INTRODUCTION

Antimicrobial resistance is often used as a definition for drug resistance which occurs when microorganisms like bacteria, viruses, fungi and parasites withstand a drug that was intended to cure the infection[1,2]. Multidrug-resistant strains of *MRSA* cause some serious infections such as pneumonia, endocarditis, skin and soft tissue infections within intensive care units[3,4]. Recent studies confirmed that indole derivatives have promising antimicrobial activity against various microorganisms including *MRSA*[5]. Studies showed that one of the main contributors to *Staphylococcus aureus* antibiotic resistance is the *NorA* efflux pump[6,7]. *NorA* is able to export a variety of structurally unrelated drugs, such as fluoroquinolones, ethidium bromide, cetrimide, benzalkonium chloride, tetraphenylphosphonium bromide, and acriflavine[8]. Indoles are one of the reported classes of *NorA* inhibitors[9] for example 5-nitro-2-phenylindole that characterizes a promising lead structure able to produce a 4-fold increase in *S. aureus* susceptibility to ciprofloxacin[10]. Tert-butyl (2-(3-hydroxyureido)-2-(1H-indol-3-yl)ethyl) carbamate, which is not toxic to human cells, was also found to be an active indolic *NorA* inhibitor[11].

Azole containing compounds such as fluconazole, ketoconazole and itraconazole are the most widely used antifungal agents in clinic[12,13]. Despite all the claims there are a lot of studies that have demonstrated ineffectiveness of fluconazole against *Candida krusei*[14,15] which has been recognized as a potentially multidrug-resistant fungal pathogen[16]. Consequently it is essential to develop new active compounds against fungal pathogens including *C. krusei*. Multidrug-resistant infection strains are diseases of emerging healthcare concern that have demanded the attention of researchers. Synthesis and antifungal activity of indole-linked triazole derivatives[17] showed that almost all the indole derivatives showed excellent antifungal activities against *Candida albicans* and *C. krusei* with low MIC values[18]. Antimicrobial activity studies of some 1,2,4-triazole and 1,3,4-thiadiazole derivatives have indicated good antimicrobial activity[19,20]. In addition it is well known that imidazoles and triazoles (azoles) make up the largest group of agents against mycosis infections[21]. Indole derivatives were found to be particularly effective and suitable for further developments in antimicrobial drug development studies[22].

This study is part of an ongoing project to search for novel antimicrobial drug candidates especially against *MRSA* and *C. krusei.* New indole derivatives substituted with triazole, thiadiazole and carbothioamide were tested against *S. aureus*, *MRSA*, *Escherichia coli*, *Bacillus subtilis*, *C. albicans* and *C. krusei* by using the 2-fold serial dilution technique. The minimum inhibitory concentration (MIC) was determined for test compounds and for the reference standards sultamicillin, ampicillin, fluconazole and ciprofloxacin.

MATERIALS AND METHODS

Chemistry

Synthesis and spectroscopic characterization of 31 indole derivatives (Table 1) were published in our earlier study[23]. Reaction of indole 3-acetylhydrazine with isothiocyanates in ethanol under reflux gave the corresponding hydrazinecarbothioamides (**1a**–**h**). Treatment of **1a–h** under acidic conditions with full region chemical control to gives the corresponding 2-aminothiadiazoles (**2a**–**h**). Conversely, treatment of **1a–h** under basic conditions (aq. NaOH) with heating produced the 3-thiotriazoles (**3a**–**h**). Triazoles (**3a**–**h**) could be further alkylated under basic conditions to yield substituted triazoles (**4a**–**g)**.

