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Synthesis, Characterization, *in vitro* Biological Evaluation of a Series of Benzothiazole Amides as Antibacterial Agents

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A series of benzothiazole amides (**VH01-06**) were synthesized by using modified Schotten-Baumann reaction conditions, all the resultant compounds were obtained in good yields and purity. The molecular structure of the compounds was characterized using physical and spectral methods. The structures of the compounds were consistent with the analytical data obtained for FT-IR, ^{1}H NMR, ^{13}C NMR and HRMS ESI-Mass spectroscopic techniques. Further, compounds were subjected to the *in vitro* antibacterial activity screening against various strains that includes Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 3359 and methicillin-sensitive *Staphylococcus aureus* (MSSA) ATCC 2592) and Gram-negative bacteria (*Escherichia coli* J53 R1, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031 and *Klebsiella pneumonia* BAA-1075). Based on the biological screening results, compounds **VH01-06** were found to possesses limited antibacterial properties against selected strains, since the recorded activity was found at 100 µM concentration. While compound **VH05** displayed specific activity against *E. coli* ATCC 25923 at 200 µg/mL.

Keywords: Benzothiazole-amide, *in vitro* antibacterial activity, Schotten-Baumann reaction, Biological activity.

INTRODUCTION

Benzothiazole is a heterocyclic compound where thiazole-fused to the benzene ring, the substitution of varied functional groups at different positions in the ring explored the chemical diversity of benzothiazole for various possible chemical modifications [1]. In addition, benzothiazole derivatives experimented by the researchers in the design, discovery and development of various classes of therapeutic agents approved for the clinical use [2]. Medicinal chemists have been continuous embarking on the synthesis of novel compounds of this chemotype due to the broad spectrum of medicinal uses associated with the benzothiazole scaffold [3]. The modified analogues of benzothiazole revealed a significant number of pharmacological properties such as antifungal [4], cyclooxygenase inhibitors [5], antimicrobial [6], anticonvulsant [7], mosquitocidal [8], anticancer [9], antioxidant [10], anti-inflammatory [11], bacterial type II topoisomerase inhibitors [12], photosensitizers [13], selective PI3Kβ inhibitors [14], topoisomerase I inhibitors [15], cytotoxic agents [16], falcipain inhibitors [17], tubulin polymerization inhibitors [18], p53 inhibitors [19], neuroprotective [20], antiacetylcholinesterase [21], vasorelaxants [22], inhibitors of the insulin releasing process [22], antiproliferative agents [23], anti-influenza [24], hemostatic agents [25], antitubercular agents [26], chemokine receptor 2 (CXCR2) inhibitors [27], EGFR inhibitors [28], cyclin-dependent kinase 2 inhibitors [29], β-amyloid imaging agents [30], α-glucosidase inhibitor [31], antidepressant [32], monoamine oxidase A/B inhibitors [33], non-carboxylic PTP1B inhibitors [34], histone deacetylase inhibitors [35], β-glucuronidase activity [36], antiviral [37], tyrosine kinase inhibitors [38], antibacterial [39], anti-infective agents [40], human DNA topoisomerase IIα inhibitors [41], 17β-HSD10 inhibitors [42], human estrogen receptor modulators [43], CK-1δ inhibitors [44], antidiabetic [45], KATP channel Openers [46], schistosomicidal agents [47], COX-2/5-LOX inhibitors [48], analgesics [49], plant growth regulators

[50], Raf-1 inhibitor [51], PPARα antagonists [52], aurora B kinase inhibitors [53], antileishmanial [54], antichagasic agents [55], diuretic [56], DNA gyrase B inhibitors [57], respectively.

The benzothiazole nucleus help in the synthesis many therapeutic agents [3]. The development of biological activities of benzothiazole derivatives has been engaging in recent years [1]. These derivatives have particular importance in the medicinal chemistry field, resulting in their remarkable pharmacological properties [2]. Based on the structure-activity relationship (SAR) reported in the literature, position 2 in the benzothiazole ring has been identified as a key structural modification carried out and resulted as a potential antimicrobial agent [58]. Therefore, we have conducted this study with the primary objective of synthesise a series of 2-substituted benzothiazoles and evaluated their *in vitro* antibacterial activity against particular Gram-positive and Gram-negative strains as part of our ongoing systematic investigation on the identification of novel antibacterial agents.

