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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 IC_{50} 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 IC_{50} values 10.33 and 9.70 µM/L comparable to doxorubicin that exerted IC_{50} values 8.55 and 8.90 µM/L against HepG-2 and MCF-7 cell lines; respectively.

Keywords: Thienopyridine, pyridothienopyridine, pyridothienopyrimidine, 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].

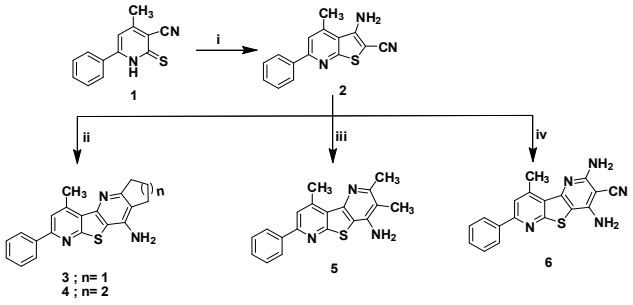
The aforementioned biological activities stimulated our interest for the synthesis of several new condensed heterocyclic compounds containing thieno[2,3-b] pyridine moiety condensed with each of pyridine, cyclopentyl, tetrahydroquinoline, pyrimidine, 1,6-naphthiridin, benzofuro[2,3-b] pyridine, imidazo[1,2-*c*] 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 o-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 o-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 pyridothienopyridine 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 *o*-aminonitrile derivative **2** was refluxed with malononitrile in presence of a catalytic amount of triethylamine [36] to yield pyridothienopyridine-3-carbonitrile derivative **6.**



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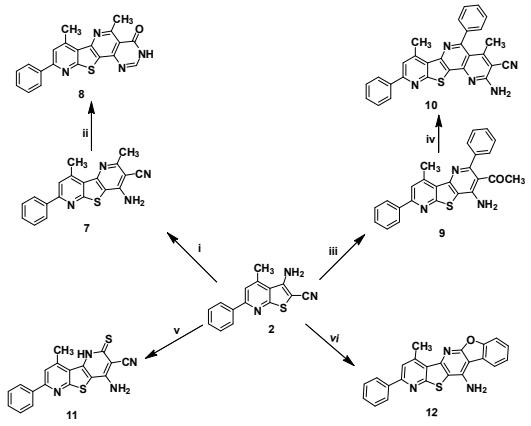
Reagents: i) $ClCH_2CN$ /NaOH / absolute EtOH; ii)  **/ $ZnCl_2$; iii) $CH_3COCH_2CH_3$ / $ZnCl_2$; iv) $CNCH_2CN$/absolute EtOH/ Et_3N.**

Scheme 1. Synthetic pathway for compounds **3**, **4**, **5** and **6.**

The interaction of o-aminonitrile derivative **2** with cyanoacetone in dry DMF [37] gave the corresponding pyridothienopyridine-3-carbonitrile derivative **7** "(Scheme **2**)". Cyclization of compound **7** upon refluxing in formic acid only [38] afforded the pyrimidinone derivative **8.** The **IR** spectrum of compound **8** 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 **2** with benzoyl acetone in presence of sodium ethoxide [39] yielded compound **9** 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 **2** and cyanothioacetamide in ethanol containing a catalytic amount of piperidine [40] afforded pyridothienopyridine-3-carbonitrile derivative **11** which its **^{1}H NMR** spectrum showed two deuterium oxide exchangeable singlets at d 4.50 and 6.90 ppm attributed to NH_2 and NH protons; respectively. When o-aminonitrile derivative **2** was treated with benzofuran-2(3H)-one in dioxane containing a catalylic amount of triethylamine, it furnished a single product identified as compound **12**.



Reagents: i) CH_3COCH_2CN /DMF; ii) HCOOH; iii) C_6H_5-$COCH_2COCH_3$/ NaOEt; iv) $CNCH_2CN$/ absolute EtOH/ piperidine; v) $CNCH_2CSNH_2$/ absolute EtOH/ piperidine; vi)  **/ dioxane/ Et_3N.**

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

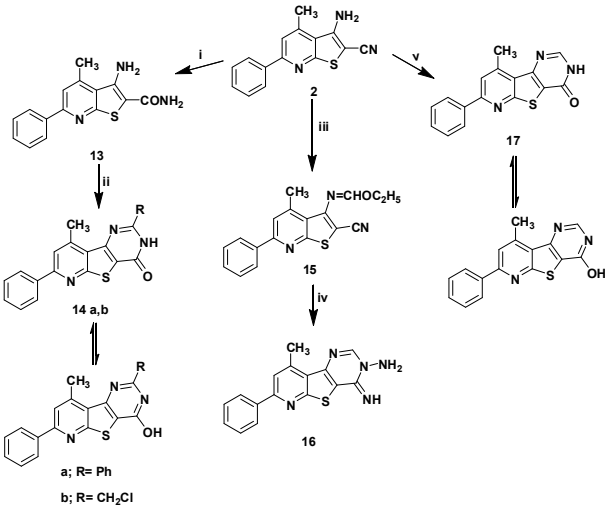
The o-aminonitrile derivative **2** was subjected to partial hydrolysis by stirring with concentrated sulphuric acid [33] to yield 2-carboxamide derivative **13** "(Scheme **3**)". The **^{1}H NMR** spectrum of compound **13** displayed two deuterium oxide exchangeable singlets at d 5.68 and d 6.92 ppm corresponding to NH_2 and $CONH_2$ protons; respectively.

Acid-catalyzed bis-nucleophilic cyclocondensation of o-aminocarboxamide derivative **13** with acid chlorides namely; benzoyl chloride and chloroacetyl chloride in glacial acetic acid [33] furnished pyrimidinone derivatives **14a** and **14b**; respectively. The **^{1}H NMR** spectra of compounds **14a** and **14b** revealed two deuterium oxide exchangeable singlets at d 12.60, 10.50 ppm corresponding to pyrimidine NH protons; respectively. However, **^{1}H NMR** spectrum of compound **14b** showed an additional singlet at d 3.07 ppm integrated for two protons corresponding to CH_2-Cl protons.

The o-aminonitrile derivative **2** 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 **15** 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 **15** upon reaction with excess hydrazine hydrate [42] yielded novel pyridothienopyrimidine derivative **16.** 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 **16**. The **^{1}H NMR** spectrum of compound **16** 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 **2** was refluxed with formic acid to yield pyridothienopyrimidinone derivative **17** 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 pyrimidinone ring [33]. The **^{1}H NMR** spectrum of the compound **17** displayed a deuterium oxide exchangeable singlet corresponding to OH proton at δ 12.80 ppm.



Reagents: i) conc.H_2SO_4 /Stirring at r.t.; ii) RCOCl / CH_3COOH; iii) $HC(OC_2H_5)_3$ / AC_2O; iv) NH_2NH_2; v) HCOOH.

