International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 4, 2016

Original Article

SYNTHESIS, STRUCTURAL ELUCIDATION AND ANTIMICROBIAL EVALUATION OF 2-{4-(T-AMINO)-2-(BUT-2-YN-1-YL)}-1, 3 BENZOTHIAZOLE DERIVATIVES

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Received: 21 Dec 2015 Revised and Accepted: 18 Feb 2016

ABSTRACT

Objective: A new series of 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives, 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole (BZ2), 2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole (BZ3), 2-[4-(piperidin-1-yl) but-2-yn-1-yl]-1,3-benzothiazole (BZ4), 2-[4-(azepan-1-yl)but-2-yn-1-yl]-1,3-benzothiazole (BZ5), 2-[4-(4-methylpiperazin-1-yl) but-2-yn-1-yl]-1,3-benzothiazole (BZ6), 2-[4-(2, 6-dimethylpiperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole (BZ7) were synthesized and screened *in vitro* as potential antimicrobial agents.

Methods: *In-vitro* antimicrobial activity evaluation was done, by agar diffusion method and broth dilution test against *Staphylococcus aureus* ATCC 6538p, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, and *Bacillus subtilis* ATCC 6633. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were determined. The results of antimicrobial testing were compared to two positive control drugs ciprofloxacin (5 µg/ml) and fluconazole (500µg/ml).

Results: Compound 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole (BZ5) showed the highest antibacterial activity against *S. aureus* with MIC value of 15.62 µg/ml while; Compound 2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole (BZ7) exhibited the highest antibacterial activity against *P. aeruginosa* with MIC value of 31.25 µg/ml. Compounds 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole (BZ2) and 2-[4-(azepan-1-yl)but-2-yn-1-yl]-1,3-benzothiazole (BZ5) showed the highest antifungal activity against *C. albicans* with MIC value of 15.62 µg/ml (for both).

Conclusion: The results obtained showed variation in the antibacterial and antifungal activity based on the structure of the cyclic amines in these amino acetylenic benzothiazole derivatives.

Keywords: Benzothiazole, Aminoacetylenic, Antibacterial, Antifungal, Mannich reaction

INTRODUCTION

Microbes are the causative agents of various types of infectious diseases like pneumonia [1], amebiasis [2], typhoid [3], malaria [4], common cold [5], influenza [6], syphilis and AIDS [7]. The majority of microbes causing these infections have been developed resistance against the commonly used antimicrobial agents [8], so searching for new antimicrobial agents is crucial, among various heterocyclic derivatives that act as antimicrobial agents were the benzothiazole derivatives. In one study [9] which compares the potency between oxazole and benzothiazole derivatives for antibacterial and antifungal activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* for antibacterial activity, and for antifungal activity against *Aspergillus niger* and *Candida albicans.* Benzothiazole derivative 1 was found more potent than oxazole derivative 2 in both antibacterial and antifungal activity.

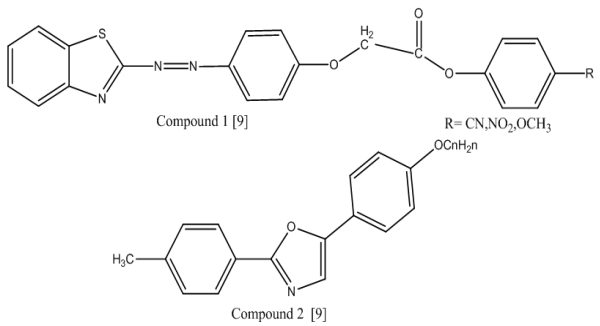


Fig. 1: The structure of benzothiazole derivative (compound 1) and oxazole derivative (compound 2)

Another series of substituted benzothiazole derivatives were found to be associated with a different antibacterial activity such as compounds 3 and 4. Both exhibited high antibacterial activity against drug-resistant Gram-positive bacteria. Some of these benzothiazoles are active against *Enterococcus faecalis* [10].

