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Original article

# Synthesis and antimicrobial activity of some new thiazole, thiophene and pyrazole derivatives containing benzothiazole moiety

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ABSTRACT

In an attempt to find a new class of antimicrobial agents, a series of thiazole, thiophene, pyrazole and other related products containing benzothiazole moiety were prepared *via* the reaction of *N*-(benzothiazol-2-yl)-2-cyanoacetamide (**1**) with appropriate chemical reagents. These compounds were screened for their antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), Gram-negative bacteria (*Pseudomonas phaseolicola* and *Pseudomonas fluorescens*) and antifungal activity against *Fusarium oxysporum* and *Aspergillus fumigatus*. Among the synthesized compounds, thiophene **13** showed equal activity with chloroamphenicol against *S. aureus* (MIC 3.125 μg/mL), while its activity was 50% lower than of chloroamphenicol against *S. pyogenes*. Thiazole **3** and pyrazolo[1,5-*a*]pyrimidine **21b** were found to exhibit the most potent in *vitro* antifungal activity with MICs (6.25 μg/mL) against *A. fumigatus* and *F. oxysporum*. Structures of the newly synthesized compounds were established by elemental analysis and spectral data.



## 1. Introduction

The benzothiazole ring is present in various marine or terrestrial natural compounds which have useful biological activities [1–5]. Benzothiazole derivatives have attracted a great deal of interest due to their anticancer [6], antitumor [7], anticonvulsant [8], antiviral [9], antibacterial [10], antimicrobial [11] and fungicidal activities [12]. They are also useful as anti-allergic [13], anti-inflammatory [14] and anthelmintic [15] agents and as appetite depressants [16], intermediates for dyes [17], plant protectants [18], histamine $H_2$ antagonists [19] and photographic sensitizers [20]. On the other hand, careful literature survey revealed that thiazole, thiophene and pyrazole ring systems have occupied a unique position in the design and synthesis of novel biological active agents with remarkable analgesic and anti-inflammatory activities [21–24], in addition to their well documented potential antimicrobial activities [25–28]. Morever, thiazoles have found application in drug development for the treatment of hypertension [29], schizophrenia [30], HIV infections [31], and as new inhibitors of bacterial DNA gyrase B [32]. Also, a large number of thiophene derivatives have found to exhibit pharmacological activity [33–35]. Furthermore, diverse chemotherapeutic activities have ascribed to pyrazoles as antimicrobial [36,37], antiparasitic [38], antivirial [39], and antineoplastic agents [40,41].

In view of the above-mentioned facts and in continuation of our interest in the synthesis of heterocycles containing benzothiazole moiety [42–44], to identify new candidates that may be value in designing new, potent, selective and less toxic antimicrobial agents, we report herein the synthesis and antimicrobial evaluation of some novel structure hybrids incorporating both the benzothiazole moiety with either the thiazole, thiophene or pyrazole ring systems through different linkages. This combination was suggested in an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules. The target compounds were rationalized so as to comprise some pharmacophores that are believed to be responsible for the biological activity of some relevant chemotherapeutic agents such as the carboxamido and thiocarbamyl functionalities [45,46]. The substitution pattern of thiazole, thiophene and pyrazole rings was carefully selected so as to confer different electronic environment to the molecules.

## 2. Results and discussion

### 2.1. Chemistry

The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in schemes 1–3. In

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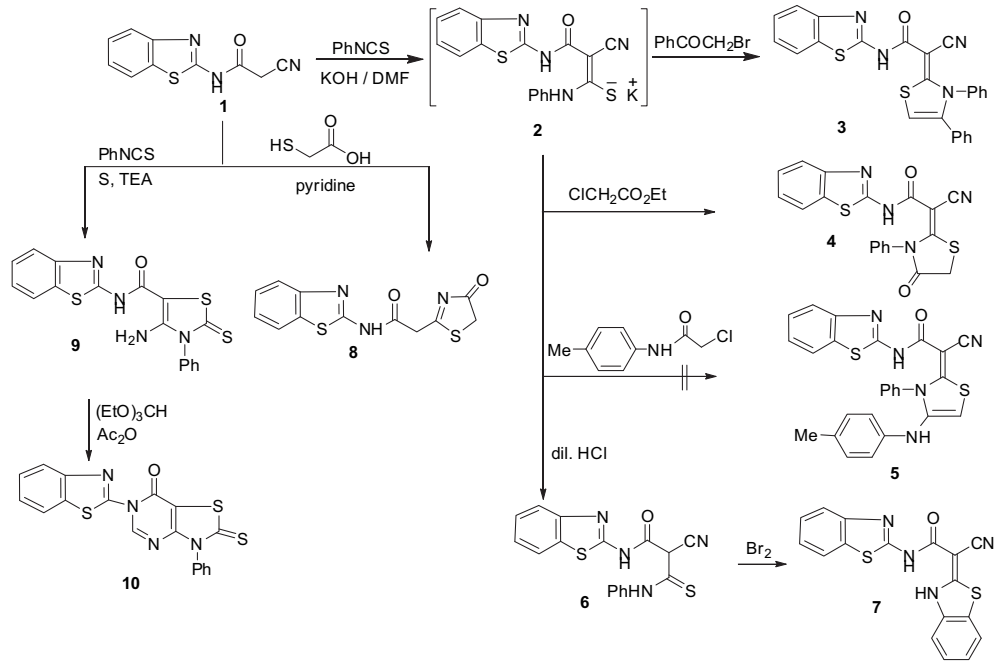
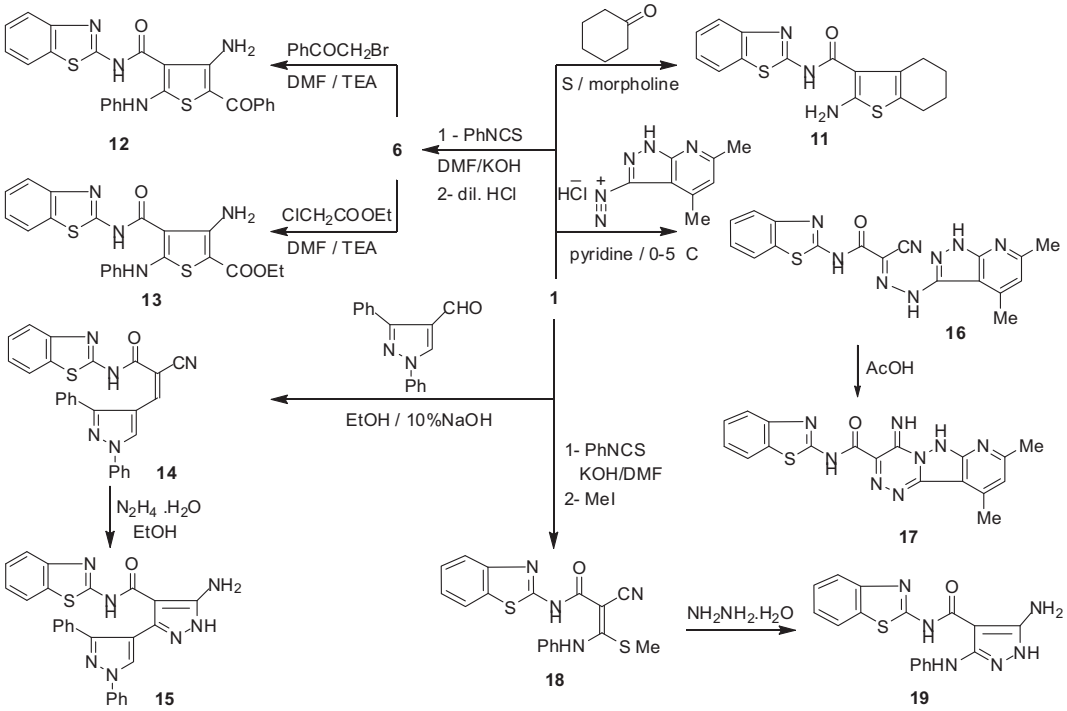
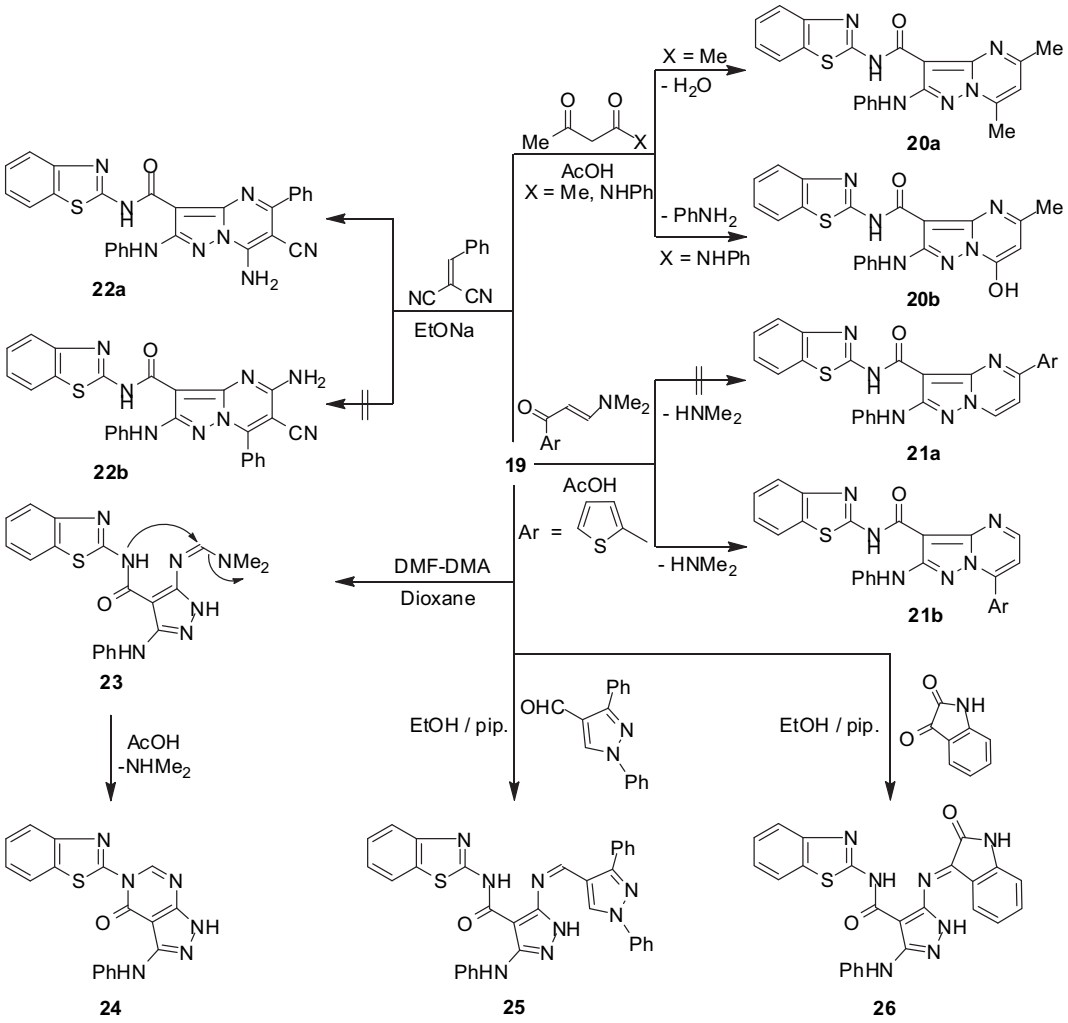



