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. α,β-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
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 K₂CO₃ 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 ν (C≡N) for 5a–c and the presence of broad band corresponding to ν (NH₂). Also, the IR spectra of 7a–c showed the absence of ν(C=O, ester) for 6a–c and the presence of a broad band corresponding to ν(NH₂).

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.) |
|-----------|-----|-----|-----------|-----------|-------------|------------------------------|
| 3a        | F   | H   | 86        | 185–187   | EtOH        | C₁₆H₁₃ClFN₂O₂ (505.73)       |
| 3b        | Cl  | H   | 82        | 155–157   | EtOH        | C₁₆H₁₃Cl₂N₂O₂ (322.19)       |
| 3c        | Cl  | Cl  | 85        | 203–205   | EtOH        | C₁₆H₁₂Cl₃N₂O₂ (356.63)       |
| 4a        | F   | H   | 65        | 192–194   | DMF/H₂O (2:1) | C₁₉H₁₃ClN₃O₃S (385.84)     |
| 4b        | Cl  | H   | 58        | 206–208   | AcOH/H₂O (2:1) | C₁₉H₁₃ClN₃O₃S (402.30)     |
| 4c        | Cl  | Cl  | 70        | 225–227   | DMF/H₂O (2:1) | C₁₉H₁₂Cl₃N₃O₃S (436.74)     |
| 5a        | F   | H   | 78        | 198–200   | EtOH/Ether (2:1) | C₂₁H₁₆ClN₄O₂S (471.93)     |
| 5b        | Cl  | H   | 76        | 189–191   | EtOH/Ether (2:1) | C₂₁H₁₆ClN₄O₂S (488.39)     |
| 5c        | Cl  | Cl  | 69        | 245–257   | EtOH/Ether (2:1) | C₂₁H₁₅ClN₃O₃S (522.83)     |
| 6a        | F   | H   | 65        | 176–178   | Dioxane    | C₂₁H₁₆ClN₄O₂S (471.93)     |
| 6b        | Cl  | H   | 70        | 214–216   | EtOH       | C₂₁H₁₆ClN₄O₂S (488.39)     |
| 6c        | Cl  | Cl  | 58        | 235–237   | DMF/EtOH (2:1) | C₂₁H₁₆ClN₄O₂S (522.83)     |
| 7a        | F   | H   | 86        | 200–202   | MeOH       | C₂₁H₁₆ClN₄O₂S (442.89)     |
| 7b        | Cl  | H   | 85        | 228–230   | AcOH       | C₂₁H₁₆ClN₄O₂S (459.35)     |
| 7c        | Cl  | Cl  | 84        | 256–258   | AcOH/H₂O (2:1) | C₂₁H₁₅ClN₄O₂S (493.79)     |
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 K₂CO₃ 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_{23}H_{15}ClFN_{3}O_{3}S (467.90) |
| 9b        | Cl | H  | 68        | 214–216   | AcOH        | C_{23}H_{15}Cl_{2}N_{3}O_{3}S (484.35) |
| 9c        | Cl | Cl | 60        | 282–284   | DMF/H_{2}O (2:1) | C_{23}H_{14}Cl_{3}N_{3}O_{3}S (518.80) |
| 10a       | F  | H  | 80        | 178–180   | AcOH/H_{2}O (2:1) | C_{23}H_{16}ClFN_{4}O_{2}S (466.92) |
| 10b       | Cl | H  | 72        | 188–190   | AcOH/H_{2}O (2:1) | C_{23}H_{16}Cl_{2}N_{4}O_{2}S (483.37) |
| 10c       | Cl | Cl | 65        | 256–258   | DMF/H_{2}O (2:1) | C_{23}H_{15}Cl_{3}N_{4}O_{2}S (517.81) |
| 11a       | F  | H  | 78        | 186–188   | AcOH/H_{2}O (2:1) | C_{23}H_{16}ClFN_{4}O_{2}S (480.94) |
| 11b       | Cl | H  | 66        | 200–202   | AcOH        | C_{23}H_{18}Cl_{2}N_{4}O_{2}S (497.40) |
| 11c       | Cl | Cl | 72        | 264–266   | DMF/H_{2}O (2:1) | C_{23}H_{17}Cl_{3}N_{4}O_{2}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).

