

Article

## Synthesis and Antimicrobial Activity of Some New Pyrimidinone and Oxazinone Derivatives Fused with Thiophene Rings Using 2-Chloro-6-ethoxy-4-acetylpyridine as Starting Material

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**Abstract:** A series of pyridines, pyrimidinones, oxazinones and their derivatives were synthesized as antimicrobial agents using citrazinic acid (2,6-dihydroxyisonicotinic acid) as a starting material.  $\alpha,\beta$ -Unsaturated ketones **3a–c** were condensed with cyanothioacetamide in the presence of ammonium acetate to give 2-cyanopyridinethiones **4a–c**, which were reacted with ethyl chloroacetate to yield the corresponding cyano esters **5a–c**. The esters **5a–c** were cyclized by action of sodium methoxide to aminoesters **6a–c**, which were aminolyzed with ammonia to corresponding aminoamide derivatives **7a–c**. Also, the esters **6a–c** were hydrolyzed with NaOH to the corresponding sodium salt **8a–c**, which were treated with acetic anhydride to afford 2-methyloxazinones **9a–c**. The latter compounds were treated with ammonium acetate to afford 2-methylpyrimidinones **10a–c**, followed by methylation with methyl iodide to yield 2,3-dimethyl-pyrimidinones **11a–c**. The antimicrobial screening showed that many of these compounds have good antibacterial and antifungal activities comparable to streptomycin and fusidic acid used as reference drugs.

**Keywords:** citrazinic acid; acryloyl candidates; oxazinone; pyrimidinone; antimicrobial agents

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## 1. Introduction

In previous work, we have found that certain substituted pyridines and their derivatives showed antimicrobial, analgesic, anticonvulsant, antiparkinsonian [1–4] and antitumor activities [5–7]. In addition, the biological and analgesic activities of many heterocyclic compounds containing a sulfur atom have been reviewed [8–10]. On the other hand, thienopyrimidine and thioxopyrimidine derivatives have promising biological [11,12] and anticancer activity [13]. Recently, some new oxazinones, thienopyrimidinones and their derivatives have been synthesized as anti-inflammatory, antimicrobial and anti-HIV agents [14–18]. In view of these observations and in continuation of our previous work in pyridine chemistry, we have now synthesized some novel heterocyclic compounds containing the thieno[2,3-*b*]pyridine moiety fused with a pyridine, oxazinone, or pyrimidinone, nucleus and tested their antimicrobial activities.

## 2. Results and Discussion

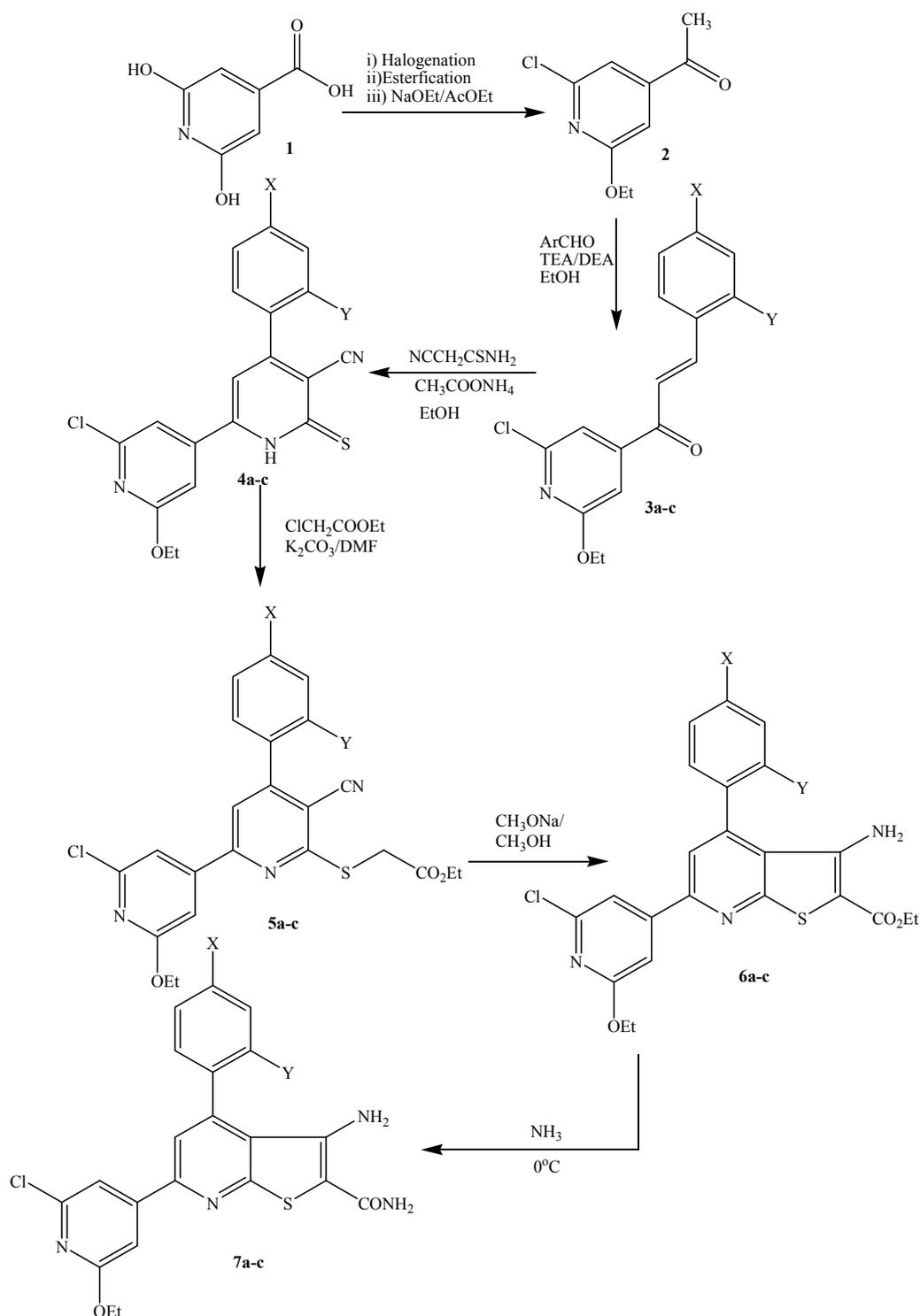
### 2.1. Synthesis

The starting materials **3a–c** (Table 1) were prepared from 2,6-dihydroxyisonicotinic acid (**1**) via the corresponding 2-chloro-6-ethoxy-4-acetylpyridine **2** according to literature methods [1,19]. Acryloyl derivatives **3a–c** were condensed with 2-cyanothioacetamide in the presence of ammonium acetate to give the corresponding cyanopyridine thione derivatives **4a–c** (Table 1). Treatment of **4a–c** with ethyl chloroacetate in the presence of anhydrous K<sub>2</sub>CO<sub>3</sub> gave the corresponding ethyl ester derivative **5a–c** (Table 1), which were cyclized by sodium methoxide in methanol to give the amino ester derivatives **6a–c** (Table 1). Aminolysis of compounds **6a–c** by action of ammonia gas afforded the corresponding aminoamide derivatives **7a–c** (Scheme 1, Table 1). The IR spectra of **6a–c** showed the absence of  $\nu$  (C≡N) for **5a–c** and the presence of broad band corresponding to  $\nu$  (NH<sub>2</sub>). Also, the IR spectra of **7a–c** showed the absence of  $\nu$  (C=O, ester) for **6a–c** and the presence of a broad band corresponding to  $\nu$  (NH<sub>2</sub>).

**Table 1.** Melting points, crystallization solvents, yields, molecular formulae and molecular weights of compounds **3–7**.

