Utility of Activated Nitriles in the Synthesis of some New Pyridine and Fused Pyridine Derivatives with Anticancer Activity

Aisha Y. Hassan¹; Marwa T. Sarg²; and Mona. S. El-Zoghi³*

¹Organic Chemistry Department, Faculty of Science (Girls) Al-Azhar University, Cairo, Egypt.  
²Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy (Girls) Al-Azhar University, Cairo, Egypt.  
³Pharmaceutical Chemistry Department, Faculty of Pharmacy, Menoufia University, Menoufia, Egypt.

*Corresponding author: Mona. S. El-Zoghi, Pharmaceutical Chemistry Department, Faculty of Pharmacy, Menoufia University, Menoufia, Egypt.  
Email address: mona_elzoghbi@yahoo.com

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ABSTRACT

Objective: This study aimed synthesis of pyridine and fused pyridine derivatives based on the importance of these heterocycles as anticancer. Method: Novel pyridine and fused pyridine derivatives 3-35 were synthesized through different chemical reactions. Results: Structures of these compounds were confirmed by spectral and elemental analyses. The obtained compounds were evaluated for their in vitro antitumor activity against liver HepG2 and breast MCF-7 cancer cell lines compared to the reference drug (5-fluorouracil). Conclusion: Compounds 4, 12, 13, 19, 21, 28 and 29 were found to be the most active against both cell lines exhibiting IC₅₀ values ranging from 3.05-11.50 μg/mL and 2.87-6.23 μg/mL against HepG-2 and MCF-7 cell lines, respectively.

Keywords: Pyridine; Anticancer; HepG2, MCF-7

INTRODUCTION

Pyridine nuclei are considered as important nitrogen containing heterocyclic systems owing to their several biological activities. Among the important biological activities are anticancer, treatment of Alzheimer's disease, antibacterial, antitubercular, antifungal, anti-inflammatory and the treatment of many cardiovascular diseases such as angina and hypertension. Moreover, pyridine derivatives were found to inhibit the growth of human MCF-7 and HepG-2 cancer cells by exhibiting G2/M phase arrest through a p53-p21-mediated pathway and apoptosis through JNK upregulation. However, numerous cyanopyridines carrying lipophilic moieties can inhibit survivin, that is considered as an inhibitor of apoptosis (IAP) family. Also, several 2-oxo-1,2-dihydropyridine-3-carbonitrile derivatives were found to inhibit the oncogenic serine/threonine kinase PIM-1, that included in cancer cell survival, differentiation, and proliferation. A series of 1-(2-methyl-6-(4-methoxy/3,4-dimethoxyphenyl)-pyridin-3-y1)-3-phenyl-ureas was developed as promising anti-proliferative agents against breast cancer cell line (MCF-7).

It was reported that hetero ring fused pyridine derivatives were found to exhibit significant anticancer activity against four human cancer cell lines such as human breast cancer MCF-7, lung carcinoma A549, melanoma B16-F10 and HeLa cervical carcinoma. In this study, several 2-oxo-1,2-dihydropyridine-3-carbonitrile derivatives were found to inhibit the oncogenic serine/threonine kinase PIM-1. Cyano derivatives were found to inhibit HIV reverse transcriptase and human topoisomerase II. Furthermore, some pyridine derivatives were found to inhibit the human cancer cell lines MCF-7 and HepG-2. Therefore, in this study, the synthesized compounds were evaluated for their in vitro antitumor activity against liver HepG2 and breast MCF-7 cancer cell lines compared to the reference drug (5-fluorouracil).
HeLa (cervical cancer, CCL-2), COLO-205 (colon cancer, CCL-222), HepG2 (liver cancer, HB-8065) and MCF7 (breast cancer, HTB-22) \cite{17,18}. A class of thieno[2,3-b]pyridine compounds have a potent anticancer activity against a variety of tumor cell lines \cite{19,20}, such as breast cancer cell line MDA-MB-231 \cite{21} and MDA-MB-435 melanoma cell line \cite{22}. While, [1,6]naphthyridines derivatives were found to have anticancer activity against melanoma cell line (MDA-MB-435) \cite{22}. Therefore, our aim was to synthesize and evaluate the anticancer activity of various fused and substituted pyridine analogues.

**MATERIAL AND METHODS**

**Chemistry**

All melting points were taken on Electro thermal LA 9000 SERIS, Digital Melting Point Apparatus and were uncorrected. IR Spectra were determined using KBr disk technique on Nikolet IR 200 FT IR Spectrophotometer at Pharmaceutical Analytical Unit, Faculty of Pharmacy, Cairo University, and values are represented in cm$^{-1}$. The $^1$H NMR Spectra was recorded in Varian Gemini EM-300 MHz, NMR Spectrometer at laboratories of the nuclear magnetic resonance, Chemical Warfare Department, Ministry of Defense, DMSO-d$_6$ was used as a solvent and Chemical shifts were measured in δ ppm, relative to TMS as internal standard. Mass Spectra were recorded at 70 ev on DI-50 unit of Schimadzu GC/MS-QP5050A Spectrometer at Regional Center for Mycology and Biotechnology, Al-Azhar University. Microanalyses were carried out at Regional Center for Mycology and Biotechnology, Al-Azhar University.

Cyanothioacetamide 1 was prepared according to the reported procedure \cite{23,24}.

**Ethyl 5-cyano-6-mercaptopo-2-oxo-1,2-dihydropyridine-3-carboxylate; 2**

To a solution of sodium metal (0.02 g, 1 mmol) in an absolute ethanol (20 mL), cyanothioacetamide 1 (1 g, 10 mmol) was added. The reaction mixture was stirred for 5 min. during which a solution was formed. After that diethyl ethoxymethylidenmalonate (2.16 g, 10 mmol) was added to the reaction mixture and stirring was continued for another 10 min. A precipitate was formed and the mixture was left for 2 h. The precipitate was filtered off, washed with ethanol and hexane and crystallized from DMF/ethanol. Orange powder, 82 % yield; m.p.; 303-305 °C as reported \cite{25}.

**Ethyl 2-acetyl-3-amino-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-5-carboxylate; 3**

An equimolar mixture of compound 2 (0.45 g, 2 mmol) and chloroacetonitrile (0.8 g, 16 mL, 2 mmol) was fused for 20 h. The reaction mixture was allowed to cool then triturated with ethanol. The obtained solid was filtered off and washed with various solvents to give compound 3.

Black powder, 38 % yield; m.p.; >360 °C, IR (KBr, cm$^{-1}$): 3421, 3414 (br. OH tautomer); 3387, 3292 (NH$_2$, NH); 3049 (C–H aromatic); 2924, 2854 (C-H aliphatic); 1720, 1710, 1701 (C=O); 1591 (C=N); 1560 (C=C); 1255, 1090 (C–S–O–C); 1236, 1090 (C–O–C). $^1$H-NMR (DMSO-d$_6$, δ ppm): 1.08-1.19 (m, 3H, –OCH$_2$CH$_3$); 2.55 (s, 3H, CH$_2$CO); 3.90-4.05 (m, 2H, –OCH$_2$CH$_3$); 6.38 (s, 2H, NH$_2$; D$_2$O exchangeable); 7.12 (s, 1H, NH; D$_2$O exchangeable); 7.95 (s, 1H, pyridine–C=H). MS m/z (relative intensity %): 280 (M$^+$, 2.53).

**Anal. Form:** C$_{12}$H$_2$N$_2$O$_2$S (280), **Calcd. (%)**: C, 51.42; H, 4.32; N, 9.99; S, 11.44. **Found (%)**: C, 51.69; H, 4.37; N, 10.12; S, 11.59.

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**Figure 1. Biologically active pyridine containing anticancer agents.**
3-Amino-5-(ethoxy carbonyl)-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylic acid; 4

A mixture of compound 2 (0.45 g, 2 mmol) and chloroacetic acid (0.19 g, 2 mmol) was refluxed for 10 h in a mixture of acetic anhydride and acetic acid (1:2) in presence of anhydrous sodium acetate (0.16 g, 2 mmol). The reaction mixture was allowed to cool then poured onto crushed ice and the obtained solid was filtered, washed with water and crystallized from ethanol to yield compound 4.

Dark brown powder, 62% yield, m.p.; > 360 °C, IR (KBr, cm⁻¹): 3446, 3421 (br. OH); 3275, 3228 (NH₂, NH); 3057 (C–H aromatic); 2926, 2840 (C–H aliphatic); 1732, 1699, 1683 (C=O); 1570 (C≡N); 1558 (C=C); 1242, 1091 (C–S–C & C–O–C). ¹H-NMR (DMSO-d₆, δ ppm): 1.20-1.25 (m, 3H, –OCH₂CH₃); 2.00-4.12 (m, 2H, –OCH₂CH₃); 6.13 (s, 2H, NH₂, D₂O exchangeable); 7.52 (s, 1H, pyridine–C₃–H); 8.15 (s, 1H, NH, D₂O exchangeable); 11.60 (s, 1H, OH, D₂O exchangeable). MS m/z (relative intensity %): 283 (M⁺+1, 3.10); 282 (M⁺, 6.45); 281(M⁺-1, 3.76); 280 (M⁺-2, 1.60). Anal. Form: C₁₃H₁₆N₂O₃S (282). Calcd. (%): C, 47.02; H, 3.61; N, 10.04; S, 11.12.

Ethyl 5-cyano-6-(2-ethoxy-2-oxoethylthio)-2-oxo-1,2-dihydropyridine-3-carboxylate; 5 and Diethyl 3-amino-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-2,5-dicarboxylate; 6

Compound 2 (0.45 g, 2 mmol) was suspended in sodium ethoxide solution [prepared by dissolving sodium (0.05 g, 2 mmol) in 10 mL absolute ethanol] and then ethyl chloroacetate (0.24 g, 0.21 mL, 2 mmol) was added and heated under reflux for 12 h. The formed solid was filtered, washed with water several times and crystallized from ethanol to give compound 5, while the alcohol insoluble part was found to be compound 6.

Ethyl 5-cyano-6-(2-ethoxy-2-oxoethylthio)-2-oxo-1,2-dihydropyridine-3-carboxylate; 5

Orange powder, 45 % yield; m.p.; 120-122°C, IR (KBr, cm⁻¹): 3429, 3323 (NH); 3049 (C–H aromatic); 2927, 2852 (C–H aliphatic); 2216 (C≡N); 1730, 1710, 1701 (C=O); 1618 (C≡N); 1580 (C≡C); 1278, 1091 (C–S–C); 1278, 1064 (C–O–C). ¹H-NMR (DMSO-d₆, δ ppm): 1.23-1.54 (m, 6H, two –OCH₂CH₃); 4.25 (q, 2H, J = 7.2 Hz, thiophene–C₃–OCH₂CH₃); 4.41 (q, 2H, J = 7.2 Hz, pyridine–C₃–OCH₂CH₃); 4.70 (s, 1H, SCH₂ tautomer); 7.51 (s, 1/2 H, SCH=C=O–H tautomer, D₂O exchangeable); 7.63 (s, 1/2 H, NH tautomer, D₂O exchangeable); 8.32 (s, 1/2 H, SCH=C=O–H tautomer, D₂O exchangeable). MS m/z (relative intensity %): 311 (M⁺+1, 2.41); 310 (M⁺, 5.21); 309 (M⁺-1, 33.96). Anal. Form: C₁₅H₁₈N₂O₅S (310). Calcd. (%): C, 50.31; H, 4.55; N, 9.03; S, 10.33. Found (%): C, 50.43; H, 4.59; N, 9.12; S, 10.46.