EXPERIMENTAL

The purity of the synthesized compounds was checked on pre-coated 60 F_{254} silica gel TLC plates (USA, Merck, 0.25 mm) thickness by means of a gradient solvent system with *n*-hexane and ethyl acetate. Flourier-transform infrared (FT-IR) spectrometer (Japan, Shimadzu, Model: MIRAffinity-1S) used to record the spectra. ^{1}H NMR & ^{13}C NMR spectra recorded on a Varian NMR System (Varian, 500 MHz, USA) using TMS (tetramethylsilane) as an internal standard, Weighing Balance (Mettler Toledo, Model: ML204, USA) was used to weigh the chemicals used in the synthetic protocols. The Electrospray Ionization mass spectra (ESI-MS) were recorded using High-resolution mass spectrometry (HRMS) (Thermo Scientific, Q Exactive Focus (Orbitrap LC-MS/MS System), USA). Melting point apparatus (Stuart Scientific, Model: SMP1, U.K.) were determined in open capillary tubes and are uncorrected.

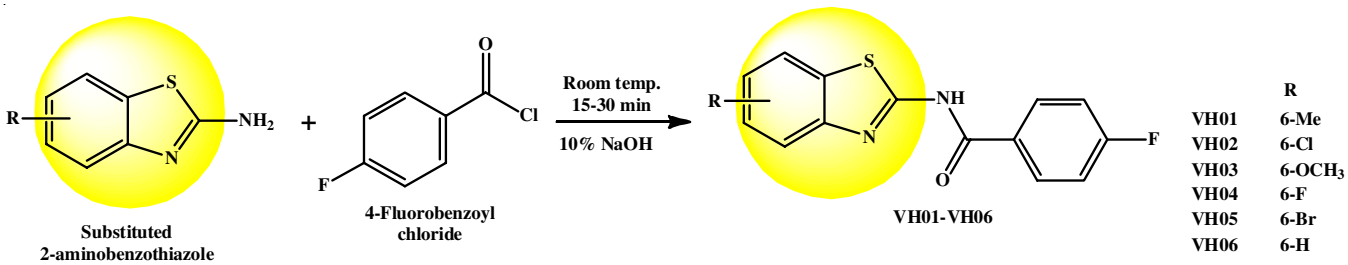
All the reagents and chemical were purchased from Sigma-Aldrich, USA, includes 4-fluorobenzoyl chloride, 2-amino-benzothiazole, 2-amino-6-chlorobenzothiazole, 2-amino-6-bromobenzothiazole, 2-amino-6-methylbenzothiazole, 2-amino-6-methoxybenzothiazole, 2-amino-6-fluorobenzothiazole, sodium hydroxide, dichloromethane, acetone, methanol, hexane, ethyl acetate, absolute ethanol respectively. The Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 3359 and methicillin-sensitive *Staphylococcus aureus* (MSSA) ATCC 2592 and Gram-negative bacteria (*Escherichia coli* J53 R1, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031 and *Klebsiella pneumonia* BAA-1075). The ATCC cultures were obtained from a commercial provider and the rest of the strains were obtained from the Research Lab Office of International Medical University (IMU).

General procedure of synthesis of benzothiazole-amides (VH01-VH06): The procedure followed for the synthesis was described by Schotten-Baumann reaction with slight modification, an equimolecular amount of substituted 2-aminobenzothiazole (0.01 mol) was added into a round-bottomed flask that dissolved in 50 mL of 10% NaOH. An equimolecular amount of reactant 4-fluorobenzoyl chloride (0.01 mol) was added dropwise to the reaction mixture with continuous stirring and the round bottomed flask (RBF) was then closed with a glass lid and then the reaction mixture was vigorously agitated for 15-30 min with occasional venting of pressure build inside the flask and the reaction mixture was then cooled to room temperature. The precipitated crude solid product was then filtered under vacuum, washed with distilled water, dried over sodium sulphate bed, further subjected for purification using recrystallization technique (**Scheme-I**).

