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# SYNTHESIS AND BIOLOGICAL ASSESSMENT OF NEW BENZOTHIAZOLOPYRIDINE AND BENZOTHIAZOLYL-TRIAZOLE DERIVATIVES AS ANTIOXIDANT AND ANTIBACTERIAL AGENTS

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**ABSTRACT**. A novel series of benzothiazolopyridine derivatives was synthesized *via* interaction of -2-(benzothiazol-2-yl)-3-(4-chlorophenyl)acrylonitrile (**2**) with a diverse of commercially available reagents (indandione, thiobarbituric acid, and malononitrile). Moreover, a novel group of benzothiazole linked substituted 1,2,3-triazole derivatives were synthesized by exploring the chemical behavior of 5-benzothiazolyl-2-(4-chlorophenyl)-triazol-4-amine through refluxing in glacial acetic acid, condensation with phthalic anhydride, and cyanoacetylation reactions. All newly synthetized compounds have been tested for their antioxidant and antibacterial activities compared with ascorbic acid and Ampicillin as reference drugs, respectively. The benzothiazolo-pyridopyrimidine compound **6** was found the most potent antioxidant agent with $IC_{50}$ = 0.015 mg/mL compared to the results of ascorbic acid ($IC_{50}$ = 0.022 mg/mL). The investigated compounds showed no antibacterial properties against Gram-negative bacterial species, *Pseudomonas aeruginosa* and *Escherichia coli*. Benzothiazolopyridine derivative **5** displayed the best growth inhibition against Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus* with inhibition zones 24 and 20 mm, respectively.

**KEY WORDS**: Benzothiazole, Pyridobenzothiazole, 1,2,3-Triazole, Naphtharidine, Antioxidant

## INTRODUCTION

Benzothiazoles are heteroaromatic scaffolds that employed in pharmaceutical manufacturing and other applications including dying and pesticides industries. Benzothiazole derivatives play a key role in a variety of medications and bio-active components [1-4]. Over span of two decades, the synthesis and functionalization of benzothiazole derivatives become hot point for synthetic organic chemists due to their numerous pharmacological functions including antitumor activity [5-8], calmodulin antagonists [9], neurotransmission blockage [10-12], antimicrobial and antifungal [13,14], anticonvulsant [15], antidiabetic [16-18], antimalarial [19], analgesic [20], antioxidant activity [21], and DNA minor groove binder [22]. In view of previously mentioned biomedical potentialities of benzothiazole, the authors have synthesized and screened novel series of benzothiazolopyridine and benzothiazolyl-triazole derivatives for their antioxidant and antibacterial activities.

## RESULTS AND DISCUSSION

The synthesis of -2-(benzo[*d*]thiazol-2-yl)-3-(4-chlorophenyl)acrylonitrile (**2**) was carried out according to reported literature [23] by treatment of benzothiazolyl-acetonitrile (**1**) with 4–chlorobenzaldehyde. Thus, the benzothiazole derivative **2** act as a precursor for the synthesis of novel benzothiazolopyridine derivatives. Synthetic route of benzothiazolopyridine derivatives is

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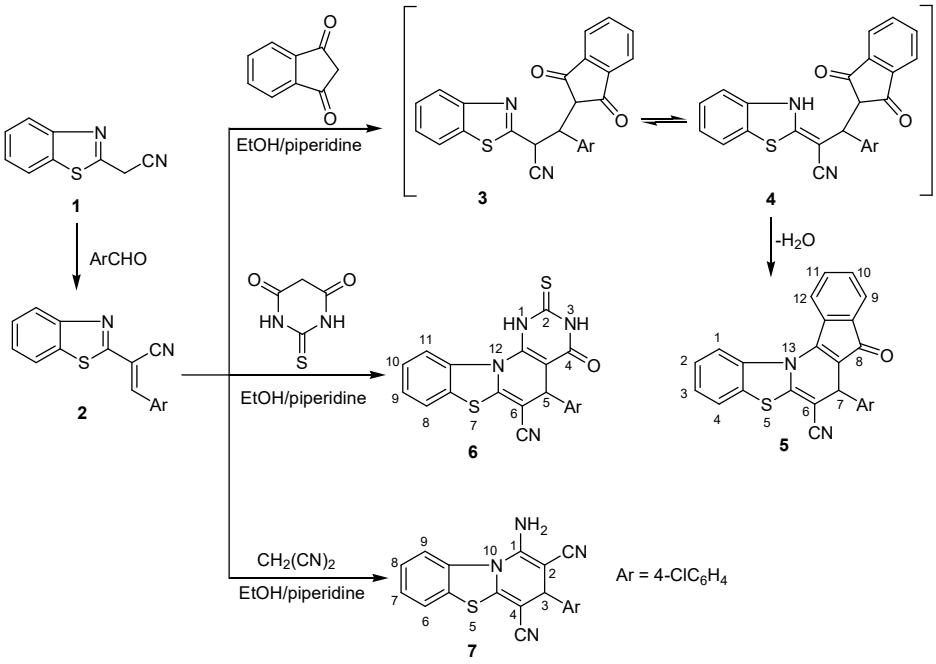
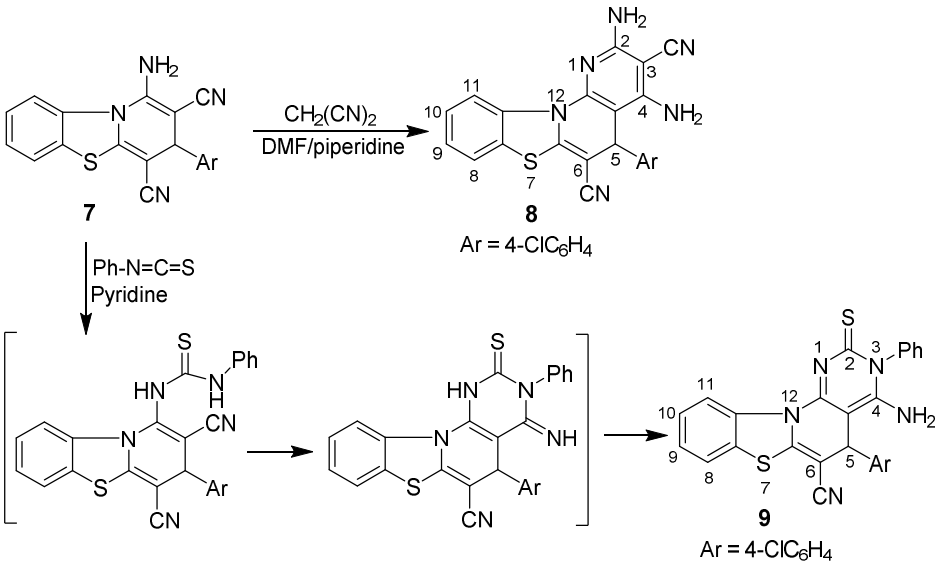
shown in Scheme 1. Accordingly, the reaction of **2** with indandione under refluxing in EtOH including catalytic drops of piperidine afforded benzothiazolopyridine derivative **5**. The formation of **5** was occurred firstly by Michael addition of the activated methylene of indanedione to the activated double bond in compound **2** to form imine Michael adduct intermediate **3**, which converted to enamine intermediate **4** that underwent an intramolecular cyclocondensation to give finally isolable product **5**. The structure of **5** was elucidated based on its analytical and spectral data. Indeed, its IR spectrum revealed two stretching frequencies at 1643 and 2260 $cm^{-1}$ owing to C=O and C≡N groups, respectively. Its $^1$H NMR spectrum displayed singlet signal at δ 4.78 ppm attributable to 4H-dihydropyridine. In a similar manner and methodology, treatment of **2** with thiobarbituric acid furnished 5-(4-chlorophenyl)-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2*H*-benzo[4',5']thiazolo[3',2':1,6]pyrido[2,3-*d*]pyrimidine-6-carbonitrile (**6**) (Scheme 1). The analytical and spectral data were in accordance with its proposed structure. The benzothiazolopyridine compound **7** (Scheme 1) was prepared as reported in literature [24].