Diethyl 3-amino-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-2,5-dicarboxylate; 6

Pale yellow powder, 38 % yield, m.p.; 240-242 °C, IR (KBr, cm⁻¹): 3446, 3311, 3201 (NH₂, NH); 2980 (C–H aromatic); 2860, 2840 (C–H aliphatic); 1720, 1700, 1670 (C=O); 1608 (C≡N); 1550 (C≡C); 1253, 1064 (C–S–C); 1253, 1037 (C–O–C). ¹H-NMR (DMSO-d₆, δ ppm): 1.32 (t, 3H, J = 7.1 Hz, thiophene–C₃–OCH₂CH₃); 1.51 (t, 3H, J = 7.1 Hz, pyridine–C₃–OCH₂CH₃); 4.30 (q, 2H, J = 7.1 Hz, thiophene–C₂–OCH₂CH₃); 4.69 (q, 2H, J = 7.1 Hz, pyridine–C₃–OCH₂CH₃); 7.63 (s, 2H, NH₂, D₂O exchangeable); 8.96 (s, 1H, pyridine–C₃–H); 8.97 (s, 1H, NH, D₂O exchangeable). MS m/z (relative intensity %): 310 (M⁺, 21.64); 309 (M⁺-1, 100); 308 (M⁺-2, 6.08). Anal. Form: C₁₃H₁₈N₂O₅S (310). Calcd. (%): C, 50.31; H, 4.55; N, 9.03; S, 10.33. Found (%): 50.45; H, 4.61; N, 9.08; S, 10.45.

Ethyl 5-cyano-6-[2-(4-methoxyphenyl)-2-oxoethythio]-2-oxo-1,2-dihydropyridin-3-carboxylate; 7 and Ethyl 3-amino-2-(4-methoxybenzoyl)-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-5-carboxylate; 8

A mixture of compound 2 (0.45 g, 2 mmol), 4-methoxycinnamyl chloride (0.45 g, 0.042 mmol) and a catalytic amount of anhydrous potassium carbonate (0.28 g, 2 mmol) in dimethylformamide (10 mL) was refluxed for 10 h. The reaction mixture was poured onto crushed ice and the precipitate formed was filtered, washed with water and crystallized from ethanol to give compound 7, while the alcohol insoluble part was found to be compound 8.

Ethyl 5-cyano-6-[2-(4-methoxyphenyl)-2-oxoethythio]-2-oxo-1,2-dihydropyridin-3-carboxylate; 7

Brown powder, 40 % yield, m.p.; 100-102 °C, IR (KBr, cm⁻¹): 3406 (br. OH tautomer); 3385, 3284, 3277 (NH); 3101, 3035 (C–H aromatic); 2922, 2835 (C–H aliphatic); 2220 (C≡N); 1690, 1670 (C=O), 1593 (C≡N); 1558 (C≡C); 1292, 1080 (C–S–C); 1251, 1060 (C–O–C). ¹H-NMR (DMSO-d₆, δ ppm): 1.20-2.14 (m, 3H, –OCH₂CH₃); 3.71 (s, 3H, –OCH₃); 3.82-3.87 (m, 2H, –OCH₂CH₃); 4.20 (s, 1H, S–CH₂–C=O tautomer); 7.06 (d, 2H, J = 8.5 Hz, 4–OCH₃–C₆H₄–CH₃–S–H); 7.75 (d, 2H, J = 8.5 Hz, 4–OCH₃–C₆H₄–CH₃–S–H); 7.86 (s, 1/2 H, –CH≡C–O–H tautomer); 7.93 (s, 1/2 H, CH≡C–OH tautomer, D₂O exchangeable); 8.05 (s, 1H, pyridine–C₃–H); 8.40 (s, 1/2 H, NH, D₂O exchangeable); 10.80 (s, 1/2 H, pyridine–C₃–OH tautomer, D₂O exchangeable). MS m/z (relative intensity %): 372 (M⁺, 21.13). Anal. Form: C₁₅H₁₄N₂O₅S (372). Calcd. (%): C, 58.05; H, 4.33; N, 7.52; S, 8.61. Found (%): C, 58.23; H, 4.41; N, 7.69; S, 8.78.
Ethyl 3-amino-2-(4-methoxybenzoyl)-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-5-carboxylate; 8

Brown powder, 32% yield, m.p.: 240-242 °C, IR (KBr, cm⁻¹): 3388, 3269, 3188 (NH₂, NH); 3070, 3061 (C–H aromatic); 2926, 2852 (C–H aliphatic); 1720, 1700, 1680 (C=O); 1593 (C=N); 1535 (C=C); 1288, 1060 (C–S–C); 1251, 1024 (C–O–C). ¹H-NMR (DMSO-d₆, δ ppm): 1.20-1.23 (m, 3H, –OCH₂CH₃ 3.51 (s, 3H, –OCH₃); 3.81-3.86 (m, 2H, –OCH₂CH₃); 5.21 (s, 2H, NH₂, D₂O exchangeable); 7.00-7.10 (m, 2H, 4- OCH₃-C₆H₄-C₃–H); 7.80-7.93 (m, 2H, 4-OCH₃-C₆H₄-C₂–H); 8.47 (s, 1H, NH, D₂O exchangeable); 8.95 (s, 1H, pyridine–C₆–H). MS m/z (relative intensity %): 372 (M⁺, 10.00). Anal. Form: C₁₆H₁₆N₂O₄S (372). Calcd. (%): C, 58.05; H, 4.33; N, 7.52; S, 8.61. Found (%): C, 58.17; H, 4.39; N, 7.64; S, 8.79.

Ethyl 3-amino-2-cyano-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-5-carboxylate; 9 and Ethyl 5-cyano-(cyanomethylthio)-2-oxo-1,2-dihydropyridine-3-carboxylate; 10

**Method A:**

To a solution of compound 2 (0.45 g, 2 mmol) in dimethylformamide (10 mL), anhydrous potassium carbonate (0.28 g, 2 mmol) was added. The reaction mixture was stirred at room temperature and chloroacetanilide (0.15 g, 0.13 mL, 2 mmol) was added dropwise while stirring. Stirring was continued at room temperature for 24 h. The reaction mixture was then poured onto cold water and acidified with diluted hydrochloric acid and the obtained product was filtered, washed with water and crystallized from ethanol giving compound 10, while the alcohol insoluble part was found to be compound 9.

**Method B for preparing compound 9**

An equimolar mixture of compound 2 (0.45 g, 2 mmol), anhydrous potassium carbonate (0.28 g, 2 mmol) and chloroacetanilide (0.15 g, 0.13 mL, 2 mmol) in dimethylformamide (10 mL) was refluxed for 12 h. The reaction mixture was then diluted with acidified water and the obtained product was filtered, washed with water to give compound 9.

Ethyl 3-amino-2-cyano-6-oxo-5,7-dihydrothieno[2,3-b]pyridine-5-carboxylate; 9

Dark brown powder, 43% yield, m.p.: > 360 °C, IR (KBr, cm⁻¹): 3421, 3408 (br. OH tautomer); 3332, 3319, 3149 (NH₂, NH); 3057 (C–H aromatic); 2922, 2852 (C–H aliphatic); 2200 (C=C); 1700, 1680 (C=O); 1593 (C=N); 1544 (C=C); 1280, 1060 (C–S–C); 1280, 1020 (C–O–C). ¹H-NMR (DMSO-d₆, δ ppm): 1.20-1.23 (m, 3H, –OCH₂CH₃); 3.10-3.30 (m, 2H, –OCH₂CH₃ under DMSO); 6.89 (s, 2H, NH₂, D₂O exchangeable); 7.23 (s, 1H, NH, D₂O exchangeable); 7.95 (s, 1H, pyridine–C₆–H). MS m/z (relative intensity %): 264 (M⁺+1, 24.16); 263 (M⁺, 4.83); 261 (M⁺–2, 2.74). Anal. Form: C₁₉H₁₄N₄O₅S (263). Calcd. (%): C, 50.18; H, 3.45; N, 15.96; S, 12.18. Found (%): C, 50.41; H, 3.43; N, 16.08; S, 12.32.

Ethyl 5-cyano-(cyanomethylthio)-2-oxo-1,2-dihydropyridine-3-carboxylate; 10

Dark brown powder, 40% yield, m.p.: 180-182 °C, IR (KBr, cm⁻¹): 3392, 3342, 3170 (NH); 3049 (C–H aromatic); 2924, 2852 (C–H aliphatic); 2191 (C≡N); 1720, 1700 (C=O); 1595 (C≡N); 1560 (C=C); 1296, 1095 (C–S–C); 1259, 1095 (C–O–C). ¹H-NMR (DMSO-d₆, δ ppm): 1.20-1.36 (m, 3H, –OCH₂CH₃); 3.47 (s, 1H, –S–CH₂–CN tautomer); 4.10-4.30 (m, 2H, –OCH₂CH₃); 6.34 (s, 1/2 H, S–CH=C=NH tautomer, D₂O exchangeable); 7.13 (s, 1/2 H, pyridine–NH, D₂O exchangeable); 7.46 (s, 1/2 H, pyridine–C₂–OH tautomer, D₂O exchangeable); 8.32 (s, 1/2 H, S–CH=C=NH tautomer); 8.62 (s, 1H, pyridine–C₆–H). MS m/z (relative intensity %): 263 (M⁺, 1.37). Anal. Form: C₁₁H₈N₄O₄S (263). Calcd. (%): C, 50.18; H, 3.45; N, 15.96; S, 12.18. Found (%): C, 50.34; H, 3.49; N, 16.04; S, 12.30.

Ethyl 3-amino-2-carbamoyl-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-5-carboxylate; 11

A mixture of 5-cyano-6-mercaptopyrindine 2 (0.45 g, 2 mmol), chloroacetamide (0.19 g, 2 mmol) and anhydrous potassium carbonate (0.28 g, 2 mmol) in dimethylformamide (5 mL) was refluxed for 20 h. The reaction mixture was diluted with water and the obtained solid was filtered, washed with water and crystallized from DMF/H₂O to give compound 11.