4-Fluoro-*N*-(6-methylbenzo[*d*]thiazol-2-yl)benzamide (VH01): Yield: 63%; slight yellowish powder; m.p.: 228-232 ºC; m.f.: $C_{15}H_{11}N_2OSF$; Relative molecular mass: 286; FT-IR (ATR, ν_{max} cm^{-1}): 3392.79 (2º amide N-H *str.*), 1708.03 (C=O *str.*), 1633.71 (aromatic C=C *str.*), 1597.06 (N-H bend.); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.18 (dd, *J* = 8.8, 5.5 Hz, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.65 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.38 (t, *J* = 8.8 Hz, 1H, Ar-H), 7.27 (d, *J* = 8.3 Hz, 2H, Ar-H), 2.41 (s, 3H, Ar-CH_3); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 164.28, 150.42, 133.66, 132.59, 131.63, 128.02, 121.79, 116.42, 116.24, 116.06, 40.33, 40.16, 39.99, 39.82, 39.66, 39.49, 39.32, 21.44; HRMS ESI-MS (*m/z*): 287.0650 $[M+H]^+$ (positive-ion mode), 285.0507 $[M-H]^-$ (negative-ion mode).

N-(6-Chlorobenzo[*d*]thiazol-2-yl)-4-fluorobenzamide (VH02): Yield: 62%; cream coloured powder; m.p.: 218-220 ºC; m.f.: $C_{14}H_8N_2OSClF$; Relative molecular mass: 306; FT-IR (ATR, ν_{max} cm^{-1}): 3267.41 (2º amide N-H *str.*), 1678.07 (C=O *str.*), 1627.92 (aromatic C=C *str.*), 1531.48 (N-H bend.); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.19 (dd, *J* = 8.8, 5.4 Hz, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 7.76 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.47 (d, *J* = 8.6, 2H, Ar-H), 7.39 (t, *J* = 8.8 Hz, 1H, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 161.36, 154.07, 131.73, 128.19, 126.99, 121.88, 116.27, 116.10, 98.87, 40.34, 40.17, 40.00, 39.84, 39.67, 39.50, 39.33; HRMS ESI-MS (*m/z*): 328.9923 $[M+Na]^+$ (positive-ion mode), 304.9960 $[M-H]^-$ (negative-ion mode).

4-Fluoro-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)benzamide (VH03): Yield: 75%; white coloured powder; m.p.: 208-212



Scheme-I: Synthesis scheme of benzothiazole-amides (**VH01-VH06**)

ºC; m.f.: $C_{15}H_{11}N_2O_2SF$; Relative molecular mass: 302; FT-IR (ATR, ν_{max} cm^{-1}): 3385.07 (2º amide N-H *str.*), 1676.14 (C=O *str.*), 1641.42 (aromatic C=C *str.*), 1544.98 (N-H bend.); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.18 (dd, *J* = 8.8, 5.4 Hz, 1H, Ar-H), 7.66 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.58 (s, 1H, Ar-H), 7.37 (t, *J* = 8.8 Hz, 1H, Ar-H), 7.04 (d, *J* = 8.8 Hz, 2H, Ar-H), 3.80 (s, 3H, Ar-OCH_3); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 167.02, 166.86, 166.19, 164.19, 156.69, 154.82, 154.66, 131.59, 118.51, 116.22, 116.13, 115.96, 115.53, 113.32, 105.93, 105.08, 56.01, 40.33, 40.16, 39.99, 39.82, 39.66, 39.49, 39.32; HRMS ESI-MS (*m/z*): 325.0419 $[M+Na]^+$ (positive-ion mode), 301.0454 $[M-H]^-$ (negative-ion mode).