**Scheme 1.** Synthetic route to thiazoles.

Scheme 1, the starting compound *N*-(benzothiazol-2-yl)-2-cyanoacetamide (**1**) was prepared by the cyanoacetylation of 2-aminobenzothiazole with 1-cyanoacetyl-3,5-dimethylpyrazole according to a literature procedure [47]. The active methylene moiety of *N*-(benzothiazol-2-yl)-2-cyanoacetamide (**1**) was allowed to react with phenyl isothiocyanate in dimethylformamide in the presence of an equimolar amount of potassium hydroxide yielded the non-isolable intermediate potassium sulphide salt **2**, which reacted *in situ* with phenacyl bromide to afford *N*-(benzothiazol-2-yl)-2-cyano-2-(3,4-diphenylthiazol-2-ylidene) acetamide (**3**). Elemental analysis and spectral data were in favor of these proposed thiazole structure. The IR spectrum of **3** showed absorption bands at 3384, 2178 and 1625 $cm^{-1}$ due to NH, CN and carboxamide groups, respectively. The $^1H$ NMR spectrum showed a multiplet signals in the region at $\delta$ 7.18–7.86 ppm corresponding to the aromatic protons together with the thiazole-H5 and a singlet signal at $\delta$ 10.59 ppm exchangeable with $D_2O$ for NH proton. The mass spectrum revealed a molecular ion peak at $m/z$ = 452




**Scheme 2.** Synthetic route to thiophenes and pyrazoles.




**Scheme 3.** Reactions of aminopyrazole with some electrophilic reagents.

corresponding to a molecular formula $C_{25}H_{16}N_4OS_2$. In contrast to the behavior of the intermediate **2** towards phenacyl bromide, it has been found that, the *in situ* reaction of **2** with 2-chloro-*N*-*p*-tolylacetamide afforded thiazolidin-4-one **4** rather than the expected thiazoline **5**. The formation of thiazolidin-4-one **4** may be rationalized through the first S-alkylation followed by nucleophilic addition of NH group to the amidic carbonyl followed by elimination of *p*-toluidine. The structure of the isolated product **4** was elucidated on the basis of their spectral data (IR, MS and $^1$H NMR) and also, by independent synthesis *via* treatment of the intermediate **2** with ethyl chloroacetate.

Treatment of the non-isolable potassium salt **2** with dilute hydrochloric acid furnished the corresponding thiocarbamoyl derivative **6**. Oxidative cyclization of thiocarbamoyl derivative **6** with bromine in ethyl acetate furnished 2-(benzothiazol-2-ylidene)-*N*-(benzothiazol-2-yl)-2-cyanoacetamide (**7**). The formation of benzothiazole **7** is in the line with previous reports [48,49].

On the other hand, thiazolidin-4-one **8** could be achieved *via* the reaction of amide **1** with thioglycolic acid in boiling pyridine. The Gewald reaction of amide **1** with elemental sulfur and phenyl isothiocyanate in a mixture of EtOH/DMF containing triethylamine as a basic catalyst led to functionalized thiazoline **9**. Cyclization of thiazoline **9** with triethyl orthoformate in acetic anhydride afforded thiazolo[4,5-*d*]pyrimidine **10** as deazapurine analogue. The structure of the prepared compounds was elucidated on the basis of elemental analysis and spectral data. The IR spectrum of thiazoline **9** revealed the absence of C≡N absorption band and the presence of new absorption bands at 3430, 3345 cm$^{-1}$ assignable to the amino group and a band at 1231 cm$^{-1}$ due to C=S group. The $^1$H NMR spectrum of compound **10** was characterized by the existence of thiazolopyrimidine H-5 at $\delta$ 9.39 ppm. Its mass spectrum showed a molecular ion peak at $m/z = 394$ corresponding to a molecular formula $C_{18}H_{10}N_4OS_3$.

Shifting to Scheme 2, 2-amino-*N*-(benzothiazol-2-yl)-4,5,6,7-tetrahydrobenzothiophene-3-carboxamide (**11**) could be achieved according to the method described by Gewald, by reacting amide **1** with sulfur and cyclohexanone in the presence of morpholine as a basic catalyst. The assignment of the structure of compound **11** was based on analytical and spectroscopic data. Thus, its IR spectrum displayed absorption bands at 3440, 3333 and 3210 cm$^{-1}$ assignable to $NH_2$ and NH groups. The mass spectrum showed a molecular ion peak at $m/z = 329$ corresponding to a molecular formula $C_{16}H_{15}N_3OS_2$.