Dark brown powder, 52% yield, m.p.: > 360 °C, IR (KBr, cm⁻¹): 3392 (br. OH tautomer); 3336, 3313, 3196 (NH₂, NH); 3064 (C–H aromatic); 2924, 2852 (C–H aliphatic); 1710, 1690, 1660 (C=O); 1604 (C≡N); 1541 (C≡C); 1286, 1060 (C–S–C); 1271, 1030 (C–O–C). ¹H-NMR (DMSO-d₆, δ ppm): 1.01-1.18 (m, 3H, –OCH₂CH₃); 4.00-4.03 (m, 2H, –OCH₂CH₃); 6.75 (s, 2H, thienopyridine–C₂–NH₂, D₂O exchangeable); 7.44 (s, 2H, thienopyridine–C₆–NH₂, D₂O exchangeable); 7.89 (s, 1H, pyridine–C₆–H); 8.15 (s, 1H, D₂O exchangeable). MS m/z (relative intensity %): 281 (M⁺, 1.87); 279 (M⁺–2, 0.57). Anal. Form: C₁₁H₁₀N₃O₃S (281). Calcd. (%): C, 46.97; H, 3.94; N, 14.94; S, 11.40. Found (%): C, 47.12; H, 3.98; N, 15.08; S, 11.51.

Ethyl 10-amino-2-oxo-1H-6,7,8,9-tetrahydropyrido[3′,2′:4,5][thieno[3,2-b]quinoline-3-carboxylate; 12 and Ethyl 4,7-dioxo-6H-2,2-hexamethylene-1,2,3,4-tetrahydropyrido [3′,2′ : 4,5][thieno[3,2-d]pyrimidine-8-carboxylate; 13

An equimolar mixture of compound 9 (0.53 g, 2 mmol) or 11 (0.56 g, 2 mmol) and cyclohexanone (0.2 g, mL, 2 mmol) in dimethylformamide (5 mL) containing

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a catalytic amount of zinc chloride (0.27 g, 2 mmol) was refluxed for 20 h. The reaction mixture was allowed to cool and the solid product obtained was filtered off and washed with water to yield compound 12. The filtrate was poured onto crushed ice and the obtained solid was collected to give compound 13.

**Ethyl 10-amino-2-oxo-1H-6,7,8,9-tetrahydropyrindro [3',2':4,5]thieno [3,2-b]quinoline; 12**

Dark brown powder, DMF/H2O, 53 % yield, m.p.; > 360 °C, IR (KBr, cm−1): 3406 (br. OH tautomeric); 3352, 3307, 3265 (NH2, NH); 3074 (C-H aromatic); 2933 (C-H aliphatic); 1700, 1660 (C=O); 1589 (C=N); 1558 (C=C); 1270, 1064 (C-S-C & C-O-C).1H-NMR (DMSO-d6, δ ppm): 1.20-1.26 (m, 3H, –O-CH2CH3); 1.80-1.85 (m, 4H, tetrahydroquinoline–C3=C=CH2); 3.11-3.19 (m, 2H, tetrahydroquinoline–C6=C=CH2); 3.40-3.50 (m, 2H, 2H, tetrahydroquinoline–C6=C=CH2); 3.55-3.61 (m, 2H, 2H, –O-CH2CH3); 5.84 (s, 2H, NH, D2O exchangeable); 7.19 (s, 1H, NH, D2O exchangeable); 8.08 (s, 1H, thienopyridine–C=N–H). MS m/z (relative intensity %): 345 (M+42, 2.23); 343 (M+, 3.76). Anal. Form: C20H12N2O2S (343). Calcld. (%): C, 59.46; H, 4.99; N, 12.24; S, 9.34. Found (%): C, 59.46; H, 4.08; N, 12.32; S, 9.49.

**Ethyl 4,7-dioxo-6H-2,2-hexamethylene-1,2,3,4-tetrahydropyrindro [3',2':4,5]thieno [3,2-d]pyrimidine-5-carboxylate; 13**

Buff powder, DMF/EtOH, 32 % yield, m.p.; > 360 °C. IR (KBr, cm−1): 3448 (br. OH tautomeric); 3207, 3192 (C-H aromatic); 2924, 2854 (C-H aliphatic); 1700, 1680, 1670 (C=O); 1587 (C=N); 1255, 1103 (C-S-C); 1255, 1043 (C-O-C).1H-NMR (DMSO-d6, δ ppm): 1.19-1.24 (m, 3H, –O-CH2CH3); 1.70-1.83 (m, 6H, cyclohexyl-C3=C=CH2); 2.20-2.27 (m, 4H, cyclohexyl-C2=C-CH3); 3.52-3.61 (m, 2H, –O-CH2CH3); 7.95 (s, 1H, pyrimidine–N=N–H, D2O exchangeable); 8.22 (s, 1H, thienopyridine–C=N–H); 12.19 (s, 2H, thienopyridine–N=C=H, H, pyrimidine–N=N–H, D2O exchangeable). MS m/z (relative intensity %): 361 (M+, 2.80). Anal. Form: C19H12N2O2S (361). Calcld. (%): C, 56.50; H, 5.30; N, 11.63; S, 8.87. Found (%): C, 56.67; H, 5.37; N, 11.80; S, 9.04.

**Ethyl 2-acetyl-3-imino-2-methyl-6-oxo-2,3,6,7-tetrahydrothieno[2,3-b]pyridine-5-carboxylate; 14**

Ethyl 3'-imino-2,6'-dioxo-6',7'-dihydro-3'H-spirocyclopentane-1,2'-thieno[2,3-b]pyridine-5'-carboxylate; 15 and Ethyl 2,2-diacyethyl-3-imino-6-oxo-2,3,6,7-tetrahydrothieno[2,3-b] pyridine-5-carboxylate; 16

**General procedure**

A mixture of 5-cyano-6-mercaptopypyridine 2 (0.45 g, 2 mmol) and the appropriate ketone (2 mmole) namely: ethylmethyl ketone, cyclopentanone and acetylacetone; respectively was refluxed in acetic acid (5 mL) containing a few drops of conc. H2SO4 (3-5 drops) for 2 h, then acetic anhydride (2 mL) was added to the reaction mixture and refluxing was continued for another 2 h. The reaction mixture was cooled and the obtained products were filtered and crystallized from the appropriate solvent to yield compounds 14, 15 and 16, respectively.
**Ethyl 5-cyano-6-(1-ethoxy-1,3-dioxobutan-2-ylthio)-2-oxo-1,2-dihydropyridine-3-carboxylate; 17, and Diethyl 2-acetyl-3-imino-6-oxo-2,3,6,7-tetrahydro thieno[2,3-b]pyridine-2,5-dicarboxylate; 18**

To a stirred solution of 5-cyano-6-mercaptopopyridine **2** (0.45 g, 2 mmol) in ethanol/dimethylformamide (4:1) (10 mL) aqueous potassium hydroxide solution (0.11 g, 2 mmol) (2 mL) was added. To the resulting solution ethyl 2-chloro-3-oxobutanolate (0.33 g, 0.28 mL, 2 mmol) was added and the reaction mixture was stirred for 24 h, then diluted with water. The precipitated solid was filtered off, washed with water, dried and crystallized from dioxane/ethanol to give compound **17** while the insoluble part was found to be compound **18**.

**Ethyl 5-cyano-6-(1-ethoxy-1,3-dioxobutan-2-ylthio)-2-oxo-1,2-dihydropyridine-3-carboxylate; 17**

Buff powder, 45% yield, m.p.: 95-97 °C, IR (KBr, cm⁻¹): 3410 (OH tautomer); 3317, 3221 (NH); 2926, 2850 (C–H aliphatic); 2206 (C≡N); 1728, 1680 (C=O); 1631 (C=O); 1539, 1512 (C=C); 1300, 1026 (C–S–C), 1261, 1026 (C–O–C). 

**1H-NMR** (DMSO-d₆, δ ppm): 1.24–1.34 (m, 4 H, two –O–CH₂CH₃); 3.71 (s, 3H, CH₃C≡O); 4.19 (q, 2H, J= 7.2 Hz, 3-oxobutanolate–C–O–CH₂CH₃); 4.29 (q, 2H, J=7.1 Hz, pyridine–C–COO–CH₂CH₃); 7.59 (s, 1H, 3-oxobutanolate–C==H); 7.29 (s, 1/2 H, NH, D₂O exchangeable); 8.15 (s, 1H, pyridine–C≡H); 12.10 (s, 1/2 H, OH tautomer, D₂O exchangeable). MS m/z (relative intensity %): 352 (M⁺, 97). **Anal. Form:** C₁₄H₁₄N₂O₅S (322). **Calcd.** (%): C, 52.17; H, 4.38; N. 8.69; S, 9.95. **Found** (%): C, 52.32; H, 4.42; N, 8.81; S, 10.03.

**Diethyl 2-acetyl-3-imino-6-oxo-2,3,6,7-tetrahydro thieno[2,3-b]pyridine-2,5-dicarboxylate; 18**

Brown powder, 40% yield, m.p.: > 360°C, IR (KBr, cm⁻¹): 3431 (OH tautomer); 3261, 3186 (NH); 3000 (C–H aromatic); 2920 (C–H aliphatic); 1730, 1670 (C=O); 1616 (C≡N); 1550 (C=C); 1301, 1093 (C–S–C); 1265, 1066 (C–O–C). **1H-NMR** (DMSO-d₆, δ ppm): 1.23-1.33 (m, 6H, two –O–CH₂CH₃); 2.71 (s, 3H, CH₃C≡O); 4.17 (q, 2H, J=6.7Hz, 3-oxobutanolate–C–O–CH₂CH₃); 4.28 (q, 2H, J=6.7Hz, pyridine–C≡COO–CH₂CH₃); 5.80 (s, 1H, C≡NH, D₂O exchangeable); 7.29 (s, 1/2 H, NH, D₂O exchangeable); 8.18 (s, 1H, pyridine–C≡H); 12.18 (s, 1/2 H, OH tautomer, D₂O exchangeable). MS m/z (relative intensity %): 352 (M⁺, 2.99). **Anal. Form:** C₁₃H₁₀N₂O₈S (352). **Calcd.** (%): C, 51.13; H, 4.58; N, 7.95; S, 9.10. **Found** (%): C, 51.29; H, 4.64; N, 8.07; S, 9.15.

**Ethyl 6-(2-chloro-2-oxoacetylthio)-5-cyano-2-oxo-1,2-dihydro pyridine-3-carboxylate; 19 and Ethyl 8-cyano-2,3,5-trioxo-3,5-dihydro-2H-thiazolo[3,2-al]pyridine-6-carboxylate; 20**

A mixture of 5-cyano-6-mercaptopopyridine **2** (0.45 g, 2 mmol) and oxalylchloride (0.25 g, 0.17 mL, 2 mmol) was fused at 160-170 °C for 30 min. The reaction mixture was then triturated with ethanol. The obtained precipitate was filtered, washed with diethyl ether and crystallized from ethanol to give compound **19** while the alcoholic insoluble part was found to be compound **20**.

