Utility of thieno[2,3-b] pyridine derivatives in the synthesis of some condensed heterocyclic compounds with expected biological activity

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Abstract
On the pharmaceutical account of the reported anticancer activity of thieno[2,3-b] pyridine and condensed thieno[2,3-b] pyridine, new compounds containing thieno[2,3-b] pyridine condensed with each of pyridine, cyclopentyl, tetrahydroquinoline, pyrimidine, 1,6-naphthiridin, benzofuro[2,3-b] pyridine, imidazo[1,2-c] pyrimidine, [1,2,3] triazolo[1,5-a] pyrimidine were synthesized through different chemical reactions. The obtained compounds were evaluated for their in vitro antitumor activity against Liver HepG-2 and Breast MCF-7 cell lines compared to the reference drug (doxorubicin). Compounds 5, 7, 12, 23, 24, 37 and 39 were found to be the most active against both cell lines exhibiting IC50 values ranging from 10.33-43.90 μM/L and 9.70-48.80 μM/L against HepG-2 and MCF-7 cell lines, respectively. From which compound 5 was the most active compound exerting comparable activity to the reference drug against both cell lines, showing IC50 values 10.33 and 9.70 μM/L comparable to doxorubicin that exerted IC50 values 8.55 and 8.90 μM/L against HepG-2 and MCF-7 cell lines, respectively.

Keywords: Thienopyridine, pyridothenopyridine, pyridothenopyrimidine, anticancer, liver HepG-2, breast MCF-7

Introduction
The thieno[2,3-b]pyridine derivatives occupy special place and have attracted considerable attention because of their broad pharmacological activities, including anticancer [1-9], antiviral [10-13], anti-inflammatory [14-17], antimicrobial [18,19], antidiabetic [20-23], antihypertensive [24-26] and osteogenic [27,28] activities, in addition to treatment of CNS disorders [29-31].

The aforementioned biological activities stimulated our interest for the synthesis of several new condensed heterocyclic compounds containing thieno[2,3-b] pyridine moiety condensed with each of pyridine, cyclopentyl, tetrahydroquinoline, pyrimidine, 1,6-naphthiridin, benzofuro[2,3-b] pyridine, imidazo[1,2-c] pyrimidine, [1,2,3] triazolo[1,5-a] pyrimidine. The new condensed heterocyclic derivatives possessing latent functional substituents appear promising to fulfill the objectives of our biological activity studies and the desired chemical transformations.

Results and discussion
Chemistry
The α-aminonitrile thienopyridine derivative 2 was synthesized through treatment of the pyridine thione derivative 1 with chloroacetonitrile in presence of sodium hydroxide [32]. *(Scheme 1)*. Fusion of the α-aminonitrile derivative 2 with cyclic ketones namely; cyclopentanone and cyclohexanone in the presence of anhydrous zinc chloride [33] yielded the tetracyclic compounds 3 and 4; respectively. Reactions utilizing cyclohexanone resulted in higher yields of the product compared to cyclopentanone, a fact that could be attributed to the steric flexibility of cyclohexanone [34]. Reactions of compound 2 with ethyl methyl ketone furnished the tricyclic pyridothenopyrimidine derivative 5. The reactions of α-aminonitriles with various cyclic ketones in presence of Lewis acid were reported to be achieved through the nucleophilic attack of the lone pair of amino nitrogen on the carbonyl carbon to form Schiff’s base, followed by complex formation between Lewis acid, zinc chloride and the nitrile triple bond that accelerates the intramolecular cyclization [35]. Furthermore, the α-aminonitrile derivative 2 was refluxed with malononitrile in presence of a catalytic amount of triethylamine [36] to yield pyridothenopyridine-3-carbonitrile derivative 6.
The interaction of o-aminonitrile derivative and cyanothioacetamide in ethanol containing a catalytic amount of piperidine [39]. Furthermore, treatment of o-aminonitrile with benzoyl acetone in dry DMF [37] gave the pyridothienonaphthyridine-3-carbonitrile derivative [10]. The IR spectrum of the compound showed a broad absorption band at 3416 cm$^{-1}$ due to the tautomeric OH group besides a band at 1719 cm$^{-1}$ corresponding to C=O function.

Moreover, treatment of o-aminonitrile with benzoyl acetone in presence of sodium ethoxide [39] yielded compound that was further refluxed with malononitrile in presence of a catalytic amount of piperidine [39] to yield pyridothienonaphthyridine-3-carbonitrile derivative [10].

Furthermore, refluxing of equimolar amounts of compounds and cyanothioacetamide in ethanol containing a catalytic amount of piperidine [40] afforded pyridothienopyrimidinone derivative [7] which its $^1$H NMR spectrum showed two deuterium oxide exchangeable singlets at δ 4.50 and 6.90 ppm attributed to ethyl protons of the ethoxymethylene group. In addition to a singlet due methine proton at δ 7.25 ppm.

The o-aminonitrile derivative was reacted with triethyl orthoformate [41] which underwent a nucleophilic substitution reaction in the presence of acetic anhydride introducing a replaceable ethoxy group furnishing the intermediate compound. The $^1$H NMR spectrum of compound displayed a triplet at δ 1.06 ppm and a quartet at δ 3.44 ppm attributed to ethanol molecules and intramolecular cyclization to yield the target compound. The $^1$H NMR spectrum of compound showed two deuterium oxide exchangeable singlets at δ 6.06 and δ 8.90 ppm corresponding to the vicinal amino and imino groups, respectively.

Similarly, compound was refluxed with formic acid to yield pyridothienopyrimidinone derivative which its synthesis was rationalized via a sequence of N-formylation followed by cyclization processes involving the interaction between nucleophilic hydroxyl group and electrophilic nitrile carbon to give the 4-iminoxazine intermediate which underwent rearrangement to yield the pyrimidine ring [33]. The $^1$H NMR spectrum of the compound displayed a deuterium oxide exchangeable singlet corresponding to OH proton at δ 12.80 ppm.

Reagents: i) CH$_3$COCH$_2$CN / DMF; ii) HCOOH; iii) C$_6$H$_5$COCH$_2$CN / NaOEt; iv) CNCH$_2$CN/ absolute EtOH/ piperidine; v) CNCH$_2$CSNH$_2$/ absolute EtOH/ piperidine; vi) dioxane/ Et$_3$N.

Scheme 2. Synthetic pathways for compounds 7, 8, 9, 10, 11 and 12.

The o-aminonitrile derivative was subjected to partial hydrolysis by stirring with concentrated sulphuric acid [33] to yield 2-carboxamide derivative [13]. The $^1$H NMR spectrum of compound displayed two deuterium oxide exchangeable singlets at δ 5.68 and δ 6.92 ppm corresponding to NH$_2$ and CONH$_2$ protons, respectively.

Acid-catalyzed bis-nucleophilic cyclocondensation of o-aminocarboxamide derivative with acid chlorides namely; benzoyl chloride and chloroacetyl chloride in glacial acetic acid [33] furnished pyrimidine derivatives 14a and 14b; respectively. The $^1$H NMR spectra of compounds 14a and 14b revealed two deuterium oxide exchangeable singlets at δ 12.60, 10.50 ppm corresponding to pyrimidine NH protons; respectively. However, the $^1$H NMR spectrum of compound showed an additional singlet at δ 3.07 ppm integrated for two protons corresponding to CH$_2$Cl protons.

The o-aminonitrile derivative was reacted with triethyl orthoformate [41] which underwent a nucleophilic substitution reaction in the presence of acetic anhydride introducing a replaceable ethoxy group furnishing the intermediate compound 15. The $^1$H NMR spectrum of compound displayed a triplet at δ 1.06 ppm and a quartet at δ 3.44 ppm attributed to ethyl protons of the ethoxymethylene group. In addition to a singlet due methine proton at δ 7.25 ppm.

