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SYNTHESIS AND REACTIONS OF SOME PYRIDINE AND THIENO [2,3-b] PYRIDINE DERIVATIVES WITH EXPECTED BIOLOGICAL ACTIVITY

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Abstract

A synthesis of pyridine-2(1H)-thione derivative **2** and thieno-[2,3-b] -pyridine derivatives **4,5** utilizing cyanothioacetamide and arylhydrazone of benzoylacetone **1** as starting compounds is described. Pyridinethione derivative **6** reacted with aromatic aldehyde to give styrylpyridinethione derivative **7**. Compound **7** was reacted with chloroacetone to obtain thienopyridine derivative **8**. Cycloaddition reaction was carried out on **8** with dienophiles, to afford the corresponding cycloadduct **9**. Pyridinethione derivative **6** reacted with halogen-containing materials to give the corresponding thienopyridine derivatives **10**, **17**. The reaction of **10** with triethyl orthoformate or formic acid led to the formation of pyridothienopyrimidine derivative **11**. The hydrazino derivative **13**, gave with triethyl orthoformate triazolo-pyrimidothienopyridine derivative **14**. Compound **10** reacted with nitrous acid to gave pyridothienotrizinone derivative **15**. Thienopyridine derivative **17** reacted with triethyl orthoformate to give **18**. Triazipinopyrimidothienopyridine derivative **20** obtained from compound **18** after reaction with hydrazine hydrate and acetylacetone. Compound **17** reacted with carbon disulfide to give pyrimidinedithione derivative **21**.

Introduction

In recent years several papers dealing with the mechanism by which anticancer drugs exert their action at the molecular level have aroused considerable interest. Many different targets have been identified and characterized, including DNA and enzymes involved in processing nucleic acids, such as topoisomerases [1-3]. Intercalation of planer aromatic or heteroaromatic compounds with DNA is one of the important modes of actions in DNA drug interaction [4,5], based on the ability of the chromophore to bind strongly between the base pairs. In this field, the promising antitumor properties shown by ellipticine [6,7], as part of a program designed to investigate the biological activity of tricyclic and tetracyclic systems containing a thiophene ring as central nucleus [8,9].

Results and Discussion

In the present paper we reported reactions of cyanothioacetamide with arylhydrazone of benzoyl acetone **1** and benzoylacetone for the synthesis of substituted pyridine-2(1H)-thione and their condensed derivatives. Thus, it has been

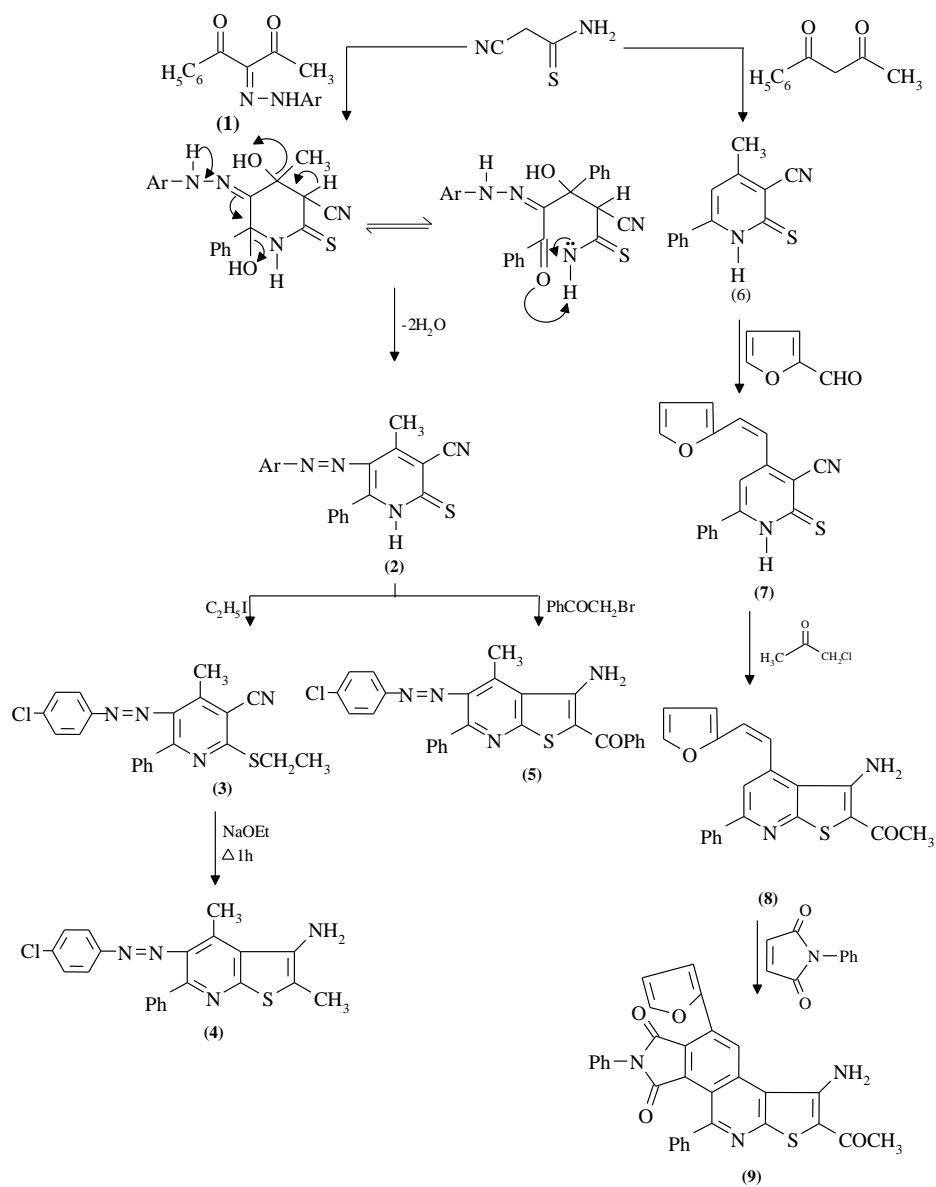
found that benzoylacetone coupled with aryldiazonium chloride in ethanol containing sodium acetate [10] to afford the corresponding monoarylhydrazone derivative **1** in good yield. Compound **1** reacts with cyanothioacetamide in refluxing ethanol sodium ethoxide for 5h to give the 3-cyanopyridine 2(1H)-thione derivative **2**. Compound **2** reacted with ethyl iodide in DMF-sodium hydroxide to afford the corresponding S-ethyl derivative **3**. The ¹H-NMR of **3** revealed a triplet at δ 1.40 ppm and a quartet at δ 4.53 ppm assignable to S-ethyl group of compound **3**. Further confirmation of the structure **3** was given via their cyclization using sodium ethoxide solution to afford thieno[2,3-b] pyridine derivative **4**. The IR spectrum of **4** showed no band of CN group and instead the band of newly NH₂ group was detected. The ¹H-NMR spectrum of **4** also revealed no signals of -SCH₂-protons but revealed signals at δ 5.27 ppm for NH₂ protons. Considering the previous results, we can conclude that both-SCH₂- and CN group in **3** may involved in the cyclization step to afford **4**. When **2** was treated with phenyl bromide in ethanol-sodium ethoxide, S-alkylated derivative could not be isolated, but their cyclizations product, thieno[2,3-b] pyridine derivative **5** was obtained. The IR spectrum of **5** revealed the absence of a CN band and ¹H-NMR spectrum showed besides the aromatic proton signals at δ 7.21-7.98 ppm, a broad band at δ 4.38 ppm assignable to an amino function.

3-Cyano-6-phenyl-2-(1H)-pyridinethione **6** as starting material was prepared via condensing benzoylacetone with cyanothioacetamide [11], and used as starting material, therefore, interaction of **6** with 2-furaldehyde afforded 3-cyano-6-phenyl-4-styryl-2(1H)-pyridinethione **7**, which was converted into 2-acetyl-3-amino-6-phenyl-4-styrylthieno[2,3-b]-pyridine **8** via reaction of **7** with chloroacetone in dimethyl formamide in presence of anhydrous potassium carbonate. IR spectrum of **8** showed disappearance of (C≡N) band and presence of bands at ν₃₃₉₀, 3330 cm⁻¹(NH₂). The dienic nature of **8** was investigated through their reaction with *N*-phenylmaleimide as dienophiles. Thus, **8** reacted with *N*-phenylmaleimide in ethanol [12] to afford cycloadducts **9**, which could be formulated as the pyrrolo[3,4-f] quinolino[2,3:6',7'] thiophene derivative. The IR spectrum showed the two widely separated band of -CO-N-(Ph) -CO- grouping at ν₁₇₅₀ and ν₁₇₀₀ cm⁻¹ characteristic of the cycloadducts [12]. Its mass spectrum gave a molecular ion peaks at m/e=529 (8.11%) (Figure I), (Scheme I).

