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Synthesis and Biological Evaluation of Thieno[2,3-c]pyridines and Related Heterocyclic Systems

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Reaction of 3,5-*bis*(pyrrolidene)-4-piperidone (**1**), with malononitrile. Ethyl cyanoacetate and benzoyl acetonitrile in basic medium to give pyrano[3,2-c]pyridine derivatives (**2a-c**). The treatment of compounds **2a-c** with acetic acid and ammonium acetate led to the conversion of pyran ring to pyridine ring in compound **3a-c**. Reaction of **1** with 2 mole of malononitrile afforded aminoquinolizinone derivative (**4a**). Reaction of **5** with thiosemicarbazide, acetylacetone and cyanothioacetamide gave pyrazolo[3,4-c]pyridine (**6**), pyrido[4,3-c]-4-cyclohexene 5-one and pyrido[2,3-c]pyridine-2-thione (**8**) derivatives. Interaction of 8 with *p*-cyanoacetophenol afforded thieno[2,3-b]-(1,6)naphthyridine derivative (**10**). The formation of thieno[2,3-c]pyridine (**11a-c**) from Gewald reaction products. On the other hand, reaction of **11a,b** with carbon disulfide gave pyridothienopyrimidine derivatives (**12a,b**). Alkylation of **12a,b** with ethyl iodide gave **13b** and **14a**. Condensation of 1-methyl-4-piperidone with malononitrile gave 1-methyl-4-piperidonylidene malononitrile (**15**). Reaction of **15** with *p*-chlorobenzaldehyde afforded 3-*p*-chlorobenzylidene-1-methyl-4-piperidonylidene malononitrile (**16**). 6-Amino-5,7,7-tricyano-8-*p*-chloro-phenyl-2-methyl-1,2,3,4,7,8-hexahydroisoquinoline (**17**) was performed by the treatment of 1-methyl-4-piperidone with a mixture of one equivalent of *p*-chlorobenzaldehyde and two equivalent of malono-nitrile. The synthesized compounds were screened for their *in vitro* antitumor activity at the National Center Institute (NCI).

Key Words: Synthesis, Biological activities, Thieno[2,3-c]pyridines, Heterocyclic systems.

INTRODUCTION

Several annulated pyridines isolated from natural sources possess a broad spectrum of therapeutic activity. Members of this class were found to be protectors against gastric erosions[1] and coronary vasodilator and blood heightening agents[2]. They also proved to be tuberculastatic, antiviral, fungicidal, insecticidal and pesticidal[3,4] agents. They were used as inhibitors or aldose reductas and inhibitors of cataract formation in diabetics[5]. In addition to the previous mentioned properties of annulated pyridines, many thienopyridines have been evaluated pharmacologically and they have been used as analgesics and anti-inflammants and showed activity against diabetes mellitus[6-9].

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Quinolizine derivatives have attracted a great deal of interest due to their biological activities such as anti-HIV[10] and as potent selective human steroid[11,12]. In junction with our interest in the synthesis of functionally substituted heteroaromatic compounds as potential pharm-aseticals[13,14].

EXPERIMENTAL

All melting points were taken on Gallen Kamp melting apparatus and are uncorrected. The IR spectra were recorded on a pye-unicam Sp-3-100 spectrophotometer using KBr Wafer Technique. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker 400 MHz with ($CDCl_3$) as solvent and tetramethylsilane as an internal standard, chemical shifts are as δ units (ppm). The mass spectra were recorded on Ms-S 988 operating at 70 eV. Elemental analysis was determined using a Perkin-Elmer 240C microanalyses. The newly synthesized compounds were screened *in vitro* antitumor activity at Cairo University, National Center Institute, Cancer Biology Department, Pharmacology unit.

Compound **5** and **11a** were prepared according to the literature procedure[15,16].

1- Methyl-3,5-*bis*(pyrrolidene)-4-piperidone (1): A mixture of 1-methyl-4-piperidone (0.01 mol) and 2-pyrrole carboxaldehyde (0.02 mol) in alcoholic NaOH (50 mL, 10 %) was stirred at room temperature for 2 h. The separated solid was filtered and recrystallized from ethanol.

General procedure for the synthesis of 2- Amino-3-(cyano/or carboxylate/or benzoyl)-6-methyl-4-(2-pyrrole)-8-(2-pyrrole methylene)-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-c]pyridine 2a-c: A mixture of (0.01 mol) and malononitrile or ethyl cyanoacetate or benzoylacetonitrile (0.01 mol) in ethanol (20 mL) and piperidine (0.5 mL) was refluxed for 4 h, then solution was evaporated under reduced pressure. The solid product were collected by filtration and recrystallized from ethanol to give **2a-c**.

General procedure for the synthesis of 3-(cyano/or carboxylate/or benzoyl)-6-methyl-4-(2-pyrrole)-8-(2-pyrrole methylene)-5,6,7,8-tetrahydropyrido-[3,2-c]pyridine-1*H*-2-one (3a-c): A solution of each **2a-c** (0.01 mol) in acetic acid (30 mL) and ammonium acetate (2 g) was heated under reflux for 2 h. The mixture allowed to cool to room temperature, then poured into ice-cold water. The solid product were collected by filtration and recrystallized from a mixture of ethanol and DMF (2:1).

3-Cyano-6-methyl-4-(2-pyrrole)-8-(2-pyrrole methylene)-5,6,7,8-tetrahydropyrido[3,2-c]pyridine-1*H*-2-thione (3d) and 3-cyano-4-(2-thienyl)-6-methyl-8-(2-thienylidene)-5,6,7,8-teterahydropyrido[3,2-c]pyridine-1*H*-2-thione (8): A mixture of **1** or **5** (0.01 mol) and cyanothioacetamide (0.01 mol) in ethanol (20 mL) and piperidine (0.5 mL) was refluxed for 3 h. The excess of solvent was evaporated under reduced pressure. The solid product was collected by filtration and crystallized from a mixture of ethanol and DMF to afford **3d**.

6-Amino-4,10-Di(2-pyrrole)-8-oxoquinolizine-5,9-dicarbonitrile derivative 4a

Method A: A mixture of compound **1** (0.01 mol), malanonitrile (0.02 mol) in ethanol (30 mL) and few drops of piperidine were refluxed for 3 h. The reaction mixture was allowed to cool to room temperature, then poured into ice cold water and neutralized with HCl. The solid product, so formed was collected by filtration and recrystallized from DMF/ETOH (2:1).

