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Synthesis, antimycobacterial and antifungal evaluation of new 4-(furan-2-ylmethyl)-6-methylpyridazin-3(2*H*)-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, ^{1}H- and ^{13}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 *Candida albicans* and *Candida 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 been delayed since the beginning of the pandemic.[1] 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.[2–5] 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, par-

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ticularly in countries with low TB incidence.[6] 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.[7] Pyridazine-3(2*H*)-ones have attracted ample attention in the field of medicinal chemistry due to their diverse pharmacological properties such as antimycobacterial,[8,9] antibacterial,[10] antifungal,[11] antiviral,[12] anti-inflammatory,[13] anticancer[14] and anticonvulsant[15] 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.[8,16–19]

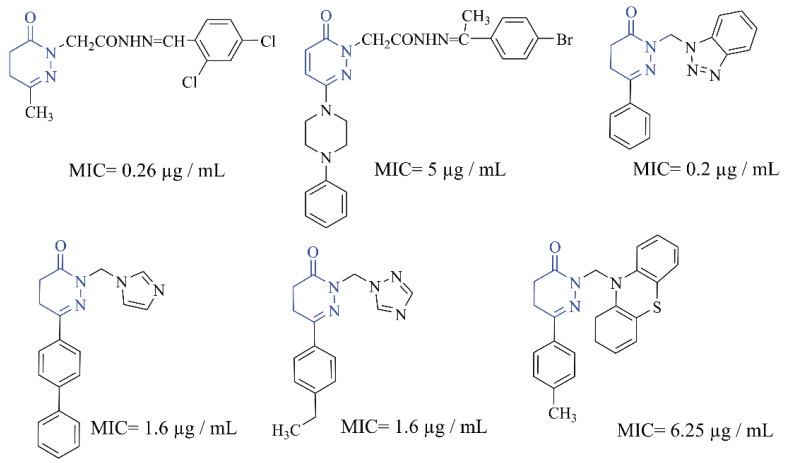


Fig. 1. Some 2,6-disubstituted pyridazin-3(2*H*)-ones with antimycobacterial.

On the other hand, its effects on various microorganisms make the furan an important moiety. Furan and nitrofuran structures have long been demonstrated to be active in the development of new compounds with antimycobacterial[20–27] and antifungal[28–30] 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(*2H*)-one ring, and test them for antimycobacterial and 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 attach-

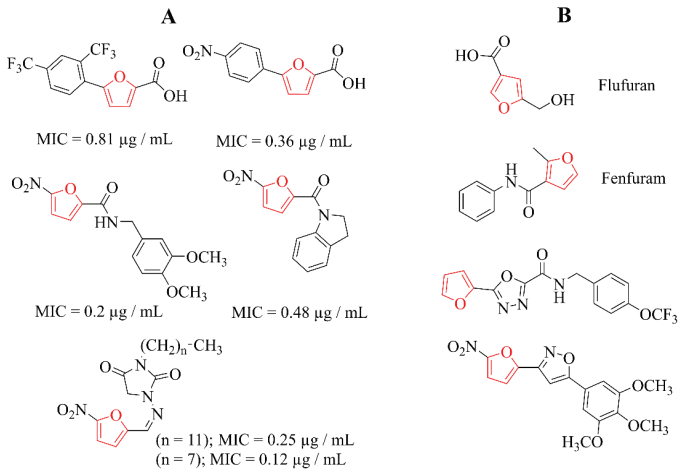


Fig. 2. Furan/nitrofuran compounds with: A) antimycobacterial and B) antifungal activity.

ment (Pike Technologies), and they were published in cm^{-1}. The ^{1}H and ^{13}C-NMR spectra (DMSO-d6/$CDCl_3$) 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 δ / ppm). The ESI-MS spectra were obtained using a micromass ZQ-4000 single quadruple 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(2*H*)-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 °C for 2 h. After evaporation of the solvent, the resulting solid was filtered and washed with ether to get the desired compound[8].

*4-(2-Furylmethyl)-6-methylpyridazin-3(2*H*)-one (**2**)*. 3.36 g (30 mmol) of **1** was dissolved in a 5 % KOH solution (35 mL) in ethanol. 2.48 mL (30 mmol) of furfural was added and refluxed for 4 h. Some of the solvent was removed and the mixture was concentrated. Subsequently, the solution poured into ice water was acidified with 2 M HCl (pH 2). The precipitated solid was crystallized from ethyl acetate. Yield, 3.51 g (62 %).

*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 min. Chloroacetyl chloride (12 mmol) was added dropwise under a fume hood. The reaction mixture was removed from the ice bath and stirred for 1 h 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.[31] The details for **3a–j** and **4a–f** are given in Supplementary material to this paper.

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

The mixture of **2** (2 mmol) and potassium carbonate (4 mmol) in 20 mL of acetonitrile was refluxed for 30 min. Subsequently, **3a–j** or **4a–f** was added and mixture was refluxed for 4–6 h. 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.

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 was used as reference compound. The minimum inhibitor concentration (*MIC*) values of the compounds are given in Table I in μg / 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 *ODλ* 600 of 0.0008, corresponding to approximately 4×10^5 CFU/mL in 200 μL per well. Plates were incubated at 37 °C for 1 week, at which point 32.5 μL 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 *Candida albicans* (ATCC 90028), *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 90018). *MIC* values (μg/mL), were determined by broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) reference documents.[32] 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 $0.5–2.5\times10^3$ cfu/mL. Fluconazole was dissolved at a concentration of 64–0.0625 μg/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––0.25 μg/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, ^{1}H- and ^{13}C-NMR, mass spectral data as well as elemental analysis. Spectral data details and spectra are presented in the Supplementary material.

