Objectives: In new antimicrobial drug development studies, indole and its derivatives create an important class of compounds. In addition, azoles and their derivatives were recognized to be associated with a variety of biologic activities such as antibacterial and antifungal. In this study antimicrobial activities of some indole derivatives mainly substituted with 1,2,4-triazole, 1,3,4-thiadiazole and hydrazinecarbothioamide were investigated to evaluate their efficacy.

Materials and Methods: The efficacy of new compounds was evaluated using 2-fold serial dilutions against Staphylococcus aureus, MRSA, Escherichia coli, Bacillus subtilis, Candida albicans, and Candida krusei.

Results: The MIC was determined for test compounds and for the reference standards sultamicillin, ampicillin, fluconazole, and ciprofloxacin.

Conclusion: The compounds possessed a broad spectrum of activity having MIC values of 3.125-50 µg/mL against the tested microorganisms. This study provides valuable evidence that the indole-triazole derivative compound 3d holds significant promise as a novel antibacterial and antifungal lead compound.

Key words: Indole, 1, 2, 4-triazole, 1, 3, 4-thiadiazole, MRSA, C. krusei
INTRODUCTION

Antimicrobial resistance is often used as a definition for drug resistance, which occurs when microorganisms such as bacteria, viruses, fungi, and parasites withstand a drug that was intended to cure the infection. Multidrug-resistant strains of Methicillin-resistant Staphylococcus aureus (MRSA) cause some serious infections such as pneumonia, endocarditis, and skin and soft tissue infections within intensive care units. Recent studies confirmed that indole derivatives have promising antimicrobial activity against various microorganisms including MRSA. Studies showed that one of the main contributors to Staphylococcus aureus antibiotic resistance is the NorA efflux pump. NorA is able to export a variety of structurally unrelated drugs, such as fluoroquinolones, ethidium bromide, cetrimide, benzalkonium chloride, tetraphenylphosphonium bromide, and acriflavine. Indoles are one of the reported classes of NorA inhibitors, for example, 5-nitro-2-phenylindole, which characterizes a promising lead structure able to produce a 4-fold increase in S. aureus susceptibility to ciprofloxacin. Tert-buty1 (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.

Azole-containing compounds such as fluconazole, ketoconazole, and itraconazole are the most widely used antifungal agents in the clinici. Despite all the claims, many studies have demonstrated the ineffectiveness of fluconazole against Candida krusei, which has been recognized as a potentially multidrug-resistant fungal pathogen. 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 and have demanded the attention of researchers. Synthesis and antifungal activity of indole-linked triazole derivatives showed that almost all indole derivatives showed excellent antifungal activities against Candida albicans and C. krusei with low minimum inhibitory concentration (MIC) values. Antimicrobial activity studies of some 1,2,4-triazole and 1,3,4-thiadiazole derivatives indicated good antimicrobial activity. In addition, it is well known that imidazoles and triazoles (azoles) make up the largest group of agents against Candida krusei,14,15 Dextrose broth (SDB) (Difco), were used for growing and diluting 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 inoculating approximately 2 mL 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 10⁶ CFU/mL for bacteria and 10⁵ CFU/mL for yeasts. The tube dilution technique was used for the determination of the MICs. Indole derivatives were investigated to evaluate their efficacy against multi-drug-resistant 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 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, 31.2, 156 and 0.78 µg/mL concentrations with MHB and SDB. 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 sulfamicillin, ampicillin, fluconazole, and ciprofloxacin.

MATERIALS AND METHODS

Chemistry

The synthesis and spectroscopic characterization of 31 indole derivatives (Table 1) were published in our earlier study. The 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 gives the corresponding 2-aminothiadiazoles (2a-h). Conversely, treatment of 1a-h under basic conditions (aq. NaOH) with heating produced 3-thiatriazoles (3a-h). Triazoles (3a-h) could be further alkylated under basic conditions to yield substituted triazoles (4a-g).
RESULTS AND DISCUSSION

Antimicrobial activity was investigated by finding the MICs of the indole derivatives against *S. aureus*, MRSA, *E. coli*, *B. subtilis*, *C. albicans* and *C. krusei* strains and comparing with ampicillin, sultamicillin, ciprofloxacin and fluconazole as standard drugs. The MIC values of the compounds and standard drugs are 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 demonstrated a good level of antifungal activity, particularly against *C. krusei*, even more effective than the standard drug fluconazole.