<<Table 1>>

Microbiology

Antibacterial and antifungal activity test was carried out against standard strains. American Type Culture Collection (ATCC) strains of the microorganisms used in this study were obtained from the culture collection of the Refik Saydam Health Institution of Health Ministry, Ankara, and maintained at the Microbiology Department of the Faculty of Pharmacy of Ankara University. Mueller–Hinton Broth (MHB)(Difco), Mueller–Hinton Agar (MHA) (Oxoid), Sabouraud Dextrose Agar (SDA), (Difco) and Sabouraud Dextrose Broth (SDB) (Difco), were applied for growing and diluting of the microorganism suspensions. The following reference strains were used for testing antimicrobial activity: **Gram positive bacteria:** *S. aureus* ATCC 25923, *MRSA* ATCC

43300, *B. subtilis* ATCC 6633. **Gram negative bacteria**: *E.coli* ATCC 25922, **Yeast:** *C. albicans* ATCC 10231 and *C. krusei ATCC 6258.*

Antibacterial and Antifungal Activity Assay

The bacterial strains were maintained on MHA medium for 24 h at 37 ◦C and fungi were maintained on SDA for 48 h at 25 ◦C. Overnight cultures were prepared by innoculating approximately 2mL Mueller Hinton broth (MHB) with 2–3 colonies of each organism taken from MHA. Inocula were prepared by diluting overnight cultures into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 Mcfarland standard having approximately 10^8 cfu/mL for bacterial and 10^7 cfu/mL for yeasts. The tube dilution technique was used for determination of the minimum inhibitory concentrations (MIC)[24,25]. Indole derivatives were investigated to evaluate their efficacy against multi-drug-resistant in microbial infections by using the 2-fold serial dilution technique against *S. aureus*, *MRSA, E. coli, B. subtilis*, *C. albicans* and *C. krusei*.

The synthesized compounds and the standards were dissolved in 12.5% DMSO at concentrations of 200 µg/mL. Further dilutions of the compounds and standard drugs in the test medium were prepared at the following quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg/mL concentrations with Mueller-Hinton Broth and Sabouraud Dextrose Broth. A set of tubes containing only inoculated broth was used as controls.

After incubation for 24 h at 37 °C for the antibacterial assay and for 48 h at 25 °C for the antifungal assay, the last tube with no growth of microorganism and/or yeast was recorded to represent the MIC (expressed in µg/mL). The MIC was determined for test compounds and for the reference standards sultamicillin, ampicillin, fluconazole and ciprofloxacin. Every experiment in the antibacterial and antifungal assays was performed in duplicate.

RESULTS AND DISCUSSION

Antimicrobial activity was investigated by finding the minimum inhibitory concentration (MIC) of the indole derivatives against *S. aureus, MRSA, E. coli, B. subtilis, C. albicans and C. krusei* strains and comparing with ampicillin, sultamicillin,

ciproflaxacin and fluconazole as standard drugs. The *MIC* value of the compounds and standard drugs is given in (Table 2). The MIC values were within the range of 3.125–50 µg/mL. Most of the compounds showed significant antibacterial activity against *S. aureus, MRSA, E. coli,* and *B. subtilis*. In addition, the compounds demonstrate a good level of antifungal activity particularly against *C. krusei* even more effective than standard drug fluconazole.

<<Table 2>>

Antibacterial activity of all the tested compounds demonstrated acceptable antibacterial effects. While **1c**, **1h**, **3h** and **4c** showed moderate activity against *S. aureus* compared with ampicillin, sultamicillin, ciprofloxacin, the most effective compounds were **2h** (indole-thiadiazole) and **3d** (indole-triazole) with an MIC value of 6.25 µg/mL (Figure 1/a).

(Figure 1/b) shows antibacterial effects of the tested compounds against *MRSA* strains. Activities of compounds **1d**, **1h**, **2b**, **2h**, **3h** were found to be at the same level as ciprofloxacin while compounds **2c** (indole-thiadiazole) and **3d** (indole-triazole) demonstrated excellent activity against *MRSA* being more effective than ciprofloxacin. The other tested compounds were found to have the same activity value or were found more active than ampicillin and sultamicillin.

<<Figure 1>>

None of the tested indole derivatives were found to be more active than ciprofloxacin that has MIC value of 0.09 µg/mL against *E. coli*. However most of the synthesized compounds demonstrated the same or lower MIC value compared with ampicillin and sultamicillin (Figure 2/a). Finally effects of the tested indole derivatives against *B. subtilis* strains (Figure 2/b) showed that the most effective compounds were found **2c** (indole-thiadiazole) and **3c** (indole-triazole) with MIC value of 3.125 µ.g/ml. Although the tested compounds were not as active as ciprofloxacin and sultamicillin, they were much more active than ampicillin.