| Comp. No. | Fungi | Yeast | Bacteria |
|-----------|-------|-------|----------|
|            |       |       | Gram – ve | Gram + ve |
|            |       |       | B.s  | S.l | E.c | P. sp |
| 3a        | 12    | 12    | 12   | 11  | 13  | 14  | 14  | 14  | 13  |
| 3b        | 12    | 12    | 10   | 11  | 9   | 8   | 7   | 9   | 11  |
| 3c        | 8     | 10    | 9    | 11  | 12  | 12  | 12  | 11  | 14  |
| 4a        | 10    | 12    | 11   | 11  | 13  | 11  | 10  | 12  | 11  |
| 4b        | 11    | 12    | 13   | 11  | 14  | 13  | 11  | 12  | 12  |
| 4c        | 10    | 12    | 12   | 13  | 13  | 12  | 10  | 12  | 11  |
| 5a        | 17    | 16    | 16   | 17  | 22  | 23  | 24  | 23  | 21  |
| 5b        | 4     | 5     | 4    | 3   | 7   | 8   | 7   | 9   | 8   |
| 5c        | 13    | 12    | 12   | 13  | 11  | 13  | 12  | 10  | 9   |
| 6a        | 10    | 13    | 10   | 11  | 21  | 20  | 21  | 23  | 23  |
| 6b        | 8     | 8     | 6    | 7   | 11  | 12  | 13  | 13  | 12  |
| 6c        | 12    | 13    | 13   | 12  | 13  | 11  | 13  | 12  | 13  |
| 7a        | 13    | 12    | 12   | 13  | 11  | 13  | 12  | 10  | 9   |
| 7b        | 7     | 5     | 8    | 9   | 6   | 12  | 13  | 13  | 12  |
| 7c        | 12    | 13    | 11   | 13  | 12  | 8   | 8   | 6   | 7   |
| 9a        | 12    | 10    | 11   | 11  | 20  | 20  | 21  | 19  | 20  |
| 9b        | 19    | 20    | 19   | 19  | 11  | 13  | 12  | 10  | 9   |
| 9c        | 10    | 11    | 11   | 12  | 10  | 11  | 10  | 12  | 11  |
| 10a       | 15    | 16    | 13   | 14  | 11  | 11  | 12  | 12  | 13  |
| 10b       | 23    | 22    | 22   | 20  | 11  | 23  | 22  | 24  | 23  |
| 10c       | 11    | 10    | 12   | 11  | 11  | 10  | 12  | 11  | 11  |
| 11a       | 13    | 12    | 12   | 13  | 11  | 13  | 12  | 10  | 9   |
| 11b       | 10    | 12    | 11   | 11  | 13  | 11  | 10  | 12  | 11  |
| 11c       | 13    | 11    | 10   | 12  | 11  | 10  | 12  | 11  | 11  |
| Streptomycin | - | - | - | - | 21 | 22 | 21 | 22 | 21 |
| Fusidic acid | 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 |
|           |      |           | B.s | S.I | E.c | Ps |
| 9a        | 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 |
|-----------|-------|-------|
|           |      | A.n | Pen. sp | C. a | R.i |
| 5a        | 1×   | 17  | 16  | 16  | 17 |
|           | 2×   | 18  | 18  | 19  | 19 |
|           | 3×   | 19  | 20  | 20  | 21 |
|           | 4×   | 20  | 22  | 20  | 21 |
| 10a       | 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. \(^1\)H- and \(^13\)C-NMR spectra were measured on a Jeol 5000 MHz spectrometer (Jeol, Tokyo, Japan) in DMSO-\(d_6\), 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\(_{254}\), 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, cm⁻¹): ν 1679 (C=O), 1607 (C=C); ¹H-NMR: δ 1.32 (t, 3H, CH₃, J = 6.95 Hz), 3.81 (q, 2H, CH₂, 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); ¹³C-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 (M⁺, 15), 184 (100); Elemental analysis for C₁₆H₁₃ClFNO₂ (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, cm⁻¹): ν 1682 (C=O), 1610 (C=C); ¹H-NMR: δ 1.33 (t, 3H, CH₃, J = 6.95 Hz), 3.92 (q, 2H, CH₂, 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); ¹³C-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, 186.86; MS, m/z (%): 322 (M⁺, 8), 165 (100); Elemental analysis for C₁₆H₁₃Cl₂NO₂ (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, cm⁻¹): ν 1678 (C=O), 1612 (C=C); ¹H-NMR: δ 1.28 (t, 3H, CH₃, J = 6.95 Hz), 3.86 (q, 2H, CH₂, 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); ¹³C-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, 187.05; MS, m/z (%): 356 [M⁺,10], 199 [100, base peak]; Elemental analysis for C₁₆H₁₂Cl₃NO₂ (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, cm⁻¹): ν 3330 (NH), 2210 (CN), 1218 (C=S); ¹H-NMR: δ 1.30 (t, 3H, CH₃, J = 6.95 Hz), 3.86 (q, 2H, CH₂, J = 6.95 Hz), 6.46 (d, 1H, CH-olefinic-H, J = 14.60 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 D₂O); ¹³C-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 [M⁺,24], 135 [100, base peak]; Elemental analysis for C₁₆H₁₃ClF₃OS (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, cm⁻¹): ν 3356 (NH), 2215 (CN), 1210 (C=S); ¹H-NMR: δ 1.34 (t, 3H, CH₃, J = 6.95 Hz),
3.86 (q, 2H, CH₂, 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 D₂O); 13C-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⁺,32], 211 [100, base peak]; Elemental analysis for C₁₉H₁₃Cl₂N₃OS (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-ethoxypyridin-4-yl)-4-(2,4-dichlorophenyl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile (4c).