Comp. No.	X	Y	Yield (%)	M.p. (°C)	Cryst. Solv.	Molecular Formula (Mol. Wt.)
<b>3a</b>	F	H	86	185–187	EtOH	C <sub>16</sub> H <sub>13</sub> ClFNO <sub>2</sub> (505.73)
<b>3b</b>	Cl	H	82	155–157	EtOH	C <sub>16</sub> H <sub>13</sub> Cl <sub>2</sub> NO <sub>2</sub> (322.19)
<b>3c</b>	Cl	Cl	85	203–205	EtOH	C <sub>16</sub> H <sub>12</sub> Cl <sub>3</sub> NO <sub>2</sub> (356.63)
<b>4a</b>	F	H	65	192–194	DMF/H <sub>2</sub> O (2:1)	C <sub>19</sub> H <sub>13</sub> ClFN <sub>3</sub> OS (385.84)
<b>4b</b>	Cl	H	58	206–208	AcOH/H <sub>2</sub> O (2:1)	C <sub>19</sub> H <sub>13</sub> Cl <sub>2</sub> N <sub>3</sub> OS (402.30)
<b>4c</b>	Cl	Cl	70	225–227	DMF/H <sub>2</sub> O (2:1)	C <sub>19</sub> H <sub>12</sub> Cl <sub>3</sub> N <sub>3</sub> OS (436.74)
<b>5a</b>	F	H	78	198–200	EtOH/Ether (2:1)	C <sub>23</sub> H <sub>19</sub> ClFN <sub>3</sub> O <sub>3</sub> S (471.93)
<b>5b</b>	Cl	H	76	189–191	EtOH/Ether (2:1)	C <sub>23</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>3</sub> S (488.39)
<b>5c</b>	Cl	Cl	69	245–257	EtOH/Ether (2:1)	C <sub>23</sub> H <sub>18</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>3</sub> S (522.83)
<b>6a</b>	F	H	65	176–178	Dioxane	C <sub>23</sub> H <sub>19</sub> ClFN <sub>3</sub> O <sub>3</sub> S (471.93)
<b>6b</b>	Cl	H	70	214–216	EtOH	C <sub>23</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>3</sub> S (488.39)
<b>6c</b>	Cl	Cl	58	235–237	DMF/EtOH (2:1)	C <sub>23</sub> H <sub>18</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>3</sub> S (522.83)
<b>7a</b>	F	H	86	200–202	MeOH	C <sub>21</sub> H <sub>16</sub> ClFN <sub>4</sub> O <sub>2</sub> S (442.89)
<b>7b</b>	Cl	H	85	228–230	AcOH	C <sub>21</sub> H <sub>16</sub> Cl <sub>2</sub> N <sub>4</sub> O <sub>2</sub> S (459.35)
<b>7c</b>	Cl	Cl	84	256–258	AcOH/H <sub>2</sub> O (2:1)	C <sub>21</sub> H <sub>15</sub> Cl <sub>3</sub> N <sub>4</sub> O <sub>2</sub> S (493.79)

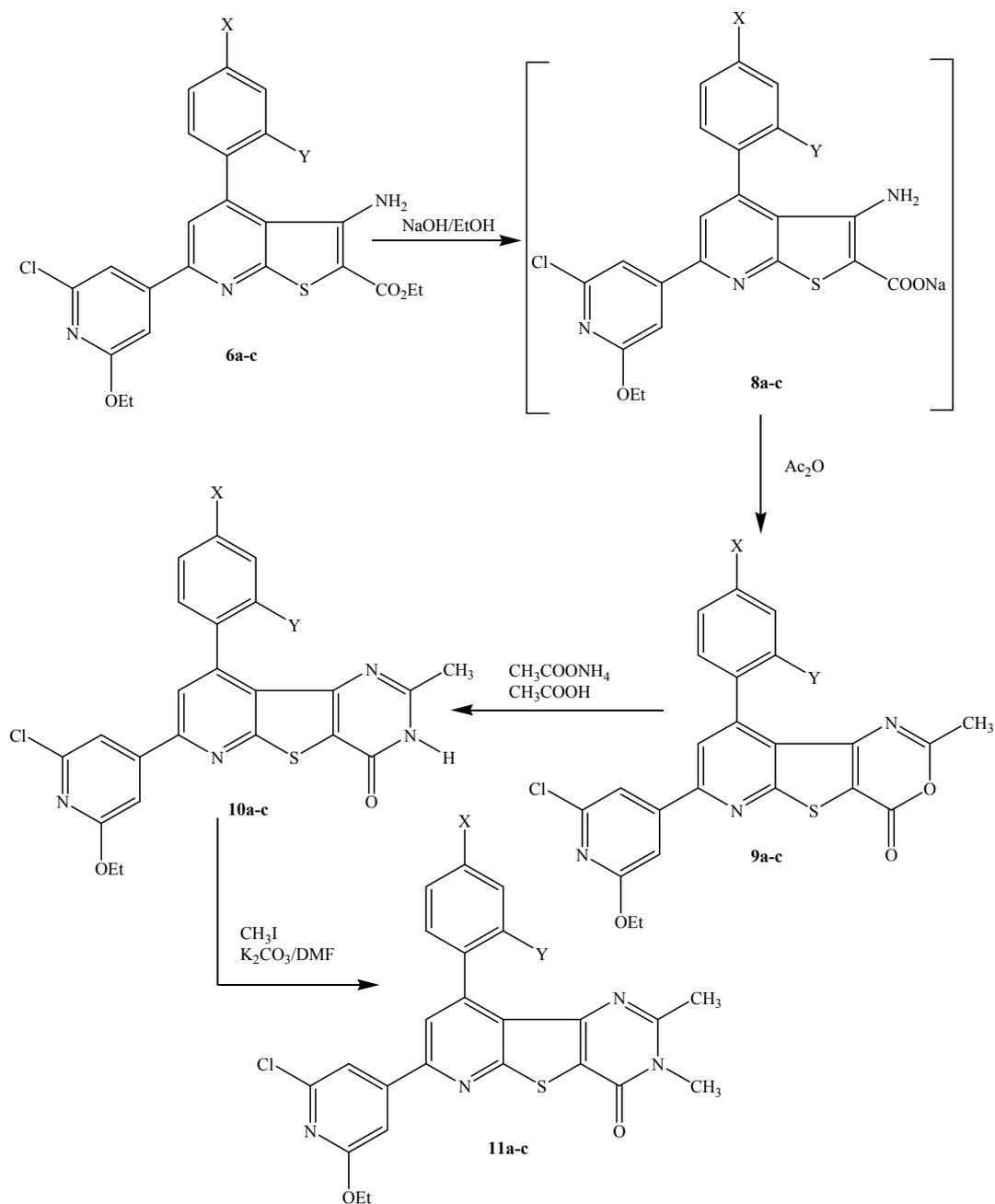
Scheme 1. Synthetic Pathway for Compound 3–7.



Compounds **6a–c** were hydrolyzed by refluxing with ethanolic sodium hydroxide (NaOH) to the corresponding sodium salts **8a–c**, which was treated *in situ* with refluxing acetic anhydride to give the corresponding oxazinone derivatives **9a–c** (Table 2). Reaction of **9a–c** with ammonium acetate in refluxing acetic acid afforded the corresponding pyrimidinone derivatives **10a–c** (Table 2), which were treated with methyl iodide in *N,N*-dimethylformamide in the presence of anhydrous K<sub>2</sub>CO<sub>3</sub> to yield the corresponding 3-methyl-pyrimidinone derivatives **11a–c** (Scheme 2, Table 2).

**Table 2.** Melting points, crystallization solvents, yields, molecular formulae and molecular weights of compounds 9–11.

Comp. No.	X	Y	Yield (%)	M.p. (°C)	Cryst. Solv.	Molecular Formula (Mol. Wt.)
9a	F	H	75	195–197	EtOH	C <sub>23</sub> H <sub>15</sub> ClFN <sub>3</sub> O <sub>3</sub> S (467.90)
9b	Cl	H	68	214–216	AcOH	C <sub>23</sub> H <sub>15</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>3</sub> S (484.35)
9c	Cl	Cl	60	282–284	DMF/H <sub>2</sub> O (2:1)	C <sub>23</sub> H <sub>14</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>3</sub> S (518.80)
10a	F	H	80	178–180	AcOH/H <sub>2</sub> O (2:1)	C <sub>23</sub> H <sub>16</sub> ClFN <sub>4</sub> O <sub>2</sub> S (466.92)
10b	Cl	H	72	188–190	AcOH/H <sub>2</sub> O (2:1)	C <sub>23</sub> H <sub>16</sub> Cl <sub>2</sub> N <sub>4</sub> O <sub>2</sub> S (483.37)
10c	Cl	Cl	65	256–258	DMF/H <sub>2</sub> O (2:1)	C <sub>23</sub> H <sub>15</sub> Cl <sub>3</sub> N <sub>4</sub> O <sub>2</sub> S (517.81)
11a	F	H	78	186–188	AcOH/H <sub>2</sub> O (2:1)	C <sub>24</sub> H <sub>18</sub> ClFN <sub>4</sub> O <sub>2</sub> S (480.94)
11b	Cl	H	66	200–202	AcOH	C <sub>24</sub> H <sub>18</sub> Cl <sub>2</sub> N <sub>4</sub> O <sub>2</sub> S (497.40)
11c	Cl	Cl	72	264–266	DMF/H <sub>2</sub> O (2:1)	C <sub>24</sub> H <sub>17</sub> Cl <sub>3</sub> N <sub>4</sub> O <sub>2</sub> S (531.84)

**Scheme 2.** Synthetic Pathway for Compound 9–11.

## 2.2. Antimicrobial Activity

The antimicrobial activities of some of the synthesized compounds were determined by the agar diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [20]. The compounds were evaluated for antimicrobial activity against bacteria, viz. *Streptomyces* sp., *Bacillus subtilis*, *Streptococcus lactis*, *Escherichia coli*, and *Pseudomonas* sp. and antifungal activity against various fungi, viz. *Aspergillus niger*, *Penicillium* sp and yeast *Candida albican* and *Rhodotorula ingeniosa*.

