Synthesis of some new thienopyridine and pyridothienopyrimidine derivatives with expected antitumor activity

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Abstract:

The thioxopyridine derivative (2) on treatment with ethyl chloroacetate gave the corresponding ethyl 3-aminothieno [2,3-b]pyridin-2-carboxylate (3). Condensation of 3 with Ac_2O gave the corresponding 4-H-pyrido[3',2':4,5]thieno[3,2-d] [1,3]oxazine derivative (4). Reaction of 4 with bifunctional groups gave 3,4-dihydropyrido[3',2':4,5]thieno[3,2-d]pyrimidines ($5_{a,b}$) and 2-carbamoyl-3-acetamidothieno[2,3-b]pyridine derivative (6). Intramolecular cyclization of 6 by Ac_2O/AcOH gave (7). Reaction of 5b with different electrophilic reagents were studied. Also, 5a upon treatment with ethyl chloroacetate and/or methyl iodide (K_2CO_3) furnished the corresponding N-ethylacetate (13) and N-methyl derivative (16) respectively. Hydrazinolysis of 13 gave the corresponding acetohydrazide (14) which on treatment with benzaldehyde furnished the corresponding 1H-pyrazol-4-yl derivative (15). The IR, ^1H NMR and MS spectra of newly derivatives were discussed. The antitumor activity against the liver tumor cell line $HepG_2$ of the prepared compounds were tested. Compounds 8a, 14 were more potent (IC_{50} = 2.75 and 2.12 μg/ml) than the standard drug (IC_{50} = 4.60 μg/ml).

Keywords — *Cyanothioxapyridine, Thienopyridine, Pyridothieno pyrimidine and anti-tumor, HepG2.*

I. INTRODUCTION

In the last several decades, pyridothienopyrimidine derivatives have received considerable attention due to their wide-range of applications.Pyridothienopyrimidines are reported to exhibit anticancer [1-3], anti-inflammatory[4,5] antimalarial[6], anti-allergy [7], anti-microbial[8-9], antifilarial [10]. Insecticidal activities [11], also as V.E. GFR-2-inhibitors[12]. Moreover, as inhibitor of CLKI and Dy RKIA kinases[13]. Encouraged by these reports, it was considered of interest to of synthesizing a new series of pyridothienopyrimidine derivatives as potential antitumor agents.

II. EXPERIMENTAL

All melting points are uncorrected. IR spectra (KBr) were recorded with a Perkin Elmer Spectrum RXIFT-IR systems. ^{1}HNMR were measured with a Varian Gemini 200 MHz instrument using TMS as internal standard and mass spectra were measured with a Shimadzu GC-MS-QP 100 EX mass spectrometer .

Synthesis of 3-(4-bromophenyl)-2-cyanoprop-2-enethioamide (1)

A mixture of 4-bromobenzaldehyde (0.01 mol), thiocyano-acetamide (0.01 mol) and (0.002 mmol) of piperidine in ethanol (50 ml) was heated under reflux for 6hr., the solid separated after concentration and cooling was filtered off, and crystallized from benzene 98% yield. m.p. 155°C IR., cm^{-1}, 3413, 2950, 2215 and 1239 for NH_2, CH aliphatic, C≡N and C=S group, respectively. Analysis for $C_{10}H_7BrN_2S$ (267.14): calcd. C 45.12, H 2.62, Br 29.95, N 10.48, S 11.98; found C 44.96, H 2.64, Br 29.91, N 10.49, S 12.00%.

Synthesis of ethyl 4(-4-bromophenyl)-5-cyano-1,6-dihydro-2-methyl-6-thioxopyridin-3-carboxylate (2).

