

## Synthesis and antimicrobial activity of novel pyrido[2,3-*d*]pyrimidin-4(1*H*)-ones, and 2-pyrazoline derivatives

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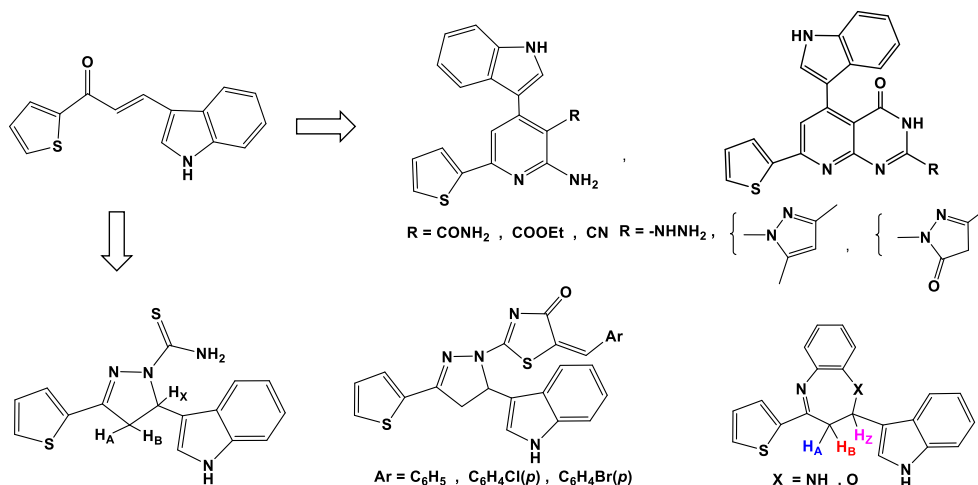
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### Abstract

The synthesis of thienyl indoyl chalcone produced a novel group of compounds bearing indole and thiophene moieties. The new series of substituted pyridines, 2-pyrazolines, and azepines were synthesized. 2-Aminopyridines were produced by reacting chalcone with active methylene reagents. The chalcone were converted to pyrimidinones. These compounds served as the initial substrate for the synthesis 1-thiocarbamoyl-2-pyrazoline. 1-(4-oxo-5*H*-thiazolyl)-2-pyrazoline and 1-(4-phenylthiazolyl)-2-pyrazoline were synthesized by treating 1-thiocarbamoyl pyrazoline with chloroacetic acid or phenacyl bromide. The chalcone underwent a reaction with bifunctional agents resulting in the formation of azepines. The compounds were structurally determined with spectral data and they were tested for antimicrobial activity giving reasonable results.



**Keywords:** Pyridines, 2-pyrazolines, azepines, indole, thiophene, antimicrobial potency

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Page 1 of 15

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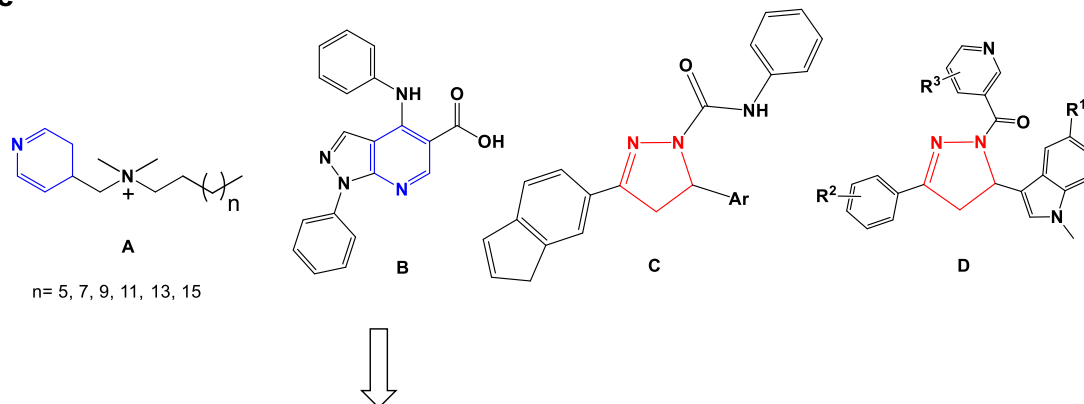
## Introduction

In 1846, Anderson discovered pyridine, an aromatic heterocyclic compound. Subsequently, Korner (1869) and Dewar (1871) determined the pyridine structure. It is a constituent of almost 7000 existing pharmacological compounds having medicinal characteristics. Pyridine has a vital role in the field of chemistry [1,2]. *N,N*-dimethyl-*N*-(4-methylpyridyl)-*N*-alkyl-ammonium chloride **A** is an example a pyridine analog that shows potency and sensitivity for fungal and bacterial strains [3]. Furthermore, clinical and theoretical research on pyrazolopyridine-5-carboxylic acids **B**, showed antibacterial action toward a drug-resistant *S. epidermidis* [4]. Studies have shown that thienopyridines are effective against the two kinds of bacteria [5]. Research findings suggest that pyridine derivatives exhibit significant effectiveness in combating *Herpes simplex* virus type 1 (HSV-1) [6]. as well as exhibiting anticancer [7-10], anti-diabetic [11,12], and anti-inflammatory properties [13-15].

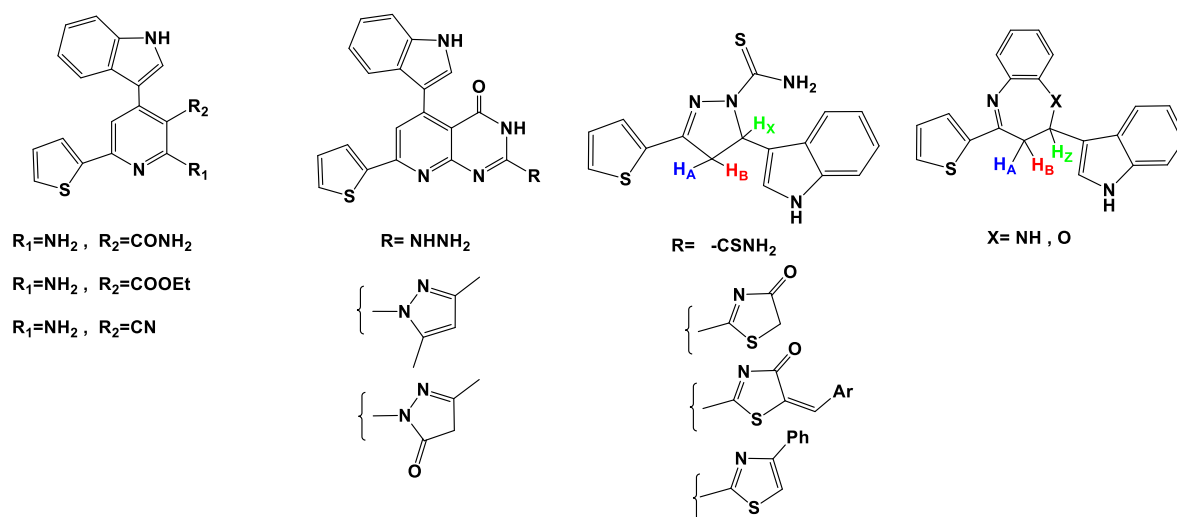
2-Pyrazoline is a saturated pyrazole distinguished by the presence of two next atoms of nitrogen and an endocyclic double bond within its ring framework. Due to its superior stability concerning the other two forms, 2-pyrazoline is the most well-researched among the three forms of pyrazolines, namely 1-pyrazoline, 2-pyrazoline, and 3-pyrazoline. Research has demonstrated that 2-pyrazolines **C** exhibits potent anticancer effects on cervical cancer cells from humans (HeLa) and breast cancer cells from humans (MCF7) [16]. In addition, nicotinoyl pyrazolines **D** showed great effectiveness on cancer cells when employed as inhibitors of tubulin assembly [17] (Figure 1). Several 2-pyrazoline structures exhibited notable biological properties, such as considerable antiproliferative activity [18-20], antibacterial and antifungal effects [21-27], anti-hyperglycemic activity [28], as well as anti-inflammatory and analgesic properties [29-32]. Moreover, it is noteworthy that 3-indolecarboxaldehyde and 2-acetyl thiophene are widely used as fundamental components for synthesizing a wide variety of heterocyclic compounds that have significant biological functionalities [33-46].

Based on the foregoing and a review of our work [31], we decided to produce a new category of pyridine, 2-pyrazoline, and azepine derivatives by reacting chalcone **1** composed of indole and thiophene moieties with different active chemical reagents. The newly synthesized compounds were evaluated for their antimicrobial activity.