The concentrations of the tested compounds (10 µg/mL) were used according to a modified Kirby-Bauer's disk diffusion method. The sterile discs were impregnated with 10 µg/disc of the tested compound. Each tested compound was performed in triplicate. The solvent DMSO was used as a negative control and streptomycin/fusidic acid were used as standard calculated average diameters (for triplicates) of the zone of inhibition (in mm) for tested samples with that produced by the standard drugs. Four of the synthesized compounds **5a**, **7b**, **9b** and **10b** exhibited potent antibacterial and antifungal bioactivity compared with the standard drug used. The other tested compounds were found to exhibit a moderate to low antibacterial activity (Table 3).

**Table 3.** Antimicrobial activities of the newly synthesized compounds 3–11.

Comp. No.	Fungi		Yeast			Bacteria			
	<i>A.n</i>	<i>Pen. sp</i>	<i>C. a</i>	<i>R.i</i>	<i>Str. sp</i>	Gram – ve		Gram + ve	
						<i>B.s</i>	<i>S.l</i>	<i>E.c</i>	<i>P. sp</i>
<b>3a</b>	12	12	12	11	13	14	14	14	13
<b>3b</b>	12	12	10	11	9	8	7	9	11
<b>3c</b>	8	10	9	11	12	12	12	11	14
<b>4a</b>	10	12	11	11	13	11	10	12	11
<b>4b</b>	11	12	13	11	14	13	11	12	12
<b>4c</b>	10	12	12	13	13	12	10	12	11
<b>5a</b>	17	16	16	17	22	23	24	23	21
<b>5b</b>	4	5	4	3	7	8	7	9	8
<b>5c</b>	13	12	12	13	11	13	12	10	9
<b>6a</b>	10	13	10	11	21	20	21	23	23
<b>6b</b>	8	8	6	7	11	12	13	13	12
<b>6c</b>	12	13	13	12	13	11	13	12	13
<b>7a</b>	13	12	12	13	11	13	12	10	9
<b>7b</b>	7	5	8	9	6	12	13	13	12
<b>7c</b>	12	13	11	13	12	8	8	6	7
<b>9a</b>	12	10	11	11	20	20	21	19	20
<b>9b</b>	19	20	19	19	11	13	12	10	9
<b>9c</b>	10	11	11	12	10	11	10	12	11
<b>10a</b>	15	16	13	14	11	11	12	12	13
<b>10b</b>	23	22	22	20	11	23	22	24	23
<b>10c</b>	11	10	12	11	11	10	12	11	11
<b>11a</b>	13	12	12	13	11	13	12	10	9
<b>11b</b>	10	12	11	11	13	11	10	12	11
<b>11c</b>	13	11	10	12	11	10	12	11	11
<b>Streptomycin</b>	-	-	-	-	21	22	21	22	21
<b>Fusidic acid</b>	17	17	18	18	-	-	-	-	-

*A.n*: *Aspergillus niger*; *Pen. sp*: *Penicillium sp*; *C. a*: *Candida albican*; *Str. sp*: *Streptomyces sp*; *R.i*: *Rhodotorula ingeniosa*; *B.s*: *Bacillus subtilis*; *S.l*: *Streptococcus lactis*; *E.c*: *Escherichia coli*; *P. sp*: *Pseudomonas sp*.

On the other hand, when different concentrations of compound **9a** were used, it was exhibited a moderate antibacterial activity, but it exhibited very good antibacterial activity at higher concentrations (3× and 4×) (Table 4), while different concentrations of compounds **5a** and **10a** exhibited very good antifungal activities (2× and 3×) (Table 5).

**Table 4.** Antibacterial activity of compound **9a** at different concentrations.

Comp. No.	Conc.	Strep. sp	Bacteria			
			Gram – ve		Gram + ve	
			<i>B.s</i>	<i>S.l</i>	<i>E.c</i>	<i>Ps</i>
<b>9a</b>	1×	20	20	21	19	20
	2×	23	23	22	23	22
	3×	25	24	24	24	26
	4×	25	25	27	25	26

Where × = 10 µg.

**Table 5.** Antifungal activity of compounds **5a** and **10a** at different concentrations.

Comp. No.	Conc.	Fungi			
		<i>A.n</i>	<i>Pen. sp</i>	<i>C. a</i>	<i>R.i</i>
<b>5a</b>	1×	17	16	16	17
	2×	18	18	19	19
	3×	19	20	20	21
	4×	20	22	20	21
<b>10a</b>	1×	15	16	13	14
	2×	16	18	18	17
	3×	18	20	20	20
	4×	20	22	20	21

Where × = 10 µg.

### 3. Experimental

#### 3.1. Chemistry

Melting points were measured using Electrothermal 9100 digital melting point apparatus (Electrothermal, Essex, UK) and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1600 FTIR (Perkin-Elmer, Downers Grove, IL, USA) in KBr discs. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were measured on a Jeol 5000 MHz spectrometer (Jeol, Tokyo, Japan) in DMSO-*d*<sub>6</sub>, and chemical shifts were recorded in δ ppm relative to the internal standard TMS. The Mass spectra were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Madison, WI, USA) using EI and the values of *m/z* are indicated in Dalton. Elemental analyses were performed on a Perkin-Elmer 2400 analyzer (Perkin-Elmer) and were found within ±0.4% of the theoretical values. All reactions were followed by TLC (Silica gel, Aluminum Sheets 60 F<sub>254</sub>, Merck, Darmstadt, Germany). Starting material **2** was prepared from citrazinic acid (**1**) according to published procedures [1,19]. Antimicrobial screening was carried out in Department of Microbial Chemistry, National Research Center, Cairo, Egypt.

*1-(2-Chloro-6-ethoxypyridin-4-yl)-3-(substituted phenyl)prop-2-en-1-ones 3a–c.* A mixture of 2-chloro-6-ethoxy-4-acetylpyridine (**2**) [19] (1 mmol) and an aromatic aldehyde, namely, 4-flouro-, 4-chloro- or 2,4-dichlorobenzaldehyde (1 mmol) in absolute ethanol (30 mL) was refluxed in the

presence of a mixture of TEA/DEA (3 mL, 1:1 v:v) for 6 h. The reaction mixture was concentrated under reduced pressure, the obtained solid was filtered off, washed with ether, dried and crystallized from the proper solvents to afford the corresponding acryloyl derivatives **3a–c**, respectively.

*1-(2-Chloro-6-ethoxypyridin-4-yl)-3-(4-fluorophenyl)prop-2-en-1-one (3a)*. IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  1679 (C=O), 1607 (C=C);  $^1\text{H-NMR}$ :  $\delta$  1.32 (t, 3H,  $\text{CH}_3$ ,  $J = 6.95$  Hz), 3.81 (q, 2H,  $\text{CH}_2$ ,  $J = 6.95$  Hz), 6.65 (d, 1H, CH-olefinic-H,  $J = 14.60$  Hz), 6.98 (d, 1H, CH-olefinic-H,  $J = 14.60$  Hz), 7.28–7.96 (m, 6H, 4 Ph-H + 2 pyr-H);  $^{13}\text{C-NMR}$ : 13.68, 64.32, 104.95, 109.56, 114.72, 121.30, 129.86, 130.05, 144.65, 145.84, 146.50, 160.95, 164.96, 186.50; MS,  $m/z$  (%): 306 ( $\text{M}^+$ , 15), 184 (100); Elemental analysis for  $\text{C}_{16}\text{H}_{13}\text{ClFNO}_2$  (305.73): calcd.: C, 62.86; H, 4.29; Cl, 11.60; N, 4.58. found: C, 62.80; H, 4.26; Cl, 11.55; N, 4.52.