To investigate the structure–activity relationship with respect to antimicrobial properties we cyclized the thiocarbamoyl functionality of compound **6** to thiophene. Thus, refluxing of thiocarbamoyl derivative **6** with phenacyl bromide and/or ethyl chloroacetate in dimethylformamide containing a catalytic amount of triethylamine afforded 4-amino-2-anilino-3-[(benzothiazol-2-ylamino)carbonyl]-5-benzoylthiophene (**12**) and ethyl 3-amino-5-

anilino-4-[(benzothiazol-2-ylamino)carbonyl]thiophene-2-carboxylate (**13**), respectively. The IR spectra of the isolated products showed the absence of C≡N group and the presence of $NH_2$ group at 3425–3370 $cm^{-1}$. The $^1H$ NMR spectrum of compound **12** showed three singlet signals at $\delta$ 13.35, 11.83 and 8.78 ppm due to amidic NH, NH and $NH_2$ protons, respectively. Moreover, the mass spectrum showed a molecular ion peak at $m/z = 470$ corresponding to a molecular formula $C_{25}H_{18}N_4O_2S_2$.

To further explore the synthetic potentially of amide **1**, the Knoevenagel condensation of amide **1** with 1,3-diphenylpyrazole-4-carboxaldehyde [50] was investigated. Heating amide **1** with 1,3-diphenylpyrazole-4-carboxaldehyde in ethanolic sodium hydroxide (10%) afforded *N*-(benzothiazol-2-yl)-2-cyano-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)acrylamide (**14**). The addition of hydrazine hydrate to the activated double bond of compound **14** in boiling ethanol afforded 5-amino-*N*-(benzothiazol-2-yl)-1′,3′-diphenyl-1*H*,1′*H*-3,4′-bipyrazole-4-carboxamide (**15**).

Coupling of amide **1** with diazotized 3-amino-4,6-dimethyl-2*H*-pyrazolo[3,4-*b*]pyridine [51] in pyridine at 0–5 °C gave the hydrazono derivative **16**. Formation of the triazine derivative **17** was achieved in an excellent yield by heating compound **16** in glacial acetic acid. Elemental analysis, IR, NMR and MS are in agreement with the proposed structure.

On the other hand, the 3-amino-5-anilinopyrazole **19** was served as key intermediate in Scheme 3. It was prepared in two consequence steps by reacting **1** with phenyl isothiocyanate and methyl iodide in basic dimethylformamide to give the ketene *N*,*S*-acetal **18** that reacted with hydrazine hydrate in boiling ethanol to give the target compound **19**. The structure of the aminopyrazole **19** was identified as the reaction product on the basis of its elemental analysis and spectroscopic data. Its IR spectrum displayed the lacks of absorption band assignable to the C≡N group and the presence of a new absorption band at 3412, 3355 $cm^{-1}$ assignable to $NH_2$ group. The $^1H$ NMR spectrum displayed a broad singlet signal at $\delta$ 6.43 ppm corresponding to the $NH_2$ protons, a multiplet signals at $\delta$ 6.86–7.84 ppm related to the aromatic protons, and another three singlet signals at $\delta$ 9.32, 11.09 and 12.99 ppm assignable to three NH protons. The $^{13}C$ NMR spectrum was characterized by signals at 116–140 ppm assignable to aromatic carbons and a signal at 166 ppm corresponding to carbonyl carbon atom. Moreover, the mass spectrum showed a molecular ion peak at ($M^+$) $m/z = 350$ corresponding to a molecular formula $C_{17}H_{14}N_6OS$.

3(5)-Aminopyrazoles are versatile reagents and have been extensively used as synthetic starting materials for the synthesis of several polysubstituted fused pyrazoles of potential biological activity [52–55]. It was thus of interest to study the reactivity of 5-aminopyrazole **19** towards a variety of chemical reagents. The general literature procedure [56–59] for the synthesis of pyrazolo[1,5-*a*]pyrimidines involves cyclocondensation of aminopyrazoles with reagents having 1,3-electrophilic centers such as $\beta$-diketones or enaminones. Cyclocondensation of compound **19** with either acetyl acetone or acetoacetanilide in boiling acetic acid produced in each case a single product, as evidenced by TLC. The reaction products can be formulated as pyrazolo[1,5-*a*]pyrimidine derivatives **20a** and **20b**, evidence for assigned structures being provided by analytical and spectroscopic data. For example, The IR spectrum of compound **20a** showed the appearance of absorption band at 1661 $cm^{-1}$ corresponding to the amidic carbonyl group. The $^1H$ NMR spectrum exhibited two additional singlet signals at $\delta$ 2.46 and 2.54 ppm assignable to the protons of two methyl groups in pyrimidine ring and a singlet signal at $\delta$ 6.82 ppm for proton in the pyrimidine ring. The mass spectrum showed a molecular ion peak at $m/z = 414$ corresponding to a molecular formula $C_{22}H_{18}N_6OS$.

Furthermore, the reaction of aminopyrazole **19** with 3-(*N*,*N*-dimethylamino)-1-(thiophen-2-yl)prop-2-en-1-one [60] in glacial acetic acid at reflux afforded pyrazolo[1,5-*a*]pyrimidine derivative **21b** rather than **21a**. The reaction product **21b** was confirmed on the basis of its elemental analysis and spectral data. The formation of compound **21b** is assumed to take place *via* an initial Michael addition of the exocyclic amino group in **19** to the activated double bond in enaminone followed by cyclization and aromatization *via* loss of both dimethylamine and water molecules.

The reactivity of aminopyrazole **19** towards benzylidene malononitrile was also investigated as an alterative route to obtain pyrazolo[1,5-*a*]pyrimidine derivative. Thus, reaction of **19** with $\alpha$-cyanocinnamonitrile in ethanolic sodium ethoxide solution yielded product for which structure **22a** or **22b** seemed possible. Structure **22a** appears more likely than **22b** on the basis that ring nitrogen is the most steric hindrance center in the molecule [61]. The structure of **22a** was assigned on the basis of elemental analysis and spectral data.

Moreover, heating **19** with dimethylformamide–dimethylacetal (DMF–DMA) in dioxane furnished azaenaminopyrazole **23** in a reasonable yield. Elemental analysis and spectral data were in agreement with the formation of the azaenamine product **23**. Its $^1H$ NMR spectrum showed a new singlet signals at $\delta$ 3.19, 3.23 and 8.33 ppm corresponding to the protons of the two methyl groups and the methine proton. The mass spectrum showed a molecular ion peak at $m/z = 405$ corresponding to a molecular formula $C_{20}H_{19}N_7OS$. Compound **23** was converted to pyrazolo[3,4-*d*]pyrimidine derivative **24** upon heating in glacial acetic acid.

In addition, the condensation of **19** with 1,3-diphenylpyrazole-4-carboxaldehyde and isatin in boiling ethanol in the presence of catalytic amount of piperidine furnished the corresponding Schiff's base **25** and **26** in excellent yield. The chemical structure of **25** and **26** were supported on the basis of elemental analysis and spectral data.

## 3. Pharmacology

### 3.1. Antimicrobial evaluation

Fourteen of the newly synthesized target compounds were evaluated for their in *vitro* antibacterial activity against *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615) as examples of Gram-positive bacteria and *Pseudomonas phaseolicola* (GSPB 2828) and *Pseudomonas fluorescens* (S 97) as examples of Gram-negative bacteria. They were also evaluated for their in *vitro* antifungal potential against *Fusarium oxysporum* and *Aspergillus fumigatus* fungal strains.

Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Chloroamphenicol, cephalothin and cycloheximide were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds showed significant growth inhibition zones (>12 mm) using twofold serial dilution method [62]. The MIC (μg/mL) and inhibition zone diameters values are recorded in Table 1.

The results depicted in Table 1 revealed that most of tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, and also against antifungal strains.

In general, most of the tested compounds revealed better activity against the Gram-positive rather than the Gram-negative bacteria. It would be also noticed that compounds belonging to the thiophene and pyrazole series (Schemes 2 and 3) exhibited better antibacterial potentials than members of the thiazole one (Scheme 1).