Furthermore, compound upon reaction with excess hydrazine hydrate [42] yielded novel pyridothienopyrimidinione derivative. The reaction mechanism was reported [41] to be accomplished through addition of a hydrazine molecule on the enamine double bond followed by elimination of an ethanol molecule and intramolecular cyclization to yield the target compound. The $^1$H NMR spectrum of compound showed two deuterium oxide exchangeable singlets at δ 6.06 and δ 8.90 ppm corresponding to the vicinal amino and imino groups, respectively.

Similarly, compound was refluxed with formic acid to yield pyridothienopyrimidinione derivative which its synthesis was rationalized via a sequence of N-formylation followed by cyclization processes involving the interaction between nucleophilic hydroxyl group and electrophilic nitrile carbon to give the 4-iminoxazine intermediate which underwent rearrangement to yield the pyrimidine ring [33]. The $^1$H NMR spectrum of the compound displayed a deuterium oxide exchangeable singlet corresponding to OH proton at δ 12.80 ppm.

Reagents: i) conc.H$_2$SO$_4$/Stirring at r.t.; ii) RCOCl / CH$_3$COOH; iii) HC(OC$_2$H$_5$)$_2$ / AC$_2$O; iv) NH$_2$NH$_2$; v) HCOOH.
Scheme 3. Synthetic pathways for compounds 13, 14a,b, 15, 16 and 17.

The interaction of the o-aminonitrile derivative 2 with phenyl isothiocyanate in presence of pyridine [36] led to the formation of pyridothienopyrimidine-2-thione 18. “(Scheme 4)” The 1H NMR spectrum of compound 18 showed two deuterium oxide exchangeable singlets at 7.10 and 9.75 ppm attributed to pyrimidine NH and imino protons, respectively.

A facile reaction occurred when o-aminonitrile derivative 2 was refluxed in excess formamide [43] to furnish the corresponding 4-aminopyrimidine derivative 19. The reaction mechanism was proposed to be proceeding first through o-cyanoformamidine formation followed by intramolecular cyclization via nucleophilic attack of the lone pair of the formamide amino group on the electrophilic nitrile carbon [43].

Diazotization of compound 2 was accomplished through its reaction with cold hydrochloric acid and saturated aqueous sodium nitrite solution to yield thienopyridine-3-diazonium chloride 20 which was further coupled with compounds bearing active methylene functions namely; malononitrile and ethyl cyanoacetate [44] to afford the corresponding hydrazono derivatives 21 and 22, respectively. The 1H NMR spectra of compounds 21 and 22 revealed deuterium oxide exchangeable singlets at d 8.50 and d 8.28 ppm attributed to NH protons, respectively.

Hydrazono derivatives 21 and 22 were cyclized upon treatment with hydrazine hydrate in boiling ethanol [44] to afford the expected 3,5-diaminopyrazole derivative 23 and 3-amino-5-oxopyrazole derivative 24. The 1H NMR spectrum of compound 23 revealed a deuterium oxide exchangeable singlet at δ 9.85 ppm integrated for four protons attributed to two NH protons. The 1H NMR spectrum of the compound 24 displayed three deuterium oxide exchangeable singlets corresponding to pyrazole-NH, pyrazole-C3-NH, and thiophene-C3-NH protons at δ 7.00, 7.22 and 8.60 ppm, respectively.

CNCH2COOC2H5/ absolute EtOH; vi) NH2NH2/ absolute EtOH.

Scheme 4. Synthetic pathways for compounds 18, 19, 20, 21, 22 and 23.

Compound 2 was also reacted with ethylene diamine in the presence of carbon disulphide disulfide [45] to afford 2-imidazolylthienopyrimidine derivative 25 “(Scheme 5)”. The reaction mechanism is proposed to proceed through addition of carbon disulphide and an ethylene diamine molecules on the nitrile function followed by the elimination of 2-thioximidazolidine moiety to yield thiocarboxamide derivative which further reacted with one molecule of ethylene diamine with simultaneous elimination of ammonia and hydrogen sulphide molecules to yield the imidazolidine ring.

The 1H NMR spectrum of compound 25 showed two deuterium oxide exchangeable singlets at δ 6.90 and 7.20 ppm attributed to NH and NH2 protons, respectively.

Compound 25 was further subjected to cyclization into tetracylic imidazopyridothienopyrimidine systems in different ways [45]. Treatment of compound 25 with triethyl orthoformate gave the unsubstituted imidazopyridothienopyrimidine 26, while refluxing of compound 25 with benzaldehyde yielded the corresponding 5-phenylimidazopyridothienopyrimidine 27. On the other hand, imidazopyridothienopyrimidine-5-thione 28 was obtained by heating compound 25 with ethanol.

The 1H NMR spectrum of compound 27 displayed a singlet at δ 8.71 ppm corresponding to pyrimidine-C3 proton besides to a deuterium oxide exchangeable singlet at δ 9.65 ppm attributed to NH proton. Furthermore, the 1H NMR spectrum of compound 28 revealed a deuterium oxide exchangeable singlet at δ 8.50 ppm attributed to NH proton.

Scheme 5. Synthetic pathways for compounds 25, 26, 27 and 28. The thienopyridine o-amoester derivative 29 was prepared through the reaction of pyridine thione 1 with ethyl chloroacetate in presence of sodium hydroxide [46] “(Scheme 6).” The amino group in o-amoester derivative 29 was diazotized by nitrososulfuric acid followed by

Reagents: i) NH2(CH2)2NH2/ CS2; ii) HCO(OC2H5)3/gl.AcOH; iii) C6H5-CHO/absolute EtOH; iv) CS2/pyridine.

Reagents: i) C3H5-NCS/ dioxane/ pyridine; ii) HCONH2; iii) NaNO2/ HCl/ 0-5°C; iv) CNCH2CN/ absolute EtOH; v)
the addition of sodium azide solution [47] to yield azide derivative 30. Compound 30 was further reacted with different nitriles namely; malononitrile and 2-(benzo[d]thiazol-2-yl) acetonitrile by refluxing in sodium methoxide [47] to yield pyridotheniotriazolopyrimidinones 31a and 31b, respectively. Moreover, methylthiocarbonothioylamino derivative 32 was prepared in a one pot reaction by treating a vigorously stirred solution of o-amino ester derivative 29 with carbon disulphide and sodium hydroxide solution [48] to yield the sodium salt of dithiocarbamic acid which was not isolated and was further treated with dimethyl sulphate. Compound 32 was further cycled by refluxing with hydrazine hydrate in ethanol [48] to yield 2-thioxopyridothienopyrimidin-4-one 33. The 1H NMR spectrum of compound 32 revealed a singlet at d 2.71 ppm attributed to S-CH3 protons. In addition to a deuterium oxide exchangeable singlet at d 7.32 ppm attributed to NH proton. However, the 1H NMR spectrum of compound 33 revealed two deuterium oxide exchangeable signlets at d 4.30 and d 6.61 ppm attributed to NH2 and pyrimidine NH protons; respectively.

Furthermore, treatment of o-aminoester derivative 29 with triethyl orthoformate in presence of sodium azide [49] afforded the 3-tetrazolylthienopyridine-2-carboxylate 34 which was further subjected to hydrazinolysis to give 2,3-diaminopyridothienopyrimidinone 35. The 1H NMR spectrum of compound 35 revealed two deuterium oxide exchangeable signlets at d 4.10 and 6.80 ppm attributed to pyrimidine-C2-NH2 and pyrimidine-N1-NH2 protons; respectively.

The 3-carbohydrazide derivative 36 was prepared by treatment of o-aminoester 29 with hydrazine hydrate [46]. “(Scheme 7)”. Compound 36 was further refluxed with triethyl orthoformate in presence acetic anhydride and with acetic anhydride only [50] to yield the formimidate derivative 37 and the acetamide derivative 38; respectively. The 1H NMR spectrum of compound 37 displayed two singlets at d 7.87 ppm and d 8.36 ppm due to N=CH proton and pyrimidine C4 proton; respectively.