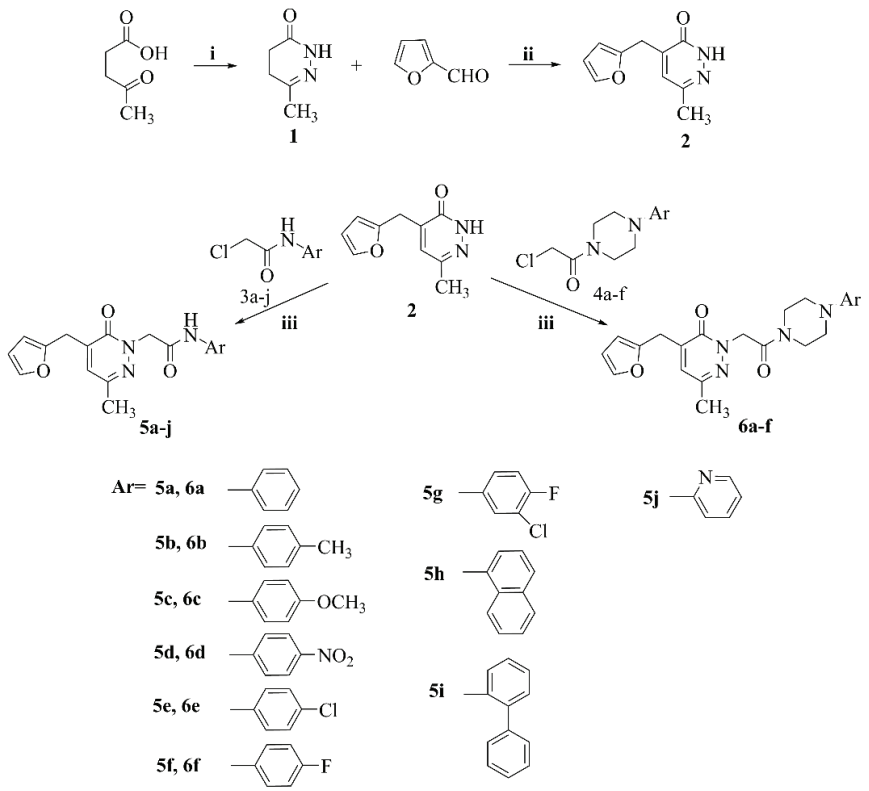
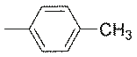
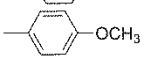
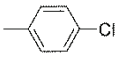
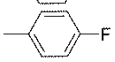
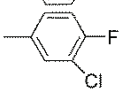
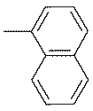
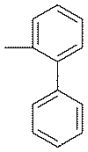
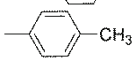
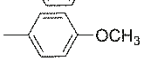
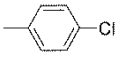
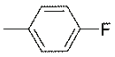


Fig. 3. Synthesis of the target compounds. Reagents and conditions: *i*. $NH_2NH_2{\cdot}H_2O$, reflux, *ii*. 5 % KOH/ethanol, HCl, reflux and *iii*. K_2CO_3, reflux.

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 *C. albicans*, *C. krusei* and *C. parapsilosis* in comparison with fluconazole. The *MIC* values of the compounds against these organisms were reported in Table I.

TABLE I. Antimycobacterial and antifungal activities of the compounds

Compd.	Ar	*MIC* / μg mL^{-1}			
		M. tuberculosis H37Rv	*C. albicans* ATCC 90028	*C. krusei* ATCC 6258	*C. parapsilosis* 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

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-yl-methyl)-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**, *MI*: 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* (*MIC* ≥ 256 μg/mL). Electron withdrawing groups (F and NO_2) 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 structures of the target compounds were characterized using elemental analysis and spectroscopic methods (IR, mass, ^{1}H- and ^{13}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 pyridazine-3-ones are required to enable even lower MICs and therapeutic potential.

SUPPLEMENTARY MATERIAL

Additional data and information 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

BURCU KARAYAVUZ[1], SIVA KRISHNA VAGOLU[2], DIDEM KART[3], TONE TØNJUM[2,4] и OYA UNSAL-TAN[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Farmacy, Hacettepe University, Ankara, Turkey, [2]Unit for Genome Dynamics, Department of Microbiology, University of Oslo, Oslo, Norway, [3]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey и [4]Unit for Genome Dynamics, Department of Microbiology, Oslo University Hospital, Oslo, Norway.

Током истраживања извршена је синтеза и евалуацији серије нових деривата 4-(фуран-2-илметил)-6-метилпиридазин-3(*2H*)-она **5a–j** и **6a–f** и испитана је њихова антимикобактеријска и антифунгална активност. Структуре циљних једињења потврђене су елементарном анализом и спектроскопским техникама (IR, масеном спектрометријом, ^{1}H и ^{13}C-NMR). Међу тестираним једињењима, **5e**, **5g**, **5i** и **6e** су показале највећу активност против *Mycobacterium tuberculosis*, док су **5h**, **6d** и **6f** показале умерену *in vitro* антифунгалну активност против *C. albicans* and *C. parapsilosis*.

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