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Original Research Article

Synthesis and evaluation of antimicrobial properties of some azole derivatives

Mohd Imran[1]*, Abdulhakim Bawadekji[2], Nawaf Alotaibi[3]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University, PO Box 840, Rafha 91911, [2]Department of Biological Sciences, College of Science, Northern Border University, PO Box 1321, Arar 91431, [3]Department of Pharmaceutics, Faculty of Pharmacy, Northern Border University, PO Box 840, Rafha 91911, Saudi Arabia

For correspondence: **Email: imran_inderlok@yahoo.co.in; **Tel:** +966-599577945*

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Abstract

Purpose: *To synthesize new azole derivatives and determine their antimicrobial properties.*
Methods: *The reaction of the intermediates (2a-2c) with 3a-3c in acetone/potassium carbonate solution yielded 4a-4i, which were characterized using Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR)) and mass spectrometry (MS). Compounds 4a-4i were assessed for their antibacterial and antifungal effects using the sequential dilution technique, relative to ofloxacin and ketoconazole.*
Results: *The spectral data for 4a-4i were consistent with the assigned structures. The MIC of compound 4h (10 µg/ml) was similar to that of ketoconazole against Aspergillus flavus, Penicillium citrinum, and Aspergillus niger. The MIC value of compound 4b (10 µg/ml) for Penicillium citrinum was comparable to that of ketoconazole while the MIC value of compound 4d against Staphylococcus aureus and Escherichia coli (20 µg/ml) was equivalent to the corresponding MIC value for ofloxacin.*
Conclusion: *The synthesized compounds bearing boronic acid moiety are good antimicrobial agents. Accordingly, further investigation into the thiazole-imidazole or thiazole-triazole derivatives bearing boronic acid moiety is suggested.*

Keywords: *Synthesis, Imidazole, Thiazole, Triazole, Antimicrobials*

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INTRODUCTION

Recent reports on microbial resistance and emergence of new microbial diseases pose serious challenges to the affected patients, as well as the health care community [1]. This challenge is further exacerbated by inappropriate antimicrobial therapy trends [2]. Therefore, scientists are making efforts to provide novel antimicrobial agents with diverse mechanisms of action from the known chemical classes of the existing antimicrobial drugs [3,4].

Azole is one of the important chemical classes of antimicrobial agents [5]. The development of azole antimicrobial agents is the focus of current research [6]. Many azole-based antimicrobial agents are already in clinical use, for example, ketoconazole, econazole, miconazole, posaconazole, fluconazole, voriconazole, and

isavuconazole. Based on these facts [5-10], the present study was carried out to synthesize new azole derivatives, and to determine their antibacterial and antifungal properties.

EXPERIMENTAL

Materials and reagents

Gallenkamp apparatus was used to determine the melting points of the synthesized compounds. The IR spectra determination (KBr; wave number in cm^{-1}), NMR analysis (DMSO-d_6; δ in ppm), mass analysis (M^+; m/z), and elemental investigation (C, H and N analysis) were performed using Shimadzu spectrophotometer, Bruker DRX-300 spectrophotometer, Jeol-JMS-D-300 spectrometer, and VARIO El Elementer apparatus, respectively. The monitoring of reactions and assessment of purity were carried out using TLC. Compounds **3a-3c** were purchased from Sigma Aldrich.

Synthesis of substituted phenacyl intermediates (2a-2c)

Compounds **2a-2c** were prepared using the prior art process [11]. In general, a mixture of acetophenone (0.1 mole) in acetic acid (20 ml) was stirred at 80°C with a solution of bromine (0.1 mole) in acetic acid (25 ml). The precipitate was filtered and recrystallized from ethanol.