*1-(2-Chloro-6-ethoxypyridin-4-yl)-3-(4-chlorophenyl)prop-2-en-1-one (3b)*. IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  1682 (C=O), 1610 (C=C);  $^1\text{H-NMR}$ :  $\delta$  1.33 (t, 3H,  $\text{CH}_3$ ,  $J = 6.95$  Hz), 3.92 (q, 2H,  $\text{CH}_2$ ,  $J = 6.95$  Hz), 6.58 (d, 1H, CH-olefinic-H,  $J = 14.60$  Hz), 7.05 (d, 1H, CH-olefinic-H,  $J = 14.60$  Hz), 7.12–7.88 (m, 6H, 4 Ph-H + 2 pyr-H);  $^{13}\text{C-NMR}$ : 13.86, 64.26, 105.78, 109.62, 121.12, 126.86, 128.25, 132.85, 132.96, 144.68, 145.78, 146.65, 164.84, 186.86; MS,  $m/z$  (%): 322 ( $\text{M}^+$ , 8), 165 (100); Elemental analysis for  $\text{C}_{16}\text{H}_{13}\text{Cl}_2\text{NO}_2$  (322.18): calcd.: C, 59.65; H, 4.07; Cl, 22.01; N, 4.35. found: C, 59.60; H, 4.00; Cl, 21.96; N, 4.30.

*1-(2-Chloro-6-ethoxypyridin-4-yl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (3c)*. IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  1678 (C=O), 1612 (C=C);  $^1\text{H-NMR}$ :  $\delta$  1.28 (t, 3H,  $\text{CH}_3$ ,  $J = 6.95$  Hz), 3.86 (q, 2H,  $\text{CH}_2$ ,  $J = 6.95$  Hz), 6.46 (d, 1H, CH-olefinic-H,  $J = 14.60$  Hz), 7.10 (d, 1H, CH-olefinic-H,  $J = 14.60$  Hz), 7.25–7.76 (m, 5H, 3 Ph-H + 2 pyr-H);  $^{13}\text{C-NMR}$ : 13.92, 64.30, 105.96, 109.46, 121.21, 125.69, 128.78, 129.56, 130.85, 132.05, 133.65, 144.86, 145.88, 146.54, 164.78, 187.05; MS,  $m/z$  (%): 356 [ $\text{M}^+$ , 10], 199 [100, base peak]; Elemental analysis for  $\text{C}_{16}\text{H}_{12}\text{Cl}_3\text{NO}_2$  (356.63): calcd.: C, 53.89; H, 3.39; Cl, 29.82; N, 3.93. found: C, 53.83; H, 3.34; Cl, 29.80; N, 3.88.

*6-(2-Chloro-6-ethoxypyridin-4-yl)-4-(substituted phenyl)-1,2-dihydro-2-thioxopyridine-3-carbonitriles 4a–c*. A mixture of **3a–c** (1 mmol), 2-cyanothioacetamide (0.10 g, 1 mmol) and ammonium acetate (0.6 g, 8 mmol) in absolute ethanol (30 mL) was refluxed for 5 h. After cooling, the formed product was collected by filtration, washed with ethanol, dried and crystallized from the proper solvents to give the corresponding thioxopyridine derivatives **4a–c**, respectively.

*6-(2-Chloro-6-ethoxypyridin-4-yl)-4-(4-fluorophenyl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile (4a)*. IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3330 (NH), 2210 (CN), 1218 (C=S);  $^1\text{H-NMR}$ :  $\delta$  1.30 (t, 3H,  $\text{CH}_3$ ,  $J = 6.95$  Hz), 3.90 (q, 2H,  $\text{CH}_2$ ,  $J = 6.95$  Hz), 6.95–7.78 (m, 6H, 4 Ph-H + 2 pyr-H), 8.46 (s, 1H, pyr-5'-H), 9.24 (s, 1H, NH exchangeable with  $\text{D}_2\text{O}$ );  $^{13}\text{C-NMR}$ : 13.66, 63.98, 103.66, 103.88, 107.89, 108.55, 114.58, 116.02, 127.50, 128.04, 145.48, 148.60, 160.56, 161.76, 164.65, 167.47, 168.05; MS,  $m/z$  (%): 386 [ $\text{M}^+$ , 24], 135 [100, base peak]; Elemental analysis for  $\text{C}_{19}\text{H}_{13}\text{ClFN}_3\text{OS}$  (385.84): calcd.: C, 59.14; H, 3.40; Cl, 9.19; N, 10.89; S, 8.31. found: C, 59.10; H, 3.35; Cl, 9.14; N, 10.85; S, 8.28.

*6-(2-Chloro-6-ethoxypyridin-4-yl)-4-(4-chlorophenyl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile (4b)*. IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3356 (NH), 2215 (CN), 1210 (C=S);  $^1\text{H-NMR}$ :  $\delta$  1.34 (t, 3H,  $\text{CH}_3$ ,  $J = 6.95$  Hz),

3.86 (q, 2H, CH<sub>2</sub>, *J* = 6.95 Hz), 7.12–7.80 (m, 6H, 4 Ph-H + 2 pyr-H), 8.52 (s, 1H, pyr-5'-H), 9.18 (s, 1H, NH exchangeable with D<sub>2</sub>O); <sup>13</sup>C-NMR: 13.92, 64.12, 103.96, 104.04, 108.14, 108.86, 115.82, 127.66, 128.10, 129.68, 132.67, 145.56, 148.72, 160.77, 164.58, 167.55, 167.86; MS, *m/z* (%): 402 [M<sup>+</sup>,32], 211 [100, base peak]; Elemental analysis for C<sub>19</sub>H<sub>13</sub>Cl<sub>2</sub>N<sub>3</sub>OS (402.29): calcd.: C, 56.73; H, 3.26; Cl, 17.63; N, 10.45; S, 7.97. found: C, 56.68; H, 3.20; Cl, 17.60; N, 10.40; S, 7.92.

*6-(2-Chloro-6-ethoxyppyridin-4-yl)-4-(2,4-dichlorophenyl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile (4c)*. *v* 3348 (NH), 2218 (CN), 1212 (C=S); <sup>1</sup>H-NMR:  $\delta$  1.32 (t, 3H, CH<sub>3</sub>, *J* = 6.95 Hz), 3.78 (q, 2H, CH<sub>2</sub>, *J* = 6.95 Hz), 6.98–7.68 (m, 5H, 3 Ph-H + 2 pyr-H), 8.64 (s, 1H, pyr-5'-H), 9.34 (s, 1H, NH exchangeable with D<sub>2</sub>O); <sup>13</sup>C-NMR: 14.14, 64.18, 103.88, 104.08, 108.22, 108.92, 115.76, 125.98, 128.56, 129.16, 131.86, 132.15, 134.86, 145.64, 148.80, 161.24, 164.32, 167.45, 168.18; MS, *m/z* (%): 436 [M<sup>+</sup>,14], 279 [100, base peak]; Elemental analysis for C<sub>19</sub>H<sub>12</sub>Cl<sub>3</sub>N<sub>3</sub>OS (436.74): calcd.: C, 52.25; H, 2.77; Cl, 24.35; N, 9.62; S, 7.34. found: C, 52.20; H, 2.71; Cl, 24.30; N, 9.57; S, 7.28.

*Ethyl 2-(6-(2-chloro-6-ethoxyppyridin-4-yl)-3-cyano-4-(substituted phenyl)pyridin-2-ylthio)acetates 5a–c*. To a mixture of **4a–c** (1 mmol) and anhydrous K<sub>2</sub>CO<sub>3</sub> (0.18 g, 1 mmol) in *N*-dimethylformamide (25 mL) was stirred at room temperature for 2 h, ethyl chloroacetate (0.18 g, 1.5 mmol) was added with stirring. The reaction mixture was heated at 60 °C for 2 h and after cooling poured into ice. The solid formed was collected by filtration, washed with water, dried and crystallized from the proper solvents to afford the corresponding pyridinethioacetate derivatives **5a–c**, respectively.

*Ethyl 2-(6-(2-chloro-6-ethoxyppyridin-4-yl)-3-cyano-4-(4-fluorophenyl)pyridin-2-ylthio)acetate (5a)*. IR (KBr, cm<sup>-1</sup>): *v* 2219 (CN), 1735 (C=O, ester); <sup>1</sup>H-NMR:  $\delta$  1.28, 1.32 (2t, 6H, 2 CH<sub>3</sub>), 3.68, 3.86 (2q, 4H, 2 CH<sub>2</sub>), 4.38 (s, 2H, S–CH<sub>2</sub>), 7.16–7.82 (m, 6H, 4 Ph-H + 2 pyr-H), 8.18 (s, 1H, pyr-5'-H); <sup>13</sup>C-NMR: 13.65, 14.05, 32.04, 59.86, 64.08, 101.36, 101.57, 102.85, 115.02, 116.75, 117.02, 128.74, 132.58, 145.65, 151.56, 153.65, 157.08, 162.15, 163.56, 163.94, 168.90; MS, *m/z* (%): 472 [M<sup>+</sup>,12], 426 [100, base peak]; Elemental analysis for C<sub>23</sub>H<sub>19</sub>ClFN<sub>3</sub>O<sub>3</sub>S (471.93): calcd.: C, 58.54; H, 4.06; Cl, 7.51; N, 8.90; S, 6.79. found: C, 58.48; H, 4.00; Cl, 7.45; N, 8.84; S, 6.72.