**Ethyl 6-(2-chloro-2-oxoacetylthio)-5-cyano-2-oxo-1,2-dihydropyridine-3-carboxylate; 19**

Dark brown powder, 36 % yield, m.p.: 117-119 °C, IR (KBr, cm⁻¹): 3425, 3414 (br. OH tautomer); 3238, 3192 (NH); 2993 (C–H aromatic); 2926, 2850 (C–H aliphatic); 2223 (C≡N); 1737, 1697 (C=O); 1602 (C≡N); 1550 (C=C); 1286, 1070 (C–S–C); 1249, 1070 (C–O–C).

**1H-NMR** (DMSO-d₆, δ ppm): 1.42 (t, 3H, J = 3.4 Hz, –OCH₂CH₃); 4.55 (q, 2H, J = 7.1 Hz, –OCH₂CH₃); 8.15 (s, 1/2 H, NH, D₂O exchangeable); 8.39 (s, 1H, pyridine–C≡H); 12.77 (s, 1/2 H, OH tautomer, D₂O exchangeable). MS m/z (relative intensity %): 314 (M⁺, 0.79). **Anal. Form:** C₁₃H₁₀C₄N₂O₈S (314). **Calcd.** (%): C, 41.98; H, 2.24; N, 8.90; S, 10.19. **Found** (%): C, 42.13; H, 2.21; N, 8.98; S, 10.31.

**Ethyl 8-cyano-2,3,5-trioxo-3,5-dihydro-2H-thiazolo[3,2-al]pyridine-6-carboxylate; 20**

Dark brown crystals, 38 % yield, m.p.: >360 °C, IR (KBr, cm⁻¹): 3068, 3016 (C–H aromatic); 2924, 2854 (C–H aliphatic); 2214 (C≡N); 1701, 1680, 1670 (C=O); 1622, 1595 (C≡N); 1558, 1535 (C=C); 1273, 1060 (C–S–C); 1244, 1060 (C–O–C). **1H-NMR** (DMSO-d₆, δ ppm): 1.21 (t, 3H, J = 6Hz, –OCH₂CH₃); 4.05-4.38 (m, 2H, –OCH₂CH₃); 8.51 (s, 1H, pyridine–C≡C–H). MS m/z (relative intensity %): 278 (M⁺, 2.01). **Anal. Form:** C₁₃H₈N₂O₈S (278). **Calcd.** (%): C, 47.48; H, 2.17; N, 10.07; S, 11.52. **Found** (%): C, 47.62; H, 2.19; N, 10.23; S, 11.60.

**Ethyl 5-cyano-6-(ethoxycarbonylthio)-2-oxo-1,2-dihydropyridine-3-carboxylate; 21**

A mixture of 5-cyano-6-mercaptopopyridine **2** (0.45 g, 2 mmol) and ethyl chloroformate (0.22 g, 0.19 mL, 2 mmol) containing a catalytic amount of anhydrous potassium carbonate (0.28 g, 2 mmol) in dry dimethylformamide (10 mL) was refluxed for 20 h. The
Ethyl 8-cyano-2-(2-methoxy-2-oxoethyldiene)-3,5-dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-6-carboxylate; 22

An equimolar mixture of 5-cyano-6-mercaptopurine 2 (0.45 g, 2 mmol) and dimethyl acetylene dicarboxylate (0.28 g, 0.25 mL, 2 mmol) was fused at 160-170 °C for 8 h. The reaction mixture was left to cool then triturated with absolute ethanol. The obtained product was filtered, washed with diethyl ether, left to dry and crystallized from ethanol to give compound 22.

Dark brown powder, 80 % yield, m.p.: 224-226 °C. IR (KBr, cm⁻¹): 3005 (C-H aromatic); 2954, 2926, 2852 (C-H aliphatic); 1690 (C=O); 1624 (C=O); 1540 (C=C); 1520, 1240, 1070 (C-O); 2922 (C-H aromatic); 2852, 2826 (C-H aliphatic); 2227 (C=O); 1710, 1660 (C=O); 1587 (C=C); 1529 (C=C); 1251, 1070 (C-S-C & C-O-C). MS m/z (relative intensity %): 344 (M⁺, 1.14). Anal. Form: Cl₄H₂N₂O₅S. Calcd. (%): C, 50.42; H, 3.05; N, 9.45; S, 10.96. Found (%): C, 50.53; H, 4.31; N, 11.89; S, 13.61.

Ethyl 3-acetyl-1-(4-chlorophenyl)-8-cyano-5-oxo-1,5-dihydro[1,2,4] triazolo[4,3-a]pyridine-6-carboxylate; 24

To a solution of compound 2 (0.45 g, 2 mmol) in dimethyl formamide (5 mL), 4-chlorophenyl-2-oxopropane hydrazonoyl chloride [26] 23 (0.46 g, 2 mmol) and a catalytic amount of anhydrous potassium carbonate (0.28 g, 2 mmol) were added. The reaction mixture was refluxed for 12 h, allowed to cool and the precipitated product was filtered off, washed with water, and crystallized from ethanol to give compound 24.

Brown powder, 43 % yield, m.p.: 240-242 °C. IR (KBr, cm⁻¹): 3051 (C-H aromatic); 2924, 2852 (C-H aliphatic); 2200 (C=N); 1700, 1680, 1670 (C=O); 1610 (C=N); 1560 (C=C); 1288, 1091 (C-O-C); 1091 (p-Cl-phenyl); 829 (C-Cl). ¹H-NMR (DMF-d₆, δ ppm): 1.12-1.30 (m, 3H, –OCH₂CH₃); 2.89 (s, 3H, CH₂CO–); 4.07-4.19 (m, 2H, –OCH₂CH₃); 7.33-7.51 (m, 2H, 4-Cl-C₆H₄–C₆H₂–H); 7.95 (s, 1H, pyridine C=H); 8.45-8.61 (m, 2H, 4-Cl-C₆H₄–C₆H₂–H). MS m/z (relative intensity %): 384 (M⁺, 1.66). Anal. Form: C₂₉H₁₅ClN₂O₅S. Calcd. (%): C, 56.19; H, 3.41; N, 14.56. Found (%): C, 56.48; H, 3.48; N, 14.72.

Ethyl 5-cyano-6-(methylthio)-2-oxo-1,2-dihydropyridine-3-carboxylate; 25

A mixture of compound 2 (0.45 g, 2 mmol) and potassium hydroxide (0.11 g, 2 mmol) in dimethylformamide (10 mL) was stirred for 2 h at room temperature, then methyl iodide (0.28 g, 0.12 mL, 2 mmol) was added and stirring was continued for another 2 h. The reaction mixture was poured on ice cold water, acidified with diluted hydrochloric acid, filtered, washed with water and crystallized from ethanol to give compound 25.

Orange powder, 55 % yield, m.p.: 205-207 °C. IR (KBr, cm⁻¹): 3404 (br. OH tautomer); 3385, 3365 (NH); 3047 (C-H aromatic); 2926, 2852 (C-H aliphatic); 2227 (C=O); 1710, 1660 (C=O); 1587 (C=N); 1529 (C=C); 1251, 1070 (C-S-C & C-O-C). MS m/z (relative intensity %): 238 (M⁺, 1.14). Anal. Form: C₉H₁₀N₂O₅S. Calcd. (%): C, 50.41; H, 4.23; N, 11.76; S, 13.46. Found (%): C, 50.53; H, 4.31; N, 11.89; S, 13.61.

5-Amino-6,8-dihydropyrazolo[3,4-b: 4',3'-e]pyridine-3(2H)-one; 26

To a suspension of the 5-methyl derivative 25 (0.48 g, 2 mmol) in absolute ethanol (10 mL), hydrazine hydrate (0.2 g, 0.19 mL, 4 mmol) was added and the reaction mixture was heated under reflux for 12 h. It was concentrated and the obtained product was filtered, washed with several solvents to yield compound 26.

Yellow powder, 38 % yield, m.p.: > 360 °C. IR (KBr, cm⁻¹): 3300, 3188, 3138 (NH₂, NH); 3000 (C-H aromatic); 2922 (C-H aliphatic); 1690 (C=O); 1624 (C=N); 1590 (C=C). ¹H-NMR (DMF-d₆, δ ppm): 5.23 (s, 2H, NH₂, D₂O exchangeable); 6.21 (s, 2H, two pyrazole–NH, D₂O exchangeable); 7.02 (s, 1H, pyridine–NH, D₂O exchangeable); 8.45 (s, 1H, pyridine–C=H). MS m/z (relative intensity %): 191 (M⁺+1, 1.97); 190 (M⁺, 2.46); 189 (M⁺-1, 5.98); 188 (M⁺-2, 3.68). Anal. Form: C₈H₁₀N₂O. Calcd. (%): C, 44.21; H, 3.18; N, 44.19. Found (%): C, 44.34; H, 3.25; N, 44.52.

Cinnamoyl chloride; 27

A mixture of cinnamic acid (1.4 g, 10 mmol) and thionyl chloride (1.7 g, 1.1 mL, 15 mmol) was stirred at room temperature for 15 min. Stirring was then completed for additional 2 h at 80 °C. The obtained solid was collected and dried to yield yellow crystals of cinnamoyl chloride.Yield %: 75 %, m.p.: 33-34°C (Lit.[27], 30-33°C).

Ethyl 6-(cinnamoylthio)-5-cyano-2-oxo-1,2-dihydropyridine-3-carboxylate; 28

and Ethyl 9-
cyano-2,6-dioxo-4-phenyl-2,3,4,6-tetrahydropyrido [2,1-b][1,3]thiazine-7-carboxylate; 29

An equimolar mixture of 5-cyano-6-mercaptopypyridine 2 (0.45 g, 2 mmol) and cinna
moyl chloride 27 (0.33 g, 2 mmol) was refluxed for 20 h in a mixture of pyridine and benzene (1:1) (10 mL). The reaction mixture was allowed to cool then poured onto crushed ice while scratching then the solid formed was filtered off, dried and crystallized from ethanol to give compound 28, while the alcoholic insoluble part was found to be compound 29.

