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## Synthesis, antimycobacterial and antifungal evaluation of new 4-(furan-2-ylmethyl)-6-methylpyridazin-3(2H)-ones

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**Abstract:** This study reports the synthesis and evaluation of a series of new pyridazin-3-ones with furan moieties **5a-j** and **6a-f**, to test for their antimycobacterial and antifungal activities. The structures of the target compounds were confirmed by elemental analysis and spectroscopic techniques (IR, mass, <sup>1</sup>H- and <sup>13</sup>C-NMR). Amongst the compounds tested, **5e**, **5g**, **5i** and **6e** exhibited highest activity against *Mycobacterium tuberculosis*, while **5h**, **6d** and **6f** showed moderate *in vitro* antifungal activities against *C. albicans* and *C. parapsilosis*.

**Keywords:** pyridazinone; furan; biological activity.

### INTRODUCTION

Tuberculosis (TB), the main leading infectious killer globally, is a contagious disease caused by *Mycobacterium tuberculosis* (Mtb). According to the WHO report in 2022, TB was responsible for the deaths of 1.6 million people in 2021. The number of TB deaths increased in 2020 and 2021, breaking the downward trend between 2005 and 2019. The fight against TB has damaged since the beginning of the pandemic<sup>1</sup>. Current treatment of drug-sensitive tuberculosis is a 6-month regimen of 4 first-line drugs (isoniazide, rifampicin, ethambutol, and pyrazinamide). Treatment of cases with drug-resistant Mtb requires 18-24 months of therapy with the addition of second-line drugs<sup>2-5</sup>. Mtb can survive in nutrient and oxygen deprived conditions, and the disease most often becomes latent. The ability of Mtb to enter latency makes it very difficult to eradicate. The reactivation of latent TB infection accounts for the majority of new TB cases, particularly in

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countries with low TB incidence<sup>6</sup>. Considering the compliance problem in treatment, the increase in drug-resistant cases and the emergence of many individuals with latent TB, new effective antitubercular agents are needed<sup>7</sup>. Pyridazine-3(2*H*)-ones have attracted ample attention in the field of medicinal chemistry due to their diverse pharmacological properties such as antimycobacterial<sup>8,9</sup>, antibacterial<sup>10</sup>, antifungal<sup>11</sup>, antiviral<sup>12</sup>, anti-inflammatory<sup>13</sup>, anticancer<sup>14</sup>, and anticonvulsant<sup>15</sup> activities. Pyridazine-3(2*H*)-ones also hold the advantage that they are easy to modify from the different positions of the ring. It has been reported in the literature that many compounds with a pyridazin-3(2*H*)-one skeleton exhibited antimycobacterial potential. In particular, 2,6-disubstituted-pyridazin-3(2*H*)-ones have been found to have strong antimycobacterial activities as summarized in Fig. 1<sup>8,16-19</sup>.

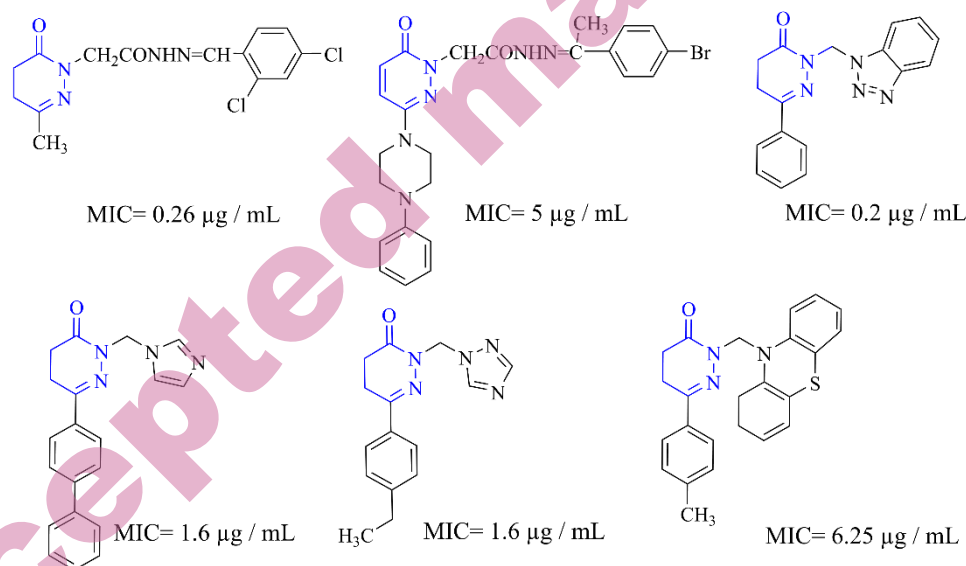


Fig. 1. Some 2,6-disubstituted-pyridazin-3(2*H*)-ones with antimycobacterial.

On the other hand, its effects on various microorganisms make the furan an important moiety. Furan and nitrofur structures have long been demonstrated to be active in the development of new compounds with antimycobacterial<sup>20-27</sup> and antifungal<sup>28-30</sup> activity (Fig. 2).

The aim of this study was to synthesize a set of new 4-(furan-2-ylmethyl)-6-methyl-pyridazin-3(2*H*)-ones, which are the hybridization product of furan and pyridazin-3(2*H*)-one ring, and test them for antimycobacterial and antifungal activity.

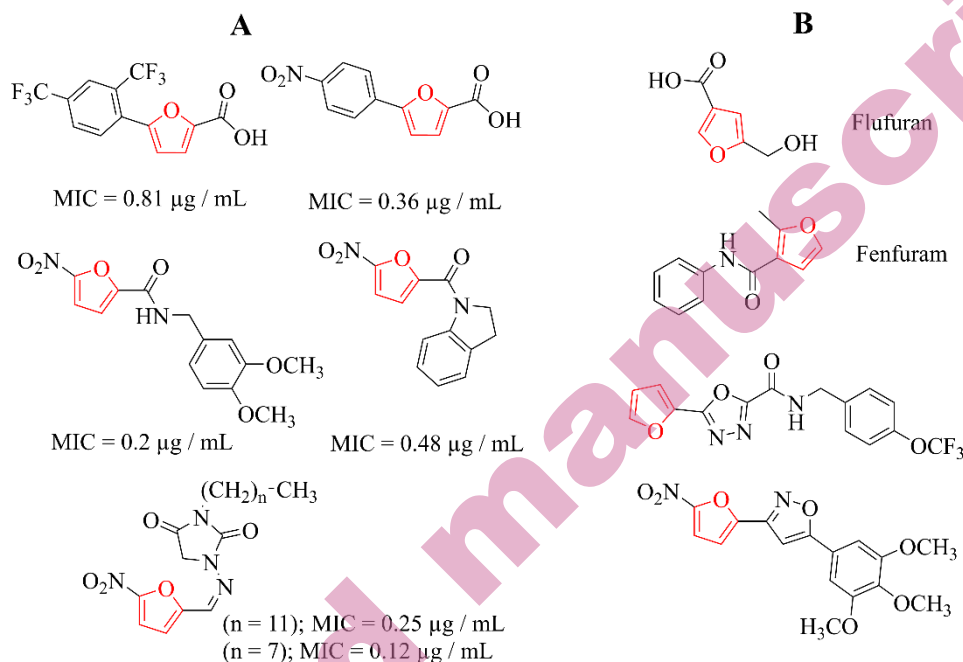


Fig. 2. Furan/nitrofurans compounds with (A) antimycobacterial and (B) antifungal activity.