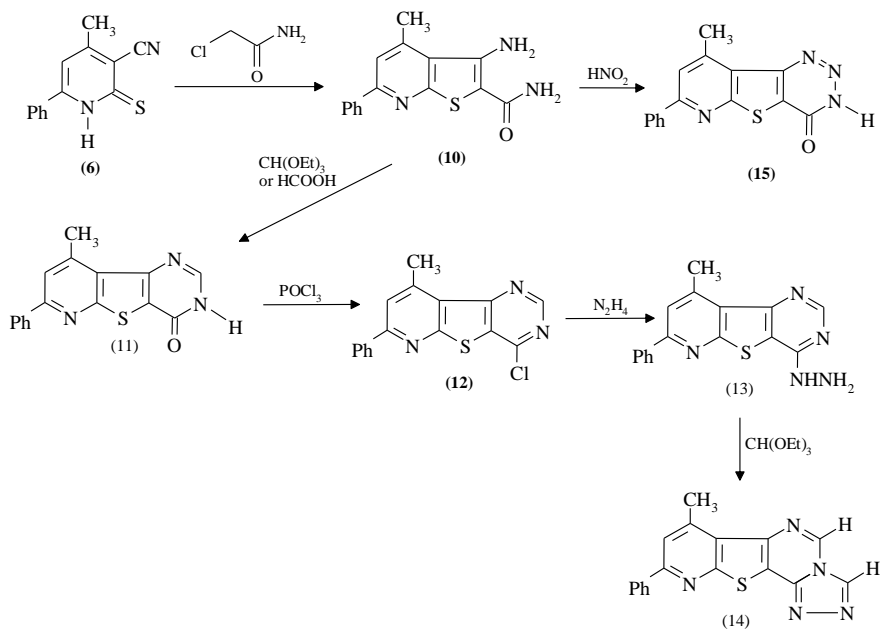
Interaction of 3-amino-4-methyl-6-phenyl thieno [2,3-b] pyridine-2-carboxamide **10** [13], with triethyl orthoformate or formic acid [5,6] gave pyrido thieno pyrimidinone derivative **11**. The chloro compound **12** was synthesized by

refluxing the pyrimidinone **11** in phosphorous oxychloride. The chlorine atom in **12** underwent displacement reaction when reacted with hydrazine hydrate to afford 4-hydrazino-9-methyl-7-phenylpyrido [3',2':4,5] thino [2,3-d] pyridine **13** in good yield. The hydrazino compound **13** was used as a key intermediate to synthesize new ring system, namely, triazolothienopyrimidopyridine derivative **14** through the reaction with triethyl orthoformate. Its ¹H-NMR spectrum revealed the signal at δ 9.86 ppm corresponding to triazolo H-4[14]. Its mass spectrum gave molecular ion peak at $m/e = 317$, (3.39%) Fig. II. Based on the above data compound **14** was formulated as 11-methyl-9-phenyl-[1,2,4] triazolo[4'',3'': 1':6'] pyrimido [4',5':,4,5' thieno [2,3-b] pyridine **14**. Moreover compound **10** reacts with nitrous acid to give the self-cyclized reaction product **15**. The IR spectrum of the latter compound showed the band of NH triazine ring at ν 3315 cm^{-1} and CO of triazine ring at ν 1668 cm^{-1} [15]. Its ¹H-NMR spectrum revealed a signal at δ 8.13 ppm corresponding to NH. Its mass spectrum gave a molecular ion peak at m/e 294 (73.87%) (Fig. III). Based on the above data compound **15** was formulated as 9-methyl-7-phenyl pyrido [5,4-b] thieno [3',2'-d] [1,2,3] triazin-4-(3H)-one **15**, (Scheme II).

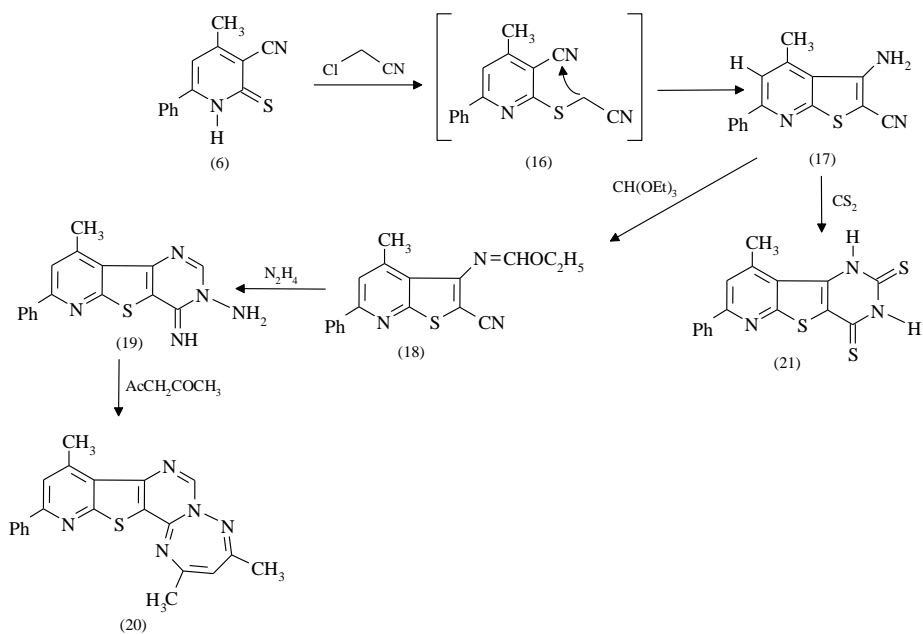
Compound **6** reacts with chloroacetonitrile to afford the isolated reaction product **17**. The IR spectrum of the latter compound showed the absorption band at ν 3335, 3231 cm^{-1} corresponding to NH_2 group, at ν 2191 cm^{-1} due to $\text{C}\equiv\text{N}$. Its ¹H-NMR spectrum revealed the signal of NH_2 . The formation of **17** most likely proceeded via the initial formation of the corresponding 2-S-alkyl pyridine intermediate **16** which under cyclization into **17** under the applied reaction conditions. Based on the above data the reaction product was identified as thieno [2,3-b] pyridine derivative **17**. The reaction of compound **17** with triethyl orthoformate in acetic anhydride afforded the ether derivative **18** which in turn was reacted with hydrazine hydrate in dioxan on cold to afford 3-amino-4-imino-9-methyl-7-phenyl pyrimido [4',5':4,5'] thieno [2,3-b] pyridine **19**. The latter compound was reacted with acetyl acetone to give **20**. Its ¹H-NMR spectrum revealed signal at δ 6.28 corresponding to triazpino H-6 [14] and the mass spectrum was compatible with the molecular formula $\text{C}_{21}\text{H}_{17}\text{N}_5\text{S}$ ($M^+ = 371$), (Fig. IV). Based on the elemental analysis and spectral data, the latter isolated product was identified as 5,7,13-trimethyl-11-phenyltriazipino [2'',3'':1',6'] pyrimido [4',5':5,5] thieno[2,3-b]pyridine **20**. the pyrimidindithione derivative **21** was obtained from the reaction of compound **17** with carbon disulfide in pyridine, (Scheme III).