Ethyl 6-(cinnamoylthio)-5-cyano-2-oxo-1,2-
dihydropyridine-3-carboxylate; 28

Yellow powder, 39 % yield, m.p.; 134-136 °C, IR (KBr, cm⁻¹): 3380, 3340 (NH); 3066, 3026 (C-H aromatic); 2924, 2852 (C-H aliphatic); 2220 (C=N); 1720, 1700, 1680 (C=O); 1629 (C=C); 1530 (C=C); 1284, 1074 (C-S-C); 1265, 1074 (C-O-C). 1H-NMR (DMSO-d₆, δ ppm): 1.21 (t, 3H, J = 7.1 Hz, -OCH₂CH₃); 4.09 (q, 2H, J = 7.1 Hz, -OCH₂CH₃); 6.52 (d, 1H, J = 15.9 Hz,  =CH₂-C=O); 7.34-7.52 (m, 3H, CH₃-C₅,4,5-H); 7.59 (d, 1H, J = 15.9 Hz, =CH₂-C₅H₃); 7.62-7.77 (m, 2H, CH₃-C₅,6-H); 7.81 (s, 1H, pyridine-C₅-H); 11.10 (s, 1H, OH tautomer, D₂O exchangeable). MS m/z (relative intensity %): 354 (M⁺, 2.32). Anal. Form: C₁₄H₁₂N₂O₂S. Calcd. (%): C, 61.01; H, 3.98; N, 7.90; S, 9.05. Found (%): C, 61.24; H, 3.96; N, 7.98; S, 9.17.

Ethyl 9-cyano-2,6-dioxo-4-phenyl-2,3,4,6-
tetrahydropyrido[2,1-b][1,3]thiazine-7-carboxylate; 29

Orange powder, 38 % yield, m.p.; > 360 °C, IR (KBr, cm⁻¹): 3410 (br. OH tautomer); 3049, 3003 (C-H aromatic); 2922, 2852 (C-H aliphatic); 2210 (C=N); 1710, 1680 (C=O); 1585 (C=C); 1269, 1091 (C=S-C & C-O-C). 1H-NMR (DMSO-d₆, δ ppm): 1.02-1.22 (m, 3H, =OCH₂CH₃); 3.81-3.85 (m, 2H, =OCH₂CH₃); 7.36-7.40 (m, 1H, thiazine-C₅-H); 7.77 (d, 1H, J = 7.5 Hz, thiazine-C₅-H); 8.25-8.34 (m, 3H, CH₃-C₅,4,5-H); 8.49-8.51 (m, 2H, CH₃-C₅,6-H); 8.59 (s, 1H, pyridine-C₅-H); 14.38 (s, 1H, OH, D₂O exchangeable). MS m/z (relative intensity %): 356 (M⁺+2, 2.10); 354 (M⁺, 2.46). Anal. Form: C₁₄H₁₄N₂O₂S (354). Calcd. (%): C, 61.01; H, 3.98; N, 7.90; S, 9.05. Found (%): C, 61.18; H, 4.03; N, 7.98; S, 9.14.

2,4-Dimethoxybenzylidenemalononitrile; 30

2,4-Dimethoxybenzaldehyde (3.32 g, 20 mmol) was added to a solution of malononitrile (1.32 mL, 20.8 mmol) in ethanol (14 mL) containing a catalytic amount of piperidine (3 drops). The reaction mixture was refluxed for one hour and allowed to cool to room temperature. The obtained solid was filtered off, washed with ethanol and dried to yield yellow crystals. Yield %; 92 %, m.p.; 144-146 °C (Lit. [28], 149-152°C).

Ethyl 3-cyano-2-(2,4-dimethoxyphenyl)-4-imino-7-
oxo-7,8-dihydro-4H-thiopyran[2,3-b]pyridine-6-
carboxylate; 31; and Ethyl 9-cyano-2-(2,4-
dimethoxyphenyl)-4-imino-6-oxo-4,6-dihydro-
pyrido[2,1-b][1,3]thiazine-7-carboxylate; 32

An equimolar mixture of 5-cyano-6-mercaptopypyridine 2 (0.45 g, 2 mmol) and 2,4-
dimethoxybenzylidenemalononitrile 30 (0.43 g, 2 mmol) was refluxed for 20 h in dimethylformamide (5 mL) containing a catalytic amount of potassium carbonate (0.28 g, 2 mmol). The reaction mixture was allowed to cool then poured onto cold water. The solid formed was filtered off, washed with water then dried and crystallized from ethanol to give compound 31, while the insoluble part was found to be compound 32.

Ethyl 3-cyano-2-(2,4-dimethoxyphenyl)-4-imino-7-
oxo-7,8-dihydro-4H-thiopyran[2,3-b]pyridine-6-
carboxylate; 31

Brown powder, 30 % yield, m.p.; 80-82 °C, IR (KBr, cm⁻¹): 3427, 3412 (br. OH tautomer); 3211, 3190 (NH); 3049, 3007 (C-H aromatic); 2926, 2852 (C-H aliphatic); 2214 (C=N); 1720, 1650 (C=O); 1606 (C=N); 1544 (C=C); 1253, 1099 (C=S-C); 1253, 1022 (C-O-C). 1H-NMR (DMSO-d₆, δ ppm): 1.10-1.23 (m, 3H, -OCH₂CH₃); 3.62-3.86 (m, 2H, -OCH₂CH₃); 4.03 (s, 6H, two OCH₃); 6.50-6.90 (m, 1H, 2,4-(OCH₃)₂-C₅H₃-C₅-H); 7.22 (s, 1H, 2,4-(OCH₃)₂-C₅H₃-C₅-H); 7.90 (s, 1H, 2,4-(OCH₃)₂-C₅H₃-C₅-H); 8.15 (s, 1H, pyridine-NH, D₂O exchangeable); 8.41 (s, 1H, pyridine-C₅-H); 8.68 (s, 1H, imino-NH, D₂O exchangeable). MS m/z (relative intensity %): 411 (M⁺, 0.88). Anal. Form: C₂₉H₂₃N₃O₅S (411). Calcd. (%): C, 58.38; H, 4.16; N, 10.21; S, 7.79. Found (%): C, 58.51; H, 4.22; N, 10.34; S, 7.84.

Ethyl 9-cyano-2-(2,4-dimethoxyphenyl)-4-imino-6-
oxo-4,6-dihydro-pyrido[2,1-b][1,3]thiazine-7-
carboxylate; 32

Orange powder, 42 % yield, m.p.; > 360 °C, IR (KBr, cm⁻¹): 3363, 3188 (NH); 3072, 3014 (C-H aromatic); 2931, 2841 (C-H aliphatic); 2214 (C=N); 1700, 1680 (C=O);1610, 1597 (C=N); 1560, 1541 (C=C); 1253, 1024 (C-S-C & C-O-C). MS m/z (relative intensity %): 411 (M⁺, 0.82). Anal. Form: C₂₉H₂₃N₃O₅S (411). Calcd. (%): C, 58.38; H, 4.16; N, 10.21; S, 7.79. Found (%): C, 58.53; H, 4.20; N, 10.32; S, 7.87.

Ethyl 2-chloromethyl-6H, 9H-4,7-dioxo-3,4-
dihydropyrido[3',2' : 4,5]thieno[3,2-d]pyrimidin-8-
carboxylate; 33

2-Aminocarboxamide derivative 11 (0.56 g, 2 mmol) was fused in a water bath for 12 h with chloroacetyl chloride (0.22 g, 0.15 mL, 2 mmol). The reaction mixture was allowed to cool and the obtained product was filtered then washed with different solvents to yield compound 33.
Dark brown crystals, 38 % yield, m.p.; > 360 °C; IR (KBr, cm⁻¹): 3327, 3292, 3170 (NH); 3051 (C=H aromatic); 2924 (C–H aliphatic); 1720, 1690, 1670 (C=O); 1604 (C=N); 1571 (C=C); 1280, 1060 (C–S–C), 1261, 1060 (C–O–C); 800 (C–Cl). 1H-NMR (DMSO-d₆, δ ppm): 1.20-1.23 (m, 3H, –OCH₂CH₃); 2.35 (s, 2H, CH₂Cl); 3.20-3.81 (m, 2H, –OCH₂CH₃, under DMSO); 8.29 (s, 1H, pyridine–NH, D₂O exchangeable); 8.42 (s, 1H, pyrimidine–NH, D₂O exchangeable); 8.82 (s, 1/2 H, pyridine–C₃–H); 11.69 (s, 1/2 H, OH tautomer, D₂O exchangeable). MS m/z (relative intensity %): 339 (M⁺, 5.96). **Anal. Form:** C₂₁H₁₅ClN₅O₂S (339). **Calcd. (%)**: C, 45.96; H, 2.97; N, 12.37; S, 9.44. **Found (%)**: C, 46.08; H, 2.99; N, 12.50; S, 9.59.

**Ethyl 4,7-dioxo-2-thioxo-6H, 9H, 1,2,3,4-tetrahydropyrido[3′,2′: 4,5] thieno[3,2-d]pyrimidine-8-carboxylate; 34**

A mixture of the α-amino carboxamide derivative 11 (0.56 g, 2 mmol), carbon disulfide (0.15 g, 0.2 mL, 2 mmol) and potassium carbonate (0.28 g, 2 mmol) in dimethylformamide (5 mL) was refluxed for 20 h. The reaction mixture was then cooled, diluted with cold water and acidified with dilute acetic acid. The obtained precipitate was collected by filtration and washed with several solvent to yield compound 34.

Brown powder, 42 % yield, m.p.; > 360 °C; IR (KBr, cm⁻¹): 3379, 3336, 3186 (NH); 3055 (C=H aromatic); 2920 (C–H aliphatic); 1700, 1680 (C=O); 1604 (C=N); 1558 (C=C); 1453, 1319, 1139, 1060 (I, II, III, IV bands N–C=S); 1269, 1060 (C–S–C); 1261, 1060 (C–O–C). 1H-NMR (DMSO-d₆, δ ppm): 1.20-1.30 (m, 3H, –OCH₂CH₃); 3.40-3.61 (m, 2H, –OCH₂CH₃); 7.95 (s, 1H, pyridine–C_=H); 8.15 (s, pyridine–NH, D₂O exchangeable); 9.57 (s, 1H, pyrimidine–N₁–H, D₂O exchangeable); 9.69 (s, 1H, pyrimidine–N₃–H, D₂O exchangeable). MS m/z (relative intensity %): 323 (M⁺, 1.33); 322 (M⁺–1, 0.73); 321 (M⁺–2, 1.05). **Anal. Form:** C₂₁H₁₅ClN₅O₂S (323). **Calcd. (%)**: C, 44.57; H, 2.81; N, 13.00; S, 19.83. **Found (%)**; C, 44.72; H, 2.81; N, 13.14; S, 19.91.

**Ethyl 4,7-dioxo-2-(4-chlorophenyl)-6H, 9H-3,4-dihydropyrido[3′,2′: 4,5]thieno[3,2-d]pyrimidine-8-carboxylate; 35**

An equimolar mixture of compound 11 (0.56 g, 2 mmol) and 4-chlorobenzaldehyde (0.28 g, 2 mmol) was fused for 2 h in presence of few drops of piperidine (2-3 drops). The formed solid was collected by filtration and crystallized from dimethylformamide to give compound 35.

