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ANADOLU UNIVERSITY
GRADUATE SCHOOL OF HEALTH SCIENCES

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Research Article

Synthesis, biological activity evaluation and molecular docking studies of novel thiazole derivatives

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ABSTRACT

Resistance to existing drugs develops because of insensible use of antibacterial and antifungal drugs. Therefore, there is a need for the development of new drug candidate compounds. The thiazole ring has many biological activities. It is possible to include antibacterial and antifungal activities among these activities. In addition to these, the thiazole ring has been preferred because it is the bioisostere of the imidazole ring in the structure of many antifungal drugs. For this purpose, within the scope of this study, 7 new thiazole compounds were synthesized, and their structure determinations were carried out using HRMS, ^{1}H-NMR, ^{13}C-NMR spectroscopic methods. Their antibacterial and antifungal activities were investigated by *in vitro* methods. As a result of activity tests, compound **3e** showed activity against *C.krusei* strain with MIC_{50}=31.25 ug/mL. The potential effectiveness of the compound **3e** on the 14alpha-demethylase enzyme (PDB ID:3LD6) was tested by *in silico* studies.

Keywords: Thiazole, Antibacterial Activity, Antifungal Activity, Molecular Docking

1. INTRODUCTION

Bacterial pathogen infections, which have become quite common in recent years, pose a danger to public health [1]. Causing some pathogens to escape the existing arsenal of antibiotics, causing health emergencies and major socio-economic impacts; The abuse or misuse of antibiotics in humans, animals, and agricultural practices perpetuates the need for new drug candidate molecules [2].

Nitrogen-containing heterocyclic analogs are of great interest in drug discovery. This is due to the well-known activities of these analogues in the pharmaceutical and medical fields [3]. Many heterocyclic compounds bearing a five-membered ring are known to be rich in biological activity. Thiazole was first reported as an effective nucleus by Hantzsch and Weber in 1887. This ring system has also attracted intense interest from industrial and pharmaceutical researchers in recent years [4]. In addition to this information, Nitrogen-based heterocycles (especially those bound to phenolic substrates) are popular for their pharmacological properties, including their anticancer, anti-malarial, antitubercular, and antimicrobial activities [5]. In the literature studies on the thiazole ring, studies on the antibacterial and antifungal activities of this ring are frequently presented [6-15]. In addition to these, the thiazole ring has been preferred because it is the bioisostere of the imidazole ring in the structure of many antifungal drugs.

The imidazole ring system is present in many antifungal drugs (miconazole, ketoconazole). This group of drugs, called azoles, constitutes a group of drugs in antifungal therapy. It was thought that an imidazole ring-like effect could be created by using the thiazole ring. At the same time, there are examples where drugs containing the azole group have an antibacterial effect (metronidazole). New derivatives showing both effects are important in this field.

In this study, 7 new compounds containing thiazole ring were synthesized, their structures were determined, and their antibacterial and antifungal effects were investigated. In addition to the thiazole ring, the piperazine ring, which is also in the structure of many antifungal agents, is also included in the structure.

2. MATERIALS AND METHODS

2.1. Chemistry

The description of the synthesis pathways of the compounds is given below. The synthesized compounds were subjected to spectral analysis for structure determination. NMR spectra (^{1}H-NMR and ^{13}C-NMR) were performed using Bruker DPX 300 FT-NMR spectrometer and Bruker DPX 75 MHz spectrometer, respectively. LCMS-IT-TOF (Shimadzu, Kyoto, Japan) device was used for high resolution Mass spectra (HRMS). Electron spray ionization (ESI) was used as the ionization technique. While the numerical data of the obtained results are presented in **Table-1**; spectra are presented in the Supporting Information file (**Figure S1-S21**).

2.1.1. Synthesis of 4-(4-acetylpiperazin-1-yl) benzaldehyde (1).

1-(Piperazin-1-yl)ethan-1-one (0.039 mol, 5 gr) and 4-fluorobenzaldehyde (0.039 mol, 4.836 gr) were dissolved in DMF (dimethylformamide) in presence of potassium carbonate. And reaction mixture was refluxed for 36h. At the end of the reaction, the product, which was cooled and poured into ice water, solidified. The solid product was filtered off, washed 3 times with water and dried.

2.1.2. Synthesis of 2-(4-(4-acetylpiperazin-1-yl) benzylidene)hydrazine-1-carbothioamide (2)

Compound **1** (0.017 mol, 3.944 gr) was dissolved in absolute ethanol. Thiosemicarbazide (0.017 mol, 1.547 gr) was added in reaction mixture and this mixture was refluxed for 12 h. The end of the reaction was checked with TLC, the product precipitated solidly in the reaction medium. This precipitate was filtered off and dried by washing with cold ethanol.

2.1.3. Synthesis of target compounds (3a-3h)

Compound **2** (0.001 mol, 0.305 gr) and suitable phenylacylbromidederivatives (0.001 mol) were refluxed in absolute ethanol for 12h. The end of the reaction was checked with TLC, the product precipitated solidly in the reaction medium. This precipitate was filtered off, dried and crystallized from hexane.

2.2. Antibacterial and anticandidal activity

The activity study of synthesized compounds (**3a-3g**) was evaluated on eight bacterial and three fungal strains according to the standart procedure of CLSI [16] as designated in the prior study [17]. The strains used for both antibacterial and antifungal activity are listed in Table-2. ATCC codes are as follows; ATCC 6051, ATCC 25922, ATCC 2942, ATCC 13883, ATCC 27853, ATCC 29213, ATCC 12228, ATCC 8100. ATCC 6258, ATCC 24433, ATCC 22019.

2.3. Prediction of ADME Parameters

SwissADME (online) were used for prediction of ADME parameters [18].

2.4. Molecular Docking Study

Molecular docking studies were performed using *in-silico* procedure to define the binding modes of compound **3e** (NO_2 containing compound) in the active regions of enzymes X-ray crystal structures of 14alpha-demethylase (PDB ID:3LD6) [19] were retrieved from Protein Data Bank server (www.pdb.org, accessed 25.02.2022). Molecular docking studies were performed as previously reported [20-22].

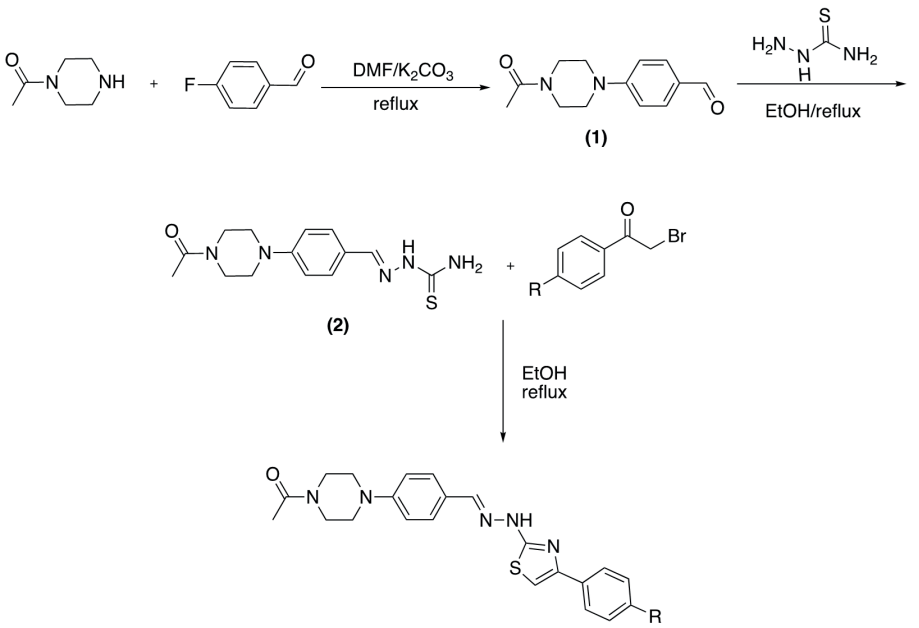
Table 1. Spectral analysis results of compounds **3a-3g**

Compounds	^{1}H-NMR	^{13}C-NMR	HRMS
3a	2.04 (3H, s, -$COCH_3$), 3.22 (2H, br.s., piperazine), 3.28 (2H, br.s., piperazine), 3.58 (4H, br.s., piperazine), 7.04 (2H, d, *J*=8.4 Hz, Ar-H), 7.29-7.33 (2H, m, Ar-H), 7.41 (2H, t, *J*=7.3 Hz, Ar-H), 7.54 (2H, d, *J*=8.5 Hz, Ar-H), 7.84 (2H, d, *J*=8.5 Hz, Ar-H), 7.99 (1H, s, Ar-H).	21.65, 45.55, 48.15, 48.49, 103.87, 115.93, 125.68, 126.08, 128.09, 128.19, 129.10, 134.52, 142.99, 149.78, 151.32, 168.79, 168.82	calcd for $C_{22}H_{23}N_5OS$: 406.1696; found 406.1686.
3b	2.05 (3H, s, -$COCH_3$), 2.32 (3H, s, -CH_3), 3.18-3.22 (2H, m, piperazine), 3.25-3.28 (2H, m, piperazine), 3.58 (4H, br.s., piperazine), 7.00 (2H, d, *J*=8.8 Hz, Ar-H), 7.20-7.22 (3H, m, Ar-H), 7.53 (2H, d, *J*=8.8 Hz, Ar-H), 7.74 (2H, d, *J*=8.1 Hz, Ar-H), 7.94 (1H, s, Ar-H).	21.27, 21.66, 45.67, 47.85, 48.21, 102.74, 115.63, 125.34, 125.93, 127.91, 129.63, 132.44, 137.24, 142.24, 150.72, 151.70, 168.71, 168.79	calcd for $C_{23}H_{25}N_5OS$: 420.1853; found 420.1860.
3c	2.05 (3H, s, -$COCH_3$), 3.18-3.21 (2H, m, piperazine), 3.25-3.28 (2H, m, piperazine), 3.56-3.58 (4H, m, piperazine), 3.78 (3H, s, -OCH_3), 6.95-7.01 (4H, m, Ar-H), 7.10 (1H, s, Ar-H), 7.51 (2H, d, *J*=8.8 Hz, Ar-H), 7.78 (2H, d, *J*=8.8 Hz, Ar-H), 7.92 (1H, s, Ar-H), 11.90 (1H, s, -NH).	21.70, 40.93, 45.70, 47.83, 48.18, 55.60, 101.52, 114.39, 115.59, 125.34, 127.27, 127.86, 128.12, 141.97, 151.75, 159.18, 159.19, 168.69, 168.78.	calcd for $C_{23}H_{25}N_5O_2S$: 436.1802; found 436.1818
3d	2.05 (3H, s, -$COCH_3$), 3.19-3.22 (2H, m, piperazine), 3.25-3.29 (2H, m, piperazine), 3.56-3.58 (4H, m, piperazine), 7.01 (2H, d, *J*=8.9 Hz, Ar-H), 7.52 (2H, d, *J*=8.9 Hz, Ar-H), 7.61 (1H, s, Ar-H), 7.87 (2H, d, *J*=8.7 Hz, Ar-H), 7.96 (1H, s, Ar-H), 8.03 (2H, d, *J*=8.5 Hz, Ar-H), 12.02 (1H, s, -NH).	21.70, 45.69, 47.77, 48.13, 107.59, 109.95, 115.54, 119.49, 125.12, 126.55, 127.98, 133.15, 139.32, 142.62, 149.28, 151.84, 168.77, 169.12	calcd for $C_{23}H_{22}N_6OS$: 431.1649; found 431.1651
3e	2.05 (3H, s, -$COCH_3$), 3.21-3.22 (2H, m, piperazine), 3.27-3.29 (2H, m, piperazine), 3.58 (4H, br.s., piperazine), 7.01 (2H, d, *J*=8.9 Hz, Ar-H), 7.53 (2H, d, *J*=8.8 Hz, Ar-H), 7.68 (1H, s, Ar-H), 7.96 (1H, s, Ar-H), 8.11 (2H, d, *J*=8.9 Hz, Ar-H), 8.26-8.29 (2H, m, Ar-H), 12.07 (1H, s, -NH).	21.65, 40.93, 45.68, 47.76, 48.11, 108.58, 115.53, 124.57, 125.08, 126.76, 127.99, 141.22, 142.74, 146.60, 148.97, 151.86, 168.78, 169.22	calcd for $C_{22}H_{22}N_6O_3S$: 389.1152; found 389.1134
3f	2.05 (3H, s, -$COCH_3$), 3.18-3.21 (2H, m, piperazine), 3.24-3.28 (2H, m, piperazine), 3.57-3.59 (4H, m, piperazine), 6.99 (2H, d, *J*=8.9 Hz, Ar-H), 7.36 (1H, s, Ar-H), 7.46 (2H, d, *J*=8.6 Hz, Ar-H), 7.53 (2H, d, *J*=8.8 Hz, Ar-H), 7.87 (2H, d, *J*=8.6 Hz, Ar-H), 7.95 (1H, s, Ar-H), 11.97 (1H, s, -NH).	21.66, 40.92, 45.68, 47.79, 48.14, 104.47, 115.56, 125.22, 127.66, 127.92, 129.07, 132.30, 134.09, 142.31, 149.71, 151.79, 168.77, 168.95	calcd for $C_{22}H_{22}N_5OSCl$: 440.1306; found 440.1299
3g	2.05 (3H, s, -$COCH_3$), 3.18-3.21 (2H, m, piperazine), 3.25-3.28 (2H, m, piperazine), 3.57-3.58 (4H, m, piperazine), 6.99 (2H, d, *J*=8.9 Hz, Ar-H), 7.36 (1H, s, Ar-H), 7.52 (2H, d, *J*=8.9 Hz, Ar-H), 7.60 (2H, d, *J*=8.6 Hz, Ar-H), 7.81 (2H, d, *J*=8.6 Hz, Ar-H), 7.94 (1H, s, Ar-H), 11.97 (1H, s, -NH).	21.67, 40.93, 45.68, 47.79, 48.14, 104.56, 115.56, 120.89, 125.21, 127.93, 127.97, 131.98, 134.43, 142.31, 149.78, 151.80, 168.77, 168.94	calcd for $C_{22}H_{22}N_5OSBr$: 484.0801; found 484.0814

Table 2. Antibacterial and anticandidal activity of synthesized compounds (**3a-3g**) and standart drugs (**SD1-SD3**)

ID	*Antibacterial activity* MIC_{50} (µg/mL) [a]								*Anticandidal activity* MIC_{50} (µg/mL) [b]		
	B.subtilis	***E.coli***	***E.faecalis***	***K.pneumoniae***	***P.aeruginosa***	***S.aureus***	***S.epidermis***	***S.marcescens***	***C. albicans***	***C. krusei***	***C. parapsilopsis***
3a	125	125	125	125	62.5	62.5	125	62.5	62.5	62.5	62.5
3b	125	62.5	125	125	62.5	62.5	125	62.5	62.5	62.5	62.5
3c	125	62.5	125	125	62.5	62.5	125	62.5	125	62.5	62.5
3d	125	125	125	125	62.5	125	125	62.5	125	62.5	62.5
3e	125	125	125	62.5	62.5	62.5	125	62.5	62.5	31.25	62.5
3f	125	125	125	125	125	125	125	62.5	125	62.5	62.5
3g	125	125	125	125	125	125	125	62.5	125	62.5	62.5
SD1	< 0.97	< 0.97	< 0.97	< 0.97	< 0.97	< 0.97	< 0.97	< 0.97	-	-	-
SD2	-	-	-		-	-	-		3.90	3.90	1.95
SD3	-	-	-		-	-	-		7.81	7.81	3.90

[a] The test results were expressed as means of triplicate assays. [b] The test results were expressed as means of triplicate assays. [c] The test results were expressed as means of quartet assays ± SEM. SD1: Azithromycin. SD2: Voriconazole. SD3: Fluconazole



Compounds	R
3a	-H
3b	-CH_3
3c	-OCH_3
3d	-CN
3e	-NO_2
3f	-Cl
3g	-Br

Scheme 1. Synthesis pathway for obtained compounds **(3a-3g)**

3. RESULTS AND DISCUSSION

3.1. Chemistry

The compounds **3a-3g** were obtained as presented in **Scheme 1**. Initially, aldehyde derivative (**1**) was obtained by means of the reaction between 1-(piperazin-1-yl)ethan-1-one and 4-fluorobenzaldehyde using potassium carbonate. Secondly, the thiosemicarbazone derivative (**2**) was obtained by means of reaction between 4-(4-acetylpiperazin-1-yl)benzaldehyde and thiosemicarbazide. The target compounds (**3a-3g**) were obtained using ring closure reaction. The structures of the compounds **3a-3g** were evaluated by using spectroscopic methods (HRMS, ^{1}H-NMR and ^{13}C-NMR).

When the NMR data of the compounds are examined, it is seen that the protons of piperazine come in the form of 2H, 2H, 4H, between 3.18 ppm and 3.59 ppm. Protons of the acetyl group attached to piperazine were recorded as singlet between 2.04 ppm and 2.05 ppm. The carbon belonging to this group was recorded between 21.27 ppm and 21.70 ppm values. In addition, the carbonyl carbon of this group was recorded between 168.78 ppm and 169.22 ppm. While the methyl group of compound **3b** was recorded as a singlet at 2.32 ppm; The methoxy group of compound **3c** was recorded as singlet at 3.78 ppm. Mass spectra were performed using high resolution liquid chromatography. In the mass spectra taken using the electron spray method, all compounds were recorded as an excess of their molecular weights.

3.2. Antibacterial and anticandidal activity

Eight bacterial strains (it can be seen in **Table-2**) were used to evaluate the antibacterial activity of the obtained compounds (**3a-3g**). Azithromycin was used as the reference drug in fluorometric measurements of which MIC_{50} values were calculated using resazurin solution [23-24]. The results are presented in **Table 2**. When **Table-2** is examined, it is seen that the antibacterial MIC_{50} values of the compounds **3a-3g** were determined between 62.5-125 ug/mL.

Three candida strains (it can be seen in **Table-2**) were used to evaluate the antifungal activity of the obtained compounds (**3a-3g**). Voriconazole and fluconazole were used as the reference drug in fluorometric measurements of which MIC_{50} values were calculated using resazurin solution [23,24]. The results are presented in **Table 2**. When **Table-2** is examined, it is seen that the antifungal MIC_{50} values of the compounds **3a-3g** were determined between 31.25-125 ug/mL. Compound **3e** was the most active compound with MIC_{50}=31.25 ug/mL against *C. krusei*. However, this activity value is not as high as predicted. When the structure of the compound is examined, the nitro group in its structure is remarkable compared to other compounds. The nitro group contributed a little to the activity. However, in general, MIC_{50} values in the series suggest that one or more of the common structures negatively affect the activity. For this purpose, *in silico* molecular docking studies of the compounds were carried out.

It is known that azole group compounds exert their antifungal activities by inhibiting ergosterol biosynthesis. They inhibit the conversion of lanosterol molecule to ergosterol by inhibition of 14alpha-demethylase enzyme. The antifungal activity of compound **3e** on *C.krusei* is promising. However, modifications can be made to the molecule to increase this efficiency. For this reason, first, how the compound acts in the active site of the enzyme should be examined. *In silico* studies were carried out using compound **3e** and 14alpha-demethylase enzyme crystal for this purpose.