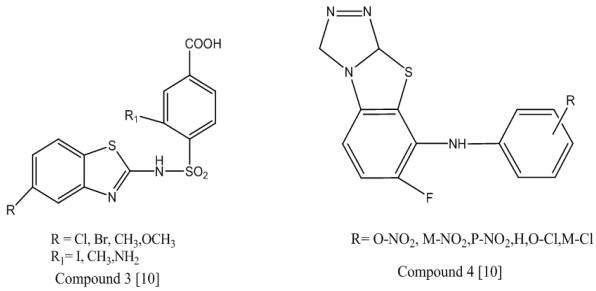


Fig. 2: The structure of substituted benzothiozole derivatives (compound 3 and 4)

As a result of reviewing the investigational drugs used as antimicrobial agents [11, 12]. We envision a unique and new series of benzothiazole derivatives, namely 2-{4-(t-amino)-2-(Burt-2-in-1-yl)}-1, 3-benzothiazoles based on the concept of the fractional based analysis with the following unique substitutes. Benzothiazole ring as a directing moiety towards different sites in bacteria and fungi that lead to antimicrobial activity, intensify the effect by the amino acetylenic link which provides the following binding interaction. The cyclic amino group provides ionic or hydrogen bonding with various

sites in bacteria or fungi, a similar acetylenic group for electrostatic interaction and the 2-butyl to provide the appropriate distance between benzothiazole moiety and the cyclic amine. These forces of interaction expected to provide the potential antimicrobial activity. Our preliminary antimicrobial evaluation was in agreement with the design of this unique series of amino acetylenic benzothiazoles.

MATERIALS AND METHODS

Experimental

Chemicals

The following chemicals and materials were used: Benzothiazole (Sigma-Aldrich, St. Louis, U. S. A), Piperidine (Sigma-Aldrich, St. Louis, U. S. A), Propargyl bromide (Sigma-Aldrich, St. Louis, U. S. A), 2-Methylpiperidine (Sigma-Aldrich, St. Louis, U. S. A), 2, 6-Dimethyl-piperidine (Sigma-Aldrich, St. Louis, U. S. A), Pyrrolidine (Sigma-Aldrich, St. Louis, U. S. A), 1-Methyl-piperazine (Sigma-Aldrich, St. Louis, U. S. A), Hexamethylenamine (Sigma-Aldrich, St. Louis, U. S. A), DMSO (Sigma-Aldrich, St. Louis, U. S. A), Potassium carbonate anhydrous, Chloroform, Ethanol (Sd Fine Chem Ltd), Cuprous Chloride, 1,4-Dioxane (Full Time Chemicals, Anqing, China), Acetonitrile (SDFCL Chemicals, Mumbai, India), Paraformaldehyde (BDH Chemicals, Pennsylvania, U. S. A), Distilled water, Dimethyl sulfoxide-d6 (Sigma-Aldrich, St. Louis, U. S. A) and Tetramethyl silane (Sigma-Aldrich, St. Louis, U. S. A).

Culture media

Muller Hinton Agar, Muller Hinton Broth, Sabouraud's Dextrose Agar, and Sabourauds Dextrose Broth were obtained from Oxoid Laboratories (Hampshire, U. K).

Instrumentation

The structures of the synthesized compounds were confirmed by IR, ^{1}H-NMR, [13]C-NMR, DSC and elemental analysis. Melting points were determined on Gallenkamp Melting point Apparatus (California, U. S. A). Infrared (IR) spectra were recorded on Bruker FT-IR spectrophotometer (Massachusetts, U. S. A), using KBr discs and values were represented in cm^{-1}. ^{1}H-NMR and [13]C-NMR spectra were measured on a Varian 300 MHz spectrometer (Illinois, U. S. A) and DMSO-d_6 as a solvent with TMS (Tetramethyl silane) as the internal standard. 1H data are reported in order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; m, multiplet). The elemental analysis was performed for C, H, N using (Euro EA elemental analyzer, Milan, Italy). The results obtained had a maximum deviation of (+2.59 to-2.95) from the theoretical value, which is considered within the acceptable variation range in results (±4. 4 %). This variation range is set according to the accuracy of Euro EA Elemental Analyzer device. DSC thermogram measurement was carried out by using the DSC 1 Stare System v.11. ox (Mettler Toledo, Zürich, Switzerland). ChemBioDraw (Massachusetts, U. S. A) was used in the drawing of our schemes.

