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# NEW PYRIDINE DERIVATIVES AS ANTIUREASE INHIBITORS: SYNTHESIS AND THEIR EVALUATION FOR ANTIMICROBIAL ACTIVITIES

Hakan BEKTAS,[a,*] Canan ALBAY,[a] Emre MENTESE[b] and Neslihan DEMİRBAS[c]

[a]Department of Chemistry, Faculty of Arts and Sciences, Giresun University, 28049, Giresun, Turkey
[b]Department of Chemistry, Faculty of Arts and Sciences, Recep Tayyip Erdogan University, 53100, Rize, Turkey
[c]Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080, Trabzon, Turkey

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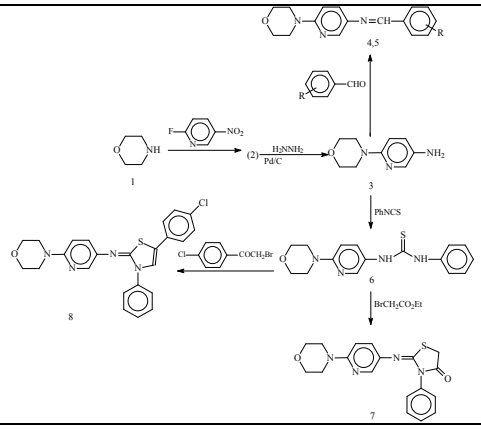
6-Morpholin-4-ylpyridin-3-amine (3) gathered starting from morpholine by two steps was converted into the corresponding arylidenehydrazides through the reaction with several aromatic aldehydes. The treatment of compound 3 with phenylisothiocyanate generated *N*-(6-morpholin-4-ylpyridin-3-yl)-*N'*-phenylthiourea (6). The synthesis of 1,3-thiazolidine (7) and 1,3-thiazole derivatives was performed from the reaction of 6 with 4-chlorophenacyl bromide and ethyl bromoacetate, respectively. All synthesized compounds were inspected for their antimicrobial and antiurease activites.



## INTRODUCTION

Upon the discovery of protonsil in 1935, clinical application for antibacterial chemotherapy were launched, which was then followed by the introduction of virtually all the major classes of antibiotics and synthetic antibacterial agents for the next thirty years. On the other hand, reports lately suggest a developing resistance among bacteria and fungi against existing drugs; and all across the world this has been recognized as a serious health problem. It is thus urgently needed to develop new antimicrobial drug targets, which can be obtained via genomics, improving the current nature of antibiotics and identifying new antibacterial agents with novel mode of action and structure.[1-13] The escalation of microbial resistance is not the only issue with the drugs used for clinical purposes. It is likewise of importance that the administration of such drugs may cause often dose-limiting, toxic side effects. Tuberculosis (TB) has once more reclaimed its former place among fatal infections. As the survey by Global Alliances reports, the world annually encounters 8-10 million new active TB cases, 3 million of which result in death.[14-16]

Urease (urea amidohydrolase; EC 3.5.1.5) is reportedly a catalyst for hydrolysis of urea to ammonia and $CO_2$. Urease activity is observed in a large variety of microorganisms and plants. Some of them are capable of producing large amounts of the enzyme. Since the isolation of *Helicobacter pylori,* evidence has accumulated that closely associates H. pylori with gastroduodenal disorders. Investigations made clear that the bacterium was

* Corresponding author: hakanbe_28@hotmail.com

capable of causing many gastroduodenal diseases ranging from gastritis to gastric and duodenal ulcers, and even gastric cancer, as well as some other various extraintestinal pathologies. It may be possible to decrease these negative effects by controlling the urease activity through inhibitors. It was highlighted that urease inhibitors lately have an important role to play in the treatment of the infections caused by urease producing bacteria. Broadly speaking, inhibitors of urease fall under two classification: organic compounds, such as acetohydroxamic acid, humic acid, and 1,4-benzoquinone; and heavy metal ions, such as $Cu^{2+}$, $Zn^{2+}$, $Pd^{2+}$, and $Cd^{2+}$.[17-21]

