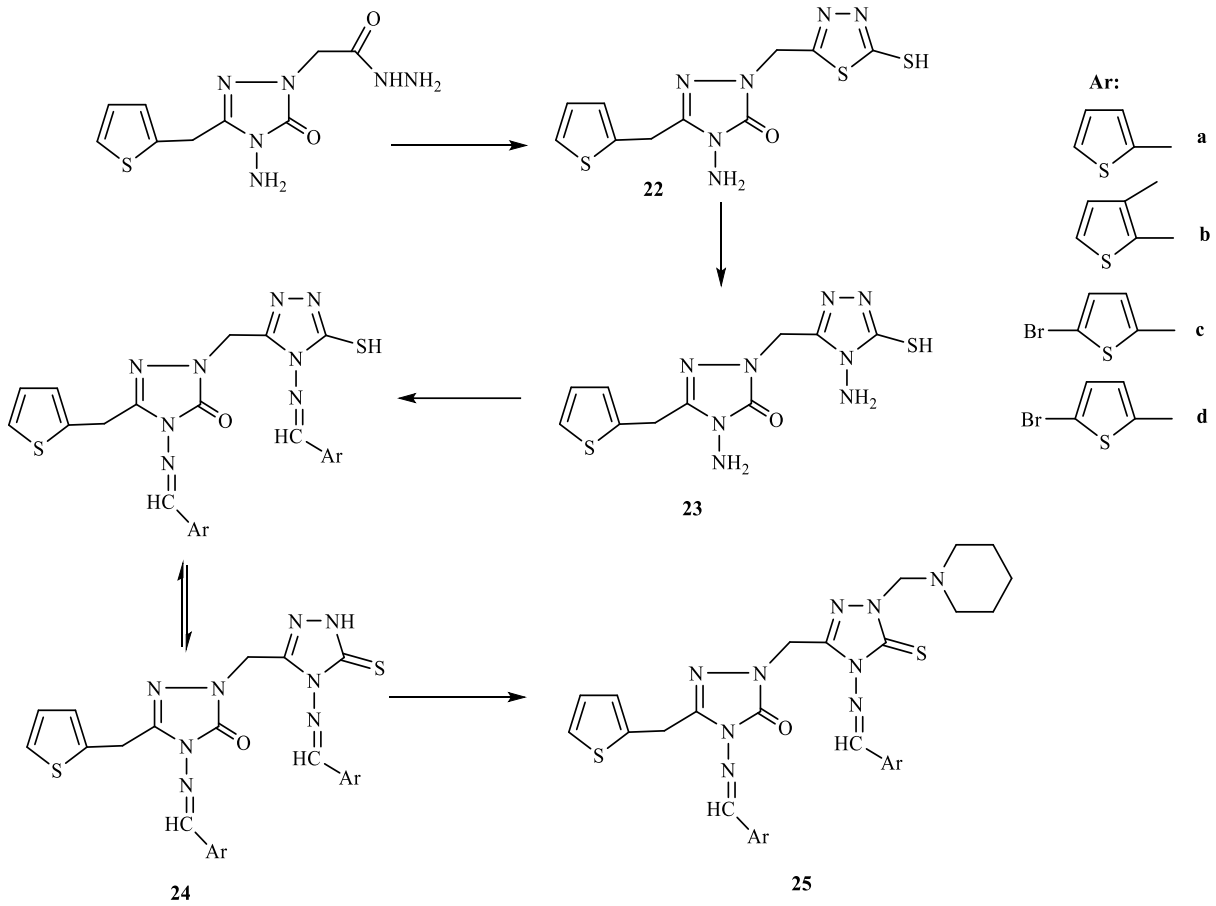

Figure 5. Reaction steps of synthesized 1,2,4-triazole derivatives, Schiff bases, Mannich bases Pathogenic Gram-negative and Gram-positive bacteria, fungi, and mycobacterium-type microorganisms were used in this large-scale study. When the results obtained from the study were presented in the form of a table, it was determined that the results were as follows.