<<Figure 2>>

The MIC values of the tested indole derivatives indicated that nearly all the indole derivatives showed excellent antifungal activities against *C. krusei* and moderate

activities against *C. albicans* compared with the standard drug fluconazole. Figure 3/a shows the MIC value of the indole compounds against *C. albicans* compared with fluconazole. The most effective compounds are **1b**, **2b, 2c, 2d, 3b, 3c** and **3d** with MIC values of 3.125 µg/mL. While the activity results against *C. albicans* are not very satisfactory, the results of antifungal activity against *C. krusei* strains were quite promising. All the tested compounds were found several times more effective than fluconazole. As seen in Figure 3/b, most of the effective compounds **1a,b,d/ 2a,b,c,d/3a,b,c,d,h** and **4a,d,e,f,g** have MIC values of 3.125 µ.g/mL where fluconazole has an MIC value of 64 µg/mL. The results were in accordance with those found by Na, 2010[17] the most potent antifungal activity against *C. krusei* is obtained with halogenated indole derivatives.

<<Figure 3>>

CONCLUSIONS

According to the activity results, all of synthesized compounds demonstrated significant antibacterial and antifungal effects, while antifungal effects of compounds **3a-h** are promising to develop into more effective new lead compounds against *C. krusei*. Roughly 17% of *Candida* isolates exhibit resistance against azoles and most probably the extensive use of fluconazole is the main reason for this resistance. *C. krusei* is one of the species showing actual resistance to fluconazole[16,26]. Therefore the search for new and more effective anti-fungal agents against *C. krusei* seems ever more important[27]. Azole compounds prevent the synthesis of ergosterol by inhibiting the cytochrome P-450 dependent enzyme lanosterol 14α-demethylase. Triazoles have a broad range of applications in the treatment of fungal infections because of their good affinity for fungal cytochrome P-450 enzymes[28]. It is reasonable to assume that the synthesized indole-triazole derivatives (**3a**-**h**, **4a**-**g**) have the same mechanism of action.

Compounds **2c** (indole-thiadiazole) and **3d** (indole-triazole) demonstrated excellent activity against *MRSA* at a much higher level than ciprofloxacin. It was observed that compounds **1h**, **2h** and **3h** have a MIC value of 6.25 µg/mL which is the same value

as for ciprofloxacin. All these compounds have a *m*-chlorophenyl group as a substituent. This shows that not only the indole ring also the side chains are important for the activity. Singh stated that chloro substituents for triazolylindole derivatives are beneficial as well as hydroxy and methoxy substitution for activity[29].

Most of the tested indole derivatives were found to be highly active against *C. krusei*. Between the tested indoles the most active compounds were found in the indole-triazole group. This was followed by indole-thiadiazole group. These results suggested that tested indole derivatives were eligible to be development candidates and especially compound **3d** is a promising lead compound mainly against *MRSA* and *C. krusei*. However, further research needs to be carried out to approve the specific mode of action of the indole derivatives as antimicrobial agents.

Antifungal molecules common use combined with insufficient treatment are responsible for promoting these microorganisms resistance to drugs used in treatment. The antibacterial and antifungal activity results were thought to be exerted through a target because the introduction of the substituent groups on the indole caused differences in the activity. Therefore, the modification of these compounds should continue to be investigated.

ACKNOWLEDGEMENTS

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FIGURES AND CAPTIONS

Figure 1. a) The MIC value of synthesized compounds against *S. aureus* compared with ampicillin, sultamicillin, ciprofloxacin **b)** The MIC value of synthesized compounds against *MRSA* compared with ampicillin, sultamicillin, and ciprofloxacin.
Figure 2. a) The MIC value of synthesized compounds against *E. coli* compared with ampicillin, sultamicillin, ciprofloxacin. **b)** The MIC value of synthesized compounds against *B. subtilis* compared with ampicillin, sultamicillin, ciprofloxacin.
Figure 3. a) The MIC value of synthesized compounds against *C. albicans* compared with fluconazole **b)** The MIC value of synthesized compounds against *C. krusei* compared with fluconazole.