## EXPERIMENTAL

### Chemistry

The melting points were measured using the Thomas-Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA); the data are uncorrected. ATR-FTIR spectra were acquired using a Spectrum BX FTIR spectrometer (Perkin Elmer) with MIRacle ATR attachment (Pike Technologies), and they were published in  $\text{cm}^{-1}$ . The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra (DMSO- $d_6$ / $\text{CDCl}_3$ ) were recorded on a Varian Mercury 400 FT NMR or Bruker Avance Neo 500 MHz spectrophotometer using TMS as an internal reference (Chemical shift represented in  $\delta$  ppm). The ESI-MS spectra were obtained using a micromass ZQ-4000 single quadrupole mass spectrometer. Elemental analyses (C, H and N) were carried out using a Leco CHNS 932 analyzer.

### Synthetic procedures

#### 6-Methyl-4,5-dihydropyridazine-3(2H)-one (**1**)

50 mmol of hydrazine hydrate was added to a solution of 50 mmol of levulinic acid in 60 mL of ethanol and heated under reflux at 120  $^\circ\text{C}$  for 2 hours. After evaporation of the solvent, the resulting solid was filtered and washed with ether to get the desired compound<sup>8</sup>.

#### 4-(2-Furylmethyl)-6-methylpyridazin-3(2H)-one (**2**)

3.36 g (30 mmol) of **1** was dissolved in a 5 % (w / v) KOH solution (35 mL) in ethanol. 2.48 mL (30 mmol) of furfural was added and refluxed for 4 hours. Some of the solvent was removed and the mixture was concentrated. Subsequently, the solution poured into ice water was acidified with 2N HCl (pH = 2). The precipitated solid was crystallized from ethyl acetate.

Yield, 3.51 g (62%); m.p. 126-127 °C. IR; 3124 (N-H), 2960, 2842 (C-H), 1648 (C=O), 1609, 1562, 1505, 1473, 1434 (C=N ve C=C). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>, 400 MHz); δ 2.18 (3H; s; -CH<sub>3</sub>), 3.80 (2H; s; -CH<sub>2</sub>-), 6.20 (1H; d; furan H<sub>3</sub>; *J* = 3.2 Hz), 6.39 (1H; dd; furan H<sub>4</sub>; *J*<sub>1</sub>=3.2 Hz, *J*<sub>2</sub>=2 Hz), 6.98 (1H; s; pyridazinone H<sub>5</sub>), 7.56 (1H; d; furan H<sub>5</sub>; *J* = 1.6 Hz), 12.76 (1H; s; NH) ppm. <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>, 100 MHz); δ 20.21 (CH<sub>3</sub>), 27.20 (CH<sub>2</sub>), 107.38, 110.61 (furan C<sub>3</sub>, C<sub>4</sub>), 131.08 (pyridazinone C<sub>5</sub>), 139.11 (pyridazinone C<sub>4</sub>), 142.17 (furan C<sub>5</sub>), 144.11 (pyridazinone C<sub>6</sub>), 150.89 (furan C<sub>2</sub>), 160.18 (pyridazinone CO) ppm. ESI-MS (*m/z*): 191.27 [M+H]<sup>+</sup>, 213.27 [M+Na]<sup>+</sup> (100%). Anal. Calcd. For C<sub>10</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>: C, 63.15; H, 5.30; N, 14.73. Found: C, 62.96; H, 5.34; N, 14.70.

*General procedure for the preparation of 2-chloro-N-arylacetamides (3a-j) and 2-chloro-1-(4-arylpiperazin-1-yl)ethanones (4a-f)*

Various aryl amines (10 mmol) or *N*-arylpiperazines were dissolved in 10 ml of acetone. Potassium carbonate (20 mmol) was added and mixed in an ice bath for 5 minutes. Chloroacetyl chloride (12 mmol) was added dropwise under a fume hood. The reaction mixture was removed from the ice bath and stirred for 1 hour at room temperature. When the reaction was complete, the mixture was poured into ice water, the precipitated solid was filtered off and desired intermediate compounds were obtained<sup>31</sup>. The details for **3a-j** and **4a-f** are given in supplementary file.

*General procedure for the preparation of 2-[5-(furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(aryl)acetamides (5a-j) and 4-(furan-2-ylmethyl)-6-methyl-2-[2-oxo-2-(4-arylpiperazin-1-yl)ethyl]pyridazin-3(2H)-ones (6a-f)*

The mixture of **2** (2 mmol) and potassium carbonate (4 mmol) in 20 mL of acetonitrile was refluxed for 30 minutes. Subsequently, **3a-j** or **4a-f** was added and mixture was reflux for 4-6 hours. When the reaction was complete, it was poured into ice water and extracted with ethyl acetate. After the ethyl acetate phase was dried with anhydrous sodium sulfate and filtered, the excess solvent was removed. It was purified by column chromatography with the appropriate ethyl acetate: *n*-hexane solvent system.

*2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-phenylacetamide (5a)*

Yield 45%; m.p. 124-125 °C. IR; 3265 (N-H), 3088 (C-H), 1671, 1658 (C=O), 1613, 1600, 1553, 1501, 1446 (C=N ve C=C). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz); δ 2.30 (3H; s; -CH<sub>3</sub>), 3.95 (2H; s; -CH<sub>2</sub>-), 4.95 (2H; s; CH<sub>2</sub>CONH), 6.21 (1H; d; furan H<sub>3</sub>; *J* = 3.2 Hz), 6.35-6.36 (1H; dd; furan H<sub>4</sub>; *J*<sub>1</sub>=3 Hz, *J*<sub>2</sub>=2 Hz), 6.85 (1H; s; pyridazinone H<sub>5</sub>), 7.05 (1H; t; Ar-H<sub>4'</sub>), 7.23-7.27 (2H; m; Ar-H<sub>3',5'</sub> ve CHCl<sub>3</sub>), 7.38 (1H; dd; furan H<sub>5</sub>; *J*<sub>1</sub>=2 Hz, *J*<sub>2</sub>=0.8 Hz), 7.48 (2H; d; Ar-H<sub>2',6'</sub>; *J*=7.6 Hz), 8.95 (1H; s; NH) ppm. <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz); δ 20.94 (CH<sub>3</sub>), 28.34 (CH<sub>2</sub>), 57.83 (CH<sub>2</sub>CO), 108.15, 110.63 (furan C<sub>3</sub>, C<sub>4</sub>), 119.83, 124.25, 128.84, 131.17, 137.70, 140.16, 142.26, 145.76 (aromatic carbons), 150.14 (furan C<sub>2</sub>), 160.59 (pyridazinone CO), 165.19 (CONH) ppm. ESI-MS (*m/z*): 324.34 [M+H]<sup>+</sup>, 346.30 [M+Na]<sup>+</sup> (% 100). Anal. Calcd. For C<sub>18</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>: C, 66.66; H, 5.30; N, 13.00. Found: C, 67.01; H, 5.43; N, 13.00.