*Ethyl 2-(6-(2-chloro-6-ethoxyppyridin-4-yl)-3-cyano-4-(4-chlorophenyl)pyridin-2-ylthio)acetate (5b)*. IR (KBr, cm<sup>-1</sup>): *v* 2222 (CN), 1732 (C=O, ester); <sup>1</sup>H-NMR:  $\delta$  1.29, 1.32 (2t, 6H, 2 CH<sub>3</sub>), 3.56, 3.84 (2q, 4H, 2 CH<sub>2</sub>), 4.42 (s, 2H, S–CH<sub>2</sub>), 7.10–7.72 (m, 6H, 4 Ph-H + 2 pyr-H), 8.64 (s, 1H, pyr-5'-H); <sup>13</sup>C-NMR: 13.78, 14.15, 32.18, 60.05, 64.18, 101.48, 101.68, 102.74, 116.88, 117.02, 127.54, 128.12, 129.57, 133.45, 145.56, 150.96, 153.64, 157.18, 163.72, 164.05, 170.04; MS, *m/z* (%): 488 [M<sup>+</sup>,32], 120 [100, base peak]; Elemental analysis for C<sub>23</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>S (488.38): calcd.: C, 56.56; H, 3.92; Cl, 14.52; N, 8.60; S, 6.57. found: C, 56.50; H, 3.88; Cl, 14.47; N, 8.55; S, 6.51.

*Ethyl 2-(6-(2-chloro-6-ethoxyppyridin-4-yl)-3-cyano-4-(2,4-dichlorophenyl)pyridin-2-ylthio)acetate (5c)*. IR (KBr, cm<sup>-1</sup>): *v* 2218 (CN), 1735 (C=O, ester); <sup>1</sup>H-NMR:  $\delta$  1.26, 1.30 (2t, 6H, 2 CH<sub>3</sub>), 3.58, 3.78 (2q, 4H, 2 CH<sub>2</sub>), 4.36 (s, 2H, S–CH<sub>2</sub>), 7.12–7.65 (m, 5H, 3 Ph-H + 2 pyr-H), 8.56 (s, 1H, pyr-5'-H); <sup>13</sup>C-NMR: 13.84, 14.18, 32.18, 59.92, 64.18, 100.98, 101.59, 102.66, 116.82, 117.06, 125.86, 128.48, 129.24, 131.92, 132.24, 134.74, 145.58, 151.08, 153.72, 157.22, 163.88, 164.15, 168.84; MS, *m/z* (%):

523 [M<sup>+</sup>,8], 247 [100, base peak]; Elemental analysis for C<sub>23</sub>H<sub>18</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>3</sub>S (522.83): C, 52.84; H, 3.47; Cl, 20.34; N, 8.04; S, 6.13. found: C, 52.78; H, 3.40; Cl, 20.28; N, 8.00; S, 6.07.

*Ethyl 3-amino-6-(2-chloro-6-ethoxypyridin-4-yl)-4-(substituted phenyl)thieno[2,3-b]pyridine-2-carboxylates 6a–c.* A mixture of **5a–c** (1 mmol) in sodium methoxide solution (20 mL, 2%) was refluxed for 1 h on a water bath at 70 °C with stirring. The reaction mixture was evaporated under reduced pressure, the obtained residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub>, washed with H<sub>2</sub>O, 10 mL 1 N HCl and then with water. The solvent was dried over anhydrous CaCl<sub>2</sub>, evaporated under reduced pressure, and the obtained product was crystallized to afford from the proper solvents to afford the corresponding ethyl thienopyridinecarboxylates **6a–c**, respectively.

*Ethyl 3-amino-6-(2-chloro-6-ethoxypyridin-4-yl)-4-(4-fluorophenyl)thieno[2,3-b]pyridine-2-carboxylate (6a).* IR (KBr, cm<sup>-1</sup>): ν 3443 (NH<sub>2</sub>), 1742 (C=O, ester); <sup>1</sup>H-NMR: δ 1.30, 1.34 (2t, 6H, 2 CH<sub>3</sub>), 3.72, 4.06 (2q, 4H, 2 CH<sub>2</sub>), 4.36 (s, 2H, NH<sub>2</sub> exchangeable with D<sub>2</sub>O), 7.24–7.75 (m, 6H, 4 Ph-H + 2 pyr-H), 8.35 (s, 1H, pyr-5'-H); <sup>13</sup>C-NMR: 13.95, 14.16, 60.24, 64.18, 101.58, 103.02, 115.16, 118.35, 120.76, 122.15, 128.66, 132.64, 134.12, 145.72, 149.65, 151.64, 154.57, 155.75, 160.12, 162.65, 164.12; MS, *m/z* (%): 472 [M<sup>+</sup>,26], 317 [100, base peak]; Elemental analysis for C<sub>23</sub>H<sub>19</sub>ClFN<sub>3</sub>O<sub>3</sub>S (471.93): calcd.: C, 58.54; H, 4.06; Cl, 7.51; N, 8.90; S, 6.79. found: C, 58.48; H, 4.00; Cl, 7.45; N, 8.86; S, 6.71.

*Ethyl 3-amino-6-(2-chloro-6-ethoxypyridin-4-yl)-4-(4-chlorophenyl)thieno[2,3-b]pyridine-2-carboxylate (6b).* IR (KBr, cm<sup>-1</sup>): ν 3452 (NH<sub>2</sub>), 1737 (C=O, ester); <sup>1</sup>H-NMR: δ 1.26, 1.31 (2t, 6H, 2 CH<sub>3</sub>), 3.78, 4.10 (2q, 4H, 2 CH<sub>2</sub>), 4.48 (s, 2H, NH<sub>2</sub> exchangeable with D<sub>2</sub>O), 7.24–7.82 (m, 6H, 4 Ph-H + 2 pyr-H), 8.72 (s, 1H, pyr-5'-H); <sup>13</sup>C-NMR: 14.08, 14.25, 60.15, 64.10, 100.42, 103.64, 118.05, 121.16, 122.25, 127.66, 128.44, 133.45, 133.95, 134.50, 146.02, 149.75, 151.18, 154.65, 156.05, 159.64, 164.15; MS, *m/z* (%): 488 [M<sup>+</sup>,8], 332 [100, base peak]; Elemental analysis for C<sub>23</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>S (488.38): calcd.: C, 56.56; H, 3.92; Cl, 14.52; N, 8.60; S, 6.57. found: C, 56.50; H, 3.88; Cl, 14.46; N, 8.55; S, 6.50.

*Ethyl 3-amino-6-(2-chloro-6-ethoxypyridin-4-yl)-4-(2,4-dichlorophenyl)thieno[2,3-b]pyridine-2-carboxylate (6c).* IR (KBr, cm<sup>-1</sup>): ν 3456 (NH<sub>2</sub>), 1735 (C=O, ester); <sup>1</sup>H-NMR: δ 1.30, 1.33 (2t, 6H, 2 CH<sub>3</sub>), 3.82, 4.15 (2q, 4H, 2 CH<sub>2</sub>), 4.56 (s, 2H, NH<sub>2</sub> exchangeable with D<sub>2</sub>O), 7.08–7.68 (m, 5H, 3 Ph-H + 2 pyr-H), 8.62 (s, 1H, pyr-5'-H); <sup>13</sup>C-NMR: 14.12, 14.26, 59.98, 64.32, 100.86, 102.72, 118.02, 121.06, 122.00, 126.16, 128.87, 129.36, 132.18, 134.05, 135.44, 136.74, 145.64, 149.85, 151.38, 154.72, 155.43, 160.04, 164.25; MS, *m/z* (%): 523 [M<sup>+</sup>,6], 177 [100, base peak]; Elemental analysis for C<sub>23</sub>H<sub>18</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>3</sub>S (522.83): calcd.: C, 52.84; H, 3.47; Cl, 20.34; N, 8.04; S, 6.13. found: C, 52.77; H, 3.42; Cl, 20.30; N, 7.97; S, 6.08.

*3-Amino-6-(2-chloro-6-ethoxypyridin-4-yl)-4-(substituted-phenyl)thieno[2,3-b]pyridine-2-carbox-amides 7a–c.* A current of ammonia gas was passed through a suspension of **6a–c** (1 mmol) in absolute ethanol (100 mL), at 0 °C till saturation. The reaction mixture was left overnight at -4 °C, evaporated under reduced pressure, the residue obtained was triturated with *n*-hexane, the formed solid was filtered off,

washed with water and crystallized from the proper solvents to give the corresponding thienopyridine carboxamides **7a–c**, respectively.