## Some of reported pyridine and 2-pyrazoline derivatives with biological importance



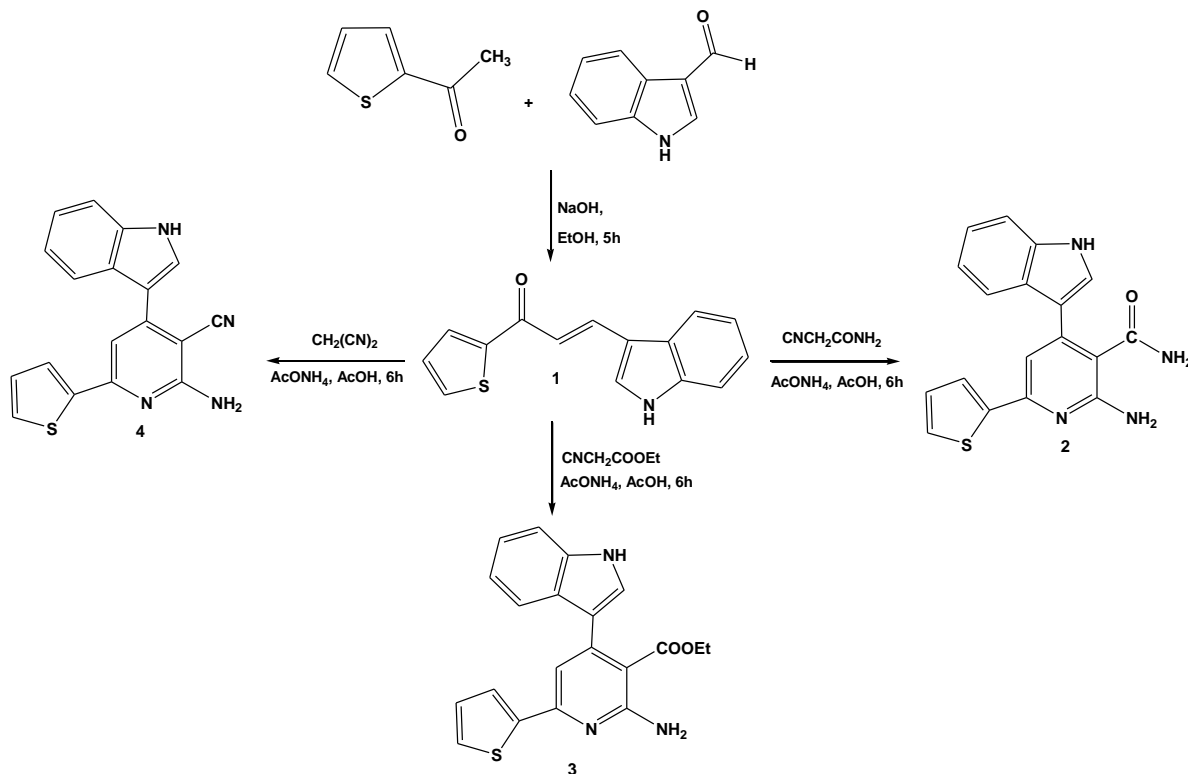
## The novel synthesized pyridine, pyridopyrimidine and 2-pyrazoline analogs



**Figure 1.** The reported and new synthesized biologically active pyridine, pyridopyrimidine, and 2-pyrazolines.

## Results and Discussion

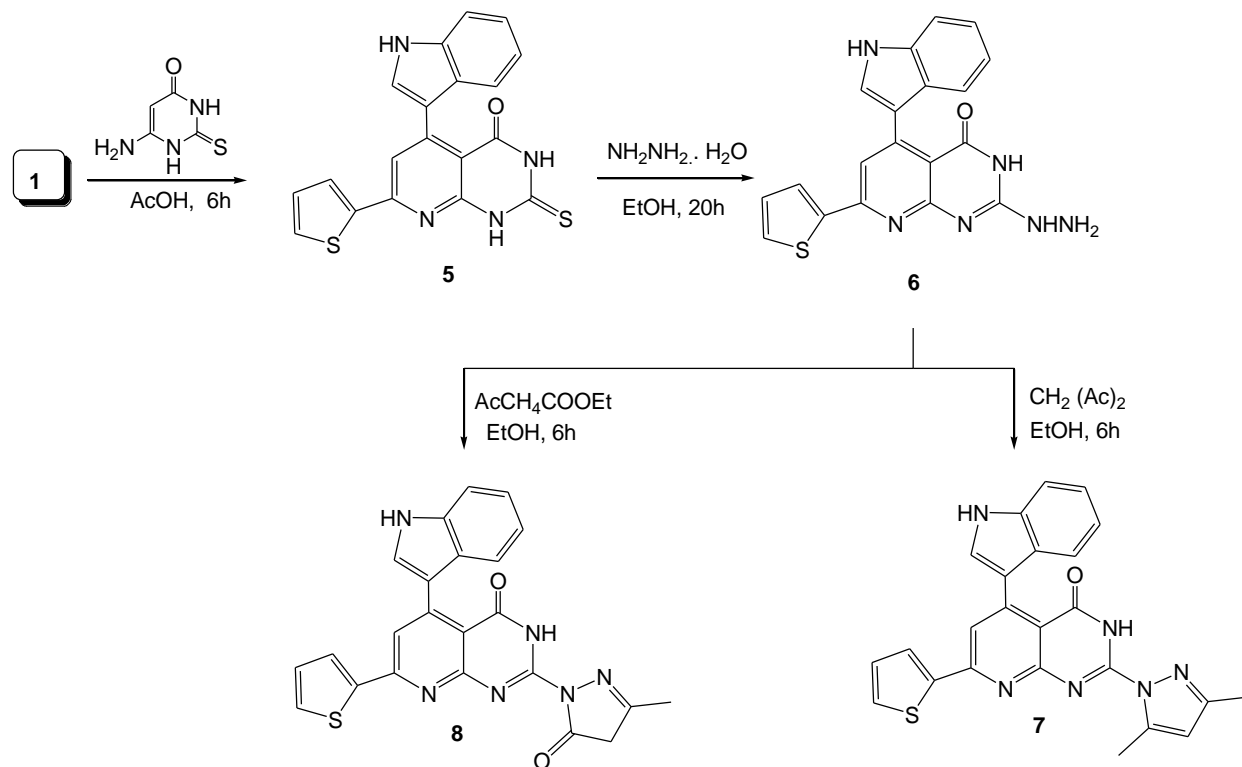
The key starting material, thienyl indolyl propenone **1**, was generated through the Claisen-Schmidt procedure by reacting 2-acetylthiophene and indole-3-carboxaldehyde [47]. The chalcone **1** was interacted with each of cyanoacetamide, ethyl cyanoacetate, and malononitrile, in the presence of ammonium acetate in hot ethanol, to provide 2-amino-3-carboxamidepyridine **2**, ethyl 2-aminothienyl indolyl pyridine-3-carboxylate **3**, and 2-amino-3-cyanothieryl indolyl pyridine **4**, respectively (Scheme 1).