Method B: To a mixture of compound **3a** (0.01 mol) and malononitrile (0.01 mol) in DMF (30 mL), few drops of piperidine were added. The mixture was refluxed for 5 h, then allowed to cool, poured into ice-cold water and neutralized with HCl. The solid product so formed was collected by filtration and recrystallized from DMF/ETOH (2:1).

2-Isothiocarbamoyl-3,3a,4,5,6,7-hexahydro-5-methyl-7-(2-thienylidene)-3-(2-thienyl)-2*H*-pyrazolo[4,3-c]pyridine (6): The mixture of thiosemicarbazide (30 mmol) and compound **5** (10 mmol) was refluxed in ethanol (110 mL) containing 9 % concentrated hydrochloric acid for 6 h. The reaction mixture was cooled down and the precipitate was filtered, washed with cold ethanol and water until neutral. The separated precipitate was filtered off. It was recrystallized from methanol.

6-Acetyl-1-methyl-3(2-thienylidene)-7-(2-thienyl)1,2,3,8-tetrahydro-1*H*-pyrido[4,3-c]-4-cyclohexene-5-one (7): A sample of compound **5** (0.01 mol) in methanolic sodium methoxide (0.25 g in 50 mL methanol) was refluxed for 8 h. The solid product separated from the hot mixture was filtered off and washed with water. The separated precipitate was filtered off and recrystallized from methanol.

3-Amino-2-(4-cyano-benzoyl)-6-methyl-4-(2-thienyl)-8-(2-thienylidene) 5,6,7,8-tetrahydrothieno [2,3-b]-(1,6)-naphthyridine (10): A mixture of **8** (10 mmol) and *p*-cyanoacetophenone (10 mmol) was refluxed in acetic acid (20 mL) containing a few drops of concentrated H_2SO_4 for 3 h. The reaction mixture was cooled and neutralized with NH_4OH solution. The resulting precipitate was collected by filteration. Washed with water several times and dried. The crude product was crystallized from ethanol.

2-Amino-6-methyl-3-substituted-4,5,6,7-tetrahydrothieno[2,3-c] pyridines (11a-c): To a stirred mixture, heated at 50-60 ºC, of 1-mehtyl-4-piperidone, active methylene nitrile and sulphur in ethanol (50 mL), morpholine (2 mL) was added dropwise. Stirring was continued at this temperature for 1 h, then the reaction mixture was left to cool and poured into ice-cold water. The solid product formed was crystallized from ethanol.

7-Methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-d]pyrimidine-(1*H*, 3*H*)-(2,4-dithione or 4-one-2-thione) (12a,b): A mixture of **11a** or **11b** (0.01 mol) and CS_2 (5 mL) in pyridine (30 mL) was refluxed for 8h. The reaction mixture was then allowed to cool and the solid product was filtered off and recrystallized from ethanol to give **12a,b**.

Alkylation of 12a,b (formation of 13b and 14a): A mixture of **12a,b** (0.01 mol), ethyl iodide (0.02 mol) and used sodium acetate (0.02 mol) [one equivalent

(0.01 mol) of both sodium acetate and ethyl iodide used for (0.01 mol) of **12b**] in ethanol (50 mL) was refluxed for 2 h. The solid product obtained after cooling was collected and recrystallized from ethanol.

1-Methyl-4-piperidylidine malononitrile (15): To a mixture of malononitrile (0.1 mol) and 1-methyl-4-piperidone (0.1 mol) in ethanol (50 mL) a few drops of diethyl amine was added. The mixture was stirred at room temperature for 1 h. The solid product was collected and recrystallized from benzene.

Condensation of compound 15 with *p*-chloro benzaldehyde (16): To a mixture of compound **15** (0.01 mol) and *p*-chloro benzaldehyde (0.01 mol) in (50 mL) ethanol, a few drops of piperidine were added. The mixture was refluxed for 1 h, then allowed to cool. The solid product was collected and recrystallized from ethanol.

6-Amino-1,3,4,8-tetrahydro-7-dicyano-2-methyl isoquinoline-5-carbonitrile (17)

Method A: A mixture of malononitrile (0.01 mol) and compound **16** (0.01 mol) and drops of piperidine in ethanol (50 mL) was stirred for 2 h at room temperature. The solid products was collected and recrystallized from ethanol.

Method B: A mixture of malononitrile (0.01 mol), 1-methyl-4-piperidone (0.005 mol), *p*-chloro benzaldehyde (0.005 mol) and drops of piperidine in ethanol (50 mL) was stirred for 1 h at room temperature. The solid product was collected and recrystallized from ethanol.

Biological activities: Antitumor activity (*in vitro*-study)[17].