Additionally, Schiff bases are known to possess antimicrobial properties apart from other biological activities.[22-27] It was reported that the existence of phenylmorpholine moiety in the structure is important for antimicrobial activity, in addition, the presence of aryliden or alkylidenhydrazide functionality causes the emergence of antibacterial and antituberculosis activity. In this connection, some compounds incorporating phenylmorpholine nucleus and arylidenhydrazide function in their structures were reported as antimicrobial and antituberculosis agents.[28-29]

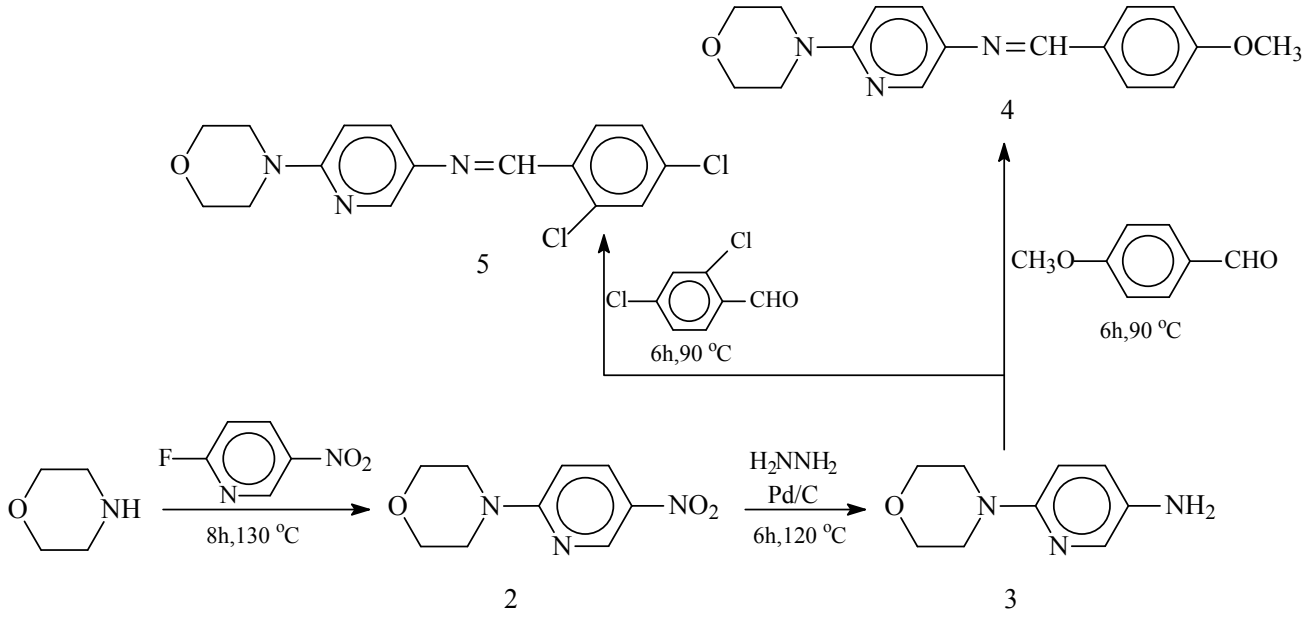
It is well documented that pyridine nucleus plays an important role in medicinal chemistry. Most tuberculosis treatment drugs are derivatives of pyridine. These include izoniazide, iproniazide, methazide, pashydrazide and pasiniazide.[30-31]

As an extension of our previous studies on the synthesis of nitrogenated heterocycles with potential chemotherapeutic activities, some new pyridin-2-ylmorpholine derivatives were synthesized as potantial antimicrobial and antiureaz agents.