4-Fluoro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)benzamide (VH04): Yield: 53%; yellowish orange coloured powder; m.p.: 180-182 ºC; m.f.: $C_{14}H_8N_2OSF_2$; Relative molecular mass: 290; FT-IR (ATR, ν_{max} cm^{-1}): 3385.07 (2º amide N-H *str.*), 1670.35 (C=O *str.*), 1635.64 (aromatic C=C *str.*), 1535.34 (N-H bend.); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.19 (dd, *J* = 8.8, 5.5 Hz, 1H, Ar-H), 7.55 (dd, *J* = 8.8, 2.7 Hz, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 7.28 (dd, *J* = 8.8, 4.9 Hz, 3H, Ar-H), 7.02 (td, *J* = 9.1, 2.7 Hz, 1H, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 166.81, 158.51, 156.63, 149.81, 132.27, 118.55, 113.27, 113.08, 108.32, 108.11, 40.33, 40.16, 40.10, 39.92, 39.92, 39.81, 39.81, 39.59, 39.50, 39.33, 7.56; HRMS ESI-MS (*m/z*): 313.0221 $[M+Na]^+$ (positive-ion mode), 289.0256 $[M-H]^-$ (negative-ion mode).

N-(6-Bromobenzo[*d*]thiazol-2-yl)-4-fluorobenzamide (VH05): Yield: 46%; light brown coloured powder; m.p.: 194-196 ºC; m.f.: $C_{14}H_8N_2OSBrF$; Relative molecular mass: 351; FT-IR (ATR, ν_{max} cm^{-1}): 3448.72 (2º amide N-H str.), 16780.14 (C=O *str.*), 1629.85 (aromatic C=C *str.*), 1525.69 (N-H bend.); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.19 (dd, *J* = 8.7, 5.5 Hz, 1H, Ar-H), 7.86 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.59 (s, 1H, Ar-H), 7.31 (d, *J* = 8.5, 2H, Ar-H), 7.23 (d, *J* = 8.5 Hz, 2H, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 167.61, 152.50, 152.35, 133.55, 131.65, 128.7, 124.62, 123.70, 119.62, 119.46, 116.18, 116.01, 112.54, 40.34, 40.17, 40.00, 39.90, 39.78, 39.67, 39.50, 39.33; HRMS ESI-MS (*m/z*): 372.9420 $[M+Na]^+$ (positive-ion mode), 350.9431 $[M-H]^-$ (negative-ion mode).

N-(Benzo[*d*]thiazol-2-yl)-4-fluorobenzamide (VH06): Yield: 55%; white coloured powder; m.p.: 212-214 ºC; m.f.: $C_{14}H_9N_2OSF$; Relative molecular mass: 272; FT-IR (ATR, ν_{max} cm^{-1}): 3226.91 (2º amide N-H str.), 1674.21 (C=O *str.*), 1600.92 (aromatic C=C *str.*), 1543.05 (N-H bend.); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.18 (dd, *J* = 8.8, 5.5 Hz, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.65 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.38 (t, *J* = 8.8 Hz, 1H, Ar-H), 7.27 (d, *J* = 8.3 Hz, 2H, Ar-H), 2.41 (s, 3H, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 164.28, 150.42, 133.66, 132.59, 131.63, 128.02, 121.79, 116.42, 116.24, 116.06, 40.33, 40.16, 39.99, 39.82, 39.66, 39.49, 39.32, 21.44; HRMS ESI-MS (*m/z*): 295.0313 $[M+Na]^+$ (positive-ion mode), 271. 0349 $[M-H]^-$ (negative-ion mode).

General procedure of *in vitro* antibacterial screening: The synthesized compounds (**VH01-06**) were investigated for antibacterial properties by determining MICs using broth micro dilution assay. The test samples were experimented in a twofold serial dilution A 96-well microtiter plate was prepared with the compounds at concentrations ranging from 0.39-200 µM. Ampicillin (Sigma-Aldrich) was used as a positive control and tested at concentrations ranging from 100-0.39 µM. Media used in the assay was Muller Hinton broth (Hi-Media). The bacterial strains for the assay were prepared by colony suspension method and dilution corresponds to the final inoculums of 5×10^5 CFU/mL upon inoculation into each well containing twofold serial dilutions of test compounds. The plates were incubated for 16 h at 37 ºC. After that, 20 µL of 0.15 mg/mL resazurin dye (Sigma-Aldrich) was added to each well and the plates were further incubated for 4 h at 37 ºC. The lowest concentration at which colour of the broth persisted as purple colour was recorded as the end point *i.e.* MIC. In presence of viable cells, the purple colour of resazurin will change into pink or colourless, this chemical reaction is due to reduction of resorufin by oxidoreductases within viable cells. General guidelines for this experiment were obtained from Clinical and Laboratory Standards Institute (CLSI 2011) with recommendations adapted from Cos *et al.* [59].