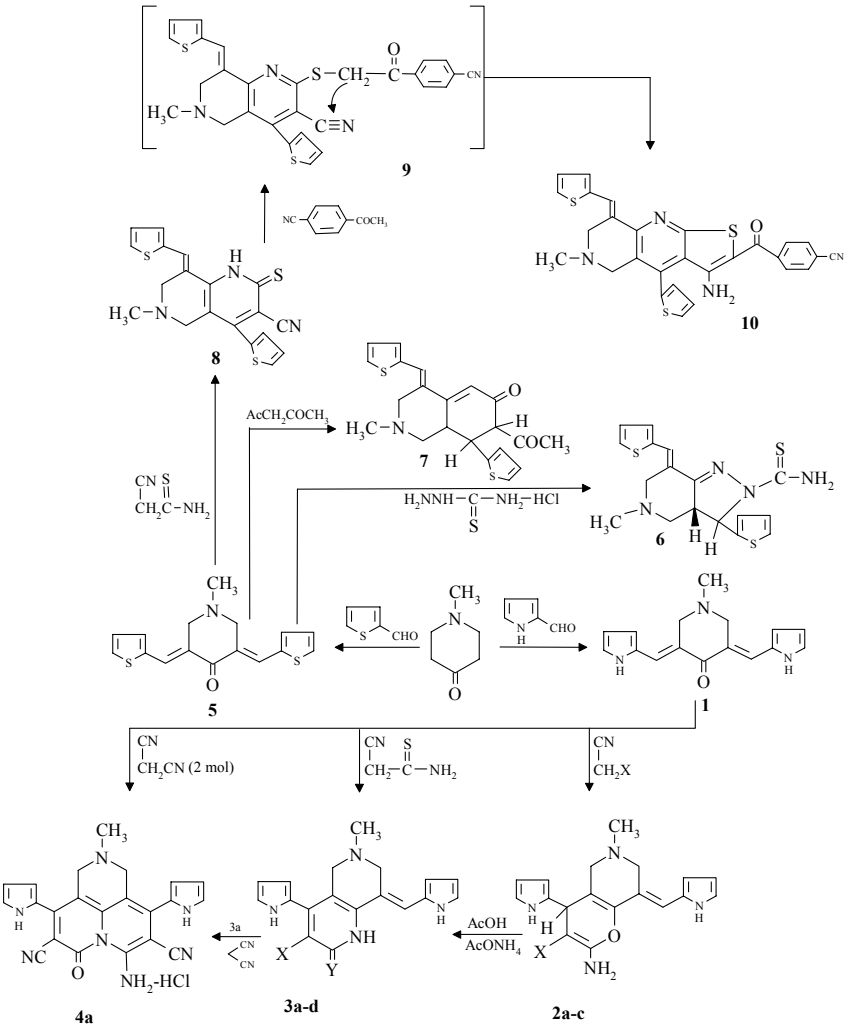
Ehrlich A sites carcinoma cells (EAC) is drawn from mice bearing, in sterile test tube, where 2.5×10^5 tumor cells/mL were suspended in phosphate buffer saline. Three different concentration for each compound (25, 50, 100 µg/mL). Add 2.5×10^5 tumor cell for each test tube at 37 °C for 2 h. The cells were tested for viability and contamination by staining, certain cell volume of this fluid by an equal volume of the working solution of trypan blue dye and examined under microscope. The dead cells stained blue and live cell not stained then carried out to calculate the percentage of nonviable cells. Compounds producing more than 70 % non-viable cell are considered active[18]

$$\% \text{ of non-viable cells} = \frac{\text{no of nonviable}}{\text{Total no. of cell}} \times 100$$

Antitumor activity (*in vitro* study): Potential cytotoxicity of the compounds was tested using the method of Skehan *et al.*[19]. Cells were plated in 96-muli well plate (10 cells/well) for 24 h before treatment with the compound to allow attachment of cell to the wall of the plate. Different concentration of the compound under test (0, 10, 25, 50 and 100 µg/m) were added to the cell monolayer triplicate wells were prepared for each individual dose. Mono layer cells were incubated with the compounds for 48 h at 37 ºC and in atmosphere of 5 % CO_2. After 48 h, cells were fixed, washed and stained with sulfo-rhodamine-B-stain. Excess stain was washed with acetic acid and attached stain was recovered with *Tris* EDTA buffer. Colour intensity was measured in an ELISA reader.

RESULTS AND DISCUSSION

The present study is a continuation of our previous efforts[20,21] aiming to locate novel synthetic compounds for future development as antitumor agents. When 1-methyl-4-piperidone was stirred with two moles of 2-pyrrole carboxaldehyde in alcoholic sodium hydroxide solution the corresponding 3,5-*bis*(pyrrolidene)-4-piperidone (**1**) was obtained (**Scheme-I**). This latter compound was treated with malononitrile, ethyl cyanoacetate and benzoyl acetonitrile in basic medium to give pyrano[3,2-c]pyridine derivative (**2a-c**) in good yield. The physical data and the elemental analysis is given in Table-1.



a : x = CN Y = O
b: x = COOEt Y = O
c: x = COPh Y = O
d: x = CN Y = S

a : x = CN
b: x = COOEt
c: x = COPh

Scheme-I

The IR spectra of isolated products showed in each case the absence of carbonyl band and revealed the presence of two bands in the regions of 3440, 3330 cm^{-1} due to the NH_2 group. Their ^1H NMR spectra showed in each case, a broad signal (D_2O exchangeable) at δ 5.21 ppm due to NH_2 protons in addition to a singlet signal at δ 4.61 ppm for pyran-4*H*. Moreover, the ^{13}C NMR of the reaction product revealed high field signals at δ 43.47 ppm corresponding to the sp^3 carbon coupled with proton[22,23].

Similar to reported[24,25] rearrangement of 2-amino pyrans **2a-c** into pyridine on refluxing in a mixture of acetic acid and ammonium acetate, compounds **2a-c** were converted into tetrahydropyrido[3,2-c] pyridine-(1*H*)-2-one derivatives (**3a-c**) by similar treatment. Similarly, compound **1** react with cyanothioacetamide in ethanol and in presence of catalytic amount of piperidine at reflux afforded product which was identified as 5,6,7,8-tetrahydropyrido[3,2-c]pyridine-(1*H*)-2-thione derivative (**3d**). Compound **1** reacts also with 2 mole of malononitrile in ethanolic piperidine at reflux in one step reaction to give a high yield of a crystalline aminoquinolizinone derivative (**4a**), for which structure **4a** as assigned. The physical data and the elemental analysis is given in Table-1.

The IR spectrum of the reaction product, showed an amino nitrile and carbonyl absorption band at 3452, 3344, 2190 and 1654 cm^{-1}, respectively, which are compatible with assigned structure. Moreover, treatment of compound **3a** with malononitrile in a basic medium afforded a product identical in all respects (m.p. and spectra) with that obtained previously from the reaction of **1** with 2 mole malononitrile.

In order to obtain potentially antitumor compound starting from 3,5-*bis*-(thienylidene)-4-piperidone (**5**) and thiosemicarbazide new bicyclic pyrazoline (**6**) has been prepared. The cyclization performed with thiosemicarbazide under acidic condition yielded only one diasteroisomer which has been separated. The physical data and the elemental analysis is given in Table-1.

The structure and the relative configuration of the compound has been determined by ^1H and ^{13}C NMR spectra in the mass spectrum revealed molecular ion peak at m/z 374 (Fig. 1). Table-2. Thus, treatment of **5** with acetylacetone in refluxing sodium methoxide yielded pyrido[4,3-c]cyclohexene-5-one derivative (**7**). Thus, the IR spectrum showed absorption hand at 1714 and 1660 cm^{-1} assignable to two carbonyl groups, respectively. It mass spectrum revealed a molecular ion peak at m/z 383 (Fig. 2). The ^1H NMR data are listed in Table-2.