**Table 1**
Minimal inhibitory concentrations (MIC, μg/mL) and inhibition zone (mm) of some new synthesized compounds.

| Compound no. | MIC[a] in μg/mL, and zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Bacteria | | | | Fungi | |
| | Gram-positive bacteria | | Gram-negative bacteria | | | |
| | *S. aureus* | *S. pyogenes* | *P. phaseolicola* | *P. fluorescens* | *F. oxysporum* | *A. fumigatus* |
| **3** | 50 (19) | 100 (15) | 100 (14) | 50 (19) | 100 (15) | 6.25 (38) |
| **4** | 100 (15) | 100 (14) | 100 (15) | 100 (17) | 100 (14) | 50 (19) |
| **7a** | 25 (25) | 25 (25) | 50 (19) | 100 (14) | 100 (15) | –[b] |
| **8** | 25 (26) | 25 (25) | 100 (14) | 6.25 (37) | 100 (16) | 50 (20) |
| **9** | 12.5 (32) | 100 (14) | 50 (20) | 100 (16) | 12.5 (31) | 100 (16) |
| **11** | 6.25 (38) | 6.25 (38) | 100 (14) | 100 (16) | 100 (15) | 50 (19) |
| **12** | 6.25 (38) | 6.25 (37) | 50 (19) | 50 (20) | 100 (14) | – |
| **13** | 3.125 (43) | 6.25 (38) | 50 (19) | – | 100 (14) | 12.5 (31) |
| **15** | 12.5 (32) | 6.25 (38) | 100 (14) | 50 (20) | 50 (19) | – |
| **17** | 100 (14) | 100 (15) | 100 (15) | 12.5 (32) | 25 (26) | 50 (19) |
| **20b** | 25 (25) | 6.25 (37) | 25 (26) | 50 (19) | 100 (15) | 100 (14) |
| **21b** | 100 (14) | 100 (15) | 100 (15) | 50 (18) | 6.25 (37) | 25 (26) |
| **24** | 100 (15) | 100 (15) | 6.25 (38) | 100 (15) | 50 (19) | 100 (15) |
| **25** | 3.125 (44) | 6.25 (37) | 100 (15) | 50 (19) | 25 (26) | 100 (14) |
| *Reference drugs* | | | | | | |
| Chloramphenicol | 3.125 (42) | 3.125 (44) | 6.25 (37) | 6.25 (38) | NT[c] | NT |
| Cephalothin | 6.25 (36) | 6.25 (37) | 6.25 (38) | 6.25 (37) | NT | NT |
| Cycloheximide | NT | NT | NT | NT | 3.125 (43) | 3.125 (42) |

[a] MIC: Minimal inhibitory concentration values with SEM = 0.02.
[b] (−): totally inactive (no inhibition zone).
[c] NT: Not tested.

Regarding the structure–activity relationship of the thiophenes against Gram-positive bacteria, the results revealed that compounds **11**, **12**, and **13** exhibited broad spectrum antibacterial profile against the tested organisms. Thiophenes with electron withdrawing groups such as $CO_2Et$ or PhCO recorded higher activity. In this view, compound **13** was equipotent to chloroamphenicol in inhibiting the growth of *S. aureus* (MIC 3.125 μg/mL), while its activity was 50% lower than of chloroamphenicol against *S. pyogenes*. Compounds **11** and **12** showed 50% of the activity of chloroamphenicol (MIC 6.25 μg/mL) but they were equipotent to cephalothin in inhibiting the growth of *S. aureus* and *S. pyogenes* (MIC 6.25 μg/mL).

On the other hand, compounds **3**, **7a**, **8**, **9**, **15**, **20b** and **24** exhibited weak to moderate growth inhibitory activity against Gram-positive bacteria as revealed from their MIC values (25–100 μg/mL). Among these compounds **9** and **15** showed relatively good growth inhibitory profiles against *S. aureus* (MIC 12.5 μg/mL) which were about 25% of the activity of chloroamphenicol and 50% of cephalothin against the same organism. Moreover, distinctive anti-Gram-positive profile was displayed by compound **25** where it proved to be equipotent as chloroamphenicol against *S. aureus* (MIC 3.125 μg/mL) together with a significant activity against *S. pyogenes* (MIC 6.25 μg/mL).

Concerning the antibacterial activity of the compounds **3**, **12**, **15**, and **20b** revealed weak growth inhibitory against the tested Gram-negative bacteria (MIC 50 μg/mL). On the other hand, compounds **8** and **24** showed equipotent activity as chloroamphenicol and cephalothin (MIC 6.25 μg/mL) against *P. fluorescens* and *P. phaseolicola*.

Regarding the activity of thiazoles, thiophenes and pyrazoles incorporating benzothiazole moiety, against antifungal strains, the results revealed that compounds **3** and **21** were 50% lower than cycloheximide in inhibitory the growth of *A. fumigatus* and *F. oxysporum* (MIC 6.25 μg/mL), while the reactivity of compound **9** was 25% lower than cycloheximide against *F. oxysporum* (MIC 12. 5 μg/mL).

The substitution pattern was also crucial. It is worth mentioning that incorporation of benzothiazole to thiophene nucleus at position 3 *via* a carboxamide linker produced a high antimicrobial activity. Conversion of aminopyrazole **19** to Schiff base **25** enhanced also the antimicrobial activity. On the other hand, incorporation of benzothiazole nucleus to thiazole derivatives at position 2 in compounds **3**, **4**, and **8** unfortunately produced weak antimicrobial activity. High biological activity can be correlated with low electron density of ring systems.

In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of some new thiazoles, thiophenes and pyrazoles incorporating benzothiazole moiety with the hope of discovering new structure leads serving as potent antimicrobial agents. Our aim has been verified by the synthesis of three different groups of structure hybrids comprising basically the benzothiazole moiety attached to either polysubstituted thiazole, thiophene or pyrazole counter parts through various linkages of synthergistic purpose. The obtained results clearly revealed that compounds derived from thiophenes and pyrazoles exhibited better antimicrobial activity than their thiazole structure variants.

## 4. Experimental

All melting points were measured on a Gallenkamp electrothermal melting point apparatus. IR spectra were recorded for KBr disc on a Mattson 5000 FTIR spectrophotometer. $^1$H NMR spectra were measured on a Bruker AC 300 (300 MHz) in $CDCl_3$ or DMSO-$d_6$ as solvent, using TMS as an internal standard, and chemical shifts are expressed as $\delta_{ppm}$. Mass spectra were determined on Finnigan Incos 500 (70 ev). Elemental analyses were carried out in the Microanalytical Unit of the Faculty of Science, Cairo University. *N*-(Benzothiazol-2-yl)-2-cyanoacetamide (**1**) [47], 1,3-diphenyl-pyrazole-4-carboxaldehyde [50], 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*] pyridin-3-amine [51], and 3-(*N*,*N*-dimethylamino)-1-(thiophen-2-yl)prop-2-en-1-one [60] were prepared following literature procedure.

### 4.1. General procedure for the synthesis of thiazoline (**3**) and thiazolidin-4-one (**4**)

To a cold suspension of powdered divided KOH (0.56 g, 0.01 mol) in DMF (20 mL) was added amide **1** (2.17 g, 0.01 mol) and

phenyl isothiocyanate (1.2 mL, 0.01 mol). The reaction mixture was stirred at room temperature for 24 h, and then treated with phenacyl bromide and/or 2-chloro-*N*-*p*-tolylacetamide (0.01 mol) and the stirring was continued at room temperature for further 5 h. The reaction mixture was poured into 50 mL of cold water. The resultant solid products were collected by filtration and recrystallized from a mixture of EtOH/DMF (1:1) to give compounds **3** and **4**.

#### 4.1.1. N-(Benzothiazol-2-yl)-2-cyano-2-(3,4-diphenylthiazol-2-ylidene)acetamide (**3**)

White powder; Yield 74%; mp 302–304 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3384 (NH), 2178 (C≡N), 1625 (C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 7.18–7.86 (m, 15H, Ar-H, thiazoline H-5), 10.59 (s, 1H, NH). MS *m*/*z* (%): 452 ($M^+$, 16.3), 304 (17.6), 303 (100), 150 (2.4), 134 (12.7), 77 (14.2). Anal. For $C_{25}H_{16}N_4OS_2$ (452.55) Calcd.: C 66.35; H 3.56; N 12.38%, Found: C 66.32; H 3.49; N 12.19%.