RESULTS AND DISCUSSION

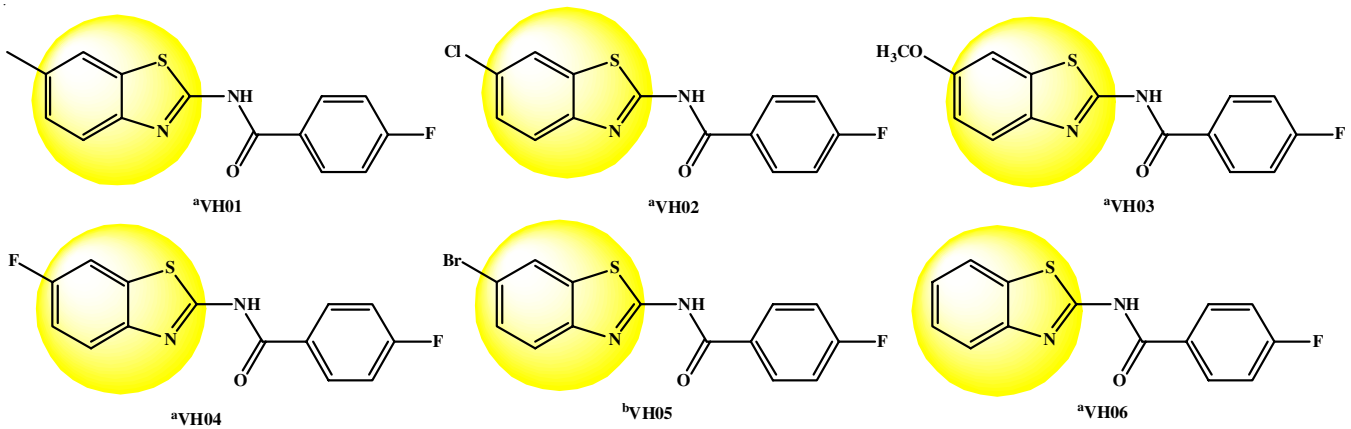
The basis for the molecular structure elucidation of the compounds **VH01-VH06** has been revealed through careful understanding of the scheme of conventional organic synthesis and theoretical chemistry associated with the reaction conditions adopted to chemically synthesise the final compounds by conducting a reaction between substituted 2-aminobenzothiazoles and an 4-fluorobenzoyl chloride which yields amide derivatives of the substituted benzothiazoles (**VH01-VH06**) as the significant final products of the reaction. In this study, a series of six amide derivatives was synthesized, the probable theoretical molecular structures of **VH01-06** were used as supporting information to interpret FT-IR, ^{1}H & ^{13}C NMR and HR-MS Mass spectra. The HRMS electrospray ionization mass spectrometry (ESI-MS) spectra was recorded in positive and negative ion modes was used to elucidate the exact mass of the samples of **VH01-06** using LC-MS grade methanol as solvent. The HRMS ESI positive ion mass spectra of **VH01-06** showed a pseudo-molecular ion signal at *m/z* 287.0650 $[M+H]^+$ for **VH01**, 328.9923 $[M+Na]^+$ for **VH02**, 325.0419 $[M+Na]^+$ for **VH03**, 313.0221 $[M+Na]^+$ for **VH04**, 372.9420 $[M+Na]^+$ for **VH05**, 295.0313 $[M+Na]^+$ for **VH06** revealing an exact molecular mass of the compounds **VH01-06**, respectively. Likewise, the HRMS-ESI negative ion mass spectra showed pseudo-molecular ion signals at *m/z* values 285.0507 for **VH01**, 304.9960 for **VH02**, 301.0454 for **VH03**, 289.0256 for **VH04**, 350.9431 for **VH05**, 271. 0349 for **VH06**, revealing $[M–H]^-$ molecular ions of the compounds **VH01-06**, respectively. The HRMS ESI-positive and HRMS ESI-negative pseudo-molecular ions were matched correspondingly to their exact masses of compounds **VH01-06**.