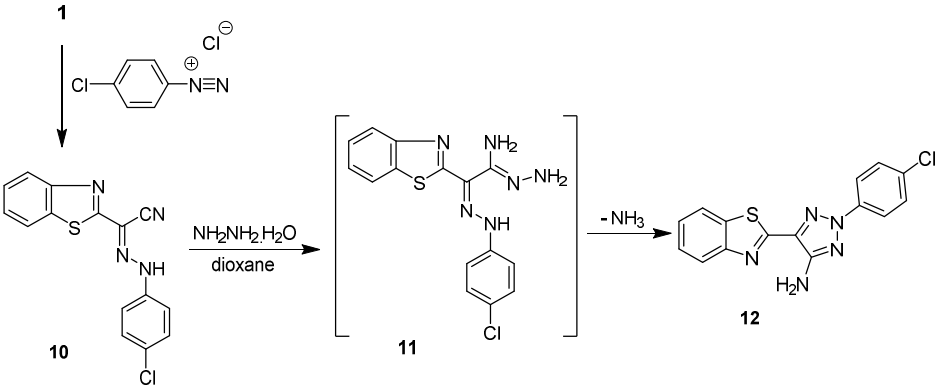

Scheme 1. Synthetic route of benzothiazolopyridine derivatives **5**, **6**, and **7**.

Benzothiazolo[3,2-*a*][1,8]naphthyridine derivatve **8** was achieved by treatment of derivative **7** with malononitrile under refluxing in DMF catalyzed by piperidine [25] (Scheme 2). The structure of **8** was clearly identified by its $^1$H NMR spectrum that exhibited singlet signal at δ 4.22 ppm attributable to $C_5$-H and two singlet signals ($D_2O$ exchangeable) at 6.45 and 6.88 ppm corresponding to two $NH_2$ protons. Interaction of **7** with phenyl isothiocyanate under refluxing in pyridine presented 4-amino-5-(4-chlorophenyl)-3-phenyl-2-thioxo-3,5-dihydro-2*H*-benzo-[4',5']thiazolo[3',2':1,6]pyrido[2,3-*d*]pyrimidine-6-carbonitrile (**9**) (Scheme 2). The structure of **9** was assigned obviously from its spectral data. Its mass spectrum offered molecular ion peak at m/z 498 that coincide with its proposed structure. Its $^1$H NMR spectrum exhibited two singlet signals at δ 4.19 and 6.68 due to the protons of C-5 and amino group ($NH_2$), respectively.




Scheme 2. Synthetic route of benzothiazolopyridine derivatives **8** and **9**.