**Scheme 1.** The synthesis of 2-aminopyridines (**2-4**).

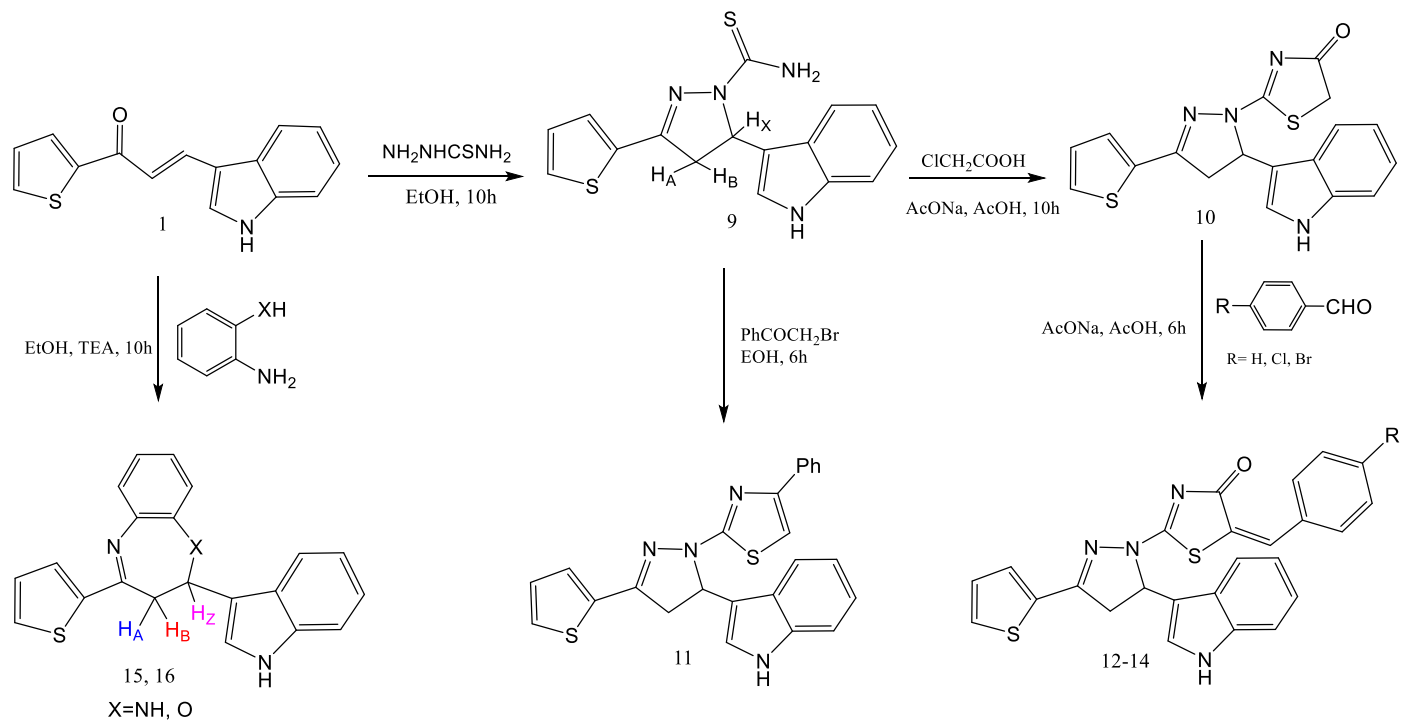
The structures of products **2-4** were verified utilizing spectral data (FTIR, NMR, and mass) and elemental analysis. Thus, Fourier Transform Infrared (FTIR) spectrum of product **2** revealed absorbance peaks at  $\nu$  3446, 3239, and 3098  $\text{cm}^{-1}$  due to pyridine-NH<sub>2</sub> and indole-NH, as well as a peak at  $\nu$  1628  $\text{cm}^{-1}$  belonging to the carbonyl group. The <sup>1</sup>H NMR spectrum indicated that signals from two amino groups come together with aromatic proton signals at  $\delta$  7.01-7.84 ppm. Similarly, the existence of carbonyl carbon was verified by signals in the <sup>13</sup>C NMR spectrum with  $\delta$  166.7 ppm. The FTIR analysis for **3** revealed bands of absorption at  $\nu$  445, 3374, and 3333  $\text{cm}^{-1}$ , which are associated with the amino group and indole-NH. Further, absorbance at  $\nu$  1711  $\text{cm}^{-1}$  corresponds to the carbonyl group of the ester. A signal at  $\delta$  176.9 ppm was detected in the <sup>13</sup>C NMR of **3**, indicating the presence of the ester carbonyl group attributable to the carbonyl group.

Chalcone **1** and 6-amino-2-thiouracil were refluxed in acetic acid to yield pyridopyrimidinone **5**. The FTIR spectrum of the latter compound exhibited peaks at  $\nu$  3390, 3307, and 3176  $\text{cm}^{-1}$  belonging to pyrimidine-NH and indole-NH, as well as absorption bands at  $\nu$  1659  $\text{cm}^{-1}$  of the pyrimidine. The <sup>1</sup>H NMR displayed peaks at  $\delta$  11.00 and 13.25 ppm, due to 2 NH of pyrimidine. The <sup>13</sup>C NMR of **5** indicated two signals at  $\delta$  165.2 and 176.0 ppm correlating to C=O and C=S of the pyrimidine ring, respectively. To examine the synthetic potential of compound **5**, it was heated with hydrazine hydrate to produce 2-hydrazinyl pyridopyrimidinone **6** that was then employed as a reactant with active methylene reagents like acetylacetone and ethyl acetoacetate, yielding 2-pyrazolyl pyridopyrimidines **7** and **8**. The product structures **6-8** were validated using elemental and spectroscopic data.



**Scheme 2.** The synthesis of pyrido[2,3-*d*]pyrimidines (**5-8**).

When chalcone **1** was heated with thiosemicarbazide, it afforded 1-thiocarbamoyl-2-pyrazoline **9**. FTIR spectroscopy of **9** showed the existence of an amino functional group, revealing two peaks at  $\nu$  3447 and 3312  $\text{cm}^{-1}$ . The <sup>1</sup>H NMR spectrum had three doublets of doublets at  $\delta$  3.68-3.76, 3.90-3.98, and 6.29-6.35 ppm, owing to the H<sub>A</sub>, H<sub>B</sub>, and H<sub>X</sub>, indicating the formation of a 2-pyrazoline nucleus. The <sup>13</sup>C NMR spectrum was consistent with FTIR and <sup>1</sup>H NMR, with three distinct signals at  $\delta$  49.1 for pyrazoline-CH<sub>2</sub>, 66.8 for pyrazoline-CH, and 177.0 ppm due to the C=S group. When  $\alpha$ -halocarbonyl reagents such as chloroacetic acid or phenacyl bromide reacted with thiocarbamoyl derivative **9**, the products 1-(4-oxo-5*H*-thiazol-2-yl)-2-pyrazoline **10** or 1-(4-phenylthiazol-2-yl)-2-pyrazoline **11** were formed. The reactivity of the methylene group at the thiazole moiety in product **10** was assessed by treating it using various aromatic aldehydes like benzaldehyde, *p*-chlorobenzaldehyde, and *p*-bromobenzaldehyde to produce the arylidenes **12-14** (Scheme 3). The products **9-14** were fully verified through elemental analysis together with spectral data such as the FTIR, NMR, with MS spectrometry. Chalcone **1** underwent treatment with *o*-phenylenediamine or *o*-aminophenol to obtain benzodiazepine **15** and benzoxazepine **16**, respectively. The formation of benzodiazepine **15** was verified through FTIR, <sup>1</sup>H- and <sup>13</sup>C NMR. The FTIR diagram of **15** indicated the lack of the chalcone carbonyl group as well as the existence of absorption bands at  $\nu$  3333 and 3172  $\text{cm}^{-1}$  belonging to diazepine-NH and indole-NH. The <sup>1</sup>H NMR spectrum of **15** verified the formation of the benzodiazepine nucleus through the appearance of three doublets of doublets at  $\delta$  3.13-3.20 for H<sub>A</sub>, 3.81-3.88 for H<sub>B</sub>, and 5.00-5.56 for H<sub>X</sub>. In addition, the <sup>13</sup>C NMR diagram confirmed the absence of a carbonyl group of ketone **1** and also the presence of two signals at 18.5 and 46.2 for CH<sub>2</sub> and CH of the dihydroazepine ring.



**Scheme 3.** The synthesis of 2-pyrazolines (9-11) and azepines (15 and 16).

### Antimicrobial screening

**Antibacterial activity.** All compounds 2-16 were evaluated for antibacterial effects against both Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*). Chloramphenicol was selected as the reference (Table 1). The minimum inhibitory concentrations (MICs) were calculated. Each of the compounds displayed considerable antibacterial effects. The compounds, 2-aminopyridines 2-4 and pyrido[2,3-*d*] pyrimidin-4(1*H*)-ones 5-8 displayed moderate antibacterial properties.

2-Pyrazolone derivatives 9-14 exhibit significant activity against tested against other antibacterial species, with MICs ranging from 4-30  $\mu\text{g}/\text{mL}$ . When compared to pyridines 2-8, the azepine analogs had significantly higher antibacterial efficacy. Compounds 10 and 11, containing 2-pyrazoline and thiazole moieties, showed high potency, and this was increased after inserting the aryl group, giving the arylidenes 12-14. 1-(5-*p*-Bromobenzyliden-4-oxothiazol-2-yl)-3-(thien-2-yl)-5-(1*H*-indol-3-yl)-2-pyrazoline (14) displayed the most potent antibacterial activity against *B. cereus*, *S. aureus*, *P. aeruginosa*, and *E. coli*, with MICs of 4, 15, 14, and 18  $\mu\text{g}/\text{ml}$ , respectively. *B. cereus* was the most sensitive bacterial species against 1-(5-*p*-bromobenzyliden-4-oxothiazol-2-yl)-3-(thien-2-yl)-5-(1*H*-indol-3-yl)-2-pyrazoline (14) possesses a MIC of 4  $\mu\text{g}/\text{mL}$ .