The FT-IR vibrational spectroscopy of compounds **VH01-06** showed characteristic bands at various vibrational frequencies that indicate characteristic bands related to the functional groups present in the series of molecules **VH01-06** such as a weak stretching frequency was recorded in all FT-IR spectra between 3340 cm^{-1} and 3300 cm^{-1} confirming the presence of 2º amido group (-CONH-). The bands accountable to the pres-

ence of carbonyl (C=O) stretching were observed at the frequencies in the proximity between 1710 cm^{-1} and 1670 cm^{-1}. Correspondingly, a strong, sharp band was identified at the same frequency in all the compounds **VH01-06** at 1650 cm^{-1} and 1600 cm^{-1} indicating the presence of aromatic (C=C stretching). In the same order, there is a strong band accountable for N-H bending frequency observed within the range of 1550 cm^{-1} and 1500 cm^{-1}, respectively. The ^{1}H NMR spectra of compounds **VH01-06** revealed the types of protons present in the compounds **VH01-06** with characteristic peaks of chemical shifts with relatively integrated protons on the ppm scale have been correlated with the molecular structures of **VH01-06**. The presence of characteristic aromatic protons (Ar-H) respectively resonated on the baseline scale between 8.20 δ ppm and 7.00 δ ppm, a set of eight protons (Ar-H, aromatic) were integrated as observed in case of compound **VH06** and seven protons (Ar-H, aromatic) were integrated as observed in case of compounds **VH01-05**. Concerning the compound **VH01**, a singlet peak was observed at 2.41 δ ppm with the integration of three protons showed that the three protons are equivalent and shielded, resonated frequency is corresponding to Ar-CH_3. In the same order **VH03**, a singlet peak was observed at 3.80 δ ppm with the integration of three protons showed that the three protons are equivalent and shielded, resonated frequency is corresponding to Ar-OCH_3. The proton integrations associated with **VH01-06**, which appeared on the chemical shift baseline, were consistent with the theoretical calculation of protons exclusive of carboxamido proton (-CON**H**-), since it is a readily exchangeable proton and does not contribute to the proton resonance due to hydrogen-deuterium exchange. Since the experimental mode present condition, the carboxamide proton of compounds **VH01-06** were disappeared on the chemical shift scale. The ^{13}C NMR spectra of compounds **VH01-06** revealed the nature and type of carbons present in the compounds **VH01-06** with characteristic peaks of chemical shifts with relatively integrated carbons on the ppm scale have been correlated with the molecular structures of compounds **VH01-06**. The presence of characteristic aromatic carbons respectively resonated on the baseline scale between 120 δ ppm and 170 δ ppm, a set of 13 aromatic carbons and one characteristic carbonyl carbon has appeared on the baseline within the range of 160-170 δ ppm in case of compounds **VH01-06**. Concerning the compound **VH01**, a characteristic peak was observed at 21.44 δ ppm showed that the carbon is shielded, the resonated frequency is corresponding to aromatic methyl carbon (Ar-**C**H_3). In same order, compound **VH03**, a singlet peak was observed at 56.01 δ ppm showed that the carbon is shielded, the resonated frequency is corresponding to methoxy carbon (Ar-O**C**H_3). The carbon chemical shifts associated with compounds **VH01-06** which appeared on the baseline, were consistent with the theoretical range of carbons. However, since the magnetic moment of ^{13}C is weaker than ^{1}H; thus, the signals that appear in ^{13}C spectra were not strong as ^{1}H spectra. The peak integration is not a reliable parameter in ^{13}C NMR spectroscopy because the natural abundance of resonating carbon signals (signal-to-noise ratio) on the chemical shift scale is very low. However, the applied magnetic field is the same as ^{1}H NMR (500 MHz).

***In vitro* antibacterial activity:** The MIC (µg/mL) values against the tested bacterial (MRSA ATCC 33591, MSSA ATCC 2592, *E. coli* J53 R1, *E. coli* ATCC 25922, *K. pneumonia* ATCC 10031 and *K. pneumoniae BAA-1075* was determined *via* broth microdilution assay. Compound **VH05** exhibited inhibition against *E. coli* ATCC 25922 at 200 µg/mL, which was the highest tested concentration. In contrast, other compounds did not exhibit inhibition on any of the tested organisms even at the highest concentration (200 µL/mL) (Fig. 1).