A mixture of 1(0.01 mol), ethyl acetoacetate (0.015 mol) and (0.002 mmol) of piperidine in dioxane (50 ml) was heated under reflux for 6 hr. The reaction mixture was concentrated and cooled. The separated solid was filtered off, and crystallized from benzene 90% yield, m.p. 100°C, IR, cm^{-1} 3340, 2962, 2214, 1753 and 1376 cm^{-1} for NH, CH aliphatic, C≡N, CO ester and C=S group, respectively. ^{1}H NMR (DMSO-d_6) δ(ppm): spectrum exhibited signals at 7.85(2H, d, J= 8.3Hz, Ar–H), 7.10 (2H, d, J = 8.2 Hz, Ar–H), 2.97 (3H, s, CH_3), 4.1 (2H, q, J= 7.2 Hz, $\underline{CH_2}CH_3$), 1.19 (3H, t, J= 7.0 Hz, $CH_2\underline{CH_3}$ and 9.84 (1H, s, NH).Analysis for $C_{16}H_{13}BrN_2O_2S$ (377.26): Calcd. C 50.89, H 3.45, Br 21.21, N 7.42, S 8.48.; found C 50.94, H 3.47, Br 21.18, N 7.43, S 8.50%

Synthesis of diethyl 3-amino-4-(4-bromophenyl)-6-methylthieno[2,3-b]pyridin-2,5-dicarboxylate (3).

By stirring at room temperature 4-5hr. compound 2(0.01 mol) , ethyl chloroacetate (0.01 mol), sodium methoxide (0.01 mol) and methanol (30 ml), the solid that separated was filtered off, washed with methanol and crystallized from ethanol, 60% yield, m.p. 190°C. IR, cm^{-1} 3393, 2915, 1730 and 1590 cm^{-1} for NH_2, CH aliphatic, CO ester and C=N group, respectively. ^{1}H NMR (DMSO-d_6) δ(ppm): spectrum exhibited signals at 7.69(2H, d, J=8.2 Hz, Ar–H), 7.12 (2H, d, J = 8.4 Hz, Ar–H), 2.17 (3H, s, CH_3), 4.10 (4H, q, J = 7.2 Hz, $\underline{CH_2}CH_3$), 1.81 (6H, t, J = 6.9 Hz, 2 x $CH_2\underline{CH_3}$) and 7.51 (2H, s, NH_2): MS (m/z, %) 463 (1.18%): Analysis for $C_{20}H_{19}BrN_2O_4S$

(463.34): calcd. C 51.79, H 4.10, Br 17.27, N 6.04, S 6.91; found C 51.84, H 4.13, Br 17.25, N, 6.06, S6.92%.

Synthesis of ethyl 9-(4-bromophenyl)-2,7-dimethyl-4-oxo-4H-pyrido [3',2': 4,5]thieno [3,2-d] [1,3]oxazine-8-carboxylate (4).

A solution of compound **3** (0.01 mol) in acetic anhydride (5 ml) was heated under reflux for 3hr. the solid that separated on cooling was filtered off and crystallized from ethanol 60% yield, m.p 280°C. IR cm^{-1}, CH aliphatic at 2892 and CO(ester) at 1752, 1718; MS (m/z, %) 459 (21.13%). Analysis for $C_{20}H_{15}BrN_2O_4S$ (459.29): calcd. C 52.25, H 3.27, Br 17.42, N 6.10, S 6.97; found C 52.50, H 3.10, Br 17.30, N 6.00, S 6.89%.

Synthesis of ethyl 9-(4-bromophenyl)-2,7-dimethyl-4-oxo-3,4-dihydropyrido [3',2':4,5] thieno [3,2-d] pyrimidin-8-carboxylate (5a).

A solution of compound **4** (0.01 mol) and formamide (15 ml) was heated under reflux for (3hr) the reaction mixture was diluted with cold water, the solid which was separated out was dried and crystallized from ethanol, 80% yield, m.p. 295°C. IR, cm^{-1} NH at 3460, CH aliphatic at 2915, CO (ester) at 1756 and CO(amidic) at 1660. ^{1}H NMR (DMSO-d_6)δ (ppm): Spectrum exhibited signals at 7.69(2H, d, J= 8.0 Hz, Ar–H), 7.35 (2H, d, J = 8.2 Hz, Ar–H), 2.57 (6H, s, 2x CH_3), 4.02 (2H, q, J = 7.3 Hz, $\underline{CH_2}CH_3$), 1.21 (3H, t, J = 7.1 Hz, $CH_2\underline{CH_3}$) and 13.55 (1H, s, NH). Analysis for $C_{20}H_{16}BrN_3O_3S$ (458.33): calcd. C 52.36, H 3.49, Br 17.45, N 9.16, S 6.98; found. C 52.41, H 3.52, Br 17.43, N 9.17, S.6.95%.