**Table 1.** The antibacterial activity of the synthesized compounds, MIC ( $\mu\text{g/mL}$ )

Compound No.	Gram positive		Gram negative	
	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas Aruginose</i>	<i>Escherichia Coli</i>
<b>2</b>	90	100	90	97
<b>3</b>	50	80	92	95
<b>4</b>	40	70	89	88
<b>5</b>	45	72	90	91
<b>6</b>	42	71	80	75
<b>7</b>	41	78	75	70
<b>8</b>	25	35	50	64
<b>9</b>	10	25	24	25
<b>10</b>	21	20	30	2
<b>11</b>	10	22	25	28
<b>12</b>	11	18	19	16
<b>13</b>	12	17	18	19
<b>14</b>	4	15	14	18
<b>15</b>	18	19	20	25
<b>16</b>	20	20	26	21
Reference*	10	10	10	10

\*Chloramphenicol was used as antibacterial standard. (–) no activity.

**Antifungal activity.** Compounds **2-16** were tested for antifungal action against the following species: *Geotrichum candidum*, *Candida albicans*, *Trichophyton rubrum*, and *Aspergillus flavus*. Clotrimazole was selected as the reference drug (Table 2). Similar to antibacterial actions, arylidene derivatives **12-14** exhibited high antifungal potency with MICs that ranged from 30-50  $\mu\text{g/mL}$ . Compound **14** showed unique MICs of 4, 15, 14, and 18  $\mu\text{g/mL}$  against fungal species; *G. candidum*, *C. albicans*, *T. rubrum*, and *A. flavus*, respectively. Compounds **15** and **16** having azepine nucleus generated acceptable results, with MICs ranging from 15 to 38  $\mu\text{g/mL}$ . *G. candidum* was one of the most inhibited fungi species from 4-(thienyl-2-yl)-2-(indol-3-yl)-2,3-dihydrobenzo [b][1,4]oxazepine (**16**) has a MIC of 15  $\mu\text{g/mL}$ .

**Table 2.** The antifungal activity of the synthesized compounds, MIC ( $\mu\text{g/mL}$ )

Compound No.	Pathogenic Fungi			
	<i>Geotrichum candidum</i>	<i>Candida albicans</i>	<i>Trichophyton rubrum</i>	<i>Aspergillus flavus</i>
<b>2</b>	-	110	100	98
<b>3</b>	115	105	95	90
<b>4</b>	100	98	-	87
<b>5</b>	94	90	82	77
<b>6</b>	88	89	76	-
<b>7</b>	74	-	-	-
<b>8</b>	70	72	64	-
<b>9</b>	67	68	60	70
<b>10</b>	64	60	57	61
<b>11</b>	50	55	52	57
<b>12</b>	42	50	50	50
<b>13</b>	38	48	49	46
<b>14</b>	30	42	40	40
<b>15</b>	25	37	38	37
<b>16</b>	15	30	30	20
Reference*	20	10	10	10

\* Clotrimazole was used as antifungal standard.

(-) no activity.

## Conclusions

This work presents a novel group of pyridines, pyridopyrimidines, 2-pyrazolines, and azepines structured on thiophene and indole nuclei. The chemical reactivity of the starting compound thienyl indolyl 2-propenone **1** was studied by its reactivity with various reagents. 2-Aminopyridines **2**, **3**, and **4** were obtained using active methylene compounds. In addition, 2-thioxopyridopyrimidinone **5** was achieved by the treatment of 6-amino-2-thiouracil with **1**. By reacting **5** with a variety of reagents, 2-substituted pyridopyrimidinones **6-8** were generated. Furthermore, 2-pyrazolines **9-14** and azepines **16**, and **17** derive from the synthesized starting compound **1**. Spectroscopic data (FTIR, NMR, and MS spectrometry) with the analysis of elements elucidated the compounds structures. The new compounds **2-16** have been evaluated for antimicrobial activity. The findings showed that 2-pyrazolines **9-14** have greater antibacterial activity than pyridine derivatives **2-4**. Furthermore, arylidenes with bromine atom **14** are more active than those with chlorine atom **13**. Azepines **15** and **16** (with seven membered rings) are more effective than compounds **2-4**, which have six members.

## Experimental Section

**General.** In this work, the analytical-quality substances were used. The melting points were determined *via* the melting point instrument, APP Digital ST 15. FTIR spectra were obtained with the Shimadzu-408 infrared

spectrophotometer and are shown in  $\text{cm}^{-1}$  mode. NMR spectra were gathered using a Bruker AV-400 spectrometer. TMS was used as a standard. Mass spectrometry was conducted with a Varian MAT 312 apparatus operating in EI mode at a scan energy of 70 eV. System GmbH vario EL V2.3 1998 CHNS Mode was used to analyze elements.

**General approach for the synthesis of 2-aminopyridines 2-4.** A mixture of **1** (0.50 g, 2 mmol), active methylene reagents (2 mmol), and  $\text{AcONH}_4$  (0.25 g, 3 mmol) was heated at reflux in AcOH (10 mL) for 6 h. Upon cooling, the separated product was filtered off, rinsed using water, and purified with ethanol.

**2-Amino-3-carboxamide-6-(thien-2-yl)-4-(indole-3-yl)pyridine (2).** Yield: 77%, white crystals: mp 180-183 °C. IR ( $\nu/\text{cm}^{-1}$ ): 3446, 3239, 3098 (br.  $\text{NH}_2$  and indole-NH), 3052 (Ar-H), 1628 (CO);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 7.01-7.84 (m, 7 H, 3 thienyl-H and  $2\text{NH}_2$ ), 7.97-8.54 (m, 6H, 5 indole-H and pyridine-H), 12.13 (s, 1H, indole-NH);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  112.9, 113.1, 115.6, 120.9, 121.6, 123.2, 125.6, 128.6, 129.1, 129.3, 132.5, 133.7, 134.4, 138.0, 138.7, 146.7, 166.7 (CO). ESIMS  $m/z$  (%) 334.2 [ $\text{M}^+$ ] (72). Anal. Calcd. For  $\text{C}_{18}\text{H}_{14}\text{N}_4\text{OS}$  (334.39): C, 64.65; H, 4.22; N, 16.75; S, 9.59. Found: C, 64.58; H, 4.16; N, 16.67; S, 9.48.

**Ethyl 2-amino-6-(thien-2-yl)-4-(indol-3-yl)pyridine-3-carboxylate (3).** Yield: 72%, orange crystals: mp 193-195°C. IR ( $\nu/\text{cm}^{-1}$ ): 3445, 3374, 3333 ( $\text{NH}_2$  and indole-NH), 3026 (Ar-H), 1711 (CO);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  1.31-1.38 (t,  $J$  24.4 Hz, 3H,  $\text{CH}_3$ ), 4.24-4.29 (q,  $J$  14.0, 7.2 Hz, 2H,  $\text{CH}_2$ ), 7.23-8.22 (m, 9 H, 3 thienyl-H, 5 indole-H and pyridine-H), 8.55 (s, 2H,  $\text{NH}_2$  vanished by  $\text{D}_2\text{O}$ ), 12.57 (s, 1H, indole-NH);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 14.5 ( $\text{CH}_3$ ), 61.8 ( $\text{CH}_2$ ), 92.8, 110.4, 112.9, 113.1, 115.6, 118.9, 120.8, 121.6, 123.2, 125.7, 128.6, 132.4, 133.0, 138.0, 138.6, 146.9, 176.9 (CO). ESIMS  $m/z$  (%) 363.2 [ $\text{M}^+$ ] (87). Anal. Calcd. For  $\text{C}_{20}\text{H}_{17}\text{N}_3\text{O}_2\text{S}$  (363.43): C, 66.10; H, 4.71; N, 11.56; S, 8.82. Found: C, 66.03; H, 4.64; N, 11.46; S, 8.70.

**2-Amino-3-cyano-6-(thien-2-yl)-4-(indole-3-yl)pyridine (4).** Yield: 68%, yellow crystals: mp 211-213 °C. IR ( $\nu/\text{cm}^{-1}$ ): 3350, 3210, 3121 ( $\text{NH}_2$  and indole-NH), 3047 (Ar-H), 2217 (CN);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  7.24-8.52 (m, 9 H, 3 thienyl-H, 5 indole-H and pyridine-H), 8.66 (s, 2H,  $\text{NH}_2$  vanished by  $\text{D}_2\text{O}$ ), 12.35 (s, 1H, indole-NH);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  98.2, 110.4, 111.4, 113.5, 116.4, 118.8, 119.2, 122.0, 123.0, 125.7, 128.6, 130.6, 133.7, 136.6, 138.6, 142.7, 153.0. ESIMS  $m/z$  (%) 316.2 [ $\text{M}^+$ ] (69). Anal. Calcd. for  $\text{C}_{18}\text{H}_{12}\text{N}_4\text{S}$  (316.38): C, 68.33; H, 3.82; N, 17.71; S, 10.13. Found: C, 68.27; H, 3.76; N, 17.60; S, 10.03.

