Synthesis and Cytotoxicity of Thieno[2,3-b]Pyridine Derivatives Toward Sensitive and Multidrug-Resistant Leukemia Cells

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Abstract

A new series of substituted ethyl 7-cyclopropyl-2-(2-aryloxo)-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylates 3a–e were prepared by utilizing ethyl 2-chloro-7-cyclopropyl-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate (1) and replacing of the 2-chlorine with anions obtained from phenol (2a), salicylaldehyde derivatives 2b–d, or thiophenol (2e), leading to the respective ethyl 7-cyclopropyl-2-(2-aryloxo)-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylates 3a–e. The new compounds were evaluated for their in vitro cytotoxicity towards sensitive CCRF-CEM and multidrug-resistant CEM/ADR5000 leukemia cells. The screening revealed that compounds 3a, 3b, and 3e inhibited the growth of both cell lines. Compound 3b, with a phenol moiety, exhibited the highest growth inhibitory activity against CEM/ADR5000 and CCRF-CEM cells with IC50 values 4.486 ± 0.286 and 2.580 ± 0.550 µM, respectively. Collectively, the presented results demonstrate that the synthesized thieno[2,3-b]pyridines warrant further exploration for potential use as anti-cancer agents.

Keywords: Thieno[2,3-b]pyridine, Multidrug resistance, Cytotoxicity.

1. Introduction

Thieno[2,3-b]pyridines were mentioned for the first time in 1913.1 The chemistry of thieno[2,3-b]pyridines has been well documented during the past decades.1 Various biological activities of this heterocyclic compounds class were described,2 such as antimicrobial,3–6 anti-inflammatory,7–9 antioxidant,6 antituberculous,8 and antimalarial activities.10 Moreover, the incorporation of the N-cyclopropyl group with 4-oxothieno[2,3-b]pyridines showed a higher potency against Escherichia coli ATCC10536 than the N-ethyl and N-tert-butyl analogs.11 It is important to mention that compounds containing thieno[2,3-b]-pyridines moieties attracted considerable interest regarding their potency as anti-cancer agents.12–14

Despite severe undesired side effects, chemotherapeutics are considered effective treatments of primary and metastatic tumors (Figure 1).15 One serious problem of cancer chemotherapy is the development of resistance towards multiple structurally and functionally unrelated anti-cancer drugs.16–18 This phenomenon defined as multidrug resistance (MDR), where chemotherapy fails even at high drug, which leads to toxic side effects.19 MDR is frequently caused by the
overexpression of membrane efflux pumps of the ATP-binding cassette (ABC) transporter family. The best characterized ABC-transporter in this context is P-glycoprotein (Pgp), which causes increased transport of chemotherapeutic agents out of the cells.\textsuperscript{20,21} Thieno[2,3-\textit{b}]pyridines have been reported to exhibit chemopreventive effects suppressing carcinogenesis of numerous tumor types including breast, prostate, non-small cell lung, melanoma, leukemia, ovarian, liver, and colon cancer.\textsuperscript{22,23} As a part of our continuing search for novel biological agents,\textsuperscript{24-27} newly synthesized 4,7-dihydrothieno[2,3-\textit{b}]pyridine derivatives were evaluated for their growth inhibitory activity towards multidrug-resistant CEM/ADR5000 cells in comparison to their pa-

**Figure 1.** Representative samples of chemotherapy in market.

**Scheme 1.** Preparation of ethyl 3-nitro-4-oxothieno[2,3-\textit{b}]pyridine-5-carboxylate 1.
rental sensitive cell line, CCRF-CEM. This is the first report on the cytotoxicity of 4,7-dihydrothieno[2,3-b]pyridine against sensitive and multidrug resistance leukemia cells. Moreover, the structure-activity relationship of the synthesized set was also studied.

2. Results and Discussion

2.1. Chemistry

The synthesis of a selected set of 4,7-dihydrothieno[2,3-b]pyridine derivatives 3a–e has been accomplished in two-step reactions as illustrated in Schemes 1 and 2. The first step involves the formation of ethyl 3-nitro-4-oxothieno[2,3-b]pyridine-5-carboxylate 1. The latter synthon was prepared by starting with 2,5-dichlorothiophene-3-carboxylic acid (4). The following successive steps were performed according to a reported procedure.\(^\text{11}\)

Freshly prepared oxyanions from phenol (2a), salicylaldehyde (2b), 5-methylsalicylaldehyde (2c), and 5-chlorosalicylaldehyde (2d) took part in subsequent nucleophilic aromatic substitution (SNAr) reactions of chloro substituent in compound 1 as shown in Scheme 2. Whereas, compound 3e was prepared according to the same procedure by sulfur anion obtained from benzenethiol (2e) and then reacted with compound 1 in the same manner as phenol derivatives (see Scheme 2).