Synthesis of 2-(prop-2-yn-1-yl)-1,3-benzothiazole (BZ1)

A mixture of Propargyl bromide (1.88 g, 15.8 mmol) in Acetonitrile (10 ml) was added to the mixture of Benzothiazole (1.93 g, 13.2 mmol) and Potassium carbonates anhydrous (2.18 g, 15.88 mmol) in Acetonitrile (20 ml). The reaction mixture was heated and stirred under reflux for 80 min. After cooling, the insoluble residue was removed by filtration, and then the solvent was removed under reduced pressure. After that, 30 ml chloroform and 20 ml Distilled water (D. W) were added, and the filtrate was extracted using a separatory funnel. The organic layer was concentrated by the removal of chloroform under reduced pressure to afford the desired orange powder compound BZ1 $C_{10}H_7NS$ in 1.4 gm, 80% yield; IR (KBr cm^{-1}): 3075, 2913, (ArH, stretch), 2294 (C≡CH, stretch), 1611, 1423 (Ar, C=C, stretch), 1252, 1109, 1019 (Ar, C=C, bending), 893, 750 (ArH, bending).^{1}H-NMR (DMSO-d_6): δ 2.49 (s, 1H, C≡ CH), 3.38 (s, 2H, CH_2-C≡), 6.8-7.1 (m, 4H, ArH).[13]C-NMR (DMSO-d_6): 62 (CH_2), 74 (CH), 81 (C≡CH), 114, 116, 122, 124, 132, 151(Ar, C), 174 (N=C-S).

Elemental analysis: Calcd. for $C_{10}H_7NS$: C, 63.47%; H, 3.73%; N, 7.40%, found C, 63.24%; H, 3.51%; N, 7.31%.

Synthesis of 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1, 3-benzothiazole derivatives (BZ2-BZ7)

A mixture of 2-(prop-2-in-1-yl)-1,3-benzothiazole BZ1 (1.5 g, 0.01 mol), Paraformaldehyde (0.5 g, 0.015 mol), the cyclic amine around (0.01 mol), and a catalytic amount of Cuprous chloride (0.03 g), in Peroxide-free 1,4-Dioxane (30 ml) was stirred and refluxed for 1 h. After cooling, the insoluble residue was removed by filtration then the solvent was distilled off under reduced pressure. Ethanol (30 ml) was added to the residue, and then ethanol was evaporated under reduced pressure. What residue left was dissolved in the least amount of ethanol, precipitated with water, filtered and dried to afford Mannich bases BZ2, BZ3, BZ4, BZ5, BZ6, BZ7 (Fig.3). Physical and spectral data for the resulting compounds (BZ1-BZ7) are listed below in the results.

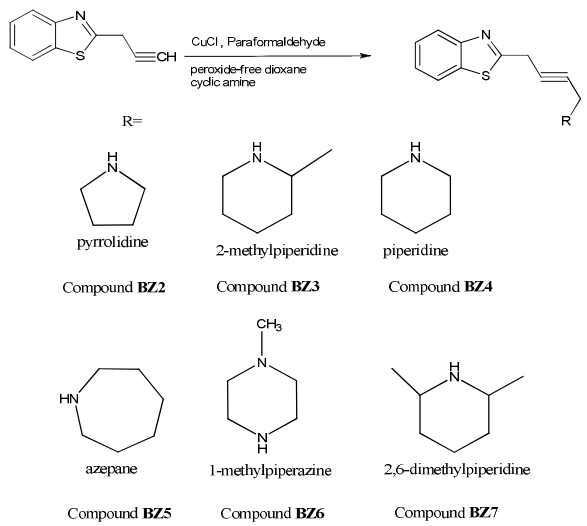


Fig. 3: Synthesis of t-amino [but-2-yn-1-yl]-1, 3-benzothiazole derivatives (BZ2-BZ7)