*2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-[4-methylphenyl]acetamide (5b)*

Yield 39%; m.p. 112 °C. IR; 3271 (N-H), 3072 (C-H), 1702, 1640 (C=O), 1607, 1546, 1515, 1446 (C=N ve C=C). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>, 400 MHz); δ 2.23 (3H; s; -CH<sub>3</sub>), 2.25 (3H; s; -CH<sub>3</sub>), 3.85 (2H; s; -CH<sub>2</sub>-), 4.83 (2H; s; CH<sub>2</sub>CONH), 6.22 (1H; d; furan H<sub>3</sub>; *J* = 2.8 Hz), 6.40-6.42 (1H; dd; furan H<sub>4</sub>; *J*<sub>1</sub>=3.2 Hz, *J*<sub>2</sub>=2 Hz), 7.05 (1H; s; pyridazinone H<sub>5</sub>), 7.11 (2H; d; Ar-H<sub>3',5'</sub>; *J* = 8.4 Hz), 7.45 (2H; d; Ar-H<sub>2',6'</sub>; *J* = 8.8 Hz), 7.58 (1H; d; furan H<sub>5</sub>; *J* = 1.2 Hz), 10.18 (1H; s; NH) ppm. <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>, 100 MHz); δ 20.27, 20.40 (CH<sub>3</sub>), 27.59 (CH<sub>2</sub>), 54.73

( $\underline{\text{CH}_2\text{CO}}$ ), 107.54, 110.64 (furan  $\text{C}_3, \text{C}_4$ ), 119.00, 129.15, 130.93, 132.27, 136.22, 138.79, 142.24, 143.85 (aromatic carbons), 150.73 (furan  $\text{C}_2$ ), 159.09 (pyridazinone CO), 164.90 ( $\underline{\text{CONH}}$ ) ppm. ESI-MS ( $m/z$ ): 338.35 [ $\text{M}+\text{H}$ ] $^+$ , 360.31 [ $\text{M}+\text{Na}$ ] $^+$  (% 100). Anal. Calcd. For  $\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_3$ : C, 67.64; H, 5.68; N, 12.46. Found: C, 67.42; H, 5.74; N, 12.41.

*2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(4-methoxyphenyl)acetamide (5c)*

Yield 38%; m.p. 73 °C. IR; 3268 (N-H), 1649 (C=O), 1600, 1546, 1463, 1440, 1415 (C=N ve C=C).  $^1\text{H-NMR}$  (DMSO- $d_6$ , 400 MHz);  $\delta$  2.23 (3H; s;  $-\text{CH}_3$ ), 3.72 (3H; s;  $-\text{OCH}_3$ ), 3.85 (2H; s;  $-\text{CH}_2-$ ), 4.81 (2H; s;  $\underline{\text{CH}_2\text{CONH}}$ ), 6.22 (1H; d; furan  $\text{H}_3$ ;  $J=3.2$  Hz), 6.41 (1H; t; furan  $\text{H}_4$ ), 6.88 (2H; d; Ar- $\text{H}_{3',5'}$ ;  $J=9.2$  Hz), 7.05 (1H; s; pyridazinone  $\text{H}_5$ ), 7.48 (2H; d; Ar- $\text{H}_{2',6'}$ ;  $J=9.2$  Hz), 7.58 (1H; m; furan  $\text{H}_5$ ), 10.12 (1H; s; NH) ppm.  $^{13}\text{C-NMR}$  (DMSO- $d_6$ , 100 MHz);  $\delta$  20.27 ( $\text{CH}_3$ ), 27.60 ( $\text{CH}_2$ ), 54.66 ( $\underline{\text{CH}_2\text{CO}}$ ), 55.12 ( $\text{OCH}_3$ ), 107.54, 110.64 (furan  $\text{C}_3, \text{C}_4$ ), 113.89, 120.51, 130.91, 131.88, 138.79, 142.24, 143.83, 155.25 (aromatic carbons), 150.74 (furan  $\text{C}_2$ ), 159.09 (pyridazinone CO), 164.65 ( $\underline{\text{CONH}}$ ) ppm. ESI-MS ( $m/z$ ): 354.29 [ $\text{M}+\text{H}$ ] $^+$ , 376.26 [ $\text{M}+\text{Na}$ ] $^+$  (% 100). Anal. Calcd. For  $\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_4$ : C, 64.58; H, 5.42; N, 11.89. Found: C, 64.31; H, 5.56; N, 11.66.

*2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(4-nitrophenyl)acetamide (5d)*

Yield 37%; m.p. 165-166 °C. IR; 3227 (N-H), 1719, 1648 (C=O), 1595, 1576, 1503 (C=N ve C=C).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz);  $\delta$  2.35 (3H; s;  $-\text{CH}_3$ ), 3.97 (2H; s;  $-\text{CH}_2-$ ), 5.02 (2H; s;  $\underline{\text{CH}_2\text{CONH}}$ ), 6.20 (1H; dd; furan  $\text{H}_3$ ;  $J_1=3.2$  Hz,  $J_2=0.8$  Hz), 6.36 (1H; dd; furan  $\text{H}_4$ ;  $J_1=1.6$  Hz,  $J_2=0.8$  Hz), 6.98 (1H; s; pyridazinone  $\text{H}_5$ ), 7.37 (1H; dd; furan  $\text{H}_5$ ;  $J_1=1.6$  Hz,  $J_2=0.8$  Hz), 7.48-7.52 (2H; m; Ar- $\text{H}_{2',6'}$ ), 7.96-8.00 (2H; m; Ar- $\text{H}_{3',5'}$ ), 9.95 (1H; s; NH) ppm.  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz);  $\delta$  20.95 ( $\text{CH}_3$ ), 28.48 ( $\text{CH}_2$ ), 58.09 ( $\underline{\text{CH}_2\text{CO}}$ ), 108.12, 110.68 (furan  $\text{C}_3, \text{C}_4$ ), 119.10, 124.64, 131.95, 139.83, 142.35, 143.28, 143.62, 146.33 (aromatic carbons), 149.91 (furan  $\text{C}_2$ ), 160.77 (pyridazinone CO), 165.79 ( $\underline{\text{CONH}}$ ) ppm. ESI-MS ( $m/z$ ): 369.27 [ $\text{M}+\text{H}$ ] $^+$ , 391.21 [ $\text{M}+\text{Na}$ ] $^+$  (% 100). Anal. Calcd. For  $\text{C}_{18}\text{H}_{16}\text{N}_4\text{O}_5$ : C, 58.69; H, 4.38; N, 15.21. Found: C, 58.26; H, 4.25; N, 15.11.

*2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(4-chlorophenyl)acetamide (5e)*

Yield 46%; m.p. 131 °C. IR; 3277 (N-H), 3082 (C-H), 1708, 1649 (C=O), 1551, 1489, 1421, 1400 (C=N ve C=C).  $^1\text{H-NMR}$  (DMSO- $d_6$ , 400 MHz);  $\delta$  2.24 (3H; s;  $-\text{CH}_3$ ), 3.85 (2H; s;  $-\text{CH}_2-$ ), 4.85 (2H; s;  $\underline{\text{CH}_2\text{CONH}}$ ), 6.22 (1H; d; furan  $\text{H}_3$ ;  $J=3.2$  Hz), 6.40-6.41 (1H; dd; furan  $\text{H}_4$ ;  $J_1=3.2$  Hz,  $J_2=2$  Hz), 7.06 (1H; s; pyridazinone  $\text{H}_5$ ), 7.37 (2H; d; Ar- $\text{H}_{3',5'}$ ;  $J=8.8$  Hz), 7.58-7.61 (3H; m; Ar- $\text{H}_{2',6'}$  and furan  $\text{H}_5$ ), 10.43 (1H; s; NH) ppm.  $^{13}\text{C-NMR}$  (DMSO- $d_6$ , 100 MHz);  $\delta$  20.24 ( $\text{CH}_3$ ), 27.59 ( $\text{CH}_2$ ), 54.86 ( $\underline{\text{CH}_2\text{CO}}$ ), 107.54, 110.64 (furan  $\text{C}_3, \text{C}_4$ ), 120.57, 126.95, 128.71, 131.02, 137.66, 138.80, 142.25, 143.95 (aromatic carbons), 150.71 (furan  $\text{C}_2$ ), 159.08 (pyridazinone CO), 165.37 ( $\underline{\text{CONH}}$ ) ppm. ESI-MS ( $m/z$ ): 358.24 [ $\text{M}+\text{H}$ ] $^+$ , 380.21 [ $\text{M}+\text{Na}$ ] $^+$  (% 100), 382.20 [ $\text{M}+\text{Na}+2$ ] $^+$ . Anal. Calcd. For  $\text{C}_{18}\text{H}_{16}\text{ClN}_3\text{O}_3$ : C, 60.42; H, 4.51; N, 11.74. Found: C, 60.12; H, 4.43; N, 11.66.