Table 7. Antimicrobial activity results of synthesized compounds

	MIC (μg/mL)								
	Gram negative			Gram positive			Fungi		Mycobacterium
	Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Bc*	*Ca*	*Sc*	*Ms*
22	125	125	125	-	-	-	125	125	-
23	-	-	500	250	-	250	125	125	62,5
24a	500	500	125	125	-	500	125	125	500
24b	-	500	500	500	-	-	500	500	500
24c	500	500	125	500	-	-	125	125	250
24d	3,9	7,8	31,25	0,98	15,6	0,98	15,6	3,9	7,8
25a	-	125	125	250	-	-	250	125	500
25b	-	500	500	-	-	-	500	500	-
25c	500	125	125	500	-	-	125	125	500
25d	31,25	31,25	62,5	7,8	62,5	15,6	31,25	31,25	15,6
Amp.	10	18	>128	35	10	15	NT	NT	NT
Strep.	NT	NT	NT	NT	NT	NT	NT	NT	4
Flu.	NT	NT	NT	NT	NT	NT	<8	<8	NT

Ec: Escherichia coli ATCC 25922; Yp: Yersinia pseudotuberculosis ATCC 911; Pa: Pseudomonas aeruginosa ATCC 27853; Sa: Staphylococcus aureus ATCC 25923; Ef: Enterococcus faecalis ATCC 29212; Bc: Bacillus cereus 702 Roma; Ms: Mycobacterium smegmatis ATCC607; Ca:Candida albicans ATCC 60193; Sc: Saccharomyces cerevisiae RSKK 251; Amp.: Ampicillin; Str.: Streptomycin; Flu.: Fluconazole; (—): no activity;NT: not tested.

All synthesized compounds were observed to have antimicrobial effects. In general, it was concluded that the activity of the synthesized compounds against Gram-negative bacteria was higher than Gram-positive bacteria. It is an important result that **24d** type Schiff Base and **25d** type Mannich Base have high efficacy against all tested bacteria, fungi and microorganisms (Unver et al., 2016).

In a recent study, 1,2,4-triazole derivatives and the antibacterial effects of Schiff and Mannich Bases obtained from these derivatives were compared. The flow chart of 27 new compounds synthesized is given below.