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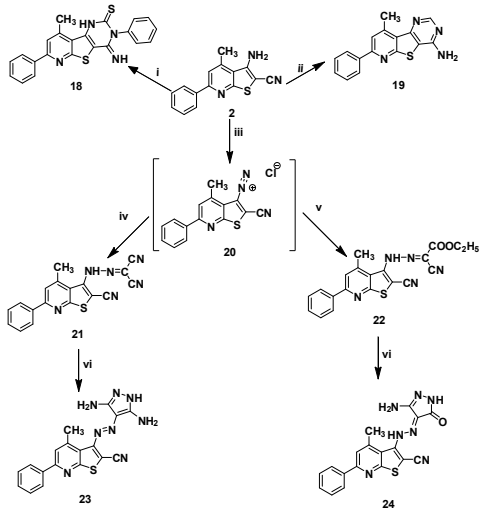
Scheme 3. Synthetic pathways for compounds **13**, **14a,b**, **15**, **16** and **17.**

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 **^{1}H NMR** spectrum of compound **18** showed two deuterium oxide exchangeable singlets at d 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 corressponding 4-aminopyrimidine derivative **19**. The reaction mechanism was reported 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 **^{1}H 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 **^{1}H NMR** spectrum of compound **23** revealed a deuterium oxide exchangeable singlet at δ 9.85 ppm integrated for four protons attributed to two NH_2 protons. The **^{1}H NMR** spectrum of the compound **24** displayed three deuterium oxide exchangeable singlets corresponding to pyrazole-NH, pyrazole-C_3-NH_2 and thiophene-C_3-NH protons at δ 7.00, 7.22 and 8.60 ppm; respectively.



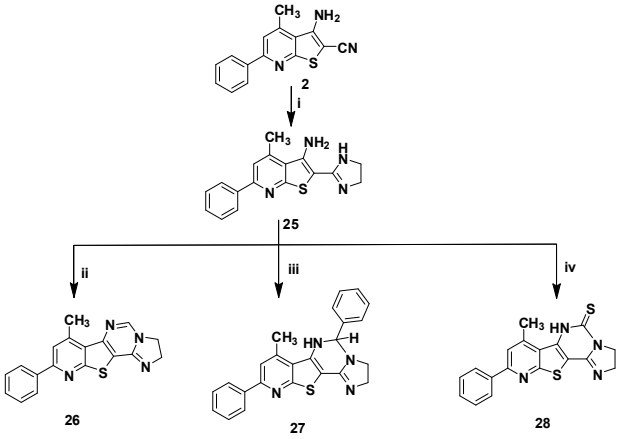
Reagents: i) C_6H_5-NCS/ dioxane/ pyridine; ii) $HCONH_2$; iii) $NaNO_2$/ HCl/ 0-5°C; iv) $CNCH_2CN$/ absolute EtOH; v) $CNCH_2COOC_2H_5$/ absolute EtOH; vi) NH_2NH_2/ absolute EtOH.

Scheme 4. Synthetic pathways for compounds **18**, **19**, **20**, **21**, **22**, **23** and **24.**

Compound **2** was also reacted with ethylene diamine in the presence of carbon carbon disulphide disulfide [45] to afford 2-imidazolylthienopyridine 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-thioxoimidazolidine 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. **^{1}H NMR** spectrum of compound **25** showed two deuterium oxide exchangeable singlets at d 6.90 and 7.20 ppm attributed to NH and NH_2 protons; respectively.

Compound **25** was further subjected to cyclization into tetracyclic 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 in boiling pyridine.

The **^{1}H NMR** spectrum of compound **27** displayed a singlet at d 8.71 ppm corresponding to pyrimidine-C_2 proton besides to a deuterium oxide exchangeable singlet at d 9.65 ppm attributed to NH proton. Furthermore, the **^{1}H NMR** spectrum of compound **28** revealed a deuterium oxide exchangeable singlet at d 9.85 ppm attributed to NH proton.



Reagents: i) $NH_2(CH_2)_2NH_2$/ CS_2; ii) $HC(OC_2H_5)_3$/gl.AcOH; iii) C_6H_5-CHO/absolute EtOH; iv) CS_2/pyridine.

Scheme 5. Synthetic pathways for compounds **25**, **26**, **27** and **28**.

The thienopyridine o-aminoester 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-aminoester derivative **29** was diazotized by nitrososulfuric acid followed by

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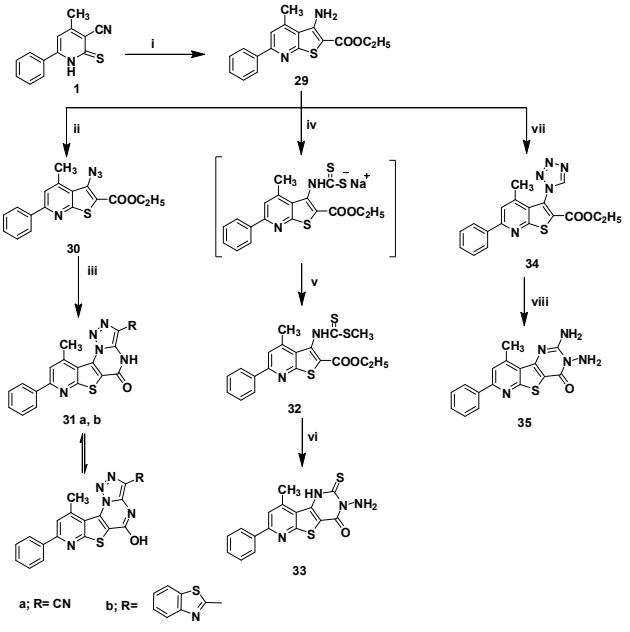
the addition of sodium azide solution [47] to yield azide derivative **30.** Compound **30** was further reacted with different nitriles namely; malononitrile and 2-(benzo[d]thiazol-2-yl) acetonitrile by refluxing in sodium methoxide [47] to yield pyridothienotriazolopyrimidinones **31a** and **31b**; 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 cyclized by refluxing with hydrazine hydrate in ethanol [48] to yield 2-thioxopyridothienopyrimidin-4-one **33**. The **^{1}H NMR** spectrum of compound **32** revealed a singlet at δ 2.71 ppm attributed to S-CH_3 protons. In addition to a deuterium oxide exchangeable singlet at d 7.32 ppm attributed to NH proton. However, the **^{1}H NMR** spectrum of compound **33** revealed two deuterium oxide exchangeable signlets at d 4.30 and d 6.61ppm attributed to NH_2 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 derivative **34** which was further subjected to hydrazinolysis to give 2,3-diaminopyridothienopyrimidinone **35**. The **^{1}H NMR** spectrum of compound **35** revealed two deuterium oxide exchangeable singlets at d 4.10 and 6.80 ppm attributed to pyrimidine-C_2-NH_2 and pyrimidine-N_3-NH_2 protons; respectively.