Scheme I



Scheme II



Scheme III

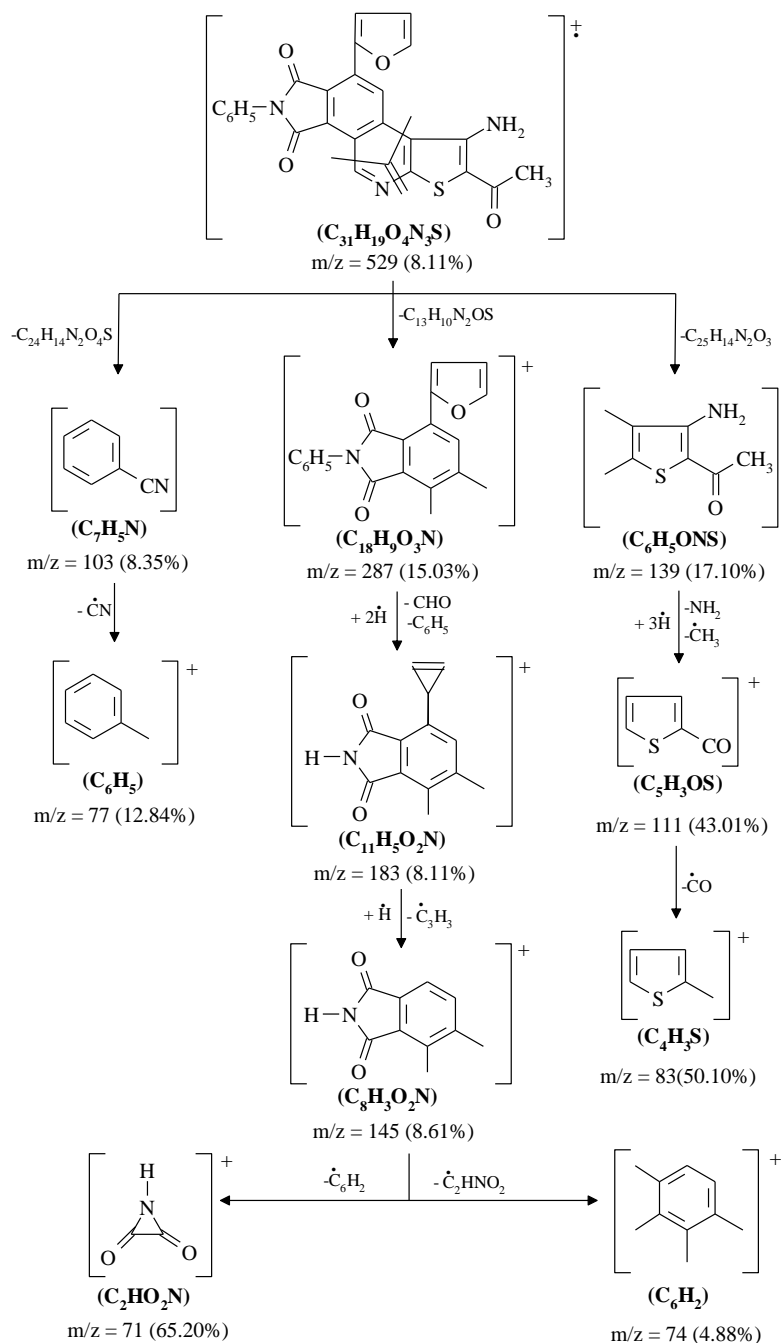


Fig. I. Mass fragmentation pattern of compound 9

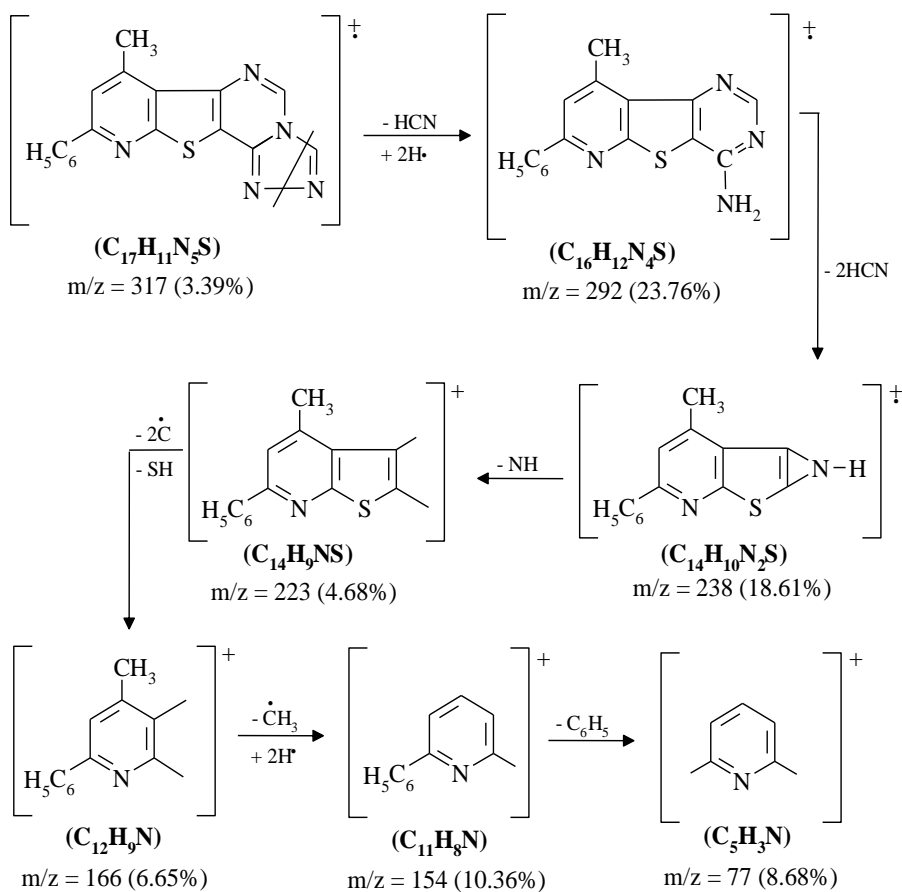


Fig. II. Mass fragmentation pattern of compound **14**.

