

# Synthesis of New Thiazole, Bithiazolidinone and Pyrano[2,3-*d*]thiazole Derivatives as Potential Antimicrobial Agents

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**Abstract:** In an attempt to find a new class of antimicrobial agents, a series of thiazole derivatives containing furan moiety **3**, **7**, **8a,b**, **10a-c** and **12** were prepared via the reaction of 2-cyano-*N*-(furan-2-ylmethyl)acetamide (**1**) with phenyl isothiocyanate and  $\alpha$ -halocarbonyl compounds. Also, many bithiazolidinone and pyranothiazole derivatives were synthesized through interaction of thiazolidinone **7** with appropriate electrophilic reagents. These compounds were screened for their antibacterial and antifungal activity. Among the synthesized compounds, thiazole derivatives **23a** and **23b** were equipotent to gentamycin against *K. pneumonia* (MIC 0.49  $\mu$ g/mL), and showed *in vitro* antifungal activity equipotent to amphotericin B versus *A. fumigatus* (MIC 0.98  $\mu$ g/mL), *A. clavatus* (MIC 0.98  $\mu$ g/mL) and *G. candidium* (MIC 0.49  $\mu$ g/mL). Also, bithiazolidinones **14** and **15** were equipotent to amphotericin B in inhibiting the growth of *A. clavatus* (MIC 0.98  $\mu$ g/mL) and *G. candidium* (MIC 0.49  $\mu$ g/mL). Structures of the newly synthesized compounds were established by elemental analysis and spectral data.

**Keywords:** thiazole, bithiazolidinone, pyranothiazole, antimicrobial activity.

## INTRODUCTION

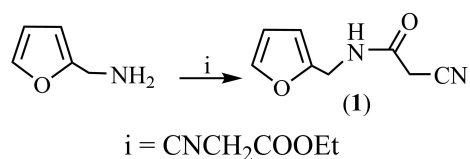
**D**ESPITE a large number of antibiotics and chemotherapeutics available for medical use, the emerging resistance to old and new antibiotics has created a substantial need for new classes of antimicrobial agents. A potential approach to overcome the problem of antibiotic resistance is to design innovative agents with different modes of action, so that no cross resistance with present drugs can occur.<sup>[1]</sup>

Thiazoles are a familiar group of heterocyclic compounds possessing a wide variety of biological activities, and their usefulness as medicines are well established. This later is also an integral part of all the available penicillins, which have revolutionized the therapy of bacterial diseases.<sup>[2]</sup> Thiazoles have also attracted continuing interest because of their varied biological activities,<sup>[3]</sup> which have found applications in the treatment of allergies,<sup>[4]</sup> hypertension,<sup>[5]</sup> inflammation,<sup>[6]</sup> schizophre-

nia,<sup>[7]</sup> microbial infections,<sup>[8,9]</sup> HIV infections,<sup>[10]</sup> hypnotics<sup>[11]</sup> and for the treatment of pain.<sup>[12]</sup> They have been also used as fibrinogen receptor antagonists with antithrombotic activity<sup>[13]</sup> and as new inhibitors of bacterial DNA gyrase B.<sup>[14]</sup>

Moreover, many biologically important derivatives of furan substituted at 2- and 5-positions are frequently observed in nature show broad-spectrum phytocidal, antibacterial and insecticidal activities<sup>[15,16]</sup> and exhibit pharmacological properties which serves as anti-depressant and anti-inflammatory agents.<sup>[17,18]</sup>

In view of the above-mentioned findings, and as a continuation of our effort to identify new candidates,<sup>[19–26]</sup> which are crucial in designing new, potent, selective, and less toxic antimicrobial agents, its report herein the synthesis and antimicrobial evaluation of some novel structure hybrids incorporating both the thiazole ring system with furan moiety. This combination was suggested in an attempt to investigate the influence of such



**Scheme 1.** Synthesis of 2-cyano-*N*-(furan-2-ylmethyl)acetamide.

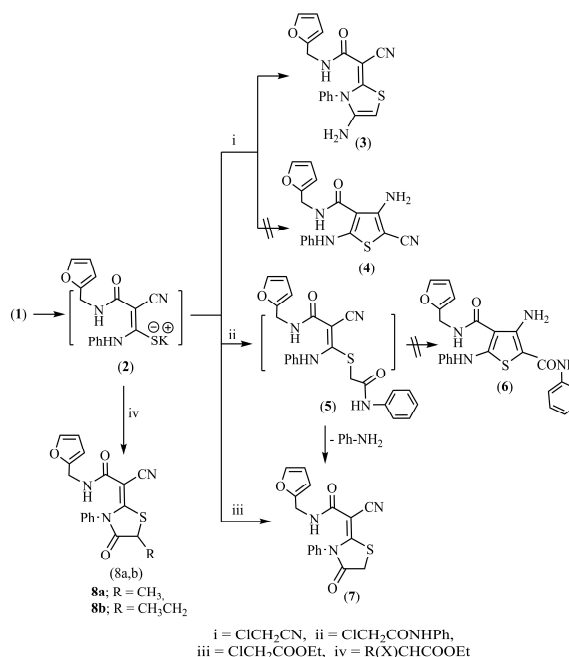
hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules. The target compounds were rationalized so as to comprise some pharmacophores that are believed to be responsible for the biological activity of some relevant chemotherapeutic such as the carboxamido, amino and cyano groups functionalities. The substitution pattern of the thiazole ring was carefully selected in order to confer different electronic environment to the molecules.

## RESULTS AND DISCUSSION

### Chemistry

The starting compound 2-cyano-*N*-(furan-2-ylmethyl)acetamide (**1**) was readily available from the solvent-free reaction of furan-2-ylmethanamine with an excess amount of ethyl cyanoacetate under fusion condition,<sup>[27]</sup> (Scheme 1).