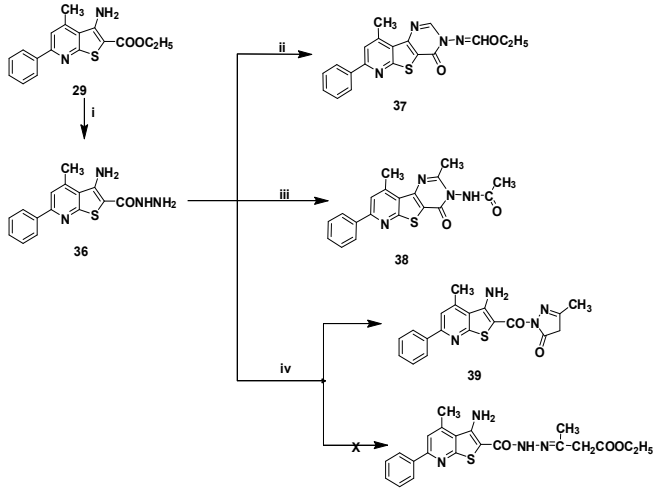


Reagents: i) $ClCH_2COOC_2H_5$/NaOH/EtOH; ii) $NaNO_2$/H_2SO_4/ NaN_3; iii) $CNCH_2R$/NaOMe; iv) CS_2/NaOH/DMSO; v) $(CH_3)_2SO_4$; vi) NH_2NH_2/absolute EtOH; vii) $CH(OC_2H_5)_3$/NaN_3/gl.AcOH; viii) NH_2NH_2.

Scheme 6. Synthetic pathways for compounds **29**, **30**, **31a,b**, **32**,**33**, **34** and **35**.

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 **^{1}H 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 C_2 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 **^{1}H NMR** spectrum revealed a singlet at d 2.26 ppm integrated for five protons corresponding to pyrazole-C_3-CH_3 and pyrazole-CH_2 protons. It also showed a deuterium oxide exchangeable singlet at d 16.53 ppm attributed to H-bonded NH_2 protons.



Reagents: i) NH_2NH_2/absolute EtOH; ii) $HC(C_2H_5)_3$/AC_2O; iii) AC_2O; iv) $CH_3COCH_2COOC_2H_5$/ NaOEt.

Scheme 7. Synthetic pathways for compounds **36**, **37**, **38** and **39**.

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 IC_{50} values 10.33 and 9.70 μM/L; respectively which represents comparable activity to the reference drug doxorubicin (IC_{50} 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

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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 N_3 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 IC_{50} values of the tested compounds against HepG2 cell line.

Sample concentration (µg/mL) Compound No.	Growth inhibition %						IC_{50} (µM/L)
	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
21	46.53	30.82	16.58	5.74	1.96	0.00	>100
22	79.84	52.77	34.59	23.21	14.54	7.49	59.31
23	88.62	80.54	56.17	37.84	19.07	8.58	27.77
24	89.59	86.21	74.38	35.5	18.38	6.86	22.90
25	84.37	46.83	30.54	18.35	10.28	3.46	87.87
26	87.88	47.72	30.13	17.27	10.04	5.87	82.91
27	67.48	38.14	26.11	15.19	6.03	2.82	88.52
28	84.15	75.07	50.41	29.18	12.37	5.26	35.38
30	56.72	38.04	19.27	7.66	2.42	0.00	>100
31a	54.38	39.25	18.57	6.74	1.87	0.00	>100
31b	58.97	27.08	16.86	8.25	3.17	0.00	92.16
32	26.16	10.82	5.18	1.87	0.00	0.00	>100
34	80.28	61.81	26.76	14.64	7.43	1.92	56.92
35	68.67	51.86	30.4	16.68	7.92	2.77	73.90
36	86.21	71.49	36.76	21.83	10.62	5.44	57.98
37	79.32	67.24	52.03	31.88	20.77	13.42	33.47
38	77.83	63.28	46.42	27.82	12.78	5.07	41.71
39	87.92	78.52	39.07	20.86	10.47	1.53	43.90
Doxorubicin	89.05	85.71	83.10	78.68	69.68	51.75	8.55

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Table 2. Six dose growth inhibition percent and IC_{50} values of the tested compounds against MCF-7 cell line.

Sample concentration (µg/mL) Compound No.	Growth inhibition %						IC_{50} (µM/L)
	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

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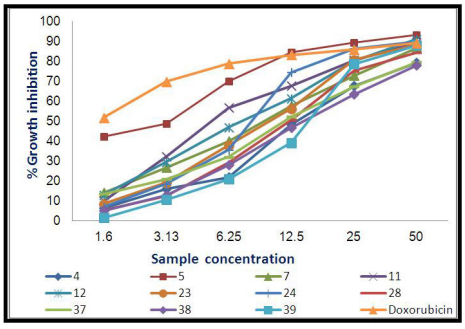

Figure 1. Percent growth inhibition curves of most active compounds and reference drug (doxorubicin) against HepG2 cell line.

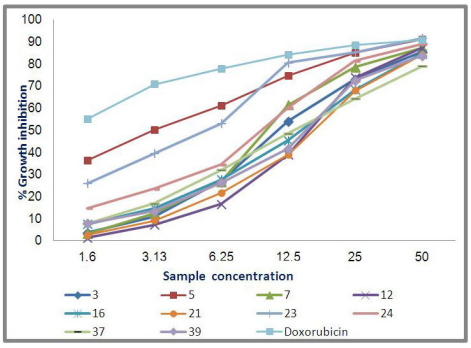

Figure 2. Percent growth inhibition curves of most active compounds and reference drug (doxorubicin) against MCF-7 cell line.

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. ^{1}H-NMR spectra were recorded in (DMSO-d_6) at 300 MHz on a Varian Gemini NMR spectrometer (δ, ppm) using TMS as an internal standard, Resarch 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 GF_{254} 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/alicyclic ketones (10 mmol), in presence of anhydrous $ZnCl_2$ (0.68g, 5 mmol) was fused at 120–130° C for 5h. The reaction mixture was allowed to cool, triturated with H_2O 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.** (%) **for** $C_{20}H_{17}N_3S$ (331.43): 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 (NH_2); 3090 (CH-aromatic); 2930 (CH-aliph.); 1644 (C=N); 1563 (C=C). **^{1}H NMR (DMSO-d_6, δppm):** 2.60-2.80 (m, 6H, cyclopentyl-C-$_{6,7,8}$-H); 3.02 (s, 3H, CH_3); 5.40 (s, 2H, NH_2, D_2O exchangeable); 7.30-7.65 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.95 (s, 1H, C_3-H); 8.10-8.15 (m, 2H, C_6H_5- $C_{2,6}$-H)**. Mass spectrum, m/z (%):** 329 ($M^{+\cdot}$-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.** (%) **for** $C_{21}H_{19}N_3S$ (345.46): C, 73.01; H, 5.54; N, 12.16. **Found** (%): C, 73.04; H, 5.52; N, 12.22. **IR (KBr, cm^{-1})**: 3430, 3300 (NH_2); 3075 (CH-aromatic); 2922, 2855 (CH-aliph.); 1647 (C=N); 1570 (C=C). **MS, m/z (%):** 345 ($M^{+\cdot}$, 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.** (%) **for** $C_{19}H_{17}N_3S$ (319.42): C, 71.44; H, 5.36. **Found** (%): C, 71.46; H, 5.39. **IR (KBr,cm^{-1}):** 3300, 3175 (NH_2); 3042 (CH-aromatic); 2961, 2922 (CH-aliph.); 1647 (C=N); 1558 (C=C); 1345, 1021 (C-S-C) .**^{1}H NMR (DMSO-d_6, δppm):** 1.91 (s, 3H, C_3-CH_3); 2.56 (s, 3H, C_2-CH_3); 2.73 (s, 3H, C_9-CH_3); 5.40 (s, 2H, NH_2, D_2O exchangeable); 7.30-7.65 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 8.10-8.28 (m, 3H, C_8-H & C_6H_5-$C_{2,6}$-H). **MS, m/z (%):** 319 ($M^{+\cdot}$, 2.13), 57 (100.00).