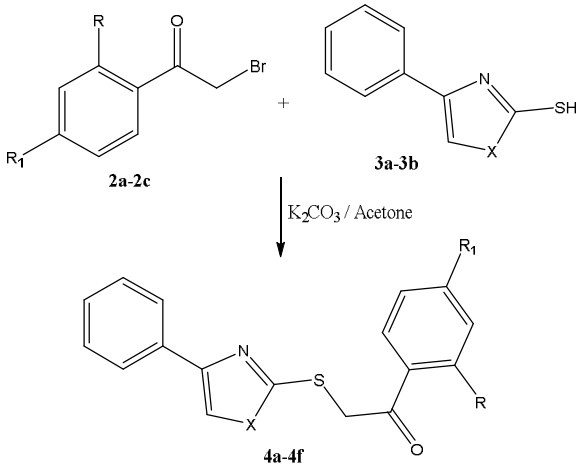
Synthesis of 4-(2-((4-phenyl-1H-imidazole-2-yl)thio)acetyl)phenyl acetate (4a)

A mixture of **2a** (0.1 mole), **3a** (0.1 mole) and potassium carbonate (0.1 mole) in 30 mL acetone was stirred at 25 °C for 15-20 h. The reaction mixture was dissolved in water (250-500 mL), and the filtered residue was purified with ethanol.

The other imidazole derivatives (**4b-4c)**, thiazole derivatives (**4d-4f)**, and triazole derivatives (**4g-4i)** were also prepared in a similar manner.

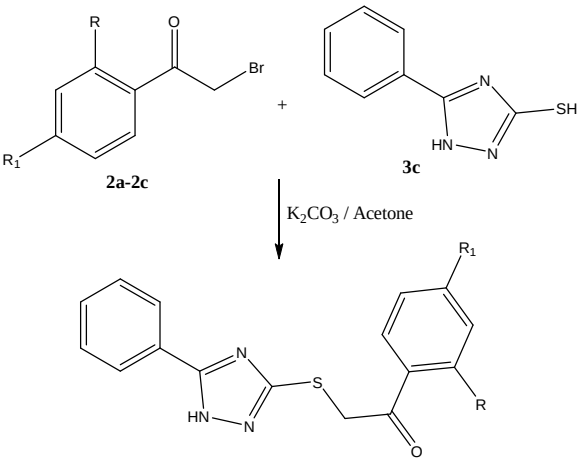
Determination of antimicrobial activity

The sequential dilution technique [12,13] was used for determination of antimicrobial effects of the synthesized compounds. A similar procedure was described in previous publications [7-10]. Different concentrations of **4a-4i**, ketoconazole, and ofloxacin were prepared and their MICs were determined using agar medium and sterile dimethyl sulfoxide (DMSO). The sterile DMSO also functioned as control or blank. The microorganisms tested are indicated in Table 2.



4a: X = -NH-, R = -H, R_1 = -$OCOCH_3$; **4b:** X = -NH-, R = -H, R_1 = -$B(OH)_2$
4c: X = -NH-, R = -Br, R_1 = -H; **4d:** X = -S-, R = -H, R_1 = -$OCOCH_3$
4b: X = -S-, R = -H, R_1 = -$B(OH)_2$; **4c:** X = -S-, R = -Br, R_1 = -H;

Figure 1: Synthesis of compounds **4a-4f**



4g: R = -H, R_1 = -$OCOCH_3$; **4h:** R = -H, R_1 = -$B(OH)_2$
4i: R = -Br, R_1 = -H

Figure 2: Synthesis of compounds **4g-4i**

Statistical analysis

The data are expressed as mean ± standard error mean (S.E.M., n=3). Statistical analysis was done using SPSS-software (version 20). Statistical significance was assumed at $p < 0.05$.

RESULTS

Figure 1 and Figure 2 depict the synthesis of compounds **4a-4f** and **4g-4i**, respectively. The reaction of **2a-2c** with **3a-3c** in acetone/K_2CO_3 yielde **4a-4i**, which were characterized through spectral analysis.

Table 1 and Table 2 display the spectral data of compounds **4a-4i**. The IR spectra of the different compounds in **4a-4i** series exhibited characteristic IR bands. These bands included

characteristics peaks for –OH groups of **4b, 4e**, and **4h**, starting from 3350 to 3360 cm^{-1}; -NH- groups of **4a-4c** and **4g-4i** from 3120 to 3125 cm^{-1}; and C=O groups of the acetoxy groups of **4a, 4d,** and **4g,** from 1730 to 1735 cm^{-1}. Compounds **4a-4i** also exhibited peaks for C=O group from 1690 to 1695; C=N group from 1640 to 1645; and C=C assemblage from 1595 to 1600 cm^{-1}.