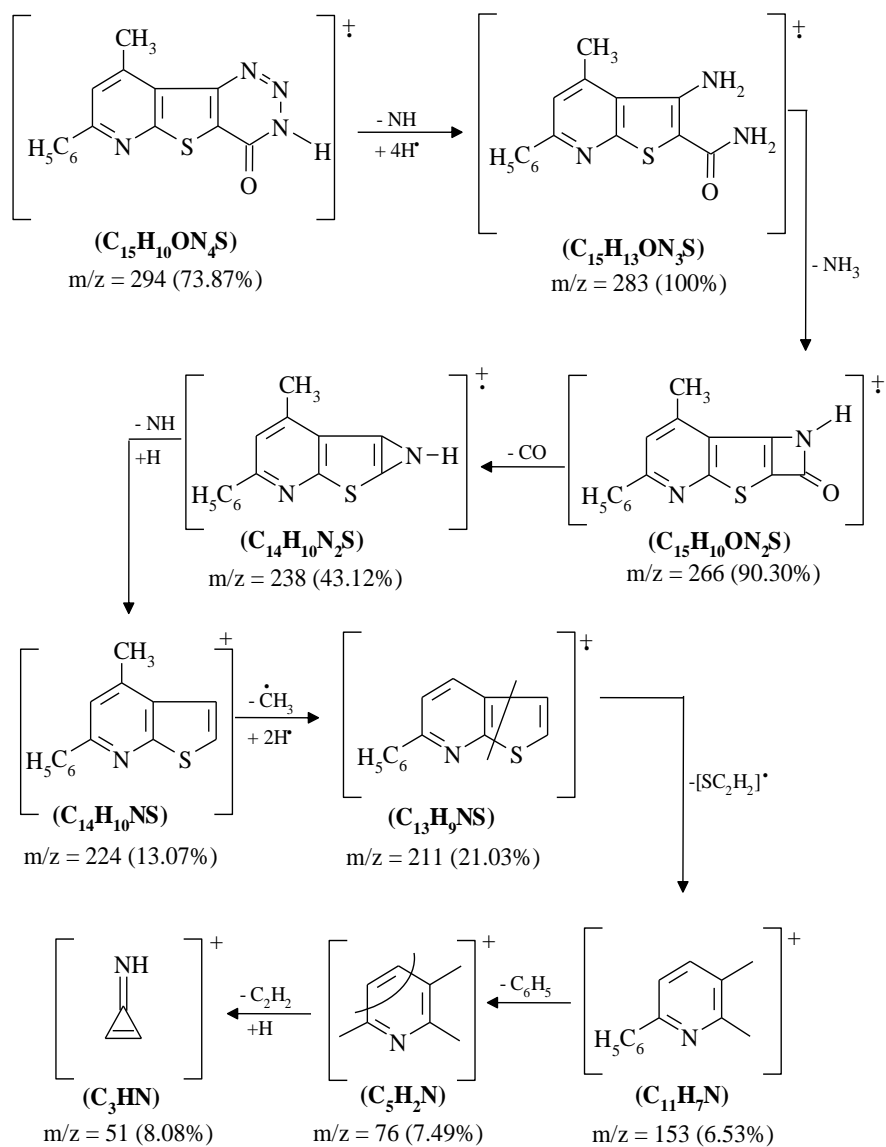


Fig. III. Mass fragmentation pattern of compound 15

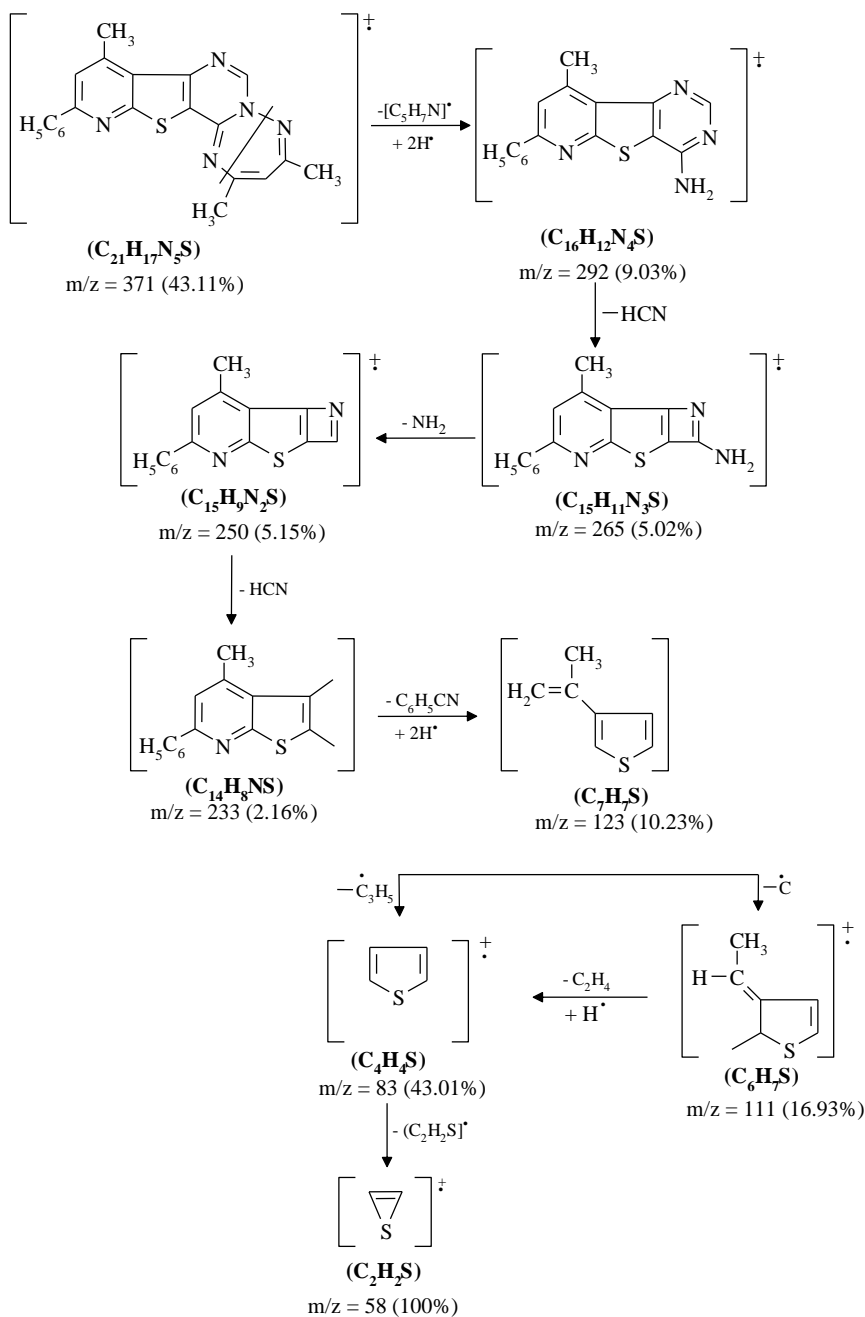


Fig. IV. Mass fragmentation pattern of compound 20.

Experimental

Melting points were taken on Gallen Kamp Melting apparatus and are uncorrected. Infrared spectra were obtained on Nexus 470-670, $^1\text{H-NMR}$ spectra and $^{13}\text{C-NMR}$ run on JEOL-400 MHZ in DMSO- d_6 . The mass spectra were recorded on Ms-S988 operating at 70eV. Microanalysis were performed by using pekin-Elmer 2400 CHN analyzer. The newly synthesized compounds were screened in vitro antitumor activity at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit.

Arylhydrazone of benzoylacetone (1):

A solution of benzoylacetone (2.24 g, 0.01 mol) in ethanol (100 ml) containing sodium acetate (3.0 g) was cooled to 0°C , stirred and treated gradually with a cooled solution of aryldiazonium chloride (prepared from 0.01 mol of amine and appropriate quantities of HCl and NaNO_2). The solid product formed on standing was collected and crystallized from ethanol; m.p. 85°C ; yield 84% IR ν (cm^{-1}): 3250 (NH), 3040 (CH-Ar), 2900 (CH aliph.), 1668 (C=O), 1640 (C=N) $^1\text{H-NMR}$ (DMSO- d_6): δ 2.34 (s, 3H, CH_3), 7.20-7.79 (m, 9H, Ar-H), 8.82 (s, 1H, NH). Anal. Calcd for $\text{C}_{16}\text{H}_{13}\text{ClN}_2\text{O}_2$: C, 63.89; H, 4.32; N, 9.31; Cl, 11.81. Found: C, 63.82; H, 4.30; N, 9.30; Cl, 11.80.

3-Cyano-5-(p-chlorophenylhydrazone)-4-methyl-6-phenyl-2(1H)-pyridinethione(2).

A mixture of 1 (0.01 mol) and cyanothioacetamide (1g, 0.01 mol) was dissolved in ethanol (30 ml) containing sodium ethoxide (0.68 g, 0.01 mol). The mixture was refluxed for 5h, and then allowed to cool at room temperature and acidified with cold dilute hydrochloric acid. The resulting solid product was collected by filtration and crystallized from dioxan; m.p $> 360^\circ\text{C}$; yield 70%.

IR ν (cm^{-1}): 3294 (NH); 3088 (CH-Ar); 2840 (CH aliph.); 2195 (C \equiv N) $^1\text{H-NMR}$ (DMSO- d_6): δ 2.51 (s, 3H, CH_3), 7.22-7.84 (m, 9H, Ar-H), 12.42 (s, 1H, NH); MS, $m/z = 364$ (M^+) Anal. Calcd. for $\text{C}_{19}\text{H}_{13}\text{ClN}_4\text{S}$: C, 62.55; H, 3.56; N, 15.36; S, 8.77, Cl, 9.73. Found: C, 62.50; H, 3.60; N, 15.30; S, 8.70; Cl, 9.70.

2- Ethylthio-5(p-chlorophenyl hydrazone)-6-phenyl-4-methyl Pyridine-3-carbonitrile (3).

A mixture of 2 (0.01 mol), NaOH (0.4 g, 0.01 mol) and ethyl iodide (0.01 mol) in dry DMF (50 ml) was stirred at room temperature for 24h, then dilute with cold water (100 ml) and the resulting solid product was collected by filtration and

crystallized from ethanol; m.p. 260°C IR $\nu(\text{cm}^{-1})$: 3022 (CH-Ar), 2936 (CH aliph.), 2219 (C \equiv N). $^1\text{H-NMR}$ (DMSO- d_6) δ 1.40 (t, 3H, CH₂CH₃, J=7.2 Hz), 2.51 (s, 3H, CH₃), 4.53 (q, 2H, CH₂CH₃, J=7.20 Hz) 7.60-8.12 (m, 9H, Ar-H), Anal. Calcd. for C₂₁H₁₇ClN₄S: C, 64.20; H, 4.33; N, 14.26; S, 8.15; Cl, 9.04 Found : C, 64.12; H, 4.30; N 14.20; S, 8.10, Cl, 9.00. ^{13}C : δ 22.24 (CH₃); 22.62 (CH₃); 60.00 (CH₂); 116.18 (CN) and 121.92; 122.24 123.40; 129.91; 130.01; 131.11; 132.30; 133.41; 134.13.