Synthesis of 2,4-diamino-9-methyl-7-phenyl-pyrido [3`,2`:4,5]thieno[3,2-b]pyridine-3-carbonitrile; 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.** (%) **for** $C_{18}H_{13}N_5S$ (331.39): C, 65.24; H, 3.95; N, 21.13. **Found** (%): C, 65.27; H, 3.96; N, 21.21. **IR (KBr, cm^{-1})**: 3407, 3332, 3240, 3174 (NH_2); 3000 (CH-aromatic); 2917, 2845 (CH-aliph.); 2213 (C≡N); 1650 (C=N); 1569 (C=C). **MS, m/z (%):** 331 ($M^{+\cdot}$, 0.19), 67 (100.00).

Synthesis of 4-amino-2,9-dimethyl-7-phenyl-pyrido [3`,2`:4,5]thieno[3,2-b]pyrid-ine-3-carbonitrile; 7

A mixture of chloroacetone (0.46 g, 0.4 mL, 5 mmol) and potassium cyanide (0.33 g, 5 mmol) was refluxed in dry DMF (10 mL) for 10 min. to which a solution of compound

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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 0.88 g (53%); m.p.: 101-102°C. **Anal. Calcd.** (%) **for** $C_{19}H_{14}N_4S$ (330.41): C, 69.07; H, 4.27; N, 16.96. **Found** (%): C, 69.12; H. 4.31; N, 17.08. **IR (KBr, cm^{-1}):** 3426, 3300 (NH_2); 3000 (CH-aromatic); 2924 (CH-aliph.); 2205 (C≡N); 1640 (C=N); 1590 (C=C). **^{1}H NMR (DMSO-d$_6$, δppm):** 2.61 (s, 3H, C_2-CH_3); 2.89 (s, 3H, C_9-CH_3); 6.60 (s, 2H, NH_2, D_2O exchangeable); 7.51–7.60 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.87 (s, 1H, C_8-H); 8.10-8.28 (m, 2H, C_6H_5-$C_{2,6}$-H). **MS, m/z (%):** 332($M^{+\cdot}$+2, 2.02), 331($M^{+\cdot}$+1, 1.52), 330 ($M^{+\cdot}$, 0.51), 328 ($M^{+\cdot}$-2, 1.77), 78 (100.00).

Synthesis of 5,7-dimethyl-9-phenylpyrido[3``,2``:4`,5`] thieno[2`,3`:5,6]-pyrido[4,3-d]pyrimidin-4(3H)-one; 8

Compound **7** (1. 65 g, 5 mmol) was refluxed in excess formic acid (20 mL) for 10h. The reaction mixture was allowed to cool then poured onto crushed ice to yield a solid product which was filtered, washed with water then allowed to dry. The product was recrystallized from DMF/ethanol.

Black crystals; yield 1.55 g (87%); m.p.: > 360°C. **Anal. Calcd.** (%) **for** $C_{20}H_{14}N_4OS$ (358.42): C, 67.02; H, 3.94; N, 15.63. **Found** (%): C, 67.04; H, 3.99; N, 15.77. **IR (KBr, cm^{-1}):** 3416 (OH tautomer); 3275 (NH); 2920, 2851 (CH-aliph.); 1719 (C=O); 1642 (C=N); 1576 (C=C). **MS, m/z (%):** 359 ($M^{+\cdot}$+1, 7.62), 357 ($M^{+\cdot}$-1, 4.85), 80 (100.00).

Synthesis of 3-acetyl-4-amino-9-methyl-2,7-diphenylpyrido[3`,2`:4,5]-thieno[3,2-b]pyridine; 9

Equimolar amounts of compound **2** (1.33 g, 5 mmol) and benzoylacetone (0.81 g, 5 mmol) in ethanolic sodium ethoxide solution [(prepared by dissolving sodium metal (0.12 g, 5 mmol) in absolute ethanol (30 mL)], were refluxed for 10 h. The reaction mixture was allowed to cool, and then poured onto ice-cold water. The obtained precipitate was filtered off and recrystallized from ethanol.

Brown powder; yield 0.93 g (45%); m.p.: 79-81°C. **Anal. Calcd.** (%) **for** $C_{25}H_{19}N_3OS$ (409.50): C, 73.32; H, 4.68; N, 10.26. **Found** (%): C, 73.39; H, 4.66; N, 10.34. **IR (KBr, cm^{-1}):** 3321, 3174 (NH_2); 3059 (CH-aromatic); 2970, 2923 (CH-aliph.); 1690 (C=O); 1580 (C=N); 1551 (C=C). **^{1}H NMR (DMSO-d$_6$, δppm):** 2.84 (s, 3H, $COCH_3$); 3.02 (s, 3H, C_9-CH_3); 7.20 (s, 2H, NH_2, D_2O exchangeable); 7.48-7.62 (m, 6H, two C_6H_5-$C_{3,4,5}$-H); 7.98(s, 1H, C_8-H); 8.16-8.22 (m, 4H, two C_6H_5-$C_{2,6}$-H). **MS, m/z (%):** 409 ($M^{+\cdot}$, 4.39), 77 (100.00).

Synthesis of 2-amino-4,7-dimethyl-5,9-diphenyl-pyrido[3`,2`:4,5]thieno[3,2-h]-[1,6]naphthyridine-3-carbonitrile; 10

Compound **9** (2.05 g, 5 mmol) and an equimolar amount of malononitrile (0.33 g, 5 mmol) were refluxed in absolute ethanol (30 mL) containing a catalytic amount of piperidine (3 drops) for 15 h. The reaction mixture was allowed to cool and the obtained solid was filtered, washed with ethanol and left to dry. It was recrystallized from DMF/ethanol.

Brown powder; yield 1.97g (86%); m.p.: > 360°C. **Anal. Calcd.** (%) **for** $C_{28}H_{19}N_5S$ (457.55): C, 73.50; H, 4.19; N, 15.31. **Found** (%): C, 73.48; H, 4.24; N, 15.52. **IR (KBr, cm^{-1}):** 3332, 3240 (NH_2); 3000 (CH-aromatic); 2919 (CH-aliph.); 2212 (C≡N); 1650 (C=N); 1567 (C=C). **MS, m/z (%):** 456 ($M^{+\cdot}$–1, 0.38), 78 (100.00).