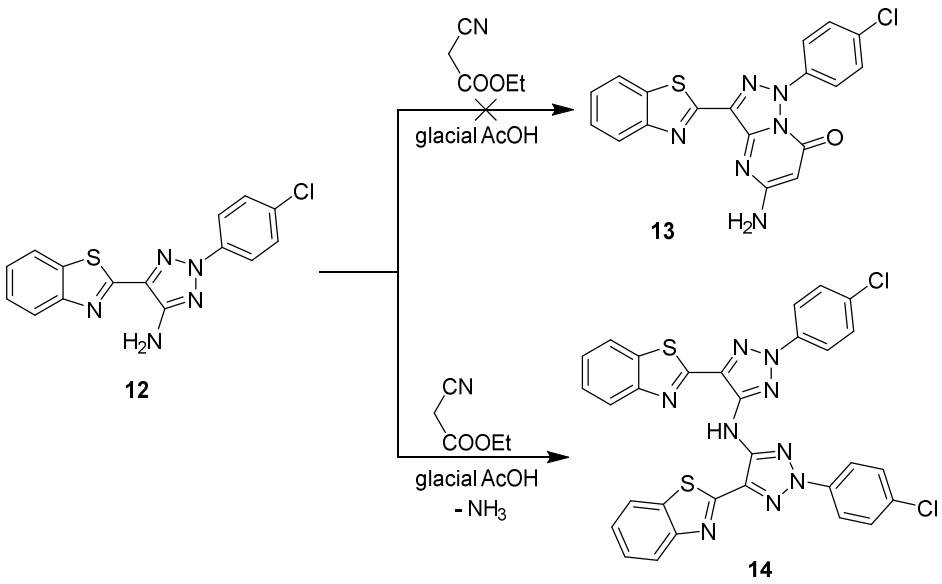
Diazo-coupling of benzothiazolyl-acetonitrile (**1**) with 4-chlorophenyl diazonium chloride in ethanolic sodium acetate afforded the corresponding hydrazone derivative **10** [26, 27]. Treatment of **10** with hydrazine hydrate under refluxing in dioxane provided the benzothiazolyl-triazole hybrid of the type **12** (Scheme 3). The chemical structure of **12** was established based on its $^1$H NMR which revealed singlet signal at δ 6.96 ppm assigned for $NH_2$ group in addition to multiplet signal at δ 7.41-8.33 ppm due to aromatic protons.




Scheme 3. Synthetic route of benzothiazolyl-triazole derivative **12**.

Attempt to prepare [1,2,3]triazolo[1,5-*a*]pyrimidine derivative **13** by reaction of benzothiazolyl-triazole hybrid **12** with ethyl cyanoacetate under refluxing in AcOH was failed and instead obtained bis(5-(benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-2*H*-1,2,3-triazol-4-

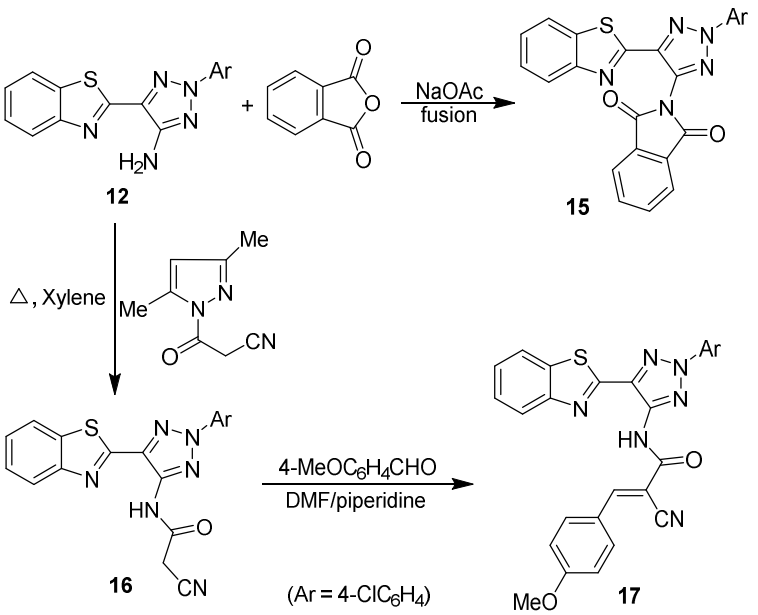
yl)amine (**14**) (Scheme 4). The identity of the reaction product was clearly demonstrated from its mass and $^1$H NMR spectral data. Its mass spectrum offered molecular ion peak at m/z 638. $^1$H NMR spectrum displayed a singlet signal for one proton at δ 13.11 ppm corresponding to NH group in addition to a massive signal assigned at δ 7.42-8.33 ppm, integrated for sixteen aromatic protons. The structure **14** was confirmed chemically through its formation by refluxing of **12** in glacial AcOH without addition ethyl cyanoacetate (Scheme 4).




Scheme 4. Synthesis of bis-(benzothiazolyl-triazolyl)amine derivative **14**.

Heating an equimolar ratio of **12** and phthalic anhydride in the presence of freshly fused sodium acetate offered isoindoline derivative **15** (Scheme 5). The possibility of formation of **15** was demonstrated from its IR and $^1$H NMR spectra. However, its IR spectrum displayed two absorption peaks at 1723 and 1752 cm$^{-1}$ attributable to two C=O groups. Moreover, absence of any singlet signal due to $NH_2$ group in its $^1$H NMR spectrum confirm that amino group was involved in the reaction. Cyanoacetylation of **12** was accomplished by its treatment with 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile in refluxing dry xylene to give cyanoacetamide derivative **16** (Scheme 5). The activity of methylene group in compound **16** was examined by its treatment with 4-anisaldehyde and phenyl isothiocyanate to offer acrylamide derivatives **17** and **18**, respectively (Scheme 5). The chemical structures of compounds **16**, **17** and **18** were assigned according to their chemical and spectral data.