3-Amino-5-(p-chlorophenyl hydrazone)-2,4-dimethyl-6-phenyl thieno[2,3-b] pyridine (4).

Compound **3** was heated under reflux in ethanolic sodium ethoxide solution (0.5g, 0.02 mol-atom of sodium in 25 ml of ethanol) for 1h. After cooling, the solid product was filter, wash with water and recrystallized from dioxan; m.p.> 360°C; yield 60% IR $\nu(\text{cm}^{-1})$: 3310, 3210 (NH₂); 3050(CH-Ar); 2700- 2900 (CH-aliph.).

$^1\text{H-NMR}$ (DMSO- d_6): δ 2.50 (s, 3H, CH₃ pyridine ring), 2.88 (s, 3H, CH₃ thiophene ring), 5.27 (s, 2H, NH₂) 7.38-7.94 (m, 9H, Ar-H)

Anal.calcd. for C₂₁H₁₇ClN₄S: C, 64.20; H, 4.33; N, 14.26, S, 8.15, Cl, 9.04 Found: C, 64.11, H, 4.32; N, 14.20; S, 8.14; Cl, 9.00

3-Amino-2-benzoyl-5-(p-chlorophenylhydrazone-)-4-methyl-6-phenylthieno[2,3-b] pyridine (5).

A mixture of **2** (0.01 mol), sodium ethoxide (0.68 g, 0.01 mol) and phenacyl bromide (2.09 g, 0.01 mol) in dry ethanol (50 ml) was refluxed for 5h and then allowed to cool to room temperature and acidified with cold dilute hydrochloric acid. The resulting solid was collected by filtration and crystallized from ethanol; m.p. 150°C; yield 60% IR $\nu(\text{cm}^{-1})$: 3435, 3317 (NH₂), 3055 (CH-Ar), 2926 (CH aliph.), 1671 (CO-H bonded), 1604 (C=C).

$^1\text{H-NMR}$ (DMSO- d_6): δ 2.50 (s, 3H, CH₃), 4.38 (s, 2H, NH₂), 7.21-7.98(m, 14H, Ar-H). Anal. Calcd. for C₂₇H₁₉ClN₄OS: C, 67.15; H, 3.93, 11.60; S, 6.63; Cl, 7.35.

Found: C, 67.10; H, 3.90; N, 11.62; S, 6.60; Cl, 7.30.

^{13}C - δ 22.24 (CH₃);121.92; 122.24; 123.40; 129.91; 130.01; 131.11; 132.30; 133.44; 134.19; 134.99; 135.11; 135.81; 136.01; 136.25 and 204.40 (CO).

3-Cyano-6-phenyl-4-styryl-2-(1H)-pyridinethione (7).

To a solution of 6 (0.01 mol) in dioxan (20 ml), 2-furaldehyde (0.01 mol) and catalytic amount of piperidine were added, the reaction mixture was refluxed for 45 hr. After cooling, the precipitate was filtered and recrystallized from ethanol, m.p. 250°C; yield 75%.

IR $\nu(\text{cm}^{-1})$: 3421(NH), 3050(CH-Ar), 2214(C \equiv N) $^1\text{H-NMR}$ (DMSO- d_6): δ 6.87 (s, 1H, pyridine H-5), 7.56-8.00 (m, 8H, Ar-H), 7.57, 8.28 (2d, 2H, CH styryl, J=5.87 Hz), 8.92 (s, 1H, NH). Anal.calcd. for C₁₈H₁₂N₂OS: C, 71.05; H, 3.94; N, 9.21; S, 10.52, Found : C, 71.00; H, 3.90, N, 9.20; S, 10.50.

2-Acetyl-3-amino-6-phenyl-4-styryl thieno[2,3-b] pyridine (8).

To a solution of 7 (0.01 mol) in dimethylformamide (50 ml), potassium carbonate anhydrous (2.76 g, 0.02 mol) and chloroacetone (0.01 mol) were added. The reaction mixture was stirred at room temperature for 7 h and then diluted with cold water (50 ml). The resulting solid product was collected by filtration, washed with water, dried and recrystallized from ethanol, m.p. 200°C; yield 72% IR $\nu(\text{cm}^{-1})$: 3390, 3300 (NH₂), 3078 (CH-Ar); 2985 (CH aliph.), 1663 (C=O Acetyl), 1604 (C=C). $^1\text{H-NMR}$ (DMSO- d_6): δ 2.32 (s, 3H, COCH₃), 4.58 (s, 2H, NH₂), 6.04 (s, 1H, pyridine H-5), 8.21-8.25 (m, 8H, Ar-H), 8.69, 9.07 (2d, 2H, CH styryl, J = 6.30 Hz).

^{13}C : δ 27.76 (CH₃), 116.74; 117.16, 117.78; 127.40; 127.95; 128.07; 132.33, 133.80, 141.00, 143.00 and 200.14 (CO).

Anal.Calcd. for C₂₁H₁₆N₂O₂S: C, 70.00; H, 4.44; N, 7.77; S, 8.88. Found: C, 70.10; H, 4.40; N, 7.75; δ , 8.90.

Pyrrolo[3,4-f] quinolino[2,3: 6',7'] thiophene derivative (9).

A solution of 8 (0.01 mol) with N-phenyl maleimide in ethanol (50 ml). The reaction mixture was heated under reflux for 7 hrs. The solid product obtained after cooling were filtered off and crystallized from dioxan, m.p. 350°C, yield 65%.

IR $\nu(\text{cm}^{-1})$: 3350, 3175 (NH₂), 3079 (CH-Ar), 2979 (CH aliph.), 1750, 1700 (-CO-NPh-CO-), 1625 (CO-H bonded), 1604 (C=C).

$^1\text{H-NMR}$ (DMSO- d_6): δ 2.88 (s, 3H, COCH₃), 4.97 (s, 2H, NH₂), 7.29-7.95 (m, 13H, ArH); MS, m/z=529(M⁺) Anal. Calcd. for C₃₁H₁₉O₄N₃S:C, 70.32; H, 3.59, N, 7.93;

S, 6.04. Found: C, 70.30, H, 3.60; N, 7.95; S, 6.00. The mass spectrum of (9) showed a molecular ion peak $m/z=529$ (Fig. I)

9-Methyl-7-phenyl pyrido [3',2':4,5] thieno[3,2-d] pyrimidine-4 (3H)-one (11).

Method A:

A mixture of **10** (0.01 mol) and triethyl orthoformate (3 ml) was refluxed for 5h in ethanol in the presence of few drops of acetic acid. The solid product separated from the hot mixture was filtered and recrystallized from Dioxan, m.p. 260°C, yield 85%.

IR $\nu(\text{cm}^{-1})$: 3434 (OH), 3046 (CH-Ar), 2920 (CH-aliph.), 1667(CO pyrimidine), 1600 (C=C).

$^1\text{H-NMR}$ (DMSO- d_6): δ 2.51 (s, 3H, CH_3), 7.53- 7.58 (m, 6H, ArH and pyridine $\text{C}_8\text{-H}$), 8.13 (s, 1H, pyrimidine CH), 8.24 (br, 1H, NH).

^{13}C : δ 21.00 (CH_3), 113.74, 119.04, 127.78(CH-pyridine), 129.62, 166.90 (CH-pyrimidine), 168.60 (C=O), 127.78, 129.62.

Anal. Calcd. for $\text{C}_{16}\text{H}_{11}\text{N}_3\text{OS}$: C, 65.52; H, 3.75; N, 14.33; S, 10.92.

Found : C, 65.50; H, 3.81. N, 14.30. S, 10.90. Mass spectrum showed a molecular ion peak $m/z = 293$ (100%), other significant peaks appear at 267(11.96%) 237 (8.01%) 193 (3.08%).

Method B

A solution of **10** (0.01 mol) and formic acid (20 ml) was heated under reflux for 4h. The solid product was collected filtrated washed with ethanol, dried then crystallized from dioxane to give **11**.