*3-Amino-6-(2-chloro-6-ethoxypyridin-4-yl)-4-(4-fluorophenyl)thieno[2,3-*b*]pyridine-2-carboxamide (7a)*. IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3460–3380 ( $\text{NH}_2$ ), 1675 ( $\text{C}=\text{O}$ , amide);  $^1\text{H-NMR}$ :  $\delta$  1.32 (t, 3H,  $\text{CH}_3$ ), 3.85 (q, 2H,  $\text{CH}_2$ ), 4.46, 6.85 (2s, 4H, 2  $\text{NH}_2$  exchangeable with  $\text{D}_2\text{O}$ ), 7.12–7.68 (m, 6H, 4 Ph-H + 2 pyr-H), 8.56 (s, 1H, pyr-5'-H);  $^{13}\text{C-NMR}$ : 14.06, 64.28, 100.96, 102.22, 115.36, 120.82, 122.45, 128.37, 128.46, 132.84, 137.15, 145.82, 149.65, 152.00, 154.74, 157.75, 161.55, 162.76, 164.30; MS,  $m/z$  (%): 443 [ $\text{M}^+$ , 8], 332 [100, base peak]; Elemental analysis for  $\text{C}_{21}\text{H}_{16}\text{ClFN}_4\text{O}_2\text{S}$  (442.89): calcd.: C, 56.95; H, 3.64; Cl, 8.00; N, 12.65; S, 7.24. found: C, 56.90; H, 3.60; Cl, 7.940; N, 12.60; S, 7.19.

*3-Amino-6-(2-chloro-6-ethoxypyridin-4-yl)-4-(4-chlorophenyl)thieno[2,3-*b*]pyridine-2-carboxamide (7b)*.  $\nu$  3456–3378 ( $\text{NH}_2$ ), 1672 ( $\text{C}=\text{O}$ , amide);  $^1\text{H-NMR}$ :  $\delta$  1.30 (t, 3H,  $\text{CH}_3$ ), 3.82 (q, 2H,  $\text{CH}_2$ ), 4.44, 6.88 (2s, 4H, 2  $\text{NH}_2$  exchangeable with  $\text{D}_2\text{O}$ ), 7.32–7.68 (m, 6H, 4 Ph-H + 2 pyr-H), 8.68 (s, 1H, pyr-5'-H);  $^{13}\text{C-NMR}$ : 13.68, 64.12, 100.00, 102.04, 121.24, 122.12, 127.85, 128.38, 128.55, 134.05, 135.15, 137.05, 146.12, 149.65, 151.00, 154.36, 156.14, 161.42, 164.04; MS,  $m/z$  (%): 459 [ $\text{M}^+$ , 25], 287 [100, base peak]; Elemental analysis for  $\text{C}_{21}\text{H}_{16}\text{Cl}_2\text{N}_4\text{O}_2\text{S}$  (459.34): calcd.: C, 54.91; H, 3.51; Cl, 15.44; N, 12.20; S, 6.98. found: C, 54.86; H, 3.45; Cl, 15.39; N, 12.16; S, 6.92.

*3-Amino-6-(2-chloro-6-ethoxypyridin-4-yl)-4-(2,4-dichlorophenyl)thieno[2,3-*b*]pyridine-2-carboxamide (7c)*. IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3456 ( $\text{NH}_2$ ), 1735 ( $\text{C}=\text{O}$ , ester);  $^1\text{H-NMR}$ :  $\delta$  1.28 (t, 3H,  $\text{CH}_3$ ), 3.86 (q, 2H,  $\text{CH}_2$ ), 4.54, 6.76 (2s, 4H, 2  $\text{NH}_2$  exchangeable with  $\text{D}_2\text{O}$ ), 7.12–7.73 (m, 5H, 3 Ph-H + 2 pyr-H), 8.48 (s, 1H, pyr-5'-H);  $^{13}\text{C-NMR}$ : 14.12, 64.33, 101.04, 102.84, 121.32, 122.40, 126.24, 128.65, 128.75, 129.72, 132.32, 135.12, 136.66, 137.22, 145.56, 149.55, 151.22, 154.44, 157.12, 161.26, 164.57; MS,  $m/z$  (%): 494 [ $\text{M}^+$ , 12], 320 [100, base peak]; Elemental analysis for  $\text{C}_{21}\text{H}_{15}\text{Cl}_3\text{N}_4\text{O}_2\text{S}$  (493.79): calcd.: C, 51.08; H, 3.06; Cl, 21.54; N, 11.35; S, 6.49. found: C, 51.00; H, 3.00; Cl, 21.50; N, 11.30; S, 6.44.

*7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(substituted-phenyl)-2-methyl-4H-pyrido[3',2':4,5]thieno[3,2-*d*]-[1,3]-oxazin-4-ones 9a–c*. A mixture of **6a–c** (1 mmol) in ethanolic NaOH (100 mL, 5%) was heated under reflux for 4 h. The solvent was evaporated under reduced pressure, the obtained sodium salt **8a–c** was dissolved in acetic anhydride (100 mL) and refluxed it for 6 h. The reaction mixture was concentrated and allowed to cool, poured onto ice water, the obtained solid was collected by filtration, washed with water, dried and crystallized from the proper solvents to afford the corresponding thienooxazinopyridine derivatives **9a–c**, respectively.

*7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(4-fluorophenyl)-2-methyl-4H-pyrido[3',2':4,5]thieno[3,2-*d*]-[1,3]oxazin-4-one (9a)*. IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  1750 ( $\text{C}=\text{O}$ );  $^1\text{H-NMR}$ :  $\delta$  1.30 (t, 3H,  $\text{CH}_3$ ), 2.03 (s, 3H,  $\text{CH}_3$ ), 3.78 (q, 2H,  $\text{CH}_2$ ), 7.04–7.58 (m, 6H, 4 Ph-H + 2 pyr-H), 8.62 (s, 1H, pyr-5'-H);  $^{13}\text{C-NMR}$ : 14.10, 18.98, 64.30, 100.86, 101.68, 116.02, 120.80, 125.85, 128.42, 132.78, 134.46, 135.35, 145.72, 150.05, 151.75, 154.90, 155.25, 158.62, 162.70, 164.08, 165.25; MS,  $m/z$  (%): 468 [ $\text{M}^+$ , 6], 217 [100, base peak]; Elemental analysis for  $\text{C}_{23}\text{H}_{15}\text{ClFN}_3\text{O}_3\text{S}$  (467.89): calcd.: C, 59.04; H, 3.23; Cl, 7.58; N, 8.98; S, 6.85. found: C, 58.96; H, 3.18; Cl, 7.52; N, 8.90; S, 6.80.

7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(4-chlorophenyl)-2-methyl-4H-pyrido[3',2':4,5]thieno[3,2-d]-[1,3]oxazin-4-one (**9b**). IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  1745 (C=O);  $^1\text{H-NMR}$ :  $\delta$  1.28 (t, 3H,  $\text{CH}_3$ ), 2.01 (s, 3H,  $\text{CH}_3$ ), 3.89 (q, 2H,  $\text{CH}_2$ ), 7.23–7.65 (m, 6H, 4 Ph-H + 2 pyr-H), 8.42 (s, 1H, pyr-5'-H);  $^{13}\text{C-NMR}$ : 14.08, 21.60, 64.18, 100.55, 101.45, 121.12, 125.68, 128.12, 128.96, 133.05, 134.58, 135.32, 135.72, 145.92, 149.75, 151.84, 154.86, 155.14, 158.70, 164.12, 165.18; MS,  $m/z$  (%): 484 [ $\text{M}^+$ ,15], 156 [100, base peak]; Elemental analysis for  $\text{C}_{23}\text{H}_{15}\text{Cl}_2\text{N}_3\text{O}_3\text{S}$  (484.35): calcd.: C, 57.03; H, 3.12; Cl, 14.64; N, 8.68; S, 6.62. found: C, 56.95; H, 3.10; Cl, 14.60; N, 8.63; S, 6.58.

7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(2,4-dichlorophenyl)-2-methyl-4H-pyrido[3',2':4,5]thieno[3,2-d]-[1,3]oxazin-4-one (**9c**). IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  1750 (C=O);  $^1\text{H-NMR}$ :  $\delta$  1.30 (t, 3H,  $\text{CH}_3$ ), 2.00 (s, 3H,  $\text{CH}_3$ ), 3.82 (q, 2H,  $\text{CH}_2$ ), 7.21–7.68 (m, 5H, 3 Ph-H + 2 pyr-H), 8.54 (s, 1H, pyr-5'-H);  $^{13}\text{C-NMR}$ : 14.10, 19.18, 64.22, 100.28, 101.15, 121.16, 125.56, 126.46, 128.58, 129.80, 132.44, 134.34, 135.18, 135.45, 136.73, 145.88, 149.72, 151.69, 154.78, 155.18, 159.06, 164.18, 165.32; MS,  $m/z$  (%): 519 [ $\text{M}^+$ ,8], 320 [100, base peak]; Elemental analysis for  $\text{C}_{23}\text{H}_{14}\text{Cl}_3\text{N}_3\text{O}_3\text{S}$  (518.79): calcd.: C, 53.25; H, 2.72; Cl, 20.50; N, 8.10; S, 6.18. found: C, 53.18; H, 2.68; Cl, 20.45; N, 8.00; S, 6.12.