3.3. Prediction of ADME Parameters

The physicochemical properties of drugs provide important information about their ability to be a drug. Thanks to these parameters that can be calculated online, an impression is gained about the drug profile of the compounds. The online SwissADME program was used and the estimated ADME parameters of the obtained compounds were calculated [18]. When **Table-3**, in which the results are presented, is examined, it is seen that none of the compounds with positive drug candidate potential violate the Lipinski rule [25]. It is also a positive feature that the Lipinski rule violation values are zero. Gastrointestinal absorption is an important parameter for oral administration of compounds. When the pharmacokinetic part is examined, the high gastrointestinal absorption profiles of all

Table 3. Predicted ADME parameters of compounds **3a-3g**

Comp	Physicochemical Properties						Pharmaco-kinetics			Drug likeness		Medicinal Chemistry
	HBA	HBD	TPSA	Log Po/w	LogS	GIA	Log Kp	BBB permeant	P-gp substrate	Lipinski	Vio	SA
3a	3	1	89.07	3.10	-4.89	High	-5.96	No	No	Yes	0	3.47
3b	3	1	89.07	3.32	-5.19	High	-5.79	No	No	Yes	0	3.59
3c	4	1	98.30	3.38	-4.96	High	-6.17	No	No	Yes	0	3.54
3d	4	1	112.86	3.09	-4.84	High	-6.31	No	No	Yes	0	3.58
3e	5	1	134.89	2.52	-4.95	High	-6.36	No	No	Yes	0	3.60
3f	3	1	89.07	3.46	-5.48	High	-5.72	No	No	Yes	0	3.46
3g	3	1	89.07	3.56	-5.80	High	-5.95	No	No	Yes	0	3.50

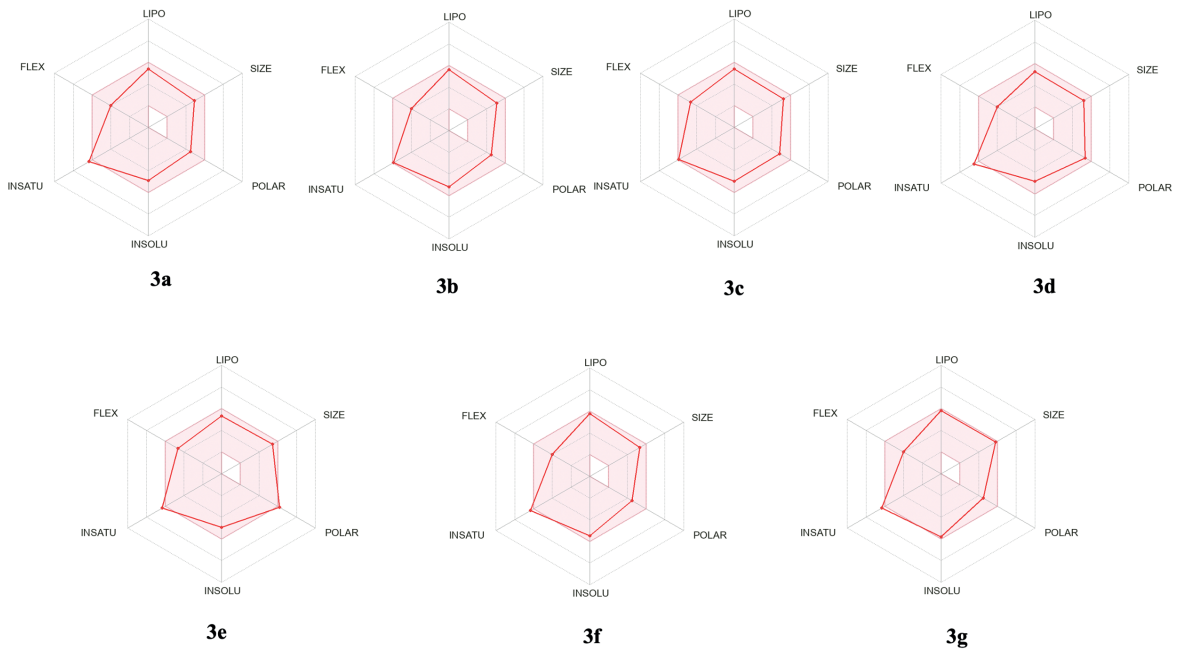


Figure 1. Oral bioavailability radars of obtained compounds **(3a-3g)**

compounds indicate the oral use potential of the compounds. The hydrophilic-lipophilic properties diagram of the compound **3e** were presented using the Molinsparition program (**Figure 1**). The synthetic accessibility (SA) value gets harder from 1 to 10 [26]. This value is an important value for synthetic chemistry. There is no compound approaching or exceeding 10 in compound **3a-3g**. The SA values of the compounds ranged between 3.47 and 3.60. P-gp causes excretion of some drugs, so the pharmacokinetic efficacy is reduced. It is also known that some cancer cells cause an increase in the amount of this protein and cause drug resistance because of drugs acting as P-gb substrate. Examining **Table-3**, none of the compounds (**3a-3g)** act as a substrate for P-gp. As a result, it is seen that all compounds have suitable physicochemical parameters. This demonstrates the value of compounds. Especially the high gastrointestinal permeability will allow the oral use of the compounds.

3.3. Molecular Docking Studies

To elucidate the antifungal action mechanism of compound **3e**, which is the most active derivative according to the activity result, it was subjected to *in silico* insertion procedure with 14alpha-demethylase (PDB ID:3LD6). The placement of the compound in the enzyme active site is shown in **Figure-2**. Compound **3e** appears to localize to the enzyme active site. The thiazole ring of the compound is located close to the HEM molecule in the enzyme active site. This may be a suitable placement for the azole group.

Docking studies were performed on the 14alpha-demethylase crystals (PDB ID:3LD6) [19] for approved binding model. **Figure-3** shows the 3D localization of compound **3e** in the enzyme active site. The bonds formed by compound **3e** at the enzyme active site can be summarized as follows. A hydrogen bond is formed between the nitro group of compound **3e** and the amino group of Ile488. Aromatic hydrogen bonds are formed between the phenyl ring of the 4-nitrophenyl group and the carbonyl group of Met487 and Pro376. Pi-pi interaction was established between the thiazole ring of compound **3e** and phenyl ring of Phe234.

In addition to these interactions, compound **3e** does not interact with the HEM group in the enzyme active site. This lack of interaction may be the reason for the low activity value compared to the reference drugs.

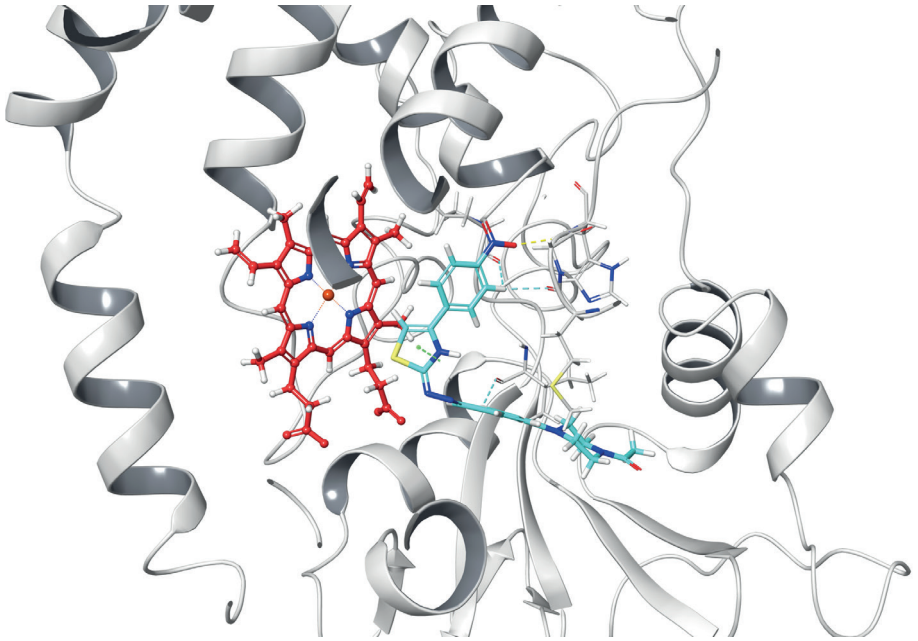


Figure 2. Localization of compound **3e** in the enzyme active site (PDB ID:3LD6)

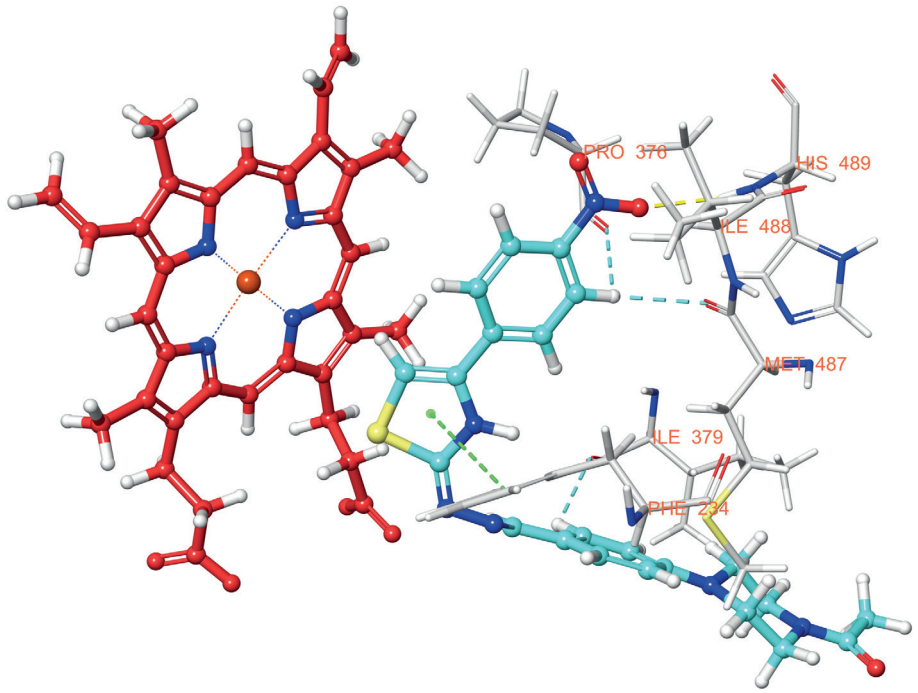


Figure 3. The three-dimensional interacting mode of compound **3e** in the active region of 14alpha-demethylase enzyme (PDB ID: 3LD6)

4. CONCLUSION

Frequent and uncontrolled use of antibiotics and antifungal drugs causes the development of resistance. Resistance to drugs with clinical use is a risk factor especially for patients in critical condition. For this reason, the need to develop new antibacterial and antifungal drugs always continues. It is known that azole group compounds constitute most antifungal drugs. These ring systems, which also have antibacterial activity, are molecules with an antimicrobial activity profile.

The strategy of designed new compounds using pharmacophore structures with proven activity are a frequently preferred method for medicinal chemists. The thiazole ring has a wide range of activity profile. Within the scope of this study, 7 compounds containing thiazole were synthesized, their structure determinations were carried out and their biological activities were determined by *in vitro* methods. Compound **3e** showed activity on the *C.krusei* line with a value of MIC_{50}=31.25 ug/ml. This activity value is 4 times lower than the reference drug fluconazole (MIC_{50}=7.81 ug/mL against *C.krusei*). No antibacterial activity was detected. While the compounds do not show antibacterial activity, they exhibit antifungal activity, suggesting that the mechanism of action may be based on the azole group. Molecular docking studies were carried out to better understand the reason for this difference and to determine the points to be considered in the design of new compounds. It is known that azole group compounds perform their antifungal activities by inhibiting ergosterol biosynthesis. The azole group antifungal agents inhibit the 14alpha-demethylase enzyme. Molecular docking studies due to the thiazole structure were carried out using 14alpha-demethylase crystal (PDB ID:3LD6). Here it is seen that the piperazine group of the compound **3e** does not contribute to the enzyme active site. The changing of more smaller volume groups can provide the compound's approximation to the HEM molecule. For this purpose, new compounds will be designed by keeping 4-nitrophenyl and thiazole structures constant based on this compound in future studies. And so, the activity is thought to increase.

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Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: DO, ZAK; Design: DO, ZAK; Supervision: YÖ, ZAK; Materials: DO, UK, ÜDG; Data Collection and/or Processing: DO, ZAK; Analysis and/or Interpretation: DO, UK, ÜDG; Literature Search: DO, ZAK; Writing: DO, YÖ; Critical Reviews: YÖ, ZAK.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Supporting Information

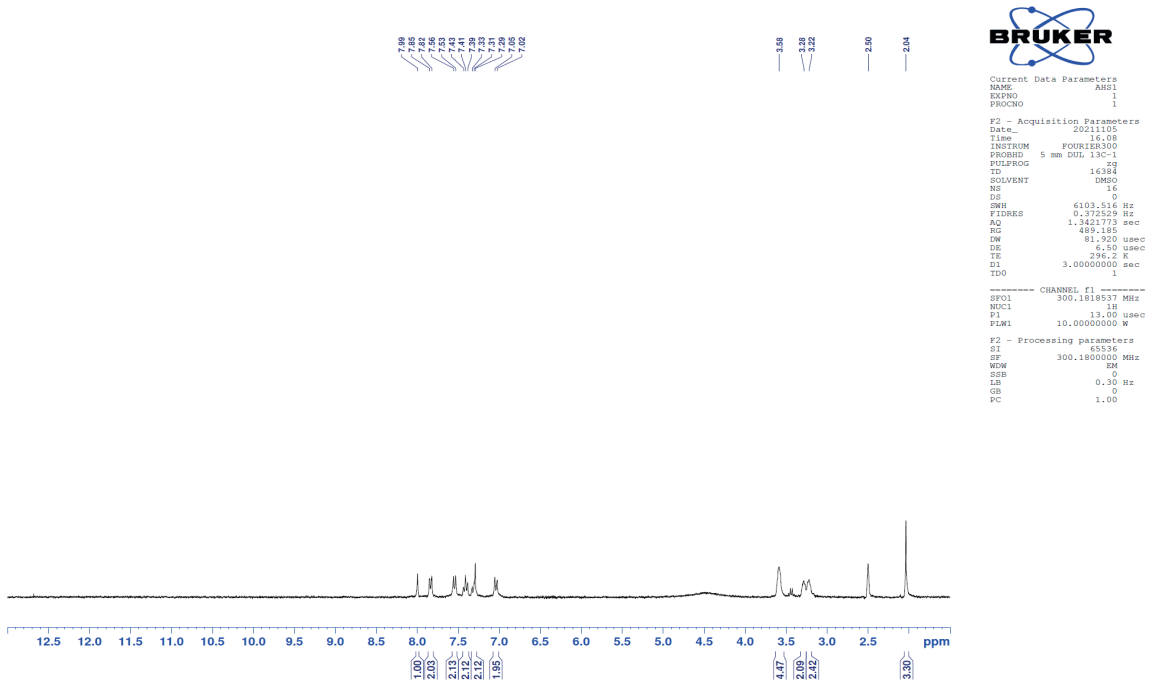


Figure S1. Compound **3a** ^{1}H-NMR spectrum

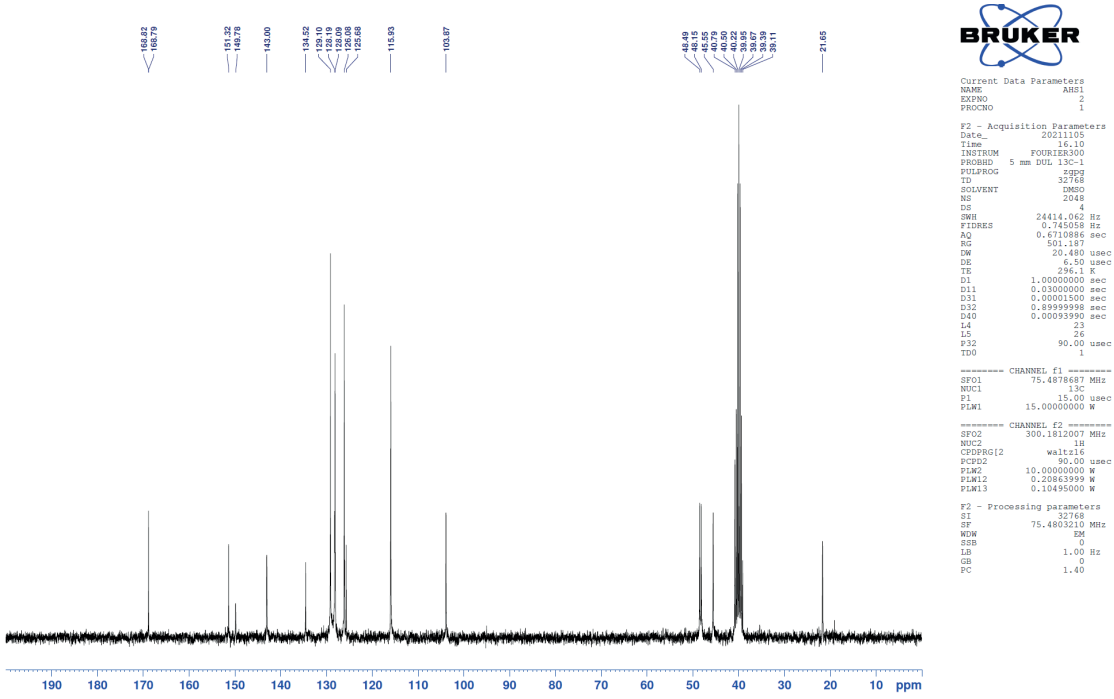


Figure S2. Compound **3a** ^{13}C-NMR spectrum

Formula Predictor Report - AHS-1_111.lcd Page 1 of 1

Data File: C:\LabSolutions\Data\Analiz\derya\AHS-1_111.lcd

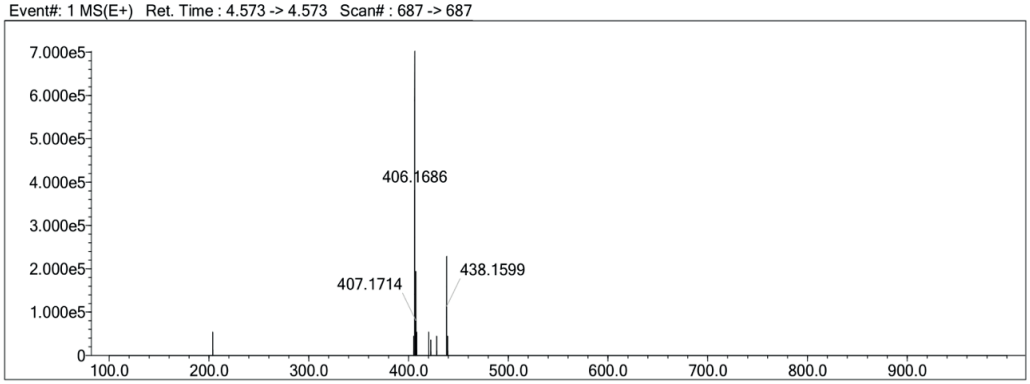
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	6	46	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	5	36	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

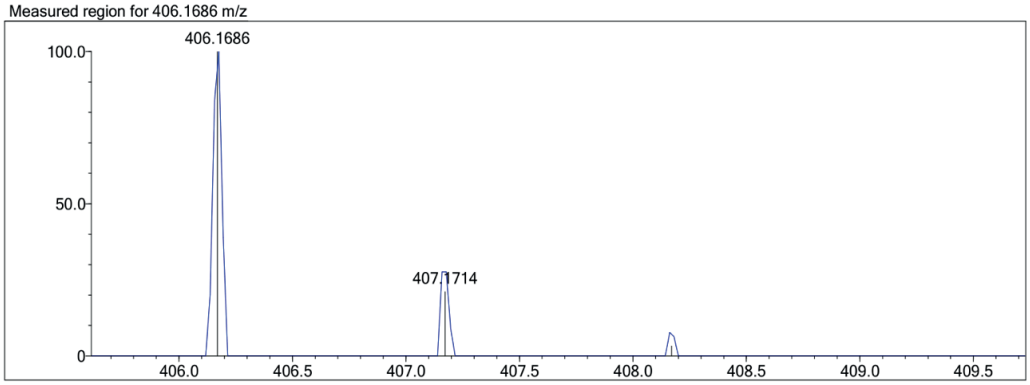
DBE Range: 0.0 - 30.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 50

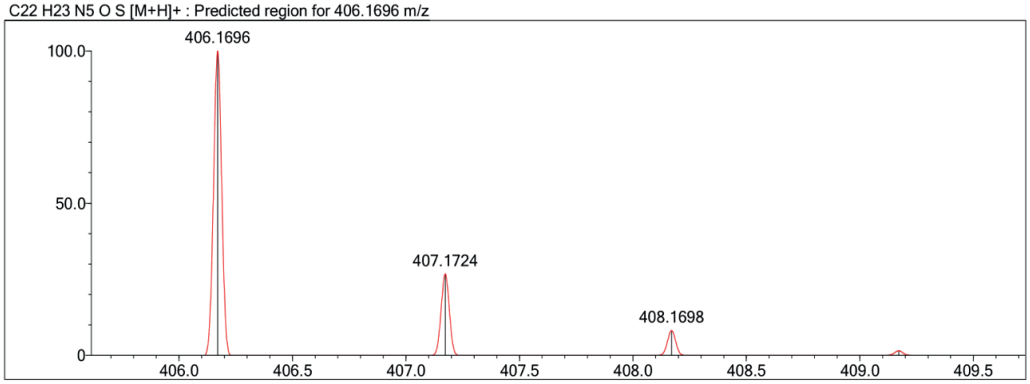
Event#: 1 MS(E+) Ret. Time : 4.573 -> 4.573 Scan# : 687 -> 687