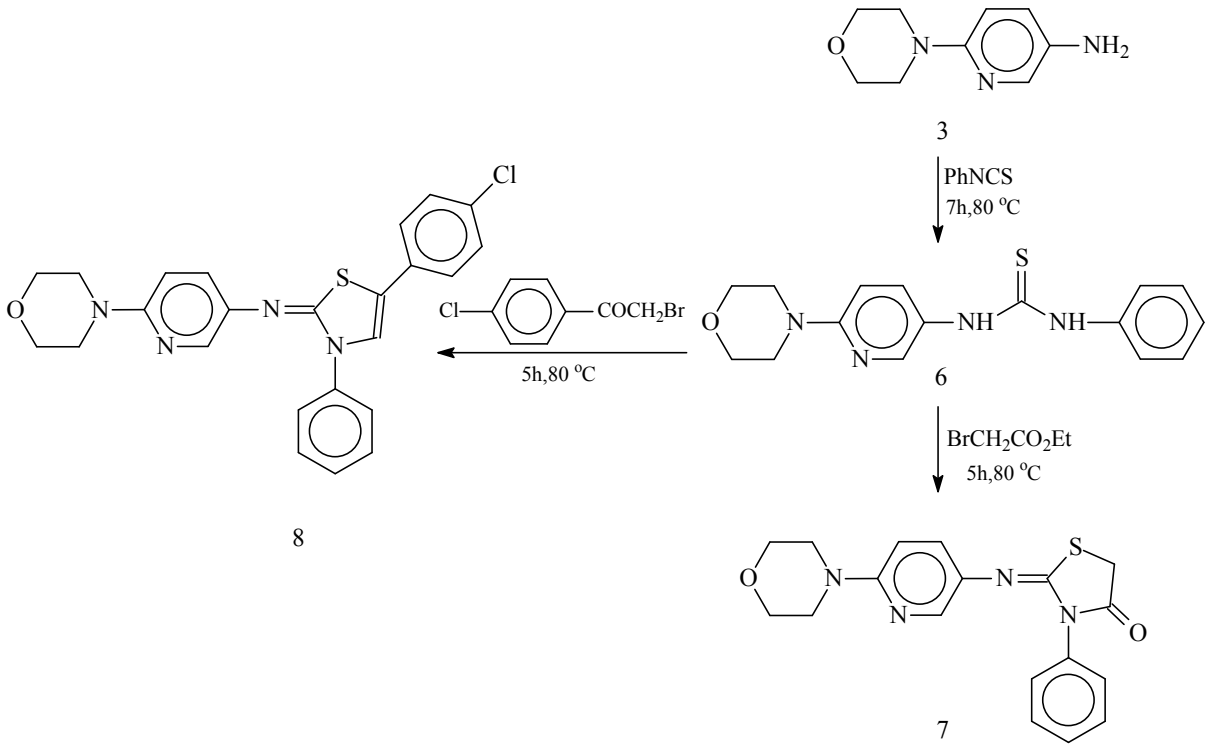
## RESULTS AND DISCUSSION

An illustration and outline of the synthetic route for the newly synthesized compounds (2-8) can be found in Scheme 1 and 2.

The synthesis of 4-(5-nitropyridin-2-yl)morpholine (2) was performed from the reaction of morpholine with 2-fluoro-5-nitropyridine, it was then converted into the corresponding hydrazide (3) by treating with hydrazine hydrate. These two compounds (2 and 3) are commercially present. With the goal of increasing the antimicrobial activity, compound 3 was converted into arylidenhydrazides by the treatment with aromatic aldehydes namely 4-methoxybenzaldehyde and 2,4-dichlorobenzaldehyde. In the $^1$H NMR spectra of these compounds, the signal derived from $NH_2$ group disappeared, and in its stead, new signals originating from aldehyde moiety were recorded at the related chemical shiff values in the $^1$H and $^{13}$C NMR spectra. Furthermore, these compounds (4 and 5) exhibited EI-MS and elemental analysis data consistent with the proposed structures.




Scheme 1 – Synthetic pathway for the preparation of compounds **2-5**.




Scheme 2 – Synthetic pathway for the preparation of compounds **6-8**.