Synthesis of ethyl 3-amino-9-(4-bromophenyl-2,7-dimethyl-4-oxo-3,4-dihydropyrido[3',2':4,5] thieno[3,2-d] pyrimidin-8-carboxylate (5_b).

A mixture of compound **4** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol (30 ml) was refluxed for (3hr). the solid which was separated out after concentration and cooling was dried and crystallized from ethanol , 90% yield, m.p. 260°C. IR, cm^{-1} NH_2 at 3430, CH aliphatic at 2899, CO(ester) at 1743 and CO (amidic) at 1633. ^{1}HNMR (DMSO-d_6) δ (ppm): spectrum exhibited signals at 7.68 (2H, d, J=8.2 Hz, Ar–H), 7.35 (2H, d, J = 8.0 Hz, Ar–H), 2.17 (3H, s, CH_3), 2.08 (3H, s, CH_3), 4.12 (2H, q, J = 7.5 Hz, $\underline{CH_2}CH_3$), 1.20 (3H, t, J = 7.2 Hz, $CH_2\underline{CH_3}$) and 3.57 (2H, s, NH_2).; MS (m/z, %) 473 (0.96%). Analysis for $C_{20}H_{17}BrN_4O_3S$ (473.34): calcd. C 50.70, H 3.59, Br 16.90, N 11.83; S, 6.76; found. C 50.69; H, 3.60; Br 16.82, N 11.80, S, 6.80%.

Synthesis of ethyl 2-(2-aminophenyl carbamoyl)-3-acetamido-4-(4-bromophenyl)-6-methylthieno [2,3-b] pyridin-5-carboxylate (6).

A mixture of compound **4** (0.01 mol) and o-phenylendiamine (0.01 mol) was stirred in chloroform (20 ml) for (16 hr) at room temp., leave aside over night to give compound **6** which was dried and crystallized from chloroform, 70% yield, m.p. 220°C. IR cm^{-1} NH, NH_2 at 3460, 3420, CH aliphatic at 2912, CO(ester) at 1733 and CO (amidic) at 1652, 1660. ^{1}H NMR (DMSO-d_6) δ (ppm): spectrum exhibited signals at 7.96-7.41 (8H, m, Ar–H), 2.92 (3H,s,CH_3), 2.50 (3H, s,$COCH_3$), 4.03 (2H, q, J= 7.4 Hz, $\underline{CH_2}CH_3$), 1.08 (3H, t, J=7.0 Hz, $CH_2\underline{CH_3}$) 5.38 (2H, s,NH_2), 9.64 (1H, s, NH) and 9.18 (1H, s, CO NH): MS (m/z, %) 567 (1.48%): Analysis for $C_{26}H_{23}BrN_4O_4S$(567.45): calcd. C 54.98, H 4.05, Br 14.10, N 9.87, S 5.64; Found . C 55.03, H 4.09, Br 14.11, N, 9.87, S5.65%.

Synthesis of ethyl 3-(2-aminophenyl)-9-(-4-bromo-phenyl)-2,7-dimethyl-4-oxo-3,4-dihydropyrido-[3',2':4,5] thieno[3,2-d]pyrimidin-8-carboxylate(7).

A solution of compound **6** (0.01 mol) in acetic anhydride and acetic acid (5 ml) was heated under reflux for (5hr). The solid that separated on cooling was filtered off and crystallized from butanol 60% yield, m.p. 350°C. IR, cm^{-1}. NH_2 at 3418, CH aliphatic at 2911, 2960, CO(ester) at 1743 and CO (amidic) at 1652. ^{1}H NMR (DMSO-d_6) δ (ppm): spectrum exhibited signals at 7.89-7.42 (8H, m, Ar–H), 2.51(6H, s, 2 x CH_3), 4.10 (2H,q, J = 7.2 Hz, $\underline{CH_2}CH_3$), 1.31 (3H, t, J = 7.0 Hz, $CH_2\underline{CH_3}$) and 5.10 (2H, s, NH_2). Analysis for $C_{26}H_{21}BrN_4O_3S$ (549.44): calcd. C56.79, H 3.82, Br 14.56, N 10.19, S 5.82; found. C 56.84, H, 3.85, Br 14.54. N 10.20, S, 5.84.