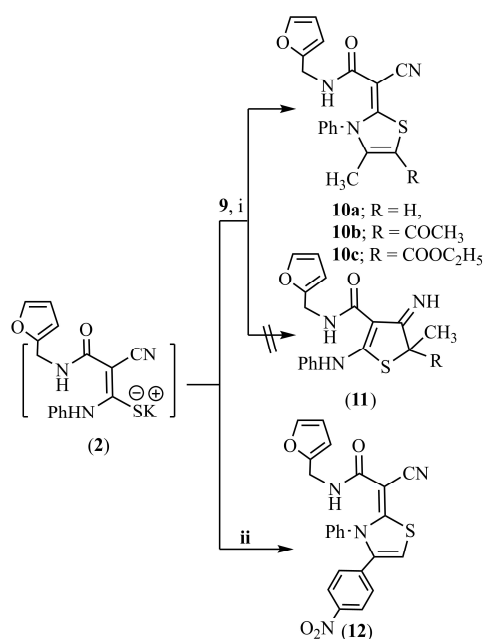
The active methylene moiety of 2-cyano-*N*-(furan-2-ylmethyl)acetamide (**1**) was allowed to react with phenyl isothiocyanate in dimethylformamide in the presence of an equimolar amount of potassium hydroxide yielded the non-isolable intermediate potassium sulphide salt **2**, which reacted in situ with chloroacetonitrile to afford the novel 4-aminothiazole derivative **3** rather than thiophene structure **4**. The reaction product **3** was confirmed on the basis of elemental analysis and spectral data. The IR spectrum of **3** showed absorption bands at 3423, 3308, 3110, 2184 and 1637  $\text{cm}^{-1}$  assignable for  $\text{NH}_2$ , NH, CN and carboxamide groups, respectively. While its  $^1\text{H}$  NMR spectrum showed doublet and singlet signals at  $\delta$  4.27, 7.16 ppm characteristic for  $\text{CH}_2$  and thiazole-H5 protons, doublet and triplet signals at  $\delta$  6.14, 6.34 ppm can be assigned to furan protons, in addition to singlet and triplet signals at  $\delta$  8.22, 10.08 ppm for  $\text{NH}_2$  and NH protons, respectively. Also, it has been found that, the in situ reaction of **2** with 2-chloro-*N*-phenylacetamide afforded thiazolidin-4-one **7** rather than the expected thiophene **6**. The formation of thiazolidin-4-one **7** may be rationalized through the first *S*-alkylation to form non-isolable intermediate **5** followed by nucleophilic addition of NH group to the amidic carbonyl with elimination of aniline.  $^1\text{H}$  NMR spectrum of compound



**Scheme 2.** Synthetic route to thiazoles.

**7** was characterized by the presence of a singlet signal at  $\delta$  3.92 ppm for thiazole methylene protons.  $^{13}\text{C}$  NMR of compound **7** revealed signals at  $\delta$  34.70 (C5 of thiazole), 36.32 ( $\text{CH}_2$ ), 165.95, 167.77 (C4, C2 of thiazole), in addition to, signals at 107.35, 110.85, 138.39 and 142.37 ppm that are characteristic for furan carbons. The structure of the isolated product **7** was elucidated also, by independent synthesis via treatment of the intermediate **2** with ethyl chloroacetate. Cycloalkylation of non-isolable salt **2** with either ethyl  $\alpha$ -chloropropionate or ethyl  $\alpha$ -bromobutyrate produced in each case, a single product. The reaction products can be formulated as thiazolidin-4-one derivatives **8a** and **8b**, evidence for assigned structures being provided by analytical and spectroscopic data. For example, the IR spectrum of compound **8a** showed the appearance of absorption band at 3326, 2203, 1733 and 1646  $\text{cm}^{-1}$  corresponding to the NH, CN, ( $\text{C}=\text{O}$ ; thiazolidinone) and carboxamide groups, respectively. The  $^1\text{H}$  NMR spectrum of compound **8a** exhibited signals at  $\delta$  1.56 (d, 3H,  $\text{CH}_3$ ), 4.25 (q, 1H, CH), 4.33 (d, 2H,  $\text{CH}_2$ ) with triplet signal at  $\delta$  7.95 ppm for the amide group proton. The formation of **8** was assumed to proceed via an initial alkylation followed by intramolecular cyclization with ethanol elimination (Scheme 2).

Next, treatment of the intermediate **2**, formed in situ under the same reaction condition, with  $\alpha$ -halogenated compounds **9** produced in each case a single product identified as 4-methylthiazole derivatives **10** instead of the thiophene derivative **11** (Scheme 3). The structure of the 4-methylthiazoles **10** was established by analytical and

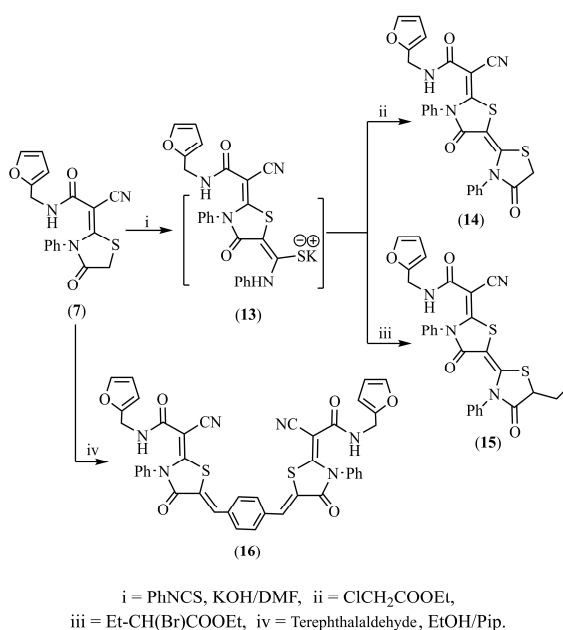


i = R-CH(Cl)COCH<sub>3</sub>, ii = 2-bromo-1-(4-nitrophenyl)ethanone

Scheme 3. Synthetic route to thiazoles.

spectroscopic data. The IR spectrum of compound **10a** showed the appearance of absorption band at 3367, 2170 and 1646 cm<sup>-1</sup> corresponding to the imino, nitrile and carboxamide groups, respectively. The <sup>1</sup>H NMR spectrum of compound **10b** was characteristic by the presence of singlet signals at δ 2.09, 2.30 for 2CH<sub>3</sub> with triplet signal at δ 9.67 ppm for NH protons. <sup>13</sup>C NMR of compound **10b** revealed signals at δ 14.31, 29.95 (2CH<sub>3</sub>), 36.10 (CH<sub>2</sub>), 115.06, 145.35, 170.21 (thiazole-carbons), in addition to two singlet at δ 163.96 and 165.22 ppm (2C=O). Similarly, cyclocondensation of the intermediate salt **2** with 2-bromo-1-(4-nitrophenyl)ethanone at room temperature afforded the corresponding 2,3-dihydrothiazole derivative **12** (Scheme 3). The structure of the latter product was confirmed by the presence of singlet signal at δ 6.87 ppm assignable for thiazole-H5 in <sup>1</sup>H NMR spectrum.

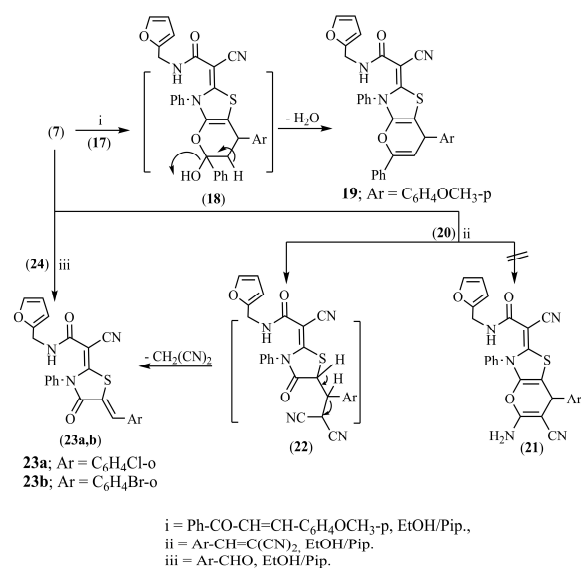
To investigate the structure reactivity relationship with respect to antimicrobial properties, the reactivity of 4-thiazolidinone **7** toward some electrophilic reagents was investigated and bithiazolidinone derivatives were obtained. Thus, the non-isolable potassium sulphide salt **13** was achieved by the nucleophilic addition of active methylene group in 4-thiazolidinone **7** to phenyl isothiocyanate in dry dimethylformamide at room temperature in the presence of potassium hydroxide (Scheme 4). Cycloalkylation of non-isolable salt **13** with ethyl chloroacetate and/or ethyl α-bromobutyrate at room temperature gave bithiazolidinone derivatives **14** and **15**.