The new compounds 1 and 3a–e were characterized by IR, MS, and NMR spectral data. These data, given in the

![Scheme 2. Synthesis of ethyl 2-(aryloxo)-7-cyclopentyl-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylic acids 3a–e.](attachment:image.png)

| compound | a | b | c | d | e |
|----------|---|---|---|---|---|
| R        | H | CHO| CHO| CHO| H |
| X        | H | H | 4'- Me | 4'- Cl | H |
| Y        | O | O | O | O | S |

Table 1. NMR spectroscopic data (500 MHz, CDCl\(_3\)) for compound 3a.

| position | \(\delta_c\) type | \(\delta_H\) (\(\text{in Hz}\)) | HMBC |
|----------|------------------|--------------------------|------|
| 1        | -                | -                        | -    |
| 2        | 130.39, C        | -                        | -    |
| 3        | 141.39, C        | -                        | -    |
| 3a       | 121.43, C        | -                        | -    |
| 4        | 167.76, C        | -                        | -    |
| 5        | 116.00, C        | -                        | -    |
| 6        | 145.73, CH       | 8.36, s                  | 3, 4, 1', CO\(_2\)Et |
| 7a       | 150.81, C        | -                        | -    |
| 1'       | 36.56, CH        | 3.47, m                  | -    |
| 2'       | 7.85, CH\(_2\)   | 1.22, m                  | 1'   |
| 3'       | 7.85, CH\(_2\)   | 1.28, m                  | 1'   |
| 1''      | 157.16, C        | -                        | -    |
| 2'', 6'' | 118.42, CH       | 7.25, d                  | 1'', 2'', 6'', 4'' |
| 3'', 5'' | 130.38, CH       | 7.45, t                  | 1'', 3'', 5'' |
| 4''      | 126.28, CH       | 7.30, t                  | 2'', 6'' |
| -OCH\(_2\)CH\(_3\) | 14.37, CH\(_3\) | 1.39, t                  | CH\(_2\) |
| -OCH\(_2\)CH\(_3\) | 61.34, CH\(_2\)  | 4.36, q                  | CO\(_2\)Et |
| C-COO\(_2\)Et| 164.81, C       | -                        | -    |
experimental section, were in compliance with the assigned structures. HMBC correlations allowed the complete assignments: the protons at position H-5' showed a common strong correlation in all compounds 3a–e with the carbons at positions C-1', C-3' and a weak one with the carbons at positions C-4' and C-6', while in compound 3c an additional correlation of the protons at δH 7.54 (d, J = 8.0 Hz, 1H, H-5') with the carbons of the methyl group at δC 20.69 (C-CH3) was observed. The presence of the aldehyde group at position C-2'' enabled to differentiate the correlation in compounds 3b–d for the protons at position H-3'' which showed a correlation with the carbons at positions C-1'', C-5'' and with the aldehyde carbon itself at δC 186.22–188.9 (C-CHO). In addition, in compound 3c the correlation between the signal at δH 7.84 (s, 1H, H-3'') and the signal at δC 20.69 (C-CH3) for the methyl group was detected. The correlation of proton at position H-4'' with the carbons at positions C-2'' and C-6'' was presented in all compounds, except for compounds 3c–d due to substituted position C-4'' to methyl and chloro, respectively. The NMR data of the compound 3a, as a representative of the title compounds 3a–e, are shown in Table 1.

Thus, the mass spectra display the correct molecular ion peaks, for which the measured high-resolution mass spectra (HRMS) data were in good agreement with the calculated values. DEPT and 2D (COSY, HMQC, HMBC) experiments showed correlations that helped in the 1H and 13C signal assignments to the different carbons, and they are attached and/or neighboring hydrogens. H-6 proton resonating at 8.36 as a sharp singlet made a long-range correlation with C-3, C-4, C-1' and CO2Et. In addition, protons 2', 3' show correlation with C-1'. On the other hand, the phenol ring correlated with aldehyde functionality (Table 1).

2. 2. Biological Evaluation. Cytotoxic Activities Against Lymphoblastic Leukemia Cells

As the first step, all compounds were tested at a fixed concentration of 10 µM (Figure 2) in CCRF-CEM and CEM/ADR5000 cells. Compounds 3a, 3e, and 3b significantly inhibited cell viability (reduction to less than 10% growth).