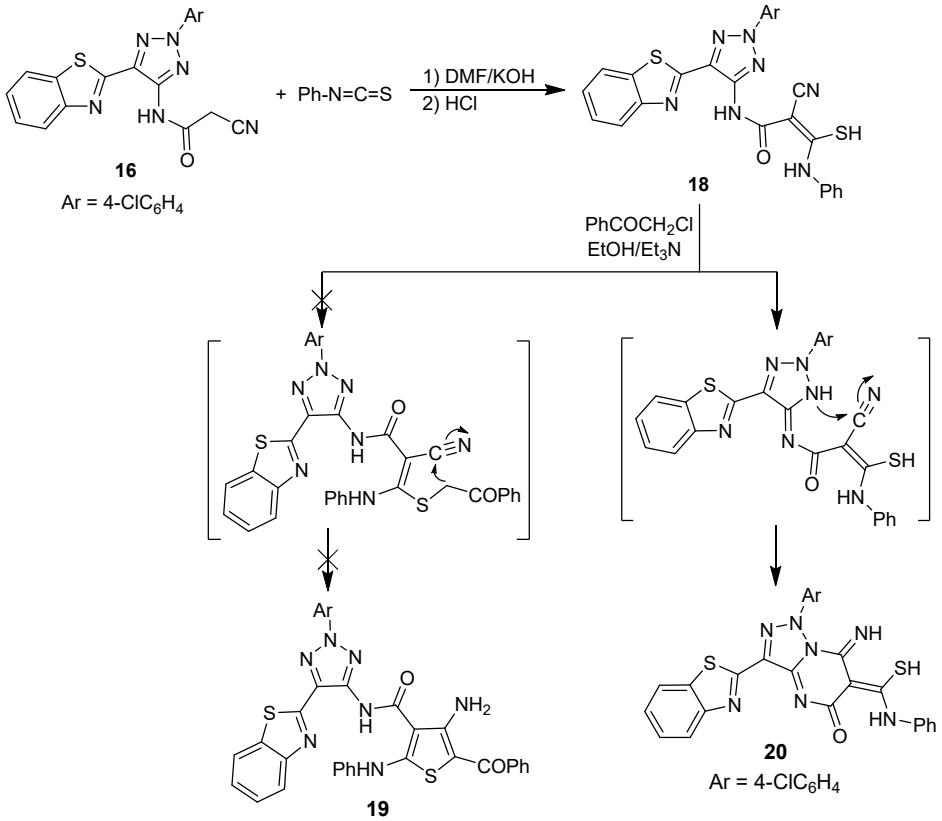
Contradictory to our exception by formation of thiophene derivative **19** *via* treatment of **18** with phenacyl bromide in refluxing EtOH enclosing drops of triethylamine, mass spectrum of reaction product provided molecular ion peak at m/z 530 identical to molecular weight of **18**, which confirms that phenacyl bromide was not involved in the reaction. The IR spectrum of reaction product showed absence of any absorption peaks in the region of 2000-2250 cm$^{-1}$ which confirms that the nitrile (-C≡N) group was involved in the cyclization reaction to give [1,2,3]triazolo[1,5-*a*]pyrimidine derivative **20** (Scheme 6). $^1$H NMR spectrum of **20** displayed three singlet signals at δ 5.43, 9.08 and 10.77 ppm corresponding to SH and two NH groups, respectively.




Scheme 5. Synthetic route of benzothiazolyl-triazole derivatives **15** and **17**.

*Antioxidant activity (DPPH assay)*

The synthesized compounds were assessed as antioxidant agents using DPPH colorimetric assay. The samples capacity as potent antioxidant agent is depending on, in the first rank, the capability of the sample to trap the free radicals of DPPH solution, and hence stabilization of the free radical. The % inhibition and $IC_{50}$ values in mg/mL "half maximal inhibitory concentration, which is the sample concentration responsible for 50% scavenge of DPPH free radical in the solution" were calculated for all the tested samples as well as for ascorbic acid, which was used as a standard compound. In order to compare the results obtained in the optimal way, we note that the calculations of the $IC_{50}$ values are more closely related to the concentration, so this method is more accurate than comparing the results with the % inhibitions, which differ automatically according to the concentration of the sample, but at the same time it is difficult to apply it to all samples due to the different concentration of each sample. Generally, the results in Table 1 demonstrated that compound **6** is the most potent antioxidant agent ($IC_{50}$ = 0.015 mg/mL) in comparison with the results of ascorbic acid ($IC_{50}$ = 0.0222 mg/mL). In addition, compounds **5**, and **17** revealed potent antioxidant capacities ($IC_{50}$ = 0.018, and 0.020 mg/mL) higher than that of ascorbic acid. Compounds **9** ($IC_{50}$ = 0.099 mg/mL), **15** ($IC_{50}$ = 0.094 mg/mL), **16** ($IC_{50}$ = 0.035 mg/mL), and **20** ($IC_{50}$ = 0.046 mg/mL) displayed good antioxidant behavior relative to ascorbic acid. The lowest antioxidant aptitude was recorded for compounds **8** ($IC_{50}$ = 0.191 mg/mL), **12** ($IC_{50}$ = 0.163 mg/mL), **14** ($IC_{50}$ = 0.138 mg/mL) although these compounds have good antioxidant activity in the same time in comparison of the $IC_{50}$ values of these compounds with that of ascorbic acid. On the other side, the % inhibition of the investigated samples were calculated at four different concentrations for each sample in serial dilutions, 0.01-0.001 or 0.039-0.005 mg/mL. Subsequently, the % inhibitions are indication for the strength of the antioxidant capacity of the tested sample, in which the % inhibition decreased by decreasing the sample concentration. The results referred to the following order of antioxidant potency: compound **6** > **5** > **17** > **Vit. C** > **16**.




Scheme 6. Synthesis of benzothiazolyl-triazolo[1,5-*a*]pyrimidine derivative **20**.

The antioxidant results could be interpreted based on the structure activity relationship (SAR's) of the tested compounds. Thus, compound **6** exhibited the most potent antioxidant activity that may be attributed to NH group which is located between carbonyl and thiocarbonyl groups which can act as hydrogen donor leaving high stable free radical by resonance on both C=O and C=S groups. Compounds **17** and **16** provided high antioxidant activity due to presence amidic NH group [28]. Compound **17** afforded antioxidant activity greater than **16** which confirm that introduce 4-methoxybenzylidene moiety on **16** gave more extra stability of resulted free radical. The potent antioxidant activity of compound **5** may be attributed to the keto-enol tautomerism of the carbonyl group in compound **5** (Figure 1). Enolic OH act as hydrogen donor forming very stable radical.

Although the tricyclic compound **8** has two free amino groups with free electron pairs, they produce a decrease in the antioxidant potency, which may have affected by the effect of nitrile substituent as good electron withdrawing group.