Compound **5** was further utilizing for another cyclocondensation reaction using cyanothioacetamide in refluxing ethanol containing catalytic amount of piperidine to afford the pyrido[3,2-c]pyridine derivative (**8**). The interaction of compound **8** with *p*-cyanoacetophenone in boiling acetic acid containing a few drops of concentrated sulfuric acid[22] for 2 h afforded 3-amino-2-(4-cyanobenzoyl)-6-methyl-4-(2-thienyl)-8-(2-thienylidene)-5,6-7,8-tetrahydrothieno[2,3-b]-1,6-naphthyridine (**10**) in promising yield (Table-1). The structure of compound **10** was confirmed based on ^1H NMR spectrum that revealed the presence of amino group signals and also ^{13}C NMR which revealed the presence of only one CN signal.

TABLE-1
ANALYTICAL DATA OF SYNTHESIZED COMPOUNDS

Compd. No.	m.p. (ºC)	Yield (%)	m.f. (m.w.)	Elemental analysis %: Calcd. (Found)			
				C	H	N	S
1	215	85	$C_{16}H_{17}N_3O$	71.91	6.36	15.73	-
			(267)	(72.00)	(6.23)	(15.90)	-
2a	190	60	$C_{19}H_{19}N_5O$	68.46	5.70	21.02	-
			(333)	(68.23)	(5.15)	(21.40)	-
2b	140	65	$C_{21}H_{24}N_4O_3$	66.31	16.31	14.73	-
			(380)	(66.50)	(6.00)	(14.60)	-
2c	180	55	$C_{25}H_{24}N_4O_2$	72.81	5.82	13.59	-
			(412)	(72.50)	(6.00)	(13.50)	-
3a	>360	62	$C_{19}H_{17}N_5O$	68.88	5.13	21.14	-
			(331)	(68.50)	(5.00)	(21.80)	-
3b	340	80	$C_{21}H_{22}N_4O_3$	66.66	5.82	14.81	-
			(378)	(66.80)	(5.32)	(15.00)	-
3c	>360	65	$C_{25}H_{22}N_4O_2$	73.17	5.36	13.65	-
			(410)	(73.75)	(5.50)	(13.70)	-
3d	>360	88	$C_{19}H_{17}N_5S$	65.70	4.89	20.17	9.22
			(347)	(65.90)	(5.01)	(20.50)	(9.00)
4a*	250	80	$C_{22}H_{18}N_7OCl$	61.18	4.17	22.71	-
			(431.5)	(61.20)	(4.00)	(22.80)	-
6	260	75	$C_{17}H_{18}N_4S_3$	54.54	4.81	14.97	25.66
			(374)	(53.82)	(4.11)	(15.00)	(25.50)
7	280	80	$C_{21}H_{21}NO_2S_2$	65.79	5.48	3.65	16.71
			(383)	(65.71)	(5.32)	(3.10)	(16.00)
8	190	90	$C_{19}H_{16}N_2S_3$	59.84	3.93	11.02	25.19
			(381)	(59.90)	(3.21)	(10.88)	(25.06)
10	>360	80	$C_{28}H_{20}N_4OS_3$	64.12	3.81	10.68	18.32
			(524)	(64.00)	(3.00)	(10.20)	(17.89)
11b	180	80	$C_9H_{13}N_3OS$	51.18	6.16	19.90	15.16
			(211)	(51.52)	(5.95)	(19.95)	(15.58)
11c	90	85	$C_{11}H_{16}O_2N_2S$	55.00	6.66	11.66	13.33
			(240)	(55.40)	(6.60)	(11.90)	(13.20)
12a	300	80	$C_{10}H_{11}N_3S_3$	44.60	4.08	15.61	35.68
			(269)	(44.68)	(3.82)	(15.95)	(35.00)
12b	240	70	$C_{10}H_{11}N_3OS_2$	47.43	4.34	16.60	25.29
			(253)	(47.00)	(4.70)	(16.30)	(25.32)
13b	>360	75	$C_{12}H_{15}N_3OS_2$	51.24	5.33	14.94	22.77
			(281)	(51.00)	(5.32)	(14.60)	(22.71)
14a	230	75	$C_{14}H_{19}N_3S_3$	51.69	5.84	12.92	29.53
			(325)	(52.00)	(5.74)	(13.00)	(29.48)
15	80	60	$C_9H_{11}N_3$	67.08	6.83	26.08	-
			(161)	(67.10)	(7.00)	(26.00)	-
16*	300	90	$C_{16}H_{14}N_3Cl$	67.72	4.93	14.81	-
			(283.5)	(67.88)	(5.00)	(15.01)	-
17*	>360	60	$C_{19}H_{16}N_5Cl$	65.23	4.57	20.02	-
			(349.5)	(65.30)	(4.11)	(20.18)	-

4a*: Cl: Calcd: 8.22; found: 8.30; **16***: Cl: Calcd: 12.52; found: 12.12;
17*: Cl: Calcd: 10.15; found: 10.00.