Ethyl 2-(6-(2-chloro-6-ethoxypyridin-4-yl)-3-cyano-4-(substituted phenyl)pyridin-2-ylthio)acetates 5a–c.

To a mixture of 4a–c (1 mmol) and anhydrous K₂CO₃ (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-ethoxypyridin-4-yl)-3-cyano-4-(4-fluorophenyl)pyridin-2-ylthio)acetate (5a).**

IR (KBr, cm⁻¹): v 2219 (CN), 1735 (C=O, ester); ¹H-NMR: δ 1.28, 1.32 (2t, 6H, 2 CH₃), 3.68, 3.86 (2q, 4H, 2 CH₂), 4.38 (s, 2H, S–CH₂), 7.16–7.82 (m, 6H, 4 Ph-H + 2 pyr-H), 8.18 (s, 1H, pyr-5’-H); ¹³C-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⁺,12], 426 [100, base peak]; Elemental analysis for C₂₃H₁₉ClFN₃O₃S (471.93): calcd.: C, 58.54; H, 4.06; Cl, 7.51; N, 8.90; 17; S, 6.79. found: C, 58.48; H, 4.00; Cl, 7.45; N, 8.84; 17; S, 6.72.

**Ethyl 2-(6-(2-chloro-6-ethoxypyridin-4-yl)-3-cyano-4-(4-chlorophenyl)pyridin-2-ylthio)acetate (5b).**

IR (KBr, cm⁻¹): v 2222 (CN), 1732 (C=O, ester); ¹H-NMR: δ 1.29, 1.32 (2t, 6H, 2 CH₃), 3.68, 3.84 (2q, 4H, 2 CH₂), 4.42 (s, 2H, S–CH₂), 7.10–7.72 (m, 6H, 4 Ph-H + 2 pyr-H), 8.64 (s, 1H, pyr-5’-H); ¹³C-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, 164.05, 170.04; MS, m/z (%): 488 [M⁺,32], 120 [100, base peak]; Elemental analysis for C₂₃H₁₉ClFN₃O₃S (487.93): 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-ethoxypyridin-4-yl)-3-cyano-4-(2,4-dichlorophenyl)pyridin-2-ylthio)acetate (5c).**