*2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(4-fluorophenyl)acetamide (5f)*

Yield 55%; m.p. 132 °C. IR; 3294 (N-H), 3095 (C-H), 1703, 1645 (C=O), 1598, 1557, 1537, 1409 (C=N ve C=C).  $^1\text{H-NMR}$  (DMSO- $d_6$ , 400 MHz);  $\delta$  2.24 (3H; s;  $-\text{CH}_3$ ), 3.85 (2H; s;  $-\text{CH}_2-$ ), 4.85 (2H; s;  $\underline{\text{CH}_2\text{CONH}}$ ), 6.22 (1H; d; furan  $\text{H}_3$ ;  $J=2.8$  Hz), 6.40-6.41 (1H; dd; furan  $\text{H}_4$ ;  $J_1=2.6$  Hz,  $J_2=1.6$  Hz), 7.06 (1H; s; pyridazinone  $\text{H}_5$ ), 7.13-7.18 (2H; t; Ar- $\text{H}_{3',5'}$ ), 7.57-

7.60 (3H; m; Ar-H<sub>2,6</sub> and furan H<sub>5</sub>), 10.34 (1H; s; NH) ppm. <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>, 100 MHz); δ 20.26 (CH<sub>3</sub>), 27.59 (CH<sub>2</sub>), 54.75 (CH<sub>2</sub>CO), 107.54, 110.64 (furan C<sub>3</sub>,C<sub>4</sub>), 115.36, 120.76, 130.99, 135.11, 138.80, 142.24, 143.92, 156.83 (aromatic carbons), 150.72 (furan C<sub>2</sub>), 159.14 (pyridazinone CO), 165.12 (CONH) ppm. ESI-MS (*m/z*): 342.29 [M+H]<sup>+</sup>, 364.26 [M+Na]<sup>+</sup> (% 100). Anal. Calcd. For C<sub>18</sub>H<sub>16</sub>FN<sub>3</sub>O<sub>3</sub>: C, 63.34; H, 4.72; N, 12.31. Found: C, 63.23; H, 4.79; N, 12.15.

*2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(3-chloro-4-fluorophenyl)acetamide (5g)*

Yield 39%; m.p. 140-141 °C. IR; 3280 (N-H), 3076 (C-H), 1711, 1644 (C=O), 1593, 1555, 1538, 1400 (C=N ve C=C). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz); δ 2.32 (3H; s; -CH<sub>3</sub>), 3.95 (2H; s; -CH<sub>2</sub>-), 4.95 (2H; s; CH<sub>2</sub>CONH), 6.19 (1H; dd; furan H<sub>3</sub>; *J*<sub>1</sub> = 3 Hz, *J*<sub>2</sub> = 0.6 Hz), 6.35 (1H; dd; furan H<sub>4</sub>; *J*<sub>1</sub> = 3 Hz, *J*<sub>2</sub> = 1.8 Hz), 6.92 (2H; ; pyridazinone H<sub>5</sub> and Ar-H<sub>6</sub>), 7.13-7.15 (1H; m; Ar-H<sub>5</sub>), 7.37 (1H; dd; furan H<sub>5</sub>; *J*<sub>1</sub> = 2 Hz, *J*<sub>2</sub> = 0.8 Hz), 7.63 (1H; dd; Ar-H<sub>2</sub>; *J*<sub>1</sub> = 6.8 Hz *J*<sub>2</sub> = 2.4 Hz), 9.41 (1H; s; NH) ppm. <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz); δ 20.92 (CH<sub>3</sub>), 28.42 (CH<sub>2</sub>), 57.84 (CH<sub>2</sub>CO), 108.09, 110.64 (furan C<sub>3</sub>,C<sub>4</sub>), 116.20, 119.16, 120.74, 121.64, 131.60, 134.46, 139.89, 142.27, 146.00, 153.27, 155.71 (aromatic carbons), 150.02 (furan C<sub>2</sub>), 160.64 (pyridazinone CO), 165.24 (CONH) ppm. ESI-MS (*m/z*): 398.18 [M+Na]<sup>+</sup> (% 100), 400.17 [M+Na+2]<sup>+</sup>. Anal. Calcd. For C<sub>18</sub>H<sub>15</sub>ClFN<sub>3</sub>O<sub>3</sub>: C, 57.53; H, 4.02; N, 11.18. Found: C, 57.41; H, 3.91; N, 11.06.

*2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(naphthalen-1-yl)acetamide (5h)*

Yield 41%; m.p. 165 °C. IR; 3303 (N-H), 2956 (C-H), 1671, 1651 (C=O), 1603, 1544, 1504, 1467, 1434 (C=N ve C=C). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz); δ 2.33 (3H; s; -CH<sub>3</sub>), 4.01 (2H; s; -CH<sub>2</sub>-), 5.10 (2H; s; CH<sub>2</sub>CONH), 6.22 (1H; d; furan H<sub>3</sub>; *J* = 3.2 Hz), 6.34 (1H; dd; furan H<sub>4</sub>; *J*<sub>1</sub> = 3.4 Hz, *J*<sub>2</sub> = 2 Hz), 6.87 (1H; s; pyridazinone H<sub>5</sub>), 7.36-7.37 (1H; dd; furan H<sub>5</sub>; *J*<sub>1</sub> = 1.8 Hz, *J*<sub>2</sub> = 1.2 Hz), 7.43-7.56 (3H; m; Ar-H), 7.65 (1H; d; Ar-H *J* = 8.4 Hz), 7.84 (1H; d; Ar-H *J* = 7.6 Hz), 7.98 (1H; d; Ar-H *J* = 8.4 Hz), 8.14 (1H; d; Ar-H *J* = 7.2 Hz), 9.39 (1H; s; NH) ppm. <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz); δ 20.96 (CH<sub>3</sub>), 28.29 (CH<sub>2</sub>), 58.34 (CH<sub>2</sub>CO), 108.22, 110.65 (furan C<sub>3</sub>,C<sub>4</sub>), 119.21, 120.73, 125.26, 125.74, 125.91, 126.12, 126.37, 128.64, 131.28, 132.47, 133.96, 140.36, 142.31, 146.10 (aromatic carbons), 150.05 (furan C<sub>2</sub>), 160.80 (pyridazinone CO), 165.79 (CONH) ppm. ESI-MS (*m/z*): 374.29 [M+H]<sup>+</sup>, 396.25 [M+Na]<sup>+</sup> (% 100). Anal. Calcd. For C<sub>22</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>: C, 70.76; H, 5.13; N, 11.25. Found: C, 70.47; H, 5.14; N, 11.23.