#### 4.1.2. N-(Benzothiazol-2-yl)-2-cyano-2-(4-oxo-3-phenylthiazolidin-2-ylidene)acetamide (**4**)

Pale yellow sheets; Yield 66%; mp 214–216 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3488 (NH), 2208 (C≡N), 1736 (C=O), 1643 (C=O). $^1$H NMR ($CDCl_3$): $\delta_{ppm}$ = 3.95 (s, 2H, $CH_2$), 7.26–7.83 (m, 9H, Ar-H), 11.35 (s, 1H, NH). MS *m*/*z* (%): 392 ($M^+$, 30.5), 244 (11.4), 243 (70.6), 217 (6.8), 215 (100), 169 (21.5), 150 (8.2), 132 (34.3), 77 (62.3). Anal. For $C_{19}H_{12}N_4O_2S_2$ (392.45) Calcd.: C 58.15; H 3.08; N 14.28%, Found: C 57.97; H 2.98; N 14.31%.

### 4.2. Synthesis of 3-anilino-N-(benzothiazol-2-yl)-2-cyano-3-thioxopropanamide (**6**)

To a stirred solution of powdered KOH (0.56 g, 0.01 mol) in DMF (20 mL) was added compound **1** (2.17 g, 0.01 mol). After the mixture was stirred for 0.5 h, phenyl isothiocyanate (1.2 mL, 0.01 mol) was added and the stirring was continued at room temperature for 24 h. The reaction mixture was poured onto (100 mL) ice-cold water containing few drops of HCl (0.1 N). The solid product that separated was filtered, washed with water and recrystallized from EtOH to give compound **6**.

Yellow crystals; Yield 82%; mp 202–204 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3440, 3227 (2NH), 2183 (C≡N), 1636 (C=O), 1288 (C=S). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 4.28 (s, 1H, CH), 7.10–7.80 (m, 9H, Ar-H), 9.85 (s, 1H, NH), 13.52 (s, 1H, CONH). Anal. For $C_{17}H_{12}N_4OS_2$ (352.43) Calcd.: C 57.93; H 3.43; N 18.90%, Found: C 57.82; H 3.37; N 18.75%.

### 4.3. Synthesis of 2-(benzothiazol-2-ylidene)-N-(benzothiazol-2-yl)-2-cyanoacetamide (**7**)

To a cold suspension of compound **6** (0.352 g, 0.001 mol) in ethyl acetate (20 mL), pyridine (1.6 mL) was added dropwise during 10 min, with stirring. The solution of bromine (0.16 g, 0.002 mol) in ethyl acetate (10 mL) was added dropwise during 10 min and the stirring was continued for further 3 h. The yellow precipitate formed was filtered and washed with ethyl acetate followed by ether. The product was dried and recrystallized from a mixture of EtOH/DMF (1:1) to give compound **7**.

Pale yellow crystals; Yield 55%; mp 238–240 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3436, 3190 (2NH), 2223 (C≡N), 1685 (C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 7.06–8.33 (m, 8H, Ar-H), 8.72 (s, 1H, NH), 10.01 (s, 1H, NH). MS *m*/*z* (%): 350 ($M^+$, 42.8), 202 (2.6), 178 (2.8), 174 (24), 150 (100), 149 (8.4), 135 (3.2), 123 (9.8), 110 (6.4), 77 (29.3). Anal. For $C_{17}H_{10}N_4OS_2$ (350.42) Calcd.: C 58.27; H 2.88; N 15.99%, Found: C 58.12; H 2.71; N 15.76%.

### 4.4. Synthesis of N-(benzothiazol-2-yl)-2-(4-oxo-4,5-dihydrothiazol-2-yl) acetamide (**8**)

A mixture of compound **1** (2.17 g, 0.01 mol) and thioglycolic acid (0.69 mL, 0.01 mol) in dry pyridine (20 mL) was heated under reflux for 3 h, allowed to cool, and poured into cold water (50 mL). The solid product obtained was collected by filtration, dried and recrystallized from ethanol to give compound **8**.

White crystals; Yield 62%; mp 350–352 °C; IR (KBr): $\upsilon_{max}$/$cm^{-1}$ = 3165 (NH), 1699, 1664 (2C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 2.77 (s, 2H, $CH_2$), 3.34 (s, 2H, $CH_2$–S), 7.23–7.86 (m, 4H, Ar-H), 11.21 (s, 1H, NH). MS *m*/*z* (%): 291 ($M^+$, 5.2), 178 (0.9), 150 (100), 114 (21.7), 105 (10.8), 98 (1.4), 77 (2.6), 68 (71.8). Anal. For $C_{12}H_9N_3O_2S_2$ (291.35) Calcd.: C 49.47; H 3.11; N 14.42%, Found: C 49.37; H 2.99; N 14.21%.

### 4.5. Synthesis of 4-amino-N-(benzothiazol-2-yl)-2,3-dihydro-3-phenyl-2-thioxo-thiazole -5-carboxamide (**9**)

To a solution of amide **1** (2.17 g, 0.01 mol) in ethanol (15 mL) containing triethylamine (0.5 mL), elemental sulfur (0.32 g, 0.01 mol) and phenyl isothiocyanate (1.35 mL, 0.01 mol) were added. The reaction mixture was heated at 60 °C for 2 h with continuous stirring and then poured into a beaker containing an ice-water mixture with few drops of HCl. The formed solid product was collected by filtration, dried and recrystallized from EtOH to give compound **9**.

Yellow sheets; Yield 72%; mp 242–244 °C, IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3430, 3345 ($NH_2$), 3253 (NH), 1690 (C=O), 1231 (C=S). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 7.14 (s, 2H, $NH_2$), 7.31–7.91 (m, 9H, Ar-H), 10.62 (s, 1H, NH). MS *m*/*z* (%): 384 ($M^+$, 100), 235 (87.8), 208 (29), 178 (10), 176 (34.7), 150 (71.1), 136 (40.8), 77 (80.6). Anal. For $C_{17}H_{12}N_4OS_3$ (384.50) Calcd.: C 53.10; H 3.15; N 14.57%, Found: C 52.84; H 2.99; N 14.23%.

### 4.6. Synthesis of 6-(benzothiazol-2-yl)-2,3-dihydro-3-phenyl-2-thioxothiazolo[4,5-d] pyrimidin-7(6H)-one (**10**)

A solution of compound **9** (3.8 g, 0.01 mol) in a mixture of triethyl orthoformate (2.5 mL) and acetic anhydride (2.5 mL) was heated under reflux for 8 h and then allowed to cool. The precipitate that formed was collected by filtration, dried and recrystallized from acetic acid to give compound **10**.

Yellow brightness crystals; Yield 43%; mp 316–318 °C, IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 1686 (C=O), 1249 (C=S). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 7.24–7.74 (m, 9H, Ar-H), 9.39 (s, 1H, thiazolopyrimidine H-5). MS *m*/*z* (%): 394 ($M^+$, 46.3), 351 (9.8), 320 (17.1), 225 (51.2), 134 (34.1), 96 (68.3), 77 (100), 69 (24.4). Anal. For $C_{18}H_{10}N_4OS_3$ (394.49) Calcd.: C 54.80; H 2.56; N 14.20%, Found: C 54.59; H 2.38; N 14.08%.

### 4.7. Synthesis of 2-amino-N-(benzothiazol-2-yl)-4,5,6,7-tetrahydrobenzothiophene-3-carboxamide (**11**)

A mixture of compound **1** (2.17 g, 0.01 mol), cyclohexanone (0.98 g, 0.01 mol), and elemental sulfur (0.35 g, 0.015 mol) in ethanol (20 mL) containing morpholine (0.87 g, 0.01 mol) was heated at 60 °C for 2 h with stirring. After the reaction mixture was allowed to cool, the precipitate that formed was collected by filtration, dried and recrystallized from chloroform to give **11**.

Pale yellow crystals; Yield 86%; mp 250–252 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3440, 3333 ($NH_2$), 3210 (NH), 1635 (C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 1.10–1.16 (m, 8H, ring 4$CH_2$), 5.98 (s, 2H, $NH_2$), 7.25–7.92 (m, 4H, Ar-H), 11.97 (s, 1H, NH). MS *m*/*z* (%): 329 ($M^+$, 30.5), 181 (11.7), 179 (100), 153 (5.5), 150 (24.3), 118 (21.2), 77 (13.2).