This study aimed to determine antibacterial activities of six compounds against a set of six bacterial strains. MRSA exhibited resistance to all the six compounds. However, the colour intensity of the formazan product for MRSA treated with 200 µg/mL of compounds **VH01**, **VH02**, **VH03** and **VH04** was lesser compared to the control untreated MRSA. This indicates the compounds have mild inhibitory activity against MRSA. The MIC for antibiotic ampicillin against MRSA was 200 µg/mL, demonstrating a significant antibiotic resistance in this strain. Testing of MSSA also showed similar results in which some inhibition may be present at 200 µg/mL based on comparison of the formazan product colour intensity between the control



a = MIC: >200 µg/mL against MSSA ATCC 25923, MRSA ATCC 33591, *E. coli* J53 R1, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 10031, *K. pneumoniae* BAA-1705'
b = MIC: 200 µg/mL against *E. coli* ATCC 25922, MIC: >200 µg/mL against MSSA ATCC 25923, MRSA ATCC 33591, *E. coli* J53 R1, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 10031, *K. pneumoniae* BAA-1705

Fig. 1. Chemical structures of compounds (**VH01-VH06**) with their *in vitro* antibacterial activity against bacterial strains

bacteria and bacteria treated with all the six compounds, whereas MIC ampicillin for MSSA was 0.78 µg/mL. Gram-negative *E. coli* ATTC 25923 showed susceptibility to compound **VH05**, in which the MIC recorded was 200 µg/mL. In contrast, all six compounds did not exhibit any activity against *E. coli* J53 R1 and ampicillin was inactive against this bacteria strain. All the synthesized compounds did not show any inhibition against *K. pneumonia* strains *(K. pneumonia* ATCC 10031 and *K. pneumonia* BAA-1075). The findings correspond to the antibacterial properties of benzothiazole derivatives studied by Talib *et al*. [6]. They have synthesized benzothiazole analogues screened for *in vitro* antibacterial activities against *S. aureus* and *E. coli*. The results showed that the compounds were active against tested bacteria although the concentration escalated to 500 µg/mL [6]. In same study, few bromo benzothiazole derivatives have also exhibited some activity against *E. coli*. In addition, another study has reported good activity of bromo group on benzene ring in comparison with choro and fluoro substituents against *E. coli* (MTCC1231) [10]. These findings agreed with the result in this study compound **VH05** showed activity against *E. coli* ATCC 25922. In another investigation, certain benzothiazole derivatives showed antibacterial action against *E. coli* and *S. aureus* [61]. However, two compounds containing either chlorine or fluorine atom in the basic nucleus as seen in compound *N*-(6-fluorobenzo[*d*]thiazol-2-yl)-4-nitrobenzene sulfonamide and *N*-(6-chlorobenzo[*d*]thiazol-2-yl)-4-nitrobenzene sulphonamide did not produce antibacterial activity against tested bacteria have similarity with two compounds in this study compounds **VH02** and **VH04** are consistent with the similar observation. Moreover, a significant number of benzothiazole derivatives were reported in the literature, with activity ranging from moderate to excellent against different strains of *E. coli* [58].

Conclusion

Present study explored the antibacterial properties of a series of benzothiazole derivatives against selected bacterial strains; based on the limited chemical diversity of benzothiazole derivatives, one could understand the modification of 2-aminobenzothiazole ring system with carboxamido linkage might not be a good contributing feature towards the observed biological activity. On the other hand, the observed antibacterial activity also suggests that the substitution of different types of chemical features such as electron-withdrawing or electron-donating could not influence the improvement of the activity as revealed based on the screening results. Based on the screening results, further modification of compound **VH05** with other substituted benzoyl chlorides may enhance the antibacterial activity. Although the compounds screened were not very potent, they exhibited some degree of activity at higher test concentrations (> 200 µg/mL), indicating that further exploration of the chemical diversity of this type of compounds is needed to fully understand its complete nature structure-activity relationship.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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