Synthesis of thienopyrimidine derivatives ($8_{a,b}$)

A mixture of compound **5b** (0.01 mol), (maleic and/or phthalic anhydride) was heated in an oil bath for (1hr). The reaction mixture was diluted with water and the obtained solid was filtered off, dried and crystallized from ethanol to give compounds **($8_{a,b}$)**, respectively.

Ethyl-9-(4-bromophenyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,7-dimethyl-4-oxo-3,4-dihydro-pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-carboxylate (8a). yield 65%; m.p. 290°C. IR, cm^{-1} CH aliphatic at 2986, CO(ester) at 1767 and CO (amidic) at 1675, 1650. MS (m/z %) 554 [M^+, 1.92%]. Analysis for $C_{24}H_{17}BrN_4O_5S$ (553.38): calcd. C 52.04, H 3.07, Br 14.46, N 10.12 S 5.78; found C 5 2.09, H 3.10, Br 14.44, N 10.12, S 5.79%.

Ethyl 9-(4-bromophenyl)-3-(1,3-dioxoisoindolin-2-yl)-2,7-dimethyl-4-oxo-3,4-dihydropyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-carboxylate (8b). yield 70%; m.p. 270°C. IR, cm^{-1}. CH aliphatic at 2986, CO(ester) at 1755 and CO(amidic) at 1643, 1655. ^{1}H NMR (DMSO-d_6) δ(ppm): spectrum exhibited signals at 7.42-6.96 (8H, m, Ar–H), 2.73(3H, s, CH_3), 2.67 (3H, s, CH_3), 3.76 (2H, q, J= 7.2 Hz, $\underline{CH_2}CH_3$) and 1.13 (3H, t, J = 7.0 Hz, $cH_2\underline{CH_3}$). Analysis for $C_{28}H_{19}BrN_4O_5S$ (603.44): calcd. C 55.68, H, 3.15, Br 13.26, N 9.28, S 5.30 ; found. C 55.73, H 3.17 , Br 13.24, N 9.28, 5.31%.

Synthesis of ethyl 9-(4-bromophenyl)-3-((4-chloro-benzelidine)amino)-2,7-dimethyl-4-oxo-3,4-dihydro-pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-carboxylate (9).

A mixture of compound **5_b** (0.01 mol), p-chlorobenzaldehyde (0.01 mol), few drops of piperidine was heated in an oil bath for (1 hr), the reaction mixture was diluted with water. The solid that separated out, dried and crystallized from ethanol 60% yield, m.p. 295°C. IR, cm^{-1} CO(amidic) at 1630 and C=N at 1610. MS (m/z, %), 595 (0.97%). CH aliphatic at 2970, CO(ester) at 1749, CO(amidic) at 1630 and C=N at 1610.MS (m/z, %) 595 (0.97%). Analysis for $C_{27}H_{20}BrClN_4O_3S$ (595.01): calcd. C 54.45, H 3.36, Br 13.45, Cl 5.88, N 9.41, S 5.38; found. C 54.42, H 3.32, Br. 13.49, Cl 5.90, N 9.43. S 5.30%.

Synthesis of ethyl 9-(4-bromophenyl)-3-(cyclohexyl-ideneamino)-2,7-dimethyl-4-oxo-3,4-dihydropyrido[3',2':4,5] thieno[3,2-d]pyrimidin-8-carboxylate (10).

A mixture of compound **5_b**with cyclohexanone(0.02 mol), few drops of piperidine was heated in an oil bath for (1hr), the reaction mixture was diluted with water. The solid that separated out, dried and crystallized from ethanol to give schiff'sbase70% yield, m.p. 125°C. IR, cm^{-1}, CH aliphatic at 2886, 2917, CO(ester) at 1739, CO(amidic) at 1640 and C=N at 1614. ^{1}H NMR (DMSO–d_6) δ (ppm): spectrum exhibited signals at 7.39 (2H, d, J = 8.0 Hz, Ar–H), 7.11 (2H, d, J=7.9 Hz, Ar–H), 2.20 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.51-1.30 (10H, m, 5 x CH_2), 3.46 (2H, q, J= 7.2 Hz, $\underline{CH_2}CH_3$) and 1.05 (3H, t, J = 7.1 Hz, $\underline{CH_2}CH_3$): Analysis for $C_{26}H_{25}BrN_4O_3S$ (553.47): calcd. C56.37, H 4.52, Br 14.45, N 10.12, S 5.78; found C 56.42, H 4.55, Br 14.44, N 10.12, S 5.79%.