*Antimicrobial activity*

The antibacterial activity of ten of the newly synthesized benzothiazole analogues against numerous Gram-positive (*B. cereus* and *S. aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacterial species by agar well diffusion assay as shown in Table 2. No anti-

bacterial activity was recorded for the investigated compounds against *P. aeruginosa* or *E. coli* (Gram-negative bacteria) using diameter of discs is 6 mm size. Also, the DMSO was used as a solvent to prepare a solution of each tested sample, in which it was applied as a control sample with no antibacterial effects on all the tested bacterial species. The results demonstrated that compounds **9**, **12**, **14**, **16**, and **20** have no antibacterial properties against all the Gram-positive or

Table 1. The DPPH antioxidant results of the synthesized benzothiazole-based samples.

| Sample | Concentration (mg/mL) | % Remaining DPPH | % Inhibition | $IC_{50}$ (mg/mL) |
|---|---|---|---|---|
| 5 | 0.039 | 24.57 | 75.43 | 0.018±0.004 |
| | 0.02 | 46.43 | 53.57 | |
| | 0.01 | 63.86 | 36.14 | |
| | 0.005 | 77.29 | 22.71 | |
| 6 | 0.01 | 62.31 | 37.69 | 0.015±0.002 |
| | 0.005 | 74.65 | 25.35 | |
| | 0.0025 | 84.79 | 15.21 | |
| | 0.00125 | 92.57 | 7.43 | |
| 8 | 0.039 | 84.71 | 15.29 | 0.191±0.015 |
| | 0.02 | 90.57 | 9.43 | |
| | 0.01 | 93.29 | 6.71 | |
| | 0.005 | 95.71 | 4.29 | |
| 9 | 0.01 | 92.18 | 7.82 | 0.099±0.007 |
| | 0.005 | 95.12 | 4.88 | |
| | 0.0025 | 96.67 | 3.33 | |
| | 0.00125 | 98.14 | 1.86 | |
| 12 | 0.039 | 85.86 | 14.14 | 0.163±0.012 |
| | 0.02 | 97.43 | 2.57 | |
| | 0.01 | 98.86 | 1.14 | |
| | 0.005 | 99.71 | 0.29 | |
| 14 | 0.01 | 95.29 | 4.71 | 0.138±0.008 |
| | 0.005 | 97.29 | 2.71 | |
| | 0.002 | 98.43 | 1.57 | |
| | 0.001 | 99.71 | 0.29 | |
| 15 | 0.01 | 92.86 | 7.14 | 0.094±0.006 |
| | 0.005 | 94.29 | 5.71 | |
| | 0.002 | 96.29 | 3.71 | |
| | 0.001 | 99.71 | 0.29 | |
| 16 | 0.039 | 47.86 | 52.14 | 0.035±0.005 |
| | 0.02 | 61 | 39 | |
| | 0.01 | 70 | 30 | |
| | 0.005 | 74.29 | 25.71 | |
| 17 | 0.039 | 22.31 | 77.69 | 0.020±0.003 |
| | 0.0195 | 55.76 | 44.24 | |
| | 0.00975 | 79.67 | 20.33 | |
| | 0.004875 | 84.27 | 15.73 | |
| 20 | 0.01 | 82.29 | 17.71 | 0.046±0.007 |
| | 0.005 | 85.33 | 14.67 | |
| | 0.0025 | 91.17 | 8.83 | |
| | 0.00125 | 92.51 | 7.49 | |
| Ascorbic acid | 0.062 | 15.27 | 84.73 | 0.022±0.004 |
| | 0.031 | 39.08 | 60.92 | |
| | 0.016 | 61.07 | 38.93 | |
| | 0.008 | 74.81 | 25.19 | |

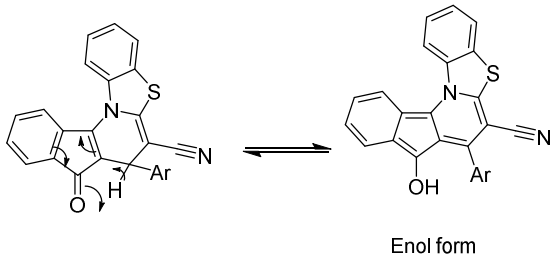



Figure 1. Antioxidant activity of compound **5**.

Table 2. The antimicrobial results of the newly prepared compounds against the diverse bacterial species.

| Compound No. | Inhibition zone (mm) [a] | | | |
|---|---|---|---|---|
| | Gram positive bacteria | | Gram negative bacteria | |
| | *Bacillus cereus* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Escherichia coli* |
| **5** | 20 | 24 | - | - |
| **6** | 8 | 16 | - | - |
| **8** | 10 | - | - | - |
| **9** | - | - | - | - |
| **12** | - | - | - | - |
| **14** | - | - | - | - |
| **15** | 11 | 11 | - | - |
| **16** | - | - | - | - |
| **17** | 19 | 22 | - | - |
| **21** | - | - | - | - |
| **Ampicillin** | 22 | 24 | 23 | 25 |
| Control [b] | - | - | - | - |

[a] The inhibition zone measured in mm, the diameter of discs is 6 mm size. [b] DMSO was used as a control.