From the structure-activity relationship (SAR) point of view, incorporating salicylaldehyde moiety to thieno[2,3-b]pyridine (3b) exhibited good activity against CCRF-CEM and CEM/ADR5000 cells with IC50 values of 4.76 and 5.11 µM, respectively. However, incorporating the phenol moiety (3a) increased activity against CCRF-CEM and CEM/ADR5000 (Table 2). Changing the hydrogen on the para position of 3b with a halogen in 3d or with methyl in 3c led to reduced biological activity against both cell lines. While replacement of the phenol moiety with thiophenol in 3e retrieved biological activity against CCRF-CEM and CEM/ADR5000 cells with IC50 values of 4.00 and 4.59 µM for 3e, respectively (Table 2). This is a remarkable result since CEM/ADR cells are more than 1000-fold resistant to the established anti-cancer drug doxorubicin. Hence, these compounds inhibited multidrug-resistant cells with similar efficacy as sensitive cells, possibly qualifying them as candidates for further development as treatment of unresponsive cancers.

Table 2. Cytotoxicity of active compounds towards sensitive human lymphoblastic CCRF-CEM and multidrug-resistant CEM/ADR5000 leukemia cells as determined by the resazurin assay.

| Compounds | CCRF-CEM | IC50 [µM]a | CEM/ADR5000 | Degree of resistanceb |
|-----------|-----------|-------------|-------------|-----------------------|
| 3a        | 2.580 ± 0.550 | 4.486 ± 0.286 | 2.580 ± 0.550 | 1.15 ± 0.017          |
| 3b        | 4.763 ± 0.160 | 5.109 ± 0.294 | 4.763 ± 0.160 | 1.07 ± 0.001          |
| 3e        | 4.009 ± 0.154 | 4.591 ± 0.017 | 4.009 ± 0.154 | 1.15 ± 0.017          |

a The lymphoblastic leukemia cells were treated with different concentrations of each compound. Mean values and standard deviation of three independent experiments with every six parallel measurements are shown. b The degrees for resistance were calculated by division of the IC50 values of the compounds for CEM/ADR5000 by the corresponding IC50 values for CCRF-CEM cells.

3. Experimental

3. 1. Chemicals and Equipment

2,5-Dichlorothiophene, cyclopropylamine, 3-acetyl-2,5-dichlorothiophene, phenol, 2-hydroxybenzaldehyde, 2-hydroxy-5-methylbenzaldehyde, 5-chloro-2-hydroxybenzaldehyde, and thiophenol were purchased from Aldrich. Sodium hydride, sodium hydroxide, magnesium sulfate, triethylamine, dimethyformamide, dimethyl sulf oxide, thionyl chloride (SOCl2), and ethyl 3-(N,N-dimethylamino)acrylate were purchased from Acros. Benze-
ene and tetrahydrofuran were dried over sodium metal and distilled, then collected under the nitrogen atmosphere. Thin-layer chromatography plates (Macherey-Nagel GmbH & Co.KG Xtra-SIL G/UV254, 20 × 20 cm, 0.20 mm silica gel 60). Silica gel 60, 0.06–0.2 mm (70–230 mesh ASTM) for column chromatography. Ultraviolet Fluorescence Analysis Cabinet was used to visualize the colorless spots. Melting points (uncorrected) were determined on the Electrothermal IA6304 Melting Point apparatus in open capillary tubes. 1H and 13C NMR spectra were recorded on a 500 MHz (Bruker 500 MHz Avance III) and 400 MHz (Bruker Avance III 400 MHz) spectrometers with TMS as the internal standard. High-resolution mass spectra (HRMS) were measured (in positive or negative ion mode) using the electrospray ion trap (ESI) technique by collision-induced dissociation on a Bruker APEX-IV (7 Tesla) instrument. IR spectra were recorded on a Nicolet Impact 400 FT-IR spectrophotometer.

3. 2. Synthesis of Ethyl 2-Chloro-7-cyclopropyl-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate (1)

A mixture of 2,5-dichlorothiophene-3-carboxylic acid 4 (5 g, 25.4 mmol), and thionyl chloride (SOCl2) (12.0 g, 101 mmol) were dissolved in dry benzene (60 mL), then refluxed for 4–5 h under anhydrous conditions. The solvent and excess thionyl chloride were distilled under reduced pressure, dry benzene (20 mL) was then added to remove the trace of thionyl chloride, and re-distilled. The resulting 2,5-dichloro-3-thionobenzoyl chloride 5 was used for the next step without further purification. To a stirred and cooled (5–10 °C) solution of ethyl 3-(N,N-dimethylamino)acrylate (4.3 g, 30 mmol) and triethylamine (5.1 g, 51 mmol) in dry benzene (50 mL) was added dropwise a solution of 5 in dry benzene (20 mL).