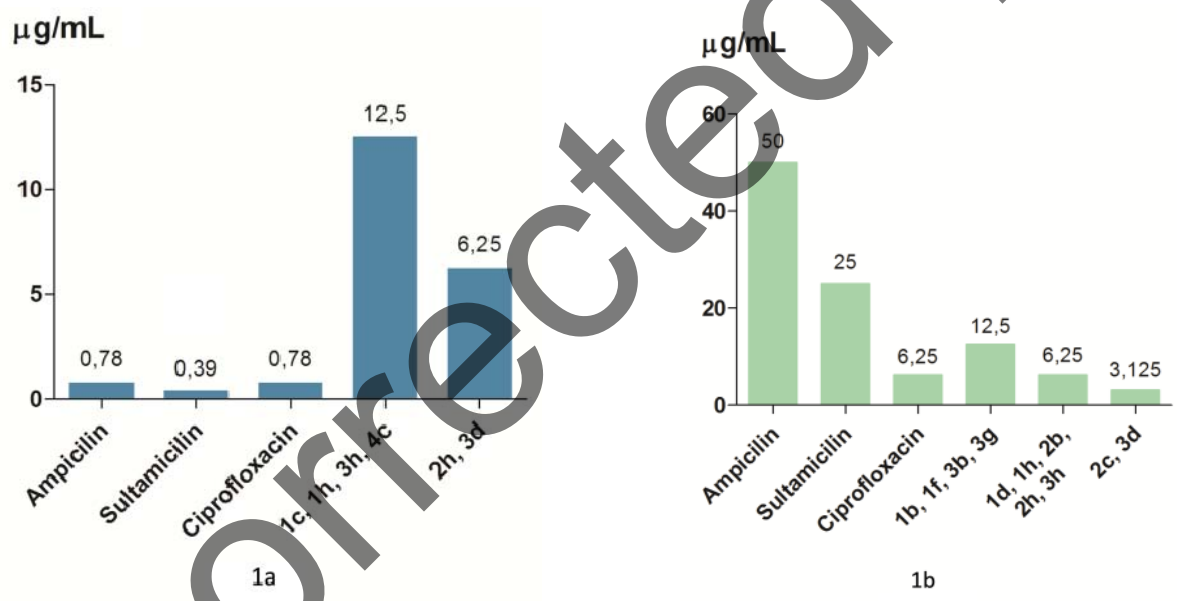
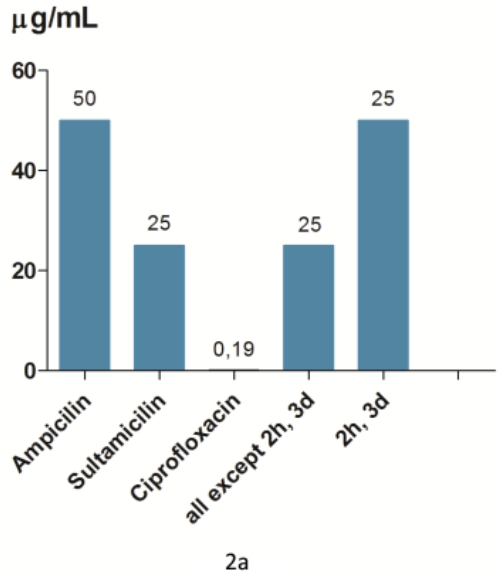


Figure 1.