Synthesis of 2-[4-(pyrrolidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole (BZ2)

The title compound BZ2 was synthesized, using a similar procedure to that described for the preparation of 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7) in 1.4 g, 54% yield as orange powder; m. p: (157-159 ^{o}C). IR (KBr cm^{-1}): 2913 (ArH, stretch), 3030, 2357 (C≡C, stretch), 1602, 1414 (Ar, C=C, stretch), 1252, 1136 (Ar, C=C, bending), 866, 759, (ArH, bending). ^{1}H-NMR (DMSO-d_6): δ, 1.6-1.7 (m, various proton of cyclic amine), 2.4 (s, 2H, C-CH_2-N), 3.8 (s, 2H, CH_2-C), 6.6-7.2 (m, 4H, ArH).

Synthesis of *2*-[4-(2-methylpiperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole (BZ3)

The title compound BZ3 was synthesized, using a similar procedure to that described for the preparation of 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7) in 1.5 g, 52% yield as orange powder; m. p (130-134 ^{o}C). IR (KBr cm^{-1}): 2915 (Ar, C-H stretch), 2800 (Ar, C-H stretch), 1480 (Ar, C=C stretch), 1250 (Ar, C-N, stretch), 1025 (Ar, C-N, stretch), 850 (Ar, C-H, bend), 750 (Ar, C-H, bend). ^{1}H-NMR (DMSO-d_6): δ, 1.1 (d, 3H, CH-CH_3), 1.34, 1.5 (m, various proton of cyclic amine), 3.4 (s, 2H, C-CH_2-N), 3.7 (s, 2H, CH_2-C), 6.6-7.2 (m, 4H, ArH).

Synthesis of 2-[4-(piperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole (BZ4)

The title compound BZ4 was synthesized, using a similar procedure to that described for the preparation of 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7) in 1.73 g, 64% yield as orange powder; m. p: (148-150 ^{o}C). IR (KBr cm^{-1}): 2915 (Ar, C-H, stretch), 2800 (Ar, C-H, stretch), 1480 (Ar, C=C, stretch), 1250 (Ar, C-

N, stretch), 1025 (Ar, C-N, stretch), 850 (Ar, C-H, bend), 750 (Ar, C-H, bend). ^{1}H-NMR (DMSO-d_6): δ, 1.5 (m, various proton of cyclic amine), 2.8 (s, 2H, C-CH_2-N), 3.7 (s, 2H, CH_2-C≡), 6.6-7.2 (m, 4H, ArH).

Synthesis of 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole (BZ5)

The title compound BZ5 was synthesized, using a similar procedure to that described for the preparation of 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7), in 1.44 g, 50% yield as orange powder; m. p: (122-125^{o}C). IR (KBr cm^{-1}): 2915 (Ar, C-H, stretch), 2800 (Ar, C-H, stretch), 1480 (Ar, C=C, stretch), 1250 (Ar, C-N, stretch), 1025 (Ar, C-N, stretch), 850 (Ar, C-H, bend), 750 (Ar, C-H, bend). ^{1}H-NMR (DMSO-d_6): δ, δ 1.6-1.7 (m, various proton of cyclic amine), 2.4 (s, 2H, C-CH_2-N), 3.6 (s, 2H, CH_2-C), 6.6-7.2 (m, 4H, ArH). ^{13}C-NMR (DMSO-d_6): δ, 26 (C[20]), 28 (C[19]), 32 (C[17,18]), 66 (C[13,15,16]), 86 (C[11,12]), 114 (C^3), 117 (C^4), 120 (C^6), 123 (C^5), 132 (C^2), 153 (C^1), 174 (C^8), 55 (C[10]).