Measured region for 406.1686 m/z



C22 H23 N5 O S [M+H]+ : Predicted region for 406.1696 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	81.39	C22 H23 N5 O S	[M+H]+	406.1686	406.1696	-1.0	-2.46	84.48	14.0

Figure S3. Compound **3a** HRMS report

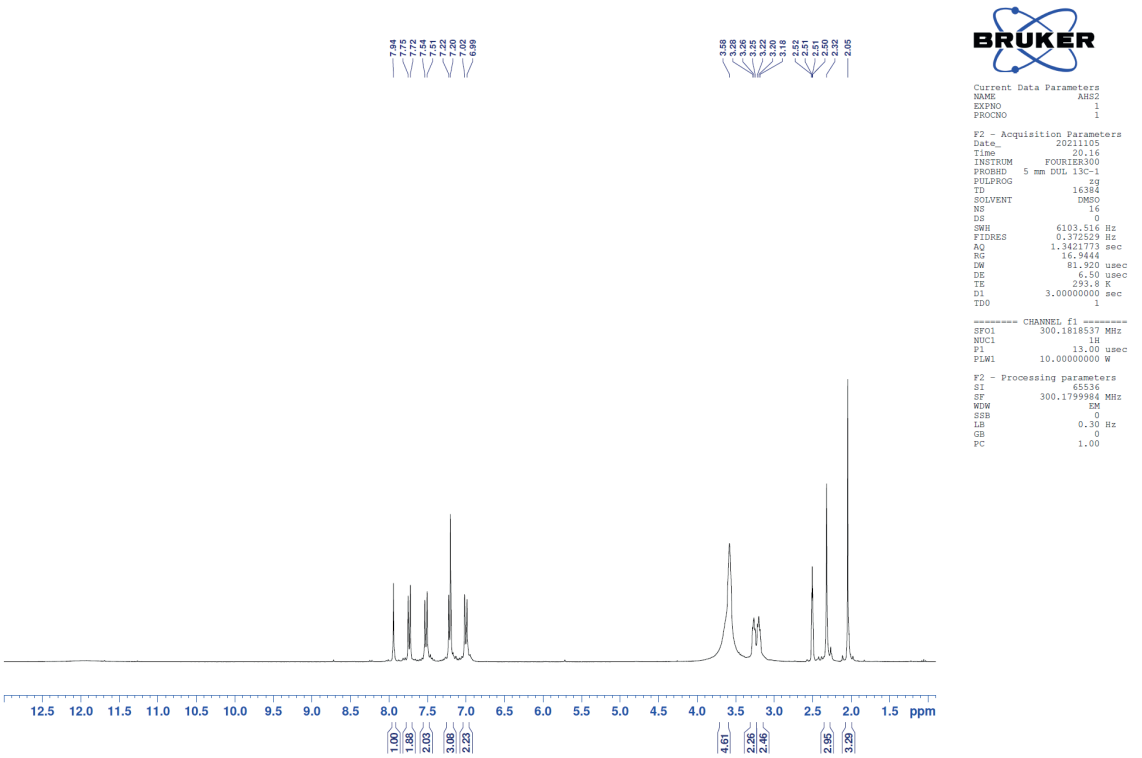


Figure S4. Compound **3b** ^{1}H-NMR spectrum

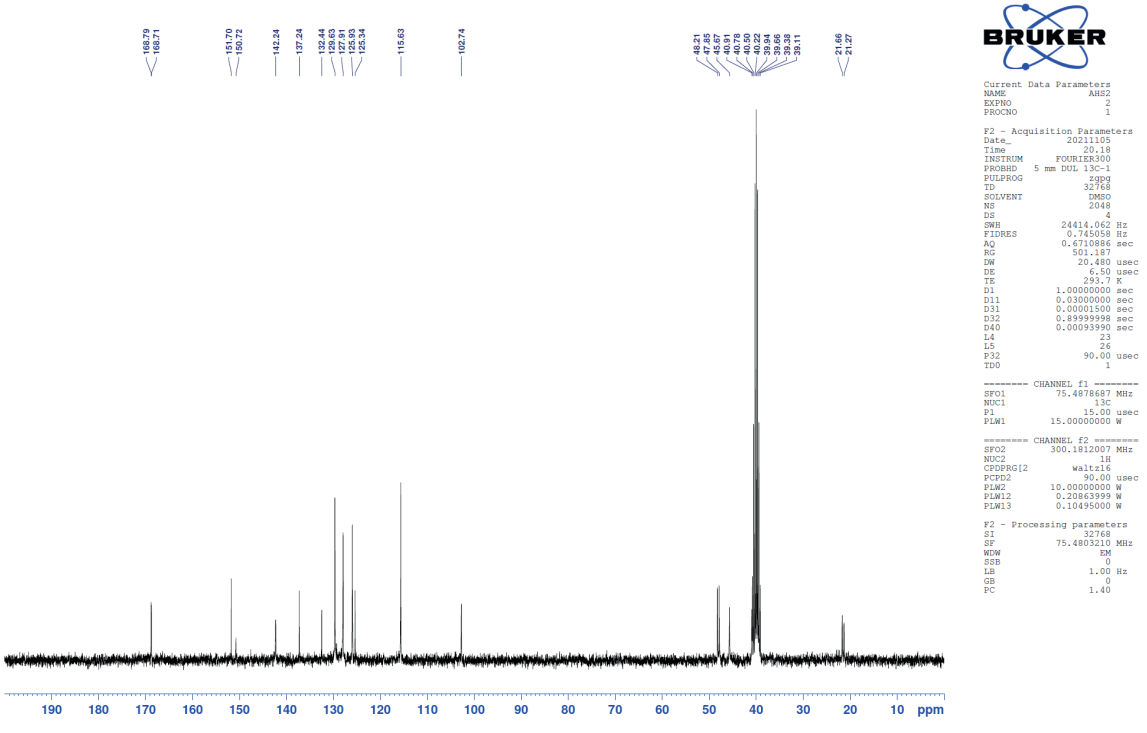


Figure S5. Compound **3b** ^{13}C-NMR spectrum

Formula Predictor Report - AHS-2_112.lcd Page 1 of 1

Data File: C:\LabSolutions\Data\Analiz\derya\AHS-2_112.lcd

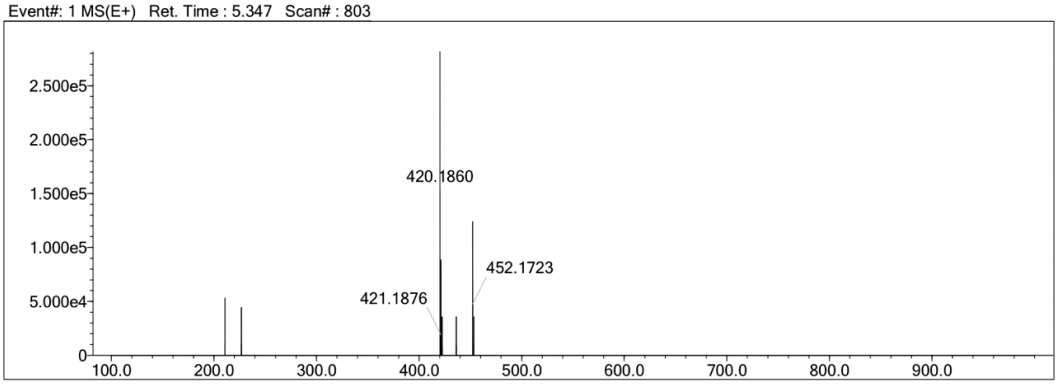
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	6	46	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	5	36	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

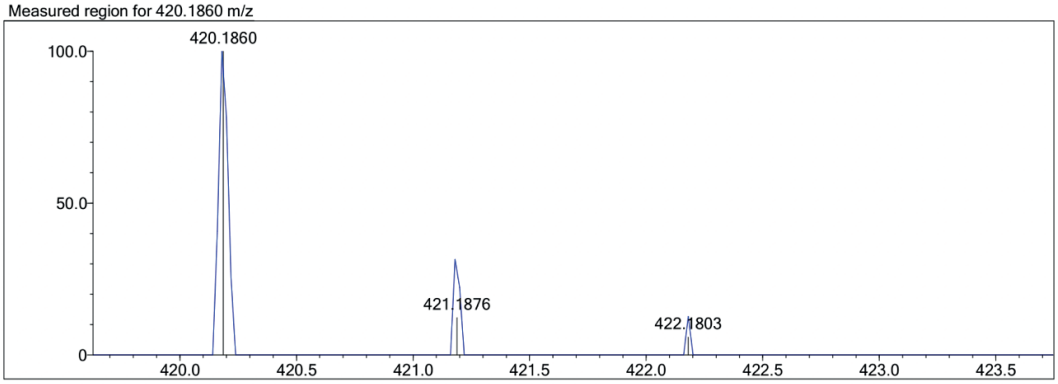
DBE Range: 0.0 - 30.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 50

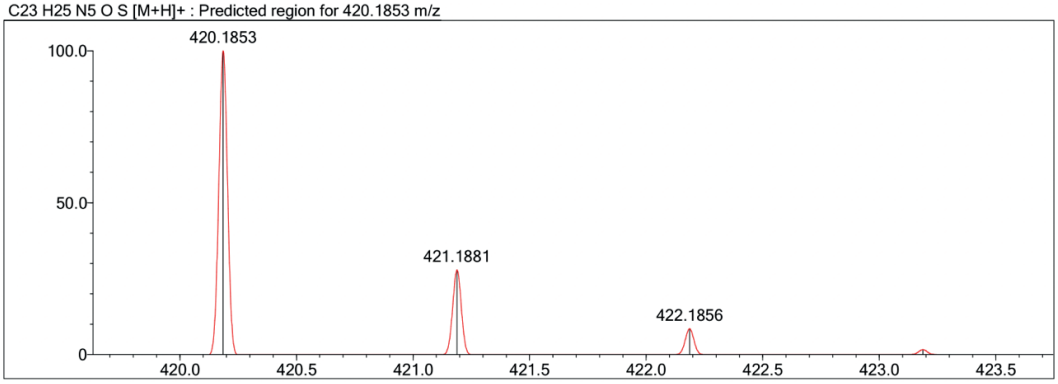
Event#: 1 MS(E+) Ret. Time : 5.347 Scan# : 803



Measured region for 420.1860 m/z



C23 H25 N5 O S [M+H]+ : Predicted region for 420.1853 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	68.79	C23 H25 N5 O S	[M+H]+	420.1860	420.1853	0.7	1.67	69.96	14.0

Figure S6. Compound **3b** HRMS report

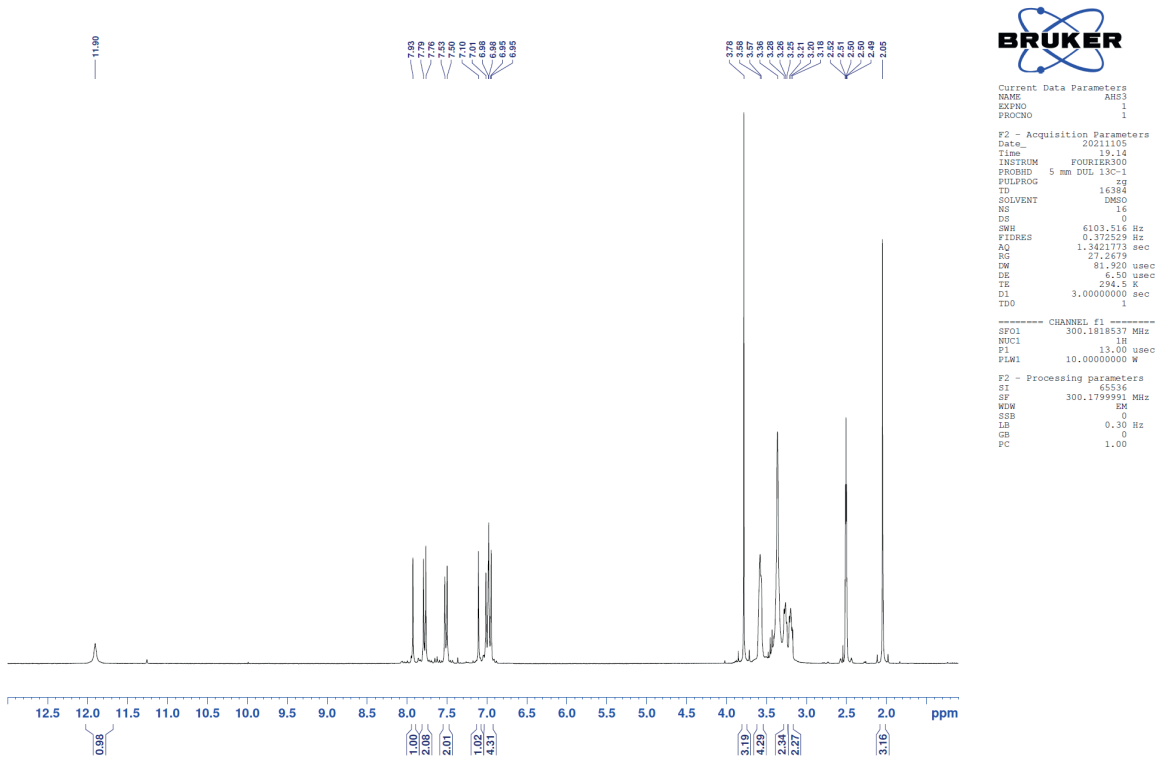


Figure S7. Compound **3c** [1]H-NMR spectrum

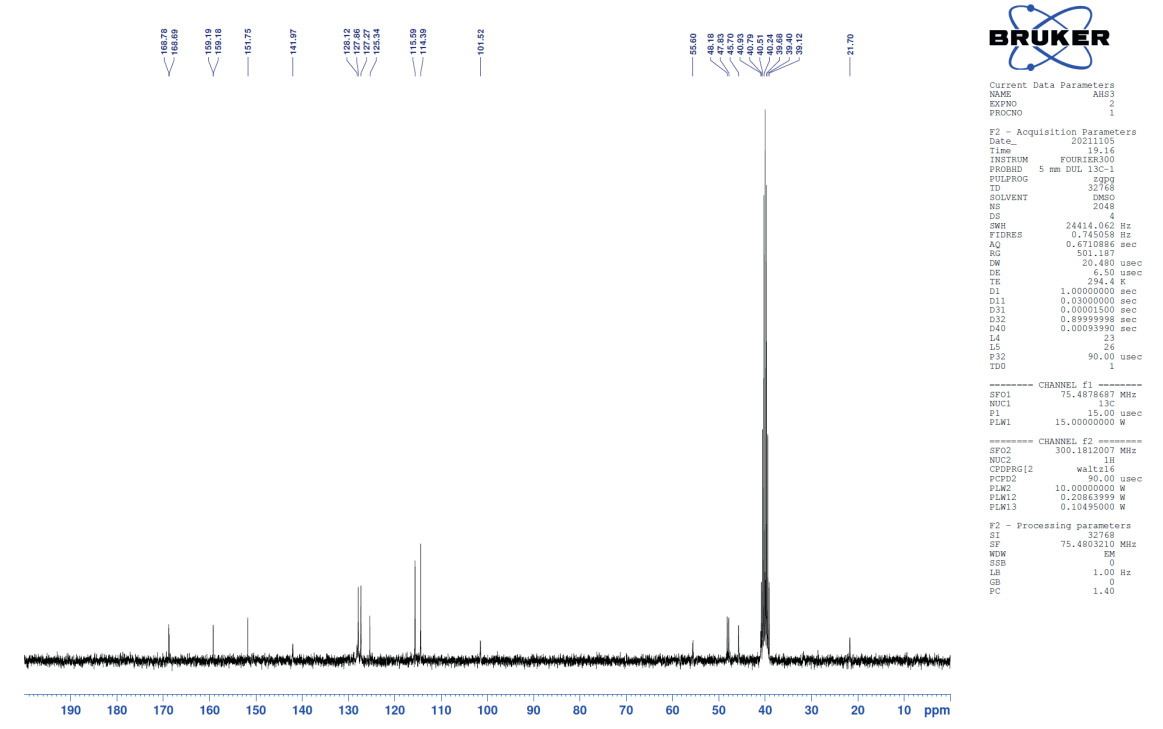


Figure S8. Compound **3c** [13]C-NMR spectrum

Formula Predictor Report - AHS-3_104.lcd Page 1 of 1

Data File: C:\LabSolutions\Data\Analiz\derya\AHS-3_104.lcd

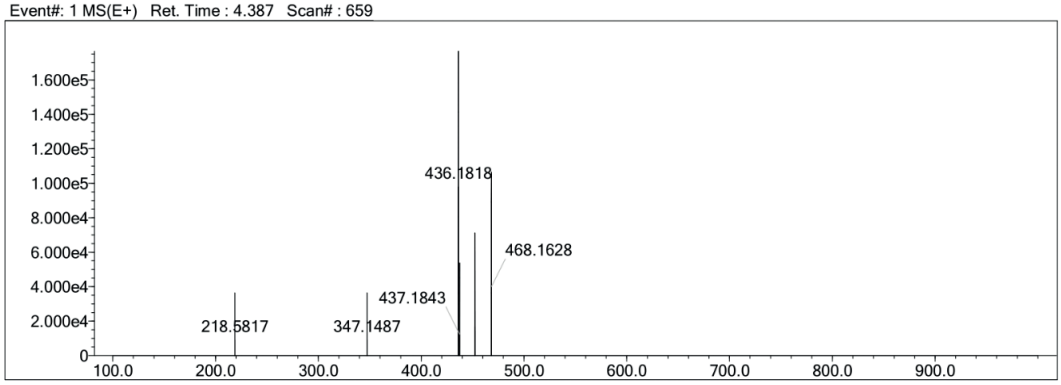
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	6	46	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	5	36	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

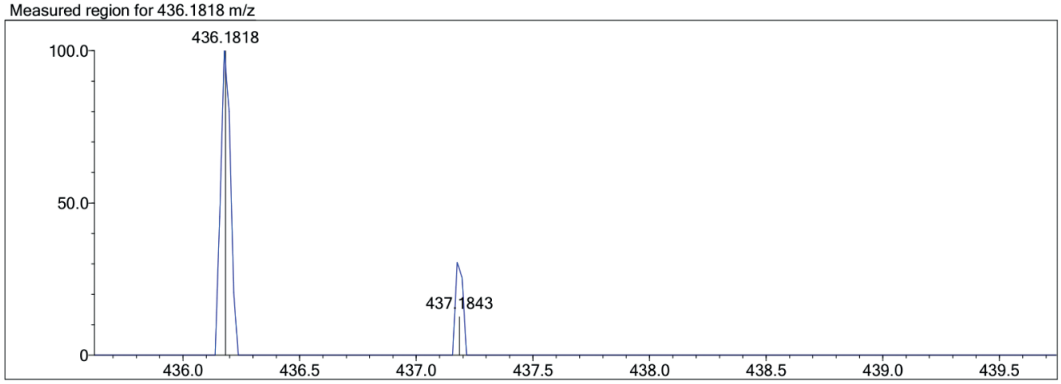
DBE Range: 0.0 - 30.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 50

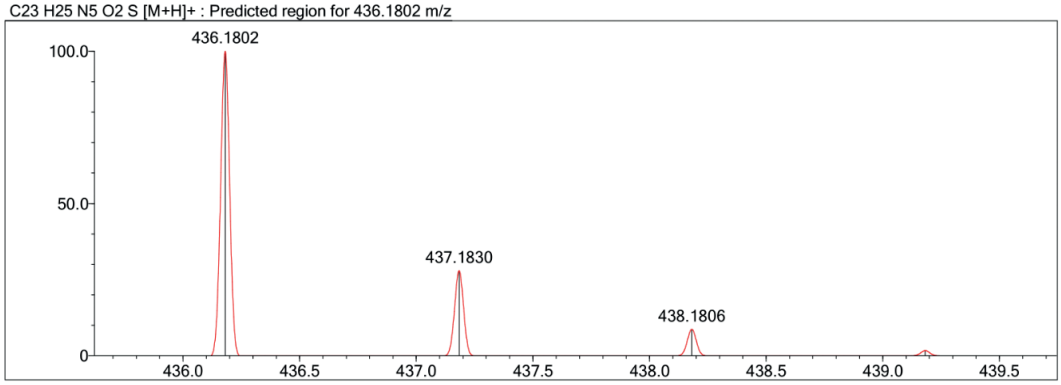
Event#: 1 MS(E+) Ret. Time : 4.387 Scan# : 659