7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(substituted-phenyl)-2-methylpyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-4(3H)-ones **10a–c**

A mixture of **9a–c** (1 mmol) and ammonium acetate (0.6 g, 8 mmol) in glacial acetic acid (100 mL) was heated under reflux for 6 h. The reaction mixture was evaporated under reduced pressure, the residue was triturated with cooled water, the solid formed was collected by filtration, washed with water, dried and crystallized from the proper solvents to afford the corresponding thienopyrimidino-pyridine 0.30 g (70%) **10a–c**, respectively.

7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(4-fluorophenyl)-2-methylpyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-4(3H)-one (**10a**). IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3420 (NH), 1650 (C=O);  $^1\text{H-NMR}$ :  $\delta$  1.28 (t, 3H,  $\text{CH}_3$ ), 2.32 (s, 3H,  $\text{CH}_3$ ), 3.86 (q, 2H,  $\text{CH}_2$ ), 7.12–7.64 (m, 6H, 4 Ph-H + 2 pyr-H), 8.58 (s, 1H, pyr-5'-H), 9.26 (s, 1H, NH exchangeable with  $\text{D}_2\text{O}$ );  $^{13}\text{C-NMR}$ : 14.08, 24.98, 64.42, 101.02, 102.54, 116.15, 121.04, 126.14, 128.85, 132.86, 136.76, 137.05, 145.88, 150.15, 151.98, 154.10, 154.86, 157.25, 160.03, 162.99, 164.28; MS,  $m/z$  (%): 467 [ $\text{M}^+$ ,18], 156 [100, base peak]; Elemental analysis for  $\text{C}_{23}\text{H}_{16}\text{ClFN}_4\text{O}_2\text{S}$  (466.91): calcd.: C, 59.16; H, 3.45; Cl, 7.59; N, 12.00; S, 6.87. found: C, 59.10; H, 3.38; Cl, 7.52; N, 11.94; S, 6.83.

7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(4-chlorophenyl)-2-methylpyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-4(3H)-one (**10b**). IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3438 (NH), 1649 (C=O);  $^1\text{H-NMR}$ :  $\delta$  1.31 (t, 3H,  $\text{CH}_3$ ), 2.24 (s, 3H,  $\text{CH}_3$ ), 3.78 (q, 2H,  $\text{CH}_2$ ), 7.33–7.72 (m, 6H, 4 Ph-H + 2 pyr-H), 8.62 (s, 1H, pyr-5'-H), 9.32 (s, 1H, NH exchangeable with  $\text{D}_2\text{O}$ );  $^{13}\text{C-NMR}$ : 13.98, 25.04, 64.53, 100.12, 101.36, 121.13, 126.45, 128.15, 129.05, 133.76, 135.99, 136.88, 145.76, 146.05, 149.85, 151.90, 154.16, 154.92, 157.48, 159.73, 164.36; MS,  $m/z$  (%): 483 [ $\text{M}^+$ ,18], 326 [100, base peak]; Elemental analysis for  $\text{C}_{23}\text{H}_{16}\text{Cl}_2\text{N}_4\text{O}_2\text{S}$  (483.36): calcd.: C, 57.15; H, 3.34; Cl, 14.67; N, 11.59; S, 6.63. found: C, 57.10; H, 3.28; Cl, 14.62; N, 11.53; S, 6.58.

7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(2,4-dichlorophenyl)-2-methylpyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-4(3H)-one (**10c**). IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3465 (NH), 1653 (C=O);  $^1\text{H-NMR}$ :  $\delta$  1.26 (t, 3H,  $\text{CH}_3$ ), 2.12 (s, 3H,

CH<sub>3</sub>), 3.80 (q, 2H, CH<sub>2</sub>), 7.21–7.70 (m, 5H, 3 Ph-H + 2 pyr-H), 8.72 (s, 1H, pyr-5'-H), 9.48 (s, 1H, NH exchangeable with D<sub>2</sub>O); <sup>13</sup>C-NMR: 13.92, 24.87, 64.42, 99.96, 101.02, 120.33, 126.32, 126.64, 128.36, 129.72, 132.88, 135.09, 136.64, 136.84, 145.86, 146.13, 149.77, 151.92, 153.96, 154.66, 157.68, 160.02, 164.48; MS, *m/z* (%): 518 [M<sup>+</sup>,5], 145 [100, base peak]; Elemental analysis for C<sub>23</sub>H<sub>15</sub>Cl<sub>3</sub>N<sub>4</sub>O<sub>2</sub>S (517.81): calcd.: C, 53.35; H, 2.92; Cl, 20.54; N, 10.82; S, 6.19. found: C, 53.30; H, 2.87; Cl, 20.50; N, 10.79; S, 6.14.

*7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(4-fluorophenyl)-2,3-dimethylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4(3H)-ones 11a–c*. A solution of **10a–c** (1 mmol) in DMF (20 mL) was stirred with anhydrous K<sub>2</sub>CO<sub>3</sub> (0.19 g, 1 mmol) for 10 min at room temperature, then methyl iodide (0.28 g, 2 mmol) in DMF (5 mL) were added. The reaction mixture was heated at 60 °C for 4 h, after cooling, poured into ice water, and the formed precipitate was filtered off, washed with water, dried and crystallized from the proper solvents to afford the corresponding thieno-*N*-methylpyrimidinopyridines **11a–c**, respectively.

*7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(4-fluorophenyl)-2,3-dimethylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4(3H)-one (11a)*. IR (KBr, cm<sup>-1</sup>): ν 1668 (C=O); <sup>1</sup>H-NMR: δ 1.28 (t, 3H, CH<sub>3</sub>), 2.32, 3.10 (2s, 6H, 2 CH<sub>3</sub>), 3.78 (q, 2H, CH<sub>2</sub>), 7.08–7.68 (m, 6H, 4 Ph-H + 2 pyr-H), 8.62 (s, 1H, pyr-5'-H); <sup>13</sup>C-NMR: 14.00, 22.14, 26.06, 64.15, 100.10, 101.32, 116.18, 120.34, 126.34, 128.95, 132.90, 136.42, 145.76, 146.15, 149.85, 151.80, 154.02, 154.77, 157.36, 159.63, 162.76, 164.30; MS, *m/z* (%): 481 [M<sup>+</sup>,4], 98 [100, base peak]; Elemental analysis for C<sub>24</sub>H<sub>18</sub>ClFN<sub>4</sub>O<sub>2</sub>S (480.94): calcd.: C, 59.94; H, 3.77; Cl, 7.37; N, 11.65; S, 6.67. found: C, 59.88; H, 3.72; Cl, 7.33; N, 11.60; S, 6.61.

*7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(4-chlorophenyl)-2,3-dimethylpyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-4(3H)-one (11b)*. IR (KBr, cm<sup>-1</sup>): ν 1670 (C=O); <sup>1</sup>H-NMR: δ 1.29 (t, 3H, CH<sub>3</sub>), 2.18, 3.06 (2s, 6H, 2 CH<sub>3</sub>), 3.82 (q, 2H, CH<sub>2</sub>), 7.28–7.68 (m, 6H, 4 Ph-H + 2 pyr-H), 8.78 (s, 1H, pyr-5'-H); <sup>13</sup>C-NMR: 13.86, 22.00, 26.28, 64.14, 99.58, 100.12, 120.56, 126.76, 128.00, 128.95, 133.45, 135.85, 136.56, 145.32, 146.75, 149.80, 150.87, 153.78, 154.65, 157.45, 160.02, 164.28; MS, *m/z* (%): 497 [M<sup>+</sup>,19], 162 [100, base peak]; Elemental analysis for C<sub>24</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>2</sub>S (497.39): calcd.: C, 57.95; H, 3.65; Cl, 14.26; N, 11.26; S, 6.45. found: C, 57.90; H, 3.59; Cl, 14.22; N, 11.20; S, 6.40.