Gram-negative bacterial species. It was noted that compounds **5**, **6**, **8**, **15**, and **17** are the most potent antibacterial agents, in which compounds **5** (IZ = 20 mm), and **17** (IZ = 19 mm) are the most potent antimicrobial agents between the other tested compounds against *B. cereus*. In addition, compounds **5**, **6**, **15**, and **17** displayed the most effective impacts for the inhibition of the growth of the bacterial strains against *S. aureus*. Therefore, compounds **5** (IZ = 24 mm), and **17** (IZ = 22 mm) have the most effective impacts for growth inhibitions against *S. aureus*. Conversely, the behavior of compound **8** against both Gram-positive bacterial species (*B. cereus,* and *S. aureus*) is completely different, in which this compound has no antibacterial activity against *S. aureus*, reflecting the factor of the bacterial species type that control the obtained results, along with the structure-activity relationships of the investigated compounds, and the sample concentration that has been prepared. The mechanism of action, in this assessment, is still not illuminated and the antibacterial activity of the investigated samples' solutions is a consequence of these points: (i) The cell membrane has electrostatic interaction with the tested samples. (ii) Cellular internalization of the tested samples. (iii) The role and impact of reactive oxygen or nitrogen sources, for example, the carbonyl groups, imino, amino, bicyclic, or binary heterocyclic systems. Briefly, the interaction could arise between the reactive sites of the investigated samples with the cell membrane resulting in a change in the permeability of the cell membrane. Consequently, induce oxidative stress was accomplished owing to the introduction of the tested samples into the cell, hereafter growth inhibition, and cell death [29]. In addition, the cell wall of the Gram-positive bacteria has a negative charge, which arise from the presence of teichoic acid that contains a negative charge on phosphate groups attached to either the peptidoglycan or the

primary plasma membrane. Teichoic acid has a valuable role hence it provides rigidity to the cell wall through the cations attractions. Thick and multilayered was noticed for the cell wall of Gram-negative bacteria. The effect of the tested samples is to inhibit the cell wall synthesis, inhibition of electron transport chain, protein synthesis, nucleic acid replication, and transcription, and plasma membrane injury.

## EXPERIMENTAL

Melting points were determined on Gallenkamp electric device and were uncorrected. The infrared spectra were recorded with KBr on Thermo Scientific Nicolet iS10 FTIR spectrometer. The $^1$H NMR spectra were recorded in DMSO-$d_6$ on Bruker's spectrometer at 400 MHz. The mass spectra were determined on Quadrupole GC-MS (DSQII) mass spectrometer at 70 eV. Elemental analyses of carbon, hydrogen, and nitrogen were estimated on Perkin Elmer 2400 analyzer. The antioxidant and antibacterial activities were performed at the microbiology unit, Cairo University, Egypt.

*Synthesis of 7-(4-chlorophenyl)-8-oxo-7,8-dihydrobenzo[4,5]thiazolo[3,2-a]indeno[2,1-e]pyridine-6-carbonitrile (**5**).* A mixture of 2-(benzo[*d*]thiazol-2-yl)-3-(4-chlorophenyl)-acrylonitrile (**2**) (0.29 g, 1 mmol) and 1,3-indandione (0.14 g, 1 mmol) in 20 ml EtOH with adding a few drops of piperidine was refluxed for 5 h. The reaction mixture was allowed to cool at room temperature. The precipitate that formed was filtered off, dried and then recrystallized from EtOH to afford the benzothiazolopyridine derivative **5** as orange powder. Yield 55%, mp 194-196 °C. IR ($\nu$, cm$^{-1}$): 1643 (C=O), 2243 (C≡N). $^1$H NMR (δ, ppm): 4.78 (s, 1H, pyridine-CH), 7.17-7.77 (m, 12H, Ar-H). Anal. calcd. for $C_{25}H_{13}ClN_2OS$: C, 70.67; H, 3.08; N, 6.59%. Found: C, 70.79; H, 3.05; N, 6.68%. MS: *m/z* 424 [M$^+$, 46%].

*Synthesis of 5-(4-chlorophenyl)-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2H-benzo[4',5']thiazolo[3',2':1,6]pyrido[2,3-d]pyrimidine-6-carbonitrile (**6**).* A mixture of 2-(benzo[*d*]thiazol-2-yl)-3-(4-chlorophenyl)acrylonitrile (**2**) (0.29 g, 1 mmol) and 2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (0.14 g, 1 mmol) in 20 mL EtOH with adding a few drops of piperidine was refluxed for 8 h. The reaction mixture was allowed to cool at room temperature. The precipitate that formed was filtered off, dried and then recrystallized from EtOH to afford pyrido[2,3-*d*]pyrimidine derivatives **6** as yellow powder. Yield 75%, mp 259–261 °C. IR ($\nu$, cm$^{-1}$): 1638 (C=O), 2220 (C≡N), broad 3390-3436 (2 N-H). $^1$H NMR (δ, ppm): 4.12 (s, 1H, CH), 7.16-7.79 (m, 8H, Ar-H), 9.14 (s, 1H, NH), 11.89 (s, 1H, NH). Anal. calcd. for $C_{20}H_{11}ClN_4OS_2$: C, 56.80; H, 2.62; N, 13.25%. Found: C, 56.65; H, 2.68; N, 13.35%. MS: *m/z* 422 [M$^+$, 28%].

*Synthesis of 2,4-diamino-5-(4-chlorophenyl)-5H-benzo[4,5]thiazolo[3,2-a]-[1,8]naphthyridine-3,6-dicarbonitrile (**8**).* A mixture of **7** (0.36 g, 1 mmol) and malononitrile (0.07 g, 1 mmol) in DMF (25 mL) in the presence of a catalytic amount of piperidine was refluxed for 7 h. Yellow precipitate was formed after cooling. The precipitate was washed with hot ethanol, and then recrystallized from DMF–ethanol. Yield 62%, mp > 300 °C. IR ($\nu$, cm$^{-1}$): 2210 (C≡N), 3319, 3428 and 3450 (2 $NH_2$). $^1$H NMR (δ, ppm): 4.22 (s, 1H, CH), 6.45 (s, 2H, $NH_2$), 6.88 (s, 2H, $NH_2$), 7.24-7.88 (m, 8H, Ar-H). Anal. calcd. for $C_{22}H_{13}ClN_6S$: C, 61.61; H, 3.06; N, 19.59%. Found: C, 61.80; H, 3.15; N, 19.71%. MS: *m/z* 428 [M$^+$, 35%].