Measured region for 436.1818 m/z



C23 H25 N5 O2 S [M+H]+ : Predicted region for 436.1802 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	0.00	C23 H25 N5 O2 S	[M+H]+	436.1818	436.1802	1.6	3.67	0.00	14.0

Figure S9. Compound **3c** HRMS report

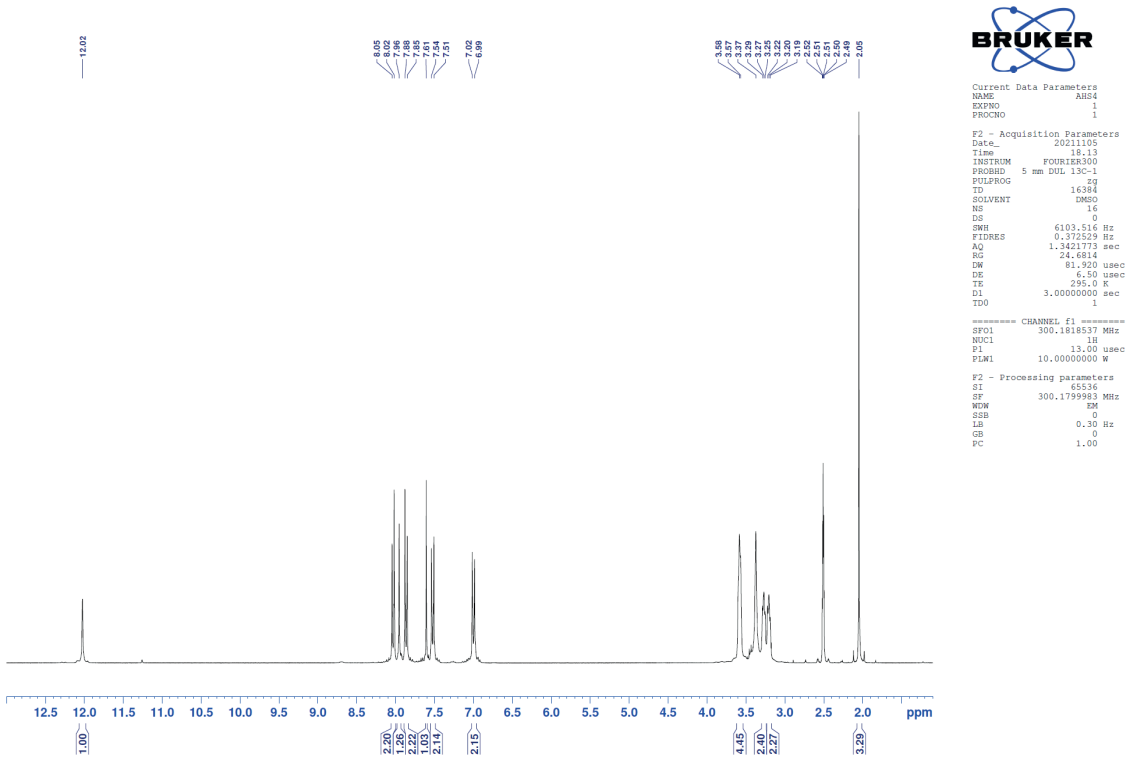


Figure S10. Compound **3d** ^{1}H-NMR spectrum

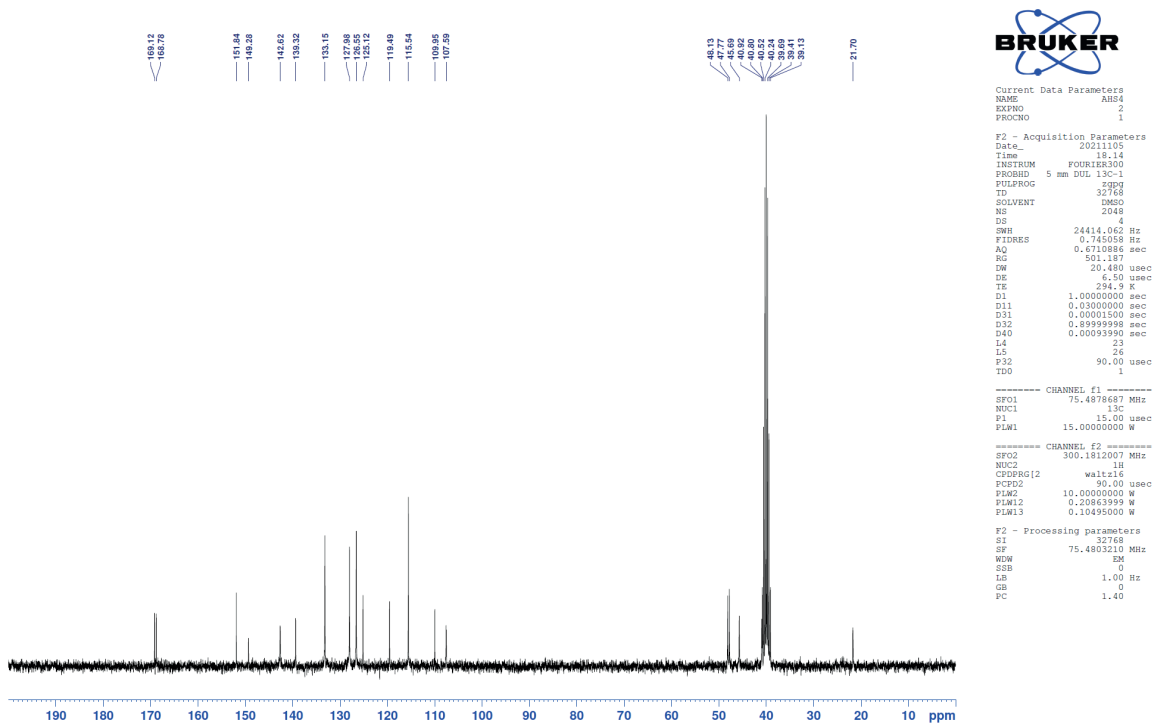


Figure S11. Compound **3d** ^{13}C-NMR spectrum

Formula Predictor Report - AHS-4_114.lcd Page 1 of 1

Data File: C:\LabSolutions\Data\Analiz\derya\AHS-4_114.lcd

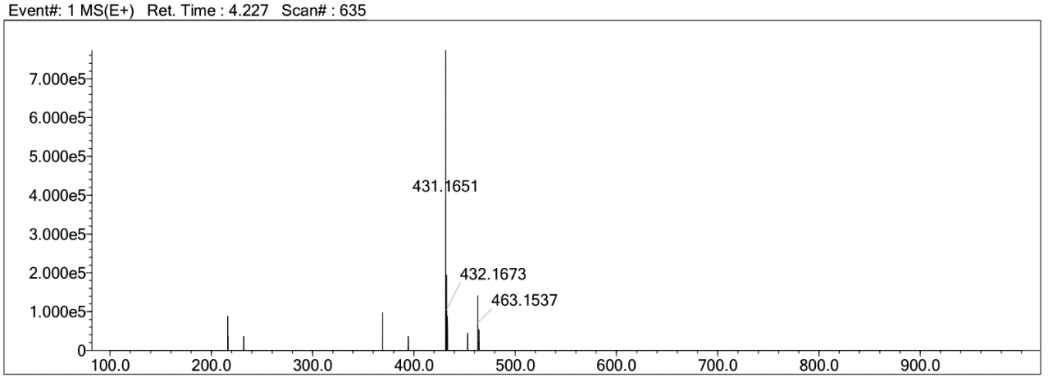
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	6	46	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	5	36	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

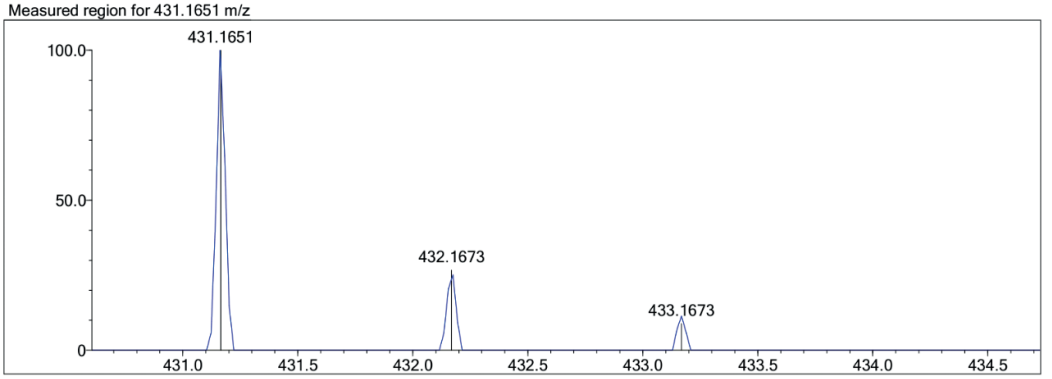
DBE Range: 0.0 - 30.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 50

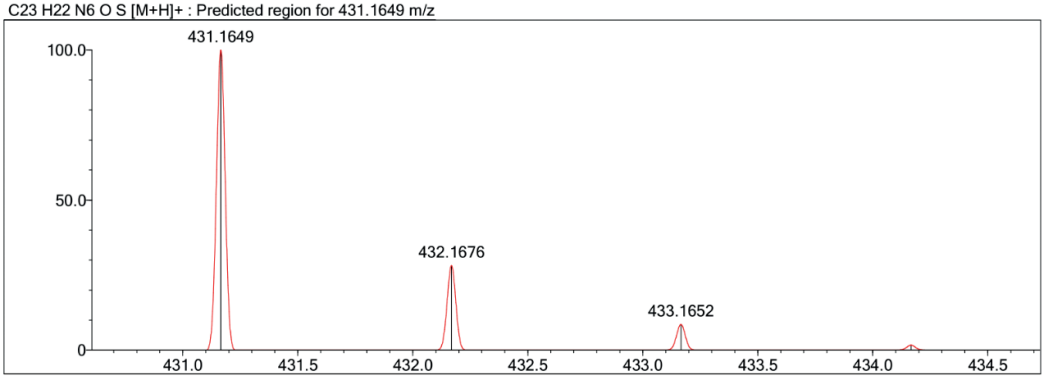
Event#: 1 MS(E+) Ret. Time : 4.227 Scan# : 635



Measured region for 431.1651 m/z



C23 H22 N6 O S [M+H]+ : Predicted region for 431.1649 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	77.87	C23 H22 N6 O S	[M+H]+	431.1651	431.1649	0.2	0.46	77.87	16.0

Figure S12. Compound **3d** HRMS report

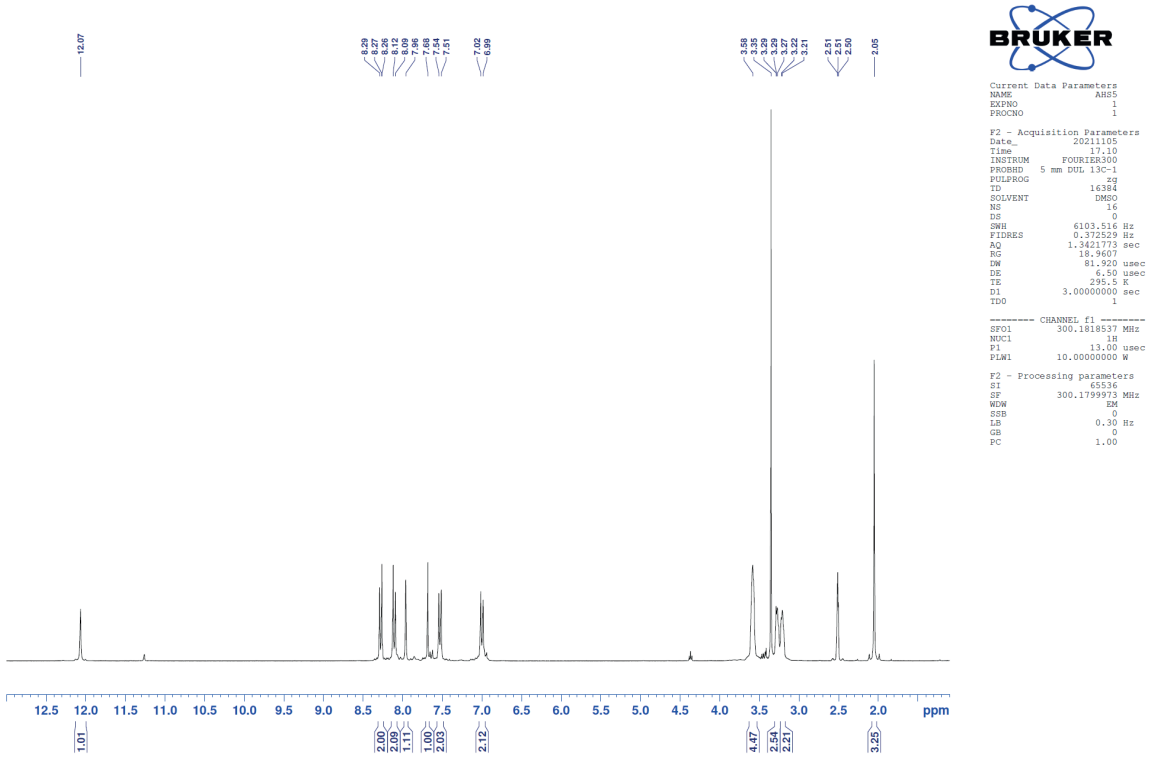


Figure S13. Compound **3e** ^{1}H-NMR spectrum

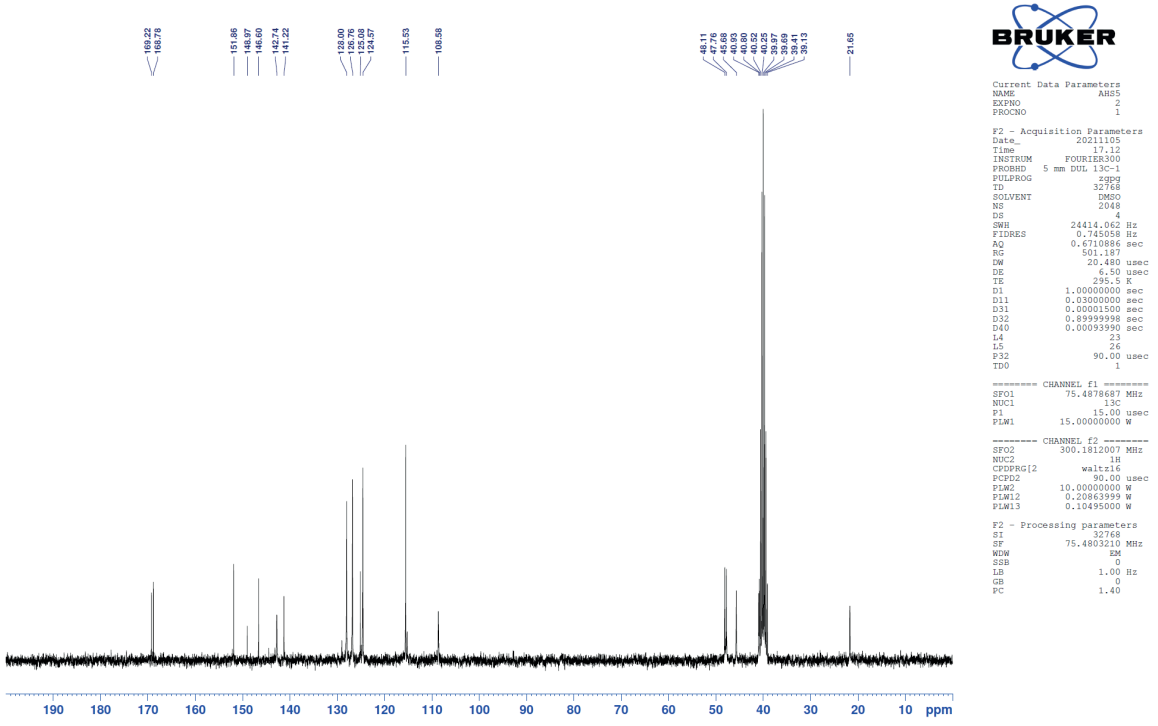


Figure S14. Compound **3e** ^{13}C-NMR spectrum

Formula Predictor Report - AHS-5_105.lcd Page 1 of 1

Data File: C:\LabSolutions\Data\Analiz\derya\AHS-5_105.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	6	46	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	5	36	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 50

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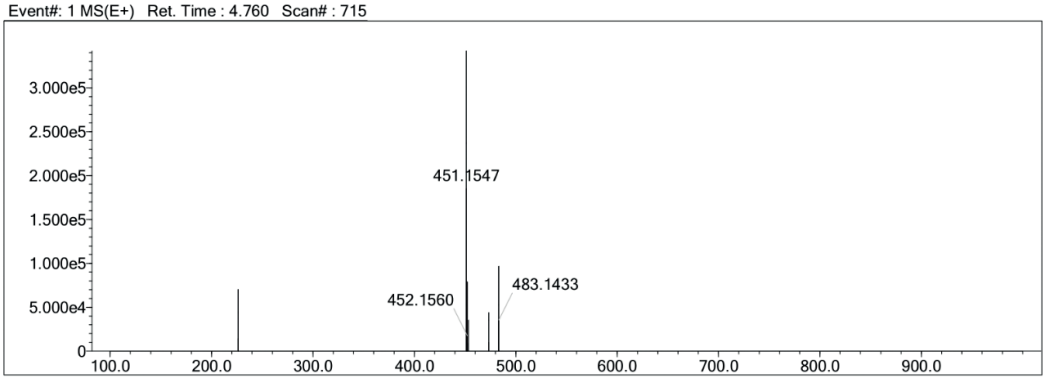
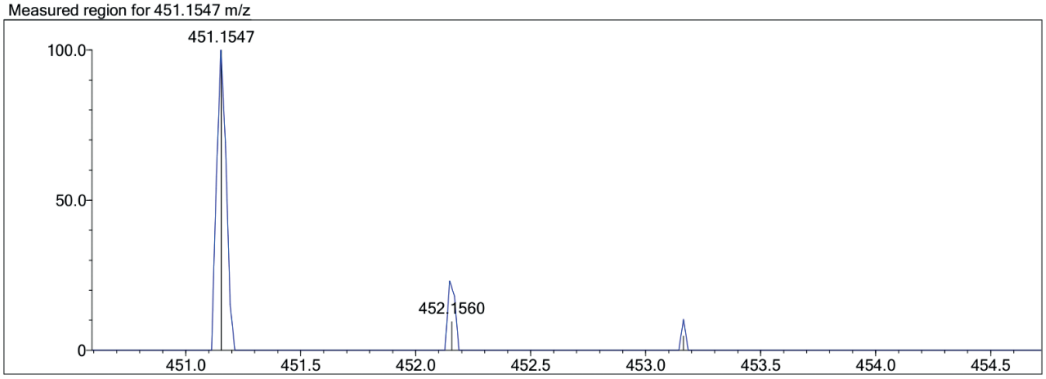
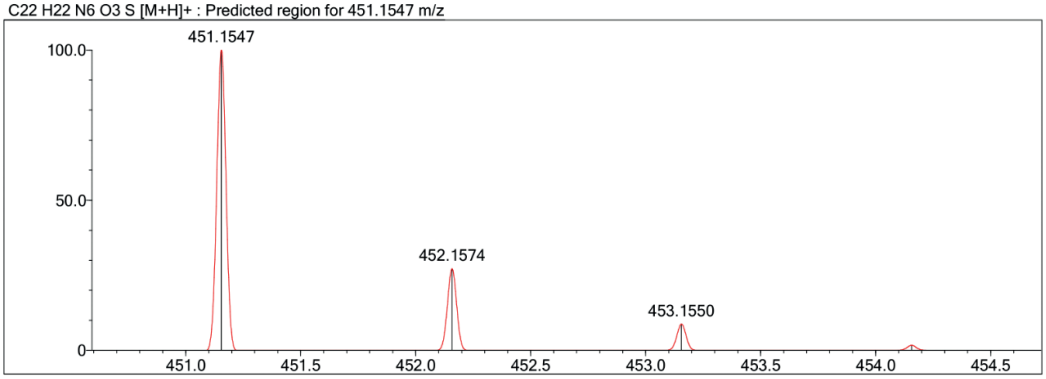