Dark brown powder, 53 % yield, m.p.; > 360 °C; IR (KBr, cm⁻¹): 3385, 3327, 3169 (NH); 3057 (C=H aromatic); 2929 (C–H aliphatic); 1710, 1680, 1660 (C=O); 1595 (C=N); 1558 (C=C); 1269, 1089 (C–S–C & C–O–C); 1089 (p-Cl-C₆H₄). 1H-NMR (DMSO-d₆, δ ppm): 1.53-1.65 (m, 3H, –OCH₂CH₃); 3.90-4.00 (m, 2H, –OCH₂CH₃); 7.20-7.50 (m, 2H, 4-Cl-C₆H₄–C₆H₄–H); 7.95 (s, 1H, pyridine–C₃–H); 8.30-8.48 (m, 2H, 4-Cl-C₆H₄–C₆H₄–H); 9.32 (s, 1H, pyridine–N₁–H, D₂O exchangeable); 9.48 (s, 1H, pyrimidine–N₃–H, D₂O exchangeable). MS m/z (relative intensity %): 402 (M⁺+1, 0.76); 399 (M⁺–2, 0.60); 398 (M⁺–3, 0.79). **Anal. Form:** C₂₁H₁₅ClN₅O₂S (401). **Calcd. (%)**: C, 53.80; H, 3.01; N, 10.46; S, 7.98. **Found (%)**: C, 54.01; H, 3.07; N, 10.57; S, 8.05.

**Anticancer screening**

Mammalian cell lines: MCF-7 cells (human breast cancer cell line) were obtained from VACSERA Tissue culture unit HepG-2 cells (human cell line of a well differentiated hepatocellular carcinoma isolated from a liver biopsy of a male Caucasian aged 15 years) were obtained from the American type culture collection (ATCC).

Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from sigma (st. louis, mo., USA) DMEM, RPMI-1640, FBS, HEPES buffer solution, L-glutamine, gentamycin and 0.25% trypsin-EDTA which were purchased from (Bio whittaker® Lonza, Belgium). Furthermore, crystal violet stains (1%): are prepared from 0.5% (w/v) crystal violet and 50% methanol then made up to volume with water and filtered through a Whatmann No. 1. Filter paper.

**Cell line Propagation**

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/mL gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were sub-cultured two times a week. Cell viability was monitored by determining the effect of the test samples on cell morphology and cell viability.

**Cytotoxicity evaluation using viability assay**

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1x10⁴ cells per well in 100µL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compounds were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO₂ for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the incubated without test sample and with or without DMSO. The little percentage of DMSO present in the incubated without test sample and with or without DMSO.

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experiment. After incubation of the cells for 24 h at 37°C, various concentrations of sample (500, 250, 125, 62.5, 31.25 & 15.6 µg) were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated.

RESULTS AND DISCUSSION

Chemistry

Literature survey revealed that, thienopyridine derivatives were reported as potent anticancer agents. Therefore, 5-cyano-6-mercaptopyrindine 2 was fused with chloroacetone to yield the cyclic thieno[2,3-b]pyridine derivative 3 as revealed in scheme 1. The IR spectrum of compound 3 lacked the absorption band due to CN group and showed absorption bands due to NH₂ function at 3387 and 3292 cm⁻¹. The ¹H-NMR spectrum of compound 3 showed a singlet signal at δ 2.55 ppm corresponding to acetyl CH₃ protons, in addition to, a deuterium oxide exchangeable singlet signal at δ 6.33 ppm due to NH₂ protons. However, compound 2 was refluxed with an equimolar amount of chloroacetic acid in a mixing solvent of acetic anhydride and acetic acid (1:2) in presence of anhydrous sodium acetate to yield thieno[2,3-b]pyridine derivative 4. The IR spectrum of compound 4 lacked the absorption band due to CN function and showed broad absorption bands due to carboxylic OH group at 3446 and 3421 cm⁻¹, in addition to; absorption bands at 3275, 3228 cm⁻¹ corresponding to NH₂ and NH functions. The ¹H-NMR spectrum of compound 4 revealed two deuterium oxide exchangeable singlet signals at δ 6.13 and δ 8.15 ppm corresponding to NH₂ and pyridine-Nı-H protons, respectively. In addition to; a deuterium oxide exchangeable singlet signal at δ 11.60 ppm due to carboxylic OH proton. While, the reaction of compound 2 with ethyl chloroacetate in ethanolic solution of sodium ethoxide yielded both the open chain S-alkylated product 5 and the fused thieno[2,3-b]pyridine derivative 6. IR spectrum of compound 6 lacked the absorption band due to CN group and showed absorption bands at 3446, 3311 and 3201 cm⁻¹ due to NH₂ and NH functions. The ¹H-NMR spectrum of compound 5 revealed two singlet signals integrated for one proton and half proton at δ 4.70 and 8.32 ppm; respectively corresponding to SCH₂ and SCH=NH tautomer, respectively. In addition to, a deuterium oxide exchangeable singlet signal at δ 7.51 ppm due to SCH=NH tautomer integrated for half proton, while the ¹H-NMR spectrum of compound 6 showed an additional deuterium oxide exchangeable singlet signal at δ 7.63 ppm due to NH₂ protons. Furthermore, 5-cyano-6-mercaptopyrindine 2 was refluxed with 4-methoxyphenyl bromide in dimethylformamide in presence of potassium carbonate as a base to yield both the open chain S-alkylated derivative 7 and the fused thienopyridine derivative 8. IR spectrum of compound 8 lacked the absorption band due to CN function and revealed absorption bands at 3388, 3269 and 3188 cm⁻¹ due to NH₂ and NH groups. The ¹H-NMR spectra of compound 7 and 8 showed singlet signals at δ 3.71 and 3.51 ppm corresponding to OCH₂ protons, respectively. The ¹H-NMR spectrum of compound 7 revealed a singlet signal at δ 4.20 ppm corresponding to S-CH₂–C=O protons. While, the ¹H-NMR spectrum of compound 8 showed a deuterium oxide exchangeable singlet signal at δ 5.21 ppm due to NH₂ protons.

In addition, the reaction of 5-cyano-6-mercaptopyrindine 2 with chloroacetanilide by stirring at room temperature in dimethylformamide containing potassium carbonate yielded both the S-alkylated derivative 10 and the α-aminonitrile thienopyridine derivative 9 as revealed in Scheme 2.

However, refluxing of compound 2 with chloroacetanilide in dimethylformamide/potassium carbonate mixture afforded only the cyclization product 9. Compound 9 prepared by the aforementioned two conditions were found to be identical as revealed by TLC, m.p. mixed m.p and IR spectrum. The ¹H-NMR spectrum of compound 9 revealed a deuterium oxide exchangeable singlet signal at δ 6.89 ppm due to NH₂ protons, while the ¹H-NMR spectrum of compound 10 showed a singlet signal at δ 3.47 ppm integrated for one proton due to S-CH=NH proton. In addition to, a deuterium oxide exchangeable singlet signal at δ 6.34 ppm integrated for half proton due to the tautomeric S-CH=C=NH proton and a singlet signal at δ 8.32 ppm integrated for half proton corresponding to S-CH=NH tautomer. However, upon refluxing of compound 2 with chloroacetamide in dimethylformamide in presence of anhydrous potassium carbonate yielded the cyclic 3-amino-2-carbamoylthieno[2,3-b]pyridine derivative 11 through the formation of a non-isolated S-alkylated derivative which in turn underwent Thorpe-Zeiglar cyclization to afford the target compound. The ¹H-NMR spectrum of compound 11 revealed two deuterium oxide exchangeable singlet signals at δ 6.75
Scheme 1

Reagents & conditions: (i) NaOCl/CH₂OH/H₂O/T. (ii) CICH₂COOH/10% ACO₂H/NaOCl/reflux; (iii) CICH₂COOH/NaOCl/reflux; (iv) 4-CH₃-O-C₆H₅-COCH₃Br/NaNH₂, K₂CO₃/DMF/reflux; (v) CICH₂COOH/CH₃OH/NaOCl/reflux.
and 7.44 ppm corresponding to thienopyridine–C₃–NH₂ and thienopyridine–C₄–NH₂, respectively. Moreover, the target aminooxindolino derivative 12 and the spiroxypiridimine analogue 13 were obtained by two methods either by refluxing of the o-aminonitrile derivative 9 or the o-aminocarboxamide compound 11 with cyclohexanone in dimethylformamide containing zinc chloride as a catalyst. The reaction mechanism for the synthesis of compounds 12 and 13 from o-aminonitrile derivative 9 was reported 35 to be achieved as follows (Figure 2).

The postulated reaction mechanism for the synthesis of compounds 12 and 13 from o-aminocarboxamide analogue 11 is illustrated as follows in Figure 3:

IR spectra of compounds 12 and 13 lacked the absorption bands due to CN group present in their precursor. However, the ¹H-NMR spectrum of compound 12 revealed multiplet signals at δ 1.80-1.85, 3.11-3.19 and 3.40-3.50 ppm corresponding to tetrahydroquinoline–C₇₋₈–CH₂, tetrahydroquinoline–C₉–CH₂ and tetrahydroquinoline–C₆–CH₃, respectively. While the ¹H-NMR spectrum of compound 13 exhibited multiplet signals due to cyclohexyl protons at their expected chemical shifts.

Furthermore, the reaction of 5-cyano-6-mercaptopopyridine derivative 2 with several aliphatic methyl ketones; namely ethyl methyl ketone, cyclopentanone and acetylacetone in glacial acetic acid and acetic anhydride containing few drops of concentrated sulphuric acid yielded their corresponding thienopyridine derivatives 14, 15 and 16, respectively as shown in Scheme 3. The reaction was suggested to proceed through the formation of a non-isolated S-alkylated intermediate which underwent intramolecular cyclo addition of active methine proton on the cyano function to form their corresponding 3-iminothienopyridine derivatives. IR spectra of compounds 14, 15 and 16 lacked the absorption bands due to cyano group of their precursor. ¹H-NMR spectra of compounds 14, 15 and 16 revealed deuterium oxide exchangeable singlet signals at δ 6.89-6.90 ppm attributed to imino proton. However, stirring of compound 2 with ethyl α-chloroacetoacetate in a mixture of ethanol and dimethylformamide (4 : 1) containing aqueous potassium hydroxide yielded the open chain S-alkylated derivative 17 and the cyclic thienopyridine derivative 18. ¹H-NMR spectra of compounds 17 and 18 revealed two singlet signals at δ 2.71 ppm attributed to the acetyl CH₃ protons. However, ¹H-NMR spectrum of compound 17 showed a singlet signal at δ 5.79 ppm due to 3-oxobutanoate-C₂-H, while, ¹H-NMR spectrum of compound 18 showed a deuterium oxide exchangeable singlet signal at δ 5.80 ppm corresponding to imino proton.