Furthermore, the reaction of acid hydrazide derivatives with ethyl acetoacetate were reported to yield either pyrazolone derivatives [50,51] or the open chain imine derivative [50]. However, compound 36 upon reaction with ethyl acetoacetate in presence of sodium ethoxide [50] yielded the pyrazolone derivative 39 which its 1H NMR spectrum revealed a singlet at d 2.26 ppm integrated for five protons corresponding to pyrazole-C2-CH3 and pyrazole-CH3 protons. It also showed a deuterium oxide exchangeable singlet at d 16.53 ppm attributed to H-bonded NH3 protons.

**Biological evaluation**

The synthesized compounds were screened for their in vitro cytotoxic activity against human hepatocellular liver carcinoma (HepG2). (Table 1) and human breast cancer cell line (MCF-7) (Table 2). Doxorubicin was used as the reference drug.

The pyrido[3′,2′:4,5]thieno[3,2-b]pyridine derivative 5 exhibited highly potent anticancer activity against both HepG2 (Figure 1) and MCF-7 cell lines (Figure 2) showing IC50 values 10.33 and 9.70 µM/L; respectively which represents comparable activity to the reference drug doxorubicin (IC50 8.555 and 8.90 µM/L). However, replacement of only one methyl group and retaining the 2- methyl function as in compound 7 resulted in decrease in the anticancer activity.
against both cell lines. Also, fusion of benzofuropyridine ring to the thieno[2,3-b]pyridine nucleus as in compound 12 resulted in potent anticancer activity against HepG2 cell line and moderate activity against MCF-7 cell line. It is to be noted that, introduction of a pyrazole ring to the thienopyridine backbone either through an azo junction as in 3,5-diaminopyrazole derivative 23 or a hydrazo function in 3-aminopyrazol-5-one derivative 24 or a carbonyl group as in3-methyl pyrazol-5-one derivative 39 afforded significant activity against both cell lines. Also, the introduction of ethoxymethyleneamino moiety at the N₃ of pyrimidine-4-one ring fused to the thieno[2,3-b]pyridine back bone as in compound 37 also resulted in marked increase in activity against both HepG2 and MCF-7 cell lines.

Table 1. Six dose growth inhibition percent and IC₅₀ values of the tested compounds against HepG2 cell line.

| Sample concentration (µg/mL) | Growth inhibition % | IC₅₀ (µM/L) |
|-----------------------------|---------------------|------------|
| Compound No. | 50 | 25 | 12.5 | 6.25 | 3.13 | 1.6 |
| 3 | 77.17 | 62.86 | 35.25 | 18.74 | 7.68 | 1.82 | 57.93 |
| 4 | 79.09 | 67.37 | 48.13 | 21.84 | 15.95 | 6.38 | 39.66 |
| 5 | 93.09 | 89.17 | 84.36 | 70.02 | 48.68 | 42.3 | 10.33 |
| 6 | 63.74 | 40.14 | 24.86 | 10.74 | 3.82 | 0.00 | >100 |
| 7 | 86.58 | 72.59 | 57.42 | 39.76 | 26.53 | 13.95 | 29.95 |
| 8 | 69.47 | 53.28 | 37.31 | 24.62 | 10.81 | 2.18 | 62.50 |
| 9 | 40.68 | 29.17 | 25.31 | 17.46 | 8.24 | 2.78 | >100 |
| 10 | 31.07 | 20.82 | 13.58 | 4.7 | 1.44 | 0.00 | >100 |
| 11 | 89.17 | 80.24 | 67.55 | 56.48 | 31.86 | 9.63 | 30.99 |
| 12 | 91.06 | 80.13 | 61.35 | 46.88 | 29.52 | 12.94 | 19.92 |
| 13 | 28.14 | 16.72 | 8.83 | 2.78 | 0.00 | 0.00 | >100 |
| 14b | 61.82 | 52.83 | 30.26 | 12.84 | 7.04 | 1.27 | 69.62 |
| 15 | 86.28 | 68.58 | 27.62 | 15.35 | 8.94 | 2.76 | 60.04 |
| 16 | 78.64 | 53.18 | 32.61 | 21.06 | 10.28 | 2.47 | 75.15 |
| 17 | 21.47 | 7.82 | 3.26 | 1.08 | 0.00 | 0.00 | >100 |
| 18 | 57.25 | 39.18 | 21.09 | 10.96 | 3.87 | 1.41 | 99.87 |
| 19 | 46.53 | 30.82 | 16.58 | 5.74 | 1.96 | 0.00 | >100 |
| 20 | 79.84 | 52.77 | 34.59 | 23.21 | 14.54 | 7.49 | 59.31 |
| 21 | 88.62 | 80.54 | 56.17 | 37.84 | 19.07 | 8.58 | 27.77 |
| 22 | 89.59 | 86.21 | 74.38 | 35.5 | 18.38 | 6.86 | 22.90 |
| 23 | 84.37 | 46.83 | 30.54 | 18.35 | 10.28 | 3.46 | 87.87 |
| 24 | 87.88 | 47.72 | 30.13 | 17.27 | 10.04 | 5.87 | 82.91 |
| 25 | 67.48 | 38.14 | 26.11 | 15.19 | 6.03 | 2.82 | 88.52 |
| 26 | 84.15 | 75.07 | 50.41 | 26.76 | 12.37 | 5.26 | 35.38 |
| 27 | 56.72 | 38.04 | 19.27 | 7.66 | 2.42 | 0.00 | >100 |
| 28 | 54.38 | 39.25 | 18.57 | 6.74 | 1.87 | 0.00 | >100 |
| 29 | 58.97 | 27.08 | 16.86 | 8.25 | 3.17 | 0.00 | 92.16 |
| 30 | 26.16 | 10.82 | 5.18 | 1.87 | 0.00 | 0.00 | >100 |
| 31b | 80.28 | 61.81 | 26.76 | 14.64 | 7.43 | 1.92 | 56.92 |
| 32 | 68.67 | 51.86 | 30.4 | 16.68 | 7.92 | 2.77 | 73.90 |
| 33 | 86.21 | 71.49 | 36.76 | 21.83 | 10.62 | 5.44 | 57.98 |
| 34 | 79.32 | 67.24 | 52.03 | 31.88 | 20.77 | 13.42 | 33.47 |
| 35 | 77.83 | 63.28 | 46.42 | 27.82 | 12.78 | 5.07 | 41.71 |
| 36 | 87.92 | 78.32 | 39.07 | 20.86 | 10.47 | 1.53 | 43.90 |
| 37 | 89.05 | 85.71 | 83.10 | 78.68 | 69.68 | 51.75 | 8.55 |

Doxorubicin
### Table 2. Six dose growth inhibition percent and IC\textsubscript{50} values of the tested compounds against MCF-7 cell line.