The resulting mixture was refluxed at 85 °C for 2 h, then cooled to room temperature, and washed with water (3 × 2 mL). The organic layer was separated, dried over MgSO4 and the solvent was then evaporated to dryness to obtain the product 6. A stirred solution of 6 in dichloromethane (50 mL) was treated dropwise with cyclopentanone amine (2.85 g, 50 mmol) at 2–4 °C. The resulting mixture was then stirred at 25 °C for 24 h. The solvent was evaporated and the residue was soaked with hexane to obtain a yellow precipitate product 7. Sodium hydride (0.8 g, 17.5 mmol, 55%) in dry THF (60 mL) was added to the pure 7. The reaction mixture was stirred at room temperature for 30 min. Then, the temperature was increased to 60 °C for 3 h. The solvent was evaporated, and the residual white precipitate 8 was washed with water and dried. The product crystallized from the CHCl3/ethanol mixture (1:2). Finally, the nitration of 8 was achieved by dissolving 8 in concentrated sulfuric acid (6 mL). The latter solution was slowly and dropwise added for 30 min to −5 °C stirred solution of fuming nitric acid (2 mL) and concentrated sulfuric acid (5 mL). The mixture was allowed to warm to 5 °C and poured into the ice bath (50 mL). The solid product 1 was filtered and crystallized from DMF/ethanol (1:9) [mp 180–182 °C dec., total yield 30%]. 1H NMR (500 MHz, CDCl3) δ 1.26 (m, 2H) and 1.29 (m, 2H) (H2-2' + H2-3'), and 1.36 (t, J = 7.1 Hz, 3H, CH3CH2O-), 3.49 (m, 1H, H-1'), 4.35 (q, J = 7.1 Hz, 2H, -OCH2CH3), 8.36 (s, 1H, H-6).11

3. 3. General Procedure for Synthesis of Ethyl 2-(Aryloxoy/arylthio)-7-cyclopropyl-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylates 3a–e

To a stirred solution of 1 (0.2 g, 0.6 mmol) in dimethyl sulfoxide (2 mL) the appropriate sodium salt of phenol (2a), salicylaldehydes 2b–d or thiophenol (2e) (0.9 mmol) [prepared from the reaction of compounds 2a–e (0.9 mmol) with sodium ethoxide (0.06 g, 0.9 mmol) in ethanol] was slowly added. The resulting mixture was then heated at 70–80 °C for 12 h. The reaction mixture was cooled and poured onto ice-water (20 mL), then extracted with chloroform (20 mL), the organic layer was washed with water (2 × 20 mL) and brine (2 × 20 mL), dried over anhydrous sodium sulfate Na2SO4 and the solvent was evaporated to yield a yellow, solid substance. This crude product was purified by column chromatography using silica gel and eluting with chloroform/ethyl acetate (1:1, v/v) to give solid products (Scheme 2).

Ethyl 7-Cyclopropyl-3-nitro-4-oxo-2-phenoxy-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate (3a). This compound was prepared from 1 (0.2 g, 0.6 mmol) and phenol (2a) (0.10 g, 0.9 mmol) by following the general procedure and reaction conditions as described above. Reaction time 12 h; yield 0.20 g (87%), mp 260–262 °C. HRMS (ESI) m/z [M+Na] calcd for C19H16N2O6SNa: 423.06213; found: 423.06182. IR 3900, 3089, 2952, 1728, 1688, 1615, 1559, 1486, 1453, 1395, 1340, 1320, 1229, 1199, 1146, 1070, 1044, 922, 875, 836, 800, 691, 597, 554 cm−1. 1H NMR (500 MHz, CDCl3) δ 1.22 (m, 2H) and 1.29 (m, 2H) (H2-2' + H2-3'), 4.35 (q, J = 7.0 Hz, 3H, CH3CH2O-), 3.47 (m, 1H, H-1'), and 8.36 (s, 1H, H-6).11