4-Chloro-9-methyl-7-phenyl pyrido[3',2':4,5]thieno[3,2-d] pyrimidine (12).

A sample of the pyrimidinone derivative **11** (3g) in phosphorous oxychloride (15 ml) was refluxed for 4h, then cool. The reaction mixture was poured into ice/water mixture and the solid product was collected by filtration and recrystallized from ethanol, m.p. 165°C, yield 65% IR $\nu(\text{cm}^{-1})$: 3032 (CH-Ar), 2957 (CH aliph.), 1640 (C=N), 1561 (C=C); MS; m/z 311(M^+).

Anal.Calcd. for $C_{16}H_{10}ClN_3S$: C, 61.63; H, 3.21; N, 13.48; S, 10.27; Cl, 11.39, Found : C, 61.60; H, 3.20; N, 13.50; S, 10.20; Cl, 11.40.

4-Hydrzino-9-methyl-7-phenyl-pyrido [3',2': 4,5] thieno [2,3-d]

pyrimidine (13).

A mixture of the chloro compound **12** (0.01 mol) and hydrazine hydrate (0.12 mol) in ethanol (50 ml) was refluxed for 2h. The solid product separated from the hot mixture was filtered off and recrystallized from dioxan; m.p. 320°C; yield 75%.

IR $\nu(\text{cm}^{-1})$: 3390, 3210, 3150 (NH, NH_2), 3050 (CHAr), 2950 (CH aliph.); 1625 (C=N); 1590 (C=C).

$^1\text{H-NMR}$ (DMSO- d_6): δ 2.50 (s, 3H, CH_3), 4.03 (s, 2H, NH_2) 6.90 (s, 1H, pyridine H-8), 7.28-7.49 (m, 5H, Ar-H) 7.70 (s, 1H, pyrimidine H-2), 10.04 (s, 1H, NH)
Anal.Calcd. for $C_{16}H_{13}N_5S$: C, 62.54; H, 4.23; N, 22.80; S,10.42. Found: C, 62.50; H, 4.25; N, 22.80, S, 10.40.

11-Methyl-9-phenyl-1,2,4-triazolo[4'',3''':1',6']pyrimido[4',5':4,5'] thieno[2,3-b] pyridine (14).

A mixture of **13** (0.01 mol) and triethyl orthoformate (2 ml) in ethanol (30 ml) in the presence of a few drops of acetic acid was refluxed for 5h. The solid crystals separated from the hot mixture was filtered off and recrystallized from acetic acid; m.p. 280°C; yield 70%.

IR $\nu(\text{cm}^{-1})$: 3046 (CH-Ar), 2890 (CH aliph.), 1654 (C=N) $^1\text{H-NMR}$ (DMSO- d_6) δ 2.50 (s, 3H, CH_3): 7.53-7.55 (m, 6H, Ar-H and C-H pyridine); 8.23, 9.86 (2s, 2H, CH-pyrimidine and CH-triazol).

^{13}C : δ 21.40 (CH_3); 127.40 (CH-pyridine), 129.90, 131.13, 132.45, 134.60 and 161.97 (CH-pyrimidine); 171.00 (CH-triazole).

Mass spectrum showed a molecular ion peak $m/z=317$ (100%) (Fig. II)

Anal. Calcd for $C_{17}H_{11}N_5S$: C, 64.35; H, 3.47; N, 22.08; S,10.09. Found: C, 64.40; H, 3.50; N, 22.01; S, 10.00.

9-Methyl-7-phenylpyrido[5,4-b]thieno[3',2'-d]-(1,2,3)-triazin-4(3H)-one (15).

A cold solution of sodium nitrite (0.01 mol) was added to a cold solution of **10**, ethanol (20 ml) and conc. Hydrochloric acid (0.5 ml) portionwise during period of 30 min. The reaction mixture was stirred for further 2h. in ice bath. After stirring was completed, the solid product obtained was collected by filtration, washed with water, dried, then crystallized from ethanol, m.p. 210°C; yield 60%.

IR $\nu(\text{cm}^{-1})$: 3432 (OH), 3315 (NH), 3070 (CH-Ar) 2942 (CH aliph.), 1668 (C=O) triazine); 1590 (C=C) $^1\text{H-NMR}$ (DMSO- d_6): δ 2.50 (s, 3H, CH₃), 7.20 (s, 1H, pyridine-H), 7.49-7.75 (m, 5H, Ar-H), 8.13 (s, 1H, NH exchangeable with D₂O); MS: m/z = 294 (73.87%) (Fig. III), Anal. Calcd. for C₁₅H₁₀N₄OS: C, 61.22; H, 3.40; N, 19.04; S, 10.88. Found : C, 61.20, H, 3.44; N, 19.00; S, 11.00.

3- Amino-2-cyano-4-methyl-6-phenyl-thieno [2,3-b] pyridine 17.

To a solution of **6** (0.005 mol) in ethanolic sodium ethoxide solution in (0.5 g, 0.02-atom of sodium 25 ml of ethanol), chloroacetonitrile (0.005 mol) was added and the mixture was heated under reflux for 1h. After cooling , the solid product was collected and recrystallized from ethanol: m.p. 130°C; yield 75%.

IR $\nu(\text{cm}^{-1})$: 3335, 3231 (NH₂), 3050 (CH-Ar), 2900 (CH-aliph.), 2191 (C≡N), 1580 (C=C). $^1\text{H-NMR}$ (DMSO- d_6): δ 2.58 (s, 3H, CH₃), 5.58 (s, 2H, NH₂), 6.58 (s, 1H, pyridine H-5), 7.17-7.84 (m, 5H-Ar-H).

^{13}C : δ 20.00(CH₃), 116.29 (C≡N), 122.67, 127.55, 129.04, 129.44, 130.53, 137.68.

Anal. Calcd. for C₁₅H₁₁N₃S: C, 67.92; H, 4.15; N, 15.84; S, 12.07. Found : C, 67.90; H, 4.00; N, 15.90; S, 12.00.

2-Cyano-3-ethoxymethyleneamino-4-methyl-6-phenylthieno[2,3-b]pyridine (18).

A mixture of **17** (0.01 mol) and triethyl orthoformate (0.02 mol) in acetic anhydride (10 ml) was refluxed for 5h, then cool. The solid product was filtered off, washed several times with cold ethanol and recrystallized from ethanol; m.p. 170°C, yield 80%.

IR $\nu(\text{cm}^{-1})$: 3050 (CH-Ar), 2985-2870 (CH-aliph.) 2194 (C≡N), 1590 (C=C).

$^1\text{H-NMR}$ (DMSO- d_6): δ 0.99 (t, 3H, CH₂CH₃, J=14.30) 2.51 (s, 3H, CH₃), 4.06 (q, 2H, CH₂ CH₃, J=11.10) 7.23-7.83 (m, 6H, Ar-H) and pyridine H-5) 8.21 (s, 1H, N=CH).

^{13}C : δ 14.63 (CH_3), 20.24 (CH_3), 63.94 (CH_2); 114.88 ($=\text{CH}$); 120.14 ($\text{C}\equiv\text{N}$); 127.00 (CH -pyridine) and 128.11, 129.00, 130.01, 137.35, 137.80. Anal. Calcd for : $\text{C}_{18}\text{H}_{15}\text{N}_3\text{OS}$: C, 67.28; H, 4.67; N, 13.08; S, 9.96.

Found : C, 67.30; H, 4.60; N, 13.00; S, 9.90.

3-Amino-4-imino-9-methyl-7-phenyl pyrimido [4',5': 4,5] thieno[2,3-b] pyridine (19).

A sample of compound **18** (0.01 mol) was dissolved in dioxan (50 ml) and then hydrazine hydrate (0.01 mol) was added drop wise while stirring . Stirring was continued for 4h, during this period of time, solid crystals were separated. The mixture was heated on water bath for 1h, cool and the solid product was collected by filtration. Recrystallized from dioxan; m.p. 270°C. yield 90 % IR (cm^{-1}): 3305, 3259, 3140 (NH_2 , NH) 1640 ($\text{C}=\text{N}$), 1605 ($\text{C}=\text{C}$).