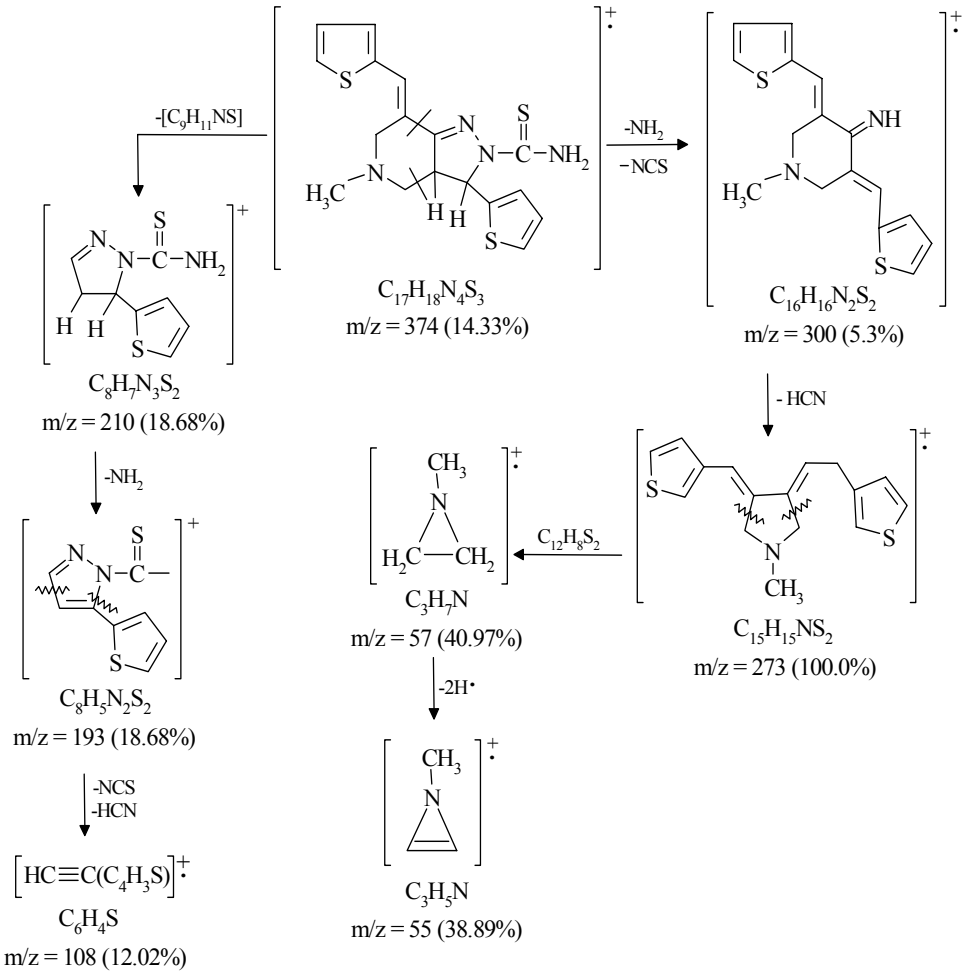


Fig. 1. Mass fragmentation pattern of compound **6**

A series of thieno[2,3-c]pyridines (**11a-c**) were prepared in one-pot reaction involving 1-methyl-4-piperidone, sulphur and an active methylene nitrile in the presence of morpholine as a catalyst (**Scheme-II**). This method is analogous to that of Gewald for the preparation of benzothiophene derivatives[23], where cyclohexanone was used instead of the 1-methyl-4-piperidone. The interaction of the *o*-amino nitrile derivative (**11a**) or the *o*-amino-carboxamide (**11b**) with carbon disulphide in pyridine gave the corresponding pyridothienopyrimidine-(1*H*, 3*H*)-2,4-dithione (**12a**) and pyridothienopyrimidine (1*H*,3*H*)-4-one-2-thione (**12b**), respectively. The ethyl thio derivatives (**13b** and **14a**) were obtained by ethylation of **12a,b** using ethyl iodide in refluxing ethanol in the presence of sodium acetate. It spectroscopic data fit perfectly with those of products (Table-2).