| Compound No. | Growth inhibition % | IC\textsubscript{50} (µM/L) |
|--------------|----------------------|-----------------------------|
| Sample concentration (µg/mL) | 50 25 12.5 6.25 3.13 1.6 |
| 3 | 85.54 73.18 54.26 27.02 10.87 3.74 | 34.69 |
| 4 | 67.38 38.26 26.03 14.62 7.83 1.94 | >100 |
| 5 | 91.22 85.08 74.66 61.22 50.18 36.44 | 9.70 |
| 6 | 60.04 42.38 16.04 28.62 5.26 1.04 | >100 |
| 7 | 87.22 78.64 61.46 26.68 12.44 3.25 | 31.47 |
| 8 | 54.61 32.18 20.62 12.46 3.82 0.00 | >100 |
| 9 | 25.74 10.57 3.72 0.00 0.00 0.00 | >100 |
| 10 | 41.38 20.55 7.68 1.87 0.00 0.00 | >100 |
| 11 | 86.24 61.42 37.81 21.73 9.52 2.84 | 54.24 |
| 12 | 87.25 73.79 38.94 16.66 7.27 1.44 | 43.25 |
| 13 | 35.49 10.28 3.85 0.00 0.00 0.00 | >100 |
| 14b | 57.16 21.27 7.82 3.85 0.00 0.00 | >100 |
| 15 | 56.36 21.08 10.82 4.57 1.28 0.00 | >100 |
| 16 | 84.64 68.31 45.47 27.74 14.81 7.57 | 48.80 |
| 17 | 26.06 13.82 5.28 1.94 0.00 0.00 | >100 |
| 18 | 85.72 52.64 27.08 16.26 7.82 2.04 | 59.17 |
| 21 | 52.47 36.16 28.91 13.66 4.15 1.78 | >100 |
| 22 | 71.62 46.91 17.13 8.71 2.59 0.88 | 72.15 |
| 23 | 91.66 85.22 80.54 52.98 39.41 25.87 | 14.95 |
| 24 | 89.11 81.38 60.26 34.42 23.57 14.81 | 26.63 |
| 25 | 63.73 32.15 17.82 8.22 3.11 0.00 | >100 |
| 26 | 70.34 36.81 17.26 8.18 3.22 0.00 | >100 |
| 27 | 56.40 19.09 7.24 1.96 0.00 0.00 | >100 |
| 28 | 82.62 63.16 34.94 20.65 12.02 4.83 | 54.78 |
| 30 | 51.71 26.82 9.24 2.78 0.00 0.00 | >100 |
| 31a | 41.28 23.44 14.07 7.22 1.94 0.00 | >100 |
| 31b | 53.46 26.84 9.58 1.89 0.00 0.00 | >100 |
| 32 | 61.37 38.65 20.28 10.96 4.15 1.26 | 93.15 |
| 34 | 67.14 29.62 12.85 6.28 1.53 0.00 | >100 |
| 35 | 40.84 31.62 23.49 16.46 9.59 2.18 | >100 |
| 36 | 74.62 48.24 27.66 10.88 3.62 0.00 | 89.48 |
| 37 | 78.93 64.36 48.62 32.04 16.93 7.84 | 37.31 |
| 38 | 53.28 26.17 7.86 3.22 0.00 0.00 | >100 |
| 39 | 83.68 72.53 41.74 26.28 13.46 7.82 | 43.63 |
| Doxorubicin | 90.76 88.45 84.26 77.78 70.82 55.16 | 8.90 |
Experimental Chemistry

All melting points were measured on Electro thermal LA 9000 SERIS, Digital Melting point Apparatus and are uncorrected. IR spectra (KBr) were recorded on FT-IR 200 spectrophotometer (υ cm⁻¹), pharmaceutical analytical unit, Faculty of Pharmacy, Al-Azhar University. ¹H-NMR spectra were recorded in (DMSO-d₆) at 300 MHz on a Varian Gemini NMR spectrometer (δ, ppm) using TMS as an internal standard, Research Service Unit, Faculty of Science, Cairo University. Mass spectra were recorded on GC Ms-QP 5050A mass spectrometer at 70 eV and microanalytical data were performed in Regional center for Mycology and Biotechnology, Al-Azhar University. Thin layer chromatography was performed on precoated (0.25mm) silica gel GF₂₅₄ plates (E. Merck, Germany). Compounds were detected with 254 nm UV lamp.

General procedure for the synthesis of compounds 3, 4 and 5

An equimolar mixture of compound 2 (2.65 g, 10 mmol) and cyclic/acyclic ketones (10 mmol), in presence of anhydrous ZnCl₂ (0.68g, 5 mmol) was fused at 120–130°C for 5h. The reaction mixture was allowed to cool, triturated with H₂O and neutralized with sodium hydroxide 10%. The separated solid was filtered off, washed with water, left to dry then recrystallized from ethanol.

9-Amino-4-methyl-2-phenyl-7,8-dihydro-6H-cyclopenta [b]pyrido[3’,2’:4,5]thieno[2,3-e]pyridine; 3
Brown powder; yield 1.70 g (51%); m.p.: >360°C. Anal. Calcd. (%): C, 72.48; H, 5.17; N, 12.68. Found (%): C, 72.53; H, 5.28, N, 12.74. IR (KBr, cm⁻¹): 3443, 3226 (NH₂); 3090 (CH-aromatic); 2930 (CH-aliph.); 1644 (C=N); 1563 (C=C).
¹H NMR (DMSO-d₆, δ ppm): 2.60-2.80 (m, 6H, cyclopentyl-C-6,7,8-H); 3.02 (s, 3H, CH₃); 5.40 (s, 2H, NH₂, D₂O exchangeable); 7.30-7.65 (m, 3H, C₆H₅-C₃,4,5-H); 7.95 (s, 1H, C₃-H ); 8.10-8.15 (m, 2H, C₆H₅- C₂,6-H). Mass spectrum, m/z (%): 329 (M⁺-2, 0.03), 55(100.00).

10-Amino-4-methyl-2-phenyl-6,7,8,9-tetra-hydropyrido [3’,2’:4,5]thieno[3,2-b]-quinoline; 4
Buff crystals; yield 2.76 g (80%); m.p.: 220–222°C. Anal. Calcd. (%): C, 73.01; H, 5.54; N, 12.16. Found (%): C, 73.04; H, 5.52, N, 12.22. IR (KBr, cm⁻¹): 3450, 3300 (NH₂); 3075 (CH-aromatic); 2922, 2855 (CH-aliph.); 1647 (C=N); 1570 (C=C). MS, m/z (%): 345 (M⁺, 3.47), 80 (100.00).

4-Amino-2,3,9-trimethyl-7-phenylpyrido[3’,2’:4,5]thieno[3,2-b]pyridine; 5
Brown powder; yield 1.75 g (55%); m.p.: >360°C. Anal. Calcd. (%): C, 71.44; H, 5.36. Found (%): C, 71.46; H, 5.39. IR (KBr, cm⁻¹): 3290, 3175 (NH₂); 3042 (CH-aromatic); 2961, 2855 (CH-aliph.); 1647 (C=N); 1570 (C=C). MS, m/z (%): 319 (M⁺, 2.13), 57 (100.00).

Synthesis of 2,4-diamino-9-methyl-7-phenyl-pyrido[3’,2’:4,5]thieno[3,2-b]pyridine; 6
Compound 2 (2.65 g, 10 mmol) and an equimolar amount of malononitrile (0.66 g, 10 mmol) were refluxed in absolute ethanol (30 mL) containing 5 drops of triethylamine for 9 h. The reaction mixture was allowed to cool and the obtained solid was filtered off, washed with ethanol and recrystallized from DMF/ethanol.
Brown powder; yield 2.76 g (83%); m.p.: >360°C. Anal. Calcd. (%): C, 65.24; H, 3.95; N, 21.13. Found (%): C, 65.27; H, 3.96; N, 21.21. IR (KBr, cm⁻¹): 3407, 3175 (NH₂); 3042 (CH-aromatic); 2961, 2922 (CH-aliph.); 1647 (C=N); 1570 (C=C). MS, m/z (%): 319 (M⁺, 2.13), 57 (100.00).
2 (1.33 g, 5 mmol) in dry DMF (20 mL) was added dropwise while stirring and reflux was continued for another 15 h. The reaction mixture was allowed to cool, then poured onto crushed ice and the product was filtered off, washed with water and recrystallized from ethanol.

Brown powder; yield 1.87 g (86%); m.p.: > 360 °C. Anal. Calcd. (%) for C₉H₇N₂O₅S (431.55): C, 65.60; H, 3.34; N, 16.95. Found (%): C, 65.24; H, 3.49; N, 16.30.

Synthesis of 4-amino-9-methyl-7-phenyl-2-thioxo-1,2-dihydropyrido [3’,2’:4,5]thieno[3,2-b]pyridine-3-carbonitrile; 11

Amixture of compound 2 (2.65 g, 10 mmol) and cyanothioacetamide (1 g, 10 mmol) was refluxed for 8 h in absolute ethanol (30 mL) containing 3 drops of piperidine. The reaction mixture was allowed to cool, then poured onto iced-cold water, and neutralized with hydrochloric acid (10%). The separated solid was filtered and recrystallized from ethanol.