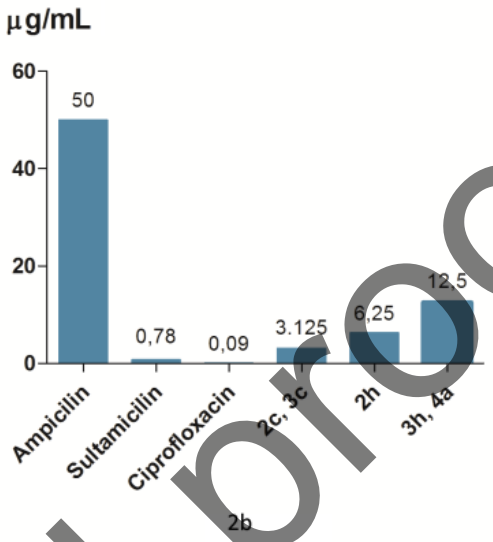
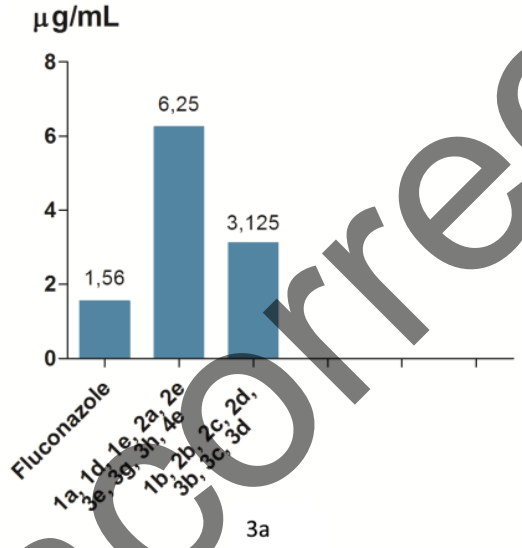


Figure 2.



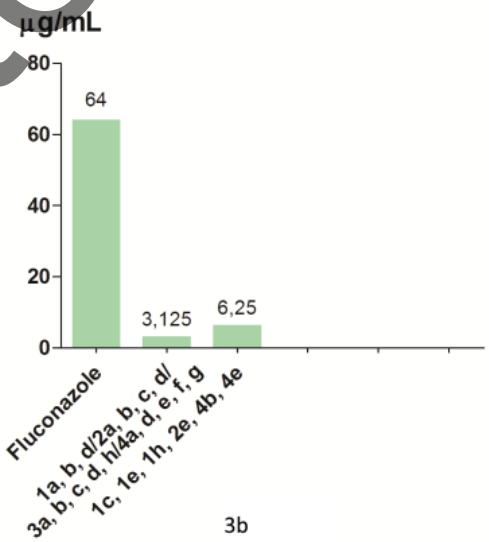


Figure 3.

Table 1. Chemical structures of new indole-based MLT analogues

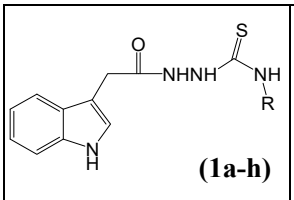
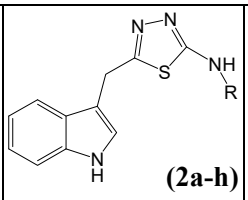
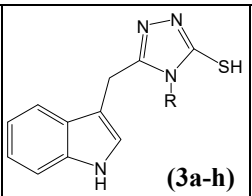
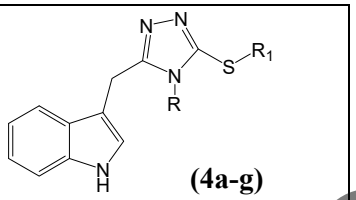
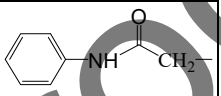
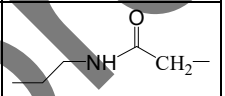
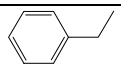
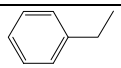
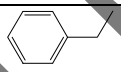
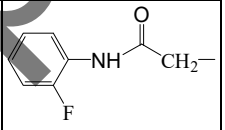
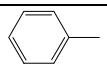
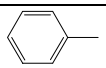
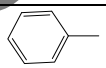
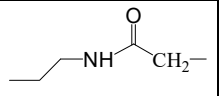
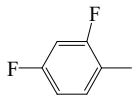
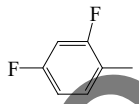
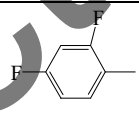
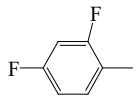
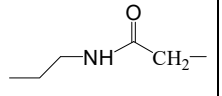
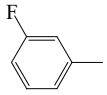
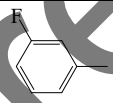
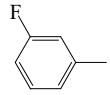
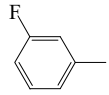
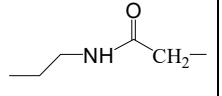
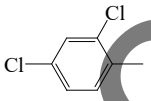
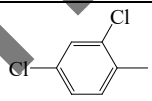
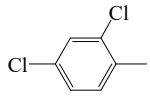
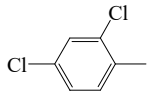
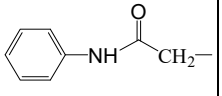
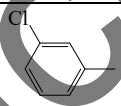
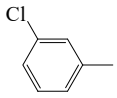
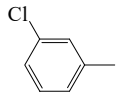
 (1a-h)		 (2a-h)		 (3a-h)		 (4a-g)		
R		R		R		R		R_1
1a	CH_3-CH_2-	**2a**	CH_3-CH_2-	**3a**	CH_3-CH_2-	**4a**	CH_3-CH_2-	
1b	CH_3-CH_2-CH_2-	**2b**	CH_3-CH_2-CH_2-	**3b**	CH_3-CH_2-CH_2-	**4b**	CH_3-CH_2-CH_2-	
1c		**2c**	H	**3c**		**4c**		
1d		**2d**		**3d**		**4d**		
1e		**2e**		**3e**		**4e**		
1f		**2f**		**3f**		**4f**		
1g		**2g**		**3g**		**4g**		
1h		**2h**		**3h**				