**7-(Thien-2-yl)-5-(1H-indol-3-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (5).** 6-Amino-2-thiouracil (0.28 g, 2 mmol) was mixed with chalcone **1** (0.50 g, 2 mmol) in glacial AcOH (10 mL) then refluxed for 15 h. Once cooled, the liquid was added to broken ice and the resulting solid filtered off and purified from acetic acid giving a brown powder. Yield 74%: mp 221-223 °C. IR ( $\nu/\text{cm}^{-1}$ ): 3390, 3307, 3176 (2 NH and indole-NH), 3026 (Ar-H), 1659 (CO);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  6.65-8.39 (m, 9 H, 3 thienyl-H, 5 indole-H and pyridine-H), 11.00 (s, 1H, NH vanished by  $\text{D}_2\text{O}$ ), 12.70 (s, 1H, indole-NH), 13.25 (s, 1H, NH vanished by  $\text{D}_2\text{O}$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  100.9, 110.9, 112.1, 116.1, 117.4, 118.0, 120.6, 121.3, 129.7, 130.6, 132.6, 135.5, 135.9, 146.3, 150.6, 155.6, 165.2 (C=O), 176.0 (C=S). ESIMS  $m/z$  (%) 376.3 ( $\text{M}^+$ , 68%). Anal. Calcd. for  $\text{C}_{19}\text{H}_{12}\text{N}_4\text{OS}_2$  (376.45): C, 60.62; H, 3.21; N, 14.88; S, 17.04. Found: C, 60.54; H, 3.13; N, 14.80; S, 16.94.

**2-Hydrazinyl-7-(thien-2-yl)-5-(1H-indol-3-yl)pyrido[2,3-d]pyrimidin-4(3H)-one (6).** A solution of 2-thioxopyridopyrimidine (0.75 g, 2 mmol) in ethanol (10 mL) was treated with hydrazine hydrate (80%, 4 mL) and kept at reflux for 20 h. Upon cooling, the precipitate was collected and purified from DMF. Yield 68%, white powder: mp 301-302 °C. IR ( $\nu/\text{cm}^{-1}$ ): 3410 and 3235 (NH and  $\text{NH}_2$ ), 3180 (indole-NH), 1665 (CO);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  6.88-8.15 (m, 9 H, 3 thienyl-H, 5 indole-H and pyridine-H), 8.21 (s, 2H,  $\text{NH}_2$  vanished by  $\text{D}_2\text{O}$ ), 12.40 (s, 1H, indole-NH vanished with  $\text{D}_2\text{O}$ ), 13.00 (s, 1H, NH vanished by  $\text{D}_2\text{O}$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  101.8, 102.6, 106.2, 108.2, 127.7, 128.2, 129.4, 129.7, 130.9, 131.2, 133.8, 134.2, 150.6, 151.9, 158.3, 159.2,

167.5, 168.7, 168.9, 177.0. ESIMS  $m/z$  (%) 374.3 ( $M^+$ , 59%). Anal. Calcd. for  $C_{19}H_{14}N_6OS$  (374.42): C, 60.95; H, 3.77; N, 22.45; S, 8.56. Found: C, 60.89; H, 3.70; N, 22.40; S, 8.46.

**General process for synthesizing 2-pyrazoloyl pyrido[2,3-*d*]pyrimidines (7 and 8).**

2-hydrazinyl

pyridopyrimidine **6** (0.74 g, 2 mmol) was mixed with acetylacetone or ethyl acetoacetate (2 mmol) in ethanol and the mixture refluxed for 8 h. Upon cooling, the formed solid was collected, dried and recrystallized with 1,4-dioxane/ethanol.

**2-(3,5-Dimethyl-1H-pyrazol-1-yl)-7-(thien-2-yl)-5-(1H-indol-3-yl)pyrido[2,3-*d*]pyrimidin-4(3H)-one (7).** Yield: 82%, white crystals: mp 204-206 °C. IR ( $\nu/cm^{-1}$ ): 3385 (NH), 3148 (indole-NH), 2982 (Aliph-H), 1656 (CO);  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  1.70 (s, 3H, CH<sub>3</sub>), 2.20 (s, 3H, CH<sub>3</sub>), 6.60 (s, 1H, pyrazole-H<sub>4</sub>), 7.24–8.22 (m, 9 H, 3 thienyl-H, 5 indole-H and pyridine-H), 12.32 (s, 1H, indole-NH), 13.12 (s, 1H, NH vanished by D<sub>2</sub>O) ppm;  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  14.1 (CH<sub>3</sub>), 15.3 (CH<sub>3</sub>), 108.3, 111.8, 113.9, 114.8, 116.6, 117.7, 118.9, 120.4, 122.3, 125.9, 127.4, 129.2, 130.8, 132.6, 134.4, 135.7, 146.4, 151.2, 152.9, 154.1, 155.8, 166.2 ppm. ESIMS  $m/z$  (%) 438.4 ( $M^+$ , 62%). Anal. Calcd for  $C_{24}H_{18}N_6OS$  (438.50): C, 65.74; H, 4.14; N, 19.17; S, 7.31. Found: C, 65.68; H, 4.08; N, 19.11; S, 7.21.

**2-(3-Methyl-5-oxo-2-pyrazolin-1-yl)-7-(thien-2-yl)-5-(1H-indol-3-yl)pyrido[2,3-*d*]pyrimidin-4(3H)-one (8).** Yield: 76%, white plates: mp 199-201 °C. IR ( $\nu/cm^{-1}$ ): 3305 (NH), 3151 (indole-NH), 3058 (Ar-H), 1675 (CO), 1645 (pyrazolone-CO);  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  2.28 (s, 3H, CH<sub>3</sub>), 2.70 (s, 2H, pyrazoline-CH<sub>2</sub>), 7.12-8.34 (m, 9 H, 3 thienyl-H, 5 indole-H and pyridine-H), 12.61 (s, 1H, indole-NH), 13.00 (s, 1H, NH vanished by D<sub>2</sub>O) ppm;  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  = 17.5 (CH<sub>3</sub>), 42.6 (CH<sub>2</sub>), 107.9, 112.1, 114.2, 115.3, 117.8, 119.2, 121.0, 122.8, 126.1, 127.8, 129.0, 130.7, 132.5, 135.8, 145.8, 150.4, 152.8, 154.2, 155.9, 165.2, 167.4 ppm. ESIMS  $m/z$  (%) 440.3 ( $M^+$ , 81%). Anal. Calcd for  $C_{23}H_{16}N_6O_2S$  (440.48): C, 62.72; H, 3.66; N, 19.08; S, 7.28. Found: C, 62.66; H, 3.60; N, 19.00; S, 7.20.

**1-Thiocarbamoyl-3-(thien-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (9).** A solution of NaOH (4 mmol) in H<sub>2</sub>O (1 mL) was introduced to chalcone **1** (0.50 g, 2 mmol) and thiosemicarbazide (2 mmol) dissolved in ethanol (10 mL). The mixture was subjected to reflux for 10 h, following which the product was transferred to crushed ice. The solid mass underwent filtration, drying, and crystallization with dioxane. Yield: 68%, yellow crystals: mp 231-232 °C. IR ( $\nu/cm^{-1}$ ): 3447, 3312 (NH<sub>2</sub>), 3180 (NH), 3039 (Ar-H);  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 3.68-3.76 (dd,  $J$  24.0, 10.0 Hz, H<sub>A</sub>), 3.90-3.98 (dd,  $J$  20.0, 14.0 Hz, H<sub>B</sub>), 6.29-6.35 (dd,  $J$  14.0, 10.0 Hz, H<sub>X</sub>), 7.12-8.28 (m, 8 H, 3 thienyl-H and 5 indole-H), 8.34 (s, 2H, NH<sub>2</sub>), 11.59 (s, 1H, indole-NH) ppm;  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  = 49.1 (pyrazoline-CH<sub>2</sub>), 66.8 (pyrazoline-CH), 111.6, 112.9, 113.1, 115.6, 120.9, 121.1, 122.5, 123.9, 124.4, 125.7, 128.6, 138.0, 141.3, 177.0 ppm. ESIMS  $m/z$  (%) 326.2 ( $M^+$ , 81%). Anal. Calcd. for  $C_{16}H_{14}N_4S_2$  (326.44): C, 58.87; H, 4.32; N, 17.16; S, 19.65. Found: C, 58.80; H, 4.25; N, 17.04; S, 19.53.