Finally, the nitration of 8 was achieved by dissolving 8 in concentrated sulfuric acid (6 mL). The latter solution was slowly and dropwise added for 30 min to −5 °C stirred solution of fuming nitric acid (2 mL) and concentrated sulfuric acid (5 mL). The mixture was allowed to warm to 5 °C and poured into the ice bath (50 mL). The solid product 1 was filtered and crystallized from DMF/ethanol (1:9) [mp 180–182 °C dec., total yield 30%]. 1H NMR (500 MHz, CDCl3) δ 1.26 (m, 2H) and 1.29 (m, 2H) (H2-2' + H2-3'), and 1.36 (t, J = 7.1 Hz, 3H, CH3CH2O-), 3.49 (m, 1H, H-1'), 4.35 (q, J = 7.1 Hz, 2H, -OCH2CH3), 8.36 (s, 1H, H-6).11
Ethyl 7-Cyclopropyl-2-(2-formylphenoxo)-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate (3b). This compound was prepared from 1 (0.2 g, 0.6 mmol) and salicylaldehyde (2b) (0.12 g, 0.9 mmol) by following the general procedure and reaction conditions as described above. Reaction time 12 h; yield 0.22 g (85%), mp 200–202 °C. HRMS (ESI) m/z [M+Na+] calcd for C_{20}H_{16}N_2O_7SNa: 451.05704; found: 451.05789. IR 3853, 3745, 2983, 2354, 1687, 1620, 1542, 1448, 1389, 1335, 1299, 1030, 835, 780, 536 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆) δ 1.05 (m, 2H) and 1.19 (m, 2H) (H₂-2' + H₂-3'), and 1.23 (t, J = 7.1 Hz, 3H, CH₂CH₂O-), 3.64 (m, 1H, H-1'), 4.18 (q, J = 7.1 Hz, 2H, -OCH₂CH₂CH₃), δ 7.39 (d, J = 8.3 Hz, 1H, H-6'), 7.46 (t, J = 7.5 Hz, 1H, H-4''), 7.76 (t, J = 7.4 Hz, 1H, H-5''), 7.90 (d, J = 7.1 Hz, 1H, H-3'), 8.32 (s, 1H, H-6), 10.26 (s, 1H, CHO). ¹³C NMR (125 MHz, DMSO-d₆) δ 16.65 (CCH₂CH₃), 14.65 (—OCH₂CH₃), 37.16 (C-1'), 60.83 (OCH₂CH₃), 116.06 (C-3), 118.66 (C-6''), 119.19 (C-2a), 126.48 (C-4''), 126.90 (C-4'), 130.46 (C-3'), 131.44 (C-2), 137.06 (C-5''), 143.72 (C-3'), 146.15 (C-6), 149.33 (C-7a), 158.24 (C-1''), 164.39 (C-COOEt), 167.56 (C-4), 188.9 (C-CHO).

Ethyl 7-Cyclopropyl-2-(2-formyl-4-methylphenoxo)-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate (3c). This compound was prepared from 1 (0.2 g, 0.6 mmol) and 2-hydroxy-5-chlorobenzaldehyde (2b) (0.14 g, 0.9 mmol) by following the general procedure and reaction conditions as described above. Reaction time 12 h; yield 0.16 g (62%), mp 203–205 °C. HRMS (ESI) m/z [M+Na+] calcd for C_{20}H_{16}ClN_2O_7SNa: 463.03928; found: 439.03896. IR 3900, 3089, 2952, 1728, 1688, 1615, 1559, 1486, 1453, 1395, 1344, 1320, 1229, 1199, 1146, 1070, 1044, 925, 836, 800, 761, 694, 597, 554 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 1.15–1.22 (m, 4H, H₂-2' + H₂-3'), and 1.45 (t, J = 7.0 Hz, 3H, -OCH₂CH₃), 2.50 (s, 3H, C(4'')-CH₃), 3.57 (m, 1H, H-1'), 4.42 (q, J = 7.0 Hz, 2H, -OCH₂CH₃), 7.38 (d, J = 8.4 Hz, 1H, H-6''), 7.54 (d, J = 8.0 Hz, 1H, H-5''), 7.84 (s, 1H, H-3'), 8.43 (s, 1H, H-6), 10.48 (s, 1H, CHO). ¹³C NMR (125 MHz, CDCl₃) δ 6.83 (C-2' + C-3'), 14.34 (—OCH₂CH₃), 20.69 (C-CH₂CH₃), 36.70 (C-1'), 61.30 (—OCH₂CH₃), 116.04 (C-3), 118.24 (C-6''), 121.21 (C-3a), 126.25 (C-2'), 129.60 (C-3'), 131.46 (C-2', C-3'), 136.65 (C-4''), 141.99 (C-3), 145.88 (C-6), 149.58 (C-7a), 156.63 (C-1''), 164.49 (C-COOEt), 167.63 (C-4), 187.66 (C-CHO).