Elemental analysis: Calcd. for **$C_{17}H_{20}N_2S$**: C, 67.97%; H, 6.71%; N, 9.32% found C, 66.3%; H, 5.89%; N, 9.3%.

Synthesis of 2-[4-(4-methylpiperazin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole (BZ6)

The title compound BZ6 was synthesized, using a similar procedure to that described for the preparation of 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1, 3-benzothiazole derivatives (BZ2-BZ7); in 1.75 g, 64% yield as orange powder; m. p: (166-170 ^{o}C). IR (KBr cm^{-1}): 2915 (Ar, C-H, stretch), 2800 (Ar, C-H, stretch), 1480 (Ar, C=C, stretch), 1250 (Ar, C-N, stretch), 1025 (Ar, C-N, stretch), 850 (Ar, C-H, bend), 750 (Ar, C-H, bend). ^{1}H-NMR (DMSO-d_6): δ, 1.8-2.1 (m, various proton of cyclic amine), 2.5 (s, 2H, C-CH_2-N), 3.7 (s, 2H, CH_2-C), 6.6-7.2 (m, 4H, ArH).

Synthesis of 2-[4-(2, 6-dimethylpiperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole (BZ7)

The title compound BZ7 was synthesized, using a similar procedure to that described for the preparation of 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7), in 1.83 g, 63% yield as orange powder; m. p: (160-163 ^{o}C). IR (KBr cm^{-1}): 2915 (Ar, C-H, stretch), 2800 (Ar, C-H, stretch), 1480 (Ar, C=C, stretch), 1250 (Ar, C-N, stretch), 1025 (Ar, C-N, stretch), 850 (Ar, C-H, bend), 750 (Ar, C-H, bend). ^{1}H-NMR (DMSO-d_6): δ, 1.1 (d, 6H, CH-CH_3), 1.4, 1.5, 1.7, 1.8, 1.9 (m, various proton of cyclic amine), 3.4 (s, 2H, C-CH_2N), 4.2 (s, 2H, CH_2-C), 7-7.6 (m, 4H, ArH).^{13}C-NMR (DMSO-d_6): δ, 23, 25, 35 (various C of cyclic amine), 66 (C-N), 80, 81 (C≡C), 114, 117, 120, 123, 132, 153 (Ar, C), 174 (N=C-S).

Antimicrobial activity testing

All the newly synthesized compounds 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7) were tested for *in vitro* antimicrobial activity, by the agar well diffusion method [13] measuring the inhibition zone diameter, using 10^5 CFU/ml microbial inoculum and 0.1 ml of serial compounds dilution, and by determination of minimum inhibitory concentration (MIC) [13], the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were also recorded against the selected microorganisms. The synthesized compounds were screened for antimicrobial activity against two Gram-negative microorganisms *E. coli* (ATCC 8739) and *P. aeruginosa* (ATCC 9027) and two Gram-positive microorganisms *S. aureus* (ATCC 6538p) and *B. subtilis* (ATCC 6633), using Muller Hinton agar medium at 200, 100, 50, 25 µg/ml concentrations. Screening of antifungal activity was done against *C. albicans* (ATCC 10231), using Sabouraud's dextrose agar medium at 200, 100, 50, 25 µg/ml.

Stock solutions of the compounds were first prepared by dissolving them in a solution of 30% DMSO in water. Then the solutions were diluted serially in the media (Muller Hinton Broth for bacteria and Sabouraud's Dextrose Broth for fungi) so as to achieve the required concentrations of the compound ranging from 200-25 µg/ml. A solution of 30% DMSO in distilled water (D. W) was employed as a solvent control. The results of antimicrobial testing are reported and compared with those of standard drugs ciprofloxacin 5 µg/ml and fluconazole 500 µg/ml. The antimicrobial activity testing was done in triplicate.