Scheme 4. Synthetic route to bithiazoles.

<sup>13</sup>C NMR of compound **14** revealed signals at δ 31.65 (CH<sub>2</sub>), 106.60 (thiazole-C5'), 163.63, 166.12 (thiazole-C4, C4'), in addition to singlet signal at 173.30 ppm (C=O). Furthermore, the Knoevenagel condensation of thiazolidinone **7** with terephthalaldehyde in ethanolic piperidine solution under reflux conditions afforded the corresponding bithiazolidinone derivative **16** (Scheme 4).

In addition, pyrano[2,3-*d*]thiazoles are useful in the treatment of diseases such as diabetes, obesity, hyperlipidemia, and atherosclerotic diseases.<sup>[28]</sup> They are also known to show bactericidal, fungicidal and molluscicidal activities.<sup>[29,30]</sup> Therefore, pyrano[2,3-*d*]thiazole of type **19** was obtained by reaction between equimolecular amounts of compound **7** and chalcone derivative **17** in the presence of a catalytic amount of piperidine. The structure formula of compound **19** resulting from this reaction has been confirmed using elemental analyses and spectral methods. <sup>1</sup>H NMR spectrum of compound **19** showed a new two doublet and singlet signals at δ 3.43, 4.94, 3.84 ppm corresponding to H4, H5 of pyran and methoxy protons, in addition to triplet signal at 8.20 ppm assignable for NH proton. The formation of **19** was assumed to proceed via Michael addition of the active methylene to the α,β-unsaturated ketone **17** to yield the Michael adduct **18** followed by intramolecular cyclization with elimination of water. In contrast to the anticipated formation of pyrano[2,3-*d*]thiazole derivative **19**, the reaction of **7** with α-cinnamionitriles **20a,b** in refluxing ethanol and in the presence of a catalytic amount of piperidine, the corresponding benzylidene thiazolidinone



**Scheme 5.** Synthetic route to pyrano[2,3-*d*]thiazole.

derivative **23** was obtained. The formation of this compound was assumed to proceed via retro Michael addition through the elimination of malononitrile from the non-isolable adduct **22**. The structure of the isolated products **23a,b** were elucidated on the basis of their spectral data (IR, MS and  $^1\text{H}$  NMR) and also, by independent synthesis via reaction of the thiazolidinone **7** with benzaldehyde derivatives **24** (Scheme 5).

## Antimicrobial Evaluation

Fourteen of the newly synthesized target compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024) and *Bacillus subtilis* (RCMB 010063) as examples of Gram-positive bacteria and *Pseudomonas aeruginosa* (RCMB 010043), *Proteus vulgaris* (RCMB 010085) and *Klebsiella pneumonia* (RCMB 010093) as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Aspergillus fumigatus*, *Aspergillus clavatus* and *Geotricum candidium* fungal strain.

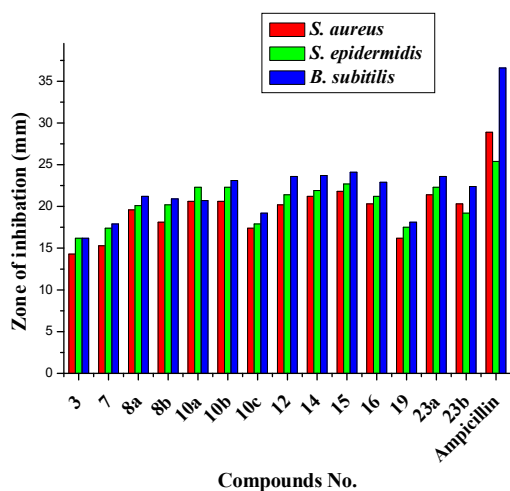
Agar-diffusion method<sup>[31]</sup> was used for the determination of the preliminary antibacterial and antifungal activity. Ampicillin, gentamycin and amphotericin B were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds with significant growth inhibition zones using twofold serial dilution method.<sup>[32]</sup> The MIC ( $\mu\text{g/mL}$ ) and inhibition zone diameters values are presented 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.

Regarding the structure reactivity relationship between the thiazoles and antibacterial activities, the

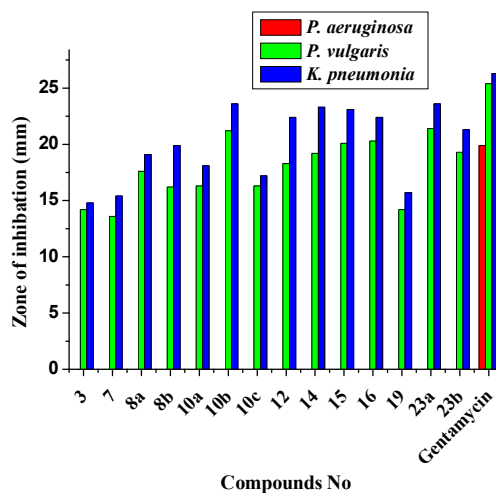
**Table 1.** Antimicrobial inhibition zone in mm and minimal inhibitory concentrations (MIC,  $\mu\text{g/ml}$ , between brackets) of some new synthesized compounds