It was reported that the compounds having an arylidene-amino structure may exist as *E/Z* geometrical isomers about the –N=CH- double band.[32-35] The literature survey revealed that compounds containing an imine bond may be present in higher percentages in dimethyl-$d_6$ sulfoxide solution in the form of geometrical *E* isomer about the –N=CH- double band.[36] The *Z* isomers can be stabilized in less polar solvents by an intramolecular hydrojen bond. However, the $^1$H- and $^{13}$C-NMR spectra of compounds 4 and 5 showed the existence of only one isomer. It can be concluded that compounds 4 and 5 exist as their *E* geometrical isomers considering bulky arylidene substituent, which is in agreement with the literature.[37-42]

The reaction of hydrazide with phenylisothiocyanate produced *N*-(6-morpholin-4-ylpyridin-3-yl)-*N*'-phenylthiourea (6). The $^1$H- and $^{13}$C NMR spectra exhibited additional signals at the aromatic region due to phenyl ring, while the signal originated from amin function was absent in the $^1$H NMR and FT-IR spectra of compound 6. In the EI-MS spectrum, molecular ion peak was seen at the corresponding *m/z* value, 314.48. Furthermore, this compound gave elemental analysis data consistent with the proposed structure.

There is a considerable interest to the chemistry of thiazolidinone ring, which constitute an important class of pharmaceuticals displaying a broad spectrum of biological activity such as anti-mycobacterial, anti-fungal, anti-cancer, anti-tuberculosis, anti-convulsant, antiinflammatory, and analgesic activities.[43-47]

The compounds that contain morpholine moiety in their structures were observed to possess antimicrobial, anti-inflammatory and central nervous system activities. To illustrate, morfazinamide is used in the treatment of tuberculosis.[48] Another important representative of this class include the morpholine derivative linezolid, currently in phase III clinical development for the treatment of Gram-positive infections.[49-50]

With the aim to introduce the 1,3-thiazole nucleus to pyridin-2-ylmorpholine skeleton as a group responsible for antimicrobial activity, compound 6 was treated with 4-chlorophenacyl bromide, thus, 2-[(6-morpholin-4-ylpyridin-3-yl)imino]-3-phenyl-1,3-thiazolidin-4-one (8) was obtained. On the other hand, the reaction of 6 with ethyl bromoacetate afforded *N*-[5-(4-chlorophenyl)-3-phenyl-1,3-thiazol-2(3*H*)-ylidene]-6-morpholin-4-ylpyridin-3-amine (7). The C-2, C-4 and C-5 atoms resonated at the related chemical shiff values in the $^{13}$C NMR spectra. The signal derived from C-5 protons of compound 7 was observed at 3.65 ppm, while the signal belonging to C-4 proton of compound 8 appeared at 7.92 ppm. The absence any signal pointing NH protons in the FT-IR and

$^1$H NMR spectra of compounds 7 and 8 is another evidence for the condensation between compound 6 and 4-chlorophenacyl bromide or ethyl bromoacetate. Moreover, these compounds exhibited EI-MS spectrum and elemental analysis data consistent with the assigned structures.

The newly synthesized compounds 2-8 (except 6) were evaluated in vitro for their antimicrobial activities. The results are presented in the Table 1. According to obtained results, compounds 2-4 and 8 displayed anti *Mycobacterium smegmatis* activity with the MIC values varying 31,3-250 µg/mL. Among these compounds, compounds 3 and 8 were the most active. All compounds were found to be active in high concentrations on yeast like fungy, *C. albicans* and *S. cerevisiae*.

Almost all of the compounds showed moderate to good urease inhibitory activity (Table 2). The inhibition was increased with increasing morpholin compound concentration. The activities of potent compound are in the range of 0.42-1.66 µM. Lower $IC_{50}$ values indicate more enzyme inhibitor activity. Compound 8 proved to be the most potent, demonstrating an enzyme inhibition activity with an $IC_{50}$ = 0.42 ± 0.038 µM. The least active compound 2 had an $IC_{50}$ = 1.66 ± 0.45 µM.