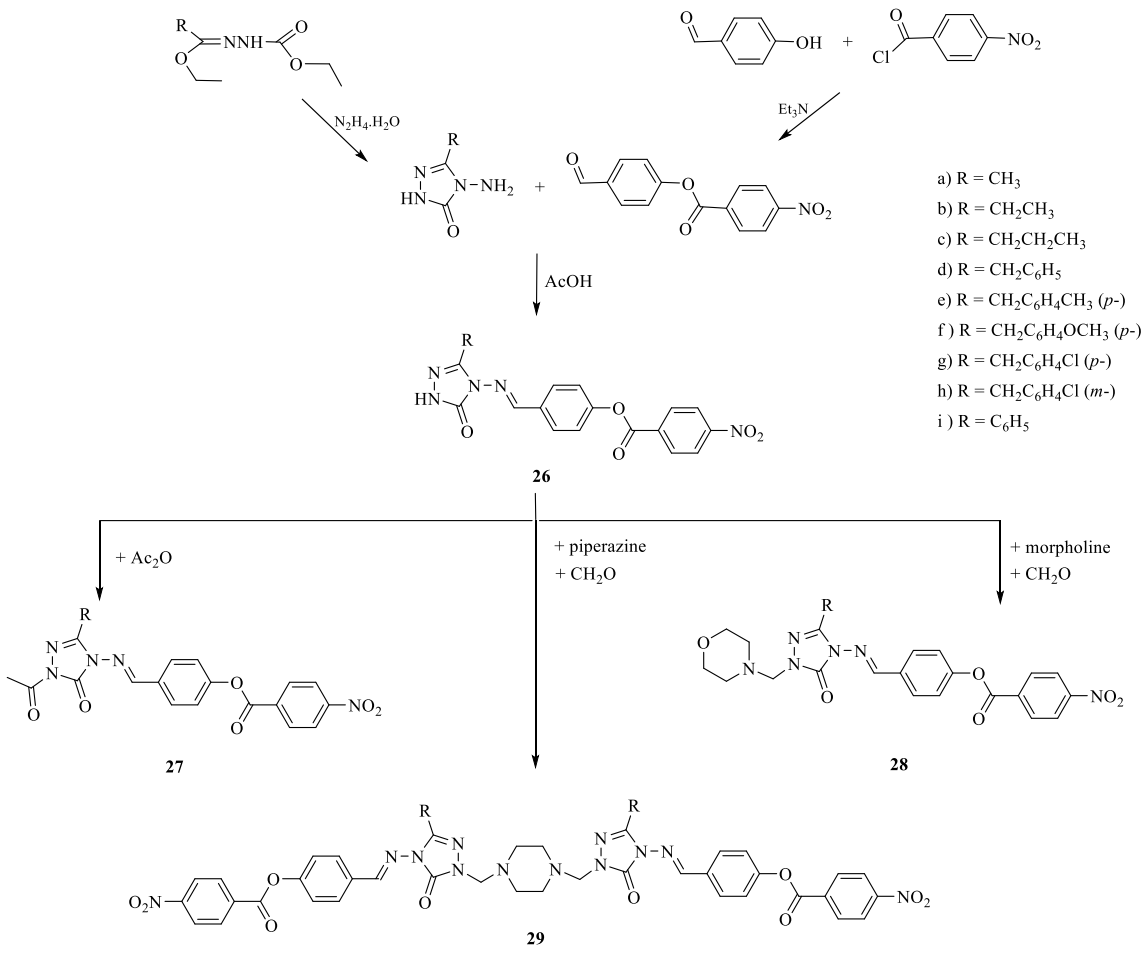

Figure 6. Reaction scheme of synthesized 1,2,4-triazole, Schiff bases, Mannich bases

The antimicrobial activity results of the synthesized compounds are given in the Table 8.

Table 8. In vitro antimicrobial activity of the compounds **3-6**.

Compound No	Microorganisms, inhibition zones (mm)					
	B. subtilis	*B. cereus*	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
26a	-	-	12	-	-	-
26b	10	-	11	-	-	11
26c	-	10	-	-	-	-
26d	12	-	13	-	-	12
26e	-	9	-	-	-	-
26f	-	-	-	-	-	-
26g	11	9	-	-	-	-
26h	-	-	-	-	-	-
26i	10	-	-	-	-	13
27a	-	10	-	-	-	-
27b	-	-	-	-	-	-

27c	-	-	-	-	-	-
27d	-	-	-	-	-	-
27e	-	-	-	-	-	-
27g	-	-	-	-	-	-
27i	-	-	-	-	-	-
28a	16	13	12	-	14	14
28b	15	13	14	-	14	14
28d	13	11	12	-	11	13
28e	12	11	11	-	11	9
28g	14	12	12	-	11	9
28i	15	13	11	-	13	19
29b	-	13	11	-	11	-
29d	-	11	11	-	12	-
29f	-	9	11	-	12	13
29g	10	11	13	-	13	13
29h	-	10	11	-	13	9
Amp.	33	36	37	34	36	35
Neo.	17	17	13	16	17	16
Str.	12	12	21	10	12	11

Evaluation of results according to inhibition diameter: <5.5 mm negative effect (-); 5.5-10mm low effect (+); 11-16mm moderate effect (++); ≥17 mm high effect (+++)

The activities of the synthesized type **26** compounds against gram positive bacteria were higher than the activities against gram negative bacteria. Compounds **26b** and **26i** showed low activity against *B. subtilis*, while the effects of compounds **26d** and **26g** were moderate. Only low level activities were obtained from compounds **26c**, **26e** and **26g** against *B. cereus* strain. Moderate effects of compounds **26a**, **26b** and **26d** were detected against *S. aureus* strain, which is a common infectious agent. Type **3** compounds had no activity against *E. coli* and *P. aeruginosa* strains. Moderate effects of **26b**, **26d** and **26i** compounds were determined against *K. pneumoniae* strain as well. No actibacterial effects were observed in type **27** acetylation derivatives synthesized from type **27** compounds. Only against *B. cereus* there was a low effect of compound **27a**.

The antibacterial effects of Mannich bases obtained by using morpholine (**28** type) and piperazine (**29** type) in the presence of formaldehyde from **26** type compounds as a different compound group were also investigated. The synthesized type **28** compounds had moderate effects on all Gram-positive bacteria studied. Although there was no activity against the Gram negative bacteria *E. coli*, compounds of type **28** were moderately active against the *P. aeruginosa* strain. Compounds **28e** and **28g** showed low activities against *K. pneumoniae* strain,

while the activity of compound **28i** was high. The effect levels of other **28** type compounds were moderate.

When the antibacterial effects of the **29** types Mannich bases were investigated, it was seen that the **29g** compound had a low effect against *B. subtilis* and no effects on the others. For *B. cereus*, moderate activities were mostly obtained from the **29** type compounds. Similarly, moderate activities were determined for *S. aureus* from **29** type compounds. Type 6 compounds had moderate effects on *P. aeruginosa* strains but had no effects on *E. coli* strain. For *K. pneumoniae*, no effects were observed from compounds **29b** and **29d**. A low level of effect was determined only for **29h**, and moderate effects were seen for **29f** and **29g**. A detailed list of the newly synthesized compounds is given in Table 8 (Alkan et al., 2022).

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