^1H -NMR (DMSO-d_6): δ 2.58 (s, 3H, CH_3), 5.31 (s, 2H, NH_2), 7.32-8.04 (m, 6H, Ar-H and pyridine H-8), 8.59 (s, 1H, pyrimidine H-2), 12.62 (s, 1H, NH). Anal. Calcd for $\text{C}_{16}\text{H}_{13}\text{N}_5\text{S}$: C, 62.54; H, 4.23; N, 22.80; S, 10.42. Found: C, 62.50; H, 4.20; N, 22.80; S, 10.40.

5,7,13-Trimethyl-11-phenyltriazipino [2'', 3'':1', 6'] pyrimido [4',5': 4,5']thieno[2,3-b] pyridine (20).

A mixture of compound **19** (0.005 mol) and acetyl acetone (0.005 mol) in ethanol (30 ml) was refluxed for 8 hrs. The solid crystals separated from the hot mixture was filtered off and recrystallized from dioxan, m.p. 180°C; yield 90% IR (cm^{-1}): 3055 (CH -Ar), 2900-2800(CH -aliph.) ^1H -NMR (DMS-d_6): δ 2.01 (s, 3H, CH_3 -triazipin ring), 2.20 (s, 3H, CH_3 -triazipin ring), 2.50 (s, 3H, CH_3 pyridine ring), 6.28 (s, 1H, triazipin-H-6), 7.49-8.34 (m, 6H, Ar-H and H-pyridine), 9.05 (s, 1H, pyrimidin, H-2); Mass spectrum m/z 371 (43.11%) (Fig. IV). ^{13}C : δ 16.30 (CH_3); 19.72 (CH_3); 20.42 (CH_3); 127.77 (CH -pyridine); 129.44 (CH -triazipin); 130.00, 130.66, 131.02, 132.68, 133.11 and 151.57 (CH -pyrimidine).

Anal, Calcd. for $\text{C}_{21}\text{H}_{17}\text{N}_5\text{S}$: C, 67.92; H, 4.58; N, 18.86; S, 8.62 Found: C, 67.90; H, 4.60; N, 18.80; S, 8.58.

9-Methyl-7-phenyl pyrimido[4',5': 4,5] thieno[2,3-b] pyridine-2,4(1H, 3H)-dithione 21.

A sample of **17** (0.5 g) and carbon disulfide (3 ml) in pyridine (10 ml) was heated on water bath until the hydrogen sulfide ceased 10hr., then, cool. The solid product was filtered off, washed several times with ethanol and recrystallized from dioxan; m.p. 280°C; yield 85%

IR $\nu(\text{cm}^{-1})$: 3412 (NH); 3050 (CH-Ar); 2950 (CH-aliph); 2700 (SH) $^1\text{H-NMR}$ - (DMSO- d_6): δ 2.50 (s, 3H, CH₃); 6.61 (s, 1H, C₃-Hpyridine); 7.42-8.02 (m, 5H, Ar-H); 8.16, 8.61 (2s, 2H, 2NH).

^{13}C : δ 21.00 (CH₃); 127.61 130.95, 136,11, 133.11; 135.95, 136.11 and 184.00 (C=S); 186.40 (C=S), Mass spectrum: m/z 341 (0.5%) with base peak m/z = 265(100%).

Anal.Calcd for: C₁₆H₁₁N₃S₃: C, 56.30; H, 3.22; N, 12.31; S, 28.15, Found: C 56.11; H, 3.50; N, 12.00; S, 28.80.

Biological Results and Structure Activity Correlation**Antitumor activity (in vitro-study)**

Potential cytotoxicity of the synthesized compounds was tested using the method of Skehan *et al.* [16]. Cells were plated in 96-multi well plate (10 cells/Well) for 24 hrs before treatment with the compound to allow attachment of cells to the wall of the plate. Different concentration of the compounds under test (0, 10, 25, 50 and 100 $\mu\text{g}/\text{m}$) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hrs at 37°C and in atmosphere of 5% CO₂. After 48 hrs, cells were fixed, washed and stained with sluo-Rhodamine-B-stains. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and compound drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound.

The relation between surviving fraction and compound concentration was plotted to obtain the survival curve of tested cell, the response parameter calculated was IC₅₀ value. The data to tested compounds are summarized in Table I and II.

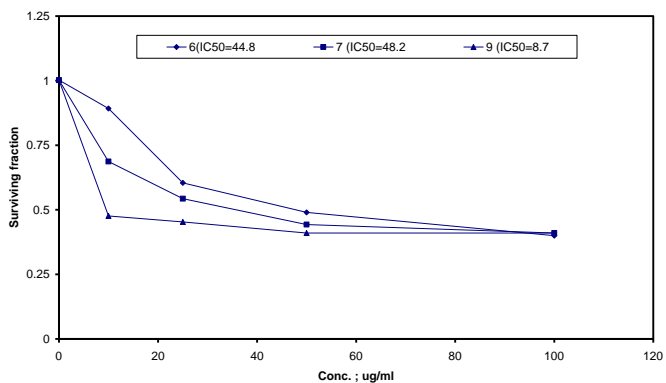
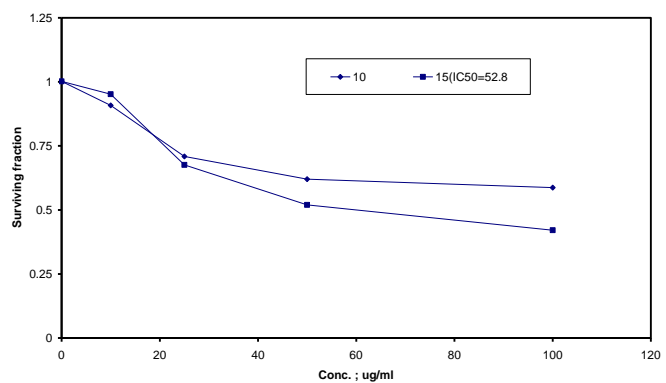
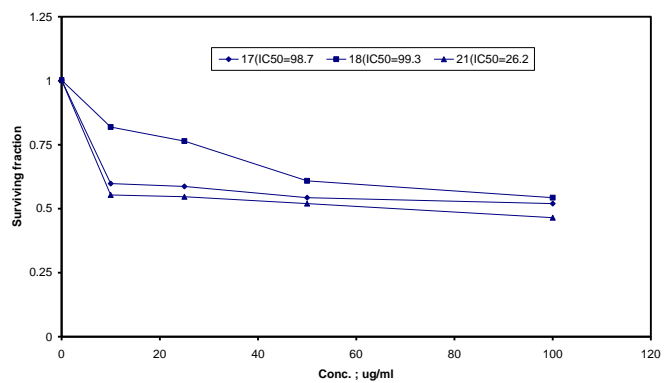
In Vitro Anti-HPG2 testing**Fig. 4****Fig. (5)****Fig. (6)**

Fig. 4,5,6: The inhibitory effect of compounds 6,7,9,10,15,17,18 and 21 concentration on HEPG2 cells activity.

Compounds **6** and **7** having thiol group are nearly active as the positive control Doxorubicin [17] with IC_{50} , 44.8 and 48.2 $\mu\text{g/ml}$. Pyrrolo [3,4-f] quinolino [2,3:6,7] thiophene **9** is more effective than the positive control (Doxorubicin) with IC_{50} 8.7 $\mu\text{g/ml}$. Compound **10** proved to posses moderate activity against HEPG2 cell lines. On the other hand cyclization of compound **10** yielded pyridothienotriazinone derivative **15** proved to be active toward the HEPG2 cell IC_{50} 52.8 $\mu\text{g/ml}$. Compound **17** and **18** showed cytotoxicity to HEPG2 cells at IC_{50} (98.7 and 99.3 $\mu\text{g/ml}$). Compound **21** proved to be active member than the positive control (Doxorubicin) among the pyrimidine dithione molecule with IC_{50} 26.2 $\mu\text{g/ml}$ (Table I).