*2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-((1,1'-biphenyl)-2-yl)acetamide (5i)*

Yield 45%; m.p. 158-159 °C. IR; 3288 (N-H), 3045 (C-H), 1697, 1642 (C=O), 1522, 1475, 1449, 1437, 1418 (C=N ve C=C). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz); δ 2.14 (3H; s; -CH<sub>3</sub>), 3.82 (2H; s; -CH<sub>2</sub>-), 4.79 (2H; s; CH<sub>2</sub>CONH), 6.22 (1H; d; furan H<sub>3</sub>; *J* = 3.2 Hz), 6.39 (1H; dd; furan H<sub>4</sub>; *J*<sub>1</sub> = 3 Hz, *J*<sub>2</sub> = 1.8 Hz), 6.66 (1H; s; pyridazinone H<sub>5</sub>), 7.14-7.19 (2H; m; Ar-H), 7.23-7.26 (2H; m; Ar-H), 7.34-7.38 (4H; m; Ar-H), 7.43 (1H; m; furan H<sub>5</sub>), 7.75 (1H; s; NH), 8.36 (1H; d; Ar-H *J* = 8.4 Hz) ppm. <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz); δ 20.92 (CH<sub>3</sub>), 28.15 (CH<sub>2</sub>), 56.20 (CH<sub>2</sub>CO), 108.32, 110.70 (furan C<sub>3</sub>,C<sub>4</sub>), 121.30, 124.51, 127.75, 128.44, 128.84, 129.17, 130.03, 130.50, 132.38, 134.28, 137.97, 140.35, 142.29, 145.50 (aromatic carbons), 150.12 (furan C<sub>2</sub>), 159.74 (pyridazinone CO), 165.03 (CONH) ppm. ESI-MS (*m/z*): 400.27 [M+H]<sup>+</sup>, 422.25 [M+Na]<sup>+</sup> (% 100). Anal. Calcd. For C<sub>24</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>: C, 72.16; H, 5.30; N, 10.52. Found: C, 72.08; H, 5.39; N, 10.30.



2-[5-(Furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(pyridin-2-yl)acetamide (**5j**)

Yield 44%; m.p. 178 °C. IR; 3264 (N-H), 3087, 2983, 2949 (C-H), 1671, 1658 (C=O), 1613, 1600, 1553, 1501, 1445 (C=N ve C=C). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz); δ 2.28 (3H; s; -CH<sub>3</sub>), 3.97 (2H; s; -CH<sub>2</sub>-), 4.99 (2H; s; CH<sub>2</sub>CONH), 6.21 (1H; dd; furan H<sub>3</sub>, *J*<sub>1</sub>= 3.2 Hz, *J*<sub>2</sub>= 0.8 Hz), 6.36 (1H; dd; furan H<sub>4</sub>, *J*<sub>1</sub>= 3 Hz, *J*<sub>2</sub>= 2 Hz), 6.79 (1H; s; pyridazinone H<sub>5</sub>), 7.03 (1H; ddd; Ar-H *J*<sub>1</sub>= 7.4 Hz, *J*<sub>2</sub>= 5 Hz, *J*<sub>3</sub>= 0.8 Hz), 7.39 (1H; m; furan H<sub>5</sub>), 7.68 (1H; ddd; Ar-H, *J*<sub>1</sub>= 8.6 Hz, *J*<sub>2</sub>= 7.2 Hz, *J*<sub>3</sub>= 1.4 Hz), 8.19 (1H; d; Ar-H *J*=8.4 Hz), 8.28 (1H; ddd; Ar-H, *J*<sub>1</sub>= 5.1 Hz, *J*<sub>2</sub>= 2 Hz, *J*<sub>3</sub>= 0.8 Hz), 9.18 (1H; s; NH) ppm. <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz); δ 21.00 (CH<sub>3</sub>), 28.23 (CH<sub>2</sub>), 56.51 (CH<sub>2</sub>CO), 108.25, 110.61 (furan C<sub>3</sub>,C<sub>4</sub>), 114.37, 120.03, 130.80, 138.42, 140.56, 142.24, 145.55, 147.77, 150.25, 151.09 (aromatic carbons), 160.32 (pyridazinone CO), 165.57 (CONH) ppm. ESI-MS (*m/z*): 325.31 [M+H]<sup>+</sup>, 347.27 [M+Na]<sup>+</sup> (% 100). Anal. Calcd. For C<sub>17</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>: C, 62.95; H, 4.97; N, 17.27. Found: C, 62.66; H, 4.92; N, 16.97.

4-(Furan-2-ylmethyl)-6-methyl-2-[2-oxo-2-(4-phenylpiperazin-1-yl)ethyl]pyridazin-3(2H)-one (**6a**)

Yield 40%; m.p. 124 °C. IR; 3116, 2982, 2828 (C-H), 1647 (C=O), 1605, 1535, 1493, 1443 (C=N ve C=C). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>, 500 MHz); δ 2.22 (3H; s; -CH<sub>3</sub>), 3.12-3.14 (4H; m; piperazine), 3.59-3.66 (4H; m; piperazine), 3.84 (2H; s; -CH<sub>2</sub>-), 4.99 (2H; s; CH<sub>2</sub>CO), 6.22 (1H; d; furan H<sub>3</sub>, *J*= 3.05), 6.41 (1H; m; furan H<sub>4</sub>), 6.83 (1H; m; Ar-H), 6.98 (2H; d; Ar-H *J*= 8.25), 7.05 (1H; s; pyridazinone H<sub>5</sub>), 7.25 (2H; m; Ar-H), 7.58 (1H; s; furan H<sub>5</sub>) ppm. <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>, 125 MHz); δ 20.73 (CH<sub>3</sub>), 28.14 (CH<sub>2</sub>), 44.43, 48.88 (piperazine carbons), 53.25 (CH<sub>2</sub>CO), 108.00, 111.13 (furan C<sub>3</sub>,C<sub>4</sub>), 116.42, 119.88, 129.48, 131.35, 139.19, 142.72, 144.17 (aromatic carbons), 151.26 (furan C<sub>2</sub>), 159.57 (pyridazinone CO), 165.20 (CH<sub>2</sub>CON) ppm. ESI-MS (*m/z*): 393.31 [M+H]<sup>+</sup>, 415.30 [M+Na]<sup>+</sup> (% 100). Anal. Calcd. For C<sub>22</sub>H<sub>24</sub>N<sub>4</sub>O<sub>3</sub>: C, 67.33; H, 6.16; N, 14.28. Found: C, 67.24; H, 6.10; N, 14.21.

4-(Furan-2-ylmethyl)-6-methyl-2-[2-oxo-2-(4-(4-methylphenyl)piperazin-1-yl)ethyl]pyridazin-3(2H)-one (**6b**)

Yield 47%; m.p. 140 °C. IR; 3127, 3105, 2982, 2810 (C-H), 1679, 1649 (C=O), 1605, 1537, 1512, 1445 (C=N ve C=C). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz); δ 2.27 (6H; d; CH<sub>3</sub>), 3.11-3.19 (4H; m; piperazine), 3.64-3.67 (2H; m; piperazine), 3.78-3.80 (2H; m; piperazine), 3.93 (2H; s; -CH<sub>2</sub>-), 4.99 (2H; s; CH<sub>2</sub>CO), 6.19 (1H; d; furan H<sub>3</sub>, *J*= 3.2), 6.35 (1H; dd; furan H<sub>4</sub>, *J*<sub>1</sub>= 2.8 Hz, *J*<sub>2</sub>= 1.6 Hz), 6.78 (1H; s; pyridazinone H<sub>5</sub>), 6.83-6.86 (2H; m; Ar-H<sub>2',6'</sub>), 7.10 (2H; d; Ar-H<sub>3',5'</sub>, *J*= 8 Hz), 7.38 (1H; m; furan H<sub>5</sub>) ppm. <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz); δ 20.46 (CH<sub>3</sub>), 21.00 (CH<sub>3</sub>), 28.25 (CH<sub>2</sub>), 42.10, 44.88, 50.00 (piperazine carbons), 52.96 (CH<sub>2</sub>CO), 108.06, 110.58 (furan C<sub>3</sub>,C<sub>4</sub>), 117.16, 130.23, 140.08, 142.11, 144.67, 148.77 (aromatic carbons), 150.57 (furan C<sub>2</sub>), 160.04 (pyridazinone CO), 164.71 (CH<sub>2</sub>CON) ppm. ESI-MS (*m/z*): 429,28 [M+Na]<sup>+</sup> (% 100). Anal. Calcd. For C<sub>23</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub>: C, 67.96; H, 6.45; N, 13.78. Found: C, 67.83; H, 6.51; N, 13.68.