Compounds no.	Gram-positive bacteria			Gram-negative bacteria			Fungi		
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>P. vulgaris</i>	<i>K. pneumonia</i>	<i>A. fumigatus</i>	<i>A. clavatus</i>	<i>G. candidium</i>
<b>3</b>	14.3	16.2	16.2	NA*	14.2	14.8	14.6	14.9	15.7
<b>7</b>	15.3	17.4	17.9	NA	13.6	15.4	12.3	14.2	14.9
<b>8a</b>	19.6	20.1	21.2	NA	17.6	19.1	17.2	19.6	20.9
<b>8b</b>	18.1	20.2	20.9	NA	16.2	19.9	16.3	18.2	20.3
<b>10a</b>	20.6	22.3	20.7	NA	16.3	18.1	16.3	19.2	19.6
<b>10b</b>	20.6	22.3	23.1	NA	21.2	23.6	18.6	19.2	20.9
<b>10c</b>	17.4	17.9	19.2	NA	16.3	17.2	13.3	15.2	17.3
<b>12</b>	20.2(3.9)	21.4(1.95)	23.6(0.98)	NA	18.3(7.81)	22.4(0.98)	18.2(7.81)	21.4(1.95)	23.2(0.98)
<b>14</b>	21.2(1.95)	21.9(0.98)	23.7(0.48)	NA	19.2(3.9)	23.3(0.98)	20.3(3.9)	22.3(0.98)	23.6(0.49)
<b>15</b>	21.8(1.95)	22.7(0.98)	24.1(0.48)	NA	20.1(3.9)	23.1(0.98)	19.7(3.9)	22.7(0.98)	23.8 (0.49)
<b>16</b>	20.3(3.9)	21.2(1.95)	22.9(0.98)	NA	20.3(3.9)	22.4(0.98)	20.1(3.9)	21.9(0.98)	23.5(0.98)
<b>19</b>	16.2	17.5	18.1	NA	14.2	15.7	14.3	16.2	15.4
<b>23a</b>	21.4(1.95)	22.3(0.98)	23.6(0.49)	NA	21.4(1.95)	23.6(0.49)	22.3(0.98)	22.7(0.98)	23.9(0.98)
<b>23b</b>	20.3(1.93)	19.2(0.98)	22.4(0.49)	NA	19.3(1.95)	21.3(0.49)	21.1(0.98)	20.7(0.98)	22.9(0.98)
Ampicillin	28.9(0.24)	25.4(0.49)	36.6(0.24)	NA*	NT	NT	NT	NT	NT
Gentamycin	NT*	NT	NT	19.9(3.9)	25.4(0.98)	26.3(0.49)	NT	NT	NT
Amphotericin B	NT	NT	NT	NT	NT	NT	23.7(0.98)	21.9(0.98)	25.4(0.49)

\*NA: no activity, \*NT: not tested



**Figure 1.** Antibacterial activity of the synthesized compounds against Gram-positive bacteria.



**Figure 2.** Antibacterial activity of the synthesized compounds against Gram-negative bacteria.

results showed that thiazole benzylidene derivatives **23a**, **23b** and bithiazolidinone derivatives **14–16** were the most active among the synthesized compounds (Figures 1 and 2). Thiazoles **10b**, **10c** and **12** containing electron withdrawing groups such as  $\text{COCH}_3$ ,  $\text{CO}_2\text{C}_2\text{H}_5$  and  $\text{NO}_2$  recorded moderate to good activity. In this view, compounds **23a** and **23b** were equipotent to gentamycin against *K. pneumonia* (MIC 0.49  $\mu\text{g}/\text{mL}$ ), while their activity were 50 % lower than both gentamycin and ampicillin in inhibiting the growth of *P. vulgaris* (MIC 1.95  $\mu\text{g}/\text{mL}$ ), *S. epidermidis* (MIC 0.98  $\mu\text{g}/\text{mL}$ ) and *B. subtilis* (MIC 0.49  $\mu\text{g}/\text{mL}$ ). Bithiazolidinone derivatives **14** and **15** showed 50 % of the activity of ampicillin and gentamycin against *S. epidermidis* (MIC 0.98  $\mu\text{g}/\text{mL}$ ), *B. subtilis* (MIC 0.49  $\mu\text{g}/\text{mL}$ ). Bithiazolidinone **20** showed relatively good growth inhibitory profiles against *K. pneumonia* (MIC 0.98  $\mu\text{g}/\text{mL}$ ) which was about 50 % of the activity of gentamycin. The 4-nitrophenylthiazole **12** showed relatively good growth inhibitory profiles against *B. subtilis* (MIC 0.98  $\mu\text{g}/\text{mL}$ ) and *K. pneumonia* (MIC 0.98  $\mu\text{g}/\text{mL}$ ), which was about 50 % of the activity of ampicillin and gentamycin. Concerning the antibacterial activity of the compounds **3**, **7**, **8a**, **8b**, **10a**, **10c** and **19** showed weak activities against the tested Gram-positive and Gram-negative bacteria. Unfortunately, all the tested compounds were completely inactive toward *P. aeruginosa* (RCMB 010043) compared to gentamycin. Gram negative bacteria have a relatively thin cell wall constituted by few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. These differences in cell wall structure can produce differences in antibacterial susceptibility and some antibiotics are ineffective against Gram-negative pathogens. [33–35]

Regarding the activity of thiazoles incorporating furan moiety versus antifungal strains, thiazole benzylidene **23a** and **23b** showed *in vitro* antifungal activity equipotent to amphotericin B against *A. fumigatus* (MIC 0.98  $\mu\text{g}/\text{mL}$ ), *A. clavatus* (MIC 0.98  $\mu\text{g}/\text{mL}$ ) and *G. candidium* (MIC 0.49  $\mu\text{g}/\text{mL}$ ). Bithiazolidinones **14** and **15** were equipotent to amphotericin B in inhibiting the growth of *A. clavatus* (MIC 0.98  $\mu\text{g}/\text{mL}$ ) and *G. candidium* (MIC 0.49  $\mu\text{g}/\text{mL}$ ). But their activities were 25 % of amphotericin B against *A. fumigatus* (MIC 3.9  $\mu\text{g}/\text{mL}$ ). indeed, Bithiazolidinones **16** displayed equipotent activity to amphotericin B versus *A. clavatus* (MIC 0.98  $\mu\text{g}/\text{mL}$ ) while its activity was 50 % lower than *G. candidium* (MIC 0.98  $\mu\text{g}/\text{mL}$ ) compared to amphotericin B. 4-Nitrophenylthiazole **12** showed relatively good growth inhibitory profiles versus *A. clavatus* (MIC 1.95  $\mu\text{g}/\text{mL}$ ) and *G. candidium* (MIC 0.98  $\mu\text{g}/\text{mL}$ ) which was about 50 % of the activity of amphotericin B. Other synthesized compounds showed weak to moderate activity against antifungal strains (Figure 3).

## CONCLUSION

The objective of the present study was to synthesize and investigate the antimicrobial activities of some new thiazoles incorporating furan moiety with the hope of discovering new structure leads serving as potent antimicrobial agents. Our aim has been verified by the synthesis of thiazole, bithiazoles, thiazole benzylidene and pyranothiazole derivatives. It is worth mentioning that, synthesis of thiazole derivative containing electron withdrawing groups such as **10b**, **10c** and **12** exhibited good antimicrobial activity. The best antimicrobial activity was

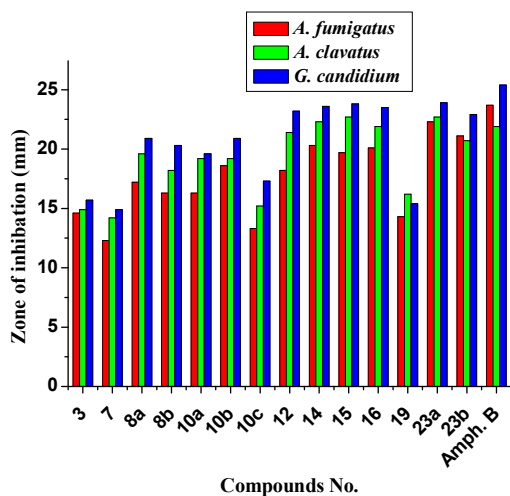


Figure 3. Antifungal activity of the synthesized compounds.

observed when 4-thiazolidinone **7** was converted into bithiazoles **14–16** or thiazole benzylidene derivatives **23a**, **23b**.