**1-(4-Oxo-5H-thiazol-2-yl)-3-(thien-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (10).** An equimolar mixture containing 1-thiocarbamoyl-2-pyrazoline **9** (0.65 g, 2 mmol), chloroacetic acid (0.18 g, 2 mmol), with anhydrous NaOAc (0.40 g) in glacial AcOH (20 mL) was refluxed for 10 h. The formed solid while cooling was crystallized with DMF. Yield: 68%, white crystals: mp 177-178 °C. IR ( $\nu/cm^{-1}$ ): 3247 (NH), 3051 (Ar-H), 1703 (CO);  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 3.50-3.57 (dd,  $J$  20.0, 8.0 Hz, H<sub>A</sub>), 3.89 (s, 2H, CH<sub>2</sub>), 4.22-4.29 (dd,  $J$  16.0, 12.0 Hz, H<sub>B</sub>), 6.18-6.23 (dd,  $J$  12.0, 8.0 Hz, H<sub>X</sub>), 7.17-8.57 (m, 8 H, 3 thienyl-H and 5 indole-H), 11.68 (s, 1H, indole-NH) ppm;  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  48.2 (pyrazoline-CH<sub>2</sub>), 60.4 (thiazole-CH<sub>2</sub>), 66.5 (pyrazoline-CH), 112.4, 121.2, 122.5, 123.2, 124.6, 125.0, 128.5, 132.1, 134.8, 137.6, 141.1, 142.4, 148.3, 167.8 (CO) ppm. ESIMS  $m/z$  (%) 366.3 ( $M^+$ , 72%). Anal. Calcd. for  $C_{18}H_{14}N_4OS_2$  (366.46): C, 58.99; H, 3.85; N, 15.29; S, 17.50. Found: C, 58.92; H, 3.78; N, 15.20; S, 17.40.

**1-(4-Phenylthiazol-2-yl)-3-(thien-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (11).** Phenacyl bromide (0.39 g, 2 mmol) was mixed with 1-thiocarbamoyl-2-pyrazoline **9** (0.65 g, 2 mmol) in ethanol (25 mL) and refluxed for 6 h. The

solid obtained upon cooling filtered off and crystallized in 1,4-dioxane. Yield: 64%, brown powder: mp 190-192 °C. IR ( $\nu/\text{cm}^{-1}$ ): 3151 (NH), 3051 (Ar-H);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 3.91-3.98 (dd,  $J$  18.0, 10.0 Hz,  $\text{H}_A$ ), 4.16-4.23 (dd,  $J$  16.0, 12.0 Hz,  $\text{H}_B$ ), 6.12-6.18 (dd,  $J$  12.0, 10.0 Hz,  $\text{H}_X$ ), 7.13-8.15 (m, 14 H, 5 Ar-H, 3 thienyl-H, 5-indole-H and thiazole-H), 9.00 (s, 1H, thiazole-H), 11.70 (s, 1H, indole-NH) ppm;  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  = 48.2 (pyrazoline- $\text{CH}_2$ ), 60.1 (thiazole- $\text{CH}_2$ ), 66.5 (pyrazoline-CH), 101.8, 102.5, 106.3, 108.2, 127.8, 128.0, 128.7, 129.6, 129.9, 130.9, 131.1, 133.9, 134.2, 150.7, 151.8, 158.0, 159.1 ppm. ESIMS  $m/z$  (%) 429.1 ( $\text{M}^+$ , 73%). Anal. Calcd. for  $\text{C}_{24}\text{H}_{18}\text{N}_4\text{S}_2$  (426.56): C, 67.58; H, 4.25; N, 13.13; S, 15.03. Found: C, 67.51; H, 4.18; N, 13.02; S, 15.13.

**General route for the synthesis of 1-arylidene-thiazolyl-2-pyrazolines (12-14).** The aromatic aldehydes (2 mmol), anhydrous AcONa (0.40 g), and compound **10** (0.73 g, 2 mmol) were refluxed in AcOH (10 mL) for 6 h. The solution was cooled before being added to broken ice. The solid was obtained through filtration, drying, and crystallization with dioxane, resulting in the formation of products **12-14**.

**1-(5-Benzyliden-4-oxothiazol-2-yl)-3-(thien-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (12).** Yield: 80%, orange powder: mp 230-231 °C. IR ( $\nu/\text{cm}^{-1}$ ): 3130 (NH), 3025 (Ar-H), 1711 (CO);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.31-3.39 (dd,  $J$  24.0, 10.0 Hz,  $\text{H}_A$ ), 3.90-3.98 (dd,  $J$  20.0, 12.0 Hz,  $\text{H}_B$ ), 6.11-6.16 (dd,  $J$  12.0, 10.0 Hz,  $\text{H}_X$ ), 6.84-8.65 (m, 14 H, 5 Ar-H, 3 thienyl-H, 5 indole-H and =CH-), 11.69 (s, 1H, indole-NH) ppm;  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  44.2 (pyrazoline- $\text{CH}_2$ ), 66.8 (pyrazoline-CH), 111.6, 112.4, 116.1, 116.5, 118.6, 119.3, 120.9, 121.1, 123.1, 125.8, 125.9, 127.8, 129.8, 130.0, 132.0, 136.6, 137.6, 152.4, 155.8, 160.3, 168.6 (CO) ppm. ESIMS  $m/z$  (%) 454.4 ( $\text{M}^+$ , 62%). Anal. Calcd. for  $\text{C}_{25}\text{H}_{18}\text{N}_4\text{OS}_2$  (454.57): C, 66.06; H, 3.99; N, 12.33; S, 14.11. Found: C, 66.00; H, 3.97; N, 12.25; S, 14.04.

**1-(5-*p*-Chlorobenzyliden-4-oxothiazol-2-yl)-3-(thien-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (13).** Yield: 69%, pale yellow crystals: mp 200-201 °C. IR ( $\nu/\text{cm}^{-1}$ ): 3130 (NH), 3068 (Ar-H), 1710 (CO);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.32-3.40 (dd,  $J$  24.0, 10.0 Hz,  $\text{H}_A$ ), 3.91-3.99 (dd,  $J$  0.0, 12.0 Hz,  $\text{H}_B$ ), 6.12-6.17 (dd,  $J$  2.0, 10.0 Hz,  $\text{H}_X$ ), 6.90-8.60 (m, 13 H, 4 Ar-H, 3 thienyl-H, 5 indole-H and =CH-), 11.71 (s, 1H, indole-NH) ppm; ESIMS  $m/z$  (%) 488.9 ( $\text{M}^+$ , 73%) and 491.0 ( $\text{M}^+ + 1$ , 24%). Anal. Calcd. for  $\text{C}_{25}\text{H}_{17}\text{ClN}_4\text{OS}_2$  (489.01): C, 61.40; H, 3.50; Cl, 7.25; N, 11.46; S, 13.11. Found: C, 61.33; H, 3.42; Cl, 7.14; N, 11.40; S, 13.01.

**1-(5-*p*-Bromobenzyliden-4-oxothiazol-2-yl)-3-(thien-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (14).** Yield: 77%, red crystals: mp 220-221 °C. IR ( $\nu/\text{cm}^{-1}$ ): 3145 (NH), 3012 (Ar-H), 1722 (CO);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.46-3.50 (dd,  $J$  15.0, 7.0 Hz,  $\text{H}_A$ ), 3.92-3.95 (dd,  $J$  10.0, 8.5 Hz,  $\text{H}_B$ ), 6.34-6.36 (dd,  $J$  8.5, 7.0 Hz,  $\text{H}_X$ ), 6.90-8.60 (m, 13 H, 4 Ar-H, 3 thienyl-H, 5 indole-H and =CH-), 11.71 (s, 1H, indole-NH) ppm; ESIMS  $m/z$  (%) 533.2 ( $\text{M}^+$ , 54%) and 534.3 ( $\text{M}^+ + 1$ , 53%). Anal. Calcd. for  $\text{C}_{25}\text{H}_{17}\text{BrN}_4\text{OS}_2$  (533.46): C, 56.29; H, 3.21; Br, 14.98; N, 10.50; S, 12.02. Found: C, 56.23; H, 3.14; Br, 14.87; N, 10.40; S, 12.18.

**General method for the synthesis of azepines (15 and 16).** A mixture of **1** (0.50 g, 2 mmol) and either *o*-phenylenediamine or *o*-aminophenol (2 mmol) in ethanol (10 mL) along with triethylamine (3-4 drops) was refluxed for 10 h. The solution was subjected to cooling at 0 °C and permitted to stand overnight. The solid product was filtered off, rinsed with water, and then purified in 1,4-dioxane.