Synthesis of diethyl2,2'-((9-(4-bromophenyl)-8-(ethoxycarbonyl)-2,7-dimethyl-4-oxopyrido[3',2':4,5] thieno[3,2-d]pyrimidin-3(4H)-yl) azanediyl) diacetate (11).

A mixture of compound **5_b** (0.01 mol), ethyl chloroacetate (0.04 mol) and anhydrous potassium carbonate (0.04 mol) in dry acetone (30 ml) was refluxed for (24hr), the excess acetone was evaporated and the reaction mixture was diluted with water, the solid that separated was crystallized from acetone, 80% yield, m.p. 200°C. IR, cm^{-1}. CH aliphatic at 2930, 2965, CO(ester) at 1743 and CO(amidic) at 1658. ^{1}H NMR (DMSO–d_6) δ (ppm): spectrum exhibited signals at 7.75 (2H, d, J = 8.3 Hz, Ar–H), 7.38 (2H, d, J = 8.4 Hz, Ar–H), 2.50 (6H, s, 2 x CH_3), 2.14 (4H, s, 2 x CH_2) 4.10 (6H, q, J = 6.0 Hz, 3 x $\underline{CH_2}CH_3$) and 1.21 (9H, t, J = 6.0 Hz, 3 x $CH_2\underline{CH_3}$): Analysis for $C_{28}H_{29}BrN_4O_7S$(645.52): calcd. C 52.05, H 4.49, Br 12.39, N 8.68, S 4.96; found. C 52.10, H 4.53, Br 12.38, N 8.68, S 4.97%.

Synthesis of diethyl9,9'-bis(4-bromophenyl)-2,2',7,7'-tetramethyl-4,4'-dioxo-4H,4'H-[3,3'-bipyrido [3',2': 4,5] thieno[3,2-d]pyrimidine]-8,8'-dicarboxylate (12).

A mixture of compounds **4** (0.01 mol), and **5_b**(0.01 mol) was fused in an oil bath for (1hr), the obtained solid was washed with ethanol, dried and recrystallized from ethanol 80% yield, m.p. 320°C. IR, cm^{-1}. CH aliphatic at 2900, 2898, CO(ester) at 1764 and CO(amidic) at 1646. MS(m/z%) 914 (0.77%): Analysis for $C_{40}H_{30}Br_2N_6O_6S_2$ (914.64); calcd.
C 52.48, H 3.28, Br 17.49, N 9.18, S 6.99; found. C 52.53, H, 3.31, Br 17.47, N 9.19, S 7.01%.

Synthesis of ethyl 9-(4-bromophenyl)-3-(2-ethoxy-2-oxoethyl)-2,7-dimethy-4-oxo-3,4-dihydropyrido [3'2': 4,5] thieno [3,2-d] pyrimidin-8-carboxylate (13).

A mixture of compound **5_a**(0.01 mol), ethyl chloroacetate (0.04 mol) and anhydrous potassium carbonate (0.04 mol) in dry acetone (30 ml) was refluxed for (24 hr), the excess acetone was removed by distillation and the residue poured with stirring into water, the obtained solid was filtered off, dried and recrystallized from ethanol 70% yield, m.p. 210°C. IR, cm^{-1} CH aliphatic at 2930, CO(ester) at 1745, 1759 and CO(amidic) at 1638. ^{1}H NMR (DMSO-d_6) δ (ppm): spectrum exhibited signals at 7.99(2H, d, J = 5.1 Hz, Ar–H), 7.56 (2H, d, J = 5.1 Hz, Ar–H), 5.30 (2H, s, NCH_2), 2.95 (6H, s, 2x CH_3), 3.94 (4H, q, J = 7.2Hz, 2 x $\underline{CH_2}CH_3$) and 1.24 (6H, t, J= 7.1Hz, 2 x $CH_2\underline{CH_3}$): Analysis for $C_{24}H_{22}BrN_3O_5S$(544.42): calcd. C 52.90, H, 4.04, Br 14.69 N 7.71, S 5.88; found . C 52.91, H 4.07, Br 14.68 , N 7.72, S 5.89%.