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

A mixture 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 ice-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** $C_{18}H_{12}N_4S_2$ (348.44): C, 62.05; H, 3.47; N, 16.08. **Found** (%): C, 62.11; H, 3.49; N, 16.22. **IR (KBr, cm^{-1}):** 3427, 3275 (NH, NH_2); 3100 (CH-aromatic); 2926 (CH-aliph.); 2213 (C≡N); 1621 (C=N); 1562 (C=C); 1526, 1358, 1120, 1079 (I, II, III, IV bands N-C=S). **^{1}H NMR (DMSO-d$_6$, δppm):** 2.84 (s, 3H, CH_3); 4.50 (s, 2H, NH_2, D_2O exchangeable.); 6.90 (s, 1H, NH, D_2O exchangeable.);7.40-7.60 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.78 (s, 1H, C_8-H); 8.15 (d, 1H, J= 8.4Hz, C_6H_5-C_2-H); 8.24 (d, 1H, J= 8.1Hz, C_6H_5-C_6-H). **MS, m/z (%):** 348 ($M^{+\cdot}$, 0.82), 53 (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 benzofuran-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 2.54g (67%); m.p.: 50-52°C. **Anal. Calcd.** (%) **for** $C_{23}H_{15}N_3OS$ (381.45): C, 72.42; H, 3.96; N, 11.02. **Found** (%): C, 72.47; H, 3.98; N, 11.13. **IR (KBr, cm^{-1})**: 3326, 3192 (NH_2); 3057 (CH-aromatic); 2925 (CH-aliph.); 1662 (C=N); 1590 (C=C); 1286, 1051 (C-O-C). **^{1}H NMR (DMSO-d$_6$, δppm):** 2.84 (s, 3H, CH_3); 6.90 (s, 2H, NH_2, D_2O exchangeable); 7.49- 7.68 (m, 5H, C_6H_5- $C_{3,4,5}$-H & C_6H_5- $C_{3`,4`}$-H); 7.69 (s, 1H, C_3-H); 7.79 (d, 2H, J=7.2Hz, C_6H_5-$C_{2`,5`}$-H); 7.82 (d, 2H, J= 9.9 Hz, C_6H_5-$C_{2,6}$-H). **MS, m/z (%):** 380 ($M^{+\cdot}$-1, 0.59), 106 (100.00).

Synthesis of 3-amino-4-methyl-6-phenylthieno[2,3-b] pyridin-2-carboxamide; 13

Compound **2** (2.65 g, 10 mmol) was stirred in 25 ml conc. H_2SO_4 for 19 h at room temperature. The reaction mixture was poured drop wise over crushed ice. The solid product was filtered, washed with water, left to dry and recrystallized

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from ethanol.

Brown powder; Yield 2.39 g (85%); m.p.: > 360°C. **Anal. Calcd.** (%) **for** $C_{15}H_{13}N_3OS$ (283.35): C, 63.58; H, 4.62; N, 14.83. **Found** (%): C, 63.60; H, 4.65; N, 14.90. **IR (KBr, cm^{-1}):** 3327, 3185 (NH_2); 3075 (CH-aromatic); 2921 (CH-aliph.); 1649 (C=O); 1603 (C=N); 1563 (C=C). **^{1}H NMR (DMSO-d$_6$, δ ppm):** 3.01 (s, 3H, CH_3); 5.68 (s, 2H, C_3-NH_2, D_2O exchangeable); 6.92 (s, 2H, $CONH_2$, D_2O exchangeable); 7.47-7.55 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.77 (s, 1H, C_5-H); 7.90-8.24 (m, 2H, C_6H_5-$C_{2,6}$-H). **MS, m/z (%):** 283 ($M^{+\cdot}$, 9.44), 44 (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 and chloroacetyl chloride was refluxed in acetic acid (10 mL) for 10 h. The reaction mixture was allowed to cool, and then poured onto ice-cold water. The separated solid was filtered, washed with water and recrystallized from ethanol.

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

Black powder; yield 1.75 g (48%); m.p.: 115-117°C. **Anal. Calcd.** (%) **for** $C_{22}H_{15}N_3OS$ (369.44): C, 71.52; H, 4.09; N, 11.37. **Found** (%): C, 71.51; H, 4.13; N, 11.42. **IR (KBr, cm^{-1}):** 3425 (OH tautomer); 3250 (NH); 3080 (CH-aromatic); 2924 (CH-aliph.); 1700 (C=O); 1564 (C=N). **^{1}H NMR (DMSO-d$_6$, δ ppm):** 3.02 (s, 3H, CH_3); 7.48-7.62 (m, 6H, two C_6H_5-$C_{3,4,5}$-H); 7.95 (d, 2H, J= 8Hz, C_2-C_6H_5-$C_{2,6}$-H); 8.00 (s, 1H, C_8-H); 8.10-8.30 (m, 2H, C_7-C_6H_5-$C_{2,6}$-H); 12.60 (s, 1H, OH, D_2O exchangeable). **MS, m/z (%):** 369 ($M^{+\cdot}$, 2.37), 53 (100.00).

2-(Chloromethyl)-9-methyl-7-phenylpyrido[3`,2`:4,5] thieno[3,2-d]pyrimidin-4(3H)-one; 14b

Black crystals; yield 2.43 g (72%); m.p.: > 360°C. **Anal. Calcd.** (%) **for** $C_{17}H_{12}ClN_3OS$ (341.81): C, 59.73; H, 3.54; N, 12.29. **Found** (%): C, 59.82; H, 3.57; N, 12.35. **IR (KBr, cm^{-1}):** 3421 (OH tautomer); 3280 (NH); 2914 (CH-aliph.); 1740 (C=O); 1636 (C=N); 1545 (C=C); 850 (C-Cl). **^{1}H NMR (DMSO-d, δ ppm):** 2.73 (s, 3H, CH_3); 3.07 (s, 2H, CH_2Cl); 7.40–7.70 (m, 4H, C_6H_5-$C_{3,4,5}$-H & C_8-H); 8.10-8.25 (m, 2H, C_6H_5- $C_{2,6}$-H); 10.50 (s, 1H, OH, D_2O exchangeable). **MS, m/z (%):** 341($M^{+\cdot}$, 1.02), 53(100.00).

Synthesis of ethyl N-(2-cyano-4-methyl-6-phenylthieno [2,3-b]pyridin-3-yl)formimidate; 15

Compound **2** (2.65 g, 10 mmol) was refluxed with an equimolar amount of triethyl orthoformate (1.48 g, 1.64 mL, 10 mmol) in acetic anhydride (15 mL) for 8 h. The reaction mixture was allowed to cool, and then poured onto crushed ice. The obtained solid was filtered, washed with water and recrystallized from ethanol.