4-(Furan-2-ylmethyl)-6-methyl-2-[2-oxo-2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl]pyridazin-3(2H)-one (**6c**)

Yield 47%; m.p. 121-122 °C. IR; 3047, 2996, 2838 (C-H), 1671, 1641 (C=O), 1599, 1510, 1463, 1447 (C=N ve C=C). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz); δ 2.26 (-CH<sub>3</sub>), 3.04-3.12 (4H; m; piperazine), 3.64-3.66 (2H; m; piperazine), 3.77-3.80 (5H; m; piperazine and -OCH<sub>3</sub>), 3.93 (2H; s; -CH<sub>2</sub>-), 4.99 (2H; s; CH<sub>2</sub>CO), 6.19 (1H; d; furan H<sub>3</sub>, *J*= 2.8 Hz), 6.35 (1H; dd; furan H<sub>4</sub>, *J*<sub>1</sub>= 3 Hz, *J*<sub>2</sub>= 1.8 Hz), 6.78 (1H; s; pyridazinone H<sub>5</sub>), 6.84-6.92 (4H; m; Ar-H), 7.37 (1H; m; furan H<sub>5</sub>) ppm. <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz); δ 20.98 (CH<sub>3</sub>), 28.24 (CH<sub>2</sub>), 42.22, 44.99, 50.93

(piperazine carbons), 52.96 ( $\underline{\text{CH}_2\text{CO}}$ ), 55.54 ( $\text{OCH}_3$ ), 108.04, 110.57 (furan  $\text{C}_3, \text{C}_4$ ), 114.54, 119.08, 130.58, 140.08, 142.10, 144.67, 145.18, 154.50 (aromatic carbons), 150.57 (furan  $\text{C}_2$ ), 160.05 (pyridazinone CO), 164.71 ( $\text{CH}_2\text{CON}$ ) ppm. ESI-MS ( $m/z$ ): 423.42  $[\text{M}+\text{H}]^+$ , 445.39  $[\text{M}+\text{Na}]^+$  (% 100). Anal. Calcd. For  $\text{C}_{23}\text{H}_{26}\text{N}_4\text{O}_4$ : C, 65.39; H, 6.20; N, 13.26 Found: C, 65.17; H, 6.20; N, 13.16.

*4-(Furan-2-ylmethyl)-6-methyl-2-[2-oxo-2-(4-(4-nitrophenyl)piperazin-1-yl)ethyl]pyridazin-3(2H)-one (6d)*

Yield 51%; m.p. 153-154 °C. IR: 2879 (C-H), 1673, 1650 (C=O), 1595, 1505, 1483, 1434 (C=N ve C=C).  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ , 500 MHz);  $\delta$  2.22 (3H; s;  $-\text{CH}_3$ ), 3.52-3.54 (2H; m; piperazine), 3.62 (4H; m; piperazine), 3.72 (2H; m; piperazine), 3.84 (2H; s;  $-\text{CH}_2-$ ), 5.00 (2H; s;  $\underline{\text{CH}_2\text{CO}}$ ), 6.22 (1H; dd; furan  $\text{H}_3$ ,  $J_1=3.2$  Hz,  $J_2=0.7$  Hz), 6.41 (1H; dd; furan  $\text{H}_4$ ,  $J_1=3.1$  Hz,  $J_2=1.9$  Hz), 7.03-7.05 (3H; m; pyridazinone  $\text{H}_5$  ve Ar- $\text{H}_{2'',6''}$ ), 7.58 (1H; dd; furan  $\text{H}_5$ ,  $J_1=1.8$  Hz,  $J_2=0.8$  Hz), 8.09 (2H; m; Ar- $\text{H}_{3'',5''}$ ) ppm.  $^{13}\text{C-NMR}$  ( $\text{DMSO-d}_6$ , 125 MHz);  $\delta$  20.72 ( $\text{CH}_3$ ), 28.14 ( $\text{CH}_2$ ), 43.71, 46.21, 46.42 (piperazine carbons), 53.25 ( $\underline{\text{CH}_2\text{CO}}$ ), 107.99, 111.13 (furan  $\text{C}_3, \text{C}_4$ ), 113.06, 126.21, 131.37, 137.50, 139.20, 142.71, 144.20, 154.80 (aromatic carbons), 151.28 (furan  $\text{C}_2$ ), 159.57 (pyridazinone CO), 165.52 ( $\text{CH}_2\text{CON}$ ) ppm. ESI-MS ( $m/z$ ): 460,38  $[\text{M}+\text{Na}]^+$  (% 100). Anal. Calcd. For  $\text{C}_{22}\text{H}_{23}\text{N}_5\text{O}_5$ : C, 60.40; H, 5.30; N, 16.01 Found: C, 60.35; H, 5.38; N, 15.79.

*4-(Furan-2-ylmethyl)-6-methyl-2-[2-oxo-2-(4-(4-chlorophenyl)piperazin-1-yl)ethyl]pyridazin-3(2H)-one (6e)*

Yield 51%; m.p. 146-147 °C. IR: 3047, 2935 (C-H), 1677, 1649 (C=O), 1537, 1497, 1467, 1451, 1432 (C=N ve C=C).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz);  $\delta$  2.25 (3H; s;  $-\text{CH}_3$ ), 3.13-3.21 (4H; m; piperazine), 3.65-3.67 (2H; m; piperazine), 3.77-3.80 (2H; m; piperazine), 3.92 (2H; s;  $-\text{CH}_2-$ ), 4.98 (2H; s;  $\underline{\text{CH}_2\text{CO}}$ ), 6.19 (1H; d; furan  $\text{H}_3$ ,  $J=2.8$  Hz), 6.35 (1H; dd; furan  $\text{H}_4$ ,  $J_1=3.2$  Hz,  $J_2=2$  Hz), 6.78 (1H; s; pyridazinone  $\text{H}_5$ ), 6.82-6.84 (2H; m; Ar- $\text{H}_{2'',6''}$ ), 7.21-7.26 (2H; m; Ar- $\text{H}_{3'',5''}$ ), 7.37 (1H; d; furan  $\text{H}_5$ ,  $J=1.6$  Hz) ppm.  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz);  $\delta$  20.98 ( $\text{CH}_3$ ), 28.23 ( $\text{CH}_2$ ), 41.90, 44.71, 49.40 (piperazine carbons), 52.92 ( $\underline{\text{CH}_2\text{CO}}$ ), 108.06, 110.57 (furan  $\text{C}_3, \text{C}_4$ ), 117.96, 125.59, 129.13, 130.61, 140.07, 142.11, 144.71, 149.46 (aromatic carbons), 150.53 (furan  $\text{C}_2$ ), 160.03 (pyridazinone CO), 164.79 ( $\text{CH}_2\text{CON}$ ) ppm. ESI-MS ( $m/z$ ): 427,38  $[\text{M}+\text{H}]^+$ , 449,35  $[\text{M}+\text{Na}]^+$  (% 100), 451,34  $[\text{M}+\text{Na}+2]^+$ . Anal. Calcd. For  $\text{C}_{22}\text{H}_{23}\text{ClN}_4\text{O}_3$ : C, 61.90; H, 5.43; N, 13.12 Found: C, 61.79; H, 5.57; N, 12.91.