## EXPERIMENTAL

### 1. General

All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland) and utilized without any further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethanol:ethyl acetate,1:1, and detection was made using UV light. FT-IR spectra were recorded as potassium bromide pellets using a *Perkine Elmer* 1600 series FTIR spectrometer. $^1$H NMR and $^{13}$C NMR spectra were registered in DMSO-$d_6$ on a *BRUKER AVENE II* 400 MHz NMR Spectrometer (400.13 MHz for $^1$H and 100.62 MHz for $^{13}$C). The chemical shifts are given in ppm relative to $Me_4Si$ as an internal reference, *J* values are given in Hz. The elemental analysis was performed on *a Costech Elemental Combustion System* CHNS-O elemental analyzer. All the compounds gave C, H and N analysis within ±0.4% of the theoretical values. The Mass spectra were obtained on a *Quattro LC-MS* (70 eV) Instrument. Compound 1 is available commercially. Compounds 2-8 (except 6) are newly synthesized.

*Table 1*

Antimicrobial activity of the compounds **2-8** (µg/mL)

| Comp. | Microorganisms and Minimal Inhibition Concentration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **No** | **Ec** | **Yp** | **Pa** | **Ef** | **Sa** | **Bc** | **Ms** | **Ca** | **Sc** |
| **2** | - | - | - | - | - | - | 250 | 500 | 1000 |
| **3** | 1000 | - | | - | - | - | 31,3 | 1000 | 1000 |
| **4** | - | - | - | - | - | - | 62,5 | 500 | 1000 |
| **5** | - | - | - | - | - | - | - | 500 | 500 |
| **6** | - | - | - | - | - | - | - | 500 | 1000 |
| **7** | - | - | - | - | - | - | - | 500 | 1000 |
| **8** | - | - | - | - | - | - | 31,3 | 500 | 1000 |
| Amp. | 8 | 32 | >128 | 2 | 2 | <1 | | | |
| Str. | | | | | | | 4 | | |
| Flu. | | | | | | | | <8 | <8 |

Ec*: Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Sc: *S. cerevisiae* RSKK 251 Amp.: Ampicillin, Str.: Streptomisin, Flu.: Fluconazole

*Table 2*

The urease inhibitory activity of different concentrations of compounds **2-8**

| Compounds | $IC_{50}$ (µM)* |
|---|---|
| **2** | 1.66 ± 0.45 |
| **3** | 1.01 ± 0.03 |
| **4** | 1.03 ± 0.07 |
| **5** | 1.06 ± 0.09 |
| **6** | 1.26 ± 0.04 |
| **7** | 1.29 ± 0.41 |
| **8** | 0.42 ± 0.04 |
| **Thiourea** | 0.61± 0.09 |

*Mean:±SD.

## 2. Synthesis

### *2.1. Synthesis of 4-(5-Nitropyridin-2-yl)morpholine* (2)

2-fluoro-5-nitropyridine (10 mmol) was refluxed with an excess amount of morpholine (20 mL) for 8 h (TLC controlled). Then, the mixture was put into ice-water. The precipitate was filtered off and recrystallized from ethanol.

Yield (1.92 g, 92%); m.p. 141°C; IR (KBr, ν, $cm^{-1}$) : 3060 (Ar CH), 1568 (C=N), 1349 and 1518 (-$NO_2$); $^1$H NMR (DMSO-$d_6$ , δ ppm): 3.66 -3.71 (m, 8H, 4$CH_2$), 6.92 ( d, 1H, ArH, *J*= 5.0 Hz), 8.20-8.24 (m, 1H, ArH), 8.95 (d, 1H, ArH, *J*= 5.0 Hz); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 45.47 (N-2$CH_2$), 66.47 (O-2$CH_2$), arC: [106.33 (CH), 127.30 (2C), 133.52 (CH), 146.59 (CH)]; LC-MS: *m/z* (%) 232.13 ([M+Na]$^+$, 14), 248.15 ([M+K]$^+$, 18), 246.15 (100), 104.91 (16); Anal.calcd (%) for $C_9H_{11}N_3O_3$ : C, 51.67; H, 5.30; N, 20.09. Found: C, 51.42; H, 5.35; N, 20.12.