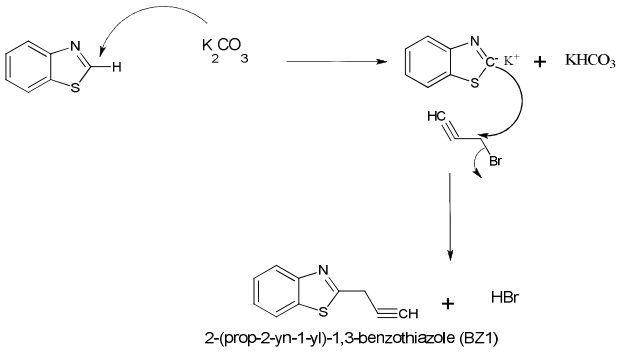
Statistical analysis

Statistical analysis was carried out using Student's t-test by statistical packages for social science software (SPSS). Values are expressed as mean±SD and values of $p<0.05$ were considered statistically significant. The relationships between variables were calculated using Pearson Correlation Coefficients.

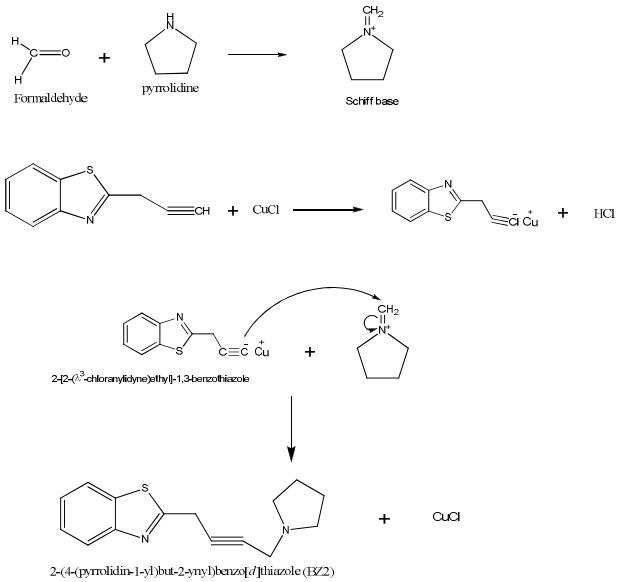
RESULTS AND DISCUSSION

Chemistry

The designed compounds were prepared as shown in schemes (1 and 2). Scheme 1 involved the alkylation of benzothiazole with 3-bromo prop-1-yne (Propargyl bromide) in the presence of acetonitrile as a solvent under basic conditions. The reaction involves direct displacement of the anionic carbon in the thiazole ring on the propargyl bromide to generate 2-(prop-2-yn-1-yl)-1, 3-benzothiazole BZ1. The Mannich reaction of 2-(prop-2-yn-1-yl)-1,3-benzothiazole BZ1, Paraformaldehyde, appropriate cyclic amine, and a catalytic amount of Cuprous chloride in Peroxide-free 1,4-Dioxane was heated to 80 ^{o}C to yield the designed compounds (BZ2-BZ7). The yield obtained ranged from 52.4 to 60.75%. The proposed mechanism for Mannich reaction is shown (scheme 2), in order for Mannich reaction to proceed, a reactive immonium cations intermediates should be formed from the condensation of the formaldehyde and the appropriate amines (Schiff base formation). The attack of the carbanion in 2-(prop-2-yn-1-yl)-1, 3-benzothiazole cuprous salt on the Schiff base generates the desired Mannich products (BZ2-BZ7). The IR, ^{1}H-NMR, ^{13}C-NMR, DSC and elemental analysis were consistent with the assigned structures.