*4-(Furan-2-ylmethyl)-6-methyl-2-[2-oxo-2-(4-(4-fluorophenyl)piperazin-1-yl)ethyl]pyridazin-3(2H)-one (6f)*

Yield 50%; m.p. 105 °C. IR: 2921, 2855 (C-H), 1667, 1644 (C=O), 1606, 1507, 1446 (C=N ve C=C).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz);  $\delta$  2.26 (3H; s;  $-\text{CH}_3$ ), 3.08-3.16 (4H; m; piperazine), 3.65-3.67 (2H; m; piperazine), 3.78-3.80 (2H; m; piperazine), 3.93 (2H; s;  $-\text{CH}_2-$ ), 4.99 (2H; s;  $\underline{\text{CH}_2\text{CO}}$ ), 6.19 (1H; d; furan  $\text{H}_3$ ,  $J=3.2$ ), 6.35 (1H; dd; furan  $\text{H}_4$ ,  $J_1=3$  Hz,  $J_2=1.8$  Hz), 6.78 (1H; s; pyridazinone  $\text{H}_5$ ), 6.87-6.90 (2H; m; Ar- $\text{H}_{2'',6''}$ ), 6.96-7.00 (2H; m; Ar- $\text{H}_{3'',5''}$ ), 7.37 (1H; dd; furan  $\text{H}_5$ ,  $J_1=2$  Hz,  $J_2=0.8$  Hz) ppm.  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz);  $\delta$  20.99 ( $\text{CH}_3$ ), 28.23 ( $\text{CH}_2$ ), 42.10, 44.88, 50.46 (piperazine carbons), 52.95 ( $\underline{\text{CH}_2\text{CO}}$ ), 108.06, 110.58 (furan  $\text{C}_3, \text{C}_4$ ), 115.73, 118.76, 130.62, 140.08, 142.12, 144.73, 147.53, 156.52, 158.90 (aromatic carbons), 150.53 (furan  $\text{C}_2$ ), 160.06 (pyridazinone CO), 164.76 ( $\text{CH}_2\text{CON}$ ) ppm. ESI-MS ( $m/z$ ): 411,46  $[\text{M}+\text{H}]^+$ , 433,43  $[\text{M}+\text{Na}]^+$  (% 100). Anal. Calcd. For  $\text{C}_{22}\text{H}_{23}\text{FN}_4\text{O}_3$ : C, 64.38; H, 5.65; N, 13.65 Found: C, 64.11; H, 5.77; N, 13.47.

### Biological Studies

#### *In-vitro M. tuberculosis MABA assay*

Antimycobacterial activities of synthesized compounds against *Mtb H37Rv* strain were tested using Microplate Alamar Blue Assay (MABA) technique. In antimycobacterial activity studies, isoniazid were used as reference compounds. The MIC (Minimum Inhibitor Concentration) values of the compounds are given in Table 1 in  $\mu\text{g} / \text{mL}$ . *Mtb H37Rv* was inoculated from a freezer stock into 7H9+OADC and grown to mid-log phase. Logarithmically growing *Mtb* was then inoculated into Sauton's medium in 96 well plates with wells containing increasing concentrations of test compounds at an  $\text{OD}_{600}$  of 0.0008, corresponding to approximately  $4 \times 10^5$  CFU/mL in 200  $\mu\text{L}$  per well. Plates were incubated at 37 °C for 1 week, at which point 32.5  $\mu\text{L}$  of a resazurin-tween mixture (8:5 ratio of 0.6 mM Resazurin in 1XPBS to 20 % Tween 80) was added and the plate was incubated at 37 °C overnight. Production of fluorescent resorufin was used as an indication to determine the MIC of the compounds.

#### *Antifungal Activity*

Antifungal activity of the target compounds and fluconazole was tested against the American Type Culture Collection (ATCC) strains of *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258), and *C. parapsilosis* (ATCC 90018). MIC values ( $\mu\text{g}/\text{mL}$ ), were determined by broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) reference documents<sup>32</sup>. Briefly, the strains which were stored at -80 °C in glycerol were thawed and subcultured twice onto Sabouraud dextrose agar before the test. Broth microdilution was performed using RPMI 1640 broth (ICN-Flow, with glutamine, without bicarbonate and with pH indicator) buffered to pH 7.0 with 3-N-morpholinopropanesulfonic acid (MOPS). The inoculum densities were prepared from 24 h subcultures. The final test concentration of fungi was 0.5 to  $2.5 \times 10^3$  cfu / mL. Fluconazole was dissolved at a concentration of 64 – 0.0625  $\mu\text{g} / \text{mL}$  in sterile deionized distilled water. The target compounds were dissolved in dimethyl sulfoxide and diluted with distilled water until their final twofold concentrations of the compounds in microtiter plate wells ranged from 1024 to 0.25  $\mu\text{g} / \text{mL}$ . After 48 h incubation at 35 °C, the plates were checked visually for the growth of fungi and the MIC values were calculated. The MIC values of the compounds were determined from three independent experiments.

## RESULTS AND DISCUSSION

### *Chemistry*

The title compounds **5a-j** and **6a-f** were synthesized via the pathway shown in Fig 3. 6-Methyl-4,5-dihydropyridazine-3(2H)-one **1**, used as starting material, was synthesized by heating levulinic acid and hydrazine hydrate in ethanol. The reaction of **1** with furfural in the presence of potassium hydroxide gave 4-(2-furylmethyl)-6-methylpyridazin-3(2H)-one **2**. The intermediate compounds (**3a-j** and **4a-f**) were gained by reacting chloroacetyl chloride with the appropriate arylamines/*N*-arylpiperazines. The target compounds, **5a-j** and **6a-f** were obtained by *N*-alkylation with **3a-j** and **4a-f** in the presence of potassium carbonate. Characterization of newly synthesized compounds (**2**, **5a-j** and **6a-f**) was performed using IR, <sup>1</sup>H and <sup>13</sup>C NMR, mass spectral data as well as elemental

analysis. Spectral data details are presented in the experimental section and spectra are presented in the supplementary file.