Ethyl 2-(4-Chloro-2-formylphenoxy)-7-cyclopropyl-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate (3d). This compound was prepared from 1 (0.2 g, 0.6 mmol) and 2-hydroxy-5-chlorobenzaldehyde (2d) (0.16 g, 0.9 mmol) by following the general procedure and reaction conditions as described above. Reaction time 12 h; yield 0.18 g (67%), mp 202–205 °C. HRMS (ESI) m/z [M+Na+] calcd for C_{19}H_{15}ClN_2O_5SNa: 485.01807; found: 485.01986; [M+H] calcd for C_{19}H_{15}ClN_2O_5S: 463.03613; found: 463.03778. IR 1683, 1623, 1555, 1526, 1487, 1448, 1394, 1323, 1245, 1160, 1136, 1067, 1030, 934, 866, 832, 797, 721, 630 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 1.18 (m, 4H, H₂-2' + H₂-3'), and 1.29 (t, J = 7.0 Hz, 3H, CH₂CH(OH)₂), 3.45 (m, 1H, H-1'), 4.25 (q, J = 6.8 Hz, 2H, —OCH₂CH₃), 7.20 (d, J = 8.4 Hz, 1H, H-6''), 7.53 (dd, J₁ = 7.4 Hz, J₂ = 8.8 Hz, 1H, H-5''), 7.83 (d, J = 2.4 Hz, 1H, H-3''), 8.28 (s, 1H, H-6), 10.31 (s, 1H, CHO). ¹³C NMR (100 MHz, CDCl₃) δ 7.92 (C-2' + C-3'), 14.35 (—OCH₂CH₃), 36.79 (C-1'), 61.41 (—OCH₂CH₃), 116.12 (C-5), 119.21 (C-6''), 121.04 (C-3a), 127.16 (C-2'), 128.99 (C-3'), 132.11 (C-4'), 135.29 (C-5'), 142.61 (C-3'), 146.12 (C-6), 147.76 (C-7a), 157.06 (C-1''), 164.37 (C-COOEt), 167.67 (C-4), 186.22 (C-CHO).
4. Conclusion

A series of novel thieno[2,3-b]pyridine derivatives have been synthesized and screened for their in vitro cytotoxicity towards sensitive CCRF-CEM and multidrug resistance CEM/ADR5000 leukemia cells. Compounds 3a, 3b, and 3e inhibited the growth of both cell lines incorporating phenol without substitution at para position, which can be considered as lead structures for further drug development.

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Appendix A. Supplementary Material

Supplementary data to this article includes 1H, 13C, DEPT, 2D NMR and HRMS spectra of the synthesized ethyl 2-(aryloxo/arylthio)-7-cyclopropyl-3-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate 3a–e compounds which are described in this article.

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Povzetek

Iz ključnega intermediata etil 7-ciklopropil-2-kloro-3-nitro-4-okso-4,7-dihidrotieno[2,3-b]piridin-5-karbonsilata (1) smo s substitucijo 2-klorovega substituenta z anioni, pripravljenimi iz fenola (2a), salicionalnih derivatov 2b–d in tiofenola (2e), sintetizirali serijo novih substituiranih 7-ciklopropil-2-(2-arilo)-3-nitro-4-okso-4,7-dihidrotieno[2,3-b]piridin-5-karbonsilatov 3a–e. Za nove spojine smo določili in vitro citotoksično delovanje proti občutljivim CCRF-CEM levkemičnim celicam in proti CEM/ADR5000 levkemičnim celicam, odpornim na več različnih učinkov. Testiranje je pokazal, da so spojine 3a, 3b in 3e inhibirale obe celične linije. Spojina 3b, ki vsebuje fenolni fragment, pa je pokazala največjo inhibitorno aktivnost rasti pri celicah CEM/ADR5000 in CCRF-CEM z IC_{50} vrednostmi 4.486 ± 0.286 µM (za prvo celično linijo) in 2.580 ± 0.550 µM (za drugo celično linijo). Skupno gledano rezultati kažejo, da pripravljeni tieno[2,3-b]piridini izkazujejo potencialno uporabnost kot protirakove učinkovine in da si zato zaslужijo, da so predmet nadaljnjih raziskav.