Black crystals; yield 2.28 g (71%); m.p.: > 360°C. **Anal. Calcd.** (%) **for** $C_{18}H_{15}N_3OS$ (321.40): C, 67.27; H, 4.70; N, 13.07. **Found** (%): C, 67.29; H, 4.68; N, 13.14. **IR (KBr, cm^{-1}**): 2919, 2850 (CH-aliph.); 2230 (C≡N); 1580 (C=N); 1462 (C=C); 1303, 1029 (C-O-C). **^{1}H NMR (DMSO-d$_6$, δ ppm):** 1.06 (t, 3H, J= 6.6 Hz, CH_2CH_3); 3.01 (s, 3H, C_4-CH_3); 3.44 (q, 2H, J=6.6 Hz, CH_2CH_3); 7.25 (s, 1H, C_3-N=CH); 7.30-8.25 (m, 6H, C_6H_5 & C_5-H). **MS, m/z (%):** 321($M^{+\cdot}$, 0.25), 319 ($M^{+\cdot}$–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.** (%) **for** $C_{16}H_{13}N_5S$ (307.37): C, 62.52; H, 4.26; N, 22.78. **Found** (%): C, 62.55; H, 4.30; N, 22.91. **IR (KBr, cm^{-1}):** 3302, 3196 (NH, NH_2); 3090 (CH-aromatic); 2921 (CH-aliph.); 1656 (C=N); 1560 (C=C). **^{1}H NMR (DMSO-d$_6$, δ ppm):** 3.02 (s, 3H, CH_3); 6.06 (s, 2H, NH_2, D_2O exchangeable); 7.40–7.60 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 8.02 (s, 1H, C_8-H); 8.21–8.24 (m, 2H, C_6H_5-$C_{2,6}$-H); 8.40 (s, 1H, C_2-H); 8.90 (s, 1H, C_4=NH, D_2O exchangeable). **MS, m/z (%):** 308 ($M^{+\cdot}$+1, 66.74), 307 ($M^{+\cdot}$, 0.30), 306 ($M^{+\cdot}$-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 by ethanol. The separated solid was filtered and recrystallized from ethanol.

Orange powder; yield 2.44 g (83%); m.p.: 270°C. **Anal. Calcd.** (%) **for** $C_{16}H_{11}N_3OS$ (293.34): C, 65.51; H, 3.78; N, 14.32. **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.); 1650 (C=O); 1614 (C=N). **^{1}H NMR (DMSO-d$_6$, δ ppm):** 2.97 (s, 3H, CH_3); 7.40-7.60 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 8.09 (s, 1H, C_8-H); 8.20- 8.30(m, 2H, C_6H_5-$C_{2,6}$-H); 8.38 (s, 1H, C_2-H); 12.80 (s, 1H, OH, D_2O exchangeable). **MS, m/z (%):** 293 ($M^{+\cdot}$, 6.98), 61 (100.00).

Synthesis of 4-imino-9-methyl-3,7-diphenyl-pyrido [3`,2`:4,5] thieno[3,2-d] pyri-midine-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.** (%) **for** $C_{22}H_{16}N_4S_2$ (400.52): C, 65.97; H, 4.03; 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.); 1644 (C=N); 1592 (C=C); 1547, 1236, 1150, 1066 (I, II, III, IV bands N-C=S). **^{1}H NMR (DMSO-d$_6$, δ ppm):** 3.00 (s, 3H, CH_3); 7.10 (s, 1H, NH, D_2O exchangeable); 7.12-7.15 (m, 6H, two C_6H_5-$C_{3,4,5}$-H);

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7.30-7.40 (m, 3H, N_3-C_6H_5-$C_{2,6}$-H & C_8-H); 7.48 (d, 2H, J=7.8Hz, C_7-C_6H_5-$C_{2,6}$-H); 9.75 (s, 1H, C_4=NH, D_2O exchangeable). **MS, m/z (%):** 401 ($M^{+\cdot}$+1, 5.56), 80 (100.00).

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** $C_{16}H_{12}N_4S$ (292.36): C, 65.73; H, 4.14; N, 19.16. **Found** (%): C, 65.77; H, 4.13; N, 19.27. **IR (KBr, cm^{-1}):** 3426, 3340 (NH_2); 2926 (CH-aliph.); 1630 (C=N); 1554 (C=C). **^{1}H NMR (DMSO-d_6, δ ppm):** 3.02 (s, 3H, CH_3); 7.42-7.60 (m, 5H, C_6H_5-$C_{3,4,5}$-H & NH_2); 8.06 (s, 1H, C_8–H); 8.22-8.24 (m, 2H, C_6H_5-$C_{2,6}$-H); 8.39 (s, 1H, C_2-H). **MS, m/z (%):** 292 ($M^{+\cdot}$, 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; malononitrile 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** $C_{18}H_{10}N_6S$ (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). **^{1}H NMR (DMSO-d_6, δ ppm):** 3.03 (s, 3H, CH_3); 7.50-7.54 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.99 (s, 1H, C_5-H); 8.20-8.23 (m, 2H, C_6H_5-$C_{2,6}$-H); 8.50 (s, 1H, NH, D_2O exchangeable). **MS, m/z (%):** 343 ($M^{+\cdot}$+1, 14.71), 342 ($M^{+\cdot}$, 2.94), 60 (100.00).

Ethyl 2-[(2-cyano-4-methyl-6-phenylthieno[2,3-b]pyridin-3-yl)hydrazono]-2-cyanoacetate; 22

Brown crystals; yield 2.68 g (69%); m.p.: 50-52°C. **Anal. Calcd.** (%) **for** $C_{20}H_{15}N_5O_2S$ (389.43): C, 61.68; H, 3.88; N, 17.98. **Found** (%): C, 61.69; H, 3.86; N, 18.12. **IR (KBr, cm^{-1}):** 3250 (NH); 3002 (CH-aromatic); 2850 (CH-aliph.); 2205 (C≡N); 1698 (C=O); 1639 (C=N); 1574 (C=C); 1250, 1019 (C-O-C). **^{1}H NMR (DMSO-d_6, δ ppm):** 1.21 (t, 3H, J= 7.2Hz, CH_2CH_3); 3.08 (s, 3H, C_4-CH_3); 3.68 (q, 2H, J= 7.2Hz, CH_2CH_3); 7.51-7.59 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 8.00 (s, 1H, C_5-H); 8.20-8.27 (m, 2H, C_6H_5-$C_{2,6}$-H); 8.28 (s, 1H, NH, D_2O exchangeable). **MS, m/z (%):** 390 ($M^{+\cdot}$+1, 5.00), 60 (100.00).

Synthesis of 3-[(3,5-diamino-1H-pyrazol-4-yl)diazenyl]-4-methyl-6-phenylthieno[2,3-b]pyridine-2-carbonitrile; 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** $C_{18}H_{14}N_8S$ (374.42): C, 57.74; H, 3.77. **Found** (%): C, 57.91; H, 3.72. **IR (KBr, cm^{-1}):** 3408, 3334, 3236, 3172 (NH, NH_2); 2924 (CH-aliph.); 2214 (C≡N); 1651 (C=N); 1565 (C=C); 1408 (N=N). **^{1}H NMR (DMSO-d_6, δ ppm):** 2.90 (s, 3H, CH_3); 6.90 (s, 1H, NH, pyrazole- N_1-H, D_2O exchangeable); 7.30-7.40 (m, 1H, C_6H_5-C_4-H); 7.41-7.58 (m, 2H, C_6H_5-$C_{3,5}$-H); 7.95 (s, 1H, C_5-H); 8.10-8.30 (m, 2H, C_6H_5-$C_{2,6}$-H); 9.85 (s, 4H, two NH_2, pyrazole-$C_{3,5}$-NH_2, D_2O exchangeable).