Black powder; yield 1.91 g (55%); m.p.: > 360 °C. Anal. Calcd. (%) for C₁₉H₁₃N₅O₅S (409.50): C, 73.50; H, 4.19; N, 15.31. Found (%): C, 73.48; H, 4.24; N, 15.52. IR (KBr, cm⁻¹): 3323, 3240 (NH₄); 3000 (CH-aromatic); 2929 (CH-aliph.-); 2212 (C≡N); 1650 (C=N); 1567 (C=C). MS, m/z (%): 456 (M⁺−1, 0.38), 78 (100.00).

Synthesis of 11-amino-4-methyl-2-phenyl[1] benzofuro[2,3-b]pyrido-[3’,2’:4,5]thieno[2,3-e]pyridine; 12

An equimolar mixture of compound 2 (2.65 g, 10 mmol) and benzo furan-2(3H)-one (1.34 g, 10 mmol) was refluxed for 14 h in dioxane (30 mL) containing a catalytic amount of triethyl amine (3 drops). The reaction mixture was allowed to cool then poured onto crushed ice to yield a solid product which was filtered and recrystallized from ethanol.

Dark yellow crystals; yield 2.54 g (67%); m.p.: 50-52 °C. Anal. Calcd. (%) for C₂₃H₁₉N₅O₅S (381.45): C, 72.42; H, 3.98; N, 11.02. Found (%): C, 72.47; H, 3.98; N, 11.13. IR (KBr, cm⁻¹): 3326, 3192 (NH); 3057 (CH-aromatic); 2925 (CH-aliph.-); 1662 (C≡N); 1590 (C=C); 1286, 1051 (C-O-C). 1H NMR (DMSO-d₆, δppm): 8.34 (s, 3H, CH₃); 4.50 (s, 2H, NH₂ D₂O exchangeable); 7.00 (s, 1H, NH D₂O exchangeable); 7.40-7.60 (m, 3H, C₆H₅-C₆-H); 7.78 (s, 1H, C=H); 8.15 (d, 1H, J= 8.4Hz, C₆H₅-C₆-H); 8.24 (d, 1H, J= 8.1Hz, C₆H₅-C₆-H). MS, m/z (%): 348 (M⁺, 0.82), 53 (100.00).
from ethanol.

Brown powder; Yield 2.39 g (85%); m.p.: > 360°C. Anal. Calcd. (%): C, 65.38; H, 4.62; N, 14.83. Found (%): C, 63.60; H, 4.65; N, 14.90. IR (KBr, cm⁻¹): 3327, 3185 (NH); 1700 (C=O); 1564 (C=N). 1H NMR (DMSO-d6, δ ppm): 1.70 (s, 3H, CH3); 3.07 (s, 2H, CH2Cl); 7.40–7.70 (m, 4H, C6H5-C3,4,5-H); 7.95 (d, 2H, J= 8Hz, C2-C6H5-C2,6-H); 8.02 (s, 1H, C8-H); 8.21–8.24 (m, 2H, C6H5-C2,6-H); 8.38 (s, 1H, C2-H); 12.80 (s, 1H, OH, D2O exchangeable). MS, m/z (%): 283 (M⁺, 94.44), 140 (100.00).

General procedure for the synthesis of compounds 14a,b

A mixture of compound 13 (2.65 g, 10 mmol) and the appropriate acid chloride (10 mmol) namely; benzoyl chloride (1.26 g, 10 mmol) and the obtained product was filtered off, washed with water and recrystallized from ethanol.

9-Methyl-2,7-diphenylpyrido[3',2':4,5]pyrimidin-4(3H)-one; 14a

Brown powder; Yield 2.43 g (72%); m.p.: 115-117°C. Anal. Calcd. (%): C, 71.51; H, 4.13; N, 14.42. Found (%): C, 71.52; H, 4.09; N, 14.37. IR (KBr, cm⁻¹): 3425 (OH tautomer); 3250 (NH); 3080 (CH-aromatic); 2921 (CH-aliph.-). 1H NMR (DMSO-d6, δ ppm): 1.70 (s, 3H, CH3); 3.01 (s, 3H, CH3); 7.10 (s, 1H, C3-N=CH); 7.25 (s, 1H, C3-N=CH); 7.40–7.60 (m, 3H, two C6H5-C3,4,5-H); 7.77 (s, 1H, C1-H); 7.90–8.24 (m, 2H, C6H5-C2,6-H). MS, m/z (%): 321 (M⁺, 0.25), 319 (M⁺–2, 0.25), 67 (100.00).

Synthesis of 3-amino-4-imino-9-methyl-7-phenyl-3,4-dihydropyrido[3',2':4,5]thieno[3,2-d]pyrimidine; 16

A mixture of compound 15 (3.21 g, 10 mmol) and excess hydrazine hydrate 99% (10 mL) was refluxed for 8 h. The reaction mixture was allowed to cool, and then triturated with ethanol. The separated solid was filtered and recrystallized from ethanol.

Brown powder; yield 2.05 g (67%); m.p.: 320-322°C. Anal. Calcd. (%): C, 65.51; H, 3.82; N, 14.39. Found (%): C, 65.53; H, 3.82; N, 14.39. IR (KBr, cm⁻¹): 3416 (OH tautomer); 3200 (NH); 3061 (CH-aromatic); 2921 (CH-aliph.-). 1H NMR (DMSO-d6, δ ppm): 2.97 (s, 3H, CH3); 7.48–7.62 (m, 6H, two C6H5-C3,4,5-H); 7.95 (d, 2H, J= 8Hz, C2-C6H5-C2,6-H); 8.00 (s, 1H, C1-H); 8.10–8.30 (m, 2H, C6H5-C2,6-H); 12.60 (s, 1H, OH, D2O exchangeable). MS, m/z (%): 308 (M⁺+1, 66.74), 307 (M⁺, 0.30), 306 (M⁺–1, 0.11), 53 (100.00).

Synthesis of 9-methyl-7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4(3H)-one; 17

Compound 2 (2.65 g, 10 mmol) was refluxed in excess formic acid (10 mL) for 16 h. The reaction mixture was allowed to cool, and then triturated with ethanol. The separated solid was filtered and recrystallized from ethanol.

Orange powder; yield 2.44 g (83%); m.p.: 270°C. Anal. Calcd. (%): C, 65.53; H, 3.78; N, 14.32. Found (%): C, 65.51; H, 3.78; N, 14.39. IR (KBr, cm⁻¹): 3416 (OH tautomer); 3200 (NH); 3061 (CH-aromatic); 2921 (CH-aliph.-). 1H NMR (DMSO-d6, δ ppm): 2.97 (s, 3H, CH3); 7.40–7.60 (m, 6H, C6H5-C3,4,5-H & C2-H); 8.00–8.20 (m, 2H, C6H5-C2,6-H); 8.38 (s, 1H, C1-H); 12.80 (s, 1H, OH, D2O exchangeable). MS, m/z (%): 293 (M⁺, 6.98), 61 (100.00).

Synthesis of 4-imino-9-methyl-3,7-diphenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidine-2(1H)-thione; 18

An equimolar mixture of compound 2 (2.65 g, 10 mmol) and phenyl isothiocyanate (1.35 g, 10 mmol) was refluxed in dry dioxane (20 mL) containing 1mL of dry pyridine for 10 h. The reaction was allowed to cool to room temperature, then poured onto crushed ice, neutralized with hydrochloric acid (10%) and the obtained product was filtered off, washed with water and left to dry. The product was recrystallized from ethanol.