**4-(Thieny-2-yl)-2-(indol-3-yl)-2,3-dihydro-1H-benzo[*b*][1,4]diazepine (15).** Yellow crystals: mp 223-225 °C. IR ( $\nu/\text{cm}^{-1}$ ): 3333 (NH), 3172 (indole-NH), 3074 (Ar-H)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 3.13-3.20 (dd,  $J$  16.0, 12.0 Hz,  $\text{H}_A$ ), 3.81-3.88 (dd,  $J$  15.2, 13.2 Hz,  $\text{H}_B$ ), 4.25 (s, 1H, NH), 5.00-5.56 (dd,  $J$  13.2, 12.0 Hz,  $\text{H}_Z$ ), 7.24-8.56 (m, 12H, thienyl-H, indole-H, and benzodiazepine-H), 12.48 (s, 1H, indole-NH) ppm;  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  = 18.5 ( $\text{CH}_2$ ), 46.2 (CH), 60.2, 68.6, 116.2, 118.4, 120.9, 121.6, 122.04, 132.87, 133.6, 137.5, 137.8, 145.5, 150.4, 153.3, 155.2, 155.9, 158.0. ESIMS  $m/z$  (%) 343.2 [ $\text{M}^+$ ] (77). Anal. Calcd. for  $\text{C}_{21}\text{H}_{17}\text{N}_3\text{S}$  (343.44): C, 73.44; H, 4.99; N, 12.23; S, 9.34. Found: C, 73.39; H, 4.93; N, 12.13; S, 9.23.

**4-(Thieny-2-yl)-2-(indol-3-yl)-2,3-dihydrobenzo[*b*][1,4]oxazepine (16).** White solid: mp 230-232 °C. IR ( $\nu$ /cm<sup>-1</sup>): 3148 (indole-NH), 3008 (Ar-H) cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 3.30-3.40 (dd, *J* 24.0, 16.0 Hz, H<sub>A</sub>), 4.10-4.19 (dd, *J* 20.0, 18.0 Hz, H<sub>B</sub>), 5.70-5.78 (dd, *J* 18.0, 16.0 Hz, H<sub>Z</sub>), 7.20-8.60 (m, 12H, thienyl-H, indole-H, and benzodioxazepine-H), 12.32 (s, 1H, indole-NH) ppm; <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 20.3 (CH<sub>2</sub>), 48.9 (CH), 62.0, 70.2, 116.4, 118.5, 120.8, 121.5, 122.4, 123.8, 125.9, 132.7, 133.5, 137.4, 137.3, 145.0, 150.2, 153.0, 155.6, 155.8, 158.2. ESIMS *m/z* (%) 344.1 [M<sup>+</sup>] (70). Anal.Calcd. for C<sub>21</sub>H<sub>16</sub>N<sub>2</sub>OS (344.43): C, 73.23; H, 4.68; N, 8.13; S, 9.31. Found: C, 73.16; H, 4.60; N, 8.02; S, 9.24.

### Biological activity

**In vitro antimicrobial assay procedure.** The studied compounds **2-16** were dissolved in DMSO resulting in a 5% solution. The resulting solution was applied to saturate filter paper discs (Whatman No. 3 and 5 mm in diameter). The discs were set on the surface of solidified nutrient agar dishes seeded with the bacteria that were examined (*Gram positive; Bacillus cereus and Staphylococcus aureus, and Gram negative; Pseudomonas aruginose or Escherichia coli*) or Czapek Dox agar dishes seeded with the fungi that were investigated (*Geotrichum candidum, Candida albicans, Trichophyton rubrum, and Aspergillus flavus*). The diameter of inhibiting zones (mm) was measured at the end of the incubation period (24-48 h) at 37 °C for bacteria and 28 °C with fungi (4-7 days) [48]. Discs soaked with DMSO were employed as controls. *Chloramphenicol* and *Clotrimazole* were chosen as references. The biologically active compounds were subsequently diluted with DMSO to produce an array of concentrations with the goal to ascertain each compound's MIC. The MIC values were calculated as  $\mu\text{g/mL}$  Tables 1 and 2 provide the antibacterial and antifungal activity data.

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## Supplementary Material

The FTIR spectra, <sup>1</sup>H NMR and <sup>13</sup>C NMR of the new compounds can be found in the supplementary material file.

## References

1. Lou, X.; Yang, Y. *W. J. Am. Chem. Soc.* **2021**, *28*, 11976-11981.  
<https://doi.org/10.1021/jacs.1c07006>

2. Henry, G. D. *Tetrahedron* **2004**, *60*, 6043.  
<https://doi.org/10.1016/j.tet.2004.04.043>
3. Brycki, B.; Małacka, I.; Koziróg, A.; Otlewska, A. *Molecules* **2017**, *22*, 130.  
<https://doi.org/10.3390/molecules22010130>
4. Leal, B.; Afonso, I. F.; Rodrigues, C. R.; Abreu, P. A.; Garrett, R.; Pinheiro, L. C. S.; Azevedo, A. R.; Borges, J. C.; Vegi, P. F.; Santos, C. C. C. *Bioorg. Med. Chem.* **2008**, *16*, 8196-8204.  
<https://doi.org/10.1016/j.bmc.2008.07.035>
5. Zav'yalova, V. K.; Zubarev, A. A.; Shestopalov, A. M. *Russ. Chem. Bull.* **2009**, *58*, 1939-1944.  
<https://doi.org/10.1007/s11172-009-0265-2>
6. Bernardino, A. M. R.; da Silva Pinheiro, L. C.; Rodrigues, C. R.; Loureiro, N. I.; Castro, H. C.; Lanfredi-Rangel, A.; Sabatini-Lopes, J.; Borges, J. C.; Carvalho, J. M.; Romeiro, G. A. *Bioorg. Med. Chem.* **2006**, *14*, 5765-5777.  
<https://doi.org/10.1016/j.bmc.2006.03.013>
7. Hayakawa, M.; Kaizawa, H.; Kawaguchi, K.; Ishikawa, N.; Koizumi, T.; Ohishi, T.; Yamano, M.; Okada, M.; Ohta, M.; Tsukamoto, S. *Bioorg. Med. Chem.* **2007**, *15*, 403-412.  
<https://doi.org/10.1016/j.bmc.2006.09.047>
8. Truong, D. T.; Ho, K.; Nhi, H. T. Y.; Nguyen, V. H.; Dang, T. T.; Nguyen, M. T. *Sci. Rep.* **2024**, *28*, 12218.  
<https://doi.org/10.1038/s41598-024-62743-3>
9. Janković, Đ. D.; Šestić, T. L.; Bekić, S. S.; Savić, M. P.; Ćelić, A. S.; Scholda, J.; Kopp, F.; Marinović, M. A.; Petri, E. T.; Ajduković, J. J. *Steroid Biochem. Mol. Biol.* **2024**, *16*, 106545.  
<https://doi.org/10.1016/j.jsbmb.2024.106545>
10. Li, A.; Huang, K.; Pan, W.; Wu, Y.; Liang, Y.; Zhang, Z.; Wu, D.; Ma, L.; Gou, Y. *J. Med. Chem.* **2024**, *67*, 9091-9103.  
<https://doi.org/10.1021/acs.jmedchem.4c00257>
11. Adib, M.; Peytam, F.; Rahmanian-Jazi, M.; Mohammadi Khanaposhtani, M.; Mahernia, S.; Bijanzadeh, H. R.; Jahani, M.; Imanparast, S.; Faramarzi, M. A.; Mahdavi, M. *New J. Chem.* **2018**, *42*, 17268-17278.  
<https://doi.org/10.1039/C8NJ02495B>
12. Suresh, L.; Kumar, P. S. V.; Onkar, P.; Srinivas, L.; Pydisetty, Y.; Chandramouli, G. V. P. *Res. Chem. Intermed.* **2017**, *43*, 5433-5451.  
<https://doi.org/10.1007/s11164-017-2938-z>
13. Podila, N.; Poddinti, N. K. Rudrapal, M. Rakshit, G.; Konidala, S. K.; Pulusu, V. S.; Bhandare, R. R.; Shaik, A. B. *Heliyon* **2024**, *10*, e29390.  
<https://doi.org/10.1016/j.heliyon.2024.e29390>
14. Bharate, S. B.; Mahajan, T. R.; Gole, Y. R.; Nambiar, M.; Matan, T. T.; Kulkarni-Almeida, A.; Balachandran, S.; Junjappa, H.; Balakrishnan, A.; Vishwakarma, R. A. *Bioorganic Med. Chem.* **2008**, *16*, 7167-7176.  
<https://doi.org/10.1016/j.bmc.2008.06.042>
15. Hamblin, J. N.; Angell, T. D. R.; Ballantine, S. P.; Cook, C. M.; Cooper, A. W. J.; Dawson, J.; Delves, C. J.; Jones, P. S.; Lindvall, M.; Lucas, F. S. *Bioorganic Med. Chem. Lett.* **2008**, *18*, 4237-4241.  
<https://doi.org/10.1016/j.bmcl.2008.05.052>
16. Tok, F.; Erdoğan, Ö.; Çevik, Ö.; Koçyiğit-Kaymakçioğlu, B. *Acta Chim. Slov.* **2022**, *69*, 293-303.  
<https://doi.org/10.17344/acsi.2021.7119>
17. Chen, K.; Zhang, Y. L.; Fan, J.; Ma, X.; Qin, Y. J.; Zhu, H. L. *Eur. J. Med. Chem.* **2018**, *156*, 722-737.  
<https://doi.org/10.1016/j.ejmech.2018.07.044>