Synthesis of 3-[2-(3-amino-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl]-4-methyl-6-phenylthieno[2,3-b] pyridine-2-carbonitrile; 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** $C_{18}H_{13}N_7OS$ (375.41): C, 57.59; H, 3.49; N, 26.12. **Found** (%): C, 57.64; H, 3.53; N, 26.29. **IR (KBr, cm^{-1}):** 3377, 3197 (NH, NH_2); 2928 (CH-aliph.); 2209 (C≡N); 1680 (C=O); 1646 (C=N); 1533 (C=C). **^{1}H NMR (DMSO-d_6, δ ppm):** 2.79 (s, 3H, CH_3); 7.00 (s, 1H, pyrazole-NH, D_2O exchangeable); 7.22 (s, 2H, pyrazole-C_3-NH_2, D_2O exchangeable); 7.51-7.59 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.82 (s, 1H, C_5-H); 8.10-8.28 (m, 2H, C_6H_5-$C_{2,6}$-H); 8.60 (s, 1H, C_3-NH, D_2O exchangeable).

Synthesis of 3-amino-2-(4,5-dihydro-1H-imidazol-2-yl)-4-methyl-6-phenyl-thieno[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** $C_{17}H_{16}N_4S$ (308.40): C, 66.21; H, 5.23; N,

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18.17. **Found** (%): C, 66.28; H, 5.28; N, 18.34. **IR (KBr, cm^{-1}):** 3320, 3248 (NH, NH_2); 3050 (CH-aromatic); 2954, 2882 (CH-aliph.); 1665 (C=N); 1509 (C=C). **^{1}H NMR (DMSO-d_6, δ ppm):** 2.84 (s, 3H, CH_3); 3.43-3.45 (m, 2H, imidazolidine-C_4-H); 3.64-3.73 (m, 2H, imidazolidine-C_5-H); 6.90 (s, 1H, NH, D_2O exchangeable); 7.20 (s, 1H, NH_2, D_2O exchangeable); 7.60-8.15 (m, 6H, C_6H_5 & C_5-H). **MS, m/z (%):** 308 ($M^{+\cdot}$, 0.23), 60 (100.00).

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, 1.1mmol), 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** $C_{18}H_{14}N_4S$ (318.40): C, 67.90; H, 4.43; N, 17.60. **Found** (%): C, 67.88; H, 4.48; N, 17.71. **IR (KBr, cm^{-1})**: 3000 (CH-aromatic); 2954, 2881 (CH-aliph.); 1508 (C=N); 1458 (C=C). **MS, m/z (%):** 318 ($M^{+\cdot}$, 19.36), 53 (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, 0.15 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** $C_{24}H_{20}N_4S$ (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}):** 3427 (NH); 3052 (CH-aromatic); 2964, 2886 (CH-aliph.); 1596 (C=N); 1484 (C=C); 1327, 1086 (C-S-C). **^{1}H NMR (DMSO-d_6, δ ppm):** 3.09 (s, 3H, CH_3); 3.56-3.76 (m, 2H, imidazo-C_2-H); 4.10-4.20 (m, 2H, imidazo- C_3-H); 7.57-7.60 (m, 3H, C_5- C_6H_5- $C_{3',4',5'}$-H); 7.69 (d, 2H, J= 8.4Hz, C_5- C_6H_5-$C_{2',6'}$-H); 7.89-7.95 (m, 4H, C_9-C_6H_5-$C_{3,4,5}$-H & C_8-H); 8.25-8.52 (m, 2H, C_9-C_6H_5-$C_{2,6}$-H); 8.71 (s, 1H, C_5-H); 9.65 (s, 1H, NH, D_2O exchangeable). **MS, m/z (%):** 394 ($M^{+\cdot}$- 2, 0.18), 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 **25** (2.28 g, 7.4 mmol) and excess carbon disulphide (10 mL) in anhydrous pyridine (20 mL) was heated under reflux for 20 h on a water bath. The reaction mixture was allowed to cool, and then poured onto crushed ice. The precipitated solid was filtered off, left to dry and recrystallized from ethanol. Brown crystals; yield 1.9 g (73%); m.p.: 108-110°C. **Anal. Calcd.** (%) **for** $C_{18}H_{14}N_4S_2$ (350.46): C, 61.69; H, 4.03; N, 15.99. **Found** (%): C, 61.73; H, 4.02; N, 16.14. **IR (KBr, cm^{-1}):** 3246 (NH); 3000 (CH-aromatic); 2881 (CH-aliph.); 1650 (C=N); 1508 (C=C); 1457, 1273, 1200, 1051 (I, II, III, IV bands N-C=S); 1306, 1273 (C-S-C). **^{1}H NMR (DMSO-d_6, δ ppm):** 3.00 (s, 3H, CH_3); 3.81-3.83 (m, 2H, imidazo-C_2-H); 4.35-4.41 (m, 2H, imidazo-C_3-H); 7.50-7.70 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.98 (s, 1H, C_8-H); 8.20-8.38 (m, 2H, C_6H_5-$C_{2,6}$-H); 9.85 (s, 1H, NH, D_2O exchangeable). **MS, m/z (%):** 350 ($M^{+\cdot}$, 0.02), 51 (100.00).

Synthesis of ethyl 3-azido-4-methyl-6-phenylthieno[2,3-b] pyridine carboxylate; 30

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** $C_{17}H_{14}N_4O_2S$ (338.38): C, 60.34; H, 4.17; N, 16.56. **Found** (%): C, 60.37; H, 4.19; N, 16.69. **IR (KBr, cm^{-1}):** 3000 (CH-aromatic); 2923, 2856 (CH-aliph.); 2128 (N_3); 1680 (C=O); 1624 (C=N); 1233, 1010 (C-O-C). **MS, m/z (%):** 339 ($M^{+\cdot}$ +1, 0.26), 338 ($M^{+\cdot}$, 0.26), 80 (100.00).

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)acetonitrile 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 recrystalized from ethanol.

3-Cyano-10-methyl-8-phenylpyrido[3',2':4,5]thieno[2,3-e] [1,2,3]triazolo-[1,5-a]pyrimidin-5(4H)-one; 31a

Dark red crystals; yield 3.21 g (90%); m.p.: > 360°C. **Anal. Calcd**, (%) **for** $C_{18}H_{10}N_6OS$ (358.38): C, 60.33; H, 2.81; N, 23.45. **Found** (%): C, 60.34; H, 2.83; N, 23.52. **IR (KBr, cm^{-1}):** 3450 (OH broad); 3350, 3200 (NH); 2975 (CH-aliph.); 2202 (C≡N); 1710 (C=O); 1621 (C=N); 1590 (C=C); 1458 (N=N). **^{1}H NMR (DMSO-d_6, δ ppm):** 2.73 (s, 3H, CH_3); 6.94-7.20 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.90 (s, 1H, C_9-H); 8.00-8.22 (m, 2H, C_6H_5-$C_{2,6}$-H); 8.52 (s, 1H, NH, D_2O exchangeable). **MS, m/z (%):** 358 ($M^{+\cdot}$, 15.11), 80 (100.00).

3-(1,3-Benzo[d]thiazol-2-yl)-10-methyl-8-phenyl pyrido [3',2':4,5]thieno[2,3-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-one; 31b

Buff crystals; yield 3.89 g (84%); m.p.: > 360°C. **Anal. Calcd.** (%) **for** $C_{24}H_{14}N_6OS_2$ (466.54): C, 61.79; H, 3.02; N, 18.01. **Found** (%): C, 61.85; H, 3.05; N, 18.12. **IR (KBr, cm^{-1}):** 3442 (OH tautomer); 3290, 3230 (NH); 3075 (CH-aromatic); 2890 (CH-aliph.); 1706

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(C=O); 1641 (C=N); 1562 (C=C); 1414 (N=N). **MS, m/z (%):** 467 ($M^{+\cdot}$+1, 1.67), 466 ($M^{+\cdot}$, 13.75), 80 (100.00).