In attempts to study the effect of fusion of different heterocyclic systems on the anticancer activity of the pyridine back bone, 5-cyano-6-mercaptopopyridine derivative 2 was fused with oxalyl chloride to yield both the S-acylated derivative 19 and the cyclic dioxo-thiazolopyridine derivative 20 as shown in Scheme 4.

IR spectra of compounds 19 and 20 showed absorption bands corresponding to carbonyl functions at 1737, 1697 and 1701, 1670 cm⁻¹, respectively. Besides, the IR spectrum of compound 20 lacked the absorption bands due to NH function. However, compound 2 was refluxed with ethyl chloroformate in dimethylformamide in presence of potassium carbonate as a base to yield the open chain S-alkylated derivative 21. IR spectrum of compound 21 showed absorption bands at 1720, 1701 and 1670 cm⁻¹ corresponding to three carbonyl functions. The fusion of compound 2 with dimethyl acetylene dicarboxylate yielded the thiazolopyridine derivative 22. The reaction mechanism is suggested to proceed through the initial nucleophilic addition of the thiol function on the acetylinic carbons followed by nucleophilic attack of pyridine–NH function on carbonyl ester group with subsequent elimination of a methanol molecule to yield the thiazole derivative 22. The postulated reaction mechanism is illustrated as follows in Figure 4.

IR spectrum of compound 22 lacked the absorption bands due to NH function. The target triazolopyridine derivative 24 was prepared by the reaction of compound 2 with N'-[(4-chlorophenyl)-2-oxopropanehydrazonyl] chloride 23 in dimethylformamide in presence of potassium carbonate as a base. The reaction mechanism is reported 36,37 to proceed as follows in Figure 5.

¹H-NMR spectrum of compound 24 showed a singlet signal at δ 2.89 ppm corresponding to –COCH₃ protons and lacked any deuterium oxide exchangeable signal due to NH proton. Furthermore, the target S-methyl analogue 25 was prepared by stirring a mixture of the thiol derivative 2 and methyl iodide in dimethylformamide in presence of potassium hydroxide as a base. The reaction of the methylthio derivative 25 with two equivalents of hydrazine hydrate in absolute ethanol yielded the dipyrzolo[3,4-b:4',3'-e]pyridine-3(2H)-one derivative 26. IR spectrum of compound 26 lacked the absorption bands due to cyano function and ester carbonyl group. ¹H-NMR spectrum of compound 26 revealed two deuterium oxide exchangeable singlet signals at δ 5.23 and δ 6.21 ppm due to NH₂ and two pyrazole NH protons, respectively.

Furthermore, the reaction of 5-cyano-6-mercaptopyrpyridine 2 with cinnamoyl chloride in a mixture of pyridine/benzene (1:1) yielded both the open chain α,β-unsaturated carbonyl derivative 28 and the cyclic pyridol[2,1-b][1,3]thiazine derivative 29 as revealed in Scheme 5.
Figure 2. Mechanistic pathway for the preparation of compounds 12 and 13 from o-aminonitrile derivative 9.

Figure 3. Mechanistic pathway for the preparation of compounds 12 and 13 from o-aminocarboxamide analogue 11.
Reagents & conditions: (i) CH₃COCH₂H₂/ACOH/H₂SO₄/AC₂O/reflux; (ii) cyclopentanone/ACOH/H₂SO₄/AC₂O/reflux; (iii) CH₃COCH₂COCH₃/ACOH/H₂SO₄/AC₂O/reflux; (iv) CH₃COCH(Cl)COOC₂H₅/EtOH/KOH/DMF/R.T.

Scheme 3

Reagents & conditions: (i) (COCl)₂/fusion; (ii) CH₃COOCH₂H₂/DMF/anhyd. K₂CO₃/reflux; (iii) DMAD/fusion; (iv) 4-ClC₆H₄-NH-N=CH(Cl)COCH₂/DMF/anhyd. K₂CO₃/reflux; (v) CH₃/DMF/KOH/R.T.; (vi) NH₂NH₂/EtOH/reflux.

Scheme 4
'\(^1\)H-NMR spectrum of compound 28 showed two doublet signals at 6.52 and 7.59 ppm corresponding to olefinic =CH⋯C=O and =CH⋯C₆H₅ protons; respectively. While the '\(^1\)H-NMR spectrum of compound 29 showed a multiplet signal at 7.36-7.40 ppm and doublet signal at 7.77 ppm due to thiazine-C₅-H and thiazine-C₆-H; respectively. Moreover, compound 2 was refluxed with 2,4-dimethoxybenzylidenemalononitrile 30 in dimethylformamide in presence of a catalytic amount of potassium carbonate to give the thioyranopyrimidine derivative 31 and the pyridothiazine analogue 32. The reaction mechanism is postulated to proceed through the reaction of thiol function with benzylidenemalononitrile to yield the Michael adduct which reacted either through the nucleophilic addition of the active methylene function on the 5-cyano group of pyridine with subsequent elimination of a hydrogen cyanide molecule to yield compound 31 or via the nucleophilic addition of pyridine NH function on one of the cyano groups of the benzylidenemalononitrile with the elimination of a hydrogen cyanide molecule to give compound 32. The postulated reaction mechanism for the synthesis of compound 31 is illustrated as follows Figure 7:

The postulated reaction mechanism for the synthesis of compound 32 is illustrated as follows Figure 8:

Figure 8: Mechanistic pathway for the preparation of compound 32.

IR spectra of compounds 31 and 32 showed absorption bands at 3211, 3190 & 3363, 3188 cm⁻¹ due to NH functions; respectively.

Our aim was also directed to study the biological activity of pyrimidine nucleus fused to the thienopyridine moiety in the field of anticancer agents. Therefore, the target pyridothienopyrimidine derivative 33 was synthesized via fusion of the o-aminoacarboxamide derivative 11 with chloroacetyl chloride as shown in scheme 6. '\(^1\)H-NMR spectrum of compound 33 revealed a deuteron oxide exchangeable singlet signal at 8.42 ppm due to pyrimidine-NH proton and lacked any deuteron oxide exchangeable singlet due to NH₂ protons of its precursor. Also, the o-aminoacarboxamide derivative 11 was refluxed with carbon disulfide in dimethylformamide containing potassium carbonate to give the corresponding 2-thioxothienopyrimidine derivative 34. '\(^1\)H-NMR spectrum of compound 34 revealed three deuteron oxide exchangeable singlet signals at 8.15, 9.57 and 9.69 ppm corresponding to pyridine⋯NH, pyrimidine⋯N₁⋯H and pyrimidine⋯N₂⋯H protons; respectively. Finally, the fusion of 2-aminoacarboxamide derivative 11 with 4-chlorobenzaldehyde in presence of piperidine yielded the corresponding dihydropyridothienopyrimidine analogue 35. '\(^1\)H-NMR spectrum of compound 35 revealed two deuteron oxide exchangeable singlet signals at 9.32 and 9.48 ppm due to pyridine-N₁⋯H and pyrimidine-N₂⋯H protons; respectively.

Anticancer screening

Thirty three synthesized compounds (3-35) were screened for their in vitro cytotoxic activity against human hepatocellular liver carcinoma (HepG2) and human breast cancer (MCF-7) cell lines in the regional center for mycology and biotechnology, at Al-Azhar University using 5-fluorouracil (5-FU) as the reference drug.

IC₅₀ values and the six dose growth inhibition percent of the tested compounds (3-35) against liver HepG2 and breast MCF-7 cell lines are represented in tables (1,2); respectively.

Table (1): IC₅₀ values and six dose growth inhibition percent of the tested compounds against liver HepG2 cell line.

As revealed from the results presented in tables (1,2) and in a trial to shed more light on the SAR of compounds bearing ethyl 6-oxothienopyridine-5-carboxylate backbone possessing different substituents in the 2-position, it is evident that the presence of a ketone function at 2-position as an acetyl group in compound 3 or 4-methoxybenzoyl as in compound 8 exhibited moderate to weak anticancer activities against both HepG2 and MCF-7 cell lines. However, replacement of the ketonic function by a carboxylic group as in compound 4 resulted in marked increase in its anticancer activities against both HepG2 and MCF-7 cell lines which is more potent than the reference drug 5-fluorouracil. Furthermore, the esterification of the carboxylic group as in compound 6 resulted in nearly equipotent activity to 5-fluorouracil against HepG2 cell line while it diminished the activity against MCF-7 cell line. However, amidation of the carboxylic group as in compound 11 or replacing the carboxylic function by cyano group as in compound 9 abolished the anticancer activities against both cell lines. Moreover, concerning the activities of different S-substituted ethyl 5-cyano-2-oxo-6-thioxopyridine-3-carboxylate backbone as in compounds 5, 10 and 21 bearing: 2-ethoxy-2-oxothiolthio, cyanothiolthio and ethoxycarbonylthio side chains; respectively exhibited nearly equipotent anticancer activities against HepG2 cell line compared to the reference drug with IC₅₀ values. However, compound 21 exerted two folds more potent activity against MCF-7 cell line, while compounds 5 and 10 showed moderate activities against MCF-7 cell line. Furthermore, compound 7 possessing 4-methoxybenzoylthiolthio side chain exerted moderate activities against both HepG2 and MCF-7 cell lines. It is worth mentioning that, S-oxalylchloride side chain as in compound 19 and S-cinnamoyl side chain as in compound 28 resulted in

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Figure 4. Mechanistic pathway for the preparation of compound 22.

Figure 5. Mechanistic pathway for the preparation of compound 24.

Figure 6. The postulated mechanism for synthesis of compound 28 and 29.

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Reagents & conditions: (i) C$_6$H$_5$CH=CHCOCl/pyridine/benzene/reflux; (ii) 2-(2,4-dimethoxybenzylidene)malononitrile/ DMF/ anh. K$_2$CO$_3$/reflux.

Scheme 5

Figure 7. Mechanistic pathway for the preparation of compound 31.