The 1H NMR spectra of **4a-4i** showed aromatic hydrogens as multiplets at δ 7.40-7.85, and the methylene protons (-CH_2-) as singlets at δ 4.60-4.63. Compounds **4a, 4d** and **4g** exhibited singlets at δ 2.12-2.15 for the methyl group. Compounds **4a-4c** and **4g-4i** showed a singlet for the –NH- group at δ 11.94-1199. The boronic acid derivatives **4b, 4e** and **4h** displayed singlet peaks for –OH group at δ 4.11-4.15 [11]. The ^{13}C NMR spectra of **4a-4i** revealed characteristic peaks of carbonyl carbon (-CH_2-CO-) at δ 195.0 - 195.1 and methylene carbon (-CH_2-CO-) at δ 39.1-38.4. Compounds **4a, 4d** and **4g** exhibited additional peaks of methyl carbon (-CH_3) at δ 21.2, and another carbonyl carbon (-CO- of acetoxy) at δ 168.1 - 168.0. Other peaks appeared in the aromatic regions of the ^{13}C NMR spectra. The mass analysis (m/z) and the elemental (C, H and N) analysis data of **4a-4i** were also in agreement with the allocated structures.

Table 3 shows the antimicrobial activities of **4a-4i**, wherein the MIC values are expressed in µg/ml. For purpose of comparison, the MIC values of ketoconazole and ofloxacin were considered as 100 %. The data revealed that compound **4h** and ketoconazole had the same MIC value (10 µg/mL) against *Aspergillus niger, Aspergillus flavus,* and *Penicillium citrinum, w*hile compound **4b** and ketoconazole had similar MIC (10 µg/mL) against *Penicillium citrinum.* Compound **4d** and ofloxacin had equivalent MIC value (20 µg/mL) against *Staphylococcus aureus* and *Escherichia coli*. It was also obvious that compounds **4d-4f** displayed very good antimicrobial activities (80 – 100 %) against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa*, relative to ofloxacin, whereas compounds **4a-4c** and **4g-4i** were moderately active.

Table 1a: Spectral data for **4a-4e**

Compound (molecular formula) (melting point)	IR (Wave number in cm-)	^{1}H NMR (δ in ppm, DMSO-d_6)	^{13}C NMR (δ in ppm, DMSO-d_6)	Mass (m/z; M^+)	C, H, N Anal., {Found (Calculated)}
4a ($C_{19}H_{16}N_2O_3S$) (178-180°C)	3120, 1730, 1690, 1640, and 1595	2.12 (s, 3H), 4.62 (s, 2H), 7.40-7.85 (m, 10H), 11.95 (s, 1H)	21.2 (-CH_3), 39.1 (-CH_2-), 120.8, 122.4 (2C), 128.4 (2C), 129.6, 130.1 (4C), 133.1, 134.0, 141.0, 141.6, 156.6, 168.1 (-CO- of acetoxy), 195.0 (-CO-)	352	C, 64.75 (64.76); H, 4.55 (4.58); N, 7.90 (7.95)
4b ($C_{17}H_{15}BN_2O_3S$) (144-146°C)	3350, 3125, 1695, 1645, 1600	4.11 (s, 2H), 4.60 (s, 2H), 7.41-7.84 (m, 10H), 11.98 (s, 1H)	39.1 (-CH_2-), 113.0, 120.8, 128.4 (2C), 129.6 (3C), 130.1 (2C), 134.0, 134.2 (2C), 136.3, 141.0, 141.6, 195.1 (-CO-).	338	C, 60.35 (60.38); H, 4.45 (4.47); N, 8.25 (8.28)
4c ($C_{17}H_{13}BrN_2OS$) (161-163°C)	3122, 1692, 1640, 1600	4.63 (s, 2H), 7.42-7.85 (m, 10H), 11.99 (s, 1H)	38.4 (-CH_2-), 120.8, 122.1, 128.2, 128.4 (3C), 129.6, 130.1 (2C), 133.1, 133.5, 134.0, 141.0, 141.2, 141.6, 195.1 (-CO-).	371 (M^+) & 373 (M^++2)	C, 54.65 (54.70); H, 3.50 (3.51); N, 7.45 (7.51)
4d ($C_{19}H_{15}NO_3S_2$) (171-173°C)	1732, 1695, 1641, 1598	2.15 (s, 3H), 4.61 (s, 2H), 7.40-7.83 (m, 10H)	21.2 (-CH_3), 39.1 (-CH_2-), 113.1, 122.4 (2C), 128.4 (2C), 129.6, 130.1 (4C), 133.1, 134.0, 155.8, 156.6, 167.6, 168.0 (-CO- of acetoxy), 195.0 (-CO-).	369	C, 61.75 (61.77); H, 4.04 (4.09); N, 3.77 (3.79)
4e ($C_{17}H_{14}BNO_3S_2$) (151-153°C)	3360, 1695, 1645, 1600	4.15 (s, 2H), 4.62 (s, 2H), 7.44-7.85 (m, 10H)	39.1 (-CH_2-), 113.0, 113.3, 128.4 (2C), 129.6 (3C), 130.1 (2C), 134.0, 134.2 (2C), 136.3, 155.8, 167.6, 195.1 (-CO-).	355	C, 57.45 (57.48); H, 3.95 (3.97); N, 3.90 (3.94)