Buff crystals; yield 2.49 g (62%); m.p.: 125-127°C. Anal. Calcd. (%): C, 63.94; H, 3.56; N, 13.99. Found (%): C, 66.01; H, 4.07; N, 14.12. IR (KBr, cm⁻¹): 3427, 3207 (NH); 3026 (CH-aromatic); 2921 (CH-aliph.-). 1H NMR (DMSO-d6, δ ppm): 3.00 (s, 3H, CH3); 7.10 (s, 1H, NH, D2O exchangeable); 7.12–7.15 (m, 6H, two C6H5-C2,6-H).
Synthesis of 4-amino-9-methyl-7-phenylpyrido[3',2':4,5]thieno[3,2-d] pyrimidine; 19
An equimolar mixture of compound 2 (2.65 g, 10 mmol) and formamide (0.45 g, 1.1 mL, 10 mmol) was fused for 10 h at 170–180°C. The reaction mixture was allowed to cool, then triturated by methanol and stirred at room temperature for 30 min. The precipitated solid was filtered, washed with cold methanol, and recrystallized from ethanol.

Black crystals; yield 1.48 g (51%); m.p.: >360°C. Anal. Calcd. (%) for C16H13N5S (292.36): C, 65.73; H, 4.13; N, 19.27. Found: C, 65.77; H, 4.13; N, 19.27. IR (KBr, cm⁻¹): 3250 (NH); 3002 (NH₂); 2926 (CH-aromatic); 2850 (CH-aliph.); 2214 (C=N); 1710 (C=O); 1646 (C=N); 1554 (C=C). 1H NMR (DMSO-d₆, δ ppm): 0.86 (s, 1H, C₂-H); 3.68 (q, 2H, J=7.2 Hz, CH₂); 5.71-5.79 (m, 3H, C₂H₅-C₃H₄ -H & NH₂); 8.00 (s, 1H, C₄-H); 8.02-8.27 (m, 2H, C₂H₅-C₂H₅-H); 8.28 (s, 1H, NH, D₂O exchangeable). MS, m/z (%): 292 (M⁺+, 10.67), 72 (100.00).

Synthesis of 2-cyano-4-methyl-6-phenylthieno[2,3-b] pyridine-3-diazonium chloride; 20
A suspension of compound 2 (2.65 g, 10 mmol) in concentrated hydrochloric acid (3 mL) was cooled to 0-5°C in an ice bath to which an ice cold solution of sodium nitrite (1.5 g, 20 mmol in 10 mL water) was added dropwise while cooling over a period of 15 minutes. The reaction mixture was then stirred for 30 minutes to yield crystals of the diazonium product 20, which was filtered and used as such in next step.

General procedure for the synthesis of compounds 21 & 22
To an ice-cold mixture of active methylene compounds namely; malonitrile and ethyl cyanoacetate (10 mmol) and anhydrous sodium acetate (4 g, 50 mmol) in absolute ethanol (50 mL), an ice cold solution of compound 20 (3.12 g, 10 mmol) in absolute ethanol (10 mL) was added dropwise over a period 15 minutes while stirring and cooling in an ice bath. Stirring was then continued for 24 h at room temperature. The reaction mixture was then filtered and the obtained product was washed with ethanol.

[(2-Cyano-4-methyl-6-phenylthieno[2,3-b]pyridin-3-yl) hydrazono]-malononitrile; 21
Brown crystals; yield 2.93 g (86%); m.p.: > 360°C. Anal. Calcd. (%) for C₁₉H₁₄N₅O₂S (342.38): C, 63.14; H, 2.94; N, 24.55. Found: C, 63.18; H, 2.95; N, 24.63. IR (KBr, cm⁻¹): 3270 (NH); 3000 (CH-aromatic); 2926 (CH-aliph.); 2203 (C≡N); 1642 (C≡N); 1566 (C≡C). 1H NMR (DMSO-d₆, δ ppm): 3.03 (s, 3H, CH₃); 7.50-7.54 (m, 3H, C₂H₅-C₃H₄ -H); 8.00 (s, 1H, NH, D₂O exchangeable). MS, m/z (%): 343 (M⁺+, 1.47), 342 (M⁺, 2.94), 60 (100.00).

Ethyl 2-(2-cyano-4-methyl-6-phenylthieno[2,3-b]pyridin-3-yl)hydrazonato-2-cyanoacetate; 22
Brown crystals; yield 2.68 g (69%); m.p.: 50-52°C. Anal. Calcd. (%) for C₁₉H₁₄N₅O₃S (389.43): C, 61.68; H, 3.36; N, 17.98. Found: C, 61.70; H, 3.63; N, 17.92. IR (KBr, cm⁻¹): 3250 (NH); 3002 (NH₂); 2926 (CH-aliph.); 2850 (CH-aliph.); 2214 (C≡N); 1710 (C=O); 1646 (C≡N); 1554 (C≡C). 1H NMR (DMSO-d₆, δ ppm): 1.21 (t, 3H, J=7.2 Hz, CH₂); 3.08 (s, 3H, CH₃); 3.68 (q, 2H, J=7.2 Hz, CH₂); 7.51-7.59 (m, 3H, C₂H₅-C₃H₄ -H & NH₂); 8.00 (s, 1H, C₄-H); 8.20-8.27 (m, 2H, C₂H₅-C₂H₅-H); 8.28 (s, 1H, NH, D₂O exchangeable). MS, m/z (%): 390 (M⁺+, 1.00), 60 (100.00).

Synthesis of 3-(3,5-diamino-1H-pyrazol-4-yl)diazonyst-4-methyl-6-phenylthieno[2,3-b]pyridine-2-carbonitrite; 23
A mixture of compound 21 (3.24 g, 10 mmol.) and excess hydrazine hydrate 99% (5 mL) in absolute ethanol (15 mL) was heated under reflux for 5 h. The reaction mixture was concentrated then allowed to cool. The obtained solid was filtered off, dried and recrystallized from ethanol.

Light brown powder; yield 2.13 g (57%); m.p.: 195-197°C. Anal. Calcd. (%) for C₁₆H₁₂N₄S (292.36): C, 65.73; H, 4.14; N, 19.27. Found: C, 65.71; H, 4.14; N, 19.27. IR (KBr, cm⁻¹): 3250 (NH); 3002 (NH₂); 2926 (CH-aromatic); 2850 (CH-aliph.); 2214 (C≡N); 1651 (C≡C); 1565 (C≡C). 1H NMR (DMSO-d₆, δ ppm): 2.90 (s, 3H, CH₃); 6.90 (s, 1H, NH, pyrazole- N, H, D₂O exchangeable); 7.30-7.40 (m, 1H, C₆H₅-C₂-H); 7.41-7.58 (m, 2H, C₂H₅-C₃H₄ -H); 7.95 (s, 1H, C₄-H); 8.10-8.30 (m, 2H, C₂H₅-C₂H₅-H); 9.85 (s, 4H, two NH₂, pyrazole- C₃H₄-NH₂, D₂O exchangeable).

Synthesis of 3-[2-(3-amino-5-oxo-1H-pyrazol-4(5H)-yldiene)hydrazinyl]-4-methyl-6-phenylthieno[2,3-b]pyridine-2-carbonitrite; 24
Compound 22 (3.89 g, 10 mmol.) and excess hydrazine hydrate 99% (5 mL) in absolute ethanol (15 mL) was heated under reflux for 5 h. The reaction mixture was concentrated then allowed to cool. The obtained solid was filtered off, dried and recrystallized from ethanol.