Anal. For $C_{16}H_{15}N_3OS_2$ (329.44) Calcd.: C 58.33; H 4.59; N 12.76%, Found: C 58.24; H 4.41; N 12.63%.

### 4.8. General procedure for synthesis of thiophene derivatives (**12**) and (**13**)

To a solution of compound **6** (3.52 g, 0.01 mol) in 20 mL ethanol, the appropriate α-halocarbonyl compounds such as phenacyl bromide and ethyl chloroacetate (0.01 mol) and few drops of triethylamine was added. The reaction mixture was refluxed for 5 h, and then allowed to cool. The formed solid product was collected by filtration, washed with ethanol and recrystallized from a mixture of EtOH/DMF (2:1) to afford the corresponding thiophene derivatives **12** and **13**.

#### 4.8.1. 4-Amino-2-anilino-3-[(benzothiazol-2-ylamino)carbonyl]-5-benzoylthiophene (**12**)

Pale yellow crystals; Yield 72%; mp 292–294 °C; IR (KBr) $\upsilon_{max}/cm^{-1}$ = 3425, 3370 ($NH_2$), 3233 (NH), 3190 (NH), 1723 (C=O), 1620 (C=O), 1562 (C=N). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 7.21–7.89 (m, 14H, Ar-H), 8.78 (br.s, 2H, $NH_2$), 11.83 (s, 1H, NH), 13.35 (s, 1H, CONH). MS *m*/*z* (%): 470 ($M^+$, 41), 454 (6.2), 350 (1.2), 322 (3.7), 320 (31.6), 319 (90), 294 (12.6), 217 (7), 202 (7.6), 178 (5.8), 177 (11.6), 176 (11.2), 150 (15.7), 135 (2.7), 106 (7.6), 98 (1.7), 92 (4.5), 84 (10.7), 77 (100). Anal. For $C_{25}H_{18}N_4O_2S_2$ (470.57) Calcd.: C 63.81; H 3.86; N 11.91%, Found: C 63.74; H 3.79; N 11.68%.

#### 4.8.2. Ethyl 3-amino-5-anilino-4-[(benzothiazol-2-ylamino) carbonyl]thiophene-2-carboxylate (**13**)

Yellow powder; Yield 76%; mp 262–264 °C; IR (KBr) $\upsilon_{max}/cm^{-1}$ = 3425, 3365 ($NH_2$), 3340, 3270 (2NH), 1660, 1645 (2C=O), 1560 (C=N). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 1.21 (t, *J* = 7.2 Hz, 3H, $CH_3$), 4.11 (q, *J* = 7.2 Hz, 2H, O–$CH_2$), 7.23–7.84 (m, 9H, Ar-H), 8.21 (s, 2H, $NH_2$), 11.74 (s, 1H, NH), 13.53 (s, 1H, CONH). MS *m*/*z* (%): 438 ($M^+$, 44), 366 (1.6), 290 (2.4), 262 (4.2), 242 (100), 178 (1.2), 151 (21.8), 84 (7.7), 77 (50.7). Anal. For $C_{21}H_{18}N_4O_3S_2$ (438.52) Calcd.: C 57.52; H 4.14; N 12.78%, Found: C 57.42; H 4.03; N 12.59%.

### 4.9. Synthesis of N-(benzothiazol-2-yl)-2-cyano-3-(1,3-diphenyl-1H-pyrazol-4-yl) acrylamide (**14**)

To a solution of compound **1** (2.17 g, 0.01 mol) in absolute ethanol (15 mL) containing 3 drops of sodium hydroxide (10%), 1,3-diphenyl-pyrazole-4-carboxaldehyde (2.48 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1 h, and then allowed to cool. The precipitate that formed was collected by filtration, washed with ethanol, dried and recrystallized from a mixture of EtOH/DMF (1:1) to give compound **14**.

Yellow powders; Yield 87%; mp 296–298 °C; IR (KBr) $\upsilon_{max}/cm^{-1}$ = 3430 (NH), 2214 (C≡N), 1628 (C=O). MS *m*/*z* (%): 447 ($M^+$, 12.8), 298 (100), 231 (48), 149 (12.8), 113 (38.3), 108 (25.5), 77 (13.8). Anal. For $C_{26}H_{17}N_5OS$ (447.51) Calcd.: C 69.78; H 3.83; N 15.65%, Found: C 69.76; H 3.68; N 15.64%.

### 4.10. Synthesis of 5-amino-N-(benzothiazol-2-yl)-1′,3′-diphenyl-1H,1′H-3,4′-bipyrazole-4-carboxamide (**15**)

To a boiling solution of compound **14** (4.47 g, 0.01 mol) in ethanol (20 mL), hydrazine hydrate (1.5 mL, 0.01 mol) was added. The reaction mixture was refluxed for 7 h and then cooled. The solid product so formed was collected by filtration, washed with ethanol and recrystallized from a mixture of DMF/EtOH (1:1) to give compound **15**.

Yellow crystals; Yield 43%; mp 228–230 °C; IR (KBr) $\upsilon_{max}/cm^{-1}$ = 3432, 3418 ($NH_2$), 3317, 3245 (2NH), 1659(C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 7.39–8.04 (m, 14H, Ar-H), 8.36 (s, 1H, pyrazole H-5), 8.68 (s, 2H, $NH_2$), 9.15 (s, 1H, NH), 10.82 (s, 1H, CONH). MS *m*/*z* (%): 477 ($M^+$, 14.2), 259 (1), 244 (5.3), 220 (1.6), 178 (3), 150 (0.8), 144 (3.2), 77 (100), 68 (0.4). Anal. For $C_{26}H_{19}N_7OS$ (477.54) Calcd.: C 65.39; H 4.01; N 20.53%, Found: C 65.21; H 3.95: N 20.42%.

### 4.11. Synthesis of N-(benzothiazol-2-yl)-2-cyano-2-[(4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine-3-yl)hydrazono]acetamide (**16**)

To a cold solution of compound **1** (2.17 g, 0.01 mol) in pyridine (15 mL), was added the diazonium salt of 4,6-dimethyl-2*H*-pyrazolo[3,4-*b*]pyridine-3-yl [prepared by dissolving sodium nitrite (0.07 g, 0.01 mol) in water (2 mL) and adding to a cold solution of 3-amino-4,6-dimethyl-2*H*-pyrazolo[3,4-*b*]pyridine (1.62 g, 0.01 mol) containing the appropriate amount of hydrochloric acid with continuous stirring] portion wise over a period of 30 min. The reaction mixture was kept in an ice bath for 24 h and then diluted with water, filtered off, dried, and recrystallized from a mixture of DMF/EtOH (2:1) to afford compound **16**.

Orange crystals; Yield 91%; mp 290–292 °C; IR (KBr) $\upsilon_{max}/cm^{-1}$ = 3367, 3287, 3190 (3NH), 2202 (C≡N), 1650 (C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 2.52 (s, 3H, $CH_3$), 2.70 (s, 3H, $CH_3$), 7.13 (s, 1H, pyridine H-5), 7.24–7.86 (m, 4H, Ar-H), 8.12 (s, 1H, NH), 8.65 (s, 1H, NH), 9.61 (s, 1H, CONH). MS *m*/*z* (%): 390 ($M^+$, 100), 358 (7.1), 346 (21), 241 (28.3), 174 (20.2), 162 (20.3), 150 (39), 147 (10), 77 (15.8). Anal. For $C_{18}H_{14}N_8OS$ (390.42) Calcd.: C 55.37; H 3.61; N 28.70%, Found: C 55.24; H 3.58; N 28.59%.

### 4.12. Synthesis of N-(benzothiazol-2-yl)-4-imino-8,10-dimethyl-4,6-dihydropyrido [2′,3′:3,4]pyrazolo[5,1-c][1,2,4]triazine-3-carboxamide (**17**)

A solution of compound **16** (3.9 g, 0.01 mol) in glacial acetic acid (15 mL) was refluxed for 2 h, and then allowed to cool. The precipitate that formed was collected by filtration, washed with ethanol and recrystallized from a mixture of DMF/EtOH (1:1) to give compound **17**.