## EXPERIMENTAL

All the melting points are uncorrected. IR (KBr) spectra were measured on Shimadzu 440 spectrometer;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were obtained in DMSO on a Varian Gemini 300 MHz spectrometer using TMS as internal standard. The chemical shifts are reported as  $\delta_{\text{ppm}}$  units. Mass spectra were obtained on GCMS\QP 1000 Ex mass spectrometer at 70 eV. Elemental analyses were carried out at the Department of Chemistry, Faculty of Science, Cairo University, Egypt. Microbiology screening was carried out in the Regional Center for Microbiology and Biotechnology (RCMB), Antimicrobial unit test organisms, Al-azhr University, Cairo, Egypt.

### General procedure for the preparation of compounds **3**, **7**, **8a,b**, **10a–c**, **12**, **14** and **15**:

To suspension of finely powdered potassium hydroxide (0.01 mol) in dry dimethylformamide (20 mL) the active methylene compound (**1** and/or **7**, 0.01 mol) and then the phenyl isothiocyanate (0.01 mol) were added in portions. The reaction mixture was stirred at room temperature for 1 h and then treated with  $\alpha$ -halogenated compound (0.01 mol) and left at room temperature for 2 h; then it was poured into ice/water and acidified with 0.1 M HCl at pH 3–4. The resulting precipitate was filtered off, dried, and recrystallized from the proper solvent.

### 2-(4-Amino-3-phenylthiazol-2(3H)-ylidene)-2-cyano-N-(furan-2-ylmethyl)acetamide (**3**)

Brown crystals (AcOH), Yield (50 %), m.p. 229–230°C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3423, 3308, 3110 (NH<sub>2</sub>/NH), 3058 (arom. CH), 2184 (C≡N) and 1637 (C=O; amide);  $^1\text{H}$  NMR (DMSO-*d*<sub>6</sub>)  $\delta/\text{ppm}$ : 4.27 (d, 2H, CH<sub>2</sub>-NH, *J* = 6 Hz), 6.14 (d, 1H, furan-H3, *J* = 3 Hz), 6.34 (t, H, furan-H4, *J* = 3 Hz), 7.16 (s, 1H, thiazole-H5), 7.30–7.60 (m, 6H, Ar-H + furan-H5), 8.22 (s, 2H, NH<sub>2</sub>), 10.08 (t, 1H, O=C-NH-CH<sub>2</sub>, *J* = 6 Hz). MS (EI, 70 eV) *m/z* (%): 338 (M<sup>+</sup>, 55), 309 (15.2), 242 (35), 215 (39.7), 135 (72.5), 64 (100) 51 (34.3). Anal. Calcd. for C<sub>17</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>S (338.38): C, 60.34; H, 4.17; N, 16.56. Found: C, 60.14; H, 4.20; N, 16.39 %.

### 2-Cyano-N-(furan-2-ylmethyl)-2-(4-oxo-3-phenylthiazolidin-2-ylidene)acetamide (**7**)

White crystals (EtOH), Yield (80 %), m.p. 108–110 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3339 (NH), 3036 (arom. CH), 2206 (C≡N), 1719 (C=O; thiazolidinone) and 1645 (C=O; amide);  $^1\text{H}$  NMR (DMSO-*d*<sub>6</sub>)  $\delta/\text{ppm}$ : 3.92 (s, 2H, CH<sub>2</sub>-thiazole), 4.27 (d, 2H, CH<sub>2</sub>-NH, *J* = 6 Hz), 6.14 (d, 1H, furan-H3, *J* = 3 Hz), 6.36 (t, H, furan-H4, *J* = 3 Hz), 7.23–8.17 (m, 6H, Ar-H + furan-H5), 8.17 (t, 1H, O=C-NH-CH<sub>2</sub>, *J* = 6 Hz);  $^{13}\text{C}$  NMR (DMSO-*d*<sub>6</sub>)  $\delta/\text{ppm}$ : 34.70 (thiazole-C5), 36.32 (CH<sub>2</sub>), 80.90 (C-CN), 107.35, 110.58, 138.39, 142.37 (furan-C), 124.40, 127.0, 128.20, 129.72, 129.87 (Ar-C), 152.63 (CN), 165.95, 167.77 (thiazole-C4, C2), 173.85 (C=O). MS (EI, 70 eV) *m/z* (%): 339 (M<sup>+</sup>, 70), 310 (9.2), 298 (18), 265 (27), 236 (22.3), 96 (37.8), 81 (100) 53 (38.3). Anal. Calcd. for C<sub>17</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S (339.37): C, 60.17; H, 3.86; N, 12.38. Found: C, 60.31; H, 3.66; N, 12.20 %.

### 2-Cyano-N-(furan-2-ylmethyl)-2-(5-methyl-4-oxo-3-phenylthiazolidin-2-ylidene)acetamide (**8a**)

White crystals (AcOH), Yield (60 %), m.p. 190–192 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3326 (NH), 3060 (arom. CH), 2915 (aliph. CH), 2203 (C≡N), 1733 (C=O; thiazolidinone) and 1646 (C=O; amide);  $^1\text{H}$  NMR (DMSO-*d*<sub>6</sub>)  $\delta/\text{ppm}$ : 1.56 (d, 3H, CH<sub>3</sub>, *J* = 9 Hz), 4.25 (q, 1H, CH, *J* = 9 Hz), 4.33 (d, 2H, CH<sub>2</sub>-NH, *J* = 6 Hz), 6.16 (d, 1H, furan-H3, *J* = 3 Hz), 6.35 (t, 1H, furan-H4, *J* = 3 Hz), 7.38–7.51 (m, 6H, Ar-H + furan-H5), 7.95 (t, 1H, O=C-NH-CH<sub>2</sub>, *J* = 6 Hz). MS (EI, 70 eV) *m/z* (%): 353 (M<sup>+</sup>, 67), 248 (33.2), 229 (58.5), 203 (17), 142 (22.3), 77 (100) 53 (65.3). Anal. Calcd. for C<sub>18</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>S (353.39): C, 61.18; H, 4.28; N, 11.89. Found: C, 60.98; H, 4.15; N, 11.72 %.