*Synthesis of 4-amino-5-(4-chlorophenyl)-3-phenyl-2-thioxo-3,5-dihydro-2H-benzo[4',5']thiazolo[3',2':1,6]pyrido[2,3-d]pyrimidine-6-carbonitrile (**9**).* A mixture of **7** (0.36 g, 1 mmol), phenyl isothiocyanate (0.12 mL, 1 mmol) in pyridine (20 mL) was refluxed for 4 h. The reaction mixture was cooled at room temperature, then poured into ice cold water, and neutralized with hydrochloric acid. The precipitated product was filtered off, washed and recrystallized from DMF–ethanol. Yield 55%, mp 295-297 °C. IR ($\nu$, cm$^{-1}$): 2211 (C≡N), 3426, 3448 ($NH_2$). $^1$H NMR

(δ, ppm): 4.19 (s, 1H, CH), 6.68 (s, 2H, $NH_2$), 7.19-7.75 (m, 13H, Ar-H). Anal. calcd for $C_{26}H_{16}ClN_5S_2$: C, 62.71; H, 3.24; N, 14.06%. Found: C, 62.58; H, 3.29; N, 13.95%. MS: *m/z* 497 [$M^+$, 21%].

*Synthesis of 5-(benzo[d]thiazol-2-yl)-2-(4-chlorophenyl)-2H-1,2,3-triazol-4-amine (**12**).* A mixture of **10** (0.31 g, 1 mmol) and $NH_2NH_2.H_2O$ (0.05 mL, 1 mmol) in 20 mL dioxane was refluxed for 9 h. The reaction mixture was allowed to cool at room temperature. The precipitate that formed was filtered off to give benzo[*d*]thiazolyl-triazole amine derivative **12** as yellow crystals. Yield 83%, mp 179–181 °C. IR (*v*, $cm^{-1}$): 3412, 3448 ($NH_2$). $^1$H NMR (δ, ppm): 6.96 (s, 2H, $NH_2$), 7.41-8.33 (m, 8H, Ar-H). Anal. calcd. for $C_{15}H_{10}ClN_5S$: C, 54.96; H, 3.08; N, 21.37%. Found: C, 54.87; H, 3.10; N, 21.43%. MS: *m/z* 329 [$M^+$ + 2, 18%], 327 [$M^+$, 64%].

*Synthesis of bis(5-(benzo[d]thiazol-2-yl)-2-(4-chlorophenyl)-2H-1,2,3-triazol-4-yl)amine (**14**).* A solution of compound **12** (0.32 g, 1 mmol) in 15 mL glacial acetic acid was heated under reflux for 6 h. The solid that formed upon cooling was collected and dried. Yield 80%; mp 239–241 °C. IR (*v*, $cm^{-1}$): 3252 (N-H). $^1$H NMR (δ, ppm): 7.42-8.33 (m, 16H, Ar-H), 13.11 (s, 1H, NH). Anal. calcd. for $C_{30}H_{17}Cl_2N_9S_2$: C, 56.43; H, 2.68; N, 19.74%. Found: C, 56.64; H, 2.60; N, 19.58%. MS: *m/z* 637 [$M^+$, 15%].

*Synthesis of 2-(5-(benzo[d]thiazol-2-yl)-2-(4-chlorophenyl)-2H-1,2,3-triazol-4-yl) isoindoline-1,3-dione (**15**).* A mixture of compound **12** (0.32 g, 1 mmol), phthalic anhydride (0.15 g, 1 mmol) and 0.5 g fused sodium acetate was heated at 140-150°C for 4 h. The oil was triturated five times with ethanol and then the solid that obtained was collected and recrystallized from acetic acid. Yield 45%; mp 284–286 °C. IR (*v*, $cm^{-1}$): 1723, 1752 (2 C=O). $^1$H NMR (δ, ppm): 7.32-8.08 (m, 12H, Ar-H). Anal. calcd. for $C_{23}H_{12}ClN_5O_2S$: C, 60.33; H, 2.64; N, 15.30%. Found: C, 60.21; H, 2.68; N, 15.37%. MS: *m/z* 457 [$M^+$, 41%].

*Synthesis of N-(5-(benzo[d]thiazol-2-yl)-2-(4-chlorophenyl)-2H-1,2,3-triazol-4-yl)-2-cyanoacetamide (**16**).* Compound **12** (0.32 g, 1 mmol) was heated under reflux with 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (0.16 g, 1 mmol) in dry xylene for 1 h. The precipitate that formed was filtered off to afford the target cyanoacetamide derivative **16**. Yield 85%; mp 279–281 °C. IR (*v*, $cm^{-1}$): 1688 (C=O), 2260 (C≡N), 3375 (N-H). $^1$H NMR (δ, ppm): 4.29 (s, 2H, $CH_2$), 7.36-7.74 (m, 8H, Ar-H), 10.39 (s, 1H, NH). Anal. calcd. for $C_{18}H_{11}ClN_6OS$: C, 54.76; H, 2.81; N, 21.29%. Found: C, 54.76; H, 2.81; N, 21.29%. MS: *m/z* 394 [$M^+$, 33%].

*Synthesis of N-(5-(benzo[d]thiazol-2-yl)-2-(4-chlorophenyl)-2H-1,2,3-triazol-4-yl)-2-cyano-3-(4-methoxyphenyl)acrylamide (**17**).* A mixture of **16** (0.39 g, 1 mmol) and *p*-anisaldehyde (0.14 mL, 1 mmol) in DMF with two drops of piperidine was refluxed for 2 h. The reaction mixture was allowed to cool at room temperature. The precipitate that formed was filtered off, afford cyanoacrylamide derivative **17** as orange crystals. Yield 78%; mp 269–271 °C. IR (*v*, $cm^{-1}$): 1636 (C=O), 2214 (C≡N), 3165 (N-H). $^1$H NMR (δ, ppm): 3.89 (s, 3H, $OCH_3$), 6.72-8.37 (m, 12H, Ar-H), 8.77 (s, 1H, CH=C), 9.01 (s, 1H, NH). Anal. calcd. for $C_{26}H_{17}ClN_6O_2S$: C, 60.88; H, 3.34; N, 16.38%. Found: C, 60.66; H, 3.41; N, 16.50%. MS: *m/z* 512 [$M^+$, 18%].