Yellow crystals; Yield 86%; mp 362–364 °C; IR (KBr) $\upsilon_{max}/cm^{-1}$ = 3437, 3394, 3158, 3201 (4NH), 1675 (C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 2.72 (s, 3H, $CH_3$), 3.01 (s, 3H, $CH_3$), 7.25 (s, 1H, pyridine H-5), 7.41–8.12 (m, 4H, Ar-H), 9.18 (s, 1H, NH), 9.62 (s, 1H, NH), 12.51 (s, 1H, CONH). Anal. For $C_{18}H_{14}N_8OS$ (390.42) Calcd.: C 55.37; H 3.61; N 28.70%, Found: C 55.29; H 3.56; N 28.62%.

### 4.13. Synthesis of 3-anilino-N-(benzothiazol-2-yl)-2-cyano-3-(methylthio)acrylamide (**18**)

To a stirred solution of potassium hydroxide (0.56 g, 0.01 mol) in dimethylformamide (20 mL) was added compound **1** (2.17 g, 0.01 mol). After the mixture was stirred for 30 min, phenyl isothiocyanate (1.2 mL, 0.01 mol) was added to the resulting mixture. Stirring was continued for 6 h, and then methyl iodide (0.62 mL, 0.01 mol) was added and stirring was continued for additional 6 h. The reaction mixture was poured onto ice-cold water. The solid product that formed was collected by filtration, dried and recrystallized from ethanol to give compound **18**.

Orange crystals; Yield 86%; mp 163–165 °C; IR (KBr) $\upsilon_{max}/cm^{-1}$ = 3447, 3395 (2NH), 2192 (C≡N), 1627 (C=O), 1600 (C=C). $^1$H NMR ($CDCl_3$): $\delta_{ppm}$ = 2.25 (s, 3H, $SCH_3$), 7.26–7.81 (m, 9H, Ar-H), 9.82 (s, 1H, NH), 12.19 (s, 1H, CONH). Anal. For $C_{18}H_{14}N_4OS_2$ (366.46) Calcd.: C 58.99; H 3.85; N 15.29%, Found: C 58.76; H 3.59; N 15.06%.

*4.14. Synthesis of 5-amino-3-anilino-N-(benzothiazol-2-yl)-1H-pyrazole-4-carboxamide (**19**)*

A mixture of compound **18** (3.66 g, 0.01 mol) and hydrazine hydrate 98% (0.01 mol) were heated on a steam bath for 1 h, then left to cool. The reaction mixture was triturated with ethanol and the resulting solid was filtered off and recrystallized from ethanol to give compound **19**.

White crystals; Yield 73%; mp 216–218 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3412, 3355 ($NH_2$), 3302, 3240, 3179 (3NH), 1620 (C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 6.43 (br.s, 2H, $NH_2$), 6.86–7.84 (m, 9H, Ar-H), 9.32 (s, 1H, NH), 11.09 (s, 1H, NH), 12.99 (s, 1H, CONH). $^{13}$C NMR (DMSO-$d_6$): $\delta_{ppm}$ = 89.9, 115.9 (2C), 116.3, 118.9, 121.8, 126.0, 129.1 (2C), 130.6, 133.2, 140.0, 142.8, 144.4, 150.5, 151.2, 166.2. MS *m*/*z* (%): 350 ($M^+$, 80), 201 (80.2), 200 (100), 174 (15.6), 159 (2.1), 151 (24.2), 150 (26.2), 145 (19.1), 117 (13.7), 77 (44.5). Anal. For $C_{17}H_{14}N_6OS$ (350.40) Calcd.: C 58.27; H 4.03; N 23.98%, Found: C 58.12; H 3.97; N 23.69%.

*4.15. General procedure for the reaction of 3(5)-aminopyrazole **19** with 1,3-dicarbonyl compounds*

To a mixture of compound **19** (3.5 g, 0.01 mol) in glacial acetic acid (25 mL), an appropriate 1,3-dicarbonyl compounds (acetylacetone or acetoacetanilide) (0.01) was added. The reaction mixture was refluxed for 12 h, and then poured into crushed ice. The solid product was collected by filtration, dried and recrystallized from a suitable solvent to give compounds **20a** and **20b**.

*4.15.1. N-(Benzothiazol-2-yl)-5,7-dimethyl-2-(phenylamino) pyrazolo[1,5-a]pyrimidine-3-carboxamide (**20a**)*

Deep brown crystals (DMF); Yield 46%; mp 242–244 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3428, 3336 (2NH), 1661 (C=O), 1601 (C=C), 1535 (Ar). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 2.46 (s, 3H, $CH_3$), 2.54 (s, 3H, $CH_3$), 6.82 (s, 1H, pyrimidine-H), 6.96–7.92 (m, 9H, Ar-H), 8.80 (s, 1H, NH), 11.08 (s, 1H, CONH). MS *m*/*z* (%): 414 ($M^+$, 15.1), 266 (17), 265 (100), 238 (5.4), 150 (1.1), 107 (3.6), 77 (5.6), 65 (10.5), 67 (4.1). Anal. For $C_{22}H_{18}N_6OS$ (414.48) Calcd.: C 63.75; H 4.38; N 20.28%, Found: C 63.42; H 4.19; N 20.08%.

*4.15.2. 2-Anilino-N-(benzothiazol-2-yl)-7-hydroxy-5-methyl-pyrazolo[1,5-a]pyrimidine-3-carboxamide (**20b**)*

Buff powder (DMF/$CHCl_3$); Yield 86%; mp 308–310 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3430 (OH), 3285, 3186 (2NH), 1665 (C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 2.46 (s, 3H, $CH_3$), 6.83 (s, 1H, pyrimidine-H), 6.96–7.96 (m, 9H, Ar-H), 9.57 (s, 1H, NH), 11.49 (s, 1H, CONH), 13.35 (s, 1H, OH). MS *m*/*z* (%): 416 ($M^+$, 93.2), 268 (34.1), 237 (34.1), 267 (100), 240 (84.1), 225 (39.4), 176 (69.7), 150 (95.5), 123 (45.5), 97 (11.4), 82 (23.5), 77 (84.1). Anal. For $C_{21}H_{16}N_6O_2S$ (416.46) Calcd.: C 60.56; H 3.87; N 20.18%, Found: C 60.42; H 3.69; N 20.20%.

*4.16. Synthesis of 2-anilino-N-(benzothiazol-2-yl)-7-(thiophen-2-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (**21b**)*

A solution of compound **19** (3.5 g, 0.01 mol) in glacial acetic acid (15 mL) and 3-(dimethylamino)-1-(thiophen-2-yl)prop-2-en-1-one (2.39 g, 0.01 mol) was heated under reflux for 3 h. The solvent was evaporated under *vacuum* and the residue was triturated with ethanol. The solid deposited was collected by filtration and recrystallized from ethanol to give compound **21b**.

Yellow crystals; Yield 90%; mp 320–322 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3435, 3264 (2NH), 1661 (C=O), 1601 (C=N). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 7.06 (t, *J* = 7.8 Hz, 1H, thiophene H-4), 7.30–7.68 (m, 9H, Ar-H), 7.74 (d, *J* = 8 Hz, 1H, thiophene H-3), 8.26 (d, *J* = 7.5 Hz, 1H, thiophene H-5), 8.53 (d, *J* = 4.8 Hz, 1H, pyrimidine H-5), 8.71 (d, *J* = 4.6 Hz, 1H, pyrimidine H-4), 9.12 (s, 1H, Ph-NH), 11.40 (s, 1H, CONH). MS *m*/*z* (%): 468 ($M^+$, 51), 319 (100), 292 (11.5), 189 (8.2), 162 (3.4), 149 (2.5), 84 (4.6), 77 (5.9), 65 (10). Anal. For $C_{24}H_{16}N_6OS_2$ (468.55) Calcd.: C 61.52; H 3.44; N 17.94%, Found: C 61.41; H 3.32; N 17.83%.