### 2-Cyano-2-(5-ethyl-4-oxo-3-phenylthiazolidin-2-ylidene)-N-(furan-2-ylmethyl)acetamide (**8b**)

White crystals (AcOH), Yield (55 %), m.p. 199–200 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3321 (NH), 3040 (arom. CH), 2916 (aliph. CH), 2203 (C≡N), 1732 (C=O; thiazolidinone) and 1646 (C=O; amide);  $^1\text{H}$  NMR (DMSO-*d*<sub>6</sub>)  $\delta/\text{ppm}$ : 1.03 (t, 3H, CH<sub>3</sub>, *J* = 6 Hz), 1.96 (p, 2H, CH<sub>2</sub>, *J* = 6 Hz), 4.19 (t, 1H, CH, *J* = 6 Hz), 4.27



(d, 2H,  $\text{CH}_2\text{-NH}$ ,  $J = 6$  Hz), 6.17 (d, 1H, furan-H3,  $J = 3$  Hz), 6.35 (t, 1H, furan-H4,  $J = 3$  Hz), 7.35–7.51 (m, 6H, Ar-H + furan-H5), 7.94 (t, 1H,  $\text{O=C-NH-CH}_2$ ,  $J = 6$  Hz). MS (EI, 70 ev)  $m/z$  (%): 367( $\text{M}^+$ , 87), 338 (7), 271 (37.2), 265 (5.5), 243 (21.5), 217 (12), 96 (50.3), 80 (100), 64 (82), 53 (25). Anal. Calcd. for  $\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}_3\text{S}$  (367.42): C, 62.11; H, 4.66; N, 11.44. Found: C, 61.90; H, 4.46; N, 11.60 %.

**2-Cyano-N-(furan-2-ylmethyl)-2-(4-methyl-3-phenylthiazol-2(3H)-ylidene)acetamide (10a)**

White powder (EtOH), Yield (70 %), m.p. 240–241 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3376 (NH), 3046 (arom. CH), 2926 (aliph. CH), 2170 ( $\text{C}\equiv\text{N}$ ) and 1646 ( $\text{C=O}$ ; amide);  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ )  $\delta/\text{ppm}$ : 1.80 (s, 3H,  $\text{CH}_3$ ), 4.29 (d, 2H,  $\text{CH}_2\text{-NH}$ ,  $J = 6$  Hz), 6.12 (d, 1H, furan-H3,  $J = 3$  Hz), 6.33 (t, 1H, furan-H4,  $J = 3$  Hz), 6.80 (s, 1H, thiazole-H5), 7.12–7.56 (m, 7H, Ar-H + furan-H5 + NH). MS (EI, 70 ev)  $m/z$  (%): 337( $\text{M}^+$ , 100), 308 (6), 241 (78.5), 214 (45), 189 (57), 118 (30.3), 96(18), 77 (64), 53 (78). Anal. Calcd. for  $\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}_2\text{S}$  (337.40): C, 64.08; H, 4.48; N, 12.45. Found: C, 64.00; H, 4.55; N, 12.33 %.

**2-(5-Acetyl-4-methyl-3-phenylthiazol-2(3H)-ylidene)-2-cyano-N-(furan-2-ylmethyl)acetamide (10b)**

White powder (AcOH), Yield (66 %), m.p. 221–222 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3316 (NH), 3061 (arom. CH), 2900 (aliph. CH), 2190 ( $\text{C}\equiv\text{N}$ ), 1699 ( $\text{C=O}$ ) and 1617 ( $\text{C=O}$ ; amide);  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ )  $\delta/\text{ppm}$ : 2.09 (s, 3H,  $\text{CH}_3$ ), 2.30 (s, 3H,  $\text{COCH}_3$ ), 4.29 (d, 2H,  $\text{CH}_2\text{-NH}$ ,  $J = 6$  Hz), 6.15 (d, 1H, furan-H3,  $J = 3$  Hz), 6.34 (t, 1H, furan-H4,  $J = 3$  Hz), 7.45–7.66 (m, 6H, Ar-H + furan-H5), 9.67 (t, 1H,  $\text{O=C-NH-CH}_2$ ,  $J = 6$  Hz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ )  $\delta/\text{ppm}$ : 14.31 ( $\text{CH}_3$ ), 29.95 ( $\text{COCH}_3$ ), 36.10 ( $\text{CH}_2$ ), 68.83 ( $\text{C-CN}$ ), 103.52, 110.32, 141.59, 141.74 (furan-C), 115.06, 145.35, 170.21 (thiazole-C), 128.90, 129.30, 129.77, 131.23, 135.97 (ph-C), 152.80 (CN), 163.96, 165.22 (2 $\text{C=O}$ ). MS (EI, 70 ev)  $m/z$  (%): 379 ( $\text{M}^+$ , 35), 350 (7), 283 (12.2), 265 (9), 241 (7.5), 231 (15), 142 (4.3), 80 (100), 64 (85), 53 (10). Anal. Calcd. for  $\text{C}_{20}\text{H}_{17}\text{N}_3\text{O}_5\text{S}$  (379.43). C, 63.31; H, 4.52; N, 11.07. Found: C, 63.20; H, 4.45; N, 10.95 %.

**Ethyl 2-(1-cyano-2-((furan-2-ylmethyl)amino)-2-oxoethylidene)-4-methyl-3-phenyl-2,3-dihydrothiazole-5-carboxylate (10c)**

White crystals (Dioxane), Yield (55 %), m.p. 231–232 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3410 (NH), 3066 (arom. CH), 2920 (aliph. CH), 2183 ( $\text{C}\equiv\text{N}$ ), 1703 ( $\text{C=O}$ ; ester) and 1633 ( $\text{C=O}$ ; amide);  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ )  $\delta/\text{ppm}$ : 1.29 (t, 3H,  $\text{CH}_3$ ,  $J = 6$  Hz), 2.12 (s, 3H,  $\text{CH}_3$ ), 3.45 (q, 2H,  $\text{CH}_2$ ,  $J = 6$  Hz), 4.30 (d, 2H,  $\text{CH}_2\text{-NH}$ ,  $J = 6$  Hz), 6.15 (d, 1H, furan-H3,  $J = 3.3$  Hz), 6.35 (t, 1H, furan-H4,  $J = 3.5$  Hz), 7.44–7.62 (m, 7H, Ar-H + furan-H5 + NH). MS (EI, 70 ev)  $m/z$  (%): 409 ( $\text{M}^+$ , 100), 380 (7), 285 (47.5), 261 (57), 212 (15), 169 (15.3), 118 (22), 77 (88), 53 (78). Anal. Calcd. for  $\text{C}_{21}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$  (409.46): C, 61.60; H, 4.68; N, 10.26. Found: C, 61.50; H, 4.57; N, 10.17 %.