Measured region for 451.1547 m/z



C22 H22 N6 O3 S [M+H]+ : Predicted region for 451.1547 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	56.75	C22 H22 N6 O3 S	[M+H]+	451.1547	451.1547	0.0	0.00	56.75	15.0

Figure S15. Compound **3e** HRMS report

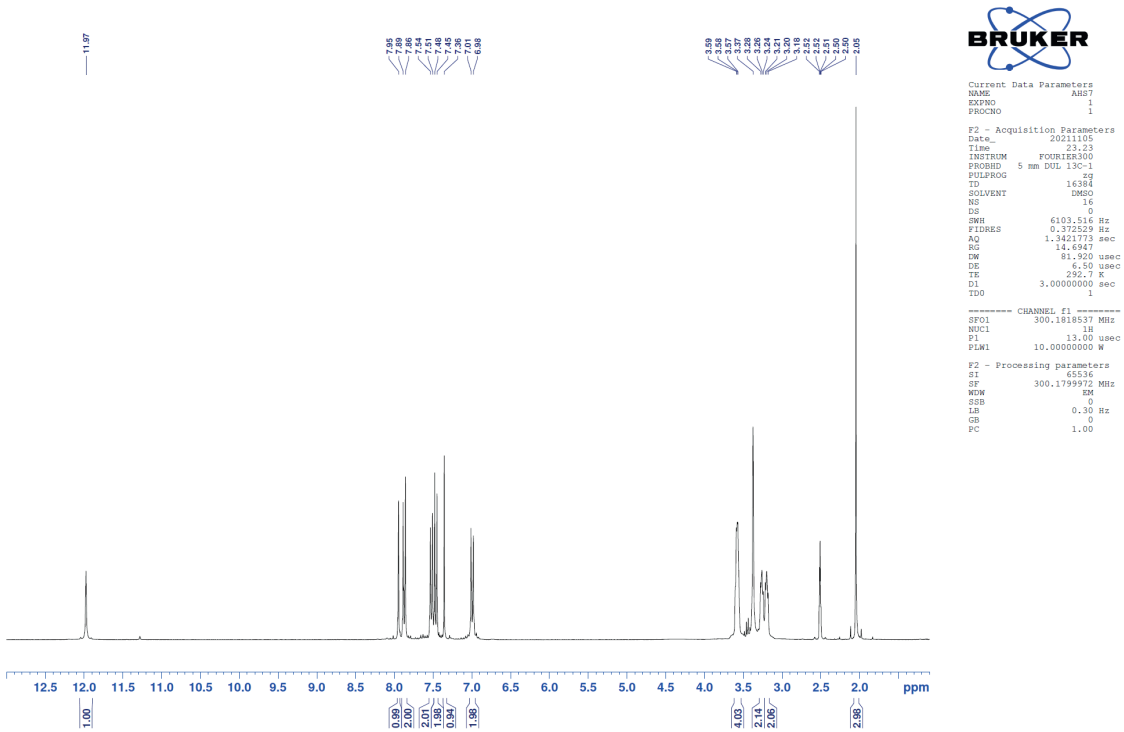


Figure S16. Compound **3f** ^{1}H-NMR spectrum

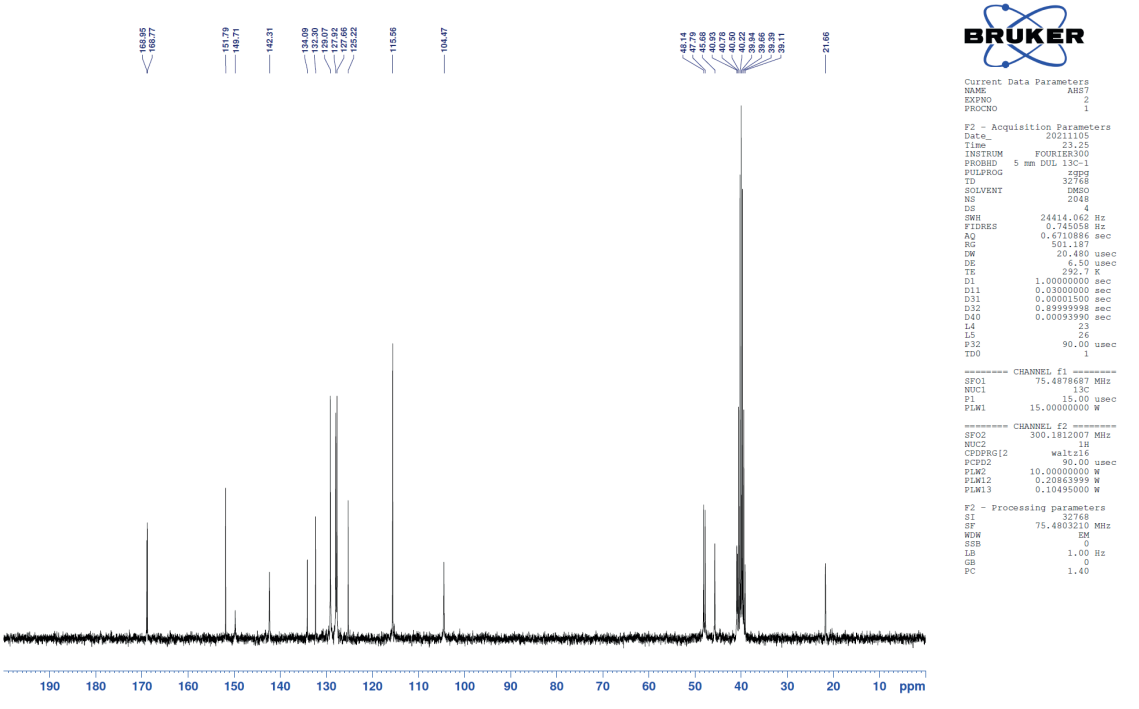


Figure S17. Compound **3f** ^{13}C-NMR spectrum

Formula Predictor Report - AHS-7_116.lcd Page 1 of 1

Data File: C:\LabSolutions\Data\Analiz\derya\AHS-7_116.lcd

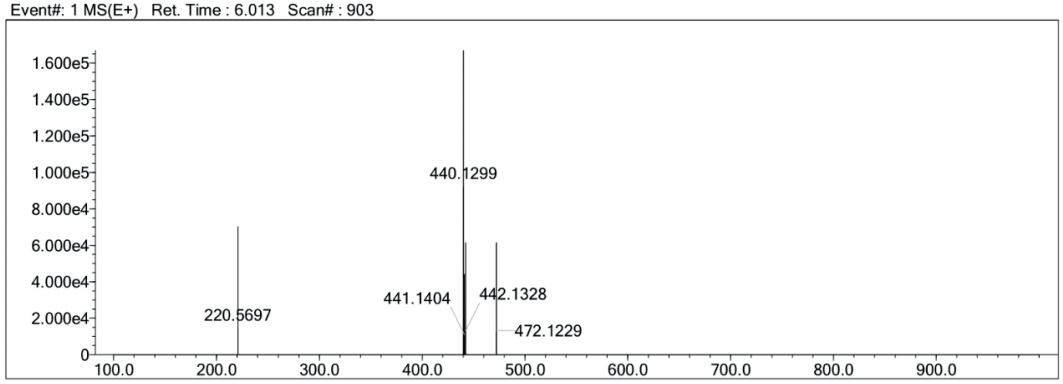
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	6	46	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	5	36	F	1	0	0	Cl	1	1	1	Pd	2	0	0	
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

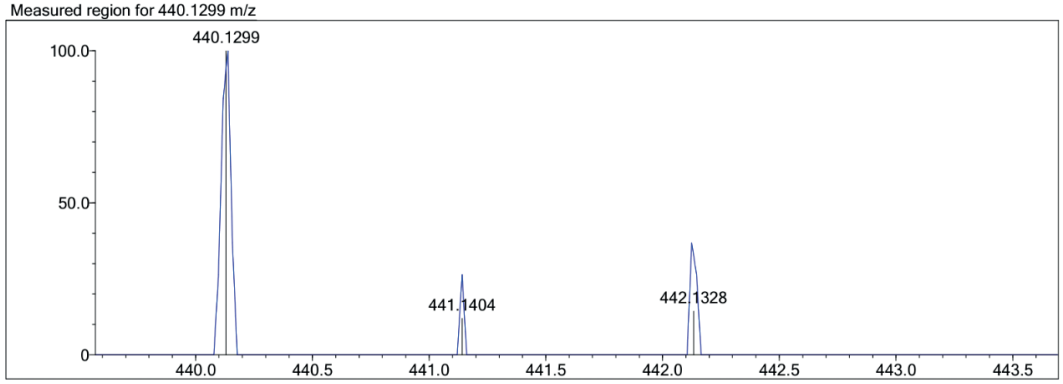
DBE Range: 0.0 - 30.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 50

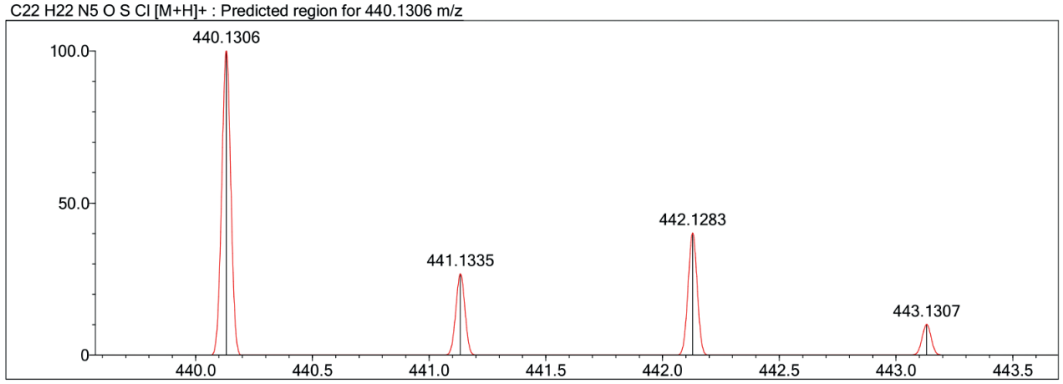
Event#: 1 MS(E+) Ret. Time : 6.013 Scan# : 903



Measured region for 440.1299 m/z



C22 H22 N5 O S Cl [M+H]+ : Predicted region for 440.1306 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	50.93	C22 H22 N5 O S Cl	[M+H]+	440.1299	440.1306	-0.7	-1.59	51.70	14.0

Figure S18. Compound **3f** HRMS report

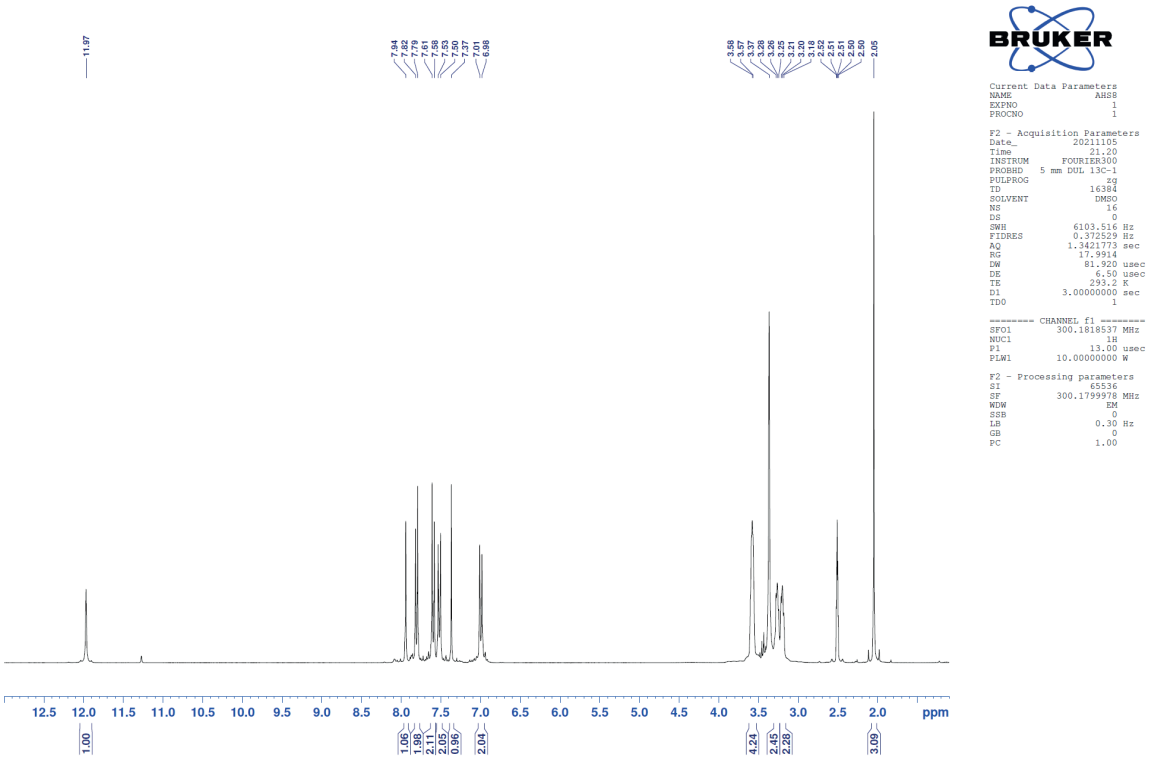


Figure S19. Compound **3g** ^{1}H-NMR spectrum

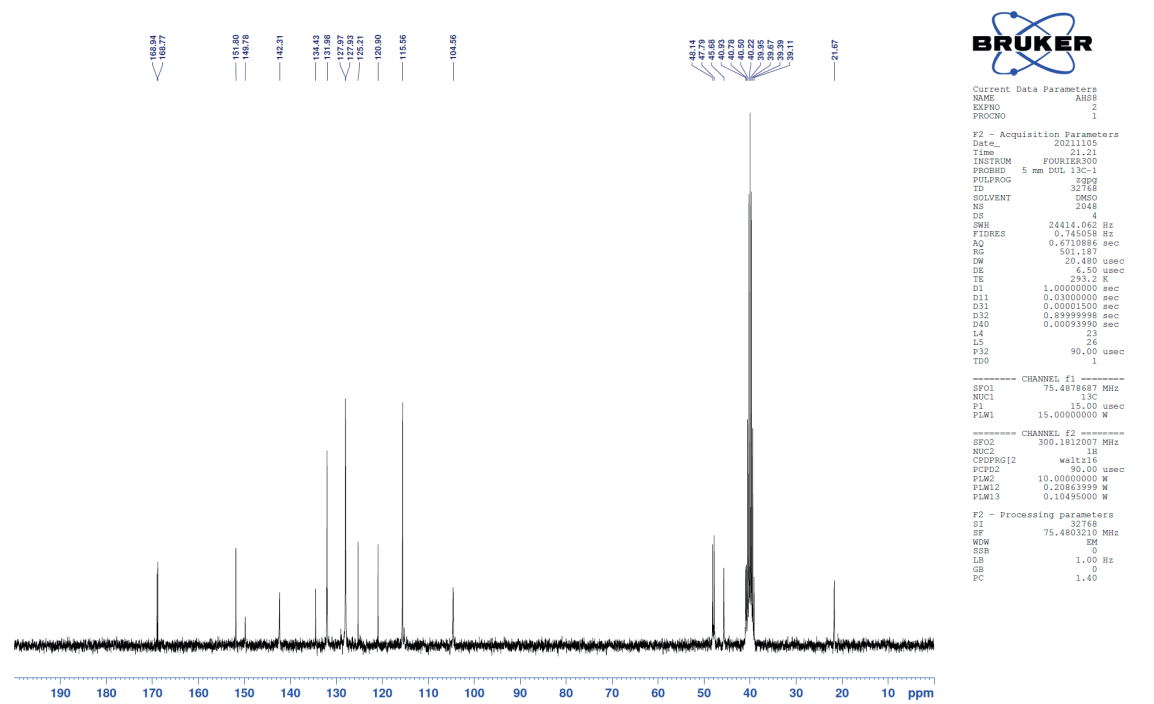


Figure S20. Compound **3g** ^{13}C-NMR spectrum

Formula Predictor Report - AHS-8_455.lcd Page 1 of 1

Data File: C:\LabSolutions\Data\Analiz\derya\AHS-8_455.lcd

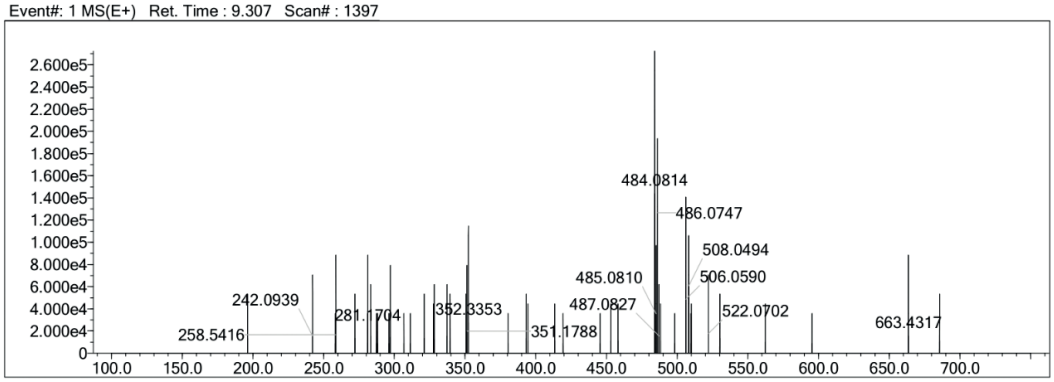
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	6	46	O	2	0	3	S	2	1	1	Ru	2	0	0	H
C	4	5	36	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	1	1	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

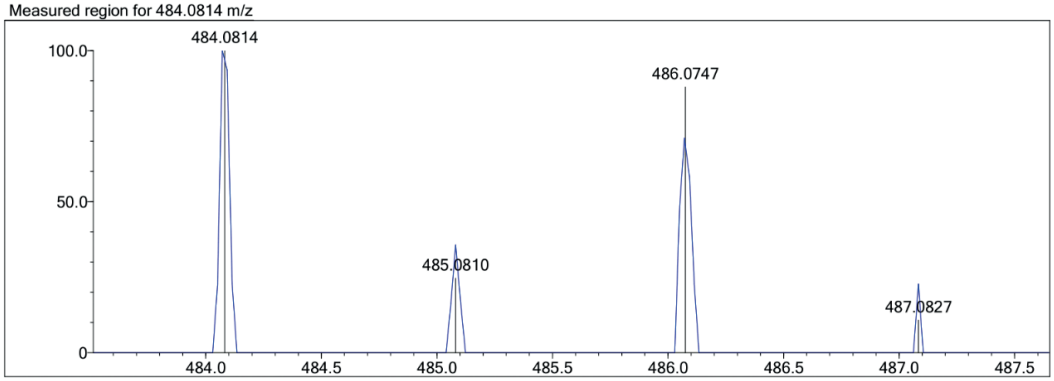
DBE Range: 0.0 - 30.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 50

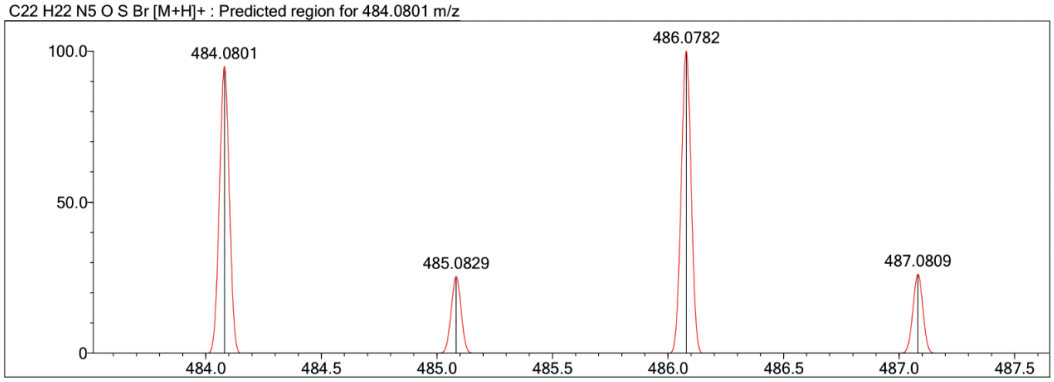
Event#: 1 MS(E+) Ret. Time : 9.307 Scan# : 1397



Measured region for 484.0814 m/z



C22 H22 N5 O S Br [M+H]+ : Predicted region for 484.0801 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	50.89	C22 H22 N5 O S Br	[M+H]+	484.0814	484.0801	1.3	2.69	53.13	14.0

Figure S21. Compound **3g** HRMS report

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GRADUATE SCHOOL OF HEALTH SCIENCES

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Research Article

An observational study on drug interactions caused by proton pump inhibitors

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ABSTRACT

Proton pump inhibitors (PPIs) are widely used to treat gastric acid-related diseases worldwide and in our country. The high reliability of PPIs also allows long-term use for appropriate indications in chronic diseases, which increases the possibility of drug-drug interactions. Therefore, it is clear that the usage of PPIs should be monitored in terms of drug-drug interactions to provide drug treatment success and patient safety. The ability of pharmacists, the closest health consultants, to identify these interactions during prescription fulfillment will significantly contribute to treatment success. Although many studies deal with the rational use of PPIs and drug interactions, the number of studies revealing observational drug interactions is minimal. This research aims to determine drug-drug interactions frequently encountered in community pharmacies for PPIs, which interact with many drug groups and are commonly prescribed. For this purpose, approximately 1700 prescriptions supplied by a selected community pharmacy, serving in Van, were examined. One hundred sixty-four of the prescriptions were evaluated by considering the study's limitations. Drug-drug interactions were checked by three different electronic database. It was determined that 73 of 164 prescriptions had interactions in at least one of the three databases. In 73 prescriptions, 86 drug interactions were observed. 34% of the interactions detected in the study were caused by lansoprazole.