IR (KBr, cm⁻¹): v 2218 (CN), 1735 (C=O, ester); ¹H-NMR: δ 1.26, 1.30 (2t, 6H, 2 CH₃), 3.58, 3.78 (2q, 4H, 2 CH₂), 4.36 (s, 2H, S–CH₂), 7.12–7.65 (m, 5H, 3 Ph-H + 2 pyr-H), 8.56 (s, 1H, pyr-5’-H); ¹³C-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 (%):
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 CH₂Cl₂, washed with H₂O, 10 mL 1 N HCl and then with water. The solvent was dried over anhydrous CaCl₂, 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⁻¹): v 3443 (NH₂), 1742 (C=O, ester); ¹H-NMR: δ 1.30, 1.34 (2t, 6H, 2 CH₃), 3.72, 4.06 (2q, 4H, 2 CH₂), 4.36 (s, 2H, NH₂ exchangeable with D₂O), 7.24–7.75 (m, 6H, 4 Ph-H + 2 pyr-H), 8.35 (s, 1H, pyr-5'-H); ¹³C-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⁺,26], 317 [100, base peak]; Elemental analysis for C₂₃H₁₉ClFN₃O₃S (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⁻¹): v 3452 (NH₂), 1737 (C=O, ester); ¹H-NMR: δ 1.26, 1.31 (2t, 6H, 2 CH₃), 3.78, 4.10 (2q, 4H, 2 CH₂), 4.48 (s, 2H, NH₂ exchangeable with D₂O), 7.24–7.82 (m, 6H, 4 Ph-H + 2 pyr-H), 8.72 (s, 1H, pyr-5'-H); ¹³C-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⁺,8], 332 [100, base peak]; Elemental analysis for C₂₃H₁₉Cl₂N₃O₃S (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⁻¹): v 3456 (NH₂), 1735 (C=O, ester); ¹H-NMR: δ 1.30, 1.33 (2t, 6H, 2 CH₃), 3.82, 4.15 (2q, 4H, 2 CH₂), 4.56 (s, 2H, NH₂ exchangeable with D₂O), 7.08–7.68 (m, 5H, 3 Pyr-H + 2 pyr-H), 8.62 (s, 1H, pyr-5'-H); ¹³C-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, 132.04, 135.44, 136.74, 145.64, 149.85, 151.38, 154.72, 155.43, 160.04, 164.25; MS, m/z (%): 523 [M⁺,6], 177 [100, base peak]; Elemental analysis for C₂₃H₁₈Cl₃N₃O₃S (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, cm⁻¹): ν 3460–3380 (NH₂), 1675 (C=O, amide); ¹H-NMR: δ 3.85 (q, 2H, CH₂), 6.85 (2s, 4H, 2 NH₂ exchangeable with D₂O), 7.12–7.68 (m, 6H, 4 Ph-H + 2 pyr-H), 8.56 (s, 1H, pyr-5'-H); ¹³C-NMR: 14.06, 64.28, 100.96, 102.22, 115.36, 120.82, 122.45, 128.37, 128.46, 132.15, 145.82, 149.65, 152.00, 154.74, 157.75, 161.55, 162.76, 164.30; MS, m/z (%): 443 [M⁺,8], 332 [100, base peak]; Elemental analysis for C₂₁H₁₆ClFN₄O₂S (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). IR (KBr, cm⁻¹): ν 3456–3378 (NH₂), 1672 (C=O, amide); ¹H-NMR: δ 1.30 (t, 3H, CH₃), 3.82 (q, 2H, CH₂), 4.44, 6.88 (2s, 4H, 2 NH₂ exchangeable with D₂O), 7.32–7.68 (m, 6H, 4 Ph-H + 2 pyr-H), 8.68 (s, 1H, pyr-5'-H); ¹³C-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, 161.42, 164.04; MS, m/z (%): 459 [M⁺,25], 287 [100, base peak]; Elemental analysis for C₂₁H₁₆Cl₂N₄O₂S (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, cm⁻¹): ν 3456 (NH₂), 1735 (C=O, ester); ¹H-NMR: δ 1.28 (t, 3H, CH₃), 3.86 (q, 2H, CH₂), 4.54, 6.76 (2s, 4H, 2 NH₂ exchangeable with D₂O), 7.12–7.73 (m, 5H, 3 Ph-H + 2 pyr-H), 8.48 (s, 1H, pyr-5'-H); ¹³C-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 [M⁺,12], 320 [100, base peak]; Elemental analysis for C₂₁H₁₅Cl₃N₄O₂S (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-(4-chlorophenyl)-2-methyl-4H-pyrido[3′,2′:4,5]thieno[3,2-d]-[1,3]oxazin-4-one (9b). IR (KBr, cm⁻¹): ν 1745 (C=O); ¹H-NMR: δ 1.28 (t, 3H, CH₃), 2.01 (s, 3H, CH₃), 3.89 (q, 2H, CH₂), 7.23–7.65 (m, 6H, 4 Ph-H + 2 pyr-H), 8.42 (s, 1H, pyr-5'-H); ¹³C-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, 149.75, 151.84, 154.86, 155.14, 158.70, 164.12, 165.18; MS, m/z (%): 484 [M⁺,15], 156 [100, base peak]; Elemental analysis for C_{23}H_{15}Cl_{2}N_{3}O_{3}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, cm⁻¹): ν 1750 (C=O); ¹H-NMR: δ 1.30 (t, 3H, CH₃), 2.00 (s, 3H, CH₃), 3.82 (q, 2H, CH₂), 7.21–7.68 (m, 5H, 3 Ph-H + 2 pyr-H), 8.54 (s, 1H, pyr-5'-H); ¹³C-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 [M⁺,8], 320 [100, base peak]; Elemental analysis for C_{23}H_{14}Cl_{3}N_{3}O_{3}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 thienopyrimidinopyridine 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, cm⁻¹): ν 3420 (NH), 1650 (C=O); ¹H-NMR: δ 1.28 (t, 3H, CH₃), 2.32 (s, 3H, CH₃), 3.86 (q, 2H, CH₂), 7.12–7.64 (m, 6H, 4 Ph-H + 2 pyr-H), 8.58 (s, 1H, pyr-5'-H); ¹³C-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, 149.75, 151.84, 154.86, 155.14, 158.70, 164.12, 165.18; MS, m/z (%): 484 [M⁺,15], 156 [100, base peak]; Elemental analysis for C_{23}H_{15}Cl_{2}N_{3}O_{3}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-(4-chlorophenyl)-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]-pyrimidin-4(3H)-one (10b). IR (KBr, cm⁻¹): ν 1745 (C=O); ¹H-NMR: δ 1.28 (t, 3H, CH₃), 3.89 (q, 2H, CH₂), 7.23–7.65 (m, 6H, 4 Ph-H + 2 pyr-H), 8.42 (s, 1H, pyr-5'-H); ¹³C-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, 149.75, 151.84, 154.86, 155.14, 158.70, 164.12, 165.18; MS, m/z (%): 484 [M⁺,15], 156 [100, base peak]; Elemental analysis for C_{23}H_{15}Cl_{2}N_{3}O_{3}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-methylpyrido[3′,2′:4,5]thieno[3,2-d]-pyrimidin-4(3H)-one (10c). IR (KBr, cm⁻¹): ν 3465 (NH), 1649 (C=O); ¹H-NMR: δ 1.26 (t, 3H, CH₃), 2.12 (s, 3H,
CH3), 3.80 (q, 2H, CH2), 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 D2O); 13C-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+5], 145 [100, base peak]; Elemental analysis for C23H15Cl3N4O2S (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 K2CO3 (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-chlorophenyl)-2,3-dimethylpyrido[3′,2′:4,5]thieno[3,2-d]-pyrimidin-4(3H)-one (11a). IR (KBr, cm⁻¹): v 1668 (C=O); 1H-NMR: δ 1.28 (t, 3H, CH3), 2.32, 3.10 (2s, 6H, 2 CH3), 3.78 (q, 2H, CH2), 7.08–7.68 (m, 6H, 4 Ph-H + 2 pyr-H), 8.62 (s, 1H, pyr-5’-H); 13C-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, 157.36, 159.63, 162.76, 164.30; MS, m/z (%): 481 [M+,4], 98 [100, base peak]; Elemental analysis for C24H18ClFN4O2S (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.

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 KNO₃, 1 g K₂HPO₄, 0.5 g KCl, 0.5 g MgSO₄, 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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Sample Availability: Samples of the compounds are available from the authors.

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