**2-Cyano-N-(furan-2-ylmethyl)-2-(4-(4-nitrophenyl)-3-phenylthiazol-2(3H)ylidene)acetamide (12)**

Yellow powder (EtOH), Yield (60 %), m.p. 210–211 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3336 (NH), 3080 (arom. CH), 2934 (aliph. CH), 2189 ( $\text{C}\equiv\text{N}$ ) and 1637 ( $\text{C=O}$ ; amide);  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ )  $\delta/\text{ppm}$ : 4.32 (d, 2H,  $\text{CH}_2\text{-NH}$ ,  $J = 6$  Hz), 6.15 (d, 1H, furan-H3,  $J = 3.5$  Hz), 6.35 (t, 1H, furan-H4,  $J = 3$  Hz), 6.87(s, 1H, thiazole-H5), 7.09–8.19 (m, 11H, Ar-H + furan-H5 + NH). MS (EI, 70 ev)  $m/z$  (%): 444( $\text{M}^+$ , 48), 364 (60), 321 (52.5), 302 (37), 274 (39), 134 (5.3), 89 (15), 80 (82), 64 (100). Anal. Calcd. for  $\text{C}_{23}\text{H}_{16}\text{N}_4\text{O}_4\text{S}$  (444.46): C, 62.15; H, 3.63; N, 12.61. Found: C, 62.04; H, 3.80; N, 12.51.

**2-Cyano-2-(4,4'-dioxo-3,3'-diphenyl-3',4,4',5-tetrahydro-2'H,3H-[2,5'-bi-thiazolylidene]-2'-ylidene)-N-(furan-2-ylmethyl)acetamide (14)**

Beige crystals (AcOH), Yield (45 %), m.p. 285–286 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3373 (NH), 3054 (arom. CH), 2932 (aliph. CH), 2190 ( $\text{C}\equiv\text{N}$ ), 1730 ( $\text{C=O}$ ; thiazolidinone) and 1646 ( $\text{C=O}$ ; amide);  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ )  $\delta/\text{ppm}$ : 4.10 (s, 2H, thiazole- $\text{CH}_2$ ), 4.17 (d, 2H,  $\text{CH}_2\text{-NH}$ ,  $J = 6$  Hz), 6.08 (d, 1H, furan-H3,  $J = 3.5$  Hz), 6.33 (t, 1H, furan-H4,  $J = 3$  Hz), 7.26–8.18 (m, 11H, Ar-H + furan-H5), 9.75 (t, 1H,  $\text{O=C-NH-CH}_2$ ,  $J = 6$  Hz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ )  $\delta/\text{ppm}$ : 31.65 (thiazole- $\text{CH}_2$ ), 35.90 ( $\text{CH}_2$ ), 73.63 ( $\text{C-CN}$ ), 106.60 (thiazole- $\text{C5}'$ ), 110.35, 152.24, 153.23 (furan-C), 113.90 (CN), 129.81, 129.92, 130.32, 133.86, 134.93 (Ar-C), 161.99 (thiazole-C2), 163.63, 166.12 (thiazole-C4, C4'), 173.30 ( $\text{C=O}$ ). MS (EI, 70 ev)  $m/z$  (%): 514( $\text{M}^+$ , 36), 434 (50.2), 391 (21.5), 221 (78), 197 (68), 103 (43), 77 (100), 64 (40). Anal. Calcd. for  $\text{C}_{26}\text{H}_{18}\text{N}_4\text{O}_4\text{S}_2$  (514.58): C, 60.69; H, 3.53; N, 10.89. Found: C, 60.49; H, 3.40; N, 10.95 %.

**2-Cyano-2-(5-ethyl-4,4'-dioxo-3,3'-diphenyl-3',4,4',5-tetrahydro-2'H,3H-[2,5'-bithiazolylidene]-2'-ylidene)-N-(furan-2-ylmethyl)acetamide(15)**

Beige crystals (AcOH), Yield (45 %), m.p. 279–280 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3340 (NH), 3025 (arom. CH), 2910 (aliph. CH), 2185 ( $\text{C}\equiv\text{N}$ ), 1732 ( $\text{C=O}$ ; thiazolidinone) and 1646 ( $\text{C=O}$ ; amide);  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ )  $\delta/\text{ppm}$ : 1.03 (t, 3H,  $\text{CH}_3$ ,  $J = 6$  Hz), 2.04 (p, 2H,  $\text{CH}_2$ ,  $J = 6$  Hz), 4.17 (d, 2H,  $\text{CH}_2\text{-NH}$ ,  $J = 6$  Hz), 4.39 (t, 1H, CH,  $J = 6$  Hz), 6.08 (d, 1H, furan-H3,  $J = 3.3$  Hz), 6.33 (t, 1H, furan-H4,  $J = 3$  Hz), 7.35–7.69 (m, 12H, Ar-H + furan-H5 + NH). MS (EI, 70 ev):  $m/z$  (%) = 542 ( $\text{M}^+$ , 25.5), 462 (23), 278 (12.5), 249 (100), 234 (8), 179 (28), 147 (32), 103 (69), 77 (100), 64 (34). Anal. Calcd. for  $\text{C}_{28}\text{H}_{22}\text{N}_4\text{O}_4\text{S}_2$  (542.63): C, 61.98; H, 4.09; N, 10.33. Found: C, 61.77; H, 3.95; N, 10.21 %.

**2,2'-(1,4-Phenylenebis(methanylylidene))bis(4-oxo-3-phenylthiazolidine-5,2-diylidene))bis(2-cyano-N-(furan-2-ylmethyl)acetamide) (16)**

To a solution of **7** (0.02 mol) in ethanol (30 mL), terephthalaldehyde (0.01 mol) and piperidine (0.5 mL) were added and the mixture was heated under reflux for 1

h; the solid product which was produced on heating was collected and recrystallized from dioxane as red solid. Yield (45 %), m.p. 290–292 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3341 (br, NH), 3028 (arom. CH), 2926 (aliph. CH), 2196 (C≡N), 1712 (C=O; thiazolidinone) and 1652 (C=O; amide);  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta_{\text{ppm}}/\text{ppm}$ : 4.34 (d, 4H, 2CH<sub>2</sub>-NH,  $J = 6$  Hz), 6.19 (d, 2H, furan-H3,  $J = 6$  Hz), 6.36 (t, 2H, furan-H4,  $J = 6$  Hz), 7.54 – 8.11 (m, 16H, Ar-H + furan-H5), 8.35 (s, 2H, =CH), 10.06 (t, 2H, 2 O=C-NH-CH<sub>2</sub>,  $J = 6$  Hz). MS (EI, 70 eV)  $m/z$  (%): 776 (M<sup>+</sup>, 28.6), 662 (8), 501 (12.5), 439 (7.3), 385 (12), 242 (8), 149 (13), 85 (45), 71 (73), 57 (100). Anal. Calcd. for C<sub>42</sub>H<sub>28</sub>N<sub>6</sub>O<sub>6</sub>S<sub>2</sub> (776.84): C, 64.94; H, 3.63; N, 10.82. Found: C, 64.72; H, 3.80; N, 10.68 %.