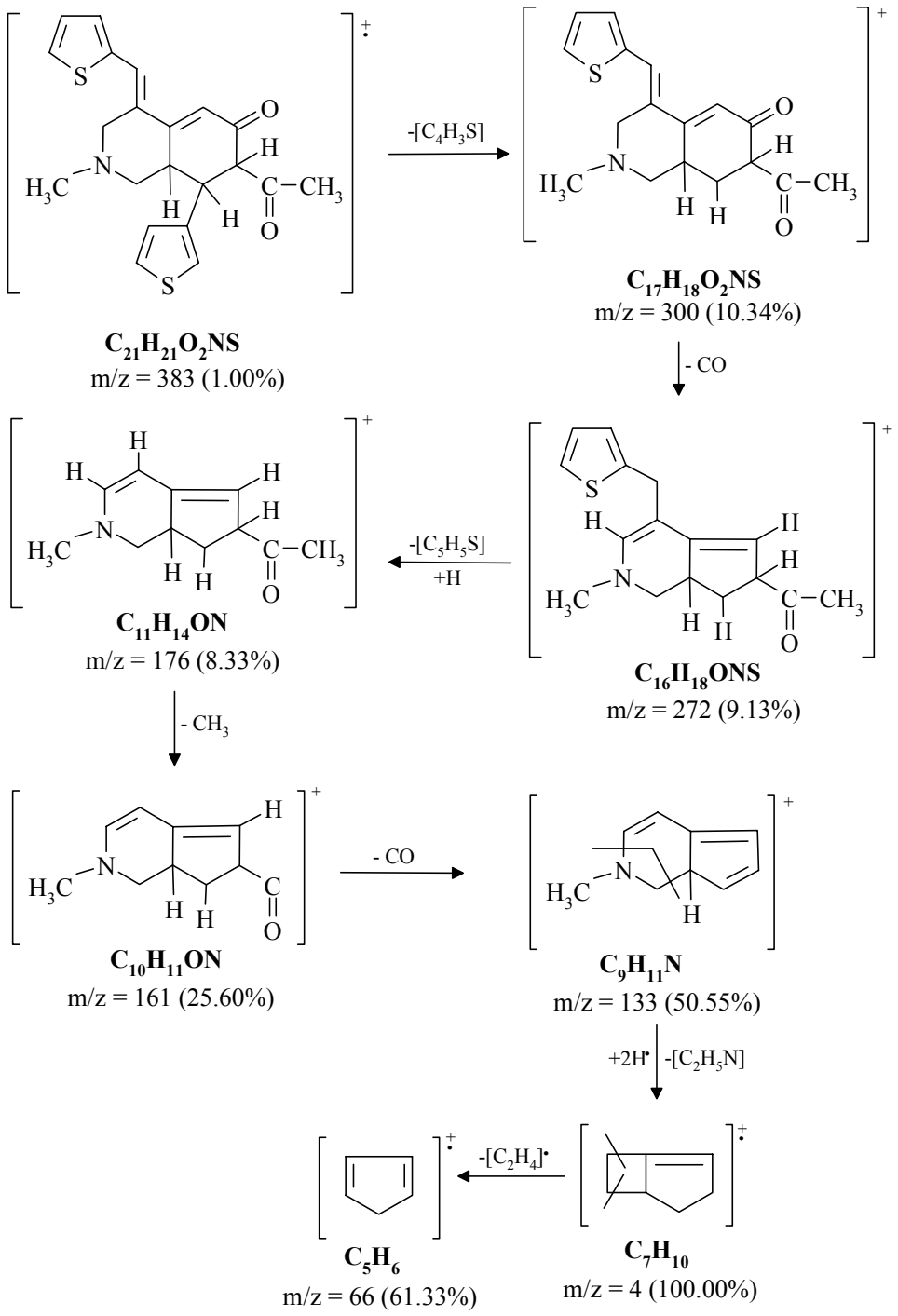


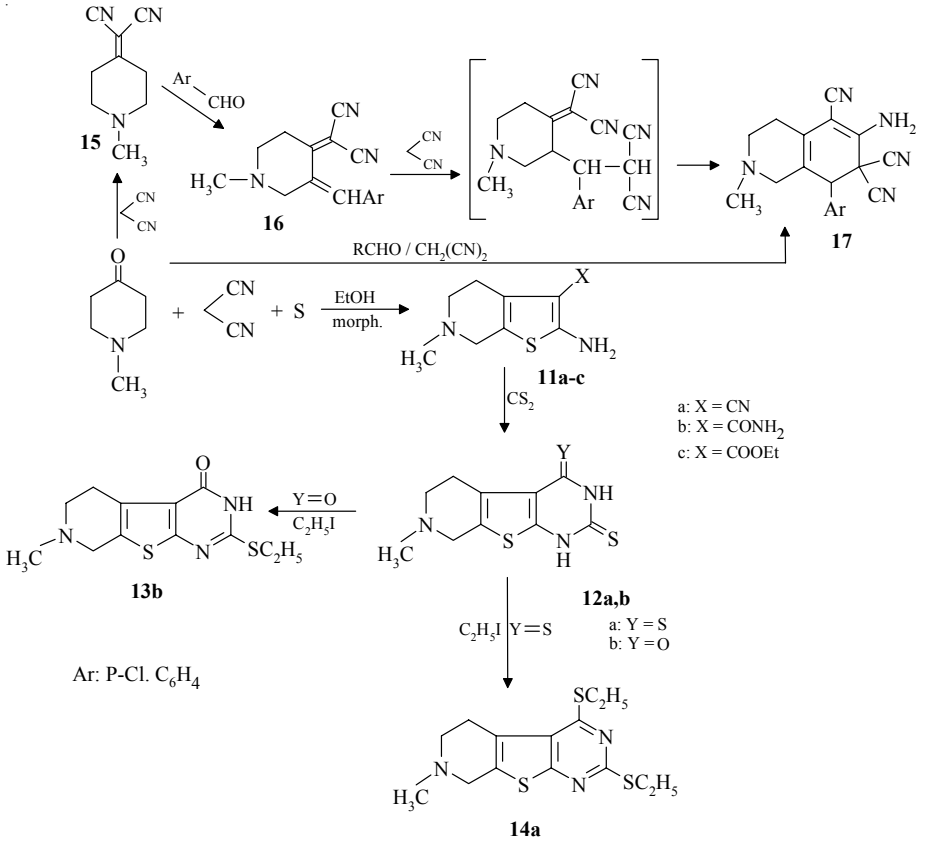
Fig. 2. Mass fragmentation pattern of compound **7**

TABLE-2
IR (cm^{-1}), ^1H NMR, ^{13}C NMR AND MASS SPECTRAL
DATA OF SYNTHESIZED COMPOUNDS

Compd. No.	IR (ν_{max}, cm^{-1})	^1H NMR δ (ppm) / ^{13}C NMR δ (ppm) / MS
1	3280 (2NH), 3050 (CH-Ar), 2900 (CH aliph.) 1650 (C=O)	2.50 (s, 3H, CH_3), 2.87, 3.09 (2s, 4H, $2CH_2$), 7.60-8.00 (m, 8H, Ar-H and ylidene CH), 7.19(s, 2H, 2NH-pyrrole ring).
2a	3440-3330 (NH_2), 3285 (2NH), 3035 (CH-Ar), 2950 (CH aliph.), 2200 (CN), 1590 (C=C)	2.22(s, 3H, CH_3), 3.00, 3.21(2s, 4H, $2CH_2$), 4.61(s, 1H, CH-pyron), 5.21 (s, 2H, NH_2), 6.50 (s, 2H, 2NH), 7.82-8.00 (m, 7H, Ar-H and ylidene CH).
2b	3424, 3332, 3280 (NH_2, 2NH), 3038 (CH-Ar), 2900-2880 (CH aliph.), 1710 (CO), 1600 (C=C).	1.50(t, 3H, CH_2CH_3, J=7.00 Hz), 2.50 (S, 3H, CH_3), 3.00, 3.38(2S, 4H, $2CH_2$) 4.20 (q, 2H, CH_2CH_3, J=8.90 Hz), 4.88 (S, 1H, CH-pyran), 5.80(S, 2H, NH_2), 6.88 (S, 2H, 2NH), 7.80-8.22 (m, 7H, Ar-H and ylidene CH).
2c	3424, 3332, 3300 (NH_2, 2NH), 3035 (CH-Ar), 2930 (CH aliph.), 1680 (CO), 1610 (C=C).	2.55 (S, 3H, CH_3), 3.22, 3.80 (2S, 4H, $2CH_2$) 4.80 (S, 1H, pyran-CH), 6.00 (S, 2H, NH_2), 6.22 (S, 2H, 2NH) 7.87-8.44 (m, 8H, Ar-H and ylidene-CH). ^{13}C: 40.11 (CH_3), 43.47 (CH), 50.00 (CH_2), 53.11(CH_2), 174.00 (C=O) and $C_{aromatic}$ 124.19, 126.32, 127.11, 128.90, 130.32, 131.00, 132.22, 133.81, 134.50, 136.90, 137.11, 137.95
3a	3300, 3180(3NH), 3050 (CH-Ar), 2910 (CH aliph.), 2221 (CN), 1660 (C=O), 1590 (C=C)	2.50 (S, 3H, CH_3), 3.20-3.65 (m, 4H, $2CH_2$), 6.38 (S, 2H, 2NH), 7.77-8.83 (m, 7H, Ar-H and ylidene CH) 12.33 (br. S, 1H, NH). ms: m/z = 331 (11.08% M^+)
3b	3270, 3200 (3NH), 3035(CH-Ar), 2950-2880 (CH aliph.), 1660 (C=O), 1720 (C=O), 1600 (C=C).	1.30 (t, 3H, CH_2CH_3, J=7.78Hz), 2.23 (S, 3H, CH_3), 3.00-3.44 (m, 4H, $2CH_2$), 4.44 (q, 2H, CH_2CH_3, J= 8.11Hz) 5.92 (S, 2H, 2NH), 7.52-8.36 (m, 7H, Ar-H and ylidene-CH), 11.80 (br.S, 1H, NH). ^{13}C: 14.00 (CH_3) 43.03 (CH_3), 50.22 (CH_2) 53.37 (CH_2), 60.11 (CH_2), 168.30 (C=O), 187(C=O), 129.11, 130.44, 130.90, 134.50, 135.00, 136.70, 137.11).
3c	3290, 3180 (3NH), 3050 (CH-Ar), 2885 (CH aliph.) 1680 (2C=O).	2.33 (S, 3H, CH_3), 3.22-3.52 (m, 4H, $2CH_2$), 6.33 (S, 2H, 2NH), 7.78-8.44 (m, 12H, Ar-H and ylidene C-H), 12.30 (br.S, 1H, NH). ^{13}C: 42.91 (CH_3), 55.01 (CH_2), 55.50 (CH_2), 168 (CO), 177 (CO) and $C_{aromatic}$: 124.07, 129.60, 131.66, 134.09, 135.50, 135.88, 136.33, 136.87, 137.11, 137.80, 138.11, 139.30.
3d	3380, 3280 (3NH), 3080 (CH-Ar), 2950 (CH aliph.), 2219 (CN), 1580 (C=C), 1330 (C=C).	2.50 (S, 3H, CH_3), 3.18-3.53 (m, 4H, $2CH_2$), 6.88 (2S, 2H, 2NH) 7.62-8.13 (m, 7H, Ar-H and ylidene C–H) ms: m/z = 347(18.02%M^+)
4a	3452, 3344, 2190 (NH_2, 2NH), 3090 (CH–Ar), 2900 (CH aliph.), 2190 (2CN), 1654 (C=O)	2.55 (S, 3H, CH_3), 4.20-4.42 (m, 4H, $2CH_2$), 5.80 (S, 2H, 2NH) 7.00-8.10 (m, 8H, Ar–H and NH_2) ms: m/z = 431(1.12%M^+)