The antibacterial activity of all tested compounds demonstrated acceptable antibacterial effects. Compounds 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 1a).

Figure 1b shows the antibacterial effects of the tested compounds against MRSA strains. The activities of compounds
1d, 1h, 2b, 2h, and 3h were found to be at the same level as ciprofloxacin, and 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 more active than ampicillin and sultamicillin.

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

The MIC values of the tested indole derivatives indicated that nearly all them showed excellent antifungal activities against *C. krusei* and moderate activities against *C. albicans* compared with the standard drug fluconazole. Figure 3a shows the MIC values of the indole compounds against *C. albicans* compared with fluconazole. The most effective compounds were 1b, 2b-d, and 3b-d, with MIC values of 3.125 µg/mL. Although the activity results against *C. albicans* were 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 3b, most of the effective compounds 1a, 1b, 1d/2a-d/3a-d, 3h, and 4a, 4d-g

| 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       | 3125    | 2a   | 25        | 25    | 25      | 25        | 6.25       | 3125    |
| 1b   | 25        | 12.5  | 25      | 25        | 3125       | 3125    | 2b   | 25        | 6.25  | 25      | 25        | 3125       | 3125    |
| 1c   | 12.5      | 25    | 25      | 25        | 12.5       | 6.25    | 2c   | 25        | 3125  | 25      | 3125       | 3125       | 3125    |
| 1d   | 25        | 6.25  | 25      | 25        | 6.25       | 3125    | 2d   | 25        | 25    | 25      | 25        | 3125       | 3125    |
| 1e   | 50        | 50    | 25      | 25        | 12.5       | 6.25    | 2e   | 50        | 50    | 25      | 25        | 6.25       | 6.25    |
| 1f   | 50        | 12.5  | 25      | 25        | 12.5       | 25      | 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       | 3125    | 4a   | 25        | 25    | 25      | 12.5       | 12.5       | 3125    |
| 3b   | 25        | 12.5  | 25      | 25        | 3125       | 3125    | 4b   | 50        | 50    | 25      | 50        | 12.5       | 6.25    |
| 3c   | 25        | 50    | 25      | 3125      | 3125       | 3125    | 4c   | 12.5      | 25    | 25      | 25        | 12.5       | 6.25    |
| 3d   | 6.25      | 3125  | 50      | 25        | 3125       | 3125    | 4d   | 50        | 50    | 25      | 50        | 12.5       | 3125    |
| 3e   | 50        | 25    | 25      | 25        | 6.25       | 3125    | 4e   | 50        | 50    | 25      | 50        | 15.6       | 3125    |
| 3f   | 25        | 25    | 25      | 25        | 12.5       | 12.5   | 4f   | 50        | 50    | 25      | 50        | 12.5       | 3125    |
| 3g   | 25        | 12.5  | 25      | 50        | 6.25       | 3125    | 4g   | 25        | 25    | 25      | 25        | 12.5       | 3125    |
| 3h   | 12.5      | 6.25  | 25      | 12.5      | 6.25       | 3125    | -    | -         | -     | -       | -         | -          | -       |

| 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      | -          | -       |

Although
had MIC values of 3.125 µg/mL, whereas fluconazole has an MIC value of 64 µg/mL. The results were in accordance with those found by Na, 2010. The most potent antifungal activity against *C. krusei* was obtained with halogenated indole derivatives.

**CONCLUSIONS**

According to the activity results, all of synthesized compounds demonstrated significant antibacterial and antifungal effects, and the antifungal effects of compounds 3a-h are promising for development into new, more effective 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 that shows actual resistance to fluconazole. Therefore, the search for new and more effective anti-fungal agents against *C. krusei* seems ever more important. 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. 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 had MIC values of 6.25 µg/mL, which is the same as for ciprofloxacin. All these compounds have a m-chlorophenyl group as a substituent. This shows that both the indole ring and the side chains are important for activity. Singh stated that chloro substituents for triazolylindole derivatives were beneficial as well as hydroxy and methoxy substitution for activity. 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, followed by the indole-thiadiazole group. These results suggest that the tested indole derivatives are eligible for development as candidates, especially compound 3d, which is a promising lead compound mainly against MRSA and *C. krusei*. However, further research needs to be performed to determine the specific mode of action and approve indole derivatives as antimicrobial agents.

The common use of antifungal molecules 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, modifications of these compounds should continue to be investigated.

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