Keywords: Comunity Pharmacy, Drug Interaction, Proton Pomp Inhibitors

1. INTRODUCTION

Proton Pump Inhibitors (PPIs) are widely used in treatment of gastro-oesophageal reflux disease (GERD), peptic ulcer, erosive esophagitis, Helicobacter pylori (HP) eradication, dyspepsia, and Zollinger-Ellison syndrome. In addition, PPIs are used to reduce the incidence of gastric ulcers due to non-steroidal anti-inflammatory drugs (NSAIDs) and to reduce the risk of gastrointestinal bleeding in intensive care patients [1]. The metabolization of the PPIs is made by hepatic cytochrome P450 (CYP) and enzymes (CYP2C19 and CYP3A4). Inhibitors and inducers of these enzyme groups can cause drug interactions with PPIs [2].

Despite the almost excellent safety profiles of the proton pump inhibitors group drugs, the treatment of patients should be monitored frequently, and caution should be exercised due to polypharmacy that may occur as a result of not being used in appropriate indications and using them for unnecessary long-term treatment. In this way, the most critical safety problem caused by excessive PPI use for a long time

is drug interactions [3]. As Johnson et al. stated, the most important reason that causes interactions in terms of PPIs is prescribing at high doses and for extended periods [4].

Encountering clinically relevant drug-drug interactions with PPIs is not common [2]. However, the ability of pharmacists, the closest health consultants, to identify these interactions during prescription fulfillment will significantly contribute to treatment success. Although many studies deal with the rational use of PPIs and drug interactions, the number of studies revealing observational drug interactions is limited. This research aims to determine drug-drug interactions frequently encountered in community pharmacies with PPIs, which interact with many drug groups and are commonly prescribed.

2. MATERIALS AND METHODS

Within the scope of this study, prescriptions containing proton pump inhibitors received between 15 December 2021 and 15 May 2022 at the "Bölge Pharmacy" serving in the Van were examined by the researchers in terms of drug interactions. In the study, the ICD-10 diagnostic code of the prescriptions, the specialty of the prescribing physician, the gender and age of the patient, how many items of medication were written on the prescription, whether there was a drug-drug interaction in the prescription, and if there was an interaction, which drugs were interacted with and the degree of interaction were collected. Potential drug interactions between prescription drugs were carried out in an electronic environment called Medscape, RxMediaPharma, and TEBRP programs.

Prescriptions containing at least one PPI and one different drug without PPI were included in the study. As a result of the evaluation with the pharmacist, it was determined that approximately 30-40 prescriptions meeting the relevant criteria were met monthly in the pharmacy. Additionally, in studies with similar study designs, the number of evaluated prescriptions was determined according to affiliated pharmacies' filling prescription rates, and almost 100-200 prescriptions were investigated. In this regard, 1700 prescriptions filled by the Bölge Pharmacy were evaluated. One hundred sixty-four prescriptions were evaluated by considering the study's inclusion criteria.

This study was conducted after Van Yüzüncü Yıl University Non-interventional Research Ethics Committee has approved the study ethically (Date:19/11/2021, Decision No: 2021/12-16).

3. RESULTS AND DISCUSSION

In the study, 164 prescriptions were evaluated, and 55 % were prescribed for women. The distribution of the prescribed physicians' specialty areas is given in Figure 1. It is seen that internal medicine specialists mainly prescribe PPIs and practitioners follow them. As presented in Öncü et al.'s study, it is known that internists frequently prescribe PPIs. It is also noteworthy that 55-80% of prescriptions do not have an appropriate indication for using PPIs [5].

Evaluated 164 prescriptions contain all PPIs available on Turkish pharmaceutical market (lansoprazole, pantoprazole, esomeprazole, omeprazole and rabeprazole). The distribution of prescribed PPIs is illustrated in Figure 2.

As can be seen from Figure 2, the most prescribed PPI was lansoprazole and followed by pantoprazole. In this study, prescription rate of the esomenprazole was low, in contrast to Arı et al. [6].

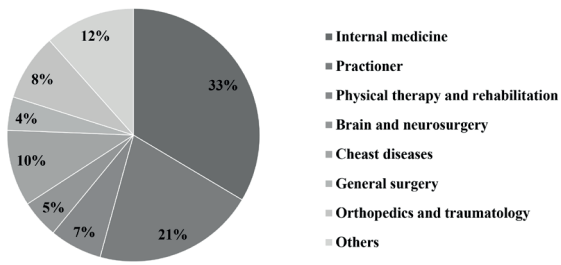

Figure 1. Prescribed physicians' specialty areas

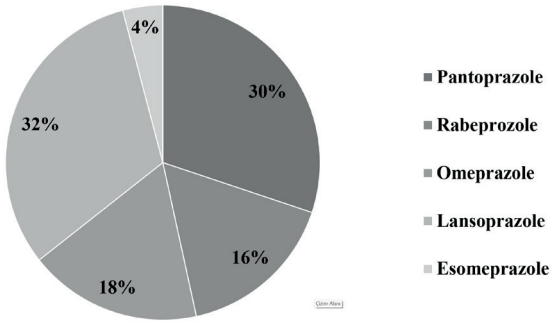

Figure 2. Distribution of the prescribed PPIs

Additionally, ICD-10 codes of these 164 prescriptions were evaluated. 60% of them had gastroesegial reflux, 18% had peptic ulcer, 8% had irreparable bowel syndrome, 4% had dyspepsia, 4% had indigestion, 1% had helicobacter pylori code.

It was determined that 73 of 164 prescriptions had interactions in at least one of the three databases. In 73 prescriptions, 86 drug interactions were observed. As stated in various literature, the findings obtained from the databases show differences. In Table 1, interactions with lansoprazole are presented.

34% of the interactions detected in the study were caused by lansoprazole. In light of the information presented in Table 1, interactions with lansoprazole were seen differently in the three databases. Among the interactions, only lansoprazole and methotrexate interaction were detected by three databases. The most common interaction between lansoprazole and acetaminophen was seen at a minor level. Table 2 summarizes detected interactions with pantoprazole.

28% of the interactions detected in the study were caused by pantoprazole. According to Table 2, no interactions were detected by Medscape; however, 24 "minor" level interactions were found by TEBRP. Only pantoprazole and rifaximin interaction was detected by RxMediaPharma and TEBRP similarly. Table 3 outlines interactions with esomeprazole.

Parallel to the prescription rate, only 7% of interactions are related to esomeprazole. Table 3 indicates that interactions caused by esomeprazole were detected only by TEBRP and were minor. Interactions with omeprazole are given in Table 4.

14% of the interactions detected in the study were caused by omeprazole. Table 4 outlined that interactions were generally at a minor level. Only interaction with ciprofloxacin (prescribed for Helicobactor pylori) was detected as monitor closely in Medscape. Lastly, interactions with rabeprazole are presented in Table 5.

Rabeprazole caused 17% of the interactions in the study. The only interaction with cefpodoxime is detected as monitor closely in Medscape. Other interactions were detected only in TEBRP and were minor.

Table 1. Interactions with lansoprazole

Drug pairs	Frequency	Medscape	RxMediaPharma	TEBRP
Lansoprazole-diclofenac	1	No interactions	No interactions	Minor
Lansoprazole-methotrexate	1	Monitor Closely	Minor	Minor
Lansoprazole-bethametazon	1	No interactions	No interactions	Minor
Lansoprazole-dextromethorpan	1	No interactions	No interactions	Minor
Lansoprazole-clarithromycin	2	Monitor Closely	No interactions	Minor
Lansoprazole-ibuprofen	3	No interactions	No interactions	Minor
Lansoprazole-prednisolone	1	No interactions	No interactions	Minor
Lansoprazole-ciprofloxacin	3	No interactions	No interactions	Minor
Lansoprazole-sucralfate	3	Minor	No interactions	Minor
Lansoprazole-acetaminophen	10	No interactions	No interactions	Minor
Lansoprazole-Vitamin D	3	No interactions	No interactions	Minor

Table 2. Interactions with pantoprazole

Drug pairs	Frequency	Medscape	RxMediaPharma	TEBRP
Pantoprazole-metilprednisolone	1	No interactions	No interactions	Minor
Pantoprazole-amoxicillin	1	No interactions	No interactions	Minor
Pantoprazole-ciprofloxacin	1	No interactions	No interactions	Minor
Pantoprazole-rifaximin	1	No interactions	Minor	Minor
Pantoprazole-diclofenac	3	No interactions	No interactions	Minor
Pantoprazole-bethametazon	1	No interactions	No interactions	Minor
Pantoprazole-metoprolol	1	No interactions	No interactions	Minor
Pantoprazole-ibuprofen	1	No interactions	No interactions	Minor
Pantoprazole-Vitamin D	3	No interactions	No interactions	Minor
Pantoprazole-famotidine	4	No interactions	No interactions	Minor
Pantoprazole-domperidone	2	No interactions	No interactions	Minor
Pantoprazole-acetaminophen	5	No interactions	No interactions	Minor

Table 3. Interactions with esomeprazole

Drug pairs	Frequency	Medscape	RxMediaPharma	TEBRP
Esomeprazole-Vitamin D	2	No interactions	No interactions	Minor
Esomeprazole-amoxicillin	2	No interactions	No interactions	Minor
Esomeprazole-diclofenac	1	No interactions	No interactions	Minor
Esomeprazole-ibuprofen	1	No interactions	No interactions	Minor

Table 4. Interactions with omeprazole

Drug pairs	Frequency	Medscape	RxMediaPharma	TEBRP
Omeprazole-piroxicam	1	No interactions	No interactions	Minor
Omeprazole-acetaminophen	1	No interactions	No interactions	Minor
Omeprazole-Vitamin D	1	No interactions	No interactions	Minor
Omeprazole-lidocaine	1	Minor	No interactions	Minor
Omeprazole-etodolac	2	No interactions	No interactions	Minor
Omeprazole-flurbiprofen	1	No interactions	No interactions	Minor
Omeprazole-acetylsalicyclic acid	1	No interactions	No interactions	Minor
Omeprazole-ciprofloxacin	1	Monitor Closely	No interactions	Minor
Omeprazole-rifampicin	2	No interactions	No interactions	Minor
Omeprazole-doxycycline	1	No interactions	No interactions	Minor

As Özdemir et al. stated, PPIs are frequently used to prevent complications related to NSAIDs, but side effects associated with concomitant use are also encountered [7]. The findings obtained in the present study also support this.

Findings from this study, similar to [8-10], suggest that omeprazole and its isomer, esomeprazole, are unlikely to cause major drug interactions especially in the treatment of Helicobacter pylori.

TEBRP was the one that gave the most interaction warnings among these three databases. In TEBRP, it was observed that all of the interactions were at the "minor" level. In the research conducted in Medscape, six interactions were detected, of which four were at the "monitor closely" level and two at the "minor" level. Two interactions at the "minor" level were detected in the search performed with RxMediaPharma. This situation is similar to many studies in the literature dealing with the consistency

Table 5. Interactions with rabeprazole

Drug pairs	Frequency	Medscape	RxMediaPharma	TEBRP
Rabeprazole-acetaminophen	3	No interactions	No interactions	Minor
Rabeprazole-ibuprofen	2	No interactions	No interactions	Minor
Rabeprazole-cefpodoxime	1	Monitor Closely	No interactions	Minor
Rabeprazole-domperidone	5	No interactions	No interactions	Minor
Rabeprazole-rifampicin	1	No interactions	No interactions	Minor
Rabeprazole-trimethoprim	1	No interactions	No interactions	Minor
Rabeprazole-azelastine	1	No interactions	No interactions	Minor
Rabeprazole-Vitamin D	1	No interactions	No interactions	Minor

of databases in detecting drug interactions [11-13]. However, it should be noted that the difference observed in this study is much more than in the literature. For this reason, it is vital to use more than one database to determine drug interactions in order to prevent possible adverse events and increase patient safety.

From a different point of view, when the interaction of PPIs is evaluated from an economic perspective, it can be said that there are some changes from the relevant literature. In a study conducted by Bilgener on PPI use between 2006 and 2011, it was emphasized that omeprazole was less costly among PPI, but physicians prescribed more expensive PPI [14]. Özdemir et al. also support this situation, in contrast a change in preference for omeprazole was found [7]. It is seen that the most preferred group after lansoprazole and pantoprazole is omeprazole (18%). However, it should be noted that the decrease in the use of lansoprazole, as stated in Bilgener [14], continues.

4. CONCLUSION

From the research that has been carried out, it is possible to conclude that PPIs appear relatively far from major drug interactions. On the other hand, however, due to the frequent prescription and irrational use of PPIs, healthcare professionals, especially pharmacists, need to be able to detect interactions caused by PPIs. It was determined that the interactions were mostly between PPIs and NSAIDs, vitamin D, and acetaminophen which are not clinically critical.

The main limitation of the observational result is evaluating only prescribed PPIs. However, it should be noted that this group is also assessed as OTC. Therefore, more deep studies should be conducted. Community pharmacists play an essential role in preventing prescribed interactions. Problems arising from drug interaction can be minimized by working in cooperation with physicians and pharmacists and allocating more time to pharmaceutical care services. In this regard, pharmacists should be a guide for patients. To increase the safety of PPIs in drug interactions, it is thought that pharmacists who have adopted themselves as lifelong learners, good healthcare providers, and good researchers will take an active role in the field. For this aim, preparing guidelines and disease algorithms will be helpful, especially for newly graduated pharmacists, and will positively affect the patients' treatment processes.

Acknowledgements

This study was created by expanding the graduation project that the first author completed at Van Yüzüncü Yıl University Faculty of Pharmacy under the supervision of the corresponding author. The initial findings of the study were presented at the "1. Ulusal ve Sosyal Eczacılık Kongresi" on 7-8 September 2022.

The authors would like to thank Pharm. Volkan AKTAŞ for providing the data used in the study.

Ethical approval

The study was approved by the Van Yüzüncü Yıl University Non-interventional Research Ethics Committee (Protocol no. 2021/12-16 / 19.11.2021).

Author contribution

Concept: BA, MA; Design: BA, MA; Supervision: MA; Materials: BA, MA; Data Collection and/or Processing: BA; Analysis and/or Interpretation: BA, MA; Literature Search: BA, MA; Writing: BA, MA; Critical Reviews: MA.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Research Article

Determination of potential probiotic properties of lactic acid bacteria isolated from colostrum milk

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ABSTRACT

Colostrum milk has been used as a source for isolating many probiotic bacteria since it contains many helpful probiotic microorganisms. Because of the huge microorganism diversity and functionality of colostrum, there is opportunity to isolate bacteria and analyze their probiotic potential. Comparing the diversity of probiotic bacteria in milk from postpartum periods is also crucial. In the current study, Lactic Acid Bacteria (LAB) cultures were isolated from cow colostrum milk. The 28 cultures were isolated, but only 2 were characterized as LAB by their colony and molecular characterization, Gram nature, catalase, antibiotics, and pepsin tolerance. As a result of molecular identification tests, the isolates were identified as *Lactobacillus casei* and *Lactobacillus paracasei* with 99-100% homology. These two isolates could survive in the presence of gastric and intestinal conditions. These isolates also showed antimicrobial and antioxidant activities. The results demonstrated that LAB species isolated from colostrum milk exhibited promising probiotic properties and seemed favorable for use in pharmaceuticals and foods.

Keywords: Colostrum, gastrointestinal tolerance, *Lactobacillus*, probiotic, postpartum milk

1. INTRODUCTION

Colostrum is the first milk produced by a female mammals (including human) after parturition. The World Health Organization suggests feeding all newborns with colostrum, a creamy yellow liquid that the mother secretes during birth and is high in many lacto-proteins [1]. It is also called postpartum or pre-milk because it is secreted by the mammary gland in the first 2-4 days of postpartum period. This fluid is essential for the newborn mammals transfer of passive immunity. Nutritionally, colostrum is rich in macronutrients (lipids, proteins, and growth factors), micronutrients (vitamins, minerals, oligosaccharides) and many unique nutritive compounds. It also plays a fundamental protection role with its valuable bioactive compound content, which includes immunoglobulins (Igs), lactoferrin (LF), lactoperoxidase, lysozyme, and cytokines. Bovine colostrum is characterised by immunoglobulin G (IgG), which is particularly important for granting passive immunity. In addition, the two most prevalent antibacterial components of colostrum are lactoferrin and lactoperoxidase. Colostrum has been utilized for the treatment and prevention of numerous infectious disorders brought on by bacteria, viruses, and protozoal pathogens due to the presence of these antimicrobial compounds. Compared to raw milk, colostrum has much more of these beneficial components.

Colostrum was utilized to cure a variety of illnesses thousands of years ago. Due to its antibacterial characteristics, doctors employed colostrum before

the discovery of antibiotics, and virologist Dr. Albert Sabin created the first polio vaccine using antibodies from cow colostrum. Because of its potent advantages as a supplementary or alternative to the medical treatment or prevention of illnesses for people of all ages, colostrum was introduced as a natural food additive. Consequently, colostrum can be applied to various products in tablet, capsule, and liquid forms to enhance the immune system, digestion, and protection against infections. Moreover, it was used commercially in food supplements in frozen, instant, or microencapsulated form. According to its useful advantages in human nutrition, colostrum is becoming more and more popular. According to latest researches, it is used to cure viral illnesses like polio, AIDS, and others and lessen the severity of their symptoms. Colostrum and its components were described by Galdino et al. [2] as a non-drug alternative to the treatment of COVID-19.

Raw colostrum contains valuable microorganisms, which are known as probiotics. Compared with milk, colostrum is a more lactose-based material that contains many bioactive compounds and also serves as a source of probiotic bacteria. Probiotics are live microorganisms that confer health benefits to the host when administered in an adequate amount [3]. Throughout the past twenty years, there have been more research showing the benefits of probiotics. As food supplements, probiotics have been shown to increase the biocompatibility and bioactivities of nutrients. Colostrum-based probiotics stimulate the immune system, control pathogen microorganisms' growth, and reduce the risk of cancer. Several studies have reported the anticarcinogenic activity of colostrum milk and its antimicrobial activity in vitro against a wide variety of bacteria and fungi. They are also known to alleviate inflammations, intestinal problems, and diarrhea [4] as well as help prevent hypercholesterolemia by deconjugating bile salts (BSH) to liberate free amino acids such as glycine [5-6]. Raw colostrum milk mainly consists of lactic acid bacteria (LAB) and *Bifidobacterium spp*. Lactic acid bacteria isolated from colostrum were 55.3% *Lactobacillus* genus [7]. There is rising interest, especially in these strains that are commonly used in food supplements due to their acclaimed health benefits. They protect against infectious diseases, improve the immune response, and reduce symptoms of irritable bowel syndrome, ulcerative colitis, allergic diseases, and atopic dermatitis associated with immunoglobulin E. The combination of probiotics' and colostrum's health advantages may help to fill new product gaps in the functional food and dietary supplement markets [8]. Only a small number of studies on probiotics made from colostrum and their positive effects have been reported, despite their wide range of therapeutic effects. The potential of probiotics of colostrum origin to exert actions in biotherapy has not been studied in detail. Due to the nutritive value of colostrum milk, this research tries to report and identify different probiotic species in the colostrum of cows and their biological activities. This research hypothesized that some colostrum-based probiotic bacteria might exhibit antimicrobial and antioxidant activities, subsequently implicating them in biotherapy as food additives. This study does not require ethics committee approval, because it does not involve an experimental process with direct animal contact. All literature sources used during the writing of this article and other similar studies confirm this situation.