Table I. In Vitro Anti-HEPG2^a Testing Results

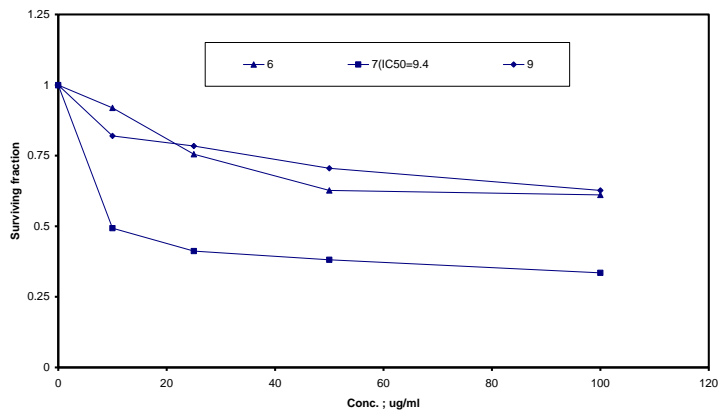
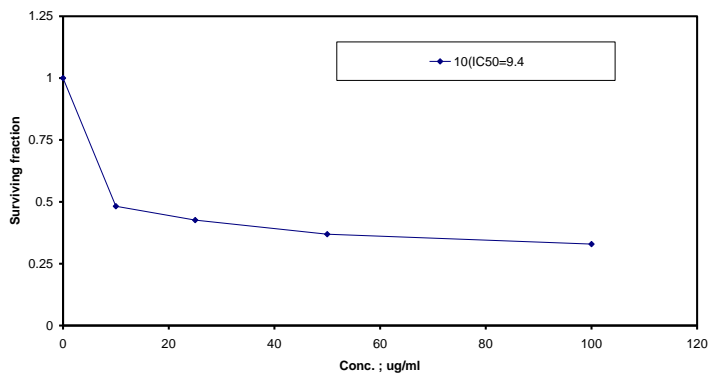
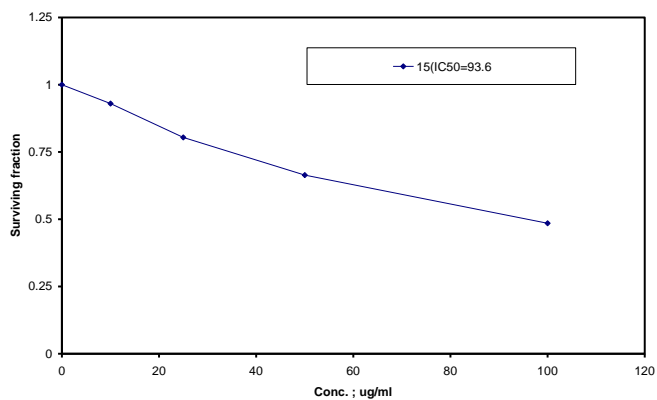
Comp	IC_{50}^b ; $\mu\text{g/ml}$
6	44.8
7	48.2
9	8.7
15	52.8
17	98.7
18	99.3
21	26.6
Doxorubicin ^c	43.6

^a Liver carcinoma cell line, ^b concentration of compounds which cause 50% inhibition of cell growth, ^c positive control^[17].

Table II. In vitro Anti-MCF7^a Testing Results.

Comp	IC_{50}^b ; $\mu\text{g/ml}$
7	9.4
10	9.4
15	93.6
17	100
21	48.3
Doxorubicin ^c	43.6

^a Breast carcinoma cell line, ^b concentration of compounds which cause 50% inhibition of cell growth, ^c positive control

In Vitro Anti-MCF7 testing**Fig. 7****Fig. (8)****Fig. (9)**

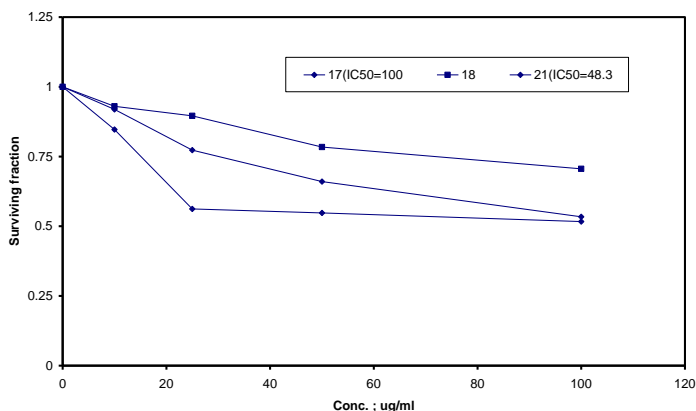


Fig. (10)

Fig. 7,8,9, 10: The inhibitory effect of compounds 6,7,9,10,15,17,18 and 21 concentration on MCF7 cells activity.

Compounds **6** and **9** proved to possess moderate activity against MCF7 cell line but compound **7** with increase magnitude of active at IC₅₀ 9.4 µg/ml is more effective than the positive control (Doxorubicin, due to olefinic function of the styryl pyridinethione [18]). MCF7 cell lines proved to be sensitive toward compound **10** than the positive control (Doxorubicin with IC₅₀ 9.4 µg/ml, while proved to be moderate activity towards compounds **15**, **17** and **18**. Also compound **21** having thiol group is nearly as active as the positive control (Doxorubicin) with IC₅₀ 48.3 µg/ml (Table II).

The attempt to connect the variation in the back bone of the synthesized heterocycles and the pattern of substitution on those heterocyclic ring system proved to be most active antitumor agent.

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الملخص العربى

تحضير وتفاعلات مشتقات البيريدين وثينو [2، 3 - ب] بيريدين المتوقع لها نشاط بيولوجى

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يتضمن هذا البحث تشييد عدد من المركبات الحلقية غير المتجانسة وذلك باستخدام مركب سيانوثنيوأسيتاميد حيث يتفاعل مع مركب أريل هيدرازون لمركب البنزويل أسيتون (1) ليعطى (2) الذى يتفاعل مع كل من فينسيل بروميد ليعطى المركب (5) ويوديد الإيثيل ليعطى المركب (3) الذى يتم تحلقه وذلك بالغليان فى أثيروكسيد الصوديوم ليعطى المركب (4). كما تمت مفاعلة مركب سيانوثنيوأسيتاميد مع البنزويل أسيتون ليعطى المركب (6) الذى يتم مفاعله مع 2-فيوالدهيد ليعطى المركب (7) الذى يتفاعل مع الكلورواسيتون ليعطى المركب (8) حيث يتفاعل مع N- فينيل ماليميد ليعطى المركب (9).

وقد تمت مفاعلة المركب (6) مع كلورواسيتاميد ليعطى المركب (10) الذى تمت مفاعله مع كل من ترائى إيثيل فورمات أو حمض الفورميك ليعطى المركب (11) الذى يتفاعل مع أوكسى كلوريد الفوسفور ليعطى المركب (12) الذى يتم مفاعله مع هيدرازين هيدرات ليعطى المركب (13) الذى يتم تحلقه وذلك بمفاعله مع ترائى إيثيل فورمات ليعطى مشتق ترائى ازول مركب (14) وأيضاً تمت مفاعلة المركب (10) مع حمض النيتروز ليعطى المركب (15).

يتفاعل مركب (6) مع كلوريد أسيتونيتريل ليعطى المركب (17) الذى يتم مفاعله مع ترائى إيثيل فورمات ليعطى مركب (18) حيث يتم تحلقه ليعطى المركب (19) وذلك بتفاعله مع هيدرازين هيدرات وأيضاً تم الحصول على المركب (20) وذلك بتفاعل المركب

19 مع اسيتيل اسيتون. كما تمت مفاعلة المركب (17) مع الكربون داى سلفيد ليعطى المركب (21).

وقد تم دراسة التأثير البيولوجى للمركبات المحضرة على خلايا الكبد والثدى المسرطنة وقد أظهرت المركبات 6، 7، 9، 15، 17، 18، 21 تأثير سُمى تجاه خلايا الكبد المسرطنة بينما أظهرت المركبات 6/9، 7 سُمية تجاه خلايا الثدى المسرطنة ولقد أظهر المركب 10 سُمية تجاه خلايا الثدى المسرطنة بينما كان متوسط التأثير تجاه خلايا الكبد المسرطنة ولقد أظهرت المركبات 15، 17، 18 تأثير متوسط تجاه السمية لخلايا الثدى المسرطنة.

ولقد أمكن اثبات التركيب البنائى لهذه المركبات اعتماداً على التحليل الكيمياءى العنصرى ودراسة أطياف الأشعة تحت الحمراء والرنين النووى المغناطيسى والكربون-13 وطيف الكتلة.