Brown crystals; yield 2.68 g (69%); m.p.: 120-122°C. Anal. Calcd. (%) for C₁₈H₁₃N₇OS (375.42): C, 57.79; H, 3.49; N, 26.12. Found: C, 57.64; H, 2.95; N, 26.29. IR (KBr, cm⁻¹): 3377, 3197 (NH, NH₂); 2928 (CH-aliph.); 2209 (C≡N); 1680 (C≡O); 1646 (C≡N); 1533 (C≡C). 1H NMR (DMSO-d₆, δ ppm): 2.79 (s, 3H, CH₃); 7.00 (s, 1H, pyrazole-NH, D₂O exchangeable); 7.22 (s, 2H, pyrazole-C₆H₅-NH₂, D₂O exchangeable); 7.51-7.59 (m, 3H, C₂H₅-C₃H₄ -H); 7.82 (s, 1H, C₄-H); 8.10-8.28 (m, 2H, C₂H₅-C₂H₅-H); 8.60 (1H, C₁-NH₂, D₂O exchangeable).

Synthesis of 3-(4,5-dihydro-1H-imidazol-2-yl)-4-methyl-6-phenylthieno[2,3-b]pyridine; 25
To a suspension of compound 2 (2.65 g, 10 mmol) in ethylene diamine (2.7 g, 3 mL, 40 mmol), carbon disulphide (1 mL) was added dropwise and the reaction mixture was heated on a water bath for 8 h. The reaction mixture was allowed to cool, and then triturated by ethanol. The obtained solid was filtered off, left to dry and recrystallized from ethanol.

Golden yellow crystals; yield 2.86g (94%); m.p.: 169-171°C. Anal. Calcd. (%) for C₁₈H₁₃N₅O₂ (308.40): C, 66.21; H, 5.23; N,
Synthesis of 7-methyl-9-phenyl-2,3-dihydroimidazo[1,2-c]pyrido[3',2':4,5]thieno[2,3-e]pyrimidine; 26
A mixture of compound 25 (0.34 g, 1.1 mmol), triethyl orthoformate (4.44 g, 5 mL, 30 mmol) and a catalytic amount of glacial acetic acid (0.2 mL) was heated under reflux for 8 h. The reaction mixture was allowed to cool and the precipitated solid was filtered off, left to dry and recrystallized from ethanol.

Buff crystals; yield 0.30 g (86%); m.p.: 165-167°C. Anal. Calcd. (%) for C_{31}H_{28}N_{4}S_{2} (350.46): C, 61.73; H, 4.02; N, 16.14. IR (KBr, cm⁻¹): 3320, 3246 (NH); 3000 (CH-aromatic); 2923, 2856 (CH-aliph.); 2128 (N₃); 1680 (C=O); 1624 (C=N); 1233, 1010 (C-O-C).

Found (%) for C_{31}H_{28}N_{4}S_{2}: C, 61.69; H, 4.03; N, 15.99. 1H NMR (DMSO-d₆, δ ppm): 3.09 (3H, CH₃); 6.94-7.20 (m, 3H, C₆H₅-C₃,4,5-H); 7.69 (d, 2H, J= 8.4Hz, C₅-C₆H₅-C₂,6-H); 7.89-7.95 (m, 4H, CH₃-C₅H₅-C₉,10-H); 8.25-8.52 (m, 2H, C₅H₅-C₉,10-H); 8.71 (s, 1H, C₅H₅); 9.65 (s, 1H, NH, D₂O exchangeable). MS, m/z (%): 394 (M⁺, 2.08), 75 (100.00).

Synthesis of 7-methyl-5,9-diphenyl-2,3,5,6-tetrahydroimidazo[1,2-c]-pyrido[3',2':4,5]thieno[2,3-e]pyrimidine; 27
To a mixture of compound 25 (0.17 g, 0.6 mmol) and benzaldehyde (0.16 g, 1.15 mL, 1.5 mmol) in absolute ethanol (5 mL), 0.1 mL concentrated hydrochloric acid was added. The mixture was heated under reflux for 14 h. The reaction mixture was allowed to cool and the precipitated solid was filtered off, left to dry and recrystallized from ethanol.

Pale yellow powder; yield 0.21 g (72%); m.p.: > 360°C. Anal. Calcd. (%) for C_{24}H_{20}N_{4}S (396.51): C, 72.70; H, 5.08; N, 14.13. Found (%): C, 72.72; H, 5.08; N, 14.22. IR (KBr, cm⁻¹): 3247 (NH); 3035, 2886 (CH-aliph.); 1596 (C≡N); 1484 (C≡C); 1327, 1086 (C≡S). 1H NMR (DMSO-d₆, δ ppm): 3.09 (3H, CH₃); 3.56-3.76 (m, 2H, imidazo-C₂-H); 4.10-4.20 (m, 2H, imidazo-C₃-H); 7.50-7.70 (m, 3H, C₆H₅-C₃,4,5-H); 7.90 (s, 1H, C₅H₅); 9.65 (s, 1H, NH, D₂O exchangeable). MS, m/z (%): 394 (M⁺, 2.08), 75 (100.00).

Synthesis of 7-methyl-9-phenyl-2,3,5,6-tetrahydroimidazo[1,2-c]-pyrido[3',2':4,5]thieno[2,3-e]pyrimidine-5-thione; 28
Compound 29 (3.12 g, 10 mmol) was dissolved in a mixture of concentrated sulphuric acid (2.5 mL) and water (7 mL) and cooled to 0-5°C in an ice bath. An aqueous solution of sodium nitrite (0.83 g, 120 mmol in 1 mL water) was added dropwise while stirring and maintaining the temperature below 5°C. Then, a solution of sodium azide (0.65 g, 10 mmol) in water (5 mL) was added dropwise to the reaction mixture while cooling. The reaction was stirred for 24 h at room temperature and the precipitated solid was filtered off, washed with water, left to dry and recrystallized from ethanol.

Light brown crystals; yield 2.56 g (76%); m.p.: > 360°C. Anal. Calcd. (%) for C_{24}H_{20}N_{4}O_{2}S (338.38): C, 60.34; H, 2.83; N, 23.52. Found (%): C, 60.37; H, 4.19; N, 16.69. IR (KBr, cm⁻¹): 3350, 3200 (NH); 2975 (CH-aliph.); 2202 (C=O); 1706 (C=O); 1621 (C≡N); 1233, 1010 (C-O-C).

General procedure for the synthesis of compounds 31a & b
Equimolar amounts of compound 30 (3.38 g, 10 mmol) and appropriate nitrile (10 mmol) namely; malononitrile and 2-(benzo[d][thiazol-2-yl]acetoni-trile were added with vigorous stirring to a solution of sodium methoxide [prepared from 0.3 g sodium and 20 mL methanol]. The reaction mixture was stirred at room temperature for 24 h. Then, poured onto crushed ice. The obtained precipitate was filtered off, washed with water, left to dry and recrystallized from ethanol.
To a vigorously stirred solution of compound 29 (3.12 g, 10 mmol) were heated under reflux in glacial acetic acid (1.6 mL, 26 mmol) and aqueous sodium hydroxide (0.8 g, 20 mmol in 2 mL water) were added simultaneously over a period of 30 min and the reaction was stirred for another 30 min. Dimethyl sulphate (2.5 g, 19 mL, 20 mmol) was then added dropwise to the reaction mixture while stirring which was continued for 24 h. The reaction mixture was poured onto crushed ice and the solid obtained was filtered, washed with water, dried and recrystallized from ethanol.

Orange powder; yield 3.52 g (81%); m.p.: > 360°C. Anal. Calcd. (%) for C16H12N4O2S2 (402.55): C, 56.53; H, 3.56; N, 6.96. Found (%): C, 56.72; H, 4.55; N, 7.08. IR (KBr, cm⁻¹): 3450 (NH); 3073 (CH-aromatic); 2920 (CH-aliph.); 1700 (C=O); 1641 (C=N); 1562 (C=C); 1414 (N=N). MS, m/z (%): 363 (M⁺, 0.07), 71 (100.00).

Synthesis of 2,3-diamino-9-phenyl-3,4-dihydropyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-one; 35

Compound 34 (3.65 g, 10 mmol.) was refluxed in excess hydrazine hydrate 99% (5 mL) for 10 h. The reaction mixture was allowed to cool, poured onto crushed ice and the solid obtained was filtered, washed with ethanol, dried and recrystallized from ethanol.