Compd. No.	IR (ν_{max}, cm^{-1})	^1H NMR δ (ppm) / ^{13}C NMR δ (ppm) / MS
6	3400, 3387, (NH_2), 3093 (CH-Ar), 2900 (CH aliph.), 1640 (C=N).	2.50 (S, 3H, CH_3), 2.87, 3.09 (2S, 4H, $2CH_2$), 4.62 (S, 2H, NH_2). 7.71 (d, 1H, C_3–H, J= 5.3Hz), 7.34 (t, 1H, C_3a–H, J=5.55 Hz), 7.90-8.01 (m, 6H, Ar–H) 8.07 (d, 1H, HC=C, J=7.71 Hz) ^{13}C: 43.03(CH_3), 55.43(CH_2), 55.80 (CH_2), 53.37 (CH), 63.01 (CH), 181.10 (C=S) and $C_{oromatic}$: 124.21, 129.60 131.66, 134.09, 135.17, 136.80, 138.90. ms: m/z = 374 (18.11% M^+)
7	3037 (CH–Ar), 2913 (CH aliph.), 1660 (C=O) 1714 (C=O).	2.30 (S, 3H, $COCH_3$), 2.55(S, 3H, CH_3) 3.50, 3.81 (2S, 4H, $2CH_2$), 4.53 (S, 1H, C_4–H), 7.33 (t, 1H, C_{7a}–H, J= 8.40.7Hz) 7.70 (d, 1H, C_7 –H, J= 5.7Hz) 8.00 (S, 1H, HC=C), 8.07 (d, 1H, C_6–H, J= 5.50 Hz), 7.72-7.94 (m, 6H, Ar–H). ms: m/z = 383 (30.14% M^+)
8	3421 (NH), 3083 (CH–Ar), 2925 (CH aliph.), 2207 (CN) 1610 (C=C), 1213 (C=S).	2.50 (S, 3H, CH_3), 3.13, 4.16 (2S, 4H, $2CH_2$) 8.24-8.71 (m, 7H, Ar–H and =CH), 9.03 (S, 1H, NH), ^{13}C: 43.03(CH_3), 53.37(CH_2), 62.98 (CH_2), 124.21 (CN), 181.70 (C=S) and $C_{aromatic}$ 129.60, 131.60, 137.30, 138.14, 139.70, 140.13, 140.90. ms: m/z = 381 (11.01% M^+)
10	3471, 3374 (NH_2), 3066 (CH–Ar), 2940 (CH aliph.), 2210 (CN), 1711 (CO) 1633 (C=N) 1588 (C=C).	2.50 (S, 3H, CH_3), 3.04, 3.10 (2S, 4H, $2CH_2$), 4.06 (br. S, 2H, NH_2), 7.35 (d, 2H, C_2–H, C_6–H, phenyl, J = 8.60 Hz), 8.00 (S, 1H, HC=C), 7.10-7.27 (m, 6H, Ar–H). ^{13}C: 43.04 (CH_3), 53.30 (CH_2), 61.98(CH_2), 118.11 (CN), $C_{aromatic}$ 128.03, 128.81, 129.11, 129.83, 130.00, 130.44, 131.08, 132.86, 133.18, 134.50 and 168(CO). ms: m/z = 524 (3.33% M^+)
11a	3330, 3240 (NH_2) 2930 (CH aliph.), 2219 (CN) 1580 (C=C).	2.33 (S, 3H, CH_3), 2.90-3.55 (m, 4H, $2CH_2$), 3.82 (S, 2H, CH_2), 4.64 (S, 2H, NH_2)
11b	3400, 3320, 3200 ($2NH_2$), 2900 (CH aliph.), 1680 (CO), 1600 (C=C)	2.55 (S, 3H, CH_3), 3.22-3.61 (m, 4H, $2CH_2$), 3.88 (S, 2H, CH_2), 5.50, 6.21 (2S, 4H, $2NH_2$).
11c	3400, 3380, (NH_2), 2930-2870 (CH aliph.), 1710 (C=O), 1610(C=C), 3421 (NH), 3083(CH–Ar), 2925 (CH aliph.) 2207 (CN), 1610 (C=C) 1213 (C=S).	1.50 (t, 3H, CH_2CH_3, J= 7.12 Hz), 2.55 (S, 3H, CH_3), 2.81--3.33 (m, 4H, $2CH_2$), 3.90 (S, 2H, CH_2), 4.22 (q, 2H, CH_2CH_3, J= 8.55Hz) 5.82 (S, 2H, NH_2).
12a	3400, 3320, (2NH), 2880 (CH aliph.), 1580 (C=C), 1320 (C=S)	2.60 (S, 3H, CH_3), 3.00-3.33 (m, 4H, $2CH_2$), 3.85 (S, 2H, CH_2), 12.80 (S, 2H, 2NH). ms : M/z = 269 (12.61% M^+
12b	3480, 3430, (2NH), 2980 (CH aliph.), 1680 (C=O), 1600 (C=C), 1300 (C=S)	2.55 (S, 3H, CH_3), 3.22-3.80 (m, 4H, $2CH_2$), 4.00 (S, 2H, CH_2), 12.50 (S, 1H, NH), 13.92 (S, 1H, NH).