### *2.2. Synthesis of 6-Morpholin-4-ylpyridin-3-amine* (3)

Pd-C (5 mmol) catalyst was added to the solution of compound 2 (10 mmol) in butanol, and the mixture was allowed to reflux in the presence of hydrazine hydrate (50 mmol) for 6 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the catalyst was removed by filtration, and the reaction solvent was removed under reduced pressure. The obtained white solid was recrystallized from ethanol to afford the desired compound.

Yield (1.32 g, 74%); m.p. 83-84°C; IR (KBr, ν, $cm^{-1}$): 3403 and 3334 ($NH_2$), 1568 (C=N); $^1$H NMR (DMSO-$d_6$, δ ppm): 3.16 (t, 4H, 2$CH_2$ *J*=8.4 Hz), 3.67 (t, 4H, 2$CH_2$, *J*=8.2 Hz), 4.62 (s, 2H, $NH_2$), 6.60 (d, 1H, ArH, *J*= 9.0 Hz), 6.94 (d, 1H, ArH, *J*= 8.6 Hz), 7.62 (s, 1H, ArH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 47.55 (N-2$CH_2$), 66.77 (O-2$CH_2$), arC: [108. 94 (CH), 125.17 (CH), 134.04 (CH), 138.23 (C), 154.03 (C)]; LC-MS: *m/z* (%) 179.28 [M]$^+$, 90), 218.15 ([M+K]$^+$, 68); Anal.calcd (%) for $C_9H_{13}N_3O_3$ : C, 60.32; H, 7.31; N, 23.45. Found: C, 60.25; H, 7.42; N, 23.40.

### *2.3. Synthesis of N-[(4-Methoxyphenyl)methylene]-6-morpholin-4-ylpyridin-3-amine* (4) *and N-[(2,4-Dichlorophenyl)methylene]-6-morpholin-4-ylpyridin-3-amine* (5)

The corresponding aldehyde (10 mmol) was added to a solution of compound 3 (10 mmol) in absolute ethanol, then the mixture was refluxed for 6 h. Then, the reaction content was let reach room temperature, and a solid was formed. This crude product was filtered off and recrystallized from acetone to obtain the desired compound.

Compound (4): Yield (2.52 g, 85%); m.p. 164-165 °C; IR (KBr, ν, $cm^{-1}$): 1553 (C=N), 1115 (C-O); $^1$H NMR (DMSO-$d_6$, δ ppm): 3.21 (t, 4H, N-2$CH_2$), 3.67 (t, 4H, O-2$CH_2$),4.12 (s, 3H, $OCH_3$), 6.70 (d, 2H, ArH, *J*= 8.8 Hz), 7.03 (brs, 3H, ArH), 7.66 (brs, 1H, ArH), 7.82 (d, 1H, ArH, *J*= 7.4 Hz), 8.63 (s, 1H, N=CH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 47.25 (N-2$CH_2$), 55.73 ($OCH_3$), 66.66 (O-2$CH_2$), arC: [109.26 (CH), 114.37 (CH), 115.08 (CH), 126.95 (CH), 127.23 (C), 128.53 (CH), 130.69 (CH), 133.99 (CH), 135.90 (C), 153.36 (C), 162.36 (C)], 161.25 (N=CH); LC-MS: *m/z* (%) 297.34 [M]$^+$, 84), 145.23 (25), 132.15 (25); Anal.calcd (%) for $C_{17}H_{19}N_3O_2$ : C, 68.67; H, 6.44; N, 14.13. Found: C, 68.45; H, 6.25; N, 14.22.