Scheme 1: Alkylation reaction of benzothiazole moiety



Scheme 2: Proposed Mannich reaction

Antimicrobial activity

The newly synthesized compounds BZ2-BZ7 showed activity against all types of the tested microorganisms, after 24 h incubation at 37 °C for bacteria and after 48 h incubation at 37 °C for fungi, the diameter zone of inhibition was measured. Compound 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole (BZ2) showed high antimicrobial activity against *P. aeruginosa* with a zone of inhibition diameter of 30, 27, 20 mm in concentrations of 200, 100, 50 µg/ml, respectively, in comparison with the positive control ciprofloxacin (5 µg/ml) which gave 21 mm. Compound 2-[4-(piperidin-1-yl) but-2-yn-1-yl]-1,3-benzothiazole (BZ4) in the concentration of 200 µg/ml showed higher antimicrobial activity against *B. subtilis*, with a zone diameter of 26 mm in comparison to ciprofloxacin (5 µg/ml) which gave 21 mm. The results obtained by comparing the MIC values for compounds BZ2-BZ7 with the MIC values of the positive control ciprofloxacin (5 µg/ml), we find that the MIC value of compound BZ5 against *S. aureus* is less than the MIC value of the positive control (15.62, 50 µg/ml respectively). The MIC of compound BZ7 against *P. aeruginosa* is less than that of the positive control ciprofloxacin in 5 µg/ml (31.25, 50 µg/ml respectively). The pattern of the results of antifungal screening was different from that of antibacterial evaluation. The results of antifungal screening showed the good antifungal activity of all the synthesized compounds against *C. albicans*. Compound 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole BZ5 also showed the highest antifungal activity among the synthesized compounds. Compounds BZ2 and BZ5 had the lowest MIC value of 15.62 µg/ml (for both) among all the synthesized compounds. The antimicrobial results of the newly synthesized compounds BZ2-BZ7 showed a broad spectrum of antibacterial and antifungal activity as shown in the table (1) and table (2).

Table 1: The diameter of the zone of inhibition (in mm) of compounds BZ2-BZ7 at 200µg/ml concentration.

Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
Concentration	**200**	**200**	**200**	**200**	**200**
BZ2	27±0.58	23±1.00	30±0.00	30±0.58	32±1.00
BZ3	25±1.00	24±0.00	27±1.15	28±0.00	30±0.00
BZ4	28±0.58	26±0.58	25±0.5	26±0.58	27±0.58
BZ5	27±1.53	24±0.58	18±0.58	24±2.00	21±0.58
BZ6	19±0.58	20±1.53	17±1.53	18±0.58	19±1.53
BZ7	17±0.00	19±1.00	18±1.73	19±1.53	20±0.00
Ciprofloxacin (5µg/ml)	30±0.58	21±1.73	30±1.53	21±0.00	-
Fluconazole (500 µg/ml)	-	-	-	-	30±0.58
Negative control	0	0	0	0	0

Values are mean±SD (n=3).

BZ2: 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1, 3-benzothiazole derivatives 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole

BZ3: 2-[4-(2-methylpiperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

BZ4: 2-[4-(piperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

BZ5: 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

BZ6: 2-[4-(4-methylpiperazin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

BZ7: 2-[4-(2, 6-dimethylpiperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

Table 2: Minimum inhibitory concentration (MIC) of compounds (BZ2-BZ7) in µg/ml against *S. aureus, B. subtilis, E. coli, P. aeruginosa* and *C. albicans*

Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
.	**MIC/MBC (µg/ml)**	**MIC/MBC (µg/ml)**	**MIC/MBC (µg/ml)**	**MIC/MBC (µg/ml)**	**MIC/MFC (µg/ml)**
BZ2	62.5/(125)	62.5/(125)	31.25/(62.5)	31.25/(62.5)	62.5/(125)
BZ3	62.5/(125)	31.25/(62.5)	31.25/(62.5)	31.25/(62.5)	62.5/(125)
BZ4	62.5/(125)	31.25/(62.5)	62.5/(125)	62.5/(125)	125/(250)
BZ5	15.62/(31.25)	31.25/(62.5)	62.5/(125)	62.5/(125)	62.5/(125)
BZ6	125/(250)	62.5/(125)	125/(250)	125/(250)	62.5/(125)
BZ7	125/(250)	31.25/(62.5)	125/(250)	125/(250)	31.25/(62.5)
ciprofloxacin (5 µg/ml)	50	25	25	50	-
fluconazole (500 µg/ml)	-	-	-	-	8
Negative control	-	-	-	-	-