*Synthesis of N-(5-(benzo[d]thiazol-2-yl)-2-(4-chlorophenyl)-2H-1,2,3-triazol-4-yl)-2-cyano-3-mercapto-3-(phenylamino)acrylamide (**18**).* To a stirred solution of **16** (0.39 g, 1 mmol) in 20 mL DMF, equivalent amount of KOH (0.06 g, 1mmol) and phenyl isothiocyanate (0.12 mL, 1 mmol) were added. The stirring was continued for additional 6 h, then the mixture was poured into ice cold water and neutralized with dilute HCl till acidic solution. The solid that formed was collected and recrystallized from ethanol. Yield 88%; mp 199–201 °C. IR (*v*, $cm^{-1}$): 1688 (C=O), 2260 (C≡N), 3376, 3456 (2 N-H). $^1$H NMR (δ, ppm): 4.89 (s, 1H, SH), 7.12-7.78 (m, 13H, Ar-H), 10.46

(s, 1H, NH), 10.82 (s, 1H, NH). Anal. calcd for $C_{25}H_{16}ClN_7OS_2$: C, 56.65; H, 3.04; N, 18.50%. Found: C, 56.42; H, 3.15; N, 18.64%. MS: *m/z* 529 [$M^+$, 14%].

*Synthesis of 3-(benzo[d]thiazol-2-yl)-1-(4-chlorophenyl)-7-imino-6-(mercapto-(phenylamino)methylene)-6,7-dihydro-[1,2,3]triazolo[1,5-a]pyrimidin-5(1H)-one* (***21***). The compound **18** (0.53 g, 1 mmol) and equivalent amount of phenacyl chloride in EtOH with adding drops of TEA was refluxed for 3-4 h. The reaction mixture was left to cool at room temperature. The precipitate that formed was filtered off and dried. Yield 64%; mp > 300 °C. IR ($\nu$, $cm^{-1}$): 1639 (C=O), 3424, 3450 (2 N-H). $^1$H NMR (δ, ppm): 5.43 (s, 1H, SH), 7.04-8.09 (m, 12|H, Ar-H), 9.08 (s, 1H, NH), 10.77 (s, 1H, NH). Anal. calcd. for $C_{25}H_{16}ClN_7OS_2$: C, 56.65; H, 3.04; N, 18.50%. Found: C, 56.78; H, 3.00; N, 18.44%. MS: *m/z* 531 [$M^+$ + 2, 22%], 529 [$M^+$, 74%].

*Antioxidant activity using DPPH assay*

The DPPH radical scavenging activity of the tested samples was determined according to the reported method [21]. The 1,1-diphenyl-2-picrylhydrazil (DPPH) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and prepared as a solution (0.012%) in methanol and then 1 mL of the solution was mixed with 1 mL of a serial dilution of the investigated samples. After incubation in the dark for 30 minutes at room temperature, the absorbance of the samples was measured against a blank sample at 517 nm. Spectrophotometric apparatus (Spekol 11 spectrophotometer, analytic Jena AG, Jena, Germany) was used to record the color intensity of the tested samples. The radical scavenging activity (RSA)% of the DPPH radicals was calculated from following equation:

$$\text{RSA (\%)} = \frac{\text{Blank Abs - Sample Abs}}{\text{Blank Abs}} \times 100$$

$IC_{50}$ (half maximal inhibitory concentration) value is the concentration of the sample that can scavenge 50% of DPPH free radical in DPPH free radical scavenging method. The $IC_{50}$ value is inversely proportional to the free radical scavenging activity/ antioxidant property of the sample. Thus, the sample will require less amount in scavenging the free radical if the $IC_{50}$ value is less or vice versa. The scavenging activity of free radicals in the sample is due to the presence of molecules which is known as antioxidants. The inhibitive concentration ($IC_{50}$) of the sample required to scavenge DPPH radical by 50% was obtained by a linear regression analysis curve plotting the different concentrations of a sample against the percentage of remaining DPPH applying the exponential curve.

*Potential antibacterial activity*

Microbial susceptibility testing: *Bacillus cereus*, *Staptylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* bacterial species were selected to assess the antibacterial activity of the investigated compounds. The antimicrobial activity of samples was estimated by filter paper disc method [30] using inoculums containing $10^6$ bacterial and fungal cells/mL to spread on Muller Hinton Agar. The discs were placed on the surface of agar plates seeded with the test organisms. The plates were incubated at 37°C. Diameters of inhibition zone (mm) were measured after 18-24 hours for bacteria, 24-48 hours for yeast [31].

## CONCLUSION

In summary, we report the synthesis, characterization, antioxidant and antimicrobial activity of some novel benzothiazole derivatives comprising pyridine and 1,2,3-triazole moieties. Pyridobenzothiazole derivatives **5-9** were synthesized in a simple and efficient methods by

allowing -2-(benzo[d]thiazol-2-yl)-3-(4-chlorophenyl)acrylonitrile (**2**) to react with different available reagents. In addition, coupling reaction of 2cyanomethylbenzothiazole with 4-chlorobenzene diazonium chloride and subsequently, refluxing the product with hydrazine hydrate afforded benzothiazolyltriazole derivative **12** which was used as precursor for the synthesis of novel benzothiazole derivatives having 1,2,3-triazole moiety **14-17** and **20**. The synthesized compounds were assessed as antioxidant agents using DPPH colorimetric. Compounds **5**, **6** and **17** exhibited potent antioxidant capacities greater than that of ascorbic acid. The antibacterial activity of the newly synthesized benzothiazole analogues against numerous Gram-positive (*B. cereus* and *S. aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) were investigated. All investigated compounds have no effect on Gram-negative bacteria, while compounds **5** and **17** were the most potent antibacterial agents versus Gram-positive bacteria.

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