Table 2. MIC values (µg/mL) of tested indole derivatives

1a-h	***S. aureus***	***MRSA***	***E. coli***	***B. subtilis***	***C. albicans***	***C. krusei***	**2a-h**	***S. aureus***	***MRSA***	***E. coli***	***B. subtilis***	***C. albicans***	***C. krusei***
1a	25	25	25	50	6.25	3.125	**2a**	25	25	25	25	6.25	3.125
1b	25	12.5	25	25	3.125	3.125	**2b**	25	6.25	25	25	3.125	3.125
1c	12.5	25	25	25	12.5	6.25	**2c**	25	3.125	25	3.125	3.125	3.125
1d	25	6.25	25	25	6.25	3.125	**2d**	25	25	25	25	3.125	3.125
1e	50	50	25	25	6.25	6.25	**2e**	50	50	25	25	6.25	6.25
1f	50	12.5	25	50	25	12.5	**2f**	25	25	25	25	12.5	12.5
1g	25	25	25	25	12.5	12.5	**2g**	50	25	25	25	12.5	12.5
1h	12.5	6.25	25	25	12.5	6.25	**2h**	6.25	6.25	50	6.25	12.5	6.25
3a-h	***S. aureus***	***MRSA***	***E. coli***	***B. subtilis***	***C. albicans***	***C. krusei***	***4a-g***	***S. aureus***	***MRSA***	***E. coli***	***B. subtilis***	***C. albicans***	***C. krusei***
3a	50	25	25	25	12.5	3.125	**4a**	25	25	25	12.5	12.5	3.125
3b	25	12.5	25	25	3.125	3.125	**4b**	50	50	25	50	12.5	6.25
3c	25	50	25	3.125	3.125	3.125	**4c**	12.5	25	25	25	12.5	6.25
3d	6.25	3.125	50	25	3.125	3.125	**4d**	50	50	25	50	12.5	3.125
3e	50	25	25	25	6.25	3.125	**4e**	50	50	25	50	6.25	3.125
3f	25	25	25	25	12.5	12.5	**4f**	50	50	25	50	12.5	3.125
3g	25	12.5	25	50	6.25	3.125	**4g**	25	25	25	25	12.5	3.125
3h	12.5	6.25	25	12.5	6.25	3.125	-	-	-	-	-	-	-

Reference Standards	***S. aureus***	***MRSA***	***E. coli***	***B. subtilis***	***C. albicans***	***C. krusei***
Sultamicillin	0.39	25	25	0.78	-	-
Ampicillin	0.78	50	50	50	-	-
Fluconazole	-	-	-	-	1.56	64
Ciprofloxacin	0.78	6.25	0.19	0.09	-	-