White crystals; yield 2.81 g (77%); m.p.: 280-282°C. Anal. Calcd. (%) for C16H15N5O2S (365.41): C, 59.16; H, 4.14; N, 19.17. Found (%): C, 59.18; H, 4.21; N, 19.25. IR (KBr, cm⁻¹): 3025 (CH-aromatic); 2857 (CH-aliph.); 1701 (C=O); 1641 (C=N); 1561 (C=C); 1414 (N=N); 1200, 1021 (C-O-C). MS, m/z (%): 368 (M⁺+3, 4.55), 51 (100.00).

Synthesis of ethyl N-(9-methyl-4-oxo-7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4(1H)-yl)formimidate; 36

The carbohydrazide derivative 36 (2.98 g, 10 mmol) was refluxed with double the amounts of triethyl orthoformate (2.9 g, 3 mL, 20 mmol) in acetic anhydride (10 mL) for 10 h. The reaction mixture was allowed to cool and the solid product was washed with ethanol, left to dry and recrystallized from ethanol.

Yellow crystals; yield 2.62 g (72%); m.p.: 108-110°C. Anal. Calcd. (%) for C19H16N5O2S (364.42): C, 62.62; H, 4.43; N, 15.37. Found (%): C, 62.69; H, 4.47; N, 15.43. IR (KBr, cm⁻¹): 3090 (CH-aromatic); 2919, 2852 (CH-aliph.); 1736 (C=O); 1660 (C=N); 1546 (C=C); 1213, 1023 (C-O-C). 'H NMR (DMSO-d₆, δ ppm): 2.71 (s, 3H, CH₃); 4.10 (s, 2H, C₂-NH₂, D₂O exchangeable); 6.80 (s, 2H, N₂-N₂H₂, D₂O exchangeable); 7.41-7.47 (m, 3H, CH₃-H₃-C₅₄₅-H); 7.52 (s, 1H, C₆-H); 8.05 (d, 2H, J=7.2Hz, C₅₄₅-H₂-C₅₄₅-H). MS, m/z (%): 323 (M⁺, 1.31), 57 (100.00).

Synthesis of 3-aminoo-9-methyl-7-phenyl-2-thioxo-2,3-dihydropyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4(1H)-one; 33

A mixture of compound 32 (4.02 g, 10 mmol) and excess hydrazine hydrate 99% (5 mL) in absolute ethanol (15 mL) was refluxed for 12 h until the methyl mercaptan evolution ceased. The reaction mixture was allowed to cool and the obtained solid was filtered off, washed with ethanol, left to dry and recrystallized from ethanol.

Yellow powder; yield 1.47 g (43%); m.p.: > 360°C. Anal. Calcd. (%) for C₁₈H₁₅N₅O₂S (364.42): C, 62.62; H, 4.43; N, 15.37. Found (%): C, 62.69; H, 4.47; N, 15.43. IR (KBr, cm⁻¹): 3090 (CH-aromatic); 2919, 2852 (CH-aliph.); 1736 (C=O); 1660 (C=N); 1546 (C=C); 1213, 1023 (C-O-C). 'H NMR (DMSO-d₆, δ ppm): 2.87 (s, 3H, CH₃); 4.30 (s, 2H, NH₂-D₂O exchangeable); 6.61 (s, 1H, NH-D₂O exchangeable); 7.48-7.54 (m, 3H, CH₃-C₅₄₅-H); 7.81 (s, 1H, C₂-H); 8.15 (d, 2H, J=7.9Hz, C₅₄₅-C₅₁₄-H). MS, m/z (%): 340 (M⁺+1, 0.71), 71 (100.00).

Synthesis of ethyl 4-methyl-6-phenyl-3-(1H-tetrazol-1-yl)thieno[2,3-b]-pyridine-2-carboxylate; 34

Equimolar amounts of compound 29 (3.12 g, 10 mmol), triethyl orthoformate (1.48 g, 1.64 mL, 10 mmol) and sodium azide (0.65 g, 10 mmol) were heated under reflux in glacial acetic acid (40 mL) for 8 h. The reaction mixture was allowed to cool then triturated with concentrated hydrochloric acid (7 mL). The solid obtained was filtered, washed with water, dried and recrystallized from ethanol.

Yellow crystals; yield 2.50 g (69%); m.p.: > 360°C. Anal. Calcd. (%) for C₁₉H₁₆N₄O₂S (364.42): C, 62.62; H, 4.43; N, 15.37.
An equimolar mixture of the carbohydrazide derivative 36 (2.98 g, 10 mmol) and ethyl acetoacetate (1.30 g, 1.3 mL, 10 mmol) in ethanolic sodium ethoxide solution [prepared by dissolving sodium metal (0.12 g, 5 mmol) in absolute ethanol 30 mL], was heated under reflux for 10 h. The reaction mixture was allowed to cool and the obtained product was filtered, washed with ethanol, left to dry and recrystallized from ethanol. Brown needle crystals; yield 2.74 g (75%); m.p.: 85-87°C. Anal. Calcld. (%) for C_{20}H_{17}N_{2}O_{2}S (364.42): C, 62.67; H, 4.45; N, 15.48. Found (%): C, 62.62; H, 4.43; N, 15.37. Synthesis of 1-(3-amino-4-methyl-6-phenylthieno[2,3-b]pyridine-2-carbonyl)-3-methyl-1H-pyrazol-5(4H)-one; 39

Brown needle crystals; yield 2.74 g (75%); m.p.: 85-87°C. Anal. Calcld. (%) for C_{20}H_{17}N_{2}O_{2}S (364.42): C, 62.62; H, 4.43; N, 15.37. Found (%): C, 62.67; H, 4.45; N, 15.48. IR (KBr, cm\(^{-1}\)): 3430, 3350 (NH); 3085 (CH-aromatic); 2927 (CH-aromatic); 1735, 1688 (two C=O); 1641 (C=N); 1553 (C=C).

Mammalian cell lines
MCF-7 cells (human breast cancer cell line) were obtained from VACSERA Tissue culture unit. HepG2 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).

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 50 µg/mL gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO\(_2\) and were subcultured two times a week.

Cytotoxicity evaluation using viability assay
For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1x10\(^4\) cells per well in 100 µL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 hof seeding. Serial two-fold dilutions of the tested chemical compounds were added to confluent cell monolayers dispersed 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% CO\(_2\) for a period of 48 h. Three wells were used for each concentration of the test sample. Control wells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment.

After incubation of the cells for 24 h at 37°C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 & 1.56 µg) were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method. 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 minutes. 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 gentle shaking 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. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated [52,53]. The cytotoxicity of the tested compounds was estimated in terms of percent growth inhibition compared to untreated control cells and their IC\(_{50}\) in µM/L which is the concentration of the compound that inhibits the tumor cell growth by 50%.

Conclusion
Most of the compounds showed better activity against liver cancer HepG2 cell line than breast cancer MCF-7 cell line. However, compounds 4, 5, 7, 11, 12, 23, 24, 28, 37, 38 and 39 showed moderate to strong activity against HepG2 with IC\(_{50}\) values ranging from 10.33-43.90 µM/L. While compounds 3, 5, 7, 12, 16, 23, 24, 37 and 39 were the most potent against MCF-7 cell line exerting IC\(_{50}\) values ranging from 9.70-48.80 µM/L. Which revealed that, compounds 5, 7, 12, 23, 24, 37 and 39 exerted potent anticancer activities against both cell lines from which the pyrido[3’,2’:4,5]thieno[3,2-b]pyridine derivative 5 was the most active compound exerting anticancer activity comparable to the reference drug doxorubicin against both HepG2 and MCF-7 cell lines showing IC\(_{50}\) values 10.33 and 9.70 µM/L; respectively, while doxorubicin exerted its IC\(_{50}\) values at 8.55 and 8.90 µM/L; respectively.

Competing interests
The authors declare that they have no competing interests.

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