Table 2: Spectral data for **4f-4i**

Compound (molecular formula) (melting point)	IR (Wave number in cm-)	^{1}H NMR (δ in ppm, DMSO-d_6)	^{13}C NMR (δ in ppm, DMSO-d_6)	Mass (m/z; M^+)	C, H, N Anal., {Found (Calculated)}
4f ($C_{17}H_{12}BrNOS_2$) (167-169^{o}C)	1690, 1640, 1595	4.62 (s, 2H), 7.41-7.83 (m, 10H).	38.4 (-CH_2-), 113.1, 122.1, 128.2, 128.4 (3C), 129.6, 130.1 (2C), 133.1, 133.5, 134.0, 141.2, 155.8, 167.6, 195.1 (-CO-).	388 (M^+) & 390 (M^++2)	C, 52.25 (52.31); H, 3.05 (3.10); N, 3.55 (3.59)
4g ($C_{18}H_{15}N_3O_3S$) (131-133^{o}C)	3125, 1735, 1695, 1645, 1600	2.14 (s, 3H), 4.62 (s, 2H), 7.41-7.83 (m, 9H), 11.95 (s, 1H)	21.2 (-CH_3), 39.1 (-CH_2-), 122.4 (2C), 128.4 (2C), 130.1 (4C), 132.0, 133.1, 133.4, 156.6, 159.8, 161.4, 168.0 (-CO- of acetoxy), 195.1 (-CO-).	353	C, 61.13 (61.18); H, 4.23 (4.28); N, 11.83 (11.89)
4h ($C_{16}H_{14}BN_3O_3S$) (140-142^{o}C)	3357, 3125, 1695, 1645, 1595	4.12 (s, 2H), 4.61 (s, 2H), 7.41-7.84 (m, 9H), 11.95 (s, 1H)	39.1 (-CH_2-), 113.0, 128.4 (2C), 129.7 (2C), 130.1 (2C), 132.0, 133.4, 134.2 (2C), 136.3, 159.5, 161.4, 195.0 (-CO-).	339	C, 56.61 (56.66); H, 4.12 (4.16); N, 12.26 (12.39)
4i ($C_{16}H_{12}BrN_3OS$) (173-175^{o}C)	3120, 1690, 1643, 1600	4.60 (s, 2H), 7.40-7.85 (m, 9H), 11.94 (s, 1H)	38.4 (-CH_2-), 122.1, 128.2, 128.4 (3C), 130.1 (2C), 132.0, 133.1, 133.4 (2C), 141.2, 159.5, 161.4, 195.1 (-CO-).	372 (M^+) & 374 (M^++2)	C, 51.31 (51.35); H, 3.20 (3.23); N, 11.18 (11.23)

Moreover, compounds **4d-4f** were least active against *Candida albicans, Aspergillus flavus, Penicillium citrinum and Aspergillus niger*, whereas compounds **4a-4c** and **4g-4i** displayed moderate-to-equivalent antifungal activities, relative to ketoconazole.