Synthesis of ethyl 9 –(4-bromophenyl)3-(2-hydra-zinyl-2-oxoethyl)-2,7-dimethyl-4-oxo-3,4-dihydro-pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-carboxylate (14).

A mixture of compound **13** (0.01 mol), and hydrazine hydrate (0.02 mol) in ethanol (20 ml) was refluxed for (6hr), the reaction mixture was concentrated cooled and the obtained was filtered off and recrystallized from ethanol 80% yield, m.p. 320°C. IR, cm^{-1}. NH, NH_2 at 3412, 3430, CH aliphatic at 2895, 2920, CO(ester) at 1742 and CO(amidic) at 1640. ^{1}H NMR (DMSO-d_6) δ (ppm): spectrum exhibited signals at 7.37 (2H, d, J= 5.1Hz, Ar–H), 7.33 (2H, d, J=5.1 Hz, Ar–H), 2.82 (3H, s, CH_3), 2.72(3H,s,CH_3), 4.07 (2H, q, J=7.0 Hz, $\underline{CH_2}CH_3$), 1.26 (3H, t, J= 6.0Hz, $CH_2\underline{CH_3}$), 5.51 (2H, s, NH_2) and 13.56 (1H, s, NH): Analysis for $C_{22}H_{20}BrN_5O_4S$(530.39): calcd. C49.77, H 3.77, Br 15.08, N 13.20, S 6.03; found C49.82, H 3.80, Br 15.07 N 13.20, S 6.05%.

Synthesis of ethyl 9-(4-bromophenyl)-3-(3-hydroxy-5-phenyl-1H-pyrazol-4-yl)-2,7-dimethyl-4-oxo-3,4-dihydropyrido[3',2': 4,5]thieno[3,2-d] pyrimidin-8-carboxylate (15).

A mixture of compound **14** (0.01 mol) and benzaldehyde (0.01 mol) in butanol (50 ml) was refluxed for (8hr), the reaction mixture was concentrated cooled and the obtained solid was filtered off and recrystallized from butanol 80% yield, m.p. 285°C. IR, cm^{-1} NH at 3350, CH aliphatic at 2917, CO(ester) at 1741 and CO(amidic) at 1665. ^1H NMR (DMSO-d_6) δ (ppm): spectrum exhibited signals at 7.98-6.02 (9H, m, Ar–H), 2.78 (3H, s, CH_3), 2.51 (3H, s, CH_3), 4.40 (2H, q, J= 7.2 Hz, $\underline{CH_2}CH_3$), 1.81 (3H, t, J= 7.0Hz, $CH_2\underline{CH_3}$), 5.55 (1H, s, NH) and 11.52 (1H, s, OH): MS (m/z%) 617 [M^+, 1.32%]. Analysis for $C_{29}H_{22}BrN_5O_4S$ (616.49): calcd. C56.45, H 3.57, Br 12.98, N 11.35, S 5.19; found C 56.43, H 3.60, Br 12.95, N 11.35, S 5.18%.

Synthesis of ethyl 9-(4-bromophenyl)-2,3,7-tri-methyl-4-oxo-3,4-ihydropyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-carboxylate (16).

A mixture of compound $\mathbf{5_a}$(0.01 mol), methyl iodide (0.02 mol) and anhydrous potassium carbonate (0.04 mol) in dry acetone (40 ml) was refluxed on water bath for (24hr), the excess acetone water removed by distillation and the residue was poured with stirring into water, the obtained solid was filtered off, dried and recrystallized from acetone 60% yield, m.p. 155°C. IR, cm^{-1}. CH aliphatic at 2971, CO(ester) at 1757 and CO(amidic) at 1639. ^1H NMR (DMSO-d_6) δ (ppm): spectrum exhibited signals at 7.52 (2H, d, J = 8.4 Hz, Ar–H), 7.39 (2H, d, J = 8.3Hz, Ar–H), 3.56(3H s,N–CH_3), 2.56 (6H, s, 2x CH_3), 4.27 (2H, q, J= 7.3Hz, $\underline{CH_2}CH_3$) and 1.11 (3H, t, J= 6.9Hz, $CH_2\underline{CH_3}$): MS (m/z%) 471[M–1, 6.35%]: Analysis for $C_{21}H_{18}BrN_3O_3S$(472.35): calcd. C 53.35, H 3.81, Br 16.94, N 8.89, S 6.77; found. C 53.40, H 3.84, Br 16.92, N 8.90, S 6.79%.