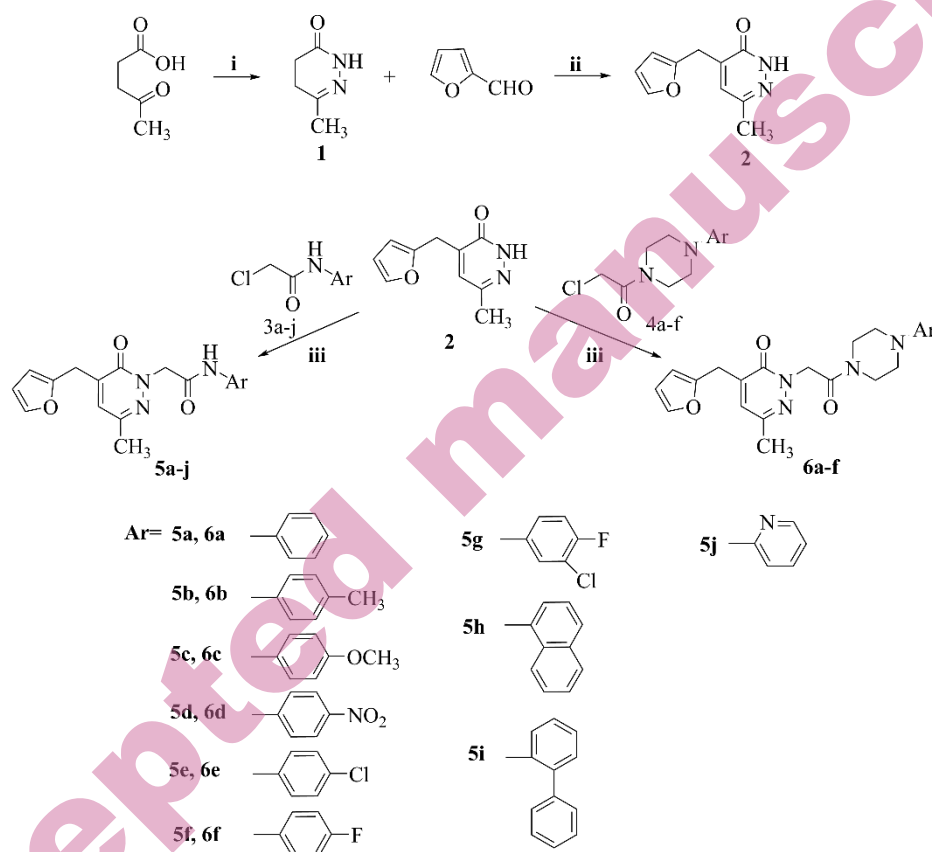

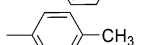
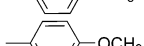
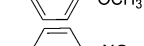
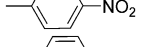
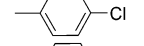
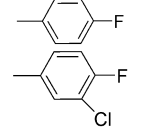
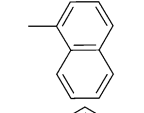
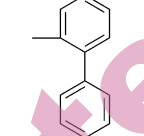
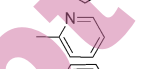
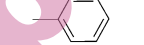
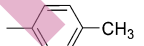
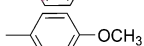
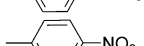
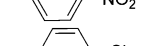
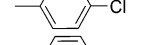


Fig. 3. Synthesis of the target compounds. Reagents and conditions: i.  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ , reflux, ii. 5 % (w/v)  $\text{KOH}$ /ethanol,  $\text{HCl}$ , reflux, iii.  $\text{K}_2\text{CO}_3$ , reflux.

#### Biological Activity

2-Substituted-4-(furan-2-ylmethyl)-6-methyl-pyridazin-3(2H)-ones (**5a-j** and **6a-f**) were tested for their antimycobacterial activities against Mtb reference strain *H37Rv* in comparison with isoniazid. These components were also tested with regard to their antifungal activities against strains of the yeast species *Candida albicans*, *C. krusei* and *C. parapsilosis* in comparison with fluconazole. The MIC values of the compounds against these organisms were reported in Table 1.

Table 1. Antimycobacterial and antifungal activities of the compounds.

Comp.	Ar	MIC ( $\mu\text{g} / \text{mL}$ )			
		<i>M. tuberculosis</i> H37Rv	<i>C. albicans</i> ATCC 90028	<i>C. krusei</i> ATCC 6258	<i>C. parapsilosis</i> ATCC 90018
5a		64.8	256	256	256
5b		67.6	256	256	256
5c		70.8	256	512	512
5d		36.9	256	256	256
5e		17.9	256	512	256
5f		34.2	256	256	256
5g		19.9	256	256	256
5h		37.4	128	256	128
5i		20.1	256	256	256
5j		65.1	256	256	128
6a		39.3	256	256	256
6b		42.9	256	256	256
6c		42.3	512	256	256
6d		46.03	128	256	64
6e		22.56	256	256	256
6f		41.1	128	256	128
isoniazid		0.02			
fluconazole			1	32	1

## CONCLUSION

The highest activity against Mtb H37Rv was found for **5e**, **5g**, **5i** and **6e** (MIC = 15-25 µg / mL). Chlorine substitution (**5e**, **5g**, **6e**) to the phenyl ring caused an increase in activity for both series (**5a-j** and **6a-f**). For 2-[5-(furan-2-ylmethyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(aryl)acetamide derivatives (**5a-j**), it was observed that the activity increased with the replacement of small groups such as phenyl (**5a**, MIC = 64.8 µg / mL) and pyridine (**5j**, MIC = 65 µg / mL) with larger groups such as biphenyl (**5i**, MIC = 20 µg / mL) and naphthyl (**5h**, MIC = 37.4 µg / mL). In addition, for the same series, introducing the electron donating groups to the phenyl ring (**5b** and **5c**, MIC = 65-70 µg / mL) did not contribute to the activity. No significant increase in the antimycobacterial activity of **6a-f** bearing the piperazine ring was observed.

The antifungal activity tests against three fungi strains (*C. albicans* ATCC 90028, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 90018) showed that **5h**, **6d** and **6f** proved to exhibit some moderate *in vitro* antifungal activities against *C. albicans* and *C. parapsilosis* (MIC = 64 - 128 µg / mL). However, all compounds were inactive against *C. krusei* (MICs ≥ 256 µg / mL). Electron withdrawing groups (F and NO<sub>2</sub>) on the phenyl ring slightly improved the activity. These activities are the only modest ones in comparison with fluconazole, the standard (MIC = 1 µg / mL).

## CONCLUSION

In summary, a series of new pyridazin-3-ones having furan moiety **5a-j** and **6a-f** was designed and synthesized to assess their antimycobacterial and antifungal activities. The structure of the target compounds were characterized using elemental analysis and spectroscopic methods (IR, mass, <sup>1</sup>H- and <sup>13</sup>C- NMR). Amongst the tested compounds, **5e**, **5g**, **5i** and **6e** showed highest activity against *M. tuberculosis* and all chlorine-bearing compounds were found to be in this group. For antifungal activity, **5h**, **6d**, and **6f** exhibited weak *in vitro* antifungal activities against *C. albicans* and *C. parapsilosis*. Further modifications of pyridazin-3-ones are required to enable even lower MICs and therapeutic potential.

## SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/12361>, or from the corresponding author on request.

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## ИЗВОД

## СИНТЕЗА И ИСПИТИВАЊЕ АНТИМИКОБАКТЕРИЈСКЕ И АНТИФУНГАЛНЕ АКТИВНОСТИ НОВИХ ДЕРИВАТА 4-(ФУРАН-2-ИЛМЕТИЛ)-6-МЕТИЛПИРИДАЗИН-3(2H)-ОНА

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Током истраживања извршена је синтеза и евалуацији серије нових деривата 4-(фуран-2-илметил)-6-метилпиридазин-3(2H)-она **5a-j** и **6a-ф** и испитана је њихова антимикобактеријска и антифунгална активност. Структуре циљних једињења потврђене су елементарном анализом и спектроскопским техникама (ИЦ, масеном спектрометријом, <sup>1</sup>H- и <sup>13</sup>C-NMP). Међу тестираним једињењима, **5e**, **5i**, **5u** и **6e** су показале највећу активност против *Mycobacterium tuberculosis*, док су **5x**, **6g** и **6ф** показале умерену ин витро антифунгалну активност против *S. albicans* and *C. parapsilosis*.

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