**2-Cyano-N-(furan-2-ylmethyl)-2-(7-(4-methoxyphenyl)-3,5-diphenyl-3,7-dihydro-2H-pyrano[2,3-d]thiazol-2-ylidene)acetamide(19)**

A mixture of **7** (0.01 mol), chalcone **21** (0.01 mol) and piperidine (0.5mL) in ethanol (30 mL) was heated under reflux for 3 h; the solid product which was produced on heating was collected and recrystallized from dioxane as yellow solid, Yield (40 %), m.p. 228-230 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3431 (br, NH), 3022 (arom. CH), 2956 (aliph. CH), 2205 (C≡N), and 1651 (C=O; amide);  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta/\text{ppm}$ : 3.43, 4.93 (2d, 2H, pyran-H4,H5,  $J = 6$  Hz), 3.84 (s, 3H, OCH<sub>3</sub>), 4.32 (d, 2H, CH<sub>2</sub>-NH,  $J = 6$  Hz), 6.09 (d, 1H, furan-H3,  $J = 6$  Hz), 6.30 (t, 1H, furan-H4,  $J = 3$  Hz), 6.85 – 7.47 (m, 16H, Ar-H + furan-H5), 8.20 (t, 1H, O=C-NH-CH<sub>2</sub>,  $J = 6$  Hz). MS (EI, 70 eV)  $m/z$  (%): 559 (M<sup>+</sup>, 37.6), 502 (6), 435 (60.5), 392 (12), 313 (5), 217 (6), 105 (13), 84 (100), 81 (84), 57 (87). Anal. Calcd. for C<sub>33</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub>S (559.63): C, 70.82; H, 4.50; N, 7.51. Found: C, 70.97; H, 4.36; N, 7.45 %.

**General Procedure for the Preparation of Compounds 23a and 23b:**

**Method A:** A mixture of **7** (0.01 mol),  $\alpha$ -cinnamitrile **20** (0.01 mol) and piperidine (0.5mL) in ethanol (30 mL) was heated under reflux for 1h; the solid product which was produced on heating was collected and recrystallized from the proper solvent to give **23**.

**Method B:** A mixture of compound **7** (0.02 mol), aromatic aldehyde (0.01 mol) and piperidine (0.01mol) in ethanol (30 mL) was heated under reflux for 1 h; the solid product which was produced on heating was collected and recrystallized from the proper solvent to give **23**.

**2-(5-(2-Chlorobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)-2-cyano-N-(furan-2-ylmethyl)acetamide (23a)**

Orange powder (AcOH), Yield (50 %), m.p. 268–270 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3325 (NH), 3060 (arom. CH), 2921 (aliph. CH), 2206 (C≡N), 1717 (C=O; thiazolidinone) and 1647 (C=O; amide);  $^1\text{H NMR}$  (DMSO- $d_6$ ):  $\delta/\text{ppm}$ : 4.31 (d, 2H, CH<sub>2</sub>-NH,  $J = 6$  Hz), 6.19 (d, 1H, furan-H3,  $J = 3$  Hz), 6.35 (t, 1H, furan-

H4,  $J = 3$  Hz), 7.55 – 7.80 (m, 10H, + Ar-H + furan-H5), 7.89 (s, 1H, =CH), 8.28 (t, 1H, O=C-NH-CH<sub>2</sub>,  $J = 6$  Hz). MS (EI, 70 eV)  $m/z$  (%): 461 (M<sup>+</sup>, 8.10), 426 (50.6), 364 (15.5), 303 (22), 265 (19.5), 168 (88), 96 (90), 77 (100), 64 (43). Anal. Calcd. for C<sub>24</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>3</sub>S (461.92): C, 62.41; H, 3.49; N, 9.10. Found: C, 62.35; H, 3.54; N, 9.00 %.

**2-(5-(2-Bromobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)-2-cyano-N-(furan-2-ylmethyl)acetamide (23b)**

Orange powder (AcOH), Yield (52 %), m.p. 265–266 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3332 (NH), 3058 (arom. CH), 2921 (aliph. CH), 2205 (C≡N), 1714 (C=O; thiazolidinone) and 1647 (C=O; amide);  $^1\text{H NMR}$  (DMSO- $d_6$ ):  $\delta/\text{ppm}$ : 4.31 (d, 2H, CH<sub>2</sub>-NH,  $J = 6$  Hz), 6.21 (d, 1H, furan-H3,  $J = 6$  Hz), 6.36 (t, 1H, furan-H4,  $J = 3$  Hz), 7.24 – 7.54 (m, 10H, + Ar-H + furan-H5), 8.11 (s, 1H, =CH), 12.26 (t, 1H, O=C-NH-CH<sub>2</sub>,  $J = 6$  Hz). MS (EI, 70 eV)  $m/z$  (%): 506 (M<sup>+</sup>, 62.5), 507 (M<sup>+</sup>, 61.5), 426 (30.6), 364 (29.5), 303 (22), 265 (19.5), 212 (30), 133 (44), 96 (93), 77 (100), 53 (61). Anal. Calcd. for C<sub>24</sub>H<sub>16</sub>N<sub>3</sub>BrO<sub>3</sub>S (506.37): C, 56.93; H, 3.18; N, 8.30. Found: C, 56.70; H, 3.00; N, 8.10 %.

**Antimicrobial Screening**

The disks of Whatman filter paper were prepared with standard size (6.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These bottles are kept into hot air oven at a temperature of 150 °C. Then, the standard sterilized filter paper disks impregnated with a solution of the test compound in DMF (100  $\mu\text{L}$ , 5 mg/mL) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard concentrations of 10<sup>6</sup> CFU/mL (Colony Forming Units/mL) and 10<sup>4</sup> CFU/mL were used for antibacterial and antifungal assay, respectively. Pyrex glass Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. The utilized test organisms were *S. aureus*, *S. epidermidis* and *B. subtilis* as examples of Gram-positive bacteria and *P. aeruginosa*, *P. vulgaris* and *K. pneumonia* as examples of Gram-negative bacteria. They were also evaluated for their in vitro antifungal potential against *A. fumigatus*, *A. clavatus* and *G. candidum* fungal strain. Ampicillin and gentamycin were used as standard antibacterial agents; while amphotericin B was used as standard antifungal agent. DMF alone was used as control at the same above-mentioned concentration and due this there was no visible change in bacterial growth. The plates were incubated at 37 °C for 24 h for bacteria and for 48 h at 25 °C for fungi. The mean zone of inhibition measured in mm  $\pm$  standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms. Compounds that showed significant growth inhibition zones using the twofold serial dilution technique were further evaluated for their minimal inhibitory concentrations (MICs).



## Minimal Inhibitory Concentration (MIC) Measurement

The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Subouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, ampicillin, gentamycin, amphotericin B and sulfoxazole were prepared in DMF at concentrations 1000 µg/mL. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500-0.007 µg/mL. 10 mL of the broth containing about 10<sup>6</sup> CFU/mL of test bacteria or 10<sup>4</sup> CFU/mL of the test fungus was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37 °C for 24 h for antibacterial activity and at 25 °C for 48 h for antifungal activity in a humid chamber. At the end of the incubation period, the minimal inhibitory concentrations (MIC) values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions.

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