Synthesis of ethyl 4-methyl-3-(methylthiocarbonothioylamino)-6-phenylthieno-[2,3-b]pyridine-2-carboxylate; 32

To a vigorously stirred solution of compound **29 (**3.12 g, 10 mmol) in dimethyl sulphoxide (5 mL) at room temperature, carbon disulphide (1.98 g, 1.6mL, 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, 1.9 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** $C_{19}H_{18}N_2O_2S_3$ (402.55): C, 56.69; H, 4.51; 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); 1470 (C=C); 1544, 1278, 1146, 1092 (N-C=S); 1278, 1092 (C-S-C); 1242, 1059 (C-O-C). **^{1}H NMR (DMSO-d$_6$, δppm):** 1.0-1.15 (m, 3H, CH_2-CH_3); 2.71 (s, 3H, S-CH_3); 2.85 (s, 3H, C_4-CH_3); 4.26-4.43 (m, 2H, CH_2-CH_3); 7.32 (s, 1H, NH, D_2O exchangeable); 7.52-7.54 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.85(s, 1H, C_5-H); 8.17(d, 2H, J=8.1Hz, C_6H_5-$C_{2,6}$-H). **MS, m/z (%):** 403 ($M^{+\cdot}$+1, 1.66), 401 ($M^{+\cdot}$-1, 0.38), 76 (100.00).

Synthesis of 3-amino-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** $C_{16}H_{12}N_4OS_2$ (340.42): C, 56.45; H, 3.55. **Found** (%):C, 56.53; H, 3.58. **IR (KBr, cm^{-1}):** 3209, 3192 (NH, NH_2); 3025 (CH-aromatic); 2923 (CH-aliph.); 1740 (C=O); 1541, 1243, 1147, 1014 (N-C=S I, II, III, IV bands); 1541 (C=C). **^{1}H NMR (DMSO-d$_6$, δppm):** 2.87 (s, 3H, CH_3); 4.30 (s, 2H, NH_2, D_2O exchangeable); 6.61 (s, 1H, NH, D_2O exchangeable); 7.48-7.54 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.81 (s, 1H, C_8-H); 8.15 (d, 2H, J=7.95Hz, C_6H_5-$C_{2,6}$-H). **MS, m/z (%):** 340 (M^+, 0.07), 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.

Buff crystals; yield 2.56 g (70%); m.p.: > 360°C. **Anal. Calcd.** (%) **for** $C_{18}H_{15}N_5O_2S$ (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 ($M^{+\cdot}$+3, 4.55), 51 (100.00).

Synthesis of 2,3-diamino-9-methyl-7-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** $C_{16}H_{13}N_5OS$ (323.37): C, 59.43; H, 4.05; N, 21.66. **Found** (%): C, 59.55; H, 4.04; N, 21.69. **IR (KBr, cm^{-1}):** 3431, 3352, 3212 (NH_2); 3054 (CH-aromatic.); 2918 (CH-aliph.); 1700 (C=O); 1673 (C=N); 1580 (C=C). **^{1}H NMR (DMSO-d$_6$, δ ppm):** 2.71 (s, 3H, CH_3); 4.10 (s, 2H, C_2-NH_2, D_2O exchangeable); 6.80 (s, 2H, N_3-NH_2, D_2O exchangeable); 7.41-7.47 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.52 (s, 1H, C_8-H); 8.05 (d, 2H, J=7.2Hz, C_6H_5-$C_{2,6}$-H). **MS, m/z (%):** 323 ($M^{+\cdot}$, 1.31), 57 (100.00).

Synthesis of ethyl N-(9-methyl-4-oxo-7-phenyl-pyrido [3`,2`:4,5]thieno[3,2-d]-pyrimidin-3(4H)-yl)formimidate; 37

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 filtered, washed with ethanol, left to dry and recrystallized from ethanol.

Brown crystals; yield 2.62 g (72%); m.p.: 108-110°C. **Anal. Calcd.** (%) **for** $C_{19}H_{16}N_4O_2S$ (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). **^{1}H NMR (DMSO-d$_6$, δ ppm):** 1.16-1.23 (m, 3H, CH_2CH_3); 2.71 (s, 3H, C_9-CH_3); 4.10-4.20 (m, 2H, CH_2-CH_3); 7.40-7.60 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.87 (s, 1H, N_3-N=CH); 7.92 (s, 1H, C_8-H); 8.10-8.30 (m, 2H, C_6H_5-$C_{2,6}$-H); 8.36 (s, 1H, C_2-H). **MS, m/z (%):** 363 ($M^{+\cdot}$-1, 0.24), 53 (100.00).

Synthesis of N-(9-methyl-4-oxo-7-phenylpyrido[3`,2`:4,5] thieno[3,2-d]pyrimi-din-3(4H)-yl)acetamide; 38

The carbohydrazide derivative **36** (2.98 g, 10 mmol) was heated under reflux in acetic anhydride (10 mL) for 10 h. The reaction mixture was allowed to cool, poured onto crushed ice and the separated solid was filtered, washed with water, dried and recrystallized from ethanol.

Yellow crystals; yield 2.50 g (69%); m.p.: >360°C. **Anal. Calcd.** (%) **for** $C_{19}H_{16}N_4O_2S$ (364.42): C, 62.62; H, 4.43; N, 15.37.

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Found (%): C, 62.61; H, 4.48; N, 15.46. **IR (KBr, cm^{-1}):** 3259 (NH); 3058 (CH-aromatic); 2919, 2852 (CH-aliph.); 1695 (C=O pyrimidinone); 1673 (C=O acetyl); 1579 (C=N); 1544 (C=C). **MS, m/z (%):** 364 (M$^{+\cdot}$, 2.43), 53 (100.00).

Synthesis of 1-(3-amino-4-methyl-6-phenylthieno[2,3-b] pyridine-2-carbonyl)-3-methyl-1H-pyrazol-5(4H)-one; 39

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. Calcd.** (%) **for** $C_{19}H_{16}N_4O_2S$ (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_2); 3085 (CH-aromatic); 2927 (CH-aliph.); 1735, 1688 (two C=O); 1641 (C=N); 1553 (C=C). **^{1}H NMR (DMSO-d$_6$, δ ppm):** 2.26 (s, 5H, pyrazole-C_3-CH_3 and pyrazole-C_4-H); 2.81(s, 3H, C_4-CH_3); 7.46-7.60 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.84 (s, 1H, C_5-H); 8.14 (d, 2H, J=7.5Hz, C_6H_5-$C_{2,6}$-H); 16.53 (s, 2H, H-bonded NH_2, D_2O exchangeable). **MS, m/z (%):** 364 (M$^{+\cdot}$, 1.30), 363 (M$^{+\cdot}$-1, 1.30), 65 (100.00).

Biological evaluation

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 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% CO_2 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 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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