*4.17. Synthesis of 7-amino-2-anilino-N-(benzothiazol-2-yl)-6-cyano-5-phenyl-pyrazolo[1,5-a]pyrimidine-3-carboxamide (**22a**)*

2-Benzylidene malononitrile (1.54 g, 0.01 mol) was added to a mixture of aminopyrazole **19** (3.5 g, 0.01 mol) in sodium ethoxide (prepared from 0.23 g of sodium metal and 20 mL of absolute ethanol). The reaction mixture was heated under reflux for 8 h, left to cool and poured onto ice-cold water containing few drops of 1 N HCl. The precipitate that formed was collected by filtration, washed with ethanol and recrystallized from ethanol to afford compound **22a**.

Yellow crystals; Yield 44%; mp 340–342 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3430, 3313 ($NH_2$), 3231, 3165 (2NH), 2216 (C≡N), 1650 (C=O). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 7.02–7.98 (m, 14H, Ar-H), 8.35 (s, 2H, $NH_2$), 9.16 (s, 1H, NH), 11.43 (s, 1H, CONH). MS *m*/*z* (%): 502 ($M^+$, 27.5), 383 (60), 354 (23.8), 326 (100), 217 (12.5), 217 (13), 77 (60). Anal. For $C_{27}H_{18}N_8OS$ (502.55) Calcd.: C 64.53; H 3.61; N 22.30%, Found: C 64.26; H 3.57; N 22.23%.

*4.18. Synthesis of 3-anilino-N-(benzothiazol-2-yl)-5-((dimethylamino)methyleneamino)-1H-pyrazole-4-carboxamide (**23**)*

To a solution of compound **19** (3.5 g, 0.01 mol) in dry dioxane (20 mL), *N*,*N*-dimethylformamide–dimethylacetal (DMF–DMA) (1.32 mL, 0.01 mol) was added. The reaction mixture was refluxed for 2 h. After cooling, the precipitated product that formed was collected by filtration, washed with petroleum ether (40–60 °C), dried and recrystallized from a mixture of EtOH/DMF (1:1) to give compound **23**.

White crystals; Yield 62%; mp 304–306 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3420, 3352, 3174 (3NH), 1649 (C=O), 1610 (C=N). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 3.19 (s, 3H, $NCH_3$), 3.23 (s, 3H, $CH_3$), 6.86–7.98 (m, 9H, Ar-H), 8.33 (s, 1H, CH=N), 8.47 (s, 1H, NH), 10.68 (s, 1H, NH), 12.07 (s, 1H, CONH). MS *m*/*z* (%): 405 ($M^+$, 20.4), 360 (40), 312 (10.6), 255 (20.8), 211 (15), 177 (6.7), 161 (9.8), 77 (100), 68 (8.2). Anal. For $C_{20}H_{19}N_7OS$ (405.48) Calcd.: C 59.24; H 4.72; N 24.18%, Found: C 59.13; H 4.72; N 24.23%.

*4.19. Synthesis of 3-anilino-5-(benzothiazol-2-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (**24**)*

A solution of compound **23** (4 g, 0.01 mol) in glacial acetic acid (20 mL), was refluxed for 1 h, and then allowed to cool. The precipitate that formed was collected by filtration, washed with ethanol, dried and recrystallized from a mixture of DMF/EtOH (1:1) to give compound **24**.

Gray sheets; Yield 47%; mp 322–324 °C; IR (KBr) $\upsilon_{max}$/$cm^{-1}$ = 3293 (NH), 1664 (C=O), 1600 (CH=N). $^1$H NMR (DMSO-$d_6$): $\delta_{ppm}$ = 6.83–7.68 (m, 9H, Ar-H), 8.03 (s, 1H, NH), 8.67 (s, 1H, CH=N). MS *m*/*z* (%): 360 ($M^+$, 45.9), 226 (2.3), 200 (11), 187 (2.3), 157 (8.7), 150 (26.1), 135 (21.6), 77 (100). Anal. for $C_{18}H_{12}N_6OS$ (360.39) Calcd.: C 59.99; H 3.36; N 23.32%, Found: C 59.73; H 3.26; N 23.19%.

*4.20. General procedure for the reaction of 3(5)-aminopyrazole **19** with 1,3-diphenylpyrazole-4-carboxaldehyde and isatin*

A mixture of compound **19** (3.5 g, 0.01 mol) and 1,3-diphenylpyrazole-4-carboxaldehyde or isatin (0.02 mol) in ethanol

(30 mL) containing a few drops of piperidine (3 drops) was refluxed for 5 h. The reaction mixture was cooled and the solid obtained was collected by filtration, washed with ethanol, dried, and recrystallized from an appropriate solvent to give compounds **25** and **26**.

*4.20.1. 3-Anilino-N-(benzothiazol-2-yl)-5-((1,3-diphenyl-1H-pyrazol-4-yl)methylene-amino)-1H-pyrazole-4-carboxamide (**25**)*

Deep yellow crystals (DMF/$CHCl_3$); Yield 57%; mp 252–254 °C; IR (KBr) $\upsilon_{max}/cm^{-1}$ = 3417, 3658, 3250 (3NH), 1662 (C=O), 1604 (C=N). $^1H$ NMR (DMSO-$d_6$): $\delta_{ppm}$ = 6.91 (s, 1H, pyrazole-H5), 7.31–8.24 (m, 19H, Ar-H), 8.51 (s, 1H, azomethine CH), 9.04 (s, 1H, Ph-*N*H), 9.31 (s, 1H, pyrazole-*N*H), 12.06 (s, 1H, CONH). MS *m*/*z* (%): 580 ($M^+$, 10.5), 446 (7.9), 431 (10.4), 246 (15.8), 220 (12.3), 150 (37.4), 145 (11.9), 77 (100). Anal. For $C_{33}H_{24}N_8OS$ (580.66) Calcd.: C 68.26; H 4.17; N 19.30%, Found: C 68.09; H 3.97; N 19.13%.

*4.20.2. 3-Anilino-N-(benzothiazol-2-yl)-5-((2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino) -1H-pyrazole-4-carboxamide (**26**)*

Deep red powder (EtOH); Yield 62%; mp 334–336 °C; IR (KBr) $\upsilon_{max}/cm^{-1}$ = 3485, 3399, 3287, 3249 (4NH), 1719, 1653 (2C=O), 1609 (C=N). $^1H$ NMR (DMSO-$d_6$): $\delta_{ppm}$ = 6.99–8.03 (m, 13H, Ar-H), 8.96 (s, 1H, NH), 11.03 (s, 1H, pyrrole -*N*H), 11.87 (s, 1H, NH), 13.19 (s, 1H, NH). MS *m*/*z* (%): 480 ($M^+$+1, 36.9), 330 (20.4), 303 (21.6), 174 (5.4), 159 (7.4), 149 (100), 77 (54.8). Anal. For $C_{25}H_{17}N_7O_2S$ (479.51) Calcd.: C 62.62; H 3.57; N 20.45%, Found: C 62.32; H 3.42; N 20.26%.

## 5. Antimicrobial evaluation

Standard sterilized filter paper disks (5 mm diameter) impregnated with a solution of the test compound in DMF (1 mg/mL) was placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were: *S. aureus* (ATCC 25923) and *S. pyogenes* (ATCC 19615) as examples of Gram-positive bacteria and *P. phaseolicola* (GSPB 2828) and *P. fluorescens* (S 97) as examples of Gram-negative bacteria. They were also evaluated for their in *vitro* antifungal potential against *F. oxysporum* and *A. fumigatus* fungal strains. Chloroamphenicol, Cephalothin and Cycloheximide were used as standard antibacterial and antifungal agents, respectively. DMF alone was used as control at the same above-mentioned concentration. The plates were incubated at 37 °C for 24 h for bacteria and for 48 days for fungi. Compounds that showed significant growth inhibition zones (>12 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

### 5.1. Minimal inhibitory concentration (MIC) measurement

The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, Streptomycin, chloroamphenicol and Treflucan were prepared in DMF at concentration of 1000 μg/mL followed by twofold dilution at concentrations of (500, 250,…, 3.125 μg/mL). The microorganism suspensions at $10^6$ CFU/mL (Colony Forming U/mL) concentration were inoculated to the corresponding wells. Plates were incubated at 36 °C for 24–48 h and the minimal inhibitory concentrations (MIC) were determined. Control experiments were also done.

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