DISCUSSION

The structure-activity analysis of compounds **4a-4i** revealed that compounds of the triazole series (**4g-4i**) were more potent antifungal agents than those of the imidazole series (**4a-4c**) and the thiazole series (**4d-4f**). This is in line with a previous report [14] which indicated that the azole ring must contain at least two nitrogen atoms for enhanced effects of the azole antifungal agents because the nitrogen atom in position 3 of imidazole ring and triazole ring is essential for the binding of fungal enzymes. This also accounts for the fact that compounds **4d-4f** which lack nitrogen at position 3, were the least potent of the thiazole compounds against the tested fungal strains. It was also obvious that the thiazole derivatives (**4d-4f**) were more potent antibacterial agents than the imidazole **(4a-4c**) and triazole (**4g-4i**) compounds.

It has been established that benzothiazole-based compounds have promising antibacterial effects [15]. There is a possibility that the 4-phenylthiazole moiety of compounds **4d-4f** might be working like benzothiazole-based compounds [15]. In addition, the most potent antifungal compound (**4h)**, and another promising antifungal agent (**4b)** also contain boronic acid moieties, which are also supposed to increase their antifungal potencies [16]. No effect on antimicrobial effect was seen with compounds containing bromine atom. However, the fluorinated derivatives of **4a-4i** must be assessed for their antimicrobial potential, since many fluorinated azole antifungal agents [16,17], and fluorinated antibacterial agents [18] are in clinical use. Finally, the presence of the acetoxy group in compounds **4a, 4d** and **4g** was not supposed to provide any additional antibacterial benefits.

CONCLUSION

Compounds **4h, 4b** and **4d** have been identified as promising antimicrobial agents. However, they need to be evaluated against other bacteria and fungi. It has been established that the presence of a fused thiazole-imidazole or thiazole-triazole ring system in the **4a-4i** types of compounds, along with a boronic acid moiety, may provide better broad-spectrum antimicrobial agents. The incorporation of fluorine in the structure may also provide beneficial outcomes.

Table 3: Antimicrobial effects of compounds **4a-4i**

Compound	MIC[a] in µg/ml (% inhibition, relative to standard)							
	Candida albicans	***Aspergillus niger***	***Aspergillus flavus***	***Penicillium citrinum***	***Staphylococcus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
4a	20 (50%)	25 (40%)	25 (40%)	20 (50%)	30 (66.66%)	40 (50%)	30 (66.66%)	30 (66.66%)
4b	12.5 (80%)	12.5 (80%)	15 (66.66%)	10 (100%)	30 (66.66%)	30 (66.66%)	30 (66.66%)	30 (66.66%)
4c	15 (66.66%)	15 (66.66%)	15 (66.66%)	15 (66.66%)	25 (80%)	25 (80%)	30 (66.66%)	25 (80%)
4d	40 (25%)	40 (25%)	40 (25%)	40 (25%)	20 (100%)	25 (80%)	20 (100%)	22.5 (88.88%)
4e	30 (33.33%)	30 (33.33%)	30 (33.33%)	30 (33.33%)	25 (80%)	25 (80%)	25 (80%)	25 (80%)
4f	30 (33.33%)	30 (33.33%)	30 (33.33%)	30 (33.33%)	25 (80%)	25 (80%)	25 (80%)	25 (80%)
4g	25 (40%)	25 (40%)	30 (33.33%)	25 (40%)	50 (40%)	40 (50%)	40 (50%)	50 (40%)
4h	12.5 (80%)	10 (100%)	10 (100%)	10 (100%)	40 (50%)	40 (50%)	40 (50%)	40 (50%)
4i	15 (66.66%)	15 (66.66%)	15 (66.66%)	15 (66.66%)	30 (66.66%)	50 (0%)	40 (50%)	30 (66.66%)
Ofloxacin	-	-	-	-	20 (100%)	20 (100%)	20 (100%)	20 (100%)
Ketoconazole	10 (100%)	10 (100%)	10 (100%)	10 (100%)	-	-	-	-
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

P <0.05, compared to control

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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