2. MATERIALS AND METHODS

2.1. Sample collection

The cow colostrum milk sample was collected under hygienic conditions from dairy farm in Üçsaray-Seyitgazi (Eskişehir, Turkey) province. Sample was collected at early morning milking time on the first day of postpartum [9]. The milk sample was transferred in 250 mL pre-sterile bottles, kept in ice-box carrier and immediately brought to the Pharmaceutical Microbiology Research Laboratory to be stored at -20°C. The dairy cow species is Brown Swiss (Montofon) and known to be 4 years old.

2.2. Isolation of Lactic Acid Bacteria

LAB were isolated by serial dilution method from fresh collected cow colostrum milk. The 1 mL colostrum sample was diluted up to 10^{-6} in 9 mL of 0.9 % NaCl (Physiological Salt Water). 100 µL of

each diluted sample was inoculated on MRS (DeMan, Rogosa, Sharpe) and M17 Agar plates and incubated at 37°C for 24-48 h [10]. Following the incubation period, colonies with various morphologies were chosen, and the purity of each was evaluated using the Gram staining technique. The pure colonies were kept at -80°C in 20% glycerol. The 28 pure bacterial colonies with various morphologies conducted to the Gram-staining and catalase tests. Only 2 Gram-positive and catalase-negative colonies were subcultured for further tests.

2.3. Identification of LAB

LAB identifcation was performed by applying the 16S rRNA sequence analysis. The choromosal DNA was extracted, and the 16S rRNA (~1.5 kb) gene was amplifed by PCR procedure. 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') universal primers were used for amplification. PCR samples were examined on 1.5 % agarose gel electrophoresis and purified using the ExoSAP-IT PCR Product Cleaning Reagent (ThermoFisher Scientific) purification kit according to the manufacturer's instructions. The BigDye Terminator v3.1 Cycle Sequencing Kit and ABI 3730XL Sanger Sequencing Device (Applied Biosystems) were used for Sanger Sequencing (Applied Biosystems). The BLAST program on the NCBI website was used to examine the homology of isolate sequences [11].

2.4. Determination of simulated gastrointestinal tolerance

The tolerance to gastrointestinal conditions was determined by using pepsin at different pH levels and incubation periods. The 3 mg/mL pepsin was added to the prepared pH 2.0 and pH 3.0 PBS buffers and filtered through a 0.22 µm cellulose acetate membrane filter.

The overnight LAB isolates were centrifuged at 10000 g for 5 min at 4ºC, and bacterial pellets were washed twice with saline solution and inoculated (10^9 cfu/mL) into prepared solutions. These suspensions were incubated at 37°C for 0, 1, and 2 hours, and after the incubation periods, LAB samples were inoculated onto MRS Agar to determine cell counts by using the plate-count method [12].

2.5. Determination of antibiotic susceptibility

The disc diffusion method was used to assess the antibiotic sensitivity of LAB isolates to standard commerially cantibiotics: Ampicillin (AM 10 µg/disc, Bioanalyse), Penicillin (P 10 U/disk, Bioanalyse), Amoxicillin (AX 10 µg/disc Bioanalyse), Teicoplanine (T 30 µg/disc Bioanalyse), Streptomycin (S 10 µg/disc Bioanalyse), Chloramphenicol (C 30 µg/disc Bioanalyse), (Erythromycin (E 15 µg/disc, Bioanalyse). The overnight LAB isolates (100 µL) at 10^8 cfu/mL were inoculated onto MRS Agar, and antibiotic discs were placed. After an incubation period, at 37ºC for 18 h, the results were determined by measuring the diameter of the inhibition zones [13]. The results were evaluated in terms of susceptibile, or resistance by the CLSI (Clinical & Laboratory Standards Institute) M02-A11 (2012) scale [14].

2.6. Determination of antimicrobial activity

The overnight LAB isolates were centrifuged at 10000 g for 15 min, and the supernatant was filtered with a 0.45 µm cellulose acetate membrane filter. The antimicrobial activity of cell-free LAB isolates was determined by the disc diffusion method against 5 standard pathogens: *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 35218, and *Campylobacter jejuni* ATCC 33560 [15]. The standard test bacteria cultures (10^8 cfu/mL) were inoculated onto MHA (Mueller Hinton Agar) and after being allowed to solidify, 6 mm wells were opened. The LAB cell-free supernatants (100 µL) were added into the wells. After 37ºC-18 h incubation period, inhibition zones were measured.

2.7. Determination of antioxidant activity by DPPH assay

The radical scavenging activity of the cell-free supernatants was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [16]. The 25 µL of dilutions of the cell-free supernatants and standards

(gallic acid and ascorbic acid) were added to 975 µL of DPPH (0.025 mg/mL). The combination was then left at room temperature in a dark place for 30 minutes. Each cell-free supernatant's percentage of DPPH radical-scavenging activity was determined using the following equation after the absorbance at 517 nm was measured: DPPH scavenging effect (%)=A0-A1/A0 × 100. A0=The absorbance of control and A1=The absorbance of standard.

3. RESULTS AND DISCUSSION

3.1. Isolation and identification of LAB

A total of 28 pure bacteria were isolated from colostrum milk samples. Only 2 isolates were Gram positive, bacilli shaped, and catalase-negative. These isolates were regarded as potential LAB isolates for testing. All 2 isolates were identified by the 16S rRNA sequencing method. By using BLAST software in the NCBI site for molecular identification, these 2 isolates showed 99-100% similarity with: C1 coded isolate is *Lactobacillus casei* (Accession Number: OP000865.1), C2 coded isolate is *Lactobacillus paracasei* (Accession Number: ON631824.1). Haghshenas et al., also reported these same strains that were isolated from different regions colostrum milks [17]. While a small number of studies have shown that breastfeeding exposes a baby to a variety of commensal LAB strains and encourages their growth and colonization, not much is known about the microbial content of human colostrum [18]. In this research, *L. paracasei* and *L.casei* were the *Lactobacillus* species obtained from the milk colostrum. In the literature, the isolates that identified by 16S rRNA sequencing: *Lactobacillus casei, L. plantarum, L. pentosus, L. kefiri, L. gasseri,* and *L. paracasei* species were generally isolated from cow colostrum with 99.8% similarity to those of the reference strains [19].

3.2. Determination of simulated gastrointestinal tolerance

The isolates were subjected to pepsin as a basic test to determine their capacity to survive passing through the gastrointestinal process. To establish sufficient numbers of active and live bacteria throughout the gastrointestinal tract, LAB isolates should be acid-resistant. In the present research, LAB isolates' viable cell counts significantly decreased when they were exposed to simulated gastric juice with a low pH. The results obtained for simulated gastric juice are given in Figure 1 (a, b). Although they retained greater viable cell counts at pH 2 compared to pH 3, isolates C1 and C2 were significantly distinct. Similar findings were made by Angmo et al., who showed that in vitro incubation at pH 2 caused a noticeably lower survival rate [20]. Also in the study of Liu et at., LAB isolates were reported to transport tolerance testing in artificial gastric juice at pH 2.5 and only *L. rhamnosus* and *L. plantarum* showed similar survival rates with current research [15].

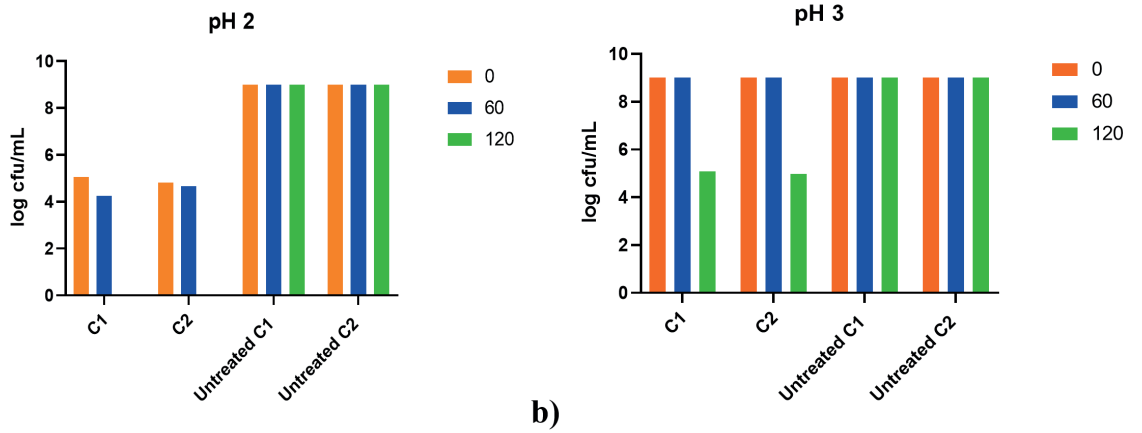

Figure 1. Gastrointestinal viability results (log cfu/mL)

The peak bacteria viability was observed at pH 3 in the simulated gastric juice. When compared with pH 2, after 120 minutes, maximum log reduction were observed for all isolates. In the literature, maximum 2 log cfu/mL reduction survival rate in gastric juice at pH 2.5-3 for 1-2 h is the standard for identifying potentially probiotic organisms [21]. The results with untreated C1 and C2 isolates reveal stabile at 9 log cfu/mL. At least, 20-40% of tests on mice show that *L. casei* and *L. paracasei* can live in the physiological conditions of the stomach and duodenum following oral administration [22].

3.3. Determination of antibiotic susceptibility

The isolated LAB strains were observed for resistance to antibiotics for safety concerns. Two functional groups of standard antibiotics are generally recommended in EFSA (European Food Safety Authority) guidelines for appropriate selection. These groups include inhibitors of cell wall synthesis (ampicillin, amoxicillin, teicoplanin, and penicillin) and inhibitors of protein synthesis (chloramphenicol, erythromycin, and streptomycin) [23]. In this research, the tested 2 isolates were susceptible to penicillin and ampicillin. These antibiotics are best known as effective inhibitors against all LAB strains. The LAB isolates were resistant to teicoplanin, chloramphenicol, erythromycin, and streptomycin. All results are given in Table 1.

Table 1. Antibiotic susceptibility results

	C1	C2
Chloramphenicol (30 µg)	R	R
Amoxicillin (10 µg)	S	R
Streptomycin (10 µg)	R	R
Ampicillin (10 µg)	S	S
Teicoplanine (30 µg)	R	R
Erythromycin (15 µg)	R	R
Penicillin (10 µg)	S	S

*R: Resistant, *S: Susceptible

3.4. Determination of antimicrobial activity

All 2 LAB isolates showed inhibition against *S.aureus*. Only *L. casei* showed antimicrobial activity against both Gram-positive (*S. aureus*) and Gram-negative (*S. typhimurim, P. aeruginosa,* and *C. jejuni*) standard pathogens (Table 2). Forestier et al., also reported that the *L. casei* have antibacterial activity against gastrointestinal pathogens [24].

And ampicillin was used as a standard antibiotic. The capacity of the LAB isolates to suppress carbohydrate fermentation is likely connected to the production of organic acids as a result of that process. Moreover, the creation of antimicrobial compounds such bacteriocins and organic acids aids LAB in its battle with the host organism for nutrients and safeguards the site of action from dangerous bacteria [25]. The antibacterial action of *Lactobacillus* strains is assumed to be caused by the release of a variety of antipathogen chemicals, including bacteriocins, biosurfactants, H_2O_2, and organic acids (hydrochloric, lactic, and acetic acids) [26].

3.5. Determination of antioxidant activity by DPPH assay

Probiotics are thought to be a new, effective source of antioxidants. In this research, antioxidant activity results were found ranged from 49.08±3.30 % (*L.*

Table 2. Antimicrobial activity of cell-free supernatants of LAB strains against standard microorganism as inhibition zone

Isolate Code – Standard Microorganisms	S. aureus	P. aeruginosa	C. jejuni	S. typhimurium	E. coli
C1	32+1.2 mm	5+0.6 mm	9+0.3 mm	8+0.9 mm	-
C2	30+1.8 mm	-	-	-	-
Ampicillin	20 mm	8 mm	10 mm	24 mm	14 mm

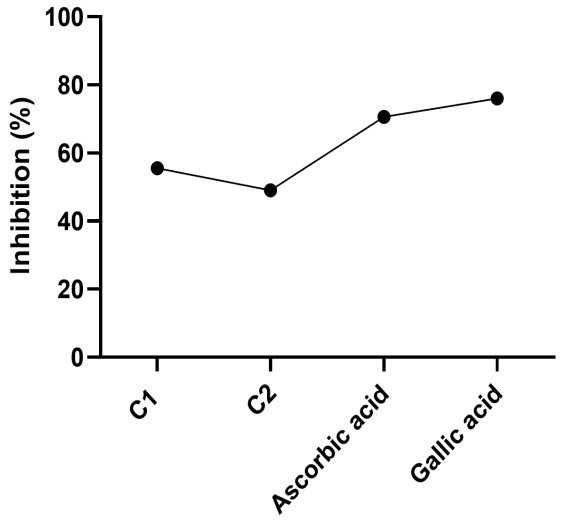

Figure 2. Antioxidant activity of cell-free supernatants of LAB isolates as percent inhibition

paracasei) to 55.51±1.7 % (*L.casei*) as shown in Figure 2. These results proved that the cell-free supernatants have good antioxidant potential when compared to standard substances (ascorbic and gallic acid). Some *Lactobacillus* species, used in the diet or as food supplements or pharmaceuticals, are known for their antioxidant effects [27]. Moreover, it has been proposed that some probiotics enhance the function of antioxidant enzymes or regulate circulatory oxidative stress, shielding cells from damage brought on by carcinogens [28]. Through the release of a peptide that can prevent oxygen radicals, recent studies have shown that *Lactobacillus* species can function as antioxidants [29].

4. CONCLUSION

Cow colostrum milk was identified as a novel source of lactic acid bacteria with the isolation of *L. casei* and *L. paracasei.* These isolates were characterized with regards to simulated gastrointestinal tolerance and antibiotic resistance. In this research, isolated LAB strains were explored by their antimicrobial and antioxidant activities as safe biotherapeutics, providing an alternative to drugs or chemicals. This research showed that *Lactobacillus casei* and *Lactobacillus paracasei* isolates can be used as single probiotic or as a contributor of synbiotic in formulations. The main difference of this research from other studies reported in scientific literature is that, LAB species were isolated for the first time from the colostrum milk of a Montofon cow species of Turkish origin, and determined by their potential probiotic properties. But, more studies are required to prove their true potential and usage areas. This phenomenon explains the growing interest in finding novel sources of probiotics that can be used in pharmaceuticals and foods.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: PS, MT; Design: PS; Supervision: PS, MT, YT; Materials: PS, MT; Data Collection and/or Processing: PS, MT; Analysis and/or Interpretation: PS, MT; Literature Search: PS, MT; Writing: PS, MT, YT; Critical Reviews: PS, MT, YT.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Research Article

Knowledge and behavior of community pharmacists towards detecting drug-drug interactions

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ABSTRACT

Drug-drug interactions (DDIs) are preventable medication errors that can cause severe adverse effects for patients, which often involve more than one mechanism. Healthcare practitioners, especially community pharmacists, must know and manage potentially significant DDIs to provide patient safety.

This paper mainly aims to determine the knowledge level of community pharmacists about DDIs and to evaluate the behavior of community pharmacists in detecting DDIs. For this aim, a face-to-face questionnaire, including a knowledge assessment test containing 20 drug pairs and ten behavior statements related to detecting DDIs, was applied to community pharmacists.

Seventy-three pharmacists participated in the study. The study's findings show that the knowledge level of community pharmacists, who are the closest health consultants, about DDIs is relatively low. In addition, responses were found to be moderate in detecting drug interactions. Although statistically significant and positive effects of vocational training on the knowledge level and behaviors of pharmacists were determined, it was revealed that education levels did not have a significant effect.

In this regard, it is essential to improve community pharmacists' DDI knowledge level through vocational training programs and encourage their interaction-detecting behavior to improve patient outcomes and patient safety.

Keywords: Community Pharmacy, Drug-Drug Interaction, Knowledge, Behavior

1. INTRODUCTION

Drug-drug interaction (DDI) is the situation in which the effect of a drug changes qualitatively and/or quantitatively in the presence of another drug, food, beverage, or some environmental chemicals [1]. DDIs are preventable medication errors that can cause severe adverse effects for patients, which often involve more than one mechanism. Three types of mechanisms are commonly seen: (i) pharmaceutical incompatibilities, (ii) pharmacokinetic drug-drug interactions, and (iii) pharmacodynamic drug-drug interactions [2]. The increased frequency of drug regimen complexity and polypharmacy in the last century and the negative outputs caused by this increase have made DDIs even more critical [3]. Priyanka et al. stated that DDIs might cause unexpected side effects of the interacting drug or a desirable enhanced action [4].

Healthcare practitioners,especially community pharmacists, are uniquely positioned with their medication knowledge and role in prescription clinical assessment and must know and manage potential significant DDIs to provide patient safety [5]. Abarca et al. and Chatsisvili et al. stated that pharmacists are vital in preventing the harmful effects of DDIs, especially for drugs with narrow therapeutic index [6,7].

Therefore, the motivation for the present study comes from filling the gap in the literature. This paper mainly aims to determine the knowledge level of community pharmacists about DDIs and to evaluate the behavior of community pharmacists in detecting DDIs. In addition, it is aimed to test the following hypotheses in the study.

H_1: Participating a vocational training on DDI affects pharmacists' DDI knowledge in a positive way

H_2: Participating a vocational training on DDI affects pharmacists' interaction-checking behavior in a positive way

H_3: Education levels of pharmacists affect their DDI knowledge in a positive way

H_4: Education levels of pharmacists affect their interaction-checking behavior in a positive way

H_5: DDI knowledge level of pharmacists affects pharmacists' interaction-checking behavior in a positive way

2. MATERIALS AND METHODS

This study was conducted after Van YüzüncüYıl University Non-interventional Research Ethics Committee approved the study ethically (Date:19/11/2021, Decision No: 2021/12-1). Within the scope of this study, a face-to-face questionnaire was applied to community pharmacists affiliated with the Van-Bitlis-Hakkari Chamber of Pharmacists.One of the authors administered the questionnaires in the working environments of pharmacists in January-May 2022.

The questionnaire consists of three parts. In the first part, the demographic characteristics of the pharmacists were asked. In the second part, a knowledge assessment test including 20 drug pairs commonly stated in the relevant literature, and pharmacists were asked to classify the drug pairs as (1) contraindication, (2) may be used together with monitoring, (3) no interaction, and (4) not sure (to avoid guessing) [8-12]. The last part of the questionnaire contains ten behavior statements for detecting DDIs prepared by 5 points Likert scale.

The population of the study is consisting nearly 222 community pharmacists. The sample size of this study was calculated using the acceptable error level method under the assumption that the sample statistics are normally distributed. The sample size was calculated as 67 by taking a 0.10 confidence level, z=1.96, d (sensitivity)=0.05, and p and q values as 0.50. To increase the reliability of the study's results, it aimed to reach the maximum number of pharmacists that can be achieved, and the participation of 73 community pharmacists was ensured.

Pharmacists' knowledge scores were calculated, giving five for each correct answer and zero for each incorrect answer in the knowledge assessment test. Knowledge scores are classified as: (i) 0-25 very low, (ii) 26-50 low, (iii) 51-75 moderate, and (iv) 76-100 high.

The statistical significance was considered as $p< 0.05$ and 95% confidence interval.

3. RESULTS AND DISCUSSION

Seventy-three pharmacists participated in the study, of which 36% were female and 64% were male. 67% have a bachelor's degree, 26% have a master's degree, and 7% have a doctorate degree. In addition, 46% of them attended a vocational training program on DDIs. Considering the frequency of encountering DDI in the prescriptions per month, it was determined that 65% had one or fewer, 22% had 2-5, and 13% had five or more interactions.