Compound (5): Yield (2.2 g, 63%); m.p. 155-156 °C; IR (KBr, ν, $cm^{-1}$): 3056 (Ar CH), 1545 (C=N); $^1$H NMR (DMSO-$d_6$, δ ppm): 3.45 (t, 4H, N-2$CH_2$), 3.68 (t, 4H, O-2$CH_2$), 6.82 (d, 1H, ArH, *J*= 8.4 Hz), 7.52-7.98 (m, 4H, ArH), 8.24 (s, 1H, ArH), 8.84 (s, 1H, N=CH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 44.56 (N-2$CH_2$), 67.85 (O-2$CH_2$), arC: [101.52(CH), 106.86 (C), 125.08 (C), 128.85 (CH), 131.56 (2CH), 132.32 (2CH), 136.84 (C), 137.24 (C), 157.56 (C)], 156.24 (N=CH); LC-MS: *m/z* (%) 336.65 [M]$^+$, 84), 338.45 (28), 215 (20); Anal.calcd (%) for $C_{16}H_{15}Cl_2N_3O$ : C, 57.16; H, 4.50; N, 12.50. Found: C, 57.28; H, 4.52; N, 12.66.

### *Synthesis of N-(6-Morpholin-4-ylpyridin-3-yl)-N'-phenylthiourea* (6)

The solution of compound 3 (10 mmol) in absolute ethanol was stirred under reflux in the presence of phenylisothiocynate (10 mmol) for 7 h. The reaction's progress was monitored by TLC. Upon cooling the reaction mixture down to room temperature, a solid formed. This crude product was separated by filtration and recrystallized from ethanol in order to obtain the target compound.[51]

Yield (2.14 g, 68%); m.p. 211-212 °C; IR (KBr, ν, $cm^{-1}$): 3206 (2NH), 3025 (Ar CH), 1545 (C=N), 1248 (C=S), 1111 (C-O); $^1$H NMR (DMSO-$d_6$, δ ppm): 3.38 (d, 4H, N-2$CH_2$ + $H_2O$, *J*= 4.6 Hz), 3.68 (brs, 4H, O-2$CH_2$), 6.80 (d, 3H, ArH, *J*= 10.0 Hz), 7.56 (d, 3H, ArH, *J*= 8 Hz ), 8.04 (s, 2H, ArH), 9.43 (s, 1H, NH), 9.74 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 46.02 (N-2$CH_2$), 66.65 (O-2$CH_2$), arC: [107.09 (2CH), 127.51 (2C), 136.42 (2CH), 138.72 (2CH), 145.15 (2CH), 157.54 (C)], 182.73 (C=S); LC-MS: *m/z* (%) 314.48 [M]$^+$ (62), 228.99 (35), 145.23 (55), 121.13 (25); Anal.calcd (%) for $C_{16}H_{18}N_4OS$ : C, 61.12; H, 5.77; N, 17.82; S, 10.20. Found: C, 61.22; H, 5.65; N, 17.92; S, 9.22.

### *Synthesis of 2-[(6-Morpholin-4-ylpyridin-3-yl)imino]-3-phenyl-1,3-thiazolidin-4-one* (7)

A mixture of compound **6** (10 mmol) and ethyl bromoacetate in absolute ethanol was allowed to reflux in the presence of dried sodium acetate (50 mmol) for 5 h (The progress of the reaction was monitored by TLC). Then, the reaction mixture was reduced to room temperature, and the salt was separated by filtration. After removing the solvent under reduced pressure, a solid was obtained. This crude product was recrystallized from dimethyl sulfoxide to afford the desired product.

Yield (3.69 g, 68%); m.p. 160-161 °C; IR (KBr, ν, $cm^{-1}$): 2922, 2852 ($CH_2$), 1732 (C=O), 1492 (C=N), 1116 (C-O); $^1$H NMR (DMSO-$d_6$, δ ppm): 3.43 (t, 4H, N-2$CH_2$ + $H_2O$, *J*= 9 Hz), 3.68 (s, 4H, O-2$CH_2$), 4.14 (s, 2H, $CH_2$), 6.81 (d, 1H, ArH, *J*= 8.6 Hz), 6.92 (d, 1H, ArH, *J*= 9 Hz ), 7.18 (d, 1H, ArH, *J*= 9 Hz ), 7.57 (d, 1H, ArH, *J*= 9 Hz ), 7.75 (s, 2H, ArH), 8.10 (s, 2H, ArH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 33.51 ($CH_2$), 56.73 (N-2$CH_2$), 66.64 (O-2$CH_2$), arC: [107.43 (CH), 107.88 (2CH), 122.87 (C), 131.48 (2CH), 136.23 (C), 138.20 (CH), 140.11 (CH), 147.54 (CH), 157.14 (C)], 159.05 (thiazolidine C-2), 172.51 (thiazolidine C-4); LC-MS: *m/z* (%) 378.40 [M+Na] (16), 354.12 [M]$^+$ (84), 355.45 [M+1] (45), 284.25 (35), 279.15 (95), 264.12 (100); Anal.calcd (%) for $C_{18}H_{18}N_4O_2S$ : C, 61.00; H, 5.12; N, 15.81; S, 9.05. Found: C, 61.22; H, 5.32; N, 15.74; S, 9.12.