*7-(2-Chloro-6-ethoxypyridin-4-yl)-9-(2,4-dichlorophenyl)-2,3-dimethylpyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-4(3H)-one (11c)*. IR (KBr, cm<sup>-1</sup>): ν 1667 (C=O); <sup>1</sup>H-NMR: δ 1.28 (t, 3H, CH<sub>3</sub>), 2.22, 2.96 (2s, 6H, 2 CH<sub>3</sub>), 3.80 (q, 2H, CH<sub>2</sub>), 7.24–7.70 (m, 5H, 3 Ph-H + 2 pyr-H), 8.65 (s, 1H, pyr-5'-H); <sup>13</sup>C-NMR: 13.90, 22.01, 26.48, 64.08, 99.86, 100.09, 120.60, 126.45, 126.58, 128.25, 129.52, 132.66, 135.14, 136.60, 136.78, 145.42, 146.78, 149.82, 151.14, 153.88, 154.72, 157.48, 159.72, 164.20; MS, *m/z* (%): 532 [M<sup>+</sup>,19], 252 [100, base peak]; Elemental analysis for C<sub>24</sub>H<sub>17</sub>Cl<sub>3</sub>N<sub>4</sub>O<sub>2</sub>S (531.84): calcd.: C, 54.20; H, 3.22; Cl, 20.00; N, 10.53; S, 6.03. found: C, 54.15; H, 3.16; Cl, 19.85; N, 10.48; S, 6.00.

### 3.2. Antimicrobial Screening Media

The following media were used:

1. PDA medium: this medium was used for fungi cultivation. It consists of 4 g dextrose/L potatoes extract.

2. Czapek Dox medium: it consists of 10 g glucose, 2 g KNO<sub>3</sub>, 1g K<sub>2</sub>HPO<sub>4</sub>, 0.5 g KCl, 0.5 g MgSO<sub>4</sub>, and 0.05 g ferrous sulphate/L distilled water. This medium is specialized for bacteria cultivation.
3. Medium 3: it consists of 10 glucose, 5 g peptone, 3 yeast extract, and 3 malt extract. It was used for yeast cultivation.

#### 4. Conclusions

A series of newly compounds **3–11** were prepared using citrazinic acid (2,6-dihydroxyisonicotinic acid) as a starting material. The obtained derivatives were screening as antimicrobial and antifungal agents. Four of the synthesized compounds **5a**, **7b**, **9b** and **10b** exhibited potent antibacterial and antifungal bioactivity compared with streptomycin and fusidic acid used as reference drugs. The other tested compounds were found to exhibit moderate to low antibacterial activity. On the other hand when higher concentrations (3× and 4×) of compound **9a**, which exhibited a moderate antibacterial activity, were used, this compound exhibited very good antibacterial activity. While different concentrations of compounds **5a** and **10a** exhibited a very good antifungal activity (2× and 3×).

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#### References

1. Amr, A.E. Synthesis of some heterocyclic compounds as potential antimicrobial agents using 2,6-diacetylpyridine as synthon. *Indian J. Heterocycl. Chem.* **2000**, *10*, 49–58.
2. Attia, A.; Abdel-Salam, O.I.; Amr, A.E.; Stibor, I.; Budesinsky, M. Synthesis and antimicrobial activity of some new chiral bridged macrocyclic pyridines. *Egypt. J. Chem.* **2000**, *43*, 187–201.
3. Nehad, A.A.; Amr, A.E.; Alhusien, A.I. Synthesis, reactions and pharmacological screening of heterocyclic derivatives using nicotinic acid as a natural synthon. *Monatsh. Chem.* **2007**, *138*, 559–567.
4. Amr, A.E.; Hegab, M.I.; Ibrahim, A.A.; Abdulla, M.M. Synthesis and reactions of some fused oxazinone, pyrimidinone, thiopyrimidone and triazinone derivatives with thiophene ring as analgesic, anticonvulsant and antiparkinsonian agents. *Monatsh. Chem.* **2003**, *134*, 1395–1409.
5. Amr, A.E.; Mohamed, A.M.; Mohamed, S.F.; Abdel-Hafez, N.A.; Hammam, A.G. Anticancer activities of some newly synthesized pyridine, pyrane and pyrimidine derivatives. *Bioorg. Med. Chem.* **2006**, *14*, 5481–5488.
6. Abou-Ghalia, M.H.; Amr, A.E. Synthesis and investigation of a new cyclo-(N<sup>α</sup>-dipicolinoyl)pentapeptide of a breast and CNS cytotoxic activity and an ionophoric specificity. *Amino Acids* **2004**, *26*, 283–289.
7. Hammam, A.G.; Fahmy, A.F.M.; Amr, A.E.; Ashraf, M.M. Synthesis of novel tricyclic heterocyclic compounds as potential anticancer agents using chromanone and thiochromanone as synthons. *Indian J. Chem. Sect. B* **2003**, *42B*, 1985–1993.

8. Chakrabarti, J.K.; Horsman, L.; Hotten, T.M.; Pullar, I.A.; Tupper, D.E.; Wright, F.C. 4-Piperazinyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepines as potential neuroleptics *J. Med. Chem.* **1980**, *23*, 878–884.
9. Ram, V.J.; Pandey, H.K.; Vlietinck, A.J. Thieno[2,3-*d*]pyrimidines as potential chemotherapeutic agents. II. *J. Heterocycl. Chem.* **1981**, *18*, 1277–1280.
10. Fahmy, H.H.; El-Eraay, W. Synthesis and evaluation of the analgesic and antiinflammatory activities of *O*-substituted salicylamides. *Arch. Pharm. Res.* **2001**, *24*, 171–179.
11. De Clercq, E. Potential of bromovinyldeoxyuridine in anticancer chemotherapy. *Anticancer Res.* **1986**, *6*, 549–556.
12. De Clercq, E.; Desgranges, C.; Herdewijn, P.; Sim, I.S.; Jones, A.S.; McLean, M.J.; Walker, R.T. Synthesis and antiviral activity of (*E*)-5-(2-bromovinyl)uracil and (*E*)-5-(2-bromovinyl)uridine. *J. Med. Chem.* **1986**, *29*, 213–217.
13. Braña, M.F.; Castellano, J.M.; Morán, M.; Pérez, de Vega M.J.; Romerdahl, C.R.; Qian, X.D.; Bousquet, P.; Emling, F.; Schlick, E.; Keilhauer, G. Bis-naphthalimides: A new class of antitumor agents. *Anticancer Drug Des.* **1993**, *8*, 257–268.
14. Amr, A.E.; Sabry, N.M.; Abdalla, M.M. Synthesis, reactions and antiinflammatory activity of heterocyclic derivatives system fused to a thiophene moiety using citrazinic acid as synthon. *Monatsh. Chem.* **2007**, *138*, 699–707.
15. Ghozlan, S.A.S.; Al-Omar, M.A.; Amr, A.E.; Khalil, K.; Abd El-Wahab, A.A. Synthesis and antimicrobial activity of some heterocyclic 2,6-bis(substituted)-1,3,4-thiadiazolo-, oxadiazolo- and oxathiazolidinopyridine derivatives from 2,6-pyridine dicarboxylic acid dihydrazide. *J. Heterocycl. Chem.* **2011**, *48*, 1103–1110.
16. Al-Omar, M.A.; Amr, A.E. Synthesis of some new pyridine-2,6-carboxamide-derived Schiff Bases as potential antimicrobial agents. *Molecules* **2010**, *15*, 4711–4721.
17. Al-Salahi, R.A.; Al-Omar, M.A.; Amr, A.E. Synthesis of chiral macro-cyclic or linear pyridine carboxamides from pyridine-2,6-dicarbonyl dichloride as anti-microbial agents. *Molecules* **2010**, *15*, 6588–6597.
18. Abdel Fattah, Y.; Amr, A.E.; Mohamed, S.F.; Abdalla, M.M.; Al-Omar, M.A.; Shafik, S.H. Cytotoxicity and anti-HIV evaluations of some new synthesized quinazoline and thioxopyrimidine derivatives using 4-(thiophen-2-yl)-3,4,5,6-tetrahydro-benzo[*h*]quinazoline-2(1*H*)-thione as synthon. *J. Chem. Sci.* **2012**, *124*, 693–702.
19. Attia, A.; Abdel-Salam, O.I.; Abo-Ghalia, M.H.; Amr, A.E. Chemical and biological reactivity of newly synthesized 2-chloro-6-ethoxy-4-acetylpyridine. *Egypt. J. Chem.* **1995**, *38*, 543–553.
20. Furtado, G.L.; Medeiros, A.A. Single-disk diffusion testing (Kirby-Bauer) of susceptibility of *Proteus mirabilis* to chloramphenicol: Significance of the intermediate category. *J. Clin. Microbiol.* **1980**, *12*, 550–553.

*Sample Availability:* Samples of the compounds are available from the authors.