Cytotoxicity assays

The cytotoxicity Was measured using the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) cell viability assay [15,16]. In brief, cells were seeded in 96-well plates and treated with graded concentrations of tested compounds. Then the medium was replaced with fresh medium and cells were incubated with MTT. Four hours later, cells were lysed by addition of dimethylsulphoxide (DMSO). The solubilized formazan products were quantified for absorbance at a wavelength of 570 nm.

Cytotoxicity against the liver tumor cell line (HepG2) in vitro.

Compounds 2,3,4,5_b,6,7,8_a,8_b, 9,10,11,12,13, 14, 15 and 16 were evaluated for their human tumor cell growth inhibitory activity against $HepG_2$(hepatocarcinoma). The measurement of cell growth and viability were determined as described previouse [15,16].

Cytotoxicity evaluation using viability assay were performed by a Regional Center for Mycology and Biotechnology (RCMP), Al-Azhar University. The inhibitory activity of the synthetic compounds against the liver carcinoma cell line (HepG2) is given in (Figs. 2, suplementry material). The IC_{50} values were in the low concentration in microgram range.

III. RESULTS AND DISCUSSION

The new derivatives were prepared following the reaction sequences depicted in schemes 1-3. Reflux of p-bromobenzaldehyde and cyanothioacetamide in dioxanewith a catalytic amount of piperidine afforded arylidene cyanothioacetamide 1 in good yield.

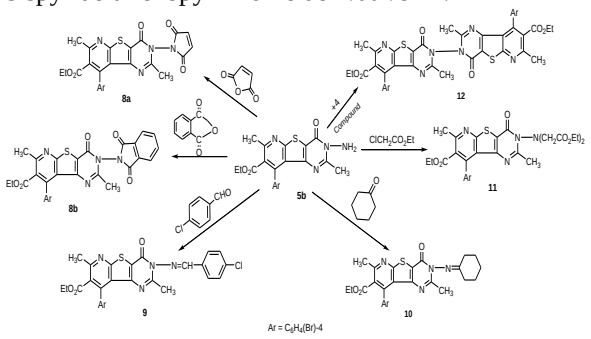
Compound 1 reacts with ethyl acetoacetate in the presence of a catalytic amount of piperidine in boiling dioxane to produce the corresponding thioxopyridine derivative 2. The reaction probably follows through mechanism followed in chart 1.

Treatment of 2 with ethyl chloroacetate, CH_3ONa gave thieno[2,3-b] pyridine 3 via the non-isolable intermediate A. Acetylation of amino ester 3 with acetic anhydride gave non-isolated N-acetylated intermediate B which cyclized under the reaction conditions to yield oxazinone derivative 4.

Condensation of oxazinone derivative 4 with formamide (Mahmoud, N., et al., 2008) afforded thieno[2,3-d] pyrimidine derivative 5a, while hydrazinolysis took place to give 3-amino-3H-thieno[2,3-d] pyrimidine-4-one 5b.

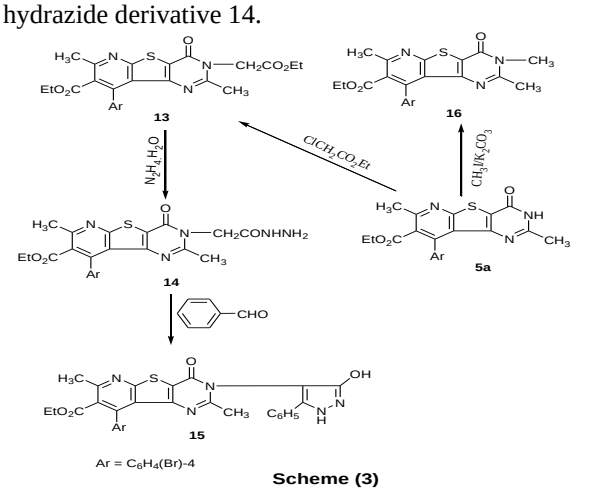
In addition the reaction of 4 with o-phenylenediamine leads to opening of the oxazinone ring and formation of ethyl 2-(2-aminophenylcarbomoyl)-3-acetamido-4-(4-bromophenyl)-6-methylthieno [2,3-b]pyridin-5-carboxylate 6. Cyclocondensation of 6 by Ac_2O/AcOH affordedthe corresponding pyridothieno pyrimidine 7.