Figure 8. Mechanistic pathway for the preparation of compound 32.
marked improvement in activity against both cell lines with two folds more potent activities than the reference drug. Moreover, the presence of ethyl 3-oxobutanoate side chain as in compound 17 diminished the anticancer activities against both HepG2 and MCF-7 cell lines, while the S-methyl side chain in compound 25 abolished the activity against HepG2 cell line while it exerted moderate activity against MCF-7 cell line. In a trial to investigate the effect of fusion of different rings to ethyl 6-oxothienopyridine-5-carboxylate backbone, it was found that, fusion of 4-aminotetrahydroquinoline to the ethyl 6-oxothienopyridine-5-carboxylate backbone as in compound 12 resulted in highly potent anticancer agent against both HepG2 and MCF-7 cell lines this might be an indication of the better binding to the protein. While fusion of 2-substituted-4-oxopyrimidine moieties to the ethyl 6-oxothienopyridine-5-carboxylate backbone as in compounds 13, 33, 34 and 35 showed variable activities. However, the replacement of spirocyclohexyl ring with chloromethyl group, thione function or 4-chlorophenyl moiety as in compounds 33, 34 and 35; respectively, abolished the anticancer activities. Moreover, modification of the 5-amino function of ethyl 6-oxothienopyridine-5-carboxylate backbone into imino group as in compounds 14, 15, 16 and 18 bearing different substituents in 6-position resulted in inactive compounds except for compound 18 bearing acetyl and ethyl carboxylate groups in 6-position exhibited good anticancer activity against MCF-7 cell line. Furthermore, concerning the various activities of different rings fused to the ethyl 5-cyano-2-oxopyridine-3-carboxylate backbone, it was found that, the fusion of substituted thiazole ring to the ethyl 5-cyano-2-oxopyridine-3-carboxylate backbone as in compounds 20 and 22 diminished the anticancer activities against both HepG2 and MCF-7 cell lines, while fusion of thiazine ring to the ethyl 5-cyano-2-oxopyridine-3-carboxylate backbone as in compounds 29 and 32 resulted in marked increase in the anticancer activities against both cell lines. However, fusion of [1,2,4]triazole ring to the ethyl 5-cyano-2-oxopyridine-3-carboxylate backbone as in compound 24 resulted in marked elevation in the anticancer activity against MCF-7 cell line but showed weak anticancer activity against HepG2 cell line. Whereas, the fusion of thiopyran ring to the ethyl 2-oxopyridine-3-carboxylate nucleus as in compound 31 led to nearly equipotent anticancer activity against HepG2 and half potent activity against MCF-7 cell lines compared to the reference drug 5-fluorouracil. On the other hand, fusion of two pyrazole rings to the pyridine nucleus as in compound 26 resulted in poor anticancer activities against both HepG2 and MCF-7 cell lines.

CONCLUSION

It can be concluded that most of the synthesized compounds showed strong anticancer activities against both liver cancer HepG2 and breast cancer MCF-7 cell lines. However, compounds 4, 5, 6, 10, 12, 13, 19, 21, 28, 29 and 31 exhibited more potent to equipotent
Table 1. IC<sub>50</sub> values and six dose growth inhibition percent of the tested compounds against liver HepG2 cell line

| Compound No. | Sample concentration (µg/mL) | IC<sub>50</sub> (µg/mL) | Growth inhibition % |
|--------------|-----------------------------|--------------------------|---------------------|
|              | 50  | 25  | 12.5 | 6.25 | 3.13 | 1.6 | |
| 3            | 65.25 | 51.93 | 24.41 | 10.82 | 3.36 | 1.59 | 24.10 |
| 4            | 91.22 | 86.53 | 76.11 | 60.55 | 41.36 | 16.43 | 4.53 |
| 5            | 89.13 | 80.82 | 61.40 | 18.77 | 7.66 | 1.79 | 10.80 |
| 6            | 74.14 | 61.28 | 53.63 | 45.28 | 35.09 | 23.32 | 9.78 |
| 7            | 78.13 | 60.92 | 40.29 | 15.08 | 3.68 | 0.00 | 18.40 |
| 8            | 51.07 | 19.66 | 7.33 | 3.85 | 0.00 | 0.00 | 49.10 |
| 9            | 31.28 | 13.44 | 4.13 | 0.92 | 0.00 | 0.00 | >50 |
| 10           | 86.33 | 70.54 | 56.09 | 21.66 | 7.44 | 1.83 | 11.40 |
| 11           | 52.11 | 15.54 | 6.19 | 1.28 | 0.00 | 0.00 | 48.60 |
| 12           | 85.83 | 76.36 | 68.95 | 43.18 | 19.26 | 8.29 | 7.90 |
| 13           | 91.59 | 86.26 | 81.04 | 65.52 | 43.04 | 21.51 | 4.09 |
| 14           | 69.41 | 58.33 | 42.86 | 26.11 | 9.29 | 4.16 | 18.30 |
| 15           | 53.19 | 17.57 | 6.11 | 2.02 | 0.00 | 0.00 | 47.80 |
| 16           | 18.42 | 5.84 | 1.26 | 0.00 | 0.00 | 0.00 | >50 |
| 17           | 65.47 | 47.02 | 16.44 | 8.28 | 2.16 | 0.00 | 29 |
| 18           | 61.48 | 48.33 | 16.96 | 5.84 | 1.63 | 0.00 | 28.2 |
| 19           | 91.51 | 85.07 | 78.18 | 71.41 | 50.64 | 21.28 | 3.09 |
| 20           | 52.14 | 20.38 | 8.42 | 2.66 | 0.00 | 0.00 | 48.30 |
| 21           | 88.46 | 80.38 | 64.57 | 23.98 | 10.79 | 3.54 | 10.30 |
| 22           | 19.37 | 5.26 | 0.85 | 0.00 | 0.00 | 0.00 | >50 |
| 24           | 68.16 | 42.85 | 21.60 | 10.71 | 4.22 | 1.54 | 32.10 |
| 25           | 70.88 | 32.62 | 10.59 | 3.61 | 0.98 | 0.00 | 36.40 |
| 26           | 60.47 | 37.26 | 20.14 | 8.53 | 1.87 | 0.00 | 38.70 |
| 28           | 91.26 | 84.52 | 73.27 | 69.49 | 51.38 | 20.87 | 3.05 |
| 29           | 80.87 | 68.26 | 56.05 | 25.42 | 8.58 | 2.84 | 11.30 |
| 31           | 85.27 | 68.92 | 57.41 | 43.32 | 18.51 | 4.77 | 9.21 |
| 32           | 83.43 | 67.06 | 51.25 | 18.33 | 5.61 | 0.43 | 12.30 |
| 33           | 59.13 | 20.48 | 8.32 | 3.06 | 0.92 | 0.00 | 44.10 |
| 34           | 63.07 | 17.31 | 8.57 | 1.55 | 0.00 | 0.00 | 42.90 |
| 35           | 73.61 | 54.52 | 20.48 | 8.36 | 1.59 | 0.00 | 23.30 |
| 5-FU         | 81.07 | 68.38 | 55.83 | 34.69 | 26.52 | 18.78 | 10.80 |
### Table 2. IC₅₀ values and six dose growth inhibition percent of the tested compounds against breast MCF-7 cell line

| Compound No. | Sample concentration (µg/mL) | IC₅₀ (µg/mL) |
|--------------|------------------------------|-------------|
|              | 50  | 25  | 12.5 | 6.25 | 3.13 | 1.6 |
| Growth inhibition % |
| 3            | 74.62 | 62.16 | 34.68 | 15.68 | 1.83 | 0.00 | 19.50 |
| 4            | 88.51 | 74.09 | 63.18 | 51.23 | 29.72 | 12.31 | 6.07 |
| 5            | 71.79 | 58.08 | 43.82 | 28.38 | 12.66 | 7.35 | 17.90 |
| 6            | 68.53 | 54.74 | 31.93 | 9.87  | 3.71  | 1.24  | 22.40 |
| 7            | 75.49 | 61.27 | 35.04 | 10.52 | 3.76  | 1.22  | 19.60 |
| 8            | 63.46 | 40.54 | 19.05 | 8.31  | 1.99  | 0.00  | 35.30 |
| 9            | 40.43 | 21.88 | 8.56  | 2.15  | 0.00  | 0.00  | >50  |
| 10           | 81.58 | 68.41 | 52.96 | 30.77 | 13.83 | 9.16  | 11.70 |
| 11           | 58.35 | 37.13 | 23.08 | 10.85 | 3.68  | 1.46  | 40.20 |
| 12           | 89.53 | 81.48 | 70.27 | 65.72 | 56.11 | 18.41 | 2.87 |
| 13           | 90.22 | 82.75 | 68.54 | 57.21 | 35.64 | 18.72 | 5.21 |
| 14           | 60.15 | 39.03 | 12.54 | 3.95  | 1.28  | 0.00  | 38.00 |
| 15           | 65.38 | 18.07 | 7.16  | 1.83  | 0.00  | 0.00  | 41.90 |
| 16           | 29.34 | 12.98 | 5.87  | 1.06  | 0.00  | 0.00  | >50  |
| 17           | 59.03 | 30.87 | 12.48 | 5.82  | 1.31  | 0.00  | 42.00 |
| 18           | 81.06 | 68.92 | 57.53 | 33.06 | 18.28 | 7.02  | 10.60 |
| 19           | 86.28 | 78.41 | 59.37 | 57.25 | 42.16 | 19.09 | 4.75 |
| 20           | 54.28 | 35.82 | 19.02 | 10.24 | 4.86  | 0.77  | 44.20 |
| 21           | 93.11 | 88.58 | 79.33 | 68.72 | 51.04 | 28.16 | 3.05 |
| 22           | 28.18 | 11.21 | 5.87  | 0.92  | 0.00  | 0.00  | >50  |
| 24           | 83.74 | 74.26 | 61.04 | 45.61 | 19.09 | 10.28 | 8.03 |
| 25           | 76.83 | 67.95 | 52.69 | 18.04 | 9.66  | 4.72  | 12.00 |
| 26           | 51.02 | 41.26 | 23.92 | 14.07 | 6.28  | 0.85  | 38.90 |
| 28           | 90.38 | 84.32 | 71.91 | 50.17 | 15.02 | 8.58  | 6.23 |
| 29           | 90.35 | 84.17 | 75.68 | 67.61 | 25.74 | 10.69 | 4.94 |
| 31           | 80.47 | 63.92 | 54.84 | 21.55 | 9.74  | 2.69  | 11.60 |
| 32           | 79.26 | 68.17 | 56.52 | 17.06 | 9.69  | 2.72  | 11.50 |
| 33           | 62.78 | 39.22 | 23.06 | 10.73 | 3.92  | 1.29  | 36.40 |
| 34           | 59.87 | 30.71 | 13.94 | 4.88  | 1.26  | 0.00  | 41.50 |
| 35           | 53.71 | 26.38 | 12.66 | 4.75  | 1.29  | 0.00  | 46.60 |
| 5-FU         | 92.13 | 83.02 | 65.35 | 47.17 | 31.83 | 19.58 | 7.22 |

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activities against HepG2 cell line with IC\textsubscript{50} values ranging from 3.05-11.40 µg/mL compared to the reference drug 5-fluorouracil (IC\textsubscript{50} value 10.80 µg/mL). While compounds 4, 12, 13, 19, 21, 24, 28 and 29 were the more potent to equipotent against MCF-7 cell line exerting IC\textsubscript{50} values ranging from 2.87-6.23 µg/mL compared to the reference drug 5-fluorouracil (IC\textsubscript{50} value 7.22 µg/mL). It is worth mentioning that, compounds 4, 12, 13, 19, 21, 24, 28 and 29 exerted highly potent anticancer activities against both HepG2 and MCF-7 cell lines.

Conflict of interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

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