18. Sever, B.; Altıntop, M. D.; Radwan, M. O.; Özdemir, A.; Otsuka, M.; Fujita, M.; Ciftci, H. I. *Eur. J. Med. Chem.* **2019**, *182*, 111648.  
<https://doi.org/10.1016/j.ejmech.2019.111648>
19. Gomha, S. M.; Abdelaziz, M. R.; Kheder, N. A.; Abdel-Aziz, H. M.; Alterary, S.; Mabkhot, Y. N. *Chem. Cent. J.* **2017**, *11*, 105.  
<https://doi.org/10.1186/s13065-017-0335-8>
20. Abdel-Wahab, B. F.; Abdel-Aziz, H. A.; Ahmed, E. M. *Eur. J. Med. Chem.* **2009**, *44*, 2632-2635.  
<https://doi.org/10.1016/j.ejmech.2008.09.029>
21. Pola, S.; Banoth, K. K.; Sankaranarayanan, M.; Ummani, R.; Garlapati, A. *Med. Chem. Res.* **2020**, *29*, 1819-1835.  
<https://doi.org/10.1007/s00044-020-02602-8>
22. Dawane, B. S.; Konda, S. G.; Mandawad, G. G.; Shaikh, B. M. *Eur. J. Med. Chem.* **2021**, *45*, 387-392.  
<https://doi.org/10.1016/j.ejmech.2009.10.015>
23. Hassan, S. Y. *Molecules* **2013**, *18*, 2683-2711.  
<https://doi.org/10.3390/molecules18032683>
24. Dhonnar, S. L.; Jagdale, B. S.; Adole, V. A.; Sadgir, N. V. *Mol. Divers.* **2023**, *27*, 2441-2452.  
<https://doi.org/10.1007/s11030-022-10562-x>
25. Karad, S. C.; Purohit, V. B.; Thakor, P.; Thakkar, V. R.; Raval, D. K. *Eur. J. Med. Chem.* **2016**, *112*, 270-279.  
<https://doi.org/10.1016/j.ejmech.2016.02.016>
26. Ramírez-Prada, J.; Robledo, S. M.; Vélez, I. D.; Crespo, M. D. P.; Quiroga, J.; Abonia, R.; Montoya, A.; Svetaz, L.; Zacchino, S.; Insuasty, B. *Eur. J. Med. Chem.* **2017**, *131*, 237-254.  
<https://doi.org/10.1016/j.ejmech.2017.03.016>
27. Deng, H.; Yu, Z. Y.; Shi, G. Y.; Chen, M. J.; Tao, K.; Hou, T. P. *Chem. Biol. Drug Des.* **2012**, *79*, 279-789.  
<https://doi.org/10.1111/j.1747-0285.2011.01308.x>
28. Ovais, S.; Pushpalatha, H.; Reddy, G. B.; Rathore, P.; Bashir, R.; Yaseen, S. Javed, K. *Eur. J. Med. Chem.* **2014**, *80*, 209-217.  
<https://doi.org/10.1016/j.ejmech.2014.04.046>
29. Mantzanidou, M.; Pontiki, E.; Hadjipavlou-Litina, D. *Molecules* **2021**, *26*, 3439.  
<https://doi.org/10.3390/molecules26113439>
30. Vasudha, D.; Jagadeesh, A.; Mali, S. N.; Bhandare, R. R.; Shaik, A. B. *Chem. Phys. Impact* **2024**, *8*, 100500.  
<https://doi.org/10.1016/j.chphi.2024.100500>
31. Abeed, A. A. O.; Abdel Jaleel, G. A.; Youssef, M. S. K. *Curr. Org. Synth.* **2019**, *16*, 921-930.  
<https://doi.org/10.2174/1570179416666190703115133>
32. Asif, M.; Almehmadi, M. M.; Alsaiari, A.; Allahyani, M. *Curr. Org. Synth.* **2023**, *21*, 858-888.  
<https://doi.org/10.2174/0115701794260444230925095804>
33. Ziarani, G. M.; Hasani, S.; Mohajer, F.; Varma, R. S.; Rafiee, F. *Top Curr. Chem.* **2022**, *380*, 24.  
<https://doi.org/10.1007/s41061-022-00379-5>
34. Venugopala, K. N.; Habeebuddin, M.; Aldhubiab, B. E.; Asif, A. H. *Molecules* **2021**, *26*, 5235.  
<https://doi.org/10.3390/molecules26175235>
35. Böttcher, C.; Chapman, A.; Fellermeier, F.; Choudhary, M.; Scheel, D.; Glawischnig, E. *Plant Physiol.* **2014**, *165*, 841-853.  
<https://doi.org/10.1104/pp.114.235630>
36. Gallego-Yerga, L.; Chiliquinga, A. J.; Peláez, R. *Int. J. Mol. Sci.* **2023**, *24*, 11093.  
<https://doi.org/10.3390/ijms241311093>

37. Chen, H.; Deng, S.; Albadari, N.; Yun, M. K.; Zhang, S.; Li, Y.; Ma, D.; Parke, D. N.; Yang, L.; Seagroves, T. N.; White, S. W.; Miller, D. D.; Li, W. *J. Med. Chem.* **2024**, *64*, 12049-12074.  
<https://doi.org/10.1021/acs.jmedchem.1c00715>
38. Song, M.; Hua, Y.; Liu, Y.; Xiao, X.; Yu, H.; Deng, X. *Molecules* **2023**, *28*, 6325.  
<https://doi.org/10.3390/molecules28176325>
39. Aboshouk, D. R.; Youssef, M. A.; Bekheit, M. S.; Hamed, A. R.; Girgis, A. S. *RSC Adv.* **2024**, *14*, 5690-5728.  
<https://doi.org/10.1039/D3RA08962B>
40. Yadav, R.; Meena, D.; Singh, K.; Tyagi, R.; Yadav, Y.; Sagar, R. *RSC Adv.* **2023**, *13*, 21890-21925.  
<https://doi.org/10.1039/D3RA03862A>
41. Rupa, S. A.; Moni, M. R.; Patwary, M. A. M.; Mahmud, M. M.; Haque, M. A.; Uddin, J.; Abedin, S. M. T. *Molecules*, **2022**, *27*, 1656.  
<https://doi.org/10.3390/molecules27051656>
42. Hassneen, H. M.; Abdallah, T. A. *Molecules*, **2003**, *8*, 333-341.  
<https://doi.org/10.3390/80300333>
43. Mabkhot, Y. N.; Alatibi, F.; El-Sayed, N. N.; Al-Showiman, S.; Kheder, N. A.; Wadood, A.; Rauf, A.; Bawazeer, S.; Hadda, T. B. *Molecules* **2016**, *21*, 222.  
<https://doi.org/10.3390/molecules21020222>
44. Mete, E.; Gul, H. I.; Kazaz, C. *Molecules* **2007**, *12*, 2579-2588.  
<https://doi.org/10.3390/12122579>
45. Akulov, A. A.; Varaksin, M. V.; Charushin, V. N.; Chupakhin, O. N. *ACS Omega* **2019**, *10*, 825-834.  
<https://doi.org/10.1021/acsomega.8b02916>
46. Meleddu, R.; Petrikaite, V.; Distinto, S.; Arridu, A.; Angius, R.; Serusi, L.; Škarnulytė, L.; Endriulaitytė, U.; Paškevičius, T. M.; Cottiglia, F.; Gaspari, M.; Taverna, D.; Deplano, S.; Fois, B.; Maccioni, E. *ACS Med. Chem. Lett.* **2018**, *10*, 571.  
<https://doi.org/10.1021/acsmchemlett.8b00596>
47. Tsukerman, S. V.; Nikitchenko, V. M.; Bugai, A. I.; Lavrushine, V. F. *Chem. Heterocycl. Comp.* **1969**, *5*, 268-272.  
<https://doi.org/10.1007/BF00475844>
48. Kwon-Chung, K. J.; Bennett, J. E. *Medical Mycology*, Lea & Febiger, Philadelphia, PA **1992**, 866.

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