BZ2: 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1, 3-benzothiazole derivatives 2-[4-(pyrrolidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

BZ3: 2-[4-(2-methylpiperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

BZ4: 2-[4-(piperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

BZ5: 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

BZ6: 2-[4-(4-methylpiperazin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

BZ7: 2-[4-(2, 6-dimethylpiperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole

MIC: Minimum inhibitory concentration.

MBC: Minimum bactericidal concentration.

MFC: Minimum fungicidal concentration

It was clear that there is variability in the susceptibilities of the different microorganisms to the different tested compounds and variability in the antibacterial activity, according to the method of investigation (agar diffusion method or broth dilution method). Many factors may influence the results, of these is the diffusion of the compound into the agar medium, which affected by the solubility of the compound [13] and the molecular weight of the compound, compounds with poor water solubility diffuse into the agar medium with difficulty giving smaller zone of inhibition, reported as low antimicrobial activity, the results obtained are often inadequate in comparison with the results of the broth dilution test which lacks these difficulties [13]. The good antibacterial activity of the compounds *2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole* (BZ5) against Gram-positive bacteria is attributed to the lipophilicity and steric effect due to the large size of azepan and methyl piperidine groups respectively. The steric effect may exert physical pressure on the cell wall of Gram-positive bacteria, and the lipophilicity effect may facilitate the diffusion of the compound through the cell wall. The outer membrane of the Gram-negative bacteria which characterize them from Gram-positive bacteria consists mainly of lipopolysaccharide, lipoproteins, and phospholipids, so the lipophilicity facilitated the entry of the compound into the microorganism which related to its antibacterial activity against Gram-negative bacteria.

Substitution on a piperidine ring with a methyl group, lead to enhanced antimicrobial activity against Gram-negative bacteria (compound 2-[4-(2-methylpiperidin-1-yl) but-2-yn-1-yl]-1, 3-benzothiazole (BZ3) when compared to compound BZ4. Also, disubstitution on ortho position of the piperidine ring with a methyl group increased the antibacterial activity (compound 2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole BZ7), in comparison with compound 2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole BZ4 against *P. aeruginosa.* Compound 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole BZ5 also showed the highest antifungal activity among the synthesized compounds, due to steric and lipophilicity effects of the large azepan group. The compounds possessed a broad spectrum of antimicrobial activity against the tested microorganisms and displayed similar antimicrobial activity against fungi, as bacteria, demonstrated by similar MIC values against the tested microorganism. Another rationalization for these compounds with the selectivity to fungal sites of action, such as inhibition of Squalene epoxidase [14-16], CYP-450 [14, 17], and others [14] more than to those associated with the bacterial transpeptidase enzyme or blockade of protein synthesis. Further investigation may verify these points.

CONCLUSION

In conclusion, we have reported the synthesis of novel series of amino acetylenic benzothiazole derivatives. These amino acetylenic benzothiazole derivatives showed promising activity against Gram positive bacteria, Gram negative bacteria, and fungi. Compound BZ5 showed the highest antibacterial activity against *S. aureus* among all the compounds with a MIC value of 15.62 µg/ml while; Compound BZ7 exhibited the highest antibacterial activity against *P. aeruginosa* with a MIC value of 31.25 µg/ml. The data on antimicrobial and antifungal activity generated from this investigation merit the generation of new derivatives amino acetylenic benzothiazoles with greater potency.

ACKNOWLEDGEMENT

The authors would like to thank the University of Petra/Faculty of Pharmacy, for providing the necessary facilities to carry out this work.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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