### *Synthesis of N-[5-(4-Chlorophenyl)-3-phenyl-1,3-thiazol-2(3H)-ylidene]-6-morpholin-4-ylpyridin-3-amine* (8)

A mixture of compound 6 (10 mmol) and 4-chlorophenacylbromide (10 mmol) in absolute ethanol was refluxed in the presence of dried sodium acetate (50 mmol) for 8 h. Then, the reaction content was cooled down to room temperature and the salt was separated by filtration. Upon evaporation of the solvent under reduced pressure, an oily

product appeared. When treated with water, a solid was formed. This crude product was recrystallized from benzene-petroleum ether (1:2) to afford the desired compound.

Yield (3.19 g, 71%); m.p. 124-126 °C; IR (KBr, ν, $cm^{-1}$): 1489 (C=N), 1116 (C-O); $^1$H NMR (DMSO-$d_6$, δ ppm): 3.37 (brs, 4H, N-2$CH_2$ + $H_2O$), 3.65 (d, 4H, O-2$CH_2$, *J*=11.4 Hz), 6.64-7.00 (m, 3H, ArH), 7.14-7.36 (m, 5H, ArH), 7.58 (d, 3H, ArH, *J*= 8.2 Hz), 7.74 (s, 1H, ArH), 7.92 (s, 1H, CH, thiazole C-4); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 56.74 (N-2$CH_2$), 66.69 (O-2$CH_2$), 108.38 (CH, thiazole C-4), arC: [125.26 (C), 128.73 (2CH), 129.18 (2CH), 129.53 (2CH), 130.46 (2C), 131.06 (2CH), 134.02 (2C), 141.33 (2CH), 148.13 (2CH)], 156.41 (thiazole C-2), 158.30, (thiazole C-5); LC-MS: *m/z* (%) 464.97 $[M]^+$ (25), 466.12 [M+1] (62), 284.25 (45), 264.12 (94); Anal.calcd (%) for $C_{24}H_{21}ClN_4O_2S$ : C, 62.00; H, 4.55; N, 12.05; S, 6.90. Found: C, 62.21; H, 4.65; N, 12.18; S, 6.85.

### 3. Antimicrobial Activity

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC35218, *Yersinia pseudotuberculosis (Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus)* 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607, *Candida albicans* (*C. albicans*) ATCC60193 and *Saccharomyces cerevisiae* (*S. cerevisia*) RSKK 251. All the newly synthesized compounds were weighed and dissolved in hexane to prepare extract stock solution of 20.000 microgram/milliliter (µg/mL).

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double microdilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined.[52] The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The micro dilution test plates were incubated for 18-24 h at 35 °C. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis,* and incubated for 48-72 h at 35 °C.[53] Ampicillin (10 µg) and fluconazole (5µg) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide with dilution of 1:10 was used as solvent control. The results are presented in Table 1.

Urease inhibitory activity was determined according to Van Slyke and Archibald[54] and results can be found on Table 2.

## CONCLUSIONS

Results of this study reveal that the synthesized new morpholine derivatives show antiurease activities. Thus, the new morpholine derivatives may be regarded as a primary urease inhibitory, which would mean that these compounds can become a source of antiurease for the pharmaceutical and agricultural industries.

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