Percentages of correct answers for drug pairs are given in Table 1.

According to Table 1, the drug pair with the highest number of correct responses by pharmacists was Sildenafil-Isosorbylmononitrate, with a 55% correct response rate. However, the least correct response rate (approximately 11%) was seen in Phenobarbital-Methyldopa and Amiodoran-Fluconazole. In the literature, the drug pairs to which pharmacists respond correctly vary in studies dealing with the knowledge level of pharmacists about DDIs. According to Alrabiah et al., pharmacists mostly answered the warfarin-cimetidine pair correctly (59.7%) [11]. Additionally, Oğuz and Arslan found that the pharmacists mostly gave correct answers for the warfarin-cimetidine pair in both the pre-test and post-test [13]. In contrast, in the study of Ko et al., this drug pair was the pair with the least correct answer [8]. As seen from Table 2, this pair's correct answer rate is relatively low in this study. Also, it is seen that the drug pairs with low correct response rates are similar to the study of Oğuz and Arslan [13].

When the knowledge scores of the pharmacists were evaluated, it was seen that the highest score was "80" and the lowest score was "0". The mean score was calculated as 24.8. The distribution of knowledge scores of pharmacists can be seen in Figure 1.

It should be noted that the knowledge score of 13 pharmacists was"0," and only four had scored over moderate level. In light of these findings, it is seen that the participants' level of knowledge about DDIs is low. This situation is paralel to the literature evaluating pharmacists' knowledge of DDIs [11,14].

In the study conducted by Oğuz and Arslan to reveal the effect of an educational intervention on DDIs for senior pharmacy faculty students, it is seen that the average knowledge scores of the students is 22.639 in the pre-test and 48.056 in the post-test [13].

In the next step of the study, the behaviors of pharmacists to identify DDIs are discussed with ten expressions, and the average response to these statements is given in Table 2.

Table 1. DDIs Knowledge Results

No	DrugPairs	Percentages of correctanswers (%)
1	warfarin -cimetidine	27.40
2	sildenafil –isosorbidemononitrate	54.79
3	alprazolam -itraconazole	24.66
4	warfarin -verapamil	24.66
5	theophylline -omeprazole	31.51
6	atenolol -ranitidine	30.14
7	digoxin - clarithromycin	19.18
8	cyclosporine -rifampicin	23.29
9	itraconazole -quinidine	21.92
10	methotrexate -probenecid	16.44
11	methyldopa -phenobarbital	10.96
12	amiodarone-simvastatin	27.40
13	pimozide -ketoconazole	17.81
14	dopamine -phenytoin	26.03
15	phenytoin -cimetidine	27.40
16	metformin -erythromycin	36.99
17	theophylline -ciprofloxacin	26.03
18	amiodarone -fluconazole	10.96
19	digoxin -warfarin	23.29
20	acyclovir -simvastatin	31.51

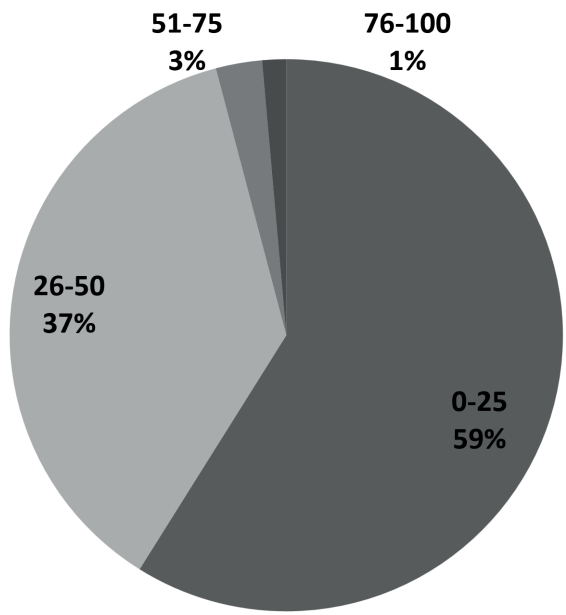

Figure 1. Knowledge scores

Table 2. Mean values

No	Items	Mean	Standard deviation
D1	I check for DDIs whilefilling prescriptions.	3.328	1.028
D2	I use electronic drug information resources while detecting DDIs.	3.521	1.203
D3	I use package inserts of the drugs while detecting DDIs.	3.164	1.106
D4	I use the internet while detecting DDIs.	3.220	1.325
D5	I counsel my patients on DDIs.	3.110	1.308
D6	I check whether there is an interaction between the drugs my patients are using and their prescribed drugs.	3.138	1.254
D7	When I encounter a DDI on a prescription, I contact the prescribing physician.	3.069	1.084
D8	When I encounter an interaction between the drugs my patients are using and their prescription drugs, I contact the prescribing physician.	2.890	1.318
D9	When I encounter a DDI in the prescription, I refer the patient to the physician.	3.178	1.124
D10	When I encounter an interaction between the drugs my patients are using and their prescription drugs, I refer them to the physician.	3.712	1.124

When Table 2 is examined, it is seen that the average of the responses given to the behavioral statements is at a moderate level. Considering the response frequencies of pharmacists, it has been determined that approximately 40% of them frequently check whether there is a drug interaction in the prescription, and they use electronic drug information sources at the highest rate in detecting drug interactions. Similarly, Dahri et al. revealed that pharmacists use different sources to detect DDIs and mostly prefer electronic databases [5]. Furthermore, parallel to Hamadouk et al. and Makkaoui et al., it is seen that pharmacists mostly prefer to refer patients to physicians when they encounter a DDI [15,16].

Following this, the data obtained from the behavioral expressions were subjected to explanatory factor analysis (EFA) by varimax rotation to determine underlying latent variables of interaction detection behavior. Five expressions were extracted from EFA, anda one-factor structure was obtained, which explained 54.532% of the variance. EFA results are given in Table 3. The Cronbach's alpha value was calculated as 0.776. This value shows that the obtained factor result is reliable.

Lastly, *t*-test and ANOVA tests were performed to determine the effects of demographic characteristics on the pharmacists' knowledge test scores and interaction detection behavior, and the results are presented in Table 4.

Table 3. EFA results

Item	Factor loadings	Cronbach's alpha
D7	0.834	
D1	0.829	
D6	0.748	0.776
D10	0.649	
D4	0.603	

Table 4. *t-test* and ANOVA results

	Participation in vocational training		Education level	
	t	**Sig.**	**F**	**Sig.**
Knowledge scores	2.590	0.012*	0.375	0.689
Interaction detection behavior	4.375	0.000*	2.610	0.0081

A statistically significant difference was found between the mean knowledge scores of pharmacists who participated in vocational training on drug interactions and those who did not, at the 95% confidence interval. It was determined that the averages of the pharmacists who participated in vocational training were higher than those who did not. In this regard, the first hypothesis of this study is confirmed. Similarly, Saverna et al., Harrington et al., and Hincapie et al. emphasized the importance of a drug-drug interaction-specific training program to improve the short-term drug-drug interaction knowledge of healthcare providers [9,17,18].

According to Table 4, it can be seen that participation in vocational training created a statistically significant difference in their interaction detection behavior. Therefore, the second hypothesis of this study is confirmed. This finding contrasts Akgöl and Baltacı Bozkurt's study, in which no relationship was found between pharmacy duties and vocational training participation [19].

Jose et al. stated that pharmacy education should encourage pharmacists about DDIs [20]. In contrast, there was no statistically significant difference in DDIs knowledge of pharmacists andtheir interaction detection behavioraccording to educational level in the 95% confidence interval. Herewith third and fourth hypotheses of the study are rejected.

In addition, it was determined that the knowledge scores of pharmacists in the 95% confidence interval did not create a statistically significant difference in interaction detection behavior. Considering that knowledge is among the antecedents that affect the behavior of individuals, it is expected that increasing the level of knowledge on this subject will contribute positively to the development of interaction detection behaviors of pharmacists. However, the findings reject the fifth hypothesis of the study.

4. CONCLUSION

With this study, the knowledge level of community pharmacists on DDIs was determined, and a measurement tool was presented to contribute to future studies in detecting drug interaction behaviors of community pharmacists. The study's findings show that the knowledge level of community pharmacists, who are the closest health consultants, about DDIs is relatively low. In addition, responses were found to be moderate in detecting drug interactions. Although the literature has stated that the knowledge level of pharmacists and other health professionals on this subject is low, the values obtained are far below expectations.

In this context, it is necessary to increase pharmacists' awareness of this issue during undergraduate education. Beside this, improving community pharmacists' DDIs knowledge level through vocational training programs and encouraging their interaction-detecting behavior to improve patient outcomes and protect patients from DDIs related problems is essential.

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Ethical approval

The study was approved by the Van YüzüncüYıl University Non-interventional Research Ethics Committee (Protocol no. 2021/12-1 / 19.11.2021).

Author contribution

Concept: FO, MA; Design: FO, MA; Supervision: MA; Materials: FO, MA; Data Collection and/or Processing: FO; Analysis and/or Interpretation: FO, MA; Literature Search: FO, MA; Writing: FO, MA; Critical Reviews: MA.

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The authors declared that there is no conflict of interest.

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Review Article

The role of immunotherapy in lung cancer: Actual scenery

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ABSTRACT

More than half of those who succumb to cancer each year also lose their battle with the disease, making cancer a leading cause of death worldwide. After surgery, hormonal therapy, radiotherapy and chemotherapy, which are preferred in cancer management, immunotherapy has revolutionized. In this mini-review, we cover the various immunotherapeutic approaches used in contemporary cancer immunotherapies. These are immune checkpoint blockade, an attemp planned to 'unleash' robust T cell responses, and adaptive cellular therapies connected on the infusion of tumor-struggling immune cells into the body. One of these attemps, Nivolumab, became the first ICI to be approved to treat lung cancer in 2014. To date, different ICIs, such as pembrolizumab, atezolizumab, and durvalumab, have been in a row introduced into clinical medicine and have shown significant effect. Therefore, in this mini-review, we present some emerging goals and attemps in cancer immunotherapy.

Keywords: Lung cancer, Checkpoint inhibitor, Chimeric antigen receptor T cells, Cytotoxic T-lymphocyte antigen 4, Programmed cell death protein 1

1. INTRODUCTION

In the world, lung cancer (LC), one of the deadliest malignancies, causes more than 25% of all cancer-related fatalities each year [1]. According to estimates, there are 1.8 million new cases of lung cancer each year, and 1.6 million people die from the disease [2-4]. The depressing survival results in patients with LC are currently is directing great try to develop new treatments for this high-risk group. Most statistics of patients with LC include both small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC sources from badly differentiated neuro endocrine cells, results in rapid metastasis, and has a weak prognosis with weak response to therapy. The incidence of small cell cancers is higher, especially among men, and is associated with a history of smoking. Generally, approximately 10% to 15% of all lung cancers are SCLC, and about 80% to 85% are NSCLC [5-8]. The current conventional treatments currently used for NSCLC are surgery, chemotherapy, radiation therapy, immunotherapy and targeted therapies [9]. Among the targeted therapies, epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), ROS1 and BRAF have provided significant improvements especially in patients with advanced course [10]. In recent years, immunotherapies such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) inhibitors have replaced chemotherapy and gained therapeutic support against these mutations [11]. Nevertheless, chemotherapy is the first choice for patients who are not suitable for unidirectional and/or combined forms of immunotherapy. These patients may have immunotherapy contraindications or conditions of concern associated with combination immunotherapy, such as efficiency or risk of toxicity

[12]. Radiation therapy, on the other hand, uses high-energy radiation such as gamma rays, X-rays, protons, or electron rays to completely destroy the tumor site. Surgical interventions are recommended as a therapy choice in early-degree NSCLC [13].

Advanced lung cancer has a poor prognosis and standard therapies with cytotoxic anticancer drugs have restricted therapeutic impacts [14,15]. Various forms of immunotherapy have been developed after the years, like immunostimulating cytokines, oncolytic viruses, and tumor-targeting antibodies, that work by enhancing the anti-neoplastic effect of the existing immune system [16]. For cancer immunotherapy, agents are used to enable or increase the activation of the immune system to offensive cancer cells [17-19]. Cancer immunotherapy has altered the paradigm for cancer treatment [20,21]. At the same time, this method purpose to enhance antitumor immune responses with less off-target effects than chemotherapies and another agents that directly destroy cancer cells [22]. In last years, the dealling of the detailed mechanism of cancer immunotherapy and the positive results of anti PD-1 antibodies, one of the immune checkpoint inhibitors (ICIs), in clinical studies have marked a new era in the therapy of lung cancer [23]. Significant research in advanced NSCLC has informed developed survival with anti-PD-1/PD-L1 antibody therapy, both single and in combination with chemotherapy. For this reason, immunotherapy is considered a encouraging approach to treat certain types of cancer and furthermore to cure the disease [24,25]. Effective results have been obtained by stimulating the immune system instead of directly destroying cancer cells, which is why immunotherapy has now become the fifth sought-after step in cancer treatment after radiotherapy, surgery, medical oncology and interventional oncology.

This mini-review will review (ICPIs) and (CAR-) T cells, the two main agents behind recent immunotherapeutic agents in NSCLC and SCLC [26,27].

1.1. Immune Checkpoint Inhibitors (ICI)

The adaptive immune system, which arises with the production of many antigens, can distinguish cancers from normal cells [28-31]. These exceptional cancer antigens are recognized by T-cell receptors (TCR) thanks to major histocompatibility complexes (MHC) on antigen-presenting cells (APCs). According to these methods, which include T cell activation, clonal proliferation of antigen-specific cells, immune effector cell aggregation, cytokine release, and ultimately cytotoxic T cell-mediated tumor cell killing, tumor growth will eventually stop. The balance of costimulatory and inhibitory molecular interactions between T cells and APCs governs all of these scenarios. However, tumor cells can evade an immune attack by increasing inhibitory signaling and decreasing costimulatory signaling. [32,33]. Given its success in treating cancer, the first approved ICPI was an antibody against CTLA-4 and PD-1 or PD-L1 [34,35].

1.1.1. CTLA4

The family of immunoglobulins includes CTLA4. Furthermore, together with the T cell co-stimulatory protein CD28, is co-expressed by activated T cells. Humans produce three isoforms as a result of gene splicing. They are the exon 1 and exon 4 versions, the soluble form of CTLA-4, and the full-length CTLA-4, respectively [36]. During T cell receptor (TCR)-mediated and CD28-mediated T cell activation, CTLA4 expression on effector T cells is increased to enable downstream regulation of immunity. The discovery of CTLA 4 as a negative regulator of T cell activation has helped to explain how it might activate the T cells' therapeutic response against cancer. CTLA 4-mediated tumor withdrawing mechanisms are pleiotropic but associated with T lymphocyte activity. T cell replies are required for the therapeutic effects of CTLA 4 directed agents. In animal models, consumed T cell abolished tumoricidal activity [37,38]. Studies have indicated the antitumor effect and clinical avails of antibodies such as ipilimumab, which block CTLA-4 interplays through ligands [39,40].

1.1.2. PD1

PD-1 and PD-L1 are members of the type I transmembrane protein class [41]. Immunity is severely inhibited by programmed cell death protein 1 (PD1). Natural killer T cell, T cell, B cell and activated monocytes all mean the PD1 protein. PDL1

and PDL2 are the two ligands for PD1, which are both related in cell death. In a research, it was shown that regenerated T cells in the peripheral blood of lung cancer patients after PD1 suppression express CD28 [42,43]. Anti-PD1/PD-L1 immunotherapy methods, blocks PD1, an inhibitory lymphocyte receptor, while releasing anti-tumor immune cytotoxicity [44,45].

1.1.3. PDL1

PDL1 causes PD1-mediated immune suppression because it is structurally expressed on T cells, B cells, macrophages, non-lymphoid organs like the heart and lungs, in parenchymal cells, and on the surface of tumor cells. PDL1 expression, but not PDL2, has also been found in the placenta, pancreatic islets, and cardiac endothelium at low levels, suggesting a function for PDL1 in immunological tolerance. In addition, PDL1 blockade is effective in the treatment of malignancies of the bladder, lung and other organs [43-46].

Biochemical experiments confirmed that [italic] in silico[/italic] nominees are true inhibitors of the PD-1/PD-L1 interplay. These results were also verified [italic]in vitro[/italic]. Additionally, the study demonstrated the capability of small molecule inhibitors to reduce tumor masses and mediate antitumor immune reactions using the PD-1/PD-L1 mouse model [47].

1.1.4. PDL2

PDL2, such as PDL1, is an immune checkpoint inhibitor. Although the activity of PDL2 is less well understood than PDL1, its clinical value is still under investigation [48]. The activation of PD1 by PDL2 significantly reduces CD4+ T cell increment and cytokine formation that is mediated by the TCR123. At the same time, novel studies revealed that PDL2 can reduce PDL1 and/or PD1 connecting and increase the expression of CD3 and excitable T-cell co-stimulator (ICOS) on T cells, perchance through conjectural second receptor. Former searches have demonstrated that PDL2 could develop T cell activity through a PD1 free method [49,50].

The promotion of ICI has certainly been the first of all oncologic accomplishment of the last ten years.

Table 1. FDA Immune Checkpoint Inhibitors Approved for NSCLC

Agent	Molecular Target
Ipilimumab	CTLA-4
Nivolumab	PD-1
Pembrolizumab	PD-1
Cemiplimab	PD-1
Atezolizumab	PD-L1
Durvalumab	PD-L1

FDA confirmations started with ipilimumab for melanoma in 2011 [51]. At present confirmed ICIs for NSCLC as of October 2022 are shown in Table 1 [52,53].

1.2. Chimeric Antigen Receptors (CARs)

CARs are at the tender spot of this grand revision of adoptive T cell treatments and concretize a pass from classic immunology to synthetic cell therapy. CARs are synthetic fusion proteins made up of a transmembrane domain, an extracellular domain that can precisely bind to a target molecule expressed on the surface of tumor cells, and an intracellular domain that sends a warning to activate T cells when the extracellular domain interacts with its target. The extracellular domain of an antibody is typically composed of the antigen-recognition regions as a single-chain unstable fragment [54,55]. CAR-T cells detect particular tumor antigens in an MHC-independent way, activating and carrying out their anticancer function [56]. Clinical application of CAR T-cell therapies against LC remnants is constrained by physical and immunological barriers, antigen escape and heterogeneity, on-target off-tumor toxicity, and a number of additional reasons [57]. With CAR T-cell therapy, the patient's own genetically altered T cells are used to find and eradicate the malignancy [58]. The complete range of activity of checkpoint blocking medications used one or in combination is now the focus of significant research due to the intricate biology of immune checkpoint pathways, which still holds plenty mysteries [58-60]. Since rerouted T cell therapy was first introduced by CAR, it has become recognized as a viable cancer treatment method. Contrary to TCRs, CARs are synthetic receptors having cytoplasmic signaling domains, transmembrane domains, and extracellular

antigen identification domains. As a result, CARs are skilled of shifting the specificity of T and NK cells to tumor-associated antigens (TAAs) produced on tumor cells by identifying the targeted antigen in an MHC-independent manner [59]. Some patients have responded highly to immunotherapy, while others do not have the same positive response. No one can predict how your body will respond to any one treatment. A doctor should definitely determine what to expect when using an immunotherapy drug and what the right treatment is for you [61].

2. CONCLUSION

The outlook for treating hematological malignancies has changed as a result of cancer immunotherapies such ICIs and CAR-T. Even if there are more individuals who have long-term survival after receiving ICI therapy than with other treatments, these examples are rare. The choice of new therapeutics and efficacy-enhancing methods, like combination therapy, remains a difficult problem to tackle. To ensure that immunotherapy is successful in the future, predictive criteria must be improved. Although PD-L1 and CARs are both useful in case selection, it is now known that resistance can arise through multiple mechanisms. Despite the fact that immuno chemotherapy has made significant progress in the treatment of LC, it is anticipated that it will continue to advance when targeted medicines or new combinations of therapies are developed. Clinical investigations are evaluating the advancement of novel therapeutic options, such as the combination of PD-1/PD-L1 inhibitors with other ICIs and DNA repair targeted medicines.

Author contribution

Concept: İE, GAÇ; Design: İE, GAÇ; Supervision: GAÇ; Data Collection and/or Processing: İE, AH, SNE, GAÇ; Literature Search: İE, AH, SNE, GAÇ; Writing: İE, GAÇ; Critical Reviews: İE, GAÇ.

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