Also, compound 5_b was used as precursor for the preparation of several new compounds. This reaction of 5_b with maleic anhydride and phthalic anhydride gave thienopyrimidine derivatives 8_a and 8_b. Condensation of $5_b$with p-chlorobenzaldehyde gave the corresponding arylidene derivative 9, similarly compound 5b condensed with cyclohexanone to give compound 10. Reaction of $5_b$with ethylchloroacetate gave the diethyl diacetate derivative 11. On the other hand, reaction of $5_b$with compound 4 gave the bispyrido thienopyrimidine derivative 12.



Scheme (2)

Treatment of ethyl 9-(4-bromophenyl)-2,7-dimethyl-4-oxo-3,4-dihydropyrido [3',2':4,5] pyrimidin-8-carboxylate 5a with ethyl chloroacetate in the presence of K2CO3 gave the corresponding 3-substituted thieno [2,3-d] pyrimdine derivative 13, which on hydrazinolysis afforded the corresponding hydrazide derivative 14.



Scheme (3)

Cyclization of carbohydrazide 14 with benzaldehyde in boiling ethanol afforded 3-(1H-pyrazol-4-yl)-4-oxo-3,4-dihydropyrido[3',2':4,5] thieno[2,3-d]pyrimidin-8-carboxylate 15. The reaction probably follows through the mechanism flowed in chart 2.

Treatment of $5_a$with methyl iodide in dry acetone/K_2CO_3 gave the corresponding 3-methylpyridothieno pyrimidine derivative 16. The structures newly synthesized derivatives were supported by correct analytical data, IR, ^{1}H NMR and mass spectra studies (cf experimental part).

Cytotoxicity against the liver tumor cell line (HepG2) in vitro

The cytotoxic antitoumer activity activity for Compounds 2,3,4,5_b,6,7,8_a,8_b, 9,10,11,12,13, 14, 15 and 16, were evaluated against $HepG_2$(hepatocarcinoma). The IC_{50} values were in the low concentration in microgram range. Compounds 8_a ,9 and 14 had the most prominent activity against the HepG2 and (IC_{50}= 2.75 µg/ml, 5.45 mg/ml and 2.12µg/ml respectively(Fig1). Surprisingly, compounds $8_a$and 14 were more potent than the standard drug (IC_{50} of standard drug = 4.60 µg/ml) in $HepG_2$ cell line. Compounds 2,6 and 9 had moderate inhibitory activity against $HepG_2$ with IC_{50} = 26.30µg/ml, 5.45 µg/ml and 11.10µg/ml respectively. While compounds 3,4,$_b$,7, 8_b, 10,11,12,13,15 and 16 had weak activity.

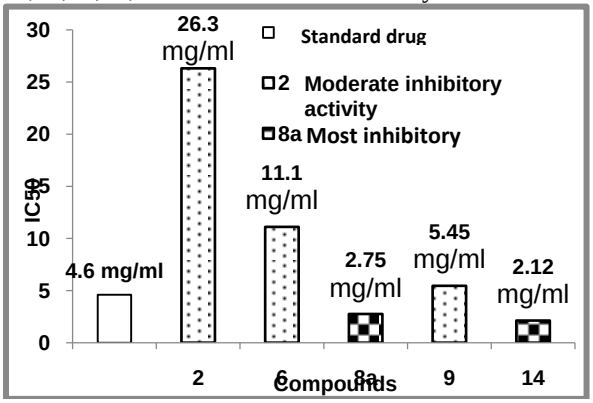


Fig. (1): IC_{50} for the Standard Drug and the Potent Compounds

IV. CONCLUSION

In conclusion, the pyridothienopyrimidine with 3-(1H-1-pyrazol-1-yl) 8a, 3-((4-chloro-benzelidine)amino) 9 and 3-(2-hydrazinyl-oxo ethyl) 14 functions exhibited of some new heterocyclic compounds. Structure of the synthesized the most active compounds against $HepG_2$ as compared with the used standard drug.However, the remaining compounds exhibited moderate to lower antitumor activities.

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