Compd. No.	IR (ν_{max},cm^{-1})	^1H NMR δ (ppm) / ^{13}C NMR δ (ppm) / MS
13b	3400 (NH), 2900-2880 (CH aliph.), 1700 (C=O), 1600 (C=C).	1.4 (t, 3H, CH_2CH_3, J= 8.55Hz), 2.55 (S, 3H, CH_3), 3.00--3.33 (m, 4H, $2CH_2$), 4.00 (S, 2H, CH_2), 4.35 (q, 2H, CH_2CH_3, J= 9.55Hz) 12.03 (S, 1H, NH). ms : M/z = 281 (3.38% M^+)
14a	2950-2870 (CH aliph), 1640 (C=N), 1590 (C=C).	1.50 (t, 3H, CH_2CH_3, J= 7.55 Hz), 2.55 (S, 3H, CH_3), 3.00--3.35 (m, 4H, $2CH_2$), 4.00 (S, 2H, CH_2), 4.82 (q, 2H, CH_2CH_3, J= 8.80Hz) ms : M/z = 325 (6.11% M^+)
15	2980-2870 (CH aliph.), 2190 (2CN), 1580 (C=C).	2.58 (S, 3H, CH_3), 3.32, 3.55 (2S, 4H, $2CH_2$), 3.80, 4.00 (2S, 4H, $2CH_2$).
16	30.55(CH–Ar), 2900-2880 (CH aliph.) 2222 (2CN), 1610 (C=C).	2.60 (S, 3H, CH_3), 3.33-3.78 (m, 4H, $2CH_2$), 4.22 (S, 2H, CH_2), 7.66-8.12 (m, 5H, Ar–H and = CH)
17	3380, 3210 (NH_2), 3050 (CH–Ar), 2920 (CH aliph), 2223 (3CN), 1575 (C=C).	2.58 (S, 3H, CH_3), 3.12-3.35 (m, 4H, $2CH_2$), 3.80 (S, 2H, CH_2), 4.80 (S, 2H, NH_2), 5.20 (S, 1H, CH) 7.25-7.60 (m, 4H, Ar–H) ms : M/z = 349 (4.11% M^+)



Scheme-II

On the other hand, when 1-methyl-4-piperidone was allowed to condense first with malononitrile followed by the interaction of the condensation product, 1-methyl-4-piperidonylidene malononitrile (**15**) with *p*-chloro benzaldehyde,3-*p*-chloro benzylidene-1-methyl-4-piperidony-lidene malononitrile (**16**) was obtained. The interaction of 1-methyl-4-piperidone with a mixture of one equivalent of *p*-chloro benzaldehyde and two equivalent of malononitrile gave a product which was identified as 6-amino-5,7,7-tricyano-8-*p*-chlorophenyl-2-methyl-1,2,3,4,7,8-hexahydroisoquinoline (**17**) (**Scheme-II**).

The antitumor activity data of the synthesized compounds are given in Table-3.

TABLE-3
In vitro CYTOTOXIC ACTIVITY OF SYNTHESIZED COMPOUNDS

Compd. No.	Non-viable cells (%) concentration (µg/mL)		
	100 %	50 %	25 %
1	85	70	80
2a	80	50	30
2b	60	40	20
2c	50	20	30
3a	80	70	50
3b	80	50	30
3c	70	30	20
3d	90	80	80
4a	90	80	80
5	80	80	90
6	90	80	80
7	60	40	20
8	80	80	70
10	70	80	80
11a	50	40	40
11b	80	80	90
12a	90	85	80
12b	85	80	60
16	80	85	80
17	80	55	40
Doxorubicin [Ref. 26]	100	55	20

The relation between surviving fraction and drug concentration was plotted to obtain this survival curse of tested cell, the response parameter calculated was IC_{50} value. The data to tested compounds are summarized in Table-4.

The α,β-unsaturated ketone found in compounds **1** and **5** proved to be essential for antitumor activity[27]. Cyclocondensation of 1,5 afforded pyrido[3,2-c]pyridine-2-thione (**3d**), **8** and pyrazolo[4,3-c]pyridine **6** at which the olefinic residue, remaining from the chalcone function, in addition to the introduction heterocyclic ring, which showed to be active toward the used tumor cell lines. Compounds **4a**,

TABLE-4
In vitro ANTI-HEPG2 AND ANTI-MCF7 TESTING
RESULTS OF SYNTHESIZED COMPOUNDS

Compd. No.	Anti-HEPG2[a] at IC^{c}_{50} (µg/mL)	Anti-MCF7[b] at IC_{50} (µg/mL)
1	22.10	78.50
3d	14.60	88.30
4a	90.80	33.20
5	16.30	28.80
6	28.40	60.70
8	66.30	54.80
10	90.10	75.20
11b	80.61	95.30
12a	50.60	46.40
12b	100.00	99.20
16	90.80	78.90
Positive control	Zerumbone = 3.45 + 0.026 µg/mL	Doxorubicin = 43.6 µg/mL

[a]Liver carcinoma cell line, [b]Breast carcinoma cell line, [c]Concentration of compounds which cause 50 % inhibition of cell growth.

9b, **10** and **14** with electron-withdrawing group such as cyano results in reduction of activity[28]. Compound **12a** is the most active member among the thiol group, while compound **12b** proved to be inactive toward the used tumor cell line due to presence of an ionizable hydrogen[29].

On the basis of the structure of tested compound, it is concluded that the structure activity relationships provided evidence, that geometery, size and shape of the compound is an important as their substituents. These heterocycles could be considered as useful template for future development and further derivatization or modification to obtain more potent and selective antitumor agents.

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